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A molecular modeling study on binding of drugs to calmodulin

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SUMMARY

Computer-graphical methods have been used to study the interaction between a series of drugs and calmodulin. Based on the X-ray crystallographic coordinates of the α-C atoms of calmodulin, a molecular model of the helical sequences was built. The model has been used to derive two possible binding sites for phenothiazines and one binding site for penfluridol. The principal binding forces occur through contacts between acidic amino acids of calmodulin and the protonated side-chain nitrogen of the drugs as well as between a basic amino acid and the electronegative substituents of the aromatic rings. Calculated interaction energies show a good correlation with experimental inhibition data.

INTRODUCTION

Calmodulin belongs to the family of Ca^{2+}-binding proteins. It modulates the activity of a number of enzymes in a Ca^{2+}-dependent manner, including a cyclic nucleotide phosphodiesterase, myosin light chain kinase, brain adenylate cyclase, and phosphorylase kinase (for a review see Ref. 1). Calmodulin is a small acidic protein consisting of a single polypeptide chain of 148 amino acids (Fig. 1). It has four calcium-binding sites, two in the N-terminal domain and two in the C-terminal domain. The Ca^{2+}-saturated form of calmodulin is the active form.

A recently described crystallographic structure [2] shows that calmodulin consists of two globular domains, each containing two calcium-binding sites, connected by a long exposed α-helix (Fig. 2).

Drugs from various pharmacological and chemical classes (phenothiazines, butyrophenones, naphthalenesulfonamides, antitumor agents, neuropeptides, etc.) inhibit the action of calmodulin on many enzymes by binding to it directly [3].

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 1                                  10
ALA-ASP-GLN-LEU-THR-GLU-GLU-GLN-ILE-ALA-GLU-PHE-LYS-GLU-ALA-
                  20                                  30
PHE-SER-LEU-PHE-ASP-LYS-ASP-GLY-ASP-GLY-THR-ILE-THR-THR-LYS-
                                    40
GLU-LEU-GLY-THR-VAL-MET-ARG-SER-LEU-GLY-GLN-ASN-PRO-THR-GLU-
                  50                                  60
ALA-GLU-LEU-GLN-ASP-MET-ILE-ASN-GLU-VAL-ASP-ALA-ASP-GLY-ASN-
                                    70
GLY-THR-ILE-ASP-PHE-PRO-GLU-PHE-LEU-THR-MET-MET-ALA-ARG-LYS-
                  80                                  90
MET-LYS-ASP-THR-ASP-SER-GLU-GLU-GLU-ILE-ARG-GLU-ALA-PHE-ARG-
                                   100
VAL-PHE-ASP-LYS-ASP-GLY-ASN-GLY-TYR-ILE-SER-ALA-ALA-GLU-LEU-
                  110                                 120
ARG-HIS-VAL-MET-THR-ASN-LEU-GLY-GLU-LYS-LEU-THR-ASP-GLU-GLU-
                                   130
VAL-ASP-GLU-MET-ILE-ARG-GLU-ALA-ASN-ILE-ASP-GLY-ASP-GLY-GLN-
                  140                                 148
VAL-ASN-TYR-GLU-GLU-PHE-VAL-GLN-MET-MET-THR-ALA-LYS
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Fig. 1. Amino acid sequence of bovine brain calmodulin. Underlined sequences represent calcium-binding loops.

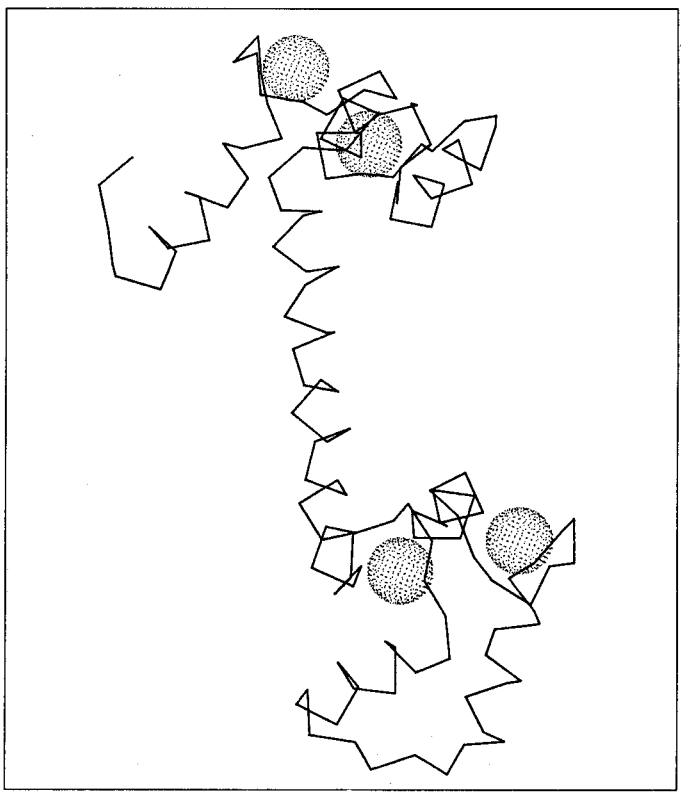


Fig. 2. Calmodulin α-C backbone. The van der Waals spheres of the calcium ions are shown as dotted surfaces.

Experimental work has indicated that there are two Ca^{2+}-dependent binding sites for phenothiazines on calmodulin, one existing on each half of the protein molecule [4].

The structural requirements for calmodulin binding of the drugs comprise an aromatic region with lipophilic substituents, and a nitrogen which is protonated at physiological pH, separated from the aromatic rings by four or more atoms [5].

The residues in the central helix seem to play an important role in the interaction of calmodulin with the drugs [6]. Gariepy and Hodges [7] as well as Reid [8] have proposed an α-helical region in the central helix nearby the third Ca^{2+}-loop as a binding site for trifluoperazine. This region consists of amino acid residues 82–92, including hydrophobic residues as well as negatively charged residues.

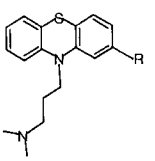
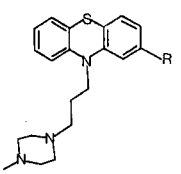
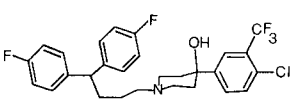
Other studies have implicated a contribution of methionine and lysine residues in the interaction of phenothiazines with calmodulin [9, 10].

Based on these experimental data, we have performed a theoretical study, using molecular mechanics and computer graphics to locate two binding sites for a series of phenothiazine drugs on calmodulin.

METHODS

A series of seven phenothiazine derivatives as well as penfluridol, representing a wide range in IC_{50} values for the inhibition of calmodulin-dependent activation of phosphodiesterase (these inhibitory effects can be attributed to direct binding of the drugs to the calmodulin molecule) was chosen for this study. Structural formulas and IC_{50} values are shown in Table 1.

TABLE 1
CALMODULIN ANTAGONISTS INVESTIGATED IN THIS STUDY

Structural formulas	R	Name	IC_{50}[a] (μM)
	-H	promazine	110
	-OH	hydroxypromazine	78
	$-SCH_3$	thiomethylpromazine	42
	-Cl	chlorpromazine	40
	$-CF_3$	trifluopromazine	28
	-Cl	prochlorperazine	22
	$-CF_3$	trifluoperazine	17
		penfluridol	3

[a]See Ref. 5 for determination of IC_{50} values.

Small molecule building

The compounds were constructed on an E&S PS390 system with the SYBYL 3.5 program [11]. The geometry of the tricyclic system was taken from crystallographic data [12], substituents and side chains were added with standard bond lengths and angles.

All calculations were done on the protonated amines. Trifluoperazine and prochlorperazine have two basic nitrogens (pK_a 8.0 and 3.9) and are monoprotonated at physiological pH. Since crystal structures only show the diprotonated molecules, we have performed MNDO and CNDO calculations to find out on which nitrogen the monoprotonation occurs.

These calculations show that the protonation of N1 nitrogen is energetically more favorable than the protonation of N2. Using the SEARCH routine of SYBYL, a conformational search for sterically-allowed, low-energy conformations was performed.

The global energy minimum corresponds to a conformation where the cationic part of the protonated side chain interacts with the electron-donating nucleus.

However, another low-energy conformation with no intramolecular interactions was chosen as starting conformation for further calculations. This is in accordance with studies of Pullman [13] who showed that the monocationic form of small biomolecules adopts a highly folded conformation in vacuo, whereas solvation results in an extension of the structure.

Since the conformation of the side chain is independent of the nature of the substituent on the tricyclic nucleus, the same conformation was retained for all phenothiazine compounds. In the case of penfluridol, the crystal conformation was used.

Macromolecular modeling

Using the coordinates of the α-carbons of calmodulin (the coordinates of the side chains were not available at that time) from the Brookhaven Data Bank [14], the α-helical sequences of the protein were constructed with the protein-modeling routine MENDYL [10].

Side chains were added in standard geometry and conformation. In order to prevent bad van der Waals contacts within the peptide, a molecular mechanics optimization (MAXIMIN) was performed.

Construction of interaction complexes

Firstly, a phenothiazine molecule was fitted manually near the postulated interacting amino-acid residues of calmodulin. Then the docking of the substrate to the receptor was carried out applying the DOCK routine of SYBYL 3.5. DOCK optimizes the interaction complex by rotating, translating, and fitting the substrate molecule to the receptor molecule. Ionic interactions, hydrogen bonding, and van der Waals contacts were taken into consideration.

In order to obtain low-energy complexes, the docking procedure was repeated several times with different initial orientations and conformations of the receptor model side chains and the substrate compounds.

The peptide model and the interaction complexes are too large to employ a quantum chemical charge calculation. Therefore we calculated the charge distribution with the topological method of Gasteiger and Marsili [15] using a parameter set that showed a good approximation to MNDO

charges for the functional groups of the amino-acid side chains and the phenothiazine compounds.

Energy calculations for the protein model, the phenothiazines, and the protein–phenothiazine complexes were performed with the ENERGY routine. Energies are calculated as sums of bond stretching, angle bending, torsional, electrostatic and van der Waals energy terms.

The interaction energy term is defined as the difference between the sum of the total energies of the isolated binding partners and the total energy of the interaction complex.

A dielectric constant of 1.0 was used since DOCK and ENERGY do not provide a distance-dependent dielectric constant. Therefore, the energy term for the electrostatic energy is too high; however, on increasing the dielectric constant the total interaction energy would remain negative. These calculated energies are not to be considered as absolute values. They have to be used as measures on a relative scale. All computations were executed on a MicroVax II.

RESULTS AND DISCUSSION

As mentioned in the Introduction, we started our study on the basis of an explicit proposal of a phenothiazine binding site on sequence 82–92 of calmodulin [8]. This work postulated that binding of the drugs in the C-terminal domain involves a hydrophobic patch as well as ionic interactions: the cationic end of the phenothiazine drugs lies in a close proximity to Glu-84 and Glu-87, whereas the aromatic moiety of the phenothiazines overlaps the π-orbitals of the two aromatic phenylalanine residues 89 and 92.

Other experimental findings indicated that methionines and lysines of the N-terminal domain might be involved in the binding of drugs [9, 10].

The docking procedures resulted in a model for two possible binding sites for phenothiazine drugs on calmodulin, shown in Fig. 3.

In agreement with the proposals described above, we found an interaction of the negatively charged amino acids Glu-84 and to a lesser extent Glu-83 as well as Glu-87 with the protonated side-chain nitrogen of the phenothiazines as the most important binding factor in the C-terminal region.

In our model, Phe-89 and Phe-92 are too far from the residues of the hydrophilic glutamate cluster to provide a sandwich-like interaction with the tricyclic nucleus. As can be derived from crystallographic data, energetically favorable sandwich complexes need intermolecular distances around 3.6 Å [16]. When such a sandwich was constructed, only weak van der Waals contacts occurred between Phe-89 and one of the aromatic rings of the phenothiazine tricyclus. However, this unspecific interaction did not account for the variation in experimental binding affinities of the phenothiazine derivatives. Gresh and Pullman [17] proposed on the basis of theoretical computations that, in fragment 82–92, the crucial residues involved in specific phenothiazine binding, are Ala-88, Val-91, Phe-89, and Phe-92. This cannot be supported by our model.

Instead of the phenylalanines, we found Arg-86 to be involved in the binding of the substrate molecules. Figure 4 shows this binding site in detail.

The second binding site shows the following features: the phenothiazine rings fit into a core formed by residues 39–51. Binding contacts occur between Met-51 and, to a lesser extent, between Leu-39 and the aromatic rings of the phenothiazines. Lys-75 forms a hydrogen bond with the substituents in position 2 of the tricyclus. The protonated side chain of the phenothiazines runs along the central helix and interacts with the acidic side chain of Glu-82 (Fig. 5).

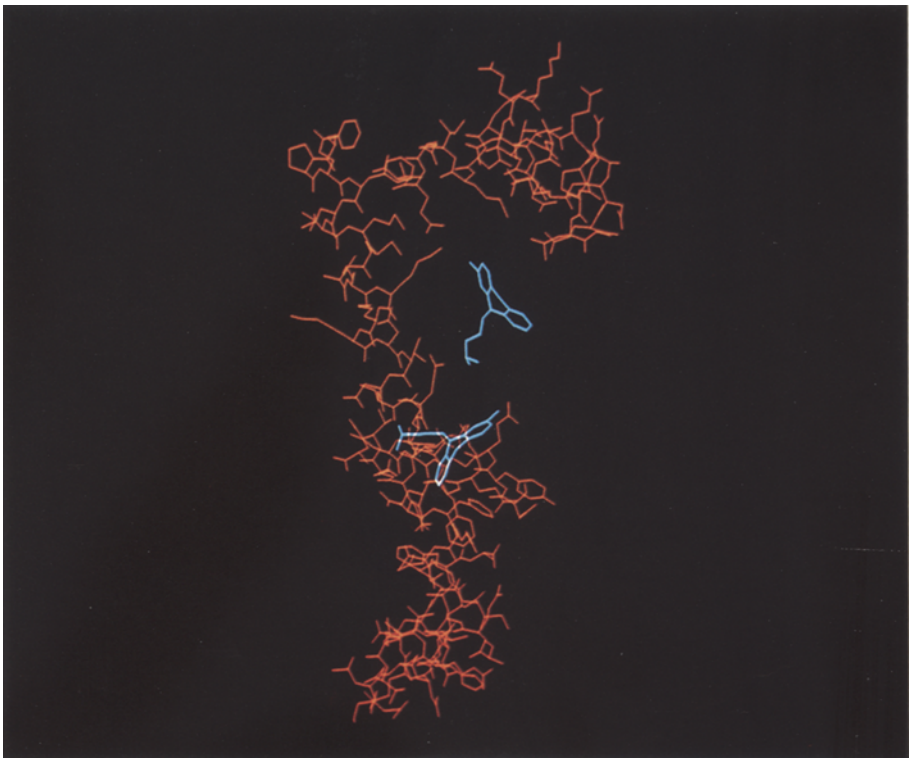


Fig. 3. The two proposed binding sites for phenothiazine drugs on calmodulin. The α-helices of calmodulin are shown in red and the drug molecules (chlorpromazine) in blue.

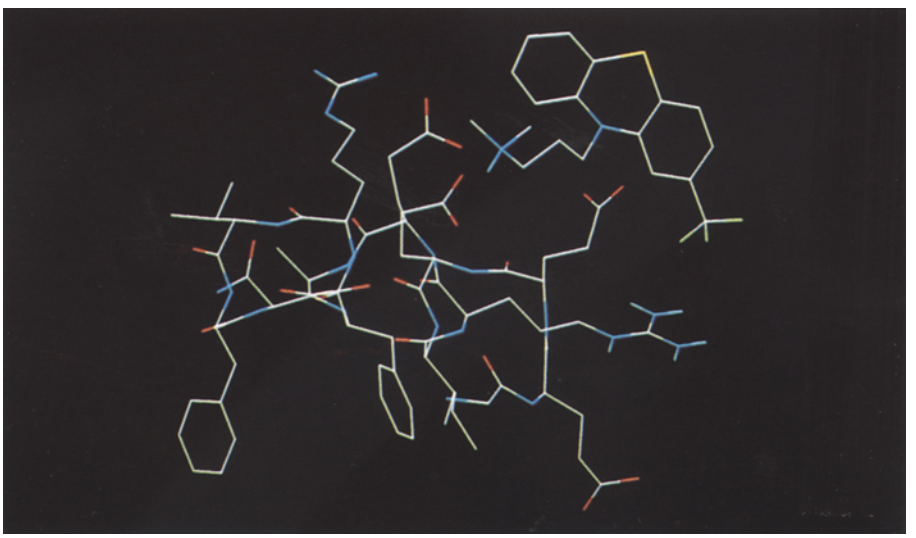


Fig. 4. The interaction between trifluprομazine and sequence 82–92 (color is coded by atom type; only the nitrogen-bound proton of the phenothiazine and the hydrogens of Arg-86 are shown).

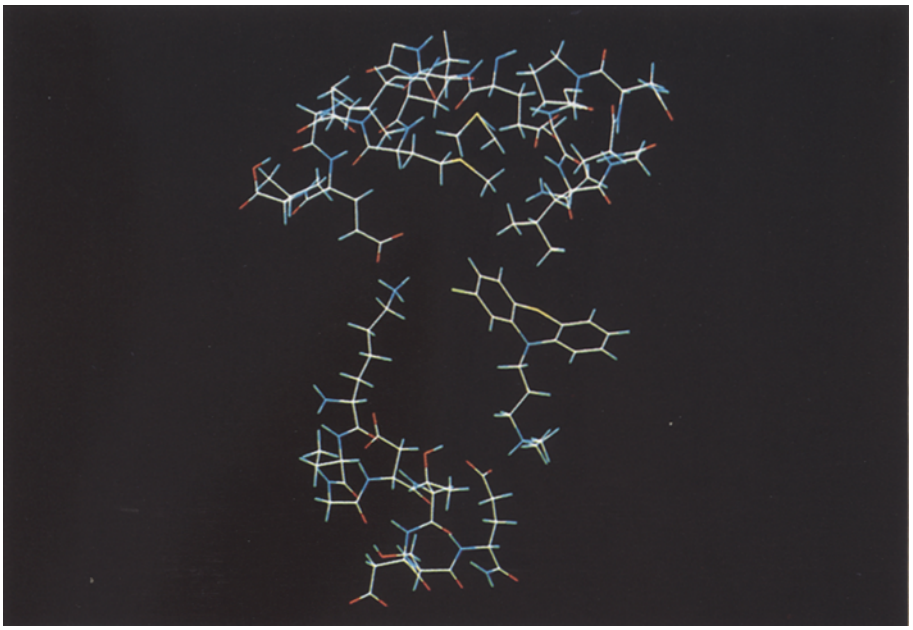


Fig. 5. The proposed second binding site for phenothiazines on calmodulin (chlorpromazine interaction complex).

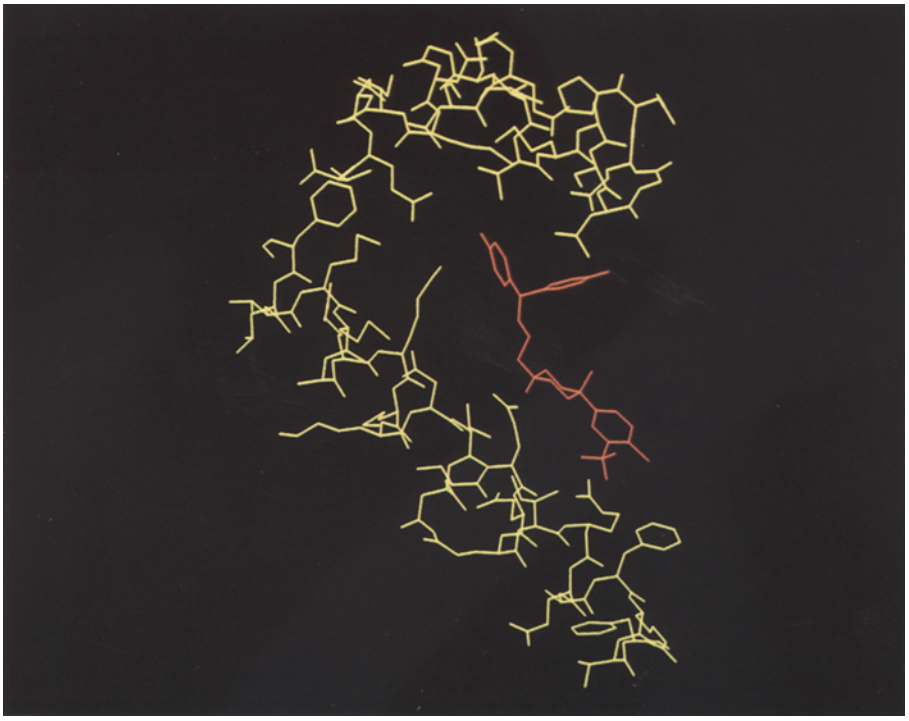


Fig. 6. Penfluridol binding site model (penfluridol = red; protein model = yellow).

Due to its size, penfluridol extends over a longer range of the central helix than the phenothiazine derivatives (Fig. 6) and, therefore, an additional binding contact to Arg-86 arises. As Arg-86 is part of the phenothiazine binding site I, this would easily explain why only one binding site was found for penfluridol experimentally [18].

Calculated interaction energies of seven phenothiazine derivatives and penfluridol for this binding site show an excellent correlation with the corresponding biological data (Fig. 7).

It was suggested that the increasing binding affinity in the series promazine > chlorpromazine > trifluprom azine is contributed by the increasing hydrophobicity [5]. However, in contradiction to this idea, the compound hydroxypromazine is less lipophilic than promazine but possesses higher calmodulin inhibitory potency than promazine. Our results indicate that the ring substituents bind to a basic amino-acid side chain protonated at a physiological pH. The formation of specific hydrogen bonds between the nitrogen-bound proton and the electron-rich substituents provides the necessary binding forces, resulting in a correct ordering of all phenothiazines according to their experimentally derived potencies.

There are some shortcomings and limitations in our interaction model. The choice of the amino-acid side chain conformations is necessarily somewhat arbitrary and does not consider their dynamic nature in solution.

The main problem with respect to the calculations of binding energies arises from the fact that the DOCK routine of SYBYL 3.5 can not perform a total relaxation of both binding partners but only a partial relaxation of the ligands. The result of this shortcoming can be studied at phenothiazine binding site I, for example. Here the DOCK routine only finds two binding contacts.

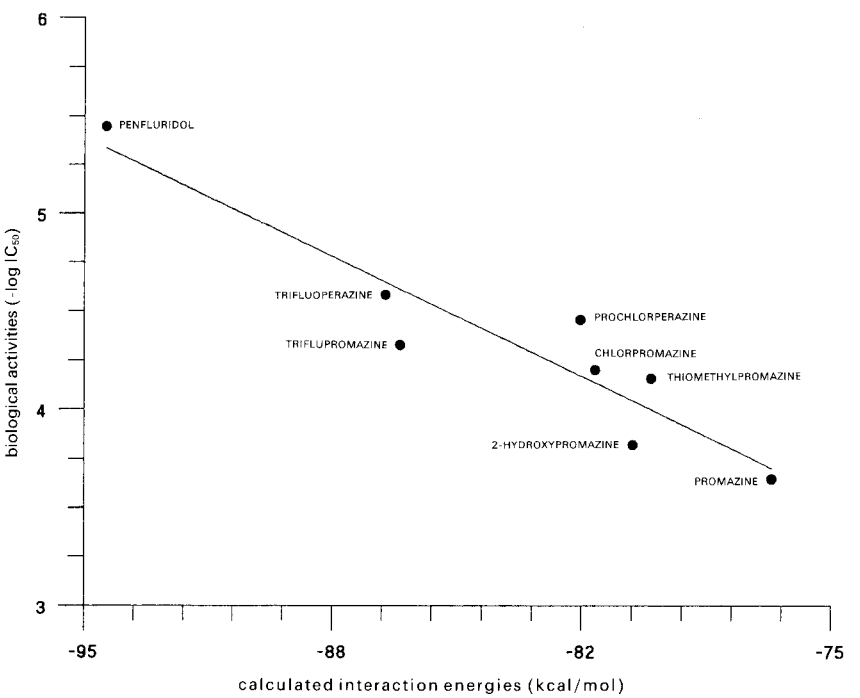


Fig. 7. Correlation between calculated interaction energies and biological activities ($-\log IC_{50}$). The correlation coefficient was 0.93.

Probably these are the most important ones but it appears to be unrealistic that other amino acids should not contribute to the overall binding. Another example of the same kind is the underestimation of the binding energies generated by the piperazine substituents of perazines at phenothiazine binding site II when compared to the binding strength of the simple dimethylamino group of promazines. Interestingly, this underestimation of binding strength due to the piperazine ring system is compensated, in the case of the trifluoperazine, by an overestimation of the contribution to binding of the trifluoromethyl group. The latter effect can be clearly recognized in the high binding energy of triflupromazine and may be accounted for by the charges at the trifluoromethyl group, which were calculated with the topological Gasteiger method.

When this paper was in preparation, Strynadka and James [19] published molecular modeling studies of troponin-C and calmodulin with two predicted binding sites for trifluoperazine on calmodulin. This study supports some of our results. Based on a highly refined X-ray structure of troponin-C and using the GROMOS molecular dynamics program, the authors postulated some extra hydrophobic contacts, in addition to the electrostatic interaction, that were also found in our study. Although these hydrophobic interactions could not be predicted by us due to the missing information about the correct positions of the side chains, our model seems to be capable of describing phenothiazine binding modes on calmodulin. It may not be correct, and is certainly not the only plausible model, but it allows us to explain satisfactorily the differences in binding energies of a series of phenothiazines and of penfluridol.

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