The Histamine H_1-Receptor Antagonist Binding Site. A Stereoselective Pharmacophoric Model Based upon (Semi-)Rigid H_1-Antagonists and Including a Known Interaction Site on the Receptor†

Anton M. ter Laak, Jennifer Venhorst, Gabriëlle M. Donné-Op den Kelder,* and Hendrik Timmerman

Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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A new pharmacophoric model for the H_1-antagonist binding site is derived which reveals that a simple atom to atom matching of compounds is not sufficient; in this model, interacting residues from the receptor need to be included. To obtain this model, the bioactive conformations of several (semi-)rigid classical histamine H_1-receptor antagonists have been investigated (cyproheptadine, phenindamine, triprolidine, epinastine, mequitazine, IBF28145, and mianserine). In general, these antihistamines contain two aromatic rings and a basic nitrogen atom. A previously derived pharmacophoric model with the nitrogen position fixed relative to the two aromatic rings is now found not to be suitable for describing the H_1-antagonist binding site. A procedure is described which allows for significant freedom in the position of the basic nitrogen of the histamine H_1-antagonist. The area accessible to the basic nitrogen is confined to the region accessible to its counterion on the histamine H_1-receptor, i.e., the carboxylate group of Asp^{116}. The basic nitrogen is assumed to form an ionic hydrogen bond with this aspartic acid which C_α- and C_β-carbons are fixed with respect to the protein backbone. Via this hydrogen bond, the direction of the acidic proton of the antagonist is taken into account. Within these computational procedures, an aspartic acid is coupled to the basic nitrogen of each H_1-antagonist considered; the carboxylate group is connected to the positively charged nitrogen via geometric H-bonding restraints obtained from a thorough database search (CSD). Also to the basic nitrogen of the pharmacophore is coupled an aspartic acid (to yield our new template). In order to derive a model for the H_1-antagonist binding site, the aromatic ring systems of the antagonists and template are matched according to a previously described procedure. Subsequently, the C_α- and C_β-carbons of the aspartic acid coupled to the H_1-antagonists are matched with those of the template in a procedure which allows the antagonist and the carboxylate group to adapt their conformation (and also their relative position) in order to optimize the overlap with the template. A six-point pharmacophoric model is derived which has stereoselective features and is furthermore able to distinguish between the so-called "cis"- and "trans"-rings mentioned in many (Q)SAR studies on H_1-antagonists. Due to its stereoselectivity, the model is able to designate the absolute bioactive configuration of antihistamines such as phenindamine (*S*), epinastine (*S*), and IBF28145 (*R*). A further merit of this study is that a model is obtained which includes an amino acid from the receptor. Since this amino acid has been identified to be Asp^{116}, tools are now available to dock the antagonists with the aspartic acid coupled to the nitrogen in a homology model of the receptor, while matching the coupled aspartate with Asp^{116} of the protein. The most likely (energetically favorable) binding site for the antagonists can then be determined by allowing for rotation around the C_α–C_β bond, while leaving the position of C_α and C_β unchanged. Since several homology models can be built for the protein depending on the alignment chosen, the likelihood of the binding site and especially the (dis)agreement with (Q)SAR and biological data will give clues for the most probable 3D-model (i.e., alignment) of the protein (studies in progress). The underlying approach which includes known interacting amino acids from the receptor into a pharmacophoric model is of general importance for verifying protein models with limited reliability, such as models derived for G-protein-coupled receptors from bacteriorhodopsin.

Introduction

For several decades, almost all compounds with known histamine H_1-blocking activity shared a common chemical structure consisting of two neighboring aromatic rings and a side chain with a basic nitrogen (see, e.g., Figure 1). Although initially the expectations for the therapeutic use of these so-called "classical" histamine H_1-antagonists against allergic disorders were very high, the application of these drugs has been rather limited, mainly because of severe central side effects, especially sedation.

In the last decade new H_1-antagonists became available which lack sedative effects, probably due to their limited passage of the blood–brain barrier.[1] The chemi-

* To whom correspondence should be addressed.

† Abbreviations: aI, angle between the planes of the fitted "cis"-rings; aII, angle between the planes of the fitted "trans"-rings; dI, distance between the centroids of the fitted "cis"-rings; dII, distance between the centroids of the fitted "trans"-rings; ΔE, ab initio internal energy relative to GES; GES, Global minimum energy structure (ab initio); GPCR, G-protein coupled receptor; QSAR, quantitative structure–activity relationship; VDW_{excl}, excluded van der Waals volume.

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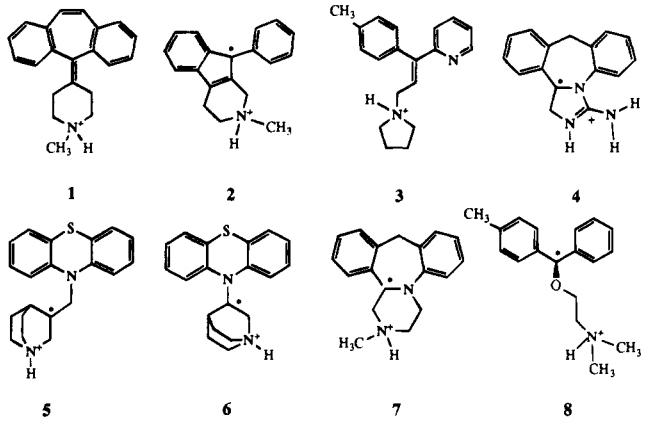


Figure 1. Histamine H_1-antagonists used for the development of the H_1-antagonist binding site model: cyproheptadine (**1**), phenindamine (**2**), *trans*-triprolidine (**3**), epinastine (**4**), mequitazine (**5**), IBF28145 (**6**), mianserine (**7**), and (*R*)-4-methyldiphenhydramine (**8**).

cal structures of these drugs (e.g., astemizole, temelastine, terfenadine, mequitazine, epinastine, cetirizine, and loratadine) often deviate from the basic structure of classical antihistamines. Recently, a study on a series of sedating and nonsedating H_1-blockers demonstrated that nonsedative H_1-antagonists fulfill specific lipophilicity criteria which prevent them from brain penetration.[2]

For the purpose of rational drug design based on structure–activity information, one is confined to data mainly concerning so called classical antagonists.[3] QSAR studies indicate that para substitution with a small lipophilic group group (i.e., CH_3, Cl) is favorable for only one of the two aromatic rings of classical H_1-antagonists (Figure 1). On the basis of a comparison with diphenylaminopropene analogues (e.g., triprolidine **3**, Figure 1) the latter ring is called the "cis"-ring.[4] The aromatic character of this ring seems to be indispensable for H_1-activity. In contrast, the second aromatic "trans"-ring can be replaced by nonaromatic lipophilic groups (e.g., cyclohexyl) without drastic effects on H_1-blocking activity. Obviously, the different receptor environment of the two ring systems is responsible for the stereoselectivity observed for many H_1-antagonists, e.g., the trans-isomer of triprolidine **3** is 1000 times more active than the cis-isomer and the *R*-isomer of **8** (4-methyldiphenhydramine, Figure 1) is 100 times more active than the *S*-isomer.[4-5]

Three-dimensional models describing the structural features of histamine H_1-receptor antagonists are generally based upon the semirigid and potent H_1-antagonist cyproheptadine **1**.[6-8] Most of the early 3D-models were derived from flexible H_1-antagonists for which only one conformation was considered (crystal structure[6] or global minimum[7]). In later years, when lack of CPU time was not so much an issue, Van Drooge *et al.* derived a five-point pharmacophore by matching low-energy conformations of the semirigid and potent H_1-antagonists phenindamine (**2**) and triprolidine (**3**) on six template conformations of cyproheptadine (**1**).[8] This nonstereoselective model describes the relative position of two aromatic rings and a basic nitrogen atom derived from **1** with the piperidylene ring in the so-called "boat3" conformation.

Initially, the present study focused upon the accommodation of (semi-)rigid H_1-antagonists in the above mentioned five-point pharmacophore. However, due to unsatisfactory results we had to establish a new pharmacophore. The final model comprises a residue from the protein (an Asp), contains six pharmacophoric recognition points, and is stereoselective. These results might have important implications for rational drug design.

Strategy

Initially, we tried to accommodate (semi-)rigid and potent H_1-antagonists in the five-point pharmacophore derived earlier by Van Drooge *et al.* (Figure 1, compounds **4**–**7**).[8] However, the basic nitrogen could not always be matched with the position of the template nitrogen in the boat3 conformation of **1**. Therefore, we allowed for a certain flexibility in the position of the nitrogen. For this purpose, an approach was developed which considers the several interaction possibilities between a basic nitrogen and an aspartic acid in the histamine H_1-receptor. The introduction of an aspartate is based upon the observation that aspartic acids are present within transmembrane domains II and III of the histamine H_1-receptor.[9-12] A recent site-directed mutagenesis study on the histaminergic H_1-receptor confirms our hypothesis by showing that the aspartate conserved in all aminergic G-protein coupled receptors (Asp^{116}) interacts with the classical H_1-antagonist mepyramine.[13] In our strategy, we further assume that the cis- and trans-rings of **1**–**7** have a preferred orientation within the antagonist binding site, resulting in a relative position toward the C_α- and C_β-carbons of the aspartate. Furthermore, we implicitly assume that all antagonists considered bind to the same site. These considerations lay the foundation for our model.

In order to derive a new pharmacophoric model for the H_1-antagonist binding site, low-energy conformations of compounds **2**–**7** (Figure 1) are superimposed on six previously derived low-energy conformations of cyproheptadine.[8] An aspartate is coupled to the basic nitrogen of each H_1-antagonist and to the template conformations of **1** (Figure 2A). For this coupling, geometric constraints are obtained from a statistical analysis of the Cambridge Structural Database with respect to the specific type of hydrogen-bonding interaction. In a first step the aromatic rings of the antagonist and the template are matched. Subsequently, a "unique" position for the C_α- and C_β-atoms of the aspartate is derived by allowing for flexibility in the asparate side chain. In this second step the positions of the ring systems of the antagonist are fixed relative to those of the pharmacophore, while the C_α's and C_β's are being matched (Figure 2C). An additional advantage of this approach compared to all previous approaches is that the directionality of the hydrogen bond between the basic nitrogen and the aspartate is automatically taken into account. The stereoselectivity of the model is determined in a final step, where the flexible and stereoselective isomers of **8** are used to designate the cis- and trans-ring of our pharmacophoric model. Our approach results in a new and stereoselective pharmacophore defined by the relative positions of the C_α- and C_β-carbons of an aspartic acid of the H_1-receptor (probably Asp^{116}) positioned relative to the cis- and trans-ring of classical histamine H_1-antagonists.

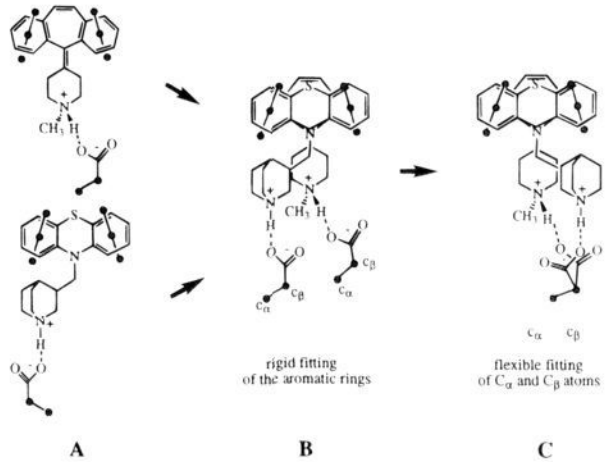


Figure 2. Strategy for fitting histamine H_1-antagonists when (A) an aspartic acid is connected to the protonated amino group of the template (cyproheptadine) and a second H_1-antagonist (here mequitazine) according to geometric constraints obtained from a statistical analysis on CSD data. (B) The aromatic rings are superimposed in a rigid fit. (C) In a flexible fit procedure the C_α- and C_β-atoms of the aspartatic acids are matched. The dotted line represents the virtual axis in the N−H···O bond.

Methods

Investigated Compounds (Figure 1). In the present modeling study we consider the (semi-)rigid histamine H_1-antagonists **1**−**7** and the stereoselective isomers of 4-methyldiphenhydramine **8**. From displacement experiments with [^{3}H]mepyramine (guinea pig cerebellum membranes, 30 min), it is evident that compounds **1**−**4** and **7**−**8** are potent H_1-antagonists with apparent pK_i values of 9.27 (**1**), 8.20 (**2**), 8.78 (**3**, cis/trans mixture), 8.85 (**4**), 9.07 (**7**), and 7.91 (**8**, *R*/*S* mixture).[14] The high potencies of these compounds have also been measured in other, different, and therefore incomparable functional systems, for example, pA_2(**1**) = 9, pA_2(**2**) = 8.8, pA_2(**3**) = 9.9, −log(IC_{50})(**4**) = 8.8, and pA_2(**8**) = 8.7 (in contrast to a value of 6.8 for the *S*-isomer of **8**).[5,15−18] The potencies for **5** and **6** are reported by Barbe *et al.* as being 7.2 and 7.3, respectively (pED_{50} values measured on guinea pig ileum).[19] However, a binding constant of 8.0 was reported for **5** by Leysen *et al.* (displacement of [^{3}H]mepyramine from guinea pig cerebellum membranes after 30 min), and even higher pIC_{50} values (up to 9.0) were measured at longer incubation times.[20]

The activities of the potent and (semi-)rigid H_1-antagonists used in this study are measured in different pharmacological systems and should therefore not be compared quantitatively. For this reason no relative weights were given to the compounds during the construction of the present H_1-antagonist model.

Conformational Analyses. All calculations on H_1-antagonists **1**−**8** were performed on the protonated species (Figure 1). The low-energy conformations of compounds **1**−**3** were obtained from Van Drooge *et al.*[8] Compounds **4**−**8** were built using the modeling package Chem-X January 1990 (Chemical Design Ltd., Oxford, U.K.). The three possible conformations of the extremely rigid compound **4** were built from scratch and optimized within Chem-X. In contrast, the starting conformations of compounds **5**−**8** were built using crystal structures (complete structures or fragments) from the Cambridge Structural Database (diphenhydramine, code JEMJOA; mianserine, code BUCVAW; phenothiazine group, code MPMPTZ; 3-quinuclidinyl group, code BEWDOW10). Subsequently, MacroModel (version 2.5) was used for molecular mechanics conformational analyses on compounds **5**−**8**. If neccesary, the ring closure bond option was used (**5**−**7**) and a large number of conformations was generated by changing all rotatable bonds with increments of 30°. The conformations were energy optimized with an MM2 force field (NBRF optimization) with the program Batchmin 2.7 in order to obtain the low-energy conformations. The partial charges used in these calculations stem from the classical definition of bond dipoles and thus correspond to the MM2 dipoles exactly. For the dielectric constant a value of 1 was used (BatchMin User Manual Version 4.0, Columbia University, New York).

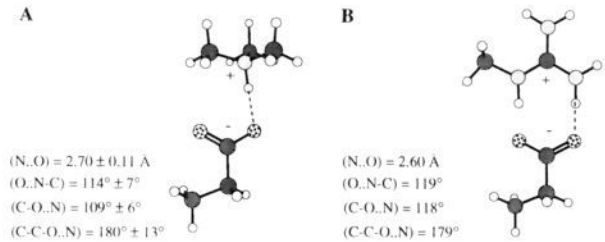


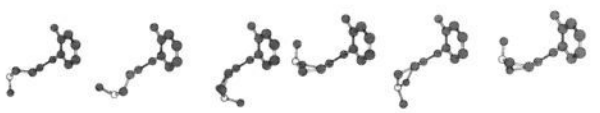
Figure 3. Representation of the applied geometric constraints for the ionic hydrogen bonding interaction between an aspartic acid and (A) a protonated tertiary amino group or (B) a protonated guanidino group. Part A represents the average hydrogen bond geometry with standard deviations (n = 23) from a statistical search (GSTAT88) in the Cambridge Structural Database. Part B represents an optimized hydrogen bond geometry using ab initio methods (GAMESS, STO-3G basis set).

Fit Procedures. In the present study two different fit procedures were used (Chem-X, January 1990). The first approach corresponds to the one described earlier in which two neighboring aromatic rings and a basic nitrogen of an H_1-antagonist are matched with a five-point pharmacophore.[8] Each aromatic ring is represented by two dummy atoms, 1.8 Å above and below the centroid of the ring. Low-energy conformations of compounds **2**−**8** are superimposed on the template conformations of **1** (the pharmacophore) using restraint constants of 10 kcal/(mol·Å^2) for the dummies and 100 for the basic nitrogen. The compounds are first matched by allowing for global rotations and translations only (rigid match). In a subsequent step (flexible fit) the internal nonbonded energy of the antagonist is minimized with respect to user-defined exocyclic bonds (compound **2**, 1 rotatable bond; **3**, 4 bonds; **5**, 2; **6**, 1; and **8**, 6) and the penalty functions (restraints). Endocyclic nitrogen inversion was considered for compounds **4**−**7**. Also the nonplanar ring system(s) of compounds **1**, **2**, **4**, and **7** can adopt several distinct low-energy conformations due to variations around endocyclic bonds. Therefore, all low-energy conformations of compounds **1**−**2** and **4**−**7** generated manually or with Macromodel were considered separately in the above procedure. For compounds **3** and **8** (only exocyclic torsions), only the GES was considered since the restraint constants are high enough to overcome the small energy barriers present.

In the second approach, an aspartic acid is coupled to the basic nitrogen of each investigated antagonist and the template conformations of **1** (Figure 2A). The carboxylate group is connected to the protonated tertiary amino group (all compounds except **4**) according to geometric constraints derived from a statistical analysis (GSTAT88) on 23 structures describing the interaction between a tertiary amino and a carboxylate (Cambridge Structural Database, Oct. 1992;[21] codes BINRIZ, BPAMAL, COYNEJ, CUXKOV, DEBALB, FAKCAV, FUGSAB, FUPDOJ, GEXWOV, JAPCAE, JEGWUN, JIDLAJ, JIFYOM, KEXXUG, KEXYAN, LOXSYC10, MPSIHF, NICSAL, PRPENG, PYCHMA, SEBROG, TALCOY, VIPRIV, Figure 3A). X-ray structures were accepted when the N···O distance was smaller than 3.4 Å.

Compound **4** contains a fused guanidino group. A carboxylate is connected using geometric constraints derived from a study on arginine−carboxylate interactions.[22] The geometry of this bidentate complex (i.e., a methylguanidinoaspartate moiety) was optimized using ab initio methods (GAMESS, STO-3G basis set). Although this so-called anti II geometry involves two hydrogen bonds, only one is used to connect the aspartate to the guanidino of **4** (Figure 3B) (see Discussion). In all cases (**1**−**8**), a virtual bond was established between the

Table 1. Low-Energy Conformations of Cyproheptadine (Side View)[a]

	chair1eq	chair2eq	boat3eq	boat4eq	boat5eq	boat6eq
						
ΔE	0.00	1.36	6.08	3.35	6.06	3.36
	chair1ax	chair2ax	boat3ax	boat4ax	boat5ax	boat6ax
						
ΔE	2.42	3.80	8.11	4.99	6.29	4.98

[a] The piperidylene ring of cyproheptadine can attain six different conformations with the *N*-methyl group in the equatorial (upper six conformations) or axial orientation (lower six conformations). The calculated ab initio energy differences relative to the global minimum (ΔE) are given in kcal/mol (GAMESS U.K., SV 4-31G basis set).

hydrogen-donating nitrogen of the antagonist and the hydrogen-accepting oxygen of the aspartate in order to allow for rotation around this bond in the flexible fit procedure.

In the first step (rigid fit) the aromatic ring systems are matched, defining each ring by two dummies (Figure 2B). In the subsequent flexible fit, the C_α- and C_β-carbons are matched with restraint constants of 50 kcal/mol·Å^2) (six-point pharmacophore, Figure 2C). In addition to the above-mentioned user-defined rotatable bonds, the above virtual bond and the C_β–C_γ bond of the aspartate group are also allowed to rotate.

Twelve different template conformations of **1** are considered (Table 1). The pharmacophore is asymmetric as the aspartate distorts the mirror symmetry in cyproheptadine. Consequently, both stereoisomers of chiral compounds **2** and **4**–**8** are considered.

The criteria used to accept matching results are as follows: at maximum 0.5 Å for the distances between the centroids of the rings (dI and dII), 0.5 Å for the distances between the basic nitrogens (five-point pharmacophore), at maximum 35° for the angles between the rings (aI and aII) and 0.3 Å for the deviation between C_α's and C_β's (six-point pharmacophore). Final conformations with an energy ($\Delta\Delta H_f$ or ΔE) >10 kcal/mol above GES (global energy structure) were rejected.

Energy Calculations. All low-energy conformations and those obtained after fitting were eventually geometry optimized with the quantum chemical program GAMESS-UK (STO-3G basis set) on an IBM-RISC System 6000.[23,24] The two aromatic rings, the basic nitrogen, and the attached proton were restrained to a fixed position during optimization of the fitted conformation in order to preserve the match with the pharmacophore. A single-point SV 4-31G calculation was done to obtain accurate ab initio energies. For compounds **1**, **2**, and **4**, all low-energy conformations were refined ab initio. For the more flexible compounds **3** and **5**–**8**, the MM2 global minimum was asumed to yield the ab initio global minimum (GES).

The ab initio energy difference (ΔE) between the fitted conformation and the corresponding global minimum was calculated. In contrast to the (semi-)rigid compounds, the GES of the flexible compound **8** contains an intramolecular hydrogen bond. As no information is available on the presence of an internal H-bond in solution, the global energy conformation of **8** is assumed to be the lowest energy conformation without such an H-bond. This energy was used for determining ΔE values.

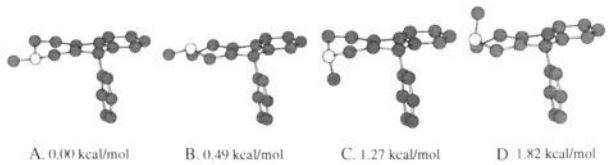


Figure 4. The fused tetrahydropyridine ring of phenindamine (**2**) can adopt two different twisted boat conformations which differ about 0.5 kcal/mol in internal energy (ΔE). The *N*-methyl group can either be in the preferred equatorial (A and B) or in the axial position (C and D).

Results and Discussion

Conformational Analysis of Cyproheptadine (1). The major reason for using the potent H_1-antagonist **1** as a template molecule is its relative rigidity. The piperidylene ring may attain two chair and four boat conformations (see also ref 8). Including the two different attachments of the *N*-methyl group being either axial or equatorial, 12 different low-energy conformations are obtained (Table 1). The relative energies of these ab initio optimized conformations indicate chair1 with the *N*-methyl group equatorial (chair1eq) to be the GES; this conformation is also found in X-ray studies on cyproheptadine hydrochloride.[25] More importantly, ^{1}H-NMR experiments show that chair1eq and chair2eq are the only conformations observed in solution ($CDCl_3$) occurring in a ratio of about 4:1.[26] This is in agreement with our data in Table 1.

Consequently, three conformations of 1 were considered as possible templates: the two conformations observed in solution (chair1eq and chair2eq) and boat3eq, since this conformation has earlier been suggested to be the bioactive conformation.[8]

Conformation Analysis of Phenindamine (2) and Triprolidine (3). The chiral molecule phenindamine **2** and the nonchiral *trans*-triprolidine **3** are two semirigid H_1-antagonists used in the study by Van Drooge *et al.*[8] It is worthwhile mentioning that the double bond of phenindamine can either be in the piperidyl ring (as in **2**, Figure 1) or in between the phenyl group and the piperidyl ring (not shown). Thus phenindamine can be isolated in two epimeric forms, their ratio depending on the salt or free base being formed.[27] The structural formula **2** depicted in Figure 1 is representative for the commercially available phenindamine tartrate salt and is reported to be the bioactive epimer of phenindamine.[28] Taking into account the likely epimerization of phenindamine, it is interesting to note that no one has ever separated stereoisomers of **2**. Since no information is available on the absolute configuration of the bioactive epimer **2**, both stereoisomers of **2** were considered in the fitting procedures.

The relative rigidity of **2** is due to the limited flexibility of the fused tetrahydropyridine ring and the presence of only one freely rotatable bond. We found that the tetrahydropyridine ring can adopt two different boatlike conformations which differ only 0.5 kcal/mol in internal energy (ab initio, SV4-31G basis). As the *N*-methyl group can either be in the favorable equatorial or in the less favorable axial position, four local minima are obtained all within 1.8 kcal/mol from the global minimum (Figure 4).

The conformational analysis for **3** was performed for the trans-isomer only as it displays a 1000-fold higher antihistaminic potency than the cis-isomer.[17] The con-

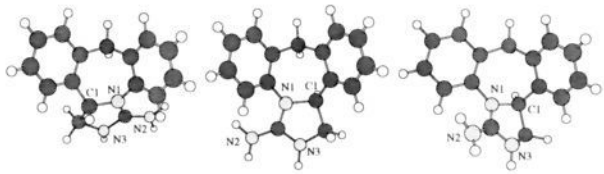


Figure 5. Three conformations of the protonated *S*-isomer of epinastine (**4**) with their ΔE values. See text for further explanation.

formational analysis resulted in a global energy structure (GES) with the α-pyridyl ring and the double bond almost in the same plane (deviation 22°); the substituted phenyl ring is rotated out of this plane by ~59°. Considering the many low-energy conformations of **3** (4 rotatable bonds) and low-energy barriers between the local minima, only the GES was used for flexible fitting purposes. Starting from other low-energy conformations for this specific compound is unlikely to influence the final results.

Conformation Analysis of Epinastine (4), Phenothiazine Derivatives 5 and 6, and Mianserine (7). Epinastine **4** is a highly rigid and basic compound with a pK_a of ~11.[2] All theoretically possible conformations and tautomers of the protonated form were built and optimized. Tautomers with two protons at nitrogen N2 (see Figure 5) are at least 36 kcal/mol more favorable (SV 4-31G basis set) than tautomers with the acidic proton attached to N1 or N3. Therefore, the tautomer depicted in Figure 2 is likely to be the bioactive protonated species of **4**. In total, six conformations are possible for **4**: A, B, and C for both the *S*- and *R*-isomer (Figure 5). The large ab initio energy differences indicate that conformation A is probably active at H_1-receptors. It is important to mention that **4** can be methylated at N3 (thereby replacing the N3-hydrogen) without losing antihistamininic activity; in fact this compound is 2 times more active than **4** itself.[29] On the basis of this observation, the ionic hydrogen bond between **4** and a protein carboxylate is assumed to be formed with N2 and not with N3.

As the tricyclic phenothiazine part of compounds **5** and **6** is symmetric, interconversion of the thiazine ring does not yield different conformations. However, inversion of the ring nitrogen can put the side chain either in an axial or equatorial orientation. The local minima resulting from the conformational analyses show that the equatorial position is energetically more favorable than the axial position (ab initio ΔE: 3.40 kcal/mol for **5** and 1.44 kcal/mol for **6**). For fitting purposes, both the equatorial and axial conformers were considered. Since the carbon connecting the tricyclic part with the 3-quinuclidinyl group is chiral, two stereoisomers were also investigated.

Although mianserine (**7**) has no freely rotatable bonds, the molecule is relatively flexible. The fused piperazinyl ring can be oriented either axial or equatorial at both the central chiral carbon and the neighboring nitrogen (nitrogen inversion). As a consequence, this ring can attain several chair, boat, and twisted boat conformations. Conformational analysis yielded six low-energy piperazinyl chair conformations. The GES is a chair with the *N*-methyl in an equatorial position (SV4-31G basis set). All low-energy conformations of both stereoisomers were considered in the fitting procedures.

Pharmacophores with a Fixed Position for the Basic Nitrogen Atom. In our present study we found that antihistaminics **4**−**7** could not be fitted on the previously assumed bioactive boat3eq conformation of cyproheptadine.[8] Although the aromatic rings of **4**−**7** match the pharmacophore well, the nitrogens of especially **4**, **5**, and **7** hardly match (distance >0.8 Å; Table 2). Since we also found that the energy difference between boat3eq and the global minimum chair1eq is substantially higher with ab initio methods (ΔE: 6.08 kcal/mol) than with semiempirical methods (MNDO: $\Delta\Delta H_f$: 2.7 kcal/mol), we questioned the validity of boat3 as a possible pharmacophore.[8]

On the basis of the above observations, we focused our attention on chair1eq and chair2eq, which are found in solution and have the lowest ab initio energies. We found that only a limited number of compounds could be fitted on the two five-point pharmacophores represented by chair1eq (compounds **2** and **3**) and chair2eq (compounds **2**, **3** and **5**). In all other combinations (i.e., **4**, **6**, and **7** on chair2eq and **4**−**7** on chair1eq), the fit of the basic nitrogens was not satisfactory (N−N distances >0.5 Å; Table 2).

In summary, the fit results, partially presented in Table 2, demonstrate that the basic nitrogen of H_1-receptor antagonists does not occupy one particular position in space with respect to the position of the aromatic rings. We therefore conclude that a five-point pharmacophore derived from either chair1eq, chair2eq, or boat3eq is not sufficient to describe the histamine H_1-antagonist binding site.

A Pharmacophore with a Variable Position for the Basic Nitrogen Atom. From the results presented in Table 2, we anticipated that the ionic interaction between the basic nitrogen and the H_1-receptor should have a relatively large positional freedom. We therefore introduced an aspartic acid into our model with which the antagonists interact and which represents an aspartic acid from the receptor. A penalty function was put on the position of the C_α- and C_β-carbons; no restraints were put on the basic nitrogens. This approach results in a six-point pharmacophore, while at the same time sufficient attention is given to the directionality of the acidic proton.

For the construction of the new pharmacophore, the experimentally observed chair1eq and chair2eq were considered as templates. In the two conformations, the acidic hydrogens have opposite directions which is illustrated by the different positions of the aspartate in Figure 6. Since preliminary fitting experiments showed that the acidic hydrogens in chair1ax and chair2ax point approximately in a similar direction as in the above-investigated chair2eq and chair1eq conformations (see Table 1), these axial conformations were not further investigated.

It was observed that when more than three molecules were fitted simultaneously according to the flexible fitting procedure described in Figure 2C, the number of restraints prevented the C_α- and C_β-atoms from converging. Therefore at maximum two compounds out of the series **2**−**7** were simultaneously fit on **1**. Each compound was combined with several other compounds (starting with the most rigid ones) in order to derive the most likely template molecule. In case a template

Table 2. Quality of the Fits of Compounds **2**–**7** Fitted on the Chair1eq, Chair2eq, and Boat3eq Conformations of Cyproheptadine

	fitted on chair1eq		fitted on chair2eq		fitted on boat3eq	
compound	N–N[a] (Å)	ΔE[b] (kcal/mol)	N–N[a] (Å)	ΔE[b] (kcal/mol)	N–N[a] (Å)	ΔE[b] (kcal/mol)
cyproheptadine (**1**)		*0.00*		*1.36*		*6.08*
phenindamine[c] (**2**)	0.25	1.82	0.26	2.47	0.17	2.93
triprolidine[c] (**3**)	0.19	11.61	0.18	10.82	0.08	10.30
epinastine (**4**)	3.62	0.00	2.57	0.00	1.64	0.00
mequitazine (**5**)	0.67	0.49	0.04	1.48	0.78	4.63
IBF28145 (**6**)	0.80	5.06	0.94	1.85	0.13	5.43
mianserine (**7**)	2.35	3.70	1.57	3.70	0.89	6.60

[a] The distance between the basic nitrogen atoms with respect to cyproheptadine. [b] Ab initio energies of the fitted conformations relative to the global minimum energy (ΔE) (Gamess U.K., SV 4-31G basis set). [c] Results for chair1eq and chair2eq are taken from Van Drooge *et al.*[8]

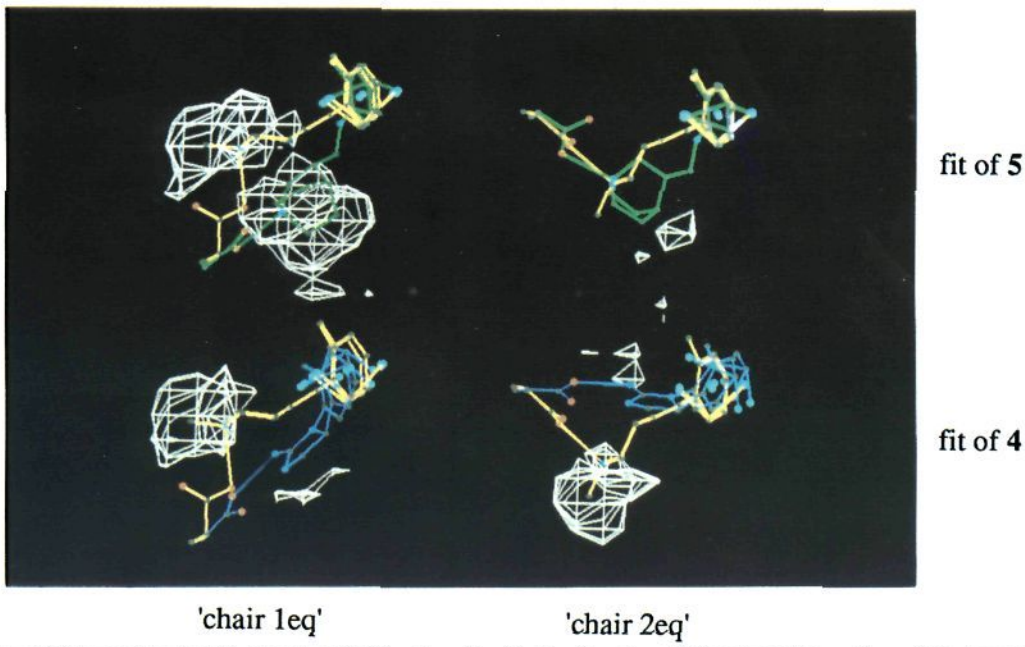


Figure 6. Fits of (*R*)-mequitazine (**5**, green) and (*S*)-epinastine (**4**, blue) on two different conformations of the template molecule cyproheptadine (**1**, yellow). Mequitazine (**5**) reveals a much better overlap with chair2eq (upper right) than with chair1eq (upper left) as illustrated by the excluded VDW volumes of the fitted molecules (white). Epinastine (**4**) reveals similar results (lower right and left). Also the quality of the fit for **4** on **1** with respect to the aromatic rings is much better for chair2eq than for chair1eq.

was rejected, it was checked whether the outcome was not an artefact of the applied restraints.

Especially in the fits of **4** and **5** on **1**, a clear distinction is present between the quality of the fits on chair1eq and chair2eq (Figure 6). Within our predefined criteria, epinastine **4** does not fit on chair1eq due to low fit quality of the aromatic rings; the centroids have at best deviations of 0.42 (ring I) and 1.00 Å (ring II), respectively. Moreover, the C_β of the aspartate coupled to **4** could not be superimposed on the C_β of chair1eq (C_β–C_β > 0.8 Å). Also the steric overlap between **4** and chair1eq is low (VDW_{excl} = 34.2 Å^3) compared to the overlap between **4** and chair2eq (VDW_{excl} = 44.7 Å^3).

Comparable results were found for mequitazine (**5**). Although the rings of **5** fitted well on both templates, the aspartate of **5** did not match the chair1eq aspartate (C_β–C_β > 0.8 Å). Moreover, the 3-quinuclidinyl side chain of **5** is forced to occupy a spatial area different from the one occupied by the piperidylene side chain of the template chair1eq (VDW_{excl} = 73.8 Å^3; Figure 6). In contrast, the fit on the chair2eq aspartate appeared to be much better (C_α–C_α = 0.07 Å, C_β–C_β = 0.15 Å) and resulted in a large steric overlap (VDW_{excl} = 8.0 Å^3).

The fit results for both **4** and **5** on **1** indicate that the bioactive conformation of **1** most probably is chair2eq. Low-energy conformations of **4** (GES) and **5** (ΔE = 1.94 kcal/mol; Table 3) have a good steric overlap with a low-energy conformation of **1** (chair2eq, ΔE = 1.36 kcal/mol) with respect to the aromatic rings and the side chains; moreover, the three compounds are able to interact with the same carboxylate group.

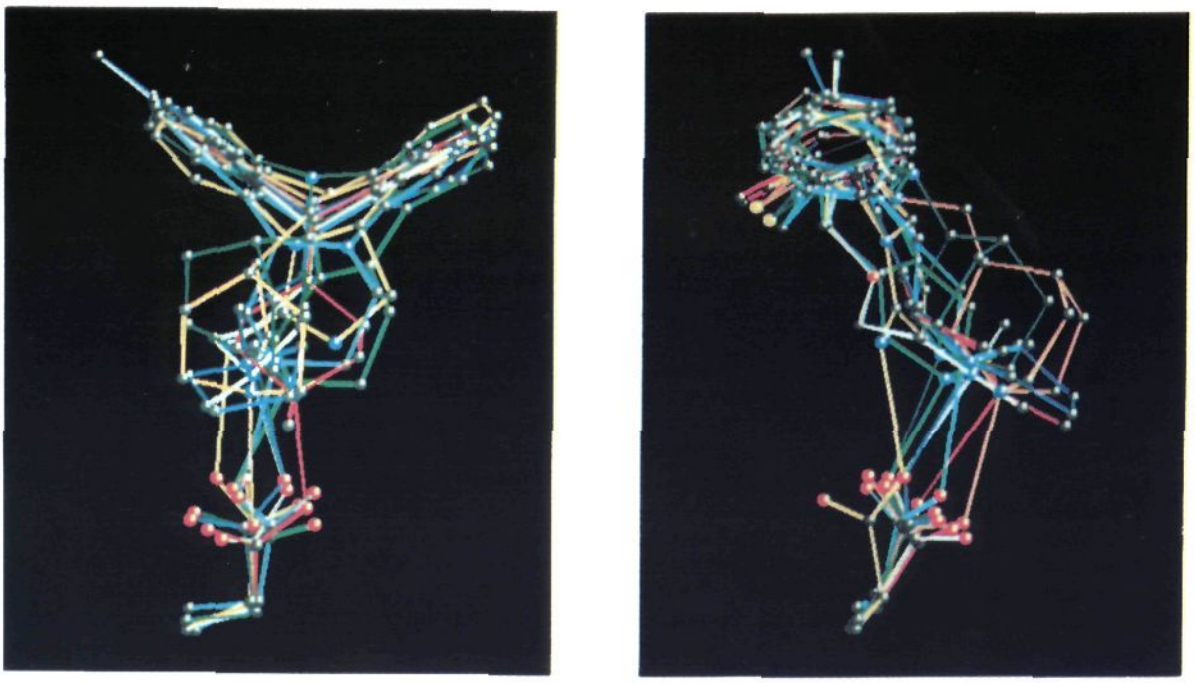
Our tentative conclusions are supported by the remarkable good fits of compounds **2**, **3**, **6**, and **7** on the six-point chair2eq pharmacophore (Table 3); because of the limited flexibility of **2**, **6**, and **7**, these results were unexpected. Figure 7 shows the conformations of **2**–**7** superimposed on chair2eq. The molecules show a good fit with respect to the aromatic rings, relatively compact steric boundaries of the various side chains and only negligible variations in the position of the C_α- and C_β-atoms of the postulated aspartic acid. The model shows that the interacting basic nitrogen occupies largely different positions within the antagonist binding site. For clarity, the fits are presented separately in Figure 8.

Bioactive Conformations of the H_1-Antagonists. In most cases the energy of the best fitting, presumably

Table 3. Quality of the Fits of Compounds **2**–**8** Fitted on the Chair2eq Conformation of Cyproheptadine with an Aspartate Coupled to a Basic Nitrogen[a]

compound	dI[b] (Å)	dII[c] (Å)	aI[d] (deg)	aII[e] (deg)	N–N[f] (Å)	C_α–C_α[g] (Å)	C_β–C_β[h] (Å)	ΔE[i] (kcal/mol)
cyproheptadine (**1**)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.36
phenindamine (**2**)	0.22	0.37	34.11	4.38	1.16	0.12	0.04	3.58
triprolidine (**3**)	0.20	0.27	20.29	7.45	1.15	0.09	0.16	7.42
epinastine (**4**)	0.43	0.29	23.03	31.07	2.04	0.10	0.26	0.00
mequitazine (**5**)	0.11	0.10	8.41	8.45	0.92	0.07	0.15	1.94
IBF28145 (**6**)	0.24	0.16	17.69	15.75	1.67	0.14	0.20	4.25
mianserine (**7**)	0.25	0.34	18.31	11.63	1.09	0.33	0.20	2.62
(*R*)-4-Me-diphenhydramine (**8**)	0.10	0.09	6.61	6.36	1.11	0.07	0.08	1.88
(*S*)-4-Me-diphenhydramine (**8**)	0.16	0.14	5.50	6.17	1.49	0.28	0.25	11.04

[a] All results are with respect to cyproheptadine. [b] Distance between the ring centroids of the cis-rings. [c] Distance between the ring centroids of the trans-rings. [d] Angle between the cis-rings. [e] Angle between the trans-rings. [f] Distance between the basic nitrogens. [g] Distance between the C_α atoms. [h] Distance between the C_β atoms. [i] Ab initio energies of the fitted conformations relative to the global minimum energy (ΔE; Gamess U.K., SV 4-31G basis set).



A B

Figure 7. The H_1-antagonists comprising the final H_1-antagonist binding site model; the aspartic acid of the protein is included: red = **1** (chair2eq); lightgreen = (*S*)-**2** (Figure 4); dark blue = *trans*-**3**; yellow = (*S*)-**4** (Figure 5A); orange = (*R*)-**5** (equatorial); dark green = (*R*)-**6** (axial); light blue = (*R*)-**7**; white ≈ (*R*)-**8**. (A) Front view of the model revealing the overlay of the cis-rings (upper left) and the trans-rings (upper right) and the fit of the C_α- and C_β-carbon atoms of the aspartate. (B) Side view (90° rotated) illustrating the variation in the position of the basic nitrogen atom interacting with the aspartic acid.

bioactive, conformation appeared to be very close to the global or a local minimum (Table 3). For example, the final conformation of phenindamine **2** is a slightly distorted version of conformation C (Figure 4). An acceptable ab initio energy of 3.58 kcal/mol above GES was found.

The internal energy of **3** is high relative to GES (ΔE = 7.42 kcal/mol, Table 3). This is caused by the inability of the Chem-X force field to treat conjugated systems in an appropriate way. The Chem-X molecular mechanics force field easy rotates the aromatic rings out of the plane of the conjugated double bond in order to improve the fit, whereas ab initio methods assign relatively high energies to these distorted systems. This phenomenon can also be observed when **3** is fitted on the three different conformations of **1** in the absence of an aspartate (Table 2). Even when the restraints on the basic nitrogen or the aspartate are removed, the ring systems of **3** and **1** are hard to match. This suggests that in order to explain the high antagonistic activity of **3**, we might have to assume that the position of one of the rings might be less restricted than that of the second ring. This suggestion is supported by QSAR studies which indicate that the steric boundaries of the trans-ring are relatively undefined compared to those of the cis-ring.[3] The probably unnecessary forced fit of the trans-ring of **3** on the trans-ring of **1** explains the relatively high internal energy of **3**.

As a consequence of the above observations, the orientation of the trans-ring in compounds **4**–**7** relative to **1** might also be too restricted. Nonetheless, the ring systems of **4**–**7** fitted extremely well on **1** (Table 3). The lower fit quality is generally found for the more rigid compounds **4** and **7**. This is probably due to the restriction of the N–H···O hydrogen bond angle and the N–H···O–C dihedral angle to their optimal values of 180° (Figure 2). Since the actual interaction is probably less restricted, it is to be expected that relative internal energies can be slightly overestimated (in the order of a few kcal/mol).

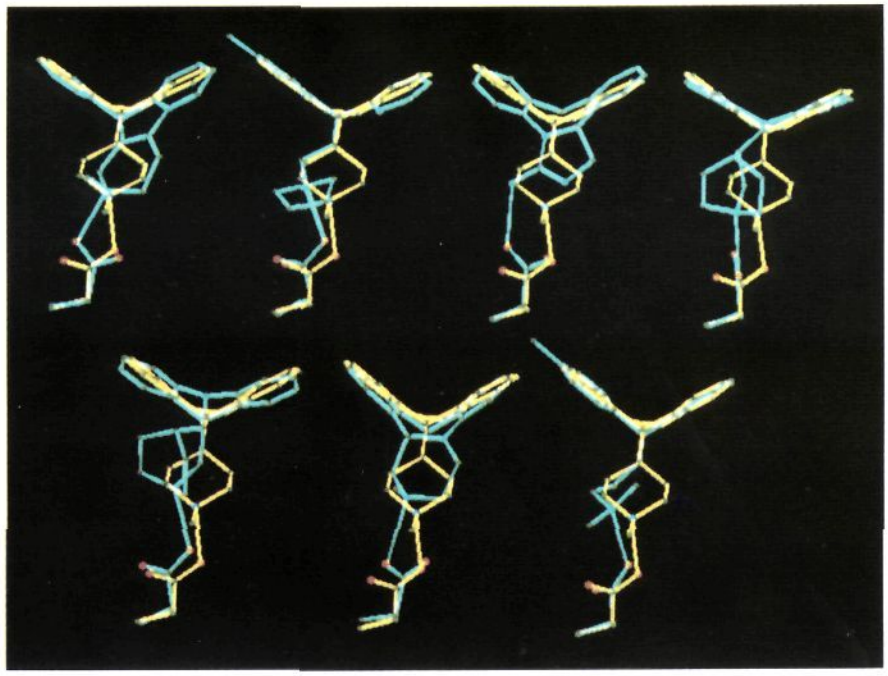


Figure 8. Separate fits of compounds **2**–**8** (blue) on chair2eq of **1** (yellow). This view shows the cis-ring at the left side and the trans-ring at the right side of the molecules.

In conclusion, the whole series of (semi-)rigid H_1-antagonists can be fitted well on a six-point pharmacophore (chair2eq coupled to an aspartate) illustrated by high-quality fits, low intramolecular energies relative to GES, and optimal interaction geometries with an H_1-receptor aspartic acid.

Definition of the Cis- and Trans-Ring. One of the aromatic rings of *trans*-triprolidine **3** is substituted with a *p*-methyl group and has been defined as the cis-ring of H_1-antagonists. We found that the cis- and trans-ring of **3** when fitted on **1** cannot be interchanged. Thus, our model confirms the substantially lower affinity of *cis*-triprolidine compared to *trans*-triprolidine **3** (see Introduction). The ring closest to the carboxylate group (smallest distances between the C_α- and C_β-atoms of the aspartic acid and the centroids of the ring systems) is defined as the cis-ring (Figure 9).

At this stage of the study, it is important to mention that the model still lacks stereoselectivity since for none of the chiral compounds (**2** and **4**–**7**) the absolute configuration of the bioactive stereoisomer is known. As a consequence, two pharmacophores are possible: the pharmacophore shown in Figure 7 and its mirror image.

The pharmacophore with the appropriate stereochemistry can be found by fitting the potent and stereoselective isomers of 4-methyldiphenhydramine **8** on chair2eq. For both isomers, the para-substituted ring was fitted on the cis-ring of chair2eq. Keeping the aromatic rings optimally fitted on the template, only one of the two mirror images of the pharmacophore appeared to be a good template for the most active *R*-isomer of **8** (1.88 kcal/mol above GES). In contrast, the 100 times less potent *S*-isomer did not match well with chair2eq unless the side chain was folded (best fit: 11.04 kcal/mol above GES). Figures 7 and 9 present the pharmacophore in the appropriate configuration. *The orientation of the cis- and trans-ring of antihistamines with respect to an aspartic acid can therefore be described in a three-dimensional and stereoselective pharmacophoric model (Figure 9).*

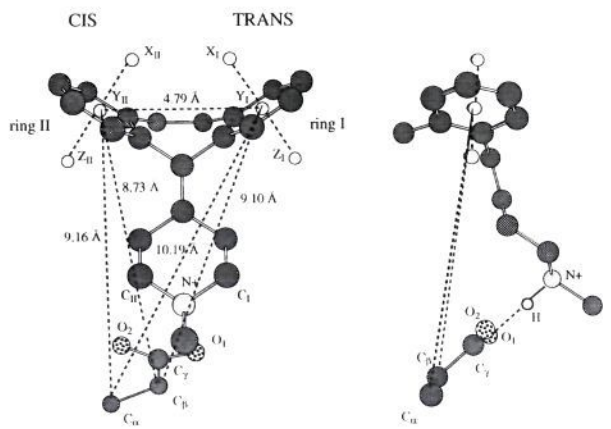


Figure 9. Pharmacophoric model of the histamine H_1-antagonist binding site based on chair2eq of **1** (left, front view; right, side view). Dotted lines connect the C_α- and C_β-atoms of the aspartate with the centroids of the cis- and trans-ring. The aspartate is defined with respect to cyproheptadine by the distance O_1–N^+ = 2.69 Å, the angles O_1–H–N^+ = 180.0° and O_1–N^+–C_{II} = 114.1° and the dihedral angles C_γ–O_1–N^+–C_I = −177.7, C_β–C_γ–O_1–N^+ = −179.7, and C_α–C_b–C_γ–O_1 = 149.0.

Qualitative Prediction of the Stereoselectivity of Antihistamines 2, 4, and 6. Compound **8** made it possible to determine the stereoselectivity of the model; now we can qualitatively predict the absolute configuration of the bioactive stereoisomers of **2**, **4**, and **6**. We found that only the *S*-isomer of **2**, the *S*-isomer of **4**, and the *R*-isomer of **6** are able to fit the present pharmacophore. The model also designates the phenyl group of phenindamine **2** as the cis-ring. Also, the aromatic ring of **4** closest in space to its guanidino group is found to be comparable to the cis-ring (Figure 9).

Unfortunately, the pharmacophore does not unambiguously predict the stereoselectivity of **5** *and* **7**.

Mequitazine **5** is represented by its *R*-isomer, whereas the *S*-isomer fits equally well. This is not surprising since the chiral center is relatively "far away" from the aromatic ring systems when compared to **6**. Mianserine (**7**) is represented by its *R*-isomer, whereas also in this case the mirror image (*S*-isomer) fits equally well. Since in the fits of the *R*- and *S*-isomers of **7** the aromatic rings are interchanged, it is not possible to predict the stereoselectivity of **7** and/or designate the putative cis- and trans-ring.

The presented pharmacophore enables us to extrapolate the structure activity relationships obtained for classical H_1-antagonists to a 3D-model for (semi-)rigid antihistamines.[3] Also less rigid and/or nonclassical H_1-antagonists can be fitted in the pharmacophoric model in order to elucidate their stereoselectivity and/or designate their cis- and trans-rings. In general, the model will be useful for understanding the binding modes of nonclassical antagonists and for the design of new, preferably nonsedating, antihistamines. In preliminary studies we have found that modern antihistamines such as terfenadine, astemizole, loratadine, and cetirizine also fit the model indeed. However, the "best" fit cannot be assigned based on these results, since these compounds usually have an additional flexible side chain attached to the basic nitrogen which occupies an area within the H_1-receptor not described by the present model.

Conclusions

A new pharmacophoric model for the histamine H_1-antagonist binding site has been derived which reveals that a simple atom to atom matching of compounds is not sufficient anymore for describing the binding of an extended series of antagonists but that interacting residues from the receptor need to be included. The new model allows for significant freedom in the position of the basic nitrogen of the histamine H_1-antagonist. The area accessible to the basic nitrogen is confined to the region accessible to its counterion on the histamine H_1-receptor, i.e., the carboxylate group of Asp^{116}. The basic nitrogen is assumed to form an ionic hydrogen bond with this aspartic acid whose C_α- and C_β-carbons are fixed with respect to the protein backbone. Via this hydrogen bond the directionality of the acidic proton of the antagonist is taken into account. A six-point pharmacophore is derived describing the bioactive conformations of cyproheptadine, phenindamine, triprolidine, epinastine, mequitazine, IBF28145, and mianserine.

The merits of this study are as follows.

(i) The pharmacophore is stereoselective and is able to designate the absolute bioactive configuration of H_1-antagonists such as phenindame (*S*), epinastine (*S*), and IBF28145 (*R*). Since we did not succeed in accommodating the opposite enantiomers of these three compounds, we conclude that the receptor is highly stereoselecive for these componds.

(ii) The model is able to distinguish between the so-called cis- and trans-rings mentioned in many (Q)SAR studies on H_1-antagonists.

(iii) The bioactive conformation of cyproheptadine is revealed not be the chair1eq[6,7] or boat3eq conformation[8] but chair2eq. This has important implications for the docking of cyproheptadine (and other classical H_1-antagonists) into three-dimensional receptor models since the directionality of the proton differs in the three low-energy conformations and cyproheptadine is the basis of our template.

(iv) The existence of several low-energy conformations for cyproheptadine indicates that this compound possibly binds to the histamine and muscarine or serotonine receptors in different conformations. This might further explain the different stereoselectivity observed for histamine and muscarine antagonists.

(v) A model is obtained which includes one amino acid of the receptor. Since this amino acid has been identified to be Asp^{116}, tools are now available to dock the antagonists in a homology model of the receptor while matching the aspartate coupled to the basic nitrogen of the antagonist with Asp^{116} of the protein. The most likely (energetically favorable) binding site for the antagonists can then be determined by allowing for rotation around the $C_\alpha-C_\beta$ bond while leaving the position of C_α and C_β unchanged. Since several homology models can be built for the protein depending on the alignment chosen (e.g., refs 30–32), a number of possible binding sites will be obtained (one for each 3D-model/alignment). The most probable binding site and therefore the most probable alignment can be attained when the energetics of the binding sites are compared, i.e., by investigating whether the model explains observed biological data such as affinity values. Furthermore, the properties of the binding site should explain the known (Q)SAR data. Both studies will give clues on the most probable 3D-model (i.e., alignment) of the protein. The underlying approach including known protein interaction sites into a pharmacophoric model is of general importance for verifying protein models with limited reliability such as models derived for GPCRs from bacteriorhodopsin. Furthermore, these interaction sites are necessary in case a simple atom to atom matching of compounds is not sufficient to derive a model which can accommodate all investigated compounds. Studies investigating several 3D-models for the H_1-receptor are in progress.

Since our model is able to assign the bioactive stereoisomers of phenindame (*S*-isomer), epinastine (*S*-isomer), and IBF28145 (*R*-isomer) and further predicts that the receptor binds these enantiomers with high stereoselectivity, we encourage chiral separation of the enantiomers of these compounds for further validation of our model.

Finally, the presented pharmacophoric model might enhance insights into the SARs of several other classes of histamine H_1-antagonists. In the near future we intend to use our model as a basis for an extensive comparative molecular field analysis (CoMFA)[33] and, when possible, determine the bioactive conformation of several classical and nonclassical H_1-antagonists.

Coordinates of the H_1-antagonist pharmacophoric model may be obtained from the authors (by e-mail: donne@chem.vu.nl).

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