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New Activation Model for the Histamine H_2 Receptor, Explaining the Activity of the Different Classes of Histamine H_2 Receptor Agonists

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SUMMARY

Recently we developed amthamine [2-amino-5-(2-aminoethyl)-4-methylthiazole]. This cyclic analogue of dimaprit proved to be the most potent and selective histamine H_2 receptor agonist of a series of substituted 4- or 5-(2-aminoethyl)thiazoles. Quantum chemical studies on histamine (N_π-H tautomer), dimaprit, and amthamine revealed that, based upon geometries of molecular electrostatic potentials, it is likely that these agonists accept a proton from the proton-donating receptor site on their double-bonded (heteroaromatic) nitrogen atoms. In contrast to reported models, this new model is able to accommodate and explain the agonistic activities of all known (including nontautomeric) histamine H_2 receptor agonists. Quantitative structure-activity relationship studies with a series of substituted histamine derivatives and heterocyclic analogues support the presented model, in which the monocations in extended conformation interact with the receptor surface; their affinities correlate with the proton association constants of the heteroaromatic nuclei. The negatively charged anchoring site for the ethylammonium side chain of these agonists in this model is a functional group with a pK_a value of 4.17.

Over the past decades much attention has been paid to the structural requirements for agonists for the various types of histamine receptors. Due to the availability of only a limited number of potent and selective agonists for the different subtypes of histamine receptors, the majority of research in this field has concentrated on the endogenous (nonspecific) agonist histamine, **1**, itself. Concerning the stimulation of the histamine H_1 receptor, only qualitative functional chemical requirements have been formulated, of which an extensive review is given by Cooper *et al.* (1). Only recently the histamine H_3 receptor (2) was identified. Progress has been made in the development of specific agonists (3–5) for this type of receptor, but studies about the mode of action are still absent from the literature.

For the histamine H_2 receptor, however, the situation is quite different. The activation model for the H_2 receptor postulated by Weinstein *et al.* (6) is generally accepted and served as an important concept for further studies (7–13). According to this model, histamine is considered to interact with three different sites of the histamine H_2 receptor, as shown in Fig. 1. The proton transfer as a result of the tautomeric shift in the imidazole system is thought to be responsible for activation of the H_2 receptor.

For the first selective histamine H_2 receptor agonist, dimaprit (*S*-[3-(*N*,*N*-dimethylamino)propyl]isothiourea) (**2**), described by Parsons *et al.* (14), the mechanism of stimulation was less clear. Both a dynamic model (N-fit) (Fig. 2) (15) and a static model (S-fit) (Fig. 3) (16) were postulated. Only the first model offers possibilities for a 1,3-prototropic shift, as with histamine. However, recently Donné-Op den Kelder and co-workers (17, 18) concluded, as a result of fit studies with dimaprit and histamine, that "the S-fit conformation is more optimally oriented for a possible proton transfer than the N-fit conformation (18)." If this proton transfer is necessary for receptor stimulation, it implies that the sulfur atom of the isothiourea moiety should act as the proton acceptor, with the NH_2 part of this group as the proton donor. Quantum chemical studies on proton-relay mechanisms involving dimaprit, by Pardo *et al.* (19, 20), Ciuffo *et al.* (21), and Haaksma *et al.* (22, 23), seem to confirm the aforementioned mode of stimulation.

A series of rigid analogues of dimaprit (**3–8**) (Fig. 4) were prepared by Impicciatore *et al.* (24) and show a stimulating effect on gastric acid secretion in the guinea pig fundus preparation and in the cat.

For these rigid dimaprit analogues (i.e., 2-aminothiazoles), Haaksma *et al.* (18, 22, 23) developed a stimulation model, closely related to the Weinstein proton-transfer model, in which a proton of the receptor interacts with the sulfur atom of the thiazole system. However, because an electron pair of

[1] John Charles Eriks died October 26, 1992; we (H.v.d.G. and H.T.) will remember him as an excellent colleague.

ABBREVIATION: MEP, molecular electrostatic potential.

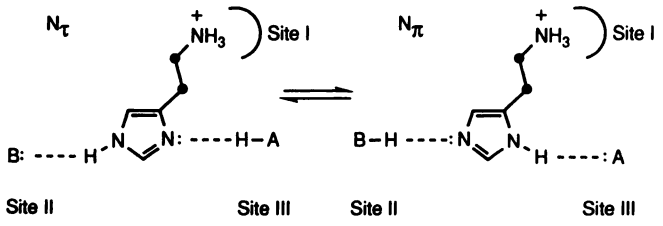


Fig. 1. Stimulation of the histamine H_2 receptor by histamine, according to the model of Weinstein *et al.* (6).

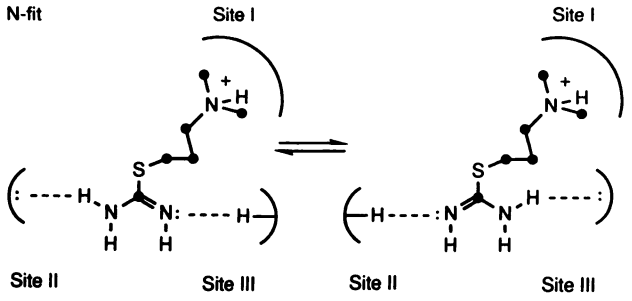


Fig. 2. Interaction of dimaprit with the histamine H_2 receptor, according to the model of Durant *et al.* (15).

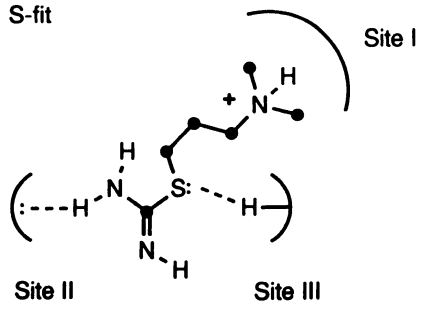


Fig. 3. Interaction of dimaprit with the histamine H_2 receptor, according to the model of Green *et al.* (16).

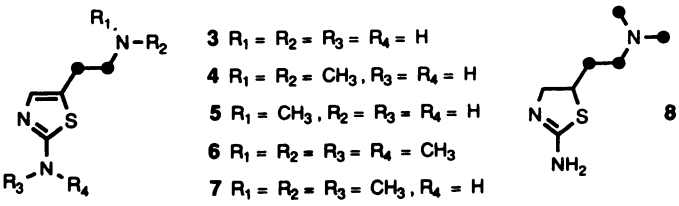


Fig. 4. Rigid analogues of dimaprit, from the report of Impicciatore *et al.* (24).

the sulfur atom is involved in the aromaticity of the thiazole nucleus, the sulfur atom bears a partially positive charge making an interaction with a proton doubtful.

Recently we prepared a series of 4- and 5-(2-aminoethyl)-thiazoles. These compounds appear to be weak to moderate full histamine H_2 agonists (25–28), with amthamine (**9**) being the most potent ($pD_2 = 6.21$; histamine $pD_2 = 6.14$).

Particularly remarkable in our new series are the histamine H_2 receptor agonistic activities of the nontautomeric 5-(2-aminoethyl)-4-methylthiazole (**10**) (Fig. 5) and 5-(2-aminoethyl)-2,4-dimethylthiazole (**11**) (Fig. 5). Both compounds are full histamine H_2 receptor agonists (27), with pD_2 values of 4.67 and 3.78, respectively. These activities are competitively antagonized by the selective histamine H_2 receptor antagonist cimetidine. Together with the reported histamine H_2 receptor agonistic activities of 2-(2-aminoethyl)thiazole (**12**) (Fig. 5) (29), 5-(2-aminoethyl)thiazole (**13**) (Fig. 5) (30), and betahistine (**14**) (Fig. 5) (29), these nontautomeric structures provide supporting evidence for the doubts of Ganellin (31), Cooper *et al.* (29), Buschauer *et al.* (32), and Van der Goot *et al.* (33), on whether tautomerism is necessarily involved in the stimulation of the histamine H_2 receptor. Obviously these histamine H_2 receptor agonists, **10–14**, first accept a proton from the proton-donating site of the receptor; the second step in the activation mechanism remains unclear, because these agonists are unable to donate a proton toward the proton-accepting site of the receptor.

Based on the aforementioned considerations and a careful analysis of the properties of a series of 4- and 5-(2-aminoethyl)thiazoles and corresponding substituted histamine analogues, we present in this paper a refined model for the stimulation of the histamine H_2 receptor.

Materials and Methods

Quantum chemical calculations. The *ab initio* calculations used in this study were performed with the Hartree-Fock method using the SV 6-31G* basis set (34). The quantum chemical program package GAMESS-UK (35–37) was used on a HP-700 computer. Geometries of compounds were fully optimized, implying variation of all bond distances, bond angles, and torsion angles. Starting geometries were obtained from a full MNDO (38) geometry optimization. The MNDO calculations were performed with a μVAX-II. MEPs were generated with the package GAMESS-UK.

Histamine H_2 receptor agonists. Histamine dihydrochloride was purchased from Janssen Chimica (Geel, Belgium), 2-aminohistamine dihydrochloride was synthesized according to the method of Vitali *et al.* (39), dimaprit dihydrobromide came from laboratory stock, and 4-methylhistamine dihydrochloride (SK&F 71517), 2-methylhistamine dihydrochloride (SK&F 91256), and 2-(2-aminoethyl)thiazole dihydrochloride (SK&F 71481) were generously provided by Smith Kline & French Laboratories (Welwyn Garden City, Herts, UK). Syntheses of 2-amino-5-(2-aminoethyl)thiazole dihydrobromide, 2-amino-5-(2-aminoethyl)-4-methylthiazole dihydrobromide (amthamine), 5-(2-aminoethyl)-4-methylthiazole dihydrobromide, 5-(2-aminoethyl)-2,4-dimethylthiazole dihydrobromide, and 2-amino-4-(2-aminoethyl)-thiazole dihydrobromide were reported earlier (25–27). [^{3}H]Tiotidine was purchased from NEN DuPont ('s Hertogenbosch, the Netherlands). All other chemicals, solvents, and reagents used are commercially available and were used without further purification, unless otherwise stated.

Determination of the macroscopic proton association constants. All compounds were titrated potentiometrically under a N_2

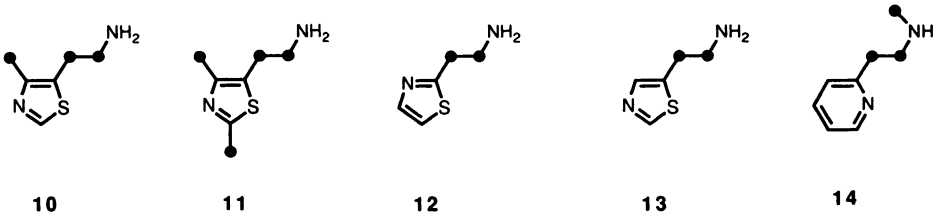


Fig. 5. Nontautomeric histamine H_2 receptor agonists.

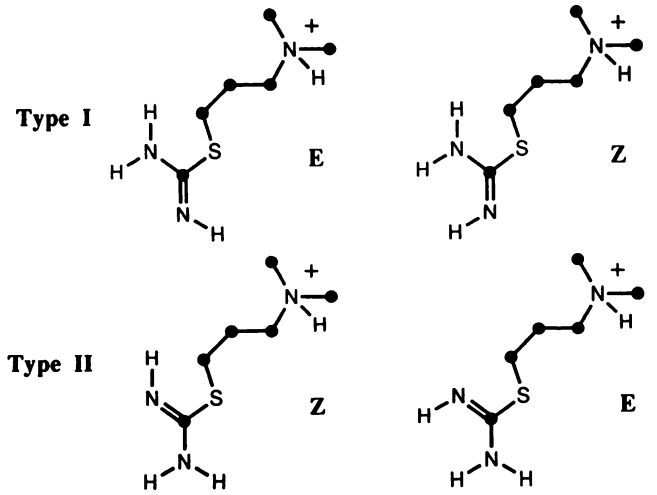


Fig. 6. Tautomeric and rotameric isomers of dimaprit, as described by Durant *et al.* (15).

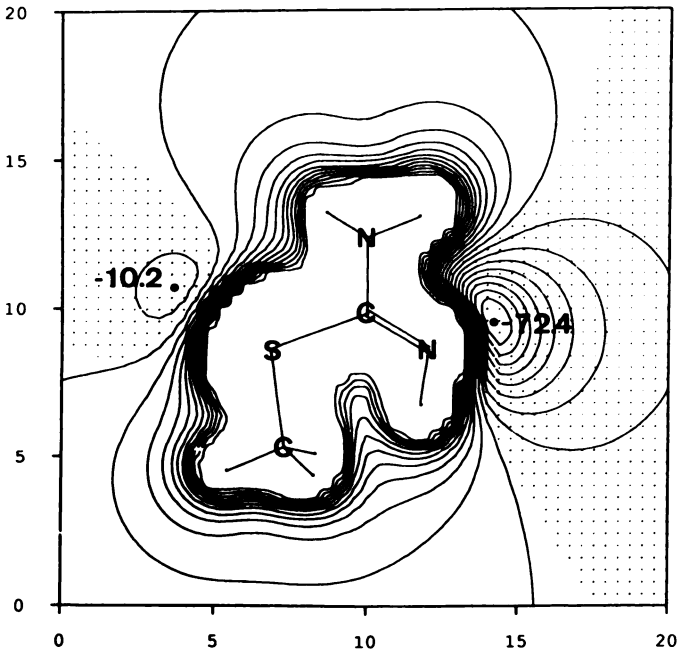


Fig. 7. MEP map for *S*-methylisothiourea (**15a**). *Dotted areas*, negative electrostatic potentials; *abscissa* and *ordinate*, atomic units.

atmosphere at 37.02 ± 0.07°, in a thermostated air-tight titration vessel (40), by using 50.00 ml of an approximately 10^{-3} M solution of the hydrobromic or hydrochloric salts in twice distilled H_2O containing 0.162 M KNO_3 (same ionic strength as in binding studies and functional experiments) with a standardized 0.1 M KOH solution (Titrisol; Merck) containing 0.062 M KNO_3, using a Mettler DV11 microburette. Measurements of the pH were performed with a Metrohm Herisau ion activity meter E 580. Calibration of the electrode system (E 202 pH electrode; Metrohm) was performed with J.T. Baker Dilut-it pH buffers (pH 4.00 and pH 7.00; I = 0.05 and 0.1, respectively).

Titration curves were analyzed using the program ASCALBASE. Log K_1 and log K_2 values (stoichiometric association constants) reported in Table 2 are mean ± standard error values of n independently performed titration experiments.

Calculations. All calculations were performed on a Macintosh Plus 4-Mb computer from Apple Computer Inc. (Cupertino, CA). Regression analyses presented were obtained with the software package Statworks (version 1.2; Cricket Software, Inc., Philadelphia, PA). Stoichiometric proton association constants (log K_n) were calculated with ASCALBASE, which is a worksheet of the spreadsheet software package

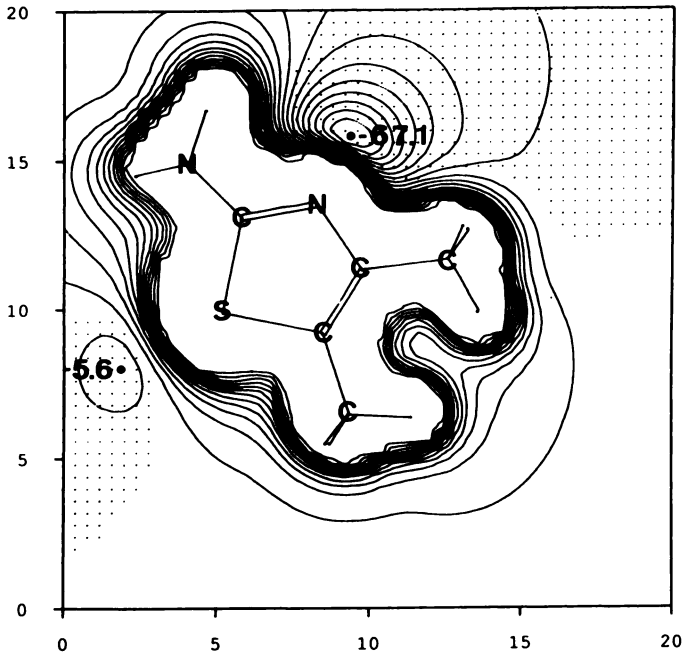


Fig. 8. MEP map for 2-amino-4,5-dimethylthiazole (**16a**). *Dotted areas*, negative electrostatic potentials; *abscissa* and *ordinate*, atomic units.

TABLE 1
Calculated total molecular energies from optimized geometries

	Total molecular energy	$E[NH^+] - E[SH^+]$
	atomic units	*kcal/mol*
S-Methylisothiourea		
Free base (**15a**)	−585.623153	
N-Protonated (**15b**)	−586.012241	−56.14
S-Protonated (**15c**)	−585.922769	
2-Amino-4,5-dimethylthiazole		
Free base (**16a**)	−700.404920	
N-Protonated (**16b**)	−700.790481	−55.01
S-Protonated (**16c**)	−700.702810	

Multiplan (version 1.02; Microsoft Corp.). Based on the equations for electroneutrality, total ligand, and stoichiometric stability constants, concentrations of all known species are calculated, taking into account volume changes during the titration experiment. The values of log K_1 and log K_2 are obtained by performing a linear regression analysis of the two titration variables of each data point resulting from a transformation of the original set of equations in such a way that one linear equation is obtained with K_1 as intercept and β_2 (= $K_1 \times K_2$) as slope. At least 25 data points of each titration curve were analyzed. Outliers among the data points were automatically removed from the original data set, using the standard error of the obtained regression equation and applying the Student t test (95% confidence limit). Log K_n values reported are stoichiometric (at a defined ionic strength) association constants.

Pharmacology. Histamine H_2 receptor agonistic properties were determined with the isolated spontaneously beating guinea pig right atrium according to the method of Sterk *et al.* (41). The pD_2 values from the chronotropic effects were evaluated not as relative activities with respect to histamine, as often is done, but taking into account that efficacies might differ for the various agonists; for statistical reasons (42), the uncorrected pD_2 values (taken at 50% maximal response of the agonistic dose-response curves) of the investigated H_2 receptor agonists are given (see Table 2). Intrinsic activities (α) were evaluated from the ratio of maximal effect from the agonistic dose-response curves and maximal effect from the histamine curves, as determined on the same right atrium preparations.

Receptor binding at H_2 receptors was determined with guinea pig

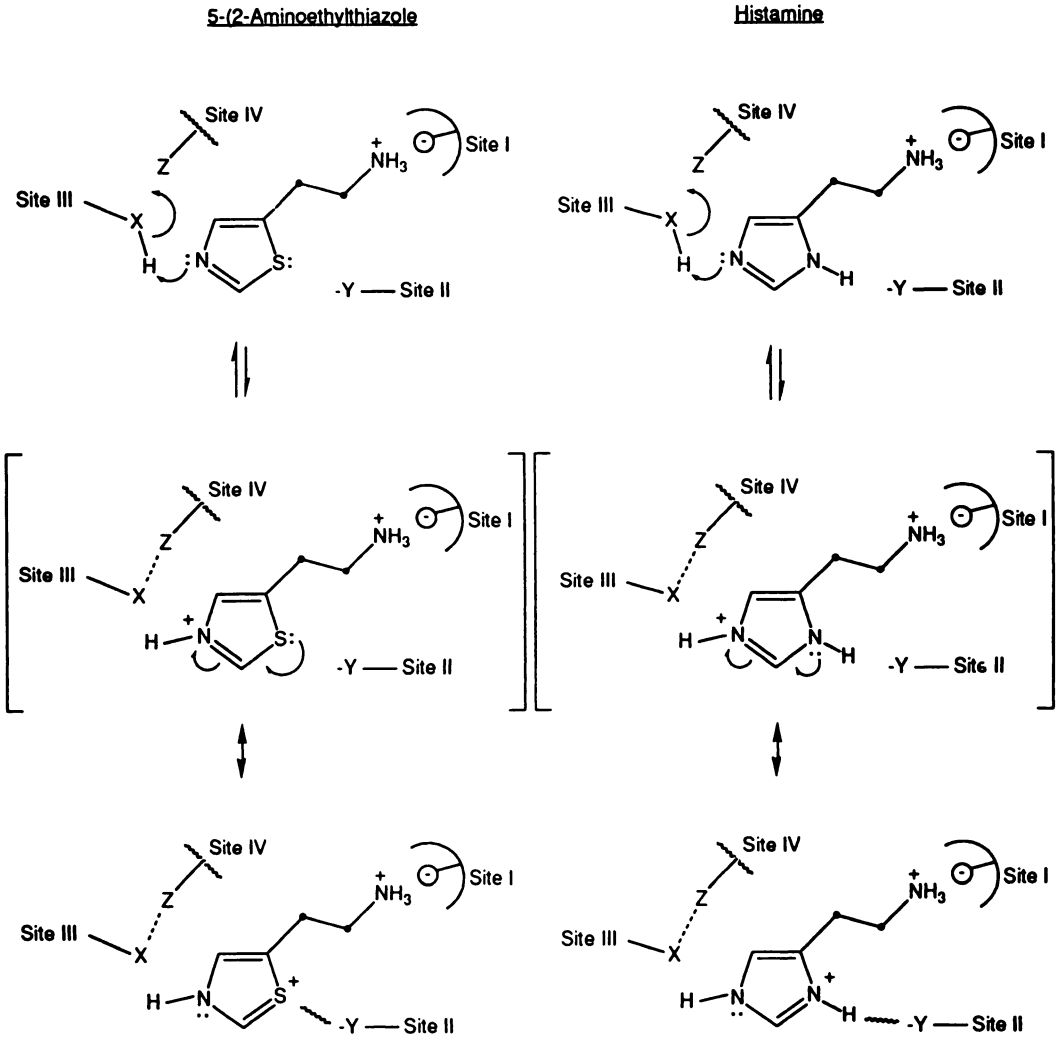


Fig. 9. Proposed model for stimulation of the histamine H_2 receptor by 5-(2-aminoethylthiazole) and histamine.

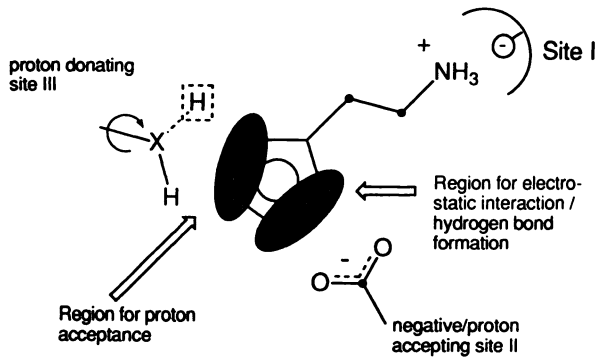


Fig. 10. Regions for proton acceptance and electrostatic interaction of agonists with the histamine H_2 receptor.

cerebral cortex preparations by the displacement of the selective histamine H_2 receptor agonist [^{3}H]tiotidine, as described by Gajtkowski *et al.* (43). The obtained displacement curves were analyzed with the nonlinear regression program LIGAND (44).

Results and Discussion

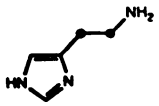
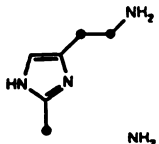
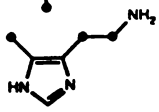
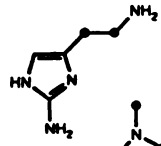
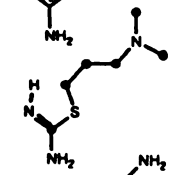
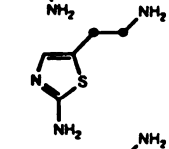
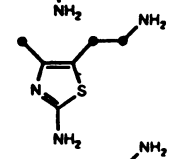
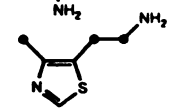
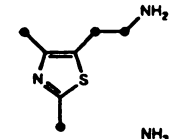
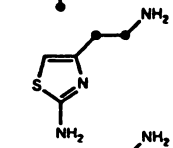
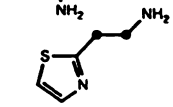
Quantum chemical calculations and qualitative considerations regarding binding and receptor stimulation. The rigid dimaprit analogues amthamine, **9**, and 2-amino-5-(2-aminoethyl)thiazole, **3**, behave both *in vitro* and *in vivo* undoubtedly as full agonists of the histamine H_2 receptor. As a consequence there should be a conformation of dimaprit (isothiourea moiety) that is comparable to the active conformation of 2-aminothiazole derivatives. Considering the four possible tautomeric and rotameric isomers of dimaprit as mentioned by Durant *et al.* (15), it appeared that the type II Z-isomer of dimaprit (Fig. 6) is a suitable candidate to be the active one. In addition, Pardo *et al.* (19) reported the type II Z-isomer to be the most stable one of the four isomers shown in Fig. 6. To confirm that this isomer can be the active form, *ab initio* quantum chemical calculations on the type II Z-isomer of dimaprit and amthamine were performed. Because upon interaction with the histamine H_2 receptor the ethylammonium side chain of these agonists is effectively neutralized, we simplified the procedure by optimizing the geometries of *S*-methylisothiourea, **15a**, and 2-amino-4,5-dimethylthiazole, **16a**. Omission of the side chain in these calculations does not lead to a change in the general shape of the potential energy curves for proton transfer for either histamine or dimaprit, as reported by Pardo *et al.* (19). Consequently, this procedure should also not affect the general shape of the MEPs.

MEPs were calculated in the plane defined by the thiazole ring of 2-amino-4,5-dimethylthiazole (**16a**). For *S*-methylisothiourea (**15a**) the plane of the MEP is taken through the C-

TABLE 2

Experimental results

All values are mean ± standard error of *n* independently performed experiments.

No.	Compound	pK_d[a]	*n*	pD_2[b]	α[c]	*n*	log K_1[d]	log K_2[e]	*n*	Monocation at pH 7.4[f]
										mol %
1.		4.16 ± 0.08	3	6.14 ± 0.04	1.00	22	9.32 ± 0.14	5.93 ± 0.14	3	95.6
18.		3.62 ± 0.11	2	4.57 ± 0.09	0.93 ± 0.03	2	9.40 ± 0.02	6.83 ± 0.01	3	78.2
19.		3.60 ± 0.10	4	5.70 ± 0.05	1.02 ± 0.04	2	9.25 ± 0.07	6.48 ± 0.01	3	88.2
20.		3.30 ± 0.03	3	5.26 ± 0.02	0.85 ± 0.02	3	9.45 ± 0.02	7.34 ± 0.01	3	53.2
2.		4.58 ± 0.11	2	5.67 ± 0.12	1.06 ± 0.03	4	8.96 ± 0.03	8.22 ± 0.04	3	13.1[g]
3.		4.82 ± 0.10	3	5.51 ± 0.05	1.00 ± 0.05	4	9.13 ± 0.01	4.94 ± 0.02	2	97.8
9.		5.30 ± 0.08	3	6.21 ± 0.09	0.95 ± 0.02	7	9.15 ± 0.02	5.40 ± 0.01	6	97.3
10.		3.45 ± 0.12	2	4.67 ± 0.12	0.96 ± 0.03	4	8.97 ± 0.05	3.23 ± 0.02	3	97.4
11.		3.76 ± 0.11	2	3.78 ± 0.13	0.97 ± 0.10	3	8.98 ± 0.01	3.74 ± 0.05	3	97.4
21.		3.96 ± 0.07	4	4.60 ± 0.09	0.94 ± 0.04	4	9.39 ± 0.02	4.36 ± 0.03	4	98.9
12.		3.99 ± 0.14	2	4.40 ± 0.05	0.91 ± 0.04	4	8.76 ± 0.01	3.36 ± 0.04	4	95.8

[a] Binding to guinea pig cortex (K_d in M).
[b] Histamine H_2 receptor agonistic activity (chronotropic effect) on isolated guinea pig right atrium (D_2 in M).
[c] Intrinsic activity.
[d] Proton association constant of side chain.
[e] Proton association constant of heteroaromatic nucleus.
[f] Calculated mole fraction of monocation.
[g] Sum of monocations (dimethylammonium and isothiouronium).

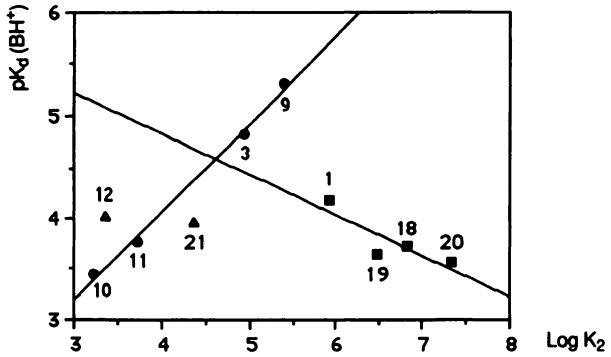


Fig. 11. Relation between binding of monocations [$pK_a(BH^+)$] and proton association constants (log K_2) of the heteroaromatic nuclei of a series of histamine and thiazole derivatives. Numbering refers to compounds mentioned in Table 2.

S-C atoms. Figs. 7 and 8 reveal that both in *S*-methylisothiourea (**15a**) and in 2-amino-4,5-dimethylthiazole (**16a**) pronounced minima are present in the vicinity of the double-bonded nitrogen atoms, of −72.4 and −67.1 kcal/mol, respectively, whereas smaller minima of −10.2 and −5.6 kcal/mol are observed in the proximity of the sulfur atoms. Both MEPs show a great congruence, indicating that the type II Z-isomer of *S*-methylisothiourea indeed must be viewed as the active conformation.

Because protonation is considered to be one of the events in stimulation of the H_2 receptor, it is interesting to compare the MEPs of histamine (**1**), *S*-methylisothiourea (**15a**) (as a substitute for dimaprit), and 2-amino-4,5-dimethylthiazole (**16a**) (as a substitute for amthamine). Weinstein *et al.* (6) reported a MEP minimum of −98.7 kcal/mol in the vicinity of the N_π nitrogen atom for the neutral free histamine, whereas Luque *et al.* (12, 13) obtained −101.37 kcal/mol for the same minimum. As stated above, our observed minima near the sulfur atoms in *S*-methylisothiourea (**15a**) and 2-amino-4,5-dimethylthiazole (**16a**) are on the order of −10 to −5 kcal/mol and those near the double-bonded nitrogen atoms are −67 to −72 kcal/mol. Therefore, protonation at the sulfur atom instead of the double-bonded nitrogen atom in these compounds has to be regarded as highly unfavorable.

Moreover, Pardo *et al.* (19) reported that a planarity constraint for protonation toward the planar *S*-methylisothiourea effectively leads to "a break of the bond between sulfur atom and the amidine moiety." These results are a good reason to abandon the idea of protonation of the sulfur atom in dimaprit and the thiazoles triggering the H_2 receptor.

Calculation of the total molecular energies (Table 1) reveals that the *N*-protonated species of *S*-methylisothiourea (**15b**) and 2-amino-4,5-dimethylthiazole (**16b**) are −56.14 and −55.01 kcal/mol, respectively, more stable than the corresponding *S*-protonated species (**15c** and **16c**). This implies that, even in the gas phase, protonation on the sulfur atom is highly unfavorable, in comparison with protonation on the nitrogen atoms.

In previous studies from our group, a proton transfer model for 2-amino-5-methylthiazole (**17**) has been proposed by Haaksma *et al.* (22, 23); this model is the only one in the literature that might explain the histamine H_2 receptor agonistic activities of the nontautomeric agonists. In this model proton-donating and accepting groups of the receptor are mimicked by an NH_4^+ group and a $HCOO^-$ group, respectively. It was suggested that, during the proton transfer, the proton followed a path along the sulfur and the exocyclic amino group, toward the endocyclic nitrogen. However, from mass spectral data it is known that in the gas phase the system NH_3/HCOOH is 561 kJ/mol more stable than NH_4^+/$HCOO^-$ (45). This implies that the high driving force for the proton transfer presented by Haaksma *et al.* (22, 23) results to a great extent from the choice of the initial gas-phase receptor conditions. The second major contribution to this high driving force results from the observed difference in stability between the *N*- and *S*-protonated species of 2-amino-5-methylthiazole. It follows from these considerations that the results of Haaksma *et al.* (22, 23) do not provide additional evidence for the proton-transfer model.

Because the sulfur atom is not likely to be protonated, attention has to be directed to the double-bonded nitrogen atom in histamine (**1**), dimaprit (**2**), and amthamine (**9**). In these three histamine H_2 receptor agonists, the double-bonded nitrogen atoms have comparable affinities for a proton (46, 47) and from a geometrical point of view the MEP minima are all situated in the same position.

The positive charge in protonated thiazoles is due to resonance distributed over both the nitrogen and sulfur atoms, as shown by Haake and Miller (48). Net atomic charges in the gas phase on nitrogen and sulfur atoms of approximately +0.4 and +0.6, respectively, as calculated by these authors, resemble the approximately 50:50 distribution that may be expected for imidazolium ions. The calculated MEPs of *N*-protonated *S*-methylisothiourea and 2-amino-4,5-dimethylthiazole fully confirm these early calculations. In both species, a uniform positive charge distribution is observed, thus enabling an ionic interaction with a negatively charged receptor site. Additionally,

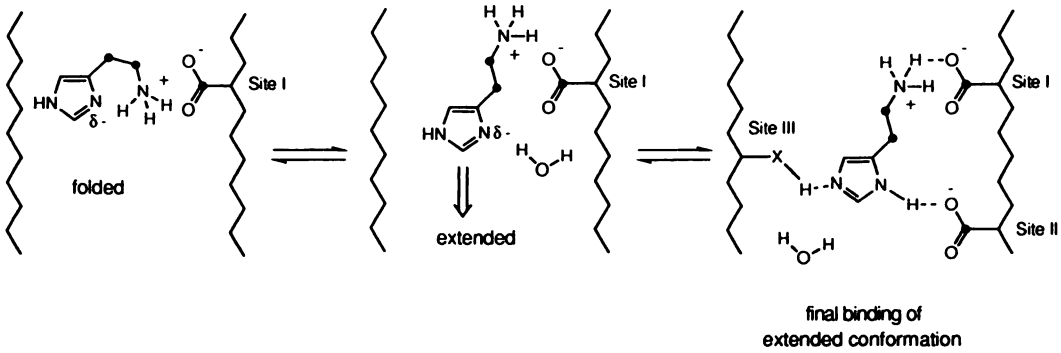


Fig. 12. Equilibrium folded and extended monocations as presumed to be present at the active site of the histamine H_2 receptor.

polarization of the sulfur atom in these structures by a negatively charged receptor site leads to a substantial stabilization of the positive charge on the sulfur atoms, thus enhancing the interaction of the agonists with the negatively charged receptor site.

The similarity of charge distribution in both protonated thiazoles and imidazoles served as the major concept in the development of a new model describing the binding and stimulation of the histamine H_2 receptor. After anchoring (Fig. 9) of the positively charged ethylammonium side chain to a negatively charged group on site I of the receptor, the thiazole ring of the agonist is protonated at the double-bonded nitrogen atom by the proton-donating site III of the receptor. The distribution of the positive charge over the heterocyclic ring enables an electrostatic interaction with the negatively charged (proton-accepting) site II. The negatively charged (deprotonated) site III is thought to interact with an additional site IV, causing the actual stimulation of the histamine H_2 receptor. Whereas in the case of thiazoles the interaction at site II is limited to a purely electrostatic one, for imidazoles the formation of a hydrogen bond between the N_π-H hydrogen atom and this site II is involved.

Possible candidates for the proton-donating group of site III are the $-NH_3^+$ group (lysine), the guanidinium group (arginine), the -OH group (serine, threonine, and tyrosine), and the -SH group (cysteine). For the negatively charged group of site II, the $-COO^-$ residues of aspartic acid and glutamic acid should be considered. Due to rotational freedom all these groups allow an interaction with the agonists in a relatively large region. Free rotation of the proton-donating group, as depicted in Fig. 10, gives the receptor freedom to donate a proton towards both *ortho*- and *meta*-position, relative to the ethylammonium side chain of the agonist. The negatively charged carboxylate (site II) offers the possibility of an electrostatic interaction with the induced positive charges in the heteroaromatic nucleus.

Gantz *et al.* (49) resolved the amino acid sequence of the histamine H_2 receptor from canine gastric parietal cells. These authors suggested that the proton-donating site of the histamine H_2 receptor is Thr^{190}, located in helix 5 of the investigated receptor protein, although replacement by Ala^{190} does not completely abolish cAMP production. For this reason proton donation by another amino acid residue cannot be excluded. On the same helix the presence of an essential Asp^{186} is reported, which can interact with the -NH group of the imidazole ring in histamine and the sulfur-atom in dimaprit and the thiazoles.

Our model is able to accommodate and explain the activities of all known (including nontautomeric) agonists. Because histamine and its heterocyclic analogues are expected to interact with the histamine H_2 receptor in extended (*trans*) conformations, rotation around the bond between the heteroaromatic carbon atom and the first carbon atom of the ethylammonium side chain gives the various types of agonists mentioned in Table 2 the ability to orientate their heteroaromatic proton-accepting nitrogen atom in the desired region, as shown in Fig. 10. Energy barriers between these *trans* rotameric conformations reported (50–53) are sufficiently low to allow these rotations. Even the weak histamine H_2 receptor agonistic activity of betahistine (**14**) (Fig. 5) ($pD_2 \approx 3.5$) can be explained by considering the fact that in this six-membered protonated pyridine system positive charges are induced in *ortho*- and *para*-positions, relative to the protonated nitrogen atom, thus again allowing a weak electrostatic interaction in the desired region.

Quantitative considerations regarding binding. Binding of an agonist to the histamine H_2 receptor is the very first step in a multicascade event (54) that ultimately leads to the observed biological effect. At a given conformation of the histamine H_2 receptor the affinity of the investigated agonists is determined by their physico-chemical properties and can be quantified by means of appropriate binding studies.

All the investigated compounds (Table 2) proved to be full agonists on the guinea pig right atrium (observed intrinsic activities, α, for all agonists are, within statistical limits, equal to 1). In the applied binding assay on guinea pig cerebral cortex, these agonists displaced the selective histamine H_2 receptor agonist [^{3}H]tiotidine in a uniform way (only one specific binding site was observed), indicating that the observed series can be treated as a homologous one.

It is well established that for histamine, its derivatives, and dimaprit the monocation is in fact the active species. In solution at physiological pH for histamine this monocation is the predominant species (96%) (55). For dimaprit, however, only 5% (15) of the monocation is present under the same conditions. Structure-activity relationship studies applied to this type of agonists should therefore make use of biological parameters that are corrected for the actual concentrations of the active species. Thus, we determined proton association constants of all investigated compounds and calculated the mole fractions of the active species (monocations) at physiological pH (Table 2). Histamine (**1**) and the thiazole derivatives **3**, **9–12**, and **21** show approximately the same fractions of monocations, ranging from 95.6 to 97.8%. This is in contrast to the more basic histamine derivatives **18–20**, where mole fractions of monocations are 78.2%, 88.2%, and 53.2%, respectively. Affinity constants (pK_d on guinea pig cortex) were converted to the corrected values for monocations [$pK_d(BH^+)$] and plotted versus the proton association constants (log K_2) of the heteroaromatic nuclei, as shown in Fig. 11.

Within the homologous series of the 5-(2-aminoethyl)thiazoles **3**, **9**, **10**, and **11**, a significant linear correlation exists between the proton association constant of the heteroaromatic nucleus (log K_2) and $pK_d(BH^+)$, as demonstrated in eq. 1.

$$pK_d(BH^+) = (0.86 \pm 0.04) \log K_2 + (0.63 \pm 0.19) \quad (1)$$

where $r = 0.997$, SE = 0.07, $F = 383$, and $n = 4$. The $pK_d(BH^+)$ values of the thiazole derivatives **12** and **21** (which both have a nitrogen atom in the *ortho*-position, relative to the ethylammonium side chain) scatter around the obtained correlation. A similar positive linear correlation was observed by Durant *et al.* (30), for the less basic 4(5)-nitrohistamine, 4(5)-chlorohistamine, and histamine, between the log K_2 of the aromatic ring and the relative potencies for the stimulation of the gastric acid secretion. In contrast, the $pK_d(BH^+)$ values of the basic histamine analogues **1** and **18–20** show a negative correlation (Fig. 11) with their proton association constants, suggesting that there might exist an optimum value for the proton association constant of the heteroaromatic nucleus of histamines. Histamine and analogues, and more generally heterocyclic ethylamines with a double-bonded nitrogen atom in the *ortho*-position toward the side chain, are known to tend to the formation of an internal hydrogen bond (56) between the ethylammonium

side chain and the electronegative nitrogen atom of the heteroaromatic nucleus. Increasing basicity of the heteroaromatic nucleus results in stabilization of this internal hydrogen bond.

When the histamine monocation approaches the active site of the receptor (Fig. 12), it is assumed that the negatively charged site I of the receptor functions as the counteranion. Interaction of the ethylammonium group with the negatively charged part at site I of the receptor results in unfolding of the ring closed structure. The equilibrium concentrations of folded (internally hydrogen bonded) and extended conformations (non-hydrogen bonded) are obviously determined by a competition between the negatively charged site I and the partially negatively charged nitrogen atom of the heteroaromatic nucleus for the positively charged ethylammonium side chain. Taking into account that all compounds possess identical side chains (dimaprit has been omitted), it is reasonable to assume that the ratio of folded/extended is predominantly determined by the ratio of the proton affinities of the heterocyclic nucleus and site I, i.e., K_2/K_1. Then the mole fraction of the extended form n_e is given by

$$n_e = \frac{K_I}{K_I + K_2} \quad (2)$$

The next logical step is to calculate the pK_d values for the monocations in the extended form, resulting in

$$pK_d(BH^+)_e = pK_d(BH^+) - \log \frac{K_I}{K_I + K_2} \quad (3)$$

and to check whether these new $pK_d(BH^+)_e$ values fit eq. 1, which was derived from compounds existing only in the extended form. Obviously, scattering of these values around the line belonging to eq. 1 depends on the value of K_I. Using simple statistical procedures this scattering was minimized, resulting in a value of 4.17 for log K_I. This value is very close to the values for aspartate and glutamate in small peptides (57, 58). A prerequisite for the validation of this procedure is that the statistics of the resulting equation after minimization are of high quality. It turned out that, indeed, very acceptable statistics were obtained, although 2-(2-aminoethyl)thiazole (**12**) proved to be an outlier; this thiazole, however, bears the aminoethyl side chain in position 2 instead of position 4 or 5. After removal of this compound the final regression equation, eq. 4, was obtained.

$$pK_d(BH^+)_e = (0.82 \pm 0.04) \log K_2 + (0.80 \pm 0.19) \quad (4)$$

where $r = 0.994$, SE = 0.13, $F = 574$, and $n = 9$.

In fact, we developed a model describing the affinity of H_2 agonists for the histamine H_2 receptor that depends only on the basicity of the heterocyclic nucleus, with interaction taking place in the extended form, whereas the occurrence of the extended forms is determined by interaction with a basic moiety at the receptor surface with log $K_I = 4.17$.

The obtained eq. 4 has very important consequences for the design of new substituted 2-(heteroaryl)ethylamines as histamine H_2 receptor agonists. In general, an increase in the basicity of the heteroaromatic nucleus of the monocations results in an increase of affinity toward the histamine H_2 receptor. If we consider the situation at physiological conditions (pH 7.40), however, log K_2 values of >6.4 result in a major decrease of the concentration of the monocations. For histamine, and more generally for internal hydrogen bond-forming agonists, we also observe an additional decrease in the concentration of the extended monocations due to the formation of the internal hydrogen bond. This effect is already observed at log K_2 values of >3.2. Particularly due to the increase in the occurrence of the cyclic conformation and the dication with increase of the basicity of the heterocyclic nucleus (log K_2), a nonlinear (parabolic) curve is obtained when plotting pK_d versus log K_2. Such an observation has been described by Luque *et al.* (13), who correlated MEP minima in the vicinity of the basic nitrogen atoms of the heteroaromatic nucleus of a series of histamine H_2 receptor agonists with their reported agonistic activity. The MEP minima reported by these authors show a fairly good linear correlation with the reported (59) pK_a values of the investigated compounds. For the series consisting of histamine, 4(5)-nitrohistamine, 4(5)-chlorohistamine, 4(5)-methylhistamine, 2-(2-aminoethyl)thiazole, and 3-(2-aminoethyl)-1,2,4-triazole, a nonlinear (parabolic) relation between the observed histamine H_2 receptor agonistic activities and the MEP minima was found. This maximum is rather broad and located at a value close to the log K_2 value of histamine. This finding is in good agreement with the structure-activity relationships we described here, showing that the more basic and more active histamine derivatives, i.e., histamine itself and 4(5)-methylhistamine, are located at the broad maximum of the parabola, whereas the less basic agonists are located in the left part of this same parabola (Fig. 11).

With these results, and in contrast to the proton-transfer models, the model described here appears to be useful for explaining quantitatively the affinity for the histamine H_2 receptor of both tautomeric and nontautomeric H_2 agonists.

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References

1. Cooper, D. G., R. C. Young, G. J. Durant, and C. R. Ganellin. Histamine receptors, in *Comprehensive Medicinal Chemistry* (J. C. Emmett, ed.), Vol. 3. Pergamon Press, Oxford, UK, 323–421 (1990).
2. Arrang, J.-M., M. Garbarg, and J.-C. Schwartz. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature (Lond.)* **302**:832–837 (1983).
3. Arrang, J.-M., M. Garbarg, J.-C. Lancelot, J.-M. Lecomte, H. Pollard, M. Robba, W. Schunack, and J.-C. Schwartz. Highly potent and selective ligands for histamine H_3-receptors. *Nature (Lond.)* **327**:117–123 (1987).
4. Howson, W., M. E. Parsons, P. Raval, and G. T. G. Swayne. Two novel, potent and selective histamine H_3-receptor agonists. *Bioorg. Med. Chem. Lett.* **2**:77–78 (1992).
5. Van der Goot, H., M. J. P. Schepers, G. J. Sterk, and H. Timmerman. Isothiourea analogues of histamine as potent agonists or antagonists of the histamine H_3-receptor. *Eur. J. Med. Chem.* **27**:511–517 (1992).
6. Weinstein, H., D. Chou, C. L. Johnson, S. Kang, and J. P. Green. Tautomerism and the receptor action of histamine: a mechanistic model. *Mol. Pharmacol.* **12**:738–745 (1976).
7. Richards, W., J. Wallis, and C. R. Ganellin. Imidazole geometry and the calculation of tautomer preference for histamine. *Eur. J. Med. Chem.* **14**:9–12 (1979).
8. Topiol, S., H. Weinstein, and R. Osman. Theoretical investigation of histamine tautomerism. *J. Med. Chem.* **27**:1531–1535 (1984).
9. Smeyers, Y. G., F. J. Romero-Sánchez, and H. Hernandez-Laguna. A quantum mechanical study of the activity of some H_2-receptor agonists of histamine, in *QSAR and Strategies in the Design of Bioactive Compounds* (J. K. Seydel, ed.). VCH Verlagsgesellschaft, Weinheim, 374–377 (1985).
10. Weinstein, H., A. P. Mazurek, R. Osman, and S. Topiol. Theoretical studies

on the activation mechanism of the histamine H_2-receptor: the proton transfer between histamine and a receptor model. *Mol. Pharmacol.* **29**:29–33 (1985).
11. Reggio, P., S. Topiol, and H. Weinstein. Molecular determinants for the agonist activity of 2-methylhistamine and 4-methylhistamine at H_2-receptors. *J. Med. Chem.* **29**:2412–2415 (1986).
12. Luque, F. J., F. Illas, and R. Pouplana. On the mechanism of histamine H_2 receptor activation. *Mol. Pharmacol.* **32**:557–563 (1987).
13. Luque, F. J., F. Sanz, F. Illas, R. Pouplana, and Y. G. Smeyers. Relationships between the activity of some H_2-receptor agonists of histamine and their *ab initio* molecular electrostatic potential (MEP) and electron density comparison coefficients. *Eur. J. Med. Chem.* **23**:7–10 (1988).
14. Parsons, M. E., D. A. A. Owen, C. R. Ganellin, and G. J. Durant. Dimaprit [*S*-[3-(*N*,*N*-dimethylamino)propyl]isothiourea], a highly specific histamine H_2-receptor agonist. 1. Pharmacology. *Agents Actions* **7**:31–37 (1977).
15. Durant, G. J., C. R. Ganellin, and M. E. Parsons. Dimaprit [*S*-[3-(*N*,*N*-dimethylamino)propyl]isothiourea], a highly specific histamine H_2-receptor agonist. 2. *Agents Actions* **7**:39–43 (1977).
16. Green, J. P., C. L. Johnson, and H. Weinstein. Histamine as a neurotransmitter, in *Psychopharmacology, A Generation of Progress* (M. A. Lipton, A. Di Mascio, and K. F. Killam, eds.). Raven Press, New York, 319–322 (1978).
17. Donné-Op den Kelder, G. M., E. E. J. Haaksma, and H. Timmerman. Theoretical considerations concerning the H_2-receptor, in *Trends in Medicinal Chemistry '88* (H. van der Goot, G. Domány, L. Pallos, and H. Timmerman, eds.). Elsevier, Amsterdam, 365–392 (1989).
18. Haaksma, E. E. J., G. M. Donné-Op den Kelder, P. Vernooijs, and H. Timmerman. A theoretical study concerning the mode of interaction of the histamine H_2-agonist dimaprit. *J. Mol. Graphics* **10**:79–87 (1992).
19. Pardo, L., A. P. Mazurek, R. Osman, and H. Weinstein. Theoretical studies of the activation mechanism of the histamine H_2-receptors: dimaprit and the receptor model. *Int. J. Quant. Chem. Quant. Biol. Symp.* **16**:281–290 (1989).
20. Pardo, L., J. Giraldo, M. Martin, and M. Campillo. Theoretical studies on the histamine H_2 receptor: construction of a receptor model based on structural properties of dimaprit and N^{ω}-guanylhistamine. *Mol. Pharmacol.* **40**:980–987 (1991).
21. Ciuffo, G. M., L. S. Mayorga, M. R. Estrada, R. R. Ibanez, M. B. Santillan, and E. A. Jauregui. Empirical atom-atom potential calculations on dimaprit, an agonist in histamine H_2 receptors. *J. Mol. Struct.* **92**:63–72 (1983).
22. Haaksma, E. E. J., G. M. Donné-Op den Kelder, H. Timmerman, and H. Weinstein. Theoretical analysis of the activity of dimaprit derivatives on the H_2-receptor. *Agents Actions Suppl.* **33**:315–324 (1991).
23. Haaksma, E. E. J. Development of theoretical models for activation and recognition of the histamine H_2-receptor by H_2-receptor agonists and antagonists. Ph.D. Thesis. Vrije Universiteit, Amsterdam, The Netherlands, 71–89 (1991).
24. Impicciatore, M., G. Morini, M. Chiavarini, E. Barocelli, F. Bordi, P. V. Plazzi, and F. Vitali. Histamine receptors and metabolism. 4. Are histamine receptors involved in the stimulant activities of thiazolylethylamines supposed as cyclic models of dimaprit? *Agents Actions* **20**:262–264 (1987).
25. Eriks, J. C., G. J. Sterk, H. van der Goot, and H. Timmerman. Preparation of substituted thiazole derivatives as drugs: Patent Cooperation Treaty International Application WO 91 10,657, July 25 (1991). *Chem. Abstr.* **115**:183285 g (1991).
26. Eriks, J. C., G. J. Sterk, E. M. van der Aar, S. A. B. E. van Acker, H. van der Goot, and H. Timmerman. 4- or 5-(ω-Aminoalkyl)thiazoles and derivatives: new selective H_2-receptor agonists. *Agents Actions Suppl.* **33**:301–314 (1991).
27. Eriks, J. C., H. van der Goot, and H. Timmerman. Histamine H_2-receptor agonists: synthesis, *in vitro* pharmacology and qualitative structure-activity relationships of substituted 4- and 5-(2-aminoethyl)thiazoles. *J. Med. Chem.* **35**:3239–3246 (1992).
28. Coruzzi, G., M. Adami, G. Bertaccini, and H. Timmerman. Amthamine: a new potent and selective histamine H_2-receptor agonist. *Pharmacol. Res.* **25**(Suppl. 2):277–278 (1992).
29. See Ref. 1, p. 353.
30. Durant, G. J., C. R. Ganellin, and M. E. Parsons. Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. Med. Chem.* **18**:905–909 (1975).
31. Ganellin, C. R. Chemistry and structure-activity relationships of drugs acting at histamine receptors, in *Pharmacology of Histamine Receptors* (C. R. Ganellin and M. E. Parsons, eds.). Wright PSG, Bristol, U.K. 10–102 (1982).
32. Buschauer, A., W. Schunack, J.-M. Arrang, M. Garbarg, J.-C. Schwartz, and J. M. Young. Histamine receptors, in *Receptor Pharmacology and Function* (M. Williams, R. A. Glennon, and P. M. W. M. Timmermans, eds.). Dekker, New York, 293–348 (1989).
33. Van der Goot, H., A. Bast, and H. Timmerman. Structural requirements for histamine H_2 agonists and H_2 antagonists. in *Handb. Exp. Pharmacol.* **97**:573–748 (1991).
34. Frisch, M. J., J. A. Pople, and J. S. Binkley. Self-consistent molecular orbital methods. 25. Supplementary functions for Gaussian basis sets. *J. Chem. Phys.* **80**:3265–3269 (1984).
35. Dupuis, M., D. Spangler, and J. Wendoloski. Program. no. QG01 (GAMESS). Natural Resource of Computational Chemistry Software Catalog, Vol. 1 (1980).
36. Guest, M. F., and J. Kendrick. *GAMESS User Manual, an Introductory Guide. CCP/86/1.* Daresbury Laboratory, Daresbury, U.K. (1986).
37. Guest, M. F., R. J. Harrison, J. H. van Lenthe, and L. C. H. Corler. Computational chemistry on the FPS-X64 scientific computers. *Theor. Chim. Acta* **71**:117–148 (1987).
38. Dewar, M. J. S., and W. Thiel. Ground states of molecules. 38. The MNDO method: approximations and parameters. *J. Am. Chem. Soc.* **99**:4899–4907 (1977).
39. Vitali, T., M. Impicciatore, P. V. Plazzi, F. Bordi, and M. Vitto. Sintesi ed attività del diclorato di 2-(2-amino-4-imidazolil)etilamina (2-aminoistaminia). *Farm. Ed. Sci.* **39**:70–80 (1984).
40. Eriks, J. C., H.-D. Gaisser, H. van der Goot, and H. Timmerman. Determination of formation constants of copper(II) complexes of Adler medium components with a solid-state copper(II) ion-selective electrode. *Pharm. Weekblad. Sci. Ed.* **5**:319–324 (1983).
41. Sterk, G. J., H. van der Goot, and H. Timmerman. Studies on histaminergic compounds. II. Synthesis and histamine H_2-activity of dimaprit and some of its analogues. *Eur. J. Med. Chem.* **19**:545–550 (1984).
42. Hancock, A. A., E. N. Bush, D. Stanisic, J. J. Kyncl, and C. T. Lin. Data normalisation before statistical analysis: keeping the horse before the cart. *Trends Pharmacol. Sci.* **9**:29–33 (1989).
43. Gajtkowski, G. A., D. B. Norris, T. J. Rising, and T. P. Wood. Specific binding of [^{3}H]-tiotidine to histamine H_2-receptors in guinea-pig cerebral cortex. *Nature (Lond.)* **304**:65–67 (1983).
44. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
45. Lias, S. G., J. E. Bartmess, J. F. Liebman, J. L. Holmes, R. D. Levin, and W. G. Mellard. Gas-phase ion and neutral thermochemistry. *J. Phys. Chem. Ref. Data* **17**(Suppl. 1):5–647 (1988).
46. Forlani, L., P. De Maria, and A. Fini. Electrical effects in substituted thiazoles: pK_a values of some 5-substituted 2-aminothiazoles and 5-substituted 2-*N*,*N*-dimethylaminothiazoles. *J. Chem. Soc. Perkin Trans. II* 1156–1158 (1980).
47. Forlani, L., and P. De Maria. Tautomerism of aminothiazoles. pK_{BH^+} values of 2-aminothiazoles and of some model imines. *J. Chem. Soc. Perkin Trans. II* 535–537 (1982).
48. Haake, P., and W. B. Miller. A comparison of thiazoles and oxazoles. *J. Am. Chem. Soc.* **85**:4044–4045 (1963).
49. Gantz, I., M. Schäffer, J. DelValle, G. Logsdon, V. Campbell, M. Uhler, and T. Yamada. Molecular cloning of a gene encoding the histamine H_2-receptor. *Proc. Natl. Acad. Sci. USA* **88**:429–433 (1991).
50. Kier, L. B. Molecular orbital calculations of the preferred conformations of histamine and a theory on its dual activity. *J. Med. Chem.* **11**:441–445 (1968).
51. Ganellin, C. R., G. N. J. Port, and W. G. Richards. Conformation of histamine derivatives. 2. Molecular orbital calculations of preferred conformations in relation to dual receptor activity. *J. Med. Chem.* **16**:616–620 (1973).
52. Ganellin, C. R. Conformation of histamine derivatives. 3. A relationship between conformation and pharmacological activity. *J. Med. Chem.* **16**:620–623 (1973).
53. Abraham, R. J., and D. Birch. The use of the counter-ion in molecular orbital calculations of histamine conformations. *Mol. Pharmacol.* **11**:663–666 (1975).
54. Golberg, N. D. Cyclic nucleotides and cell function, in *Cell Membranes: Biochemistry, Cell Biology & Pathology* (G. Weissman and R. Claiborn, eds.). HP Publishing Co., New York, 185–201 (1975).
55. Ganellin, C. R. The tautomer ratio of histamine. *J. Pharm. Pharmacol.* **25**:787–792 (1973).
56. Niemann, C., and J. T. Hays. The relation between structure and histamine-like activity. *J. Am. Chem. Soc.* **64**:2288–2289 (1942).
57. Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution.* Butterworths, London, 368–375 (1965).
58. Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972.* Butterworths, London, 7586 (1972).
59. See Ref. 1, p. 351.

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