Novel Nonimidazole Histamine H_3 Receptor Antagonists: 1-(4-(Phenoxymethyl)benzyl)piperidines and Related Compounds

Tibor Mikó,[§] Xavier Ligneau,[||] Heinz H. Pertz,[§] C. Robin Ganellin,[⊥] Jean-Michel Arrang,[†] Jean-Charles Schwartz,[||] Walter Schunack,[§] and Holger Stark*[,‡]

Institut für Pharmazie, Freie Universität Berlin, Königin-Luise-Strasse 2+4, 14195 Berlin, Germany, Bioprojet Biotech, 4 rue du Chesnay Beauregard BP 96205, 35762 Saint Grégoire Cedex, France, Department of Chemistry, Christopher Ingold Laboratories, University College London, 20 Gordon Street, London WC1H 0AJ, U.K.; Unité de Neurobiologie et Pharmacologie Moléculaire, Centre Paul Broca de l'INSERM, 2ter rue d'Alésia, 75014 Paris, France, and Institut für Pharmazeutische Chemie, Johann Wolfgang Goethe-Universität, Biozentrum, Marie-Curie-Strasse 9, 60439 Frankfurt am Main, Germany

Received October 30, 2002

In an extension of very recently published studies on successful imidazole replacements in some series of histamine H_3 receptor antagonists, we report on a new class of lipophilic nonimidazole antagonist having an aliphatic tertiary amino moiety connected to a benzyl template substituted in the 4-position by a phenoxymethyl group. The structural modifications were performed with the intention to avoid possible negative side effects reported for other series of antagonists. The novel compounds combine different characteristics of recently developed histamine H_3 receptor antagonists. The compounds were screened for their affinity in a binding assay for the human histamine H_3 receptor stably expressed in CHO−K1 cells and tested for their in vivo potency in the central nervous system of mice after oral administration. Different substitution patterns on the phenoxy group were used to optimize in vitro and/or in vivo potency leading to some compounds with low nanomolar affinity and high oral in vivo potency. Modifications of the basic piperidino moiety were performed by ring expansion, contraction, and opening. Selected compounds exhibited selectivity in functional assays on isolated organs of guinea-pig for H_3 vs H_1 and H_2 receptors. Unexpectedly, some of the novel antagonists also showed a slight preference for the human histamine H_3 receptor compared to their affinities for the guinea-pig H_3 receptor.

Introduction

Histamine H_3 receptors were first identified by Arrang et al. as autoreceptors on histaminergic neurons in rat brain cortex.[1] While histamine acts as a neurotransmitter at postsynaptic H_1 and H_2 receptors, activation of the presynaptically localized histamine H_3 receptor exerts a negative feedback on histamine synthesis and release in histaminergic neurons.[2,3] In addition, H_3 receptors were found to be presynaptic heteroreceptors modulating the release of other neurotransmitters.[4−7] The physiological role of the recently cloned H_4 receptor is currently under investigation in different laboratories.[8,9] Due to the important role of the H_3 receptor in (patho)physiological conditions, antagonists are proposed to have a therapeutic use in neurological disorders and psychiatric diseases such as schizophrenia, attention-deficit hyperactivity disorder (ADHD), dementia, epilepsy, obesity, and narcolepsy.[10] To support this suggestion GT-2331 (cipralisant, formerly Perceptin) has been described as entering phase II in clinical trials for the treatment of ADHD.[11,12]

The recent cloning of rodent and especially human H_3 receptors and the identification of different isoforms in rodents as well as in man has opened a new chapter in the understanding of the role of the third histamine receptor subtype.[13−18] There are also new insights in receptor−ligand interactions.[19,20] For the last years several attempts to replace the imidazole moiety common to most classes in this field have been carried out.[21−24] We reported the successful discovery of UCL 1972 (K_i (rat) = 39 ± 11 nM; ED_{50} (mouse) = 1.1 ± 0.6 mg/kg po; Figure 1) as a novel prototype for nonimidazole histamine H_3 receptor antagonists with relatively high in vivo activity.[25] These results inspired our investigations to replace imidazole by a piperidino group in different classes of known H_3 receptor antagonists[26]

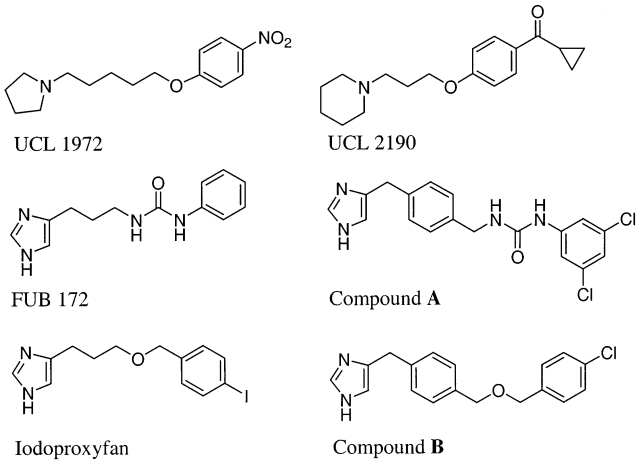


Figure 1.

* To whom correspondence should be addressed. Tel.: +49-69-798 29302. Fax.: +49-69-798 29258. E-mail: h.stark@pharmchem.uni-frankfurt.de.
[§] Freie Universität Berlin.
[||] Bioprojet Biotech.
[⊥] University College London.
[†] Centre Paul Broca de l'INSERM.
[‡] Johann Wolfgang Goethe-Universität Frankfurt am Main.

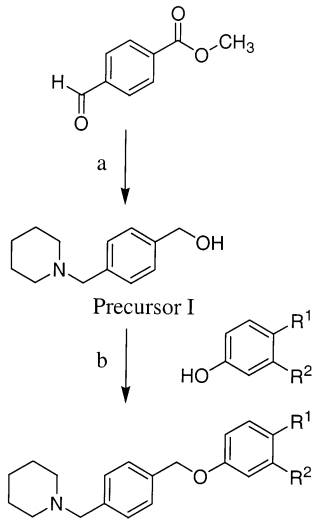
10.1021/jm021084k CCC: $25.00
Published on Web 03/07/2003

to reveal general patterns for their pharmacological behavior and to avoid possible therapeutic drawbacks such as poor absorption, poor penetration through the blood−brain barrier, interaction with CYP 450 isoenzymes, and/or hepatic toxicity, although these parameters are beyond the scope of this paper. Compared to the related imidazole-containing H_3 receptor antagonists, almost all of these novel nonimidazole compounds have an aminoalkyl moiety in common but with different chain length and chain structures. The development of UCL 2190 is one example of this replacement strategy, ciproxifan[27] being lead structure (Figure 1).[26] Recently another scaffold was synthesized containing a 4-((1*H*-imidazol-4-yl)methy)benzyl template (compounds **A**, **B**; Figure 1).[28] These imidazole-containing analogues with different functionalities (from Schering Plough) show strong similarities to our own 3-(1*H*-imidazol-4-yl)propyl series. The trimethylene chain was exchanged with a *p*-xylene-α,α′-diyl moiety. Urea derivative compound **A** can be compared to FUB 172,[29] and the benzyl ether compound **B** can be compared to the proxyfan series, iodoproxyfan being the most prominent reference compound.[30] Since iodoproxyfan shows partial agonist properties, we followed the replacement strategy in the proxifan series: a phenoxy moiety instead of a benzyloxy moiety. Supported by the promising results of UCL 2190, this series seemed more encouraging than other leads. Here we report on the development of a novel class of nonimidazole histamine H_3 receptor antagonists based on a 4-(phenoxymethyl)benzylamine template. As in the related imidazole-containing series where optimized substitution patterns were described to be different to that found in the proxifan series, we investigated numerous substituents and substitution patterns on the phenoxy group. In addition, some changes were performed on the alkyl groups at their amino functionality. Displacement experiments were performed with all compounds at human histamine H_3 receptors stably expressed in CHO−K1 cells.[19,31] In vivo screening was performed after oral administration to mice, measuring the level of the main histamine metabolite, N^{τ}-methylhistamine, in the cerebral cortex.[32] Results from this in vivo screening reflect numerous pharmacodynamic and pharmacokinetic parameters leading to a general rating of compounds. The failure of in vivo potency can have different reasons which were not investigated. The most interesting compounds were also tested in functional models on isolated organs of guinea-pig for H_3[33] and/or for H_1 and H_2 receptor potencies.[34]

Chemistry

Synthesis. The key intermediate for the preparation of different ethers was 4-(piperidinomethyl)benzyl alcohol, which was prepared in two steps from 4-formylbenzoic acid methyl ester. First reductive amination was carried out with piperidine and titanium(IV) isopropoxide as Lewis acid catalyst,[35] a method that provides a mild and effective way to alkylate amines with aldehydes. The remaining ester group of the resulting 4-(piperidinomethyl)benzoic acid methyl ester was reduced to the corresponding benzyl alcohol (Precursor I, Scheme 1) with $NaBH_4$ in diglyme in the presence of aluminum chloride.[36] The combination of a borohydride with aluminum chloride results in a system with increased reducing power, which is as potent as lithium aluminum hydride, but relatively nonhazardous. Ether formation with the corresponding commercially available phenol derivatives was then performed using a Mitsunobu-type procedure.[37] Although this reaction provides a convenient way for variations in the substitution pattern on the phenoxy group and for purification of the products due to small amounts of side products, different methods for preparation were performed. For amine variations, compounds **28** and **36**−**38** were prepared by S_N2 reaction of *p*-α,α′-dibromoxylene in excess with 4-hydroxyacetophenone, resulting in 1-(4-(4-(bromomethyl)benzyloxy)phenyl)ethanone (Precursor II) as main product (Scheme 2), and then the final compounds were obtained by alkylation of the related amines. Compound **29** was synthesized by S_NAR reaction of 4-fluorophenyl-cyclopropylmethanone with 1,4-benzenedimethanol to the intermediate cyclopropyl-(4-(4-(hydroxymethyl)benzyloxy)phenyl)methanone. Con-

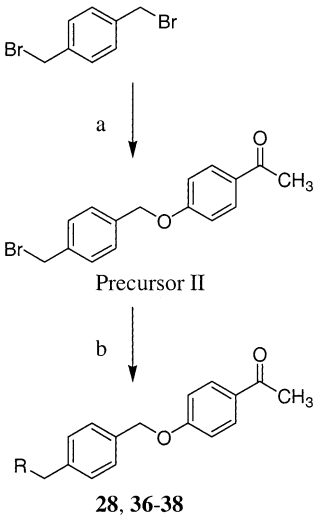
Scheme 1. General Procedure for Mitsunobu Type Phenyl Ethers[a]



1-**27**, **30**, **33**-**35**

[a] (a) (i) Piperidine, $Ti[OCH(CH_3)_2]_4$, $NaBH_3CN$, MeOH, (ii) $NaBH_4$, $AlCl_3$, diglyme; (b) Ph_3P, DEAD, THF.

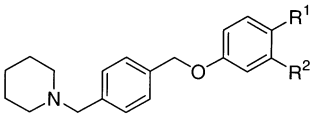
Scheme 2. General Procedure for Compounds with Different Amine Moieties[a]



28, **36**-**38**

[a] (a) 4-Hydroxyacetophenone, K_2CO_3, acetone; (b) K_2CO_3, corresponding secondary amine, EtOH.

Table 1. Structures, Chemical Data, and Pharmacological Results of Substituted 1-(4-(Phenoxymethyl)benzyl)piperidines for in Vitro Affinity at Human Histamine H_3 Receptor and Oral Antagonist Potency in Vivo in Mice



no.	R^1	R^2	formula	M_r	yield (%)	mp (°C)	K_i[a] (nM)	ED_{50}[b] (mg/kg) $\bar{x} \pm s_{\bar{x}}$
1	H	H	$C_{19}H_{23}NO \cdot C_2H_2O_4 \cdot 0.25H_2O$	375.6	31	135	227	≈30
2	F	H	$C_{19}H_{22}FNO \cdot C_2H_2O_4$	389.2	20	143	123	≈15
3	Cl	H	$C_{19}H_{22}ClNO \cdot C_2H_2O_4$	405.6	60	165	124	>10
4	Cl	F	$C_{19}H_{21}ClFNO \cdot C_2H_2O_4$	423.6	55	150	95	>10
5	Cl	Cl	$C_{19}H_{21}Cl_2NO \cdot C_2H_2O_4 \cdot 0.25H_2O$	444.6	52	121	167	>10
6	Cl	CH_3	$C_{20}H_{24}ClNO \cdot C_2H_2O_4$	419.6	27	113	42	>10
7	Br	H	$C_{19}H_{22}BrNO \cdot C_2H_2O_4$	450.0	42	178	114	>10
8	I	H	$C_{19}H_{22}INO \cdot C_2H_2O_4 \cdot 0.25H_2O$	501.6	10	176−177	107	>10
9	CH_3	H	$C_{20}H_{25}NO \cdot C_2H_2O_4$	385.2	43	162−164	126	1.8 ± 0.4
10	CF_3	H	$C_{20}H_{22}F_3NO \cdot C_2H_2O_4 \cdot 0.25H_2O$	443.6	19	139	125	>10
11	CH_3	CH_3	$C_{21}H_{27}NO \cdot C_2H_2O_4$	399.2	41	147−148	149	4.8 ± 0.8
12	H	CH_3	$C_{20}H_{25}NO \cdot C_2H_2O_4$	385.2	51	147	334	≈20
13	C_2H_5	H	$C_{21}H_{27}NO \cdot C_2H_2O_4$	399.2	20	136	163	2.2 ± 0.5
14	C_3H_7	H	$C_{22}H_{29}NO \cdot C_2H_2O_4$	413.2	26	142−143	257	4.3 ± 0.9
15	$-CH_2CH_2CH_2-$		$C_{22}H_{27}NO \cdot C_2H_2O_4$	411.2	16	154−155	87	1.9 ± 0.4
16	$CH(CH_3)_2$	H	$C_{22}H_{29}NO \cdot C_2H_2O_4$	413.2	13	136−137	253	3.0 ± 1.0
17	C_4H_9	H	$C_{23}H_{31}NO \cdot C_2H_2O_4 \cdot 0.5H_2O$	436.2	46	131	169	2.8 ± 1.2
18	$-CH_2CH_2CH_2CH_2-$		$C_{23}H_{29}NO \cdot C_2H_2O_4$	425.2	44	165−167	92	3.5 ± 1.5
19	C_5H_{11}	H	$C_{24}H_{33}NO \cdot C_2H_2O_4 \cdot 0.5H_2O$	450.2	43	125	1130	3.0 ± 1.1
20	cyclopentyl	H	$C_{24}H_{31}NO \cdot C_2H_2O_4$	439.2	32	159	201	3.1 ± 0.6
21	$CH_2C_6H_5$	H	$C_{26}H_{29}NO \cdot C_2H_2O_4 \cdot 0.75H_2O$	474.7	26	116−117	238	>10
22	$CH_2CH_2C_6H_5$	H	$C_{27}H_{31}NO \cdot C_2H_2O_4$	475.2	29	170−171	890	≈30
23	$CH{=}CHC_6H_5$	H	$C_{27}H_{29}NO \cdot C_2H_2O_4$	473.2	50	190	761	>10
24	OCH_3	H	$C_{20}H_{24}NO_2 \cdot C_2H_2O_4$	400.1	40	145	119	≈20
25	OC_3H_7	H	$C_{22}H_{29}NO_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$	419.7	47	145	88	≈10
26	OC_6H_5	H	$C_{25}H_{27}NO_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$	467.7	49	151	530	>10
27	$OCH_2C_6H_5$	H	$C_{26}H_{29}NO_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$	481.7	35	159−160	550	>30
28	$C(=O)CH_3$	H	$C_{21}H_{25}NO_2 \cdot C_2H_2O_4$	413.2	19	136−137	84	3.9 ± 1.9
29	C(=O)-cyclopropyl	H	$C_{23}H_{27}NO_2 \cdot C_2H_2O_4$	439.2	9	194	66	5.9 ± 1.2
30	C(=O)-cyclopentyl	H	$C_{25}H_{31}NO_2 \cdot C_2H_2O_4$	467.2	40	144−145	157	2.8 ± 0.9
31	$C(=NOH)\text{-}CH_3$	H	$C_{21}H_{26}N_2O_2 \cdot 0.25H_2O$	342.7	95	156−157	55	≈5
32	$C(=NOCH_3)\text{-}CH_3$	H	$C_{22}H_{28}N_2O_2 \cdot C_2H_2O_4$	442.2	89	158	129	6.4 ± 0.6
33	imidazol-1-yl	H	$C_{22}H_{25}N_3O \cdot {}_2C_2H_2O_4 \cdot 0.5H_2O$	536.2	37	155−156	27	4.0 ± 1.3
34	piperidinomethyl	H	$C_{23}H_{34}N_2O \cdot {}_2C_2H_2O_4$	558.2	20	198−199	2.8	9.6 ± 4.5
35	(phenylamino)methyl	H	$C_{25}H_{30}N_2O \cdot {}_2C_2H_2O_4 \cdot 0.5H_2O$	563.2	19	136	90	≈10
thioperamide							60[c]	1.0 ± 0.5[d]
clobenpropit							2.4[c]	26 ± 7[d]
ciproxifan							46[c]	0.14 ± 0.03[d]
acetoproxifan							87[c]	0.24 ± 0.06[e]

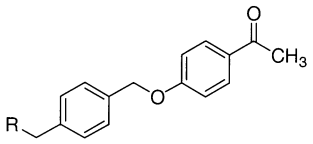
[a] [^{125}I]Iodoproxyfan binding assay with membranes from CHO−K1 cells stably expressing the human H_3 receptor ($\log K_i$ SEM ≤ 0.2).[19,31] [b] Central H_3 receptor screening in vivo after oral administration to mice ± SEM.[32] [c] Reference 39. [d] Reference 27. [e] Reference 47.

version of alcohol into chloride was performed with thionyl chloride followed by piperidine alkylation. Cyclopentyl-(4-hydroxyphenyl)methanone, as an intermediate for **30**, was prepared by Friedel−Crafts-acylation of phenol with $AlCl_3$ at ambient temperature in nitrobenzene. A mixture of ortho- and para-isomers was separated by column chromatography. The phenol derivative for the synthesis of **22** was obtained by catalytic hydrogenation of 4-hydroxystilbene according to standard procedures. Methoxime and oxime derivatives **31** and **32** were achieved by heating compound **28** in absolute solvent with *O*-methylhydroxylammonium chloride or hydroxylammonium chloride under basic conditions, respectively. The phenol precursor for **34** and **35** was obtained by reductive amination of piperidine or aniline, respectively, with 4-hydroxybenzaldehyde according to standard procedures.

Pharmacological Results and Discussion

In Vitro Binding Assay at Cloned Human H_3 Receptors. The affinity of compounds was determined by measuring the displacement curves of [^{125}I]iodoproxyfan at human histamine H_3 receptors stably expressed in CHO cells.[19,31] Influence of the substitution pattern on the phenoxy group is shown in Table 1, whereas the influence of the amino-containing moiety is shown in Table 2. The parent compound **1** showed moderate nanomolar affinity at the human H_3 receptor. Halogen, small alkyl, or methoxy substitutions in the 4-position increased affinities, demonstrating that electron-withdrawing properties (cf. substituents on phenyl group of compounds in Figure 1) had limited, if any, influence on receptor binding (**2**, **3**, **7**−**10**, **13**, and **24**). Effects of additional substitution in the meta-position to *p*-monochloro **3** can be examined with **4**−**6**. Additional chlorination (**5**) decreased affinity, whereas fluorination (**4**) and methylation (**6**) gave an increase. Comparing the mono- and dimethylated derivatives **9**, **11**, and **12** showed a beneficial effect of substitution in the para-position. A tendency for decreased affinities is seen in the series of para-alkylated compounds roughly in order of increasing steric bulk (**9** → **13** ≈ **17** → **20** → **14** ≈

Table 2. Structures, Physical Data, and Pharmacological Screening Results of 4-(4-(Acetyl)phenoxymethyl)benzyl Derivatives for in Vitro Affinity at the Human Histamine H_3-Receptor and Oral Antagonist Potency in Vivo in Mice



no.	R	formula	M_r	yield (%)	mp (°C)	K_i[a] (nM)	ED_{50}[b] (mg/kg) $\bar{x} \pm s_{\bar{x}}$
36	azepan-1-yl	$C_{22}H_{27}NO_2 \cdot HCl \cdot 0.25H_2O$	378.2	34	198−199	37	4.3 ± 2.2
37	pyrrolidino	$C_{20}H_{23}NO_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$	404.0	10	157−158	26	1.9 ± 1.0
38	$(H_5C_2)_2N$-	$C_{20}H_{25}NO_2 \cdot HCl$	347.6	26	254−255	91	3.4 ± 0.8

[a] [^{125}I]Iodoproxyfan binding assay with membranes from CHO−K1 cells stably expressing the human H_3 receptor (log K_i SEM ≤ 0.2).[19,31] [b] Central H_3 receptor screening in vivo after oral administration to mice ± SEM.[32]

16 → **19**). In contrast to the weak influence of an additional *m*-methyl substituent (**11**) to **9** or that of chain elongations (**13**, **14**, **17**, and **19**) of **9**, ring formation to 5-indanyl (**15**) or 1,2,3,4-tetrahydronaphthyl moieties (**18**) clearly enhanced affinities comparable to that of the dihalogenated derivative **4**. An even larger difference was seen by comparison of compounds **19** and **20**, both having pentyl groups. Whereas the *n*-alkyl compound **19** showed a dramatic loss in affinity, the related cycloalkyl compound **20** was active in almost the same concentration range as the smaller alkyl derivatives **16**, **14**, and the parent compound **1**. Introduction of phenylalkyl substituents to create even bulkier moieties (**21**−**23**) led to results comparable to those obtained for the alkyl series. Conversion into dialkylated *p*-hydroquinone compounds (**24**−**27**) was effective for alkyl but not for phenylalkyl substituents. Surprisingly, the propyloxy compound **25** showed higher affinity than that of the shorter methoxy compound **24**. In the series of compounds containing an acyl moiety (**28**−**30**) the ciproxifan analogue showed low nanomolar affinity at the human receptor. Conversion of acetoproxifan (FUB 372) into the corresponding oxime (imoproxifan) or methoxime derivative resulted in a drastic increase in affinity.[38] Unfortunately, the oxime derivatives **31** and **32** did not become more active than the corresponding acetyl derivative **28**. One might speculate that the differences in structure−activity relationships between the series described here and the proxifan series are strong indications for differences in receptor−ligand interactions and a different mode of binding. Compounds **33**−**35** containing substituents with basic properties showed good affinities. Compound **34**, as the most basic compound, exhibited the highest affinity in this series, displaying a similar value to that of clobenpropit.[39] Compound **34**, with two benzylpiperidine structures, may possess two potential bioisosteric interaction areas to the imidazole binding site of the H_3 receptor protein and therefore may display higher affinity. In a second approach we determined the influence of replacing the piperidine ring by similar structures (Table 2). While an expansion of the ring size to a seven-membered azepane (**36**) had almost no influence on affinity, decreased ring size to a pyrrolidine ring tended to slightly enhance activity. Keeping the same number of carbon atoms, but opening the ring to a diethylamino moiety (**38**), was not advantageous.

In Vivo Screening on Swiss Mice. For drug design of centrally active drugs, it is essential that these compounds are distributed after absorption into their target area and influence the central nervous system (CNS). All novel compounds were screened for their modulating effects on N^{τ}-methylhistamine levels in the brain cortex of Swiss mice after oral administration (Tables 1, 2). Parent compound **1** as well as the halogenated derivatives **2**−**8** showed weak or lacking in vivo potency as was also the case for the compounds of the proxyfan series in this test model. The same was true for the electron-withdrawing substituted trifluoromethyl derivative **10** and the *m*-methyl-substituted derivative **12**. Aromatic moieties and/or ether structures (**21**−**27**, and **35**) led to weak or missing in vivo potency with the exception of **33**. Relatively high antagonist in vivo potencies below 5 mg/kg were achieved with the alkylated compounds **9**, **11**, and **13**−**20**. Although the differences were not statistically significant, the smaller alkylated derivatives showed higher in vivo potency compared to the compounds with longer alkyl chains. A direct correlation between chain length and in vivo results was not detectable (results not shown). It might be concluded that lipophilicity, metabolic stability, and steric receptor−ligand interaction are three important parameters among others which have an important influence on in vivo potency. The calculated partition coefficient values (LogP)[40] varied in a range from 3.21 (**37**) to 6.51 (**23**) ($\bar{x} = 4.86 \pm 0.62$) and showed no direct correlation to in vitro or in vivo potencies (results not shown). Surprisingly, in vivo the pentyl derivative **19** was as potent as the related cyclopentyl derivative **20** which showed more than 5 times higher binding affinity than **19**. One might argue that these findings are caused by species differences in the test models, but many other reasons can also been taken into account. Compounds **28**−**34** showed moderate to good antagonist in vivo potencies without having a correlation to their in vitro affinities. The substituent on the phenoxy group and its position had marked influence on in vivo potency. On the other hand, it seems remarkable that all acylated derivatives, despite the structure of their amino group (**28**, **36**−**38**), showed any in vivo potency (Tables 1, 2). This result is somewhat surprising since, for a long time, the imidazole nucleus seemed to be essential for H_3 receptor affinity, and now we find numerous bioisosteric replacements with relative small changes in affinities and in vivo potencies. To exclude effects on the main histamine-metabolizing enzyme in brain, histamine *N*-methyltransferase [EC 2.1.1.8], which may interfere with the in vivo data, compound **28** was tested as the single example, showing relative weak affinity

Table 3. Potency of Selected Compounds at Histamine Receptor Subtypes

no.	H_3 pA_2[a]	H_2 pA_2[b]	H_1 pA_2[c]
28	7.21	4.99	5.34
30	7.51		
31	7.07	4.85	5.10
32	6.54	5.40	5.46
33	7.80		
34	7.42		
36	7.51		
37	7.74	5.11	5.03

[a] Functional H_3 receptor assay on guinea-pig ileum (SEM ≤ 0.2).[33] [b] Functional H_2 receptor test on guinea-pig atrium (SEM ≤ 0.2).[34] [c] Functional H_1 receptor test on guinea-pig ileum (SEM ≤ 0.2).[34]

on rat enzyme (IC_{50} value of 10.4 ± 1.3 μM) (for test assay and discussion, see ref 41).

Screening of Selected Compounds at Other Functional Histamine Subreceptor Models. Selected compounds have been evaluated for their activity in a functional model for the histamine H_3 receptor on the guinea-pig ileum (Table 3).[33] Activity of compounds **1**, **9**, **11**, **14**, **15**, **21**, **22**, and **26** was not exactly determined due to high muscarinic M_3 receptor antagonist activity in this test model[42] but were calculated to have pA_2 values ≤6.8. The results on guinea-pig ileum mostly showed slightly lower values than the binding affinities at human H_3 receptors, which is in accordance with previously reported results. Only imidazolyl compound **33** showed slightly higher potency in the guinea-pig assay than in the human test model. The number of results is much too low to draw any conclusions on species specificity.

Furthermore, compounds **28**, **31**, **32**, and **37** have been tested for their antagonist activity at H_1 and H_2 receptors in functional tests on isolated organs of guinea-pig (Table 3).[34] Results indicate moderate to good preference of the H_3 vs H_1 and H_2 receptors.

Conclusions

We have successfully replaced the imidazole ring of compounds of the 4-((1*H*-imidazol-4-yl)methy)benzyl series by a piperidino group or related secondary amino groups. The compounds represent a new class of nonimidazole histamine H_3 receptor antagonists, some of which display low nanomolar affinities at the human H_3 receptor and high antagonist in vivo potencies in the CNS of mice after oral application. Structure−activity relationships are similar but somewhat different to those obtained in the previously reported series of imidazole-containing H_3 receptor antagonists. Species differences have to be taken into account with the pharmacological data. The compounds tested showed moderate to good selectivity for the H_3 receptor vs H_1 and H_2 receptors.

Experimental Section

Chemistry. General Procedures. Melting points were determined on an Electrothermal Büchi 512 or 545 apparatus and are uncorrected. For all compounds, ^{1}H NMR spectra were recorded on a Bruker DPX 400 (400 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from internal standard Me_4Si as a reference. ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; and m, multiplet), approximate coupling constants in hertz, and the number of protons (Az, azepanyl; Cyp, cyclopropyl; Im, imidazolyl; Ind, indanyl; Pip, piperidino; Ph, phenyl; Pyr, pyrrolidino; Xyl, *para*-α,α′-xylenediyl). Mass spectra were obtained on EI-MS Finnigan MAT CH7A and Finnigan MAT 711 (high-resolution mass spectra) spectrometers (resolving power 12 500). 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 for all compounds. Column chromatography or flash column chromatography were carried out using silica gel 62−200 μm or 20−63 μm (Merck), respectively. TLC was performed on silica gel PF_{254} plates (Merck), and the spots were detected by UV spectroscopy (wavelength 254 nm) and visualized with Dragendorff reagent. Abbreviations for solvents are the following: Et_2O, diethyl ether; EtOH, ethanol; EtOAc, ethyl acetate; MeOH, methanol; PE, petroleum ether (bp 40−60 °C); TEA, triethylamine; THF, tetrahydrofuran. Only spectra of prominet intermediate products and the most interesting compounds are shown (**9**, **15**, **28**−**29**, **31**, **33**−**34**, **36**, and **37**).

4-(Piperidinomethyl)benzoic Acid Methyl Ester. A mixture of 4-formylbenzoic acid methyl ester (3.2 g, 20 mmol), piperidine (1.7 g, 20 mmol), and titanium(IV) isopropoxide (7.4 mL, 25 mmol) in 20 mL of MeOH was stirred at room temperature. After 3 h, sodium cyanoborohydride (0.84 g, 13.4 mmol) was added, and the solution was stirred overnight. Water was added, and the resulting inorganic precipitate was filtered and washed with EtOH. The filtrate was then concentrated in vacuo, and the residue was purified by flash column chromatography (eluent: EtOAc/hexane, 1:1, saturated with ammonia) to give a colorless oil. Yield 65%; ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 8.0 Hz, 2H, Ph-2-H, Ph-6-H), 7.39 (d, J = 8.0 Hz, 2H, Ph-3-H, Ph-5-H), 3.90 (s, 3H, CH_3), 3.50 (s, 2H, CH_2N), 2.33−2.39 (m, 4H, Pip-2-H, Pip-6-H), 1.50−1.61 (m, 4H, Pip-3-H, Pip-5-H), 1.38−1.45 (m, 2H, Pip-4-H); MS m/z 233 ([M$^{\bullet+}$], 64), 202 (9), 149 (62), 121 (15), 98 (100), 84 (97), 42 (21). Anal. ($C_{14}H_{19}NO_2$).

4-(Piperidinomethyl)phenylmethanol (Precursor I). 4-(Piperidinomethyl)benzoic acid methyl ester (3 g, 15 mmol) was dissolved in 50 mL of diglyme together with sodium borohydride (0.95 g, 15 mmol). A freshly prepared solution of aluminum chloride in diglyme was then slowly added dropwise. Then the solution was heated under reflux for 24 h. The solution was poured into ice and filtered. The filtrate was then concentrated in vacuo to give a colorless oil, which was additionally purified by flash column chromatography (eluent: EtOAc/hexane:1/1, saturated with ammonia). Yield 82%; ^{1}H NMR (CF_3COOD) δ 7.58 (d, J = 8.2 Hz, 2H, Ph-2-H, Ph-6-H), 7.48 (d, J = 8.2 Hz, 2H, Ph-3-H, Ph-5-H), 6.54−6.84 (m, 1H, Pip-N$^+$), 4.92 (s, 2H, CH_2O), 4.38 (d, J = 9.3 Hz, 2H, CH_2N), 3.67 (d, J = 11.5 Hz, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.05 (s, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.80 (d, J = 14.9 Hz, 2H, Pip-3-H_{eq}, Pip-5-H_{eq}), 1.99 (d, J = 13.8 Hz, 1H, Pip-4-H_{eq}), 1.82−1.91 (m, 2H, Pip-3-H_{ax}, Pip-5-H_{ax}), 1.54−1.64 (m, 1H, Pip-4-H_{ax}); MS m/z 205 ([M$^{\bullet+}$], 27), 188 (26), 121 (100), 98 (77), 84 (64), 42 (15). Anal. ($C_{13}H_{19}NO$).

General Synthetic Procedure for Mitsunobu-Type Ether Formation (1−27, 30, and 33−35). Triphenylphosphine (0.65 g, 2.5 mmol) was dissolved with 4-(piperidinomethyl)phenylmethanol (0.51 g, 2.5 mmol) and the corresponding phenol (2.5 mmol) in 15 mL of dry THF under argon atmosphere. After the mixture was cooled for 5 min in an ice bath, diethyl azodicarboxylate (DEAD, 0.48 mL, 2.6 mmol) was slowly added, and the solution was stirred for 72 h at room temperature. The solvent was removed under reduced pressure and the product purified via flash column chromatography (eluent: EtOAc/hexane, 1:1, saturated with ammonia). The oil obtained was dried in vacuo and then crystallized as hydrogen oxalate in EtOH/Et_2O.

1-(4-(4-Methylphenoxymethyl)benzyl)piperidine (9). ^{1}H NMR (CF_3COOD) δ 7.64 (d, J = 7.9 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.49 (d, J = 7.9 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.17 (d, J = 8.3 Hz, 2H, Ph-3-H, Ph-5-H), 6.99 (d, J = 8.3 Hz, 2H, Ph-2-H, Ph-6-H), 6.77−6.81 (m, 1H, Pip-N$^+$), 5.29 (s, 2H, CH_2O), 4.37

(d, J = 5.3 Hz, 2H, CH_2N), 3.67 (d, J = 11.9 Hz, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.00−3.08 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.32 (s, 3H, CH_3), 2.09 (d, J = 14.9 Hz, 2H, Pip-3-H_{eq}, Pip-5-H_{eq}), 1.99 (d, J = 13.8 Hz, 1H, Pip-4-H_{eq}), 1.82−1.92 (m, 2H, Pip-3-H_{ax}, Pip-5-H_{ax}), 1.55−1.64 (m, 1H, Pip-4-H_{ax}); MS m/z 295 ([$M^{\bullet+}$], 23), 188 (100), 104 (27), 98 (7), 84 (11), 45 (16). Anal. C, H, N.

1-(4-(Indan-5-yloxymethyl)benzyl)piperidine (15). ^{1}H NMR (DMSO-d_6) δ 7.48 (s, 4H, Xyl-2-H, Xyl-3-H, Xyl-5-H, Xyl-6-H), 7.10 (d, J = 8.2 Hz, 1H, Ind-6-H), 6.88 (s, 1H, Ind-2-H), 6.75 (dd, J_1 = 2.3 Hz, J_2 = 5.8 Hz, 1H, Ind-7-H), 5.07 (s, 2H, CH_2O), 4.13 (s, 2H, CH_2N), 2.90−3.05 (m, 4H, Ind-3-H, Ind-5-H), 2.74−2.82 (m, 4H, Pip-2-H, Pip-6-H), 1.95−2.03 (m, 2H, Ind-4-H), 1.64−1.72 (m, 4H, Pip-3-H, Pip-5-H), 1.45−1.54 (m, 2H, Pip-4-H); MS m/z 321 ([$M^{\bullet+}$], 25), 188 (100), 104 (17), 98 (3), 84 (6), 45 (7). Anal. C, H, N.

1-(4-(4-(Piperidinomethyl)benzyloxy)phenyl)-1*H*-imidazole (33). ^{1}H NMR (CF_3COOD) δ 8.84 (s, 1H, Im-2-H), 7.68 (s, 1H, Im-5-H), 7.63−7.66 (m, 3H, Xyl-2-H, Xyl-6-H, Im-4-H), 7.51−7.54 (m, 4H, Xyl-3-H, Xyl-5-H, Ph-3-H, Ph-5-H), 7.29 (d, J = 8.7 Hz, 2H, Ph-2-H, Ph-6-H), 6.77−6.81 (m, 1H, Pip-N^+), 5.31 (s, 2H, CH_2O), 4.38 (d, J = 4.6 Hz, 2H, CH_2N), 3.71 (d, J = 12.0 Hz, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.02−3.10 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.09 (d, J = 14.8 Hz, 2H, Pip-3-H_{eq}, Pip-5-H_{eq}), 2.02 (d, J = 13.8 Hz, 1H, Pip-4-H_{eq}), 1.83−1.93 (m, 2H, Pip-3-H_{ax}, Pip-5-H_{ax}), 1.59−1.65 (m, 1H, Pip-4-H_{ax}); MS m/z 347 ([$M^{\bullet+}$], 9), 264 (57), 188 (100), 160 (32), 104 (46), 84 (22). Anal. C, H, N.

1-(4-(4-(Piperidinomethyl)phenoxymethyl)benzyl)piperidine (34). ^{1}H NMR (CF_3COOD) δ 7.63 (d, J = 8.0 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.50 (d, J = 8.0 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.41 (d, J = 8.6 Hz, 2H, Ph-3-H, Ph-5-H), 7.17 (d, J = 8.6 Hz, 2H, Ph-2-H, Ph-6H), 6.69−6.80 (m, 2H, Pip-N^+), 5.28 (s, 2H, CH_2O), 4.38 (d, J = 5.4 Hz, 2H, CH_2N), 4.31 (d, J = 5.3 Hz, 2H, Ph-CH_2N), 3.60−3.75 (m, 4H, Pip-2-H_{eq}, Pip-6-H_{eq}), 2.97−3.09 (m, 4H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.09 (d, J = 14.7 Hz, 4H, Pip-3-H_{eq}, Pip-5-H_{eq}), 1.99 (d, J = 13.8 Hz, 2H, Pip-4-H_{eq}), 1.84−1.94 (m, 4H, Pip-3-H_{ax}, Pip-5-H_{ax}), 1.54−1.64 (m, 2H, Pip-4-H_{ax}); MS m/z 378 ([$M^{\bullet+}$], 12), 295 (49),188 (100), 104 (36), 98 (18), 84 (18), 45 (66). Anal. C, H, N.

1-(4-(4-(Bromomethyl)benzyloxy)phenyl)ethanone (Precursor II). *p*-α,α′-Dibromoxylene (5.88 g, 20 mmol) was stirred in 100 mL of acetone together with 4-hydroxyacetophenone (0.68 g, 5 mmol) and potassium carbonate (0.82 g, 6 mmol) under reflux for 12 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (eluent: CH_2Cl_2/PE/MeOH, 60:38:2). Yield 71%; mp 105 °C; ^{1}H NMR ($CDCl_3$): δ 7.91−7.95 (m, 2H, Ph-3-H, Ph-5-H), 7.36−7.44 (m, 4H, Xyl-2-H, Xyl-3-H, Xyl-5-H, Xyl-6-H), 6.97−7.01 (m, 2H, Ph-2-H, Ph-6-H), 5.12 (s, 2H, CH_2O), 4.50 (s, 2H, CH_2−Br), 2.55 (s, 3H, CH_3), MS m/z 318 ([$M^{\bullet+}$], 7), 239 (12), 104 (100), 78 (10), 43 (9). Anal. ($C_{16}H_{15}BrO_2$).

General Synthetic Procedure for Compounds with Different Amine Moieties (28, 36−38). 1-(4-(4-(Bromomethyl)benzyloxy)phenyl)ethanone (Precursor II, 0.95 g, 3 mmol) and the corresponding amine (6 mmol) were dissolved in 20 mL of EtOH and heated for 12 h in the presence of potassium carbonate (1.38 g, 10 mmol). The solvent was evaporated and the residue dissolved in 30 mL of EtOAc. The organic layer was washed with water and extracted with 1 N hydrochloric acid (3 × 15 mL). In the case of compounds **36** and **38** a white precipitate was formed. This precipitate was collected and dried to give the hydrochloride salts. In the case of compounds **28** and **37** the aqueous layer was alkalized and extracted with EtOAc (3 × 20 mL). The solvent was removed and the resulting crude product crystallized as a salt of oxalic acid from EtOH/Et_2O after purification by column chromatography (eluent: EtOAc/TEA, 99:1).

1-(4-(4-(Piperidinomethyl)benzyloxy)phenyl)ethanone (28). ^{1}H NMR (CF_3COOD) δ 8.12 (d, J = 8.7 Hz, 2H, Ph-2-H, Ph-6-H), 7.64 (d, J = 7.8 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.49 (d, J = 7.8 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.16 (d, J = 8.7 Hz, 2H, Ph-3-H, Ph-5-H), 6.77−6.81 (m, 1H, Pip-N^+), 5.32 (s, 2H, CH_2O), 4.37 (d, J = 5.2 Hz, 2H, CH_2N), 3.71 (d, J = 11.9 Hz, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.00−3.08 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.77 (s, 3H, CO−CH_3), 2.09 (d, J = 14.9 Hz, 2H, Pip-3-H_{eq}, Pip-5-H_{eq}), 1.99 (d, J = 13.5 Hz, 1H, Pip-4-H_{eq}), 1.82−1.92 (m, 2H, Pip-3-H_{ax}, Pip-5-H_{ax}), 1.55−1.64 (m, 1H, Pip-4-H_{ax}); MS m/z 323 ([$M^{\bullet+}$], 24), 188 (100), 153 (13), 104 (33), 98 (17), 84 (24), 45 (10). Anal. C, H, N.

1-(4-(4-(Azepane-1-ylmethyl)benzyloxy)phenyl)ethanone (36). ^{1}H NMR (CF_3COOD) δ 8.12 (d, J = 8.9 Hz, 2H, Ph-2-H, Ph-6-H), 7.64 (d, J = 8.1 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.52 (d, J = 8.1 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.16 (d, J = 8.9 Hz, 2H, Ph-3-H, Ph-5-H), 6.99−7.11 (m, 1H, Az-N^+), 5.32 (s, 2H, CH_2O), 4.44 (d, J = 5.6 Hz, 2H, CH_2N), 3.67−3.72 (m, 2H, Az-2-H_{eq}, Az-7-H_{eq}), 3.26−3.34 (m, 2H, Az-2-H_{ax}, Az-7-H_{ax}), 2.76 (s, 3H, CO−CH_3), 2.06−2.11 (m, 2H, Az-3-H_{eq}, Az-6-H_{eq}), 1.95−2.01 (m, 2H, Az-3-H_{ax}, Az-6-H_{ax}), 1.80−1.92 (m, 4H, Az-4-H, Az-5-H); MS m/z 337 ([$M^{\bullet+}$], 35), 239 (7), 202 (100), 104 (67), 98 (62), 42 (36). Anal. C, H, N.

1-(4-(4-(Pyrrolidinomethyl)benzyloxy)phenyl)ethanone (37). ^{1}H NMR (CF_3COOD) δ 8.12 (d, J = 8.8 Hz, 2H, Ph-2-H, Ph-6-H), 7.65−7.8 (m, 1H, Pyr-N^+), 7.64 (d, J = 7.9 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.52 (d, J = 7.9 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.16 (d, J = 8.8 Hz, 2H, Ph-3-H, Ph-5-H), 5.32 (s, 2H, CH_2O), 4.46 (d, J = 5.9 Hz, 2H, CH_2N), 3.74−3.79 (m, 2H, Pyr-2-H_{eq}, Pyr-5-H_{eq}), 3.26−3.35 (m, 2H, Pyr-2-H_{ax}, Pyr-5-H_{ax}), 2.77 (s, 3H, CO−CH_3), 2.27−2.36 (m, 2H, Pyr-3-H_{eq}, Pyr-4-H_{eq}), 2.19−2.25 (m, 2H, Pyr-3-H_{ax}, Pyr-4-H_{ax}); MS m/z 309 ([$M^{\bullet+}$], 13), 174 (100), 104 (31), 84 (14), 70 (14), 45 (12). Anal. C, H, N.

Cyclopropyl-(4-(4-(piperidinomethyl)benzyloxy)phenyl)methanone (29). 1,4-Benzenedimethanol (4.14 g, 30 mmol) in 30 mL of THF was added dropwise to a suspension of sodium hydride (25 mmol, 60% dispersion in paraffin oil) in 30 mL of THF with a catalytic amount of tetrabutylammonium iodide and 15-crown-5. After stirring for 15 min, cyclopropyl-(4-fluorophenyl)methanone (3.28 g, 20 mmol) was added dropwise, and the solution was refluxed for 2 days. Water was added carefully and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2/MeOH, 98:2) resulting in **cyclopropyl-(4-(4-(hydroxymethyl)benzyloxy)phenyl)methanone**. Yield 38%; mp 100 °C; ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 8.8 Hz, 2H, Ph-3-H, Ph-5-H), 7.41 (d, J = 7.9 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.33 (d, J = 7.9 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.12 (d, J = 8.8 Hz, 2H, Ph-2-H, Ph-6-H), 5.19 (s, 2H, CH_2O), 4.49 (s, 2H, CH_2OH), 2.82−2,86 (m, 1H, Cyp-1-H), 0.97 (d, J = 6.1 Hz, 4H, Cyp-2-H, Cyp-3-H); MS m/z 282 ([$M^{\bullet+}$], 4), 121 (100), 104 (1), 93 (18), 41 (3). Anal. ($C_{18}H_{18}O_3$). The resulting ether (1.12 g, 4 mmol) was dissolved in 30 mL of dry THF, stirred at 0 °C, and thionyl chloride (0.95 g, 8 mmol) was added dropwise. After 1 h at ambient temperature, the mixture was warmed to 60 °C for 2 h. The solvent and the excess of thionyl chloride were evaporated, and the residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2/MeOH, 95:5) resulting in **cyclopropyl-(4-(4-(chlormethyl)benzyloxy)phenyl)methanone**. Yield 96%; mp 111−112 °C; ^{1}H NMR (DMSO-d_6) δ 8.03 (d, J = 9.3 Hz, 2H, Ph-3-H, Ph-5-H), 7.4 (d, J = 7.9 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.34 (d, J = 7.9 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.13 (d, J = 9.3 Hz, 2H, Ph-2-H, Ph-6-H), 5.21 (s, 2H, CH_2O), 4.49 (s, 2H, CH_2−Cl), 2.82−2.87 (m, 1H, Cyp-1-H), 0.97 (d, J = 6.1 Hz, 4H, Cyp-2-H, Cyp-3-H); MS m/z 300 ([$M^{\bullet+}$], 7), 265 (2), 139 (100), 104 (27), 77 (9), 41 (4). Anal. ($C_{18}H_{17}ClO_2$). The product (0.6 g, 2 mmol) was dissolved in 40 mL of EtOH and heated with piperidine (0.34 g, 4 mmol) and potassium carbonate (0.82 g, 6 mmol) for 12 h under reflux. The solvent was removed and EtOAc (50 mL) added and washed with 0.5 N sodium carbonate solution (15 mL). The organic layer was extracted with 0.5 N hydrochloric acid (3 × 15 mL). The aqueous layer was alkalized and extracted with EtOAc. The solvent was removed and the residue crystallized as a salt of oxalic acid from EtOH/Et_2O. ^{1}H NMR (DMSO-d_6) δ 8.04 (d, J = 8.3 Hz, 2H, Ph-3-H, Ph-5-H), 7.52 (d, J = 3.8 Hz, 4H, Xyl-2-H, Xyl-3-H, Xyl-5-H, Xyl-6-H), 7.14 (d, J = 8.3 Hz, 2H, Ph-2-H, Ph-6-H), 5.24 (s, 2H, CH_2O), 4.13 (s, 2H, CH_2N), 2.84−2.89 (m, 5H, Pip-2-H, Pip-6-H, Cyp-1-H), 1.67−

1.69 (m, 4H, Pip-3-H, Pip-5-H), 1.45−1.49 (m, 2H, Pip-4-H), 0.99 (d, J = 5.8 Hz, 4H, Cyp-2-H, Cyp-3-H); MS m/z 349 ([$M^{\bullet+}$], 23), 265 (<1), 188 (100), 153 (8), 104 (43), 98 (15), 84 (20), 69 (10), 42 (7). Anal. C, H, N.

1-(4-(4-Piperidinomethylbenzyloxy)phenoxy)ethanonoxime (31). Compound **28** (0.2 g, 0.5 mmol) was dissolved together with hydroxylamine hydrochloride (0.7 g, 1 mmol) and K_2CO_3 (1.6 g, 1.2 mmol) in 20 mL of absolute EtOH and heated under reflux until no initial compound was detectable with TLC. The solvent was removed and the remaining material purified by flash column chromatography (eluent: EtOAc) to give a white solid. ^{1}H NMR (CF_3COOD) δ 7.81 (d, J = 7.7 Hz, 2H, Ph-3-H, Ph-5-H), 7.63 (d, J = 7.5 Hz, 2H, Xyl-2-H, Xyl-6-H), 7.51 (d, J = 7.5 Hz, 2H, Xyl-3-H, Xyl-5-H), 7.25 (d, J = 7.7 Hz, 2H, Ph-2-H, Ph-6-H), 6.77−6.81 (m, 1H, Pip-N^+), 5.31 (s, 2H, CH_2O), 4.38 (s, 2H, CH_2N), 3.71 (d, J = 11.9 Hz, 2H, Pip-2-H_{eq}, Pip-6-H_{eq}), 3.01−3.09 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 2.86 (s, 3H, CNOHCH_3), 2.10 (d, J = 14.7 Hz, 2H, Pip-3-H_{eq}, Pip-5-H_{eq}), 1.99 (d, J = 13.6 Hz, 1H, Pip-4-H_{eq}), 1.83−1.93 (m, 2H, Pip-3-H_{ax}, Pip-5-H_{ax}), 1.55−1.64 (m, 1H, Pip-4-H_{ax}); MS m/z 338 ([$M^{\bullet+}$], 10), 321 (37), 188 (100), 104 (25), 98 (6), 84 (7). Anal. C, H, N.

Pharmacology. General Methods. Histamine H_3 Receptor Antagonist Activity In Vivo in Mouse. In vivo testing was performed after oral administration of the compound to Swiss mice as described by Garbarg et al.[32] 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 treatment. Animals were decapitated 90 min after treatment, and the cerebral cortex was isolated. The cortex was homogenized in 10 volumes of ice-cold perchloric acid (0.4 M). The N^τ-methylhistamine level was measured by radioimmunoassay.[43] By oral treatment with 3 mg/kg of ciproxifan the maximum 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 values with SEM.[44] The pharmacological tests were performed at least in triplicate.

[^{125}I]Iodoproxyfan Binding Assay on Stably Transfected CHO−K1 Cells. Potency of the novel compounds **7**−**18** was investigated in a radioligand binding assay described by Ligneau et al.[31] Transfected CHO−K1 cells were washed and harvested with a PBS medium. They were centrifuged (140 g, 10 min, +4 °C) and then homogenized with a Polytron in the ice-cold binding buffer (Na_2HPO_4/KH_2PO_4, c = 50 mmol/L, pH = 7.5). The homogenate was centrifuged (23 000 × g, 30 min, +4 °C) and the pellet obtained resuspended in the binding buffer to constitute the membrane preparation used for the binding assays. Aliquots of the membrane suspension (5−15 μg protein) were incubated for 60 min at 25 °C with [^{125}I]-iodoproxyfan (c = 25 pmol/L) alone, or together with competing drugs dissolved in the same buffer to give a final volume of 200 μL. Incubations were performed in triplicate and stopped by four additions (5 mL) of ice-cold medium, followed by rapid filtration through glass microfiber filters (GF/B Whatman, Clifton, NJ) presoaked in polyethylene imine (ω = 0.3%). Radioactivity trapped on the filters was measured with a LKB (Rockville, MD) gamma counter (efficiency: 82%). Specific binding was defined as that inhibited by imetit (c = 1 μmol/L), a specific H_3 receptor agonist.[31] K_i values were determined according to the Cheng−Prusoff equation.[45] Data are presented as the mean of experiments performed at least in triplicate.

Histamine H_3 Receptor Antagonist Activity on Guinea-Pig Ileum.[33] Strips of guinea-pig ileal longitudinal muscle with adhering myenteric plexus, approximately 2 cm in length and proximal to the ileocaecal junction, were prepared as described previously.[46] The strips were mounted isometrically under a tension of approximately 7.5 ± 2.0 mN in 20 mL of organ baths filled with modified Krebs−Henseleit solution of the following composition (mM): NaCl 117.9, KCl 5.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.3, $NaHCO_3$ 25.0, D-glucose 5.5, and choline chloride 0.001, aerated with 95% O_2/5% CO_2 (V/V) and kept at 37 °C. Mepyramine (1 μM) was present throughout the experiment to block ileal H_1 receptors. After an equilibration period of 1 h with washings every 10 min, the preparations were stimulated for 30 min with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. Viability of the muscle strips was monitored by addition of the H_3 receptor agonist (R)-α-methylhistamine (100 nM), which caused a relaxation of the twitch response of more than 50 up to 100%. After wash out, reequilibration, and 30 min field stimulation, a cumulative concentration−response curve to (R)-α-methylhistamine (1−1000 nM) was constructed. Subsequently, the preparations were washed intensively and reequilibrated for 20−30 min in the absence of the antagonist under study. During the incubation period, the strips were stimulated continuously for 30 min. Finally, a second concentration−response curve to (R)-α-methylhistamine was obtained.[31,33] The rightward displacement of the curve to the H_3 receptor agonist evoked by the antagonist under study was corrected with the mean shift monitored by daily control preparations in the absence of the antagonist. All compounds were tested in concentrations not blocking ileal M_3 receptors. The pharmacological tests were performed at least in triplicate.

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.[34] Each pharmacological test was performed at least in triplicate, but the exact type of interaction was not determined in each case. The given values represent the mean.

Acknowledgment. This work was supported by the Fonds der Chemischen Industrie, Verband der Chemischen Industrie, Frankfurt/Main, Germany. We thank I. Walter for carrying out pharmacological testing for guinea-pig H_1, H_2, and H_3 values and M. Berg for synthesizing the first batches of compounds **28** and **37**.

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JM021084K