Novel Carbamates as Potent Histamine H_3 Receptor Antagonists with High *in Vitro* and Oral *in Vivo* Activity[†,⊥]

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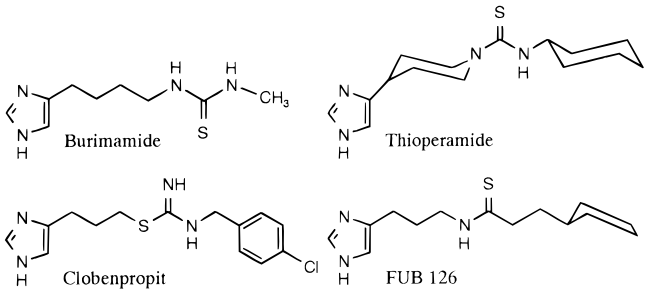
The known histamine H_3 receptor antagonists burimamide, thioperamide, clobenpropit, and a related homohistamine thioamide derivative were taken as templates in search for new leads. Novel histamine H_3 receptor antagonists structurally described as carbamate derivatives of 3-(1*H*-imidazol-4-yl)propanol were prepared in high yields by treatment of the alcohol with corresponding isocyanates or carbamoyl chlorides and investigated for their H_3 receptor antagonist activity. Different chain lengths and various substituents possessing different electronic and steric parameters were introduced and structure−activity relationships established. In different functional tests, the new antagonists showed high H_3 receptor antagonist potencies *in vitro* (−log K_i values of 6.4−8.4) at synaptosomes of rat cerebral cortex and low activities at histamine H_1 and H_2 receptor subtypes. They were also screened for their central *in vivo* activity in mice after peroral administration. The most promising compounds (**2**, **16**, **19**) showed ED_{50} values of about 1−2 mg/kg and thus are potential drugs for the therapy of H_3 receptor dependent diseases. Some of the novel carbamate derivatives are H_3 receptor selective compounds with high *in vitro* and *in vivo* activity.

Introduction

While the function of histamine as an autacoid has been known for a long time, the neurotransmitter function of this biogenic amine has been established especially during the last decade.[1] With the identification of the third histamine receptor subtype acting as a presynaptically located autoreceptor on histaminergic nerve endings, the neurotransmitter function of histamine was manifested.[2,3] Activation of histamine H_3 receptors leads to both an inhibition of histamine synthesis from L-histidine and an inhibition of histamine release from vesicles in histaminergic neurons. Histamine H_3 receptor antagonists increase histamine synthesis and release by inhibiting a presently unknown negative feedback mechanism.[4] Although H_3 receptors were identified in peripheral tissues in different species, the highest density of this receptor is generally identified in the central nervous system (CNS) where it was detected first on rat cerebral cortex[2] and afterwards also in the human brain.[5] Histamine H_3 receptors are not only autoreceptors; they also act as heteroreceptors. A modulating effect on the release of several neurotransmitters was an indication for the general regulatory role of H_3 receptors in neurotransmission, e.g., the influence of the release of noradrenaline,[6] serotonin,[7] acetylcholine,[8] dopamine,[9] and neuropeptides from nonadrenergic noncholinergic (NANC) nerves.[10] On account of the highest density of H_3 receptors in the CNS and the importance of these neurotransmitters, the therapeutic use of H_3 receptor ligands seems to be especially useful for different CNS disorders. By increasing the brain histamine level, H_3 receptor antagonists have a stimulating effect on different cerebral functions and therefore influence different psychic diseases or disorders, e.g., epilepsy,[11] stress,[12] food intake,[13] sleeping,[14] vertigo,[15] or Morbus Alzheimer. At present the indications for the therapeutic use are not totally clear.

Chart 1. Sulfur-Containing Histamine H_3 Receptor Antagonists



New pharmacological tools are highly required to clarify these pharmacological problems and clinical usefulness. With regard to further drug development, the new ligands should be highly potent and selective for histamine H_3 receptors and avoid any predictable toxicity.

A number of potent and selective H_3 receptor antagonists possess sulfur-containing functionalities, e.g., the thiourea derivatives burimamide[2] and thioperamide,[16] the isothiourea derivative clobenpropit,[17] and FUB 126,[18] a thioamide derivative of homohistamine (Chart 1). Thioperamide is active in nanomolar and clobenpropit in subnanomolar concentration *in vitro*. Both compounds are also active *in vivo*, whereby clobenpropit is clearly less effective than thioperamide (cf. Table 1). None of these compounds have ever been reported to

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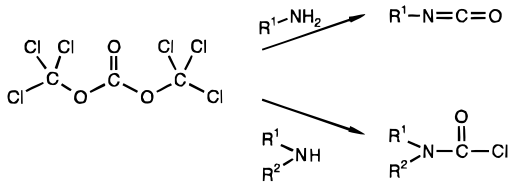
be used in clinical trials. Most probably toxicological problems might have stopped further development. Undesirable side effects like hepatotoxicity depend presumably on the thiourea group and related moieties. Thiourea-containing drug molecules are used to treat hyperthyroidism. Agranulocytosis is a serious toxic effect with these drugs. The clinical use of metiamide, a thiourea-containing H_2 receptor antagonist, was stopped due to some incidence of granulocytopenia.[19,20] This would have prevented the development of H_2 receptor antagonists if cimetidine and later on other compounds had not been found showing a better pharmacological side-effect profile.

Therefore, histamine H_3 receptor antagonists without sulfur-containing functionalities have been developed to avoid toxicity problems.[21] Amides,[22,23] guanidines,[24] and heteroaryl compounds,[25] developed by derivatization of the endogenous ligand histamine and further optimized, showed moderate to high activity *in vitro*, but they were less effective *in vivo*. The reason for lacking activity *in vivo* may be a metabolic one or might be caused by these drugs' inability to cross the blood−brain barrier. In previous reports it had been shown that a general construction scheme belongs to all potent H_3 receptor antagonists known so far. They are all imidazole derivatives connected with a polar group by a chain. By another chain, this polar group may be connected with a lipophilic moiety.[26] In this paper the imidazole chain consisting of three methylene groups was kept constant. In earlier reports this moiety had been shown to produce highest activity with compounds containing bulky substituents on the polar moiety.[23−25] As polar groups, an N-monosubstituted thiocarbamate **1** as well as N-monosubstituted (**2**−**40**, **45**) and N,N-disubstituted (**41**−**44**) carbamates had been used. Different chain lengths between the polar group and the lipophilic moiety and the influence of the lipophilic moiety itself were also investigated. The thiocarbamate **1** was prepared as a structure analogous to thiourea derivatives. It is quasi the link between the functionalities of thioureas and carbamates. Because of the low activity and expected toxicological problems, only one thiocarbamate was prepared. All compounds were screened in functional tests for their histamine H_3 receptor antagonist activity on synaptosomes of rat cerebral cortex by measuring the amount of the released tritium-labeled histamine.[27] Regarding the potential therapeutic use of H_3 receptor antagonists, the central activity *in vivo* was investigated in mice.[27] It could be shown that a great number of the novel carbamate derivatives are also active *in vivo* after peroral administration, thus demonstrating their ability to cross biological membranes like the blood−brain barrier. This biological barrier to the main therapeutic target seems to be a problem for many histamine H_3 receptor antagonists known so far. The activity at other histamine receptor subtypes was determined for most of the carbamates in functional tests on isolated organs of the guinea pig (cf. Table 2).

Chemistry

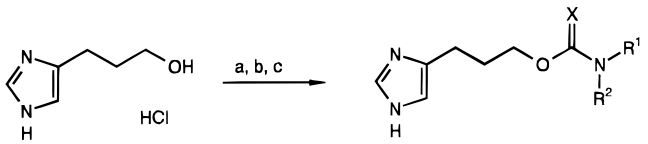
3-(1*H*-Imidazol-4-yl)propanol[28] is the key intermediate for all novel synthesized carbamates. It is prepared from urocanic acid by esterification, hydrogenation,[29] imidazole protection by tritylation, reduction by complex hydrides, and consequent deprotection.[30] The tritylation increases lipophilicity, reduces side reactions, and facilitates the isolation of the product after reduction, thus increasing the overall yield.[30] In contrast to other synthetic approaches,[31,32] 3-(1*H*-imidazol-4-yl)propanol can thus be obtained in high yield and high purity.

Scheme 1. Synthesis of Isocyanates and Carbamoyl Chlorides[a]



[a] R^1 = alkyl, aryl, alkylaryl; R^2 = alkyl, aryl.

Scheme 2. Synthesis of a Thiocarbamate as Well as of Mono- and Dialkylated Carbamates[a]



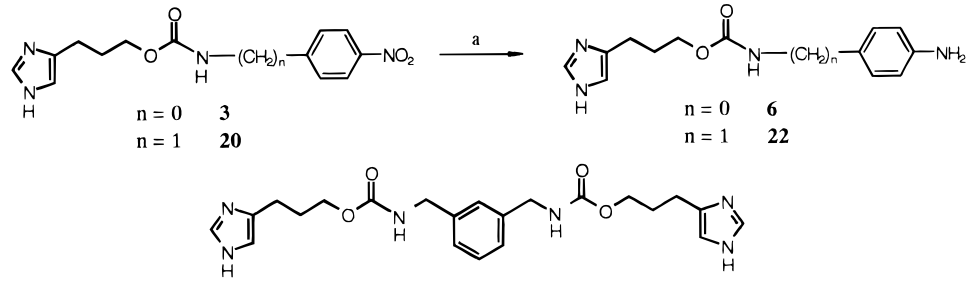
[a] (a) i, NaH in toluene, ii, acetonitrile, R^1−N=C=S (X = S, R^1 = Ph, R^2 = H); (b) acetonitrile, R^1−N=C=O (X = O, R^1 = alkyl, aryl, alkylaryl, R^2 = H); (c) i, NaH in toluene, ii, acetonitrile, R^1R^2−N−CO−Cl (X = O, R^1 = alkyl, aryl, alkylaryl, R^2 = alkyl, aryl).

Because of the low reactivity of isothiocyanates, the thiocarbamate **1** was synthesized by treatment of phenyl isothiocyanate with the freshly prepared sodium 3-(1*H*-imidazol-4-yl)propanolate. For the synthesis of isocyanates and carbamoyl chlorides the use of gaseous phosgene was avoided due to toxicity reasons. The phosgene substitutes trichloromethyl chloroformate (diphosgene) and bis(trichloromethyl) carbonate (triphosgene) were used.[33,34] The corresponding amines were allowed to react with an excess of the phosgene surrogate to result in the reactive precursor molecules (Scheme 1). The advantage of the phosgene surrogates is that they are easy to handle and to measure out. For the synthesis of N-monosubstituted carbamates **2**−**5**, **7**−**21**, **23**−**40**, and **45** (Schemes 2 and 3), the corresponding isocyanates were dissolved in an aprotic, polar solvent, e.g., acetonitrile, and then 3-(1*H*-imidazol-4-yl)-propanol hydrochloride was added. The protonated imidazolium ring does not have nucleophilic properties, and therefore the alcoholic functionality is the preferred moiety for electrophilic attack by the isocyanates.

The synthesis of the N,N-disubstituted carbamates **41**−**44** was modified because of the lower reactivity of carbamoyl chlorides.[35] 3-(1*H*-Imidazol-4-yl)propanol was added in the form of sodium alcoholate to raise the nucleophilicity of this functionality. An excess of base resulted in the dianion of the imidazolylalkyl alcohol. In all cases the carbamate derivatives were obtained in high yields and high purity.

The synthesis of the aminophenyl-substituted carbamate derivatives **6** and **22** was carried out starting from the corresponding nitro derivatives **3** and **20**, respectively. Aromatic nitro groups can easily be hydrogenated under mild conditions. The hydrogenation of the nitro group was completed before the carbamate group is attacked (Scheme 3). The chiral compounds **37** and **38** were prepared from the chiral 1-phenylethanamine precursors.

Scheme 3. Synthesis of Aminophenyl-Substituted Carbamates and Structure of **45**[a]



45

[a] (a) Pd/C (10%), 1 bar of H_2.

Biological Results and Discussion

***In Vitro* Testing on Synaptosomes of Rat Cerebral Cortex and *in Vivo* Results on Mice.** The synthesized compounds were tested for their histamine H_3 receptor antagonist activity in the model of Arrang et al.,[16] thereby investigating their influence on K^+-induced [^{3}H]histamine release from rat brain synaptosomes. Results are presented in Tables 1 and 2.

Thiocarbamate **1** was prepared to prove a possible bioisosterism with the thiourea group. Thereafter the sulfur atom of the thiocarbamate moiety was exchanged by oxygen. The conversion led to the carbamates as new leads for histamine H_3 receptor antagonists. The transition to carbonates was not examined because these compounds are generally considered to be inactive under *in vivo* conditions and therefore cannot be used for drug development.

All novel compounds showed moderate to high antagonist activity at histamine H_3 receptors. The *N*-phenyl-substituted thiocarbamate **1** is moderately potent *in vitro* and *in vivo*. The oxy-analogous carbamate **2** showed higher potency *in vitro* and also higher activity *in vivo*. The increased lipophilicity of the sulfur-containing **1** does not result in increased activity, especially *in vivo*. Due to the higher activity of the carbamate **2**, further investigations had the aim to show structure−activity relationships and to develop potential drugs in the class of this new lead.

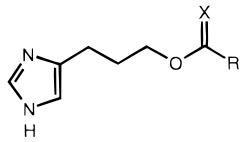
Introduction of substituents with electron-withdrawing properties on the phenyl ring (**11**, **14**−**16**) led to similar or, in the case of nitro and trifluoromethyl substituents (**3**−**5**, **8**−**10**), decreased activity *in vivo*. The position of the substituent in the ring system does not seem to be important for H_3 receptor antagonist activity in this class of compounds since there are no significant differences in the $-\log K_i$ values of *ortho*-, *meta*-, or *para*-substituted derivatives. A slight preference for the *para*-position is given. Reduction of the 4-nitro group (**3**) to the corresponding amino compound **6** leads to a loss in the *in vitro* activity. This may not only be caused by the newly introduced basic properties because the alkaline dimethylamino-substituted derivative **7** is similarly active to the corresponding nitro derivative **3**. The activity *in vitro* does not depend on electron-withdrawing or electron-releasing substituents (**12** → **2**; **12** → **8**). Some halogen substituents increase the *in vitro* activity (**15**, chlorine; **13**, iodine). At present the 4-chlorophenyl-substituted carbamate **15** is the most potent carbamate *in vitro* ($-\log K_i = 8.4$). The *in vivo* activity of **15**, however, is lower than that of the unsubstituted derivative **2**. This example shows that there is no direct correlation between *in vitro* and *in vivo* activity. This result is not surprising since the functional test represents more or less the receptor−ligand interaction, whereas the *in vivo* screening represents the sum of pharmacodynamic and pharmacokinetic processes of a given compound. Further investigations are necessary to clarify the exact reason for the loss of *in vivo* activity of some compounds. For drug development this screening is satisfactory and, as shown in this series, successful.

Introduction of the bulky naphthyl residue results in a marked decrease in activity *in vitro* and *in vivo* (**2** → **17**). Steric parameters of the substituents on the carbamate moiety seem to be important for the antagonists' receptor binding. Replacement of the aromatic ring system of **2** by a cyclohexyl residue as is present in the lipophilic structure of thioperamide also leads to a less potent compound (**18**).

The activity *in vitro* of the benzylic compound **19** is in the same range as the phenylic one (**2**), whereas a slight decrease in activity *in vivo* of **19** is observed. In comparison with the phenylic compounds **2**−**16**, similar structure−activity relationships were obtained for benzylic compounds (**19**−**27**, **36**−**38**) with comparable substituents. The carbamate with a *p*-chlorobenzyl substituent (**26**) is equipotent to the unsubstituted derivative **19**. In the class of isothiourea derivatives, this substitution pattern led to an increase in activity of more than 1 order of magnitude.[17] This is a strong evidence for different receptor binding of the two chemically different classes of histamine H_3 receptor antagonists. Replacement of the benzylic ring system by a 2-furylmethyl (**28**) or a 2-thienylmethyl (**29**) substituent led to less potent compounds. In comparison to **19**, the introduction of a ferrocenylmethyl system enhances the activity *in vitro* slightly (**30**).The ferrocene moiety is a possible bioisosteric replacement of a phenyl substituent. The introduction of cycloalkyl groups instead of the aromatic ring in the case of the cyclohexyl derivative **31** leads to equipotency compared to the benzyl derivative **19**. Compounds with a ring size larger than four carbon atoms are active *in vivo* with ED_{50} values between 3.1 and 9.5 mg/kg po. Replacement of the methylene spacer by a carbonyl group decreases activity (**35**).

The introduction of a methyl group into the methylene spacer leads to chiral compounds. The enantiomers **37** and **38** and the racemic mixture **36** were tested for their H_3 receptor antagonist activity, but there was no significant difference to be observed. This lack of chiral discrimination is consistent with the lack of pronounced substituent effects in the aryl group of the carbamates.

Table 1. Structures, Chemical Data, and Results of Screening for Histamine H_3 Receptor Antagonist Activity *in Vitro* and *in Vivo* in Rodents



no.	X	R	yield (%)	mp[a] (°C)	formula	M_r	K_i[b] (nM), $\bar{X} \pm S_{\bar{X}}$	ED_{50}[c] (mg kg^{-1}), $\bar{X} \pm S_{\bar{X}}$
1	S	NH-Ph	54	129−131	$C_{13}H_{15}N_3OS \cdot C_4H_4O_4$	377.4	56 ± 12	19 ± 5
2	O	NH-Ph	89	115	$C_{13}H_{15}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	365.9	14 ± 8	1.3 ± 0.6
3	O	NH-(4-NO_2-Ph)	80	235	$C_{13}H_{14}N_4O_4 \cdot HCl \cdot 0.25H_2O$	331.2	21 ± 9	6 ± 3
4	O	NH-(3-NO_2-Ph)	81	162	$C_{13}H_{14}N_4O_4 \cdot C_4H_4O_4 \cdot 0.25H_2O$	410.9	22 ± 7	>10
5	O	NH-(2-NO_2-Ph)	83	160	$C_{13}H_{14}N_4O_4 \cdot HCl \cdot 0.25H_2O$	331.2	30 ± 28	>10
6	O	NH-(4-NH_2-Ph)	55	160	$C_{13}H_{16}N_4O_2 \cdot HCl \cdot 0.25H_2O$	301.3	355 ± 239	nd[d]
7	O	NH-(4-N(CH_3)$_2$-Ph)	79	124−125	$C_{15}H_{20}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	408.9	34 ± 11	9.8 ± 0.9
8	O	NH-(4-CF_3-Ph)	92	129	$C_{14}H_{14}F_3N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	433.9	15 ± 7	≈10
9	O	NH-(3-CF_3-Ph)	90	128	$C_{14}H_{14}F_3N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	433.9	19 ± 9	≈10
10	O	NH-(2-CF_3-Ph)	94	83	$C_{14}H_{14}F_3N_3O_2 \cdot C_4H_4O_4 . 0.25H_2O$	433.9	24 ± 17	≈10
11	O	NH-(4-OCF_3-Ph)	95	119−121	$C_{14}H_{14}F_3N_3O_3 \cdot C_4H_4O_4 \cdot 0.25H_2O$	444.9	26 ±10	6.3 ± 1.5
12	O	NH-(4-CH_3-Ph)	97	142	$C_{14}H_{17}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	379.9	11 ± 6	2.1 ± 0.9
13[e]	O	NH-(4-I-Ph)	81	139	$C_{13}H_{14}IN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	491.8	11 ± 4	15 ± 5
14	O	NH-(4-Br-Ph)	71	144−145	$C_{13}H_{14}BrN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	440.2	18 ± 4	1.9 ± 0.4
15	O	NH-(4-Cl-Ph)	87	132−134	$C_{13}H_{14}ClN_3O_2 \cdot C_4H4O_4 \cdot 0.25H_2O$	400.3	4.2 ± 0.4	3.5 ± 1
16	O	NH-(4-F-Ph)	86	137−138	$C_{13}H_{14}FN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	383.8	24 ± 4	1.3 ± 0.9
17	O	NH-2-naphthyl	82	138−139	$C_{17}H_{17}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	415.9	114 ± 46	>10
18	O	NH-cyclohexyl	82	76	$C_{13}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	371.9	69 ± 37	>10
19	O	NH-CH_2-Ph	92	123	$C_{14}H_{17}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	379.9	11 ± 6	1.8 ± 0.4
20	O	NH-CH_2-(4-NO_2-Ph)	86	129	$C_{14}H_{16}N_4O_4 \cdot C_4H_4O_4 \cdot 0.25H_2O$	424.9	15 ± 6	17 ± 2
21	O	NH-CH_2-(3-NO_2-Ph)	79	126	$C_{14}H_{16}N_4O_4 \cdot C_4H_4O_4 \cdot 0.25H_2O$	424.9	13 ± 3	>10
22	O	NH-CH_2-(4-NH_2-Ph)	50	120	$C_{14}H_{18}N_4O_2 . C_4H_4O_4 \cdot 0.25H_2O$	394.9	541 ± 69	≫10
23	O	NH-CH_2-(4-CF_3-Ph)	78	97	$C_{15}H_{16}F_3N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	447.9	12 ± 6	3.9 ± 0.3
24	O	NH-CH_2-(3-CF_3-Ph)	20	98	$C_{15}H_{16}F_3N_3O_2 \cdot C_4H_4O_4$	443.4	8.1 ± 2.7	2.6 ± 1.1
25[e]	O	NH-CH_2-(4-I-Ph)	75	111	$C_{14}H_{16}IN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	505.8	12 ± 2	nd
26	O	NH-CH_2-(4-Cl-Ph)	85	133−134	$C_{14}H_{16}ClN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	414.3	14 ± 5	3.0 ± 0.7
27	O	NH-CH_2-(4-F-Ph)	82	137−138	$C_{14}H_{16}FN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	397.9	23 ± 2	2.2 ± 1
28	O	NH-CH_2-2-furyl	55	78−80	$C_{12}H_{15}N_3O_3 \cdot C_4H_4O_4 \cdot 0.25H_2O$	369.8	42 ± 16	>10
29	O	NH-CH_2-2-thienyl	79	103−105	$C_{12}H_{15}N_3O_2S \cdot C_4H_4O_4 \cdot 0.25H_2O$	385.9	18 ± 3	3.0 ± 0.8
30	O	NH-CH_2-2-ferrocenyl	52	104−106	$C_{18}H_{21}FeN_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	487.8	5.8 ± 1	nd
31	O	NH-CH_2-cyclohexyl	85	106	$C_{14}H_{23}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	385.9	11 ± 6	3.1 ± 1.6
32	O	NH-CH_2-cyclopentyl	86	71	$C_{13}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	371.9	32 ± 11	9.5 ± 2.9
33	O	NH-CH_2-cyclobutyl	96	93−94	$C_{12}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	357.9	14 ± 6	≈10
34	O	NH-CH_2-cyclopropyl	78	93	$C_{11}H_{17}N_3O_2 \cdot C_4H_4O_4 \cdot 0.5H_2O$	348.4	52 ± 16	>10
35	O	NH-CO-Ph	68	150	$C_{14}H_{15}N_3O_3 \cdot 0.25H_2O$	277.8	95 ± 42	>10
36	O	(*R*/*S*)-NH-$CHCH_3$-Ph	*f*	105	$C_{15}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	393.9	49 ± 17	13 ± 6
37[g]	O	(*R*)-NH-$CHCH_3$-Ph	81	105	$C_{15}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	393.9	64 ± 21	≈10
38[h]	O	(*S*)-NH-$CHCH_3$-Ph	83	105	$C_{15}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	393.9	95 ± 42	≈10
39	O	NH-CH_2-CH_2-Ph	78	107−109	$C_{15}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	393.9	10 ± 3	2.5 ± 0.9
40	O	NH-CH_2-CH_2-CH_2-Ph	80	90	$C_{16}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	407.9	26 ± 5	6.9 ± 0.3
41	O	4-Ph-piperidino	68	125−126	$C_{18}H_{23}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	434.0	105 ± 31	>10
42	O	N(CH_3)CH_2-Ph	45	70	$C_{15}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	393.9	42 ± 18	2.9 ± 0.6
43	O	N(C_2H_5)CH_2-Ph	78	64−65	$C_{16}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	407.9	69 ± 12	5.9 ± 2.5
44	O	NPh_2	72	105	$C_{19}H_{19}N_3O_2 \cdot C_4H_4O_4$	437.5	165 ± 47	>10
45	O	"dicarbamate"	68	126	$C_{22}H_{28}N_6O_4 \cdot 2C_4H_4O_4 \cdot 0.25H_2O$	677.2	50 ± 15	>10
thioperamide							4 ± 1[i]	1.0 ± 0.5
clobenpropit							0.6 ± 0.1[j]	26 ± 7

[a] Crystallization solvent: Et_2O/EtOH. [b] Functional H_3 receptor test *in vitro* on synaptosomes of rat cerebral cortex.[26] [c] *In vivo* screening on central H_3 receptor activity after po application in mouse. [d] nd = not determined. [e] Reference 30. [f] Racemic mixture of **37** and **38**. [g] $[\alpha]^{20}_{546} = 47.4°$ (c = 10.8 mg/mL in EtOH). [h] $[\alpha]^{20}_{546} = 45.8°$ (c = 10.4 mg/mL in EtOH). [i] Reference 16. [j] Reference 41.

This structural element does not seem to be involved in a close ligand−receptor molecular interaction.

Prolongation of the spacer by one to three methylene groups led to compounds with lowered activity *in vivo* (**2** → **19** → **39** → **40**). When introducing a piperidine ring as a spacer, a further decrease in activity is obtained (**41**). To clarify if this decrease is caused by the steric variation or the disubstitution of the carbamate nitrogen, additional disubstituted derivatives were tested. N-Disubstitution of **19** by a methyl or ethyl group leads to compounds with lowered activity *in vitro* and *in vivo* (**42**, **43**). An important loss in activity is observed in the case of disubstitution by two phenyl rings (**44**). Analogous to the voluminous naphthyl residue, the histamine H_3 receptor activity of the bulky disubstituted carbamate **44** also seems to be negatively influenced.

The structural requirements for H_3 receptor antagonist activity were introduced twice into one molecule (**45**, Scheme 3). Dicarbamate **45** with a phenyl ring as the central structural element is not favorable compared to the monocarbamate derivatives but still possesses activity *in vitro* in the same range as the first lead, the thiocarbamate **1**.

It is remarkable that a large number of chemical variations can be performed by maintaining the *in vitro* and often the *in vivo* activity of the histamine H_3 receptor antagonists. The introduced substituents in-

Table 2. Activity of Selected Compounds at Histamine Receptor Subtypes

	−log K_i		
no.	H_3 receptor[a]	H_2 receptor[b]	H_1 receptor[c]
2	7.9	<4.0	5.0
3	7.7	4.9	4.7
4	7.7	4.9	4.9
5	7.5	4.3	4.5
8	7.8	4.4	4.8
9	7.7	4.9	4.2
10	7.6	4.0	4.4
13	8.0	5.3	4.4
14	7.7	4.8	4.6
15	8.4	4.1	4.2
16	7.6	4.2	4.1
17	6.9	5.1	5.1
19	8.0	4.8	4.4
20	7.8	4.8	4.9
21	7.9	6.0	4.3
22	6.3	4.0	4.0
23	7.9	4.9	4.8
25	7.9	4.5	5.1
26	7.9	4.1	4.8
27	7.6	5.4	4.3
28	7.4	<4.0	<4.0
31	8.0	4.2	3.9
32	7.5	<4.0	4.3
33	7.9	4.0	<4.0
37	7.2	<4.0	4.3
39	8.0	<4.0	4.2
40	7.6	4.1	4.1
41	7.0	4.8	5.5
42	7.4	<4.0	4.8
43	7.2	4.4	4.9
45	7.3	<4.0	<4.0

[a] Functional H_3 receptor test on synaptosomes of rat cerebral cortex.[26] [b] Functional H_2 receptor test on guinea pig atrium.[40] [c] Functional H_1 receptor test on guinea pig ileum.[40]

fluence the antagonist−receptor binding, but a definite conclusion concerning electronic or steric parameters could not be detected. Small or bulky groups as well as electron-withdrawing or electron-releasing substituents could be introduced by maintaining moderate to high H_3 receptor antagonist activity. The largest number of carbamate derivatives show a certain homogeneity in their receptor activity *in vitro*, differing <1 order of magnitude. The histamine H_3 receptor antagonist activity of compounds **2**−**5**, **7**−**16**, **18**−**21**, **23**−**40**, **42**, **43**, and **45** is in the nanomolar concentration range. The kind of substitution and the substitution pattern on the lipophilic residue seem to have minor influence on histamine H_3 receptor antagonist activity *in vitro* and, therefore, minor influence on close ligand−receptor interaction.

A few structural limitations could be claimed. The lipophilicity of the residue should be enhanced but by avoiding steric hindrance. The substituents decrease activity if they are too bulky (e.g., two phenyl groups) or too hydrophilic (e.g., an amino substituent).

These structural requirements concern the *in vitro* activity, but similar statements can be made for the *in vivo* activity. The screening on central histamine H_3 receptor antagonist activity after po administration determines the necessary data for a first evaluation of a drug selection dealing with a lot of pharmacokinetic parameters. The *in vivo* activity at the H_3 receptor is one point; the activity at other receptor subtypes is another one. For compounds with H_3 receptor activity, the activity at other histamine receptor subtypes is of special interest.

Screening of Compounds at Other Histamine Receptors. The compounds were tested for their H_1 receptor activity on the guinea pig ileum and for their H_2 receptor activity at the guinea pig right atrium. All newly designed compounds show selectivity for the histamine H_3 receptor (cf. Table 2). The activity at the H_3 receptor clearly predominates. The worst selectivity ratio is of 1.5:1 log units for H_3 vs H_1/H_2 potency. In most cases the selectivity ratio is about 3 log units for H_3 vs H_1/H_2 potency. This shows that the novel carbamates, additionally to their pronounced activity *in vitro* and *in vivo*, are also selective compounds for the histamine H_3 receptor.

Conclusions

The new carbamates are potent and selective histamine H_3 receptor antagonists. Compounds with a substituted or an unsubstituted phenyl moiety at the nitrogen of the carbamate functionality are the most effective compounds *in vitro* and *in vivo*. Concerning the substitution pattern, a slight preference for the *para*-position could be observed. Aromatic and alicyclic substituents are well accepted by the H_3 receptor. Introduction or prolongation of a chain between the nitrogen of the carbamate moiety and the lipophilic residue did not improve activity. In general one surprising result is the activity of a large number of compounds being relatively constant within a series of compounds with different lipophilic moieties, different substituents, different positions of the substituents, and different chains. This homogeneity in the *in vitro* activity could be used for improving the pharmacokinetic properties of selected compounds *in vivo* for drug development. A lot of structural variations are tolerated by the receptor, which allows variation of absorption, metabolism, half-life, distribution, and affinity to other receptor subtypes. The new antagonists are useful pharmacological tools and potential drugs for histamine H_3 receptor dependent diseases.

Experimental Section

Chemistry. General Procedures. Melting points were determined on an Electrothermal IA 9000 digital or a Büchi 512 apparatus and are uncorrected. For all compounds ^{1}H NMR spectra were recorded on a Bruker AC 300 (300 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS as reference. ^{1}H NMR data are reported in the order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; asterisk, exchangeable by D_2O), number of protons, and approximate coupling constants in hertz. For example the spectra of parent compounds obtained by different synthesis (**1**, **2**, **22**, **42**, **45**) and of a substituted compound with advantageous pharmacological properties (**16**) are shown. Mass spectra were obtained on an EI-MS Finnigan MAT CH7A and a Finnigan MAT 711 or, in the case of FAB^+ spectra, a Finnigan MAT CH5DF instrument (xenon/DMSO/glycerol). Optical rotation was determined on a Perkin-Elmer 241 MC polarimeter. Elemental analyses (C, H, N) for all compounds were measured on Perkin-Elmer 240 B or Perkin-Elmer 240 C instruments and are within ±0.4% of the theoretical values. TLC was performed on silica gel PF_{254} plates (Merck). Preparative, centrifugally accelerated, radial thin layer chromatography was performed using a Chromatotron 7924T instrument (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 63−200 μm (Macherey, Nagel & Co.).

3-(1*H*-Imidazol-4-yl)propyl *N*-Phenylthiocarbamate (1). A solution of phenyl isothiocyanate (0.14 g, 5 mmol) in 20 mL

of dry acetonitrile was added to a prepared mixture of 3-(1*H*-imidazol-4-yl)propanol hydrochloride[30] (0.8 g, 5 mmol) and NaH (0.6 g, 15 mmol, suspension in toluene) in 50 mL of toluene. After 4 h 20 mL of MeOH was added, and the mixture was stirred for 30 min. The mixture was evaporated under reduced pressure, and the resulting oil was purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (gradient from 99:1 to 90:10), ammonia atmosphere]. Compound **1** was crystallized as hydrogen maleate from Et_2O/EtOH: ^1H NMR (Me_2SO-d_6) δ 11.1 (s*, 1H, CS-NH), 8.82 (s, 1H, Im-2-H), 7.37 (m, 6H, Im-5-H, 5Ph-H), 6.04 (s, 2H, Mal), 4.51 (br, 2H, CH_2-O), 2.73 (br, 2H, Im-CH_2), 2.07 (m, 2H, C*H*$_2$-CH_2-O); MS *m*/*z* 261 ($M^{\bullet+}$, <1), 135 (100), 109 (12), 108 (21), 107 (16), 95 (21), 93 (18), 91 (11), 82 (22), 81 (35), 77 (83). Anal. ($C_{13}H_{15}N_3OS\cdot C_4H_4O_4$) C, H, N.

N-Monosubstituted Carbamates 2−5, 7−21, and 23−40. A solution of the corresponding amine (5 mmol) in dry ethyl acetate was added to a solution of trichloromethyl chloroformate (1.19 g, 6 mmol) in 20 mL of dry ethyl acetate with a catalytic amount of charcoal. The reaction mixture was stirred at ambient temperature for 5 min and then heated to reflux until the mixture was getting clear. The solution was cooled and filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in 40 mL of dry acetonitrile, and 3-(1*H*-imidazol-4-yl)propanol hydrochloride[30] (0.8 g, 5 mmol) was added. The reaction mixture was refluxed for 2−4 h and concentrated in vacuo, and the residue was purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (95:5), ammonia atmosphere] to afford a colorless oil which was crystallized as hydrogen maleate from Et_2O/EtOH.

3-(1*H*-Imidazol-4-yl)propyl *N*-phenylcarbamate (2): ^1H NMR (Me_2SO-d_6) δ 9.65 (s*, 1H, CO-NH), 8.92 (s, 1H, Im-2-H), 7.45 (m, 3H, Im-5-H, Ph-2-H, Ph-6-H), 7.28 (m, 2H, Ph-3-H, Ph-5-H), 6.99 (m, 1H, Ph-4-H), 6.07 (s, 2H, Mal), 4.12 (m, 2H, CH_2-O), 2.75 (m, 2H, Im-CH_2), 1.99 (m, 2H, C*H*$_2$-CH_2-O); MS *m*/*z* 245 ($M^{\bullet+}$, <1), 173 (59), 119 (26), 109 (11), 108 (11), 95 (19), 91 (21), 82 (31), 81 (27), 72 (13), 55 (13), 54 (100), 53 (21). Anal. ($C_{13}H_{15}N_3O_2\cdot C_4H_4O_4\cdot 0.25\ H_2O$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-(4-fluorophenyl)carbamate (16): ^1H NMR (Me_2SO-d_6) δ 9.69 (s*, 1H, CO-NH), 8.88 (s, 1H, Im-2-H), 7.46 (m, 3H, Im-5-H, Ph-2-H, Ph-6-H), 7.13 (m, 2H, Ph-3-H, Ph-5-H), 6.05 (s, 2H, Mal), 4.12 (t, J = 6.5 Hz, 2H, CH_2-O), 2.74 (t, J = 7.6 Hz, 2H, Im-CH_2), 1.97 (m, 2H, C*H*$_2$-CH_2-O); MS *m*/*z* 263 ($M^{\bullet+}$, 3), 191 (45), 137 (64), 111 (100), 109 (41), 108 (19), 98 (12), 96 (23), 95 (59), 84 (39), 80 (11), 64 (14), 54 (39). Anal. ($C_{13}H_{14}FN_3O_2\cdot C_4H_4O_4\cdot 0.25\ H_2O$) C, H, N.

4-Aminophenyl-Substituted Carbamates 6 and 22. To a solution of the corresponding nitro derivatives **3** and **20** (5 mmol) in 20 mL of MeOH was added palladium on charcoal (10%, 0.4 g), and the mixture was hydrogenated (1 bar). When the calculated amount of hydrogen was absorbed, the reaction mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (95:5), ammonia atmosphere] to afford a colorless oil which was crystallized as hydrogen maleate from Et_2O/EtOH.

3-(1*H*-Imidazol-4-yl)propyl *N*-[(4-aminophenyl)methyl]carbamate (22): ^1H NMR (Me_2SO-d_6) δ 8.87 (s, 1H, Im-2-H), 7.48 (m*, 1H, CO-NH), 7.40 (s, 1H, Im-5-H), 6.91 (d, J = 8.2 Hz, 2H, Ph-2-H, Ph-6-H), 6.50 (d, J = 8.2 Hz, 2H, Ph-3-H, Ph-5-H), 6.05 (s, 2H, Mal), 3.98 (m, 4H, CH_2-O, NH-C*H*$_2$), 2.68 (t, J = 7.4 Hz, 2H, Im-CH_2), 1.88 (m, 2H, C*H*$_2$-CH_2-O); MS *m*/*z* 274 ($M^{\bullet+}$, 8), 148 (10), 126 (11), 121 (23), 109 (27), 108 (29), 107 (36), 106 (54), 99 (11), 98 (30), 96 (18), 95 (79), 88 (10), 82 (62), 81 (100), 80 (21), 53 (14), 51 (20). Anal. ($C_{14}H_{18}N_4O_2\cdot C_4H_4O_4\cdot 0.25H_2O$) C, H, N.

N,N-Disubstituted Carbamates 41−44. The corresponding amine (5 mmol) was added to a solution of trichloromethyl chloroformate (1.2 g, 6 mmol) in 20 mL of dry toluene with a catalytic amount of charcoal. The reaction mixture was stirred at ambient temperature for 5 min and then heated to reflux until the mixture was getting clear (8−12 h). The solution was cooled and filtered, and the solvent was evaporated under reduced pressure. The oily residue was dissolved in 40 mL of dry acetonitrile, and the solution was added to a prepared mixture of 3-(1*H*-imidazol-4-yl)propanol hydrochloride[30] (0.8 g, 5 mmol) and NaH (0.6 g, 15 mmol, 60% suspension in toluene) in 50 mL of toluene. After 4 h 20 mL of MeOH was added, and the mixture was stirred for 30 min. The mixture was evaporated under reduced pressure, and the resulting oil was purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (gradient from 99:1 to 90:10), ammonia atmosphere] to afford a colorless oil which was crystallized as hydrogen maleate from Et_2O/EtOH.

3-(1*H*-Imidazol-4-yl)propyl *N*-methyl-*N*-(phenylmethyl)carbamate (42): ^1H NMR (Me_2SO-d_6) δ 8.88 (s, 1H, Im-2-H), 7.21−7.43 (m, 6H, Im-5-H, 5Ph-H), 6.05 (s, 2H, Mal), 4.42 (s, 2H, N-CH_2), 4.07 (br, 2H, CH_2-O), 2.63−2.79 (m, 5H, Im-CH_2, CH_3), 1.94 (br, 2H, C*H*$_2$-CH_2-O); MS *m*/*z* 273 ($M^{\bullet+}$, 28), 109 (44), 108 (100), 107 (39), 98 (16), 95 (51), 91 (44), 82 (31), 81 (49), 80 (12), 54 (17). Anal. ($C_{15}H_{19}N_3O_2\cdot C_4H_4O_4\cdot 0.25\ H_2O$) C, H, N.

1,3-Phenylenebis[3-(1*H*-imidazol-4-yl)propyl *N*-methylcarbamate] (45). A solution of 1,3-bis(aminomethyl)-benzene (0.7 g, 5 mmol) in dry ethyl acetate was added to a solution of trichloromethyl chloroformate (2.4 g, 12 mmol) in 20 mL of dry ethyl acetate with a catalytic amount of charcoal. The same procedure as described for N-monosubstituted carbamates was performed, but a doubled amount of 3-(1*H*-imidazol-4-yl)propanol hydrochloride[30] (1.6 g, 10 mmol) was added. The reaction mixture was refluxed for 2−4 h and concentrated in vacuo, and the residue was purified by rotatory chromatography [eluent: $CHCl_3$/MeOH (95:5), ammonia atmosphere] to afford a colorless oil which was crystallized as hydrogen maleate from Et_2O/EtOH: ^1H NMR (Me_2SO-d_6) δ 8.88 (s, 2H, Im-2-H, Im-2'-H), 7.71 (m*, 2H, 2CO-NH), 7.40 (s, 2H, Im-5-H, Im-5'-H), 7.12 (m, 4H, 4Ph-H), 6.05 (s, 4H, Mal), 4.16 (m, 4H, NH-C*H*$_2$, NH-C*H*$_2$'), 3.99 (t, J = 6.5 Hz, 4H, CH_2-O, CH_2-O'), 2.69 (t, J = 7.5 Hz, 4H, Im-CH_2, Im-CH_2'), 1.91 (m, 4H, C*H*$_2$-CH_2-O, C*H*$_2$-CH_2-O'); MS *m*/*z* (FAB$^+$) 441 (M + $H^{+\cdot}$, 14), 109 (33). Anal. ($C_{22}H_{28}N_6O_4\cdot 2C_4H_4O_4\cdot 0.25\ H_2O$) C, H, N.

Pharmacology. General Methods: Histamine H_3 Receptor Assay on Synaptosomes from Rat Cerebral Cortex. The new compounds were tested for their H_3 receptor antagonist activity in an assay with K^+-evoked depolarization-induced release of [^{3}H]histamine from rat synaptosomes according to Garbarg et al.[27] A synaptosomal fraction from rat cerebral cortex prepared according to the method of Whittaker[36] was preincubated for 30 min with L-[^{3}H]histidine (0.4 μM) at 37 °C in a modified Krebs-Ringer solution. Then 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). Drugs 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] The K_i values were determined according to the Cheng−Prusoff equation.[37] The data presented are given as mean values with standard error of the mean for a minimum of three separate determinations each.

Histamine H_3 Receptor Antagonist Activity *in Vivo* in Mouse. *In vivo* testing for some selected compounds was performed after peroral administration to Swiss mice described by Garbarg et al.[27] The brain histamine turnover was determined by measuring the level of the main metabolite of histamine, N^τ-methylhistamine. Mice were fasted for 24 h before po treatment; 90 min after treatment animals were decapitated, and the cerebral cortex was prepared out. The cortex was homogenized in 10 vol of ice-cold perchloric acid (0.4 M). The N^τ-methylhistamine level was measured by a radioimmunoassay described by Garbarg et al.[38] Through treatment with 10 mg/kg thioperamide, the maximal N^τ-methylhistamine level was obtained and related to the level reached with the administered drug, and the ED_{50} value was calculated.[39]

***In Vitro* Screening at Other Histamine Receptors.** Selected compounds were screened for histamine H_2 receptor activity at isolated spontaneously beating guinea pig right atrium as well as for H_1 receptor activity at isolated guinea

pig ileum by standard methods described by Hirschfeld et al.[40] Each pharmacological test was performed at least in triplicate, but the exact type of receptor interaction was not determined in each case. The given values represent the mean.

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