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Conformational analyses on histamine H_2-receptor antagonists

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SUMMARY

In a series of compounds with H_2-antihistaminic activity, a conformational analysis was performed based on force field calculations. The drugs studied were cimetidine, ranitidine, famotidine, roxatidine and the conformationally more restricted ICI127032. For the compounds containing a flexible chain, the local minima conformations and the global minimum conformation were calculated. These conformations were used for a systematic structural comparison with all energetically allowed conformations of the ICI derivative, with regard to the best fit of the common structural features. In this way a pharmacophore could be developed consisting of four parts: (1) a polar planar group, uncharged at physiological pH; (2) a hydrophobic part formed by aromatic systems or flexible chains; (3) an – under physiological conditions – protonated nitrogen atom; and (4) a substructure, which contains a hydrogen bond donor site and a hydrogen bond acceptor site in a specific spatial arrangement.

INTRODUCTION

The discovery of histamine H_2-receptor antagonists by Black et al. in 1972 [1] opened a new era in the treatment of peptic ulcer and other diseases connected with gastric acid hypersecretion. The basis for this treatment is the concept that blockade of H_2-receptors causes, beside other effects, an inhibition of gastric acid secretion.

Several papers have appeared showing that the H_2-receptor is coupled to an adenylate cyclase system and that the elevated c-AMP level is responsible for the acid secretion, relaxation of, for example, the uterus, and cardiac effects. Topiol suggested the 5-HT_2-receptor and the H_2-receptor to have a similar origin [2]. Donné-Op den Kelder et al. proposed a similar activation mechanism for both receptors [3]. Thus it is probable that the H_2-receptor operates in a way comparable to the 5-HT_2-receptor or other G-protein-coupled membrane receptors.

The actually known H_2-receptor antagonists have a considerable diversity of chemical struc-

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tures but they all appear to contain at least one aromatic system and one planar π-electron system which is polar and has a propensity towards hydrogen-bonding. These two substructures are connected by another aromatic system, which generates diaryl structures (e.g. ICI127032), or by a flexible chain.

With respect to the aromatic ring system H_2-antagonists with a flexible chain can be divided into four main series, the imidazole (cimetidine), aminomethylfurane (ranitidine), guanidinothiazole (famotidine) and piperidinomethylphenoxy (roxatidine) series (see Fig. 1) [4].

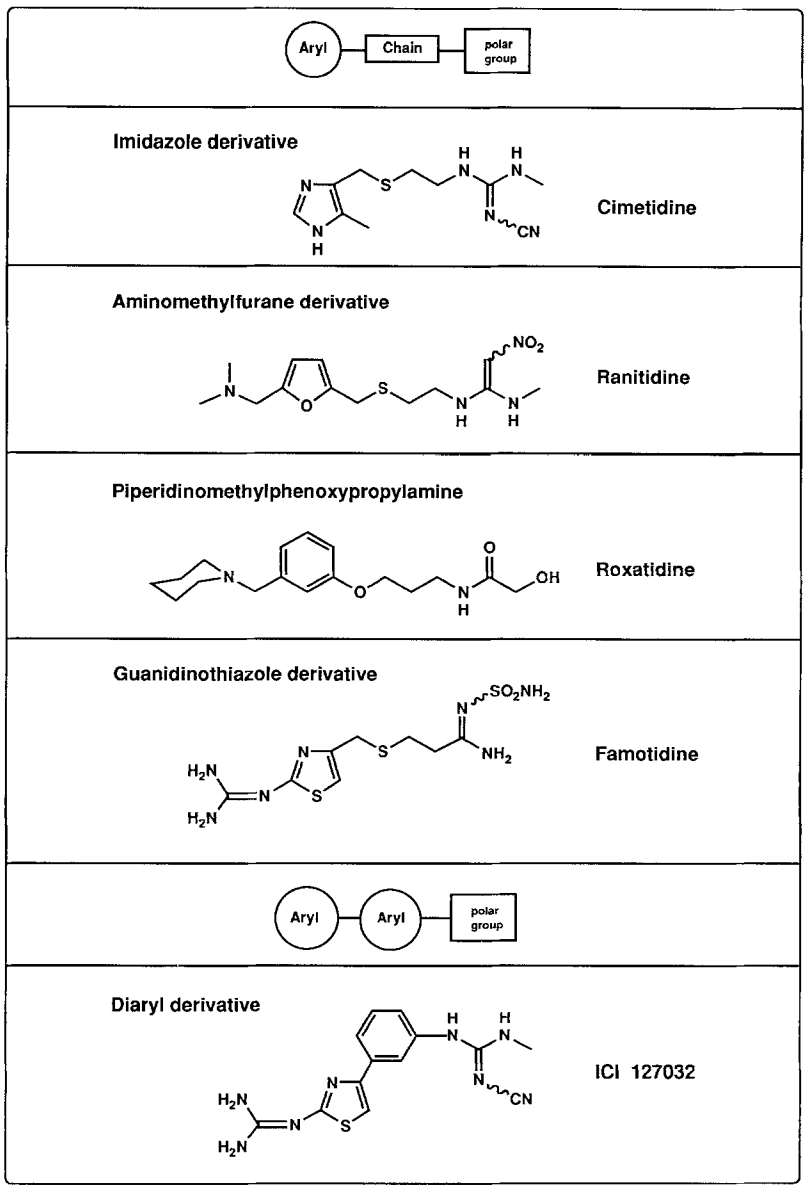


Fig. 1. Structures of the investigated H_2-receptor antagonists.

Because of the large number of conformers which can be expected in case of several rotational axes, it is necessary in order to be able to find an active conformation for H_2-antagonists with a flexible chain to consider more rigid but nevertheless active H_2-receptor antagonists. ICI127032 possessing a diaryl structure answers these demands. Therefore the first step of the work presented here was to calculate the conformational properties of this derivative and subsequently search for energetically allowed conformations of the more flexible structures fitting one of the ICI127032 conformations.

METHODS

1. General procedure

The molecular structures of cimetidine, ranitidine, roxatidine, famotidine, and ICI127032 were constructed on an Evans & Sutherland PS 390 applying the molecular modeling package SYBYL 5.1 (TRIPOS Associates Inc., St. Louis, MO, U.S.A.) and the standard geometries implemented in this program. The geometries of substructures consisting of conjugated double-bond systems were calculated with the semiempirical quantum chemistry program AM1. To verify the results the geometries obtained were compared with the corresponding parts of the crystal structures.

Using the SEARCH option within SYBYL 5.1 a systematical conformational search for sterically allowed low-energy conformations was performed.

SEARCH is a conformational analysis method based on the screening of van der Waals' contact distances. Conformations with interatomic distances shorter than the sum of the respective van der Waals' radii are rejected. As rotational increment a step size of 30° or 10° was chosen. Conformations with an energy more than 21 kJ/mol (5 kcal/mol) above absolute energy minimum were discarded.

Despite these restrictions the list of allowed conformations was too large. Therefore this list was subdivided into similarity groups and the lowest energy conformations of each group were determined. For this purpose the program GROSCON was used, which is explicitly described in a recent publication [5]. Conformations found by GROSCON are situated in a local energy minimum. The program STAR [6] served to visualize positions of defined structural elements found by SEARCH.

Using MAXIMIN, a molecular mechanics minimization program in the SYBYL 5.1 package, minimum energy geometries of the GROSCON conformations were obtained. MAXIMIN performs bond length and valence angle minimization simultaneously with slight variations of the torsional angles yielding a minimum energy geometry of a certain basic conformer.

All calculations and molecular manipulations were carried out on the Evans & Sutherland PS 390 graphics system and MicroVAX II as well as MicroVAX 2000 microcomputers at the Pharmaceutical Institute of the Free University of Berlin.

2. Conformational analysis of ICI127032

The diaryl structure of ICI127032 was analyzed with an increment of 10°. The energy of the conformations obtained is a function of two important torsion angles, τ 1 and τ 2, respectively (see Fig. 2).

The phenyl ring, which connects the guanidinothiazole moiety to the cyanoguanidine, will influence the geometry and the electronic properties of these groups only to some degree. Therefore

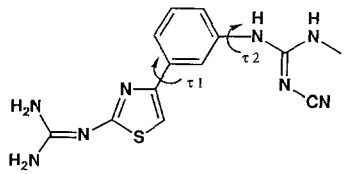


Fig. 2. Structure of ICI127032 with two important torsion angles marked as τ 1 and τ 2.

three substructures I, II and III were used to study the conformational behavior of ICI127032 (see Fig. 3). All parts were calculated by AM1, because the SYBYL force field is not able to treat conjugated double-bond systems.

3. Conformational analysis of H_2-antagonists containing a flexible chain

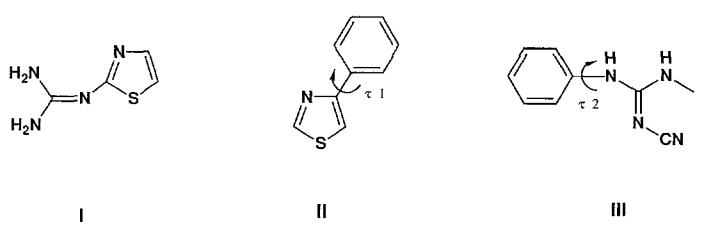
The polar planar endgroups, which contain double bonds, were calculated by AM1. The results were found to be in accordance with corresponding crystal structures. Subsequently the endgroups were defined as aggregates to prevent them from being affected by the SYBYL force field. So SEARCH only treated the five remaining rotatable bonds.

After calculating all energetically allowed conformations the corresponding GROSCON conformations were determined. Each GROSCON conformation was used as a basic conformation for MAXIMIN. The lowest energy conformation found by this procedure is supposed to be the global minimum conformation. The energy value of this conformer serves as reference value for the estimation of conformers obtained by the multifit comparison (see the next section). With the help of STAR those GROSCON conformations were chosen in which the polar planar endgroups and the aromatic systems possess similar positions with respect to the ICI derivative.

4. The multifit procedure

The SYBYL multifit procedure is a minimization method similar to MAXIMIN. It can be used in two alternative ways: a set of flexible molecules can be mapped onto a rigid reference compound, or all the molecules are treated as flexible entities and the treatment is directed towards the minimization of the whole set.

In the case reported here, it was obvious to choose ICI127032 as the rigid reference compound, whereas bonds and angles of all other congeners were allowed to move. However, the polar endgroups were defined as aggregates (see section 1). The minimization was directed on one hand towards the geometry-optimization of all flexible molecules and simultaneously towards the best fit



I II III

Fig. 3. Substructures of ICI127032 used for investigations.

with the rigid ICI127032. Thus the energy is not only a function of the atomic coordinates but also of the spring tension between the points, which are to be superimposed.

The multifit option finds only the local energy minimum next to the starting point and therefore each single energetically allowed ICI127032 conformation was used as a basic reference conformation in a multifit run.

5. Potentials

The molecular electrostatic potentials were calculated using the potential option of SYBYL on the basis of point charges calculated by AM1.

A positive test charge placed near the molecule will be attracted or rejected, depending on the character of the molecular potential in the pertinent space segment. The resulting electrostatic potential was calculated for every point of a grid with an interval of 1 Å.

RESULTS AND DISCUSSION

The guanidinothiazole substructure of ICI127032 was calculated by the semiempirical quantum chemistry program AM1.

The tautomer with the double bond in conjugation with the thiazole ring is the most stable form. The position of the guanidino part is syn with respect to the nitrogen atom of the thiazole. This is in accordance with the studies by Donetti et al. [7] and with previous work based on *p*Ka studies [8].

Molecular mechanics calculations demonstrate the presence of a hydrogen-bridge between one of the guanidino NH_2-groups and the thiazole nitrogen. The crystal structure of tiotidine [8] shows this bridge as well. The N-inversion is of minor importance because of the high inversion barrier [7].

From these considerations it follows that the guanidino group and the thiazole may be considered essentially coplanar.

The preferred value for the torsional angle τ 2 (see Fig. 2) is 60°. The position of the center of the polar endgroup is almost not influenced by the value of this torsion angle, so it was fixed at 60°.

The rotation around τ 1 is of most importance: it determines the orientation of the aromatic system in relation to the polar planar endgroup. Molecular mechanics calculations show two energy minima, one for τ 1 = 0° and one for τ 1 = 180°. With AM1 four energy minima were found: τ 1 = 30°, τ 1 = 150° and, correspondingly, τ 1 = 210° and τ 1 = 330°. As can be seen in Fig. 4 only slight energy differences occur during a full 360° rotation. For this reason all conformations within the 21 kJ (5 kcal) range, i.e., ICI127032 conformations with τ 1 values from 0° to 60°, 120° to 180° and, correspondingly, 180° to 240° and 300° to 360° were used for further investigations.

The preliminary visual inspection of the compounds with a flexible chain was supported by STAR. In Fig. 5 the results for famotidine are presented: every cross represents an energetically allowed position of the center of the polar planar endgroup. The magenta-colored crosses belong to conformations used for the multifit comparison.

During the multifit procedure the conformations of the flexible molecules were allowed to change in order to fit the common structural features of ICI127032, whereas the ICI derivative in its templet function was defined as an aggregate. The variable spring constant which causes the con-

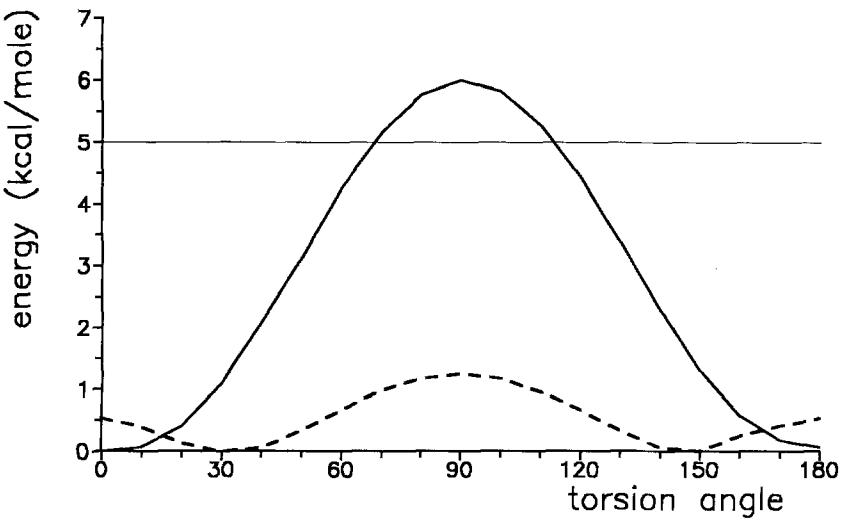


Fig. 4. Conformational energy of 4-phenyl-^{1}H-imidazole as a function of τ 1. The global energy minimum is taken as energy zero. The dashed line corresponds to the AM1 calculation, the solid line to the molecular mechanics calculation. The energy cut-off is marked at 21 kJ (5 kcal)/mol.

formational change was set to 84 kJ (20 kcal)/Å^2. Based on the constant chosen, the force exerted on the flexible molecules is comparable to the force of a hydrogen-bond recognition between a receptor and its ligand. Thus the multifit comparison can be considered as the simulation of possible conformational changes of ligands when approaching the receptor.

Each visually chosen conformation was compared with the 26 allowed conformations of ICI127032. On each compound under study about 260 multifit calculations were performed. The procedure was automatized using collect files.

Figure 6 shows the results for the best fitting conformations of famotidine, cimetidine, roxatidine, and ranitidine. For the ICI127032 conformations with τ 1 = 160° and τ 1 = 340° the multifit conformations of each compound under study are situated in a local energy minimum. During this multifit procedure none of the two ICI127032 conformers was especially favoured.

Gilman et al. [8] analyzed some conformationally more restricted tiotidine analogues (see Fig. 7). Only compound II has considerable H_2-antagonistic activity. Based on these experimental data the ICI127032 conformation with τ 1 = 160° can be supported. The conformations yielded by this procedure neither correspond to the calculated global minimum conformation nor to the crystal structures – they are more elongated. From the literature it is known that G-protein-coupled membrane receptors, e.g., the adrenergic β-receptor, the cholinergic muscarine receptor and the 5-HT_2-receptor consist of 7 α-helices forming a hydrophilic channel through the membrane [9]. The H_2-receptor belongs to the same class of membrane receptors and is considered to operate in a similar way as the receptors mentioned above. The H_2-receptor probably forms a similar channel. More elongated conformations as presented here would therefore more easily cross such a channel.

Figure 8 shows that all H_2-antagonists are able to meet the same relative spatial arrangement

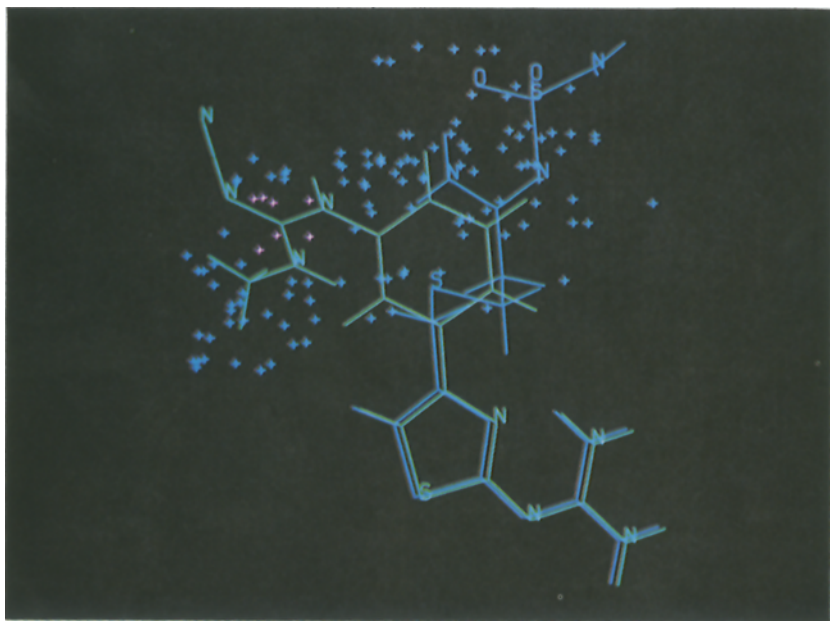


Fig. 5. Superposition of ICI127032 (cyan) and famotidine (blue). The possible conformations of famotidine are displayed by crosses representing the center of the polar planar endgroup of famotidine. The magenta-colored crosses represent the conformations which were used for the multifit comparison.

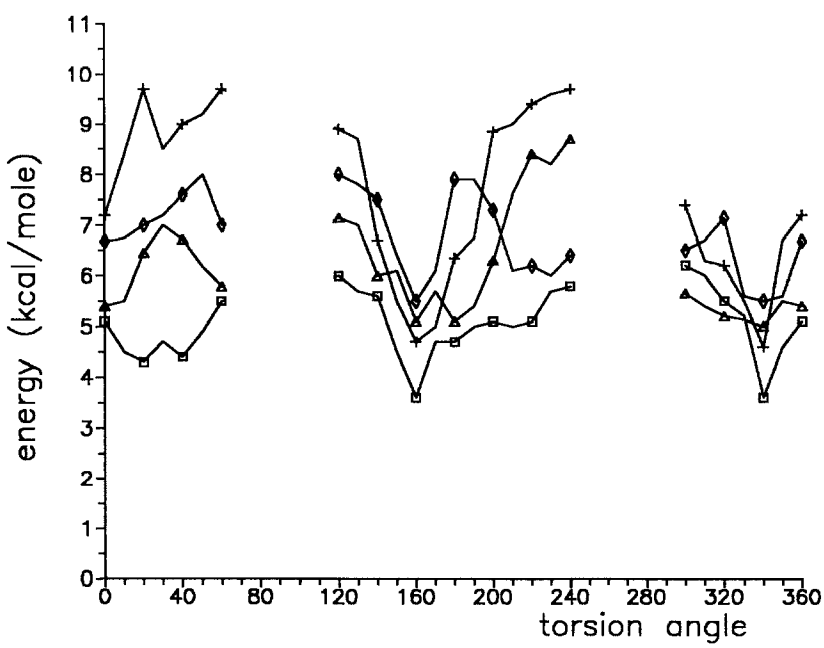
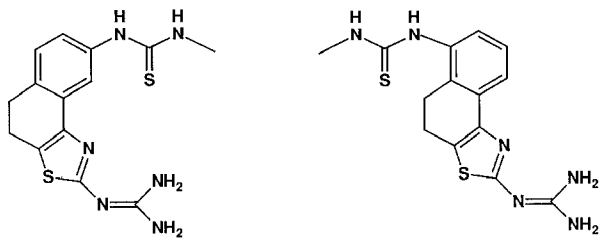


Fig. 6. Structural comparison of cimetidine (rhombus), ranitidine (triangle), roxatidine (cross), and famotidine (square). The base conformation of ICI127032 is characterized by τ 1 (x-axis)., The y-axis presents the difference between the energy of the resulting multifit-conformations and the calculated global energy minima of each compound, which are taken as energy zero.



I II

Fig. 7. Conformationally restricted tiotidine analogues.

of the polar planar endgroup and the central hydrophobic part of the molecule, which is formed by a flexible chain or aromatic systems. However, there is no agreement between the nitrogen substituents of the different heterocyclic systems. Ranitidine and roxatidine which possess a nitrogen atom protonated under physiological conditions fit neither one of the nitrogens of the guanidino part of famotidine nor one of the nitrogen atoms of the imidazole of cimetidine. This means it was not possible to superimpose these atoms. Therefore, from the conformational point of view, H_2-antagonists cannot meet the same binding site in this domain of the molecule.

This result is supported by consideration of the electronic properties in this region. The potentials of the guanidinothiazole and of the imidazole are shown in Figs. 9 and 10. Because the interaction with the H_2-receptor is of the competitive type, the antagonists under study should occupy

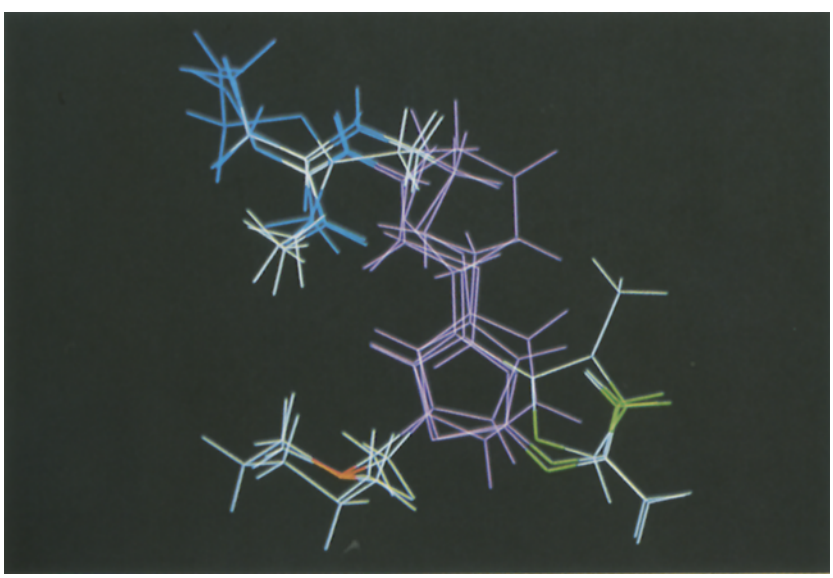


Fig. 8. Superposition of all conformations obtained by the multifit comparison. The hydrophobic aromatic systems or flexible chains are violet; the electron-rich centers of the polar planar endgroups are blue; the nitrogen substituents, which probably form hydrogen bonds, are green; and the protonated nitrogens are red.

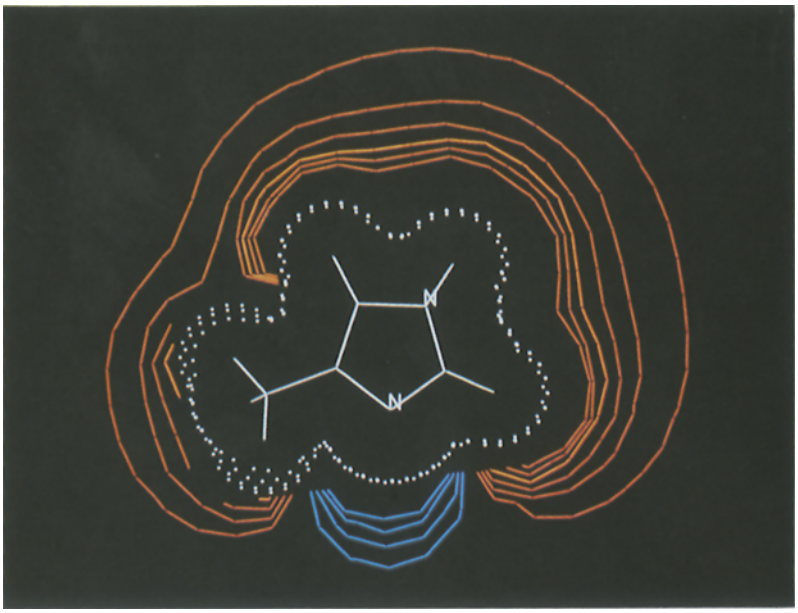


Fig. 9. Electrostatic potential of imidazole. The red isopotential lines represent values between 8 and 50 kJ (2 and 12 kcal)/mol. The blue lines represent values between −8 and −33 kJ (−2 and −8 kcal)/mol. The white points symbolize the volume of the molecule.

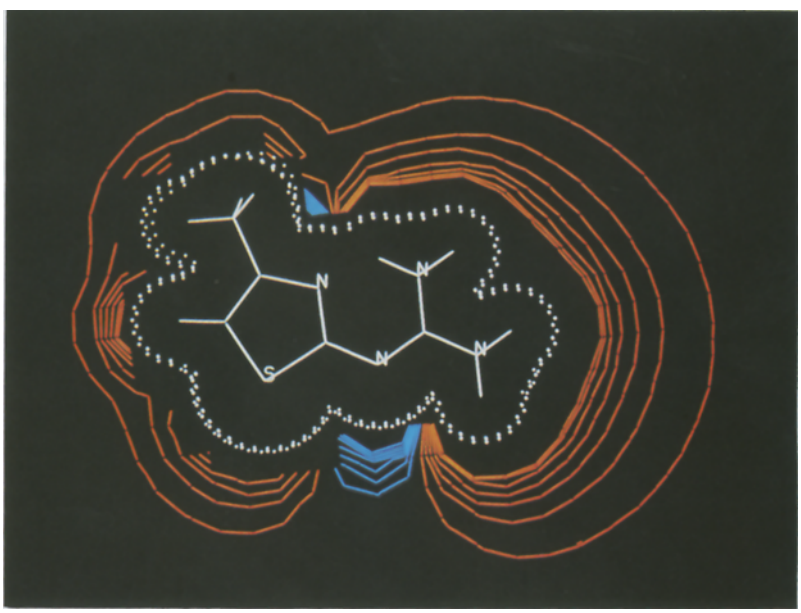


Fig. 10. Electrostatic potential of guanidinothiazole. Red lines represent values between 8 and 50 kJ (2 and 12 kcal)/mol, blue lines correspond to values between −8 and −42 kJ (−2 and −10 kcal)/mol. The white points symbolize the volume of the molecule.

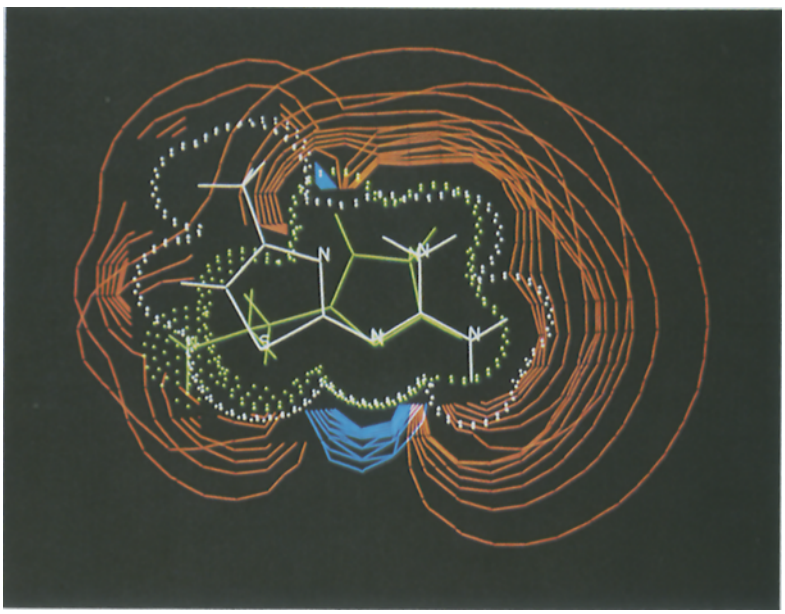


Fig. 11. Superposition of histamine (green) and guanidinothiazole (white) in order to reach the best agreement of their electrostatic potentials. (The nitrogen of the histamine side chain was treated in a neutralized form.) The points correspond to the volumes of the molecules.

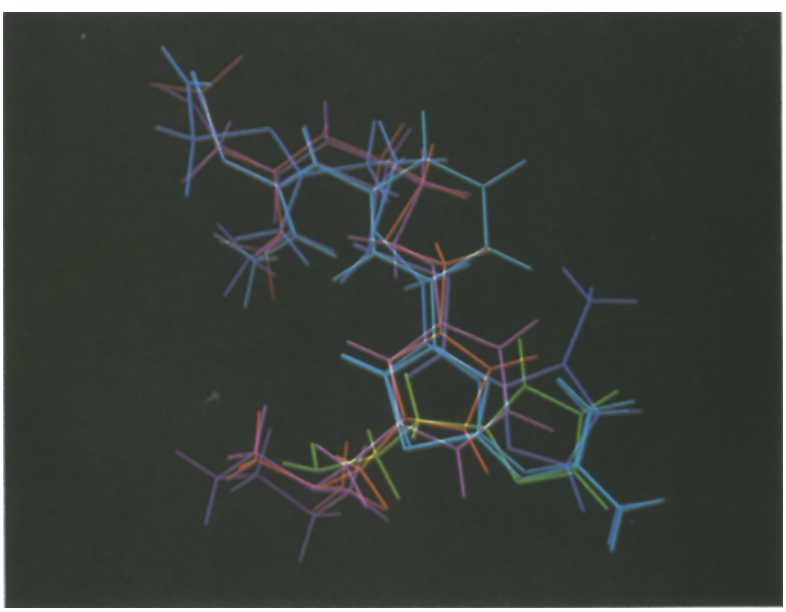


Fig. 12. Superposition of all compounds and histamine: cimetidine (purple), ranitidine (red), roxatidine (magenta), famotidine (blue), ICI127032 (cyan), and histamine (green).

at least one of the histamine binding sites. Therefore the molecular electrostatic potential of histamine was calculated as well. The histamine conformation used is the trans-trans conformation which is postulated to be the bioactive one [10].

The guanidinothiazole substructure and histamine were arranged in order to reach the best superposition of the potentials (see Fig. 11).

The MEP minimum of the guanidinothiazole is in the same position as the MEP minimum of histamine which is considered as essential for the receptor recognition [11]. One nitrogen atom of the guanidino moiety of the guanidinothiazole corresponds to the τ N of histamine which is supposed to interact with a hydrogen bond acceptor site of the receptor; the other corresponds to the π N of histamine which assumingly binds to a hydrogen bond donor site [12].

Figure 12 shows the superposition with all the other compounds. The protonated nitrogen atom of the histamine side chain is located in a similar position as the protonated nitrogens of ranitidine and roxatidine. Weinstein et al. [12] postulated an anionic binding site for this nitrogen atom. Figure 13 shows the resulting pharmacophore.

Cimetidine is not able to meet the hydrogen bond donor site, the hydrogen bond acceptor site and the common position of the polar endgroup simultaneously. If the polar planar endgroup is oriented in the same way as the corresponding endgroups of all other compounds cimetidine looses the possibility to meet both of the imidazole binding sites. Only the hydrogen bond donor site can be occupied. On the other hand, if cimetidine contacts both of the imidazole binding sites it cannot occupy the binding site of the polar planar endgroup. This might be the reason for the lower activity of cimetidine.

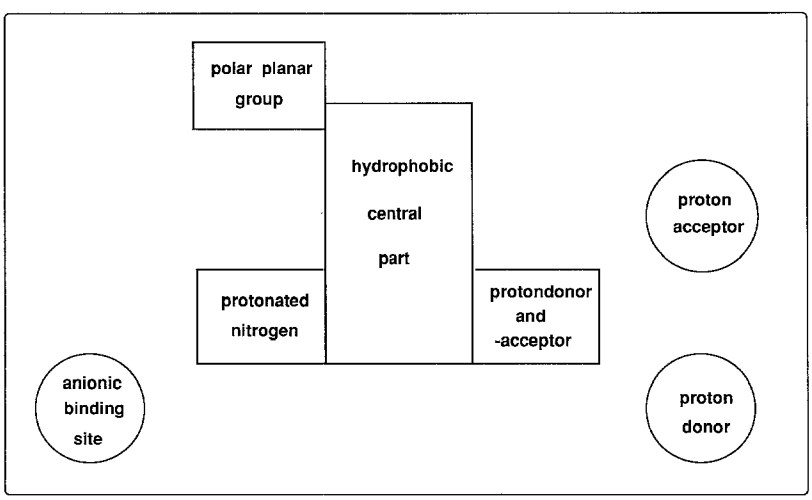


Fig. 13. Postulated pharmacophore for H_2-receptor antagonists consisting of four parts: a protonated nitrogen substituent (for ranitidine and roxatidine) that probably interacts with an anionic binding site; a hydrogen bond donor and acceptor site (for ICI127032, famotidine and cimetidine), which meet a hydrogen-acceptor and -donor site of the receptor; a hydrophobic central part; and a polar planar group.

CONCLUSION

In this study one representative of each structural class of H_2-receptors was taken into consideration. On the basis of the results of our theoretical study we conclude that H_2-receptor antagonists bind to their receptor in the following way:

(1) All compounds are able to meet the same binding site with their polar planar endgroup.

(2) The central hydrophobic substructure which is formed by a flexible chain with or without a sulfur atom or by aromatic systems is located similarly for all molecules.

(3) Compounds possessing (under physiological conditions) a protonated nitrogen substituent at the aromatic ring bind to the proposed anionic binding site.

(4) Compounds containing substituents which are predominantly uncharged under physiological conditions occupy the hydrogen-donor and -acceptor sites of the receptor.

Although there is not yet enough information about the H_2-receptor, several papers postulate that it operates in a way comparable to the β-receptor or the 5-HT_2-receptor [2,3] and therefore belongs to the G-protein-coupled membrane receptor class.

The results presented here support the prevalent ideas of G-protein-coupled membrane receptors and their activation mechanism.

Note

The cartesian coordinates of the postulated biological active conformations of cimetidine, ranitidine, roxatidine, famotidine and ICI127032 are available on request.

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