Synthesis and structure-activity relationships of N^G-acylated arylalkylguanidines and related compounds as histamine receptor ligands: Searching for selective H_4R agonists

Dissertation

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Everything should be made as simple as possible, but not simpler.

Albert Einstein (1879 – 1955)

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Contents

Abbreviations

abs	absolute
Anal.	analysis
aq.	aqueous
Ar	aromatic
ATP	adenosine triphosphate
Boc	*tert*-butoxycarbonyl
B_{max}	the maximal specific binding of a ligand
bp	boiling point
BRET	bioluminescence resonance energy transfer
n-BuLi	*n*-butyl lithium
cAMP	cyclic 3', 5'-adenosine monophosphate
cat.	catalytical amounts
Cbz	benzyloxycarbonyl
CDI	*N*,*N*'-carbonyldiimidazole
cHex	cyclohexyl
CI	chemical ionization
CNS	central nervous system
COSY	correlated spectroscopy
C_{quat}	quaternary carbon atom
CREB	cAMP response element binding protein
d	day(s) or doublet
DAG	diacylglycerol
DCM	dichloromethane
dec.	decomposition
DIAD	diisopropyl azodicarboxylate
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*, *N*-dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	per-deuterated DMSO
DNABP	DNA binding protein
E1, E2	1st and 2nd extracellular loop of a GPCR
EDC	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide
EC_{50}	molar concentration of the agonist causing 50 % of the maximal response

EDTA	ethylendiaminetetraacetic acid
EI	electron impact ionization
E_{max}	maximal response relative to histamine (1.00)
eq	equivalents
ES	electrospray ionization
FRET	fluorescence resonance energy transfer
Fur	furanyl
G	G-protein
GDP	guanosine diphosphate
GPCR	G-protein coupled receptor
gp	guinea pig
gpH_1R	guinea pig histamine H_1 receptor
gpH_2R	guinea pig histamine H_2 receptor
GTP	guanosine triphosphate
h	hour(s) or human
HR	histamine receptor
hH_1R	human histamine H_1 receptor
hH_2R	human histamine H_2 receptor
hH_2R-$G_{s\alpha S}$	fusion protein between the hH_2R and short splice variant of $G_{s\alpha}$
hH_3R	human histamine H_3 receptor
hH_4R	human histamine H_4 receptor
hH_4R-RGS19	fusion protein between the hH_4R and RGS19
HMBC	heteronulcear multiple bond correlation
HPLC	high performance (pressure) liquid chromatography
HR-MS	high resolution mass spectroscopy
H_1R, H_2R, H_3R, H_4R	histamine receptor subtypes
HSQC	heteronuclear single quantum coherence
IC_{50}	functional assay: antagonist (inverse agonist) concentration suppressing 50 % of an agonist induced effect radioligand binding assay: ligand concentration inhibiting the binding of a radioligand by 50 %
IgE	immunoglobuline E
Im	imidazolyl
IP_3	inositol-1,4,5-trisphosphate
IP_3R	inositol trisphosphate receptor
IR	infrared spectroscopy
J	coupling constant

k’	capacity factor
K_B	dissociation constant (functional assay)
K_D	dissociation constant (saturation binding)
K_I	dissociation constant (competition binding)
k_{ob}	observed rate constant
k_{on}	association rate constant
k_{off}	dissociation rate constant
LSI	liquid secondary ion
m	multiplet
MAPK	mitogen-activated proteine kinase
min	minute(s)
mp	melting point
MS	mass spectrometry
N^G	guanidino-nitrogen
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
PE	petroleum ether
Ph	phenyl
P_i	inorganic phosphate
PIP_2	phosphatidylinositol-4,5-bisphosphate
PKA	protein kinase A
PKC	protein kinase C
PLC_β	phospholipase C_β
ppm	part per million
Pyr	pyridyl
Pyraz	pyrazolyl
Phth	phthalimide
pEC_{50}	negative decadic logarithm of the molar concentration of the agonist causing 50 % of the maximal response
q	quartet
quin	quintet
r^2	coefficient of determination
R	inactive state of a GPCR
R*	active state of a GPCR
RGS	regulator of G-protein signaling
RP	reversed phase
rt	room temperature

rpm	revolutions per minute
s	singlet
SEM	standard error of the mean
sep	septet
Sf9	*Spodoptera frugiperda* insect cell line
t	triplet
t_0	dead time
TBME	*tert*-butylmethylether
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thio	thiophenyl
Thiaz	thiazolyl
TLC	thin layer chromatography
TM	transmembrane
TMEDA	N^1,N^1,N^2,N^2-tetramethylethane-1,2-diamine
t_R	retention time
Triaz	1*H*-1,2,4-triazolyl
Tris	tris(hydroxymethyl)aminomethane

Chapter 1

Introduction

1.1 G-protein coupled receptors

1.1.1 GPCRs as drug targets and their classification

G-protein coupled receptors (GPCRs) represent the largest group of integral membrane receptors.[1-6] About 800 identified GPCRs correspond to about 2 % of the human genome.[1] Due to the multitude of important physiological functions influenced by GPCRs, these receptors are also involved in a variety of diseases including pain, asthma, inflammation, obesity, cancer, as well as cardiovascular, metabolic, gastrointestinal and CNS diseases.[7] This makes GPCRs one of the most important class of drug targets. Approximately 30 % of the currently marketed drugs address about 40 GPCRs.[1,5] This offers a large space for the further development of new drugs targeting this class of receptors.

A protein has to fulfill two requirements to be classified as a GPCR. One requirement is the existence of seven α-helical transmembrane (TM) domains, each consisting of about 25 to 35 amino acids. Besides this structural requirement, the receptor has to interact with intracellular heterotrimeric G-proteins. The GPCR is able to bind an extracellular ligand and transduce the signal *via* the G-protein into the cell.[2]

GPCRs can be divided in two groups: “endoGPCRs” (≈ 400 GPCRs) that are addressed by endogenous ligands like peptides, lipids, prostanoids, neurotransmitters, nucleosides and nucleotides and “csGPCRs” (chemosensory GPCRs, ≈ 400 GPCRs) that respond to external signals like odors, tastes, photons or pheromones.[8, 9] Endogenous ligands have been identified for more than 260 endoGPCRs. The remaining GPCRs are so-called “orphan” receptors as their endogenous ligands are not known to date. Based on structural differences, mammalian GPCRs can be grouped in following receptor families: rhodopsin, secretin, adhesion, glutamate and frizzled/taste2 receptor families.[5] All families share a common architecture: an extracellular N-terminus and intracellular C-terminus and seven transmembrane α-helices that are connected by three extracellular and three intracellular loops. The rhodopsin receptor family (class A) represents the largest subfamily comprising 672 GPCRs (including 388 csGPCRs). Opsins, olfactory GPCRs, small-molecule/peptide

hormone GPCRs and glycoprotein hormone GPCRs belong to this class. Numerous highly conserved amino acids in the seven TM domains, a short N-terminal tail and a disulfide bridge linking the extracellular loops E1 and E2 are characteristic features of the class A GPCRs. Small molecules bind to these receptors within the seven TM domains, whereas more space filling ligands like peptides and glycoproteins interact with the N-terminus, extracellular loops and amino acids located at the top of the TM helices. The secretin-like receptor family (class B) includes GPCRs for peptides like secretin, calcitonin and parathyroid hormone. This class of GPCRs has 15 members and is characterized by a N-terminus that contains a network of three disulfide bridges forming a globular domain structure. Activation of the receptor obviously occurs by bridging the N-terminus with extracellular loops and TM segments *via* the ligand. The 33 members of the adhesion GPCR family usually possess long and highly glycosylated N-termini and are thought to participate in cell adhesion. The metabotropic glutamate receptors (mGluRs), the γ-aminobutyric acid type B receptors ($GABA_BRs$) and Ca^{2+}-sensing receptors (CaRs) belong to the glutamate receptor family (class C). The 22 members of this GPCR-family are usually characterized by very large N- and C-terminal tails, a disulfide bridge connecting E1 and E2 and a very short third intracellular loop. The N-terminus, containing a Venus flytrap module, forms the ligand binding site. The 11 frizzled and smoothened receptors play a role in cell development and proliferation. The taste2 receptor family comprises 25 members that are crucial for the detection of the bitter taste of compounds.[1, 2, 5, 9]

A milestone in GPCR research was the determination of the crystal structure of bovine rhodopsin by Palczewski in 2000 which provided first insight into the three dimensional arrangement of a mammalian class A GPCR. This structure served as template for the generation of numerous GPCR homology models. Such homology models are powerful tools to study GPCR conformations and ligand-receptor interaction on the molecular level and for virtual screening of compound libraries. The recently solved crystal structures of the human β_2-adrenergic receptor[10-12] and the turkey β_1-adrenergic receptor[13] revealed some unexpected differences relative to bovine rhodopsin. In contrast to bovine rhodopsin in both aminergic GPCRs the ionic-lock (salt bridge between $Arg^{3.50}$ and $Glu^{6.30}$ stabilizing the inactive state of rhodopsin) is absent and the E2 contains an α-helix.[11, 12] The recent crystal structures and the resulting improved homology models will facilitate the target-based drug design for many GPCRs.

1.1.2 G-protein cycle and signal transduction

GPCRs in the active conformation (can be agonist-free considering constitutively active GPCRs or stabilized by an agonist) are able to activate heterotrimeric G-proteins which transduce the external signal into the cell. These G-proteins consist of a G_α-subunit and a

$G_{\beta\gamma}$-complex (Figure 1.1).[14, 15] Binding of the G-protein to the GPCR induces a conformational change of the G-protein and results in the release of GDP from the G_{α}-subunit and the formation of the ternary complex. The ternary complex consisting of the agonist, the receptor and the nucleotide free G-protein is characterized by high affinity for agonists. Binding of GTP to the G_{α}-subunit causes a conformational change of the G-protein and disrupts the ternary complex. The $G_{\alpha\text{-GTP}}$-subunit and the $G_{\beta\gamma}$-complex dissociate from the receptor and from each other and interact with effector proteins like enzymes or ion-channels resulting in cellular biological responses. After a certain period of time, the G_{α}-induced effector modulation is terminated due to the intrinsic GTPase activity of G_{α}. GTP bound to G_{α} is hydrolyzed to GDP and phosphate, and the GDP-bound G_{α}-subunit re-associates with the $G_{\beta\gamma}$-subunit allowing the next G-protein cycle.[16] Besides the GPCRs, the activity of G-proteins is also receptor independently modulated by a family of proteins named regulators of G-protein signaling (RGS). These proteins stimulate the GTPase activity of the α-subunit.[17-19]

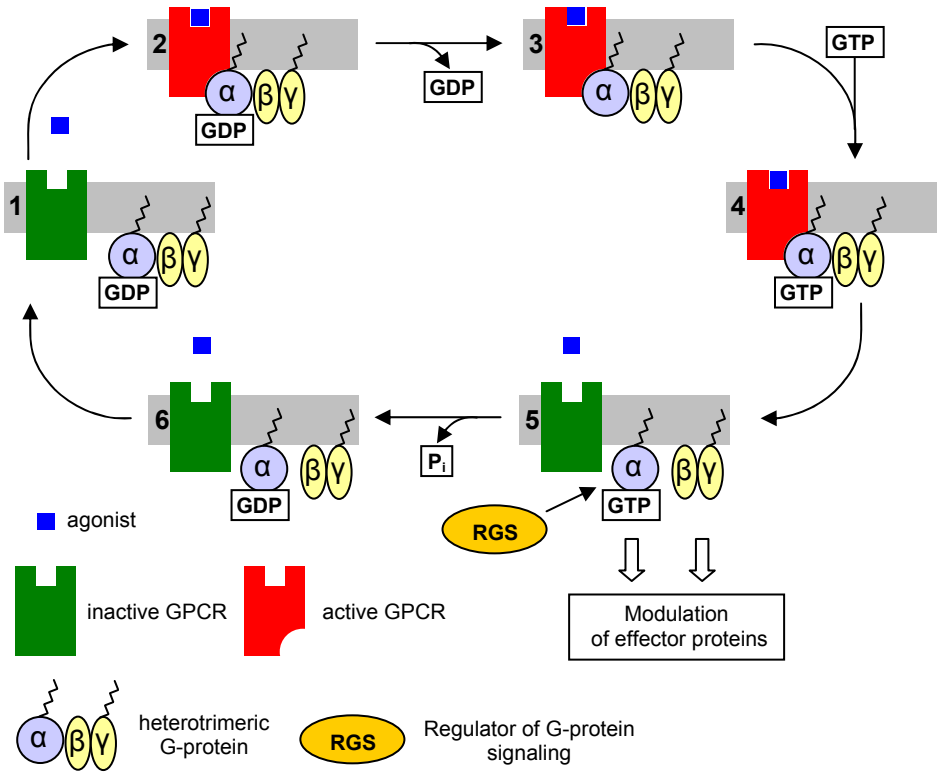


Figure 1.1. The G-protein cycle.

The 16 genes encoding for α-subunits of G-proteins result in minimum 28 distinct subunits that are divided in four subfamilies, termed G_s, $G_{i/o}$, $G_{q/11}$, $G_{12/13}$[9, 20], based on their structure and signaling pathway. While some α-subunits are very restrictedly expressed, others are localized in numerous tissues or are more or less ubiquitously found.[15] Five different β- and

12 different γ-subunits have been reported (splice variants are not included).[20] All α- and γ-subunits hold lipid anchors keeping the G-proteins in proximity to the membrane and therefore facilitate interactions with membrane proteins such as GPCRs.[21, 22]

Members of the $G_{\alpha s}$-subfamiliy activate adenylyl cyclases (AC 1 – 9) resulting in increased cellular cAMP turnover (Figure 1.2). In contrast, $G_{\alpha i}$ inhibits AC (5 and 6) activity.[9] The second messenger cAMP exerts various effects on effector proteins as activation of the protein kinase A (PKA) or the mitogen-activated protein kinase (MAPK) pathway both modulating gene expression.[23] Phosphodiesterases inactivate cAMP and terminate the signal transduction. $G_{\alpha o}$-proteins for example inhibit voltage dependent Ca^{2+}-channels.[24] Activated $G_{\alpha q/11}$-subunits stimulate the phospholipase C_β (PLC_β) resulting in hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) and formation of the second messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor (ligand-gated Ca^{2+}-ion channel) which is located in the membrane of the endoplasmatic reticulum and promotes the release of Ca^{2+}-ions from this intracellular store into the cytoplasm.[25] DAG activates the protein kinase C (PKC) and modulates the function of cellular proteins by phosphorylation. IP_3 is inactivated by dephosphorylation, whereas DAG is degraded by lipases or inactivated by phosphorylation.[26, 27] Finally, the $G_{\alpha 12/13}$-proteins have been shown to interact with Ras homology guanine nucleotide exchange factors (RhoGEFs).[9] Besides the G_α-subunit, also the $G_{\beta\gamma}$-complex has been shown to be involved in signal transduction. Examples for $G_{\beta\gamma}$-regulated effectors are the G-protein regulated inwardly rectifying K^+-channels[28], isoforms of the adenylyl cyclase[29] and the PLC[30]. Meanwhile also G-protein independent signaling pathways of GPCRs have been reported.[23] However, GPCR signal transduction is much more complex than the presented classical pathways and the insight into these networks rapidly increases.

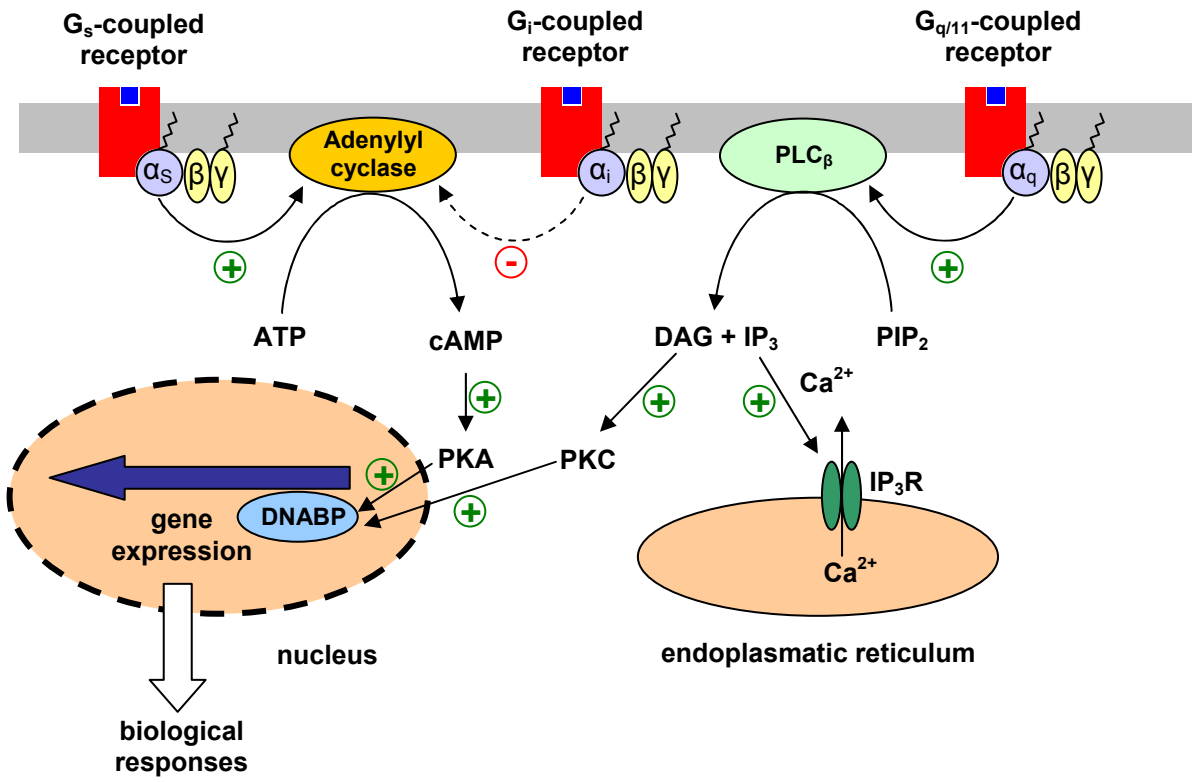


Figure 1.2. Classical signal transduction pathways of G-protein coupled receptors. Adapted from Jacoby et al.[1] (DNABP: DNA binding protein, PKA/PKC: protein kinase A/C, DAG: diacylglycerol, IP_3: inositol-1,4,5-trisphosphate, PIP_2: phosphatidylinositol-4,5-bisphosphate, PLC_β: phospholipase C_β, IP_3R: IP_3 receptor).

1.1.3 Receptor models of GPCR activation and ligand classification

Different models have been developed to describe the interaction between a GPCR, the G-protein and the ligand. The most simple one is the ternary complex model (Figure 1.3).[31] However, in this model agonist binding to the receptor is condition to activate the G-protein and therefore, constitutive activity and inverse agonistic activity of ligands can not be explained. This resulted in the extended ternary complex model which describes an equilibrium between two distinct receptor states: an inactive and an active receptor state.[32, 33] The inactive receptor (R) state is allowed to isomerize to an active receptor state (R*) independently from agonist binding. The ratio between [R*] and [R] describes the degree of constitutive activity of a GPCR. The active state of the receptor is able to bind G-proteins and promote GDP/GTP exchange resulting in signal transduction. In contrast to the extended ternary complex model, the thermodynamically more complete cubic ternary model additionally includes the formation of non-signaling complexes (RG and ARG) between the G-protein and the inactive state of the receptor.[34-36]

Full agonists preferentially bind to and stabilize the active state of the receptor and produce a maximal biological response (efficacy). Inverse agonists particularly bind and stabilize the inactive state of the receptor and reduce the basal G-protein activity. Partial agonists and partial inverse agonists are less effective in stabilizing the active and the inactive state of the

receptor, respectively. Neutral antagonists do not distinguish between the different receptor states and do not affect the equilibrium.[37]

However, the two-state model of GPCR activation can not sufficiently describe all observed experimental findings. GPCRs are assumed to exist not just in one active conformation, but to form multiple active state receptor conformations. There is increasing evidence that structurally different agonists stabilize distinct receptor conformations which can result in diverse biological responses.[38] Development of such ligands acting on one and the same GPCR but modulating different signal transduction pathways may become promising for the fine-tuning of drug action. However, further studies are required to elaborate the potential therapeutic relevance of distinct GPCR phenotypes.[9, 39-42]

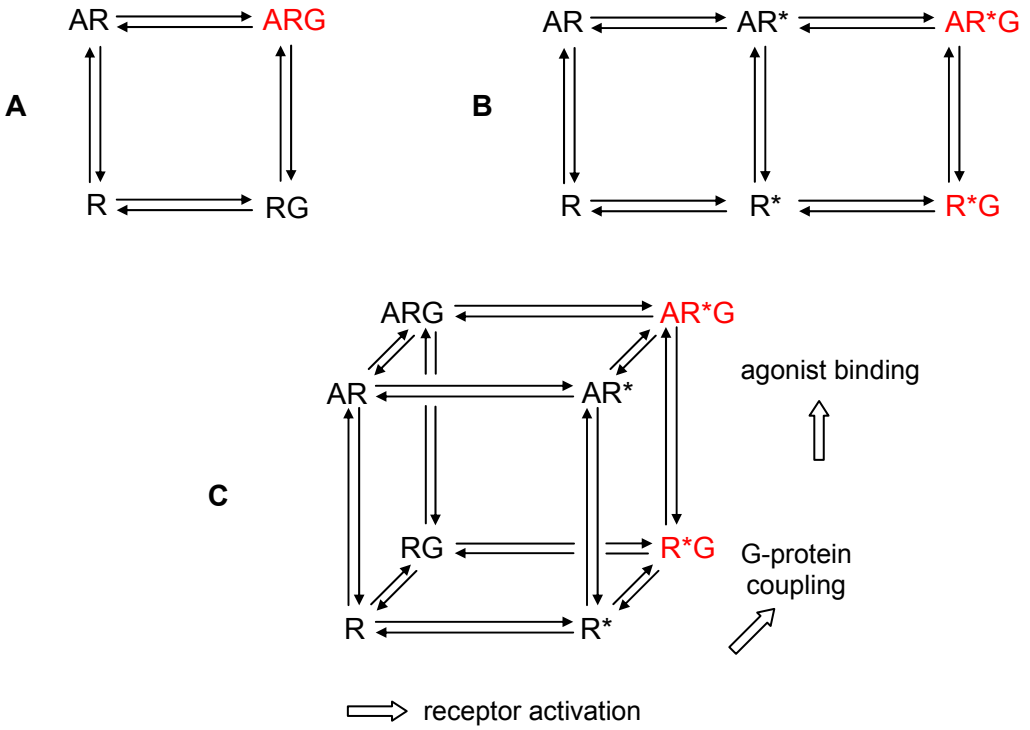


Figure 1.3. Models describing the interaction between GPCR, agonist and G-protein (R: inactive state of the receptor; R*: active state of the receptor; G: G-protein; A: agonist). A, Ternary complex model: This model allows no discrimination between R and R*; B, Extended ternary complex model: This model accounts for R and R*; C, Cubic ternary complex model: This model permits the interaction of G with R resulting in non-signaling complexes. Signaling complexes mediating GDP/GTP exchange are highlighted in red.

1.1.4 GPCR oligomerization and "bivalent ligands"

Over a long period of time GPCRs were considered to act as monomeric entities in a 1:1:1 stoichiometry with the G-protein and the ligand.[43] However, experiments employing different techniques like cross-linking, immunoblotting and co-immunoprecipitation as well as FRET (fluorescence resonance energy transfer) and BRET (bioluminescence resonance energy transfer) investigations in living cells provided convincing evidence for GPCRs to form dimers or higher order oligomers.[1, 44, 45] The existence of homodimers has been demonstrated for

several class A and C GPCRs including the dopamine D_2 and D_3 receptors,[46, 47] the β_2-adrenoceptor,[48] the H_1Rs, H_2Rs and H_4Rs,[49-51] opioid receptors[52-54], the mGluRs[55, 56] and CaRs[57]. Besides homodimers also heterodimers like the δ/κ-opioid receptors,[53] the somatostatine $SSTR_{1B}$/dopamine D_2 receptors[58] and the $GABA_{B1}$/$GABA_{B2}$ receptors[59] have been identified. The GPCRs are believed to interact *via* their extracellular loops, transmembrane helices and intracellular loops, forming covalent or non-covalent interactions.[44] Examples for covalently linked receptors are the mGluRs and CaRs providing dimers *via* disulfide bonds. Conversely, the $GABA_{B1}$R/$GABA_{B2}$R heterodimer forms non-covalent interactions through a C-terminal coiled-coil domain.[9] Although, few is known about the physiological role of GPCR dimerization, several investigations suggest a crucial role in GPCR trafficking, folding, activation and internalization.[44] The $GABA_{B1}$R/$GABA_{B2}$R heterodimer is a demonstrative example: The presence of $GABA_{B2}$R is prerequisite for a proper transfer of $GABA_{B1}$R to the cell surface. Moreover, the $GABA_{B1}$R binds its agonist but does not couple to the G-protein, whereas the $GABA_{B2}$R stimulates G-protein signaling but does not bind the ligand.[59-61] There is growing evidence that GPCR dimerization, in particular heterodimerization, results in complexes with modified ligand binding and signal transduction properties relative to the individual receptors.[9, 54] Distinct characteristics arising from heterodimerization have been shown for the κ- and δ-opioid receptors[53], the μ- and δ-opioid receptors[54] or the angiotensin AT_1 and bradykinin B_2 receptors[62]. However, as many experiments have been performed in recombinant cell systems, the physiological relevance of GPCR oligomerization has to be further elucidated in ongoing studies.

The bivalent ligand approach in the design of ligands targeting GPCRs has proven to be promising to improve not only potency and selectivity but also the pharmacokinetic profile of compounds.[63] Usually bivalent ligands are characterized by a molecule containing two sets of pharmacophoric entities linked through a spacer. However, in the broader sense bivalent ligands can be divided in molecules comprising two sets of pharmacophoric groups or a single pharmacophore connected to a non-pharmacophoric recognition unit.[64, 65] Two different binding modes for bivalent ligands at the receptor(s) are imaginable (Figure 1.4). If the spacer is of sufficient length the bivalent ligand may bridge two neighboring receptors, each pharmacophoric entitiy interacting with the binding site of one receptor. Molecular modeling studies with μ-opioid receptor dimers estimate a distance between the binding sites of approximately 27 Å.[65] For bivalent ligands with shorter linkers, next to the binding site an accessory recognition site at a single receptor is probable. The existence of accessory binding sites has been demonstrated for bivalent opioid receptor antagonists and explains increased affinities of bivalent ligands, that are not capable to link two receptors, relative to the monovalent counterparts.[63-66] According to Portoghese,[64] such bivalent ligands containing spacers of insufficient length for bridging two receptors fit to the “message-address” concept

proposed by Schwyzer[67]. The pharmacophore can be considered as the “message” which is recognized by a family of receptors and, in case of agonists, is responsible for receptor activation, whereas the second pharmacophoric or non-pharmacophoric entity is considered as the “address” conferring additional affinity.

The increase in affinity of bivalent ligands relative to their monovalent analogs can be explained by the assumption that the bivalent ligands first bind in a univalent manner to the receptor (Figure 1.4). Thereby, the second recognition unit of the bivalent ligand achieves closer proximity to the second binding site (neighboring receptor or accessory binding site) corresponding to a high local concentration of the second recognition unit. This should afford an increase in affinity greater than expected from the sum of its two monovalent pharmacophores.[64, 65] The spacer length of the bivalent ligands plays a crucial role with respect to affinity as too short spacers prevent bridging of the binding sites, whereas too long linkers reduce the residence time of the recognition unit in vicinity to the binding site. Moreover, immobilization of the flexible linker of the bivalent ligand upon receptor binding results in a decrease in entropy. That means, the improved affinity of a bivalent ligand is enthalpy driven and to some extent compensated through the loss of entropy (ignoring a possible increase in entropy due to linker-mediated dehydration of a receptor surface).[68, 69] For many bivalent ligands containing a linker of insufficient length to bridge neighboring receptors an increase in selectivity relative to the monovalent counterparts is observed.[63] This can be explained with an accessory binding site for the second pharmacophore (Figure 1.4 A) present only on one receptor subtype. The affinity of bivalent ligands can also be influenced by cooperativity effects.[64, 65]

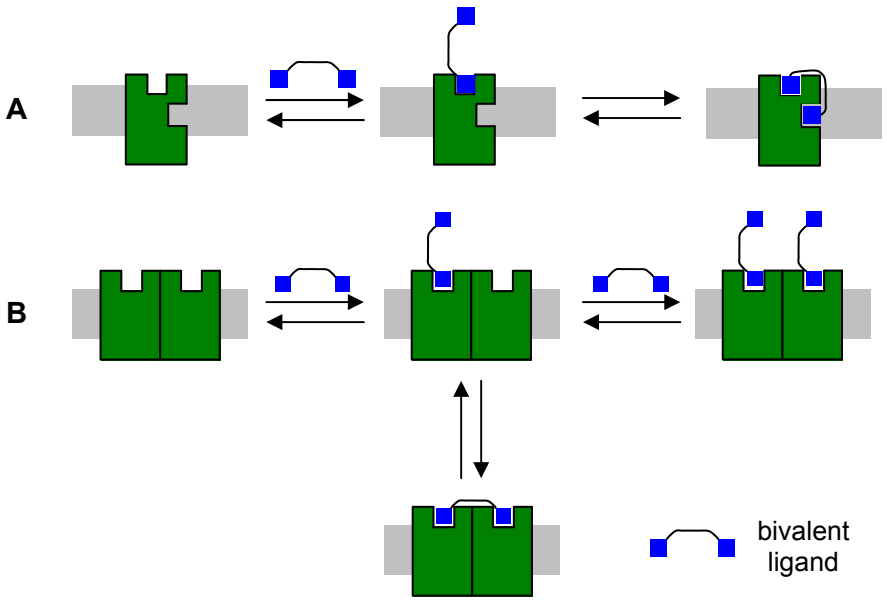


Figure 1.4. Bivalent ligand binding to A, a GPCR with an accessory binding site, or to B, a GPCR dimer. The bivalent ligand is believed to bind first in a univalent manner before addressing the second binding site. Concerning the receptor dimer (B) univalent binding of a second bivalent ligand is possible. However, bridging neighboring receptors *via* the bivalent ligand is favored. Adapted from Portoghese et al.[65]

Taken together, the bivalent ligand approach is a promising way not just to design highly potent and selective compounds. The recent advances in studying GPCR dimerization and the rising evidence for heterodimers to form a kind of new receptor subtypes with modified pharmacological behavior will promote the demand for molecules addressing neighboring receptors simultaneously.[9, 43] Such bivalent ligands are required as pharmacological tools to study receptor dimerization and the potential of GPCR dimers and higher order oligomers as drug targets.

1.2 Histamine and histamine receptor subtypes

1.2.1 The biogenic amine histamine: An overview

The first synthesis of the biogenic amine histamine (**1.1**, 2-(1*H*-imidazol-4-yl)ethanamine) has been reported by Windaus and Vogt in 1907.[70] Three years later, Sir Henry Dale and his colleagues at the Wellcome Laboratories were able to isolate this amine from *Secale cornutum*.[71, 72]

Histamine contains an imidazole ring that forms the two possible tautomers, **1.1a** and **1.1b** (Figure 1.5). The monocation of histamine preferentially forms the N^{τ}-tautomer (τ from the Greek telos) in aqueous solution (≈ 80 %)[73, 74] and in the crystal (100 %)[75], whereas the histamine base crystallizes in the N^{π}-tautomeric (π from the Greek pros) form (100 %)[76]. With the imidazole ring (pK_a = 5.8) and the primary amino group (pK_a = 9.4) histamine contains two basic centers[77] with the monocation predominating at physiological pH.[78]

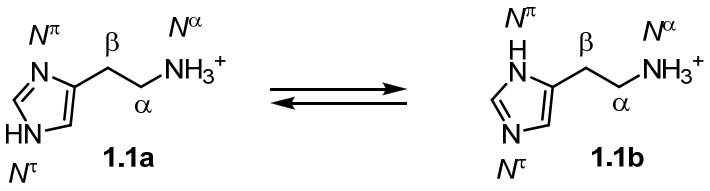


Figure 1.5. Tautomeric forms of the histamine monocation.

Histamine is formed in the body by decarboxylation of the amino acid L-histidine (**1.2**) catalyzed by the enzyme L-histidine-decarboxylase (HDC) or L-aromatic amino acid decarboxylase under participation of the cofactor pyridoxalphosphate (Figure 1.6).[79, 80] Released histamine is rapidly inactivated by two pathways. In one route the primary amino group is oxidized in two steps catalyzed by the enzymes diamine oxidase and xanthine oxidase to imidazole-4-acetic acid (**1.7**). This acid is further metabolized by ribosylation of the imidazole ring. The major metabolic pathway in humans starts with the histamine *N*-methyltransferase (HNMT) catalyzed methylation (cofactor *S*-adenosyl-methionin) of the

imidazole N^{τ}-nitrogen followed by subsequent oxidation of the primary amine to the corresponding carboxylic acid.[81] Two possible models for the inactivation of released histamine *via* the HNMT are discussed. According to the plasma membrane hypothesis the HNMT can be translocated from the cytosol to the cell membrane and act on the cell surface. The transporter hypothesis considers histamine to enter the cell by using organic cation transporters (OCTs) before being inactivated in the cytosol by the HNMT.[82] The half-life of histamine is very short with less than 10 s in the rat and 20 – 30 s in the dog.[72]

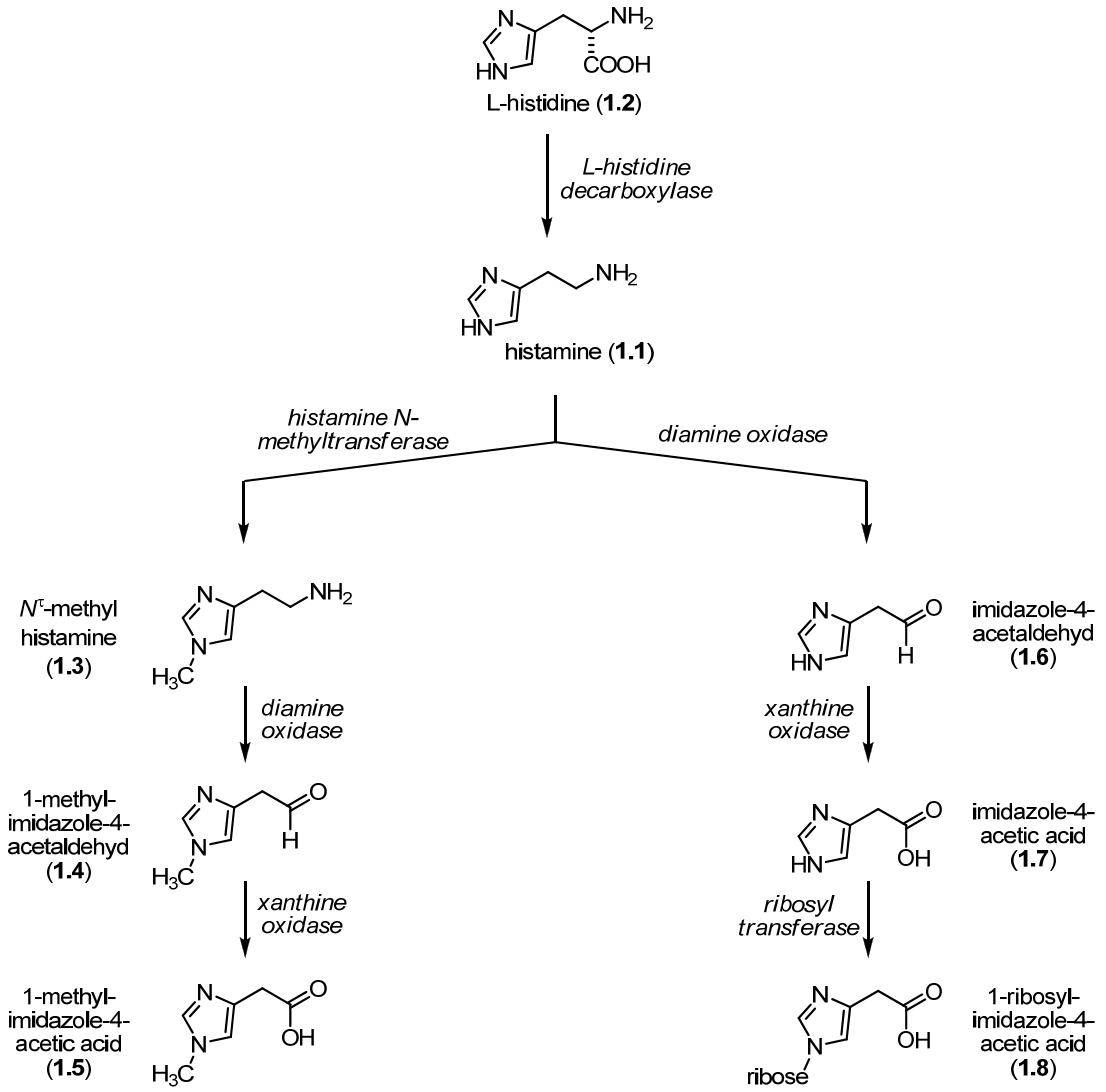


Figure 1.6. Biosynthesis and biotransformation of histamine.

High tissue concentrations of histamine are found in particular in the lungs, the skin, connective tissues and gastrointestinal tract.[72] From the cellular point of view histamine is located in mast cells[83], blood basophils[84], blood platelets[85], enterochromaffin-like (ECL) cells of the stochmach[86], endothelial cells[87] and also in neurons[88]. In mast cells and basophils histamine is stored in secretory granules and released during allergic conditions resulting in smooth muscle contraction, vasodilatation and an increase in vascular permeability.[89]

Besides the IgE-mediated histamine liberation, also peptides, drugs, toxins or chemicals like substance P, mastoparan, morphine or compound 48/80 can trigger histamine release from mast cells and basophils.[90-92] Histamine released from ECL cells controls gastric acid secretion from parietal cells.[93] In histaminergic neurons that are mainly located in the tuberomamillary nucleus of the posterior hypothalamus, histamine acts as a neurotransmitter playing a crucial role in sleep/waking cycle, learning and memory, anxiety, locomotion, feeding and drinking and neuroendocrine regulation.[94] Certain cells like macrophages, dendritic cells, neutrophils and T-cells also have been found to release histamine after *de-novo* synthesis without prior storage in granules.[89]

The mentioned effects of histamine are all mediated by four histamine receptor (HR) subtypes: the H_1R, H_2R, H_3R and H_4R. All these receptors are members of class A or rhodopsin-like GPCRs.[95, 96]

1.2.2 The histamine H_1 receptor

The H_1R mediates typical pathophysiological histamine effects that have been known for almost 100 years. Even if not aware of the target, the first classical “antihistamines” addressing this HR subtype were developed in the 1930s and 1940s and employed for the treatment of allergic conditions.[97] However, these antihistamines could not antagonize all effects provoked by histamine and, therefore, Ash and Schild introduced in 1966 for the first time the denotation of the H_1R.[98] In 1991 Yamashita and colleagues were able to clone the bovine H_1R[99],two years later the cloning of the human H_1R was reported[100].

The human H_1R represents a 487 amino acid protein that preferentially couples to a pertussis-toxin insensitive $G_{q/11}$-protein.[101] The receptor is expressed in numerous tissues like the brain, smooth muscles from airways, blood vessels and gastrointestinal tract, the cardiovascular system, endothelial cells and lymphocytes. H_1R receptor activation in smooth muscle cells results in a contraction due to calcium mobilization from intracellular stores.[102, 103] Vascular permeability increases upon H_1R stimulation as a result of endothelial cell contraction.[104-106] Furthermore, the H_1R triggers the release of nitric oxide from endothelial cells which induces dilatation of vascular smooth muscles.[107, 108] The aforementioned H_1R mediated histamine effects promote the typical allergic reactions as urticaria, bronchoconstriction and decrease in blood pressure. In the CNS the H_1R is involved in the modulation of a multitude of functions like the circadian rhythm of sleep and wakefulness[109, 110], cognitive processes[111, 112], thermoregulation[113] and pain[114].

The histamine H_1R is characterized by a large third intracellular loop and a relatively short C-terminal tail.[95] Molecular modeling investigations and site-directed mutagenesis suggest histamine to bind to the hH_1R by forming an ionic interaction between its protonated amino group and the conserved Asp-107 of TM3. Lys-191 in TM5 is considered to interact with N^{π}

of the imidazole ring, whereas the Asn-198 is supposed to form a hydrogen bond with N^{τ}-H. In place of Asn-198, N^{τ}-H may interact with Thr-194 (Figure 1.7).[115-118]

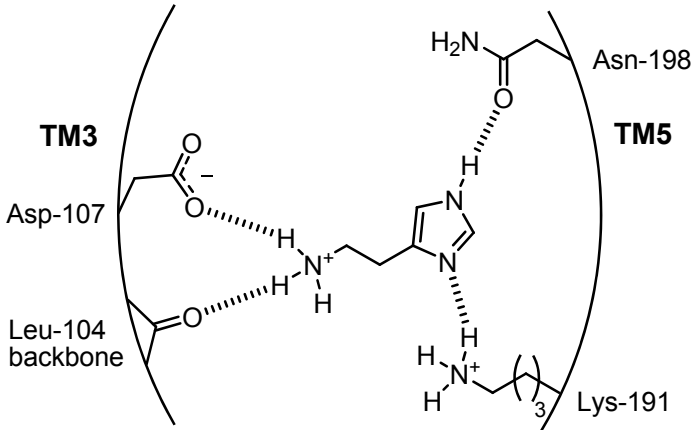
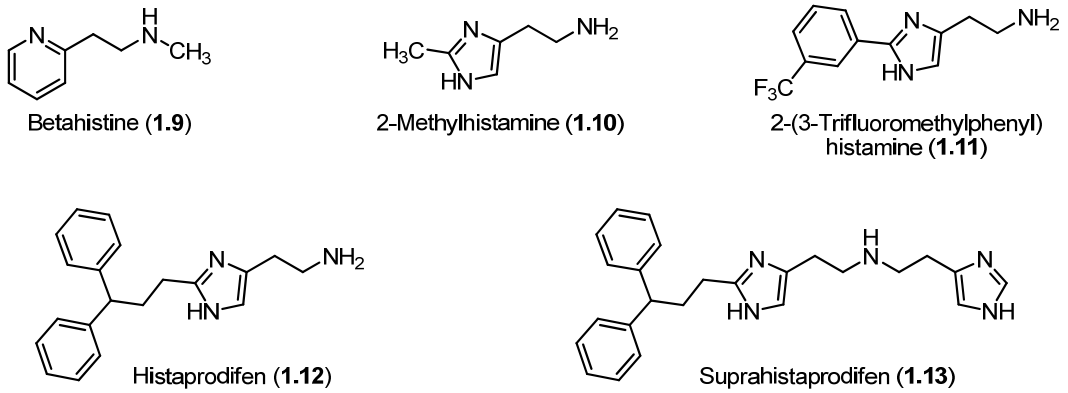


Figure 1.7. Proposed binding mode of histamine at the hH_1R.

Many efforts have been spent to obtain H_1R subtype selective agonists. The first H_1R agonists displaying some selectivity over the H_2R were betahistine (**1.9**) and 2-methyl-histamine (**1.10**). However, the potencies of these histamine analogs were rather poor (5 – 20 % relative to histamine).[119, 120] Potencies similar and even superior to histamine were achieved in the series of the 2-phenylhistamines, in particular when a halogen or a halogen-substituted group was introduced in the *meta* position of the phenyl ring (**1.11**).[121, 122] The most successful approach in this field led to the histaprodifens (**1.12**) and especially the suprahistaprodifens (**1.13**) which are about 36 times more potent H_1R agonists than histamine[118, 123] and are highly interesting pharmacological tools. The only H_1R agonist used in therapy is betahistine (Aequamen®) which has some relevance for the therapy of Menière's disease.[124]

The first generation H_1R antagonists like mepyramine (**1.14**, Pyrilamine®) or diphen-hydramine (**1.15**, Dolestan®) have already been developed more than 50 years ago. Drawback of these antiallergic drugs is their high lipophilicity that enables penetration through the blood-brain barrier and causes sedation. To reduce this side effect more polar H_1R antagonists such as cetirizine (**1.16**, Zyrtec®) and fexofenadine (**1.17**, Telfast®) have been developed which belong to top selling blockbuster drugs.[95] The most commonly used radioligand to label the H_1R is the high affinity antagonist [^{3}H]mepyramine.[125]

H_1R agonists



H_1R antagonists

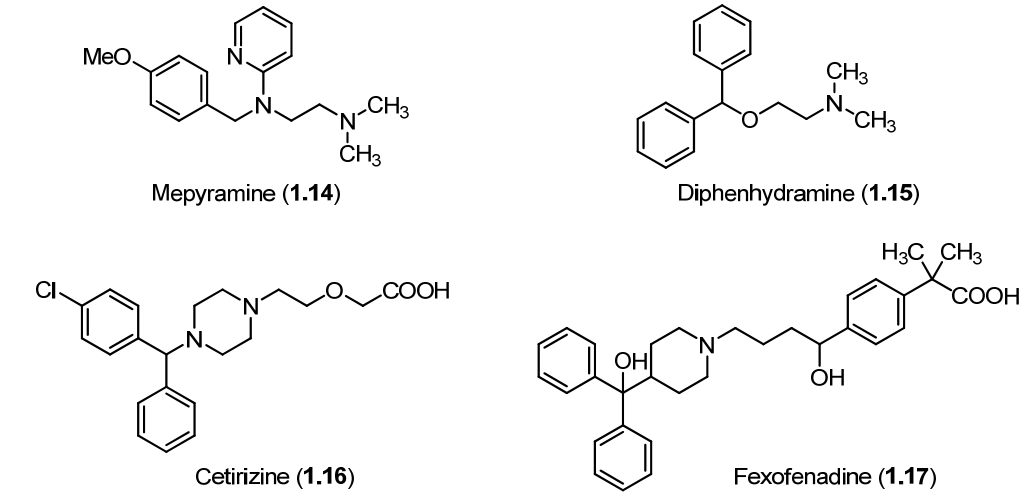


Figure 1.8. Structures of H_1R ligands.

1.2.3 The histamine H_2 receptor

Ash and Schild had suggested the existence of an additional HR subtype in 1966.[98] In 1972 Black and colleagues confirmed this prediction by pharmacological characterization of the second histamine receptor using the first H_2R antagonist burimamide. Contrary to the classical antihistamines, burimamide was able to block the histamine mediated gastric acid secretion and positive chronotropic effect on the heart.[126] In 1991, Gantz and co-workers were able to clone the intronless canine and humane H_2Rs.[127, 128]

The human H_2R consists of 359 amino acids and couples to a G_s-protein resulting in an increase in cAMP turnover.[128-130] High expression levels of the receptor are found in the heart[131], in gastric parietal cells[132], neurons[133], vascular[134, 135], airway[136] and uterine[137] smooth muscle cells and immune cells[89]. Activation of cardiac H_2Rs results in a positive chronotropic and inotropic response.[126, 138, 139] An essential physiological function of the H_2R is the control of gastric acid secretion from parietal cells.[126, 140] Moreover, histamine mediates smooth

muscle relaxation in airway, uterine and blood vessels *via* the H_2R.[126, 134, 136, 137] H_2Rs have numerous functions in the immune system. For example H_2Rs have been shown to inhibit T-cell proliferation[141-143], to block the histamine release from mast cells and to modulate cytokine production[144-146]. Additionally, stimulation of H_2Rs expressed on promyelocytic leukemic cells triggers the functional differentiation to mature granulocytes.[147] In the CNS, H_2Rs inhibit the long-lasting afterhyperpolarization after Ca^{2+} influx and block the accommodation of action potential after firing.[133]

In contrast to the H_1R, the H_2R possesses a substantially shorter third intracellular loop and a longer palmitoylated C-terminus.[95] The endogenous ligand histamine is assumed to bind in its N^{π}-tautomeric form to amino acids located in TM3 and TM5 of the hH_2R. The protonated amino group interacts with the conserved Asp-98 of TM3 and the imidazole N^{π}-H forms a hydrogen bond with Asp-186 of TM5. Imidazole N^{τ} interacts with Tyr-182 likewise located in TM5 (Figure 1.9).[148, 149] Alternatively to Tyr-182, Thr-190 may participate in histamine binding.[150]

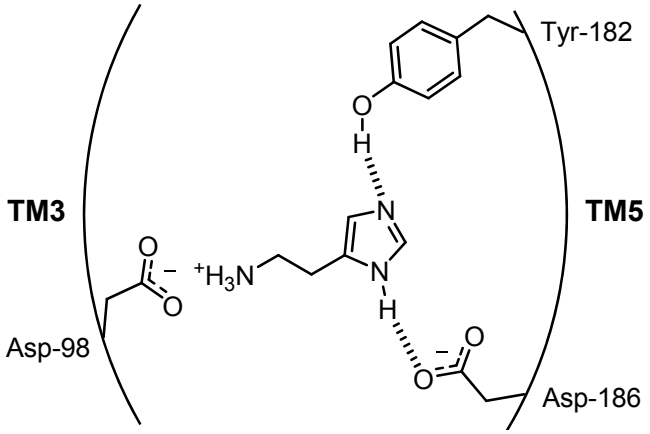


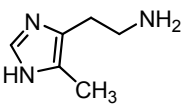
Figure 1.9. Proposed binding mode of histamine at the hH_2R.

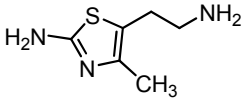
H_2R agonists can be roughly divided in amine-type H_2R agonists and guanidine-type H_2R agonists (Figure 1.10). The first amine-type H_2R agonist showing selectivity over the H_1R was 5-methylhistamine (**1.18**, former nomenclature: 4-methylhistamine).[120] However, very recently this compound turned out to be selective for the H_4R.[151] Further examples of amine-type H_2R agonists are the non-imidazoles amthamine (**1.19**) and dimaprit (**1.20**).[152-154] Contrary to 5-methylhistamine and dimaprit, the aminothiazole analog amthamine is devoid of agonistic activity at the H_3R and H_4R.[151] The first guanidine-type H_2R agonist was imidazolylpropylguanidine (**1.21**, SK&F 91486), exerting just poor partial agonistic activity at the guinea pig (gp) right atrium.[155] Introduction of a cimetidine-like substituent at the guanidine group resulted in impromidine (**1.22**) which displays a drastically increased potency at the gpH_2R (50 times the potency of histamine at the gpH_2R).[156, 157] The imidazolyl-propylguanidine moiety is considered to be crucial for H_2R agonistic activity, whereas the additional substituent is regarded as affinity conferring group.[158] Exchanging the cimetidine-

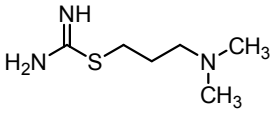
like portion with pheniramine-like moieties led to arpromidine (**1.23**) and analogs, which belong to the most potent guanidine-type H_2R agonists (up to 400 times the potency of histamine at the gpH_2R).[159, 160] The chiral impromidine isomer sopromidine is of special interest as the (*R*)-configurated compound (**1.24**, sopromidine) behaves as gpH_2R agonist, whereas the (*S*)-configurated counterpart is devoid of agonist activity.[161] Due to the positive inotropic and chronotropic activity of H_2R agonists, impromidine has been investigated in the clinic for the therapy of patients suffering from severe catcholamine-insensitive congestive heart failure.[162, 163] However, the strong basic guanidine moiety of these compounds is responsible for pharmacokinetic drawbacks. The guanidine group is nearly quantitatively protonated under physiological conditions, which results in a lack of oral bioavailability and CNS penetration.[164] To overcome the unfavorable pharmacokinetic properties of arpromidine the strong basic guanidine group was replaced with an acylguanidine group which is by approximately five orders of magnitude less basic ($pK_a \approx 13 \rightarrow pK_a \approx 8$).[164] The obtained N^G-acylated imidazolylpropylguanidines turned out to be very potent H_2R agonists with slightly reduced potencies relative to their guanidine analogs.[164] Moreover, acylguanidine-type H_2R agonists like UR-AK24 (**1.25**) proved to be orally bioavailable and brain-penetrating compounds.[164] H_2R agonists capable to pass the blood brain barrier are considered as promising pharmacological tools to evaluate the function of H_2Rs in the CNS. Though, more detailed pharmacological investigations of these acylguanidine-type H_2R agonists revealed these compounds to be even more potent at the hH_3R and hH_4R. Obviously, the imidazolyl-propylguanidine portion is a “privileged structural motif” for HR binding, and replacing this moiety may be a promising initial point for the development of subtype selective compounds.[164] In terms of the H_2R, selective acylguanidine-type compounds like UR-PG267 (**1.26**) could be generated by replacing the imidazole with a 2-aminothiazole ring.[165]

For more than three decades H_2R antagonists have been successfully used in the clinic for the treatment of gastroduodenal ulcer and gastroesophageal reflux disease. After the discovery of burimamide[126] a structural analog, cimetidine (**1.27**, Tagamet®), became the first marketed drug, before other H_2R antagonists such as ranitidine (**1.28**, Zantic®) and famotidine (**1.29**, Pepdul®) were introduced in therapy.[166, 167] [^{3}H]Tiotidine and [^{125}I]iodoamino-potentidine are the most commonly used H_2R ligands for radioligand binding assays.[166]

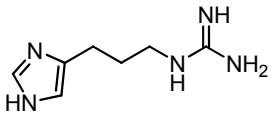
Amine-type H_2R agonists

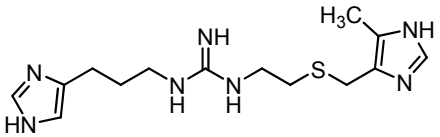

5-Methylhistamine (**1.18**)

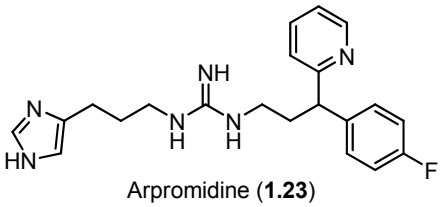

Amthamine (**1.19**)

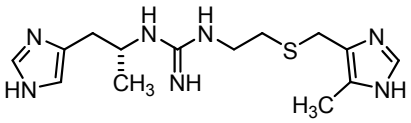

Dimaprit (**1.20**)

Guanidine-type H_2R agonists

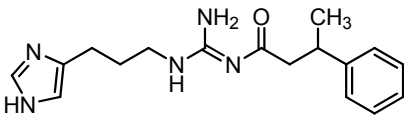

SK&F 91486 (**1.21**)

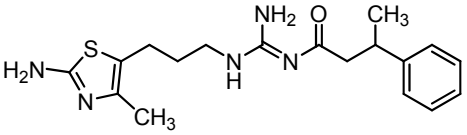

Impromidine (**1.22**)


Arpromidine (**1.23**)

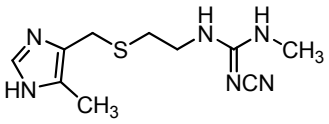

Sopromidine (**1.24**)

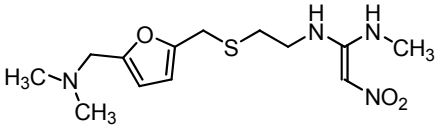
Acylguanidine-type H_2R agonists

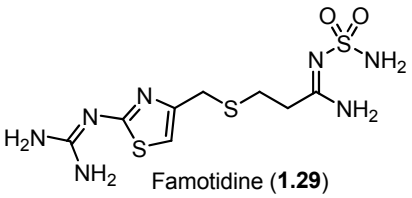

UR-AK24 (**1.25**)

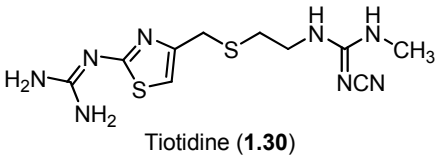

UR-PG276 (**1.26**)

H_2R antagonists


Cimetidine (**1.27**)


Ranitidine (**1.28**)


Famotidine (**1.29**)


Tiotidine (**1.30**)

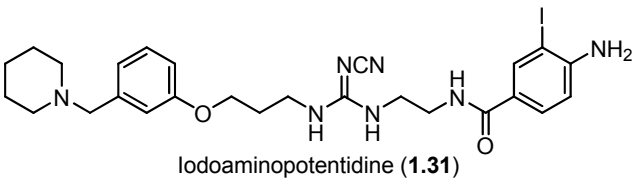

Iodoaminopotentidine (**1.31**)

Figure 1.10. Structures of H_2R ligands.

1.2.4 The histamine H_3 receptor

In 1983 Schwartz and his co-workers evaluated the liberation of histamine from neurons of the rat cortex and showed histamine to inhibit its own release.[168] Unexpectedly, the H_2R antagonist burimamide suppressed the histamine release at nanomolar concentrations which was far below the concentration necessary for blocking the H_2R. This observation prompted Arrang and colleagues to conclude the existence of a third HR subtype.[169] The development of the selective H_3R agonist (*R*)-α-methylhistamine and of the selective H_3R antagonist thioperamide in 1987 further confirmed the third HR subtype.[170] Finally, after numerous homology-based (relative to the $H_{1/2}Rs$) cloning approaches failed, Lovenberg and co-workers were able to clone the hH_3R in 1999.[171] The cloned receptor displays only low sequence homology with the $H_{1/2}Rs$ of about 20 % which is comparable to its homology with other aminergic GPCRs.[171] This explains the failure of cloning this receptor based on the sequence homology of the known HR subtypes. In contrast to the H_1R and H_2R, the gene encoding the H_3R contains two[172] or perhaps three[173] introns resulting in at least 20 hH_3R isoforms.[174] The best characterized isoform is the 445 amino acids containing hH_3R described by Lovenberg et al.[171] The H_3R has been shown to display constitutive activity in recombinant systems as well as in brain membranes of the rat.[175] The H_3R couples to $G_{i/o}$-proteins[176] and has been demonstrated to influence several signal transduction pathways. For instance, inhibition of adenylyl cyclase (AC), activation of mitogen-activated protein kinase (MAPK), activation of phospholipase A_2 (PLA_2), lowering of intracellular Ca^{2+}-levels and inhibition of the Na^+/H^+ exchanger are reported.[174]

The H_3R is preferentially localized in the CNS and acts as an autoreceptor regulating the synthesis and release of histamine from histaminergic neurons.[177, 178] In addition, as a presynaptic heteroreceptor the H_3R controls the neuronal release of dopamine[179], serotonin[180], noradrenaline[181], acetylcholine[182] and γ-amino butyric acid[183]. The H_3R is supposed to be involved in a multitude of CNS functions like wakefulness, locomotor activity, thermoregulation, food intake and memory.[184]

According to Yao and coworkers histamine is assumed to interact with the conserved Asp-114 of TM3 *via* the protonated amino group. Asp-80 in TM2 obviously forms a hydrogen bridge with N^{τ}-H, which is crucial for receptor activation. The imidazole N^{π} is likely to interact with Asn-404 in TM7 (Figure 1.11).[185] In contrast, Uveges and colleagues suggest the imidazole ring to interact with Glu-206 in TM5.[186]

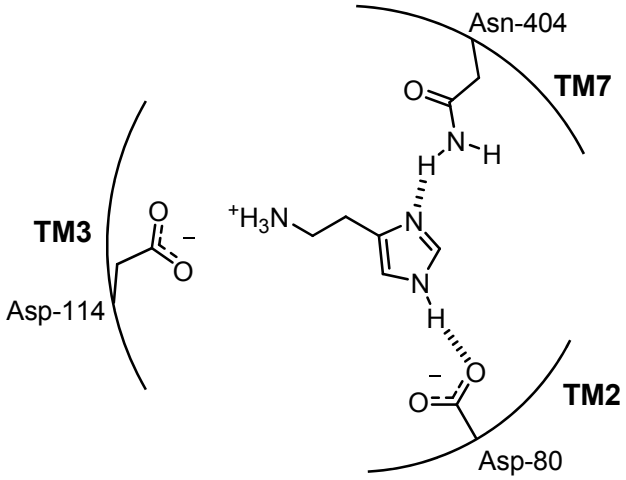
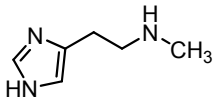


Figure 1.11. Proposed binding mode of histamine at the hH_3R according to Yao et al.[185]

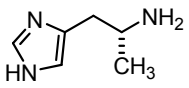
Typical H_3R agonists are N^{α}-methylhistamine (**1.32**) and the highly selective (*R*)-α-methylhistamine (**1.33**, Figure 1.12).[170] Structurally less related to histamine are the H_3R agonists imetit (**1.34**)[187] and methimepip (**1.35**)[188]. The latter shows high selectivity for the H_3R[188]. An interesting approach to increase bioavailability and CNS penetration of the very polar (*R*)-α-methylhistamine was achieved by generation of more lipophilic azomethine prodrugs such as BP 2-94 (**1.36**) and analogs.[189] H_3R agonists may be of therapeutic value for the treatment of insomnia[190], pain[191], inflammation[192, 193] or migraine[194].

However, it has to be considered that due to the high sequence homology of the H_3R and H_4R many imidazole-containing H_3R ligands are also active at the H_4R.[151] For example the classical H_3R antagonist (inverse agonist) thioperamide (**1.37**) displays similar affinity for both HR subtypes.[151] Further examples of imidazole-containing H_3R antagonists are clobenpropit (**1.38**) or iodophenpropit (**1.39**), which are active at the H_4R as well.[151] Meanwhile, several non-imidazole H_3R antagonists such as A-349821 (**1.40**)[195] or JNJ 10181457 (**1.41**)[196] have been developed. Contrary to the imidazoles, these compounds are inactive at the H_4R and do not bind to off-targets like cytochrome P450 and therefore have improved drug-like properties. H_3R antagonists have been extensively studied by several pharmaceutical companies as drug candidates for the treatment of obesity[197], schizophrenia[198], attention-deficit hyperactivity disorder[199], narcolepsy[200] or Alzheimer's disease[201, 202]. In addition, H_3R antagonists may be of interest to relieve nasal congestion, since H_3R activation blocks noradrenalin release from sympathetic nerve ends.[203] For radio-ligand binding studies particularly the high affinity H_3R agonists [^{3}H]N^{α}-methylhistamine and [^{3}H](*R*)-α-methylhistamine or the inverse agonist [^{125}I]iodophenpropit have been used.[166]

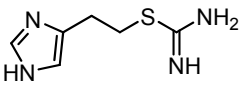
H_3R agonists



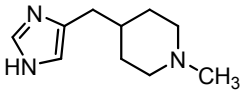
N^{α}-Methylhistamine (**1.32**)



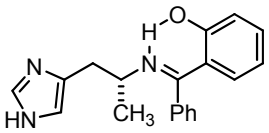
(*R*)-α-Methylhistamine (**1.33**)



Imetit (**1.34**)

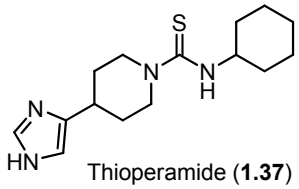


Methimepip (**1.35**)

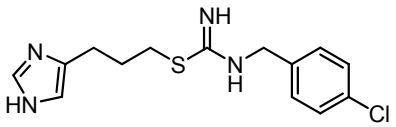


BP 2-94 (**1.36**, prodrug)

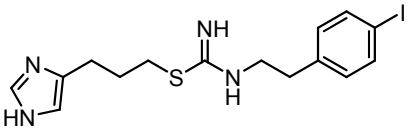
Imidazole-containing H_3R antagonists (inverse agonists)



Thioperamide (**1.37**)

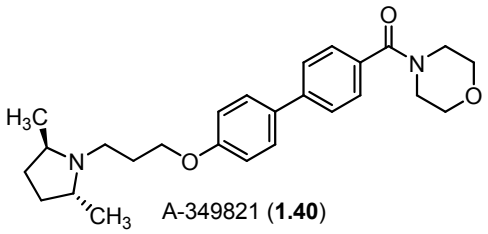


Clobenpropit (**1.38**)

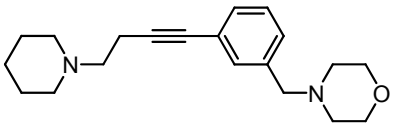


Iodophenpropit (**1.39**)

Non-imidazole-containing H_3R antagonists (inverse agonists)



A-349821 (**1.40**)



JNJ 10181457 (**1.41**)

Figure 1.12. Structures of H_3R ligands.

1.2.5 The histamine H_4 receptor

Already in 1994 Raible and colleagues predicted the existence of an additional HR subtype. They observed that the histamine triggered calcium mobilization in human eosinophils could not be blocked by H_1R and H_2R antagonists, but with the H_3R antagonist thioperamide. However, the potent H_3R agonist (*R*)-α-methylhistamine was less potent than histamine in inducing calcium mobilization, which disagrees with an H_3R mediated effect. Therefore, Raible and co-workers suggested a novel HR subtype on human eosinophils.[204, 205] After cloning of the hH_3R by Lovenberg and co-workers[171], in the years 2000 and 2001 several research groups were able to identify and clone a gene encoding for a new HR subtype due to the high sequence homology with the H_3R (58 % sequence identity within the TM domains).[206-212] This new hH_4R consists of 390 amino acids and – like the H_3R – displays a rather high constitutive activity.[210] The gene encoding the receptor consists of two introns and three exons[207]. Very recently, the existence of two splice variants of the H_4R has been

reported. These H_4R isoforms were mainly localized intracellularly, failed ligand binding and prevented the translocation of the coexpressed full-length H_4R to the cell membrane.[213] Like the H_3R, the H_4R couples to pertussis toxin sensitive $G_{i/o}$-proteins resulting in AC inhibition as well as in activation of the MAPK pathway.[207, 210] Furthermore, H_4R activation in mast cells results in Ca^{2+}-mobilization which is sensitive to pertussis-toxin and the PLC_β inhibitor U73122. Most likely, the $G_{\beta\gamma}$-subunits of the $G_{i/o}$-proteins are involved in PLC_β-activation (Figure 1.13).[214, 215]

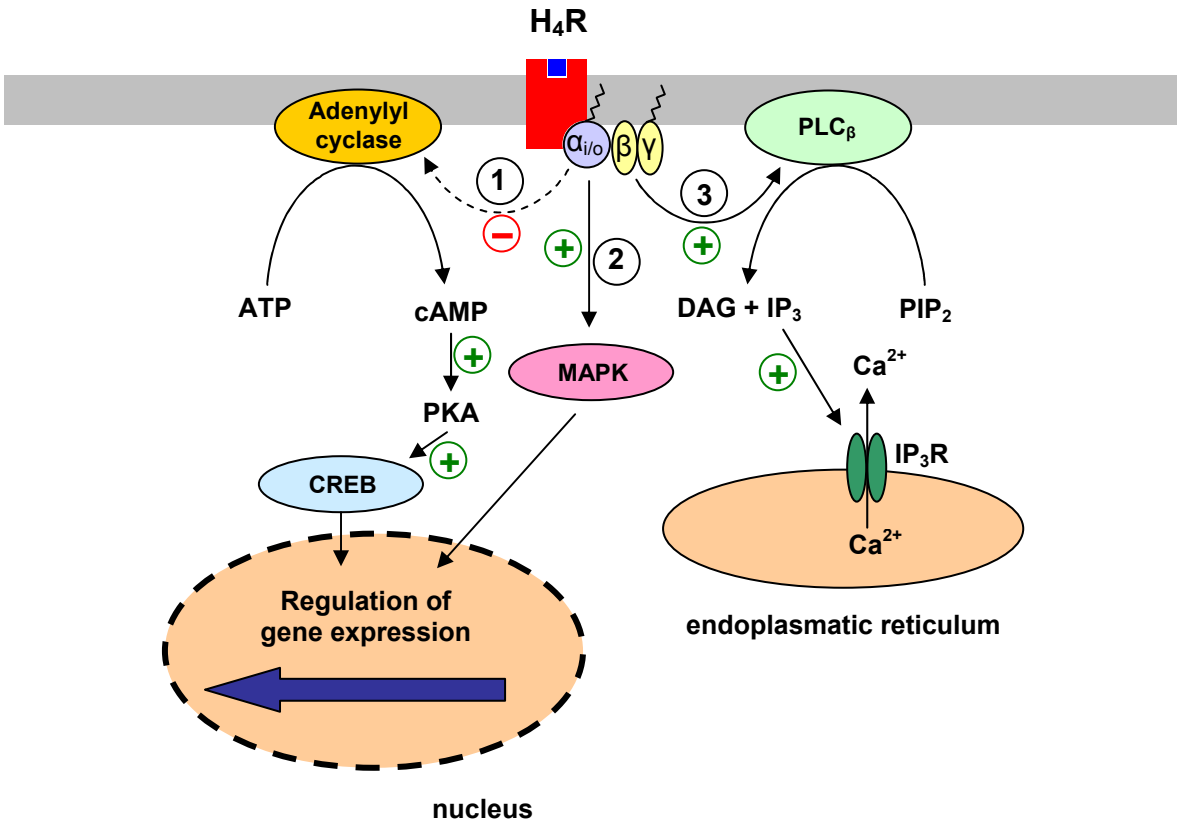


Figure 1.13. Signal transduction pathways of the H_4R. 1, Inhibition of the adenylyl cyclase *via* the α-subunit of the G-protein resulting in decreased cAMP turnover. cAMP stimulates PKA activity resulting in phosphorylation of CREB and modulation of gene expression. 2, Activation of $G_{\alpha i/o}$ is also considered to stimulate the MAPK activity. 3, The $G_{\beta\gamma}$-subunit of the G-protein obviously activates the PLC_β causing hydrolysis of PIP_2 to IP_3 and DAG and Ca^{2+}-mobilization from intracellular stores. (CREB: cAMP-responsive element binding protein, MAPK: mitogen-activated protein kinase, PKA/PKC: protein kinase A/C, DAG: diacylglycerol, IP_3: inositol-1,4,5-trisphosphate, PIP_2: phosphatidylinositol-4,5-bisphosphate, PLC_β: phospholipase C_β, IP_3R: IP_3 receptor).

High expression levels of the H_4R have been described in immune cells like eosinophils, T-cells, dendritic cells, mast cells and basophils.[207, 209, 210, 212, 214, 216] In addition, H_4Rs have been shown to be localized on neurons together with H_1Rs and H_3Rs.[217] Little is known about the exact physiological and pathophysiological roles of the H_4R. The H_4R has been shown to trigger chemotaxis of mast cells and eosinophils[214, 218] and to induce Ca^{2+}-mobilization in mast cells[214], monocytes[219] and eosinophils[220]. Furthermore, stimulation of the H_4R results in actin polymerization, shape change and upregulation of adhesion proteins in eosinophils.[218,

[220] The release of inflammatory mediators like interleukin-16[216], leukotriene B_4[221] and chemokine ligand 2[219] is modulated *via* this HR subtype. Expression levels of the H_4R in monocytes have been demonstrated to depend on interferon-γ, interleukin-10 and interleukin-13.[210, 219] Besides the listed *in vitro* evaluations, the role of the H_4R has been investigated in several animal models. H_4R-deficient mice as well as mice treated with H_4R antagonists displayed a reduced allergic airway inflammation.[222] Moreover, H_4R antagonists proved to be beneficial in the mouse zymosan-induced peritonitis model.[223] Inhibition of the H_4R turned out to be beneficial in the carrageenan-induced paw edema in rats[224, 225] and had a protective effect in the rat colitis model.[226] In mice, H_4R antagonists were superior to H_1R antagonists in attenuation of experimental pruritus.[227] Additionally, the H_4R was found to be expressed in synovial cells of patients suffering from rheumatoid arthritis. The observed variations in the expression levels of the H_4R may be related to severity and duration of the rheumatoid arthritis.[217] These findings as well as the expression pattern of the H_4R suggest this HR subtype to play a crucial role in inflammatory and immunological processes.

Like all other HR subtypes, the H_4R is a class A GPCR which contains typical motifs of biogenic amine receptors: a conserved Asp in TM3, a DRY-motif at the end of TM3, a disulfide bridge linking the extracellular loops E1 and E2 and a possible palmitoylation site in the C-terminal tail.[206] Molecular modeling and site-directed mutagenesis performed by Shin et al.[228] and Jongejan et al.[229] assume histamine to interact with the hH_4R *via* its protonated amino group with Asp-94 of TM3, whereas the imidazole ring is considered to mainly interact with Glu-182 of TM5. Jongejan et al. propose the imidazole N^{π} to form a hydrogen bond with the protonated Glu-182 and the imidazole N^{τ}-H to interact with Ser-320 of TM6 (Figure 1.14 A). In contrast, Kiss and colleagues suggest a reversed binding mode of histamine at the hH_4R to be more favorable.[230] They expect the protonated amino group to interact with Glu-182. Asp-94 is presumed to form a hydrogen bond with the imidazole N^{τ}-H and Thr-323 of TM6 to interact with the imidazole N^{π} (Figure 1.14 B). Moreover, the authors describe a possible binding mode of the cyanoguanidine-type H_4R agonist OUP-16 at the hH_4R. The imidazole N^{τ}-H of this compound is considered to interact like histamine with Asp-94, whereas an *N*-H group of the cyanoguanidine moiety presumably forms a hydrogen bond with Glu-182. In addition, a hydrogen bond between the nitrile group and Thr-323 is expected (Figure 1.14 C). Furthermore, very recently the importance of Phe-169 in the second extracellular loop of the hH_4R for agonist binding has been demonstrated.[231]

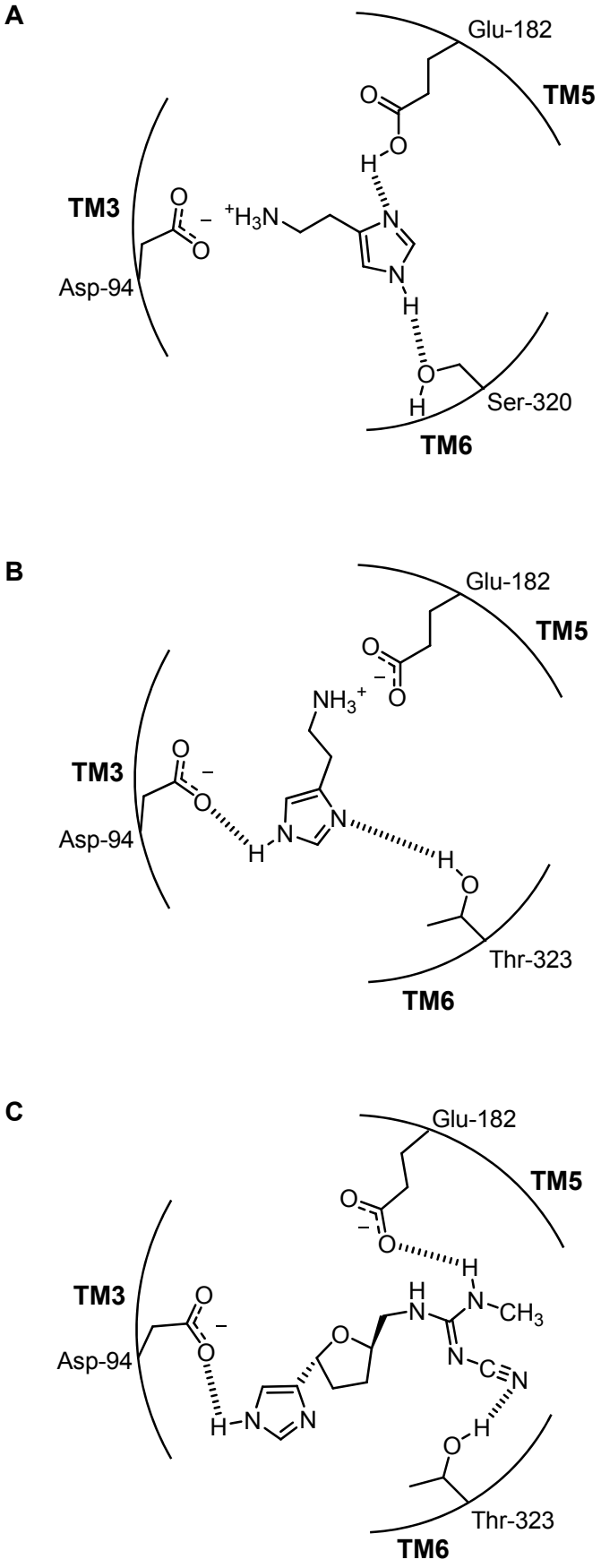
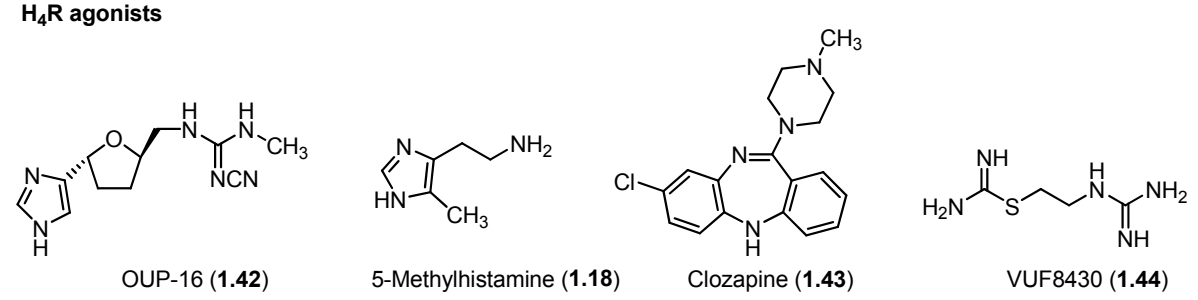


Figure 1.14. Proposed binding modes of histamine (A, B) and OUP-16 (C) at the hH_4R according to A, Jongejan et al.[229] (A) and Kiss et al.[230] (B, C)

The successful cloning and expression of the H_4R stimulated the search for selective agonists and antagonists. Numerous known GPCR ligands were pharmacologically studied on the new HR. Several H_2R and H_3R ligands in particular imidazole-containing compounds such as clobenpropit (**1.38**) or imetit (**1.34**) as well as the isothiourea dimaprit (**1.20**) proved to exert agonistic activity at the H_4R.[151] Even the antipsychotic drug clozapine (**1.43**) was active as an agonist at H_4R (Figure 1.15).[207] The first reported selective H_4R agonist was the chiral tetrahydrofuran analog OUP-16 (**1.42**) which was derived from the H_3R agonist imifuramine.[232] Thereafter, 5-methylhistamine (**1.18**), originally considered a H_2R selective agonist, turned out to be a more potent and selective agonist at the H_4R.[151] The H_4R agonist VUF8430 (**1.44**) was developed based on the H_2R agonist dimaprit.[233] In addition, N^G-acylated imidazolylpropylguanidines like UR-AK24 (**1.25**) – originally designed as H_2R agonists – turned out to be highly potent and almost full H_4R agonists. However, the residual agonistic activities of these compounds at the H_2R and H_3R compromise their use as H_4R agonists. Selective H_4R agonists are required as pharmacological tools for further studies on the biological role of this new HR subtype.

The H_3R inverse agonists thioperamide (**1.37**) and iodophenpropit (**1.39**) were identified as potent H_4R inverse agonist and neutral antagonist, respectively. Meanwhile, highly selective H_4R antagonists such as the indole-2-carboxamide JNJ 7777120 (**1.45**)[234], the quinoxaline analog **1.46**[225] and the 2-aminopyrimidines **1.47**[235] and **1.48**[236] have been developed. With the conformationally constrained compound **1.48** the lack of selectivity of **1.47** toward the 5-HT_{1A} and 5-HT_{1D} receptors could be overcome.[236] In particular, JNJ 7777120 is a valuable pharmacological tool and has already been employed in several animal models to study the biological function of the H_4R.[222-224, 227] Encouraged by the promising results from animal models, H_4R antagonists are discussed as potential drugs for the treatment of inflammatory and autoimmune diseases like allergic rhinitis, asthma bronchiale, rheumatoid arthritis or pruritus.[237] For radioligand binding studies [^{3}H]histamine and [^{3}H]JNJ 7777120 as well the iodinated H_3R ligand [^{125}I]iodophenpropit have been employed.[151]

H_4R agonists



H_4R selective antagonists

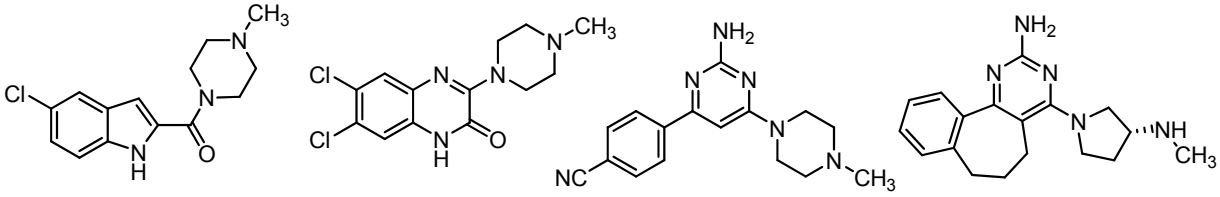


Figure 1.15. Structures of H_4R ligands.

1.3 References

(1) Jacoby, E., Bouhelal, R., Gerspacher, M., Seuwen, K., The 7 TM G-Protein-Coupled Receptor Target Family. *ChemMedChem* **2006**, 1, 760-782.

(2) Fredriksson, R., Lagerstrom, M. C., Lundin, L.-G., Schioth, H. B., The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. *Mol. Pharmacol.* **2003**, 63, 1256-1272.

(3) Wise, A., Jupe, S. C., Rees, S., The identification of ligands at orphan G-protein coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* **2004**, 44, 43-66.

(4) Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., et al., Initial sequencing and analysis of the human genome. *Nature* **2001**, 409, 860-921.

(5) Lagerström, M. C., Schiöth, H. B., Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discov.* **2008**, 7, 339-357.

(6) Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. H., Chen, L., Skupski, M., et al., The Sequence of the Human Genome. *Science* **2001**, 291, 1304-1351.

(7) Fang, Y., Lahiri, J., Picard, L., G protein-coupled receptor microarrays for drug discovery. *Drug Discov. Today* **2003**, 8, 755-761.

(8) Vassilatis, D. K., Hohmann, J. G., Zeng, H., Li, F., Ranchalis, J. E., Mortrud, M. T., Brown, A., Rodriguez, S. S., Weller, J. R., Wright, A. C., Bergmann, J. E., Gaitanaris, G. A., The G protein-coupled receptor repertoires of human and mouse. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, 100, 4903-4908.

(9) Kristiansen, K., Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol. Ther.* **2004**, 103, 21-80.

(10) Rosenbaum, D. M., Cherezov, V., Hanson, M. A., Rasmussen, S. G. F., Thian, F. S., Kobilka, T. S., Choi, H.-J., Yao, X.-J., Weis, W. I., Stevens, R. C., Kobilka, B. K., GPCR Engineering Yields High-Resolution Structural Insights into 2-Adrenergic Receptor Function. *Science* **2007**, 318, 1266-1273.

(11) Cherezov, V., Rosenbaum, D. M., Hanson, M. A., Rasmussen, S. G. F., Thian, F. S., Kobilka, T. S., Choi, H.-J., Kuhn, P., Weis, W. I., Kobilka, B. K., Stevens, R. C., High-Resolution Crystal Structure of an Engineered Human 2-Adrenergic G Protein Coupled Receptor. *Science* **2007**, 318, 1258-1265.

(12) Rasmussen, S. G. F., Choi, H.-J., Rosenbaum, D. M., Kobilka, T. S., Thian, F. S., Edwards, P. C., Burghammer, M., Ratnala, V. R. P., Sanishvili, R., Fischetti, R. F., Schertler, G. F. X., Weis, W. I., Kobilka, B. K., Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature* **2007**, 1-6.

(13) Warne, T., Serrano-Vega, M. J., Baker, J. G., Moukhametzianov, R., Edwards, P. C., Henderson, R., Leslie, A. G., Tate, C. G., Schertler, G. F., Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature* **2008**, 454, 486-491.

(14) Gilman, A. G., G Proteins: Transducers of Receptor-Generated Signals. *Annu. Rev. Biochem.* **1987**, 56, 615-649.

(15) Offermanns, S., G-proteins as transducers in transmembrane signalling. *Prog. Biophys. Mol. Biol.* **2003**, 83, 101-130.

(16) Seifert, R. G Protein-Coupled Receptors as Drug Targets: Analysis of Activation and constitutive Activity. In *G Protein-coupled Receptors as Drug Targets*, Seifert, R., Wieland, T., Eds. Wiley-VCH, Weinheim: **2005**; pp 122-140.

(17) Ross, E. M., Wilkie, T. M., GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu. Rev. Biochem.* **2000**, 69, 795-827.

(18) De Vries, L., Zheng, B., Fischer, T., Elenko, E., Farquhar, M. G., The Regulator of G Protein Signaling Family. *Annu. Rev. Pharmacol. Toxicol.* **2000**, 40, 235-271.

(19) Neubig, R. R., Siderovski, D. P., Regulators of G-protein signalling as new central nervous system drug targets. *Nat. Rev. Drug Discov.* **2002**, 1, 187-197.

(20) Milligan, G., Kostenis, E., Heterotrimeric G-proteins: a short history. *Br. J. Pharmacol.* **2006**, 147 Suppl 1, S46-S55.

(21) Zhang, F. L., Casey, P. J., Protein prenylation: molecular mechanisms and functional consequences. *Annu. Rev. Biochem.* **1996**, 65, 241-269.

(22) Milligan, G., Grassie, M. A., How do G-proteins stay at the plasma membrane? *Essays Biochem.* **1997**, 32, 49-60.

(23) Marinissen, M. J., Gutkind, J. S., G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol. Sci.* **2001**, 22, 368-376.

(24) Kinoshita, M., Nukada, T., Asano, T., Mori, Y., Akaike, A., Satoh, M., Kaneko, S., Binding of $G_{\alpha q}$ N Terminus Is Responsible for the Voltage-resistant Inhibition of α_{1A} (P/Q-type, $Ca_v2.1$) Ca^{2+} Channels. *J. Biol. Chem.* **2001**, 276, 28731-28738.

(25) Mikoshiba, K., IP_3 receptor/Ca^{2+} channel: from discovery to new signaling concepts. *J. Neurochem.* **2007**, 102, 1426-1446.

(26) Payrastre, B., Missy, K., Giuriato, S., Bodin, S., Plantavid, M., Gratacap, M.-P., Phosphoinositides: key players in cell signalling, in time and space. *Cell. Signal.* **2001**, 13, 377-387.

(27) Berridge, M. J., Inositol Trisphosphate and Diacylglycerol: Two Interacting Second Messengers. *Annu. Rev. Biochem.* **1987**, 56, 159-193.

(28) Yamada, M., Inanobe, A., Kurachi, Y., G Protein Regulation of Potassium Ion Channels. *Pharmacol. Rev.* **1998**, 50, 723-757.

(29) Sunahara, R. K., Dessauer, C. W., Gilman, A. G., Complexity and Diversity of Mammalian Adenylyl Cyclases. *Annu. Rev. Pharmacol. Toxicol.* **1996**, 36, 461-480.

(30) Exton, J. H., Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annu. Rev. Pharmacol. Toxicol.* **1996**, 36, 481-509.

(31) De Lean, A., Stadel, J. M., Lefkowitz, R. J., A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *J. Biol. Chem.* **1980**, 255, 7108-7117.

(32) Samama, P., Cotecchia, S., Costa, T., Lefkowitz, R. J., A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. *J. Biol. Chem.* **1993**, 268, 4625-4636.

(33) Leff, P., The two-state model of receptor activation. *Trends Pharmacol. Sci.* **1995**, 16, 89-97.

(34) Weiss, J. M., Morgan, P. H., Lutz, M. W., Kenakin, T. P., The Cubic Ternary Complex Receptor-Occupancy Model I. Model Description. *J. Theor. Biol.* **1996**, 178, 151-167.

(35) Weiss, J. M., Morgan, P. H., Lutz, M. W., Kenakin, T. P., The Cubic Ternary Complex Receptor-Occupancy Model II. Understanding Apparent Affinity. *J. Theor. Biol.* **1996**, 178, 169-182.

(36) Weiss, J. M., Morgan, P. H., Lutz, M. W., Kenakin, T. P., The cubic ternary complex receptor-occupancy model. III. resurrecting efficacy. *J. Theor. Biol.* **1996**, 181, 381-397.

(37) Kenakin, T., Drug efficacy at G-protein coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* **2002**, 42, 349-379.

(38) Takasu, H., Gardella, T. J., Luck, M. D., Potts, J. T., Bringhurst, F. R., Amino-Terminal Modifications of Human Parathyroid Hormone (PTH) Selectively Alter Phospholipase C Signaling via the Type 1 PTH Receptor: Implications for Design of Signal-Specific PTH Ligands. *Biochemistry* **1999**, 38, 13453-13460.

(39) Seifert, R., Wenzel-Seifert, K., Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn. Schmiedebergs Arch. Pharmacol.* **2002**, 366, 381-416.

(40) Kenakin, T., Ligand-selective receptor conformations revisited: the promise and the problem. *Trends Pharmacol. Sci.* **2003**, 24, 346-354.

(41) Schöneberg, T., Schulz, A., Gudermann, T., The structural basis of G-protein-coupled receptor function and dysfunction in human diseases. *Rev. Physiol. Biochem. Pharmacol.* **2002**, 144, 143-227.

(42) Perez, D. M., Karnik, S. S., Multiple Signaling States of G-Protein-Coupled Receptors. *Pharmacol. Rev.* **2005**, 57, 147-161.

(43) George, S. R., O'Dowd, B. F., Lee, S. P., G-Protein-Coupled Receptor Oligomerisation and its Potential for Drug Discovery. *Nat. Rev. Drug Discov.* **2002**, 1, 808-820.

(44) Nikbin, N., Edwards, C., Reynolds, C. A., G-protein Coupled Receptor Dimerization. *IJPT* **2003**, 1-11.

(45) Szidonya, L., Cserzo, M., Hunyady, L., Dimerization and oligomerization of G-protein-coupled receptors: debated structures with established and emerging functions. *J. Endocrinol.* **2008**, 196, 435-453.

(46) Lee, S. P., O'Dowd, B. F., Ng, G. Y., Varghese, G., Akil, H., Mansour, A., Nguyen, T., George, S. R., Inhibition of cell surface expression by mutant receptors demonstrates that D_2 dopamine receptors exist as oligomers in the cell. *Mol. Pharmacol.* **2000**, 58, 120-128.

(47) Nimchinsky, E. A., Hof, P. R., Janssen, W. G., Morrison, J. H., Schmauss, C., Expression of dopamine D_3 receptor dimers and tetramers in brain and in transfected cells. *J. Biol. Chem.* **1997**, 272, 29229-29237.

(48) Angers, S., Salahpour, A., Joly, E., Hilairet, S., Chelsky, D., Dennis, M., Bouvier, M., Detection of β_2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). *Proc. Natl. Acad. Sci. U. S. A.* **2000**, 97, 3684-3689.

(49) Bakker, R. A., Dees, G., Carrillo, J. J., Booth, R. G., Lopez-Gimenez, J. F., Milligan, G., Strange, P. G., Leurs, R., Domain Swapping in the Human Histamine H_1 Receptor. *J. Pharmacol. Exp. Ther.* **2004**, 311, 131-138.

(50) Fukushima, Y., Asano, T., Saitoh, T., Anai, M., Funaki, M., Ogihara, T., Katagiri, H., Matsuhashi, N., Yazaki, Y., Sugano, K., Oligomer formation of histamine H_2 receptors expressed in Sf9 and COS7 cells. *FEBS Lett.* **1997**, 409, 283-286.

(51) van Rijn, R. M., Chazot, P. L., Shenton, F. C., Sansuk, K., Bakker, R. A., Leurs, R., Oligomerization of Recombinant and Endogenously Expressed Human Histamine H_4 Receptors. *Mol. Pharmacol.* **2006**, 70, 604-615.

(52) Cvejic, S., Devi, L. A., Dimerization of the δ-opioid receptor: implication for a role in receptor internalization. *J. Biol. Chem.* **1997**, 272, 26959-26964.

(53) Jordan, B. A., Devi, L. A., G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* **1999**, 399, 697-700.

(54) George, S. R., Fan, T., Xie, Z., Tse, R., Tam, V., Varghese, G., O'Dowd, B. F., Oligomerization of μ- and δ-opioid receptors. Generation of novel functional properties. *J. Biol. Chem.* **2000**, 275, 26128-26135.

(55) Romano, C., Yang, W. L., O'Malley, K. L., Metabotropic glutamate receptor 5 is a disulfide-linked dimer. *J. Biol. Chem.* **1996**, 271, 28612-28616.

(56) Kunishima, N., Shimada, Y., Tsuji, Y., Sato, T., Yamamoto, M., Kumasaka, T., Nakanishi, S., Jingami, H., Morikawa, K., Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* **2000**, 407, 971-977.

(57) Bai, M., Trivedi, S., Brown, E. M., Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. *J. Biol. Chem.* **1998**, 273, 23605-23610.

(58) Rocheville, M., Lange, D. C., Kumar, U., Patel, S. C., Patel, R. C., Patel, Y. C., Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. *Science* **2000**, 288, 154-157.

(59) Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., Mosbacher, J., Bischoff, S., Kulik, A., Shigemoto, R., Karschin, A., Bettler, B., GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* **1998**, 396, 683-687.

(60) Kuner, R., Kohr, G., Grunewald, S., Eisenhardt, G., Bach, A., Kornau, H. C., Role of heteromer formation in $GABA_B$ receptor function. *Science* **1999**, 283, 74-77.

(61) White, J. H., Wise, A., Main, M. J., Green, A., Fraser, N. J., Disney, G. H., Barnes, A. A., Emson, P., Foord, S. M., Marshall, F. H., Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* **1998**, 396, 679-682.

(62) Abdalla, S., Lother, H., Quitterer, U., AT_1-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration. *Nature* **2000**, 407, 94-98.

(63) Halazy, S., G-protein coupled receptors bivalent ligands and drug design. *Exp. Opin. Ther. Patents* **1999**, 9, 431-446.

(64) Portoghese, P. S., Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. *Trends Pharmacol. Sci.* **1989**, 10, 230-235.

(65) Portoghese, P. S., From Models to Molecules: Opioid Receptor Dimers, Bivalent Ligands, and Selective Opioid Receptor Probes. *J. Med. Chem.* **2001**, 44, 2259-2269.

(66) Jones, R. M., Hjorth, S. A., Schwartz, T. W., Portoghese, P. S., Mutational Evidence for a Common κ Antagonist Binding Pocket in the Wild-Type κ and Mutant μ[K303E] Opioid Receptors. *J. Med. Chem.* **1998**, 41, 4911-4914.

(67) Schwyzer, R., ACTH: a short introductory review. *Ann. N. Y. Acad. Sci.* **1977**, 297, 3-26.

(68) Contreras, J.-M., Sippl, W. Homo and Heterodimer Ligands: the Twin Drug Approach. In *The Practice of Medicinal Chemistry*, Wermuth, C. G., Ed. Academic press: Oxford, **2008**.

(69) Mammen, M., Choi, S.-K., Whitesides, G. M., Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors. *Angew. Chem. Int. Ed.* **1998**, 37, 2754-2794.

(70) Windaus, A., Vogt, W., Synthesis of Imidazolylethylamine. *Ber. Dtsch. Ges.* **1908**, 40, 3691-3685.

(71) Barger, G., Dale, H. H., 4-β-Aminoethylglyoxaline (β-Aminazolylethylamine) and the other Active Principles of Ergot. *J. Chem. Soc.* **1910**, 97, 2592-2595.

(72) Parsons, M. E., Ganellin, C. R., Histamine and its receptors. *Br. J. Pharmacol.* **2006**, 147, S127-S135.

(73) Ganellin, C. R., The tautomer ratio of histamine. *J. Pharm. Pharmacol.* **1973**, 25, 787-792.

(74) Ganellin, C. R. Imidazole tautomerism of histamine derivatives. In *Molecular and Quantum Pharmacology*, Bergmann, E. D., Pullman, B., Eds. D. Reidel: Dordrecht-Holland, **1974**; pp 43-53.

(75) Prout, K., Critchley, S. R., Ganellin, C. R., 2-(4-Imidazolyl)-ethylammonium bromide (histamine monohydrobromide). *Acta Crystallogr. Sect. B* **1974**, 30, 2884-2886.

(76) Bonnet, J. J., Ibers, J. A., The structure of histamine. *J. Am. Chem. Soc.* **1973**, 95, 4829-4833.

(77) Paiva, T. B., Tominaga, M., Paiva, A. C., Ionization of histamine, *N*-acetylhistamine, and their iodinated derivatives. *J. Med. Chem.* **1970**, 13, 689-692.

(78) Ganellin, C. R. Chemistry and Structure-Activity Relationsships of Drugs Acting at Histamine Receptors. In *Pharmacology of Histamine Receptors*, Ganellin, C. R., Parsons, M. E., Eds. Wright PSG: Bristol, London, Boston, **1982**; pp 10-102.

(79) Schayer, R. W., Origin and fate of histamine in the body. *Ciba Foundation Symposium, Histamine* **1956**, 183-188.

(80) Aures, D., Hakanson, R., Clark, W. G., Histidine decarboxylase and DOPA decarboxylase. *Handb. Neurochem.* **1970**, 4, 165-196.

(81) Beaven, M. A. Factors Regulating Availability of Histamine at Tissue Receptors. In *Pharmacology of Histamine Receptors*, Ganellin, C. R., Parsons, M. E., Eds. Wright PSG: Bristol, London, Boston, **1982**; pp 102-145.

(82) Ogasawara, M., Yamauchi, K., Satoh, Y., Yamaji, R., Inui, K., Jonker, J. W., Schinkel, A. H., Maeyama, K., Recent advances in molecular pharmacology of the histamine systems: organic cation transporters as a histamine transporter and histamine metabolism. *J. Pharmacol. Sci.* **2006**, 101, 24-30.

(83) Riley, J. F., West, G. B., Histamine in tissue mast cells. *J. Physiol. (London)* **1952**, 117, 72P-73P.

(84) Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A., Parish, H. H., Jr., Distribution of histamine among leukocytes and platelets. *Blood* **1955**, 10, 467-481.

(85) Saxena, S. P., Brandes, L. J., Becker, A. B., Simons, K. J., LaBella, F. S., Gerrard, J. M., Histamine is an intracellular messenger mediating platelet aggregation. *Science* **1989**, 243, 1596-1599.

(86) Hakanson, R., Larsson, L. I., Sundler, F., Endocrine-like cells in rat stomach: effects of 6-hydroxydopa on amine stores and amino acid decarboxylase activities. A chemical, fluorescence histochemical and electron microscopic study. *J. Pharmacol. Exp. Ther.* **1974**, 191, 92-101.

(87) Karnushina, I. L., Palacios, J. M., Barbin, G., Dux, E., Joo, F., Schwartz, J. C., Studies on a capillary-rich fraction isolated from brain: histaminic components and characterization of the histamine receptors linked to adenylate cyclase. *J. Neurochem.* **1980**, 34, 1201-1208.

(88) Schwartz, J. C., Pollard, H., Quach, T. T., Histamine as a neurotransmitter in mammalian brain: neurochemical evidence. *J. Neurochem.* **1980**, 35, 26-33.

(89) Thurmond, R. L., Gelfand, E. W., Dunford, P. J., The role of histamine H_1 and H_4 receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug Discov.* **2008**, 7, 41-53.

(90) Church, M. K., Okayama, Y., el-Lati, S., Mediator secretion from human skin mast cells provoked by immunological and non-immunological stimulation. *Skin Pharmacol.* **1991**, 4 Suppl 1, 15-24.

(91) Chahdi, A., Fraundorfer, P. F., Beaven, M. A., Compound 48/80 Activates Mast Cell Phospholipase D via Heterotrimeric GTP-Binding Proteins. *J. Pharmacol. Exp. Ther.* **2000**, 292, 122-130.

(92) Klinker, J. F., Seifert, R., Rezeptor-unabhängige Aktivierung von G-Proteinen. *Pharm. Unserer Zeit* **1995**, 24, 250-263.

(93) Moessner, J., Caca, K., Developments in the inhibition of gastric acid secretion. *Eur. J. Clin. Invest.* **2005**, 35, 469-475.

(94) Passani, M. B., Giannoni, P., Bucherelli, C., Baldi, E., Blandina, P., Histamine in the brain: Beyond sleep and memory. *Biochem. Pharmacol.* **2007**, 73, 1113-1122.

(95) Hill, S. J., Ganellin, C. R., Timmerman, H., Schwartz, J.-C., Shankley, N. P., Young, J. M., Schunack, W., Levi, R., Haas, H. L., International Union of Pharmacology. XIII. Classification of Histamine Receptors. *Pharmacol. Rev.* **1997**, 49, 253-278.

(96) Hough, L. B., Genomics Meets Histamine Receptors: New Subtypes, New Receptors. *Mol. Pharmacol.* **2001**, 59, 415-419.

(97) Ulrich, M., Die Geschichte der Antihistaminika. *Pharm. Unserer Zeit* **2004**, 33, 86-91.

(98) Ash, A. S., Schild, H. O., Receptors mediating some actions of histamine. *Br. J. Pharmacol. Chemother.* **1966**, 27, 427-439.

(99) Yamashita, M., Fukui, H., Sugama, K., Horio, Y., Ito, S., Mizuguchi, H., Wada, H., Expression cloning of a cDNA encoding the bovine histamine H_1 receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, 88, 11515-11519.

(100) De Backer, M. D., Gommeren, W., Moereels, H., Nobels, G., Van Gompel, P., Leysen, J. E., Luyten, W. H. M. L., Genomic cloning, heterologous expression and pharmacological characterization of a human histamine H_1 receptor. *Biochem. Biophys. Res. Commun.* **1993**, 197, 1601-1608.

(101) Hill, S. J., Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.* **1990**, 42, 45-83.

(102) Matsumoto, T., Kanaide, H., Nishimura, J., Shogakiuchi, Y., Kobayashi, S., Nakamura, M., Histamine activates H_1-receptors to induce cytosolic free calcium transients in cultured vascular smooth muscle cells from rat aorta. *Biochem. Biophys. Res. Commun.* **1986**, 135, 172-177.

(103) Kotlikoff, M. I., Murray, R. K., Reynolds, E. E., Histamine-induced calcium release and phorbol antagonism in cultured airway smooth muscle cells. *Am. J. Physiol.* **1987**, 253, 561-566.

(104) Majno, G., Palade, G. E., Studies on inflammation. 1. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J. Biophys. Biochem. Cytol.* **1961**, 11, 571-605.

(105) Majno, G., Shea, S. M., Leventhal, M., Endothelial contraction induced by histamine-type mediators: An electron microscopic study. *J. Cell Biol.* **1969**, 42, 647-672.

(106) Killackey, J. J., Johnston, M. G., Movat, H. Z., Increased permeability of microcarrier-cultured endothelial monolayers in response to histamine and thrombin. A model for the in vitro study of increased vasopermeability. *Am. J. Pathol.* **1986**, 122, 50-61.

(107) Toda, N., Endothelium-dependent relaxation induced by angiotensin II and histamine in isolated arteries of dog. *Br. J. Pharmacol.* **1984**, 81, 301-307.

(108) Van De Voorde, J., Leusen, I., Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur. J. Pharmacol.* **1983**, 87, 113-120.

(109) Monti, J. M., Involvement of histamine in the control of the waking state. *Life Sci.* **1993**, 53, 1331-1338.

(110) Monti, J. M., Jantos, H., Leschke, C., Elz, S., Schunack, W., The selective histamine H_1-receptor agonist 2-(3-trifluoromethylphenyl)histamine increases waking in the rat. *Eur. Neuropsychopharmacol.* **1994**, 4, 459-462.

(111) Malmberg-Aiello, P., Ipponi, A., Bartolini, A., Schunack, W., Antiamnesic effect of metoprine and of selective histamine H_1 receptor agonists in a modified mouse passive avoidance test. *Neurosci. Lett.* **2000**, 288, 1-4.

(112) Nakazato, E., Yamamoto, T., Ohno, M., Watanabe, S., Cholinergic and glutamatergic activation reverses working memory failure by hippocampal histamine H_1 receptor blockade in rats. *Life Sci.* **2000**, 67, 1139-1147.

(113) Chen, Z., Sugimoto, Y., Kamei, C., Effects of intracerebroventricular injection of histamine and its related compounds on rectal temperature in mice. *Methods Find. Exp. Clin. Pharmacol.* **1995**, 17, 669-675.

(114) Mobarakeh, J. I., Sakurada, S., Katsuyama, S., Kutsuwa, M., Kuramasu, A., Lin, Z. Y., Watanabe, T., Hashimoto, Y., Yanai, K., Role of histamine H_1 receptor in pain perception: a study of the receptor gene knockout mice. *Eur. J. Pharmacol.* **2000**, 391, 81-89.

(115) Ohta, K., Hayashi, H., Mizuguchi, H., Kagamiyama, H., Fujimoto, K., Fukui, H., Site-Directed Mutagenesis of the Histamine H_1 Receptor: Roles of Aspartic Acid[107], Asparagine[198] and Threonine[194]. *Biochem. Biophys. Res. Commun.* **1994**, 203, 1096-1101.

(116) Leurs, R., Smit, M. J., Meeder, R., Terlaak, A. M., Timmerman, H., Lysine[200] Located in the Fifth Transmembrane Domain of the Histamine H_1 Receptor Interacts with Histamine but Not with All H_1 Agonists. *Biochem. Biophys. Res. Commun.* **1995**, 214, 110-117.

(117) Jongejan, A., Leurs, R., Delineation of Receptor-Ligand Interactions at the Human Histamine H_1 Receptor by a Combined Approach of Site-Directed Mutagenesis and Computational Techniques - or - How to Bind the H_1 Receptor. *Arch. Pharm. (Weinheim).* **2005**, 338, 248-259.

(118) Elz, S., Kramer, K., Pertz, H. H., Detert, H., ter Laak, A. M., Kuhne, R., Schunack, W., Histaprodifens: Synthesis, Pharmacological in Vitro Evaluation, and Molecular Modeling of a New Class of Highly Active and Selective Histamine H_1-Receptor Agonists. *J. Med. Chem.* **2000**, 43, 1071-1084.

(119) Durant, G. J., Emmett, J. C., Ganellin, C. R., Roe, A. M., Slater, R. A., Potential histamine H_2-receptor antagonists. 3. Methylhistamines. *J. Med. Chem.* **1976**, 19, 923-928.

(120) Durant, G. J., Ganellin, C. R., Parsons, M. E., Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. Med. Chem.* **1975**, 18, 905-909.

(121) Leschke, C., Elz, S., Garbarg, M., Schunack, W., Synthesis and Histamine H_1 Receptor Agonist Activity of a Series of 2-Phenylhistamines, 2-Heteroarylhistamines, and Analogs. *J. Med. Chem.* **1995**, 38, 1287-1294.

(122) Zingel, V., Elz, S., Schunack, W., Histamine analogues. 33rd communication: 2-phenylhistamines with high histamine H_1-agonistic activity. *Eur. J. Med. Chem.* **1990**, 25, 673-680.

(123) Menghin, S., Pertz, H. H., Kramer, K., Seifert, R., Schunack, W., Elz, S., N^{α}-Imidazolylalkyl and Pyridylalkyl Derivatives of Histaprodifen: Synthesis and in Vitro Evaluation of Highly Potent Histamine H_1-Receptor Agonists. *J. Med. Chem.* **2003**, 46, 5458-5470.

(124) Barak, N., Betahistine: what's new on the agenda? *Expert Opin. Investig. Drugs* **2008**, 17, 795-804.

(125) Hill, S. J., Young, J. M., Marrian, D. H., Specific binding of ^{3}H-mepyramine to histamine H_1 receptors in intestinal smooth muscle. *Nature* **1977**, 270, 361-363.

(126) Black, J. W., Duncan, W. A., Durant, C. J., Ganellin, C. R., Parsons, E. M., Definition and antagonism of histamine H_2-receptors. *Nature* **1972**, 236, 385-390.

(127) Gantz, I., Schaffer, M., DelValle, J., Logsdon, C., Campbell, V., Uhler, M., Yamada, T., Molecular cloning of a gene encoding the histamine H_2 receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, 88, 429-433.

(128) Gantz, I., Munzert, G., Tashiro, T., Schaffer, M., Wang, L., DelValle, J., Yamada, T., Molecular cloning of the human histamine H_2 receptor. *Biochem. Biophys. Res. Commun.* **1991**, 178, 1386-1392.

(129) Klein, I., Levey, G. S., Activation of myocardial adenyl cyclase by histamine in guinea pig, cat, and human heart. *J. Clin. Invest.* **1971**, 50, 1012-1015.

(130) Johnson, C. L., Mizoguchi, H., The interaction of histamine and guanylnucleotides with cardiac adenylate cyclase and its relationship to cardiac contractility. *J. Pharmacol. Exp. Ther.* **1977**, 200, 174-186.

(131) Johnson, C. L., Weinstein, H., Green, J. P., Studies on histamine H_2 receptors coupled to cardiac adenylate cyclase. Blockade by H_2 and H_1 receptor antagonists. *Mol. Pharmacol.* **1979**, 16, 417-428.

(132) Soll, A. H., Wollin, A., Histamine and cyclic AMP in isolated canine parietal cells. *Am. J. Physiol.* **1979**, 237, E444-450.

(133) Haas, H., Panula, P., The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat. Rev. Neurosci.* **2003**, 4, 121-130.

(134) Reinhardt, D., Ritter, E., Hypothermia-induced potentiation of histamine H_2-receptor-mediated relaxation and cyclic AMP increase in the isolated mesenteric artery of the rabbit. *Agents Actions* **1979**, 9, 9-14.

(135) Ottosson, A., Jansen, I., Edvinsson, L., Characterization of histamine receptors in isolated human cerebral arteries. *Br. J. Pharmacol.* **1988**, 94, 901-907.

(136) Eyre, P., Chand, N. Histamine receptor mechanisms of the lung. In *Pharmacology of Histamine Receptors*, Ganellin, C. R., Parsons, M. E., Eds. Wright PSG: Bristol, London, Boston, **1982**; pp 298-322.

(137) Verma, S. C., McNeill, J. H., The effect of histamine, isoproterenol and tyramine on rat uterine cyclic AMP. *Res. Commun. Chem. Pathol. Pharmacol.* **1976**, 13, 55-64.

(138) Inui, J., Imamura, H., Restoration by histamine of the calcium-dependent electrical and mechanical response in the guinea-pig papillary muscle partially depolarized by potassium. *Naunyn. Schmiedebergs Arch. Pharmacol.* **1976**, 294, 261-269.

(139) Levi, R., Owen, D. A. A., Trzeciakowski, J. Actions of histamine on the heart and vasculature. In *Pharmacology of Histamine Receptors*, Ganellin, C. R., Parsons, M. E., Eds. Wright PSG: Bristol, London, Boston, **1982**; pp 236-297.

(140) Black, J. W., Shankley, N. P., The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis. *Br. J. Pharmacol.* **1985**, 86, 571-579.

(141) Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O. A., Malolepszy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., Akdis, C. A., Histamine regulates T-cell and antibody responses by differential expression of H_1 and H_2 receptors. *Nature* **2001**, 413, 420-425.

(142) Rezai, A. R., Salazar-Gonzalez, J. F., Martinez-Maza, O., Bramhall, J., Afrasiabi, R., Kermani-Arab, V., Histamine blocks interleukin 2 (IL-2) gene expression and regulates IL-2 receptor expression. *Immunopharmacol. Immunotoxicol.* **1990**, 12, 345-362.

(143) Kunzmann, S., Mantel, P. Y., Wohlfahrt, J. G., Akdis, M., Blaser, K., Schmidt-Weber, C. B., Histamine enhances TGF-β_1-mediated suppression of Th2 responses. *FASEB J.* **2003**, 17, 1089-1095.

(144) Lippert, U., Moller, A., Welker, P., Artuc, M., Henz, B. M., Inhibition of cytokine secretion from human leukemic mast cells and basophils by H_1- and H_2-receptor antagonists. *Exp. Dermatol.* **2000**, 9, 118-124.

(145) Lichtenstein, L. M., Gillespie, E., The effects of the H_1 and H_2 antihistamines on "allergic" histamine release and its inhibition by histamine. *J. Pharmacol. Exp. Ther.* **1975**, 192, 441-450.

(146) Bissonnette, E. Y., Histamine inhibits tumor necrosis factor alpha release by mast cells through H_2 and H_3 receptors. *Am. J. Respir. Cell Mol. Biol.* **1996**, 14, 620-626.

(147) Seifert, R., Höer, A., Schwaner, I., Buschauer, A., Histamine increases cytosolic Ca^{2+} in HL-60 promyelocytes predominantly via H_2 receptors with an unique agonist/antagonist profile and induces functional differentiation. *Mol. Pharmacol.* **1992**, 42, 235-241.

(148) Nederkoorn, P. H. J., van Lenthe, J. H., van der Goot, H., Donné-Op den Kelder, G. M., Timmermann, H., The agonistic binding site at the histamine H_2 receptor. I. Theoretical investigations of histamine binding to an oligopeptide mimicking a part of the fifth transmembrane α-helix. *J. Comput. Aided Mol. Des.* **1996**, 10, 461-478.

(149) Nederkoorn, P. H. J., van Gelder, E. M., Donné-Op den Kelder, G. M., Timmermann, H., The agonistic binding site of the histamine H_2 receptor. II. Theoretical investigations of histamine binding to receptor models of the seven α-helical transmembrane domain. *J. Comput. Aided Mol. Des.* **1996**, 10, 479-489.

(150) Gantz, I., DelValle, J., Wang, L. D., Tashiro, T., Munzert, G., Guo, Y. J., Konda, Y., Yamada, T., Molecular basis for the interaction of histamine with the histamine H_2 receptor. *J. Biol. Chem.* **1992**, 267, 20840-20843.

(151) Lim, H. D., van Rijn, R. M., Ling, P., Bakker, R. A., Thurmond, R. L., Leurs, R., Evaluation of Histamine H_1-, H_2-, and H_3-Receptor Ligands at the Human Histamine H_4 Receptor: Identification of 4-Methylhistamine as the First Potent and Selective H_4 Receptor Agonist. *J. Pharmacol. Exp. Ther.* **2005**, 314, 1310-1321.

(152) Eriks, J. C., Van der Goot, H., Sterk, G. J., Timmerman, H., Histamine H_2-Receptor Agonists. Synthesis, in Vitro Pharmacology, and Qualitative Structure-Activity Relationships of Substituted 4- and 5-(2-Aminoethyl)thiazoles. *J. Med. Chem.* **1992**, 35, 3239-3246.

(153) Durant, G. J., Ganellin, C. R., Parsons, M. E., Dimaprit, (*S*-[3-(*N*,*N*-dimethylamino)propyl]isothiourea). A highly specific histamine H_2-receptor agonist. Part 2. Structure-activity considerations. *Agents Actions* **1977**, 7, 39-43.

(154) Parsons, M. E., Owen, D. A., Ganellin, C. R., Durant, G. J., Dimaprit -(*S*-[3-(*N*,*N*-dimethylamino)prophyl]isothiourea) - a highly specific histamine H_2 -receptor agonist. Part 1. Pharmacology. *Agents Actions* **1977**, 7, 31-37.

(155) Parsons, M. E., Blakemore, R. C., Durant, G. J., Ganellin, C. R., Rasmussen, A. C., 3-[4(5)-Imidazolyl]propylguanidine (SK&F 91486) - a partial agonist at histamine H_2-receptors. *Agents Actions* **1975**, 5, 464.

(156) Durant, G. J., Duncan, W. A., Ganellin, C. R., Parsons, M. E., Blackmore, R. C., Rasmussen, A. C., Impromidine (SK&F 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature* **1978**, 276, 403-405.

(157) Durant, G. J., Ganellin, C. R., Hills, D. W., Miles, P. D., Parsons, M. E., Pepper, E. S., White, G. R., The Histamine H_2 Receptor Agonist Impromidine: Synthesis and Structure-Activity Considerations. *J. Med. Chem.* **1985**, 28, 1414-1422.

(158) Dove, S., Elz, S., Seifert, R., Buschauer, A., Structure-Activity Relationsships of Histamine H_2 Receptor Ligands. *Mini Rev. Med. Chem.* **2004**, 4, 941-954.

(159) Buschauer, A., Synthesis and in Vitro Pharmacology of Arpromidine and Related Phenyl(pyridylalkyl)guanidines, a Potential New Class of Positive Inotropic Drugs. *J. Med. Chem.* **1989**, 32, 1963-1970.

(160) Buschauer, A., Friese-Kimmel, A., Baumann, G., Schunack, W., Synthesis and histamine H_2 agonistic activity of arpromidine analogues: replacement of the pheniramine-like moiety by non-heterocyclic groups. *Eur. J. Med. Chem.* **1992**, 27, 321-330.

(161) Elz, S., Gerhard, G., Schunack, W., Histamine analogues. 32nd communication: synthesis and pharmacology of sopromidine, a potent and stereoselective isomer of the achiral H_2-agonist impromidine. *Eur. J. Med. Chem.* **1989**, 24, 259-262.

(162) Baumann, G., Felix, S. B., Heidecke, C. D., Riess, G., Loher, U., Ludwig, L., Blomer, H., Apparent superiority of H_2-receptor stimulation and simultaneous beta-blockade over conventional treatment with beta-sympathomimetic drugs in post-acute myocardial infarction: cardiac effects of impromidine-a new specific H_2-receptor agonist-in the surviving catecholamine-insensitive myocardium. *Inflamm. Res.* **1984**, 15, 216-228.

(163) Baumann, G., Permanetter, B., Wirtzfeld, A., Possible value of H_2-receptor agonists for treatment of catecholamine-insensitive congestive heart failure. *Pharmacol. Ther.* **1984**, 24, 165-177.

(164) Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press, DOI: 10.1021/jm800841w. Epub ahead of print on Oct 800825, 802008.

(165) Kraus, A., Ghorai, P., Birnkammer, T., Schnell, D., Elz, S., Seifert, R., Dove, S., Bernhardt, G., Buschauer, A., N^G-Acylated Aminothiazolylpropylguanidines as Potent and Selective Histamine H_2 Receptor Agonists. *ChemMedChem* **2008**, accepted.

(166) van der Goot, H., Timmerman, H., Selective ligands as tools to study histamine receptors. *Eur. J. Med. Chem.* **2000**, 35, 5-20.

(167) Yanagisawa, I., Hirata, Y., Ishii, Y., Studies on histamine H_2 receptor antagonists. 2. Synthesis and pharmacological activities of *N*-sulfamoyl and *N*-sulfonyl amidine derivatives. *J. Med. Chem.* **1987**, 30, 1787-1793.

(168) Schwartz, J. C., Histamine as a transmitter in brain. *Life Sci.* **1975**, 17, 503-517.

(169) Arrang, J. M., Garbarg, M., Schwartz, J. C., Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **1983**, 302, 832-837.

(170) Arrang, J. M., Garbarg, M., Lancelo, J. C., Lecomte, J. M., Pollard, H., Robba, M., Schunack, W., Schwartz, J. C., Highly potent and selective ligands for histamine H_3-receptors. *Nature* **1987**, 327, 117-123.

(171) Lovenberg, T. W., Roland, B. L., Wilson, S. J., Jiang, X., Pyati, J., Huvar, A., Jackson, M. R., Erlander, M. G., Cloning and functional expression of the human histamine H_3 receptor. *Mol. Pharmacol.* **1999**, 55, 1101-1107.

(172) Wiedemann, P., Bonisch, H., Oerters, F., Bruss, M., Structure of the human histamine H_3 receptor gene (HRH_3) and identification of naturally occurring variations. *J. Neural Transm.* **2002**, 109, 443-453.

(173) Coge, F., Guenin, S. P., Audinot, V., Renouard-Try, A., Beauverger, P., Macia, C., Ouvry, C., Nagel, N., Rique, H., Boutin, J. A., Galizzi, J. P., Genomic organization and characterization of splice variants of the human histamine H_3 receptor. *Biochem. J.* **2001**, 355, 279-288.

(174) Bongers, G., Bakker, R. A., Leurs, R., Molecular aspects of the histamine H_3 receptor. *Biochem. Pharmacol.* **2007**, 73, 1195-1204.

(175) Rouleau, A., Ligneau, X., Tardivel-Lacombe, J., Morisset, S., Gbahou, F., Schwartz, J. C., Arrang, J. M., Histamine H_3-receptor-mediated [^{35}S]GTP γ[S] binding: evidence for constitutive activity of the recombinant and native rat and human H_3 receptors. *Br. J. Pharmacol.* **2002**, 135, 383-392.

(176) Clark, E. A., Hill, S. J., Sensitivity of histamine H_3 receptor agonist-stimulated [^{35}S]GTP γ[S] binding to pertussis toxin. *Eur. J. Pharmacol.* **1996**, 296, 223-225.

(177) Arrang, J. M., Devaux, B., Chodkiewicz, J. P., Schwartz, J. C., H_3-receptors control histamine release in human brain. *J. Neurochem.* **1988**, 51, 105-108.

(178) Arrang, J. M., Garbarg, M., Schwartz, J. C., Autoinhibition of histamine synthesis mediated by presynaptic H_3-receptors. *Neuroscience* **1987**, 23, 149-157.

(179) Schlicker, E., Fink, K., Detzner, M., Gothert, M., Histamine inhibits dopamine release in the mouse striatum via presynaptic H_3 receptors. *J. Neural Transm. Gen. Sect.* **1993**, 93, 1-10.

(180) Schlicker, E., Betz, R., Gothert, M., Histamine H_3 receptor-mediated inhibition of serotonin release in the rat brain cortex. *Naunyn. Schmiedebergs Arch. Pharmacol.* **1988**, 337, 588-590.

(181) Schlicker, E., Fink, K., Hinterthaner, M., Gothert, M., Inhibition of noradrenaline release in the rat brain cortex via presynaptic H_3 receptors. *Naunyn. Schmiedebergs Arch. Pharmacol.* **1989**, 340, 633-638.

(182) Clapham, J., Kilpatrick, G. J., Histamine H_3 receptors modulate the release of [^{3}H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H_3 receptor subtypes. *Br. J. Pharmacol.* **1992**, 107, 919-923.

(183) Garcia, M., Floran, B., Arias-Montano, J. A., Young, J. M., Aceves, J., Histamine H_3 receptor activation selectively inhibits dopamine D_1 receptor-dependent [^{3}H]GABA release from depolarization-stimulated slices of rat substantia nigra pars reticulata. *Neuroscience* **1997**, 80, 241-249.

(184) Bakker, R. A., Histamine H_3-receptor isoforms. *Inflamm. Res.* **2004**, 53, 509-516.

(185) Yao, B. B., Hutchins, C. W., Carr, T. L., Cassar, S., Masters, J. N., Bennani, Y. L., Esbenshade, T. A., Hancock, A. A., Molecular modeling and pharmacological analysis of species-related histamine H_3 receptor heterogeneity. *Neuropharmacology* **2003**, 44, 773-786.

(186) Uveges, A. J., Kowal, D., Zhang, Y., Spangler, T. B., Dunlop, J., Semus, S., Jones, P. G., The Role of Transmembrane Helix 5 in Agonist Binding to the Human H_3 Receptor. *J. Pharmacol. Exp. Ther.* **2002**, 301, 451-458.

(187) van der Goot, H., Schepers, M. J. P., Sterk, G. J., Timmerman, H., Isothiourea analogues of histamine as potent agonists or antagonists of the histamine H_3-receptor. *Eur. J. Med. Chem.* **1992**, 27, 511-517.

(188) Kitbunnadaj, R., Hashimoto, T., Poli, E., Zuiderveld, O. P., Menozzi, A., Hidaka, R., de Esch, I. J. P., Bakker, R. A., Menge, W. M. P. B., Yamatodani, A., Coruzzi, G., Timmerman, H., Leurs, R., *N*-Substituted Piperidinyl Alkyl Imidazoles: Discovery of Methimepip as a Potent and Selective Histamine H_3 Receptor Agonist. *J. Med. Chem.* **2005**, 48, 2100-2107.

(189) Krause, M., Rouleau, A., Stark, H., Luger, P., Lipp, R., Garbarg, M., Schwart, J. C., Schunack, W., Synthesis, X-ray crystallography, and pharmacokinetics of novel azomethine prodrugs of (*R*)-α-methylhistamine: highly potent and selective histamine H_3 receptor agonists. *J. Med. Chem.* **1995**, 38, 4070-4079.

(190) Lin, J. S., Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med. Rev.* **2000**, 4, 471-503.

(191) Cannon, K. E., Nalwalk, J. W., Stadel, R., Ge, P., Lawson, D., Silos-Santiago, I., Hough, L. B., Activation of spinal histamine H_3 receptors inhibits mechanical nociception. *Eur. J. Pharmacol.* **2003**, 470, 139-147.

(192) Matsubara, T., Moskowitz, M. A., Huang, Z., UK-14,304, *R*(-)-α-methyl-histamine and SMS 201-995 block plasma protein leakage within dura mater by prejunctional mechanisms. *Eur. J. Pharmacol.* **1992**, 224, 145-150.

(193) Ichinose, M., Belvisi, M. G., Barnes, P. J., Histamine H_3-receptors inhibit neurogenic microvascular leakage in airways. *J. Appl. Physiol.* **1990**, 68, 21-25.

(194) Millan-Guerrero, R. O., Pineda-Lucatero, A. G., Hernandez-Benjamin, T., Tene, C. E., Pacheco, M. F., N^{α}-methylhistamine safety and efficacy in migraine prophylaxis: phase I and phase II studies. *Headache* **2003**, 43, 389-394.

(195) Esbenshade, T. A., Fox, G. B., Krueger, K. M., Baranowski, J. L., Miller, T. R., Kang, C. H., Denny, L. I., Witte, D. G., Yao, B. B., Pan, J. B., Faghih, R., Bennani, Y. L., Williams, M., Hancock, A. A., Pharmacological and behavioral properties of A-349821, a selective and potent human histamine H_3 receptor antagonist. *Biochem. Pharmacol.* **2004**, 68, 933-945.

(196) Bonaventure, P., Letavic, M., Dugovic, C., Wilson, S., Aluisio, L., Pudiak, C., Lord, B., Mazur, C., Kamme, F., Nishino, S., Carruthers, N., Lovenberg, T., Histamine H_3 receptor antagonists: from target identification to drug leads. *Biochem. Pharmacol.* **2007**, 73, 1084-1096.

(197) Hancock, A. A., H_3 receptor antagonists/inverse agonists as anti-obesity agents. *Curr. Opin. Investig. Drugs* **2003**, 4, 1190-1197.

(198) Ito, C., The role of the central histaminergic system on schizophrenia. *Drug News Perspect.* **2004**, 17, 383-387.

(199) Howard, H. R., Agents for attention-deficit hyperactivity disorder – an update. *Expert Opin. Ther. Patents* **2004**, 14, 983-1008.

(200) Leurs, R., Bakker, R. A., Timmerman, H., de Esch, I. J. P., The histamine H_3 receptor: From gene cloning to H_3 receptor drugs. *Nat. Rev. Drug Discov.* **2005**, 4, 107-120.

(201) Giovannini, M. G., Bartolini, L., Bacciottini, L., Greco, L., Blandina, P., Effects of histamine H_3 receptor agonists and antagonists on cognitive performance and scopolamine-induced amnesia. *Behav. Brain Res.* **1999**, 104, 147-155.

(202) Prast, H., Argyriou, A., Philippu, A., Histaminergic neurons facilitate social memory in rats. *Brain Res.* **1996**, 734, 316-318.

(203) Varty, L. M., Gustafson, E., Laverty, M., Hey, J. A., Activation of histamine H_3 receptors in human nasal mucosa inhibits sympathetic vasoconstriction. *Eur. J. Pharmacol.* **2004**, 484, 83-89.

(204) Raible, D. G., Schulman, E. S., DiMuzio, J., Cardillo, R., Post, T. J., Mast cell mediators prostaglandin-D_2 and histamine activate human eosinophils. *J. Immunol.* **1992**, 148, 3536-3542.

(205) Raible, D. G., Lenahan, T., Fayvilevich, Y., Kosinski, R., Schulman, E. S., Pharmacologic characterization of a novel histamine receptor on human eosinophils. *Am. J. Respir. Crit. Care Med.* **1994**, 149, 1506-1511.

(206) Nakamura, T., Itadani, H., Hidaka, Y., Ohta, M., Tanaka, K., Molecular Cloning and Characterization of a New Human Histamine Receptor, hH_4R. *Biochem. Biophys. Res. Commun.* **2000**, 279, 615-620.

(207) Oda, T., Morikawa, N., Saito, Y., Masuho, Y., Matsumoto, S., Molecular Cloning and Characterization of a Novel Type of Histamine Receptor Preferentially Expressed in Leukocytes. *J. Biol. Chem.* **2000**, 275, 36781-36786.

(208) Coge, F., Guenin, S.-P., Rique, H., Boutin, J. A., Galizzi, J.-P., Structure and expression of the human histamine H_4-receptor gene. *Biochem. Biophys. Res. Commun.* **2001**, 284, 301-309.

(209) Liu, C., Ma, X.-J., Jiang, X., Wilson, S. J., Hofstra, C. L., Blevitt, J., Pyati, J., Li, X., Chai, W., Carruthers, N., Lovenberg, T. W., Cloning and Pharmacological Characterization of a Fourth Histamine Receptor (H_4) Expressed in Bone Marrow. *Mol. Pharmacol.* **2001**, 59, 420-426.

(210) Morse, K. L., Behan, J., Laz, T. M., West, R. E., Jr., Greenfeder, S. A., Anthes, J. C., Umland, S., Wan, Y., Hipkin, R. W., Gonsiorek, W., Shin, N., Gustafson, E. L., Qiao, X., Wang, S.,

Hedrick, J. A., Greene, J., Bayne, M., Monsma, F. J., Jr., Cloning and Characterization of a Novel Human Histamine Receptor. *J. Pharmacol. Exp. Ther.* **2001**, 296, 1058-1066.

(211) Nguyen, T., Shapiro, D. A., George, S. R., Setola, V., Lee, D. K., Cheng, R., Rauser, L., Lee, S. P., Lynch, K. R., Roth, B. L., O'Dowd, B. F., Discovery of a Novel Member of the Histamine Receptor Family. *Mol. Pharmacol.* **2001**, 59, 427-433.

(212) Zhu, Y., Michalovich, D., Wu, H.-L., Tan, K. B., Dytko, G. M., Mannan, I. J., Boyce, R., Alston, J., Tierney, L. A., Li, X., Herrity, N. C., Vawter, L., Sarau, H. M., Ames, R. S., Davenport, C. M., Hieble, J. P., Wilson, S., Bergsma, D. J., Fitzgerald, L. R., Cloning, Expression, and Pharmacological Characterization of a Novel Human Histamine Receptor. *Mol. Pharmacol.* **2001**, 59, 434-441.

(213) vanRijn, R. M., vanMarle, A., Chazot, P. L., Langemeijer, E., Qin, Y., Shenton, F. C., Lim, H. D., Zuiderveld, O. P., Sansuk, K., Dy, M., Smit, M. J., Tensen, C. P., Bakker, R. A., Leurs, R., Cloning and characterization of dominant negative splice variants of the human histamine H_4 receptor. *Biochem. J.* **2008**, 414, 121-131.

(214) Hofstra, C. L., Desai, P. J., Thurmond, R. L., Fung-Leung, W.-P., Histamine H_4 Receptor Mediates Chemotaxis and Calcium Mobilization of Mast Cells. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1212-1221.

(215) de Esch, I. J. P., Thurmond, R. L., Jongejan, A., Leurs, R., The histamine H_4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol. Sci.* **2005**, 26, 462-469.

(216) Gantner, F., Sakai, K., Tusche, M. W., Cruikshank, W. W., Center, D. M., Bacon, K. B., Histamine H_4 and H_2 Receptors Control Histamine-Induced Interleukin-16 Release from Human $CD8^+$ T Cells. *J. Pharmacol. Exp. Ther.* **2002**, 303, 300-307.

(217) Nakaya, M., Takeuchi, N., Kondo, K., Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann. Otol. Rhinol. Laryngol.* **2004**, 113, 552-557.

(218) Ling, P., Ngo, K., Nguyen, S., Thurmond, R. L., Edwards, J. P., Karlsson, L., Fung-Leung, W.-P., Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.* **2004**, 142, 161-171.

(219) Dijkstra, D., Leurs, R., Chazot, P., Shenton, F. C., Stark, H., Werfel, T., Gutzmer, R., Histamine downregulates monocyte CCL2 production through the histamine H_4 receptor. *J. Allergy Clin. Immunol.* **2007**, 120, 300-307.

(220) Buckland, K. F., Williams, T. J., Conroy, D. M., Histamine induces cytoskeletal changes in human eosinophils via the H_4 receptor. *Br. J. Pharmacol.* **2003**, 140, 1117-1127.

(221) Takeshita, K., Sakai, K., Bacon, K. B., Gantner, F., Critical Role of Histamine H_4 Receptor in Leukotriene B_4 Production and Mast Cell-Dependent Neutrophil Recruitment Induced by Zymosan in Vivo. *J. Pharmacol. Exp. Ther.* **2003**, 307, 1072-1078.

(222) Dunford, P. J., O'Donnell, N., Riley, J. P., Williams, K. N., Karlsson, L., Thurmond, R. L., The Histamine H_4 Receptor Mediates Allergic Airway Inflammation by Regulating the Activation of $CD4^+$ T Cells. *J. Immunol.* **2006**, 176, 7062-7070.

(223) Thurmond, R. L., Desai, P. J., Dunford, P. J., Fung-Leung, W.-P., Hofstra, C. L., Jiang, W., Nguyen, S., Riley, J. P., Sun, S., Williams, K. N., Edwards, J. P., Karlsson, L., A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.* **2004**, 309, 404-413.

(224) Coruzzi, G., Adami, M., Guaita, E., de Esch, I. J. P., Leurs, R., Antiinflammatory and antinociceptive effects of the selective histamine H_4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. *Eur. J. Pharmacol.* **2007**, 563, 240-244.

(225) Smits, R. A., Lim, H. D., Hanzer, A., Zuiderveld, O. P., Guaita, E., Adami, M., Coruzzi, G., Leurs, R., de Esch, I. J. P., Fragment Based Design of New H_4 Receptor-Ligands with Anti-inflammatory Properties in Vivo. *J. Med. Chem.* **2008**, 51, 2457-2467.

(226) Varga, C., Horvath, K., Berko, A., Thurmond, R. L., Dunford, P. J., Whittle, B. J. R., Inhibitory effects of histamine H_4 receptor antagonists on experimental colitis in the rat. *Eur. J. Pharmacol.* **2005**, 522, 130-138.

(227) Dunford, P. J., Williams, K. N., Desai, P. J., Karlsson, L., McQueen, D., Thurmond, R. L., Histamine H_4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. *J. Allergy Clin. Immunol.* **2007**, 119, 176-183.

(228) Shin, N., Coates, E., Murgolo, N. J., Morse, K. L., Bayne, M., Strader, C. D., Monsma, F. J., Jr., Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H_4 receptor. *Mol. Pharmacol.* **2002**, 62, 38-47.

(229) Jongejan, A., Lim, H. D., Smits, R. A., de Esch, I. J. P., Haaksma, E., Leurs, R., Delineation of Agonist Binding to the Human Histamine H_4 Receptor Using Mutational Analysis, Homology Modeling, and ab Initio Calculations. *J. Chem. Inf. Model.* **2008**, 48, 1455-1463.

(230) Kiss, R., Noszál, B., Rácz, Á., Falus, A., Erós, D., Keseru, G. M., Binding mode analysis and enrichment studies on homology models of the human histamine H_4 receptor. *Eur. J. Med. Chem.* **2008**, 43, 1059-1070.

(231) Lim, H. D., Jongejan, A., Bakker, R. A., Haaksma, E., de Esch, I. J. P., Leurs, R., Phenylalanine^{169} in the second extracellular loop of the human histamine H_4 receptor is responsible for the difference in agonist binding between human and mouse H_4 receptors. *J. Pharmacol. Exp. Ther.* **2008**, 327, 88-96.

(232) Hashimoto, T., Harusawa, S., Araki, L., Zuiderveld, O. P., Smit, M. J., Imazu, T., Takashima, S., Yamamoto, Y., Sakamoto, Y., Kurihara, T., Leurs, R., Bakker, R. A., Yamatodani, A., A Selective Human H_4-Receptor Agonist: (-)-2-Cyano-1-methyl-3-{(2*R*,5*R*)-5-[1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl}methylguanidine. *J. Med. Chem.* **2003**, 46, 3162-3165.

(233) Lim, H. D., Smits, R. A., Bakker, R. A., van Dam, C. M. E., de Esch, I. J. P., Leurs, R., Discovery of *S*-(2-Guanidylethyl)-isothiourea (VUF 8430) as a Potent Nonimidazole Histamine H_4 Receptor Agonist. *J. Med. Chem.* **2006**, 49, 6650-6651.

(234) Jablonowski, J. A., Grice, C. A., Chai, W., Dvorak, C. A., Venable, J. D., Kwok, A. K., Ly, K. S., Wei, J., Baker, S. M., Desai, P. J., Jiang, W., Wilson, S. J., Thurmond, R. L., Karlsson, L., Edwards, J. P., Lovenberg, T. W., Carruthers, N. I., The First Potent and Selective Non-Imidazole Human Histamine H_4 Receptor Antagonists. *J. Med. Chem.* **2003**, 46, 3957-3960.

(235) Altenbach, R. J., Adair, R. M., Bettencourt, B. M., Black, L. A., Fix-Stenzel, S. R., Gopalakrishnan, S. M., Hsieh, G. C., Liu, H., Marsh, K. C., McPherson, M. J., Milicic, I., Miller, T. R., Vortherms, T. A., Warrior, U., Wetter, J. M., Wishart, N., Witte, D. G., Honore, P., Esbenshade, T. A., Hancock, A. A., et al., Structure-Activity Studies on a Series of a 2-Aminopyrimidine-Containing Histamine H_4 Receptor Ligands. *J. Med. Chem.* **2008**, 51, 6571-6580.

(236) Cowart, M. D., Altenbach, R. J., Liu, H., Hsieh, G. C., Drizin, I., Milicic, I., Miller, T. R., Witte, D. G., Wishart, N., Fix-Stenzel, S. R., McPherson, M. J., Adair, R. M., Wetter, J. M., Bettencourt, B. M., Marsh, K. C., Sullivan, J. P., Honore, P., Esbenshade, T. A., Brioni, J. D., Rotationally Constrained 2,4-Diamino-5,6-disubstituted Pyrimidines: A New Class of Histamine H_4 Receptor Antagonists with Improved Druglikeness and in Vivo Efficacy in Pain and Inflammation Models. *J. Med. Chem.* **2008**, 51, 6547-6557.

(237) Jablonowski, J. A., Carruthers, N. I., Thurmond, R. L., The Histamine H_4 Receptor and Potential Therapeutic Uses for H_4 Ligands. *Mini Rev. Med. Chem.* **2004**, 4, 993-1000.

Chapter 2

Scope and objectives

Arpromidine belongs to the most potent guanidine-type histamine H_2 receptor (H_2R) agonists reported in literature.[1] However, drawbacks of this class of compounds are the very poor bioavailability after oral administration and lack of CNS penetration. This resulted in the development of N^G-acylated imidazolylpropylguanidines with substantially reduced basicity relative to the guanidines. Depending on the acyl residue, these compounds show a comparable or just slight reduction in H_2R agonistic activity relative to the guanidine-type H_2R agonists which demonstrates the alkylguanidines and acylguanidines to conduct as bioisosteres.[2-4]

Surprisingly, more detailed pharmacological investigations of the acylguanidine-type H_2R agonists at other HR subtypes revealed these compounds to be also active at the hH_3R and hH_4R. In general, the compounds displayed high potencies at both HR subtypes along with rather low efficacies at the hH_3R but high efficacies at the hH_4R.

The aim of this thesis was the design, synthesis and pharmacological characterization of novel N^G-acylated imidazolylpropylguanidines and related compounds to elucidate structure-activity and structure-selectivity relationships at the different HR subtypes and to obtain HR subtype selective compounds. As the biological role of the recently reported H_4R is far from being understood,[5-11] a major goal was the development of potent and selective H_4R agonists as pharmacological tools. Therefore, the structural components of the N^G-acylated imidazolylpropylguanidines should be systematically modified resulting in four series of compounds (Figure 2.1).

Cyanoguanidines like OUP-16[12] display agonistic potency at the H_4R, whereas in the series of guanidine-type H_2R agonists the introduction of a cyanoguanidine group was not tolerated in terms of H_2R activation.[13] Therefore, in the first series of compounds the basic acylguanidine group should be replaced by a non-basic cyanoguanidine group with the intent to dispose agonistic activity at the H_2R and retain agonistic potency at the H_4R. In addition, the acylguanidine group should be replaced with a basic carbamoylguanidine and a non-basic sulfonylguanidine moiety to obtain information about the structural and physicochemical requirements of the central group for HR activity.

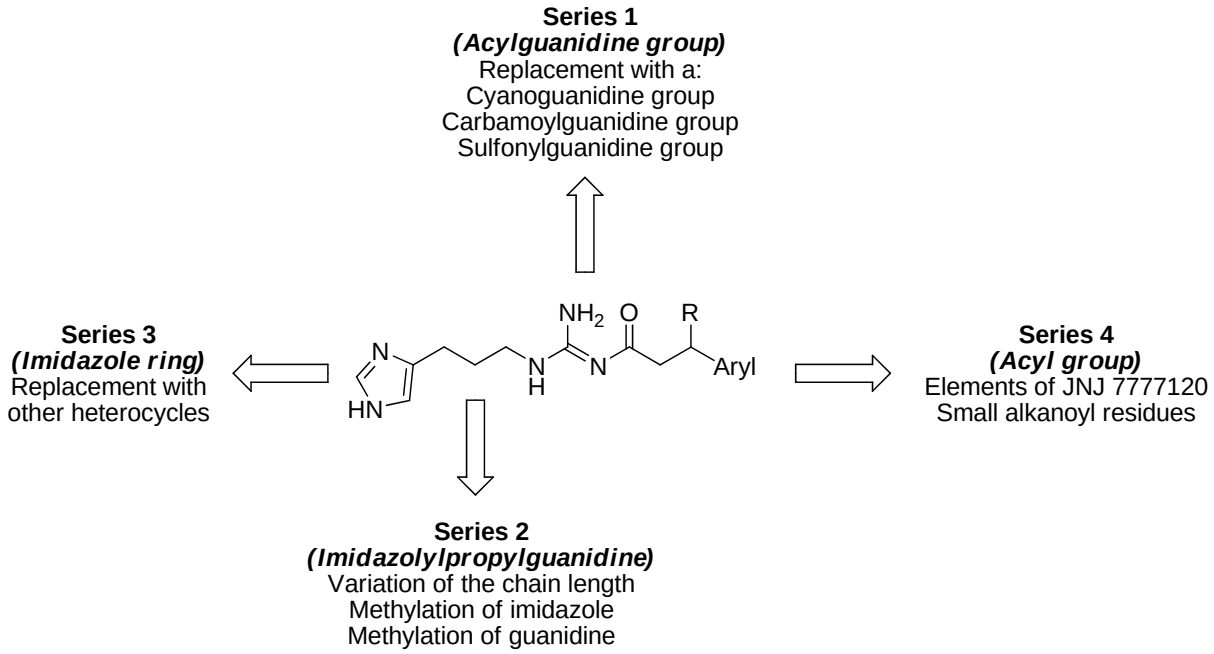


Figure 2.1. Overview about the intended modifications of the N^G-acylated imidazolylpropylguanidines resulting in four series of compounds.

The second series of compounds should focus on the imidazolylpropylguanidine portion. A three membered carbon chain connecting the imidazole-ring with the (acyl)guanidine moiety has been demonstrated to be optimal for H_2R agonistic activity.[2, 14] To elucidate if this is also true for H_3R and H_4R activity, the chain length should be varied. As methylation in position 5 of the imidazole ring in histamine substantially increased selectivity for the H_4R relative to the H_3R,[15] the same strategy should be employed for the N^G-acylated imidazolylpropyl-guanidines. Methylation of the guanidine group in arpromidine was incompatible with high H_2R agonistic potency.[1] Therefore, N^G-alkylation should be investigated with respect to shifting the selectivity towards the H_3R or H_4R at the expense of H_2R agonistic activity.

Replacing the imidazole ring in acylguanidine-type H_2R agonists with aminothiazoles resulted in remarkably enhanced selectivity for the H_2R relative to the H_3R and H_4R.[16] To evaluate if this concept of improving HR subtype selectivity can be transferred to other heterocycles, in the third series the imidazole ring should be substituted with aromatic groups.

The last series of compounds should focus on the acyl residue. Two strategies should be employed in order to achieve an increase in selectivity for the H_4R: Structural moieties of the selective H_4R antagonist JNJ 7777120 or small alkanoyl residues, respectively, were attached to the imidazolylpropylguanidine component. The latter approach was stimulated by the observation that high H_2R potency is favored by more bulky N^G-acyl residues in guanidine-type HR ligands,[14] whereas the small endogenous ligand histamine is approximately 50 times more potent at the H_3R and H_4R than at the H_2R. Obviously, an additional affinity conferring moiety is not required to obtain high potencies at the H_3R and

H_4R. Hence, small alkanoyl residues should be introduced with the aim to shift selectivity from the H_2R toward the H_3R and in particular H_4R.

Moreover, the prepared compounds should be evaluated for the potential to convert promising HR ligands to pharmacological tools with special qualities as for example radioligands.

The synthesized compounds should be pharmacologically investigated for agonism and antagonism at the different HR subtypes in membrane steady-state GTPase activity assays. In addition, selected compounds should be investigated at the guinea-pig ileum for H_1R activity and at the guinea-pig right atrium for H_2R activity.

In summary, the aim of this thesis was to design, synthesize and pharmacologically characterize new N^G-acylated imidazolylpropylguanidines and related compounds to get more information about structure-activity and structure-selectivity relationships and to obtain HR subtype selective compounds – in particular H_4R agonists – as pharmacological tools.

References

(1) Buschauer, A., Synthesis and in Vitro Pharmacology of Arpromidine and Related Phenyl(pyridylalkyl)guanidines, a Potential New Class of Positive Inotropic Drugs. *J. Med. Chem.* **1989**, 32, 1963-1970.

(2) Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press, DOI: 10.1021/jm800841w. Epub ahead of print on Oct 800825, 802008.

(3) Xie, S.-X., Ghorai, P., Ye, Q.-Z., Buschauer, A., Seifert, R., Probing Ligand-Specific Histamine H_1- and H_2-Receptor Conformations with N^G-Acylated Imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006**, 317, 139-146.

(4) Xie, S.-X., Kraus, A., Ghorai, P., Ye, Q.-Z., Elz, S., Buschauer, A., Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-AK57), a Potent Partial Agonist for the Human Histamine H_1- and H_2-Receptors. *J. Pharmacol. Exp. Ther.* **2006**, 317, 1262-1268.

(5) Nakamura, T., Itadani, H., Hidaka, Y., Ohta, M., Tanaka, K., Molecular Cloning and Characterization of a New Human Histamine Receptor, hH_4R. *Biochem. Biophys. Res. Commun.* **2000**, 279, 615-620.

(6) Oda, T., Morikawa, N., Saito, Y., Masuho, Y., Matsumoto, S., Molecular Cloning and Characterization of a Novel Type of Histamine Receptor Preferentially Expressed in Leukocytes. *J. Biol. Chem.* **2000**, 275, 36781-36786.

(7) Coge, F., Guenin, S.-P., Rique, H., Boutin, J. A., Galizzi, J.-P., Structure and expression of the human histamine H_4-receptor gene. *Biochem. Biophys. Res. Commun.* **2001**, 284, 301-309.

(8) Liu, C., Ma, X.-J., Jiang, X., Wilson, S. J., Hofstra, C. L., Blevitt, J., Pyati, J., Li, X., Chai, W., Carruthers, N., Lovenberg, T. W., Cloning and Pharmacological Characterization of a Fourth Histamine Receptor (H_4) Expressed in Bone Marrow. *Mol. Pharmacol.* **2001**, 59, 420-426.

(9) Morse, K. L., Behan, J., Laz, T. M., West, R. E., Jr., Greenfeder, S. A., Anthes, J. C., Umland, S., Wan, Y., Hipkin, R. W., Gonsiorek, W., Shin, N., Gustafson, E. L., Qiao, X., Wang, S.,

Hedrick, J. A., Greene, J., Bayne, M., Monsma, F. J., Jr., Cloning and Characterization of a Novel Human Histamine Receptor. *J. Pharmacol. Exp. Ther.* **2001**, 296, 1058-1066.

(10) Nguyen, T., Shapiro, D. A., George, S. R., Setola, V., Lee, D. K., Cheng, R., Rauser, L., Lee, S. P., Lynch, K. R., Roth, B. L., O'Dowd, B. F., Discovery of a Novel Member of the Histamine Receptor Family. *Mol. Pharmacol.* **2001**, 59, 427-433.

(11) Zhu, Y., Michalovich, D., Wu, H.-L., Tan, K. B., Dytko, G. M., Mannan, I. J., Boyce, R., Alston, J., Tierney, L. A., Li, X., Herrity, N. C., Vawter, L., Sarau, H. M., Ames, R. S., Davenport, C. M., Hieble, J. P., Wilson, S., Bergsma, D. J., Fitzgerald, L. R., Cloning, Expression, and Pharmacological Characterization of a Novel Human Histamine Receptor. *Mol. Pharmacol.* **2001**, 59, 434-441.

(12) Hashimoto, T., Harusawa, S., Araki, L., Zuiderveld, O. P., Smit, M. J., Imazu, T., Takashima, S., Yamamoto, Y., Sakamoto, Y., Kurihara, T., Leurs, R., Bakker, R. A., Yamatodani, A., A Selective Human H_4-Receptor Agonist: (-)-2-Cyano-1-methyl-3-{(2*R*,5*R*)-5-[1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl}methylguanidine. *J. Med. Chem.* **2003**, 46, 3162-3165.

(13) Durant, G. J., Ganellin, C. R., Hills, D. W., Miles, P. D., Parsons, M. E., Pepper, E. S., White, G. R., The Histamine H_2 Receptor Agonist Impromidine: Synthesis and Structure-Activity Considerations. *J. Med. Chem.* **1985**, 28, 1414-1422.

(14) Dove, S., Elz, S., Seifert, R., Buschauer, A., Structure-Activity Relationsships of Histamine H_2 Receptor Ligands. *Mini Rev. Med. Chem.* **2004**, 4, 941-954.

(15) Lim, H. D., van Rijn, R. M., Ling, P., Bakker, R. A., Thurmond, R. L., Leurs, R., Evaluation of Histamine H_1-, H_2-, and H_3-Receptor Ligands at the Human Histamine H_4 Receptor: Identification of 4-Methylhistamine as the First Potent and Selective H_4 Receptor Agonist. *J. Pharmacol. Exp. Ther.* **2005**, 314, 1310-1321.

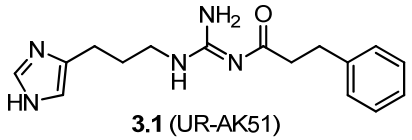
(16) Kraus, A., Ghorai, P., Birnkammer, T., Schnell, D., Elz, S., Seifert, R., Dove, S., Bernhardt, G., Buschauer, A., N^G-Acylated Aminothiazolylpropylguanidines as Potent and Selective Histamine H_2 Receptor Agonists. *ChemMedChem* **2008**, accepted.

Chapter 3

Imidazolylbutylcyanoguanidines and analogs: Identification of potent and selective histamine H_3R and H_4R ligands

3.1 Introduction

Among the HR subtypes, the H_4R shows the highest degree of homology with the H_3R. Therefore it is not surprising that many H_3R ligands also exhibit high affinity for the H_4R.[1] Especially for imidazole containing compounds such as thioperamide or proxyfan an overlap in the pharmacological profiles is observed.[1] At present, only a confined number of selective H_4R agonists, including 5-methylhistamine,[1] OUP-16 (**3.2**)[2] or VUF 8430,[3] has been reported. However, these compounds have limitations since they also activate other HR subtypes.

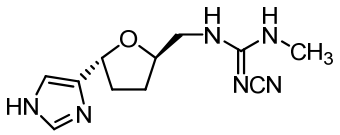


3.1 (UR-AK51)

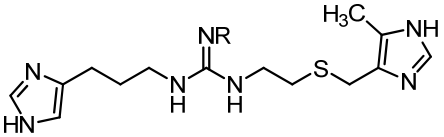
	EC_{50} [nM]	E_{max}
hH_2R	100	0.84
hH_3R	2.8	0.52
hH_4R	17	0.74

Figure 3.1. The acylguanidine UR-AK51 and its pharmacological profile at HRs.

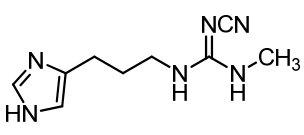
Initial point for the design of new selective H_4R agonists were N^G-acylated imidazolylpropyl-guanidines, originally developed as potent H_2R agonists.[4-6] More detailed pharmacological investigations revealed that several of these compounds are also active at the H_3R and H_4R. For example UR-AK51 (**3.1**)[5] shows high potencies both at the H_3R and H_4R but exhibits lower efficacy at the H_3R relative to the H_4R (Figure 3.1). With the aim to increase selectivity for the H_4R over the H_2R and H_3R, the presented study was focused on the central acyl-



3.2 (OUP-16)
EC_{50} (hH_4R) = 78 nM
E_{max} = 0.99
40 fold selectivity over hH_3R



R = H: **3.3** (Impromidine) gpH_2R agonist
rel. potency 48 x histamine, E_{max} = 0.99
R = CN: **3.4** gpH_2R antagonist, pA_2 = 6.24



3.5 (Improgan)
K_i (hH_4R) = 6,000 nM
inactive at $H_{1/2/3}Rs$

Figure 3.2. Cyanoguanidines described in literature and their pharmacological activities at HRs.

guanidine moiety. The first reported selective H_4R agonist OUP-16[2] contains – compared to the N^G-acylated imidazolylpropylguanidines ($pK_a \approx 8.0$)[7] – a non-basic cyanoguanidine moiety ($pK_a \approx -0.4$)[8]. Obviously, for H_4R activation a second basic component besides the imidazole ring is not essential. In contrast, when replacing the strongly basic guanidine group in the potent H_2R agonist impromidine (**3.3**), H_2R agonistic activity is abolished (Figure 3.2).[9] Moreover, the antinociceptive drug improgan (**3.5**) – as well containing a cyanoguanidine moiety – displays low affinity for the H_4R and lacks affinity for other HRs.[10] Therefore, cyanoguanidine analogs of the N^G-acylated imidazolylpropylguanidines were prepared with the goal to obtain compounds that still exhibit agonistic activity at the H_4R but are devoid of agonistic activity at other HR subtypes. Besides the cyanoguanidines, a couple of compounds were synthesized containing a carbamoylguanidine ($pK_a \approx 8.0$)[8] or a sulfonylguanidine ($pK_a \approx 0.7$)[7] moiety instead of the acylguanidine group (Figure 3.3). These central groups with different basicities may reveal more information about structure-activity relationships at the distinct HR subtypes. As acylguanidine-type H_2R agonists containing two sets of pharmacophoric groups (bivalent ligands) turned out to be considerably more potent than the monovalent counterparts,[11] this bivalent approach was also investigated in the cyanoguanidine series with respect to H_4R activity.

In this chapter the development of a new class of potent and selective hH_4R agonists and the unexpected discovery of a highly potent and selective carbamoylguanidine-type hH_3R inverse agonist is reported.

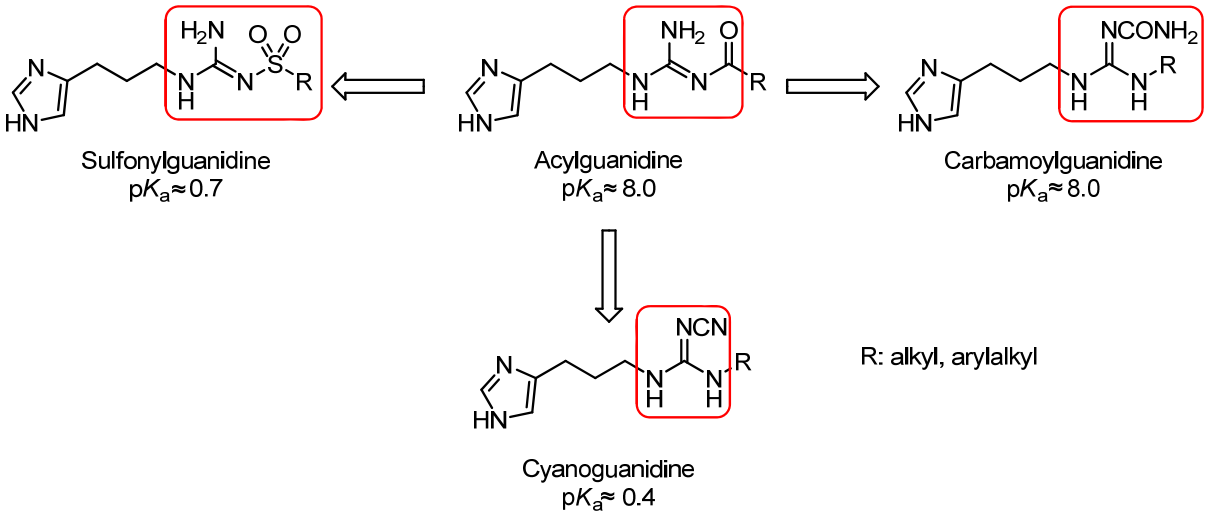
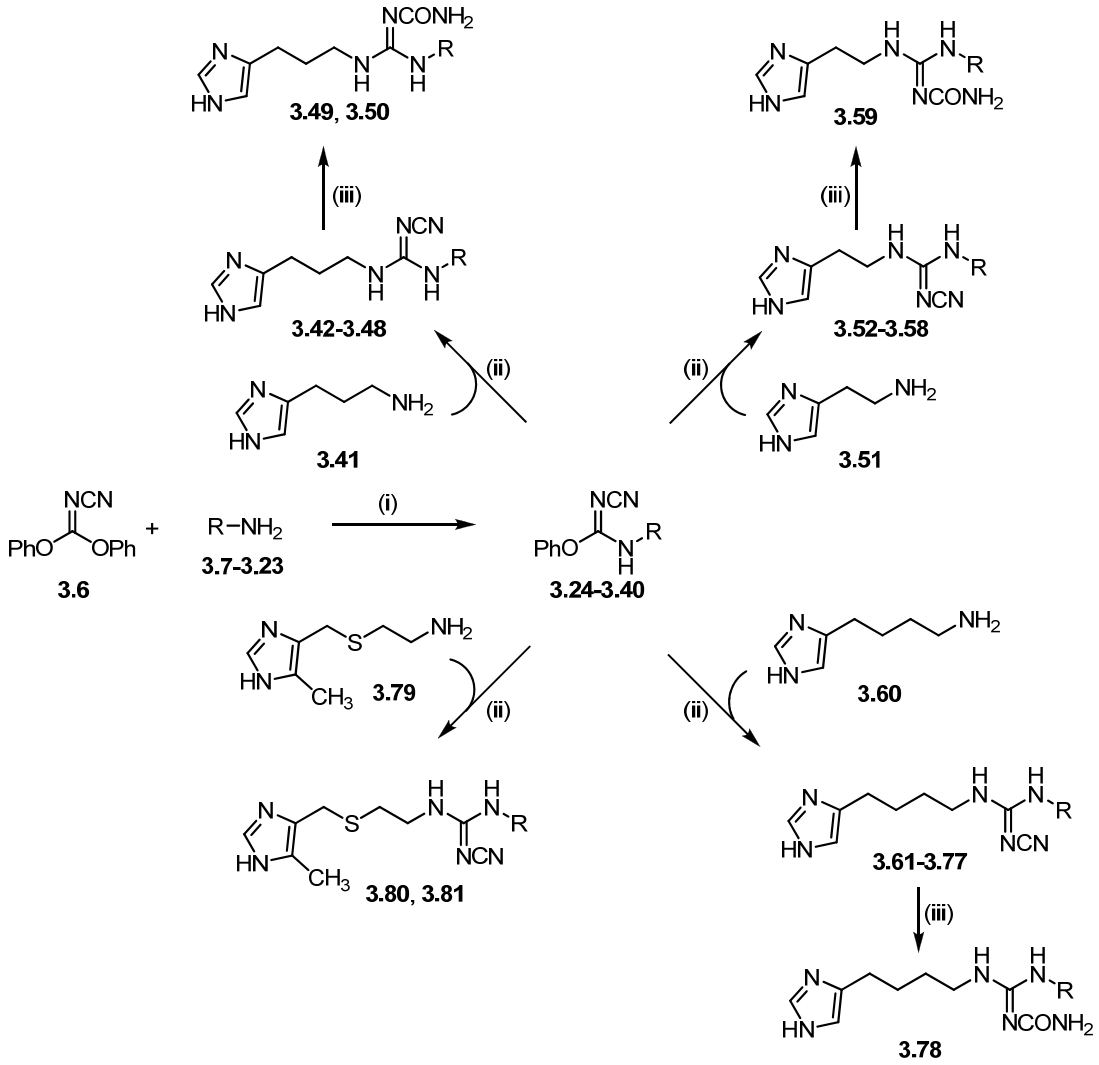


Figure 3.3. Replacement of the central acylguanidine group with a non-basic cyanoguanidine or sulfonylguanidine group and a basic carbamoylguanidine group.

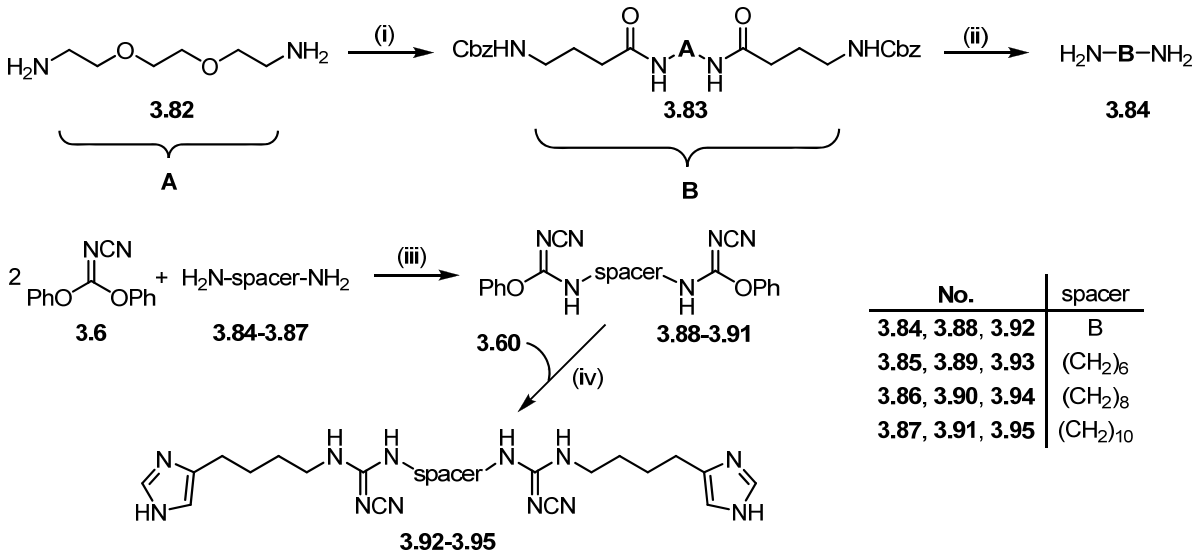
3.2 Chemistry



No.	R	No.	R
3.7, **3.24**, **3.42**, **3.49**, **3.52**, **3.59**, **3.61**, **3.78**, **3.80**	Ph-$(CH_2)_3$	**3.16**, **3.33**, **3.70**	Pyridin-3-yl-$(CH_2)_3$
3.8, **3.25**, **3.43**, **3.53**, **3.62**	Ph-$(CH_2)_2$	**3.17**, **3.34**, **3.71**	Pyridin-4-yl-$(CH_2)_3$
3.9, **3.26**, **3.44**, **3.50**, **3.54**, **3.63**	Ph-$(CH_2)_4$	**3.18**, **3.35**, **3.72**	Ph-$CH(CH_3)$-$(CH_2)_2$
3.10, **3.27**, **3.45**, **3.55**, **3.64**, **3.81**	Ph_2CH-$(CH_2)_2$	**3.19**, **3.36**, **3.73**	H
3.11, **3.28**, **3.46**, **3.56**, **3.65**	cHex-$(CH_2)_3$	**3.20**, **3.37**, **3.74**	CH_3
3.12, **3.29**, **3.47**, **3.57**, **3.66**	Ph-S-$(CH_2)_2$	**3.21**, **3.38**, **3.75**	CH_2CH_3
3.13, **3.30**, **3.48**, **3.58**, **3.67**	1*H*-Indol-3-yl-$(CH_2)_2$	**3.22**, **3.39**, **3.76**	$CH(CH_3)_2$
3.14, **3.31**, **3.68**	4-F-Ph-$(CH_2)_3$	**3.23**, **3.40**, **3.77**	CH_2-$CH(CH_3)_2$
3.15, **3.32**, **3.69**	Pyridin-2-yl-$(CH_2)_3$		

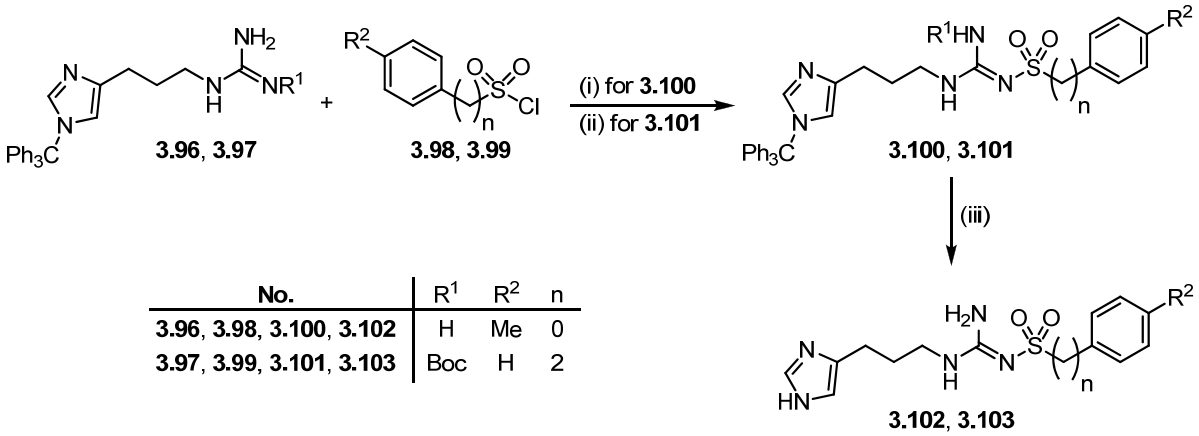
Scheme 3.1. Synthesis of the isourea precursors **3.24-3.40**, the cyanoguanidines **3.42-3.48**, **3.52-3.58**, **3.61-3.77**, **3.80** and **3.81**, and the carbamoylguanidines **3.49**, **3.50**, **3.59** and **3.78**. Reagents and conditions: (i) DCM, 1 h, rt; (ii) MeCN, overnight, reflux; (iii) HCl 1 M, 14 d, rt.

The cyanoguanidines **3.42-3.48**, **3.52-3.58**, **3.61-3.77**, **3.80** and **3.81** were synthesized according to a synthetic routine previously described by Buschauer and colleagues (Scheme 3.1).[12] Aminolysis of diphenyl cyanocarbonimidate (**3.6**)[13, 14] with the primary amines **3.7-3.23** at ambient temperature gave the isourea intermediates **3.24-3.40** that crystallized from diethylether. Conversion of the isoureas to the cyanoguanidines was performed by refluxing with histamine (**3.51**) or the analogous primary amines **3.41**, **3.60** or **3.79** in acetonitrile. Acidic hydrolysis of the cyanoguanidines[8] resulted in the carbamoylguanidines **3.49**, **3.50**, **3.59** and **3.78**.



Scheme 3.2. Synthesis of the bivalent cyanoguanidines **3.92-3.95**. Reagents and conditions: (i) 4-(benzyloxycarbonylamino)butanoic acid (2 eq), CDI (2.2 eq), THF, overnight, rt; (ii) H_2, Pd/C (10 %), MeOH, 12h, rt; (iii) DCM/MeOH, 1 h, rt; (iv) MeCN, 12 h, reflux.

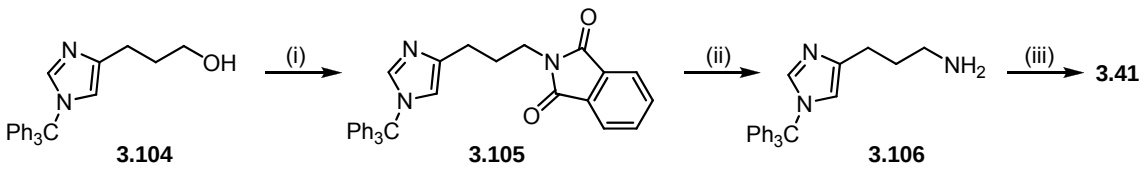
For the preparation of the bivalent cyanoguanidines **3.92-3.95** the diamine spacers **3.84-3.87** were treated with 2 equivalents of **3.6** providing the bis-isoureas **3.88-3.91**, followed by aminolysis of these intermediates with **3.60** (Scheme 3.2). The spacer **3.84** was formed by coupling of the diamine **3.82** with two equivalents of Cbz-protected γ-aminobutyric acid and subsequent catalytic hydrogenation.



No.	R^1	R^2	n
3.96, **3.98**, **3.100**, **3.102**	H	Me	0
3.97, **3.99**, **3.101**, **3.103**	Boc	H	2

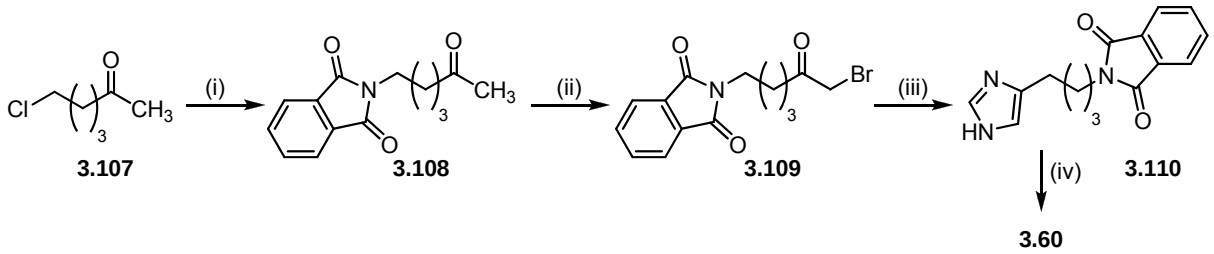
Scheme 3.3. Synthesis of the N^G-sulfonated imidazolylpropylguanidines **3.102** and **3.103**. Reagents and conditions: (i) NaH (60 % dispersion in mineral oil) (2 eq), NEt_3 (2 eq), THF, overnight, 0 °C → rt; (ii) DIEA (3 eq), THF, overnight, 0 °C → rt; (iii) TFA (20 %), DCM, 5h, rt.

The sulfonylguanidines **3.102** and **3.103** were prepared employing two different synthetic pathways (Scheme 3.3). **3.102** was synthesized by reaction of imidazolylpropylguanidine **3.96**[4] with tosylchloride (**3.98**) followed by detritylation under acidic conditions. As several attempts to prepare **3.103** in the same manner failed, **3.96** was replaced with its less basic *tert*-butoxycarbonyl (Boc) protected analog **3.97**. This method had already been employed for the preparation of N^G-acylated imidazolylpropylguanidines (cf. Chapter 6) and turned out to be also appropriate for the preparation of **3.103**.



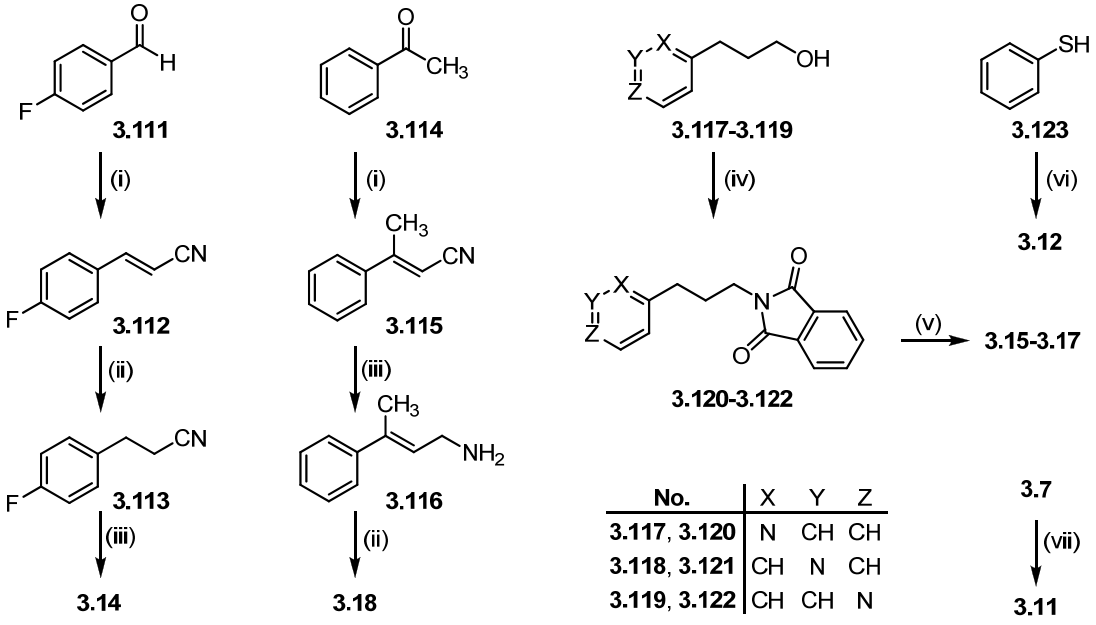
Scheme 3.4. Synthesis of 3-(1*H*-imidazol-4-yl)propan-1-amine **3.41**. Reagents and conditions: (i) phthalimide (1.5 eq), PPh_3 (1.5 eq), DIAD (2 eq), THF, 1 h, 0 °C → rt; (ii) $N_2H_4 \cdot H_2O$ (6.3 eq), *n*-BuOH, 1h, reflux; (iii) HCl 1N, 30 min, reflux.

Amines not commercially available (**3.11**, **3.12**, **3.14-3.18**, **3.41** and **3.60**) were synthesized as shown in Schemes 3.4 – 3.6. **3.41** was synthesized starting from the imidazolylpropanol **3.104**[4, 15] (Scheme 3.4). Conversion of the alcohol to the primary amine **3.106** was carried out by introduction of a phthalimide group under *Mitsunobu*-conditions[16] to give **3.105** followed by hydrazinolysis.[17] Acidic hydrolysis of the trityl protecting group produced **3.41**.



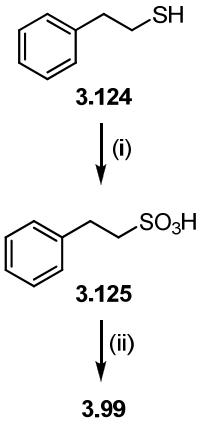
Scheme 3.5. Synthesis of 4-(1*H*-imidazol-4-yl)butan-1-amine (**3.60**). Reagents and conditions: (i) phthalimide (1 eq), K_2CO_3 (1.5 eq), KI (cat.), 12 h, 100 °C; (ii) urea (1 eq), Br_2 (1 eq), MeOH, 24h, rt; (iii) formamidine, 5 h, 160 °C; (iv) HCl 20 %, overnight, reflux.

The higher homolog of **3.41**, 4-(1*H*-imidazol-4-yl)butan-1-amine (**3.60**), was prepared as outlined in Scheme 3.5 by analogy with a synthetic pathway proposed by Buschauer and colleagues for the synthesis of homohistamine.[18] 6-Phthalimidohexan-2-one (**3.108**) was obtained from the reaction of 6-chlorohexan-2-one (**3.107**) with phthalimide.[19] Regioselective bromination of **3.108** was achieved by employing methanol as solvent in the presence of urea according to Zav'yalov and Kravchenko.[20] Cyclization of formamidine with the α-bromoketone **3.109** in a *Bredereck* synthesis[21] gave the imidazole **3.110** which was converted to **3.60** by acidic hydrolysis of the phthalimide group.



Scheme 3.6. Synthesis of the arylalkylamines and analogs **3.11**, **3.12** and **3.14-3.18**. Reagents and conditions: (i) diethyl cyanomethylphosphonate (1.2 eq), NaH (60 % dispersion in mineral oil) (1.2 eq), THF, 6 h, rt; (ii) H_2, Pd/C (10 %) (cat.), MeOH, 6h, rt; (iii) $LiAlH_4$ (2 eq), Et_2O, 2 h, reflux; (iv) phthalimide (1.1 eq), PPh_3 (1.1 eq), DIAD (1.1 eq.), THF, overnight, 0 °C → rt; (v) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, overnight, rt; (vi) bromoethylamine · HBr (1 eq), K^{t}BuO (2 eq), MeOH, 48 h, rt; (vii) H_2, Rh/C (5 %) (cat.), EtOH, 75 bar, 24 h, rt.

The amines **3.11**, **3.12** and **3.14-3.18** were synthesized as presented in Scheme 3.6. Starting from the aldehyde **3.111** or ketone **3.114**, the nitriles **3.112** and **3.115** were synthesized by chain elongation using a *Horner-Wadsworth-Emmons* reaction.[22] **3.14** was obtained by hydrogenation of the double bond in **3.112** followed by reduction of the nitrile **3.113** with $LiAlH_4$, whereas **3.18** was generated contrariwise. Starting from the pyridylpropyl-alcohols **3.117-3.119**, the pyridylpropylamine isomers **3.15-3.17** were synthesized *via* conversion into the phthalimides **3.120-3.122** under *Mitsunobu* conditions[16] followed by hydrazinolysis.[23] **3.12** was obtained by nucleophilic substitution of bromoethylamine with thiophenol.[24] Hydrogenation of the phenyl ring in **3.7** over Rh/C gave **3.11**.



Scheme 3.7. Synthesis of **3.99**. Reagents and conditions: (i) CH_3CO_3H (3 eq), DCM, 2 h, 0 °C → rt; (ii) $SOCl_2$ (20 eq), DMF (cat.), DCM, 16 h, 0 → 35 °C.

Phenylethylsulfonyl chloride **3.99** required for the synthesis of **3.103** was synthesized according to the procedure described by Hannedouche and co-workers (Scheme 3.7).[25] 2-Phenylethanethiol (**3.124**) was oxidized to the sulfonic acid **3.125** by treatment with peracetic acid and transformed to the sulfonyl chloride **3.99** by employing thionyl chloride.

3.3 Pharmacological results and discussion

3.3.1 Potencies and efficacies of the synthesized compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase activity assay

The prepared compounds were investigated for agonism and antagonism at the four hHR subtypes in steady-state GTPase assays measuring the enzymatic hydrolysis of radioactively labeled [γ-^{32}P]GTP after G-protein activation *via* the respective HR subtype. These investigations were performed using membrane preparations of Sf9 insect cells coexpressing the hH_1R plus RGS4, expressing the hH_2R-$G_{s\alpha S}$ fusion protein, coexpressing the hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4 or coexpressing the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ (Table 3.1).[4, 26, 27]

Cyanoguanidines 3.42-3.48, 3.52-3.58, 3.61-3.77, 3.80 and 3.81. The initial investigation focused on the replacement of the acylguanidine moiety in UR-AK51 with a cyanoguanidine group to evaluate if this modification is tolerated by the hH_4R with respect to agonistic activity. Indeed, the resulting compound **3.42** displayed partial agonism at the hH_4R (EC_{50} = 760 nM, E_{max} = 0.53). Moreover, **3.42** was almost inactive at the hH_1R and exhibited just weak partial agonism at the hH_2R (EC_{50} = 14 µM). At the hH_3R, **3.42** behaved as moderate inverse agonist (K_B = 1,900 nM, E_{max} = -0.37). However, compared to the reference

compound UR-AK51 (hH_4R: EC_{50} = 17 nM, E_{max} = 0.74), efficacy and in particular potency at the hH_4R drastically decreased (almost 50-fold). The phenylpropyl portion was varied to investigate the influence of the substituent in the "eastern part" of the molecule on hH_4R activity. Shortening (**3.43**) as well as elongation (**3.44**) of the carbon chain resulted in considerably reduced potencies and efficacies. The space filling diphenylpropyl residue in **3.45** caused a complete loss of activity, and substitution of the phenyl with a cyclohexyl ring (**3.46**) was not tolerated concerning hH_4R agonism (E_{max} = -0.20). Similar impact was observed for the indole **3.48**. Only the imidazolylpropylcyanoguanidine **3.47**, bearing a phenylthioethyl substituent, retained some hH_4R agonistic activity (EC_{50} = 1,300 nM, E_{max} = 0.33).

Since an improvement of the hH_4R agonistic potency was not achieved by modifying the N-substituent (the "eastern part") of **3.42**, the attention was turned on the carbon chain connecting the imidazole ring with the cyanoguanidine group. This connecting chain proved to be critical for hH_4R agonism. The lower homologs of **3.42-3.48**, the compounds **3.52-3.58**, were substantially less potent. For instance, **3.52** was almost inactive (K_B > 10 µM) compared to **3.42**. By contrast, increasing the chain length turned out to be a key step in structural optimization. The higher homolog of **3.42** (**3.61**) displayed 5-fold higher potency and essentially improved efficacy at the hH_4R (EC_{50} = 150 nM, E_{max} = 0.90). As expected from the impromidine analog **3.4**[9] (cf. Figure 3.2), **3.61** showed only low potency and no significant efficacy at the hH_2R (EC_{50} = 5,600 nM, E_{max} = 0.08). **3.61** was found to be almost inactive at the hH_1R (K_B > 10 µM) and only poorly active at the hH_3R (K_B = 2,300 nM, E_{max} = -0.03). Notably, **3.61** is a potent and selective hH_4R agonist devoid of agonistic activity at other HRs. Further elongation to a five membered carbon chain was not tolerated with respect to potency and agonistic activity at the hH_4R.[28]

Assured from these results, **3.61** was used as new lead structure to further improve hH_4R agonistic potency and selectivity. Keeping the imidazolylbutyl portion constant, structural modifications were again focused on the "eastern part" of the cyanoguanidine-type compounds. Besides the residues employed in the homohistamine (**3.42-3.48**) and histamine (**3.52-3.58**) series, additional groups were introduced. Modifying the carbon spacer separating the phenyl ring and the cyanoguanidine group was found to be critical. Reducing the chain length just by one methylene group (**3.62**) clearly reduced potency and efficacy (EC_{50} = 1,100 nM, E_{max} = 0.36). Elongation of the spacer (**3.63**) even caused a loss of efficacy (E_{max} = 0.05). Apparently, a definite distance between the cyanoguanidine moiety and the phenyl ring is required for hH_4R activation. The "eastern part" also turned out to be sensitive towards steric effects. A bulky diphenylpropyl residue (**3.64**) generated a weak inverse agonist at the hH_4R (K_B = 2,500 nM, E_{max} = -0.43). By contrast, a less space filling methyl group in α-position to the phenyl ring (**3.72**) had no significant influence on potency

and efficacy relative to **3.61** (EC_{50} = 140 nM, E_{max} = 0.79). Replacing the phenyl with a cyclohexyl (**3.65**) or an indole ring (**3.67**) was not tolerated in terms of hH_4R activity. Notably, **3.67** was 10-fold more potent than **3.61** at the hH_3R (K_B = 170 nM). Surprisingly, a fluorine substituent in position 4 of the phenyl ring (**3.68**) was found to abolish hH_4R agonistic efficacy (K_B = 260 nM, E_{max} = -0.10). Since the van der Waals radii for fluorine and hydrogen are comparable, obviously the negative polarization of the fluorine avoids the stabilization of an active hH_4R conformation. Replacement of the phenyl carbocycle with a 2-, 3- or 4-pyridyl ring (**3.69-3.71**) also resulted in lower potencies and efficacies at the hH_4R compared to **3.61**. Interestingly, potencies decreased in the rank order from the 2-pyridyl to the 4-pyridyl analog (EC_{50} = 580 nM → EC_{50} = 2,600 nM). The lone pair of the nitrogens in the pyridine rings cause a negative electrostatic potential that evidently is unfavorable for receptor binding, in particular for **3.71** with the nitrogen in position 4. In contrast, formation of a thioether in the chain connecting the phenyl ring and the cyanoguanidine group (**3.66**, laboratory code: UR-PI376) afforded a considerable increase in potency and retained high efficacy at the hH_4R (EC_{50} = 34 nM, E_{max} = 0.93) relative to compound **3.61**. Introduction of the sulfur atom in the chain slightly enlarges the distance between the phenyl carbocycle and the cyano-guanidine group. As this chain length proved to be critical in terms of potency at the hH_4R (cf. **3.62** and **3.63**), this fine tuning of the spacer length may be an explanation for the increased potency. At the hH_3R, UR-PI376 showed no agonism but moderate antagonistic/inverse agonistic activity (K_B = 990 nM, E_{max} = -0.30). Activities of UR-PI376 at the hH_1R and hH_2R were negligible (K_B > 10 µM, E_{max} ≈ 0.07).

Thus minor structural modifications in the "eastern part" of the imidazolylbutylcyano-guanidines can drastically change potency and efficacy. To obtain additional information about the structural requirements for the substituent in this position, a number of analogs bearing small alkyl residues (**3.74-3.77**) as well as the unsubstituted parent compound (**3.73**) were prepared. Leaving out the substituent (**3.73**) was well tolerated in terms of hH_4R agonistic activity. **3.73** was essentially equipotent and equiefficacious with **3.61** (EC_{50} = 170 nM, E_{max} = 0.91). However, compound **3.73** possessed similar potency at the hH_3R and in contrast to the other imidazolylbutylcyanoguanidines exhibited partial agonistic activity at this HR subtype (EC_{50} = 250 nM, E_{max} = 0.42). The residue in the "eastern part" appears to be indispensable to discriminate between the hH_3R and hH_4R. This assumption is supported by the data on the alkylated analogs **3.74-3.77**. Introduction of a methyl group (**3.74**) resulted in 10-fold lower potency at the hH_3R (K_B = 2,100 nM, E_{max} = 0.16) but also hH_4R potency decreased (EC_{50} = 960 nM, E_{max} = 0.75). However, enlarging the alkyl substituent increased potency at the hH_4R (Me < Et < iPr < iBu, EC_{50} = 960 nM → EC_{50} = 120 nM) without remarkably affecting efficacy (E_{max} = 0.75 – 0.90). By contrast, at the hH_3R, with increasing alkyl residues partial agonistic activity was abolished and potency slightly decreased (K_B =

2,100 nM → K_B = 3,400 nM). Reason for the pharmacological behavior at the $hH_{3/4}Rs$ may be that the unsubstituted compound **3.73** is able to form interactions – possibly a hydrogen bond – with amino acid residues at both receptors. Presumably, introduction of alkyl residues may prevent this kind of interaction, which may be compensated at the hH_4R by hydrophobic interactions with the alkyl residues of the compounds. Since compounds with lager alkyl substituents are less potent at the hH_3R, apparently, hydrophobic interactions are not feasible or steric factors may disturb binding. Nevertheless, the pharmacological data show that larger alkyl substituents are required for hH_4R selectivity over the hH_3R and for high hH_4R potencies. For example an almost 30-fold selectivity in combination with high potency and efficacy at the hH_4R was achieved for compound **3.77**, characterized by a bulky isobutyl residue (EC_{50} = 120 nM, E_{max} = 0.90). The activities of **3.73-3.77** at the hH_1R and hH_2R were negligible (very weak antagonism, K_B > 10 µM).

Recently, 5-methylhistamine was identified as a selective hH_4R agonist (Table 6.1).[1] Hence, two compounds (**3.80** and **3.81**) bearing a methyl group at position 5 of the imidazole ring were prepared to evaluate if this modification can also improve selectivity of cyanoguanidine-type hH_4R agonists. Due to the availability of the corresponding amine, the prepared compounds contain a sulfur atom in the chain separating the imidazole ring from the cyano-guanidine group. Unfortunately, both synthesized compounds (**3.80** and **3.81**) including the analog of lead compound **3.61** were almost inactive at the hH_4R (K_B > 10 µM). Provided that the sulfur atom in the spacer does not substantially change the pharmacological properties of **3.80** and **3.81**, in contrast to histamine introduction of a methyl group at position 5 of the imidazole ring seems to be not tolerated in cyanoguanidine-type hH_4R agonists. This finding suggests the imidazole rings of the imidazolylbutylcyanoguanidines and histamine to exhibit distinct binding modes at the hH_4R.

Bivalent cyanoguanidines 3.92-3.95. Acylguanidine-type H_2R agonists containing two pharmacophores linked through a spacer ("bivalent ligands") have been shown to be substantially more potent relative to the monovalent compounds.[11] Hence, this bivalent ligand approach was applied to the imidazolylbutylcyanoguanidines, aiming at compounds with improved hH_4R agonistic potencies. In the series of bivalent acylguanidines, spacers consisting of 4 to 20 methylene groups between the two pharmacophores were employed. In the case of bivalent acylguanidine-type hH_2R agonists the optimal spacer length was around eight methylene groups.[11] Therefore, 6, 8 and 10-membered carbon chains were selected for connecting the cyanoguanidine moieties (**3.93-3.95**). However, the length of these spacers is presumably insufficient to bridge neighboring GPCRs, since molecular modeling investigations for *µ*-opioid receptor homodimers predict a distance between the binding sites of around 27 Å for the TM 5,6 interface.[29] In **3.92**, the distance between the equivalent pharmacophoric groups (cyanoguanidines ≈ 25 Å , imidazoles ≈ 40 Å) probably holds an

adequate length for bridging two receptors which should result in a potency greater than that caused from the sum of its two monovalent pharmacophores.[29] This may be of interest as homo-oligomerization has been reported for the hH_4R.[30] To increase hydrophilicity and thereby prevent unspecific membrane effects or micelle formation, ether and amide groups were inserted into the spacer (**3.92**).

However, none of the prepared bivalent cyanoguanidines **3.92-3.95** had agonistic activity at the hH_4R. The poor potency observed for **3.93** with the six-membered methylene spacer was slightly enhanced when the spacer length was extended as in **3.94** and **3.95** (K_B = 1,800 nM → K_B = 390 nM). **3.92** – containing the longest spacer – turned out to be virtually inactive at the hH_4R (K_B > 10 µM). As the potencies of the bivalent ligands **3.93-3.95** at the hH_4R did not clearly exceed the potencies of the monovalent ligands **3.74** and **3.75** (EC_{50} = 960 nM, EC_{50} = 700 nM), evidently the second pharmacophore was not able to address an additional binding site. Moreover, agonistic activities were abolished. Obviously, the spacer prevents an appropriate binding of the imidazolylbutylcyanoguanidine group to stabilize an active hH_4R conformation.

By contrast, at the hH_3R the bivalent ligands **3.93-3.95** showed a significant increase in potency relative to the monovalent counterparts **3.74** and **3.75**. Potencies peaked for the compounds with octamethylene (**3.94**) and decamethylene (**3.95**) chain (K_B = 17 nM, K_B = 16 nM), corresponding to a more than 100-fold increase in potency compared to **3.74** and **3.75** (K_B = 2,100 nM, K_B = 2,900 nM). The efficacies remained essentially unchanged. The strong enhancement of potency suggests one of the pharmacophores in the bivalent ligands **3.93-3.95** to address the identical binding site as the corresponding monovalent ligands, and the second pharmacophore – as the spacer lengths presumably are too short for bridging vicinal receptors – to increase affinity by addressing a second binding site on the same hH_3R molecule.[31, 32] This putative accessory recognition site for the imidazolylbutylcyanoguanidine pharmacophore present at the hH_3R, but not at the hH_4R, would also explain the switch from hH_4R to hH_3R selective compounds. Such an additional binding site on GPCRs for bivalent ligands has been described for the *κ*-opioid receptor antagonist *nor*-BNI. Mutational studies revealed an additional interaction of this ligand with a glutamate residue located at the top of TM6 to be primarily responsible for the high affinity and selectivity toward the *μ*-opioid receptor.[33] Moreover, many examples of bivalent GPCR-ligands with drastically increased activities relative to the monovalent parent compounds despite insufficient linker chain lengths for addressing neighboring receptors have been reported.[29, 34-36] Likewise, the most potent bivalent acylguanidine-type H_2R agonists contain linkers which are presumably too short for interacting with two receptors simultaneously.[11]

The imidazolylbutylcyanoguanidine group seems to be a versatile structural motif to address an accessory recognition site at the hH_3R. Therefore, the synthesis of bivalent ligands

containing an H_3R pharmacophore linked *via* a spacer to an imidazolylbutylcyanoguanidine group may be an appropriate approach to improve selectivity and affinity of hH_3R ligands. Furthermore, such compounds may be interesting pharmacological tools to study the accessory binding site of this HR subtype. For **3.92** a 10-fold higher potency at the hH_3R compared to the monovalent compounds **3.74** and **3.75** was determined (K_B = 250 nM). Due to sufficient spacer length in **3.92**, in addition to addressing a possible accessory binding site on the same receptor, bridging of two hH_3R is conceivable.

Carbamoylguanidines 3.49, 3.50, 3.59 and 3.78. Although potent and selective hH_4R agonists were obtained in the cyanoguanidine series, the potencies of these compounds at the hH_4R were lower than those of the corresponding acylguanidine-type compounds (cf. Chapter 6). To study to which extent the lack of basicity of the cyanoguanidine group accounts for the differences in hH_4R potencies, a handful of structurally related but basic carbamoylguanidine-type compounds were prepared (**3.49**, **3.50**, **3.59** and **3.78**; $pK_a \approx 8.0$)[8]. In analogy to the cyanoguanidine-series, compounds with a two- (**3.59**), three (**3.49** and **3.50**) and four-membered (**3.78**) carbon chain separating the imidazole ring from the carbamoylguanidine group were investigated for their pharmacological activities at the hHRs. In contrast to the cyanoguanidine **3.42** that showed partial agonistic activity at the hH_4R (E_{max} = 0.53), the carbamoylguanidine analog **3.49** exerted considerable inverse agonistic efficacy (E_{max} = -0.75). Potency of **3.49** at the hH_4R was moderate (K_B = 170 nM). The phenylbutyl analog **3.50** exhibited comparable potency and efficacy. Like at the hH_4R, **3.49** and **3.50** were moderately potent inverse agonists at the hH_3R. Interestingly, both carbamoyl-guanidines showed moderate partial agonistic activity at hH_2R ($E_{max} \approx 0.60$). As expected from **3.52**, the histamine analog **3.59** (laboratory code: UR-PI97) exhibited weak inverse agonistic activity at the hH_4R (K_B = 1,300 nM, E_{max} = -0.51). Analogous to **3.49** and **3.50**, UR-PI97 behaved as weak partial agonist at the hH_2R, even though efficacy was remarkably reduced (EC_{50} = 2,000 nM, E_{max} = 0.26) and showed negligible activity at the hH_1R (K_B > 10,000 nM). Surprisingly, this compound turned out to be a highly potent hH_3R inverse agonist (K_B = 3.8 nM, E_{max} = -0.97). Compared to the cyanoguanidine analog **3.52**, introduction of the carbamoylguanidine moiety resulted in a more than 1,500-fold increase in potency. In addition, UR-PI97 is highly selective over the hH_1R, hH_2R and hH_4R (> 2,500, > 500 and > 300-fold selectivity, respectively). The high selectivity of UR-PI97 over the hH_4R is untypical as many imidazole containing H_3R ligands as thioperamide or iodophenpropit also have high affinity for the H_4R.[1] In the cyanoguanidine series highest hH_4R agonistic potency resided in compounds having a tetramethylene chain connecting the imidazole ring and the cyanoguanidine group. By contrast, the imidazolylbutylcarbamoylguanidine **3.78** showed no agonistic activity at the hH_4R and had similar pharmacological activities as the carbamoylguanidines **3.49** and **3.50** at the HR subtypes.

Substitution of the cyanoguanidine group with a carbamoylguanidine moiety resulted in reversed pharmacological activities concerning the hH_2R and hH_4R. All carbamoylguanidines exerted partial agonistic activity at the hH_2R, whereas most cyanoguanidines showed very low efficacies at this HR subtype. Moreover, the carbamoylguanidines exhibited higher potencies at the hH_2R than the corresponding cyanoguanidines. Concerning the hH_4R, the exchange of the cyanoguanidine moiety with a carbamoylguanidine group resulted in a loss of agonistic potency and produced inverse agonists. Since the cyanoguanidine and carbamoylguanidine groups have closely related structures, the different physicochemical properties may be an explanation for the essential differences in the pharmacological profile at the HRs. In contrast to the cyanoguanidines the basicity of the carbamoylguanidines is high enough to allow protonation under physiological conditions. Obviously, when comparing the cyanoguanidines and carbamoylguanidines a charged central group is not compatible with hH_4R agonistic activity, whereas for hH_2R agonistic activity protonation is favorable. In the case of UR-PI97, the capability to form an ionic interaction between the positively charged carbamoylguanidine group and a negatively charged amino acid residue (possibly the conserved Asp114 of TM3 that is crucial for histamine binding at the hH_3R)[37] may be the reason for the high increase in potency at the hH_3R. However, the discrepancies in the pharmacological activities at the HRs can not just be explained by differences in basicity. Compared to the likewise basic acylguanidines, the carbamoylguanidines exhibit considerably lower potencies at $hH_{2/3/4}Rs$ and have no agonistic activity at the hH_4R. Obviously, the variations in the structural arrangements and sizes of the central groups also contribute to the observed differences in HR subtype activity.

Sulfonylguanidines 3.102 and 3.103. Additional investigations were carried out to evaluate the influence of the basicity on HR subtype activity and selectivity using the sulfonylguanidines **3.102** and **3.103**. Unlike the acylguanidine group ($pK_a \approx 8$)[7], the sulfonylguanidine group ($pK_a \approx 0.4$)[7] is virtually uncharged at physiological pH. The sulfonylguanidine analog of UR-AK51 (**3.103**) showed a more than 50-fold decrease in potency at the hH_4R (K_B = 1,400 nM) and, contrary to UR-AK51, had no agonistic activity. The same was observed for the sulfonylguanidine **3.102** (acylguanidine analog at the hH_4R: EC_{50} = 6.1 nM, E_{max} = 0.40). **3.102** and **3.103** acted as moderate inverse agonists at the hH_3R and showed partial agonism at the hH_2R ($E_{max} \approx 0.50$). However, potencies were reduced by two orders of magnitude relative to the acylated guanidines at the H_2R subtype (EC_{50} = 12,000 nM). These findings show that removing basicity from the acylguanidines is not tolerated at the different HR subtypes. In terms of hH_2R activity, this can be well explained by the putative interaction hH_2R model. An ionic interaction between the positively charged acylguanidine group and the highly conserved aspartate residue of helix 3 is assumed.[38] Similar interactions can be expected for the other HR subtypes. Obviously, for the acylguanidines an ionic interaction is

crucial for potent $hH_{2/3/4}R$ binding since the structurally closely related, non-protonated sulfonylguanidine analogs **3.102** and **3.103** display drastically reduced potencies. In contrast, likewise non-basic cyanoguanidines such as UR-PI376 are able to potently activate the hH_4R. Apparently, for these cyanoguanidine-type compounds an ionic interaction with the receptor is not required. Therefore, distinct binding modes for the acylguanidine-type and cyanoguanidine-type agonists at the hH_4R can be presumed. Nevertheless, it has to be considered that next to the physicochemical differences of the acylguanidines and sulfonyl-guanidines also steric factors, as for example the different orientation of the oxygen atoms, may play a role in altering the pharmacological activities at the HR subtypes.

Table 3.1. Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay.[a]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
histamine	190[6]	1.00		1,200[6]	1.00		25 ± 3.1	1.00	3	12 ± 2.5[39]	1.00	8
thioperamide	-	-		-	-		97 ± 18[40]	-0.71 ± 0.06[40]	5	110 ± 16[39]	-0.95 ± 0.07[39]	6
5-methyl-histamine	16,000 ± 540	0.90 ± 0.02	3	2,900 ± 1,200	1.01 ± 0.03	4	[b]	0.12 ± 0.02	3	70 ± 18	0.90 ± 0.06	5
3.42	(>10,000)	n.d.	3	14,000 ± 1,100	0.39 ± 0.01	2	(1,900 ± 350)	-0.37 ± 0.01	2	760 ± 250	0.53 ± 0.06	3
3.43	n.d.	n.d.		inactive		1	n.d.	n.d.		(4,800 ± 3,400)	0.13 ± 0.08	2
3.44	n.d.	n.d.		9,000 ± 2,600	0.17 ± 0.01	2	n.d.	n.d.		(6,000 ± 730)	-0.15 ± 0.03	3
3.45	n.d.	n.d.		(>10,000)	n.d.	2	n.d.	n.d.		(>10,000)	n.d.	3
3.46	n.d.	n.d.		(>10,000)	n.d.	1	n.d.	n.d.		(1,500 ± 100)	-0.20 ± 0.01	2
3.47	n.d.	n.d.		7,200 ± 1,300	0.57 ± 0.02	2	n.d.	n.d.		1,300 ± 550	0.33 ± 0.05	2
3.48	n.d.	n.d.		inactive		1	n.d.	n.d.		(4,300 ± 800)	-0.01 ± 0.09	2
3.49	(>10,000)	n.d.	2	650 ± 36	0.65 ± 0.01	2	(590 ± 90)	-0.67 ± 0.03	2	(170 ± 13)	-0.75 ± 0.06	2
3.50	n.d.	n.d.		380 ± 16	0.55 ± 0.01	2	(310 ± 25)	-0.75 ± 0.02	2	(470 ± 23)	-0.76 ± 0.03	2
3.52	(>10,000)	n.d.	2	>10,000	n.d.	2	(6,600 ± 820)	-0.31 ± 0.02	2	(>10,000)	n.d.	2
3.53	n.d.	n.d.		inactive		1	n.d.	n.d.		(6,600 ± 1,000)	0.23 ± 0.05	3
3.54	n.d.	n.d.		(>10,000)	n.d.	1	n.d.	n.d.		(>10,000)	n.d.	4
3.55	n.d.	n.d.		(>10,000)	n.d.	3	n.d.	n.d.		(>10,000)	n.d.	1
3.56	n.d.	n.d.		3,900 ± 1,200	0.14 ± 0.01	2	n.d.	n.d.		(2,700 ± 450)	-0.23 ± 0.06	3

Table 3.1. (continued)

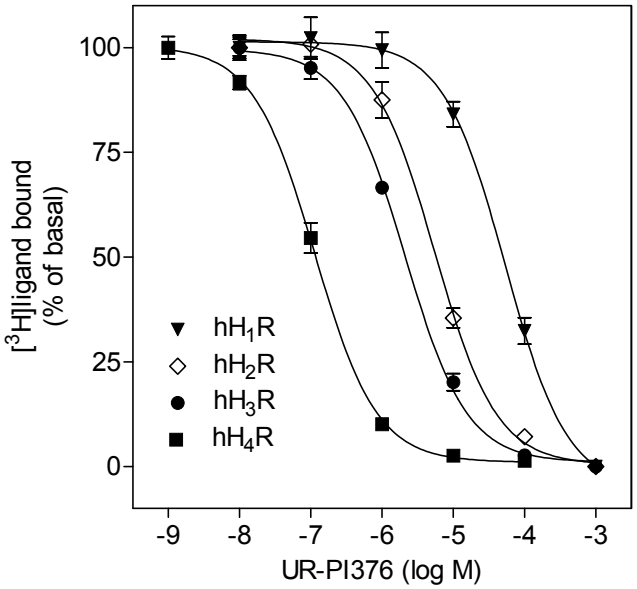
Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
3.57	n.d.	n.d.		(>10,000)	n.d.	1	n.d.	n.d.		(8,900 ± 3,400)	-0.04 ± 0.09	2
3.58	n.d.	n.d.		(>10,000)	n.d.	1	n.d.	n.d.		(>10,000)	n.d.	1
3.59 (UR-PI97)	(>10,000)	-0.02 ± 0.01	3	2,000 ± 280	0.26 ± 0.01	2	(3.8 ± 0.12)	-0.97 ± 0.04	3	(1,300 ± 63)	-0.51 ± 0.02	2
3.61	(>10,000)	n.d.	2	(5,600 ± 900)	0.08 ± 0.05	3	(2,300 ± 500)	-0.03 ± 0.06	2	150 ± 47	0.90 ± 0.11	3
3.62	n.d.	n.d.		(>10,000)	n.d.	3	(1,500 ± 160)	-0.23 ± 0.00	2	1,100 ± 700	0.36 ± 0.05	2
3.63	n.d.	n.d.		(9,300 ± 250)	0.11 ± 0.01	2	(2,900 ± 500)	-0.13 ± 0.04	2	(460 ± 46)	0.05 ± 0.01	2
3.64	n.d.	n.d.		(>10,000)	n.d.	2	n.d.	n.d.		(2,500 ± 690)	-0.43 ± 0.11	2
3.65	n.d.	n.d.		(>10,000)	n.d.	2	(1,500 ± 350)	-0.21 ± 0.01	2	(450 ± 22)	-0.15 ± 0.01	2
3.66 (UR-PI376)	(>10,000)	0.07 ± 0.05	2	(>10,000)	0.08 ± 0.01	2	(990 ± 120)	-0.30 ± 0.02	3	34 ± 0.80	0.93 ± 0.02	3
3.67	n.d.	n.d.		(>10,000)	0.11 ± 0.02	2	(170 ± 27)	-0.02 ± 0.02	2	(1,600 ± 38)	-0.25 ± 0.05	2
3.68	n.d.	n.d.		(5,200 ± 120)	0.17 ± 0.00	2	(770 ± 110)	-0.28 ± 0.02	2	(260 ± 5.2)	-0.10 ± 0.02	2
3.69	n.d.	n.d.		(3,300 ± 240)	0.14 ± 0.01	2	(1,300 ± 200)	-0.44 ± 0.01	2	580 ± 130	0.57 ± 0.03	2
3.70	n.d.	n.d.		(1,200 ± 97)	0.15 ± 0.00	2	(650 ± 28)	-0.13 ± 0.01	2	1,200 ± 76	0.34 ± 0.04	2
3.71	n.d.	n.d.		(7,700 ± 2,200)	0.19 ± 0.00	2	(770 ± 120)	-0.29 ± 0.00	2	2,600 ± 290	0.42 ± 0.05	2
3.72	(>10,000)	n.d.	2	(>10,000)	0.16 ± 0.01	2	(640 ± 20)	-0.29 ± 0.02	2	140 ± 13	0.79 ± 0.03	3
3.73	n.d.	n.d.		(>10,000)	0.03 ± 0.00	2	250 ± 63	0.42 ± 0.02	2	170 ± 45	0.91 ± 0.00	3
3.74	(>10,000)	n.d.	2	(>10,000)	0.03 ± 0.01	2	(2,100 ± 660)	0.16 ± 0.02	2	960 ± 140	0.75 ± 0.03	2

Table 3.1. (continued)

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
3.75	(>10,000)	n.d.	2	(>10,000)	0.05 ± 0.01	2	(2,900 ± 610)	0.09 ± 0.00	2	700 ± 147	0.82 ± 0.01	3
3.76	(>10,000)	n.d.	2	(>10,000)	0.07 ± 0.02	2	(3,200 ± 530)	0.07 ± 0.05	2	330 ± 33	0.86 ± 0.02	3
3.77	(>10,000)	n.d.	2	(>10,000)	0.07 ± 0.01	2	(3,400 ± 1000)	-0.05 ± 0.03	2	120 ± 26	0.90 ± 0.02	3
3.78	n.d.	n.d.		440 ± 120	0.46 ± 0.01	2	(360 ± 12)	-0.52 ± 0.01	2	(85 ± 1.9)	-0.68 ± 0.06	2
3.80	n.d.	n.d.		(1,800 ± 700)	-0.15 ± 0.04	2	n.d.	n.d.		(>10,000)	n.d.	2
3.81	n.d.	n.d.		(1,600 ± 550)	-0.11 ± 0.06	2	n.d.	n.d.		(>10,000)	n.d.	2
3.92	n.d.	n.d.		inactive		2	(250 ± 42)	-0.17 ± 0.02	2	(>10,000)		1
3.93	(8,600 ± 1,700)	0.01 ± 0.04	2	(>10,000)	0.12 ± 0.00	2	(50 ± 1.0)	-0.35 ± 0.03	2	(1,800 ± 280)	-0.15 ± 0.07	2
3.94	n.d.	n.d.		(3,800 ± 950)	0.08 ± 0.02	2	(17 ± 1.5)	-0.25 ± 0.01	2	(530 ± 36)	-0.05 ± 0.02	2
3.95	n.d.	n.d.		(2,400 ± 280)	0.04 ± 0.04	2	(16 ± 0.45)	-0.06 ± 0.01	2	(390 ± 24)	-0.07 ± 0.03	2
3.102	(>10,000)	n.d.	2	12,000 ± 720	0.47 ± 0.07	2	(780 ± 47)	-0.33 ± 0.03	2	(1,400 ± 340)	0.26 ± 0.02	2
3.103	n.d.	n.d.		12,000 ± 2,900	0.53 ± 0.04	3	(270 ± 8.2)	-0.67 ± 0.02	2	(1,400 ± 320)	-0.14 ± 0.08	2

[a] Steady-state GTPase activity in Sf9 insect cell membranes expressing the hH_1R + RGS4, hH_2R-$G_{s\alpha S}$ fusion protein, hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ + RGS4 or hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ was determined as described under *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Typical basal GTPase activities in the hH_1R assay ranged between ≈ 1.0 and 1.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 100 to 150 % above basal. Typical basal GTPase activities in the hH_2R assay ranged between ≈ 1.0 and 2.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 250 to 300 % above basal. Typical basal GTPase activities in the hH_3R assay ranged between ≈ 3.0 and 4.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to ≈ 60 % above basal. Typical basal GTPase activities in the hH_4R assay ranged between ≈ 2.5 and 3.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to 60 to 70 % above basal. n gives the number of independent experiments performed in duplicate each. For compounds investigated in the antagonist mode (K_B values), E_{max} values were determined at a concentration of 10 µM.[b] no agonistic activity up to a concentration of 1 mM. n.d.: not determined.

3.3.2 Affinities of UR-PI376 (3.66) for the hH_1R, hH_2R, hH_3R and hH_4R subtypes in radioligand binding experiments



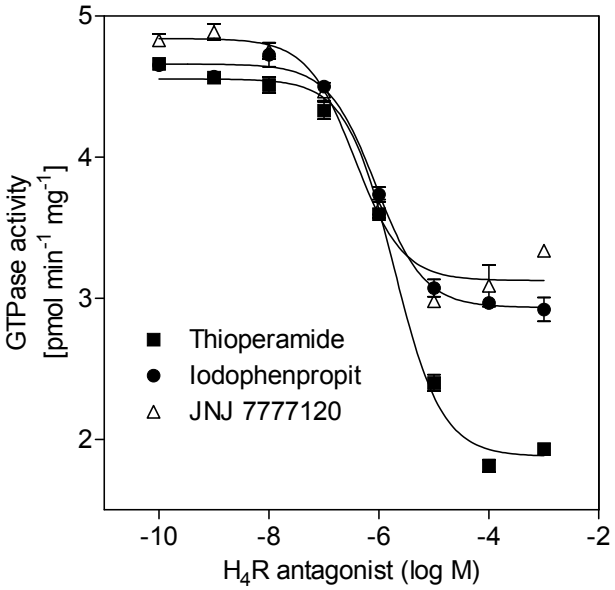
	K_I (UR-PI376) [nM]	n
hH_1R	26,000 ± 6,600	2
hH_2R	4,100 ± 780	3
hH_3R	530 ± 43	3
hH_4R	58 ± 13	3

Figure 3.4. Displacement of [^{3}H]mepyramine (5 nM), [^{3}H]tiotidine (10 nM), [^{3}H]N^{α}-methylhistamine (3 nM) and [^{3}H]histamine (10 nM) with UR-PI376 from Sf9 insect cell membranes expressing the hH_1R + RGS4, hH_2R-$G_{s\alpha S}$ fusion protein, hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ + RGS4 or hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$. Radioligand binding was determined as described under *Pharmacological methods*. Data were analyzed for best fit to one site (monophasic) competition curves. Data points shown are the means of n independent experiments each performed in duplicate.

UR-PI376 (**3.66**) was identified as the most potent and selective hH_4R agonist in the cyanoguanidine series by using steady-state GTPase assays (EC_{50} = 34 nM, E_{max} = 0.93, ≈ 30-fold selectivity toward the hH_3R). Nevertheless, agonist potencies determined in functional assays depend on different factors as for example the G-protein availability.[6] This can cause deviations between potencies received from functional experiments and binding studies. For this reason dissociation constants (K_I values) of UR-PI376 at the different hHR subtypes were determined. UR-PI376 was found to displace [^{3}H]mepyramine from the hH_1R, [^{3}H]tiotidine from the hH_2R, [^{3}H]N^{α}-methylhistamine from the hH_3R and [^{3}H]histamine from the hH_4R giving monophasic competition binding curves (Figure 3.4). In accordance with the results from the GTPase assay, UR-PI376 bound with the highest affinity to the hH_4R (K_I = 58 nM) and, as expected, showed a remarkably lower affinity for the hH_3R (K_I = 530 nM). However, the affinity of UR-PI376 for the hH_4R was slightly reduced compared to the EC_{50} value determined in the GTPase activity assay, while the contrary was observed for the affinity of UR-PI376 to the hH_3R. Therefore, selectivity of UR-PI376 for the hH_4R over the hH_3R is about three times lower than in the functional experiments. At the hH_1R and hH_2R, the compound displayed low affinity resulting in a 450- and 70-fold selectivity for the hH_4R,

respectively. Overall, the determined K_I values and their rank orders are in agreement with the potencies evaluated in the functional GTPase assays and confirm UR-PI376 to be a highly affine and selective ligand for hH_4R.

3.3.3 Inhibition of the UR-PI376 (3.66) stimulated GTP hydrolysis at the hH_4R by standard H_4R antagonists



Compound	K_B [nM]	K_I [nM][a]
Thioperamide	62 ± 4.6	125
Iodophenpropit	30 ± 2.4	13
JNJ 7777120	12 ± 2.5	16

Figure 3.5. Inhibition of the UR-PI376 stimulated GTP hydrolysis at the hH_4R by the H_4R antagonists thioperamide, iodophenpropit and JNJ 7777120. Steady-state GTPase activity in Sf9 insect cell membranes expressing the hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ was determined as described under *Pharmacological methods*. Reaction mixtures contained 1 µM of UR-PI376. Data were analyzed by nonlinear regression and were best fit to sigmoid concentration/response curves. Data points shown are the means of two independent experiments each performed in duplicate.[a] K_I values of the reference H_4R antagonists were taken from Lim et al.[1]

The functional pharmacological activities of all compounds were determined in steady-state GTPase activity assays using membranes of Sf9 insect cells expressing the respective HR subtype. When using membranes instead of intact cells, G-proteins are directly accessible to the evaluated compounds. Therefore, the possibility of direct, receptor independent G-protein activation has to be taken into account. For many compounds like peptides, local anesthetics, β-adrenoceptor antagonists but also for cationic-amphiphilic HR ligands, direct G-protein activation has been reported.[41-43] UR-PI376 with its polar and basic imidazole ring and more lipophilic side chain somehow features cationic-amphiphilic properties. To exclude a direct G-protein activation, GTPase activity was stimulated with UR-PI376 (1 µM) and the effect of increasing concentrations of the H_4R antagonists thioperamide, iodophenpropit and JNJ 7777120 was evaluated. As shown in Figure 3.5, all antagonists suppressed the UR-PI376 induced GTP hydrolysis in a dose-dependent manner. Thioperamide was more

effective than the other standard H_4R antagonists due to its pronounced inverse agonistic activity at the hH_4R (Table 3.1). The K_B values determined for thioperamide, iodophenpropit and JNJ 7777120 against UR-PI376 in the functional assay were in good accordance with K_I values from binding studies reported in literature (Figure 3.5). These results indicate the H_4R antagonists to compete with UR-PI376 for the same binding site at the hH_4R and suggest the GTPase activation through UR-PI376 to be receptor mediated. Direct G-protein activation by HR receptor ligands was reported to occur at concentrations > 10 µM.[41-43] By contrast, UR-PI376 stimulated GTPase activation in membranes expressing the hH_4R was detectable at concentrations ≥ 10 nM. The inverse agonistic activity of UR-PI376 at the hH_3R also supports a receptor dependent G-protein activation as both the hH_3R and hH_4R were coexpressed with $G_{i\alpha2}$. Finally, UR-PI376 was found to displace [^{3}H]histamine from the hH_4R (Figure 3.4). These data confirm UR-PI376 to act as a hH_4R agonist in the GTPase assay, whereas direct G-protein stimulation can definitely be ruled out.

3.3.4 Potencies and efficacies of selected compounds at the guinea pig ileum (gpH_1R) and guinea pig right atrium (gpH_2R)

Selected compounds were investigated on the isolated guinea pig (gp) ileum for H_1R activity and on the isolated spontaneously beating guinea pig right atrium for H_2R activity using histamine as the reference agonist (Table 3.2).

The sulfonylguanidines **3.102** and **3.103** both showed very poor activities at the gpH_2R. Contrary to **3.102**, which was a very weak antagonist, the sulfonyl analog of UR-AK51 (**3.103**) exerted weak partial agonism (E_{max} = 0.38). As observed for the hH_2R in the GTPase assay, replacing the basic acylguanidine moiety with a non-basic sulfonylguanidine group was not tolerated at gpH_2R. The potent cyanoguanidine-type hH_4R agonist UR-PI376 (**3.66**) was a very poor gpH_2R antagonist. This result is consistent with the poor hH_2R activity determined in the GTPase assay. At the guinea pig ileum, all evaluated compounds acted as weak gpH_1R antagonists.

Table 3.2. Pharmacological activities of selected compounds at the guinea pig ileum (gpH_1R) and the guinea pig right atrium (gpH_2R).

Compound	gpH_1R		gpH_2R		
	pA_2	n[a]	pEC_{50}[b] (pA_2) [pD_2']	E_{max}[c]	n[a]
histamine	-	-	6.00 ± 0.10	1.00 ± 0.02	> 50
UR-AK51[4]	5.67 ± 0.14	5	6.78 ± 0.10	0.97 ± 0.05	3
3.66 (UR-PI376)	n.d.		(4.24 ± 0.12)	[d]	2
3.102	4.97 ± 0.08	12	(< 4.5) [< 4.5]	< 5[e]	2
3.103	< 4.5	12	4.42	0.38 ± 3	2

[a] number of experiments, [b] pEC_{50} were calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$. Summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, n = 16). The SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50}, [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine; [d] E_{max} (histamine) at 50 µM **3.66**: 0.80 ± 0.01; [e] E_{max} (histamine) at 30 µM **3.102**: 0.93 ± 0.02; n.d.: not determined.

3.3.5 Summary and Conclusion

Starting from the N^G-acylated imidazolylpropylguanidine UR-AK51, which is lacking HR subtype selectivity and shows high activities at $hH_{2/3/4}Rs$, several classes of compounds with structurally related polar central groups were prepared. The aim was to obtain potent hH_4R agonists with improved selectivity compared to UR-AK51 and to acquire information about structure-activity and structure-selectivity relationships of the compounds at the distinct HR subtypes.

Replacing the acylguanidine moiety in UR-AK51 with a cyanoguanidine group produced **3.42** that exhibited moderate partial agonism at the hH_4R. Altering the chain length between the imidazole ring and the cyanoguanidine group revealed a tetramethylene spacer to be optimal for high potency and efficacy at the hH_4R. By contrast, two- and five-membered carbon chains were not tolerated with respect to hH_4R agonistic activity. The residue in the "eastern part" of the imidazoylbutylcyanoguanidines was found to be sensitive toward variations. Minor modifications, for example altering the chain length or fluorine substitution of the phenyl ring, substantially reduced hH_4R activity. Otherwise, introduction of small alkyl residues was well accepted and revealed that larger substituents in the "eastern part" of the compounds are required for selectivity over the hH_3R. At the hH_1R and hH_2R, most cyano-guanidines showed negligible efficacies and just low potencies. Compared to hH_1R and hH_2R higher activities were observed for the hH_3R, but almost all imidazolylbutylcyanoguanidines were most potent at the hH_4R. Introduction of a methyl group at position 5 of the imidazole ring resulted in nearly total loss of hH_4R activity. The possibility of direct, receptor

independent G-protein activation in the steady-state GTPase assay by these compounds could be excluded by investigating UR-PI376 in the presence of different hH_4R antagonists.

The application of the bivalent ligand approach to imidazolylbutylcyanoguanidines was unsuccessful with respect to hH_4R agonism. However, a substantial increase in potency compared to the corresponding "monovalent" ligands was observed at the hH_3R. As the distances between the pharmacophores of the bivalent ligands presumably are too short to enable simultaneous occupation of both ("orthosteric") binding pockets of a receptor dimer, the interaction of the second pharmacophore with an accessory binding site at the same hH_3R molecule can be assumed.[31, 32]

Exchange of the cyanoguanidine with a carbamoylguanidine group drastically changed the pharmacological activities at the HRs. All compounds displayed inverse agonistic activity at the hH_4R, whereas partial agonism was observed at the hH_2R. Unexpectedly, with UR-PI97 a highly potent hH_3R inverse agonist was identified. Compared to the acylguanidines, the sulfonylguanidine analogs suffered a considerable decrease in activity at all HR subtypes.

Substitution of the acylguanidine component with a cyanoguanidine, carbamoylguanidine or sulfonylguanidine moiety had great impact on potency and efficacy at the HR subtypes. Basicity of the groups turned out to play a crucial role for the pharmacological properties as the structurally related basic acylguanidines and the non-basic sulfonylguanidine analogs as well as the basic carbamoylguanidines and non-basic cyanoguanidines had considerably different activities at the HR subtypes. However, great differences are also obvious when comparing the pharmacological profiles of the compounds with similar basicities (acylguanidines *versus* carbamoylguanidines and cyanoguanidines *versus* sulfonyl-guanidines, respectively). Thus, next to the physicochemical differences, certainly, the varying structures of the central groups largely influence the biological activities at the distinct HR subtypes.

In summary, with UR-PI97 unexpectedly a new highly potent carbamoylguanidine-type hH_3R inverse agonist was discovered that is 25 times more potent than the standard H_3R ligand thioperamide. In contrast to thioperamide, that is essentially equipotent at the hH_3R and hH_4R, for UR-PI97 a more than 300-fold selectivity over the hH_4R was found. Since many reported imidazole containing H_3R antagonists/inverse agonists also bind with high affinity to the H_4R,[1] UR-PI97 belongs to the most selective imidazole containing hH_3R inverse agonists known so far.

Furthermore, new potent and selective cyanoguanidine-type hH_4R agonists were obtained. UR-PI376 turned out to be the most potent hH_4R agonist in this series. Compared to the lead compound UR-AK51 (Figure 3.1), UR-PI376 is devoid of agonistic activities at the hH_2R and hH_3R, and the selectivity for the hH_4R toward the hH_2R and hH_3R subtype considerably increased (approximately 300- and 30-fold selectivity in the GTPase assay, respectively).

Radioligand binding studies confirmed data from the functional GTPase assays. In contrast to UR-PI376 other selective hH_4R agonists, such as 5-methylhistamine[1] (Table 3.1), VUF 8430[3] or OUP-16[2] have agonistic activities at other HR subtypes. Therefore the new cyanoguanidine-type H_4R agonist UR-PI376 will be a valuable additonal pharmacological tool to study the biological functions of the hH_4R.

3.4 Experimental section

3.4.1 Chemistry

3.4.1.1 General conditions

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany), TCI Europe (Zwijndrecht, Belgium) and used without further purification. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen). Anhydrous DMF was purchased from Sigma-Aldrich Chemie GmbH. 3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol (**3.104**) was a gift from Prof. Dr. Sigurd Elz, Department of Pharmaceutical/Medicinal Chemistry I, University of Regensburg. Flash chromatography was performed on silica gel (Merck silica gel 60, 40 - 63 µM). Reactions were monitored by TLC on aluminum plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm). The compounds were detected by UV light (254 nm), a 0.3 % solution of ninhydrine in *n*-butanol (amines) or a 1.0 % solution of Fast Blue B salt (imidazole containing compounds) in EtOH/H_2O = 30/70 (v/v). All melting points are uncorrected and were measured on a Büchi 530 (Büchi GmbH, Essen, Germany) apparatus.

Nuclear Magnetic Resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz), Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz) or Bruker Avance 600 (^{1}H: 600.1 MHz; ^{13}C: 150.9 MHz) NMR spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts are given in δ (ppm) relative to external standards. Abbreviations for the multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sep (septet), m (multiplet), brs (for broad singlet) and combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) was determined by DEPT 135 (distortionless enhancement by polarization transfer): “+” primary and tertiary carbon atom (positive DEPT 135 signal), “-“ secondary carbon atom (negative DEPT 135 signal), “quat” quaternary carbon atom. In certain cases 2D-NMR techniques (COSY, HMQC, HSQC, HMBC, NOESY) were used to assign ^{1}H and ^{13}C chemical shifts. Infrared spectra (IR) were

measured on a Bruker Tensor 27 spectrometer equipped with an ATR (attenuated total reflexion) unit from Harrick Scientific Products Inc. (Ossining/NY, US). Mass spectra (MS) were recorded on a Finnigan MAT 95 (EI-MS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ES-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Elemental analysis (C, H, N, Heraeus Elementar Vario EL III) were performed by the Analytical Department of the University Regensburg and are within ± 0.4 % unless otherwise noted.

Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was Eurosphere-100 (250 x 32 mm) (Knauer), which was attached to a UV-detector model K-2000 (Knauer). UV detection of the compounds was done at 210 nm. The temperature was 25 °C and the flow rate 37 ml/min. The mobile phase was 0.1 % TFA in millipore water and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products (TSP, Egelsbach, Germany) equipped with a SN 400 controller, P4000 pump, an AS3000 autosampler and a Spectra Focus UV/VIS detector. Stationary phase was a Eurosphere-100 C-18 (250 x 4.0 mm, 5 µm) column (Knauer) thermostated at 30 °C. The flow rate was 0.8 ml/min and the dead time (t_0) was 3.32 min. For some compounds analytical HPLC was performed on a Merck-Hitachi system equipped with a L-5000 LC controller, 655A-12 LC pump, a 655A-40 autosampler and a L-4250 UV/VIS detector. Stationary phase was a Eurosphere-100 C-18 (250 x 4.0 mm, 5 µm) column (Knauer) thermostated at 25 °C. The flow rate was 0.7 ml/min and the dead time (t_0) was 2.54 min. As mobile phase gradients of MeCN/0.05 % TFA (aq.) were used and the absorbance was detected at 210 nm. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 210 nm. HPLC conditions, retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and purities of the synthesized compounds are listed in the appendix (Chapter 9).

3.4.1.2 Preparation of the isoureas 3.24-3.40

General procedure

A solution of the pertinent primary amine (1 eq) and diphenyl cyanocarbonimidate (**3.6**, 1 eq) in DCM was stirred for 1 h. After evaporation of the solvent, the product was crystallized from Et_2O.

1-Cyano-2-phenyl-3-(3-phenylpropyl)isourea (3.24)

The title compound was prepared from **3.7** (0.68 g, 5.0 mmol) and **3.6** (1.19 g, 5.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.2 g, 86 %); mp 92 – 95 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.91 – 2.10 (m, 2H, Ph-CH_2-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.6 Hz, Ph-C***H***$_2$), 3.39 – 3.54 (m, 2H, Ph-$(CH_2)_2$-C***H***$_2$), 6.86 (t, 1H, ^3J = 6.0 Hz, N-***H***), 7.00

– 7.51 (m, 10H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.97 (-, Ph-CH_2-***C***H_2), 32.88 (-, Ph-***C***H_2), 42.27 (-, Ph-$(CH_2)_2$-***C***H_2), 115.68 (C_{quat}, ***C***≡N), 121.48 (+, 2 Ph-***C***), 126.30 (+, 1 Ph-***C***), 126.67 (+, 1 Ph-***C***), 128.36 (+, 2 Ph-***C***), 128.64 (+, 2 Ph-***C***), 129.60 (+, 2 Ph-***C***), 140.66 (C_{quat}, Ph-***C***), 151.04 (C_{quat}, 1 Ph-***C***), 164.05 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3190 (N-H), 3061, 2942, 2187 (C≡N), 1635 (C=N), 1598, 1419, 1203. CI-MS (NH_3) *m/z* (%): 280 (100) $[M + H]^+$. Anal. ($C_{17}H_{17}N_3O$) C, H, N. $C_{17}H_{17}N_3O$ (279.34).

1-Cyano-2-phenyl-3-phenethylisourea (3.25)[13]

The title compound was prepared from **3.8** (0.97 g, 8.0 mmol) and **3.6** (1.91 g, 8.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.3 g, 85 %); mp 126 – 128 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.96 (t, 2H, ^3J = 7.1 Hz, Ph-C***H***$_2$), 3.63 – 3.78 (m, 2H, Ph-CH_2-C***H***$_2$), 6.90 (d, 2H, ^3J = 7.8 Hz, Ph-***H***), 7.07 (t, 1H, ^3J = 6.1 Hz, N-***H***), 7.19 – 7.44 (m, 8H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 36.18 (-, Ph-***C***H_2), 43.93 (-, Ph-CH_2-***C***H_2), 115.69 (C_{quat}, ***C***≡N), 121.45 (+, 2 Ph-***C***), 126.63 (+, 1 Ph-***C***), 126.93 (+, 1 Ph-***C***), 128.87 (+, 2 Ph-***C***), 128.97 (+, 2 Ph-***C***), 129.53 (+, 2 Ph-***C***), 137.81 (C_{quat}, 1 Ph-***C***), 151.96 (C_{quat}, Ph-***C***), 164.03 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3188 (N-H), 3063, 2189 (C≡N), 1646 (C=N), 1448, 1419, 1196. CI-MS (NH_3) *m/z* (%): 264 (100) $[M - H^+]^-$. Anal. ($C_{16}H_{15}N_3O$) C, H, N. $C_{16}H_{15}N_3O$ (265.31).

1-Cyano-2-phenyl-3-(4-phenylbutyl)isourea (3.26)

The title compound was prepared from **3.9** (1.19 g, 8.0 mmol) and **3.6** (1.91 g, 8.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (2.0 g, 85 %); mp 134 – 136 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.61 – 1.81 (m, 4H, Ph-CH_2-(C***H***$_2$)$_2$, 2.67 (t, 2H, ^3J = 6.7 Hz, Ph-C***H***$_2$), 3.38 – 3.50 (m, 2H, Ph-$(CH_2)_3$-C***H***$_2$), 6.99 (t, 1H, ^3J = 5.8 Hz, N-***H***), 7.01 – 7.51 (m, 10H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.32, 29.06 (-, Ph-CH_2-(***C***H_2)$_2$), 35.34 (-, Ph-***C***H_2), 42.56 (-, Ph-$(CH_2)_3$-***C***H_2), 115.77 (C_{quat}, ***C***≡N), 121.51 (+, 2 Ph-***C***), 125.99 (+, 1 Ph-***C***), 126.63 (+, 1 Ph-***C***), 128.45 (+, 2 Ph-***C***), 128.47 (+, 2 Ph-***C***), 129.58 (+, 2 Ph-***C***), 141.73 (C_{quat}, 1 Ph-***C***), 151.09 (C_{quat}, 1 Ph-***C***), 164.04 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3190 (N-H), 3057, 2934, 2187 (C≡N), 1644 (C=N), 1446, 1417, 1192. EI-MS (70 eV) *m/z* (%): 293 (10) $[M^{+\bullet}]$, 91 (100) $[C_7H_7^+]$. Anal. ($C_{18}H_{19}N_3O$) C, H, N. $C_{18}H_{19}N_3O$ (293.36).

1-Cyano-3-(3,3-diphenylpropyl)-2-phenylisourea (3.27)[44]

The title compound was prepared from **3.10** (1.06 g, 4.0 mmol) and **3.6** (0.95 g, 4.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.3 g, 91 %); mp 166 – 168 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.37 – 2.52 (m, 2H, Ph_2CH-C***H***$_2$), 3.33 – 3.46 (m, 2H, Ph_2CH-CH_2-C***H***$_2$), 4.03 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 6.97 – 7.11 (m, 3H, 2 Ph-***H*** + N-***H***), 7.13 – 7.49 (m, 13H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 35.11 (-, Ph_2CH-

CH_2-***C***H_2), 41.48 (-, Ph_2CH-***C***H_2), 48.63 (+, Ph_2***C***H), 115.62 (C_{quat}, ***C***≡N), 121.46 (+, 2 Ph-***C***), 126.66 (+, 3 Ph-***C***), 127.70 (+, 4 Ph-***C***), 128.78 (+, 4 Ph-***C***), 129.57 (+, 2 Ph-***C***), 143.61 (C_{quat}, 2 Ph-***C***), 151.03 (C_{quat}, 1 Ph-***C***), 163.93 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3184 (N-H), 3061, 2187 (C≡N), 1641 (C=N), 1447, 1208. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 356 (100) $[M + H]^+$. Anal. ($C_{23}H_{21}N_3O$) C, H, N. $C_{23}H_{21}N_3O$ (355.43).

1-Cyano-3-(3-cyclohexylpropyl)-2-phenylisourea (3.28)

The title compound was prepared from **3.11** (1.13 g, 8.0 mmol) and **3.6** (1.91 g, 8.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.75 g, 77 %); mp 101 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.76 – 1.03 (m, 2H), 1.04 – 1.42 (m, 6H), 1.54 – 1.94 (m, 7H), 3.31 – 3.58 (m, 2H, cHex-$(CH_2)_2$-C***H***$_2$), 6.80 (t, 1H, ^3J = 5.8 Hz, N-***H***), 7.03 – 7.61 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.33 (-, 2 ***C***H_2), 26.61 (-, ***C***H_2), 26.98 (-, ***C***H_2), 33.30 (-, 2 ***C***H_2), 34.32 (-, ***C***H_2), 37.30 (+, ***C***H), 43.13 (-, ***C***H_2), 115.77 (C_{quat}, ***C***≡N), 121.49 (+, 2 Ph-***C***), 126.62 (+, 1 Ph-***C***), 129.59 (+, 2 Ph-***C***), 151.11 (C_{quat}, 1 Ph-***C***), 164.05 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3183 (N-H), 3063, 2918, 2851, 2189 (C≡N), 1640 (C=N), 1446, 1416, 1207. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 286 (100) $[M + H]^+$. Anal. ($C_{17}H_{23}N_3O$) C, H, N. $C_{17}H_{23}N_3O$ (285.38).

1-Cyano-2-phenyl-3-[2-(phenylthio)ethyl]isourea (3.29)

The title compound was prepared from **3.12** (1.53 g, 10.0 mmol) and **3.6** (2.38 g, 10.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (2.6 g, 86 %); mp 110 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.18 (t, 2H, ^3J = 7.0 Hz, Ph-S-C***H***$_2$), 3.55 – 3.69 (m, 2H, Ph-S-CH_2-C***H***$_2$), 7.05 (d, 2H, ^3J = 7.8 Hz, Ph-***H***), 7.15 – 7.50 (m, 8H, Ph-***H***), 7.57 (t, 1H, ^3J = 6.1 Hz, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 33.22 (-, Ph-S-***C***H_2), 41.68 (-, Ph-S-CH_2-***C***H_2), 115.55 (C_{quat}, ***C***≡N), 121.44 (+, 2 Ph-***C***), 126.71 (+, 1 Ph-***C***), 126.79 (+, 1 Ph-***C***), 129.26 (+, 2 Ph-***C***), 128.62 (+, 2 Ph-***C***), 129.92 (+, 2 Ph-***C***), 134.60 (C_{quat}, 1 Ph-***C***), 150.98 (C_{quat}, 1 Ph-***C***), 163.88 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3252 (N-H), 3212, 3065, 2190 (C≡N), 1635 (C=N), 1433, 1207. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 298 (100) $[M + H]^+$. Anal. ($C_{16}H_{15}N_3OS$) C, H, N. $C_{16}H_{15}N_3OS$ (297.37).

1-Cyano-3-(1*H*-indol-3-yl)ethyl-2-phenylisourea (3.30)

The title compound was prepared from **3.13** (1.28 g, 8.0 mmol) and **3.6** (1.91 g, 8.0 mmol) in DCM (50 mL) according to the general procedure. The obtained solid was recrystallized from Et_2O/MeCN yielding a beige solid (2.1 g, 86 %); mp 167 – 168 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) isomers**:** δ [ppm] = 2.95 (t, 0.8H, ^3J = 7.4 Hz, indole-3-C***H***$_2$), 3.03 (t, 1.2H, ^3J = 7.0 Hz, indole-3-C***H***$_2$), 3.44 – 3.67 (m, 2H, indole-3-CH_2-C***H***$_2$), 6.77 – 7.62 (m, 10H, Ph-***H*** + indole-***H***), 8.49 (t, 0.4H, ^3J = 5.4 Hz, N-***H***), 8.88 (t, 0.6H, ^3J = 5.7 Hz, N-***H***), 10.89 (s, 1H,

indole-N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6) isomers**:** δ [ppm] = 24.06, 25.44 (-, indole-3-***C***H_2), 42.81, 43.06 (-, ***C***H_2-NH), 110.66, 110.84 (C_{quat}, indole-***C***-3), 111.33 (+, indole-***C***-7), 114.77 (C_{quat}, ***C***≡N), 118.13, 118.27, 118.31, 119.84 (+, indole-***C***-4,5,6), 120.91, 121.46 (+, Ph-***C***), 123.00, 123.07 (+, indole-***C***-2), 126.01, 126.04 (+, Ph-***C***), 127.05, 127.11 (C_{quat}, indole-***C***-3a), 129.24, 130.14 (+, Ph-***C***), 136.15, 136.21 (C_{quat}, indole-***C***-7a), 150.97, 151.42 (C_{quat}, Ph-***C***), 159.50, 162.62 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3354 (N-H), 3205 (N-H), 3058, 2180 (C≡N), 1640 (C=N), 1445, 1424, 1198. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 305 (100) $[M + H]^+$. Anal. ($C_{18}H_{16}N_4O$) C, H, N. $C_{18}H_{16}N_4O$ (304.35).

1-Cyano-3-[3-(4-fluorophenyl)propyl]-2-phenylisourea (3.31)

The title compound was prepared from **3.14** (0.92 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.2 g, 67 %); mp 104 – 106 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.88 – 2.06 (m, 2H, Ph-CH_2-C***H***$_2$), 2.69 (t, 2H, ^3J = 7.6 Hz, Ph-C***H***$_2$), 3.37 – 3.53 (m, 2H, Ph-$(CH_2)_2$-C***H***$_2$), 6.89 – 7.53 (m, 10H, Ph-***H*** + N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 31.10, 32.07 (-, 4-F-Ph-***C***H_2-***C***H_2), 42.13 (-, 4-F-Ph-$(CH_2)_2$-***C***H_2), 115.38 (+, ^2J = 21.1 Hz, 4-F-Ph-***C***-3,5), 115.71 (C_{quat}, ***C***≡N), 121.47 (+, 2 Ph-***C***), 126.69 (+, 1 Ph-***C***), 129.62 (+, 2 Ph-***C***), 129.73 (+, ^3J = 7.9 Hz, 4-F-Ph-***C***-2,6), 136.36 (C_{quat}, ^4J = 3.2 Hz, 4-F-Ph-***C***-1), 151.05 (C_{quat}, 1 Ph-***C***), 161.46 (C_{quat}, ^2J = 244.0 Hz, 4-F-Ph-***C***-4), 164.04 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3205 (N-H), 3064, 2944, 2187 (C≡N), 1647 (C=N), 1448, 1429, 1203. CI-MS (NH_3) *m/z* (%): 298 (100) $[M + H]^+$. Anal. ($C_{17}H_{16}FN_3O$) C, H, N. $C_{17}H_{16}FN_3O$ (297.33).

1-Cyano-2-phenyl-3-[3-(pyridin-2-yl)propyl]isourea (3.32)

The title compound was prepared from **3.15** (0.82 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.2 g, 71 %); mp 96 – 98 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.07 – 2.21 (m, 2H, Pyr-2-CH_2-C***H***$_2$), 3.04 (t, 2H, ^3J = 6.2 Hz, Pyr-2-C***H***$_2$), 3.54 – 3.64 (m, 2H, Pyr-2-$(CH_2)_2$-C***H***$_2$), 6.95 – 7.88 (m, 8H, Ph-***H*** + Pyr-3,4,5-***H***), 8.49 (dd, 1H, ^3J = 5.9 Hz, ^4J = 1.6 Hz, Pyr-6-***H***), 9.41 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.94 (-, Pyr-2-CH_2-***C***H_2), 35.97 (-, Pyr-2-***C***H_2), 43.57 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 115.89 (C_{quat}, ***C***≡N), 121.61 (+, Pyr-***C***-5 + 2 Ph-***C***), 123.05 (+, Pyr-***C***-3), 126.41 (+, 1 Ph-***C***), 129.50 (+, 2 Ph-***C***), 136.97 (+, Pyr-***C***-4), 149.76 (+, Pyr-***C***-6), 151.29 (C_{quat}, 1 Ph-***C***), 160.12 (C_{quat}, Pyr-***C***-2), 163.44 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3060, 2190 (C≡N), 1607 (C=N), 1326, 1208. CI-MS (NH_3) *m/z* (%): 281 (100) $[M + H]^+$. Anal. ($C_{16}H_{16}N_4O$) C, H, N. $C_{16}H_{16}N_4O$ (280.32).

1-Cyano-2-phenyl-3-[3-(pyridin-3-yl)propyl]isourea (3.33)

The title compound was prepared from **3.16** (0.82 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.1 g, 65 %); mp 90 – 91 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.91 – 2.09 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.72 (t, 2H, ^{3}J = 7.7 Hz, Pyr-3-C***H***$_2$), 3.38 – 3.53 (m, 2H, Pyr-3-$(CH_2)_2$-C***H***$_2$), 7.00 – 7.58 (m, 8H, Ph-***H*** + Pyr-4,5-***H*** + N-***H***), 8.42 – 8.50 (m, 2H, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.07, 30.67 (-, Pyr-3-***C***H_2-***C***H_2), 42.06 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 115.71 (C_{quat}, ***C***≡N), 121.46 (+, 2 Ph-***C***), 123.50 (+, Pyr-***C***-5), 126.67 (+, 1 Ph-***C***), 129.61 (+, 2 Ph-***C***), 135.81 (+, Pyr-***C***-4), 136.17 (C_{quat}, Pyr-***C***-3), 147.79 (+, Pyr-***C***-6), 149.86 (+, Pyr-***C***-2), 151.06 (C_{quat}, 1 Ph-***C***), 163.99 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3185 (N-H), 3056, 2937, 2187 (C≡N), 1632 (C=N), 1421, 1198, 1190. CI-MS (NH_3) *m/z* (%): 281 (100) $[M + H]^+$. Anal. ($C_{16}H_{16}N_4O$) C, H, N. $C_{16}H_{16}N_4O$ (280.32).

1-Cyano-2-phenyl-3-[3-(pyridin-4-yl)propyl]isourea (3.34)

The title compound was prepared from **3.17** (0.82 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.34 g, 80 %); mp 120 – 121 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.86 – 2.11 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.71 (t, 2H, ^{3}J = 7.7 Hz, Pyr-4-C***H***$_2$), 3.39 – 3.50 (m, 2H, Pyr-4-$(CH_2)_2$-C***H***$_2$), 7.02 – 7.18 (m, 4H, Ph-***H*** + Pyr-3,5-***H***), 7.24 – 7.46 (m, 3H, Ph-***H***), 7.71 (t, 1H, ^{3}J = 5.6 Hz, N-***H***), 8.49 (dd, 2H, ^{3}J = 4.4 Hz, ^{4}J = 1.6 Hz, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.82, 32.21 (-, Pyr-4-***C***H_2-***C***H_2), 42.02 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 115.76 (C_{quat}, ***C***≡N), 121.44 (+, 2 Ph-***C***), 123.82 (+, Pyr-***C***-3,5), 126.69 (+, 1 Ph-***C***), 129.62 (+, 2 Ph-***C***), 149.88 (+, Pyr-***C***-2,6), 149.93 (C_{quat}, Pyr-***C***-4), 151.06 (C_{quat}, Ph-***C***), 163.99 (C_{quat}, ***C***=N). IR (cm^{-1}) = 2959, 2823, 2181 (C≡N), 1600 (C=N), 1452, 1416, 1200. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 281 (100) $[M + H]^+$. Anal. ($C_{16}H_{16}N_4O$) C, H, N. $C_{16}H_{16}N_4O$ (280.32).

1-Cyano-2-phenyl-3-(3-phenylbutyl)isourea (3.35)

The title compound was prepared from **3.18** (0.90 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (1.5 g, 82 %); mp 132 – 134 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.31 (d, 3H, ^{3}J = 6.9 Hz, PhC***H***$_3$CH), 1.91 – 2.03 (m, 2H, $PhCH_3CH$-C***H***$_2$), 2.75 – 2.90 (m, 1H, $PhCH_3$C***H***), 3.18 – 3.42 (m, 2H, $PhCH_3CH$-CH_2-C***H***$_2$), 6.83 (t, 1H, ^{3}J = 5.2 Hz, N-***H***), 6.99 – 7.52 (m, 10H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 22.62 (+, Ph***C***H_3CH), 37.49, (-, ***C***H_2), 37.56 (+, $PhCH_3$***C***H), 41.25 (-, ***C***H_2), 115.62 (C_{quat}, ***C***≡N), 121.46 (+, 2 Ph-***C***), 126.54 (+, 1 Ph-***C***), 126.60 (+, 1 Ph-***C***), 126.87 (+, 2 Ph-***C***), 128.76 (+, 2 Ph-***C***), 129.56 (+, 2 Ph-***C***), 145.71 (C_{quat}, 1 Ph-***C***), 151.05 (C_{quat}, 1 Ph-***C***), 163.91 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3185 (N-H), 3055, 2955, 2184 (C≡N), 1642 (C=N), 1450,

1427, 1201. CI-MS (NH_3) *m/z* (%): 294 (100) $[M + H]^+$. Anal. ($C_{18}H_{19}N_3O$) C, H, N. $C_{18}H_{19}N_3O$ (293.36).

1-Cyano-2-phenylisourea (3.36)[45]

The title compound was prepared from a 7 M solution of ammonia (**3.19**) in MeOH (0.86 mL, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (0.8 g, 83 %); mp 149 – 151 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.13 – 7.20 (m, 2H, Ph-***H***), 7.24 – 7.32 (m, 1H, Ph-***H***), 7.37 – 7.46 (m, 2H, Ph-***H***), 8.58 (brs, 2H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 114.86 (C_{quat}, ***C***≡N), 121.60 (+, 2 Ph-***C***), 126.09 (+, 1 Ph-***C***), 129.50 (+, 2 Ph-***C***), 151.03 (C_{quat}, 1 Ph-***C***), 164.53 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3321 (N-H), 3174, 3056, 2200 (C≡N), 1646 (C=N), 1576, 1452, 1203. CI-MS (NH_3) *m/z* (%): 179 (100) $[M + NH_4]^+$, 162 (71) $[M + H]^+$. Anal. ($C_8H_7N_3O$) C, H, N. $C_8H_7N_3O$ (161.16).

1-Cyano-3-methyl-2-phenylisourea (3.37)[46]

The title compound was prepared from a 8 M solution of methylamine (**3.20**) in MeOH (0.75 mL, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (0.8 g, 76 %); mp 125 – 126 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) isomers: δ [ppm] = 2.76 (s, 1.4 H, C***H***$_3$), 2.91 (s, 1.6 H, C***H***$_3$), 7.14 – 7.54 (m, 5H, Ph-***H***), 8.19 (brs, 0.45H, N-***H***), 8.66 (brs, 0.55H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6) isomers: δ [ppm] = 28.69, 29.15 (+, ***C***H$_3$), 114.43, 114.85 (C_{quat}, ***C***≡N), 120.12, 121.70 (+, 2 Ph-***C***), 126.13, 126.26 (+, 1 Ph-***C***), 129.39, 130.22 (+, 2 Ph-***C***), 151.14, 151.29 (C_{quat}, 1 Ph-***C***), 160.06, 163.16 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3189 (N-H), 3015, 2186 (C≡N), 1656 (C=N), 1628, 1386. CI-MS (NH_3) *m/z* (%): 193 (100) $[M + NH_4]^+$, 176 (68) $[M + H]^+$. Anal. ($C_9H_9N_3O$) C, H, N. $C_9H_9N_3O$ (175.19).

1-Cyano-3-ethyl-2-phenylisourea (3.38)[47]

The title compound was prepared from a 30 – 40 % solution of ethylamine (**3.21**) in MeOH (0.90 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (0.5 g, 45 %); mp 117 – 118 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.29 (t, 3H, ^3J = 7.2 Hz, C***H***$_3$), 3.41 – 3.55 (m, 2H, C***H***$_2$), 6.99 (brs, 1H, N-***H***), 7.09 (d, 2H, ^3J = 7.8 Hz, Ph-***H***), 7.24 – 7.33 (m, 1H, Ph-***H***), 7.34 – 7.50 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.93 (+, ***C***H$_3$), 37.74, (-, ***C***H$_2$), 115.84 (C_{quat}, ***C***≡N), 121.50 (+, 2 Ph-***C***), 126.60 (+, 1 Ph-***C***), 129.57 (+, 2 Ph-***C***), 151.11 (C_{quat}, 1 Ph-***C***), 163.97 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3259 (N-H), 2979, 2182 (C≡N), 1626 (C=N), 1419, 1208. CI-MS (NH_3) *m/z* (%): 190 (100) $[M + H]^+$. Anal. ($C_{10}H_{11}N_3O$) C, H, N. $C_{10}H_{11}N_3O$ (189.21).

1-Cyano-3-isopropyl-2-phenylisourea (3.39)[48]

The title compound was prepared from **3.22** (0.35 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (0.8 g, 68 %); mp 124 – 126 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.33 (d, 6H, ^{3}J = 6.0 Hz, C***H***$_3$), 4.04 – 4.22 (m, 1H, C***H***), 6.17 (brs, 1H, N-***H***), 7.09 (d, 2H, ^{3}J = 7.8 Hz, Ph-***H***), 7.30 (d, 1H, ^{3}J = 7.2 Hz, Ph-***H***), 7.35 – 7.48 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 22.62 (+, ***C***H$_3$), 45.73, (+, ***C***H), 115.44 (C_{quat}, ***C***≡N), 121.43 (+, 2 Ph-***C***), 126.70 (+, 1 Ph-***C***), 129.60 (+, 2 Ph-***C***), 151.05 (C_{quat}, 1 Ph-***C***), 163.22 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3201 (N-H), 3068, 2980, 2189 (C≡N), 1642 (C=N), 1431, 1200. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 204 (100) [M + H]$^{+}$. Anal. ($C_{11}H_{13}N_3O$) C, H, N. $C_{11}H_{13}N_3O$ (203.24).

1-Cyano-3-isobutyl-2-phenylisourea (3.40)

The title compound was prepared from **3.23** (0.44 g, 6.0 mmol) and **3.6** (1.43 g, 6.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (0.8 g, 59 %); mp 103 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.00 (d, 6H, ^{3}J = 6.7 Hz, C***H***$_3$), 1.86 – 2.03 (m, 1H, C***H***), 3.21 – 3.30 (m, 2H, CH_2), 6.91 (brs, 1H, N-***H***), 7.07 (d, 2H, ^{3}J = 7.8 Hz, Ph-***H***), 7.23 – 7.33 (m, 1H, Ph-***H***), 7.34 – 7.46 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 19.92 (+, ***C***H$_3$), 28.79, (+, ***C***H), 49.90 (-, CH_2), 115.68 (C_{quat}, ***C***≡N), 121.46 (+, 2 Ph-***C***), 126.58 (+, 1 Ph-***C***), 129.56 (+, 2 Ph-***C***), 151.10 (C_{quat}, 1 Ph-***C***), 164.16 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3194 (N-H), 3063, 2955, 2190 (C≡N), 1635 (C=N), 1423, 1207. CI-MS (NH_3) *m/z* (%): 218 (100) [M + H]$^{+}$. Anal. ($C_{12}H_{15}N_3O$) C, H, N. $C_{12}H_{15}N_3O$ (217.27).

3.4.1.3 Preparation of the cyanoguanidines 3.42-3.48, 3.52-3.58, 3.61-3.77, 3.80 and 3.81

General procedure

Hydrochlorides of **3.41** and **3.51** were converted into the bases by passing a basic ion exchanger (Merck, ion exchanger III, mobile phase: MeOH). The isourea (1 eq) and the pertinant amine (1.1 eq) were refluxed in MeCN for 12 h. After removal of the solvent *in vacuo*, the crude product was purified by flash chromatography. For analytical purposes a small amount of most compounds was converted to the hydrogenoxalate by addition of a saturated solution of oxalic acid in Et_2O to a solution of the cyanoguanidine in EtOH.

2-Cyano-1-[3-(1*H*-imidazol-4-yl)propyl]-3-(3-phenylpropyl)guanidine (3.42)

The title compound was prepared from **3.24** (0.56 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.45 g, 72 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.80 – 1.95 (m, 4H,

Im-4-CH_2-C***H***$_2$ + Ph-CH_2-C***H***$_2$), 2.59 – 2.72 (m, 4H, Im-4-C***H***$_2$ + Ph-C***H***$_2$), 3.17 – 3.28 (m, 4H, Im-4-$(CH_2)_2$-C***H***$_2$ + Ph-$(CH_2)_2$-C***H***$_2$), 7.04 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.11 – 7.29 (m, 5H, Ph-***H***), 8.12 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 23.87 (-, Im-4-***C***H_2), 29.87 (-, Im-4-CH_2-***C***H_2), 32.23 (-, Ph-CH_2-***C***H_2), 33.98 (-, Ph-***C***H_2), 41.98 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.42 (-, Ph-$(CH_2)_2$-***C***H_2), 117.06 (+, Im-***C***-5), 120.18 (C_{quat}, ***C***≡N), 127.03 (+, Ph-***C***-4), 129.45 (+, 2 Ph-***C***), 129.50 (+, 2 Ph-***C***), 135.39 (+, Im-***C***-2), 136.74 (C_{quat}, Im-***C***-4), 142.84 (C_{quat}, Ph-***C***-1), 161.26 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3250 (N-H), 2937 (C-H), 2857 (C-H), 2157 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 311 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{22}N_6$ [$M^{+\bullet}$] 310.1906; found 310.1903. Anal. ($C_{17}H_{22}N_6$ · 0.25 H_2O) C, H, N. $C_{17}H_{22}N_6$ (310.40).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)propyl]-3-(2-phenylethyl)guanidine (3.43)

The title compound was prepared from **3.25** (0.53 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.37 g, 62 %); mp (hydrogenoxalate) 146 – 149 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.78 – 1.91 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.69 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 2.84 (t, 2H, ^3J = 7.2 Hz, Ph-C***H***$_2$), 3.22 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.44 (t, 2H, ^3J = 7.2 Hz, Ph-CH_2-C***H***$_2$), 7.11 – 7.33 (m, 6H, Ph-***H***, Im-5-***H***), 8.58 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 22.96 (-, Im-4-***C***H_2), 29.47 (-, Im-4-CH_2-***C***H_2), 36.68 (-, Ph-***C***H_2), 41.80 (-, Im-4-$(CH_2)_2$-***C***H_2), 44.27 (-, Ph-CH_2-***C***H_2), 116.91 (+, Im-***C***-5), 120.02 (C_{quat}, ***C***≡N), 127.58 (+, Ph-***C***-4), 129.63 (+, 2 Ph-***C***), 130.05 (+, 2 Ph-***C***), 134.93 (+, Im-***C***-2), 135.43 (C_{quat}, Im-***C***-4), 140.12 (C_{quat}, Ph-***C***-1), 161.20 (C_{quat}, ***C***=N), 167.90 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3249 (N-H), 2940 (C-H), 2854 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (MeOH + NH_4OAc) *m/z* (%): 297 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{16}H_{20}N_6$ [$M^{+\bullet}$] 296.1749; found 296.1747. Anal. ($C_{16}H_{20}N_6$ · 0.7 $C_2H_2O_4$ · 0.5 H_2O) C, H, N. $C_{16}H_{20}N_6$ (296.37).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)propyl]-3-(4-phenylbutyl)guanidine (3.44)

The title compound was prepared from **3.26** (0.59 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.51 g, 79 %); mp (hydrogenoxalate) 128 – 130 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.49 – 1.72 (m, 4H, Ph-CH_2-C***H***$_2$-C***H***$_2$), 1.82 – 1.96 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.63 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 2.71 (t, 2H, ^3J = 7.5 Hz, Ph-C***H***$_2$), 3.17 – 3.30 (m, 4H, Im-4-$(CH_2)_2$-C***H***$_2$ + Ph-$(CH_2)_3$-C***H***$_2$), 7.08 – 7.27 (m, 6H, Ph-***H*** + Im-5-***H***), 8.50 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] =

23.09 (-, Im-4-***C***H_2), 29.63 (-, Im-4-CH_2-***C***H_2), 29.75, 30.03 (-, Ph-CH_2-***C***H_2-***C***H_2), 36.50 (-, Ph-***C***H_2), 41.82 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.65 (-, Ph-$(CH_2)_3$-***C***H_2), 116.91 (+, Im-***C***-5), 120.19 (C_{quat}, ***C***≡N), 126.84 (+, Ph-***C***-4), 129.38 (+, 2 Ph-***C***), 129.48 (+, 2 Ph-***C***), 134.97 (+, Im-***C***-2), 135.59 (C_{quat}, Im-***C***-4), 143.51 (C_{quat}, Ph-***C***-1), 161.23 (C_{quat}, ***C***=N), 168.22 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3245 (N-H), 2934 (C-H), 2858 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 325 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{24}N_6$ $[M^{+\bullet}]$ 324.2062; found 324.2058. Anal. ($C_{18}H_{24}N_6 \cdot 0.85\ C_2H_2O_4$) C, H, N. $C_{18}H_{24}N_6$ (324.42).

2-Cyano-1-(3,3-diphenylpropyl)-3-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.45)

The title compound was prepared from **3.27** (0.71 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 95/3/2 v/v/v) yielding a colorless foam-like solid (0.51 g, 66 %); mp (hydrogenoxalate) 156 – 158 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.80 – 1.93 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.28 – 2.39 (m, 2H, Ph_2CH-C***H***$_2$), 2.70 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.15 (t, 2H, ^3J = 7.3 Hz, Ph_2CH-CH_2-C***H***$_2$), 3.21 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 4.00 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 7.31 – 7.11 (m, 11H, Ph-***H*** + Im-5-***H***), 8.59 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 22.90 (-, Im-4-***C***H_2), 29.46 (-, Im-4-CH_2-***C***H_2), 35.98 (-, Ph_2CH***C***H_2), 41.73, 41.78 (-, Im-4-$(CH_2)_2$-***C***H_2 + Ph_2CHCH_2-***C***H_2), 50.05 (+, Ph_2***C***H), 116.87 (+, Im-***C***-5), 120.12 (C_{quat}, ***C***≡N), 127.43 (+, 2 Ph-***C***-4), 128.89 (+, 4 Ph-***C***), 129.63 (+, 4 Ph-***C***), 134.89 (+, Im-***C***-2), 135.30 (C_{quat}, Im-***C***-4), 145.81 (C_{quat}, 2 Ph-***C***-1), 161.21 (C_{quat}, ***C***=N), 167.51 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3242 (N-H), 2989 (C-H), 2901 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 387 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{23}H_{26}N_6$ $[M^{+\bullet}]$ 386.2219; found 386.2214. Anal. ($C_{23}H_{26}N_6 \cdot 0.25\ H_2O$) C, H, N. $C_{23}H_{26}N_6$ (386.49).

2-Cyano-1-(3-cyclohexylpropyl)-3-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.46)

The title compound was prepared from **3.28** (0.57 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 95/3/2 v/v/v) yielding a colorless foam-like solid (0.35 g, 55 %); mp (hydrogenoxalate) 144 – 146 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 0.81 – 1.00 (m, 2H, cHex-C***H***$_2$), 1.10 – 1.35 (m, 6H), 1.49 – 1.79 (m, 7H), 1.85 – 1.99 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.75 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 3.16 (t, 2H, ^3J = 7.2 Hz, cHex-$(CH_2)_2$-C***H***$_2$), 3.28 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.25 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 8.58 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 23.03 (-, Im-4-***C***H_2), 27.54 (-, 2 cHex-***C***), 27.83 (-, ***C***H_2), 27.87 (-, ***C***H_2), 29.64 (-, Im-4-CH_2-***C***H_2), 34.58 (-, 2 cHex-***C***), 35.63 (-, ***C***H_2), 38.80 (+, cHex-***C***), 41.81

(-, Im-4-$(CH_2)_2$-***C***H_2), 43.21 (-, cHex-$(CH_2)_2$-***C***H_2), 116.93 (+, Im-***C***-5), 120.20 (C_{quat}, ***C***≡N), 134.97 (+, Im-***C***-2), 135.47 (C_{quat}, Im-***C***-4), 161.23 (C_{quat}, ***C***=N), 167.72 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3276 (N-H), 2919 (C-H), 2849 (C-H), 2159 (C≡N), 1580 (C=N). ES-MS (MeOH + NH_4OAc) *m/z* (%): 317 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{28}N_6$ $[M^{+\bullet}]$ 316.2375; found 316.2374. Anal. ($C_{17}H_{28}N_6 \cdot 0.8\ C_2H_2O_4$) C, H, N. $C_{17}H_{28}N_6$ (316.44).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)propyl]-3-[2-(phenylthio)ethyl]guanidine (3.47)

The title compound was prepared from **3.29** (0.60 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.52 g, 78 %); mp (hydrogenoxalate) 126 – 129 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.83 – 1.96 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.11 (t, 2H, ^3J = 6.9 Hz, S-CH_2-C***H***$_2$), 3.22 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.42 (t, 2H, ^3J = 6.9 Hz, S-C***H***$_2$), 7.13 – 7.21 (m, 1H, Ph-4-***H***), 7.22 – 7.34 (m, 3H, Ph-***H*** + Im-5-***H***), 7.34 – 7.43 (m, 2H, Ph-***H***), 8.65 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 22.90 (-, Im-4-***C***H_2), 29.36 (-, Im-4-CH_2-***C***H_2), 33.62 (-, S-***C***H_2), 41.90 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.32 (-, S-CH_2-***C***H_2), 116.91 (+, Im-***C***-5), 119.82 (C_{quat}, ***C***≡N), 127.38 (+, Ph-***C***-4), 130.20 (+, 2 Ph-***C***), 130.48 (+, 2 Ph-***C***), 134.91 (+, Im-***C***-2), 135.22 (C_{quat}, Im-***C***-4), 136.97 (C_{quat}, Ph-***C***-1), 161.18 (C_{quat}, ***C***=N), 167.52 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3247 (N-H), 2938 (C-H), 2847 (C-H), 2160 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 329 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{16}H_{20}N_6S$ $[M^{+\bullet}]$ 328.1470; found 328.1467. Anal. ($C_{16}H_{20}N_6S \cdot 0.75\ C_2H_2O_4 \cdot 0.25\ H_2O$) C, H, N. $C_{16}H_{20}N_6S$ (328.44).

2-Cyano-1-[3-(1*H*-imidazol-4-yl)propyl]-3-[2-(1*H*-indol-3-yl)ethyl]guanidine (3.48)

The title compound was prepared from **3.30** (0.61 g, 2.0 mmol) and **3.41** (0.28 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.53 g, 79 %); mp (hydrogenoxalate) 160 – 164 °C. ^{1}H-NMR (400 MHz, COSY, CD_3OD, hydrogenoxalate): δ [ppm] = 1.65 – 1.76 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.58 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.00 (t, 2H, ^3J = 6.9 Hz, indole-3-C***H***$_2$), 3.13 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.50 (t, 2H, ^3J = 6.9 Hz, indole-3-CH_2-C***H***$_2$), 6.99 (ddd, 1H, ^3J = 7.8 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, indole-5-***H***), 7.05 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.3 Hz, indole-6-***H***), 7.10 (s, 1H, indole-2-***H***), 7.15 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.29 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.1 Hz, ^5J = 0.8 Hz, indole-7-***H***), 7.56 (ddd, 1H, ^3J = 7.8 Hz, ^4J = 1.3 Hz, ^5J = 0.8 Hz, indole-4-***H***), 8.64 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (100 MHz, HMQC, CD_3OD, hydrogenoxalate): δ [ppm] = 22.63 (-, Im-4-***C***H_2), 26.40 (-, indole-3-***C***H_2), 29.20 (-, Im-4-CH_2-***C***H_2), 41.61 (-, Im-

4-$(CH_2)_2$-***C***H_2), 43.71 (-, indole-3-CH_2-***C***H_2), 112.37 (+, indole-***C***-7), 112.70 (C_{quat}, indole-***C***-3), 116.82 (+, Im-***C***-5), 119.33 (+, indole-***C***-4), 119.82 (+, indole-***C***-5), 120.12 (C_{quat}, ***C***≡N), 122.47 (+, indole-***C***-6), 123.98 (+, indole-***C***-2), 128.75 (C_{quat}, indole-***C***-3a), 134.74 (+, Im-***C***-2), 135.04 (C_{quat}, Im-***C***-4), 138.22 (C_{quat}, indole-***C***-7a), 161.29 (C_{quat}, ***C***=N), 167.11 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3280 (N-H), 2936 (C-H), 2854 (C-H), 2160 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 336 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{21}N_7$ $[M^{+\bullet}]$ 335.1858; found 335.1854. Anal. ($C_{18}H_{21}N_7 \cdot C_2H_2O_4$) C, H, N. $C_{18}H_{21}N_7$ (335.41).

2-Cyano-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-(3-phenylpropyl)guanidine (3.52)

The title compound was prepared from **3.24** (0.56 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.44 g, 75 %); mp (hydrogenoxalate) 149 – 151 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.77 – 1.89 (m, 2H, Ph-CH_2-C***H***$_2$), 2.62 (t, 2H, ^3J = 7.7 Hz, Ph-C***H***$_2$), 2.93 (t, 2H, ^3J = 6.9 Hz, Im-4-C***H***$_2$), 3.19 (t, 2H, ^3J = 7.2 Hz, Ph-$(CH_2)_2$-C***H***$_2$), 3.51 (t, 2H, ^3J = 6.9 Hz, Im-4-CH_2-C***H***$_2$), 7.11 – 7.30 (m, 6H, Ph-***H*** + Im-5-***H***), 8.56 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 26.29 (-, Im-4-***C***H_2), 32.14 (-, Ph-CH_2-***C***H_2), 33.93 (-, Ph-***C***H_2), 41.57 (-, Im-4-CH_2-***C***H_2), 42.46 (-, Ph-CH_2-CH_2-***C***H_2), 117.69 (+, Im-***C***-5), 119.86 (C_{quat}, ***C***≡N), 127.06 (+, Ph-***C***-4), 129.45 (+, 2 Ph-***C***), 129.52 (+, 2 Ph-***C***), 133.20 (C_{quat}, Im-***C***-4), 135.15 (+, Im-***C***-2), 142.78 (C_{quat}, Ph-***C***-1), 161.21 (C_{quat}, ***C***=N), 167.65 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3266 (N-H), 2972 (C-H), 2901 (C-H), 2160 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 297 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{16}H_{19}N_6$ $[M – H]^+$ 295.1671; found 295.1675. Anal. ($C_{16}H_{20}N_6 \cdot 0.9$ $C_2H_2O_4 \cdot 0.25$ H_2O) C, H, N. $C_{16}H_{20}N_6$ (296.37).

2-Cyano-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-(2-phenylethyl)guanidine (3.53)

The title compound was prepared from **3.25** (0.53 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.40 g, 72 %); mp (hydrogenoxalate) 149 – 151 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 2.78 – 2.92 (m, 4H, Im-4-C***H***$_2$ + Ph-C***H***$_2$), 3.38 – 3.50 (m, 4H, Im-4-CH_2-C***H***$_2$ + Ph-CH_2-C***H***$_2$), 7.14 – 7.32 (m, 6H, Ph-***H***, Im-5-***H***), 8.56 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 26.26 (-, Im-4-***C***H_2), 36.59 (-, Ph***C***H_2), 41.59 (-, Im-4-CH_2-***C***H_2), 44.26 (-, Ph-CH_2-***C***H_2), 117.71 (+, Im-***C***-5), 119.76 (C_{quat}, ***C***≡N), 127.61 (+, Ph-***C***-4), 129.65 (+, 2 Ph-***C***), 130.03 (+, 2 Ph-***C***), 133.19 (C_{quat}, Im-***C***-4), 135.21 (+, Im-***C***-2), 140.03 (C_{quat}, Ph-***C***-1), 161.16 (C_{quat}, ***C***=N), 167.73 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3256 (N-H), 2939 (C-H), 2854 (C-H), 2160 (C≡N), 1576 (C=N).

ES-MS (MeOH + NH_4OAc) *m/z* (%): 283 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{15}H_{18}N_6$ $[M^{+\bullet}]$ 282.1593; found 282.1588. Anal. ($C_{15}H_{18}N_6 \cdot 0.8\ C_2H_2O_4 \cdot 0.25\ H_2O$) C, H, N. $C_{15}H_{18}N_6$ (282.34).

2-Cyano-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-(4-phenylbutyl)guanidine (3.54)

The title compound was prepared from **3.26** (0.59 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.46 g, 74 %); mp (hydrogenoxalate) 138 – 140 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.47 – 1.69 (m, 4H, Ph-CH_2-C***H***$_2$-C***H***$_2$), 2.62 (t, 2H, ^3J = 7.3 Hz, Ph-C***H***$_2$), 2.93 (t, 2H, ^3J = 6.8 Hz, Im-4-C***H***$_2$), 3.19 (t, 2H, ^3J = 6.9 Hz, Ph-$(CH_2)_3$-C***H***$_2$), 3.51 (t, 2H, ^3J = 6.8 Hz, Im-4-CH_2-C***H***$_2$), 7.08 – 7.29 (m, 6H, Ph-***H***, Im-5-***H***), 8.57 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 26.31 (-, Im-4-***C***H_2), 29.75, 29.96 (-, Ph-CH_2-***C***H_2-***C***H_2), 36.51 (-, Ph-***C***H_2), 41.58 (-, Im-4-CH_2-***C***H_2), 42.70 (-, Ph-$(CH_2)_3$-***C***H_2), 117.73 (+, Im-***C***-5), 119.92 (C_{quat}, ***C***≡N), 126.90 (+, Ph-***C***-4), 129.43 (+, 2 Ph-***C***), 129.52 (+, 2 Ph-***C***), 133.16 (C_{quat}, Im-***C***-4), 135.15 (+, Im-***C***-2), 143.51 (C_{quat}, Ph-***C***-1), 161.22 (C_{quat}, ***C***=N), 167.44 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3257 (N-H), 2971 (C-H), 2930 (C-H), 2161 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 311 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{22}N_6$ $[M^{+\bullet}]$ 310.1906; found 310.1897. Anal. ($C_{17}H_{22}N_6 \cdot C_2H_2O_4$ $\cdot$ 0.25 H_2O) C, H, N. $C_{17}H_{22}N_6$ (310.40).

2-Cyano-1-(3,3-diphenylpropyl)-3-[2-(1*H*-imidazol-4-yl)ethyl]guanidine (3.55)

The title compound was prepared from **3.27** (0.71 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 95/3/2 v/v/v) yielding a colorless resin that solidified over Et_2O (0.54 g, 72 %); mp 178 – 180 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.23 – 2.34 (m, 2H, Ph_2CH-C***H***$_2$), 2.76 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 3.12 (t, 2H, ^3J = 7.3 Hz, Ph_2CH-CH_2-C***H***$_2$), 3.36 (t, 2H, ^3J = 7.2 Hz, Im-4-CH_2-C***H***$_2$), 3.97 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 6.84 (br s, 1H, Im-5-***H***), 7.10 – 7.31 (m, 10H, Ph-***H***), 7.55 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.07 (-, Im-4-***C***H_2), 36.01 (-, Ph_2CH-***C***H_2), 41.68 (-, Im-4-CH_2-***C***H_2), 42.67 (-, Ph_2CH-CH_2***C***H_2), 50.09 (+, Ph_2***C***H), 120.16 (C_{quat}, ***C***≡N), 127.41 (+, 2 Ph-***C***-4), 128.89 (+, 4 Ph-***C***), 129.62 (+, 4 Ph-***C***), 136.28 (+, Im-***C***-2), 145.84 (C_{quat}, 2 Ph-***C***-1), 161.19 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3314 (N-H), 2939 (C-H), 2841 (C-H), 2172 (C≡N), 1584 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 273 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{22}H_{24}N_6$ $[M^{+\bullet}]$ 372.2062; found 372.2056. Anal. ($C_{22}H_{24}N_6 \cdot 0.25\ H_2O$) C, H, N. $C_{22}H_{24}N_6$ (372.47).

2-Cyano-1-(3-cyclohexylpropyl)-3-[2-(1*H*-imidazol-4-yl)ethyl]guanidine (3.56)

The title compound was prepared from **3.28** (0.57 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.37 g, 60 %); mp (hydrogenoxalate) 160 – 162 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 0.80 – 0.99 (m, 2H, cHex-C***H***$_2$), 1.08 – 1.35 (m, 6H, cHex), 1.46 – 1.60 (m, 2H, cHex-CH_2-C***H***$_2$), 1.61 – 1.79 (m, 5H, cHex), 2.96 (t, 2H, ^3J = 6.8 Hz, Im-4-C***H***$_2$), 3.14 (t, 2H, ^3J = 7.3 Hz, cHex-$(CH_2)_2$-C***H***$_2$), 3.53 (t, 2H, ^3J = 6.8 Hz, Im-4-CH_2-C***H***$_2$), 7.30 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 8.68 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 26.19 (-, Im-4-***C***H_2), 27.53 (-, 2 cHex-***C***), 27.78 (-, ***C***H_2), 27.82 (-, ***C***H_2), 34.57 (-, 2 cHex-***C***), 35.60 (-, ***C***H_2), 38.79 (+, cHex-***C***), 41.49 (-, Im-4-CH_2-***C***H_2), 43.24 (-, cHex-$(CH_2)_2$-***C***H_2), 117.75 (+, Im-***C***-5), 119.91 (C_{quat}, ***C***≡N), 132.97 (C_{quat}, Im-***C***-4), 135.09 (+, Im-***C***-2), 161.20 (C_{quat}, ***C***=N), 167.13 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3277 (N-H), 2921 (C-H), 2848 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (MeOH + NH_4OAc) *m/z* (%): 303 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{16}H_{26}N_6$ $[M^{+\bullet}]$ 302.2219; found 302.2218. Anal. ($C_{16}H_{26}N_6 \cdot C_2H_2O_4$) C, H, N. $C_{16}H_{26}N_6$ (302.42).

2-Cyano-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-[2-(phenylthio)ethyl]guanidine (3.57)

The title compound was prepared from **3.29** (0.60 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.51 g, 82 %); mp (hydrogenoxalate) 133 – 135 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 2.94 (t, 2H, ^3J = 6.8 Hz, Im-4-C***H***$_2$), 3.10 (t, 2H, ^3J = 6.9 Hz, S-CH_2-C***H***$_2$), 3.40 (t, 2H, ^3J = 6.9 Hz, S-C***H***$_2$), 3.49 (t, 2H, ^3J = 6.8 Hz, Im-4-CH_2-C***H***$_2$), 7.15 – 7.23 (m, 1H, Ph-4-***H***), 7.25 – 7.33 (m, 3H, Ph-***H*** + Im-5-***H***), 7.35 – 7.42 (m, 2H, Ph-***H***), 8.71 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 25.95 (-, Im-4-***C***H_2), 33.61 (-, S-***C***H_2), 41.49 (-, Im-4-CH_2-***C***H_2), 42.28 (-, S-CH_2-***C***H_2), 117.79 (+, Im-***C***-5), 119.50 (C_{quat}, ***C***≡N), 127.47 (+, Ph-***C***-4), 130.23 (+, 2 Ph-***C***), 130.59 (+, 2 Ph-***C***), 132.70 (C_{quat}, Im-***C***-4), 135.08 (+, Im-***C***-2), 136.85 (C_{quat}, Ph-***C***-1), 161.16 (C_{quat}, ***C***=N), 166.73 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3246 (N-H), 2989 (C-H), 2900 (C-H), 2162 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 315 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{15}H_{18}N_6S$ $[M^{+\bullet}]$ 314.1314; found 314.1310. Anal. ($C_{15}H_{18}N_6S \cdot C_2H_2O_4$) C, H, N. $C_{15}H_{18}N_6S$ (314.41).

2-Cyano-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-[2-(1*H*-indol-3-yl)ethyl]guanidine (3.58)

The title compound was prepared from **3.30** (0.61 g, 2.0 mmol) and **3.51** (0.24 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by

flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless solid (0.41 g, 64 %); mp 78 – 82 °C. ^{1}H-NMR (600 MHz, NOESY, CD_3OD): δ [ppm] = 2.69 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 2.99 (t, 2H, ^3J = 7.1 Hz, indole-3-C***H***$_2$), 3.34 (t, 2H, ^3J = 7.3 Hz, Im-4-CH_2-C***H***$_2$), 3.48 (t, 2H, ^3J = 7.1 Hz, indole-3-CH_2-C***H***$_2$), 6.82 (brs, 1H, Im-5-***H***), 7.03 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz , ^4J = 1.0 Hz, indole-5-***H***), 7.08 – 7.12 (m, 2H, indole-6-***H*** + indole-2-***H***), 7.34 (ddd, 1H, ^3J = 8.1 Hz, ^4J = 1.0 Hz, ^5J = 1.0 Hz, indole-7-***H***), 7.57 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.2 Hz, ^5J = 1.0 Hz, indole-4-***H***), 7.59 (brs, 1H, Im-2-***H***). ^{13}C-NMR (150 MHz, HSQC, HMBC, CD_3OD): δ [ppm] = 26.33 (-, indole-3-***C***H_2), 27.88 (-, Im-4-***C***H_2), 42.58 (-, Im-4-CH_2-***C***H_2), 43.60 (-, indole-3-CH_2-***C***H_2), 112.32 (+, indole-***C***-7), 112.73 (C_{quat}, indole-***C***-3), 117.35 (+, Im-***C***-5), 119.28 (+, indole-***C***-4), 119.76 (+, indole-***C***-5), 120.19 (C_{quat}, ***C***≡N), 122.45 (+, indole-***C***-6), 123.77 (+, indole-***C***-2), 128.69 (C_{quat}, indole-***C***-3a), 136.21 (+, Im-***C***-2), 138.22 (C_{quat}, indole-***C***-7a), 161.21 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3253 (N-H), 2927 (C-H), 2847 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 322 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{19}N_7$ $[M^{+\bullet}]$ 321.1702; found 321.1698. Anal. ($C_{17}H_{22}N_6 \cdot 0.25\ H_2O$) C, H, N. $C_{17}H_{19}N_7$ (321.38).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-(3-phenylpropyl)guanidine (3.61)

The title compound was prepared from **3.24** (0.56 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.47 g, 72 %); mp (hydrogenoxalate) 134 – 137 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.52 – 1.77 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 1.78 – 1.92 (m, 2H, Ph-CH_2-C***H***$_2$), 2.63 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.74 (t, 2H, ^3J = 7.3 Hz, Ph-C***H***$_2$), 3.15 – 3.27 (m, 4H, Im-4-$(CH_2)_3$-C***H***$_2$ + Ph-$(CH_2)_2$-C***H***$_2$), 7.10 – 7.30 (m, 6H, Im-5-***H*** + Ph-***H***), 8.61 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 25.20 (-, Im-4-***C***H_2), 26.87 (-, Im-4-CH_2-***C***H_2), 29.84 (-, Im-4-$(CH_2)_2$-***C***H_2), 32.30 (-, Ph-CH_2-***C***H_2), 34.00 (-, Ph***C***H_2), 42.17 (-, Im-4-$(CH_2)_3$-***C***H_2), 42.43 (-, Ph-$(CH_2)_2$-***C***H_2), 116.87 (+, Im-***C***-5), 120.29 (C_{quat}, ***C***≡N), 127.05 (+, Ph-***C***-4), 129.47 (+, 2 Ph-***C***), 129.53 (+, 2 Ph-***C***), 134.80 (+, Im-***C***-2), 135.69 (C_{quat}, Im-***C***-4), 142.87 (C_{quat}, Ph-***C***-1), 161.22 (C_{quat}, ***C***=N), 167.65 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3250 (N-H), 2937 (C-H), 2857 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 325 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{24}N_6$ $[M^{+\bullet}]$ 324.2062; found 324.2055. Anal. ($C_{18}H_{24}N_6 \cdot 0.9\ C_2H_2O_4$) C, H, N. $C_{18}H_{24}N_6$ (324.42).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-(2-phenylethyl)guanidine (3.62)

The title compound was prepared from **3.25** (0.53 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless

foam-like solid (0.55 g, 88 %); mp (hydrogenoxalate) 142 – 144 °C. ^{1}H-NMR (400 MHz, CD_3OD, COSY): δ [ppm] = 1.45 – 1.56 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.56 – 1.67 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.58 (t, 2H, ^{3}J = 7.3 Hz, Im-4-C***H***$_2$), 2.82 (t, 2H, ^{3}J = 7.2 Hz, Ph-C***H***$_2$), 3.14 (t, 2H, ^{3}J = 7.1 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.42 (t, 2H, ^{3}J = 7.2 Hz, Ph-CH_2-C***H***$_2$), 6.76 (s, 1H, Im-5-***H***), 7.15 – 7.30 (m, 5H, Ph-***H***), 7.53 (d, 1H, ^{4}J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (100 MHz, CD_3OD, HSQC): δ [ppm] = 27.14 (-, Im-4-***C***H_2), 27.66 (-, Im-4-CH_2-***C***H_2), 29.86 (-, Im-4-$(CH_2)_2$-***C***H_2), 36.67 (-, Ph***C***H_2), 42.47 (-, Im-4-$(CH_2)_3$-***C***H_2), 44.15 (-, Ph-CH_2-***C***H_2), 117.74 (+, Im-***C***-5), 120.19 (C_{quat}, ***C***≡N), 127.51 (+, Ph-***C***-4), 129.56 (+, 2 Ph-***C***), 129.95 (+, 2 Ph-***C***), 135.73 (+, Im-***C***-2), 137.97 (C_{quat}, Im-***C***-4), 140.11 (C_{quat}, Ph-***C***-1), 161.13 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3245 (N-H), 2935 (C-H), 2857 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 311 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{22}N_6$ $[M^{+\bullet}]$ 310.1906; found 310.1912. Anal. ($C_{17}H_{22}N_6 \cdot 0.75\ C_2H_2O_4 \cdot 0.5\ H_2O$) C, H, N. $C_{17}H_{22}N_6$ (310.40).

2-Cyano-1-{1-[4-(1*H*-imidazol-4-yl)butyl]}-3-(4-phenylbutyl)guanidine (3.63)

The title compound was prepared from **3.26** (0.59 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.45 g, 66 %); mp (hydrogenoxalate) 125 – 128 °C. ^{1}H-NMR (600 MHz, CD_3OD, NOESY): δ [ppm] = 1.54 – 1.61 (m, 4H, Im-4-$(CH_2)_2$-C***H***$_2$ + Ph-$(CH_2)_2$-C***H***$_2$), 1.62 – 1.69 (m, 4H, Im-4-CH_2-C***H***$_2$ + Ph-CH_2-C***H***$_2$), 2.61 (t, 2H, ^{3}J = 7.4 Hz, Im-4-C***H***$_2$), 2.63 (t, 2H, ^{3}J = 7.5 Hz, Ph-C***H***$_2$), 3.18 – 3.24 (m, 4H, Im-4-$(CH_2)_3$-C***H***$_2$ + Ph-$(CH_2)_3$-C***H***$_2$), 6.77 (s, 1H, Im-5-***H***), 7.13 – 7.21 (m, 5H, Ph-***H***), 7.55 (d, 1H, ^{4}J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, CD_3OD, HSQC): δ [ppm] = 27.23 (-, Im-4-***C***H_2), 27.71 (-, Im-4-CH_2-***C***H_2), 29.73, 29.99, 30.05 (-, Im-4-$(CH_2)_2$-***C***H_2 + Ph-CH_2-***C***H_2-***C***H_2), 36.49 (-, Ph***C***H_2), 42.48, 42.54 (-, Im-4-$(CH_2)_3$-***C***H_2, Ph-$(CH_2)_3$-***C***H_2), 120.39 (C_{quat}, ***C***≡N), 126.83 (+, Ph-***C***-4), 129.37 (+, 2 Ph-***C***), 129.47 (+, 2 Ph-***C***), 135.74 (+, Im-***C***-2), 143.50 (C_{quat}, Ph-***C***-1), 161.19 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3266 (N-H), 2937 (C-H), 2858 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 339 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{19}H_{26}N_6$ $[M^{+\bullet}]$ 338.2219; found 338.2224. Anal. ($C_{19}H_{26}N_6 \cdot 0.85\ C_2H_2O_4$) C, H, N. $C_{19}H_{26}N_6$ (338.45).

2-Cyano-1-(3,3-diphenylpropyl)-3-[4-(1*H*-imidazol-4-yl)butyl]guanidine (3.64)

The title compound was prepared from **3.27** (0.71 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless resin that solidified over Et_2O (0.46 g, 57 %); mp 80 – 84 °C. ^{1}H-NMR (400 MHz, COSY, CD_3OD): δ [ppm] = 1.48 – 1.58 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.58 – 1.68 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.27 – 2.36 (m, 2H, Ph_2CH-C***H***$_2$), 2.59 (t, 2H, ^{3}J = 7.2 Hz, Im-4-C***H***$_2$), 3.10 – 3.19 (m,

4H, Im-4-$(CH_2)_3$-C***H***$_2$ + Ph_2CH-CH_2-C***H***$_2$), 3.99 (t, 1H, ^3J = 7.8 Hz, Ph_2C***H***), 6.76 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.11 – 7.19 (m, 2H, Ph-4-***H***), 7.23 – 7.30 (m, 8H, Ph-***H***), 7.52 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (100 MHz, HMQC, CD_3OD): δ [ppm] = 27.21 (-, Im-4-***C***H_2), 27.75 (-, Im-4-CH_2-***C***H_2), 29.96 (-, Im-4-$(CH_2)_2$-***C***H_2), 36.13 (-, Ph_2CH-***C***H_2), 41.70, 42.52 (-, Im-4-$(CH_2)_3$-***C***H_2 + Ph_2CH-CH_2-***C***H_2), 50.12 (+, Ph_2***C***H), 117.82 (+, Im-***C***-5), 120.39 (C_{quat}, ***C***≡N), 127.43 (+, 2 Ph-***C***-4), 128.91 (+, 4 Ph-***C***), 129.64 (+, 4 Ph-***C***), 135.79 (+, Im-***C***-2), 138.33 (C_{quat}, Im-***C***-4), 145.87 (C_{quat}, Ph-***C***-1), 161.21 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3277 (N-H), 2936 (C-H), 2858 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN + NH_4OAc) *m/z* (%): 401 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{24}H_{28}N_6$ $[M^{+\bullet}]$ 400.2375; found 400.2372. Anal. ($C_{24}H_{28}N_6$ · 0.25 H_2O) C, H, N. $C_{24}H_{28}N_6$ (400.52).

2-Cyano-1-(3-cyclohexylpropyl)-3-[4-(1*H*-imidazol-4-yl)butyl]guanidine (3.65)

The title compound was prepared from **3.28** (0.57 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.49 g, 75 %); mp (hydrogenoxalate) 141 – 143 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.80 – 0.99 (m, 2H, cHex-C***H***$_2$), 1.09 – 1.35 (m, 6H), 1.46 – 1.79 (m, 11H), 2.60 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.15 (t, 2H, ^3J = 7.2 Hz, cHex-$(CH_2)_2$-C***H***$_2$), 3.21 (t, 2H, ^3J = 6.8 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 6.77 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.54 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.23 (-, Im-4-***C***H_2), 27.54 (-, 2 cHex-***C***), 27.76 (-, Im-4-CH_2-***C***H_2), 27.83 (-, ***C***H_2), 27.90 (-, ***C***H_2), 30.07 (-, Im-4-$(CH_2)_2$-***C***H_2), 34.57 (-, 2 c-Hex-***C***), 35.61 (-, ***C***H_2), 38.79 (+, cHex-***C***), 42.48 (-, Im-4-$(CH_2)_3$-***C***H_2), 43.09 (-, cHex-$(CH_2)_2$-***C***H_2), 117.81 (+, Im-***C***-5), 120.44 (C_{quat}, ***C***≡N), 135.77 (+, Im-***C***-2), 138.00 (C_{quat}, Im-***C***-4), 161.19 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3265 (N-H), 2922 (C-H), 2848 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 331 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{30}N_6$ $[M^{+\bullet}]$ 330.2532; found 330.2533. Anal. ($C_{18}H_{30}N_6$ · 0.8 $C_2H_2O_4$ · 0.25 H_2O) C, H, N. $C_{18}H_{30}N_6$ (330.47).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[(2-phenylthio)ethyl]guanidine (3.66)

The title compound was prepared from **3.29** (0.60 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.42 g, 62 %); mp (hydrogenoxalate) 130 – 133 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.45 – 1.70 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.58 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.07 (t, 2H, ^3J = 7.0 Hz, S-CH_2-C***H***$_2$), 3.13 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.39 (t, 2H, ^3J = 7.0 Hz, S-C***H***$_2$), 6.76 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.10 – 7.41 (m, 5H, Ph-***H***), 7.53 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.17 (-, Im-4-***C***H_2),

27.67 (-, Im-4-CH_2-***C***H_2), 29.80 (-, Im-4-$(CH_2)_2$-***C***H_2), 33.53 (-, S-***C***H_2), 42.27 (-, Im-4-$(CH_2)_3$-***C***H_2), 42.61 (-, S-CH_2-***C***H_2), 117.88 (+, Im-***C***-5), 120.17 (C_{quat}, ***C***≡N), 127.30 (+, Ph-***C***-4), 130.16 (+, 2 Ph-***C***), 130.35 (+, 2 Ph-***C***), 135.76 (+, Im-***C***-2), 136.94 (C_{quat}, Ph-***C***-1), 138.10 (C_{quat}, Im-***C***-4), 161.02 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3267 (N-H), 2989 (C-H), 2901 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (MeCN + TFA) *m/z* (%): 343 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{22}N_6S$ $[M^{+\bullet}]$ 342.1627; found 342.1625. Anal. ($C_{17}H_{22}N_6S \cdot 0.75\ C_2H_2O_4$) C, H, N. $C_{17}H_{22}N_6S$ (342.46).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[2-(1*H*-indol-3-yl)ethyl]guanidine (3.67)

The title compound was prepared from **3.30** (0.61 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a beige foam-like solid (0.58 g, 83 %); mp (hydrogenoxalate) 154 – 156 °C. ^{1}H-NMR (400 MHz, COSY, CD_3OD): δ [ppm] = 1.33 – 1.44 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.48 – 1.60 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.52 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 2.97 (t, 2H, ^3J = 7.0 Hz, indole-3-C***H***$_2$), 3.06 (t, 2H, ^3J = 7.2 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.47 (t, 2H, ^3J = 7.0 Hz, indole-3-CH_2-C***H***$_2$), 6.73 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.00 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.05 – 7.10 (m, 2H, indole-6-***H*** + indole-2-***H***), 7.32 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.0 Hz, ^5J = 0.8 Hz, indole-7-***H***), 7.52 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.55 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.2 Hz, ^5J = 0.8 Hz, indole-4-***H***). ^{13}C-NMR (100 MHz, CD_3OD, HSQC): δ [ppm] = 26.37 (-, indole-3-***C***H_2), 27.07 (-, Im-4-***C***H_2), 27.60 (-, Im-4-CH_2-***C***H_2), 29.74 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.43 (-, Im-4-$(CH_2)_3$-***C***H_2), 43.59 (-, indole-3-CH_2-***C***H_2), 112.34 (+, indole-***C***-7), 112.73 (C_{quat}, indole-***C***-3), 117.85 (+, Im-***C***-5), 119.26 (+, indole-***C***-4), 119.79 (+, indole-***C***-5), 120.41 (C_{quat}, ***C***≡N), 122.47 (+, indole-***C***-6), 123.80 (+, indole-***C***-2), 128.68 (C_{quat}, indole-***C***-3a), 135.69 (+, Im-***C***-2), 138.09 (C_{quat}, Im-***C***-4), 138.20 (C_{quat}, indole-***C***-7a), 161.20 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3249 (N-H), 2936 (C-H), 2849 (C-H), 2158 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 350 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{19}H_{23}N_7$ $[M^{+\bullet}]$ 349.2015; found 349.2010. Anal. ($C_{19}H_{23}N_7 \cdot C_2H_2O_4$) calcd. C: 57.39, H: 5.73, N: 22.31, found C: 56.50, H: 6.37, N: 21.57. $C_{19}H_{23}N_7$ (349.43).

2-Cyano-1-[3-(4-fluorophenyl)propyl]-3-[4-(1*H*-imidazol-4-yl)butyl]guanidine (3.68)

The title compound was prepared from **3.31** (0.59 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a pale yellow foam-like solid (0.55 g, 80 %); mp (hydrogenoxalate) 132 – 134 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.49 – 1.73 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 1.76 – 1.90 (m, 2H, Ph-CH_2-C***H***$_2$), 2.54 – 2.67 (m, 4H, Im-4-C***H***$_2$ + Ph-C***H***$_2$), 3.12 – 3.26 (m, 4H, Im-4-$(CH_2)_3$-C***H***$_2$ +

Ph-$(CH_2)_2$-C***H***$_2$), 6.77 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 6.92 – 7.03 (m, 2H, Ph-***H***), 7.14 – 7.23 (m, 2H, Ph-***H***), 7.53 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.29 (-, Im-4-***C***H_2), 27.76 (-, Im-4-CH_2-***C***H_2), 30.01 (-, Im-4-$(CH_2)_2$-***C***H_2), 32.36 (-, 4-F-Ph-CH_2-***C***H_2), 33.15 (-, 4-F-Ph***C***H_2), 42.30 (-, Im-4-$(CH_2)_3$-***C***H_2), 42.53 (-, 4-F-Ph-$(CH_2)_2$-***C***H_2), 116.04 (+, ^2J = 21.3 Hz, 4-F-Ph-***C***-3,5), 120.34 (C_{quat}, ***C***≡N), 131.04 (+, ^3J = 7.8 Hz, 4-F-Ph-***C***-2,6), 135.78 (+, Im-***C***-2), 138.79 (C_{quat}, ^4J = 3.1 Hz, Ph-***C***-1), 161.22 (C_{quat}, ***C***=N), 162.83 (C_{quat}, ^1J = 271.7 Hz, 4-F-Ph-***C***-4). IR (cm^{-1}) = 3256 (N-H), 2970 (C-H), 2865 (C-H), 2158 (C≡N), 1576 (C=N), 1219 (C-F). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 343 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{23}FN_6$ [$M^{+\bullet}$] 342.1968; found 342.1969. Anal. ($C_{18}H_{23}FN_6 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N. $C_{18}H_{23}FN_6$ (342.41).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[3-(pyridin-2-yl)propyl]guanidine (3.69)

The title compound was prepared from **3.32** (0.56 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.59 g, 91 %); mp (hydrogenoxalate) 156 – 158 °C. ^{1}H-NMR (600 MHz, CD_3OD, COSY): δ [ppm] = 1.54 – 1.61 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.61 – 1.70 (m, 2H, Im-4-CH_2-C***H***$_2$), 1.90 – 1.97 (m, 2H, Pyr-2-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 2.81 (t, 2H, ^3J = 7.5 Hz, Pyr-2-C***H***$_2$), 3.21 (t, 2H, ^3J = 7.1 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.24 (t, 2H, ^3J = 7.0 Hz, Pyr-2-$(CH_2)_2$-C***H***$_2$), 6.76 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.24 (ddd, 1H, ^3J = 7.5 Hz, ^3J = 5.0 Hz, ^4J = 1.2 Hz, Pyr-5-***H***), 7.30 (ddd, 1H, ^3J = 7.8 Hz, ^4J = 1.2 Hz, ^5J = 1.0 Hz, Pyr-3-***H***), 7.53 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***), 7.74 (ddd, 1H, ^3J = 7.8 Hz, ^3J = 7.5 Hz, ^4J = 1.8 Hz, Pyr-4-***H***), 7.74 (ddd, 1H, ^3J = 5.0 Hz, ^4J = 1.8 Hz, ^5J = 1.0 Hz, Pyr-6-***H***). ^{13}C-NMR (150 MHz, CD_3OD, HSQC, HMBC): δ [ppm] = 27.14 (-, Im-4-***C***H_2), 27.72 (-, Im-4-CH_2-***C***H_2), 29.99 (-, Im-4-$(CH_2)_2$-***C***H_2), 30.48 (-, Pyr-2-***C***H_2), 35.61 (-, Pyr-2-CH_2-***C***H_2), 42.20 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 42.52 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.87 (+, Im-***C***-5), 120.24 (C_{quat}, ***C***≡N), 122.94 (+, Pyr-***C***-5), 124.73 (+, Pyr-***C***-3), 135.73 (+, Im-***C***-2), 138.20 (C_{quat}, Im-***C***-4), 138.83 (+, Pyr-***C***-4),149.64 (+, Pyr-***C***-6), 161.23 (C_{quat}, ***C***=N), 162.37 (C_{quat}, Pyr-***C***-2). IR (cm^{-1}) = 3242 (N-H), 2938 (C-H), 2857 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 326 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_7$ [$M^{+\bullet}$] 325.2015; found 325.2009. Anal. ($C_{17}H_{23}N_7$ · 2 $C_2H_2O_4 \cdot H_2O$) C, H, N. $C_{17}H_{23}N_7$ (325.41).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[3-(pyridin-3-yl)propyl]guanidine (3.70)

The title compound was prepared from **3.33** (0.56 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.34 g, 52 %); mp (hydrogenoxalate) 156 – 158 °C. ^{1}H-NMR (400 MHz,

CD_3OD, COSY): δ [ppm] = 1.52 – 1.61 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.61 – 1.70 (m, 2H, Im-4-CH_2-C***H***$_2$), 1.82 – 1.93 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 2.68 (t, 2H, ^3J = 7.8 Hz, Pyr-3-C***H***$_2$), 3.19 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.24 (t, 2H, ^3J = 7.1 Hz, Pyr-3-CH_2-CH_2-C***H***$_2$), 6.76 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.35 (ddd, 1H, ^3J = 7.9 Hz, ^3J = 4.9 Hz, ^5J = 0.9 Hz, Pyr-5-***H***), 7.53 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***), 7.70 (ddd, 1H, ^3J = 7.9 Hz, ^4J = 2.3 Hz, ^4J = 1.6 Hz, Pyr-4-***H***), 8.35 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.6 Hz, Pyr-6-***H***), 8.39 (dd, 1H, ^4J = 2.3 Hz, ^5J = 0.9 Hz, Pyr-2-***H***). ^{13}C-NMR (100 MHz, CD_3OD, HSQC, HMBC): δ [ppm] = 27.17 (-, Im-4-***C***H_2), 27.73 (-, Im-4-CH_2-***C***H_2), 29.94 (-, Im-4-$(CH_2)_2$-***C***H_2), 30.94 (-, Pyr-3-***C***H_2), 31.81 (-, Pyr-3-CH_2-***C***H_2), 42.17 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 42.52 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.72 (+, Im-***C***-5), 120.20 (C_{quat}, ***C***≡N), 125.22 (+, Pyr-***C***-5), 135.75 (+, Im-***C***-2), 138.24 (+, Pyr-***C***-4), 139.31 (C_{quat}, Pyr-***C***-3), 147.73 (+, Pyr-***C***-6), 150.06 (+, Pyr-***C***-2), 161.22 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3265 (N-H), 2931 (C-H), 2863 (C-H), 2160 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 326 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_7$ $[M^{+\bullet}]$ 325.2015; found 325.2014. Anal. ($C_{17}H_{23}N_7$ · 2 $C_2H_2O_4$ · 1.3 H_2O) C, H, N. $C_{17}H_{23}N_7$ (325.41).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[3-(pyridin-4-yl)propyl]guanidine (3.71)

The title compound was prepared from **3.34** (0.56 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.34 g, 52 %); mp (hydrogenoxalate) 155 – 157 °C. ^{1}H-NMR (400 MHz, CD_3OD, COSY): δ [ppm] = 1.51 – 1.61 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.61 – 1.70 (m, 2H, Im-4-CH_2-C***H***$_2$), 1.84 – 1.95 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 2.69 (t, 2H, ^3J = 7.8 Hz, Pyr-4-C***H***$_2$), 3.19 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.24 (t, 2H, ^3J = 7.1 Hz, Pyr-4-$(CH_2)_2$-C***H***$_2$), 6.77 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.28 (dd, 2H, ^3J = 4.5 Hz, ^5J = 1.6 Hz, Pyr-3,5-***H***), 7.53 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***), 8.39 (dd, 2H, ^3J = 4.5 Hz, ^5J = 1.6 Hz, Pyr-2,6-***H***). ^{13}C-NMR (100 MHz, CD_3OD, HSQC, HMBC): δ [ppm] = 27.17 (-, Im-4-***C***H_2), 27.72 (-, Im-4-CH_2-***C***H_2), 29.94 (-, Im-4-$(CH_2)_2$-***C***H_2), 31.02 (-, Pyr-4-CH_2-***C***H_2), 33.25 (-, Pyr-4-***C***H_2), 42.18 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 42.51 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.58 (+, Im-***C***-5), 120.18 (C_{quat}, ***C***≡N), 125.62 (+, Pyr-***C***-3,5), 135.75 (+, Im-***C***-2), 138.26 (C_{quat}, Im-***C***-4), 149.95 (+, Pyr-***C***-2,6), 153.69 (C_{quat}, Pyr-***C***-4), 161.20 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3244 (N-H), 2933 (C-H), 2864 (C-H), 2160 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 326 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_7$ $[M^{+\bullet}]$ 325.2015; found 325.2007. Anal. ($C_{17}H_{23}N_7$ · 2 $C_2H_2O_4$ · 2 H_2O) C, H, N. $C_{17}H_{23}N_7$ (325.41).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-(3-phenylbutyl)guanidine (3.72)

The title compound was prepared from **3.35** (0.59 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.53 g, 78 %); mp (hydrogenoxalate) 135 – 137 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.25 (d, 3H, ^3J = 6.9 Hz, PhC***H***$_3$CH), 1.54 – 1.60 (m, 2H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 1.78 – 1.90 (m, 2H, PhCH_3CH-C***H***$_2$), 2.59 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 2.68 – 2.83 (m, 1H, PhCH_3C***H***), 2.97 – 3.20 (m, 4H, PhCH_3CH-CH_2-C***H***$_2$ + Im-4-$(CH_2)_3$-C***H***$_2$), 6.76 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.11 – 7.32 (m, 5H, Ph-***H***), 7.53 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 23.09 (+, ***C***H_3), 27.21 (-, Im-4-***C***H_2), 27.74 (-, Im-4-CH_2-***C***H_2), 29.97 (-, Im-4-$(CH_2)_2$-***C***H_2), 38.58, (-, PhCH_3CH-***C***H_2), 38.93 (+, PhCH_3***C***H), 41.43 (-, PhCH_3CH-CH_2-***C***H_2), 42.48 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.69 (+, Im-***C***-5), 120.39 (C_{quat}, ***C***≡N), 127.32 (+, Ph-***C***-4), 128.05 (+, 2 Ph-***C***), 129.64 (+, 2 Ph-***C***), 135.77 (+, Im-***C***-2), 138.03 (C_{quat}, Im-***C***-4), 147.85 (C_{quat}, Ph-***C***-1), 161.14 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3250 (N-H), 2930 (C-H), 2866 (C-H), 2159 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 339 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{19}H_{26}N_6$ $[M^{+\bullet}]$ 338.2219; found 338.2210. Anal. ($C_{19}H_{26}N_6$ · 0.85 $C_2H_2O_4$) C, H, N. $C_{19}H_{26}N_6$ (338.45).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]guanidine (3.73)

The title compound was prepared from **3.36** (0.32 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 87.5/10.5/2 v/v/v) yielding a colorless foam-like solid (0.35 g, 85 %); mp (hydrogenoxalate) 172 – 174 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.46 – 1.74 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.61 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 3.17 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 6.80 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.62 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.05 (-, Im-4-***C***H_2), 27.71 (-, Im-4-CH_2-***C***H_2), 29.95 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.23 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.81 (+, Im-***C***-5), 120.22 (C_{quat}, ***C***≡N), 135.72 (+, Im-***C***-2), 137.99 (C_{quat}, Im-***C***-4), 161.13 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3312 (N-H), 2940 (C-H), 2858 (C-H), 2164 (C≡N), 1558 (C=N). ES-MS (MeCN + TFA) *m/z* (%): 207 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_9H_{14}N_6$ $[M^{+\bullet}]$ 206.1280; found 206.1281. Anal. ($C_9H_{14}N_6$ · $C_2H_2O_4$ · 0.1 H_2O) C, H, N. $C_9H_{14}N_6$ (206.25).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-methylguanidine (3.74)[8]

The title compound was prepared from **3.37** (0.35 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielding a colorless foam-like solid (0.34 g, 77 %); mp (hydrogenoxalate) 143 – 145 °C. ^{1}H-NMR (300 MHz,

CD_3OD): δ [ppm] = 1.49 – 1.73 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 2.77 (s, 3H, C***H***$_3$), 3.20 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 6.77 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.55 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.18 (-, Im-4-***C***H_2), 27.75 (-, Im-4-CH_2-***C***H_2), 28.73 (+, C***H***$_3$), 30.07 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.50 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.89 (+, Im-***C***-5), 120.32 (C_{quat}, ***C***≡N), 135.77 (+, Im-***C***-2), 138.21 (C_{quat}, Im-***C***-4), 161.99 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3277 (N-H), 2972 (C-H), 2901 (C-H), 2158 (C≡N), 1577 (C=N). ES-MS (MeCN + TFA) *m/z* (%): 221 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{10}H_{16}N_6$ [$M^{+\bullet}$] 220.1436; found 220.1439. Anal. ($C_{10}H_{16}N_6 \cdot C_2H_2O_4 \cdot 0.4\ H_2O$) C, H, N. $C_{10}H_{16}N_6$ (220.27).

2-Cyano-1-ethyl-3-[4-(1*H*-imidazol-4-yl)butyl]guanidine (3.75)

The title compound was prepared from **3.38** (0.38 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.39 g, 83 %); mp (hydrogenoxalate) 130 – 133 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.14 (t, 3H, ^3J = 7.2 Hz, C***H***$_3$), 1.50 – 1.72 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.16 – 3.27 (m, 4H, CH_3C***H***$_2$ + Im-4-$(CH_2)_3$-***C***H_2), 6.77 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.55 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 15.13 (+, ***C***H_3), 27.20 (-, Im-4-***C***H_2), 27.77 (-, Im-4-CH_2-***C***H_2), 30.04 (-, Im-4-$(CH_2)_2$-***C***H_2), 37.62 (-, ***C***H_2-CH_3), 42.51 (-, Im-4-$(CH_2)_3$-***C***H_2), 117.95 (+, Im-***C***-5), 120.48 (C_{quat}, ***C***≡N), 135.79 (+, Im-***C***-2), 138.15 (C_{quat}, Im-***C***-4), 161.10 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3264 (N-H), 2934 (C-H), 2858 (C-H), 2155 (C≡N), 1572 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 235 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{11}H_{18}N_6$ [$M^{+\bullet}$] 234.1593; found 234.1590. Anal. ($C_{11}H_{18}N_6 \cdot 0.75\ C_2H_2O_4$) C, H, N. $C_{11}H_{18}N_6$ (234.30).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-isopropylguanidine (3.76)

The title compound was prepared from **3.39** (0.41 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.39 g, 79 %); mp (hydrogenoxalate) 129 – 131 °C. ^{1}H-NMR (600 MHz, CD_3OD): δ [ppm] = 1.25 (d, 6H, ^3J = 6.5 Hz, C***H***$_3$), 1.46 – 1.69 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 1.62 – 1.69 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 3.22 (t, 2H, ^3J = 7.4 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.86 (sep, 1H, ^3J = 6.5 Hz, C***H***), 6.77 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.54 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 22.83 (+, ***C***H_3), 27.20 (-, Im-4-***C***H_2), 27.75 (-, Im-4-CH_2-***C***H_2), 30.02 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.50 (-, Im-4-$(CH_2)_3$-***C***H_2), 45.06 (+, ***C***H), 117.87 (+, Im-***C***-5), 120.42 (C_{quat}, ***C***≡N), 135.78 (+, Im-***C***-2), 138.15 (C_{quat}, Im-***C***-4), 160.37 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3241 (N-H), 2937 (C-H), 2860 (C-H),

2157 (C≡N), 1569 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 249 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{12}H_{20}N_6$ $[M^{+\bullet}]$ 248.1749; found 248.1746. Anal. ($C_{12}H_{20}N_6 \cdot 0.75\ C_2H_2O_4 \cdot 0.25\ H_2O$) C, H, N. $C_{12}H_{20}N_6$ (248.33).

2-Cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-isobutylguanidine (3.77)

The title compound was prepared from **3.40** (0.43 g, 2.0 mmol) and **3.60** (0.31 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielding a colorless foam-like solid (0.28 g, 53 %); mp (hydrogenoxalate) 140 – 143 °C. ^{1}H-NMR (600 MHz, CD_3OD, COSY): δ [ppm] = 0.90 (d, 6H, ^3J = 6.7 Hz, C***H***$_3$), 1.54 – 1.60 (m, 2H, Im-4-$(CH_2)_2$-***C***H_2), 1.62 – 1.69 (m, 2H, Im-4-CH_2-C***H***$_2$), 1.79 – 1.87 (m, 1H, C***H***), 2.60 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 2.99 (d, 2H, ^3J = 7.2 Hz, $(CH_3)_2$CHC***H***$_2$), 3.21 (t, 2H, ^3J = 7.1 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 6.76 (d, 1H, ^4J = 1.1 Hz, 1H, Im-5-***H***), 7.54 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, CD_3OD, HSQC): δ [ppm] = 20.83 (+, ***C***H_3), 27.15 (-, Im-4-***C***H_2), 27.70 (-, Im-4-CH_2-***C***H_2), 29.55 (+, ***C***H), 30.00 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.47 (-, Im-4-$(CH_2)_3$-***C***H_2), 50.07 (+, $(CH_3)_2$CH-***C***H_2)), 117.65 (+, Im-***C***-5), 120.36 (C_{quat}, ***C***≡N), 135.72 (+, Im-***C***-2), 138.10 (C_{quat}, Im-***C***-4), 161.28 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3280 (N-H), 2955 (C-H), 2871 (C-H), 2157 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%): 263 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{13}H_{22}N_6$ $[M^{+\bullet}]$ 262.1906; found 262.1906. Anal. ($C_{13}H_{22}N_6 \cdot 0.75\ C_2H_2O_4 \cdot 0.25\ H_2O$) C, H, N. $C_{13}H_{22}N_6$ (262.35).

2-Cyano-1-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-3-(3-phenylpropyl)-guanidine (3.80)

The title compound was prepared from **3.24** (0.56 g, 2.0 mmol) and **3.79**[8] (0.38 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 95/3/2 v/v/v) yielding a pale yellow foam-like solid (0.42 g, 61 %); mp (hydrogenoxalate) 148 – 150 °C. ^{1}H-NMR (300 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 1.79 – 1.93 (m, 2H, Ph-CH_2-C***H***$_2$), 2.34 (s, 3H, Im-5-C***H***$_3$), 2.58 – 2.69 (m, 4H, Ph-C***H***$_2$ + Im-4-CH_2-S-C***H***$_2$), 3.20 (t, 2H, ^3J = 7.1 Hz, Ph-$(CH_2)_2$-C***H***$_2$), 3.36 (t, 2H, ^3J = 7.1 Hz, Im-4-CH_2-S-CH_2-C***H***$_2$), 3.84 (s, 2H, Im-4-C***H***$_2$), 7.10 – 7.30 (m, 5H, Ph-***H***), 8.61 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, hydrogenoxalate): δ [ppm] = 9.12 (+, ***C***H_3), 24.74 (-, Im-4-***C***H_2), 31.99 (-, Im-4-CH_2-S-***C***H_2), 32.16, 33.96 (-, Ph***C***H_2-***C***H_2), 41.95 (-, Im-4-CH_2-S-CH_2-***C***H_2), 42.48 (-, Ph-$(CH_2)_2$-***C***H_2), 119.95 (C_{quat}, ***C***≡N), 127.08 (+, Ph-***C***-4), 127.86, 128.03 (C_{quat}, Im-***C***-4,5), 128.03 (+, 2 Ph-***C***), 129.54 (+, 2 Ph-***C***), 133.92 (+, Im-***C***-2), 142.80 (C_{quat}, Ph-***C***-1), 161.14 (C_{quat}, ***C***=N), 167.00 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3253 (N-H), 2989 (C-H), 2901 (C-H), 2161 (C≡N), 1576 (C=N). ES-MS (H_2O/MeCN) *m/z* (%):

357 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{24}N_6S$ $[M^{+\bullet}]$ 356.1783; found 356.1781. Anal. ($C_{18}H_{24}N_6S \cdot 0.85\ C_2H_2O_4 \cdot 0.25\ H_2O$) C, H, N. $C_{18}H_{24}N_6S$ (356.49).

2-Cyano-1-(3,3-diphenylpropyl)-3-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-guanidine (3.81)

The title compound was prepared from **3.27** (0.71 g, 2.0 mmol) and **3.79**[8] (0.38 g, 2.2 mmol) in MeCN (50 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/7 M NH_3 in MeOH 98/2 v/v) yielding a pale yellow foam-like solid (0.63 g, 73 %); mp (hydrogenoxalate) 144 – 146 °C. ^{1}H-NMR (400 MHz, COSY, CD_3OD, hydrogenoxalate): δ [ppm] = 2.29 – 2.38 (m, 5H, Ph_2CH-C***H***$_2$ + Im-5-C***H***$_3$), 2.60 (t, 2H, ^3J = 7.0 Hz, Im-4-CH_2-S-C***H***$_2$), 3.14 (t, 2H, ^3J = 7.2 Hz, Ph_2CH-CH_2-C***H***$_2$), 3.32 (t, 2H, ^3J = 7.0 Hz, Im-4-CH_2-S-CH_2-C***H***$_2$), 3.81 (s, 2H, Im-4-C***H***$_2$), 4.01 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 7.12 – 7.19 (m, 2H, Ph-4-***H***), 7.23 – 7.31 (m, 8H, Ph-***H***), 8.60 (s, 1H, Im-2-***H***). ^{13}C-NMR (100 MHz, HMQC, CD_3OD, hydrogenoxalate): δ [ppm] = 9.05 (+, Im-5-***C***H_3), 24.66 (-, Im-4-***C***H_2), 31.94 (-, Im-4-CH_2-S-***C***H_2), 35.84 (-, Ph_2CH-***C***H_2), 41.70 (-, Ph_2CH-CH_2-***C***H_2), 41.94 (-, Im-4-CH_2-S-CH_2-***C***H_2), 49.96 (+, Ph_2***C***H), 119.92 (C_{quat}, ***C***≡N), 127.45 (+, 2 Ph-***C***-4), 127.76, 128.01 (C_{quat}, Im-***C***-4,5), 128.89 (+, 4 Ph-***C***), 129.64 (+, 4 Ph-***C***), 133.87 (+, Im-***C***-2), 145.74 (C_{quat}, 2 Ph-***C***-1), 161.11 (C_{quat}, ***C***=N), 166.77 (C_{quat}, hydrogenoxalate). IR (cm^{-1}) = 3245 (N-H), 2927 (C-H), 2822 (C-H), 2161 (C≡N), 1576 (C=N). ES-MS (MeOH + NH_4OAc) *m/z* (%): 433 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{24}H_{28}N_6S$ $[M^{+\bullet}]$ 432.2096; found 432.2101. Anal. ($C_{24}H_{28}N_6S \cdot C_2H_2O_4 \cdot 0.5\ H_2O$) C, H, N. $C_{24}H_{28}N_6S$ (432.58).

3.4.1.4 Preparation of the carbamoylguanidines 3.49, 3.50, 3.59 and 3.78

General procedure

The pertinent cyanoguanidine was dissolved in 1 M HCl (25 mL) and left for 14 d at room temperature. After removing the solvent *in vacuo*, the crude product was purified by preparative HPLC, followed by flash chromatography under basic conditions to remove possible impurities with the corresponding guanidine.

2-Carbamoyl-1-[3-(1*H*-imidazol-4-yl)propyl]-3-(3-phenylpropyl)guanidine (3.49)

The title compound was prepared from **3.42** (0.20 g, 0.64 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) followed by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielded a colorless semisolid compound (0.20 g, 95 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.79 – 1.94 (m, 4H, Im-4-CH_2-C***H***$_2$ + Ph-CH_2-C***H***$_2$), 2.57 – 2.72 (m, 4H, Im-4-C***H***$_2$ + Ph-C***H***$_2$), 3.12 – 3.27 (m, 4H, Im-4-$(CH_2)_2$-C***H***$_2$ + Ph-CH_2-CH_2-C***H***$_2$), 6.79 (s, 1H, Im-5-***H***), 7.10 – 7.31 (m, 5H, Ph-***H***), 7.55 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 24.83 (-, Im-4-***C***H_2),

30.46 (-, Im-4-CH_2-***C***H_2) 32.34 (-, Ph-CH_2-***C***H_2), 34.05 (-, Ph-***C***H_2), 41.31, 41.38 (-, Im-4-$(CH_2)_2$-***C***H_2 + Ph-CH_2-CH_2-***C***H_2), 117.87 (+, Im-***C***-5), 127.02 (+, Ph-***C***-4), 129.50 (+, 2 Ph-***C***), 129.53 (+, 2 Ph-***C***), 135.93 (+, Im-***C***-2), 137.62 (C_{quat}, Im-***C***-4), 142.90 (C_{quat}, Ph-***C***-1), 160.96 (C_{quat}, ***C***=N), 169.32 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3206, 2933, 2864, 1570, 1397, 1369. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 329 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{17}H_{24}N_6O$ $[M^{+\bullet}]$ 328.2012; found 328.2088. $C_{17}H_{24}N_6O$ (328.41).

2-Carbamoyl-1-[3-(1*H*-imidazol-4-yl)propyl]-3-(4-phenylbutyl)guanidine (3.50)

The title compound was prepared from **3.44** (0.20 g, 0.62 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) followed by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielded a colorless semisolid compound (0.17 g, 80 %). ^{1}H-NMR (600 MHz, COSY, CD_3OD, trifluoroacetate): δ [ppm] = 1.62 – 1.74 (m, 4H, Ph-CH_2-C***H***$_2$-C***H***$_2$), 1.96 – 2.03 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.66 (t, 2H, ^3J = 7.7 Hz, Ph-C***H***$_2$), 2.80 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 3.31 (t, 2H, ^3J = 7.0 Hz, Ph-$(CH_2)_3$-C***H***$_2$), 3.38 (t, 2H, ^3J = 6.6 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.12 – 7.27 (m, 5H, Ph-***H***), 7.33 (brs, 1H, Im-5-***H***), 8.79 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, HSQC, HMBC, CD_3OD, trifluoroacetate): δ [ppm] = 22.56 (-, Im-4-***C***H_2), 28.59 (-, Im-4-CH_2-***C***H_2), 29.17, 29.52 (-, Ph-CH_2-***C***H_2-***C***H_2), 36.29 (-, Ph***C***H_2), 41.76 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.75 (-, Ph-$(CH_2)_3$-***C***H_2), 117.02 (+, Im-***C***-5), 126.98 (+, Ph-***C***-4), 129.43 (+, 2 Ph-***C***), 129.45 (+, 2 Ph-***C***), 134.35 (C_{quat}, Im-***C***-4), 134.96 (+, Im-***C***-2), 143.09 (C_{quat}, Ph-***C***-1), 154.55 (C_{quat}, ***C***=N), 156.86 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3192, 2934, 2858, 1723, 1576, 1594, 1399, 1370. ES-MS (H_2O/MeCN) *m/z* (%): 343 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{18}H_{26}N_6O$ $[M + H]^+$ 343.2241; found 343.2250. $C_{18}H_{26}N_6O$ (342.44).

2-Carbamoyl-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-(3-phenylpropyl)guanidine (3.59)

The title compound was prepared from **3.52** (0.41 g, 1.38 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 20/80) followed by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 90/8/2 v/v/v) yielded a colorless semisolid compound (0.35 g, 81 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.89 – 2.04 (m, 2H, Ph-CH_2-C***H***$_2$), 2.70 (t, 2H, ^3J = 7.6 Hz, Ph-C***H***$_2$), 3.07 (t, 2H, ^3J = 6.9 Hz, Im-4-C***H***$_2$), 3.24 – 3.37 (m, 2H, overlap with solvent, Ph-CH_2-CH_2-C***H***$_2$), 3.65 (t, 2H, ^3J = 6.9 Hz, Im-4-CH_2-C***H***$_2$), 7.14 – 7.32 (m, 5H, Ph-***H***), 7.43 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 8.84 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 25.27 (-, Im-4-***C***H_2), 31.20 (-, Ph-CH_2-***C***H_2), 33.69 (-, Ph-***C***H_2), 41.37 (-, Im-4-***C***H_2), 42.45 (-, Ph-$(CH_2)_2$-***C***H_2), 118.19 (+, Im-***C***-5), 127.34 (+, Ph-***C***-4), 129.47 (+, 2 Ph-***C***), 129.67 (+, 2 Ph-***C***), 131.56 (C_{quat}, Im-***C***-4), 135.37 (+, Im-***C***-2), 142.07 (C_{quat}, Ph-***C***-1), 154.71 (C_{quat}, ***C***=N), 156.86 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3153, 2930, 1662, 1603, 1181, 1128. ES-MS (H_2O/MeCN) *m/z* (%): 315

(100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{16}H_{22}N_6O$ $[M^{+\bullet}]$ 314.1855; found 314.1860. $C_{16}H_{22}N_6O$ (314.39).

2-Carbamoyl-1-[4-(1*H*-imidazol-4-yl)butyl]-3-(3-phenylpropyl)guanidine (3.78)

The title compound was prepared from **3.61** (0.48 g, 1.48 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 10/90, 20 min: 40/60) followed by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 92.5/5.5/2 v/v/v) yielded a colorless foam-like solid (0.29 g, 57 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.49 – 1.74 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 1.78 – 1.93 (m, 2H, Ph-CH_2-C***H***$_2$), 2.55 – 2.70 (m, 4H, Im-4-C***H***$_2$ + Ph-C***H***$_2$), 3.11 – 3.27 (m, 4H, Im-4-$(CH_2)_3$-C***H***$_2$ + Ph-$(CH_2)_2$-C***H***$_2$), 6.76 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.10 – 7.29 (m, 5H, Ph-***H***), 7.53 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.17 (-, Im-4-***C***H_2), 27.88 (-, Im-4-CH_2-***C***H_2), 30.14 (-, Im-4-$(CH_2)_2$-***C***H_2), 32.38 (-, Ph-CH_2-***C***H_2), 34.06 (-, Ph***C***H_2), 41.40, 41.69 (-, Im-4-$(CH_2)_3$-C***H***$_2$ + Ph-$(CH_2)_2$-C***H***$_2$), 117.61 (+, Im-***C***-5), 127.00 (+, Ph-***C***-4), 129.49 (+, 2 Ph-***C***), 129.53 (+, 2 Ph-***C***), 135.75 (+, Im-***C***-2), 142.95 (C_{quat}, Ph-***C***-1), 160.96 (C_{quat}, ***C***=N), 169.38 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3200, 2989, 2932, 1569, 1394, 1367. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 343 (100) $[M + H]^+$. HRMS (LSI-MS) calcd. for $C_{18}H_{27}N_6O$ $[M + H]^+$ 343.2241; found 343.2255. $C_{18}H_{26}N_6O$ (342.43).

3.4.1.5 Preparation of the diamine 3.84

4,15-Dioxo-8,11-dioxa-5,14-diazaoctadecane-1,18-diyldicarbamic acid dibenzyl ester (3.83)

4-(Benzyloxycarbonylamino)butanoic acid (8.54 g, 36.0 mmol) and CDI (6.42 g, 39.5 mmol) were dissolved in THF_{abs} (150 mL). After stirring for 1 h, **3.82** (2.67 g, 18.0 mmol) were added and stirred overnight. The precipitated product was filtered and washed with THF (2 x 15 mL) yielding a white solid (8.5 g, 80 %); mp 127 – 129 °C. ^{1}H-NMR (400 MHz, DMSO-d_6, COSY): δ [ppm] = 1.54 – 1.66 (m, 4H, CO-CH_2-C***H***$_2$), 2.06 (t, 4H, ^3J = 7.4 Hz, CO-C***H***$_2$), 2.93 – 3.01 (m, 4H, CO-$(CH_2)_2$-C***H***$_2$), 3.13 – 3.21 (m, 4H, CONH-C***H***$_2$), 3.38 (t, 4H, ^3J = 5.9 Hz, CONH-CH_2-C***H***$_2$), 3.48 (s, 4H, O-C***H***$_2$-C***H***$_2$-O), 4.99 (s, 4H, Ph-C***H***$_2$), 7.21 (t, 2H, ^3J = 5.5 Hz, N-***H***), 7.26 – 7.40 (m, 10H, Ph-***H***), 7.82 (t, 2H, ^3J = 5.5 Hz, N-***H***). ^{13}C-NMR (100 MHz, DMSO-d_6, HSQC): δ [ppm] = 25.70 (-, CO-CH_2-***C***H_2), 32.68 (-, CO-***C***H_2), 39.98 (-, CONH-***C***H_2), 40.21 (-, CO-$(CH_2)_2$-***C***H_2), 65.16 (-, Ph-***C***H_2), 69.13 (-, CONH-CH_2-***C***H_2), 69.51 (-, O-***C***H_2-***C***H_2-O), 127.74 (+, 6 Ph-***C***), 128.35 (+, 4 Ph-***C***), 137.24 (C_{quat}, Ph-***C***-1), 156.07 (C_{quat}, O-***C***=O), 171.81 (C_{quat}, CH_2***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 587 (100) $[M + H]^+$. Anal. ($C_{30}H_{42}N_4O_8$) C, H, N. $C_{30}H_{42}N_4O_8$ (586.68).

***N*,*N*'-(Ethylenedioxydiethyl)di-4-aminobutanamide (3.84)**

3.83 (7.2 g, 12.3 mmol) was hydrogenated over Pd/C (10 %) (0.72 g, cat.) in MeOH (150 mL) at room temperature for 12 h. The catalyst was removed by filtration over Celite and after evaporation of the solvent, a colorless semisolid compound was received that was used without further purification (3.8 g, 97 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.47 – 1.61 (m, 4H, CO-CH_2-C***H***$_2$), 2.08 (t, 4H, ^3J = 7.4 Hz, CO-C***H***$_2$), 2.42 – 2.53 (m, 4H, CO-$(CH_2)_2$-C***H***$_2$), 2.84 (brs, 4H, N-***H***), 3.12 – 3.23 (m, 4H, CONH-C***H***$_2$), 3.38 (t, 4H, ^3J = 5.9 Hz, CONH-CH_2-C***H***$_2$), 3.49 (s, 4H, O-C***H***$_2$-C***H***$_2$-O), 7.91 (t, 2H, ^3J = 5.0 Hz, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 29.38 (-, CO-CH_2-***C***H_2), 32.86 (-, CO-***C***H_2), 38.37 (-, CONH-***C***H_2), 41.09 (-, CO-$(CH_2)_2$-***C***H_2), 69.07 (-, CONH-CH_2-***C***H_2), 69.44 (-, O-***C***H_2-***C***H_2-O), 172.25 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 319 (100) $[M + H]^+$. $C_{14}H_{30}N_4O_4$ (318.41).

3.4.1.6 Preparation of the bivalent isourea precursors 3.88-3.91

General procedure

A solution of the primary amine (1 eq) and diphenyl cyanocarbonimidate (2 eq) in DCM or DCM/MeOH was stirred for 1 h at room temperature. After evaporation of the solvent, the product was crystallized from Et_2O.

1,1'-Dicyano-2,2'-diphenyl-3,3'-[*N*,*N*'-(ethylendioxydiethyl)di(aminocarbonylpropyl)]-diisourea (3.88)

The title compound was prepared from **3.84** (1.91 g, 6.0 mmol) and **3.6** (2.86 g, 12.0 mmol) in DCM/MeOH (60 mL 1/1 v/v) according to the general procedure yielding a white solid (3.5 g, 96 %); mp 155 – 158 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.66 – 1.75 (m, 2H, CO-CH_2-C***H***$_2$), 1.76 – 1.86 (m, 2H, CO-CH_2-C***H***$_2$), 2.09 (t, 2H, ^3J = 7.2 Hz, CO-C***H***$_2$), 2.16 (t, 2H, ^3J = 7.2 Hz, CO-C***H***$_2$), 3.14 – 3.22 (m, 6H, CO-$(CH_2)_2$-C***H***$_2$ + CONH-C***H***$_2$), 3.30 (t, 2H, ^3J = 6.7 Hz, CONH-C***H***$_2$), 3.35 – 3.41 (m, 4H, O-C***H***$_2$-C***H***$_2$-O), 3.44 – 3.51 (m, 4H, CONH-CH_2-C***H***$_2$), 7.12 – 7.33 (m, 6H, Ph-***H***), 7.37 – 7.50 (m, 4H, Ph-***H***), 7.87 – 7.95 (m, 2H, N-***H***), 8.62 (brs, 2H, N-***H***). ^{13}C-NMR (150 MHz, DMSO-d_6): δ [ppm] = 24.25, 25.17 (-, CO-CH_2-***C***H_2), 32.32, 32.35 (-, CO-***C***H_2), 38.51 (-, CONH-***C***H_2), 41.76, 42.10 (-, CO-$(CH_2)_2$-***C***H_2), 69.12 (-, CONH-CH_2-***C***H_2), 69.50 (-, O-***C***H_2-***C***H_2-O), 119.90, 121.72 (C_{quat}, ***C***≡N), 126.14 (+, Ph-***C***), 126.19 (+, Ph-***C***), 129.46 (+, Ph-***C***), 130.26 (+, Ph-***C***), 151.21 (C_{quat}, Ph-***C***-1), 151.57 (C_{quat}, Ph-***C***-1), 159.56 (C_{quat}, ***C***=N), 162.66 (C_{quat}, ***C***=N), 171.60 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3310 (N-H), 3198 (N-H), 2924, 2857, 2190 (C≡N), 1639 (C=N), 1444, 1420, 1200. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 607 (100) $[M + H]^+$. Anal. ($C_{30}H_{38}N_8O_6$ · 0.5 H_2O) C, H, N. $C_{30}H_{38}N_8O_6$ (606.67).

1,1'-Dicyano-2,2'-diphenyl-3,3'-hexamethylendiisourea (3.89)

The title compound was prepared from **3.85** (0.70 g, 6.0 mmol) and **3.6** (2.86 g, 12.0 mmol) in DCM/MeOH (60 mL 1/1 v/v) according to the general procedure yielding a white solid (2.2 g, 91 %); mp 172 – 173 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.21 – 1.73 (m, 8H, NH-CH_2-(C***H***$_2$)$_4$-CH_2-NH), 3.16 – 3.42 (m, 4H, C***H***$_2$NH), 7.11 – 7.53 (m, 10H, Ph-***H***), 8.54 (brs, 2H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 25.64 (-, NH-$(CH_2)_2$-(***C***H_2)$_2$-$(CH_2)_2$-NH), 27.92, 29.00 (-, NH-CH_2-***C***H_2-$(CH_2)_2$-***C***H_2-CH_2-NH), 41.91, 42.21 (-, ***C***H_2NH), 114.28, 114.70 (C_{quat}, ***C***≡N), 119.68, 121.60 (+, 4 Ph-***C***), 125.98, 126.12 (+, 2 Ph-***C***), 129.44, 130.17 (+, 4 Ph-***C***), 151.11, 151.61 (C_{quat}, 2 Ph-***C***), 159.42, 162.55 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3186 (N-H), 2936, 2187 (C≡N), 1635 (C=N), 1441, 1416, 1204. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 405 (100) $[M + H]^+$. Anal. ($C_{22}H_{24}N_6O_2 \cdot 0.25\ H_2O$) C, H, N. $C_{22}H_{24}N_6O_2$ (404.46).

1,1'-Dicyano-2,2'-diphenyl-3,3'-octamethylendiisourea (3.90)

The title compound was prepared from **3.86** (0.87 g, 6.0 mmol) and **3.6** (2.86 g, 12.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (2.5 g, 96 %); mp 150 – 152 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.17 – 1.40 (m, 8H, NH-$(CH_2)_2$-(C***H***$_2$)$_4$-$(CH_2)_2$-NH), 1.41 – 1.67 (m, 4H, NH-CH_2-C***H***$_2$-$(CH_2)_4$-C***H***$_2$-CH_2-NH), 3.13 – 3.34 (m, 4H, C***H***$_2$NH), 7.11 – 7.53 (m, 10H, Ph-***H***), 8.56 (brs, 2H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 25.64 (-, NH-$(CH_2)_3$-(***C***H_2)$_2$-$(CH_2)_3$-NH), 27.98, 29.01 (-, NH-CH_2-C***H***$_2$-$(CH_2)_4$-C***H***$_2$-CH_2-NH), 28.42 (-, NH-$(CH_2)_2$-C***H***$_2$-$(CH_2)_2$-C***H***$_2$-$(CH_2)_2$-NH), 41.92, 42.23 (-, ***C***H_2NH), 114.30, 114.71 (C_{quat}, ***C***≡N), 119.69, 121.56 (+, 4 Ph-***C***), 125.97, 126.11 (+, 2 Ph-***C***), 129.44, 130.17 (+, 4 Ph-***C***), 151.10, 151.59 (C_{quat}, 2 Ph-***C***), 159.41, 162.54 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3177 (N-H), 3068, 2930, 2193 (C≡N), 1627 (C=N), 1418, 1202. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 433 (100) $[M + H]^+$. Anal. ($C_{24}H_{28}N_6O_2$) C, H, N. $C_{24}H_{28}N_6O_2$ (432.52).

1,1'-Dicyano-2,2'-diphenyl-3,3'-decamethylendiisourea (3.91)

The title compound was prepared from **3.87** (1.03 g, 6.0 mmol) and **3.6** (2.86 g, 12.0 mmol) in DCM (50 mL) according to the general procedure yielding a white solid (2.54 g, 92 %); mp 155 – 157 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.15 – 1.40 (m, 12H, NH-$(CH_2)_2$-(C***H***$_2$)$_6$-$(CH_2)_2$-NH), 1.39 – 1.67 (m, 4H, NH-CH_2-C***H***$_2$-$(CH_2)_6$-C***H***$_2$-CH_2-NH), 3.13 – 3.42 (m, 4H, C***H***$_2$NH), 7.10 – 7.54 (m, 10H, Ph-***H***), 8.50 (brs, 2H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 25.99 (-, NH-$(CH_2)_4$-(***C***H_2)$_2$-$(CH_2)_4$-NH), 28.00, 29.00 (-, NH-CH_2-C***H***$_2$-$(CH_2)_6$-C***H***$_2$-CH_2-NH), 28.50, 28.80 (-, NH-$(CH_2)_2$-(C***H***$_2$)$_2$-$(CH_2)_2$-(C***H***$_2$)$_2$-$(CH_2)_2$-NH), 41.91, 42.25 (-, ***C***H_2NH), 114.30, 114.70 (C_{quat}, ***C***≡N), 119.66, 121.55 (+, 4 Ph-***C***), 125.94, 126.11 (+, 2 Ph-***C***), 129.43, 130.15 (+, 4 Ph-***C***), 151.10, 151.62 (C_{quat}, 2 Ph-***C***), 159.39, 162.53 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3198 (N-H), 2928, 2857, 2183 (C≡N), 1634 (C=N), 1417, 1201. ES-MS

(DCM/MeOH + NH_4OAc) *m/z* (%): 461 (100) $[M + H]^+$. Anal. ($C_{26}H_{32}N_6O_2$) C, H, N. $C_{26}H_{32}N_6O_2$ (460.57).

3.4.1.7 Preparation of the bivalent cyanoguanidines 3.92-3.95

General procedure

The pertinent isourea (1 eq) and **3.60** (2.2 eq) were refluxed in MeCN for 12 h. After removal of the solvent *in vacuo*, the crude product was purified by flash chromatography.

2,2'-Dicyano-3,3'-bis[4-(1*H*-imidazol-4-yl)butyl]-1,1'-[*N*,*N*'-(ethylendioxydiethyl)di-(aminocarbonylpropyl)]diguanidine (3.92)

The title compound was prepared from **3.88** (1.21 g, 2.0 mmol) and **3.60** (0.61 g, 4.4 mmol) in MeCN (120 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 85/13/2 v/v/v) yielding a colorless foam-like solid (0.63 g, 45 %). ^{1}H-NMR (400 MHz, CD_3OD, COSY): δ [ppm] = 1.53 – 1.72 (m, 8H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 1.76 – 1.86 (m, 4H, CO-CH_2-C***H***$_2$), 2.24 (t, 4H, ^3J = 7.2 Hz, CO-C***H***$_2$), 2.61 (t, 4H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 3.17 – 3.25 (m, 8H, Im-4-$(CH_2)_3$-C***H***$_2$ + CO-$(CH_2)_2$-C***H***$_2$), 3.35 (t, 4H, ^3J = 5.6 Hz, CONH-C***H***$_2$), 3.53 (t, 4H, ^3J = 5.6 Hz, CONH-CH_2-C***H***$_2$), 3.60 (s, 4H, O-C***H***$_2$-C***H***$_2$-O), 6.79 (d, 2H, ^4J = 1.1 Hz, Im-5-***H***), 7.58 (d, 2H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (100 MHz, CD_3OD, HSQC, HMBC): δ [ppm] = 26.51 (-, CO-CH_2-***C***H_2), 27.06 (-, Im-4-***C***H_2), 27.67 (-, Im-4-CH_2-***C***H_2), 29.91 (-, Im-4-$(CH_2)_2$-***C***H_2), 33.76 (-, CO-***C***H_2), 40.37 (-, CONH-***C***H_2), 42.18 (-, CO-$(CH_2)_2$-***C***H_2), 42.52 (-, Im-4-$(CH_2)_3$-***C***H_2), 70.60 (-, CONH-CH_2-***C***H_2), 71.32 (-, O-***C***H_2-***C***H_2-O), 117.90 (+, Im-***C***-5), 120.22 (C_{quat}, ***C***≡N), 135.72 (+, Im-***C***-2), 137.97 (C_{quat}, Im-***C***-4), 161.23 (C_{quat}, ***C***=N), 175.52 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3279 (N-H), 2927 (C-H), 2866 (C-H), 2159 (C≡N), 1646 (C=O), 1575 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 695 (100) $[M - H]^-$. HRMS (LSI-MS) calcd. for $C_{32}H_{53}N_{14}O_4$ $[M + H]^+$ 697.4369; found 697.4368. $C_{32}H_{52}N_{14}O_4$ (696.85).

2,2'-Dicyano-3,3'-bis[4-(1*H*-imidazol-4-yl)butyl]-1,1'-hexamethylendiguanidine (3.93)

The title compound was prepared from **3.89** (0.81 g, 2.0 mmol) and **3.60** (0.61 g, 4.4 mmol) in MeCN (120 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 85/13/2 v/v/v) yielding a colorless foam-like solid (0.58 g, 58 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.29 – 1.40 (m, 4H, NH-$(CH_2)_2$-(C***H***$_2$)$_2$-$(CH_2)_2$-NH), 1.46 – 1.72 (m, 12H, Im-4-CH_2-C***H***$_2$-C***H***$_2$ + NH-CH_2-C***H***$_2$-$(CH_2)_2$-C***H***$_2$-CH_2-NH), 2.61 (t, 4H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.13 – 3.26 (m, 8H, Im-4-$(CH_2)_3$-C***H***$_2$ + NH-C***H***$_2$-$(CH_2)_4$-C***H***$_2$-NH), 6.78 (d, 2H, ^4J = 1.1 Hz, Im-5-***H***), 7.59 (d, 2H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.14 (-, Im-4-***C***H_2), 27.53 (-, NH-$(CH_2)_2$-(***C***H_2)$_2$-$(CH_2)_2$-NH), 27.74 (-, Im-4-CH_2-***C***H_2), 30.04 (-, Im-4-$(CH_2)_2$-***C***H_2), 30.48 (-, NH-CH_2-

CH_2-$(CH_2)_2$-***C***H_2-CH_2-NH), 42.52 (-, Im-4-$(CH_2)_3$-***C***H_2), 42.72 (-, NH-***C***H_2-$(CH_2)_4$-***C***H_2-NH), 117.89 (+, Im-***C***-5), 120.48 (C_{quat}, ***C***≡N), 135.76 (+, Im-***C***-2), 138.07 (C_{quat}, Im-***C***-4), 161.19 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3249 (N-H), 2933 (C-H), 2857 (C-H), 2155 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 495 (100) $[M + H]^+$. HRMS (LSI-MS) calcd. for $C_{24}H_{39}N_{12}$ $[M + H]^+$ 495.3415; found 495.3406. $C_{24}H_{38}N_{12}$ (494.64).

2,2'-Dicyano-3,3'-bis[4-(1*H*-imidazol-4-yl)butyl]-1,1'-octamethylendiguanidine (3.94)

The title compound was prepared from **3.90** (0.87 g, 2.0 mmol) and **3.60** (0.61 g, 4.4 mmol) in MeCN (120 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 85/13/2 v/v/v) yielding a colorless foam-like solid (0.64 g, 62 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.26 – 1.39 (m, 8H, NH-$(CH_2)_2$-(C***H***$_2$)$_4$-$(CH_2)_2$-NH), 1.45 – 1.73 (m, 12H, Im-4-CH_2-C***H***$_2$-C***H***$_2$ + NH-CH_2-C***H***$_2$-$(CH_2)_4$-C***H***$_2$-CH_2-NH), 2.60 (t, 4H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.13 – 3.25 (m, 8H, Im-4-$(CH_2)_3$-C***H***$_2$ + NH-C***H***$_2$-$(CH_2)_6$-C***H***$_2$-NH), 6.77 (d, 2H, ^4J = 1.1 Hz, Im-5-***H***), 7.55 (d, 2H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.20 (-, Im-4-***C***H_2), 27.73, 27.75 (-, Im-4-CH_2-***C***H_2 + NH-$(CH_2)_2$-***C***H_2-$(CH_2)_2$-***C***H_2-$(CH_2)_2$-NH), 30.06 (-, Im-4-$(CH_2)_2$-***C***H_2), 30.33, 30.49 (-, NH-CH_2-***C***H_2-CH_2-(***C***H_2)$_2$-CH_2-***C***H_2-CH_2-NH), 42.50 (-, Im-4-$(CH_2)_3$-***C***H_2), 42.76 (-, NH-***C***H_2-$(CH_2)_6$-***C***H_2-NH), 117.85 (+, Im-***C***-5), 120.46 (C_{quat}, ***C***≡N), 135.77 (+, Im-***C***-2), 138.16 (C_{quat}, Im-***C***-4), 161.20 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3263 (N-H), 2971 (C-H), 2901 (C-H), 2155 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 523 (100) $[M + H]^+$. HRMS (LSI-MS) calcd. for $C_{26}H_{43}N_{12}$ $[M + H]^+$ 523.3728; found 523.3725. $C_{26}H_{42}N_{12}$ (522.69).

2,2'-Dicyano-3,3'-bis[4-(1*H*-imidazol-4-yl)butyl]-1,1'-decamethylendiguanidine (3.95)

The title compound was prepared from **3.91** (0.92 g, 2.0 mmol) and **3.60** (0.61 g, 4.4 mmol) in MeCN (120 mL) according to the general procedure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH/7 M NH_3 in MeOH 85/13/2 v/v/v) yielding a colorless foam-like solid (0.88 g, 80 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.22 – 1.40 (m, 12H, NH-$(CH_2)_2$-(C***H***$_2$)$_6$-$(CH_2)_2$-NH), 1.45 – 1.74 (m, 12H, Im-4-CH_2-C***H***$_2$-C***H***$_2$ + NH-CH_2-C***H***$_2$-$(CH_2)_6$-C***H***$_2$-CH_2-NH), 2.60 (t, 4H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.12 – 3.27 (m, 8H, Im-4-$(CH_2)_3$-C***H***$_2$ + NH-C***H***$_2$-$(CH_2)_8$-C***H***$_2$-NH), 6.77 (d, 2H, ^4J = 1.1 Hz, Im-5-***H***), 7.55 (d, 2H, ^4J = 1.1 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.22 (-, Im-4-***C***H_2), 27.76 (-, Im-4-CH_2-***C***H_2), 27.81 (-, NH-$(CH_2)_2$-***C***H_2-$(CH_2)_4$-***C***H_2-$(CH_2)_2$-NH), 30.07 (-, Im-4-$(CH_2)_2$-***C***H_2), 30.41, 30.52, 30.62 (-, NH-CH_2-***C***H_2-CH_2-(***C***H_2)$_4$-CH_2-***C***H_2-CH_2-NH), 42.52 (-, Im-4-$(CH_2)_3$-***C***H_2), 42.80 (-, NH-***C***H_2-$(CH_2)_8$-***C***H_2-NH), 117.90 (+, Im-***C***-5), 120.51 (C_{quat}, ***C***≡N), 135.78 (+, Im-***C***-2), 138.16 (C_{quat}, Im-***C***-4), 161.19 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3263 (N-H), 2972 (C-H), 2856 (C-H), 2156 (C≡N), 1576 (C=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 549 (100) $[M - H]^-$. HRMS (LSI-MS) calcd. for $C_{28}H_{47}N_{12}$ $[M + H]^+$ 551.4041; found 551.4034. $C_{28}H_{46}N_{12}$ (550.75).

3.4.1.8 Preparation of the trityl-protected sulfonylguanidines 3.100 and 3.101

1-(4-Tosyl)-2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.100)

A mixture of **3.96**[4] (0.41 g, 1.0 mmol) and NaH (60 % dispersion in mineral oil) (0.08 g, 2.0 mmol) in THF_{abs} (15 mL) was stirred 45 min at 50 °C. After cooling to 0 °C, subsequently NEt_3 (0.28 mL, 2.0 mmol) and **3.98** (0.19 g, 1.0 mmol) in THF_{abs} (15 mL) were added. Stirring was continued overnight at ambient temperature. After removing the solvent *in vacuo*, the crude product was purified by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 80/9/1 v/v/v) yielding a colorless foam-like solid (0.22 g, 39 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.73 – 1.94 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.36 (s, 3H, C***H***$_3$), 2.44 – 2.79 (m, 2H, Im-4-C***H***$_2$), 3.25 – 3.42 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 6.72 (s, 1H, Im-5-***H***), 7.06 – 7.15 (m, 6H, Ph-***H***), 7.19 (d, 2H, ^3J = 8.0 Hz, Ph-3,5-***H***), 7.30 – 7.45 (m, 10H, Ph-***H*** + Im-2-***H***), 7.74 (d, 2H, ^3J = 8.0 Hz, Ph-2,6-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 564 (100) $[M + H]^+$. $C_{33}H_{33}N_5O_2S$ (563.71).

1-(*tert*-Butoxycarbonyl)-2-(2-phenylethylsulfonyl)-1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.101)

To a solution of **3.97** (cf. Chapter 6, **6.30**) (0.51 g, 1.0 mmol) and DIEA (0.39 g, 3.0 mmol) in DCM (20 mL), **3.99** (0.21 g, 1.0 mmol) dissolved in DCM (10 mL) was added dropwise at 0 °C. After 1 h, stirring was continued overnight at room temperature and the solvent was evaporated. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielding the title compound as colorless oil (0.34 g, 50 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.47 (s, 3H, C(C***H***$_3$)$_3$), 1.49 (s, 6H, C(C***H***$_3$)$_3$), 1.86 – 1.91 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.51 – 2.63 (m, 2H, Im-4-C***H***$_2$), 3.04 – 3.17 (m, 2H, Ph-C***H***$_2$), 3.23 – 3.35 (m, 2H, Ph-CH_2-C***H***$_2$), 3.76 – 3.85 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 6.53 (d, 0.7H, ^4J = 1.3 Hz, Im-5-***H***), 6.55 (d, 0.3H, ^4J = 1.3 Hz, Im-5-***H***), 7.06 – 7.38 (m, 21H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 678 (100) $[M + H]^+$. $C_{39}H_{43}N_5O_4S$ (677.85).

3.4.1.9 Preparation of the sulfonylguanidines 3.102 and 3.103

1-[3-(1*H*-Imidazol-4-yl)propyl]-2-(4-tosyl)guanidine (3.102)

A mixture of **3.100** (0.19 g, 0.34 mmol) and TFA (5 mL) in DCM (20 mL) was stirred for 5 h. After evaporation of the solvent, the crude product was purified by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielding the title compound as colorless semisolid (0.12 g, 82 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.76 – 1.92 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.38 (s, 3H, C***H***$_3$), 2.70 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 3.23 (t, 2H, ^3J = 6.7 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.29 (d, 2H, ^3J = 8.3 Hz, Ph-3-***H***), 7.33 (s, 1H, Im-5-***H***), 7.72 (d, 2H, ^3J = 8.3 Hz, Ph-2-***H***), 8.76 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ

[ppm] = 21.46 (+, ***C***H_3), 22.35 (-, Im-4-***C***H_2), 29.64 (-, Im-4-CH_2-***C***H_2), 40.67 (-, Im-4-$(CH_2)_2$-***C***H_2), 117.02 (+, Im-***C***-5), 127.21 (+, 2 Ph-***C***-2), 130.41 (+, 2 Ph-***C***-3), 134.67 (+, Im-***C***-2), 134.85 (C_{quat}, Im-***C***-4), 141.99 (C_{quat}, Ph-***C***-1), 143.83 (C_{quat}, Ph-***C***-4), 158.70 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3159, 2989, 2901, 1663, 1624, 1548, 1166, 1128, 1081. HRMS (EI-MS) calcd. for $C_{14}H_{19}N_5O_2S$ [$M^{+\bullet}$] 321.1259; found 321.1257. $C_{14}H_{19}N_5O_2S$ · TFA (435.42).

1-[3-(1*H*-Imidazol-4-yl)propyl]-2-(2-phenylethylsulfonyl)guanidine (3.103)

A mixture of **3.101** (0.30 g, 0.44 mmol) and TFA (5 mL) in DCM (20 mL) was stirred for 5 h. After evaporation of the solvent, the crude product was purified by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielding the title compound as colorless semisolid (0.14 g, 71 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.82 – 1.96 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.76 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 3.01 – 3.11 (m, 2H, Ph-C***H***$_2$), 3.19 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.23 – 3.29 (m, 2H, Ph-CH_2-C***H***$_2$), 7.15 – 7.31 (m, 5H, Ph-***H***), 7.37 (s, 1H, ^4J = 1.3 Hz, Im-5-***H***), 8.76 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 22.42 (-, Im-4-***C***H_2), 29.48 (-, Im-4-CH_2-***C***H_2), 31.42 (-, Ph-***C***H_2), 40.63 (-, Im-4-$(CH_2)_2$-***C***H_2), 56.24 (-, Ph-CH_2-***C***H_2), 117.07 (+, Im-***C***-5), 127.66 (+, Ph-***C***-4), 129.44 (+, 2 Ph-***C***), 129.77 (+, 2 Ph-***C***), 134.70 (+, Im-***C***-2), 134.89 (C_{quat}, Im-***C***-4), 140.27 (C_{quat}, Ph-***C***-1), 158.95 (C_{quat}, ***C***=N). IR (cm^{-1}) = 3149, 2972, 2901, 1666, 1625, 1554, 1186, 1131, 1101. HRMS (EI-MS) calcd. for $C_{15}H_{21}N_5O_2S$ [$M^{+\bullet}$] 335.1416; found 335.1421. $C_{15}H_{21}N_5O_2S$ · TFA (449.45).

3.4.1.10 Preparation of 3-(1H-imidazol-4-yl)propan-1-amine 3.41

2-[3-(1-Trityl-1*H*-imidazol-4-yl)propyl]isoindoline-1,3-dione (3.105)[17]

To a cold solution (0 °C) of **3.104**[4, 15] (10.00 g, 27.1 mmol) in THF_{abs} (250 mL), phthalimide (6.00 g, 40.8 mmol) and triphenylphosphine (10.7 g, 40.8 mmol) were added. Under ice cooling DIAD (11.00 g, 54.2 mmol) in THF_{abs} (200 mL) was added dropwise. The mixture was allowed to warm to ambient temperature, stirred for 1 h and concentrated *in vacuo*. The precipitated product was filtered, washed twice with cold THF (20 mL) and recrystallized from THF/MeCN yielding the title compound as white solid (13.0 g, 96 %); mp 212 °C (ref.[17]: 217 – 219 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.93 – 2.09 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 3.73 (t, 2H, ^3J = 7.2 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.57 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.28 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***), 7.64 – 7.72 (m, 2H, Phth-***H***), 7.77 – 7.85 (m, 2H, Phth-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.03 (-, Im-4-CH_2-***C***H_2), 28.34 (-, Im-4-***C***H_2), 37.70 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.11 (C_{quat}, ***C***Ph_3), 118.04 (+, Im-***C***-5), 123.15 (+, Phth-***C***-4, 7), 127.95 (+, 3 Ph-***C***-4), 128.02 (+, 6 Ph-***C***), 129.84 (+, 6 Ph-***C***), 132.23 (C_{quat}, Im-***C***-4), 133.82 (+, Phth-***C***-5, 6), 138.39 (+, Im-***C***-2), 140.65 (C_{quat}, Phth-***C***-

3a, 7a), 142.61 (C_{quat}, 3 Ph-***C***-1), 168.39 (C_{quat}, 2 ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 498 (100) $[M + H]^+$. Anal. ($C_{33}H_{27}N_3O_2$) C, H, N. $C_{33}H_{27}N_3O_2$ (497.59).

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-amine (3.106)[49]

A mixture of **3.105** (12.80 g, 25.7 mmol) and hydrazine monohydrate (8.0 mL, 164.6 mmol) in *n*-butanol was refluxed for 1 h. After removal of insoluble material, the filtrate was evaporated giving a pale brownish oil that solidified (9.11 g, 96 %); mp 106 – 108 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.70 – 1.82 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.57 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 2.70 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.52 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.08 – 7.18 (m, 6H, Ph-***H***), 7.27 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 25.83 (-, Im-4-***C***H_2), 33.42 (-, Im-4-CH_2-***C***H_2), 41.85 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.09 (C_{quat}, ***C***Ph_3), 117.79 (+, Im-***C***-5), 127.97 (+, 3 Ph-***C***-4), 128.00 (+, 6 Ph-***C***), 129.81 (+, 6 Ph-***C***), 138.35 (+, Im-***C***-2), 141.45 (C_{quat}, Im-***C***-4), 142.63 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 368 (100) $[M + H]^+$. Anal. ($C_{25}H_{25}N_3 \cdot 0.5\ H_2O$) C, H, N. $C_{25}H_{25}N_3$ (367.49).

3-(1*H*-Imidazol-4-yl)propan-1-amine (3.41)

3.106 (8.00 g, 21.8 mmol) was heated under reflux in 1M HCl for 30 min. The cooled mixture was filtered and the solvent was removed *in vacuo.* The obtained residue was recrystallized from EtOH yielding a white solid (3.37 g, 78 %); mp (**3.41** · 2 HCl) 151 °C (ref.[50]: 155 – 157 °C). ^{1}H-NMR (300 MHz, D_2O, dihydrochloride): δ [ppm] = 1.89 – 2.03 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.76 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.96 (t, 2H, ^3J = 7.8 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.19 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 8.51 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, dihydrochloride): δ [ppm] = 20.93 (-, Im-4-***C***H_2), 25.62 (-, Im-4-CH_2-***C***H_2), 38.49 (-, Im-4-$(CH_2)_2$-***C***H_2), 115.57 (+, Im-***C***-5), 131.98 (C_{quat}, Im-***C***-4), 133.10 (+, Im-***C***-2). EI-MS (70 eV) *m/z* (%): 125 (11) $[M^{+\bullet}]$, 95 (100) $[M - CH_2\text{-}NH_2]^+$. Anal. ($C_6H_{11}N_3$ · 2 HCl) C, H, N. $C_6H_{11}N_3$ · 2 HCl (198.09).

3.4.1.11 Preparation of 4-(1H-imidazol-4-yl)butane-1-amine 3.60

2-(5-Oxohexyl)isoindoline-1,3-dione (3.108)

A mixture of **3.107** (25.00 g, 185.7 mmol), finely powdered K_2CO_3 (38.50 g, 277.8 mmol), phthalimide (27.32 g, 185.7 mmol) and catalytical amounts of KI (0.1 g, 0.6 mmol) in DMF was stirred for 12 h at 100 °C. The cooled mixture was poured in ice-cold water (1000 mL) and extracted with $CHCl_3$ (1 x 600 mL, 2 x 200 mL). The combined organic layers were washed with saturated $NaHCO_3$ (400 mL), 2 % HCl (2 x 400 mL), brine (400 mL) and dried over Na_2SO_4. Evaporation to dryness yielded a yellowish oil that crystallized from EtOH/H_2O as white solid (41.1 g, 91 %); mp 63 °C (ref.[51]: 57 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.54 – 1.77 (m, 4H, Phth-CH_2-C***H***$_2$-C***H***$_2$), 2.13 (s, 3H, CH_3), 2.49 (t, 2H, ^3J = 7.0 Hz, Phth-

$(CH_2)_3$-C***H***$_2$), 3.69 (t, 2H, ^3J = 6.9 Hz, Phth-C***H***$_2$), 7.67 – 7.75 (m, 2H, Phth-***H***), 7.79 – 7.87 (m, 2H, Phth-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 20.82 (-, ***C***H_2), 27.97 (-, ***C***H_2), 30.00 (+, ***C***H_3), 37.53 (-, ***C***H_2), 42.91 (-, ***C***H_2), 123.24 (+, Phth-***C***-4,7), 132.12 (C_{quat}, Phth-***C***-3a, 7a), 133.95 (+, Phth-***C***-5,6), 168.43 (C_{quat}, Phth-***C***-1,3), 208.38 (C_{quat}, ***C***OCH_3). CI-MS (NH_3) *m/z* (%): 264 (100) $[M + NH_4]^+$. Anal. ($C_{14}H_{15}NO_3$) C, H, N. $C_{14}H_{15}NO_3$ (245.27).

2-(6-Bromo-5-oxohexyl)isoindoline-1,3-dione (3.109)[52]

To a solution of **3.108** (93.30 g, 380.4 mmol) and urea (22.85 g, 380.4 mmol) in MeOH (400 mL), bromine (60.79 g, 380.4 mmol) was rapidly added and stirred for 24 h. The precipitated product was filtered, washed with MeOH (20 mL), dried and crystallized from DCM/hexane yielding the title compound as a white solid (50.7 g, 41 %); mp 112 – 113 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.58 – 1.79 (m, 4H, Phth-CH_2-C***H***$_2$-C***H***$_2$), 2.72 (t, 2H, ^3J = 6.9 Hz, Phth-$(CH_2)_3$-C***H***$_2$), 3.70 (t, 2H, ^3J = 6.8 Hz, Phth-C***H***$_2$), 3.88 (s, 2H, C***H***$_2$Br), 7.68 – 7.75 (m, 2H, Phth-***H***), 7.80 – 7.88 (m, 2H, Phth-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 20.89 (-, ***C***H_2), 27.81 (-, ***C***H_2), 34.20 (-, ***C***H_2), 37.36 (-, ***C***H_2), 38.98 (-, ***C***H_2), 123.28 (+, Phth-***C***-4,7), 132.10 (C_{quat}, Phth-***C***-3a, 7a), 133.99 (+, Phth-***C***-5,6), 168.42 (C_{quat}, Phth-***C***-1,3), 201.55 (C_{quat}, ***C***OCH_3). CI-MS (NH_3) *m/z* (%): 343 (13) $[M + NH_4]^+$, 341 (13) $[M + NH_4]^+$, 263 (100) $[M – Br + H + NH_4]^+$. Anal. ($C_{14}H_{14}BrNO_3$) C, H, N. $C_{14}H_{14}BrNO_3$ (324.17).

2-(4-(1*H*-Imidazol-4-yl)butyl)isoindoline-1,3-dione (3.110)

3.109 (50.00 g, 154.2 mmol) was stirred for 5 h at 160 °C in formamidine (300 mL). After removal of the solvent *in vacuo*, the residue was basified with saturated $NaHCO_3$ (400 mL) and extracted with $CHCl_3$ (1 x 600 mL, 2 x 200 mL). The combined organic layers were extracted with 5 % HCl (3 x 300 mL), the aqueous solution alkalized with $NaHCO_3$ and extracted with $CHCl_3$ (3 x 200 mL). After drying the combined organic layers over Na_2SO_4, the solvent was evaporated yielding a beige solid that was used with further purification (21.5 g, 52 %); mp 158 – 160 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.54 – 1.82 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.67 (t, 2H, ^3J = 6.9 Hz, Im-4-C***H***$_2$), 3.71 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 6.78 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.54 (d, 1H, ^4J = 0.9 Hz, Im-2-***H***), 7.67 – 7.88 (m, 4H, Phth-***H***). CI-MS (NH_3) *m/z* (%): 270 (100) $[M + H]^+$. $C_{15}H_{15}N_3O_2$ (269.30).

4-(1*H*-Imidazol-4-yl)butan-1-amine (3.60)[53]

3.110 (17.00 g, 63.1 mmol) was refluxed overnight in 20 % HCl (350 mL). Insoluble material was filtered off and the solvent was evaporated. The remaining dark oil was basified by addition of NH_3 (aq.) 32 %, evaporated to dryness and purified by flash chromatography (DCM/MeOH/NH_3 (aq.) 32 % 67/25/8 v/v/v) yielding the title compound as brownish oil (6.7 g, 76 %). For analytical purposes a small amount was converted to the dihydrobromide and

crystallized from EtOAc/EtOH (beige solid); mp (**3.60** · 2 HBr) 176 – 177 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dihydrobromide): δ [ppm] = 1.49 – 1.74 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.67 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 2.73 – 2.89 (m, 2H, Im-4-$(CH_2)_3$-C***H***$_2$), 7.48 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.82 (brs, 3H, N***H***$_3^+$), 9.09 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 14.14 (brs, 2H, Im-N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dihydrobromide): δ [ppm] = 23.05, 24.68, 25.90 (-, Im-4-***C***H_2-***C***H_2-***C***H_2), 38.22 (-, Im-4-$(CH_2)_3$-***C***H_2), 115.54 (+, Im-***C***-5), 132.73 (C_{quat}, Im-***C***-4), 133.62 (+, Im-***C***-2). CI-MS (NH_3) *m/z* (%): 140 (100) $[M + H]^+$. Anal. ($C_7H_{13}N_3$ · 2 HBr) C, H, N. $C_7H_{13}N_3$ (139.20).

3.4.1.12 Synthesis of the amines 3.11, 3.12 and 3.14-3.18

(*E*)-3-(4-Fluorophenyl)acrylonitrile (3.112)

To a solution of diethyl cyanomethylphosphonate (8.60 g, 48.5 mmol) in THF_{abs} (100 mL), NaH (60 % dispersion in mineral oil) (1.94 g, 48.5 mmol) was added in portions. After stirring for 1 h at ambient temperature, **3.111** (5.00 g, 40.3 mmol) in THF_{abs} (50 mL) was added dropwise and stirred for 5 h. The solvent was evaporated and the crude product purified by flash chromatography (PE/EtOAc 95/5 v/v) yielding a beige solid that was recrystallized from hexane (colorless needles, 3.9 g, 55 %); mp 66 – 68 °C (ref.[54]: 64 – 66 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 5.81 (dd, 1H, ^3J = 16.6 Hz, ^5J = 0.6 Hz, C***H***CN), 7.05 – 7.16 (m, 2H, Ph-***H***), 7.37 (d, 1H, ^3J = 16.6 Hz, PhC***H***), 7.41 – 7.50 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 96.17 (+, ^6J = 2.6 Hz, ***C***HCN), 116.42 (+, ^2J = 22.1 Hz, 4-F-Ph-***C***-3,5), 118.03 (C_{quat}, ***C***≡N), 129.43 (+, ^3J = 8.7 Hz, 4-F-Ph-***C***-2,6), 129.86 (C_{quat}, ^4J = 3.4 Hz, 4-F-Ph-***C***-1), 149.26 (+, Ph-***C***H), 164.63 (C_{quat}, ^1J = 253.1 Hz, 4-F-Ph-***C***-4). EI-MS (70 eV) *m/z* (%): 146 (100) $[M^{+\bullet}]$. Anal. (C_9H_6FN) C, H, N. C_9H_6FN (147.15).

3-(4-Fluorophenyl)propanenitrile (3.113)

To a solution of **3.112** (4.00 g, 26.8 mmol) in MeOH (150 mL), Pd/C (10%) (0.40 g, cat.) was added. After stirring for 6 h at room temperature under a hydrogen atmosphere, the catalyst was removed by filtration over Celite and the solvent was evaporated. The obtained yellowish oil was purified by distillation yielding a colorless oil (3.4 g, 84 %); bp (0.5 mbar) 80 °C (ref.[55] (0.05 mm): 73 – 76 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.60 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 2.93 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$), 6.98 – 7.08 (m, 2H, Ph-***H***), 7.17 – 7.24 (m, 2H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 19.61 (+, ^6J = 1.3 Hz, ***C***H_2CN), 30.83 (+, Ph-***C***H_2), 115.79 (+, ^2J = 22.1 Hz, 4-F-Ph-***C***-3,5), 118.98 (C_{quat}, ***C***≡N), 129.91 (+, ^3J = 8.1 Hz, 4-F-Ph-***C***-2,6), 133.76 (C_{quat}, ^4J = 3.3 Hz, 4-F-Ph-***C***-1), 162.06 (C_{quat}, ^1J = 245.6 Hz, 4-F-Ph-***C***-4). EI-MS (70 eV) *m/z* (%): 149 (17) $[M^{+\bullet}]$, 109 (100) $[M - CH_2CN]^+$. Anal. (C_9H_8FN · 0.1 H_2O) C, H, N. C_9H_8FN (149.16).

3-(4-Fluorophenyl)propan-1-amine (3.14)[56]

To a solution of **3.113** (3.30 g, 22.1 mmol) in anhydrous Et_2O (150 mL), $LiAlH_4$ (1.68 g, 44.2 mmol) was added in portions. After refluxing for 2 h, the mixture was cooled externally with ice and 1.7 mL H_2O, 1.7 mL NaOH 15 % and 5.1 mL H_2O were consecutively added. The insoluble material was removed by filtration, washed with Et_2O (2 x 50 mL) and the solvent of the combined organic layers was evaporated. The remaining oil was purified by distillation yielding a colorless oil (2.2 g, 65 %); bp (0.5 mbar) 50 – 54 °C. For analytical purposes a small amount was converted to the hydrochloride (addition of 5 – 6 M HCl in 2-propanol to a solution of **3.14** in EtOH) and recrystallized from TBME/EtOH (white solid); mp (**3.14** · 2 HCl) 142 – 144 °C (ref.[24]: 151 °C). ^{1}H-NMR (300 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 1.78 – 1.92 (m, 2H, C***H***$_2$CH$_2$NH$_3^+$), 2.64 (t, 2H, ^3J = 7.7 Hz, C***H***$_2$), 2.74 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$), 7.07 – 7.17 (m, 2H, Ph-***H***), 7.21 – 7.31 (m, 2H, Ph-***H***), 8.13 (brs, 3H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 28.72 (-, Ph-CH$_2$-***C***H$_2$), 30.87 (-, Ph-***C***H$_2$), 38.06 (-, C***H***$_2$NH$_3^+$), 114.99 (+, ^2J = 21.0 Hz, 4-F-Ph-***C***-3,5), 129.95 (+, ^3J = 7.9 Hz, 4-F-Ph-***C***-2,6), 136.94 (C$_{quat}$, ^4J = 3.1 Hz, 4-F-Ph-***C***-1), 160.63 (C$_{quat}$, ^1J = 241.3 Hz, 4-F-Ph-***C***-4). EI-MS (70 eV) *m/z* (%): 153 (10) [M$^{+\bullet}$], 44 (100). Anal. (C$_9$H$_{12}$FN · HCl) C, H, N. C$_9$H$_{12}$FN (153.20).

3-Phenylbut-2-enenitrile (3.115)

To a solution of diethyl cyanomethylphosphonate (14.2 g, 80 mmol) in THF$_{abs}$ (100 mL), NaH (60 % dispersion in mineral oil) (3.2 g, 40 mmol) was added in portions. After stirring for 1 h at ambient temperature, **3.114** (8.0 g, 67 mmol) in THF$_{abs}$ (50 mL) was added dropwise and stirred for 5 h. The solvent was evaporated and the obtained oil was purified by distillation yielding a colorless oil (7.6 g, 79 %); bp (0.5 mbar) 78 – 80 °C (ref.[24] bp$_{0.05}$ 120 °C). ^{1}H-NMR (300 MHz, CDCl$_3$): δ [ppm] = 2.48 (d, 3H, ^4J = 1.1 Hz, C***H***$_3$), 5.62 (q, 1H, ^4J = 1.1 Hz, C***H***CN), 7.37 – 7.50 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, CDCl$_3$): δ [ppm] = 20.27 (+, ***C***H$_3$), 95.59 (+, ***C***HCN), 117.69 (C$_{quat}$, ***C***≡N), 125.90 (+, 2 Ph-***C***), 128.88 (+, 2 Ph-***C***), 130.31 (+, Ph-***C***), 138.26 (C$_{quat}$, Ph-***C***), 159.83 (C$_{quat}$, ***C***CH$_3$). EI-MS (70 eV) *m/z* (%): 143 (100) [M$^{+\bullet}$]. C$_{10}$H$_9$N (143.19).

3-Phenylbut-2-en-1-amine (3.116)

To a solution of **3.115** (3.8 g, 26.5 mmol) in anhydrous Et_2O (50 mL), $LiAlH_4$ (0.52 g, 13.3 mmol) was added in portions. After refluxing for 2 h, the mixture was cooled externally with ice and 0.6 mL H_2O, 0.6 mL NaOH 15 % and 2.4 mL H_2O were consecutively added. The insoluble material was removed by filtration, washed with Et_2O (2 x 20 mL) and the solvent of the combined organic layers was evaporated. The remaining oil was purified by distillation (bp (0.5 mbar) 75 – 77 °C) yielding a colorless oil which was converted to the hydrochloride (addition of 5 – 6 M HCl in 2-propanol to a solution of **3.116** in EtOH) and recrystallized from

EtOH/Et_2O yielding a white solid (2.4 g, 46 %); mp (**3.116** · HCl) 216 – 217 °C (ref.[24]: 214 °C). ^{1}H-NMR (300 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 2.06 (d, 3H, ^4J = 1.2 Hz, C***H***$_3$), 3.62 (d, 2H, ^3J = 7.0 Hz, C***H***$_2$), 5.81 – 5.90 (m, 1H, C***H***), 7.26 – 7.46 (m, 5H, Ph-***H***), 8.30 (brs, 3H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 15.86 (+, ***C***H$_3$), 36.95 (-, ***C***H$_2$), 119.86 (-, ***C***H), 125.47 (+, 2 Ph-***C***), 127.59 (+, 1 Ph-***C***), 128.37 (+, 2 Ph-***C***), 139.46 (C_{quat}, ***C***=CH), 141.73 (C_{quat}, Ph-***C***-1). CI-MS (NH_3) *m/z* (%): 148 (100) $[M + H]^+$. Anal. ($C_{10}H_{13}N$ · HCl) C, H, N. $C_{10}H_{13}N$ · HCl (183.68).

3-Phenylbutan-1-amine (3.18)[24]

To a solution of **3.116** · HCl (2.30 g, 12.5 mmol) in EtOH (100 mL), Pd/C (10%) (0.23 g, cat.) was added. After stirring for 6 h at room temperature under a hydrogen atmosphere, the catalyst was removed by filtration over Celite and the solvent was evaporated. The residue was dissolved in H_2O (20 mL) and dried by lyophilization yielding a white solid (2.2 g, 95 %); mp (**3.18** · HCl) 128 – 129 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 1.21 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$), 1.77 – 1.90 (m, 2H, PhCH$_3$CH-C***H***$_2$), 2.44 – 2.74 (m, 2H, C***H***$_2$-NH_3^+), 2.74 – 2.89 (m, 1H, PhCH$_3$C***H***), 7.16 – 7.37 (m, 5H, Ph-***H***), 8.02 (brs, 3H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 21.93 (+, ***C***H$_3$), 34.98 (-, PhCH$_3$CH-***C***H$_2$), 36.30 (+, PhCH$_3$***C***H), 37.23 (-, ***C***H$_2$$NH_3^+$), 126.17 (-, Ph-***C***-4), 126.70 (-, 2 Ph-***C***), 128.40 (-, 2 Ph-***C***), 145.74 (C_{quat}, Ph-***C***-1). CI-MS (NH_3) *m/z* (%): 150 (100) $[M + H]^+$. Anal. ($C_{10}H_{15}N$ · HCl) C, H, N. $C_{10}H_{15}N$ · HCl (185.69).

General procedure for the synthesis the 2-[3-(pyridyl)propyl]isoindoline-1,3-diones (3.120-3.122)

To a cold solution (0 °C) of the pertinent 3-pyridylpropan-1-ol (5.0 g, 36.4 mmol) in THF_{abs} (100 mL), phthalimide (5.9 g, 40.1 mmol) and triphenylphosphine (10.52 g, 40.1 mmol) were added. Under external cooling with ice, DIAD (8.11 g, 40.1 mmol) in THF_{abs} (50 mL) was added dropwise. The mixture was allowed to warm and stirred overnight at ambient temperature. After concentration *in vacuo*, the crude product was subjected to flash chromatography. For analytical purposes a small amount of the purified product was converted into the picrate by addition of a saturated solution of picric acid in EtOH and recrystallized from EtOH/H_2O.

2-[3-(Pyridin-2-yl)propyl]isoindoline-1,3-dione (3.120)[23]

The title compound was prepared from **3.117** according to the general procedure and purified by flash chromatography (PE/EtOAc 70/30 v/v) yielding a brownish oil (7.3 g, 75 %); mp (**3.120** · $C_6H_3N_3O_7$) 139 – 140 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 2.00 – 2.13 (m, 2H, Pyr-2-CH$_2$-C***H***$_2$), 3.04 (t, 2H, ^3J = 8.0 Hz, Pyr-2-C***H***$_2$), 3.67 (t, 2H, ^3J = 6.5 Hz,

Pyr-2-$(CH_2)_2$-C***H***$_2$), 7.81 – 7.92 (m, 5H, Pyr-5-***H*** + Phth-***H***), 7.98 (d, 1H, ^3J = 8.0 Hz, Pyr-3-***H***), 8.44 – 8.52 (m, 1H, Pyr-4-***H***), 8.58 (s, 2H, picrate-***H***), 8.79 (dd, 1H, ^3J = 5.8 Hz, ^4J = 1.7 Hz, Pyr-6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 27.39 (-, Pyr-2-CH_2-***C***H_2), 30.49 (-, Pyr-2-***C***H_2), 36.64 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 122.95 (-, Phth-***C***-4,7), 124.08 (C_{quat}, picrate-***C***-4), 124.47 (+, Pyr-***C***-5), 125.10 (+, picrate-***C***-3,5), 126.56 (+, Pyr-***C***-3), 131.58 (C_{quat}, Phth-***C***-3a,7a), 134.34 (+, Phth-***C***-5,6), 141.75 (C_{quat}, picrate-***C***-2,6), 141.85 (+, Pyr-***C***-6), 145.73 (+, Pyr-***C***-4), 156.28 (C_{quat}, Pyr-***C***-2), 160.69 (C_{quat}, picrate-***C***-1), 167.91 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 267 (100) $[M + H]^+$. Anal. ($C_{16}H_{14}N_2O_2 \cdot C_6H_3N_3O_7$) C, H, N. $C_{16}H_{14}N_2O_2$ (266.29).

2-[3-(Pyridin-3-yl)propyl]isoindoline-1,3-dione (3.121)[23]

The title compound was prepared from **3.118** according to the general procedure and purified by flash chromatography (PE/EtOAc 60/40 v/v) yielding a pale yellow solid (5.4 g, 56 %); mp (**3.121** · $C_6H_3N_3O_7$) 145 – 146 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.91 – 2.06 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.85 (t, 2H, ^3J = 7.9 Hz, Pyr-3-C***H***$_2$), 3.64 (t, 2H, ^3J = 6.7 Hz, Pyr-3-$(CH_2)_2$-C***H***$_2$), 7.80 – 7.91 (m, 4H, Phth-***H***), 7.99 (dd, 1H, ^3J = 8.0 Hz, ^3J = 5.7 Hz, Pyr-5-***H***), 8.52 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.9 Hz, ^4J = 1.4 Hz, Pyr-4-***H***), 8.58 (s, 2H, picrate-***H***), 8.77 (dd, 1H, ^3J = 5.7 Hz, ^4J = 1.4 Hz, Pyr-6-***H***), 8.84 (d, 1H, ^4J = 1.9 Hz, Pyr-2-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 28.76 (-, Pyr-3-CH_2-***C***H_2), 28.99 (-, Pyr-3-***C***H_2), 36.72 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 122.92 (-, Phth-***C***-4,7), 124.06 (C_{quat}, picrate-***C***-4), 125.10 (+, picrate-***C***-3,5), 126.54 (+, Pyr-***C***-5), 131.60 (C_{quat}, Phth-***C***-3a,7a), 134.29 (+, Phth-***C***-5,6), 139.99 (+, Pyr-***C***-6), 141.03 (C_{quat}, Pyr-***C***-3), 141.71 (+, Pyr-***C***-2), 141.76 (C_{quat}, picrate-***C***-2,6), 145.60 (+, Pyr-***C***-4), 160.70 (C_{quat}, picrate-***C***-1), 167.94 (C_{quat}, ***C***=O). ES-MS (H_2O/MeCN) *m/z* (%): 267 (100) $[M + H]^+$. Anal. ($C_{16}H_{14}N_2O_2 \cdot C_6H_3N_3O_7$) C, H, N. $C_{16}H_{14}N_2O_2$ (266.29).

2-[3-(Pyridin-4-yl)propyl]isoindoline-1,3-dione (3.122)[23]

The title compound was prepared from **3.119** according to the general procedure and purified by flash chromatography (PE/EtOAc 60/40 v/v) yielding a beige solid (7.4 g, 90 %); mp (**3.122** · $C_6H_3N_3O_7$) 180 – 182 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, picrate): δ [ppm] = 1.95 – 2.07 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.95 (t, 2H, ^3J = 7.8 Hz, Pyr-4-C***H***$_2$), 3.64 (t, 2H, ^3J = 6.7 Hz, Pyr-4-$(CH_2)_2$-C***H***$_2$), 7.80 – 7.90 (m, 4H, Phth-***H***), 7.98 (dd, 2H, ^3J = 6.7 Hz, ^4J = 1.5 Hz, Pyr-3,5-***H***), 8.57 (s, 2H, picrate-***H***), 8.81 (dd, 2H, ^3J = 6.7 Hz, ^4J = 1.5 Hz, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, picrate): δ [ppm] = 27.99 (-, Pyr-4-CH_2-***C***H_2), 32.33 (-, Pyr-4-***C***H_2), 36.77 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 122.90 (-, Phth-***C***-4,7), 124.07 (C_{quat}, picrate-***C***-4), 125.11 (+, picrate-***C***-3,5), 126.79 (+, Pyr-***C***-3,5), 131.59 (C_{quat}, Phth-***C***-3a,7a), 134.26 (+, Phth-***C***-5,6), 141.36 (+, Pyr-***C***-2,6), 141.74 (C_{quat}, picrate-***C***-2,6), 160.70 (C_{quat}, picrate-***C***-1), 162.35 (C_{quat}, Pyr-***C***-

4), 167.94 (C_{quat}, 2 ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 267 (100) $[M + H]^+$. Anal. ($C_{16}H_{14}N_2O_2 \cdot C_6H_3N_3O_7$) C, H, N. $C_{16}H_{14}N_2O_2$ (266.29).

General procedure for the preparation of the 3-pyridylpropan-1-amines (3.15-3.17)

A mixture of the pertinent 2-(3-pyridylpropyl)isoindoline-1,3-dione (1 eq) and hydrazine monohydrate (6 eq) in EtOH was stirred overnight at room temperature. After removal of insoluble material and concentration *in vacuo*, the crude product was subjected to flash chromatography. For analytical purposes a small amount of the purified product was converted into the dipicrate by addition of a saturated solution of picric acid in EtOH and recrystallized from $EtOH/H_2O$.

3-(Pyridin-2-yl)propan-1-amine (3.15)[23]

The title compound was prepared from **3.120** (7.00 g, 26.3 mmol) and hydrazine monohydrate (7.7 mL, 157.8 mmol) in EtOH (200 mL) according to the general procedure and purified by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 80/18/2 v/v/v) yielding a brownish oil (3.6 g, 85 %); mp (**3.15** · 2 $C_6H_3N_3O_7$) 188 – 190 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.91 – 2.04 (m, 2H, Pyr-2-CH_2-C***H***$_2$), 2.79 – 2.93 (m, 2H, Pyr-2-$(CH_2)_2$-C***H***$_2$), 3.03 (t, 2H, ^3J = 7.7 Hz, Pyr-2-C***H***$_2$), 7.72 (brs, 3H, N***H***$_3^+$), 7.82 – 7.93 (m, 2H, Pyr-3,5-***H***), 8.40 – 8.49 (m, 1H, Pyr-4-***H***), 8.59 (s, 2H, picrate-***H***), 8.82 (dd, 1H, ^3J = 5.7 Hz, ^4J = 1.7 Hz, Pyr-6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 26.21 (-, Pyr-2-CH_2-***C***H_2), 30.28 (-, Pyr-2-***C***H_2), 38.03 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 124.17 (C_{quat}, picrate-***C***-4), 124.37 (+, Pyr-***C***-5), 125.12 (+, picrate-***C***-3,5), 126.14 (+, Pyr-***C***-3), 141.75 (C_{quat}, picrate-***C***-2,6), 142.85 (+, Pyr-***C***-6), 144.97 (+, Pyr-***C***-4), 156.07 (C_{quat}, Pyr-***C***-2), 160.71 (C_{quat}, picrate-***C***-1). ES-MS (MeCN + TFA) *m/z* (%): 137 (100) $[M + H]^+$. Anal. ($C_8H_{12}N_2$ · 2 $C_6H_3N_3O_7$) C, H, N. $C_8H_{12}N_2$ (136.19).

3-(Pyridin-3-yl)propan-1-amine (3.16)[23]

The title compound was prepared from **3.121** (5.20 g, 19.5 mmol) and hydrazine monohydrate (5.7 mL, 117.0 mmol) in EtOH (150 mL) according to the general procedure and purified by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 80/18/2 v/v/v) yielding a pale yellow oil (2.6 g, 88 %); mp (**3.16** · 2 $C_6H_3N_3O_7$) 218 °C (dec.). ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.83 – 1.97 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.76 – 2.90 (m, 4H, Pyr-3-C***H***$_2$-CH_2-C***H***$_2$), 7.69 (brs, 3H, N-***H***), 7.99 (dd, 1H, ^3J = 8.1 Hz, ^3J = 6.0 Hz, Pyr-5-***H***), 8.42 (ddd, 1H, ^3J = 8.1 Hz, ^4J = 2.0 Hz, ^4J = 1.4 Hz, Pyr-4-***H***), 8.59 (s, 2H, picrate-***H***), 8.78 – 8.82 (m, 2H, Pyr-6-***H*** + Pyr-2-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 27.70 (-, Pyr-3-CH_2-***C***H_2), 28.39 (-, Pyr-3-***C***H_2), 38.01 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 124.15 (C_{quat}, picrate-***C***-4), 125.12 (+, picrate-***C***-3,5), 126.56 (+, Pyr-***C***-5), 140.16 (C_{quat}, Pyr-***C***-3), 140.69 (+, Pyr-***C***-6),

141.75 (C_{quat}, picrate-***C***-2,6), 142.10 (+, Pyr-***C***-2), 144.98 (+, Pyr-***C***-4), 160.72 (C_{quat}, picrate-***C***-1). ES-MS (H_2O/MeOH + NH_4OAc) *m/z* (%): 137 (100) $[M + H]^+$. Anal. ($C_8H_{12}N_2$ · 2 $C_6H_3N_3O_7$) C, H, N. $C_8H_{12}N_2$ (136.19).

3-(Pyridin-4-yl)propan-1-amine (3.17)[23]

The title compound was prepared from **3.122** (7.20 g, 27.0 mmol) and hydrazine monohydrate (7.9 mL, 162.0 mmol) in EtOH (250 mL) according to the general procedure and purified by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32% 80/18/2 v/v/v) yielding a colorless oil (2.9 g, 79 %); mp (**3.17** · 2 $C_6H_3N_3O_7$) 210 – 211 °C (dec.). ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.86 – 1.99 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.77 – 2.90 (m, 2H, Pyr-4-CH_2-CH_2-C***H***$_2$), 2.94 (t, 2H, ^3J = 7.8 Hz, Pyr-4-C***H***$_2$), 7.71 (brs, 3H, N-***H***), 7.92 (dd, 2H, ^3J = 6.7 Hz, ^4J = 1.4 Hz, Pyr-3,5-***H***), 8.59 (s, 2H, picrate-***H***), 8.84 (d, 2H, ^3J = 6.7 Hz, ^4J = 1.4 Hz, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 26.92 (-, Pyr-4-CH_2-***C***H_2), 31.62 (-, Pyr-4-***C***H_2), 38.11 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 124.17 (C_{quat}, picrate-***C***-4), 125.13 (+, picrate-***C***-3,5), 126.62 (+, Pyr-***C***-3,5), 141.74 (C_{quat}, picrate-***C***-2,6), 141.90 (+, Pyr-***C***-2,6), 160.72 (C_{quat}, picrate-***C***-1), 161.17 (C_{quat}, Pyr-***C***-4). ES-MS (H_2O/MeOH + NH_4OAc) *m/z* (%): 137 (100) $[M + H]^+$. Anal. ($C_8H_{12}N_2$ · 2 $C_6H_3N_3O_7$) C, H, N. $C_8H_{12}N_2$ (136.19).

2-(Phenylthio)ethanamine (3.12)

A solution of **3.123** (2.2 g, 20 mmol), bromoethylamine · HBr (4.1 g, 20 mmol) and K^{t}BuO (4.5 g, 40 mmol) in anhydrous MeOH (160 mL) was stirred for 48 h at room temperature under a nitrogen atmosphere. The solvent was removed *in vacuo,* 1 M NaOH (30 mL) was added to the residue and extracted with Et_2O (3 x 80 mL). After evaporation of the combined organic layers, 1 M HCl (50 mL) was added, the solvent was removed under reduced pressure and H_2O (20 mL) was added. The solvent was removed by lyophilization giving a white solid (3.4 g, 89 %); mp (**3.12** · HCl) 116 – 117 °C (ref.[57]: 100 – 102 °C). ^{1}H-NMR (300 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 2.85 – 3.00 (m, 2H, C***H***$_2$-NH_3^+), 3.28 – 3.29 (m, 2H, S-C***H***$_2$), 7.21 – 7.29 (m, 1H, Ph-4-***H***), 7.31 – 7.48 (m, 4H, Ph-***H***), 8.32 (brs, 3H, N***H***$_3^+$). ^{13}C-NMR (75 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 29.00 (-, S-***C***H_2), 37.97 (-, ***C***H_2-NH_3^+), 126.27 (+, Ph-***C***-4), 128.50 (+, 2 Ph-***C***), 129.21 (+, 2 Ph-***C***), 134.21 (C_{quat}, Ph-***C***-1). CI-MS (NH_3) *m/z* (%): 154 (100) $[M + H]^+$. Anal. ($C_8H_{11}NS$ · HCl) C, H, N. $C_8H_{11}NS$ · HCl (189.71).

3-Cyclohexylpropan-1-amine hydrochloride (3.11)[24]

To a solution of **3.7** · HCl (2.58 g, 15.0 mmol) in EtOH (50 mL), Rh/C (5 %) (0.45 g, cat.) was added. After hydrogenation for 24 h at room temperature at 75 bar, the catalyst was removed by filtration over Celite and the solvent was concentrated *in vacuo*. The title compound was

crystallized by addition of Et_2O yielding a white solid (2.5 g, 94 %); mp (**3.11** · HCl) 217 – 219 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 0.73 – 0.96 (m, 2H, C***H***$_2$), 1.05 – 1.30 (m, 6H), 1.48 – 1.74 (m, 7H), 2.63 – 2.78 (m, 2H, C***H***$_2$-NH_3^+), 8.07 (brs, 3H, N***H***$_3^+$). ^{13}C-NMR (75 MHz, DMSO-d_6, hydrochloride): δ [ppm] = 24.21 (-, ***C***H_2), 25.63 (-, 2 ***C***H_2), 26.01 (-, ***C***H_2), 32.53 (-, 2 ***C***H_2), 33.35 (-, ***C***H_2), 36.46 (+, ***C***H), 38.83 (-, ***C***H_2-NH_3^+). CI-MS (NH_3) *m/z* (%): 142 (100) $[M + H]^+$. Anal. ($C_9H_{19}N$ · HCl · 0.25 H_2O) C, H, N. $C_9H_{19}N$ · HCl (177.71).

3.4.1.13 Preparation of 2-phenylethanesulfonic acid (3.125) and 2-phenylethanesulfonyl chloride (3.99)

2-Phenylethanesulfonic acid (3.125)[25]

To a cold solution (0 °C) of **3.124** (4.5 g, 32.5 mmol) in DCM (140 mL), a 32 % solution of peracetic acid in acetic acid (w/w) (20.5 mL, 97.5 mmol) was added dropwise. The solution was allowed to warm to ambient temperature and stirred for additional 2 h. The solvent was removed under reduced pressure yielding a dark red oil that was used without further purification. (6.0 g, 99 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.05 – 3.16 (m, 2H, Ph-C***H***$_2$), 3.27 – 3.38 (m, 2H, C***H***$_2SO_3H$), 7.12 – 7.36 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.92 (-, Ph-***C***H_2), 52.99 (-, ***C***H_2SO_3H), 126.99 (-, Ph-***C***-4), 128.42 (-, 2 Ph-***C***), 128.85 (-, 2 Ph-***C***), 137.53 (C_{quat}, Ph-***C***-1). EI-MS (70 eV) *m/z* (%): 186 (4) [$M^{+\bullet}$], 104 (100) [M – H_2SO_3]$^{+\bullet}$. $C_8H_{10}O_3S$ (186.23).

2-Phenylethanesulfonyl chloride (3.99)[25]

To a solution of **3.125** (1.86 g, 10.0 mmol) in DCM (135 mL), thionylchloride (14.2 mL, 200 mmol) was added at 0 °C. After addition of DMF (1.5 mL), the mixture was warmed to 35 °C and stirred for 16 h. The solvent was removed *in vacuo* and the crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) yielding a colorless oil (1.3 g, 63 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.29 – 3.39 (m, 2H, Ph-C***H***$_2$), 3.86 – 3.95 (m, 2H, C***H***$_2SO_3Cl$), 7.21 – 7.41 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.48 (-, Ph-***C***H_2), 66.22 (-, ***C***H_2SO_3Cl), 127.68 (-, Ph-***C***-4), 128.54 (-, 2 Ph-***C***), 129.21 (-, 2 Ph-***C***), 135.61 (C_{quat}, Ph-***C***-1). EI-MS (70 eV) *m/z* (%): 204 (9) [$M^{+\bullet}$], 104 (100) [M – HSO_2Cl]$^{+\bullet}$. $C_8H_9ClO_2S$ (204.67).

3.4.2 Pharmacological methods

3.4.2.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide maleate and iodophenpropit dihydrobromide were from Tocris Bioscience (Ellisville, USA). The H_4R antagonist JNJ 7777120 was a gift from Dr. R.

Thurmond (Department of Immunology, Johnson & Johnson Pharmaceutical R&D, San Diego, CA). [^{3}H]mepyramine, [^{3}H]tiotidine, [^{3}H]*N*-α-methylhistamine and [^{3}H]histamine were from PerkinElmer Life Sciences (Boston, MA). [γ-^{32}P]GTP was synthesized according to a previously described method.[58] [^{32}P]P_i (8,500 – 9,100 Ci/mmol orthophosphoric acid) was from PerkinElmer Life Sciences (Boston, MA). All unlabeled nucleotides, glycerol-3-phosphate dehydrogenase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were from Roche (Mannheim, Germany). 3-Phosphoglycerate kinase and L-α-glycerol phosphate were from Sigma. GF/C filters were from Whatman (Maidstone, UK).

3.4.2.2 Steady-state GTPase activity assay

GTPase activity assays were performed as previously described for the H_1R[27], H_2R[26], H_3R and H_4R[4]. H_1R assays: Sf9 insect cell membranes coexpressing the hH_1R and RGS4 were employed, H_2R assays: Sf9 insect cell membranes expressing the hH_2R-$G_{s\alpha S}$ fusion protein were employed, H_3R assays: Sf9 insect cell membranes coexpressing the hH_3R, mammalian $G_{i\alpha 2}$, $G_{\beta 1\gamma 2}$ and RGS4 were employed, H_4R assays: Sf9 insect cell membranes coexpressing the hH_4R-RGS19 fusion protein, mammalian $G_{i\alpha 2}$ and $G_{\beta 1\gamma 2}$ were employed. The respective membranes were thawed, sedimented by a 10 min centrifugation at 4 °C and 13,000*g*. Membranes were resuspended in 10 mM Tris/HCl, pH 7.4. Each assay tube contained Sf9 membranes expressing the respective HR subtype (10 – 20 µg protein/tube), $MgCl_2$ ($H_{1/2}R$ assays: 1.0 mM, $H_{3/4}R$ assays: 5.0 mM), 100 µM EDTA, 100 µM ATP, 100 nM GTP, 100 µM adenylyl imidophosphate, 5 mM creatine phosphate, 40 µg creatine kinase and 0.2 % (w/v) bovine serum albumin in 50 mM Tris/HCl, pH 7.4 and the investigated ligands at various concentrations. All H_4R assays additionally contained 100 mM NaCl. For the determination of K_B values (antagonist mode of the GTPase activity assay) histamine was added to the reaction mixtures (final concentrations: H_1R: 200 nM; H_2R: 1 µM; $H_{3/4}R$: 100 nM).

Reaction mixtures (80 µL) were conducted for 2 min at 25 °C. After the addition of 20 µl of [γ-^{32}P]GTP (0.1 µCi/tube), reaction mixtures were incubated for 20 min at 25 °C. Reactions were terminated by the addition of 900 µL slurry consisting of 5% (w/v) activated charcoal and 50 mM NaH_2PO_4, pH 2.0. Charcoal absorbs nucleotides but not P_i. Charcoal-quenched reaction mixtures were centrifuged for 7 min at room temperature at 13000*g*. 600 µL of the supernatant were removed and $^{32}P_i$ was determined by liquid scintillation counting. Spontaneous [γ-^{32}P]GTP degradation was determined in tubes containing all components described above, plus a high concentration of unlabeled GTP (1 mM) that due to competition with [γ-^{32}P]GTP prevents [γ-^{32}P]GTP hydrolysis by enzymatic activities present in Sf9 membranes. Spontaneous [γ-^{32}P]GTP degradation was <1 % of the total amount of

radioactivity added. The experimental conditions chosen ensured that not more than 10 % of the total amount of [γ-^{32}P]GTP added was converted to $^{32}P_i$.

3.4.2.3 Radioligand binding assays[26, 27]

For the binding experiments the Sf9 insect cell membranes described in 3.4.2.3 were employed. The respective membranes were thawed and sedimented by a 10 min centrifugation at 4 °C and 13,000*g.* Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4). Each tube (total volume 500 µL) contained 50 µg (hH_1R, hH_3R), 120 µg (hH_4R) or 250 µg (hH_2R) of membrane protein. Competition binding experiments were performed in the presence of 5 nM [^{3}H]mepyramine (hH_1R), 10 nM [^{3}H]tiotidine (hH_2R), 3 nM [^{3}H]*N*-α-methylhistamine (hH_3R) or 10 nM [^{3}H]histamine (hH_4R) and increasing concentrations of unlabeled ligands. Incubations were conducted for 60 min at 25 °C and shaking at 250 rpm. Bound radioligand was separated from free radioligand by filtration through 0.3 % polyethyleneimine-pretreated GF/C filters, followed by three washes with 2 mL of cold binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting.

3.4.2.4 Histamine H_1R assay on guinea pig ileum[4]

Guinea pigs of either sex (250 – 500 g) were stunned by a blow on the neck and exsanguinated. The ileum was rapidly removed, rinsed and cut into segments of 1.5 – 2.0 cm length. The tissues were mounted isotonically (preload of 5 mN) in a jacketed 20-mL organ bath that was filled with Tyrode's solution of the following composition [mM]: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, and glucose 5.0. The solution additionally contained atropine to block cholinergic M receptors at a concentration not affecting H_1 receptors (0.05 µM). The solution was aerated with 95% O_2 – 5% CO_2 and warmed to a constant temperature of 37 °C. During an equilibration period of 80 min, the tissues were stimulated three times with histamine (1 µM, then 10 µM) followed by washout. Up to four cumulative concentration-response curves were determined on each tissue: a first to histamine (0.01 – 30 µM), and the 2nd – 4th to histamine in the presence of increasing concentrations of antagonist (incubation time 10 – 15 min). pEC_{50} differences were not corrected since four successive curves for histamine were super imposable under these conditions (n > 10).

3.4.2.5 Histamine H_2R assay on the isolated spontaneously beating guinea pig right atrium[4]

Hearts were rapidly removed from guinea pigs used for studies on the ileum (see above). The right atrium was quickly dissected and set up isometrically in Krebs-Henseleit solution under a diastolic resting force of 5 mN in a jacketed 20 mL organ bath of 32.5 °C as previously described.[59] The bath fluid (composition [mM]: NaCl 118.1, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.64, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.0, sodium pyruvate 2.0) was gassed with 95% O_2 – 5% CO_2 and additionally contained (*RS*)-propranolol (0.3 µM) and mepyramine (1 µM). Experiments were started after 30 min of continuous washing and an additional equilibration period of 15 min. *Antagonists*: Two successive concentration-frequency curves to histamine (0.1 – 30 µM) were established, the first in the absence and the second in the presence of the compound under study (incubation time 30 min). *Agonists*: Two successive concentration-frequency curves were established, the first to histamine (0.1 – 30 µM) and the second for the agonist under study. Furthermore, the sensitivity to 30 µM cimetidine was routinely checked at the end of each H_2R agonist concentration-effect curve established in the absence of an H_2R antagonist, and a significant reduction of frequency was always observed after 15 – 30 min.

3.5 References

(1) Lim, H. D., van Rijn, R. M., Ling, P., Bakker, R. A., Thurmond, R. L., Leurs, R., Evaluation of Histamine H_1-, H_2-, and H_3-Receptor Ligands at the Human Histamine H_4 Receptor: Identification of 4-Methylhistamine as the First Potent and Selective H_4 Receptor Agonist. *J. Pharmacol. Exp. Ther.* **2005**, 314, 1310-1321.

(2) Hashimoto, T., Harusawa, S., Araki, L., Zuiderveld, O. P., Smit, M. J., Imazu, T., Takashima, S., Yamamoto, Y., Sakamoto, Y., Kurihara, T., Leurs, R., Bakker, R. A., Yamatodani, A., A Selective Human H_4-Receptor Agonist: (-)-2-Cyano-1-methyl-3-{(2*R*,5*R*)-5-[1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl}methylguanidine. *J. Med. Chem.* **2003**, 46, 3162-3165.

(3) Lim, H. D., Smits, R. A., Bakker, R. A., van Dam, C. M. E., de Esch, I. J. P., Leurs, R., Discovery of *S*-(2-Guanidylethyl)-isothiourea (VUF 8430) as a Potent Nonimidazole Histamine H_4 Receptor Agonist. *J. Med. Chem.* **2006**, 49, 6650-6651.

(4) Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press, DOI: 10.1021/jm800841w. Epub ahead of print on Oct 800825, 802008.

(5) Xie, S.-X., Kraus, A., Ghorai, P., Ye, Q.-Z., Elz, S., Buschauer, A., Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-AK57), a Potent Partial Agonist for the Human Histamine H_1- and H_2-Receptors. *J. Pharmacol. Exp. Ther.* **2006**, 317, 1262-1268.

(6) Xie, S.-X., Ghorai, P., Ye, Q.-Z., Buschauer, A., Seifert, R., Probing Ligand-Specific Histamine H_1- and H_2-Receptor Conformations with N^G-Acylated Imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006**, 317, 139-146.

(7) pK_a-value was calculated with ACD Labs 9.0, Advanced Chemistry Development, Toronto (Canada).

(8) Durant, G. J., Emmett, J. C., Ganellin, C. R., Miles, P. D., Parsons, M. E., Prain, H. D., White, G. R., Cyanoguanidine-Thiourea Equivalence in the Development of the Histamine H_2-Receptor Antagonist, Cimetidine. *J. Med. Chem.* **1977**, 20, 901-906.

(9) Durant, G. J., Ganellin, C. R., Hills, D. W., Miles, P. D., Parsons, M. E., Pepper, E. S., White, G. R., The Histamine H_2 Receptor Agonist Impromidine: Synthesis and Structure-Activity Considerations. *J. Med. Chem.* **1985**, 28, 1414-1422.

(10) Zhu, Y., Michalovich, D., Wu, H.-L., Tan, K. B., Dytko, G. M., Mannan, I. J., Boyce, R., Alston, J., Tierney, L. A., Li, X., Herrity, N. C., Vawter, L., Sarau, H. M., Ames, R. S., Davenport, C. M., Hieble, J. P., Wilson, S., Bergsma, D. J., Fitzgerald, L. R., Cloning, Expression, and Pharmacological Characterization of a Novel Human Histamine Receptor. *Mol. Pharmacol.* **2001**, 59, 434-441.

(11) Kraus, A., Highly Potent, Selective Acylguanidine-Type Histamine H_2 Receptor Agonists: Synthesis and Structure-Activity Relationships. *Doctoral thesis*, University of Regensburg, Germany **2007**.
http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=904&la=de.

(12) Buschauer, A., Synthese und pharmakologische Wirkung von Arylmethylthioethylguanidinen. *Arzneim.-Forsch.* **1987**, 37 (II), 1008-1012.

(13) Webb, R. L., Eggleston, D. S., Labaw, C. S., Lewis, J. J., Wert, K., Diphenyl Cyancarbonimidate and Dichlorodiphenoxymethane as Synthons for the Construction of Heterocyclic Systems of Medicinal Interest. *J. Heterocycl. Chem.* **1987**, 24, 275 - 278.

(14) Webb, R. L., Labaw, C. S., Diphenyl Cyanocarbonimidate. A Versatile Synthon for the Construction of Heterocyclic Systems. *J. Heterocycl. Chem.* **1982**, 19, 1205 - 1206.

(15) Stark, H., Purand, K., Hüls, A., Ligneau, X., Garbarg, M., Schwartz, J.-C., Schunack, W., [^{125}I]Iodoproxyfan and Related Compounds: A Reversible Radioligand and Novel Classes of Antagonists with High Affinity and Selectivity for the Histamine H_3 Receptor. *J. Med. Chem.* **1996**, 39, 1220-1226.

(16) Mitsunobu, O., Yamada, M., Mukaiyama, T., Preparation of esters of phosphoric acid by the reaction of trivalent phosphorus compounds with diethyl azodicarboxylate in the presence of alcohols. *Bull. Chem. Soc. Jpn.* **1967**, 40, 935-939.

(17) Bertinaria, M., Stilo, A. D., Tosco, P., Sorba, G., Poli, E., Pozzoli, C., Coruzzi, G., Fruttero, R., Gasco, A., [3-(1*H*-Imidazol-4-yl)propyl]guanidines Containing Furoxan Moieties: A New Class of H_3-Antagonists Endowed with NO-Donor Properties. *Bioorg. Med. Chem.* **2003**, 11, 1197-1205.

(18) Buschauer, A., Lachenmeyr, F., Schunack, W., Synthesis and histamine H_2-receptor activity of heterocyclic impromidine analogues. *Pharmazie* **1992**, 47, 86-91.

(19) Lee, C. H., Lee, J. S., Na, H. K., Yoon, D. W., Miyaji, H., Cho, W. S., Sessler, J. L., Cis- and Trans-Strapped Calix[4]pyrroles Bearing Phthalamide Linkers: Synthesis and Anion-Binding Properties. *J. Org. Chem.* **2005**, 70, 2067-2074.

(20) Zav'yalov, S. I., Kravchenko, N. E., *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1984**, 33, 418.

(21) Bredereck, H., Theilig, G., Untersuchungen in der Oxazolreihe und Umwandlungen von Oxazolen in Imidazole mittels Formamids (Formamid-Reaktionen, I. Mitteil.). *Chem. Ber.* **1953**, 86, 96-109.

(22) Wadsworth, W. S., Emmons, W. D., The Utility of Phosphonate Carbanions in Olefin Synthesis. *J. Am. Chem. Soc.* **1961**, 83, 1733-1738.

(23) Allen, J. R., Hitchcock, S. A., Liu, B., William Wilson, J. T. Preparation of 2-hydroxyindanylamide derivatives as muscarinic receptor agonists. WO 2005009941, **2005**. *Chem. Abstr.* 142:197700.

(24) Buschauer, A., Friese-Kimmel, A., Baumann, G., Schunack, W., Synthesis and histamine H_2 agonistic activity of arpromidine analogues: replacement of the pheniramine-like moiety by non-heterocyclic groups. *Eur. J. Med. Chem.* **1992**, 27, 321-330.

(25) Hannedouche, J., Clarkson, G. J., Wills, M., A New Class of "Tethered" Ruthenium(II) Catalyst for Asymmetric Transfer Hydrogenation Reactions. *J. Am. Chem. Soc.* **2004**, 126, 986-987.

(26) Kelley, M. T., Bürckstümmer, T., Wenzel-Seifert, K., Dove, S., Buschauer, A., Seifert, R., Distinct Interaction of Human and Guinea Pig Histamine H_2-Receptor with Guanidine-Type Agonists. *Mol. Pharmacol.* **2001**, 60, 1210-1225.

(27) Seifert, R., Wenzel-Seifert, K., Bürckstümmer, T., Pertz, H. H., Schunack, W., Dove, S., Buschauer, A., Elz, S., Multiple Differences in Agonist and Antagonist Pharmacology between Human and Guinea Pig Histamine H_1-Receptor. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1104-1115.

(28) Geyer, R., Imidazolylalkylguanidinderivate als Histamin H_4 Rezeptor Agonisten: Synthese und pharmakologische Charakterisierung. *Diploma Thesis*, Department of Pharmaceutical and Medicinal Chemistry II, University of Regensburg: Regensburg (Germany) **2008**.

(29) Portoghese, P. S., From Models to Molecules: Opioid Receptor Dimers, Bivalent Ligands, and Selective Opioid Receptor Probes. *J. Med. Chem.* **2001**, 44, 2259-2269.

(30) van Rijn, R. M., Chazot, P. L., Shenton, F. C., Sansuk, K., Bakker, R. A., Leurs, R., Oligomerization of Recombinant and Endogenously Expressed Human Histamine H_4 Receptors. *Mol. Pharmacol.* **2006**, 70, 604-615.

(31) Portoghese, P. S., Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. *Trends Pharmacol. Sci.* **1989**, 10, 230-235.

(32) Halazy, S., G-protein coupled receptors bivalent ligands and drug design. *Exp. Opin. Ther. Patents* **1999**, 9, 431-446.

(33) Jones, R. M., Hjorth, S. A., Schwartz, T. W., Portoghese, P. S., Mutational Evidence for a Common κ Antagonist Binding Pocket in the Wild-Type κ and Mutant μ[K303E] Opioid Receptors. *J. Med. Chem.* **1998**, 41, 4911-4914.

(34) Turnheim, K., Kraupp, O., Pulmonary and systemic circulatory effects and β-adrenergic selectivity of hexoprenaline, salbutamol, oxyfedrine, and isoproterenol. *Eur. J. Pharmacol.* **1971**, 15, 231-239.

(35) Perez, M., Pauwels, P. J., Fourrier, C., Chopin, P., Valentin, J.-P., John, G. W., Marien, M., Halazy, S., Dimerization of sumatriptan as an efficient way to design a potent, centrally and orally active 5-HT_{1B} agonist. *Bioorg. Med. Chem. Lett.* **1998**, 8, 675-680.

(36) Perez, M., Jorand-Lebrun, C., Pauwels, P. J., Pallard, I., Halazy, S., Dimers of $5HT_1$ ligands preferentially bind to $5HT_{1B/1D}$ receptor subtypes. *Bioorg. Med. Chem. Lett.* **1998**, 8, 1407-1412.

(37) Yao, B. B., Hutchins, C. W., Carr, T. L., Cassar, S., Masters, J. N., Bennani, Y. L., Esbenshade, T. A., Hancock, A. A., Molecular modeling and pharmacological analysis of species-related histamine H_3 receptor heterogeneity. *Neuropharmacology* **2003**, 44, 773-786.

(38) Preuss, H., Species-selective Interactions of Histamine H_2 Receptors with Guanidine-type Agonists: Molecular Modelling, Site-directed Mutagenesis and Pharmacological Analysis. *Doctoral thesis*, University of Regensburg, Germany **2007**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=843&la=de.

(39) Schneider, E., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(40) Schnell, D., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(41) Klinker, J. F., Seifert, R., Rezeptor-unabhängige Aktivierung von G-Proteinen. *Pharm. Unserer Zeit* **1995**, 24, 250-263.

(42) Hagelüken, A., Grünbaum, L., Klinker, J. F., Nürnberg, B., Harhammer, R., Schultz, G., Leschke, C., Schunack, W., Seifert, R., Histamine receptor-dependent and/or -independent activation of guanine nucleotide-binding proteins by histamine and 2-substituted histamine derivatives in human leukemia (HL-60) and human erythroleukemia (HEL) cells. *Biochem. Pharmacol.* **1995**, 49, 901-914.

(43) Seifert, R., Hageluken, A., Hoer, A., Hoer, D., Grunbaum, L., Offermanns, S., Schwaner, I., Zingel, V., Schunack, W., Schultz, G., The H_1 receptor agonist 2-(3-chlorophenyl)histamine activates G_i proteins in HL-60 cells through a mechanism that is independent of known histamine receptor subtypes. *Mol. Pharmacol.* **1994**, 45, 578-586.

(44) Eriks, J. C., Sterk, G. J., Van der Goot, H., Timmerman, H. Preparation of substituted thiazole derivative as drugs. WO 9110657, **1991**, *Chem. Abstr.* 115:183285.

(45) Garratt, P. J., Hobbs, C. J., Wrigglesworth, R., One-carbon compounds as synthetic intermediates. The synthesis of hydropyrimidines and hydroquinazolines by sequential nucleophilic addition to diphenyl cyanocarbonimidate with concomitant cyclization. **1989**, 54, 1062-1069.

(46) Citterio, A., Minisci, F. Process for the preparation of cimetidine. DE 3148103, **1983**, *Chem. Abstr.* 99:88200.

(47) Martin, D., Witke, K., Reich, P., Nadolski, K., Cyanic acid esters. XVI. Cleavage of the silicon-nitrogen bond by cyanic acid esters. Synthesis of *N*-trimethylsilyl- and *N*-cyanoisoureas. **1968**, 101, 3185-3200.

(48) Martin, D., Tittelbach, F., Nadolski, K., Cyanic acid ester. 32. Simple syntheses for aminoaryloxy-1,3,5-triazines. *Journal für Praktische Chemie (Leipzig)* **1981**, 323, 694-699.

(49) Wolin, R., Connolly, M., Afonso, A., Hey, J. A., She, H., Rivelli, M. A., Willams, S. M., West, R. E., Novel H_3 receptor antagonists. Sulfonamide homologs of histamine. *Bioorg. Med. Chem. Lett.* **1998**, 8, 2157-2162.

(50) Sellier, C., Buschauer, A., Elz, S., Schunack, W., Zur Synthese von (*Z*)- und (*E*)-3-(1*H*-Imidazol-4-yl)-2-propenamin und einigen 3-(1*H*-Imidazol-4-yl)propanaminen. *Liebigs Ann. Chem.* **1992**, 1992, 317-323.

(51) Ghorai, P., Arpromidine-related acylguanidines: synthesis and structure activity relationships of a new class of guanidine-type histamine H_2 receptor agonists with reduced basicity. *Doctoral thesis*, University of Regensburg, Germany **2005**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=561&la=de.

(52) Pioch, R. P. 2,4-Disubstituted thiazole derivatives. EP 49618, **1982**, *Chem. Abstr.* 97:72354.

(53) Akabori, S., Kaneko, T., Amino acids and their derivatives. V. 3. Synthesis of 2 higher homologs of histamine. *J. Chem. Soc. Jpn.* **1932**, 53, 207-212 (*Chem. Abstr.* 227:2500).

(54) DiBiase, S. A., Lipisko, B. A., Haag, A., Wolak, R. A., Gokel, G. W., Direct synthesis of α,β-unsaturated nitriles from acetonitrile and carbonyl compounds: survey, crown effects, and experimental conditions. *J. Org. Chem.* **1979**, 44, 4640-4649.

(55) McFarland, J. W., Howes, H. L., Conover, L. H., Lynch, J. E., Austin, W. C., Morgan, D. H., Novel anthelmintic agents. V. Thiazoline and dihydrothiazine analogs of pyrantel. *J. Med. Chem.* **1970**, 13, 113-119.

(56) Pryde, D. C., Cook, A. S., Burring, D. J., Jones, L. H., Foll, S., Platts, M. Y., Sanderson, V., Corless, M., Stobie, A., Middleton, D. S., Foster, L., Barker, L., Van Der Graaf, P., Stacey, P., Kohl, C., Coggon, S., Beaumont, K., Novel selective inhibitors of neutral endopeptidase for the treatment of female sexual arousal disorder. *Bioorg. Med. Chem.* **2007**, 15, 142-159.

(57) Pathak, U., Raza, S. K., Kulkarni, A. S., Vijayaraghvan, R., Kumar, P., Jaiswal, D. K., Novel S-Substituted Aminoalkylamino Ethanethiols as Potential Antidotes against Sulfur Mustard Toxicity. *J. Med. Chem.* **2004**, 47, 3817-3822.

(58) Walseth, T. F., Johnson, R. A., The enzymatic preparation of [α-^{32}P] nucleoside triphosphates, cyclic [^{32}P] AMP, and cyclic [^{32}P] GMP. *Biochim. Biophys. Acta* **1979**, 562, 11-31.

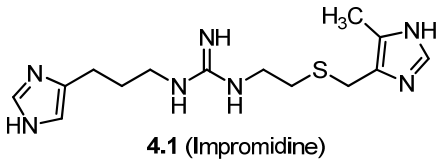
(59) Black, J. W., Duncan, W. A., Durant, C. J., Ganellin, C. R., Parsons, E. M., Definition and antagonism of histamine H_2-receptors. *Nature* **1972**, 236, 385-390.

Chapter 4

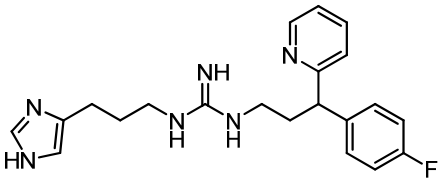
N^G-Acylated imidazolylalkylguanidines: synthesis and structure-activity relationships at the histamine receptor subtypes

4.1 Introduction

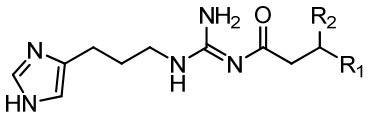
The development of less basic H_2R agonists relative to established guanidine-type compounds like impromidine (**4.1**)[1, 2] or arpromidine (**4.2**)[3, 4] (Figure 4.1) resulted in N^G-acylated imidazolylpropyl-guanidines[5-7], a new class of potent H_2R agonists (Figure 4.1). The acylguanidine moiety was found to be a useful bioisosteric replacement of the strongly basic guanidine group to obtain potent H_2R agonists with more favorable pharmacokinetic properties.[7] However, various of these acylguanidine-type H_2R agonists even exhibited higher activities at the hH_3R and hH_4R relative to the hH_2R (Figure 4.1 and Table 4.1). Mostly, the efficacies of these compounds were high at the hH_2R and hH_4R but rather low at the hH_3R.



4.1 (Impromidine)



4.2 (Arpromidine)



N^G-acylated imidazolylpropylguanidines

4.3 (UR-AK24): R^1 = Me, R^2 = Ph
4.4: R^1 = Me, R^2 = 2-thienyl
4.5 (UR-PG80): R^1 = R^2 = Ph
4.6: R^1 = 2-thiazolyl, R^2 = Ph

Figure 4.1. Structures of the guanidine-type H_2R agonists impromidine and arpromidine and N^G-acylated imidazolyl-propylguanidines with activity at the H_2R, H_3R and H_4R.

The unexpected pharmacological activities of these compounds at HR subtypes other than H_2R prompted us to modify the structures of the evaluated N^G-acylated imidazolylpropylguanidines with the aim to elaborate the potential of generating selective acylguanidine-type HR ligands and to investigate structure-activity and structure-selectivity relationships.

In this medicinal chemistry project we focused on the imidazolylpropylguanidine part and left the acyl residues unaffected (Figure 4.2). The three-membered carbon chain separating the imidazole ring from the acylguanidine or guanidine group has been shown to be optimal for H_2R agonistic activity.[1, 7] To elucidate the optimal distance of the linker for H_3R and H_4R activity, the higher and lower homologs were investigated. Since a central methyl branch in the alkyl chain was not well tolerated with respect to H_2R activity,[8] we examined this modification as a possibility to increase selectivity for other HR subtypes. The same consideration was the rationale for the preparation of N^G-methylated guanidine analogs.[3] As 5-methylhistamine has proven to be a selective H_4R agonist (cf. Chapter 3),[9] substitution of the imidazole ring with a methyl group in position 5 was regarded as an opportunity for shifting selectivity toward the H_4R.

This chapter presents the synthesis of N^G-acylated imidazolylalkylguanidines structurally modified according to Figure 4.2 and the functional pharmacological activities of these compounds at the different HR subtypes. The structure-activity and structure-selectivity relationships at the HR subtypes will be discussed.

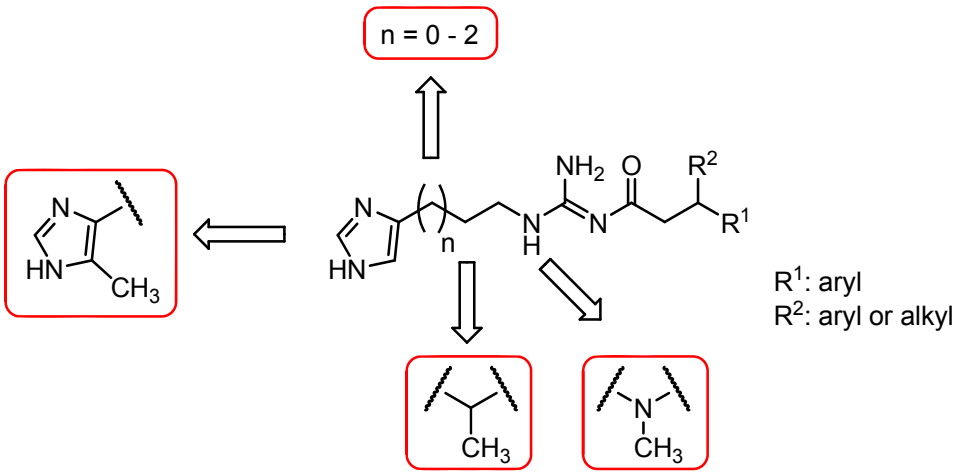
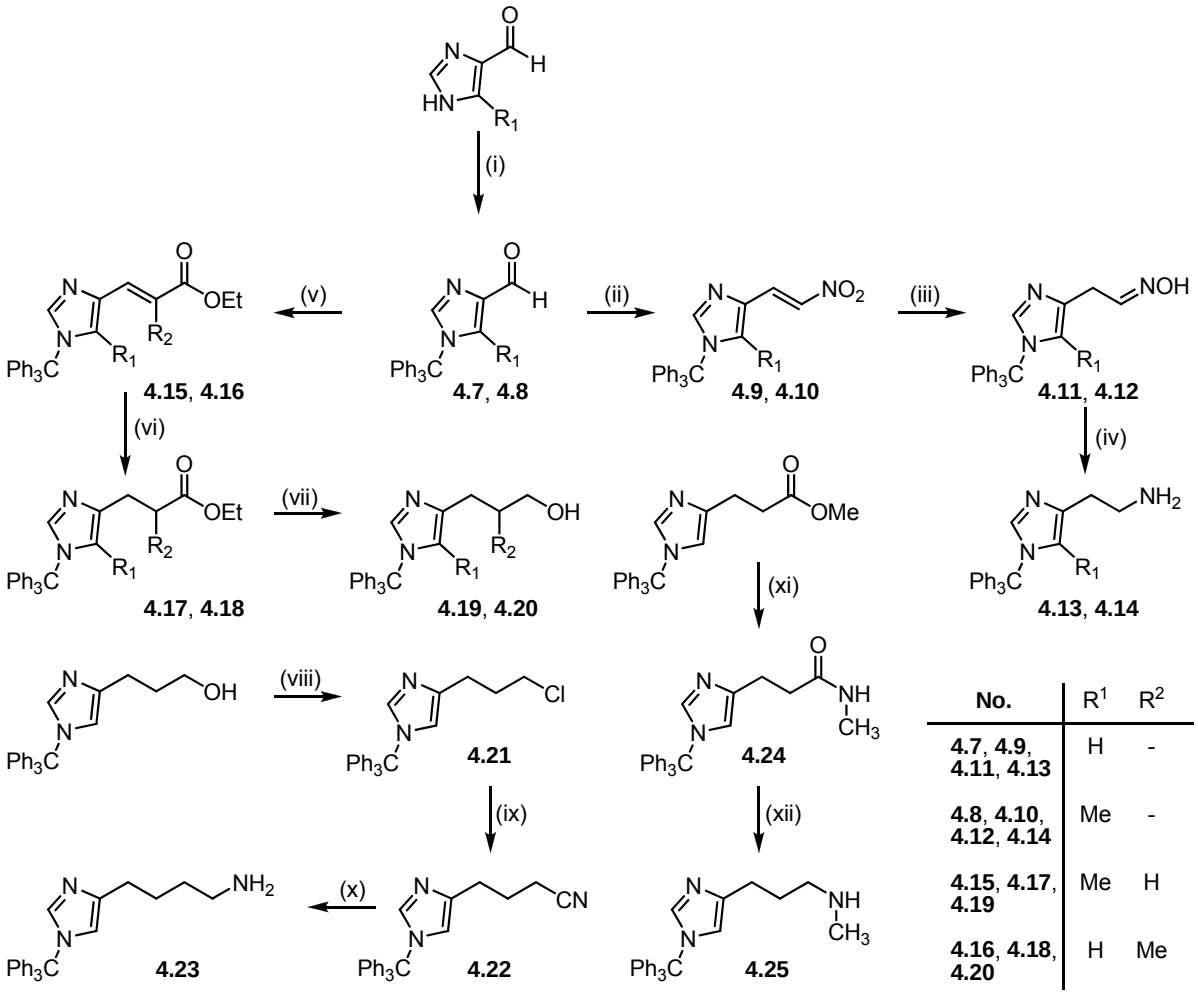


Figure 4.2. Overview about the structural modifications of N^G-acylated imidazolylpropylguanidines described in this chapter.

4.2 Chemistry

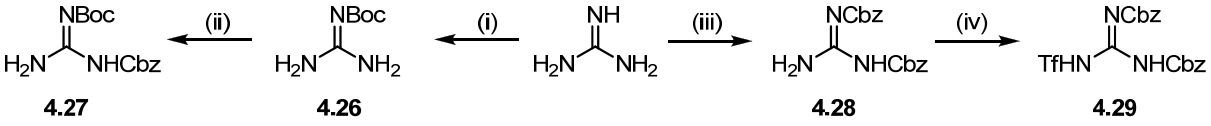
The imidazolylalkylamines **4.13**, **4.14**, **4.23** and **4.25**, and imidazolylalkylalcohols **4.19** and **4.20** that are precursors for the synthesis of the corresponding guanidines were prepared according to Scheme 4.1. To reduce polarity and facilitate separation of the intermediates, the imidazole ring was initially trityl-protected in each case. The imidazolylethylamines **4.13** and **4.14** were prepared by analogy with a synthetic pathway described for the synthesis of 5-methylhistamine by Davey and colleagues.[10] Reaction of the aldehydes **4.7** and **4.8** with

nitromethane in the presence of ammonia acetate in a *Henry* reaction[11] formed the nitroalkenes **4.9** and **4.10**. Reduction in two steps *via* the aldoximes **4.11** and **4.12** provided the imidazolylethylamines **4.13** and **4.14**. The imidazolylpropanols **4.19** and **4.20** were obtained *via Horner-Wadsworth-Emmons* reaction[12] of the aldehydes **4.7** and **4.8** with triethyl phosphonoacetate followed by hydrogenation of the C=C double bond and reduction of the ester functionality with $LiAlH_4$. 3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol was treated with thionyl chloride to yield the chloride **4.21**.[13] Elongation of the carbon chain by *Kolbe* nitrile synthesis[14] and reduction of the nitrile **4.22** with $LiAlH_4$ gave the amine **4.23**. The *N*-methylated imidazolylpropylamine **4.25** was prepared in two steps by aminolysis of methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate followed by reduction of the amide **4.24**.



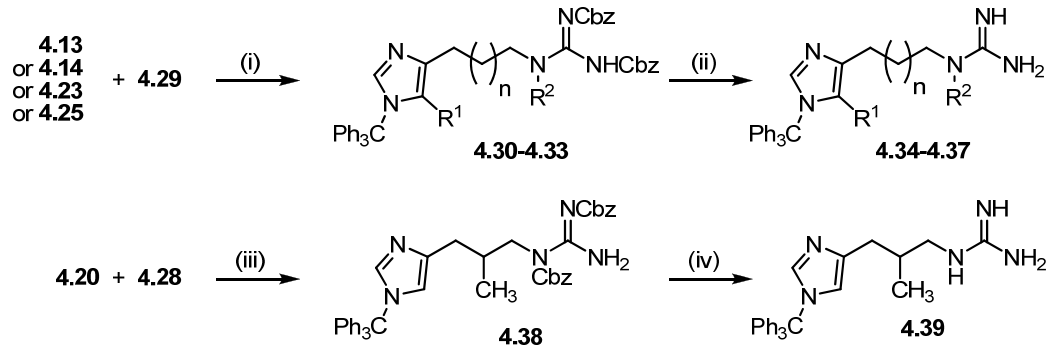
Scheme 4.1. Synthesis of the imidazolylalkylamines **4.13**, **4.14**, **4.23** and **4.25**, and imidazolylalkyl-alcohols **4.19** and **4.20**. Reagents and conditions: (i) TrCl (1.1 eq), NEt_3 (1.8 eq), MeCN, 20 h, rt; (ii) NH_4OAc (1.1 eq), $MeNO_2$ (excess), 5 h, 50 °C; (iii) Pd/C (10 %) (cat.), $NaH_2PO_2 \cdot H_2O$ (11 eq), THF, 1 h, rt; (iv) $LiAlH_4$ (3 eq), THF, 3 h, 0 °C → rt; (v) triethyl phosphonoacetate (1.5 eq), NaH (60 % dispersion in mineral oil) (1.5 eq), THF, overnight, reflux; (vi) Pd/C (10 %) (cat.), EtOH, 3 h, rt; (vii) $LiAlH_4$ (2 eq), THF/Et_2O, 2 h, reflux; (viii) $SOCl_2$ (2 eq), THF, 3 h, 0 °C → rt; (ix) KCN (2 eq), KI (cat.), DMSO, 24 h, 80 °C; (x) $LiAlH_4$ (2 eq), THF/Et_2O, 3h, reflux; (xi) $MeNH_2$ (50 eq), MeOH, overnight, rt; (xii) $LiAlH_4$ (2 eq), THF/Et_2O, 3h, reflux.

The preparation of the guanidinylation reagents **4.27-4.29** is depicted in Scheme 4.2. Successive reactions of guanidine with Boc-anhydride[15] and benzyl chloroformate produced **4.27**[7]. Acylation of guanidine with benzyl chloroformate under basic conditions gave the di-Cbz-protected guanidine **4.28**. Compound **4.29** was obtained by further reaction with triflic anhydride.[16, 17] **4.27**[18] and **4.28** are versatile reagents for the guanidinylation of alcohols applying *Mitsunobu* conditions,[16, 19] whereas **4.29** is useful for the conversion of amines to guanidines.



Scheme 4.2. Synthesis of the guanidinylation reagents **4.27-4.29**. Reagents and conditions: (i) NaOH (2 eq), Boc_2O (0.8 eq), 1,4-dioxane/H_2O, overnight, 0 °C → rt. (ii) benzyl succinimidyl carbonate (1 eq), DMF, overnight, rt; (iii) benzyl chloroformate (3 eq), NaOH (5 eq), H_2O/DCM, 20 h, 0 °C; (iv) Tf_2O (1 eq), NaH (60 % dispersion in mineral oil) (2 eq), chlorobenzene, overnight, - 45 °C → rt.

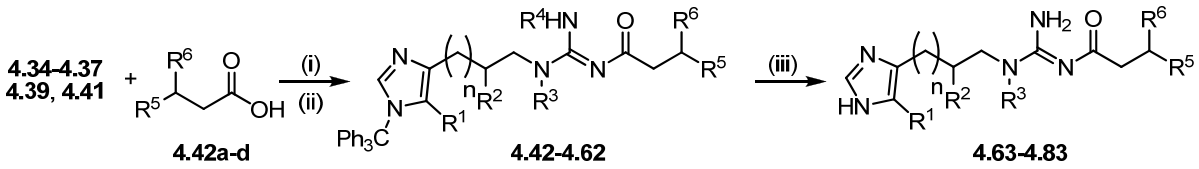
The imidazolylalkylamines **4.13**, **4.14**, **4.23** and **4.**25 were treated with the guanidinylation reagent **4.29** to yield the corresponding di-Cbz protected guanidines **4.30-4.33** (Scheme 4.3).[17] Removing the Cbz protection groups by catalytic hydrogenation gave the guanidines **4.34-4.37**. The imidazolylalkylalcohols **4.19** and **4.20** were converted to the diurethane-protected guanidines **4.38** and **4.40** under *Mitsunobu* conditions.[7, 16, 19] Cleavage of the Cbz-groups by hydrogenolysis afforded the guanidine **4.39** and the Boc-protected guanidine **4.41**, respectively. As in the *Mitsunobu* reaction alkylation of **4.27** can occur at the nitrogen bearing the Boc- or the Cbz-group, regioisomers of **4.40** and **4.41** were obtained.



No.	R^1	R^2	n
4.30, 4.34	H	H	0
4.31, 4.35	Me	H	0
4.32, 4.36	H	H	2
4.33, 4.37	H	Me	1

Scheme 4.3. Synthesis of the imidazolylalkylguanidines **4.34-4.37**, **4.39** and **4.41**. Reagents and conditions: (i) NEt_3 (1 eq), DCM, overnight, rt; (ii) H_2, Pd/C (10 %) (cat.), MeOH, 3 h, rt; (iii) PPh_3 (1.5 eq), DIAD (1.5 eq), THF, overnight, 0 °C → rt; (iv) H_2, Pd/C (10 %) (cat.), MeOH, 3 h, rt.

N^G-acylated imidazolylalkylguanidines **4.42-4.52** and **4.57-4.62** were obtained by N^G-acylation of the free guanidine group of **4.34-4.37** and **4.39** with the carboxylic acids **4.42a-d**.[7, 20, 21] **4.53-4.56** were synthesized by coupling the carboxylic acids **4.42a-d** to N^G-Boc-protected guanidine **4.41** (cf. Chapter 6). Cleavage of the protective groups was performed with trifluoroacetic acid giving the N^G-acylated imidazolylalkylguanidines **4.63-4.83** (Scheme 4.4).



No.	R^1	R^2	R^3	R^4	R^5	R^6	n
4.42a	-	-	-	-	Ph	Me	-
4.42b	-	-	-	-	2-thienyl	Me	-
4.42c	-	-	-	-	Ph	Ph	-
4.42d	-	-	-	-	2-thiazolyl	Ph	-
4.42, **4.63**	H	H	H	H	Ph	Me	0
4.43, **4.64**	H	H	H	H	2-thienyl	Me	0
4.44, **4.65**	H	H	H	H	Ph	Ph	0
4.45, **4.66**	H	H	H	H	Ph	Me	2
4.46, **4.67**	H	H	H	H	2-thienyl	Me	2
4.47, **4.68**	H	H	H	H	Ph	Ph	2
4.48, **4.69**	H	H	H	H	2-thiazolyl	Ph	2
4.49, **4.70**	H	Me	H	H	Ph	Me	1
4.50, **4.71**	H	Me	H	H	2-thienyl	Me	1
4.51, **4.72**	H	Me	H	H	Ph	Ph	1
4.52, **4.73**	H	Me	H	H	2-thiazolyl	Ph	1
4.53, **4.74**	Me	H	H[a]	Boc[a]	Ph	Me	1
4.54, **4.75**	Me	H	H[a]	Boc[a]	2-thienyl	Me	1
4.55, **4.76**	Me	H	H[a]	Boc[a]	Ph	Ph	1
4.56, **4.77**	Me	H	H[a]	Boc[a]	2-thiazolyl	Ph	1
4.57, **4.78**	Me	H	H	H	Ph	Me	0
4.58, **4.79**	Me	H	H	H	2-thienyl	Me	0
4.59, **4.80**[7]	Me	H	H	H	Ph	Ph	0
4.60, **4.81**	Me	H	H	H	2-thiazolyl	Ph	0
4.61, **4.82**	H	H	Me	H	Ph	Me	1
4.62, **4.83**	H	H	Me	H	Ph	Ph	1

Scheme 4.4. Synthesis of the N^G-acylated imidazolylalkylguanidines **4.63-4.83** and the corresponding protected intermediates **4.42-4.62**. Reagents and conditions: (i) for **4.42-4.52** and **4.57-4.62**: CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF, 5 h, rt. (ii) for **4.53-4.56**: EDC · HCl (1.2 eq), DMAP (1.1 eq), DCM, 24 h, 0 °C → rt. (iii) TFA (20 %), DCM, 5 h, rt. [a] or R^3 = Boc and R^4 = H (mixture of isomers).

4.3 Pharmacological results and discussion

The potencies and efficacies of the synthesized compounds were evaluated on the four human HR subtypes in steady-state GTPase assays determining the enzymatic hydrolysis of radioactively labeled [γ-^{32}P]GTP after G-protein activation *via* the respective HR subtype (Table 4.1). These pharmacological studies were performed using membrane preparations of Sf9 insect cells expressing the hH_1R plus RGS4, the hH_2R-$G_{s\alpha S}$ fusion protein, the hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4 or the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$.[7, 22, 23]
In addition, selected compounds were evaluated for H_1R and H_2R activity at the guinea pig (gp) ileum and guinea pig right atrium, respectively (Table 4.2).

4.3.1 Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay

Modification of the chain connecting the imidazole ring with the acylguanidine moiety in **4.63-4.73** considerably changed the pharmacological activities of the compounds at the HR subtypes relative to the reference compounds **4.3-4.6**, containing a three-membered carbon chain. Shortening the chain length in **4.63** and **4.64** substantially increased potency at the hH_1R and yielded partial agonists. In particular **4.63**, with an efficacy of 0.5, confirmed the potential of some N^G-acylated imidazolylalkylguanidines to activate the hH_1R and to provide possible lead structures for the development of hH_1R agonists, as previously described by Xie et al. for the acylguanidine UR-AK57.[6] The decrease in efficacy of the diarylpropanoyl analog **4.65** at the hH_2R relative to **4.5** has already been reported (E_{max} = 0.69 → E_{max} = 0.17).[7] In contrast, **4.63** and **4.64**, containing smaller 3-arylbutanoyl residues, retained higher efficacies at this HR subtype (E_{max} ≈ 0.50). Potencies of the N^G-acylated imidazolylethyl-guanidines **4.63-4.65** at the hH_2R moderately decreased. Obviously, reducing chain length is better tolerated in terms of efficacy at the hH_2R for less bulky substituents, but is generally unfavorable with respect to both potency and efficacy. At the hH_3R, the activities of **4.63** and **4.64** were not substantially different from those of the reference compounds **4.3** and **4.4**. However, the diphenylpropanoyl analog **4.65** had drastically increased efficacy (E_{max} = 0.04 → E_{max} = 0.66) at this HR subtype. Apparently, the combination of an ethylene spacer between the imidazole ring and acylguanidine group and a bulky acyl substituent opens the route to acylguanidine-type compounds displaying highest efficacy at the hH_3R. At the hH_4R, **4.63-4.65** were moderate inverse agonists (K_B = 150 – 240 nM, E_{max} = -0.07 – -0.30).
The substantial influence of the chain length on the pharmacological activities at the HRs is also visible for **4.66-4.69**, containing a four-membered carbon chain. Contrary to the acylguanidines with an ethylene linker (**4.63-4.65**), **4.66-4.69** were devoid of hH_2R agonistic activity and showed neutral antagonist properties. The potencies of **4.66** and **4.67** carrying

3-arylbutanoyl residues were lowered, whereas potencies of the diarylpropanoyl analogs **4.68** and **4.69** were not affected relative to the reference compounds **4.3-4.6**. At the hH_3R, increasing chain length diminished efficacy and produced – with exception of **4.67** – inverse agonists. For the potencies a similar trend as that at the hH_2R was observed. Regarding the hH_4R, for **4.66-4.69** agonistic activity was shifted toward inverse agonism and potencies were 5 to 20 times lower compared to **4.3-4.6**.

Taken together, these findings evidence the distance between the imidazole ring and the acylguanidine group to largely affect HR activity. As already observed for the guanidine-type H_2R agonists,[1] a three-membered carbon chain proved to be favored for acylguanidine-type H_2R agonists, underlining the bioisosteric quality of the guanidine and the acylguanidine group.[7] Likewise, at the hH_3R, potencies in general peaked with a trimethylene spacer which also was required for agonistic activity of the compounds at the hH_4R. By contrast, a two-membered carbon chain was optimal for hH_1R activity.

The compounds chain-branched β to the imidazole ring (**4.70-4.73**) exhibited a certain decrease in potency at the hH_2R and low efficacies (E_{max} = 0.10 – 0.31). At the hH_3R, the 3-arylbutanoyl analogs **4.70** and **4.71** displayed 5- to 10-fold reduced potencies relative to the reference compounds **4.3** and **4.4** and were neutral antagonists. Contrary, the pharmacological properties of the diarylpropanoyl analogs **4.72** and **4.73** were just slightly affected. Regarding the hH_4R, β-methylation of the propyl chain was deleterious for agonistic efficacy and substantially lowered potencies. Obviously, the carbon chain of the N^G-acylated imidazolylpropylguanidines is sensitive toward substitution with respect to hH_2R and hH_4R activation. Steric factors may prevent the stabilization of an active receptor conformation with compounds **4.70-4.73**.

Besides methyl-branching of the spacer, a methyl substitution was introduced in position 5 of the imidazole ring (**4.74-4.77**). Potencies of **4.74-4.77** at the hH_2R slightly increased, whereas efficacies were remarkably lower compared to those of the reference compounds **4.3-4.6**. The activities of **4.74-4.77** at the hH_3R strongly depended on the acyl residue. **4.76** and **4.77**, containing bulky diarylpropanoyl moieties, displayed substantially reduced potencies and low efficacies (E_{max} = 0.03 – 0.16). In contrast, for the 3-arylbutanoyl analogs **4.74** and **4.75** potencies decreased just slightly relative to the reference compounds **4.3** and **4.4**, but an essential increase in efficacy was observed (E_{max} ≈ 0.85). Apparently, depending on the acyl residue, methyl substitution of the imidazole ring in position 5 provides highly potent hH_3R ligands with high efficacies. Unexpectedly, at the hH_4R this modification was not well tolerated since the compounds showed reduced potencies and drastically decreased efficacies relative to **4.3-4.6**. These results are in contrast to the pharmacological profile of 5-methylhistamine which binds > 500 times less potent to the hH_3R relative to histamine and was found to be a potent and selective full hH_4R agonist.[9] Obviously, identical structural

modifications at the imidazole ring of the N^G-acylated imidazolylpropylguanidines and histamine do not shift the pharmacological properties at the HRs toward the same direction. Therefore, different binding modes for the imidazole ring of the acylguanidine-type compounds and histamine can be expected both at the hH_3R and hH_4R.

Contrary to the selective hH_4R agonist 5-methylhistamine,[9] the evaluated acylguanidine analogs **4.78-4.81** exhibited no agonistic activity at the hH_2R and hH_4R. Moreover, in general the potencies at these HR subtypes were notedly decreased relative to the higher homologs **4.74-4.77**, again confirming a three-membered carbon chain to be more favorable for high potency and efficacy at the hH_2R and hH_4R. Interestingly, similar to **4.74-4.77**, the compounds bearing 3-arylbutanoyl residues (**4.78** and **4.79**) behaved as rather potent partial hH_3R agonists with up to 40-fold selectivity over all other HR subtypes, whereas **4.80** and **4.81**, carrying more bulky acyl groups, exerted considerably reduced activities devoid of agonistic efficacy.

Methylation of the guanidine-NH bearing the imidazolylpropyl portion in **4.82** and **4.83** resulted in dramatically reduced activities and a loss of agonistic efficacy at all HR subtypes. Obviously, this guanidine-NH group is crucial for the interaction with amino acid residues of the HRs, for example by forming a hydrogen bond. This experimental finding confirms results from an H_2R homology model, suggesting the acylguanidine group of N^G-acylated imidazolylpropylguanidines to form charge-assisted hydrogen bonds with the conserved Asp-98 in transmembrane domain 3 (TM3).[7, 24] As in the case of the hH_2R, the basic amino group of histamine is assumed to interact with the corresponding conserved Asp (Asp-94) in TM3 at the hH_4R.[25] Since the hH_4R agonistic efficacy is lost as well upon N-methylation of the guanidine moiety in **4.82** and **4.83**, a comparable interaction between the receptor protein and the acylguanidine group of N^G-acylated imidazolylpropylguanidines can be expected at both the hH_4R and the hH_2R.

Table 4.1. Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay.[a]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
histamine	190[5]	1.00		1,200[5]	1.00		25 ± 3.1	1.00	3	12 ± 2.5[26]	1.00	8
thioperamide	-	-		-	-		97 ± 18[27]	-0.71 ± 0.06[27]	5	110 ± 16[26]	-0.95 ± 0.07[26]	6
4.3 (UR-AK24)[6]	(>10,000)[7]	n.d.		67[6]	0.87[6]		2.5 ± 0.65	0.24 ± 0.02	2	15 ± 0.3	0.84 ± 0.06	2
4.4[7]	(>10,000)[7]	n.d.		110[7]	0.97[7]		1.6 ± 0.96	0.29 ± 0.01	2	6.3 ± 1.2	0.76 ± 0.03	3
4.5 (UR-PG80)[5]	(3,000)[7]	0.19[7]		78[5]	0.69[5]		(17 ± 1.5)	0.04 ± 0.05	2	8.6 ± 0.9	0.76 ± 0.03	2
4.6[7]	(>10,000)[7]	n.d.		550[7]	0.93[7]		(110 ± 4.5)	-0.20 ± 0.01	2	66 ± 10	0.47 ± 0.03	2
4.63	460 ± 24	0.50 ± 0.02	2	160 ± 19	0.52 ± 0.04	2	9.8 ± 3.4	0.22 ± 0.02	2	(240 ± 96)	-0.23 ± 0.08	2
4.64	440 ± 70	0.29 ± 0.02	2	410 ± 10	0.45 ± 0.06	2	9.6 ± 1.9	0.30 ± 0.06	2	(150 ± 21)	-0.07 ± 0.05	2
4.65[5]	2,300[5]	0.35[5]		190[5]	0.17[5]		17 ± 2.4	0.66 ± 0.02	2	(220 ± 29)	-0.30 ± 0.11	2
4.66	(8,000 ± 260)	0.02 ± 0.07	2	(230 ± 100)	0.02 ± 0.01	2	(9.6 ± 0.9)	-0.27 ± 0.04	2	(100 ± 25)	-0.72 ± 0.08	3
4.67	n.d.	n.d.		(590 ± 11)	-0.01 ± 0.04	2	(12 ± 2.1)	0.09 ± 0.01	2	(140 ± 1.6)	-0.58 ± 0.20	2
4.68	(8,500 ± 250)	0.00 ± 0.02	2	(79 ± 14)	-0.09 ± 0.03	2	(11 ± 3.4)	-0.58 ± 0.02	2	(140 ± 69)	-0.75 ± 0.25	2
4.69	n.d.	n.d.		(870 ± 31)	-0.01 ± 0.04	2	(62 ± 8.1)	-0.54 ± 0.01	2	(500 ± 154)	-0.30 ± 0.15	2
4.70	(5,300 ± 450)	0.08 ± 0.05	2	490 ± 53	0.31 ± 0.01	3	(24 ± 5.5)	-0.01 ± 0.01	2	(280 ± 86)	-0.07 ± 0.03	2
4.71	n.d.	n.d.		420 ± 46	0.30 ± 0.03	3	(11 ± 2.9)	-0.01 ± 0.02	2	(56 ± 18)	0.13 ± 0.00	2
4.72	(4,200 ± 890)	0.04 ± 0.02	2	200 ± 11	0.10 ± 0.01	3	(18 ± 5.4)	-0.23 ± 0.03	2	(330 ± 140)	-0.54 ± 0.09	2
4.73	n.d.	n.d.		790 ± 250	0.18 ± 0.01	3	(75 ± 7.6)	-0.26 ± 0.00	2	(1,000 ± 170)	-0.22 ± 0.03	2
4.74	(>10,000)	0.11 ± 0.06	2	34 ± 8.0	0.44 ± 0.01	2	8.7 ± 1.1	0.84 ± 0.10	2	(88 ± 43)	0.24 ± 0.03	3

Table 4.1. (continued)

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
4.75	n.d.	n.d.		20 ± 0.9	0.40 ± 0.01	2	6.1 ± 0.5	0.88 ± 0.00	2	(35 ± 22)	0.13 ± 0.11	2
4.76	(5,200 ± 1,300)	0.03 ± 0.01	2	17 ± 2.8	0.24 ± 0.02	3	(120 ± 11)	0.03 ± 0.05	2	(260 ± 130)	-0.27 ± 0.08	2
4.77	n.d.	n.d.		230 ± 49	0.31 ± 0.01	2	(490 ± 30)	0.16 ± 0.03	2	(230 ± 83)	0.29 ± 0.09	2
4.78	(>10,000)	0.01 ± 0.02	2	(550 ± 130)	-0.06 ± 0.04	2	9.7 ± 0.7	0.56 ± 0.04	2	(380 ± 160)	-0.55 ± 0.03	3
4.79	n.d.	n.d.		(350 ± 49)	-0.06 ± 0.02	3	12 ± 1.5	0.55 ± 0.05	2	(350 ± 170)	-0.33 ± 0.10	3
4.80	(>10,000)	0.02 ± 0.01	2	(240 ± 67)	-0.06 ± 0.05	2	(470 ± 200)	0.02 ± 0.03	2	(170 ± 57)	-0.65 ± 0.09	3
4.81	n.d.	n.d.		(1,400 ± 160)	-0.06 ± 0.00	2	(230 ± 15)	-0.19 ± 0.02	2	(460 ± 33)	0.04 ± 0.06	2
4.82	inactive		1	(>10,000)	n.d.	2	(180 ± 2.9)	-0.56 ± 0.02	2	(6,900 ± 2,600)	-0.10 ± 0.05	2
4.83	n.d.	n.d.		(>10,000)	-0.03 ± 0.04	2	(340 ± 67)	-0.46 ± 0.02	2	(9,600 ± 3,600)	-0.12 ± 0.04	2

[a] Steady-state GTPase activity in Sf9 insect cell membranes expressing the hH_1R + RGS4, hH_2R-$G_{s\alpha S}$ fusion protein, hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ + RGS4 or hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ was determined as described under *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Typical basal GTPase activities in the hH_1R assay ranged between ≈ 1.0 and 1.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 100 to 150 % above basal. Typical basal GTPase activities in the hH_2R assay ranged between ≈ 1.0 and 2.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 250 to 300 % above basal. Typical basal GTPase activities in the hH_3R assay ranged between ≈ 3.0 and 4.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to ≈ 60 % above basal. Typical basal GTPase activities in the hH_4R assay ranged between ≈ 2.5 and 3.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to 60 to 70 % above basal. n gives the number of independent experiments performed in duplicate each. For compounds investigated in the antagonist mode (K_B values), E_{max} values were determined at a concentration of 10 µM. n.d.: not determined.

4.3.2 Potencies and efficacies of selected compounds at the guinea pig ileum (gpH_1R) and guinea pig right atrium (gpH_2R)

The investigated N^G-acylated imidazolylalkylguanidines (Table 4.2) behaved as weak antagonists with pA_2 values in the range of 5 – 6 at the guinea pig ileum.

At the gpH_2R, all investigated compounds suffered a decrease in potency relative to the compounds **4.3-4.6** but usually partial agonistic activity retained. Increasing the chain length between the imidazole ring and the acylguanidine group by one methylene group (**4.66**) substantially lowered potency and efficacy at the gpH_2R relative to **4.3**. This observation is in agreement with data for the higher homolog of impromidine (**4.1**) that likewise proved to be significantly less potent and efficacious at the gpH_2R.[1] In contrast, at the human isoform the agonistic efficacy of **4.66** determined in GTPase assays was completely abolished.

Methylation of the carbon chain in β-position position to the imidazole ring in **4.70** decreased potency at the gpH_2R compared to compound **4.3**, whereas efficacy was just moderately reduced. This corresponds to the behavior of the impromidine-like counterpart that also showed a diminished potency and efficacy at the gpH_2R when a methyl group was introduced.[8] At the hH_2R, the decrease in efficacy of compound **4.70** was considerably more pronounced.

A methyl group in position 5 of the imidazole ring resulted in compounds **4.74-4.77**, displaying comparably high efficacies relative to **4.3-4.6** at the gpH_2R. However, the potencies were about 5-fold lower. This is contrary to the human isoform, where efficacy substantially decreased, whereas potency even slightly increased. Obviously, an active conformation of the gpH_2R can be well stabilized regardless of the presence of a methyl group at the imidazole ring, whereas at the human receptor the 5-methyl group is deleterious for agonistic efficacy.

Compared to the reference compounds **4.3-4.6**, potencies of the 5-methylhistamine analogs **4.78-4.81** dropped by almost two orders of magnitude at the gpH_2R and also efficacies decreased, in particular for **4.80** and **4.81**, bearing a bulky diarylpropanoyl residue. In contrast, at the hH_2R, **4.78-4.81** exhibited no agonistic efficacy.

Methylation of the guanidine-NH adjacent to the imidazolylpropyl group (**4.82**) yielded a virtually inactive compound at the guinea pig right atrium. As observed at the hH_2R, the unsubstituted guanidine-NH is crucial for receptor binding. In the arpromidine (**4.2**) series, methylation of the opposite guanidine-NH, bearing the diarylpropyl residue, caused a drastic reduction of potency at the gpH_2R but the compound still displayed nearly full agonistic efficacy (E_{max} = 0.8).[3] Obviously, the guanidine-NH attached to the imidazolylpropyl group seems to be more critical for the interaction with the gpH_2R, in particular, with respect to receptor activation.

The evaluated compounds turned out to be more potent at the hH_2R than at the gpH_2R. This may appear exceptional as guanidine- and acylguanidine-type H_2R agonists have usually been more potent at the gpH_2R.[5, 6, 28] However, it has to be considered that the guinea pig right atrium and the GTPase activity assay are different pharmacological analysis systems, in particular with regard to the receptor system (H_2R-$G_{s\alpha S}$ fusion protein *versus* native non-fused receptor), read-out or different access to the receptors in membrane preparations and isolated organs. Actually, potencies of acylguanidine-type H_2R agonists determined at the guinea pig right atrium were lower than potencies determined in the GTPase assay on the gpH_2R-$G_{s\alpha S}$ fusion protein.[29] This observation may explain the lower potencies of the evaluated compounds at the gpH_2R (guinea pig right atrium) relative to the hH_2R (GTPase assay).

Table 4.2. Pharmacological activities of selected compounds at the guinea pig ileum (gpH_1R) and the guinea pig right atrium (gpH_2R).

Compound	gpH_1R		gpH_2R		
	pA_2	n[a]	pEC_{50}[b] /(pA_2)/[pD_2']	E_{max}[c]	n[a]
histamine	-	-	6.00 ± 0.10	1.00 ± 0.02	> 50
4.3 (UR-AK24)[7]	5.87 ± 0.14	2	7.80 ± 0.07	0.99 ± 0.02	4
4.4[7]	n.d.		7.42 ± 0.10	0.99 ± 0.02	3
4.5 (UR-PG80)[7]	6.13 ± 0.05	10	7.55 ± 0.09	0.85 ± 0.03	5
4.6[7]	5.33 ± 0.05	18	7.42 ± 0.03	1.00 ± 0.01	3
4.66	5.66 ± 0.04	18	5.92 ± 0.11	0.47 ± 0.01	2
4.70	5.88 ± 0.12	18	6.11 ± 0.16	0.80 ± 0.04	3
4.74	5.50 ± 0.02	12	7.11 ± 0.08	0.98 ± 0.03	3
4.75	5.77 ± 0.03	12	6.68 ± 0.11	0.94 ± 0.03	2
4.76	5.65 ± 0.05	8	6.75 ± 0.23	0.73 ± 0.04	4
4.77	5.45 ± 0.04	12	6.80 ± 0.02	1.03 ± 0.09	2
4.78	5.51 ± 0.04	6	5.71 ± 0.04	0.78 ± 0.02	2
4.79	5.49 ± 0.03	12	5.69 ± 0.11	0.72 ± 0.05	2
4.80	5.30 ± 0.03	12	5.71 ± 0.01	0.44 ± 0.01	2
4.81	4.88 ± 0.08	8	5.40 ± 0.13	0.43 ± 0.05	2
4.82	< 4.5	18	(< 4.3) [4.08 ± 0.05]	< 0.10	3

[a] number of experiments, [b] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$. Summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, n = 16). The SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50}, [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine; n.d.: not determined.

4.3.3 Summary and conclusion

Regarding the hH_1R, potencies and agonistic efficacies could be increased by shortening the chain length separating the imidazole ring from the acylguanidine group (**4.63-4.64**). All other modifications produced just poorly active compounds.

A three-membered carbon chain separating the imidazole ring from the acylguanidine group proved to be crucial for high hH_2R agonistic potency. Higher (**4.66-4.69**) and lower homologs (**4.63-4.65**) as well as methyl-branched analogs (**4.70-4.73**) were unfavorable with respect to both potency and efficacy, confirming findings from guanidine-type H_2R agonists.[1, 8] Methylation of the imidazole ring in position 5 (**4.74-4.77**) slightly increased potency, but considerably reduced efficacy at the hH_2R. Taken together, in this series of compounds all performed modifications significantly reduced efficacy at the hH_2R confirming an imidazolylpropylguanidine group to be essential for potent acylguanidine-type hH_2R agonists.

Concerning gpH_2R agonism, the structural modifications reduced potencies relative to **4.3-4.6**. However, the imidazolylpropylguanidine portion was less sensitive toward modifications concerning agonistic activity at this species isoform than at the human counterpart. This offers the opportunity to obtain compounds with species-dependent efficacies. Such compounds may be of interest as tools to study differences in the activation of both H_2R species isoforms.

Many of the structural modifications affected H_2R activity in a similar way as previously observed in the class of guanidine-type H_2R agonists.[1, 3, 8] This further confirms the acylguanidine group to be an appropriate bioisostere with reduced basicity for the guanidine moiety.[7]

Most striking concerning the hH_3R was the ability to essentially increase efficacy of acylguanidine-type compounds by modifying the imidazolylpropylguanidine portion. In particular, introduction of a methyl group to the imidazole ring in position 5 opened access to compounds showing – in contrast to the reference compounds – both highest potency and efficacy at this HR subtype (**4.74**, **4.75**, **4.78** and **4.79**). These findings disclose the possibility to develop selective acylguanidine-type hH_3R agonists. In contrast to standard H_3R agonists such as (*R*)-α-methylhistamine, which features insufficient peroral absorption and poor brain penetration[30], N^G-acylated imidazolylpropylguanidines have been shown to be orally bioavailable and to penetrate the blood-brain barrier in mice.[7] Therefore, such acylguanidine-type H_3R agonists may become useful pharmacological probes for further studying the role of the H_3R in the CNS. In addition, H_3R agonists have been discussed as possible drugs for the treatment of insomnia[31], pain[32], myocardial ischaemic arrhythmias[33] and neurogenic inflammation[34, 35].

Concerning the hH_4R, the imidazolylpropylguanidine group was extremely sensitive toward structural variations. All compounds showed lower potencies compared to the reference compounds and negligible agonistic efficacies.

In summary, potencies, efficacies and HR subtype selectivities of the N^G-acylated imidazolylpropylguanidines **4.3-4.6** could be broadly altered by modifying the imidazolylpropylguanidine portion. The ability of N^G-acylated imidazolylalkylguanidines to provide ligands for all four HR subtypes further confirms this moiety to serve as a "privileged structure".[7] Therefore, this structural motif is a promising building block for the development of potent and selective HR ligands as pharmacological tools, including compounds with combined activities at certain HR subtypes.

4.4 Experimental section

4.4.1 Chemistry

4.4.1.1 General conditions

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany), TCI Europe (Zwijndrecht, Belgium) and used without further purification. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen). Anhydrous DMF was purchased from Sigma-Aldrich Chemie GmbH. 3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol was a gift from Prof. Dr. Sigurd Elz, Department of Pharmaceutical/Medicinal Chemistry I, University of Regensburg. Flash chromatography was performed on silica gel (Merck silica gel 60, 40 - 63 µM). Reactions were monitored by TLC on aluminum plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm). The compounds were detected by UV light (254 nm), a 0.3 % solution of ninhydrine in *n*-butanol (amines) or a 1.0 % solution of Fast Blue B salt (Sigma-Aldrich Chemie GmbH) in $EtOH/H_2O$ = 30/70 (v/v). All melting points are uncorrected and were measured on a Büchi 530 apparatus (Büchi GmbH, Essen, Germany).

Nuclear Magnetic Resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz) or Bruker Avance 600 (^{1}H: 600.1 MHz; ^{13}C: 150.9 MHz) NMR spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts are given in δ (ppm) relative to external standards. Abbreviations for the multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), brs (for

broad singlet) and combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) was determined by DEPT 135 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. In certain cases 2D-NMR techniques (COSY, HMQC, HSQC, HMBC, NOESY) were used to assign ^{1}H and ^{13}C chemical shifts. Infrared spectra (IR) were measured on a Bruker Tensor 27 spectrometer equipped with an ATR (attenuated total reflexion) unit from Harrick Scientific Products Inc. (Ossining/NY, US). Mass spectra (MS) were recorded on a Finnigan MAT 95 (EI-MS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ES-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Elemental analysis (C, H, N, Heraeus Elementar Vario EL III) were performed by the Analytical Department of the University Regensburg and are within ± 0.4 % unless otherwise noted.

Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was Eurosphere-100 (250 x 32 mm) (Knauer), which was attached to a UV-detector model K-2000 (Knauer). UV detection of the compounds was done at 210 nm. The temperature was 25 °C and the flow rate 37 ml/min. The mobile phase was 0.1 % TFA in millipore water and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products (TSP) equipped with a SN 400 controller, P4000 pump, an AS3000 autosampler and a Spectra Focus UV/VIS detector. Stationary phase was a Eurosphere-100 C-18 (250 x 4.0 mm, 5 µm) column (Knauer) thermostated at 30 °C. The flow rate was 0.8 ml/min and the dead time (t_0) was 3.32 min. As mobile phase gradients of MeCN/0.05 % TFA (aq.) were used and the absorbance was detected at 210 nm. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 210 nm. HPLC conditions, retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and purities of the synthesized compounds are listed in the appendix (Chapter 9).

4.4.1.2 Preparation of the 2-(1-trityl-1H-imidazol-4-yl)ethanamines 4.13 and 4.14

1-Trityl-1*H*-imidazole-4-carbaldehyde[36] (4.7)

To a solution of 1*H*-imidazole-4-carbaldehyde (5.0 g, 52.0 mmol) and trityl chloride (16.0 g, 57.2 mmol) in MeCN (165 mL), NEt_3 (13.0 mL, 93.6 mmol) was added dropwise. After 20 h, hexane (17 mL) and H_2O (170 mL) were added and the mixture was stirred for 30 min. The precipitated product was filtered and washed with H_2O (2 x 20 mL). Recrystallization from EtOAc/hexane yielded a beige solid (15.1 g, 86 %); mp 192 – 194 °C (ref.[36]: 193 – 196 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.06 – 7.16 (m, 6H, Ph-***H***), 7.31 – 7.41 (m, 9H, Ph-***H***), 7.53 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.61 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 9.88 (s, 1H, CO***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 76.39 (C_{quat}, ***C***Ph_3), 126.80 (+, Im-***C***-5), 128.42 (+, 6 Ph-***C***),

128.57 (+, 3 Ph-***C***-4), 129.69 (+, 6 Ph-***C***), 140.65 (+, Im-***C***-2), 140.90 (C_{quat}, Im-***C***-2), 141.56 (C_{quat}, 3 Ph-***C***-1), 186.61 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 677 (4) [2M + H]$^+$, 243 (100) [CPh_3^+]. Anal. ($C_{23}H_{18}N_2O$) C, H, N. $C_{23}H_{18}N_2O$ (338.40).

5-Methyl-1-trityl-1*H*-imidazole-4-carbaldehyde (4.8)[37]

To a solution of 5-methyl-1-trityl-1*H*-imidazole-4-carbaldehyde (5.3 g, 48.1 mmol) and trityl chloride (14.7 g, 52.9 mmol) in MeCN (160 mL), NEt_3 (12.1 mL, 86.6 mmol) was added dropwise. After 20 h, hexane (16 mL) and H_2O (160 mL) were added and the mixture was stirred for 30 min. The precipitated product was filtered and washed with H_2O (2 x 20 mL). Recrystallization from EtOAc/hexane yielded a beige solid (15.3 g, 90 %); mp 162 – 165 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.55 (s, 3H, C***H***$_3$), 7.12 – 7.21 (m, 6H, Ph-***H***), 7.30 – 7.39 (m, 10H, Ph-***H*** + Im-2-***H***), 9.11 (s, 1H, CO***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 15.77 (+, ***C***H$_3$), 76.19 (C_{quat}, ***C***Ph$_3$), 128.43 (+, 6 Ph-***C***), 128.47 (+, 3 Ph-***C***-4), 128.58 (C_{quat}, Im-***C***-4), 129.59 (+, 6 Ph-***C***), 141.53 (+, Im-***C***-2), 141.58 (C_{quat}, 3 Ph-***C***-1), 151.68 (C_{quat}, Im-***C***-5), 181.17 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 353 (9) [M + H]$^+$, 243 (100) [CPh_3^+]. Anal. ($C_{24}H_{20}N_2O$) C, H, N. $C_{24}H_{20}N_2O$ (352.43).

(*E*)-4-(2-Nitrovinyl)-1-trityl-1*H*-imidazole (4.9)[38]

A mixture of **4.7** (6.8 g, 20.1 mmol) and NH_4OAc (1.7 g, 22.1 mmol) in an excess of nitromethane (55 mL) was stirred at 50 °C for 5 h (light protection!). After removing the solvent *in vacuo*, the remaining residue was triturated with H_2O (60 mL), filtered and washed with Et_2O (2 x 20 mL) yielding a yellow solid (6.4 g, 84 %); mp 218 – 220 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.07 – 7.17 (m, 6H, Ph-***H***), 7.23 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.32 – 7.41 (m, 9H, Ph-***H***), 7.52 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.76 (d, 1H, ^3J = 13.1 Hz, vinyl-C***H***), 7.83 (d, 1H, ^3J = 13.1 Hz, vinyl-C***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 76.22 (C_{quat}, ***C***Ph$_3$), 127.38 (+, Im-***C***-5), 128.40 (+, 6 Ph-***C***), 128.56 (+, 3 Ph-***C***), 129.65 (+, 6 Ph-***C***), 130.88 (+, vinyl-***C***), 133.01 (C_{quat}, Im-***C***-4), 135.81 (+, vinyl-***C***), 141.29 (+, Im-***C***-2), 141.64 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 382 (3) [M + H]$^+$, 243 (100) [CPh_3^+]. Anal. ($C_{24}H_{19}N_3O_2$) C, H, N. $C_{24}H_{19}N_3O_2$ (381.43).

(*E*)-5-Methyl-4-(2-nitrovinyl)-1-trityl-1*H*-imidazole (4.10)

A mixture of **4.8** (8.5 g, 24.2 mmol) and NH_4OAc (2.1 g, 26.6 mmol) in an excess of nitromethane (65 mL) was stirred at 50 °C for 5 h (light protection!). After removing the solvent *in vacuo*, the remaining residue was taken up in DCM (100 mL) and extracted with water (2 x 30 mL). The organic layer was evaporated and the crude product was crystallized from MeOH/Et_2O yielding a yellow solid (7.9 g, 82 %); mp 176 – 178 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.62 (s, 3H, C***H***$_3$), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.33 – 7.41 (m, 9H, Ph-***H***),

7.46 (s, 1H, Im-2***H***), 7.83 (d, 1H, ^3J = 13.0 Hz, vinyl-C***H***), 7.88 (d, 1H, ^3J = 13.0 Hz, vinyl-C***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 12.21 (+, ***C***H_3), 75.88 (C_{quat}, ***C***Ph_3), 128.38 (+, 6 Ph-***C***), 128.40 (+, 3 Ph-***C***-4), 129.91 (+, 6 Ph-***C***), 130.13 (+, vinyl-***C***), 132.84 (C_{quat}, Im-***C***-4), 134.99 (+, vinyl-***C***), 137.15 (C_{quat}, Im-***C***-5), 140.40 (+, Im-***C***-2), 140.94 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 396 (100) $[M + H]^+$. Anal. ($C_{25}H_{21}N_3O_2$) C, H, N. $C_{25}H_{21}N_3O_2$ (395.45).

2-(1-Trityl-1*H*-imidazol-4-yl)acetaldehyde oxime (4.11)

To a solution of **4.9** (6.3 g, 16.6 mmol) and Pd/C (10 %) (0.40 g, cat.) in THF (100 mL), a solution of $NaH_2PO_2 \cdot H_2O$ (19.2 g, 181 mmol) in H_2O (50 mL) was added dropwise. After addition was complete, the mixture was stirred for 1 h. The catalyst was removed by filtration over Celite and EtOAc (50 mL) was added to the filtrate. The filtrate was washed with a saturated K_2CO_3 solution (100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Crystallization from MeCN gave a beige solid (3.5 g, 57 %); mp 182 – 184 °C (dec.). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 3.46 (d, 2H, ^3J = 5.0 Hz, C***H***$_2$), 6.75 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 6.81 (t, 1H, ^3J = 5.0 Hz, C***H***NOH), 7.05 – 7.15 (m, 6H, Ph-***H***), 7.30 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.31 – 7.47 (m, 9H, Ph-***H***), 10.90 (s, 1H, O***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 24.40 (-, Im-4-***C***H_2), 74.45 (C_{quat}, ***C***Ph_3), 118.15 (+, Im-***C***-5), 127.89 (+, 3 Ph-***C***-4), 128.12 (+, 6 Ph-***C***), 129.13 (+, 6 Ph-***C***), 136.53 (C_{quat}, Im-***C***-4), 137.90 (+, Im-***C***-2), 142.21 (C_{quat}, 3 Ph-***C***-1), 148.04 (+, ***C***HNOH). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 368 (56) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{24}H_{21}N_3O \cdot 0.25\ H_2O$) C, H, N. $C_{24}H_{21}N_3O$ (367.44).

2-(5-Methyl-1-trityl-1*H*-imidazol-4-yl)acetaldehyde oxime (4.12)

To a solution of **4.10** (6.3 g, 15.9 mmol) and Pd/C (10 %) (0.38 g, cat.) in THF (100 mL), a solution of $NaH_2PO_2 \cdot H_2O$ (18.3 g, 173 mmol) in H_2O (50 mL) was added dropwise. After addition was complete, the mixture was stirred for 1 h. The catalyst was removed by filtration over Celite and EtOAc (50 mL) was added to the filtrate. The filtrate was washed with a saturated K_2CO_3 solution (100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. After addition of MeCN, the product precipitated and was recrystallized from MeCN/EtOAc yielding a beige solid (4.1 g, 68 %); mp 188 – 189 °C (dec.). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.39 (s, 3H, C***H***$_3$), 3.42 (d, 0.6H, ^3J = 6.2 Hz, C***H***$_2$), 3.63 (d, 1.4 H, ^3J = 5.1 Hz, C***H***$_2$), 6.91 (t, 0.7H, ^3J = 5.1 Hz, C***H***NOH), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.28 – 7.36 (m, 10H, Ph-***H*** + Im-2-***H***), 7.50 (t, 0.3H, ^3J = 6.2 Hz, C***H***NOH), 10.44 (brs, 1H, O***H***). ^{13}C-NMR (75 MHz, $CDCl_3$) dominating isomer: δ [ppm] = 11.62 (+, ***C***H_3), 23.68 (-, Im-4-***C***H_2), 75.06 (C_{quat}, ***C***Ph_3), 126.13 (C_{quat}, Im-***C***), 127.93 (+, 3 Ph-***C***-4), 128.05 (+, 6 Ph-***C***), 130.09 (+, 6 Ph-***C***), 135.36 (C_{quat}, Im-***C***), 137.15 (+, Im-***C***-2), 141.77 (C_{quat}, 3 Ph-***C***-1), 149.88 (+, ***C***HNOH). ES-MS (DCM/MeOH +

NH_4OAc) *m/z* (%): 382 (100) $[M + H]^+$. Anal. ($C_{25}H_{23}N_3O \cdot 0.75\ H_2O$) C, H, N. $C_{25}H_{23}N_3O$ (381.47).

2-(1-Trityl-1*H*-imidazol-4-yl)ethanamine (4.13)[39]

To a mixture of $LiAlH_4$ (0.99 g, 26.1 mmol) in THF_{abs} (60 mL), **4.11** (3.2 g, 8.7 mmol) was added in portions at 0 °C. The mixture was allowed to warm to room temperature. After stirring for 3 h, the mixture was cooled externally with ice and 2.6 mL H_2O, 2.6 mL NaOH 15 % and 10.4 mL H_2O were consecutively added. The insoluble material was removed by filtration and washed with THF (2 x 20 mL). The combined organic layers were washed with a saturated solution of K_2CO_3, dried over Na_2SO_4 and evaporation of the solvent yielded a brownish foam-like solid that was used without further purification. (2.8 g, 91 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.66 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 2.96 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$-NH_2), 6.59 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.07 – 7.18 (m, 6H, Ph-***H***), 7.24 – 7.39 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 32.61 (-, Im-4-***C***H_2), 41.94 (-, ***C***H_2-NH_2), 75.15 (C_{quat}, ***C***Ph_3), 118.62 (+, Im-***C***-5), 128.02 (+, 3 Ph-***C***-4), 128.04 (+, 6 Ph-***C***), 129.79 (+, 6 Ph-***C***), 138.70 (+, Im-***C***-2), 139.50 (C_{quat}, Im-***C***-4), 142.58 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 354 (26) $[M + H]^+$, 243 (100) $[CPh_3^+]$. $C_{24}H_{23}N_3$ (353.46).

2-(5-Methyl-1-trityl-1*H*-imidazol-4-yl)ethanamine (4.14)

To a mixture of $LiAlH_4$ (0.87 g, 22.8 mmol) in THF_{abs} (50 mL), **4.12** (2.9 g, 7.6 mmol) was added in portions at 0 °C. The mixture was allowed to warm to room temperature. After stirring for 3 h, the mixture was cooled externally with ice and 2.3 mL H_2O, 2.3 mL NaOH 15 % and 9.2 mL H_2O were consecutively added. The insoluble material was removed by filtration and washed with THF (2 x 15 mL). The combined organic layers were washed with a saturated solution of K_2CO_3, dried over Na_2SO_4 and evaporation of the solvent yielded a pale yellow foam-like solid that was used without further purification. (2.2 g, 76 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.32 (s, 3H, C***H***$_3$), 2.49 (t, 2H, overlap with solvent, ^3J = 7.0 Hz, Im-4-C***H***$_2$), 2.79 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$-NH_2), 7.04 – 7.13 (m, 6H, Ph-***H***), 7.33 – 7.46 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 11.27 (+, ***C***H_3), 29.57 (-, Im-4-***C***H_2), 41.20 (-, ***C***H_2-NH_2), 74.02 (C_{quat}, ***C***Ph_3), 124.83 (C_{quat}, Im-***C***-5), 127.81 (+, 3 Ph-***C***-4), 128.07 (+, 6 Ph-***C***), 129.44 (+, 6 Ph-***C***), 136.31 (+, Im-***C***-2), 137.67 (C_{quat}, Im-***C***-4), 141.54 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 368 (100) $[M + H]^+$. $C_{25}H_{25}N_3$ (367.49).

4.4.1.3 Preparation of the 3-(1-trityl-1H-imidazol-4-yl)propan-1-ols 4.19 and 4.20

(*E*)-Ethyl 3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)acrylate (4.15)[40]

To a solution of triethyl phosphonoacetate (14.6 g, 13.0 mL, 65.1 mmol) in THF_{abs} (250 mL), NaH (60 % dispersion in mineral oil) (2.60 g, 65.1 mmol) was added in portions. After stirring for 1 h at ambient temperature, a solution of **4.8** (15.3 g, 43.4 mmol) in THF_{abs} (150 mL) was added dropwise. When addition was complete, the mixture was refluxed overnight. The solvent was evaporated and the crude product was dissolved in EtOAc (200 mL) and washed with water (3 x 60 mL). The organic layer was dried over Na_2SO_4, evaporated and the crude product purified by flash chromatography (PE/EtOAc 80/20 v/v). Recrystallization from EtOAc/hexane yielded the *E*-isomer as white solid (13.2 g, 72 %); mp 175 – 176 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.29 (t, 3H, ^3J = 7.1 Hz, CH_2CH_3), 1.57 (s, 3H, Im-5-C***H***$_3$), 4.22 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 6.58 (d, 1H, ^3J = 15.4 Hz, C***H***CO), 7.09 – 7.19 (m, 6H, Ph-***H***), 7.28 – 7.41 (m, 10H, Ph-***H*** + Im-2-***H***), 7.56 (d, 1H, ^3J = 15.4 Hz, Im-4-C***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 11.99 (+, Im-5-***C***H$_3$), 14.44 (+, CH_2***C***H$_3$), 60.12 (-, ***C***H$_2$CH$_3$), 75.40 (C_{quat}, ***C***Ph$_3$), 115.36 (+, ***C***HCO), 128.15 (+, 3 Ph-***C***-4), 128.21 (+, 6 Ph-***C***), 129.99 (+, 6 Ph-***C***), 132.90 (C_{quat}, Im-***C***), 135.11 (+, Im-4-***C***H), 136.40 (C_{quat}, Im-***C***), 139.43 (+, Im-***C***-2), 141.38 (C_{quat}, 3 Ph-***C***-1), 167.95 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 423 (100) $[M + H]^+$. Anal. ($C_{28}H_{26}N_2O_2$) C, H, N. $C_{28}H_{26}N_2O_2$ (422.52).

(*E*)-Ethyl 2-methyl-3-(1-trityl-1*H*-imidazol-4-yl)acrylate (4.16)

To a solution of triethyl phosphonoacetate (9.0 g, 8.1 mL, 37.8 mmol) in THF_{abs} (150 mL), NaH (60 % dispersion in mineral oil) (1.51 g, 37.8 mmol) was added in portions. After stirring for 1 h at ambient temperature, a solution of **4.7** (8.54 g, 25.2 mmol) in THF_{abs} (75 mL) was added dropwise. When addition was complete, the mixture was refluxed overnight. The solvent was evaporated and the crude product was taken up in EtOAc (150 mL) and washed with water (3 x 50 mL). The organic layer was dried over Na_2SO_4, evaporated and the crude product purified by flash chromatography (PE/EtOAc 70/30 v/v). Recrystallization from EtOAc/hexane yielded the *E*-isomer as white solid (7.9 g, 74 %); mp 158 – 159 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.30 (t, 3H, ^3J = 7.1 Hz, CH_2CH_3), 2.20 (d, 3H, ^4J = 1.8 Hz, CH=CC***H***$_3$), 4.21 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 7.02 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.11 – 7.19 (m, 6H, Ph-***H***), 7.30 – 7.39 (m, 9H, Ph-***H***), 7.51 – 7.53 (m, 2H, Im-2-***H*** + C***H***=C). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.14 (+, ***C***H$_3$), 14.40 (+, ***C***H$_3$), 60.61 (-, ***C***H$_2$CH$_3$), 75.71 (C_{quat}, ***C***Ph$_3$), 124.61 (+, Im-***C***-5), 125.47 (C_{quat}, CH=***C***), 128.22 (+, 6 Ph-***C***), 128.29 (+, 3 Ph-***C***-4), 129.75 (+, 6-Ph-***C***), 130.47 (+, ***C***H=CCH$_3$), 138.03 (C_{quat}, Im-***C***-4), 139.51 (+, Im-***C***-2), 142.07 (C_{quat}, 3 Ph-***C***-4), 169.00 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 423 (56) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{28}H_{26}N_2O_2$) C, H, N. $C_{28}H_{26}N_2O_2$ (422.52).

Ethyl 3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propanoate (4.17)

To a solution of **4.15** (6.7 g, 15.9 mmol) in EtOH (150 mL), Pd/C (10 %) (0.67 g, cat.) was added. After stirring for 3 h at room temperature (TLC control) under a hydrogen atmosphere, the catalyst was removed by filtration over Celite and the solvent was evaporated. The crude product was purified by flash chromatography (PE/EtOAc 60/40 v/v) yielding a colorless oil that solidified *in vacuo* (white solid, 6.4 g, 94 %); mp 94 – 95 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.24 (t, 3H, ^{3}J = 7.1 Hz, $CH_2C\boldsymbol{H}_3$), 1.39 (s, 3H, Im-5-C$\boldsymbol{H}_3$), 2.68 (t, 2H, ^{3}J = 7.4 Hz, C$\boldsymbol{H}_2$), 2.79 (t, 2H, ^{3}J = 7.4 Hz, C$\boldsymbol{H}_2$), 4.11 (q, 2H, ^{3}J = 7.1 Hz, C$\boldsymbol{H}_2CH_3$), 7.08 – 7.17 (m, 6H, Ph-***H***), 7.22 (s, 1H, Im-2-***H***), 7.28 – 7.37 (m, 9H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 11.63 (+, Im-5-***C***H_3), 14.31 (+, $CH_2\boldsymbol{C}H_3$), 22.73 (-, Im-4-***C***H_2), 34.25 (-, Im-4-CH_2-***C***H_2), 60.22 (-, ***C***H_2CH_3), 74.78 (C_{quat}, ***C***Ph_3), 125.22 (C_{quat}, Im-***C***), 127.81 (+, 3 Ph-***C***-4), 127.95 (+, 6 Ph-***C***), 130.11 (+, 6 Ph-***C***), 137.42 (+, Im-***C***-2), 138.54 (C_{quat}, Im-***C***), 142.04 (C_{quat}, 3 Ph-***C***-1), 173.45 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 425 (100) $[M + H]^+$. Anal. ($C_{28}H_{28}N_2O_2$) C, H, N. $C_{28}H_{28}N_2O_2$ (424.53).

Ethyl 2-methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanoate (4.18)

To a solution of **4.16** (7.5 g, 17.8 mmol) in a mixture of EtOH (200 mL) and THF (50 mL), Pd/C (10 %) (0.75 g, cat.) was added. After stirring for 3 h at room temperature (TLC control) under a hydrogen atmosphere, the catalyst was removed by filtration over Celite and the solvent was evaporated. The crude product was purified by flash chromatography (PE/EtOAc 60/40 v/v) yielding a white solid. (7.0 g, 93 %); mp 138 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.15 (d, 3H, ^{3}J = 6.9 Hz, Im-4-CH_2-CH-C$\boldsymbol{H}_3$), 1.16 (t, 3H, ^{3}J = 7.1 Hz, $CH_2C\boldsymbol{H}_3$), 2.61 (dd, 1H, ^{2}J = 13.8 Hz, ^{3}J = 6.5 Hz, Im-4-C$\boldsymbol{H}_2$), 2.76 – 2.89 (m, 1H, Im-4-CH_2-C***H***), 2.93 (dd, 1H, ^{2}J = 13.8 Hz, ^{3}J = 7.2 Hz, Im-4-C$\boldsymbol{H}_2$), 3.97 – 4.10 (m, 2H, C$\boldsymbol{H}_2CH_3$), 6.53 (d, 1H, ^{4}J = 1.4 Hz, Im-5-***H***), 7.08 – 7.17 (m, 6H, Ph-***H***), 7.28 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.27 (+, $CH_2\boldsymbol{C}H_3$), 16.98 (+, Im-4-CH_2-CH-***C***H_3), 32.44 (-, Im-4-***C***H_2-CH), 39.95 (+, Im-4-CH_2-C***H***), 60.16 (-, ***C***H_2CH_3), 75.12 (C_{quat}, ***C***Ph_3), 118.86 (+, Im-***C***-5), 128.01 (+, 9 Ph-***C***), 129.80 (+, 6 Ph-***C***), 138.36 (+, Im-***C***-2), 139.12 (C_{quat}, Im-***C***-4), 142.54 (C_{quat}, 3 Ph-***C***-4), 176.37 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 425 (55) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{28}H_{26}N_2O_2$) C, H, N. $C_{28}H_{28}N_2O_2$ (424.53).

3-(5-Methyl-1-trityl-1*H*-imidazol-4-yl)propan-1-ol (4.19)[41]

To a solution of **4.17** (5.2 g, 12.2 mmol) in a mixture of THF_{abs} (100 mL) and anhydrous Et_2O (30 mL), $LiAlH_4$ (0.93 g, 24.4 mmol) was added in portions at 0 °C. After stirring for 15 min, the mixture was allowed to warm to room temperature and refluxed for 2 h. The mixture was cooled externally with ice and 1.0 mL H_2O, 1.0 mL NaOH 15 % and 4.0 mL H_2O were consecutively added. Insoluble material was removed by filtration and washed with THF (2 x

30 mL). After removing the solvent *in vacuo*, the crude product was subjected to flash chromatography ($CHCl_3$/MeOH 95:5 v/v) and recrystallized from MeCN yielding a white solid (3.0 g, 64 %); mp 157 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (s, 3H, Im-5-C***H***$_3$), 1.81 – 1.92 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.65 (t, 2H, ^3J = 6.5 Hz, Im-4-C***H***$_2$), 3.71 (t, 2H, ^3J = 5.6 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 4.97 (brs, 1H, O***H***), 7.07 – 7.17 (m, 6H, Ph-***H***), 7.28 (s, 1H, Im-2-***H***), 7.29 – 7.36 (m, 9H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 11.64 (+, Im-5-***C***H_3), 24.51 (-, Im-4-***C***H_2), 31.68 (-, Im-4-CH_2-***C***H_2), 62.55 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.19 (C_{quat}, ***C***Ph_3), 125.25 (C_{quat}, Im-***C***), 128.02 (+, 3 Ph-***C***-4), 128.11 (+, 6 Ph-***C***), 130.04 (+, 6 Ph-***C***), 136.55 (+, Im-***C***-2), 138.78 (C_{quat}, Im-***C***), 141.62 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 383 (100) $[M + H]^+$. Anal. ($C_{26}H_{26}N_2O$) C, H, N. $C_{26}H_{26}N_2O$ (382.50).

2-Methyl-3-(1-trityl-1*H*-imidazol-4-yl)propan-1-ol (4.20)

To a solution of **4.18** (6.5 g, 15.3 mmol) in a mixture of THF_{abs} (80 mL) and anhydrous Et_2O (40 mL), $LiAlH_4$ (1.16 g, 30.6 mmol) was added in portions at 0 °C. After stirring for 15 min, the mixture was allowed to warm to room temperature and refluxed for 2 h. The mixture was cooled externally with ice and 1.2 mL H_2O, 1.2 mL NaOH 15 % and 4.8 mL H_2O were consecutively added. Insoluble material was removed by filtration and washed with THF (2 x 30 mL). After removing the solvent *in vacuo*, the crude product was subjected to flash chromatography ($CHCl_3$/MeOH 95:5 v/v) yielding a white solid (3.5 g, 60 %); mp 132 – 133 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.87 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$), 1.93 – 2.07 (m, 1H, Im-4-CH_2-C***H***), 2.52 (dd, 1H, ^2J = 14.6 Hz, ^3J = 7.0 Hz, Im-4-C***H***$_2$), 2.64 (dd, 1H, ^2J = 14.6 Hz, ^3J = 5.0 Hz, Im-4-C***H***$_2$), 3.44 (dd, 1H, ^2J = 11.2 Hz, ^3J = 7.1 Hz, Im-4-CH_2-CH-C***H***$_2$), 3.59 (dd, 1H, ^2J = 11.2 Hz, ^3J = 4.4 Hz, Im-4-CH_2-CH-C***H***$_2$), 4.56 (brs, 1H, O***H***), 6.54 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.08 – 7.19 (m, 6H, Ph-***H***), 7.28 – 7.41 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 17.15 (+, ***C***H_3), 33.04 (-, Im-4-CH_2), 35.72 (+, Im-4-CH_2-***C***H), 67.98 (-, Im-4-CH_2-CH-C***H***$_2$), 75.21 (C_{quat}, ***C***Ph_3), 118.90 (+, Im-***C***-5), 128.06 (+, 9 Ph-***C***), 129.79 (+, 6 Ph-***C***), 138.19 (+, Im-***C***-2), 139.53 (C_{quat}, Im-***C***-4), 142.46 (C_{quat}, 3 Ph-***C***-4). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 383 (75) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{26}H_{26}N_2O$) C, H, N. $C_{26}H_{26}N_2O$ (382.50).

4.4.1.4 Preparation of 4-(1-trityl-1H-imidazol-4-yl)butan-1-amine 4.23

4-(3-Chloropropyl)-1-trityl-1*H*-imidazole (4.21)

To a solution of 3-(1-trityl-1*H*-imidazol-4-yl)propan-1-ol[7, 42] (20.0 g, 54.3 mmol) in THF_{abs} (300 mL), thionylchloride (7.9 mL, 12.9 g, 108.6 mmol) was added dropwise at 0 °C. After stirring for 1 h at 0 °C and 2 h at ambient temperature, the solvent was concentrated *in vacuo*. The product was crystallized by addition of Et_2O (100 mL) yielding a white solid. (22.3 g, 97 %).

To obtain the free base a 1 M solution of NaOH was added to the hydrochloride and extracted with DCM. The organic layer was dried over Na_2SO_4 followed by evaporation of the solvent; mp (**4.21** · HCl) 143 °C (ref.[42]: 147°C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.05 – 2.16 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.69 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 3.54 (t, 2H, ^3J = 6.5 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.57 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.28 – 7.35 (m, 9H, Ph-***H***), 7.36 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 25.55 (-, Im-4-***C***H_2), 32.11 (-, Im-4-CH_2-***C***H_2), 44.60 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.17 (C_{quat}, ***C***Ph_3), 118.35 (+, Im-***C***-5), 128.02 (+, 3 Ph-***C***-4), 128.04 (+, 6 Ph-***C***), 129.78 (+, 6 Ph-***C***), 138.60 (+, Im-***C***-2), 140.07 (C_{quat}, Im-***C***-4), 142.57 (C_{quat}, 3 Ph-***C***-1). ES-MS (MeOH + NH_4OAc) *m/z* (%): 387 (74) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{25}H_{23}ClN_2$ · HCl) C, H, N. $C_{25}H_{23}ClN_2$ (386.92).

4-(1-Trityl-1*H*-imidazol-4-yl)butanenitrile (4.22)

To a mixture of KCN (3.36 g, 51.6 mmol) and KI (0.2 g, cat.) in anhydrous DMSO (200 mL) at 80 °C, a solution of **4.21** (10.0 g, 25.8 mmol) in anhydrous DMSO (50 mL) was added dropwise. When the addition was complete, the mixture was stirred for 24 h at 80 °C. After cooling to room temperature, the mixture was poured in water/NH_3 (800 mL, pH = 8 – 9) and extracted with DCM (3 x 250 mL). The organic phase was washed with water (3 x 250 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (DCM/MeOH/NH_3 (aq.) 32 % 95.5/2.5/2 v/v/v) yielding a brownish solid (6.5 g, 67 %); mp 100 – 102 °C (ref.[43]: yellow oil). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.97 – 2.09 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.35 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 2.71 (t, 2H, ^3J = 7.2 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.62 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.08 – 7.18 (m, 6H, Ph-***H***), 7.29 – 7.39 (m, 9H, Ph-***H***), 7.47 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 16.45 (-, Im-4-$(CH_2)_2$-***C***H_2), 24.97 (-, Im-4-CH_2-***C***H_2), 26.59 (-, Im-4-***C***H_2), 75.71 (C_{quat}, ***C***Ph_3), 118.85 (+, Im-***C***-5), 119.64 (C_{quat}, ***C***≡N), 128.21 (+, 3 Ph-***C***-4), 128.27 (+, 6 Ph-***C***), 129.70 (+, 6 Ph-***C***), 138.34 (+, Im-***C***-2), 138.43 (C_{quat}, Im-***C***-4), 142.06 (C_{quat}, 3 Ph-***C***-1). IR (cm^{-1}) = 3059, 2936, 2245 (C≡N), 1735, 1492, 1445. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 378 (78) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{26}H_{23}N_3$) C, H, N. $C_{26}H_{23}N_3$ (377.48).

4-(1-Trityl-1*H*-imidazol-4-yl)butan-1-amine (4.23)[44]

To a solution of **4.22** (6.0 g, 15.9 mmol) in a mixture of THF_{abs} (75 mL) and anhydrous Et_2O (75 mL), $LiAlH_4$ (1.21 g, 31.8 mmol) was added in portions at 0 °C. After stirring for 15 min, the mixture was allowed to warm to room temperature and refluxed for 3 h. The mixture was cooled externally with ice and 1.2 mL H_2O, 1.2 mL NaOH 15 % and 4.8 mL H_2O were consecutively added. Insoluble material was removed by filtration and washed with THF (2 x 30 mL). The organic layer was washed with a saturated solution of $NaHCO_3$, water and brine and dried over Na_2SO_4. After removing the solvent *in vacuo*, a pale yellow oil was obtained

that was used without further purification (5.2 g, 86 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.40 – 1.76 (m, 2H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.54 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 2.68 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.52 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.25 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.66 (-, ***C***H_2), 28.36 (-, ***C***H_2), 33.63 (-,***C***H_2), 42.09 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.08 (C_{quat}, ***C***Ph_3), 117.72 (+, Im-***C***-5), 127.95 (+, 3 Ph-***C***-4), 127.99 (+, 6 Ph-***C***), 129.82 (+, 6 Ph-***C***), 138.31 (+, Im-***C***-2), 141.88 (C_{quat}, Im-***C***-4), 142.67 (C_{quat}, 3 Ph-***C***-1). ES-MS (MeOH + NH_4OAc) *m/z* (%): 382 (100) $[M + H]^+$. $C_{26}H_{27}N_3$ (381.51).

4.4.1.5 Preparation of N-methyl-3-(1-trityl-1H-imidazol-4-yl)propan-1-amine 4.25

N-Methyl-3-(1-trityl-1*H*-imidazol-4-yl)propanamide (4.24)

To a solution of methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate[7, 42] (8.0 g, 20.2 mmol) in MeOH (250 mL), a 8 M solution of methylamine in EtOH (126 mL, 1.01 mol) was added and stirred overnight. After removing the solvent *in vacuo*, the crude product was purified by flash chromatography ($CHCl_3$/MeOH 95/5 v/v) and recrystallized from MeCN/EtOAc yielding a white solid (6.5 g, 81 %); mp 181 – 182 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.54 (t, 2H, ^3J = 7.0 Hz, Im-4-CH_2-C***H***$_2$), 2.74 (d, 3H, ^3J = 4.8 Hz, C***H***$_3$), 2.84 (t, 2H, ^3J = 7.0 Hz, Im-4-C***H***$_2$), 6.51 (brs, 1H, N-***H***), 6.58 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.07 – 7.16 (m, 6H, Ph-***H***), 7.27 – 7.39 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.39 (-, Im-4-***C***H_2), 26.39 (-, ***C***H_3), 36.58 (-, Im-4-CH_2-***C***H_2), 75.23 (C_{quat}, ***C***Ph_3), 118.43 (+, Im-***C***-5), 128.07 (+, 9 Ph-***C***), 129.77 (+, 6 Ph-***C***), 138.33 (+, Im-***C***-2), 140.24 (C_{quat}, Im-4-***C***), 142.43 (C_{quat}, Ph-***C***), 173.59 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 396 (100) $[M + H]^+$. Anal. ($C_{26}H_{25}N_3O$) C, H, N. $C_{26}H_{25}N_3O$ (395.50).

N-Methyl-3-(1-trityl-1*H*-imidazol-4-yl)propan-1-amine (4.25)[45]

To a solution of **4.24** (6.0 g, 15.2 mmol) in THF_{abs} (100 mL) and anhydrous Et_2O (75 mL), $LiAlH_4$ (1.15 g, 30.3 mmol) was added in portions at 0 °C. After stirring for 15 min, the mixture was allowed to warm to room temperature and refluxed for 3 h. The mixture was cooled externally with ice and 1.2 mL H_2O, 1.2 mL NaOH 15 % and 4.8 mL H_2O were consecutively added. Insoluble material was removed by filtration and washed with THF (2 x 30 mL). The organic layer was washed with a saturated solution of $NaHCO_3$, water and brine and dried over Na_2SO_4. After removing the solvent *in vacuo*, a light yellow oil was obtained that was used without further purification (5.1 g, 88 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.74 – 1.90 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.40 (s, 3H, C***H***$_3$), 2.53 – 2.62 (m, 4H, Im-4-C***H***$_2$-CH_2-C***H***$_2$), 6.52 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.08 – 7.37 (m, 16H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.24, 29.51 (-, Im-4-***C***H_2-***C***H_2), 36.53 (+, ***C***H_3), 51.67 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.08 (C_{quat}, ***C***Ph_3), 117.88 (+, Im-***C***-5), 127.97 (+, 3 Ph-***C***), 128.01 (+, 6 Ph-***C***), 129.82 (+, 6

Ph-***C***), 138.30 (+, Im-***C***-2), 141.51 (C_{quat}, Im-***C***-4), 142.61 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + TFA) *m/z* (%): 382 (100) $[M + H]^+$. $C_{26}H_{27}N_3$ (381.51).

4.4.1.6 Preparation of the guanidinylation reagents 4.26-4.29

N^1-(*tert*-Butoxycarbonyl)guanidine (4.26)[15]

To a solution of guanidine · HCl (7.5 g, 78.9 mmol) in 4 M NaOH (40 mL, 160 mmol), a solution of di-*tert*-butyl dicarbonate (13.71 g, 62.8 mmol) in 1,4-dioxane (80 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the crude product was suspended in H_2O (20 mL), stirred for 15 min and filtered off. This procedure was repeated with Et_2O (20 mL) yielding a white solid. (6.6 g, 66 %); mp > 280 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.34 (s, 9H, C***H***$_3$), 6.83 (brs, 4H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 28.17 (+, ***C***H$_3$), 75.41 (C_{quat}, ***C***$(CH_3)_3$), 162.29, 163.19 (C_{quat}, ***C***=O + ***C***=N). CI-MS (NH_3) *m/z* (%): 160 (100) $[M + H]^+$. Anal. ($C_6H_{13}N_3O_2$) C, H, N. $C_6H_{13}N_3O_2$ (159.19).

N^1-(Benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)guanidine (4.27)[18]

To a solution of **4.26** (4.5 g, 28.3 mmol) in anhydrous DMF (70 mL), benzyl succinimidyl carbonate (7.1 g, 28.3 mmol) was added in portions. After stirring overnight, crushed ice (20 g) was added and sonicated for 10 min. The precipitate was filtered off, washed with MeOH (10 mL) and recrystallized from MeOH yielding a white solid (7.0 g, 84 %); mp 124 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.43 (s, 9H, C***H***$_3$), 5.06 (s, 2H, C***H***$_2$), 7.27 – 7.42 (m, 5H, Ph-***H***), 8.52 (brs, 1H, N-***H***), 8.71 (brs, 1H, N-***H***), 10.61 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 27.65 (+, ***C***H$_3$), 65.73 (-, ***C***H$_2$), 80.95 (C_{quat}, ***C***$(CH_3)_3$), 127.53 (+, 2 Ph-***C***), 127.67 (+, Ph-***C***-4), 128.28 (+, 2 Ph-***C***), 136.87 (C_{quat}, Ph-***C***-1), 155.37, 158.82, 161.26 (C_{quat}, 2 ***C***=O + ***C***=N). CI-MS (NH_3) *m/z* (%): 294 (100) $[M + H]^+$. Anal. ($C_{14}H_{19}N_3O_4$) C, H, N. $C_{14}H_{19}N_3O_4$ (293.32).

N^1,N^2-Bis(benzyloxycarbonyl)guanidine (4.28)

To a solution of guanidine · HCl (8.4 g, 87.9 mmol) and NaOH (17.6 g, 439.5 mmol) in H_2O (90 mL), DCM (175 mL) was added and benzyl chloroformate (37.6 mL, 45.0 g, 263.7 mmol) was added dropwise at 0 °C. After stirring for 20 h at 0 °C, DCM (200 mL) was added to the mixture and the layers were separated. The aqueous layer was extracted with DCM (200 mL) and the combined organic layers were washed with H_2O and dried over $MgSO_4$. After removing the solvent *in vacuo*, the crude product was recrystallized from MeOH giving colorless crystals. (21.3 g, 74 %); mp 145 – 146 °C. (ref.[16]: 149 – 150°C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 5.11 (s, 4H, C***H***$_2$), 7.27 – 7.45 (m, 10H, Ph-***H***), 8.69 (brs, 2H, N-***H***), 10.89 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 66.15 (-, 2 ***C***H$_2$), 127.66 (+, 4

Ph-***C***), 127.86 (+, 2 Ph-***C***-4), 128.32 (+, 4 Ph-***C***), 136.32 (C_{quat}, 2 Ph-***C***-1), 158.78 (C_{quat}, ***C***=O). ES-MS (MeCN/H_2O + TFA) *m/z* (%): 328 (100) $[M + H]^+$. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N. $C_{17}H_{17}N_3O_4$ (327.33).

N^1,N^2-Bis(benzyloxycarbonyl)-N^3-trifluoromethanesulfonylguanidine (4.29)[16]

To a solution of **4.28** (13.5 g, 41.2 mmol) in anhydrous chlorobenzene (350 mL), NaH (60 % dispersion in mineral oil) (3.30 g, 82.4 mmol) was added in portions at 0 °C (argon atmosphere). After stirring for 1 h at 0 °C, the mixture was cooled to –45 °C and trifluoromethanesulfonic anhydride (6.9 mL, 11.62 g, 41.2 mmol) was added. The mixture was allowed to warm to ambient temperature and stirred overnight. After evaporation of the solvent, EtOAc (300 mL) and a 2 M solution of $KHSO_4$ (75 mL) were added to the residue. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and removed *in vacuo*. Purification was performed by flash chromatography (PE/EtOAc 80/20 v/v) yielding a colorless semisolid compound (16.2 g, 86 %). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.33 – 7.46 (m, 10H, Ph-***H***), 11.56 (brs, 2H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 67.78 (-, 2 ***C***H_2), 128.22 (+, 4 Ph-***C***), 128.40 (+, 2 Ph-***C***), 128.42 (+, 4 Ph-***C***), 134.93 (C_{quat}, 2 Ph-***C***-1), 151.37, 152.12 (C_{quat}, ***C***=O, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 460 (100) $[M + H]^+$. $C_{18}H_{16}F_3N_3O_6S$ (459.40).

4.4.1.7 Preparation of the diurethane-protected 1-trityl-1H-imidazol-4-ylalkyl-guanidines 4.30-4.33

General procedure

To a solution of the pertinent amine (1 eq) and **4.29** (0.9 eq) in DCM, NEt_3 (1 eq) was added. After stirring overnight at room temperature, the organic layer was washed with saturated $NaHCO_3$ solution, water and brine and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography.

***N^1,N^2*-Bis(benzyloxycarbonyl)-*N^3*-[2-(1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.30)**[7]

The title compound was prepared from **4.13** (6.8 g, 19.2 mmol), **4.29** (7.95 g, 17.3 mmol) and NEt_3 (2.7 mL, 1.94 g, 19.2 mmol) in DCM (100 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 60/40 v/v) yielded a pale yellow foam-like solid (5.6 g, 49 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.79 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.69 – 3.79 (m, 2H, Im-4-CH_2-C***H***$_2$), 5.11 (s, 2H, Ph-C***H***$_2$), 5.15 (s, 2H, Ph-C***H***$_2$), 6.62 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.23 – 7.43 (m, 20H, Ph-***H*** + Im-2-***H***), 8.63 (t, 1H, ^3J = 5.0 Hz, N-***H***), 11.73 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.76 (-, Im-4-***C***H_2), 40.82 (-, Im-4-CH_2-***C***H_2), 67.15 (-, Ph-***C***H_2), 68.01 (-, Ph-***C***H_2), 75.26 (C_{quat}, ***C***Ph_3),

118.80 (+, Im-***C***-5), 127.90 (+, 1 Ph-***C***), 128.04 (+, 3 Ph-***C***), 128.07 (+, 6 Ph-***C***), 128.20 (+, 2 Ph-***C***), 128.42 (+, 4 Ph-***C***), 128.72 (+, 2 Ph-***C***), 128.78 (+, 1 Ph-***C***), 129.84 (+, 6 Ph-***C***), 134.71 (C_{quat}, 1 Ph-***C***-1), 136.87 (C_{quat}, 1 Ph-***C***-1), 138.04 (C_{quat}, Im-***C***-4), 138.91 (+, Im-***C***-2), 142.47 (C_{quat}, 3 Ph-***C***-1), 153.59 (C_{quat}, ***C***=O), 155.91 (C_{quat}, ***C***=O), 163.75 (C_{quat}, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 664 (100) $[M + H]^+$. $C_{41}H_{37}N_5O_4$ (663.76).

***N*^{1},*N*^{2}-Bis(benzyloxycarbonyl)-*N*^{3}-[2-(5-methyl-1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.31)

The title compound was prepared from **4.14** (4.0 g, 10.9 mmol), **4.29** (4.5 g, 9.8 mmol) and NEt_3 (1.5 mL, 1.10 g, 10.9 mmol) in DCM (60 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 60/40 v/v) yielded a pale yellow foam-like solid (5.6 g, 84 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.38 (s, 3H, C***H***$_3$), 2.87 (t, 2H, ^3J = 6.2 Hz, Im-4-C***H***$_2$), 3.77 – 3.87 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 5.09 (s, 2H, Ph-C***H***$_2$), 5.19 (s, 2H, Ph-C***H***$_2$), 7.05 – 7.17 (m, 6H, Ph-***H***), 7.24 – 7.43 (m, 20H, Ph-***H*** + Im-2-***H***), 8.62 (t, 1H, ^3J = 5.0 Hz, N-***H***), 11.72 (brs, 1H, N-***H***). ^{13}C-NMR (150 MHz, $CDCl_3$): δ [ppm] = 11.50 (+, ***C***H$_3$), 25.41 (-, Im-4-***C***H$_2$), 40.66 (-, Im-4-CH$_2$-***C***H$_2$), 67.11 (-, Ph-***C***H$_2$), 68.11 (-, Ph-***C***H$_2$), 76.15 (C_{quat}, ***C***Ph$_3$), 127.85 (+, 2 Ph-***C***), 128.01 (+, 4 Ph-***C***), 128.35 (+, 6 Ph-***C***), 128.46 (+, 3 Ph-***C***), 128.71 (+, 3 Ph-***C***), 128.78 (+, 1 Ph-***C***), 129.83 (C_{quat}, Im-***C***), 129.94 (+, 6 Ph-***C***), 134.64 (C_{quat}, 1 Ph-***C***-1), 136.24 (+, Im-***C***-2), 136.82 (C_{quat}, 1 Ph-***C***-1), 140.80 (C_{quat}, 3 Ph-***C***-1), 141.47 (C_{quat}, Im-***C***), 153.53 (C_{quat}, ***C***=O), 156.00 (C_{quat}, ***C***=N), 163.69 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 678 (100) $[M + H]^+$. $C_{42}H_{39}N_5O_4$ (677.79).

***N*^{1},*N*^{2}-Bis(benzyloxycarbonyl)-*N*^{3}-[4-(1-trityl-1*H*-imidazol-4-yl)butyl]guanidine (4.32)

The title compound was prepared from **4.23** (4.5 g, 11.8 mmol), **4.29** (4.9 g, 10.6 mmol) and NEt_3 (1.6 mL, 1.19 g, 11.8 mmol) in DCM (60 mL) according to the general procedure. Purification by flash chromatography (DCM/MeOH 99/1 v/v) yielded a pale yellow foam-like solid (3.8 g, 52 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.51 – 1.74 (m, 4H, Im-4-CH$_2$-C***H***$_2$-C***H***$_2$), 2.56 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.35 – 3.37 (m, 2H, Im-4-(CH$_2$)$_3$-C***H***$_2$), 5.11 (s, 2H, Ph-C***H***$_2$), 5.16 (s, 2H, Ph-C***H***$_2$), 6.52 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.27 – 7.43 (m, 20H, Ph-***H*** + Im-2-***H***), 8.29 (t, 1H, ^3J = 5.0 Hz, N-***H***), 11.74 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.58, 27.98, 28.55 (-, Im-4-***C***H$_2$-***C***H$_2$-***C***H$_2$), 41.08 (-, Im-4-(CH$_2$)$_3$-***C***H$_2$), 67.14 (-, Ph-***C***H$_2$), 68.12 (-, Ph-***C***H$_2$), 75.13 (C_{quat}, ***C***Ph$_3$), 117.96 (+, Im-***C***-5), 127.87 (+, 1 Ph-***C***), 127.98 (+, 3 Ph-***C***), 128.02 (+, 6 Ph-***C***), 128.13 (+, 2 Ph-***C***), 128.40 (+, 2 Ph-***C***), 128.47 (+, 2 Ph-***C***), 128.72 (+, 2 Ph-***C***), 128.78 (+, 1 Ph-***C***), 129.81 (+, 6 Ph-***C***), 134.71 (C_{quat}, 1 Ph-***C***-1), 136.90 (C_{quat}, 1 Ph-***C***-1), 138.39 (+, Im-***C***-2), 141.25 (C_{quat}, Im-***C***-4), 142.61 (C_{quat}, 3 Ph-***C***-1), 153.88, 155.93, 163.76 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 692 (100) $[M + H]^+$. $C_{43}H_{41}N_5O_4$ (691.82).

N^1,N^2-Bis(benzyloxycarbonyl)-N^3-methyl-N^3-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (4.33)

The title compound was prepared from **4.25** (4.0 g, 10.5 mmol), **4.29** (4.4 g, 9.5 mmol) and NEt_3 (1.5 mL, 1.06 g, 10.5 mmol) in DCM (60 mL) according to the general procedure. Purification by flash chromatography (DCM/MeOH 99/1 v/v) yielded a pale yellow foam-like solid (4.5 g, 62 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.77 – 1.98 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.50 (t, 2H, ^3J = 6.5 Hz, Im-4-C***H***$_2$), 2.99 (s, 3H, C***H***$_3$), 3.57 (t, 2H, ^3J = 6.8 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 5.08 (brs, 4H, PhC***H***$_2$), 6.53 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.03 – 7.48 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 692 (100) $[M + H]^+$. $C_{43}H_{41}N_5O_4$ (691.82).

4.4.1.8 Preparation of the diurethane-protected 3-(1-trityl-1H-imidazol-4-yl)-propylguanidines 4.38 and 4.40

General procedure

To a solution of the pertinent alcohol **4.19** or **4.20** (1 eq), the diurethane-protected guanidine **4.27** or **4.28** (1.75 eq) and PPh_3 (1.5 eq) in THF_{abs}, DIAD (1.5 eq) in THF_{abs} was added dropwise at 0 °C. After the addition was complete, the solution was allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the crude product purified by flash chromatography.

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)-2-methylpropyl]guanidine (4.38)

The title compound was prepared from a solution of **4.20** (4.0 g, 10.5 mmol), **4.28** (6.02 g, 18.4 mmol), PPh_3 (4.14 g, 15.8 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.1 mL, 3.19 g, 15.8 mmol) in THF_{abs} (25 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) yielded a colorless foam-like solid. (6.1 g, 84 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.81 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$), 2.27 – 2.42 (m, 2H, Im-4-C***H***$_2$), 2.50 – 2.65 (m, 1H, Im-4-CH_2-C***H***), 3.92 – 4.04 (d, 2H, ^3J = 6.3 Hz, Im-4-CH_2-CH-C***H***$_2$), 5.10 (s, 2H, Ph-C***H***$_2$), 5.18 (s, 2H, Ph-C***H***$_2$), 6.50 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.06 – 7.16 (m, 6H, Ph-***H***), 7.20 – 7.39 (m, 20H, Ph-***H*** + Im-2-***H***), 9.26 (brs, 1H, N-***H***), 9.44 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 17.34 (+, ***C***H_3), 33.04 (+, Im-4-CH_2-***C***H), 33.40 (-, Im-4-***C***H_2), 49.89 (-, Im-4-CH_2-CH-***C***H_2), 66.97 (-, Ph-***C***H_2), 68.84 (-, Ph-***C***H_2), 75.07 (C_{quat}, ***C***Ph_3), 118.75 (+, Im-***C***-5), 127.69 (+, 1 Ph-***C***), 127.78 (+, 2 Ph-***C***), 127.99 (+, 3 Ph-***C***), 128.01 (+, 6 Ph-***C***), 128.37 (+, 4 Ph-***C***), 128.66 (+, 1 Ph-***C***), 128.71 (+, 2 Ph-***C***), 129.82 (+, 6 Ph-***C***), 134.80 (C_{quat}, Ph-***C***), 137.18 (C_{quat}, Ph-***C***), 138.31 (+, Im-***C***-2), 139.80 (C_{quat}, Im-***C***-4),

142.62 (C_{quat}, 3 Ph-***C***-1), 156.24 (C_{quat}, ***C***=N), 161.20 (C_{quat}, ***C***=O), 163.90 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 692 (100) $[M + H]^+$. $C_{43}H_{41}N_5O_4$ (691.82).

N^1-(Benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)-N^1-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]guanidine and N^1-(Benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)-N^3-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.40)

The title compound (mixture of isomers) was prepared from a solution of **4.19** (4.6 g, 12.0 mmol), **4.27** (6.16 g, 21.0 mmol), PPh_3 (4.72 g, 18.0 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.6 mL, 3.64 g, 18.0 mmol) in THF_{abs} (25 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) yielded a colorless foam-like solid. (6.3 g, 80 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.31 (s, 0.8H, Im-5-C***H***$_3$), 1.35 (s, 2.2H, Im-5-C***H***$_3$), 1.45 (s, 2.4H, C(C***H***$_3$)$_3$), 1.50 (s, 6.6H, C(C***H***$_3$)$_3$), 1.86 – 2.00 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.42 – 2.53 (m, 2H, Im-4-C***H***$_2$), 3.90 – 4.01 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 5.11 (s, 1.5H, PhC***H***$_2$), 5.20 (s, 0.5H, PhC***H***$_2$), 7.06 – 7.39 (m, 21H, Ph-***H*** + Im-2-***H***), 9.38 (brs, 1H, N-***H***), 9.42 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 658 (100) $[M + H]^+$. $C_{39}H_{41}N_5O_4$ (643.77).

4.4.1.9 Preparation of the trityl-protected imidazolylalkylguanidines 4.34-4.37, 4.39 and 4.41

General procedure

A mixture of the pertinent diurethane-protected guanidine and catalytical amounts of Pd/C (10 %) in MeOH was stirred under a hydrogen atmosphere at room temperature for approximately 3 h (TLC control). After the Cbz-groups were quantitatively cleaved, the catalyst was removed by filtration over Celite and the solvent was evaporated.

N-(2-(1-Trityl-1*H*-imidazol-4-yl)ethyl)guanidine (4.34)[7]

The title compound was prepared from **4.30** (5.5 g, 8.3 mmol) and Pd/C (10 %) (0.55 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (3.1 g, 94 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 2.77 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.40 (t, 2H, ^3J = 7.1 Hz, Im-4-CH_2-C***H***$_2$), 6.80 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.10 – 7.18 (m, 6H, Ph-***H***), 7.34 – 7.40 (m, 9H, Ph-***H***), 7.43 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 28.71 (-, Im-4-***C***H_2), 42.34 (-, Im-4-CH_2-***C***H_2), 76.99 (C_{quat}, ***C***Ph_3), 120.86 (+, Im-***C***-5), 129.35 (+, 6 Ph-***C***), 129.45 (+, 3 Ph-***C***), 130.92 (+, 6 Ph-***C***), 138.51 (C_{quat}, Im-***C***-4), 139.78 (+, Im-***C***-2), 143.69 (C_{quat}, 3 Ph-***C***-1), 158.86 (C_{quat}, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 396 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{25}H_{25}N_5$ $[M^{+\bullet}]$ 395.2110; found 395.2109. $C_{25}H_{25}N_5$ (395.50).

***N*-[2-(5-Methyl-1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.35)**

The title compound was prepared from **4.31** (5.4 g, 8.0 mmol) and Pd/C (10 %) (0.54 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (3.1 g, 95 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.43 (s, 3H, C***H***$_3$), 2.72 (t, 2H, ^3J = 7.0 Hz, Im-4-C***H***$_2$), 3.42 (t, 2H, ^3J = 7.0 Hz, Im-4-CH$_2$-C***H***$_2$), 7.10 – 7.19 (m, 6H, Ph-***H***), 7.25 (s, 1H, Im-2-***H***), 7.34 – 7.43 (m, 9H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 11.99 (+, ***C***H$_3$), 29.58 (-, Im-4-***C***H$_2$), 42.22 (-, Im-4-CH$_2$-***C***H$_2$), 76.64 (C$_{quat}$, ***C***Ph$_3$), 128.47 (C$_{quat}$, Im-***C***), 129.37 (+, 9 Ph-***C***), 131.22 (+, 6 Ph-***C***), 137.01 (C$_{quat}$, Im-***C***), 138.60 (+, Im-***C***-2), 143.01 (C$_{quat}$, 3 Ph-***C***-1), 158.65 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 410 (100) [M + H]$^+$. HRMS (EI-MS) calcd. for $C_{26}H_{27}N_5$ [M$^{+\bullet}$] 409.2267; found 409.2270. $C_{26}H_{27}N_5$ (409.53).

***N*-[4-(1-Trityl-1*H*-imidazol-4-yl)butyl]guanidine (4.36)**

The title compound was prepared from **4.32** (3.7 g, 5.3 mmol) and Pd/C (10 %) (0.37 g, cat.) in MeOH (100 mL) according to the general procedure yielding a colorless foam-like solid (2.2 g, 98 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.47 – 1.74 (m, 4H, Im-4-CH$_2$-C***H***$_2$-C***H***$_2$), 2.55 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 3.15 (t, 2H, ^3J = 6.9 Hz, Im-4-(CH$_2$)$_3$-C***H***$_2$), 6.66 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.19 (m, 6H, Ph-***H***), 7.33 – 7.42 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.54, 28.31, 29.43 (+, Im-4-***C***H$_2$-***C***H$_2$-***C***H$_2$), 42.26 (-, Im-4-(CH$_2$)$_3$-***C***H$_2$), 76.83 (C$_{quat}$, ***C***Ph$_3$), 119.75 (+, Im-***C***-5), 129.30 (+, 6 Ph-***C***), 129.40 (+, 3 Ph-***C***), 130.90 (+, 6 Ph-***C***), 139.38 (+, Im-***C***-2), 142.06 (C$_{quat}$, Im-***C***-4), 143.80 (C$_{quat}$, 3 Ph-***C***-1), 158.88 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 424 (97) [M + H]$^+$, 243 (100) [CPh$_3^+$]. HRMS (EI-MS) calcd. for $C_{27}H_{29}N_5$ [M$^{+\bullet}$] 423.2423; found 423.2417. $C_{27}H_{29}N_5$ (423.55).

***N*-Methyl-*N*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.37)**

The title compound was prepared from **4.33** (4.4 g, 6.4 mmol) and Pd/C (10 %) (0.44 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (2.6 g, 96 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.82 – 1.97 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.54 (t, 2H, ^3J = 7.3 Hz, Im-4-C***H***$_2$), 2.98 (s, 3H, C***H***$_3$), 3.38 (t, 2H, ^3J = 7.5 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.71 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.07 – 7.19 (m, 6H, Ph-***H***), 7.27 – 7.43 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 25.19 (-, Im-4-CH$_2$-***C***H$_2$), 27.81 (-, Im-4-***C***H$_2$), 36.28 (+, ***C***H$_3$), 50.99 (-, Im-4-***C***H$_2$), 76.89 (C$_{quat}$, ***C***Ph$_3$), 119.83 (+, Im-***C***-5), 129.35 (+, 6 Ph-***C***), 129.42 (+, 3 Ph-***C***), 130.91 (+, 6 Ph-***C***), 138.48 (+, Im-***C***-2), 141.32 (C$_{quat}$, Im-***C***-4), 143.76 (C$_{quat}$, 3 Ph-***C***-1), 158.68 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 424 (100) [M + H]$^+$. HRMS (EI-MS) calcd. for $C_{27}H_{29}N_5$ [M$^{+\bullet}$] 423.2423; found 423.2424. $C_{27}H_{29}N_5$ (423.55).

***N*-[2-Methyl-3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.39)**

The title compound was prepared from **4.38** (6.0 g, 8.7 mmol) and Pd/C (10 %) (0.60 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (3.5 g, 95 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 0.90 (d, 3H, ^{3}J = 6.7 Hz, C***H***$_3$), 1.96 – 2.11 (m, 1H, C***H***), 2.39 (dd, 1H, ^{2}J = 14.5 Hz, ^{3}J = 7.0 Hz, Im-4-C***H***$_2$), 2.58 (dd, 1H, ^{2}J = 14.5 Hz, ^{3}J = 6.1 Hz, Im-4-C***H***$_2$), 3.00 (dd, 1H, ^{2}J = 13.5 Hz, ^{3}J = 6.9 Hz, Im-4-CH$_2$-CH-C***H***$_2$), 3.14 (dd, 1H, ^{2}J = 13.5 Hz, ^{3}J = 6.3 Hz, Im-4-CH$_2$-CH-C***H***$_2$), 6.69 (d, 1H, ^{4}J = 1.4 Hz, Im-5-***H***), 7.10 – 7.19 (m, 6H, Ph-***H***), 7.33 – 7.40 (m, 9H, Ph-***H***), 7.41 (d, 1H, ^{4}J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 17.61 (+, ***C***H$_3$), 32.84 (-, Im-4-***C***H$_2$), 34.73 (+, Im-4-CH$_2$-***C***H), 47.88 (-, Im-4-CH$_2$-CH-***C***H$_2$), 76.86 (C$_{quat}$, ***C***Ph$_3$), 120.91 (+, Im-***C***-5), 129.33 (+, 6 Ph-***C***), 129.42 (+, 3 Ph-***C***), 130.88 (+, 6 Ph-***C***), 139.47 (+, Im-***C***-2), 140.00 (C$_{quat}$, Im-***C***-4), 143.76 (C$_{quat}$, 3 Ph-***C***-4), 159.20 (C$_{quat}$, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 424 (100) [M + H]$^+$. HRMS (EI-MS) calcd. for $C_{27}H_{29}N_5$ [M$^{+\bullet}$] 423.2423; found 423.2415. $C_{27}H_{29}N_5$ (423.55).

***N*^{1}-(*tert*-Butoxycarbonyl)-*N*^{1}-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]guanidine and *N*^{1}-(*tert*-Butoxycarbonyl)-*N*^{2}-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.41)**

The title compound (mixture of isomers) was prepared from **4.40** (6.2 g, 9.4 mmol) and Pd/C (10 %) (0.62 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (4.7 g, 98 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers**:** δ [ppm] = 1.35 (s, 0.8H, Im-5-C***H***$_3$), 1.36 (s, 2.2H, Im-5-C***H***$_3$), 1.47 (s, 2.4H, C***H***$_3$), 1.48 (s, 6.6H, C***H***$_3$), 1.81 – 2.05 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.38 (t, 0.5H, ^{3}J = 6.8 Hz, Im-4-C***H***$_2$), 2.49 (t, 1.5H, ^{3}J = 7.0 Hz, Im-4-C***H***$_2$), 3.34 (t, 0.5H, ^{3}J = 6.7 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 3.66 – 3.82 (m, 1.5H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 7.09 – 7.41 (m, 16H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 510 (100) [M + H]$^+$. HRMS (EI-MS) calcd. for $C_{31}H_{35}N_5O_2$ [M$^{+\bullet}$] 523.2947; found 523.2960. $C_{31}H_{35}N_5O_2$ (509.64).

4.4.1.10 Preparation of the trityl-protected N^G-acylated imidazolylalkyl-guanidines 4.42-4.52 and 4.57-4.62

General procedure

A solution of the pertinent carboxylic acid (1 eq) and CDI (1.2 eq) in THF$_{abs}$ (15 mL) was stirred for 1 h under argon atmosphere at room temperature. In a separate flask NaH (60 % dispersion in mineral oil) (2 eq) was added to a solution of the pertinent guanidine (1 eq) in THF$_{abs}$ (15 mL) under argon atmosphere, stirred for 45 min at 30 – 35 °C and allowed to cool to ambient temperature. Both mixtures were united and stirred for 5 h under argon atmosphere. EtOAc (50 mL) was added and the organic phase was washed with H_2O (3 x 20

mL) and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by flash chromatography.

N^1-(3-Phenylbutanoyl)-N^2-[2-(1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.42)

The title compound was prepared from **4.42a** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.34** (396 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (230 mg, 42 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (d, 3H, ^3J = 6.9 Hz, PhC***H***$_3$CH), 2.56 (dd, 1H, ^2J = 14.6 Hz, ^3J = 8.4 Hz, PhCH$_3$CH-C***H***$_2$), 2.64 – 2.87 (m, 3H, Im-4-C***H***$_2$ + PhCH$_3$CH-C***H***$_2$), 3.28 – 3.39 (m, 1H, PhCH$_3$C***H***), 3.56 (t, 2H, ^3J = 6.1 Hz, Im-4-CH$_2$-C***H***$_2$), 6.62 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.06 – 7.39 (m, 21H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 542 (100) $[M + H]^+$. $C_{35}H_{35}N_5O$ (541.69).

N^1-[3-(Thiophen-2-yl)butanoyl]-N^2-[2-(1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.43)

The title compound was prepared from **4.42b**[7] (170 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.34** (396 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow oil (250 mg, 46 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.39 (d, 3H, ^3J = 6.9 Hz, ThioC***H***$_3$CH), 2.49 (dd, 1H, ^2J = 15.0 Hz, ^3J = 8.5 Hz, ThioCH$_3$CH-C***H***$_2$), 2.67 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.3 Hz, ThioCH$_3$CH-C***H***$_2$), 2.82 (t, 2H, ^3J = 6.1 Hz, Im-4-C***H***$_2$), 3.55 – 3.70 (m, 3H, Im-4-CH$_2$-C***H***$_2$ + ThioCH$_3$C***H***), 6.63 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 6.83 – 6.93 (m, 2H, Thio-3,4-***H***), 7.04 – 7.15 (m, 6H, Ph-***H***), 7.28 – 7.41 (m, 11H, Ph-***H*** + Thio-5-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 548 (100) $[M + H]^+$. $C_{33}H_{33}N_5OS$ (547.71).

N^1-(3,3-Diphenylpropanoyl)-N^2-[2-(1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.44)[7]

The title compound was prepared from **4.42c** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.34** (396 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (360 mg, 60 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.75 (t, 2H, ^3J = 5.8 Hz, Im-4-C***H***$_2$), 3.02 (d, 2H, ^3J = 7.8 Hz, Ph$_2$CH-C***H***$_2$), 3.53 (t, 2H, ^3J = 6.6 Hz, Im-4-CH$_2$-C***H***$_2$), 4.60 (t, 1H, ^3J = 7.8 Hz, Ph$_2$C***H***), 6.58 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.03 – 7.40 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 604 (100) $[M + H]^+$. $C_{40}H_{37}N_5O$ (603.75).

N^1-(3-Phenylbutanoyl)-N^1-[4-(1-trityl-1*H*-Imidazol-4-yl)butyl]guanidine (4.45)

The title compound was prepared from **4.42a** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.36** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (340 mg, 60 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.57 – 1.76 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.52 – 2.66 (m, 3H, Im-4-C***H***$_2$ + $PhCH_3$CH-C***H***$_2$), 2.73 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.8 Hz, $PhCH_3$CH-C***H***$_2$), 3.21 – 3.40 (m, 3H, Im-4-$(CH_2)_3$-C***H***$_2$ + $PhCH_3$C***H***), 6.55 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.06 – 7.40 (m, 21H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 570 (100) $[M + H]^+$. $C_{37}H_{39}N_5O$ (569.74).

N^1-[3-(Thiophen-2-yl)butanoyl]-N^2-[4-(1-trityl-1*H*-imidazol-4-yl)butyl]guanidine (4.46)

The title compound was prepared from **4.42b**[7] (170 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.36** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (280 mg, 49 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.36 (d, 3H, ^3J = 6.9 Hz, ThioC***H***$_3$CH), 1.63 – 1.72 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.50 (dd, 1H, ^2J = 14.8 Hz, ^3J = 8.6 Hz, ThioCH_3CH-C***H***$_2$), 2.57 (t, 2H, ^3J = 6.5 Hz, Im-4-C***H***$_2$), 2.70 (dd, 1H, ^2J = 14.8 Hz, ^3J = 6.1 Hz, ThioCH_3CH-C***H***$_2$), 3.23 (t, 2H, ^3J = 7.6 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.57 – 3.71 (m, 1H, ThioCH_3C***H***), 6.55 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 6.84 (ddd, 1H, ^3J = 3.5 Hz, ^4J = 1.2 Hz, ^4J = 1.0 Hz, Thio-3-***H***), 6.89 (dd, 1H, ^3J = 5.1 Hz, ^3J = 3.5 Hz, Thio-4-***H***), 7.08 (dd, 1H, ^3J = 5.1 Hz, ^4J = 1.2 Hz, Thio-5-***H***), 7.09 – 7.16 (m, 6H, Ph-***H***), 7.29 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 576 (100) $[M + H]^+$. $C_{35}H_{37}N_5OS$ (575.77).

N^1-(3,3-Diphenylpropanoyl)-N^2-[4-(1-trityl-1*H*-imidazol-4-yl)butyl]guanidine (4.47)

The title compound was prepared from **4.42c** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.36** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow foam-like solid (230 mg, 36 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.54 – 1.69 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.54 (t, 2H, ^3J = 6.2 Hz, Im-4-C***H***$_2$), 3.08 (d, 2H, ^3J = 7.9 Hz, Ph_2CHC***H***$_2$), 3.20 (t, 2H, ^3J = 7.9 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 4.62 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 6.54 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.05 – 7.39 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 632 (100) $[M + H]^+$. $C_{42}H_{41}N_5O$ (631.81).

N^1-[3-Phenyl-3-(thiazol-2-yl)propanoyl]-N^2-[4-(1-trityl-1*H*-Imidazol-4-yl)butyl]guanidine (4.48)

The title compound was prepared from **4.42d**[7] (233 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.36** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a brownish oil (220 mg, 34 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.58 – 1.67 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.55 (t, 2H, ^3J = 6.3 Hz, Im-4-C***H***$_2$), 3.04 (dd, 1H, ^2J = 15.9 Hz, ^3J = 7.0 Hz, PhThiazCH-C***H***$_2$), 3.19 (t, 2H, ^3J = 7.3 Hz, Im-4-$(CH_2)_3$-C***H***$_2$), 3.38 (dd, 1H, ^2J = 15.9 Hz, ^3J = 8.1 Hz, PhThiazCH-C***H***$_2$), 4.96 – 5.04 (m, 1H, PhThiazC***H***), 6.53 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.07 – 7.41 (m, 22H, Ph-***H*** + Thiaz-5-***H*** + Im-2-***H***), 7.66 (d, 1H, ^3J = 3.3 Hz, Thiaz-4-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 639 (100) $[M + H]^+$. $C_{39}H_{38}N_6OS$ (638.82).

N^1-(3-Phenylbutanoyl)-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)-2-methylpropyl]guanidine (4.49)

The title compound was prepared from **4.42a** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.39** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (270 mg, 47 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.84 – 0.99 (m, 3H, Im-4-CH_2-CH-C***H***$_3$), 1.27 – 1.37 (m, 3H, PhC***H***$_3$CH), 1.99 – 2.15 (m, 1H, Im-4-CH_2-C***H***), 2.27 – 2.91 (m, 4H, Im-4-C***H***$_2$ + PhCH_3CH-C***H***$_2$), 3.09 – 3.26 (m, 1H, Im-4-CH_2-CH-C***H***$_2$), 3.29 – 3.43 (m, 1H, PhCH_3C***H***), 3.42 – 3.57 (m, 1H, Im-4-CH_2-CH-C***H***$_2$), 6.52 – 6.61 (m, 1H, Im-5-***H***), 7.06 – 7.41 (m, 21H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 570 (100) $[M + H]^+$. $C_{37}H_{39}N_5O$ (569.74).

N^1-[3-(Thiophen-2-yl)butanoyl]-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)-2-methylpropyl]-guanidine (4.50)

The title compound was prepared from **4.42b**[7] (170 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.39** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (320 mg, 56 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.87 – 0.99 (m, 3H, Im-4-CH_2-CH-C***H***$_3$), 1.37 – 1.44 (m, 3H, ThioC***H***$_3$CH), 2.01 – 2.18 (m, 1H, Im-4-CH_2-C***H***), 2.28 – 2.98 (m, 4H, Im-4-C***H***$_2$ + ThioCH_3CH-C***H***$_2$), 3.10 – 3.28 (m, 1H, Im-4-CH_2-CH-C***H***$_2$), 3.42 – 3.58 (m, 1H, Im-4-CH_2-CH-C***H***$_2$), 3.59 – 3.74 (m, 1H, ThioCH_3C***H***), 6.52 (m, 1H, Im-5-***H***), 6.84 – 6.94 (m, 2H, Thio-3,4-***H***), 7.05 – 7.12 (m, 7H, Ph-***H*** + Thio-5-***H***), 7.30 – 7.40 (m, 10H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 576 (67) $[M + H]^+$, 482 (100). $C_{35}H_{37}N_5OS$ (575.77).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)-2-methylpropyl]guanidine (4.51)

The title compound was prepared from **4.42c** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.39** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (230 mg, 40 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.79 – 1.00 (m, 3H, C***H***$_3$), 1.94 – 2.16 (m, 1H, Im-4-CH$_2$-C***H***), 2.22 – 2.69 (m, 2H, Im-4-C***H***$_2$), 2.99 – 3.26 (m, 3H, Im-4-CH$_2$-CH-C***H***$_2$ + Ph$_2$CH-C***H***$_2$), 3.34 – 3.61 (m, 1H, Im-4-CH$_2$-CH-C***H***$_2$), 4.53 – 4.70 (m, 1H, Ph$_2$C***H***), 6.49 – 6.61 (m, 1H, Im-5-***H***), 7.05 – 7.41 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 632 (100) $[M + H]^+$. $C_{42}H_{41}N_5O$ (631.81).

N^1-[3-Phenyl-3-(thiazol-2-yl)propanoyl]-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)-2-methylpropyl]-guanidine (4.52)

The title compound was prepared from **4.42d**[7] (233 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.39** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a brownish foam-like solid (230 mg, 36 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.84 – 1.02 (m, 3H, Im-4-CH$_2$-CH-C***H***$_3$), 1.95 – 2.17 (m, 1H, Im-4-CH$_2$-C***H***), 2.27 – 2.71 (m, 2H, Im-4-C***H***$_2$), 2.99 – 3.13 (m, 1H, PhThiazCH-C***H***$_2$), 3.13 – 3.26 (m, 1H, Im-4-CH$_2$-CH-C***H***$_2$), 3.34 – 3.47 (m, 1H, PhThiazCH-C***H***$_2$), 3.47 – 3.61 (m, 1H, Im-4-CH$_2$-CH-C***H***$_2$), 4.98 – 5.07 (m, 1H, PhThiazC***H***), 6.52 – 6.60 (m, 1H, Im-5-***H***), 7.27 – 7.38 (m, 22H, Ph-***H***, Im-2-***H***, Thiaz-5-***H***), 7.67 (d, 1H, ^3J = 3.3 Hz, Thiaz-4-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 639 (75) $[M + H]^+$, 482 (100). $C_{39}H_{38}N_6OS$ (638.82).

N^1-(3-Phenylbutanoyl)-N^2-[2-(5-methyl-1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.57)

The title compound was prepared from **4.42a** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.35** (410 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (390 mg, 70 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.30 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.33 (s, 3H, Im-5-C***H***$_3$), 2.68 – 2.81 (m, 3H, Im-4-C***H***$_2$ + PhCH$_3$CH-C***H***$_2$), 2.87 (dd, 1H, ^2J = 15.6 Hz, ^3J = 7.6 Hz, PhCH$_3$CH-C***H***$_2$), 3.32 – 3.45 (m, 1H, PhCH$_3$C***H***), 3.69 (t, 2H, ^3J = 5.7 Hz, Im-4-CH$_2$-C***H***$_2$), 7.03 – 7.39 (m, 20H, Ph-***H***), 7.72 (s, 1H, Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 556 (100) $[M + H]^+$. $C_{36}H_{37}N_5O$ (555.71).

N^1-[2-(5-Methyl-1-trityl-1*H*-imidazol-4-yl)ethyl]-N^2-[3-(thiophen-2-yl)butanoyl]guanidine (4.58)

The title compound was prepared from **4.42b**[7] (170 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.35** (410 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow oil (210 mg, 37 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.35 (s, 3H, Im-5-C***H***$_3$), 1.39 (d, 3H, ^3J = 6.9 Hz, ThioC***H***$_3$CH), 2.73 – 2.84 (m, 3H, ThioCH$_3$CH-C***H***$_2$ + Im-4-C***H***$_2$), 2.92 (dd, 1H, ^2J = 15.6 Hz, ^3J = 7.4 Hz, ThioCH$_3$CH-C***H***$_2$), 3.52 – 3.72 (m, 3H, Im-4-CH$_2$-C***H***$_2$ + ThioCH$_3$C***H***), 6.82 – 6.94 (m, 2H, Thio-***H***), 7.04 – 7.18 (m, 7H, Ph-***H*** + Thio-***H***), 7.26 – 7.41 (m, 10H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 562 (100) $[M + H]^+$. $C_{34}H_{35}N_5OS$ (561.74).

N^1-(3,3-Diphenylpropanoyl)-N^2-[2-(5-methyl-1-trityl-1*H*-imidazol-4-yl)ethyl]guanidine (4.59)

The title compound was prepared from **4.42c** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.35** (410 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (400 mg, 65 %).^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.32 (s, 3H, Im-5-C***H***$_3$), 2.73 (t, 2H, ^3J = 5.4 Hz, Im-4-C***H***$_2$), 3.25 (d, 2H, ^3J = 7.9 Hz, Ph$_2$CH-C***H***$_2$), 3.66 (t, 2H, ^3J = 5.4 Hz, Im-4-CH$_2$-C***H***$_2$), 4.67 (t, 1H, ^3J = 7.9 Hz, Ph$_2$C***H***), 7.05 – 7.41 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 618 (100) $[M + H]^+$. $C_{41}H_{39}N_5O$ (617.78)

N^1-[2-(5-Methyl-1-trityl-1*H*-imidazol-4-yl)ethyl]-N^2-[3-phenyl-3-(thiazol-2-yl)propanoyl]-guanidine (4.60)

The title compound was prepared from **4.42d**[7] (233 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.35** (410 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a brownish oil (290 mg, 46 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.31 (s, 3H, Im-5-C***H***$_3$), 2.66 (t, 2H, ^3J = 6.0 Hz, Im-4-C***H***$_2$), 3.06 (dd, 1H, ^2J = 16.0 Hz, ^3J = 7.0 Hz, PhThiazCH-C***H***$_2$), 3.41 (dd, 1H, ^2J = 16.0 Hz, ^3J = 8.0 Hz, PhThiazCH-C***H***$_2$), 3.52 (t, 2H, ^3J = 6.0 Hz, Im-4-CH$_2$-C***H***$_2$), 4.97 – 5.05 (m, 1H, PhThiazC***H***), 7.16 – 7.41 (m, 22H, Ph-***H*** + Im-2-***H*** + Thiaz-5-***H***), 7.66 (d, 1H, ^3J = 3.3 Hz, Thiaz-4-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 625 (100) $[M + H]^+$. $C_{38}H_{36}N_6OS$ (624.80).

N^1-Methyl-N^2-(3-phenylbutanoyl)-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.61)

The title compound was prepared from **4.42a** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.37** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (380 mg, 67 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (d, 3H, ^3J = 6.9 Hz, PhC***H***$_3$CH), 1.84 – 2.01 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.45 – 2.59 (m, 3H, Im-4-C***H***$_2$ + PhCH_3CH-C***H***$_2$), 2.66 (dd, 1H, ^2J = 14.6 Hz, ^3J = 6.4 Hz, PhCH_3CH-C***H***$_2$), 3.03 (brs, 3H, NC***H***$_3$), 3.30 – 3.49 (m, 3H, Im-4-$(CH_2)_2$-C***H***$_2$ + PhCH_3C***H***), 6.54 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.07 – 7.39 (m, 21H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 570 (100) $[M + H]^+$. $C_{37}H_{39}N_5O$ (569.74).

N^1-(3,3-Diphenylpropanoyl)-N^2-methyl-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.62)

The title compound was prepared from **4.42c** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **4.37** (424 mg, 1.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (410 mg, 65 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.78 – 2.00 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.49 (t, 2H, ^3J = 6.3 Hz, Im-4-C***H***$_2$), 2.97 (brs, 3H, NC***H***$_3$), 3.06 (d, 2H, ^3J = 7.8 Hz, Ph_2CHC***H***$_2$), 3.33 – 3.47 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 4.69 (t, 1H, ^3J = 7.8 Hz, Ph_2C***H***), 6.53 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.04 – 7.41 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 632 (100) $[M + H]^+$. $C_{42}H_{41}N_5O$ (631.81).

4.4.1.11 Preparation of the Boc/trityl-protected N^G-acylated imidazolylpropyl-guanidines 4.53-4.56

General procedure

To a solution of the pertinent carboxylic acid (1 eq) and of Boc-protected guanidine **4.41** (1 eq) in DCM (20 mL), EDC · HCl (1.2 eq) and DMAP (1.1 eq) were added at 0 °C. After stirring for 4 h at 0 °C, the solution was allowed to warm to ambient temperature and stirred for additional 20 h. DCM (20 mL) was added and the organic phase was washed with water and brine and dried over Na_2SO_4. The solvent was evaporated and the crude product purified by flash chromatography.

N^1-(*tert*-Butoxycarbonyl)-N^1-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine and N^1-(*tert*-Butoxycarbonyl)-N^2-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]-N^3-(3-phenylbutanoyl)guanidine (4.53)

The title compound (mixture of isomers) was prepared from **4.42a** (164 mg, 1.0 mmol), **4.41** (524 mg, 1.0 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (450 mg, 67 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.22 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.37 (s, 3H, Im-5-C***H***$_3$), 1.49 (s, 7H, C(C***H***$_3$)$_3$), 1.50 (s, 2H, C(C***H***$_3$)$_3$), 1.86 – 1.99 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.45 – 2.66 (m, 4H, Im-4-C***H***$_2$ + PhCH$_3$CH-C***H***$_2$), 3.25 – 3.37 (m, 1H, PhCH$_3$C***H***), 3.88 – 3.99 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 7.08 – 7.36 (m, 21H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 670 (100) [M + H]$^+$. $C_{42}H_{47}N_5O_3$ (669.85).

N^1-(*tert*-Butoxycarbonyl)-N^1-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]-N^2-[3-(thiophen-2-yl)butanoyl]guanidine and N^1-(*tert*-Butoxycarbonyl)-N^2-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]-N^3-[3-(thiophen-2-yl)butanoyl]guanidine (4.54)

The title compound (mixture of isomers) was prepared from **4.42b**[7] (170 mg, 1.0 mmol) (164 mg, 1.0 mmol), **4.41** (524 mg, 1.0 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (490 mg, 72 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.30 (d, 3H, ^3J = 6.9 Hz, ThioC***H***$_3$CH), 1.37 (s, 3H, Im-5-C***H***$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.86 – 2.00 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.44 – 2.72 (m, 4H, Im-4-C***H***$_2$ + ThioCH$_3$CH-C***H***$_2$), 3.36 – 3.49 (m, 1H, ThioCH$_3$C***H***), 3.89 – 4.00 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.75 – 6.93 (m, 2H, Thio-3,4-***H***), 7.01 – 7.39 (m, 17H, Ph-***H*** + Im-2-***H*** + Thio-5-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 676 (100) [M + H]$^+$. $C_{40}H_{45}N_5O_3S$ (675.88).

N^1-(*tert*-Butoxycarbonyl)-N^2-(3,3-diphenylpropanoyl)-N^1-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]guanidine and N^1-(*tert*-Butoxycarbonyl)-N^2-(3,3-diphenylpropanoyl)-N^3-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (4.55)

The title compound (mixture of isomers) was prepared from **4.42c** (226 mg, 1.0 mmol), **4.41** (524 mg, 1.0 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (390 mg, 53 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.39 (s, 3H, Im-5-C***H***$_3$), 1.48 (s, 6H, C(C***H***$_3$)$_3$), 1.48 (s, 3H, C(C***H***$_3$)$_3$), 1.87 – 2.01 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.43 – 2.57 (m, 2H, Im-4-C***H***$_2$), 3.07 (d, 1.3H, ^3J = 7.9 Hz, Ph$_2$CH-C***H***$_2$), 3.12 (d, 0.7H, ^3J = 8.1 Hz, Ph$_2$CH-C***H***$_2$), 3.86 – 3.97 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 4.56 (t, 1H, ^3J

= 7.8 Hz, Ph_2CH), 4.61 (t, 1H, ^3J = 7.8 Hz, Ph_2CH), 7.04 – 7.44 (m, 26H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 732 (100) $[M + H]^+$. $C_{47}H_{49}N_5O_3$ (731.92).

N^1-(*tert*-Butoxycarbonyl)-N^1-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]-N^2-[3-phenyl-3-(thiazol-2-yl)propanoyl]guanidine and N^1-(*tert*-Butoxycarbonyl)-N^2-[3-(5-methyl-1-trityl-1*H*-imidazol-4-yl)propyl]-N^3-[3-phenyl-3-(thiazol-2-yl)propanoyl]guanidine (4.56)

The title compound (mixture of isomers) was prepared from **4.42d**[7] (233 mg, 1.0 mmol), **4.41** (524 mg, 1.0 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a brownish oil (410 mg, 55 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.38 (s, 3H, Im-5-C***H***$_3$), 1.48 (s, 7.5H, C(C***H***$_3$)$_3$), 1.48 (s, 1.5H, C(C***H***$_3$)$_3$) 1.84 – 1.97 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.44 – 2.56 (m, 2H, Im-4-C***H***$_2$), 3.05 – 3.16 (m, 1H, PhThiazCH-C***H***$_2$), 3.38 – 3.49 (m, 1H, PhThiazCH-C***H***$_2$), 3.86 – 3.95 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 4.97 – 5.05 (m, 1H, PhThiazC***H***), 7.09 – 7.37 (m, 22H, Ph-***H*** + Im-2-***H*** + Thiaz-5-***H***), 7.63 (d, 0.85H, ^3J = 3.3 Hz, Thiaz-4-***H***), 7.67 (d, 0.15H, ^3J = 3.3 Hz, Thiaz-4-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 739 (100) $[M + H]^+$. $C_{44}H_{46}N_6O_3S$ (738.94).

4.4.1.12 Preparation of the N^G-acylated imidazolylpropylguanidines 4.63-4.83

General procedure

The pertinent Boc/trityl-protected N^G-acylated imidazolylpropylguanidine was stirred for 5 h in a mixture of TFA (5.0 mL) and DCM (20 mL). After removing the solvent *in vacuo*, the crude product was purified by preparative HPLC. All compounds were dried by lyophilization and obtained as trifluoroacetates.

N^1-[2-(1*H*-Imidazol-4-yl)ethyl]-N^2-(3-phenylbutanoyl)guanidine (4.63)

The title compound was prepared from **4.42** (220 mg, 0.41 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 22.5/72.5) yielded a colorless semisolid compound (80 mg, 37 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.19 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.59 (dd, 1H, ^2J = 14.8 Hz, ^3J = 9.2 Hz, $PhCH_3CH$-C***H***$_2$), 2.69 (dd, 1H, ^2J = 14.8 Hz, ^3J = 6.3 Hz, $PhCH_3CH$-C***H***$_2$), 2.93 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.03 – 3.21 (m, 1H, $PhCH_3$C***H***), 3.47 (t, 2H, ^3J = 6.6 Hz, Im-4-CH_2-C***H***$_2$), 7.10 – 7.32 (m, 6H, Ph-***H*** + Im-5-***H***), 8.50 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 21.18 (+, Ph***C***H_3CH), 22.80 (-, Im-4-***C***H_2), 36.61 (+, $PhCH_3$***C***H), 39.85 (-, Im-4-CH_2-***C***H_2), 45.06 (-, $PhCH_3CH$-***C***H_2), 116.63 (+, Im-***C***-5), 126.88 (+, 2 Ph-***C***), 126.99 (+, Ph-***C***-4), 128.89 (+, 2 Ph-***C***), 129.38 (C_{quat}, Im-***C***-4), 133.56 (+, Im-***C***-2), 144.84 (C_{quat}, Ph-***C***-1), 152.97 (C_{quat}, ***C***=N), 176.02 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3144, 3034, 2847, 1662,

1630, 1177, 1131. HRMS (EI-MS) calcd. for $C_{16}H_{21}N_5O$ [$M^{+\bullet}$] 299.1746; found 299.1744. $C_{16}H_{21}N_5O \cdot 2$ TFA (527.42).

***N*^{1}-[2-(1*H*-Imidazol-4-yl)ethyl]-*N*^{2}-[3-(thiophen-2-yl)butanoyl]guanidine (4.64)**

The title compound was prepared from **4.43** (240 mg, 0.44 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a colorless semisolid compound (62 mg, 27 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.26 (d, 3H, ^3J = 7.0 Hz, ThioC***H***$_3$CH), 2.60 (dd, 1H, ^2J = 15.0 Hz, ^3J = 9.0 Hz, ThioCH$_3$CH-C***H***$_2$), 2.72 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.2 Hz, ThioCH$_3$CH-C***H***$_2$), 2.95 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.40 – 3.56 (m, 3H, Im-4-CH$_2$-C***H***$_2$ + ThioCH$_3$C***H***), 6.81 (ddd, 1H, ^3J = 3.5 Hz, ^4J = 1.2 Hz, ^4J = 0.7 Hz, Thio-3-***H***), 6.88 (dd, 1H, ^3J = 5.0 Hz, ^3J = 3.5 Hz, Thio-4-***H***), 7.14 – 7.20 (m, 2H, Thio-5-***H*** + Im-5-***H***), 8.51 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 22.12 (+, ThioCH***C***H$_3$), 22.82 (-, Im-4-***C***H$_2$), 31.95 (+, ThioCH$_3$***C***H), 39.92 (-, Im-4-CH$_2$-***C***H$_2$), 46.06 (-, ThioCH$_3$CH-***C***H$_2$), 116.69 (+, Im-***C***-5), 123.67, 123.90 (+, Thio-***C***-3,4), 127.15 (+, Thio-***C***-5), 129.40 (C$_{quat}$, Im-***C***-4), 133.61 (+, Im-***C***-2), 148.70 (C$_{quat}$, Thio-***C***-2), 153.02 (C$_{quat}$, ***C***=N), 175.52 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3139, 3023, 2852, 1662, 1626, 1182, 1126. HRMS (EI-MS) calcd. for $C_{14}H_{19}N_5OS$ [$M^{+\bullet}$] 305.1310; found 305.1312. $C_{14}H_{19}N_5OS \cdot 2$ TFA (533.44).

***N*^{1}-(3,3-Diphenylpropanoyl)-*N*^{2}-[2-(1*H*-imidazol-4-yl)ethyl]guanidine (4.65)**

The title compound was prepared from **4.44** (350 mg, 0.58 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a white solid (80 mg, 23 %); mp 69 – 72 °C. ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 2.90 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.16 (d, 2H, ^3J = 8.2 Hz, Ph$_2$CH-C***H***$_2$), 3.45 (t, 2H, ^3J = 6.6 Hz, Im-4-CH$_2$-C***H***$_2$), 4.42 (t, 1H, ^3J = 8.2 Hz, Ph$_2$C***H***), 7.10 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.13 – 7.31 (m, 10H, Ph-***H***), 8.47 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 22.78 (-, Im-4-***C***H$_2$), 39.88 (-, Im-4-CH$_2$-***C***H$_2$), 42.25 (-, Ph$_2$CH-***C***H$_2$), 46.52 (+, Ph$_2$***C***H), 116.59 (+, Im-***C***-5), 127.16 (+, 2 Ph-***C***-4), 127.40 (+, 4 Ph-***C***), 129.04 (+, 4 Ph-***C***), 129.35 (C$_{quat}$, Im-***C***-4), 133.53 (+, Im-***C***-2), 142.82 (C$_{quat}$, 2 Ph-***C***-1), 152.93 (C$_{quat}$, ***C***=N), 175.22 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3141, 2989, 2900, 1667, 1594, 1190, 1131. HRMS (EI-MS) calcd. for $C_{21}H_{23}N_5O$ [$M^{+\bullet}$] 361.1903; found 310.1905. $C_{21}H_{23}N_5O \cdot 2$ TFA (589.49).

***N*^{1}-[4-(1*H*-Imidazol-4-yl)butyl]-*N*^{2}-(3-phenylbutanoyl)guanidine (4.66)**

The title compound was prepared from **4.45** (330 mg, 0.58 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a colorless semisolid compound (160 mg, 50 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ

[ppm] = 1.22 (d, 3H, ^{3}J = 7.0 Hz, PhC***H***$_3$CH), 1.47 – 1.65 (m, 4H, Im-4-CH$_2$-C***H***$_2$-C***H***$_2$), 2.57 – 2.67 (m, 3H, Im-4-C***H***$_2$ + PhCH$_3$CH-C***H***$_2$), 2.73 (dd, 1H, ^{2}J = 14.7 Hz, ^{3}J = 6.3 Hz, PhCH$_3$CH-C***H***$_2$), 3.11 – 3.26 (m, 3H, Im-4-(CH$_2$)$_3$-C***H***$_2$ + PhCH$_3$C***H***), 7.10 (d, 1H, ^{4}J = 1.4 Hz, Im-5-***H***), 7.13 – 7.32 (m, 5H, Ph-***H***), 8.25 (d, 1H, ^{4}J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 21.19 (+, Ph***C***H$_3$CH), 23.33, 24.81, 26.46 (-, Im-4-***C***H$_2$-***C***H$_2$-***C***H$_2$), 36.71 (+, PhCH$_3$***C***H), 40.94 (-, Im-4-(CH$_2$)$_3$-***C***H$_2$), 45.17 (-, PhCH$_3$CH-***C***H$_2$), 115.27 (+, Im-***C***-5), 126.93 (+, 2 Ph-***C***), 127.02 (+, Ph-***C***-4), 128.94 (+, 2 Ph-***C***), 132.78 (+, Im-***C***-2), 133.42 (C$_{quat}$, Im-***C***-4), 144.92 (C$_{quat}$, Ph-***C***-1), 152.72 (C$_{quat}$, ***C***=N), 176.06 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3140, 2969, 2868, 1662, 1627, 1178, 1130. HRMS (EI-MS) calcd. for C$_{18}$H$_{25}$N$_5$O [M$^{+\bullet}$] 327.2059; found 327.2059. C$_{18}$H$_{25}$N$_5$O · 2 TFA (555.47).

***N*^{1}-[4-(1*H*-Imidazol-4-yl)butyl]-*N*^{2}-[3-(thiophen-2-yl)butanoyl]guanidine (4.67)

The title compound was prepared from **4.46** (270 mg, 0.47 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a colorless semisolid compound (150 mg, 57 %). Prep. HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5). ^{1}H-NMR (300 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 1.27 (d, 3H, ^{3}J = 7.0 Hz, ThioC***H***$_3$CH), 1.46 – 1.64 (m, 4H, Im-4-CH$_2$-C***H***$_2$-C***H***$_2$), 2.55 – 2.67 (m, 3H, Im-4-C***H***$_2$ + ThioCH$_3$CH-C***H***$_2$), 2.72 (dd, 1H, ^{2}J = 14.9 Hz, ^{3}J = 6.2 Hz, ThioCH$_3$CH-C***H***$_2$), 3.18 (t, 2H, ^{3}J = 6.2 Hz, Im-4-(CH$_2$)$_3$-C***H***$_2$), 3.42 – 3.56 (m, 1H, ThioCH$_3$C***H***), 6.82 (ddd, 1H, ^{3}J = 3.5 Hz, ^{4}J = 1.3 Hz, ^{4}J = 0.6 Hz, Thio-3-***H***), 6.85 (dd, 1H, ^{3}J = 5.0 Hz, ^{3}J = 3.5 Hz, Thio-4-***H***), 7.10 (d, 1H, ^{4}J = 1.4 Hz, Im-5-***H***), 7.15 (dd, 1H, ^{3}J = 5.0 Hz, ^{4}J = 1.3 Hz, Thio-5-***H***), 8.43 (d, 1H, ^{4}J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 22.13 (+, Thio***C***H$_3$CH), 23.30, 24.81, 26.43 (-, Im-4-***C***H$_2$-***C***H$_2$-***C***H$_2$), 32.00 (+, ThioCH$_3$***C***H), 40.94 (-, Im-4-(CH$_2$)$_3$-***C***H$_2$), 46.08 (-, ThioCH$_3$CH-***C***H$_2$), 115.24 (+, Im-***C***-5), 123.67, 123.88 (+, Thio-***C***-3,4), 127.13 (+, Thio-***C***-5), 132.73 (+, Im-***C***-2), 133.38 (C$_{quat}$, Im-***C***-4), 148.73 (C$_{quat}$, Thio-***C***-2), 152.70 (C$_{quat}$, ***C***=N), 175.50 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3133, 2989, 2900, 1662, 1627, 1178, 1129. HRMS (EI-MS) calcd. for C$_{16}$H$_{23}$N$_5$OS [M$^{+\bullet}$] 333.1623; found 333.1618. C$_{16}$H$_{23}$N$_5$OS · 2 TFA (561.50).

***N*^{1}-(3,3-Diphenylpropanoyl)-*N*^{2}-[4-(1*H*-imidazol-4-yl)butyl]guanidine (4.68)

The title compound was prepared from **4.47** (220 mg, 0.35 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a white solid (96 mg, 44 %); mp 78 – 82 °C. ^{1}H-NMR (300 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 1.48 – 1.64 (m, 4H, Im-4-CH$_2$-C***H***$_2$-C***H***$_2$), 2.64 (t, 2H, ^{3}J = 6.8 Hz, Im-4-C***H***$_2$), 3.15 – 3.26 (m, 4H, Im-4-(CH$_2$)$_3$-C***H***$_2$ + Ph$_2$CHC***H***$_2$), 4.50 (t, 1H, ^{3}J = 6.8 Hz, Ph$_2$C***H***), 7.10 (d, 1H, ^{4}J = 1.4 Hz, Im-5-***H***), 7.15 – 7.34 (m, 10H, Ph-***H***), 8.47 (d, 1H, ^{4}J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 23.34, 24.81, 26.44 (-, Im-4-***C***H$_2$-***C***H$_2$-***C***H$_2$), 41.01 (-, Im-4-

(CH_2)$_3$-***C***H_2), 42.38 (-, Ph_2CH-***C***H_2), 46.66 (+, Ph_2***C***H), 115.28 (+, Im-***C***-5), 127.22 (+, 2 Ph-***C***-4), 127.57 (+, 4 Ph-***C***), 129.11 (+, 4 Ph-***C***), 132.79 (+, Im-***C***-2), 133.42 (C_{quat}, Im-***C***-4), 142.95 (C_{quat}, Ph-***C***-1), 152.71 (C_{quat}, ***C***=N), 175.30 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3027, 2866, 1665, 1601, 1181, 1128. HRMS (EI-MS) calcd. for $C_{23}H_{27}N_5O$ [$M^{+\bullet}$] 389.2216; found 389.2220. $C_{23}H_{27}N_5O$ · 2 TFA (617.54).

N^1-[4-(1*H*-Imidazol-4-yl)butyl]-N^2-[3-phenyl-3-(thiazol-2-yl)propanoyl]guanidine (4.69)

The title compound was prepared from **4.48** (210 mg, 0.33 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a pale yellow semisolid compound (170 mg, 70 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.41 – 1.62 (m, 4H, Im-4-CH_2-C***H***$_2$-C***H***$_2$), 2.59 (t, 2H, ^3J = 6.8 Hz, Im-4-C***H***$_2$), 3.15 (t, 2H, ^3J = 6.3 Hz, Im-4-(CH_2)$_3$-C***H***$_2$), 3.31 (dd, 1H, ^2J = 16.6 Hz, ^3J = 8.1 Hz, PhThiazCH-C***H***$_2$), 3.46 (dd, 1H, ^2J = 16.6 Hz, ^3J = 7.3 Hz, PhThiazCH-C***H***$_2$), 5.04 (dd, 1H, ^3J = 8.1 Hz, ^3J = 7.3 Hz, PhThiazC***H***), 7.04 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.21 – 7.35 (m, 5H, Ph-***H***), 7.65 (d, 1H, ^3J = 3.7 Hz, Thiaz-5-***H***), 7.79 (d, 1H, ^3J = 3.7 Hz, Thiaz-4-***H***), 8.41 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 23.26, 24.77, 26.37 (-, Im-4-***C***H_2-***C***H_2-***C***H_2), 41.00 (-, Im-4-(CH_2)$_3$-***C***H_2), 41.50 (-, PhThiazCH***C***H_2), 42.95 (+, PhThiaz***C***H), 115.19 (+, Im-***C***-5), 122.41 (+, Thiaz-***C***-5), 127.85 (+, 2 Ph-***C***), 127.85 (+, Ph-***C***-4), 128.73 (+, 2 Ph-***C***), 132.69 (+, Im-***C***-2), 133.32 (C_{quat}, Im-***C***-4), 137.14 (+, Thiaz-***C***-4), 138.11 (C_{quat}, Ph-***C***-1), 152.51 (C_{quat}, ***C***=N), 172.92, 175.30 (C_{quat}, Thiaz-***C***-2 + ***C***=O). IR (cm^{-1}) = 3133, 2989, 2901, 1663, 1627, 1131. HRMS (EI-MS) calcd. for $C_{20}H_{24}N_6OS$ [$M^{+\bullet}$] 396.1735; found 396.1732. $C_{20}H_{24}N_6OS$ · 3 TFA (738.58).

N^1-[3-(1*H*-Imidazol-4-yl)-2-methylpropyl]-N^2-(3-phenylbutanoyl)guanidine (4.70)

The title compound was prepared from **4.49** (250 mg, 0.44 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 30/70) yielded a colorless semisolid compound (106 mg, 43 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 0.98 (d, 3H, ^3J = 6.7 Hz, Im-4-CH_2-CH-C***H***$_3$), 1.32 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.09 – 2.26 (m, 1H, Im-4-CH_2-C***H***), 2.59 (dd, 1H, ^2J = 15.0 Hz, ^3J = 8.7 Hz, Im-4-C***H***$_2$), 2.74 (dd, 1H, ^2J = 15.2 Hz, ^3J = 7.4 Hz, PhCH_3CH-C***H***$_2$), 2.81 (dd, 1H, ^2J = 15.2 Hz, ^3J = 7.7 Hz, PhCH_3CH-C***H***$_2$), 2.86 (ddd, 1H, ^2J = 15.0 Hz, ^3J = 8.7 Hz, ^4J = 1.7 Hz, Im-4-C***H***$_2$), 3.14 – 3.39 (m, 3H, Im-4-CH_2-CH-C***H***$_2$ + PhCH_3C***H***), 7.11 – 7.32 (m, 5H, Ph-***H***), 7.37 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 8.79 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 17.23 (+, Im-4-CH_2-CH***C***H_3), 22.32 (+, PhC***H***$_3$CH), 29.83 (-, Im-4-***C***H_2), 33.76 (+, Im-4-CH_2-***C***H), 37.75 (+, PhCH_3***C***H), 46.17 (-, PhCH_3CH-***C***H_2), 47.57 (-, Im-4-CH_2-CH-***C***H_2), 117.97 (+, Im-***C***-5), 127.72 (+, Ph-***C***-4), 127.97 (+, 2 Ph-***C***), 129.68 (+, 2 Ph-***C***), 133.13 (C_{quat}, Im-***C***-4), 134.99 (+, Im-***C***-2), 146.42 (C_{quat}, Ph-***C***-1), 155.45 (C_{quat}, ***C***=N), 176.23 (C_{quat}, ***C***=O).

IR (cm^{-1}) = 3139, 2972, 2901, 1663, 1627, 1174, 1136. HRMS (LSI-MS) calcd. for $C_{18}H_{26}N_5O$ $[M + H]^+$ 328.2132; found 328.2137. $C_{18}H_{26}N_5O \cdot 2$ TFA (555.47).

N^1-[3-(1*H*-Imidazol-4-yl)-2-methylpropyl]-N^2-[3-(thiophen-2-yl)butanoyl]guanidine (4.71)

The title compound was prepared from **4.50** (250 mg, 0.43 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a colorless semisolid compound (90 mg, 37 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 0.99 (d, 3H, ^3J = 6.7 Hz, Im-4-CH_2-CH-C***H***$_3$), 1.40 (d, 3H, ^3J = 7.0 Hz, ThioC***H***$_3$CH), 2.09 – 2.30 (m, 1H, Im-4-CH_2-C***H***), 2.61 (dd, 1H, ^2J = 15.0 Hz, ^3J = 8.7 Hz, Im-4-C***H***$_2$), 2.77 (dd, 1H, ^2J = 15.4 Hz, ^3J = 7.2 Hz, ThioCH_3CH-C***H***$_2$), 2.84 (dd, 1H, ^2J = 15.4 Hz, ^3J = 7.5 Hz, ThioCH_3CH-C***H***$_2$), 2.88 (dd, 1H, ^2J = 15.0 Hz, ^3J = 5.3 Hz, Im-4-C***H***$_2$), 3.17 – 3.31 (m, 2H, Im-4-CH_2-CH-C***H***$_2$), 3.57 – 3.71 (m, 1H, ThioCH_3C***H***), 6.87 – 6.93 (m, 2H, Thio-3,4-***H***), 7.19 (dd, 1H, ^3J = 4.1 Hz, ^4J = 2.2 Hz, Thio-5-***H***), 7.37 (s, 1H, Im-5-***H***), 8.79 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 17.25 (+, Im-4-CH_2-CH-***C***H_3), 23.22 (+, ThioC***H***$_3$CH), 29.85 (-, Im-4-***C***H_2), 33.00 (+, ThioCH_3***C***H), 33.78 (+, Im-4-CH_2-***C***H), 47.09 (-, ThioCH-***C***H_2), 47.63 (-, Im-4-CH_2-CH-***C***H_2), 117.98 (+, Im-***C***-5), 124.24, 124.48 (+, Thio-***C***-3,4), 127.77 (+, Thio-***C***-5), 133.13 (C_{quat}, Im-***C***-4), 134.99 (+, Im-***C***-2), 149.96 (C_{quat}, Thio-***C***-2), 155.46 (C_{quat}, ***C***=N), 175.73 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3133, 2970, 2901, 1662, 1627, 1184, 1127. HRMS (EI-MS) calcd. for $C_{16}H_{24}N_5OS$ $[M + H]^+$ 334.1696; found 334.1692. $C_{16}H_{23}N_5OS \cdot 2$ TFA (561.50).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1*H*-imidazol-4-yl)-2-methylpropyl]guanidine (4.72)

The title compound was prepared from **4.51** (210 mg, 0.33 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a white solid (100 mg, 49 %); mp 54 – 57 °C. ^{1}H-NMR (600 MHz, D_2O, trifluoroacetate, COSY): δ [ppm] = 0.81 (d, 3H, ^3J = 6.7 Hz, Im-4-CH_2-CH-C***H***$_3$), 2.00 – 2.11 (m, 1H, Im-4-CH_2-C***H***), 2.48 (dd, 1H, ^2J = 15.2 Hz, ^3J = 8.4 Hz, Im-4-C***H***$_2$), 2.63 (dd, 1H, ^2J = 15.2 Hz, ^3J = 5.8 Hz, Im-4-C***H***$_2$), 3.07 (dd, 1H, ^2J = 13.9 Hz, ^3J = 6.8 Hz, Im-4-CH_2-CH-C***H***$_2$), 3.11 (dd, 1H, ^2J = 13.9 Hz, ^3J = 7.0 Hz, Im-4-CH_2-CH-C***H***$_2$), 3.20 (d, 2H, ^3J = 8.2 Hz, Ph_2CH-C***H***$_2$), 4.47 (t, 1H, ^3J = 8.2 Hz, Ph_2C***H***), 7.10 (s, 1H, Im-5-***H***), 7.15 – 7.30 (m, 10H, Ph-***H***), 8.36 (s, 1H, Im-2-***H***). ^{13}C-NMR (150 MHz, D_2O, trifluoroacetate, HSQC, HMBC): δ [ppm] = 16.02 (+, Im-4-CH_2-CH-C***H***$_3$), 28.25 (-, Im-4-***C***H_2), 31.86 (+, Im-4-CH_2-***C***H), 42.29 (-, Ph_2CH-***C***H_2), 46.31 (-, Im-4-CH_2-CH-***C***H_2), 46.56 (+, Ph_2***C***H), 116.26 (+, Im-***C***-5), 127.18 (+, 2 Ph-***C***-4), 127.50 (+, 4 Ph-***C***), 129.06 (+, 4 Ph-***C***), 131.13 (C_{quat}, Im-***C***-4), 133.02 (+, Im-***C***-2), 142.86 (C_{quat}, 2 Ph-***C***-1), 152.90 (C_{quat}, ***C***=N), 175.29 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3182, 3033, 2858, 1663, 1629, 1188, 1130. HRMS (LSI-MS) calcd. for $C_{23}H_{28}N_5O$ $[M + H]^+$ 390.2288; found 390.2285. $C_{23}H_{27}N_5O \cdot 2$ TFA (617.54).

N^1-[3-(1*H*-Imidazol-4-yl)-2-methylpropyl]-N^2-[3-phenyl-3-(thiazol-2-yl)propanoyl]-guanidine (4.73)

The title compound was prepared from **4.52** (220 mg, 0.34 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a pale yellow semisolid (79 mg, 31 %). ^{1}H-NMR (600 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.00 (d, 3H, ^3J = 6.7 Hz, Im-4-CH_2-CH-C***H***$_3$), 2.16 – 2.25 (m, 1H, Im-4-CH_2-C***H***), 2.62 (dd, 1H, ^2J = 15.1 Hz, ^3J = 8.8 Hz, Im-4-C***H***$_2$), 2.89 (ddd, 1H, ^2J = 15.1 Hz, ^3J = 5.4 Hz, ^4J = 2.8 Hz, Im-4-C***H***$_2$), 3.19 – 3.30 (m, 3H, Im-4-CH_2-CH-C***H***$_2$ + PhThiazCH-C***H***$_2$), 3.61 (dd, 1H, ^2J = 16.4 Hz, ^3J = 8.4 Hz, PhThiazCH-C***H***$_2$), 4.99 (dd, 1H, ^3J = 8.4 Hz, ^3J = 6.7 Hz, PhThiazC***H***), 7.27 – 7.38 (m, 5H, Ph-***H***), 7.39 (s, 1H, Im-5-***H***), 7.48 (d, 1H, ^3J = 3.3 Hz, Thiaz-5-***H***), 7.71 (d, 1H, ^3J = 3.3 Hz, Thiaz-4-***H***), 8.81 (d, 1H, ^4J = 1.0 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, CD_3OD, trifluoroacetate, HSQC): δ [ppm] = 17.18 (+, Im-4-CH_2-CH-***C***H_3), 29.80 (-, Im-4-***C***H_2), 33.74 (+, Im-4-CH_2-***C***H), 43.55 (-, PhThiazCH***C***H_2), 45.94 (+, PhThiaz***C***H), 47.58 (-, Im-4-CH_2-CH-***C***H_2), 117.94 (+, Im-***C***-5), 121.20 (+, Thiaz-***C***-5), 128.91 (+ , Ph-***C***-4), 129.05 (+, 2 Ph-***C***), 130.09 (+, 2 Ph-***C***), 133.09 (C_{quat}, Im-***C***-4), 134.98 (+, Im-***C***-2), 142.31 (C_{quat}, Ph-***C***-1), 142.83 (+, Thiaz-***C***-4), 155.32 (C_{quat}, ***C***=N), 174.29, 174.93 (C_{quat}, ***C***=O + Thiaz-***C***-2). IR (cm^{-1}) = 3131, 3030, 2853, 1663, 1627, 1170, 1130. HRMS (EI-MS) calcd. for $C_{20}H_{24}N_6OS$ [$M^{+\bullet}$] 396.1732; found 396.1731. $C_{20}H_{24}N_6OS \cdot 3$ TFA (738.58).

N^1-[3-(5-Methyl-1*H*-imidazol-4-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine (4.74)

The title compound was prepared from **4.53** (340 mg, 0.43 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 30/70) yielded a colorless semisolid compound (147 mg, 62 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.20 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.76 – 1.88 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.09 (s, 3H, Im-5-C***H***$_3$), 2.58 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 2.62 (dd, 1H, ^2J = 15.0 Hz, ^3J = 9.2 Hz, PhCH_3CH-C***H***$_2$), 2.62 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.4 Hz, PhCH_3CH-C***H***$_2$), 3.09 – 3.26 (m, 3H, Im-4-$(CH_2)_2$-C***H***$_2$ + PhCH_3C***H***), 7.12 – 7.33 (m, 5H, Ph-***H***), 8.25 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 8.78 (+, Im-5-***C***H_3), 21.55 (-, Im-4-***C***H_2), 22.37 (+, Ph***C***H_3CH), 28.22 (-, Im-4-CH_2-***C***H_2), 37.69 (+, PhCH_3***C***H), 41.65 (-, Im-4-$(CH_2)_2$-***C***H_2), 46.10 (-, PhCH_3CH-***C***H_2), 126.85 (C_{quat}, Im-***C***-5), 127.73 (+, Ph-***C***-4), 127.97 (+, 2 Ph-***C***), 129.03 (C_{quat}, Im-***C***-4), 129.68 (+, 2 Ph-***C***), 133.32 (+, Im-***C***-2), 146.45 (C_{quat}, Ph-***C***-1), 155.26 (C_{quat}, ***C***=N), 176.09 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3027, 2901, 1663, 1603, 1178, 1127. HRMS (EI-MS) calcd. for $C_{18}H_{25}N_5O$ [$M^{+\bullet}$] 327.2059; found 327.2052. $C_{18}H_{25}N_5O \cdot 2$ TFA (555.47).

N^1-[3-(5-Methyl-1*H*-imidazol-4-yl)propyl]-N^2-[3-(thiophen-2-yl)butanoyl]guanidine (4.75)

The title compound was prepared from **4.54** (470 mg, 0.70 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a

colorless semisolid compound (220 mg, 56 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.29 (d, 3H, ^{3}J = 7.0 Hz, ThioC***H***$_3$CH), 1.79 – 1.94 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.12 (s, 3H, Im-5-C***H***$_3$), 2.62 (t, 2H, ^{3}J = 7.4 Hz, Im-4-C***H***$_2$), 2.65 (dd, 1H, ^{2}J = 15.0 Hz, ^{3}J = 9.0 Hz, ThioCH$_3$CH-C***H***$_2$), 2.75 (dd, 1H, ^{2}J = 15.0 Hz, ^{3}J = 6.2 Hz, ThioCH$_3$CH-C***H***$_2$), 3.20 (t, 2H, ^{3}J = 6.7 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 3.45 – 3.60 (m, 1H, ThioCH$_3$C***H***), 6.86 (ddd, 1H, ^{3}J = 3.5 Hz, ^{4}J = 1.3 Hz, ^{4}J = 0.7 Hz, Thio-3-***H***), 6.90 (dd, 1H, ^{3}J = 5.0 Hz, ^{3}J = 3.5 Hz, Thio-4-***H***), 7.20 (dd, 1H, ^{3}J = 5.0 Hz, ^{4}J = 1.3 Hz, Thio-5-***H***), 8.28 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 7.94 (+, Im-5-***C***H$_3$), 20.01 (-, Im-4-***C***H$_2$), 22.25 (+, ThioCH***C***H$_3$), 26.26 (-, Im-4-CH$_2$-***C***H$_2$), 31.93 (+, ThioCH$_3$***C***H), 40.44 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 45.98 (-, ThioCH$_3$CH-***C***H$_2$), 123.69, 123.93 (+, Thio-***C***-3,4), 125.44 (C$_{quat}$, Im-***C***-5), 127.04 (+, Thio-***C***-5), 127.20 (C$_{quat}$, Im-***C***-4), 131.37 (+, Im-***C***-2), 148.80 (C$_{quat}$, Thio-***C***-2), 152.83 (C$_{quat}$, ***C***=N), 175.50 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3036, 2876, 1662, 1177, 1128. HRMS (EI-MS) calcd. for $C_{16}H_{23}N_5OS$ [M$^{+\bullet}$] 333.1623; found 333.1621. $C_{16}H_{23}N_5OS \cdot 2$ TFA (561.50).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(5-methyl-1*H*-imidazol-4-yl)propyl]guanidine (4.76)

The title compound was prepared from **4.55** (260 mg, 0.36 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a white solid (120 mg, 54 %); mp 54 – 57 °C. ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.74 – 1.89 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.06 (s, 3H, Im-5-C***H***$_3$), 2.57 (t, 2H, ^{3}J = 7.4 Hz, Im-4-C***H***$_2$), 3.12 – 3.25 (m, 4H, Im-4-(CH$_2$)$_2$-C***H***$_2$ + Ph$_2$CH-C***H***$_2$), 4.47 (t, 1H, ^{3}J = 8.2 Hz, Ph$_2$C***H***), 7.12 – 7.32 (m, 10H, Ph-***H***), 8.18 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 8.78 (+, Im-5-***C***H$_3$), 21.52 (-, Im-4-***C***H$_2$), 28.16 (-, Im-4-CH$_2$-***C***H$_2$), 41.65 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 43.80 (-, Ph$_2$CH-***C***H$_2$), 48.06 (+, Ph$_2$***C***H), 126.85 (C$_{quat}$, Im-***C***-5), 127.83 (+, 2 Ph-***C***-4), 128.83 (+, 4 Ph-***C***), 128.99 (C$_{quat}$, Im-***C***-4), 129.73 (+, 4 Ph-***C***), 133.31 (+, Im-***C***-2), 144.53 (C$_{quat}$, 2 Ph-***C***-1), 155.17 (C$_{quat}$, ***C***=N), 175.53 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3031, 2909, 1664, 1599, 1180, 1129. HRMS (EI-MS) calcd. for $C_{23}H_{27}N_5O$ [M$^{+\bullet}$] 389.2216; found 389.2216. $C_{23}H_{27}N_5O \cdot 2$ TFA (617.54).

N^1-[3-(5-Methyl-1*H*-imidazol-4-yl)propyl]-N^2-[3-phenyl-3-(thiazol-2-yl)propanoyl]-guanidine (4.77)

The title compound was prepared from **4.56** (390 mg, 0.53 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a white solid (150 mg, 38 %); mp 58 – 61. ^{1}H-NMR (600 MHz, D_2O, trifluoroacetate, COSY): δ [ppm] = 1.78 – 1.88 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.08 (s, 3H, Im-5-C***H***$_3$), 2.59 (t, 2H, ^{3}J = 7.3 Hz, Im-4-C***H***$_2$), 3.18 (t, 2H, ^{3}J = 6.5 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 3.23 (dd, 1H, ^{2}J = 16.3 Hz, ^{3}J = 8.0 Hz, PhThiazCH-C***H***$_2$), 3.41 (dd, 1H, ^{2}J = 16.3 Hz, ^{3}J = 7.7 Hz, PhThiazCH-C***H***$_2$), 4.90 (dd, 1H, ^{3}J = 8.0 Hz, ^{3}J = 7.7 Hz, PhThiazC***H***), 7.20 – 7.35 (m, 5H, Ph-***H***), 7.40 (d, 1H, ^{3}J = 3.3 Hz,

Thiaz-5-***H***), 7.61 (d, 1H, ^3J = 3.3 Hz, Thiaz-4-***H***), 8.25 (s, 1H, Im-2-***H***). ^{13}C-NMR (150 MHz, D_2O, trifluoroacetate, HSQC, HMBC): δ [ppm] = 7.90 (+, Im-5-***C***H_3), 19.95 (-, Im-4-***C***H_2), 26.20 (-, Im-4-CH_2-***C***H_2), 40.40 (-, Im-4-$(CH_2)_2$-***C***H_2), 42.06 (-, PhThiazCH-***C***H_2), 44.17 (+, PhThiaz***C***H), 120.61 (+, Thiaz-***C***-5), 125.41 (C_{quat}, Im-***C***-5), 127.04 (C_{quat}, Im-***C***-4), 127.75 (+, 2 Ph-***C***), 128.09 (+, Ph-***C***-4), 129.28 (+, 2 Ph-***C***), 131.37 (+, Im-***C***-2), 140.29 (C_{quat}, Ph-***C***-1), 141.64 (+, Thiaz-***C***-4), 152.99 (C_{quat}, ***C***=N), 173.17 (C_{quat}, Thiaz-***C***-2), 174.24 (C_{quat}, ***C***=O). IR (cm^{-1}) = 2989, 2901, 1668, 1652, 1179, 1129. HRMS (LSI-MS) calcd. for $C_{20}H_{25}N_6OS$ [M + H]$^+$ 397.1805; found 397.1803. $C_{20}H_{24}N_6OS \cdot 3$ TFA (738.58).

***N*^{1}-[2-(5-Methyl-1*H*-imidazol-4-yl)ethyl]-*N*^{2}-(3-phenylbutanoyl)guanidine (4.78)**

The title compound was prepared from **4.57** (360 mg, 0.65 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a white solid (150 mg, 43 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.30 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.23 (s, 3H, Im-5-C***H***$_3$), 2.73 (dd, 1H, ^2J = 15.3 Hz, ^3J = 7.4 Hz, PhCH_3CH-C***H***$_2$), 2.79 (dd, 1H, ^2J = 15.3 Hz, ^3J = 7.8 Hz, PhCH_3CH-C***H***$_2$), 2.98 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.22 – 3.36 (m, 1H, PhCH_3C***H***), 3.56 (t, 2H, ^3J = 6.6 Hz, Im-4-CH_2-C***H***$_2$), 7.14 – 7.32 (m, 5H, Ph-***H***), 8.72 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 8.82 (+, Im-5-***C***H_3), 22.43 (+, Ph***C***H_3CH), 23.45 (-, Im-4-***C***H_2), 37.67 (+, PhCH_3***C***H), 41.23 (-, Im-4-CH_2-***C***H_2), 46.03 (-, PhCH_3CH-***C***H_2), 126.32 (C_{quat}, Im-***C***-5), 127.75 (+, Ph-***C***-4), 127.97 (+, 2 Ph-***C***), 128.32 (C_{quat}, Im-***C***-4), 129.69 (+, 2 Ph-***C***), 133.80 (+, Im-***C***-2), 146.40 (C_{quat}, Ph-***C***-1), 155.30 (C_{quat}, ***C***=N), 176.11 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3036, 2876, 1670, 1602, 1179, 1128. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_5O$ [$M^{+\bullet}$] 313.1903; found 310.1899. $C_{17}H_{23}N_5O \cdot 2$ TFA (541.44).

***N*^{1}-[2-(5-Methyl-1*H*-imidazol-4-yl)ethyl]-*N*^{2}-[3-(thiophen-2-yl)butanoyl]guanidine (4.79)**

The title compound was prepared from **4.58** (200 mg, 0.36 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a colorless semisolid compound (51 mg, 26 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.38 (d, 3H, ^3J = 7.0 Hz, ThioC***H***$_3$CH), 2.26 (s, 3H, Im-5-C***H***$_3$), 2.76 (dd, 1H, ^2J = 15.6 Hz, ^3J = 7.1 Hz, ThioCH_3CH-C***H***$_2$), 2.82 (dd, 1H, ^2J = 15.6 Hz, ^3J = 7.6 Hz, ThioCH_3CH-C***H***$_2$), 3.00 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.58 (t, 2H, ^3J = 6.6 Hz, Im-4-CH_2-C***H***$_2$), 3.55 – 3.70 (m, 1H, ThioCH_3C***H***), 6.89 (ddd, 1H, ^3J = 3.5 Hz, ^4J = 1.4 Hz, ^4J = 0.7 Hz, Thio-3-***H***), 6.91 (dd, 1H, ^3J = 4.9 Hz, ^3J = 3.5 Hz, Thio-4-***H***), 7.20 (dd, 1H, ^3J = 4.9 Hz, ^4J = 1.4 Hz, Thio-5-***H***), 8.73 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 8.85 (+, Im-5-***C***H_3), 23.27 (+, ThioCH***C***H_3), 23.47 (-, Im-4-***C***H_2), 32.93 (+, ThioCH_3***C***H), 41.31 (-, Im-4-CH_2-***C***H_2), 46.99 (-, ThioCH_3CH-***C***H_2), 124.27, 124.48 (+, Thio-***C***-3,4), 126.34 (C_{quat}, Im-***C***-5), 127.79 (+, Thio-***C***-5), 128.34 (C_{quat}, Im-***C***-4), 133.83 (+, Im-***C***-2), 149.93 (C_{quat}, Thio-***C***-2), 155.28 (C_{quat},

C=N), 175.59 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3035, 2905, 1663, 1180, 1128. HRMS (EI-MS) calcd. for $C_{15}H_{21}N_5OS$ [$M^{+\bullet}$] 319.1467; found 319.1465. $C_{15}H_{21}N_5OS \cdot 2$ TFA (547.47).

N^1-(3,3-Diphenylpropanoyl)-N^2-[2-(5-methyl-1*H*-imidazol-4-yl)ethyl]guanidine (4.80)

The title compound was prepared from **4.59** (360 mg, 0.58 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a white solid (182 mg, 52 %); mp 58 – 61 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 2.18 (s, 3H, Im-5-C***H***$_3$), 2.96 (t, 2H, ^3J = 6.5 Hz, Im-4-C***H***$_2$), 3.25 (d, 2H, ^3J = 8.0 Hz, Ph_2CH-C***H***$_2$), 3.54 (t, 2H, ^3J = 6.5 Hz, Im-4-CH_2-C***H***$_2$), 4.58 (t, 1H, ^3J = 8.0 Hz, Ph_2C***H***), 7.13 – 7.31 (m, 10H, Ph-***H***), 8.71 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 8.80 (+, Im-5-***C***H_3), 23.43 (-, Im-4-***C***H_2), 41.24 (-, Im-4-CH_2-***C***H_2), 43.78 (-, Ph_2CH-***C***H_2), 48.04 (+, Ph_2***C***H), 126.30 (C_{quat}, Im-***C***-5), 127.84 (+, 2 Ph-***C***-4), 128.34 (C_{quat}, Im-***C***-4) 128.82 (+, 4 Ph-***C***), 129.73 (+, 4 Ph-***C***), 133.79 (+, Im-***C***-2), 144.49 (C_{quat}, 2 Ph-***C***-1), 155.24 (C_{quat}, ***C***=N), 175.57 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3151, 2901, 1665, 1599, 1183, 1129. HRMS (EI-MS) calcd. for $C_{22}H_{25}N_5O$ [$M^{+\bullet}$] 375.2059; found 375.2051. $C_{22}H_{25}N_5O \cdot 2$ TFA (603.51).

N^1-[2-(5-Methyl-1*H*-imidazol-4-yl)ethyl]-N^2-[3-phenyl-3-(thiazol-2-yl)propanoyl]guanidine (4.81)

The title compound was prepared from **4.60** (270 mg, 0.43 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a colorless semisolid compound (33 mg, 11 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 2.23 (s, 3H, Im-5-C***H***$_3$), 2.99 (t, 2H, ^3J = 6.6 Hz, Im-4-C***H***$_2$), 3.21 (dd, 1H, ^2J = 16.4 Hz, ^3J = 6.6 Hz, PhThiazCH-C***H***$_2$), 3.51 – 3.63 (m, 3H, Im-4-CH_2-C***H***$_2$ + PhThiazCH-C***H***$_2$), 4.95 (dd, 1H, ^3J = 8.5 Hz, ^3J = 6.6 Hz, PhThiazC***H***), 7.24 – 7.37 (m, 5H, Ph-***H***), 7.47 (d, 1H, ^3J = 3.4 Hz, Thiaz-5-***H***), 7.69 (d, 1H, ^3J = 3.4 Hz, Thiaz-4-***H***), 8.72 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 8.83 (+, Im-5-***C***H_3), 23.46 (-, Im-4-***C***H_2), 41.35 (-, Im-4-CH_2-***C***H_2), 43.51 (-, PhThiazCH-***C***H_2), 45.92 (+, PhThiaz***C***H), 121.24 (+, Thiaz-***C***-5), 126.32 (C_{quat}, Im-***C***-5), 128.36 (C_{quat}, Im-***C***-4), 128.93 (+, Ph-***C***-4), 129.08 (+, 2 Ph-***C***), 130.13 (+, 2 Ph-***C***), 133.81 (+, Im-***C***-2), 142.33 (C_{quat}, Ph-***C***-1), 142.87 (+, Thiaz-***C***-4), 155.23 (C_{quat}, ***C***=N), 174.29, 174.89 (C_{quat}, Thiaz-***C***-2 + ***C***=O). IR (cm^{-1}) = 3155, 2989, 2901, 1663, 1178, 1127. HRMS (EI-MS) calcd. for $C_{19}H_{22}N_6OS$ [$M^{+\bullet}$] 382.1576; found 382.1582. $C_{19}H_{22}N_6OS \cdot 3$ TFA (724.55).

N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^1-methyl-N^2-(3-phenylbutanoyl)guanidine (4.82)

The title compound was prepared from **4.61** (370 mg, 0.65 mmol) according to the general procedure. Purification by preparative HPLC (0.1 % TFA (aq.): 0 min: 15/85, 20 min: 35/65) yielded a colorless semisolid compound (189 mg, 52 %). ^{1}H-NMR (600 MHz, D_2O,

trifluoroacetate, COSY, 358 K): δ [ppm] = 1.89 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.45 – 2.53 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 3.20 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.42 (d, 1H, ^3J = 7.8 Hz, PhCH$_3$CH-C***H***$_2$), 3.55 (s, 3H, NC***H***$_3$), 3.83 – 3.90 (m, 1H, PhCH$_3$C***H***), 3.91 – 4.00 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 7.78 (s, 1H, Im-5-***H***), 7.79 – 7.96 (m, 5H, Ph-***H***), 9.15 (s, 1H, Im-2-***H***). ^{13}C-NMR (150 MHz, D$_2$O, trifluoroacetate, HSQC, HMBC, 358 K): δ [ppm] = 21.51 (-, Im-4-***C***H$_2$), 22.03 (+, Ph***C***H$_3$CH), 25.40 (-, Im-4-CH$_2$-***C***H$_2$), 37.20 (+, N***C***H$_3$), 37.26 (+, PhCH$_3$***C***H), 45.35 (-, PhCH$_3$CH-***C***H$_2$), 50.95 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 116.21 (+, Im-***C***-5), 127.57 (+, 2 Ph-***C***), 127.62 (+, Ph-***C***-4), 129.58 (+, 2 Ph-***C***), 133.06 (C$_{quat}$, Im-***C***-4), 134.00 (+, Im-***C***-2), 145.92 (C$_{quat}$, Ph-***C***-1), 153.46 (C$_{quat}$, ***C***=N), 175.54 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3128, 2989, 2901, 1659, 1620, 1176, 1129. HRMS (EI-MS) calcd. for $C_{18}H_{25}N_5O$ [M$^{+\bullet}$] 327.2059; found 327.2064. $C_{18}H_{25}N_5O \cdot 2$ TFA (555.47).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]-N^2-methylguanidine (4.83)

The title compound was prepared from **4.62** (400 mg, 0.63 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 20/80, 20 min: 45/65) yielded a white solid (202 mg, 52 %); mp 52 – 56 °C. ^{1}H-NMR (600 MHz, D$_2$O, trifluoroacetate, 358 K): δ [ppm] = 2.42 – 2.50 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 3.15 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 3.55 (s, 3H, NC***H***$_3$), 3.93 (d, 2H, ^3J = 8.0 Hz, Ph$_2$CHC***H***$_2$), 3.95 (t, 2H, ^3J = 7.2 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 5.15 (t, 1H, ^3J = 8.0 Hz, Ph$_2$C***H***), 7.76 (s, 1H, Im-5-***H***), 7.80 – 7.96 (m, 10H, Ph-***H***), 9.15 (s, 1H, Im-2-***H***). ^{13}C-NMR (150 MHz, D$_2$O, trifluoroacetate, HSQC, HMBC, 358 K): δ [ppm] = 21.50 (-, Im-4-***C***H$_2$), 25.37 (-, Im-4-CH$_2$-***C***H$_2$), 37.24 (+, N***C***H$_3$), 42.89 (-, Ph$_2$CH-***C***H$_2$), 47.53 (-, Ph$_2$***C***H), 51.10 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 116.1 7 (-, Im-***C***-5), 127.78 (+, 4 Ph-***C***), 128.27 (+, 2 Ph-***C***-4), 129.68 (+, 4 Ph-***C***), 133.04 (C$_{quat}$, Im-***C***-4), 133.98 (+, Im-***C***-2), 143.74 (C$_{quat}$, Ph-***C***-1), 153.45 (C$_{quat}$, ***C***=N), 174.77 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3136, 3028, 2868, 1659, 1620, 1177, 1126. HRMS (EI-MS) calcd. for $C_{23}H_{27}N_5O$ [M$^{+\bullet}$] 389.2216; found 389.2218. $C_{23}H_{27}N_5O \cdot 2$ TFA (617.54).

4.4.2 Pharmacological methods

4.4.2.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide maleate was from Tocris Bioscience (Ellisville, USA). [γ-^{32}P]GTP was synthesized according to a previously described method.[46] [^{32}P]P$_i$ (8,500 – 9,100 Ci/mmol orthophosphoric acid) was from PerkinElmer Life Sciences (Boston, MA). All unlabeled nucleotides, glycerol-3-phosphate dehydrogenase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were from Roche

(Mannheim, Germany). 3-Phosphoglycerate kinase and L-α-glycerol phosphate was from Sigma.

4.4.2.2 Steady-state GTPase activity assay

See section 3.4.2.2.

4.4.2.3 Histamine H_1R assay on guinea pig ileum[7]

See section 3.4.2.4.

4.4.2.4 Histamine H_2R assay on the isolated spontaneously beating guinea pig right atrium[7]

See section 3.4.2.5.

4.5 References

(1) Durant, G. J., Ganellin, C. R., Hills, D. W., Miles, P. D., Parsons, M. E., Pepper, E. S., White, G. R., The Histamine H_2 Receptor Agonist Impromidine: Synthesis and Structure-Activity Considerations. *J. Med. Chem.* **1985**, 28, 1414-1422.

(2) Durant, G. J., Duncan, W. A., Ganellin, C. R., Parsons, M. E., Blackmore, R. C., Rasmussen, A. C., Impromidine (SK&F 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature* **1978**, 276, 403-405.

(3) Buschauer, A., Synthesis and in Vitro Pharmacology of Arpromidine and Related Phenyl(pyridylalkyl)guanidines, a Potential New Class of Positive Inotropic Drugs. *J. Med. Chem.* **1989**, 32, 1963-1970.

(4) Buschauer, A., Friese-Kimmel, A., Baumann, G., Schunack, W., Synthesis and histamine H_2 agonistic activity of arpromidine analogues: replacement of the pheniramine-like moiety by non-heterocyclic groups. *Eur. J. Med. Chem.* **1992**, 27, 321-330.

(5) Xie, S.-X., Ghorai, P., Ye, Q.-Z., Buschauer, A., Seifert, R., Probing Ligand-Specific Histamine H_1- and H_2-Receptor Conformations with N^G-Acylated Imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006**, 317, 139-146.

(6) Xie, S.-X., Kraus, A., Ghorai, P., Ye, Q.-Z., Elz, S., Buschauer, A., Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-AK57), a Potent Partial Agonist for the Human Histamine H_1- and H_2-Receptors. *J. Pharmacol. Exp. Ther.* **2006**, 317, 1262-1268.

(7) Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press, DOI: 10.1021/jm800841w. Epub ahead of print on Oct 800825, 802008.

(8) Sellier, C., Elz, S., Buschauer, A., Schunack, W., Histamine analogues: imidazolylalkylguanidines, synthesis and *in vitro* pharmacology. *Eur. J. Med. Chem.* **1992**, 27, 27-32.

(9) Lim, H. D., van Rijn, R. M., Ling, P., Bakker, R. A., Thurmond, R. L., Leurs, R., Evaluation of Histamine H_1-, H_2-, and H_3-Receptor Ligands at the Human Histamine H_4 Receptor: Identification of 4-Methylhistamine as the First Potent and Selective H_4 Receptor Agonist. *J. Pharmacol. Exp. Ther.* **2005**, 314, 1310-1321.

(10) Davey, D. D., Lumma, W. C., Convenient synthesis of 4-methylhistamine and racemic α,4-dimethylhistamine and α,4-dimethylhistidine. *J. Org. Chem.* **1989**, 54, 3211-3213.

(11) Henry, L., Nitro-alcohols. *C. R. Hebd. Acad. Sci.* **1895**, 120, 1265-1268.

(12) Wadsworth, W. S., Emmons, W. D., The Utility of Phosphonate Carbanions in Olefin Synthesis. *J. Am. Chem. Soc.* **1961**, 83, 1733-1738.

(13) Stark, H., Hüls, A., Ligneau, X., Purand, K., Pertz, H., Arrang, J.-M., Schwartz, J.-C., Schunack, W., Development of FUB 181, a Selective Histamine H_3-Receptor Antagonist of High Oral *in Vivo* Potency with 4-(ω-(Arylalkyloxy)alkyl)-1*H*-imidazole Structure. *Arch. Pharm. (Weinheim).* **1998**, 331, 211-218.

(14) Friedman, L., Shechter, H., Preparation of Nitriles from Halides and Sodium Cyanide. An Advantageous Nucleophilic Displacement in Dimethyl Sulfoxide. *J. Org. Chem.* **1960**, 25, 877-879.

(15) Zapf, C. W., Creighton, C. J., Tomioka, M., Goodman, M., A Novel Traceless Resin-Bound Guanidinylating Reagent for Secondary Amines To Prepare N,N-Disubstituted Guanidines. *Org. Lett.* **2001**, 3, 1133-1136.

(16) Feichtinger, K., Sings, H. L., Baker, T. J., Matthews, K., Goodman, M., Triurethane-Protected Guanidines and Triflyldiurethane-Protected Guanidines: New Reagents for Guanidinylation Reactions. *J. Org. Chem.* **1998**, 63, 8432-8439.

(17) Feichtinger, K., Zapf, C., Sings, H. L., Goodman, M., Diprotected Triflylguanidines: A New Class of Guanidinylation Reagents. *J. Org. Chem.* **1998**, 63, 3804-3805.

(18) Brennauer, A., Acylguanidines as bioisosteric groups in argininamide-type neuropeptide Y Y_1 and Y_2 receptor antagonists: synthesis, stability and pharmacological activity. *Doctoral thesis*, University of Regensburg, Germany **2006**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=742&la=de.

(19) Mitsunobu, O., Yamada, M., Mukaiyama, T., Preparation of esters of phosphoric acid by the reaction of trivalent phosphorus compounds with diethyl azodicarboxylate in the presence of alcohols. *Bull. Chem. Soc. Jpn.* **1967**, 40, 935-939.

(20) Paul, R., Anderson, G. W., *N*,*N*'-Carbonyldiimidazole, a New Peptide Forming Reagent. *J. Am. Chem. Soc.* **1960**, 82, 4596-4600.

(21) Paul, R., Anderson, G. W., *N*,*N*'-Carbonyldiimidazole in Peptide Synthesis. III. A Synthesis of Isoleucine-5 Angiotensin II Amide-I. *J. Org. Chem.* **1962**, 27, 2094-2099.

(22) Kelley, M. T., Bürckstümmer, T., Wenzel-Seifert, K., Dove, S., Buschauer, A., Seifert, R., Distinct Interaction of Human and Guinea Pig Histamine H_2-Receptor with Guanidine-Type Agonists. *Mol. Pharmacol.* **2001**, 60, 1210-1225.

(23) Seifert, R., Wenzel-Seifert, K., Bürckstümmer, T., Pertz, H. H., Schunack, W., Dove, S., Buschauer, A., Elz, S., Multiple Differences in Agonist and Antagonist Pharmacology between Human and Guinea Pig Histamine H_1-Receptor. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1104-1115.

(24) Preuss, H., Species-selective Interactions of Histamine H_2 Receptors with Guanidine-type Agonists: Molecular Modelling, Site-directed Mutagenesis and Pharmacological Analysis. *Doctoral thesis*, University of Regensburg, Germany **2007**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=843&la=de.

(25) Jongejan, A., Lim, H. D., Smits, R. A., de Esch, I. J. P., Haaksma, E., Leurs, R., Delineation of Agonist Binding to the Human Histamine H_4 Receptor Using Mutational Analysis, Homology Modeling, and ab Initio Calculations. *J. Chem. Inf. Model.* **2008**, 48, 1455-1463.

(26) Schneider, E., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(27) Schnell, D., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(28) Xie, S.-X., Schalkhausser, F., Ye, Q.-Z., Seifert, R., Buschauer, A., Effects of Impromidine- and Arpromidine-Derived Guanidines on Recombinant Human and Guinea Pig Histamine H_1 and H_2 Receptors. *Arch. Pharm. (Weinheim).* **2007**, 340, 9-16.

(29) Kraus, A., Highly Potent, Selective Acylguanidine-Type Histamine H_2 Receptor Agonists: Synthesis and Structure-Activity Relationships. *Doctoral thesis*, University of Regensburg, Germany **2007**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=904&la=de.

(30) Krause, M., Rouleau, A., Stark, H., Luger, P., Lipp, R., Garbarg, M., Schwart, J. C., Schunack, W., Synthesis, X-ray crystallography, and pharmacokinetics of novel azomethine prodrugs of (*R*)-α-methylhistamine: highly potent and selective histamine H_3 receptor agonists. *J. Med. Chem.* **1995**, 38, 4070-4079.

(31) Lin, J. S., Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med. Rev.* **2000**, 4, 471-503.

(32) Cannon, K. E., Nalwalk, J. W., Stadel, R., Ge, P., Lawson, D., Silos-Santiago, I., Hough, L. B., Activation of spinal histamine H_3 receptors inhibits mechanical nociception. *Eur. J. Pharmacol.* **2003**, 470, 139-147.

(33) Levi, R., Smith, N. C., Histamine H_3-receptors: a new frontier in myocardial ischemia. *J. Pharmacol. Exp. Ther.* **2000**, 292, 825-830.

(34) Rouleau, A., Garbarg, M., Ligneau, X., Mantion, C., Lavie, P., Advenier, C., Lecomte, J. M., Krause, M., Stark, H., Schunack, W., Schwartz, J. C., Bioavailability, antinociceptive and antiinflammatory properties of BP 2-94, a histamine H_3 receptor agonist prodrug. *J. Pharmacol. Exp. Ther.* **1997**, 281, 1085-1094.

(35) McLeod, R. L., Aslanian, R., del Prado, M., Duffy, R., Egan, R. W., Kreutner, W., McQuade, R., Hey, J. A., Sch 50971, an orally active histamine H_3 receptor agonist, inhibits central neurogenic vascular inflammation and produces sedation in the guinea pig. *J. Pharmacol. Exp. Ther.* **1998**, 287, 43-50.

(36) Chen, B. C., Skoumbourdis, A. P., Sundeen, J. E., Rovnyak, G. C., Traeger, S. C., Efficient Molar-Scale Synthesis of 1-Methyl-5-acylimidazole Triflic Acid Salts. *Org. Process Res. Dev.* **2000**, 4, 613-614.

(37) Geerts, J.-P., Motte, G., Differding, E., Henichart, J.-P. Preparation of substituted 4-(1,2,3,4-tetrahydro-1-naphthalenyl)-1*H*-imidazoles and 4-(2,3-dihydro-1*H*-inden-1-yl)-1*H*-imidazoles and their formulations as antiischemics and antihypertensives. EP 717037, **1996**, *Chem. Abstr.* 125:114632.

(38) Brana, M. F., Guisado, C., Sanz, F., Synthesis of new 4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine derivatives. **2003**, 40, 917-923.

(39) Nguyen, D. N., Stump, C. A., Walsh, E. S., Fernandes, C., Davide, J. P., Ellis-Hutchings, M., Robinson, R. G., Williams, T. M., Lobell, R. B., Huber, H. E., Buser, C. A., Potent inhibitors of farnesyltransferase and geranylgeranyltransferase-I. **2002**, 12, 1269-1273.

(40) Bermudez, J., King, F. D., Sanger, G. J., Indazole and indoline as aromatic bioisosteres in the imidazole class of serotonin 5-HT_3 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1992**, 2, 1509-1512.

(41) Rudolf, K., Hurnaus, R., Stenkamp, D., Mueller, S., Krist, B. Preparation and use of substituted imidazoles as selective histamine H_3 receptor agonists. WO 2003040106, **2003**, *Chem. Abstr.* 138:368893.

(42) Stark, H., Purand, K., Hüls, A., Ligneau, X., Garbarg, M., Schwartz, J.-C., Schunack, W., [^{125}I]Iodoproxyfan and Related Compounds: A Reversible Radioligand and Novel Classes of Antagonists with High Affinity and Selectivity for the Histamine H_3 Receptor. *J. Med. Chem.* **1996**, 39, 1220-1226.

(43) Menghin, S., Pertz, H. H., Kramer, K., Seifert, R., Schunack, W., Elz, S., N^{α}-Imidazolylalkyl and Pyridylalkyl Derivatives of Histaprodifen: Synthesis and in Vitro Evaluation of Highly Potent Histamine H_1-Receptor Agonists. *J. Med. Chem.* **2003**, 46, 5458-5470.

(44) Wolin, R., Connolly, M., Afonso, A., Hey, J. A., She, H., Rivelli, M. A., Willams, S. M., West, R. E., Novel H_3 receptor antagonists. Sulfonamide homologs of histamine. *Bioorg. Med. Chem. Lett.* **1998**, 8, 2157-2162.

(45) Graßmann, S., Apelt, J., Sippl, W., Ligneau, X., Pertz, H. H., Zhao, Y. H., Arrang, J.-M., Ganellin, C. R., Schwartz, J.-C., Schunack, W., Stark, H., Imidazole Derivatives as a Novel Class of Hybrid Compounds with Inhibitory Histamine *N*-Methyltransferase Potencies and Histamine hH_3 Receptor Affinities. *Bioorg. Med. Chem.* **2003**, 11, 2163-2174.

(46) Walseth, T. F., Johnson, R. A., The enzymatic preparation of [α-^{32}P] nucleoside triphosphates, cyclic [^{32}P] AMP, and cyclic [^{32}P] GMP. *Biochim. Biophys. Acta* **1979**, 562, 11-31.

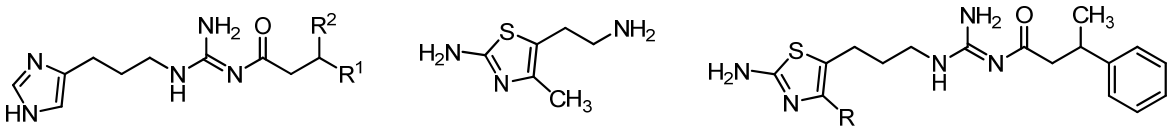
Chapter 5

Synthesis and structure-activity relationships of N^G-acylated arylpropylguanidines at the histamine receptor subtypes

5.1 Introduction

Guanidine-type compounds like arpromidine and analogs represent highly potent H_2R agonists.[1, 2] However, the strong basic guanidine group that is crucial for receptor activation also provides unfavorable pharmacokinetic properties of the compounds, for instance, very low absorption after oral administration and lack of penetration across the blood brain barrier.[3] These drawbacks could be resolved by replacing the guanidine group ($pK_a \approx 13$) with a considerably less basic acylguanidine moiety ($pK_a \approx 8$, Figure 5.1: **5.1-5.4**).[3-6]

However, these N^G-acylated imidazolylpropylguanidines developed as H_2R agonists lacked selectivity toward the hH_3R and hH_4R (Table 5.1). This drawback could be overcome in a recent study by replacing the imidazole ring with other heterocycles. Since the histamine analog and H_2R agonist amthamine (**5.5**) had been shown to be inactive at the H_3R,[7] substitution of the imidazole ring in the acylguanidine-type H_2R agonists with an aminothiazole heterocycle was elucidated as possibility to increase H_2R selectivity. Indeed, introduction of 2-aminothiazole or 2-amino-4-methylthiazole rings revealed selective acylguanidine-type H_2R agonists devoid of agonistic activity at other HRs (**5.6** and **5.7**).[3] As this bioisosteric replacement of the 1*H*-imidazol-4-yl ring was successful to increase selectivity for an HR subtype, in the present study additional heterocycles were introduced with the aim to identify further bioisosteres for the 1*H*-imidazol-4-yl ring and thereby improve HR subtype selectivity of N^G-acylated imidazolylpropylguanidines. Moreover, structure-activity relationships of N^G-acylated hetarylpropylguanidines were analyzed.



5.1 (UR-AK24): R^1 = Me, R^2 = Ph
5.2: R^1 = Me, R^2 = 2-thienyl
5.3 (UR-PG80): R^1 = R^2 = Ph
5.4: R^1 = 2-thiazolyl, R^2 = Ph

5.5
(Amthamine)

5.6 (R: Me, UR-PG276)
5.7 (R: H, UR-AK470)

Figure 5.1. Structures of N^G-acylated imidazolylpropylguanidines, which are active at the H_2R, H_3R and H_4R (**5.1-5.4**), the H_2R agonist amthamine (**5.5**) and related potent and selective acylguanidine-type H_2R agonists (**5.6** and **5.7**).

The attention was turned to heterocycles that had already been employed in the development of HR ligands, especially for the preparation of hetaryl analogs of histamine (Figure 5.2).[8, 9] Several of these histamine analogs proved to be just poorly active at the H_1R and the H_2R and therefore, the bioisosteric approach was also tried with respect to shift the selectivity of some N^G-acylated arylpropylguanidines toward the H_3R or the H_4R.

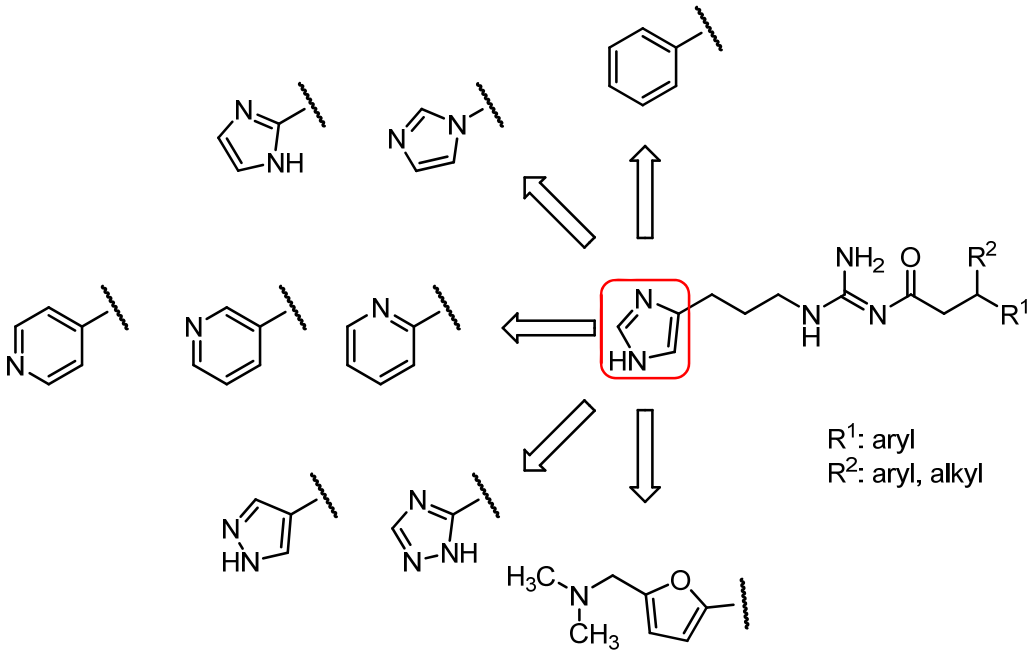
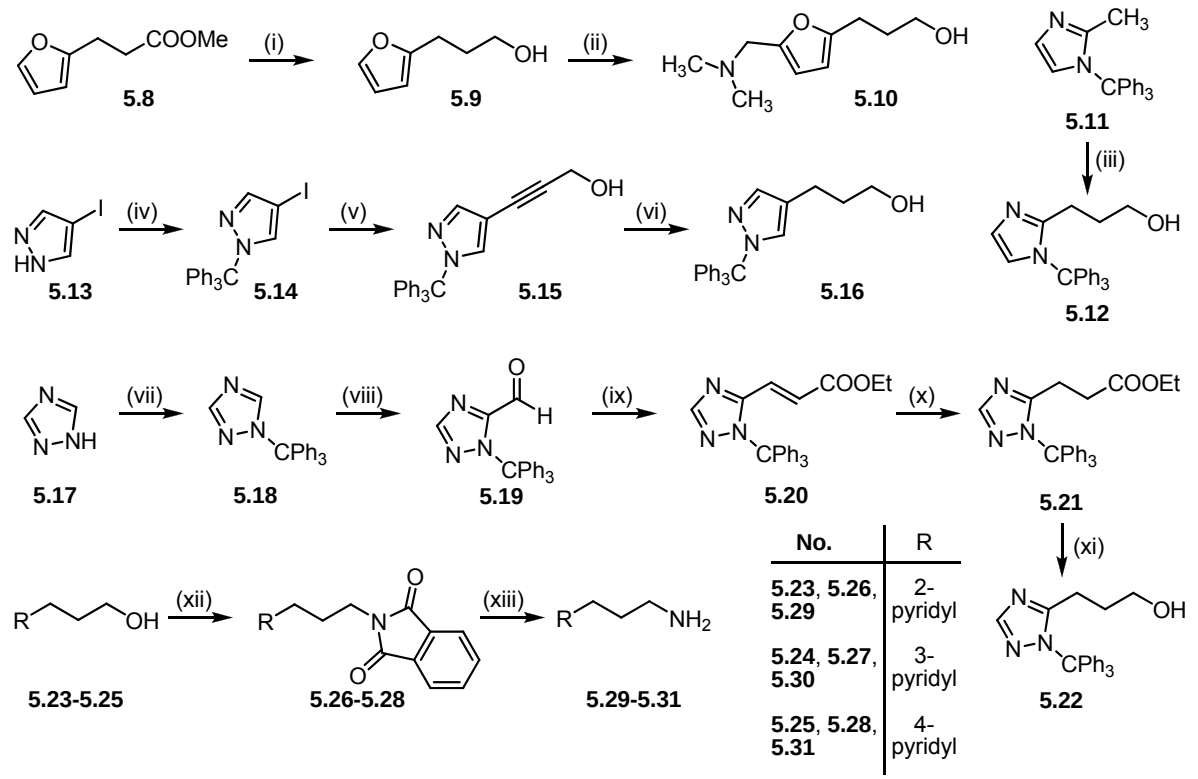


Figure 5.2. Imidazole replacement in acylguanidine-type non-selective H_2R agonists. Overview about the introduced aromatic rings.

5.2 Chemistry

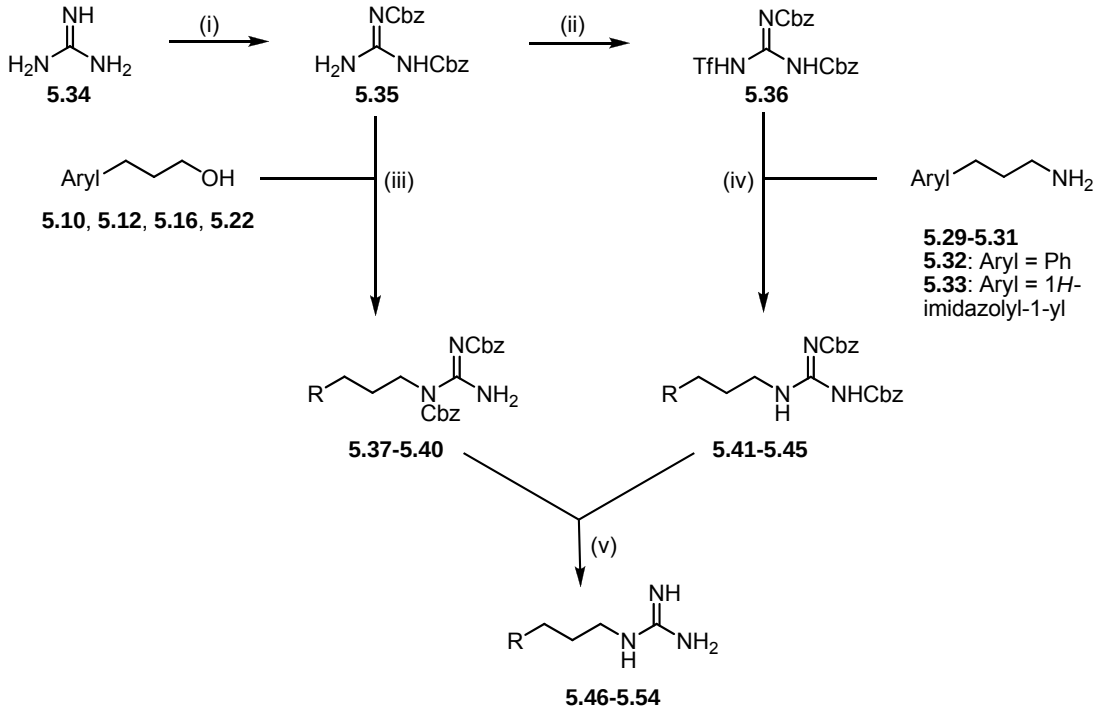
The amines and alcohols required for the preparation of the arylpropylguanidines **5.46-5.54** were synthesized as depicted in Scheme 5.1. Reduction of **5.8** with $LiAlH_4$ followed by aminomethylation in a *Mannich* reaction[10] gave the furan analog **5.10**. The imidazolylpropanol **5.12** was prepared by deprotonation of the methyl group in **5.11** with

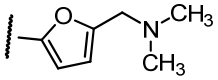
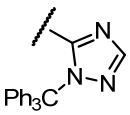
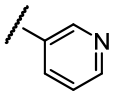
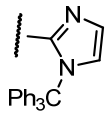
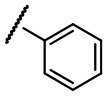
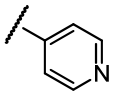
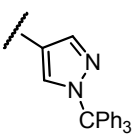
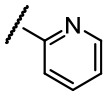
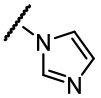
n-BuLi in THF and treatment with oxirane as electrophile.[11] Introduction of a three-membered carbon chain to the pyrazole core was performed by C-C coupling of the trityl-protected iodopyrazole **5.13** with propargyl alcohol under *Sonogashira* conditions[12] using $Pd(PPh_3)_2Cl_2$ and CuI as catalyst. Hydrogenation of the triple bond over Pd/C (10 %) provided the pyrazolylpropanol **5.16**. The triazolylpropanol **5.22** was obtained in five steps starting from 1*H*-1,2,4-triazole (**5.17**). After trityl-protection of **5.17**,[13, 14] **5.18** was treated with *n*-BuLi and DMF in THF to afford the aldehyde **5.19**.[15] Elongation of the side chain by two carbon atoms was carried out *via Horner-Wadsworth-Emmons* reaction employing triethyl phosphonoacetate.[16] Subsequent hydrogenation of the C=C double bond and reduction of the ethyl ester yielded **5.22**. Conversion of the pyridylpropanols **5.23-5.25** to the corresponding phthalimides **5.26-5.28** under *Mitsunobu* conditions[17] followed by hydrazinolysis gave the pyridylpropylamines **5.29-5.31**.[18]



Scheme 5.1. Synthesis of the arylpropyl alcohols **5.10**, **5.12**, **5.16** and **5.22**, and the pyridylpropylamines **5.29-5.31**. Reagents and conditions: (i) $LiAlH_4$ (2 eq), Et_2O, overnight, 0 °C → rt; (ii) $NH(CH_3)_2 \cdot HCl$ (1.6 eq), $(CH_2O)_n$ (1.6 eq), EtOH, overnight, reflux; (iii) oxirane (5 eq), *n*-BuLi (1.1 eq), THF, overnight, - 78 °C → rt; (iv) TrCl (1 eq), NEt_3 (1.2 eq), DCM, 12 h, 0 °C → rt; (v) $Pd(PPh_3)_2Cl_2$ (0.03 eq), CuI (0.05 eq), DIPA (4.5 eq), propargyl alcohol (1.1 eq), DMF, 48 h, - 15 °C → rt; (vi) H_2, Pd/C (10 %) (cat.), MeOH, overnight, rt; (vii) TrCl (1 eq), NEt_3 (1 eq), DCM, overnight, rt; (viii) TMEDA (1 eq), *n*-BuLi (1.1 eq), DMF (0.9 eq), THF, 12 h, -78 °C; (ix) triethyl phosphonoacetate (1.2 eq), NaH (60 % dispersion in mineral oil) (1.2 eq), THF, overnight, rt; (x) H_2, Pd/C (10 %) (cat.), EtOH/THF, overnight, rt; (xi) $LiAlH_4$ (2 eq), THF, 2 h, 0 °C → reflux; (xii) phthalimide (1.1 eq), PPh_3 (1.1 eq), DIAD (1.1 eq), THF, overnight, 0 °C → rt. (xiii) $N_2H_4 \cdot H_2O$ (6 eq), EtOH, overnight, rt.

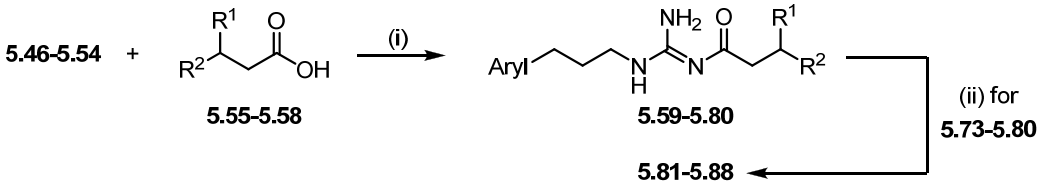
The arylpropylguanidines **5.46-5.54** were synthesized starting from the corresponding arylpropyl alcohols **5.10**, **5.12**, **5.16** and **5.22** or arylpropylamines **5.29-5.33** (Scheme 5.2). Conversion of the alcohol functionality to the di-Cbz-protected guanidines **5.37-5.40** was accomplished under *Mitsunobu* conditions[17] according to Feichtinger and colleagues.[19] Treatment of the arylpropylamines **5.29-5.33** with the triflyl-di-Cbz-protected guanidine **5.36**[19] provided the di-Cbz protected arylpropylguanidines **5.41-5.45**. Finally, the arylpropyl-guanidines **5.46-5.54** were obtained by hydrogenolytic cleavage of the Cbz groups.

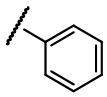
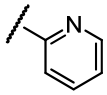
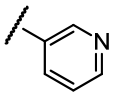
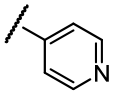
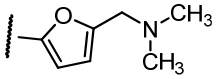
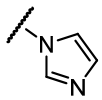


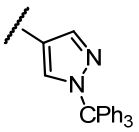
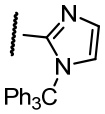
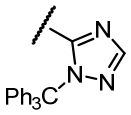
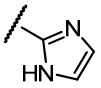
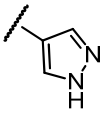
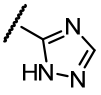
No.	R	No.	R	No.	R
5.37, **5.46**		**5.40**, **5.49**		**5.43**, **5.52**	
5.38, **5.47**		**5.41**, **5.50**		**5.44**, **5.53**	
5.39, **5.48**		**5.42**, **5.51**		**5.45**, **5.54**	

Scheme 5.2. Synthesis of the arylpropylguanidines **5.46-5.54**. Reagents and conditions: (i) benzyl chloroformate (3 eq), NaOH (5 eq), H_2O/DCM, 20 h, 0 °C; (ii) Tf_2O (1 eq), NaH (60 % dispersion in mineral oil) (2 eq), chlorobenzene, overnight, - 45 °C → rt; (iii) PPh_3 (1.5 eq), DIAD (1.5 eq), THF, overnight, 0 °C → rt; (iv) NEt_3 (1 eq), DCM, overnight, rt; (v) H_2, Pd/C (10 %) (cat.), MeOH, 3 h, rt.

The designated N^G-acylated arylpropylguanidines were prepared following the synthetic pathway outlined in Scheme 5.3. Coupling of the CDI-activated carboxylic acids[20, 21] **5.55-5.58** to the arylpropylguanidines **5.46-5.54** gave the acylguanidines **5.59-5.80**.[3] Heterocycles bearing a trityl group were deprotected under acidic conditions yielding **5.81-5.88**.



No.	Aryl	R^1	R^2
5.55	-	Me	Ph
5.56	-	Me	2-thienyl
5.57	-	Ph	Ph
5.58	-	Ph	2-thiazolyl
5.59		Me	Ph
5.60		Ph	Ph
5.61		Me	Ph
5.62		Ph	Ph
5.63		Me	Ph
5.64		Ph	Ph
5.65		Me	Ph
5.66		Ph	Ph
5.67		Me	Ph
5.68		Me	2-thienyl
5.69		Ph	Ph
5.70		Ph	2-thiazolyl
5.71		Me	Ph
5.72		Ph	Ph

No.	Aryl	R^1	R^2
5.73		Me	Ph
5.74		Ph	Ph
5.75		Me	Ph
5.76		Me	2-thienyl
5.77		Ph	Ph
5.78		Ph	2-thiazolyl
5.79		Me	Ph
5.80		Me	2-thienyl
5.81		Me	Ph
5.82		Me	2-thienyl
5.83		Ph	Ph
5.84		Ph	2-thiazolyl
5.85		Me	Ph
5.86		Ph	Ph
5.87		Me	Ph
5.88		Ph	Ph

Scheme 5.3. Synthesis of the N^G-acylated arylpropylguanidines **5.59-5.72** and **5.81-5.88**, and the trityl-protected intermediates **5.73-5.80**. Reagents and conditions: (i) CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF, 5 h, rt. (ii) TFA (20 %), DCM, 5 h, rt.

5.3 Pharmacological results and discussion

The potencies and efficacies of the synthesized compounds were evaluated for all HR subtypes in steady-state GTPase assays determining the enzymatic hydrolysis of radioactively labeled [γ-^{32}P]GTP after G-protein activation *via* the respective HR subtype (Table 5.1). These pharmacological studies were performed using membrane preparations of Sf9 insect cells expressing the hH_1R plus RGS4, the hH_2R-$G_{s\alpha S}$ fusion protein, the hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4 or the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$.[3, 22, 23]
Selected compounds were additionally investigated for H_1R and H_2R activity at the guinea pig (gp) ileum and guinea pig right atrium, respectively (Table 5.2).

5.3.1 Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay

First it was verified that – with respect to pharmacological activity at the HR subtypes – the imidazole ring of the N^G-acylated imidazolylpropylguanidines can not just be substituted by an arbitrary aromatic ring. Therefore, the imidazole ring was replaced with a phenyl ring (**5.59** and **5.60**). As expected, potencies at the $hH_{2/3/4}Rs$ dramatically decreased and agonistic activity was lost. However, at the hH_2R, the potencies of the compounds (K_B = 1,300 nM) were comparable to that of the H_2R antagonists cimetidine or ranitidine.[24]
Replacing the imidazole ring in UR-AK24 with a 2-pyridyl ring resulted in a hH_2R partial agonist (**5.61**) with a potency comparable to that of the endogenous ligand histamine (EC_{50} = 840 nM, E_{max} = 0.30). At the hH_1R, this compound also behaved as weak partial agonist. These findings are in agreement with data for the 2-pyridyl analog of histamine, betahistine, which has been found to exert weak agonism at the hH_1R and hH_2R.[23, 24] The bulky diphenylpropanoyl residue in **5.62** was not tolerated in terms of agonistic potency. At the hH_3R and hH_4R, **5.61** and **5.62** were almost inactive. The 3- and 4-pyridyl-analogs **5.63-5.66** displayed moderate antagonistic activity at the hH_2R and negligible activities at the other HR subtypes.
Replacement of the imidazole ring with the 5-[(dimethylamino)methyl]furan-2-yl group from the H_2R antagonist ranitidine afforded rather potent hH_2R antagonists (**5.67-5.70**). All prepared compounds turned out to be superior to ranitidine (K_B = 840 nM)[24] at the hH_2R, highest potency was observed for the diphenylpropanoyl analog **5.68** (K_B = 94 nM). **5.67-5.70** were weak inverse agonists at the hH_3R and almost inactive at the hH_1R and hH_4R.
Next to new heterocycles also isomers of the 1*H*-imidazol-4-yl ring were investigated. The 1*H*-imidazol-1-yl isomer **5.71** turned out to be essentially equiefficacious (E_{max} = 0.77) at the hH_4R relative to the reference compound UR-AK24 (E_{max} = 0.84), but potency suffered a 65-fold decrease (EC_{50} = 15 nM → EC_{50} = 1,000 nM). At all other HRs, **5.71** bound very

weakly (K_B > 10 µM). However, these findings show that in contrast to other HR subtypes an accessible NH-group in the imidazole ring obviously is not required to stabilize an active hH_4R conformation, but is crucial for high potency at this HR subtype. As visible with the diphenylpropanoyl analog **5.72**, that is almost inactive at the hH_4R, the ability to activate the hH_4R strongly depends on the constitution of the acyl residue.

For the 1*H*-imidazol-2-yl isomers **5.81-5.84** similar pharmacological activities at the hH_4R were observed as for the 1*H*-imidazol-1-yl isomers **5.71** and **5.72**. Both 3-arylbutanoyl analogs (**5.81** and **5.82**) exhibited moderate partial agonistic potency, however the efficacies were substantially lower (**5.81**: E_{max} = 0.35, **5.82**: E_{max} = 0.50) relative to **5.1** and **5.2**. Like for the isomer **5.72**, a diarylpropanoyl residue in **5.83** and **5.84** abolished agonism at the hH_4R. At the other HR subtypes, **5.81-5.84** were very weak antagonists or inverse agonists.

Both imidazole isomers demonstrate that the hH_4R with respect to agonistic activity better tolerates modifications in the arrangement of the nitrogen atoms in the imidazole ring than other HR subtypes.

Replacing the imidazole ring in UR-AK24 with a 1*H*-pyrazol-4-yl ring (**5.85**) resulted in a moderate decrease in potency and efficacy at the hH_2R (EC_{50} = 240 nM, E_{max} = 0.44). Interestingly, this compound was virtually inactive at all other HRs. Therefore, replacing the imidazole ring with a pyrazole ring turned out to be a promising approach for shifting selectivity toward the hH_2R. However, contrary to the reference compound UR-PG80, the bulky diphenylpropanoyl residue in **5.86** was deleterious for agonistic activity at the hH_2R.

Compared to the pyrazole **5.85**, the 1*H*-1,2,4-triazol-3-yl analog **5.87** was more efficacious, but exerted a moderately reduced potency (EC_{50} = 740 nM, E_{max} = 0.66). In contrast to the pyrazole **5.86**, the triazole analog **5.88** exhibited partial agonistic activity at the hH_2R and showed a slightly increased potency relative to **5.87** (EC_{50} = 410 nM, E_{max} = 0.42). This observation suggests the triazole and the pyrazole containing acylguanidines to feature different binding modes at the hH_2R. Obviously, triazole compounds offer more chemical space for the modification of the acyl residue than the pyrazole analogs which may be favorable with respect to the further development of potent hH_2R agonists. Like the pyrazole analogs **5.85** and **5.86**, the triazoles **5.87** and **5.88** were almost inactive at the other HRs.

Next to the distinct position of the nitrogen atoms in the five membered ring, the main difference between the imidazole ring ($pK_a \approx 7$)[6] and the pyrazole ($pK_a \approx 3$)[6] and triazole ($pK_a \approx 3$)[6] cycles is the drastically decreased basicity. Obviously, for hH_2R activity the presence of an heterocycle that is protonable under physiological conditions is not required. This constitutes an opportunity to increase H_2R selectivity.

Table 5.1. Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay.[a]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
histamine	190[4]	1.00		1,200[4]	1.00		25 ± 3.1	1.00	3	12 ± 2.5[25]	1.00	8
thioperamide	-	-		-	-		97 ± 18[26]	-0.71 ± 0.06[26]	5	110 ± 16[25]	-0.95 ± 0.07[25]	6
5.1 (UR-AK24)[5]	(>10,000)[3]			67[5]	0.87[5]		2.5 ± 0.65	0.24 ± 0.02	2	15 ± 0.3	0.84 ± 0.06	2
5.2[3]	(>10,000)[3]			110[3]	0.97[3]		1.6 ± 0.96	0.29 ± 0.01	2	6.3 ± 1.2	0.76 ± 0.03	3
5.3 (UR-PG80)[4]	(3,000)[3]	0.19[3]		78[4]	0.69[4]		(17 ± 1.5)	0.04 ± 0.05	2	8.6 ± 0.9	0.76 ± 0.03	2
5.4[3]	(>10,000)[3]			550[3]	0.93[3]		(110 ± 4.5)	-0.20 ± 0.01	2	66 ± 10	0.47 ± 0.03	2
5.59	(9,000 ± 1,300)	-0.02 ± 0.05	2	(1,300 ± 130)	-0.10 ± 0.00	2	(>10,000)	-0.19 ± 0.03	2	(>10,000)	n.d.	2
5.60	(5,500 ± 410)	-0.01 ± 0.01	2	(1,300 ± 250)	-0.12 ± 0.01	2	(7,900 ± 53)	-0.24 ± 0.01	2	(>10,000)	n.d.	2
5.61	(4,600 ± 130)	0.21 ± 0.03	2	840 ± 210	0.30 ± 0.01	3	(>10,000)	-0.17 ± 0.01	2	(>10,000)	n.d.	2
5.62	(3,900 ± 160)	0.10 ± 0.02	2	(2,100 ± 58)	-0.00 ± 0.03	2	(4,500 ± 380)	-0.44 ± 0.01	2	(>10,000)	n.d.	2
5.63	(>10,000)	n.d.	2	(5,400 ± 57)	-0.11 ± 0.05	2	(>10,000)	-0.01 ± 0.01	2	(>10,000)	n.d.	2
5.64	(9,100 ± 790)	0.06 ± 0.04	2	(730 ± 140)	-0.16 ± 0.05	2	(>10,000)	-0.14 ± 0.01	2	(>10,000)	n.d.	2
5.65	(>10,000)	0.12 ± 0.06	2	(2,900 ± 42)	0.00 ± 0.09	2	(>10,000)	-0.07 ± 0.06	2	(>10,000)	n.d.	2
5.66	(>10,000)	0.08 ± 0.07	2	(730 ± 6.8)	-0.12 ± 0.06	2	(8,800 ± 230)	-0.08 ± 0.07	2	(>10,000)	n.d.	2
5.67	(>10,000)	0.04 ± 0.01	2	(300 ± 19)	-0.12 ± 0.02	3	(1,200 ± 31)	-0.64 ± 0.00	2	(>10,000)	n.d.	2
5.68	n.d.	n.d.		(310 ± 84)	-0.12 ± 0.02	2	(1,700 ± 170)	-0.73 ± 0.01	2	(>10,000)	-0.14 ± 0.05	2
5.69	(7,800 ± 83)	-0.02 ± 0.00	2	(94 ± 29)	-0.16 ± 0.02	2	(1,300 ± 200)	-0.65 ± 0.01	2	(>10,000)	-0.16 ± 0.09	2

Table 5.1. (continued)

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
5.70	n.d.	n.d.		(740 ± 200)	-0.13 ± 0.02	2	(3,100 ± 180)	-0.62 ± 0.04	2	(>10,000)	n.d.	2
5.71	(>10,000)	n.d.	2	(>10,000)	n.d.	2	(>10,000)	-0.05 ± 0.03	2	1,000 ± 290	0.77 ± 0.01	2
5.72	(>10,000)	0.01 ± 0.01	2	(1,500 ± 99)	-0.07 ± 0.02	2	(>10,000)	-0.07 ± 0.02	2	(>10,000)	n.d.	2
5.81	(>10,000)	0.09 ± 0.06	2	(5,000 ± 200)	0.03 ± 0.03	2	(3,900 ± 1,000)	-0.32 ± 0.02	2	2,900 ± 1,300	0.35 ± 0.01	2
5.82	n.d.	n.d.		(5,500 ± 1,800)	0.04 ± 0.00	2	(4,500 ± 1,400)	-0.46 ± 0.02	2	770 ± 220	0.50 ± 0.04	3
5.83	(8,500 ± 1,800)	0.04 ± 0.01	2	(740 ± 200)	-0.04 ± 0.06	2	(3,400 ± 340)	-0.33 ± 0.00	2	(1,400 ± 480)	-0.08 ± 0.19	2
5.84	n.d.	n.d.		(6,500 ± 1,700)	-0.00 ± 0.00	2	(5,200 ± 470)	-0.46 ± 0.01	2	(>10,000)	n.d.	3
5.85	(>10,000)	n.d.	4	240 ± 59	0.44 ± 0.01	2	(>10,000)	0.04 ± 0.01	2	(>10,000)	n.d.	2
5.86	(8,800 ± 600)	0.06 ± 0.02	2	(3,800 ± 750)	0.08 ± 0.04	2	(>10,000)	-0.02 ± 0.01	2	(>10,000)	n.d.	2
5.87	(>10,000)	n.d.	2	740 ± 57	0.66 ± 0.02	3	(>10,000)	-0.03 ± 0.00	2	inactive		2
5.88	(>10,000)	0.06 ± 0.02	2	410 ± 7.0	0.42 ± 0.01	2	(>10,000)	-0.03 ± 0.02	2	inactive		3

[a] Steady-state GTPase activity on Sf9 membranes expressing the hH_1R + RGS4, hH_2R-$G_{s\alpha S}$ fusion protein, hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ + RGS4 or hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ was determined as described under *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Typical basal GTPase activities in the hH_1R assay ranged between ≈ 1.0 and 1.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 100 to 150 % above basal. Typical basal GTPase activities in the hH_2R assay ranged between ≈ 1.0 and 2.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 250 to 300 % above basal. Typical basal GTPase activities in the hH_3R assay ranged between ≈ 3.0 and 4.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to ≈ 60 % above basal. Typical basal GTPase activities in the hH_4R assay ranged between ≈ 2.5 and 3.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to 60 to 70 % above basal. n gives the number of independent experiments performed in duplicate each. For compounds investigated in the antagonist mode (K_B values), E_{max} values were determined at a concentration of 10 µM. n.d.: not determined.

5.3.2 Potencies and efficacies of selected compounds at the guinea pig ileum (gpH_1R) and guinea pig right atrium (gpH_2R)

At the guinea-pig ileum the investigated N^G-acylated arylpropylguanidines (Table 5.2) usually acted as moderate H_1R antagonists.

Like at the hH_2R, introduction of a phenyl (**5.60**), 3-pyridyl (**5.63**), 4-pyridyl (**5.65**), 5-[(dimethylamino)methyl]furan-2-yl (**5.67**), 1*H*-imidazol-1-yl (**5.71**) and 1*H*-imidazol-2-yl ring (**5.81**) resulted in a loss of agonistic efficacy at the gpH_2R relative to the reference compounds **5.1-5.4** and yielded weakly active gpH_2R antagonists. Remarkably, the H_2R antagonistic activity of compound **5.67** was comparable to that of cimetidine at the guinea pig right atrium.[27]

Substitution of the 1*H*-imidazol-4-yl ring with a 2-pyridyl ring (**5.61**) produced a partial gpH_2R agonist with increased potency relative to histamine, but, as at the hH_2R, rather low efficacy (E_{max} = 0.26). This corresponds to the histamine analogs 2-(2-pyridyl)ethanamine and betahistine that likewise display weak partial agonistic activity at the guinea pig right atrium.[8]

Replacing the 1*H*-imidazol-4-yl ring with a 1*H*-pyrazol-4-yl ring (**5.85** and **5.86**) resulted in compounds retaining partial agonistic activity at the gpH_2R. However, relative to UR-AK24 and UR-PG80, potency decreased by about one order of magnitude and efficacy was reduced. With respect to efficacy the introduction of a 1*H*-1,2,4-triazol-3-yl ring in **5.87** and **5.88** was considerably more favorable. These compounds turned out to be equiefficacious to their 1*H*-imidazol-4-yl analogs UR-AK24 and UR-PG80, whereas potency moderately decreased by a factor of about 6. These findings show the 1*H*-1,2,4-triazol-3-yl ring to be a possible bioisostere for the 1*H*-imidazol-4-yl ring with respect to gpH_2R activity. At the hH_2R, both potency and efficacy of **5.87** and **5.88** were reduced relative to UR-AK24 and UR-PG80, demonstrating the gpH_2R to better tolerate the substitution of the imidazole with a triazole ring.

Table 5.2. Pharmacological activities of selected compounds at the guinea pig ileum (gpH_1R) and the guinea pig right atrium (gpH_2R).

compound	gpH_1R		gpH_2R		
	pA_2	n[a]	pEC_{50}[b]/(pA_2)/[pD'_2][c]	E_{max}[d]	n[a]
Histamine	-	-	6.00 ± 0.10	1.00 ± 0.02	> 50
5.1 (UR-AK24)[3]	5.87 ± 0.14	4	7.80 ± 0.07	0.99 ± 0.02	4
5.3 (UR-PG80)[3]	6.13 ± 0.05	10	7.55 ± 0.09	0.85 ± 0.03	5
5.60	n.d.		(< 4.5) [< 4.5]	-	2
5.61	n.d.		6.71 ± 0.04	0.26 ± 0.03	3
5.63	n.d.		(< 4.5) [4.22 ± 0.04]	-	2
5.65	n.d.		(4.72 ± 0.34) [4.54 ± 0.03]	-	2
5.67	5.52 ± 0.06	18	(6.28 ± 0.13)	0[e]	2
5.71	5.95 ± 0.05	18	(< 4.5) [4.16 ± 0.05]	0[f]	2
5.81	5.63 ± 0.04	18	(4.90 ± 0.16) [4.44 ± 0.15]	< 10[g]	2
5.85	5.42 ± 0.10	15	6.33 ± 0.07	0.54 ± 0.03	3
5.86	5.59 ± 0.09	17	6.44 ± 0.11	0.41 ± 0.05	3
5.87	5.83 ± 0.07	16	7.00 ± 0.07[h]	1.00 ± 0.02	3
5.88	5.79 ± 0.04	16	6.69 ± 0.02	0.83 ± 0.06	3

[a] number of experiments, [b] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$. Summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, n = 16). The SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50}, [c] pD'_2 values given in brackets for compounds producing a significant, concentration-dependent reduction of the maximal response of histamine; [d] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine, [e] E_{max} (histamine) at 10 µM **5.67**: 0.68 ± 0.01; [f] E_{max} (histamine) at 30 µM **5.71**: 0.69 ± 0.03; [g] E_{max} (histamine) at 30 µM **5.81**: 0.54 ± 0.09; [h] pA_2 of cimetidine (10 µM, n = 2): 6.32 ± 0.06; n.d.: not determined.

5.3.3 Summary and conclusion

For most investigated acylguanidines replacement of the 1*H*-imidazol-4-yl ring with other heterocycles or isomers resulted in considerably reduced potency and efficacy at the hH_2R, hH_3R and hH_4R. This underlines the substantial contribution of an appropriate arrangement of the nitrogen atoms in the heterocycle for binding to the H_2R, H_3R and H_4R and for stabilizing an active conformation of the H_2R and H_4R. Only very restricted modifications of

this structural moiety of the acylguanidine-type compounds were tolerated with respect to HR activity.

The phenyl- (**5.59** and **5.60**), 3-pyridyl- (**5.63** and **5.64**), 4-pyridyl- (**5.65** and **5.66**) or substituted furan (**5.67-5.70**) analogs were virtually inactive at all HR subtypes. Replacement of the 1*H*-imidazol-4-yl ring with the 1*H*-imidazol-1-yl (**5.71** and **5.72**) and 1*H*-imidazol-2-yl (**5.81-5.84**) isomers afforded compounds (**5.71**, **5.82** and **5.83**) exhibiting partial agonistic activity only at the hH_4R. Most striking was **5.71** that displayed a comparably high efficacy as the reference compound UR-AK24 at this HR subtype. However, for all compounds potencies were substantially reduced. As these acylguanidines were poorly active at the other HR subtypes and the hH_4R activity largely depended on the type of acyl residue, further modifications in this area appear promising with respect to the development of more potent and selective hH_4R agonists.

Introduction of a 2-pyridyl (**5.61** and **5.62**), a 1*H*-pyrazol-4-yl (**5.85** and **5.86**) or a 1*H*-1,2,4-triazol-3-yl (**5.87** and **5.88**) ring provided compounds exhibiting partial to full agonistic activity at the hH_2R and gpH_2R. Except to the 2-pyridyl analogs, these compounds had negligible activities at the other hHR subtypes. In particular, the triazole ring was identified as a promising bioisostere for the imidazole ring with respect to H_2R activity. At the gpH_2R, the N^G-acylated triazolylpropylguanidines (**5.87** and **5.88**) were equiefficacious to UR-AK24 and UR-PG80. In contrast to UR-AK24 and UR-PG80, **5.87** and **5.88** proved to be virtually inactive at all other hHR subtypes, demonstrating the 1*H*-1,2,4-triazol-3-yl ring to be a versatile heterocycle toward H_2R selectivity. However, the reported bioisosteric replacement of the imidazole ring with a 2-aminothiazole or a 2-amino-4-methylthiazole ring (Figure 5.1) yielded selective H_2R agonists with higher potencies relative to the triazole analogs at the H_2R.[28] Nevertheless, N^G-acylated triazolylpropylguanidines may be of interest as a replacement of the corresponding aminothiazoles since the latter raised suspicion to have toxic effects in cellular assays. Additionally, thiazoles have been reported to form toxic metabolites after oxidative cleavage of the ring.[29-31] In contrast, the triazole ring is considered as relatively stable to oxidation by oxygenases[32] and therefore may be a promising alternative bioisostere for the imidazole ring to obtain selective acylguanidine-type H_2R agonists.

5.4 Experimental section

5.4.1 Chemistry

5.4.1.1 General conditions

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany), TCI Europe (Zwijndrecht, Belgium) and used without further purification. 2-Methyl-1-trityl-1*H*-imidazole was a gift from Dr. Birgit Striegl, Department of Pharmaceutical/ Medicinal Chemistry I, University of Regensburg. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen). Anhydrous DMF was purchased from Sigma-Aldrich Chemie GmbH. Flash chromatography was performed on silica gel (Merck silica gel 60, 40 - 63 µM). Reactions were monitored by TLC on aluminum plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm). The compounds were detected by UV light (254 nm) or a 0.3 % solution of ninhydrine in *n*-butanol (amines). All melting points are uncorrected and were measured on a Büchi 530 apparatus (Büchi GmbH, Essen, Germany).

Nuclear Magnetic Resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded with Bruker Avance 300, Bruker Avance 400 or Bruker Avance 600 NMR spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts are given in δ (ppm) relative to external standards. Abbreviations for the multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), brs (for broad singlet) and combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) was determined by DEPT 135 (distortionless enhancement by polarization transfer): "+" primary and tertiary carbon atom (positive DEPT 135 signal), "-" secondary carbon atom (negative DEPT 135 signal), "quat" quaternary carbon atom. In certain cases 2D-NMR techniques (COSY, HMQC, HSQC, HMBC, NOESY) were used to assign ^{1}H and ^{13}C chemical shifts. Infrared spectra (IR) were measured on a Bruker Tensor 27 spectrometer equipped with an ATR (attenuated total reflexion) unit from Harrick Scientific Products Inc. (Ossining/NY, US). Mass spectra (MS) were recorded on a Finnigan MAT 95 (EI-MS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ES-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Elemental analysis (C, H, N, Heraeus Elementar Vario EL III) were performed by the Analytical Department of the University Regensburg and are within ± 0.4 % unless otherwise noted.

Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was Eurosphere-100 (250 x 32 mm) (Knauer), which was attached to a UV-detector model K-2000 (Knauer). UV detection of the compounds was done at 210 nm. The temperature was 25 °C and the flow rate 37 ml/min. The mobile phase was 0.1 % TFA in millipore water and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products (TSP) equipped with a SN 400 controller, P4000 pump, an AS3000 autosampler and a Spectra Focus UV/VIS detector. Stationary phase was a Eurosphere-100 C-18 (250 x 4.0 mm, 5 µm) column (Knauer) thermostated at 30 °C. The flow rate was 0.8 ml/min and the dead time (t_0) was 3.32 min. As mobile phase gradients of MeCN/0.05 % TFA (aq.) were used and the absorbance was detected at 210 nm. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 210 nm. HPLC conditions, retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and purities of the synthesized compounds are listed in the appendix (Chapter 9).

5.4.1.2 Preparation of the arylpropylalcohols 5.10, 5.12, 5.16 and 5.22, and arylpropylamines 5.29-5.31

3-(Furan-2-yl)propan-1-ol (5.9)[33]

To a solution of **5.8** (10.0 g, 59.4 mmol) in Et_2O (200 mL), $LiAlH_4$ (4.51 g, 118.8 mmol) was added in portions at 0 °C. After addition was complete, the mixture was allowed to warm to ambient temperature and stirred overnight. The mixture was cooled externally with ice and subsequently 4.5 mL H_2O, 4.5 mL NaOH 15 % and 18 mL H_2O were added. Insoluble material was removed by filtration and washed with Et_2O (2 x 50 mL). The combined organic layers were washed with H_2O and brine and dried over Na_2SO_4. Evaporation of the solvent provided a colorless oil that was used without further purification (6.1 g, 81 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.85 – 1.96 (m, 2H, Fur-2-CH_2-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.4 Hz, Fur-2-C***H***$_2$), 3.68 (t, 2H, ^3J = 6.4 Hz, Fur-2-$(CH_2)_2$-C***H***$_2$), 6.01 (m, 1H, Fur-3-***H***), 6.28 (dd, 1H, ^3J = 3.1 Hz, ^3J = 1.9 Hz, Fur-4-***H***), 6.28 (dd, 1H, ^3J = 1.9 Hz, ^4J = 0.8 Hz, Fur-5-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.34 (-, Fur-2-CH_2-***C***H_2), 31.03 (-, Fur-2-***C***H_2), 62.09 (-, Fur-2-$(CH_2)_2$-***C***H_2), 105.07 (+, Fur-***C***-3), 110.17 (+, Fur-***C***-4), 140.99 (+, Fur-***C***-5), 155.59 (C_{quat}, Fur-***C***-2). CI-MS (NH_3) *m/z* (%): 127 (100) $[M + H]^+$. $C_7H_{10}O_2$ (126.15).

3-{5-[(Dimethylamino)methyl]furan-2-yl}propan-1-ol (5.10)[10]

A solution of **5.9** (5.51 g, 43.7 mmol), dimethylamine · HCl (5.55 g, 68.1 mmol) and paraformaldehyde (2.05 g, 68.1 mmol) in EtOH (100 mL) was refluxed overnight. After removing the solvent under reduced pressure, an aqueous solution of NaOH (1 M, 100 mL) was added and the aqueous layer was extracted with Et_2O (3 x 80 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo* yielding a colorless

oil (6.8 g, 85 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.82 – 1.94 (m, 2H, Fur-2-CH_2-C***H***$_2$), 2.23 (s, 6H, C***H***$_3$), 2.70 (t, 2H, ^3J = 7.4 Hz, Fur-2-C***H***$_2$), 3.39 (s, 2H, Fur-5-C***H***$_2$), 3.64 (t, 2H, ^3J = 6.4 Hz, Fur-2-$(CH_2)_2$-C***H***$_2$), 5.91 (d, 1H, ^3J = 3.0 Hz, Fur-3-***H***), 6.06 (d, 1H, ^3J = 3.0 Hz, Fur-4-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 24.47 (-, Fur-2-CH_2-***C***H_2), 31.14 (-, Fur-2-***C***H_2), 44.92 (+, 2 ***C***H_3), 55.92 (-, Fur-5-***C***H_2), 61.91 (-, Fur-2-$(CH_2)_2$-***C***H_2), 105.45 (+, Fur-***C***-3), 109.20 (+, Fur-***C***-4), 150.99 (C_{quat}, Fur-***C***-5), 155.59 (C_{quat}, Fur-***C***-2). CI-MS (NH_3) *m/z* (%): 184 (100) $[M + H]^+$. $C_{10}H_{17}NO_2$ (183.25).

3-(1-Trityl-1*H*-imidazol-2-yl)propan-1-ol (5.12)[11]

5.11[11] (8.0 g, 23.4 mmol) was dissolved in THF_{abs} (240 mL) under an argon atmosphere and cooled to – 78 °C. *n*-BuLi 1.6 M in hexane (15.6 mL, 25.0 mmol) was added dropwise (internal temperature < – 65 °C) and stirred for 1 h at – 78 °C. Oxirane (ca. 5.9 mL, 5.15 g, 117 mmol) was condensed into THF_{abs} (5 mL) at – 78 °C and added to the mixture. The reaction mixture was allowed to slowly warm to ambient temperature and stirred overnight. After addition of NH_4Cl (1 M, 150 mL), the product was extracted with EtOAc (4 x 70 mL) and the combined organic layers dried over Na_2SO_4. The solvent was removed *in vacuo* and purification by flash chromatography (DCM/MeOH 97.5/2.5 v/v) yielded a white solid (4.5 g, 52 %); mp 154 – 155 °C (ref.[11]: 157.5 – 157.9 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.31 – 1.42 (m, 2H, Im-2-CH_2-C***H***$_2$), 2.09 (t, 2H, ^3J = 6.3 Hz, Im-2-C***H***$_2$), 3.50 (t, 2H, ^3J = 5.3 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 6.68 (d, 1H, ^3J = 1.5 Hz, Im-4-***H***), 6.91 (d, 1H, ^3J = 1.5 Hz, Im-5-***H***), 7.08 – 7.17 (m, 6H, Ph-***H***), 7.29 – 7.38 (m, 9H, Ph-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.18, 29.49 (-, Im-2-***C***H_2-***C***H_2), 62.87 (-, Im-2-$(CH_2)_2$-***C***H_2), 75.01 (C_{quat}, ***C***Ph_3), 121.34 (+, Im-***C***-5), 124.73 (+, Im-***C***-4), 127.95 (+, 3 Ph-***C***-4), 128.11 (+, 6 Ph-***C***), 129.89 (+, 6 Ph-***C***), 142.46 (C_{quat}, 3 Ph-***C***-1), 150.45 (C_{quat}, Im-***C***-2). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 369 (44) $[M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{25}H_{24}N_2O$) C, H, N. $C_{25}H_{24}N_2O$ (368.47)

4-Iodo-1-trityl-1*H*-pyrazole (5.14)[34]

To a solution of **5.13** (5.0 g, 25.8 mmol) and tritylchloride (7.19 g, 25.8 mmol) in DCM (100 mL), NEt_3 (4.3 mL, 3.13 g, 31.0 mmol) was added dropwise at 0 °C. After addition was complete, the mixture was allowed to warm to ambient temperature and stirred for 12 h. The organic layer was washed with H_2O (2 x 30 mL) and dried over Na_2SO_4. After concentration *in vacuo*, the product was crystallized from DCM/hexane and washed with hexane yielding a white solid (7.3 g, 65 %); mp 189 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 6.99 – 7.07 (m, 6H, Ph-***H***), 7.32 – 7.41 (m, 9H, Ph-***H***), 7.44 (d, 1H, ^4J = 0.5 Hz, Pyraz-3-***H***), 7.74 (d, 1H, ^4J = 0.5 Hz, Pyraz-5-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 57.42 (C_{quat}, Pyraz-***C***-4), 78.53 (C_{quat}, ***C***Ph_3), 127.81 (+, 9 Ph-***C***), 129.49 (+, 6 Ph-***C***), 135.99 (+, Pyraz-***C***-5), 142.36

(C_{quat}, 3 Ph-***C***-1), 144.23 (+, Pyraz-***C***-3). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 437 (1) [M + H]$^+$, 243 (100) [CPh_3^+]. Anal. ($C_{22}H_{17}IN_2$) C, H, N. $C_{22}H_{17}IN_2$ (436.29).

3-(1-Trityl-1*H*-pyrazol-4-yl)prop-2-yn-1-ol (5.15)

To a solution of **5.14** (10.6 g, 24.3 mmol), $Pd(PPh_3)_2Cl_2$ (0.51 g, 0.7 mmol), CuI (0.23 g, 1.2 mmol) and diisopropylamine (15.5 mL, 11.1 g, 109.4 mmol) in degassed DMF, a solution of propargylalcohol (1.5 g, 26.7 mmol) in THF (10 mL) was added at –15 °C. After addition was complete, the mixture was stirred for 48 h at room temperature. The solvent was removed *in vacuo*, the residue dissolved in 200 mL EtOAc and washed with water (2 x 80 mL). After drying over $MgSO_4$, the solvent was evaporated and the crude product purified by flash chromatography (PE/EtOAc 80/20 v/v) yielding a beige solid (7.2 g, 81 %); mp 194 – 196 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.68 (brs, 1H, O***H***), 4.40 (s, 2H, C$\boldsymbol{H}_2$), 7.09 – 7.17 (m, 6H, Ph-***H***), 7.27 – 7.36 (m, 9H, Ph-***H***), 7.52 (d, 1H, ^4J = 0.5 Hz, Pyraz-***H***), 7.74 (d, 1H, ^4J = 0.5 Hz, Pyraz-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 51.70 (-, ***C***H_2), 77.45 (C_{quat}, ***C***Ph_3), 79.18 (C_{quat}, Pyraz-4-***C***≡C), 88.31 (C_{quat}, Pyraz-4-C≡***C***), 101.26 (C_{quat}, Pyraz-***C***-4), 127.88 (+, 6 Ph-***C***), 127.98 (+, 3 Ph-***C***), 130.12 (+, 6 Ph-***C***), 135.61 (+, Pyraz-***C***-5), 142.22 (+, Pyraz-***C***-3), 142.64 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 729 (12) [2M + H]$^+$, 243 (100) [CPh_3^+]. Anal. ($C_{25}H_{20}N_2O \cdot 0.25\ H_2O$) C, H, N. $C_{25}H_{20}N_2O$ (364.44).

3-(1-Trityl-1*H*-pyrazol-4-yl)propan-1-ol (5.16)

5.15 (7.1 g, 19.5 mmol) was dissolved in MeOH (200 mL) and hydrogenated over Pd/C (10 %) (0.71 g) at room temperature overnight. After removing the catalyst by filtration over Celite, the solvent was evaporated and the crude product recrystallized from MeCN yielding a beige solid (6.8 g, 95 %); mp 94 – 97 °C (ref.[35]: 129 – 131 °C (Et_2O)). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.55 – 1.68 (m, 2H, Pyraz-4-CH_2-C$\boldsymbol{H}_2$), 2.42 (t, 2H, ^3J = 7.6 Hz, Pyraz-4-C$\boldsymbol{H}_2$), 3.37 (t, 2H, ^3J = 6.4 Hz, Pyraz-4-$(CH_2)_2$-C$\boldsymbol{H}_2$), 4.39 (brs, 1H, O***H***), 6.98 – 7.42 (m, 16H, Ph-***H*** + Pyraz-***H***), 7.47 (s, 1H, Pyraz-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 19.84 (-, Pyraz-4-***C***H_2), 33.64 (-, Pyraz-4-CH_2-***C***H_2), 59.83 (-, Pyraz-4-$(CH_2)_2$-***C***H_2), 77.51 (C_{quat}, ***C***Ph_3), 119.74 (C_{quat}, Pyraz-***C***-4), 127.52 (+, 3 Ph-***C***-4), 127.62 (+, 6 Ph-***C***), 129.54 (+, 6 Ph-***C***), 130.17 (+, Pyraz-***C***-5), 138.98 (+, Pyraz-***C***-3), 143.12 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 369 (30) [M + H]$^+$, 243 (100) [CPh_3^+]. Anal. ($C_{25}H_{24}N_2O$) C, H, N. $C_{25}H_{24}N_2O$ (368.47).

1-Trityl-1*H*-1,2,4-triazole (5.18)

To a solution of **5.17** (6.91 g, 100 mmol) and tritylchloride (27.9 g, 100 mmol) in DCM (100 mL), NEt_3 (13.8 mL, 10.1 g, 100 mmol) was added dropwise. After addition was complete, the mixture was stirred overnight. The organic layer was washed with a saturated solution of

NaCl (3 x 30 mL) and dried over Na_2SO_4. After concentration *in vacuo*, the residue was recrystallized from MeCN yielding a white solid (27.2 g, 87 %); mp 208 °C (ref.[15]: 213 - 214 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.09 – 7.19 (m, 6H, Ph-***H***), 7.29 – 7.39 (m, 9H, Ph-***H***), 8.03 (s, 1H, Triaz-***H***), 8.08 (s, 1H, Triaz-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 78.14 (C_{quat}, ***C***Ph_3), 128.09 (+, 6 Ph-***C***), 128.31 (+, 3 Ph-***C***), 130.00 (+, 6 Ph-***C***), 141.92 (C_{quat}, 3 Ph-***C***-1), 145.75 (+, Triaz-***C***-5), 151.91 (+, Triaz-***C***-3). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 623 (22) $[2M + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{21}H_{17}N_3$) C, H, N. $C_{21}H_{17}N_3$ (311.38).

1-Trityl-1*H*-1,2,4-triazole-5-carbaldehyde (5.19)[15]

5.18 (10.0 g, 32.1 mmol) and TMEDA (4.8 mL, 3.73 g, 32.1 mmol) were dissolved in THF_{abs} (200 mL) under an argon atmosphere and cooled to – 78 °C. *n*-BuLi 1.6 M in hexane (22.1 mL, 35.3 mmol) was added dropwise (internal temperature < – 65 °C) and stirred for 1 h at – 78 °C. Anhydrous DMF (22.6 mL, 21.42 g, 29.3 mmol) was added dropwise to the mixture. After addition was complete, the mixture was stirred for 12 h at – 78 °C. The reaction mixture was allowed to warm to – 30 °C and poured in 200 mL of ice cold H_2O. Extraction with EtOAc (3 x 150 mL), drying over Na_2SO_4 and evaporation of the volatiles gave the crude product. Purification by flash chromatography (PE/EtOAc 70/30 v/v) followed by recrystallization from EtOAc/hexane yielded a white solid (8.8 g, 25.8 mmol); mp 150 – 152 °C (ref.[14]: 156 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.05 – 7.17 (m, 6H, Ph-***H***), 7.29 – 7.40 (m, 9H, Ph-***H***), 8.10 (s, 1H, Triaz-3-***H***), 9.15 (s, 1H, CO***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 80.29 (C_{quat}, ***C***Ph_3), 128.12 (+, 6 Ph-***C***), 128.52 (+, 3 Ph-***C***), 129.83 (+, 6 Ph-***C***), 141.65 (C_{quat}, 3 Ph-***C***-1), 150.02 (+, Triaz-C-3), 151.67 (C_{quat}, Triaz-***C***-5), 177.98 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 372 (2) $[M + MeOH + H]^+$, 243 (100) $[CPh_3^+]$. Anal. ($C_{22}H_{17}N_3O$) C, H, N. $C_{22}H_{17}N_3O$ (339.39).

Ethyl 3-(1-trityl-1*H*-1,2,4-triazol-5-yl)acrylate (5.20)

To a solution of triethyl phosphonoacetate (6.67 g, 5.90 mL, 29.8 mmol) in THF_{abs} (150 mL) NaH (60 % dispersion in mineral oil) (1.19 g, 29.8 mmol) was added in portions. After stirring for 1 h at ambient temperature, a solution of **5.19** (8.43 g, 24.8 mmol) in THF_{abs} (75 mL) was added dropwise. When addition was complete, the mixture was stirred overnight at room temperature. The solvent was evaporated and the crude product was taken up in EtOAc (150 mL) and washed with water (3 x 50 mL). The organic layer was dried over Na_2SO_4, evaporated and the crude product purified by flash chromatography (PE/EtOAc 60/40 v/v) giving the *E*- and *Z*-isomer. Recrystallization from EtOAc/hexane yielded the *E*-isomer and the *Z*-isomer as white solid (*E*: 6.4 g, *Z*: 1.8 g, 67 %); mp (*E*) 184 – 186 °C, mp (*Z*) 145 – 146 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) (*E*)-isomer: δ [ppm] = 1.17 (t, 3H, ^3J = 7.1 Hz, C***H***$_3$), 4.06 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$), 6.59 (dd, 1H, ^3J = 15.4 Hz, ^5J = 0.6 Hz, Triaz-5-C***H***), 6.69 (d, 1H, ^3J

= 15.4 Hz, C***H***CO), 7.06 – 7.17 (m, 6H, Ph-***H***), 7.28 – 7.38 (m, 9H, Ph-***H***), 7.97 (d, 1H, ^5J = 0.6 Hz, Triaz-3-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$) (*E*)-isomer: δ [ppm] = 14.12 (+, ***C***H_3), 60.65 (-, ***C***H_2), 79.02 (C_{quat}, ***C***Ph_3), 124.59 (+, vinyl-***C***), 128.01 (+, 6 Ph-***C***), 128.23 (+, 3 Ph-***C***), 129.32 (+, vinyl-***C***), 129.97 (+, 6 Ph-***C***), 141.89 (C_{quat}, 3 Ph-***C***-1), 149.59 (+, Triaz-***C***-3), 152.39 (C_{quat}, Triaz-***C***-5), 165.47 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 410 (1) $[M + H]^+$, 243 (100) [CPh_3^+]. Anal. *E*-isomer ($C_{26}H_{23}N_3O_2$) C, H, N. $C_{26}H_{23}N_3O_2$ (409.48).
^{1}H-NMR (300 MHz, $CDCl_3$) (*Z*)-isomer: δ [ppm] = 1.24 (t, 3H, ^3J = 7.2 Hz, C***H***$_3$), 4.19 (q, 2H, ^3J = 7.2 Hz, C***H***$_2$), 5.56 (d, 1H, ^3J = 12.0 Hz, Triaz-5-C***H***), 5.68 (d, 1H, ^3J = 12.0 Hz, C***H***CO), 7.10 – 7.20 (m, 6H, Ph-***H***), 7.26 – 7.38 (m, 9H, Ph-***H***), 7.96 (s, 1H, Triaz-3-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$) (*Z*)-isomer: δ [ppm] = 14.02 (+, ***C***H_3), 60.84 (-, ***C***H_2), 78.66 (C_{quat}, ***C***Ph_3), 125.09 (+, vinyl-***C***), 125.45 (+, vinyl-***C***), 127.85 (+, 6 Ph-***C***), 128.16 (+, 3 Ph-***C***), 130.29 (+, 6 Ph-***C***), 141.72 (C_{quat}, 3 Ph-***C***-1), 149.29 (+, Triaz-***C***-3), 151.66 (C_{quat}, Triaz-***C***-5), 165.52 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *Z*-isomer *m/z* (%): 410 (100) $[M + H]^+$. Anal. *Z*-isomer ($C_{26}H_{23}N_3O_2$) C, H, N. $C_{26}H_{23}N_3O_2$ (409.48).

Ethyl 3-(1-trityl-1*H*-1,2,4-triazol-5-yl)propanoate (5.21)[36]

5.20 (8.0 g, 19.5 mmol) was dissolved in a mixture of EtOH (120 mL) / THF (30 mL) and hydrogenated over Pd/C (10 %) (0.80 g) at room temperature overnight. After removing the catalyst by filtration over Celite, the solvent was evaporated and the crude product recrystallized from hexane/EtOAc yielding a white solid (6.3 g, 79 %); mp 140 – 141 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.19 (t, 3H, ^3J = 7.1 Hz, C***H***$_3$), 2.17 – 2.25 (m, 2H, C***H***$_2$), 2.28 – 2.35 (m, 2H, C***H***$_2$), 4.06 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$$CH_3$), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.28 – 7.39 (m, 9H, Ph-***H***), 7.87 (s, 1H, Triaz-3-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 14.20 (+, ***C***H_3), 24.38 (-, ***C***H_2), 31.09 (-, ***C***H_2), 60.47 (-, ***C***$H_2$$CH_3$), 77.89 ($C_{quat}$, ***C***$Ph_3$), 127.90 (+, 6 Ph-***C***), 127.93 (+, 3 Ph-***C***), 130.04 (+, 6 Ph-***C***), 141.78 (C_{quat}, 3 Ph-***C***-1), 148.63 (+, Triaz-***C***-3), 156.60 (C_{quat}, Triaz-***C***-5), 172.18 (C_{quat}, ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 412 (100) $[M + H]^+$. Anal. ($C_{26}H_{25}N_3O_2$) C, H, N. $C_{26}H_{25}N_3O_2$ (411.50).

3-(1-Trityl-1*H*-1,2,4-triazol-5-yl)propan-1-ol (5.22)[36]

To a solution of **5.21** (6.0 g, 14.6 mmol) in THF_{abs}, $LiAlH_4$ (1.11 g, 29.2 mmol) was added in portions at 0 °C. After addition was complete, the mixture was allowed to warm to room temperature and refluxed for 2 h. Subsequently, under external ice cooling, H_2O (1.1 mL), NaOH 15 % (1.1 mL) and H_2O (4.4 mL) were added, insoluble material filtered off and washed with THF (2 x 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent evaporated *in vacuo*. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) followed by recrystallization from hexane/EtOAc yielded a white solid (4.1 g, 76 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.34 – 1.47 (m, 2H, Triaz-5-CH_2-C***H***$_2$), 2.18 (t, 2H, ^3J =

6.7 Hz, Triaz-5-C***H***$_2$), 3.39 – 3.51 (m, 2H, Triaz-5-(CH$_2$)$_2$-C***H***$_2$), 3.75 (brs, 1H, O-***H***), 7.06 – 7.17 (m, 6H, Ph-***H***), 7.28 – 7.38 (m, 9H, Ph-***H***), 7.88 (s, 1H, Triaz-3-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 27.27, 29.07 (-, Triaz-5-***C***H$_2$-***C***H$_2$), 62.30 (-, Triaz-5-(CH$_2$)$_2$-***C***H$_2$), 77.93 (C$_{quat}$, ***C***Ph$_3$), 127.91 (+, 6 Ph-***C***), 127.94 (+, 3 Ph-***C***), 129.89 (+, 6 Ph-***C***), 141.92 (C$_{quat}$, 3 Ph-***C***-1), 148.13 (+, Triaz-***C***-3), 157.82 (C$_{quat}$, Triaz-***C***-5). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 370 (100) $[M + H]^+$. Anal. ($C_{24}H_{23}N_3O$) C, H, N. $C_{24}H_{23}N_3O$ (369.46).

Preparation of the 2-[3-(pyridyl)propyl]isoindoline-1,3-diones 5.26-5.28

For the preparation of **5.26-5.28** see section 3.4.1.12 (compounds **3.120-3.122**).

Preparation of the 3-pyridylpropan-1-amines 5.29-5.31

For the preparation of **5.29-5.31** see section 3.4.1.12 (compounds **3.15-3.17**).

5.4.1.3 Preparation of the guanidinylation reagents 5.35 and 5.36

For the preparation of **5.35** and **5.36** see section 4.4.1.6 (compounds **4.28** and **4.29**).

5.4.1.4 Preparation of the di-Cbz-protected arylpropylguanidines 5.37-5.40

General procedure

To a solution of the pertinent alcohol (1 eq), the di-Cbz-protected guanidine **5.35** (1.5 eq) and PPh_3 (1.5 eq) in THF_{abs}, DIAD (1.5 eq) in THF_{abs} was added dropwise at 0 °C. After the addition was complete, the solution was allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the crude product purified by flash chromatography.

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-(3-{5-[(dimethylamino)methyl]furan-2-yl}propyl)guanidine (5.37)

The title compound was prepared from a solution of **5.10** (2.18 g, 11.9 mmol), **5.35** (5.84 g, 17.9 mmol), PPh_3 (4.68 g, 17.9 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.5 mL, 3.61 g, 17.9 mmol) in THF_{abs} (30 mL) according to the general procedure. Purification by flash chromatography (DCM/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow oil. (4.1 g, 70 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.88 – 2.02 (m, 2H, Fur-2-CH$_2$-C***H***$_2$), 2.22 (s, 6H, C***H***$_3$), 2.61 (t, 2H, ^3J = 7.5 Hz, Fur-2-C***H***$_2$), 3.35 (s, 2H, Fur-5-C***H***$_2$), 4.06 (t, 2H, ^3J = 7.5 Hz, Fur-2-(CH$_2$)$_2$-C***H***$_2$), 5.15 (s, 2H, Ph-C***H***$_2$), 5.23 (s, 2H, Ph-C***H***$_2$), 5.93 (d, 1H, ^3J = 3.0 Hz, Fur-3-***H***), 6.00 (d, 1H, ^3J = 3.0 Hz, Fur-4-***H***), 9.25 (brs, 1H, N-***H***), 9.44 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 25.38 (-, ***C***H$_2$), 26.86 (-, ***C***H$_2$), 44.47 (-, Fur-2-(CH$_2$)$_2$-***C***H$_2$), 44.97 (+, 2 ***C***H$_3$), 55.96 (-, Fur-5-***C***H$_2$), 67.06 (-, Ph-***C***H$_2$), 68.89 (-, Ph-***C***H$_2$), 105.35 (+, Fur-

C-3), 109.12 (+, Fur-***C***-4), 127.82 (+, 1 Ph-***C***), 127.95 (+, 2 Ph-***C***), 128.30 (+, 2 Ph-***C***), 128.41 (+, 2 Ph-***C***), 128.80 (+, 3 Ph-***C***), 134.75 (C_{quat}, 1 Ph-***C***-1), 137.06 (C_{quat}, 1 Ph-***C***-1), 150.58 (C_{quat}, Fur-***C***-5), 154.90 (C_{quat}, Fur-***C***-2), 155.96, 160.62, 163.97 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 493 (100) $[M + H]^+$. $C_{27}H_{32}N_4O_5$ (492.57).

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[3-(1-trityl-1*H*-imidazol-2-yl)propyl]guanidine (5.38)

The title compound was prepared from a solution of **5.12** (3.9 g, 10.6 mmol), **5.35** (5.20 g, 15.9 mmol), PPh_3 (4.17 g, 15.9 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.1 mL, 3.22 g, 15.9 mmol) in THF_{abs} (30 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 60/40 v/v) yielded a colorless foam-like solid. (5.6 g, 78 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.59 – 1.74 (m, 2H, Im-2-CH_2-C***H***$_2$), 1.93 (t, 2H, ^3J = 8.2 Hz, Im-2-C***H***$_2$), 3.75 (t, 2H, ^3J = 6.8 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 5.07 (s, 2H, Ph-C***H***$_2$), 5.08 (s, 2H, Ph-C***H***$_2$), 6.66 (d, 1H, ^3J = 1.5 Hz, Im-4-***H***), 6.94 (d, 1H, ^3J = 1.5 Hz, Im-5-***H***), 7.03 – 7.09 (m, 6H, Ph-***H***), 7.20 – 7.42 (m, 19H, Ph-***H***), 9.08 (brs, 1H, N-***H***), 9.36 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.95, 27.83 (-, Im-2-***C***H_2-***C***H_2), 44.10 (-, Im-2-$(CH_2)_2$-***C***H_2), 67.10 (+, Ph-***C***H_2), 68.59 (+, Ph-***C***H_2), 74.70 (C_{quat}, ***C***Ph_3), 121.18 (+, Im-***C***-5), 125.49 (+, Im-***C***-4), 127.78 (+, 3 Ph-***C***), 127.83 (+, 1 Ph-***C***), 128.00 (+, 6 Ph-***C***), 128.07 (+, 2 Ph-***C***), 128.10 (+, 2 Ph-***C***), 128.40 (+, 2 Ph-***C***), 128.63 (+, 1 Ph-***C***), 128.73 (+, 2 Ph-***C***), 129.74 (+, 6 Ph-***C***), 134.81 (C_{quat}, Ph-***C***-1), 137.09 (C_{quat}, Ph-***C***-1), 142.53 (C_{quat}, 3 Ph-***C***-1), 149.74 (C_{quat}, Im-***C***-2), 155.80, 160.49, 163.75 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 678 (100) $[M + H]^+$. $C_{42}H_{39}N_5O_4$ (677.79).

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[3-(1-trityl-1*H*-pyrazol-4-yl)propyl]guanidine (5.39)

The title compound was prepared from a solution of **5.16** (4.4 g, 11.9 mmol), **5.35** (5.86 g, 17.9 mmol), PPh_3 (4.69 g, 17.9 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.5 mL, 3.62 g, 17.9 mmol) in THF_{abs} (30 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) followed by crystallization from DCM/hexane yielded a white solid. (6.1 g, 76 %); mp 144 – 145 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.73 – 1.89 (m, 2H, Pyraz-4-CH_2-C***H***$_2$), 2.40 (t, 2H, ^3J = 7.9 Hz, Pyraz-4-C***H***$_2$), 4.02 (t, 2H, ^3J = 7.4 Hz, Pyraz-4-$(CH_2)_2$-C***H***$_2$), 5.13 (s, 2H, Ph-C***H***$_2$), 5.19 (s, 2H, Ph-C***H***$_2$), 7.09 – 7.18 (m, 7H, Ph-***H*** + Pyraz-3-***H***), 7.24 – 7.42 (m, 19H, Ph-***H***), 7.44 (s, 1H, Pyraz-5-***H***), 9.26 (brs, 1H, N-***H***), 9.44 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 21.66 (-, Pyraz-4-***C***H_2), 29.87 (-, Pyraz-4-CH_2-***C***H_2), 44.65 (-, Pyraz-4-$(CH_2)_2$-***C***H_2), 67.06 (+, Ph-***C***H_2), 68.82 (+, Ph-***C***H_2), 78.37 (C_{quat}, ***C***Ph_3), 119.39 (C_{quat}, Pyraz-***C***-4), 127.64 (+, 3 Ph-***C***), 127.70 (+, 6 Ph-***C***), 127.85 (+, 1 Ph-***C***), 127.96 (+, 2 Ph-***C***), 128.30 (+, 2 Ph-***C***), 128.44 (+, 2 Ph-***C***), 128.82 (+, 2 Ph-***C***), 128.85 (+, 1 Ph-***C***), 130.17 (+, 6 Ph-***C***), 130.36 (+, Pyraz-***C***-5), 134.72 (C_{quat}, 1 Ph-***C***-1), 137.05 (C_{quat}, 1 Ph-***C***-1), 139.10 (+, Pyraz-***C***-3), 143.48 (C_{quat}, 3 Ph-***C***-1), 155.96, 160.67,

163.98 (C_{quat}, 2 ***C***=O **+** ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 678 (100) $[M + H]^+$. Anal. ($C_{42}H_{39}N_5O_4$) C, H, N. $C_{42}H_{39}N_5O_4$ (677.79).

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[3-(1-trityl-1*H*-1,2,4-triazol-5-yl)propyl]guanidine (5.40)

The title compound was prepared from a solution of **5.22** (3.86 g, 10.4 mmol), **5.35** (5.14 g, 15.7 mmol), PPh_3 (4.12 g, 15.7 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.1 mL, 3.17 g, 15.7 mmol) in THF_{abs} (50 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) followed by recrystallization from EtOAc/hexane yielded a white solid. (6.2 g, 87 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.59 – 1.71 (m, 2H, Triaz-5-CH_2-C***H***$_2$), 2.02 (t, 2H, ^3J = 8.1 Hz, Triaz-5-C***H***$_2$), 3.77 (t, 2H, ^3J = 6.9 Hz, Triaz-5-$(CH_2)_2$-C***H***$_2$), 5.09 (s, 2H, Ph-C***H***$_2$), 5.10 (s, 2H, Ph-C***H***$_2$), 7.01 – 7.10 (m, 6H, Ph-***H***), 7.19 – 7.42 (m, 19H, Ph-***H***), 7.86 (s, 1H, Triaz-5-***H***), 9.11 (brs, 1H, N-***H***), 9.37 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.37, 26.56 (-,Triaz-5-***C***H_2-***C***H_2), 43.83 (-, Triaz-5-$(CH_2)_2$-***C***H_2), 67.10 (+, Ph-***C***H_2), 68.77 (+, Ph-***C***H_2), 77.66 (C_{quat}, ***C***Ph_3), 127.81 (+, 3 Ph-***C***), 127.83 (+, 6 Ph-***C***), 127.93 (+, 1 Ph-***C***), 128.11 (+, 2 Ph-***C***), 128.20 (+, 2 Ph-***C***), 128.45 (+, 2 Ph-***C***), 128.79 (+, 3 Ph-***C***), 129.75 (+, 6 Ph-***C***), 134.66 (C_{quat}, 1 Ph-***C***-1), 136.97 (C_{quat}, 1 Ph-***C***-1), 141.94 (C_{quat}, 3 Ph-***C***-1), 148.71 (+, Triaz-***C***-3), 155.73, 160.43, 163.76 (C_{quat}, 2 ***C***=O **+** ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 679 (100) $[M + H]^+$. Anal. ($C_{41}H_{38}N_6O_4$) C, H, N. $C_{41}H_{38}N_6O_4$ (678.78).

5.4.1.5 Preparation of the di-Cbz-protected arylpropylguanidines 5.41-5.45

General procedure

To a solution of the pertinent amine (1 eq) and **5.36** (0.9 eq) in DCM NEt_3 (1 eq) was added. After stirring overnight at room temperature, the organic layer was washed with saturated $NaHCO_3$ solution, water and brine and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography.

N^1,N^2-Bis(benzyloxycarbonyl)-N^3-(3-phenylpropyl)guanidine (5.41)

The title compound was prepared from **5.32** (0.78 g, 5.8 mmol), **5.36** (2.39 g, 5.2 mmol) and NEt_3 (0.8 mL, 0.59 g, 5.8 mmol) in DCM (50 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) yielded a colorless oil (1.8 g, 70 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.86 – 1.99 (m, 1H, Ph-CH_2-C***H***$_2$), 2.68 (t, 2H, ^3J = 7.7 Hz, Ph-C***H***$_2$), 3.42 – 3.52 (m, 2H, Ph-$(CH_2)_2$-C***H***$_2$), 5.14 (s, 2H, Ph-C***H***$_2$-O), 5.19 (s, 2H, Ph-C***H***$_2$-O), 7.14 – 7.45 (m, 15H, Ph-***H***), 8.36 (t, 1H, ^3J = 4.8 Hz, N-***H***), 11.76 (s, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.56 (-, Ph-CH_2-***C***H_2), 33.03 (-, Ph-***C***H_2), 40.57 (-, Ph-CH_2-CH_2-***C***H_2), 67.21 (-, Ph-***C***H_2-O), 68.19 (-, Ph-***C***H_2-O), 126.08 (+, 1 Ph-***C***), 127.92 (+, 1 Ph-***C***), 128.16 (+, 2 Ph-***C***), 128.38 (+, 2 Ph-***C***), 128.42 (+, 2 Ph-***C***), 128.48 (+, 2 Ph-***C***),

128.50 (+, 2 Ph-***C***), 128.73 (+, 2 Ph-***C***), 128.81 (+, 1 Ph-***C***), 134.69 (C_{quat}, 1 Ph-***C***), 136.88 (C_{quat}, 1 Ph-***C***), 141.07 (C_{quat}, 1 Ph-***C***), 153.94 (C_{quat}, ***C***=O), 156.05 (C_{quat}, ***C***=N), 163.78 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 446 (99) $[M + H]^+$, 312 (100) $[M – Ph\text{-}CH_2\text{-}OCO + H]^+$. $C_{26}H_{27}N_3O_4$ (445.51).

***N*^{1},*N*^{2}-Bis(benzyloxycarbonyl)-*N*^{3}-[3-(pyridin-2-yl)propyl]guanidine (5.42)

The title compound was prepared from **5.29** (1.71 g, 12.6 mmol), **5.36** (5.19 g, 11.3 mmol) and NEt_3 (1.7 mL, 1.27 g, 12.6 mmol) in DCM (100 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) yielded a pale yellow oil (4.3 g, 84 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.99 – 2.11 (m, 2H, Pyr-2-CH_2-C***H***$_2$), 2.85 (t, 2H, ^3J = 7.6 Hz, Pyr-2-C***H***$_2$), 3.45 – 3.54 (m, 2H, Pyr-2-$(CH_2)_2$-C***H***$_2$), 5.12 (s, 2H, PhC***H***$_2$), 5.17 (s, 2H, PhC***H***$_2$), 7.08 (ddd, 1H, ^3J = 7.5 Hz, ^3J = 4.9 Hz, ^4J = 1.2 Hz, Pyr-5-***H***), 7.15 (ddd, 1H, ^3J = 7.8 Hz, ^4J = 1.2 Hz, ^5J = 0.9 Hz, Pyr-3-***H***), 7.56 (ddd, 1H, ^3J = 7.8 Hz, ^3J = 7.5 Hz, ^4J = 1.8 Hz, Pyr-4-***H***), 8.44 (t, 1H, ^3J = 5.0 Hz, N-***H***), 8.51 (ddd, 1H, ^3J = 4.9 Hz, ^4J = 1.8 Hz, ^5J = 0.9 Hz, Pyr-6-***H***), 11.72 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 28.67 (-, Pyr-2-CH_2-***C***H_2), 35.34 (-, Pyr-2-***C***H_2), 40.63 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 67.17 (-, Ph***C***H_2), 68.16 (-, Ph***C***H_2), 121.26 (+, Pyr-***C***-5), 122.93 (+, Pyr-***C***-3), 127.93 (+, 1 Ph-***C***), 128.18 (+, 2 Ph-***C***), 128.43 (+, 2 Ph-***C***), 128.52 (+, 2 Ph-***C***), 128.73 (+, 2 Ph-***C***), 128.81 (+, 1 Ph-***C***), 134.70 (C_{quat}, 1 Ph-***C***-1), 136.46 (+, Pyr-***C***-4), 136.86 (C_{quat}, 1 Ph-***C***-1), 149.41 (+, Pyr-***C***-6), 153.82, 156.03, 163.76 (C_{quat}, 2 ***C***=O + ***C***=N), (C_{quat}, ***C***=O), 160.77, (C_{quat}, Pyr-***C***-2). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 447 (100) $[M + H]^+$. $C_{25}H_{26}N_4O_4$ (446.50).

***N*^{1},*N*^{2}-Bis(benzyloxycarbonyl)-*N*^{3}-[3-(pyridin-3-yl)propyl]guanidine (5.43)

The title compound was prepared from **5.30** (1.93 g, 14.2 mmol), **5.36** (5.86 g, 12.8 mmol) and NEt_3 (2.0 mL, 1.44 g, 14.2 mmol) in DCM (100 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 60/40 v/v) yielded a colorless oil (4.4 g, 76 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.86 – 1.99 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.67 (t, 2H, ^3J = 7.8 Hz, Pyr-3-C***H***$_2$), 3.43 – 3.54 (m, 2H, Pyr-3-$(CH_2)_2$-C***H***$_2$), 5.13 (s, 2H, PhC***H***$_2$), 5.18 (s, 2H, PhC***H***$_2$), 7.19 (dd, 1H, ^3J = 7.8 Hz, ^3J = 4.8 Hz, ^5J = 0.6 Hz, Pyr-5-***H***), 7.27 – 7.43 (m, 10H, Ph-***H***), 7.51 (ddd, 1H, ^3J = 7.8 Hz, ^4J = 2.1 Hz, ^4J = 1.8 Hz, Pyr-4-***H***), 8.38 (t, 1H, ^3J = 5.1 Hz, N-***H***), 8.42 – 8.49 (m, 2H, Pyr-2,6-***H***), 11.75 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.19, 30.32 (-, Pyr-3-***C***H_2-***C***H_2), 40.38 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 67.23 (-, Ph***C***H_2), 68.26 (-, Ph***C***H_2), 123.40 (+, Pyr-***C***-5), 127.98 (+, 1 Ph-***C***), 128.19 (+, 2 Ph-***C***), 128.45 (+, 2 Ph-***C***), 128.51 (+, 2 Ph-***C***), 128.74 (+, 2 Ph-***C***), 128.86 (+, 1 Ph-***C***), 134.60 (C_{quat}, 1 Ph-***C***-1), 135.78 (+, Pyr-***C***-4), 136.34 (C_{quat}, Pyr-***C***-3), 136.78 (C_{quat}, 1 Ph-***C***-1), 147.72 (+, Pyr-***C***-6), 149.91 (+, Pyr-***C***-2), 153.95, 156.10, 163.73 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 447 (100) $[M + H]^+$. $C_{25}H_{26}N_4O_4$ (446.50).

N^1,N^2-Bis(benzyloxycarbonyl)-N^3-[4-(pyridin-4-yl)propyl]guanidine (5.44)

The title compound was prepared from **5.31** (1.43 g, 10.5 mmol), **5.36** (4.34 g, 9.5 mmol) and NEt_3 (1.5 mL, 1.06 g, 10.5 mmol) in DCM (100 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 40/60 v/v) yielded a colorless oil (3.7 g, 88 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.85 – 2.01 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.66 (t, 2H, ^3J = 7.7 Hz, Pyr-4-C***H***$_2$), 3.40 – 3.54 (m, 2H, Pyr-4-$(CH_2)_2$-C***H***$_2$), 5.13 (s, 2H, PhC***H***$_2$), 5.18 (s, 2H, PhC***H***$_2$), 7.12 (dd, 2H, ^3J = 4.4 Hz, ^4J = 1.6 Hz, Pyr-3,5-***H***), 7.28 – 7.43 (m, 10H, Ph-***H***), 8.37 (t, 1H, ^3J = 5.0 Hz, N-***H***), 7.12 (dd, 2H, ^3J = 4.4 Hz, ^4J = 1.6 Hz, Pyr-2,6-***H***), 11.74 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 29.49, 32.32 (-, Pyr-4-***C***H_2-***C***H_2), 40.35 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 67.25 (-, Ph***C***H_2), 68.29 (-, Ph***C***H_2), 123.81 (+, Pyr-***C***-3,5), 128.01 (+, 1 Ph-***C***), 128.20 (+, 2 Ph-***C***), 128.46 (+, 2 Ph-***C***), 128.51 (+, 2 Ph-***C***), 128.75 (+, 2 Ph-***C***), 128.87 (+, 1 Ph-***C***), 134.58 (C_{quat}, 1 Ph-***C***-1), 136.74 (C_{quat}, 1 Ph-***C***-1), 149.91 (+, Pyr-***C***-2,6), 150.00 (C_{quat}, Pyr-***C***-4), 153.96, 156.10, 163.73 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 447 (100) $[M + H]^+$. $C_{25}H_{26}N_4O_4$ (446.50).

N^1,N^2-Bis(benzyloxycarbonyl)-N^3-[3-(1*H*-imidazol-1-yl)propyl]guanidine (5.45)

The title compound was prepared from **5.33** (2.09 g, 16.7 mmol), **5.36** (6.90 g, 15.0 mmol) and NEt_3 (2.3 mL, 1.69 g, 16.7 mmol) in DCM (100 mL) according to the general procedure. Purification by flash chromatography (DCM/MeOH 97.5/2.5 v/v) yielded a pale yellow oil (5.4 g, 83 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 2.00 – 2.14 (m, 2H, Im-1-CH_2-C***H***$_2$), 3.39 – 3.49 (m, 2H, Im-1-$(CH_2)_2$-C***H***$_2$), 4.00 (t, 2H, ^3J = 7.0 Hz, Im-1-C***H***$_2$), 5.13 (s, 2H, Ph-C***H***$_2$), 5.18 (s, 2H, Ph-C***H***$_2$), 6.94 – 6.96 (m, 1H, Im-4-***H***), 7.04 – 7.06 (m, 1H, Im-5-***H***), 7.27 – 7.43 (m, 10H, Ph-***H***), 7.50 – 7.52 (m, 1H, Im-2-***H***), 8.38 (t, 1H, ^3J = 5.6 Hz, N-***H***), 11.72 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 30.73 (-, Im-1-CH_2-***C***H_2), 37.93 (-, Im-1-$(CH_2)_2$-***C***H_2), 44.43 (-, Im-1-***C***H_2), 67.29 (-, Ph-***C***H_2), 68.41 (-, Ph-***C***H_2), 118.81 (+, Im-***C***-5), 128.05 (+, 1 Ph-***C***), 128.15 (+, 2 Ph-***C***), 128.49 (+, 2 Ph-***C***), 128.55 (+, 2 Ph-***C***), 128.77 (+, 2 Ph-***C***), 128.92 (+, 1 Ph-***C***), 129.67 (+, Im-***C***-4), 134.50 (C_{quat}, 1 Ph-***C***-1), 136.64 (C_{quat}, 1 Ph-***C***-1), 137.11 (+, Im-***C***-2), 153.94, 156.30, 163.65 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 436 (100) $[M + H]^+$. $C_{23}H_{25}N_5O_4$ (435.48).

5.4.1.6 Preparation of the arylpropylguanidines 5.46-5.54

General procedure

A mixture of the pertinent di-Cbz-protected guanidine and catalytical amounts of Pd/C (10 %) in MeOH was stirred under an hydrogen atmosphere at room temperature for approximately 3 h (TLC control). After the Cbz-groups were quantitatively cleaved, the catalyst was removed by filtration over Celite and the solvent was evaporated. For analytical purposes a

small amount of some compounds (**5.37**, **5.41**, **5.43-5.45**) was converted into the picrate by addition of a saturated solution of picric acid in EtOH and recrystallization from EtOH/H_2O.

_N_-(3-{5-[(Dimethylamino)methyl]furan-2-yl}propyl)guanidine (5.46)

The title compound was prepared from **5.37** (3.0 g, 6.1 mmol) and Pd/C (10 %) (0.30 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless oil (1.3 g, 95 %); mp (**5.46** · 2 $C_6H_3N_3O_7$) 185 – 187 °C (dec.). ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.72 – 1.86 (m, 2H, Fur-2-CH_2-C***H***$_2$), 2.65 (t, 2H, ^3J = 7.7 Hz, Fur-2-C***H***$_2$), 2.73 (s, 6H, C***H***$_3$), 3.09 – 3.22 (m, 2H, Fur-2-$(CH_2)_2$-C***H***$_2$), 4.31 (s, 2H, Fur-5-C***H***$_2$), 6.23 (d, 1H, ^3J = 3.2 Hz, Fur-3-***H***), 6.60 (d, 1H, ^3J = 3.2 Hz, Fur-4-***H***), 7.02 (brs, 4H, N-***H***), 7.48 (t, 1H, ^3J = 5.6 Hz, N-***H***), 8.60 (s, 4H, picrate-***H***), 9.73 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 24.46 (-, ***C***H_2), 26.72 (-, ***C***H_2), 40.01 (-, Fur-2-$(CH_2)_2$-***C***H_2), 41.52 (+, 2 ***C***H_3), 51.80 (-, Fur-5-***C***H_2), 106.77 (+, Fur-***C***-3), 114.94 (+, Fur-***C***-4), 124.14 (C_{quat}, picrate-***C***-4), 125.14 (+, picrate-***C***-3,5), 141.75 (C_{quat}, picrate-***C***-2,6), 142.69 (C_{quat}, Fur-***C***-5), 156.55 (C_{quat}, ***C***=N), 156.81 (C_{quat}, Fur-***C***-2) 160.73 (C_{quat}, picrate-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 225 (100) $[M + H]^+$. Anal. ($C_{11}H_{20}N_4O$ · 2 $C_6H_3N_3O_7$) C, H, N. $C_{11}H_{20}N_4O$ (224.30).

_N_-[3-(1-Trityl-1_H_-imidazol-2-yl)propyl]guanidine (5.47)

The title compound was prepared from **5.38** (5.57 g, 8.2 mmol) and Pd/C (10 %) (0.56 g, cat.) in MeOH (150 mL) according to the general procedure yielding a white solid (3.2 g, 95 %); mp 158 – 162 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.23 – 1.35 (m, 2H, Im-2-CH_2-C***H***$_2$), 2.05 (t, 2H, ^3J = 7.5 Hz, Im-2-C***H***$_2$), 2.85 (t, 2H, ^3J = 7.1 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 6.80 (d, 1H, ^3J = 1.6 Hz, Im-4-***H***), 6.95 (d, 1H, ^3J = 1.6 Hz, Im-5-***H***), 7.09 – 7.19 (m, 6H, Ph-***H***), 7.32 – 7.44 (m, 9H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.72, 28.60 (-, Im-2-***C***H_2-***C***H_2), 41.77 (-, Im-2-$(CH_2)_2$-***C***H_2), 76.50 (C_{quat}, ***C***Ph_3), 122.68 (+, Im-***C***-5), 125.94 (+, Im-***C***-4), 129.33 (+, 3 Ph-***C***), 129.39 (+, 6 Ph-***C***), 131.05 (+, 6 Ph-***C***), 143.67 (C_{quat}, 3 Ph-***C***-1), 150.71 (C_{quat}, Im-***C***-2), 158.74 (C_{quat}, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 410 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{26}H_{27}N_5$ $[M^{+\bullet}]$ 409.2267; found 409.2266. $C_{26}H_{27}N_5$ (409.53).

_N_-(3-(1-Trityl-1_H_-pyrazol-4-yl)propyl)guanidine (5.48)

The title compound was prepared from **5.39** (5.5 g, 8.1 mmol) and Pd/C (10 %) (0.55 g, cat.) in MeOH (150 mL) according to the general procedure yielding a white solid (3.2 g, 96 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.72 – 1.87 (m, 2H, Pyraz-4-CH_2-C***H***$_2$), 2.53 (t, 2H, ^3J = 7.6 Hz, Pyraz-4-C***H***$_2$), 3.14 (t, 2H, ^3J = 7.0 Hz, Pyraz-4-$(CH_2)_2$-C***H***$_2$), 7.05 – 7.17 (m, 6H, Ph-***H***), 7.24 – 7.38 (m, 10H, Ph-***H*** + Pyraz-3-***H***), 7.49 (s, 1H, Pyraz-5-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 22.08 (-, Pyraz-4-***C***H_2), 31.20 (-, Pyraz-4-CH_2-***C***H_2), 41.85 (-, Pyraz-4-

$(CH_2)_2$-***C***H_2), 79.93 (C_{quat}, ***C***Ph_3), 120.83 (C_{quat}, Pyraz-***C***-4), 128.90 (+, 6 Ph-***C***), 128.98 (+, 3 Ph-***C***), 131.22 (+, 6 Ph-***C***), 132.31 (+, Pyraz-***C***-5), 140.31 (+, Pyraz-***C***-3), 144.56 (C_{quat}, 3 Ph-***C***-1), 158.94 (C_{quat}, ***C***=N). EI-MS (70 eV) *m/z* (%): 409 (16) [$M^{+\bullet}$], 243 (100) [CPh_3^+]. HRMS (EI-MS) calcd. for $C_{26}H_{27}N_5$ [$M^{+\bullet}$] 409.2267; found 409.2265. $C_{26}H_{27}N_5$ (409.53).

_N_-[3-(1-Trityl-1*H*-1,2,4-triazol-5-yl)propyl]guanidine (5.49)

The title compound was prepared from **5.40** (4.4 g, 6.5 mmol) and Pd/C (10 %) (0.44 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (2.6 g, 98 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.31 – 1.44 (m, 2H, Triaz-5-CH_2-C***H***$_2$), 2.14 (t, 2H, ^3J = 7.5 Hz, Triaz-5-C***H***$_2$), 2.91 (t, 2H, ^3J = 7.0 Hz, Triaz-5-$(CH_2)_2$-C***H***$_2$), 7.06 – 7.15 (m, 6H, Ph-***H***), 7.30 – 7.40 (m, 9H, Ph-***H***), 7.92 (s, 1H, Triaz-5-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 27.22, 27.33 (-, Triaz-5-***C***H_2-***C***H_2), 41.53 (-, Triaz-5-$(CH_2)_2$-***C***H_2), 79.53 (C_{quat}, ***C***Ph_3), 129.11 (+, 6 Ph-***C***), 129.15 (+, 3 Ph-***C***), 131.09 (+, 6 Ph-***C***), 143.21 (C_{quat}, 3 Ph-***C***-1), 149.01 (+, Triaz-***C***-3), 158.55, 158.74 (C_{quat}, ***C***=N + Triaz-***C***-5). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 411 (100) [M + H]$^+$. HRMS (EI-MS) calcd. for $C_{25}H_{26}N_6$ [$M^{+\bullet}$] 410.2219; found 410.2214. $C_{25}H_{26}N_6$ (410.51).

3-Phenylpropylguanidine (5.50)[37]

The title compound was prepared from **5.41** (1.72 g, 3.9 mmol) and Pd/C (10 %) (0.17 g, cat.) in MeOH (80 mL) according to the general procedure yielding a colorless foam-like solid (0.65 g, 94 %); mp (**5.50** · $C_6H_3N_3O_7$) 146 – 148 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, picrate): δ [ppm] = 1.71 – 1.84 (m, 1H, Ph-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.8 Hz, Ph-C***H***$_2$), 3.06 – 3.16 (m, 2H, Ph-$(CH_2)_2$-C***H***$_2$), 7.00 (brs, 4H, N-***H***), 7.15 – 7.35 (m, 5H, Ph-***H***), 7.45 (t, 1H, ^3J = 5.4 Hz, N-***H***), 8.59 (s, 2H, picrate-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, picrate): δ [ppm] = 30.09 (-, Ph-CH_2-***C***H_2), 31.95 (-, Ph-***C***H_2), 40.30 (-, Ph-CH_2-CH_2-***C***H_2), 124.12 (C_{quat}, picrate-***C***-4), 125.13 (+, picrate-***C***-3,5), 125.85 (+, 1 Ph-***C***), 128.14 (+, 2 Ph-***C***), 128.30 (+, 2 Ph-***C***), 141.00 (C_{quat}, Ph-***C***), 141.75 (C_{quat}, picrate-***C***-2,6), 156.57 (C_{quat}, ***C***=N), 160.73 (C_{quat}, picrate-***C***-1). CI-MS (NH_3) *m/z* (%): 178 (100) [M + H]$^+$. Anal. ($C_{10}H_{15}N_3$ · $C_6H_3N_3O_7$) C, H, N. $C_{10}H_{15}N_3$ (177.25).

3-(Pyridin-2-yl)propylguanidine (5.51)[38]

The title compound was prepared from **5.42** (4.2 g, 9.4 mmol) and Pd/C (10 %) (0.42 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (1.6 g, 96 %); mp (**5.51** · 2 $C_6H_3N_3O_7$) 194 – 195 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.85 – 1.99 (m, 2H, Pyr-2-CH_2-C***H***$_2$), 2.98 (t, 2H, ^3J = 7.8 Hz, Pyr-2-C***H***$_2$), 3.12 – 3.24 (m, 2H, Pyr-2-$(CH_2)_2$-C***H***$_2$), 7.05 (brs, 4H, N-***H***), 7.50 (t, 1H, ^3J = 5.6 Hz, N-***H***), 7.81 – 7.92 (m, 2H, Pyr-3,5-***H***), 8.40 – 8.49 (m, 1H, Pyr-4-***H***), 8.59 (s, 2H, picrate-***H***), 8.80 (dd, 1H, ^3J = 5.8 Hz, ^4J = 1.6 Hz, Pyr-6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm]

= 27.70 (-, Pyr-2-CH_2-***C***H_2), 30.35 (-, Pyr-2-***C***H_2), 39.93 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 124.20 (C_{quat}, picrate-***C***-4), 124.43 (+, Pyr-***C***-5), 125.15 (+, picrate-***C***-3,5), 126.39 (+, Pyr-***C***-3), 141.73 (C_{quat}, picrate-***C***-2,6), 142.29 (+, Pyr-***C***-6), 145.36 (+, Pyr-***C***-4), 156.30 (C_{quat}, Pyr-***C***-2), 156.53 (C_{quat}, ***C***=N), 160.71 (C_{quat}, picrate-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 179 (100) $[M + H]^+$. Anal. ($C_9H_{14}N_4 \cdot 2\ C_6H_3N_3O_7$) C, H, N. $C_9H_{14}N_4$ (178.23).

3-(Pyridin-3-yl)propylguanidine (5.52)

The title compound was prepared from **5.43** (4.31 g, 9.7 mmol) and Pd/C (10 %) (0.43 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (1.7 g, 97 %); mp (**5.52** · 2 $C_6H_3N_3O_7$) 186 – 187 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.76 – 1.94 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.80 (t, 2H, ^3J = 7.8 Hz, Pyr-3-C***H***$_2$), 3.08 – 3.22 (m, 2H, Pyr-3-$(CH_2)_2$-C***H***$_2$), 7.03 (brs, 4H, N-***H***), 7.48 (t, 1H, ^3J = 5.5 Hz, N-***H***), 7.99 (dd, 1H, ^3J = 8.0 Hz, ^3J = 5.9 Hz, Pyr-5-***H***), 8.43 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.9 Hz, ^4J = 1.5 Hz, Pyr-4-***H***), 8.59 (s, 2H, picrate-***H***), 8.77 – 8.81 (m, 2H, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 28.77 (-, Pyr-3-CH_2-***C***H_2), 29.09 (-, Pyr-3-***C***H_2), 40.03 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 124.16 (C_{quat}, picrate-***C***-4), 125.13 (+, picrate-***C***-3,5), 126.54 (+, Pyr-***C***-5), 140.40 (+, Pyr-***C***-6), 140.62 (C_{quat}, Pyr-***C***-3), 141.74 (C_{quat}, picrate-***C***-2,6), 141.91 (+, Pyr-***C***-2), 145.14 (+, Pyr-***C***-4), 156.53 (C_{quat}, ***C***=N), 160.72 (C_{quat}, picrate-***C***-1). ES-MS (H_2O/MeCN) *m/z* (%): 179 (100) $[M + H]^+$. Anal. ($C_9H_{14}N_4 \cdot 2\ C_6H_3N_3O_7$) C, H, N. $C_9H_{14}N_4$ (178.23).

3-(Pyridin-4-yl)propylguanidine (5.53)

The title compound was prepared from **5.44** (3.65 g, 8.2 mmol) and Pd/C (10 %) (0.37 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (1.4 g, 96 %); mp (**5.53** · 2 $C_6H_3N_3O_7$) 205 – 207 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.78 – 1.95 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.66 (t, 2H, ^3J = 7.8 Hz, Pyr-4-C***H***$_2$), 3.09 – 3.22 (m, 2H, Pyr-4-$(CH_2)_2$-C***H***$_2$), 7.03 (brs, 3H, N-***H***), 7.49 (t, 1H, ^3J = 5.5 Hz, N-***H***), 7.92 (dd, 2H, ^3J = 6.7 Hz, ^4J = 1.4 Hz, Pyr-3,5-***H***), 8.59 (s, 4H, picrate-***H***), 8.82 (d, 2H, ^3J = 6.7 Hz, ^4J = 1.4 Hz, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 28.41 (-, Pyr-4-CH_2-***C***H_2), 32.04 (-, Pyr-4-***C***H_2), 40.14 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 124.15 (C_{quat}, picrate-***C***-4), 125.14 (+, picrate-***C***-3,5), 126.61 (+, Pyr-***C***-3,5), 141.75 (C_{quat}, picrate-***C***-2,6), 141.83 (+, Pyr-***C***-2,6), 156.52 (C_{quat}, ***C***=N), 160.72 (C_{quat}, picrate-***C***-1), 161.58 (Cquart, Pyr-***C***-4). ES-MS (H_2O/MeCN) *m/z* (%): 179 (100) $[M + H]^+$. Anal. ($C_9H_{14}N_4 \cdot 2\ C_6H_3N_3O_7$) C, H, N. $C_9H_{14}N_4$ (178.23).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)guanidine (5.54)**[39]

The title compound was prepared from **5.45** (5.3 g, 12.2 mmol) and Pd/C (10 %) (0.53 g, cat.) in MeOH (150 mL) according to the general procedure yielding a colorless foam-like solid (1.9 g, 95 %); mp (**5.54** · 2 $C_6H_3N_3O_7$) 190 – 191 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dipicrate): δ [ppm] = 1.97 – 2.09 (m, 2H, Im-1-CH_2-C***H***$_2$), 3.08 – 3.18 (m, 2H, Im-1-$(CH_2)_2$-C***H***$_2$), 4.21 (t, 2H, ^3J = 7.1 Hz, Im-1-C***H***$_2$), 7.07 (brs, 4H, N-***H***), 7.48 (t, 1H, ^3J = 5.7 Hz, N-***H***), 7.70 – 7.73 (m, 1H, Im-4-***H***), 7.75 – 7.78 (m, 1H, Im-5-***H***), 8.60 (s, 4H, picrate-***H***), 9.08 – 9.11 (m, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6, dipicrate): δ [ppm] = 28.81 (-, Im-1-CH_2-***C***H_2), 37.79 (-, Im-1-$(CH_2)_2$-***C***H_2), 46.02 (-, Im-1-***C***H_2), 120.03, 121.03 (+, Im-***C***-4,5), 124.17 (C_{quat}, picrate-***C***-4), 125.14 (+, picrate-***C***-3,5), 135.35 (+, Im-***C***-2), 141.75 (C_{quat}, picrate-***C***-2,6), 156.55 (C_{quat}, ***C***=N), 160.73 (C_{quat}, picrate-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 168 (100) $[M + H]^+$. Anal. ($C_7H_{13}N_5$ · 2 $C_6H_3N_3O_7$) C, H, N. $C_7H_{13}N_5$ (167.21).

5.4.1.7 Preparation of the N^G-acylated arylpropylguanidines 5.59-5.80

General procedure 1

A solution of the pertinent carboxylic acid (1 eq) and CDI (1.2 eq) in THF_{abs} (15 mL) was stirred for 1 h under argon atmosphere at room temperature. In a separate vessel, NaH (60 % dispersion in mineral oil) (2 eq) was added to a solution of the pertinent guanidine (1 eq) in THF_{abs} (15 mL) under argon atmosphere, stirred for 45 min at 30 – 35 °C and allowed to cool to ambient temperature. Both mixtures were merged and stirred for 5 h under argon atmosphere. EtOAc (50 mL) was added and the organic phase was washed with H_2O (3 x 20 mL) and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by flash chromatography or preparative HPLC. Compounds purified by preparative HPLC were dried by lyophilization and obtained as trifluoroacetates.

General procedure 2

A solution of the pertinent carboxylic acid (1 eq) and CDI (1.2 eq) in anhydrous DMF (10 mL) was stirred for 1 h under argon atmosphere at room temperature. In a separate vessel, NaH (60 % dispersion in mineral oil) (2 eq) was added to a solution of the pertinent guanidine (1 eq) in anhydrous DMF (10 mL) under argon atmosphere, stirred for 45 min at 30 – 35 min and allowed to cool to ambient temperature. Both mixtures were merged and stirred for 5 h under argon atmosphere. After evaporation of the solvent, the crude product was subjected to flash chromatography or preparative HPLC. Compounds purified by preparative HPLC were dried by lyophilization and obtained as trifluoroacetic acid salts.

N^1-(3-Phenylbutanoyl)-N^2-(3-phenylpropyl)guanidine (5.59)

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.50** (177 mg, 1.0 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 50/50) yielded a white solid (92 mg, 21 %); mp 118 – 119 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$, trifluoroacetate): δ [ppm] = 1.30 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.95 – 2.05 (m, 2H, Ph-CH_2-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.8 Hz, Ph-C***H***$_2$), 2.75 (dd, 1H, ^2J = 15.0 Hz, ^3J = 7.7 Hz, $PhCH_3CH$-C***H***$_2$), 2.87 (dd, 1H, ^2J = 15.0 Hz, ^3J = 7.5 Hz, $PhCH_3CH$-C***H***$_2$), 3.27 – 3.39 (m, 1H, PhC***H***), 3.40 (t, 2H, ^3J = 7.1 Hz, Ph-CH_2-CH_2-C***H***$_2$), 7.14 – 7.36 (m, 10H, Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 22.28 (+, Ph***C***H_3CH), 30.76, 33.66 (-, Ph-***C***H_2-***C***H_2), 37.67 (+, $PhCH_3$***C***H), 42.06 (-, Ph-CH_2-CH_2-***C***H_2), 46.29 (-, $PhCH_3CH$-***C***H_2), 127.31 (+, Ph-***C***-4), 127.77 (+, Ph-***C***-4), 127.93 (+, 2 Ph-***C***), 129.46 (+, 2 Ph-***C***), 129.65 (+, 2 Ph-***C***), 129.72 (+, 2 Ph-***C***), 142.02 (C_{quat}, Ph-***C***-1), 146.41 (C_{quat}, Ph-***C***-1), 152.27 (C_{quat}, ***C***=N), 175.91 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3245, 2960, 1706, 1656, 1594, 1197, 1140. HRMS (EI-MS) calcd. for $C_{20}H_{25}N_3O$ [$M^{+\bullet}$] 323.1998; found 323.1995. $C_{20}H_{25}N_3O$ · TFA (437.46).

N^1-(3,3-Diphenylpropanoyl)-N^2-(3-phenylpropyl)guanidine (5.60)

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.50** (177 mg, 1.0 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 55/45) yielded a white solid (141 mg, 28 %); mp 138 – 140 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$, trifluoroacetate): δ [ppm] = 1.92 – 2.03 (m, 2H, Ph-CH_2-C***H***$_2$), 2.70 (t, 2H, ^3J = 7.7 Hz, Ph-C***H***$_2$), 3.34 (d, 2H, ^3J = 8.1 Hz, Ph_2CH-C***H***$_2$), 3.39 (t, 2H, ^3J = 7.0 Hz, Ph-CH_2-CH_2-C***H***$_2$), 4.65 (t, 1H, ^3J = 8.1 Hz, Ph_2C***H***), 7.12 – 7.42 (m, 15H, Ph-***H***). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$, trifluoroacetate): δ [ppm] = 30.36, 33.40 (-, Ph-***C***H_2-***C***H_2), 41.60 (-, Ph-CH_2-CH_2-***C***H_2), 43.08 (-, Ph_2CH-***C***H_2), 47.67 (+, Ph_2***C***H), 126.90 (+, 1 Ph-***C***-4), 127.44 (+, 2 Ph-***C***-4), 128.64 (+, 4 Ph-***C***), 129.30 (+, 2 Ph-***C***), 129.32 (+, 2 Ph-***C***), 129.43 (+, 4 Ph-***C***), 142.00 (C_{quat}, 1 Ph-***C***-1), 144.35 (C_{quat}, 2 Ph-***C***-1), 155.47 (C_{quat}, ***C***=N), 175.81 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3247, 2971, 1704, 1661, 1598, 1193, 1142. HRMS (EI-MS) calcd. for $C_{25}H_{27}N_3O$ [$M^{+\bullet}$] 385.2154; found 385.2168. $C_{25}H_{27}N_3O$ · TFA (499.52).

N^1-(3-Phenylbutanoyl)-N^2-[3-(pyridin-2-yl)propyl]guanidine (5.61)

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.51** (178 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 35/65) yielded a colorless oil (421 mg, 76 %). ^{1}H-NMR (400 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.31 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.07 – 2.16 (m, 2H,

Pyr-2-CH_2-C***H***$_2$), 2.73 (dd, 1H, ^2J = 15.3 Hz, ^3J = 7.3 Hz, $PhCH_3CH$-C***H***$_2$), 2.79 (dd, 1H, ^2J = 15.3 Hz, ^3J = 7.8 Hz, $PhCH_3CH$-C***H***$_2$), 3.12 (t, 2H, ^3J = 8.0 Hz, Pyr-2-C***H***$_2$), 3.26 – 3.36 (m, 1H, overlap with solvent, $PhCH_3$C***H***), 3.39 (t, 2H, ^3J = 6.8 Hz, Pyr-2-$(CH_2)_2$-C***H***$_2$), 7.14 – 7.30 (m, 5H, Ph-***H***), 7.86 (ddd, 1H, ^3J = 7.7 Hz, ^3J = 5.9 Hz, ^4J = 1.2 Hz, Pyr-5-***H***), 7.94 (ddd, 1H, ^3J = 8.1 Hz, ^4J = 1.2 Hz, ^5J = 0.8 Hz, Pyr-3-***H***), 8.46 (ddd, 1H, ^3J = 8.1 Hz, ^3J = 7.7 Hz, ^4J = 1.6 Hz, Pyr-4-***H***), 8.71 (ddd, 1H, ^3J = 5.9 Hz, ^4J = 1.6 Hz, ^5J = 0.8 Hz, Pyr-6-***H***). ^{13}C-NMR (100 MHz, CD_3OD, trifluoroacetate, HSQC, HMBC): δ [ppm] = 22.28 (+, Ph***C***H_3CH), 28.39 (-, Pyr-2-CH_2-***C***H_2), 31.84 (-, Pyr-2-***C***H_2), 37.59 (+, $PhCH_3$***C***H), 41.62 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 46.07 (-, $PhCH_3CH$-***C***H_2), 126.09 (+, Pyr-***C***-5), 127.66 (+, Ph-***C***-4), 127.92 (+, 2 Ph-***C***), 128.23 (+, Pyr-***C***-3), 129.63 (+, 2 Ph-***C***), 143.29 (+, Pyr-***C***-6), 146.43 (C_{quat}, Ph-***C***-1), 147.33 (+, Pyr-***C***-4), 155.29 (C_{quat}, ***C***=N), 157.88 (C_{quat}, Pyr-***C***-2), 175.91 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3066, 2964, 1668, 1602, 1178, 1127. HRMS (EI-MS) calcd. for $C_{19}H_{24}N_4O$ [$M^{+\bullet}$] 324.1950; found 324.1951. $C_{19}H_{24}N_4O \cdot 2$ TFA (552.47).

***N*^{1}-(3,3-Diphenylpropanoyl)-*N*^{2}-[3-(pyridin-2-yl)propyl]guanidine (5.62)**

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.51** (178 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 25/75, 20 min: 45/55) yielded a semisolid compound (264 mg, 43 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 2.02 – 2.16 (m, 2H, Pyr-2-CH_2-C***H***$_2$), 3.08 (t, 2H, ^3J = 7.9 Hz, Pyr-2-C***H***$_2$), 3.26 (d, 2H, ^3J = 8.0 Hz, Ph_2CHC***H***$_2$), 3.39 (t, 2H, ^3J = 6.8 Hz, Pyr-2-$(CH_2)_2$-C***H***$_2$), 4.59 (t, 1H, ^3J = 8.0 Hz, Ph_2C***H***), 7.14 – 7.33 (m, 10H, Ph-***H***), 7.82 (ddd, 1H, ^3J = 7.7 Hz, ^3J = 5.8 Hz, ^4J = 1.2 Hz, Pyr-5-***H***), 7.90 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 1.2 Hz, ^5J = 0.8 Hz, Pyr-3-***H***), 8.46 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.7 Hz, ^4J = 1.7 Hz, Pyr-4-***H***), 8.71 (ddd, 1H, ^3J = 5.8 Hz, ^4J = 1.6 Hz, ^5J = 0.8 Hz, Pyr-6-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 28.41 (-, Pyr-2-CH_2-***C***H_2), 32.10 (-, Pyr-2-***C***H_2), 41.71 (-, Pyr-2-$(CH_2)_2$-***C***H_2), 43.81 (-, Ph_2CH-***C***H_2), 48.00 (+, Ph_2***C***H), 125.94 (+, Pyr-***C***-5), 127.82 (+, 2 Ph-***C***-4), 128.03 (+, Pyr-***C***-3), 128.84 (+, 4 Ph-***C***), 129.73 (+, 4 Ph-***C***), 143.77 (-, Pyr-***C***-6), 144.56 (C_{quat}, 2 Ph-***C***-1), 146.81 (+, Pyr-***C***-4), 155.21 (C_{quat}, ***C***=N), 158.15 (C_{quat}, Pyr-***C***-2), 175.42 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3064, 2971, 2901, 1668, 1599, 1179, 1127. HRMS (EI-MS) calcd. for $C_{24}H_{26}N_4O$ [$M^{+\bullet}$] 386.2107; found 386.2105. $C_{24}H_{26}N_4O \cdot 2$ TFA (614.54).

***N*^{1}-(3-Phenylbutanoyl)-*N*^{2}-[3-(pyridin-3-yl)propyl]guanidine (5.63)**

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.52** (178 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 35/65) yielded a colorless oil (355 mg, 64 %). ^{1}H-NMR (300 MHz,

CD_3OD, trifluoroacetate): δ [ppm] = 1.32 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.95 – 2.09 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.73 (dd, 1H, ^2J = 15.3 Hz, ^3J = 7.4 Hz, PhCH_3CH-C***H***$_2$), 2.80 (dd, 1H, ^2J = 15.3 Hz, ^3J = 7.7 Hz, PhCH_3CH-C***H***$_2$), 2.90 (t, 2H, ^3J = 8.0 Hz, Pyr-3-C***H***$_2$), 3.24 – 3.33 (m, 1H, overlap with solvent, PhCH_3C***H***), 3.35 (t, 2H, ^3J = 7.0 Hz, Pyr-3-$(CH_2)_2$-C***H***$_2$), 7.12 – 7.32 (m, 5H, Ph-***H***), 7.86 (dd, 1H, ^3J = 8.0 Hz, ^3J = 5.8 Hz, Pyr-5-***H***), 8.39 (d, 1H, ^3J = 8.0 Hz, Pyr-4-***H***), 8.56 – 8.80 (m, 2H, Pyr-2-***H*** + Pyr-6-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 22.36 (+, Ph***C***H_3CH), 29.79 (-, Pyr-3-CH_2-***C***H_2), 30.61 (-, Pyr-3-***C***H_2), 37.68 (+, PhCH_3***C***H), 41.83 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 46.10 (-, PhCH_3CH-***C***H_2), 127.72 (+, Ph-***C***-4), 127.97 (+, 2 Ph-***C***), 128.31 (+, Pyr-***C***-5), 129.68 (+, 2 Ph-***C***), 141.22 (+, Pyr-***C***-6), 142.88 (+, Pyr-***C***-2), 143.15 (C_{quat}, Pyr-***C***-3), 146.46 (C_{quat}, Ph-***C***-1), 147.62 (+, Pyr-***C***-4), 155.23 (C_{quat}, ***C***=N), 176.05 (C_{quat}, ***C***=O). IR (cm^{-1}) = 2971, 2901, 1668, 1603, 1177, 1130. HRMS (EI-MS) calcd. for $C_{19}H_{24}N_4O$ [$M^{+\bullet}$] 324.1950; found 324.1946. $C_{19}H_{24}N_4O \cdot 2$ TFA (552.47).

***N*^{1}-(3,3-Diphenylpropanoyl)-*N*^{2}-[3-(pyridin-3-yl)propyl]guanidine (5.64)**

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.52** (178 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 45/55) yielded a white solid (384 mg, 62 %); mp 48 – 52 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.94 – 2.08 (m, 2H, Pyr-3-CH_2-C***H***$_2$), 2.88 (t, 2H, ^3J = 8.0 Hz, Pyr-3-C***H***$_2$), 3.26 (d, 2H, ^3J = 8.0 Hz, Ph_2CHC***H***$_2$), 3.33 (t, 2H, overlap with solvent, ^3J = 6.9 Hz, Pyr-3-$(CH_2)_2$-C***H***$_2$), 4.59 (t, 1H, ^3J = 8.0 Hz, Ph_2C***H***), 7.12 – 7.34 (m, 10H, Ph-***H***), 7.87 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 6.0 Hz, ^5J = 0.7 Hz, Pyr-5-***H***), 8.37 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 1.4 Hz, Pyr-4-***H***), 8.64 (dd, 1H, ^3J = 6.0 Hz, ^4J = 1.4 Hz, Pyr-6-***H***), 8.71 (dd, 1H, ^4J = 2.0 Hz, ^5J = 0.7 Hz, Pyr-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 29.74 (-, Pyr-3-CH_2-***C***H_2), 30.60 (-, Pyr-3-***C***H_2), 41.84 (-, Pyr-3-$(CH_2)_2$-***C***H_2), 43.79 (-, Ph_2CH-***C***H_2), 48.07 (+, Ph_2***C***H), 127.81 (+, Ph-***C***-4), 128.27 (+, Pyr-***C***-5), 128.85 (+, 2 Ph-***C***), 129.72 (+, 2 Ph-***C***), 141.30 (+, Pyr-***C***-6), 142.96 (+, Pyr-***C***-2), 143.07 (C_{quat}, Pyr-***C***-3), 144.55 (C_{quat}, Ph-***C***-1), 147.51 (+, Pyr-***C***-4), 155.16 (C_{quat}, ***C***=N), 175.52 (C_{quat}, ***C***=O). IR (cm^{-1}) = 2973, 2901, 1668, 1599, 1180, 1128. HRMS (EI-MS) calcd. for $C_{24}H_{26}N_4O$ [$M^{+\bullet}$] 386.2107; found 386.2110. $C_{24}H_{26}N_4O \cdot 2$ TFA (614.54).

***N*^{1}-(3-Phenylbutanoyl)-*N*^{2}-[3-(pyridin-4-yl)propyl]guanidine (5.65)**

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.53** (178 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 35/65) yielded a colorless semisolid compound (312 mg, 56 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.31 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH),

1.99 – 2.12 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.73 (dd, 1H, ^2J = 15.2 Hz, ^3J = 7.4 Hz, $PhCH_3CH$-C***H***$_2$), 2.80 (dd, 1H, ^2J = 15.2 Hz, ^3J = 7.7 Hz, $PhCH_3CH$-C***H***$_2$), 3.01 (t, 2H, ^3J = 8.0 Hz, Pyr-4-C***H***$_2$), 3.20 – 3.33 (m, 1H, overlap with solvent, $PhCH_3$C***H***), 3.37 (t, 2H, ^3J = 7.0 Hz, Pyr-4-$(CH_2)_2$-C***H***$_2$), 7.13 – 7.31 (m, 5H, Ph-***H***), 7.98 (d, 2H, ^3J = 6.8 Hz, Pyr-3,5-***H***), 8.74 (d, 2H, ^3J = 6.8 Hz, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 22.34 (+, Ph***C***H_3CH), 29.07 (-, Pyr-4-CH_2-***C***H_2), 33.97 (-, Pyr-4-***C***H_2), 37.70 (+, $PhCH_3$***C***H), 41.84 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 46.11 (-, $PhCH_3CH$-***C***H_2), 127.71 (+, Ph-***C***-4), 127.99 (+, 2 Ph-***C***), 128.58 (+, Pyr-***C***-3,5), 129.68 (+, 2 Ph-***C***), 142.53 (+, Pyr-***C***-2,6), 146.46 (C_{quat}, Ph-***C***-1), 155.28 (C_{quat}, ***C***=N), 164.69 (C_{quat}, Pyr-***C***-4), 176.11 (C_{quat}, ***C***=O). IR (cm^{-1}) = 2969, 2901, 1666, 1639, 1177, 1128. HRMS (EI-MS) calcd. for $C_{19}H_{24}N_4O$ [$M^{+\bullet}$] 324.1950; found 324.1960. $C_{19}H_{24}N_4O$ · 2 TFA (552.47).

***N*^{1}-(3,3-Diphenylpropanoyl)-*N*^{2}-[3-(pyridin-4-yl)propyl]guanidine (5.66)**

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.53** (178 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 45/55) yielded a white solid (388 mg, 63 %); mp 93 – 94 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.95 – 2.10 (m, 2H, Pyr-4-CH_2-C***H***$_2$), 2.95 (t, 2H, ^3J = 8.0 Hz, Pyr-4-C***H***$_2$), 3.26 (d, 2H, ^3J = 8.0 Hz, Ph_2CHC***H***$_2$), 3.34 (t, 2H, overlap with solvent, ^3J = 7.0 Hz, Pyr-4-$(CH_2)_2$-C***H***$_2$), 4.59 (t, 1H, ^3J = 8.0 Hz, Ph_2C***H***), 7.13 – 7.33 (m, 10H, Ph-***H***), 7.88 (d, 1H, ^3J = 6.6 Hz, Pyr-3,5-***H***), 8.69 (d, 1H, ^3J = 6.6 Hz, Pyr-2,6-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 29.09 (-, Pyr-4-CH_2-***C***H_2), 33.82 (-, Pyr-4-***C***H_2), 41.88 (-, Pyr-4-$(CH_2)_2$-***C***H_2), 43.82 (-, Ph_2CH-***C***H_2), 48.08 (+, Ph_2***C***H), 127.82 (+, 2 Ph-***C***-4), 128.19 (+, Pyr-***C***-3,5), 128.85 (+, 4 Ph-***C***), 129.72 (+, 4 Ph-***C***), 143.50 (+, Pyr-***C***-2,6), 145.54 (C_{quat}, 2 Ph-***C***-1), 155.17 (C_{quat}, ***C***=N), 163.19 (C_{quat}, Pyr-***C***-4), 175.52 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3091, 1677, 1596, 1594, 1199, 1133. HRMS (EI-MS) calcd. for $C_{24}H_{26}N_4O$ [$M^{+\bullet}$] 386.2107; found 386.2105. $C_{24}H_{26}N_4O$ · 2 TFA (614.54).

***N*^{1}-(3-{5-[(Dimethylamino)methyl]furan-2-yl}propyl)-*N*^{2}-(3-phenylbutanoyl)guanidine (5.67)**

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.46** (224 mg, 1.0 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 30/70) yielded a pale brownish oil (208 mg, 35 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.24 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.82 – 1.95 (m, 2H, Fur-2-CH_2-C***H***$_2$), 2.63 (t, 2H, ^3J = 7.4 Hz, Fur-2-C***H***$_2$), 2.68 – 2.79 (m, 8H, N(C***H***$_3$)$_2$ + $PhCH_3CH$-C***H***$_2$), 3.15 – 3.28 (m, 3H, Fur-2-$(CH_2)_2$-C***H***$_2$ + $PhCH_3$C***H***), 4.14 (d, 1H, ^2J = 14.9 Hz, Fur-5-

C***H***$_2$), 4.19 (d, 1H, ^2J = 14.9 Hz, Fur-5-C***H***$_2$), 6.10 (d, 1H, ^3J = 3.2 Hz, Fur-3-***H***), 6.50 (d, 1H, ^3J = 3.2 Hz, Fur-4-***H***), 7.17 – 7.37 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 21.29 (+, Ph***C***H$_3$CH), 24.53 (-, Fur-2-***C***H$_2$), 25.43 (-, Fur-2-CH$_2$-***C***H$_2$), 36.61 (+, PhCH$_3$***C***H), 40.66 (-, Fur-2-(CH$_2$)$_2$-***C***H$_2$), 41.93 (+, N(***C***H$_3$)$_2$), 45.07 (-, PhCH$_3$CH-***C***H$_2$), 53.03 (-, Fur-5-***C***H$_2$), 107.23 (+, Fur-***C***-3), 115.59 (+, Fur-***C***-4), 126.98 (+ , 2 Ph-***C***), 127.06 (+, Ph-***C***-4), 128.99 (+, 2 Ph-***C***), 142.04 (C$_{quat}$, Fur-***C***-5), 145.02 (C$_{quat}$, Ph-***C***-1), 152.76 (C$_{quat}$, ***C***=N), 157.65 (C$_{quat}$, Fur-***C***-2), 176.00 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3032, 2964, 1669, 1602, 1594, 1177, 1132. HRMS (EI-MS) calcd. for $C_{21}H_{30}N_4O_2$ [M$^{+\bullet}$] 370.2369; found 370.2370. $C_{21}H_{30}N_4O_2 \cdot 2$ TFA (598.54).

***N*^{1}-(3-{5-[(Dimethylamino)methyl]furan-2-yl}propyl)-*N*^{2}-[3-(thiophen-2-yl)butanoyl]-guanidine (5.68)**

The title compound was prepared from **5.56**[3] (170 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.46** (224 mg, 1.0 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a pale brownish oil (72 mg, 12 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.33 (d, 3H, ^3J = 7.0 Hz, ThioC***H***$_3$CH), 1.84 – 1.97 (m, 2H, Fur-2-CH$_2$-C***H***$_2$), 2.69 – 2.87 (m, 10H, Fur-2-C***H***$_2$ + + ThioCH$_3$CH-C***H***$_2$ + N(C***H***$_3$)$_2$), 3.36 (t, 2H, ^3J = 6.7 Hz, Fur-2-(CH$_2$)$_2$-C***H***$_2$), 3.48 – 3.62 (m, 1H, ThioCH$_3$C***H***), 4.17 (d, 1H, ^2J = 14.7 Hz, Fur-5-C***H***$_2$), 4.22 (d, 1H, ^2J = 14.7 Hz, Fur-5-C***H***$_2$), 6.13 (d, 1H, ^3J = 3.2 Hz, Fur-3-***H***), 6.52 (d, 1H, ^3J = 3.2 Hz, Fur-4-***H***), 6.89 (ddd, 1H, ^3J = 3.5 Hz, ^4J = 1.3 Hz, ^4J = 0.7 Hz, Thio-3-***H***), 6.94 (dd, 1H, ^3J = 5.0 Hz, ^3J = 3.5 Hz, Thio-4-***H***), 7.24 (dd, 1H, ^3J = 5.0 Hz, ^4J = 1.3 Hz, Thio-5-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 22.28 (+, Thio***C***H$_3$CH), 24.55 (-, Fur-2-***C***H$_2$), 25.44 (-, Fur-2-CH$_2$-***C***H$_2$), 31.96 (+, ThioCH$_3$***C***H), 40.75 (-, Fur-2-(CH$_2$)$_2$-***C***H$_2$), 41.92 (+, N(***C***H$_3$)$_2$), 46.02 (-, ThioCH$_3$CH-***C***H$_2$), 53.03 (-, Fur-5-***C***H$_2$), 107.27 (+, Fur-***C***-3), 115.58 (+, Fur-***C***-4), 123.74, 123.97 (+, Thio-***C***-3,4), 127.25 (+, Thio-***C***-5), 142.04 (C$_{quat}$, Fur-***C***-5), 148.87 (C$_{quat}$, Thio-***C***-2), 152.75 (C$_{quat}$, ***C***=N), 157.64 (C$_{quat}$, Fur-***C***-2), 175.47 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 2989, 2901, 1663, 1178, 1130. HRMS (EI-MS) calcd. for $C_{19}H_{28}N_4O_2S$ [M$^{+\bullet}$] 376.1933; found 376.1930. $C_{19}H_{28}N_4O_2S \cdot 2$ TFA (604.56).

***N*^{1}-(3-{5-[(Dimethylamino)methyl]furan-2-yl}propyl)-*N*^{2}-(3,3-diphenylpropanoyl)-guanidine (5.69)**

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.46** (224 mg, 1.0 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a brownish oil (176 mg, 27 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.89 – 2.02 (m, 2H, Fur-2-CH$_2$-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.5 Hz, Fur-

2-C***H***$_2$), 2.83 (s, 6H, N(C***H***$_3$)$_2$), 3.22 – 3.33 (m, 4H, overlap with solvent, Fur-2-(CH$_2$)$_2$-C***H***$_2$ + Ph$_2$CH-C***H***$_2$), 4.30 (s, 2H, Fur-5-C***H***$_2$), 4.58 (t, 1H, ^3J = 8.0 Hz, Ph$_2$C***H***), 6.18 (d, 1H, ^3J = 3.2 Hz, Fur-3-***H***), 6.58 (d, 1H, ^3J = 3.2 Hz, Fur-4-***H***), 7.13 – 7.34 (m, 10H, Ph-***H***). ^{13}C-NMR (75 MHz, CD$_3$OD, trifluoroacetate): δ [ppm] = 25.97 (-, Fur-2-***C***H$_2$), 27.34 (-, Fur-2-CH$_2$-***C***H$_2$), 41.82 (-, Fur-2-(CH$_2$)$_2$-***C***H$_2$), 42.76 (+, N(***C***H$_3$)$_2$), 43.86 (-, Ph$_2$CH***C***H$_2$), 48.08 (+, Ph$_2$***C***H), 54.09 (-, Fur-5-***C***H$_2$), 108.48 (+, Fur-***C***-3), 116.61 (+, Fur-***C***-4), 127.83 (+ , 2 Ph-***C***-4), 128.84 (+, 4 Ph-***C***), 129.73 (+, 4 Ph-***C***), 143.86 (C$_{quat}$, Fur-***C***-5), 144.53 (C$_{quat}$, 2 Ph-***C***-1), 155.09 (C$_{quat}$, ***C***=N), 158.84 (C$_{quat}$, Fur-***C***-2), 175.54 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 2989, 2901, 1663, 1599, 1174, 1126. HRMS (EI-MS) calcd. for C$_{26}$H$_{32}$N$_4$O$_2$ [M$^{+\bullet}$] 432.2525; found 432.2531. C$_{26}$H$_{32}$N$_4$O$_2$ · 2 TFA (660.60).

***N*^{1}-(3-{5-[(Dimethylamino)methyl]furan-2-yl}propyl)-*N*^{2}-[3-phenyl-3-(thiazol-2-yl)-propanoyl]guanidine (5.70)**

The title compound was prepared from **5.58**[3] (233 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.46** (224 mg, 1.0 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a brownish semisolid compound (134 mg, 17 %). ^{1}H-NMR (400 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 1.82 – 1.93 (m, 2H, Fur-2-CH$_2$-C***H***$_2$), 2.63 (t, 2H, ^3J = 7.3 Hz, Fur-2-C***H***$_2$), 2.73 (s, 6H, N(C***H***$_3$)$_2$), 3.23 (t, 2H, ^3J = 6.8 Hz, Fur-2-(CH$_2$)$_2$-C***H***$_2$), 3.34 (dd, 1H, ^2J = 16.6 Hz, ^3J = 8.2 Hz, PhThiazCH-C***H***$_2$), 3.48 (dd, 1H, ^2J = 16.6 Hz, ^3J = 7.2 Hz, PhThiazCH-C***H***$_2$), 4.13 (d, 1H, ^2J = 14.5 Hz, Fur-5-C***H***$_2$), 4.17 (d, 1H, ^2J = 14.5 Hz, Fur-5-C***H***$_2$), 5.05 (dd, 1H, ^3J = 8.2 Hz, ^3J = 7.2 Hz, PhThiazC***H***), 6.08 (d, 1H, ^3J = 3.2 Hz, Fur-3-***H***), 6.47 (d, 1H, ^3J = 3.2 Hz, Fur-4-***H***), 7.29 – 7.40 (m, 5H, Ph-***H***), 7.64 (d, 1H, ^3J = 3.6 Hz, Thiaz-5-***H***), 7.80 (d, 1H, ^3J = 3.6 Hz, Thiaz-4-***H***). ^{13}C-NMR (100 MHz, D$_2$O, trifluoroacetate, HSQC, HMBC): δ [ppm] = 24.44 (-, Fur-2-***C***H$_2$), 25.33 (-, Fur-2-CH$_2$-***C***H$_2$), 40.75 (-, Fur-2-(CH$_2$)$_2$-***C***H$_2$), 41.57 (-, PhThiazCH***C***H$_2$), 41.84 (+, N(***C***H$_3$)$_2$), 43.19 (+, PhThiaz***C***H), 52.94 (-, Fur-5-***C***H$_2$), 107.17 (+, Fur-***C***-3), 115.48 (+, Fur-***C***-4), 122.05 (+, Thiaz-***C***-5), 127.86 (+ , 2 Ph-***C***), 128.65 (+, Ph-***C***-4), 129.52 (+, 2 Ph-***C***), 138.19 (+, Thiaz-***C***-4), 138.62 (C$_{quat}$, Ph-***C***-1), 141.93 (C$_{quat}$, Fur-***C***-5), 152.55 (C$_{quat}$, ***C***=N), 157.54 (C$_{quat}$, Fur-***C***-2) 173.07 (C$_{quat}$, ***C***=O), 175.11 (C$_{quat}$, Thiaz-***C***-2). IR (cm^{-1}) = 2989, 2901, 1669, 1173, 1130. HRMS (EI-MS) calcd. for C$_{23}$H$_{29}$N$_5$O$_2$S [M$^{+\bullet}$] 439.2042; found 439.2040. C$_{23}$H$_{29}$N$_5$O$_2$S · 3 TFA (781.64).

***N*^{1}-[3-(1*H*-Imidazol-1-yl)propyl]-*N*^{2}-(3-phenylbutanoyl)guanidine (5.71)**

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.54** (167 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 35/65) yielded a colorless oil (275 mg, 51 %). ^{1}H-NMR (600 MHz,

D_2O, trifluoroacetate): δ [ppm] = 1.24 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.11 – 2.19 (m, 2H, Im-1-CH_2-C***H***$_2$), 2.68 (dd, 1H, ^2J = 15.0 Hz, ^3J = 9.1 Hz, $PhCH_3CH$-C***H***$_2$), 2.75 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.4 Hz, $PhCH_3CH$-C***H***$_2$), 3.18 – 3.25 (m, 1H, $PhCH_3$C***H***), 3.28 (t, 2H, ^3J = 6.7 Hz, Im-1-$(CH_2)_2$-C***H***$_2$), 4.22 (t, 2H, ^3J = 7.2 Hz, Im-1-C***H***$_2$), 7.20 – 7.34 (m, 5H, Ph-***H***), 7.35 – 7.36 (m, 1H, Im-4-***H***), 7.41 – 7.43 (m, 1H, Im-5-***H***), 8.66 – 8.67 (m, 1H, Im-2-***H***). ^{13}C-NMR (150 MHz, D_2O, trifluoroacetate, HSQC, HMBC): δ [ppm] = 21.23 (+, Ph***C***H_3CH), 27.64 (-, Im-1-CH_2-***C***H_2), 36.43 (+, $PhCH_3$***C***H), 38.30 (-, Im-1-$(CH_2)_2$-***C***H_2), 44.90 (-, $PhCH_3CH$-***C***H_2), 46.53 (-, Im-1-***C***H_2), 120.01 (+, Im-***C***-4), 121.64 (+, Im-***C***-5), 126.88 (+, 2 Ph-***C***), 126.97 (+, Ph-***C***-4), 128.90 (+, 2 Ph-***C***), 134.54 (+, Im-***C***-2), 144.97 (C_{quat}, Ph-***C***-1), 152.92 (C_{quat}, ***C***=N), 175.91 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3138, 2989, 2901, 1667, 1601, 1174, 1132. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_5O$ [$M^{+\bullet}$] 313.1903; found 313.1899. $C_{17}H_{23}N_5O$ · 2 TFA (541.44).

***N*^{1}-(3,3-Diphenylpropanoyl)-*N*^{2}-[3-(1*H*-imidazol-1-yl)propyl]guanidine (5.72)**

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.54** (224 mg, 1.0 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 20/80, 20 min: 40/60) yielded a white solid (162 mg, 27 %); mp 48 – 52 °C. ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 2.01 – 2.13 (m, 2H, Im-1-CH_2-C***H***$_2$), 3.19 (d, 2H, ^3J = 8.1 Hz, Ph_2CHC***H***$_2$), 3.21 (t, 2H, ^3J = 6.8 Hz, Im-1-$(CH_2)_2$-C***H***$_2$), 4.14 (t, 2H, ^3J = 7.2 Hz, Im-1-C***H***$_2$), 4.45 (t, 1H, ^3J = 8.1 Hz, Ph_2C***H***), 7.12 – 7.29 (m, 11H, Ph-***H*** + Im-4-***H***), 7.32 – 7.35 (m, 1H, Im-5-***H***), 8.58 – 8.61 (m, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 27.60 (-, Im-1-CH_2-***C***H_2), 38.32 (-, Im-1-$(CH_2)_2$-***C***H_2), 42.10 (-, Ph_2CH-***C***H_2), 46.38 (+, Ph_2***C***H), 46.50 (-, Im-1-***C***H_2), 119.98 (+, Im-***C***-4), 121.60 (+, Im-***C***-5), 127.16 (+, 2 Ph-***C***-4), 127.45 (+, 4 Ph-***C***), 129.05 (+, 4 Ph-***C***), 134.51 (+, Im-***C***-2), 142.91 (C_{quat}, 2 Ph-***C***-1), 152.87 (C_{quat}, ***C***=N), 175.09 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3149, 3068, 2969, 1665, 1603, 1178, 1132. HRMS (EI-MS) calcd. for $C_{22}H_{25}N_5O$ [$M^{+\bullet}$] 375.2059; found 375.2055. $C_{22}H_{25}N_5O$ · 2 TFA (603.51).

***N*^{1}-(3-Phenylbutanoyl)-*N*^{2}-[3-(1-trityl-1*H*-pyrazol-4-yl)propyl]guanidine (5.73)**

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.48** (409 mg, 1.0 mmol) according to the general procedure 2. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (410 mg, 74 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.27 (d, 3H, ^3J = 6.9 Hz, PhC***H***$_3$CH), 1.75 – 1.89 (m, 2H, Pyraz-4-CH_2-C***H***$_2$), 2.43 – 2.56 (m, 3H, Pyraz-4-C***H***$_2$ + $PhCH_3CH$-C***H***$_2$), 2.62 (dd, 1H, ^2J = 14.6 Hz, ^3J = 6.2 Hz, $PhCH_3CH$-C***H***$_2$), 3.07 (t, 2H, ^3J = 6.9 Hz, Pyraz-4-$(CH_2)_2$-C***H***$_2$), 3.24

– 3.38 (m, 1H, $PhCH_3C\boldsymbol{H}$), 7.08 – 7.37 (m, 21H, Ph-***H*** + Pyraz-3-***H***), 7.48 (s, 1H, Pyraz-5-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 556 (66) $[M + H]^+$, 466 (100). $C_{36}H_{37}N_5O$ (555.71).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1-trityl-1*H*-pyrazol-4-yl)propyl]guanidine (5.74)

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.48** (409 mg, 1.0 mmol) according to the general procedure 2. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless oil (430 mg, 70 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.69 – 1.86 (m, 2H, Pyraz-4-CH_2-C***H***$_2$), 2.48 (t, 2H, ^3J = 7.4 Hz, Pyraz-4-C***H***$_2$), 2.97 – 3.08 (m, 4H, Pyraz-4-$(CH_2)_2$-C***H***$_2$ + $Ph_2CHC\boldsymbol{H}_2$), 4.63 (t, 1H, ^3J = 7.8 Hz, $Ph_2C\boldsymbol{H}$), 7.05 – 7.37 (m, 26H, Ph-***H*** + Pyraz-3-***H***), 7.46 (s, 1H, Pyraz-5-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 618 (83) $[M + H]^+$, 528 (100). $C_{41}H_{39}N_5O$ (617.78).

N^1-(3-Phenylbutanoyl)-N^2-[3-(1-trityl-1*H*-imidazol-2-yl)propyl]guanidine (5.75)

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.47** (409 mg, 1.0 mmol) according to the general procedure 1. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow oil (290 mg, 52 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.28 (d, 3H, ^3J = 6.9 Hz, PhC***H***$_3$CH), 1.36 – 1.52 (m, 2H, Im-2-CH_2-C***H***$_2$), 1.84 (t, 2H, ^3J = 5.7 Hz, Im-2-C***H***$_2$), 2.55 (dd, 1H, ^2J = 14.8 Hz, ^3J = 8.2 Hz, $PhCH_3CH$-C***H***$_2$), 2.69 (dd, 1H, ^2J = 14.8 Hz, ^3J = 6.4 Hz, $PhCH_3CH$-C***H***$_2$), 3.07 (t, 2H, ^3J = 7.4 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 3.26 – 3.38 (m, 1H, $PhCH_3C\boldsymbol{H}$), 6.78 (d, 1H, ^3J = 1.5 Hz, Im-4-***H***), 6.89 (d, 1H, ^3J = 1.5 Hz, Im-5-***H***), 7.05 – 7.39 (m, 20H, Ph-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 556 (100) $[M + H]^+$. $C_{36}H_{37}N_5O$ (555.71).

N^1-[3-(Thiophen-2-yl)butanoyl]-N^2-[3-(1-trityl-1*H*-Imidazol-2-yl)propyl]guanidine (5.76)

The title compound was prepared from **5.56**[3] (170 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.47** (409 mg, 1.0 mmol) according to the general procedure 1. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow foam-like solid (310 mg, 55 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.34 – 1.54 (m, 5H, ThioC***H***$_3$CH + Im-2-CH_2-C***H***$_2$), 1.85 (t, 2H, ^3J = 7.1 Hz, Im-2-C***H***$_2$), 2.60 (dd, 1H, ^2J = 15.0 Hz, ^3J = 8.4 Hz, $ThioCH_3CH$-C***H***$_2$), 2.78 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.6 Hz, $ThioCH_3CH$-C***H***$_2$), 3.09 (t, 2H, ^3J = 7.4 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 3.55 – 3.70 (m, 1H, $ThioCH_3C\boldsymbol{H}$), 6.77 – 6.94 (m, 4H, Thio-3,4-***H*** + Im-4,5-***H***), 7.04 – 7.16 (m, 7H, Ph-***H***, Thio-5-***H***), 7.27 – 7.39 (m, 9H, Ph-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 562 (100) $[M + H]^+$. $C_{34}H_{35}N_5OS$ (561.74).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1-trityl-1*H*-imidazol-2-yl)propyl]guanidine (5.77)

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.47** (409 mg, 1.0 mmol) according to the general procedure 1. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow foam-like solid (380 mg, 62 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.30 – 1.42 (m, 2H, Im-2-CH_2-C***H***$_2$), 1.81 (t, 2H, ^3J = 5.6 Hz, Im-2-C***H***$_2$), 3.03 (t, 2H, ^3J = 7.1 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 3.12 (d, 2H, ^3J = 7.9 Hz, Ph_2CHC***H***$_2$), 4.62 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 6.77 (d, 1H, ^3J = 1.5 Hz, Im-4-***H***), 6.85 (d, 1H, ^3J = 1.5 Hz, Im-5-***H***), 7.04 – 7.40 (m, 25H, Ph-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 618 (100) $[M + H]^+$. $C_{41}H_{39}N_5O$ (617.78).

N^1-[3-Phenyl-3-(thiazol-2-yl)propanoyl]-N^2-[3-(1-trityl-1*H*-imidazol-2-yl)propyl]guanidine (5.78)

The title compound was prepared from **5.58**[3] (233 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.47** (409 mg, 1.0 mmol) according to the general procedure 1. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a brownish oil (300 mg, 48 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.35 – 1.50 (m, 2H, Im-2-CH_2-C***H***$_2$), 1.81 (t, 2H, ^3J = 5.7 Hz, Im-2-C***H***$_2$), 2.96 – 3.10 (m, 3H, Im-2-$(CH_2)_2$-C***H***$_2$ + PhThiazCH-C***H***$_2$), 3.39 (dd, 1H, ^2J = 16.0 Hz, ^3J = 8.2 Hz, PhThiazCH-C***H***$_2$), 5.00 (dd, 1H, ^3J = 8.2 Hz, ^3J = 7.2 Hz, PhThiazC***H***), 6.76 (d, 1H, ^3J = 1.5 Hz, Im-4-***H***), 6.87 (d, 1H, ^3J = 1.5 Hz, Im-5-***H***), 7.14 – 7.41 (m, 21H, Ph-***H*** + Thiaz-5-***H***), 7.66 (d, 1H, ^3J = 3.5 Hz, Thiaz-4-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 625 (100) $[M + H]^+$. $C_{38}H_{36}N_6OS$ (624.80).

N^1-(3-Phenylbutanoyl)-N^2-[3-(1-trityl-1*H*-1,2,4-triazol-3-yl)propyl]guanidine (5.79)

The title compound was prepared from **5.55** (164 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.49** (411 mg, 1.0 mmol) according to the general procedure 2. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (470 mg, 84 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.27 (d, 3H, ^3J = 6.9 Hz, PhC***H***$_3$CH), 1.41 – 1.57 (m, 2H, Triaz-5-CH_2-C***H***$_2$), 1.95 (t, 2H, ^3J = 5.9 Hz, Triaz-5-C***H***$_2$), 2.48 (dd, 1H, ^2J = 14.6 Hz, ^3J = 8.9 Hz, $PhCH_3CH$-C***H***$_2$), 2.62 (dd, 1H, ^2J = 14.6 Hz, ^3J = 8.5 Hz, $PhCH_3CH$-C***H***$_2$), 3.04 (t, 2H, ^3J = 7.5 Hz, Triaz-5-$(CH_2)_2$-C***H***$_2$), 3.24 – 3.39 (m, 1H, $PhCH_3$C***H***), 7.05 – 7.14 (m, 6H, Ph-***H***), 7.23 – 7.38 (m, 14H, Ph-***H***), 7.88 (s, 1H, Triaz-3-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 557 (100) $[M + H]^+$. $C_{35}H_{36}N_6O$ (556.70).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1-trityl-1*H*-1,2,4-triazol-3-yl)propyl]guanidine (5.80)

The title compound was prepared from **5.57** (226 mg, 1.0 mmol), CDI (195 mg, 1.2 mmol), NaH (60 % dispersion in mineral oil) (80 mg, 2.0 mmol) and **5.49** (411 mg, 1.0 mmol) according to the general procedure 2. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (580 mg, 94 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.37 – 1.51 (m, 2H, Triaz-5-CH_2-C***H***$_2$), 2.88 (t, 2H, ^3J = 5.8 Hz, Triaz-5-C***H***$_2$), 2.99 (t, 2H, ^3J = 7.6 Hz, Triaz-5-$(CH_2)_2$-C***H***$_2$), 3.04 (d, 2H, ^3J = 7.8 Hz, Ph_2CHCH_2), 4.63 (t, 1H, ^3J = 7.8 Hz, Ph_2C***H***), 7.00 – 7.40 (m, 25H, Ph-***H***), 7.86 (s, 1H, Triaz-3-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 619 (100) $[M + H]^+$. $C_{40}H_{38}N_6O$ (618.77).

5.4.1.8 Preparation of the N^G-acylated arylpropylguanidines 5.81-5.88

The pertinent trityl-protected N^G-acylated arylpropylguanidine was stirred for 5 h in a mixture of TFA (5.0 mL) and DCM (20 mL). After removing the solvent *in vacuo*, the crude product was purified by preparative HPLC. All compounds were dried by lyophilization and obtained as trifluoroacetic acid salts.

N^1-[3-(1*H*-Imidazol-2-yl)propyl]-N^2-(3-phenylbutanoyl)guanidine (5.81)

The title compound was prepared from **5.75** (280 mg, 0.50 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a colorless semisolid compound (79 mg, 29 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.20 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.90 – 2.05 (m, 2H, Im-2-CH_2-C***H***$_2$), 2.64 (dd, 1H, ^2J = 15.0 Hz, ^3J = 8.9 Hz, $PhCH_3CH$-C***H***$_2$), 2.71 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.4 Hz, $PhCH_3CH$-C***H***$_2$), 2.91 (t, 2H, ^3J = 7.7 Hz, Im-2-C***H***$_2$), 3.10 – 3.22 (m, 1H, $PhCH_3$C***H***), 3.26 (t, 2H, ^3J = 6.7 Hz, Im-2-$(CH_2)_2$-C***H***$_2$), 7.13 (s, 2H, Im-4,5-***H***), 7.14 – 7.33 (m, 5H, Ph-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 21.36 (+, Ph***C***H_3CH), 22.58 (-, Im-2-***C***H_2), 24.95 (-, Im-2-CH_2-***C***H_2), 36.41 (+, $PhCH_3$***C***H), 40.33 (-, Im-2-$(CH_2)_2$-***C***H_2), 44.88 (-, $PhCH_3CH$-***C***H_2), 118.59 (+, Im-***C***-4,5), 126.93 (+, 2 Ph-***C***), 127.01 (+, Ph-***C***-4), 128.95 (+, 2 Ph-***C***), 145.04 (C_{quat}, Ph-***C***-1), 146.18 (C_{quat}, Im-***C***-2), 152.92 (C_{quat}, ***C***=N), 175.95 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3114, 2939, 2719, 1662, 1626, 1179, 1128. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_5O$ $[M^{+\bullet}]$ 313.1903; found 313.1906. $C_{17}H_{23}N_5O \cdot 2$ TFA (541.44).

N^1-[3-(1*H*-Imidazol-2-yl)propyl]-N^2-[3-(thiophen-2-yl)butanoyl]guanidine (5.82)

The title compound was prepared from **5.76** (280 mg, 0.50 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a beige semisolid compound (99 mg, 36 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.28 (d, 3H, ^3J = 7.0 Hz, ThioC***H***$_3$CH), 1.94 – 2.07 (m, 2H, Im-2-CH_2-C***H***$_2$), 2.65 (dd, 1H, ^2J

= 15.2 Hz, ^3J = 8.8 Hz, ThioCH$_3$CH-C***H***$_2$), 2.74 (dd, 1H, ^2J = 15.2 Hz, ^3J = 6.3 Hz, ThioCH$_3$CH-C***H***$_2$), 2.94 (t, 2H, ^3J = 7.7 Hz, Im-2-C***H***$_2$), 3.29 (t, 2H, ^3J = 6.8 Hz, Im-2-(CH$_2$)$_2$-C***H***$_2$), 3.44 – 3.58 (m, 1H, ThioCH$_3$C***H***), 6.85 (ddd, 1H, ^3J = 3.5 Hz, ^4J = 1.2 Hz, ^4J = 0.6 Hz, Thio-3-***H***), 6.90 (dd, 1H, ^3J = 5.0 Hz, ^3J = 3.5 Hz, Thio-4-***H***), 7.16 (s, 2H, Im-4,5-***H***), 7.19 (dd, 1H, ^3J = 5.0 Hz, ^4J = 1.2 Hz, Thio-5-***H***). ^{13}C-NMR (75 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 22.26 (+, ThioCH***C***H$_3$), 22.59 (-, Im-2-***C***H$_2$), 24.96 (-, Im-2-CH$_2$-***C***H$_2$), 31.79 (+, ThioCH$_3$***C***H), 40.36 (-, Im-2-(CH$_2$)$_2$-***C***H$_2$), 45.89 (-, ThioCH$_3$CH-***C***H$_2$), 118.59 (+, Im-***C***-4,5), 123.67, 123.90 (+, Thio-***C***-3,4), 127.17 (+, Thio-***C***-5), 146.19 (C$_{quat}$, Im-***C***-2), 148.88 (C$_{quat}$, Thio-***C***-2), 152.94 (C$_{quat}$, ***C***=N), 175.42 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3121, 2989, 2901, 1663, 1625, 1594, 1180, 1128. HRMS (EI-MS) calcd. for C$_{15}$H$_{21}$N$_5$OS [M$^{+\bullet}$] 319.1467; found 319.1472. C$_{15}$H$_{21}$N$_5$OS · 2 TFA (547.47).

N^1-(3,3-Diphenylpropanoyl)-*N*^{2}-[3-(1*H*-imidazol-2-yl)propyl]guanidine (5.83)

The title compound was prepared from **5.77** (370 mg, 0.60 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 35/65) yielded a colorless semisolid compound (115 mg, 32 %). ^{1}H-NMR (300 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 1.86 – 2.02 (m, 2H, Im-2-CH$_2$-C***H***$_2$), 2.88 (t, 2H, ^3J = 7.7 Hz, Im-2-C***H***$_2$), 3.17 (d, 2H, ^3J = 8.1 Hz, Ph$_2$CHC***H***$_2$), 3.23 (t, 2H, ^3J = 6.7 Hz, Im-2-(CH$_2$)$_2$-C***H***$_2$), 4.44 (t, 1H, ^3J = 8.1 Hz, Ph$_2$C***H***), 7.06 (s, 2H, Im-4,5-***H***), 7.10 – 7.30 (m, 10H, Ph-***H***). ^{13}C-NMR (75 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 22.58 (-, Im-2-***C***H$_2$), 24.90 (-, Im-2-CH$_2$-***C***H$_2$), 40.37 (-, Im-2-(CH$_2$)$_2$-***C***H$_2$), 42.09 (-, Ph$_2$CH-***C***H$_2$), 46.33 (+, Ph$_2$***C***H), 118.54 (+, Im-***C***-4,5), 127.17 (+, 2 Ph-***C***-4), 127.48 (+, 4 Ph-***C***), 129.07 (+, 4 Ph-***C***), 142.95 (C$_{quat}$, 2 Ph-***C***-1), 146.14 (C$_{quat}$, Im-***C***-2), 152.85 (C$_{quat}$, ***C***=N), 175.08 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3114, 2990, 2902, 1663, 1623, 1180, 1129. HRMS (EI-MS) calcd. for C$_{22}$H$_{25}$N$_5$O [M$^{+\bullet}$] 375.2059; found 375.2059. C$_{22}$H$_{25}$N$_5$O · 2 TFA (603.51).

N^1-[3-(1*H*-Imidazol-2-yl)propyl]-*N*^{2}-[3-phenyl-3-(thiazol-2-yl)propanoyl]guanidine (5.84)

The title compound was prepared from **5.78** (270 mg, 0.43 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 27.5/72.5) yielded a pale yellow semisolid compound (140 mg, 45 %). ^{1}H-NMR (300 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 1.91 – 2.06 (m, 2H, Im-2-CH$_2$-C***H***$_2$), 2.92 (t, 2H, ^3J = 7.7 Hz, Im-2-C***H***$_2$), 3.24 – 3.37 (m, 3H, Im-2-(CH$_2$)$_2$-C***H***$_2$ + PhThiazCH-C***H***$_2$), 3.45 (dd, 1H, ^2J = 16.6 Hz, ^3J = 7.4 Hz, PhThiazCH-C***H***$_2$), 4.90 (dd, 1H, ^3J = 7.7 Hz, ^3J = 7.4 Hz, PhThiazC***H***), 7.11 (s, 2H, Im-4,5-***H***), 7.24 – 7.37 (m, 5H, Ph-***H***), 7.52 (d, 1H, ^3J = 3.5 Hz, Thiaz-5-***H***), 7.70 (d, 1H, ^3J = 3.5 Hz, Thiaz-4-***H***). ^{13}C-NMR (75 MHz, D$_2$O, trifluoroacetate): δ [ppm] = 22.57 (-, Im-2-***C***H$_2$), 24.92 (-, Im-2-CH$_2$-***C***H$_2$), 40.41 (-, Im-2-(CH$_2$)$_2$-***C***H$_2$), 41.67 (-, PhThiazCH-***C***H$_2$), 43.43 (+, PhThiaz***C***H), 118.54 (+, Im-***C***-4,5), 121.49 (+, Thiaz-***C***-5), 127.83 (+, 2 Ph-***C***), 128.44 (+, Ph-

C-4), 129.44 (+, 2 Ph-***C***), 139.31 (C_{quat}, Ph-***C***-1), 139.53 (+, Thiaz-***C***-4), 146.16 (C_{quat}, Im-***C***-2), 152.81 (C_{quat}, ***C***=N), 173.41, 174.37 (C_{quat}, Thiaz-***C***-2 + ***C***=O). IR (cm^{-1}) = 3117, 2902, 1663, 1624, 1181, 1129. HRMS (EI-MS) calcd. for $C_{19}H_{22}N_6OS$ [$M^{+\bullet}$] 382.1576; found 382.1577. $C_{19}H_{22}N_6OS \cdot 2$ TFA (724.55).

N^1-(3-Phenylbutanoyl)-N^2-[3-(1*H*-pyrazol-4-yl)propyl]guanidine (5.85)

The title compound was prepared from **5.73** (400 mg, 0.72 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 20/80, 20 min: 45/65) yielded a pale yellow semisolid compound (210 mg, 54 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.32 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 1.82 – 1.97 (m, 2H, Pyraz-4-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.6 Hz, Pyraz-4-C***H***$_2$), 2.60 (dd, 2H, ^2J = 15.1 Hz, ^3J = 7.6 Hz, PhCH_3CH-C***H***$_2$), 2.75 (dd, 1H, ^2J = 15.1 Hz, ^3J = 7.5 Hz, PhCH_3CH-C***H***$_2$), 3.23 – 3.37 (m, 3H, overlap with solvent, PhCH_3C***H*** + Pyraz-4-$(CH_2)_2$-C***H***$_2$), 7.13 – 7.33 (m, 5H, Ph-***H***), 7.62 (s, 2H, Pyraz-3,5-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 21.87 (-, Pyraz-4-***C***H_2), 22.30 (+, Ph***C***H_3CH), 30.22 (-, Pyraz-4-CH_2-***C***H_2), 37.69 (+, PhCH_3***C***H), 41.92 (-, Pyraz-4-$(CH_2)_2$-***C***H_2), 46.21 (-, PhCH_3CH-***C***H_2), 121.22 (C_{quat}, Pyraz-***C***-4), 127.75 (+, Ph-***C***-4), 127.95 (+, 2 Ph-***C***), 129.70 (+, 2 Ph-***C***), 133.85 (+, Pyraz-***C***-3,5), 146.43 (C_{quat}, Ph-***C***-1), 155.08 (C_{quat}, ***C***=N), 176.00 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3092, 2968, 1662, 1594, 1176, 1133. HRMS (EI-MS) calcd. for $C_{17}H_{23}N_5O$ [$M^{+\bullet}$] 313.1903; found 313.1898. $C_{17}H_{23}N_5O \cdot 2$ TFA (541.44).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1*H*-pyrazol-4-yl)propyl]guanidine (5.86)

The title compound was prepared from **5.74** (420 mg, 0.68 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 30/70, 20 min: 50/50) yielded a pale yellow semisolid compound (205 mg, 50 %). ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.80 – 1.94 (m, 2H, Pyraz-4-CH_2-C***H***$_2$), 2.56 (t, 2H, ^3J = 7.6 Hz, Pyraz-4-C***H***$_2$), 3.24 (t, 2H, ^3J = 7.0 Hz, Pyraz-4-$(CH_2)_2$-C***H***$_2$), 3.25 (d, 2H, ^3J = 8.0 Hz, Ph_2CHC***H***$_2$), 4.59 (t, 1H, ^3J = 8.0 Hz, Ph_2C***H***), 7.11 – 7.34 (m, 10H, Ph-***H***), 7.51 (s, 2H, Pyraz-3,5-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 21.87 (-, Pyraz-4-***C***H_2), 30.27 (-, Pyraz-4-CH_2-***C***H_2), 41.94 (-, Pyraz-4-$(CH_2)_2$-***C***H_2), 43.90 (-, Ph_2CH-***C***H_2), 48.06 (-, Ph_2***C***H), 120.76 (C_{quat}, Pyraz-***C***-4), 127.84 (+, 2 Ph-***C***-4), 128.84 (+, 4 Ph-***C***), 129.74 (+, 4 Ph-***C***), 133.82 (+, Pyraz-***C***-3,5), 144.51 (C_{quat}, Ph-***C***-1), 154.98 (C_{quat}, ***C***=N), 175.46 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3026, 2923, 1663, 1599, 1183, 1129. HRMS (EI-MS) calcd. for $C_{22}H_{25}N_5O$ [$M^{+\bullet}$] 375.2059; found 375.2049. $C_{22}H_{25}N_5O \cdot 2$ TFA (603.51).

N^1-(3-Phenylbutanoyl)-N^2-[3-(1*H*-1,2,4-triazol-3-yl)propyl]guanidine (5.87)

The title compound was prepared from **5.79** (450 mg, 0.81 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 15/85, 20 min: 40/60) yielded a white solid (220 mg, 50 %); mp 58 – 62 °C. ^{1}H-NMR (400 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.32 (d, 3H, ^3J = 7.0 Hz, PhC***H***$_3$CH), 2.01 – 2.11 (m, 2H, Triaz-3-CH_2-C***H***$_2$), 2.69 – 2.80 (m, 2H, PhCH_3CH-C***H***$_2$), 2.88 (t, 2H, ^3J = 7.3 Hz, Triaz-3-C***H***$_2$), 3.26 – 3.36 (m, 1H, overlap with solvent, PhCH_3C***H***), 3.37 (t, 2H, ^3J = 7.1 Hz, Triaz-3-$(CH_2)_2$-C***H***$_2$), 7.14 – 7.31 (m, 5H, Ph-***H***), 8.39 (s, 1H, Triaz-5-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate, HSQC): δ [ppm] = 22.29 (+, Ph***C***H_3CH), 24.20 (-, Triaz-3-***C***H_2), 26.94 (-, Triaz-3-CH_2-***C***H_2), 37.60 (+, PhCH_3***C***H), 41.69 (-, Triaz-3-$(CH_2)_2$-***C***H_2), 46.25 (-, PhCH_3CH-***C***H_2), 127.75 (+, Ph-***C***-4), 127.92 (+, 2 Ph-***C***), 129.72 (+, 2 Ph-***C***), 146.45 (C_{quat}, Ph-***C***-1), 146.91 (+, Triaz-***C***-5), 155.22 (C_{quat}, ***C***=N), 159.51 (C_{quat}, Triaz-***C***-3), 175.80 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3086, 2971, 2931, 1669, 1602, 1178, 1130. HRMS (EI-MS) calcd. for $C_{16}H_{22}N_6O$ [$M^{+\bullet}$] 314.1855; found 314.1853. $C_{16}H_{22}N_6O \cdot 2$ TFA (542.43).

N^1-(3,3-Diphenylpropanoyl)-N^2-[3-(1*H*-1,2,4-triazol-3-yl)propyl]guanidine (5.88)

The title compound was prepared from **5.80** (560 mg, 0.93 mmol) according to the general procedure. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 20/80, 20 min: 50/50) yielded a white solid (228 mg, 41 %); mp 65 – 68 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.98 – 2.12 (m, 2H, Triaz-3-CH_2-C***H***$_2$), 2.88 (t, 2H, ^3J = 7.3 Hz, Triaz-3-C***H***$_2$), 3.25 (d, 2H, ^3J = 7.9 Hz, Ph_2CHC***H***$_2$), 3.36 (t, 2H, ^3J = 7.1 Hz, Triaz-3-$(CH_2)_2$-C***H***$_2$), 4.59 (t, 1H, ^3J = 7.9 Hz, Ph_2C***H***), 7.11 – 7.32 (m, 10H, Ph-***H***), 8.42 (s, 1H, Triaz-5-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 24.19 (-, Triaz-3-***C***H_2), 26.89 (-, Triaz-3-CH_2-***C***H_2), 41.70 (-, Triaz-3-$(CH_2)_2$-***C***H_2), 43.92 (-, Ph_2CH-***C***H_2), 47.96 (+, Ph_2***C***H), 127.83 (+, 2 Ph-***C***-4), 128.82 (+, 4 Ph-***C***), 129.74 (+, 4 Ph-***C***), 144.54 (C_{quat}, 2 Ph-***C***-1), 146.87 (+, Triaz-***C***-5), 155.14 (C_{quat}, ***C***=N), 159.50 (C_{quat}, Triaz-***C***-3), 175.28 (C_{quat}, ***C***=O). IR (cm^{-1}) = 2970, 2901, 1662, 1598, 1183, 1129. HRMS (EI-MS) calcd. for $C_{21}H_{24}N_6O$ [$M^{+\bullet}$] 376.2012; found 376.2004. $C_{21}H_{24}N_6O \cdot 2$ TFA (604.50).

5.4.2 Pharmacological methods

5.4.2.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide maleate was from Tocris Bioscience (Ellisville, USA). [γ-^{32}P]GTP was synthesized according to a previously described method.[40] [^{32}P]P_i (8,500 – 9,100 Ci/mmol orthophosphoric acid) was from PerkinElmer Life Sciences (Boston, MA). All unlabeled nucleotides, glycerol-3-phosphate dehydrogenase, triose phosphate isomerase,

glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were from Roche (Mannheim, Germany). 3-Phosphoglycerate kinase and L-α-glycerol phosphate was from Sigma.

5.4.2.2 Steady-state GTPase activity assay

See section 3.4.2.2.

5.4.2.3 Histamine H_1R assay on guinea pig ileum[3]

See section 3.4.2.4.

5.4.2.4 Histamine H_2R assay on the isolated spontaneously beating guinea pig right atrium[3]

See section 3.4.2.5.

5.5 References

(1) Buschauer, A., Synthesis and in Vitro Pharmacology of Arpromidine and Related Phenyl(pyridylalkyl)guanidines, a Potential New Class of Positive Inotropic Drugs. *J. Med. Chem.* **1989**, 32, 1963-1970.

(2) Buschauer, A., Friese-Kimmel, A., Baumann, G., Schunack, W., Synthesis and histamine H_2 agonistic activity of arpromidine analogues: replacement of the pheniramine-like moiety by non-heterocyclic groups. *Eur. J. Med. Chem.* **1992**, 27, 321-330.

(3) Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press, DOI: 10.1021/jm800841w. Epub ahead of print on Oct 800825, 802008.

(4) Xie, S.-X., Ghorai, P., Ye, Q.-Z., Buschauer, A., Seifert, R., Probing Ligand-Specific Histamine H_1- and H_2-Receptor Conformations with N^G-Acylated Imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006**, 317, 139-146.

(5) Xie, S.-X., Kraus, A., Ghorai, P., Ye, Q.-Z., Elz, S., Buschauer, A., Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-AK57), a Potent Partial Agonist for the Human Histamine H_1- and H_2-Receptors. *J. Pharmacol. Exp. Ther.* **2006**, 317, 1262-1268.

(6) pK_a-value was calculated with ACD Labs 9.0, Advanced Chemistry Development, Toronto (Canada).

(7) Coruzzi, G., Timmerman, H., Adami, M., Bertaccini, G., The new potent and selective histamine H_2 receptor agonist amthamine as a tool to study gastric secretion. *Naunyn. Schmiedebergs Arch. Pharmacol.* **1993**, 348, 77-81.

(8) Ganellin, C. R. Chemistry and Structure-Activity Relationsships of Drugs Acting at Histamine Receptors. In *Pharmacology of Histamine Receptors*, Ganellin, C. R., Parsons, M. E., Eds. Wright PSG: Bristol, London, Boston, **1982**; pp 10-102.

(9) Ganellin, C. R., Pharmacochemistry of H_1 and H_2 Receptors, in *The Histamine Receptor.*, (Schwartz, J. C., Haas, H. L. ed.) **1992**, pp 1-56, Wiley-Liss, Inc.

(10) Navarro, J., Synthesis and pharmacology of two new histamine receptor antagonists related to ranitidine. *Pharmazie* **1995**, 50, 12-15.

(11) Striegl, B., Synthese und funktionelle *in-vitro*-Pharmakologie neuer Histamin-H_1-Rezeptoragonisten aus der Suprahistaprodifen-Reihe. *Doctoral thesis*, University of Regensburg, Germany **2006**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=681&la=de.

(12) Sonogashira, K., Tohda, Y., Hagihara, N., A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, 16, 4467-4470.

(13) Bulger, P. G., Cottrell, I. F., Cowden, C. J., Davies, A. J., Dolling, U.-H., An investigation into the alkylation of 1,2,4-triazole. *Tetrahedron Lett.* **2000**, 41, 1297-1301.

(14) Kunze, M., Histamin-H_1-Rezeptoragonisten vom Suprahistaprodifen- und 2-Phenylhistamin-Typ und 2-substituierte Imidazolylpropan-Derivate als Liganden für H_1/H_2/H_3/H_4-Rezeptoren. *Doctoral thesis*, University of Regensburg, Germany **2006**. http://www.opus-bayern.de/uni-regensburg/frontdoor.php?source_opus=711&la=de.

(15) Cox, J. M., Bellini, P., Barrett, R., Ellis, R. M., Hawkes, T. R. Preparation of triazolylalkylphosphonic acids as herbicides. WO 9315610, **1993**, *Chem. Abstr*. 120:134813.

(16) Wadsworth, W. S., Emmons, W. D., The Utility of Phosphonate Carbanions in Olefin Synthesis. *J. Am. Chem. Soc.* **1961**, 83, 1733-1738.

(17) Mitsunobu, O., Yamada, M., Mukaiyama, T., Preparation of esters of phosphoric acid by the reaction of trivalent phosphorus compounds with diethyl azodicarboxylate in the presence of alcohols. *Bull. Chem. Soc. Jpn.* **1967**, 40, 935-939.

(18) Allen, J. R., Hitchcock, S. A., Liu, B., William Wilson, J. T. Preparation of 2-hydroxyindanylamide derivatives as muscarinic receptor agonists. WO 2005009941, **2005**. *Chem. Abstr.* 142:197700.

(19) Feichtinger, K., Sings, H. L., Baker, T. J., Matthews, K., Goodman, M., Triurethane-Protected Guanidines and Triflyldiurethane-Protected Guanidines: New Reagents for Guanidinylation Reactions. *J. Org. Chem.* **1998**, 63, 8432-8439.

(20) Paul, R., Anderson, G. W., *N*,*N*'-Carbonyldiimidazole, a New Peptide Forming Reagent. *J. Am. Chem. Soc.* **1960**, 82, 4596-4600.

(21) Paul, R., Anderson, G. W., *N*,*N*'-Carbonyldiimidazole in Peptide Synthesis. III. A Synthesis of Isoleucine-5 Angiotensin II Amide-I. *J. Org. Chem.* **1962**, 27, 2094-2099.

(22) Kelley, M. T., Bürckstümmer, T., Wenzel-Seifert, K., Dove, S., Buschauer, A., Seifert, R., Distinct Interaction of Human and Guinea Pig Histamine H_2-Receptor with Guanidine-Type Agonists. *Mol. Pharmacol.* **2001**, 60, 1210-1225.

(23) Seifert, R., Wenzel-Seifert, K., Bürckstümmer, T., Pertz, H. H., Schunack, W., Dove, S., Buschauer, A., Elz, S., Multiple Differences in Agonist and Antagonist Pharmacology between Human and Guinea Pig Histamine H_1-Receptor. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1104-1115.

(24) Preuss, H., Ghorai, P., Kraus, A., Dove, S., Buschauer, A., Seifert, R., Constitutive Activity and Ligand Selectivity of Human, Guinea Pig, Rat, and Canine Histamine H_2 Receptors. *J. Pharmacol. Exp. Ther.* **2007**, 321, 983-995.

(25) Schneider, E., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(26) Schnell, D., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(27) Durant, G. J., Emmett, J. C., Ganellin, C. R., Miles, P. D., Parsons, M. E., Prain, H. D., White, G. R., Cyanoguanidine-Thiourea Equivalence in the Development of the Histamine H_2-Receptor Antagonist, Cimetidine. *J. Med. Chem.* **1977**, 20, 901-906.

(28) Kraus, A., Ghorai, P., Birnkammer, T., Schnell, D., Elz, S., Seifert, R., Dove, S., Bernhardt, G., Buschauer, A., N^G-Acylated Aminothiazolylpropylguanidines as Potent and Selective Histamine H_2 Receptor Agonists. *ChemMedChem* **2008**, accepted.

(29) Mizutani, T., Suzuki, K., Relative hepatotoxicity of 2-(substituted phenyl)thiazoles and substituted thiobenzamides in mice: evidence for the involvement of thiobenzamides as ring cleavage metabolites in the hepatotoxicity of 2-phenylthiazoles. *Toxicol. Lett.* **1996**, 85, 101-105.

(30) Mizutani, T., Yoshida, K., Kawazoe, S., Possible role of thioformamide as a proximate toxicant in the nephrotoxicity of thiabendazole and related thiazoles in glutathione-depleted mice: Structure-toxicity and metabolic studies. *Chem. Res. Toxicol.* **1993**, 6, 174-179.

(31) Mizutani, T., Yoshida, K., Kawazoe, S., Formation of toxic metabolites from thiabendazole and other thiazoles in mice. Identification of thioamides as ring cleavage products. *Drug Metab. Dispos.* **1994**, 22, 750-755.

(32) Dalvie, D. K., Kalgutkar, A. S., Khojasteh-Bakht, S. C., Obach, R. S., O'Donnell, J. P., Biotransformation Reactions of Five-Membered Aromatic Heterocyclic Rings. *Chem. Res. Toxicol.* **2002**, 15, 269-299.

(33) Alonso, D., Perez, M., Gomez, G., Covelo, B., Fall, Y., The furan approach to oxacycles. Part 3: Stereoselective synthesis of 2,3-disubstituted tetrahydropyrans. *Tetrahedron* **2005**, 61, 2021-2026.

(34) Lee, C. S., Allwine, D. A., Barbachyn, M. R., Grega, K. C., Dolak, L. A., Ford, C. W., Jensen, R. M., Seest, E. P., Hamel, J. C., Schaadt, R. D., Stapert, D., Yagi, B. H., Zurenko, G. E., Genin, M. J., Carbon-carbon-linked (pyrazolylphenyl)oxazolidinones with antibacterial activity against multiple drug resistant gram-positive and fastidious gram-negative bacteria. *Bioorg. Med. Chem.* **2001**, 9, 3243-3253.

(35) Kieć-Kononowicz, K., Ligneau, X., Schwartz, J.-C., Schunack, W., Pyrazoles as potential histamine H_3-receptor antagonists. *Arch. Pharm. (Weinheim).* **1995**, 328, 469-472.

(36) Nara, H., Kaieda, A., Sato, K., Terauchi, J. Preparation of heterocyclic amide compounds as matrix metalloproteinase inhibitors. JP322043, **2007**, *Chem. Abstr.* 146:482087.

(37) Stanford, D. J., Fernandez, R. A., Zeller, M., Hunter, A. D., *N*-(3-Phenylpropyl)guanidinium bromide. *Acta Crystallographica, Section E: Structure Reports Online* **2007**, E63, 1934-1936.

(38) Black, J. W., Durant, G. J., Emmett, J. C., Ganellin, C. R. Amidine derivatives and their pharmacologically active addition salts. DE 2053175, **1971**, *Chem. Abstr.* 75:118317.

(39) Castillo-Melendez, J. A., Golding, B. T., Optimization of the synthesis of guanidines from amines *via* nitroguanidines using 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboxamidine. **2004**, 1655-1663.

(40) Walseth, T. F., Johnson, R. A., The enzymatic preparation of [α-^{32}P] nucleoside triphosphates, cyclic [^{32}P] AMP, and cyclic [^{32}P] GMP. *Biochim. Biophys. Acta* **1979**, 562, 11-31.

Chapter 6

N^G-Acylated imidazolylpropylguanidines as potent histamine H_4 receptor agonists: selectivity by variation of the N^G-substituent

6.1 Introduction

Among a series of N^G-acylated imidazolylpropylguanidines[1-3] originally developed by our group as potent H_2R agonists, several compounds were serendipitously found to be even more potent at the hH_3R and the hH_4R (for example UR-AK24 (**6.1**), Figure 6.1).[2, 3] Most of the investigated compounds displayed high efficacies at the hH_4R, but low efficacies at the hH_3R. Subsequent evaluation of the parent structure, 3-(1*H*-imidazol-4-yl)propylguanidine (**6.2**, SK&F 91486, Figure 6.1), a weak partial H_2R agonist,[4] surprisingly revealed this compound as a highly potent partial agonist at the hH_3R and hH_4R (Table 6.1).

Starting from this model compound, the goal of the present study was to elaborate the possibility to develop more selective hH_4R agonists by acylation of the guanidine group in SK&F 91486. One strategy was to introduce acyl residues containing structural motifs of the potent and selective hH_4R antagonist JNJ 7777120

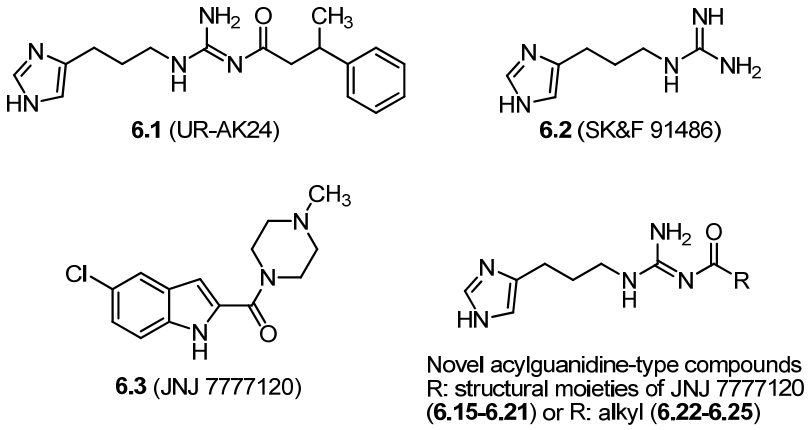


Figure 6.1. Structures of the acylguanidine UR-AK24 (**6.1**), SK&F 91486 (**6.2**), the selective H_4R antagonist JNJ 7777120 (**6.3**), and the novel N^G-acylated imidazolylpropyl-guanidines (**6.15-6.25**) presented in this study.

(**6.3**, Figure 6.1).[5] The resulting hybrid molecules (**6.15-6.21**) consisting of an H_4R agonistic and antagonistic moiety could be favorable for hH_4R selectivity and activity, provided that the acyl residue can additionally interact with the binding site of JNJ 7777120. The second approach focused on the size of the acyl residues with respect to selectivity for H_4R over H_2R. The imidazolylpropylguanidine portion is considered to be responsible for H_2R agonistic activity, whereas the N^G-acyl residue is assumed to contribute to H_2R affinity.[3] However, the endogenous ligand histamine, lacking additional affinity conferring residues, is about 50 times more potent at the hH_3R and the hH_4R relative to the hH_2R (Table 6.1). As bulky N^G-acyl groups may be dispensable or unfavorable to obtain highly potent compounds at the hH_3R and hH_4R, acylation of SK&F 91486 with small alkanoyl groups (**6.22-6.25**) was explored to switch selectivity toward the hH_3R and hH_4R and to gain more insight into the structure-activity relationships of the N^G-acylated imidazolylpropylguanidines at the distinct histamine receptor subtypes.

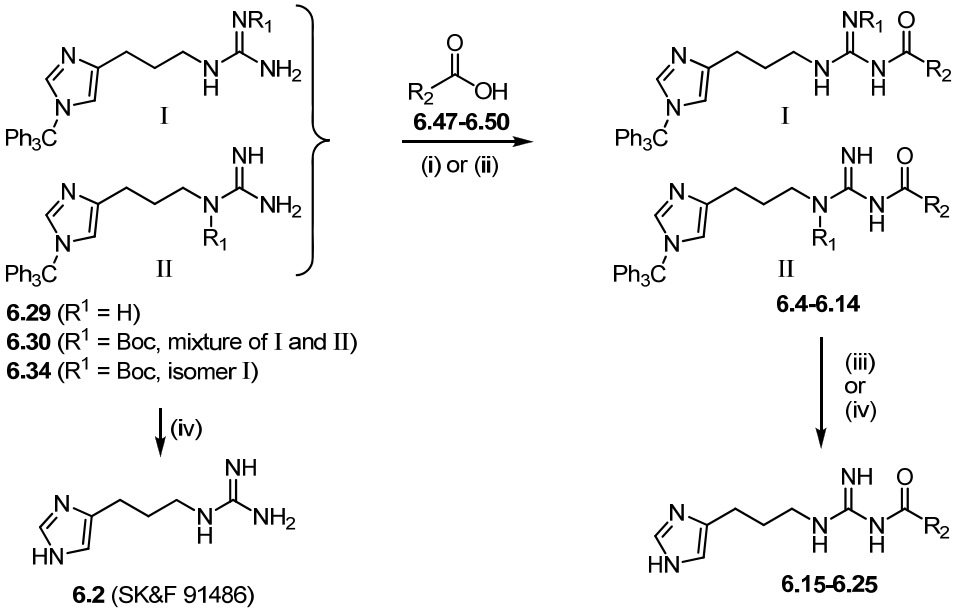
6.2 Chemistry

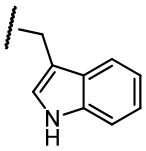
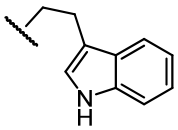
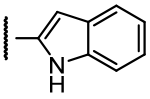
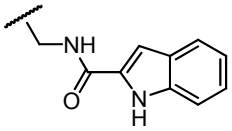
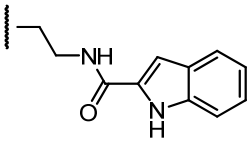
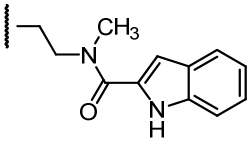
The N^G-acylated imidazolylpropylguanidines **6.4**, **6.6** and **6.8** were synthesized by acylation of the guanidine base **6.29** with CDI-activated carboxylic acids[6, 7] according to the method described by Ghorai et al. (Scheme 6.1).[3] However, preparation of the acylguanidines *via* this route showed drawbacks. The synthesis of **6.7** failed since under the strongly basic conditions (NaH) required for the deprotonation of the guanidine moiety an intramolecular cyclization of the carboxylic acid **6.47** (Scheme 6.4) occurred (acylation of the indole nitrogen giving a six-membered ring; analysed by mass spectrometry). Therefore, a modified synthetic pathway was employed. To avoid the strongly basic conditions, instead of the free guanidine ($pK_a \approx 13$)[8] the Boc-protected derivatives ($pK_a \approx 5$)[8] **6.30** or **6.34** (**6.30**: mixture of isomers I and II, **6.34**: single isomer; c.f. Scheme 6.2) were used,[9] yielding the trityl- and Boc-protected intermediates **6.5**, **6.7** and **6.9-6.14**. This procedure can be performed in a one-pot reaction without pre-activation of the carboxylic acids. Moreover, the isolation and purification of the less polar and less basic twofold acylated intermediates was facilitated, fewer side reactions were observed and excessive acylation of the guanidine moiety was prevented. Nevertheless, as marked cleavage of one acyl-group was detectable by NMR after storage in solution for few days, the intermediates **6.5**, **6.7**, **6.9-6.14** should be deprotected timely. Detritylation of the imidazole ring was routinely performed in a mixture of 20 % TFA in dichloromethane (**6.15**, **6.17**, **6.19**).[3] However, under these conditions the deprotection of compound **6.8** gave **6.19** in only 3 % yield and produced a large number of side products, whereas refluxing in hydrochloric acid resulted in 10 to 20 times higher yields. In contrast to the TFA/dichloromethane mixture, side reactions were reduced in aqueous medium, presumably, because the intermediate trityl cation was trapped and precipitated as

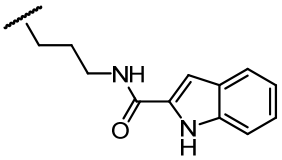
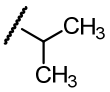
triphenylmethanol. This procedure was applied for the preparation of the N^G-acylated imidazolylpropylguanidines **6.16**, **6.18**, **6.20-6.25** and for **6.2** (SK&F 91486).[35]

In theory, acylation of the Boc-protected guanidine is possible at the nitrogen adjacent to the carbon chain or at the unsubstituted nitrogen. However, 2D-NMR experiments (HMBC, NOESY) with compound **6.24**, synthesized from building block **6.34**, i. e. isomer I, bearing the Boc-group at the terminal nitrogen, confirmed the acylation at the designated third guanidine nitrogen.

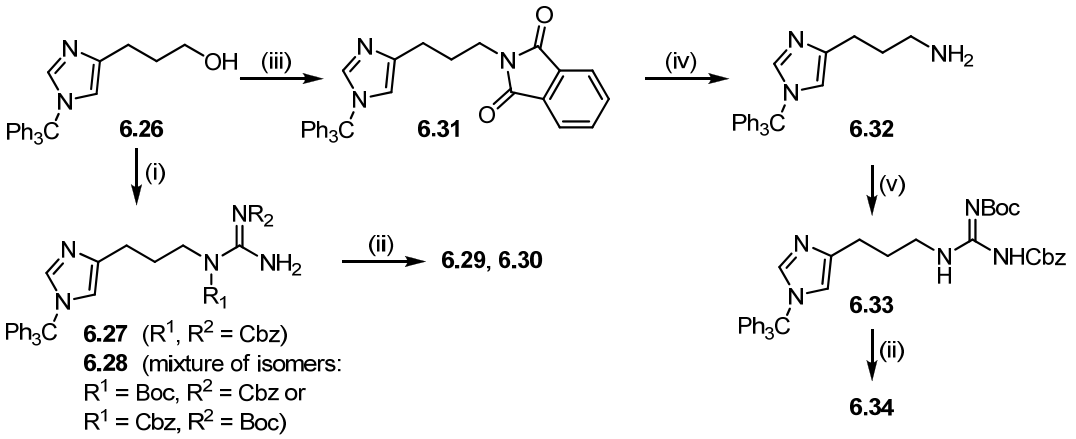
The trityl-protected imidazolylpropylguanidine **6.29** was synthesized *via Mitsunobu* reaction[10] followed by hydrogenation over Pd/C as previously described.[3] The Boc-protected guanidines **6.30** (mixture of isomers I and II) and **6.34** (isomer I, Scheme 6.1), respectively, were obtained depending on the employed procedure (Scheme 6.2). Condensation of trityl-protected imidazolylpropanol **6.26**[3, 11] with Cbz/Boc-diprotected guanidine **6.37** under *Mitsunobu* conditions[10] yielded the diurethane-protected guanidine **6.28**. Hydrogenolytic cleavage of the Cbz group provided the Boc-protected imidazolylpropylguanidine **6.30**. As the alkylation of the substituted guanidine **6.37** (Scheme 6.3) can occur alternatively at the nitrogens bearing electron-withdrawing groups (Cbz or Boc), **6.28** and **6.30** were obtained as mixtures of regioisomers. The formation of isomers was avoided by an alternative convenient route: guanidinylation of the amine **6.32** with Boc/Cbz/triflyl-protected guanidine (**6.38**) gave **6.33**, and subsequent hydrogenation afforded the designated Boc-protected imidazolylpropylguanidine **6.34** (Scheme 6.2).



No.	R^1	R^2
6.4, 6.15	H	
6.5[c], **6.16**	Boc	
6.6, 6.17	H	
6.7[c], **6.18**	Boc	
6.8, 6.19	H	
6.9[c], **6.20**	Boc	

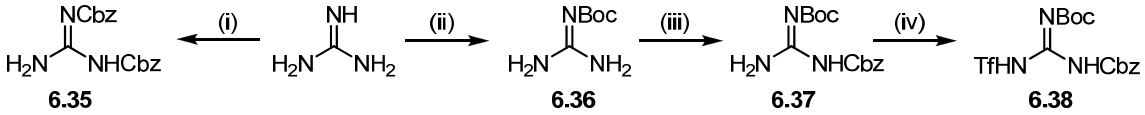
No.	R^1	R^2
6.10[c], **6.21**	Boc	
6.11[d], **6.22**[e]	Boc	CH_3
6.12[d], **6.23**[f]	Boc	CH_2CH_3
6.13[d], **6.24**[g]	Boc	$CH_2CH_2CH_3$
6.14[d], **6.25**[h]	Boc	

Scheme 6.1. Synthesis of the N^G-acylated imidazolylpropylguanidines **6.15-6.25** and **6.2** (SK&F 91486). Reagents and conditions: (i) for **6.4**, **6.6**, **6.8**: CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF, 5 h, rt; (ii) for **6.5**, **6.7**, **6.9-6.14**: EDC · HCl (1.2 eq), DMAP (1.1 eq), DCM, 24 h, 0 °C → rt; (iii) for **6.15**, **6.17**, **6.19**: TFA (20 %), DCM, 5 h, rt; (iv) for **6.2, 6.16, 6.18, 6.20-6.25**: HCl 1 M, 30 min, reflux. [b] c.f. Scheme 6.2. [c] mixture of I and II. [d] isomer I. [e] UR-PI288. [f] UR-PI294. [g] UR-PI295. [h] UR-PI287.



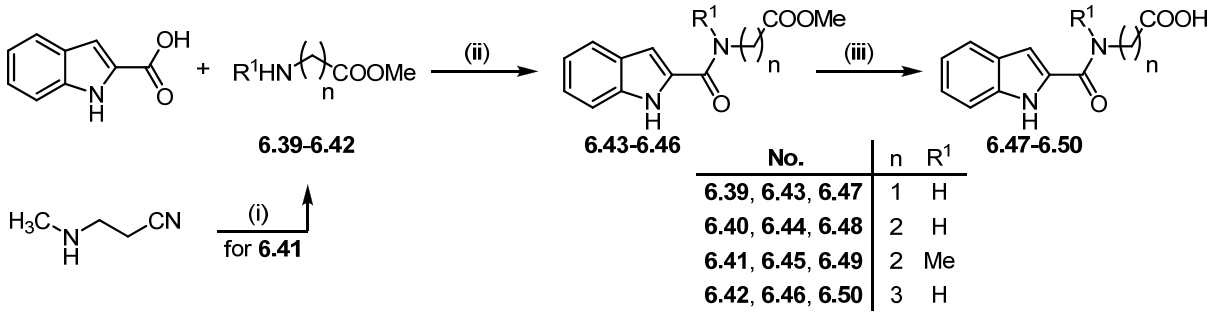
Scheme 6.2. Preparation of the imidazolylpropylguanidines **6.29**, **6.30**, **6.34**. Reagents and conditions: (i) **6.35** or **6.37** (1.75 eq), PPh_3 (1.5 eq), DIAD (1.5 eq), THF, overnight, 0 °C → rt; (ii) H_2, Pd/C (10 %) (cat.), MeOH, 3 h, rt; (iii) phthalimide (1.5 eq), PPh_3 (1.5 eq), DIAD (2 eq), THF, 1 h, 0 °C → rt; (iv) $N_2H_4 \cdot H_2O$ (6.4 eq), *n*-BuOH, 1 h, reflux; (v) **6.38** (0.9 eq), NEt_3 (1 eq), DCM, overnight, rt.

Preparation of the guanidinylation reagents employed in Scheme 6.2 was achieved by successive introduction of the protective groups starting from guanidine as described by Feichtinger et al. (Scheme 6.3).[12]



Scheme 6.3. Preparation of the guanidinylation reagents **6.35-6.38**. Reagents and conditions: (i) benzyl chloroformate (3 eq), NaOH (5 eq), H_2O/DCM, 20 h, 0 °C; (ii) NaOH (2 eq), Boc_2O (0.8 eq), 1,4-dioxane/H_2O, overnight, 0 °C → rt; (iii) benzyl succinimidyl carbonate (1 eq), DMF, overnight, rt; (iv) Tf_2O (1 eq), NaH (60 % dispersion in mineral oil) (2 eq), chlorobenzene, overnight, - 45 °C → rt.

Not commercially available carboxylic acids (**6.47-6.50**) were synthesized as outlined in Scheme 6.4. EDC-activated 1*H*-indole-2-carboxylic acid was coupled to the pertinent amino acid methyl ester followed by saponification of the ester with LiOH in EtOH. Treatment of 3-(methylamino)propanenitrile with HCl in MeOH provided the corresponding methyl ester **6.36**.[13]



No.	n	R^1
6.39, **6.43**, **6.47**	1	H
6.40, **6.44**, **6.48**	2	H
6.41, **6.45**, **6.49**	2	Me
6.42, **6.46**, **6.50**	3	H

Scheme 6.4. Preparation of the carboxylic acids **6.47-6.50**. Reagents and conditions: (i) HCl (gas), Na_2SO_4, MeOH, 5 h, rt; (ii) EDC · HCl (1.2 eq), DMAP (1.3 eq), DCM, 24 h, 0 °C → rt; (iii) $LiOH \cdot H_2O$ (2 eq), EtOH, 20 h, rt.

6.3 Pharmacological results and discussion

The synthesized compounds were investigated for agonism and antagonism at the four human histamine receptor subtypes in steady-state GTPase assays using membrane preparations of Sf9 insect cells expressing the hH_1R plus RGS4 (regulator of G-protein signaling), the hH_2R-$G_{s\alpha S}$ fusion protein, the hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4, or the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ (Table 6.1).[3, 14, 15] In addition, selected compounds were investigated at the guinea pig ileum and the guinea pig right atrium, respectively, for activity at gpH_1R and gpH_2R.

6.3.1 Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase activity assay

The reference acylguanidine UR-AK24 was similarly potent as histamine at the hH_4R,[3] had high affinity but low efficacy at the hH_3R, was about 35 times more potent than histamine at the hH_2R and a weak antagonist at the hH_1R. SK&F 91486, which is considered as essential moiety for agonistic activity of such acylguanidine-type H_2R agonists,[3] was about half as potent as histamine at the hH_2R and acted as partial agonist (EC_{50} = 2,600 nM, E_{max} = 0.66). This is in accordance with previous findings at the guinea pig right atrium (H_2R).[4] At the hH_3R and hH_4R, SK&F 91486 showed similarly high potencies (EC_{50} ≈ 8 nM) and efficacies of ≈ 0.7 to 0.8, whereas the compound was almost ineffective at the hH_1R. These results suggest the imidazolylpropylguanidine moiety to be a suitable agonist structure not just at the hH_2R, but also at the hH_3R and hH_4R.

Most of the new acylguanidines (**6.15-6.25**) listed in Table 6.1, except compounds **6.17** and **6.21**, which had negligible efficacy if any, proved to be hH_4R agonists achieving low nanomolar potencies. However, the two groups of compounds, **6.15-6.21** and **6.22-6.25**, differed in particular in terms of efficacy and hH_4R selectivity. In compounds **6.15-6.21**, the guanidine group of SK&F 91486 was acylated with an indole-3-alkanoyl or indole-2-carbonyl residue reminiscent of the core structure of the selective hH_4R antagonist JNJ 7777120.[5] Compared to SK&F 91486, the acylation of the guanidine group with indole-3-acetic or -propanoic acid (**6.15**, **6.16**) substantially increased potency at the hH_2R and thereby produced diminished discrimination between the hH_2R and hH_4R. Agonistic efficacy at the hH_3R was abolished. In compounds **6.17-6.21**, indole-2-carboxylic acid is attached to the imidazolylpropylguanidine portion either directly (**6.17**) or *via* amino acid linkers (**6.18-6.21**). The amino acid spacers were considered as “ring-opened equivalents” to the piperazine ring in JNJ 7777120, and the basic tertiary amine, which is regarded as crucial for H_4R affinity of JNJ 7777120,[16] may be mimicked by the acylguanidine group. Direct attachment of the indole-2-carboxylic acid (**6.17**) significantly changed the pharmacological profile at H_xRs

relative to the reference compound SK&F 91486. Compound **6.17** showed moderate partial agonism at hH_1R (EC_{50} = 1,700 nM, E_{max} = 0.42). At hH_2R, **6.17** was about 10 times more potent than SK&F 91486 and showed slightly elevated efficacy (EC_{50} = 290 nM, E_{max} = 0.87). In contrast, at the hH_3R and hH_4R, **6.17** was > 10 times less potent and efficacy substantially decreased. The incorporation of a glycine spacer (**6.18**) was not tolerated in terms of hH_2R activity; compared to **6.17** efficacy and potency remarkably dropped (EC_{50} = 950 nM, E_{max} = 0.51). At the hH_3R and hH_4R, **6.18** was more potent than compound **6.17** and displayed partial agonistic activity at both receptors. Replacing the glycine spacer with β-alanine (**6.19**) and 4-aminobutyric acid (**6.21**) increased potency at the hH_2R by a factor of 10 (EC_{50} = 83 nM) and 30 (EC_{50} = 30 nM), respectively. Obviously, the increased flexibility of the higher homologs favored high hH_2R potency. The hH_3R and hH_4R affinities remained largely unaffected, whereas the efficacies at these receptors were significantly reduced. At all hH_xR, especially at the hH_2R, *N*-methylation of the amide nitrogen (**6.20**) increased efficacies, but the potencies at the hH_2R and hH_4R (EC_{50} values: 350 nM and 150 nM) were reduced, suggesting the amide NH group to contribute to hH_2R and hH_4R binding, although steric factors may also play a role. In contrast, at the hH_3R, **6.20** was more potent as a partial agonist than the unmethylated analog **6.19**. Taken together, the hybrid approach, combining hH_4R agonistic (SK&F 91486) and antagonistic (JNJ 7777120) structural elements, was unsuccessful with respect to improving the hH_4R selectivity.

The second approach to increase selectivity for the hH_4R, in particular over the hH_2R, was focused on the introduction of small N^G-alkanoyl residues instead of the larger arylalkanoyl groups found to be useful in H_2R agonists.[3] This idea was stimulated by the fact that the natural agonist, histamine, is 100 times more potent at hH_4R than hH_2R (EC_{50} values: 12 nM vs. 1200 nM). Obviously, additional affinity-conferring substituents are not required to achieve low nanomolar hH_4R potencies. Acetylation of the guanidine group yielding **6.22** (UR-PI288) provided a moderate increase in potency and efficacy (EC_{50} = 730 nM, E_{max} = 0.76) relative to SK&F 91486 at the hH_2R. As expected, the potencies of the acylguanidines at the hH_2R further increased (EC_{50} = 730 nM → EC_{50} = 110 nM) with the extension of the alkanoyl residue (**6.23**, UR-PI294; **6.24**, UR-PI295; **6.25**, UR-PI287), whereas the efficacy remained unaffected. However, with the small N^G-alkanoyl residues in **6.22-6.25** the hH_2R agonistic potency was kept considerably lower than with larger arylalkanoyl groups.[3] The N^G-alkanoylguanidines **6.22-6.25** were very weak partial agonists at the hH_1R. Regarding the hH_3R, acetylation of the guanidine group in SK&F 91486 slightly increased potency, but substantially decreased efficacy (EC_{50} = 3.6 nM, E_{max} = 0.27). Likewise, substitution of the guanidine group with larger alkanoyl residues was poorly tolerated in terms of hH_3R efficacy. Obviously, acylation of the guanidine group modifies the interaction of the imidazolylpropyl-guanidine moiety with the hH_3R in a manner that impedes the stabilization of an active

receptor conformation. Contrary to the structure-activity relationships at the hH_3R, the alkanoyl residue was beneficial for the activation of the hH_4R. The N^G-alkanoyl imidazolylpropylguanidines **6.22-6.25** were highly potent and (almost) full agonists at the hH_4R (EC_{50} values: 3.0 – 4.9 nM, E_{max}: 0.90 – 1.00). This demonstrates that acylation of the guanidine group in SK&F 91486 is a successful way to shift the efficacy to the hH_4R at the expense of the hH_3R agonism.

To visualize the impact of the size and lipophilicity of the acyl group on the potency at the different HR subtypes, the clogP values of the compounds bearing small alkanoyl residues (**6.22-6.25**) were calculated and plotted against their pEC_{50} values. As shown in Figure 6.2, concerning the hH_2R, the potencies of the compounds tended to increase with their lipophilicity, suggesting hydrophobic interactions of the alkyl chains with amino acid residues of the hH_2R. Contrary to the potencies, the efficacies at the hH_2R were not remarkably affected (E_{max}: 0.76 – 0.86). A similar H_2R structure-activity relationship was observed for **6.22-6.25** in functional studies using isolated, spontaneously beating guinea pig right atria (c.f. 6.3.2). This confirms the observation from guanidine-type H_2R agonists that the imidazolylpropylguanidine part is crucial for agonistic activity, whereas the second substituent at the guanidine moiety is necessary as affinity-conferring moiety.[17, 18] The potencies at the hH_3R and hH_4R were almost independent of compound lipophilicity. Obviously, in contrast to the hH_2R, larger N^G-acyl substituents are not required for gaining additional affinity for the $hH_{3/4}Rs$. Therefore, acylation of the guanidine moiety with small residues enables a shift of selectivity from the hH_2R toward the hH_3R and hH_4R.

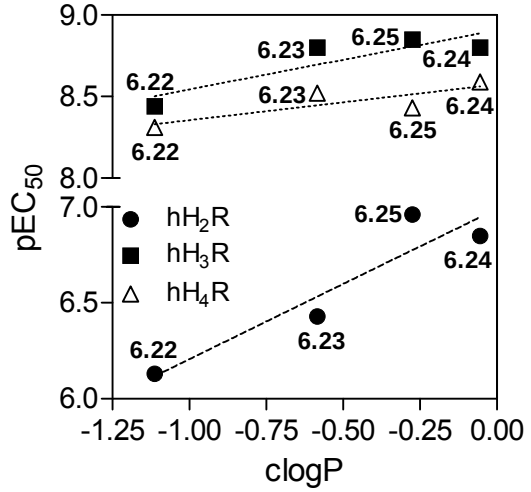


Figure 6.2. Relation between the pEC_{50} values of the N^G-alkanoyl imidazolylpropylguanidines **6.22-6.25** at the hH_2R, hH_3R and hH_4R and their clogP values (calculated with ChemDraw Ultra 10.0, CambridgeSoft, Cambridge, UK). The lines, representing the trend, were obtained from linear regression (hH_2R: slope, 0.783 ± 0.208; r^2, 0.877; p = 0.0636. hH_3R: slope, 0.364 ± 0.139; r^2, 0.774; p = 0.120. hH_4R: slope, 0.219 ± 0.104; r^2, 0.688; p = 0.170).

Table 6.1. Potencies and efficacies of the prepared compounds at the hH_1R, hH_2R, hH_3R and hH_4R in the steady-state GTPase assay.[a]

Compound	hH_1R			hH_2R			hH_3R			hH_4R		
	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n	EC_{50} (K_B) [nM]	E_{max}	n
histamine	190 ± 8.6	1.00		1,200 ± 300	1.00		25 ± 3.1	1.00	3	12 ± 2.5[19]	1.00	8
thioperamide	-	-		-	-		97 ± 18[20]	-0.71 ± 0.06[20]	5	110 ± 16[19]	-0.95 ± 0.07[19]	6
6.1 (UR-AK24)[2]	(>14,000)	0.35[2]		67[2]	0.87[2]		2.5 ± 0.65	0.24 ± 0.02	2	15 ± 0.3	0.84 ± 0.06	2
6.2 (SK&F 91486)	(>10,000)	n.d.	2	2,600 ± 63	0.66 ± 0.02	3	7.6 ± 1.8	0.69 ± 0.04	3	8.1 ± 0.80	0.83 ± 0.01	3
6.15	(2,100 ± 610)	0.00 ± 0.08	2	36 ± 4.0	0.81 ± 0.02	2	(31 ± 8.8)	-0.15 ± 0.02	2	19 ± 6.1	0.70 ± 0.12	3
6.16	(3,900 ± 14)	0.09 ± 0.02	2	82 ± 5.9	0.77 ± 0.02	2	(27 ± 9.1)	-0.18 ± 0.02	2	7.1 ± 2.8	0.45 ± 0.08	3
6.17	1,700 ± 110	0.42 ± 0.00	2	290 ± 11	0.87 ± 0.00	2	(110 ± 10)	-0.38 ± 0.01	2	(370 ± 14)	0.21 ± 0.03	2
6.18	n.d.	n.d.		950 ± 350	0.51 ± 0.01	3	16 ± 0.10	0.45 ± 0.03	2	28 ± 13	0.71 ± 0.05	3
6.19	n.d.	n.d.		83 ± 0.70	0.33 ± 0.04	2	(67 ± 7.3)	0.10 ± 0.02	2	33 ± 9.0	0.37 ± 0.02	2
6.20	n.d.	n.d.		350 ± 60	0.68 ± 0.04	3	11 ± 2.0	0.30 ± 0.01	2	150 ± 23	0.52 ± 0.06	4
6.21	(11,000 ± 120)	0.16 ± 0.08	2	30 ± 7.6	0.51 ± 0.01	2	(67 ± 9.4)	-0.18 ± 0.02	2	(96 ± 12)	-0.06 ± 0.14	2
6.22 (UR-PI288)	13,000 ± 800	0.24 ± 0.02	2	730 ± 60	0.76 ± 0.02	2	3.6 ± 1.2	0.27 ± 0.02	3	4.9 ± 0.40	1.00 ± 0.02	3
6.23 (UR-PI294)	3,500 ± 1,200	0.30 ± 0.01	2	370 ± 23	0.83 ± 0.08	2	1.6 ± 0.22	0.39 ± 0.03	3	3.0 ± 0.30	0.90 ± 0.02	3
6.24 (UR-PI295)	2,200 ± 290	0.33 ± 0.03	2	140 ± 9.1	0.77 ± 0.02	2	1.6 ± 0.24	0.37 ± 0.00	3	2.6 ± 0.10	0.96 ± 0.03	3
6.25 (UR-PI287)	2,600 ± 250	0.35 ± 0.01	2	109 ± 34	0.86 ± 0.01	2	1.4 ± 0.58	0.26 ± 0.02	3	3.7 ± 0.20	0.94 ± 0.04	3

[a] Steady-state GTPase activity in Sf9 membranes expressing the hH_1R + RGS4, hH_2R-$G_{s\alpha S}$, hH_3R + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ + RGS4 or hH_4R-RGS19 fusion protein + $G_{i\alpha 2}$ + $G_{\beta 1\gamma 2}$ was determined as described under *Pharmacological methods*. Reaction mixtures contained ligands at concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Typical basal GTPase activities in the hH_1R assay ranged between ≈ 1.0 and 1.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 100 to 150 % above basal. Typical basal GTPase activities in the hH_2R assay ranged between ≈ 1.0 and 2.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (100 µM) amounted to 250 to 300 % above basal. Typical basal GTPase activities in the hH_3R assay ranged between ≈ 3.0 and 4.5 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to ≈ 60 % above basal. Typical basal GTPase activities in the hH_4R assay ranged between ≈ 2.5 and 3.0 pmol·mg^{-1}·min^{-1}, and the maximal stimulatory effect of histamine (10 µM) amounted to 60 to 70 % above basal. n gives the number of independent experiments performed in duplicates each. For compounds investigated in the antagonist mode (K_B values), E_{max} values were determined at a concentration of 10 µM. n.d.: not determined.

6.3.2 Potencies and efficacies of selected compounds at the guinea pig ileum (gpH_1R) and guinea pig right atrium (gpH_2R)

Table 6.2. Pharmacological activities of selected compounds at the guinea pig ileum (gpH_1R) and the guinea pig right atrium (gpH_2R).

compound	gpH_1R		gpH_2R		
	pA_2	n[a]	pEC_{50}[b]	E_{max}[c]	n[a]
histamine	-	-	6.00 ± 0.10	1.00 ± 0.02	> 50
6.1 (UR-AK24)[3]	5.87 ± 0.14	4	7.80 ± 0.07	0.99 ± 0.02	4
6.15	7.41 ± 0.04	39	6.74 ± 0.13	0.91 ± 0.04	4
6.16	6.40 ± 0.05	11	7.11 ± 0.10[d]	1.01 ± 0.01	3
6.20	5.52 ± 0.07	12	5.45 ± 0.18	0.84 ± 0.04	4
6.21	6.13 ± 0.03	8	6.31 ± 0.16	0.90 ± 0.04	3
6.22 (UR-PI288)	5.07 ± 0.09	6	6.09 ± 0.09[d]	0.83 ± 0.01	3
6.23 (UR-PI294)	5.29 ± 0.09	12	6.52 ± 0.09[d]	0.94 ± 0.03	3
6.24 (UR-PI295)	5.45 ± 0.07	10	6.73 ± 0.17[d]	0.97 ± 0.01	4
6.25 (UR-PI287)	5.11 ± 0.08	12	6.96 ± 0.04[d]	0.98 ± 0.04	3

[a] number of experiments, [b] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$. Summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, n = 16). The SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50}, [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine, [d] pA_2 of cimetidine (10 μM), n = 3: 6.44 ± 0.04 (agonist **6.22**), 6.46 ± 0.02 (**6.23**), 6.39 ± 0.09 (**6.24**), 6.52 ± 0.02 (**6.25**), n = 2: 6.36 ± 0.20 (**6.16**).

The investigated N^G-acylated imidazolylpropylguanidines usually acted as weak to moderate H_1R antagonists at the guinea pig ileum (Table 6.2). Species-dependent differences were most striking for compound **6.15**, which turned out to be a fairly potent gpH_1R antagonist with a pA_2 value of 7.41, but displayed just poor antagonistic activity at the hH_1R in the GTPase assay.

At the guinea pig right atrium, all evaluated compounds bearing an indole residue (**6.15**, **6.16**, **6.20**, **6.21**) turned out to be partial to full H_2R agonists. However, in comparison to the parent compound UR-AK24 the potencies of the compounds were reduced as also found in the hH_2R GTPase assay. In particular for **6.20**, containing an indole-2-carboxylic acid, linked to the guanidine group *via* an *N*-methylated β-alanine spacer, potency dropped by more than two orders of magnitude. Otherwise, **6.21** demonstrates that a rather extended acyl residue is also tolerated in terms of gpH_2R agonistic activity. The alkanoyl analogs **6.22-6.25** exerted a similar structure-activity relationship at the gpH_2R as at the human isoform, i.e., the potency increased with size and lipophilicity of the alkanoyl residue, confirming the observation that the N^G-substituent in acylguanidine-type and guanidine-type H_2R agonists is

crucial for gaining gpH_2R affinity.[3, 17, 18] In the presence of the H_2R antagonist cimetidine the concentration-response curves of the compounds **6.22-6.25** were shifted rightward and the calculated pA_2 values for cimetidine ($pA_2 \approx 6.4$) were in accordance with reference data for antagonism of cimetidine against histamine at the gpH_2R.[21]

6.3.3 Summary and conclusion

Starting from the potent nonselective acylguanidine-type hH_4R agonist UR-AK24, which was initially designed and synthesized as an H_2R agonist, the parent compound SK&F 91486 (weak partial agonist at the H_2R[4]) was identified as a highly potent hH_3R and hH_4R partial agonist. With the aim to increase the selectivity of the acylguanidine-type compounds for the hH_4R, two distinct strategies were explored.

In the first approach the guanidine group in SK&F 91486 was acylated with indolealkanoic and indole-2-carboxylic acid moieties as structural motifs derived from the selective and potent hH_4R antagonist JNJ 7777120. Depending on the residues and amino acid spacers, compounds with varying potencies and efficacies (GTPase assay) at the different histamine receptor subtypes were obtained. Obviously, the indole substructures were not suitable to confer additional affinity by interaction with the binding site of JNJ 7777120, as neither potency nor selectivity for the hH_4R were substantially improved. An explanation therefore may be provided by an hH_4R homology model, suggesting both histamine and JNJ 7777120 to mainly interact with Asp-94 of TM3 and Glu-182 of TM5.[16] Presumably, as previously described for the H_2R,[3, 22] histamine and N^G-acylated imidazolylpropylguanidines also predominantly interact with identical amino acid residues at the hH_4R, resulting in overlapping binding sites of the imidazolylpropylguanidine group and JNJ 7777120.

In the second approach small alkanoyl residues were attached to SK&F 91486 (**6.22-6.25**). With increasing lipophilicity of the alkanoyl residues, potencies at the hH_2R increased, too, confirming the importance of the acyl group as an affinity-conferring moiety at the hH_2R. At the hH_3R, acylation drastically lowered efficacy, whereas the same compounds turned out to be potent and full (or nearly full) hH_4R agonists. Thus, although the imidazolylpropyl-guanidine portion is capable of stimulating hH_2R, hH_3R and hH_4R, selectivity can be achieved by appropriate N^G-acylation.

A major advantage of the test systems applied in this study is that for any given H_xR subtype, we used an identical read-out, namely steady-state GTP hydrolysis. This is a very proximal read-out in G-protein-mediated signal transduction, reducing bias of agonist evaluation usually introduced by downstream measurements of second messenger generation, changes in cell function or gene transcription. Thus, the receptor profiles described herein for acylguanidines reflect true differences in pharmacology and not differences in read-out between various receptors.

The hH_4R agonists **6.22-6.25** belong to the most potent hH_4R agonists reported up to now and may become useful experimental tools in addition to selective hH_4R antagonists like JNJ 7777120 to analyze the as yet incompletely understood (patho)physiological functions of the H_4R. In most immune cells like mast cells or eosinophils, where the H_4R is mainly located, H_3Rs are not expressed.[23-25] Therefore, based on the > 100-fold selectivity over the hH_1R and hH_2R subtypes, N^G-alkanoyl imidazolylpropylguanidines like **6.22** may also be applicable for investigating the function of the hH_4R in hH_3R deficient immune cells. Moreover, these potent hH_4R agonists may be suitable pharmacological probes for desensitization studies with the hH_4R.

Taken together, as previously observed, from the medicinal chemistry point of view, the imidazolylpropylguanidine scaffold may be considered a “privileged structure” providing ligands for several histamine receptor subtypes.[3] However, as demonstrated in the present study, potencies, efficacies and receptor selectivities of the imidazolylpropylguanidines can be triggered by variation of the N^G-acyl substituent.

6.4 Experimental section

6.4.1 Chemistry

6.4.1.1 General conditions

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), IRIS Biotech GmbH (Marktredwitz, Germany), Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany), TCI Europe (Zwijndrecht, Belgium) and used without further purification. Deuterated solvents for NMR spectroscopy were from Deutero GmbH (Kastellaun, Germany). All solvents were of analytical grade or distilled prior to use. If moisture-free conditions were required, reactions were performed in dried glassware under inert atmosphere (argon or nitrogen). Anhydrous DMF was purchased from Sigma-Aldrich Chemie GmbH. Flash chromatography was performed on silica gel (Merck silica gel 60, 40 - 63 µM). Reactions were monitored by TLC on aluminum plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm). The compounds were detected by UV light (254 nm), a 0.3 % solution of ninhydrine in *n*-butanol (amines) or a 1.0 % solution of Fast Blue B salt (Sigma-Aldrich Chemie GmbH) in $EtOH/H_2O$ = 30/70 (v/v). All melting points are uncorrected and were measured on a Büchi 530 apparatus (Büchi GmbH, Essen, Germany).

Nuclear Magnetic Resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded with Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz) or Bruker Avance 600 (^{1}H: 600.1 MHz; ^{13}C: 150.9 MHz) NMR spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany). Chemical shifts are given in δ (ppm) relative to external standards. Abbreviations for the

multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sep (septet), m (multiplet), brs (for broad singlet) and combinations thereof. In certain cases 2D-NMR techniques (HSQC, HMBC, NOESY) were used to assign ^{1}H and ^{13}C chemical shifts. Infrared spectra (IR) were measured on a Bruker Tensor 27 spectrometer equipped with an ATR (attenuated total reflexion) unit from Harrick Scientific Products Inc. (Ossining/NY, US). Mass spectra (MS) were recorded on a Finnigan MAT 95 (EI-MS 70 eV, HR-MS), Finnigan SSQ 710A (CI-MS (NH_3)) and on a Finnigan ThermoQuest TSQ 7000 (ES-MS) spectrometer. The peak-intensity in % relative to the strongest signal is indicated in parenthesis. Elemental analysis (C, H, N, Elementar Vario EL, Hanau, Germany) were performed by the Analytical Department of the University Regensburg and are listed in the appendix (Chapter 9).

Preparative HPLC was performed with a pump model K-1800 (Knauer, Berlin, Germany), the column was Eurosphere 100 (250 x 32 mm) (Knauer), which was attached to a UV-detector model K-2000 (Knauer). UV detection of the compounds was performed at 210 nm. The temperature was 25 °C and the flow rate 37 ml/min. The mobile phase was 0.1 % TFA in millipore water and MeCN. Analytical HPLC was performed on a system from Thermo Separation Products (TSP, Egelsbach, Germany) equipped with an SN 400 controller, P4000 pump, an AS3000 autosampler and a Spectra Focus UV/VIS detector. Stationary phase was a Eurosphere-100 C-18 (250 x 4.0 mm, 5 µm) column (Knauer) thermostated at 30 °C. The flow rate was 0.8 ml/min and the dead time (t_0) was 3.32 min. As mobile phase gradients of MeCN/0.05 % TFA (aq.) were used and the absorbance was detected at 210 nm. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 210 nm. HPLC conditions, retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and purities of the target compounds are listed in the appendix (Chapter 9).

6.4.1.2 Preparation of 1-(3-(1H-imidazol-4-yl)propyl)guanidine 6.2 (SK&F 91486)[26]

A suspension of **6.34** (600 mg, 1.18 mmol) in HCl (1 M, 30 mL) was refluxed for 1 h. After removing insoluble material by filtration, the solvent was evaporated. The obtained residue was recrystallized from EtOAc/EtOH yielding a white solid (162 mg, 59 %). mp (**6.2** · 2 HCl) 149 – 150 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, dihydrochloride): δ [ppm] = 1.76 – 1.91 (m, 2H), 2.71 (t, 2H, ^3J = 7.5 Hz), 3.09 – 3.21 (m, 2H), 7.33 (brs, 4H), 7.47 (d, 1H, ^4J = 1.3 Hz), 8.12 (t, 1H, ^3J = 5.7 Hz), 9.03 (d, 1H, ^4J = 1.3 Hz), 14.60 (brs, 2H). ^{13}C-NMR (75 MHz, DMSO-d_6, dihydrochloride): δ [ppm] = 20.94, 27.26, 39.57, 115.49, 132.24, 133.46, 157.07. IR (cm^{-1}) = 3112, 2989, 2901, 1662, 1616, 1096. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 168 (100) [M + H]$^+$. HRMS (EI-MS) calcd. for $C_7H_{13}N_5$ [$M^{+\bullet}$] 167.1171; found 167.1166. Anal. ($C_7H_{13}N_5$ · 2 HCl · 0.5 H_2O) C, H, N. $C_7H_{13}N_5$ · 2 HCl (240.13).

6.4.1.3 Preparation of the trityl-protected N^G-acylated imidazolylpropyl-guanidines 6.4, 6.6 and 6.8

General procedure

A solution of the pertinent carboxylic acid (1 eq) and CDI (1.2 eq) in THF_{abs} (15 mL) was stirred for 1 h under argon atmosphere at room temperature. Parallel to this, NaH (60 % dispersion in mineral oil) (2 eq) was added to a solution of **6.29** (1 eq) in THF_{abs} (15 mL) under argon atmosphere, stirred for 45 min at 30 – 35 °C and allowed to cool to ambient temperature. Both mixtures were united and stirred for 5 h under argon atmosphere. EtOAc (50 mL) was added and the organic phase was washed with H_2O (3 x 20 mL) and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by flash chromatography.

N^1-[2-(1*H*-Indol-3-yl)acetyl]-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.4)

The title compound was prepared from 2-(1*H*-indol-3-yl)acetic acid (350 mg, 2.0 mmol), CDI (390 mg, 2.4 mmol), NaH (60 % dispersion in mineral oil) (160 mg, 4.0 mmol) and **6.29** (819 mg, 2.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a pale yellow foam-like solid (530 mg, 47 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.74 – 1.90 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.51 (t, 2H, ^3J = 6.0 Hz, Im-4-C***H***$_2$), 3.50 (t, 2H, ^3J = 6.4 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.92 (s, 2H, indole-4-C***H***$_2$), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.00 – 7.46 (m, 20H, Ph-***H*** + indole-***H*** + Im-2-***H***), 7.66 (d, 1H, ^3J = 7.7 Hz, indole-4-***H***), 11.57 (indole-N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 567 (100) $[M + H]^+$. $C_{36}H_{34}N_6O$ (566.69).

N^1-(1*H*-Indole-2-carbonyl)-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.6)

The title compound was prepared from 1*H*-indole-2-carboxylic acid (322 mg, 2.0 mmol), CDI (390 mg, 2.4 mmol), NaH (60 % dispersion in mineral oil) (160 mg, 4.0 mmol) and **6.29** (819 mg, 2.0 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH/NH_3 (aq.) 32 % 95/3/2 v/v/v) yielded a colorless foam-like solid (600 mg, 54 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.83 – 2.00 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.79 (t, 2H, ^3J = 5.9 Hz, Im-4-C***H***$_2$), 3.51 (t, 2H, ^3J = 6.5 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.59 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 7.04 – 7.17 (m, 7H, Ar-***H***), 7.20 – 7.42 (m, 13H, Ar-***H***), 7.66 (d, 1H, ^3J = 8.3 Hz, indole-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 553 (100) $[M + H]^+$. $C_{35}H_{32}N_6O$ (552.67).

N^1-[3-(1*H*-Indole-2-carbonylamino)propanoyl]-N^2-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.8)

The title compound was prepared from **6.48** (464 mg, 2.0 mmol), CDI (390 mg, 2.4 mmol), NaH (60 % dispersion in mineral oil) (160 mg, 4.0 mmol) and **6.29** (819 mg, 2.0 mmol)

according to the general procedure. The precipitated product was filtered, washed with THF (2 x 5 mL) and used without further purification (white solid, 842 mg, 67 %); mp 214 – 215 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.62 – 1.83 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.32 – 2.55 (m, 4H, overlap with solvent, Im-4-C***H***$_2$ + indole-2-CO-NH-CH_2-C***H***$_2$), 3.01 – 3.27 (m, 2H, indole-2-CO-NH-C***H***$_2$), 3.42 – 3.56 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 6.63 (s, 1H, Im-5-***H***), 6.95 – 7.49 (m, 20H, Ph-***H*** + Im-2-***H***, indole-***H***), 7.57 (d, 1H, ^3J = 7.9 Hz, indole-***H***), 11.57 (s, 1H, indole-N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 624 (100) $[M + H]^+$. $C_{38}H_{37}N_7O_2$ (623.75).

6.4.1.4 Preparation of the Boc/trityl-protected N^G-acylated imidazolylpropyl-guanidines 6.5, 6.7 and 6.9-6.14

General procedure

To a solution of the pertinent carboxylic acid (1 eq) and Boc-protected guanidine **6.30** or **6.34** (1 eq) in DCM (20 mL), EDC · HCl (1.2 eq) and DMAP (1.1 eq) were added at 0 °C. After stirring for 4 h at 0 °C, the solution was allowed to warm to ambient temperature and stirred for additional 20 h. DCM (20 mL) was added and the organic phase was washed with water and brine and dried over Na_2SO_4. The solvent was evaporated and the crude product purified by flash chromatography.

N^1-(*tert*-Butoxycarbonyl)-N^2-[3-(1*H*-indol-3-yl)propanoyl]-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.5)

The title compound was prepared from 3-(1*H*-indol-3-yl)propanoic acid (189 mg, 1.0 mmol), **6.30** (510 mg, 1.0 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless foam-like solid (350 mg, 51 %). ^{1}H-NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9H, C(C***H***$_3$)$_3$), 1.72 – 1.89 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.45 (t, 2H, ^3J = 7.1 Hz, Im-4-C***H***$_2$), 2.56 (t, 2H, ^3J = 7.5 Hz, indole-3-CH_2-C***H***$_2$), 2.92 (t, 2H, ^3J = 7.5 Hz, indole-3-C***H***$_2$), 3.86 (t, 2H, ^3J = 7.1 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.60 (s, 1H, Im-5-***H***), 6.83 – 7.55 (m, 21H, Ph-***H*** + indole-***H*** + Im-2-***H***), 10.72 (indole-N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 681 (100) $[M + H]^+$. $C_{42}H_{44}N_6O_3$ (680.84).

N^1-(*tert*-Butoxycarbonyl)-N^2-[2-(1*H*-indole-2-carbonylamino)acetyl]-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.7)

The title compound was prepared from **6.47** (164 mg, 0.75 mmol), **6.30** (382 mg, 0.75 mmol), EDC · HCl (173 mg, 0.9 mmol) and DMAP (101 mg, 0.83 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a

colorless oil (200 mg, 38 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.48 (s, 9H, C(C***H***$_3$)$_3$), 1.91 – 2.04 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.41 – 2.65 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 3.42 – 3.52 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 4.18 (d, 1.6H, ^3J = 4.7 Hz, indole-2-CO-NH-C***H***$_2$), 4.28 (d, 0.4H, ^3J = 5.2 Hz, indole-2-CO-NH-C***H***$_2$), 6.57 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 6.93 – 6.96 (m, 1H, indole-***H***), 7.06 – 7.48 (m, 20H, Ph-***H*** + indole-***H*** + Im-2-***H*** + N-***H***), 7.58 – 7.70 (m, 1H, indole-7-***H***), 8.66 (t, 1H, ^3J = 5.6 Hz, N-***H***), 9.61 (brs, 1H, N-***H***), 11.86 (s, 1H, indole-N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 710 (100) $[M + H]^+$. $C_{42}H_{43}N_7O_4$ (709.84).

N^1-(*tert*-Butoxycarbonyl)-N^2-[3-(*N*-methyl-1*H*-indole-2-carboxamido)propanoyl]-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.9)

The title compound was prepared from **6.49** (246 mg, 1.0 mmol), **6.30** (510 mg, 1.0 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless foam-like solid (440 mg, 60 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.42 (s, 9H, C(C***H***$_3$)$_3$), 1.85 – 2.07 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.46 – 3.51 (m, 9H, Im-4-C***H***$_2$ + indole-2-CO-NC***H***$_3$C***H***$_2$-C***H***$_2$), 3.95 (t, 2H, ^3J = 7.3 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.60 (s, 1H, Im-5-***H***), 7.01 – 7.71 (m, 21H, Ph-***H*** + indole-***H*** + Im-2-***H***), 10.33 (brs, 1H, indole-N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 738 (100) $[M + H]^+$. $C_{44}H_{47}N_7O_4$ (737.89).

N^1-(*tert*-Butoxycarbonyl)-N^2-[4-(1*H*-indole-2-carboxamido)butanoyl]-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.10)

The title compound was prepared from **6.50** (185 mg, 0.75 mmol), **6.30** (382 mg, 0.75 mmol), EDC · HCl (173 mg, 0.9 mmol) and DMAP (101 mg, 0.83 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (280 mg, 51 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers**:** δ [ppm] = 1.47 (s, 5.3H, C(C***H***$_3$)$_3$), 1.47 (s, 3.7H, C(C***H***$_3$)$_3$), 1.82 – 2.08 (m, 4H, Im-4-CH$_2$-C***H***$_2$ + indole-2-CO-NH-CH$_2$-C***H***$_2$), 2.41 – 2.65 (m, 4H, Im-4-C***H***$_2$ + indole-2-CO-NH-(CH$_2$)$_2$-C***H***$_2$), 3.36 – 3.60 (m, 4H, Im-4-(CH$_2$)$_2$-C***H***$_2$ + indole-2-CO-NH-C***H***$_2$), 6.54 (d, 0.4H, ^4J = 1.3 Hz, Im-5-***H***), 6.58 (d, 0.6 H, ^4J = 1.3 Hz, Im-5-***H***), 6.63 (t, 0.4H, ^3J = 5.7 Hz, N***H***), 6.85 – 6.92 (m, 1H, indole-***H***), 7.03 – 7.70 (m, 20.6H, Ph-***H*** + indole-***H*** + Im-2-***H*** + N-***H***), 8.49 (t, 0.6H, ^3J = 5.6 Hz, CON-***H***), 8.92 (t, 0.4H, ^3J = 5.3 Hz, CON-***H***), 9.25 (brs, 0.6H, N-***H***), 9.69 (brs, 0.4H, N-***H***), 12.22 (s, 0.6H, indole-N-***H***), 12.50 (s, 0.4H, indole-N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 738 (100) $[M + H]^+$. $C_{44}H_{47}N_7O_4$ (737.89).

N^1-Acetyl-N^2-(*tert*-butoxycarbonyl)-N^3-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.11)

The title compound was prepared from acetic acid (60 mg, 1.0 mmol), **6.34** (510 mg, 1.00 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (360 mg, 65 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.85 – 1.99 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.16 (s, 3H, C***H***$_3$), 2.59 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.39 – 3.49 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.53 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.08 – 7.17 (m, 6H, Ph-***H***), 7.29 – 7.39 (m, 10H, Ph-***H*** + Im-2-***H***), 8.96 (t, 1H, ^3J = 5.2 Hz, N-***H***), 12.41 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 552 (100) $[M + H]^+$. $C_{33}H_{37}N_5O_3$ (551.68).

N^1-(*tert*-Butoxycarbonyl)-N^2-propionyl-N^3-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.12)

The title compound was prepared from propanoic acid (74 mg, 1.0 mmol), **6.34** (510 mg, 1.00 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (320 mg, 57 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.18 (t, 3H, ^3J = 7.5 Hz, C***H***$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.85 – 1.98 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.41 (q, 2H, ^3J = 7.5 Hz, C***H***$_2$CH$_3$), 2.60 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.39 – 3.50 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.53 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.29 – 7.38 (m, 10H, Ph-***H*** + Im-2-***H***), 9.01 (t, 1H, ^3J = 5.0 Hz, N-***H***), 12.42 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 566 (100) $[M + H]^+$. $C_{34}H_{39}N_5O_3$ (565.71).

N^1-(*tert*-Butoxycarbonyl)-N^2-butyryl-N^3-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.13)

The title compound was prepared from butyric acid (88 mg, 1.0 mmol), **6.34** (510 mg, 1.00 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (320 mg, 55 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 0.97 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.59 – 1.76 (m, 2H, C***H***$_2$CH$_3$), 1.85 – 1.98 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.35 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$CH$_2$CH$_3$), 2.60 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 3.39 – 3.50 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.53 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.17 (m, 6H, Ph-***H***), 7.29 – 7.39 (m, 10H, Ph-***H*** + Im-2-***H***), 9.01 (t, 1H, ^3J = 5.0 Hz, N-***H***), 12.40 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 580 (100) $[M + H]^+$. $C_{35}H_{41}N_5O_3$ (579.73).

N^1-(*tert*-Butoxycarbonyl)-N^2-isobutyryl-N^3-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.14)

The title compound was prepared from isobutyric acid (88 mg, 1.0 mmol), **6.34** (510 mg, 1.00 mmol), EDC · HCl (230 mg, 1.2 mmol) and DMAP (134 mg, 1.1 mmol) according to the general procedure. Purification by flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v) yielded a colorless oil (320 mg, 55 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.21 (d, 6H, ^3J = 6.9 Hz, CH(C***H***$_3$)$_2$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.85 – 1.99 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.42 – 2.66 (m, 3H, C***H*** + Im-4-C***H***$_2$), 3.39 – 3.50 (m, 2H, Im-4-(CH$_2$)$_2$-C***H***$_2$), 6.53 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.17 (m, 6H, Ph-***H***), 7.29 – 7.38 (m, 10H, Ph-***H*** + Im-2-***H***), 9.03 (t, 1H, ^3J = 5.0 Hz, N-***H***), 12.50 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 580 (100) $[M + H]^+$. $C_{35}H_{41}N_5O_3$ (579.73).

6.4.1.5 Preparation of the N^G-acylated imidazolylpropylguanidines 6.15-6.25

General procedure 1

The pertinent trityl-protected N^G-acylated imidazolylpropylguanidine was stirred for 5 h in a mixture of TFA (5.0 mL) and DCM (20 mL). After removing the solvent *in vacuo*, the crude product was purified by preparative HPLC. The solvent was removed by lyophilization and the compounds were obtained as trifluoroacetates.

General procedure 2

The pertinent Boc-trityl-protected N^G-acylated imidazolylpropylguanidine was refluxed for 30 min in HCl (1 M, 20 mL). After removing the precipitated trityl alcohol by filtration, the solvent was removed *in vacuo*. Purification of the crude product was performed by preparative HPLC. The solvent was removed by lyophilization and the compounds obtained as trifluoroacetates.

N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-[2-(1*H*-indol-3-yl)acetyl]guanidine (6.15)

The title compound was prepared from **6.4** (520 mg, 0.92 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 20/80) yielded a pale yellow solid (80 mg, 16 %); mp 69 – 73 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.91 – 2.06 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.79 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 3.34 (t, 2H, ^3J = 6.9 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 3.93 (s, 2H, indole-4-C***H***$_2$), 7.03 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.12 (ddd, 1H, ^3J = 8.1 Hz, ^3J = 7.0 Hz, ^4J = 1.2 Hz, indole-6-***H***), 7.28 (s, 1H, indole-2-***H***), 7.33 (s, 1H, Im-5-***H***), 7.37 (ddd, 1H, ^3J = 8.1 Hz, ^4J = 1.0 Hz, ^5J = 0.8 Hz, indole-7-***H***), 7.55 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.2 Hz, ^5J = 0.8 Hz, indole-4-***H***), 8.76 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 22.58 (-, Im-4-***C***H$_2$), 27.93 (-, Im-4-CH$_2$-***C***H$_2$), 35.31 (-, indole-4-***C***H$_2$), 41.63 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 106.84

(C_{quat}, indole-***C***-3), 112.58 (+, indole-***C***-7), 117.11 (+, Im-***C***-5), 119.36 (+, indole-***C***-4), 120.33 (+, indole-***C***-5), 122.91 (+, indole-***C***-6), 125.81 (+, indole-***C***-2), 128.40 (C_{quat}, indole-***C***-3a), 134.30 (C_{quat}, Im-***C***-4), 134.98 (+, Im-***C***-2), 138.20 (C_{quat}, indole-***C***-7a), 155.52 (C_{quat}, ***C***=N), 175.87 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3133, 3028, 2814, 1662, 1628, 1181, 1125. HRMS (EI-MS) calcd. for $C_{17}H_{20}N_6O$ [$M^{+\bullet}$] 324.1699; found 324.1696. $C_{17}H_{20}N_6O \cdot 2$ TFA (552.43).

N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-[3-(1*H*-indol-3-yl)propanoyl]guanidine (6.16)

The title compound was prepared from **6.5** (330 mg, 0.48 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a white solid (147 mg, 54 %); mp 54 – 57 °C. ^{1}H-NMR (600 MHz, NOESY, CD_3OD, trifluoroacetate): δ [ppm] = 1.95 – 2.05 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.80 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.84 (t, 2H, ^3J = 7.4 Hz, indole-3-CH_2-C***H***$_2$), 3.12 (t, 2H, ^3J = 7.4 Hz, indole-3-C***H***$_2$), 3.34 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.98 (ddd, 1H, ^3J = 7.9 Hz, ^3J = 7.1 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.05 – 7.09 (m, 2H, indole-6-***H*** + indole-2-***H***), 7.31 (ddd, 1H, ^3J = 8.1 Hz, ^4J = 1.0 Hz , ^5J = 0.9 Hz, indole-7-***H***), 7.34 (s, 1H, Im-5-***H***), 7.54 (ddd, 1H, 1H, ^3J = 7.9 Hz, ^4J = 1.2 Hz , ^5J = 0.9 Hz, indole-4-***H***), 8.78 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, HMBC, CD_3OD, trifluoroacetate): δ_C [ppm] = 21.34 (-, indole-3-***C***H_2), 22.54 (-, Im-4-***C***H_2), 27.93 (-, Im-4-CH_2-***C***H_2), 39.18 (-, indole-3-CH_2-***C***H_2), 41.56 (-, Im-4-$(CH_2)_2$-***C***H_2), 112.30 (+, indole-***C***-7), 114.14 (C_{quat}, indole-***C***-3), 117.13 (+, Im-***C***-5), 119.19 (+, indole-***C***-4), 119.69 (+, indole-***C***-5), 122.44 (+, indole-***C***-6), 123.34 (+, indole-***C***-2), 128.40 (C_{quat}, indole-***C***-3a), 134.30 (C_{quat}, Im-***C***-4), 134.95 (+, Im-***C***-2), 138.16 (C_{quat}, indole-***C***-7a), 155.27 (C_{quat}, ***C***=N), 177.14 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3142, 3034, 2962, 1662, 1628, 1182, 1127. HRMS (EI-MS) calcd. for $C_{18}H_{22}N_6O$ [$M^{+\bullet}$] 338.1855; found 338.1852. $C_{18}H_{22}N_6O \cdot 2$ TFA (566.45).

N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-(1*H*-indole-2-carbonyl)guanidine (6.17)

The title compound was prepared from **6.6** (500 mg, 0.90 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 20/80) yielded a white solid (120 mg, 25 %); mp 62 – 65 °C. ^{1}H-NMR (300 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 2.02 – 2.16 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.88 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 3.47 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.13 (ddd, 1H, ^3J = 8.1 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.33 (ddd, 1H, ^3J = 8.3 Hz, ^3J = 7.0 Hz, ^4J = 1.2 Hz, indole-6-***H***), 7.39 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.45 – 7.52 (m, 2H, indole-3-***H*** + indole-7-***H***), 7.68 (ddd, 1H, ^3J = 8.1 Hz, ^4J = 1.2 Hz, ^4J = 1.0 Hz, indole-4-***H***), 8.82 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 22.65 (-, Im-4-***C***H_2), 28.11 (-, Im-4-CH_2-***C***H_2), 41.83 (-, Im-4-$(CH_2)_2$-***C***H_2), 109.06 (+, indole-***C***-3), 113.51 (+, indole-***C***-7), 117.16 (+, Im-***C***-5), 122.15 (+, indole-***C***-5), 123.81 (+, indole-***C***-4), 127.34 (+, indole-***C***-6), 128.65 (C_{quat}, indole-***C***-3a), 129.10 (C_{quat}, indole-***C***-2), 134.37 (C_{quat}, Im-***C***-4), 135.07 (+, Im-***C***-2), 139.92 (C_{quat}, indole-***C***-

7a), 155.79 (C_{quat}, ***C***=N), 163.33 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3132, 2989, 1665, 1635, 1199, 1131. HRMS (EI-MS) calcd. for $C_{16}H_{18}N_6O$ [$M^{+\bullet}$] 310.1542; found 310.1541. $C_{16}H_{18}N_6O \cdot 2$ TFA (538.40).

***N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl]-*N*^{2}-[2-(1*H*-indole-2-carbonylamino)acetyl]guanidine (6.18)

The title compound was prepared from **6.7** (190 mg, 0.27 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a white solid (53 mg, 33 %); mp 85 – 89 °C. ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.78 – 1.93 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.65 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.20 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 4.14 (s, 2H, indole-2-CO-NH-***C***H_2), 7.05 – 7.13 (m, 3H, Im-5-***H*** + indole-3-***H*** + indole-5-***H***), 7.25 (ddd, 1H, ^3J = 8.2 Hz ,^3J = 7.0 Hz , ^4J = 1.1 Hz, indole-6-***H***), 7.43 (d, 1H, ^3J = 8.2 Hz, indole-7-***H***), 7.64 (d, 1H, ^3J = 8.1 Hz, indole-4-***H***), 8.44 (s, 1H, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 21.04 (-, Im-4-***C***H_2), 25.94 (-, Im-4-CH_2-***C***H_2), 40.54 (-, Im-4-$(CH_2)_2$-***C***H_2), 43.53 (-, indole-2-CO-NH-***C***H_2), 105.12 (+, indole-***C***-3), 112.38 (+, indole-***C***-7), 115.45 (+, Im-***C***-5), 120.84 (+, indole-***C***-5), 122.30 (+, indole-***C***-4), 125.16 (+, indole-***C***-6), 127.09 (C_{quat}, indole-***C***-3a), 129.42 (C_{quat}, indole-***C***-2), 132.36 (C_{quat}, Im-***C***-4), 133.02 (+, Im-***C***-2), 136.89 (C_{quat}, indole-***C***-7a), 152.72 (C_{quat}, ***C***=N), 164.35 (indole-2-***C***O), 172.37 (C_{quat}, guanidine-***C***O). IR (cm^{-1}) = 3235, 3142, 3034, 2869, 1664, 1634, 1554, 1197, 1128. HRMS (LSI-MS) calcd. for $C_{18}H_{22}N_7O_2$ [M + H]$^+$ 368.1829; found 368.1831. $C_{18}H_{21}N_7O_2 \cdot 2$ TFA (595.45).

***N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl]-*N*^{2}-[3-(1*H*-indole-2-carbonylamino)propanoyl]guanidine (6.19)

The title compound was prepared from **6.8** (480 mg, 0.77 mmol) according to the general procedure 1. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a beige solid (15 mg, 3 %); mp 63 – 67 °C. ^{1}H-NMR (600 MHz, CD_3OD, trifluoroacetate): δ [ppm] = 1.98 – 2.06 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.80 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.85 (t, 2H, ^3J = 6.4 Hz, indole-2-CO-NH-CH_2-C***H***$_2$), 3.39 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.75 (t, 2H, ^3J = 6.4 Hz, indole-2-CO-NH-C***H***$_2$), 7.05 (d, 1H, ^4J = 0.8 Hz, indole-3-***H***), 7.06 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.2 Hz, ^4J = 0.9 Hz, indole-5-***H***), 7.22 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.2 Hz, ^4J = 1.1 Hz, indole-6-***H***), 7.33 (s, 1H, Im-5-***H***), 7.43 (dd, 1H, ^3J = 8.2 Hz, ^4J = 0.9 Hz, indole-7-***H***), 7.59 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.1 Hz, ^4J = 0.8 Hz, indole-4-***H***), 8.79 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, HSQC, HMBC, CD_3OD, trifluoroacetate): δ [ppm] = 22.51 (-, Im-4-***C***H_2), 27.97 (-, Im-4-CH_2-***C***H_2), 36.07 (-, indole-2-CO-NH-***C***H_2), 38.08 (-, indole-2-CO-NH-CH_2-***C***H_2), 41.65 (-, Im-4-$(CH_2)_2$-***C***H_2), 104.64 (+, indole-***C***-3), 113.08 (+, indole-***C***-7), 117.10 (+, Im-***C***-5), 121.26 (+, indole-***C***-5), 122.79 (+, indole-***C***-4), 125.20 (+, indole-***C***-6), 128.97

(C_{quat}, indole-***C***-3a), 131.94 (C_{quat}, indole-***C***-2), 134.29 (C_{quat}, Im-***C***-4), 134.94 (+, Im-***C***-2), 138.37 (C_{quat}, indole-***C***-7a), 155.24 (C_{quat}, ***C***=N), 164.42 (C_{quat}, indole-2-***C***O), 175.48 (C_{quat}, guanidine-***C***O). IR (cm^{-1}) = 3274, 3152, 2986, 1668, 1623, 1557, 1181, 1126. HRMS (LSI-MS) calcd. for $C_{19}H_{24}N_7O_2$ [$M^{+\bullet}$] 382.1986; found 382.1993. $C_{19}H_{23}N_7O_2 \cdot 2$ TFA (609.48).

***N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl]-*N*^{2}-[3-(*N*-methyl-1*H*-indole-2-carbonylamino)propanoyl]guanidine (6.20)**

The title compound was prepared from **6.9** (400 mg, 0.54 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a pale pink solid (215 mg, 64 %); mp 89 – 93 °C. ^{1}H-NMR (600 MHz, COSY, NOESY, CD_3OD, trifluoroacetate): δ [ppm] = 1.91 – 2.00 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.73 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.87 (t, 2H, ^3J = 6.6 Hz, indole-2-CO-NCH_3CH_2-C***H***$_2$), 3.24 – 3.55 (m, 5H, Im-4-$(CH_2)_2$-C***H***$_2$ + C***H***$_3$), 3.93 (brs, 2H, indole-2-CO-NCH_3C***H***$_2$), 6.89 (s, 1H, indole-3-***H***), 7.04 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J =0.9 Hz, indole-5-***H***), 7.19 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J =1.1 Hz, indole-6-***H***), 7.28 (s, 1H, Im-5-***H***), 7.40 (dd, 1H, ^3J = 8.2 Hz, ^4J = 0.9 Hz, indole-7-***H***), 7.59 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.1 Hz, ^4J = 0.9 Hz, indole-4-***H***), 8.75 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, HSQC, HMBC, CD_3OD, trifluoroacetate): δ [ppm] = 22.45 (-, Im-4-***C***H_2), 27.89 (-, Im-4-CH_2-***C***H_2), 36.28 (-, indole-2-CO-NCH_3CH_2-***C***H_2), 38.35 (+, ***C***H_3), 41.51 (-, Im-4-$(CH_2)_2$-***C***H_2), 46.26 (-, indole-2-CO-NCH_3***C***H_2), 107.15 (+, indole-***C***-3), 112.82 (+, indole-***C***-7), 117.07 (+, Im-***C***-5), 121.20 (+, indole-***C***-5), 122.79 (+, indole-***C***-4), 125.17 (+, indole-***C***-6), 128.81 (C_{quat}, indole-***C***-3a), 130.66 (C_{quat}, indole-***C***-2), 134.24 (C_{quat}, Im-***C***-4), 134.86 (+, Im-***C***-2), 137.66 (C_{quat}, indole-***C***-7a), 155.23 (C_{quat}, ***C***=N), 166.01 (indole-2-***C***O), 175.43 (C_{quat}, guanidine-***C***O). IR (cm^{-1}) = 3278, 2989, 1658, 1609, 1181, 1133. HRMS (EI-MS) calcd. for $C_{20}H_{25}N_7O_2$ [$M^{+\bullet}$] 395.2070; found 395.2070. $C_{20}H_{25}N_7O_2 \cdot 2$ TFA (623.50).

***N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl]-*N*^{2}-[4-(1*H*-indole-2-carbonylamino)butanoyl]guanidine (6.21)**

The title compound was prepared from **6.10** (270 mg, 0.37 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 25/75) yielded a pale pink solid (103 mg, 45 %); mp 102 – 105 °C. ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.28 – 1.43 (m, 2H, indole-2-CO-NH-CH_2-C***H***$_2$), 1.89 – 2.01 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.26 (t, 2H, ^3J = 7.7 Hz, C***H***$_2$), 2.41 (t, 2H, ^3J = 6.2 Hz, C***H***$_2$), 2.63 (t, 2H, ^3J = 7.2 Hz, Im-4-C***H***$_2$), 3.35 (t, 2H, ^3J = 6.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.88 (d, 1H, ^4J = 0.8 Hz, indole-3-***H***), 6.92 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 6.97 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.1 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.12 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.1 Hz, ^4J = 1.2 Hz, indole-6-***H***), 7.31 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.0 Hz, indole-7-***H***), 7.52 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.2 Hz, ^4J = 0.8 Hz, indole-4-***H***), 8.43 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 20.78 (-, Im-

4-***C***H_2), 23.90 (-, indole-2-CO-NH-CH_2-***C***H_2), 25.75 (-, Im-4-CH_2-***C***H_2), 34.93, 38.92 (-, indole-2-CO-NH-***C***H_2-CH_2-***C***H_2), 40.19 (-, Im-$(CH_2)_2$-***C***H_2), 103.88 (+, indole-***C***-3), 112.14 (+, indole-***C***-7), 115.35 (+, Im-***C***-5), 120.61 (+, indole-***C***-5), 121.99 (+, indole-***C***-4), 124.69 (+, indole-***C***-6), 127.09 (C_{quat}, indole-***C***-3a), 130.18 (C_{quat}, indole-***C***-2), 132.12 (C_{quat}, Im-***C***-4), 132.87 (+, Im-***C***-2), 136.62 (C_{quat}, indole-***C***-7a), 152.55 (C_{quat}, ***C***=N), 163.54 (C_{quat}, indole-2-***C***O), 177.14 (C_{quat}, guanidine-***C***O). IR (cm^{-1}) = 3232, 3134, 2988, 1684, 1620, 1558, 1178, 1132. HRMS (LSI-MS) calcd. for $C_{20}H_{26}N_7O_2$ $[M + H]^+$ 396.2142; found 396.2141. $C_{20}H_{25}N_7O_2 \cdot 2$ TFA (623.50).

***N*^{1}-Acetyl-*N*^{2}-[3-(1*H*-imidazol-4-yl)propyl]guanidine (6.22)**

The title compound was prepared from **6.11** (350 mg, 0.63 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 4/96) yielded a colorless semisolid compound (150 mg, 54 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.84 – 1.97 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.08 (s, 3H, C***H***$_3$), 2.69 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 3.26 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.12 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 8.46 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 21.06 (-, Im-4-***C***H_2), 23.69 (+, ***C***H_3), 26.04 (-, Im-4-CH_2-***C***H_2), 40.38 (-, Im-4-$(CH_2)_2$-***C***H_2), 115.48 (+, Im-***C***-5), 132.44 (C_{quat}, Im-***C***-4), 133.03 (+, Im-***C***-2), 152.98 (C_{quat}, ***C***=N), 174.73 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3139, 3035, 2854, 1662, 1629, 1179, 1124. HRMS (EI-MS) calcd. for $C_9H_{15}N_5O$ $[M^{+\bullet}]$ 209.1277; found 209.1275. $C_9H_{15}N_5O \cdot 2$ TFA (437.30).

***N*^{1}-[3-(1*H*-Imidazol-4-yl)propyl]-*N*^{2}-propionylguanidine (6.23)**

The title compound was prepared from **6.12** (310 mg, 0.55 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 5/95, 20 min: 15/85) yielded a colorless semisolid compound (144 mg, 58 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 0.98 (t, 3H, ^3J = 7.5 Hz, C***H***$_3$), 1.84 – 1.97 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.36 (q, 2H, ^3J = 7.5 Hz, C***H***$_2$$CH_3$), 2.69 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 3.26 (t, 2H, ^3J = 6.9 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 7.11 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 8.45 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 7.70 (+, ***C***H_3), 21.07 (-, Im-4-***C***H_2), 26.06 (-, Im-4-CH_2-***C***H_2), 30.16 (-, ***C***$H_2$$CH_3$), 40.37 (-, Im-4-$(CH_2)_2$-***C***$H_2$), 115.48 (+, Im-***C***-5), 132.44 (C_{quat}, Im-***C***-4), 133.04 (+, Im-***C***-2), 153.07 (C_{quat}, ***C***=N), 178.27 (C_{quat}, ***C***=O). IR (cm^{-1}) = 3133, 2989, 2901, 1662, 1628, 1180, 1128. HRMS (EI-MS) calcd. for $C_{10}H_{17}N_5O$ $[M^{+\bullet}]$ 233.1433; found 233.1430. $C_{10}H_{17}N_5O \cdot 2$ TFA (451.32).

***N*^{1}-Butyryl-*N*^{2}-[3-(1*H*-imidazol-4-yl)propyl]guanidine (6.24)**

The title compound was prepared from **6.13** (310 mg, 0.55 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 5/95, 20 min:

20/80) yielded a colorless semisolid compound (156 mg, 61 %). ^{1}H-NMR (600 MHz, D_2O, trifluoroacetate): δ [ppm] = 0.86 (t, 3H, ^3J = 7.5 Hz, C***H***$_3$), 1.54 – 1.62 (m, 2H, C***H***$_2$CH$_3$), 1.94 – 2.01 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.38 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$CH$_2$CH$_3$), 2.76 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 3.33 (t, 2H, ^3J = 6.9 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 7.19 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 8.52 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (150 MHz, D_2O, trifluoroacetate): δ [ppm] = 12.60 (+, ***C***H$_3$), 17.68 (-, ***C***H$_2$CH$_3$), 21.07 (-, Im-4-***C***H$_2$), 26.04 (-, Im-4-CH$_2$-***C***H$_2$), 38.58 (-, ***C***H$_2$CH$_2$CH$_3$), 40.41 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 115.48 (+, Im-***C***-5), 132.44 (C$_{quat}$, Im-***C***-4), 133.04 (+, Im-***C***-2), 153.04 (C$_{quat}$, ***C***=N), 177.59 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3109, 2973, 2901, 1662, 1629, 1172, 1125. HRMS (EI-MS) calcd. for $C_{11}H_{19}N_5O$ [M$^{+\bullet}$] 237.1590; found 237.1592. $C_{11}H_{19}N_5O \cdot 2$ TFA (465.35).

N^1-[3-(1*H*-Imidazol-4-yl)propyl]-N^2-isobutyrylguanidine (6.25)

The title compound was prepared from **6.14** (310 mg, 0.53 mmol) according to the general procedure 2. Purification by preparative HPLC (MeCN/0.1 % TFA (aq.): 0 min: 10/90, 20 min: 20/80) yielded a colorless semisolid compound (166 mg, 67 %). ^{1}H-NMR (300 MHz, D_2O, trifluoroacetate): δ [ppm] = 1.03 (d, 6H, ^3J = 6.9 Hz, 2 C***H***$_3$), 1.85 – 1.98 (m, 2H, Im-4-CH$_2$-C***H***$_2$), 2.53 (sep, 1H, ^3J = 6.9 Hz, C***H***), 2.70 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 3.27 (t, 2H, ^3J = 6.8 Hz, Im-4-(CH$_2$)$_2$-C***H***$_2$), 7.12 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 8.46 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***). ^{13}C-NMR (75 MHz, D_2O, trifluoroacetate): δ [ppm] = 17.87 (+, ***C***H$_3$), 21.10 (-, Im-4-***C***H$_2$), 26.05 (-, Im-4-CH$_2$-***C***H$_2$), 36.20 (+, ***C***H), 40.42 (-, Im-4-(CH$_2$)$_2$-***C***H$_2$), 115.48 (+, Im-***C***-5), 132.45 (C$_{quat}$, Im-***C***-4), 133.06 (+, Im-***C***-2), 153.31 (C$_{quat}$, ***C***=N), 181.44 (C$_{quat}$, ***C***=O). IR (cm^{-1}) = 3129, 2989, 2901, 1662, 1628, 1180, 1127. HRMS (EI-MS) calcd. for $C_{11}H_{19}N_5O$ [M$^{+\bullet}$] 237.1590; found 237.1589. $C_{11}H_{19}N_5O \cdot 2$ TFA (465.35).

6.4.1.6 Preparation of the diurethane-protected 3-(1-trityl-1H-imidazol-4-yl)-propylguanidines 6.27 and 6.28

General procedure

To a solution of **6.26**[3, 11] (1 eq), the diurethane-protected guanidine **6.35** or **6.37** (1.75 eq) and PPh_3 (1.5 eq) in THF_{abs}, DIAD (1.5 eq) in THF_{abs} was added dropwise at 0 °C. After the addition was complete, the solution was allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the crude product purified by flash chromatography.

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.27)[3]

The title compound was prepared from a solution of **6.26**[3, 11] (4.4 g, 11.9 mmol), **6.35** (6.82 g, 20.8 mmol), PPh_3 (4.68 g, 17.9 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.6 mL, 3.62 g, 17.9 mmol) in THF_{abs} (30 mL) according to the general procedure. Purification by

flash chromatography (PE/EtOAc 80/20 v/v) yielded a colorless foam-like solid. (7.3 g, 91 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.89 – 2.04 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 4.02 (t, 2H, ^3J = 7.3 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 5.07 (s, 2H, PhC***H***$_2$), 5.21 (s, 2H, PhC***H***$_2$), 6.59 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.03 – 7.17 (m, 6H, Ph-***H***), 7.21 – 7.40 (m, 19H, Ph-***H***), 7.43 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 9.26 (s, 1H, N-***H***), 9.42 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 678 (100) $[M + H]^+$. $C_{42}H_{39}N_5O_4$ (677.79).

N^1-(Benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)-N^1-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.28)

The title compound was prepared from a solution of **6.26**[3, 11] (4.42 g, 12.0 mmol), **6.37** (6.16 g, 21.0 mmol), PPh_3 (4.72 g, 18.0 mmol) in THF_{abs} (100 mL) and a solution of DIAD (3.6 mL, 3.64 g, 18.0 mmol) in THF_{abs} (25 mL) according to the general procedure. Purification by flash chromatography (PE/EtOAc 80/20 v/v) yielded a colorless foam-like solid. (5.8 g, 75 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers: δ [ppm] = 1.47 (s, 2.8H, C***H***$_3$), 1.48 (s, 6.2H, C***H***$_3$), 1.85 – 1.99 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.51 – 2.61 (m, 2H, Im-4-C***H***$_2$), 3.92 – 4.06 (m, 2H, Im-4-$(CH_2)_2$-C***H***$_2$), 5.10 (s, 1.4H, PhC***H***$_2$), 5.17 (s, 0.6H, PhC***H***$_2$), 6.49 (d, 0.3H, ^4J = 1.1 Hz, Im-5-***H***), 6.54 (d, 0.7H, ^4J = 1.1 Hz, Im-5-***H***), 7.07 – 7.17 (m, 6H, Ph-***H***), 7.23 – 7.39 (m, 15H, Ph-***H*** + Im-2-***H***), 9.35 (brs, 1H, N-***H***), 9.40 (brs, 1H, N-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 644 (100) $[M + H]^+$. $C_{39}H_{41}N_5O_4$ (643.77).

6.4.1.7 Preparation of the diurethane-protected 3-(1-trityl-1H-imidazol-4-yl)-propylguanidine 6.33

2-[3-(1-Trityl-1*H*-imidazol-4-yl)propyl]isoindoline-1,3-dione (6.31)

To a cold solution (0 °C) of **6.26**[3, 11] (10.00 g, 27.1 mmol) in THF_{abs} (250 mL), phthalimide (6.00 g, 40.8 mmol) and triphenylphosphine (10.7 g, 40.8 mmol) were added. Under external ice cooling DIAD (11.00 g, 54.2 mmol) in THF_{abs} (200 mL) was added dropwise. The mixture was allowed to warm to ambient temperature, stirred for 1 h and concentrated *in vacuo*. The precipitated product was filtered, washed twice with cold THF (20 mL) and recrystallized from THF/MeCN yielding the title compound as white solid (13.0 g, 96 %); mp 212 °C (ref.[27]: 217 – 219 °C). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.93 – 2.09 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 3.73 (t, 2H, ^3J = 7.2 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.57 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.09 – 7.18 (m, 6H, Ph-***H***), 7.28 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***), 7.64 – 7.72 (m, 2H, Phth-***H***), 7.77 – 7.85 (m, 2H, Phth-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 26.03 (-, Im-4-CH_2-***C***H_2), 28.34 (-, Im-4-***C***H_2), 37.70 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.11 (C_{quat}, ***C***Ph_3), 118.04 (+, Im-***C***-5), 123.15 (+, Phth-***C***-4, 7), 127.95 (+, 3 Ph-***C***-4), 128.02 (+, 6 Ph-***C***), 129.84 (+, 6 Ph-***C***), 132.23 (C_{quat}, Im-***C***-4), 133.82 (+, Phth-***C***-5, 6), 138.39 (+, Im-***C***-2), 140.65 (C_{quat}, Phth-***C***-

3a, 7a), 142.61 (C_{quat}, 3 Ph-***C***-1), 168.39 (C_{quat}, 2 ***C***=O). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 498 (100) $[M + H]^+$. Anal. ($C_{33}H_{27}N_3O_2$) C, H, N. $C_{33}H_{27}N_3O_2$ (497.59).

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-amine (6.32)[27]

A mixture of **6.31** (12.80 g, 25.7 mmol) and hydrazine monohydrate (8.0 mL, 164.6 mmol) in *n*-butanol was refluxed for 1 h. After removal of insoluble material, the filtrate was evaporated giving a pale brownish oil that solidified (9.11 g, 96 %); mp 106 – 108 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.70 – 1.82 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.57 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 2.70 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.52 (d, 1H, ^4J = 1.4 Hz, Im-5-***H***), 7.08 – 7.18 (m, 6H, Ph-***H***), 7.27 – 7.37 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 25.83 (-, Im-4-***C***H_2), 33.42 (-, Im-4-CH_2-***C***H_2), 41.85 (-, Im-4-$(CH_2)_2$-***C***H_2), 75.09 (C_{quat}, ***C***Ph_3), 117.79 (+, Im-***C***-5), 127.97 (+, 3 Ph-***C***-4), 128.00 (+, 6 Ph-***C***), 129.81 (+, 6 Ph-***C***), 138.35 (+, Im-***C***-2), 141.45 (C_{quat}, Im-***C***-4), 142.63 (C_{quat}, 3 Ph-***C***-1). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 368 (100) $[M + H]^+$. Anal. ($C_{25}H_{25}N_3 \cdot 0.5\ H_2O$) C, H, N. $C_{25}H_{25}N_3$ (367.49).

***N*^{1}-(Benzyloxycarbonyl)-*N*^{2}-(*tert*-butoxycarbonyl)-*N*^{3}-[3-(1-trityl-1*H*-imidazol-4-yl)-propyl]guanidine (6.33)**

To a solution of **6.38** (10.17 g, 23.9 mmol) and **6.32** (9.78 g, 26.6 mmol) in DCM (200 mL) NEt_3 (3.7 mL, 2.69 g, 26.6 mmol) was added. After stirring overnight at room temperature, the organic layer was washed with saturated $NaHCO_3$ solution, water and brine and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (PE/EtOAc 60/40 v/v) yielding a colorless foam-like solid (13.6 g, 88 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.46 (s, 9H, C(C***H***$_3$)$_3$), 1.84 – 1.98 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.59 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 3.43 (t, 2H, ^3J = 7.2 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 5.13 (s, 2H, PhC***H***$_2$), 6.54 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.08 – 7.17 (m, 6H, Ph-***H***), 7.24 – 7.43 (m, 15H, Ph-***H*** + Im-2-***H***), 8.46 (brs, 1H, N-***H***), 11.36 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 25.61 (-, ***C***H_2), 28.07 (+, ***C***H_3), 28.58 (-, ***C***H_2), 40.71 (-, Im-4-$(CH_2)_2$-***C***H_2), 67.00 (-, Ph***C***H_2), 75.22 (C_{quat}, ***C***Ph_3), 83.32 (C_{quat}, ***C***$(CH_3)_3$), 118.10 (+, Im-***C***-5), 127.85 (+, 1 Ph-***C***), 128.07 (+, 11 Ph-***C***), 128.42 (+, 2 Ph-***C***), 129.79 (+, 6 Ph-***C***), 137.07 (C_{quat}, 1 Ph-***C***-1), 138.50 (+, Im-***C***-2), 140.30 (C_{quat}, Im-***C***-4), 142.46 (C_{quat}, 3 Ph-***C***-1), 153.11, 156.49, 163.65 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 644 (100) $[M + H]^+$. $C_{39}H_{41}N_5O_4$ (643.77).

6.4.1.8 Preparation of the trityl protected imidazolylpropylguanidines 6.29, 6.30 and 6.34

General procedure

A mixture of the pertinent diurethane-protected guanidine and catalytical amounts of Pd/C (10 %) in MeOH was stirred under a hydrogen atmosphere at room temperature for approximately 3 h (TLC control). After the Cbz-groups were quantitatively cleaved, the catalyst was removed by filtration over Celite and the solvent was evaporated.

***N*-[3-(1-Trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.29)**[3]

The title compound was prepared from **6.27** (7.0 g, 10.3 mmol) and Pd/C (10 %) (0.7 g, cat.) in MeOH (200 mL) according to the general procedure yielding a colorless foam-like solid (4.1 g, 97 %). ^{1}H-NMR (300 MHz, CD_3OD): δ [ppm] = 1.80 – 1.92 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.58 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 3.17 (t, 2H, ^3J = 7.0 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.70 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.10 – 7.19 (m, 6H, Ph-***H***), 7.33 – 7.43 (m, 10H, Im-2-***H*** + Ph-***H***). ^{13}C-NMR (75 MHz, CD_3OD): δ [ppm] = 25.66 (-, Im-4-***C***H_2), 29.76 (-, Im-4-CH_2-***C***H_2), 41.83 (-, Im-4-$(CH_2)_2$-***C***H_2), 76.87 (C_{quat}, ***C***Ph_3), 119.93 (+, Im-***C***-5), 129.32 (+, 6 Ph-***C***), 128.42 (+, 3 Ph-***C***-4), 130.90 (+, 6 Ph-***C***), 139.53 (+, Im-***C***-2), 141.21 (C_{quat}, Im-***C***-4), 143.75 (C_{quat}, 3 Ph-***C***-1), 158.89 (C_{quat}, ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 410 (100) $[M + H]^+$. $C_{26}H_{27}N_5$ (409.53).

***N*^{1}-(*tert*-Butoxycarbonyl)-*N*^{1}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.30)**

The title compound was prepared from **6.28** (5.6 g, 8.7 mmol) and Pd/C (10 %) (0.56 g, cat.) in MeOH (200 mL) according to the general procedure yielding a colorless foam-like solid (4.3 g, 97 %). ^{1}H-NMR (300 MHz, $CDCl_3$) isomers**:** δ [ppm] = 1.47 (s, 2.8H, C***H***$_3$), 1.50 (s, 6.2H, C***H***$_3$), 1.80 – 2.04 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.48 – 2.63 (m, 2H, Im-4-C***H***$_2$), 3.34 (t, 0.6H, ^3J = 6.7 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 3.85 (t, 1.4H, ^3J = 7.7 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.53 – 6.58 (d, 1H, ^4J = 1.3 Hz, Im-5-***H***), 7.07 – 7.16 (m, 6H, Ph-***H***), 7.28 – 7.39 (m, 10H, Ph-***H*** + Im-2-***H***). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 510 (100) $[M + H]^+$. $C_{31}H_{35}N_5O_2$ (509.64).

***N*^{1}-(*tert*-Butoxycarbonyl)-*N*^{2}-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (6.34)**

The title compound was prepared from **6.33** (13.5 g, 21.0 mmol) and Pd/C (10 %) (1.35 g, cat.) in MeOH (200 mL) according to the general procedure. Purification by flash chromatography yielded a colorless foam-like solid (10.6 g, 93 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 1.47 (s, 9H, C(C***H***$_3$)$_3$), 1.80 – 1.93 (m, 2H, Im-4-CH_2-C***H***$_2$), 2.57 (t, 2H, ^3J = 6.1 Hz, Im-4-C***H***$_2$), 3.35 (t, 2H, ^3J = 6.7 Hz, Im-4-$(CH_2)_2$-C***H***$_2$), 6.55 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 7.07 – 7.16 (m, 6H, Ph-***H***), 7.30 – 7.38 (m, 10H, Ph-***H*** + Im-2-***H***). ^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 23.30 (-, ***C***H_2), 28.54 (+, ***C***H_3), 29.50 (-, ***C***H_2), 40.27 (-, Im-4-$(CH_2)_2$-***C***H_2),

75.28 (C_{quat}, ***C***Ph_3), 77.60 (C_{quat}, ***C***$(CH_3)_3$), 118.31 (+, Im-***C***-5), 128.11 (+, 9 Ph-***C***), 129.76 (+, 6 Ph-***C***), 138.06 (+, Im-***C***-2), 140.48 (C_{quat}, Im-***C***-4), 142.38 (C_{quat}, 3 Ph-***C***-1), 162.56, 163.98 (C_{quat}, ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 510 (100) $[M + H]^+$. HRMS (EI-MS) calcd. for $C_{31}H_{35}N_5O_2$ $[M^{+\bullet}]$ 509.2791; found 509.2786. Anal. ($C_{31}H_{35}N_5O_2 \cdot 0.5\ H_2O$) C, H, N. $C_{31}H_{35}N_5O_2$ (509.64).

6.4.1.9 Preparation of the guanidinylation reagents 6.35-6.38

N^1,N^2-Bis(benzyloxycarbonyl)guanidine (6.35)[12]

For the preparation of **6.35** see section 4.4.1.6 (compound **4.28**).

N^1-(*tert*-Butoxycarbonyl)guanidine (6.36)[28]

For the preparation of **6.36** see section 4.4.1.6 (compound **4.26**).

N^1-(Benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)guanidine (6.37)

For the preparation of **6.37** see section 4.4.1.6 (compound **4.27**).

N^1-Benzyloxycarbonyl-N^2-(*tert*-butoxycarbonyl)-N^3-trifluoromethanesulfonylguanidine (6.38)

To a solution of **6.37** (10.0 g, 34.1 mmol) in anhydrous chlorobenzene (250 mL) NaH (60 % dispersion in mineral oil) (2.73 g, 68.2 mmol) was added in portions at 0 °C (argon atmosphere). After stirring for 1 h at 0 °C, the mixture was cooled to –45 °C and trifluoromethanesulfonic anhydride (5.7 mL, 9.62 g, 34.1 mmol) was added. The mixture was allowed to warm to ambient temperature and stirred overnight. After evaporation of the solvent, a 2 M solution of $KHSO_4$ (60 mL) was added to the residue and extracted with EtOAc (250 mL). The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and removed *in vacuo*. Purification was performed by flash chromatography (PE/EtOAc 80/20 v/v) yielding a colorless semisolid compound (10.9 g, 75 %). ^{1}H-NMR (600 MHz, DMSO-d_6): δ [ppm] = 1.45 (s, 9H, C***H***$_3$), 5.22 (s, 2H, C***H***$_2$), 7.33 – 7.47 (m, 5H, Ph-***H***), 11.13 (brs, 1H, N-***H***), 11.42 (brs, 1H, N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 27.40 (+, ***C***H_3), 67.74 (-, ***C***H_2), 83.37 (C_{quat}, ***C***$(CH_3)_3$), 118.99 (C_{quat}, ^1J = 320.6 Hz, CF_3), 128.21 (+, 2 Ph-***C***), 128.38 (+, Ph-***C***), 128.41 (+, 2 Ph-***C***), 134.97 (C_{quat}, Ph-***C***), 149.85, 151.46, 152.12 (C_{quat}, 2 ***C***=O + ***C***=N). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 443 (100) $[M + NH_4]^+$, 426 (78) $[M + H]^+$. $C_{15}H_{18}F_3N_3O_6S$ (425.38).

6.4.1.10 Preparation of methyl 3-(methylamino)propanoate 6.41

Methyl 3-(methylamino)propanoate (6.41)[13]

To a solution of 3-(methylamino)propanenitrile (3.36 g, 40.0 mmol) in anhydrous MeOH (40 mL), Na_2SO_4 (1.0 g) was added. HCl gas was bubbled through the solution for 5 h. After concentration of the solvent to approximately 20 mL, the residue was cooled to – 78 °C and filtered to remove precipitated NH_4Cl. The filtrate was evaporated to dryness yielding a pale yellow oil that was used without further purification (2.8 g, 60 %). ^{1}H-NMR (300 MHz, D_2O): δ [ppm] = 2.62 (s, 3H, NHC***H***$_3$), 2.73 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$CO), 3.20 (t, 2H, ^3J = 6.5 Hz, NHC***H***$_2$), 3.61 (s, 3H, OC***H***$_3$). ^{13}C-NMR (75 MHz, D_2O): δ [ppm] = 30.04 (-, ***C***H_2CO), 33.04 (+, NH***C***H_3), 44.38 (-, NH***C***H_2), 52.65 (+, O***C***H_3), 172.88 (C_{quat}, ***C***=O). CI-MS (NH_3) *m/z* (%): 118 (100) $[M + H]^+$. $C_5H_{11}NO_2 \cdot HCl$ (153.61).

6.4.1.11 Preparation of the methyl esters 6.43-6.46

General procedure

To a solution of 1*H*-indole-2-carboxylic acid (1 eq) and the methyl ester of the amino acid (hydrochloride) (1.2 eq) in DCM, EDC · HCl (1.2 eq) and DMAP (1.6 eq) were added at 0 °C. After stirring for 4 h at 0 °C, the solution was allowed to warm to ambient temperature and stirred for additional 20 h. The organic layer was washed with H_2O and 10 % HCl, dried over Na_2SO_4 and evaporated *in vacuo*.

Methyl 2-(1*H*-indole-2-carbonylamino)acetate (6.43)

The title compound was prepared from 1*H*-indole-2-carboxylic acid (1.61 g, 10.0 mmol), methyl 2-aminoacetate · HCl (1.51 g, 12.0 mmol), EDC · HCl (2.30 g, 12.0 mmol) and DMAP (1.95 g, 16.0 mmol) in DCM (100 mL) according to the general procedure. Recrystallization from MeOH yielded a white solid (1.7 g, 73 %); mp 209 – 211 °C (dec.). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 3.67 (s, 3H, C***H***$_3$), 4.06 (d, 2H, ^3J = 6.0 Hz, C***H***$_2$), 7.04 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.16 (dd, 1H, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-3-***H***), 7.19 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, indole-6-***H***), 7.44 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-7-***H***), 7.63 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 1.1 Hz, indole-4-***H***), 8.96 (t, 1H, ^3J = 6.0 Hz, CON***H***), 11.63 (brs, 1H, indole-N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 40.72 (+, ***C***H_3), 51.72 (-, ***C***H_2), 102.92 (+, indole-***C***-3), 112.26 (+, indole-***C***-7), 119.72 (+, indole-***C***-5), 121.54 (+, indole-***C***-4), 123.42 (+, indole-***C***-6), 126.93 (C_{quat}, indole-***C***-3), 130.93 (C_{quat}, indole-***C***-2), 136.43 (C_{quat}, indole-***C***-7), 161.45 (C_{quat}, indole-2-***C***O), 170.37 (C_{quat}, ***C***$OOCH_3$). EI-MS (70 eV) *m/z* (%): 232 (100) [$M^{+\bullet}$]. Anal. ($C_{12}H_{12}N_2O_3$) C, H, N. $C_{12}H_{12}N_2O_3$ (232.24).

Methyl 3-(1*H*-indole-2-carboxamido)propanoate (6.44)

The title compound was prepared from 1*H*-indole-2-carboxylic acid (0.81 g, 5.0 mmol), methyl 3-aminopropanoate · HCl (0.84 g, 6.0 mmol), EDC · HCl (1.15 g, 6.0 mmol) and DMAP (0.98 g, 8.0 mmol) in DCM (50 mL) according to the general procedure. Recrystallization from MeOH yielded a white solid (0.78 g, 63 %); mp 148 – 150 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.62 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$CO), 3.48 – 3.57 (m, 2H, NHC***H***$_2$), 3.62 (s, 3H, C***H***$_3$), 7.02 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.09 (dd, 1H, ^4J = 2.1 Hz, ^4J = 0.9 Hz, indole-3-***H***), 7.17 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, indole-6-***H***), 7.42 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-7-***H***), 7.60 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.1 Hz, ^4J = 1.1 Hz, indole-4-***H***), 8.59 (t, 1H, ^3J = 5.6 Hz, CON***H***), 11.59 (brs, 1H, indole-N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 33.61 (-, ***C***H$_2$), 35.03 (-, ***C***H$_2$), 51.35 (+, ***C***H$_3$), 102.40 (+, indole-***C***-3), 112.19 (+, indole-***C***-7), 119.61 (+, indole-***C***-5), 121.39 (+, indole-***C***-4), 123.18 (+, indole-***C***-6), 126.94 (C_{quat}, indole-***C***-3), 131.45 (C_{quat}, indole-***C***-2), 136.29 (C_{quat}, indole-***C***-7), 161.10 (C_{quat}, indole-2-***C***O), 171.68 (C_{quat}, ***C***OOCH$_3$). CI-MS (NH_3) *m/z* (%): 247 (100) $[M + H]^+$. Anal. ($C_{13}H_{14}N_2O_3$) C, H, N. $C_{13}H_{14}N_2O_3$ (246.26).

Methyl 3-(*N*-methyl-1*H*-indole-2-carbonylamino)propanoate (6.45)

The title compound was prepared from 1*H*-indole-2-carboxylic acid (1.61 g, 10.0 mmol), **6.41** · HCl (1.84 g, 12.0 mmol), EDC · HCl (2.30 g, 12.0 mmol) and DMAP (1.95 g, 16.0 mmol) in DCM (100 mL) according to the general procedure. Recrystallization from MeOH yielded a white solid (1.24 g, 73 %); mp 119 – 121 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.70 (t, 2H, ^3J = 7.2 Hz, C***H***$_2$CO), 3.28 (brs, 3H, NC***H***$_3$), 3.61 (s, 3H, COOC***H***$_3$), 3.78 (brs, 2H, NC***H***$_2$), 6.87 (s, 1H, indole-3-***H***), 7.04 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, indole-5-***H***), 7.19 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-6-***H***), 7.43 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, ^4J = 1.1 Hz, indole-7-***H***), 7.60 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.9 Hz, ^4J = 1.0 Hz, indole-4-***H***), 11.56 (brs, 1H, indole-N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 31.85 (-, ***C***H$_2$CO), 37.07 (+, N***C***H$_3$), 44.78 (-, NC***H***$_2$), 51.44 (+, OC***H***$_3$), 104.74 (+, indole-***C***-3), 111.98 (+, indole-***C***-7), 119.59 (+, indole-***C***-5), 121.40 (+, indole-***C***-4), 123.26 (+, indole-***C***-6), 126.92 (C_{quat}, indole-***C***-3), 129.91 (C_{quat}, indole-***C***-2), 135.68 (C_{quat}, indole-***C***-7), 162.48 (C_{quat}, indole-2-***C***O), 171.61 (C_{quat}, ***C***OOCH$_3$). CI-MS (NH_3) *m/z* (%): 261 (100) $[M + H]^+$. Anal ($C_{14}H_{16}N_2O_3$) C, H, N. $C_{14}H_{16}N_2O_3$ (260.29).

Methyl 4-(1*H*-indole-2-carbonylamino)butanoate (6.46)

The title compound was prepared from 1*H*-indole-2-carboxylic acid (1.61 g, 10.0 mmol), methyl 4-aminobutanoate · HCl (1.84 g, 12.0 mmol), EDC · HCl (2.30 g, 12.0 mmol) and DMAP (1.95 g, 16.0 mmol) in DCM (100 mL) according to the general procedure. Recrystallization from MeOH yielded a white solid (1.63 g, 63 %); mp 140 – 141 °C. ^{1}H-NMR

(300 MHz, DMSO-d_6): δ [ppm] = 1.74 – 1.87 (m, 2H, C***H***$_2$CH$_2$CO), 2.39 (t, 2H, ^3J = 7.4 Hz, C***H***$_2$CO), 3.26 – 3.36 (m, 2H, NHC***H***$_2$), 3.62 (s, 3H, C***H***$_3$), 7.02 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, indole-5-***H***), 7.10 (dd, 1H, ^4J = 2.0 Hz, ^4J = 0.9 Hz, indole-3-***H***), 7.17 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-6-***H***), 7.42 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, ^4J = 1.1 Hz, indole-7-***H***), 7.60 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-4-***H***), 8.49 (t, 1H, ^3J = 5.7 Hz, CON***H***), 11.55 (brs, 1H, indole-N-***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 24.54 (-, ***C***H$_2$CH$_2$CO), 30.66 (-, ***C***H$_2$CO), 37.98 (-, NH***C***H$_2$), 51.19 (+, ***C***H$_3$), 102.19 (+, indole-***C***-3), 112.18 (+, indole-***C***-7), 119.56 (+, indole-***C***-5), 121.34 (+, indole-***C***-4), 123.08 (+, indole-***C***-6), 126.99 (C$_{quat}$, indole-***C***-3), 131.67 (C$_{quat}$, indole-***C***-2), 136.27 (C$_{quat}$, indole-***C***-7), 161.05 (C$_{quat}$, indole-2-***C***O), 173.05 (C$_{quat}$, ***C***OOCH$_3$). CI-MS (NH$_3$) *m/z* (%): 261 (100) [M + H]$^+$. Anal. (C$_{14}$H$_{16}$N$_2$O$_3$) C, H, N. C$_{14}$H$_{16}$N$_2$O$_3$ (260.29).

6.4.1.12 Preparation of the carboxylic acids 6.47-6.50

General procedure

A solution of the pertinent methyl ester (1 eq) and LiOH · H$_2$O (2 eq) in EtOH was stirred for 20 h at ambient temperature. The solvent was removed *in vacuo*, the residue taken up in H$_2$O and adjusted to pH = 2 with concentrated HCl. After extraction of the precipitated product with EtOAc, washing with H$_2$O and drying over Na$_2$SO$_4$, the solvent was evaporated giving the respective carboxylic acid.

2-(1*H*-Indole-2-carbonylamino)acetic acid (6.47)

The title product was prepared from **6.43** (1.65 g, 7.1 mmol) and LiOH · H$_2$O (0.60 g, 14.2 mmol) in EtOH (80 mL) according to the general procedure. Extraction with EtOAc (3 x 80 mL). Recrystallization from MeOH yielded a white solid (0.76 g, 49 %); mp 220 – 222 °C (dec.) (ref.[29]: 158 – 159 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 3.97 (d, 2H, ^3J = 6.0 Hz, C***H***$_2$), 7.04 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.15 (dd, 1H, ^4J = 2.0 Hz, ^4J = 0.9 Hz, indole-3-***H***), 7.19 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-6-***H***), 7.44 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, ^4J = 1.0 Hz, indole-7-***H***), 7.63 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-4-***H***), 8.84 (t, 1H, ^3J = 6.0 Hz, CON***H***), 11.61 (brs, 1H, indole-N-***H***), 12.65 (brs, 1H, COO***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 40.72 (-, ***C***H$_2$), 102.75 (+, indole-***C***-3), 112.24 (+, indole-***C***-7), 119.67 (+, indole-***C***-5), 121.49 (+, indole-***C***-4), 123.32 (+, indole-***C***-6), 126.96 (C$_{quat}$, indole-***C***-3), 131.16 (C$_{quat}$, indole-***C***-2), 136.40 (C$_{quat}$, indole-***C***-7), 161.35 (C$_{quat}$, indole-2-***C***O), 171.30 (C$_{quat}$, ***C***OOH). ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 219 (100) [M + H]$^+$. Anal. (C$_{11}$H$_{10}$N$_2$O$_3$) C, H, N. C$_{11}$H$_{10}$N$_2$O$_3$ (218.21).

3-(1*H*-Indole-2-carbonylamino)propanoic acid (6.48)

The title product was prepared from **6.44** (0.76 g, 3.1 mmol) and LiOH · H_2O (0.26 g, 6.2 mmol) in EtOH (100 mL) according to the general procedure. Extraction with EtOAc (3 x 50 mL). Recrystallization from MeOH yielded a white solid (0.54 g, 75 %); mp 163 – 164 °C. (ref.[30]: 163 – 165 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.55 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$CO), 3.44 – 3.55 (m, 2H, NHC***H***$_2$), 7.02 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.10 (dd, 1H, ^4J = 2.1 Hz, ^4J = 0.9 Hz, indole-3-***H***), 7.17 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.1 Hz, indole-6-***H***), 7.42 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, ^4J = 1.0 Hz, indole-7-***H***), 7.60 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.1 Hz, ^4J = 1.1 Hz, indole-4-***H***), 8.55 (t, 1H, ^3J = 5.6 Hz, CON***H***), 11.57 (brs, 1H, indole-N-***H***), 12.28 (brs, 1H, COO***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 33.81 (-, ***C***H$_2$), 35.11 (-, ***C***H$_2$), 102.37 (+, indole-***C***-3), 112.19 (+, indole-***C***-7), 119.60 (+, indole-***C***-5), 121.38 (+, indole-***C***-4), 123.16 (+, indole-***C***-6), 126.96 (C_{quat}, indole-***C***-3), 131.54 (C_{quat}, indole-***C***-2), 136.28 (C_{quat}, indole-***C***-7), 161.06 (C_{quat}, indole-2-***C***O), 172.81 (C_{quat}, ***C***OOH). CI-MS (NH_3) *m/z* (%): 233 (100) $[M + H]^+$. Anal. ($C_{12}H_{12}N_2O_3$) C, H, N. $C_{12}H_{12}N_2O_3$ (232.24).

Methyl 3-(*N*-methyl-1*H*-indole-2-carbonylamino)propanoate (6.49)

The title product was prepared from **6.45** (1.17 g, 4.5 mmol) and LiOH · H_2O (0.38 g, 9.0 mmol) in EtOH (80 mL) according to the general procedure. Extraction with EtOAc (3 x 60 mL). Evaporation of the solvent gave a white solid (1.0 g, 90 %); mp 155 – 157 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.55 – 2.72 (m, 2H, C***H***$_2$CO), 3.36 (brs, 3H, NC***H***$_3$), 3.62 – 3.95 (brs, 2H, NC***H***$_2$), 6.86 (s, 1H, indole-3-***H***), 7.04 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-5-***H***), 7.19 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-6-***H***), 7.43 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-7-***H***), 7.60 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 1.9 Hz, ^4J = 1.0 Hz, indole-4-***H***), 11.56 (brs, 1H, indole-N-***H***), 12.39 (brs, 1H, COO***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 32.36 (-, ***C***H$_2$CO), 37.85 (+, N***C***H$_3$), 44.85 (-, NC***H***$_2$), 104.79 (+, indole-***C***-3), 111.98 (+, indole-***C***-7), 119.58 (+, indole-***C***-5), 121.39 (+, indole-***C***-4), 123.24 (+, indole-***C***-6), 126.93 (C_{quat}, indole-***C***-3), 129.99 (C_{quat}, indole-***C***-2), 135.67 (C_{quat}, indole-***C***-7), 162.44 (C_{quat}, indole-2-***C***O), 172.81 (C_{quat}, ***C***OOH). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 247 (100) $[M + H]^+$. Anal. ($C_{14}H_{16}N_2O_3$) C, H, N. $C_{14}H_{16}N_2O_3$ (260.29).

4-(1*H*-Indole-2-carbonylamino)butanoic acid (6.50)

The title product was prepared from **6.46** (1.5 g, 5.8 mmol) and LiOH · H_2O (0.49 g, 11.6 mmol) in EtOH (100 mL) according to the general procedure. Extraction with EtOAc (3 x 80 mL). Recrystallization from MeOH yielded a white solid (0.78 g, 58 %); mp 148 – 150 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 1.70 – 1.85 (m, 2H, C***H***$_2$CH$_2$CO) 2.30 (t, 2H, ^3J = 7.4 Hz, C***H***$_2$CO), 3.25 – 3.36 (m, 2H, NHC***H***$_2$), 7.02 (ddd, 1H, ^3J = 8.0 Hz, ^3J = 7.0 Hz, ^4J = 1.1

Hz, indole-5-***H***), 7.11 (dd, 1H, ^4J = 2.0 Hz, ^4J = 0.9 Hz, indole-3-***H***), 7.17 (ddd, 1H, ^3J = 8.2 Hz, ^3J = 7.0 Hz, ^4J = 1.0 Hz, indole-6-***H***), 7.42 (ddd, 1H, ^3J = 8.2 Hz, ^4J = 1.9 Hz, ^4J = 1.1 Hz, indole-7-***H***), 7.60 (ddd, 1H, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 1.0 Hz, indole-4-***H***), 8.50 (t, 1H, ^3J = 5.7 Hz, CON***H***), 11.55 (brs, 1H, indole-N-***H***), 12.17 (brs, 1H, COO***H***). ^{13}C-NMR (75 MHz, DMSO-d_6): δ [ppm] = 24.61 (-, ***C***$H_2$$CH_2$CO), 31.07 (-, ***C***$H_2$CO), 38.14 (-, NH***C***$H_2$), 102.20 (+, indole-***C***-3), 112.18 (+, indole-***C***-7), 119.56 (+, indole-***C***-5), 121.33 (+, indole-***C***-4), 123.07 (+, indole-***C***-6), 127.00 (C_{quat}, indole-***C***-3), 131.72 (C_{quat}, indole-***C***-2), 136.27 (C_{quat}, indole-***C***-7), 161.02 (C_{quat}, indole-2-***C***O), 174.19 (C_{quat}, ***C***OOH). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 247 (100) $[M + H]^+$. Anal. ($C_{13}H_{14}N_2O_3 \cdot 0.1\ H_2O$) C, H, N. $C_{13}H_{14}N_2O_3$ (246.26).

6.4.2 Pharmacological methods

6.4.2.1 Materials

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide maleate was from Tocris Bioscience (Ellisville, USA). [γ-^{32}P]GTP was synthesized according to a previously described method.[31] [^{32}P]P_i (8,500 – 9,100 Ci/mmol orthophosphoric acid) was from PerkinElmer Life Sciences (Boston, MA). All unlabeled nucleotides, glycerol-3-phosphate dehydrogenase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were from Roche (Mannheim, Germany). 3-Phosphoglycerate kinase and L-α-glycerol phosphate was from Sigma.

6.4.2.2 Steady-state GTPase activity assay

See section 3.4.2.2.

6.4.2.3 Histamine H_1R assay on guinea pig ileum[3]

See section 3.4.2.4.

6.4.2.4 Histamine H_2R assay on the isolated spontaneously beating guinea pig right atrium[3]

See section 3.4.2.5.

6.5 References

(1) Xie, S.-X., Ghorai, P., Ye, Q.-Z., Buschauer, A., Seifert, R., Probing Ligand-Specific Histamine H_1- and H_2-Receptor Conformations with N^G-Acylated Imidazolylpropylguanidines. *J. Pharmacol. Exp. Ther.* **2006**, 317, 139-146.

(2) Xie, S.-X., Kraus, A., Ghorai, P., Ye, Q.-Z., Elz, S., Buschauer, A., Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (UR-AK57), a Potent Partial Agonist for the Human Histamine H_1- and H_2-Receptors. *J. Pharmacol. Exp. Ther.* **2006**, 317, 1262-1268.

(3) Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press, DOI: 10.1021/jm800841w. Epub ahead of print on Oct 800825, 802008.

(4) Parsons, M. E., Blakemore, R. C., Durant, G. J., Ganellin, C. R., Rasmussen, A. C., 3-[4(5)-Imidazolyl]propylguanidine (SK&F 91486) - a partial agonist at histamine H_2-receptors. *Agents Actions* **1975**, 5, 464.

(5) Jablonowski, J. A., Grice, C. A., Chai, W., Dvorak, C. A., Venable, J. D., Kwok, A. K., Ly, K. S., Wei, J., Baker, S. M., Desai, P. J., Jiang, W., Wilson, S. J., Thurmond, R. L., Karlsson, L., Edwards, J. P., Lovenberg, T. W., Carruthers, N. I., The First Potent and Selective Non-Imidazole Human Histamine H_4 Receptor Antagonists. *J. Med. Chem.* **2003**, 46, 3957-3960.

(6) Paul, R., Anderson, G. W., *N*,*N*'-Carbonyldiimidazole, a New Peptide Forming Reagent. *J. Am. Chem. Soc.* **1960**, 82, 4596-4600.

(7) Paul, R., Anderson, G. W., *N*,*N*'-Carbonyldiimidazole in Peptide Synthesis. III. A Synthesis of Isoleucine-5 Angiotensin II Amide-I. *J. Org. Chem.* **1962**, 27, 2094-2099.

(8) pK_a-value was calculated with ACD Labs 9.0, Advanced Chemistry Development, Toronto (Canada).

(9) Schneider, E., Keller, M., Brennauer, A., Hoefelschweiger, B. K., Gross, D., Wolfbeis, O. S., Bernhardt, G., Buschauer, A., Synthesis and Characterization of the First Fluorescent Nonpeptide NPY Y_1 Receptor Antagonist. *ChemBioChem* **2007**, 8, 1981-1988.

(10) Mitsunobu, O., Yamada, M., Mukaiyama, T., Preparation of esters of phosphoric acid by the reaction of trivalent phosphorus compounds with diethyl azodicarboxylate in the presence of alcohols. *Bull. Chem. Soc. Jpn.* **1967**, 40, 935-939.

(11) Stark, H., Purand, K., Hüls, A., Ligneau, X., Garbarg, M., Schwartz, J.-C., Schunack, W., [^{125}I]Iodoproxyfan and Related Compounds: A Reversible Radioligand and Novel Classes of Antagonists with High Affinity and Selectivity for the Histamine H_3 Receptor. *J. Med. Chem.* **1996**, 39, 1220-1226.

(12) Feichtinger, K., Sings, H. L., Baker, T. J., Matthews, K., Goodman, M., Triurethane-Protected Guanidines and Triflyldiurethane-Protected Guanidines: New Reagents for Guanidinylation Reactions. *J. Org. Chem.* **1998**, 63, 8432-8439.

(13) Vohra, S. K., Harrington, G. W., Swern, D., Reversible interconversion of *N*-nitroso(2-methylamino)acetonitrile and 3-methyl-5-amino-1,2,3-oxadiazolium chloride and related reactions. *J. Org. Chem.* **1978**, 43, 1671-1673.

(14) Kelley, M. T., Bürckstümmer, T., Wenzel-Seifert, K., Dove, S., Buschauer, A., Seifert, R., Distinct Interaction of Human and Guinea Pig Histamine H_2-Receptor with Guanidine-Type Agonists. *Mol. Pharmacol.* **2001**, 60, 1210-1225.

(15) Seifert, R., Wenzel-Seifert, K., Bürckstümmer, T., Pertz, H. H., Schunack, W., Dove, S., Buschauer, A., Elz, S., Multiple Differences in Agonist and Antagonist Pharmacology between Human and Guinea Pig Histamine H_1-Receptor. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1104-1115.

(16) Kiss, R., Noszál, B., Rácz, Á., Falus, A., Erós, D., Keseru, G. M., Binding mode analysis and enrichment studies on homology models of the human histamine H_4 receptor. *Eur. J. Med. Chem.* **2008**, 43, 1059-1070.

(17) Durant, G. J., Duncan, W. A., Ganellin, C. R., Parsons, M. E., Blackmore, R. C., Rasmussen, A. C., Impromidine (SK&F 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature* **1978**, 276, 403-405.

(18) Buschauer, A., Synthesis and in Vitro Pharmacology of Arpromidine and Related Phenyl(pyridylalkyl)guanidines, a Potential New Class of Positive Inotropic Drugs. *J. Med. Chem.* **1989**, 32, 1963-1970.

(19) Schneider, E., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(20) Schnell, D., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(21) Durant, G. J., Emmett, J. C., Ganellin, C. R., Miles, P. D., Parsons, M. E., Prain, H. D., White, G. R., Cyanoguanidine-Thiourea Equivalence in the Development of the Histamine H_2-Receptor Antagonist, Cimetidine. *J. Med. Chem.* **1977**, 20, 901-906.

(22) Nederkoorn, P. H. J., van Lenthe, J. H., van der Goot, H., Donné-Op den Kelder, G. M., Timmermann, H., The agonistic binding site at the histamine H_2 receptor. I. Theoretical investigations of histamine binding to an oligopeptide mimicking a part of the fifth transmembrane α-helix. *J. Comput. Aided Mol. Des.* **1996**, 10, 461-478.

(23) Thurmond, R. L., Gelfand, E. W., Dunford, P. J., The role of histamine H_1 and H_4 receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug Discov.* **2008**, 7, 41-53.

(24) Lippert, U., Artuc, M., Grutzkau, A., Babina, M., Guhl, S., Haase, I., Blaschke, V., Zachmann, K., Knosalla, M., Middel, P., Kruger-Krasagakis, S., Henz, B. M., Human Skin Mast Cells Express H_2 and H_4, but not H_3 Receptors. *J. Investig. Dermatol.* **2003**, 123, 116-123.

(25) Ling, P., Ngo, K., Nguyen, S., Thurmond, R. L., Edwards, J. P., Karlsson, L., Fung-Leung, W.-P., Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.* **2004**, 142, 161-171.

(26) Black, J. W., Parsons, M. E. Pharmaceutical compositions and methods of inhibiting H-1 and H-2 histamine receptors. US 4000302, 1976. *Chem. Abstr.* 86:145927.

(27) Bertinaria, M., Stilo, A. D., Tosco, P., Sorba, G., Poli, E., Pozzoli, C., Coruzzi, G., Fruttero, R., Gasco, A., [3-(1*H*-Imidazol-4-yl)propyl]guanidines Containing Furoxan Moieties: A New Class of H_3-Antagonists Endowed with NO-Donor Properties. *Bioorg. Med. Chem.* **2003**, 11, 1197-1205.

(28) Zapf, C. W., Creighton, C. J., Tomioka, M., Goodman, M., A Novel Traceless Resin-Bound Guanidinylating Reagent for Secondary Amines To Prepare N,N-Disubstituted Guanidines. *Org. Lett.* **2001**, 3, 1133-1136.

(29) González, J. F., de la Cuesta, E., Avendaño, C., From cyclic dehydrodipeptides to uncommon acyclic peptide mimetics. *Tetrahedron Lett.* **2006**, 47, 6711-6714.

(30) Kaiser, H. M., Zenz, I., Lo, W. F., Spannenberg, A., Schroder, K., Jiao, H., Gordes, D., Beller, M., Tse, M. K., Preparation of Novel Unsymmetrical Bisindoles under Solvent-Free Conditions: Synthesis, Crystal Structures, and Mechanistic Aspects. *J. Org. Chem.* **2007**, 72, 8847-8858.

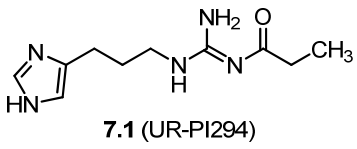
(31) Walseth, T. F., Johnson, R. A., The enzymatic preparation of [α-^{32}P] nucleoside triphosphates, cyclic [^{32}P] AMP, and cyclic [^{32}P] GMP. *Biochim. Biophys. Acta* **1979**, 562, 11-31.

Chapter 7

Tritium-labeled N^1-[3-(1*H*-imidazol-4-yl)propyl]-N^2-propionylguanidine ([^{3}H]UR-PI294), a high affinity histamine H_3 and H_4 receptor radioligand

7.1 Introduction

To investigate the biological role of the human (h) H_3R, and in particular that of the hH_4R, selective agonists and antagonists as well as special ligands including radioligands are required as pharmacological tools. The N^G-alkanoylimidazolylpropylguanidines described in Chapter 6 are high affinity hH_3R antagonists (partial agonists) and highly potent full hH_4R agonists, which belong to the most potent hH_4R agonists described so far. For example, UR-PI294 (**7.1**), bearing an N^G-propionyl group (Figure 7.1), possesses more than 1000- and 100-fold selectivity for the $hH_{3,4}Rs$ relative to the hH_1R and hH_2R, respectively.



7.1 (UR-PI294)

	EC_{50} [nM]	E_{max}
hH_1R	3,500	0.30
hH_2R	370	0.83
hH_3R	1.6	0.39
hH_4R	3.0	0.90

Figure 7.1. The acylguanidine **7.1** (UR-PI294): structure and pharmacological profile at the four histamine receptors, determined in steady-state GTPase assays using membrane preparations of Sf9 cells expressing the respective human histamine receptor. Agonistic activity expressed as EC_{50}; E_{max} = efficacy relative to histamine = 1.00.

The propionyl group in UR-PI294 is of special interest, as it offers the possibility to introduce a commercially available tritiated propionyl residue. The resulting radioligand, [^{3}H]UR-PI294 (**7.10**), may be a valuable pharmacological tool for the labeling of the hH_3R and in particular of the hH_4R. With respect to the hH_3R, the low efficacy of UR-PI294 at this histamine receptor subtype may be of interest, because partial agonistic radioligands with low intrinsic activities have been shown to behave as antagonists.[1-3] Therefore, [^{3}H]UR-PI294 may complement the commercially available and preferably used full hH_3R agonists [^{3}H](*R*)-α-methylhistamine[4] and [^{3}H]N^α-methylhistamine[5, 6] (Figure 7.2) in pharmacological studies at the hH_3R.

Concerning the hH_4R, only three radioligands have been reported: [^{3}H]histamine,[7-10] [^{3}H]JNJ 7777120[7, 11] and [^{125}I]iodophenpropit (Figure 7.2).[7] The low specific activity (10 - 25 Ci/mmol),[7, 9, 11-14] of the commonly commercially available H_4R radioligand [^{3}H]histamine brings about low sensitivity and requires high concentrations of either the radioligand or the receptor protein. The use of iodinated compounds is compromised by potential chemical instability,[15] the shorter half-life of ^{125}I-labeled (59.4 days) compared to ^{3}H-labeled ligands (12.4 years) and the need for special safety precautions (shielding) during preparation and handling. By contrast, tritium-labeled UR-PI294 is considered a valuable novel radioligand in particular for the hH_4R.

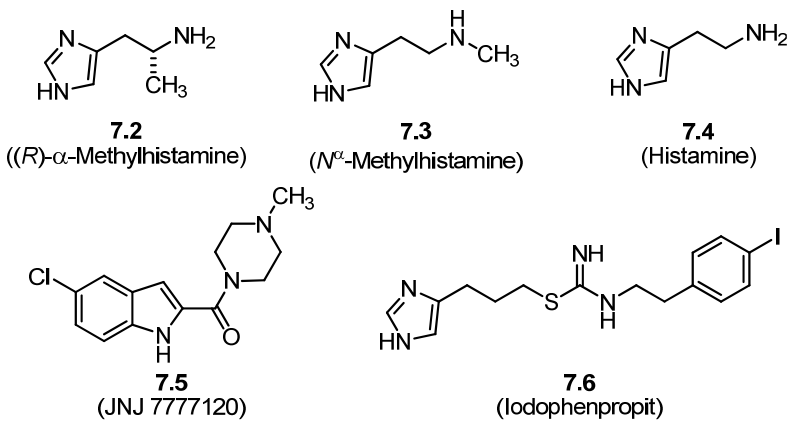
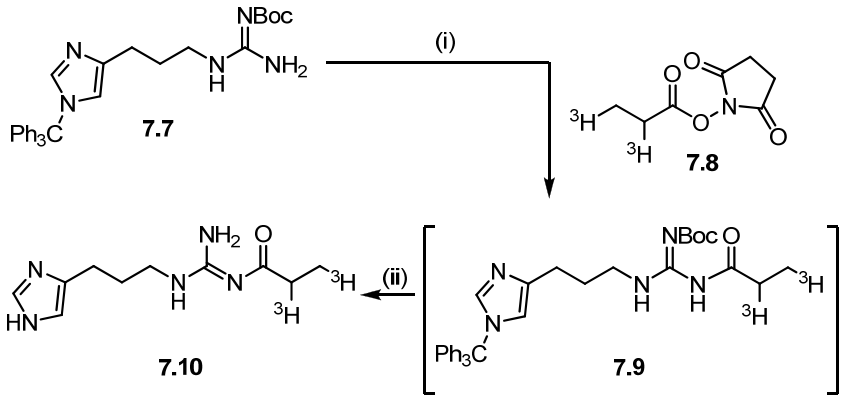


Figure 7.2. Structures of hH_3R and hH_4R ligands reported as radioligands.

This study presents the synthesis and pharmacological characterization of [^{3}H]UR-PI294, a new high-affinity hH_3R and hH_4R radioligand.

7.2 Chemistry

The radioligand **7.10** ([^{3}H]UR-PI294) was prepared as depicted in Scheme 7.1. Acylation of Boc-guanidine **7.7** (c.f. Chapter 6) with tritiated propionic acid was achieved by employing the commercially available reactive succinimidyl ester (**7.8**). Intermediate **7.9** was not isolated and directly deprotected under acidic conditions. After purification by HPLC, the designated radioligand **7.10** ([^{3}H]UR-PI294) was obtained in high radiochemical purity



Scheme 7.1. Synthesis of the radioligand [^{3}H]UR-PI294 (**7.10**). Reagents and conditions: (i) NEt_3, $CHCl_3$, 16 h, rt; (ii) TFA 23 %, $CHCl_3$, 5 h, rt.

(97.8 %) with a specific activity of 41.8 Ci/mmol. The identity of the radioligand was confirmed by HPLC analysis of the labeled (**7.10**) and unlabeled (**7.1**) UR-PI294 by identical retention times (Figure 7.3).

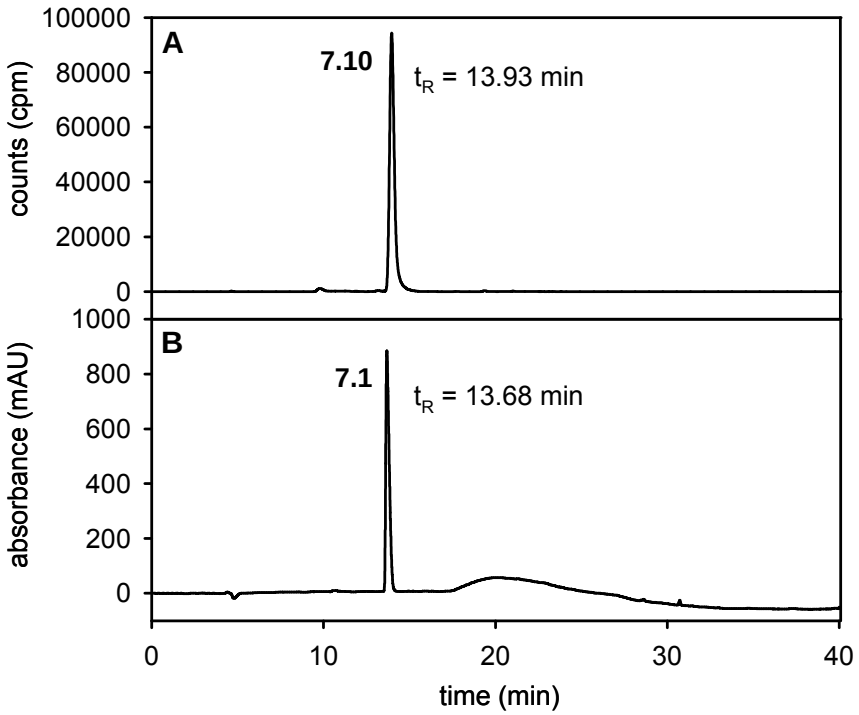


Figure 7.3. Identity and purity control of [^{3}H]UR-PI294 (**7.10**). A, radiochromatogram of the prepared radioligand [^{3}H]UR-PI294 (**7.10**), c: 0.8 µM. B, UV (λ = 210 nm) chromatogram of unlabeled UR-PI294 (**7.1**), c: 100 µM. Conditions: injection volume: 100 µL, gradient: 0.05 % TFA in MeCN (v/v) / 0.05 % TFA in H_2O (v/v): 0 min: 5/95, 13 min: 11.5/88.5, 25 min: 95/5, 40 min: 95/5), flow: 0.8 mL/min. The minor difference in t_R (13.68 vs. 13.93 min) results from the setup of UV and radiodetector in series.

7.3 Results and discussion

7.3.1 Saturation binding analysis of [^{3}H]UR-PI294 at the hH$_3$R and hH$_4$R

The specific binding of [^{3}H]UR-PI294 to membranes of Sf9 insect cells co-expressing hH$_3$R plus $G_{i\alpha2}$ plus $G_{\beta1\gamma2}$ plus RGS4 (regulator of G-protein signaling 4) was saturable (Figure 7.4 A). Up to a radioligand concentration of 5 nM, nonspecific binding, determined in the presence of thioperamide (10 µM) was low, amounting to 5 to 10 % of total binding. Specific binding *versus* [^{3}H]UR-PI294 concentrations was best fitted by nonlinear regression to a one-site binding model (Figure 7.4 A). The determined K_D value of 1.1 nM (Table 7.1) is consistent with the EC_{50} value determined in functional GTPase assays (EC_{50} = 1.6 nM, Figure 7.1) confirming that [^{3}H]UR-PI294 acts as a high-

Table 7.1. Saturation binding parameters of [^{3}H]UR-PI294 at the hH$_3$R and hH$_4$R.[a]

	K_D	B_{max}
	[nM]	[pmol · mg protein^{-1}]
hH$_3$R	1.1 ± 0.2	1.4 ± 0.3
hH$_4$R	5.1 ± 1.9	2.0 ± 0.1

[a] Data are the means of five independent experiments each performed in duplicate.

affinity radioligand at the hH_3R. The estimated B_{max} value was 1.4 pmol per mg of membrane protein. Within the investigated concentration range, the Scatchard plot was linear, which is in agreement with the binding of [^{3}H]UR-PI294 to a single binding site (Figure 7.4 B).

Binding of [^{3}H]UR-PI294 to the hH_4R was determined in Sf9 insect cell membranes co-expressing the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$. Nonspecific binding was determined in the presence of an excess of thioperamide (10 µM). Similar to its binding to the hH_3R, [^{3}H]UR-PI294 bound specifically to the hH_4R (Figure 7.4 C) in a saturable manner. Specific binding *versus* the concentration of [^{3}H]UR-PI294 was best described by a one-site model and afforded a K_D value of 5.1 nM (Table 7.1). This fits very well to the potency determined in the functional GTPase assay (EC_{50} = 3.0 nM, Figure 7.1). The calculated B_{max} value of 2.0 pmol per mg membrane protein reveals similar expression levels of the hH_3R and hH_4R in the Sf9 insect cell membranes. The corresponding Scatchard plot of the ratio bound/free *versus* bound [^{3}H]UR-PI294 revealed a straight line indicating that the radioligand binds to a single binding site (Figure 7.4 D).

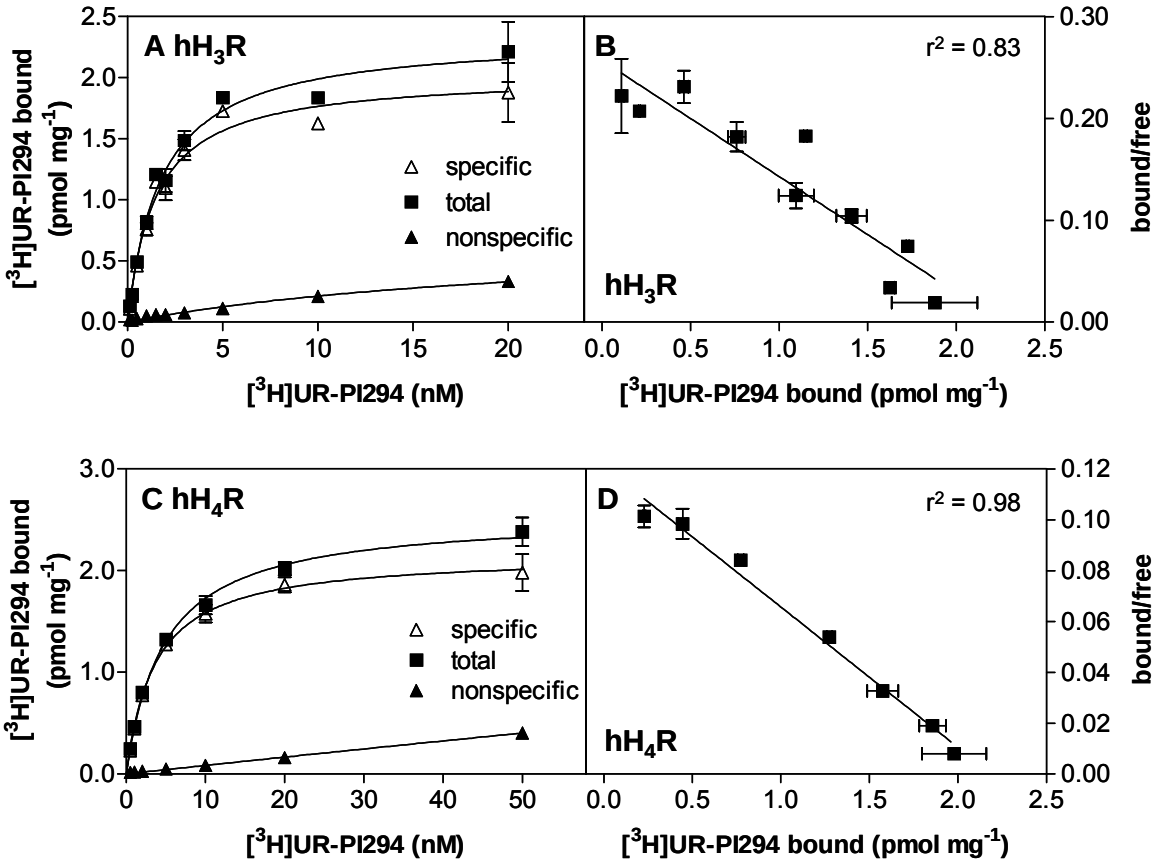


Figure 7.4. Representative [^{3}H]UR-PI294 saturation binding experiments in Sf9 insect cell membranes expressing the hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4 (A, B) or the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ (C, D) performed in duplicate. Membranes were incubated with increasing concentrations of [^{3}H]UR-PI294 as described in the *Pharmacological methods section*. Nonspecific binding was determined in the presence of 10 µmol/L of thioperamide. Specific binding is the difference between the total and nonspecific binding of [^{3}H]UR-PI294 at a given concentration. A, C, data were best fitted by nonlinear regression to a one-site model. B, D, corresponding Scatchard plots of saturation binding data, best fitted by linear regression.

7.3.2 Analysis of the association and dissociation kinetic of [^{3}H]UR-PI294 at the hH_3R and hH_4R

Association and dissociation experiments were carried out with Sf9 insect cell membranes expressing the hH_3R at a [^{3}H]UR-PI294 concentration of 2 nM at 22 °C. As already observed for other hH_3R radioligands,[16, 17] the association and dissociation kinetics of [^{3}H]UR-PI294 at the hH_3R were very rapid (Figure 7.5 A). The specific binding of [^{3}H]UR-PI294 achieved equilibrium after approximately 10 minutes corresponding to an association half-life ($t_{½}$) of 1.4 min. Displacement of [^{3}H]UR-PI294 by thioperamide (10 µM) was complete within 20 min, indicating that [^{3}H]UR-PI294 binds reversibly to the receptor ($t_{½}$ = 3.2 min). The resulting association rate constant (k_{on}) was 0.14 min^{-1} nM^{-1}, and the dissociation rate constant (k_{off}) was 0.21 min^{-1}. The K_D value calculated from the ratio of the k_{off} and k_{on} values (1.5 nM) is in very good agreement with the K_D value determined from the saturation binding isotherms (Table 7.2).

Due to the lower affinity of [^{3}H]UR-PI294 for the hH_4R, a concentration of 10 nM was employed for recording the association and dissociation rates of the radioligand at Sf9 insect cell membranes, expressing the hH_4R-RGS19 fusion protein (22 °C). Compared to the hH_3R, [^{3}H]UR-PI294 showed remarkably slower association and dissociation kinetics at the hH_4R (Figure 7.5 C, D). The specific binding of [^{3}H]UR-PI294 reached equilibrium within 60 min. Association kinetics of [^{3}H]UR-PI294 was almost 10 times slower compared to the hH_3R ($t_{½}$ = 11.4 min). Likewise, the dissociation of [^{3}H]UR-PI294 from the hH_4R, initiated by thioperamide (10 µM), was slow, and not terminated after 1 h. The resulting k_{on} for [^{3}H]UR-PI294 was 0.0027 min^{-1} nM^{-1} and the k_{off} 0.033 min^{-1} yielding a K_D value of 12 nM, which is consistent with the K_D value from the saturation binding studies (Table 7.2). The rather slow association rate of the radioligand at the hH_4R required an incubation period of the membranes with [^{3}H]UR-PI294 of at least 60 min for saturation and competition experiments to ensure measurements at equilibrium.

Table 7.2. Parameters of the kinetic analysis of [^{3}H]UR-PI294 binding in Sf9 insect cell membranes expressing the hH_3R plus $G_{iα2}$ plus $β_1γ_2$ plus RGS4 or the hH_4R-GAIP plus $G_{iα2}$ plus $β_1γ_2$.

	k_{ob}	$t_{½}$ (association)	k_{on}	k_{off}	$t_{½}$ (dissociation)	K_D (kinetic)	K_D (saturation)[a]
	[min^{-1}]	[min]	[min^{-1}·nM^{-1}]	[min^{-1}]	[min]	[nM]	[nM]
hH_3R	0.50	1.4	0.14	0.21	3.2	1.5	1.1
hH_4R	0.06	11.5	0.0027	0.033	20.7	12	5.1

[a] the dissociation constants taken from Table 7.1 determined in saturation binding experiments are listet for comparison.

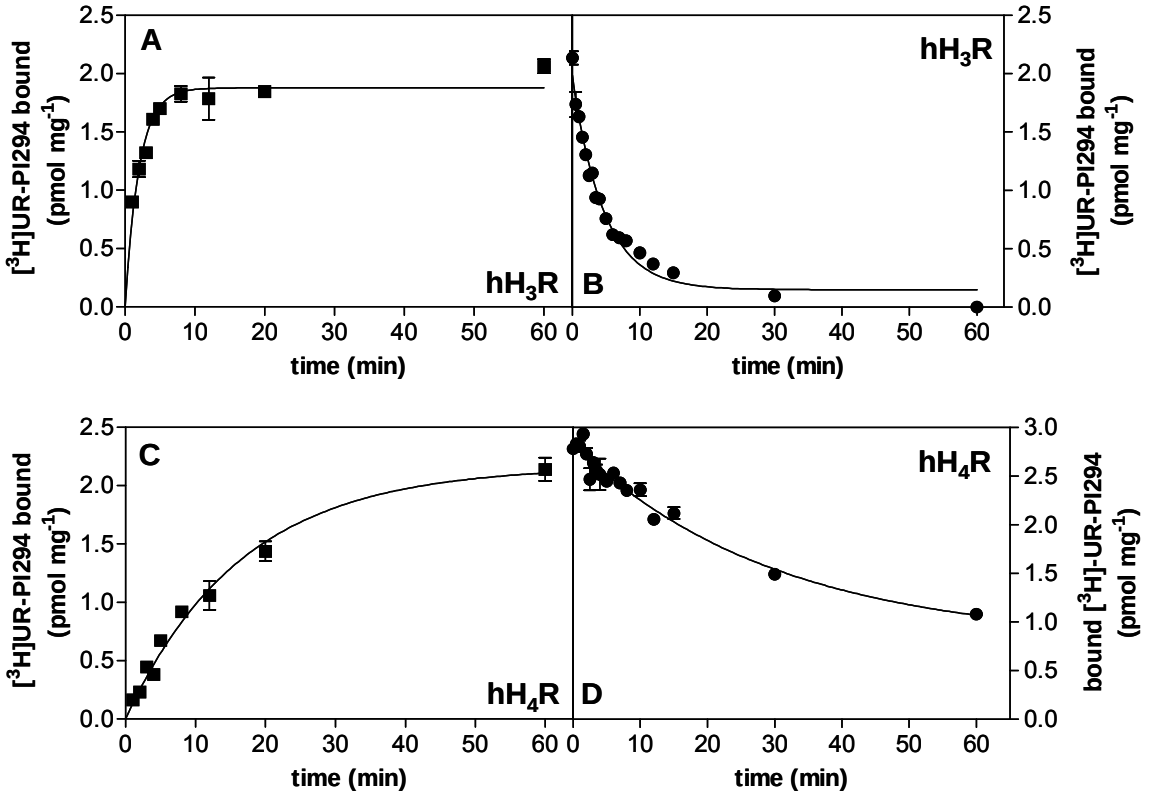


Figure 7.5. Specific binding kinetics of [^{3}H]UR-PI294 to Sf9 insect cell membranes expressing the hH_3R plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ plus RGS4 (A, B) or the hH_4R-RGS19 fusion protein plus $G_{i\alpha 2}$ plus $G_{\beta 1\gamma 2}$ (C, D) were measured as described under *Pharmacological methods*. A, C, association kinetics for 2 nM (A) and 10 nM (C) of [^{3}H]UR-PI294, respectively. Nonspecific binding was determined for each time point in the presence of thioperamide (10 µM). Specific binding is the difference between the total [^{3}H]UR-PI294 and nonspecific [^{3}H]UR-PI294 binding for a given time point. Data were best fitted by nonlinear regression to a one-phase exponential association model. B, D, dissociation kinetics for 2 nM (B) and 10 nM (D) of [^{3}H]UR-PI294, respectively. Displacement of [^{3}H]UR-PI294 was induced by thioperamide (10 µM). Nonspecific binding was determined by incubation of [^{3}H]UR-PI294 for 60 min with the respective membrane in the presence of thioperamide (10 µM). Specific binding is the difference between the total and nonspecific [^{3}H]UR-PI294 binding. Data were best fitted by nonlinear regression to a one-phase exponential decay model. All experiments were performed in duplicate at 22 °C.

7.3.3 Competition binding experiments of [^{3}H]UR-PI294 with reference ligands at the hH_3R and hH_4R

A number of standard ligands for the hH_3R and hH_4R were evaluated for their ability to inhibit specific binding of [^{3}H]UR-PI294 to Sf9 insect cell membranes expressing the hH_3R or hH_4R-RGS19 fusion protein. The resulting K_i values were determined and compared with affinities reported from competition binding experiments using other radioligands for the hH_3R and hH_4R. All obtained competition curves were monophasic and best described by a one-site competition model.

[^{3}H]UR-PI294 was displaced from the hH_3R by the H_3R agonists histamine and (*R*)-α-methylhistamine as well as by the H_3R antagonists (inverse agonists) thioperamide and clobenpropit (Figure 7.6 A). All resulting K_i values were in good agreement with reported K_i values determined with established H_3R radioligands (Table 7.3).

The H_4R ligands histamine, thioperamide, clobenpropit and JNJ 7777120 inhibited specific binding of [^{3}H]UR-PI294 to the hH_4R in a concentration-dependent manner (Figure 7.6 B). As for the hH_3R, the determined K_I values for hH_4R binding were in good agreement with K_I values from literature (Table 7.3).

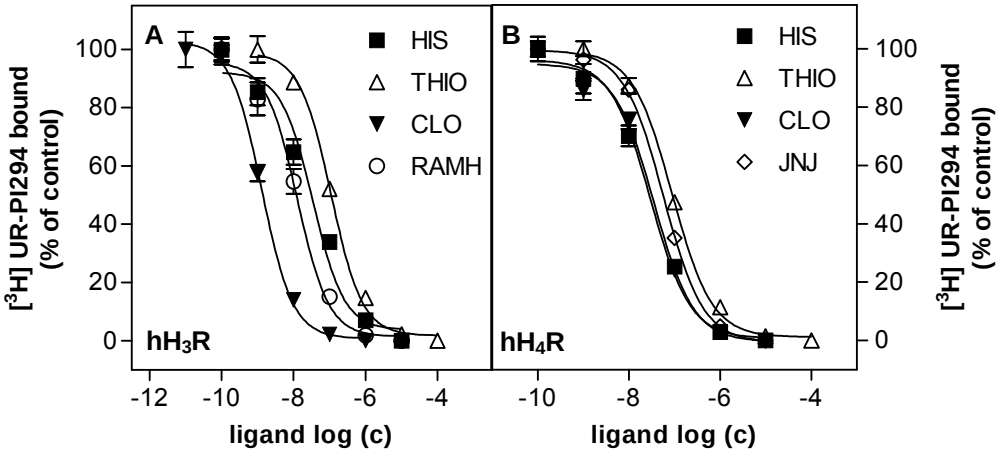


Figure 7.6. Competition of [^{3}H]UR-PI294 binding by reference H_3R and H_4R ligands in Sf9 insect cell membranes expressing the hH_3R plus $G_{i\alpha2}$ plus $G_{\beta1\gamma2}$ plus RGS4 (A) or the hH_4R-RGS19 fusion protein plus $G_{i\alpha2}$ plus $G_{\beta1\gamma2}$ (B). [^{3}H]UR-PI294 concentrations were 2 nM for membranes expressing the hH_3R and 5 nM for membranes expressing the hH_4R. Data were analyzed by nonlinear regression and best fitted to one-site (monophasic) competition curves. Data points shown are the mean of three independent experiments, each performed in duplicate. (HIS: histamine, THIO: thioperamide, CLO: clobenpropit, RAMH: (*R*)-α-methylhistamine, JNJ: JNJ 7777120).

Table 7.3. K_I values of reference H_3R and H_4R ligands from competition binding experiments using [^{3}H]UR-PI294 in Sf9 insect cell membranes expressing the hH_3R plus $G_{i\alpha2}$ plus $G_{\beta1\gamma2}$ plus RGS4 or the hH_4R-RGS19 fusion protein plus $G_{i\alpha2}$ plus $G_{\beta1\gamma2}$ in comparison to K_I values reported in literature.

compound	hH_3R		hH_4R	
	K_I [^{3}H]UR-PI294[a]	K_I reported[b]	K_I [^{3}H]UR-PI294[a]	K_I reported[c]
	[nM]	[nM]	[nM]	[nM]
Histamine	11 ± 3.0	2.8 - 15	15.0 ± 2.0	8.1 ± 17
(*R*)-α-Methyl-histamine	4.5 ± 1.1	0.56 - 6.3	-	-
Thioperamide	38 ± 2.0	25 - 76	45 ± 1.9	27 - 210
Clobenpropit	0.46 ± 0.02	0.34 - 3.8	18 ± 2.2	7.2 - 13
JNJ 7777120	-	-	30 ± 3.8	4.0 - 32

[a] Mean K_I values determined in three independent experiments, each performed in duplicate. [b] ref.[7, 18-23] [c] ref.[7, 12, 14, 21, 24-27]

7.3.4 Summary and conclusions

Binding of [^{3}H]UR-PI294 to Sf9 insect cell membranes expressing the hH_3R or the hH_4R-RGS19 fusion protein was saturable and highly specific. [^{3}H]UR-PI294 showed high affinities for both receptors. However, affinity for the hH_3R was five times higher relative to the hH_4R (K_D = 1.1 nM (hH_3R), K_D = 5.1 nM (hH_4R)). The saturation binding curves were best described by a one-site model and the Scatchard plots appeared to be linear, suggesting [^{3}H]UR-PI294 to bind to a single binding site both at membranes expressing the hH_3R and the hH_4R, respectively. Kinetic experiments showed a rapid association and dissociation of the radioligand from the hH_3R, whereas these processes were about 10 times slower for the hH_4R. The resulting dissociation constants agreed very well with the K_D values obtained from the saturation binding studies for both histamine receptor subtypes. Binding constants determined for H_3R and H_4R reference ligands were consistent with data reported in literature, confirming [^{3}H]UR-PI294 to be a suitable radioligand for the determination of affinities of unlabeled H_3R and H_4R ligands.

Due to the lack of selectivity between the hH_3R and hH_4R, the radioligand is primarily valuable for application in recombinant systems expressing only one histamine receptor subtype. However, in most immune cells such as mast cells or eosinophils, where the H_4R is mainly located, H_3Rs are not expressed.[28-30] Therefore, due to the more than 100-fold selectivity over the hH_1R and hH_2R subtypes, [^{3}H]UR-PI294 may also be applicable for labeling the hH_4R in these native cells devoid of hH_3Rs.

Taken together, [^{3}H]UR-PI294 is a new highly potent acylguanidine-type radioligand for both the hH_3R and hH_4R. The facile preparation and rather low costs employing commercially available tritiated succinimidyl propionate make [^{3}H]UR-PI294 readily accessible under common laboratory conditions. The low efficacy of [^{3}H]UR-PI294 at the hH_3R may result in similar affinities for both the high-affinity and low-affinity binding site of the receptor. Such radioligands are required for studying constitutive activity of GPCRs.[31] Therefore, [^{3}H]UR-PI294 may be a promising tool for investigating constitutive activity of the hH_3R. The high specific activity of [^{3}H]UR-PI294 (41.8 Ci/mmol) compared to commonly available [^{3}H]histamine combined with high affinity and low nonspecific binding at the hH_4R evidence this radioligand in particular as promising pharmacological probe for the hH_4R, which is a promising new target, for instance, for the development of anti-inflammatory drugs.[28]

7.4 Experimental section

7.4.1 Synthesis

7.4.1.1 General conditions

Commercial reagents and chemicals were purchased from Acros Organics (Geel, Belgium), Merck KGaA (Darmstadt, Germany), Sigma-Aldrich Chemie GmbH (Munich, Germany) and used without further purification. Succinimidyl [2,3-$^3H_2$]propionate solution in ethyl acetate (60 Ci/mmol, 5 mCi/mL) was from Biotrend, American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). All solvents were of analytical grade or distilled prior to use. Scintillation cocktail was from Carl Roth GmbH (Rotiszint eco plus, Karlsruhe, Germany). Analytical HPLC was performed on a system from Waters (Waters GmbH, Eschborn, Germany) equipped with a Waters pump control module, Waters 510 HPLC pump, a Waters 486 UV/VIS detector and a Packard radiomatic Flo-one beta series A-500 radiodetector. Stationary phase was an Agilent Scalar C18 (250 x 4.6 mm, 5 µm) column. The flow rate was 0.8 mL/min. As mobile phase gradients of MeCN/TFA 0.05 % (v/v) and H_2O/TFA 0.05 % (v/v) were used. The absorbance was detected at 210 nm or the radioactivity was measured with the radiodetector by liquid scintillation counting. Radiochemical purity of [^{3}H]UR-PI294 was calculated as the percentage peak area from the radiochromatogram.

7.4.1.2 Preparation of N^1-[3-(1H-imidazol-4-yl)propyl]-N^2-[2,3-$^3H_2$]propionyl-guanidine ([^{3}H]UR-PI294, 7.10)

To a solution of **7.7** (preparation c.f. Chapter 6, compound **6.34**) (2.0 µmol, 1.02 mg, 40 eq) and NEt_3 (4.0 µmol, 0.405 mg, 80 eq) in $CHCl_3$ (300 µL), a solution of succinimidyl [2,3-$^3H_2$]propionate in ethyl acetate (600 µL, 50 nmol, 1 eq, 8.76 µg, 3 mCi) was added. The solvent was removed by a rotary evaporator and the residue taken up in $CHCl_3$ (100 µL). After the mixture was stirred for 16 h at room temperature, TFA (30 µL) was added and stirring was continued for additional 5 h. The solvent was removed under reduced pressure and the residue taken up in 500 µL of a mixture of MeCN/H_2O/TFA 5/94.9/0.1 (v/v/v). Insoluble material was removed by centrifugation for 10 min at 13,000 rpm and aliquots (5 x 100 µL) of the supernatant purified by HPLC. The fractions containing the radioligand were collected at approximately 14 min (gradient: 0.05 % TFA in MeCN/0.05 % TFA in H_2O: 0 min: 5/95 (v/v), 13 min: 11.5/88.5 (v/v), 25 min: 95/5 (v/v), 40 min: 95/5 (v/v)). The solvent of the combined fractions was evaporated and the residue was taken up in 450 µL of a mixture of EtOH/H_2O 1/1 (v/v). The concentration of the radioligand in this stock solution was determined after recording a calibration curve with the unlabeled ligand UR-PI294 (HPLC, λ = 210 nm) from the peak area (11.89 µM, 5.35 nmol, yield: 10.7 %). The total activity of

[^{3}H]UR-PI294 was determined by dilution of 1.5 µL of the stock solution with 448.5 µL MeCN/TFA (aq.) 0.1 % 5/95 (v/v) and counting 9 µL of this dilution (three times in duplicate) in 3 mL scintillation cocktail (total activity in 450 µL stock solution: 223.6 µCi). This results in a specific activity for [^{3}H]UR-PI294 of 41.8 Ci/mmol. The activity concentration of the stock solution was adjusted to 250 µCi/mL with a mixture of EtOH/H_2O 1/1 (v/v) (5.98 µM). HPLC analysis showed a radiochemical purity of 97.8 %. The identity of the radioligand was confirmed by HPLC analysis of the labeled and unlabeled UR-PI294 under same conditions resulting in nearly identical retention times (Figure 7.3).

7.4.2 Pharmacological methods

7.4.2.1 General

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). Thioperamide maleate, (*R*)-α-methylhistamine dihydrobromide and clobenpropit dihydrobromide were from Tocris Bioscience (Ellisville, MO, USA). The H_4R antagonist JNJ 7777120 was a gift from Dr. R. Thurmond (Department of Immunology, Johnson & Johnson Pharmaceutical R&D, San Diego, CA, USA). GF/C filters were from Whatman (Maidstone, UK). Radioactivity was determined by liquid scintillation counting in a Beckman LS-6500 device.

7.4.2.2 [^{3}H]UR-PI294 binding assay

Radioligand binding experiments were performed with a similar procedure described for the H_1R and H_2R by Seifert et al. and Kelley et al.[32, 33] For [^{3}H]UR-PI294 binding experiments Sf9 insect cell membranes co-expressing the hH_3R, mammalian $G_{i\alpha 2}$, $G_{\beta 1\gamma 2}$ and RGS4 or the hH_4R-GAIP fusion protein, mammalian $G_{i\alpha 2}$ and $G_{\beta 1\gamma 2}$ were employed. The respective membranes were thawed and sedimented by a 10 min centrifugation at 4 °C and 13,000 g. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4). Each tube (total volume 250 µL) contained 25 – 50 µg (hH_3R) or 50 µg (hH_4R) of membrane protein. Saturation binding experiments were performed in a concentration range from 0.125 – 20 nM (hH_3R) and 0.5 – 50 nM (hH_4R) of [^{3}H]UR-PI294. Nonspecific binding was determined in the presence of 10 µM thioperamide. Competition binding experiments were performed with 2 nM [^{3}H]UR-PI294 (hH_3R) or 5 nM [^{3}H]UR-PI294 (hH_4R) and increasing concentrations of unlabeled ligands. Incubations were conducted for 60 min (hH_3R) or 60 – 90 min (hH_4R) at 22 °C and shaking at 250 rpm. Bound radioligand was separated from free radioligand by filtration through 0.3 % (m/v) polyethyleneimine-pretreated GF/C filters followed by three washes with 2 mL of cold binding buffer (4 °C) using a Brandel Harvester. Pretreating the filter with polyethyleneimine reduced filter binding of

[^{3}H]UR-PI294 to 0.14 %. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting.

7.4.2.3 [^{3}H]UR-PI294 kinetic studies

For the kinetic experiments the membranes were prepared as described for the [^{3}H]UR-PI294 binding assays. Tubes containing [^{3}H]UR-PI294 at a concentration of 2 nM (hH_3R) or 10 nM (hH_4R) and the respective hH_3R or hH_4R membrane at a concentration of 200 µg/mL (hH_3R and hH_4R) in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4) were employed. The reaction mixtures were agitated with a magnetic stir bar and incubated at ambient temperature (22 °C). Association kinetic experiments were started by addition of membrane suspension to the tube. At each time point 250 µL aliquots (50 µg membrane protein) were withdrawn from the tube. Nonspecific binding was determined for each time point in the presence of thioperamide (10 µM). Bound [^{3}H]UR-PI294 was separated from free [^{3}H]UR-PI294 by filtration through GF/C filters (pretreated with 0.3 % polyethyleneimine solution) using a Millipore 1225 vacuum sampling manifold followed by three washes with 2 mL binding buffer (4 °C). Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting. For the dissociation kinetics the tubes containing [^{3}H]UR-PI294 and the respective membrane were incubated for 60 min (hH_3R) or 90 min (hH_4R), respectively before starting dissociation by addition of thioperamide (10 µM final concentration). By analogy with the association kinetics, for each time point, a 250 µL aliquot was removed. Nonspecific binding was determined by incubation [^{3}H]UR-PI294 for 60 min in the presence of thioperamide (10 µM). Bound [^{3}H]UR-PI294 was measured as described for the association kinetics.

7.5 References

(1) Seifert, R., Monovalent Anions Differentially Modulate Coupling of the beta 2-Adrenoceptor to G_{salpha} Splice Variants. *J. Pharmacol. Exp. Ther.* **2001**, 298, 840-847.

(2) Seifert, R., Gether, U., Wenzel-Seifert, K., Kobilka, B. K., Effects of Guanine, Inosine, and Xanthine Nucleotides on beta 2-Adrenergic Receptor/G_s Interactions: Evidence for Multiple Receptor Conformations. *Mol. Pharmacol.* **1999**, 56, 348-358.

(3) Seifert, R., Lee, T. W., Lam, V. T., Kobilka, B. K., Reconstitution of β_2-adrenoceptor-GTP-binding-protein interaction in Sf9 cells . High coupling efficiency in a β_2-adrenoceptor-$G_{s\alpha}$ fusion protein. *Eur. J. Biochem.* **1998**, 255, 369-382.

(4) Arrang, J. M., Garbarg, M., Lancelo, J. C., Lecomte, J. M., Pollard, H., Robba, M., Schunack, W., Schwartz, J. C., Highly potent and selective ligands for histamine H_3-receptors. *Nature* **1987**, 327, 117-123.

(5) West, R. E., Jr., Zweig, A., Shih, N. Y., Siegel, M. I., Egan, R. W., Clark, M. A., Identification of two H_3-histamine receptor subtypes. *Mol. Pharmacol.* **1990**, 38, 610-613.

(6) Korte, A., Myers, J., Shih, N.-Y., Egan, R. W., Clark, M. A., Characterization and tissue distribution of H_3 histamine receptors in guinea pigs by N^{α}-methylhistamine. *Biochem. Biophys. Res. Commun.* **1990**, 168, 979-986.

(7) Lim, H. D., van Rijn, R. M., Ling, P., Bakker, R. A., Thurmond, R. L., Leurs, R., Evaluation of Histamine H_1-, H_2-, and H_3-Receptor Ligands at the Human Histamine H_4 Receptor: Identification of 4-Methylhistamine as the First Potent and Selective H_4 Receptor Agonist. *J. Pharmacol. Exp. Ther.* **2005**, 314, 1310-1321.

(8) Gbahou, F., Vincent, L., Humbert-Claude, M., Tardivel-Lacombe, J., Chabret, C., Arrang, J.-M., Compared pharmacology of human histamine H_3 and H_4 receptors: structure-activity relationships of histamine derivatives. *Br. J. Pharmacol.* **2006**, 147, 744-754.

(9) Hashimoto, T., Harusawa, S., Araki, L., Zuiderveld, O. P., Smit, M. J., Imazu, T., Takashima, S., Yamamoto, Y., Sakamoto, Y., Kurihara, T., Leurs, R., Bakker, R. A., Yamatodani, A., A Selective Human H_4-Receptor Agonist: (-)-2-Cyano-1-methyl-3-{(2*R*,5*R*)-5-[1*H*-imidazol-4(5)-yl]tetrahydrofuran-2-yl}methylguanidine. *J. Med. Chem.* **2003**, 46, 3162-3165.

(10) Oda, T., Morikawa, N., Saito, Y., Masuho, Y., Matsumoto, S., Molecular Cloning and Characterization of a Novel Type of Histamine Receptor Preferentially Expressed in Leukocytes. *J. Biol. Chem.* **2000**, 275, 36781-36786.

(11) Thurmond, R. L., Desai, P. J., Dunford, P. J., Fung-Leung, W.-P., Hofstra, C. L., Jiang, W., Nguyen, S., Riley, J. P., Sun, S., Williams, K. N., Edwards, J. P., Karlsson, L., A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.* **2004**, 309, 404-413.

(12) Morse, K. L., Behan, J., Laz, T. M., West, R. E., Jr., Greenfeder, S. A., Anthes, J. C., Umland, S., Wan, Y., Hipkin, R. W., Gonsiorek, W., Shin, N., Gustafson, E. L., Qiao, X., Wang, S., Hedrick, J. A., Greene, J., Bayne, M., Monsma, F. J., Jr., Cloning and Characterization of a Novel Human Histamine Receptor. *J. Pharmacol. Exp. Ther.* **2001**, 296, 1058-1066.

(13) Venable, J. D., Cai, H., Chai, W., Dvorak, C. A., Grice, C. A., Jablonowski, J. A., Shah, C. R., Kwok, A. K., Ly, K. S., Pio, B., Wei, J., Desai, P. J., Jiang, W., Nguyen, S., Ling, P., Wilson, S. J., Dunford, P. J., Thurmond, R. L., Lovenberg, T. W., Karlsson, L., et al., Preparation and Biological Evaluation of Indole, Benzimidazole, and Thienopyrrole Piperazine Carboxamides: Potent Human Histamine H_4 Antagonists. *J. Med. Chem.* **2005**, 48, 8289-8298.

(14) van Rijn, R. M., Chazot, P. L., Shenton, F. C., Sansuk, K., Bakker, R. A., Leurs, R., Oligomerization of Recombinant and Endogenously Expressed Human Histamine H_4 Receptors. *Mol. Pharmacol.* **2006**, 70, 604-615.

(15) Mantyh, P. W. Autoradiographic Localization and Characterization of Receptor Binding Sites in the Brain and Peripheral Tissues. In *Receptor Localization: Ligand Autoradiography*, Leslie, F. M., Altar, C. A., Eds. Alan R. Liss: New York, **1988**; Vol. 13, pp 9-36.

(16) Witte, D., Yao, B. B., Miller, T. R., Carr, T. L., Cassar, S., Sharma, R., Faghih, R., Surber, B. W., Esbenshade, T. A., Hancock, A. A., Krueger, K. M., Detection of multiple H_3 receptor affinity states utilizing [^{3}H]A-349821, a novel, selective, non-imidazole histamine H_3 receptor inverse agonist radioligand. *Br. J. Pharmacol.* **2006**, 148, 657-670.

(17) Harper, E. A., Shankley, N. P., Black, J. W., Characterization of the binding of [^{3}H]-clobenpropit to histamine H_3-receptors in guinea-pig cerebral cortex membranes. *Br. J. Pharmacol.* **1999**, 128, 881-890.

(18) Ligneau, X., Morisset, S., Tardivel-Lacombe, J., Gbahou, F., Ganellin, C. R., Stark, H., Schunack, W., Schwartz, J. C., Arrang, J. M., Distinct pharmacology of rat and human histamine H_3 receptors: role of two amino acids in the third transmembrane domain. *Br. J. Pharmacol.* **2000**, 131, 1247-1250.

(19) Lovenberg, T. W., Pyati, J., Chang, H., Wilson, S. J., Erlander, M. G., Cloning of Rat Histamine H_3 Receptor Reveals Distinct Species Pharmacological Profiles. *J. Pharmacol. Exp. Ther.* **2000**, 293, 771-778

(20) Wieland, K., Bongers, G., Yamamoto, Y., Hashimoto, T., Yamatodani, A., Menge, W. M. B. P., Timmerman, H., Lovenberg, T. W., Leurs, R., Constitutive Activity of Histamine H_3 Receptors Stably Expressed in SK-N-MC Cells: Display of Agonism and Inverse Agonism by H_3 Antagonists. *J. Pharmacol. Exp. Ther.* **2001**, 299, 908-914.

(21) Liu, C., Ma, X.-J., Jiang, X., Wilson, S. J., Hofstra, C. L., Blevitt, J., Pyati, J., Li, X., Chai, W., Carruthers, N., Lovenberg, T. W., Cloning and Pharmacological Characterization of a Fourth Histamine Receptor (H_4) Expressed in Bone Marrow. *Mol. Pharmacol.* **2001**, 59, 420-426.

(22) Yao, B. B., Hutchins, C. W., Carr, T. L., Cassar, S., Masters, J. N., Bennani, Y. L., Esbenshade, T. A., Hancock, A. A., Molecular modeling and pharmacological analysis of species-related histamine H_3 receptor heterogeneity. *Neuropharmacology* **2003**, 44, 773-786.

(23) Medhurst, A. D., Atkins, A. R., Beresford, I. J., Brackenborough, K., Briggs, M. A., Calver, A. R., Cilia, J., Cluderay, J. E., Crook, B., Davis, J. B., Davis, R. K., Davis, R. P., Dawson, L. A., Foley, A. G., Gartlon, J., Gonzalez, M. I., Heslop, T., Hirst, W. D., Jennings, C., Jones, D. N. C., et al., GSK189254, a Novel H_3 Receptor Antagonist That Binds to Histamine H_3 Receptors in Alzheimer's Disease Brain and Improves Cognitive Performance in Preclinical Models. *J. Pharmacol. Exp. Ther.* **2007**, 321, 1032-1045.

(24) Zhu, Y., Michalovich, D., Wu, H.-L., Tan, K. B., Dytko, G. M., Mannan, I. J., Boyce, R., Alston, J., Tierney, L. A., Li, X., Herrity, N. C., Vawter, L., Sarau, H. M., Ames, R. S., Davenport, C. M., Hieble, J. P., Wilson, S., Bergsma, D. J., Fitzgerald, L. R., Cloning, Expression, and Pharmacological Characterization of a Novel Human Histamine Receptor. *Mol. Pharmacol.* **2001**, 59, 434-441.

(25) Jablonowski, J. A., Grice, C. A., Chai, W., Dvorak, C. A., Venable, J. D., Kwok, A. K., Ly, K. S., Wei, J., Baker, S. M., Desai, P. J., Jiang, W., Wilson, S. J., Thurmond, R. L., Karlsson, L., Edwards, J. P., Lovenberg, T. W., Carruthers, N. I., The First Potent and Selective Non-Imidazole Human Histamine H_4 Receptor Antagonists. *J. Med. Chem.* **2003**, 46, 3957-3960.

(26) Terzioglu, N., van Rijn, R. M., Bakker, R. A., De Esch, I. J. P., Leurs, R., Synthesis and structure-activity relationships of indole and benzimidazole piperazines as histamine H_4 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004**, 14, 5251-5256.

(27) Schneider, E., *Personal communication*, Department of Pharmacology and Toxicology, University of Regensburg: Regensburg (Germany), **2008**.

(28) Thurmond, R. L., Gelfand, E. W., Dunford, P. J., The role of histamine H_1 and H_4 receptors in allergic inflammation: the search for new antihistamines. *Nat. Rev. Drug Discov.* **2008**, 7, 41-53.

(29) Lippert, U., Artuc, M., Grutzkau, A., Babina, M., Guhl, S., Haase, I., Blaschke, V., Zachmann, K., Knosalla, M., Middel, P., Kruger-Krasagakis, S., Henz, B. M., Human Skin Mast Cells Express H_2 and H_4, but not H_3 Receptors. *J. Investig. Dermatol.* **2003**, 123, 116-123.

(30) Ling, P., Ngo, K., Nguyen, S., Thurmond, R. L., Edwards, J. P., Karlsson, L., Fung-Leung, W.-P., Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.* **2004**, 142, 161-171.

(31) Wieland, T., Seifert, R. Methological Approaches. In *G Protein-coupled Receptors as Drug Targets* Seifert, R., Wieland, T., Eds. Wiley-VCH: Weinheim, **2005**; pp 81-120.

(32) Seifert, R., Wenzel-Seifert, K., Bürckstümmer, T., Pertz, H. H., Schunack, W., Dove, S., Buschauer, A., Elz, S., Multiple Differences in Agonist and Antagonist Pharmacology between Human and Guinea Pig Histamine H_1-Receptor. *J. Pharmacol. Exp. Ther.* **2003**, 305, 1104-1115.

(33) Kelley, M. T., Bürckstümmer, T., Wenzel-Seifert, K., Dove, S., Buschauer, A., Seifert, R., Distinct Interaction of Human and Guinea Pig Histamine H_2-Receptor with Guanidine-Type Agonists. *Mol. Pharmacol.* **2001**, 60, 1210-1225.

Chapter 8

Summary

In previous studies N^G-acylated imidazolylpropylguanidines, originally developed as histamine H_2 receptor (H_2R) agonists with reduced basicity relative to guanidine-type H_2R agonists, turned out to be also highly potent at the histamine H_3 receptor (H_3R) and histamine H_4 receptor (H_4R). Particularly at the H_4R, the compounds showed high agonistic efficacies.

This thesis aimed at the design, synthesis and pharmacological characterization of novel acylguanidine-type compounds and analogs to evaluate structure-activity relationships (SAR) at the histamine receptors (HRs) and to obtain HR subtype selective ligands. As the biological role of the recently discovered H_4R is far from being understood, a major interest was the development of potent and selective H_4R agonists as pharmacological tools.

The prepared compounds were investigated for their pharmacological activities at the human (h) H_1R, H_2R, H_3R and H_4R in steady-state GTPase assays, employing membrane preparations of Sf9 insect cells expressing the distinct HR subtype. In addition, selected compounds were evaluated at the guinea pig (gp) ileum for H_1R activity and at the guinea pig right atrium for H_2R activity (in cooperation with Prof. Seifert (Medical School Hannover) and Prof. Elz (University of Regensburg)).

In the first series of compounds the basic central group of the N^G-acylated imidazolylpropyl-guanidines was replaced with a non-basic cyanoguanidine moiety resulting in moderate partial agonists at the hH_4R. As efforts to improve potency and efficacy by modifying the second alkyl substituent besides the imidazolylpropyl group were unsuccessful, the focus was turned on the carbon chain connecting the imidazole ring and the cyanoguanidine group. Elongation of the spacer length by one methylene group was the key step toward potent and selective hH_4R agonists. Out of these cyanoguanidines, 2-cyano-1-[4-(1*H*-imidazol-4-yl)butyl]-3-[(2-phenylthio)ethyl]guanidine (UR-PI376) was the most potent hH_4R agonist (hH_4R: EC_{50} = 34 nM, E_{max} = 0.93). Additionally, UR-PI376 showed a more than 25-fold selectivity over the hH_3R in the GTPase assay and negligible activities at the hH_1R and hH_2R. In contrast to other reported selective H_4R agonists, UR-PI376 exerted no agonistic activity at the other HR subtypes which makes this compound a promising pharmacological tool for studying the biological function of the hH_4R. In addition, the carbamoylguanidine-type

compound 2-carbamoyl-1-[2-(1*H*-imidazol-4-yl)ethyl]-3-(3-phenylpropyl)guanidine (UR-PI97) was identified as highly potent hH_3R inverse agonist (K_B = 3.8 nM, E_{max} = -0.97) showing a more than 300-fold selectivity over the hH_4R.

The next series was focused on the imidazolylpropylguanidine portion of the acylguanidines. Minor structural modifications of this group essentially changed the pharmacological activities at the HR subtypes. For example, all performed modifications abolished hH_4R agonistic activity, whereas introduction of a methyl group in position 5 of the imidazole ring remarkably improved efficacy at the hH_3R. The study showed the imidazolylalkylguanidine part to be a promising structural motif for the development of HR ligands with new pharmacological profiles.

Substitution of the 4-imidazolyl ring with isomers and other heterocycles was critical for the activity at all HRs. The most promising compounds in this series were the 1*H*-1,2,4-triazol-3-yl analogs that showed (partial) agonistic activity at the hH_2R and gpH_2R and negligible activities at the other HR subtypes. This suggests the triazole ring to be promising bioisosteric imidazole replacement for the generation of new selective H_2R agonists.

Attaching small N^G-alkanoyl residues to the imidazolylpropylguanidine moiety turned out to be favorable for hH_4R activity. Potent (partial) hH_4R agonists with up to 1000- and 100-fold selectivity relative to hH_1R and hH_2R, respectively, and only low efficacy at the hH_3R were obtained.

Due to the high potency of N^1-[3-(1*H*-imidazol-4-yl)propyl]-N^2-propionylguanidine at the hH_3R and hH_4R, this ligand was prepared as tritiated radioligand ([^{3}H]UR-PI294). This readily available new radioligand specifically bound to the hH_3R and hH_4R with high affinities (K_D (hH_3R) = 1.1 nM, K_D (hH_4R) = 5.1 nM) and proved to be a valuable pharmacological tool for the determination of binding constants of H_3R and H_4R ligands.

In conclusion, the structural modifications of N^G-acylated imidazolylpropylguanidines yielded valuable information about SAR and structure-selectivity relationships and revealed new promising pharmacological tools for HR. The imidazolylbutylcyanoguanidines represent a new class of potent and selective hH_4R agonists. Moreover, the carbamoylguanidine UR-PI97 was identified as a highly potent and selective hH_3R inverse agonist. The synthesized N^G-alkanoyl imidazolylpropylguanidines are among the most potent hH_4R agonists and paved the way to the new high affinity hH_3R and hH_4R radioligand [^{3}H]UR-PI294.

Chapter 9

Appendix

9.1 Data analysis and pharmacological parameters

All data are presented as mean of n independent experiments ± SEM. Agonist potencies were given as EC_{50} values (molar concentration of the agonist causing 50 % of the maximal response) or as pEC_{50} values (negative decadic logarithm of the EC_{50} value). pEC_{50} values obtained from the guinea pig right atrium were corrected according to the long term mean value of the reference agonist histamine in our laboratory (guinea pig atrium (gpH_2R): pEC_{50} = 6.00 for histamine). Maximal responses were expressed as E_{max} values. The E_{max} value of histamine was set to 1.00, E_{max} values of other compounds were referred to this value.

IC_{50} values were converted to K_I and K_B values using the Cheng-Prussoff equation.[1] K_I values were analyzed by nonlinear regression and best fit to one-site (monophasic) competition curves. EC_{50}/K_B values from the functional GTPase assays were analyzed by nonlinear regression and best fit to sigmoidal dose-response curves (GraphPad Prism 4.02 software, San Diego, CA).

Antagonist potencies determined on isolated organs were expressed as apparent pA_2. The apparent pA_2 value was calculated according to following equation: $pA_2 = \log (r - 1) - \log [B]$, where [B] is the molar concentration of antagonist and r the ratio of agonist EC_{50} measured in the presence and absence of antagonist.[2] Noncompetitive antagonists were characterized at the guinea pig ileum and atrium by estimation of a pD'_2 value according to the equation: $pD'_2 = -\log c(B) + \log(100/E_{max} - 1)$.[3]

Observed rate constants (k_{ob}) were analyzed by nonlinear regression and best fit to one-phase exponential association curves. Dissociation constants (k_{off}) were analyzed by nonlinear regression and best fit to one-phase exponential decay curves. (GraphPad Prism 4.02 software, San Diego, CA, USA). k_{ob} is the observed association rate constant. Association rate constants (k_{on}) were calculated according to following equation: $k_{on} = (k_{ob} - k_{off})/[\text{radioligand}]$.[4] Dissociation constants (K_D) from the kinetic experiments were calculated according to following equation: $K_D = k_{off}/k_{on}$.[4]

9.2 Elemental analysis data

No.	formula	calculated			found		
		C	H	N	C	H	N
3.11	$C_9H_{19}N \cdot HCl \cdot 0.25\ H_2O$	59.32	11.34	7.69	59.64	11.25	7.93
3.12	$C_8H_{11}NS \cdot HCl$	50.65	6.38	7.38	50.49	6.36	7.30
3.14	$C_9H_{12}FN \cdot HCl$	57.00	6.91	7.39	56.96	6.95	7.26
3.15	$C_8H_{12}N_2 \cdot 2\ C_6H_3N_3O_7$	40.41	3.05	18.85	40.42	3.15	19.20
3.16	$C_8H_{12}N_2 \cdot 2\ C_6H_3N_3O_7$	40.41	3.05	18.85	40.23	2.91	19.08
3.17	$C_8H_{12}N_2 \cdot 2\ C_6H_3N_3O_7$	40.41	3.05	18.85	40.37	2.91	19.19
3.18	$C_{10}H_{15}N \cdot HCl$	64.68	8.68	7.54	64.81	8.86	7.23
3.24	$C_{17}H_{17}N_3O$	73.10	6.13	15.04	73.02	6.07	15.12
3.25	$C_{16}H_{15}N_3O$	72.43	5.70	15.84	72.37	5.67	15.95
3.26	$C_{18}H_{19}N_3O$	73.69	6.53	14.32	73.41	6.57	14.25
3.27	$C_{23}H_{21}N_3O$	77.72	5.96	11.82	77.83	5.99	11.91
3.28	$C_{17}H_{23}N_3O$	71.55	8.12	14.72	71.56	7.95	14.89
3.29	$C_{16}H_{15}N_3OS$	64.62	5.08	14.13	64.57	5.07	14.16
3.30	$C_{18}H_{16}N_4O$	71.04	5.30	18.41	70.81	5.39	18.40
3.31	$C_{17}H_{16}FN_3O$	68.67	5.42	14.13	68.49	5.17	14.04
3.32	$C_{16}H_{16}N_4O$	68.55	5.75	19.99	68.51	5.69	20.29
3.33	$C_{16}H_{16}N_4O$	68.55	5.75	19.99	68.31	5.84	20.19
3.34	$C_{16}H_{16}N_4O$	68.55	5.75	19.99	68.32	5.43	20.39
3.35	$C_{18}H_{19}N_3O$	73.69	6.53	14.32	73.67	6.76	14.39
3.36	$C_8H_7N_3O$	59.62	4.38	26.07	59.68	4.34	26.32
3.37	$C_9H_9N_3O$	61.70	5.18	23.99	61.53	5.08	24.09
3.38	$C_{10}H_{11}N_3O$	63.48	5.86	22.21	63.37	5.82	22.35
3.39	$C_{11}H_{13}N_3O$	65.01	6.45	20.68	65.17	6.21	20.97

Elemental analysis data (continued)

3.40	$C_{12}H_{15}N_3O$	66.34	6.96	19.34	66.11	7.00	19.63
3.41	$C_6H_{11}N_3 \cdot 2\ HCl$	36.38	6.61	21.21	36.09	6.65	21.39
3.42	$C_{17}H_{22}N_6 \cdot 0.25\ H_2O$	64.84	7.20	26.69	64.93	7.50	26.85
3.43	$C_{16}H_{20}N_6 \cdot 0.7\ C_2H_2O_4 \cdot 0.5\ H_2O$	56.73	6.13	22.81	56.97	6.45	22.50
3.44	$C_{18}H_{24}N_6 \cdot 0.85\ C_2H_2O_4$	59.01	6.46	20.96	58.98	6.81	21.30
3.45	$C_{23}H_{26}N_6 \cdot 0.25\ H_2O$	70.65	6.83	21.49	70.33	6.89	21.64
3.46	$C_{17}H_{28}N_6 \cdot 0.8\ C_2H_2O_4$	57.51	7.68	21.63	57.18	7.45	21.40
3.47	$C_{16}H_{20}N_6S \cdot 0.75\ C_2H_2O_4 \cdot 0.25\ H_2O$	52.49	5.54	20.99	52.20	5.61	21.14
3.48	$C_{18}H_{21}N_7 \cdot C_2H_2O_4$	56.46	5.45	23.05	56.51	5.20	22.82
3.52	$C_{16}H_{20}N_6 \cdot 0.9\ C_2H_2O_4 \cdot 0.25\ H_2O$	55.98	5.89	22.01	55.71	5.72	22.39
3.53	$C_{15}H_{18}N_6 \cdot 0.8\ C_2H_2O_4 \cdot 0.25\ H_2O$	55.56	5.65	23.42	55.23	5.76	23.68
3.54	$C_{17}H_{22}N_6 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$	56.36	6.10	20.75	56.28	6.05	20.93
3.55	$C_{22}H_{24}N_6 \cdot 0.25\ H_2O$	70.09	6.55	22.29	70.39	6.85	22.68
3.56	$C_{16}H_{26}N_6 \cdot C_2H_2O_4$	55.09	7.19	21.41	55.34	7.29	21.67
3.57	$C_{15}H_{18}N_6S \cdot C_2H_2O_4$	50.48	4.98	20.78	50.51	5.20	20.89
3.58	$C_{17}H_{22}N_6 \cdot 0.25\ H_2O$	62.65	6.03	30.09	62.58	6.31	29.91
3.60	$C_7H_{13}N_3 \cdot 2\ HBr$	27.93	5.02	13.96	27.83	5.24	13.81
3.61	$C_{18}H_{24}N_6 \cdot 0.9\ C_2H_2O_4$	58.65	6.41	20.73	58.73	6.64	20.83
3.62	$C_{17}H_{22}N_6 \cdot 0.75\ C_2H_2O_4 \cdot 0.5\ H_2O$	57.45	6.08	21.95	57.43	6.38	21.72
3.63	$C_{19}H_{26}N_6 \cdot 0.85\ C_2H_2O_4$	59.91	6.73	20.25	59.71	6.90	20.44
3.64	$C_{24}H_{28}N_6 \cdot 0.25\ H_2O$	71.17	7.09	20.75	70.89	7.24	20.94

Elemental analysis data (continued)

3.65	$C_{18}H_{30}N_6 \cdot 0.8\ C_2H_2O_4 \cdot 0.25\ H_2O$	57.84	7.95	20.65	58.20	8.27	20.45
3.66	$C_{17}H_{22}N_6S \cdot 0.75\ C_2H_2O_4$	54.20	5.78	20.50	53.94	6.16	20.75
3.67	$C_{19}H_{23}N_7 \cdot C_2H_2O_4$	57.39	5.73	22.31	56.50	6.37	21.57
3.68	$C_{18}H_{23}FN_6 \cdot C_2H_2O_4 \cdot H_2O$	53.33	6.04	18.66	53.07	6.10	18.28
3.69	$C_{17}H_{23}N_7 \cdot 2\ C_2H_2O_4 \cdot H_2O$	48.18	5.58	18.73	48.30	5.92	18.49
3.70	$C_{17}H_{23}N_7 \cdot 2\ C_2H_2O_4 \cdot 1.3\ H_2O$	47.69	5.64	18.54	47.94	5.95	18.14
3.71	$C_{17}H_{23}N_7 \cdot 2\ C_2H_2O_4 \cdot 2\ H_2O$	46.58	5.77	18.11	46.97	5.94	17.92
3.72	$C_{19}H_{26}N_6 \cdot 0.85\ C_2H_2O_4$	59.91	6.73	20.25	59.58	7.04	20.39
3.73	$C_9H_{14}N_6 \cdot C_2H_2O_4 \cdot 0.1\ H_2O$	44.32	5.48	28.19	44.08	5.76	28.36
3.74	$C_{10}H_{16}N_6 \cdot C_2H_2O_4 \cdot 0.4\ H_2O$	45.39	5.97	26.47	45.66	6.22	26.28
3.75	$C_{11}H_{18}N_6 \cdot 0.75\ C_2H_2O_4$	49.74	6.51	27.84	49.79	6.87	27.64
3.76	$C_{12}H_{20}N_6 \cdot 0.75\ C_2H_2O_4 \cdot 0.25\ H_2O$	50.61	6.92	26.23	50.26	7.17	26.16
3.77	$C_{13}H_{22}N_6 \cdot 0.75\ C_2H_2O_4 \cdot 0.25\ H_2O$	52.08	7.23	25.13	51.88	7.60	25.47
3.80	$C_{18}H_{24}N_6S \cdot 0.85\ C_2H_2O_4 \cdot 0.25\ H_2O$	54.08	6.04	19.21	53.76	6.21	19.58
3.81	$C_{24}H_{28}N_6S \cdot C_2H_2O_4 \cdot 0.5\ H_2O$	58.74	5.88	15.81	59.01	6.24	15.62
3.83	$C_{30}H_{42}N_4O_8$	61.42	7.22	9.55	61.35	7.51	9.48
3.88	$C_{30}H_{38}N_8O_6 \cdot 0.5\ H_2O$	58.52	6.38	18.20	58.65	6.58	18.36
3.89	$C_{22}H_{24}N_6O_2 \cdot 0.25\ H_2O$	64.61	6.04	20.55	64.37	5.92	20.56
3.90	$C_{24}H_{28}N_6O_2$	66.65	6.53	19.43	66.35	6.45	19.13
3.91	$C_{26}H_{32}N_6O_2$	67.80	7.00	18.25	67.49	7.10	18.09
3.105	$C_{33}H_{27}N_3O_2$	79.66	5.47	8.44	79.35	5.31	8.10

Elemental analysis data (continued)

3.106	$C_{25}H_{25}N_3 \cdot 0.5\ H_2O$	79.75	6.96	11.16	80.15	6.67	11.06
3.108	$C_{14}H_{15}NO_3$	68.56	6.16	5.71	68.63	6.25	5.61
3.109	$C_{14}H_{14}BrNO_3$	51.87	4.35	4.32	51.86	4.38	4.32
3.112	C_9H_6FN	73.46	4.11	9.52	73.41	3.92	9.57
3.113	$C_9H_8FN \cdot 0.1\ H_2O$	71.60	5.47	9.28	71.77	5.22	9.17
3.116	$C_{10}H_{13}N \cdot HCl$	65.39	7.68	7.63	65.39	7.82	7.38
3.120	$C_{16}H_{14}N_2O_2 \cdot C_6H_3N_3O_7$	53.34	3.46	14.14	53.10	3.20	14.28
3.121	$C_{16}H_{14}N_2O_2 \cdot C_6H_3N_3O_7$	53.34	3.46	14.14	53.24	3.12	14.42
3.122	$C_{16}H_{14}N_2O_2 \cdot C_6H_3N_3O_7$	53.34	3.46	14.14	53.21	3.42	14.22
4.7	$C_{23}H_{18}N_2O$	81.63	5.36	8.28	81.56	5.25	8.18
4.8	$C_{24}H_{20}N_2O$	81.79	5.72	7.95	81.80	5.46	7.78
4.9	$C_{24}H_{19}N_3O_2$	75.57	5.02	11.02	75.47	5.23	10.86
4.10	$C_{25}H_{21}N_3O_2$	75.93	5.35	10.63	75.78	5.60	10.33
4.11	$C_{24}H_{21}N_3O \cdot 0.25\ H_2O$	77.50	5.83	11.30	77.80	5.91	10.98
4.12	$C_{25}H_{23}N_3O \cdot 0.75\ H_2O$	76.02	6.25	10.64	76.16	6.27	10.62
4.15	$C_{28}H_{26}N_2O_2$	79.59	6.20	6.63	79.40	6.28	6.36
4.16	$C_{28}H_{26}N_2O_2$	79.59	6.20	6.63	79.59	6.37	6.47
4.17	$C_{28}H_{28}N_2O_2$	79.22	6.65	6.60	78.99	6.95	6.39
4.18	$C_{28}H_{26}N_2O_2$	79.22	6.65	6.60	79.17	6.71	6.43
4.19	$C_{26}H_{26}N_2O$	81.64	6.85	7.32	81.46	6.93	7.34
4.20	$C_{26}H_{26}N_2O$	81.64	6.85	7.32	81.36	7.01	7.10
4.21	$C_{25}H_{23}ClN_2 \cdot HCl$	70.92	5.71	6.62	70.85	5.87	6.54
4.22	$C_{26}H_{23}N_3$	82.73	6.14	11.13	82.68	6.19	10.94
4.24	$C_{26}H_{25}N_3O$	78.96	6.37	10.62	79.01	6.44	10.69
4.26	$C_6H_{13}N_3O_2$	45.27	8.23	26.40	45.12	8.52	26.31

Elemental analysis data (continued)

4.27	$C_{14}H_{19}N_3O_4$	57.33	6.53	14.33	57.06	6.80	14.29
4.28	$C_{17}H_{17}N_3O_4$	62.38	5.23	12.84	62.40	5.57	13.03
5.12	$C_{25}H_{24}N_2O$	81.49	6.57	7.60	81.54	6.71	7.40
5.14	$C_{22}H_{17}IN_2$	60.56	3.93	6.42	60.49	3.92	6.41
5.15	$C_{25}H_{20}N_2O \cdot 0.25\ H_2O$	81.39	5.60	7.59	81.34	5.67	7.34
5.16	$C_{25}H_{24}N_2O$	81.49	6.57	7.60	81.31	6.80	7.70
5.18	$C_{21}H_{17}N_3$	81.00	5.50	13.49	80.60	5.68	13.46
5.19	$C_{22}H_{17}N_3O$	77.86	5.05	12.38	77.69	5.12	12.22
5.20 (*E*)	$C_{26}H_{23}N_3O_2$	76.26	5.66	10.26	76.04	5.65	10.03
5.20 (*Z*)	$C_{26}H_{23}N_3O_2$	76.26	5.66	10.26	76.08	5.97	10.03
5.21	$C_{26}H_{25}N_3O_2$	75.89	6.12	10.21	75.98	5.95	10.11
5.22	$C_{24}H_{23}N_3O$	78.02	6.27	11.37	77.79	6.41	11.39
5.39	$C_{42}H_{39}N_5O_4$	74.43	5.80	10.33	74.27	5.93	10.13
5.40	$C_{41}H_{38}N_6O_4$	72.55	5.64	12.38	72.47	5.56	12.42
5.46	$C_{11}H_{20}N_4O \cdot 2\ C_6H_3N_3O_7$	40.47	3.84	20.52	40.52	3.81	20.59
5.50	$C_{10}H_{15}N_3 \cdot C_6H_3N_3O_7$	47.29	4.46	20.68	47.01	4.38	20.65
5.51	$C_9H_{14}N_4 \cdot 2\ C_6H_3N_3O_7$	39.63	3.17	22.01	39.46	2.83	22.40
5.52	$C_9H_{14}N_4 \cdot 2\ C_6H_3N_3O_7$	39.63	3.17	22.01	39.45	2.81	22.41
5.53	$C_9H_{14}N_4 \cdot 2\ C_6H_3N_3O_7$	39.63	3.17	22.01	39.36	2.79	22.30
5.54	$C_7H_{13}N_5 \cdot 2\ C_6H_3N_3O_7$	36.49	3.06	24.64	36.51	2.84	24.81
6.2	$C_7H_{13}N_5 \cdot 2\ HCl \cdot 0.5\ H_2O$	33.75	6.47	28.46	33.36	6.72	28.52
6.31	$C_{33}H_{27}N_3O_2$	79.66	5.47	8.44	79.35	5.31	8.10
6.32	$C_{25}H_{25}N_3 \cdot 0.5\ H_2O$	79.75	6.96	11.16	80.15	6.67	11.06

Elemental analysis data (continued)

6.34	$C_{31}H_{35}N_5O_2 \cdot 0.5\ H_2O$	71.79	7.00	13.50	71.72	6.95	13.44
6.43	$C_{12}H_{12}N_2O_3$	62.06	5.21	12.06	62.09	5.03	12.09
6.44	$C_{13}H_{14}N_2O_3$	63.40	5.73	11.38	63.45	5.87	11.30
6.45	$C_{14}H_{16}N_2O_3$	64.60	6.20	10.76	64.64	6.46	11.04
6.46	$C_{14}H_{16}N_2O_3$	64.60	6.20	10.76	64.67	6.16	10.68
6.47	$C_{11}H_{10}N_2O_3$	60.55	4.62	12.84	60.34	4.72	12.83
6.48	$C_{12}H_{12}N_2O_3$	62.06	5.21	12.06	61.87	5.56	11.95
6.49	$C_{14}H_{16}N_2O_3$	63.40	5.73	11.38	63.25	5.90	11.23
6.50	$C_{13}H_{14}N_2O_3 \cdot 0.1\ H_2O$	62.94	5.77	11.29	62.79	5.64	11.10

9.3 HPLC purity data

No.	t_R (min)	k'	purity (%)	No.	t_R (min)	k'	purity (%)
3.49[b]	14.58	3.39	99.5	**5.60**[e]	19.82	4.97	97.0
3.50[b]	16.10	3.85	99.7	**5.61**[c]	10.14	2.05	99.9
3.59[b]	13.60	3.10	100	**5.62**[c]	15.04	3.53	98.7
3.78[b]	14.73	3.44	99.6	**5.63**[c]	10.07	2.03	99.4
3.92[f]	6.84	1.69	99.7	**5.64**[c]	14.82	3.46	96.5
3.93[f]	10.61	3.18	98.1	**5.65**[c]	9.94	1.99	99.6
3.94[f]	13.44	4.29	97.6	**5.66**[c]	14.60	3.40	99.8
3.95[f]	16.43	5.47	94.4	**5.67**[b]	17.65	4.32	99.6
3.102[a]	15.08	3.54	99.7	**5.68**[b]	17.12	4.16	99.2
3.103[b]	15.42	3.64	97.8	**5.69**[b]	21.02	5.33	99.0
4.63[b]	15.28	3.60	99.3	**5.70**[b]	17.68	4.33	99.8
4.64[b]	14.48	3.36	99.7	**5.71**[b]	15.95	3.80	99.4
4.65[b]	18.84	4.67	99.8	**5.72**[b]	19.32	4.82	98.9
4.66[b]	16.31	3.91	99.8	**5.81**[b]	15.72	3.73	99.9
4.67[b]	15.36	3.63	99.0	**5.82**[b]	15.17	3.57	98.4
4.68[b]	19.72	4.94	99.9	**5.83**[b]	19.18	4.78	98.4
4.69[b]	16.67	4.02	100	**5.84**[b]	16.15	3.86	100
4.70[b]	16.58	3.99	99.8	**5.85**[c]	13.70	3.13	97.6
4.71[b]	15.94	3.80	98.5	**5.86**[c]	18.74	4.64	94.3
4.72[b]	19.78	4.96	100	**5.87**[c]	11.39	2.43	99.7
4.73[b]	16.63	4.01	100	**5.88**[c]	16.67	4.02	96.4
4.74[b]	16.59	4.00	100	**6.15**[a]	14.71	3.43	95.4
4.75[b]	15.69	3.73	98.5	**6.16**[a]	16.23	3.89	99.8
4.76[b]	19.79	4.96	99.6	**6.17**[a]	15.61	3.70	99.7
4.77[b]	16.58	3.99	97.8	**6.18**[b]	14.44	3.35	98.7
4.78[a]	16.64	4.01	99.7	**6.19**[a]	15.43	3.65	100
4.79[a]	16.07	3.84	98.0	**6.20**[a]	16.52	3.98	99.1
4.80[a]	20.63	5.21	99.2	**6.21**[b]	14.55	3.38	96.2
4.81[b]	15.91	3.79	100	**6.22**[d]	6.12	0.84	100
4.82[b]	15.20	3.58	99.7	**6.23**[d]	9.44	1.84	99.8
4.83[b]	18.81	4.67	100	**6.24**[d]	14.18	3.27	99.9
5.59[e]	14.23	3.29	98.1	**6.25**[d]	13.46	3.05	99.9

[a] TSP-system, gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 5/95, 25 min: 50/50, 30 min: 95/5, 35 min: 95/5. [b] TSP-system, gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 5/95, 25 min: 55/45, 30 min: 90/10, 40 min: 90/10. [c] TSP-system, gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 20/80, 25 min: 55/45, 30 min: 90/10, 40 min: 90/10. [d] TSP-system, gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 5/95, 25 min: 20/80, 30 min: 90/10, 40 min: 90/10. [e] TSP-system, gradient mode: MeCN/0.05 % TFA (aq.): 0 min: 40/60, 25 min: 60/40, 30 min: 90/10, 40 min: 90/10. [f] Merck-Hitachi-system, gradient mode: 0.025 % TFA in MeCN/0.025 % TFA (aq.): 0 min: 20/80, 25 min: 65/35, 30 min: 90/10, 40 min: 90/10.

9.4 Short lectures and poster presentations

Igel P., Schneider E., Schnell D., Elz S., Seifert R., Buschauer A., "*Imidazolylbutylcyanoguanidines: Synthesis and pharmacological characterization of new potent and selective H_4R agonists*", 4th Summer School Medicinal Chemistry, University of Regensburg, September 29 – October 01, 2008.

Igel P., Schneider E., Schnell D., Elz S., Seifert R. and Buschauer A., "*UR-PI376: A new potent and selective H_4 receptor agonist*", Abstract published in: Drugs of the Future 33 (Suppl. A), 189, XXth International Symposium on Medicinal Chemistry, Vienna, August 31 – September 04, 2008.

Igel P., Ghorai P., Kraus A., Schneider E., Schnell D., Elz S., Seifert R., and Buschauer A., „*Imidazolylalkylcyanoguanidines: Towards new selective histamine H_4 receptor agonists*", Annual meeting "Frontiers in Medicinal Chemistry", University of Regensburg, March 02 – 05, 2008.

„Towards selective H_4R agonists: Synthesis and pharmacological properties of N^G-acylated arylalkylguanidines and analogs" short lecture in occasion of the joint meeting of the GRK 760 and GRK 677 (Bonn), Nuremberg, October 08th – 10th, 2007 and of the Christmas Colloquium of the Department of Organic Chemistry, University of Regensburg, December 12, 2007.

Igel P., Ghorai P., Kraus A., Schneider E., Schnell D., Elz S., Seifert R., and Buschauer A. „*N^G-Acylated imidazolylalkylguanidines and analogs as histamine ($H_{2,3}$ or H_4) receptor ligands*", Annual meeting of the German Pharmaceutical Society (DPhG), Erlangen, October 10 – 13, 2007.

Igel P., Ghorai P., Kraus A., Schneider E., Elz S., Seifert R., Buschauer A., "*Design and synthesis of novel histamine H_4 receptor agonists*", 3rd Summer School Medicinal Chemistry, University of Regensburg, September 25 – 27, 2006.

Igel P., Ghorai P., Kraus A., Schneider E., Elz S., Seifert R. and Buschauer A., "*Design and synthesis of novel histamine H_4 receptor agonists*", Abstract published in: Drugs of the Future 31 (Suppl. A), 135, XIXth International Symposium on Medicinal Chemistry, Istanbul, August 29 – September 02, 2006.

9.5 Publications

Igel P., Schnell D., Bernhardt G., Seifert R., Buschauer A., Tritium-labeled N^1-[3-(1*H*-imidazol-4-yl)propyl]-N^2-propionylguanidine ([^{3}H]UR-PI294), a high affinity histamine H_3 and H_4 receptor radioligand, *ChemMedChem* **2008**, in press.

Igel P., Schneider E., Schnell D., Elz S., Seifert R., Buschauer A., N^G-Acylated imidazolylpropylguanidines as potent histamine H_4 receptor agonists: selectivity by variation of the N^G-substituent, submitted to *J. Med. Chem.*, **2008**.

Ghorai, P., Kraus, A., Keller, M., Götte, C., Igel, P., Schneider, E., Schnell, D., Bernhardt, G., Dove, S., Zabel, M., Elz, S., Seifert, R., Buschauer, A., Acylguanidines as Bioisosteres of Guanidines: N^G-Acylated Imidazolylpropylguanidines, a New Class of Histamine H_2 Receptor Agonists. *J. Med. Chem.* **2008**, in press.

9.6 References

(1) Cheng, Y.-C., Prusoff, W. H., Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, 22, 3099-3108.

(2) Furchgott, R. F. The Classification of Adrenoceptors (Adrenergic Receptors). In *Catecholamines, Handbook of Experimental Pharmacology*, Blaschko, H., Muscholl, E., Eds. Springer-Verlag: Berlin, **1972**; Vol. 33, pp 283-335.

(3) Van Rossum, J. M., Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn. Ther.* **1963**, 143, 299-330.

(4) Lazareno, S., Quantification of receptor interactions using binding methods. *J. Recept. Signal Transduct. Res.* **2001**, 21, 139-165.

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Patrick Igel