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UNSYMMETRICALLY SUBSTITUTED GUANIDINES AS POTENT HISTAMINE H_3-RECEPTOR ANTAGONISTS

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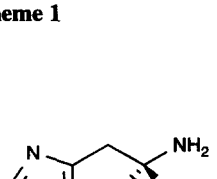
Abstract: Unsymmetrically trisubstituted and disubstituted guanidine derivatives of (1*H*-imidazol-4-yl)alkyl amines were synthesized and investigated for histamine H_3-receptor activity. Electron-withdrawing substitution of the guanidino group resulted in antagonists with a potential prodrug character. The H_3-receptor selective N^1-cyclohexylmethyl-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine possesses a -log K_i of 9.1.

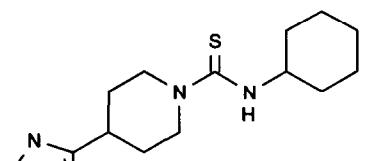
The discovery of a third histamine receptor subtype gave fresh impetus to an old field of research.[1,2] By means of a negative feed back mechanism histamine modulates its own synthesis and release via presynaptic histamine H_3-autoreceptors. Moreover H_3-receptors have been proved to be heteroreceptors as well. They modulate the release of a number of different neurotransmitters.[3,4,5] H_3-receptors are found in highest density in the central nervous system but this type of receptor is also found in many peripheral tissues.

H_3-Receptor antagonists influence peripheral cholinergic transmission[6] and potentiate gastrin/cholecystokinin stimulated histamine release.[7] In the cardiovascular system decrease in heart rate, blood pressure and total peripheral resistance evoked by the H_3-receptor agonist (R)-α-methylhistamine (**1**) are abolished by the H_3-receptor antagonist thioperamide (**2**).[8] Therefore, the availability of peripherally active H_3-receptor antagonists which could not penetrate the blood-brain barrier and thus are avoid of any central effects is highly recommended. These antagonists are pharmacological tools for investigations on peripheral H_3-receptors as well as potential drugs for a therapy of peripheral H_3-receptor dependent diseases.

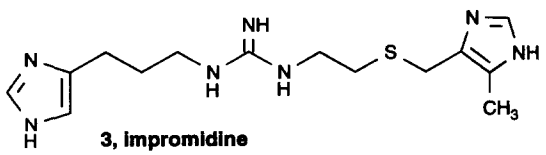
Scheme 1



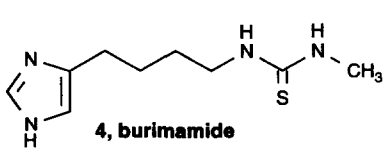
1, (R)-α-methylhistamine



2, thioperamide



3, impromidine



4, burimamide

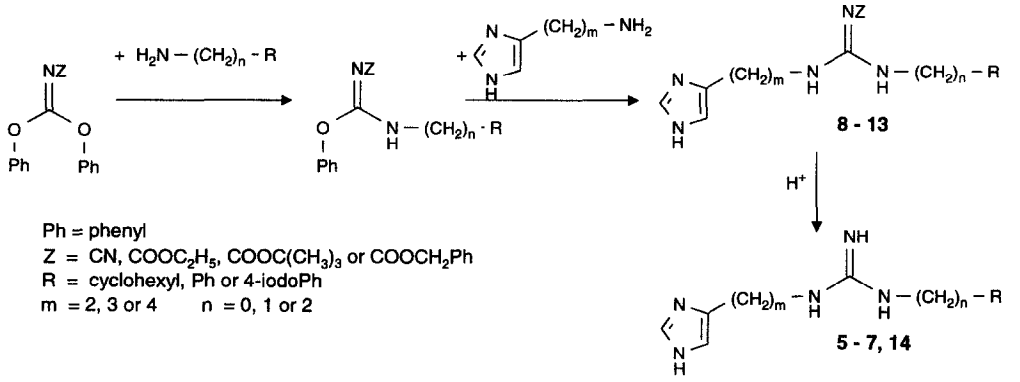
Although one of the first known histamine H_3-receptor antagonists is a guanidine derivative, only a small number of compounds containing this structure element are described. The aforesaid guanidino H_3-receptor antagonist is impromidine (**3**) which was primarily proved to be a potent agonist at H_2-receptors. In addition to impromidine, the thiourea derivative burimamide (**4**), a compound with a combined H_2-/H_3-antagonistic profile, was used for the first characterization of histamine H_3-receptors[1] (Scheme 1).

In search for selective and potent H_3-receptor ligands we have synthesized guanidine derivatives of (1*H*-imidazol-4-yl)alkyl amines (Scheme 2). In order to optimize the structure, the distance between the imidazole ring and the guanidine functionality has been varied (m = 2, 3, 4) as well as the distance between the lipophilic residue (R) and the guanidino group (n = 0, 1, 2). Moreover, we have reduced the basicity of the guanidines by triple substitution (**8-13**) for improved pharmacokinetics e.g. absorption. The iodinated compounds (**6**, **11**), however, have been prepared as potential radioligands for H_3-receptor characterization.

The effects of the new compounds were tested on potassium evoked [^{3}H]histamine release from synaptosomes of rat cerebral cortex which had been preincubated for half an hour with tritium-marked histidine using the procedure of Arrang et al.[1,2] For the determination of -log K_i-values, according to Cheng-Prusoff equation,[9] three separate experiments were at least carried out. The trisubstituted guanidines were tested as free bases while the disubstituted guanidines were dissolved as dihydrochlorides in water. Test results are presented in Table 1. None of the cyclohexyl substituted compounds produced a significant effect on H_1-receptor guinea-pig ileum[10] or on H_2-receptor guinea-pig atrium preparations[11] up to a concentration of 10^{-5} M, except the most potent compound **7** in this series. N^1-Cyclohexylmethyl-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (**7**) showed at H_1-receptors a -log K_i of 5.1 and at H_2-receptors a pD_2-value of 6.4.

Synthesis is done by successive nucleophilic exchange of phenoxy groups of different N-substituted diphenyl carbonimidates[12] with primary amines to afford unsymmetrically N^1,N^2,N^3-trisubstituted guanidines (Scheme 2). Because of the two nucleophilic centres of imidazole alkyl amines, the non-imidazole containing amine has first of all to react with the synthon. The first aminolysis is performed at ambient temperature in acetonitrile, whereas the second step is carried out at reflux after having added the imidazole alkyl amine. Chromatographic separation is facilitated due to the electron-withdrawing substituent which drastically reduces the basicity of the guanidino group. The yield of this one-pot synthesis varied between 35 to 60%.

Scheme 2

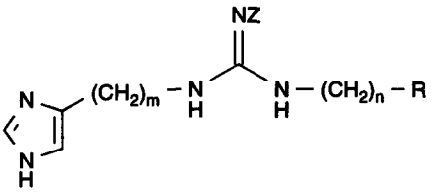


Ph = phenyl
Z = CN, $COOC_2H_5$, $COOC(CH_3)_3$ or $COOCH_2Ph$
R = cyclohexyl, Ph or 4-iodoPh
m = 2, 3 or 4 n = 0, 1 or 2

The *tert.*-butyl substituted guanidino-N-carboxylates of the trisubstituted guanidines (**10**, **11**, **13**) were comfortably hydrolyzed to afford the corresponding unsymmetrically disubstituted guanidines. This was performed in quantitative yields under mild acidic conditions. All compounds were characterized by ^{1}H NMR (not shown), mp, FAB$^+$ mass spectra and elemental analyses of C, H, and N (Table 2). Most of the guanidines, even the dihydrochlorides (**6**, **7**), crystallized after stirring in Et_2O for 24 hours. Extremely hygroscopic compounds (**5**, **14**) were transformed into picrate salts for characterization.

All compounds are potent histamine H_3-receptor antagonists.[13] Their affinity to other histamine receptors, H_1 and H_2 respectively, is negligible. Thus the guanidines can be described as selective H_3-receptor antagonists. This selectivity has great advantage for further pharmacological testing.

Table 1. Structure and histamine H_3-receptor antagonist activity of the guanidines **5** - **14**



Compound	m	Z	n	R	K_i [M]	-log K_i
5	2	H	2	phenyl	$1.1 \pm 0.2 \times 10^{-7}$	7.0[a]
6	3	H	1	4-iodophenyl	$1.6 \pm 0.2 \times 10^{-9}$	8.8
7	3	H	1	cyclohexyl	$7.4 \pm 2.7 \times 10^{-10}$	9.1
8	3	CN	1	cyclohexyl	$2.2 \pm 1.1 \times 10^{-8}$	7.7
9	3	$COOC_2H_5$	1	cyclohexyl	$3.6 \pm 0.7 \times 10^{-8}$	7.4
10	3	$COOC(CH_3)_3$	1	cyclohexyl	$9.5 \pm 2.1 \times 10^{-9}$	8.0
11	3	$COOC(CH_3)_3$	1	4-iodophenyl	$1.2 \pm 0.2 \times 10^{-8}$	7.9
12	3	$COOCH_2$phenyl	1	cyclohexyl	$1.3 \pm 0.9 \times 10^{-8}$	7.9
13	4	$COOC(CH_3)_3$	0	cyclohexyl	$7.0 \pm 3.0 \times 10^{-9}$	8.2
14	4	H	0	cyclohexyl	$5.0 \pm 3.0 \times 10^{-9}$	8.3

[a] -log K_i-value of 6.3 on electrically evoked guinea-pig ileum contraction[14]

With regard to structure-activity relationships the total distance between the imidazole nucleus and the lipophilic ring was kept constant with a 7-membered chain. In previous studies the distance of about 7 atoms was found to have the best antagonistic histamine H_3-receptor fit.[15,16,17] In the series of disubstituted guanidines the position of the polar guanidino group finds an optimum in three methylene groups from the imidazole ring (**7**). The difference in activity between three and four methylene groups is smaller than the difference between three and two methylene groups. This may be caused by a larger steric variability of the butylene spacer compared to an ethylene one. The longer chain possesses a higher degree of flexibility than the shorter spacer. Compound **7** is about 5 times more potent than the reference thioperamide (**2**; -log K_i = 8.4[2]).

Although the unsymmetrically dialkyl substituted guanidines have higher receptor activity than the trisubstituted guanidines with electron-withdrawing groups, compounds **8** - **13** show only minor lowering in affinity. These results are unexpected, because the electronic parameters of the central polar group are totally different. While compound **7** is diprotonated (imidazole and guanidino group) under physiological conditions, **8** - **13** do not possess any more basic characteristics at the guanidino functionality. Furthermore, the different electron-withdrawing groups have totally different steric parameters. The small cyano group (**8**) has to be compared to the bulky benzoxycarbonyl (**12**) or *tert.*-butoxycarbonyl group (**10**, **11**). Their activities differ 0.3 orders of magnitude or less which means that the volume of the substituents at the guanidino nitrogen is less important for receptor binding. The equipotent effects of the cyclohexyl and the iodophenyl derivatives (**10**, **11**) underlines this conclusion. In contrast to steric and electronic parameters the polarity of the functional group seems to be an essential element of histamine H_3-receptor antagonism.

Due to the high affinity of N^1-4-iodobenzyl-N^2-[3-(1*H*-imidazol-4-yl)propyl]guanidine (**6**) and the *tert.*-butoxycarbonyl derivative **11** these compounds should have been potential candidates for H_3-receptor characterization in a [^{125}I]iodo radiolabelled form of **6** or **11**. In binding experiments with [^{3}H](R)-α-methyl-histamine, **6** and **11** show a K_i at 3.1±0.6 nM and 3.3±0.2 nM, respectively. Unfortunately, the unspecific binding of the tested guanidines is too high for a characterization of H_3-receptors in the brain. It seems to be obvious that this problem may be caused by the large number of possible hydrogen bonds of the polar group. The same difficulties may raise with the comparable compound [^{125}I]iodophenpropit which belongs to an isothiourea series.[18] The problem of the radioligands was solved with a compound belonging to a different chemical class of H_3-receptor antagonists.[19]

The disubstituted guanidine impromidine (**3**) is ineffective *per os* due to its strong basicity. The major advantage of the slightly less potent trisubstituted guanidines should be their potential prodrug character for improved pharmacokinetics, because a similar prodrug approach of guanidines has already been described in the field of H_2-receptor agonists.[20] Absorption of the trisubstituted guanidines should be increased compared to the disubstituted ones because **8** - **13** are much more lipophilic and less basic. These guanidines which are substituted with ester functionalities or a cyano group may liberate the more potent disubstituted guanidines by hydrolysis after absorption. This hypothesis has to be proved by detection of peripheral H_3-receptor mediated actions *in vivo*, but unfortunately an *in vivo* test system does not exist up to now to confirm this statement for histamine H_3-receptor antagonists.

In conclusion, the new guanidines are potent and selective histamine H_3-receptor antagonists. Electron-withdrawing substitution slightly decreases the activity but should increase *per os* bioavalibility and the effect *in vivo* due to their prodrug character. Steric and electronic parameters seem to be less important than polarity and lipophilicity on H_3-antagonistic activity of (1*H*-imidazol-4-yl)propyl amine derivatives. The disubstituted guanidine **7** with the optimal chain length is about 5 times more potent than the reference thioperamide. The new antagonists are useful pharmacological tools and potential drugs for peripheral H_3-receptor ligand applications.

Table 2. Analytical data of the guanidines **5** - **14**

Compound	formula	molecular weight	mp [°C]	cryst. solvent	elemental analyses	mass spectrum $[M+H]^{\cdot+}$
5	$C_{14}H_{19}N_5 \times 2C_6H_3N_3O_7$	715.5	191-193[a]	H_2O	C, H, N	258[b]
6	$C_{14}H_{18}IN_5 \times 2HCl \times {}^5/_4H_2O$	456.2	92-94	Et_2O	C, H, N[c]	384
7	$C_{14}H_{25}N_5 \times 2HCl \times H_2O$	354.3	76	Et_2O	C, H, N	264
8	$C_{15}H_{24}N_6 \times {}^1/_4H_2O$	292.9	103	Et_2O	C, H, N	289
9	$C_{17}H_{25}N_5O_2$	335.5	118-119	Et_2O	C, H, N	336
10	$C_{19}H_{33}N_5O_2$	363.5	137	Et_2O	C, H, N	364
11	$C_{19}H_{32}IN_5O_2$	483.4	155	Et_2O	C, H, N	484
12	$C_{22}H_{31}N_5O_2$	397.5	120	EtOAc	C, H, N	398
13	$C_{19}H_{33}N_5O_2$	363.5	96-98	Et_2O	C, H, N[c]	364
14	$C_{14}H_{25}N_5 \times 2C_6H_3N_3O_7 \times H_2O$	739.6	157	H_2O	C, H, N	264[b]

[a] 196-198 °C,[14] [b] Compounds measured as dihydrochloride salts, [c] Difference for N is 0.7 and 1.0 for compounds **6** and **13**, respectively

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