Eur J Med Chem (1995) 30, 881–889

Heteroarylaminoethyl and heteroarylthioethyl imidazoles. Synthesis and H_3-receptor affinity

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(Received 8 April 1995; accepted 10 July 1995)

Summary — The synthesis of new H_3-receptor antagonists, 4-(2-heteroarylaminoethyl) and 4-(2-heteroarylthioethyl) imidazoles and their H_3-receptor affinity obtained from competitive binding curves *vs* [^{3}H]-N^α-methylhistamine ([^{3}H]NAMHA) on rat brain cortex membranes are described. These compounds are derived from structural modulations of thioperamide and were synthesized in order to study binding interactions with H_3-receptors and find alternative lead compounds with H_3-receptor antagonist activity. The new compounds differ from thioperamide by the following features: 1) the *N*-cyclohexylcarbothioamide moiety of thioperamide has been replaced by a benzothiazole (**1**); 2) the piperidine ring has been replaced by more flexible aminoethyl and thioethyl chains, in order to lower the excessive rigidity of **1** and to test the importance of the tertiary piperidine nitrogen; and 3) the benzothiazole moiety of **1** has been replaced by other heterocyclic nuclei, endowed with different lipophilic, steric and hydrogen-bonding features. Some of the compounds tested showed good affinity for central H_3-receptors (pK_i range: 5.89–7.96) and can be considered as lead compounds for further optimization studies. The most lipophilic compounds showed higher affinities among benzo-condensed compounds, while imidazolylthioethyl imidazoles were more potent in displacing [^{3}H]NAMHA than thiazolylthioethyl and thiazolylaminoethyl imidazoles which suggests an interaction between the annular NH of the imidazolylthioethyl moiety and the binding site.

heteroarylglaminoethylimidazole / heteroarylthioethylimidazole / histamine H_3-receptor affinity / [^{3}H]-N^α-methylhistamine / rat brain cortex membrane

Introduction

Besides its known actions on H_1 and H_2 receptors, histamine acts on a third class of receptors, named H_3, which have been discovered in the CNS and in peripheral sites of various mammalians, as well as in human tissues [1, 2]. There is evidence that H_3-receptors are G-protein-coupled presynaptic autoreceptors, inhibiting the release of histamine itself from nerve endings; they have also been observed to modulate the release of other neurotransmitters (NA, D, 5HT), thus being involved in several physiological functions [3]. Several effects have already been observed both *in vitro* and *in vivo* for H_3-receptor agonists and antagonists [4–9], but for future therapeutical uses of these compounds a deeper knowledge of the pathophysiological roles of H_3-receptors has still to be attained. The availability of potent and selective H_3-receptor antagonists, in particular, could be useful in elucidating their roles, because these compounds can modulate the tonic effects of histamine on physiological systems regulated by H_3-receptors.

New compounds with H_3-receptor antagonist activity are needed not only as new pharmacological tools, but also to provide information on structure–activity relationships for this class of molecule. This information could in turn help to understand drug–receptor interactions.

Different approaches have been used for the synthesis of new H_3-receptor antagonists, although some common requirements for H_3-receptor antagonism can be recognized. Indeed, most of the H_3-receptor antagonists synthesized so far are characterized by the presence of an imidazole ring, connected by means of an alkyl chain, generally with two or three carbon atoms or a piperidine ring, to a polar group (amides or amines [10], isothioureas [11], guanidines, esters, guanidine esters, ureas or thioamides [12]), in turn linked to a lipophilic moiety.

Starting from these observations, we have synthesized new compounds [13–15] with H_3-receptor antagonist activity by introducing some structural variations to the known antagonist thioperamide. This compound has a pA_2 value of 8.96 on K^+-induced release of [^{3}H]histamine from slices of rat cerebral cortex [16], and a pA_2 value on electrically stimulated guinea-pig ileum of 9.05 [14]. For what concerns H_3-receptor binding of thioperamide, its inhibition

curve depends on experimental conditions, such as the ionic composition of the solution and the presence of guanine nucleotides [17] (see *Experimental protocols* for further details). Under the conditions employed in the present study, we obtained a pK_i value of 8.49 [13].

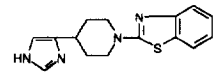
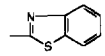
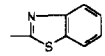
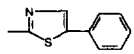
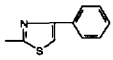
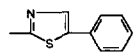
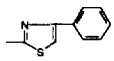
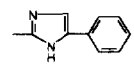
The thioperamide derivatives we recently studied included a series of benzothiazoles, related to thioperamide. Compound **1** (table I) was the lead compound. A QSAR analysis of the receptor affinity for a series of derivatives of compound **1**, 6-substituted on the benzothiazole ring, showed that lipophilicity and/or steric hindrance of the substituents caused a decrease in H_3-receptor affinity [15]. Although the results reported in the literature [15] referred only to position 6 of benzothiazole, they prompted us to believe that excessive rigidity of the whole molecule **1**, as well as its high apparent lipophilicity, were unfavourable features for binding to H_3-receptors.

In order to find new lead compounds for the H_3-receptor antagonist class, a new series of compounds, listed in table I, was designed, with the aim of maintaining the general characteristics of compound **1** (presence of an imidazole ring connected, by a chain in position 4(5), to another heterocycle by means of a heteroatom) but at the same time changing some physicochemical features of the molecules. In particular, the piperidine ring of **1** was replaced with more flexible amino and thioethyl chains to reduce the rigidity of the molecule, while amine and thioether bridges were introduced in order to test the importance of a hydrogen-donor centre in the chain.

The benzothiazole nucleus, on the other hand, was either maintained or replaced by other heterocycles. Monocyclic groups were chosen in order to obtain compounds with reduced lipophilicity and steric hindrance compared with **1**, which appeared unfavourable for H_3-receptor affinity. In addition, in accordance with the rules of isosteric replacement, both imidazole and thiazole derivatives were synthesized, to evaluate the possibility of hydrogen bonding with the receptor in that part of the molecule. The hydrophobicity of this moiety was modulated by synthesizing benzo-condensed or phenyl-substituted heterocyclic nuclei.

Referring to the chosen structures listed in table I, it can be observed that 4(5)-[2-(imidazol-2-yl)thioethyl]imidazole **12** and its phenyl derivative **13** show some resemblance to isothioureic H_3-receptor antagonists, which are derivatives of the agonist imetit [11, 18–20]. In particular, the alkylthioimidazole group of **12** could be considered as a cyclic model of the isothiourea group in imetit and its derivatives. One of the main differences between **12** and imetit derivatives is the limited possibility of the former to form hydrogen bonds compared with the isothiourea group of the latter. To test the possibility that the imidazole NH of **12** is involved in hydrogen bonding, we also tested compound **14**, which completely lacks hydrogen donor centres in that part of the molecule.

Table I. Histamine H_3-receptor affinity of tested compounds.

Compound	X	Y	pK_i	nH
1			6.58±0.03	1.17±0.06
2	-NH-		7.73±.0.04	1.05±0.08
3	-NH-		7.14±0.05	1.15±0.13
4	-S-		7.96±0.08	0.93±0.11
5	-S-		7.26±0.06	0.85±0.10
6	-NH-		5.89±0.07	0.94±0.17
7	-NH-		7.00±0.05	0.84±0.10
8	-NH-		7.49±0.07	0.88±0.10
9	-S-		6.04±0.05	1.18±0.16
10	-S-		7.67±0.06	1.19±0.16
11	-S-		7.77±0.07	0.90±0.11
12	-S-		7.24±0.07	0.90±0.10
13	-S-		7.83±0.07	0.93±0.12
14	-S-		6.30±0.08	0.98±0.10

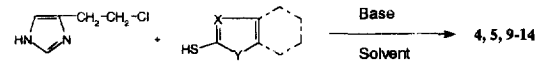
[1]A two-site model was significantly better than a one-site model with nH constrained to 1 in a relative goodness-of-fit test [27], but both models were acceptable according to an absolute goodness-of-fit test [27] (see *Experimental protocols*): apparent pK_is for one-site model are reported; [2]concentrations up to 10 μM of cold ligand were tested, and so B_{asp} could not be accurately estimated; this could affect the precision of pK_i; [3]increasing the concentration of [^{3}H]NAMHA a non-proportional shift of displacement curves was observed, revealing a not fully competitive interaction.

Chemistry

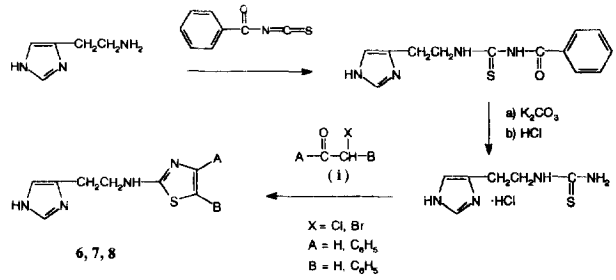
The compounds listed in table I were prepared following various synthetic routes. The synthesis of compound **1** has been described in a previous paper [15]. During the preparation of this manuscript, compounds **2**, **6** and **12** were described in a patent [21], with different synthetic routes.

Compounds **2** and **3** were prepared by condensation of histamine with 2-chlorobenzothiazole and benzimidazole-2-sulfonic acid, respectively. 2-[4(5)-Imidazolyl]ethylthio-heterocylic products (**4**, **5** and **9–14**) were synthesized according to scheme 1, by condensation of 4(5)-(2-chloroethyl)imidazole with the appropriate 2-mercapto heterocycle, employing different acceptor bases and experimental conditions for the different compounds (see *Experimental protocols* and table II).

Since 2-halogenated thiazoles are relatively inert to substitution with alkyl amines, compared with the corresponding benzothiazoles, 2-thiazolylaminoethyl derivatives (**6–8**) were prepared in good yields starting from 2-[4(5)-imidazolyl]ethylthiourea, as described in scheme 2. Following this scheme, *N*-



Scheme 1.



Scheme 2.

Table II. Reagents, conditions, isolation and purification methods for compounds **4**, **5** and **9–15**, according to scheme 1.

Product	*Nucleophile*	*Base*	*Solvent*	*Temperature*	*Duration*	*Isolation and purification*
4	2-Mercaptobenzothiazole	20% NaOH	EtOH	Boiling	1 h 30	Evaporation of the solvent, chromatographic separation CH_2Cl_2/CH_3OH (9:1) + $NH3_3$(g)
5	2-Mercaptobenzoimidazole	NaOH	H_2O	40°C	2 h	Precipitation on cooling
9	2-Mercaptothiazole	EtONa	EtOH	55°C	24 h	Evaporation of the solvent, chromatographic separation CH_2Cl_2/CH_3OH (9:1) + NH_3(g)
10	5-Phenyl-2-mercapto-thiazole[a]	EtONa	DMSO	20°C	18 h	Chromatographic separation $CHCl_3/CH_3OH$ (15:1) + NH_3(g)
11	4-Phenyl-2-mercapto-thiazole[b]	EtONa	DMSO	20°C	18 h	Chromatographic separation $CHCl_3/CH_3OH$ (15:1) + NH_3(g)
12	2-Mercaptoimidazole	EtONa	EtOH	45°C	24 h	Evaporation of the solvent, chromatographic separation CH_2Cl_2/CH_3OH (9:1)
13	4-Phenyl-2-mercapto-imidazole	EtONa	EtOH	45°C	24 h	Evaporation of the solvent, chromatographic separation CH_2Cl_2/CH_3OH (9:1)
14	1-Methyl-2-mercapto-imidazole	EtONa	DMSO	20°C	20 h	Chromatographic separation $CHCl_3/CH_3OH$ (10:1) + NH_3(g)

[a]Described in reference [31]; prepared from α-chloroacetaldehyde following the method described in reference [25] for 4-substituted-2-mercaptothiazoles, mp 208–209°C (208–209°C [31]); [b]prepared according to reference [25], mp 177–178°C (177–178°C [32]).

benzoyl-*N'*-2-[4(5)-imidazolyl]ethylthiourea (which was cited in reference [22]) was prepared by the addition of histamine to benzoylisothiocyanate. The product was then hydrolyzed to 2-[4(5)-imidazolyl]ethylthiourea. Thiazole and phenylthiazole rings (compounds **6–8**) were obtained by cyclization of the thiourea group with the appropriate α-halogenated carbonyl compound, which was, in turn, α-chloroacetaldehyde, ω-bromoacetophenone, or α-chlorophenylacetaldehyde (see *Experimental protocols* and table III).

Results and discussion

The compounds described were submitted to preliminary functional tests for their activity on peripheral H_1-, H_2- and H_3-receptors, in order to ascertain their antagonist activity; a detailed discussion of these results are presented elsewhere [23].

As regards H_3-receptor activity, most of the tested compounds antagonised the (*R*)-α-methylhistamine-induced reduction of twitches in electrically stimulated guinea-pig ileum in a competitive manner, with the exception of compounds **6** and **9** which displayed a non-surmountable antagonism at micromolar concentrations. Compound **13** showed the highest pA_2 of 8.19; two compounds, **7** and **8**, gave pA_2 values lower than 7 (6.94 and 6.91, respectively), while pA_2 values for the other compounds ranged from 7.14 to 7.69. H_1-receptor antagonism was only observed for compounds **2**, **4**, **5**, **10** and **11** at concentrations higher than 10^{-6} M, and, as regards H_2-receptor antagonism, compounds **4**, **5**, **7**, **10**, **11** and **13** reduced maximal atrial chronotropic response to dimaprit at the concentration of 10^{-5} M [23].

The aim of the present work was to study the effects of structural variations on the binding to H_3-receptors. On this basis, competitive binding studies on rat brain membranes, employing [^{3}H]NAMHA at concentrations that guaranteed a selective binding on H_3-receptors (see *Experimental protocols*), were chosen as appropriate biological tests, seeing that all the compounds belonged to the H_3-receptor antagonist class.

All the compounds tested showed medium to high affinity for rat cerebral H_3-receptors, as can be observed from the pK_i values reported in table I and from the inhibition curves represented in figure 1. With increasing concentrations of [^{3}H]NAMHA, compounds **10** and **11** gave a right shift of the inhibition curves not proportional to the concentration of the labelled ligand. Despite their good affinity for the binding site (apparent pK_i of 7.67 and 7.77 respectively), therefore, they did not seem to inhibit the binding of [^{3}H]NAMHA in a fully competitive manner. The analysis of the competitive binding curves gave pseudo-Hill coefficients lower than unity for some compounds, although these differences were not significant at *t*-test ($p = 0.05$). For three of these compounds with nH < 1 (**5**, **8**, **11**) a two-site model gave a significantly better fit than the one-site model (with nH forced to 1), but in all cases the residuals around the regression model were lower than the intrinsic error of the single points (see *Experimental protocols*), so that the presence of multiple binding sites cannot be stated from the observed data.

Comparing the pK_i values of compounds **1** and **2**, it appears that the replacement of the rigid piperidine ring with the flexible aminoethyl chain leads to an increment of affinity, which is generally retained by other bicyclic compounds **3–5**. Flexible chains thus seem to allow better accommodation of the terminal moiety than piperidine. This is in contrast with the lower affinity of the flexible *N*-cyclohexyl-*N'*-{2-[4(5)-

Table III. Reagents and conditions for the preparation of compounds **6–8**, referring to the last step represented in scheme 2.

Product	*Reagent*	*A*	*B*	*X*	*Solvent*	*Temperature*	*Duration*
6	α-Chloroacetaldehyde diethylacetale	H	H	Cl	H_2O	100°C	12 h
7	α-Chlorophenyl-acetaldehyde	H	C_6H_5	Cl	Dioxane/H_2O 9:6	Boil.	6 h
8	ω-Bromo-acetophenone	C_6H_5	H	Br	EtOH	70°C	30 min

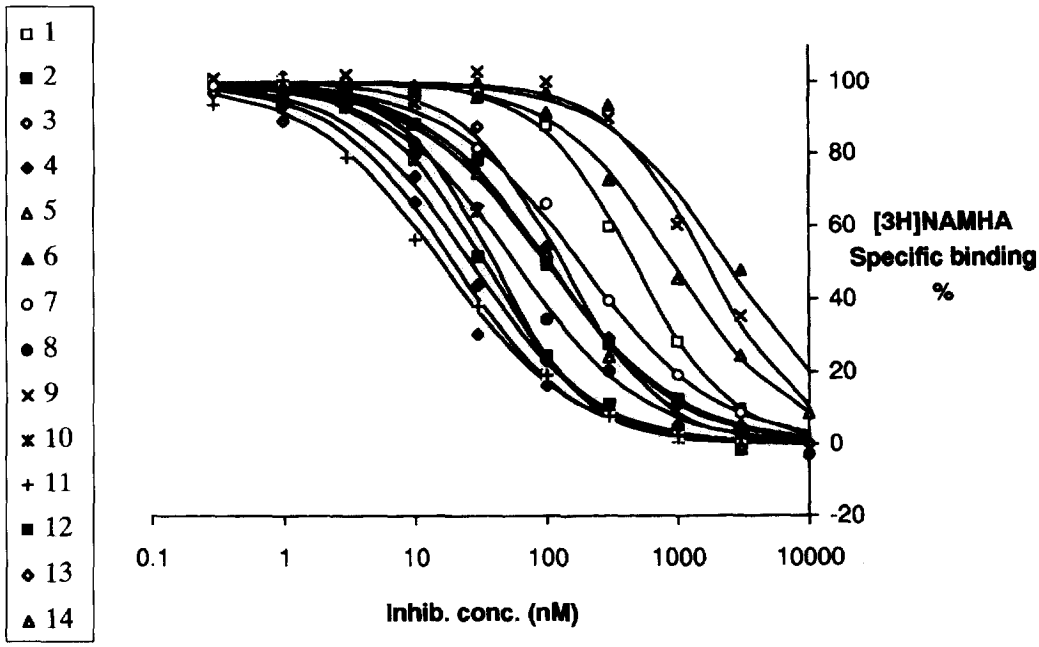


Fig 1. Inhibition of 0.5 nM [^{3}H]NAMHA binding on rat cortex membranes. Percent of specific binding *vs* inhibitor concentration on a log scale is represented. Symbols represent mean values obtained for tested compounds, as illustrated in the key.

imidazolyl]ethyl}thiourea compared with the rigid analogue thioperamide, as we have observed in competitive binding assays *vs* [^{3}H]histamine on rat cerebral cortex membranes [24]. This difference could be attributed to an optimal accommodation of both the thiourea group and the cyclohexyl residue of thioperamide at the receptor site, while the heteroaromatic nuclei of **1** and other compounds now described need a flexible chain to establish good interactions with the receptor. For this reason, contrary to our expectations [15], although we considered the benzothiazole nucleus to be a rigid model of the cycloalkylcarbothioamide group in thioperamide, it cannot be considered as its bioisoster.

To mimic the tertiary piperidine nitrogen of **1**, a secondary NH bridge or a thioether group was introduced. For the two resulting classes of compounds, a slight but systematic difference in affinity was observed. In fact, compared with the corresponding ones bearing an NH group, compounds bearing a sulphur in their chain showed higher pK_i values for amounts which varied from 0.12 (**5**–**3**) to 0.67 (**10**–**7**). From this observation it can be concluded that, while a bioisosterism for these two groups can be detected, the presence of a hydrogen bond donor such as NH is not required in that position of the molecule. This can also be argued from the presence of the piperidine tertiary nitrogen in thioperamide. The differences observed in affinity between ethylamino and ethylthio derivatives could be explained by a different lipophilic contribution or a different electronic effect for NH and S.

Another difference in affinity that could be related to the lipophilic contribution is that observed for benzothiazole and benzimidazole derivatives. Compounds **2** and **4** actually showed higher values of pK_i than compounds **3** and **5** respectively, with differences of 0.6–0.7 log units. A dramatic drop in affinity (and in H_3-receptor antagonist activity) was also observed when the benzothiazole was replaced by monocyclic thiazole, in compounds **6** and **9**, which is compatible with the decrease in lipophilicity. The introduction of a phenyl ring on the thiazole nucleus again led to a general rise in affinity, although the affinity of benzothiazole derivatives was never reached. In this case the higher steric hindrance of benzocondensed nuclei is not relevant for ligand binding, probably owing to chain flexibility.

An important exception to this behaviour was compound **12**, which, despite its hydrophilicity, was

as active as its benzocondensed analogue (**5**) in displacing [^{3}H]NAMHA from its binding sites. The introduction of a phenyl ring, moreover, maintained a positive effect, so that compound **13** was one of the more active in the series. This exception could be due to an interaction of the imidazole NH and a hypothetical accepting site in the receptor. This secondary interaction seems to be performed by compounds **12** and **13**, and not by the benzimidazole derivatives **3** and **5**, since the characteristics of benzocondensed rings could be unfavourable for such a hydrogen bond. Another proof of the importance of the imidazole NH is the fact that *N*-methylation led to compound **14**, which was much less active than **12** in displacing [^{3}H]NAMHA. This hypothetical interaction between the annular NH and the receptor site could be comparable to an analogous interaction involving the thiourea NH of thioperamide.

Although the contribution of phenyl rings to affinity was positive, it was not constant over the whole series. In particular, it reached a maximum for thiazolylthioethyl derivatives **10** and **11**, but for these compounds a shift to a not fully competitive behaviour was also observed. However, while the phenyl effect was exactly the same for 4- and 5-substitution in these compounds, a different picture emerged for thiazolylaminoethyl derivatives **7** and **8**. 4-Phenyl derivative **8** displaced [^{3}H]NAMHA at significantly lower concentrations than 5-phenyl derivative **7**. As for the displacement curves, as already stated, the better description given by a two-site model for compound **8**, not observed for **7**, seemed to be an artefact, because the intrinsic error of measurements made it possible for this difference to be attributable to chance. The pseudo-Hill coefficients are smaller than unity for both the compounds, which therefore had very similar displacement curves (see fig 1). Apart from site heterogeneity, which cannot be resolved with the present data, the difference in apparent pK_is could be related to the possibility of tautomerism of the aminothiazole, which would bring about the existence of a minimal fraction of a compound with an NH in the heterocycle. If this tautomer contributed to the binding, the position of the phenyl ring would be determining, as the orientation of the cycle would be fixed by hydrogen bonding. For mercaptothiazole derivatives, on the contrary, the position of the phenyl ring would not be relevant, the heterocycle being allowed to rotate in the binding site if no directional interaction could be undertaken with the receptor.

The high affinity of phenylimidazole derivative **13** is consistent with the lipophilic contribution of the phenyl ring (different from **12**) and with the possibility to interact with the receptor through the imidazole NH, which for compounds **3** and **5** was hampered by the steric hindrance of the benzocondensed ring.

Conclusions

In the series of heteroarylaminoethyl and heteroarylthioethyl imidazoles, there are compounds endowed with good affinity for central H_3-receptors. A positive lipophilic contribution of the ending moiety and a direct interaction of the annular NH with the binding site for non-benzocondensed nuclei could explain the differences in affinity observed over the series, although different electronic distribution or steric features have to be invoked in order to explain higher pK_i values for benzothiazole derivatives compared with the corresponding phenylthiazoles.

The original aim of the study was achieved, as the replacement of piperidine ring in **1** with flexible chains gave a number of compounds interesting both for their H_3-receptor affinity and for the possibility of their being considered as new lead compounds which can be submitted to derivatization to optimize their pharmacological properties. In particular, there are compounds in this series which deserve more attention for their affinities, such as compound **13**, which is also endowed with good H_3-receptor antagonist potency on guinea-pig ileum and with good selectivity [23], and compound **4**, whose structure will be optimized in order to increase its antagonist potency and selectivity. Furthermore, compounds such as **4**, **10** and **11** lack centres with strong hydrogen bond acidity or basicity. Such centres have been recognized as unfavourable features for the crossing of the blood-brain barrier [25], and so these compounds could provide new H_3-receptor antagonists able to perform strong central actions.

Experimental protocols

Chemistry

Melting points were not corrected and were measured with a Büchi instrument (Tottoli). The newly synthesized substances were analyzed for C, H and N. The percentages we found were within ±0.4% of the theoretical values. Characteristic data and yields of final products are reported in table IV. The ^{1}H-NMR spectra were recorded on a Bruker 300 spectrometer (300 MHz) with tetramethylsilane (TMS) as an internal standard. Samples were dissolved in DMSO-d_6, unless otherwise indicated. Spectral data of synthesized compounds are reported in table V. Reactions were followed by TLC, on Kieselgel 60 F 254 (DC-Alufolien, Merck).

Compounds and intermediates were purified by chromatography on preparative Gilson HPLC using a SiO_2 column (LiChroprep, Si 60, 15–25 μm, Merck); the eluents were mixtures of methylene chloride/methanol or chloroform/methanol at various volume ratios.

N-(2-Benzothiazolyl)-2-[4(5)-imidazolyl]ethylamine **2**

Histamine dihydrochloride (0.5 g, 2.72 mmol), 2-chlorobenzothiazole (0.5 g, 2.95 mmol) and triethylamine (0.8 g, 8 mmol) were dissolved in 20 ml methanol and stirred at 50°C for 24 h.

Table IV. Yield and characteristics of final products.

Compound	*Yield*	*Crystallization solvent*	*Melting point*	*Anal*
1	75%	EtOH/Petroleum ether	191-193°C	$C_{15}H_{16}N_4S$ (ref. 15)
2	62%	EtOH/Petroleum ether	191-192°C	$C_{12}H_{12}N_4S$
3	65%	EtOH/Et_2O	223-225°C [a]	$C_{12}H_{13}N_5 \cdot 2HCl \cdot 0.5H_2O$ [a]
4	70%	EtOH/Et_2O	168-170°C [b]	$C_{12}H_{11}N_3S_2 \cdot HCl$ [b]
5	65%	EtOH/H_2O	188-189°C	$C_{12}H_{12}N_4S$
6	62%	EtOH	242-243°C [c]	$C_8H_{10}N_4S \cdot 2C_6H_3N_3O_7$ [c]
7	70%	i.PrOH	195-196°C [d]	$C_{14}H_{14}N_4S \cdot 1.5C_2H_2O_4 \cdot 0.5H_2O$ [d]
8	65%	i.PrOH	80-82°C [e]	$C_{14}H_{14}N_4S \cdot 1.5C_2H_2O_4$ [e]
9	68%	EtOH/Et_2O	161-163°C [f]	$C_8H_9N_3S_2 \cdot 2HCl \cdot 0.5H_2O$ [f]
10	55%	EtOH/Petroleum ether	126-128°C	$C_{14}H_{13}N_3S_2$
11	60%	EtOH/Et_2O	146-148°C	$C_{14}H_{13}N_3S_2$
12	72%	EtOH/Et_2O	205-206°C [g]	$C_8H_{10}N_4S \cdot 2HCl \cdot 0.5H_2O$ [g]
13	65%	PrOH	243-246°C [h]	$C_{14}H_{14}N_4S \cdot 2HCl$ [h]
14	58%	EtOH/Et_2O	173-175°C [i]	$C_9H_{12}N_4S \cdot 2HCl$ [i]

[a] **3** dihydrochloride•0.5H_2O, free base: mp 88–91°C; [b] **4** hydrochloride; free base: mp 99–100°C; [c] **6** picrate; free base: mp 134–138°C; [d] **7**•1.5 oxalate•0.5H_2O, free base: mp 167–170°C; [e] **8**•1.5 oxalic acid; free base: mp 150–153°C; [f] **9** dihydrochloride•0.5H_2O; free base is an oil; [g] **12** dihydrochloride•0.5H_2O: free base is an oil; [h] **13** dihydrochloride; free base is an oil; [i] dihydrochloride.

The solvent was then evaporated under reduced pressure and the residue was dissolved in water. Extraction with chloroform and evaporation of the dried organic phase gave a powder of **2**, which was purified by column chromatography (SiO_2, $CHCl_3$/MeOH 8:1.5) and crystallization. The yield and characteristics of compound **2** are reported in table IV.

N-(2-Benzoimidazolyl)-2-[4(5)-imidazolyl]ethylamine ***3***

A mixture of 0.61 g histamine (5.5 mmol) and 0.99 g benzimidazole-2-sulphonic acid (5 mmol, prepared according to reference [20]) was heated at 130°C for 2 h. After cooling, the solid product was treated with an aqueous solution of Na_2CO_3 and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and evaporated under reduced pressure; the oily residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 10:1.5 + NH_3(g)). The yield and characteristics of compound **3** are reported in table IV.

General method of condensation of mercapto-heterocycles with 2-chloroethylimidazole (preparation of compounds ***4, 5, 9–14)***

Compounds **4**, **5**, and **9–14** were prepared, according to scheme 1, starting from 2-chloroethylimidazole and the appropriate mercapto-heterocycles as a nucleophile. The reagents, conditions, and isolation and purification methods are reported in table II. Equimolar ratios of 2-chloroethylimidazole/nucleophile were used, with double molar ratio of base, because 2-chloroethylimidazole hydrochloride was employed as a starting compound. Reactions were followed by TLC (SiO_2,

Table V. ^{1}H-NMR data for compounds **2–15** (δ in ppm). TMS as internal standard.

2	2.84 (t, 2H, CH_2), 3.60 (m, 2H, CH_2), 6.84 (s, 1H, Im-5-H), 7.02 (t, 1H, Benzoth), 7.21 (t, 1H, Benzoth), 7.38 (d, 1H, Benzoth), 7.54 (s, 1H, Im-2-H), 7.64 (d, 1H, Benzoth), 8.07 (t[a], 1H, NH), 11.83 (br[a], 1H, Im-1-H).
3	2.80 (t, 2H, CH_2), 3.51 (m, 2H, CH_2), 6.52 (br[a], 2H, chain NH and Benzoim-1-H), 6.83-6.86 (m, 3H, Im-5-H and Benzoim), 7.09-7.12 (m, 2H, Benzoim), 7.54 (s, 1H, Im-2-H).
4[b]	3.14 (t, J=7.2 Hz, 2H, CH_2), 3.61 (t, J=7.2 Hz, 2H, CH_2), 6.88 (s, 1H, Im-5-H), 7.26 (t, 1H, Benzoth), 7.38 (t, 1H, Benzoth), 7.60 (s, 1H, Imi-2-H), 7.71 (d, 1H, Benzoth), 7.82 (d, 1H, Benzoth), 10.13 (br, 1H, Im-1-H)
5	2.97 (t, J=7.3 Hz, 2H, CH_2), 3.53 (t, J=7.3 Hz, 2H, CH_2), 6.87 (s, 1H, Im-5-H), 7.10 (m, 2H, Benzoim-5-H, Benzoim-6-H), 7.43 (br, 2H, Benzoim-4-H, Benzoim-7-H), 7.54 (s, 1H, Im-2-H), 12.20 (br[a], 2H, NH).
6	2.78 (t, J=7.2 Hz, 2H, CH_2), 3.42 (m, 2H, CH_2), 6.58 (m, 1H, Th-5-H), 6.82 (s, 1H, Im-5-H), 7.00 (m, 1H, Th-4-H), 7.58 (s, 1H, Im-2-H).
7	2.80 (t, J=7.3 Hz, 2H, CH_2), 3.48 (m, 2H, CH_2), 6.83 (s, 1H, Im-5-H), 7.16 (t, 1H, Ph-4-H), 7.31 (t, 2H, Ph-3-H), 7.41 (d, 2H, Ph-2-H), 7.46 (s, 1H, Th-4-H), 7.50 (s, 1H, Im-2-H), 7.82 (t, 1H, Th-NH).
8	2.84 (t, J=7.2 Hz, 2H, CH_2), 3.51 (m, 2H, CH_2), 6.90 (s, 1H, Im-5-H), 7.00 (s, 1H, Th-5-H), 7.24 (t, 1H, Ph-4-H), 7.36 (t, 2H, Ph-3-H), 7.70 (s, 1H, Im-2H), 7,73 (t, 1H, Th-NH), 7.82 (d, 2H, Ph-2-H).
9	2.93 (t, J=7.3 Hz, 2H, CH_2), 3.45 (t, J=7.3 Hz, 2H, CH_2), 6.86 (s, 1H, Im-5-H), 7.54 (s, 1H, Im-2-H), 7.62 (d, J=3.2 Hz, 1H, Th-5-H), 7.72 (d, J=3.2 Hz, 1H, Th-4-H).
10	2.96 (t, J=7.3 Hz, 2H, CH_2), 3.48 (t, J=7.3 Hz, 2H, CH_2), 6.88 (s, 1H, Im-5-H), 7.35 (t, 1H, Ph-4-H), 7.43 (t, 2H, Ph-3-H), 7.55 (s, 1H, Im-2-H), 7.61 (d, 2H, Ph-2-H), 8.11 (s, 1H, Th-4-H), 11.82 (br, 1H, Im-1-H).
11	2.99 (t, J=7.3 Hz, 2H, CH_2), 3.51 (t, J=7.3 Hz, 2H, CH_2), 6.88 (s, 1H, Im-5-H), 7.34 (t, 1H, Ph-4-H), 7.43 (t, 2H, Ph-3-H), 7.55 (s, 1H, Im-2-H), 7.93 (d, 2H, Ph-2-H), 8.01 (s, 1H, Th-5-H), 11.87 (br, 1H, Im-1-H).
12	3.02 (t, J=7.0 Hz, 2H, CH_2), 3.70 (t, J=7.0 Hz, 2H, CH_2), 7.56 (s, 1H, Im-5-H), 7.69 (s, 2H, Im-4(5)-H), 9.05 (s, 1H, Im-2-H).
13	3.07 (t, J=7.0 Hz, 2H, CH_2), 3.70 (t, J=7.0 Hz, 2H, CH_2), 7.39 (t, 1H, Ph-4-H), 7.48 (t, 2H, Ph-3-H), 7.57 (s, 1H, Im-5-H), 7.92 (d, 2H, Ph-2-H), 8.15 (s, 1H, Ph-Im-5-H), 9.03 (s, 1H, Im-2-H).
14[b]	2.80 (t, J=7.0 Hz, 2H, CH_2), 3.11 (t, J=7.0 Hz, 2H, CH_2), 3.49 (s, 3H, CH_3), 6.67 (s, 1H, Im-5-H), 6.84 (d, 1H, N-Me-Im-5H), 6.92 (d, 1H, N-Me-Im-4H), 7.43 (s, 1H, Im-2-H).

[a]Exchangeable with D_2O; [b]in $CDCl_3$. Abbreviations: Im = imidazole, Th = thiazole, Benzoim = benzoimidazole, Benzoth = benzothiazole, Ph = phenyl.

$CH_3COOEt/EtOH/NH_3$ (aq) 6:1:1). The final products were characterized as free bases or their salts. The characteristics of these compounds are reported in table IV.

*General method of preparation of aminothiazole derivatives (compounds **6–8**)*

Compounds **6–8** were prepared as described in scheme 2, by condensation of 2-[4(5)-imidazolyl]ethylthiourea hydrochloride with the appropriate α-chloro or α-bromo carbonyl compound in equimolar ratio. The reagents and conditions of the last step described in scheme 2 are reported in table III. Reactions were followed by TLC (SiO_2, $CH_3COOEt/EtOH/NH_3$ (aq) 6:1:1). The final products were collected from the reaction mixtures by evaporation of the solvents, and the free bases were purified by means of chromatography on a silica-gel column, employing a mixture of CH_2Cl_2/MeOH 9:1, saturated with NH_3 (g). The yields and characteristics of the final compounds are reported in table IV.

N-Benzoyl-N'-2-[4(5)-imidazolyl]ethylthiourea

A solution of 3.0 g of benzoylisothiocyanate (18 mmol) in 100 ml of chloroform was added over 1 h, to a boiling suspension of 2.0 g histamine (18 mmol) in 500 ml chloroform. The reaction was followed by TLC (SiO_2, $CH_3COOEt/EtOH/NH_3$ (aq) 6:1:1) and, after 1 h at boiling temperature, the reaction mixture was decoloured, filtered, washed with 500 ml

water and dried with Na_2SO_4. Evaporation of the chloroform under reduced pressure gave *N*-benzoyl-*N'*-2-[4(5)-imidazolyl]-ethylthiourea as a white powder, which was crystallized from chloroform. Yield 58%. Melting point: 165–167°C. Mass: 274 (M^+).

N-2-[4(5)-Imidazolyl]ethylthiourea hydrochloride

N-Benzoyl-*N'*-2-[4(5)-imidazolyl]ethylthiourea (3.5 g, 12.7 mmol) was suspended in 200 ml of an aqueous solution of Na_2CO_3 (20%), and heated at 60–70°C until dissolution was complete (about 1 h; TLC: SiO_2, $CH_3COOEt/EtOH/NH_3$ (aq) 6:1:1). The solution was then decoloured, filtered and evaporated under reduced pressure, and the residue was extracted with absolute EtOH. The evaporation of the alcoholic solution gave the solid base, which was treated with dilute HCl to give the title compound. Yield 65%. Melting point >300°C. Mass: 170 (M^+).

Biological evaluation

Binding assays were conducted on rat brain cortex membranes, in accordance with a previously described method [13]. Wistar rats were killed and their brains were immediately removed and mechanically homogenized in Tris-HCl 50 mM, pH 7.6. The homogenate was centrifuged at 1000 *g* for 10 min, the pellet was discarded and the resulting supernatant was centrifuged at 10 000 *g* for 30 min. The pellet was resuspended in a solution of Tris-HCl 50 mM pH 7.4, NaCl 50 mM.

Membranes (0.5 mg protein) were incubated for 30 min with the labelled ligand ([^{3}H]NAMHA) in 1 ml of Tris-HCl 50 mM, pH 7.4, NaCl 50 mM, EDTA 1 mM. Incubation was ended by addition of 4 ml of ice-cold Tris-HCl NaCl solution and rapid filtration on AAWP Millipore filters (pore diameter = 0.8 μM) under vacuum. Filters were rinsed twice with 10 ml ice-cold buffer. Filter-bound radioactivity was measured at 43% efficiency.

Under these experimental conditions, within the [^{3}H]NAMHA concentration range of 0.2–3.0 nM, 90% of the labelled ligand binding was displaced by 1 μM thioperamide, and we have observed that competition by 1 μM thioperamide had the same effect as unlabelled histamine in reducing binding of [^{3}H]NAMHA. Specific binding was thus defined as the binding inhibited by 1 μM thioperamide. Moreover, in the presence of 1 μM thioperamide, the addition of the tested compounds, mepiramine or cimetidine gave no further displacement of the binding. The competition of these compounds was therefore only effective on H_3-binding sites.

Protein concentration was assayed with the Biorad method using bovine serum albumin as a standard.

The pK_is were calculated from the inhibition curves of the compounds tested *vs* 0.5 nM [^{3}H]-N^{α}-methylhistamine ([^{3}H]NAMHA), according to the equation of Cheng and Prusoff [26]: $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of the labelled ligand and K_d is its dissociation constant, measured by means of saturation binding experiments (for [^{3}H]NAMHA, $K_d = 0.62 \pm 0.07$ [13]). The IC_{50} and nH values were obtained by logistic regression of the binding data (four experiments). The differences of the pseudo-Hill coefficients (nH) from unity were tested with a *t*-test. When the presence of multiple binding sites was suspected, a regression with a two-site model, $B = B_{max,1}/(1 + IC_{50,1}) + B_{max,2}/(1 + IC_{50,2}) + B_{asp}$, was performed, and the choice of the most adequate model was made on the basis of two *F*-tests, for the relative and absolute goodness of fit respectively [27].

Under the present experimental conditions, competitive binding assays with thioperamide gave a monophasic curve with pK_i = 8.49, nH = 1.01 [13]; this result was in agreement with the K_i value reported by Arrang *et al* [28], who employed [^{3}H]-(*R*)-α-methylhistamine (K_i = 2.1 nM) as labelled ligand. West *et al* reported for thioperamide a biphasic inhibition of [^{3}H]NAMHA binding, with two K_i values of 4.9 nM (K_{iA}) and 64 nM (K_{iB}) respectively [29], and a single K_i value of 16 nM *vs* [^{3}H]-(*R*)-α-methylhistamine [30]. As these differences can be ascribed to different ionic composition of the solution [17], we kept constant ionic composition during all the experiments, in order to make comparisons only on strictly consistent data.

In the competitive binding experiments, concentrations of the inhibitors up to 10 μM were tested, and therefore pK_is lower than 6 could not be accurately calculated.

Acknowledgments

Financial support from MURST and CNR is gratefully acknowledged.

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