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N-Alkenyl and cycloalkyl carbamates as dual acting histamine H_3 and H_4 receptor ligands

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ABSTRACT

Previous studies have shown that several imidazole derivatives posses affinity to histamine H_3 and H_4 receptors. Continuing our study on structural requirements responsible for affinity and selectivity for H_3/H_4 receptor subtypes, two series of 3-(1*H*-imidazol-4-yl)propyl carbamates were prepared: a series of unsaturated alkyl derivatives (**1**–**9**) and a series possessing a cycloalkyl group different distances to the carbamate moiety (**10**–**13**). The compounds were tested for their affinities at the human histamine H_3 receptor, stably expressed in CHO-K1 cells. Compounds **1**, **2**, **5**–**7**, **10**–**13** were investigated for their affinities at the human histamine H_4 receptor co-expressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$ subunits in Sf9 cells. To expand the pharmacological profile, compounds were further tested for their H_3 receptor antagonist activity on guinea pig ileum and in vivo after oral administration to mice. All tested compounds exhibited good affinity for the human histamine H_3 receptor with K_i values in the range from 14 to 194 nM. All compounds were active in vivo after peroral administration (p.o.) to Swiss mice, thus demonstrating their ability to cross the blood-brain barrier. The most potent H_3 receptor ligand of these series was compound **5**, 3-(1*H*-imidazol-4-yl)propyl pent-4-enylcarbamate with the highest affinity (K_i = 14 nM). Additionally, compound **3** showed remarkable central nervous system (CNS) H_3R activity, increasing the N^{τ}-methylhistamine levels in mice with an ED_{50} value of 0.55 mg/kg, p.o. evidencing therefore, a twofold increase of inverse agonist/antagonist potency compared to the reference inverse agonist/antagonist thioperamide. In this study, the imidazole propyloxy carbamate moiety was kept constant. The different lipophilic moieties connected to the carbamate functionality in the eastern part of the molecule had a range of influences on the human H_4 receptor affinity (154–1326 nM).

1. Introduction

Histamine has been found to exert tremendous influence over variety of physiological and pathophysiological processes. It mediates its effects through binding to four, so far known histamine receptor subtypes, designated H_1 to H_4 (H_1R–H_4R). All of them belong to the family of G-protein-coupled receptors, and are differentially expressed in various cell types. The H_1R and H_2R antagonists are for many years in clinical use, in the treatment of allergic conditions and gastric ulcers, respectively. The potential clinical applications for ligands of H_3R and the H_4R are being studied intensively. The H_3R initially described in 1983 was found to be a constitutively active receptor mostly expressed in the brain.[1,2] H_3Rs are identified as presynaptic autoreceptors, regulating the synthesis and the release of histamine, as well as heteroreceptors on non-histaminergic neurons controlling the release of many other important neurotransmitters, such as acetylcholine, norepinephrine, dopamine and serotonin.[3] Given their localization and their ability to affect multiple neurotransmitter systems it is supposed that H_3R antagonists/inverse agonists could be useful for the treatment of central nervous system (CNS) disorders such as Alzheimer's disease, schizophrenia, attention-deficit and hyperactivity disorder (ADHD), narcolepsy, pain and obesity.[4–6]

The H_4R is the latest identified receptor belonging to the histamine receptor family. The human H_3R and H_4R have about 31% sequence homology (54% in the transmembrane domains) and display similar genomic structures.[7] In contrast to these structural similarities, the expression patterns of both receptors strongly differ. Whereas the H_3R is predominantly localized in the brain, the H_4R is expressed mainly in the peripheral tissues and cells of the immune system.[7] Distinct expression patterns of H_4R on various

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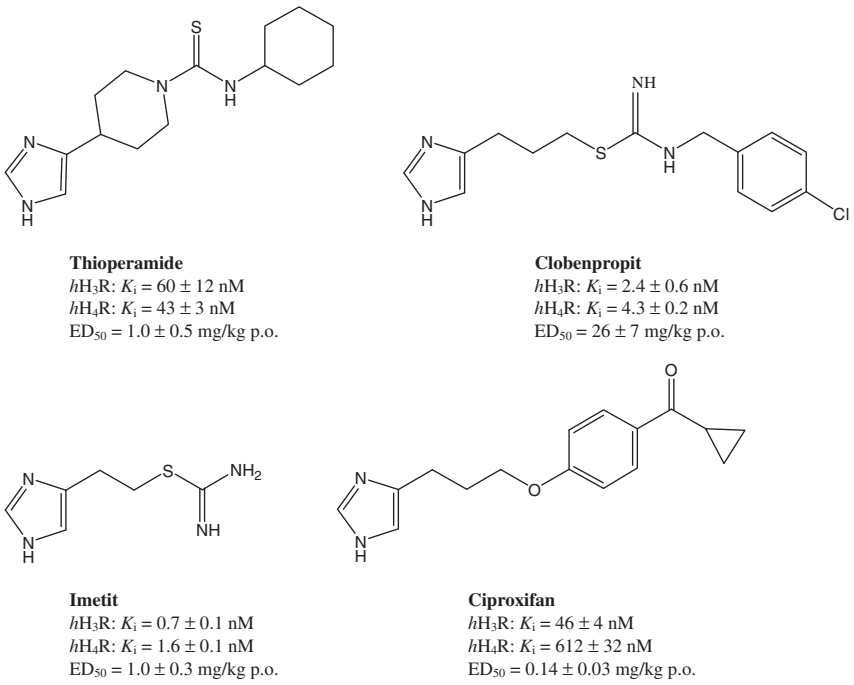


Figure 1. Structures and pharmacological data of some reference imidazole-containing histamine H_3R ligands (all K_i values are taken from Ref. 32, ED_{50} values are taken from Ref. 20 for thioperamide and clobenpropit, Ref. 37 for imetit and from Ref. 47 for ciproxifan).

hematopoietic cells such as mast cells, basophils, eosinophils, T-cells and dendritic cells suggests it has an important role in the regulation of immune responses and inflammation.[6,8]

Histamine H_3R inverse agonists/antagonists belong to two general groups: imidazole containing compounds and non-imidazole derivatives.[4,9–11] Due to some structural similarities between human H_3R and H_4R, it is not surprising that numerous imidazole containing H_3R ligands, that is, clobenpropit, imetit and thioperamide, have also significant affinity for the human H_4R (Fig. 1). Many dual-acting H_3R and H_4R ligands have been successfully used as reference compounds and pharmacological tools in H_3R and H_4R research. It is supposed that such compounds with hybrid structures showing dual H_3R and H_4R antagonistic activity could possess therapeutic profile in the therapy of pain and cancer.[6]

While the current medicinal chemistry efforts are mainly focused on selective acting GPCRs ligands, and particularly on histamine H_3 and H_4 selective agonists/antagonists, there are also several efforts to develop dual acting H_3/H_4 compounds.[12–19] The identification of novel selective H_4R ligands is still of interest to enlarge the knowledge of the pharmacological profile of the hH4R and distinct ligand-receptor interactions. Tritium-labeled N^1-[3-(1*H*-imidazol-4-yl)propyl]-N^2-propionylguanidine ([^{3}H]UR-PI294), was discovered as a high-affinity histamine H_3 and H_4 receptor radioligand.[12] Cyclopropane-based conformationally restricted analogs of 4-methylhistamine were designed and investigated as histamine H_3/H_4 receptor ligands. Pharmacological profiles of these analogs were shown to be different depending on the stereochemical character of the cyclopropane backbone and potent H_3 and/or H_4 receptor antagonists with low nanomolar K_i values were identified.[13,14] N^G-Acylated imidazolyl-propylguanidines, originally described several years ago as H_2R agonists[15–17] were shown to display greater activity at H_3R and H_4Rs.[18] Recently, Lim et al.[19] has described a series of clobenpropit analogs as dual activity ligands for the histamine H_3R and H_4Rs. These compounds showed moderate to high affinity for both the human H_3R and H_4R but different intrinsic activity at the H_4R from neutral antagonism to full agonism.[19] General structures of dual acting H_3/H_4R ligands are shown in Fig 2.

In our studies, we focused on the synthesis and pharmacological evaluation of 3-(1*H*-imidazol-4-yl)propyl carbamates. This structural element had been intensively characterized as a potential H_3R antagonist pharmacophore in different series of imidazole

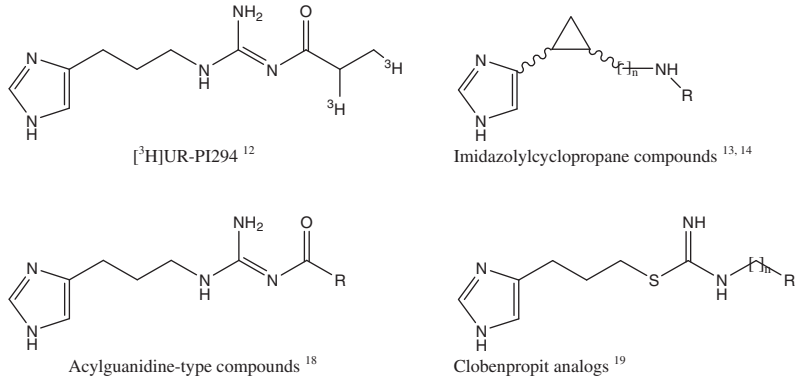


Figure 2. General structures of high-affinity histamine H_3 and/or H_4R ligands.

containing ligands.[20–24] Recently, we reported about an expanded structure affinity/efficacy relationship (SAR) of branched alkyl carbamates of 3-(1*H*-imidazol-4-yl)propanol, that showed both high H_3R antagonist activity and improved H_4R affinity.[25] To continue the investigation on dual H_3/H_4 receptor affinity and the structural requirements for affinity at the H_3R and H_4R, we introduced different substituents in the eastern part of the molecule. Slight structural variations on sterically restricted alkenyl and cycloalkyl residues caused subtle differences in ligand-receptor interactions that might influence the H_3R and H_4R potencies.

This paper describes the synthesis and pharmacological evaluations of two series of 3-(1*H*-imidazol-4-yl)propyl carbamates: a series of unsaturated alkyl derivatives (**1**–**9**) and a series possessing a cycloalkyl group at variable distances to the carbamate moiety (**10**–**13**) (Table 1)

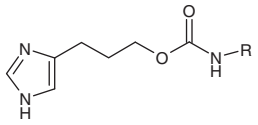
2. Results

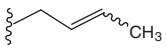
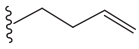
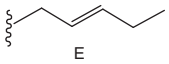
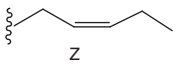
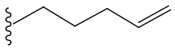
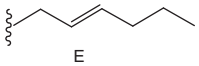
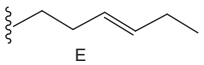
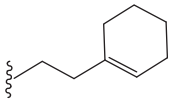
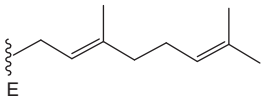
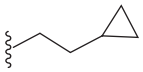
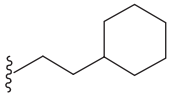
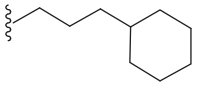
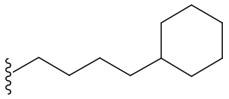
2.1. Chemistry

3-(1*H*-imidazol-4-yl)propanol was the key intermediate for all of the novel synthesized carbamates. It was prepared starting from urocanic acid in its trityl-protected and deprotected form as described by Stark et al.[20] Carbamates **1**, **4–6** and **9–13** were prepared by the method described earlier[20,21,23] from appropriate amines by their reaction with excess of diphosgene, forming an intermediate isocyanate, which subsequently reacted with 3-(1*H*-imidazol-4-yl)propanol hydrochloride to furnish the desired products (Scheme 1, route 1).

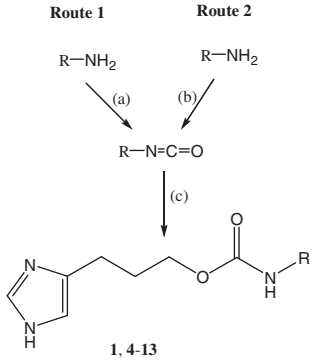
Compounds **2** and **3** were synthesized from the corresponding (commercially available) carboxylic acids by modified Curtius

Table 1
Structures and pharmacological results of compounds **1**–**13**

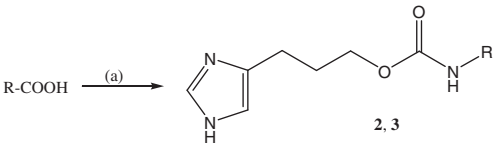


Compounds	R	ED_{50}[a] (mg/kg p.o.)	IC_{50}[b] (nM)	hH_3R K_i[d] (nM)	hH_4R K_i[e] (nM)	Selectivity ratio hH_4R/hH_3R
1		3.8 ± 0.6	128	54	1053 ± 123	20
2		2.7 ± 1.0	68	55	378 ± 14	7
3		0.55 ± 0.16	51	27	nt	
4		1.4 ± 0.7	52	27	nt	
5		2.3 ± 0.6	nt[c]	14	421 ± 72	30
6		0.56 ± 0.06	nt	24 ± 5	703 ± 54	29
7		1.9 ± 0.8	nt	26 ± 6	1326 ± 76	51
8		1.3 ± 0.3	29	26	nt	
9		7.3 ± 1.4	nt	20 ± 5	nt	
10		1.4 ± 0.8	nt	17	155 ± 24	9
11		3.5 ± 1.4	33	25	154 ± 15	6
12		4.6 ± 2.2	43	86	447 ± 41	5
13		4.7 ± 1.6	32	194	500 ± 105	2.5

[a] Central H_3R test in vivo: measurement of N^{τ}-methylhistamine in brain after oral administration to mouse[37] mean ± SEM.
[b] H_3R assay on guinea pig ileum[35,36] (number of experiments (*n*): for compd **2**–**4** *n* = 6; for compd **1** *n* = 7, for compd **10** *n* = 8).
[c] Not tested.
[d] Affinity of the test compounds determined by displacement of [^{125}I]iodoproxyfan binding to membranes of CHO-K1 cells expressing the human H_3R,[31] data from a single experiment with each concentrations tested at least in triplicate, except for compounds **6**, **7** and **9** with mean ± SEM of three independent experiments.
[e] Affinity of the test compounds determined by displacement of [^{3}H]histamine binding to membranes of Sf9 cells expressing the human H_4R, co-expressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$ subunits,[33,34] mean ± SD of at least three independent experiments.



Scheme 1. Synthesis of carbamates **1**, **4**–**13**. Reagents and conditions: (a) $ClCOOCCl_3$, charcoal (cat.), ethyl acetate, 4–5 h reflux; (b) di-*tert*-butyldicarbonate, DMAP, acetonitrile, 10 min, rt; (c) 3-(1*H*-imidazol-4-yl)propanol·HCl, acetonitrile, 4–5 h reflux (for route 1), 24 h reflux (for route 2).



Scheme 2. Synthesis of carbamates **2** and **3**. Reagents and condiotns: (a) DPPA, Et_3N, 3-(1*H*-imidazol-4-yl)propanol·HCl, acetonitrile, 24 h reflux.

reaction. In this reaction, the carboxylic acid was reacted with diphenyl phosphorazidate (DPPA) under basic conditions (TEA), leading to an intermediate carboxylic acid azide, which is converted by nitrogen liberation to the corresponding isocyanate.[26,27] These in situ formed isocyanates were reacted with 3-(1*H*-imidazol-4-yl)propanol hydrochloride yielding the appropriate carbamates (Scheme 2).

An alternative approach was used for the synthesis of compounds **7** and **8**. In these cases, the isocyanates were prepared under mild conditions by DMAP-catalyzed reaction of amines with di-*tert*-butyldicarbonate.[28,29] The isocyanates without isolation were added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride and converted into the appropriate carbamates (Scheme 1, route 2).

The synthesis and physicochemical data of compounds **5** and **10** were described previously.[23]

Compounds **2**, **3** and **8** were prepared from commercially available intermediates (carboxylic acids for **2** and **3** and amine for **8**). Non-commercially available amines (**1b**–**8b**) were prepared by hydrazinolysis of the corresponding N-substituted phthalimides and isolated as hydrochlorides (Scheme 3). N-Substituted phthalimides (**1a–8a**) were prepared using the Mitsunobu reaction, from appropriate alcohols and phthalimide in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine[30] (Scheme 3). The corresponding alcohols for the synthesis of these N-substituted phthalimides were commercially available.

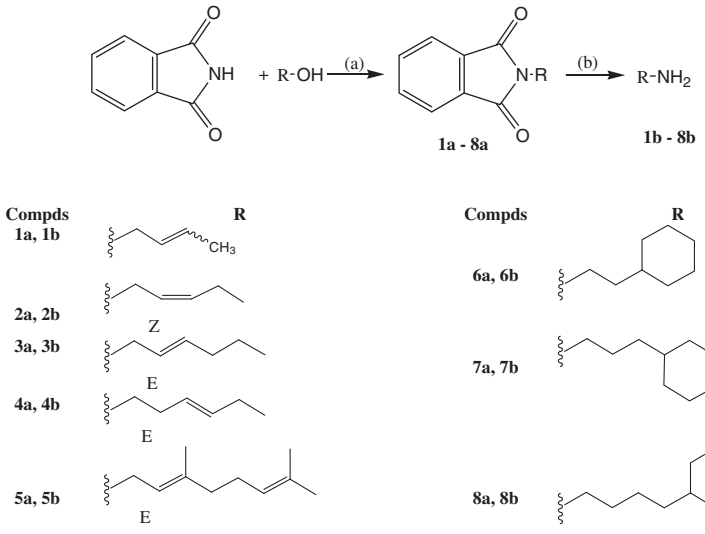
All final compounds **1–13** were isolated as hydrogen maleates. Their purity was checked by TLC, and their structures were confirmed by standard spectral techniques (^{1}H NMR, IR, MS) and elemental analysis (Section 5.1.)

2.2. Pharmacology

Human H_3R (hH_3R) affinities of the described compounds were evaluated by competition binding experiments with [^{125}I]iodoproxyfan on membranes of CHO-K1 cells stably expressing the hH_3R.[31,32] Compounds (**1**, **2**, **5**–**7**, **10**–**13**) were further tested regarding their affinities at human H_4R (hH_4R) by a [^{3}H]histamine replacement assay on membranes of Sf9 cells, co-expressing the hH_4R with $G\alpha_{i2}$ and $G\beta_1\gamma_2$ subunits.[33,34] In addition, these ligands were characterized for H_3R antagonist activity with a functional in vitro test on the isolated guinea-pig ileum (compd **1**–**4**, **8**, **11**–**13**) and in vivo in the brain of Swiss mice. The in vitro potency was determined by the concentration-dependent inhibition of electrically evoked twitches of guinea pig ileum by (*R*)-α-methylhistamine in the presence of test compound.[35,36] The in vivo tests were performed by quantification of endogenous N^{τ}-methylhistamine level in the brain of Swiss mice after oral (p.o.) treatment with test compounds.[37] Selected compounds (**1**, **2**, **4**, **8**, **11**–**13**) were additionally investigated for their affinities at H_1R and H_2R on isolated organs of the guinea-pig.[38] Results of pharmacological screening are summarized in Tables 1 and 2.

3. Pharmacological effects and discussion

All tested compounds exhibited histamine H_3/H_4 receptor affinity. Good to moderate affinity was observed for the hH_3R with K_i values in the range from 14 to 194 nM and moderate to weak affinity for the hH_4R (K_i values from 154 to 1326 nM). Among carba-



Scheme 3. Synthesis of N-substituted phthalimides **1a**–**8a** and amines **1b**–**8b**. Reagents and conditions: (a) Ph_3P, DEAD, THF, 20 h, rt; (b) H_2N–NH_2, EtOH, 3 h reflux; HCl concd.

Table 2
Activity of selected compounds at histamine H_1 and H_2 receptor subtypes

Compounds	H_1R pA_2[a]	H_2R pD_2[b]
1	4.16	3.75
2	<4.0	<4.0
4	4.1	3.85
8	4.33	4.35
11	3.96	4.49
12	4.97	4.94
13	<5.52	4.61

[a] Functional H_1R assay on guinea-pig ileum (SEM < 0.2).[38]
[b] Functional H_2R assay on guinea-pig atrium (SEM < 0.2).[38]

mates possessing an unsaturated moiety (**1**–**9**), the *h*H_3R affinity depended mainly on the chain length and on the position of unsaturated double bond. The weakest affinity is exhibited by butenyl derivatives (**1** and **2**) and elongation of the chain length to five, six and even eight carbon atoms or the introduction of bulky cyclohexenylethyl substituent led to about 2–3-fold increase of in vitro affinity. The highest affinity is shown by the 4-pentenyl derivative **5** (K_i = 14 nM) and 2,6-octadienyl derivative **9** (K_i = 20 nM).

Subtle differences in lipophilic alkenyl residue in compounds **1**, **2**–**9** and **3**–**5** caused distinct changes in *h*H_4R affinity, leading to reduced affinities in the submicromolar concentration range. Compounds **10** and **11** with an ethyl chain between cycloalkyl and carbamate moiety showed the highest affinity for both *h*H_3R and *h*H_4Rs. Elongation of the carbon chain between carbamate group and the cyclohexyl ring resulted in a decrease of H_3R and H_4R affinities (**11**→**12**→**13**).

In addition, compounds were evaluated for their antagonist activity for H_3R in the functional in vitro test in the guinea pig ileum (selected compounds) and in vivo in mouse brain after p.o. administration. All compounds investigated showed moderate H_3R in vitro antagonist activities. Compounds with a cyclohexyl moiety showed the highest potency, independently of its distance from the carbamate moiety (**11**–**13**) or the existence of double bond (**8**). Carbamates with a short unsaturated chain (**1**–**4**) exhibited somewhat lower activity in this in vitro model. However, compounds showed moderate to good inverse agonist/antagonist in vivo potency with ED_{50} values in the range from 0.55 to 7.3 mg/kg p.o. In the group of unsaturated derivatives the activity was dependent on the chain length and on the place of unsaturated bond. In our series of compounds, the optimal chain length was 5–6 carbon atoms. *N*-Butenyl derivatives (**1** and **2**) showed slightly lower activity. Elongation of the chain to octenyl (**9**) moiety resulted in significant decrease of the in vivo potency. Activity in this group of compounds was determined also by the position of unsaturated bond. Presented data indicated that β-position was preferred over δ and γ position, respectively (**3**, **4**→**5**; **6**→**7**, **8**). Nevertheless, the most actives among investigated compounds were *E*-isomers (**3**, **6**) which exhibited an in vivo potency about twofold higher than the potency of the reference inverse agonist/antagonist thioperamide (ED_{50} 1.0 mg/kg p.o.). Comparison of the in vivo activity of these compounds with previously described by our group their saturated analogs[21] indicated, that straight *n*-alkyl chains at the carbamate nitrogen were more beneficial to activity than the corresponding to them rigid unsaturated ones. Only *N*-allyl derivative (ED_{50} = 0.54 mg/kg p.o.)[23] was about fivefold more active than its *N*-*n*-propyl analog (ED_{50} = 2.7 mg/kg p.o.).[21] The *E*-isomers of the five- and six-membered alkenyl chains with double bond in β position (compounds **3** and **6**) showed the potency in the same range as their saturated versions (ED_{50} = 0.69 mg/kg and 0.88 mg/kg, respectively[21]). Cyclohexylalkyl carbamates showed moderate in vivo activity with ED_{50} ranging from 3.5 to 4.7 mg/kg p.o. In the group of cyclohexyl derivatives the change of the alkyl linker between the carbamate group and the cyclohexyl moiety from 1 (compound described in lit. 21) to 4 carbon atoms was without the significant influence on the in vivo potency, but compound devoid of this linker showed significantly lower activity (ED_{50} > 10 mg/kg).[20]

Selected compounds (**1**, **2**, **4**, **8**, **11**–**13**) were additionally tested for their H_1R activity on guinea-pig ileum as well as for their H_2R potency on guinea-pig atrium[38] (Table 2). All tested compounds showed low affinity for these receptors with pA_2 values below 5.0 (except of **13**, pA_2 <5.52 for H_1R).

4. Conclusions

As a continuation of the search for 3-(1*H*-imidazol-4-yl) propanol carbamates with dual histamine H_3R/H_4R affinity, unsaturated alkyl derivatives (**1**–**9**) and those possessing a cycloalkyl group in different distances to the carbamate moiety (**10**–**13**) were synthesized. Tested compounds exhibited good affinity for the *h*H_3R and moderate to weak affinity for the *h*H_4R. These results indicate that restricted alkenyl analogs may provide greater selectivity for H_3R over H_4R. For example, the hex-3-enyl analog (compound **7**) was 51-fold selective for the H_3R over the H_4R.

5. Experimental

5.1. Chemistry

Melting points were determined on a MEL-TEMP II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian-Mercury 300 MHz spectrometer or on a Bruker AC 300 (300 MHz) in DMSO-d_6 or $CDCl_3$. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane as reference. Data are reported in the following order: multiplicity (br, broad; def, deformed; s, singlet; d, doublet; dq, doublet of quartets; t, triplet; m, multiplet; qu, quintet; q, quartet; sc, sextet; Cyhexl, cyclohexyl; Im, imidazol-4-yl; Mal, maleic acid; Pht, phthalimide; Ph, phenyl), approximate coupling constants *J* in Hertz (Hz), number of protons, ∗, exchangeable by D_2O. Mass spectra (MS) were obtained on an EI-MS Finnigan MAT CH7A and Finnigan MAT 711 (high-resolution mass spectra). Spectrometers resolving power 12,500. Elemental analyses were measured on Perkin–Elmer 240 B or Perkin–Elmer 240 C instruments and are within ±0.4% of the theoretical values. Preparative, centrifugally accelerated, rotatory chromatography was performed using a Chromatotron 7924T (Harrison Research) and glass rotors with 4 mm layers of Silica Gel 60 PF_{254} containing gypsum (Merck). Column chromatography was performed using Silica Gel 60 (0.063–0.20 mm; Merck). TLC was carried out using Silica Gel F_{254} plates (Merck), and the spots were visualized with fast blue salt BB (Aldrich) (carbamates **1**–**13**), ninhydrin (amines **1b**–**8b**) or by UV absorption at 254 nM (phthalimides **1a**–**8a**). The following abbreviations are used: DEAD, diethyl azodicarboxylate; DMAP, 4-dimethylaminopyridine; DPPA, diphenyl phosphoryl azide; TEA, triethylamine.

5.1.1. General synthetic procedure for the preparation of *N*-substituted phthalimides (1a–8a)

The N-substituted phthalimides were prepared according to literature.[30] A mixture of phthalimide (2.21 g, 15 mmol), triphenylphosphine (3.93 g, 15 mmol), and appropriate alcohol (15 mmol) in 20 mL of dry THF was cooled to 0 °C. Diethyl azodicarboxylate (DEAD) (2.61 g, 15 mmol) in 15 mL of dry THF was slowly added dropwise (1 h). The reaction mixture was then allowed to warm to room temperature and stirred overnight. Solvent was evaporated under reduced pressure and the residue suspended in Et_2O.

After the precipitate was filtered, the solvent was evaporated and the residue purified by column chromatography [eluent CH_2Cl_2] to afford appropriate N-substituted phthalimide.

5.1.1.1. *N*-((*E*/*Z*)-But-2-enyl)phthalimide (1a). From (*E*/*Z*)-but-2-enol (crotyl alcohol) (15 mmol, 1.1 g). Yield: 73%, mp 75 °C (mp 77.5–79.5 °C, Ref. 39).

5.1.1.2. *N*-((*Z*)-Pent-2-enyl)phthalimide (2a). From (*Z*)-pent-2-enol (15 mmol, 1.3 g). Colorless oil. Yield: 91%. ^{1}H NMR [$CDCl_3$]: δ = 7.84–7.81 (m, 2H, Pht-3,6-*H*), 7.72–7.68 (m, 2H, Pht-4,5-*H*), 5.63–5.54 (m, 1H, N–CH_2–C*H*=CH), 5.46–5.38 (m, 1H, CH=C*H*–CH_2–CH_3), 4.31 (d, *J* = 5.8 Hz, 2H, N–CH_2), 2.27 (qu, *J* = 7.4 Hz, 2H, CH_2–CH_3), 1,03 (t, *J* = 7.4 Hz, 3H, CH_3).

5.1.1.3. *N*-((*E*)-Hex-2-enyl)phthalimide (3a). From (*E*)-hex-2-enol (30 mmol, 3.0 g). Yield: 77%, mp 60–61 °C (described in Ref. 40).

5.1.1.4. *N*-((*E*)-Hex-3-enyl)phthalimide (4a). From (*E*)-hex-3-enol (30 mmol, 3.0 g). Yield: 81%, mp 49–50 °C (described in Ref. 40).

5.1.1.5. *N*-((*E*)-3,7-Dimethyl-2,6-octadienyl)phthalimide (5a). From (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol) (50 mmol, 7.7 g). Yield: 74%, mp 57–58 °C (described in Ref. 40).

5.1.1.6. *N*-[1-(2-Cyclohexyl)ethyl]phthalimide (6a). From 2-cyclohexylethanol (15 mmol, 1.92 g). Mp 48–51 °C, Yield: 75%. ^{1}H NMR [$CDCl_3$]: δ = 7.82 (m, 2H, Pht-3,6-*H*), 7,70 (m, 2H, Pht-4,5-*H*), 3.70 (t, *J* = 7.6 Hz, 2H, N–CH_2), 1.81–1.53 (m, 7H, Cyhexl-1,3,4,5-*H*), 1.37–1.10 (m, 4H, Cyhexl-2,6-*H*), 0.96 (q, *J* = 11.6 Hz, 2H, CH_2-Cyhexl); MS (70 eV); *m*/*z* (%) = 257 ([M$^+$], 92), 229 (10), 162 (21), 161 (100), 160 (84), 149 (25), 148 (28), 133 (18), 130 (22), 105 (17), 104 (19), 98 (24), 81 (16), 77 (22), 76 (15), 67 (15), 55 (31), 41 (30), 30 (11); IR (KBr) (cm^{-1}) 2922, 2849 *v* ([C–H] CH_2), 1710 *v* [C=O], 1396 δ ([C–H], *N*–CH_2)

Anal. Calcd for $C_{16}H_{19}NO_2$ (M_r: 257.33): C, 74.68; H, 7.44; N, 5.44. Found: C, 74.75; H, 7.56; N, 5.36.

5.1.1.7. *N*-[1-(3-Cyclohexyl)propyl]phthalimide (7a). From 3-cyclohexyl-1-propanol (15 mmol, 2.13 g). Yield: 85%, mp 47–48 °C (mp 51 °C, Ref. 41).

5.1.1.8. *N*-[1-(4-Cyclohexyl)butyl]phthalimide (8a). From 4-cyclohexyl-1-butanol (15 mmol, 2.34 g). Mp 86–88 °C, yield: 79%. ^{1}H NMR [$CDCl_3$]: δ = 7.89–7.82 (m, 2H, Pht-3,6-*H*), 7.72–7.64 (m, 2H, Pht-4,5-*H*), 3.67 (t, *J* = 7.3 Hz, 2H, N–CH_2), 1.68–1.58 (m, 7H, Cyhexl-1,3,4,5-*H*), 1.38–1.30 (m, 2H, N–CH_2–CH_2) 1.25–1.07 (m, 6H, Cyhexl-2,6-*H* + CH_2–CH_2-Cyhexl), 0.96 (q, *J* = 10.0 Hz, 2H, CH_2-Cyhexl); MS (70 eV); *m*/*z* (%) = 285 ([M$^+$], 100), 202 (6), 175 (13), 174 (17), 162 (16), 161 (63), 160 (78), 148 (22), 133 (17), 130 (17), 105 (15), 104 (12), 83 (8); IR (KBr) (cm^{-1}) 2925, 2849 *v* ([C–H] CH_2), 1704 *v* [C=O], 1400 δ ([C–H], *N*–CH_2)

Anal. Calcd for $C_{18}H_{23}NO_2$ (M_r: 285.17): C, 75.76; H, 8.12; N, 4.91. Found: C, 75.37; H, 8.00; N, 4.77.

5.1.2. General synthetic procedure for the preparation of amines (1b–8b)

Appropriate N-substituted phthalimide (5 mmol) and hydrazine hydrate (5 mmol, 0.3 g) in 20 mL of EtOH were refluxed for 3 h. The suspension was cooled, filtered, acidified with hydrochloric acid, and once more filtered. The filtrate was concentrated under reduced pressure and the products were crystallized as hydrochloride salts from Et_2O and used for further synthesis.

5.1.2.1. (*E*/*Z*)-But-2-enamine hydrochloride (1b). From **1a** (5 mmol, 1.0 g). Mp 110–112 °C. Yield: 98%. ^{1}H NMR [DMSO-d_6]: δ = 8.24 (br s, 3H, NH_3^+), 5.87–5.76 (m, 1H, C*H*=CH–CH_3), 5.60–5.49 (m, 1H, CH=C*H*–CH_3), 3.37–3.31 (m, 2H, N–CH_2), 1.70–1.67 (dq, *J* = 6,5 Hz, *J* = 1,7 Hz, 3H, CH_3), FAB-MS *m*/*z* (%): 72 ([M+H]$^+$, 100), 63 (16), 56 (11), 55 (90), 39 (20), 30 (12), 29 (19), 27 (12)

5.1.2.2. (*Z*)-Pent-2-enamine hydrochloride (2b). From **2a** (13 mmol, 2.8 g). Mp 134–135 °C. Yield: 74%. ^{1}H NMR [DMSO-d_6]: δ = 8.08 (br s, 3H, NH_3^{+*}), 5.69–5.62 (m, 1H, N–CH_2–C*H*=C), 5.44–5.38 (m, 1H, C=C*H*–CH_2–CH_3), 3.48 (d, *J* = 7.1 Hz, 2H, N–CH_2), 2.07 (qu, *J* = 7.4 Hz, 2H, CH_2CH_3), 0.95 (t, *J* = 7,5 Hz, 3H, CH_3); MS (70 eV); *m*/*z* (%) = 84 ([M−H]$^+$, 4), 70 (12), 68 (40), 67 (19), 56 (100); IR (KBr) (cm^{-1}) 3406, 3017 *v* ([N–H], 2972 *v* ([C–H] CH_2), 1477 δ ([C–H], N–CH_2).

5.1.2.3. (*E*)-Hex-2-enamine hydrochloride (3b). From **3a** (20 mmol, 4.6 g). Mp 180–185 °C. Yield: 48%. ^{1}H NMR [DMSO-d_6]: δ = 8.06 (br s, 3H, NH_3^+), 5.83–5.73 (m, 1H, N–CH_2–C*H*=CH), 5.52–5.43 (m, 1H, CH=C*H*–CH_2–CH_2), 3.34 (d, *J* = 6.6 Hz, 2H, N–CH_2), 1.98 (q, *J* = 6.9 Hz, 2H, CH_2–CH_2–CH_3), 1.35 (sc, *J* = 7.4 Hz, 2H, CH_2-CH_3), 0.84 (t, *J* = 7.4 Hz, 3H, CH_3)

5.1.2.4. (*E*)-Hex-3-enamine (4b). From **4a** (23 mmol, 5.3 g). After concentration under reduced pressure the residue was alkalized with 20 mL of 10% KOH and extracted with Et_2O. The organic solution was dried (Na_2SO_4), filtered and after concentration the crude product was used. Yield: 44%.

5.1.2.5. (*E*)-3,7-Dimethyl-2,6-octadienyl amine hydrochloride (geranylamine hydrochloride) (5b). From **5a** (33 mmol, 8.5 g). Yield: 68%, mp 155–160 °C (mp 146 °C, Ref. 42).

5.1.2.6. 2-Cyclohexylethanamine hydrochloride (6b). From (**6a**) (10 mmol, 2.57 g). Yield: 50%, mp 230 °C (mp 253–254 °C, Ref. 43).

5.1.2.7. 3-Cyclohexylpropanamine hydrochloride (7b). From (**7a**) (10 mmol, 2.71 g). Yield: 59%, mp 215 °C (mp 224–228 °C, Ref. 43).

5.1.2.8. 4-Cyclohexylbutanamine hydrochloride (8b). From (**8a**) (10 mmol, 2.85 g). Yield: 86%, mp 160–162 °C (mp 165 °C, Ref. 41).

5.1.3. General procedure for the synthesis of carbamates (1, 4–6, 9–13)

To the solution of trichloromethyl chloroformate (0.6 g, 3 mmol) and the catalytic amount of activated charcoal in 20 mL of dry ethyl acetate was added rapidly the corresponding amine hydrochloride (2.5 mmol) in 10 mL of dry ethyl acetate. The reaction mixture was heated to reflux for 4–5 h, the black solution was cooled and filtered, and the solvent was evaporated carefully under reduced pressure. The freshly prepared isocyanate was dissolved in 20 mL of dry acetonitrile and added to a mixture of 3-(1*H*-imidazol-4-yl)propanol hydrochloride (2.5 mmol, 0.4 g) in 10 mL of dry acetonitrile. The solution was refluxed for 4–5 h and concentrated in vacuo. The residue was purified by rotatory chromatography [eluent: CH_2Cl_2/MeOH (gradient from 99:1 to 90:10), ammonia atmosphere]. Separation was monitored by TLC. The products were obtained as colorless oils and crystallized as hydrogen maleates in Et_2O/EtOH.

5.1.3.1. (*E*/*Z*)-3-(1*H*-Imidazol-4-yl)propyl but-2-enylcarbamate hydrogen maleate (1). From (**1b**) (2.5 mmol, 0.27 g). Yield 18.8%. Mp 96 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.85 (s, 1H, Im-2-*H*),

7.39 (s, 1H, Im-5-*H*), 7.21 (s, 1H, CON*H**), 6.04 (s, 2H, Mal), 5.54–5.36 (m, 2H, *HC*=*CH*), 3.97 (t, *J* = 6.4 Hz, 2H, *CH*$_2$O), 3.53 (br s, 2H, N–*CH*$_2$), 2.67 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 1.88 (qu, *J* = 7.2 Hz, 2H, Im-CH$_2$*CH*$_2$), 1.62 (d, *J* = 6.4 Hz, 3H, *CH*$_3$); FAB-MS; *m/z* (%) = 224 ([M+H]$^+$, 100), 185 (20), 127 (13),109 (57), 95 (6), 93 (52), 82 (11), 81 (10), 75 (18), 57 (14); IR (KBr) (cm^{-1}): 1701 (*ν*[C=O]). Anal. Calcd for $C_{11}H_{17}N_3O_2 \times C_4H_4O_4 \times 0.25\ H_2O$ (M_r: 343.85): C, 52.40; H, 6.30; N, 12.22. Found: C, 52.62; H, 6.11; N, 12.27.

5.1.3.2. (*Z*)-3-(1*H*-Imidazol-4-yl)propyl pent-2-enylcarbamate hydrogen maleate (4). From (**2b**) (3.0 mmol, 0.36 g). Yield 11.4%. Mp 89 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.83 (s, 1H, Im-2-*H*), 7.38 (s, 1H, Im-5-*H*), 7.21 (s, 1H, CON*H**), 6.04 (s, 2H, Mal), 5.44–5.38 (m, 1H, C*H*=CH), 5.31–5.25 (m, 1H, CH=C*H*), 3.97 (t, *J* = 6.4 Hz, 2H, *CH*$_2$O), 3.61 (t, *J* = 5.4 Hz, 2H, N–*CH*$_2$), 2.66 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 2.03 (qu, *J* = 7,4 Hz, 2H, CH$_3$*CH*$_2$), 1.88 (qu, *J* = 7.2 Hz, 2H, Im-CH$_2$*CH*$_2$), 0.92 (t, *J* = 7.5 Hz, 3H, *CH*$_3$). FAB-MS *m/z* (%) = 238 ([M+H]$^+$, 41), 127 (23),109 (100), 95 (9), 82 (17), 81 (22), 63 (9); IR (KBr) (cm^{-1}): 1696 (*ν*[C=O]). Anal. Calcd for $C_{12}H_{19}N_3O_2 \times C_4H_4O_4$ (M_r: 353.37): C, 54.38; H, 6.56; N, 11.89. Found: C, 54.17; H, 6.56; N, 11.88.

5.1.3.3. Synthesis and physicochemical data of compound 5. It was described previously.[23]

5.1.3.4. (*E*)-3-(1*H*-Imidazol-4-yl)propyl hex-2-enylcarbamate hydrogen maleate (6). From (**3b**) (5 mmol, 0.7 g). Yield 36.1%. Mp 82 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.81 (s, 1H, Im-2-*H*), 7.36 (s, 1H, Im-5-*H*), 7.20 (s, 1H, CON*H*), 6.01 (s, 2H, Mal), 5.54–5.31 (m, 2H, C*H*=C*H*), 3.94 (t, *J* = 6.3 Hz, 2H, *CH*$_2$O), 3.55 (t, *J* = 5.5 Hz, 2H, N–*CH*$_2$), 2.64 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 1.98–1.84 (m, 4H, CH=CH–*CH*$_2$ + Im-CH$_2$*CH*$_2$), 1.30 (sc, *J* = 7.4 Hz, 2H, *CH*$_2$–CH$_3$), 0.83 (t, *J* = 7.4 Hz, 3H, *CH*$_3$). MS (70 eV); *m/z* (%) = 251 ([M]$^+$, 14), 109 (37), 108 (100), 107 (37), 98 (19), 95 (64), 82 (31), 81 (59), 80 (10), 72 (21), 69 (15); IR (KBr) (cm^{-1}): 1700 (*ν*[C=O]). Anal. Calcd for $C_{13}H_{21}N_3O_2 \times C_4H_4O_4$ (M_r: 367.41): C, 55.58; H, 6.86; N, 11.44. Found: C, 55.36; H, 6.64; N, 11.32.

5.1.3.5. (*E*)-3-(1*H*-Imidazol-4-yl)propyl 3,7-dimethylocta-2,6-dienylcarbamate hydrogen maleate (9). From (**5b**) (2.5 mmol, 0.47 g). Yield 10.8%. Mp 68–70 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.80 (s, 1H, Im-2-*H*), 7.35 (s, 1H, Im-5-*H*), 7.12 (s, 1H, CON*H*), 6.02 (s, 2H, Mal), 5.11–5.05 (m, 2H, 2 × C*H*=C), 3.94 (t, *J* = 6.6 Hz, 2H, *CH*$_2$O), 3.57–3.53 (m, 2H, N–*CH*$_2$), 2.65 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 2.02–1.84 (m, 6H, Im-CH$_2$*CH*$_2$ + *CH*$_2$–*CH*$_2$–CH=C(CH$_3$)$_2$), 1.61–1.52 (m, 9H, 3 × CH$_3$). MS (70 eV); *m/z* (%) = 305 ([M]$^+$, 20), 237 (16), 183 (6), 127 (15), 109 (100), 108(52), 107 (24), 98 (31), 95 (50), 82 (34), 81 (51), 80 (13), 71 (10), 69 (36); IR (KBr) (cm^{-1}): 1698 (*ν*[C=O]). Anal. Calcd for $C_{17}H_{27}N_3O_2 \times C_4H_4O_4 \times 1.25\ H_2O$ (M_r: 443.74): C, 56.84; H, 7.61; N, 9.47. Found: C, 56.86; H, 7.74; N, 9.49.

5.1.3.6. Synthesis and physicochemical data of compound 10. It was described previously.[23]

5.1.3.7. 3-(1*H*-Imidazol-4-yl)propyl 2-cyclohexylethylcarbamate hydrogen maleate (11). From (**6b**) (2.5 mmol, 0.40 g). Yield 24.2%. Mp 107–108 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.84 (s, 1H, Im-2-*H*), 7.38 (s, 1H, Im-5-*H*), 7.03 (s, 1H, CON*H**), 6.04 (s, 2H, Mal), 3.96 (t, *J* = 6.4 Hz, 2H, *CH*$_2$O), 2.98 (q, *J* = 6.6 Hz, 2H, N–*CH*$_2$), 2.66 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 1.88 (qu, *J* = 7.2 Hz, 2H, Im-CH$_2$*CH*$_2$), 1.66–1.59 (m, 5H, Cyhexl-1,2,6-*H*), 1.31–1.07 (m, 6H, Cyhexl-3,4,5-*H*), 0.85 (q, *J* = 10.5 Hz, 2H, CH$_2$-cyclohex). MS (70 eV); *m/z* (%) = 279 ([M]$^+$, 9), 109 (25), 108 (100), 107 (26), 95 (54), 82 (20), 81 (40), 80 (8); IR (KBr) (cm^{-1}): 1704 (*ν*[C=O]). Anal. calcd for $C_{15}H_{25}N_3O_2 \times C_4H_4O_4 \times 0.5\ H_2O$ (M_r: 404,46): C, 56.42; H, 7.48; N, 10.39. Found: C, 56.80; H, 7.18; N, 10.36.

5.1.3.8. 3-(1*H*-Imidazol-4-yl)propyl 2-cyclohexylpropylcarbamate hydrogen maleate (12). From (**7b**) (2.5 mmol, 0.44 g). Yield 53.9%. Mp 95–96 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.90 (s, 1H, Im-2-*H*), 7.40 (s, 1H, Im-5-*H*), 7.06 (t, *J* = 5.4 Hz, 1H, CON*H**), 6.05 (s, 2H, Mal), 3.96 (t, *J* = 6.4 Hz, 2H, *CH*$_2$O), 2.92 (q, *J* = 6.4 Hz, 2H, N–*CH*$_2$), 2.68 (t, *J* = 7.5 Hz, 2H, Im-*CH*$_2$), 1.89 (qu, *J* = 7.2 Hz, 2H, Im-CH$_2$*CH*$_2$), 1.65 (d, *J* = 11.6 Hz, 5H, Cyhexl-1,2,6-*H*), 1.37 (q, *J* = 7.4 Hz, 2H, *CH*$_2$–CH$_2$-Cyhexl), 1.22–1.08 (m, 6H, Cyhexl-3,4,5-*H*), 0.82 (q, *J* = 10.3 Hz, 2H, *CH*$_2$-Cyhexl). MS (70 eV); *m/z* (%) = 293 ([M]$^+$, 9), 109 (25), 108 (100), 107 (27), 95 (60), 82 (21), 81 (36); IR (KBr) (cm^{-1}): 1699 (*ν*[C=O]). Anal. Calcd for $C_{16}H_{27}N_3O_2 \times C_4H_4O_4 \times 0.25\ H_2O$ (M_r: 413,98): C, 58.03; H, 7.67; N, 10.15. Found: C, 57.91; H, 7.44; N, 10.03.

5.1.3.9. 3-(1*H*-Imidazol-4-yl)propyl 2-cyclohexylbutylcarbamate hydrogen maleate (13). From (**8b**) (2.5 mmol, 0.48 g). Yield 37.7%. Mp 112–113 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.83 (s, 1H, Im-2-*H*), 7.38 (s, 1H, Im-5-*H*), 7.05 (t, *J* = 5.3 Hz, 1H, CON*H**), 6.04 (s, 2H, Mal), 3.96 (t, *J* = 6.5 Hz, 2H, *CH*$_2$O), 2.94 (q, *J* = 6.2 Hz, 2H, N–*CH*$_2$), 2.66 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 1.88 (qu, *J* = 7.1 Hz, 2H, Im-CH$_2$*CH*$_2$), 1.63 (d, *J* = 12.0 Hz, 5H, Cyhexl-1,2,6-*H*), 1.35 (q, *J* = 7.2 Hz, 2H, NH–CH$_2$–*CH*$_2$–), 1.27–1.05 (m, 8H, Cyhexl-3,4,5-*H* + CH$_2$–*CH*$_2$–CH$_2$-Cyhexl), 0.83 (q, *J* = 10.0 Hz, 2H, *CH*$_2$-Cyhexl). MS (70 eV); *m/z* (%) = 307 ([M]$^+$, 9), 109 (26), 108 (100), 107 (25), 98 (13), 95 (65), 82 (19), 81 (42); IR (KBr) (cm^{-1}): 1698 (*ν*[C=O]).

Anal. Calcd for $C_{17}H_{29}N_3O_2 \times C_4H_4O_4$ (M_r: 423,51): C, 59.56; H, 7.85; N, 9.92. Found: C, 59.41; H, 7.80; N, 9.88.

5.1.4. Synthesis of carbamates by modified Curtius reaction (2 and 3)

5.1.4.1. 3-(1*H*-Imidazol-4-yl)propyl but-3-enylcarbamate hydrogen maleate (2). The mixture of 4-pentenoic acid (2.5 mmol, 0.25 g), triethylamine (2.5 mmol, 0.25 g), diphenylphosphoryl azide (2.5 mmol, 0.69 g), and 3-(1*H*-imidazol-4-yl)propanol hydrochloride (2.5 mmol, 0.4 g) was refluxed for 24 h in 20 mL of dry acetonitrile. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate and the white precipitate was filtered. The filtrate was washed twice with saturated K_2CO_3 and the organic fraction after concentration was purified by rotatory chromatography [eluent: AcOEt/*i*-PrOH (60:40). Separation was monitored by TLC. Obtained colorless oil was crystallized as hydrogen maleate in Et$_2$O/EtOH. Yield 43.5%. Mp 95 °C. ^{1}H NMR [DMSO-d_6]: δ = 8.83 (s, 1H, Im-2-*H*), 7.37 (s, 1H, Im-5-*H*), 7.10 (s, 1H, CON*H**), 6.04 (s, 2H, Mal), 5.81–5.71 (m, 1H, C*H*=CH$_2$), 5.07–4.99 (m, 2H, CH=C*H*$_2$), 3.96 (t, *J* = 6.5 Hz, 2H, *CH*$_2$O), 3.02 (q, *J* = 6.3 Hz, 2H, N–*CH*$_2$), 2.66 (t, *J* = 7.4 Hz, 2H, Im-*CH*$_2$), 2.15 (q, *J* = 7.0 Hz, 2H, *CH*$_2$CH=CH$_2$), 1.88 (qu, *J* = 7.2 Hz, 2H, Im-CH$_2$*CH*$_2$); MS (70 eV); *m/z* (%) = 223 ([M$^+$], 17), 182 (17), 153 (5), 109 (64), 108 (100), 107 (40), 95 (85), 82 (26); IR (KBr) (cm^{-1}): 1696 (*ν*[C=O]).

Anal. Calcd for $C_{11}H_{17}N_3O_2 \times C_4H_4O_4$ (M_r: 339.35): C, 52.81; H, 6.24; N, 12.38. Found: C, 52.81; H, 6.23; N, 12.09.

5.1.4.2. (*E*)-3-(1*H*-Imidazol-4-yl)propyl pent-2-enylcarbamate hydrogen maleate (3). The mixture of (E)-3-hexenoic acid (2.5 mmol, 0.29 g), triethylamine (2.5 mmol, 0.25 g), diphenylphosphoryl azide (2.5 mmol, 0.69 g), and 3-(1*H*-imidazol-4-yl)propanol hydrochloride (2.5 mmol, 0.4 g) was refluxed for 24 h in 20 mL of dry acetonitrile. The solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane and washed successively with 5% citric acid, water and saturated solution of NaHCO$_3$. After concentration of the combined organic fractions, the residue was purified by rotatory chromatography

[eluent: CH_2Cl_2/MeOH (gradient from 99:1 to 90:10), ammonia atmosphere]. Separation was monitored by TLC. The product was obtained as colorless oil and crystallized as hydrogen maleate in Et_2O/EtOH. Yield 7.4%. Mp 94 °C. ^1H NMR [DMSO-d_6]: δ = 8.84 (s, 1H, Im-2-*H*), 7.37 (s, 1H, Im-5-*H*), 7.20 (s, 1H, CON*H**), 6.02 (s, 2H, Mal), 5.39–5.30 (m, 1H, C*H*=CH), 5.59–5.45 (m, 1H, CH=C*H*), 3.97 (t, *J* = 6.3 Hz, 2H, CH_2O), 3.61 (t, *J* = 5.5 Hz, 2H, N–CH_2), 2.66 (t, *J* = 7.4 Hz, 2H, Im-CH_2), 1.99–1.84 (m, 4H, CH_3CH_2 + Im-CH_2CH_2), 0.91 (t, *J* = 7.4 Hz, 3H, CH_3). FAB-MS *m/z* (%) = 238 ([M·+H]$^+$, 100), 237 (4), 185 (15), 127 (18), 109 (77), 95 (9), 82 (17); IR (KBr) (cm^{-1}): 1700 (*v*[C=O]). Anal. Calcd for $C_{12}H_{19}N_3O_2 \times C_4H_4O_4 \times H_2O$ (M_r: 371.40): C, 51.74; H, 6.79; N, 11.31. Found: C, 52.06; H, 6.37; N, 10.95.

5.1.5. General procedure for the synthesis of carbamates (7 and 8)

To a solution of di-*tert*-butyldicarbonate (7 mmol, 1.52 g) in 10 mL of anhydrous acetonitrile were added successively a solution of 4-dimethylaminopyridine (5 mmol, 0.62 g) in 10 mL of anhydrous acetonitrile and a solution of appropriate amine (free base) (5 mmol) in 5 mL of anhydrous acetonitrile. After stirring for 10 min at room temperature 3-(1*H*-imidazol-4-yl)propanol hydrochloride (5 mmol, 0.8 g) was added. The reaction mixture was refluxed for 24 h. The solvent was removed under reduced pressure, and the residue was alkalized by NH_3/MeOH and dissolved in dichloromethane. The precipitate was filtered and the filtrate was purified first by rotatory chromatography [eluent: CH_2Cl_2/MeOH (gradient from 99:1 to 90:10)], and next by column chromatography [eluent: CH_2Cl_2:MeOH:NH_3/MeOH (95:5:1)]. Separation was monitored by TLC. The products were obtained as colorless oils and crystallized as hydrogen maleates in Et_2O/EtOH.

5.1.5.1. (*E*)-3-(1*H*-Imidazol-4-yl)propyl hex-3-enylcarbamate hydrogen maleate (7). From (**4b**) (5 mmol, 0.5 g). Yield 12.8%. Mp 109–110 °C. ^1H NMR [DMSO-d_6]: δ = 8.79 (s, 1H, Im-2-*H*), 7.34 (s, 1H, Im-5-*H*), 7.00 (s, 1H, CON*H*), 6.01 (s, 2H, Mal), 5.43–5.34 (m, 2H, C*H*=C*H*), 3.94 (t, *J* = 6.3 Hz, 2H, CH_2O), 2.95 (q, *J* = 5.5 Hz, 2H, N–CH_2), 2.64 (t, *J* = 7.4 Hz, 2H, Im-CH_2), 1.98–1.84 (m, 4H, CH=CH–CH_2 + Im-CH_2CH_2), 1.30 (m, *J* = 7.4 Hz, 2H, CH_2–CH_3), 0.83 (t, *J* = 7.4 Hz, 3H, CH_3). MS (70 eV); *m/z* (%) = 251 ([M]$^+$, 26), 109 (85), 108 (100), 107 (29), 98 (16), 95 (55), 82 (25); IR (KBr) (cm^{-1}): 1692 (*v*[C=O]). Anal. Calcd for $C_{13}H_{21}N_3O_2 \times C_4H_4O_4$ (M_r: 367.41): C, 55.58; H, 6.86; N, 11.44. Found: C, 55.24; H, 7.19; N, 11.31

5.1.5.2. 3-(1*H*-Imidazol-4-yl)propyl 2-cyclohexenylethylcarbamate hydrogen maleate (8). From 2-(1-cyclohexenyl)ethylamine (2,5 mmol, 0.31 g). Yield 10.2%. Mp 85 °C. ^1H NMR [DMSO-d_6]: δ = 8.90 (s, 1H, Im-2-*H*), 7.40 (s, 1H, Im-5-*H*), 7.00 (s, 1H, CON*H*), 6.04 (s, 2H, Mal), 5.35 (s, 1H, C*H*=C), 3.94 (t, *J* = 6.6 Hz, 2H, CH_2O), 2.99 (q, *J* = 6.3 Hz, 2H, N–CH_2), 2.65 (t, *J* = 7.4 Hz, 2H, Im-CH_2), 1.99 (t, 2H, Im-CH_2CH_2), 1.87 (m, 6H, CH_2-Cyhexl + Cyhexl-3,6-*H*), 1.57–1.44 (m, 4H, Cyhexl-3,6-*H*). MS (70 eV); *m/z* (%) = 277 ([M]$^+$, 23), 183 (11), 127 (16), 109 (100), 108(32), 107 (13) 95 (25), 82 (28); IR (KBr) (cm^{-1}): 1691 (*v*[C=O]). Anal. Calcd for $C_{15}H_{23}N_3O_2 \times C_4H_4O_4 \times 0.5\ H_2O$ (M_r: 402,45): C, 56.71; H, 7.01; N, 10.44. Found: C, 56.41; H, 6.68; N, 10.37.

5.2. Pharmacology

5.2.1. In vitro [^{125}I]iodoproxyfan binding assay on *h*H_3R

Potency of the synthesized compounds was investigated in a radioligand binding assay described by Ligneau et al.[31] CHO-K1 cells stably expressing the *h*H_3R were washed and harvested with a PBS medium. They were centrifuged (140*g*, 10 min, +4 °C), and then homogenized with a Polytron in an ice-cold binding buffer (Na_2HPO_4/KH_2PO_4, *c* = 50 mM, pH 7.5). The homogenate was centrifuged (23,000*g*, 30 min, +4 °C) and the pellet obtained was resuspended in the binding buffer to constitute the membrane preparation used for the binding assay. For competition binding experiments, membrane suspension (5–15 μg protein/well) was incubated for 60 min at +25 °C with [^{125}I]iodoproxyfan (*c* = 25 pM) alone, or together with competing drugs dissolved in the same buffer to give a final volume of 200 μL. Incubations were performed in triplicate and stopped by four additions (5 mL) of ice-cold medium, followed by rapid filtration through glass microfibre filters (GF/B Whatman, Clifton, NJ) presoaked in polyethyleneimine (PEI) (ω = 0.3%). Radioactivity trapped on the filters was measured with a LKB (Rockville, MD) gamma counter (efficiency: 82%). Specific binding was defined as that inhibited by imetit (*c* = 1 μM), a specific histamine H_3 receptor agonist.[44] Assays were run in triplicates at least with 9 concentrations between 0.1 nM and 1 μM. The corresponding K_i values were determined from the IC_{50} value according to the Cheng–Prusoff equation.[45]

5.2.2. In vitro [^{3}H]histamine binding assay on *h*H_4R

Prior to the experiments, cell membranes were sedimented by a 10-min centrifugation at +4 °C and 16,000*g* and resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4). Competition binding experiments were carried out by incubating membranes, 35 μg/well (prepared from Sf9 cells expressing *h*H_4R, co-expressed with G- protein $G\alpha_{i2}$ and $G\beta_1\gamma_2$ subunits) in a final volume of 200 μL containing binding buffer and [^{3}H]histamine (10 nM, 566.1 GBq/mmol). Assays were run in triplicates with seven appropriate concentrations between 0.1 nM and 100 μM of the test compound. Incubations were performed for 60 min at +25 °C and shaking at 250 rpm. Non-specific binding was determined in the presence of 100 μM unlabeled histamine. Bound radioligand was separated from free radioligand by filtration through GF/B filters pretreated with 0.3% (mass/vol) PEI and washed three times with 0.3 mL of ice-cold binding buffer (+4 °C). The amount of radioactivity collected on the filter was determined by liquid scintillation counting. Competition binding data were analyzed by the software GraphPad Prism™ (2000, version 3.02, San Diego, CA, USA) using non-linear least squares fit. Affinity values (K_i) were expressed as mean ± standard deviation (SD). K_i values were calculated from the IC_{50} values according to Cheng–Prusoff equation.

5.2.3. Histamine H_3 receptor antagonist activity on guinea pig ileum

For selected compounds, H_3 receptor activity was measured by concentration-dependent inhibition of electrically evoked twitches of isolated guinea pig ileum segments induced by (*R*)-α-methylhistamine in the presence of the antagonist according to Ligneau et al.[44] Longitudinal muscle strips were prepared from the small intestine, 20–50 cm proximal to the ileocecal valve. The muscle strips were mounted between two platinum electrodes (4 mm apart) in 20 mL of Krebs buffer, containing 1 μM mepyramine, connected to an isometric transducer, continuously gassed with oxygen containing 5% CO_2 at 37 °C. After equilibration of the muscle segments for 1 h with washing every 10 min, they were stimulated continuously with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. After 30 min of stimulation, a cumulative concentration–response curve with increasing concentrations of (*R*)-α-methylhistamine was recorded. Subsequently the preparations were washed three times every 10 min without stimulation. Then, the antagonist was incubated for 20–30 min before redetermination of the dose–response curve of (*R*)-α-methylhistamine.[35,36] All compounds were tested in concentrations that did not block the ileal muscarinic M_3 receptors.

5.2.4. Histamine H_3-receptor antagonist potency in vivo in the mouse

In vivo testing was performed after oral administration of test compound to male Swiss mice according to Garbarg et al.[37] Brain histaminergic neuronal activity was assessed by measuring the level of the main metabolite of histamine, N^{τ}-methylhistamine. Mice were fasted for 24 h before p.o. treatment. Animals were decapitated 90 min after treatment, and the cerebral cortex was homogenized in 10 volumes (w/v) of ice-cold perchloric acid (0.4 M). The N^{τ}-methylhistamine level was measured by radioimmunoassay.[46] By treatment with 3 mg/kg ciproxifan the maximal increase in N^{τ}-methylhistamine level was obtained[47] and compared to that level reached with the administered drug. Dose–response curves were established with at least 6 mice per dose. The ED_{50} values were calculated as mean with SEM.[48]

5.2.5. In vitro screening at histamine H_1 and H_2 receptors

Selected compounds were screened for histamine H_2 receptor potency on the isolated spontaneously beating guinea pig right atrium as well as for H_1 receptor potency on the isolated guinea pig ileum by standard methods described by Hirschfeld et al.[38] Each pharmacological test was performed at least in triplicate. The values that are given represent means.

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Supplementary data

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