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Importance of the lipophilic group in carbamates having histamine H_3-receptor antagonist activity

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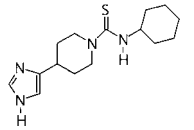
In order to evaluate changes in the lipophilic part of designed carbamates concerning their potential histamine H_3-receptor antagonist properties a new series of *O*-[3-(1*H*-imidazol-4-yl)propanol]carbamates was derived containing *N*-mono- or di-alkenyl, alkynyl, cycloalkyl, or double-branched alkyl substituents. The compounds were tested *in vitro* for their H_3-receptor antagonist activity on synaptosomes of rat cerebral cortex and shared moderate to high antagonist activity *in vitro*. In this series 3-(1*H*-imidazol-4-yl)propyl *N*-(4-pentenyl)carbamate (**4**) was the most potent compound *in vitro* (K_i = 6.3 nM). H_3-receptor antagonist activity in the central nervous system (CNS) was detected for most compounds in the *in vivo* H_3-receptor assay based upon measurement of brain N^{τ}-methylhistamine levels after p.o. administration to mice. The most effective carbamate *in vivo*, 3-(1*H*-imidazol-4-yl)propyl *N*-(allyl)carbamate (**3**), showed higher CNS potency (ED_{50} = 0.48 mg/kg p.o.) than the reference antagonist thioperamide. For some novel carbamates their histamine H_1- and H_2-receptor activities were determined on isolated organs of guinea-pig thereby demonstrating their high H_3-receptor selectivity.

1. Introduction

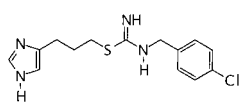
The existence of a third histamine receptor subtype, the so-called histamine H_3-receptor, was first reported in 1983 [1]. Histamine H_3-receptors are presynaptically located on histaminergic neurones, mainly in the central nervous system (CNS). Their activation causes inhibition of histamine synthesis in and histamine release from histaminergic neurones [1, 2]. Apart from their activity as autoreceptors H_3 receptors have also been found to act as heteroreceptors located on non-histaminergic neurones in the CNS [3] and to a minor extent in peripheral tissues [4–6], thus modulating the effect of a number of different neurotransmitters, e.g., dopamine [7], serotonin [8], acetylcholine [9], and noradrenaline [10]. Histamine H_3-receptor antagonists have not yet been used clinically, but therapeutic indications for H_3-receptor antagonists have been proposed for several diseases and conditions of the CNS like epilepsy [11, 12], cognitive and sleep disorders [13], memory and learning deficits [14, 15], and Alzheimer's disease [16, 17].

During the last few years several synthetic attempts have been carried out to develop potent and selective antagonists of the histamine H_3 receptor. The first prototypic agent to be reported was thioperamide, showing high potency *in vitro* as well as *in vivo* [18]. Clobenpropit was identified as even more potent *in vitro*, but less effective *in vivo* [19]. Neither thioperamide nor clobenpropit have been introduced into clinical trials, presumably due to hepatotoxicity caused by their thiourea or isothiourea moieties. In the course of further extensive studies a general construction pattern for H_3-receptor antagonists was evaluated [20]. It consists of a nitrogen-containing heterocycle connected to a polar moiety by an alkyl spacer, itself connected to a lipophilic residue directly or by another spacer. The particular elements of this model were recently checked leading to new efficient histamine H_3-receptor antagonists [21, 22]. Several compounds, possessing a carbamate group as the polar part of the moieties conforming to the general H_3-receptor antagonist pattern, were synthesized, and they showed high *in vitro* and *in vivo* activity [23–25]. The carbamate function was selected because it retains electronic properties similar to those of thioperamide and clobenpropit, but without possible toxic side effects due to sulphur-containing groups [20]. As a lipophilic element of this pattern a large variety of structurally different moieties was investigated with (un)substituted aryl, arylalkyl [23, 24] (type A), and (branched)alkyl substituents (type B) with, or without, heteroatomic (O, S) bridges as potential bioisosteric methylene group replacements [25].

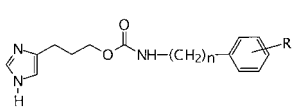
The lipophilic part is believed to enhance the activity of H_3-receptor antagonists since it plays an important role in modulating the physicochemical properties of the investigated compounds by enabling them to cross the blood-brain barrier. In order to further study the steric requirements of the lipophilic part of H_3-receptor antagonists a new series of carbamates was designed containing *N*-mono- or di-alkenyl, alkynyl, cycloalkyl, or double-branched alkyl substituents (Table 1). Their physicochemical properties were predicted theoretically [26]. All compounds were screened for their histamine H_3-receptor antagonist activity in functional tests on synaptosomes of rat cerebral cortex by measuring the amount of released tritium-labeled histamine [27]. Selected compounds were tested on the guinea-pig ileum, in a second functional his-



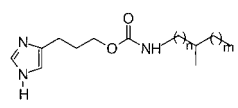
Thioperamide



Clobenpropit



Type A



Type B

tamine H_3-receptor assay [28]. Bearing in mind the potential therapeutic use of H_3-receptor antagonists, the CNS antagonist potency was determined *in vivo* after p.o. administration to Swiss mice [27]. The affinity for H_1 and H_2 histamine receptors was tested on the isolated spontaneously beating guinea-pig right atrium and the isolated guinea-pig ileum, respectively [29] (Table 2).

2. Investigations, results and discussion

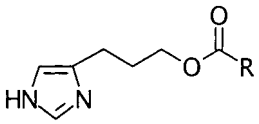
2.1. Chemistry

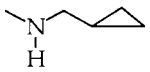
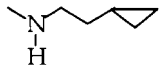
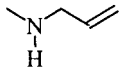
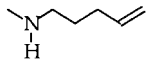
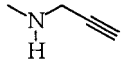
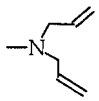
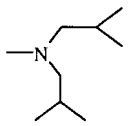
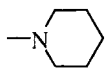
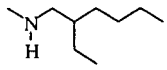
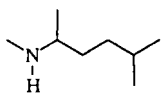
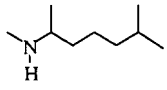
3-(1*H*-Imidazol-4-yl)propanol [31], used as starting material for the synthesis of all the described compounds, was prepared in a five-step synthesis starting from urocanic acid and consisting in its esterification, reduction of the double bond by hydrogenation over Pd/C [30], imidazole ring protection by tritylation followed by reduction of the ester functional group with complex hydrides. Prior to generation of the carbamate product the protecting trityl group was cleaved with acid [31].

For the synthesis of the *N*-monosubstituted carbamates **1** to **5** and **10**–**12** the corresponding isocyanates were used, while for the synthesis of the *N,N*-disubstituted carbamates **6**–**9** the appropriate carbamoyl chlorides were prepared (Scheme 2, Table 1).

The isocyanates were obtained from the corresponding amines by reaction with an excess of trichloromethyl chloroformate (diphosgene), a liquid substitute for phos-

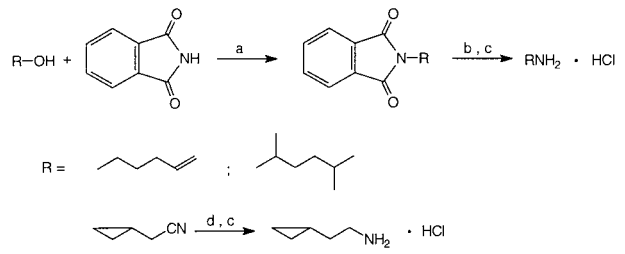
Table 1: Structures, physicochemical properties, and results of the screening on histamine H_3-receptor antagonist potency *in vitro* and *in vivo* on rodents



Compd.	R	Calculated value		K_i[a] (nM)	ED_{50}[b] (mg/kg)
		log P	log D	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$
1		1.05	0.96	52 ± 16[c]	>10[c]
2		1.56	1.47	10 ± 2	1.4 ± 0.8
3		0.84	0.75	85 ± 27	0.54 ± 0.17
4		1.86	1.77	6.3 ± 1.6	2.3 ± 0.6
5		0.33	0.24	131 ± 33	~10
6		1.49	1.41	51 ± 15	~10
7		2.20	2.11	64 ± 10	3.0 ± 1.2
8		2.79	2.70	72 ± 17	5.2 ± 3.2
9		1.28	1.19	61 ± 17	3.1 ± 1.2
10		3.53	3.44	n.d.[d]	8.1 ± 2.2
11		3.04	2.95	n.d.	3.4 ± 0.6
12		3.55	3.46	22 ± 5	1.6 ± 0.8
Thioperamide		1.83	1.73	4 ± 1[e]	1.0 ± 0.5[c]
Clobenpropit		3.02	−0.09	0.6 ± 0.1[f]	26 ± 7[c]

[a] functional H_3-receptor assay *in vitro* on synaptosomes of rat cerebral cortex [27]; [b] central H_3-receptor test *in vivo* after p.o. administration to mice [27]; [c] reference [24]; [d] n.d. = not determined; [e] reference [18]; [f] reference [35].

Scheme 1



(a) DEAD, triphenylphosphine, THF; (b) H_2NNH_2, EtOH; (c) HCl, EtOH; (d) Na, toluene, dodecanoic acid, EtOH.

Table 2: Activity of selected compounds at histamine receptor subtypes

Compd.	H_3		H_2	H_1
	pK_i[a]	pK_B[b]	pK_B[c]	pK_B[d]
1	7.3	7.3		
2	8.0		~3.5	3.8
3	7.1	6.6	<4.0	4.0
4	8.2		3.6	3.9
5	6.9	6.4	<4.3	<4.0
7	7.2	7.2	3.5	4.1
8	7.1		3.5	4.7
11			≤4.7	4.5
12	7.7		4.8	4.8

[a] functional H_3-receptor assay on synaptosomes of rat cerebral cortex [27]; [b] functional H_3-receptor assay on guinea-pig ileum [28, 34]; [c] functional H_2-receptor assay on guinea-pig right atrium [29]; [d] functional H_1-receptor test on guinea-pig ileum [29].

gene. The intermediate isocyanates were carefully separated by distillation from the excess of diphosgene in order to avoid reaction of diphosgene with the alcohol functionality; then they were added to 3-(1*H*-imidazol-4-yl)propanol·HCl. The use of 3-(1*H*-imidazol-4-yl)propanol in the form of its hydrochloride salt reduced likely side reactions (the protonated imidazolium ring being attacked to a smaller extent by the isocyanate, thus resulting in increased yields [28]). In the preparation sequence of **3** and **5** isolation of the isocyanates was not possible because their boiling points were close to that of diphosgene. In this case the crude reaction mixture containing the isocyanate was added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride in acetonitrile. An attempt to avoid this inconvenience by using an excess of phosgene solution in toluene (synthesis of compound **11**) instead of diphosgene did not, however, increase the yield of the final product. Compounds **1**, **3**, **5**, **10**, and **12** were obtained from commercially available amines. Amines for the synthesis of **4** and **11** were prepared starting with the appropriate alcohols (Scheme 1). Alcohols were transformed in a Mitsunobu protocol-adapted Gabriel synthesis into the corresponding *N*-alkylphthalimides [32] and subsequently, by means of hydrazinolysis, into the desired amines, which were isolated as hydrochlorides. The amine for synthesis of **2** was isolated as hydrochloride after Bouveault-Blanc reduction of cyclopropylacetonitrile by means of hydrogenation with a sodium/ethanol system [33]. The reaction was carried out in toluene with a catalytic amount of dodecanoic acid used as emulsifier.

The carbamoyl chlorides needed for the synthesis of the *N,N*-disubstituted carbamates **6**–**9** were obtained by heating a toluene solution of the corresponding commercially available amine with an excess of diphosgene. The carbamoyl chlorides in toluene solution were treated with 3-(1*H*-imidazol-4-yl)propanol·hydrochloride to yield compounds **6**–**8**. Carbamate **9** was similarly prepared in acetonitrile.

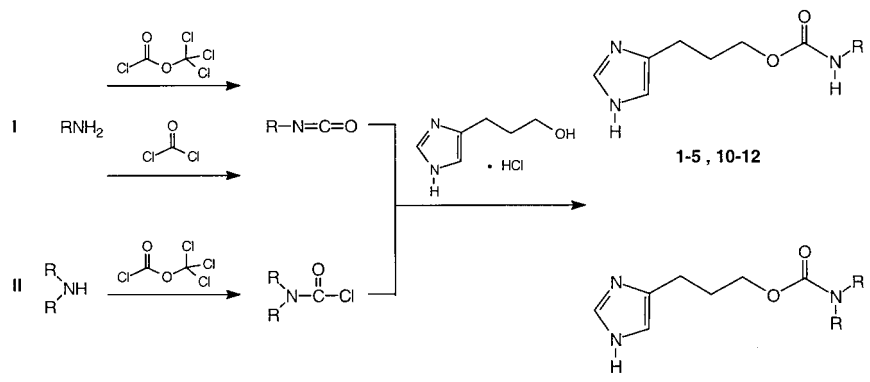
For compounds **1**–**12** predicted pK_a values and log partition (P) and distribution (D) coefficients were calculated and given partly in Table 1.

2.2. Pharmacological results and discussion

2.2.1. Histamine H_3-receptor antagonist in vitro assay on synaptosomes of rat cerebral cortex

Compounds **2**–**9** and **12** were tested for their histamine H_3-receptor antagonist potency in a functional assay on synaptosomes of rat cerebral cortex [27] (Table 1). The cyclopropylmethyl derivative **1** was selected as the lead structure from the previously examined compounds [24] to allow a direct comparison of the changes in activity of the newly designed compounds with the modification of the lipophilic group of the carbamate moieties. All the examined compounds showed antagonist properties at the histamine H_3 receptor in the range of $K_i = 6–131$ nM. An improvement in activity over lead **1** was observed when the cyclopropyl ring was placed at a greater distance to the polar part of the carbamate moiety (homologisation, **1** → **2**). Replacement of the cyclopropylmethyl substituent by an allyl group (**3**) reduced potency. Compound **4** with a longer five-carbon-atom chain, containing a terminal double bond, was the most active compound *in vitro* in this series of derivatives with a K_i value comparable to that of thioperamide. Activity was further reduced when the allyl substituent was replaced by a propynyl group (compound **5**). As *in vitro* tests reflect the ligand-receptor interaction it may be speculated that by digonal hybridisation directed substituent with steric restrictions does not favour binding

Scheme 2



with a hydrophobic pocket of the receptor in the same manner as it is the case with compound **3**. On the other hand, it must be stressed that the differences in the *in vitro* activities of **3** and **5** are not significant. The *N,N*-disubstituted derivatives **6**–**9** were all similarly active but less active than **4**. Activity was a little improved for the double-branched alkyl derivative **12**.

2.2.2. Histamine H_3-receptor antagonist in vivo assay on mice brain after p.o. administration.

For rational drug development the *in vivo* potency of novel compounds must be regarded as a crucial prerequisite. For this reason the pharmacological effect of all compounds designed was screened *in vivo* at histamine H_3 receptors [27]. Lead **1** belongs to the group of compounds showing *in vitro* activity, but is almost ineffective *in vivo* (cf. Table 1, clobenpropit). Allyl derivative **3** (ED_{50} = 0.48 mg/kg) was the most active compound in the whole series examined with an activity higher than that of thioperamide and clobenpropit. When the double bond was shifted to the end of a five-membered chain (**4**) a significant (ca. 4-fold) decrease of activity was observed. Because of these differences in receptor affinities (K_i values) this change must most probably be caused by pharmacokinetic reasons. The digonally hybridised **5**, which showed very low activity *in vitro,* also showed low potency *in vivo*. Similarly, the reduction of p.o. activity was observed for the diallyl substituted derivative **6**. In the cyclopropyl group spacer elongation from one to two methylene groups resulted in an increase in *in vivo* activity parallel to the increase in *in vitro* affinity. A cyclopropyl ring connected with the polar part of moiety by a spacer longer than in **1**, as in compound **2**, resulted in a seven-fold increase in activity. Disubstitution of the carbamate nitrogen (**7**, **8**) with alkyl groups or when included in a piperidine ring (**9**) was well tolerated. Introduction of substituents with higher steric demands at the position β to the carbamate function, as in **10**, or with double-branching at the beginning or at the end of the alkyl chain (**11**, **12**) also provided active compounds.

2.2.3. Screening of selected compounds at other functional histamine receptor models.

The affinity of the compounds obtained was determined not only at synaptosomes of rat cerebral cortex but for selected compounds **3**, **5** and **7** also at the guinea-pig ileum, another functional model for the histamine H_3 receptor (Table 2) [28, 34]. This test allows the determination of antagonist activity in a peripheral tissue model, measuring H_3-heteroreceptor function. In this test only the *N,N*-dipropyl derivative (**7**) showed high antagonist activity ($pK_B \sim 7.2$) comparable to that of the lead structure **1** with $pK_B \sim 7.3$. The lowest pK_B value found was that for the propynyl derivative **5**. The data obtained on the guinea-pig ileum for H_3 receptors are in reasonable agreement with the data on rat synaptosomes. Small differences like those for **3** and **5** are often found with a variety of compounds [25].

In addition, selected compounds **2**–**5**, **7**, **8**, **11**, and **12** were tested for their H_1-receptor activity on guinea-pig ileum as well as for their H_2-receptor activity on guinea-pig atrium [29] (Table 2). All compounds showed low affinity for H_1 as well for H_2 receptors with K_B values higher than the micromolar concentration range ($pK_B < 5$) demonstrating their high selectivity for the third histamine receptor.

2.2.4. Prediction of physicochemical properties

The aim of this work was to design new carbamate H_3-receptor antagonists showing high *in vitro* and especially *in vivo* activity by modification of the alkyl substituent of the lead structure **1**. Since these modifications were only concerned with the lipophilic part of the molecules, the prediction of physicochemical properties is important in this context. The only pK_A value of physiological importance is that for the imidazolium cation ($pK_A = 6.76$) and this is unlikely to change much among the various carbamate compounds **1**–**12**.

It is well accepted that compounds expected to act in the CNS tissue should possess log P ∼ 2 [36]. The calculated values of partition (P) and distribution (D) factors vary from 0.33 to 3.55 for log P and from 0.24 to 3.46 for log D. The calculated log P and log D values apparently cannot explain the differences observed in the *in vivo* activities of the examined compounds since the most active structure compound **3** has log P (log D) values equal to 0.84 (0.75), far from the expected log P ∼ 2. However, the compound with lowest activity, **5,** as may be expected has the lowest log P (log D) values = 0.33 (0.24). Nevertheless, the prediction of *in vivo* CNS activity based on log P or log D values seems to be too simplistic since this calculation does not imply hydrogen bonds or steric demands for different pharmacokinetic behaviour like distribution or metabolism.

In conclusion, it may be noticed that the lipophilic properties do not decide only on the activity of this series of carbamates derived in their lipophilic *N*-substituent, more probably spatial properties greatly influence pharmacodynamic and pharmacokinetic behaviour as histamine H_3-receptor antagonists.

3. Experimental

3.1. Chemistry

3.1.1. Methods

Chemical yields: non-optimized reaction conditions. M.p.'s were determined on an Electrothermal IA 9000 digital apparatus and are uncorrected. ^{1}H NMR spectra were recorded in DMSO d_6 solution on a Bruker AC 300 (300 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS as reference (Im: imidazolyl, Mal: maleic acid, cyclopr: cyclopropyl, d: doublet, s: singlet, t: triplet, q: quartet, qu: quintet, sc: sextet, m: multiplet, br: broad, def: deformed, asterisk: exchangeable by D_2O); approximate coupling constants in hertz (Hz). MS were obtained on EI-MS Finnigan MAT CH7A, FAB ($^+$FAB,xenon,DMSO/glycerol) on Finnigan MAT CH5 DF. IR spectra were recorded on Perkin-Elmer 1420 Ratio Recording IR-spectrometer in KBr discs, ν: (cm^{-1}). Elemental analyses (C, H, N) were measured on Perkin-Elmer 240B or Perkin-Elmer 240C instruments and were within ± 0.4% of the theoretical values. TLC was performed on precoated TLC aluminium sheets silica gel 60 F_{254} layer, thickness 0.2 mm, using the following solvent systems: I: $CHCl_3$/MeOH (9 : 1) ammonia atmosphere (for amines and carbamates), II: toluene/acetone (20 : 1.5) for phthalimides, III: $CHCl_3$/MeOH (95 : 5) ammonia atmosphere for carbamates. 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 carried out using silica gel 63 to 200 μm (Macherey, Nagel & Co.). The predictions of pK_a, log P combined, and log D values were determined with the PALLAS program [26]. *3-(1H-Imidazol-4-yl)propyl N-(cyclopropylmethyl)carbamate* (**1**) was obtained as described in the literature [24].

3.1.2. General procedure for the synthesis of N-monosubstituted carbamates **2**–**5**, **10**–**12**

A catalytic amount of activated charcoal was suspended in 20 ml of dry ethyl acetate and trichloromethyl chloroformate (1.09 g, 5.5 mmol). To the reaction mixture was added the solution of corresponding amine (5 mmol) in 10 ml of dry ethyl acetate or rapidly corresponding amine · hydrochloride (5 mmol) as solid. The reaction mixture was heated under reflux for 4 – 5 h; the black solution was then cooled and filtered, and the solvent was

evaporated carefully under reduced pressure. The freshly prepared isocyanate was dissolved in 40 ml of dry CH_3CN and added to 3-(1*H*-imidazol-4-yl)propanolhydrochloride [31]. The solution was heated under reflux for 2 – 4 h and then concentrated *in vacuo*. The residue was purified by rotatory chromatography [eluent: CH_2Cl_2/MeOH (gradient from 99 : 1 to 90 : 10); ammonia atmosphere was used for **3** and **5**.
The products were obtained as colourless oils and crystallized as hydrogen maleates from Et_2O/EtOH (**2**–**4** and **10**–**12**); **5** as hydrogen oxalate.

3.1.3. 3-(1 H-Imidazol-4-yl)propyl N-(cyclopropylethyl)carbamate (**2**)

2-Cyclopropylethylamine · hydrochloride was obtained using the method described for pentylamine [33].
Dry toluene (10 ml) was added to Na (1.24 g, 54 mmol) and one drop of dodecanoic acid; the mixture was rapidly stirred for 3 h. To the emulsion obtained cyclopropylacetonitrile (0.99 g, 12 mmol) dissolved in dry EtOH (1.93 g, 42 mmol) was added dropwise. The mixture was allowed to cool down, MeOH (10 ml) and then H_2O were added. After the layers had separated the amine was extracted (×4) with ethyl ether, the organic layer was dried (Na_2SO_4) and evaporated. A few drops of concentrated HCl were added, and the amine was separated as hydrochloride, m.p. 181–184 °C; TLC-(I); yield 55%; ^{1}H NMR: δ = 8.04 (s, 3 H, NH_3^+), 2.82 (sc, J = 6.5, 3 H, C*H*$_2$–NH_2), 1.47 (q, J = 7.3, 2H, cyclopr-C*H*$_2$–), 0.73 (qu, J = 7.3, 1 H, C*H*), 0.43 (qu, J = 4.1, 2 H, cyclopr), 0.07 (q, J = 5.1, 2 H, cyclopr).
$C_5H_{11}N$ · HCl (121.6)
The amine obtained was used for the synthesis of carbamate **2** without further purification. Compound **2** was obtained as described in 3.1.2.
2 · hydrogen maleate: m.p. 102–103 °C (colourless crystals); TLC-(I); yield 23%; EI-MS: m/z (rel. Int. [%]): 237 (28, [M$^{+\cdot}$]), 182 (6), 153 (2), 108 (100), 95 (58), 81 (42), 54 (14), 41 (14), 28 (18); ^{1}H NMR: δ = 8.85 (s, 1 H, Im-2-H), 7.39 (s, 1 H, Im-5-H), 7.09 (t, 1 H, NH*), 6.04 (s, 2 H, Mal.), 3.98 (t, J = 6.5, 2 H, CH_2–O), 3.01 (q, J = 5.0, 2 H, NH–C*H*$_2$), 2.67 (t, J = 7.6, 2 H, Im-CH_2), 1.88 (qu, J = 7.2, 2 H, Im-CH_2-C*H*$_2$), 1.30 (q, J = 7.1, 2 H, CH_2-cyclopr), 0.65 (m, 1 H, C*H*), 0.37 (m, 2 H, cyclopr), 0.01 (q, J = 5.5, 2 H, cyclopr); IR: 1701 (C=O).
$C_{12}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25\ H_2O$ (357.9).

3.1.4. 3-(1 H-Imidazol-4-yl)propyl N-allylcarbamate (**3**)

Compound **3** was obtained as described in 3.1.2. The isocyanate was not separated by distillation from the excess of diphosgene, but the crude reaction product was directly added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride in dry CH_3CN.
3 · hydrogen maleate: m.p. 88 °C (colourless crystals); TLC–(III); yield 93%; EI-MS: m/z (rel. Int. [%]): 209 (25, [M$^{+\cdot}$]), 108 (100), 95 (100), 81 (65), 54 (15), 41 (26), 28 (19); ^{1}H-NMR: δ = 8.91 (s, 1 H, Im-2-H), 7.42 (s, 1 H, Im-5-H), 7.31 (t, 1 H, NH*), 6.05 (s, 2 H, Mal.), 5.86–5.73 (m, 1 H, CH=), 5.15–5.03 (m, 2H, NH–C*H*$_2$), 3.99 (t, J = 6.5, 2H, CH_2–O), 3.61 (m, 2H, CH=C*H*$_2$), 2.70 (t, J = 7.5, 2H, Im-CH_2), 1.92 (m, 2H, Im-CH_2-C*H*$_2$); IR: 1695 (C=O).
$C_{10}H_{15}N_3O_2 \cdot C_4H_4O_4$ (325.3)

3.1.5. 3-(1 H-Imidazol-4-yl)propyl N-(4-pentenyl)carbamate (**4**)

A mixture of phthalimide (2.21 g, 15 mmol), triphenylphosphine (3.93 g, 15 mmol) and 4-penten-1-ol (1.29 g, 15 mmol) in 10 ml of dry THF was cooled to 0 °C. Diethyl azodicarboxylate (DEAD) (2.61 g, 15 mmol) in 10 ml of dry THF was slowly added dropwise (30 min); 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. Then the precipitate was filtered, the solvent was evaporated and the residue purified by CC (eluent: CH_2Cl_2) to afford *N*-(4-pentenyl)phthalimide; TLC-(II); yield 96%; EI-MS: m/z (rel. Int. [%]): 215 (13, [M$^{+\cdot}$]); ^{1}H NMR: δ = 7.89 – 7.82 (m, 4 H, aromat H), 5.88–5.75 (m, 1 H, CH=), 5.07–4.94 (m, 2H, C*H*$_2$–CH=), 3.58 (t, J = 7.1, 2 H, N–CH_2), 2.09–2.02 (def q, 2 H, =CH_2), 4.29 (qu, J = 7.1, 2 H, CH_2C*H*$_2$$CH_2$).
$C_{13}H_{13}NO_2$ (215.2)

N-(4-Pentenyl)phthalimide (2.15 g, 15 mmol) and hydrazine hydrate (0.62 g, 10 mmol) in 40 ml of EtOH were refluxed for 2 h. The suspension was cooled down, filtered, acidified with HCl and filtered once more. The filtrate was concentrated under reduced pressure; the crystalline 4-pentenamine · hydrochloride was washed with diethyl ether. M.p. 160–161 °C (white crystals); TLC–(I); yield 80%; $^+$FAB-MS: m/z (rel. Int. [%]): 86 (100, [M + H]$^+$); ^{1}H-NMR: δ = 8.19 (br s, 3 H (NH_3^+)*), 5.85–5.72 (m, 1 H, CH=), 5.08–4.98 (m, 2 H, CH_2=), 2.76–2.68 (m, 2 H, NCH_2), 2.12–2.05 (q, J = 7.3, 2H, C*H*$_2$CH=), 1.66 (qu, J = 7.6, 2 H, NCH_2C*H*$_2$).
$C_5H_{11}N$ · HCl (121.6)

Compound **4** was obtained as described in 3.1.2. using 4-pentenamine · hydrochloride.
4 · hydrogen maleate: m.p. 80–81 °C (colourless crystals); TLC-(I); yield 43%; EI-MS: m/z (rel. Int. [%]): 237 (13, [M$^{+\cdot}$]), 126 (2), 108 (100), 95 (76), 81 (50), 54 (17), 41 (23); ^{1}H NMR: δ = 8.67 (s, 1 H, Im-2-H), 7.25 (s, 1 H, Im-5-H), 7.11 (t, 1 H, NH*), 6.05 (s, 2 H, Mal.), 5.86–5.74 (m, 1 H, C*H*=CH_2), 5.07–4.94 (m, 2 H, CH=C*H*$_2$), 3.97 (t, J = 6.4, 2 H, CH_2–O), 2.96 (q, J = 6.6, 2 H, NH–CH_2), 2.68 (t, J = 7.5, 2 H, Im-CH_2), 2.01 (q, J = 7.3, 2 H, NH–CH_2–C*H*$_2$), 1.89 (qu, J = 7.2, 2 H, Im-CH_2–C*H*$_2$), 1.48 (qu, J = 7.2, 2 H, C*H*$_2$–CH=CH_2); IR: 1701 (C=O).
$C_{12}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25\ H_2O$ (357.9)

3.1.6. 3-(1 H-Imidazol-4-yl)propyl N-(propargyl)carbamate (**5**)

Compound **5** was obtained as described for **3**.
5 · hydrogen oxalate: m.p. 153–155 °C (colourless crystals); TLC-(III); yield 4%; EI-MS: m/z (rel. Int. [%]): 207 (12, [M$^{+\cdot}$]), 108 (76), 95 (100), 81 (85); ^{1}H-NMR: δ = 8.50 (s, 1 H, Im-2-H), 7.57 (s, 1 H, NH*), 7.22 (s, 1 H, Im-5-H), 3.99 (t, J = 6.3, 2 H, O–CH_2), 3.76 (m, 2 H, NH–C*H*$_2$), 3.08 (s, 1 H, CH), 2.64 (t, J = 7.1, 2 H, Im-CH_2), 1.89 (m, 2 H, Im-CH_2–C*H*$_2$); IR: 1700 (C=O).
$C_{10}H_{13}N_3O_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$ (301.8)

3.1.7. General procedure for the synthesis of the N,N-disubstituted carbamates (**6**–**9**)

The corresponding amine (5 mmol) was added dropwise to the suspension of catalytic amount of active charcoal and trichloromethyl chloroformate (5.5 mmol) in 20 ml of dry toluene. The reaction mixture was refluxed until it was getting clear (8–12 h) and filtered. To the carbamoyl chlorides thus obtained 5 mmol of 3-(1*H*-imidazol-4-yl)propanol · hydrochloride was added, the suspension was refluxed for 6 h to 2 days. The solution was cooled down, filtered, and the solvent was evaporated under reduced pressure. The oil residue was purified by rotatory chromatography [eluent: CH_2Cl_2/MeOH (gradient from 95 : 5 to 90 : 10) ammonia atmosphere]. The carbamates obtained were crystallized from EtOH/Et_2O as hydrogen oxalates **6**, **7**, as hydrogen maleate **9,** or as a free base **8**.

3.1.8. 3-(1 H-Imidazol-4-yl)propyl N,N-diallylcarbamate (**6**)

Compound **6** was obtained as described in 3.1.7.
6 · hydrogen oxalate: m.p. 57 °C, (colourless crystals); TLC-(III); yield 20%; $^+$FAB-MS m/z (rel. Int. [%]): 250 (18, [M + H]$^+$), 187 (10), 159 (11), 109 (28), 74 (17), 57 (18), 45 (12), 39 (100); ^{1}H NMR: δ = 8.86 (s, 1 H, Im-2-H), 7.40 (s, 1 H, Im-5-H), 6.04 (s, 2 H, Mal), 5.76 (m, 2 H, 2CH=), 5.12 (m, 4 H, 2C*H*$_2$=CH), 4.04 (t, J = 5.9, 2H, CH_2–O), 3.78 (br., 4 H, 2 N–CH_2), 2.68 (t, J = 7.4, 2 H, Im-CH_2), 1.91 (m, 2 H, Im-CH_2–C*H*$_2$); IR: 1690 (C=O).
$C_{13}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25\ H_2O$ (369.9)

3.1.9. 3-(1 H-Imidazol-4-yl)propyl N,N-dipropylcarbamate (**7**)

Compound **7** was obtained as described in 3.1.7.
7 · hydrogen oxalate: m.p. 142 °C (colourless crystals); TLC-(III); yield 37%; El-MS: m/z (rel. Int. [%]): 253 (18, [M$^{+\cdot}$]), 108 (100), 95 (27), 81 (41), 45 (42), 28 (13); ^{1}H-NMR: δ = 8.59 (s, 1 H, Im-2-H), 7.25 (s, 1 H, Im-5-H), 4.00 (t, J = 6.3, 2 H, CH_2–O), 3.11 (t, J = 7.4, 4 H, 2N–CH_2), 2.68 (t, J = 7.6, 2 H, Im-CH_2), 1.91 (m, 2 H, Im-CH_2–C*H*$_2$), 1.54–1.41 (m, 4 H, 2C*H*$_2$–CH_3), 0.82 (t, J = 7.4, 6 H, 2CH_3); IR: 1700 (C=O).
$C_{13}H_{23}N_3O_2 \cdot C_2H_2O_4$ (343.4)

3.1.10. 3-(1 H-Imidazol-4-yl)propyl N,N-diisopropylcarbamate (**8**)

Compound **8** was obtained as described in 3.1.7.
8: m.p. 106 °C (colourless crystals), TLC-(III); yield 45%; El-MS: m/z (rel. Int. [%]): 253 (17, [M$^{+\cdot}$]), 109 (100), 95 (23), 81 (44), 43 (24), 28 (11); ^{1}H-NMR: δ = 7.54 (s, 1 H, Im-2-H), 6.76 (s, 1 H, Im-5-H), 3.99 (t, J = 6.4, 2 H, O–CH_2), 3.86 (br., 2 H, Im-CH_2), 2.57 (m, 2 H, 2CH), 1.87 (m, Im-CH_2–C*H*$_2$), 1.15 (br., 12 H, 4 CH_3); IR: 1680 (C=O).
$C_{13}H_{23}N_3O_2 \cdot 0.25H_2O$ (257.9)

3.1.11. 4-[3-(Piperidinocarbonyloxy)propyl)]-1 H-imidazole (**9**)

Compound **9** was obtained as described in 3.1.7. with the following difference: after the carbamoyl chloride was obtained, the solvent was evaporated, and the residue was dissolved in CH_3CN.
9 · hydrogen maleate: m.p. 99 °C (colourless crystals); TLC-(III); yield 69%; El-MS: 237 (15, [M$^{+\cdot}$]), 108 (100), 98 (12), 95 (44), 91 (54), 81 (44), 54 (13); ^{1}H NMR: δ = 8.88 (s, 1 H, Im-2-H), 7.41 (s, 1 H, Im-5-H), 6.05 (s, 2 H, Mal.), 4.01 (t, J = 6.2, 2H, CH_2O), 3.30 (m, 4 H, 2 CH_2N), 2.69 (t, J = 7.5, 2 H, Im-CH_2), 1.92 (m, 2 H, Im-CH_2–CH_2), 1.23–1.54 (m, 6 H, 3 CH_2).
$C_{12}H_{19}N_3O_2 \cdot C_4H_4O_4$ (353.4)

3.1.12. 3-(1 H-Imidazol-4-yl)propyl N-(2-ethylhexyl)carbamate (**10**)

Compound **10** was obtained as described in 3.1.2.
10 · hydrogen maleate: m.p. 66–71 °C (colourless crystals); TLC-(I); yield 41%; El-MS: 281 (11, [M$^{+\cdot}$]), 182 (3), 174 (2), 108 (100), 95 (54), 81 (37), 54 (27), 43 (13); ^{1}H NMR: δ = 8.86 (s, 1 H, Im-2-H), 7.37 (s, 1 H, Im-5-H), 7.04 (s, 1 H, NH*), 6.06 (s, 2 H, Mal.), 3.96 (t, J = 6.2, 2 H,

CH_2-O), 2. 87 (t, 2 H, NH$-CH_2$), 2.68 (t, J = 7.2, 2 H, Im-CH_2), 1.90 (t, J = 6.9, 2 H, Im-CH_2-CH_2), 1.30 (m, 9 H, $CH_3-(CH_2)_3$), 0.82 (m, 6 H, CH_3-CH_2-CH); IR: 1696 (C=O).
$C_{15}H_{27}N_3O_2 \cdot C_4H_4O_4 \cdot 0.75\ H_2O$ (411.0)

3.1.13. 3-(1 H-Imidazol-4-yl)propyl N-(5-methyl-2-hexyl)carbamate (**11**)

N-(5-Methyl)-2-hexylphthalimide and 5-methyl-2-hexylamine · HCl were obtained as described in 3.1.5. (yields 65% and 63%, respectively).
5-Methyl-2-hexylamine · HCl: m.p. 120–125 °C; TLC-(I); yield 63%; $^+$FAB-MS: 116 (100, $[M+H]^+$), 79 (4), 57 (21), 44 (14), 43 (14), 29 (9); ^{1}H-NMR: δ = 0.86 (d, J = 6.6, 6H, $CH(CH_3)_2$), 1.16–1.22 (m, 5 H, $H_2NCH(CH_3)CH_2CH_2-$), 1.33–1.55 (m, 2 H, $H_2NCH(CH_3)CH_2-$), 1.59 to 1.68 (m, 1 H, $CH(CH_3)_2$), 3.07 (qu, J = 6.5, 1 H, H_2N-CH), 8.09 (s, 3 H, NH_3^+*).
$C_7H_{14}N \cdot HCl$ (151.7)
Compound **11** was obtained as described in 3.1.2. Phosgene (20% in toluene) was used instead of diphosgene.
11 · hydrogen maleate: m.p. 98–100 °C (colourless crystals); TLC-(I); yield 7%;. El-MS: m/z (rel. Int. [%]): 267 (12, $[M^+]$), 196 (7), 126 (4), 108 (100), 95 (59), 81 (37), 54 (38). ^{1}H NMR: δ = 8.85 (s, 1 H, Im-2-H), 7.38 (s, 1 H, Im-5-H), 6.92 (d, J = 8.1, 1 H, NH*), 6.04 (s, 2 H, Mal), 3.96 (t, J = 6.2, 2 H, $O-CH_2$), 3.4 (m, 1 H, NH$-CH$), 2.68 (t, J = 7.5, 2 H, Im-CH_2), 1.89 (t, J = 7.2, 2 H, Im-CH_2-CH_2), 1.53–1.43 (m, 1 H, $CH(CH_3)_2$), 1.36–1.30 (m, 2 H, NH$-CH(CH_3)CH_2$), 1.15–1.12 (m, 2 H, $CH_2-CH(CH_3)_2$), 1.02 (d, J = 6.6, 3 H, NH$-CH(CH_3)$), 0.84 (d, J = 6.6, 6 H, $CH(CH_3)_2$); IR: 1693 (C=O).
$C_{14}H_{25}N_3O_2 \cdot C_4H_4O_4 \cdot 0.5\ H_2O$ (392.5)

3.1.14. 3-(1 H-Imidazol-4-yl)propyl N-(6-methyl-2-heptyl)carbamate (**12**)

Compound **12** was obtained as described in 3.1.2.
12 · hydrogen maleate: m.p. 103–106 °C (colourless crystals); TLC-(I); yield 9%;. El-MS: m/z (rel. Int. [%]): 281 (13, $[M^+]$), 196 (6), 126 (4), 108 (100), 95 (59), 81 (35), 54 (13), 31 (13); ^{1}H-NMR: δ = 8.84 (s, 1 H, Im-2-H), 7.62 (s, 1 H, Im-5-H), 6.94 (d, J = 8.2, 1 H, NH*), 6.04 (s, 2 H, Mal), 3.95 (t, J = 6.4, 2H, CH_2-O), 3.45 (t, J = 6.6, 1 H, NH$-CH$), 2.67 (t, J = 7.5, 2 H, Im-CH_2), 1.89 (qu, J = 7.2, 2 H, Im-CH_2-CH_2), 1.53 to 1.43 (m, 1 H, $CH(CH_3)_2$), 1.40–1.10 (m, 6 H, $CH_2-CH_2-CH_2$), 1.00 (d, J = 6.6, 3 H, NH$-CH(CH_3)$), 0.83 (d, J = 6.6, 6 H, $CH(CH_3)_2$); IR: 1695 (C=O).
$C_{15}H_{27}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25\ H_2O$ (402.0)

3.2. Pharmacology

3.2.1. Histamine H_3-receptor assay on synaptosomes of rat cerebral cortex

The compounds were tested for their H_3-receptor antagonist activity in an assay with K^+-evoked depolarization-induced release of [^{3}H]histamine from synaptosomes of rat cerebral cortex according to Garbarg et al. [27]. A synaptosomal fraction from rat cerebral cortex prepared according to the method of Whittaker [37] was preincubated for 30 min with L-[^{3}H]histidine (0.4 μM) at 37 °C in a modified Krebs-Ringer solution. The synaptosomes were washed extensively, resuspended in fresh 2 mM K^+ Krebs-Ringer's medium, and incubated for 2 min with 2 or 30 mM K^+ (final concentration). Compounds and 1 μM histamine were added 5 min before the depolarization stimulus. Incubations were stopped by rapid centrifugation, and [^{3}H]histamine levels were determined after purification by liquid scintillation spectrometry [27]. K_i values were determined according to the Cheng-Prusoff equation [38]. The data presented are given as mean values with standard error of the mean (SEM) for a minimum of three separate determinations each.

3.2.2. 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. [35]. 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 of 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 frequence of 0.1 Hz. After 30 min of stimulation, a cumulative dose–response curve was recorded. Subsequently the preparations were washed three times every 10 min without stimulation. The antagonist was incubated for 20–30 min before redetermination of the dose–response curve of (*R*)-α-methylhistamine [28].

3.2.3. Histamine H_3-receptor antagonist potency in vivo in mice

In vivo testing was performed after peroral administration to Swiss mice as described by Garbarg et al. [27]. Brain histamine turnover 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 dissected out. The cortex was homogenized in 10 vol of ice-cold perchloric acid (0.4 M). The N^τ-methylhistamine level was measured by radioimmunoassay [39]. By treatment with 3 mg/kg ciproxifan [40] the maximal N^τ-methylhistamine level was obtained and related to the level reached with the administered compounds, and the ED_{50} value was calculated as a mean with SEM [41].

3.2.4. In vitro screening at other histamine receptors

Selected compounds were screened for histamine H_2-receptor activity on the isolated spontaneously beating guinea-pig right atrium as well as for H_1-receptor activity on the isolated guinea-pig ileum by standard methods described by Hirschfeld et al. [29]. Each pharmacological test was performed at least in triplicate, but the exact type of interaction had not been determined in each case. The given values represent the mean.

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