Highly Potent, Selective Acylguanidine-Type Histamine H_2 Receptor Agonists: Synthesis and Structure-Activity Relationships

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer.nat.) der Naturwissenschaftlichen Fakultät IV - Chemie und Pharmazie – der Universität Regensburg

vorgelegt von

Anja Kraus

aus Neumarkt i. d. OPf.

2007

Die vorliegende Arbeit entstand in der Zeit von April 2004 bis November 2007 unter der Leitung von Herrn Prof. Dr. A. Buschauer am Institut für Pharmazie der Naturwissenschaftlichen Fakultät IV – Chemie und Pharmazie – der Universität Regensburg.

Das Promotionsgesuch wurde eingereicht im November 2007.

Tag der mündlichen Prüfung: 14. Dezember 2007

Prüfungsausschuß:	Prof. Dr. A. Göpferich	(Vorsitzender)
	Prof. Dr. A. Buschauer	(Erstgutachter)
	Prof. Dr. S. Elz	(Zweitgutachter)
	Prof. Dr. H.-A. Wagenknecht	(Prüfer)

Es irrt der Mensch, solang´ er strebt.

Johann Wolfgang von Goethe, Faust I

Danksagung

An dieser Stelle möchte ich mich bedanken bei:

Herrn Prof. Dr. A. Buschauer für die interessante Themenstellung, seine wissenschaftlichen Anregungen und Förderung sowie für seine konstruktive Kritik bei der Durchsicht dieser Arbeit,

Herrn Prof. Dr. G. Bernhardt für seine entgegengebrachte Hilfsbereitschaft bei analytischen Problemen,

Herrn Prof. Dr. S. Elz und seinen Mitarbeitern für die Durchführung der organpharmakologischen Untersuchungen am Meerschweinchen und für die Erstellung des Zweitgutachtens,

Herrn Prof. Dr. R. Seifert und seinen Mitarbeitern für die Möglichkeit zur Durchführung der GTPase Assays und die Testung von Substanzen,

Frau K. Fisch für die zuverlässige und schnellstmögliche Durchführung der GTPase Assays,

Frau E. Schreiber für die Durchführung der Ca^{2+} Assays,

Frau S. Bollwein für die Durchführung der CE - Experimente,

Herrn D. Schnell und seiner Praktikantin Stefanie Reeh für die Testung der Substanzen an rekombinanten H_3 und H_4 Membranen,

Herrn M. Keller für die Hilfe bei Problemen an der HPLC-Anlage,

allen Mitarbeitern der analytischen Abteilungen der Fakultät für die Aufnahme der NMR- und Massenspektren, sowie der Durchführung der Elementaranalysen,

der „Histamin-Gruppe“ (Herrn T. Birnkammer, Frau D. Erdmann, Herrn Dr. P. Ghorai, Herrn P. Igel, Herrn J. Mosandl, Herrn Dr. H. Preuß und Herrn D. Schnell) für die gute, interdisziplinäre Zusammenarbeit,

Herrn T. Birnkammer, Frau D. Erdmann, Herrn Dr. P. Ghorai und Frau G. Petrache für eine schöne und entspannte Atmosphäre im gemeinsamen Labor,

Herrn M. Lopuch für die Synthese von H_2-Agonisten im Rahmen seiner Masterarbeit und die Bereitstellung dieser Daten,

meinen studentischen Hilfskräften und Schwerpunktpraktikanten für ihre engagierte Mitarbeit im Labor,

Frau S. Heinrich, Herrn P. Richthammer und Frau M. Wechler für die Hilfsbereitschaft und Unterstützung bei vielen organisatorischen und technischen Dingen,

allen Mitgliedern des Lehrstuhls für ihre Kollegialität, Hilfsbereitschaft und ein gutes Arbeitsklima,

dem Gaduiertenkolleg 760 der DFG für die finanzielle Unterstützung und wissenschaftliche Förderung,

all meinen Freunden für ihren Beistand und den Spaß, auch außerhalb der Uni, vor allem Frau S. Schmidt für eine einmalige, neunjährige WG-Zeit und Frau K. Wiesler für die Unterstützung, den Zusammenhalt und die unzähligen Mittags- und Kaffeepausen während der letzten neun Jahre,

meiner Familie, ganz besonders meinen Eltern für ihre Geduld, Unterstützung und Hilfe in jeglicher Art und Weise in all den Jahren,

und vor allem meinem Freund Martin.

Contents

Chapter 1
Introduction

1.1. Histamine receptors

Histamine (**HIS**) exerts its effects through four receptor subtypes, termed histamine H_1 (H_1R), H_2 (H_2R), H_3 (H_3R) and H_4 receptors (H_4R), which all belong to class 1 (rhodopsin-like) G protein coupled receptors (GPCRs)[1-3] (for a description of the classification of GPCRs see chapter 1.3.1). Long before their respective genes were cloned[4-6], the H_1R, H_2R and H_3R were pharmacologically identified. More recently, the gene encoding the H_4R based on the sequence homology to the H_3R gene (37-43 %) was determined[7, 8]. Unlike the H_3R and H_4R, the first two histamine receptor subtypes have shown to be targets for blockbuster drugs. H_1R antagonists (antihistamines) are used to treat allergic conditions like hay fever and H_2R antagonists have been developed as antiulcer drugs[1].

The cellular expression of the 487 amino acids containing human H_1R includes airway smooth muscles, endothelial cells, neurons, dendritic cells, mast cells, monocytes/macrophages and lymphocytes[9, 10]. Stimulation of the H_1R activates phospholipase C *via* a pertussis toxin-insensitive $G_{q/11}$ protein and leads to the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), which yields in calcium mobilization from intracellular stores and activation of protein kinase C[9, 11]. H_1R antagonists are commonly divided into sedating first-generation antagonists like chlorpheniramin and chlorpromazine, and non-sedating second-generation antagonists like cetirizine (Figure 1.1). Today, especially the second-generation H_1R antagonists are used in the therapy of allergic diseases like urticaria, allergic rhinitis and bronchial asthma[1]. Important experimental tools to analyze H_1R function in cellular and organ systems are H_1R agonists, which are divided into three groups[1, 12]. 1) Small agonists derived from **HIS** such as 2-methylhistamine and betahistine, 2) **HIS** derivatives with bulkier aromatic substituents at position 2 of the imidazole ring such as 2-(3-trifluoromethylphenyl)histamine and 3) the histaprodifens such as suprahistaprodifen[13-16] (Figure 1.1). In our group, fluorescent H_1R antagonists were developed[17, 18].

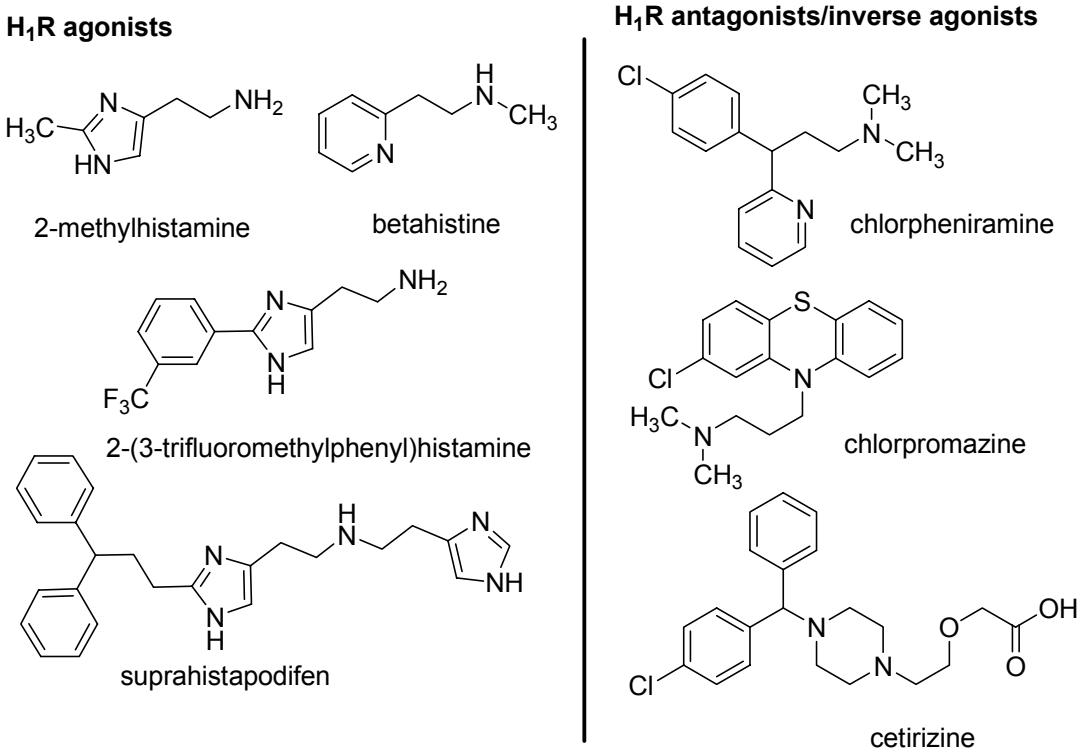


Figure 1.1. Structures of selected H_1R ligands.

A detailed description for the H_2R is given in chapter 1.2.

The human H_3R consists of 445 amino acids and activates $G_{i/o}$ proteins, which in turn inhibit of adenylyl cyclase (AC), thereby lowering the cAMP level, and activate other effector pathways including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways[19, 20]. Mainly expressed in the CNS, the H_3R acts as a pre-synaptic autoreceptor that regulates the synthesis and release of **HIS** from histaminergic neurons and has also been found in low densities in some peripheral tissues[1, 21]. Furthermore, the H_3R can regulate serotonergic, noradrenergic, cholinergic and dopaminergic neurotransmitter release in mammalian brain[1]. Because all these neurotransmitter are involved in vigilance, attention and cognition enhancement, food intake and sleep-wake cycles, H_3R antagonists are proposed as potential tools for the treatment of neurological dysfunctions and psychiatric diseases like schizophrenia, attention-deficit hyperactivity disorder (ADHD), dementia, epilepsy, obesity and narcolepsy[19, 22, 23]. H_3R antagonists can be divided into imidazole-containing antagonists, like thioperamide and clobenprobit, and non-imidazole antagonists with improved oral bioavailability and blood brain penetration, like JNJ-5207852[19, 24-26] (Figure 1.2). Recent studies with the non-imidazole containing H_3R antagonist/inverse agonist BF2.649 may indicate a

potential to represent a new class of antipsychotics[27, 28]. Selective agonists like (*R*)-α-methylhistamine, imetit, immepip and methylimmepip (Figure 1.2) are pharmacological tools and potential drugs for insomnia, myocardial ischaemic arrythmias as well as inflammatory and gastric acid related diseases[19, 29, 30].

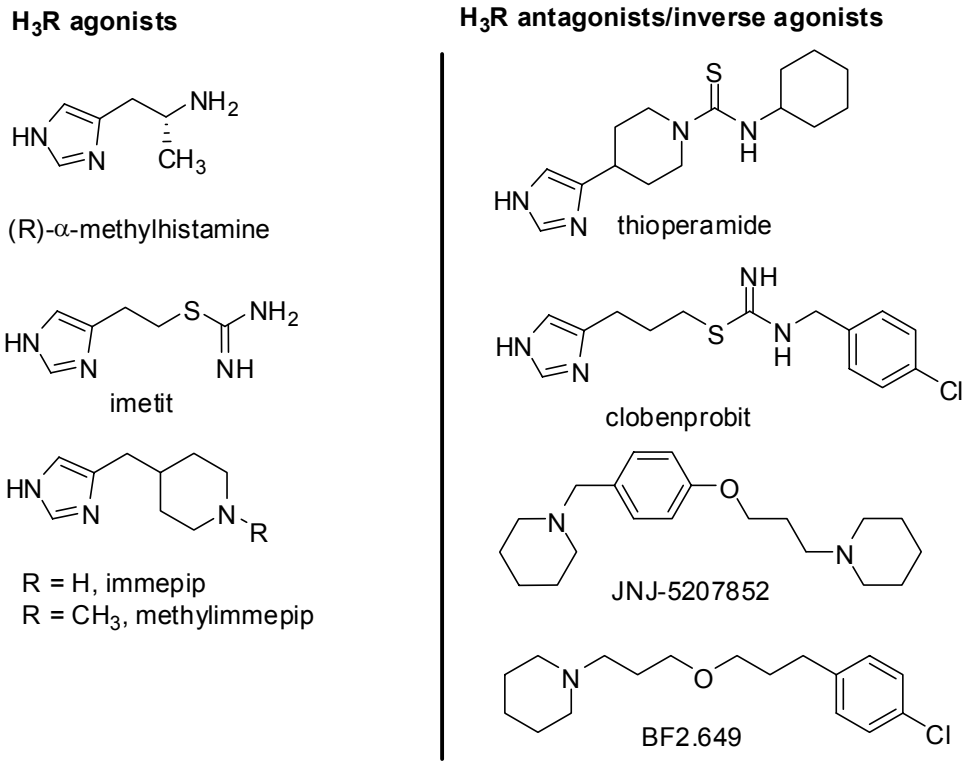


Figure 1.2. Structures of selected H_3R agonists and antagonists/inverse agonists.

The human H_4R is a 390 amino acid protein and shares the highest homology sequence to the H_3R of about 40 % (58 % homology in the transmembrane regions) among the histamine receptors. The homology to H_1R and H_2R is actually lower than to other GPCRs[7]. H_4Rs are predominantly expressed in bone marrow and peripheral hematopoietic cells, most convincingly shown for eosinophils, mast cells, basophils, dendritic cells and T cells suggesting that it plays a role in the inflammatory response[31]. Like the H_3R, the H_4R couples to pertussis toxin-sensitive $G_{i/o}$ proteins and thereby inhibits forskolin-induced cAMP production[8], activates mitogen-activated protein kinase[32] and mobilizes calcium in eosinophils and mast cells[33]. Due to the high homology to the H_3R, the pharmacologies of the H_3R and H_4R overlap and many H_3R ligands also bind to the H_4R. The first highly selective H_4R agonist was OUP-16[34] and later 4-methylhistamine[35] (Figure 1.3) as a high-affinity H_4R ligand with a >100-fold selectivity for the hH_4R over the other histamine receptor subtypes was

identified. The first selective H_4R antagonist turned out to be the indolylpiperazine derivative JNJ-7777120 (Figure 1.3)[36]. Selective H_4R agonists and antagonists may be useful for the treatment of allergic rhinitis, asthma, dermatitis and autoimmune diseases like rheumatoid arthritis, multiple sclerosis, type I diabetes and systemic lupus erythematosus[31].

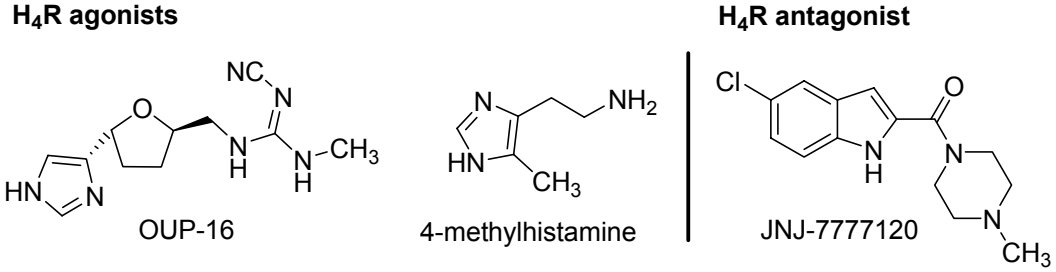


Figure 1.3. Structures of selected H_4R ligands.

1.2. Histamine H_2 receptor

To date, H_2R isoforms of canine[5], human[37], rat[38], guinea pig[39] and mouse[40] have been cloned, exhibiting an overall amino acid sequence identity of more than 80 %. Within the α-helical transmembrane domains exists a sequence identity of 90 % whereas the N-terminal domain and the C-terminus are the least conserved regions. As recently demonstrated, the cH_2R exhibit an increased constitutive activity compared to hH_2R, gpH_2R and rH_2R[41, 42]. The DNA sequence encodes for 358 (rat, mouse) and 359 (human, canine, guinea pig) amino acids.

1.2.1. Distribution and function

H_2Rs are located on gastric parietal cells, exerting a potent effect on gastric acid secretion[43]. H_2R antagonists like metiamide, cimetidine, ranitidine, famotidine (Figure 1.4), tiotidine, nizatidine and roxatidine became blockbuster drugs for the treatment of gastric and duodenal ulcer[1]. The high affinity (K_D = 0.3 nM) H_2R radioligand [^{125}I]iodoaminopotentidine[44-47] was used for audioradiographic mapping of H_2Rs in mammalian brain. The H_2R was found with highest densities in the basal ganglia, hippocampus, amygdala and cerebral cortex and with lowest densities in the cerebellum and hypothalamus[45]. The direct action of H_2R stimulation on neuronal membranes is usually excitatory or potentiates excitation[48]. In hippocampal pyramidal cells, **HIS** blocks directly the calcium-dependent potassium conductance, which causes a long-lasting afterhyperpolarization and affects the accommodation and

firing[48]. Synaptic transmission in the hippocampus is potentiated and the firing of several types of neurons is enhanced for many hours after they have been exposed to **HIS** or the H_2R agonist impromidine for only a few minutes[49, 50]. Due to the fact that the function of the H_2Rs in the brain has not been identified[51], because most of the available H_2R ligands do not really penetrate the blood brain barrier, centrally active H_2R agonists and antagonists will be useful pharmacological tools to study the role of these receptors in the brain.

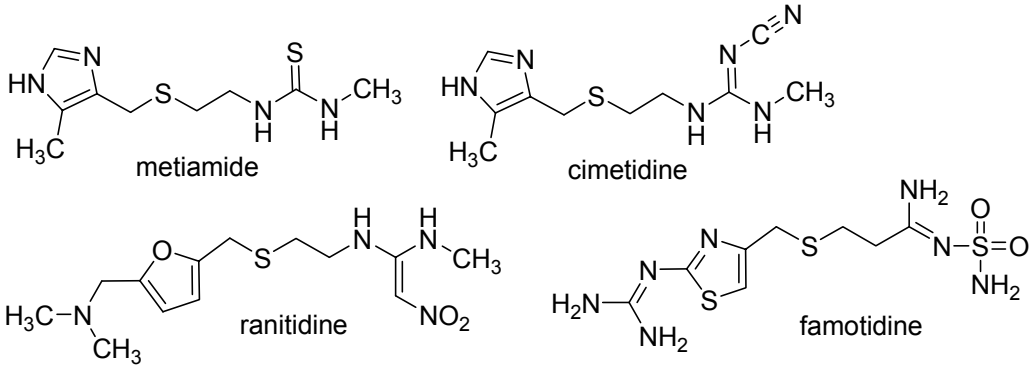


Figure 1.4. Structures of H_2R antagonists/inverse agonists.

H_2Rs are also present in cardiac tissues. Stimulation of the H_2R can mediate chronotropic and inotropic effects on atrial and ventricular tissues[52]. Thus, the spontaneously beating guinea pig right atrium was used as a standard H_2R model in organ pharmacology while measuring the change in the heart rate upon H_2R ligand exposure. H_2R-mediated smooth muscle relaxation has been documented in airway, uterine, and vascular smooth muscle[53]. Promyelocytic leukemic cells express H_2Rs and activation leads to functional differentiation to mature granulocytes[54, 55], and therefore H_2R agonists are discussed to be useful for the treatment of acute promyelocytic leukemia. Finally, the histaminergic system exerts various regulatory functions affecting the immune response and certain aspects of hematopoiesis[56-58]. Different patterns of histamine receptor expression on T helper 1 (TH1) and T helper 2 (TH2) cells induces converse T cell responses following histamine stimulation. TH1 cells show predominantly, but not exclusive, expression of H_1R, while TH2 cells show increased expression of H_2R, acting as the negative regulator of proliferation, IL-4 and IL-13 production[56]. Additionally, **HIS** directly causes B cell antibody production as a co-stimulatory receptor on B cells. Whereas the humoral immune responses may

be blocked by **HIS** through the H_1R mainly expressed on TH1 cells, the humoral immune response is enhanced through the H_2R[56].

1.2.2. Signal transduction mechanisms

The H_2R is a G_s coupled protein and therefore activates adenylyl cyclase, resulting in an increase in cAMP production[1, 59]. Thus, cAMP activates protein kinases, which phosphorylate regulatory proteins, leading, for instance, to an influx of Ca^{2+} in cardiac myocytes. Furthermore, there are reports of H_2Rs coupling to other signalling systems. H_2R stimulation has been shown to increase the intracellular concentration of free calcium ions mediated by action of phospholipase C[60, 61]. Some reports demonstrated that the H_2R couples to both the G_s and $G_{q/11}$ signalling pathways[62, 63].

1.2.3. Histamine H_2 receptor agonists

The H_2R agonists can be divided into two structural classes, the small amine-type agonists corresponding to histamine-like structures and the guanidine-type agonists corresponding to impromidine-like structures[64].

1.2.3.1. Small molecule histamine H_2 receptor agonists

5-Methylhistamine (Figure 1.5) was the first H_2R agonist described in literature that exhibited any selectivity for the H_2R[65, 66]. Further selective H_2R agonists devoid of an imidazole ring are dimaprit[67, 68], amthamine[69] and amselamine[70] (Figure 1.5). These compounds are similar to **HIS** concerning both structural criteria and H_2R-agonistic activity, but are selective to the H_2R.

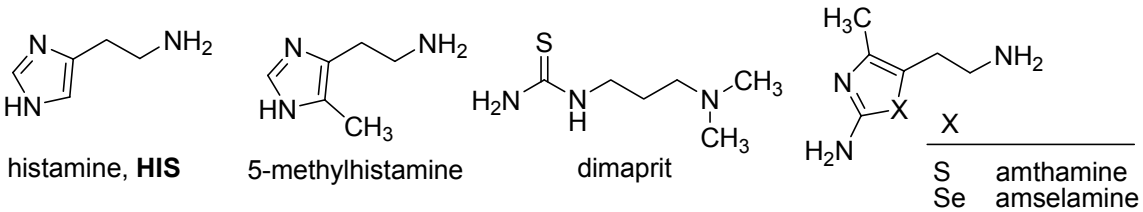


Figure 1.5. Structures of histamine and small molecule H_2R agonists.

During a long time a tautomeric shift of the ligand has been thought to be a structural requirement for the stimulation of the H_2R[71]. This tautomerism process involved two proton transfers, the donation of a proton from the receptor to the agonist on one side and on the other side a proton would be donated from the agonist to the receptor. To

explain the binding modes of either tautomeric or non-tautomeric ligands such as dimaprit and amthamine, a new activation model was suggested[72]. The partially positively charged sulphur atom of amthamine interacts with a negatively charged site of the receptor whereas the double bonded nitrogen atom accepts a proton from the proton donating site of the receptor[72]. This model implies that a tautomeric shift is no prerequisite for H_2R stimulation. Moreover, it was shown that the two mechanistic models of H_2R activation are compatible if the amino acid residues which constitute the recognition centre of the receptor are present in different acid-base conjugate forms[73].

In vitro mutagenesis studies and modelling approaches based on rhodopsin pointed out the histamine binding site of the H_2R. H_2R mutants indicated an ionic interaction of the protonated amino group with Asp-98 in TM3[74]. The second and third site of the widely accepted three-point model could prinicipally be formed with the couples Asp-186/Thr-190 or Asp-186/Tyr-182in TM5[75, 76]. Subsequently, it was revealed that the proposed two H-bonds of the imidazole ring with H_2R are only possible with Tyr-182 and Asp-186[76].

1.2.3.2. Imidazolylpropylguanidines

The first highly potent H_2R agonist was the guanidine derivative impromidine (Figure 1.6) which combines potent H_2R agonism with moderate and potent H_1R and H_3R antagonistic activity, respectively[11, 77]. Impromidine is about 50 times more potent than **HIS** and a full agonist on the spontaneously beating guinea pig right atrium (chronotropic response), but in several other H_2R containing tissues its relative potency and efficacy was lower[11, 77].

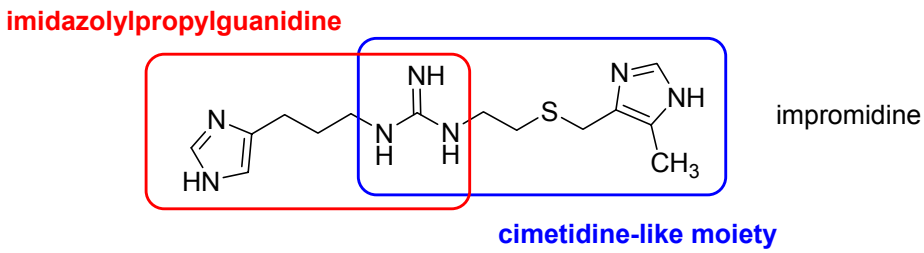


Figure 1.6. Structure of the first highly potent H_2R agonist impromidine.

This prototypical guanidine-type H_2R agonist is composed of the weak partial agonist imidazolylpropylguanidine (SK&F 91486)[78] and a cimetidine-like moiety devoid of

H_2R agonistic activity. A large number of impromidine analogues have been synthesized and evaluated for H_2R agonism[64]. Impromidine was the first clinically tested specific H_2R agonist and turned out to be a potent inotropic stimulator in patients suffering from severe catecholamine-insensitive congestive heart failure[79-81].

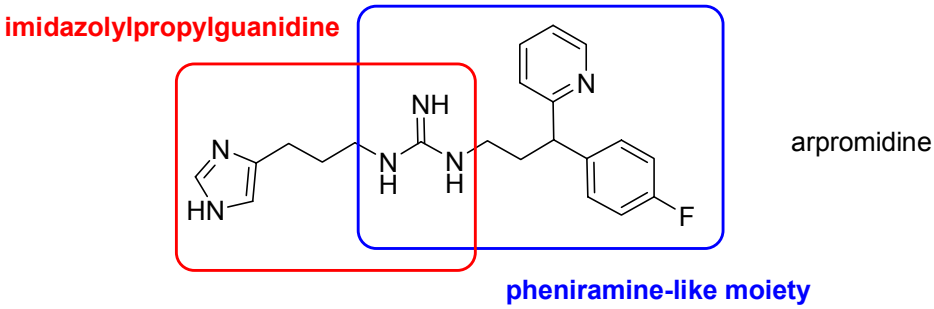


Figure 1.7. Structure of the H_2R agonist arpromidine.

The replacement of the cimetidine-like moiety in impromidine by a more lipophilic pheniramine-like structure resulted in arpromidine (**ARP**)[82] (Figure 1.7) which became a promising new chemical lead for the development of ´cardiohistaminergics`[83, 84]. This pharmacological hybrid molecule is about 100 times more potent than **HIS** on the guinea pig right atrium and turned out to be an H_1R antagonist which is about equipotent with pheniramine on the guinea pig ileum. A large series of arpromidine-related imidazolylpropylguanidines were developed as positive inotropic vasodilators[82-85]. The most potent arpromidine analogues achieve about 400 times the activity of **HIS** on the isolated spontaneously beating guinea pig right atrium. These arpromidine-like compounds, in particular the 3,4- and 3,5-difluorinated analogues proved to be superior to impromidine in potency, hemodynamic profile and side effects when tested in the guinea pig under physiological conditions and in a pathophysiological model of severe congestive heart failure (vasopressin-induced acute heart failure)[84]. Furthermore, such compounds were described as first competitive non-peptide neuropeptide Y (NPY) receptor antagonists with moderately low potency at the Y_1 receptor (pK_i up to 6.5)[86, 87].

The interaction of guanidine-type agonists may be interpreted by analogy with the model proposed for **HIS**[64]. Most amino acids interacting with guanidine-type agonists are identical in hH_2R and gpH_2R species isoforms[88]. The complex of **ARP** in the binding site of the hH_2R is shown in Figure 1.8. The imidazole moiety in the

imidazolylpropylguanidines interacts by building H-bonds with Asp-186 and Tyr-182 in TM5 and the strongly basic guanidino group underwents an ionic interaction with Asp-98 in TM3[75]. Moreover, residues in TM6 face the imidazolylpropylguanidine moiety of **ARP** in the H_2R model[41, 88].

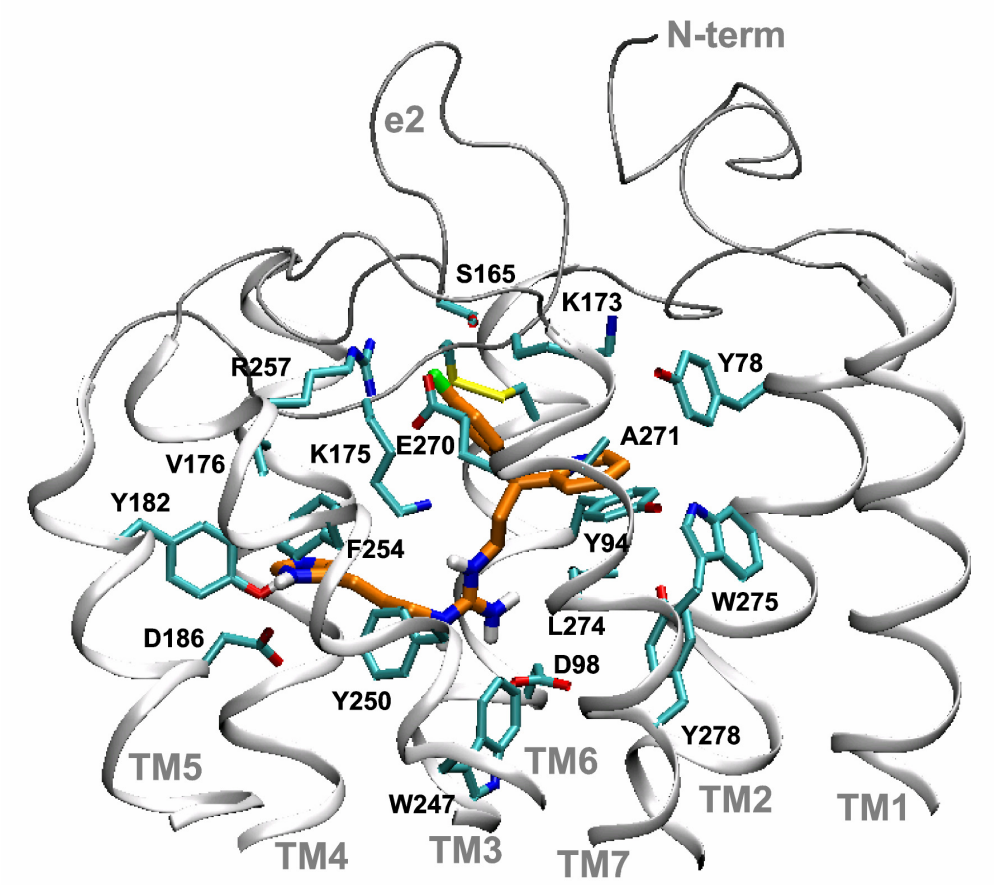


Figure 1.8. Side view of the hH_2R model in complex with **ARP**. The putative agonist binding site and the extracellular components of the hH_2R are shown. The carbon atoms of **ARP** are shown in orange, grey ribbons illustrate transmembrane domains TM1-TM7, thin grey lines the extracellular loops e1, e2 and e3 and the N-terminus. Taken from Preuss, 2007[41].

In the hH_2R model, the pyridyl ring of **ARP**, which was predicted to point into a "lower" binding region where bulk mainly decreases activity[64], was surrounded by a cluster of aromatic and hydrophobic residues in TM2, TM3 and TM7. The 4-F-phenyl group was proposed to point into an "upper" binding region where bulk may enhance agonist activity[89] and amino acids in TM6 and TM7 are involved[64, 88]. Furthermore, based on the crystal structure of rhodopsin[90] the participation of amino acids in the e2 loop to the binding pocket was proposed for members of class 1 GPCRs, as experimentally demonstrated for the dopamine D_2 receptor[91], the adenosine A_{2a} receptor[92] and the muscarinic M_3 receptor[93]. Very recently, site-directed mutagenesis studies revealed that the residues of the e2 loop presumably do not directly face the

binding pocket and do not contribute to the species-selective interactions of *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines and N^G-acylated analogues[41, 94]. Hence, participation of the e2 loop to the binding pocket may apply for many[95] but possibly not for all class 1 GPCRs.

In contrast to small H_2R agonists, guanidines are less potent and efficient agonists at the H_2R of human neutrophils than at the H_2R of the guinea pig right atrium[96, 97]. This effect could also be confirmed with a membrane steady-state GTPase activity assay using fusion proteins of the H_2R and the short splice variant of G_α, $G_{\alpha S}$. The compounds proved to be less potent and efficacious at the hH_2R-$G_{s\alpha S}$ than at the gpH_2R-$G_{s\alpha S}$[88]. Considerations from a sequence alignment suggested that the nonconserved Asp-271 (gpH_2R) in TM7 confers to this species-selectivity and can directly participate in ligand binding. Moreover, an interhelical H-bond between Tyr-17 in TM1 and Asp-271 was predicted from a three-dimensional homology model of the gpH_2R stabilizing an active guanidine bound conformation[88]. Such interactions cannot occur with Cys-17 and Ala-271 in hH_2R[88]. These data was initially confirmed by an Ala-271→Asp-271 mutation in hH_2R-$G_{s\alpha S}$ (hH_2R-A271D-$G_{s\alpha S}$)[88] and subsequently by an Ala-271→Asp-271 and Cys-17→Tyr-17 double mutation in hH_2R-$G_{s\alpha S}$ (hH_2R-C17Y-A271D-$G_{s\alpha S}$)[41, 98]. Hence, guanidines stabilize an active conformation in gpH_2R more efficiently and potently than in hH_2R.

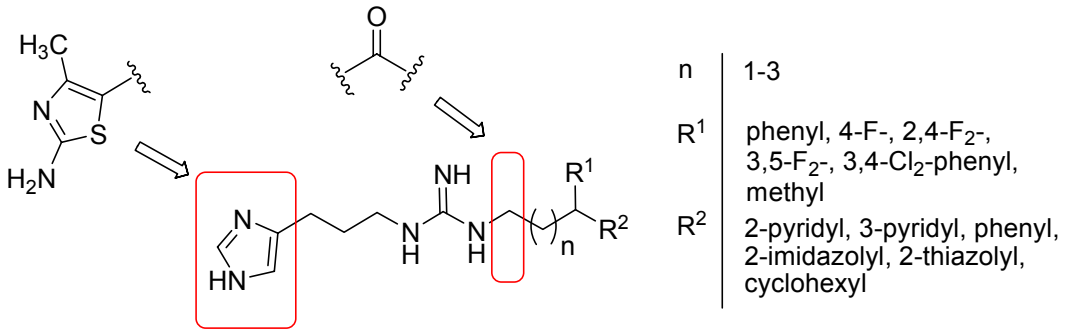


Figure 1.9. General structure of guandine-type H_2R agonists and N^G-acylated analogues.

The guanidine-type agonists are strong bases (pK_a~13) and are nearly quantitatively protonated at physiological pH. Recently, compounds with improved pharmacokinetic properties, particularly with regards to oral bioavailability and penetration across the blood brain barrier, were developed in our workgroup[99]. The introduction of a carbonyl function adjacent to the guanidine moiety reduces the basicity by 4-5 orders of magnitude (pK_a~8). A series of N^G-acylated guandines was synthesized and

pharmacologically characterized[99-101]. Such compounds are absorbed from the gastrointestinal tract and are capable of penetrating across the blood brain barrier after administration to mice[102]. Centrally active H_2R agonists may be useful pharmacological tools to study the role of H_2Rs in the brain. Furthermore, the common imidazole ring of the guanidines and N^G-acylated analogues was bioisosterically replaced by a 2-amino-4-methylthiazole group without affecting the H_2R agonistic activity, but resulting in increased selectivity for H_2R over H_3R[99, 103].

1.3. G protein coupled receptors and oligomerization

1.3.1. Classification and activation of G protein coupled receptors

G protein coupled receptors (GPCRs) represent the largest class of cell surface receptors and are the most important group of targets for a large number of therapeutic agents. GPCRs share a similar architecture of seven hydrophobic, membrane-spanning α-helices forming a receptor which binds an extracellular ligand that exert a specific effect into the cell and is able to interact with a G protein[104]. For many GPCRs G protein independent signalling pathways have also been reported[104, 105]. GPCRs are known as extremely versatile receptors, hence it is difficult to obtain diffraction-quality crystals from a native unbound receptor[106]. A broad variety of ligands such as biogenic amines, purines and nucleic acid derivatives, lipids, peptides and proteins, odorants, pheromones, tastants, ions like calcium and protons, and even photons in the case of rhodopsin are able to mediate their message through these proteins[104, 107]. Based on phylogenetic and ligand-receptor relationships human GPCRs are divided into three main subclasses/families, 1, 2, 3. The class 1 or rhodopsin-like familiy is the largest subgroup and contains receptors for odorants, small molecules such as catecholamines and amines, some peptides and glycoprotein hormones. Endogenous small-molecule ligands interact with a binding pocket located within the TM bundle and binding of peptides and glycoproteins occurs at the N-terminus, the extracellular loop regions and the upper part of the TM helices[108]. Furthermore, class 1 receptors are characterized by several highly conserved amino acids and a disulphide bridge that connects the first and second extracellular loop[109]. Rhodopsin was the first GPCR for which a high-resolution crystal structure had been determined[90, 110, 111]. Very recently, the crystal structure of the β_2-adrenergic receptor was reported[112-114]. The class 2 or secretin-like receptors are characterized by a relatively long N-terminus containing several

cystein residues, which presumably form a network of disulphide bridges, and is involved in ligand binding[109]. Ligands for class 2 receptors include hormones such as glucagons and parathyroid hormone. The small glutamate class 3 contains the metabotropic glutamate, the Ca^{2+}-sensing and the γ–aminobutyric acid $(GABA)_B$ receptors and are characterized by a very long N-terminus and C-tail, a disulphide bridge connecting the first and second extracellular loop and a very short and well-conserved third intracellular loop. The ligand binding-site is located in the N-terminal domain, which is often described as being like a ´Venus fly trap`[107, 109]. Finally, a separate group includes the frizzled/smoothened family receptors[105]. For many of the identified GPCRs the natural ligands are unknown so far and are still "orphan" receptors.

GPCRs function through heterotrimeric G proteins, consisting of a G_α-subunit and a $G_{\beta\gamma}$-heterodimer[115, 116]. Connected with a G protein on the intracellular side, GPCRs interact with an extracellular ligand and undergo a conformational change into an active state. The α-subunit normally binds GDP in the resting state. Upon activation, GDP is replaced by GTP, which binds to G_α inducing another conformational change in this subunit leading to dissociation of the heterotrimer in $G_{\alpha\text{-GTP}}$ and $G_{\beta\gamma}$. Both subunits now can interact and activate a secondary messenger system such as adenylate cyclase. There are several types of G proteins such as G_s, G_i and $G_{q/11}$ which use different secondary messenger pathways[115, 117]. The intrinsic GTPase activity of the G_α-subunit converts GTP to GDP and P_i and the dissociated subunits are allowed to recombine to start a new cycle.

Some GPCRs are known to be constitutively active. This stands for the ability of a GPCR to adopt an active conformation in the absence of an agonist[118, 119]. Many drugs which were traditionally classified as competitive antagonists are in fact inverse agonists and a large number of disease-causing GPCR mutations with increased constitutive activity had been identified[105, 120].

1.3.2. GPCR oligomerization

Almost all therapeutic agents that are directed towards GPCRs have been designed using assays that presume that these receptors are monomeric. Now it is widely accepted that these receptors exist and are active as homo-oligomeric and hetero-oligomeric complexes and could have important implications for the development and screening for new drugs[109, 121]. Because of the fact that GPCRs exist of seven

hydrophobic transmembrane domains, incomplete solubilization can lead to aggregation and be misunderstood for dimerization[117]. Thus, methods verifying the existence of oligomers in living cells were developed. Using bioluminescence resonance energy transfer (BRET) and photobleaching and time-resolved fluorescence resonance energy transfer (FRET), dimerization in living cells for different GPCRs were shown, including the β_2-adrenergic receptor[122], the δ-opioid receptor[123], the thyrotropin-releasing hormone receptor[124] and the SSTR5-somatostatin receptor[125]. The most direct demonstration of GPCR oligomerization provides the atomic force microscopy, which reveals that rhodopsin exists in an oligomeric arrangement in its native environment[126-128]. Support for the oligomerization of GPCRs has been obtained experimentally by co-immunoprecipitation, energy transfer between two tagged receptors, disulfide cross-linking, pharmacological enhancement of the signal arising from one receptor by the agonist of the second receptor in the heterodimeric complex, radiation inactivation, gel exclusion chromatography, and interference with intracellular trafficking[129]. Moreover, recent results pointed out that the homo-dimer of a mammalian class 1 GPCR requires binding of only one agonist to induce internalization and therefore supports the idea that GPCRs internalize as dimers as it was demonstrated for the β_2-adrenoceptor[130].

There is an evidence that GPCR oligomers are already formed in the endoplasmatic reticulum (ER) before they are transported to the cell surface[131]. Although it is unclear whether oligomerization during biogenesis is directed towards all GPCRs, BRET and FRET studies have revealed that several receptors dimerize in the ER[132, 133]. Moreover, some GPCRs oligomerize at the cell surface in a process regulated by ligands[131, 134, 135].

Various regions of GPCRs are thought to form an interface between monomeric units[135, 136]. Thus for the dimerization of class 3 receptors, an intermolecular disulphide bond between the amino termini has been shown to be crucial[109]. Because there are great differences in the structure of the amino termini of class 1 receptors compared to those of the class 3 receptors, the sites of intermolecular interaction for class 1 receptors were found elsewhere[109]. For rhodopsin-like receptors, the transmembrane domains are thought to be involved in oligomerization include transmembrane helices 1 and 4-6 and cytoplasmic loops formed between transmembrane helices 1 and 2 and transmembrane helices 5 and 6[135-137].

The fact that GPCRs are able to form oligomeric entities opens the possibility to improve drugs by the development of dimeric ligands acting as bivalent ligands[109]. The term “bivalent ligand” stands for a molecule that contains two pharmacophores linked through a spacer and also describes compounds that are not dimeric[138, 139]. One aim of developing bivalent ligands is to enhance the affinity of the fully bound dimeric compound compared to a monovalent ligand or a univalently bound bivalent ligand. The length and chemical properties of the spacer are crucial factors concerning the ability to bridge two neighbouring receptors. A very short or a very long spacer decreases the potential to occupy two vicinal receptors[139]. Binding of one pharmacophore of a dimeric ligand exhibiting an optimal spacer length to one recognition site of a receptor dimer might have increased affinity compared to their monovalent ligands because the unbound partner is arranged in closer proximity to neighboring binding sites[109]. An alternative theory for increasing affinity and/or potency of dimeric ligands is that these ligands might more readily induce or stabilize the dimeric conformation of the receptors as it was proposed for the bivalent endothelin peptide[109, 140]. In addition to increased affinity, changes in selectivity for bivalent opioid receptor ligands has been observed[138]. Changes in κ-opioid receptor selectivity were monitored in dependence on the spacer length [138].

There is evidence that many rhodopsin-like GPCRs exist and act as oligomeric entities including the histamine H_1[141, 142], H_2[143], H_3[144, 145] and H_4[7, 146] receptors. The synthesis and pharmacological investigations of double pharmacophore ligands with different spacer lengths for bridging hypothetical dimeric histamine receptors are subject of this work (chapter 5).

1.4. References

(1) 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, (3), 253-278.

(2) Hough, L. B., Genomics Meets Histamine Receptors: New Subtypes, New Receptors. *Mol. Pharmacol.* **2001,** 59, (3), 415-419.

(3) Foord, S. M.; Bonner, T. I.; Neubig, R. R.; Rosser, E. M.; Pin, J.-P.; Davenport, A. P.; Spedding, M.; Harmar, A. J., International Union of Pharmacology. XLVI. G Protein-Coupled Receptor List. *Pharmacol. Rev.* **2005,** 57, (2), 279-288.

(4) 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, (24), 11515-19.

(5) 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, (2), 429-433.

(6) 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, (6), 1101-1107.

(7) 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, (3), 427-433.

(8) Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S.-i., Molecular Cloning and Characterization of a Novel Type of Histamine Receptor Preferentially Expressed in Leukocytes. *J. Biol. Chem.* **2000,** 275, (47), 36781-36786.

(9) Hill, S. J., Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.* **1990,** 42, (1), 45-83.

(10) Togias, A., H_1-receptors: Localization and role in airway physiology and in immune functions. *J. Allergy Clin. Immunol.* **2003,** 112, (4, Supplement 1), S60-S68.

(11) Leurs, R.; Smit, M. J.; Timmerman, H., Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther.* **1995,** 66, (3), 413-463.

(12) Seifert, R.; Wenzel-Seifert, K.; Burckstummer, 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 H1-Receptor. *J. Pharmacol. Exp. Ther.* **2003,** 305, (3), 1104-1115.

(13) Elz, S.; Kramer, K.; Leschke, C.; Schunack, W., Ring-substituted histaprodifen analogues as partial agonists for histamine H_1 receptors: synthesis and structure-activity relationships. *Eur. J. Med. Chem.* **2000,** 35, (1), 41-52.

(14) 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, (6), 1071-1084.

(15) 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, (8), 1287-1294.

(16) Zingel, V.; Schunack, W., Agonists of the histamine-H_1-receptors: structure and pharmacology. *Pharmazie* **1993,** 48, (7), 483-93.

(17) Li, L.; Kracht, J.; Peng, S.; Bernhardt, G.; Buschauer, A., Synthesis and pharmacological activity of fluorescent histamine H_1 receptor antagonists related to mepyramine. *Bioorg. Med. Chem. Lett.* **2003,** 13, (7), 1245-1248.

(18) Schneider, E. Development of fluoresence-based methods for the determination of ligand affinity, selectivity and activity at G protein coupled receptors. Doctoral Thesis, University of Regensburg, Germany, 2005.

(19) 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, (2), 107-120.

(20) Bongers, G.; Bakker, R. A.; Leurs, R., Molecular aspects of the histamine H_3 receptor. *Biochem. Pharmacol.* **2007,** 73, (8), 1195-1204.

(21) Haaksma, E. E. J.; Leurs, R.; Timmerman, H., Histamine receptors: subclasses and specific ligands. *Pharmacol. Ther.* **1990,** 47, (1), 73-104.

(22) Leurs, R.; Blandina, P.; Tedford, C.; Timmerman, H., Therapeutic potential of histamine H_3 receptor agonists and antagonists. *Trends Pharmacol. Sci.* **1998,** 19, (5), 177-184.

(23) Parmentier, R.; Anaclet, C.; Guhennec, C.; Brousseau, E.; Bricout, D.; Giboulot, T.; Bozyczko-Coyne, D.; Spiegel, K.; Ohtsu, H.; Williams, M.; Lin, J. S., The brain H_3-receptor as a novel therapeutic target for vigilance and sleep-wake disorders. *Biochem. Pharmacol.* **2007,** 73, (8), 1157-1171.

(24) Bertoni, S.; Flammini, L.; Manenti, V.; Ballabeni, V.; Morini, G.; Comini, M.; Barocelli, E., In vitro pharmacology at human histamine H_3 receptors and brain access of non-imidazole alkylpiperidine derivatives. *Pharmacol. Res.* **2007,** 55, (2), 111-116.

(25) Stark, H.; Kathmann, M.; Schlicker, E.; Schunack, W.; Schlegel, B.; Sippl, W., Medicinal Chemical and Pharmacological Aspects of Imidazole-Containing Histamine H_3 Receptor Antagonists. *Mini Rev. Med. Chem.* **2004,** 4, 965-977.

(26) Cowart, M.; Altenbach, R.; Black, L.; Faghih, R.; Zhao, C.; Hancock Arthur, A., Medicinal chemistry and biological properties of non-imidazole histamine H_3 antagonists. *Mini Rev. Med. Chem.* **2004,** 4, (9), 979-92.

(27) Ligneau, X.; Landais, L.; Perrin, D.; Piriou, J.; Uguen, M.; Denis, E.; Robert, P.; Parmentier, R.; Anaclet, C.; Lin, J.-S.; Burban, A.; Arrang, J.-M.; Schwartz, J.-C., Brain histamine and schizophrenia: Potential therapeutic applications of H_3-receptor inverse agonists studied with BF2.649. *Biochem. Pharmacol.* **2007,** 73, (8), 1215-1224.

(28) Ligneau, X.; Perrin, D.; Landais, L.; Camelin, J. C.; Calmels, T. P. G.; Berrebi-Bertrand, I.; Lecomte, J. M.; Parmentier, R.; Anaclet, C.; Lin, J. S.; Bertaina-Anglade, V.; la Rochelle, C. D.; d'Aniello, F.; Rouleau, A.; Gbahou, F.; Arrang, J. M.; Ganellin, C. R.; Stark, H.; Schunack, W.; Schwartz, J. C., BF2.649 [1-{3-[3-(4-Chlorophenyl)propoxy]propyl}piperidine, Hydrochloride], a

Nonimidazole Inverse Agonist/Antagonist at the Human Histamine H3 Receptor: Preclinical Pharmacology. *J. Pharmacol. Exp. Ther.* **2007,** 320, (1), 365-375.

(29) de Esch, I. J. P.; Belzar, K. J., Histamine H_3 receptor agonists. *Mini Rev. Med. Chem.* **2004,** 4, (9), 955-963.

(30) Kitbunnadaj, R.; Zuiderveld, O. P.; DeEsch, I. J. P.; Vollinga, R. C.; Bakker, R.; Lutz, M.; Spek, A. L.; Cavoy, E.; Deltent, M. F.; Menge, W. M. P. B.; Timmerman, H.; Leurs, R., Synthesis and Structure-Activity Relationships of Conformationally Constrained Histamine H_3 Receptor Agonists. *J. Med. Chem.* **2003,** 46, (25), 5445-5457.

(31) 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, (9), 993-1000.

(32) 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, (3), 1058-66.

(33) 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, (6), 1117-1127.

(34) 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, (14), 3162-3165.

(35) 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 H4 Receptor: Identification of 4-Methylhistamine as the First Potent and Selective H_4 Receptor Agonist. *J. Pharmacol. Exp. Ther.* **2005,** 314, (3), 1310-1321.

(36) 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, (19), 3957-3960.

(37) 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, (3), 1386-1392.

(38) Ruat, M.; Traiffort, E.; Arrang, J.-M.; Leurs, R.; Schwartz, J.-C., Cloning and tissue expression of a rat histamine H_2-receptor gene. *Biochem. Biophys. Res. Commun.* **1991,** 179, (3), 1470-1478.

(39) Traiffort, E.; Vizuete, M. L.; Tardivellacombe, J.; Souil, E.; Schwartz, J. C.; Ruat, M., The Guinea Pig Histamine H_2 Receptor: Gene Cloning, Tissue Expression and Chromosomal Localization of Its Human Counterpart. *Biochem. Biophys. Res. Commun.* **1995,** 211, (2), 570-577.

(40) Kobayashi, T.; Inoue, I.; Jenkins, N. A.; Gilbert, D. J.; Copeland, N. G.; Watanabe, T., Cloning, RNA Expression, and Chromosomal Location of a Mouse Histamine H_2 Receptor Gene. *Genomics* **1996,** 37, (3), 390-394.

(41) 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/volltexte/2007/843/

(42) 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, (3), 983-995.

(43) Gespach, C.; Bouhours, D.; Bouhours, J.-F.; Rosselin, G., Histamine interaction on surface recognition sites of H_2-type in parietal and non-parietal cells isolated from the guinea pig stomach. *FEBS Lett.* **1982,** 149, (1), 85-90.

(44) Hirschfeld, J.; Buschauer, A.; Elz, S.; Schunack, W.; Ruat, M.; Traiffort, E.; Schwartz, J. C., Iodoaminopotentidine and related compounds: a new class of ligands with high affinity and selectivity for the histamine H_2 receptor. *J. Med. Chem.* **1992,** 35, (12), 2231-2238.

(45) Traiffort, E.; Pollard, H.; Moreau, J.; Ruat, M.; Schwartz, J. C.; Martinez-Mir, M. I.; Palacios, J. M., Pharmacological Characterization and Autoradiographic Localization of Histamine H_2 Receptors in Human Brain Identified with [125I]Iodoaminopotentidine. *J. Neurochem.* **1992,** 59, (1), 290-299.

(46) Ruat, M.; Traiffort, E.; Bouthenet, M. L.; Schwartz, J. C.; Hirschfeld, J.; Buschauer, A.; Schunack, W., Reversible and Irreversible Labeling and Autoradiographic Localization of the Cerebral Histamine H_2 Receptor Using [^{125}I]iodinated Probes. *Proc. Natl. Acad. Sci. U. S. A.* **1990,** 87, (5), 1658-1662.

(47) Martinez-Mir, M. I.; Pollard, H.; Moreau, J.; Arrang, J. M.; Ruat, M.; Traiffort, E.; Schwartz, J. C.; Palacios, J. M., Three histamine receptors (H_1, H_2 and H_3) visualized in the brain of human and non-human primates. *Brain Res.* **1990,** 526, (2), 322-327.

(48) Haas, H.; Panula, P., The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat. Rev. Neurosci.* **2003,** 4, (2), 121-130.

(49) Brown, R. E.; Stevens, D. R.; Haas, H. L., The physiology of brain histamine. *Prog. Neurobiol.* **2001,** 63, (6), 637-672.

(50) Selbach, O.; Brown, R. E.; Haas, H. L., Long-term increase of hippocampal excitability by histamine and cyclic AMP. *Neuropharmacology* **1997,** 36, (11-12), 1539-1548.

(51) Parsons, M. E.; Ganellin, C. R., Histamine and its receptors. *Br. J. Pharmacol.* **2006,** 147, (S1), S127-S135.

(52) Levi, R. C.; Alloatti, G., Histamine modulates calcium current in guinea pig ventricular myocytes. *J. Pharmacol. Exp. Ther.* **1988,** 246, (1), 377-383.

(53) Jolly, S.; Desmecht, D., Functional identification of epithelial and smooth muscle histamine-dependent relaxing mechanisms in the bovine trachea, but not in bronchi. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2003,** 134, (1), 91-100.

(54) Klinker, J. F.; Wenzel-Seifert, K.; Seifert, R., G-Protein-coupled Receptors in HL-60 Human Leukemia Cells. *Gen. Pharmacol.* **1996,** 27, (1), 33-54.

(55) Seifert, R.; Hoer, 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, (2), 235-241.

(56) Akdis, C. A.; Simons, F. E. R., Histamine receptors are hot in immunopharmacology. *Eur. J. Pharmacol.* **2006,** 533, (1-3), 69-76.

(57) Dy, M.; Schneider, E., Histamine-cytokine connection in immunity and hematopoiesis. *Cytokine Growth Factor Rev.* **2004,** 15, (5), 393-410.

(58) Schneider, E.; Rolli-Derkinderen, M.; Arock, M.; Dy, M., Trends in histamine research: new functions during immune responses and hematopoiesis. *Trends Immunol.* **2002,** 23, (5), 255-263.

(59) Del Valle, J.; Gantz, I., Novel insights into histamine H_2 receptor biology. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1997,** 273, (5), G987-996.

(60) Delvalle, J.; Wang, L.; Gantz, I.; Yamada, T., Characterization of H_2 histamine receptor: linkage to both adenylate cyclase and $[Ca^{2+}]_i$ signaling systems. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1992,** 263, (6), G967-972.

(61) Wang, L.; Gantz, I.; DelValle, J., Histamine H_2 receptor activates adenylate cyclase and PLC via separate GTP-dependent pathways. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1996,** 271, (4), G613-620.

(62) Leopoldt, D.; Harteneck, C.; Nürnberg, B., G Proteins endogenously expressed in Sf 9 cells: interactions with mammalian histamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997,** 356, (2), 216-224.

(63) Kuhn, B.; Schmid, A.; Harteneck, C.; Gudermann, T.; Schultz, G., G proteins of the G_q family couple the H_2 histamine receptor to phospholipase C. *Mol. Endocrinol.* **1996,** 10, (12), 1697-1707.

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

(65) 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, (7), 923-928.

(66) 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, (9), 905-909.

(67) 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. *Inflamm. Res.* **1977,** 7, (1), 39-43.

(68) Parsons, M. E.; Owen, D. A. A.; Ganellin, C. R.; durant, G. J., Dimaprit-[S-[3-(N,N-dimethylamino)propyl]isothiourea] —A highly specific histamine H_2-receptor agonist. Part 1. Pharmacology. *Inflamm. Res.* **1977,** 7, (1), 31-37.

(69) 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, (17), 3239-3246.

(70) van der Goot, H.; Eriks, J. C.; Leurs, R.; Timmerman, H., Amselamine, a new selective histamine H_2-receptor agonist. *Bioorg. Med. Chem. Lett.* **1994,** 4, (16), 1913-1916.

(71) Weinstein, H.; Chou, D.; Johnson, C. L.; Kang, S.; Green, J. P., Tautomerism and the receptor action of histamine: a mechanistic model. *Mol. Pharmacol.* **1976,** 12, (5), 738-45.

(72) Eriks, J. C.; van der Goot, H.; Timmerman, H., New activation model for the histamine H_2 receptor, explaining the activity of the different classes of histamine H_2 receptor agonists. *Mol. Pharmacol.* **1993,** 44, (4), 886-894.

(73) Giraldo, J., A pH-dependent model of the activation mechanism of the histamine H_2 receptor. *Biochem. Pharmacol.* **1999,** 58, (2), 343-353.

(74) 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, (29), 20840-20843.

(75) Nederkoorn, P. H. J.; Gelder, E. M.; Donné-Op den Kelder, G. M.; Timmerman, H., The agonistic binding site at 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, (5), 479-489.

(76) Nederkoorn, P. H. J.; Lenthe, J. H.; Goot, H.; Donné-Op den Kelder, G. M.; Timmerman, 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, (5), 461-478.

(77) Durant, G. J.; Duncan, W. A. M.; Ganellin, C. R.; Parsons, M. E.; Blakemore, R. C.; Rasmussen, A. C., Impromidine (SKF 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature (London, United Kingdom)* **1978,** 276, (5686), 403-5.

(78) 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. *Inflamm. Res.* **1975,** 5, (5), 464-464.

(79) Baumann, G.; Felix, S. B.; Heidecke, C. D.; Rieß, G.; Loher, U.; Ludwig, L.; Blömer, H., Apparent superiority of H_2-receptor stimulation and simultaneous β-blockade over conventional treatment with β-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, (3), 216-228.

(80) 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, (2), 165-177.

(81) Baumann, G.; Riess, G.; Erhardt, W. D.; Felix, S. B.; Ludwig, L.; Blumel, G.; Blomer, H., Impaired beta-adrenergic stimulation in the uninvolved ventricle post-acute myocardial infarction: Reversible defect due to excessive circulating catecholamine-induced decline in number and affinity of beta-receptors. *Am. Heart J.* **1981,** 101, (5), 569-581.

(82) 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, (8), 1963-70.

(83) Buschauer, A.; Baumann, G., Structure-activity relationships of histamine H_2-agonists, a new class of positive inotropic drugs. *Agents Actions Suppl.* **1991,** 33, (New Perspect. Histamine Res.), 231-56.

(84) Felix, S. B.; Buschauer, A.; Baumann, G., Therapeutic value of H_2-receptor stimulation in congestive heart failure. Hemodynamic effects of BU-E-76, BU-E-75 and arpromidine (BU-E-50) in comparison to impromidine. *Agents Actions Suppl.* **1991,** 33, (New Perspect. Histamine Res.), 257-69.

(85) 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, (4), 321-330.

(86) Dove, S.; Michel, M. C.; Knieps, S.; Buschauer, A., Pharmacology and quantitative structure-activity relationships of imidazolylpropylguanidines with mepyramine-like substructures as non-peptide neuropeptide Y Y_1 receptor antagonists. *Can. J. Physiol. Pharmacol.* **2000,** 78, (2), 108-15.

(87) Knieps, S.; Michel, M. C.; Dove, S.; Buschauer, A., Non-peptide neuropeptide Y antagonists derived from the histamine H_2 agonist arpromidine: role of the guanidine group. *Bioorg. Med. Chem. Lett.* **1995,** 5, (18), 2065-70.

(88) 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, (6), 1210-1225.

(89) Dove, S.; Buschauer, A., Improved alignment by weighted field fit in CoMFA of histamine H_2 receptor agonistic imidazolylpropylguanidines. *Quant. Struct.-Act. Rel.* **1999,** 18, (4), 329-341.

(90) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Trong, I. L.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M., Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science* **2000,** 289, (5480), 739-745.

(91) Shi, L.; Javitch, J. A., The second extracellular loop of the dopamine D_2 receptor lines the binding-site crevice. *Proc. Natl. Acad. Sci. U. S. A.* **2004,** 101, (2), 440-445.

(92) Kim, J.; Jiang, Q.; Glashofer, M.; Yehle, S.; Wess, J.; Jacobson, K. A., Glutamate residues in the second extracellular loop of the human A2a adenosine receptor are required for ligand recognition. *Mol. Pharmacol.* **1996,** 49, (4), 683-691.

(93) Scarselli, M.; Li, B.; Kim, S.-K.; Wess, J., Multiple Residues in the Second Extracellular Loop Are Critical for M_3 Muscarinic Acetylcholine Receptor Activation. *J. Biol. Chem.* **2007,** 282, (10), 7385-7396.

(94) Preuss, H.; Ghorai, P.; Kraus, A.; Dove, S.; Buschauer, A.; Seifert, R., Point mutations in the second extracellular loop of the histamine H_2 receptor do not affect the species-selective activity of guanidine-type agonists. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2007**, DOI 10.1007/s00210-007-0204-4.

(95) Shi, L.; Javitch, J. A., The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu. Rev. Pharmacol. Toxicol.* **2002,** 42, (1), 437-467.

(96) Burde, R.; Buschauer, A.; Seifert, R., Characterization of histamine H_2-receptors in human neutrophils with a series of guanidine analogues of impromidine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1990,** 341, (5), 455-461.

(97) Burde, R.; Seifert, R.; Buschauerz, A.; Schultz, G., Histamine inhibits activation of human neutrophils and HL-60 leukemic cells via H_2-receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1989,** 340, (6), 671-678.

(98) Preuss, H.; Ghorai, P.; Kraus, A.; Dove, S.; Buschauer, A.; Seifert, R., Mutations of Cys-17 and Ala-271 in the Human Histamine H_2 Receptor Determine the Species Selectivity of Guanidine-Type Agonists and Increase Constitutive Activity. *J. Pharmacol. Exp. Ther.* **2007,** 321, (3), 975-982.

(99) 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/volltexte/2006/561/

(100) 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, (1), 139-146.

(101) Xie, S.-X.; Kraus, A.; Ghorai, P.; Ye, Q.-Z.; Elz, S.; Buschauer, A.; Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1H-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, (3), 1262-1268.

(102) Keller, M. HPLC-MS investigations on absorption and brain penetration of histamine H_2 receptor agonists and NPY antagonists. Diploma Thesis, University of Regensburg, Germany, 2004.

(103) Eriks, J. C.; Sterk, G. J.; Van der Aar, E. M.; Van Acker, S. A. B. E.; Van der Goot, H.; Timmerman, H., 4- Or 5-(w-Aminoalkyl)thiazoles and derivatives; new selective H_2-receptor agonists. *Agents Actions Suppl.* **1991,** 33, (New Perspect. Histamine Res.), 301-14.

(104) 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, (6), 1256-1272.

(105) Fredholm, B. B.; Hokfelt, T.; Milligan, G., G-protein-coupled receptors: an update. *Acta Physiologica* **2007,** 190, (1), 3-7.

(106) Kobilka, B. K.; Deupi, X., Conformational complexity of G-protein-coupled receptors. *Trends Pharmacol. Sci.* **2007,** 28, (8), 397-406.

(107) Jacoby, E.; Bouhelal, R.; Gerspacher, M.; Seuwen, K., The 7 TM G-Protein-Coupled Receptor Target Family. *Chem.Med.Chem.* **2006,** 1, (8), 760-782.

(108) 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, (1), 21-80.

(109) George, S. R.; O'Dowd, B. F.; Lee, S. P., G-Protein-coupled receptor oligomerization and its potential for drug discovery. *Nat. Rev. Drug Discov.* **2002,** 1, (10), 808-820.

(110) Teller, D. C.; Okada, T.; Behnke, C. A.; Palczewski, K.; Stenkamp, R. E., Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-protein-coupled receptors (GPCRs). *Biochemistry* **2001,** 40, (26), 7761-72.

(111) Okada, T.; Palczewski, K., Crystal structure of rhodopsin: implications for vision and beyond. *Curr. Opin. Struct. Biol.* **2001,** 11, (4), 420-426.

(112) 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 b2-Adrenergic G Protein Coupled Receptor. *Science* **2007**, DOI: 10.1126/science.1150577

(113) 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 b2 adrenergic G-protein-coupled receptor. *Nature* **2007**, DOI: 10.1038/nature.06325.

(114) 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 b2 Adrenergic Receptor Function. *Science* **2007**, DOI: 10.1126/science.1150609.

(115) Hamm, H. E., The Many Faces of G Protein Signaling. *J. Biol. Chem.* **1998,** 273, (2), 669-672.

(116) Milligan, G.; Kostenis, E., Heterotrimeric G-proteins: a short history. *Br. J. Pharmacol.* **2006,** 147, (1), 46-55.

(117) Nikbin, N.; Edwards, C.; Reynolds, C. A., G-protein coupled receptor dimerization. *IJPT* **2003,** 2, (1), 1-11.

(118) Leurs, R.; Pena, M. S. R.; Bakker, R. A.; Alewijnse, A. E.; Timmerman, H., Constitutive activity of G protein coupled receptors and drug action. *Pharm. Acta Helv.* **2000,** 74, (2-3), 327-331.

(119) Samama, P.; Cotecchia, S.; Costa, T.; Lefkowitz, R. J., A mutation-induced activated state of the b_2-adrenergic receptor. Extending the ternary complex model. *J. Biol. Chem.* **1993,** 268, (7), 4625-4636.

(120) Seifert, R.; Wenzel-Seifert, K., Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2002,** 366, (5), 381-416.

(121) Salahpour, A.; Angers, S.; Bouvier, M., Functional Significance of Oligomerization of G-protein-coupled Receptors. *Trends Endocrinol. Metab.* **2000,** 11, (5), 163-168.

(122) Angers, S.; Salahpour, A.; Joly, E.; Hilairet, S.; Chelsky, D.; Dennis, M.; Bouvier, M., Detection of b_2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). *Proc. Natl. Acad. Sci. U. S. A.* **2000,** 97, (7), 3684-3689.

(123) McVey, M.; Ramsay, D.; Kellett, E.; Rees, S.; Wilson, S.; Pope, A. J.; Milligan, G., Monitoring Receptor Oligomerization Using Time-resolved Fluorescence Resonance Energy Transfer and Bioluminescence Resonance Energy Transfer. . *J. Biol. Chem.* **2001,** 276, (17), 14092-14099.

(124) Kroeger, K. M.; Hanyaloglu, A. C.; Seeber, R. M.; Miles, L. E. C.; Eidne, K. A., Constitutive and Agonist-dependent Homo-oligomerization of the Thyrotropin-releasing Hormone Receptor. . *J. Biol. Chem.* **2001,** 276, (16), 12736-12743.

(125) Rocheville, M.; Lange, D. C.; Kumar, U.; Sasi, R.; Patel, R. C.; Patel, Y. C., Subtypes of the Somatostatin Receptor Assemble as Functional Homo- and Heterodimers. *J. Biol. Chem.* **2000,** 275, (11), 7862-7869.

(126) Palczewski, K., G protein-coupled receptor rhodopsin. *Annu. Rev. Biochem.* **2006,** 75, (1), 743-767.

(127) Fotiadis, D.; Jastrzebska, B.; Philippsen, A.; Muller, D. J.; Palczewski, K.; Engel, A., Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. *Curr. Opin. Struct. Biol.* **2006,** 16, (2), 252-259.

(128) Liang, Y.; Fotiadis, D.; Filipek, S.; Saperstein, D. A.; Palczewski, K.; Engel, A., Organization of the G Protein-coupled Receptors Rhodopsin and Opsin in Native Membranes. *J. Biol. Chem.* **2003,** 278, (24), 21655-21662.

(129) Filipek, S.; Krzysko, K. A.; Fotiadis, D.; Liang, Y.; Saperstein, D. A.; Engel, A.; Palczewski, K., A concept for G protein activation by G protein-coupled receptor dimers: the transducin/rhodopsin interface. *Photochem. Photobiol. Sci.* **2004,** 3, (6), 628-638.

(130) Sartania, N.; Appelbe, S.; Pediani, J. D.; Milligan, G., Agonist occupancy of a single monomeric element is sufficient to cause internalization of the dimeric b_2-adrenoceptor. *Cell. Signal.* **2007,** 19, (9), 1928-1938.

(131) Terrillon, S.; Bouvier, M., Roles of G-protein-coupled receptor dimerization. *EMBO Reports* **2004,** 5, (1), 30-34.

(132) Salahpour, A.; Angers, S.; Mercier, J.-F.; Lagace, M.; Marullo, S.; Bouvier, M., Homodimerization of the b_2-Adrenergic Receptor as a Prerequisite for Cell Surface Targeting. *J. Biol. Chem.* **2004,** 279, (32), 33390-33397.

(133) Terrillon, S.; Durroux, T.; Mouillac, B.; Breit, A.; Ayoub, M. A.; Taulan, M.; Jockers, R.; Barberis, C.; Bouvier, M., Oxytocin and Vasopressin V1a and V2 Receptors Form Constitutive Homo- and Heterodimers during Biosynthesis. *Mol. Endocrinol.* **2003,** 17, (4), 677-691.

(134) Milligan, G., G Protein-Coupled Receptor Dimerization: Function and Ligand Pharmacology. *Mol. Pharmacol.* **2004,** 66, (1), 1-7.

(135) Park, P. S. H.; Filipek, S.; Wells, J. W.; Palczewski, K., Oligomerization of G Protein-Coupled Receptors: Past, Present, and Future. *Biochemistry* **2004,** 43, (50), 15643-15656.

(136) Javitch, J. A., The Ants Go Marching Two by Two: Oligomeric Structure of G-Protein-Coupled Receptors. *Mol. Pharmacol.* **2004,** 66, (5), 1077-1082.

(137) Fotiadis, D.; Liang, Y.; Filipek, S.; Saperstein, D. A.; Engel, A.; Palczewski, K., The G protein-coupled receptor rhodopsin in the native membrane. *FEBS Lett.* **2004,** 564, (3), 281-288.

(138) Portoghese, P. S., Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. *Trends Pharmacol. Sci.* **1989,** 10, (6), 230-235.

(139) Portoghese, P. S., From Models to Molecules: Opioid Receptor Dimers, Bivalent Ligands, and Selective Opioid Receptor Probes. *J. Med. Chem.* **2001,** 44, (14), 2259-2269.

(140) Himeno, A.; Shigematsu, K.; Taguchi, T.; Niwa, M., Endothelin-1 Binding to Endothelin Receptors in the Rat Anterior Pituitary Gland: Interaction in the Recognition of Endothelin-1 Between ETA and ETB Receptors. *Cell. Mol. Neurobiol.* **1998,** 18, (4), 447-452.

(141) 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, (1), 131-138.

(142) Carrillo, J. J.; Pediani, J.; Milligan, G., Dimers of Class A G Protein-coupled Receptors Function via Agonist-mediated Trans-activation of Associated G Proteins. *J. Biol. Chem.* **2003,** 278, (43), 42578-42587.

(143) 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, (2), 283-286.

(144) Shenton, F. C.; Hann, V.; Chazot, P. L., Evidence for native and cloned H_3 Histamine receptor higher oligomers. *Inflamm. Res.* **2005,** 54, (0), S48-S49.

(145) Bakker, R. A.; Lozada, A. F.; van Marle, A.; Shenton, F. C.; Drutel, G.; Karlstedt, K.; Hoffmann, M.; Lintunen, M.; Yamamoto, Y.; van Rijn, R. M.; Chazot, P. L.; Panula, P.; Leurs, R., Discovery of Naturally Occurring Splice Variants of the Rat Histamine H_3 Receptor That Act as Dominant-Negative Isoforms. *Mol. Pharmacol.* **2006,** 69, (4), 1194-1206.

(146) 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, (2), 604-615.

Chapter 2

Scope of the Thesis

N-[3-(1*H*-Imidazol-4-yl)propyl)guanidines such as arpromidine are the most potent histamine H_2 receptor agonists known so far. From a therapeutic point of view, these compounds are possibly useful as positive inotropic and vasodilatory drugs (´cardiohistaminergics`) for the treatment of severe congestive heart failure, as agents inducing cell differentiation in acute myelogenous leukaemia and as anti-inflammatory drugs. The strongly basic guanidino group, which is supposed to interact with Asp-98 in TM3 of the H_2R, is essential for the agonistic activity. However, the guanidino group is also responsible for very low oral bioavailability, non-H_2R-mediated effects and lack of penetration across the blood brain barrier. Very recently, a novel class of N^G-acylated analogues with reduced basicity and therefore improved pharmacokinetic properties such as oral bioavailability and capability of penetrating across the blood brain barrier, was developed in our workgroup[1, 2]. As demonstrated for *N*-[3-(1*H*-imidazol-4-yl)propyl)guanidines, the acylated derivatives are less potent and efficacious at $gpH_2R\text{-}G_{s\alpha S}$ than at $hH_2R\text{-}G_{s\alpha S}$ fusion proteins in a membrane steady-state GTPase assay[3-5]. By contrast, histamine and small H_2R agonists do not exhibit species-selectivity. Recently, *N*-(3-cyclohexylbutanoyl)-*N´*-[3-(1H-imidazol-4-yl)propyl]guanidine (Figure 2.1, UR-AK57), a compound which was synthesized during the preceding diploma work[6], was identified as the most potent hH_2R agonist so far (EC_{50} = 23 nM in a GTPase activity assay at $hH_2R\text{-}G_{s\alpha S}$ fusion proteins). Moreover, unlike most related acylguanidines, UR-AK57 exhibits H_1R agonistic activity[5].

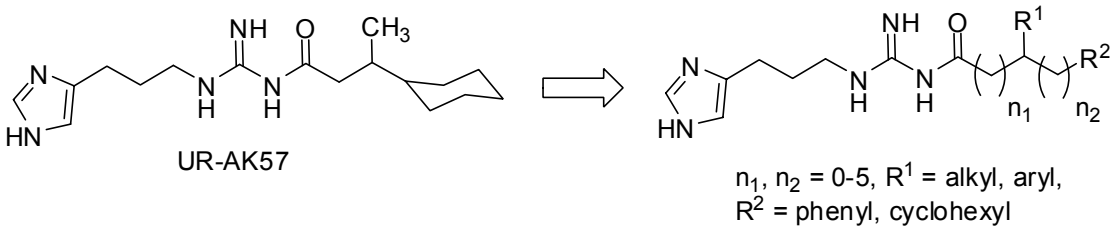


Figure 2.1. Structure of the H_2R agonist UR-AK57 and the general structure of N^G-acylated imidazolylpropylguanidines to be synthesized.

The aim of this work was to synthesize and characterize N^G-acylated imidazolylpropylguanidines with different chain lengths and alkyl/aryl substituted side chains to identify selective and potent agonists particularly at the human histamine H_2 receptor. Previously, the bioisosteric replacement of the imidazole ring by a 2-amino-4-methylthiazole group in the series of N^G-acylguanidine resulted in similarly potent and efficacious agonists at the H_2R, but with a remarkably increased selectivity over the H_3R[1]. To further elucidate the structure-activity relationships and the selectivity towards the H_2R *versus* the H_3R and the H_4R, a serie of N^G-acylated 3-(2-amino-4-methylthiazol-5-yl)- and 3-(2-aminothiazol-5-yl)propylguanidines (Figure 2.2) was designed and synthesized. The new compounds were investigated for H_2R agonistic potency on the isolated guinea pig right atrium, for species selective H_2R agonism and for histamine receptor subtype selectivity in GTPase assays using membranes of Sf9 cells expressing human and guinea pig H_2R-$G_{s\alpha S}$ fusion proteins, human H_3R+$G\alpha_o$+$\beta_1\gamma_2$+RGS4 membranes and human H_4R-GAIP+$G\alpha_{i2}$+$\beta_1\gamma_2$ membranes.

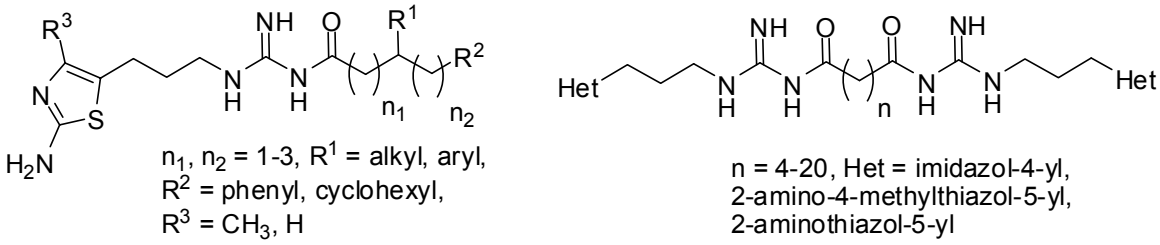


Figure 2.2. General structure of N^G-acylated 2-aminothiazolylpropylguanidines and bivalent ligands.

Histamine H_1-H_4 receptors are known to exist as oligomeric entities[7-12]. Hence, as part of this work prototypical bivalent H_2R ligands with different spacer lengths (Figure 2.2) were synthesized to specify the distinct interactions with the human and guinea pig H_2R and to investigate the activity at H_3R and H_4R in the steady-state GTPase activity assay.

In summary, as part of a programme aiming at the development of centrally active guanidine-type selective agonists for the human histamine H_2 receptor the aim of this thesis was to synthesize and pharmacologically characterize N^G-acylated hetarylpropylguanidines in order to further elaborate the structure-activity relationships and to improve the H_2R subtype selectivity.

2.1. References

(1) 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/volltexte/2006/561/

(2) Götte, C. Synthese, Enantiomerentrennung und Struktur-Wirkungsbeziehungen neuer Histamin H_2 Rezeptoragonisten des Arpromidin Typs. Doctoral Thesis, University of Regensburg, Germany, 2001.

(3) 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, (6), 1210-1225.

(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, (1), 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-(1H-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, (3), 1262-1268.

(6) Kraus, A. Histamin-H_2-agonistisch wirksame N^G-acylierte Imidazolylpropylguanidine: Synthese und pharmakologische Charakterisierung. Diploma thesis, University of Regensburg, Germany, 2004.

(7) 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, (1), 131-138.

(8) Carrillo, J. J.; Pediani, J.; Milligan, G., Dimers of Class A G Protein-coupled Receptors Function via Agonist-mediated Trans-activation of Associated G Proteins. *J. Biol. Chem.* **2003,** 278, (43), 42578-42587.

(9) 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, (2), 283-286.

(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, (3), 427-433.

(11) Shenton, F. C.; Hann, V.; Chazot, P. L., Evidence for native and cloned H_3 Histamine receptor higher oligomers. *Inflamm. Res.* **2005,** 54, (0), S48-S49.

(12) 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, (2), 604-615.

Chapter 3

N^G-Acylated Imidazolylpropylguanidines – Synthesis and Histamine H_2 Receptor Agonistic Activity

3.1. Introduction

The histamine H_2 receptor (H_2R) is a member of the "class A" or "rhodopsin family" of G protein-coupled receptors (GPCRs). H_2Rs were detected in numerous peripheral tissues and cells, for example, in leukocytes, the heart, airways, uterus and vascular smooth muscles and in the brain[1, 2]. In the human brain, the histamine H_2R is widely distributed in the basal ganglia, hippocampus, amygdala, and cerebral cortex as well as, with lower densities, in cerebellum and hypothalamus[3]. As H_2Rs couple to the G_s protein, receptor stimulation results in an increase in adenylyl cylase activity. H_2R-mediated biological responses are, for instance, the stimulation of gastric acid secretion, an increase in cardiac contractility and the induction of myeloid cell differentiation[1, 4].

N-[3-(1*H*-Imidazol-4-yl)propyl]guanidines like arpromidine (**ARP**) (Figure 3.1) and analogues[5] are the most potent H_2R agonists known so far. In addition to their value as pharmacological tools, such compounds could be useful as drugs, for instance, for the treatment of severe congestive heart failure, acute myelogenous leukaemia and inflammatory diseases[2]. The major disadvantages of the arpromidine-type H_2R agonists result from the strongly basic guanidine moiety: insufficient oral bioavailability and lack of penetration across the blood brain barrier. Surprisingly, the H_2R agonistic potency was retained when the methylene group adjacent to the guanidine was replaced with a carbonyl group, even though the basicity was thereby reduced by 4-5 orders of magnitude (for comparison: pK_a (guanidine) ≈ 12.5, pK_a (acetylguanidine) ≈ 7.6[6])[7]. Obviously, the acylguanidine group in this new class of H_2R agonists, the N^G-acylated imidazolylpropylguanidines, is sufficiently basic to interact with Asp-98 of the histamine H_2R by analogy with the suggested binding mode for arpromidine-like compounds. Moreover, a considerable portion of the

acylguanidines remains uncharged under physiological conditions so that these compounds are able to overcome the blood brain barrier. The brain penetration of *N*-[3-(3,4-difluorophenyl)-3-(thiazol-2-yl)propanoyl]- (**I**) and *N*-[3-phenylbutanoyl]-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (**II**) (Figure 3.1) were studied in nude mice after peroral and intravenous administration. In contrast to arpromidine-type H_2R agonists the acylated guanidines were absorbed from the gastrointestinal tract and detected in the brain by HPLC-MS analysis[8]. A slight to moderate decrease in H_2R agonistic potency, as found *in vitro* for the first series of acylguanidines compared to the corresponding alkylguanidines, can be accepted due to improved pharmacokinetic properties. Centrally active H_2R agonists will be useful tools to study the role of histamine H_2 receptors in the brain.

Guanidine-type H_2R agonists show different potencies depending on the considered species and tissue, for instance, the potency of the guanidines is different on human neutrophils compared to the guinea pig right atrium. Both the guanidines and the acylguanidines proved to be less potent and efficacious at the human (hH_2R) compared to the guinea pig (gpH_2R) histamine H_2 receptor[9, 10]. A homology model of the gpH_2R suggested that an H-bond between Tyr-17 and Asp-271 stabilizes an active receptor conformation of the gpH_2R. In particular, the presence of Ala-271 in the hH_2R instead of Asp-271 (gpH_2R) in transmembrane domain 7 accounts for this species-dependent difference[9].

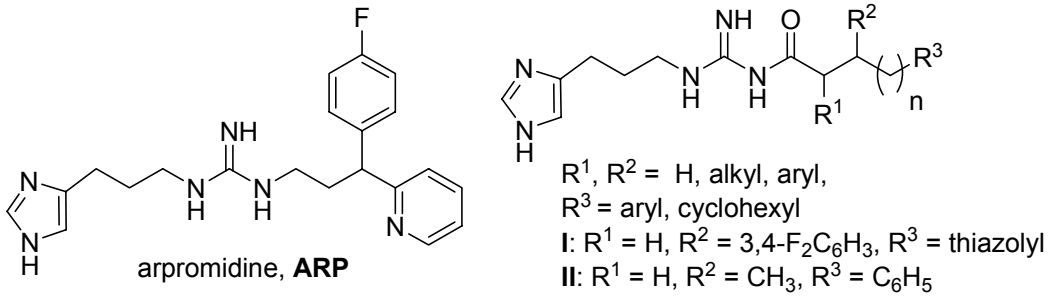
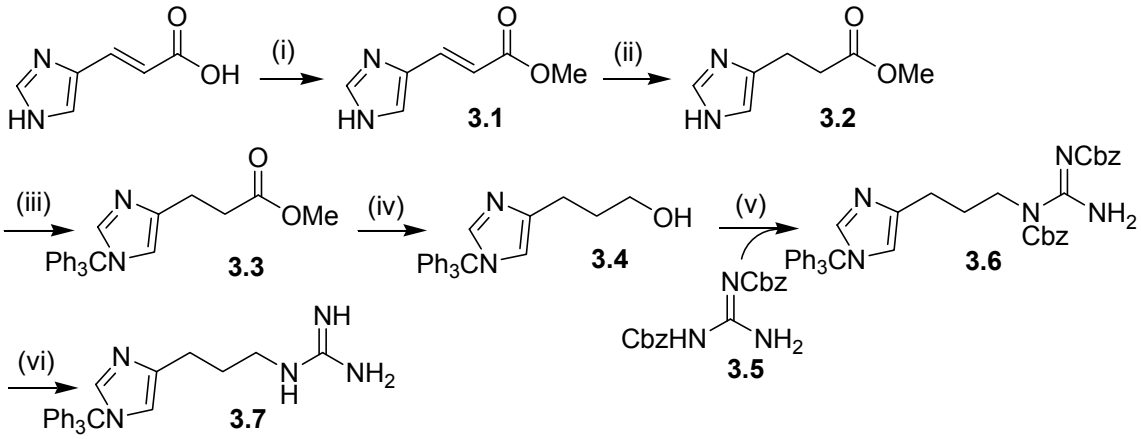


Figure 3.1. Structure of arpromidine, the general structure of N^G-acylated imidazolylpropylguanidines and structure of N^G-acylated imidazolylpropylguanidines **I** and **II** absorbed from the gastrointestinal tract of nude mice.

Previously it was shown that the alkylguanidines can be bioisosterically replaced by acylguanidines with a moderate decrease in potency at the guinea pig right atrium but improved pharmacokinetic properties[7]. In order to further study the structure-activity relationships, various N^G-acylated imidazolylpropylguanidines were synthesized and pharmacologically characterized.

3.2. Chemistry

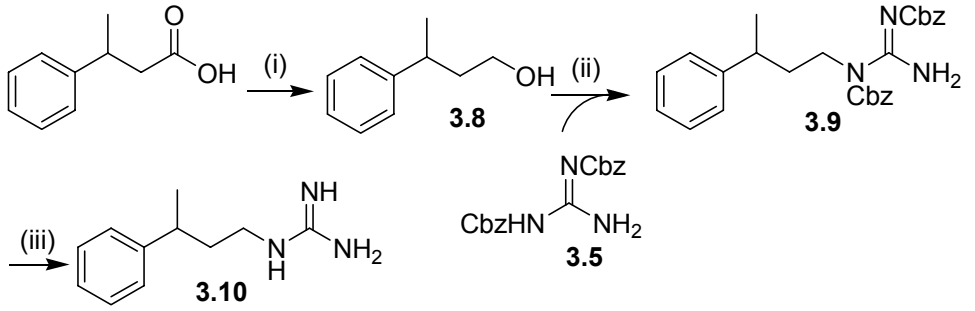
Towards the synthesis of N^G-acylated imidazolylpropylguanidines, a guanidine building block is coupled to carboxylic acids to make use of common coupling reagents. *N*-[3-(1-Trityl-1*H*-imidazol-4-yl)propyl]guanidine **3.7** was synthesized with minor modifications as previously described[7] starting from urocanic acid. After esterification, hydrogenation of the double bond and trityl-protection of the imidazole NH, the ester function was reduced with $LiAlH_4$. The alcohol **3.4** was coupled to the di-Cbz-protected guanidine **3.5** under Mitsunobu conditions[11] and, subsequently, the protecting groups were cleaved to yield the imidazolylpropylguanidine building block **3.7** (Scheme 3.1).



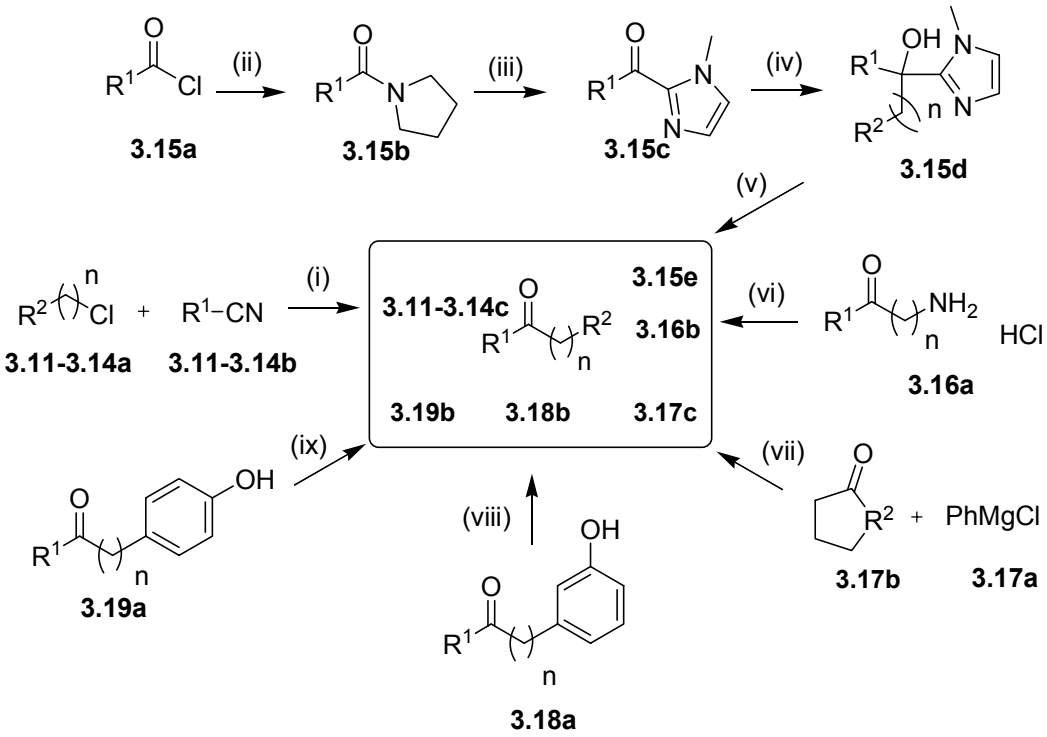
Scheme 3.1. Synthesis of the imidazolylpropylguanidine building block **3.7**. Reagents and conditions: (i) anhydrous Na_2SO_4, H_2SO_4/conc., MeOH/abs, 30 h, reflux[12]; (ii) H_2, Pd/C (10 %) (cat.), MeOH, 5 bar, 24 h, rt; (iii) CPh_3Cl (1.1 eq) , NEt_3 (2.8 eq), MeCN, 12h, rt; (iv) $LiAlH_4$ (2 eq), THF/abs, Et_2O/abs, 2 h, reflux; (v) **3.5** (1.8 eq), PPh_3 (1.5 eq), DIAD (1.5 eq), THF/abs, 24 h, rt; (vi) H_2, Pd/C (10 %), MeOH/THF (1:1), 5 bar, 24 h, rt.

The arylalkylguanidine building block **3.10** was synthesized according to Scheme 3.2 starting from 3-phenylbutyric acid. After reduction of the carboxylic acid with $LiAlH_4$, the free alcohol **3.8** was coupled to di-Cbz-protected guanidine **3.5** as described in Scheme 3.1 to obtain 3-phenylbutylguanidine **3.10**.

Scheme 3.3 summarizes the synthesis of ketones which were required as starting material for the Wittig-Horner reaction. The ketones **3.11-3.14c** were synthesized by reaction of Grignard reagents **3.11-3.14a** with nitriles **3.11-3.14b**.



Scheme 3.2. Synthesis of the arylalkylguanidine building block **3.10**; Reagents and conditions: (i) $LiAlH_4$ (1.2 eq), THF/abs, 2 h, rt; (ii) **3.5** (1.8 eq), PPh_3 (1.5 eq), DIAD (1.4 eq), THF/abs, 24 h, rt; (iii) H_2, Pd/C (10 %), MeOH/THF (1:1), 5 bar, 24 h, rt.



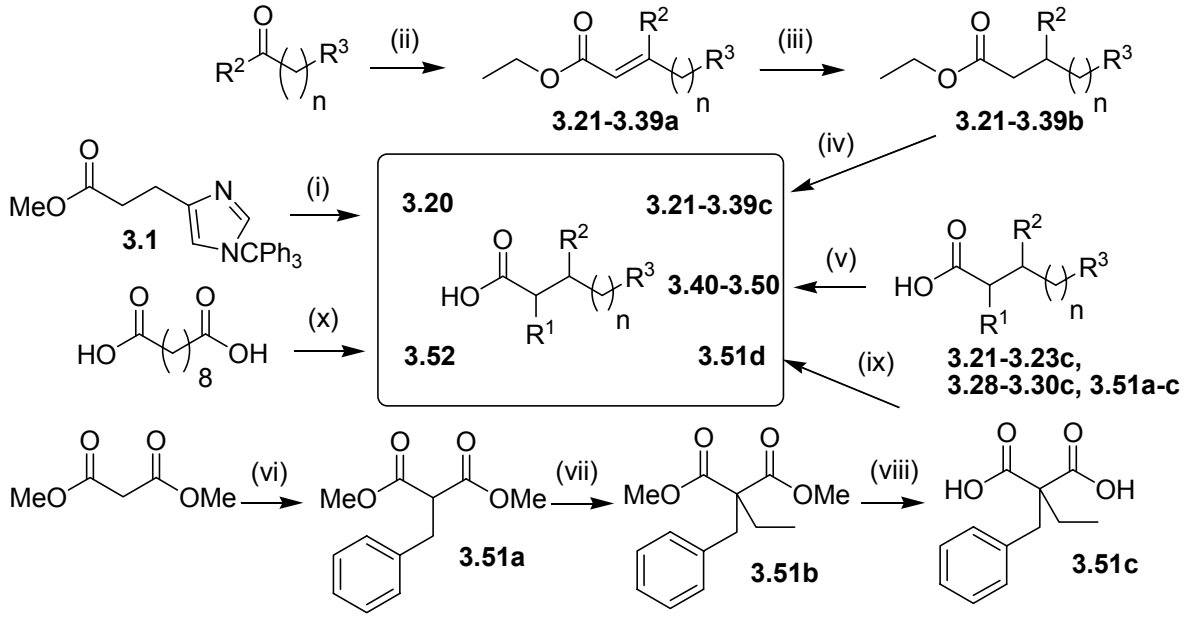
No	R^1	R^2	n	No	R^1	R^2	n
3.11a-c	C_6H_5	C_6H_5	1	**3.16a-b**	C_6H_5	NHBoc	1
3.12a-c	C_6H_5	4-CH_3-C_6H_4	1	**3.17a-c**	C_6H_5	NHBoc	3
3.13a-c	CH_3	C_6H_5	1	**3.18a-b**	CH_3	3-OBn-C_6H_4	0
3.14a-c	CH_3	C_6H_5	3	**3.19a-b**	CH_3	4-MPM-C_6H_4	0
3.15a-e	cHex	C_6H_5	1				

Scheme 3.3. Synthesis of ketones. Reagents and conditions: (i) Mg (1.2 eq), Et_2O, HCl, 24 h, reflux; (ii) pyrrolidine (0.8 eq), DMAP (1.5 eq), DCM, 20 min, rt; (iii) 1-methyl-1*H*-imidazole (1 eq), n-BuLi (1 eq), THF/abs, 10 min, -78 °C, **3.15b** (1 eq) , 30 min, rt; (iv) Mg (2 eq), BnCl (2 eq), Et_2O, 2-3 h, rt; (v) MeI (11 eq), EtOAc, 3-4 h, reflux, benzene, K_2CO_3 (1.2 eq), 2 h, 60 °C; (vi)$(Boc)_2O$ (1.2 eq), NEt_3 (1 eq), THF/abs, 20 h, rt; (vii) THF/abs, 1 h, -78 °C, rt; (viii) BnCl (1 eq), KOH (1.1 eq), EtOH, 5 h, reflux; (ix) 4-MPMCl (1.5 eq), K_2CO_3 (4 eq), acetone, overnight, reflux.

The synthesis of **3.15e** started from cyclohexanecarbonyl chloride **3.15a**, which was converted into the amide **3.15b** with pyrrolidine and DMAP as a base. The ketone **3.15c** was obtained in good yields by treating 1-acylpyrrolidine **3.15b** with 2-lithio-1-methyl-1*H*-imidazole, which was synthesized from 1-methyl-1*H*-imidazole and n-BuLi. The 2-acylimidazole **3.15c** was converted into the alcohol **3.15d** by Grignard reaction. Compound **3.15d** was refluxed in EtOAc solution with an excess of MeI to give an imidazolium salt which was immediately treated with K_2CO_3 at elevated temperature to yield **3.15e** in a retro-aldol reaction with the imidazolium group acting as a leaving group[13-15]. Ketone **3.16b** was easily synthesized by *tert*-butoxycarbonyl protection of the free amine **3.16a**. The selective ring-opening of lactam **3.17b** with phenylmagnesium chloride produced the ketone **3.17c**. Benzyl- and *p*-methoxybenzyl protection, respectively, of the *p*- and *m*-hydroxy groups resulted in the ketones **3.18a** and **3.19a**.

The pertinent carboxylic acids were synthesized using different standard methods as shown in Scheme 3.4. 3-(1-Trityl-1*H*-imidazol-4yl)propanoic acid **3.20** was synthesized by hydrolysis of the methyl ester **3.1**[16]. Compounds **3.21-3.39c** were obtained *via* Wittig-Horner reaction of synthesized (**3.11-3.19c**) or commercially available ketones with triethyl phosphonoacetate, subsequent hydrogenation of the double bond and hydrolysis of the ethyl ester.

The cyclohexylalkanoic acids **3.40-3.50** were prepared from the corresponding synthesized (**3.21-3.23c, 3.28-3.30c, 3.51a-d**) or commercially available phenyl-alkanoic acids by hydrogenation of the phenyl ring with Rh/Al_2O_3 or Rh/C as a catalyst and AcOH as a solvent[17]. The α-substituted alkanoic acid **3.51d** was prepared starting from dimethyl malonate. After treating with BnBr and EtBr, the methyl ester was hydrolyzed and the free dicarboxylic acid was decarboxylated at 170-200 °C to obtain 2-benzylbutanoic acid **3.51d**. 10-Benzyloxy-10-oxodecanoic acid **3.52** was synthesized by esterification of decanedioic acid with BnOH in the presence of DCC as coupling reagent.



No	R^1	R^2	R^3	n
3.20	H	H	1-trityl-2-imidazolyl	0
3.21a-c	H	CH_2CH_3	C_6H_5	0
3.22a-c	H	$CH(CH_3)_2$	C_6H_5	0
3.23a-c	H	$CH_2CH(CH_3)_2$	C_6H_5	0
3.24a-c	H	$CH_2(C_6H_5)$	C_6H_5	0
3.25a-c	H	$CH_2(4\text{-}CH_3\text{-}C_6H_4)$	C_6H_5	0
3.26a-c	H	$CH_2(C_6H_5)$	cHex	0
3.27a-c	H	CH_3	$4\text{-}CH_3\text{-}C_6H_4$	0
3.28a-c	H	CH_3	C_6H_5	1
3.29a-c	H	CH_3	C_6H_5	2
3.30a-c	H	CH_3	C_6H_5	3
3.31a-c	H	CH_3	$3\text{-}CH_3C_6H_4$	1
3.32a-c	H	CH_3	$4\text{-}CH_3\text{-}C_6H_4$	1
3.33a-c	H	CH_3	$3\text{-}F\text{-}C_6H_4$	1
3.34a-c	H	CH_3	$4\text{-}F\text{-}C_6H_4$	1
3.35a-c	H	CH_3	$3\text{-}OCH_3\text{-}C_6H_4$	1
3.36a-c	H	CH_3	$4\text{-}OCH_3\text{-}C_6H_4$	1
3.37a-c	H	CH_3	$4\text{-}CH_2CH_3\text{-}C_6H_4$	1
3.38a-c	H	CH_2NHBoc	C_6H_5	0
3.39a-c	H	$(CH_2)_3NHBoc$	C_6H_5	0
3.40	H	(*R*) CH_3	cHex	0
3.41	H	(*S*) CH_3	cHex	0
3.42	CH_3	H	cHex	0

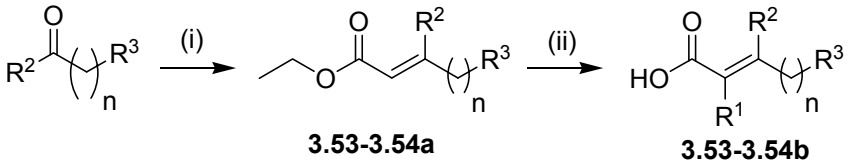
Scheme 3.4

Scheme 3.4 (continued)

3.43	H	CH_2CH_3	cHex	0
3.44	CH_2CH_3	H	cHex	0
3.45	H	$CH(CH_3)_2$	cHex	0
3.46	H	$CH_2CH(CH_3)_2$	cHex	0
3.47	H	CH_3	cHex	1
3.48	H	CH_3	cHex	2
3.49	H	CH_3	cHex	3
3.50	H	CH_2CH_3	cHex	1
3.51a-d	CH_2CH_3	H	C_6H_5	0
3.52	H	H	COBn	6
3.53a, b	H	CH_3	3-OBn-C_6H_4	0
3.54a, b	H	CH_3	4-MPM-C_6H_4	0

Scheme 3.4. Synthesis of alkanoic acids. Reagents and conditions: (i) LiOH/aq (1N) (1.2 eq), THF, 24 h, rt; (ii) NaH (60 % dispersion in mineraloil) (1.56 eq), triethyl phosphonoacetate (1.4-1.5 eq), THF/abs, 24 h, reflux; (iii) H_2, Pd/C (10 %) (cat.), EtOH, 24 h, rt; (iv) 20 % NaOH/aq, 2-3 h, reflux; (v) H_2, Rh/C or Rh/Al_2O_3 (cat.), AcOH, 8 bar, 48 h, rt; (vi) BnBr (1 eq), NaH (60 % dispersion in mineral oil) (1 eq) , THF/abs, overnight, reflux; (vii) EtBr (1.2 eq), NaH (60 % dispersion in mineral oil) (1.2 eq), THF/abs, overnight, reflux; (viii) KOH (10 eq), H_2O, EtOH, overnight, reflux; (ix) Δ, 3-4 h, 170-200 °C; (x) BnOH (1 eq), DCC (1.2 eq), DMAP (cat.), THF/abs, 48 h, rt.

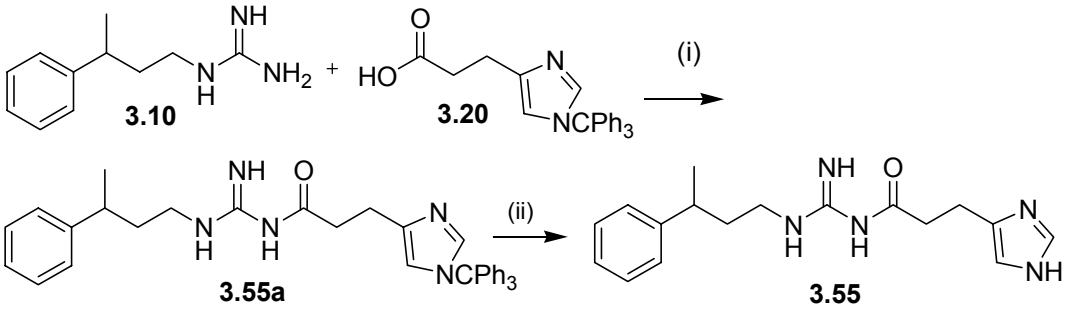
In the case of carboxylic acids **3.53-3.54b** with a benzyl- and *p*-methoxybenzyl protected hydroxy substituted phenyl ring, the hydrogenation step in the Wittig-Horner reaction was skipped to avoid deprotection and the (*E*/*Z*) ethyl ester was directly converted into the free acid (Scheme 3.5).



3.53-3.54a **3.53-3.54b**

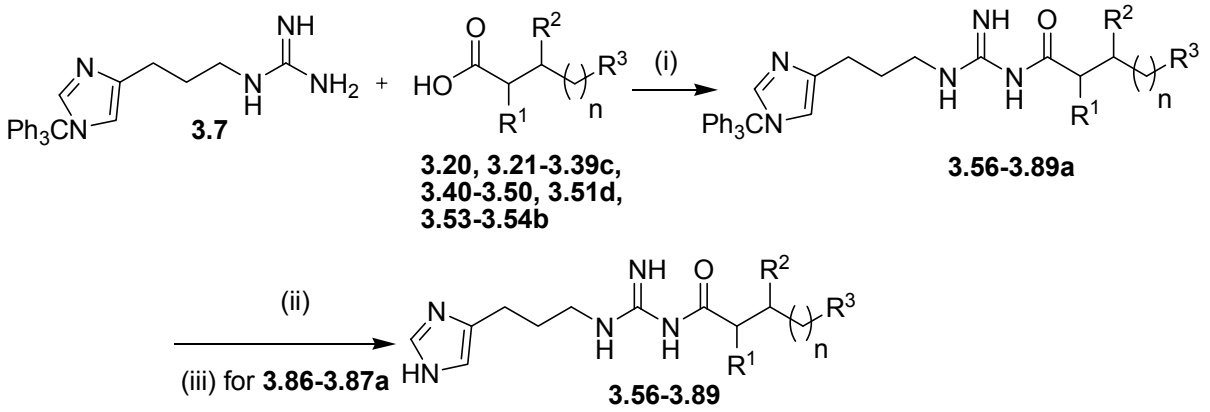
Scheme 3.5. Synthesis of unsaturated carboxylic acids **3.53-3.54b**. Reagents and conditions: (i) NaH (60 % dispersion in mineral oil) (1.56 eq), triethyl phosphonoacetate (1.4-1.5 eq), THF/abs, 24 h, reflux; (ii) 20 % NaOH/aq, 2-3 h, reflux.

3-Phenylbutylguanidine **3.10** was coupled with 3-(1-trityl-1*H*-imidazol-4yl)propanoic acid **3.20** using *N*,*N´*-carbonyldiimidazole as an acylation activating agent[18] (Scheme 3.6). Subsequently, compound **3.55a** was deprotected by treatment with TFA in DCM to yield *N*-[3-(1*H*-imidazol-4-yl)propanoyl]-*N´*-(3-phenylbutyl)guanidine **3.55**.



Scheme 3.6. Synthesis of the N^G-acylated arylalkylpropylguanidine **3.55**. (i) CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF/abs, 3-4 h, rt; (ii) 20 % TFA, DCM, 5-6 h, rt.

According to Scheme 3.7, the N^G-acylated imidazolylpropylguanidines **3.56-3.89** were synthesized by a reaction of the trityl-protected imidazolylpropylguanidine building block **3.7** with synthesized (**3.20**, **3.21-3.39c**, **3.40-3.50**, **3.51d**, **3.52**, **3.53b** and **3.54b**) and commercially available carboxylic acids. The guanidine building block **3.7** was deprotonated with NaH and coupled to the pertinent carboxylic acid, which was activated by CDI, to yield the building trityl-protected precursors **3.56-3.89a**.



No	R^1	R^2	R^3	n
3.56	H	CH_2CH_3	C_6H_5	0
3.57	CH_2CH_3	H	C_6H_5	0
3.58	H	$CH(CH_3)_2$	C_6H_5	0
3.59	H	$CH_2CH(CH_3)_2$	C_6H_5	0
3.60	H	$CH_2(C_6H_5)$	C_6H_5	0
3.61	H	$CH_2(4\text{-}CH_3\text{-}C_6H_4)$	C_6H_5	0
3.62	H	$CH_2(C_6H_5)$	cHex	0
3.63	H	H	$4\text{-}CH_3\text{-}C_6H_4$	0
3.64	H	CH_3	$4\text{-}CH_3\text{-}C_6H_4$	0

Scheme 3.7

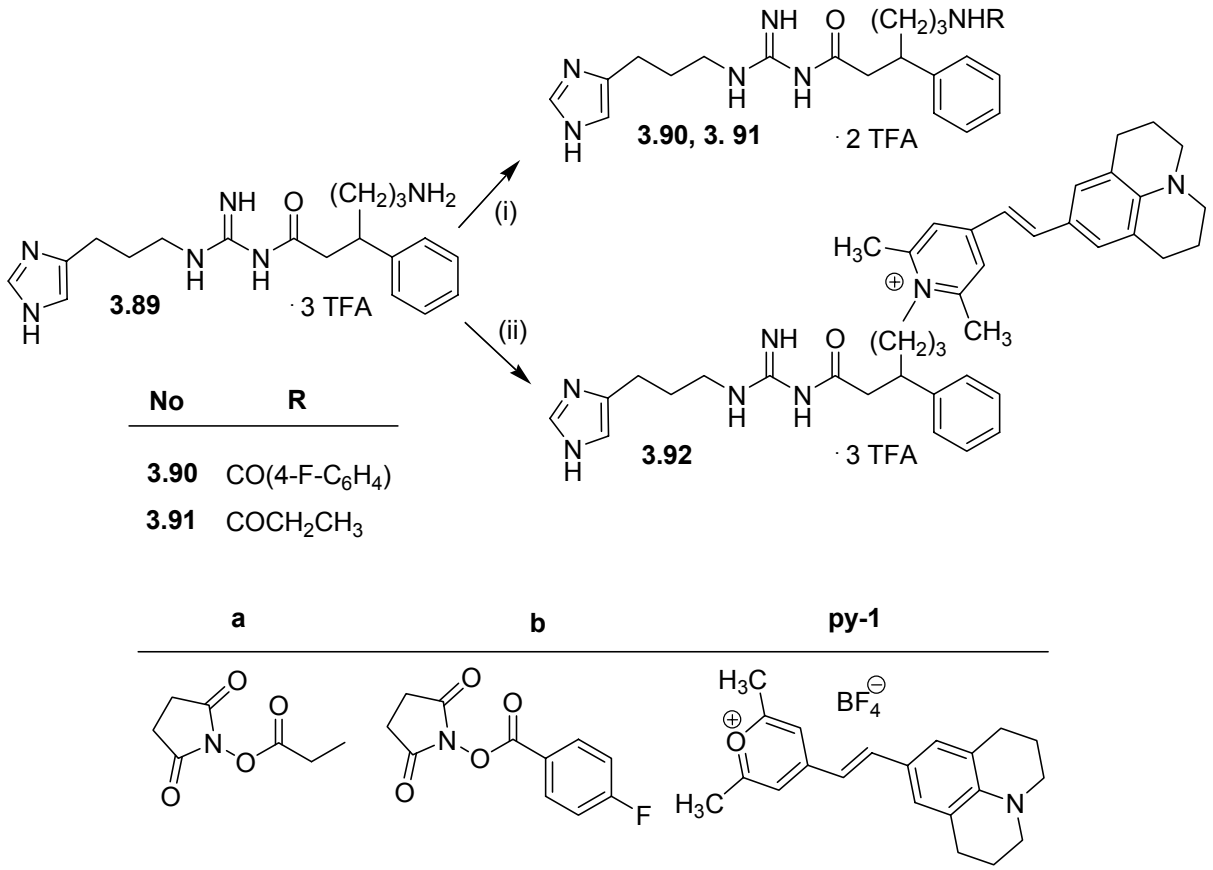
Scheme 3.7 (continued)

3.65	H	CH_3	C_6H_5	1
3.66	H	CH_3	C_6H_5	2
3.67	H	CH_3	C_6H_5	3
3.68	H	CH_3	3-CH_3-C_6H_4	1
3.69	H	CH_3	4-CH_3-C_6H_4	1
3.70	H	CH_3	3-F-C_6H_4	1
3.71	H	CH_3	4-F-C_6H_4	1
3.72	H	CH_3	3-OCH_3-C_6H_4	1
3.73	H	CH_3	4-OCH_3-C_6H_4	1
3.74	H	CH_3	4-CH_2CH_3-C_6H_4	1
3.75	H	(*R*) CH_3	cHex	0
3.76	H	(*S*) CH_3	cHex	0
3.77	CH_3	H	cHex	0
3.78	H	CH_2CH_3	cHex	0
3.79	CH_2CH_3	H	cHex	0
3.80	H	$CH(CH_3)_2$	cHex	0
3.81	H	$CH_2CH(CH_3)_2$	cHex	0
3.82	H	CH_3	cHex	1
3.83	H	CH_3	cHex	2
3.84	H	CH_3	cHex	3
3.85	H	CH_2CH_3	cHex	1
3.86	H	CH_3	3-OH-C_6H_4	0
3.87	H	CH_3	4-OH-C_6H_4	0
3.88	H	CH_2NH_2	C_6H_5	0
3.89	H	$(CH_2)_3NH_2$	C_6H_5	0

Scheme 3.7. General procedure for the coupling of carboxylic acids with the imidazolylpropyl-guanidine building block **3.7**. Reagents and conditions: (i) CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF/abs, 3-4 h, rt, (ii) 20 % TFA, DCM, 5-6 h, rt, (iii) H_2, Pd/C, EtOH, 8 bar, 6 d or 50 % TFA, DCM, 4 h, rt

The trityl group was removed under acidic conditions to give the N^G-acylated imidazolylpropylguanidines **3.56-3.89**. Prior to TFA treatment, the benzyl- and MPM protecting group in **3.86a** and **3.87a** were cleaved off by hydrogenation over Pd/C (10 %). Unfortunately, the hydrogenolysis was rather inefficient: the benzyl group could be removed after six days at 8 bar in low yield, and the reaction time was not shorter with the MPM protecting group. Finally, MPM was removed under acidic conditions with 50 % TFA together with the trityl-protecting group.

The free amino group in compound **3.89** was acylated by stirring with the pertinent succinimidyl ester for a few hours at room temperature yielding the compounds **3.90-3.91** according to Scheme 3.8.



Scheme 3.8. Synthesis of compounds **3.90**-**3.92**. Reagents and conditions: (i) **3.89** (1 eq), **a** or **b** (0.8 eq), NEt_3 (3 eq), MeCN, 4-5 h, rt; (ii) **py-1** (1 eq), **3.89** (2 eq), NEt_3 (7.5 eq), MeCN, DMF, 1 h, rt.

The fluorescent compound **3.92** was easily synthesized from **3.89** and the fluorescent pyrylium dye py-1 by ring transformation within one hour at room temperature.

3.3. Pharmacological results and discussion

Since the definition of H_2R by Black et al. in 1972[19], the isolated guinea pig right atrium is widely accepted as a pharmacological standard model for the functional characterisation of H_2R ligands. For the investigation of the H_2R agonists the positive chronotropic response was determined *versus* histamine as the reference compound (Table 3.1). In addition to the new chemical entities previously reported H_2R agonists are included in Table 3.1 with respect to the discussion of the structure-activity

relationships. Additionally, all substances were investigated in GTPase assays on human and guinea pig histamine H_2R-$G_{s\alpha S}$ fusion proteins with respect to possible species selective ligand H_2R interactions. These studies were performed using membrane preparations of hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ expressing Sf9 insect cells (Table 3.2). As H_2R agonists such as arpromidine are known to have weak to moderate H_1R antagonistic activity, selected compounds were also investigated for H_1R antagonism in a calcium assay (fura-2 assay) on human U-373 glioma cells (Table 3.3).

To further study the histamine receptor subtype selectivity, selected compounds were also tested for histamine H_3 and H_4 receptor activity in the GTPase assay (Table 3.4).

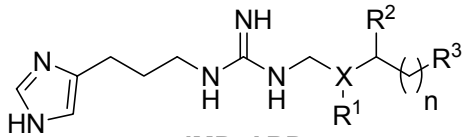
3.3.1. Histamine H_2 receptor agonism

3.3.1.1. H_2R agonism on the isolated guinea pig right atrium

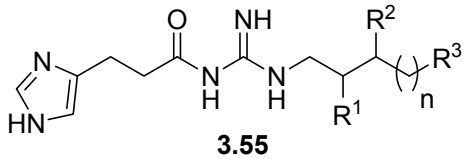
Most of the synthesized acylguanidines proved to be full or nearly full histamine H_2 receptor agonists on the spontaneously beating guinea pig right atrium. “Oxo-impromidine” (**3.93**) and “oxo-arpromidine” (**3.94**) show 2- to 5-fold lower potency at the guinea pig right atrium than the alkylguanidines impromidine and arpromidine[7]. But, with respect to *in vivo* application, this minor decrease in potency is acceptable due to improved pharmacokinetic properties.

The change of the position of the carbonyl function results in a loss of agonistic activity. Compound **3.55** is a weak antagonist (pA_2 = 5.10) on the guinea pig right atrium as well as in the GTPase assay (Table 3.2). The most potent compound in this series is the 3-phenylbutanoylguanidine **3.96**, a full agonist which is 28 times more potent than histamine at the guinea pig right atrium. Substitution in α-postion is also well tolerated. The 2-methyl-3-phenylpropanoyl derivative **3.97** shows a slight decrease in potency whereas efficacy is not affected. Compared to the corresponding phenylalkanoylguanidines the potency is decreased for the racemic 3-cyclohexylbutanoyl and 2-methyl-3-cyclohexylpropanoyl analogues. The (*S*)-configured enantiomer (**3.76**) is three times more potent than the (*R*) enantiomer (**3.75**) and about equipotent with the chiral phenyl analogue **3.96**. By contrast, in the corresponding phenylalkanoylguanidines, higher potency resides in the (*R*)-configured enantiomer (data not shown)[7]. However, this difference should not be overinterpreted as the eudismic ratios are low for both the phenyl and the cyclohexyl substituted compounds.

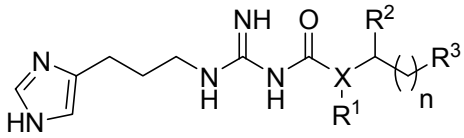
Table 3.1. Histamine H_2 receptor agonism on the guinea pig right atrium.



IMP, ARP



3.55



3.56-3.102

						H_2R agonism - isolated guinea pig right atrium		
No	**X**	**R^1**	**R^2**	**R^3**	**n**	**pEC_{50} ± SEM[a]**	**rel. pot.[b]**	**E_{max} ± SEM (%)[c]**
HIS	-	-	-	-	-	6.00 ± 0.10	100	100 ± 2
IMP	S	H	-	5-methyl-4-imidazolyl	1	7.70 ± 0.10	5,000	100
ARP	CH	H	2-pyridyl	4-F- C_6H_4	0	8.01 ± 0.10	10,200	100
3.93[7]	S	H	-	5-methyl-4-imidazolyl	1	7.38 ±0.09	2,370	101± 2
3.94[7]	CH	H	2-pyridyl	4-F-C_6H_4	0	7.34 ± 0.12	2,170	100 ± 1
3.55	CH	H	CH_3	C_6H_5	0	5.10 ± 0.19[d]	-	7 ± 1[e]
3.96[20]	CH	H	CH_3	C_6H_5	0	7.43 ± 0.07	2,710	99 ± 2
3.97[21]	CH	CH_3	H	C_6H_5	0	7.30 ± 0.10	1,980	96 ± 2
3.56	CH	H	CH_2CH_3	C_6H_5	0	6.97 ± 0.13	927	93 ± 5
3.57	CH	CH_2CH_3	H	C_6H_5	0	7.03 ± 0.15	1,060	101 ± 2
3.58	CH	H	$CH(CH_3)_2$	C_6H_5	0	6.98 ± 0.14	955	99 ± 7
3.59	CH	H	$CH_2CH(CH_3)_2$	C_6H_5	0	6.73 ± 0.10	541	88 ± 3
3.60	CH	H	$CH_2(C_6H_5)$	C_6H_5	0	6.30 ± 0.12	200	81 ± 4
3.101[7]	CH	H	C_6H_5	C_6H_5	0	7.22 ± 0.09	1650	85 ± 3
3.61	CH	H	CH_2(4-CH_3-C_6H_4)	C_6H_5	0	6.55 ± 0.12	357	82 ± 5
3.62	CH	H	$CH_2(C_6H_5)$	cHex	0	6.12 ± 0.02	132	83 ± 6
3.63	CH	H	H	4-CH_3-C_6H_4	0	6.52 ± 0.03	327	101 ± 2
3.64	CH	H	CH_3	4-CH_3-C_6H_4	0	7.10 ± 0.09	1,270	97 ± 1
3.99[21]	CH	H	CH_3	4-$CH(CH_3)_2$-C_6H_4	0	6.14 ± 0.11	137	85 ± 4
3.65	CH	H	CH_3	C_6H_5	1	7.11 ± 0.14	1,300	101 ± 3
3.66	CH	H	CH_3	C_6H_5	2	6.07 ± 0.19	117	80 ± 3
3.67	CH	H	CH_3	C_6H_5	3	6.08 ± 0.13	119	80 ± 4
3.95[21]	CH	H	H	C_6H_5	3	5.67 ± 0.17	46	62 ± 6
3.98[21]	CH	H	CH_2CH_3	C_6H_5	1	6.39 ± 0.11	247	86 ± 3
3.100[21]	CH	H	$CH_2(C_6H_5)$	C_6H_5	1	5.97 ± 0.02	92	67 ± 4

Table 3.1. (continued)

3.68	CH	H	CH_3	3-CH_3-C_6H_4	1	6.49 ±0.02	311	85 ± 4
3.69	CH	H	CH_3	4-CH_3-C_6H_4	1	6.21 ± 0.08	162	95 ± 4
3.70	CH	H	CH_3	3-F-C_6H_4	1	6.86 ± 0.11	724	100 ± 2
3.71	CH	H	CH_3	4-F-C_6H_4	1	6.32 ± 0.08	209	90 ± 1
3.72	CH	H	CH_3	3-OCH_3-C_6H_4	1	6.69 ± 0.08	490	92 ± 5
3.73	CH	H	CH_3	4-OCH_3-C_6H_4	1	6.44 ± 0.09	277	81 ± 4
3.74	CH	H	CH_3	4-CH_2CH_3-C_6H_4	1	5.84 ± 0.01	69	72 ± 2
3.102[20]	CH	H	CH_3	cHex	0	6.81 ± 0.07	641	101 ± 3
3.75	CH	H	(*R*) CH_3	cHex	0	6.86 ± 0.07	721	82 ± 4
3.76	CH	H	(*S*) CH_3	cHex	0	7.35 ± 0.12	2,230	103 ± 2
3.77	CH	CH_3	H	cHex	0	6.88 ± 0.15	764	94 ± 2
3.78	CH	H	CH_2CH_3	cHex	0	6.46 ± 0.04	290	95 ± 1
3.79	CH	CH_2CH_3	H	cHex	0	6.21 ± 0.13	161	77 ± 5
3.80	CH	H	$CH(CH_3)_2$	cHex	0	6.42 ± 0.05	263	85 ± 5
3.81	CH	H	$CH_2CH(CH_3)_2$	cHex	0	6.70 ± 0.09	501	83 ± 3
3.82	CH	H	CH_3	cHex	1	6.11 ± 0.10	128	85 ± 3
3.83	CH	H	CH_3	cHex	2	5.97 ± 0.15	94	59 ± 4
3.84	CH	H	CH_3	cHex	3	4.93 ± 0.09	9	74 ± 10
3.85	CH	H	CH_2CH_3	cHex	1	6.32 ± 0.08	210	79 ± 6
3.86	CH	H	CH_3	3-OH-C_6H_4	0	nd	nd	nd
3.87	CH	H	CH_3	4-OH-C_6H_4	0	nd	nd	nd
3.88	CH	H	CH_2NH_2	C_6H_5	0	6.42 ± 0.05	261	100 ± 3
3.89	CH	H	$(CH_2)_3NH_2$	C_6H_5	0	7.20 ± 0.05	1,580	102 ± 3

[a] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + \Delta pEC_{50}$; data shown are the ± SEM of three to seven experiments; [b] potency, relative to histamine = 100 %; [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine; [d] antagonist (pA_2); [e]E_{max} at 100 µM, E_{max} of histamine in the presence of 100 µM **3.55** was 45 ± 5 %.

The introduction of bulkier side chains reduces the potency as well as efficacy (**3.56**-**3.62**) for cyclohexyl- and phenylalkanoylguanidines. Substances bearing methyl, fluoro or methoxy substituent in *meta* position of the phenyl ring are slightly superior to the corresponding *para* substituted analogues (**3.68**-**3.73**). A 3-phenyl- or 3-benzylbutanoyl moiety, respectively, is optimal in affording high potency and efficacy at the guinea pig right atrium, whereas a cyclohexyl substituent is less well tolerated (except the (*S*)-enantiomer **3.76**). Interestingly, in the GTPase assay (Table 3.2) the 3-cyclohexylpentanoylguanidine (**3.77**) turned out to be one of the most potent compounds in this series, whereas at the guinea pig right atrium 3-phenyl- (**3.96**) and (*S*)-3-cyclohexylbutanoyl (**3.76**) substituted compounds proved to be the most potent ones. 3-Phenyl-6-aminohexanoyl guanidine (**3.89**) is about six times more potent

than the lower homologue, the 3-phenyl-4-aminobutanoylguanidine **3.88**. Both amino-functionalized compounds are full H_2R agonists.

3.3.1.2. Agonist potencies and efficacies at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins

To study species selectivity, the compounds were tested at human and guinea pig H_2R-$G_{s\alpha S}$ fusion proteins in the GTPase assay. The results are summerized in Table 3.2. N^G-acylated imidazolylpropylguanidines with two aromatic ringsystems exhibit high agonistic potency at H_2R membranes[7, 22]. However, acylguanidines with only one aromatic or cyclohexyl ring, respectively, and aliphatic side chains show similar unless higher agonistic potency at both, human and guinea pig H_2R as well[23]. At compounds with a phenyl ring and a connecting chain ranging from none to four methylene groups (**3.103**-**3.107**), minor changes in potency at hH_2R or gpH_2R, respectively, but different effects on the efficacy were noted (data not shown)[23]. Further increase of the chain (**3.95**) results in a tenfold increase in potency at the hH_2R, whereas the potency at the gpH_2R only triplicates. Compound **3.95** turned out to be the first guanidine-type (acylguanidine and alkylguanidine) H_2R agonist which has a higher potency at the human H_2 receptor (EC_{50} = 6.3 nM) than at the guinea pig H_2 receptor (EC_{50} = 18.9 nM). This data also supports the concept of a bigger and more flexible binding pocket in hH_2R than in gpH_2R[9]. Though, the efficacy is decreasing, shifting from strong partial to weak partial agonists (eff. = 0.44 for **3.95**) at hH_2R and from full to partial agonists (eff. = 0.42 for **3.95**) at the gpH_2R with increasing the chain length. At both species, the potency slightly increases by introduction of a methyl group in β-position (**3.105**→**3.96**, **3.106**→**3.65**, **3.107**→**3.66**), but again the efficacy drops. However, this is not true for compound **3.95**. In this case, a methyl introduction decreases potency fivefold at the hH_2R, whereas at the gpH_2R potency is nearly the same. An alkyl substituent in α-position (**3.97**, **3.57**, **3.77** and **3.79**) was tolerated as well with similar potency and similar (**3.97** and **3.57**) or slight decrease in efficacy (**3.77** and **3.79**) compared to the β-substituted analogues.

Table 3.2. Agonist efficacies and potencies at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ expressed in Sf9 cell membranes.

No	hH_2R-$G_{s\alpha S}$			gpH_2R-$G_{s\alpha S}$			EC_{50} hH_2R-$G_{s\alpha S}$/ EC_{50} gpH_2R-$G_{s\alpha S}$
	efficacy	EC_{50} [nM]	rel. pot.	efficacy	EC_{50} [nM]	rel. pot.	
His[9]	1.00	1260 ± 250	100	1.00	1200 ± 240	100	1.05
IMP[9]	0.84 ± 0.04	200 ± 20	641	1.00 ± 0.12	40 ± 10	3,060	5.00
ARP[9]	0.79 ± 0.07	190 ± 0.04	659	1.02 ± 0.04	70 ± 10	1,600	2.71
3.93[22]	0.79 ± 0.04	270 ± 38	440	0.93 ± 0.01	60 ± 1	2,000	4.44
3.94[22]	0.73 ± 0.03	420 ± 90	290	0.93 ± 0.04	45 ± 4	2,700	9.21
3.55	-	(1220)[a]	-	-	(1240)[a]	-	-
3.96[23]	0.87 ± 0.01	67 ± 2	1,800	1.03 ± 0.06	12 ± 1	10,000	5.58
3.97[21]	0.85 ± 0.04	22.8 ± 2.9	5,530	0.92 ± 0.11	7.9 ± 3.4	15,190	2.89
3.56	0.64 ± 0.18	36.9 ± 20.6	3,420	0.78 ± 0.08	11 ± 4.2	10,910	3.35
3.57	0.71 ± 0.05	31.3 ± 3.1	4,025	0.80	6.2	19,354	5.04
3.58	0.49	33.2 ± 2.3	3,800	0.72 ± 0.08	32.4 ± 0.8	3,700	1.02
3.59	0.3 ± 0.04	24.3 ± 3.9	5,190	0.72 ± 0.08	13.5 ± 7.9	8,890	1.8
3.60	0.34 ± 0.02	41.2 ± 12.1	3,060	0.65 ± 0.01	22.7 ± 13.7	5,290	1.81
3.61	0.48 ± 0.01	42.7 ± 2	2,950	0.46 ± 0.03	19.8 ± 11.9	6,060	2.16
3.62	0.35 ± 0.09	58.9 ± 1.4	2,140	0.68 ± 0.09	30.4 ± 23.3	3,950	1.94
3.63	0.87 ± 0.05	58.8 ± 22.5	2,140	0.9 ± 0.02	28.6 ± 4.6	4,200	2.06
3.64	0.95 ± 0.04	78.8 ± 21.3	1,600	0.85 ± 0.05	31.5 ± 4	3,810	2.5
3.99[21]	0.70 ± 0.12	50.2 ± 23.9	2,510	0.5 ± 0.2	9.1 ± 0.75	13,190	5.52
3.65	0.61 ± 0.06	26.4 ± 3	4,770	0.86 ± 0.06	9.9 ± 1.4	12,120	2.67
3.66	0.46 ± 0.1	45.8 ± 26.5	2,750	0.59 ± 0.14	23.2 ± 8.4	5,170	1.97
3.67	0.31 ± 0.01	30.5 ± 16.4	4,130	0.58 ± 0.1	12.4 ± 2.4	9,680	2.46
3.95[21]	0.44 ± 0.16	6.3 ± 0.35	20,000	0.42 ± 0.07	18.9 ± 9.9	6,350	0.33
3.98[21]	0.51 ± 0.02	38.1 ± 2.6	3,310	0.73 ± 0.01	24.8 ± 9.9	4,840	1.54
3.100[21]	(-0.06)[b]	-	-	0.32	19.5	6,154	-
3.68	0.58 ± 0.17	34.8 ± 5.9	3,620	0.77 ± 0.01	56 ± 39.5	2,140	0.6
3.69	0.34 ± 0.08	51.1 ± 32.1	2,465	0.58 ± 0.05	78.9 ± 56	1,520	0.65
3.70	0.67 ± 0.01	16.2 ± 2.4	7,780	0.98 ± 0.02	13.3 ± 11.2	9,020	1.22
3.71	0.86 ± 0.07	96.9 ± 54.4	1,300	0.84 ± 0.01	40.7 ± 18.3	2,950	2.38
3.72	0.84 ± 0.02	22.2 ± 0.75	5,675	0.80 ± 0.09	18.5 ± 5.6	6,490	1.2
3.73	0.68 ± 0.03	43 ± 21.6	2,930	0.57 ± 0.09	67.8 ± 4.7	1,770	0.63
3.74	0.34 ± 0.02	38.4 ± 20.5	3,280	0.19 ± 0.04	33.9 ± 3.4	3,540	1.13
3.102[23]	0.87 ± 0.05	23 ± 3	5,200	1.11 ± 0.16	9 ± 1	13,300	2.56
3.75	0.83 ± 0	18.6 ± 3.8	6,775	0.9 ± 0.03	23.5 ± 6.1	5,110	0.79
3.76	0.99 ± 0.01	6.5 ± 0.45	19,385	0.93 ± 0	8.5 ± 2.8	14,120	0.76
3.77	0.61 ± 0.06	9.7 ± 0.8	12,990	0.71 ± 0.06	4.1 ± 1.5	29,270	2.37
3.78	0.65 ± 0.1	7.4 ± 3.4	17,030	0.89 ± 0.05	3.5 ± 0.70	34,290	2.11

Table 3.2 (continued)

3.79	0.34 ± 0.09	21.4 ± 10.9	5,888	0.54 ± 0.06	6.4 ± 0.2	18,750	3.34
3.80	0.76 ± 0.2	67.6 ± 0	1,860	0.58 ± 0.09	22.4 ± 14.8	5,360	3.02
3.81	0.56 ± 0.04	105.1 ± 9.7	1,200	0.69 ± 0.01	10.8 ± 7.1	11,110	9.73
3.82	0.72 ± 0.04	21.6 ± 8.7	5,830	0.82 ± 0.05	16.4 ± 2.3	7,320	1.32
3.83	0.55 ± 0.03	17.2 ± 5.2	7,325	0.36 ± 0.08	15.5 ± 2	7,740	1.11
3.84	(-0.05)[b]	-	-	0.18	nd	nd	-
3.85	0.40 ± 0.04	12.6 ± 2.5	10,000	0.41 ± 0.01	10.2 ± 3	11,760	1.24
3.86	0.82 ± 0.09	46.8 ± 29	2,690	0.97 ± 0.01	4 ± 0.9	30,000	11.7
3.87	0.84 ± 0.15	30.9 ± 10.5	4,078	0.73	1.7	70,588	18.2
3.88	0.62 ± 0.05	752 ± 295	170	0.82 ± 0.03	102 ± 46	1,180	7.37
3.89	0.68 ± 0.01	172 ± 57	730	0.86 ± 0.06	21 ± 6	5,710	8.19
3.90	0.75 ± 0.16	58.3 ± 9.4	2,161	0.60	6.3	19,048	7.76
3.91	0.59	91.2	1,380	0.61	19.5	6,154	4.68
3.92	0.48	25.9	4,865	0.66	19.5	6,154	1.32

[a] Antagonist, IC_{50}; [b] no agonistic activity at a concentration of 10 µM; for structures of previously synthesized compounds, which are included in Table 3.2 with respect to the discussion of structure-acitivity relationships, see Table 3.1.

Steady state GTPase activity in Sf9 membranes expressing hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ was determined as described in the literature[9]. Reaction mixtures contained ligands at concentrations from 1 nM to 10 µM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 0.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (100 µM) ranged between ~ 2 and 13 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the ± SEM of two to three experiments or one experiment performed in duplicates each. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value. The ratio of the EC_{50} values of H_2R agonists for hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ were also continued.

Concerning efficacy β-substituents larger than methyl (**3.96**) are unfavourable at both hH_2R and gpH_2R. However, for more space-filling substituents in β-position, such as ethyl (**3.56**), isopropyl (**3.58**), isobutyl (**3.59**), benzyl (**3.60**), *p*-methylbenzyl (**3.61**) and cyclohexylmethyl (**3.62**) the influence on agonistic potency at human and guinea pig H_2R is different. There appears to be an optimum size with an isobutyl residue at the hH_2R: compound **3.59** is more potent than 3-phenylbutanoylguanidine **3.96**, whereas further increase in bulk (cf. **3.61** and **3.62**) results in reduced potency. At the gpH_2R, generally, the potency slightly decreases with introducing bulky side chains. Consequently, for these compounds the ratio of EC_{50} values (hH_2R *versus* gpH_2R) is smaller. There appears to be a different conformational flexibility of human and guinea pig H_2R. Compared to the 3-phenylpentanoylguanidine **3.56**, the introduction of a 3-benzylpentanoyl substituent (**3.98**) leads to nearly the same potency and efficacy at the hH_2R, but to only about half the potency at the gpH_2R. The 3-benzyl-4-phenylbutanoyl compound (**3.100**) is inactive at the hH_2R but not at the gpH_2R. Introduction of a methyl group at the phenyl ring of the 3-phenylpropanoyl (**3.105**)

and 3-phenylbutanoyl compound (**3.96**) has no significant effect on the potency at the hH_2R, but decreases the potency at the gpH_2R and the efficacy at both receptors. An isopropyl residue at the phenyl ring (**3.99**, EC_{50} = 9.1 nM) is tolerated, resulting in about the same gpH_2R agonistic potency as that of the 3-phenylbutanoylguanidine **3.96** (EC_{50} = 12 nM) but with lower efficacy (eff. = 0.5 for **3.99**, compared to 1.03 for **3.96**). Analogues of 3-methyl-4-phenylbutanoylguanidine **3.65** bearing a methyl, fluoro or methoxy substituent in *meta* or *para* position of the phenyl ring (except *p*-F-substituted compound **3.71**) show similar potencies at hH_2R and gpH_2R. *Meta* substitution is more favourable at both receptors compared to *para* substitution. Again at the gpH_2R, the introduction of a substituent at the phenyl ring led to a significant drop in potency; this effect is not as much pronounced at the hH_2R. Changing the position of the carbonyl function results in a complete loss of agonistic activity (**3.55**) at the hH_2R (antagonism, IC_{50} = 1.22 µM) as well as at the gpH_2R (antagonism, IC_{50} = 1.24 µM). Exchange of the phenyl against a cyclohexyl ring increases in the main the potency at both, hH_2R and gpH_2R without affecting efficacy. Compound **3.78** (hH_2R: EC_{50} = 7.4 nM, gpH_2R: EC_{50} = 3.5 nM) with a 3-cyclohexylpentanoyl moiety is about five and three times more potent than its phenyl analogue (**3.56**) at the hH_2R (EC_{50} = 36.9 nM) and the gpH_2R (EC_{50} = 11 nM), respectively, whereas the efficacy remains unchanged compared to the 3-phenyl-pentanoyl compound. The enantiomers of the 3-cyclohexylbutanoyl- and the 3-phenylbutanoylguanidine[7] derivative show opposing preference for H_2Rs: higher potency resides in the (*S*)-configured cyclohexyl substituted enantiomer (**3.76**) with a eudismic ratio of about three, whereas for the phenyl substituted analogues higher potency was found for the (*R*)-configured enantiomer. Whereas in the arpromidine series of H_2R agonists eudismic ratios up to 40 were found in favour of the (*S*)-enantiomer[24], the acylguanidine-type H_2R agonists show rather low eudismic ratios in the range of 2 - 3.5. Thus the stereochemistry of the acyl moiety seems to play only a minor role in this series of H_2R agonists. The introduction of a methyl group in β-position of the acyl group (**3.111**→**3.102**, **3.112**→**3.82**) has no significant influence on potency and efficacy in the cyclohexyl series (data for **3.102**, **3.111** and **3.112**: see reference[23]). The same is found for bulkier substituents (**3.80**, **3.81**; except for **3.78**). A primary amine group at the end of the alkanoyl side chain (**3.88** and **3.89**) results in poor agonistic activity, especially at hH_2R. Introduction of a hydroxy function at the phenyl ring (**3.86** and **3.87**) is much better tolerated. Unlike the methyl-, fluoro- or

methoxy-substituted compounds, the *para* position was preferred for the phenolic OH group. Polar groups in compounds **3.86**-**3.89** explicitly increased the selectivity towards the gpH_2R. In a first attempt to modify the structure of the acylguanidines with respect to the future development of radiotracers and fluorescence-labelled agonists, the free amino group in compound **3.89** was acylated with succinimidyl proprionate and 4-F-benzoate, respectively, and derivatized with the fluorescent pyrylium dye py-1. The decrease in basicity due to conversion of the amine to the 4-F-benzamide **3.90** and the propionamide **3.91** results in a significant increase in potency, whereas the efficacy is lowered (except for **3.90**, hH_2R) at both receptors. The fluorescent pyridinium compound **3.92** is considerably more potent than the amine **3.89** at the hH_2R (EC_{50} 29 vs. 172 nM) but equipotent with **3.89** at the gpH_2R (EC_{50} about 20 nM).

In agreement with previous results for alkylguanidines and other acylguanidines, the potencies and efficacies for most of the compounds of this new series are higher at gpH_2R-$G_{s\alpha S}$ than at hH_2R-$G_{s\alpha S}$[9, 22, 23]. Figure 3.2 shows the correlation between potencies and efficacies of a selected number of acyguanidines (data taken from Table 3.2). However, surprisingly some compounds exhibit slightly higher potency at the human compared to the guinea pig H_2 receptor. These exceptions are very stimulating with respect to the search for potent and selective agonists for the hH_2R.

Generally, compared to the results from GTPase assay on gpH_2R-$G_{s\alpha S}$ fusion proteins (Table 3.2) the potencies of acylated compounds are lower at the guinea pig right atrium (Figure 3.3), but the orders of potencies are in good agreement.

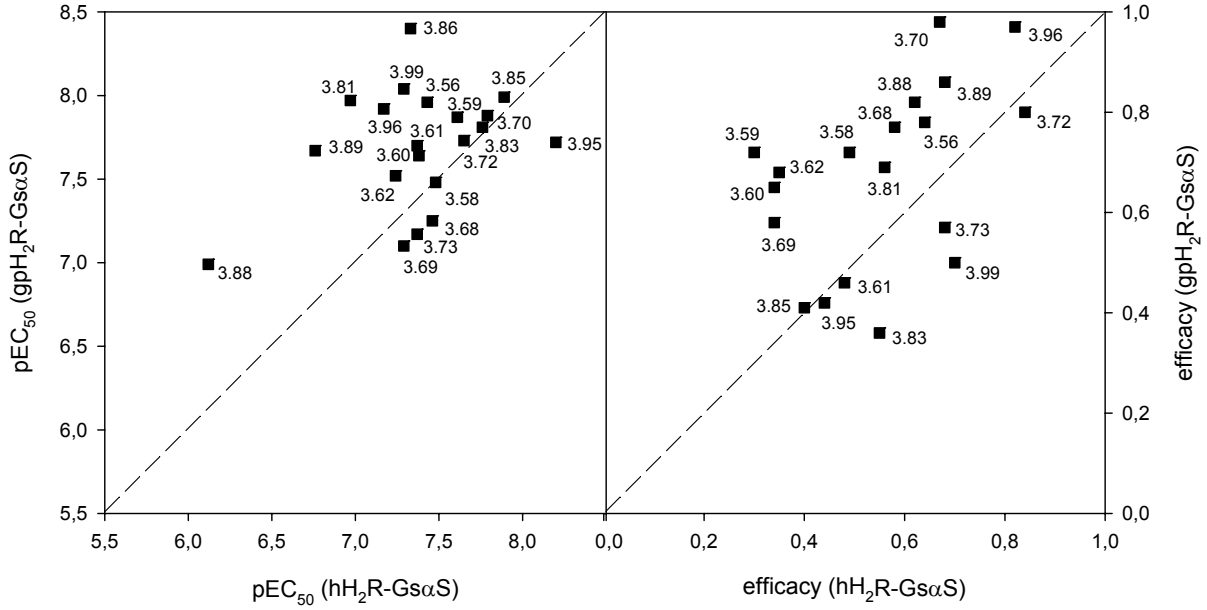


Figure 3.2. Correlation between the potencies and efficacies of selected acylated imidazolylpropylguanidines at the hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins in the GTPase assay. Agonist potencies and efficacies were taken from Table 3.2. The pEC_{50} values were derived from the EC_{50} shown in Table 3.2. The straight dashed line represents the correlation that would have been obtained if pEC_{50} values had been identical in the two systems.

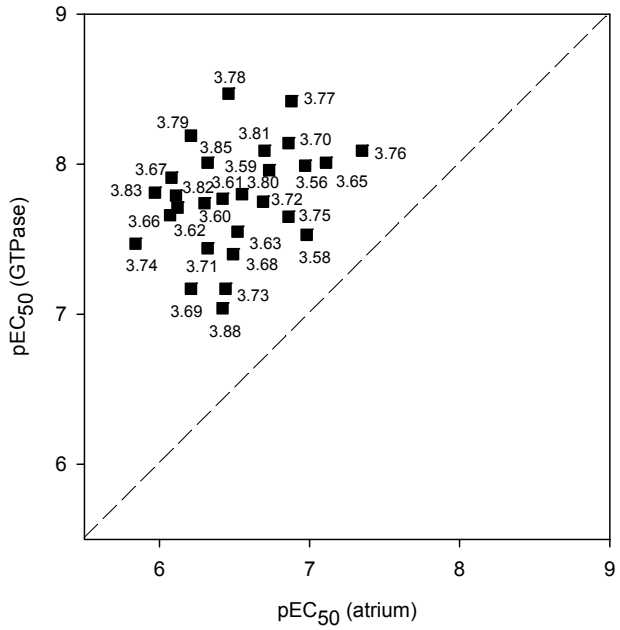


Figure 3.3. Correlation between the potencies of acylated imidazolylpropylguanidines at the guinea pig right atrium and in GTPase assay on gpH_2R-$G_{s\alpha S}$ fusion proteins. Agonist potencies were taken from Table 3.1 and Table 3.2 and pEC_{50} values were derived from the EC_{50} shown in Table 3.2. The straight dashed line represents the correlation that would have been obtained if pEC_{50} values had been identical in the two systems.

3.3.2. Receptor selectivity

3.3.2.1. Activity on the human histamine H_1 receptor on U-373 MG human cells

In the calcium assay on human U-373 MG cells all investigated acylguanidines proved to be weak histamine H_1R antagonists (Table 3.3). This is in accordance with previous investigations on the guinea pig ileum, where N^G-acylated imidazolylpropylguanidines proved to be weak H_1R antagonists, too (pA_2 values ~ 5-6)[7, 20]. Most strikingly, **3.102** turned out to be a potent partial agonist at the hH_1R with much higher efficacy than at gpH_1R (GTPase assay)[23]. This is surprising since antagonistic rather than agonistic effects were found at the guinea pig ileum and human H_1R expressing cells. In terms of efficacy and potency, **3.102** (eff. = 0.56, EC_{50} = 280 nM) is comparable to classic H_1R agonists like the most potent derivatives of 2-phenylhistamines[25]. Direct G protein activation can be excluded and the agonistic effect can be blocked by H_1 antagonists[23]. Possibly, the observed agonism is dependent on the artificial assay system (Sf9 insect cells, membrane preparations).

Table 3.3. H_1 receptor antagonism on U-373 MG human cells (Ca^{2+}-assay).

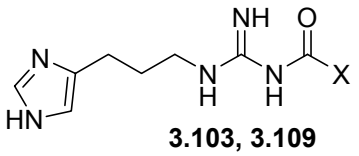
Histamine H_1 receptor antagonism U-373 MG cells (Ca^{2+}-assay)					
No	**IC_{50} [µM][a]**	**No**	**IC_{50} [µM][a]**	**No**	**IC_{50} [µM][a]**
3.93[7]	56	**3.65**	16.5	**3.77**	0.95
3.94[7]	34	**3.66**	7	**3.78**	5
3.55	61	**3.67**	9	**3.79**	0.5
3.96[20]	19.5	**3.95**[21]	7	**3.80**	5
3.97[21]	18	**3.98**[21]	16	**3.81**	4
3.56	30	**3.100**[21]	5	**3.82**	4
3.57	>30	**3.68**	14	**3.83**	7
3.58	24	**3.69**	17	**3.84**	7
3.59	16	**3.70**	19	**3.85**	7
3.60	6	**3.71**	14	**3.86**	nd
3.101[7]	nd	**3.72**	12	**3.87**	nd
3.61	12	**3.73**	24	**3.88**	95
3.62	12	**3.74**	18	**3.89**	>100
3.63	12	**3.102**[20]	0.95	**3.90**	nd
3.64	36	**3.75**	5	**3.91**	nd
3.99[21]	>30	**3.76**	0.5	**3.92**	nd

[a] IC_{50} values for the inhibition of the histamine (30 µM) induced increase in cellular calcium, one experiment or the mean of two experiments, SEM < 10 %; procedure as described from Kracht, 2001[26].

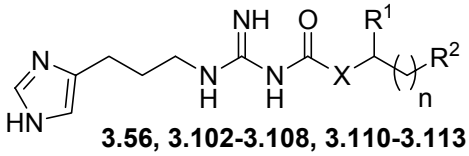
3.3.2.2. Agonist potencies and efficacies at the hH_3R and hH_4R

Surprisingly, most of the compounds (except for **3.103**, **3.106**, **3.107** and **3.112**) show agonistic activities at the $hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4 membrane in GTPase assay in the subnanomolar range.

Table 3.4. Agonist/antagonist activity on $hH_3+G\alpha_o+\beta_1\gamma_2$+RGS4- and hH_4-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$ receptor membranes.



3.103, 3.109



3.56, 3.102-3.108, 3.110-3.113

					$hH_3R + G\alpha_o + \beta_1\gamma_2$ + RGS4			hH_4R-GAIP + $G\alpha_{i2} + \beta_1\gamma_2$		
No	**X**	**R^1**	**R^2**	**n**	**efficacy**	**EC_{50} [nM]/ (K_B [nM])**	**rel. pot.**	**effi-cacy**	**EC_{50} [nM]/ (K_B [nM])**	**rel. pot.**
His	-	-	-	-	1.00	25.8 ± 3.1	100	1.00	11.6 ± 2.5	100
3.103	C_6H_5	-	-	-	-	(8.8 ± 0.4)	-	0.7	13.3	87
3.104	-	H	C_6H_5	0	0.49 ± 0.02	2.0 ± 0.6	1290	0.7	7.0	166
3.105	CH_2	H	C_6H_5	0	0.41 ± 0.04	0.6 ± 0.2	4300	0.7	17.5	66
3.106	CH_2	H	C_6H_5	1	-	(2.7 ± 0.6)	-	0.9	7.8	149
3.107	CH_2	H	C_6H_5	2	-	(3.5 ± 0.5)	-	0.8	3.6	322
3.108	-	CH_3	C_6H_5	-	0.28 ± 0.04	1.2 ± 0.3	2150	1.1	19.7	59
3.96	CH_2	CH_3	C_6H_5	0	0.27 ± 0.03	0.9 ± 0.3	2867	0.9	15.2	76
3.109	-	-	cHex	-	0.48 ± 0.01	0.7 ± 0.1	3886	0.9	5.6	207
3.110	-	H	cHex	0	0.69 ± 0.09	0.8 ± 0.2	3225	1.0	6.5	179
3.111	CH_2	H	cHex	0	0.74 ± 0.05	2.1 ± 0.9	1229	0.91	11.6	100
3.112	CH_2	H	cHex	1	-	(2.3 ± 0.1)	-	-	(8.8)	-
3.113	-	CH_3	cHex	-	0.71 ± 0.03	3.4 ± 1.0	759	1.2	20.5	57
3.102	CH_2	CH_3	cHex	0	0.44 ± 0.06	1.0 ± 0.4	2580		(16.1)	-
3.56	CH_2	CH_2CH_3	C_6H_5	0	nd	nd	nd	0.56	9.7	119

Steady state GTPase activity in Sf9 membranes expressing $hH_3R + G\alpha_o + \beta_1\gamma_2$ + RGS4 and hH_4R-GAIP + $G\alpha_{i2} + \beta_1\gamma_2$ was determined as described in literature[9]. Reaction mixtures contained ligands at concentrations from 0.1 nM to 100 µM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 1.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (10 µM) ranged between ~ 3.5 and 4.5 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the ± SEM of one to three experiments performed in duplicates each. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value. For antagonism, reaction mixtures contained histamine (100 nM) and ligands at concentrations from 0.1 nM to 100 µM.

In large part, these acylguanidines exhibit higher potencies for the hH_3R than for the hH_2R (data not shown)[23], but lower efficacies. The most potent substance in this series is the 3-phenylpropanoyl analogue which is 43 times more potent than

histamine at the hH_3R and 12 times more potent at the hH_2R. In contrast, acylated imidazolylpropylguanidines proved to be moderate to potent H_3R antagonists on the guinea pig ileum (data not shown)[7]. The agonistic response may also be due to the artifical assay conditions; this effect was never observed on the guinea pig ileum. At the hH_4R, all compounds are nearly full agonists (except for **3.112** and **3.102**) with high potencies. Hence, imidazolylpropylguanidines are not only H_2 receptor agonists, but show also agonistic (or antagonistic) activity, respectively, on human H_3R and H_4R in the GTPase assay.

3.4. Summary

A variety of N^G-acylated imidazolylpropylguanidines was synthesized in order to study the species selectivity for human *versus* guinea pig histamine H_2 receptors and to elaborate the structure-activity relationships. All compounds proved to be full or nearly full H_2R agonists at the spontaneously beating guinea pig right atrium. The potencies are somewhat lower for all acylated imidazolylpropylguanidines compared to the corresponding alkylated imidazolylpropylguanidines on the guinea pig right atrium, however the order of potency on the atrium is generally in good agreement with the data derived from $gpH_2R\text{-}G_{s\alpha S}$ in GTPase assay. The 3-phenylbutanoyl- (**3.96**) and the (*S*)-3-cyclohexylbutanoylguanidine (**3.76**) derivatives are the most active compounds at the guinea pig right atrium, achieving 27 and 22 times higher potency than histamine. At the $gpH_2R\text{-}G_{s\alpha S}$ fusion protein, the 2-methyl-3-cyclohexylpropanoyl- (**3.77**), 3-cyclohexylpentanoyl- (**3.78**) and 3- and 4-(hydroxyphenyl)butanoylguanidine (**3.86** and **3.87**) derivatives are the most potent compounds in the series of acylated imidazolylpropylguanidines. This result indicates that a methyl or ethyl substituted three-membered carbon chain between the guanidine moiety and the ring system favours high potencies at the guinea pig H_2R. As also found for the alkylguanidines, the acylguanidines are more potent and efficious at the $gpH_2R\text{-}G_{s\alpha S}$ compared to the $hH_2R\text{-}G_{s\alpha S}$[9, 22, 23]. Interestingly, the 6-phenylhexanoyl (**3.95**) analogue shows the highest agonistic activity at the $hH_2R\text{-}G_{s\alpha S}$, stimulating speculations about a higher conformational flexibility of the hH_2R[9]. This compound turned out to be the first one among all investigated alkyl- and acylguanidines which possesses a slightly higher potency at the human compared to the guinea pig H_2 receptor in the GTPase assay. In addition, the introduction of ring substituents such as methyl, ethyl, methoxy or fluoro at the 3-methyl-4-

phenylbutanoyl moiety (**3.65**) is better tolerated by the hH_2R-$G_{s\alpha S}$. Again, this may be interpreted a hint to a bigger and more flexible binding pocket[9]. The correlation of potencies and efficacies of all acylated imidazolylpropylguanidines between the human and guinea pig H_2 receptor suggests that some compounds show a similar or even slight higher potency or efficacy at the human receptor. This result suggests the development of potent and selective compounds for the hH_2R.

The insertion of a primary amino group in the side chain (**3.88**-**3.89**) results in a considerable decrease in potency at the hH_2R, whereas hydroxy functions (**3.86**-**3.87**) at the phenyl ring were much better tolerated. These compounds were 7-12 times more potent at the gpH_2R. Hence, the introduction of polar substituents (**3.86**-**3.89**) strongly enhanced the selectivity for guinea pig *versus* human H_2R. The gpH_2R model, suggesting interactions between Asp-271, Tyr-17, Trp-275 and Lys-173 in TM1, TM7 and e2, is supported by these results while a positively charged amino group or H-bond donor may directly or indirectly participate in this network[9, 27]. Aliphatic substituents are more preferred in the case of the hH_2R due to a more hydrophobic binding pocket[27].

Furthermore, selected compounds were tested for receptor selectivity at human H_1R (calcium assay on U373 cells) as well as human H_3R and H_4R expressed in Sf9 insect cells. Generally, on the human H_1R the compounds proved to be weakly active antagonists. Most strikingly, **3.102** turned out to be a potent partial agonist at the hH_1R in the GTPase assay[23]. An imidazol-4-yl ring favours binding to the histamine H_3 receptor. The endogenous ligand histamine has higher affinity for H_3R than for the other histamine receptors, numerous imidazole-type H_3R antagonists[28, 29] and agonists[30] are described in literature, and the H_2R agonists arpromidine and impromidine are also known as H_3R antagonists. As expected, the N^G-acylated imidazolylpropylguanidines synthesized as H_2R agonists, proved to be moderate to potent H_3R antagonists at the guinea pig ileum (data not shown[7]) and, surprisingly, exhibit H_3R agonism (except for **3.103**, **3.106**, **3.107** and **3.112**) at the $hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4 membrane in the GTPase assay. Moreover, the compounds also proved to be potent agonists (except for **3.112** and **3.102**) at the hH_4-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$- and hH_1R[23] expressing Sf9 membranes in the GTPase assay, and the 3-cyclohexylpropanoyl derivative (**3.111**) was discovered as the first nonpeptidic NPY Y_4 receptor antagonist (pK_i = 4.17)[31, 32].

3.5. Experimental section

3.5.1. General conditions

Commercially available reagents were purchased from Acros Organics (Belgium), Lancaster Synthesis GmbH (Germany), Sigma-Aldrich Chemie GmbH (Germany), Alfa Aesar GmbH & Co KG (Germany) or Merck (Germany) and used as received. Where indicated, reactions were carried out under a dry, oxygen-free argon atmosphere. All solvents used were of analytical grade or distilled before use. THF and Et_2O were distilled over Na, CH_2Cl_2 was predried over $CaCl_2$ or distilled from P_2O_5 and stored under argon atmosphere over molecular sieves 3 Å. Column chromatography was carried out using Merck silica gel Geduran 60 (0.063-0.200) and Merck silica gel 60 (0.040-0.063) for flash column chromatography. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminium sheets and spots were visualized with UV light at 254 nm.

Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on a Bruker Avance 300 spectrometer with per-deuterated solvents. The chemical shift δ is given in parts per million (ppm) with reference to the chemical shift of the residual protic solvent compared to tetramethylsilane (δ = 0 ppm). Multiplicities were specified with the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad signal) as well as combinations thereof. The multiplicity of carbon atoms (^{13}C-NMR) were determined by DEPT 135 and DEPT 90 (distortionless enhancement by polarization transfer): “+” primary and tertiary carbon atom (positive DEPT 135 signal), “-“ secondary carbon atom (negative DEPT 135 signal), “quart” quaternary carbon atom. Mass spectrometry analysis (MS) was performed on a Finnigan MAT 95, a Finnigan SSQ 710A and on a Finnigan ThermoQuest TSQ 7000 spectrometer. Melting points (mp) were measured on a BÜCHI 530 electrically heated copper block apparatus using an open capillary and are uncorrected. The Department of Microanalysis, University of Regensburg, carried out elemental analysis. Compounds were dried *in vacuo* at room temperature or with heating up to 50 °C for at least 24 h prior to submission for elemental analysis. 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 the UV-detector model K-2000 (Knauer). UV-detection was done at 254 and 210 or 220 nm, respectively. 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 purity data: see appendix (chapter 8).

3.5.2. Preparation of ketones 3.11-3.14c, 3.15e, 3.16b, 3.17c and 3.18-3.19b

General procedure

To a Grignard reagent, prepared from Mg and the pertinent chloride in diethyl ether/abs, was added under stirring a solution of benzonitrile or acetonitrile in diethyl ether/abs under argon and refluxed for 16 h. After cooling, the mixture was poured into a mixture of concentrated HCl and ice and extracted three to five times with diethyl ether. The organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was subjected to flash chromatography (PE/EtOAc 100/0-90/10 v/v) to obtain the ketones.

1,2-Diphenylethanone (3.11c) [33]

The title compound was prepared from Mg (1.16 g, 48 mmol), benzyl chloride (6.15 g, 4.6 ml, 48 mmol) in 40 ml diethyl ether and benzonitrile (4 g, 4 ml, 39 mmol) in 10 ml diethyl ether to obtain **3.11c** (3.41 g, 41 %) as a pale yellow solid. mp = 47 °C (ref.[33]**:** mp = 55-56 °C); ^{1}H-NMR ($CDCl_3$) δ ppm: 8.02 (m, 2H, Ar-***H***), 7.56-7.45 (m, 3H, Ar-***H***), 7.35-7.28 (m, 4H, Ar-***H***), 4.29 (s, 2H, C***H***$_2$-Ar); PI-EIMS (70 eV) *m/z* (%): 196 ($M^{+\bullet}$, 6). $C_{14}H_{12}O$ (196.24)

2-(4-Methylphenyl)-1-phenylethanone (3.12c)[34]

The title compound was prepared from Mg (1.2 g, 48 mmol), *p*-methylbenzyl chloride (6.75 g, 6.3 ml, 48 mmol) in 40 ml diethyl ether and benzonitrile (4 g, 4 ml, 39 mmol) in 10 ml diethyl ether to obtain **3.12c** (6.22 g, 76 %) as a pale yellow solid. mp = 87-88 °C (ref.[34]: 95-96 °C); ^{1}H-NMR ($CDCl_3$) δ ppm: 8.01 (m, 2H, Ar-***H***), 7.49 (m, 3H, Ar-***H***), 7.13 (m, 4H, Ar-***H***), 4.24 (s, 2H, C***H***$_2$-Ar), 2.32 (s, 3H, (*p*-C***H***$_3$)Ar); EI-MS (70 eV) *m/z* (%): 210 ($M^{+\bullet}$, 8). $C_{15}H_{14}O$ (210.27)

1-Phenylpropan-2-one (3.13c)[35]

The title compound was prepared from Mg (3.5 g, 140 mmol), benzyl chloride (18.7 g, 17 ml, 150 mmol) in 50 ml diethyl ether and acetonitrile (4.91 g, 6.3 ml, 120 mmol) in 10 ml diethyl ether to obtain **3.13c** (1.76 g, 11 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.21 (m, 5H, Ar-***H***), 3.69 (s, 2H, C***H***$_2$-Ar), 2.15 (s, 3H, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 134 ($M^{+\bullet}$, 19). $C_9H_{10}O$ (134.17)

5-Phenylpentan-2-one (3.14c)[36]

The title compound was prepared from Mg (5.6 g, 230 mmol), 1-(3-chloropropyl)-benzene (36.2 g, 35 ml, 230 mmol) in 200 ml diethyl ether and acetonitrile (7.8 g, 10 ml, 190 mmol) in 50 ml diethyl ether to obtain **3.14c** (4.33 g, 14 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.29-7.16 (m, 5H, Ar-***H***), 2.62 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$-Ar), 2.43 (t, 2H, ^3J = 7.4 Hz, COC***H***$_2$), 2.11 (s, 3H, C***H***$_3$), 1.91 (m, 2H, $COCH_2C$***H***$_2$); PI-EIMS (70 eV) *m/z* (%): 162 ($M^{+\bullet}$, 26). $C_{11}H_{14}O$ (162.23)

***N*-(Cyclohexylcarbonyl)pyrrolidine (3.15b)[13]**

To a solution of cyclohexancarbonyl chloride (2.5 g, 17 mmol) in 10 ml DCM/abs was added under argon a solution of pyrrolidine (0.92 g, 1.1 ml, 13 mmol) and DMAP (3.1 g, 25 mmol) in 15 ml DCM/abs and stirred for 20 min at room temperature. Subsequently, brine was added and the organic layer separated. The aqueous phase was extracted three times with EtOAc and the combined organic layers washed with 3 N HCl, 1 N NaOH and brine. After drying over $MgSO_4$, the solvent was removed under reduced pressure and the crude product subjected to flash chromatography (PE/EtOAc 70/30-50/50 v/v) to obtain **3.15b** (2.03 g, 86 %) as a white solid. mp = 63 °C (ref.[13]: mp = 66-67 °C); ^{1}H-NMR ($CDCl_3$) δ ppm: 3.46 (m, 4H, pyrrolidine-C***H***$_2$), 2.33 (tt, 1H, ^3J = 3.3 Hz, ^3J = 11.5 Hz, cHex-C***H***), 1.94-1.28 (m, 14H, cHex-C***H***, cHex-C***H***$_2$, pyrrolidine-C***H***$_2$); EI-MS (70 eV) *m/z* (%): 181 ($M^{+\bullet}$, 33). $C_{11}H_{19}NO$ (181.27)

Cyclohexyl(1-methyl-1*H*-imidazol-2-yl)methanone (3.15c)[14]

To a solution of 1-methyl-1*H*-imidazole (1.92 g, 1.86 ml, 23.4 mmol) in 40 ml THF/abs was added n-BuLi/15 % (14.6 ml, 23.4 mmol) at -78 °C. After 10 min, **3.15b** (4.24 g, 23.4 mmol) in 30 ml THF/abs was added and stirred for 30 min at room temperature. Subsequently, 50 ml diethyl ether and 50 ml 10 % HCl/aq were added. The aqueous phase was washed with diethyl ether and K_2CO_3 was added to adjust to pH > 10. The aqueous phase was extracted three times with EtOAc and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product purified with flash chromatography (PE/EtOAc 70/30 v/v) to obtain **3.15c** (4.29 g, 95 %) as a colourless oil. mp = 49-50 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.14 (d, 1H, ^4J = 0.8 Hz, Im-5-***H***), 7.02 (s, 1H, Im-4-***H***), 3.99 (s, 3H, C***H***$_3$), 1.93-1.71 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.47-1.23 (m, 6H, cHex-C***H***$_2$); PI-EIMS (70 eV) *m/z* (%): 192 ($M^{+\bullet}$, 38). $C_{11}H_{16}N_2O$ (192.26)

1-Cyclohexyl-1-(1-methyl-1*H*-imidazol-2-yl)-2-phenylethanol (3.15d)[37]

To a Grignard reagent, prepared from Mg (0.505 g, 20.8 mmol) and benzyl chloride (2.64 g, 2.4 ml, 20.8 mmol) in 60 ml diethyl ether/abs, was added **3.15c** (2 g, 10.4 mmol) in 20 ml diethyl ether/abs under ice cooling and stirred for 2-3 h at room temperature. Subsequently, 10 % HCl/aq was added to this mixture until pH < 3, washed with diethyl ether and K_2CO_3 was added. After extracting three times with EtOAc, the organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo* to obtain the crude product as a white solid, which was recrystallised from toluene to obtain **3.15d** (1.54 g, 60 %) as a white cotton-like solid. mp = 170-171 °C (ref.[37]: mp = 185-186 °C); ^{1}H-NMR ($CDCl_3$) δ ppm: 7.15 (m, 6H, Ar-***H***, Im-5-***H***), 6.88 (d, 1H, ^4J = 1.1 Hz, Im-4-***H***), 3.45 (s, 3H, C***H***$_3$), 3.16 (d, 2H, ^3J = 13.5 Hz, C***H***$_2$-Ar), 2.05 (s, 1H, O***H***), 1.84-1.22 (m, 11H, cHex-C***H***, cHex-C***H***$_2$); CI-MS (NH_3) *m/z* (%): 285 (MH^+, 100). $C_{18}H_{24}N_2O$ (284.39)

1-Cyclohexyl-2-phenylethanone (3.15e)[37]

MeI (12 ml, 79 mmol) was added to a solution of **3.15d** (1.53 g, 6.16 mmol) in 40 ml EtOAc and refluxed for 3-4 h. The solvent was removed under reduced pressure, 40 ml benzene and K_2CO_3 (1 g, 7.2 mmol) were added under argon resulting and the mixture was heated to 60 °C for 2 h. After drying over $MgSO_4$, the solvent was removed *in vacuo* and the crude product subjected to flash chromatography (PE/EtOAc 95/5 v/v) to abtain **3.15e** (0.7 g, 56 %) as a light brown oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.32-7.19 (m, 5H, Ar-***H***), 3.73 (s, 2H, C***H***$_2$-Ar), 2.46 (tt, 1H, ^3J = 3.3 Hz, ^2J = 11.4 Hz, cHex-C***H***), 1.73-1.26 (m, 10H, cHex-C***H***$_2$); EI-MS (70 eV) *m/z* (%): 202 ($M^{+\bullet}$, 8). $C_{14}H_{18}O$ (202.29)

(2-Oxo-2-phenylethyl)carbamic acid *tert*-butyl ester (3.16b)[38]

To a solution of 2-amino-1-phenylethanone hydrochloride (4.52 g, 26.4 mmol) in 80 ml THF/abs was added NEt_3 (2.67 g, 3.66 ml, 26.4 mmol) and $(Boc)_2O$ (7.02 g, 32 mmol) in 20 ml THF/abs and stirred for 20 h at room temperature. Subsequently, water and EtOAc were added and the aqueous layer was extracted three times with EtOAc. The organic phase was washed with 1N NaOH, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by flash chromatography (PE/EtOAc 80/20 v/v) to obtain **3.16b** (5.56 g, 90 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.96 (m, 2H, Ar-***H***), 7.62-7.49 (m, 3H, Ar-***H***), 5.55 (br s, 1H, N***H***), 4.67 (d, 2H, ^3J = 4.5 Hz, C***H***$_2$NH), 1.48 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 253 ($M+NH_4^+$, 15). $C_{13}H_{16}NO_3$ (235.28)

(5-Oxo-5-phenylbutyl)carbamic acid *tert*-butyl ester (3.17c)[39]

A solution of *tert*-butyl 2-oxopyrrolidine-1-carboxylate (9.3 g, 8.5 ml, 50 mmol) in 200 ml THF/abs was cooled to -78 °C and PhMgBr in diethyl ether (20 ml of 3M solution corresponding to 60 mmol) was added dropwise within 1 h (the temperature in the flask should be kept at -70 °C during the addition). At the end of the addition of PhMgBr, the mixture is allowed to warm up to room temperature. Subsequently, the solution was adjusted to pH = 1-3 by addition of 2M HCl. The aqueous layer was separated and extracted three times with CH_2Cl_2. After drying over $MgSO_4$, the solvent was removed *in vacuo* and the crude product subjected to flash chromatography (PE/EtOAc 90/10-85/15) to obtain **3.17c** (8.95 g, 68 %) as a white solid. mp = 88 °C (ref.[39]: 95-96 °C); ^{1}H-NMR ($CDCl_3$) δ ppm: 7.96 (m, 2H, Ar-***H***), 7.56-7.45 (m, 3H, Ar-***H***), 4.69 (br s, 1H, N***H***), 3.23 (m, 2H, C***H***$_2$NH), 3.03 (t, 2H, ^3J = 7.1 Hz, COC***H***$_2$), 1.95 (m, 2H, COCH$_2$C***H***$_2$), 1.42 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 281 ($M+NH_4^+$, 13). $C_{15}H_{21}NO_3$ (263.15)

1-(3-Benzyloxyphenyl)ethanone (3.18b)[40]

To a solution of 1-(3-hydroxyphenyl)ethanone (3 g, 22 mmol) and KOH (1.35 g, 24.2 mmol) in 15 ml EtOH was added BnCl (2.77 g, 2.5 ml, 22 mmol) and refluxed for 5 h. The solvent was removed under reduced pressure. Subsequently, water was added and extracted three times with $CHCl_3$. After drying over $MgSO_4$, the solvent was removed *in vacuo* and the crude product subjected to flash chromatography (PE/EtOAc 80/20 v/v) to obtain **3.18b** (4.19 g, 84 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.56-7.21 (m, 9H, Ar-***H***), 5.10 (s, 1H, C***H***$_2$-Ar), 2.57 (s, 3H, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 226 ($M^{+\bullet}$, 43). $C_{15}H_{14}O_2$ (226.27)

1-[4-(4-Methoxybenzyloxy)phenyl]ethanone (3.19b)[41]

To a solution of 1-(3-hydroxyphenyl)ethanone (3 g, 22 mmol) and K_2CO_3 (12.2 g, 88 mmol) in 150 ml acetone/pA was added *p*-methoxybenzyl chloride (5.17 g, 4.5 ml, 33 mmol) and refluxed overnight. After filtration of the precipitate, the solvent was removed under reduced pressure and subjected to flash chromatography (PE/EtOAc 80/20-60/40 v/v) to obtain **3.19b** (4.14 g, 73 %) as a white solid. mp = 118 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.93 (m, 2H, Ar-***H***), 7.36 (m, 2H, Ar-***H***), 6.96 (m, 4H, Ar-***H***), 5.05 (s, 2H, C***H***$_2$-Ar), 3.82 (s, 3H, (*p*-OC***H***$_3$)-Ar), 2.55 (s, 3H, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 256 ($M^{+\bullet}$, 1). $C_{16}H_{16}O_3$ (256.11)

3.5.3. Preparation of ethyl (*E,Z*)-alkenoates 3.21-3.39a and 3.53-3.54a

General procedure

To a stirred suspension of NaH (60 % dispersion in mineral oil) (1.56 eq) in THF/abs was carefully added triethyl phosphonoacetate (1.4 - 1.5 eq) under argon and was stirred at 30-35 °C for 1.5 h. Subsequently, the pertinent ketone (1 eq) was added dropwise and the mixture refluxed overnight (12-16 h). After cooling to room temperature, the mixture was poured into ice water, extracted three times with diethyl ether and the organic phase dried over $MgSO_4$. After removing the solvent under reduced pressure, the crude product was subjected to flash chromatography (PE/EtOAc 100/0-95/5 v/v) to obtain a mixture of *E*/*Z* isomeric ethyl ester.

Ethyl (*E,Z*)- 3-phenylpent-2-enoate (3.21a)[42, 43]

The title compound was prepared from propiophenone (2 g, 1.98 ml, 14.1 mmol), triethyl phosphonoacetate (4.4 ml, 20 mmol) and NaH (60 % dispersion in mineral oil) (0.6 g, 22 mmol) in 25 ml THF/abs according to the general procedure yielding **3.21a** (2.9 g, 100 %) as a colourless liquid. EI-MS (70 eV) *m/z* (%): 204 ($M^{+\bullet}$, 87). $C_{13}H_{16}O_2$ (204.27)

Ethyl (*E,Z*)-4-methyl-3-phenylpent-2-enoate (3.22a)[44]

The title compound was prepared from 2-methyl-1-phenylpropan-1-one (2 g, 2.1 ml, 13.5 mmol), triethyl phosphonoacetate (4.08 ml, 20 mmol) and NaH (60 % dispersion in mineral oil) (0.84 g, 21 mmol) in 25 ml THF/abs according to the general procedure yielding **3.22a** (2.5 g, 85 %) as a colourless liquid. PI-EIMS (70 eV) *m/z* (%): 218 ($M^{+\bullet}$, 100). $C_{14}H_{18}O_2$ (218.29)

Ethyl (*E,Z*)-5-methyl-3-phenylhex-2-enoate (3.23a)[45]

The title compound was prepared from 3-methyl-1-phenylbutan-1-one (2.27 g, 2.35 ml, 14 mmol), triethyl phosphonoacetate (4.08 ml, 20.9 mmol) and NaH (60 % dispersion in mineral oil) (0.87 g, 21.8 mmol) in 25 ml THF/abs according to the general procedure yielding **3.23a** (2.75 g, 84 %) as a colourless liquid. EI-MS (70 eV) *m/z* (%): 232 ($M^{+\bullet}$, 100). $C_{15}H_{20}O_2$ (232.32)

Ethyl (*E,Z*)-3,4-diphenylbut-2-enoate (3.24a)[46]

The title compound was prepared from **3.11c** (2.75 g, 14 mmol), triethyl phosphonoacetate (4.08 ml, 20.9 mmol) and NaH (60 % dispersion in mineral oil) (0.84 g, 21 mmol) in 25 ml THF/abs according to the general procedure yielding **3.24a** (3.21 g, 86 %) as a colourless liquid. PI-EIMS (70 eV) *m/z* (%): 266 ($M^{+\bullet}$, 100). $C_{18}H_{18}O_2$ (266.33)

Ethyl (*E*,*Z*)-3-phenyl-4-(4-methylphenyl)but-2-enoate (3.25a)

The title compound was prepared from **3.12c** (4 g, 19 mmol), triethyl phosphonoacetate (5.5 ml, 28 mmol) and NaH (60 % dispersion in mineral oil) (1.18 g, 29.4 mmol) in 60 ml THF/abs according to the general procedure yielding **3.25a** (2.64 g, 50 %) as a yellow solid. EI-MS (70 eV) *m/z* (%): 280 ($M^{+\bullet}$, 100). $C_{19}H_{20}O_2$ (280.36)

Ethyl (*E*,*Z*)-3-cyclohexyl-4-phenylbut-2-enoate (3.26a)

The title compound was prepared from **3.15e** (0.7 g, 3.5 mmol), triethyl phosphonoacetate (1.03 ml, 5.2 mmol) and NaH (60 % dispersion in mineral oil) (0.22 g, 5.46 mmol) in 10 ml THF/abs according to the general procedure yielding **3.26a** (0.67 g, 70 %) as a yellow liquid. EI-MS (70 eV) *m/z* (%): 272 ($M^{+\bullet}$, 26). $C_{18}H_{24}O_2$ (272.18)

Ethyl (*E*,*Z*)-3-(4-methylphenyl)but-2-enoate (3.27a)[47]

The title compound was prepared from 1-(4-methylphenyl)ethanone (4.6 g, 4.6 ml, 4.5 mmol), triethyl phosphonoacetate (9.6 ml, 49 mmol) and NaH (60 % dispersion in mineral oil) (2.23 g, 55.7 mmol) in 60 ml THF/abs according to the general procedure yielding **3.27a** (7.2 g, 100 %) as a yellow oil. CI-MS (NH_3) *m/z* (%): 222 ($M+NH_4^+$, 100). $C_{13}H_{16}O_2$ (204.27)

Ethyl (*E*,*Z*)-3-methyl-4-phenylbut-2-enoate (3.28a)[48]

The title compound was prepared from **3.13c** (1.5 g, 11 mmol), triethyl phosphonoacetate (3.1 ml, 15.6 mmol) and NaH (60 % dispersion in mineral oil) (0.7 g, 17.6 mmol) in 25 ml THF/abs according to the general procedure yielding **3.28a** (2.05 g, 91 %) as a pale yellow liquid. PI-EIMS (70 eV) *m/z* (%): 204 ($M^{+\bullet}$, 57). $C_{13}H_{16}O_2$ (204.27)

Ethyl (*E*,*Z*)-3-methyl-5-phenylpent-2-enoate (3.29a)[49]

The title compound was prepared from 4-phenylbutan-2-one (2.1 g, 2.1 ml, 14 mmol), triethyl phosphonoacetate (4.08 ml, 20.9 mmol) and NaH (60 % dispersion in mineral oil) (0.84 g, 21 mmol) in 25 ml THF/abs according to the general procedure yielding **3.29a** (3.09 g, 99 %) as a colourless liquid. EI-MS (70 eV) *m/z* (%): 218 ($M^{+\bullet}$, 3). $C_{14}H_{18}O_2$ (218.29)

Ethyl (*E*,*Z*)-3-methyl-6-phenylhex-2-enoate (3.30a)

The title compound was prepared from **3.14c** (2.88 g, 14 mmol), triethyl phosphonoacetate (4.08 ml, 20.9 mmol) and NaH (60 % dispersion in mineral oil) (0.84 g, 21 mmol) in 25 ml THF/abs according to the general procedure yielding

3.30a (2.18 g, 67 %) as a colourless liquid. CI-MS (NH_3) *m/z* (%): 250 ($M+NH_4^+$, 100). $C_{15}H_{20}O_2$ (232.32)

Ethyl (*E,Z*)-3-methyl-4-(3-methylphenyl)but-2-enoate (3.31a)

The title compound was prepared from 1-(3-methylphenyl)propan-2-one (2.5 g, 16.9 mmol), triethyl phosphonoacetate (4.5 ml, 23.2 mmol) and NaH (60 % dispersion in mineral oil) (1 g, 25.3 mmol) in 40 ml THF/abs according to the general procedure yielding **3.31a** (1.25 g, 34 %) as a colourless liquid. EI-MS (70 eV) *m/z* (%): 218 ($M^{+\bullet}$, 16). $C_{14}H_{18}O_2$ (218.13)

Ethyl (*E,Z*)-3-methyl-4-(4-methylphenyl)but-2-enoate (3.32a)[50]

The title compound was prepared from 1-(4-methylphenyl)propan-2-one (2.5 g, 16.9 mmol), triethyl phosphonoacetate (4.5 ml, 23.2 mmol) and NaH (60 % dispersion in mineral oil) (1 g, 25.3 mmol) in 40 ml THF/abs according to the general procedure yielding **3.32a** (1.9 g, 58 %) as a colourless oil. EI-MS (70 eV) *m/z* (%): 218 ($M^{+\bullet}$, 45). $C_{14}H_{18}O_2$ (218.13)

Ethyl (*E,Z*)-3-(3-fluorobenzyl)but-2-enoate (3.33a)

The title compound was prepared from 1-(3-fluorophenyl)propan-2-one (2.5 g, 16.4 mmol), triethyl phosphonoacetate (4.4 ml, 22.7 mmol) and NaH (60 % dispersion in mineral oil) (0.99 g, 24.8 mmol) in 40 ml THF/abs according to the general procedure yielding **3.33a** (2.03 g, 56 %) as a pale yellow oil. CI-MS (NH_3) *m/z* (%): 240 ($M+NH_4^+$, 100). $C_{13}H_{15}FO_2$ (222.25)

Ethyl (*E,Z*)-3-(4-fluorobenzyl)but-2-enoate (3.34a)

The title compound was prepared from 1-(4-fluorophenyl)propan-2-one (2 g, 1.8 ml, 13.1 mmol), triethyl phosphonoacetate (3.5 ml, 18.1 mmol) and NaH (60 % dispersion in mineral oil) (0.79 g, 19.8 mmol) in 35 ml THF/abs according to the general procedure yielding **3.34a** (1.32 g, 45 %) as a pale yellow liquid. EI-MS (70 eV) *m/z* (%): 222 ($M^{+\bullet}$, 55). $C_{13}H_{15}FO_2$ (222.25)

Ethyl (*E,Z*)-3-(3-methoxybenzyl)but-2-enoate (3.35a)[51]

The title compound was prepared from 1-(3-methoxyphenyl)propan-2-one (1.91 g, 1 ml, 11.6 mmol), triethyl phosphonoacetate (3.1 ml, 15.9 mmol) and NaH (60 % dispersion in mineral oil) (0.7 g, 17.4 mmol) in 35 ml THF/abs according to the general procedure yielding **3.35a** (1.03 g, 38 %) as a pale yellow oil. EI-MS (70 eV) *m/z* (%): 234 ($M^{+\bullet}$, 69). $C_{14}H_{18}O_3$ (234.29)

Ethyl (*E*,*Z*)-3-(4-methoxybenzyl)but-2-enoate (3.36a)[52]

The title compound was prepared from 1-(4-methoxyphenyl)propan-2-one (2 g, 1.87 ml, 12.2 mmol), triethyl phosphonoacetate (3.3 ml, 16.8 mmol) and NaH (60 % dispersion in mineral oil) (0.73 g, 18.3 mmol) in 35 ml THF/abs according to the general procedure yielding **3.36a** (1.18 g, 41 %) as a colourless liquid. EI-MS (70 eV) *m/z* (%): 234 ($M^{+\bullet}$, 79). $C_{14}H_{18}O_3$ (234.29)

Ethyl (*E*,*Z*)-3-(3-ethylbenzyl)but-2-enoate (3.37a)[53]

The title compound was prepared from 1-(3-ethylphenyl)propan-2-one (2.5 g, 15.4 mmol), triethyl phosphonoacetate (4.12 ml, 21.1 mmol) and NaH (60 % dispersion in mineral oil) (0.92 g, 23 mmol) in 40 ml THF/abs according to the general procedure yielding **3.37a** (1.86 g, 52 %) as a pale yellow liquid. EI-MS (70 eV) *m/z* (%): 232 ($M^{+\bullet}$, 72). $C_{15}H_{20}O_2$ (232.15)

Ethyl (*E*,*Z*)-4-(*tert*-butoxycarbonylamino)-3-phenylbut-2-enoate (3.38a)[54]

The title compound was prepared from **3.16b** (2 g, 8.54 mmol), triethyl phosphonoacetate (2.4 ml, 12 mmol) and NaH (60 % dispersion in mineral oil) (0.52 g, 13 mmol) in 25 ml THF/abs according to the general procedure yielding **3.38a** (0.31 g, 12 %) as a yellow oil. CI-MS (NH_3) *m/z* (%): 323 ($M+NH_4^+$, 81). $C_{17}H_{23}NO_4$ (305.37)

Ethyl (*E*,*Z*)-6-(*tert*-butoxycarbonylamino)-3-phenylhex-2-enoate (3.39a)[55]

The title compound was prepared from **3.17c** (4 g, 15 mmol), triethyl phosphonoacetate (4.1 ml, 21 mmol) and NaH (60 % dispersion in mineral oil) (0.92 g, 23 mmol) in 60 ml THF/abs according to the general procedure yielding **3.39a** (1.21 g, 24 %) as a yellow oil. CI-MS (NH_3) *m/z* (%): 351 ($M+NH_4^+$, 57). $C_{19}H_{27}NO_4$ (333.19)

Ethyl (*E*,*Z*)-3-(3-benzyloxyphenyl)but-2-enoate (3.53a)[56]

The title compound was prepared from **3.18b** (3 g, 13 mmol), triethyl phosphonoacetate (3.6 ml, 18 mmol) and NaH (60 % dispersion in mineral oil) (0.79 g, 19.7 mmol) in 45 ml THF/abs according to the general procedure yielding **3.53a** (1.51 g, 40 %) as a pale yellow liquid. EI-MS (70 eV) *m/z* (%): 296 ($M^{+\bullet}$, 11). $C_{19}H_{20}O_3$ (296.14)

Ethyl (*E*,*Z*)-3-[4-(4-methoxybenzyloxy)phenyl]but-2-enoate (3.54a)[57]

The title compound was prepared from **3.19b** (3 g, 11.8 mmol), triethyl phosphonoacetate (3.4 ml, 17.5 mmol) and NaH (60 % dispersion in mineral oil) (0.73 g, 18.3 mmol) in ml THF/abs according to the general procedure yielding **3.54a**

(2.14 g, 56 %) as a pale yellow oil. EI-MS (70 eV) *m/z* (%): 326 ($M^{+\bullet}$, 1). $C_{20}H_{22}O_4$ (326.15)

3.5.4. Preparation of ethyl alkanoates 3.21-3.39b

General procedure

The ethyl (*E,Z*)-alkenoates were dissolved in EtOH, Pd/C (10 %) was added and stirred for 24 h at room temperature under hydrogen atmosphere. The catalyst was removed by filtration over Celite and washed with EtOH. The solvent was removed *in vacuo* to obtain the ethyl alkanoates.

Ethyl 3-phenylpentanoate (3.21b)[42, 58]

The title compound was prepared from **3.21a** (2.9 g, 14.1 mmol), Pd/C (10 %) (0.3 g, cat.) and 20 ml EtOH according to the general procedure yielding **3.21b** (2.9 g, 100 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.31-7.18 (m, 5H, Ar-***H***), 4.03 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.00 (m, 1H, CH$_2$C***H***), 2.64 (dd, 1H, ^3J = 7.1 Hz, ^2J = 15.0 Hz, COC***H***H), 2.55 (dd, 1H, ^3J = 8.2 Hz, ^2J = 15.0 Hz, COC***H***H), 1.66 (m, 2H, C***H***$_2$CH$_3$), 1.13 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.79 (t, 3H, ^3J = 7.4 Hz, CH$_2$C***H***$_3$); EI-MS (70 eV) *m/z* (%): 206 ($M^{+\bullet}$, 29). $C_{13}H_{18}O_2$ (206.28)

Ethyl 4-methyl-3-phenylpentanoate (3.22b)[59]

The title compound was prepared from **3.22a** (2.5 g, 11.4 mmol), Pd/C (10 %) (0.3 g, cat.) and 20 ml EtOH according to the general procedure yielding **3.22b** (2.5 g, 99 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.25-7.16 (m, 5H, Ar-***H***), 3.95 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.88 (m, 1H, CH$_2$C***H***), 2.77 (dd, 1H, ^3J = 5.5 Hz, ^3J = 14.9 Hz, COC***H***H), 2.58 (dd, 1H, ^3J = 9.9 Hz, ^3J = 14.9 Hz, COC***H***H), 1.85 (m, 1H, C***H***(CH$_3$)$_2$), 1.06 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.95 (d, 3H, ^3J = 6.7 Hz, ***C***H$_3$), 0.76 (d, 3H, ^3J = 6.7 Hz, ***C***H$_3$); PI-EIMS (70 eV) *m/z* (%): 220 ($M^{+\bullet}$, 42). $C_{14}H_{20}O_2$ (220.31)

Ethyl 5-methyl-3-phenylhexanoate (3.23b)[60]

The title compound was prepared from **3.23a** (2.67 g, 11.4 mmol), Pd/C (10 %) (0.3 g, cat.) and 20 ml EtOH according to the general procedure yielding **3.23b** (2.7 g, 100 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.30 - 7.19 (m, 5H, Ar-***H***), 4.02 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.19 (m, 1H, CH$_2$C***H***), 2.59 (dd, 1H, ^3J = 5.5 Hz, ^3J = 13.4 Hz, COC***H***H), 2.52 (dd, 1H, ^3J = 6.6 Hz, ^3J = 13.4 Hz, COCH***H***), 1.41-1.30 (m, 2H, C***H***$_2$CH(CH$_3$)$_2$), 1.30 (m, 1H, C***H***(CH$_3$)$_2$), 1.13 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.88 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$), 0.83 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 234 ($M^{+\bullet}$, 69). $C_{15}H_{22}O_2$ (234.15)

Ethyl 3,4-diphenylbutanoate (3.24b)[58]

The title compound was prepared from **3.24a** (3.21 g, 12 mmol), Pd/C (10 %) (0.3 g, cat.) and 30 ml EtOH according to the general procedure yielding **3.24b** (2.98 g, 92 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.25-7.05 (m, 10H, Ar-***H***)), 3.97 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.41 (m, 1H, CH$_2$C***H***), 2.91 (m, 2H, C***H***$_2$-Ar), 2.67 (dd, 1H, ^3J = 5.8 Hz, ^3J = 14.4 Hz, COC***H***H), 2.59 (dd, 1H, *3J* = 7.5 Hz, ^3J = 14.4 Hz, COCH***H***), 1.10 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$); PI-EIMS (70 eV) *m/z* (%): 268 ($M^{+\bullet}$, 23). $C_{18}H_{20}O_2$ (268.35)

Ethyl 3-phenyl-4-(4-methylphenyl)butanoate (3.25b)

The title compound was prepared from **3.25a** (2.5 g, 8.9 mmol), Pd/C (10 %) (0.3 g, cat.) and 30 ml EtOH according to the general procedure yielding **3.25b** (2.06 g, 82%) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.13-6.96 (m, 9H, Ar-***H***), 3.97 (q, 2H, ^3J = 7.1Hz, C***H***$_2$CH$_3$), 3.38 (m, 1H, CH$_2$C***H***), 2.90 (dd, 1H, ^3J = 6.9 Hz, ^3J = 13.2 Hz, CH***H***-Ar), 2.83 (dd, 1H, ^3J = 5.6 Hz, ^3J = 11.44 Hz, C***H***H-Ar), 2.61 (m, 2H, COC***H***$_2$), 2.28 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.09 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$); EI-MS (70 eV) *m/z* (%): 282 ($M^{+\bullet}$, 10). $C_{19}H_{22}O_2$ (282.38)

Ethyl 3-cyclohexyl-4-phenylbutanoate (3.26b)

The title compound was prepared from **3.26a** (0.61 g, 2.24 mmol), Pd/C (10 %) (0.1 g, cat.) and 10 ml EtOH according to the general procedure yielding **3.26b** (0.6 g, 98 %) as a yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.25 (m, 5H, Ar-***H***), 4.02 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.87 (dd, 1H, ^3J = 3.4 Hz, ^2J = 13.6 Hz, C***H***H-Ar), 2.72 (dd, 1H, ^3J = 6.0 Hz, ^2J = 13.6 Hz, CH***H***-Ar), 2.59 (dd, 1H, ^3J = 9.5 Hz, ^3J = 13.6 Hz, COC***H***H), 2.44 (dd, 1H, ^3J = 8.0 Hz, ^3J = 13.5 Hz, COCH***H***), 2.12 (m, 1H, CH$_2$C***H***), 1.72 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.32 (m, 2H, cHex-C***H***$_2$), 1.20 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 1.08 (m, 2H, cHex-C***H***$_2$). $C_{18}H_{26}O_2$ (274.30)

Ethyl 3-(4-methylphenyl)butanoate (3.27b)[61, 62]

The title compound was prepared from **3.27a** (7 g, 34.3 mmol), Pd/C (10 %) (0.7 g, cat.) and 50 ml EtOH according to the general procedure yielding **3.27b** (6.58 g, 93 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.11 (m, 4H, Ar-***H***), 4.07 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.24 (m, 1H, CH$_2$C***H***), 2.59 (dd, 1H, ^3J = 7.0 Hz, ^3J = 15.0 Hz, COC***H***H), 2.50 (dd, 1H, ^3J = 8.2 Hz, ^3J = 15.0 Hz, COCH***H***), 2.31 (s, 3H, (p-C***H***$_3$)-Ar), 1.28 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$) ppm 1.19 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$); EI-MS (70 eV) *m/z* (%): 206 ($M^{+\bullet}$, 23). $C_{13}H_{18}O_2$ (206.28)

Ethyl 3-methyl-4-phenylbutanoate (3.28b)[63]

The title compound was prepared from **3.28a** (2 g, 9.8 mmol), Pd/C (10 %) (0.2 g, cat.) and 20 ml EtOH according to the general procedure yielding **3.28b** (1.9 g, 94 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.27-7.16 (m, 5H, Ar-***H***), 4.11 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.63 (dd, 1H, ^3J = 6.4 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.49 (dd, 1H, ^3J = 7.3 Hz, ^3J = 13.4 Hz, CH***H***-Ar), 2.29 (m, 2H, COC***H***$_2$), 2.14 (m, 1H, CH$_2$C***H***), 1.25 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.94 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 206 ($M^{+\bullet}$, 28). $C_{13}H_{18}O_2$ (206.28)

Ethyl 3-methyl-5-phenylpentanoate (3.29b)[64]

The title compound was prepared from **3.29a** (3.09 g, 14 mmol), Pd/C (10 %) (0.3 g, cat.) and 30 ml EtOH according to the general procedure yielding **3.29b** (2.9 g, 94 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.28-7.17 (m, 5H, Ar-***H***), 4.13 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.63 (m, 2H, C***H***$_2$-Ar), 2.35 (dd, 1H, ^3J = 6.1 Hz, ^3J = 14.6 Hz, COC***H***H), 2.17 (dd, 1H, ^3J = 7.9 Hz, ^3J = 14.6 Hz, COCH***H***), 2.03 (m, 1H, CH$_2$C***H***), 1.68-1.51 (m, 2H, C***H***$_2$CH$_2$-Ar), 1.26 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 1.02 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); PI-EIMS (70 eV) *m/z* (%): 220 ($M^{+\bullet}$, 11). $C_{14}H_{20}O_2$ (220.31)

Ethyl 3-methyl-6-phenylhexanoate (3.30b)

The title compound was prepared from **3.30a** (2.11 g, 9.1 mmol), Pd/C (10 %) (0.2 g, cat.) and 20 ml EtOH according to the general procedure yielding **3.30b** (2.11 g, 99 %) as a colourless liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.27-7.16 (m, 5H, Ar-***H***) 4.11 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.59 (m, 2H, ***C***H$_2$-Ar), 2.28 (dd, 1H, ^3J = 6.0 Hz, ^3J = 14.4 Hz, COC***H***H), 2.09 (dd, 1H, ^3J = 8.0 Hz, ^3J = 14.5 Hz, COCH***H***), 1.94 (m, 1H, CH$_2$C***H***), 1.62 (m, 4H, CH$_2$C***H***$_2$CH$_2$-Ar, C***H***$_2$CH$_2$CH$_2$-Ar), 1.23 (t, 3H, ^3J = 7.0 Hz, CH$_2$C***H***$_3$), 0.93 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); CI-MS (NH_3) *m/z* (%): 252 ($M+NH_4^+$, 100). $C_{15}H_{22}O_2$ (234.33)

Ethyl 3-methyl-4-(3-methylphenyl)butanoate (3.31b)

The title compound was prepared from **3.31a** (1.2 g, 5.5 mmol), Pd/C (10 %) (0.1 g, cat.) and 15 ml EtOH according to the general procedure yielding **3.31b** (1.16 g, 96 %) as a colourless liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.17 (m, 1H, Ar-***H***), 6.99 (m, 3H, Ar-***H***), 4.12 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.60 (dd, 1H, ^3J = 6.5 Hz, ^3J = 13.3 Hz, C***H***H-Ar), 2.46 (dd, 1H, ^3J = 7.3 Hz, ^3J = 13.3 Hz, CH***H***-Ar), 2.33 (s, 3H, (*m*-C***H***$_3$)Ar), 2.34-2.13 (m, 3H, COC***H***$_2$, C***H***CH$_3$), 1.26 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.95 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); CI-MS (NH_3) *m/z* (%): 238 ($M+NH_4^+$, 100). $C_{14}H_{20}O_2$ (220.31)

Ethyl 3-methyl-4-(4-methylphenyl)butanoate (3.32b)[65]

The title compound was prepared from **3.32a** (1.84 g, 8.4 mmol), Pd/C (10 %) (0.2 g, cat.) and 30 ml EtOH according to the general procedure yielding **3.32b** (1.84 g, 84 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.08 (m, 4H, Ar-***H***), 4.12 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.60 (dd, 1H, ^3J = 6.5 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.46 (dd, 1H, ^3J = 7.2 Hz, ^3J = 13.4 Hz, CH***H***-Ar), 2.33 (s, 3H, (*p*-C***H***$_3$)Ar) 2.34-2.12 (m, 3H, COC***H***$_2$, C***H***CH$_3$), 1.25 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.94 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 220 ($M^{+\bullet}$, 14). $C_{14}H_{20}O_2$ (220.31)

Ethyl 3-(3-fluorobenzyl)butanoate (3.33b)

The title compound was prepared from **3.33a** (2.03 g, 9.1 mmol), Pd/C (10 %) (0.2 g, cat.) and 25 ml EtOH according to the general procedure yielding **3.33b** (1.97 g, 96 %) as a colourless liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.22 (m, 1H, Ar-***H***), 6.90 (m, 3H, Ar-***H***), 4.12 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.64 (dd, 1H, ^3J = 6.3 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.48 (dd, 1H, ^3J = 7.3 Hz, ^3J = 13.4 Hz, CH***H***-Ar), 2.31-2.14 (m, 3H, COC***H***$_2$, C***H***CH$_3$), 1.25 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.94 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$). $C_{13}H_{17}FO_2$ (224.27)

Ethyl 3-(4-fluorobenzyl)butanoate (3.34b)

The title compound was prepared from **3.34a** (1.26 g, 5.7 mmol), Pd/C (10 %) (0.1 g, cat.) and 15 ml EtOH according to the general procedure yielding **3.34b** (1.29 g, 100 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.11 (m, 2H, Ar-***H***), 6.96 (m, 2H, Ar-***H***), 4.11 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.61 (dd, 1H, ^3J = 6.3 Hz, ^3J = 13.5 Hz, C***H***H-Ar), 2.46 (dd, 1H, ^3J = 7.2 Hz, ^3J = 13.5 Hz, CH***H***-Ar), 2.29-2.14 (m, 3H, COC***H***$_2$, C***H***CH$_3$), 1.25 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.93 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 224 ($M^{+\bullet}$, 20). $C_{13}H_{17}FO_2$ (224.27)

Ethyl 3-(3-methoxybenzyl)butanoate (3.35b)[51]

The title compound was prepared from **3.35a** (1 g, 4.3 mmol), Pd/C (10 %) (0.1 g, cat.) and 20 ml EtOH according to the general procedure yielding **3.35b** (0.99 g, 97 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.20 (m, 1H, Ar-***H***), 6.75 (m, 3H, Ar-***H***), 4.12 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.80 (s, 3H, (m-OC***H***$_3$)Ar), 2.61 (dd, 1H, ^3J = 6.5 Hz, ^3J = 13.3 Hz, COC***H***H), 2.47 (dd, 1H, ^3J = 7.3 Hz, ^3J = 13.3 Hz, COCH***H***), 2.33-2.13 (m, 3H, C***H***$_2$-Ar, C***H***CH$_3$), 1.25 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.95 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$). $C_{14}H_{20}O_3$ (236.31)

Ethyl 3-(4-methoxybenzyl)butanoate (3.36b)[66]

The title compound was prepared from **3.36a** (1.1 g, 4.7 mmol), Pd/C (10 %) (0.1 g, cat.) and 15 ml EtOH according to the general procedure yielding **3.36b** (1.13 g, 95 %) as a pale yellow liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.07 (m, 2H, Ar-***H***), 6.82 (m, 2H, Ar-***H***), 4.11 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.79 (s, 3H, (p-OC***H***$_3$)Ar), 2.57 (dd, 1H, ^3J = 6.5 Hz, ^3J = 13.5 Hz, C***H***H-Ar), 2.45 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.5 Hz, CH***H***-Ar), 2.30-2.11 (m, 3H, COC***H***$_2$, C***H***CH$_3$), 1.25 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$), 0.93 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 236 ($M^{+\bullet}$, 24). $C_{14}H_{20}O_3$ (236.31)

Ethyl 3-(3-ethylbenzyl)butanoate (3.37b)

The title compound was prepared from **3.37a** (1.81 g, 7.84 mmol), Pd/C (10 %) (0.2 g, cat.) and 30 ml EtOH according to the general procedure yielding **3.37b** (1.79 g, 98 %) as a colourless liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.10 (m, 4H, Ar-***H***), 4.11 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 2.61 (m, 3H, C***H***H-Ar, (*p*-C***H***$_2$CH$_3$)Ar), 2.47 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.4 Hz, CH***H***-Ar), 2.33-2.12 (m, 3H, COC***H***$_2$, C***H***CH$_3$), 1.24 (m, 6H, CH$_2$C***H***$_3$, (*p*-CH$_2$C***H***$_3$)Ar), 0.95 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 234 ($M^{+\bullet}$, 21). $C_{15}H_{22}O_2$ (234.33)

Ethyl 4-(*tert*-butoxycarbonylamino)-3-phenylbutanoate (3.38b)[67]

The title compound was prepared from **3.38a** (0.31 g, 0.98 mmol), Pd/C (10 %) (cat.) and 15 ml EtOH according to the general procedure yielding **3.38b** (0.3 g, 97 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.20 (m, 5H, Ar-**H**), 4.05 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.48 (m, 1H, CH$_2$C***H***), 3.33 (dd, 1H, ^3J = 5.6 Hz, ^3J = 13.0 Hz, C***H***HNH), 3.26 (dd, 1H, ^3J = 7.0 Hz, ^3J = 12.1 Hz, CH***H***NH), 2.70 (dd, 1H, ^3J = 6.4 Hz, ^3J = 15.5 Hz, COC**H**H), 2.60 (dd, 1H, ^3J = 7.8 Hz, ^3J = 15.5 Hz, COCH***H***), 1.41 (s, 9H, C(C***H***$_3$)$_3$), 1.14 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$); CI-MS (NH_3) *m/z* (%): 325 (M+NH_4^+, 4). $C_{17}H_{25}NO_4$ (307.38)

Ethyl 6-(*tert*-butoxycarbonylamino)-3-phenylhexanoate (3.39b)[55]

The title compound was prepared from **3.39a** (1.21 g, 9.8 mmol), Pd/C (10 %) (0.1 g, cat.) and 25 ml EtOH according to the general procedure yielding **3.39b** (1.17 g, 97 %) as a white solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.29-7.18 (m, 5H, Ar-***H***), 4.03 (q, 2H, ^3J = 7.1 Hz, C***H***$_2$CH$_3$), 3.06 (m, 3H, CH$_2$C***H***, C***H***$_2$NH), 2.62 (dd, 1H, ^3J = 5.9 Hz, ^3J = 13.8 Hz, COC***H***H), 2.55 (dd, 1H, ^3J = 6.6 Hz, ^3J = 13.8 Hz, COCH***H***), 1.61 (m, 4H, C***H***$_2$CH$_2$CH$_2$NH, CH$_2$C***H***$_2$CH$_2$NH), 1.42 (s, 9H, C(C***H***$_3$)$_3$), 1.14 (t, 3H, ^3J = 7.1 Hz, CH$_2$C***H***$_3$); CI-MS (NH_3) *m/z* (%): 353 (M+NH_4^+, 29). $C_{19}H_{29}NO_4$ (335.44)

3.5.5. Preparation of phenylalkanoic acids 3.20, 3.21-3.39c and 3.53-3.54b

3-(1-Trityl-1*H*-imidazol-4-yl)propanoic acid (3.20)[68]

To a solution of Methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate (1 g, 2.5 mmol) in 25 ml THF were added 4 ml of a 1M solution of LiOH/aq and stirred overnight. Subsequently, 10 % HCl/aq were added under ice cooling to adjust the solution to pH 4-5. The product was extracted with EtOAc, the organic layer washed with saturated NaCl/aq, dried over $MgSO_4$ and evaporated in *vacuo*. The crude product was recrystallised from EtOAc/THF to obtain **3.20** (0.79 g, 83 %) as a pale yellow solid. mp = 185 °C (ref.[68]: 188-190 °C); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.41 (d, 1H, J^4 = 1.4 Hz, Im-2-***H***), 7.37-7.13 (m, 15H, CPh_3), 6.70 (s, 1H, Im-5-***H***), 2.82 (t, 2H, J^3 = 7.4 Hz, Im-4-C***H***$_2$), 2.58 (t, 2H, J^3 = 7.3 Hz, C***H***$_2$CO); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 383 (MH^+, 100); anal ($C_{25}H_{22}N_2O_2$) C, H, N. $C_{25}H_{22}N_2O_2$ (382.1)

General procedure for the preparation of 3.21-3.39c and 3.53-3.54b

The ethyl alkanoates were suspended in 20 % NaOH/aq and refluxed for 2-4 h (unless otherwise indicated). After cooling, 10 % HCl/aq was added to adjust to pH < 2. Subsequently, EtOAc was added and the water phase extracted three times with EtOAc. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. For combustion analysis data, a small amount of acid was recrystallized from hexane or precipitated as salt with dicyclohexylamine.

3-Phenylpentanoic acid (3.21c)[69]

The title compound was prepared from **3.21b** (2.85 g, 13.8 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.21c** (2.3 g, 91 %) as a pale yellow solid. mp = 44 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 10.33 (br s, 1H, COO***H***), 7.29-7.16 (m, 5H, Ar-***H***), 3.00 (m, 1H, CH_2C***H***), 2.68 (dd, 1H, ^3J = 6.4 Hz, ^3J = 14.8 Hz, COC***H***H), 2.60 (dd, 1H, ^3J = 7.2 Hz, ^3J = 14.8 Hz, COC***H***H), 1.67 (m, 2H, C***H***$_2$$CH_3$), 0.79 (t, 3H, ^3J = 7.4 Hz, CH_2C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 178.96 (quat. ***C***=O), 143.63 (quat. Ar-***C***), 128.58 (+, 2 Ar-***C***H), 128.44 (+, 2 Ar-***C***H), 127.49 (+, Ar-***C***H), 43.50 (+, CH_2***C***H), 41.16 (-, CO***C***H_2), 29.12 (-, ***C***$H_2$$CH_3$), 11.85(+, CH_2***C***H_3); PI-EIMS (70 eV) *m/z* (%): 178 ($M^{+\bullet}$, 45); anal. ($C_{11}H_{14}O_2$) C, H. $C_{11}H_{14}O_2$ (178.23)

4-Methyl-3-phenylpentanoic acid (3.22c)[44]

The title compound was prepared from **3.22b** (2.1 g, 9.5 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.22c** (1.63 g, 89 %) as a pale yellow solid. mp = 47 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.26-7.14 (m, 5H, Ar-***H***), 2.87 (m, 1H, CH_2C***H***), 2.79 (dd, 1H, ^3J = 5.4 Hz, ^3J = 15.3 Hz, COC***H***H), 2.61 (dd, 1H, ^3J = 9.4

Hz, ^3J = 15.3 Hz, COC***H***H), 1.84 (m, 1H, C***H***$(CH_3)_2$), 0.93 (d, 3H, ^3J = 6.7 Hz, ***C***H_3), 0.75 (d, 3H, ^3J = 6.7 Hz, ***C***H_3); ^{13}C-NMR ($CDCl_3$) δ ppm: 178.11 (quat. ***C***=O), 142.52 (quat. Ar-***C***), 128.18 (+, 2 Ar-***C***H), 128.11 (+, 2 Ar-***C***H), 126.41 (+, Ar-***C***H), 48.42 (+, CH_2***C***H), 37.95 (-, CO***C***H_2), 33.08 (+, ***C***H$(CH_3)_2$), 20.54 (+, ***C***H_3), 20.17 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 192 ($M^{+\bullet}$, 27); anal. ($C_{12}H_{16}O_2$) C, H. $C_{12}H_{16}O_2$ (192.25)

5-Methyl-3-phenylhexanoic acid (3.23c)[70]

The title compound was prepared from **3.23b** (2.63 g, 11.2 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.23c** (1.81 g, 78 %) as a pale yellow solid. mp = 58 °C (ref.[70]: 52-55 °C); ^{1}H-NMR ($CDCl_3$) δ ppm: 10.37 (br s, 1H, COO***H***), 7.28-7.18 (m, 5H, Ar-***H***), 3.18 (m, 1H, C***H***CH_2), 2.63 (dd, 1H, ^3J = 4.4 Hz, ^3J = 12.8 Hz, COC***H***H), 2.56 (dd, 1H, ^3J = 5.2 Hz, ^3J = 12.8 Hz, COCH***H***), 1.44-1.29 (m, 2H, C***H***$_2$CH$(CH_3)_2$), 1.31 (m, 1H, C***H***$(CH_3)_2$), 0.88 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$), 0.83 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 177.58 (quat. ***C***=O), 143.79 (quat. Ar-***C***), 128.47 (+, 2 Ar-***C***H), 127.93 (+, Ar-***C***H), 127.42 (+, 2 Ar-***C***H), 45.34 (-, ***C***H_2CH$(CH_3)_2$), 41.87 (-, CO***C***H_2), 39.63 (+,CH_2***C***H), 25.24 (+, ***C***H$(CH_3)_2$), 22.25 (-, ***C***H_3), 21.55 (-, ***C***H_3); PI-EIMS (70 eV) *m/z* (%): 206 ($M^{+\bullet}$, 53); anal. ($C_{13}H_{18}O_2$) C, H. $C_{13}H_{18}O_2$ (206.28)

3,4-Diphenylbutanoic acid (3.24c)[71]

The title compound was prepared from **3.24b** (2.95 g, 11 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.24c** (2.4 g, 90 %) as a pale yellow solid. mp = 93 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.25-7.05 (m, 10H, Ar-***H***), 3.38 (m, 1H, CH_2C***H***), 2.90 (m, 2H, C***H***$_2$-Ar), 2.70 (dd, 1H, ^3J = 5.3 Hz, ^3J = 14.5 Hz, COC***H***H), 2.62 (dd, 1H, ^3J = 6.9 Hz, ^3J = 14.5 Hz, COCH***H***); ^{13}C-NMR ($CDCl_3$) δ ppm: 177.78 (quat. ***C***=O), 143.09 (quat. Ar-***C***), 139.28 (quat. Ar-***C***), 129.20 (+, 2 Ar-***C***H), 128.42 (+, 2 Ar-***C***H), 128.22 (+, 2 Ar-***C***H), 127.44 (+, 2 Ar-***C***H), 126.67 (+, Ar-***C***H), 126.22 (+, Ar-***C***H), 43.57 (+, CH_2***C***H), 42.96 (-, ***C***H_2), 39.60 (-, ***C***H_2); PI-EIMS (70 eV) *m/z* (%): 240 ($M^{+\bullet}$, 13); anal. ($C_{16}H_{16}O_2$) C, H. $C_{16}H_{16}O_2$ (240.30)

3-Phenyl-4-(4-methylphenyl)butanoic acid (3.25c)[72]

The title compound was prepared from **3.25b** (2 g, 7.1 mmol) and 25 ml 20 % NaOH/aq according to the general procedure yielding **3.25c** (1.75 g, 99 %) as a white solid. mp = 106 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.20-6.97 (m, 9H, Ar-***H***), 3.35 (m, 1H, CH_2C***H***), 2.84 (m, 2H, C***H***$_2$-Ar), 2.64 (m, 2H, COC***H***$_2$), 2.27 (s, 3H, (*p*-C***H***$_3$)-Ar); ^{13}C-NMR ($CDCl_3$) δ ppm: 178.18 (quat. ***C***=O), 143.24 (quat. Ar-***C***), 136.16 (quat. Ar-***C***), 129.06 (+, Ar-C***H***), 128.99 (+, Ar-C***H***), 128.91 (+, Ar-C***H***), 128.40 (+, 2 Ar-C***H***),

128.28 (+, Ar-C***H***), 127.45 (+, Ar-C***H***), 126.61 (+, Ar-C***H***), 43.59 (+, CH_2C***H***), 42.51 (-, ***C***H_2-Ar), 39.61 (-, CO***C***H_2), 21.01 (+, (*p*-***C***H_3)-Ar); EI-MS (70 eV) *m/z* (%): 254 ($M^{+\bullet}$, 16); anal. ($C_{17}H_{18}O_2$) C, H. $C_{17}H_{18}O_2$ (254.34)

3-Cyclohexyl-4-phenylbutanoic acid (3.26c)[73]

The title compound was prepared from **3.26b** (0.6 g, 2.19 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.26c** (0.5 g, 92 %) as a brown oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.26 (m, 5H, Ar-***H***), 2.76 (dd, 1H, ^3J = 6.2 Hz, ^2J = 13.6 Hz, C***H***H-Ar), 2.46 (m, 2H, CH***H***-Ar, COC***H***H), 2.36 (dd, 1H, ^3J = 6.5 Hz, ^2J = 15.5 Hz, COCH***H***), 2.12 (m, 1H, CH_2C***H***), 1.81-1.65 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.35 (m, 2H, cHex-C***H***$_2$), 0.88 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 178.76 (quat. ***C***=O), 139.69 (quat. Ar-***C***), 128.44 (+, Ar-***C***H), 128.18 (+, Ar-***C***H), 127.56 (+, Ar-***C***H), 127.28 (+, Ar-***C***H), 46.83 (-, CO***C***H_2), 41.33 (+, cHex-***C***H), 38.92 (+, ***C***HCH_2-Ar), 36.27 (-, ***C***H_2-Ar), 28.90 (-, cHex-***C***H_2), 28.25 (-, cHex-***C***H_2), 27.74 (-, cHex-***C***H_2), 27.53 (-, cHex-***C***H_2), 25.62 (-, cHex-***C***H_2); EI-MS (70 eV) *m/z* (%): 246 ($M^{+\bullet}$, 20); anal. ($C_{16}H_{22}O_2 \cdot C_{12}H_{23}N \cdot 0.25\ H_2O$) C, H, N. $C_{16}H_{22}O_2$ (246.34)

3-(4-Methylphenyl)butanoic acid (3.27c)[62]

The title compound was prepared from **3.27b** (6.5 g, 31.5 mmol) and 45 ml 20 % NaOH/aq according to the general procedure yielding **3.27c)** (5.09 g, 91 %) as a white solid. mp = 88 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.11 (m, 4H, Ar-***H***), 3.24 (m, 1H, CH_2C***H***), 2.65 (dd, 1H, ^3J = 6.9 Hz, ^3J = 15.5 Hz, COC***H***H), 2.55 (dd, 1H, ^3J = 8.2 Hz, ^3J = 15.5 Hz, COCH***H***), 2.32 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.30 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 178.33 (quat. ***C***=O), 142.42 (quat. Ar-***C***), 136.01 (quat. Ar-***C***), 129.23 (+, 2 Ar-C***H***), 126.55 (+, 2 Ar-C***H***), 42.59 (-, CO***C***H_2), 35.74 (+, ***C***HCH_3), 21.95 (+, (*p*-***C***H_3)-Ar), 21.00 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 178 ($M^{+\bullet}$, 28); anal. ($C_{11}H_{14}O_2$) C, H. $C_{11}H_{14}O_2$ (178.23)

3-Methyl-4-phenylbutanoic acid (3.28c)[74]

The title compound was prepared from **3.28b** (1.85 g, 8.97 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.28c** (1.5 g, 94 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 9.23 (br s, 1H, COO***H***), 7.29-7.16 (m, 5H, Ar-***H***), 2.64 (dd, 1H, ^3J = 6.6 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.52 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.4 Hz, CH***H***-Ar), 2.32 (m, 2H, COC***H***$_2$), 2.18 (m, 1H, CH_2C***H***), 0.98 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.48 (quat. ***C***=O), 140.06 (quat. Ar-***C***), 129.22 (+, 2 Ar-***C***H), 128.32 (+, 2 Ar-***C***H), 126.13 (+, Ar-***C***H), 42.92 (-, CO***C***H_2), 40.75 (-, ***C***H_2-Ar),

32.10 (+, ***C***HCH_3), 19.60 (+, ***C***H_3); PI-EIMS (70 eV) *m/z* (%): 178 ($M^{+\bullet}$, 23); anal. ($C_{11}H_{14}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{11}H_{14}O_2$ (178.23)

3-Methyl-5-phenylpentanoic acid (3.29c)[75]

The title compound was prepared from **3.29b** (2.7 g, 12.3 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.29c** (2.08 g, 88 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.29-7.18 (m, 5H, Ar-***H***), 2.64 (m, 2H, C***H***$_2$-Ar), 2.41 (dd, 1H, ^3J = 5.9 Hz, ^3J = 15.0 Hz, COC***H***H), 2.21 (dd, 1H, ^3J = 8.0 Hz, ^3J = 15.0 Hz, COCH***H***), 2.03 (m, 1H, CH_2C***H***), 1.69-1.54 (m, 2H, C***H***$_2$$CH_2$-Ar), 1.05 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.07 (quat. ***C***=O), 142.21 (quat. Ar-***C***), 128.36 (+, 2 Ar-***C***H), 128.30 (+, 2 Ar-***C***H), 125.78 (+, Ar-***C***H), 41.36 (-, CO***C***H_2), 38.43 (-, ***C***H_2CH_2-Ar), 33.29 (-, ***C***H_2-Ar), 29.91 (+, ***C***HCH_3), 19.59 (+, ***C***H_3); PI-EIMS (70 eV) *m/z* (%): 192 ($M^{+\bullet}$, 30); anal. ($C_{12}H_{16}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{12}H_{16}O_2$ (192.25)

3-Methyl-6-phenylhexanoic acid (3.30c)

The title compound was prepared from **3.30b** (2.05 g, 8.75 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.30c** (1.64 g, 91 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.28-7.18 (m, 5H, Ar-***H***), 2.61 (m, 2H, ***C***H_2-Ar), 2.36 (dd, 1H, ^3J = 5.9 Hz, ^3J = 15.0 Hz, COC***H***H), 2.16 (dd, 1H, ^3J = 8.1 Hz, ^3J = 14.9 Hz, COCH***H***), 1.99 (m, 1H, CH_2C***H***), 1.64 (m, 2H, C***H***$_2$$CH_2$-Ar), 1.45-1.25 (m, 2H, C***H***$_2$$CH_2CH_2$-Ar), 0.98 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.05 (quat. ***C***=O), 142.48 (quat. Ar-***C***), 128.38 (+, 2 Ar-***C***H), 128.30 (+, 2 Ar-***C***H), 125.72 (+, Ar-***C***H), 41.45 (-, CO***C***H_2), 36.24 (-, CH_2CH_2***C***H_2-Ar), 35.97 (-, ***C***$H_2CH_2CH_2$-Ar), 30.05 (+, CH_2***C***H), 28.83 (-, CH_2***C***H_2CH_2-Ar), 19.64 (+, ***C***H_3); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 205 ([M-H]$^-$, 100); anal. ($C_{13}H_{18}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{13}H_{18}O_2$ (206.28)

3-Methyl-4-(3-methylphenyl)butanoic acid (3.31c)[76]

The title compound was prepared from **3.31b** (1.11 g, 5 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.31c** (0.87 g, 90 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 10.73 (br s, 1H, COO***H***), 7.18 (m, 1H, Ar-***H***), 7.00 (m, 3H, Ar-***H***), 2.61 (dd, 1H, ^3J = 6.7 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.49 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.3 Hz, CH***H***-Ar), 2.34 (s, 1H, (*m*-C***H***$_3$)Ar), 2.39 (dd, 1H, ^3J = 5.1 Hz, ^3J = 14.4 Hz, COC***H***H), 2.21 (m, 2H, COCH***H***, C***H***CH_3), 0.99 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.54 (quat. ***C***=O), 140.02 (quat. Ar-***C***), 137.85 (quat. Ar-***C***), 129.99 (+, Ar-***C***H), 128.19 (+, Ar-***C***H), 126.86 (+, Ar-***C***H), 126.25 (+, Ar-***C***H), 42.89 (-, CO***C***H_2), 40.81 (-, ***C***H_2-Ar), 32.09 (+, ***C***HCH_3), 21.42 (+, (m-***C***H_3)Ar), 19.64

(+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 192 ($M^{+\bullet}$, 36); anal. ($C_{12}H_{16}O_2 \cdot C_{12}H_{23}N \cdot H_2O$) C, H, N. $C_{12}H_{16}O_2$ (192.25)

3-Methyl-4-(4-methylphenyl)butanoic acid (3.32c)[77]

The title compound was prepared from **3.32b** (1.8 g, 8.2 mmol) and 30 ml 20 % NaOH/aq according to the general procedure yielding **3.32c** (1.5 g, 95 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 11.14 (br s, 1H, COO***H***), 7.07 (m, 4H, Ar-***H***), 2.61 (dd, 1H, ^3J = 6.7 Hz, ^3J = 13.5 Hz, C***H***H-Ar), 2.49 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.5 Hz, CH***H***-Ar), 2.38 (dd, 1H, ^3J = 5.1 Hz, ^3J = 14.4 Hz, COC***H***H), 2.32 (s, 3H, (*p*-C***H***$_3$)Ar), 2.21 (m, 2H, COCH***H***, C***H***CH_3), 0.98 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.21 (quat. ***C***=O), 136.95 (quat. Ar-***C***), 135.56 (quat. Ar-***C***), 129.09 (+, 2 Ar-***C***H), 128.98 (+, 2 Ar-***C***H), 42.48 (-, CO***C***H_2), 40.69 (-, ***C***H_2-Ar), 32.13 (+, ***C***HCH_3), 21.00 (+, (*p*-***C***H_3)Ar), 19.57 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 192 ($M^{+\bullet}$, 22); anal. ($C_{12}H_{16}O_2 \cdot C_{12}H_{23}N \cdot 0.5\ H_2O$) calculated C (75.35), H (10.54), N (3.66), found C (75.36), H (10.20), N (3.14). $C_{12}H_{16}O_2$ (192.25)

3-(3-Fluorobenzyl)butanoic acid (3.33c)

The title compound was prepared from **3.33b** (1.94 g, 8.66 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.33c** (1.6 g, 94 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 9.87 (br s, 1H, COO***H***), 7.24 (m, 1H, Ar-***H***), 6.91 (m, 3H, Ar-***H***), 2.66 (dd, 1H, ^3J = 6.3 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.51 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.4 Hz, CH***H***-Ar), 2.29-2.14 (m, 3H, COC***H***$_2$, C***H***CH_3), 0.98 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.19 (quat. ***C***=O), 162.87 (d, quart., ^1J = 245.4 Hz, Ar-***C***F), 142.65 (d, quart., ^3J = 7.0 Hz, Ar-***C***), 129.72 (+, d, ^3J = 8.2 Hz, Ar-***C***H), 124.85 (+, d, ^4J = 2.8 Hz, Ar-***C***H), 115.98 (+, d, ^2J = 20.8 Hz, Ar-***C***H), 113.05 (+, d, ^2J = 20.9 Hz, Ar-***C***H), 42.53 (-, CO***C***H_2), 40.65 (-, ***C***H_2-Ar), 31.92 (+, C***H***CH_3), 19.50 (+, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 196 ($M^{+\bullet}$, 26); anal. ($C_{11}H_{13}FO_2 \cdot C_{12}H_{23}N \cdot 0.25\ H_2O$) C, H, N. $C_{11}H_{13}FO_2$ (196.22)

3-(4-Fluorobenzyl)butanoic acid (3.34c)[78]

The title compound was prepared from **3.34cb** (1.24 g, 5.5 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.34c** (0.99 g, 92 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 11.46 (br s, 1H, COO***H***), 7.12 (m, 2H, Ar-***H***), 6.97 (m, 2H, Ar-***H***), 2.63 (dd, 1H, ^3J = 6.6 Hz, ^3J = 13.6 Hz, C***H***H-Ar), 2.49 (dd, 1H, ^3J = 7.0 Hz, ^3J = 13.5 Hz, CH***H***-Ar), 2.36-2.18 (m, 3H, COC***H***$_2$, C***H***CH_3), 0.97 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.42 (quat. ***C***=O), 161.46 (d, quart., ^1J = 247.6 Hz, Ar-***C***F), 135.66 (d, quart., ^4J = 3.5 Hz, Ar-***C***), 130.54 (+, d, ^3J = 7.9 Hz, 2

Ar-***C***H), 115.07 (+, d, ^2J = 20.9 Hz, 2 Ar-***C***H), 42.02 (-, CO***C***H_2), 40.66 (-, ***C***H_2-Ar), 32.17 (+, ***C***HCH_3), 19.49 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 196 ($M^{+\bullet}$, 25); anal. ($C_{11}H_{13}FO_2 \cdot C_{12}H_{23}N \cdot 0.15\ H_2O$) C, H, N. $C_{11}H_{13}FO_2$ (196.22)

3-(3-Methoxybenzyl)butanoic acid (3.35c)[51]

The title compound was prepared from **3.35b** (0.97 g, 4.1 mmol) and 15 ml 20 % NaOH/aq according to the general procedure yielding **3.35c** (0.82 g, 91 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.20 (m, 1H, Ar-***H***), 6.75 (m, 3H, Ar-***H***), 3.80 (s, 3H, (*m*-OC***H***$_3$)Ar), 2.63 (dd, 1H, ^3J = 6.7 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.50 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.3 Hz, CH***H***-Ar), 2.39 (dd, 1H, ^3J = 5.1 Hz, ^3J = 14.3 Hz, COC***H***H), 2.28 (m, 1H, C***H***CH_3), 2.16 (dd, 1H, ^3J = 7.7 Hz, ^3J = 14.3 Hz, COCH***H***), 0.98 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.43 (quat. ***C***=O), 159.57 (quat. Ar-***C***), 141.70 (quat. Ar-***C***), 129.27 (+, Ar-***C***H), 121.66 (+, Ar-***C***H), 114.94 (+, Ar-***C***H), 111.40 (+, Ar-***C***H), 55.15 (+, (m-O***C***H_3)Ar), 42.94 (-, CO***C***H_2), 40.74 (-, ***C***H_2-Ar), 32.00 (+, ***C***HCH_3), 19.63 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 208 ($M^{+\bullet}$, 36); anal. ($C_{12}H_{16}O_3 \cdot C_{12}H_{23}N \cdot 0.5\ H_2O$) C, H, N. $C_{12}H_{16}O_3$ (208.25)

3-(4-Methoxybenzyl)butanoic acid (3.36c)[79]

The title compound was prepared from **3.36b** (1.06 g, 4.5 mmol) and 10 ml 20 % NaOH/aq according to the general procedure yielding **3.36c** (0.89 g, 95 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.08 (m, 2H, Ar-***H***), 6.83 (m, 2H, Ar-***H***), 3.79 (s, 3H, (*p*-OC***H***$_3$)Ar), 2.58 (dd, 1H, ^3J = 6.7 Hz, ^3J = 13.5 Hz, C***H***H-Ar), 2.48 (dd, 1H, ^3J = 7.0 Hz, ^3J = 13.5 Hz, CH***H***-Ar) 2.38-2.15 (m, 3H, COC***H***$_2$, C***H***CH_3), 0.97 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.53 (quat. ***C***=O), 158.00 (quat. Ar-***C***), 132.12 (quat. Ar-***C***), 130.13 2 (+, 2 Ar-***C***H), 113.72 (+, 2 Ar-***C***H), 55.24 (+, (p-O***C***H_3)Ar), 42.02 (-, CO***C***H_2), 40.69 (-, ***C***H_2-Ar), 32.26 (+, ***C***HCH_3), 19.55 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 208 ($M^{+\bullet}$, 16); anal. ($C_{12}H_{16}O_3 \cdot C_{12}H_{23}N \cdot 0.75H_2O$) C, H, N. $C_{12}H_{16}O_3$ (208.25)

3-(3-Ethylbenzyl)butanoic acid (3.37c)[80]

The title compound was prepared from **3.37b** (1.77 g, 7.56 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.37c** (1.45 g, 93 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 11.21 (br s, 1H, COO***H***), 7.10 (m, 4H, Ar-***H***), 2.62 (m, 3H, C***H***H-Ar, (*p*-C***H***$_2$CH$_3$)Ar), 2.52 (dd, 1H, ^3J = 7.0 Hz, ^3J = 13.5 Hz, CH***H***-Ar), 2.39 (dd, 1H, ^3J = 5.1 Hz, ^3J = 14.5 Hz, COC***H***H), 2.22 (m, 2H, COCH***H***, C***H***CH_3), 1.23 (t, 3H, ^3J = 7.6 Hz, (*p*-CH_2C***H***$_3$)Ar), 0.99 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.42 (quat. ***C***=O), 141.99 (quat. Ar-***C***), 137.19 (quat. Ar-***C***), 129.14

(+, 2 Ar-***C***H), 127.77 (+, 2 Ar-***C***H), 42.52 (-, CO***C***H_2), 40.73 (-, ***C***H_2-Ar), 32.11 (+, ***C***HCH_3), 28.44 (-, (*p*-***C***H_2CH_3)Ar), 19.61 (+, ***C***H_3), 15.59 (+, (*p*-CH_2***C***H_3)Ar); EI-MS (70 eV) *m/z* (%): 206 ($M^{+\bullet}$, 30); anal. ($C_{13}H_{18}O_2 \cdot C_{12}H_{23}N \cdot 0.3\ H_2O$) calculated C (76.40), H (10.67), N (3.56), found C (76.61), H (10.23), N (3.14). $C_{13}H_{18}O_2$ (206.28)

4-(*tert*-Butoxycarbonylamino)-3-phenylbutanoic acid (3.38c)[81]

The title compound was prepared from **3.38b** (0.28 g, 9.11 mmol), 5 ml 1N NaOH/aq and 5 ml THF. The mixture was stirred by room temperature overnight yielding **3.38c** (0.26 g, 93 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.20 (m, 5H, Ar-***H***), 3.48 (m, 1H, CH_2C***H***), 3.32 (m, 2H, C***H***$_2$NH), 2.74 (dd, 1H, ^{3}J = 5.8 Hz, ^3J = 15.9 Hz, COC***H***H), 2.65 (dd, 1H, ^3J = 6.8 Hz, ^3J = 15.7 Hz, COCH***H***), 1.41 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 278 ([M-H]$^-$, 100). $C_{15}H_{21}NO_4$ (279.33)

6-(*tert*-Butoxycarbonylamino)-3-phenylhexanoic acid (3.39c)

The title compound was prepared from **3.39b** (1.15 g, 3.43 mmol), 20 ml 1N NaOH/aq and 20 ml THF. The mixture was stirred by room temperature overnight yielding **3.39c** (0.55 g, 52 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.29-7.17 (m, 5H, Ar-***H***), 3.06 (m, 3H, CH_2C***H***, C***H***$_2$NH), 2.66 (dd, 1H, ^3J = 4.0 Hz, ^3J = 12.7 Hz, COC***H***H), 2.59 (dd, 1H, ^3J = 4.5 Hz, ^3J = 12.6 Hz, COCH***H***), 1.62 (m, 2H, C***H***$_2$$CH_2CH_2$NH), 1.41 (s, 9H, C(C***H***$_3$)$_3$), 1.33 (m, 2H, CH_2C***H***$_2$$CH_2$NH); ^{13}C-NMR ($CDCl_3$) δ ppm: 176.98 (quat. ***C***=O), 143.41 (quat. Ar-***C***), 128.59 (+, 2 Ar-***C***H), 127.41 (+, 2 Ar-***C***H), 126.68 (+, Ar-***C***H), 79.19 (quat. ***C***$(CH_3)_3$), 41.50 (+, CH_2***C***H), 41.36 (-, ***C***H_2NH), 40.34 (-, CO***C***H_2), 33.18 (***C***$H_2CH_2CH_2$NH), 28.39 (+, 3 C(***C***H$_3$)$_3$), 27.82 (-, CH_2***C***H_2CH_2NH); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 306 ([M-H]$^-$, 100). $C_{17}H_{25}NO_4$ (307.38)

(*E,Z*)-3-(3-Benzyloxyphenyl)but-2-enoic acid (3.53b)

The title compound was prepared from **3.53a** (1.47 g, 4.96 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.53b** (0.8 g, 60 %) as a white solid. mp = 116 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.43-7.08 (m, 9H, Ar-***H***), 6.17 (d, 1H, ^4J = 1.3 Hz, COC***H***), 5.10 (s, 2H, C***H***$_2$-Ar), 2.58 (d, 3H, ^4J = 1.2 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 171.41 (quat. ***C***=O), 158.87 (quat. Ar-***C***), 158.24 (quat. ***C***CH_3), 143.55 (quat. Ar-***C***), 136.72 (quat. Ar-***C***), 129.61 (+, Ar-***C***H), 128.65 (+, 2 Ar-***C***H), 128.10 (+, Ar-***C***H), 127.53 (+, 2 Ar-***C***H), 119.14 (+, Ar-***C***H), 116.45 (+, Ar-***C***H), 115.54 (+, CO***C***H), 113.31 (+, Ar-***C***H), 70.17 (-, ***C***H_2-Ar), 18.36 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 268 ($M^{+\bullet}$, 15); anal ($C_{17}H_{16}O_3 \cdot 0.25\ H_2O$) C, H. $C_{17}H_{16}O_3$ (268.31)

(*E,Z*)-3-[4-(4-Methoxybenzyloxy)phenyl]but-2-enoic acid (3.54b)

The title compound was prepared from **3.54a** (2.1 g, 6.4 mmol) and 20 ml 20 % NaOH/aq according to the general procedure yielding **3.54b** (1.5 g, 79 %) as a white solid. mp = 148-151 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.50-6.94 (m, 8H, Ar-***H***), 6.07 (d, 1H, ^4J = 1.2 Hz, COC***H***), 5.05 (s, 2H, C***H***$_2$-Ar), 3.75 (s, 3H, (*p*-OC***H***$_3$)-Ar), 2.47 (d, 3H, ^4J = 1.0 Hz, C***H***$_3$); EI-MS (70 eV) *m/z* (%): 298 ($M^{+\bullet}$, 1). $C_{18}H_{18}O_4$ (298.12)

Dimethyl 2-benzylmalonate (3.51a)[82]

To a solution of dimethyl malonate (2.64 g, 2.28 ml, 20 mmol) in 50 ml THF/abs was added NaH (60 % dispersion in mineral oil) (0.8 g, 20 mmol) in small amounts and stirred for 30-45 min at room temperature. Subsequently, benzyl bromide (3.42 g, 2.4 ml, 20 mmol) was added and refluxed overnight. After cooling, water was added and the mixture was extracted three times with diethyl ether. After drying over $MgSO_4$, the solvent was removed *in vacuo* and the crude product subjected to flash chromatography (PE) to obtain **3.51a** 2.13 g, 48 %) as a colourless liquid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.28-7.18 (m, 5H, Ar-***H***), 3.69 (m, 7H, C***H***CH_2, C***H***$_3$), 3.22 (d, 2H, ^3J = 7.8 Hz, C***H***$_2$-Ar); EI-MS (70 eV) *m/z* (%): 222 ($M^{+\bullet}$, 53). $C_{12}H_{14}O_4$ (222.24)

Dimethyl 2-benzyl-2-ethylmalonate (3.51b)[83]

To a solution of **3.51a** (2.1 g, 9.4 mmol) in 40 ml THF/abs was added NaH (60 % dispersion in mineral oil) (0.45 g, 11.3 mmol) in small amounts and stirred for 3-4 h at room temperature. Subsequently, ethyl bromide (1.22 g, 0.84 ml, 11.3 mmol) was added and refluxed over night. After cooling, water was added and the mixture extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was subjected to flash chromatography (PE/EtOAc 100/0-95/5 v/v) to obtain **3.51b** (1.33 g, 56 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.62-7.06 (m, 5H, Ar-***H***), 3.72 (s, 6H, C***H***$_3$), 3.24 (s, 2H, C***H***$_2$-Ar), 1.84 (q, 2H, ^3J = 7.5 Hz, C***H***$_2$CH$_3$), 0.92 (t, 3H, ^3J = 7.5 Hz, CH$_2$C***H***$_3$); EI-MS (70 eV) *m/z* (%): 250 ($M^{+\bullet}$, 31). $C_{14}H_{18}O_4$ (250.29)

2-Benzyl-2-ethylmalonic acid (3.51c)[84]

3.51b (1.32 g, 5.27 mmol) was dissolved in 15 ml EtOH, KOH (2.96 g, 52.7 mmol) and 15 ml H_2O were added and the solution was refluxed overnight. After cooling, EtOH was removed in vacuo, H_2O was added and extracted with diethylether. The aqueous layer was acidified with 6 N HCl/aq and extracted three times with EtOAc. The solvent was removed under reduced pressure to obtain **3.51c** (1.17 g, 100 %) as a pale yellow oil, which was used in the next step without further purification. ^{1}H-NMR

($CDCl_3$) δ ppm: 9.51 (br s, 2H, COO***H***), 7.26-7.18 (m, 5H, Ar-***H***), 3.29 (s, 1H, C***H***$_2$-Ar), 2.04 (q, 2H, ^3J = 7.4 Hz, C***H***$_2$$CH_3$), 1.26 (t, 3H, ^3J = 7.1 Hz, CH_2C***H***$_3$). $C_{12}H_{14}O_4$ (222.24)

2-Benzylbutanoic acid (3.51d)[85]

3.51c (1.45 g, 6.5 mmol) was heated to 170-200 °C in a sandbath for 3-4 h. After cooling, the crude product was subjected to flash chromatography (PE) to obtain **3.51d** (0.75 g, 65 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 11.33 (br s, 1H, COO***H***), 7.24 (m, 5H, Ar-***H***), 2.99 (dd, 1H, ^3J = 7.8 Hz, ^3J = 13.6 Hz, C***H***H-Ar), 2.76 (dd, 1H, ^3J = 6.9 Hz, ^3J = 13.7 Hz, CH***H***-Ar), 2.62 (m, 1H, COC***H***), 1.64 (m, 2H, C***H***$_2$$CH_3$), 0.97 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 181.68 (quat. ***C***OOH), 139.16 (quat. Ar-***C***), 128.91 (+, 2 Ar-***C***H), 128.44 (+, 2 Ar-***C***H), 126.40 (+, Ar-***C***H), 48.86 (-, CO***C***H), 37.71 (-, ***C***H_2-Ar), 24.78 (-, ***C***$H_2$$CH_3$), 11.63 (+, ***C***$H_3$); EI-MS (70 eV) *m/z* (%): 178 ($M^{+\bullet}$, 7); anal. ($C_{11}H_{14}O_2$ ·$C_{12}H_{23}N$·0.25 H_2O) C, H, N. $C_{11}H_{14}O_2$ (178.23)

9-(Benzyloxycarbonyl)nonanoic acid (3.52)[86]

Phenylmethanol (0.27 g, 0.25 ml, 2.47 mmol) was dropwise added to a cooled suspension of decanedioic acid (0.5 g, 2.47 mmol) and DMAP (cat.) in 3 ml THF/abs. A solution of DCC (0.61 g, 2.96 mmol),in 3 ml THF/abs was dropwise added to this mixture and stirred over the weekend at ambient temperature. Subsequently, DCU was filtered and the solvent removed under reduced pressure. The crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v)) to obtain **3.52** (0.34 g, 47 %) as a colourless solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 10.88 (s, 1H, COO***H***), 7.34 (m, 5H, Ar-***H***), 5.11 (s, 2H, C***H***$_2$-Ar), 2.34 (m, 4H, COC***H***$_2$), 1.61 (m, 4H, $COCH_2$C***H***$_2$), 1.29 (s, 8H, C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.80 (quat. ***C***OOH), 173.72 (quat. ***C***=O), 136.12 (quat. Ar-***C***), 128.55 (+, 2 Ar-***C***H), 128.18 (+, 3 Ar-***C***H), 66.11 (-, ***C***H_2-Ar), 34.30 (-, COOH-***C***H_2), 34.04 (-, ***C***H_2CO), 29.02 (-, 3 ***C***H_2), 28.96 (-, ***C***H_2), 24.90 (-, ***C***$H_2$$CH_2$CO), 24.64 (-, COOH-$CH_2$***C***$H_2$); EI-MS (70 eV) *m/z* (%): 292 ($M^{+\bullet}$, 3). $C_{17}H_{24}O_4$ (292.37)

3.5.6. Preparation of cyclohexylalkanoic acids 3.40-3.50

General procedure

To a solution of the pertinent phenylalkanoic acid in AcOH was added a catalytic amount of Rh/C or Rh/Al_2O_3 and hydrogenated at 6-8 bar for 48 h. The catalyst was removed by filtration over Celite and washed with AcOH. The solvent was removed *in*

vacuo and the crude product purified by flash chromatography (PE/EtOAc 90/10 v/v). For combustion analysis data, a small amount of acid was precipitated as salt with dicyclohexylamine.

(*R*)-3-Cyclohexylbutanoic acid (3.40)[87]

The title compound was prepared from (*R*)-3-phenylbutanoic acid (0.5 g, 3 mmol) by hydrogenation over Rh/C (0.25 g) in 15 ml AcOH according to the general procedure yielding **3.40** as a colourless oil (0.49 g, 96 %). ^{1}H-NMR ($CDCl_3$): δ (ppm): 2.43 (dd, 1H, ^3J = 5.10 Hz, ^2J = 14.9 Hz, COCH***H***), 2.11 (dd, 1H, ^3J = 9.3 Hz, ^2J = 14.9 Hz, COC***H***H), 1.87 (m, 1H, CH_3C***H***), 1.74-1.64 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.01-1.20 (m, 6H, cHex-C***H***$_2$), 0.93 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 180.30 (quat. ***C***=O), 42.54 (+, cHex-C***H***), 39.00 (-, CO***C***H_2), 35.19 (+, CH_2C***H***), 30.25 (-, cHex-***C***H_2), 28.92 (-, cHex-***C***H_2), 26.66 (-, cHex-***C***H_2), 26.62 (-, cHex-***C***H_2), 26.56 (-, cHex-***C***H_2), 16.48 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 170 ($M^{+\bullet}$, 1); anal. ($C_{10}H_{18}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{10}H_{18}O_2$ (170.11)

(*S*)-3-Cyclohexylbutanoic acid (3.41)[88]

The title compound was prepared from (*S*)-3-phenylbutanoic acid (0.51 g, 3.1 mmol) by hydrogenation over Rh/C (0.25 g) in 15 ml AcOH according to the general procedure yielding **3.41** as a colourless oil (0.49 g, 93 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.43 (dd, 1H, ^3J = 5.0 Hz, ^2J = 14.9 Hz, COCH***H***), 2.11 (dd, 1H, ^3J = 9.3 Hz, ^2J = 14.9 Hz, COC***H***H), 1.86 (m, 1H, CH_3C***H***), 1.69 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.24-1.01 (m, 6H, cHex-C***H***$_2$), 0.93 (d, 3H, ^3J = 6.8 Hz, ***C***H_3); ^{13}C-NMR ($CDCl_3$) δ ppm: 180.64 (quat. ***C***=O), 42.54 (+,cHex-C***H***), 39.06 (-, CO***C***H_2), 35.19 (+,CH_2C***H***), 30.26 (-, cHex-***C***H_2), 28.93 (-, cHex-***C***H_2), 26.67 (-, cHex-***C***H_2), 26.63 (-, cHex-***C***H_2), 26.57 (-, cHex-***C***H_2), 16.48 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 170 ($M^{+\bullet}$, 1); anal. ($C_{10}H_{18}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{10}H_{18}O_2$ (170.11)

2-(Cyclohexylmethyl)propanoic acid (3.42)[89]

The title compound was prepared from 2-benzylpropanoic acid (0.5 g, 3.04 mmol) by hydrogenation over Rh/C (0.25 g) in 25 ml AcOH according to the general procedure yielding **3.42** as a yellow oil (0.51 g, 98 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.57 (m, 1H, C***H***CH_3), 1.64 (m, 6H, cHex-C***H***$_2$), 1.29 (m, 5H, cHex-C***H***$_2$, cHex-C***H***, C***H***$_2$-cHex), 1.16 (d 3H, ^3J = 6.9 Hz, C***H***$_3$), 0.87 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 183.70 (quat. ***C***=O), 41.26 (-, ***C***H_2-cHex), 36.66 (+, ***C***HCH_3), 35.25 (+, cHex-***C***H), 33.30 (-, cHex-***C***H_2), 33.08 (-, cHex-***C***H_2), 26.54 (-, cHex-***C***H_2), 26.22 (-, cHex-***C***H_2), 26.19 (-,

cHex-***C***H_2), 17.37 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 170 ($M^{+\bullet}$, 1); anal. ($C_{10}H_{18}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{10}H_{18}O_2$ (170.25)

3-Cyclohexylpentanoic acid (3.43)[90]

The title compound was prepared from **3.21c** (1 g, 5.61 mmol) by hydrogenation over Rh/C (0.46 g) in 30 ml AcOH according to the general procedure yielding **3.43** as a colourless oil (0.8 g, 77 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.37 (dd, 1H, ^3J = 6.1 Hz, ^2J = 15.4 Hz, COC***H***H), 2.19 (dd, 1H, ^3J = 7.6 Hz, ^2J = 15.4 Hz, COCH***H***), 1.68 (m, 6H, CH_2C***H***, cHex-C***H***, cHex-C***H***$_2$), 1.41-1.02 (m, 8H, cHex-C***H***$_2$, C***H***$_2$$CH_3$), 0.89 (t, 3H, ^3J = 7.4 Hz, CH_2C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 180.59 (quat. ***C***=O), 41.86 (+, cHex-***C***H), 40.03 (+, CH_2***C***H), 35.87 (-, CO***C***H_2), 30.07 (-, cHex-***C***H_2), 29.14 (-, cHex-***C***H_2), 26.76 (-, cHex-***C***H_2), 26.72 (-, cHex-***C***H_2), 26.70 (-, cHex-***C***H_2), 23.80 (-, ***C***H_2CH_3), 11.65 (+, CH_2***C***H_3); EI-MS (70 eV) *m/z* (%): 184 ($M^{+\bullet}$, 3); anal. ($C_{11}H_{20}O_2 \cdot C_{12}H_{23}N \cdot 0.1$ H_2O) C, H, N. $C_{11}H_{20}O_2$ (184.27)

2-(Cyclohexylmethyl)butanoic acid (3.44)[89]

The title compound was prepared from **3.51d** (0.39 g, 2.2 mmol) by hydrogenation over Rh/Al_2O_3 (0.15 g) in 15 ml AcOH according to the general procedure yielding **3.44** as a colourless oil (0.3 g, 74 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.40 (m, 1H, COC***H***), 1.60 (m, 9H, cHex-C***H***$_2$, C***H***$_2$-cHex, C***H***$_2$$CH_3$, cHex-C***H***), 1.20 (m, 4H, cHex-C***H***$_2$), 0.94 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$), 0.83 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 183.03 (quat. ***C***=O), 44.35 (+, CO***C***H), 39.57 (-, ***C***H_2-cHex), 35.57 (+, cHex-***C***H), 33.58 (-, cHex-***C***H_2), 32.98 (-, cHex-***C***H_2), 26.54 (-, cHex-***C***H_2), 26.22 (-, cHex-***C***H_2), 26.18 (-, cHex-***C***H_2), 25.77 (-, ***C***H_2CH_3), 11.75 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 184 ($M^{+\bullet}$, 4); anal. ($C_{11}H_{20}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{11}H_{20}O_2$ (184.27)

3-Cyclohexyl-4-methylpentanoic acid (3.45)[73]

The title compound was prepared from **3.22c** (0.51 g, 2.65 mmol) by hydrogenation over Rh/C (0.26 g) in 15 ml AcOH according to the general procedure yielding **3.45** as a pale yellow oil (0.42 g, 80 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.24 (d, 2H, ^3J = 5.8 Hz, COC***H***$_2$), 1.83 (m, 1H, C***H***$(CH_3)_2$), 1.67 (m, 6H, CH_2C***H***, cHex-C***H***, cHex-C***H***$_2$), 1.35-1.02 (m, 6H, cHex-C***H***$_2$), 0.91 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$), 0.82 (d, 3H, ^3J = 6.8Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 181.37 (quat. ***C***=O), 45.90 (+, cHex-***C***H), 39.99 (+,CH_2***C***H), 33.20 (-, CO***C***H_2), 31.60 (-, cHex-***C***H_2), 29.47 (-, cHex-***C***H_2), 28.63 (+,***C***$H(CH_3)_2$), 26.78 (-, cHex-***C***H_2), 26.68 (-, cHex-***C***H_2), 26.62 (-, cHex-***C***H_2), 21.37 (+, ***C***H_3), 18.49 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 198 ($M^{+\bullet}$, 5); anal. ($C_{12}H_{22}O_2$ $\cdot C_{12}H_{23}N$) C, H, N. $C_{12}H_{22}O_2$ (198.30)

3-Cyclohexyl-5-methylhexanoic acid (3.46)[91]

The title compound was prepared from **3.23c** (1 g, 4.85 mmol) by hydrogenation over Rh/C (0.47 g) in 30 ml AcOH according to the general procedure yielding **3.46** as a pale yellow oil (0.85 g, 83 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 10.67 (br s, 1H, COO***H***), 2.34 (dd, 1H, ^3J = 6.6 Hz, ^2J = 15.3 Hz, COC***H***H), 2.17 (dd, 1H, ^3J = 6.9 Hz, ^2J = 15.3 Hz, COCH***H***), 1.86 (m, 1H, C***H***$(CH_3)_2$), 1.62 (m, 6H, CH_2C***H***, cHex-C***H***, cHex-C***H***$_2$), 1.34-1.01 (m, 8H, C***H***$_2$CH$(CH_3)_2$, cHex-C***H***$_2$), 0.89 (d, 3H, ^3J = 3.5 Hz, C***H***$_3$), 0.87 (d, 3H, ^3J = 3.4 Hz, C***H***$_3$); ^{13}C-NMR ($CDCl_3$) δ ppm: 180.86 (quat. ***C***=O), 40.70 (-, ***C***H_2CH$(CH_3)_2$, 40.61 (+, cHex-***C***H), 37.84 (+, CH_2***C***H), 36.53 (-, CO***C***H_2), 29.81 (-, cHex-***C***H_2), 28.91 (-, cHex-***C***H_2), 26.82 (-, cHex-***C***H_2), 26.75 (-, 2 cHex-***C***H_2), 25.46 (+, ***C***H$(CH_3)_2$), 23.01 (+, ***C***H_3), 22.51 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 212 ($M^{+\bullet}$, 2); anal. ($C_{13}H_{24}O_2 \cdot C_{12}H_{23}N \cdot 0.25H_2O$) calcd. C (75.42), H (12.02), N (3.52), found C (75.71), H, (11.20), N (3.44). $C_{13}H_{24}O_2$ (212.33)

3-(Cyclohexylmethyl)butanoic acid (3.47)[87]

The title compound was prepared from **3.28c** (0.51 g, 2.86 mmol) by hydrogenation over Rh/Al_2O_3 (0.29 g) in 15 ml AcOH according to the general procedure yielding **3.47** as a colourless oil (0.27 g, 51 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.31 (dd, 1H, ^3J = 6.8 Hz, ^2J = 11.5 Hz, COC***H***H), 2.11 (dd, 1H, ^3J = 5.7 Hz, ^2J = 16.1 Hz, COCH***H***), 1.91 (m, 1H, CH_2C***H***), 1.66 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.28-1.07 (m, 6H, cHex-C***H***$_2$, CHC***H***$_2$), 0.95 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$), 0.86 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.62 (quat. ***C***=O), 44.67 (-, CO***C***H_2), 41.92 (-, CH***C***H_2), 34.82 (+, cHex-C***H***), 33.89 (-, cHex-***C***H_2), 32.98 (-, cHex-***C***H_2), 28.09 (-, cHex-***C***H_2), 27.11 (+, ***C***HCH_3), 26.39 (-, cHex-***C***H_2), 26.30 (-, cHex-***C***H_2), 19.91 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 184 ($M^{+\bullet}$, 7); anal. ($C_{11}H_{20}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{11}H_{20}O_2$ (184.27)

5-Cyclohexyl-3-methylpentanoic acid (3.48)[92]

The title compound was prepared from **3.29c** (0.5 g, 2.6 mmol) by hydrogenation over Rh/Al_2O_3 (0.25 g) in 20 ml AcOH according to the general procedure yielding **3.48** as a pale yellow oil (0.3 g, 58 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 8.85 (br s, 1H, COO***H***), 2.36 (dd, 1H, ^3J = 5.9 Hz, ^3J = 14.9 Hz, COC***H***H), 2.14 (dd, 1H, ^3J = 7.1 Hz, ^3J = 13.9 Hz, COCH***H***), 1.92 (m, 1H, CH_2C***H***), 1.65 (m, 5H, cHex-C***H***, cHex-CH_2), 1.33-1.16 (m, 8H, CHC***H***$_2$CH$_2$, CHCH$_2$C***H***$_2$, cHex-C***H***$_2$) 0.96 (d, 3H, ^3J = 6.6 Hz, ***C***H_3), 0.88 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 180.10 (quat. ***C***=O), 44.67 (-, CO***C***H_2), 41.99 (-, CH***C***H_2), 34.82 (+, cHex-***C***H), 33.89 (-, CHCH$_2$***C***H_2), 32.98 (-, cHex-***C***H_2), 29.72 (-, cHex-***C***H_2), 27.10 (+, ***C***HCH_3), 26.66 (-, cHex-***C***H_2), 26.39 (-,

cHex-***C***H_2), 26.30 (-, cHex-***C***H_2), 19.91 (+, ***C***H_3); ES-MS (AcN/H_2O + TFA)) *m/z* (%): 197 ([M-H]$^-$, 100); anal. ($C_{12}H_{22}O_2 \cdot C_{12}H_{23}N \cdot 0.1\ H_2O$) C, H, N. $C_{12}H_{22}O_2$ (198.30)

6-Cyclohexyl-3-methylhexanoic acid (3.49)[93]

The title compound was prepared from **3.30c** (0.5 g, 2.42 mmol) by hydrogenation over Rh/Al_2O_3 (0.25 g) in 15 ml AcOH according to the general procedure yielding **3.49** as a colourless oil (0.32 g, 62 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 9.96 (br s, 1H, COO***H***), 2.35 (dd, 1H, ^3J = 5.9 Hz, ^2J = 14.9 Hz, COC***H***H), 2.14 (dd, 1H, ^3J = 8.2 Hz, ^2J = 14.9 Hz, COCH***H***), 1.95 (m, 1H, CH_2C***H***), 1.65 (m, 5H, cHex-C***H***, cHex-CH_2), 1.31-1.15 (m, 10H, CHC***H***$_2$, $CHCH_2$C***H***$_2$, $CHCH_2CH_2$C***H***$_2$, cHex-C***H***$_2$), 0.96 (d, 3H, ^3J = 6.6 Hz, ***C***H_3), 0.86 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 179.91 (quat. ***C***=O), 41.64 (-, CO***C***H_2), 37.62 (+, cHex-C***H***), 37.52 (-, CH***C***H_2), 36.95 (-, $CHCH_2CH_2$***C***H_2), 33.46 (-, cHex-***C***H_2), 33.37 (-, cHex-***C***H_2), 30.17 (+, ***C***HCH_3), 26.75 (-, cHex-***C***H_2), 26.44 (-, cHex-***C***H_2), 24.10 (-, $CHCH_2$***C***H_2), 19.69 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 212 ($M^{+\bullet}$, 20); anal. ($C_{13}H_{24}O_2 \cdot C_{12}H_{23}N \cdot 0.5\ H_2O$) C, H, N. $C_{13}H_{24}O_2$ (212.32)

3-(Cyclohexylmethyl)pentanoic acid (3.50)

The title compound was prepared from 3-benzylpentanoic acid[21] (0.55 g, 3.04 mmol) by hydrogenation over Rh/C (0.13 g) in 20 ml AcOH according to the general procedure yielding **3.50** as a colourless oil (0.48 g, 85 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.26 (d, 2H, ^3J = 6.8 Hz, COC***H***$_2$), 1.91 (m, 1H, C***H***CH_2CH_3), 1.67 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.23 (m, 8H, CHC***H***$_2CH_3$, cHex-C***H***$_2$), 0.87 (m, 5H, C***H***$_3$, C***H***$_2$-cHex); ^{13}C-NMR ($CDCl_3$) δ ppm: 180.12 (quat. ***C***=O), 41.56 (-, CO***C***H_2), 38.85 (-, ***C***H_2-cHex), 34.82 (+, cHex-***C***H), 33.56 (-, cHex-***C***H_2), 33.53 (-, cHex-***C***H_2), 33.19 (+, ***C***HCH_2CH_3), 26.67 (-, cHex-***C***H_2), 26.53 (-, CH***C***H_2CH_3), 26.37 (-, 2 cHex-***C***H_2), 10.59 (+, ***C***H_3); EI-MS (70 eV) *m/z* (%): 198 ($M^{+\bullet}$, 5); anal. ($C_{12}H_{22}O_2 \cdot C_{12}H_{23}N$) C, H, N. $C_{12}H_{22}O_2$ (198.30)

3.5.7. Preparation of the imidazolylpropylguanidine building blocks 3.7 and 3.10

(*E*)-Methyl 3-(1*H*-imidazol-4-yl)propenoate (3.1)[12]

To a solution of urocanic acid (14 g, 101.3 mmol) and anhydrous Na_2SO_4 (2 g) in 150 ml MeOH/abs was added H_2SO_4 (conc.) (8 ml), and the mixture was heated to reflux for 30 h. The solvent was removed under reduced pressure after cooling to room temperature. The residue was dissolved in a small amount of water, neutralized with

saturated $NaHCO_3$/aq and extracted three times with EtOAc. After drying over $MgSO_4$, the solvent was evaporated *in vacuo* yielding **3.1** (14.5 g, 94 %) as a white solid. ^{1}H-NMR (CD_3OD) δ ppm: 6.33 (s, 1H, Im-2-***H***), 6.18 (d, 1H, ^3J = 15.9 Hz, Im-4-C***H***CH), 5.93 (s, 1H, Im-5-***H***), 5.02 (d, 1H, ^3J = 15.8 Hz, Im-4-CHC***H***), 2.37 (s, 3H. OC***H***$_3$); EI-MS (70 eV) *m/z* (%): 152 ($M^{+\bullet}$, 50). $C_7H_8N_2O_2$ (152.15)

Methyl 3-(1*H*-imidazol-4-yl)propanoate (3.2)[94]

To a solution of **1** (9.9 g, 65.1 mmol) in 120 ml MeOH was added Pd/C (10 %) (1 g) under stirring. The mixture was hydrogenated at 5 bar for 24 h, subsequently filtered through a small pad of Celite and evaporated *in vacuo* to obtain **3.2** (10 g, 100 %) as a white solid. ^{1}H-NMR (DMSO-d_6) δ ppm: 7.51 (d, 1H, ^4J = 1.0 Hz, Im-2-***H***), 6.75 (d, 1H, ^4J = 0.8 Hz, Im-5-***H***), 3.59 (s, 3H, OC***H***$_3$), 2.75 (t, 2H, ^3J = 7.5 Hz, Im-4-C***H***$_2$), 2.60 (t, 2H, ^3J = 7.3 Hz, Im-4-CH_2C***H***$_2$); EI-MS (70 eV) *m/z* (%): 154 ($M^{+\bullet}$, 35). $C_7H_{10}N_2O_2$ (154.17)

Methyl 3-(1-trityl-1*H*-imidazol-4-yl)propanoate (3.3)[95]

Trityl chloride (15 g, 53.8 mmol) was dissolved in 150 ml MeCN and added dropwise to a suspension of **3.2** (9.2 g, 48.3 mmol) and NEt_3 (19 ml, 136 mmol) in 140 ml MeCN under external ice-cooling. After the addition, the mixture was allowed to warm to room temperature and stirring was continued for 12 h.The solvent was removed *in vauo*, the resulting solid suspended in H_2O and stirred for 1 h. The solid was filtrated and recrystallized from EtOH yielding **3.3** (15.4 g, 80 %) as a white solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 8.07 (d, 1H, ^4J = 1.6 Hz, Im-2-***H***), 7.41-7.07 (m, 15H, CPh_3), 6.77 (d, 1H, ^4J = 1.5 Hz, Im-5-***H***), 3.62 (s, 3H, OC***H***$_3$), 3.08 (t, 2H, ^3J = 6.9 Hz, Im-4-C***H***$_2$), 2.87 (t, 2H, ^3J = 6.9 Hz, Im-4-CH_2C***H***$_2$); EI-MS (70 eV) *m/z* (%): 396 ($M^{+\bullet}$, 10). $C_{26}H_{24}N_2O_2$ (396.24)

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol (3.4)[95]

3.3 (10.06 g, 25.4 mmol) was added under argon atmosphere and external ice-cooling to a suspension of $LiAlH_4$ (1.9 g, 50 mmol) in 75 ml freshly distilled THF and 25 ml Et_2O/abs. After the addition was complete, the mixture was allowed to warm to room temperature and refluxed for 2 h. To destroy excess $LiAlH_4$, 0.1N NaOH was carefully added. The mixture was extracted several times with DCM, dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by flash chromatography ($CHCl_3$/MeOH 95/5 v/v) to obtain **3.4** (6.89 g, 74 %) as a white solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.76 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 7.38-7.11 (m, 15H, CPh_3), 6.65 (d, 1H, ^4J = 1.5 Hz, Im-5-***H***), 3.71 (t, 2H, ^3J = 5.7 Hz, C***H***$_2$NH), 2.80

(t, 2H, ^3J = 6.9 Hz, Im-4-C***H***$_2$), 1.91 (m, 2H, Im-4-CH$_2$C***H***$_2$); EI-MS (70 eV) *m/z* (%): 369 ($M^{+\bullet}$, 60). $C_{25}H_{24}N_2O$ (368.24)

N,N´-Bis(benzyloxycarbonyl)guanidine (3.5)[11]

Guanidine hydrochloride (7.71 g, 80.7 mmol) and NaOH (16.15 g, 40.4 mmol) were dissolved in 40 ml H_2O and 80 ml DCM and cooled to 0 °C. Benzyloxycarbonyl-chloride (34.5 ml, 24.3 mmol) was added dropwise over 45 min and stirred at 0 °C for 20 h. The mixture was allowed to warm to ambient temperature, extracted three times with DCM and washed with H_2O. After drying over $MgSO_4$, the solvent was removed *in vacuo* and the crude product recrystallized from MeOH to obtain **3.5** (15.9 g, 60 %) as colourless crystals. ^{1}H-NMR (DMSO-d_6) δ ppm: 10.88 (br s, 1H, N***H***), 8.69 (br s, 2H, NH_2), 7.34 (m, 10H, Ar-***H***), 5.11 (s, 4H, C***H***$_2$-Ar); EI-MS (70 eV) *m/z* (%): 328 ($M^{+\bullet}$, 100). $C_{17}H_{17}N_3O_5$ (327.13)

3-Phenylbutan-1-ol (3.8)[96]

To a suspension of $LiAlH_4$ (0.57 g, 15 mmol) in 30 ml THF/abs was slowly added under argon atmosphere a solution of 3-phenylbutyric acid (2 g, 12.2 mmol) in 20 ml THF/abs, and the mixture was stirred for 2 h. After cooling of the flask (icebath), water was added cautiously to decompose excess hydride. Then 20 ml of 10 % H_2SO_4 was added, resulting in a clear solution. The solution was extracted three times with $CHCl_3$, washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash-chromatography ($CHCl_3$/MeOH 95/5 v/v) to obtain **3.8** (0.82 g, 45 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.23 (m, 5H, Ar-***H***), 3.54 (m, 2H, C***H***$_2$OH), 2.88 (m, 1H, CH$_3$C***H***), 1.85 (m, 2H, CH$_3$CHC***H***$_2$), 1.27 (d, 3H, J^3 = 7.0 Hz, CH_3); PI-EIMS *m/z* (%): 150 ($M^{+\bullet}$, 22). $C_{10}H_{14}O$ (150.22)

General procedure for the preparation of N^1,N^2-Bis(benzyloxycarbonyl)-guanidines

A solution of the alcohol (1 eq), di-Cbz protected guanidine (1.8 eq) and PPh_3 (1.5 eq) in THF/abs was cooled to -5 °C under argon atmosphere. DIAD (1.4-1.5 eq) was added dropwise at such a rate that the reaction mixture was completely colourless before addition of the next drop. After the addition was complete, the reaction mixture was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude product subjected to flash chromatography.

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-[3-(1-trityl-1*H*-imidazolyl-4-yl)propyl]guanidine (3.6)

The title compound was prepared from **3.4** (4.4 g, 11.9 mmol), di-Cbz protected guanidine (6.9 g, 21.1 mmol), PPh_3 (4.7 g, 18 mmol) in 100 ml THF/abs and DIAD (3.5 ml, 18 mmol) in 30 ml THF/abs. The crude product was subjected to flash chromatography (PE/EtOAc 60/40 v/v) to obtain **3.6** (1.22 g, 70 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.02 (d, 1H, ^4J = 1.6 Hz, Im-2-***H***), 6.91 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 5.29 (s, 2H, C***H***$_2$-Ar), 5.21 (s, 2H, C***H***$_2$-Ar), 4.30 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 3.02 (t, 2H, ^3J = 8.1 Hz, Im-4-C***H***$_2$), 2.27 (m, 2H, Im-4-CH_2C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 678 (MH^+, 100). $C_{42}H_{39}N_5O_4$ (677.46)

N^1,N^2-Bis(benzyloxycarbonyl)-N^1-(3-phenylbutyl)guanidine (3.9)

The title compound was prepared from **3.8** (0.57 g, 3.8 mmol), di-Cbz protected guanidine (2.2 g, 6.7 mmol), PPh_3 (1.5 g, 5.7 mmol) in 20 ml THF/abs and DIAD (1.1 ml, 5.4 mmol) in THF/abs. The crude product subjected to flash chromatography (PE/EtOAc 90/10 v/v) to obtain **3.9** (1.22 g, 70 %) as a colourless oil. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.36-7.12 (m, 15H, Ar-***H***), 5.16 (s, 2H, C***H***$_2$-Ar), 5.15 (s, 2H, C***H***$_2$-Ar), 3.99-3.77 (m, 2H, C***H***$_2$N), 2.74 (m, 1H, CH_3C***H***), 1.89 (m, 2H, CH_3CHC***H***$_2$), 1.19 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 460 (MH^+, 100). $C_{27}H_{29}N_3O_4$ (459.31)

General procedure for the preparation of the deprotected guanidines

To a solution of **3.6** or **3.9** in THF: MeOH (1:1) was added Pd/C (10 %) and the mixture was hydrogenated at 5 bar overnight. The catalyst was filtered off through a small pad of Celite and washed with MeOH, and the solvent was removed *in vacuo*.

***N*-[3-(1-Trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.7)**

The title compound was prepared from **3.6** (6.59 g, 9.7 mmol) by hydrogenation over Pd/C (10 %) (0.6 g, cat.) in 165 ml THF: MeOH (1:1). **3.7** (3.9 g, 98 %) was obtained as a pale yellow foam-loke solid. ^{1}H-NMR (CD_3OD) δ (ppm): 7.38-7.14 (m, 16H, CPh_3, Im-2-***H***), 6.70 (s, 1H, Im-5-***H***), 3.17 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.58 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 1.86 (m, 2H, Im-4-CH_2C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 410 (MH^+, 100). $C_{26}H_{27}N_5$ (409.32)

3-Phenylbutylguanidine (3.10)

The title compound was prepared from **3.9** (1.13 g, 2.46 mmol) in 40 ml THF: MeOH (1:1) was added Pd/C (10 %) (0.1 g, cat). **3.10** (0.47 g, 100 %) was obtained as a yellow amorphous solid. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.26 (m, 5H, Ar-***H***), 3.03 (t, 2H, J^3

= 7.0 Hz, NHC***H***$_2$), 2.81 (m, 1H, CH_3C***H***), 1.88 (m, 2H, CH_3CHC***H***$_2$), 1.29 (d, 3H, J^3 = 6.9 Hz, CH_3); CI-MS (NH_3) m/z (%): 192 (MH^+, 100). $C_{11}H_{17}N_3$ (191.27)

3.5.8. Preparation of trityl-protected imidazolylpropylguanidines 3.55a-3.89a

General procedure

To a solution of CDI (1.1 eq) in THF/abs (5-7 ml) was added under argon the carboxylic acid (1 eq) and the mixture was stirred for 1 h. In a second flask, **3.7** or **3.10** (1 eq) and NaH (60 % dispersion in mineral oil) (2 eq) in THF/abs (5-7 ml) under argon was heated to 30-35 °C for 30 min and was then allowed to cool to room temperature. The two mixtures were combined and stirred for 2 h at ambient temperature. Subsequently, water was added and extracted three times with EtOAc. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo* and the crude product purified by flash chromatography ($CHCl_3$/MeOH/NH_3 96/3/1 v/v/v).

***N*-(3-Phenylbutyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propanoyl]guanidine (3.55a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.20** (380 mg, 1 mmol), **3.10** (190 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.55a** as a pale yellow foam-like solid (130 mg, 23 %). ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.31-7.10 (m, 16H, CPh_3, Im-2-***H***), 6.58 (d, 1H, J^4= 1.0 Hz, Im-5-***H***), 3.05 (t, 2H, J^3 = 7.1 Hz, Im-4-C***H***$_2$$CH_2$), 2.87 (m, 2H, NHC***H***$_2$), 2.75 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.87 (m, 2H, $NHCH_2$C***H***$_2$), 1.23 (d, 3H, J^3 = 7.0, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 556 (MH^+, 100). $C_{36}H_{37}N_5O$ (555.54)

***N*-(3-Phenylpentanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.56a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.21c** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.56a** as a pale yellow foam-like solid (370 mg, 65 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.24 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.41 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 3.09 (m, 1H, C***H***CH_2CH_3), 2.75-2.53 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 1.85 (m, 2H, Im-4-CH_2C***H***$_2$), 1.66 (m, 2H, CHC***H***$_2CH_3$), 0.78 (t, 3H, ^3J = 7.3 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 570 (MH^+, 65). $C_{37}H_{39}N_5O$ (569.74)

***N*-(2-Benzylbutanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.57a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.51d** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.57a** as a pale yellow foam-like solid (370 mg, 35 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.11 (m, 21H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5- ***H***), 3.35 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 3.02 (m, 1H, C***H***CH_2CH_3), 2.62 (m, 4H, Im-4-C***H***$_2$, C***H***$_2$-Ar), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 1.62-1.44 (m, 2H, C***H***$_2CH_3$), 0.88 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 570 (MH^+, 100). $C_{37}H_{39}N_5O$ (569.73)

***N*-(4-Methyl-3-phenylpentanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.58a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.22c** (190 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.58a** as a pale yellow foam-like solid (310 mg, 53 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.13 (m, 16H, Im-2-***H***, CPh_3), 6.65 (d, 1H, ^4J = 0.9 Hz, Im-5-***H***), 3.12 (m, 2H, C***H***$_2$NH), 2.90 (m, 1H, CH_2C***H***), 2.72 (m, 2H, COC***H***$_2$), 2.50 (t, 2H, ^3J = 7.3Hz, Im-4-C***H***$_2$), 1.83 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***$(CH_3)_2$), 0.93 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$), 0.72 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 584 (MH^+, 100). $C_{38}H_{41}N_5O$ (583.77)

***N*-(5-Methyl-3-phenylhexanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.59a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.23c** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.59a** as a colourless foam-like solid (340 mg, 57 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.12 (m, 21H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.40 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 3.29 (m, 1H, CH_2C***H***), 2.71 (d, 2H, ^3J = 7.5 Hz, COC***H***$_2$), 2.54 (m, 2H, Im-4-C***H***$_2$), 1.85 (m, 2H, Im-4-CH_2C***H***$_2$), 1.60-1.42 (m, 2H, C***H***$_2CH(CH_3)_2$), 1.32 (m, 1H, C***H***$(CH_3)_2$), 0.88 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$), 0.81 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 598 (MH^+, 30). $C_{39}H_{43}N_5O$ (597.79)

***N*-(3,4-Diphenylbutanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.60a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.24c** (240 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2

mmol) in THF/abs according to the general procedure yielding **3.60a** as a pale yellow foam-like solid (310 mg, 49 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.11 (m, 26H, Im-2-***H***, CPh_3, Ar-***H***) 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.57 (m, 1H, C***H***CH_2), 3.43 (m, 2H, C***H***$_2$NH), 2.90 (m, 2H, C***H***$_2$-Ar), 2.53 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 1.85 (m, 2H, Im-4-CH_2C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 632 (MH^+, 80). $C_{42}H_{41}N_5O$ (631.81)

N-[3-Phenyl-4-(4-methylphenyl)butanoyl]-N´-[3-(1-trityl-1H-imidazol-4-yl)propyl]-guanidine (3.61a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.25c** (250 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.61a** as a colourless foam-like solid (170 mg, 26 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.08 (m, 26H, Im-2-***H***, CPh_3, Ar-***H***), 6.54 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 3.44 (m, 1H, C***H***CH_2), 3.33 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.86 (m, 2H, C***H***$_2$-Ar), 2.69 (m, 2H, COC***H***$_2$), 2.52 (m, 2H, Im-4-C***H***$_2$), 2.24 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.83 (m, 2H, Im-4-CH_2C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 646 (MH^+, 100). $C_{43}H_{43}N_5O$ (645.83)

N-(3-Cyclohexyl-4-phenylbutanoyl)-N´-[3-(1-trityl-1H-imidazol-4-yl)propyl]-guanidine (3.62a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.26c** (250 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.62a** as a colourless foam-like solid (210 mg, 33 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.11 (m, 21H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.37 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$NH), 2.68 (m, 2H, Im-4-C***H***$_2$), 2.54 (m, 2H, C***H***$_2$-Ar), 2.33-2.16 (m, 3H, C***H***CH_2-Ar, COC***H***$_2$), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 1.72-1.60 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.29-1.08 (m, 6H, cHex-C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 638 (MH^+, 100). $C_{42}H_{47}N_5O$ (637.85)

N-[3-(4-Methylphenyl)propanoyl]-N´-[3-(1-trityl-1H-imidazol-4-yl)propyl]-guanidine (3.63a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), 3-(4-methylphenyl)propanoic acid (160 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.63a** as a colourless foam-like solid (240 mg, 43 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.10 (m, 21H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-

5-***H***), 3.35 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.92 (m, 2H, COCH$_2$C***H***$_2$), 2.57 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$CH$_2$), 2.29 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.87 (m, 2H, Im-4-CH$_2$C***H***$_2$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 556.4 (MH$^+$, 100). $C_{36}H_{37}N_5O$ (555.71)

N-[3-(4-Methylphenyl)butanoyl]-N´-[3-(1-trityl-1H-imidazol-4-yl)propyl]guanidine (3.64a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.27c** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.64a** as a colourless foam-like solid (250 mg, 44 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.11 (m, 21H, Im-2-***H***, CPh$_3$, Ar-***H***), 6.56 (d, 1H, ^4J = 1.0 Hz, Im-5-***H***), 3.30 (m, 3H, C***H***$_2$NH, C***H***CH$_3$), 2.54 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 2.29 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.86 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.25 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 570 (MH$^+$, 100). $C_{37}H_{39}N_5O$ (569.74)

N-(3-Methyl-4-phenylbutanoyl)-N´-[3-(1-trityl-1H-imidazol-4-yl)propyl]guanidine (3.65a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.28c** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.65a** as a colourless foam-like solid (370 mg, 65 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.11 (m, 21H, Im-2-***H***, CPh$_3$, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.41 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.71 (dd, 1H, ^3J = 5.3 Hz, ^2J = 12.8 Hz, C***H***H-Ar), 2.57 (m, 2H, Im-4-C***H***$_2$), 2.41-2.26 (m, 4H, CH***H***-Ar, COC***H***$_2$, C***H***CH$_3$), 1.88 (m, 2H, Im-4-CH$_2$C***H***$_2$), 0.91 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 570 (MH$^+$, 100). $C_{37}H_{39}N_5O$ (569.74)

N-(3-Methyl-5-phenylpentanoyl)-N´-[3-(1-trityl-1H-imidazol-4-yl)propyl]guanidine (3.66a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.29c** (220 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.66a** as a colourless foam-like solid (340 mg, 58 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.53-7.08 (m, 21H, Im-2-***H***, CPh$_3$, Ar-***H***), 6.58 (s, 1H, Im-5- ***H***), 3.47 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.69-2.50 (m, 5H, Im-4-C***H***$_2$, C***H***$_2$-Ar, COCH***H***), 2.30 (dd, 1H, ^3J = 7.9 Hz, ^3J = 15.1 Hz, COC***H***H), 1.91 (m, 3H, Im-4-CH$_2$C***H***$_2$, C***H***CH$_3$), 1.72-1.53 (m, 2H, C***H***$_2$CH$_2$-Ar), 0.97 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 584 (MH$^+$, 100). $C_{38}H_{41}N_5O$ (583.76)

***N*-(3-Methyl-6-phenylhexanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.67a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.30c** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.67a** as a colourless foam-like solid (320 mg, 54 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.11 (m, 21H, Im-2-***H***, CPh_3, Ar-***H***), 6.58 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 2.58 (m, 4H, C***H***$_2$NH, Im-4-C***H***$_2$), 2.47 (dd, 1H, ^3J = 6.0 Hz, ^2J = 14.7 Hz, COC***H***H), 2.27 (dd, 1H, ^3J = 8.1 Hz, ^2J = 14.7 Hz, COCH***H***), 2.06 (m, 2H, C***H***$_2$-Ar), 1.88 (m, 2H, Im-4-CH_2C***H***$_2$), 1.69-1.56 (m, 3H, C***H***$_2$$CH_2$-Ar, C***H***$CH_3$), 1.42 (m, 2H, C***H***$_2$$CH_2$$CH_2$-Ar), 0.95 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 598 (MH^+, 100). $C_{39}H_{43}N_5O$ (597.79)

***N*-[3-Methyl-4-(3-methylphenyl)butanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.68a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.31c** (190 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.68a** as a colourless oil (310 mg, 53 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-6.97 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5- ***H***), 3.40 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.62 (m, 3H, Im-4-C***H***$_2$, C***H***H-Ar), 2.41-2.29 (m, 7H, CH***H***-Ar, C***H***CH_3, COC***H***$_2$, (*m*-C***H***$_3$)-Ar), 1.88 (m, 2H, Im-4-CH_2C***H***$_2$), 0.90 (d, 3H, ^3J = 5.9 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 584 (MH^+, 100). $C_{38}H_{41}N_5O$ (583.76)

***N*-[3-Methyl-4-(4-methylphenyl)butanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.69a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.32c** (190 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.69a** as a yellow oil (480 mg, 82 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.06 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5- ***H***), 3.40 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.62 (m, 3H, Im-4-C***H***$_2$, C***H***H-Ar), 2.35-2.52 (m, 7H, CH***H***-Ar, C***H***CH_3, COC***H***$_2$, (*p*-C***H***$_3$)-Ar), 1.88 (m, 2H, Im-4-CH_2C***H***$_2$), 0.90 (d, 3H, ^3J = 6.0 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 584 (MH^+, 100). $C_{38}H_{41}N_5O$ (583.76)

***N*-[4-(3-Fluorophenyl)-3-methylbutanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.70a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.33c** (200 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.70a** as a colourless foam-like solid (130 mg, 22 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-6.89 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5- ***H***), 3.36 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.72 (dd, 1H, ^3J = 4.7 Hz, ^2J = 12.7 Hz, C***H***H-Ar), 2.58 (m, 2H, Im-4-C***H***$_2$), 2.33 (m, 3H, COC***H***$_2$, CH***H***-Ar), 2.14 (m, 1H, C***H***CH_3), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 0.89 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 588 (MH^+, 100). $C_{37}H_{38}FN_5O$ (587.73)

***N*-[4-(4-Fluorophenyl)-3-methylbutanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.71a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.34c** (200 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.71a** as a colourless foam-like solid (370 mg, 63 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-6.91 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.0 Hz, Im-5- ***H***), 3.38 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.67 (dd, 1H, ^3J = 5.3 Hz, 2^3J = 13.1 Hz, C***H***H-Ar), 2.57 (m, 2H, Im-4-C***H***$_2$), 2.37-2.22 (m, 4H, CH***H***-Ar, $COCH_2$, C***H***CH_3), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 0.88 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 588 (MH^+, 100). $C_{37}H_{38}FN_5O$ (587.73)

***N*-[4-(3-Methoxyphenyl)-3-methylbutanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)-propyl]guanidine (3.72a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.35c** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.72a** as a colourless foam-like solid (230 mg, 38 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-6.72 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5- ***H***), 3.77 (s, 3H, (*m*-OC***H***$_3$)-Ar), 3.43 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.68-2.56 (m, 3H, Im-4-C***H***$_2$, C***H***H-Ar), 2.37 (m, 4H, CH***H***-Ar, C***H***CH_3, COC***H***$_2$), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 0.91 (d, 3H, ^3J = 5.9 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 600 (MH^+, 100). $C_{38}H_{41}N_5O_2$ (599.76)

_N_-[4-(4-Methoxyphenyl)-3-methylbutanoyl]-_N´_-[3-(1-trityl-1_H_-imidazol-4-yl)-propyl]guanidine (3.73a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.36c** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.73a** as a colourless foam-like solid (310 mg, 52 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-6.79 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.0 Hz, Im-5- ***H***), 3.76 (s, 3H, (*p*-OC***H***$_3$)-Ar), 3.37 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.61 (m, 4H, Im-4-C***H***$_2$, C***H***$_2$Ar), 2.31 (m, 3H, C***H***CH_3, COC***H***$_2$), 1.88 (m, 2H, Im-4-CH_2C***H***$_2$), 0.88 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 600 (MH^+, 100). $C_{38}H_{41}N_5O_2$ (599.76)

_N_-[4-(4-Ethylphenyl)-3-methylbutanoyl)-_N´_-[3-(1-trityl-1_H_-imidazol-4-yl)propyl]-guanidine (3.74a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.37c** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.74a** as a yellow oil (380 mg, 64 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.10 (m, 20H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 0.7 Hz, Im-5- ***H***), 3.42 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$NH), 2.65-2.11 (m, 9H, Im-4-C***H***$_2$, C***H***$_2$-Ar, (*p*-C***H***$_2CH_3$)-Ar, C***H***CH_3, COC***H***$_2$), 1.88 (m, 2H, Im-4-CH_2C***H***$_2$), 1.20 (t, 3H, ^3J = 7.6 Hz, (*p*-CH_2C***H***$_3$)-Ar), 0.91 (d, 3H, ^3J = 5.9 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 598 (MH^+, 100). $C_{39}H_{43}N_5O$ (597.79)

(_R_)-_N_-(3-Cyclohexylbutanoyl)-_N´_-[3-(1-trityl-1_H_-imidazol-4-yl)propyl]guanidine (3.75a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.40** (170 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.75a** as a colourless foam-like solid (260 mg, 46 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.0 Hz, Im-5-***H***), 3.44 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$NH), 2.57 (m, 2H, Im-4-C***H***$_2$), 2.49 (dd, 1H, ^3J = 5.0 Hz, ^2J = 14.4 Hz, COCH***H***), 2.19 (dd, 1H, ^3J = 9.4 Hz, ^2J = 14.2 Hz, COC***H***H), 1.90 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.73-1.62 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.23-1.03 (m, 6H, cHex-C***H***$_2$), 0.88 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 562 (MH^+, 100). $C_{36}H_{43}N_5O$ (561.76)

(*S*)-*N*-(3-Cyclohexylbutanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.76a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.41** (170 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.76a** as a colourless foam-like solid (190 mg, 34 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.37 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.58 (m, 2H, Im-4-C***H***$_2$), 2.41 (dd, 1H, ^3J = 4.8 Hz, ^2J = 14.2 Hz, COCH***H***), 2.07 (dd, 1H, ^3J = 9.6 Hz, ^2J = 14.1 Hz, COC***H***H), 1.89 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.72-1.62 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.21-1.00 (m, 6H, cHex-C***H***$_2$), 0.88 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 562 (MH^+, 100). $C_{36}H_{43}N_5O$ (561.76)

***N*-(3-Cyclohexyl-2-methylpropanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.77a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.42** (170 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.77a** as a colourless foam-like solid (380 mg, 68 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.0 Hz, Im-5- ***H***), 3.37 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.57 (m, 2H, Im-4-C***H***$_2$), 1.88 (m, 2H, Im-4-CH_2C***H***$_2$), 1.73-1.58 (m, 6H, cHex-C***H***$_2$), 1.23-1.08 (m, 8H, cHex-C***H***, cHex-C***H***$_2$, C***H***$_3$), 0.86 (m, 2H, C***H***$_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 562 (MH^+, 100). $C_{36}H_{43}N_5O$ (561.80)

***N*-(3-Cyclohexylpentanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.78a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.43** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.78a** as a colourless foam-like solid (260 mg, 45 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.11 (m, 16H, Im-2-***H***, CPh_3), 6.58 (s, 1H, Im-5-***H***), 3.51 (t, 2H, ^3J = 6.3 Hz, C***H***$_2$NH), 2.54 (m, 2H, Im-4-C***H***$_2$), 2.38 (dd, 1H, ^3J = 7.7 Hz, ^2J = 15.5 Hz, COC***H***H), 2.27 (dd, 1H, ^3J = 6.0 Hz, ^2J = 14.6 Hz, COCH***H***), 1.91 (m, 2H, Im-4-CH_2C***H***$_2$), 1.78-1.62 (m, 6H, C***H***CH_2CH_3, cHex-C***H***, cHex-C***H***$_2$), 1.40-1.03 (m, 8H, CHC***H***$_2CH_3$, cHex-C***H***$_2$), 0.88 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 576 (MH^+, 100). $C_{37}H_{45}N_5O$ (575.79)

***N*-[2-(Cyclohexylmethyl)butanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.79a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.44** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.79a** as a colourless foam-like solid (310 mg, 54 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.11 (m, 16H, Im-2-***H***, CPh_3), 6.55 (d, 1H, ^4J = 0.9 Hz, Im-5- ***H***), 3.35 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.57 (m, 2H, Im-4-C***H***$_2$), 2.30 (m, 1H, C***H***CH_2CH_3), 1.87 (m, 2H, Im-4-CH_2C***H***$_2$), 1.67-1.48 (m, 8H, C***H***$_2CH_3$, cHex-C***H***$_2$), 1.24-1.14 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 0.87 (m, 5H, C***H***$_3$, C***H***$_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 562 (MH^+, 100). $C_{36}H_{43}N_5O$ (561.76)

***N*-(3-Cyclohexyl-4-methylpentanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.80a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.45** (200 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.80a** as a pale yellow foam-like solid (180 mg, 31 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.11 (m, 16H, Im-2-***H***, CPh_3), 6.57 (d, 1H, ^4J = 0.7 Hz, Im-5-***H***), 3.49 (m, 2H, C***H***$_2$NH), 2.56 (m, 2H, Im-4-C***H***$_2$), 2.45 (d, 2H, ^3J = 5.6Hz, COC***H***$_2$), 1.86-1.63 (m, 7H, C***H***$(CH_3)_2$, C***H***$CH(CH_3)_2$, cHex-C***H***, cHex-C***H***$_2$), 1.25-1.04 (m, 6H, cHex-C***H***$_2$), 0.89 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$), ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 590 (MH^+, 100), $C_{38}H_{47}N_5O$ (589.81)

***N*-(3-Cyclohexyl-5-methylhexanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.81a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.46** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.81a** as a colourless foam-like solid (340 mg, 56 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.0 Hz, Im-5-***H***), 3.37 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.57 (m, 3H, Im-4-C***H***$_2$, COC***H***H), 2.34 (dd, 1H, ^3J = 7.0 Hz, ^2J= 15.8 Hz, COCH***H***), 2.11 (m, 2H, Im-4-CH_2C***H***$_2$), 1.89 (m, 1H, C***H***$(CH_3)_2$), 1.71-1.58 (m, 6H, C***H***CH_2, cHex-C***H***, cHex-C***H***$_2$), 1.21-1.03 (m, 8H, C***H***$_2CH(CH_3)_3$, cHex-C***H***$_2$), 0.87 (m, 6H, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 604 (MH^+, 100). $C_{39}H_{49}N_5O$ (603.83)

***N*-(4-Cyclohexyl-3-methylbutanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.82a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.47** (180 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.82a** as a orange foam-like solid (270 mg, 46 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 3.39 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.57 (m, 2H, Im-4-C***H***$_2$), 2.33-2.05 (m, 3H, COC***H***$_2$, C***H***CH_3), 1.89 (m, 2H, Im-4-CH_2C***H***$_2$), 1.71-1.58 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.26-1.04 (m, 6H, cHex-C***H***$_2$), 0.90 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$), 0.84 (m, 2H, C***H***$_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 576 (MH^+, 100). $C_{37}H_{45}N_5O$ (575.79)

***N*-(5-Cyclohexyl-3-methylpentanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.83a)**

The title compound was prepared from CDI (140 mg, 0.89 mmol), **3.48** (160 mg, 0.81 mmol), **3.7** (330 mg, 0.81 mmol) and NaH (60 % dispersion in mineral oil) (60 mg, 1.6 mmol) in THF/abs according to the general procedure yielding **3.83a** as a yellow oil (160 mg, 34 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.11 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.42 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.56 (m, 2H, Im-4-C***H***$_2$), 2.42 (dd, 1H, ^3J = 5.9 Hz, ^2J = 14.4 Hz, COC***H***H), 2.26 (dd, 1H, ^3J = 5.5 Hz, ^2J = 13.7 Hz, COCH***H***), 1.89 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.70-1.61 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.32-1.14 (m, 8H, CH_2C***H***$_2$-cHex, cHex-C***H***$_2$), 0.92 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$), 0.84 (m, 2H, C***H***$_2CH_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 590 (MH^+, 100). $C_{38}H_{47}N_5O$ (589.81)

***N*-(6-Cyclohexyl-3-methylhexanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.84a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.49** (210 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.84a** as a yellow oil (260 mg, 43 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.36 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.57 (m, 2H, Im-4-C***H***$_2$), 2.32 (dd, 1H, ^3J = 5.9 Hz, ^2J = 13.8 Hz, COC***H***H), 2.10 (dd, 1H, ^3J = 8.2 Hz, ^2J = 13.9 Hz, COCH***H***), 1.87 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.70-1.61 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.31-1.13 (m, 10H, C***H***$_2CH_2CH_2$-cHex, CH_2CH_2C***H***$_2$-cHex, cHex-C***H***$_2$),

0.91 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$), 0.83 (m, 2H, CH_2C***H***$_2CH_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 604 (MH^+, 100). $C_{39}H_{49}N_5O$ (603.83)

N-[3-(Cyclohexylmethyl)pentanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.85a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.50** (200 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.85a** as a colourless foam-like solid (320 mg, 56 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.12 (m, 16H, Im-2-***H***, CPh_3), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5- ***H***), 3.40 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.58 (m, 2H, Im-4-C***H***$_2$), 2.29 (m, 2H, COC***H***$_2$), 1.92 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_2CH_3), 1.72-1.62 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.34-1.11 (m, 8H, C***H***$_2CH_3$, cHex-C***H***$_2$), 0.86 (C***H***$_3$, C***H***$_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 590 (MH^+, 100). $C_{38}H_{47}N_5O$ (589.81)

N-[3-(3-hydroxyphenyl)butanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]-guanidine (3.86a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.53b** (270 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.86a-Bn** as a violett foam-like solid (270 mg, 41 %). ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 660.4 (MH^+, 100). $C_{43}H_{41}N_5O_2$ (659.33). Subsequently, **3.86a-Bn** was dissolved in 10 ml EtOH, Pd/C (10 %) (cat) was added and hydrogenated at 8 bar for 6 days (TLC-control). The catalyst was filtered over Celite, the solvent removed *in vacuo* and **3.86a** (160 mg) used in the next step without further purification.

N-[3-[4-(4-Methoxybenzyloxy)phenyl]butanoyl]-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)-propyl]guanidine (3.87a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.54b** (300 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure. Subsequently, the resulting foam-like solid was dissolved in 10 ml MeOH, Pd/C (10 %) (cat) was added and hydrogenated at room temperature overnight (TLC-control). The catalyst was filtered over Celite, the solvent removed in vacuo and **3.87a** (310 mg, 45 %) used in the next step without further purification. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 692 (MH^+, 25). $C_{44}H_{45}N_5O_3$ (691.86).

***N*-(4-*tert*-Butoxycarbonylamino-3-phenylbutanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.88a)**

The title compound was prepared from CDI (160 mg, 0.99 mmol), **3.38c** (240 mg, 0.86 mmol), **3.7** (350 mg, 0.86 mmol) and NaH (60 % dispersion in mineral oil) (70 mg, 1.72 mmol) in THF/abs according to the general procedure yielding **3.88a** as a colourless foam-like solid (220 mg, 38 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.67 (s, 1H, Im-2-***H***), 7.34-7.11 (CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 3.49 (m, 1H, C***H***CH_2NH_2), 3.30 (m, 4H, C***H***$_2$NH, C***H***$_2NH_2$), 2.63-2.56 (m, 4H, $COCH_2$, Im-4-C***H***$_2$), 1.85 (m, 2H, Im-4-CH_2C***H***$_2$), 1.38 (s, 9H, C(C***H***$_3)_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 671 (MH^+, 100). $C_{41}H_{46}N_6O_3$ (670.84)

***N*-(6-*tert*-Butoxycarbonylamino-3-phenylhexanoyl)-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (3.89a)**

The title compound was prepared from CDI (180 mg, 1.1 mmol), **3.39c** (310 mg, 1 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **3.89a** as a colourless foam-like solid (260 mg, 40 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.35-7.11 (m, 21H, Im-2-***H***, CPh_3, Ar-***H***), 6.56 (d, 1H, ^4J = 1.1 Hz, Im-5- ***H***), 3.34 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$NH), 3.17-3.05 (m, 3H, C***H***$CH_2CH_2CH_2NH_2$, C***H***$_2NH_2$), 2.56 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 1.86 (m, 2H, Im-4-CH_2C***H***$_2$), 1.73-1.57 (m, 2H, C***H***$_2CH_2CH_2NH_2$), 1.40 (s, 9H, C(C***H***$_3)_3$), 1.29 (m, 2H, CH_2C***H***$_2CH_2NH_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 699 (MH^+, 100). $C_{43}H_{50}N_6O_3$ (698.89)

3.5.9. Preparation of acylguanidines 3.55-3.92

General procedure

To a solution of the protected acylguanidine in CH_2Cl_2/abs were added TFA (20 %) and stirred at ambient temperatur until the detritylation was complete (5-7 h). Subsequently, the solvent was removed *in vacuo* and the residue was purified by preparative HPLC. All compounds were obtained as trifluoroacetic acid salts.

***N*-[3-(1*H*-Imidazol-4-yl)propanoyl]-*N´*-(3-phenylbutyl)guanidine (3.55)**

The title compound was prepared from **3.55a** (110 mg, 0.19 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.55** as a colourless oil (90 mg, 84 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.78 (d, 1H, J^4 = 1.3 Hz, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 7.23 (m, 5H, Ar-***H***), 3.17 (t, 2H, J^3 = 7.1 Hz, Im-4-C***H***$_2CH_2$), 3.06 (t, 2H, J^3 = 7.1 Hz, NHC***H***$_2$), 2.90 (t, 2H, J^3 = 7.0 Hz, Im-4-CH_2C***H***$_2$),

2.82 (m, 1H, C***H***CH_3), 1.96 (m, 2H, $NHCH_2C$***H***$_2$) 1.28 (d, 3H, J^3 = 7.0, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.89 (quart, ***C***=O), 157.44 (quart, ***C***=NH), 149.54 (quat. Ar-***C***), 137.24 (+, Im-2-***C***), 136.38 (quart, Im-4-***C***), 132.28 (+, 2 Ar-***C***H), 130.53 (+, 2 Ar-***C***H), 130.02 (+, Ar-***C***H), 119.92 (+, Im-5-***C***), 43.70 (-, Im-4-CH_2***C***H_2), 41.34 (+, ***C***HCH_3), 39.41 (-, NH***C***H_2), 38.53 (-, $NHCH_2$***C***H_2), 25.46 (+, ***C***H_3), 22.35 (-, Im-4-***C***H_2); HRMS: EI-MS: *m/z* for ($C_{17}H_{23}N_5O$) calcd. 313.1903, found 313.1902; prep. HPLC: MeOH/0.1% TFA/aq (60/40). $C_{17}H_{23}N_5O$ · 2 TFA (541.37)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(3-phenylpentanoyl)guanidine (3.56)**

The title compound was prepared from **3.56a** (270 mg, 0.47 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.56** as a colourless oil (60 mg, 23 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.76 (d, 1H, ^4J = 0.9 Hz, Im-2-***H***), 7.33 (s, 1H, Im-5-***H***), 7.22 (m, 5H, Ar-***H***), 3.31 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$NH), 3.03 (m, 1H, C***H***CH_2CH_3), 2.80 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 1.98 (m, 2H, Im-4-CH_2C***H***$_2$), 1.70 (m, 2H, CHC***H***$_2CH_3$), 0.78 (t, 3H, ^3J = 7.3 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.22 (quart, ***C***=O), 155.19 (quart, ***C***=NH), 144.47 (quat. Ar-***C***), 134.93 (+, Im-2-***C***), 134.28 (quat. Im-4-***C***), 129.57 (+, 2 Ar-***C***H), 128.79 (+, 2 Ar-***C***H), 127.77 (+, Ar-***C***H), 117.12 (+, Im-5-***C***), 45.13 (+, ***C***HCH_2CH_3), 44.75 (-, ***C***H_2NH), 41.47 (-, CO***C***H_2), 30.20 (-, CH***C***H_2CH_3), 27.87 (-, Im-4-CH_2***C***H_2), 22.51 (-, Im-4-***C***H_2), 12.25 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{18}H_{25}N_5O$] calcd. 327.20591, found 327.20547; prep. HPLC: MeOH/0.1% TFA/aq (45/55). $C_{18}H_{25}N_5O$ · 2 TFA (555.40)

***N*-(2-Benzylbutanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.57)**

The title compound was prepared from **3.57a** (350 mg, 0.61 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.57** as a colourless oil (90 mg, 27 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.77 (s, 1H, Im-2-***H***), 7.33 (s, 1H, Im-5-***H***), 7.19 (m, 5H, Ar-***H***), 3.31 (t, 2H, ^3J = 6.2 Hz, C***H***$_2$NH), 2.90-2.78 (m, 5H, Im-4-C***H***$_2$, C***H***$_2$-Ar, C***H***CH_2CH_3), 1.98 (m, 2H, Im-4-CH_2C***H***$_2$), 1.65 (m, 2H, C***H***$_2CH_3$), 0.94 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 179.93 (quart, ***C***=O), 155.10 (quart, ***C***=NH), 140.05 (quat. Ar-***C***), 134.91 (+, Im-2-***C***), 134.28 (quat. Im-4-***C***), 130.08 (+, 2 Ar-***C***H), 129.54 (+, 2 Ar-***C***H), 127.63 (+, Ar-***C***H), 117.13 (+, Im-5-***C***), 52.51 (+, ***C***HCH_2CH_3), 41.48 (-, ***C***H_2-Ar), 39.27 (-, ***C***H_2NH), 27.87 (-, Im-4-CH_2***C***H_2), 26.24 (-, CH***C***H_2CH_3), 22.53 (-, Im-4-***C***H_2), 11.89 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{18}H_{25}N_5O$] calcd. 327.2059, found 327.20549; prep. HPLC: MeCN/0.1% TFA/aq (25/75-45/55). $C_{18}H_{25}N_5O$ · 2 TFA (555.40)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(4-methyl-3-phenylpentanoyl)guanidine (3.58)**

The title compound was prepared from **3.58a** (290 mg, 0.49 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.58** as a colourless oil (90 mg, 32 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.77 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.51 (s, 1H, Im-5-***H***), 7.20 (m, 5H, Ar-***H***), 3.26 (m, overlap with solvent), 2H, C***H***$_2$NH), 2.93 (m, 3H, CH_2C***H***, COC***H***$_2$), 2.77 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 1.94 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***$(CH_3)_2$), 0.99 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$), 0.74 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.57 (quart, ***C***=O), 155.10 (quart, ***C***=NH), 143.54 (quat. Ar-***C***), 134.96 (+, Im-2-***C***), 134.25 (quat. Im-4-***C***), 129.51 (+, 2 Ar-***C***H), 129.33 (+, 2 Ar-***C***H), 127.73 (+, Ar-***C***H), 117.12 (+, Im-5-***C***), 50.32 (+, CH_2***C***H), 42.08 (-, ***C***H_2NH), 41.45 (-, CO***C***H_2), 34.43 (+, ***C***H$(CH_3)_2$), 27.83 (-, Im-4-CH_2***C***H_2), 22.50 (-, Im-4-***C***H_2), 21.05 (+, ***C***H_3), 20.86 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{19}H_{27}N_5O$] calcd. 341.22156, found 341.22193; prep. HPLC: MeOH/0.1% TFA/aq (55/45). $C_{19}H_{27}N_5O$ · 2 TFA (569.43)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(5-methyl-3-phenylhexanoyl)guanidine (3.59)**

The title compound was prepared from **3.59a** (300 mg, 0.50 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.59** as a colourless oil (80 mg, 27 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.77 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.33 (s, 1H, Im-5-***H***), 7.20 (m, 5H, Ar-***H***), 3.31 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 3.21 (m, 1H, CH_2C***H***), 2.74 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 1.98 (m, 2H, Im-4-CH_2C***H***$_2$), 1.65-1.44 (m, 2H, C***H***$_2$CH$(CH_3)_2$), 1.30 (m, 1H, C***H***$(CH_3)_2$), 0.88 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$), 0.83 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.14 (quart, ***C***=O), 155.19 (quart, ***C***=NH), 144.64 (quat. Ar-***C***), 134.92 (+, Im-2-***C***), 134.28 (quat. Im-4-***C***), 129.62 (+, 2 Ar-***C***H), 128.76 (+, 2 Ar-***C***H), 127.77 (+, Ar-***C***H), 117.13 (+, Im-5-***C***), 46.47 (-, ***C***H_2CH$(CH_3)_2$), 45.62 (-, ***C***H_2NH), 41.47 (-, CO***C***H_2), 41.19 (+, CH_2***C***H), 27.89 (-, Im-4-CH_2***C***H_2), 26.48 (+, ***C***H$(CH_3)_2$), 23.79 (+, ***C***H_3), 22.51 (-, Im-4-***C***H_2), 21.97 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{20}H_{29}N_5O$] calcd. 355.2372, found 355.23723; prep. HPLC: MeOH/0.1% TFA/aq (55/45). $C_{20}H_{29}N_5O$ · 2 TFA (583.46)

***N*-(3,4-Diphenylbutanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.60)**

The title compound was prepared from **3.60a** (280 mg, 0.44 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.60** as a colourless oil (60 mg, 22 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.76 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.32 (s, 1H, Im-5-***H***), 7.21-7.09 (m, 10H, Ar-***H***), 3.48 (m, 1H, C***H***CH_2), 3.28 (m, overlap with solvent), 2H, C***H***$_2$NH), 2.93 (m, 2H, C***H***$_2$-Ar), 2.80 (m, 4H, Im-4-C***H***$_2$,

COC***H***$_2$), 1.96 (m, 2H, Im-4-CH$_2$C***H***$_2$); ^{13}C-NMR (CD$_3$OD) δ (ppm): 176.08 (quart, ***C***=O), 155.11 (quart, ***C***=NH), 144.28 (quat. Ar-***C***), 140.82 (quat. Ar-***C***), 134.94 (+, Im-2-***C***), 134.27 (quat. Im-4-***C***), 130.39 (+, 2 Ar-***C***H), 129.51 (+, 2 Ar-***C***H), 129.26 (+, 2 Ar-***C***H), 128.82 (+, 2 Ar-***C***H), 127.83 (+, Ar-***C***H), 127.29 (+, Ar-***C***H), 117.11 (+, Im-5-***C***), 45.27 (+, ***C***HCH$_2$), 43.97 (-, ***C***H$_2$-Ar), 43.78 (-, ***C***H$_2$NH), 41.44 (-, CO***C***H$_2$), 27.84 (-, Im-4-CH$_2$***C***H$_2$), 22.51 (-, Im-4-***C***H$_2$); HRMS: EI-MS: *m/z* for [C$_{23}$H$_{27}$N$_5$O] calcd. 389.22156, found. 389.22127; prep. HPLC: MeOH/0.1% TFA/aq (55/45). C$_{23}$H$_{27}$N$_5$O · 2 TFA (617.47)

N-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[3-phenyl-4-(4-methylphenyl)butanoyl)-guanidine (3.61)

The title compound was prepared from **3.61a** (150 mg, 0.23 mmol) in 10 ml CH$_2$Cl$_2$/abs and 2 ml TFA according to the general procedure yielding **3.61** as a colourless oil (110 mg, 76 %), ^{1}H-NMR (CD$_3$OD) δ ppm: 8.75 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.32 (d, 1H, ^4J = 0.9 Hz, Im-5-***H***), 7.17 (m, 5H, Ar-***H***), 6.97 (m, 4H, Ar-***H***), 3.44 (m, 1H, C***H***CH$_2$), 3.27 (t, 2H, ^3J = 6.1 Hz, C***H***$_2$NH), 2.88 (d, 2H, ^3J = 7.6 Hz, C***H***$_2$-Ar), 2.78 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 2.23 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.96 (m, 2H, Im-4-CH$_2$C***H***$_2$); ^{13}C-NMR (CD$_3$OD) δ (ppm): 176.15 (quart, ***C***=O), 155.09 (quart, ***C***=NH), 144.42 (quat. Ar-***C***), 137.65 (quat. Ar-***C***), 136.84 (quat. Ar-***C***), 134.91 (+, Im-2-***C***), 134.26 (quat. Im-4-***C***), 130.31 (+, 2 Ar-***C***H), 129.88 (+, 2 Ar-***C***H), 129.50 (+, 2 Ar-***C***H), 128.80 (+, 2 Ar-***C***H), 127.79 (+, Ar-***C***H), 117.11 (+, Im-5-***C***), 45.34 (+, ***C***HCH$_2$), 43.73 (-, ***C***H$_2$-Ar), 43.62 (-, ***C***H$_2$NH), 41.43 (-, CO***C***H$_2$), 27.86 (-, Im-4-CH$_2$***C***H$_2$), 22.50 (-, Im-4-***C***H$_2$), 21.11 (+, (*p*-***C***H$_3$)-Ar); HRMS: EI-MS: *m/z* for [C$_{24}$H$_{29}$N$_5$O] calcd. 403.2372, found 403.23712; prep. HPLC: MeCN/0.1% TFA/aq (40/60). C$_{24}$H$_{29}$N$_5$O · 2 TFA (631.22)

N-(3-Cyclohexyl-4-phenylbutanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.62)

The title compound was prepared from **3.62a** (180 mg, 0.28 mmol) in 10 ml CH$_2$Cl$_2$/abs and 2 ml TFA according to the general procedure yielding **3.62** as a colourless oil (30 mg, 17 %), ^{1}H-NMR (CD$_3$OD) δ ppm: 8.82 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.37 (s, 1H, Im-5-***H***), 7.18 (m, 5H, Ar-***H***), 3.32 (m, overlap with solvent), 2H, C***H***$_2$NH), 2.80 (m, 3H, Im-4-C***H***$_2$, C***H***CH$_2$-Ar), 2.48 (m, 2H, C***H***$_2$-Ar), 2.30 (m, 2H, COC***H***$_2$), 2.00 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.71 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.40-1.15 (m, 6H, cHex-C***H***$_2$); ^{13}C-NMR (CD$_3$OD) δ (ppm): 177.38 (quart, ***C***=O), 155.79 (quart, ***C***=NH), 141.86 (quat. Ar-***C***), 135.00 (+, Im-2-***C***), 134.29 (quat. Im-4-***C***), 130.51 (+, 2

Ar-***C***H), 129.37 (+, 2 Ar-***C***H), 127.17 (+, Ar-***C***H), 117.12 (+, Im-5-***C***), 43.57 (+, cHex-***C***H), 42.26 (+, ***C***HCH$_2$), 41.48 (-, CO***C***H$_2$), 39.68 (-, ***C***H$_2$-Ar), 38.75 (-, ***C***H$_2$NH), 31.18 (-, cHex-***C***H$_2$), 30.37 (-, cHex-***C***H$_2$), 27.87 (-, Im-4-CH$_2$***C***H$_2$, cHex-***C***H$_2$), 27.77 (-, cHex-***C***H$_2$), 22.56 (-, Im-4-***C***H$_2$); HRMS: EI-MS: *m/z* for [$C_{23}H_{33}N_5O$] calcd. 395.2685, found 395.26886; prep. HPLC: MeCN/0.1% TFA/aq (35/65). $C_{23}H_{33}N_5O$ · 2 TFA (623.25)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[3-(4-methylphenyl)propanoyl]guanidine (3.63)**

The title compound was prepared from **3.63a** (220 mg, 0.40 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.63** as a colourless oil (110 mg, 51 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (d, 1H, ^4J = 0.9 Hz, Im-2-***H***), 7.35 (s, 1H, Im-5-***H***), 7.08 (m, 4H, Ar-***H***), 3.36 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$NH), 2.91 (t, 2H, ^3J = 7.3 Hz, COCH$_2$C***H***$_2$), 2.79 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$CH$_2$), 2.27 (s, 3H, (*p*-C***H***$_3$)-Ar), 2.01 (m, 2H, Im-4-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.64 (quart, ***C***=O), 155.33 (quart, ***C***=NH), 138.23 (quat. Ar-***C***), 137.06 (quat. Ar-***C***), 134.94 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 130.20 (+, 2 Ar-***C***H), 129.37 (+, 2 Ar-***C***H), 117.13 (+, Im-5-***C***), 41.53 (-, ***C***H$_2$NH), 39.62 (-, CO***C***H$_2$), 30.92 (-, COCH$_2$***C***H$_2$), 27.95 (-, Im-4-CH$_2$***C***H$_2$), 22.54 (-, Im-4-***C***H$_2$), 21.09 (+, (*p*-***C***H$_3$)-Ar); HRMS: EI-MS: *m/z* for [$C_{17}H_{23}N_5O$] calcd. 313.1903, found 313.1902; prep. HPLC: MeCN/0.1% TFA/aq (25/75). $C_{17}H_{23}N_5O$ · 2 TFA (541.17)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[3-(4-methylphenyl)butanoyl)guanidine (3.64)**

The title compound was prepared from **3.64a** (230 mg, 0.40 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.64** as a colourless oil (90 mg, 40 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.75 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.33 (d, 1H, ^4J = 0.9 Hz, Im-5-***H***), 7.09 (m, 4H, Ar-***H***), 3.33 (m, overlap with solvent), 2H, C***H***$_2$NH), 3.25 (m, 1H, C***H***CH$_3$), 2.80 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 2.73 (m, 2H, COC***H***$_2$), 2.25 (s, 3H, (*p*-C***H***$_3$)-Ar), 1.99 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.27 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.21 (quart, ***C***=O), 155.25 (quart, ***C***=NH), 143.32 (quat. Ar-***C***), 137.25 (quat. Ar-***C***), 134.90 (+, Im-2-***C***), 134.29 (quat. Im-4-***C***), 130.22 (+, 2 Ar-***C***H), 127.81 (+, 2 Ar-***C***H), 117.12 (+, Im-5-***C***), 46.21 (-, CO***C***H$_2$), 41.48 (-, ***C***H$_2$NH), 37.30 (+, ***C***HCH$_3$), 27.91 (-, Im-4-CH$_2$***C***H$_2$), 22.52 (-, Im-4-***C***H$_2$), 22.36 (+, (*p*-***C***H$_3$)-Ar), 21.08 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{18}H_{25}N_5O$] calcd. 327.2059, found 327.20512; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{18}H_{25}N_5O$ · 2 TFA (555.18)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(3-methyl-4-phenylbutanoyl)guanidine (3.65)**

The title compound was prepared from **3.65a** (320 mg, 0.56 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.65** as a colourless oil (100 mg, 32 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.80 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.36 (d, 1H, ^4J = 0.9 Hz, Im-5- ***H***), 7.20 (m, 5H, Ar-***H***), 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.64 (dd, 1H, ^3J = 6.6 Hz, ^3J = 13.3 Hz, C***H***H-Ar), 2.54-2.31 (m, 4H, CH***H***-Ar, COC***H***$_2$, C***H***CH_3), 2.02 (m, 2H, Im-4-CH_2C***H***$_2$), 0.96 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.82 (quart, ***C***=O), 155.28 (quart, ***C***=NH), 141.40 (quat. Ar-***C***), 134.95 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 130.37 (+, 2 Ar-***C***H), 129.36 (+, 2 Ar-***C***H), 127.25 (+, Ar-***C***H), 117.13 (+, Im-5-***C***), 44.56 (-, CO***C***H_2), 43.92 (-, ***C***H_2-Ar), 41.52 (-, ***C***H_2NH), 33.42 (+, ***C***HCH_3), 27.94 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 19.91 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{18}H_{25}N_5O$] calcd. 327.2059, found 327.20554; prep. HPLC: MeOH/0.1% TFA/aq (45/55). $C_{18}H_{25}N_5O$ · 2 TFA (555.40)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(3-methyl-5-phenylpentanoyl)guanidine (3.66)**

The title compound was prepared from **3.66a** (310 mg, 0.53 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.66** as a colourless oil (120 mg, 40 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.77 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.33 (s, 1H, Im-5- ***H***), 7.17 (m, 5H, Ar-***H***), 3.37 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.82 (m, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.66-2.53 (m, 3H, C***H***$_2$-Ar, COCH***H***), 2.31 (dd, 1H, ^3J = 7.9 Hz, ^3J = 15.1 Hz, COC***H***H), 2.02 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.67-1.52 (m, 2H, C***H***$_2$$CH_2$-Ar), 1.02 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.93 (quart, ***C***=O), 155.37 (quart, ***C***=NH), 143.56 (quat. Ar-***C***), 134.89 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 129.41 (+, 4 Ar-***C***H), 126.82 (+, Ar-***C***H), 117.13 (+, Im-5-***C***), 45.02 (-, CO***C***H_2), 41.54 (-, ***C***$H_2$$CH_2$-Ar), 39.52 (-, ***C***$H_2$NH), 34.21 (-, CH_2***C***H_2-Ar), 31.06 (+, ***C***HCH_3), 27.97 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 19.79 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{19}H_{27}N_5O$] calcd. 341.2216, found 341.2216; prep. HPLC: MeOH/0.1% TFA/aq (55/45). $C_{19}H_{27}N_5O$ · 2 TFA (569.43)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(3-methyl-6-phenylhexanoyl)guanidine (3.67)**

The title compound was prepared from **3.67a** (280 mg, 0.47 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.67** as a colourless oil (100 mg, 36 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.80 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 7.36 (d, 1H, ^4J = 1.0 Hz, Im-5-***H***), 7.18 (m, 5H, Ar-***H***), 3.38 (t, 2H, ^3J =7.0 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.59 (m, 2H, C***H***$_2$-Ar), 2.46 (dd, 1H, ^3J =

6.1 Hz, ^3J = 15.0 Hz, COC***H***H), 2.26 (dd, 1H, ^3J = 7.9 Hz, ^3J = 15.0 Hz, COCH***H***), 2.02 (m, 3H, Im-4-CH_2C***H***$_2$, C***H***CH_3), 1.64 (m, 2H, C***H***$_2CH_2$-Ar), 1.41-1.24 (m, 2H, C***H***$_2CH_2CH_2$-Ar), 0.96 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.91 (quart, ***C***=O), 155.31 (quart, ***C***=NH), 143.67 (quat. Ar-***C***), 135.00 (+, Im-2-***C***), 134.34 (quat. Im-4-***C***), 129.45 (+, 2 Ar-***C***H), 129.33 (+, 2 Ar-***C***H), 126.77 (+, Ar-***C***H), 117.12 (+, Im-5-***C***), 45.16 (-, CO***C***H_2), 41.60 (-, ***C***H_2NH), 37.11 (-, CH_2CH_2***C***H_2-Ar), 36.89 (-, ***C***$H_2CH_2CH_2$-Ar), 31.26 (+, ***C***HCH_3), 29.94 (-,CH_2***C***H_2CH_2-Ar), 27.96 (-, Im-4-CH_2***C***H_2), 22.59 (-, Im-4-***C***H_2), 19.86 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{20}H_{29}N_5O$] calcd. 355.23721, found 355.23701; prep. HPLC: MeOH/0.1% TFA/aq (55/45). $C_{20}H_{29}N_5O \cdot 2$ TFA (583.46)

N-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[3-methyl-4-(3-methylphenyl)butanoyl]-guanidine (3.68)

The title compound was prepared from **3.68a** (290 mg, 0.50 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.68** as a colourless oil (90 mg, 32 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.34 (s, 1H, Im-5-***H***), 7.11 (m, 1H, Ar-***H***), 6.95 (m, 3H, Ar-***H***), 3.34 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.82 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.57-2.45 (m, 3H, C***H***$_2$-Ar, C***H***CH_3), 2.34 (m, 2H, COC***H***$_2$), 2.28 (s, 3H, (*m*-C***H***$_3$)-Ar), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$), 0.95 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.93 (quart, ***C***=O), 155.32 (quart, ***C***=NH), 141.29 (quat. Ar-***C***), 138.99 (quat. Ar-***C***), 134.90 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 131.07 (+, Ar-***C***H), 129.25 (+, Ar-***C***H), 127.90 (+, Ar-***C***H), 127.43 (+, Ar-***C***H), 117.14 (+, Im-5-***C***), 44.62 (-, CO***C***H_2), 43.94 (-, ***C***H_2-Ar), 41.52 (-, ***C***H_2NH), 33.41 (+, ***C***HCH_3), 27.97 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 21.50 (+, (*m*-***C***H_3)-Ar), 20.03 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{19}H_{27}N_5O$] calcd. 341.2216, found 341.22153; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{19}H_{27}N_5O \cdot 2$ TFA (569.20)

N-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[3-methyl-4-(4-methylphenyl)butanoyl]-guanidine (3.69)

The title compound was prepared from **3.69a** (460 mg, 0.79 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.69** as a colourless oil (160 mg, 35 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.76 (d, 1H, ^4J = 0.7 Hz, Im-2-***H***), 7.33 (s, 1H, Im-5-***H***), 7.03 (m, 4H, Ar-***H***), 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.82 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.56-2.43 (m, 3H, C***H***$_2$Ar, C***H***CH_3), 2.31 (m, 2H, COC***H***$_2$), 2.25 (s, 3H, (*p*-C***H***$_3$)-Ar), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$), 0.93 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.96 (quart, ***C***=O), 155.31 (quart, ***C***=NH),

138.21 (quat. Ar-***C***), 136.77 (quat. Ar-***C***), 134.88 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 130.29 (+, 2 Ar-***C***H), 129.96 (+, 2 Ar-***C***H), 117.14 (+, Im-5-***C***), 44.58 (-, CO***C***H_2), 43.55 (-, ***C***H_2-Ar), 41.53 (-, ***C***H_2NH), 33.48 (+, ***C***HCH_3), 28.00 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 21.14 (+, (*p*-***C***H_3)-Ar), 20.00 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{19}H_{27}N_5O$] calcd. 341.2216, found 341.2209; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{19}H_{27}N_5O$ · 2 TFA (569.20)

_N_-[4-(3-Fluorophenyl)-3-methylbutanoyl]-_N´_-[3-(1_H_-imidazol-4-yl)propyl]-guanidine (3.70)

The title compound was prepared from **3.70a** (120 mg, 0.20 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.70** as a colourless oil (50 mg, 44 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.81 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.36 (m, 1H, Im-5-***H***), 7.27 (m, 1H, Ar-***H***), 6.93 (m, 3H, Ar-***H***), 3.36 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.68 (dd, 1H, ^3J = 6.3 Hz, ^3J = 13.4 Hz, C***H***H-Ar), 2.55 (dd, 1H, ^3J = 5.2 Hz, ^3J = 11.5 Hz, CH***H***-Ar), 2.48 (dd, 1H, ^3J = 7.2 Hz, ^3J = 16.1 Hz, COC***H***H), 2.34 (m, 2H, COCH***H***, C***H***CH_3), 2.03 (m, 2H, Im-4-CH_2C***H***$_2$), 0.96 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.65 (quart, ***C***=O), 164.33 (d, quart., ^1J = 244.1 Hz, Ar-***C***F), 155.28 (quart, ***C***=NH), 144.35 (d, quart., ^3J = 7.2 Hz, Ar-***C***), 134.97 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 131.02 (d, ^3J = 8.34 Hz, Ar-***C***H), 126.27 (d, ^4J = 2.8 Hz, Ar-***C***H), 117.13 (+, Im-5-***C***), 116.92 (d, ^2J = 21.0 Hz, Ar-***C***H), 113.94 (d, ^2J = 21.4 Hz, Ar-***C***H), 44.44 (-, CO***C***H_2), 43.46 (-, ***C***H_2-Ar), 41.55 (-, ***C***H_2NH), 33.22 (+, ***C***HCH_3), 27.93 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 19.79 (+, ***C***H_3); HRMS: FAB-MS: *m/z* for [$C_{18}H_{24}FN_5O$ + H^+] calcd. 346.1965, found 346.20379; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{18}H_{24}FN_5O$ · 2 TFA (573.17)

_N_-[4-(4-Fluorophenyl)-3-methylbutanoyl]-_N´_-[3-(1_H_-imidazol-4-yl)propyl] - guanidine (3.71)

The title compound was prepared from **3.71a** (340 mg, 0.58 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.71** as a colourless oil (130 mg, 39 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (d, 1H, ^4J = 1.1 Hz, Im-2-***H***), 7.34 (s, 1H, Im-5-***H***), 7.17 (m, 2H, Ar-***H***), 6.97 (m, 2H, Ar-***H***), 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.82 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.62 (dd, 1H, ^3J = 6.5 Hz, ^3J = 13.5 Hz, C***H***H-Ar), 2.49 (m, 2H, CH***H***-Ar, COC***H***H), 2.32 (m, 2H, COCH***H***, C***H***CH_3), 2.02 (m, 2H, Im-4-CH_2C***H***$_2$), 0.94 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.82 (quart, ***C***=O), 162.94 (d, quart., ^1J = 242.6 Hz, Ar-***C***F), 155.32 (quart,

C=NH), 137.33 (d, quart., ^4J = 3.2 Hz, Ar-***C***), 134.89 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 131.99 (d, +, ^3J = 7.7 Hz, 2 Ar-***C***H), 117.13 (+, Im-5-***C***), 115.90 (d, +, ^2J = 21.3 Hz, 2 Ar-***C***H), 44.48 (-, CO***C***H_2), 43.02 (-, ***C***H_2-Ar), 41.51 (-, ***C***H_2NH), 33.46 (+, ***C***HCH_3), 27.97 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 19.84 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{18}H_{24}FN_5O$] calcd. 345.1965, found 345.19673; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{18}H_{24}FN_5O$ · 2 TFA (573.17)

N-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[4-(3-methoxyphenyl)-3-methylbutanoyl]-guanidine (3.72)

The title compound was prepared from **3.72a** (230 mg, 0.38 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.72** as a colourless oil (110 mg, 49 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.79 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.35 (s, 1H, Im-5-***H***), 7.15 (m, 1H, Ar-***H***), 6.73 (m, 3H, Ar-***H***), 3.75 (s, 3H, (m-OC***H***$_3$)-Ar), 3.34 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.82 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.54 (m, 3H, C***H***$_2$Ar, C***H***CH_3), 2.32 (m, 2H, COC***H***$_2$), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$), 0.96 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.87 (quart, ***C***=O), 161.18 (quat. Ar-***C***), 155.28 (quart, ***C***=NH), 142.98 (quat. Ar-***C***), 134.92 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 130.33 (+, Ar-***C***H), 122.74 (+, Ar-***C***H), 117.13 (+, Im-5-***C***), 115.91 (+, Ar-***C***H), 112.69 (+, Ar-***C***H), 55.59 (+, (m-O***C***H_3)-Ar), 44.55 (-, CO***C***H_2), 43.99 (-, ***C***H_2-Ar), 41.51 (-, ***C***H_2NH), 33.34 (+, ***C***HCH_3), 27.95 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 20.08 (+, ***C***H_3); HRMS: FAB-MS: *m/z* for [$C_{19}H_{27}N_5O_2$] calcd. 357.2165, found 357.21574; prep. HPLC: MeCN/0.1% TFA/aq (25/75-40/60). $C_{19}H_{27}N_5O_2$ · 2 TFA (585.50)

N-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-[4-(4-methoxyphenyl)-3-methylbutanoyl]-guanidine (3.73)

The title compound was prepared from **3.73a** (300 mg, 0.50 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.73** as a colourless oil (160 mg, 55 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.35 (s, 1H, Im-5-***H***), 7.07 (m, 2H, Ar-***H***), 6.79 (m, 2H, Ar-***H***), 3.73 (s, 3H, (p-OC***H***$_3$)-Ar), 3.34 (t, 2H, ^3J = 7.1 Hz, C***H***$_2$NH), 2.82 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.53 (m, 2H, C***H***$_2$Ar), 2.42 (m, 1H, C***H***CH_3), 2.29 (m, 2H, COC***H***$_2$), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$), 0.95 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.96 (quart, ***C***=O), 159.63 (quat. Ar-***C***), 155.29 (quart, ***C***=NH), 134.91 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 133.31 (quat. Ar-***C***), 131.37 (+, 2 Ar-***C***H), 117.13 (+, Im-5-***C***), 114.74 (+, 2 Ar-***C***H), 55.70 (+, (p-O***C***H_3)-Ar), 44.58 (-, CO***C***H_2), 43.15 (-, ***C***H_2-Ar), 41.51 (-,

CH_2NH), 33.64 (+, ***C***HCH_3), 27.96 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 20.11 (+, ***C***H_3); HRMS: FAB-MS: *m/z* for [$C_{19}H_{27}N_5O_2$ + H^+] calcd. 358.2165, found 358.22353; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{19}H_{27}N_5O_2$ · 2 TFA (585.50)

_N_-[4-(4-Ethylphenyl)-3-methylbutanoyl]-_N´_-[3-(1_H_-imidazol-4-yl)propyl]-guanidine (3.74)

The title compound was prepared from **3.74a** (360 mg, 0.60 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.74** as a colourless oil (100 mg, 28 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.76 (d, 1H, ^4J = 0.7 Hz, Im-2-***H***), 7.33 (s, 1H, Im-5-***H***), 7.06 (m, 4H, Ar-***H***), 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.82 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.50 (m, 5H, C***H***$_2$-Ar, (*p*-C***H***$_2$$CH_3$)-Ar, C***H***$CH_3$), 2.30 (m, 2H, COC***H***$_2$), 2.01 (m, 2H, Im-4-CH_2C***H***$_2$), 1.17 (t, 3H, ^3J = 7.6 Hz, (*p*-CH_2C***H***$_3$)-Ar), 0.94 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.95 (quart, ***C***=O), 155.31 (quart, ***C***=NH), 143.34 (quat. Ar-***C***), 138.48 (quat. Ar-***C***), 134.88 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 130.35 (+, 2 Ar-***C***H), 128.78 (+, 2 Ar-***C***H), 117.14 (+, Im-5-***C***), 44.58 (-, CO***C***H_2), 43.57 (-, ***C***H_2-Ar), 41.54 (-, ***C***H_2NH), 33.45 (+, ***C***HCH_3), 29.48 (-, (*p*-***C***H_2CH_3)-Ar), 27.99 (-, Im-4-CH_2***C***H_2), 22.56 (-, Im-4-***C***H_2), 19.99 (+, (*p*-CH_2***C***H_3)-Ar), 16.25 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{20}H_{29}N_5O$] calcd. 355.2372, found 355.23757; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{20}H_{29}N_5O$ · 2 TFA (583.45)

(_R_)-_N_-(3-Cyclohexylbutanoyl)-_N´_-[3-(1_H_-imidazol-4-yl)propyl]guanidine (3.75)

The title compound was prepared from **3.75a** (240 mg, 0.43 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.75** as a colourless oil (80 mg, 34 %). ee = 95.65 %; ^{1}H-NMR (CD_3OD) δ (ppm): 8.80 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.38 (t, 2H, ^3J = 6.90 Hz, C***H***$_2$NH), 2.84 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.56 (dd, 1H, ^3J = 5.1 Hz, ^3J = 15.0 Hz, COCH***H***), 2.23 (dd, 1H, ^3J = 9.1 Hz, ^3J = 15.0 Hz, COC***H***H), 2.03 (m, 2H, Im-4-CH_2C***H***$_2$), 1.90 (m, 1H, C***H***CH_3), 1.75-1.66 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.23-1.05 (m, 6H, cHex-C***H***$_2$), 0.91 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.51 (quart, ***C***=O), 155.38 (quart, ***C***=NH), 134.96 (+, Im-2-***C***), 134.34 (quat. Im-4-***C***), 117.14 (+, Im-5-***C***), 43.94 (+, cHex-***C***H), 42.69 (-, ***C***H_2NH), 41.55 (-, CO***C***H_2), 36.43 (+, CH_3***C***H), 31.48 (-, cHex-***C***H_2), 30.02 (-, cHex-***C***H_2), 27.97 (-, Im-4-CH_2***C***H_2), 27.85 (-, cHex-***C***H_2), 27.74 (-, 2 cHex-***C***H_2), 22.57 (-,Im-4-***C***H_2), 16.60 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{17}H_{29}N_5O$] calcd. 319.2372, found 319.23666; prep. HPLC: MeCN/0.1% TFA/aq (35/65). $C_{17}H_{29}N_5O$ · 2 TFA (547.42)

(*S*)-*N*-(3-Cyclohexylbutanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.76)

The title compound was prepared from **3.76a** (160 mg, 0.29 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.76** as a colourless oil (140 mg, 88 %). ee = 98.17 %; ^{1}H-NMR (CD_3OD) δ (ppm): 8.81 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 7.37 (s, 1H, Im-5-***H***), 3.39 (t, 2H, ^3J = 6.90 Hz, C***H***$_2$NH), 2.84 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.57 (dd, 1H, ^3J = 5.1 Hz, ^3J = 15.0 Hz, COCH***H***), 2.23 (dd, 1H, ^3J J = 9.1 Hz, ^3J = 15.0 Hz, COC***H***H), 2.04 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.90 (m, 1H, C***H***CH$_3$), 1.76-1.66 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.24-1.05 (m, 6H, cHex-C***H***$_2$), 0.91 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.65 (quart, ***C***=O), 154.52 (quart, ***C***=NH), 134.06 (+, Im-2-***C***), 133.45 (quat. Im-4-***C***), 116.26 (+, Im-5-***C***), 43.05 (+, cHex-CH), 41.81 (-, ***C***H$_2$NH), 40.66 (-, CO***C***H$_2$), 35.55 (+, CH$_3$***C***H), 30.60 (-, cHex-***C***H$_2$), 29.13 (-, cHex-***C***H$_2$), 27.10 (-, Im-4-CH$_2$***C***H$_2$), 26.97 (-, cHex-***C***H$_2$), 26.87 (-, 2 cHex-***C***H$_2$), 21.86 (-,Im-4-***C***H$_2$), 15.72 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{17}H_{29}N_5O$] calcd. 319.2372, found 319.23674; prep. HPLC: MeCN/0.1% TFA/aq (35/65). $C_{17}H_{29}N_5O \cdot 2$ TFA (547.42)

***N*-(3-Cyclohexyl-2-methylpropanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.77)**

The title compound was prepared from **3.77a** (370 mg, 0.66 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.77** as a colourless oil (130 mg, 36 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.79 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.39 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.84 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.71 (m, 1H, C***H***CH$_3$), 2.04 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.66 (m, 6H, cHex-C***H***$_2$), 1.25 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.15 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$), 0.90 (m, 2H, C***H***$_2$-cHex); ^{13}C-NMR (CD_3OD) δ (ppm): 181.40 (quart, ***C***=O), 155.62 (quart, ***C***=NH), 134.92 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 117.14 (+, Im-5-***C***), 42.25 (-, ***C***H$_2$-chex), 41.60 (-, ***C***H$_2$NH), 40.27 (+, ***C***HCH$_3$), 36.65 (+, cHex-***C***H), 34.52 (-, cHex-***C***H$_2$), 34.25 (-, cHex-***C***H$_2$), 27.98 (-, Im-4-CH$_2$***C***H$_2$), 27.61 (-, cHex-***C***H$_2$), 27.35 (-, cHex-***C***H$_2$), 27.32 (-, cHex-***C***H$_2$), 22.58 (-, Im-4-***C***H$_2$), 17.93 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{17}H_{29}N_5O$] calcd. 319.2372, found 319.23686; prep. HPLC: MeCN/0.1% TFA/aq (25/75-45-55). $C_{17}H_{29}N_5O \cdot 2$ TFA (547.42)

***N*-(3-Cyclohexylpentanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.78)**

The title compound was prepared from **3.78a** (210 mg, 0.37 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.78** as a colourless oil (60 mg, 29 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.81 (d, 1H, ^4J = 1.3 Hz, Im-2-

H), 7.37 (d, 1H, ^4J = 0.8 Hz, Im-5-***H***), 3.38 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.50 (dd, 1H, ^3J = 6.2 Hz, ^3J = 15.6 Hz, COC***H***H), 2.32 (dd, 1H, ^3J = 7.5 Hz, ^3J = 15.6 Hz, COCH***H***), 2.03 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.77-1.61 (m, 6H, C***H***CH$_2$CH$_3$, cHex-C***H***, cHex-C***H***$_2$) 1.43-1.07 (m, 8H, cHex-C***H***$_2$), 0.89 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C-NMR (CD$_3$OD) δ (ppm): 177.83 (quart, ***C***=O), 155.37 (quart, ***C***=NH), 134.97 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 42.96 (+, cHex-***C***H), 41.56 (-, CO***C***H$_2$), 41.41 (+, ***C***HCH$_2$CH$_3$), 39.67 (-, ***C***H$_2$NH), 31.15 (-, cHex-***C***H$_2$), 30.34 (-, cHex-***C***H$_2$), 27.91 (-, Im-4-CH$_2$***C***H$_2$, cHex-***C***H$_2$), 27.89 (-, cHex-***C***H$_2$), 27.79 (-, cHex-***C***H$_2$), 24.72 (-, ***C***H$_2$CH$_3$), 22.56 (-, Im-4-***C***H$_2$), 12.05 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [C$_{18}$H$_{31}$N$_5$O] calcd. 333.2528, found 333.25275; prep. HPLC: MeCN/0.1% TFA/aq (35/65). C$_{18}$H$_{31}$N$_5$O · 2 TFA (561.23)

N-[2-(Cyclohexylmethyl)butanoyl]-N´-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.79)

The title compound was prepared from **3.79a** (290 mg, 0.50 mmol) in 10 ml CH$_2$Cl$_2$/abs and 2 ml TFA according to the general procedure yielding **3.79** as a colourless oil (40 mg, 14 %), ^{1}H-NMR (CD$_3$OD) δ ppm: 8.81 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.37 (d, 1H, ^4J = 0.8 Hz, Im-5-***H***), 3.39 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 2.84 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 2.56 (m, 1H, C***H***CH$_2$CH$_3$), 2.04 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.63 (m, 8H, C***H***$_2$CH$_3$, cHex-C***H***$_2$), 1.26 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 0.92 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$), 0.88 (m, 2H, C***H***$_2$-cHex); ^{13}C-NMR (CD$_3$OD) δ (ppm): 180.85 (quart, ***C***=O), 155.36 (quart, ***C***=NH), 134.96 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 47.84 (+, ***C***HCH$_2$CH$_3$)41.66 (-, ***C***H$_2$NH), 40.64 (-, ***C***H$_2$-Ar), 36.96 (+, cHex-***C***H), 34.79 (-, cHex-***C***H$_2$), 34.19 (-, cHex-***C***H$_2$), 27.92 (-, Im-4-CH$_2$***C***H$_2$), 27.58 (-, cHex-***C***H$_2$), 27.35 (-, cHex-***C***H$_2$), 27.32 (-, cHex-***C***H$_2$), 27.18 (-, CH***C***H$_2$CH$_3$), 22.60 (-, Im-4-***C***H$_2$), 11.88 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [C$_{18}$H$_{31}$N$_5$O] calcd. 333.2529, found 333.25315; prep. HPLC: MeCN/0.1% TFA/aq (30/70-45/55). C$_{18}$H$_{31}$N$_5$O · 2 TFA (561.23)

N-(4-Cyclohexyl-3-methylbutanoyl)-N´-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.80)

The title compound was prepared from **3.80a** (170 mg, 0.29 mmol) in 10 ml CH$_2$Cl$_2$/abs and 2 ml TFA according to the general procedure yielding **3.80** as a colourless oil (110 mg, 66 %), ^{1}H-NMR (CD$_3$OD) δ ppm: 8.80 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.38 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.40 (d, 2H, ^3J = 5.6 Hz, COC***H***$_2$), 2.03 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.84 (m,

1H, C***H***$(CH_3)_2$), 1.73-1.62 (m, 6H, C***H***$CH(CH_3)_2$, cHex-C***H***, cHex-C***H***$_2$), 1.39-0.99 (m, 6H, cHex-C***H***$_2$), 0.91 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$), 0.83 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 178.23 (quart, ***C***=O), 155.41 (quart, ***C***=NH), 134.95 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 46.46 (+, ***C***$HCH(CH_3)_2$), 41.56 (-, ***C***H_2NH), 41.25 (+, cHex-***C***H), 36.91 (-, CO***C***H_2), 32.85 (-, cHex-***C***H_2), 30.81 (-, cHex-***C***H_2), 29.81 (+, ***C***$H(CH_3)_2$), 27.97 (-, cHex-***C***H_2), 27.89 (-, Im-4-CH_2***C***H_2), 27.79 (-, cHex-***C***H_2), 27.72 (-, cHex-***C***H_2), 22.56 (-, Im-4-***C***H_2), 21.76 (+, ***C***H_3), 19.05 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{19}H_{33}N_5O$] calcd. 347.26851, found 347.26819; prep. HPLC: MeCN/0.1% TFA/aq (40/60). $C_{19}H_{33}N_5O$ · 2 TFA (575.25)

_N_-(3-Cyclohexyl-5-methylhexanoyl)-_N´_-[3-(1_H_-imidazol-4-yl)propyl]guanidine (3.81)

The title compound was prepared from **3.81a** (300 mg, 0.50 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.81** as a colourless oil (110 mg, 37 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.80 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.36 (d, 1H, ^4J = 0.6 Hz, Im-5-***H***), 3.38 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.49 (dd, 1H, ^3J = 6.6 Hz, ^3J = 15.6 Hz, COC***H***H), 2.29 (dd, 1H, ^3J = 6.9 Hz, ^3J = 15.6 Hz, COCH***H***), 2.05 (m, 2H, Im-4-CH_2C***H***$_2$), 1.94 (m, 1H, C***H***$(CH_3)_2$), 1.75-1.57 (m, 6H, C***H***CH_2, cHex-C***H***, cHex-C***H***$_2$), 1.30-1.06 (m, 8H, C***H***$_2CH(CH_3)_3$, cHex-C***H***$_2$), 0.88 (d, 3H, ^3J = 3.2 Hz, C***H***$_3$), 0.86 (d, 3H, ^3J = 3.2 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.81 (quart, ***C***=O), 155.39 (quart, ***C***=NH), 134.93 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 42.03 (+, cHex-***C***H), 41.78 (-, ***C***$H_2CH(CH_3)_2$), 41.53 (-, CO***C***H_2), 40.24 (-, ***C***H_2NH), 38.87 (+, ***C***$HCH_2CH(CH_3)_2$), 30.92 (-, cHex-***C***H_2), 30.12 (-, cHex-***C***H_2), 27.97 (-, 2 cHex-*C*H_2), 27.92 (-, cHex-***C***H_2), 27.84 (-, Im-4-CH_2***C***H_2), 26.68 (+, ***C***$H(CH_3)_2$), 23.39 (-, Im-4-***C***H_2), 22.97 (+, ***C***H_3), 22.55 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{20}H_{35}N_5O$] calcd. 361.28416, found 361.28366; prep. HPLC: MeCN/0.1% TFA/aq (45/55). $C_{20}H_{35}N_5O$ · 2 TFA (589.26)

_N_-(4-Cyclohexyl-3-methylbutanoyl)-_N´_-[3-(1_H_-imidazol-4-yl)propyl]guanidine (3.82)

The title compound was prepared from **3.82a** (230 mg, 0.39 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.82** as a colourless oil (110 mg, 50 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.79 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.38 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.44 (dd, 1H, ^3J = 5. 6Hz, ^3J = 14.7 Hz, COC***H***H), 2.23 (dd, 1H, ^3J = 8.1

Hz, ^3J = 14.7 Hz, COCH***H***), 2.07 (m, 3H, Im-4-$CH_2C\boldsymbol{H}_2$, C***H***C), 1.69 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.31-1.08 (m, 6H, cHex-C***H***$_2$), 0.93 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$), 0.84 (m, 2H, C***H***$_2$-cHex); ^{13}C-NMR (CD_3OD) δ (ppm): 177.05 (quart, ***C***=O), 155.38 (quart, ***C***=NH), 134.93 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.14 (+, Im-5-***C***), 45.80 (-, CO***C***H$_2$), 45.56 (-, ***C***H$_2$-cHex), 41.54 (-, ***C***H$_2$NH), 36.08 (+, cHex-***C***H), 35.11 (-, cHex-***C***H$_2$), 34.13 (-, cHex-***C***H$_2$), 28.31 (+, ***C***HCH$_3$), 27.98 (-, cHex-***C***H$_2$), 27.78 (-, Im-4-$CH_2\boldsymbol{C}H_2$), 27.47 (-, cHex-***C***H$_2$), 27.40 (-, cHex-***C***H$_2$), 22.56 (-, Im-4-***C***H$_2$), 20.06 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{18}H_{31}N_5O$] calcd. 333.25286, found 333.25247; prep. HPLC: MeCN/0.1% TFA/aq (40/60). $C_{18}H_{31}N_5O \cdot 2$ TFA (561.23)

N-(5-Cyclohexyl-3-methylpentanoyl)-N´-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.83)

The title compound was prepared from **3.83a** (140 mg, 0.24 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.83** as a colourless oil (70 mg, 51 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.79 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.37 (d, 1H, ^4J = 0.9 Hz, Im-5-***H***), 3.38 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.47 (dd, 1H, ^3J = 6.1 Hz, ^3J = 15.0 Hz, COC***H***H), 2.25 (dd, 1H, ^3J = 8.0 Hz, ^3J = 15.0 Hz, COCH***H***), 2.04 (m, 2H, Im-4-$CH_2C\boldsymbol{H}_2$), 1.94 (m, 1H, C***H***CH$_3$), 1.69 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.36-1.16 (m, 8H, $CH_2C\boldsymbol{H}_2$-cHex, cHex-C***H***$_2$), 0.95 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$), 0.88 (m, 2H, C***H***$_2$CH$_2$-cHex); ^{13}C-NMR (CD_3OD) δ (ppm): 177.08 (quart, ***C***=O), 155.38 (quart, ***C***=NH), 134.96 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 45.21 (-, CO***C***H$_2$), 41.53 (-, ***C***H$_2$NH), 39.15 (+, cHex-***C***H), 35.80 (-, ***C***H$_2$CH$_2$-cHex), 34.90 (-, cHex-***C***H$_2$), 34.72 (-, cHex-***C***H$_2$), 34.45 (-, CH$_2$***C***H$_2$-cHex), 31.69 (+, ***C***HCH$_3$), 27.97 (-, cHex-***C***H$_2$), 27.83 (-, Im-4-CH$_2$***C***H$_2$), 27.53 (-, 2 cHex-***C***H$_2$), 22.56 (-, Im-4-***C***H$_2$), 19.88 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{19}H_{33}N_5O$] calcd. 347.2685, found 347.2684; prep. HPLC: MeCN/0.1% TFA/aq (45/55). $C_{19}H_{33}N_5O \cdot 2$ TFA (575.25)

N-(6-Cyclohexyl-3-methylhexanoyl)-N´-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.84)

The title compound was prepared from **3.84a** (200 mg, 0.33 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.84** as a colourless oil (90 mg, 46 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.81 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.37 (s, 1H, Im-5-***H***), 3.38 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.84 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.47 (dd, 1H, ^3J = 6.1 Hz, ^3J = 15.0 Hz, COC***H***H), 2.26 (dd, 1H, ^3J = 8.0 Hz, ^3J = 15.0 Hz, COCH***H***), 2.03 (m, 3H, Im-4-$CH_2C\boldsymbol{H}_2$, C***H***CH$_3$), 1.68 (m, 5H, cHex-

C***H***$_2$, cHex-C***H***), 1.33-1.18 (m, 10H, C***H***$_2$CH$_2$CH$_2$-cHex, CH$_2$CH$_2$C***H***$_2$-cHex, cHex-C***H***$_2$), 0.96 (d, 3H, ^3J = 6.7 Hz, C***H***$_3$), 0.85 (m, 2H, CH$_2$C***H***$_2$CH$_2$-cHex); ^{13}C-NMR (CD_3OD) δ (ppm): 177.03 (quart, ***C***=O), 155.37 (quart, ***C***=NH), 134.97 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 45.23 (-, CO***C***H$_2$), 41.56 (-, ***C***H$_2$NH), 38.97 (+, cHex-***C***H), 38.76 (-, ***C***H$_2$CH$_2$CH$_2$-cHex), 37.99 (-, CH$_2$CH$_2$***C***H$_2$-cHex), 34.66 (-, cHex-***C***H$_2$), 34.55 (-, cHex-***C***H$_2$), 31.42 (+, ***C***HCH$_3$), 27.97 (-, cHex-***C***H$_2$), 27.85 (-, Im-4-CH$_2$***C***H$_2$), 27.56 (-, 2 cHex-***C***H$_2$), 25.18 (-, CH$_2$***C***H$_2$CH$_2$-cHex), 22.57 (-, Im-4-***C***H$_2$), 19.88 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{20}H_{359}N_5O$] calcd. 361.28416, found 361.28314; prep. HPLC: MeCN/0.1% TFA/aq (45/55). $C_{20}H_{35}N_5O$ · 2 TFA (589.26)

N-[3-(Cyclohexylmethyl)pentanoyl]-N´-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.85)

The title compound was prepared from **3.85a** (310 mg, 0.54 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.85** as a colourless oil (90 mg, 29 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.80 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 7.36 (d, 1H, ^4J = 1.0 Hz, Im-5-***H***), 3.38 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.84 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.42 (dd, 1H, ^3J = 5.2 Hz, ^3J = 13.4 Hz, COC***H***H), 2.35 (dd, 1H, ^3J = 4.5 Hz, ^3J = 13.3 Hz, COCH***H***), 2.01 (m, 3H, Im-4-CH$_2$C***H***$_2$, C***H***CH$_2$CH$_3$), 1.68 (m, 5H, cHex-C***H***, cHex-C***H***$_2$), 1.38-1.10 (m, 8H, C***H***$_2$CH$_3$, cHex-C***H***$_2$), 0.88 (t, 3H, ^3J =7.4Hz, C***H***$_3$), 0.85 (m, 2H, C***H***$_2$-cHex); ^{13}C-NMR (CD_3OD) δ (ppm): 177.42 (quart, ***C***=O), 155.39 (quart, ***C***=NH), 134.94 (+, Im-2-***C***), 134.32 (quat. Im-4-***C***), 117.13 (+, Im-5-***C***), 42.65 (-, 2 CO***C***H$_2$, ***C***H$_2$-cHex), 41.53 (-, ***C***H$_2$NH), 36.13 (+, ***C***HCH$_2$CH$_3$), 34.80 (-, cHex-***C***H$_2$), 34.70 (-, cHex-***C***H$_2$), 34.38 (+, cHex-***C***H), 27.97 (-, Im-4-CH$_2$***C***H$_2$), 27.78 (-, cHex-***C***H$_2$), 27.54 (-, CH***C***H$_2$CH$_3$), 27.46 (-, 2 cHex-***C***H$_2$), 22.55 (-, Im-4-***C***H$_2$), 10.92 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{19}H_{33}N_5O$] calcd. 347.2685, found 347.26848; prep. HPLC: MeCN/0.1% TFA/aq (35/65-45/55). $C_{19}H_{33}N_5O$ · 2 TFA (575.47)

N-[3-(3-Hydroxyphenyl)butanoyl]-N´-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.86)

The title compound was prepared from **3.86a** (160 mg) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.86** as a colourless oil (30 mg, 13 %, based on **3.86-Bn**), ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (d, 1H, ^4J = 1.2 Hz, Im-2-***H***), 7.34 (s, 1H, Im-5-***H***), 7.08 (m, 1H, Ar-***H***), 6.64 (m, 3H, Ar-***H***), 3.34 (m, overlap with solvent), 2H, C***H***$_2$NH), 3.21 (m, 1H, C***H***CH$_3$), 2.80 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 2.71 (d, 2H, ^3J = 7.5 Hz, COC***H***$_2$), 2.00 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.29 (d, 3H, ^3J = 7.0 Hz,

C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.07 (quart, ***C***=O), 158.68 (quat. Ar-***C***), 155.25 (quart, ***C***=NH), 148.00 (quat. Ar-***C***), 130.66 (+, Ar-***C***H), 118.98 (+, Ar-***C***H), 117.15 (+, Im-5-***C***), 114.85 (+, Ar-***C***H), 114.55 (+, Ar-***C***H), 46.11 (-, CO***C***H$_2$), 41.55 (-, ***C***H$_2$NH), 37.58 (+, ***C***HCH$_3$), 27.90 (-, Im-4-CH$_2$***C***H$_2$), 22.56 (-, Im-4-***C***H$_2$), 22.23 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for [$C_{17}H_{23}N_5O_2$] calcd. 329.1852, found 329.18541; prep. HPLC: MeCN/0.1% TFA/aq (20/80). $C_{17}H_{23}N_5O_2$ · 2 TFA (557.37)

***N*-[3-(4-Hydroxyphenyl)butanoyl]-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.87)**

The title compound was prepared from **3.87a** (280 mg, 0.40 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.87** as a colourless oil (20 mg, 9 %, based on **3.87a**), ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (s, 1H, Im-2-***H***), 7.35 (s, 1H, Im-5-***H***), 7.06 (m, 2H, Ar-***H***), 6.69 (m, 2H, Ar-***H***), 3.33 (m, 2H, C***H***$_2$NH), 3.22 (m, 1H, C***H***CH$_3$), 2.79 (t, 3H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 2.69 (d, 2H, ^3J = 7.5 Hz, COC***H***$_2$), 1.99 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.27 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); HRMS: EI-MS: *m/z* for [$C_{17}H_{23}N_5O_2$] calcd. 329.3968, found 329.18525; prep. HPLC: MeCN/0.1% TFA/aq (20/80-30/70). $C_{17}H_{23}N_5O_2$ · 2 TFA (557.37)

***N*-(4-Amino-3-phenylbutanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.88)**

The title compound was prepared from **3.88a** (200 mg, 0.30 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.88** as a colourless oil (60 mg, 30 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.77 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.33 (m, 6H, Im-5-***H***, Ar-***H***), 3.53 (m, 1H, C***H***CH$_2$NH$_2$), 3.34-3.21 (m, 4H, C***H***$_2$NH, C***H***$_2$NH$_2$), 3.01 (dd, 1H, ^3J = 6.2 Hz, ^2J = 16.3 Hz, COC***H***H), 2.92 (dd, 1H, ^3J = 8.4 Hz, ^2J = 16.3 Hz, COCH***H***), 2.80 (t, 2H, ^3J = 7.7 Hz, Im-4-C***H***$_2$), 1.99 (m, 2H, Im-4-CH$_2$C***H***$_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 174.77 (quart, ***C***=O), 155.13 (quart, ***C***=NH), 139.92 (quat. Ar-***C***), 134.90 (+, Im-2-***C***), 134.29 (quat. Im-4-***C***), 130.39 (+, 2 Ar-***C***H), 129.29 (+, Ar-***C***H), 129.08 (+, 2 Ar-***C***H), 117.13 (+, Im-5-***C***), 44.93 (-, ***C***H$_2$NH$_2$), 41.71 (-, ***C***H$_2$NH), 41.49 (-, CO***C***H$_2$), 41.19 (+, ***C***HCH$_2$NH$_2$), 27.87 (-, Im-4-CH$_2$***C***H$_2$), 22.50 (-, Im-4-***C***H$_2$); HRMS: FAB-MS: *m/z* for [$C_{17}H_{24}N_6O$ + H^+] calcd. 329.2012, found 329.20864; prep. HPLC: MeCN/0.1% TFA/aq (12.5/87.5). $C_{17}H_{24}N_6O$ · 3 TFA (670.17)

***N*-(6-Amino-3-phenylhexanoyl)-*N´*-[3-(1*H*-imidazol-4-yl)propyl]guanidine (3.89)**

The title compound was prepared from **3.89a** (260 mg, 0.37 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **3.89** as a colourless oil (120 mg, 46 %), ^{1}H-NMR (CD_3OD) δ ppm: 8.74 (s, 1H, Im-2-***H***), 7.31 (s,

1H, Im-5-***H***), 7.21 (m, 5H, Ar-***H***), 3.30 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$NH), 3.17 (m, 1H, C***H***$CH_2CH_2CH_2NH_2$), 2.83 (m, 6H, Im-4-C***H***$_2$, COC***H***$_2$, C***H***$_2NH_2$), 1.97 (m, 2H, Im-4-CH_2C***H***$_2$), 1.76 (m, 2H, C***H***$_2CH_2CH_2NH_2$), 1.49 (m, 2H, CH_2C***H***$_2CH_2NH_2$); ^{13}C-NMR (CD_3OD) δ (ppm): 175.85 (quart, ***C***=O), 155.20 (quart, ***C***=NH), 143.72 (quat. Ar-***C***), 134.87 (+, Im-2-***C***), 134.28 (quat. Im-4-***C***), 129.82 (+, 2 Ar-***C***H), 128.80 (+, 2 Ar-***C***H), 128.12 (+, Ar-***C***H), 117.12 (+, Im-5-***C***), 44.81 (+, ***C***HCH_2), 42.84 (-, ***C***H_2NH_2), 41.45 (-, ***C***H_2NH), 40.54 (-, CO***C***H_2), 33.77 (-, ***C***$H_2CH_2CH_2NH_2$), 27.88 (-, Im-4-CH_2***C***H_2), 26.58 (-, CH_2***C***$H_2CH_2NH_2$), 22.49 (-, Im-4-***C***H_2); HRMS: EI-MS: *m/z* for [$C_{19}H_{28}N_6O$ + H^+] calcd. 357.2325, found 357.24061; prep. HPLC: MeCN/0.1% TFA/aq (12.5/87.5). $C_{19}H_{28}N_6O$ · 3 TFA (698.20)

General procedure for the preparation of compounds 3.90-3.91

To a solution of **3.89** (1 eq) in MeCN was added NEt_3 (3 eq). Subsequently, a solution of NHS-4-F-benzoate or –propionate (0.8 eq), respectively, was added and stirred for 4-5 h at room temperature. The solvent was removed in *vacuo* and purified by preperative HPLC.

***N*-[6-(4-Fluorobenzamido)-3-phenylhexanoyl]-*N´*-[3-(1*H*-imidazol-4-yl)propyl]-guanidine (3.90)**

The title compound was prepared from **3.89** (43.5 mg, 0.0623 mmol) in 1.5 ml MeCN, NEt_3 (0.026 ml, 0.187 mmol) and NHS-4-F-benzoate (11.8 mg, 0.0498 mmol) in 0.5 ml MeCN according to the general procedure yielding **3.90** (30 mg, 85 %) as a colourless oil. ^{1}H-NMR (CD_3OD) δ ppm: 8.77 (d, 1H, ^4J = 1.4 Hz, Im-2-***H***), 7.81 (m, 2H, Ar-***H***), 7.29-7.16 (m, 8H, Ar-***H***), 3.32 (m, 4H, C***H***$_2$NHCO, C***H***$_2$NH), 3.19 (m, 1H, C***H***$CH_2CH_2CH_2NH$), 2.81 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 1.96 (m, 2H, Im-4-CH_2C***H***$_2$), 1.74 (m, 2H, C***H***$_2CH_2CH_2NH$), 1.48 (m, 2H, CH_2C***H***$_2CH_2NH$); ^{13}C-NMR (CD_3OD) δ (ppm): 176.01 (quart, ***C***=O), 169.09 (quart, ***C***=O), 166.16 (d, quart., ^1J = 250.16 Hz, Ar-***C***F), 155.19 (quart, ***C***=NH), 144.39 (quat. Ar-***C***), 134.96 (+, Im-2-***C***), 134.29 (quat. Im-4-***C***), 132.19 (d, +, ^4J = 3.2 Hz, Ar-**C**H), 130.83 (d, +, ^3J = 8.9 Hz, Ar-**C**H), 129.71 (+, 2 Ar-***C***H), 128.78 (+, 2 Ar-***C***H), 127.91 (+, Ar-***C***H), 117.12 (+, Im-5-***C***), 116.35 (d, +, ^2J = 22.13 Hz, Ar-**C**H), 45.05 (-, CO***C***H_2), 42.96 (+, ***C***HCH_2), 41.52 (-, ***C***H_2NH), 40.71 (-, ***C***H_2NHCO), 34.45 (-, ***C***$H_2CH_2CH_2NH_2$), 28.38 (-, CH_2***C***H_2CH_2NH), 27.87 (-, Im-4-CH_2***C***H_2), 22.53 (-, Im-4-***C***H_2); HRMS: EI-MS: *m/z* for [$C_{26}H_{31}FN_5O_2$] calcd. 478.24925, found 478.24833; prep. HPLC: MeCN/0.1% TFA/aq (30/70-50/50). $C_{26}H_{31}FN_6O_2$ · 2 TFA (706.54)

***N*-[3-(1*H*-Imidazol-4-yl)propyl]-*N´*-(3-phenyl-6-propionamidohexanoyl)guanidine (3.91)**

The title compound was prepared from **3.89** (43.5 mg, 0.0623 mmol) in 1.5 ml MeCN, NEt_3 (0.026 ml, 0.187 mmol) and NHS-propionate (8.79 mg, 0.0498 mmol) in 0.5 ml MeCN according to the general procedure yielding **3.91** (40 mg, 94 %) as a colourless oil. ^{1}H-NMR (CD_3OD) δ ppm: 8.78 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.24 (m, 6H, Ar-***H***), 3.32 (m, 2H, C***H***$_2$NHCO), 3.15 (m, 3H, C***H***$_2$NH, C***H***$CH_2CH_2CH_2NH$), 2.79 (m, 4H, Im-4-C***H***$_2$, COC***H***$_2$), 2.14 (q, 2H, COC***H***$_2CH_3$), 1.97 (m, 2H, Im-4-CH_2C***H***$_2$), 1.69 (m, 2H, C***H***$_2CH_2CH_2NH$), 1.34 (m, 2H, CH_2C***H***$_2CH_2NH$), 1.08 (t, 3H, ^3J = 7.6 Hz, $COCH_2$C***H***$_3$); ^{13}C-NMR (CD_3OD) δ (ppm): 177.05 (quart, ***C***=O), 175.99 (quart, ***C***=O), 155.20 (quart, ***C***=NH), 144.37 (quat. Ar-***C***), 134.96 (+, Im-2-***C***), 134.30 (quat. Im-4-***C***), 129.69 (+, 2 Ar-***C***H), 128.76 (+, 2 Ar-***C***H), 127.90 (+, Ar-***C***H), 117.13 (+, Im-5-***C***), 45.02 (-, CO***C***H_2), 42.97 (+, ***C***HCH_2), 41.52 (-, ***C***H_2NH), 40.06 (-, ***C***H_2NHCO), 34.42 (-, ***C***$H_2CH_2CH_2NH_2$), 30.24 (-, ***C***H_2CH_3), 28.37 (-, CH_2***C***H_2CH_2NH), 27.88 (-, Im-4-CH_2***C***H_2), 22.53 (-, Im-4-***C***H_2), 10.60 (+, ***C***H_3); HRMS: EI-MS: *m/z* for [$C_{22}H_{32}N_6O_2$] calcd. 412.25867, found 412.25828; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50). $C_{22}H_{32}N_6O_2$ · 2 TFA (640.51)

(*E*)-4-[2-(1,2,3,5,6,7-Hexahydropyrido[3,2,1-ij]quinolin-9-yl)ethenyl]-2,6-dimethyl-1-[7-[[3-(1*H*-Imidazol-4-yl)propyl]carbamimidoyl]amino-7-oxo-4-phenylhexyl]-pyridinium trifluoroacetate (3.92)

To a solution of **3.89** (5.53 mg, 7.9 µmol) in 800 µl MeCN was added NEt_3 (4 µl, 29.7 µmol). Subsequently, a solution of the dye py-1[97] ((*E*)-4-[2-(1,2,3,5,6,7-hexahydro-pyrido[3,2,1-ij]quinolin-9-yl)ethenyl]-2,6-dimethylpyrylium tetrafluoroborate, 1.54 mg, 3.96 µmol) in 50 µl DMF and 100 µl MeCN was added. After 1-2 min a red colour occurred and after 1 h 10% TFA/aq (35 µl) was added and purified by preperative HPLC (MeCN/0.1% TFA/aq (40/60-70/30)). **3.92** was obtained as a red oil (1.84 mg, 53 %). HRMS: PI-LSIMS: *m/z* for [$C_{40}H_{50}N_7O$] calcd. 644.40714, found 644.4050. $C_{40}H_{50}N_7O$ · 3 TFA (986.84).

3.4. References

(1) 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, (3), 253-278.

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

(3) Traiffort, E.; Pollard, H.; Moreau, J.; Ruat, M.; Schwartz, J. C.; Martinez-Mir, M. I.; Palacios, J. M., Pharmacological Characterization and Autoradiographic Localization of Histamine H_2 Receptors in Human Brain Identified with [125I]Iodoaminopotentidine. *J. Neurochem.* **1992,** 59, (1), 290-299.

(4) Del Valle, J.; Gantz, I., Novel insights into histamine H_2 receptor biology. *Am J Physiol Gastrointest Liver Physiol* **1997,** 273, (5), G987-996.

(5) 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, (8), 1963-70.

(6) Schmuck, C.; Lex, J., Acetate Binding within a Supramolecular Network Formed by a Guanidiniocarbonyl Pyrrole Cation in the Solid State. *Org. Lett.* **1999,** 1, (11), 1779-1781.

(7) 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/volltexte/2006/561/

(8) Keller, M. HPLC-MS investigations on absorption and brain penetration of histamine H_2 receptor agonists and NPY antagonists. Diploma Thesis, University of Regensburg, Germany, 2004.

(9) 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, (6), 1210-1225.

(10) Burde, R.; Buschauer, A.; Seifert, R., Characterization of histamine H_2-receptors in human neutrophils with a series of guanidine analogues of impromidine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1990,** 341, (5), 455-461.

(11) 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, (23), 8432-8439.

(12) Pirrung, M. C.; Pei, T., Synthesis of (+/-)-Homohistidine. *J. Org. Chem.* **2000,** 65, (7), 2229-2230.

(13) Matsuda, F.; Itoh, S.; Hattori, N.; Yanagiya, M.; Matsumoto, T., A simple method for synthesis of amides and peptides through acyl chlorides : A rapid synthesis of thyrotropin releasing hormone. *Tetrahedron* **1985,** 41, (18), 3625-3631.

(14) Ohta, S.; Hayakawa, S.; Moriwaki, H.; Tsuboi, S.; Okamoto, M., Synthesis of 2-acyl-1-methyl-1*H*-imidazoles and reactivity of the acyl group. *Heterocycles* **1985,** 23, (7), 1759-64.

(15) Ohta, S.; Hayakawa, S.; Nishimura, K.; Okamoto, M., Synthesis and application of imidazole derivatives. Synthesis of (1-methyl-1*H*-imidazol-2-yl)methanol derivatives and conversion into carbonyl compounds. *Chem. Pharm. Bull. (Tokyo).* **1987,** 35, (3), 1058-69.

(16) Jansen, M.; Potschka, H.; Brandt, C.; Loscher, W.; Dannhardt, G., Hydantoin-Substituted 4,6-Dichloroindole-2-carboxylic Acids as Ligands with High Affinity for the Glycine Binding Site of the NMDA Receptor. *J. Med. Chem.* **2003,** 46, (1), 64-73.

(17) Cantor, S. E.; Tarbell, D. S., The Formation of cis- and trans-Perhydrobenzofurans from 2-(2-Methoxycyclohexyl)ethanol Derivatives. Reactions Proceeding through Methoxyl Participation. *J. Am. Chem. Soc.* **1964,** 86, (14), 2902-2909.

(18) Staab, H. A., Neuere Methoden der präparativen organischen Chemie IV Synthesen mit heterocyclischen Amiden (Azoliden). *Angew. Chem.* **1962,** 74, (12), 407-423.

(19) Black, J. W.; Duncan, W. A. M.; Durant, C. J.; Ganellin, C. R.; Parsons, E. M., Definition and Antagonism of Histamine H_2-receptors. *Nature* **1972,** 236, (5347), 385-390.

(20) Kraus, A. Histamin-H_2-agonistisch wirksame N^G-acylierte Imidazolylpropylguanidine: Synthese und pharmakologische Charakterisierung. Diploma thesis, University of Regensburg, Germany, 2004.

(21) Lopuch, M. G-Protein coupled receptors: histamine receptors - New N^G-acylated imidazolylpropylguanidines as potential selective histamine H_2 receptor agonists – synthesis and pharmacological investigation. Master Thesis, Jagiellonian University, Kraków, 2006.

(22) 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, (1), 139-146.

(23) Xie, S.-X.; Kraus, A.; Ghorai, P.; Ye, Q.-Z.; Elz, S.; Buschauer, A.; Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1H-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, (3), 1262-1268.

(24) Götte, C. Synthese, Enantiomerentrennung und Struktur-Wirkungsbeziehungen neuer Histamin H_2 Rezeptoragonisten des Arpromidin Typs. Doctoral Thesis, University of Regensburg, Germany, 2001.

(25) Seifert, R.; Wenzel-Seifert, K.; Burckstummer, 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 H1-Receptor. *J. Pharmacol. Exp. Ther.* **2003,** 305, (3), 1104-1115.

(26) Kracht, J. Bestimmung der Affinität und Aktivität subtypselektiver Histamin- und Neuropeptid Y-Rezeptorliganden an konventionellen und neuen pharmakologischen In-vitro-Modellen. Doctoral Thesis, University of Regensburg, Germany, 2001.

(27) 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/volltexte/2007/843/

(28) Stark, H.; Kathmann, M.; Schlicker, E.; Schunack, W.; Schlegel, B.; Sippl, W., Medicinal Chemical and Pharmacological Aspects of Imidazole-Containing Histamine H_3 Receptor Antagonists. *Mini Rev. Med. Chem.* **2004,** 4, 965-977.

(29) Stark, H.; Lipp, R.; Arrang, J. M.; Garbarg, M.; Schwartz, J. C.; Schunack, W., Acylated and alkylated histamine derivatives as new histamine H_3-receptor antagonists. *Eur. J. Med. Chem.* **1994,** 29, (9), 695-700.

(30) de Esch, I. J. P.; Belzar, K. J., Histamine H_3 receptor agonists. *Mini Rev. Med. Chem.* **2004,** 4, (9), 955-963.

(31) Ziemek, R. Development of binding and functional assays for the neuropeptide Y Y_2 and Y_4 receptors. Doctoral Thesis, University of Regensburg, Germany, 2006. http://www.opus-bayern.de/uni-regensburg/volltexte/2006/679/

(32) Ziemek, R.; Schneider, E.; Kraus, A.; Cabrele, C.; Beck-Sickinger, A.; Bernhard, G.; Buschauer, A., Dertermination of affinity and activity of ligands at the human neuropeptide Y Y_4 receptor by flow cytometry and aequorin luminescence. *J. Recept. Signal Transduct. Res.* **2007,** 27, (4), 217-33.

(33) Kim, S. H.; Rieke, R. D., Benzylic Manganese Halides, Sulfonates, and Phosphates: Preparation, Coupling Reactions, and Applications in Organic Synthesis. *J. Org. Chem.* **2000,** 65, (8), 2322-2330.

(34) Suh, Y.; Lee, J.-s.; Kim, S.-H.; Rieke, R. D., Direct preparation of benzylic manganese reagents from benzyl halides, sulfonates, and phosphates and their reactions: applications in organic synthesis. *J. Organomet. Chem.* **2003,** 684, (1-2), 20-36.

(35) Hirabayashi, T.; Okimoto, Y.; Saito, A.; Morita, M.; Sakaguchi, S.; Ishii, Y., Iridium complex-catalyzed addition of water and alcohols to non-activated terminal alkynes. *Tetrahedron* **2006,** 62, (10), 2231-2234.

(36) Donkor, I. O.; Li, H.; Queener, S. F., Synthesis and DHFR inhibitory activity of a series of 6-substituted-2,4-diaminothieno[2,3-d]pyrimidines. *Eur. J. Med. Chem.* **2003,** 38, (6), 605-611.

(37) Ota, S.; Hayakawa, S.; Nishimura, K.; Okamoto, M., Synthesis and application of imidazole derivatives. Synthesis of (1-methyl-1H-imidazol-2-yl)methanol derivatives and conversion into carbonyl compounds. *Chem. Pharm. Bull.* **1987,** 35, (3), 1058-69.

(38) Gilmore, N. J.; Jones, S.; Muldowney, M. P., Synthetic Applicability and *in Situ* Recycling of a *B*-Methoxy Oxazaborolidine Catalyst Derived from *cis*-1-Amino-indan-2-ol. *Org. Lett.* **2004,** 6, (16), 2805-2808.

(39) Williams, G. D.; Pike, R. A.; Wade, C. E.; Wills, M., A One-Pot Process for the Enantioselective Synthesis of Amines via Reductive Amination under Transfer Hydrogenation Conditions. *Org. Lett.* **2003,** 5, (22), 4227-4230.

(40) Lin, C.-F.; Yang, J.-S.; Chang, C.-Y.; Kuo, S.-C.; Lee, M.-R.; Huang, L.-J., Synthesis and anticancer activity of benzyloxybenzaldehyde derivatives against HL-60 cells. *Bioorg. Med. Chem.* **2005,** 13, (5), 1537-1544.

(41) Kawamatsu, Y.; Sohda, T.; Imai, Y., 2-Amino-4-phenylthiazole derivatives as anti-atherogenic agents. *Eur. J. Med. Chem.* **1981,** 16, (4), 355-62.

(42) Jurkauskas, V.; Sadighi, J. P.; Buchwald, S. L., Conjugate Reduction of a, b-Unsaturated Carbonyl Compounds Catalyzed by a Copper Carbene Complex. *Org. Lett.* **2003,** 5, (14), 2417-2420.

(43) Appella, D. H.; Moritani, Y.; Shintani, R.; Ferreira, E. M.; Buchwald, S. L., Asymmetric Conjugate Reduction of a, b-Unsaturated Esters Using a Chiral Phosphine-Copper Catalyst. *J. Am. Chem. Soc.* **1999,** 121, (40), 9473-9474.

(44) Budhram, R. S.; Palaniswamy, V. A.; Eisenbraun, E. J., Steric effects in the synthesis of 1,7-dialkylindans. *J. Org. Chem.* **1986,** 51, (9), 1402-6.

(45) Huang, X.; Zhao, C.-Q., Stereoselecive Synthesis of 3-Alkylcinnamic Esters *via* Coupling Reaction of (2Z)-3-(Aryltelluro)cinnamic Esters in Presence of CuI. *Synth. Commun.* **1997,** 27, (2), 237 - 241.

(46) Duan, X.-G.; Duan, X.-L.; Rees, C. W., Reaction of trithiazyl trichloride with active methylene compounds. *J. Chem. Soc., Perkin Trans. 1* **1997**, (19), 2831-2836.

(47) Srikrishna, A.; Reddy, T. J.; Kumar, P. P.; Vijaykumar, D., A methodology for spirocyclopentannulation. Stereoselective total synthesis of (+/-)-tochuinyl acetate and (+/-)-dihydrotochuinyl acetate. *Synlett* **1996**, (1), 67-8.

(48) Cavero, M.; Motherwell, W. B.; Potier, P.; Weibel, J.-M., Thioepoxide formation by ring closure of allylthiyl radicals-a novel rearrangement of allylic thionitrites. *Chem. Commun. (Camb.)* **2002**, (20), 2394-2395.

(49) Zhou, C.; Shao, Y.; Gibbs, R. A., Aromatic farnesyl diphosphate analogues: vinyl triflate-mediated synthesis and preliminary enzymatic evaluation. *Bioorg. Med. Chem. Lett.* **2002,** 12, (10), 1417-1420.

(50) Valcavi, U.; Gaudenzi, L., Synthetic anticholesterolemic compounds. IV. Parasubstituted derivatives of b-benzylidenebutyric acid. *Farmaco, Ed. Sci.* **1961,** 16, 571-6.

(51) Horii, Z.; Hanaoka, M.; Kim, S.; Tamura, Y., Synthesis of musizin. *J. Chem. Soc.* **1963**, (Aug.), 3940-5.

(52) Yamamoto, Y.; Hatsuya, S.; Yamada, J., Stabilization and activation of dienolates with germanium and tin. Stereo- and regioselective aldol reactions, regioselective coupling reactions, and regioselective synthesis of amino acid derivatives. *J. Org. Chem.* **1990,** 55, (10), 3118-28.

(53) Kuchar, M.; Brunova, B.; Rejholec, V.; Jelinkova, M.; Holubek, J.; Nemecek, O., Quantitative relations between structure and activation of fibrinolysis in selected series of arylaliphatic acids. *Collect. Czech. Chem. Commun.* **1984,** 49, (1), 122-36.

(54) Steinhuebel, D.; Baxter, J. M.; Palucki, M.; Davies, I. W., Enol Tosylates as Viable Partners in Pd-Catalyzed Cross-Coupling Reactions. *J. Org. Chem.* **2005,** 70, (24), 10124-10127.

(55) Alig, L.; Edenhofer, A.; Hilpert, K.; Weller, T. Preparation of thiazolecarboxamides as vitronectin receptor antagonists. EP 928790, **1999**, CAN 131:116229.

(56) Galemmo, R. A., Jr.; Johnson, W. H., Jr.; Learn, K. S.; Lee, T. D. Y.; Huang, F. C.; Campbell, H. F.; Youssefyeh, R.; O'Rourke, S. V.; Schuessler, G.; et al., The development of a novel series of (quinolin-2-ylmethoxy)phenyl-containing compounds as high-affinity leukotriene receptor antagonists. 3. Structural variation of the acidic side chain to give antagonists of enhanced potency. *J. Med. Chem.* **1990,** 33, (10), 2828-41.

(57) Srikrishna, A.; Viswajanani, R.; Sattigeri, J. A.; Vijaykumar, D., A new, convenient reductive procedure for the deprotection of 4-methoxybenzyl (MPM) ethers to alcohols. *J. Org. Chem.* **1995,** 60, (18), 5961-2.

(58) Katritzky, A. R.; Feng, D.; Lang, H., Benzotriazole- and 1,2,4-Triazole-Stabilized Allylic Anions: Applications in Syntheses of Functionalized a, b-Unsaturated Ketones, g-Lactones, g-Lactams, and b-Substituted Esters. *J. Org. Chem.* **1997,** 62, (3), 706-714.

(59) Tsuchiya, Y.; Kanazawa, Y.; Shiomi, T.; Kobayashi, K.; Nishiyama, H., Asymmetric conjugate reduction of a,b-unsaturated esters with chiral rhodium(bisoxazolinylphenyl) catalysts. *Synlett* **2004**, (14), 2493-2496.

(60) Gaudiano, G.; Merlini, L., Synthesis of 2-amino-4-phenylisocaprylic acid. *Gazz. Chim. Ital.* **1961,** 91, 400-11.

(61) Rousseau, G.; Blanco, L., Reaction of silylketene acetals with 3,3-dimethylacryloyl chloride. *Tetrahedron Lett.* **1985,** 26, (35), 4195-4196.

(62) Sato, S.; Matsuda, I.; Izumi, Y., Rhodium-catalyzed isomerization of 1,3-diene monoepoxides to a, b-unsaturated carbonyl compounds. *J. Organomet. Chem.* **1989,** 359, (2), 255-266.

(63) Kowalski, C. J.; Haque, M. S.; Fields, K. W., Ester homologation via a-bromo a-keto dianion rearrangement. *J. Am. Chem. Soc.* **1985,** 107, (5), 1429-30.

(64) Hata, T.; Nakajima, M.; Sekine, M., Facile synthesis of b-alkyl-substituted esters from a, b-unsaturated aldehydes. *Tetrahedron Lett.* **1979,** 20, (22), 2047-2050.

(65) Baddar, F. G.; Dwidar, I. M.; Gindy, M., b-Aroylpropionic acids. IX. Their conversion into 1,2-benzanthraquinones. *J. Chem. Soc.* **1959**, 1002-9.

(66) Scopton, A.; Kelly, T. R., Synthesis of HKI 0231B. *J. Org. Chem.* **2005,** 70, (24), 10004-10012.

(67) Meyer, O.; Becht, J.-M.; Helmchen, G., Enantioselective synthesis of (-)-(R)-Baclofen and analogues via rhodium-catalyzed conjugate addition of boronic acids. *Synlett* **2003**, (10), 1539-1541.

(68) Menghin, S.; Pertz, H. H.; Kramer, K.; Seifert, R.; Schunack, W.; Elz, S., *N*a-Imidazolylalkyl and Pyridylalkyl Derivatives of Histaprodifen: Synthesis and in Vitro Evaluation of Highly Potent Histamine H_1Receptor Agonists. *J. Med. Chem.* **2003,** 46, (25), 5458-5470.

(69) Aurell, M. J.; Domingo, L. R.; Mestres, R.; Munoz, E.; Zaragoza, R. J., Conjugate addition of organolithium reagents to a,b-unsaturated carboxylic acids. *Tetrahedron* **1999,** 55, (3), 815-830.

(70) Reed, P. E.; Katzenellenbogen, J. A., b-Substituted b-phenylpropionyl chymotrypsins. Structural and stereochemical features in stable acyl enzymes. *J. Med. Chem.* **1991,** 34, (3), 1162-1176.

(71) Vebrel, J.; Carrie, R., Synthesis of methoxycarbonylated indenes, 1,2-dihydronaphthalenes, and benzocycloheptene. Preparation of the starting 1-indanones, 1-tetralones, and benzosuberone. *Bull. Soc. Chim. Fr.* **1982**, (3-4, Pt. 2), 116-24.

(72) Wali, M. A.; Khalil, A. K.; Bhatia, R. L.; Ahmad, S. S., Friedel-Crafts reaction. V. Effect of polar substituents on the reactivity of *p*-substituted phenylsuccinic anhydrides with simple aromatic hydrocarbons. *Proceedings - Indian Academy of Sciences, Section A* **1941,** 14A, 139-50.

(73) Nelson, S. G.; Wan, Z.; Stan, M. A., S_N2 Ring Opening of ^{b}-Lactones: An Alternative to Catalytic Asymmetric Conjugate Additions. *J. Org. Chem.* **2002,** 67, (14), 4680-4683.

(74) O'Brien, E. M.; Bercot, E. A.; Rovis, T., Decarbonylative Cross-Coupling of Cyclic Anhydrides: Introducing Stereochemistry at an sp3 Carbon in the Cross-Coupling Event. *J. Am. Chem. Soc.* **2003,** 125, (35), 10498-10499.

(75) Wu, B.; Avery, B. A.; Avery, M. A., Conjugate addition of a primary carbon radical to a,b-unsaturated carboxylic acids. *Tetrahedron Lett.* **2000,** 41, (20), 3797-3800.

(76) Galust'yan, G. G.; Rashkes, A. M.; Barashkin, V. A.; Kadyrov, C. S.; Seitanidi, K. L., Free-radical addition of alkylbenzenes to a,b-unsaturated acids. *Uzb. Khim. Zh.* **1979**, (4), 25-33.

(77) Bymaster, D. L.; Pickering, R. E.; Dobbs, T. K.; Eisenbraun, E. J., Synthesis of 2,6-dimethyl-1,2,3,4-tetrahydronaphthalene-4-^{13}C and 2,6-dimethylnaphthalene-4-^{13}C. *J. Labelled Comp. Radiopharm.* **1986,** 23, (6), 657-65.

(78) Kelley, J. L.; Rigdon, G. C.; Cooper, B. R.; McLean, E. W.; Musso, D. L.; Orr, G. F.; Selph, J. L.; Styles, V. L. Preparation of indan-1-ylideneacetamides and analogs as muscle relaxants. WO 9426692, **1994**, CAN 123:143454.

(79) Muller, A.; Meszaros, M.; Lempert-Sreter, M.; Szara, I., Dimeric propenyl phenol ethers. XIII. On metanethole and its tetralin isomers. *J. Org. Chem.* **1951,** 16, 1003-24.

(80) Brunner, O.; Grof, F., Methylethylnaphthalenes. III. Synthesis of 2-methyl-6-ethylnaphthalene. *Monatsh. Chem.* **1935,** 66, 433-7.

(81) Zelle, R. E., Resolution of 4-phenyl-2-pyrrolidinone: a versatile synthetic intermediate. *Synthesis* **1991**, (11), 1023-6.

(82) Rotthaus, O.; LeRoy, S.; Tomas, A.; Barkigia, K. M.; Artaud, I., A new family of low-spin CoIII bis(amidate) complexes with two cis or trans cyanides. *Eur. J. Inorg. Chem.* **2004**, (7), 1545-1551.

(83) Katritzky, A. R.; Aurrecoechea, J. M., Alkylation of monosubstituted malonate anions with pyridinium and quinolinium salts. *Synthesis* **1987**, (4), 342-5.

(84) Sugasawa, S.; Ushioda, S., Extension of the Bischler-Napieralski reaction--II : Synthesis of pyrroline derivatives. *Tetrahedron* **1959,** 5, (1), 48-52.

(85) Yamada, I.; Ohkouchi, M.; Yamaguchi, M.; Yamagishi, T., Asymmetric hydrogenation of acrylic acid derivatives with novel chiral rhodium-phosphinediamine complex catalysts by selective ligation between two amino units of the ligand and electrostatic interaction. *J. Chem. Soc., Perkin Trans. 1* **1997**, (12), 1869-1873.

(86) Iso, Y.; Shindo, H.; Hamana, H., Efficient Synthesis of Resin-Bound a-TMSdiazoketones and Their Use in Solid-Phase Organic Synthesis. *Tetrahedron* **2000,** 56, (30), 5353-5361.

(87) Levene, P. A.; Marker, R. E., The maximum rotations of configurationally related carboxylic acids containing a phenyl or a cyclohexyl group *J. Biol. Chem.* **1935,** 110, (2), 329-342.

(88) Tomioka, K.; Suenaga, T.; Koga, K., Asymmetric conjugate addition reaction by the use of ()-g-trityloxymethyl-g-butyrolactam as a chiral auxiliary. *Tetrahedron Lett.* **1986,** 27, (3), 369-372.

(89) Ogawa, K.; Terada, T.; Muranaka, Y.; Hamakawa, T.; Fujii, S., Studies on hypolipidemic agents. III. Synthesis and esterase-inhibitory activity of w-cycloalkyl-2-oxoalkylarenesulfonates. *Chem. Pharm. Bull. (Tokyo).* **1987,** 35, (6), 2426-36.

(90) Levene, P. A.; Marker, R. E., Maximum rotations of phenyl compounds. *J. Biol. Chem.* **1933,** 100, 685-94.

(91) Giese, B.; Harnisch, H.; Lachhein, S., C-C bond formation by radical addition to alkenes. *Synthesis* **1983**, (9), 733.

(92) Levene, P. A.; Marker, R. E., The configurational relationship of acids of the phenethyl series to those of the normal series *J. Biol. Chem.* **1935,** 110, (2), 311-321.

(93) Streinz, L.; Romanuk, M.; Sorm, F., Natural and synthetic materials with insect hormone activity. XXI. Preparation of derivatives of w-cyclohexyl-2-alkenoic acids. *Collect. Czech. Chem. Commun.* **1974,** 39, (7), 1898-1904.

(94) Schunack, W., Äther und Ester des 4-(2-Hydroxyaethyl)-imidazols und Ester der 4-Imidazolpropionsäure 4. Mitt. über Struktur-Wirkungs-Beziehungen bei Histaminanaloga. *Arch. Pharm. (Weinheim).* **1974,** 307, (7), 517-523.

(95) Stark, H.; Purand, K.; Huls, A.; Ligneau, X.; Garbarg, M.; Schwartz, J. C.; Schunack, W., [125I]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, (6), 1220-1226.

(96) Nystrom, R. F.; Brown, W. G., Reduction of Organic Compounds by Lithium Aluminum Hydride. II. Carboxylic Acids. *J. Am. Chem. Soc.* **1947,** 69, (10), 2548-2549.

(97) Höfelschweiger, B. The Pyrilium Dyes: A New Class of Biolabels. Synthesis, Spectroscopy, and Application as Labels and in General Protein Assays. Doctoral Thesis, University of Regensburg, Germany, 2005.
http://www.opus-bayern.de/uni-regensburg/volltexte/2005/540/

Chapter 4

N^G-Acylated Aminothiazolylpropylguanidines: Towards Selective Histamine H_2R Agonists

4.1. Introduction

The N^G-acylated imidazolylpropylguanidines described in chapter 3 are highly potent histamine H_2 receptor (H_2R) agonists with improved pharmacokinetic properties compared to the corresponding strongly basic N^G-alkylated analogues. However, depending on the substitution pattern, these compounds turned out to be also more or less active on other histamine receptors, in particular histamine H_3 (H_3R) and H_4 receptors (H_4R). H_3R affinity is very often found in compounds having an imidazol-4-yl moiety, which is also present in numerous highly potent and selective histamine H_3R ligands. Therefore, the bioisosteric replacement of the imidazolyl ring is the key to improve the selectivity for H_2R over H_3R.

Amthamine (**AMT**) (Figure 4.1), a thiazole analogue of histamine (**HIS**) and a cyclic analogue of dimaprit (**DIM**), is a full histamine H_2R agonist and exhibits a slightly higher potency than histamine (**HIS**) at the guinea pig right atrium[1]. Moreover, amthamine proved to be devoid of histamine H_1R and H_3R stimulatory activities[2, 3]. In contrast to histamine with an aromatic imidazole ring, amthamine is not qualified to exist in two tautomeric forms which were thought to be responsible for H_2R activation[4]. A new activation model, based on quantum chemical studies, suggests that H_2R agonists accept a proton from the proton-donating receptor site on their double-bonded (heteroaromatic) nitrogen atom. According to this model agonistic activity of tautomeric as well as nontaumeric histamine H_2R agonists can be explained[5].

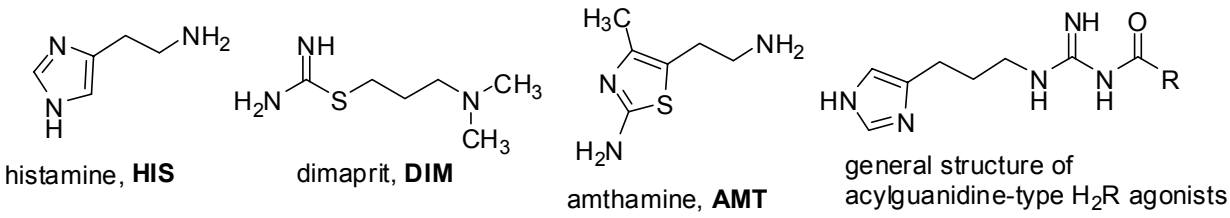


Figure 4.1. Structures of histamine and selective H_2 receptor agonists.

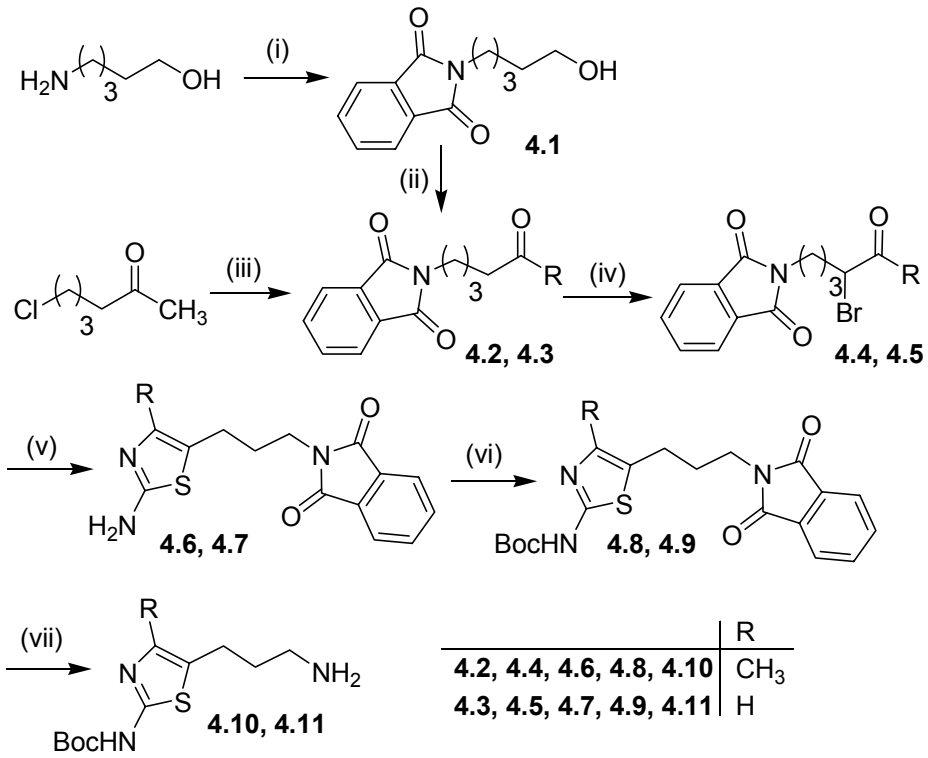
In the 1970s methyl groups were introduced at different positions of imidazole ring and side chain in order to study the structure-acitivity relationships of histamine[6, 7]. 4(5)-Methylhistamine was the first H_2R selective agonist described at that time, when only H_1R and H_2R were known. Meanwhile 4(5)-methylhistamine turned out to be a high affinity H_4R agonist. However, by analogy with the SAR of natural ligand the introduction of a 4-methyl substituent in 2-amino-5-(2-aminoethyl)thiazole led to an increase in agonistic activity at the guinea pig right atrium[1] and the H_2R selectivity of amthamine might be attributed to this methyl group.

Preliminary investigations revealed that the bioisosteric replacement of the imidazole ring in N^G-acylated imidazolylpropylguanidines against a thiazole moiety is possible without affecting the potency, but with increasing the selectivity for the H_2R, particularly over the H_3R[8]. This approach was further explored as a sub-project of this thesis. In this chapter, the synthesis and pharmacological investigation of N^G-acylated 2-amino- and 2-amino-4-methylthiazol-5-ylpropylguanidines is reported. Furthermore, the role of the 4-methyl substituent in the thiazole ring will be discussed.

4.2. Chemistry

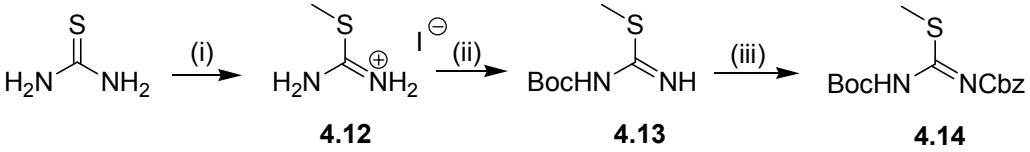
The thiazole building blocks were synthesized from thiourea and N-protected α-halo-ω-amino ketones or aldehydes, respectively. 6-Phthalimidohexan-2-one (**4.2**) was prepared from 6-chloro-2-hexanone and phthalimide (Scheme 4.1). The synthesis of 5-phthalimidopentanal (**4.3**) started from 5-aminopentanol which was treated with phthalic anhydride to give 5-phthalimidopentanol (**4.1**) and then subjected to Swern[9] oxidation to yield the corresponding aldehyde **4.3**. The compounds **4.2** and **4.3** were α-brominated in dioxane and DCM/abs, and the ring-closure reaction was carried out with thiourea in DMF. After *tert*-butoxycarbonyl (Boc) protection of the free amine function, the phthalimide group was cleaved by hydrazinolysis to give *tert*-butyl 5-(3-

aminopropyl)-4-methylthiazol-2-ylcarbamate **4.10** and *tert*-butyl-5-(3-aminopropyl)-thiazol-2-ylcarbamate **4.11**, respectively (Scheme 4.1)[1].



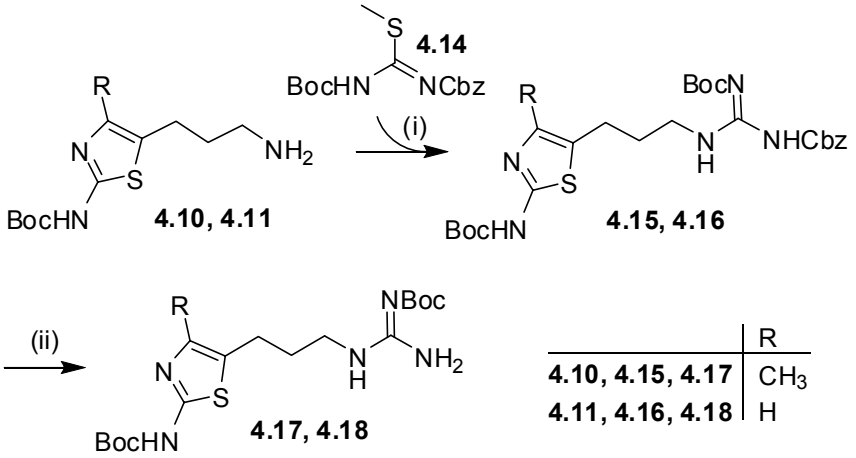
Scheme 4.1. Synthesis of the N^2-Boc-protected 2-aminothiazol-5-ylpropylamines **4.10** and **4.11**; Reagents and conditions: (i) phthalic anhydride (1 eq), 3 h, 80-100 °C; (ii) $C_2Cl_2O_2$ (1.25 eq), DMSO (2.65 eq), NEt_3 (5.5 eq), DCM/abs, -50 °C, 45 min; (iii) phthalimide (0.5 eq), K_2CO_3 (0.75 eq), DMF, 24 h, 80 °C; (iv) Br_2 (1 eq), dioxane, DCM/abs, 1h, rt; (v) thiourea (1 eq), DMF, 3 h, 100 °C; (vi) $(Boc)_2O$ (1.08 eq), NEt_3 (1.16 eq), DMAP (cat.), $CHCl_3$, overnight, rt; (vii) $N_2H_2{\cdot}H_2O$ (5 eq), EtOH, overnight, rt.

N,*N´*-Diprotected S-methylthioureas[10-12] are used for guanidinylation reactions with amines. By the use of electron withdrawing substituents, like Boc- or Cbz-protecting groups, at the N-atoms, the reactivity of the S-methylthiourea can be increased. *N*-Boc-*N´*-Cbz-S-methylthiourea **4.14** was prepared according to Scheme 4.2 by stepwise acylation of S-methylthiourea **4.12** using standard methods.



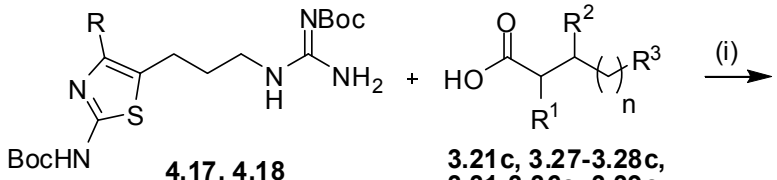
Scheme 4.2. Synthesis of *N*-tert-butoxycarbonyl-*N´*-benzyloxycarbonyl-S-methylthiourea **4.14**; Reagents and conditions: (i) MeI (1 eq), MeOH, 1h, reflux; (ii) $(Boc)_2O$ (1 eq), NEt_3 (1 eq), DCM/abs, overnight, rt; (iii) CbzOSu (1 eq), DCM/abs, 20 h, rt.

The guanidinylating reagent **4.14** was treated with the amines **4.10** and **4.11** in the presence of $HgCl_2$, whereby the metal ion acts as a desulfurizing agent *via* a complex formation[13, 14]. Hydrogenolytic cleavage of the Cbz-protecting group yielded the Boc-protected aminothiazolylpropylguanidine building blocks **4.17** and **4.18** in good yields (Scheme 4.3).



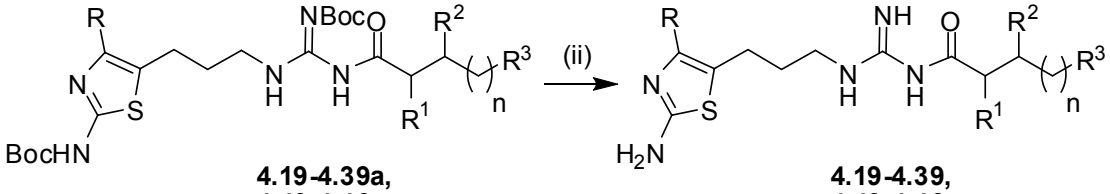
Scheme 4.3. General procedure for the preparation of Boc-protected aminothiazolylpropylguanidines **4.17**, **4.18**; Reagents and conditions: (i) **4.14** (1 eq), $HgCl_2$ (2 eq), NEt_3 (3 eq), DCM/abs, 48 h, rt; (ii) H_2, Pd/C (10 %), MeOH/THF (1:1), 8 bar, 3-4 d, rt.

Previously, attempts to couple *tert*-butyl 5-(3-guanidinopropyl)-4-methylthiazol-2-ylcarbamate to different carboxylic acids under similar conditions as described for the imidazolylpropylguanidine revealed unfavourable side reactions[8]. Due to the free guanidine moiety, reaction of carboxylic acids with the guanidine building block gave diacylated byproducts in addition to the desired monoacylated ones. To avoid diacylation, the synthetic route was changed and the Boc protected guanidines **4.17** and **4.18** were used as building blocks (Scheme 4.4). *tert*-Butyl 5-[3-[*N*-*tert*-butoxy-carbonylguanidino]propyl]-4-methylthiazol-2-ylcarbamate (**4.17**) and the analogue **4.18**, respectively, were coupled to synthesized (**3.21c**, **3.27-3.28c**, **3.31-3.36c**, **3.39**, **3.40-3.44**, **3.47**, **3.50**, **3.52**, **3.54b**, substitution pattern see chapter 3) and commercially available carboxylic acids using standard coupling reagents (EDAC, HOBt and DIEA) to obtain the N^G-acylated di-Boc-protected aminothiazolylpropylguanidines **4.19-4.39a** and **4.40-4.46a**. Both Boc groups can be easily removed in a few hours by treating with TFA in DCM to obtain the N^G-acylated 2-aminothiazolylpropylguanidines **4.19-4.46**.



4.17, 4.18

3.21c, 3.27-3.28c, 3.31-3.36c, 3.39c, 3.40-3.44, 3.47, 3.50, 3.52, 3.54b



4.19-4.39a, 4.40-4.46a

4.19-4.39, 4.40-4.46

No	R	R^1	R^2	R^3	n
4.19a, 4.19	CH_3	H	CH_3	4-CH_3-C_6H_4	0
4.20a, 4.20	CH_3	CH_3	H	C_6H_5	0
4.21a, 4.21	CH_3	H	CH_2CH_3	C_6H_5	0
4.22a, 4.22	CH_3	CH_2CH_3	H	C_6H_5	0
4.23a, 4.23	CH_3	H	CH_3	C_6H_5	1
4.24a, 4.24	CH_3	H	CH_3	3-CH_3-C_6H_4	1
4.25a, 4.25	CH_3	H	CH_3	4-CH_3-C_6H_4	1
4.26a, 4.26	CH_3	H	CH_3	3-OCH_3-C_6H_4	1
4.27a, 4.27	CH_3	H	CH_3	4-OCH_3-C_6H_4	1
4.28a, 4.28	CH_3	H	CH_3	3-F-C_6H_4	1
4.29a, 4.29	CH_3	H	CH_3	4-F-C_6H_4	1
4.30a, 4.30	CH_3	H	(*R*)CH_3	cHex	0
4.31a, 4.31	CH_3	H	(*S*)CH_3	cHex	0
4.43a, 4.32	CH_3	CH_3	H	cHex	0
4.33a, 4.33	CH_3	H	CH_2CH_3	cHex	0
4.34a, 4.34	CH_3	CH_2CH_3	H	cHex	0
4.35a, 4.35	CH_3	H	CH_3	cHex	1
4.36a, 4.36	CH_3	H	CH_2CH_3	cHex	1
4.37a, 4.37	CH_3	H	CH_3	4-OH-C_6H_4	0
4.38a, 4.38	CH_3	H	$(CH_2)_3NH_2$	C_6H_5	0
4.39a, 4.39	CH_3	H	H	COOH	6
4.40a, 4.40	H	H	H	C_6H_5	0
4.41a, 4.41	H	H	CH_3	C_6H_5	0
4.42a, 4.42	H	H	CH_2CH_3	C_6H_5	0

Scheme 4.4

Scheme 4.4 (continued)

4.43a, 4.43	H	H	C_6H_5	C_6H_5	0
4.44a, 4.44	H	H	CH_3	cHex	0
4.45a, 4.45	H	CH_3	H	cHex	0
4.46a, 4.46	H	H	CH_2CH_3	cHex	0

Scheme 4.4. General procedure for the coupling of carboxylic acids with aminothiazolylpropyl-guanidine building blocks; Reagents and conditions: (i) EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 24 h, rt; (ii) 20 % TFA, DCM/abs, 3-5 h, rt.

4.3. Pharmacological results and discussion

All compounds were tested for histamine H_2R agonism on the isolated spontaneously beating guinea pig right atrium (positive chronotopic response) (Table 4.1) and in the GTPase assay on hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins expressed in Sf9 insect cells (Table 4.2). To study receptor selectivity, the compounds were tested for histamine H_1R antagonism in the Ca^{2+}-assay on U-373 MG human cells (Table 4.3) and selected compounds were investigated in GTPase assay on human histamine H_3 and H_4 receptors (Table 4.4).

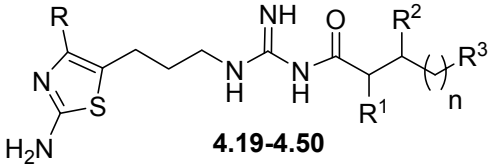
4.3.1. Histamine H_2 receptor agonism

4.3.1.1. Agonist potencies and efficacies at the guinea pig right atrium

The synthesized compounds proved to be partial to nearly full agonists at the spontaneously beating guinea pig right atrium. As also found for the N^G-acylated imidazolylpropylguanidines, the potencies of the aminothiazole derivatives are lower at the guinea pig right atrium than at gpH_2R-$G_{s\alpha S}$ fusion protein but the structure-acitivity relationships derived from both assays and the order of potencies are in good agreement. Substitution in α-position is much better tolerated in the phenyl substituted compounds (**4.20** and **4.22**) compared to the cyclohexyl derivatives (**4.32** and **4.34**). The 3-Phenylbutanoyl- (**4.47**), 3-cyclohexylbutanoyl- (**4.50**) and 3-cyclohexylpentanoyl-substituted (**4.33**) guanidines are two times more potent and have higher intrinsic activities than the corresponding 3-methyl-4-phenylbutanoyl- (**4.23**), 4-cyclohexyl-3-methylbutanoyl- (**4.35**) and 3-(cyclohexylmethyl)pentanoyl-substituted (**4.36**) analogues. Hence, three carbon atoms between the guanidine moiety and the ring system are the optimum for high potencies on the guinea pig right atrium. In contrast to the imidazole serie, the (*R*)-enantiomer (**4.30**) of the 3-cyclohexylbutanoyl derivative (**4.50**) is 1.6 times more potent than the (*S*)-enantiomer

(**4.31**), whereas for enantiomeric pair of the phenyl analogue the (*S*)-configured compoud represents the eutomer (data not shown[8]).

Table 4.1. Histamine H_2 receptor agonism on the guinea pig right atrium.



4.19-4.50

						H_2R agonism - isolated guinea pig right atrium		
No	**R**	**R^1**	**R^2**	**R^3**	**n**	**pEC_{50} ± SEM[a]**	**rel. pot. (%)[b]**	**E_{max} ± SEM (%)[c]**
HIS	-	-	-	-	-	6.00 ± 0.10	100	100 ± 2
AMT[5]	-	-	-	-	-	6.21 ± 0.09	162	95 ± 2
4.48[15]	CH_3	H	H	C_6H_5	0	nd	nd	nd
4.47[8]	CH_3	H	CH_3	C_6H_5	0	7.18 ± 0.17	1520	89 ± 3
4.19	CH_3	H	CH_3	4-CH_3-C_6H_4	0	7.04 ± 0.03	1100	64 ± 4
4.20	CH_3	CH_3	H	C_6H_5	0	6.98 ± 0.12	948	74 ± 6
4.21	CH_3	H	CH_2CH_3	C_6H_5	0	6.84 ± 0.05	697	71 ± 3
4.22	CH_3	CH_2CH_3	H	C_6H_5	0	6.69 ± 0.13	493	79 ± 4
4.23	CH_3	H	CH_3	C_6H_5	1	6.88 ± 0.06	759	89 ± 2
4.50[8]	CH_3	H	CH_3	cHex	1	7.03 ± 0.06	1070	74 ± 8
4.49[8]	CH_3	H	C_6H_5	C_6H_5	1	7.14 ± 0.11	1370	71 ± 4
4.24	CH_3	H	CH_3	3-CH_3-C_6H_4	1	6.23 ± 0.06	169	55 ± 3
4.25	CH_3	H	CH_3	4-CH_3-C_6H_4	1	6.25 ± 0.07	177	45 ± 5
4.26	CH_3	H	CH_3	3-OCH_3-C_6H_4	1	nd	nd	nd
4.27	CH_3	H	CH_3	4-OCH_3-C_6H_4	1	nd	nd	nd
4.28	CH_3	H	CH_3	3-F-C_6H_4	1	7.16 ± 0.16	1440	75 ± 3
4.29	CH_3	H	CH_3	4-F-C_6H_4	1	6.63 ± 0.07	423	46 ± 6
4.30	CH_3	H	(*R*)CH_3	cHex	0	7.11 ± 0.14	1280	80 ± 4
4.31	CH_3	H	(*S*)CH_3	cHex	0	6.91 ± 0.11	804	78 ± 3
4.32	CH_3	CH_3	H	cHex	0	6.62 ± 0.10	420	65 ± 3
4.33	CH_3	H	CH_2CH_3	cHex	0	7.12 ± 0.12	1300	70 ± 3
4.34	CH_3	CH_2CH_3	H	cHex	0	6.24 ± 0.16	173	67 ± 5
4.35	CH_3	H	CH_3	cHex	1	6.68 ± 0.18	482	49 ± 7
4.36	CH_3	H	CH_2CH_3	cHex	1	6.76 ± 0.14	571	26 ± 7
4.37	CH_3	H	CH_3	4-OH-C_6H_4	0	7.49 ± 0.12	3050	87 ± 4
4.38	CH_3	H	$(CH_2)_3NH_2$	C_6H_5	0	7.42 ± 0.03	2620	97 ± 2
4.40	H	H	H	C_6H_5	0	7.05 ± 0.04	1130	92 ± 2
4.41	H	H	CH_3	C_6H_5	0	7.42 ± 0.10	2600	92 ± 4

Table 4.1 (continued)

4.42	H	H	CH_2CH_3	C_6H_5	0	7.02 ± 0.06	1040	65 ± 6
4.43	H	H	C_6H_5	C_6H_5	0	7.41 ± 0.08	2570	74 ± 5
4.44	H	H	CH_3	cHex	0	7.14 ± 0.12	1370	84 ± 5
4.45	H	CH_3	H	cHex	0	6.92 ± 0.11	828	73 ± 2
4.46	H	H	CH_2CH_3	cHex	0	7.48 ± 0.12	3020	74 ± 4

[a] 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 + ΔpEC_{50}; data shown are the ± SEM of three to seven experiments; [b] relative potency to histamine = 100 %; [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine;

The most potent compounds among the aminothiazoles listed in Table 4.1 are **4.37** and **4.38** containing a *p*-hydroxyphenyl moiety and a free amino group, respectively. These derivatives are full and nearly full agonists and 26 and 30 times more potent than histamine.

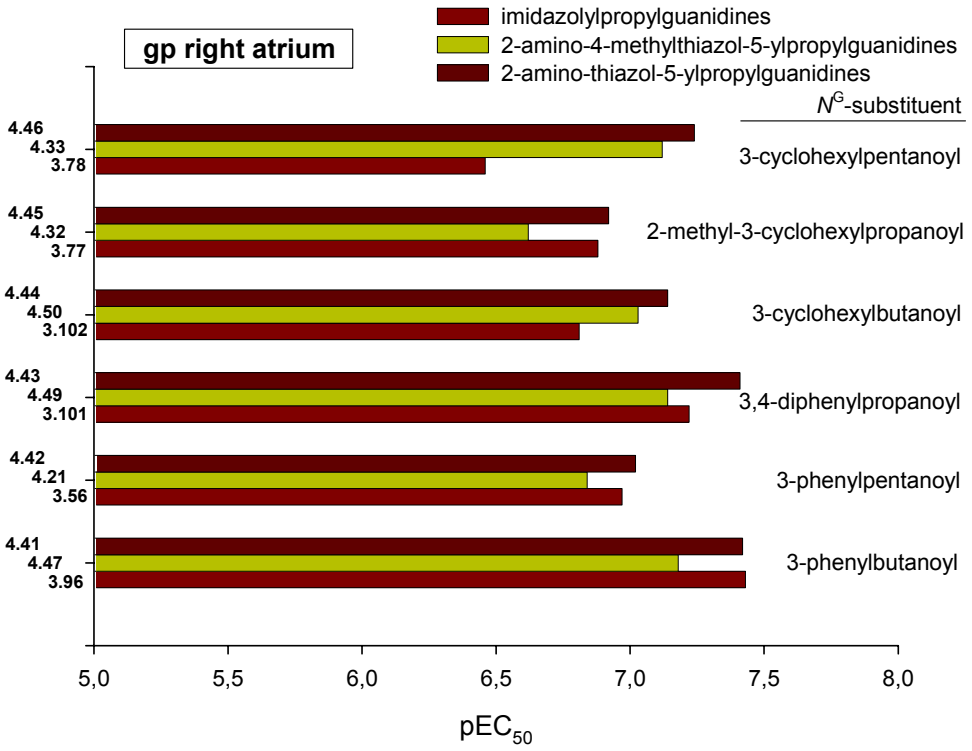


Figure 4.2. Comparison of the potencies of N^G-acylated imidazolyl- (**3.56**, **3.77**-**3.78**, **3.96**, **3.101** and **3.102**), 2-amino-4-methylthiazol-5-yl- (**4.21**, **4.32**-**4.33** and **4.47**, **4.49** and **4.50**) and 2-aminothiazol-5-ylpropylguanidines (**4.41**-**4.46**) with the same substitution patterns.

Figure 4.2 demonstrates the potencies of close structural analogues at the spontaneously beating guinea pig right atrium: N^G-acylated imidazolyl- (**3.56**, **3.77**-**3.78**, **3.96**, **3.101** and **3.102**), 2-amino-4-methylthiazolyl- (**4.21**, **4.32**-**4.33** and **4.47**, **4.49** and **4.50**) and 2-aminothiazolylpropylguanidines (**4.41**-**4.46**) lacking the 4-

methyl substituent. Except for the 3-cyclohexylbutanoyl and 3-cyclohexylpentanoyl derivatives the "demethylated" aminothiazolylpropylguanidines show similar potencies as the corresponding imidazolylpropylguanidines and both are more potent than the 2-amino-4-methylthiazolylpropylguanidines. In contrast to amthamine, the introduction of a methyl group results in a slight decrease in both potency and efficacy of the N^G-acylated guanidines at the guinea pig right atrium.

4.3.1.2. Agonist potencies and efficacies at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ in the GTPase assay

All compounds proved to be full or partial agonists in the GTPase assay at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins expressed in Sf9 cell membranes (Table 4.2). Amthamine (**AMT**) is a nearly full to full agonist at both, the human and guinea pig receptor in the GTPase assay and is slightly more potent than histamine (**HIS**). In the N^G-acylated serie the replacement of the imidazole ring against a 2-amino-4-methylthiazole moiety results in a decrease in efficacy at both hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins (Figure 4.3, **A** and **B**), whereas the potencies are in good agreement (Figure 4.3, **C** and **D**). Again, in accordance with the results for alkylated and acylated imidazolylpropylguanidines[16-18], the 2-amino-4-methylthiazol-5-ylpropylguanidines exhibit higher potencies and efficacies at gpH_2R-$G_{s\alpha S}$ than at hH_2R-$G_{s\alpha S}$.

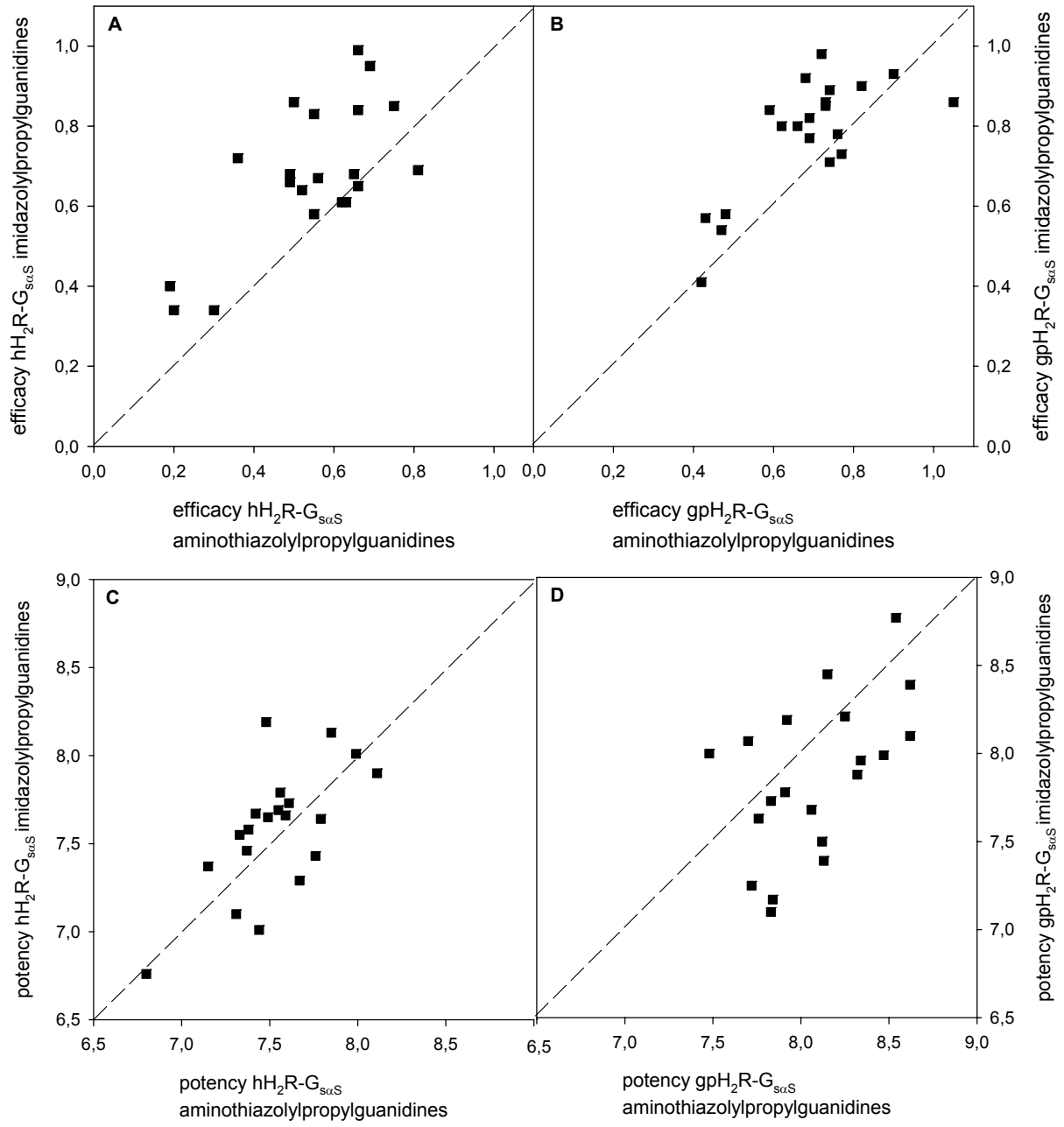


Figure 4.3. Comparison of efficacies (**A** (hH_2R-$G_{s\alpha S}$) and **B** (gpH_2R-$G_{s\alpha S}$)) and potencies (**C** (hH_2R-$G_{s\alpha S}$) and **D** (gpH_2R-$G_{s\alpha S}$) of N^G-acylated 2-amino-4-methylthiazolylpropyl-guanidines (**4.19**-**4.38**) and the corresponding imidazolylpropylguanidines (**3.56**-**3.57**, **3.64**-**3.65**, **3.68**-**3.73**, **3.75**-**3.79**, **3.82**, **3.85**, **3.87**, **3.89** and **3.97**); pEC_{50} values derived from EC_{50} values listed in Table 4.2 and Table 3.2) The straight dotted lines represent the theoretical correlations that would have been obtained if pEC_{50} values and efficacies had been identical in the two systems compared with each other.

The 2-amino-4-methylthiazol-5-ylpropylguanidines proved to be weak to strong partial agonists at both, the hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$. Introduction of a *para* methyl group in the aromatic ring system at the 3-phenylbutanoyl analogue (**4.47**→**4.19**) results in about the same potency at the human receptor, whereas potency at the

guinea pig receptor increased by a factor of three. The potency of the 3-phenylpentanoyl analogue (**4.21**) was increased at both, the hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$. A methyl substituent in α-postion was tolerated in the phenyl (**4.20**) as well as in the cyclohexyl (**4.32**) compounds, whereas an ethyl substituent in α-position (**4.22** and **4.34**) results in a decrease in potency and efficacy especially at hH_2R-$G_{s\alpha S}$. The 3-methyl-4-phenylbutanoyl compound (**4.23**) exhibits nearly the same potency as the 3-phenylbutanoyl derivative (**4.47**) but with lower efficacy at both receptors. However, the introduction of *meta* or *para* methyl, methoxy or fluoro substituents, respectively, results in an increase in potency at the guinea pig, but not at the hH_2R-$G_{s\alpha S}$ with slightly lower efficacies. The *meta* position of methoxy and fluoro substituents is more favourable at both receptors, whereas for the methyl substituted derivative the *para* position was preferred, but not in terms of efficacy. An electron withdrawing substituent (**4.28** and **4.29**) increases the potency by a factor of two to three at gpH_2R-$G_{s\alpha S}$ (EC_{50} (**4.28**) = 4.8 nM, EC_{50} (**4.29**) = 7.4 nM), but not at hH_2R-$G_{s\alpha S}$ (EC_{50} (**4.28**) = 27.4 nM, EC_{50} (**4.29**) = 36.2 nM). Compound **4.37** containing a *p*-hydroxyphenyl group the 3-phenylbutanoyl derivative (**4.47**) is about ten times more potent at gpH_2R-$G_{s\alpha S}$ acting as a strong partial agonist and the selectivity towards the guinea pig receptor was strongly increased.

In accordance with the structure-activity relationships of N^G-acylated imidazolylpropylguanidines, the exchange of a phenyl ring against a cyclohexyl moiety results in the main in higher potency and efficacy at both receptors. The 3-methyl-4-cyclohexylbutanoyl derivative is three and two times more potent at gpH_2R-$G_{s\alpha S}$ (EC_{50} (**4.35**) = 12.4 nM, EC_{50} (**4.23**) = 33.2 nM) and hH_2R-$G_{s\alpha S}$ (EC_{50} (**4.35**) = 25.5 nM, EC_{50} (**4.23**) = 41.2 nM), respectively, compared to its phenyl analogue. The enantiomers of the 3-cyclohexylbutanoyl derivative show an inverse preference (R>S) to the H_2R than its corresponding imidazolylpropyl analogue (S>R) and also than its phenyl substituted analogue (S>R, data not shown[8]). Rather low eudismic ratios were found for the 2-amino-4-methylthiazol-5-ylpropylguanidines, too.

The ratio of EC_{50} values, EC_{50} hH_2R-$G_{s\alpha S}$ to EC_{50} gpH_2R-$G_{s\alpha S}$, was highest for compound **4.38** with a free amino group in the side chain. This compound exhibits moderate agonistic activity at hH_2R-$G_{s\alpha S}$ with EC_{50} = 158.4 nM, whereas it is 18 times more potent at gpH_2R-$G_{s\alpha S}$ (EC_{50} = 8.6 nM).

Table 4.2. Agonist efficacies and potencies at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ expressed in Sf9 cell membranes.

No	hH_2R-$G_{s\alpha S}$			gpH_2R-$G_{s\alpha S}$			EC_{50} hH_2R-$G_{s\alpha S}$/ EC_{50} gpH_2R-$G_{s\alpha S}$
	efficacy	EC_{50} [nM]	rel. pot.	efficacy	EC_{50} [nM]	rel. pot.	
HIS[16]	1.00	1260 ± 250	100	1.00	1200 ± 240	100	1.05
AMT[16]	0.90 ± 0.06	450 ± 40	280	1.04 ± 0.05	440 ± 40	271	1.02
4.47[8]	0.82	47.9	2,630	0.98	22.4	5,370	2.04
4.19	0.69 ± 0.06	49.35 ± 33.9	2,553	0.73 ± 0.07	7.6 ± 3.6	15,789	6.49
4.20	0.75 ± 0.03	16.3 ± 6.1	7,730	0.68	2.4	50,000	6.79
4.21	0.52 ± 0.04	20.4 ± 3.0	6,176	0.76	4.5	26,667	4.53
4.22	0.49 ± 0.01	46.5 ± 19.6	2,709	0.62	5.6	21,428	8.30
4.23	0.63 ± 0.03	41.2 ± 1.4	3,058	0.73 ± 0.03	33.2 ± 20.6	3,614	1.24
4.24	0.55 ± 0.03	42.2 ± 9.1	2,985	0.69 ± 0.14	19.2 ± 1.8	6,250	2.19
4.25	0.30 ± 0.05	21.2 ± 3.9	5,943	0.48 ± 0.09	14.9 ± 1.6	8,053	1.42
4.26	0.66 ± 0.01	32.6 ± 17.5	3,865	0.66 ± 0.02	14.6 ± 5.3	8,219	2.23
4.27	0.49 ± 0.02	71.4 ± 17.7	1,765	0.43	14.4	8,333	4.96
4.28	0.56 ± 0.06	27.4 ± 2.9	4,598	0.72 ± 0.04	4.8 ± 0.2	25,000	5.71
4.29	0.50 ± 0.06	36.2 ± 4.6	3,480	0.59 ± 0.04	7.4 ± 1.4	16,216	4.89
4.50[8]	0.56	9.1	13,804	0.81	7.1	16,982	1.23
4.30	0.55 ± 0.02	24.3 ± 12.0	5,185	0.82 ± 0.05	17.5 ± 8.8	6,857	1.39
4.31	0.66 ± 0.01	33.2 ± 7.5	3,795	0.90 ± 0.14	19.8 ± 4.3	6,060	1.68
4.32	0.62 ± 0.04	10.1 ± 2.9	12,475	0.53 ± 0.22	8.6 ± 6.2	13,953	1.17
4.33	0.65	14.0	9,000	0.77	7.1	16,901	1.97
4.34	0.20 ± 0.01	38.3 ± 2.2	3,290	0.47	12.0	10,000	3.19
4.35	0.40	25.5	4,941	0.56	12.4	9,677	2.05
4.36	0.19 ± 0.02	10.8 ± 3.0	11,667	0.42	3.4	35,294	3.18
4.37	0.84 ± 0.03	31.9 ± 3.7	3,949	0.77	2.9	41,379	11.00
4.38	0.65 ± 0.07	158.4 ± 23.5	795	1.05 ± 0.18	8.6 ± 5.6	13,953	18.42
4.40	0.83 ± 0.03	21.9 ± 0.5	5,753	0.76 ± 0.32	13.5 ± 6.1	8,888	1.62
4.41	0.84 ± 0.11	20.3 ± 1.7	6,207	0.81 ± 0.01	5.3 ± 2.1	22,641	3.83
4.42	0.68 ± 0.09	24.3 ± 1.4	5,185	0.89 ± 0.35	29.2 ± 18.7	4,109	0.83
4.43	0.67 ± 0.09	33.9 ± 8.8	3,717	0.83 ± 0.05	7.9 ± 5.6	15,189	4.29
4.44	0.71 ± 0.12	11.05 ± 5.9	11,403	1.02	2.2	57,273	5.02
4.45	0.70 ± 0.12	15.9 ± 6.6	7,925	0.83	11.2	11,250	1.42
4.46	0.66 ± 0.08	12.5 ± 5.7	10,080	0.97	18.6	6,452	0.67

Steady state GTPase activity in Sf9 membranes expressing hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ was determined as described in literature[16]. Reaction mixtures contained ligands at concentrations from 1 nM to 10 µM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 0.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (100 µM) ranged between ~ 2 and 13 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the ± SEM of two to three experiments or one experiment performed in duplicates each. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value. The ratio of the EC_{50} values of H_2R agonists for hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ were also calculated.

In Figure 4.4 the potencies of N^G-acylated imidazolyl- (**3.56**, **3.77**-**3.78**, **3.96**, **3.101**, **3.102** and **3.105**), 2-amino-4-methylthiazolyl- (**4.21**, **4.32**-**4.33** and **4.47**-**4.50**) and 2-aminothiazolylpropylguanidines (**4.40**-**4.46**) at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ are graphically presented to illustrate the contribution of the 4-methyl substituent. The 2-aminothiazolylpropylguanidines lacking the 4-methyl substituent exhibit similar or even higher potencies and similar efficacies at hH_2R-$G_{s\alpha S}$ compared to the 2-amino-4-methylthiazolyl derivatives and both exhibit similar or higher potencies compared to the imidazole analogues. At gpH_2R-$G_{s\alpha S}$ 3-phenyl- and 3-cyclohexylpentanoyl and 2-methyl-3-cyclohexylpropanoyl substituted 2-aminothiazolylpropylguanidines are less potent than the ring-methylated analogues and the corresponding imidazolylpropyl-guanidines, but show higher efficacies. For compounds with a 3-phenylbutanoyl, 3,4-diphenylpropanoyl and 3-cyclohexylbutanoyl moiety, the “demethylated” aminothiazoles have higher H_2R agonistic potencies than the corresponding 2-amino-4-methylthiazolyl- and imidazolylpropylguanidines. Thus, the introduction of a methyl group in position 4 of the thiazole ring does not generally increase the agonistic activity of the N^G-acylguanidines, i. e. the influence of the ring-methylation on the H_2R agonistic potency is different in the acylguanidine and the thiazolylethylamine series.

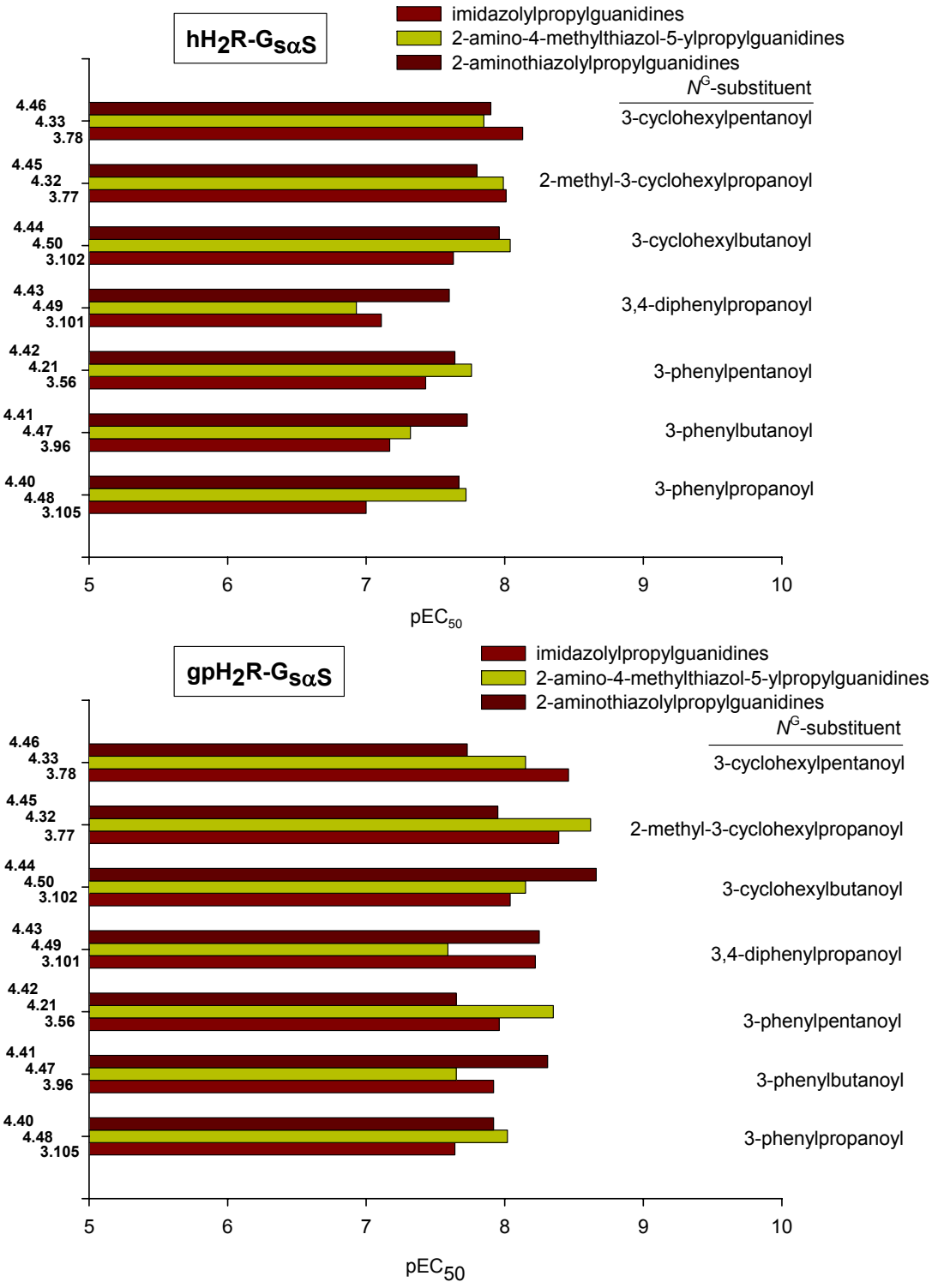


Figure 4.4. Comparison of the potencies of N^G-acylated imidazolyl- (**3.56, 3.77-3.78, 3.96, 3.101-3.102** and **3.105**), 2-amino-4-methylthiazol-5-yl- (**4.21, 4.32-4.33** and **4.47-4.50**) and 2-aminothiazol-5-ylpropylguanidines (**4.40-4.46**) with same substitution patterns.

4.3.2. Receptor selectivity

To study the histamine receptor selectivity, selected compounds were tested in the Ca^{2+}-assay on human U-373 MG cells expressing the H_1 receptor and in GTPase assays using membrane preparations of Sf9 insect cells espressing human H_3 and H_4 receptors.

4.3.2.1. Histamine H_1 receptor antagonism on U-373 MG cells (Ca^{2+}-assay)

On human U-373 cells, all compounds proved to be weak or very weak histamine H_1R antagonists (activities in the micromolar range). A polar group in the side chain (**4.37** and **4.38**) seems to disfavour activity at the H_1 receptor.

Table 4.3. Histamine H_1 receptor antagonism on U-373 MG human cells (Ca^{2+}-assay).

		Histamine H_1 receptor antagonism U-373 MG cells (Ca^{2+}-assay)			
No	**IC_{50} [µM][a]**	**No**	**IC_{50} [µM][a]**	**No**	**IC_{50} [µM][a]**
HIS	-	**4.27**	37	**4.37**	>100
AMT[5]	-	**4.28**	8	**4.38**	>100
4.47[8]	18	**4.29**	12	**4.40**	35
4.19	28	**4.50[8]**	10	**4.41**	44
4.20	65	**4.30**	16	**4.42**	50
4.21	31	**4.31**	7	**4.43**	17
4.22	47	**4.32**	47	**4.44**	23
4.23	11	**4.33**	12	**4.45**	34
4.24	9	**4.34**	22	**4.46**	16
4.25	17	**4.35**	10		
4.26	4	**4.36**	5		

[a] IC_{50} values for the inhibition of the histamine (30 µM) induced increase in cellular calcium, one experiment; procedure as described from Kracht, 2001[19].

4.3.2.2. Activities on the human H_3 and H_4 receptors (GTPase assay)

The N^G-acylated imidazolylpropylguanidines described in chapter 3 show also affinity to the H_3R due to the imidazol-4-yl moiety often found in highly potent H_3R ligands. By the replacement of the imidazole ring against an 2-aminothiazole ring, these compounds are devoid of or exhibit low (**4.45** and **4.46**) H_3R stimulatory effects at the concentration tested. Equally, the N^G-acylated imidazolylpropylguanidines are full or nearly full H_4R agonists with EC_{50} values in the nM range, the analogous aminothiazolylpropylguanidines proved to be very weak antagonists at hH_4R membranes.

Table 4.4. Agonist efficacies and potencies at hH_3R + $G\alpha_o$ + $\beta_1\gamma_2$ + RGS4 and hH_4R-GAIP + $G\alpha_{i2}$ + $\beta_1\gamma_2$ expressed in Sf9 cell membranes.

No	hH_3R + $G\alpha_o$ + $\beta_1\gamma_2$ + RGS4			hH_4R-GAIP + $G\alpha_{i2}$ + $\beta_1\gamma_2$		
	efficacy	EC_{50} [nM]	rel. pot.	efficacy	EC_{50} [nM] (IC_{50} [nM])	rel. pot.
His	1.00	25.8 ± 3.1	100	1.00	11.6 ± 2.5	100
4.32	-0.19	nd	nd	-	(>10,000)	-
4.33	-0.16	nd	nd	-	(>6000)	-
4.35	-0.17	nd	nd	-	(>10,000)	-
4.38	0.06	nd	nd	-	(>10,000)	-
4.43	0.03	nd	nd	-	(>10,000)	-
4.44	-0.12	nd	nd	-	(>10,000)	-
4.45	0.39	nd	nd	-	(>10,000)	-
4.46	0.15	nd	nd	-	(>10,000)	-

Steady state GTPase activity in Sf9 membranes expressing $hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4 and hH_4R-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$ was determined as described in literature[16]. Reaction mixtures contained ligands at a concentration 10 µM ($hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4). Typical basal GTPase activities ranged between ~ 1.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (10 µM) ranged between ~ 3.5 and 4.5 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are one experiment performed in duplicates each. The relative potency of histamine was set to 100. For antagonism, reaction mixtures contained histamine (100 nM) and ligands at concentrations from 0.1 nM to 100 µM.

4.4. Summary

Based on previous preliminary studies[8], N^G-acylated 2-amino-4-methylthiazol-5-ylpropylguanidines were synthesized and investigated for H_2R agonism at the guinea pig right atrium and on hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins expressed in Sf9 insect cells. Moreover, the compounds were studied on H_1R of U-373 MG cells and in GTPase assays on membranes of Sf9 cells expressing $hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4 and hH_4R-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$ to figure out the receptor selectivity for H_2R especially over H_3R and H_4R in comparison to the analogous N^G-acylated imidazol-4-ylpropyl-guanidines. N^G-acylated 2-amino-4-methylthiazol-5-ylpropylguanidines proved to be strong partial to full agonists at the guinea pig right atrium. By contrast to the imidazolylpropylguanidines derivatives containing a hydroxy or free amine function are 26 and 30 times more potent than histamine and turned out to be the most potent compounds in this series. The replacement of the imidazole ring against a 2-amino-4-methylthiazol ring results in a slight decrease in efficacy but in similar or even higher potency at both hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$. In agreement with the structure-activity relationships of imidazole series, the exchange of the phenyl against a cyclohexyl ring in the N^G-alkanoyl substituent results in an increase in potency. The enantiomeric 3-cyclohexylbutanoyl derivatives show inverse preference (R>S) for the

H_2R than the 3-phenylbutanoyl substituted[8] and than corresponding analogue of the imidazolylpropylguanidine series (S>R), but again with a low eudismic ratio. In agreement with the imidazole series, a very high selectivity towards gpH_2R-$G_{s\alpha S}$ was found for compounds with polar groups in the side chain (**4.37**-**4.38**). Particularly a free amino group (**4.38**) enhanced the species selectivity (gpH_2R-$G_{s\alpha S}$: EC_{50} = 8.6 nM; hH_2R-$G_{s\alpha S}$: EC_{50} = 158 nM). To proof the influence of the methyl substituent at the aminothiazole ring on the receptor selectivity, 2-aminothiazol-5-ylpropylguanidines lacking the 4-methyl group were investigated. In contrast to amthamine, the methyl group does neither enhance the agonistic activity for N^G-acylated compounds in the GTPase assay nor at the guinea pig right atrium.

As reported in chapter 3, N^G-acylated imidazolylpropylguanidines are also potent agonists (or antagonists) at H_3R and H_4R in the GTPase assay. By contrast, the N^G-acylated 2-amino-4-methylthiazol-5-ylpropylguanidines turned out to be very weak antagonists at the hH_1R (calcium assay), the gpH_3R (guinea pig ileum)[8] and the hH_4R (GTPase assay). At the concentration tested, the 2-amino-4-methylthiazol-5-ylpropylguanidines are also devoid of or exhibit low stimulatory (**4.45** and **4.46**) activities at the hH_3R (GTPase assay). In summary, the bioisosteric replacement of the imidazole against an 2-aminothiazole ring represents a successful approach to the development of highly potent and selective H_2R agonists.

4.5. Experimental section

4.5.1. General conditions

See Chapter 3.

4.5.2. Preparation of the building blocks 4.17 and 4.18

S-Methylthiouronium iodide (4.12)[20]

Thiourea (10 g, 131 mmol) and methyl iodide (18.6 g, 131 mmol) in MeOH (100 ml) were refluxed for 1 h. After evaporation, the crude product was taken up in diethyl ether, sucked off and washed twice with diethyl ether to yield **4.12** as a white solid (27.96 g, 128 mmol, 98 %). The crude product was used in the next step without further purification. ^{1}H-NMR (DMSO-d_6) δ ppm: 8.88 (br s, 4H, N***H***$_2$), 2.57 (s, 3H, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 91 (M^+, 100). $C_2H_7IN_2S$ (218.06)

***N*-tert-Butoxycarbonyl-S-methylthiourea (4.13)**

To a solution of **4.12** (27.74 g, 127 mmol) in DCM (200 ml) were added triethylamine (17.6 ml, 127 mmol) and Boc_2O (27.76 g, 127 mmol) in DCM (50 ml) and stirred for 24 h at room temperature. The mixture was subsequently washed with water and brine and the organic phase dried over $MgSO_4$. After removing the solvent under reduced pressure, the crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) yielding **4.13** as a white solid (18.13 g, 75 %). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.54 (br s, 2H, N***H***$_2$), 2.31 (s, 3H, C***H***$_3$), 1.40 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 191 (MH^+, 69). $C_7H_{14}N_2O_2S$ (190.26)

***N*-Benzyloxycarbonyl-*N*`-tert-butoxycarbonyl-S-methyl-thiourea (4.14)**

To a solution of **4.13** (8.55 g, 44.9 mmol) in DCM/abs (100 ml) was added benzyl succinimidyl carbonate (CbzOSu) (11.2 g, 44.9 mmol) and stirred for 20 h at ambient temperature. The mixture was subsequently extracted with DCM and basified with Na_2CO_3 (pH 9-10). The organic phase was washed with water, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was subjected to flash chromatography (PE/EtOAc 90/10 v/v) yielding **4.14** as a white solid (12.41 g, 85 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 11.58 (br s, 1H, N***H***), 7.37 (m, 5H, Ar-***H***), 5.19 (s, 2H, C***H***$_2$-Ar), 2.40 (s, 3H, C***H***$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 325 (MH^+, 100). $C_{15}H_{20}N_2O_4S$ (324.40)

2-(5-Hydroxypentyl)-1,3-dihydro-2*H*-isoindol-1,3-dione (4.1)[1, 21]

5-Amino-1-pentanol (7 g, 67.5 mmol) and phthalic anhydride (10 g, 67.5 mmol) were heated to 80-100 °C for 3 h. After cooling, 40 ml ice cold water was added and

extracted three times with $CHCl_3$. The organic phase was washed with 5 % $NaHCO_3$ and three times with H_2O and the organic phase was dried over $MgSO_4$. After removing the solvent under reduced pressure, the crude product was subjected to flash chromatography (PE/EtOAc 70/30 v/v) yielding **4.1** as a pale yellow solid (12.84 g, 81 %). mp = 43 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.75 (m, 2H, Ar-***H***), 7.64 (m, 2H, Ar-***H***), 3.58 (m, 4H, Pht-C***H***$_2$, C***H***$_2$OH), 2.16 (s, 1H, O***H***), 1.63 (m, 2H, Pht-CH_2C***H***$_2$), 1.53 (m, 2H, C***H***$_2CH_2OH$), 1.34 (m, 2H, C***H***$_2CH_2CH_2OH$); CI-MS (NH_3) *m/z* (%): 251 ($M+NH_4^+$, 100). $C_{13}H_{15}NO_3$ (233.36)

2-(5-Oxohexyl)-1,3-dihydro-2*H*-isoindol-1,3-dione (4.2)[8]

A mixture of phthalimide (5.5 g, 37 mmol), 6-chlorohexan-2-one (10.06 g, 75 mmol), potassium carbonate (7.7 g, 56 mmol) in 90 ml DMF were heated to 80 °C for 24 h. After cooling to room temperature, the mixture was added to ice cold water and extracted with $CHCl_3$, the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was subjected to flash chromatography (PE/EtOAc 90/10 to 70/30 v/v) yielding **4.2** as a colourless solid (7.18 g, 79 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.84 (m, 2H, Ar-***H***), 7.71 (m, 2H, Ar-***H***), 3.69 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-Pht), 2.50 (t, 2H, ^3J = 7.0 Hz, COC***H***$_2$), 2.14 (s, 3H, C***H***$_3$), 1.66 (m, 4H, $COCH_2$C***H***$_2$, $COCH_2CH_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 263 ($M+NH_4^+$, 100). $C_{14}H_{15}NO_3$ (245.10)

5-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentanal (4.3)[1]

Oxalyl chloride (2.9 ml, 33.75 mmol) in 65 ml CH_2Cl_2/abs was cooled to -50 °C and DMSO (5.1 ml, 71.6 mmol) in 25 ml CH_2Cl_2/abs was added under stirring and argon at such a rate that the temperature was maintained at -50 °C. After the addition was complete, stirring was continued for 15 min. A solution of **4.1** (6.33 g, 27 mmol) in 30 ml CH_2Cl_2/abs was added slowly and stirring was continued for another 15 min. After the addition of NEt_3 (20 ml, 148.5 mmol), the mixture was allowed to warm to room temperature, 80 ml H_2O was added and stirring continued for 30 min. The organic phase was separated and washed with H_2O to almost neutral reaction. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure yielding crude **4.3** as a yellow oil (5.81 g, 93 %) which was stored under argon and used without further purification. ^{1}H-NMR ($CDCl_3$) δ ppm: 9.75 (t, 1H, ^3J = 1.5 Hz, CO***H***), 7.82 (m, 2H, Ar-H), 7.70 (m, 2H, Ar-H), 3.70 (t, 2H, ^3J = 6.8 Hz, Pht-C***H***$_2$), 2.50 (m, 2H, C***H***$_2$COH), 1.69 (m, 4H, Pht-CH_2C***H***$_2CH_2$, Pht-CH_2CH_2C***H***$_2$); CI-MS (NH_3) *m/z* (%): 249 ($M+NH_4^+$, 100). $C_{13}H_{13}NO_3$ (231.25)

General procedure for the bromination of 4.2 and 4.3

To a solution of **4.2** or **4.3** (1 eq) in dioxane and DCM/abs bromine (1 eq) was slowly added so that the brown colour always disappeared and then stirred for 1 h at room temperature. Subsequently, the mixture was washed two times with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was obtained as a yellow oil and used in the next step without further purification.

2-(4-Bromo-5-oxohexyl)-1,3-dihydro-2*H*-isoindol-1,3-dione (4.4)[22]

The title compound was prepared from **4.2** (16.42 g, 67 mmol) in 340 ml dioxane and 220 ml DCM/abs and bromine (3.4 ml, 67 mmol) according to the general procedure yielding **4.4** as a yellow oil (21.6 g, 100 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.85 (m, 2H, Ar-***H***), 7.73 (m, 2H, Ar-***H***), 4.34 (m, 1H, COC***H***), 3.74 (t, 2H, ^3J = 6.7 Hz, C$\boldsymbol{H}_2$-Pht), 2.37 (s, 3H, Thiaz-4-C$\boldsymbol{H}_3$), 1.95 (m, 4H, COCHC$\boldsymbol{H}_2$CH$_2$, COCHCH$_2$C$\boldsymbol{H}_2$). $C_{14}H_{14}NBrO_3$ (323.01)

2-Bromo-5-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)pentanal (4.5)[1]

The title compound was prepared from **4.3** (5.77 g, 25 mmol) in 150 ml dioxane and 100 ml DCM/abs and bromine accoding to the general procedure yielding **4.5** as a yellow oil (7.86 g, 100 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 9.44 (d, 1H, ^3J = 2.3 Hz, CO***H***), 7.83 (m, 2H, Ar-***H***), 7.71 (m, 2H, Ar-***H***), 4.34 (m, 1H, Br-***H***), 3.73 (t, 2H, ^3J = 6.6 Hz, Pht-C$\boldsymbol{H}_2$), 1.87 (m, 4H, Pht-CH$_2$C$\boldsymbol{H}_2$CH$_2$, Pht-CH$_2$CH$_2$C$\boldsymbol{H}_2$), CI-MS (NH_3) *m/z* (%): 329 (MNH_4^+, 100). $C_{13}H_{12}NO_3Br$ (310.14)

General procedure for the synthesis of the thiazoles 4.6 and 4.7

To a stirred solution of crude **4.4** or **4.5** (1 eq) in DMF, a solution of thiourea (1 eq) in DMF was added and the mixture was heated at 100 °C for 3 h. After cooling and removing the solvent in vacuo, a mixture of EtOAc/MeOH (1:1 v/v) was added and stirred for 30 min. Subsequently, the precipitate was filtered off, washed with EtOAc and diethylether and the solid dried *in vacuo*.

2-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-1,3-dihydro-2*H*-isoindol-1,3-dione (4.6)[1]

The title compound was prepared from crude **4.4** (24.5 g, 75.8 mmol) in 90 ml DMF and a solution of thiourea (5.77 g, 75.8 mmol) in 90 ml DMF according to the general procedure yielding **4.6** as a fawn solid (14.71 g, 64 %). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.84 (m, 4H, Ar-***H***), 3.61 (t, 2H, ^3J = 7.0 Hz, C$\boldsymbol{H}_2$-Pht), 2.71 (t, 2H, ^3J = 7.5 Hz, Thiaz-

5-C***H***$_2$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 302 (MH^+, 100). $C_{15}H_{15}N_3O_2S$ (301.09)

2-[3-(2-Aminothiazol-5-yl)propyl]-1,3-dihydro-2*H*-isoindol-1,3-dione (4.7)[1]

The title compound was prepared from crude **4.5** (7.86 g, 25.3 mmol) in 20 ml DMF and a solution of thiourea (1.9 g, 25.3 mmol) in 20 ml DMF according to the general procedure yielding **4.7** as a light brown solid (14.71 g, 64 %). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.84 (m, 4H, Ar-***H***), 7.20 (s, 1H, Thiaz-4-***H***), 3.62 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-Pht), 2.79 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$); CI-MS (NH_3) *m/z* (%): 288 (MH^+, 100). $C_{14}H_{13}N_3O_2S$ (287.34)

General procedure for *tert*-butyoxycarbonyl protection

4.6 or **4.7** (1 eq), respectively, was dissolved in $CHCl_3$ and Boc_2O (1.08 eq) , NEt_3 (1.16) and DMAP (cat.) were added. The mixture was stirred over night at ambient temperature. The mixture was extracted with DCM, washed with 0.1N HCl, brine and water, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography.

***tert*-Butyl 4-methyl-5-[3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]thiazol-2-ylcarbamate (4.8)[8]**

The title compound was prepared from **4.6** (7.22 g, 24 mmol) in 60 ml $CHCl_3$, Boc_2O (5.8 g, 26 mmol), NEt_3 (4 ml, 28 mmol) and DMAP (cat.) according to the general procedure (PE/EtOAc 80/20 v/v) to obtain **4.8** as a colourless foam-like solid (6.06 g, 63 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.84 (m, 2H, Ar-***H***), 7.71 (m, 2H, Ar-***H***), 3.74 (t, 2H, ^3J = 7.1 Hz, C***H***$_2$-Pht), 2.71 (t, 2H, ^3J = 7.75 Hz, Thiaz-5-C***H***$_2$), 2.22 (s, 3H, Thiaz-4-C***H***$_3$), 1.98 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.51 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 402 (MH^+, 100). $C_{20}H_{23}N_3O_4S$ (401.14)

***tert*-Butyl 5-[3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl]thiazol-2-yl-carbamate (4.9)**

The title compound was prepared from **4.7** (24.48 g, 85.2 mmol) in 220 ml $CHCl_3$, Boc_2O (20.1 g, 91.9 mmol), NEt_3 (13.8 ml, 99.3 mmol) and DMAP (cat.) according to the general procedure (PE/EtOAc 60/40 v/v) to obtain **4.9** as a colourless foam-like solid (16.83 g, 51 %). mp = 166 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.83 (m, 2H, Ar-***H***), 7.71 (m, 2H, Ar-***H***), 7.06 (s, 1H, Thiaz-4-***H***), 3.76 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$-Pht), 2.79 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.04 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.57 (s, 9H, Boc-C***H***$_3$); CI-MS (NH_3) *m/z* (%): 388 (MH^+, 100). $C_{19}H_{21}N_3O_4S$ (387.45)

General procedure for hydrazinolysis of the phthalimides 4.8 and 4.9

To a suspension of **4.8** or **4.9** (1 eq), respectively, in EtOH was added hydrazine-monohydrate (5 eq). After stirring for 30 min at room temperature, the solution became clear and stirring was continued overnight. The mixture was cooled in an ice bath, the precipitate was removed by filtration and the filtrate concentrated to dryness. The crude product was subjected to flash chromatography ($CHCl_3$/MeOH/ NEt_3 94/5/1 v/v/v).

***tert*-Butyl 5-(3-aminopropyl)-4-methylthiazol-2-ylcarbamate (4.10)[8]**

The title compound was prepared from **4.8** (6.4 g, 15.9 mmol) in 70 ml EtOH and hydrazine-monohydrate (3.9 ml, 79.8 mmol) according to the general procedure yielding **4.10** as a yellow oil (4.3 g, 100 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.73 (m, 4H, C***H***$_2$NH$_2$, Thiaz-5-C***H***$_2$), 2.23 (s, 3H, Thiaz-4-C***H***$_3$), 1.75 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.51 (s, 9H, C(C***H***$_3$)$_3$); CI-MS (NH_3) *m/z* (%): 272 (MH^+, 100). $C_{12}H_{21}N_3O_2S$ (271.14)

***tert*-Butyl 5-(3-aminopropyl)thiazol-2-ylcarbamate (4.11)**

The title compound was prepared from **4.9** (17.24 g, 44.5 mmol) in 170 ml EtOH and hydrazine-monohydrate (10.8 ml, 223.3 mmol) according to the general procedure yielding **4.11** as a pale yellow solid (7.07 g, 62 %). mp = 109 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 7.02 (s, 1H, Thiaz-4-***H***), 2.77 (m, 4H, Thiaz-5-C***H***$_2$, C***H***$_2$NH$_2$), 1.78 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.56 (s, 9H, Boc-C***H***$_3$); CI-MS (NH_3) *m/z* (%): 258 (MH^+, 100). $C_{11}H_{19}N_3O_2S$ (257.35)

General procedure for the guanidinylation reaction

To a suspension of **4.10** or **4.11** (1 eq) **4.14** (1 eq) and $HgCl_2$ (2 eq) in DCM/abs was added NEt_3 (3 eq) and stirred at ambient temperature for 48 h. Subsequently, EtOAc was added and the precipitate filtered over Celite. The crude product was purified by flash chromatography (PE/EtOAc 80/20 v/v).

***tert*-Butyl 5-[3-(*N*-benzyloxycarbonyl-*N'*-*tert*-butyloxycarbonylguanidino)-propyl]-4-methylthiazol-2-ylcarbamate (4.15)**

The title compound was prepared from **4.10** (4.08 g, 15 mmol), **4.14** (4.88 g, 15 mmol), $HgCl_2$ (8.2 g, 30 mmol) and NEt_3 (4.55 g, 6.24 ml, 45 mmol) in 500 ml DCM/abs and 500 ml EtOAc according to the general procedure yielding **4.15** as a colourless foam-like solid (6.3 g, 77 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 11.35 (br s, 1H, N***H***), 8.47 (t, 1H, ^3J = 5.3 Hz, CH$_2$N***H***), 7.34 (m, 5H, Ar-***H***), 5.13 (s, 2H, C***H***$_2$-Ar), 3.46 (m, 2H, C***H***$_2$NH), 2.69 (t, 2H, ^3J =7.6 Hz, Thiaz-5-C***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$),

1.88 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.52 (s, 9H, C(C$\boldsymbol{H}_3$)$_3$), 1.48 (s, 9H, C(C$\boldsymbol{H}_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 548 (MH^+, 100). $C_{26}H_{37}N_5O_6S$ (547.25)

***tert*-Butyl 5-[3-(*N*-benzyloxycarbonyl-*N'*-tert-butyloxycarbonylguanidino)-propyl]thiazol-2-ylcarbamate (4.16)**

The title compound was prepared from **4.11** (4 g, 15.5 mmol), **4.14** (5 g, 15.5 mmol), $HgCl_2$ (8.42 g, 31 mmol) and NEt_3 (6.4 ml, 46.5 mmol) in 500 ml DCM/abs and 500 ml EtOAc according to the general procedure yielding **4.16** as a colourless foam-like solid (8.48 g, 100 %). mp = 140-142 °C; ^{1}H-NMR ($CDCl_3$) δ ppm: 11.35 (s, 1H, N***H***), 8.47 (t, 1H, ^3J = 5.4 Hz, $CH_2N\boldsymbol{H}$), 7.34 (m, 5H, Ar-***H***), 7.05 (s, 1H, Thiaz-4-***H***), 5.13 (s, 2H, C$\boldsymbol{H}_2$-Ph), 3.47 (m, 2H, C$\boldsymbol{H}_2$NH), 2.77 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C$\boldsymbol{H}_2$),1.92 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.55 (s, 9H, Boc-C$\boldsymbol{H}_3$), 1.48 (s, 9H, Boc-C$\boldsymbol{H}_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 534 (MH^+, 100). $C_{25}H_{35}N_5O_6S$ (533.64)

General procedure for the hydrogenolytic cleavage of Cbz groups

To a solution of **4.15** or **4.16** in a mixture of THF/MeOH (1:1) was added Pd/C (10 %) and hydrogenated by 8 bar for 3-4 days. The catalyst was removed by filtration over Celite and washed with MeOH. The solvent was removed *in vacuo*.

***tert*-Butyl 5-[3-(*N-tert*-butoxycarbonylguanidino)propyl]-4-methylthiazol-2-yl-carbamate (4.17)**

The title compound was prepared from **4.15** (5.8 g, 10.6 mmol) and 6 g of Pd/C (10 %) in a mixture of 160 ml THF/MeOH (1:1) according to the general procedure yielding **4.17** as a colourless foam-like solid (4.38 g, 100 %). mp = 113 °C; ^{1}H-NMR (CD_3OD) δ ppm: 3.23 (t, 2H, ^3J = 6.9 Hz, C$\boldsymbol{H}_2$NH), 2.75 (t, 2H, ^3J = 7.5Hz, Thiaz-5-C$\boldsymbol{H}_2$), 2.17 (s, 3H, Thiaz-4-C$\boldsymbol{H}_3$), 1.86 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.52 (s, 9H, C(C$\boldsymbol{H}_3$)$_3$), 1.47 (s, 9H, C(C$\boldsymbol{H}_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 414 (MH^+, 100). $C_{18}H_{31}N_5O_4S$ (413.53)

***tert*-Butyl 5-[3-(*N-tert*-butoxycarbonylguanidino)propyl]thiazol-2-ylcarbamate (4.18)**

The title compound was prepared from **4.16** (5.8 g, 10.6 mmol) and 6 g of Pd/C (10 %) in a mixture of 160 ml THF/MeOH (1:1) according to the general procedure yielding **4.18** as a colourless foam-like solid (3.39 g, 75 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.03 (s, 1H, Thiaz-4-***H***), 3.26 (t, 2H, ^3J = 6.9 Hz, C$\boldsymbol{H}_2$NH), 2.84 (t, 2H, ^3J = 7.2 Hz, Thiaz-5-C$\boldsymbol{H}_2$), 1.95 (m, 2H, Thiaz-5-$CH_2C\boldsymbol{H}_2$), 1.55 (s, 9H, Boc-C$\boldsymbol{H}_3$), 1.47 (s, 9H, C(C$\boldsymbol{H}_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 400 (MH^+, 100). $C_{18}H_{31}N_5O_4S$ (399.50)

4.5.3. Preparation of Boc-protected aminothiazolylpropylguanidines 4.19a-4.46a

General procedure

To a solution of carboxylic acid (1 eq), EDAC (1 eq) and HOBt-monohydrate (1 eq) in DCM/abs was added DIEA (1 eq) under argon and stirred for 15 min. To this mixture a solution of **4.17** or **4.18** (1 eq), respectively, in DCM/abs was added and stirred over night at room temperature. The solvent was removed under reduced pressure and EtOAc and water was added to the resulting crude mixture. The organic phase was separated and the aqueous phase extracted two times with EtOAc. After drying over $MgSO_4$, the solvent removed *in vacuo*. The crude product was purified by flash-chromatography (PE/EtOAc 80/20 v/v) unless otherwise indicated.

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-(4-methylphenyl)butanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.19a)**

The title compound was prepared from 3-*p*-tolylbutanoic acid **3.27c** (180 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.19a** as a colourless foam-like solid (450 mg, 78 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.37 (s, 1H, N***H***), 8.94 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.11 (m, 4H, Ar-***H***), 3.42 (m, 2H, C***H***$_2$NH), 3.28 (m, 1H, C***H***CH_3), 2.69-2.55 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 2.30 (s, 3H, (p-C***H***$_3$)-Ar), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.31 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 574 (MH^+, 100). $C_{29}H_{43}N_5O_5S$ (573.75)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(2-methyl-3-phenylpropanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.20a)**

The title compound was prepared from 2-methyl-3-phenylpropanoic acid (160 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.20a** as a yellow foam-like solid (400 mg, 71 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.44 (s, 1H, N***H***), 8.99 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.28-7.16 (m, 5H, Ar-***H***), 3.43 (m, 2H, C***H***$_2$NH), 3.06 (m, 1H, COC***H***), 2.74-2.59 (m, 4H, Thiaz-5-C***H***$_2$, C***H***$_2$-Ar), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.19 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$),; ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 560 (MH^+, 100). $C_{28}H_{41}N_5O_5S$ (559.72)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-phenylpentanoyl)guanidino]-propyl]-4-methylthiazol-2-ylcarbamate (4.21a)**

The title compound was prepared from 3-phenylpentanoic acid **3.21c** (90 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.21a** as a colourless foam-like solid (170 mg, 59 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.34 (s, 1H, N***H***), 8.90 (t, 1H, ^3J = 5.1 Hz, CH_2N***H***) 7.32-7.17 (m, 5H, Ar-***H***), 3.40 (m, 2H, C***H***$_2$NH), 3.03 (m, 1H, CH_2C***H***), 2.76-2.62 (m, 4H, COC***H***$_2$, Thiaz-5-C***H***$_2$), 2.19 (s, 3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH_2C***H***$_2$) 1.74-1.60 (m, 2H, C***H***$_2CH_3$), 1.53 (s, 9H, C(C***H***$_3)_3$), 1.50 (s, 9H, C(C***H***$_3)_3$), 0.81 (t, 3H, ^3J = 7.3 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 574 (MH^+, 100). $C_{29}H_{43}N_5O_5S$ (573.75)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(2-benzylbutanoyl)guanidino]prop-yl]-4-methylthiazol-2-ylcarbamate (4.22a)**

The title compound was prepared from 2-benzylbutanoic acid **3.51d** (90 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.22a** as a colourless foam-like solid (180 mg, 63 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.34 (s, 1H, N***H***), 8.99 (t, 1H, ^3J = 5.1 Hz, CH_2N***H***), 7.26-7.17 (m, 5H, Ar-***H***), 3.42 (m, 2H, C***H***$_2$NH), 2.93 (dd, 1H, ^3J = 8.7 Hz, ^2J = 13.9 Hz, C***H***H-Ar), 2.81 (dd, 1H, ^3J = 6.5 Hz, ^2J = 13.7 Hz, CH***H***-Ar), 2.69 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.54 (m, 1H, COC***H***), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.67 (m, 2H, C***H***$_2CH_3$), 1.52 (s, 9H, C(C***H***$_3)_3$), 1.48 (s, 9H, C(C***H***$_3)_3$), 0.96 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 574 (MH^+, 100). $C_{29}H_{43}N_5O_5S$ (573.75)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-methyl-4-phenylbutanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.23a)**

The title compound was prepared from 3-methyl-4-phenylbutanoic acid **3.28c** (190 mg, 1.1 mmol), EDAC (210 mg, 1.1 mmol), HOBt-monohydrate (170 mg, 1.1 mmol), DIEA (0.18 ml, 1.1 mmol) in 5 ml DCM/abs and **4.17** (450 mg, 1.1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.23a** as a colourless foam-like solid (360 mg, 63 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.40 (s, 1H, N***H***), 8.99 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.29-7.17 (m, 5H, Ar-***H***), 3.44 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.63 (dd, ^3J = 6.8 Hz, ^2J = 13.6 Hz, C***H***H-Ar), 2.55 (dd, ^3J = 6.8 Hz, ^2J

= 13.6 Hz, CH***H***-Ar), 2.38 (m, 2H, COC***H***$_2$, C***H***CH$_3$), 2.22 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.51 (s, 9H, C(C***H***$_3$)$_3$), 0.99 (d, 3H, ^3J = 6.4 Hz); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 574 (MH$^+$, 100). $C_{29}H_{43}N_5O_5S$ (573.74)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-methyl-4-(3-methylphenyl)butanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.24a)**

The title compound was prepared from 3-methyl-4-*m*-tolylbutanoic acid **3.31c**(190 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.24a** as a pale yellow foam-like solid (500 mg, 85 %). ^{1}H-NMR (CDCl$_3$) δ ppm: 12.39 (s, 1H, N***H***), 9.00 (t, 1H, ^3J = 5.2 Hz, CH$_2$N***H***), 7.15 (m, 1H, Ar-***H***), 6.98 (t, 3H, Ar-***H***), 3.44 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.60-2.40 (m, 5H, COC***H***$_2$, CH$_3$C***H***, C***H***$_2$-Ar), 2.32 (s, 3H, (m-C***H***$_3$)-Ar), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.51 (s, 9H, C(C***H***$_3$)$_3$), 0.98 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 588 (MH$^+$, 100). $C_{30}H_{45}N_5O_5S$ (587.77)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-methyl-4-(4-methylphenyl)butanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.25a)**

The title compound was prepared from 3-methyl-4-*p*-tolylbutanoic acid **3.32c** (190 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.25a** as a pale yellow foam-like solid (480 mg, 82 %). ^{1}H-NMR (CDCl$_3$) δ ppm: 12.38 (s, 1H, N***H***), 9.00 (t, 1H, ^3J = 5.2 Hz, CH$_2$N***H***), 7.07 (m, 4H, Ar-***H***), 3.44 (m, 2H, C***H***$_2$NH), 2.69 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.55 (m, 2H, C***H***$_2$-Ar), 2.41-2.28 (m, 6H, COC***H***$_2$, CH$_3$C***H***, (m-C***H***$_3$)-Ar), 2.19 (s, 3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 0.97 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH$_4$OAc) *m/z* (%): 588 (MH$^+$, 100). $C_{30}H_{45}N_5O_5S$ (587.77)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[4-(3-methoxyphenyl)-3-methylbutanoyl]guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.26a)**

The title compound was prepared from 4-(3-methoxyphenyl)-3-methylbutanoic acid **3.35c** (105 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.26a** as a

colourless foam-like solid (200 mg, 66 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.40 (s, 1H, N***H***), 9.00 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.18 (m, 1H, Ar-***H***), 6.73 (m, 3H, Ar-***H***), 3.79 (s, 3H, (p-OC***H***$_3$)-Ar), 3.44 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.56 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$-Ar), 2.39 (m, 3H, C***H***CH_2, COC***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 0.99 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 604 (MH^+, 100). $C_{30}H_{45}N_5O_6S$ (603.77)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[4-(4-methoxyphenyl)-3-methylbutanoyl]guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.27a)**

The title compound was prepared from 4-(4-methoxyphenyl)-3-methylbutanoic acid **3.36c** (105 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.27a** as a colourless foam-like solid (200 mg, 66 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.39 (s, 1H, N***H***), 9.00 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.08 (m, 2H, Ar-***H***), 6.81 (m, 2H, Ar-***H***), 3.78 (s, 3H, (m-OC***H***$_3$)-Ar), 3.44 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.53 (t, 2H, ^3J = 6.5 Hz, C***H***$_2$-Ar), 2.36 (m, 3H, C***H***CH_2, COC***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.51 (s, 9H, C(C***H***$_3$)$_3$), 0.97 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 604 (MH^+, 100). $C_{30}H_{45}N_5O_6S$ (603.77)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[4-(3-fluorophenyl)-3-methylbutano-yl]-guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.28a)**

The title compound was prepared from 4-(3-fluorophenyl)-3-methylbutanoic acid **3.33c** (100 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.28a** as a colourless foam-like solid (200 mg, 68 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.38 (s, 1H, N***H***), 8.91 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.16 (m, 1H, Ar-***H***), 6.84 (m, 3H, Ar-***H***), 3.38 (m, 2H, C***H***$_2$NH), 2.61 (m, 3H, Thiaz-5-C***H***$_2$, C***H***H-Ar), 2.46 (dd, ^3J = 7.1 Hz, ^2J = 13.5 Hz, CH***H***-Ar), 2.28 (m, 3H, C***H***CH_2, COC***H***$_2$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.80 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.45 (s, 9H, C(C***H***$_3$)$_3$), 1.44 (s, 9H, C(C***H***$_3$)$_3$), 0.91 (d, 3H, ^3J = 6.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 592 (MH^+, 100). $C_{29}H_{42}FN_5O_5S$ (591.74)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[4-(4-fluorophenyl)-3-methylbutanoyl]guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.29a)**

The title compound was prepared from 4-(4-fluorophenyl)-3-methylbutanoic acid **3.34c** (100 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.29a** as a colourless foam-like solid (220 mg, 74 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.43 (s, 1H, N***H***), 8.98 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.12 (m, 2H, Ar-***H***), 6.95 (m, 2H, Ar-***H***), 3.45 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.61 (dd, 1H, ^3J = 6.6 Hz, ^2J = 13.4 Hz, C***H***H-Ar), 2.50 (dd, 1H, ^3J = 6.9 Hz, ^2J = 13.5 Hz, CH***H***-Ar), 2.35 (m, 3H, C***H***CH_2, COC***H***$_2$), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3)_3$), 1.51 (s, 9H, C(C***H***$_3)_3$), 0.97 (d, 3H, ^3J = 6.3 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 592 (MH^+, 100). $C_{29}H_{42}FN_5O_5S$ (591.74)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-((*R*)-3-cyclohexylbutanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.30a)**

The title compound was prepared from (*R*)-3-cyclohexylbutanoic acid **3.40** (170 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.30a** as a colourless foam-like solid (270 mg, 48 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.38 (s, 1H, N***H***), 9.05 (t, 1H, ^3J = 5.1 Hz, CH_2N***H***), 3.46 (m, 2H, C***H***$_2$NH), 2.71 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.48 (dd, 1H, ^3J = 5.1 Hz, ^2J = 14.9 Hz, COCH***H***), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 2.13 (dd, 1H, ^3J = 9.1 Hz, ^2J = 14.9 Hz, COC***H***H), 1.89 (m, 3H, Thiaz-5-CH_2C***H***$_2$, CH_3C***H***), 1.74-1.63 (m, 6H, cHex-C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3)_3$), 1.50 (s, 9H, C(C***H***$_3)_3$), 1.23-1.01 (m, 7H, cHex-C***H***$_2$, cHex-C***H***), 0.92 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 566 (MH^+, 100). $C_{28}H_{47}N_5O_5S$ (565.54)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-((*S*)-3-cyclohexylbutanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.31a)**

The title compound was prepared from (*S*)-3-cyclohexylbutanoic acid **3.41** (170 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.31a** as a colourless foam-like solid (360 mg, 64 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.35 (s, 1H, N***H***), 9.05 (t, 1H, ^3J = 5.1 Hz, CH_2N***H***), 3.47 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.49 (dd, 1H, ^3J = 5.1

Hz, ^2J = 14.9 Hz, COCH***H***), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 2.12 (dd, 1H, ^3J = 9.1 Hz, ^2J = 14.9 Hz, COC***H***H), 1.90 (m, 3H, Thiaz-5-CH$_2$C***H***$_2$, CH$_3$C***H***), 1.75-1.60 (m, 6H, cHex-C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.22-1.02 (m, 7H, cHex-C***H***$_2$, cHex-C***H***), 0.92 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 566 (MH^+, 100). $C_{28}H_{47}N_5O_5S$ (565.54)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-cyclohexyl-2-methylpropanoyl)-guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.32a)**

The title compound was prepared from 3-cyclohexyl-2-methylpropanoic acid **3.42** (170 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.32a** as a pale yellow foam-like solid (390 mg, 69 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.48 (s, 1H, N***H***), 9.05 (t, 1H, ^3J = 5.1 Hz, CH$_2$N***H***), 3.45 (m, 2H, C***H***$_2$NH), 2.70 (t, 3H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.52 (m, 1H, COC***H***), 2.21 (s, 3H, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.75-1.62 (m, 6H, cHex-C***H***, C***H***$_2$-cHex, cHex-C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.22 (m, 4H, cHex-C***H***$_2$), 1.14 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$), 0.87 (m, 2H, cHex-C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 566 (MH^+, 100). $C_{28}H_{47}N_5O_5S$ (565.75)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-cyclohexylpentanoyl)guanidino]-propyl]-4-methylthiazol-2-ylcarbamate (4.33a)**

The title compound was prepared from 3-cyclohexylpentanoic acid **3.43** (180 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.33a** as a pale yellow foam-like solid (420 mg, 83 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.41 (s, 1H, N***H***), 9.05 (t, 1H, ^3J = 5.2 Hz, CH$_2$N***H***), 3.45 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.41 (dd, 1H, ^3J = 5.9 Hz, ^2J = 15.4 Hz, COC***H***H), 2.21 (m, 3H, COCH***H***, Thiaz-4-C***H***$_3$), 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.68 (m, 6H, cHex-C***H***, cHex-C***H***$_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.34–1.03 (m, 8H, cHex-C***H***$_2$, C***H***$_2$CH$_3$, CH$_2$C***H***), 0.89 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 580 (MH^+, 100). $C_{29}H_{49}N_5O_5S$ (579.79)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[2-(cyclohexylmethyl)butanoyl]-guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.34a)**

The title compound was prepared from 2-(cyclohexylmethyl)butanoic acid **3.44** (90 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.34a** as a colourless foam-like solid (190 mg, 66 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.53 (s, 1H, N***H***), 9.55 (s, 1H, CH_2N***H***), 3.55 (m, 2H, C***H***$_2$NH), 2.73 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.39 (m, 1H, COC***H***), 2.30 (s, 3H, Thiaz-4-C***H***$_3$), 1.96 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 175-1.61 (m, 8H, cHex-C***H***$_2$, C***H***$_2$$CH_3$), 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.35-1.15 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 0.89 (m, 5H, C***H***$_3$, CHC***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 580 (MH^+, 100). $C_{29}H_{49}N_5O_5S$ (579.79)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(4-cyclohexyl-3-methylbutanoyl)-guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.35a)**

The title compound was prepared from 4-cyclohexyl-3-methylbutanoic acid **3.47** (180 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **4.35a** as a pale yellow foam-like solid (370 mg, 64 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.40 (s, 1H, N***H***), 9.04 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 3.45 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.34 (dd, 1H, ^3J = 8.6 Hz, ^3J = 17.5 Hz, COC***H***H), 2.22 (s, 3H, Thiaz-4-C***H***$_3$), 2.15 (dd, 1H, ^3J = 8.2 Hz, ^2J = 18.4 Hz, COCH***H***)1.87 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.72-1.64 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.27-1.09 (m, 6H, cHex-C***H***$_2$), 0.94 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$), 0.87 (m, 2H, C***H***$_2$-cHex); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 580 (MH^+, 100). $C_{29}H_{49}N_5O_5S$ (579.79)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[3-(cyclohexylmethyl)pentanoyl]-guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.36a)**

The title compound was prepared from 3-(cyclohexylmethyl)pentanoic acid **3.50** (100 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.36a** as a colourless foam-like solid (250 mg, 84 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.42 (s, 1H, N***H***), 9.05 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 3.46 (m, 2H, C***H***$_2$NH), 2.69 (m, 2H, Thiaz-5-C***H***$_2$), 2.27 (m, 2H, COC***H***$_2$), 2.20 (s, 3H, Thiaz-4-C***H***$_3$), 1.92 (m, 2H, Thiaz-5-CH_2C***H***$_2$, CH_2C***H***), 1.69 (m, 5H,

cHex-C***H***$_2$, cHex-C***H***), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.32-1.14 (m, 8H, cHex-C***H***$_2$, C***H***$_2$CH$_3$), 0.88 (m, 5H, C***H***$_2$-cHex, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 594 (MH^+, 100). $C_{30}H_{51}N_5O_5S$ (593.82)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-[3-(4-hydroxyphenyl)propanoyl]-guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.37a)**

The title compound was prepared from **3.54b** (300 mg, 1 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (1580 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure. ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 694.3 (MH^+, 100), $C_{36}H_{49}N_5O_7S$ (693.85). Subsequently, the yellow foam-like solid (480 mg, 69 %) was dissolved in 20 ml EtOH, Pd/C (10 %) (0.45 g, cat.) was added and hydrogenated at 8 bar for 6 days (TLC-control). The catalyst was filtered over Celite, the solvent removed in vacuo and **4.37a** (340 mg) used in the next step without further purification (only the double bond was hydrogenated).

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(6-*tert*-butyloxycarbonylamino-3-phenylhexanoyl)guanidino]propyl]-4-methylthiazol-2-ylcarbamate (4.38a)**

The title compound was prepared from **3.39c** (150 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding **4.38a** as a colourless foam-like solid (240 mg, 68 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.35 (s, 1H, N***H***), 8.86 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.21 (m, 5H, Ar-***H***), 3.41 (m, 2H, C***H***$_2$NH), 3.08 (m, 3H, CH_2C***H***, C***H***$_2$$NH_2$), 2.68 (m, 4H, COC***H***$_2$, Thiaz-5-C***H***$_2$), 2.19 (s, 3H, Thiaz-4-C***H***$_3$), 1.85 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.71-1.61 (m, 2H, C***H***$_2$$CH_2CH_2NH_2$), 1.52 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.41 (s, 9H, C(C***H***$_3$)$_3$), 1.24 (m, 2H, C***H***$_2$$CH_2NH_2$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 703 (MH^+, 100). $C_{35}H_{54}N_6O_7S$ (702.90)

10-(2-(*tert*-Butoxycarbonyl)-3-{3-[2-(*tert*-butoxycarbonylamino)-4-methylthiazol-5-yl]propyl}guanidino)-10-oxodecanoic acid (4.39a)

The title compound was prepared from **3.52** (150 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (80 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.17** (210 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure yielding the Cbz-protected compound as a yellow oil, which was immediately dissolved in 10 ml MeOH and hydrogenated with Pd/C (cat.) as catalyst for 1 h at room temperature. After filtration over Celite, the solvent was removed

under reduced pressure to obtain **4.39a** (0.21 g, 70 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 3.47 (m, 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.1 Hz, Thiaz-5-C***H***$_2$), 2.33 (m, 4H, C***H***$_2$COOH, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 4H, COCH$_2$C***H***$_2$, C***H***$_2$CH$_2$COOH), 1.53 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$), 1.33 (s, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 598 (MH^+, 100). $C_{28}H_{47}N_5O_7S$ (597.77)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-phenylpropanoyl)guanidino]-propyl]thiazol-2-ylcarbamate (4.40a)**

The title compound was prepared from 3-phenylpropanoic acid (75 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.40a** as a colourless foam-like solid (160 mg, 60 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.47 (s, 1H, N***H***), 8.98 (t, 1H, ^3J = 5.2 Hz, CH$_2$N***H***), 7.25 (m, 5H, Ar-***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.47 (m, 2H, C***H***$_2$NH), 3.00 (t, 2H, ^3J = 7.7 Hz, COCH$_2$C***H***$_2$), 2.72 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 1.92 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.49 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 532 (MH^+, 100). $C_{26}H_{37}N_5O_5S$ (531.67)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-phenylbutanoyl)guanidino]-propyl]-thiazol-2-ylcarbamate (4.41a)**

The title compound was prepared from 3-phenylbutanoic acid (80 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.41a** as a colourless foam-like solid (210 mg, 77 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.39 (s, 1H, N***H***), 8.93 (t, 1H, ^3J = 5.3 Hz, CH$_2$N***H***), 7.26 (m, 5H, Ar-***H***), 7.03 (s, 1H, Thiaz-4-***H***), 3.44 (m, 2H, C***H***$_2$NH), 3.30 (m, 1H, CH$_2$C***H***), 2.77 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.63 (m, 2H, COC***H***$_2$), 1.89 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.57 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.33 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 546 (MH^+, 100). $C_{27}H_{39}N_5O_5S$ (545.69)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-phenylpentanoyl)guanidino]propyl]thiazol-2-ylcarbamate (4.42a)**

The title compound was prepared from 3-phenylpentanoic acid **3.21c** (90 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA

(0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.42a** as a colourless foam-like solid (250 mg, 89 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.34 (s, 1H, N***H***), 8.90 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.02 (s, 1H, Thiaz-4-***H***), 3.42 (m, 2H, C***H***$_2$NH), 3.04 (m, 1H, C***H***CH_2CH_3), 2.76-2.65 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 1.89 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.76-1.63 (m, 2H, C***H***$_2CH_3$), 1.56 (s, 9H, C(C***H***$_3)_3$), 1.50 (s, 9H, C(C***H***$_3)_3$), 0.81 (t, 3H, ^3J = 7.3 Hz, C***H***$_3$); CI-MS (NH_3) *m/z* (%): 560 (MH^+, 100). $C_{28}H_{41}N_5O_5S$ (559.72)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3,3-diphenylpropanoyl)guanidino]-propyl]thiazol-2-ylcarbamate (4.43a)**

The title compound was prepared from 3,3-diphenylpropanoic acid (110 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.43a** as a colourless foam-like solid (230 mg, 76 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.51 (s, 1H, N***H***), 8.84 (t, 1H, ^3J = 5.4 Hz, CH_2N***H***), 7.29-7.15 (m, 10H, Ar-***H***), 7.02 (s, 1H, Thiaz-4-***H***), 4.58 (t, 1H, ^3J = 7.7 Hz, CH_2C***H***), 3.42 (m, 2H, C***H***$_2$NH), 3.13 (dd, 2H, ^3J = 7.9 Hz, ^2J = 15.1 Hz, COC***H***$_2$), 2.76 (m, 2H, Thiaz-5-C***H***$_2$), 1.87 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.56 (s, 9H, C(C***H***$_3)_3$), 1.50 (s, 9H, C(C***H***$_3)_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 608 (MH^+, 100). $C_{32}H_{41}N_5O_5S$ (607.76)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-cyclohexylbutanoyl)guanidino]-propyl]thiazol-2-ylcarbamate (4.44a)**

The title compound was prepared from 3-cyclohexylbutanoic acid (85 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.44a** as a colourless foam-like solid (220 mg, 58 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.38 (s, 1H, N***H***), 9.06 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.48 (m, 2H, C***H***$_2$NH), 2.79 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.49 (dd, 1H, ^3J = 5.1 Hz, ^2J = 14.9 Hz, COC***H***H), 2.12 (dd, 1H, ^3J = 9.2 Hz, ^2J = 14.9 Hz, COCH***H***), 1.92 (m, 3H, Thiaz-5-CH_2C***H***$_2$, CH_2C***H***), 1.74-1.63 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.20-1.02 (m, 6H, cHex-C***H***$_2$), 1.56 (s, 9H, C(C***H***$_3)_3$), 1.50 (s, 9H, C(C***H***$_3)_3$), 0.92 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 552 (MH^+, 100). $C_{27}H_{45}N_5O_5S$ (551.74)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-cyclohexyl-2-methylpropanoyl)-guanidino]propyl]thiazol-2-ylcarbamate (4.45a)**

The title compound was prepared from 3-cyclohexyl-2-methylpropanoic acid **3.42** (85 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.45a** as a colourless foam-like solid (210 mg, 76 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.48 (s, 1H, N***H***), 9.07 (t, 1H, ^3J = 5.2 Hz, CH_2N***H***), 7.04 (s, 1H, Thiaz-4-***H***), 3.48 (m, 2H, C***H***$_2$NH), 2.80 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.52 (m, 1H, C***H***CH_3), 1.92 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.75-1.61 (m, 6H, cHex-C***H***$_2$, C***H***$_2$-Ar), 1.57 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), 1.29-1.17 (m, 8H, cHex-C***H***$_2$, cHex-C***H***, C***H***$_3$), 0.89 (m, 2H, cHex-C***H***$_2$); CI-MS (NH_3) *m/z* (%): 552 (MH^+, 100). $C_{27}H_{45}N_5O_5S$ (551.72)

***tert*-Butyl 5-[3-[*N*-*tert*-butoxycarbonyl-*N´*-(3-cyclohexylpentanoyl)guanidino]-propyl]thiazol-2-ylcarbamate (4.46a)**

The title compound was prepared from 3-cyclohexylpentanoic acid **3.43**(80 mg, 0.5 mmol), EDAC (95 mg, 0.5 mmol), HOBt-monohydrate (800 mg, 0.5 mmol), DIEA (0.09 ml, 0.5 mmol) in 2.5 ml DCM/abs and **4.18** (200 mg, 0.5 mmol) in 2.5 ml DCM/abs according to the general procedure (PE/EtOAC 70/30 v/v) yielding **4.46a** as a colourless foam-like solid (80 mg, 28 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 12.41 (s, 1H, N***H***), 9.06 (t, 1H, ^3J = 5.1 Hz, CH_2N***H***), 7.05 (s, 1H, Thiaz-4-***H***), 3.48 (m, 2H, C***H***$_2$NH), 2.79 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.42 (dd, 1H, ^3J = 5.9 Hz, ^2J = 15.5 Hz, COC***H***H), 2.21 (dd, 1H, ^3J = 7.7 Hz, ^2J = 15.4 Hz, COCH***H***), 1.92 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.75-1.60 (m, 6H, cHex-C***H***$_2$, CH_2C***H***, cHex-C***H***), 1.31-1.05 1.56 (s, 9H, C(C***H***$_3$)$_3$), 1.50 (s, 9H, C(C***H***$_3$)$_3$), (m, 8H, cHex-C***H***$_2$, C***H***$_2$$CH_3$), 0.89 (t, 3H, ^3J = 7.5 Hz, C***H***$_3$); CI-MS (NH_3) *m/z* (%): 566 (MH^+, 100). $C_{28}H_{47}N_5O_5S$ (565.76)

4.5.4. Preparation of the deprotected acylguanidines 4.19-4.46

General procedure

To a solution of the protected acylguanidine in CH_2Cl_2/abs was added TFA (20 %) and the mixture was stirred at ambient temperatur until the Boc groups were removed (3-5 h). Subsequently, the solvent was removed *in vacuo* and the residue was purified by preparative RP-HPLC (for general conditions see chapter 3). All compounds were obtained as trifluoroacetic acid salts.

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-(3-(4-methylphenyl)butanoyl)-guanidine (4.19)

The title compound was prepared from **4.19a** (430 mg, 0.75 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.19** as a colourless oil (340 mg, 75 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.07 (m, 4H, Ar-***H***), 3.29 (m, 3H, C***H***$_2$NH, CH_3C***H***), 2.69 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 2.23 (s, 3H, (*p*-C***H***$_3$)-Ar), 2.12 (s, 3H, Thiaz-4-C***H***$_3$), 1.85 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.26 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.35 (quat. ***C***=O), 170.42 (quat. Thiaz-2-***C***), 155.26 (quat. ***C***=NH), 143.27 (quat. Thiaz-4-***C***), 137.24 (quat. Ar-***C***), 132.58 (quat. Ar-***C***), 130.25 (+, 2 Ar-***C***H), 127.82 (+, 2 Ar-***C***H), 118.24 (quat. Thiaz-5-***C***), 46.25 (-, CO***C***H_2), 41.47 (-, ***C***H_2NH), 37.34 (+, ***C***HCH_3), 29.62 (-, Thiaz-5-CH_2***C***H_2), 23.57 (-, Thiaz-5-***C***H_2), 22.37 (+, (*p*-***C***H_3)-Ar), 21.16 (+, ***C***H_3), 11.48 (+, Thiaz-4-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{19}H_{27}N_5OS$) calcd. 373.1936, found 373.19282; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{19}H_{27}N_5OS \cdot 2$TFA (601.5)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-(2-methyl-3-phenylpropanoyl)-guanidine (4.20)

The title compound was prepared from **4.20a** (390 mg, 0.69 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.20** as a colourless oil (340 mg, 83 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.22 (m, 5H, Ar-***H***), 3.32 (m (overlap with solvent peaks), 2H, C***H***$_2$NH) , 2.99 (dd, 1H, ^3J = 7.6 Hz, ^2J = 12.7 Hz, C***H***H-Ar), 2.89 (m, 1H, COC***H***), 2.70 (m, 3H, Thiaz-5-C***H***$_2$, CH***H***-Ar), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.19 (d, 3H, ^3J = 6.6 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 180.28 (quat. ***C***=O), 170.37 (quat. Thiaz-2-***C***), 155.29 (quat. ***C***=NH), 140.03 (quat. Thiaz-4-***C***), 132.60 (quat. Ar-***C***), 130.13 (+, 2 Ar-***C***H), 129.55 (+, 2 Ar-***C***H), 127.69 (+, Ar-***C***H), 118.41 (quat. Thiaz-5-***C***), 45.04 (+, CO***C***H), 41.58 (-, ***C***H_2-Ar), 40.62 (-, ***C***H_2NH), 29.65 (-, Thiaz-5-CH_2***C***H_2), 23.60 (-, Thiaz-5-***C***H_2), 17.09 (+, ***C***H_3), 11.46 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{25}N_5OS$) calcd. 359.1779, found 359.17716; prep. HPLC: MeCN/0.1% TFA/aq (20/80-40/60). $C_{18}H_{25}N_5OS \cdot$ 2TFA (587.47)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-(3-phenylpentanoyl)guanidine (4.21)

The title compound was prepared from **4.21a** (150 mg, 0.26 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.21** as a colourless oil (60 mg, 38 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.22 (m, 5H, Ar-***H***), 3.27 (t,

2H, ^3J = 6.1 Hz, C***H***$_2$NH), 3.04 (m, 1H, CH$_2$C***H***), 2.83 (dd, 1H, ^3J = 6.2 Hz, ^2J = 15.1 Hz, COC***H***H), 2.74 (dd, 1H, ^3J = 9.1 Hz, ^2J = 15.1 Hz, COCH***H***), 2.66 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.85 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$) 1.68 (m, 2H, C***H***$_2$CH$_3$), 0.78 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C (CD$_3$OD) δ ppm: 176.24 (quat. ***C***=O), 170.38 (quat. Thiaz-2-***C***), 155.17 (quat. ***C***=NH), 144.45 (quat. Ar-***C***), 132.59 (quat. Thiaz-4-***C***), 129.58 (+, 2 Ar-***C***H), 128.79 (+, 2 Ar-***C***H), 127.78 (+,Ar-***C***H), 118.33 (quat. Thiaz-5-***C***), 45.17 (+, CH$_2$***C***H), 44.76 (-, ***C***H$_2$NH), 41.44 (-, CO***C***H$_2$), 30.21 (-, Thiaz-5-CH$_2$***C***H$_2$), 29.60 (-, Thiaz-5-***C***H$_2$), 23.52 (-, ***C***H$_2$CH$_3$), 12.26 (+, ***C***H$_3$), 11.44 (+, Thiaz-***C***H$_3$); HRMS: EI-MS: *m/z* for (C$_{18}$H$_{25}$N$_5$OS) calcd. 373.1936, found 373.19443; prep. HPLC: MeCN/0.1% TFA/aq (25/75-40/60). C$_{19}$H$_{27}$N$_5$OS · 2TFA (601.53)

N-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N´-(2-benzylbutanoyl)guanidine (4.22)

The title compound was prepared from **4.22a** (170 mg, 0.29 mmol) in 5 ml CH$_2$Cl$_2$/abs and 1 ml TFA according to the general procedure yielding **4.22** as a colourless oil (70 mg, 38 %). ^{1}H-NMR (CD$_3$OD) δ ppm: 7.19 (m, 5H, Ar-***H***), 3.28 (m (overlap with solvent peaks), 2H, C***H***$_2$NH), 2.85 (m, 3H, COC***H***, C***H***$_2$-Ar), 2.65 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.85 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.72 (m, 1H,C***H***HCH$_3$), 1.60 (m, 1H, CH***H***CH$_3$), 0.94 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C (CD$_3$OD) δ ppm: 179.97 (quat. ***C***=O), 170.40 (quat. Thiaz-2-***C***), 155.07 (quat. ***C***=NH), 140.04 (quat. Thiaz-4-***C***), 132.59 (quat. Ar-***C***), 130.08 (+, 2 Ar-***C***H), 129.55 (+, 2 Ar-***C***H), 127.65 (+, Ar-***C***H), 118.26 (quat. Thiaz-5-***C***), 52.51 (+, CO***C***H), 41.47 (-, CH***C***H$_2$), 39.30 (-, ***C***H$_2$NH), 29.58 (-, Thiaz-5-CH$_2$***C***H$_2$), 26.30 (-, Thiaz-5-***C***H$_2$), 23.57 (-, ***C***H$_2$CH$_3$), 11.92 (+, ***C***H$_3$), 11.47 (+, Thiaz-***C***H$_3$); HRMS: EI-MS: *m/z* for (C$_{19}$H$_{27}$N$_5$OS) calcd. 373.1936, found 373.19376; prep. HPLC: MeCN/0.1% TFA/aq (25/75-40/60). C$_{19}$H$_{27}$N$_5$OS · 2TFA (615.52)

N-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N´-(3-methyl-4-phenylbutanoyl)-guanidine (4.23)

The title compound was prepared from **4.23a** (340 mg, 0.59 mmol) in 10 ml CH$_2$Cl$_2$/abs and 2 ml TFA according to the general procedure yielding **4.23** as a pale yellow oil (240 mg, 68 %). ^{1}H-NMR (CD$_3$OD) δ ppm: 7.19 (m, 5H, Ar-***H***), 3.31 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.67 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.51 (m, 3H, C***H***$_2$-Ar, CH$_3$C***H***), 2.30 (m, 2H, COC***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$) ppm 1.87 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 0.94 (d, 3H, ^3J = 6.2Hz); ^{13}C (CD$_3$OD) δ ppm: 176.95 (quat. ***C***=O), 170.42

(quat. Thiaz-2-***C***), 155.29 (quat. ***C***=NH), 141.39 (quat. Thiaz-4-***C***), 132.58 (quat. Ar-***C***), 130.38 (+, 2 Ar-***C***H), 129.37 (+, 2 Ar-***C***H), 127.25 (+,Ar-***C***H), 118.29 (quat. Thiaz-5-***C***), 44.59 (-, CO***C***H_2), 43.96 (-, ***C***H_2-Ar), 41.54 (-, ***C***H_2NH), 33.43 (+, ***C***HCH_3), 29.69 (-, Thiaz-5-CH_2***C***H_2), 23.64 (-, Thiaz-5-***C***H_2), 19.97 (+, ***C***H_3), 11.49 (+, Thiaz-4-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{19}H_{27}N_5OS$) calcd. 373.1936, found 373.19356; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{19}H_{27}N_5OS \cdot 2TFA$ (601.49)

N-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N´-(3-methyl-4-(3-methylphenyl)-butanoyl)guanidine (4.24)

The title compound was prepared from **4.24a** (410 mg, 0.69 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.24** as a pale yellow oil (290 mg, 68 %). $C_{20}H_{29}N_5OS$ * 2TFA (), ^{1}H-NMR (CD_3OD) δ ppm: 7.13 (m, 1H, Ar-***H***), 6.97 (m, 3H, Ar-***H***), 3.33 (m (overlap with solvent peaks), 2H, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.6 Hz), 2.57-2.38 (m, 5H, COC***H***$_2$, CH_3C***H***, C***H***$_2$-Ar), 2.29 (s, 3H, (m-C***H***$_3$)-Ar), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.96 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.82 (quat. ***C***=O), 170.36 (quat. Thiaz-2-***C***), 155.20 (quat. ***C***=NH), 141.28 (quat. Thiaz-4-***C***), 139.00 (quat. ***C***-Ph), 132.58 (quat. Ar-***C***), 131.06 (+, Ar-***C***H), 129.26 (+, Ar-***C***H), 127.92 (+, Ar-***C***H), 127.42 (+, Ar-***C***H), 118.41 (quat. Thiaz-5-***C***), 44.57 (-, CO***C***H_2), 43.92 (-, ***C***H_2-Ar), 41.57 (-, ***C***H_2NH), 33.42 (+, ***C***HCH_3), 29.72 (-, Thiaz-5-CH_2***C***H_2), 23.61 (-, Thiaz-5-***C***H_2), 21.50 (+, (m-***C***H_3)-Ar), 20.03 (+, ***C***H_3), 11.45 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{20}H_{29}N_5OS$) calcd. 387.20928, found 387.20964; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{20}H_{29}N_5OS \cdot 2TFA$ (615.52)

N-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N´-(3-methyl-4-(4-methylphenyl)-butanoyl)guanidine (4.25)

The title compound was prepared from **4.25a** (460 mg, 0.78 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.25** as a pale yellow oil (320 mg, 67 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.01 (m, 4H, Ar-***H***), 3.30 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$NH), 2.66 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.48 (m, 3H, COC***H***$_2$, CH_3C***H***), 2.29 (m, 2H, C***H***$_2$-Ar), 2.24 (s, 3H, (*p*-C***H***$_3$)-Ar), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.92 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 177.06 (quat. ***C***=O), 170.40 (quat. Thiaz-2-***C***), 155.27 (quat. ***C***=NH), 138.19 (quat. Thiaz-4-***C***), 136.76 (quat. ***C***-Ph), 132.56 (quat. Ar-***C***), 130.31 (+, 2 Ar-***C***H), 129.99 (+, 2 Ar-***C***H), 118.24 (quat. Thiaz-5-***C***), 44.58 (-, CO***C***H_2), 43.58 (-, ***C***H_2-Ar), 41.54 (-, ***C***H_2NH), 33.50 (+, ***C***HCH_3), 29.71 (-, Thiaz-5-CH_2***C***H_2), 23.64 (-, Thiaz-5-***C***H_2), 21.23

(+, (*p*-***C***H_3)-Ar), 20.07 (+, ***C***H_3), 11.49 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{20}H_{29}N_5OS$) calcd. 387.2093, found 387.20934; prep. HPLC: MeCN/0.1% TFA/aq (30/70). $C_{20}H_{29}N_5OS \cdot 2TFA$ (615.52)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-[4-(3-methoxyphenyl)-3-methyl-butanoyl]guanidine (4.26)

The title compound was prepared from **4.26a** (180 mg, 0.29 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.26** as a pale yellow oil (80 mg, 44 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.15 (m, 1H, Ar-***H***), 6.73 (m, 3H, Ar-***H***), 3.75 (s, 3H, (*p*-OC***H***$_3$)-Ar), 3.31 (t, 2H, ^3J = 6.6 Hz, C***H***$_2$NH), 2.69 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.53 (m, 3H, C***H***CH_2, C***H***$_2$-Ar), 2.32 (m, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.96 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.88 (quat. ***C***=O), 170.39 (quat. Thiaz-2-***C***), 161.18 (quat. Ar-***C***), 155.25 (quat. ***C***=NH), 142.97 (quat. Thiaz-4-***C***), 132.60 (quat. Ar-***C***H), 130.33 (+, Ar-***C***H), 122.74 (+, Ar-***C***H), 118.36 (quat. Thiaz-5-***C***), 115.92 (+, Ar-***C***H), 112.69 (+, Ar-***C***H), 55.60 (+, O***C***H_3), 44.54 (-, CO***C***H_2), 44.00 (-, CH***C***H_2), 41.54 (-, ***C***H_2NH), 33.35 (+, ***C***HCH_3), 29.68 (-, Thiaz-5-CH_2***C***H_2), 23.62 (-, Thiaz-5-***C***H_2), 20.09 (+, ***C***H_3), 11.46 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{20}H_{29}N_5O_2S$) calcd. 403.2042, found 403.20446; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{20}H_{29}N_5O_2S \cdot 2TFA$ (631.52)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-[4-(4-methoxyphenyl)-3-methyl-butanoyl]-guanidine (4.27)

The title compound was prepared from **4.27a** (180 mg, 0.29 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.27** as a pale yellow oil (110 mg, 60 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.07 (m, 2H, Ar-***H***), 6.80 (m, 2H, Ar-***H***), 3.73 (s, 3H, (m-OC***H***$_3$)-Ar), 3.32 (m (overlap with solvent peaks), 2H, C***H***$_2$NH), 2.69 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.48 (m, 3H, C***H***CH_2, C***H***$_2$-Ar), 2.28 (m, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.95 (d, 3H, ^3J = 6.2 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.98 (quat. ***C***=O), 170.39 (quat. Thiaz-2-***C***), 159.62 (quat. Ar-***C***), 155.24 (quat. ***C***=NH), 133.29 (quat. Thiaz-4-***C***), 132.57 (quat. Ar-***C***), 131.37 (+, 2 Ar-***C***H), 118.32 (quat. Thiaz-5-***C***), 114.73 (+, 2 Ar-***C***H), 55.69 (+, O***C***H_3), 44.56 (-, CO***C***H_2), 43.16 (-, CH***C***H_2), 41.53 (-, ***C***H_2NH), 33.67 (+, ***C***HCH_3), 29.68 (-, Thiaz-5-CH_2***C***H_2), 23.63 (-, Thiaz-5-***C***H_2), 20.13 (+, ***C***H_3), 11.46 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{20}H_{29}N_5O_2S$) calcd. 403.2042, found 403.20523; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{20}H_{29}N_5O_2S \cdot 2TFA$ (631.52)

***N*-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N´*-[4-(3-fluorophenyl)-3-methyl-butanoyl]guanidine (4.28)**

The title compound was prepared from **4.28a** (190 mg, 0.32 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.28** as a pale yellow oil (140 mg, 71 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.24 (m, 1H, Ar-***H***), 6.91 (m, 3H, Ar-***H***), 3.32 (m (overlap with solvent peaks), 2H, C***H***$_2$NH), 2.69 (t, 2H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.53 (m, 3H, C***H***CH_2, C***H***$_2$-Ar), 2.35 (m, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.86 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.94 (d, 3H, ^3J = 6.1 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.78 (quat. ***C***=O), 170.41 (quat. Thiaz-2-***C***), 164.29 (d, quart., ^1J = 243.9 Hz, Ar-***C***F), 155.28 (quat. ***C***=NH), 144.36 (d, quart., ^3J = 7.2 Hz, Ar-***C***), 132.58 (quat. Thiaz-4-***C***), 131.01 (d, +, ^3J = 8.3 Hz, Ar-***C***H), 126.24 (+, d, ^4J = 2.5 Hz, Ar-***C***H), 118.31 (quat. Thiaz-5-***C***), 116.94 (+, d, ^3J = 20.9 Hz, Ar-***C***H), 113.91 (+, d, ^3J = 21.1 Hz, Ar-***C***H), 44.44 (-, CO***C***H_2), 43.51 (-, CH***C***H_2), 41.55 (-, ***C***H_2NH), 33.24 (+, ***C***HCH_3), 29.68 (-, Thiaz-5-CH_2***C***H_2), 23.63 (-, Thiaz-5-***C***H_2), 19.81 (+, ***C***H_3), 11.46 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{19}H_{26}FN_5OS$) calcd. 391.1842, found 391.1842; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{19}H_{26}FN_5OS \cdot 2TFA$ (619.49)

***N*-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N´*-[4-(4-fluorophenyl)-3-methyl-butanoyl]guanidine (4.29)**

The title compound was prepared from **4.29a** (200 mg, 0.34 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.29** as a pale yellow oil (100 mg, 47 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.17 (m, 2H, Ar-***H***), 6.96 (m, 2H, Ar-***H***), 3.32 (m (overlap with solvent peaks), 2H, C***H***$_2$NH), 2.69 (t, 2H, ^3J = 7.3 Hz, Thiaz-5-C***H***$_2$), 2.51 (m, 3H, C***H***CH_2, C***H***$_2$-Ar), 2.30 (m, 2H, COC***H***$_2$), 2.16 (s, 3H, Thiaz-4-C***H***$_3$), 1.89 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 0.94 (d, 3H, ^3J = 5.4 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.84 (quat. ***C***=O), 170.40 (quat. Thiaz-2-***C***), 162.93 (d, quart., ^1J = 242.5 Hz, Ar-***C***F), 155.27 (quat. ***C***=NH), 137.35 (d, quart., ^4J = 3.3 Hz, Ar-***C***), 132.58 (quat. Thiaz-4-***C***), 132.00 (d, +, ^3J = 7.8 Hz, 2 Ar-***C***H), 118.32 (quat. Thiaz-5-***C***), 115.91 (d, +, ^2J = 21.3 Hz, 2 Ar-***C***H), 44.46 (-, CO***C***H_2), 43.02 (-, CH***C***H_2), 41.54 (-, ***C***H_2NH), 33.48 (+, ***C***HCH_3), 29.69 (-, Thiaz-5-CH_2***C***H_2), 23.63 (-, Thiaz-5-***C***H_2), 19.85 (+, ***C***H_3), 11.46 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{19}H_{26}FN_5OS$) calcd. 391.1842, found 391.18328; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{19}H_{26}FN_5OS \cdot 2TFA$ (619.49)

***N*-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N´*-((*R*)-3-cyclohexylbutanoyl)-guanidine (4.30)**

The title compound was prepared from **4.30a** (250 mg, 0.44 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.30** as a colourless oil (240 mg, 92 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.70 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.55 (dd, 1H, ^3J = 5.1 Hz, ^2J = 15.0 Hz, COCH***H***), 2.23-2.17 (m, 4H, COC***H***H, Thiaz-4-C***H***$_3$), 1.90 (m, 3H, Thiaz-5-CH_2C***H***$_2$, CH_3C***H***), 1.68 (m, 6H, cHex-C***H***$_2$), 1.09 (m, 7H, cHex-C***H***$_2$, cHex-C***H***), 0.90 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 177.61 (quat. ***C***=O), 170.42 (quat. Thiaz-2-***C***), 155.38 (quat. ***C***=NH), 132.58 (quat. Thiaz-4-***C***), 118.31 (quat. Thiaz-5-***C***), 43.94 (+, cHex-***C***H), 42.71 (-, CO***C***H_2), 41.56 (-, ***C***H_2NH), 36.46 (+, ***C***HCH_3), 31.47 (-, cHex-***C***H_2), 30.02 (-, cHex-***C***H_2), 29.69 (-, Thiaz-5-CH_2***C***H_2), 27.84 (-, cHex-***C***H_2), 27.74 (-, 2 cHex-***C***H_2), 23.63 (-, Thiaz-5-***C***H_2), 16.62 (+, ***C***H_3), 11.46 (+, Thiaz-4-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{31}N_5OS$) calcd. 365.2249, found 365.22467; prep. HPLC: MeCN/0.1% TFA/aq (35/65). $C_{18}H_{31}N_5OS \cdot 2TFA$ (593.52)

***N*-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N´*-((*S*)-3-cyclohexylbutanoyl)guanidine (4.31)**

The title compound was prepared from **4.31a** (340 mg, 0.60 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.31** as a colourless oil (320 mg, 90 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 2H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.69 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.54 (dd, 1H, ^3J = 5.0 Hz, ^2J = 15.0 Hz, COCH***H***), 2.23-2.16 (m, 4H, COC***H***H, Thiaz-4-C***H***$_3$), 1.89 (m, 3H, Thiaz-5-CH_2C***H***$_2$, CH_3C***H***), 1.69 (m, 6H, cHex-C***H***$_2$), 1.09 (m, 7H, cHex-C***H***$_2$, cHex-C***H***), 0.88 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 177.67 (quat. ***C***=O), 170.43 (quat. Thiaz-2-***C***), 155.39 (quat. ***C***=NH), 132.58 (quat. Thiaz-4-***C***), 118.27 (quat. Thiaz-5-***C***), 43.93 (+, cHex-***C***H), 42.72 (-, CO***C***H_2), 41.56 (-, ***C***H_2NH), 36.37 (+, ***C***HCH_3), 31.46 (-, cHex-***C***H_2), 30.02 (-, cHex-***C***H_2), 29.70 (-, Thiaz-5-CH_2***C***H_2), 27.84 (-, cHex-***C***H_2), 27.74 (-, 2 cHex-***C***H_2), 23.65 (-, Thiaz-5-***C***H_2), 16.64 (+, ***C***H_3), 11.48 (+, Thiaz-4-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{31}N_5OS$) calcd. 365.2249, found 365.22575; prep. HPLC: MeCN/0.1% TFA/aq (35/65). $C_{18}H_{31}N_5OS \cdot 2TFA$ (593.52)

***N*-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-*N´*-(3-cyclohexyl-2-methylpropanoyl)guanidine (4.32)**

The title compound was prepared from **4.32a** (370 mg, 0.65 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.32** as a

colourless oil (320 mg, 82 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.36 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.69 (m, 3H, Thiaz-5-C***H***$_2$, COC***H***), 2.17 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.66 (m, 7H, cHex-C***H***, C***H***$_2$-cHex, cHex-C***H***$_2$), 1.23 (m, 4H, cHex-C***H***$_2$), 1.14 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$), 0.88 (m, 2H, cHex-C***H***$_2$); ^{13}C (CD_3OD) δ ppm: 181.53 (quat. ***C***=O), 170.43 (quat. Thiaz-2-***C***), 155.60 (quat. ***C***=NH), 132.57 (quat. Thiaz-4-***C***), 118.27 (quat. Thiaz-5-***C***), 42.28 (-, CH***C***H$_2$), 41.59 (-, ***C***H$_2$NH), 40.26 (+, ***C***HCH$_3$), 36.65 (+, cHex-***C***H), 34.51 (-, cHex-***C***H$_2$), 34.24 (-, cHex-***C***H$_2$), 29.67 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.60 (-, cHex-***C***H$_2$), 27.35 (-, cHex-***C***H$_2$), 27.32 (-, cHex-***C***H$_2$), 23.65 (-, Thiaz-5-***C***H$_2$), 17.96 (+, ***C***H$_3$), 11.48 (+, Thiaz-***C***H$_3$); HRMS: EI-MS: *m/z* for ($C_{18}H_{31}N_5OS$) calcd. 365.22493, found 365.22560; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{18}H_{31}N_5OS \cdot 2TFA$ (593.52)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-(3-cyclohexylpentanoyl)guanidine (4.33)

The title compound was prepared from **4.33a** (400 mg, 0.65 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.33** as a pale yellow oil (320 mg, 76 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.71 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.50 (dd, 1H, ^3J = 6.2 Hz, ^2J = 15.6 Hz, COC***H***H), 2.32 (dd, 1H, ^3J = 7.5 Hz, ^2J = 15.6 Hz, COCH***H***), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.68 (m, 6H, cHex-C***H***, cHex-C***H***$_2$), 1.39 – 1.07 (m, 8H, cHex-C***H***$_2$, C***H***$_2$CH$_3$, CH$_2$C***H***), 0.89 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 177.82 (quat. ***C***=O), 170.38 (quat. Thiaz-2-***C***), 155.32 (quat. ***C***=NH), 132.59 (quat. Thiaz-4-***C***), 118.41 (quat. Thiaz-5-***C***), 42.99 (-, CO***C***H$_2$), 41.59 (+, cHex-***C***H), 41.45 (-, ***C***H$_2$NH), 39.71 (+, CH$_2$***C***H), 31.18 (-, cHex-***C***H$_2$), 30.35 (-, cHex-***C***H$_2$), 29.72 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.91 (-, cHex-***C***H$_2$), 27.89 (-, cHex-***C***H$_2$), 27.79 (-, cHex-***C***H$_2$), 24.75 (-, ***C***H$_2$CH$_3$), 23.60 (-, Thiaz-5-***C***H$_2$), 12.05 (+, ***C***H$_3$), 11.44 (+, Thiaz-***C***H$_3$); HRMS: EI-MS: *m/z* for ($C_{19}H_{33}N_5OS$) calcd. 379.2405, found 379.24061; prep. HPLC: MeCN/0.1% TFA/aq (25/75-40/60). $C_{19}H_{33}N_5OS \cdot 2TFA$ (607.54)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-[2-(cyclohexylmethyl)butanoyl]-guanidine (4.34)

The title compound was prepared from **4.34a** (180 mg, 0.31 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.34** as a pale yellow oil (90 mg, 48 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.36 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.72 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.55 (m, 1H, COC***H***), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.92 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 8H, cHex-C***H***$_2$, C***H***$_2$CH$_3$), 1.27 (m, 5H,

cHex-C***H***$_2$, cHex-C***H***), 0.89 (m, 5H, C***H***$_3$, CHC***H***$_2$); ^{13}C (CD_3OD) δ ppm: 180.86 (quat. ***C***=O), 170.38 (quat. Thiaz-2-***C***), 155.30 (quat. ***C***=NH), 132.60 (quat. Thiaz-4-***C***), 118.39 (quat. Thiaz-5-***C***), 47.86 (+, CO***C***H), 41.64 (-, ***C***H_2NH), 40.67 (-, CH***C***H_2), 36.99 (+, cHex-***C***H), 34.79 (-, cHex-**C**H_2), 34.19 (-, cHex-**C**H_2), 29.66 (-, Thiaz-5-CH_2***C***H_2), 27.57 (-, cHex-**C**H_2), 27.36 (-, cHex-**C**H_2), 27.33 (-, cHex-**C**H_2), 27.20 (-, ***C***H_2CH_3), 23.62 (-, Thiaz-***C***H_2), 11.90 (+, Thiaz-***C***H_3), 11.46 (+, ***C***H_3); HRMS: EI-MS: *m/z* for ($C_{19}H_{33}N_5OS$) calcd. 379.2406, found 379.24131; prep. HPLC: MeCN/0.1% TFA/aq (30/70-50/50). $C_{19}H_{33}N_5OS \cdot 2TFA$ (607.54)

N-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N´-(4-cyclohexyl-3-methylbutanoyl)-guanidine (4.35)

The title compound was prepared from **4.35a** (340 mg, 0.59 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.35** as a pale yellow oil (300 mg, 84 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 2.71 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.44 (dd, 1H, ^3J = 5.7 Hz, ^2J = 14.8 Hz, COC***H***H), 2.25-2.18 (m, 4H, COCH***H***, Thiaz-4-C***H***$_3$), 2.10 (m, 1H, C***H***CH_3), 1.91 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.72 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.20 (m, 6H, cHex-C***H***$_2$), 0.94 (d, 3H, ^3J = 6.5 Hz, C***H***$_3$), 0.83 (m, 2H, C***H***$_2$); ^{13}C (CD_3OD) δ ppm: 176.96 (quat. ***C***=O), 170.36 (quat. Thiaz-2-***C***), 155.28 (quat. ***C***=NH), 132.59 (quat. Thiaz-4-***C***), 118.44 (quat. Thiaz-5-***C***), 45.80 (-, CO***C***H_2), 45.59 (-, CH***C***H_2), 41.61 (-, ***C***H_2NH), 36.10 (-, cHex-***C***H_2), 35.13, (+, cHex-***C***H), 34.14 (-, cHex-***C***H_2), 29.73 (-, Thiaz-5-CH_2***C***H_2), 28.32 (+, ***C***HCH_3), 27.78 (-, cHex-***C***H_2), 27.48 (-, cHex-***C***H_2), 27.40 (-, cHex-***C***H_2), 23.61 (-, Thiaz-5-***C***H_2), 20.06 (+, ***C***H_3), 11.46 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{19}H_{33}N_5OS$) calcd. 379.2405, found 379.24108; prep. HPLC: MeCN/0.1% TFA/aq (30/70-50/50). $C_{19}H_{33}N_5OS \cdot 2TFA$ (607.54)

N-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-N´-[3-(cyclohexylmethyl)pentanoyl]-guanidine (4.36)

The title compound was prepared from **4.36a** (340 mg, 0.59 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **4.36** as a pale yellow oil (300 mg, 84 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.71 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.38 (m, 2H, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.91 (m, 3H, Thiaz-5-CH_2C***H***$_2$, CH_2C***H***), 1.71 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.24 (m, 8H, cHex-C***H***$_2$, C***H***$_2CH_3$), 0.89 (m, 5H, C***H***$_2$-cHex, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 177.37 (quat. ***C***=O), 170.38 (quat. Thiaz-2-***C***), 155.32 (quat. ***C***=NH), 132.58 (quat. Thiaz-4-***C***), 118.41 (quat. Thiaz-5-***C***), 42.66 (-, CO***C***H_2, CH***C***H_2), 41.57 (-,

CH_2NH), 36.14 (+, ***C***HCH_2), 34.82 (-, cHex-***C***H_2), 34.69 (-, cHex-***C***H_2), 34.40 (+, cHex-***C***H), 29.73 (-, Thiaz-5-CH_2***C***H_2), 27.78 (-, cHex-***C***H_2), 27.55 (-, ***C***H_2CH_3), 27.46 (-, 2 cHex-***C***H_2), 23.59 (-, Thiaz-5-***C***H_2), 11.45 (+, ***C***H_3), 10.92 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{20}H_{35}N_5OS$) calcd. 393.2562, found 393.25625; prep. HPLC: MeCN/0.1% TFA/aq (35/65-55/45). $C_{20}H_{35}N_5OS \cdot 2TFA$ (621.57)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-[3-(4-hydroxyphenyl)butanoyl]-guanidine (4.37)

The title compound was prepared from **4.37a** (340 mg) in 5 ml CH_2Cl_2/abs and 5 ml TFA according to the general procedure yielding **4.37** as a pale yellow oil (200 mg, 51 %, over two steps). ^{1}H-NMR (CD_3OD) δ ppm: 7.06 (m, 2H, Ar-***H***), 6.69 (m, 2H, Ar-***H***), 5.49 (s, 1H, (*p*-O***H***)-Ar), 3.30 (t, 2H, ^3J = 5.8 Hz, C***H***$_2$NH), 3.22 (m, 1H, C***H***CH_3), 2.68 (m, 4H, COC***H***$_2$, Thiaz-5-C***H***$_2$), 2.15 (s, 3H, Thiaz-4-C***H***$_3$), 1.88 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.28 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); ^{13}C (CD_3OD) δ ppm: 176.22 (quat. ***C***=O), 170.36 (quat. Thiaz-2-***C***), 157.15 (quat. Ar-***C***), 155.16 (quat. ***C***=NH), 137.04 (quat. Thiaz-4-***C***), 132.59 (quat. Ar-***C***), 128.85 (+, 2 Ar-***C***H), 118.40 (quat. Thiaz-5-***C***), 116.32 (+, 2 Ar-***C***H), 46.60 (-, CO***C***H_2), 41.51 (-, ***C***H_2NH), 37.10 (+, ***C***HCH_3), 29.64 (-, Thiaz-5-CH_2***C***H_2), 23.53 (-,Thiaz-5-***C***H_2), 22.46 (+, ***C***H_3), 11.44 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{25}N_5O_2S$) calcd. 375.1729, found 375.17203; prep. HPLC: MeCN/0.1% TFA/aq (15/85-40/60). $C_{18}H_{25}N_5O_2S \cdot 2TFA$ (603.47)

_N_-[3-(2-Amino-4-methylthiazol-5-yl)propyl]-_N´_-[6-amino-3-phenylhexanoyl]-guanidine (4.38)

The title compound was prepared from **4.38a** (220 mg, 0.31 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.38** as a pale yellow oil (190 mg, 82 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.25 (m, 5H, Ar-***H***), 3.27 (m (overlap with solvent peaks), 2H, C***H***$_2$NH), 3.18 (m, 1H, CH_2C***H***), 2.84 (m, 4H, C***H***$_2NH_2$, COC***H***$_2$), 2.66 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.14 (s, 3H, Thiaz-4-C***H***$_3$), 1.77 (m, 4H, Thiaz-5-CH_2C***H***$_2$, C***H***$_2CH_2CH_2NH_2$), 1.49 (m, 2H, C***H***$_2CH_2NH_2$); ^{13}C (CD_3OD) δ ppm: 174.99 (quat. ***C***=O), 169.56 (quat. Thiaz-2-***C***), 142.85 (quat. ***C***=NH), 131.76 (quat. Ar-***C***), 129.02 (+, 2 Ar-***C***H), 128.00 (+, 2 Ar-***C***H), 127.35 (+, Ar-***C***H), 117.53 (quat. Thiaz-5-***C***), 43.98 (-, ***C***H_2NH_2), 42.08 (+, CH_2***C***H), 40.62 (-, ***C***H_2NH), 39.73 (-, CO***C***H_2), 33.03 (-, ***C***$H_2CH_2CH_2NH_2$), 28.77 (-, Thiaz-5-CH_2***C***H_2), 25.81 (-, ***C***$H_2CH_2NH_2$), 22.71 (-, Thiaz-5-***C***H_2), 10.61 (+, Thiaz-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{20}H_{30}N_6OS$) calcd. 402.22018, found 402.22013; prep. HPLC: MeCN/0.1% TFA/aq (10/90-35/65). $C_{20}H_{30}N_6OS \cdot 3TFA$ (744.53)

10-{3-[3-(2-Amino-4-methylthiazol-5-yl)propyl]guanidino}-10-oxodecanoic acid (4.39)

The title compound was prepared from **4.39a** (190 mg, 0.32 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.39** as a pale yellow oil (100 mg, 50 %). ^{1}H-NMR (CD_3OD) δ (ppm): 3.35 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$NH), 2.71 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.47 (t, 2H, ^3J = 7.4 Hz, C***H***$_2$COOH), 2.27 (t, 2H, ^3J = 7.4 Hz, COC***H***$_2$), 2.18 (s, 3H, Thiaz-4-C***H***$_3$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.62 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2CH_2$COOH), 1.34 (s, 8H, (C***H***$_2)_4$); ^{13}C (CD_3OD) δ (ppm): 177.75 (quat. ***C***OOH), 177.51 (quat. ***C***=O), 170.40 (quart., Thiaz-2-***C***), 155.39 (quart., ***C***=NH), 132.61 (quart., Thiaz-4-***C***), 118.39 (quart., Thiaz-5-***C***), 41.58 (-, ***C***H_2NH), 37.76 (-, CO***C***H_2), 34.97 (-, ***C***H_2COOH), 30.19 (-, 3 ***C***H_2), 29.92 (-, ***C***H_2), 29.70 (-, Thiaz-5-***C***H_2), 26.07 (-, ***C***H_2), 25.46(-, Thiaz-5-CH_2***C***H_2), 23.63 (-, ***C***H_2), 11.47 (+, Thiaz-5-***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{31}N_5O_3S$) calcd. 397.21476, found 397.21411; prep. HPLC: MeCN/0.1% TFA/aq (20/80-40/60). $C_{18}H_{31}N_5O_3S \cdot 2TFA$ (625.51)

***N*-[3-(2-Aminothiazol-5-yl)propyl]-*N´*-(3-phenylpropanoyl)guanidine (4.40)**

The title compound was prepared from **4.40a** (150 mg, 0.28 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.40** as a colourless oil (70 mg, 45 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.22 (m, 5H, Ph), 7.00 (s, 1H, Thiaz-4-***H***), 3.35 (t, 2H, ^3J = 6.9 Hz, $COCH_2$C***H***$_2$), 2.97 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$NH), 2.77 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 1.94 (m, 2H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ ppm: 176.47 (quat. ***C***=O), 171.83 (quat. Thiaz-2-***C***), 155.25 (quat. ***C***=NH), 141.35 (quat. ***C***-Ph), 129.63 (+, 2 ***C***H-Ph), 129.46 (+, 2 ***C***H-Ph), 127.52 (+, ***C***H-Ph), 126.38 (quat. Thiaz-5-***C***), 123.37 (+, Thiaz-4-***C***), 41.52 (-, Thiaz-5-***C***H_2), 39.54 (-, ***C***H_2NH), 31.27 (-, CO***C***H_2), 29.49 (-, $COCH_2$***C***H_2), 24.88 (-, Thiaz-5-CH_2***C***H_2); HRMS: EI-MS: *m/z* for ($C_{16}H_{21}N_5OS$) calcd. 331.14668, found 331.14624; prep. HPLC: MeCN/0.1% TFA/aq (20/80-50/50). $C_{16}H_{21}N_5OS \cdot 2TFA$ (559.43)

***N*-[3-(2-Aminothiazol-5-yl)propyl]-*N´*-(3-phenylbutanoyl)guanidine (4.41)**

The title compound was prepared from **4.41a** (190 mg, 0.35 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.41** as a colourless oil (120 mg, 60 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.22 (m, 5H, Ph), 6.99 (s, 1H, Thiaz-4-***H***), 3.31 (m, 3H, C***H***$_2$NH, CH_2C***H***), 2.74 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 1.92 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.31 (d, 3H, ^3J = 7.0 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ ppm: 176.03 (quat. ***C***=O), 171.84 (quat. Thiaz-2-***C***), 155.20 (quat. ***C***=NH), 146.42

(quat. ***C***-Ph), 129.67 (+, 2 ***C***H-Ph), 127.93 (+, 2 ***C***H-Ph), 127.71 (+, ***C***H-Ph), 126.35 (quat. Thiaz-5-***C***), 123.32 (+, Thiaz-4-***C***), 46.15 (-, Thiaz-5-***C***H_2), 41.46 (-, CO***C***H_2), 37.65 (+, CH_2***C***H), 29.44 (-, ***C***H_2NH), 24.85 (-, Thiaz-5-CH_2***C***H_2), 22.26 (+, ***C***H_3); HRMS: EI-MS: *m/z* for ($C_{17}H_{23}N_5OS$) calcd. 345.16233, found 345.16249; prep. HPLC: MeCN/0.1% TFA/aq (20/80-40/60). $C_{17}H_{23}N_5OS \cdot 2TFA$ (573.44)

***N*-[3-(2-Aminothiazol-5-yl)propyl]-*N´*-(3-phenylpentanoyl)guanidine (4.42)**

The title compound was prepared from **4.42a** (230 mg, 0.41 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.42** as a colourless oil (110 mg, 46 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.23 (m, 5H, Ar-***H***), 6.96 (s, 1H, Thiaz-4-***H***), 3.29 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 3.03 (m, 1H, C***H***CH_2CH_3), 2.83-2.70 (m, 4H, Thiaz-5-C***H***$_2$, COC***H***$_2$), 1.89 (m, 2H, Thiaz-5-CH_2C***H***$_2$), 1.76-1.61 (m, 2H, C***H***$_2CH_3$), 0.78 (t, 3H, ^3J = 7.3 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ ppm: 176.26 (quat. ***C***=O), 171.86 (quat. Thiaz-2-***C***), 155.18 (quat. ***C***=NH), 144.46 (quat. Ar-***C***), 129.57 (+, 2 Ar-***C***H), 128.80 (+, 2 Ar-***C***H), 127.77 (+, Ar-***C***H), 126.24 (quat. Thiaz-5-***C***), 123.27 (+, Thiaz-4-***C***), 45.17 (+, ***C***HCH_2CH_3), 44.76 (-, Thiaz-5-***C***H_2), 41.33 (-, ***C***H_2NH), 30.19 (-, CO***C***H_2), 29.40 (-, Thiaz-5-CH_2***C***H_2), 24.82 (-, ***C***H_2CH_3), 12.27 (+, ***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{25}N_5OS$) calcd. 359.17798, found 359.17797; prep. HPLC: MeCN/0.1% TFA/aq (20/80-40/60). $C_{18}H_{25}N_5OS \cdot 2TFA$ (587.47)

***N*-[3-(2-Aminothiazol-5-yl)propyl]-*N´*-(3,3-diphenylpropanoyl)guanidine (4.43)**

The title compound was prepared from **4.43a** (210 mg, 0.34 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.43** as a colourless oil (90 mg, 41 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.21 (m, 10H, Ar-***H***), 6.97 (s, 1H, Thiaz-4-***H***), 4.59 (t, 1H, ^3J = 8.0 Hz, CH_2C***H***), 3.26 (m, 4H, C***H***$_2$NH, COC***H***$_2$), 2.71 (t, 2H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 1.90 (m, 2H, Thiaz-5-CH_2C***H***$_2$); ^{13}C-NMR (CD_3OD) δ ppm: 175.58 (quat. ***C***=O), 171.84 (quat. Thiaz-2-***C***), 155.14 (quat. ***C***=NH), 144.51 (quat. ***C***-Ph), 129.70 (+, 4 ***C***H-Ph), 128.84 (+, 4 ***C***H-Ph), 127.79 (+, 2 ***C***H-Ph), 126.23 (quat. Thiaz-5-***C***), 123.28 (+, Thiaz-4-***C***), 48.10 (+, CH_2***C***H), 43.78 (-, CO***C***H_2), 41.36 (-, Thiaz-5-***C***H_2), 29.37 (-, ***C***H_2NH), 24.80 (-, Thiaz-5-CH_2***C***H_2); HRMS: EI-MS: *m/z* for ($C_{22}H_{25}N_5OS$) calcd. 407.17798, found 407.17921, prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{22}H_{25}N_5OS \cdot 2TFA$ (635.51)

***N*-[3-(2-Aminothiazol-5-yl)propyl]-*N´*-(3-cyclohexylbutanoyl)guanidine (4.44)**

The title compound was prepared from **4.44a** (200 mg, 0.36 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.44** as a colourless oil (110 mg, 53 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.01 (s, 1H, Thiaz-4-***H***), 3.37

(m, 2H, C***H***$_2$NH), 2.77 (t, 2H, ^3J = 7.3 Hz, Thiaz-5-C***H***$_2$), 2.56 (dd, 1H, ^3J = 5.1Hz, ^2J = 15.1Hz, COC***H***H), 2.22 (dd, 1H, ^3J = 9.1 Hz, ^2J = 15.0 Hz, COCH***H***), 1.92 (m, 3H, Thiaz-5-CH$_2$C***H***$_2$, CH$_2$C***H***), 1.77-1.65 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.28-1.04 (m, 6H, cHex-C***H***$_2$), 0.92 (d, 3H, ^3J = 6.8 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ ppm: 177.38 (quat. ***C***=O), 171.80 (quat. Thiaz-2-***C***), 155.28 (quat. ***C***=NH), 126.36 (quat. Thiaz-5-***C***), 123.52 (+, Thiaz-4-***C***), 43.95 (+, cHex-***C***H), 42.72 (-, CO***C***H$_2$), 41.53 (-, Thiaz-5-***C***H$_2$), 36.40 (+, CH$_2$***C***H), 31.47 (-, ***C***H$_2$NH), 30.04 (-, cHex-***C***H$_2$), 29.51 (-, cHex-***C***H$_2$), 27.84 (-, Thiaz-5-CH$_2$***C***H$_2$), 27.73 (-, 2 cHex-***C***H$_2$), 24.90 (-, cHex-***C***H$_2$), 16.61 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for ($C_{17}H_{29}N_5OS$) calcd. 351.20928, found 351.20847; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{17}H_{29}N_5OS \cdot 2TFA$ (579.49)

_N_-[3-(2-Aminothiazol-5-yl)propyl]-_N´_-(3-cyclohexyl-2-methylpropanoyl)guanidine (4.45)

The title compound was prepared from **4.45a** (200 mg, 0.36 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.45** as a colourless oil (170 mg, 81 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.00 (s, 1H, Thiaz-4-***H***), 3.37 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.77 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.68 (m, 1H, C***H***CH$_3$), 1.96 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.75-1.60 (m, 6H, cHex-C***H***$_2$, C***H***$_2$-Ar), 1.24 (m, 5H, cHex-C***H***$_2$, cHex-C***H***), 1.15 (d, 3H, ^3J = 6.9 Hz, C***H***$_3$), 0.89 (m, 2H, cHex-C***H***$_2$); ^{13}C-NMR (CD_3OD) δ ppm: 180.52 (quat. ***C***=O), 170.98 (quat. Thiaz-2-***C***), 154.68 (quat. ***C***=NH), 125.37 (quat. Thiaz-5-***C***), 122.39 (+, Thiaz-4-***C***), 41.36 (-, Thiaz-5-***C***H$_2$), 40.61 (-, ***C***H$_2$-cHex), 39.37 (+, CO***C***H), 35.75 (+, cHex-***C***H), 33.63 (-, ***C***H$_2$NH), 33.35 (-, cHex-***C***H$_2$), 28.57 (-, Thiaz-5-CH$_2$***C***H$_2$), 26.71 (-, cHex-***C***H$_2$), 26.46 (-, cHex-***C***H$_2$), 26.43 (-, cHex-***C***H$_2$), 24.03 (-, cHex-***C***H$_2$), 17.03 (+, ***C***H$_3$); HRMS: EI-MS: *m/z* for ($C_{17}H_{29}N_5OS$) calcd. 351.20928, found 351.20880; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{17}H_{29}N_5OS \cdot 2TFA$ (579.49)

_N_-[3-(2-Aminothiazol-5-yl)propyl]-_N´_-(3-cyclohexylpentanoyl)guanidine (4.46)

The title compound was prepared from **4.46a** (80 mg, 0.14 mmol) in 5 ml CH_2Cl_2/abs and 1 ml TFA according to the general procedure yielding **4.46** as a colourless oil (70 mg, 84 %). ^{1}H-NMR (CD_3OD) δ ppm: 7.01 (s, 1H, Thiaz-4-***H***), 3.37 (t, 2H, ^3J = 7.0 Hz, C***H***$_2$NH), 2.77 (t, 2H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.50 (dd, 1H, ^3J = 6.1 Hz, ^2J = 15.7 Hz, COC***H***H), 2.32 (dd, 1H, ^3J = 7.5 Hz, ^2J = 15.7 Hz, COCH***H***), 1.96 (m, 2H, Thiaz-5-CH$_2$C***H***$_2$), 1.69 (m, 6H, cHex-C***H***$_2$, CH$_2$C***H***, cHex-C***H***), 1.19 (m, 8H, cHex-C***H***$_2$, C***H***$_2$CH$_3$), 0.89 (t, 3H, ^3J = 7.4 Hz, C***H***$_3$); ^{13}C-NMR (CD_3OD) δ ppm: 177.73 (quat. ***C***=O), 170.60 (quat. Thiaz-2-***C***), 155.06 (quat. ***C***=NH), 126.35 (quat. Thiaz-5-

C), 123.36 (+, Thiaz-4-***C***), 42.94 (+, cHex-***C***H), 41.52 (-, Thiaz-5-***C***H_2), 41.41 (+, CH_2***C***H), 39.71 (-, CO***C***H_2), 31.19 (-, ***C***H_2NH), 30.34 (-, cHex-***C***H_2), 29.51 (-, cHex-***C***H_2), 27.88 (-, 2 cHex-***C***H_2), 27.79 (-, Thiaz-5-CH_2***C***H_2), 24.89 (-, cHex-***C***H_2), 24.75 (-, ***C***H_2CH_3), 12.05 (+, ***C***H_3); HRMS: EI-MS: *m/z* for ($C_{18}H_{31}N_5OS$) calcd. 365.22493, found 365.22445; prep. HPLC: MeCN/0.1% TFA/aq (30/70-50/50). $C_{18}H_{31}N_5OS$ · 2TFA (593.51)

4.6. References

(1) 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, (17), 3239-3246.

(2) 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-Schmiedeberg's Arch. Pharmacol.* **1993,** 348, (1), 77-81.

(3) Poli, E.; Pozzoli, C.; Coruzzi, G.; Bertaccini, G.; Timmerman, H., In vitro cardiac pharmacology of the new histamine H_2-receptor agonist amthamine: Comparisons with histamine and dimaprit. *Inflamm. Res.* **1993,** 40, (1), 44-49.

(4) Weinstein, H.; Chou, D.; Johnson, C. L.; Kang, S.; Green, J. P., Tautomerism and the receptor action of histamine: a mechanistic model. *Mol. Pharmacol.* **1976,** 12, (5), 738-45.

(5) Eriks, J. C.; van der Goot, H.; Timmerman, H., New activation model for the histamine H_2 receptor, explaining the activity of the different classes of histamine H_2 receptor agonists. *Mol. Pharmacol.* **1993,** 44, (4), 886-894.

(6) 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, (7), 923-928.

(7) 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, (9), 905-909.

(8) 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/volltexte/2006/561/

(9) Omura, K.; Swern, D., Oxidation of alcohols by "activated" dimethyl sulfoxide. a preparative, steric and mechanistic study. *Tetrahedron* **1978,** 34, (11), 1651-1660.

(10) Rasmussen, C. R.; Villani, F. J., Jr.; Reynolds, B. E.; Plampin, J. N.; Hood, A. R.; Hecker, L. R.; Nortey, S. O.; Hanslin, A.; Costanzo, M. J.; et al., A versatile synthesis of novel N,N, N-trisubstituted guanidines. *Synthesis* **1988**, (6), 460-6.

(11) Iwanowicz, E. J.; Poss, M. A.; Lin, J., Preparation of *N*,*N*'-bis-*tert*-Butoxycarbonylthiourea. *Synth. Commun.* **1993,** 23, (10), 1443 - 1445.

(12) Poss, M. A.; Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z., A mild and efficient method for the preparation of guanidines. *Tetrahedron Lett.* **1992,** 33, (40), 5933-5936.

(13) Kim, K. S.; Qian, L., Improved method for the preparation of guanidines. *Tetrahedron Lett.* **1993,** 34, (48), 7677-7680.

(14) DeMong, D. E.; Williams, R. M., The asymmetric synthesis of (2S,3R)-capreomycidine. *Tetrahedron Lett.* **2001,** 42, (21), 3529-3532.

(15) Birnkammer, T. Synthese und Histamin H_2 Rezeptor agonistische Aktivität von N^1-Acyl-N^2-[3-(2-amino-4-methylthiazol-5-yl)propyl]guanidinen. Diploma Thesis, Universitiy of Regensburg, Germany, 2007.

(16) 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, (6), 1210-1225.

(17) 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, (1), 139-146.

(18) Xie, S.-X.; Kraus, A.; Ghorai, P.; Ye, Q.-Z.; Elz, S.; Buschauer, A.; Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1H-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, (3), 1262-1268.

(19) Kracht, J. Bestimmung der Affinität und Aktivität subtypselektiver Histamin- und Neuropeptid Y-Rezeptorliganden an konventionellen und neuen pharmakologischen In-vitro-Modellen. Doctoral Thesis, University of Regensburg, Germany, 2001.

(20) Götte, C. Synthese, Enantiomerentrennung und Struktur-Wirkungsbeziehungen neuer Histamin H_2 Rezeptoragonisten des Arpromidin Typs. Doctoral Thesis, University of Regensburg, Germany, 2001.

(21) Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M., Design, synthesis, and biological evaluation of cytotoxic 11-aminoalkenylindenoisoquinoline and 11-diaminoalkenylindenisoquinoline topoisomerase I inhibitors. *Bioorg. Med. Chem.* **2004,** 12, 5147-5160.

(22) Salmen, S. Inhibitors of bacterial and mammalian hyaluronidase - synthesis and structure activity relationships. Doctoral Thesis, University of Regensburg, Germany, 2003. http://www.opus-bayern.de/uni-regensburg/volltexte/2004/320/

Chapter 5

“Bivalent” Histamine H_2 Receptor Agonists

5.1. Introduction

G-protein coupled receptors (GPCRs) are integral membrane proteins, representing the largest class of cell surface receptors and provide the targets of an increasing number of therapeutic agents. For a long time, GPCRs were regarded to function as monomeric entities, but now it is widely accepted that they form homo- and hetero-oligomeric complexes[1-4]. Histamine H_1-H_4 receptors are also known to exist as oligomers[5-11]. This prompted us to design bivalent ligands with different spacer lengths as pharmacological tools for the investigation of hypothetical dimeric histamine receptors. Bivalent ligands are thought to exhibit a greater potency than that corresponding to double concentration of a monovalent ligand. This concept has been studied, for instance, for opioid receptors in more detail[12]. The ligands have to display a correct spacer length to be able to bridge neighbouring receptors. For opioid receptors, the distance between the recognition sites of a contact dimer with a TM5/TM6 interface is about 27 Å as suggested from molecular modelling[13]. Therefore, ligands with spacer lengths between 6 and 27 Å were synthesized and investigated for histamine H_2 receptor (H_2R) agonism at the spontaneously beating guinea pig right atrium as well as hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ in a membrane steady-state GTPase assay.

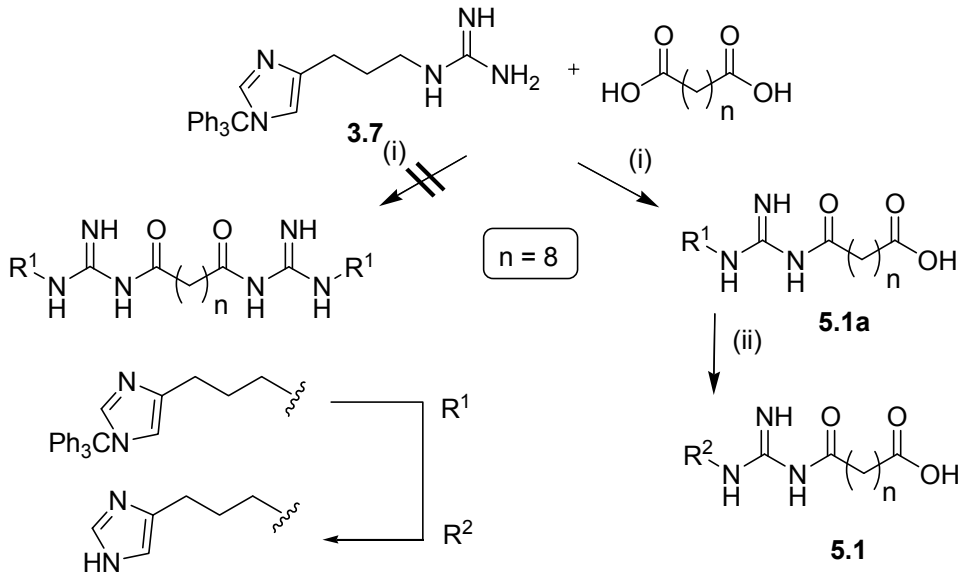
The histamine H_2 receptor isoforms of human[14], guinea pig[15], rat[16] and canine[17] are closely related to each other with an overall amino acid sequence identity of more than 80 % and more than 90 % within the seven α-helical transmembrane domains. Recent studies showed that the cH_2R exhibits an increased constitutive activity compared to hH_2R, gpH_2R and rH_2R and differences in potencies and efficacies of compounds between hH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$[18, 19]. To further study the different activation of histamine H_2R isoforms, the synthesized bivalent agonists were investigated in GTPase assays at cH_2R-$G_{s\alpha S}$ and mutant membranes (hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$).

Furthermore, in addition to amino acids in the TM domains, residues of the second extracellular (e2) loop were suggested to contribute to the ligand binding pocket of

class 1 GPCRs[20, 21]. Very recently, the high-resolution crystal structure of the human β_2-adrenergic receptor pointed out that part of the e2 loop is held out of the binding cavity by a pair of disulfide bridges and a short helical segment within the loop[22, 23]. Recent studies revealed that the e2 loop of H_2R does not contribute to the interactions of *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines and N^G-acylated analogues, an indication that participation of the e2 loop may apply not for all class 1 GPCRs[18, 24]. As an alternative to binding to receptor dimers, it is conceivable that bivalent ligands interact with two different binding sites at one and the same receptor molecule. An interaction with the e2 loop region cannot be ruled out. Therefore, the “double pharmacophore ligands” were investigated at chimeric receptors combining gpH_2R and an e2 loop containing four amino acids of hH_2R and *vice versa* using membrane preparations of Sf9 cells expressing hH_2R-gpE2-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$ (GTPase assay). Additionally, the compounds were tested at hH_3R and hH_4R for receptor selectivity.

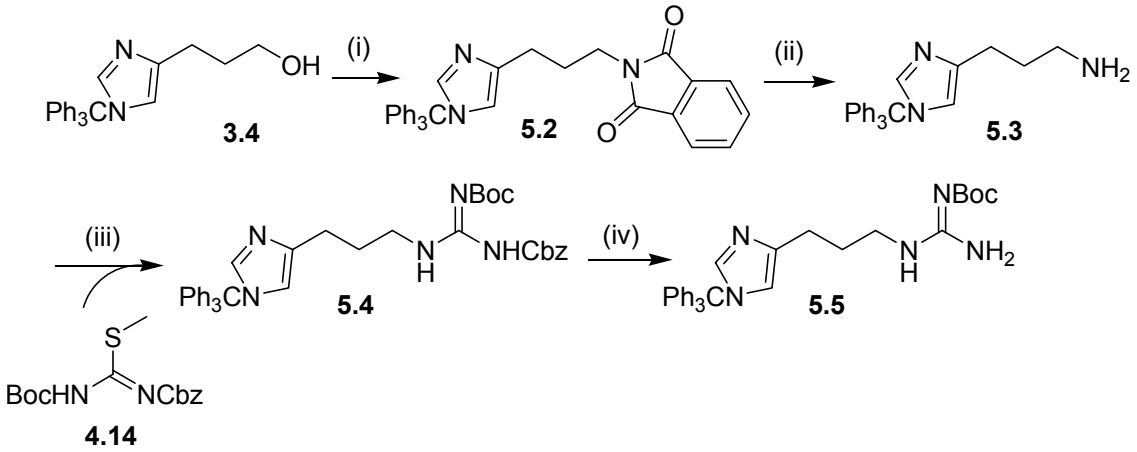
5.2. Chemistry

In principle, the coupling of two equivalents of trityl-protected imidazolylpropyl-guanidine **3.7** with one equivalent of different alkanedioic acids appears feasible to synthesize a library of bivalent acylguanidine-type H_2R agonists. However, the reaction of **3.7** with dicarboxylic acids in the presence of NaH and CDI as a coupling reagent failed as only mono-acylation (**5.1a**) occurred. The trityl group was removed by treatment of **5.1a** with TFA in DCM to obtain **5.1** (Scheme 5.1).



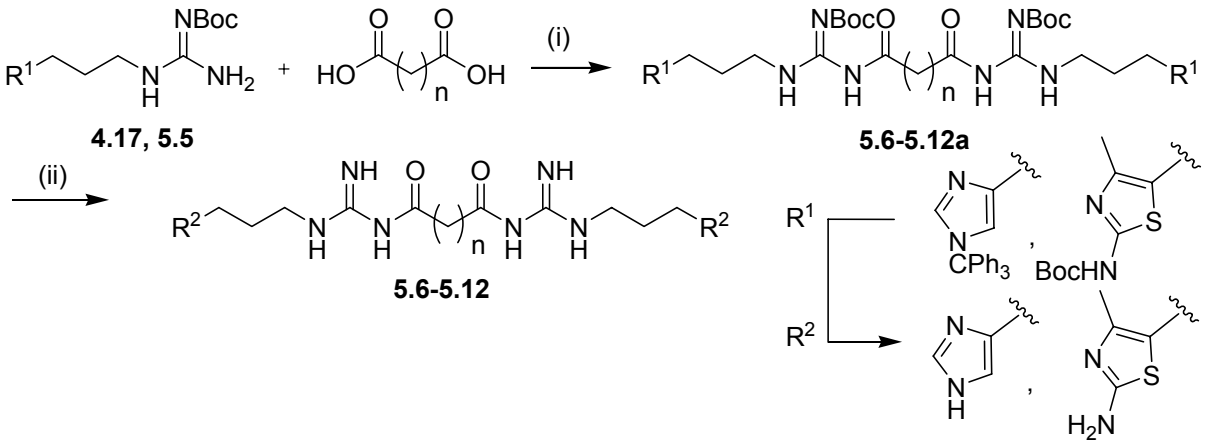
Scheme 5.1. Coupling of imidazolylpropylguanidine **3.7** with alkanedioic acids; Reagents and conditions: (i) CDI (1.2 eq), NaH (60 % dispersion in mineral oil) (2 eq), THF/abs, 3-4h, rt; (ii) 20 % TFA, DCM, 5-6 h, rt.

Mono-Boc protected guanidines can be coupled to carboxylic acids with EDAC, HOBt and DIEA in a one-pot reaction at high yields. This method was applied to the synthesis of bivalent H_2R agonists. The required building block **5.5** was prepared from the alcohol **3.4** which was converted to the primary amine **5.3** *via* the phthalimide **5.2** and subsequent treatment with hydrazine monohydrate. The free amine was then coupled to the guanidinylating reagent **4.14** by analogy with the procedure described for the aminothiazoles in chapter 4. The Cbz-group was cleaved by hydrogenation to yield the mono-Boc protected guanidine **5.5** (Scheme 5.2).



Scheme 5.2. Synthesis of *N-tert*-butoxycarbonyl-*N*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine **5.5**; Reagents and conditions: (i) phthalimide (1 eq), PPh_3 (1 eq), DIAD (1 eq), THF/abs, 24 h, rt; (ii) $N_2H_2{\cdot}H_2O$ (5 eq), EtOH, 1 h, reflux; (iii) **4.14** (1 eq), $HgCl_2$ (2 eq), NEt_3 (3 eq), DCM/abs, 48 h, rt; (v) H_2, Pd/C (10 %), MeOH/THF (1:1), 8 bar, 8-9 d, rt.

The mono-Boc-protected guanidine building blocks **4.17** and **5.5**, respectively, were coupled to different alkanedioic acids using EDAC, HOBt and DIEA as standard coupling reagents. Removal of the protecting groups under acidic conditions gave the bivalent ligands **5.6**-**5.12** (Scheme 5.3).



No	R^2	n
5.6	imidazol-4-yl	8
5.7	2-amino-4-methylaminothiazol-5-yl	4
5.8	2-amino-4-methylaminothiazol-5-yl	6
5.9	2-amino-4-methylaminothiazol-5-yl	8
5.10	2-amino-4-methylaminothiazol-5-yl	10
5.11	2-amino-4-methylaminothiazol-5-yl	14
5.12	2-amino-4-methylaminothiazol-5-yl	20

Scheme 5.3. Synthesis of bivalent N^G-acylated hetarylpropylguanidines **5.6-5.12**; Reagents and conditions: (i) EDAC (1 eq), HOBt (1 eq), DIEA (1 eq), DCM/abs, 24 h, rt; (ii) 20 % TFA, DCM/abs, 3-5 h, rt.

5.3. Pharmacological results and discussion

All compounds were tested for histamine H_2R agonism at the spontaneously beating guinea pig right atrium (positive chronotropic response) (Table 5.1) and in a membrane steady-state GTPase assay at hH_2R-$G_{s\alpha S}$, gpH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$ fusion proteins expressed in Sf9 insect cells (Table 5.2). Furthermore, the bivalent ligands were tested at an hH_2R-$G_{s\alpha S}$ double mutant with Cys17→Tyr17 and Ala-271→Asp271 exchanges (hH_2R-C17Y-A271D-$G_{s\alpha S}$) and a mutant with Cys17→Tyr17 exchange (hH_2R-C17Y-$G_{s\alpha S}$) in the sequence of the hH_2R as well as mutant hH_2R-$G_{s\alpha S}$ with an exchange of four amino acids in the e2 loop against amino acids derived from the gpH_2R (hH_2R-gpE2-$G_{s\alpha S}$) and a reverse mutant gpH_2R-$G_{s\alpha S}$

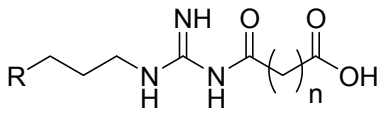
with the corresponding amino acids derived from the hH_2R (gpH_2R-hE2-$G_{s\alpha S}$) (Table 5.3 and Table 5.4) to study species-selectivity and the role of the e2 loop. The compounds were tested for histamine H_1R antagonism on U-373 MG human cells in the Ca^{2+}-assay (Table 5.5) and on the human histamine H_3 and H_4 receptor in GTPase assay (Table 5.6).

5.3.1. Histamine H_2 receptor agonism

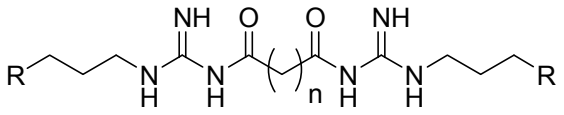
5.3.1.1. Agonistic activity on the spontaneously beating guinea pig right atrium

The bivalent ligands proved to be strong partial to full agonists at the spontaneously beating guinea pig right atrium (Table 5.1).

Table 5.1. Histamine H_2 receptor agonism at the spontaneously beating guinea pig right atrium.



5.1, 4.39



5.6-5.12

No.	R	n	H_2R agonism - isolated guinea pig right atrium		
			pEC_{50} ± SEM[a]	rel. pot.[b]	E_{max} ± SEM (%)[c]
HIS	-	-	6.00 ± 0.10	100	100 ± 2
AMT[25]	-	-	6.21 ± 0.09	160	95 ± 2
5.1	imidazol-4-yl	8	6.36 ± 0.12	231	90 ± 1
4.39[d]	2-amino-4-methylthiazol-5-yl	8	6.92 ± 0.10	826	63 ± 5
5.6	imidazol-4-yl	8	9.09 ± 0.06	122,000	87 ± 2
5.7	2-amino-4-methylthiazol-5-yl	4	nd	nd	nd
5.8	2-amino-4-methylthiazol-5-yl	6	9.48 ± 0.03	302,000	64 ± 3
5.9	2-amino-4-methylthiazol-5-yl	8	8.84 ± 0.14	69,200	64 ± 4
5.10	2-amino-4-methylthiazol-5-yl	10	nd	nd	nd
5.11	2-amino-4-methylthiazol-5-yl	14	6.13 ± 0.14	135	53 ± 11
5.12	2-amino-4-methylthiazol-5-yl	20	nd	nd	nd

[a] 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 + ΔpEC_{50}; data shown are the ± SEM of three to five experiments; [b] relative potency to histamine = 100 %; [c] efficacy, maximal response (%), relative to the maximal increase in heart rate induced by the reference compound histamine; [d] for experimental data see chapter 4.

Compound **5.8** with a spacer of six carbon atoms between the carbonyl groups is about 3000 times more potent than histamine and turned out to be the most potent agonist at the guinea pig right atrium known so far. With increasing the spacer length, potency and efficacy are decreasing. It is more likely that **5.8** containing an

insufficient spacer length to occupy two vicinal receptors assign a second binding site at the same receptor. Compounds **5.1** and **4.39** with only one pharmacophore and a carboxylic acid function are significantly less potent than the bivalent analogues.

5.3.1.2. Agonistic activity at hH_2R-$G_{s\alpha S}$, gpH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$ fusion proteins

The synthesized compounds were potent full or partial H_2 receptor agonists at hH_2R-$G_{s\alpha S}$, gpH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$ membranes in the GTPase assay (Table 5.2). With increasing the spacer length from four (~6 Å) to twenty (~27 Å) C-atoms between the carbonyl functions, the potency peaks with an octamethylene chain (~11 Å) at both, hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$. In agreement with previous studies, these compounds exhibit higher potencies and efficacies at gpH_2R-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$[27-29].
Figure 5.1 shows the correlation between potencies and efficacies at gpH_2R-$G_{s\alpha S}$ and hH_2R-$G_{s\alpha S}$ of the “double pharmacophore ligands”. Interestingly, compounds **5.7**-**5.9** are 27 to 49 times more potent at the gpH_2R-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$ and therefore exhibit the highest selectivities towards gpH_2R-$G_{s\alpha S}$ among acylguanidines known so far. Moreover, compound **5.9** has an EC_{50} value of 0.46 nM at gpH_2R-$G_{s\alpha S}$ and is among the most potent acylguanidine-type H_2R agonists identified in the GTPase assay. Further extension of the spacer length results in a significant drop in potency up to a complete loss of agonistic acitivity at hH_2R-$G_{s\alpha S}$. These results challenge the model of bivalent ligandes as compounds bridging the binding pockets of receptor dimers in the case of the H_2R agonistic acylguanidines. The occupation of two neighbouring receptors is impossible because of an insufficient spacer length in the case of the highly potent agonist **5.9**, whereas an estimated optimal spacer length results in weak agonism (**5.12**, gpH_2R-$G_{s\alpha S}$) or loss of activity (**5.12**, hH_2R-$G_{s\alpha S}$).

Table 5.2. Agonist efficacies and potencies of bivalent agonists at hH_2R-$G_{s\alpha S}$, gpH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$ expressed in Sf9 cell membranes.

No.	hH_2R-$G_{s\alpha S}$			gpH_2R-$G_{s\alpha S}$			EC_{50} hH_2R-$G_{s\alpha S}$/ EC_{50} gpH_2R-$G_{s\alpha S}$
	efficacy	EC_{50} [nM]	rel. pot.	efficacy	EC_{50} [nM]	rel. pot.	
HIS[26]	1.00	990 ± 92	100	1.00	850 ± 340	100	1.16
AMT[26]	0.91 ± 0.02	190 ± 50	521	1.04 ± 0.01	190 ± 42	447	1.00
5.1	0.67 ± 0.03	81.3 ± 12.1	1,218	0.97 ± 0.04	182.5 ± 99.3	465	0.44
4.39[a]	0.51	48.9	2,024	0.61	35.5	2,394	1.38
5.6	0.82 ± 0.04	6.3 ± 0.9	20,000	0.98 ± 0.05	1.2 ± 0.4	100,000	5.25
5.7	0.63 ± 0.03	62.4 ± 39.9	2020	0.91 ± 0.01	1.9 ± 1.2	63,158	32.8
5.8	0.58 ± 0.02	32.6 ± 22.4	3,865	0.81 ± 0.03	0.66 ± 0.2	181,818	49.4
5.9	0.53 ± 0.04	12.4 ± 6.4	10,161	0.79 ± 0.07	0.46 ± 0.1	260,869	26.9
5.10	0.44 ± 0.06	14.6 ± 6.8	8,630	0.66 ± 0.05	3.5 ± 2.2	34,285	4.17
5.11	0.12 ± 0.02	32.8 ± 15.6	3,841	0.51 ± 0.02	23.3 ± 11.0	5,150	1.4
5.12	(-	-	-)[b]	0.58 ± 0.02	537.3 ± 239.4	223	-

No.	cH_2R-$G_{s\alpha S}$		
	efficacy	EC_{50} [nM]	rel. pot.
HIS[19]	1.00	290 ± 50	100
AMT[19]	0.90 ± 0.04	110 ± 66	264
5.6	1.21 ± 0.18	151.8 ± 150.2	191
5.7	0.85 ± 0.07	31.6 ± 9.2	918
5.8	0.82 ± 0.07	5.2 ± 3.9	5,577
5.9	0.79 ± 0.10	5.5 ± 1.4	5,273
5.10	0.81 ± 0.04	9.8 ± 5.4	2,959
5.11	0.39 ± 0.04	19.7 ± 0.2	1,472
5.12	0.22 ± 0.00	285.9 ± 131	101

[a] for experimental data see chapter 4; [b] no agonistic acitvity.

Steady state GTPase activity in Sf9 membranes expressing hH_2R-$G_{s\alpha S}$, gpH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$ was determined as described in the literature[19, 27]. Reaction mixtures contained ligands at concentrations from 1 nM to 10 µM or 10 pM to 100 nM, respectively, as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 0.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (100 µM) ranged between ~ 2 and 13 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the mean ± SEM of two to five experiments performed in duplicates each. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value. The ratios of the EC_{50} values of H_2R agonists for hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ were also calculated.

The exchange of the aminothiazole ring against an imidazole ring in derivative **5.9** decreased potency about three-fold at gpH_2R-$G_{s\alpha S}$ (EC_{50} (**5.9**) = 0.46 nM, EC_{50} (**5.6**) = 1.2 nM), whereas the potency at hH_2R-$G_{s\alpha S}$ is two-fold increased (EC_{50} (**5.9**) =

12.4 nM, EC_{50} (**5.6**) = 6.3 nM). Compounds having a free carboxylic acid (**5.1** and **4.39**) exhibit lower potencies compared to their double pharmacophore analogues. Here in turn the aminothiazole derivative (**4.39**) shows higher agonistic activity than the imidazole analogue (**5.1**) but lower efficacy. Compounds **5.7**-**5.12** exhibited higher EC_{50} values and efficacies at cH_2R-$G_{s\alpha S}$ than at hH_2R-$G_{s\alpha S}$. This result is in agreement with recent studies[19]. An exception is compound **5.6**, which is more efficacious but was less potent at cH_2R-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$.

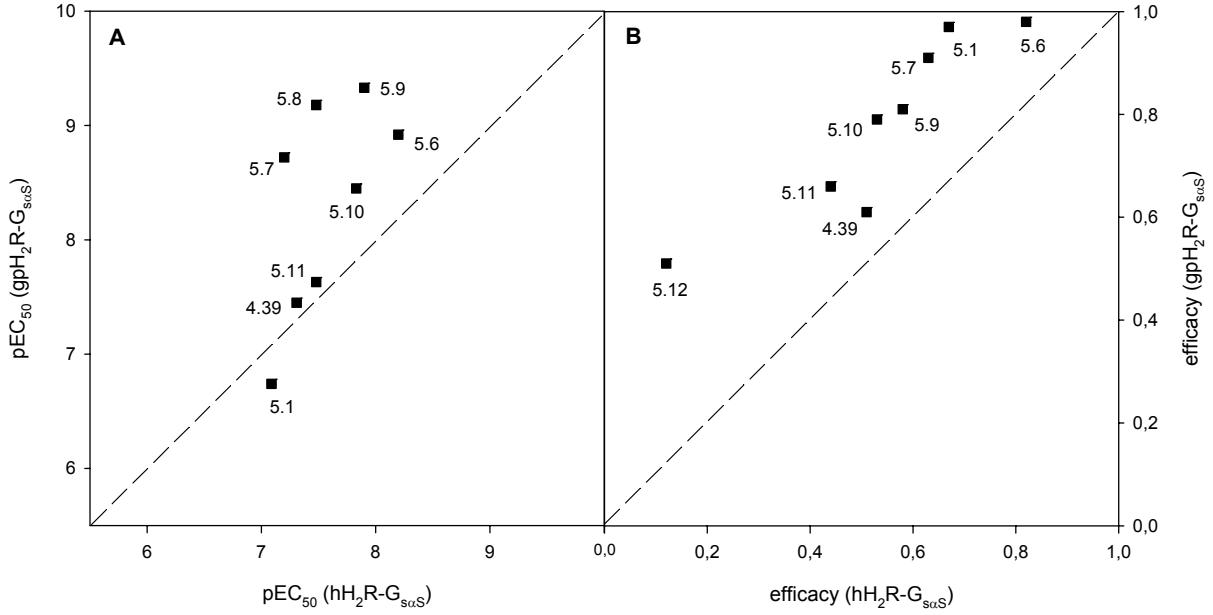


Figure 5.1. Correlation between the potencies (A) and efficacies (B) of acylated bivalent ligands at hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ fusion proteins in the GTPase assay. Agonist potencies and efficacies were taken from Table 5.1. The pEC_{50} values were derived from the EC_{50} shown in Table 5.1. The straight dotted line represents the correlation that would have been obtained if pEC_{50} values had been identical in the two systems.

5.3.1.3. Agonistic activity on different histamine H_2 receptor mutants

5.3.1.3.1. Potencies and efficacies on hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$

The data of compounds **5.6**-**5.12,** which were investigated for agonism in the GTPase assay on the hH_2R-$G_{s\alpha S}$ mutants hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$, are summarized in Table 5.3. Except for compounds **5.10** and **5.11**, the ligands showed enhanced potencies and efficacies on hH_2R-C17Y-A271D-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$. In accordance with the data for hH_2R-$G_{s\alpha S}$, the optimal spacer was an 8- to 10-membered carbon chain, and further extension of the distance (up to ~27 Å) between the two pharmacophores resulted in total loss of agonistic activity.

Generally, potencies and efficacies of the agonists were still lower at hH_2R-C17Y-A271D-$G_{s\alpha S}$ than at gpH_2R-$G_{s\alpha S}$ except for **5.10**, which was nearly equieffective at both fusion proteins. Thus, the sensitivity of the hH_2R-C17Y-A271D-$G_{s\alpha S}$ double mutant against agonist stimulation is close to that of the guinea pig H_2R isoform although not exactly the same. These results further support the hypothesis that both Tyr-17 and Asp-271 contribute largely to enhanced potencies and efficacies of acylguanidines at gpH_2R by forming an H-Bond stabilizing an active receptor confirmation[26, 27]. However, the residual differences in potencies and efficacies suggest that additional amino acids are involved in the species-selective activation of histamine H_2 receptors.

Table 5.3. Efficacies and potencies of bivalent agonists at hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$ expressed in Sf9 cell membranes.

No.	**hH_2R-C17Y-A271D-$G_{s\alpha S}$**			**hH_2R-C17Y-$G_{s\alpha S}$**		
	efficacy	**EC_{50} [nM]**	**rel. pot.**	**efficacy**	**EC_{50} [nM]**	**rel. pot.**
HIS[26]	1.00	320 ± 9	100	1.00	260 ± 110	100
AMT[26]	0.97 ± 0.01	65 ± 6	490	0.86 ± 0.19	120 ± 20	217
5.6	0.97 ± 0.07	0.65 ± 0.15	49,230	0.99	7	3,714
5.7	0.81 ± 0.00	7.6 ± 1.6	4,210	0.49	65	400
5.8	0.83 ± 0.02	2.5 ± 0.5	12,800	0.31	10	2,600
5.9	0.78 ± 0.01	2.0 ± 0.6	16,000	0.23	46	565
5.10	0.71 ± 0.01	8.8 ± 3.9	3,636	0.27	14	1,857
5.11	0.17 ± 0.01	41.8 ± 19.8	765	(-	-	-)[a]
5.12	(-	-	-)[a]	(-	-	-)[a]

[a] no agonistic activity.

Steady state GTPase activity in Sf9 membranes expressing hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$ was determined as described in literature[18, 26]. Reaction mixtures contained ligands at concentrations from 1 nM to 10 µM or 0.1 nM to 100 nM, respectively, as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 2.5 and 3.0 (1.25 for hH_2R-C17Y-$G_{s\alpha S}$) pmol/mg/min, and activities stimulated by histamine (100 µM) ranged between ~ 12.5 and 13 (1.8 for hH_2R-C17Y-$G_{s\alpha S}$) pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the ± SEM of one to two experiments performed in duplicates each. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value.

A single point mutation of Cys17→Tyr17 significantly decreased the potencies and efficacies of the bivalent agonists compared to “wild-type” hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$. These results are opposite to recent studies where alkyl- and acylguanidines showed enhanced or similar potencies and efficacies at hH_2R-C17Y-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$[18].

5.3.1.3.2. Potencies and efficacies on hH_2R-gpE2-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$

To study the influence of the e2 loop on the species selectivity of bivalent N^G-acylated guanidines, the synthesized compounds were tested on hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ which are reciprocally mutated at four positions in the e2 loop (hH_2R-gpE2-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$). The efficacies and potencies at hH_2R-gpE2-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$ are listed in Table 5.4.

Table 5.4. Efficacies and potencies of bivalent agonists at hH_2R-gpE2-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$ expressed in Sf9 cell membranes.

No	hH_2R-gpE2-$G_{s\alpha S}$			gpH_2R-hE2-$G_{s\alpha S}$		
	efficacy	EC_{50} [nM]	rel. pot.	efficacy	EC_{50} [nM]	rel. pot.
HIS[18]	1.00	700 ± 190	100	1.00	1400 ± 280	100
AMT[18]	0.94 ± 0.05	140 ± 30	500	0.94 ± 0.06	310 ± 110	452
5.6	0.81	3.6	19,444	1.11	1.2	116,666
5.7	0.63	55	1,273	0.96	7.9	17,721
5.8	0.64	49	1,428	1.00	2.2	63,636
5.9	0.65	7	10,000	0.88	3	46,667
5.10	0.54	8	8,750	0.75	12	11,667
5.11	0.21	49	1,428	0.49	32	4,375
5.12	(-	-	-)[a]	0.29	645	217

[a] no agonistic activity.

Steady state GTPase activity on Sf9 membranes expressing hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$ was determined as described in literature[18, 24]. Reaction mixtures contained ligands at concentrations from 1 nM to 10 µM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fit to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 0.5 and 1.0 pmol/mg/min, and activities stimulated by histamine (100 µM) ranged between ~ 2.8 and 4.5 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the ± SEM of one experiment performed in duplicates. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value.

The compounds **5.6**-**5.12** were similarly potent and efficacious at hH_2R-gpE2-$G_{s\alpha S}$ and “wild-type” hH_2R-$G_{s\alpha S}$ and somewhat different between gpH_2R-hE2-$G_{s\alpha S}$ and “wild-type” gpH_2R-$G_{s\alpha S}$. Figure 5.2 displays the correlation of pEC_{50} values between wild-type and e2 mutated H_2R. At gpH_2R-hE2-$G_{s\alpha S}$, ligands **5.7**-**5.10** were three to five times less potent than at wild-type gpH_2R-$G_{s\alpha S}$ and **5.6**, **5.11** and **5.12** showed similar potencies at wild-type and mutant gpH_2R. As demonstrated recently, the second extracellular loop does not contribute to species-selectivity of small histamine H_2R agonists as well as *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines and N^G-acylated derivatives at human and guinea pig H_2R isoforms[18, 24]. This appears to apply to the bivalent ligands, too. The slightly reduced potencies of compounds **5.7**-**5.11** at

gpH_2R-hE2-$G_{s\alpha S}$ *vs*. gpH_2R-$G_{s\alpha S}$ may derive from minor conformational changes of the gpH_2R binding pocket.

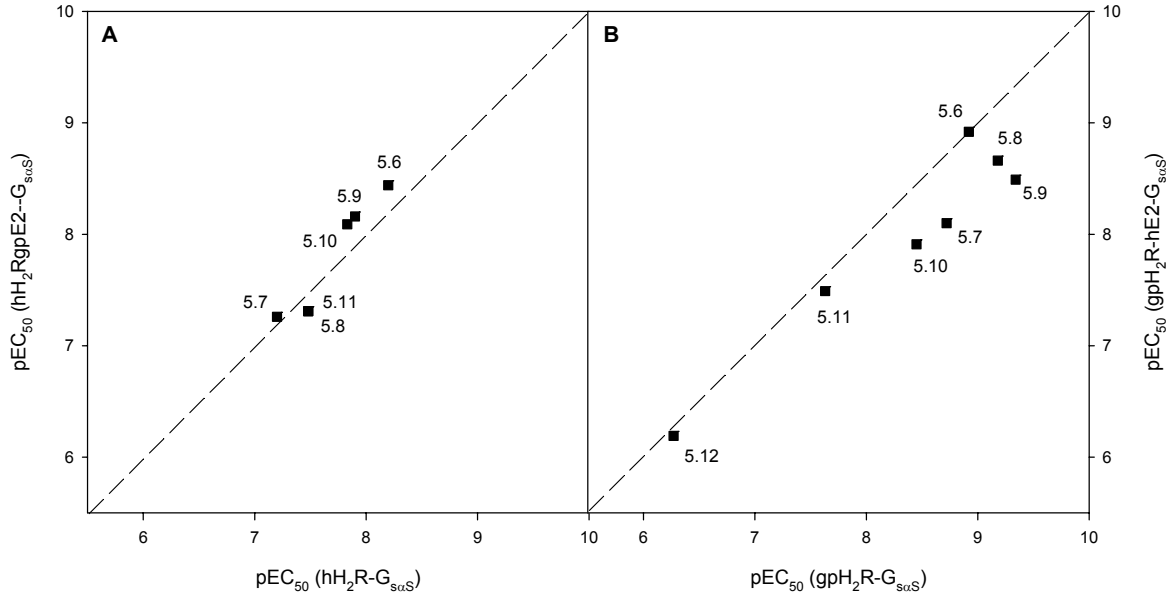


Figure 5.2. Correlation between the potencies of acylated bivalent ligands at hH_2R-$G_{s\alpha S}$ and hH_2R-gpE2-$G_{s\alpha S}$ (A) and gpH_2R-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$ (B) fusion proteins in the GTPase assay. Agonist potencies were taken from Table 5.2 and Table 5.4. The pEC_{50} values were derived from the EC_{50} shown in Table 5.2 and Table 5.4. The straight dotted line represents the correlation that would have been obtained if pEC_{50} values had been identical in the two systems.

5.3.2. Receptor selectivity

5.3.2.1. Activity at histamine H_1 receptors (Ca^{2+} assay on U-373 MG cells)

On human U-373 cells, all investigated compounds proved to be weak or very weak histamine H_1R antagonists (Table 5.5).

Table 5.5. Histamine H_1 receptor antagonism on U-373 MG human cells (Ca^{2+}-assay).

Histamine H_1 receptor antagonism U-373 MG cells (Ca^{2+}-assay)					
No	**IC_{50} [µM][a]**	**No.**	**IC_{50} [µM][a]**	**No.**	**IC_{50} [µM][a]**
HIS	-	**5.9**	5	**5.10**	7
AMT[25]	-	**5.6**	31	**5.11**	2
5.1	27	**5.7**	31	**5.12**	>100
4.39	>100	**5.8**	24		

[a] IC_{50} values for the inhibition of the histamine (30 µM) induced increase in cellular calcium, one experiment; procedure as described from Kracht, 2001[30].

5.3.2.2. Agonistic potency and efficacy at hH_3R and hH_4R membranes

As shown in chapter 4 for “monovalent” H_2R agonists, the exchange of imidazole with a 2-amino-4-methylthiazole ring favours the selectivity for H_2R, in particular over H_3R and H_4R. This is also true for the bivalent ligands. The compounds containing two 2-amino-4-methylthiazole rings are devoid of or exhibit low (**5.8-5.10**) H_3R stimulatory effects at the concentration tested. Furthermore, these compounds proved to be very weak H_4R antagonists in the GTPase assay (Table 5.6) whereas the imidazole analogue **5.6** is a potent partial agonist with an EC_{50} value of 2.8 nM.

Table 5.6. Agonist efficacies and potencies at $hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4 and hH_4R-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$ expressed in Sf9 cell membranes for bivalent ligands.

No	$hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4			hH_4R-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$		
	efficacy	EC_{50} [nM]	rel. pot.	efficacy	EC_{50} [nM] (IC_{50} [nM])	rel. pot.
His	1.00	25.8 ± 3.1	100	1.00	11.6 ± 2.5	100
5.6	- 0.25	nd	nd	0.36	2.8	414
5.7	- 0.25	nd	nd	-	(>10,000)	-
5.8	0.24	nd	nd	-	(>7,000)	-
5.9	0.36	nd	nd	-	(>10,000)	-
5.10	0.27	nd	nd	-	(>10,000)	-
5.11	- 0.47	nd	nd	-	(>10,000)	-
5.12	- 0.17	nd	nd	-	(>10,000)	-

Steady state GTPase activity in Sf9 membranes expressing $hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4 and hH_4R-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$ was determined as described[27]. Reaction mixtures contained ligands at concentrations from 0.1 nM to 100 µM as appropriate to generate saturated concentration-response curves. Data were analyzed by nonlinear regression and were best fitted to sigmoidal concentration-response curves. Typical basal GTPase activities ranged between ~ 1.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (10 µM) ranged between ~ 3.5 and 4.5 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values were referred to this value. Data shown are one experiment performed in duplicates each. The relative potency of histamine was set to 100, and the potencies of other agonists were referred to this value. For antagonism, reaction mixtures contained histamine (100 nM) and ligands at concentrations from 0.1 nM to 100 µM (hH_4R-GAIP+$G\alpha_{i2}+\beta_1\gamma_2$). For efficacy, reaction mixtures contained ligands at a concentration of 10 µM ($hH_3R+G\alpha_o+\beta_1\gamma_2$+RGS4).

5.4. Summary

Bivalent ligands were synthesized by connecting the essential partial structures, i. e. the hetarylpropylguanidine portion, of guanidine-type histamine H_2R agonists by N^G-acylation with alkanedioic acids of different length. The resulting symmetric compounds, containing either two imidazol-4-ylpropylguanidine or two 2-amino-4-methylthiazol-5-ylpropylguanidine moieties, were tested for H_2R agonism on the spontaneously beating guinea pig right atrium as well as in GTPase assay at hH_2R-$G_{s\alpha S}$, gpH_2R-$G_{s\alpha S}$ and cH_2R-$G_{s\alpha S}$ fusion proteins to study the species-selectivity.

Most strikingly, the combination of two 2-amino-4-methylthiazol-5-ylpropylguanidine moieties with an octanedioyl spacer (compound **5.8**) resulted in the most potent agonist at the guinea pig right atrium known so far: This compound proved to be about 3000 times more potent than histamine. As previously found for all *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines and N^G-acylated derivatives in the GTPase assay, the highly potent H_2R agonist **5.8** turned out to be more potent at gpH_2R-$G_{s\alpha S}$ than at hH_2R-$G_{s\alpha S}$. Interestingly, the substances with hexanedioyl to decanedioyl spacer moieties displayed the highest selectivity for the guinea pig receptor among all known acylguanidine-type H_2R agonists. Furthermore, **5.9** with a spacer length of about 11 Å is one of the most potent agonists at gpH_2R-$G_{s\alpha S}$ with an EC_{50} value of 0.46 nM. Increasing the spacer length resulted in a significant decrease in potency and efficacy up to a total loss in potency at hH_2R-$G_{s\alpha S}$. Due to insufficient spacer length for interaction with both binding pockets of H_2R dimers in the case of the most potent H_2R agonists and lack of potency in the case of compounds supposed to have optimal spacer lengths, it is concluded that only one receptor is occupied by one ligand. The dramatic increase in potency results from interaction with an additional (allosteric?) binding site rather than from occupation of a receptor dimer. The EC_{50} values and efficacies are higher at cH_2R-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$. In agreement with previous studies[19], this is not specific for the guanidine-type agonists but due to the increased constitutive activity of cH_2R-$G_{s\alpha S}$ compared to hH_2R-$G_{s\alpha S}$

As predicted by molecular modelling and verified by site-directed mutagenesis studies, the preference of the guanidine-type agonists for the gpH_2R is strongly dependent on two amino acids, Tyr-17 and Asp-271 in TM1 and TM7, which are thought to form an H-bond stabilizing an active receptor conformation[26, 27]. The bivalent ligands were tested at hH_2R-$G_{s\alpha S}$ mutants, hH_2R-C17Y-A271D-$G_{s\alpha S}$ and hH_2R-C17Y-$G_{s\alpha S}$. In agreement with recent data for monovalent ligands, the bivalent acylguanidine-type agonists turned out to be more potent and efficient at the double mutant hH_2R-C17Y-A271D-$G_{s\alpha S}$ but still less potent than at gpH_2R-$G_{s\alpha S}$. This indicates that the species-selectivity is not determined by these two amino acids alone. The compounds were less active on hH_2R-C17Y-$G_{s\alpha S}$.

Based on the crystal structure of rhodopsin[21], the participation of residues of the e2 loop to the binding pocket was proposed for some members of class 1 GPCRs[20] and experimentally demonstrated for the dopamine D_2 receptor[31], the adenosine A_{2a} receptor[32] and the M_3 muscarinic acetylcholine receptor[33]. However, the very recently

resolved crystal structure of the human β_2-adrenergic receptor did not confirm a contribution of the e2 loop to ligand binding[22, 23]. Concerning human and guinea pig histamine H_2 receptors, there is no hint from recent site-directed mutagenesis studies that the e2 loop contributes to the species-selectivity of *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines and N^G-acylated analogues[18, 24]. This is also true for the bivalent ligands presented in this study, i. e. there is no hint to an interaction with the e2 loop, too. All investigated compounds exhibited similar potencies and efficacies at “wild-type” hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ compared to the corresponding mutants in which four “critical” amino acids in the e2 loop were reciprocally mutated (hH_2R-gpE2-$G_{s\alpha S}$ and gpH_2R-hE2-$G_{s\alpha S}$).

In order to explore in more detail the high degree of species-selectivity of the bivalent ligands towards gpH_2R-$G_{s\alpha S}$ and to elaborate the structure-activity relationships with respect to the role and the interaction site of the second hetarylalkylguanidine moiety, it is necessary to synthesize and pharmacologically characterize additional compounds with different spacer lengths, more bulky spacers and distinct pharmacophores as well as non-H_2R-specific groups.

5.5. Experimental section

5.5.1. General conditions

see chapter 3.

5.5.2. Preparation of N^G-Boc-protected imidazolylpropylguanidine 5.5

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-amine (5.3)

3-(1-Trityl-1*H*-imidazol-4-yl)propan-1-ol (4 g, 11 mmol), phthalimide (1.6 g, 11 mmol) and PPh_3 (2.8 g, 11 mmol) were suspended in 100 ml THF/abs and cooled to 0 °C. DIAD (2.2 g, 2.1 ml, 11 mmol) was slowly added drop by drop, so that the yellow colour always disappeared. The mixture was allowed to warm to room temperature and stirred for 24 h. The solvent was evaporated *in vacuo* and the crude product suspended in 60 ml EtOH. After addition of hydrazine hydrate (2.7 ml, 55 mmol) the mixture was heated at refluxed for 1 h, cooled to room temperature and filtered to remove the precipitate. The solvent was evaporated under reduced pressure and the residue was subjected to flash chromatography to obtain **5.3** (2.34 g, 58 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.32 (m, 10H, CPh_3, Im-2-***H***), 7.12 (m, 6H, CPh_3), 6.52 (d, 1H, ^4J = 1.2 Hz, Im-5-***H***), 2.74 (t, 2H, ^3J = 6.9 Hz, C***H***$_2$NH$_2$), 2.59 (t, 2H, ^3J = 7.4 Hz, Im-4-C***H***$_2$), 1.78 (m, 2H, Im-4-CH$_2$C***H***$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 368 (MH^+, 100). $C_{25}H_{25}N_3$ (367.48)

***N*-Benzyloxycarbonyl-*N´*-*tert*-butoxycarbonyl-*N´´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (5.4)**

The title compound was prepared by analogy with the procedure described for **4.15** and **4.16** (Chapter 4) from **5.3** (2.03 g, 5.5 mmol), **4.14** (1.79 g, 5.5 mmol), $HgCl_2$ (3.05 g, 11.2 mmol) and NEt_3 (1.7 g, 2.33 ml, 17 mmol) in 250 ml DCM/abs. Flash chromatography ($CHCl_3$/MeOH 97.5/2.5 v/v)) yielded **5.4** as a colourless foam-like solid (3.54 g, 100 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.47 (d, 1H, ^{4}J = 1.2 Hz, Im-2-***H***), 7.34-7.10 (m, 20H, Ar-***H***, CPh_3), 6.58 (d, 1H, ^4J = 0.9 Hz, Im-5-***H***), 5.11 (s, 2H, C***H***$_2$), 3.40 (m, 2H, C***H***$_2$NH$_2$), 2.60 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 1.87 (m, 2H, Im-4-CH$_2$C***H***$_2$), 1.45 (s, 9H, Boc-C***H***$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 644 (MH^+, 100). $C_{39}H_{41}N_5O_4$ (643.77)

***N*-*tert*-Butoxycarbonyl-*N´*-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidine (5.5)**

The title compound was prepared by analogy with the procedure described for **4.17** and **4.18** (Chapter 4) from **5.4** (1.5 g, 2.33 mmol) by hydrogenation over Pd/C (10 %) (1 g) in a mixture of 60 ml THF/MeOH (1:1) at 8 bar for 8-9 days yielding **5.5** as a

colourless foam-like solid (1.05 g, 88 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.34-7.10 (m, 16H, Im-2-***H***, CPh_3), 6.57 (s, 1H, Im-5-***H***), 3.41 (m, 2H, C***H***$_2$$NH_2$), 2.56 (m, 2H, Im-4-C***H***$_2$), 1.86 (m, 2H, Im-4-CH_2C***H***$_2$), 1.46 (s, 9H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 510 (MH^+, 100). $C_{31}H_{35}NO_2$ (509.64)

5.5.3. Preparation of N^G-*tert*-butoxycarbonyl-protected acylguanidines 5.1a, 5.6a-5.12a

General procedure

To a solution of carboxylic acid (0.5 eq), EDAC (1 eq) and HOBt-monohydrate (1 eq) in DCM/abs was added DIEA (1 eq) under argon and stirred for 15 min. To this mixture a solution of **4.17** or **5.5** (1 eq) DCM/abs was added and stirred overnight at room temperature. The solvent was removed under reduced pressure, EtOAc and water were added to the residue,, the organic phase was separated, and the aqueous layer extracted two times with EtOAc. After drying over $MgSO_4$, the organic solvent was removed *in vacuo*. The crude product was purified by flash chromatography (PE/EtOAc 70/30-50/50 v/v) unless otherwise indicated.

10-Oxo-10-{3-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]guanidino}decanoic acid (5.1a)

The title compound was prepared from CDI (180 mg, 1.1 mmol), decanedioic acid (100 mg, 0.5 mmol), **3.7** (410 mg, 1 mmol) and NaH (60 % dispersion in mineral oil) (80 mg, 2 mmol) in THF/abs according to the general procedure yielding **5.1a** as a colourless foam-like solid (140 mg, 47 %). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.67 (s, 1H, Im-2-***H***), 7.35-7.11 (m, 15H, CPh_3), 6.56 (d, 1H, ^4J = 1.1 Hz, Im-5-***H***), 3.75 (m, 2H, C***H***$_2$NH), 2.56 (m, 2H, Im-4-C***H***$_2$), 2.46 (t, 2H, ^3J = 7.5 Hz, C***H***$_2$COOH), 2.25 (m, 2H, COC***H***$_2$), 1.86 (m, 4H, Im-4-CH_2C***H***$_2$, $COCH_2$C***H***$_2$), 1.68 (m, 2H, C***H***$_2$$CH_2$COOH), 1.33 (m, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) *m/z* (%): 594 (MH^+, 100). $C_{36}H_{43}N_5O_3$ (593.75)

***N*^{1},*N*^{10}-Bis{*N*-(*tert*-butoxycarbonyl)-[3-(1-trityl-1*H*-imidazol-4-yl)propyl]carbamimidoyl}decanediamide (5.6a)**

The title compound was prepared from decanedioic acid (100 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **5.5** (510 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure (flash chromatography $CHCl_3$/MeOH 95/5 v/v) yielding **5.6a** (0.18 g, 30 %) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.33-7.12 (m, 32H, Im-2-***H***, CPh_3), 6.53 (d, 2H, ^4J = 1.0 Hz, Im-5-***H***), 3.43 (m, 4H, C***H***$_2$NH), 2.59 (t, 4H, ^3J = 7.6 Hz, Im-4-C***H***$_2$),

2.34 (m, 4H, COC***H***$_2$), 1.90 (m, 4H, Im-4-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2C$***H***$_2$), 1.49 (s, 18H, C(C***H***$_3$)$_3$), 1.27 (m, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1185 (MH^+, 100). $C_{72}H_{84}N_{10}O_5$ (1184.66)

N^1,N^6-Bis(*N*-(*tert*-butoxycarbonyl)-*N´*-{3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propyl}carbamimidoyl)hexanediamide (5.7a)

The title compound was prepared from hexanedioic acid (75 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **5.7a** (0.21 g, 45 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 12.46 (s, 2H, N***H***), 8.91 (t, 2H, ^3J = 5.4 Hz, CH_2N***H***), 3.46 (m, 4H, C***H***$_2$NH), 2.72 (m, 4H, Thiaz-5-C***H***$_2$), 2.29 (m, 4H, COC***H***$_2$), 2.21 (s, 6H, Thiaz-4-C***H***$_3$), 1.88 (td, 4H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, (C***H***$_2$)$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.49 (s, 18H, C(C***H***$_3$)$_3$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 937 (MH^+, 100). $C_{42}H_{68}N_{10}O_{10}S_2$ (936.46)

N^1,N^8-Bis(*N*-(*tert*-butyoxycarbonyl)-*N´*-{3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propyl}carbamimidoyl)octanediamide (5.8a)

The title compound was prepared from octanedioic acid (90 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **5.8a** (0.21 g, 43 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 12.42 (s, 2H, N***H***), 9.01 (t, 2H, ^3J = 5.4 Hz, CH_2N***H***), 3.45 (m, 4H, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.35 (m, 4H, ^3J = 7.5 Hz, COC***H***$_2$), 2.22 (s, 6H, Thiaz-4-C***H***$_3$), 1.88 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2C$***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.49 (s, 18H, C(C***H***$_3$)$_3$), 1.36 (m, 4H, (C***H***$_2$)$_2$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 965 (MH^+, 100). $C_{44}H_{72}N_{10}O_{10}S_2$ (964.49)

N^1,N^{10}-Bis(*N*-(*tert*-butyoxycarbonyl)-*N´*-{3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propyl}carbamimidoyl)decanediamide (5.9a)

The title compound was prepared from decanedioic acid (100 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **5.9a** (0.23 g, 46 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 12.40 (s, 2H, N***H***), 9.02 (t, 2H, ^3J = 5.2 Hz, CH_2N***H***), 3.45 (m, 4H, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.35 (t, 4H, ^3J = 7.5 Hz, COC***H***$_2$), 2.21 (s, 6H, Thiaz-4-C***H***$_3$), 1.87 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2C$***H***$_2$),

1.51 (s, 18H, C(C***H***$_3$)$_3$), 1.49 (s, 18H, C(C***H***$_3$)$_3$), 1.32 (m, 8H, (C***H***$_2$)$_4$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 993 (MH^+, 100). $C_{46}H_{76}N_{10}O_{10}S_2$ (992.5)

N^1,N^{12}-Bis(*N*-(*tert*-butyoxycarbonyl)-*N´*-{3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propyl}carbamimidoyl)dodecanediamide (5.10a)

The title compound was prepared from dodecanedioic acid (120 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **5.10a** (0.34 g, 66 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 12.39 (s, 2H, N***H***), 9.02 (t, 2H, ^3J = 5.2 Hz, CH_2N***H***), 3.45 (m, 4H, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.2 Hz, Thiaz-5-C***H***$_2$), 2.34 (t, 4H, ^3J = 7.5 Hz, COC***H***$_2$), 2.21 (s, 6H, Thiaz-4-C***H***$_3$), 1.87 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.27 (m, 12H, (C***H***$_2$)$_6$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1021 (MH^+, 100). $C_{48}H_{80}N_{10}O_{10}S_2$ (1020.55)

N^1,N^{16}-Bis(*N*-(*tert*-butyoxycarbonyl)-*N´*-{3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propyl}carbamimidoyl)hexadecanediamide (5.11a)

The title compound was prepared from hexadecanedioic acid (140 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **5.11a** (0.29 g, 54 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 12.39 (s, 2H, N***H***), 9.03 (t, 2H, ^3J = 5.2 Hz, CH_2N***H***), 3.44 (m, 4H, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.39 (t, 4H, ^3J = 7.5 Hz, COC***H***$_2$), 2.22 (s, 6H, Thiaz-4-C***H***$_3$), 1.87 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.27 (m, 20H, (C***H***$_2$)$_{10}$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1077 (MH^+, 85). $C_{52}H_{88}N_{10}O_{10}S_2$ (1076.61)

N^1,N^{22}-Bis(*N*-(*tert*-butyoxycarbonyl)-*N´*-{3-[2-(*tert*-butoxycarbonyl)amino-4-methylthiazol-5-yl]propyl}carbamimidoyl)docosanediamide (5.12a)

The title compound was prepared from docosanedioic acid (185 mg, 0.5 mmol), EDAC (190 mg, 1 mmol), HOBt-monohydrate (150 mg, 1 mmol), DIEA (0.17 ml, 1 mmol) in 5 ml DCM/abs and **4.17** (410 mg, 1 mmol) in 5 ml DCM/abs according to the general procedure yielding **5.12a** (0.35 g, 60 %) as a colourless foam-like solid. ^{1}H-NMR ($CDCl_3$) δ ppm: 12.40 (s, 2H, N***H***), 9.04 (t, 2H, ^3J = 5.2 Hz, CH_2N***H***), 3.45 (m, 4H, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.39 (t, 4H, ^3J = 7.5 Hz, COC***H***$_2$), 2.21 (s, 6H, Thiaz-4-C***H***$_3$), 1.87 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.67 (m, 4H, $COCH_2$C***H***$_2$),

1.52 (s, 18H, C(C***H***$_3$)$_3$), 1.50 (s, 18H, C(C***H***$_3$)$_3$), 1.27 (m, 32H, (C***H***$_2$)$_{16}$); ES-MS (DCM/MeOH + NH_4OAc) m/z (%): 1161 (MH^+, 85). $C_{58}H_{100}N_{10}O_{10}S_2$ (1160.71)

5.5.4. Preparation of deprotected acylguanidines 5.1, 5.6-5.12

General procedure

To a solution of the Boc-protected acylguanidine in CH_2Cl_2/abs were added TFA and stirred at ambient temperatur until the Boc groups were removed (3-5 h). Subsequently, the solvent was removed *in vacuo* and the residue was purified by preparative RP-HPLC (for general conditions see chapter 3). All compounds were obtained as trifluoroacetic acid salts.

10-{3-[3-(1*H*-imidazol-4-yl)propyl]guanidino}-10-oxodecanoic acid (5.1)

The title compound was prepared from **5.1a** (130 mg, 0.22 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.1** as a white solid (90 mg, 71 %). ^{1}H-NMR (CD_3OD) δ (ppm): 8.80 (s, 1H, Im-2-***H***), 7.36 (s, 1H, Im-5-***H***), 3.38 (t, 2H, ^3J = 6.8 Hz, C***H***$_2$NH), 2.83 (t, 2H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 2.47 (t, 2H, ^3J = 7.3 Hz, C***H***$_2$COOH), 2.27 (t, 2H, ^3J = 7.4 Hz, COC***H***$_2$), 2.03 (m, 2H, Im-4-CH_2C***H***$_2$), 1.62 (m, 4H, $COCH_2$C***H***$_2$, C***H***$_2$$CH_2$COOH), 1.34 (m, 8H, (C***H***$_2$)$_4$); ^{13}C (CD_3OD) δ (ppm): 177.74 (quat. ***C***OOH), 177.45 (quat. ***C***=O), 155.40 (quart., ***C***=NH), 134.97(+, Im-2-***C***), 134.32 (quart., Im-4-***C***), 117.13 (+, Im-5-***C***), 41.55 (-, ***C***H_2NH), 37.75 (-, CO***C***H_2), 34.98 (-, ***C***H_2COOH), 30.20 (-, Im-4-***C***H_2, ***C***H_2), 30.16 (-, ***C***H_2), 29.92 (-, Im-4-***C***H_2), 27.97 (-, ***C***H_2), 26.07 (-, ***C***H_2), 25.44 (-, ***C***H_2), 22.56 (-, ***C***H_2); HRMS: EI-MS: *m/z* for ($C_{17}H_{29}N_5O_3$) calcd. 351.22704, found 351.22665; prep. HPLC: MeCN/0.1% TFA/aq (25/75). $C_{17}H_{29}N_5O_3$ · 2TFA (579.21)

***N*^{1},*N*^{10}-Bis{*N*-[3-(1*H*-imidazol-4-yl)propyl]carbamimidoyl}decanediamide (5.6)**

The title compound was prepared from **5.6a** (160 mg, 0.13 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.6** as a pale yellow oil (30 mg, 23 %). ^{1}H-NMR (CD_3OD) δ ppm: 8.81 (d, 1H, ^4J = 1.3 Hz, Im-2-***H***), 7.37 (d, 1H, ^4J = 0.9 Hz, Im-5-***H***), 3.38 (t, 4H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.84 (t, 4H, ^3J = 7.6 Hz, Im-4-C***H***$_2$), 2.47 (t, 4H, ^3J = 7.4 Hz, COC***H***$_2$), 2.03 (m, 4H, Im-4-CH_2C***H***$_2$), 1.65 (m, 4H, $COCH_2$C***H***$_2$), 1.35 (m, 8H, (C***H***$_2$)$_4$); ^{13}C (CD_3OD) δ (ppm): 177.45 (quat. ***C***=O), 155.42 (quat. ***C***=NH), 134.97 (+, Im-2-***C***), 134.33 (quat. Im-4-***C***), 117.14 (+, Im-5-***C***), 41.54 (-, ***C***H_2NH), 37.75 (-, CO***C***H_2), 30.17 (-, Im-4-***C***H_2), 29.95 (-, Im-4-CH_2***C***H_2), 27.98 (-, $COCH_2CH_2CH_2$***C***H_2), 25.45 (-, $COCH_2CH_2$***C***H_2), 22.56 (-, $COCH_2$***C***H_2); HRMS: FAB-MS: *m/z* for ($[C_{24}H_{40}N_{10}O_2 + H]^+$) calcd. 501.33357, found

501.34199; prep. HPLC: MeCN/0.1% TFA/aq (15/85-30/70). $C_{24}H_{40}N_{10}O_2 \cdot 4TFA$ (956.60)

N^1,N^6-Bis{*N*-[3-(2-amino-4-methylthiazol-5-yl)propyl]carbamimidoyl}hexane-diamide (5.7)

The title compound was prepared from **5.7a** (190 mg, 0.20 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.7** as a pale yellow oil (130 mg, 64 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 4H, ^3J = 6.8 Hz, C***H***$_2$NH), 2.71 (t, 4H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.52 (m, 4H, COC***H***$_2$), 2.17 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.71 (m, 4H, (C***H***$_2$)$_2$); ^{13}C (CD_3OD) δ (ppm): 177.09 (quart., ***C***=O), 170.42 (quart., Thiaz-2-***C***), 155.37 (quart., ***C***=NH), 132.59 (quart., Thiaz-4-***C***), 118.35 (quart., Thiaz-5-***C***), 41.58 (-, ***C***H_2NH), 37.20 (-, CO***C***H_2), 29.67 (-, Thiaz-5-***C***H_2), 24.51 (-, Thiaz-5-CH_2***C***H_2), 23.64 (-, $COCH_2$***C***H_2), 11.45 (+, Thiaz-5-***C***H_3); HRMS: FAB-MS: *m/z* for ($[C_{22}H_{36}N_{10}O_2S_2 + H]^+$) calcd. 537.2464, found 537.25507; prep. HPLC: MeCN/0.1% TFA/aq (10/90-30/70). $C_{22}H_{36}N_{10}O_2S_2 \cdot 4TFA$ (992.68)

N^1,N^8-Bis{*N*-[3-(2-amino-4-methylthiazol-5-yl)propyl]carbamimidoyl}octane-diamide (5.8)

The title compound was prepared from **5.8a** (190 mg, 0.19 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.8** as a colourless foam-like solid (110 mg, 57 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 4H, ^3J = 6.9 Hz, C***H***$_2$NH), 2.71 (t, 4H, ^3J = 7.6 Hz, Thiaz-5-C***H***$_2$), 2.48 (t, 4H, ^3J = 7.4 Hz, COC***H***$_2$), 2.18 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH_2C***H***$_2$), 1.66 (m, 4H, $COCH_2$C***H***$_2$), 1.39 (m, 4H, (C***H***$_2$)$_2$); ^{13}C (CD_3OD) δ (ppm): 177.39 (quart., ***C***=O), 170.40 (quart., Thiaz-2-***C***), 155.38 (quart., ***C***=NH), 132.60 (quart., Thiaz-4-***C***), 118.40 (quart., Thiaz-5-***C***), 41.58 (-, ***C***H_2NH), 37.64 (-, CO***C***H_2), 29.70 (-, Thiaz-5-***C***H_2), 29.59 (-, $COCH_2CH_2$***C***H_2), 25.23 (-, Thiaz-5-CH_2***C***H_2), 23.63 (-, $COCH_2$***C***H_2), 11.45 (+, Thiaz-5-***C***H_3); HRMS: FAB-MS: *m/z* for ($[C_{24}H_{40}N_{10}O_2S_2 + H]^+$) calcd. 565.27771, found 565.28476; prep. HPLC: MeCN/0.1% TFA/aq (15/85-40/60). $C_{24}H_{40}N_{10}O_2S_2 \cdot 4TFA$ (1020.73)

N^1,N^{10}-Bis{*N*-[3-(2-amino-4-methylthiazol-5-yl)propyl]carbamimidoyl}decane-diamide (5.9)

The title compound was prepared from **5.9a** (200 mg, 0.19 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.9** as a colourless foam-like solid (120 mg, 57 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 4H, ^3J =

6.8 Hz, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.45 (t, 4H, ^3J = 7.4 Hz, COC***H***$_2$), 2.16 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.63 (m, 4H, COCH$_2$C***H***$_2$), 1.33 (m, 8H, (C***H***$_2$)$_4$); ^{13}C (CD_3OD) δ (ppm): 177.57 (quart., ***C***=O), 170.43 (quart., Thiaz-2-***C***), 155.41 (quart., ***C***=NH), 132.59 (quart., Thiaz-4-***C***), 118.33 (quart., Thiaz-5-***C***), 41.56 (-, ***C***H$_2$NH), 37.75 (-, CO***C***H$_2$), 30.13 (-, Thiaz-5-***C***H$_2$), 29.90 (-, ***C***H$_2$), 29.68 (-, ***C***H$_2$), 25.46 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.64 (-, COCH$_2$***C***H$_2$), 11.46 (+, Thiaz-5-***C***H$_3$); HRMS: FAB-MS: *m/z* for ([$C_{26}H_{44}N_{10}O_2S_2$ + H]$^+$) calcd. 593.309, found 593.3161; prep. HPLC: MeCN/0.1% TFA/aq (25/75). $C_{26}H_{44}N_{10}O_2S_2$ · 4TFA (1048.78)

N^1,N^{12}-Bis{*N*-[3-(2-amino-4-methylthiazol-5-yl)propyl]carbamimidoyl}dodecanediamide (5.10)

The title compound was prepared from **5.10a** (320 mg, 0.31 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.10** as a colourless foam-like solid (180 mg, 53 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 4H, ^3J = 6.7 Hz, C***H***$_2$NH), 2.70 (t, 4H, ^3J = 7.4 Hz, Thiaz-5-C***H***$_2$), 2.45 (t, 4H, ^3J = 7.3 Hz, COC***H***$_2$), 2.16 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.63 (m, 4H, COCH$_2$C***H***$_2$), 1.30 (m, 12H, (C***H***$_2$)$_6$); ^{13}C (CD_3OD) δ (ppm): 177.66 (quart., ***C***=O), 170.43 (quart., Thiaz-2-***C***), 155.42 (quart., ***C***=NH), 132.59 (quart., Thiaz-4-***C***), 118.31 (quart., Thiaz-5-***C***), 41.57 (-, ***C***H$_2$NH), 37.80 (-, CO***C***H$_2$), 30.47 (-, Thiaz-5-***C***H$_2$), 30.32 (-, ***C***H$_2$) , 29.98 (-, ***C***H$_2$), 29.70 (-, ***C***H$_2$), 25.51 (-, Thiaz-5-CH$_2$***C***H$_2$), 23.65 (-, COCH$_2$***C***H$_2$), 11.48 (+, Thiaz-5-***C***H$_3$); HRMS: FAB-MS: *m/z* for ([$C_{28}H_{48}N_{10}O_2S_2$ + H]$^+$) calcd. 621.34031, found 621.34693; prep. HPLC: MeCN/0.1% TFA/aq (25/75-40/60). $C_{28}H_{48}N_{10}O_2S_2$ · 4TFA (1076.84)

N^1,N^{16}-Bis{*N*-[3-(2-amino-4-methylthiazol-5-yl)propyl]carbamimidoyl}hexadecanediamide (5.11)

The title compound was prepared from **5.11a** (260 mg, 0.24 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.11** as a pale yellow oil (170 mg, 62 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 4H, ^3J = 6.8 Hz, C***H***$_2$NH), 2.71 (t, 4H, ^3J = 7.5 Hz, Thiaz-5-C***H***$_2$), 2.46 (t, 4H, ^3J = 7.4 Hz, COC***H***$_2$), 2.17 (s, 6H, Thiaz-4-C***H***$_3$), 1.90 (m, 4H, Thiaz-5-CH$_2$C***H***$_2$), 1.64 (m, 4H, COCH$_2$C***H***$_2$), 1.27 (m, 20H, (C***H***$_2$)$_{10}$); ^{13}C (CD_3OD) δ (ppm): 177.61 (quart., ***C***=O), 170.42 (quart., Thiaz-2-***C***), 155.41 (quart., ***C***=NH), 132.59 (quart., Thiaz-4-***C***), 118.33 (quart., Thiaz-5-***C***), 41.57 (-, ***C***H$_2$NH), 37.80 (-, CO***C***H$_2$), 30.78 (-, Thiaz-5-***C***H$_2$), 30.73 (-, ***C***H$_2$), 30.60 (-, ***C***H$_2$), 30.40 (-, ***C***H$_2$), 30.03 (-, ***C***H$_2$), 29.70 (-, ***C***H$_2$), 25.54 (-, Thiaz-5-

$CH_2\boldsymbol{C}H_2$), 23.65 (-, $COCH_2\boldsymbol{C}H_2$), 11.48 (+, Thiaz-5-$\boldsymbol{C}H_3$); HRMS: FAB-MS: *m/z* for ($[C_{32}H_{56}N_{10}O_2S_2 + H]^+$) calcd. 677.4029, found 677.40894; prep. HPLC: MeCN/0.1% TFA/aq (25/75-50/50). $C_{32}H_{56}N_{10}O_2S_2 \cdot 4TFA$ (1132.94)

N^1,N^{22}-Bis{*N*-[3-(2-amino-4-methylthiazol-5-yl)propyl]carbamimidoyl}docosane-diamide (5.12)

The title compound was prepared from **5.12a** (330 mg, 0.24 mmol) in 10 ml CH_2Cl_2/abs and 2 ml TFA according to the general procedure yielding **5.12** as a pale yellow foam-like solid (150 mg, 43 %). ^{1}H-NMR (CD_3OD) δ ppm: 3.35 (t, 4H, ^3J = 6.8 Hz, C$\boldsymbol{H}_2$NH), 2.71 (t, 4H, ^3J = 7.6 Hz, Thiaz-5-C$\boldsymbol{H}_2$), 2.46 (t, 4H, ^3J = 7.4 Hz, COC$\boldsymbol{H}_2$), 2.17 (s, 6H, Thiaz-4-C$\boldsymbol{H}_3$), 1.90 (m, 4H, Thiaz-5-CH_2C$\boldsymbol{H}_2$), 1.64 (m, 4H, $COCH_2$C$\boldsymbol{H}_2$), 1.27 (m, 32H, (C$\boldsymbol{H}_2$)$_{16}$); ^{13}C (CD_3OD) δ (ppm): 177.58 (quart., ***C***=O), 170.42 (quart., Thiaz-2-***C***), 155.42 (quart., ***C***=NH), 132.59 (quart., Thiaz-4-***C***), 118.34 (quart., Thiaz-5-***C***), 41.56 (-, ***C***H_2NH), 37.79 (-, CO***C***H_2), 30.87 (-, 4 ***C***H_2), 30.78 (-, Thiaz-5-***C***H_2), 30.64 (-, ***C***H_2), 30.43 (-, ***C***H_2), 30.06 (-, ***C***H_2) , 29.71 (-, ***C***H_2), 25.55 (-, Thiaz-5-$CH_2\boldsymbol{C}H_2$), 23.65 (-, $COCH_2\boldsymbol{C}H_2$), 11.48 (+, Thiaz-5-$\boldsymbol{C}H_3$); HRMS: FAB-MS: *m/z* for ($[C_{38}H_{68}O_2N_{10}S_2 + H]^+$) calcd. 761.4968, found 761.50289; prep. HPLC: MeCN/0.1% TFA/aq (35/65-60/40). $C_{38}H_{68}N_{10}O_2S_2 \cdot 4TFA$ (1217.1)

5.6. References

(1) Terrillon, S.; Bouvier, M., Roles of G-protein-coupled receptor dimerization. *EMBO Reports* **2004,** 5, (1), 30-34.

(2) George, S. R.; O'Dowd, B. F.; Lee, S. P., G-Protein-coupled receptor oligomerization and its potential for drug discovery. *Nat. Rev. Drug Discov.* **2002,** 1, (10), 808-820.

(3) Nikbin, N.; Edwards, C.; Reynolds, C. A., G-protein coupled receptor dimerization. *IJPT* **2003,** 2, (1), 1-11.

(4) Pin, J.-P.; Neubig, R.; Bouvier, M.; Devi, L.; Filizola, M.; Javitch, J. A.; Lohse, M. J.; Milligan, G.; Palczewski, K.; Parmentier, M.; Spedding, M., International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the Recognition and Nomenclature of G Protein-Coupled Receptor Heteromultimers. *Pharmacol. Rev.* **2007,** 59, (1), 5-13.

(5) 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, (2), 283-286.

(6) 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, (3), 427-433.

(7) 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, (1), 131-138.

(8) Bakker, R. A.; Lozada, A. F.; van Marle, A.; Shenton, F. C.; Drutel, G.; Karlstedt, K.; Hoffmann, M.; Lintunen, M.; Yamamoto, Y.; van Rijn, R. M.; Chazot, P. L.; Panula, P.; Leurs, R., Discovery of Naturally Occurring Splice Variants of the Rat Histamine H_3 Receptor That Act as Dominant-Negative Isoforms. *Mol. Pharmacol.* **2006,** 69, (4), 1194-1206.

(9) Carrillo, J. J.; Pediani, J.; Milligan, G., Dimers of Class A G Protein-coupled Receptors Function via Agonist-mediated Trans-activation of Associated G Proteins. *J. Biol. Chem.* **2003,** 278, (43), 42578-42587.

(10) Shenton, F. C.; Hann, V.; Chazot, P. L., Evidence for native and cloned H_3 Histamine receptor higher oligomers. *Inflamm. Res.* **2005,** 54, (0), S48-S49.

(11) 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, (2), 604-615.

(12) Erez, M.; Takemori, A. E.; Portoghese, P. S., Narcotic antagonistic potency of bivalent ligands which contain b-naltrexamine. Evidence for simultaneous occupation of proximal recognition sites. *J. Med. Chem.* **1982,** 25, (7), 847-849.

(13) Portoghese, P. S., From Models to Molecules: Opioid Receptor Dimers, Bivalent Ligands, and Selective Opioid Receptor Probes. *J. Med. Chem.* **2001,** 44, (14), 2259-2269.

(14) 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, (3), 1386-1392.

(15) Traiffort, E.; Vizuete, M. L.; Tardivellacombe, J.; Souil, E.; Schwartz, J. C.; Ruat, M., The Guinea Pig Histamine H_2 Receptor: Gene Cloning, Tissue Expression and Chromosomal Localization of Its Human Counterpart. *Biochem. Biophys. Res. Commun.* **1995,** 211, (2), 570-577.

(16) Ruat, M.; Traiffort, E.; Arrang, J.-M.; Leurs, R.; Schwartz, J.-C., Cloning and tissue expression of a rat histamine H_2-receptor gene. *Biochem. Biophys. Res. Commun.* **1991,** 179, (3), 1470-1478.

(17) 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, (2), 429-433.

(18) 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/volltexte/2007/843/

(19) 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, (3), 983-995.

(20) Shi, L.; Javitch, J. A., The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu. Rev. Pharmacol. Toxicol.* **2002,** 42, (1), 437-467.

(21) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Trong, I. L.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M., Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science* **2000,** 289, (5480), 739-745.

(22) 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 b2-Adrenergic G Protein Coupled Receptor. *Science* **2007**, DOI: 10.1126/science.1150577

(23) 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 b2 Adrenergic Receptor Function. *Science* **2007**, DOI: 10.1126/science.1150609.

(24) Preuss, H.; Ghorai, P.; Kraus, A.; Dove, S.; Buschauer, A.; Seifert, R., Point mutations in the second extracellular loop of the histamine H_2 receptor do not affect the species-selective activity of guanidine-type agonists. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2007**, DOI 10.1007/s00210-007-0204-4.

(25) Eriks, J. C.; van der Goot, H.; Timmerman, H., New activation model for the histamine H_2 receptor, explaining the activity of the different classes of histamine H_2 receptor agonists. *Mol. Pharmacol.* **1993,** 44, (4), 886-894.

(26) Preuss, H.; Ghorai, P.; Kraus, A.; Dove, S.; Buschauer, A.; Seifert, R., Mutations of Cys-17 and Ala-271 in the Human Histamine H_2 Receptor Determine the Species Selectivity of Guanidine-Type Agonists and Increase Constitutive Activity. *J. Pharmacol. Exp. Ther.* **2007,** 321, (3), 975-982.

(27) 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, (6), 1210-1225.

(28) 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, (1), 139-146.

(29) Xie, S.-X.; Kraus, A.; Ghorai, P.; Ye, Q.-Z.; Elz, S.; Buschauer, A.; Seifert, R., N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1H-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, (3), 1262-1268.

(30) Kracht, J. Bestimmung der Affinität und Aktivität subtypselektiver Histamin- und Neuropeptid Y-Rezeptorliganden an konventionellen und neuen pharmakologischen In-vitro-Modellen. Doctoral Thesis, University of Regensburg, Germany, 2001.

(31) Shi, L.; Javitch, J. A., The second extracellular loop of the dopamine D_2 receptor lines the binding-site crevice. *Proc. Natl. Acad. Sci. U. S. A.* **2004,** 101, (2), 440-445.

(32) Kim, J.; Jiang, Q.; Glashofer, M.; Yehle, S.; Wess, J.; Jacobson, K. A., Glutamate residues in the second extracellular loop of the human A2a adenosine receptor are required for ligand recognition. *Mol. Pharmacol.* **1996,** 49, (4), 683-691.

(33) Scarselli, M.; Li, B.; Kim, S.-K.; Wess, J., Multiple Residues in the Second Extracellular Loop Are Critical for M_3 Muscarinic Acetylcholine Receptor Activation. *J. Biol. Chem.* **2007,** 282, (10), 7385-7396.

Chapter 6

Summary

Potent and selective histamine H_2 receptor (H_2R) agonists are valuable pharmacological tools and might be of therapeutic value as drugs, for example in the treatment of severe congestive heart failure, acute myelogenous leukaemia and inflammatory diseases. At the beginning of this project arpromidine and related *N*-[3-(1*H*-imidazol-4-yl)propyl]guanidines were known as the most potent H_2R agonists, achieving up to 400 times the potency of histamine on the isolated spontaneously beating guinea pig right atrium. Previous studies from our group have shown that the strong basicity of those compounds is considerably reduced by introduction of a carbonyl group adjacent to the guanidine moiety while the agonistic acitivity is more or less retained. Investigations of prototypical acylguanidines revealed improved pharmacokinetic properties, in particular oral bioavailability and penetration across the blood brain barrier. However, the H_2R agonistic activity turned out to be species-dependent: guanidines and N^G-acylated guanidines were less potent and efficacious at the human (h) than at the guinea pig (gp) H_2R. Moreover, the imidazolylpropylguanidines had considerable activity at H_3R and H_4R.

The aim of this thesis was to synthesize and elaborate the structure-activity relationships, in particular to improve the potency and selectivity of acylguanidine-type H_2R agonist. Therefore, numerous N^G-acylated [3-(1*H*-imidazol-4-yl)propyl]guanidines and [3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines with different alkanoyl substituents including “bivalent ligands” were prepared and pharmacologically characterized (cooperation with Prof. Dr. S. Elz and Prof. Dr. R. Seifert, University of Regensburg).

<u>N^G-Acylated [3-(1*H*-imidazol-4-yl)propyl]guanidines.</u> The synthesized compounds were investigated for agonism on the gp right atrium and in a steady-state GTPase activity assay using membranes with fusion proteins of hH_2R and gpH_2R and the short splice variant of $G_{s\alpha}$, $G_{s\alpha S}$. In general, a methyl or ethyl branched three-membered alkanoyl chain between the guanidine moiety and an aromatic or alicyclic

ring system favours high potency at the gpH_2R. By contrast, the hH_2R prefers/tolerates compounds with longer and more ramified N^G-alkanoyl chains, presumably due to a more flexible binding pocket. Polar substituents strongly shifted the species selectivity towards gpH_2R, whereas aliphatic substituents were preferred by the hH_2R. In accordance with previous data of alkyl- and acylguanidines, most compounds proved to be more potent and efficacious at the gpH_2R compared to the hH_2R. Strikingly, also compounds with similar or even slightly higher potency and efficacy at the hH_2R were found, indicating that, in principle, potent and selective compounds for the human receptor can be developed. For receptor selectivity, representative compounds were tested at hH_1R (calcium assay on U-373 MG cells) as well as in GTPase assays on hH_3R and hH_4R expressed in Sf9 cells. On human U-373 cells only weak antagonistic activity was found. Surprisingly, several N^G-acylated imidazolylpropylguanidines turned out to be potent agonists on the hH_3R and hH_4R in the GTPase assay (pEC_{50} values around 8-9).

<u>N^G-Acylated [3-(2-amino-4-methylthiazol-5-yl)propyl]guanidines.</u> The bioisosteric replacement of the imidazole against a 2-amino-4-methylthiazole ring led to equipotent or even more potent partial H_2R agonists and turned out to be key to obtain highly H_2R-selective agonists, devoid of activity on H_3R and H_4R. In contrast to the H_2R agonist amthamine (2-amino-4-methylthiazol-5-ethanamine), the 4-methyl group in the thiazole ring of acylguanidine-type H_2R agonists did enhance the agonistic potency neither in the GTPase assay nor at the gp right atrium.

<u>“Bivalent” H_2R agonists.</u> According to the “bivalent ligand approach”, symmetric compounds were synthesized by connecting either two imidazol-4-ylpropylguanidine or two 2-amino-4-methylthiazol-5-ylpropylguanidine moieties by N^G-acylation with alkanedioic acids of different length. Most strikingly, the combination of two 2-amino-4-methylthiazol-5-ylpropylguanidine moieties with an octanedioyl spacer resulted in the most potent agonist at the gp atrium known so far (pEC_{50} 9.45; 3000-fold potency of histamine). This result is in good agreement with the data from the GTPase assay. However, the spacer groups of these bivalent H_2R agonists are too short to allow simultaneous binding to both receptor molecules of a H_2R dimer. Elongation of the spacer up to a length predicted to be optimal for binding to receptor dimers (22-27 Å) resulted in a dramatic decrease or total loss of H_2R agonism. The exceptionally high

potency of the most prominent bivalent H_2R agonists can be explained by binding to an additional (allosteric?) binding site at the same receptor molecule rather than by “bridging” of a receptor dimer. Further investigations of the bivalent ligands on mutant H_2R-$G_{s\alpha S}$ fusion proteins (transmembrane domains, e2 loop) confirmed the key role of the non-conserved Tyr-17 and Asp-271 in TM1 and TM7 in the gpH_2R for species-selective H_2R activation and suggest that the second extracellular loop does not participate in direct ligand-receptor interactions.

In conclusion, numerous potent histamine H_2R agonists were identified among the synthesized N^G-acylated hetarylpropylguanidines. A major problem, selectivity for H_2R over H_3R and H_4R, was solved by bioisosteric replacement of the imidazol-4-yl with a 2-amino-4-methylthiazol-5-yl ring. The most potent and selective H_2R agonists were obtained according to the bivalent ligand approach by combining two guanidine-type H_2R agonist pharmacophores by alkanedioic acids. The results of this thesis represent a promising step towards the development of guanidine-type H_2R agonists for *in vivo* investigations.

Chapter 7

Appendix

Appendix 1: Abbreviations

abs	absolute
AMT	amthamine
aq	aqueous
Ar	aryl
ARP	arpromidine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
cAMP	cyclic 3´,5´-adenosine monophosphate
Cbz	benzyloxycarbonyl
CDI	*N*, *N´*-carbonyldiimidazole
cHex	cyclohexyl
cH_2R	canine histamine H_2 receptor
conc	concentrated
d	day
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIEA	*N*,*N*-diisopropylethylamine
DMAP	*p*-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxid
e2	second extracellular loop of a G-protein coupled receptor
EC_{50}	agonist concentration which induces 50 % of the maximum effect
EDAC	*N*-(3-dimethylaminopropyl)-*N´*-ethylcarbodiimide hydrochloride
GDP	guanosine diphosphate
GPCR	G-protein coupled receptor

gpH_2R	guinea pig histamine H_2 receptor
gpH_2R-hE2-$G_{s\alpha S}$	fusion protein of the guinea pig H_2 receptor bearing Asp-167→Gly-167, Asp-169→His-169, Ile-171→Thr-171 and Val-172→Ser-172 mutations and the short splice variant of $G_{s\alpha}$
$G_{s\alpha S}$	short splice variant of $G_{s\alpha}$
GTP	guanosine triphosphate
h	hours
H_1R, H_2R, H_3R, H_4R	histamine receptor subtypes
(c/gp/h/r)H_2R-$G_{s\alpha S}$	fusion proteins of histamine H_2 receptor species isoforms and the short splice variant of $G_{s\alpha}$
hH_2R	human histamine H_2 receptor
hH_2R-C17Y-A271D-$G_{s\alpha S}$	fusion protein of the human histamine H_2 receptor bearing a Cys-17→Tyr-17 and Ala-271→Asp-271 mutation and the short splice variant of $G_{s\alpha}$
hH_2R-C17Y-$G_{s\alpha S}$	fusion protein of the human histamine H_2 receptor bearing a Cys-17→Tyr-17 mutation and the short splice variant of $G_{s\alpha}$
hH_2R-gpE2-$G_{s\alpha S}$	fusion protein of the guinea pig H_2 receptor bearing Gly-167→Asp-167, His-169→Asp-169, Thr-171→Ile-171 and Ser-172→Val-172 mutations and the short splice variant of $G_{s\alpha}$
HIS	histamine
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high-pressure liquid spectroscopy
HR-MS	high resolution mass spectroscopy
IMP	impromidine
min	minutes
MPM	*p*-methoxybenzyl
NMR	nuclear magnetic resonance
MS	mass spectroscopy
ppm	parts per million

py-1	2,6-dimethyl-4-[(E)-2-(2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-ij]quinolin-9-yl)-vinyl]pyranylium tetrafluoro borate
rt	room temperature
SEM	standard error of the mean
Sf9	*Spodoptera frugiperda* insect cell line
TFA	trifluoroacetic acid
TM	transmembrane domain of a G-protein coupled receptor
TM1-TM7	numbering of transmembrane domains of a G-protein coupled receptor

Appendix 2: Combustion analysis data

No	Formula	Calculated			Found		
		C	H	N	C	H	N
3.20	$C_{25}H_{22}N_2O_2$	78.51	5.90	7.32	78.37	5.88	7.27
3.21c	$C_{11}H_{14}O_2$	74.13	7.92		74.26	7.59	
3.22c	$C_{12}H_{16}O_2$	74.97	8.39		74.73	8.19	
3.23c	$C_{13}H_{18}O_2$	75.69	8.80		75.77	8.78	
3.24c	$C_{16}H_{16}O_2$	79.97	6.71		80.08	6.75	
3.28c	$C_{11}H_{14}O_2 \cdot C_{12}H_{23}N$	76.83	10.37	3.90	76.45	10.46	3.75
3.29c	$C_{12}H_{16}O_2 \cdot C_{12}H_{23}N$	77.16	10.52	3.75	76.90	10.41	3.50
3.30c	$C_{13}H_{18}O_2 \cdot C_{12}H_{23}N$	77.47	10.66	3.61	77.23	10.57	3.23
3.26c	$C_{16}H_{22}O_2 \cdot C_{12}H_{23}N \cdot 0.25\ H_2O$	77.82	10.61	3.24	77.79	10.38	3.03
3.25c	$C_{17}H_{18}O_2$	80.28	7.13		80.13	6.96	
3.27c	$C_{11}H_{14}O_2$	74.13	7.92		74.06	8.29	
3.39c	$C_{17}H_{25}NO_4 \cdot C_{12}H_{23}N$	71.27	9.96	5.73	71.24	9.57	5.59
3.33c	$C_{11}H_{13}FO_2 \cdot C_{12}H_{23}N \cdot 0.25\ H_2O$	72.31	9.63	3.67	72.54	9.36	3.49
3.34c	$C_{11}H_{13}FO_2 \cdot C_{12}H_{23}N \cdot 0.15\ H_2O$	72.65	9.62	3.68	72.74	9.43	3.37
3.36c	$C_{12}H_{16}O_3 \cdot C_{12}H_{23}N \cdot 0.75 H_2O$	71.51	10.13	3.47	71.11	9.75	3.36
3.35c	$C_{12}H_{16}O_3 \cdot C_{12}H_{23}N \cdot 0.5\ H_2O$	72.65	10.11	3.53	72.36	9.87	3.18
3.31c	$C_{12}H_{16}O_2 \cdot C_{12}H_{23}N \cdot H_2O$	73.61	10.55	3.58	73.56	10.23	3.34
3.32c	$C_{12}H_{16}O_2 \cdot C_{12}H_{23}N \cdot 0.5\ H_2O$	75.35	10.54	3.66	75.36	10.20	3.14
3.37c	$C_{13}H_{18}O_2 \cdot C_{12}H_{23}N \cdot 0.3\ H_2O$	76.40	10.67	3.56	76.61	10.23	3.14
3.43	$C_{11}H_{20}O_2 \cdot C_{12}H_{23}N \cdot 0.1\ H_2O$	75.19	11.85	3.81	75.05	11.85	3.56
3.45	$C_{12}H_{22}O_2 \cdot C_{12}H_{23}N$	75.93	11.95	3.69	76.06	12.19	3.49

Table (continued)

3.46	$C_{13}H_{24}O_2 \cdot C_{12}H_{23}N \cdot 0.25H_2O$	75.42	12.02	3.52	75.71	11.30	3.44
3.47	$C_{11}H_{20}O_2 \cdot C_{12}H_{23}N$	75.56	11.86	3.83	75.34	11.75	3.51
3.48	$C_{12}H_{22}O_2 \cdot C_{12}H_{23}N \cdot 0.1\ H_2O$	75.57	11.94	3.67	75.22	11.96	3.63
3.49	$C_{13}H_{24}O_2 \cdot C_{12}H_{23}N \cdot 0.5\ H_2O$	74.75	12.02	3.48	74.66	11.89	3.20
3.40	$C_{10}H_{18}O_2 \cdot C_{12}H_{23}N$	75.16	11.75	3.98	74.96	11.67	3.65
3.41	$C_{10}H_{18}O_2 \cdot C_{12}H_{23}N$	75.16	11.75	3.98	75.06	11.59	3.73
3.42	$C_{10}H_{18}O_2 \cdot C_{12}H_{23}N$	75.16	11.75	3.98	75.10	11.43	3.76
3.50	$C_{12}H_{22}O_2 \cdot C_{12}H_{23}N$	75.93	11.95	3.69	75.72	11.97	3.44
3.44	$C_{11}H_{20}O_2 \cdot C_{12}H_{23}N$	75.56	11.86	3.83	75.19	11.48	3.60
3.51d	$C_{11}H_{14}O_2 \cdot C_{12}H_{23}N \cdot 0.25\ H_2O$	75.88	10.38	3.85	75.50	10.41	3.62
3.53b	$C_{17}H_{16}O_3 \cdot 0.25H_2O$	74.84	6.10		75.17	6.08	

Appendix 3: HPLC purity data

No	t_R [min]	k`	purity [%]	No	t_R [min]	k`	purity [%]
3.55	12.77	2.85	100	**4.19**	15.23	3.59	100
3.57	13.88	3.18	100	**4.20**	14.08	3.24	100
3.58[a]	14.83	3.47	92	**4.21**	15.27	3.60	98
3.59[a]	16.47	3.96	100	**4.22**	15.03	3.53	99
3.60[a]	16.32	3.92	100	**4.23**	15.22	3.59	100
3.61[a]	17.55	4.29	95	**4.24**	16.63	4.01	100
3.62	18.92	4.70	84	**4.25**	16.43	3.95	99
3.63[a]	13.26	2.99	100	**4.26**	15.31	3.61	99
3.64[a]	13.98	3.21	100	**4.27**	15.22	3.59	100
3.65[a]	13.90	3.19	100	**4.28**	15.79	3.76	98
3.66[a]	15.43	3.65	100	**4.29**	15.72	3.74	93
3.67[a]	16.79	4.06	97	**4.30**	16.85	4.08	98
3.68[a]	15.23	3.59	100	**4.31**	16.82	4.07	97
3.69	15.48	3.67	98	**4.32**	16.85	4.08	100
3.70[a]	14.43	3.35	100	**4.33**	18.08	4.45	100
3.71[a]	14.38	3.33	100	**4.34**	17.84	4.38	96
3.72	14.31	3.31	100	**4.35**	18.52	4.58	100
3.73[a]	13.82	3.16	100	**4.36**	19.64	4.92	100
3.74[a]	16.68	4.03	99	**4.37**	11.39	2.43	100
3.75[a]	15.59	3.69	100	**4.38**	9.14	1.75	99
3.76[a]	15.62	3.71	100	**4.39**	12.94	2.90	94
3.77	15.72	3.74	89	**4.40**	12.80	2.86	100
3.78	16.97	4.11	96	**4.41**	13.61	3.10	99

Table (continued)

3.79	16.74	4.05	97	**4.42**	14.83	3.47	95
3.80	17.99	4.42	99	**4.43**	16.35	3.93	99
3.81	19.50	4.88	100	**4.44**	16.54	3.98	98
3.82	17.41	4.25	97	**4.45**	16.34	3.92	95
3.83[a]	19.91	5.00	100	**4.46**	17.64	4.32	100
3.84[b]	18.28	4.51	99	**5.1**	11.97	2.61	97
3.85	18.54	4.59	98	**5.6**	9.66	1.91	96
3.86	10.60	2.19	83	**5.7**	8.27	1.49	93
3.87	10.21	2.08	97	**5.8**	9.51	1.87	89
3.88	6.29	0.89	100	**5.9**	10.90	2.28	100
3.89	7.85	1.37	97	**5.10**	12.77	2.85	95
3.90	15.22	3.59	100	**5.11**	15.89	3.79	96
3.91	11.83	2.57	100	**5.12**[b]	18.53	4.58	100
3.92[b]	18.03	4.43	66				

[a] 0.02 % TFA/aq; [b] gradient: 0 min: MeCN/0.05% TFA/aq 10/90, 20 min: 70/30;

Analytical HPLC was performed on a system from Thermo Separation Products equipped with a SN 400 controller, P4000 pump, an AS3000 autosampler and a Spectra Focus UV-VIS detector. Stationary phase was an Eurosphere-100 C-18 (250 x 4.0, 5 µm) column (Knauer) thermostated at 30 °C. As mobile phase gradients of MeCN/0.02 or 0.05 % TFA/aq were used (flow rate = 0.7 ml/min). Absorbance was detected at 210 nm.

The following gradient mode was used: 0 min: MeCN/0.05% TFA/aq 10/90, 20 min: 60/40, 20-23 min: 95/5, -33 min 95/5.

t_0 (Eurosphere-100 C-18) = 3.318 min; $k' = (t_R - t_0)/t_0$.

Appendix 4: List of poster presentations and publications

Short lecture and poster presentations:

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, Buschauer, A, N^G-acylated hetarylpropylguanidines-centrally active selective histamine H_2 receptor agonists, poster contribution, abstract no. A17, Annual Meeting of the German Pharmaceutical Society (DPhG), Erlangen, October 10-13, **2007**.

"N^G-acylated hetarylpropylguanidines-centrally active selective histamine H_2 receptor agonists", short lecture at the workshop Graduiertenkolleg GRK 760 and Graduiertenkolleg GRK 677 (Bonn) by the Deutsche Forschungsgemeinschaft, Nuremberg, October 8-10, **2007**.

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, Buschauer, A, Synthesis, pharmacological activity and brain penetration of N^G-acylated hetarylpropylguanidines-Histamine H_2-receptor agonists, poster contribution, abstract no. HMC13, Annual Meeting "Frontiers in Medicinal Chemistry", Berlin, March 18-21, **2007**.

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Kunze, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, Buschauer, A, N^G-Acylated Hetarylpropylguanidines: Histamine H_2 Receptor Agonism, Selectivity and Brain Penetration, poster contribution, abstract no. 37, 3rd Summer School Medicinal Chemistry, University of Regensburg, September 25-27, **2006**.

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Kunze, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, and Buschauer, A, Histamine H_2 Receptor Agonists: Synthesis, Pharmacological Activity and Brain Penetration of N^G-acylated Hetarylpropylguanidines, poster contribution, abstracts published in: *Drugs of the Future* **31** (Suppl. A), 135, XIXth International Symposium on Medicinal Chemistry, Istanbul, Turkey, August 29-September 2, **2006**.

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, and Buschauer, A, A New Class of Potent Histamine H_2 Receptor Agonists: Acylguanidines derived from Arpromidine, poster contribution, abstract no. C55, Annual Meeting of the German Pharmaceutical Society (DPhG), Johannes Gutenberg University of Mainz, October 5-8, **2005**.

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, and Buschauer, A, Synthesis and Pharmacological Investigation of N^G-acylated Imidazolylpropylguanidines: a Novel Class of Histamine H_2 Receptor Agonists, poster

contribution, abstract no. 22, Summer School “Medicinal Chemistry”, EU-ASIA-Link Medicinal Chemistry, SIOC, Shanghai, China, September 25-28, **2005**.

Kraus, A, Ghorai, P, Preuss, H, Keller, M, Bernhardt, G, Dove, S, Elz, S, Seifert, R, and Buschauer, A, N^G-acylated Imidazolylpropylguanidines: a Novel Class of Histamine H_2 Receptor Agonists, poster contribution, abstract no. PSA03, Annual Meeting “Frontiers in Medicinal Chemistry”, Leipzig, March 13-16, **2005**.

Publications:

Preuss, H, Ghorai, P, Kraus, A, Dove, S, Buschauer, A, Seifert, R, Point Mutations in the Second Extracellular Loop of the Histamine H_2 Receptor do not affect the Species-Selective Activity of Guanidine-Type Agonists. *Naunyn Schmiedeberg`s Arch Pharmac*ol, **2007**, DOI 10.1007/s00210-007-0204-4.

Ziemek, R, Schneider, E, Kraus, A, Cabrele, C, Beck-Sickinger, AG, Bernhardt, G, Buschauer, A, Determination of Affinity and Activity of Ligands at the Human Neuropeptide Y Y_4 Receptor by Flow Cytometry and Aequorin Luminescence, *J Recept Signal Transduct Res,* **2007**, 27 (4), 217-233.

Preuss, H, Ghorai, P, Kraus, A, Dove, S, Buschauer, A, Seifert, R, Constiutive Activity and Ligand Selectivity of Human, Guinea Pig, Rat, and Canine Histamine H_2 Receptors. *J Pharmacol Exp Ther,* **2007**, 321(3), 983-995.

Preuss, H, Ghorai, P, Kraus, A, Dove, S, Buschauer, A, Seifert, R, Mutations of Cys-17 and Ala-271 in the Human Histamine H_2 Receptor Determine the Species-Selectivity of Guanidine-Type Agonists and Increase Constitutive Activity. *J Pharmacol Exp Ther,* **2007**, 321(3), 975-982.

Xie, S-X, Kraus, A, Ghorai, P, Ye, Q-Z, Elz, S, Buschauer, A, Seifert, R, N^1-(3-Cyclohexylbutanoyl)-N^2-[3-(1H-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(3), 1262-1268.

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Regensburg, im November 2007

(Anja Kraus)