In chapter 3 and 4 we have discussed the conformational and mechanistic aspects of 1,4-DHP's. In this chapter we will discuss the other subclass of Ca\(^{2+}\) channel blockers, known as Benzothiazepines (BTZ’s). Diltiazem is prototype for BTZ’s. This subclass has received lesser attention in the past.

### 6.1 Previous Conformational Studies on Benzothiazepines:

Diltiazem is clinically proven drug of this subclass. For the standard numbering of this compound please refer to Fig. 2. Some experimental studies have been done regarding the conformational features of this class of compounds, including NMR and X-ray crystallographic studies.

A new pharmacophoric model of the Benzothiazepine (Diltiazem) binding site on the L-type Ca\(^{2+}\) channel was derived by
using the SYBYL software package [1]. It was hypothesized that the essential pharmacophoric elements are two aromatic rings and protonated amine nitrogen. Several lines of evidence later supported this pharmacophore hypothesis. Ansari et al [2] also studied crystal structure of diastereomers of 4-(2-hydroxyphenyl)-2-phenyl-2, 3,4,5-tetrahydro-1, 5-benzothiazepine, C$_{21}$H$_{19}$NOS that are cocrystallized. They reported that the asymmetric unit contains two molecules. One S and two C atoms of the heterocyclic ring in one molecule are disordered, resulting in two different conformers. The H atoms at the chiral centers in the ordered molecule are ‘trans’ with respect to each other, ‘cis’ in the other conformer.

Manivannam Muthukumar et al. [3] reported the molecular and supramolecular structure of the three N-acyl-C-phenylated tetrahydrobenzothiazepines, and compared these with the simpler analogue, which is unsubstituted at the N atom and whose structure has been reported by Laavanya et al. [4]. By synthesis of analogs of Benzothiazepines and study on them by various research workers has given some information regarding conformational features of this drug [5].
Theoretical study was performed to generate a pharmacophore model for chemically diverse structures that specifically interact with the Diltiazem binding site of L-type calcium channel [6]. This yielded a pharmacophore model with three crucial pharmacophoric characteristics (i) two aromatic ring systems in a distance of about 6.7 Å, (ii) a basic side chain with pKa in the physiological range and (iii) a 4'-methoxy moiety. In addition a strong MEP in 4-position (carbonyl oxygen) and hydrophobic electron-rich features in the position equivalent to the sulphur atom of BTZ derivatives were explored to be favourable for receptor binding and calcium antagonistic effect.

**6.2 Receptor Bound Drug and Its Antagonistic Activity:**

Each class of Ca$$^{2+}$$ channel antagonist exhibits distinct characteristics in their tissue selectivity [7]. They 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 [8]. A detailed structural study has been done, using spectroscopic techniques on calcium channel antagonists and their interactions with Ca$$^{2+}$$ in low-dielectric
media [9]. V.S. Ananthanarayanan et al. [10] analyzed the Drug : Ca\(^{2+}\) complex by using method of Ruben [11], which provides for the following multiple equilibria:

\[
2(\text{drug}) + \text{Ca}^{2+} \rightleftharpoons [(\text{drug})_2 \cdot \text{Ca}^{2+}]
\]

\[
[(\text{drug})_2 \cdot \text{Ca}^{2+}] + \text{Ca}^{2+} \rightleftharpoons 2[\text{drug} \cdot \text{Ca}^{2+}]
\]

This is 2:1 drug-Ca\(^{2+}\) complex and known as the “ion sandwich” complex [12]. The physiological relevance for the Ca\(^{2+}\) bound structure of Diltiazem may be sought in terms of the role of Ca\(^{2+}\) in the drug’s interaction with the receptor (namely, the Ca\(^{2+}\) channel). They also suggested that the drug is capable of entering the lipid bilayer with the cation bound in 2:1 drug-Ca\(^{2+}\) complex. They had visualized that the drug could therefore interact at the receptor site, presumed to be hydrophobic from the temperature-dependent binding data in the Ca\(^{2+}\) bound form.

The binding site for BTZ has not been identified because of lack of high affinity ligands for the BTZ site. Results of some
photoaffinity labeling and immunoprecipitation studies have suggested that the binding site for diltiazem-like Ca^{2+} channel blockers is located in the linker region between segments S5 and S6 of domain IV [13]. Previous functional studies revealed that a membrane permeable Diltiazem analogue can access its site only via an extracellular permeation pathway [14]. Each drug type has separate but overlapping or allosterically linked binding sites in Ca^{2+} channels. Many residues important for binding of Ca^{2+} channel antagonists have been identified and substantial progress has been made in characterizing the Ca^{2+} channel pore structure. If available pore models are accurate then they should predict a drug binding region that can be experimentally tested.

Extensive analysis of Benzothiazepine’s structure-activity relationships identified the methoxy group (hydrogen acceptor) on the 2-aryl ring and the basic amine in ethyl linkage at N₅ as the critical pharmacophores for high affinity interaction with the channels BTZ-binding domain [5b]. Branus et al. [15] have shown that introduction of bulky side chains at some distance from the basic amine yield
potent Ca\(^{2+}\) antagonists that bind with high affinity to partially purified L-type Ca\(^{2+}\) channels.

Benzothiazepines exist in the protonated form at physiological pH, and its pKa have been reported to be 8.91 \[16\].

IC\(_{50}\) value of Diltiazem in µM is 56 ± 8; which is the mean ± standard error of four cells \[17\].

### 6.3 Our Results and Discussion:

#### 6.3.1 Conformational and Electrostatic Aspects:

The ORTEP plots of completely optimized conformations of Diltiazem in both protonated and unprotonated forms have been developed and are shown in Fig. 21. The energetically optimized global minimum in both the cases is (2R, 3R) conformation, which can also be called as the ordered molecule according to Ansari et al \[2\]. In absence of decisive knowledge on bioactive conformation we shall consider the global minimum on potential energy hypersurface as the bioactive form. Protonation/deprotonation has been considered at the basic amine in the ethyl linkage side chain on N\(_5\) as it was found to be the critical pharmacophore in earlier studies \[5b\].
Protonated Diltiazem

Unprotonated Diltiazem

Fig. 21 ORTEP Plots of optimized conformations of Diltiazem
We have now mapped the unprotonated form of Diltiazem onto protonated form to observe the effect of deprotonation on diltiazem's conformational features (Fig. 22). Mapping indicates very little overall conformational change as it only affects locally the side chains. In the optimized conformation of Diltiazem the two aromatic rings are 6.5 Å apart in conformity with pharmacophore modeling studies of Schleifer [6]. Based on our optimized conformations we also predict that the two aryl rings should be disposed at an angle of 50.41°.

The charge environment of the drug was studied using MESP map. The MESP indicates an overall negatively charged environment on drug as shown in Fig. 23. Charge complementarity helps us understand that drug will interact with receptor residues having slightly positive or neutral charge environment.

The drug of this subgroup exists in protonated form at physiological pH. It may be anchored to the receptor via H-bonding to some proton acceptor group (e.g. tyrosine in ionized form). The H-bonding is suggested via basic amine in ethyl linkage at N₅ in conformity with earlier studies.
Fig. 22 Unprotonated Diltiazem Mapped onto Protonated Diltiazem

Fig. 23 Molecular Electrostatic Potential Maps
6.3.2 Ca\textsuperscript{2+} Ion Holding Capacity:

The Ca\textsuperscript{2+} ion holding capacity of the drug has been studied via intermolecular interaction calculations. The relative capacity of the drugs to hold the Ca\textsuperscript{2+} ion has been investigated by calculating intermolecular interaction energies at the 3-21G level using a supermolecular approach. The geometry of the complex has been completely optimized. The lowest energy complexes and corresponding interaction energies are collected in Fig. 24. The ion can be seen approaching the drug towards carbonyl at C\textsubscript{4} and its ester substituent at 3 position. Ion should not be covalently bound as we are mimicking the fact that drug can hold the ion and also release the ion if needed (only this constraint has been applied while optimizing the position of Ca\textsuperscript{2+} ion). Results indicate that Ca\textsuperscript{2+} ion prefers to be held in a position where it can optimally electrostatically interact with all the neighbouring oxygen atoms. Such ideal location is between ester substituent at C\textsubscript{3} and the carbonyl at C\textsubscript{4}. In this position it shows maximum attractive interaction energy -101.1 kCal/mol, that is, the most favourable position for the ion.
Fig. 24 Intermolecular Interaction Energy Calculation for Diltiazem...Ca^{2+} Complex
6.4 Conclusion:

1. The most stable conformation of Diltiazem is the (2R, 3R) conformation, which is also referred to as the ordered conformation.

2. The pharmacophoric features extracted indicate that two aromatic rings should be about 6.5 Å apart in conformity with earlier studies. The aromatic rings should also preferably be disposed at an angle of 50.41°.

3. The drug exists in protonated form at physiological pH. It may be anchored to the receptor via H-bonding to some proton acceptor group. It is suggested that the basic amine in the side chain at N₅ could act as H bond donor keeping the upper part of the drug free for holding the calcium ion.

4. Unprotonated form is capable of trapping Ca²⁺ ion utilizing strong electrostatic interactions via carbonyl at C₄ and ester substituent at 3 positions.
References:


   


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