Development of Chiral *N*-Alkylcarbamates as New Leads for Potent and Selective H_3-Receptor Antagonists: Synthesis, Capillary Electrophoresis, and in Vitro and Oral in Vivo Activity†

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Novel carbamates as derivatives of 3-(1*H*-imidazol-4-yl)propanol with an *N*-alkyl chain were prepared as histamine H_3-receptor antagonists. Branching of the *N*-alkyl side chain with methyl groups led to chiral compounds which were synthesized stereospecifically by a Mitsunobu protocol adapted Gabriel synthesis. The optical purity of some of the chiral compounds was determined (ee > 95%) by capillary electrophoresis (CE). The investigated compounds showed pronounced to high antagonist activity (K_i values of 4.1−316 nM) in a functional test for histamine H_3 receptors on rat cerebral cortex synaptosomes. Similar H_3-receptor antagonist activities were observed in a peripheral model on guinea pig ileum. No stereoselective discrimination for the H_3 receptor for the chiral antagonists was found with the in vitro assays. All compounds were also screened for central H_3-receptor antagonist activity in vivo in mice after po administration. Most compounds were potent agents of the H_3-receptor-mediated enhancement of brain N^{τ}-methylhistamine levels. The enantiomers of the *N*-2-heptylcarbamate showed a stereoselective differentiation in their pharmacological effect in vivo (ED_{50} of 0.39 mg/kg for the (*S*)-derivative vs 1.5 mg/kg for the (*R*)-derivative) most probably caused by differences in pharmacokinetic parameters. H_1- and H_2-receptor activities were determined for some of the novel carbamates, demonstrating that they have a highly selective action at the histamine H_3 receptor.

Introduction

Histamine H_3 receptors were first identified by Arrang et al. as autoreceptors on histaminergic neurons in slices of rat brain cortex.[5] On H_3-receptor activation, inhibition of histamine release[6] and histamine synthesis[7] have been observed on histaminergic axon terminals. H_3 receptors also occur on nonhistaminergic nerve endings, thus modulating the release of different neurotransmitters, e.g., dopamine,[8] serotonin,[9] acetylcholine,[10] and noradrenaline.[11] These neurotransmitters including histamine play an important part in the modulation and regulation of various physiological processes,[12] pathological conditions, and diseases in the central nervous system (CNS).[13] The highest density of histamine H_3 receptors was found in the brain,[14] implying a potential use of histamine H_3-receptor antagonists[13] inhibiting the negative feedback loop activated by endogenous histamine on auto- and heteroreceptors. Therapeutic applications of histamine H_3-receptor antagonists have been proposed for several diseases and conditions of the CNS, e.g., epilepsy,[15,16] schizophrenia,[17] arousal and sleep disorders,[18,19] eating[20] and drinking behavior,[21] memory and learning deficits,[22−24] and Alzheimer's disease.[25,26]

At present, thioperamide, the first potent and selective histamine H_3-receptor antagonist,[14] is a prototypic agent for this receptor system, showing high potency in vitro as well as in vivo (Chart 1, Table 1). Clobenpropit[27] is even more active in vitro, in subnanomolar concentrations, but is clearly less effective in vivo (Chart 1, Table 1). Neither thioperamide nor clobenpropit have been introduced into clinical trials, presumably due to hepatotoxicity caused by the thiourea or isothiourea moieties.

Stark et al. have described compounds having a carbamate moiety in order to retain electronic properties similar to those of thioperamide and clobenpropit but at the same time to decrease toxic side effects by avoiding sulfur-containing groups.[28] In this series, one compound [3-(1*H*-imidazol-4-yl)propyl *N*-(4-chlorophenyl)carbamate] showed an antagonist effect equal to that of thioperamide in vitro. The corresponding *N*-4-fluorophenyl and *N*-phenyl derivatives were almost equipotent with thioperamide in vivo.[28] Hence, these carbamates have provided promising leads for further

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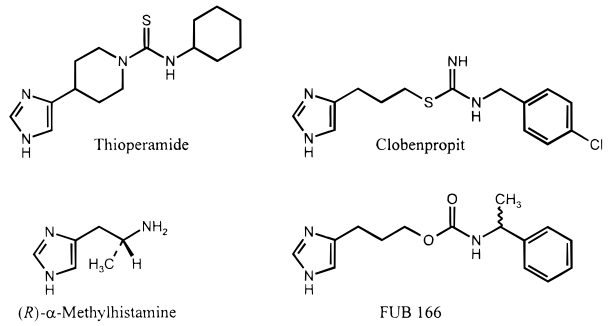
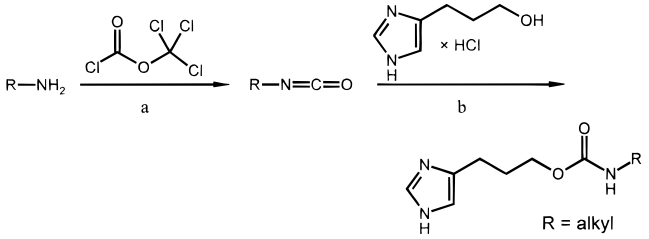
Chart 1



Scheme 1. General Synthesis of Carbamates[a]



[a] (a) Ethyl acetate, catalytic amount of charcoal, 4−5 h reflux; (b) acetonitrile, 4−5 h reflux.

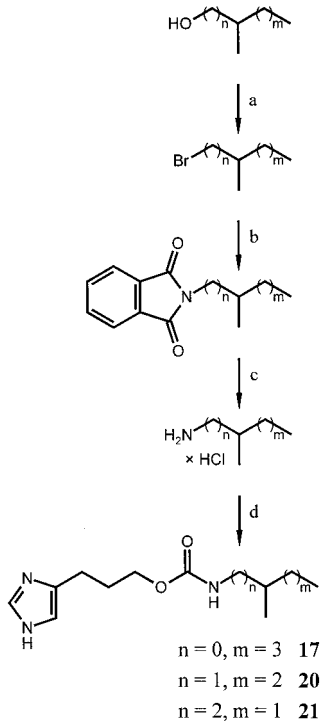
development. No stereochemical discrimination was observed for the only derivative containing a chiral center in this study (FUB 166, Chart 1). With an antagonist activity in the high nanomolar range ($K_i \approx$ 60 nM) the racemate as well as both enantiomers did not show any significant difference in vitro, and in vivo they were only moderately active with an ED_{50} of ca. 10 mg/kg po.[28]

In contrast, high stereoselective discrimination has been observed for histamine H_3-receptor agonists. For example, the prototypic agonist (*R*)-α-methylhistamine (Chart 1) is 2 orders of magnitude more active than the corresponding (*S*)-enantiomer.[29]

We have synthesized novel carbamates in the search for nontoxic, centrally acting, highly potent, and selective histamine H_3-receptor antagonists. We have extensively covered the field of *N*-alkylcarbamates by introducing *n*-alkyl chains with varying chain lengths, branching methyl groups in various positions, or heteroatomic (O, S) bridges as potential bioisosteric methylene replacements. Branching the side chain with methyl groups introduced a chiral center into some of the compounds. All the novel products were tested on the [^{3}H]histamine release from synaptosomes of rat cerebral cortex,[30] and selected compounds were tested in a second functional histamine H_3-receptor assay on guinea pig ileum.[31] Bearing in mind the potential use of H_3-receptor antagonists, we determined the central antagonist in vivo potency after po administration to mice.[30] Since some of the chiral compounds were highly potent in vitro and in vivo, we also prepared the pure enantiomers by stereospecific synthesis when the amine precursors were not commercially available (Scheme 3). In vitro and in vivo antagonist activity of the enantiomers was determined in order to assess a possible stereoselective ligand−receptor interaction.

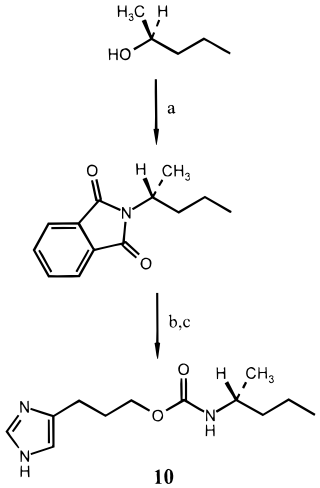
Additionally, some optically active isomers were analyzed by capillary electrophoresis to determine the enantiomeric excess (ee). Thus, a cyclodextrin-based

Scheme 2. Synthesis of Nonchiral Carbamates Starting from Alcohols[a]



[a] (a) 48% HBr, H_2SO_4 (concd); (b) potassium phthalimide, K_2CO_3, benzyltriethylammonium chloride, acetone, 4 h reflux; (c) (i) H_2NNH_2, EtOH, 15 min reflux, (ii) HCl, EtOH; (d) (i) diphosgene, ethyl acetate, catalytic amount of charcoal, 4−5 h reflux, (ii) 3-(1*H*-imidazol-4-yl)propanol hydrochloride, acetonitrile, 4−5 h reflux.

Scheme 3. Stereospecific Synthesis of Chiral Carbamates[a]



[a] (a) Phthalimide, DEAD, triphenylphosphine, THF, 3 days at rt; (b) (i) H_2NNH_2, EtOH, 15 min reflux, (ii) HCl, EtOH; (c) (i) diphosgene, ethyl acetate, catalytic amount of charcoal, 4−5 h reflux; (ii) 3-(1*H*-imidazol-4-yl)propanol hydrochloride, acetonitrile, 4−5 h reflux.

method for the separation of selected chiral compounds was developed (Figure 1). The activity at histamine H_1 and H_2 receptors for selected compounds was determined on guinea pig functional models (Table 2).

Chemistry

Synthesis. 3-(1*H*-Imidazol-4-yl)propanol[32] was used as the central building block for all of the title carbam-

ates. It was synthesized from urocanic acid by esterification followed by hydrogenation of the double bond over Pd/C.[33] To achieve higher yields in the synthetic sequence to this key intermediate, the imidazole ring was protected with triphenylmethyl chloride, followed by reduction of the ester moiety with complex hydrides and subsequent acidolytic cleavage of the protecting group.[32] *N*-Alkylcarbamates were constructed by the reaction of 3-(1*H*-imidazol-4-yl)propanol hydrochloride in acetonitrile with the appropriate isocyanate, which in turn was prepared from the corresponding amine by reaction with excess trichloromethyl chloroformate (diphosgene),[34] a liquid phosgene substitute (Scheme 1). The intermediate isocyanate was carefully separated from excess diphosgene by distillation in order to avoid reaction of excess diphosgene with the alcoholic function; then it was added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride. The use of 3-(1*H*-imidazol-4-yl)propanol in the form of its hydrochloride salt reduced side reactions, as the protonated imidazolium ring was not attacked by the isocyanate, thus resulting in increased yields.[28] Compounds **2**, **3**, **5**−**9**, **11**−**16**, and **22**−**28** were synthesized from commercially available amines (Scheme 1). Compound **1** was synthesized from the commercially available isocyanate. Racemic mixture **4** was obtained by mixing both enantiomers **5** and **6**. In the preparation sequence of **3** and **8** isolation of the isocyanates was not possible because of the similar boiling points of the isocyanate and diphosgene. In this case the crude reaction mixture containing the isocyanate was added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride in acetonitrile.

The appropriate amines for the synthesis of **17**, **20**, and **21** were obtained from the alkyl bromides via conventional Gabriel synthesis under phase-transfer conditions[35,36] and subsequent hydrazinolysis (Scheme 2). Alkyl bromides for **20** and **21** were prepared from the corresponding alcohols by standard procedures (Scheme 2).

Chiral amines for the synthesis of **10**, **18**, and **19** were prepared by stereospecific synthesis starting with the optically active alcohol. Mitsunobu protocol-adapted Gabriel synthesis transformed these optically active alcohols into the corresponding chiral *N*-alkylphthalimides by Walden inversion,[37] thus determining the absolute configuration of the enantiomers. Enantiomeric *N*-alkylphthalimides were treated as described before yielding the carbamates exemplified by **10** (Scheme 3).

Optical Purity. The optical rotation of optically active compounds was determined first, and then the optical purity of selected chiral compounds (**10**, **18**, **19**, **24**, **25**) was determined more precisely using capillary electrophoresis. A method based on cyclodextrins (CD) as chiral selectors was developed and optimized for separation of the corresponding racemates **9**, **17**, and **23**. Investigation of the potential for chiral separation with different cyclodextrins (α-CD, various substituted *β*-CDs) finally led to trimethyl-*β*-cyclodextrin (TM-*β*-CD), which allowed a separation of all the racemates investigated (Figure 1). TM-*β*-CD contains seven α-D-glucopyranose units and is methylated in positions 2, 3, and 6. Chiral CDs form diastereomeric inclusion complexes with the optically active analyte, leading to differing mobilities of the analyte in the electric field,

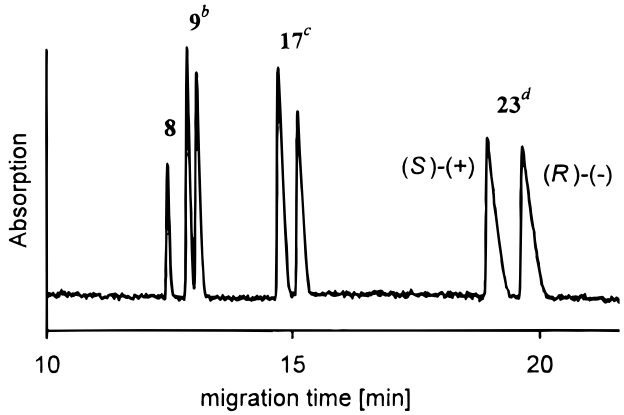


Figure 1. Separation of racemates **9**, **17,** and **23** with CE using TM-*β*-CD[a], taking **8** as internal standard. (a) BGE: 20 mg/mL TM-*β*-CD in 150 mM phospate buffer at pH = 2.7. Sample concentration: **8**, 0.5 mg/mL; **9**, **17**, **23**, 2.5 mg/mL. (b) $R_s = 1.30$. (c) $R_s = 1.80$. (d) $R_s = 1.87$.

thus resulting in separation of enantiomers. Under optimized conditions, simultaneous determination of all the compounds investigated was achieved with baseline resolution ($R_s \geq 1.3$). With increasing chain length (**9** → **17** → **23**) the resolution and migration time increased (Figure 1), presumably due to stronger interactions with TM-*β*-CD. Resolutions (R_s) were calculated using eq 1:

$$R_s = 1.177 \times \frac{(t_2 - t_1)}{(W_{h/2})_1 + (W_{h/2})_2} \qquad (1)$$

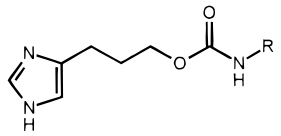
where t_2 is the migration time of the slower and t_1 the migration time of the faster moving enantiomer. $(W_{h/2})_1$ and $(W_{h/2})_2$ are the peak widths determined at half-peak height. By spiking racemates prior to injection with one enantiomer, we could determine the migration order of the enantiomers. In all cases the (*S*)-(+)-enantiomer displayed shorter migration time, indicating less interaction with TM-*β*-CD than the corresponding (*R*)-(−)-enantiomer (Figure 1).

For quantitative measurements the nonchiral compound **8** with similar structure was added as an internal standard. A calibration was performed to establish linearity of detector signal and sufficient resolution for a wide concentration range (not shown). Enantiomeric purity of **10**, **18**, **19**, **24**, and **25** was determined to be high with a minimum enantiomeric excess (ee) of 95%. The amount of unwanted enantiomer was determined to be 2.5% for **10** and **24**, 1.5% for **19**, and outside the limits of quantitation (LOQ = 1%) for **18** and **25**.

Pharmacological Results and Discussion

In Vitro Assay on Synaptosomes of Rat Cerebral Cortex and in Vivo Testing in Mouse CNS. The novel compounds were tested for their effect at histamine H_3 receptors in vitro on synaptosomes of rat cerebral cortex[30] (Tables 1 and 2) and in vivo on mice after po administration[30] (Table 1). All the compounds had antagonist properties at the histamine H_3 receptor. Whereas the in vitro model reflects the ligand−receptor interaction, the in vivo assay represents a combination of the pharmacodynamic interactions with the histaminergic system in the CNS and the pharmacokinetic properties of the compounds. Previously highly active compounds in vitro have been found to be much less

Table 1. Structures, Physical Data, and Results of the Screening on Histamine H_3-Receptor Antagonist Potency in Vitro and in Vivo in Rodents



no.	R	formula	M_r	yield (%)	mp[a] (°C)	$[\alpha]^{20}_D$[b] (deg)	K_i[c] (nM), $\bar{X} \pm S_{\bar{X}}$	ED_{50}[d] (mg/kg), $\bar{X} \pm S_{\bar{X}}$
1	CH_2-CH_3	$C_9H_{15}N_3O_2 \cdot 0.25H_2O$	201.7	20	40		316 ± 82	10 ± 2
2	$CH(CH_3)_2$	$C_{10}H_{17}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	331.8	45	113		82 ± 16	15 ± 5
3	$(CH_2)_2$-CH_3	$C_{10}H_{17}N_3O_2 \cdot C_4H_4O_4$	327.3	17	99		45 ± 9	2.7 ± 0.6
4	(*R*/*S*)-$CH(CH_3)CH_2$-CH_3	$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4$	341.4	*e*	103		20 ± 5	1.2 ± 0.3
5	(*R*)-$CH(CH_3)CH_2$-CH_3	$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.5H_2O$	350.4	31	104	−8.60	19 ± 5	2.2 ± 0.6
6	(*S*)-$CH(CH_3)CH_2$-CH_3	$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4$	341.4	24	103	+8.48	23 ± 5	5.3 ± 1.3
7	CH_2-$CH(CH_3)_2$	$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4$	341.4	48	105		30 ± 10	≥10
8	$(CH_2)_3$-CH_3	$C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4$	341.4	24	96		17 ± 2	1.0 ± 0.3
9	(*R*/*S*)-$CH(CH_3)(CH_2)_2$-CH_3	$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	359.9	37	109		25 ± 4	1.4 ± 0.4
10	(*R*)-$CH(CH_3)(CH_2)_2$-CH_3	$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4$	355.4	33	110	−5.25	12 ± 2	3.5 ± 1.1
11	(*R*/*S*)-CH_2-$CH(CH_3)CH_2$-CH_3	$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	359.9	53	97		14 ± 3	>10
12	(*S*)-CH_2-$CH(CH_3)CH_2$-CH_3	$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4$	355.4	36	96	+2.54	18 ± 4	>10
13	$(CH_2)_2$-$CH(CH_3)_2$	$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	359.9	32	100		6.5 ± 1.1	5.7 ± 0.8
14	$(CH_2)_4$-CH_3	$C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	359.9	85	91		8.5 ± 1.7	0.69 ± 0.37
15	$(CH_2)_3$-O-CH_3	$C_{11}H_{19}N_3O_3 \cdot C_4H_4O_4$	357.4	22	93		155 ± 40	>10
16	$(CH_2)_3$-S-CH_3	$C_{11}H_{19}N_3O_2S \cdot C_4H_4O_4$	373.4	26	84		22 ± 2	>10
17	(*R*/*S*)-$CH(CH_3)(CH_2)_3$-CH_3	$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$	369.4	47	109		8.7 ± 2.9	2.4 ± 0.7
18	(*R*)-$CH(CH_3)(CH_2)_3$-CH_3	$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$	369.4	41	110	−3.53	19 ± 4	1.8 ± 0.8
19	(*S*)-$CH(CH_3)(CH_2)_3$-CH_3	$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$	369.4	49	109	+3.74	12 ± 5	1.5 ± 0.3
20	(*R*/*S*)-CH_2-$CH(CH_3)(CH_2)_2$-CH_3	$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$	369.4	29	94		15 ± 5	2.6 ± 0.9
21	(*R*/*S*)-$(CH_2)_2$-$CH(CH_3)CH_2$-CH_3	$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$	369.4	46	82		5.1 ± 1.9	2.0 ± 0.3
22	$(CH_2)_5$-CH_3	$C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	373.9	82	86		8.6 ± 1.1	0.88 ± 0.62
23	(*R*/*S*)-$CH(CH_3)(CH_2)_4$-CH_3	$C_{14}H_{25}N_3O_2 \cdot C_4H_4O_4$	383.4	66	115		18 ± 4	1.1 ± 0.3
24	(*R*)-$CH(CH_3)(CH_2)_4$-CH_3	$C_{14}H_{25}N_3O_2 \cdot C_4H_4O_4$	383.4	44	115	−2.66	23 ± 8	1.5 ± 0.4
25	(*S*)-$CH(CH_3)(CH_2)_4$-CH_3	$C_{14}H_{25}N_3O_2 \cdot C_4H_4O_4$	383.4	34	115	+2.43	18 ± 3	0.39 ± 0.08
26	$(CH_2)_6$-CH_3	$C_{14}H_{25}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	388.0	38	102		4.1 ± 1.1	1.2 ± 0.3
27	(*R*/*S*)-$CH(CH_3)(CH_2)_5$-CH_3	$C_{15}H_{27}N_3O_2 \cdot C_4H_4O_4$	397.5	78	115		18 ± 5	3.2 ± 1.3
28	$(CH_2)_7$-CH_3	$C_{15}H_{27}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	402.0	37	104		5.1 ± 0.9	1.1 ± 0.2
thioperamide							4 ± 1[f]	1.0 ± 0.5[g]
clobenpropit							0.6 ± 0.1[h]	26 ± 7[g]

[a] Crystallization solvent: Et_2O/EtOH. [b] *c* 1.00, EtOH. [c] Functional H_3-receptor assay in vitro on synaptosomes of rat cerebral cortex.[30] [d] Central H_3-receptor test in vivo after po administration to mouse.[30] [e] Mixture of **5** and **6**. [f] Reference 14. [g] Reference 28. [h] Reference 44.

Table 2. Activity of Selected Compounds at Histamine Receptor Subtypes

	H_3		H_2	H_1
no.	pK_i[a]	pK_B[b]	pK_B[c]	pK_B[d]
2	7.1	7.0	4.0	<4.0
3	7.4	6.8	4.5	<3.5
7	7.5	7.4	<4.0	<4.5
8	7.8	7.2	3.4	3.8
9	7.6	7.7	3.6[e]	3.7
11	7.9	7.7	3.7[e]	4.0
13	8.2	7.2	3.8[e]	<4.0
14	8.1	7.3	4.2	4.7
22	8.1	7.5	4.0	4.5
23	7.7	7.8	4.3[e]	<4.5
26	8.4	7.7	4.9[e]	4.8[e]
27	7.7	7.3	4.8[e]	4.9
28	8.3	7.7	4.4	4.6

[a] Functional H_3-receptor assay on synaptosomes of rat cerebral cortex.[30] [b] Functional H_3-receptor assay on guinea pig ileum.[31,38] [c] Functional H_2-receptor test on guinea pig atrium.[40] [d] Functional H_1-receptor test on guinea pig ileum.[40] [e] pD'_2 value.

potent in vivo (cf. Table 1, clobenpropit).[32] For rational drug development the in vivo potency of a novel compound must be regarded as a crucial prerequisite. For this reason we have investigated the pharmacological effect at H_3 receptors of all of the title compounds not only in vitro but also in vivo.

In the series of the *n*-alkylcarbamates (**1**, **3**, **8**, **14**, **22**, **26**, **28**) the in vitro antagonist effect shows a tendency to improve with increasing chain length, with the highest activity occurring with a heptyl or octyl chain (**26**, **28**). The in vitro potencies of **26** and **28** were comparable to that of the reference ligand thioperamide. However, in vivo antagonist potency was not dependent on the chain length in the same way as in the functional in vitro assay. The optimum chain length for *n*-alkyl derivatives was achieved in vivo with the pentyl compound **14** showing an ED_{50} value below 1 mg/kg po. Introducing a terminal methoxy (**15**) or methylsulfanyl group (**16**) led to a decrease in activity in vitro and complete loss of antagonist potency in vivo.

Analogues of all the straight chain compounds were also synthesized wherein the side chain was branched with a methyl group in the α-position (**2**, **4**, **9**, **17**, **23**, **27**). Thus (apart from **2**), a chiral center was introduced into the molecule, and in order to investigate the stereochemical requirements of these chiral H_3-receptor antagonists, the enantiomers were prepared in high optical purity (**5**, **6**, **10**, **18**, **19**, **24**, **25**). The isopropyl derivative **2** showed lower activity in vitro compared to the other compounds in this α-branched series. Racemic **4** and its optical antipodes **5** and **6** showed no differences in vitro. Most other compounds branched in the α-position (**9**, **10**, **18**, **19**, **23**−**25**, **27**) were also within the same range of in vitro activity (K_i = 12−25 nM). For racemate **17** a slightly higher in vitro activity was found (K_i = 8.7 nM). Thus, no stereochemical differentiation was observed in vitro. Compound **2**, which was less active in vitro, also showed very low antagonist potency in vivo. Other compounds branched in the α-position (**4**−**6**, **9**, **10**, **17**−**19**, **27**) showed moderate to high activity after po administration to mice. Significant differences were only observed for **25**, the (*S*)-enantiomer of **23**

containing a *N*-2-heptyl side chain. This compound was remarkably potent in vivo with an ED_{50} of 0.39 mg/kg and is 2.5 times more active than thioperamide. In vivo compound **25** showed the highest potency of all the carbamates described. Surprisingly, **25** did not show any higher activity in vitro; thus it can be concluded that the affinity of **25** at the H_3 receptor has not increased. Pharmacokinetic factors must therefore account for the high antagonist potency observed in vivo.

Changing the branching position from α to *β* (**7**, **11**, **12**, **20**) led to a complete loss of the antagonist effect in vivo for **7**, **11**, and **12**. Compound **20**, the 2-methylpentyl derivative, retained its in vivo potency which was equipotent with the α-branched derivative **17** having the same chain length. In vitro the isobutyl derivative **7** is slightly less active than **11**, **12**, and **20**. Yet no chiral discrimination was observed, either in vivo or in vitro, for the *β*-branched series (**11**, **12**).

Introduction of the methyl side-chain branch into the *γ*-position (**13**, **21**) led to moderately potent compounds in vivo, whereas the in vitro activity was higher than that of compounds with the same chain length but with branching methyl groups in different positions (**9** → **11** → **13**; **17** → **20** → **21**).

All the novel carbamates show a high antagonist effect in vitro on synaptosomes of rat cerebral cortex with K_i values in the nanomolar range. Some compounds (**13**, **21**, **26**, **28**) can be regarded as being as active as the standard antagonist thioperamide. Moreover, under in vivo conditions several compounds (**4**, **8**, **9**, **14**, **19**, **22**−**26**, **28**) are at least equipotent to thioperamide. The chiral compound **25** is of particular interest due to its extremely high antagonist potency in vivo yet inconspicuous effect in vitro. The stereocenter of **25** and of the other optically active compounds does not seem to be involved in a close ligand−receptor interaction. The strikingly high potency under in vivo conditions of **25** must therefore be caused by pharmacokinetic properties of this compound. Although interactions with other receptor systems which might influence the in vivo effect cannot be excluded, this is unlikely to occur because of the high selectivity for the H_3 receptor of this class of compounds observed in vitro. Pharmacokinetic parameters which could differ regarding the (*S*)-enantiomer **25** could be absorption, metabolism, binding to plasma proteins, or crossing blood−brain barrier. Absorption and crossing blood−brain barrier are usually passive processes, being based on diffusion and mainly dependent on lipophilicity and lack of hydrogen-bonding capacity rather than stereochemical properties. Nevertheless participation of carrier-mediated processes may be involved in the membrane penetration and could conceivably be stereoselective. In general, processes involving proteins/enzymes leading to the formation of diastereomeric complexes with a chiral drug molecule could be responsible for the effect observed with **25**. The exact cause of the high in vivo potency of **25** was not investigated further but is still of interest inasmuch as, until now, no other histamine H_3-receptor antagonist having such a striking property has been described.

Screening of Selected Compounds at Other Functional Histamine Receptor Models. The potency of the title compounds was determined not only on synaptosomes of rat cerebral cortex but also for selected compounds on the guinea pig ileum, another functional model for the histamine H_3 receptor (Table 2).[31,38] Most compounds tested also showed pronounced to high antagonist activity ($pK_B > 7.0$) in the peripheral tissue model, measuring H_3-heteroreceptor function; only **3** was slightly less active. For compounds **3**, **8**, **14**, **22**, and **26**−**28** the observed antagonist activity is about 0.5 log unit lower in the assay on guinea pig ileum than in the functional model on synaptosomes of rat cerebral cortex. For **13** the difference in antagonist effect on these two functional models is as much as 1 log unit (pK_i = 8.2 vs $pK_B = 7.2$). Consistent pharmacological data were observed for **2**, **7**, **9**, **11**, and **23** with no significant difference between the two assays. Compound **23** ($pK_B = 7.8$) is the most active compound in the functional model on the guinea pig ileum. Relatively inconsistent values in these two assays have already been described for a group of 3-(1*H*-imidazol-4-yl)propyl ethers[39] and may be related to differences in animal species, incubation conditions (e.g., composition of media, duration), and access to the H_3 receptor within the preparations.

The activity at histamine H_1 and H_2 receptors was measured for selected compounds in functional tests on isolated organs (Table 2).[40] All compounds show pronounced selectivity for the histamine H_3 receptor. For most compounds the pK_B values at H_1 or H_2 receptors are below 4 (all of them are below $pK_B = 5$). This is negligible, especially when compared to the H_3-receptor affinity, the latter being 2.3−4.4 log units higher. For most compounds the difference is higher than 3 log units, demonstrating high selectivity for the third histamine receptor.

Conclusions

The title carbamates are highly potent antagonists at the histamine H_3 receptor in vitro and in vivo, and certain compounds have been shown to be selective for the H_3-receptor subtype. The highest in vitro activity on rat cerebral cortex synaptosomes was observed for straight chain *N*-heptyl and *N*-octyl derivatives, which are equipotent with thioperamide. In the *n*-alkyl series, the highest in vivo antagonist activity was found for pentyl- and hexyl-substituted carbamates, being slightly more active than thioperamide. Branching the alkyl chain with methyl groups in various positions introduced a chiral center into the molecule. Enantiomers of high optical purity were prepared using stereospecific synthesis. The enantiomeric excess of the chiral compounds was determined by capillary electrophoresis in a short time with low probe amounts. This is the first CE application to H_3-receptor ligands. For the methyl-branched compounds the highest in vitro activity was observed when branched in the *γ*-position. In vitro potency on rat synaptosomes was not influenced by stereochemical properties of the compounds since optical isomers did not show any significant differences. On the contrary, stereochemical differentiation was observed in vivo for only one enantiomeric pair. The eutomer **25** showed remarkably high in vivo antagonist activity, being 2.5 times more potent than thioperamide and 3.8 times more potent than its distomer **24**. The high activity of this enantiomer was most probably caused by pharmacokinetic differences, which have not been investigated further but are still of great interest.

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 following order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet; asterisk, exchangeable by D_2O; Im, imidazole; Mal, maleic acid), number of protons, and approximate coupling constants in hertz (Hz). Mass spectra were obtained on EI-MS Finnigan MAT CH7A and Finnigan MAT 711 (high-resolution mass spectra), spectrometers resolving power 12 500. Only spectral data of parent compounds, obtained by different synthesis, and of selected optically active compounds are shown (**1**, **3**, **5**, **8**, **10**, **12**, **16**−**20**, **24**). 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, unless otherwise stated. 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 63−200 μm (Macherey, Nagel & Co.). TLC was performed on silica gel PF_{254} plates (Merck), and the spots were visualized with iodine vapor or fast blue salt BB.

General Synthetic Procedure for Carbamates 1−28 (unless otherwise stated). To a solution of trichloromethyl chloroformate (0.6 g, 3 mmol) and a catalytic amount of activated charcoal in 20 mL of dry ethyl acetate was added rapidly the corresponding amine hydrochloride (2.5 mmol) as a solid or a solution of the corresponding amine (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 3-(1*H*-imidazol-4-yl)-propanol hydrochloride[32] (0.4 g, 2.5 mmol) 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 controlled by thin-layer chromatography [solvent: $CHCl_3$/MeOH (9:1), ammonia atmosphere]. The products were obtained as colorless oils and crystallized as hydrogen maleates in Et_2O/EtOH. Yields: 17−85%.

3-(1*H*-Imidazol-4-yl)propyl *N*-Ethylcarbamate (1). The carbamate was synthesized directly from ethyl isocyanate and crystallized as free base: ^{1}H NMR (Me_2SO-d_6) δ 7.36 (s, 1H, Im-2-H), 6.97 (t, 1H, CONH*), 6.51 (s, 1H, Im-5-H), 3.82 (t, J = 6.5 Hz, 2H, CH_2-O), 3.17 (m, 2H, NH-CH_2), 2.85 (m, 2H, Im-CH_2), 2.19 (m, 2H, Im-CH_2-CH_2), 0.64 (t, J = 7.2 Hz, 3H, CH_3); MS m/z 197 ($M^{\bullet+}$, 29), 108 (44), 95 (47), 81 (35); HRMS; calcd 197.11643, found 197.11648. Anal. ($C_9H_{15}N_3O_2 \cdot 0.5H_2O$) C, H; N: calcd, 20.83; found, 19.86.

3-(1*H*-Imidazol-4-yl)propyl *N*-Propylcarbamate (3). The isocyanate was not separated by distillation from excess diphosgene, but the crude reaction product was directly added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride[32] in dry acetonitrile: ^{1}H NMR (Me_2SO-d_6) δ 8.87 (s, 1H, Im-2-H), 7.40 (s, 1H, Im-5-H), 7.10 (m, CONH*), 6.05 (s, 2H, Mal), 3.96 (t, J = 6.5 Hz, 2H, CH_2-O), 2.92 (m, 2H, NH-CH_2), 2.67 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2-CH_2), 1.39 (m, 2H, CH_2-CH_3), 0.83 (t, J = 7.4 Hz, 3H, CH_3); MS m/z 211 ($M^{\bullet+}$, 13), 108 (100), 95 (89), 81 (92), 54 (29), 41 (29), 28 (35). Anal. ($C_{10}H_{17}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*R*)-(−)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylpropyl)-carbamate (5): ^{1}H NMR (Me_2SO-d_6) δ 8.80 (s, 1H, Im-2-H), 7.37 (s, 1H, Im-5-H), 6.95 (d, J = 7.7 Hz, 1H, CONH*), 6.00 (s, 2H, Mal), 4.00 (t, J = 6.50 Hz, 2H, CH_2-O), 3.30 (m, 1H, CH), 2.70 (t, J = 7.7 Hz, 2H, Im-CH_2), 1.90 (m, 2H, Im-CH_2-CH_2), 1.40−1.30 (m, 2H, CH_2-CH_3), 1.05 (d, J = 6.59 Hz, 3H, CH-CH_3), 0.80 (t, J = 7.4 Hz, 3H, CH_2-CH_3); MS m/z 225 ($M^{\bullet+}$, 12), 108 (100), 98 (17), 95 (43), 81 (44), 54 (16). Anal. ($C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4 \cdot 0.5\ H_2O$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-Butylcarbamate (8). The isocyanate was not separated by distillation from excess diphosgene, but the crude reaction product was directly added to 3-(1*H*-imidazol-4-yl)propanol hydrochloride[32] in dry acetonitrile: ^{1}H NMR (Me_2SO-d_6) δ 8.89 (s, 1H, Im-2-H), 7.40 (s, 1H, Im-5-H), 7.08 (m, 1H, CONH*), 6.06 (s, 2H, Mal), 3.96 (t, J = 6.4 Hz, 2H, CH_2-O), 2.96 (m, 2H, NH-CH_2), 2.67 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2-CH_2), 1.41−1.21 (m, 4H, CH_2-CH_2-CH_3), 0.86 (t, J = 7.2 Hz, 3H, CH_3); MS m/z 225 ($M^{\bullet+}$, 12), 108 (100), 95 (99), 81 (85), 72 (59), 54 (57), 45 (51), 26 (86). Anal. ($C_{11}H_{19}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*R*)-(−)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-Methylbutyl)-carbamate (10). A mixture of phthalimide (1.47 g, 10 mmol), triphenylphosphine (2.62 g, 10 mmol), and (*S*)-(+)-2-pentanol (0.88 g, 10 mmol) in 10 mL of dry THF was cooled to 0 °C. Diethyl azodicarboxylate (DEAD) (1.74 g, 10 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. After the precipitate was filtered, the solvent was evaporated and the residue purified by column chromatography (eluent: CH_2Cl_2) to afford (*R*)-(−)-*N*-(1-methylbutyl)phthalimide (yield 75%): $[\alpha]^{20}_{546}$ −11.93° (*c* 3.00, EtOH).

(*R*)-(−)-*N*-(1-Methylbutyl)phthalimide (2.3 g, 10 mmol) and hydrazine hydrate (0.62 g, 10 mmol) in 40 mL of EtOH were refluxed for 15 min. The suspension was cooled, filtered, acidified with hydrochloric acid, and once more filtered. The filtrate was concentrated under reduced pressure; the crystalline (*R*)-(+)-(1-methylbutyl)amine hydrochloride ($[\alpha]^{20}_{546}$ +3.00° (*c* 1.50, MeOH)) was washed with diethyl ether and used for further synthesis.

(*R*)-(−)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylbutyl)carbamate (**10**) was prepared following the general procedure described above: ^{1}H NMR (Me_2SO-d_6) δ 8.80 (s, 1H, Im-2-H), 7.39 (s, 1H, Im-5-H), 6.90 (d, J = 8.2 Hz, 1H, CONH*), 6.05 (s, 2H, Mal), 3.96 (t, J = 6.4 Hz, 2H, CH_2-O), 3.47 (m, 1H, CH), 2.68 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.90 (m, 2H, Im-CH_2-CH_2), 1.30−1.20 (m, 4H, CH_2-CH_2-CH_3), 1.00 (d, J = 6.6 Hz, 3H, CH-CH_3), 0.85 (t, J = 7.0 Hz, 3H, CH_2-CH_3); MS m/z 239 ($M^{\bullet+}$, 21), 108 (100), 98 (17), 95 (63), 81 (38), 54 (14). Anal. ($C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*S*)-(+)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylpropyl)-carbamate (12): ^{1}H NMR (Me_2SO-d_6) δ 8.90 (s, 1H, Im-2-H), 7.40 (s, 1H, Im-5-H), 7.10 (m, 1H, CONH*), 6.00 (s, 2H, Mal), 4.00 (t, J = 6.6 Hz, 2H, CH_2-O), 2.90 (m, 2H, NH-CH_2), 2.70 (t, J = 7.6 Hz, Im-CH_2), 1.90 (m, 2H, Im-CH_2-CH_2), 1.40−1.30 (m, 2H, CH_2-CH_3), 1.10−1.00 (m, 1H, CH), 0.90 (m, 6H, 2•CH_3); MS m/z 239 ($M^{\bullet+}$, 18), 109 (28), 108 (100), 95 (58), 81 (37), 72 (17). Anal. ($C_{12}H_{21}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

3-(1*H*-Imidazol-4-yl)propyl *N*-(3-methylsulfanyl-1-propyl)carbamate (16): ^{1}H NMR (Me_2SO-d_6) δ 8.84 (s, 1H, Im-2-H), 7.38 (s, 1H, Im-5-H), 7.14 (s, 1H, CONH*), 6.04 (s, 2H, Mal), 3.96 (t, J = 6.5 Hz, 2H, CH_2-O), 3.04 (m, 2H, NH-CH_2), 2.66 (t, J = 7.6 Hz, 2H, Im-CH_2), 2.44 (t, J = 7.3 Hz, 2H, CH_2-S), 2.02 (s, 3H, CH_3), 1.88 (m, 2H, Im-CH_2-CH_2), 1.65 (m, 2H, CH_2-CH_2-S); MS m/z 257 ($M^{\bullet+}$, 29), 242 (40), 211 (22), 183 (31), 109 (100), 98 (29), 81 (47), 72 (36), 54 (29). Anal. ($C_{11}H_{19}N_3O_2S \cdot C_4H_4O_4$) C, H, N.

(*R*/*S*)-(±)- 3-(1*H*-Imidazol-4-yl)propyl *N*-(1-Methylpentyl)carbamate (17). A mixture of potassium phthalimide (1.85 g, 10 mmol), K_2CO_3 (4.0 g, 30 mmol), and benzyltriethylammonium chloride (0.3 g, 1 mmol) in 50 mL of acetone was refluxed for 40 min. (*R*/*S*)-(±)-1-Methylpentyl bromide (1.65 g, 10 mmol) in 10 mL of acetone was added dropwise and refluxed for additional 4 h. The precipitate was filtered and the solvent evaporated under reduced pressure. The oily residue was dissolved in 50 mL of CH_2Cl_2 and washed twice with NaOH solution (2%) and water. The organic phase was dried with anhydrous sodium sulfate and concentrated in

vacuo. The residue was purified by column chromatography (eluent: CH_2Cl_2) to afford (*R*/*S*)-(±)-*N*-(1-methylpentyl)phthalimide.

(*R*/*S*)-(±)-*N*-(1-Methylpentyl)phthalimide was cleaved as described for **10**, affording (*R*/*S*)-(±)-(1-methylpentyl)amine hydrochloride. (*R*/*S*)-(±)- 3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylpentyl)carbamate (**17**) was prepared following the general procedure described above: ^{1}H NMR (Me_2SO-d_6) δ 8.80 (s, 1H, Im-2-H), 7.40 (s, 1H, Im-5-H), 6.95 (d, J = 9.2 Hz, 1H, CONH*), 6.00 (s, 2H, Mal), 4.00 (t, J = 6.4 Hz, 2H, CH_2-O), 3.45 (m, 1H, CH), 2.68 (t, J = 7.4 Hz, 2H, Im-CH_2), 1.90 (m, 2H, Im-CH_2-CH_2), 1.40−1.10 (m, 6H, (CH_2)$_3$-CH_3), 1.00 (d, J = 6.6 Hz, 3H, CH-CH_3), 0.85 (t, J = 7.00 Hz, 3H, (CH_2)$_3$-CH_3); MS *m*/*z* 253 ($M^{\bullet+}$, 25), 108 (100), 95 (53), 81 (45), 54 (20). Anal. ($C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*R*)-(−)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylpentyl)-carbamate (18): Synthetic procedure as described for **10** starting with (*S*)-(+)-2-hexanol; (*R*)-(−)-*N*-(1-methylpentyl)-phthalimide (yield 69%; $[\alpha]^{20}_{546}$ −9.00° (*c* 3.00, EtOH); (*R*)-(+)-(1-methylpentyl)amine hydrochloride ($[\alpha]^{20}_{546}$ +4.39° (*c* 1.50, MeOH)); ^{1}H NMR and MS spectral data for **18**, see **17**. Anal. ($C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*S*)-(+)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylpentyl)-carbamate (19). Synthetic procedure as described for **10** starting with (*R*)-(−)-2-hexanol; (*S*)-(+)-*N*-(1-methylpentyl)-phthalimide (yield 73%; $[\alpha]^{20}_{546}$ +19.60° (*c* 3.00, EtOH); (*S*)-(−)-(1-Methylpentyl)amine hydrochloride[41] ($[\alpha]^{20}_{546}$ −4.30° (*c* 1.50, MeOH)); ^{1}H NMR and MS spectral data for **19**, see **17**. Anal. ($C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*R*/*S*)-(±)-3-(1*H*-Imidazol-4-yl)propyl *N*-(2-Methylpentyl)carbamate (20). (*R*/*S*)-(±)-2-Methylpentanol (0.1 g, 10 mmol) was treated with 48% HBr in concentrated H_2SO_4 to afford (*R*/*S*)-(±)-2-methylpentyl bromide. (*R*/*S*)-(±)-*N*-(2-Methylpentyl)phthalimide was prepared as described for **17**. (*R*/*S*)-(±)-*N*-(2-Methylpentyl)phthalimide was cleaved as described for **10**, affording (*R*/*S*)-(±)-(2-methylpentyl)amine hydrochloride: ^{1}H NMR (Me_2SO-d_6) δ 8.87 (s, 1H, Im-2-H), 7.39 (s, 1H, Im-5-H), 7.11 (m, 1H, CONH*), 6.04 (s, 2H, Mal), 3.97 (t, J = 6.5 Hz, 2H, CH_2-O), 2.90−2.73 (m, 2H, NH-CH_2), 2.67 (t, J = 7.6 Hz, 2H, Im-CH_2), 1.90 (m, 2H, Im-CH_2-CH_2), 1.50 (m, 1H, CH), 1.40−1.00 (m, 4H, CH_2-CH_2-CH_3), 0.85 (m, 6H, 2·CH_3); MS *m*/*z* 253 ($M^{\bullet+}$, 25), 108 (100), 95 (49), 81 (27). Anal. ($C_{13}H_{23}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

(*R*)-(−)-3-(1*H*-Imidazol-4-yl)propyl *N*-(1-methylhexyl)-carbamate (24): ^{1}H NMR (Me_2SO-d_6) δ 8.87 (s, 1H, Im-2-H), 7.39 (s, 1H, Im-5-H), 6.95 (d, J = 8.3 Hz, 1H, CONH*), 6.05 (s, 2H, Mal), 3.95 (t, J = 6.5 Hz, 2H, CH_2-O), 3.44 (m, 1H, CH), 2.67 (t, J = 7.5 Hz, 2H, Im-CH_2), 1.89 (m, 2H, Im-CH_2-CH_2), 1.31−1.22 (m, 8H, (CH_2)$_4$), 1.01 (d, J = 6.6 Hz, 3H, CH-CH_3), 0.85 (t, J = 6.5 Hz, 3H, (CH_2)$_4$-CH_3); MS *m*/*z* 267 ($M^{\bullet+}$, 11), 108 (100), 95 (60), 81 (77), 54 (48), 41 (33), 26 (54). Anal. ($C_{14}H_{25}N_3O_2 \cdot C_4H_4O_4$) C, H, N.

Capillary Electrophoresis (CE). CE separations were carried out using an automated CE apparatus (Beckman P/ACE 2100); Software Gold 7.11, capillary 57 cm × 50 μm i.d. (Grohm), 50 cm to the detector; UV detection at 200 nm; voltage 25 kV; capillary temperature thermostated at 25.0 °C; sample injection 2 s with pressure (5 bar). Composition of the background electrolyte (BGE): 150 mM phosphate buffer (H_3PO_4, 85%) adjusted to pH = 2.7 with triethanolamine, 20 mg/mL trimethyl-β-cyclodextrin (Fluka); I = 65 μA. At the beginning of each working day the capillary was rinsed for 15 min with 0.1 N NaOH, for 5 min with H_2O, and for 5 min with BGE. Between each run the capillary was rinsed for 1 min with 0.1 N NaOH and for 3 min with BGE. Each experiment was performed at least in triplicate.

Pharmacology. General Methods. Histamine H_3-Receptor Assay on Synaptosomes of Rat Cerebral Cortex. 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.[30] A synaptosomal fraction from rat cerebral cortex prepared according to the method of Whittaker[42] 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). 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.[30] K_i values were determined according to the Cheng−Prusoff equation.[43] The data presented are given as mean values with standard error of the mean (SEM) for a minimum of three separate determinations each.

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 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.[31]

Histamine H_3-Receptor Antagonist Potency in Vivo in Mouse. In vivo testing was performed after peroral administration to Swiss mice as described by Garbarg et al.[30] 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 po treatment. Animals were decapitated 90 min after treatment, 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 radioimmunoassay.[45] By 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 as mean with SEM.[46]

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.[40] 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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