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Original article

Mechanistic aspects of benzothiazepines: A class of antiarrhythmic drugs

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Abstract

The authors have presented ab initio Hartree Fock calculations coupled with intermolecular interaction calculations to study mechanistic aspects of benzothiazepine class of calcium channel blockers. A channel model has been taken containing pore region glutamates and all three classes' sensing residues. Benzothiazepine drugs have been docked in and ternary complex (that is, drug …Ca^{2+}… channel model) stability has been studied and related to mechanistic aspects of these drugs.

Keywords: Benzothiazepines; Ternary complex; Ab initio; Hartree Fock; Diltiazem; DTZ323

1. Introduction

Three major classes of chemical compounds have been identified as antiarrhythmic drugs. These are phenylalkylamines, benzothiazepines and dihydropyridines. All three classes are calcium channel blockers and have been widely used as cardio-vascular drugs. Benzothiazepines have received lesser attention in the past and only few clinically proven drugs are known from this class (Fig. 1). Previous studies indicated the fused bicyclic structure and the amine nitrogen on the side chain of benzothiazepines as the essential pharmacophoric elements [1,2]. Two conformers have been reported in the X-ray crystallographic studies [3]. The H atoms on the chiral carbons 2 and 3 (cf. Fig. 1) in the seven membered heterocycle are in the *trans* and *cis* orientation in these conformers [3]. Both *cis* and *trans* conformers are biologically active; one being more potent than the other [4]. In the past some theoretical [5] and synthetic studies [2,6] have been performed to study their conformational aspects and point out pharmacophoric features. Lack of high affinity ligands has made it difficult to identify the binding site of BTZs. Each class of Ca^{2+} channel antagonist are known to bind to distinct binding sites within the $\alpha 1$ subunit of the L-type Ca^{2+} channel and to have a reciprocal allosteric interaction [7–9]. Calcium channel antagonists and their interaction with Ca^{2+} in low dielectric media has been studied using spectroscopic studies [10]. Past studies have observed and analyzed the formation of 2:1 drug–Ca^{2+} complex [11–13].

Regarding binding site, results of some photoaffinity labeling and immunoprecipitation studies have suggested that the BTZ's binding site is located in the linker region between segments S5 and S6 of domain IV [14]. SAR studies have indicated the importance of 2-aryl ring and basic amine in the side chain at N5 [2]. As indicated above, very little is known about the actual mechanism of action and potency regulation in benzothiazepines.

We wish to report in this study state of the art quantum mechanical calculations coupled with modeling techniques and intermolecular interaction calculations to arrive at some mechanistic aspects, which may help in understanding potency regulation in these drugs. We have explored the possibility of drug being anchored by a BTZ sensing residue and at the same time regulating ion flow by forming ternary complex. The stability of the ternary complex determines the possibility of such a mechanism and seems to be related to drug's potency.

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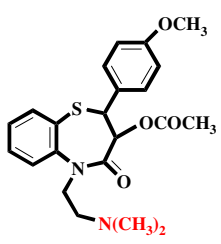
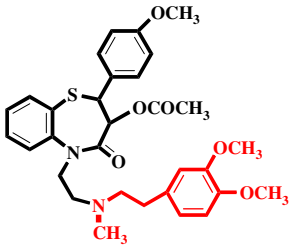


Diltiazem (DTZ)
IC_{50} = 337± 11nM



DTZ323
IC_{50} = 6.5 ± 0.2 nM

Fig. 1. Benzothiazepines as antiarrhythmic drugs.

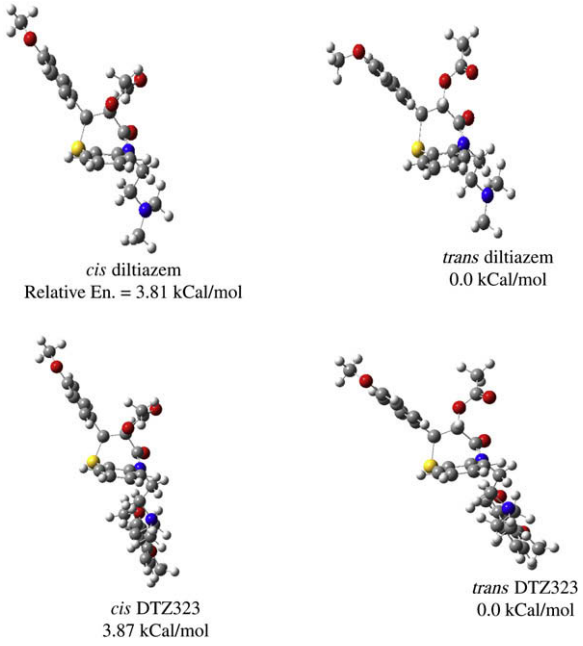


Fig. 2. Optimized conformations of DTZ and DTZ323.

2. Methodology

Ab initio Hartree Fock (HF) molecular orbital (MO) calculations have been performed on clinically used drugs (Fig. 1) from bezothiazepine subclass utilizing 6-31G basis set [15]. Complete geometry optimizations were performed using Berny's optimization procedure utilizing redundant internal coordinates [16,17]. These calculations were performed using Gaussian 2003 window's version [18]. To understand mechanistic aspects of these drugs a model of part of human calcium channel has been made by utilizing global multialignment of human (Q13936) [19–34] and rabbit (BL8) [35] calcium channel using Clustalw [36]. Homology modeling was done in collaboration with Dr. Sastry's group at Hyderabad [37]. Segments 5 and 6 of domains I–IV were modeled along with the pore region. These segments contain the sensing residues for the three subclasses of antiarrhythmic drugs. After making the model, hydrogens were minimized using Insight II program. The drugs were docked in one by one. Ca^{2+} ion was allowed in the channel. Ca^{2+} ion … channel … drug interaction energy was calculated using ab initio intermolecular interaction calculations (supermolecule approach, that is, interaction energy $= E_{\text{complex}} - (E_{Ca^{2+}} + E_{\text{channel}} + E_{\text{drug}})$). The interaction energy calculated indicates the stability of the ternary complex. The basis set superposition error has been corrected by Boy's Bernardi counterpoise correction method [38].

3. Results and discussion

Conformations obtained after complete geometry optimizations are shown in Fig. 2. Both the conformations 2,3 *cis*/*trans* are active; *cis* being more active as compared to *trans*.

A model of part of human calcium channel containing the sensing residues was made by homology modeling as explained in methodology section. Resulting, channel model is shown in Fig. 3. The pore region glutamates and the sensing residues for different classes of antiarrhythmic drugs are shown in the same figure.

For ab initio drug receptor interaction calculations we have extracted pore region of the channel, which is crucial for drug's activity containing pore region glutamates, benzothiazepine (BTZ), dihydropyridine (DHP) and phenylalkylamine (PAA) sensing residues. The extracted portion maintains the pore region so that the drug is docked in the natural environment and artificial accessibility is thus avoided. The extracted channel model, which is used in our calculations, is shown in Fig. 4. The criteria for extraction as mentioned above is to maintain benzothiazepine sensing residues' neighbouring environment as in the channel and be able to dock the drug in natural environment without removing any important binding interactions. It is to be noted that glutamate is ionized at physiological pH. Next we dock in the drugs one by one using Gaussview through the accessibility pathway.

After docking, the drug is allowed to be anchored to a BTZ sensing residue. The orientation of the drug is allowed to be changed several times and interaction energy is evaluated ab initio again and again until best interaction is observed with the channel model. Ca^{2+} is now allowed to flow in. The orientations of Ca^{2+} ion and drug are allowed to be changed (keeping the channel model frozen) until best ternary complex is observed. Huge amounts of reorganization of channel would be required only if drug undergoes conformational changes before binding to sensing residue. We have shown in our previous studies on phenylalkyl amines [39] that in phenylalkyl amines channel reorganization energy may be involved and may be important mechanistically as the drug undergoes conformational change induced by deprotonation when it diffuses through cell membrane to bind to intracellular sensing residue. In this study on benzothiazepines channel reorganization has not been considered. Channel reorganization here appears to be unlikely as there is practically no conformational change associated with protonation/deprotonation of benzothiazepines [40]. Past mechanistic probes have been concentrated in two directions, that is, drug being involved either in pore blocking [41] or drug involved in capturing calcium ions [42]. Before proceeding further with mechanistic investigations it is important to discuss the hydration state of ion inside the channel.

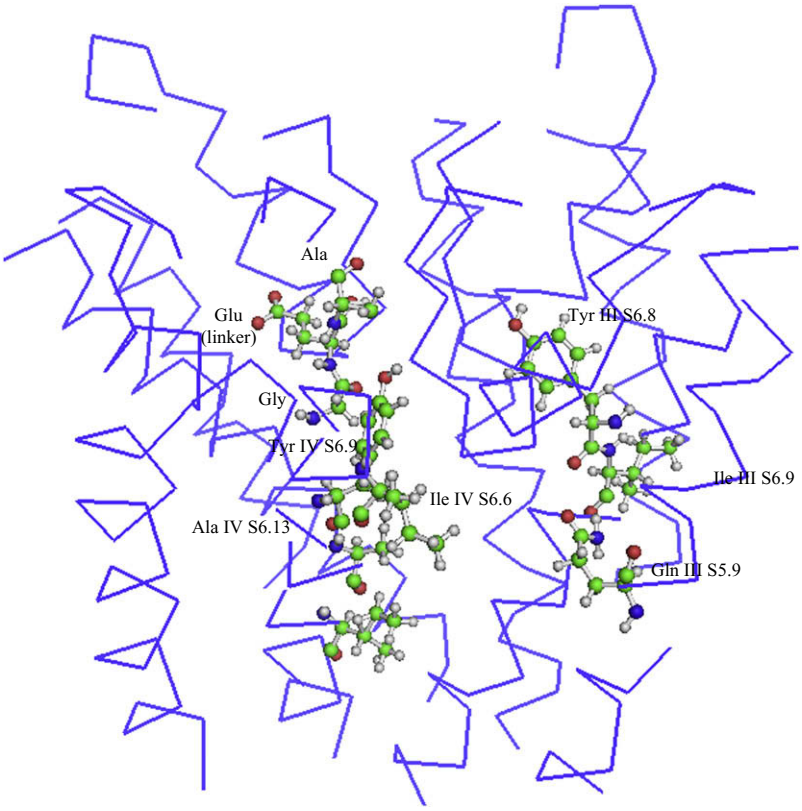


Fig. 3. Segments S5 and S6 of domain III and IV of L-type human calcium channel.

The concept of hydrated ion is only important/relevant at the stage of ion selectivity by selectivity filter. Any channel Na^+, K^+ or Ca^{2+} channel is permeable to both monovalent and divalent ions. The Ca^{2+} channel is able to select Ca^{2+} ions as it contains negatively charged glutamates in the pore region. The monovalent cations are not able to compete with divalent cations for this site and are hence left out. It has been observed experimentally that mutating pore lining glutamate to lysine in calcium channel changes selectivity of channel [32]. The converse has also been observed through mutational experiments [43] in sodium channel. In light of above discussion it is easy to visualize Ca^{2+} influx in channel.

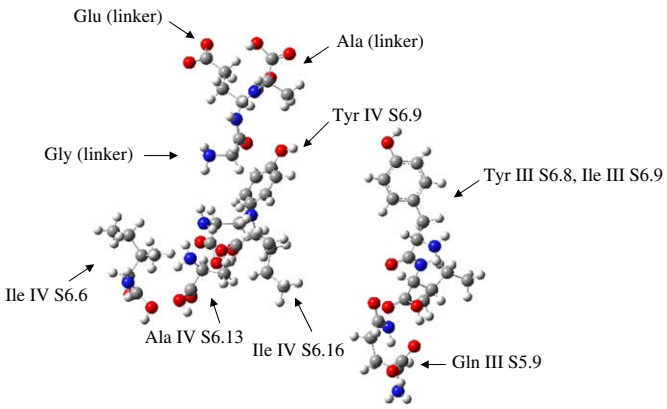
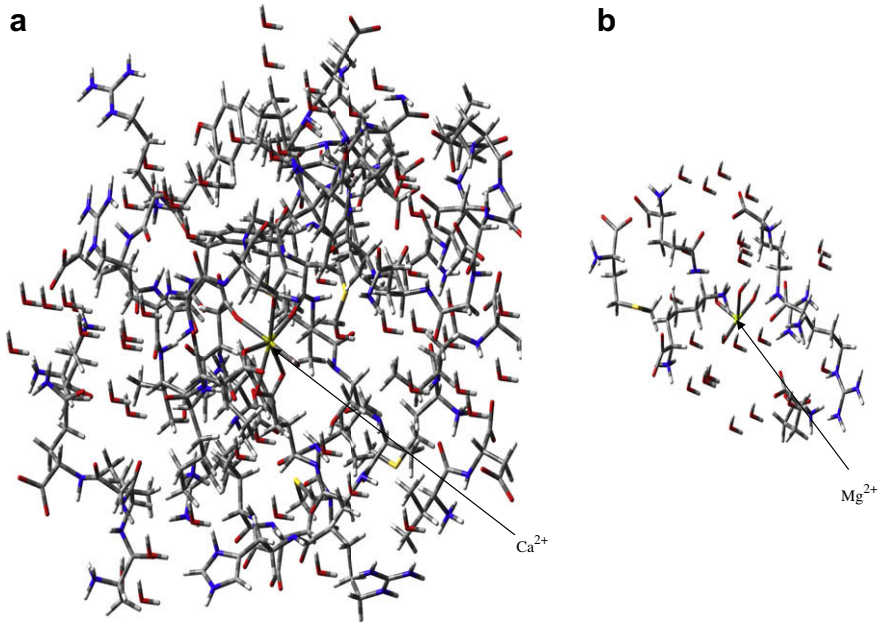


Fig. 4. Extracted channel model.

Some ions interacting with glutamate sites, rest maintaining high throughput flow through channel due to ion–ion repulsion. This has also been observed through electrophysiological measurements [44]. Calcium ions held by glutamates inside channel can also be seen/viewed through GAUSSVIEW in X-ray data available for calmodulin bound to cardiac calcium voltage gated channel [45]. Fig. 5 depicts two snapshots from this pdb file; 5a shows Ca^{2+} ion held inside the voltage gated channel and 5b shows hydrated Mg^{2+} ion outside the channel. We have calculated hydration energy for Ca^{2+} ion (cf. Fig. 6b) and also interaction energy of Ca^{2+} ion held inside the same voltage gated channel (2F3Y.pdb). Fig. 6 clearly indicates the benefit acquired by the ion on entering the voltage gated channel. Hence, the ion prefers to be desolvated first and then stabilized on entering the channel. The same has been observed in the past by other workers [46,47].

In ligand gated channels the drug is anchored to sensing residue. It is therefore obvious to foresee a ternary complex being involved mechanistically which would be experimentally very difficult to explore. In this study we have theoretically explored the possibility and stability of such a complex.

The most stable ternary complex formed with DTZ is shown in Fig. 7. Exactly the same procedure is followed to study the ternary complex formed between DTZ323, channel model and calcium ion. The best ternary complex observed is shown in Fig. 8. The calculated interaction energy between the drug, channel model and the Ca^{2+} ion indicates the stability of the ternary complex. The magnitude of interaction



Ca^{2+} ion held inside channel (2F3Y. pdb)

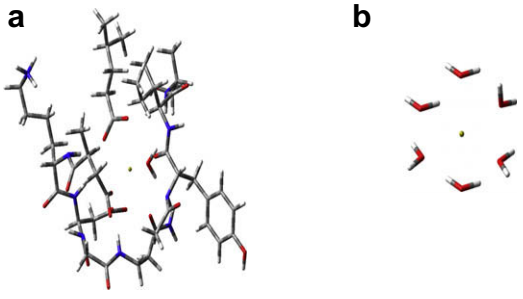
Mg^{2+} ion left outside channel (2F3Y. pdb)

Fig. 5. Divalent cation held inside and outside voltage gated channel.

energies observed is indicative of electrostatic interaction between the divalent ion doubly charged and the pore region glutamate (which is ionized at physiological pH) supplemented by the drug molecule's ion holding capacity and ability to interact with the channel (for example, Drug DTZ's ion holding capacity is in the range −81 to −101 kcal/mol). The interaction energies after basis set superposition error (BSSE) correction are also given in brackets. It is encouraging to note that BSSE is about the same in the two cases. The drug molecule conformation also helps in coordinating the ion while remaining anchored to a sensing residue. The interaction energies observed reaffirm the suggestion that ternary complex stability could be related to mechanistic aspects of these drugs as compared to earlier suggestions of single role of drug that is, either pore blocking or ion holding. At this stage we are not trying to correlate IC_{50} with ternary complex stability. More work is in progress along these lines.

4. Conclusions

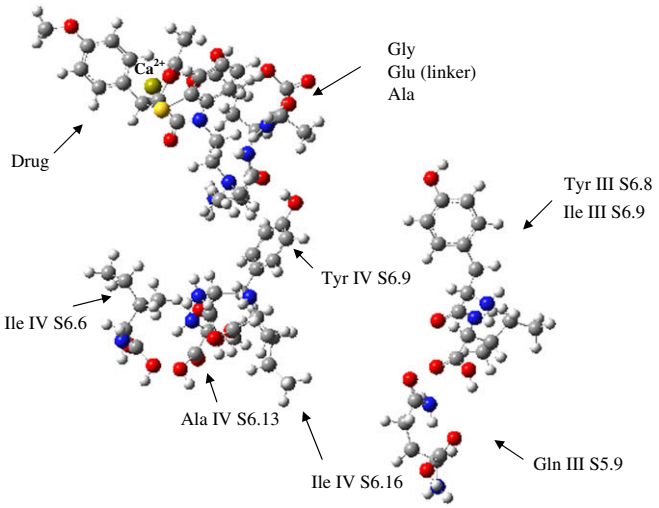
The authors have reported state of the art ab initio Hartree Fock M.O. calculations coupled with accurate intermolecular interaction calculations to gain some insight into mechanistic aspects of benzothiazepine subclass of Ca^{2+} channel blockers. It is suggested that ternary complex stability (i.e. stability of complex formed by drug, channel model and ion) could be related to mechanistic aspects of these drugs.



Int. En. = -621.59 kCal/mol

Int. En. = -178.95 kCal/mol

Fig. 6. Solvation of ion inside and outside channel.



Int. En. = -351.48 kCal/mol (-281.37) with BSSE

Fig. 7. Ternary complex formed by DTZ, channel model and Ca^{2+}.

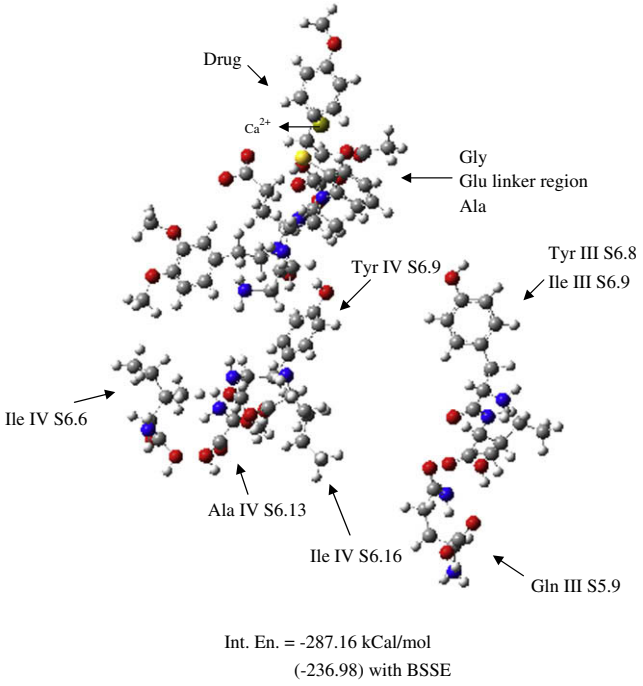


Fig. 8. Ternary complex formed by DTZ323, channel model and Ca^{2+}.

Acknowledgements

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