# A new model for the agonistic binding site on the histamine $H_2$-receptor: The catalytic triad in serine proteases as a model for the binding site of histamine $H_2$-receptor agonists

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*The historical model for the agonistic binding site on the histamine $H_2$-receptor is based on a postulated activation mechanism: it has been suggested that the histamine monocation binds to the histamine $H_2$-receptor via the formation of three hydrogen bonds. The cationic ammonium group in the side chain and the —NH— group in the τ-position of the imidazole act as proton donors, whereas the =N— atom in the π-position of the imidazole acts as a proton acceptor. Participation of the ammonium group in H-bonding with a presumed negative charge on the receptor leads to a decrease in positive charge, which is thought to induce a tautomeric change in the imidazole ring system from $N^{\tau}$-H to $N^{\pi}$-H. A consequence of this tautomeric shift is the donation of a proton from the receptor to the agonist on one side, while on the other side a proton is donated from the agonist to the receptor. The proposed tautomeric shift has been suggested to trigger the $H_2$-stimulating effect.*

*However, this model for the constitution of the agonistic binding site and the accessory activation mechanism cannot explain the weak histamine $H_2$-activity of β-histine and the activity of several other recently synthesized $H_2$-agonists. Based on a thorough literature study and with the aid of molecular electrostatic potentials (MEPs) we demonstrate that the sulphur atom present in histamine $H_2$-agonists as dimaprit and 2-amino-5-(2-aminoethyl)thiazole does not function as a proton acceptor, which implicitly means that a tautomeric shift is not a prerequisite for $H_2$-stimulation. As a consequence, the model for the agonistic binding site is adjusted, resulting in a strong resemblance to the nature and orientation of the amino acids constituting the catalytic triad in serine proteases. Within this concept, the $N^{\pi}$-H tautomer of histamine is the biologically active form, in contrast with the existing model in which the $N^{\tau}$-H tautomer is the active form.*

*Keywords: histamine $H_2$-receptor, $H_2$-agonist, agonistic binding site, activation mechanism, MEP, intramolecular hydrogen bond, serine protease, deletion model Topiol*

## INTRODUCTION

Three types of histamine receptors, i.e., the histamine $H_1$-, $H_2$-, and $H_3$-receptors, are known. With the introduction of burimamide[1] the existence of the histamine $H_2$-receptor was firmly established. In general histamine $H_2$-agonistic activity is either measured as an increase in gastric acid secretion or as a chronotropic effect on the isolated guinea pig right atrium. The antagonists developed for the histamine $H_2$-receptor are widely used in the treatment of duodenal ulcer disease.

Several authors[2–6] have proposed similar models for both the agonistic binding site on the histamine $H_2$-receptor and the accessory activation mechanism. This model is based on results from classical quantitative structure–activity relationships and on *ab initio* calculations in which a series of histamine $H_2$-receptor agonists was considered.

The currently widely accepted model is based on a tautomeric shift, which occurs after binding of an agonist: i.e., on one side of the receptor surface the agonist accepts a proton, while on the opposite side it donates one (Figure 1). For the

*ADF program, Amsterdam Density Functional program.
**DZD, Double ζ basis with *d*-functions for polarization on heavy atoms.
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histamine $H_2$-agonists 2-amino-5-(2-aminoethyl)thiazole and dimaprit in its presumed bioactive conformation (S-fit,[6] Figure 2, conformer b), this implies that in both compounds the sulphur atom acts as a proton acceptor. During the proton transfer, the proton follows a path along the sulphur toward the basic nitrogen at the opposite side of the molecular skeleton, which is present in dimaprit as well as in 2-amino-5-(2-aminoethyl)thiazole. To complete the proposed activation mechanism, the proton is finally donated to a proton-accepting group on the receptor. The net result on the receptor is the same as for the activation by histamine. However, in view of the weak electronegativity of sulphur in these molecules, it is not very likely that this atom could accept a proton from the receptor surface. Moreover, the recently synthesized and tested 2-amino-4-methyl-5-(2-aminoethyl)selenazole appears to be a more potent histamine $H_2$-agonist than its sulphur analogue, although selenium is less electronegative than sulphur. A second compound which does not fit into the proposed model is the weak histamine $H_2$-agonist β-histine (2-(2-aminoethyl)pyridine). Although β-histine can accept a proton, it cannot donate one at the opposite side of the molecular skeleton.

In this paper, we will first discuss the main features of the historical model for the histamine $H_2$-agonistic binding site and its accessory mechanism of activation. The arguments which have led to the proposition of this model will be given followed by our criticism. Based on our critical arguments a more satisfying model is proposed. The resulting constitution of the histamine $H_2$-receptor agonistic binding site then resembles the nature and relative orientation of the amino acids constituting the catalytic triad in serine proteases. To support our proposition we will be calculating MEPs for a series of twelve histamine $H_2$-receptor agonists, including several newly synthesized and tested compounds.[7,8] Finally, the new model will be discussed upon its merits.

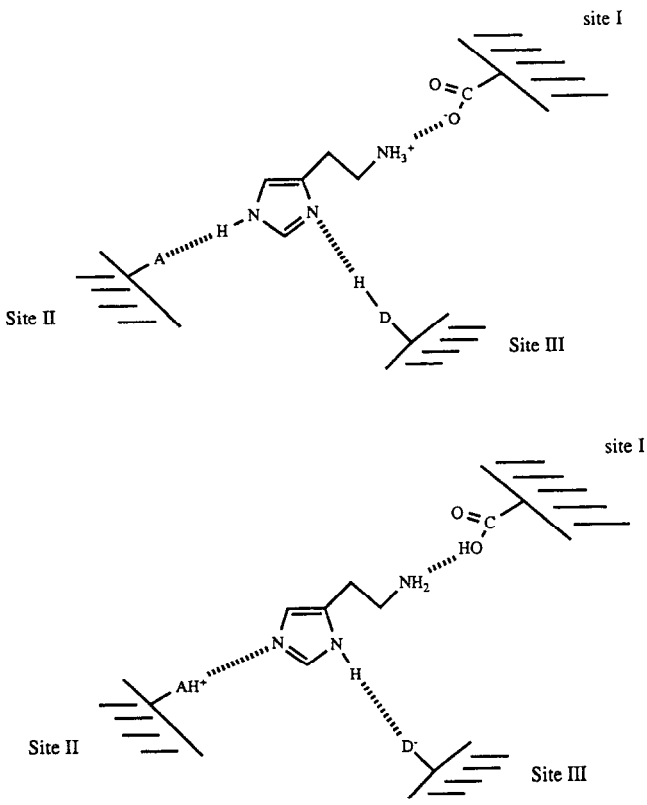



*Figure 1. Histamine before and immediately after stimulation of the histamine $H_2$-receptor. Site I is a negatively-charged group on the receptor. Sites II and III are a proton acceptor and donor, respectively.*

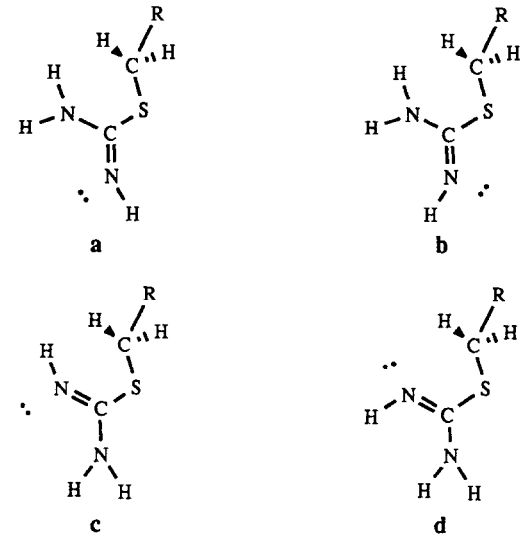



*Figure 2. Four conformers of dimaprit in which R = $CH_2CH_2N^+H(CH_3)_2$.[12] Dimaprit is drawn in its so-called S-fit conformation[4–6] in which the sulphur atom coincides with the proximal nitrogen of the histamine heterocycle and either the $NH_2$ (isomer* **a,b***) or NH (***c,d***) group of dimaprit coincides with the tele nitrogen in the imidazole ring.*

## EXISTING MODEL FOR THE HISTAMINE $H_2$-RECEPTOR AGONISTIC BINDING SITE AND ITS ACCESSORY ACTIVATION MECHANISM

### Structural requirements for histamine $H_2$-agonistic activity

Within the historical concept, structural requirements for histamine to display $H_2$-agonistic activity were considered to be the protonated side chain amino function and the ability of the imidazole system to undergo a tautomeric shift. We will summarize several arguments, which have led to a widely accepted model for the agonistic binding site and its activation mechanism. Subsequent sections briefly outline how the $H_2$-agonist dimaprit—which is structurally very dissimilar to histamine—and cyclic analogues of dimaprit were fitted into this model. MEP data seem to support the model.

### $N^\tau$-H tautomer population in relation to biological activity

Histamine can exist in different ionic forms. The first $pK_a$ value equals 5.80 and corresponds to dissociation of a proton from either of the two ring NH-groups to give the monoca-

tion.[2] The second ionization constant is found at pH 9.40 and corresponds to dissociation of the side chain amino to give the neutral species.[2] At high pH (14) the anionic species is formed;[9] at physiological pH (7.4) 96% of histamine is present as monocation.

For the determination of the relative populations of $N^{\tau}/N^{\pi}$ tautomers for each ionic species, the microscopic dissociation constants are needed. Since these constants cannot be determined from the available titration data, the relative $N^{\tau}/N^{\pi}$ populations for the monocationic species of histamine and a series of strongly related compounds (a.o. 4-methyl-, 4-chloro-, and 4-nitrohistamine) have been estimated from a Hammett equation assuming that electronic effects can be combined linearly.[2] From the results it was concluded that since "the reductions in the population of the $N^{\tau}$-H tautomer approximately parallel the changes in $H_2$-receptor agonist activities (apart from other effects, e.g., steric, polarity, and lipid-water distribution), the $N^{\tau}$-H tautomer is likely to be the biologically active form at the histamine $H_2$-receptor." Furthermore, it was suggested that when the difference in free energy between the two tautomers appeared to be small, a proton transfer might be involved in receptor activation.

## Theoretical model for the agonistic binding site and its activation mechanism

Weinstein et al.[3] have developed a model for the activation of the histamine $H_2$-receptor in line with aforementioned conclusions derived by Ganellin. In their model, histamine enters the receptor as a monocationic species in its $N^{\tau}$-H tautomeric form. *Ab initio* calculations (in the Whitman and Hornback basis with 5*s* 3*p* Gaussian orbitals on the heavy atoms, and two *s*-type Gaussians on hydrogens[10]) show that the $N^{\tau}$-H monocation is 13.1 kcal/mol more stable than the $N^{\pi}$-H form.[11] After entering the receptor, the cationic side chain of histamine interacts with a negative region on the receptor (Site I in Figure 1), which results in a neutralization of the side chain. This neutralization shifts the tautomeric preference from $N^{\tau}$-H toward $N^{\pi}$-H, as now the $N^{\pi}$-H form of the neutral species becomes 7.3 kcal/mol more stable than the $N^{\tau}$-H form. This change in tautomeric preference induced by neutralization of the side chain is assumed to cause a proton shift on the receptor surface which is responsible for triggering the biological response.[3]

## Dimaprit and cyclic analogues fitted into the model

Dimaprit (S-[3-(*N*,*N*-dimethylamino)-propyl]-isothiourea), a highly specific histamine $H_2$-receptor agonist, has been fitted into this model for the agonistic binding site, by Pardo et al.[12] and Haaksma et al.[4–6] As a histamine, dimaprit has been found experimentally to be active in its monocationic form.[13]

The monocationic form of dimaprit has four possible conformers (Figure 2). For a model compound of dimaprit ($R$ = H in Figure 2) the four conformers **a–d** have been optimized in a Hartree-Fock (HF) calculation with a 3-21G basis set.[12] As has been demonstrated for histamine,[3] replacement of the ethylamine side chain by a methyl group is allowed as it doesn't seem to affect the general shape of the potential energy curve for the suggested proton shifts. Conformer **c**, in which the lone pairs of the double-bonded nitrogen and the sulphur atom point in opposite directions, appears to be the most stable, whereas conformer **b** is the least stable (conformer **b** is 7 kcal/mol less stable than **c**).[12] Yet conformer **b** was chosen for proton transfer simulation studies, as it was found to have the largest interaction energy when placed in the receptor model.[6,12] In both simulation studies,[6,12] dimaprit was assumed to bind in the so-called S-fit conformation,[4,5] in which the sulphur atom coincides with the position of the proximal nitrogen in the imidazole ring of histamine.[4,5] Pardo et al.[12] calculated only the first proton transfer step (movement from site III to the sulphur atom in Figure 1) whereas Haaksma[6] allowed the proton to follow a path along the sulphur toward the double-bonded basic nitrogen. In Haaksma's model, a proton can be donated from either of the two $NH_2$ groups to site II on the receptor to accomplish a tautomeric shift.

Based on these theoretical studies,[4–6] cyclic dimaprit analogues (a series of thiazoles) were predicted to be potent histamine $H_2$ agonists. Subsequent synthesis and pharmacological testing[7,8] confirmed this prediction. In simulation studies on the cyclic analogue 2-amino-5-(2-aminoethyl)thiazole,[6] a proton moves from site III along the sulphur toward the nitrogen at the opposite side of the molecule and from there to the acceptor group (site II, Figure 1) on the receptor.

## Molecular electrostatic potentials

Pardo et al.[12] have calculated MEP values (HF, 3-21G) around the sulphur for the model compound methylisothiourea in its four possible conformers (**a–d** in Figure 2). The results demonstrate that conformer **b**, with the highest interaction energy for the proposed agonistic binding site, generates the deepest MEP minimum in the surroundings of the sulphur atom (–37 kcal/mol in the direction of a free electron pair), and hence has the highest proton affinity. No MEP values around the double-bonded nitrogen were reported. Also, Haaksma et al.[4–6] have reported MEP values (density functional program ADF* with a DZD** basis set[14]) for this model compound (conformer **a**, however) and for model compounds of histamine and 2-amino-5-(2-aminoethyl)thiazole (all side chains replaced by a methyl group). The MEP values calculated for dimaprit in the plane of the molecule near the basic nitrogen are only 10 kcal/mol more negative than those near the sulphur atom, whereas for the thiazole compound the MEP values near the basic nitrogen are 20 kcal/mol more negative than those in the surroundings of the sulphur atom. These values support the suggestion that the sulphur atom in these agonists is not unlikely to act as a proton acceptor.

## CRITICISM OF THE HISTORICAL HISTAMINE $H_2$-RECEPTOR MODEL

We have noticed several problems related to the model for the $H_2$-receptor.

## The $H_2$-receptor agonist β-histine

β-Histine is a weak histamine $H_2$-receptor agonist.[15] Although this molecule might be able to accept a proton from the receptor, it is unable to transfer a proton over its molecular skeleton towards the opposite side of the ring and donate it to the receptor surface. Apparently, tautomerism in the sense of a proton moving from one side of the molecule to another does not seem to be necessary for stimulation of the $H_2$-receptor.

## MEP values of dimaprit

Pardo et al.[12] have reported MEP values for methylisothiourea, but limit their data to the surroundings of the sulphur atom. From ADF electronic densities Haaksma et al.[4-6] calculated MEP data both near the sulphur and close to the double bonded nitrogen. The MEP values for dimaprit in the surroundings of these two atoms differ by no more than 10 kcal/mol. In view of the large difference in hydrogen-accepting properties of these two atoms in this molecule, this small difference in MEP values is unexpected. However, very recently Eriks et al.[7] performed HF calculations in a 6-31G* basis set on the methylisothiourea compound in its most stable conformer[12] **c**, and observed a MEP minima near the sulphur of −10 kcal/mol and near the double bonded nitrogen of −70 kcal/mol. This relatively large difference is in agreement with the expected relative proton-accepting abilities of these two atoms in dimaprit.

The unexpected large discrepancy between references 4–6 and reference 7 prompted us to thoroughly reexamine the calculation of MEPs from ADF densities (see Methods). A renewed investigation of dimaprit revealed acceptable differences from HF data (vide infra). The latter results, however, lead to the conclusion that the S atom in dimaprit and thiazol analogues is less likely to be a proton acceptor than previously assumed.[4-6]

As the MEP value near sulphur is observed to be substantially less negative than the MEP value near the basic nitrogen,[7] the sulphur atom probably more readily interacts with a (possibly negative) proton-accepting site on the receptor than with a proton donor site. In the literature,[16] it has been suggested that this proton acceptor group might be the negatively charged aspartic acid ($Asp^{186}$) present in transmembrane helix V. As the arguments which have led to the S-fit conformation of dimaprit remain valid (*ergo,* optimal geometrical resemblance, internal energy),[4-6] not only the sulphur atom of dimaprit (and thiazoles) possibly interacts with this aspartic acid, but also the proximal nitrogen atom of histamine. This concept implies that this nitrogen of histamine should be protonated, and hence that the biologically active form of histamine for the histamine $H_2$-receptor is not the $N^{\tau}$-H monocationic form but the $N^{\pi}$-H form. Also, the receptor groups which are supposed to interact with the imidazole moiety (Figure 1) need to be interchanged with respect to the previously published receptor model: the proximal position now interacts with a (negative) proton acceptor site ($Asp^{186}$),[16] whereas the tele position interacts with a proton donor ($Thr^{190}$).[16] This revised model leads to an activation mechanism in which a proton is donated from the receptor to the $=N^{\tau}$- of the heterocycle and (if possible) a proton is transferred from the π-position to the proton acceptor on the receptor (Figure 3).

This adjusted mechanism also implies that the most stable dimaprit conformer **c** can be modeled into the binding site instead of the least stable conformer **b**. Furthermore, the basic HN= function of dimaprit coincides with the $=N^{\tau}$- of histamine, whereas the sulphur atom coincides with the $N^{\pi}$-H group. As a consequence the atom with the *most negative* MEP values in its surroundings both in dimaprit and in histamine interacts with a proton-*donating* group on the receptor. Remarkably, in this revised model tautomerism is not a prerequisite for histamine $H_2$-agonistic activity.

## Agonistic activity of 2-amino-4-methyl-5-(2-aminoethyl)thiazole compared with its selenium analogue

Besides the recently published thiazole compounds,[7,8] Eriks et al. also synthesized and tested a selenazole derivative, i.e., 2-amino-4-methyl-5-(2-aminoethyl)selenazole. When fitted into the Weinstein model this compound is expected to be less potent than the corresponding thiazole, as a Se atom is even less electronegative than a sulphur atom. However, in our model the compound is expected to display higher potency which is confirmed experimentally (selenazole: $pD_2 = 6.52$ and $pK_d \approx 5.7$; corresponding thiazole: $pD_2 = 6.21$ and $pK_d = 5.30$).

## It is not necessarily the τ-form of histamine which is the pharmacologically active species

Although Ganellin[2] reports a qualitative relationship between the $N^{\tau}$-H tautomer population and $H_2$-agonistic activity, the conclusion that the $N^{\tau}$-H tautomeric form is thus the biologically active form might not be justified. For although electron-withdrawing substituents decrease the $N^{\tau}$-H population and increase the $N^{\pi}$-H mole fraction, reduced activity might also be due to a diminished ability to accept a proton from the receptor. So more than one explanation is possible for the reduced activity of 4-chlorohistamine and 4-nitrohistamine, for example.

## Intramolecular hydrogen bond

Reggio et al.[17] have ascribed the reduction in activity of 4-methylhistamine (43% relative to histamine[2]) to a decrease in mole fraction monocation. As the imidazole in 4-methylhistamine relative to histamine has a higher $pK_a$ value, the mole fraction of the dication is increased. Recently, Eriks et al.[7] actually measured the mole fraction monocation for 4-methylhistamine and for histamine, finding 88.2% and 95.6%, respectively (see Tables 1 and 2). These data cannot explain the largely reduced potency of 4-methylhistamine. This observation holds for all histamine analogues substituted with (sterically allowed) electron-releasing groups (compounds **1** to **4**, Table 1). The formation of an intramolecular hydrogen bond enhancement by the presence of electron-releasing substituents might explain these observations.

The presence of such an intramolecular H-bond was sug-

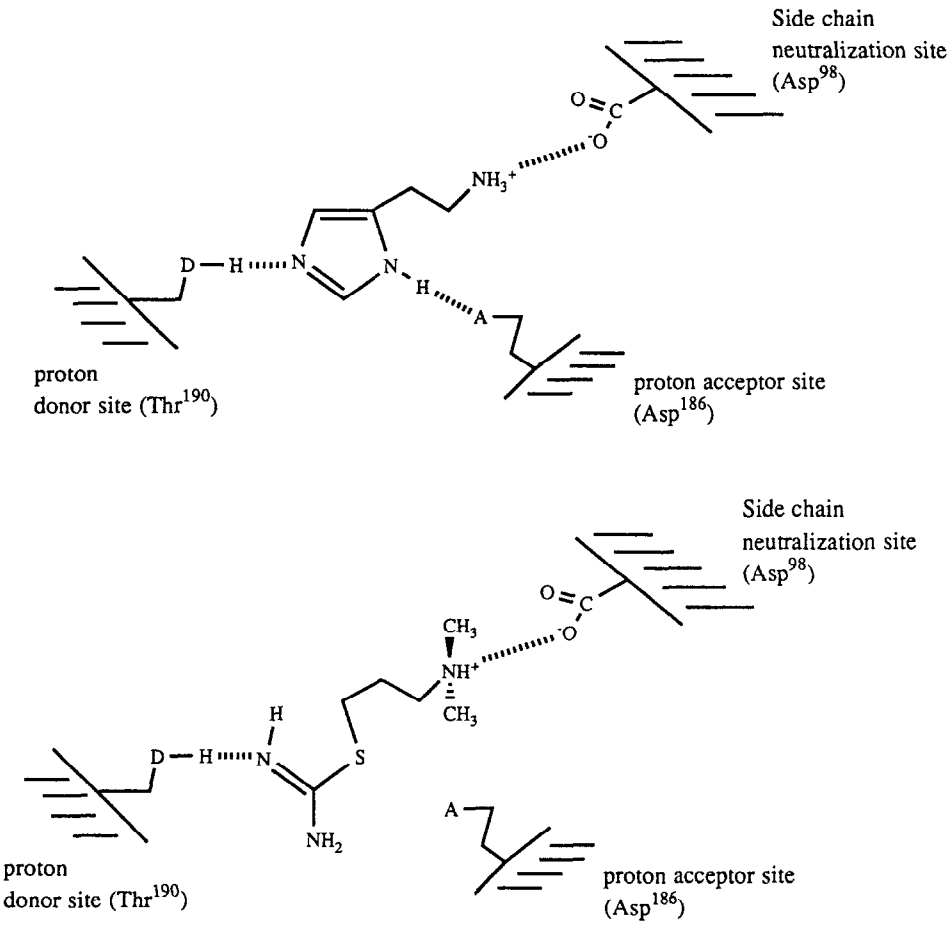


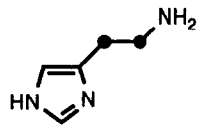
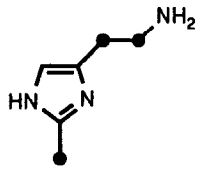
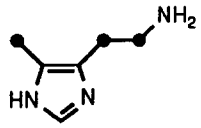
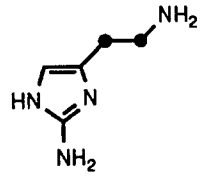
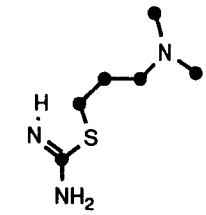

*Figure 3. Histamine ($N^{\pi}$-H form) and dimaprit fitted in our revised model; the amino acids probably interacting with the ligands are denoted with the characterization taken from Gantz et al.*[16]

gested by Niemann and Hays.[18] Histamine molecules with an intramolecular H-bond are not expected to fit into the agonistic binding site and will therefore be biologically inactive. The H-bond stabilizes the $N^{\tau}$-H form, and substituents might either increase (electron-releasing substituents) or decrease (electron-withdrawing substituents) the H-bond formation. If intramolecular H-bond formation is neglected and the aforementioned Hammett equation is applied[2] to 4-methylhistamine, this compound is expected to be equally as active as histamine, since approximately equal amounts of the monocationic $N^{\tau}$-H tautomer (or $N^{\pi}$-H tautomer) are calculated to be present (histamine: 71% $N^{\tau}$-H form of 95.6% monocation $\approx$ 68% $N^{\tau}$-H monocation; 4-methylhistamine: 80% $N^{\tau}$-H form of 88.2% monocation $\approx$ 70% $N^{\tau}$-H monocation).[2,6] Furthermore, since the heterocycle of 4-methylhistamine is more basic than the one of histamine ($pk_{a,Im}$ = 6.48, 5.93, respectively, Table 1) acceptance of a proton from the receptor is facilitated, and actually an enhancement in activity for 4-methylhistamine, relative to histamine, is expected. However, as 4-methylhistamine has a substantially lower activity than histamine (43% relative to histamine[2]), the concept of an intramolecular H-bond stabilized by electron-releasing substituents to explain the reduced activity is highly probable.

Strong evidence for this concept was recently obtained by Eriks et al.[7] These authors find a negative correlation between the proton association constant of the imidazole ring of a series of substituted histamine analogues (only electron-releasing groups; **1–4** in Table 1) and the affinity of the monocation for the histamine $H_2$-receptor. An explanation can be found when considering the molecular partition function used in statistical mechanics, which is the sum over states of an individual molecule. If only the extended $N^{\pi}$-H form of the molecule is recognized by the receptor and not the extended or folded $N^{\tau}$-H conformations, enhanced intramolecular H-bond formation by introduction of electron-releasing substituents will diminish the amount of active states, thereby possibly explaining the observed negative correlation.

More evidence for the existence of an intramolecular H-bond in histamine and ring-substituted analogues is provided by a series of thiazoles (see also Table 2, compounds **6–9**).[7] The thiazoles in Table 2 cannot form intramolecular H-bonds; nor do they give rise to any tautomeric equilibrium. Therefore, electron-releasing substituents will not influence the partition function in the same way as expected for imidazole-containing agonists. This suggests a positive slope for the relation between binding of the monocation and log $K_2$ values. Indeed, a linear relationship with a positive slope between $pK_d(BH^+)$ and log $K_2$ is found.[7] Upon introducing electron-releasing groups in these thiazoles, their ability to accept a proton ($K_2$) as well as their potency

**Table 1. Biological activities and titration data for histamine, substituted histamines, and dimaprit**

| No. | Compound | $pK_d$ [a] | $n$ | $pD_2$ [b] | $\alpha$ [c] | $n$ | log $K_1$ [d] | log $K_2$ [e] | $n$ | Mol %[f] Monocation at pH = 7.4 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. |  | 4.16 ± 0.08 | 3 | 6.14 ± 0.04 | 1.00 | 22 | 9.32 ± 0.14 | 5.93 ± 0.14 | 3 | 95.6 |
| 2. |  | 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 |
| 3. |  | 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 |
| 4. |  | 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 |
| 5. |  | 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] |

[a]Binding to guinea pig *cortex*.
[b]Histamine $H_2$-receptor activity on isolated guinea pig right *atrium* (chronotropic effect).
[c]Intrinsic activity.
[d]Proton association constant of side chain.
[e]Proton association constant of hetero aromatic nucleus.
[f]Calculated molfractions based on Log $K_1$ and Log $K_2$.
[g]Sum of monocations (dimethyl-ammonium and isothiouronium). All values given are mean values ± S.E.M. of n independently performed experiments.

increases (see Table 2 and compare $pK_2$, $pK_d$, and $pD_2$ values for compounds **6** and **7**).[7,8] This observation suggests that the ability of a compound to accept a proton (in the tele position) determines its potency. Whether a proton can be donated to the receptor in the proximal position is not of crucial importance.

## PROPOSED REFINEMENT OF THE HISTAMINE $H_2$-RECEPTOR AGONIST BINDING SITE MODEL

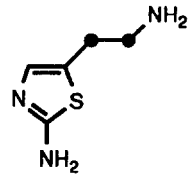
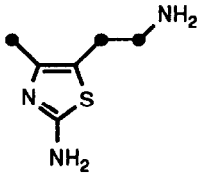
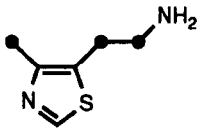
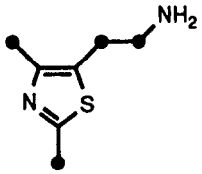
### Refined model

In order to overcome the problems noticed in relation to the earlier model for the histamine $H_2$-agonistic binding site we propose a refined model for this site with an accessory mechanism of activation. The relative position of the groups on the receptor interacting with the imidazole moiety in histamine is interchanged with respect to the Weinstein model: now a (negative) proton acceptor group on the receptor interacts with the proximal $N^{\pi}$-H group of the heterocycle, whereas a proton-donating group on the receptor interacts with the double bonded nitrogen in the tele position (compare Figures 1 and 3). Inherent to this model is the concept that the $N^{\pi}$-H tautomer is the active species.

### Validation of the proposed model

In this report the proposed refinement of the agonist binding site and its activation mechanism will be further tested by calculating MEP values for a series of histamine $H_2$-agonists using a density functional approach. Based on our thorough literature study, only the $N^{\pi}$-H tautomeric form of the histamine analogues will be considered.

**Table 2. Biological activities and titration data for substituted thiazole analogues[h]**

| No. | Compound | $pK_d$[a] | n | $pD_2$[b] | $\alpha$[c] | n | log $K_1$[d] | log $K_2$[e] | n | Mol %[f] pH = 7.4 |
|---|---|---|---|---|---|---|---|---|---|---|
| 6. |  | 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 |
| 7. |  | 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 |
| 8. |  | 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 |
| 9. |  | 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 |

[h]For further details see legend Table 1.

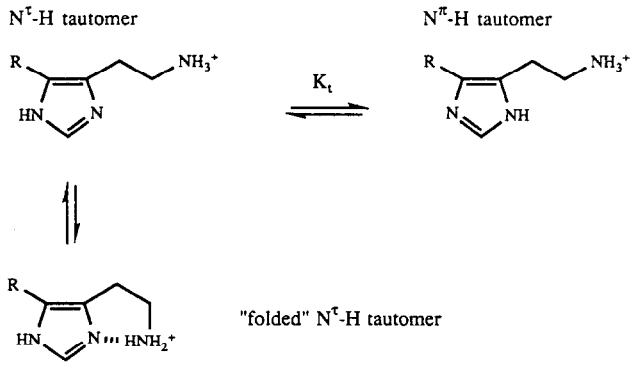



*Figure 4.* R-*substituted histamine and the intramolecular hydrogen bond.*

## METHODS OF CALCULATION

The MEP can be calculated from the nuclear and electronic charge distributions:

$$V^{MEP}(\mathbf{r}) = + \sum_{\alpha}^{N_{(all\ nuclei)}} \frac{Z_\alpha}{|\mathbf{R}_\alpha - \mathbf{r}|} - \int |\mathbf{r} - \mathbf{r}'|^{-1} \rho(\mathbf{r}')\, d\mathbf{r}' \quad (1)$$

The MEP value in point **r** gives the electrostatic interaction energy of a positive point charge (+1 a.u.) with the unperturbed molecule.

For calculating MEP values, we used the density functional program ADF.[14] Since the ADF program contains no option for calculating MEP values we have embedded a MEP option in this program.

A density functional method uses a local potential for the exchange/correlation operator. We have used the Vosko-Wilk-Nussair[19] parametrization of electron gas data for exchange and correlation. As expansion functions Slater-type orbitals (STOs) are used; hence the one-electron density is equal to:

$$\rho = \sum_{ij} R_{ij} \chi_i^* \chi_j \quad (2)$$

The STO $\chi_i$ has the form $r^{k+1}e^{-\alpha r}$. $Y_{lm}(\Omega)$, with varying radial behavior $(k,\alpha)$ and angular quantum numbers $l$ and $m$. $R_{ij}$ is the density matrix in the representation of the basis functions $\chi_i$. The Coulomb potential now equals

$$V^C(\mathbf{r}) = \int \rho(\mathbf{r}')|\mathbf{r} - \mathbf{r}'|^{-1} d\mathbf{r} = \sum_{ij} R_{ij} \int \chi_i^*(\mathbf{r}')\chi_j(\mathbf{r}')|\mathbf{r} - \mathbf{r}'|^{-1} d\mathbf{r}' \quad (3)$$

The integrals on the very right-hand side of Equation (3) are hard to evaluate when $\chi_i$ and $\chi_j$ are located on different atoms. Therefore, a set of fit functions $f_i$ is introduced such that the true density is accurately approximated by a linear combination of them and such that their Coulomb potentials $f_i^C$ can be computed. As fit functions, once again STOs centered on one atom are chosen: $\zeta^C(\mathbf{r}) = Z_{lm}(\Omega) \cdot r^{l+k} \cdot e^{-\alpha r} = Z_{lm}(\Omega) \cdot P(\mathbf{r})$. For this purpose, the already existing integral evaluation—applying incomplete gamma functions—for calculating the expectation value of the Coulomb potential operator was used.[20]

The ADF program further contains an option for freezing

the core. Core states can be calculated from atoms, and by using the frozen core approximation these core states are assumed to remain fixed during the self-consistent field (SCF) calculation on the valence electrons of the molecule. Core electrons contribute to the one-electron density via an additional potential term interpolated from the corresponding atomic potential.

Using this frozen core approximation, the MEP operator equals

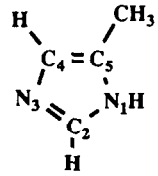
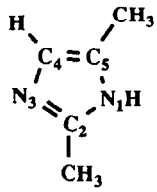
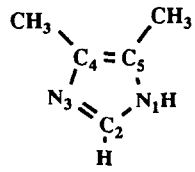
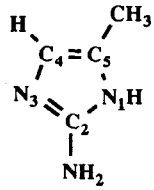
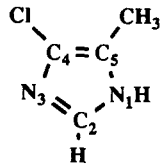
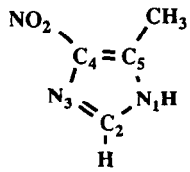
$$V^{\mathrm{MEP}}(\mathbf{r}) = + \sum_{\alpha}^{N_{(\text{all nuclei})}} \frac{Z_{\alpha}^{\mathrm{eff}}}{|\mathbf{R}_{\alpha} - \mathbf{r}|} - \int |\mathbf{r} - \mathbf{r}'|^{-1} \rho_{\mathrm{core}}(\mathbf{r}')\, d\mathbf{r}' - \int |\mathbf{r} - \mathbf{r}'|^{-1} \rho_{\mathrm{val}}(\mathbf{r}')\, d\mathbf{r}' \quad (4)$$

Here $Z_{\alpha}^{\mathrm{eff}}$ equals the number of valence electrons used for atom $\alpha$. For the term with $\rho_{\mathrm{core}}$, the interpolation from the atomic potential is used. For the contribution of the term with $\rho_{\mathrm{val}}$, the same fit procedure is followed as described above for the all-electron calculations.

## RESULTS

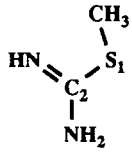
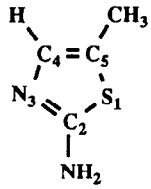
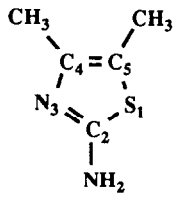
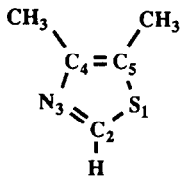
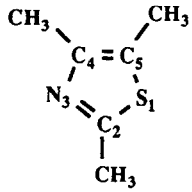
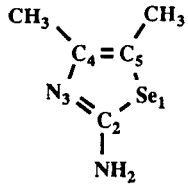
MEP values are calculated for the compounds given in Tables 3–5, i.e., histamine and ring-substituted analogues, dimaprit and its cyclic analogues (thiazoles), and a thiazole analogue in which the sulphur is replaced by a selenium (selenazole). In all calculations the side chain is replaced by a methyl. In total, 12 compounds were considered. After a geometry optimization, MEPs were calculated in the plane of the ring. All calculations were done in a DZD basis set using the Vosko-Wilk-Nussair (VWN) density functional. For the atoms C, N, and O, the 1*s* core was kept frozen. For S, the core was frozen up to the 2*p* orbitals, and for Se, up

**Table 3. Values for MEP minimum in ring substituted histamine analogues[a]**

| No. | Compound | Δ(MIN-$N_3$) | ∠(MIN-$N_3$-$C_2$) | MIN($N_3$) |
|---|---|---|---|---|
| 1 |  | 1.341 | 127.96 | −57.5 |
| 2 |  | 1.362 | 128.98 | −57.5 |
| 3 |  | 1.355 | 125.40 | −56.6 |
| 4 |  | 1.331 | 132.36 | −56.8 |
| 10 |  | 1.393 | 128.96 | −49.2 |
| 11 |  | 1.491 | 138.19 | −47.1 |

[a]The ethylammonium side chain has been replaced by a methyl group. The MEP minimum: MIN(N) (in kcal/mol) located close to the $=N_3-$ atom, is quantified by its distance to the $=N_3-$ atom: Δ(MIN-$N_3$) (in Å), together with the angle between this minimum, atom $=N_3-$, and $=C_2-$: ∠(MIN-$N_3$-$C_2$) (in degrees).

**Table 4. Values for MEP minima of dimaprit, cyclic analogues of dimaprit (thiazoles) and a thiazole derivative (selenazole)[a]**

| No. | Compound | $\Delta$(MIN-$N_3$)/ $\Delta$(MIN-S(e)$_1$) | $\angle$(MIN-$N_3$-$C_2$)/ $\angle$(MIN-S(e)$_1$-$C_2$) | MIN($N_3$)/ MIN(S(e)$_1$) |
|---|---|---|---|---|
| 5 |  | 1.344/ 2.221 | 130.82/ 128.68 | −48.1/ −6.0 |
| 6 |  | 1.373/ 2.669 | 125.86/ 137.48 | −44.5/ +0.0 |
| 7 |  | 1.354/ 2.575 | 125.24/ 138.05 | −43.2/ −1.5 |
| 8 |  | 1.367/ 2.863 | 120.09/ 132.38 | −45.3/ +0.0 |
| 9 |  | 1.379/ 2.608 | 121.48/ 136.57 | −43.2/ −1.5 |
| 12 |  | 1.327 | 125.13 No minimum near Se | −41.8 |

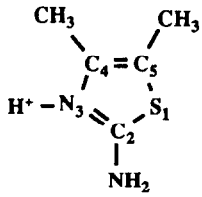
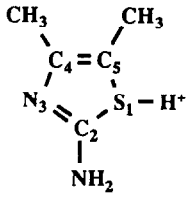
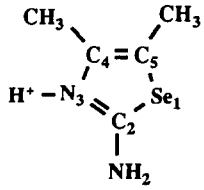
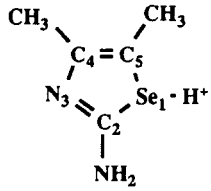
[a]In all compounds the side chain has been replaced by a methyl group. The MEP minimum close to the double bonded nitrogen =N(H)-: MIN($N_3$) (in kcal/mol) is quantified by its distance to $N_3$: $\Delta$(MIN-$N_3$) (in Å) together with the angle between this minimum, $N_3$ and $C_2$: $\angle$(MIN-$N_3$-$C_2$) (in degrees). The minimum close to the sulphur: MIN($S_1$) (in kcal/mol) is quantified by corresponding $\Delta$(MIN-$S_1$) (in Å) and $\angle$(MIN-$S_1$-$C_2$) (in degrees). No minimum was found in the surroundings of $Se_1$.

to 3*p*. The outcome of the geometry optimization on 5-methylimidazole was compared with X-ray diffraction data on histamine dihydrochloride[21] with an R-factor of 0.0340 Å. The largest absolute deviation between calculation and experiment appeared to be 0.052 Å. Thus, the location of the MEP minima can be used for conclusions concerning possible interaction points on the receptor surface.

In Table 3, values for the MEP minima and their positions relative to the double-bonded $N^{\tau}$-atom are given for histamine and a series of ring-substituted analogues. Since we have proposed a (negative) proton-accepting group on the receptor to interact with the proximal $N^{\pi}$-H group, we evaluated the $N^{\pi}$-H tautomers of histamine and its substituted analogues. In Table 4 MEP minima and related data are summarized for methylisothiourea in its most stable conformer **c**, thiazoles, and one selenazole compound. As an illustration the MEP contour diagrams for three histamine $H_2$-agonists are depicted in Figures 5–7 (compounds **1**, **6**, and **12**).

For 2-amino-4,5-dimethylselenazole (**12**) and 2-amino-4,5-dimethylthiazole (**7**) we compared energies of protonation on the nitrogen and on the sulphur c.q. selenium in the ring systems. In Table 5 energy values relative to nitrogen protonation are reported. We can protonate the sulphur or

**Table 5. (VWN) Energies (energy values relative to nitrogen protonation) in kcal/mol for 2-amino-4,5-dimethylthiazole and 2-amino-4,5-dimethylselenazole[a]**

| No. | Compound | Δ | ∠ | τ | Δ(E)[b] |
|---|---|---|---|---|---|
| | | $\Delta(H^+$ and $N_3)$ | $\angle(H^+\text{-}N_3\text{-}C_2)$ | $\tau(H^+\text{-}N_3\text{-}C_2\text{-}S_1)$ | |
| 7 |  | 1.020 | 123.34 | 179.57 | 0 |
| | | $\Delta(H^+$ and $S_1)$ | $\angle(H^+\text{-}S_1\text{-}C_2)$ | $\tau(H^+\text{-}S_1\text{-}C_2\text{-}N_3)$ | |
| 7 |  | 1.343<br>1.366 | 138.99<br>93.92 | 177.55<br>−104.05 | +57.95/<br>+40.37 |
| | | $\Delta(H^+$ and $N_3)$ | $\angle(H^+\text{-}N_3\text{-}C_2)$ | $\tau(H^+\text{-}N_3\text{-}C_2\text{-}Se_1)$ | |
| 12 |  | 1.034 | 121.48 | 179.50 | 0 |
| | | $\Delta(H^+$ and $Se_1)$ | $\angle(H^+\text{-}Se_1\text{-}C_2)$ | $\tau(H^+\text{-}Se_1\text{-}C_2\text{-}N_3)$ | |
| 12 |  | 1.448<br>1.464 | 155.68<br>94.19 | 171.15<br>−101.02 | +64.35/<br>+42.94 |

[a]The position of the proton relative to the site of protonation is quantified by the distance to this side, i.e., $\Delta(H^+\text{-}N_3)$ or $\Delta(H^+\text{-}S(e)_1)$, the angle $\angle(H^+\text{-}N_3\text{-}C_2)$ or $\angle(H^+\text{-}S(e)_1\text{-}C_2)$, and the dihedral angle $\tau(H^+\text{-}N_3\text{-}C_2\text{-}S(e)_1)$ or $\tau(H^+\text{-}N_3\text{-}C_2\text{-}S(e)_1)$.
[b]S(e)-protonation has been calculated in two ways: a protonation in the plane of the ring (dihedral angle ≈ 180°) and a protonation below (or above) the ring (dihedral angle ≈ −90°) in the direction of a lone pair of S(e).

selenium either in the plane of the ring, like an imidazole would be protonated, or in the direction of a lone pair. Adding a proton in the plane of the ring and performing a geometry optimization results in a local energy minimum; energy values relative to N-protonation are given in Table 5 (upper *E* value). Protonation on the nitrogen is favored by 58 kcal/mol for compound **7** and by 64 kcal/mol for compound **12**. More likely protonation of a sulphur or selenium atom in these ring systems will occur in the direction of a free electron pair. Therefore, we also calculated a protonation in the direction of the lone pair at a dihedral angle of about 90° out of the ring plane (lower *E* values, Table 5). Then nitrogen protonation is favored by 40 kcal/mol for compound **7** and 43 kcal/mol for compound **12**.

## DISCUSSION

### Validation of the proposed model

Examining the MEP data in Tables 3 and 4 and comparing the contour diagrams in Figures 5–7 in a qualitative way, we conclude that the sulphur atom in the investigated compounds has more the character of an —NH— group in an imidazole ring than of an =N—. For the selenium atom in compound **12** (Table 4, Figure 7) this is even more obvious. The sulphur or selenium atoms are located in the proximal position with regard to the 2-aminoethyl side chain. If the S-fit conformation of dimaprit and cyclic analogues is still assumed to be valid,[4–6] these results confirm the $N^{\pi}$-H tautomer of histamine to be active at the histamine $H_2$-receptor. The (negatively charged) proton accepting group on the receptor surface therefore interacts with the entity in the proximal position towards the 2-aminoethyl side chain, whereas in the distal ($N^{\tau}$) position a proton can be accepted. As all considerations used to derive the bioactive conformation of dimaprit and thiazole derivatives are still valid, these data validate the newly proposed model for the agonistic binding site on the histamine $H_2$-receptor and its mechanism of activation. This refined model explains the pharmacological behavior of all known histamine $H_2$-agonists. In the following sections the $H_2$-agonistic behavior of several com-

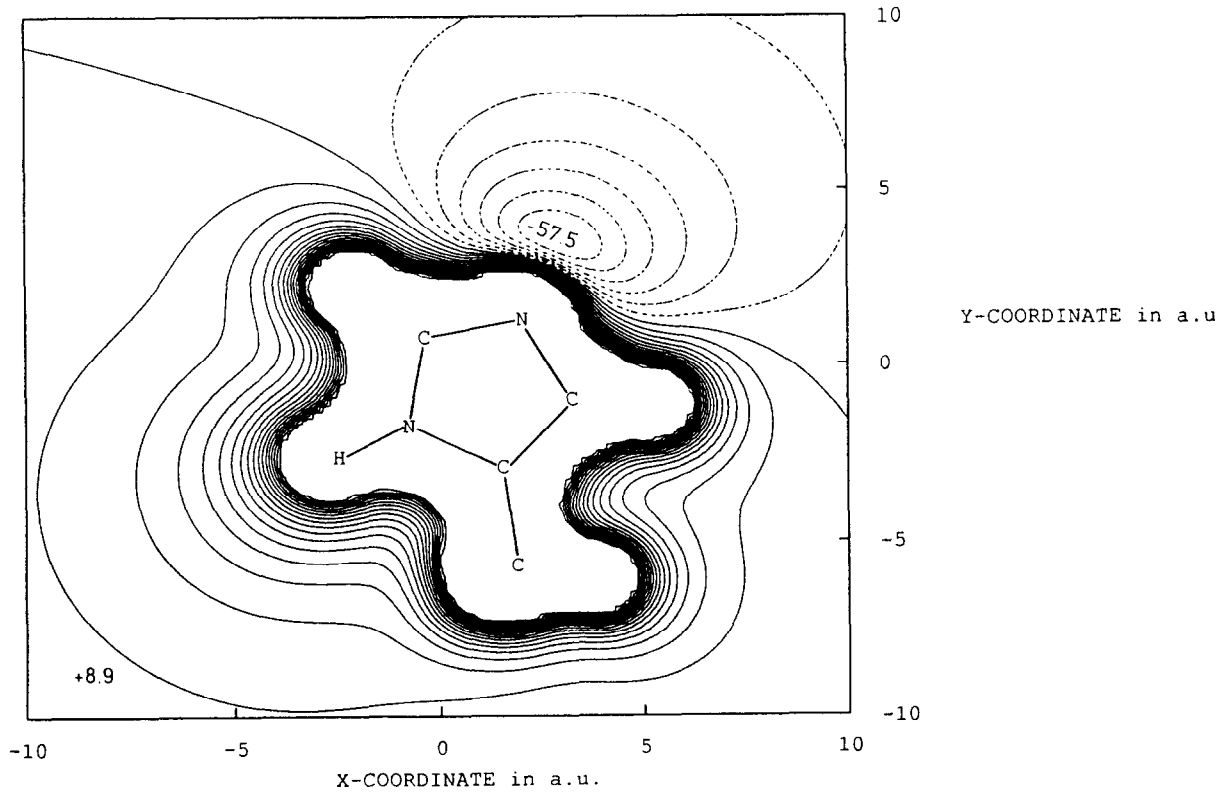



*Figure 5. MEP contours for 5-methylimidazole ($N^{\pi}$-H tautomer, Compound **1** in Table 3); the lowest MEP value is −57.5 kcal/mol. The contour line increment is 8.30 kcal/mol, and no contours drawn above +150.0 kcal/mol. Hydrogens are not drawn except for the $N^{\pi}$-H hydrogen. Negative values are indicated by dashed lines, positive values by solid lines.*

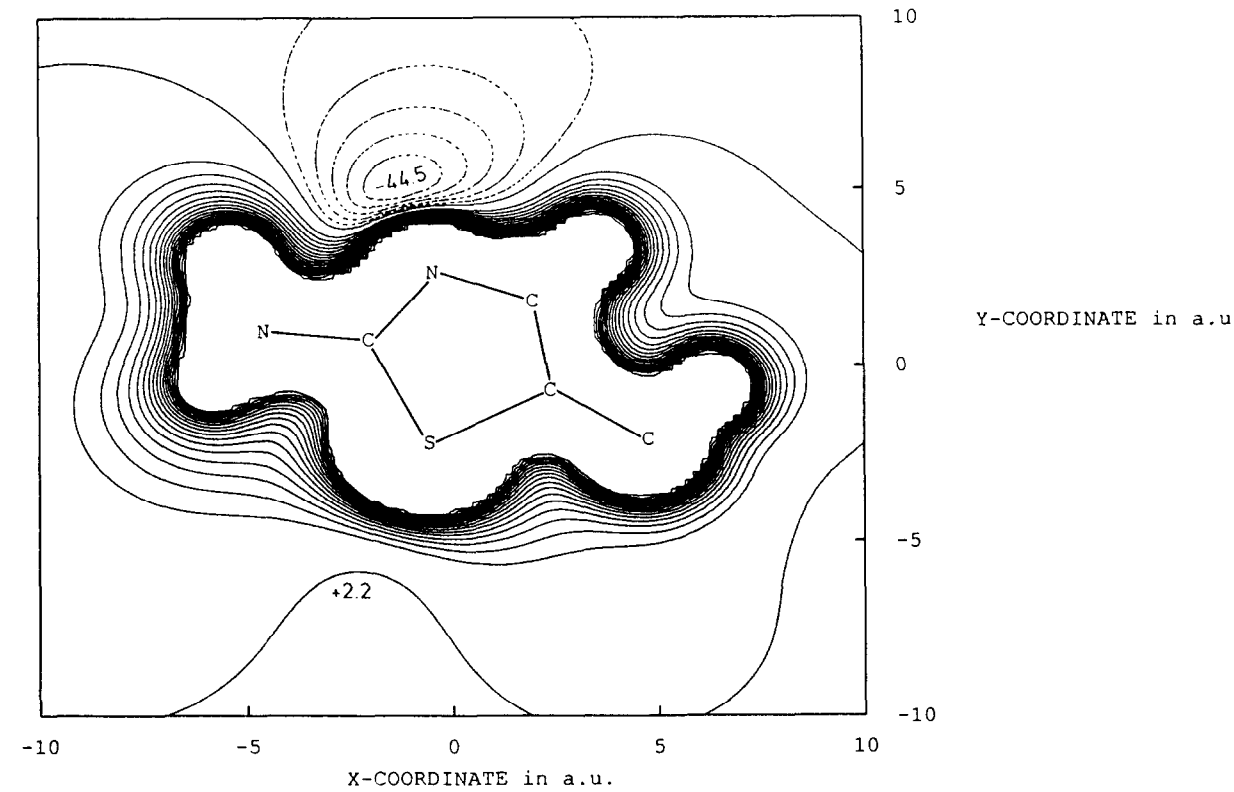



*Figure 6. MEP contours for 2-amino-5-methylthiazole (Compound **6**, Table 4); the lowest MEP value is −44.5 kcal/mol. The contour line increment is 7.78 kcal/mol, and no contours drawn above +150.0 kcal/mol. For further details see Figure 5.*

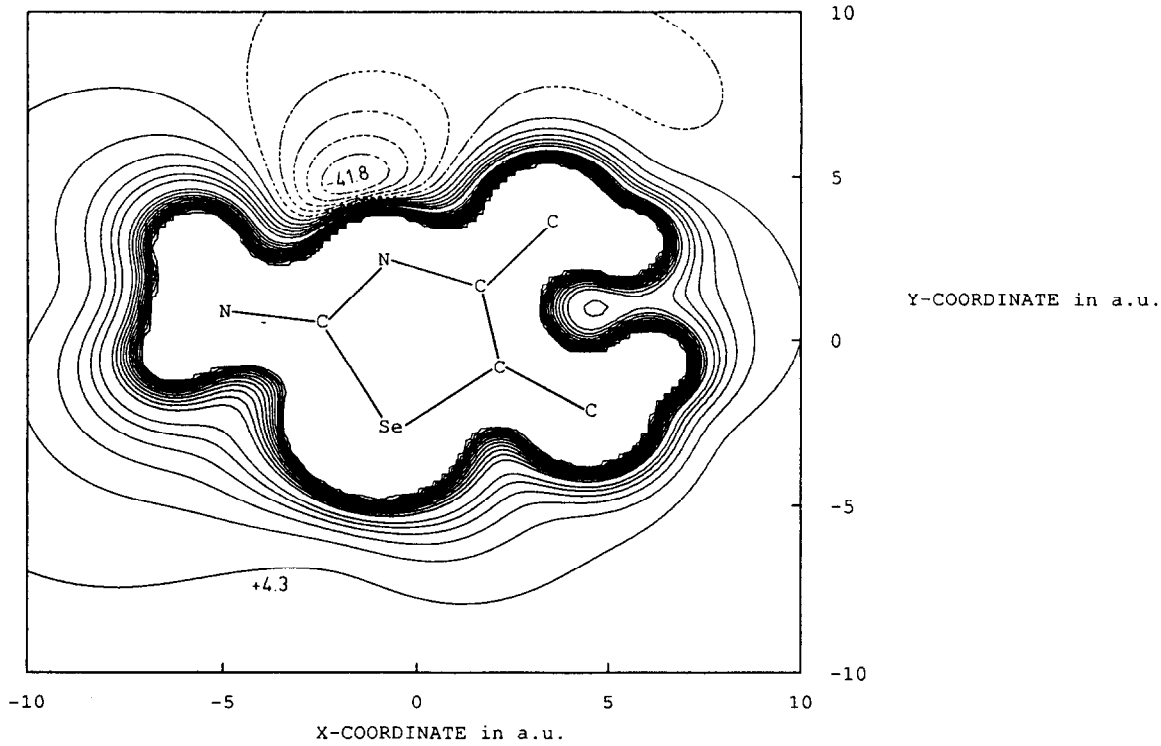



*Figure 7. MEP contours for 2-amino-4,5-dimethylselenazole (Compound 12, Table 4); the lowest MEP value is −41.8 kcal/mol. The contour line increment is 7.67 kcal/mol, and no contours drawn above + 150.0 kcal/mol. For further details see Figure 5.*

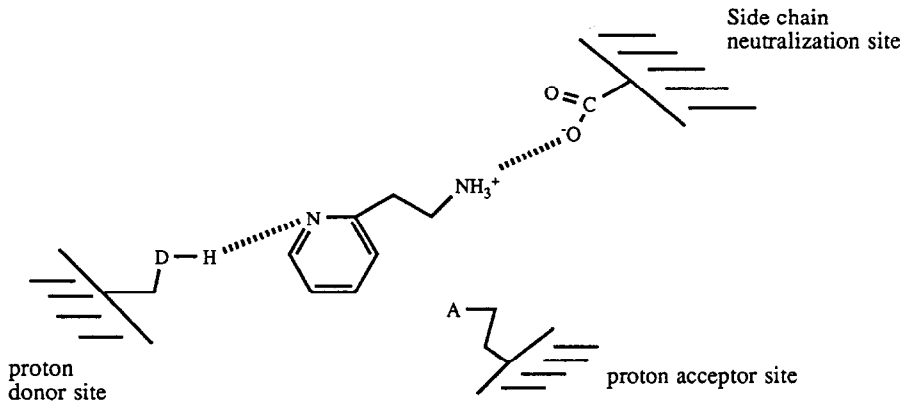



*Figure 8. Draft of the interaction of β-histine with the $H_2$-receptor surface.*

pounds, which previously could not be explained in a satisfactory way, is discussed with reference to the newly proposed model. At the same time, the several points of criticism mentioned earlier will be discussed again in the light of the revised model.

## The $H_2$-receptor agonist β-histine

In the newly proposed model, a proton can be accepted at the tele position relative to the side chain. For dimaprit, thiazole and selenazole compounds, the acceptance of this proton is sufficient to ensure $H_2$-agonistic activity, although these compounds do not participate in a tautomeric shift. The positive charge accepted from the receptor at the tele position is stabilized with the aid of the (negatively charged) proton-accepting group on the receptor surface in the proximal position. β-histine is also able to accept a proton, but the position of the basic nitrogen in the pyridine ring differs from the $=N^{\tau}$- position in an imidazole ring system. The presumed proton-donating group on the receptor surface[16] ($Thr^{190}$) is attached via a flexible side chain to the protein backbone. The proton-donating group is therefore not fixed in space, and is probably able to interact with the basic nitrogen in the pyridine ring to which the receptor can donate its proton. However, the pyridine ring system will not fit perfectly in the $H_2$-receptor agonistic binding site (Figure 8), which explains its moderate activity.

## MEP values of dimaprit

Our MEP values (Table 4) for the dimaprit model compound (methylisothiourea) in its conformer **c** are in agreement with the suspected relative proton-accepting properties of the sulphur and the double bonded nitrogen atom and with previously published HF values.[7] In our revised model the double bonded nitrogen atom, which is the most electronegative, will accept a proton from the receptor.

## The activities of 4-chloro-, and 4-nitrohistamine explained

Upon introducing the electronwithdrawing groups Cl and $NO_2$ (Table 3: **10**, **11**) the electron density near the =N- in the imidazole ring system strongly decreases (MEP value decreases by ~ 10 kcal/mol with respect to histamine). 4-Chlorohistamine and 4-nitrohistamine are therefore less able to accept a proton from the receptor and will be less biologically active. The strongly reduced activities of these compounds do not necessarily have to be attributed to decreasing amounts of the previously assumed biologically active $N^{\tau}$-H tautomer.

## Reduced $H_2$-activity of 4-methylhistamine with regard to histamine

As mentioned previously, the reduced histamine $H_2$-activity of 4-methylhistamine can only be explained by an enhanced tendency of 4-methylhistamine to form an intramolecular H-bond and the resulting effect on the equilibrium between $N^{\pi}$-H and $N^{\tau}$-H tautomers in imidazole containing molecules. As demonstrated in Table 3, histamine (**1**) and 4-methylhistamine (**3**) have comparable MEP values in the surroundings of the $=N^{\tau}$- nitrogen. The electron-releasing effect of a methyl cannot be revealed by just looking at a MEP minimum in the plane of the imidazole ring. Therefore we have used these MEP values only to obtain a qualitative picture of the histamine $H_2$-agonistic binding site.

## The importance of the first proton transfer step: evidence for the intramolecular hydrogen bond

In the previous section, it was implicitly assumed that the increased $pK_a$ of the imidazole ring in 4-methylhistamine (and of other histamine analogues with electron-releasing substituents, Table 2[7]) affects only the proton transfer from the receptor toward the agonist; i.e., acceptance of a proton is facilitated and influences $H_2$-activity, whereas the negative effect on the release of a proton from the agonist toward the receptor is not of importance. That, indeed, only the proton transfer from the receptor toward the agonist is essential for displaying $H_2$-activity is demonstrated in a series of substituted thiazole analogues (Table 2[7]). These compounds are able to accept a proton only at the double-bonded nitrogen. It is evident that the agonistic activity in this series is related to the nature of the substituent groups. For the thiazole series, $H_2$-activity can be enhanced upon introducing electron-releasing groups in the 2- or 4-position, as can be seen when the compounds **6**, **7**, and **8** in Table 2 are compared. Compound **7** is substituted with two electron-releasing groups, and has the best $H_2$-binding and -activating properties. Since these compounds cannot form intramolecular H-bonds and do not participate in a tautomeric equilibrium, these complicating factors are eliminated. Electron-releasing groups enhance the ability to accept a proton ($pK_a$ increase), and potency increases. The data obtained from the thiazole series therefore stress the importance of the first proton transfer, and indicate that the assumption used in the case of 4-methylhistamine and of other substituted histamine analogues with electron-releasing groups that the second proton step is not important for determining activity, is valid. This underlines the important conclusion that the reduced activity of substituted histamine analogues with sterically allowed electron releasing groups (e.g., of 4-methylhistamine) can only be explained by enhanced intramolecular H-bond formation, implicitly resulting in less available monocationic $N^{\pi}$-H species. Once again, the electron releasing properties of a methyl or amino group are not reproduced in a quantitative way by the MEP minima in the plane of the thiazole ring.

In our model the lack of activity of $\tau$-methylhistamine can easily be explained. This compound lacks an essential property for displaying $H_2$-activity, for it cannot accept a proton from the receptor surface in the distal position of the imidazole ring. Also this compound points out the importance of the first proton transfer step.

## The agonistic activity of a selenazole compound versus the comparable thiazole

The MEP data for the model compounds of 2-amino-4-methyl-5-(2-aminoethyl)thiazole (compound **7**, Table 4) and 2-amino-4-methyl-5-(2-aminoethyl)selenazole (compound **12**, Table 4) validate our model: the MEP values close to the selenium atom in compound **12** are positive, whereas a weak potential minimum (−1.5 kcal/mol) is present close to the sulphur in compound **7**. For compounds **7** and **12**, protonation energies have been calculated to support our conclusions (Table 5). Indeed, protonation of the =N- in the selenazole compared to protonation on Se is favored by 43 kcal/mol, while protonation of =N- in the thiazole relative to S protonation is favored only by 40 kcal/mol. For both S and Se, the energies for protonation in the direction of a lone pair is taken. The data indicate that for both compounds protonation most likely will occur at the double-bonded nitrogen, whereas the selenazole is expected to have a higher $H_2$-agonistic activity, as is found experimentally: the Se atom is more difficult to protonate when compared to S (2.57 kcal/mol) and will therefore have a more optimal interaction with a (negatively charged) proton-accepting receptor site; moreover, selenium can be better polarized (*vide infra*).

## Deletion model Topiol

With respect to the origin of receptors Topiol[22] has suggested a so-called deletion model, in which receptors together with their complementary ligands are considered to be derived from a common parent system. The deletion model can be arrived at by taking the function of a complete dynamical biochemical system composed of a collection of entities necessary for its functioning. Removal or absence of

one such an entity ($\alpha_i$) generates an inactive subsystem. Thus, $\alpha_i$ and the inactive subsystem together are proposed to constitute a ligand–receptor pair which together can form the active system. The relationships of endogenous ligands as histamine and serotonine to the biological building blocks such as histidine and tryptophan thus follows directly from this deletion model. Our newly proposed model for the histamine $H_2$-agonistic binding site strongly resembles this active system in which the amino acid histidine should be replaced by the endogenous ligand histamine (compare Figures 3 and 9). Our model also strongly resembles the catalytic triad in serine proteases. This suggestion has already been presented in literature previously,[22] although the author incorrectly uses Weinstein's data to validate his hypothesis: in Weinstein's calculations moiety $\alpha_1$ and $\alpha_3$ are interchanged.[3]

In the active form of the system depicted in Figure 9 a proton-relay process occurs whereby a proton is transferred from donor $\alpha_3$ (SER in a serine protease, THR in the $H_2$-receptor) to the $N^{\tau}$-atom of a histidine. The histidine (or histamine) in turn transfers a proton from the $N^{\pi}$-H group to acceptor $\alpha_1$ (ASP in serine proteases and $H_2$-receptor). Topiol[22] assumes the process to be modulated by the indole portion of a nearby tryptophan. The tryptophan forms a stacking complex with the imidazole ring of the histidine (histamine), as is indicated by experiment.[23] Moreover, calculations have been carried out on an imidazolium in the presence of 5-hydroxytryptomine (5-HT, serotonine).[24] On forming such a stacking complex, one of the nitrogens of the imidazolium became more strongly polarized by 5-HT than the other. This indicates that the proton affinity of one of the nitrogens could be changed. If this nitrogen is involved in a hydrogen bond, the change in proton affinity could trigger a proton transfer from the imidazolium to a proton acceptor: the 5-HT molecule (or tryptophan) alters the relative energy of the initial and the final states of the proton transfer process.

Summarizing, in agreement with suggestions in literature,[22] the catalytic triad in the serine proteases can function as a model system for the $H_2$-receptor.

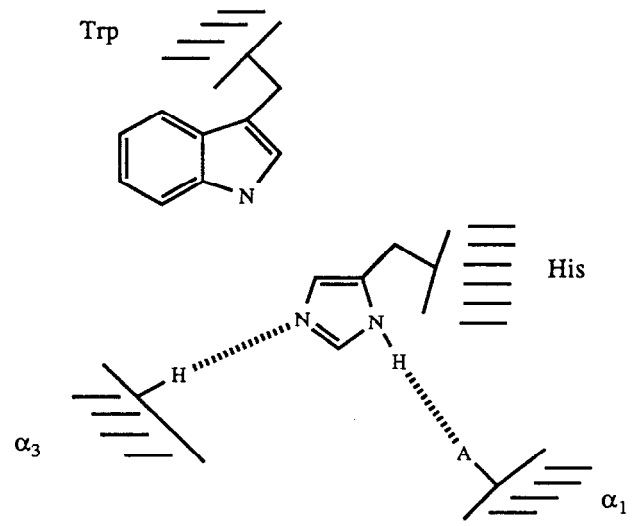



*Figure 9. Critical portion of an active system (in casu a part of a serine protease) as suggested by Topiol.*[22]

## Activation mechanism

The new model for the $H_2$-agonistic binding site already exists in nature. The $H_2$-receptor can be activated in a similar way as the serine proteases where the first step consists of a proton acceptation in the distal ($N^{\tau}$) position (from the $\alpha_3$ moiety in Figure 9). The possible nearby presence of an indole portion of a tryptophan could lower the barrier of activation for the first proton transfer step from the receptor to the $=N^{\tau}$- position of the ligand. The resulting $N^{\tau}$-H form might be stabilized by the receptor, whereas in the original model[3,11] the shift in tautomeric preference from the $N^{\tau}$-H to the $N^{\pi}$-H upon neutralization of the side chain was assumed to drive and accelerate the process.

## Future calculations

As no simple quantitative relationship can be established between the most negative MEP values and $H_2$-receptor activity, our future investigations will concern proton-relay studies within our newly proposed model. Polarization effects due to the presence of a negatively charged group $\alpha_1$ (Figure 9) can then be taken into account (*vide supra*), which is impossible for isolated compounds alone. Moreover X-ray structures for the catalytic triad[25] are available, which provide a good starting point for studying the histamine $H_2$-agonistic binding site and its accessory activation mechanism.

## Conclusions

From MEP values calculated in the plane of the molecule for a series of histamine $H_2$-agonists we conclude that a sulphur in dimaprit and thiazoles or a selenium atom in a selenazole compound have more the character of an —NH- group in an imidazole than that of an =N-, whereas the basic nitrogen in these compounds shows great resemblance to the =N- in an imidazole ring. The sulphur or selenium atoms are located in the proximal position with regard to the side chain. As the S-fit conformation of dimaprit and cyclic analogues is still considered to be valid based on geometrical considerations, this conclusion confirms the $N^{\pi}$-H tautomer of histamine to be active on the $H_2$-receptor. In serine proteases the histidine in the catalytic triad is also found in its $N^{\pi}$-H tautomer, which supports Topiol's deletion model. In the original Ganellin/Weinstein model the negative effect of substituents has been related to a decreasing $N^{\tau}/N^{\pi}$ ratio or percentage monocation. In our model, this decrease in activity is either thought to be due to a decreased $pK_{a,Im}$ value (electron-withdrawing substituents) or enhanced intramolecular H-bond formation in combination with an increased $pK_{a,Im}$ value and $N^{\tau}/N^{\pi}$ ratio (electron-donating substituents). Furthermore, we are able to explain the moderate activity of β-histine on the $H_2$-receptor, which was not possible in the original model.

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