Iodoaminopotentidine and Related Compounds: A New Class of Ligands with High Affinity and Selectivity for the Histamine H_2 Receptor

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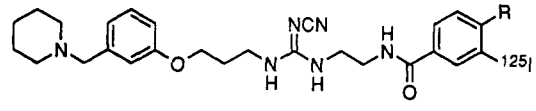
The synthesis and biological evaluation of a new class of histamine H_2 antagonists with *N*-cyano-*N'*-[ω-[3-(1-piperidinylmethyl)phenoxy]alkyl]guanidine partial structure are described as part of an extensive research program to find model compounds for the development of new radioligands with high H_2 affinity and specific activity. High receptor affinity is achieved by an additional (substituted) aromatic ring, which is connected with the third guanidine N by a carbon chain spacer and an amine, carboxamide, ester, or sulfonamide link ("polar group"). In functional studies for H_2 antagonistic activity and other pharmacological actions [e.g. H_1 antihistaminic, antimuscarinic, antiadrenergic (α_1, β_1), 5-HT_2 blocking activity] in the isolated guinea pig atrium and ileum and rat aorta and tail artery, the compounds proved to be highly potent and selective histamine H_2 receptor antagonists. The H_2 antagonistic activity is mainly depending on the length of both the *N'*-alkyl chain (chain A) and the *N''*-spacer (chain B). Compounds with a C_3 chain A and a C_2 chain B are most potent in the preferred group of substances, i.e., the carboxamide series. A wide variety of substituents at the aromatic ring is tolerated, among them iodine, amino, and azido groups. These compounds are up to 32 times more potent than cimetidine in the isolated guinea pig right atrium. The replacement of the carboxamide by an ester group (**44c**) is well tolerated, while replacement of the cyanoguanidine by an urea group results in nearly 100-fold decrease in activity (**46c,e**). The iodinated benzamides are among the most potent H_2 antagonists known so far. The [^{125}I]-labeled form of **31f** ([^{125}I]iodoaminopotentidine, [^{125}I]-*N*-[2-(4-amino-3-iodobenzamido)ethyl]-*N'*-cyano-*N''*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine) and its photolabile analogue **31h** ([^{125}I]iodoazidopotentidine, [^{125}I]-*N*-[2-(4-azido-3-iodobenzamido)ethyl]-*N'*-cyano-*N''*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine) proved to be useful probes for reversible and irreversible labeling of the histamine H_2 receptor. Radioligand binding studies in guinea pig cerebral membranes revealed considerably higher H_2 receptor affinity for **31f** (pK_i = 9.15), **31h** (pK_i = 8.58), and some analogues than functional experiments (guinea pig atrium), presumably reflecting an easier access to the H_2 receptors in membranes.

Since the characterization of the H_2 receptor in 1972 by Black et al.,[1] a huge number of potent histamine H_2 antagonists has been synthesized.[2] Nevertheless, trials to radiolabel the H_2 receptor itself failed for a long time, mainly due to the very low specific binding of the compounds with H_2 activity known so far. [^{3}H]Tiotidine was the first compound useful for specific labeling,[3] though the compound could only be used in the guinea pig brain. The detection in other tissues and species failed owing to high unspecific binding of [^{3}H]tiotidine and low abundance of H_2 receptors. No compound, however, has been known for irreversible labeling.

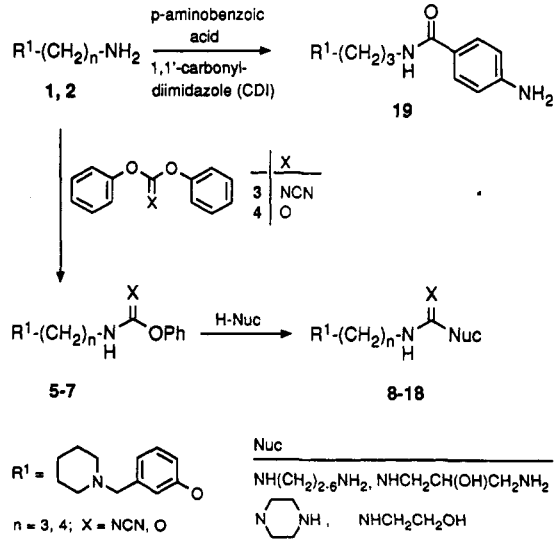
In 1990, the development of the new class of histamine H_2 antagonists, described in this paper, led to the first autoradiographic localization, high specific binding studies, and photoaffinity labeling of the H_2 receptor using the radioactive probes [^{125}I]iodoaminopotentidine (^{125}IAPT) and its photolabile azido analogue [^{125}I]iodoazidopotentidine (^{125}IAZPT)[4,5] (Scheme I). These substances are among the most potent H_2 antagonists known so far. Moreover, they also proved to be useful in species other than the guinea pig (e.g., monkey, rat, man) and allowed for the first time a characterization and a mapping of H_2 receptors in the human brain.[6,7]

Scheme I



[^{125}I]Iodoaminopotentidine (^{125}IAPT): R = NH_2
[^{125}I]Iodoazidopotentidine (^{125}IAZPT): R = N_3

Scheme II



Chemistry

The synthesis of the title compounds started from the requisite ω-[3-(1-piperidinylmethyl)phenoxy]alkanamines **1** and **2** prepared according to known methods, which were acylated (**19**) or treated with either diphenyl cyanocarbonimidate (**3**) or diphenyl carbonate (**4**) to form the reactive intermediates **5–7** (Scheme II).

The urethanes or isoureas **5–7** were allowed to react with an excess of an alkanediamine or an amino alcohol affording the nucleophilic intermediates **8–18** (Table I), which were converted in high yields into nitroanilines **22–26**, amides **19, 31–43, 45**, and **46**, ester **44c**, or aromatic

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(1) Black, J. W.; Duncan, W. A. M.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E. Definition and Antagonism of Histamine H_2 Receptors, *Nature (London)* 1972, *236*, 385–390.

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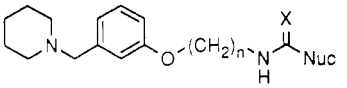
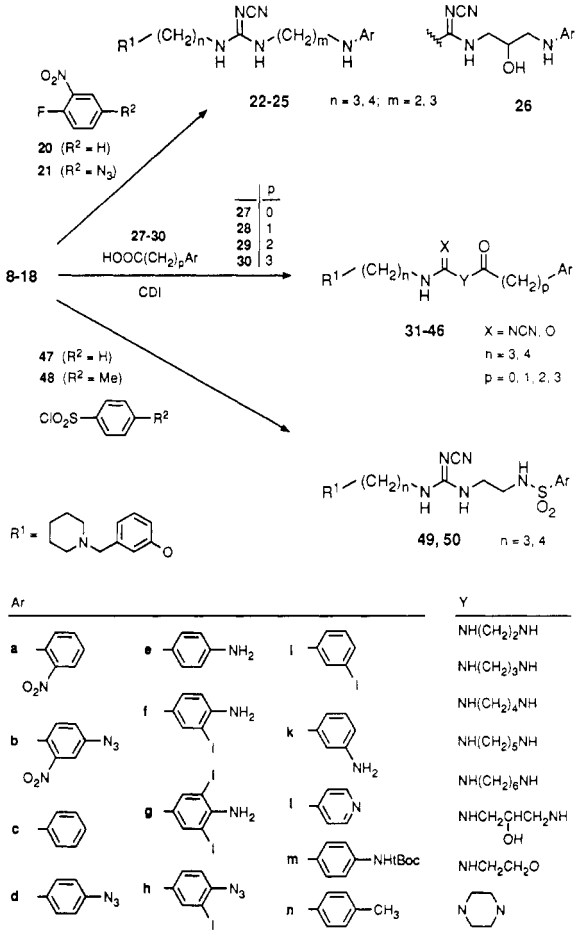
Table I. Structures and Formulas of Intermediates 8–18



no.	n	X	Nuc	% yield	mp,[a] °C	formula[b]
8	3	NCN	$NH(CH_2)_2NH_2$	90	138–140	$C_{19}H_{30}N_6O{\cdot}2C_4H_4O_4$[c]
9	3	NCN	$NH(CH_2)_3NH_2$	63	150–153	$C_{20}H_{32}N_6O{\cdot}2C_{20}H_{18}O_8{\cdot}2H_2O{\cdot}CH_3CN$[d]
10	3	NCN	$NH(CH_2)_4NH_2$	63	oil	$(C_{21}H_{34}N_6O)$[e]
11	3	NCN	$NH(CH_2)_5NH_2$	47	oil	$(C_{22}H_{36}N_6O)$[e,f]
12	3	NCN	$NH(CH_2)_6NH_2$	46	136–137	$C_{23}H_{38}N_6O{\cdot}2C_{20}H_{18}O_8{\cdot}H_2O$[d]
13	3	NCN	$NHCH_2CH(OH)CH_2NH_2$	83	86–88[g]	$C_{20}H_{32}N_6O_2$
14	4	NCN	$NH(CH_2)_2NH_2$	96	105[h]	$C_{20}H_{32}N_6O{\cdot}2C_4H_4O_4$[c]
15	4	NCN	$NH(CH_2)_3NH_2$	76	126–129	$C_{21}H_{34}N_6O{\cdot}2C_4H_4O_4$[c]
16	3	NCN	1-piperazinyl	56	oil	$(C_{21}H_{32}N_6O)$[e]
17	3	NCN	$NH(CH_2)_2OH$	83	142–145	$C_{19}H_{29}N_5O_2{\cdot}C_{20}H_{18}O_8$[i]
18	3	O	$NH(CH_2)_2NH_2$	83	oil	$(C_{18}H_{30}N_4O_2)$[e]

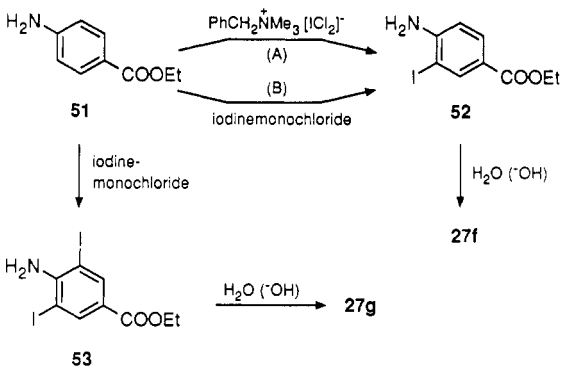
[a] Recrystallized from MeCN–EtOH, unless otherwise indicated. [b] All compounds analyzed for C, H, N except formulas in parentheses. [c] $C_4H_4O_4$ = maleic acid. [d] Bis[(2*R*,3*R*)-*O*,*O*′-ditoluoyltartrate]. [e] Used directly for further reaction without purification for analysis, structures confirmed by ^{1}H NMR. [f] $^+$FAB-MS, m/z 401 (M + H$^+$). [g] From iPrOH–Et_2O. [h] From Et_2O–EtOH–MeOH. [i] (2*R*,3*R*)-*O*,*O*′-Ditoluoyltartrate.

Scheme III



sulfonamides **49** and **50** by derivatization with fluoronitrobenzenes, by acylation via the carbonyldiimidazole (CDI) method, or by reaction with aromatic sulfonylchlorides (Scheme III). The requisite iodinated aminobenzoic acids **27f,g** were prepared by iodination of ethyl *p*-aminobenzoate (**51**), followed by alkaline hydrolysis (Scheme IV). Benzyltrimethylammonium dichloroiodate[8] proved to be a very effective reagent, in particular, for the monoiodination of **51** in high yield. In contrast to the preparation of unlabeled IAPT (**31f**) from **27f**, the radioligand [^{125}I]APT was prepared from **31e** by iodination according to the chloramine T method.[4] The mono-

Scheme IV



(2) For a recent review see: Van der Goot, H.; Bast, A.; Timmerman, H. Structural Requirements for Histamine H_2 Agonists and H_2 Antagonists. In *Histamine and Histamine Antagonists*; Uvnäs, B., Ed.; Handbook of Experiment Pharmacology, Vol. 97; Springer: Berlin Heidelberg, 1991; pp 573–748.

(3) Gajtkowski, A. G.; Norris, D. B.; Rising, T. J.; Wood, T. P. Specific Binding of ^{3}H-Tiotidine to Histamine H_2 Receptors in Guinea Pig Cerebral Cortex. *Nature (London)* **1983**, *304*, 65–67.

(4) Ruat, M.; Traiffort, E.; Bouthenet, M. L.; Schwartz, J. C.; Hirschfeld, J.; Buschauer, A.; Schunack, W. Reversible and Irreversible Labeling and Autoradiographic Localization of the Cerebral Histamine H_2 Receptor Using [^{125}I]Iodinated Probes. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1638–1662.

(5) Traiffort, E.; Ruat, M.; Schwartz, J. C. Interaction of Mianserin, Amitriptyline and Haloperiodol with Guinea Pig Cerebral Histamine H_2 Receptors Studied with [^{125}I]Iodoaminopotentidine. *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1991**, *207*, 143–148.

(6) Martinez-Mir, M. I.; Pollard, H.; Moreau, J.; Arrang, J. M.; Ruat, M.; Traiffort, E.; Schwartz, J. C.; Palacios, J. M. Three Histamine Receptors (H_1, H_2, and H_3), Visualized in the Brain of Human and Non Human Primates. *Brain Res.* **1990**, *256*, 322–327.

(7) Traiffort, E.; Pollard, H.; Moreau, J.; Ruat, M.; Schwartz, J. C.; Martinez-Mir; M. I.; Palacios, J. M. Pharmacological Characterization and Autoradiographic Localization of Histamine H_2 Receptors in Human Brain Identified with [^{125}I]-Iodoaminopotentidine. *J. Neurochem.*, in press.

(8) Kajigaeshi, S.; Kakinami, T.; Yamasaki, H.; Fujisaki, S.; Okamoto, T. Halogenation Using Quaternary Ammonium Polyhalides. VII. Iodination of Aromatic Amines by Use of Benzyltrimethylammonium Dichloroiodate(I$^-$). *Bull. Chem. Soc. Jpn.* **1988**, *61*, 600–602.

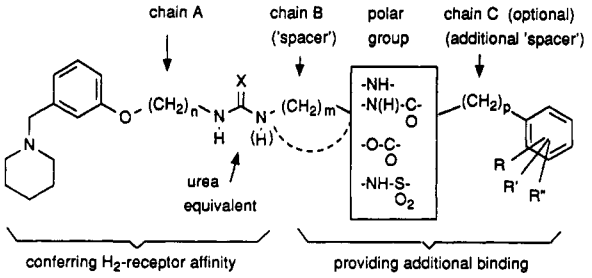


Figure 1.

iodinated *p*-azidobenzamide **31h** was obtained by the reaction of the corresponding primary amine **31f** with sodium nitrite and sodium azide in acetic acid.

Biological Results and Discussion

Intermediate **8** and the final compounds **19**, **22–26**, **31–46**, **49**, and **50** were tested for histamine H_2 antagonism (inhibition of the positive chronotropic response) with the spontaneously beating right atrium of the guinea pig according to the procedure described by Black et al.[1] (Table II). Specificity for H_2 receptors was evaluated for selected compounds (**31e–i**, **33i**) on the basis of their potency to antagonize the positive chronotropic response to isoproterenol in the guinea pig atrium (β_1 adrenergic receptors), the contractile response to histamine and carbachol in the guinea pig ileum (H_1 and muscarinic M_3 receptors), and the vasoconstricting response in the rat isolated aorta and tail artery (α_1 adrenergic and 5-HT_2 receptors, respectively) (Table III). The binding studies at histamine H_2 receptors were performed with homogenized guinea pig striatal membranes. The antagonist activity, expressed as pK_B, pA_2, or pK_i, respectively, is reported in Tables II and III.

All compounds investigated showed moderate to high H_2 antagonistic potency, ranging from 6% (**45c**) to about 4400% (**24a**) (relative to cimetidine = 100%), and pronounced H_2 selectivity in both functional pharmacological tests and binding studies.

Structure–Activity Relationships. The H_2 antagonistic potency of all tested compounds is considerably dependent on the length of the connecting carbon chains (see Figure 1). Generally, the influence of ring substituents appears to be less important. Nevertheless, there is an interdependence between substitution pattern and optimal chain length.

Nitroanilines 22–26. These H_2 antagonists are about 1.4–44 times more active than cimetidine. The highest potency is found in cyanoguanidines linked with a four-membered chain A (Figure 1) to the phenoxy moiety and a two-membered carbon chain B to the nitroanilino portion ("C_4/C_2" chain, **24a**). The incorporation of a *p*-azido group is always associated with reduced potency (**22b**, **23b**, **24b**, **25b**). The decrease in activity is remarkably high (about 1 order of magnitude) in the nitroanilino*propyl*guanidine series (see **23a** versus **23b**, and **25a** versus **25b**).

Carboxamides 31–43, 45, and 46 and Ester 44c. The H_2 antagonistic potency of these compounds in functional experiments is in the range of 0.06–32-fold that of cimetidine. The most active compounds are characterized by C_3 and C_2 carbon chains A and B, respectively ("C_3/C_2" chain). In this series iodine atoms in the 3-position or 3,5-positions (**31f,g**) favorably affect the activity relative to the *p*-amino-substituted compound **31e**. Monosubstitution with an azido group in the para-position (**31d**) or an iodine atom in meta-position (**31i**) is well tolerated, but the combination of both results in a 5–10-fold decrease in activity (**31h**), indicating the optimal orientation of the antagonist molecule at the H_2 receptor to be disturbed. In contrast to the compounds mentioned above, in the series with C_4 and C_2 chains A and B, respectively, the iodination in the meta-position generally results in a considerable decrease in potency (**33i**), although an activity-enhaning effect of iodine is found in *p*-amino-substituted compounds (**33f,g** versus **33e**). The level of potency achieved in the "C_4/C_2" series of cyanoguanidines (**33a–i**) is generally lower than in the corresponding "C_3/C_2" homologues (**31c–m**), and this tendency is even more evident in the "C_4/C_3" (**34c,d**) and in the "C_3/C_3" series (**32c,d**) as well as in compounds with a longer chain B (**35c,e**, **36c**, **37c**). Thus, the combination of a three-membered carbon chain A and a two-membered carbon chain B ("C_3/C_2" series) proved to be the optimum, which was chosen for further structural variations. Aromatic amides, such as **31c**, are more potent than the corresponding arylalkanamides (**38c**, **39c**, **40c**, **41c**), indicating that an "additional spacer" (chain C) between the carboxamide group and aromatic ring (Figure 1) should be omitted. As shown by compounds **31c** and **46c**, cyanoguanidines are about 100 times more active than the corresponding ureas, which may be the result of different conformations induced by both "urea equivalents".[9] The carboxamide nitrogen may be replaced by oxygen, resulting in an ester (**44c**), without remarkably affecting H_2 antagonistic potency, suggesting an H-donor group to be not required in this position. Comparison of the apparent dissociation constants of some compounds as antagonists of the histamine-induced chronotropic response and as inhibitors of ^{125}IAPT binding to cerebral membranes indicated some marked differences. Whereas the apparent potencies of cimetidine, ranitidine, or tiotidine were similar in the two tests, reflecting interactions with H_2 receptors, the presently described compounds were 6 (**31e**) to 54 times (**33i**) more potent in the binding test. It is unlikely that this reflects H_2 receptor heterogeneity since the same H_2 receptor mRNA is present in guinea pig heart and brain.[7] The higher apparent potency of these compounds in the binding assay presumably reflects their easier access to the H_2 receptor in membranes (as opposed to intact isolated heart) after a long, i.e. 2-h, equilibration period.[4]

Sulfonamides 49, 50. The replacement of the carbonyl function in **31c** and **33c** by a sulfonyl group leads to a decrease in H_2 activity (**49c,n**, **50c,n**). However, in contrast to the carboxamides, the "C_4/C_2" series is more potent than the compounds with a "C_3/C_2" chain.

Starting from the primary aliphatic amine **8**, which is a moderately active H_2 antagonist ($pK_B \approx 6$), the receptor affinity could be increased by all the derivatizations described above. However, the most pronounced increase in affinity was achieved by additionally introducing lipophilic substituents such as iodine. This may be due to the presence of a hydrophobic accessory binding area some distance from the binding site for the urea equivalent as recently proposed for some H_2 antagonists with cyclic polar groups, e.g., diaminofurazans, diaminothiadiazoles, or squaramides.[10] This assumption is supported by the fact that the H_2 antagonistic activity is markedly affected by (1) the nature of the urea equivalent (*N*-cyanoguanidines are more active than ureas), (2) the length of the flexible chain (optimum of the chain length of C_4/C_3 or C_3/C_2,

(9) Ganellin, C. R. Chemistry of Drugs Acting at Histamine Receptors. In *Pharmacology of Histamine Receptors*; Ganellin, C. R.; Parsons, M. E., Eds.; Wright PSG: Bristol, 1982; pp 10–102.

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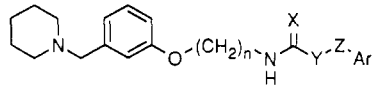
Table II. Structures, Formulas, Results of the Pharmacological Screening for H_2 Antagonism on the Isolated Guinea Pig Right Atrium, and H_2 Receptor Binding Data (Guinea Pig Cerebral Membranes) of Selected Compounds



	X
8, 22-45, 47-50	NCN
19, 46	O

no.	n	Y	Z	Ar	% yield	mp,[a] °C	formula[b]	H_2 antagonism for guinea pig atrium: $pK_B \pm 0.2$[c]	H_2 antagonism for guinea pig atrium: % rel pot.[d]	receptor binding $pK_i \pm 0.1$[e]
8[f]	3	$NH(CH_2)_2NH_2$						5.95[g]	36	
19	3	–	–	$4\text{-}NH_2C_6H_4$	54	131–134	$C_{22}H_{29}N_3O_2\cdot{}^1/_4H_2O$	6.82	263	
22a	3	$NH(CH_2)_2NH$	–	$2\text{-}NO_2C_6H_4$	65	131[h]	$C_{25}H_{33}N_7O_3$	7.45[i]	1122	
22b	3	$NH(CH_2)_2NH$	–	$4\text{-}N_3\text{-}2\text{-}NO_2C_6H_3$	45	130[j]	$C_{25}H_{32}N_{10}O_3$	7.39	977	
23a	3	$NH(CH_2)_3NH$	–	$2\text{-}NO_2C_6H_4$	63	90	$C_{26}H_{35}N_7O_3$	7.52[k]	1318	
23b	3	$NH(CH_2)_3NH$	–	$4\text{-}N_3\text{-}2\text{-}NO_2C_6H_3$	44	121[j]	$C_{26}H_{34}N_{10}O_3\cdot H_2O$	6.79[l]	245	
24a	4	$NH(CH_2)_2NH$	–	$2\text{-}NO_2C_6H_4$	68	127–128	$C_{26}H_{35}N_7O_3\cdot H_2O$	8.04[m]	4365	
24b	4	$NH(CH_2)_2NH$	–	$4\text{-}N_3\text{-}2\text{-}NO_2C_6H_3$	44	130–133[h]	$C_{26}H_{34}N_{10}O_3\cdot H_2O$	7.47[l]	1175	
25a	4	$NH(CH_2)_3NH$	–	$2\text{-}NO_2C_6H_4$	73	78–80[j]	$C_{27}H_{37}N_7O_3$	7.85	2818	
25b	4	$NH(CH_2)_3NH$	–	$4\text{-}N_3\text{-}2\text{-}NO_2C_6H_3$	43	103–105	$C_{27}H_{36}N_{10}O_3$	6.56	145	
26a	3	$NHCH_2CH(OH)CH_2NH$	–	$2\text{-}NO_2C_6H_4$	73	oil	$(C_{26}H_{35}N_7O_4)^n$	7.34[k]	871	
31c	3	$NH(CH_2)_2NH$	CO	Ph	93	139–140	$C_{26}H_{34}N_6O_2$	7.82[l]	2630	
31d	3	$NH(CH_2)_2NH$	CO	$4\text{-}N_3C_6H_4$	46	152–153[j]	$C_{26}H_{33}N_9O_2$	7.90	3162	
31e	3	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2C_6H_4$	96	92–95	$C_{26}H_{35}N_7O_2\cdot C_2H_2O_4\cdot{}^1/_2H_2O$	$pA_2 = 7.22$	660	8.01
31f	3	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2\text{-}3\text{-}IC_6H_3$	81	114–117	$C_{26}H_{34}IN_7O_2$	$pA_2 = 7.56$	1445	9.15
31g	3	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2\text{-}3,5\text{-}I_2C_6H_2$	87	131–134	$C_{26}H_{33}I_2N_7O_2$	$pA_2 = 7.79$	2455	
31h	3	$NH(CH_2)_2NH$	CO	$4\text{-}N_3\text{-}3\text{-}IC_6H_3$	57	134–136[j]	$C_{26}H_{32}IN_9O_2$	$pA_2 = 6.81$	257	7.92
31i	3	$NH(CH_2)_2NH$	CO	$3\text{-}IC_6H_4$	71	134–136	$C_{26}H_{33}IN_6O_2$	7.57[l]	1479	8.58
31k	3	$NH(CH_2)_2NH$	CO	$3\text{-}NH_2C_6H_4$	81	115–116[o]	$C_{26}H_{35}N_7O_2\cdot{}^1/_2H_2O$	7.29[p]	776	
31l	3	$NH(CH_2)_2NH$	CO	4-pyridyl	47	155–156	$C_{25}H_{33}N_7O_2\cdot{}^1/_2H_2O$	6.89	309	
31m	3	$NH(CH_2)_2NH$	CO	$4\text{-}NHtBocC_6H_4$	57	134–136[j]	$C_{31}H_{43}N_7O_4$	6.87[p]	295	
32c	3	$NH(CH_2)_3NH$	CO	Ph	73	120	$C_{27}H_{36}N_6O_2$	7.14[l]	550	
32d	3	$NH(CH_2)_3NH$	CO	$4\text{-}N_3C_6H_4$	43	73–75[j]	$C_{27}H_{35}N_9O_2\cdot{}^1/_2H_2O$	6.36	91	
33a	4	$NH(CH_2)_2NH$	CO	$2\text{-}NO_2C_6H_4$	67	114[j]	$C_{27}H_{35}N_7O_4$	7.52	1318	
33c	4	$NH(CH_2)_2NH$	CO	Ph	67	135–137[j]	$C_{27}H_{36}N_6O_2$	7.67	1862	
33d	4	$NH(CH_2)_2NH$	CO	$4\text{-}N_3C_6H_4$	67	153[j]	$C_{27}H_{35}N_9O_2$	7.54[l]	1380	
33e	4	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2C_6H_4$	88	114–115	$C_{27}H_{37}N_7O_2$	7.17[l]	589	
33f	4	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2\text{-}3\text{-}IC_6H_3$	95	132–135[j]	$C_{27}H_{36}IN_7O_2$	7.35[l]	891	
33g	4	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2\text{-}3,5\text{-}I_2C_6H_2$	83	140–146	$C_{27}H_{35}I_2N_7O_2$	7.86[p]	2884	
33i	4	$NH(CH_2)_2NH$	CO	$3\text{-}IC_6H_4$	74	137–140	$C_{27}H_{35}IN_6O_2$	6.56	145	8.28
34c	4	$NH(CH_2)_3NH$	CO	Ph	57	97–98[j]	$C_{28}H_{38}N_6O_2\cdot{}^1/_2H_2O$	6.60	158	
34d	4	$NH(CH_2)_3NH$	CO	$4\text{-}N_3C_6H_4$	63	115–117[j]	$C_{28}H_{37}N_9O_2\cdot{}^1/_2H_2O$	6.63[l]	170	
35c	3	$NH(CH_2)_4NH$	CO	Ph	76	122–123	$C_{28}H_{38}N_6O_2\cdot C_{20}H_{18}O_8\cdot 2H_2O$	6.81	257	
35e	3	$NH(CH_2)_4NH$	CO	$4\text{-}NH_2C_6H_4$	53	163	$C_{28}H_{39}N_7O_2\cdot C_{20}H_{18}O_8\cdot H_2O$	6.31	81	
36c	3	$NH(CH_2)_5NH$	CO	Ph	82	123–125	$C_{29}H_{40}N_6O_2\cdot C_{20}H_{18}O_8\cdot H_2O$	6.58	151	
37c	3	$NH(CH_2)_6NH$	CO	Ph	62	118–119[o]	$C_{30}H_{42}N_6O_2\cdot C_{20}H_{18}O_8\cdot H_2O$	6.60	158	
38c	3	$NH(CH_2)_2NH$	$C(O)CH_2$	Ph	77	126–127	$C_{27}H_{36}N_6O_2$	6.93	339	
39c	3	$NH(CH_2)_2NH$	$C(O)(CH_2)_2$	Ph	85	122–124	$C_{28}H_{38}N_6O_2$	6.27	74	
40c	3	$NH(CH_2)_2NH$	$C(O)(CH_2)_3$	Ph	98	75–78[h]	$C_{29}H_{40}N_6O_2$	6.45	112	
41c	3	$NH(CH_2)_3NH$	$C(O)(CH_2)_2$	Ph	71	104–106	$C_{29}H_{40}N_6O_2$	6.07	47	
42m	4	$NH(CH_2)_2NH$	$C(O)CH_2$	$4\text{-}NHtBocC_6H_4$	84	105–111[j]	$C_{33}H_{47}N_7O_4$	6.04	44	
43c	3	$NHCH_2CH(OH)CH_2NH$	CO	Ph	85	138–141[j]	$C_{27}H_{36}N_6O_3$	7.31[p]	813	
44c	3	$NH(CH_2)_2O$	CO	Ph	45	115[q]	$C_{26}H_{33}N_5O_3\cdot C_{20}H_{18}O_8\cdot H_2O$	7.64	1738	
45c	3	1,4-piperazinediyl	CO	Ph	70	136–140	$C_{28}H_{36}N_6O_2\cdot C_{20}H_{18}O_8\cdot H_2O$	5.16[r]	6	
46c	3	$NH(CH_2)_2NH$	CO	Ph	79	109–113	$C_{25}H_{34}N_4O_3$	6.10	50	
46e	3	$NH(CH_2)_2NH$	CO	$4\text{-}NH_2C_6H_4$	74	115–118[j]	$C_{25}H_{35}N_5O_3$	7.23	676	
49c	3	$NH(CH_2)_2NH$	SO_2	Ph	82	147–148[j]	$C_{25}H_{34}N_6O_3S$	6.67[p]	186	
49n	3	$NH(CH_2)_2NH$	SO_2	$4\text{-}MeC_6H_4$	54	128-130[s]	$C_{26}H_{36}N_6O_3S$	6.13	54	
50c	4	$NH(CH_2)_2NH$	SO_2	Ph	65	80–82	$C_{26}H_{36}N_6O_3S$	7.28	759	
50n	4	$NH(CH_2)_2NH$	SO_2	$4\text{-}MeC_6H_4$	50	110–112[t]	$C_{27}H_{38}N_6O_3S\cdot{}^1/_2H_2O$	7.29	776	
cimetidine								6.40	100	6.52
ranitidine								7.20	631	7.27
tiotidine								7.80	2512	8.25

[a] Recrystallization solvent Et_2O–EtOH unless otherwise indicated. [b] All compounds analyzed for C, H, N, except formulas in parentheses. $C_2H_2O_4$ = oxalic acid, $C_{20}H_{18}O_8$ = (2*R*,3*R*)-*O,O'*-ditoluoyl-L-tartaric acid. [c] pK_B calculated for 1 μM antagonist concentration, unless otherwise indicated. Mean of at least three independent experiments, SEM < ±0.2; for pA_2 values, also see Table III. [d] Potency relative to cimetidine = 100%. [e] Assessed as inhibition of ^{125}IAPT binding at H_2 receptors in guinea pig brain. [f] For physical properties, see Table I. [g] Antagonist concentration of 10 μM. [h] From petroleum ether–Et_2O–EtOH. [i] Antagonist concentration of 0.1 μM. [j] From Et_2O. [k] Antagonist concentration of 0.3 and 0.1 μM. [l] Antagonist concentration of 0.3 μM. [m] Antagonist concentration of 0.03 and 1 μM. [n] $^+$FAB-MS *m/z* (relative intensity) 510 (34, M + H^+). [o] From Et_2O–CH_2Cl_2. [p] Antagonist concentration of 0.3 and 1 μM. [q] From anhydrous EtOH. [r] Antagonist concentration of 30 μM. [s] From cyclohexane–EtOH. [t] From cyclohexane.

Table III. Antagonistic Activity of Selected Carboxamides at H_2 (Guinea Pig Right Atrium), H_1 (Guinea Pig Ileum), M_3 (Guinea Pig Ileum), β_1 (Guinea Pig Right Atrium), α_1 (Rat Aorta), and 5-HT_2 Receptors (Rat Tail Artery)

compd	receptor	pK_B ● SEM[a]	pA_2 ● SEM[b]	slope ± SEM[b]	N[c]
31e	H_2	7.28 ± 0.07[d]	7.22 ± 0.64	1.09 ± 0.07	7
	H_1		4.57 ± 0.26	0.88 ± 0.04	12
	M_3	5.16 ± 0.07[e]			8
31f	H_2	7.52 ± 0.07[f]	7.56 ± 0.72	0.90 ± 0.07	9
	H_1	5.96 ± 0.02[g]			4
	M_3	5.49 ± 0.09[e]			4
	β_1	4.77[e]			2
	α_1		5.59 ± 0.12	1.29 ± 0.02	12
	5-HT_2		6.06 ± 0.23	1.09 ± 0.03	13
31g	H_2	7.79 ± 0.07[h]			7
	H_1		5.80 ± 0.21	0.91 ± 0.03	9
	M_3		5.77 ± 0.18	1.04 ± 0.03	12
31h	H_2	6.98 ± 0.08[i]	6.81 ± 0.57	1.20 ± 0.08	7
	H_1	5.62 ± 0.05[g]			8
	M_3	5.48 ± 0.09[g]			6
	α_1	6.17 ± 0.07[j]			6
	β_1	5.12[e]			2
	5-HT_2	6.05 ± 0.09[k]			4
31i	H_2	7.62 ± 0.05[l]			4
	H_1		5.65 ± 0.22	1.25 ± 0.04	9
	M_3	5.61 ± 0.04[m]			4
33i	H_2	6.56[l]			2
	H_1	6.15 ± 0.09[n]			8
	M_3	5.59 ± 0.07[o]			8

[a] pK_B (mean ± SEM) calculated from the expression pK_B = −log [antagonist] + log (concentration ratio − 1). Antagonist concentrations used are indicated as footnotes. [b] pA_2 values and slope determined by a Schild plot; at least three different antagonist concentrations used. [c] Number of experiments. [d–o] Antagonist concentrations: [d] 0.1, 0.3, and 1 μM. [e] 10 and 30 μM; [f] 0.03, 0.1, and 0.3 μM; [g] 3 and 10 μM. [h] 0.03 and 0.1 μM. [i] 0.3, 1, and 3 μM. [j] 3, 10, 30, and 100 μM. [k] 3, 10, and 30 μM. [l] 1 μM. [m] 10 μM. [n] 1, 3, and 10 μM. [o] 1 and 3 μM.

depending on the nature of the polar group), (3) the nature of the polar group acting as the link between the aromatic ring and chain B, (4) an additional spacer (chain C), and (5) the substituents of the aromatic ring.

Although the contributions of the several substructures may not be attributed to a single parameter, there appears to be a general tendency toward higher H_2 affinity in compounds characterized by more hydrophobic groups. As the genes encoding the histamine H_2 receptors in dog, man, and rat have recently been cloned,[11] speculations on pharmacophores[12] and corresponding crucial receptor domains may be substantiated in the future by means of molecular modeling methods.

Histamine H_2 Receptor Selectivity. The affinities for β_1, α_1, and H_1 receptors in functional studies are all in the same range of about 3 μM ($pK_B \approx 5.5$) (Table III). The affinity for 5-HT_2 receptors is nearly $^1/_2$ order of magnitude higher than for the other non-H_2 receptors. It is evident that the selectivity for H_2 receptors—at least in functional experiments—is decreased by introducing iodine into the molecule and thereby increasing lipophilicity, reflecting an enhanced influence of non-H_2-specific drug–receptor interactions. Nevertheless, the most potent H_2 antagonists (**31f,g**) are 30–100 times more active in functional experiments at H_2 receptors than at β_1, α_1, muscarinic, and 5-HT_2 receptors.

The aim of this study was to find model compounds for the development of ^{125}I radioactive probes for reversible and irreversible labeling of the H_2 receptor. The highly potent H_2 antagonist **31c** was selected as a chemical lead, because this benzamide could be substituted over a wide range without loss of H_2 receptor affinity. The *p*-amino analogue **31e** proved to be a suitable precursor for iodination (**31f**), and furthermore, the amino group could be converted into a photolabile azide (**31h**). Thus, the synthesis of the (benzamidoalkyl)cyanoguanidines led to the development of the first reversibly and irreversibly binding [^{125}I]-radioligands for the H_2 receptor, i.e., [^{125}I]iodoaminopotentidine and [^{125}I]iodoazidopotentidine, which are useful tools for the characterization and localization of H_2 receptors.

Experimental Section

Chemistry. Melting points were determined with a Büchi apparatus and are not corrected. Elemental analyses (C, H, N), performed by the analytical department of the Institute of Pharmacy, Freie Universität Berlin, were within ±0.4% of the theoretical values unless otherwise noted. IR spectra (KBr) were measured with a Perkin-Elmer IR 1420. UV spectra were obtained on a Hewlett-Packard HP 8451 A. ^{1}H-NMR spectra were recorded on a Bruker WM 250 (250 MHz) and a Bruker AC 300 (300 MHz) spectrometer using tetramethylsilane as internal reference. Electron-impact mass spectra (EI) were measured with a Finnigan MAT CH7A (170 °C, 70 eV) and a MAT 711 mass spectrometer (200 °C, 80 eV); fast atom bombardment mass spectra ($^+$FAB, xenon, DMSO/glycerin) were recorded with a Finnigan MAT CH5DF mass spectrometer. Chromatographic purifications on a preparative scale were performed with a Chromatotron 7924T (Harrison Research), using glass rotors with 4-mm layers of silica gel PF_{254} containing gypsum (Merck) or by column chromatography on silica gel (63–200, Merck).

^{125}IAPT was prepared using the chloramine T method and purified by HPLC (C18 μBondapack, Waters) as described.[4] Azidation of this radioactive compound was detailed in the same reference. 3-[3-(1-Piperidinylmethyl)phenoxy]propanamine (**1**),[13] 4-[3-(1-piperidinylmethyl)phenoxy]butanamine (**2**),[14] *N*-cyano-*O*-phenyl-*N'*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]isourea (**5**), *N*-cyano-*O*-phenyl-*N'*-[4-[3-(1-piperidinylmethyl)phenoxy]-butyl]isourea (**6**), and phenyl *N*-[3-[3-(1-piperidinylmethyl)-phenoxy]propyl]carbamate (**7**)[15] were prepared according to known procedures. The benzoic acids and phenylalkanoic acids used (**27–30**) were either commercially available or prepared according to known methods (**27d**, *p*-azidobenzoic acid[16]) or as described below (**27f,g**), respectively.

Iodinated 4-Aminobenzoic Acids 27f,g. 4-Amino-3-iodobenzoic Acid (27f). Iodination of ethyl *p*-aminobenzoate, Method A. Iodine monochloride (ICl) (4.91 g, 30.27 mmol) was added to a stirred solution of ethyl 4-amino-3-iodobenzoate (10 g, 60.54 mmol) in CH_2Cl_2. After refluxing for 1 h, the reaction mixture was cooled and discolored by adding an aqueous solution of sodium thiosulfate (10%). The organic layer was separated, washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on a silica gel column (chloroform–petroleum ether, 8:2, v/v) affording 8.29 g (47% yield)

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of ethyl 4-amino-3-iodobenzoate (**52**) as light yellow crystals.

Method B. Benzyltrimethylammonium dichloroiodate (13.7 g, 39.19 mmol) was added to a stirred solution of ethyl 4-aminobenzoate (5 g, 30.27 mmol) and $CaCO_3$ (9.01 g, 90.8 mmol) in 250 mL of CH_2Cl_2 and 100 mL of MeOH. After 48 h insoluble material was removed by filtration and the solution was concentrated. The resulting oily residue was diluted with CH_2Cl_2 and discolored by adding an aqueous 5% solution of sodium thiosulfate. The organic layer was separated and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The resulting brown residue was recrystallized from H_2O/EtOH to furnish 8.55 g of **52** (yield 97%) as red brown crystals: mp 79–81 °C (lit.[17] mp 81–82 °C). Anal. ($C_9H_{10}INO_2$) C, H, N.

The ester **52** was converted into **27f** by alkaline hydrolysis (10% KOH, reflux 1 h) followed by acidification with HCl, yield 98%: mp 193–194 °C (sublimation) (from EtOH/H_2O) (lit.[18] mp 203–204 °C). Anal. ($C_7H_6INO_2$) C, H, N.

Ethyl 4-amino-3,5-diiodobenzoate (**53**) and 4-amino-3,5-diiodobenzoic acid (**27g**) were analogously prepared according to the procedure described above (method A for the ester) by using a double amount of ICl. **53**: yield 87%, mp 143–145 °C (from EtOH/H_2O) (lit.[19] mp 148 °C). **27g**: yield 98%, mp >350 °C (lit.[19] mp >350 °C).

Cyanoguanidines 8–17 and Urea 18. *N*-(2-Aminoethyl)-*N'*-cyano-*N''*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine (8). A 20-fold excess of ethylenediamine (12 g) was added to a suspension of *N*-cyano-*O*-phenyl-*N'*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]isourea (**5**) (7 g, 17.9 mmol) in CH_2Cl_2. The mixture was stirred overnight and subsequently poured into H_2O. The organic layer was separated and washed consecutively with H_2O, 10% NaOH, and H_2O, dried over Na_2SO_4, and evaporated in vacuo. The resulting oil (6.4 g, 90%) was pure enough for further reactions: MS (70 eV) *m/z* (relative intensity) 358 (1, $M^{+\bullet}$), 84 (100); ^1H NMR ($CDCl_3$) δ 7.23 (1 H, exchangeable, NH), 7.20 (t, *J* = 8 Hz, 1 H, 3-H), 7.16 (s, 1 H, 6-H), 6.93 (d, *J* = 8 Hz, 1 H, 2-H), 6.86 (d, *J* = 8 Hz, 1 H, 4-H), 6.32 (br, 1 H, exchangeable, NH), 4.02 (t, *J* = 5.5 Hz, 2 H, OCH_2), 3.42–2.62 (m, 10 H, 4 CH_2, 2 H exchangeable, NH_2), 2.37 (s, 4 H, 2 CH_2), 2.00 (t, *J* = 5 Hz, 2 H, CH_2), 1.54 (m, 4 H, 2 CH_2), 1.40 (m, 2 H, CH_2). An analytical sample of amine **8** was purified by preparative chromatography using a Chromatotron ($CHCl_3$–MeOH, 95:5, ammonia atmosphere). After removal of the solvents in vacuo the remaining oil was dissolved in anhydrous EtOH and converted into the maleic acid adduct by addition of an ethanolic solution of maleic acid. The salt was isolated by evaporation to dryness and crystallization from Et_2O and a few drops of EtOH: mp 138–140 °C (from MeCN–EtOH). Anal. ($C_{19}H_{30}N_6O{\cdot}2C_4H_4O_4$) C, H, N.

Cyanoguanidines **9–17** were analogously prepared starting from the requisite isoureas **5** or **6** and urea **18** starting from carbamate **7**. For the preparation of **16** iPrOH was used as the solvent instead of CH_2Cl_2. Compound **16** was isolated in the same way as **8** after removal of the solvent in vacuo. For analysis **9** and **12** were crystallized as bis[(2*R*,3*R*)-*O,O'*-ditoluoyltartrates], **17** was isolated as (2*R*,3*R*)-*O,O'*-ditoluoyltartrate [recrystallization from MeCN–EtOH (**9**), Et_2O–EtOH (**12**), anhydrous EtOH–Et_2O (**17**)], **13** was isolated as the free base (recrystallization from iPrOH–Et_2O), **14** and **15** were isolated as maleates as described above for **8** [recrystallization from Et_2O–EtOH–MeOH (**14**) or MeCN–EtOH (**15**)].

4-Amino-*N*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]benzamide (19). *p*-Aminobenzoic acid (0.55 g, 4 mmol) was added to a stirred solution of *N,N'*-carbonyldiimidazole (0.65 g, 4 mmol) in anhydrous THF. After 1 h 1.0 g (4 mmol) of amine **1** was added and the mixture was allowed to react overnight at ambient temperature. Subsequently the solution was poured into H_2O and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The remaining oil was treated with Et_2O affording 0.8 g (54%) of **19** as a white solid: mp 131–134 °C dec; MS (70 eV) *m/z* (relative intensity) 367 (2 $M^{+\bullet}$), 31 (100); ^1H NMR ($CDCl_3$) δ 7.60 (d, *J* = 8.5 Hz, 2 H, 2'-H, 6'-H), 7.21 (t, *J* = 8 Hz, 1 H, 3-H), 6.90 (m, 2 H, 6-H, 2-H), 6.78 (dd, *J* = 8, 2 Hz, 1 H, 4-H), 6.64 (d, *J* = 8.5 Hz, 2 H, 3'-H, 5'-H), 6.60 (br, 1 H, exchangeable, NH), 4.10 (t, *J* = 6 Hz, 2 H, OCH_2), 3.98 (s, 2 H, exchangeable, NH_2), 3.63 (q, *J* = 6 Hz, 2 H, CH_2NH), 3.44 (s, 2 H, NCH_2Ar), 2.37 (s, 4 H, 2 CH_2), 2.09 (quint, *J* = 6 Hz, 2 H, CH_2), 1.54 (m, 4 H, 2 CH_2), 1.43 (m, 2 H, CH_2). Anal. ($C_{22}H_{29}N_3O_2{\cdot}{}^1/_4H_2O$) C, H, N.

Nitroanilines 22–26. *N*-Cyano-*N'*-[2-(2-nitrophenylamino)ethyl]-*N''*-[4-[3-(1-piperidinylmethyl)phenoxy]butyl]guanidine (24a). A mixture of **14** (1.43 g, 4.12 mmol), *o*-fluoronitrobenzene (0.58 g, 4.12 mmol), and anhydrous Na_2CO_3 (650 mg) in 30 mL of EtOH was refluxed for 15 h. Subsequently, the hot mixture was filtered and the filtrate was evaporated in vacuo. Compound **24a** was isolated by chromatography (Chromatotron, $CHCl_3$–MeOH, 97:3, ammonia atmosphere) (yield 1.37 g, 68%): mp 127–128 °C (from Et_2O–EtOH); $^+$FAB-MS *m/z* (relative intensity) 494 (34, M + H^+), 98 (100); ^1H NMR ($CDCl_3$) δ 8.21 (br, 1 H, exchangeable, NH), 8.03 (d, *J* = 8 Hz, 1 H, 3'-H), 7.44 (t, *J* = 8 Hz, 1 H, 5'-H), 7.21 (t, *J* = 8 Hz, 1 H, 3-H), 6.95–6.88 (m, 3 H, 6-H, 2-H, 6'-H), 6.75–6.68 (m, 2 H, 4-H, 4'-H), 6.66–6.61 (br, 1 H, exchangeable, NH), 6.19–5.75 (br, 1 H, exchangeable, NH), 3.97 (t, *J* = 5.5 Hz, 2 H, OCH_2), 3.54 (br, 4 H, 2 CH_2NH), 3.42 (s, 2 H, NCH_2Ar), 3.30 (d, *J* = 6 Hz, 2 H, CH_2NH), 2.37 (s, 4 H, 2 CH_2), 1.99–1.70 (br, 4 H, CH_2CH_2), 1.57 (m, 4 H, 2 CH_2), 1.43–1.41 (m, 2 H, CH_2). Anal. ($C_{26}H_{34}N_{10}O_3{\cdot}H_2O$) C, H, N.

Nitroanilines **22a,b**, **23a,b**, **24b**, **25a,b**, and **26a** were prepared in the same way. Compound **22a** crystallized from the ethanolic solution obtained after removal of the unsoluble material by filtration of the hot reaction mixture and cooling to ambient temperature. For the azido compounds **22b**, **23b**, **24b**, and **25b** all steps were performed under dim light.

Amides 31–43, 45, and 46 and Ester 44c. *N*-[2-(4-Amino-3-iodobenzamido)ethyl]-*N'*-cyano-*N''*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine (31f). A solution of 0.34 g (2.08 mmol) of *N,N'*-carbonyldiimidazole (CDI) and 0.55 g (2.08 mmol) **27f** in 5 mL of anhydrous THF was stirred for 1 h. Subsequently 1.0 g (2.08 mmol) of amine **8** was added and the mixture was stirred for 8 h at ambient temperature. After addition of H_2O, **31f** was isolated by extraction with CH_2Cl_2 as an oil, which was purified by chromatography (Chromatotron, $CHCl_3$–MeOH, 96:4, ammonia atmosphere). The oily residue obtained after removal of the solvents was treated with Et_2O, affording 1.01 g (81%) of crystalline **31f**: mp 114–117 °C dec (from Et_2O–EtOH); MS (80 eV) *m/z* (relative intensity) 603 (1, $M^{+\bullet}$), 84 (100); ^1H NMR ($CDCl_3$) δ 8.12 (s, 1 H, 2'-H), 7.56 (d, *J* = 8.5 Hz, 1 H, 6'-H, and br, 1 H, exchangeable, NH), 7.18 (t, *J* = 7.5 Hz, 1 H, 3-H), 6.88 (d, *J* = 7.5 Hz, 2 H, 2-H, 6-H), 6.79 (d, *J* = 7.5 Hz, 1 H, 4-H), 6.64 (d, *J* = 8.5 Hz, 1 H, 5'-H, and br, 1 H, exchangeable, NH), 6.41 (br, 1 H, exchangeable, NH), 4.56 (s, 2 H, exchangeable, NH_2), 3.99 (t, *J* = 5.5 Hz, 2 H, OCH_2), 3.60–3.43 (m, 8 H, NCH_2Ar, 3 CH_2NH), 2.37 (s, 4 H, 2 CH_2), 2.03 (m, 2 H, CH_2), 1.56 (m, 4 H, 2 CH_2), 1.42 (m, 2 H, CH_2). Anal. ($C_{26}H_{34}IN_7O_2$) C, H, N.

Carboxamides **31c–e,g–m**, **32c,d**, **33a,c–g,i**, **34c,d**, **35c,e**, **36c**, **37c**, **38c**, **39c**, **40c**, **41**, **42m**, **43c**, **45c**, and **46c** were prepared in the same way. In case of carbonic acids with low solubility in THF, such as **27g**, a sufficient amount of DMF was added to the reaction mixture. For analysis some compounds had to be converted into a salt by addition of an ethanolic solution of oxalic acid (for **31e**) or a solution of (2*R*,3*R*)-*O,O'*-ditoluoyltartaric acid in Et_2O (see Table II). All azido compounds were synthesized and isolated under dim light.

Ester **44c** was obtained analogously by refluxing alcohol **17** for 3 h with the imidazolide freshly prepared from benzoic acid and *N,N'*-carbonyldiimidazole in THF. An analytical sample was crystallized as the *O,O'*-ditoluoyltartrate from EtOH.

***N*-[2-(4-Azido-3-iodobenzamido)ethyl]-*N'*-cyano-*N''*-[3-[3-(1-piperidinylmethyl)phenoxy]propyl]guanidine (Iodoazidopotentidine, IAZPT, 31h).** A 5% aqueous solution of $NaNO_2$ (0.06 g, 0.83 mmol) was added at 0 °C to a stirred solution of **31f** (0.50 g, 0.83 mmol) in 30 mL of 8 N acetic acid. After 4

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min a 5% aqueous solution of 0.05 g (0.83 mmol) of NaN_3 was added. The mixture was stirred for 3 min further at ambient temperature. After basification with 10% aqueous NaOH (cooling with ice) **31h** was isolated by extraction with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was solidified by treating the remaining yellow oil with Et_2O. Subsequently, **31h** was purified chromatographically (Chromatotron, $CHCl_3$–MeOH, 98:2, ammonia atmosphere) and crystallized from Et_2O (yield 0.3 g, 57%) (all steps were performed under dim light): mp 134–136 °C (from Et_2O); IR (KBr) 2166 (NCN), 2120 (N_3) cm^{-1}; UV (MeOH) λ_{max} 222, 272 nm (ϵ 21 377); $^+$FAB-MS m/z (relative intensity) 630 (18, M + H^+), 84 (100); ^{1}H NMR ($CDCl_3$) δ 8.27 (d, J = 2 Hz, 1 H, 2′-H), 7.91 (s, 1 H, exchangeable, NH), 7.86 (dd, J = 8.5, 2 Hz, 1 H, 6′-H), 7.21 (t, J = 8 Hz, 1 H, 3-H), 7.09 (d, J = 8.5 Hz, 1 H, 5′-H), 7.00 (s, 1 H, 6-H), 6.89 (d, J = 8 Hz, 1 H, 2-H), 6.83 (d, J = 8 Hz, 1 H, 4-H), 6.63 (m, 1 H, exchangeable, NH), 6.31 (m, 1 H, exchangeable, NH), 4.07 (t, J = 5.5 Hz, 2 H, OCH_2), 3.52–3.47 (m, 8 H, NCH_2Ar, 3 CH_2NH), 2.46 (s, 4 H, 2 CH_2), 2.07 (t, J = 6 Hz, 2 H, CH_2), 1.60 (m, 4 H, 2 CH_2), 1.45 (m, 2 H, CH_2). Anal. ($C_{26}H_{32}IN_9O_2$) C, H, N.

Sulfonamides 49, 50. *N*-(2-Benzenesulfonamidoethyl)-*N*′-cyano-*N*″-[4-[3-(1-piperidinylmethyl)phenoxy]butyl]guanidine (50c). To a stirred solution of primary amine **14** (0.6 g, 1.61 mmol) in CH_2Cl_2 was added 3 mL of Et_3N and 0.28 g (1.61 mmol) of benzenesulfonyl chloride (dissolved in CH_2Cl_2). After 5 min the mixture was poured into H_2O. The organic layer was separated, dried, over Na_2SO_4, and evaporated. The remaining oil was purified chromatographically (Chromatotron, $CHCl_3$–MeOH, 97:3, ammonia atmosphere). Sulfonamide **50c** crystallized after addition of anhydrous Et_2O (yield 0.54 g, 65%): mp 80–82 °C (from Et_2O–EtOH); MS (70 eV) m/z (relative intensity) 512 (1, $M^{+\cdot}$), 84 (100); ^{1}H NMR ($CDCl_3$) δ 7.84 (d, J = 7 Hz, 2 H, 2′-H, 6′-H), 7.60–7.49 (m, 3 H, 3′-H, 4′-H, 5′-H), 7.28 (br, 1 H, exchangeable, NH), 7.20 (t, J = 8 Hz, 1 H, 3-H), 6.99 (s, 1 H, 6-H), 6.87 (d, J = 8 Hz, 1 H, 2-H), 6.78 (d, J = 8 Hz, 1 H, 4-H), 6.11 (m, 1 H, exchangeable, NH), 5.72 (m, 1 H, exchangeable, NH), 4.01 (t, J = 5.5 Hz, 2 H, OCH_2), 3.46 (s, 2 H, NCH_2Ar), 3.31–3.06 (m, 6 H, 3 CH_2NH), 2.38 (s, 4 H, 2 CH_2), 1.84–1.78 (m, dt, J = 5.5, 6.5 Hz, 4 H, CH_2CH_2), 1.52 (m, 4 H, 2 CH_2), 1.43 (m, 2 H, CH_2). Anal. ($C_{26}H_{36}H_6O_3S$) C, H, N.

Sulfonamide **49c** was prepared in the same way; compounds **49n** and **50n** were analogously prepared at 0 °C using anhydrous pyridine instead of CH_2Cl_2–Et_3N as solvent and base. The mixture was poured onto ice. The workup followed the procedure described for **50c**. The amorphous solid was recrystallized from cyclohexane.

Pharmacology. Histamine H_2 Antagonistic Activity on the Isolated Guinea Pig Right Atrium.[1] Male guinea pigs (350–400 g) were sacrified by a blow on the head and exsanguinated. Right atria were rapidly removed, attached to a tissue holder in an organ bath (32.5 °C) containing 20 mL of Krebs–Henseleit solution, and gassed with 95% O_2/5% CO_2. The antagonistic potency was determined from isometrically recorded cumulative concentration–response curves[20] using histamine dihydrochloride (0.1–10 μM) as the reference substance. The time of incubation was generally 30 min, except for the iodinated compounds (60 min). For the pharmacological screening most compounds were tested at one or two concentrations (see Table II), and pK_B values[20] (mean of three to nine independent experiments) were calculated from the expression pK_B = −log [antagonist] + log (concentration ratio − 1), as the compounds produced a dose-dependent depression of the concentration–response curves. The pA_2 values were determined by a Schild plot[21] from EC_{50} ratios of histamine for at least three concentrations of the antagonist in seven to nine independent experiments.

β_1 Adrenoceptor Blocking Activity on the Isolated Guinea Pig Right Atrium. The procedure followed the method described above for the evaluation of H_2 antagonistic activity using isoprenaline (0.1–30 nM) as the reference agonist.

Histamine H_1 Antagonistic Activity on the Isolated Guinea Pig Ileum. The H_1 antagonistic activity was determined from isotonically recorded (load, 10 mN) cumulative concentration–response curves[20] as described[22] using histamine (0.01–3 μM) as the reference agonist. Whole segments of proximal ileum were mounted in an organ bath containing 20 mL of Tyrode's solution aerated with 95% O_2/5% CO_2. The compounds were tested at antagonist concentrations of 1 μM to 0.1 mM versus histamine after 10 min at incubation. pK_B and pA_2 values[22] were calculated as above.

Antimuscarinic Activity on the Isolated Guinea Pig Ileum. The procedure followed the method described above for histamine H_1 antagonism using carbachol (3–300 nM) as the reference agonist.

5-HT_2 Antagonistic Activity on the Rat Tail Artery. Male Wistar rats (280–350 g) were sacrificed by rapid decapitation. The ventral tail artery was quickly dissected and thoroughly cleared of connective tissue. A steel rod (diameter, 0.3 mm) was inserted into the vessel to rub off the endothelium. Up to 16 segments of 5–6 mm length were cut. Two L-shaped stainless steel hooks (diameter 0.15 mm) were gently pushed into the lumen. Organs were mounted isometrically in 20-mL organ baths containing (mM) (118) NaCl, (4.7) KCl, (2.5) $CaCl_2$, (1.2) $MgSO_4$, (1.2) KH_2PO_4, (25) $NaHCO_3$, (10) D-glucose, gassed with 95% O_2/5% CO_2 and held at 37 °C. The resting force was 5 mN. During an equilibration period of 120 min organs were stimulated once (after 60 min) with 1 μM serotonin (5-HT). Two to three cumulative concentration–response curves for 5-HT were determined for each preparation. In control experiments (n = 25) the second and third curve for 5-HT (0.01–10 μM) in the absence of antagonist were nearly superimposable. Antagonists were allowed to equilibrate for 30 min. pK_B[20] or pA_2[22] values were calculated from EC_{50} ratios of 5-HT for least three concentrations of antagonist.

α_1 Antagonistic Activity on the Rat Aorta. The thoracic aorta was obtained from rats used for 5-HT_2 experiments (see above). When cleared of connective tissue the aorta was cut into six to eight rings of 4–6 mm length. Each segment was rolled with a pair of tweezers to destroy the endothelium, subsequently pushed over two L-shaped stainless steel hooks (diameter, 0.3 mm), and mounted isometrically as described for the tail artery experiments. Resting force was 20 mN. During an equilibration period of 140 min organs were stimulated twice (after 60 and 100 min) with 0.1 μM (R)-phenylephrine. Cumulative concentration–response curves were recorded in the presence of 1 μM (±)-propranolol. Two to three curves for (R)-phenylephrine were run on each preparation. In control experiments (n = 25) the second and third curve for (R)-phenylephrine (0.003–10 μM) in the absence of antagonist showed a slight rightward shift of 0.1 log unit and an increase in maximal response of up to 10%. Antagonists were incubated for 30 min. pK_B and pA_2 values were calculated as mentioned above.

Binding Studies. Male Hartley guinea pigs (200–300 g) were sacrificed by rapid decapitation and striata homogenized with a Polytron blender in 60 volumes of cold 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 7.5. After centrifugation at 260g for 1 min, the resulting supernatant was recentrifuged at 20000g for 30 min. The final pellets were resuspended in phosphate buffer for ^{125}IAPT binding. Triplicate assays were performed in polypropylene tubes in presence of 0.1% gelatin to prevent ^{125}IAPT adsorption. Membranes (50 ng of protein) were incubated with ^{125}IAPT for 120 min at 25 °C in a final volume of 400 μL. Incubations were stopped by addition of cold buffer with 0.1% bovine serum albumin followed by rapid filtration through glass-fiber filters (GF/B) treated with 0.3% polyethylenimine using an automatic apparatus (Brandel). Bound radioactivity was measured with a LKB γ counter (82% efficiency). Specific binding was determined as that inhibited by 1 μM tiotidine.

All azido compounds were tested under dim light.

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Registry No. **1**, 73278-98-5; **2**, 73279-05-7; **5**, 98078-91-2; **6**, 117078-41-8; **7**, 140872-98-6; **8**, 140873-00-3; **9**, 140873-02-5; **10**, 140873-03-6; **11**, 140873-04-7; **12**, 140873-06-9; **13**, 140873-07-0; **14**, 140900-94-3; **15**, 140873-09-2; **16**, 140873-10-5; **17**, 140873-12-7; **18**, 140873-13-8; **19**, 140873-14-9; **20**, 1493-27-2; **21**, 28166-06-5; **22a**, 140873-15-0; **22b**, 140873-16-1; **23a**, 140873-17-2; **23b**, 140873-18-3; **24a**, 140873-19-4; **24b**, 140873-20-7; **25a**, 140873-21-8; **25b**, 140873-22-9; **26a**, 140873-23-0; **27e**, 150-13-0; **27f**, 2122-63-6; **27g**, 2122-61-4; **31c**, 140873-24-1; **31d**, 140873-25-2; **31e**, 140873-27-4; **31f**, 126632-01-7; **31g**, 140873-28-5; **31h**, 126632-02-8; **31i**, 140873-29-6; **31k**, 140873-30-9; **31l**, 140873-31-0; **31m**, 140925-90-2; **32c**, 140873-32-1; **32d**, 140873-33-2; **33a**, 140873-34-3; **33c**, 140873-35-4; **33d**, 140873-36-5; **33e**, 140873-37-6; **33f**, 140873-38-7; **33g**, 140873-39-8; **33i**, 140873-40-1; **34c**, 140873-41-2; **34d**, 140873-42-3; **35c**, 140873-44-5; **35e**, 140873-46-7; **36c**, 140873-48-9; **37c**, 140873-50-3; **38c**, 140873-51-4; **39c**, 140873-52-5; **40c**, 140873-53-6; **41c**, 140900-95-4; **42m**, 140873-54-7; **43c**, 140873-55-8; **44c**, 140873-57-0; **45c**, 140873-59-2; **46c**, 140873-60-5; **46e**, 140873-61-6; **47**, 98-09-9; **48**, 98-59-9; **49c**, 140873-62-7; **49n**, 140873-63-8; **50c**, 140873-64-9; **50n**, 140873-65-0; **51**, 94-09-7; **52**, 62875-84-7; **53**, 5400-81-7; $PhCH_2NMe_3^+[ICl_2]^-$, 114971-52-7; $H_2N(CH_2)_2NH_2$, 107-15-3.

Synthesis, Configuration, and Calcium Modulatory Properties of Enantiomerically Pure 5-Oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates[1]

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Enantiomerically pure hexahydroquinolinones of the structural type **9** were prepared by a variation of the Hantzsch synthesis in which an optically active acetoacetate served as a chiral auxiliary reagent. Determinations of the de and ee values are described. The absolute configurations of the optically pure products were characterized by single-crystal X-ray analysis. The antipodes **9a** and **9b** exhibited calcium antagonistic activities on smooth musculature; the (*S*)-(−)-enantiomer **9b** was the more potent compound with regard to the EC_{50} values which differed by a factor of 100; the intrinsic activity of **9b** was 1.2, compared with a value of 0.54 for **9a**. On the other hand, *R*-(+)-**9a** exerted positive inotropic effects on electrically stimulated atria. The cause of these effects is discussed.

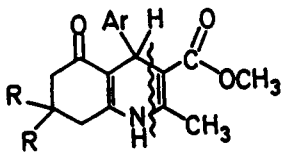
In contrast to the effects of known calcium channel blockers of the nifedipine-type, the so-called calcium agonists, such as Bay K 8644 and CGP 28392, increase calcium influx by binding at the same receptor regions.[2–5] As a physiological consequence, positive inotropic, chronotropic, and vasoconstrictive activities have been observed in vitro and in vivo.[6,7]

An essential prerequisite for a therapeutic application in cases of cardiac insufficiency, however, is cardioselectivity. Investigations on structure–activity relationships with regard to "agonism–antagonism" still do not allow any unequivocal conclusions to be drawn about the molecular requirements for selectivity.[8]

Our previous work in this area was to fix the carbonyl groups in an antiperiplanar position by anellation at the dihydropyridine ring, since it had been proposed[9] that the quality of action is associated with the respective position of the ester carbonyl group.

The racemic hexahydroquinolinones **10** and **11**[10–12] as well as the 5-oxoindeno-1,4-dihydropyridines **12**[13] exhibited simultaneous calcium antagonistic effects on smooth musculature and positive inotropic activities on electrically stimulated left atria of guinea pigs. Furthermore, the anellated lactone **11** effected an increase in contractility of isolated ventricular papillary muscle.[14]

It is now known that the dihydropyridine (DHP) receptors exhibit stereospecificity: the optical antipodes of asymmetrical dihydropyridines often possess not only differing receptor affinities[15] but sometimes also generate opposing effects.[16,17] To better differentiate the above-described anellated structures, further synthetic work was carried out to prepare the optical antipodes of hexahydroquinolinones.



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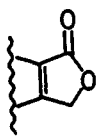

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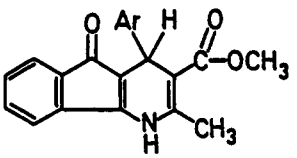

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