Special Report

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Future Medicinal Chemistry

Histamine H_2 receptor radioligands: triumphs and challenges

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Since the discovery of the histamine H_2 receptor (H_2R), radioligands were among the most powerful tools to investigate its role and function. Initially, radiolabeling was used to investigate human and rodent tissues regarding their receptor expression. Later, radioligands gained increasing significance as pharmacological tools in *in vitro* assays. Although tritium-labeling was mainly used for this purpose, labeling with carbon-14 is preferred for metabolic studies of drug candidates. After the more-or-less successful application of numerous labeled H_2R antagonists, the recent development of the G protein-biased radioligand [^{3}H]UR-KAT479 represents another step forward to elucidate the widely unknown role of the H_2R in the central nervous system through future studies.

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For more than 50 years now, the histamine H_2 receptor (H_2R) has been subject of drug research in academia and the pharmaceutical industry. While in 1966 Ash and Schild speculated about its existence [1], the SK&F research group around Sir James W Black was able to prove the presence of the H_2R in 1972 with their experiments on histamine-induced gastric acid release [2]. From today's perspective, a rather small number of 700 tested compounds within 9 years of drug development (1964–1972) [3] led via burimamide and metiamide to the world's first blockbuster drug, cimetidine (Figure 1). Cimetidine is known to be the first H_2 receptor antagonist on the market in the treatment of peptic ulcer and gastroesophageal reflux disease [3,4]. To date, five additional H_2R antagonists (ranitidine [5], famotidine [6], nizatidine [7], roxatidine [8] and lafutidine [9]; Figure 1) with increased potency and better pharmacokinetic properties have been successfully approved [10]. In contrast, H_2 receptor agonists have not yet found their way into the world's drug portfolio. Although a large number of highly potent and subtype-selective agonists has already been published, these ligands are predominantly applied in basic research studies within academia [11–18]. However, the aforementioned properties turn them into valuable pharmacological tools that could be of great importance to elucidate the largely unknown role of the H_2R in the central nervous system (CNS) [3,15]. Such molecules would be an important addition as counterparts to zolantidine (Figure 1), the only described CNS-penetrable H_2R antagonist until today [19]. In addition to this application, there are several studies discussing possible peripheral (acute myeloid leukemia) [14,20] and central (memory and learning) [21,22] therapeutic indications for H_2R agonists, although cardiac and gastric side effects should be considered. In particular, the effect on learning and memory occurs via the stimulation of postsynaptic H_2Rs, which so far have only been shown via the use of dual-acting acetylcholinesterase inhibitors and H_3R antagonists as these molecules initiate this process through inhibition of presynaptic H_3-autoreceptors [21,22]. For that reason, the use of CNS-penetrating H_2R agonists is of great interest.

Numerous publications with elaborate pharmacological studies and dedicated research were necessary to write the preceding story of success about the H_2R. In this regard, radiolabeled compounds played an important role for the examination of histamine receptor expression in the different tissues of the human body. Moreover, characterizations

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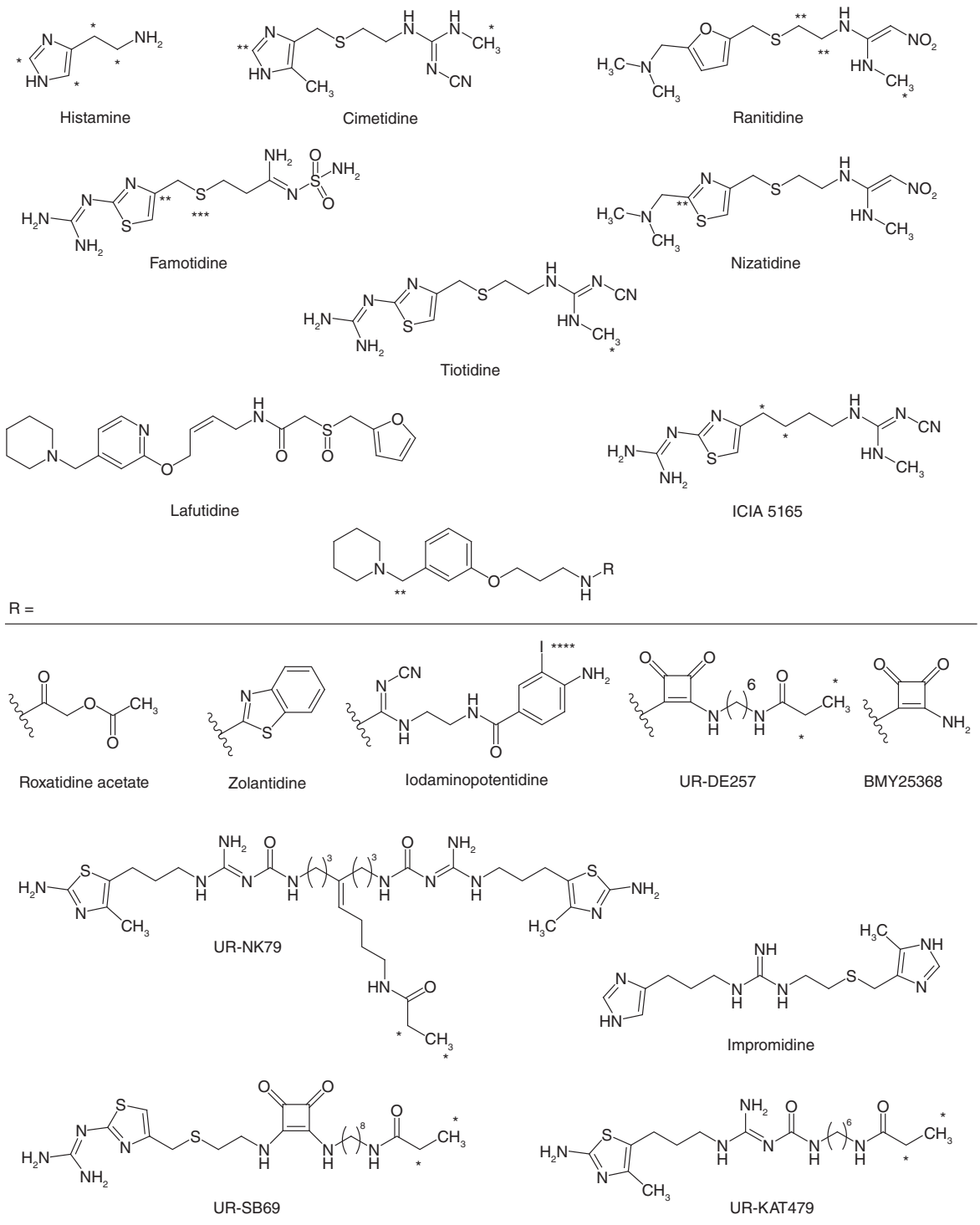

Figure 1. Structures of histamine H $_2$ receptor radioligands and the precursor BMY25368. For impromidine and lafutidine, the exact location of the tritium label was not indicated in the literature. BMY-25368 has not been synthesized in radiolabeled form.
*^{3}H-labeled position(s); **^{14}C-labeled position(s); ***^{35}S-labeled position; ****^{125}I-labeled position.

of ligand–receptor interactions and metabolic studies for potential drug candidates were facilitated. Although the number of publications of modern FRET/BRET-based methods for G-protein-coupled receptors (GPCRs) is increasing rapidly, radiolabeled compounds are still an integral part of the pharmacological repertoire of medicinal chemists.

Radiolabeling

In principle, there are several ways to introduce a radioisotope into a molecule. First, a decision has to be made on the choice of the isotope. Due to the high prevalence of hydrogen and carbon in organic compounds, their isotopes – namely, tritium (^{3}H) and carbon-14 (^{14}C) – are the first to be considered for labeling. On the other hand, compounds with radioactive isotopes of phosphorus (^{32}P, ^{33}P), sulfur (^{35}S) and iodine (^{125}I) are less frequently encountered. Except for the gamma emitter iodine-125, all isotopes listed here are beta emitters (negative beta decay).

The decisive factor for the choice of the specific radioisotope is the type of study which should be performed. It is of particular importance whether the radioligand should be applied for a preliminary *in vitro* characterization in the early phase of drug development or whether the drug candidate is already in a preclinical phase and a metabolic study (e.g., human ADME study) is required. Therefore, depending on the purpose, a thorough assessment of the situation must be made to decide which labeling is the right one. Excellent guidance to make a decision between ^{3}H- and ^{14}C-labeling is provided in the review article by Joel A Krauser, which served as a reference for some of the following points [23]. When comparing the radiochemical properties of the two isotopes, it is obvious that tritium has a significantly higher specific activity (^{3}H: 28.7 Ci/mmol vs ^{14}C: 0.0624 Ci/mmol) is cheaper to acquire and more readily available. In addition, the synthesis of tritiated compounds is much simpler since nowadays the labelling reagents are more easily to handle. Whereas tritium gas was used almost exclusively in the past, highly reactive *N*-hydroxysuccinimide (NHS) esters are now available, most of which containing a tritiated alkylic side chain. Because of their nongaseous state, these NHS esters can be used easily with exact stoichiometry delivering very high yields due to their reactivity. A disadvantage compared with tritium gas is certainly the dependence of commercially available tritiated side chains of NHS esters, which may not be compatible with the structure of an already existing drug candidate. In addition to the significantly lower acquisition costs, the lower half-life (^{3}H: 12.32 vs ^{14}C: 5730 years) also allows simpler and more economical management of radioactive waste disposal. All these aspects provide reasons to use tritiated compounds in the early phase of drug development, which is why academia works almost exclusively with [^{3}H]radioligands. Although ^{3}H-labeled compounds possess high radiochemical stability, there is a high risk of biological degradation by oxidative cleavage of the ^{3}H label mediated by cytochrome P450 enzymes. Because ^{14}C is integrated into the molecular scaffold, there is a reduced risk of direct label cleavage, which is beneficial for metabolic studies. Furthermore, the isotopic signature of ^{14}C can be analyzed and detected very specifically by mass spectrometry. Conversely, the aforementioned metabolic instability of ^{3}H-labeled compounds inevitably negates their use in such studies. Accordingly, the revealed profile of carbon-14 labeling leads to the fact that studies with ^{14}C-labeled compounds can be predominantly found in the literature in preclinical studies of the pharmaceutical industry. Nevertheless, one should not blindly follow this classification but rather make a detailed and individual evaluation when choosing the right label related to the respective project. Compounds labeled with ^{35}S are rare, certainly also due to the low sulfur content in drugs. However, the application of this isotope is often found in functional GPCR assays, in which [^{35}S]GTPγS is used. ^{35}S is also a beta emitter and possesses a much lower half-life of 87.1 days compared with ^{3}H and ^{14}C. The isotopes ^{32}P and ^{33}P are mainly used to label guanosine-5′-triphosphate (GTP) in functional assays, for example, the [γ-^{32}P]-GTPase or [γ-^{33}P]-GTPase assay, respectively. Both isotopes have extremely short half-lives (^{32}P: 14.3 and ^{33}P: 25.4 days) but show significant differences in decay energy. While phosphorus-32 has a comparatively high decay energy of 1.71 MeV, the energy beta emission of phosphorus-33 is only 0.25 MeV.

Radioligands for the histamine H_2 receptor

The most versatile molecular tools which successfully promoted pharmacological research on histamine receptors were radioligands. Therefore, in the early 1980s, the well-established H_2R antagonists [^{3}H]cimetidine and [^{3}H]ranitidine (Figure 1) were mainly employed [24–27]. However, the general drawback of these ligands was their comparatively low affinity for the H_2R (Table 1) despite their high efficacy [28–30]. In addition to the described effect at the H_2R, [^{3}H]cimetidine also exhibited considerable affinity for another imidazole recognition site, which precluded selective investigations of the H_2R [24,25]. Although [^{3}H]tiotidine (Figure 1) is routinely used in biological

Table 1. Binding data (pK_i values) of selected H_2R ligands at the human histamine H_2 receptor.

Compound	pK_i				
	hH_1R	hH_2R	hH_3R	hH_4R	Ref.
Histamine	4.6	5.1	8.2	8.1	[51]
Cimetidine	4.75	5.84	4.69	5.03	[51]
Ranitidine	4.47	6.67	4.89	<5	[51]
Famotidine	<5†	7.56	–	<5	[51]
Tiotidine	<4	7.77	<4‡	<5	[51]
Nizatidine	–	6.92§	–	–	[52]
Lafutidine	–	7.92¶	–	–	[53]
ICIA 5165	–	7.89#	–	–	[35]
Roxatidine acetate	–	7.41††	–	–	[54]
Zolantidine	5.1‡‡	7.40	–	–	[55,28]
Iodaminopotentidine	–	9.51	–	–	[29]
UR-DE257	<5	7.55	5.42	<5	[29]
BMY25368	–	7.72	4.66	–	[29,40]
UR-NK79	6.36	7.94	6.08	5.96	[14]
Impromidine	5.2	7.64	6.99	7.76	[51]
UR-SB69	<4	7.65	5.3	4.4	[40]
UR-KAT479	<5	7.59§§/7.78¶¶	4.91	<5	[17]

Binding data (pK_i) were determined at human H_xRs in different assay systems, unless otherwise stated.
†gpH_1R.
‡rH_3R.
§pK_d from measurements of CRE-SPAP production from CHO-H_2-SPAP cells in the presence of histamine.
¶Data (pK_i) from radioligand competition binding experiments performed in guinea pig cerebral cortex synaptic membrane with [^{3}H]tiotidine.
#Data (pK_i) from radioligand competition binding experiments performed at guinea pig gastric mucosa with [^{3}H]ICIA5165.
††H_2R antagonistic activity (pA_2) on the isolated guinea pig right atrium.
‡‡Data (pK_i) from competition binding experiments with [^{3}H]mepyramine performed at homogenates of guinea pig cerebral cortex.
§§Data (pK_i) from radioligand competition binding experiments performed on membrane preparations of Sf9 insect cells expressing the hH_2R-$G_{s\alpha S}$ with [^{3}H]UR-KAT479.
¶¶Data (pK_i) from radioligand competition binding experiments performed at HEK293T-hH_2R-qs5-HA cells with [^{3}H]UR-KAT479.

in vitro assays, it was reported to have a very high nonspecific binding and to address only a subpopulation of the H_2R [31,32]. Similar problems were faced in studies with the endogenous ligand [^{3}H]histamine (Figure 1), which was also found to have low affinity (Table 1) and high nonspecific binding [15,33]. Consequently, large quantities of radioligand had to be used to obtain meaningful results leading to higher risks to health and the environment, as well as inefficiency. Furthermore, [^{3}H]histamine does not exhibit any selectivity within the histamine receptor family showing even higher affinities at the H_3R and H_4R. This lack of selectivity automatically disqualified [^{3}H]histamine for selective studies at the H_2R [15]. The same disadvantage applied for [^{3}H]impromidine (Figure 1), rendering this agonist unsuitable as an appropriate H_2R radioligand, as well [34]. Unfortunately, also radiolabeling of the antagonist [^{3}H]ICIA 5165 (Figure 1), an analog of tiotidine, did not lead to any significant improvements over the drawbacks already mentioned [35].

In the 1990s, some experiments with ^{125}I-labeled compounds were performed, resulting in the versatile H_2R antagonist [^{125}I]iodoaminopotentidine (Figure 1) [28,36,37]. Thus, [^{125}I]iodoaminopotentidine constituted a potent radioligand at the H_2R (Table 1), which could also be used for autoradiography experiments in primates (human/nonhuman brain) and rodents (brain/heart) [36,37]. Its most obvious disadvantage is, however, the use of the iodine-125 isotope (gamma emitter), which involves significantly higher safety precautions during the preparation and usage of the radioligand, compared with tritiated compounds. Moreover, due to the short half-life of the isotope (59.5 days) [38], the radioligand can only be used for 4–6 weeks after preparation [29]. To improve the handling and the safety aspects, the tritiated compound [^{3}H]UR-DE257 (Figure 1) was synthesized, which exhibited a new structural motif with the squaramide modification derived from H_2R antagonist BMY25368 (Figure 1 & Table 1) [29]. [^{3}H]UR-DE257 was very well suited to determine pK_i values but turned out to be an insurmountable antagonist in functional and kinetic assays, which limited its applicability as a pharmacological tool [29]. The radioligand also possesses high affinity to histamine H_2 receptor orthologs (rat, guinea pig) beside the human H_2R [29]. Furthermore, [^{3}H]UR-DE257 could be successfully employed for autoradiography of the heart sections of transgenic mice overexpressing the human histamine H_2 receptor [39]. The synthesis of [^{3}H]UR-SB69

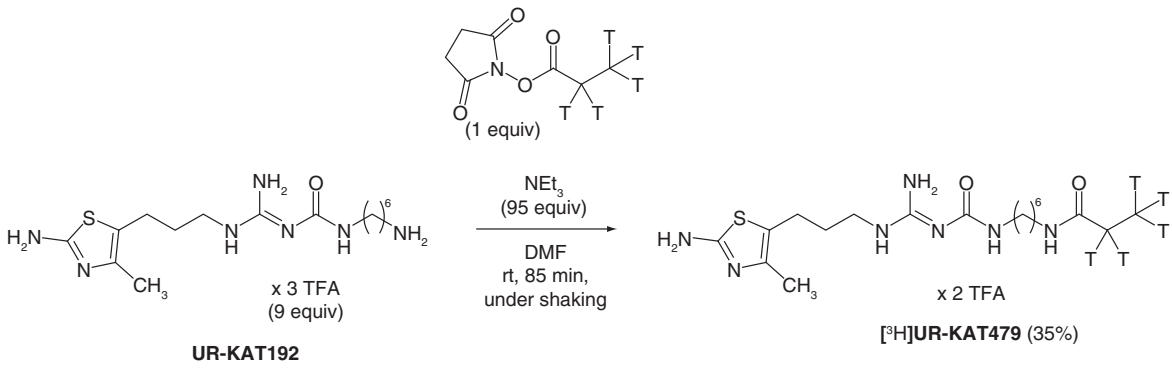

Figure 2. Synthesis of [^{3}H]UR-KAT479 by propionylation of precursor UR-KAT192 with *N*-succinimidyl [2,3-^{3}H]propionate. Radiochemical yield 35%.
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(Figure 1 & Table 1), a structural combination of the two antagonists famotidine (Figure 1 & Table 1) and UR-DE257, also failed to remedy this situation [40]. In addition to high nonspecific binding, complete displacement of the radioligand could not be achieved in dissociation experiments in this case either [40]. Furthermore, a long-term stability study showed that after 15 months, approximately 50% of the radioligand were already degraded [40]. To circumvent these problems, several attempts were made to radiolabel high affinity H_2R agonists with different structural features. A first experiment, starting from the agonistic dimer UR-NK22, resulted in [^{3}H]UR-NK79 (Figure 1 & Table 1) [14]. However, also this ligand did not bind in a saturable manner and showed high non-specific binding in saturation binding experiments [14]. Furthermore, confocal microscopy studies with fluorescent ligands of similar structure indicated that such compounds tend to a pronounced, receptor-independent cellular accumulation [14]. However, since some observations from Kagermeier *et al.* indicated an improved kinetic behavior, investigations of monomeric carbamoylguanidine-containing amines as promising precursors for H_2R radioligands were performed. This led to the development of the highly stable high affinity agonist [^{3}H]UR-KAT479 (Figures 1 & 2, & Table 1), a G protein-biased H_2R radioligand with a very good selectivity within the histamine receptor family [17]. In addition to significantly improved kinetics (including complete dissociation from the receptor), [^{3}H]UR-KAT479 showed excellent properties in saturation and competition binding assays [17]. Furthermore, its applicability was demonstrated on H_2R orthologs (guinea pig, mouse) by reporting pK_i values of standard H_2R ligands in whole cells for the first time [17]. Its G protein bias also eliminates the need for specifically adjusted assay conditions (e.g., hypotonic buffers), which can be used to prevent receptor internalization. Finally, the investigated low binding of [^{3}H]UR-KAT479 to red blood cells provides a promising basis for the use of unlabeled carbamoylguanidine-type ligands in animal studies to further explore the role of the H_2R in the CNS [17].

In addition to the application of radioligands in basic research, potential H_2R drug candidates were also investigated in preclinical studies with regard to their pharmacokinetic properties. In this case, the literature mainly consists of studies with the marketed H_2R antagonists. As discussed at the beginning, the selection of the label plays a central role, which is why publications with ^{14}C-labeled compounds are found almost exclusively for metabolic studies. The first work on this topic was published in the early 1980s, studying the excretion and metabolism of [^{14}C]cimetidine in male volunteers [41]. Extensive studies with urinary and fecal samples revealed a comprehensive picture of the metabolic spectrum of cimetidine showing that more than 70% of the ^{14}C was excreted in the urine after 24 h and 5% in the feces [41]. Unchanged cimetidine was the largest urinary component (63%), followed by cimetidine *N*′-glucuronide (24%) [41]. In addition to the synthesis of ^{35}S- and ^{14}C-labeled famotidine [42], metabolic studies with orally administered [^{14}C]famotidine in man have also been published [43]. Also in this case, urinary and fecal recovery were specifically investigated [43]. Again, renal excretion was the major route of elimination with a recovery of unchanged famotidine of approximately 67% after intravenous administration [43]. The bioavailability averaged 43% of the applied famotidine dose [43]. An interesting *ex vivo* animal model is provided by the isolated perfused kidney in rats, in which the effect of cationic drugs on the renal secretion of tritiated ranitidine ([^{3}H]ranitidine) was examined [44]. The results indicate that at clinically relevant concentrations the renal tubular secretion of ranitidine is inhibited by trimethoprim, but not by amantadine, pseudoephedrine or triamterene [44]. Besides the classical pharmacokinetic profile in human, pharmacodynamic and hormonal effects of

healthy and renally or hepatically impaired patients were also studied with [^{14}C]nizatidine [45]. Nizatidine showed high bioavailability (>90%) with more than 90% being recovered in urine within 16 h of dosing [45]. The drug did not alter hormone concentrations in plasma; however, renal impairment decreased the elimination of nizatidine [45]. Further studies deal with the secretion in human breast milk [46], as well as the localization of the active drug in the parietal cells of gastric mucosa, also here using [^{14}C]nizatidine [47]. The disposition of nizatidine was similar in lactating and nonlactating women with less than 0.1% of the maternal dose being secreted into milk during a 12-h interval after either single or multiple doses [46]. Furthermore, nizatidine was confirmed to be retained as an unchanged drug in the gastric mucosa, supporting the duration of pharmacological effects of nizatidine [47]. Among structurally novel H_2R antagonists, metabolic studies with [^{3}H]- and [^{14}C]roxatidine acetate [48], as well as autoradiographic studies of [^{3}H]lafutidine on CGRP-immunoreactive nerves and gastric parietal cells are reported [49]. Roxatidine acetate was almost completely absorbed after oral administration (>95%) and was rapidly converted to roxatidine, its major active plasma and urinary metabolite [48], whereas autoradiographic studies have shown that lafutidine effector sites coincided with the CGRP-immunoreactive nerves as well as the parietal cells [49].

In addition to the dominant number of pharmacokinetic reports, CNS permeability studies using radiolabeled H_2R antagonists have also been performed. Foremost among these is the study by Young *et al.* that attempts to establish a working model for the passage of histamine H_2 receptor antagonists across the blood–brain barrier, where [^{14}C]zolantidine (Table 1), [^{14}C]cimetidine, [^{14}C]ranitidine and [^{3}H]tiotidine, among others, were used [50].

Conclusion & future perspective

The aim of this special report was to provide an overview of the use of radioligands at the H_2R over the past five decades. Although a lot of progress was made in the field of H_2R radioligands, some challenges remain. For example, a recently published study suggests that ligands with similar structural features as [^{3}H]UR-KAT479 possess a high affinity for dopamine receptors of the D_2-like family, specifically for the dopamine D_3 receptor [18]. This drawback might hamper a possible application in autoradiography experiments.

Even though H_2R agonists have not yet found their way on the drug market, research in this field remains exciting. As described earlier, there are several unanswered questions regarding the human histamine H_2 receptor that relate to effects in both the periphery (acute myeloid leukemia) and the CNS (memory and learning), emphasizing the need for the development of appropriate pharmacological tools in this area. Continued efforts in the field of H_2R research might eventually lead to the discovery of an agonistic drug for therapeutic purposes, although some studies with regard to bioavailability and blood–brain barrier permeability of the compounds are still pending.

Executive summary

Radiolabeling

- ^{3}H and ^{14}C isotopes are most frequently used for the labeling of potential drug candidates.
- ^{3}H labeling is mainly used in preliminary *in vitro* characterization, while ^{14}C labeling is mainly required for metabolic/pharmacokinetic studies (preclinical/clinical phase).

Radioligands for the histamine H_2 receptor

- ^{3}H-labeled H_2R antagonists like [^{3}H]cimetidine, [^{3}H]ranitidine or [^{3}H]tiotidine display comparatively low affinity and high nonspecific binding at the H_2R.
- Squaramide-type H_2R antagonist [^{3}H]UR-DE257 with high affinity and lower non-specific binding but insurmountable antagonism.
- Development of the G protein-biased radioligand [^{3}H]UR-KAT479 (H_2R agonist) led to improved kinetic binding properties, beside high affinity and very good receptor subtype selectivity.
- Recent H_2R study shows high affinities of carbamoylguanidine-type agonists such as [^{3}H]UR-KAT479 at dopamine receptors of the D_2-like family (mainly D_3R).
- Pharmacokinetic studies of marketed H_2R antagonists are mainly performed with ^{14}C-labeled radioligands by pharmaceutical industry in preclinical/clinical stage.

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