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# Quantitative structure–retention relationships in the examination of the topography of the binding site of antihistamine drugs on $\alpha_1$ -acid glycoprotein

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## Abstract

Quantitative relationships between the structure of antihistamine drugs (AHD) and their retention on an  $\alpha_1$ -acid glycoprotein (AGP) HPLC column (QSRR) were studied in order to identify characteristic structural features of the binding site for AHD on AGP. The hydrophobicity of AHD was determined by HPLC on an immobilized artificial membrane (IAM) column. A highly significant QSRR equation was obtained which describes the retention of AHD on AGP in terms of the chromatographically determined hydrophobicity parameter, electron excess charge on the aliphatic nitrogen and a molecular size descriptor. The topography of the AHD-binding site on AGP was suggested to be a conical pocket with lipophilic regions at the mouth of the receptor and an anionic region close to the spike of the cone. Protonated aliphatic nitrogen is supposed to guide a drug molecule towards the anionic region of the binding site. Hydrophobic aryl moieties provide anchoring of the molecule in the lipophilic regions of the binding site. Steric hindrance prevents the molecule from plunging into the binding site.

## 1. Introduction

The biological processes of absorption, distribution, excretion and receptor activation are dynamic in nature as are the solute's distribution processes in chromatography. None of the essential pharmacological or chromatographic processes (except metabolism) implies the breaking-up of existing bonds in a drug (solute) molecule or the formation of new bonds. The same fundamental intermolecular interactions determine the behaviour of chemical compounds in both biological and chromatographic environments

[1]. However, the extreme stereospecificity of biological interactions is the feature differentiating them from the interactions determining regular chromatographic separations.

Modern techniques and procedures in high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) allow for the inclusion of biomacromolecules as active components in chromatographic systems. This approach is now termed "biochromatography".

There are numerous reports regarding biochromatographic separations. Also, the chemometric analysis of various chromatographic data has been the subject of many publications [1,2]. However, the combination of biochromatography and chemometrics is a research strategy only recently introduced and developed. The

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chemometric methodology applied is a linear free-energy relationship (LFER)-based approach, i.e. the quantitative structure–retention relationships (QSRR) [1].

Hydrophobic effects are assumed to determine passive diffusion of drugs through biological membranes and their binding to pharmacological receptors. The *n*-octanol/water partition system is the reference system commonly used for the determination of hydrophobicity which is widely employed in medicinal chemistry and molecular pharmacology; the parameter used is the logarithm of the partition coefficient,  $\log P$  [3]. Since its introduction, reversed-phase HPLC, which has been described in terms of partition of a solute between a polar, aqueous mobile phase and a nonpolar stationary phase, appeared especially suited for the determination of hydrophobicity. If the hydrophobicity measuring system is to model a given biological phenomenon, then close similarity with the “real” system is a prerequisite. For example, a partition system expected to model transport through biological membranes should exist of an aqueous phase and an organized phospholipid layer. The immobilized artificial membranes (IAMs) recently introduced as chromatographic packing materials, appear to present reliable and convenient models of natural membranes [4].

Relationships between  $\log k'$  data determined on IAM columns and  $\log P$  values, appropriately corrected for ionization, were studied for several sets of drug solutes. Poor correlations ( $r < 0.9$ ) were found for phenothiazine neuroleptics, antihistamines [5] and imidazoline derivative drugs [6] but a relatively good correlation ( $r = 0.96$ ) was noted for a series of  $\beta$ -adrenolytics [7]. Nevertheless,  $\log k'$  data from IAM columns are an interesting alternative to the classical  $\log P$  values, even more since, contrary to  $\log P$ , the chromatographic parameter  $\log k'$  can be derived in a simple, fast and reproducible manner [5–7].

After absorption into the general circulation system, drugs interact with serum proteins. There are two main drug-binding serum proteins: human serum albumin (HSA) binds acidic and neutral compounds and  $\alpha_1$ -acid glycoprotein

(AGP) is a serum protein binding mainly basic drugs, such as antihistamines [8]. Standard biochemical methods used for the determination of drug–protein binding are quite tedious. Special problems are encountered when working with chiral drugs. The ability of HSA to bind differentially to stereoisomers of a compound was demonstrated in the late 1950s [9]. To obtain enantiospecific protein-binding data by biochemical procedures, substantial amounts of pure, isolated optical isomers of a drug are needed. Moreover, fast racemization processes often make such attempts impractical.

Promising perspectives opened after the introduction of HPLC columns packed with stationary-phase materials covered with chemically immobilized HSA [10] and AGP [11]. These columns were successfully applied in chiral separations of a wide variety of drugs. Even more fascinating appeared the idea to employ the chromatographic approach to measure drug–protein interactions [12].

The question arises whether chromatographic data obtained on an immobilized serum protein correlate with biochemical protein binding data. Jewell et al. [13] reported poor correlation of HPLC retention data of propranolol obtained on an immobilized AGP column with propranolol displacement from AGP determined in an equilibrium dialysis system. On the other hand, Noctor and co-workers [14,15] found good correlation between the capacity factors of drugs determined on an HSA column and their slow equilibrium binding data. More recently a good correlation was also found between chromatographic data determined on an AGP column and the physiological AGP binding data for a series of  $\beta$ -adrenolytic drugs [16]. Chu et al. [17] postulate that the biological activity of a library of peptides could be predicted using capillary electrophoresis for the measurement of binding constants.

The unique advantage of the HPLC approach to protein-binding studies is the opportunity to readily produce quantitatively comparable binding-related retention data for larger sets of chemical derivatives. If a series of congeneric solutes is analyzed then attempts can be under-

taken to relate the retention data quantitatively to some structural data. If the structural parameters describing retention are successfully identified then it is possible to use respective QSRR for predicting the retention of a nonanalyzed solute and for elucidation of the molecular separation mechanism operating in a given chromatographic system [18]. This research methodology has previously been applied to define the topography of benzodiazepine binding sites on HSA used as an HPLC stationary phase [19].

Recently, we succeeded in deriving the QSRR for a series of 12 antihistamine drugs (AHD) chromatographed on an AGP column [16]. However, the higher-order structure of human AGP and details on its binding site(s) are not yet known. Thus we performed chromatographic measurements, molecular modeling and chemometrical analysis for extended series of available agents aiming at the elucidation of the topography of the binding site for AHD on AGP.

## 2. Experimental

### 2.1. Materials

Drug solutes were provided by the respective producers: antazoline hydrochloride (Polfa, Warszawa, Poland), chloropyramine hydrochloride (Polfa, Kraków, Poland), chlorpheniramine maleate (Sigma, St. Louis, MO, USA), cinnarizine (Polfa, Warszawa, Poland), dimethindene maleate (Zyma, Munich, Germany), diphenhydramine hydrochloride (Polfa, Kraków, Poland), isothipendyl hydrochloride (Astra Pharma AG, Frankfurt am Main, Germany), ketotifen fumarate (Polfa, Warszawa, Poland), mepyramine maleate (Rhône-Poulenc, Dagenham, UK), pheniramine hydromaleate (Hoechst, Frankfurt am Main, Germany), pizotifen hydrochloride (Sandomigran, Sandoz AG, Nürnberg, Germany), promethazine hydrochloride (Polfa, Jelenia Góra, Poland), roxatidine acetate hydrochloride (Roxit, Albert-Roussel Pharma, Wiesbaden, Germany), tripeleminamine hydrochloride (Teva Pharmaceutical Industries, Petah

Tiqua, Israel), triprolidine hydrochloride (Sigma, St. Louis, MO, USA) and tymazoline hydrochloride (Polfa, Warszawa, Poland).

### 2.2. Methods

A Merck-Hitachi (Wien, Austria) HPLC system was employed for chromatographic measurements.

#### *Measurement of interactions between the drugs and $\alpha_1$ -acid glycoprotein*

The column used was the Chiral AGP column  $100 \times 4$  mm I.D. (ChromTech, Norsborg, Sweden) packed with  $\alpha_1$ -acid glycoprotein chemically bound to silica particles of  $5 \mu\text{m}$  diameter.

A 1-mg amount of drug solute was dissolved in 4 ml of methanol. The solution was diluted 10-fold with methanol and  $20 \mu\text{l}$  of the final solution was injected onto the column.

The mobile phase was isopropanol–0.01 M Sörensen phosphate buffer pH 6.5 (5:95, v/v). The mobile phase was filtered through a membrane filter before use.

The mobile phase flow-rate was 0.5 ml/min. The detection wavelength was 215 nm. Capacity factors,  $k'_{\text{AGP}}$ , were calculated taking the sodium nitrate peak as a measure of dead volume. Retention data are collected in Table 1. Under the HPLC conditions applied only little separation of enantiomers was observed for the chiral racemates of pheniramine and promethazine. Means of two peaks were taken as  $\log k'_{\text{AGP}}$  for the QSRR study.

#### *Measurement of hydrophobicity of the drugs [16]*

The column used was of the so-called immobilized artificial membrane type. It was formed by lecithin-COOH bound to silica-propylamine with the unreacted propylamine moieties endcapped with methylglycolate. A commercial column IAM.PC.MG  $150 \times 4.6$  mm I.D. was purchased from Regis Chemical Company (Morton Grove,

Table 1

HPLC retention data determined on an immobilized artificial membrane column ( $\log k'_{IAM}$ ) and on an  $\alpha_1$ -acid glycoprotein column ( $\log k'_{AGP}$ ) and structural parameters: electron excess charge on aliphatic nitrogen ( $N_{ch}$ ) and surface area of the molecular size triangle ( $S_T$ ), for a series of antihistamine drugs

No.	Solute	$\log k'_{AGP}$	$\log k'_{IAM}$	$S_T$ ( $\text{\AA}^2$ )	$N_{ch}$ (electrons)
1	Antazoline	1.154	1.043	25.23	-0.2848
2	Chlorpheniramine	1.202	1.055	30.92	-0.2600
3	Chloropyramine	1.431	1.330	31.11	-0.2683
4	Dimethindene	1.382	1.194	29.76	-0.2666
5	Diphenhydramine	1.140	1.006	32.78	-0.2655
6	Isothipendyl	1.580	1.210	20.41	-0.2596
7	Ketotifen	1.459	1.168	25.54	-0.2448
8	Mepyramine	1.113	0.935	32.81	-0.2719
9	Pheniramine	0.926	0.602	27.37	-0.2604
10	Promethazine	1.833	1.508	20.73	-0.2572
11	Tymazoline	1.306	1.024	17.07	-0.2865
12	Tripelennamine	1.066	0.887	30.37	-0.2643
13	Tripolidine	1.185	1.084	24.02	-0.2401
14	Pizotifen	1.898	1.588	29.13	-0.2465
15	Cinnarizine	2.148	2.250	32.18	-0.2477
16	Roxatidine	0.773	0.359	28.99	-0.2794

IL, USA). The column was characterized by a particle diameter of 12  $\mu\text{m}$  and a pore diameter of 300  $\text{\AA}$ .

The mobile phase was acetonitrile-0.1 M Sørensen phosphate buffer pH 7.0 (20:80, v/v); the flow-rate was 1.0 ml/min; UV detection was performed at 254 nm. Retention data are given in Table 1.

#### Structural analysis

Molecular modeling software HyperChem (Autodesk, Sausalito, CA, USA) was employed. The structure of the solutes was optimized initially by the molecular mechanics MM + force-field method and then by the quantum chemical AM1 method using the Polak-Ribiere algorithm.

Among the variety of structural parameters tested, the following appeared meaningful to describe antihistamine retention on the AGP column: (i) electron excess charge on the nitrogen atom in the aliphatic chain ( $N_{ch}$ ), and (ii) surface area (in  $\text{\AA}^2$ ) of the triangle ( $S_T$ ) having one vertex on the aliphatic nitrogen and the two remaining vertices on the extreme atoms of the

aromatic substituents in the molecule (Fig. 1). Numerical values of the two structural descriptors are given in Table 1.

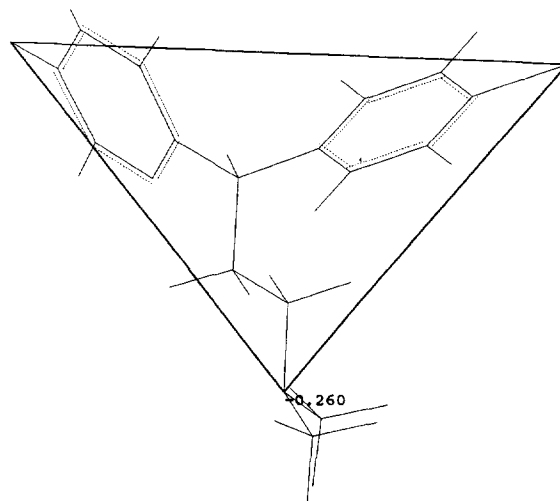


Fig. 1. Determination of the molecular size descriptor,  $S_T$ , for chlorpheniramine. To determine  $S_T$  a triangle is drawn with one vertex on the aliphatic nitrogen and the two remaining vertices on the extreme atoms of the aromatic substituents in the geometry optimized structure. The parameter  $S_T$  is the area (in  $\text{\AA}^2$ ) of the triangle.

### 3. Results and discussion

Chromatographic and structural data determined for the series of antihistamine drugs presented in Fig. 2 are given in Table 1. Drugs nos. 1–15 are classical antihistamines (antagonists of the H<sub>1</sub> receptor) and no. 16 is an antagonist of the histamine H<sub>2</sub> receptor.

There are only few quantitative data available on the binding of antihistamine drugs to serum proteins. However, the percents of drug bound in plasma for chlorpheniramine (70%) and promethazine (93%) seem to agree generally with the HPLC log  $k'_{AGP}$  data: 1.202 and 1.833, respectively [20].

QSRR of data from Table 1 resulted in several statistically significant and physically meaningful equations. In the course of the study it appeared that triprolidine was an outlier (at least in case of multiparameter regression equations). The reason may be the inadequacy of the molecular modeling parameters to account specifically for the chromatographic behaviour of this compound. For the sake of consistency the agent was excluded from further analysis.

The HPLC log  $k'_{AGP}$  values correlate significantly with the chromatographic hydrophobicity parameter determined on an immobilized artificial membrane column, log  $k'_{IAM}$ :

$$\begin{aligned} \log k'_{AGP} = & \\ & 0.413(\pm 0.085) + 0.828(\pm 0.069) \log k'_{IAM} \\ & n = 15, R = 0.957, \\ & s = 0.113, F = 142, p < 10^{-4} \end{aligned} \quad (1)$$

where  $n$  is the number of compounds used to derive the regression equation,  $R$  is a multiple correlation coefficient,  $s$  is a standard error of estimate,  $F$  is a value of the statistical significance test, and  $p$  is a significance level. The numbers in parenthesis denote the standard deviations of the regression coefficients.

In the QSRR analysis significant for log  $k'_{AGP}$  description, the structural parameters reflecting the molecular size of a solute,  $S_T$ , and the electron excess charge on the aliphatic nitrogen,  $N_{ch}$  also appeared. The resulting equation has the form:

$$\begin{aligned} \log k'_{AGP} = & 2.448(\pm 0.560) + 0.743(\pm 0.053) \\ & \times \log k'_{IAM} + 5.557(\pm 1.845)N_{ch} \\ & - 0.0170(\pm 0.0040)S_T \\ & n = 15, R = 0.986, \\ & s = 0.071, F = 130, p < 10^{-4} \end{aligned} \quad (2)$$

The predictive power of Eq. 2 is illustrated in Fig. 3.

Eq. 2 shows that the binding site on the AGP stationary phase interacting with antihistamines has lipophilic character. This is true because log  $k'_{IAM}$  is a measure of the hydrophobicity of the agents studied. The positive sign at log  $k'_{IAM}$  in Eqs. 1 and 2 demonstrates the expected increase of the binding of drug solutes by AGP with their increasing hydrophobicity. Although hydrophobicity is a structural feature most important for interactions of AHD with AGP, Eq. 2 also shows the significance of two additional parameters of the solutes with respect to the retention. The negative sign at  $S_T$ , which parameter may be viewed as a measure of molecular size, suggests steric restrictions at the AGP binding site. The parameter  $N_{ch}$  confirms the significance of electrostatic interactions caused by the protonated nitrogen atom of AHD. We assume that possible  $\pi$ – $\pi$  interactions due to the aryl groups on the drugs [21] are accounted for by the hydrophobicity parameter log  $k'_{IAM}$ .

The characteristics of the binding site for AHD on AGP provided by Eq. 2 are certainly more comprehensive than the so far accepted qualitative descriptions such as the one given by Kremer et al. [22]: “it is reasonable to assume that the aryl system contributes to hydrophobic forces, and that the positively charged tertiary nitrogen contributes to ionic forces”.

The QSRR equation derived here and the reported qualitative characteristics of the mode of binding of xenobiotics by AGP allow for an indirect definition of the topography of the AHD binding site on AGP (Fig. 4). We suggest that this site is formed by a conical pocket. Its wall (internal surface) comprises lipophilic regions at the base of the cone (the mouth of the binding site). There is an anionic region close to the spike of the cone. Electron excess charge (negative) on

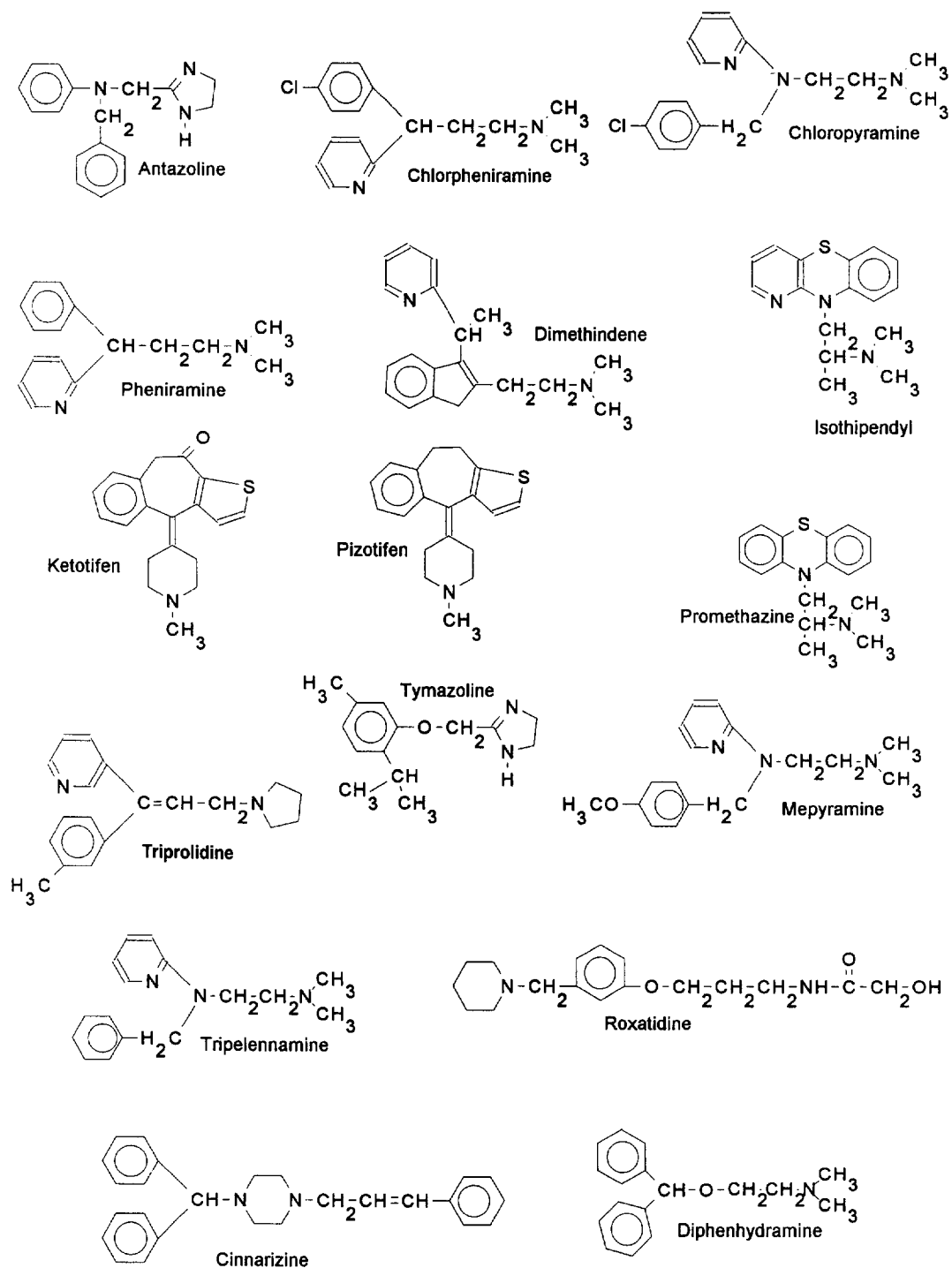


Fig. 2. Chemical structure of antihistamine drugs.

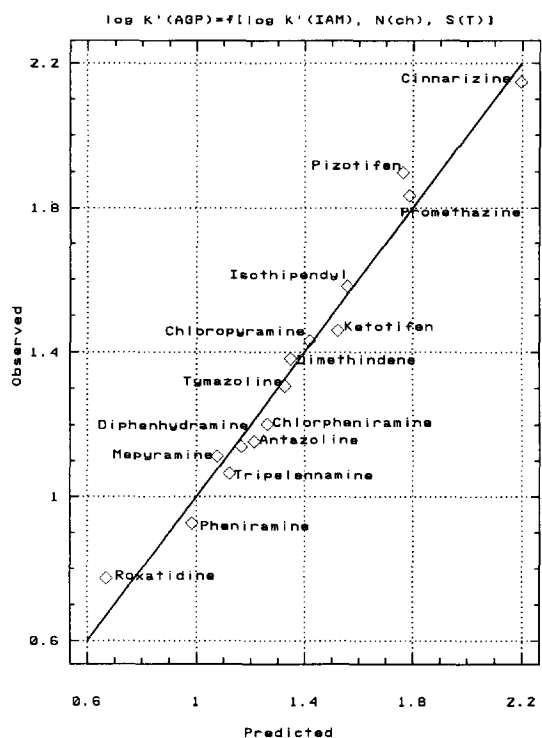


Fig. 3. Relationship between experimental  $\log k'_{AGP}$  and the values calculated by Eq. 2. The drugs are numbered as in Table 1.

the aliphatic nitrogen inversely reflects the positive charge of the protonated species. The protonated aliphatic nitrogen is supposed to guide the molecule towards the anionic region in the binding site. Hydrophobic aryl moieties are decisive for the anchoring of a drug molecule in the lipophilic regions of the binding site. Steric restriction prevents the molecule from plunging into the binding site. Supposedly, an asymmetric shape of the proposed binding site and a negative charge distribution would account for the reported enantioselective binding of some drugs by AGP [23]. The model obtained in this work supports the view [23,24] that there may be one single class of binding sites for basic drugs on AGP. To test this hypothesis work is in progress in our laboratory on agents belonging to pharmacological classes other than AHD but known also to bind to AGP.

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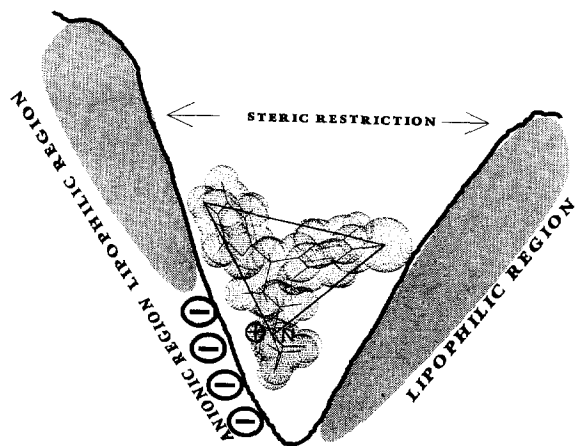


Fig. 4. Proposed topography of the binding site for antihistamine drugs on  $\alpha_1$ -acid glycoprotein. For details see the text.

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