Molecular Modeling Study of Diltiazem Mimics at L-Type Calcium Channels

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Purpose. A 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 channels.

Methods. Via molecular mechanics and quantum chemical methods solvation energies, logP values, conformational and electronic features of classical 1,5-benzoazepin-4(5H)-one (BTZ, e.g., diltiazem), 1-benzazepin-2-one (BZ), pyrrolo[2,1-d][1,5]benzoazepine, pyrrolo[2,1-c][1,4]benzoiazine, and benzocyclrocyclo[2.2.2]octyl amines derivatives were determined. Furthermore, the molecular electrostatic potentials (MEPs) and common interaction fields derived from use of the GRID program were compared.

Results. This yielded a pharmacophore model with three crucial pharmacophoric characteristics, (1) two aromatic ring systems in a distance of about 6.7 Å, (2) a basic side chain with pK_a in the physiological range, and (3) a 4'-methoxy moiety. In addition, a strong negative 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. Moreover, the stabilizing effect of substituents in 3-position of BZs on the bioactive “M” twist-conformation of the heptagonal ring could be demonstrated by molecular dynamics simulations.

Conclusions. Based on these molecular descriptors, the quinazolinone derivative MCI-176 is predicted to be a potential ligand of the diltiazem binding site.

KEY WORDS: molecular modelling; calcium entry blocker; 1,5-benzoazepin-4(5H)-one; quinazolinone; MCI-176; voltage-gated calcium channels.

INTRODUCTION

Calcium entry blockers, such as 1,4-dihydropyridines (DHPs, e.g., nifedipine), phenylalkylamines (PAA, e.g., verapamil) and benzoazepines (BTZs, e.g., diltiazem) inhibit the intracellularly directed calcium flux through L-type voltage-gated calcium channels (VGCCs) (1,2). This feature makes them indispensable in the therapy of cardiovascular diseases like hypertension or angina pectoris. Their molecular targets are distinct, but allosterically coupled, high-affinity binding sites on the a1-subunit of VGCCs where they induce stabilization of the inactivated-closed channel mode (3). While DHP and PAA binding sites, respectively, are only sensitive to structurally closely related congeners, a competitive character to the BTZ binding site is described for a multitude of chemically diverse compounds. Besides naturally occurring benzylisoquinolines (e.g., papaverine) (4) or aporphine derivatives like apomorphine (5–7), special effort was directed towards the synthesis of new ligands by structural variation of the benzoazepine scaffold of diltiazem leading to sulphur-free benzazepines, pyrrolo-fused BTZ and benzoazepine derivatives. A further simplification of these heterocyclic compounds yielded the carbocyclic benzobicyclo[2.2.2]octyl amines that still produce full BTZ-like activity. Due to many cases of serious adverse effects that have been reported during the therapy with diltiazem, including cutaneous vasculitis (8), thrombocytopenia (9), heart block (10), parkinsonism (11) and even fatal renal and hepatic toxicity (12), the development of novel drugs with enhanced efficacy is justified.

In order to explore the critical determinants of BTZ-like derivatives that contribute to high-affinity binding with the binding site, a molecular modeling study will be presented describing the generation and detailed characterization of a BTZ pharmacophore model.

MATERIALS AND METHODS

Ligands

The X-ray structure of diltiazem hydrochloride (Fig. 1) was extracted from Cambridge Structural Database (13,14). To eliminate short atom–atom contacts and conformational distortions produced by intermolecular interactions in the crystal lattice, diltiazem was geometry optimized using 100 iterations of the steepest descent algorithm.

For construction of all 1,5-benzoazepin-4(5H)-one (BTZ, Table I), 1-benzoazepin-2-one (BZ, Table I) and pyrrolo[2,1-d][1,5]benzoazepine derivatives (PBTZ, Table II) the “M” twist-conformation was fixed for the heptagonal rings (see Fig. 1), since this conformation is consistently found in all derivatives with a 3-substituent cis-oriented relative to the adjacent phenyl ring.

Pyrrolo[2,1-c][1,4]benzoazaines (P6Zs, Table II), benzocyclo[2.2.2]octyl amines (BOAs, Table III) and the quinazolone derivative MCI-176 were generated employing the BUILDER module of the SYBYL software package (21). All investigated compounds were considered in the protonated cationic form, because a pK_a of 7.7 was detected experimentally for diltiazem (22), and substitution of the basic amine against a neutral amide function decreases binding affinity of BZs by approximately two orders of magnitude (16).

Conformational Analysis

Applying the SEARCH option within SYBYL a systematic conformational search for low-energy conformations was performed. To avoid exclusion of potential conformations in this early stage, electrostatic interactions have been neglected (i.e., atomic charges were not considered). In addition, the general and the 1–4 vdW-factor were scaled down to 75% and the vdW radii of hydrogen atoms were set to 1.2 Å. Flexible bonds were fully rotated using rotational increments of 1° or 10°. Conformations with energy higher than 5 kcal/mol above the absolute energy minimum were discarded. Using the programme IXGROS (23) the remaining conformers were grouped into common families belonging to identical local minima. The lowest energy member of each family was subsequently energy...
ring B, the spiro-linked compound 5e as the most rigid derivative was subjected to a systematic conformational search yielding an optimum dihedral angle \( \phi \) of \(-42^\circ\) (Fig. 2a).

Subsequently, BTZ, BZ, PBTZ and B6Z derivatives were superimposed onto ring A of 5e and all conformations derived from the search procedure were screened (e.g., Fig. 2b) to find a common energetically favourable arrangement of all B rings. Furthermore, this analysis yielded low-energy conformations placing the ammonium functions almost identically when compared to the template (Fig. 3). For this sterical fit only 4’-methoxyxylated derivatives were used, since structure-affinity/activity relationships (SAR) of BZs reveal this particular substituent as being optimal (compare 2h-2l in Table I).

Molecular Characterization of the BTZ Pharmacophore Model

To explore essential molecular features of the preliminary sterical pharmacophore model and for characterizing potential non-covalent interactions with the binding site, hydrophobic (DRY) and alcoholic (O1_{OAc}/O1_{Ac}) probe molecules of programme GRID (26) were employed. Application of the DRY probe (Fig. 4a) indicates favourable hydrophobic interactions parallel to the aromatic rings and close to the sulphur atom of BTZ, PBTZ and B6Z derivatives. With the O1_{OAc} probe a potential hydrogen bond acceptor region is observed that is connected with the free electron pairs of the 4′-ether oxygens (Fig. 4b). Comparison of BZs, which possess H-bond capacity (2h-2k) with the 4′-ethyalted derivative 2l indicates this property to be more essential for calcium antagonism than for binding (Table I). On the other hand, lack of the terminal methyl group (i.e., 2i) leads to a pronounced loss of binding affinity, implicating both hydrogen bonding and hydrophobic contacts to be important for an accurate description of the ligand/receptor interaction.

Analysis of the fields obtained with O1_{Ac} probe reveals a common potential hydrogen bond donor region at the protonated ammonium functions (Fig. 4b). By the same token, these catonic groups cause strong positive MEPs (Fig. 5). Therefore, hydrogen bonding and/or electronic forces might be involved in interactions with the binding site in this region. For BZ derivatives with structurally modified side chains, a remarkable decrease in activity is observed if the dimethylamino function (2g) is substituted against amide (2q), aldehyde (2r), hydroxyl (2s), or carboxylate (2t) moieties (see Table I). Although the negative charge of 2t at pH 7.4 (carboxylate anion) might prevent access to the lipophilic binding site, the neutral derivatives 2q and 2s should be able to reach their molecular targets. However no substantial difference is detected for these potential hydrogen donors (2q and 2s) compared to moieties, which lack this feature (2r). Furthermore, it was shown that in preparations of fragmented cell membranes receptor affinity of the quaternary BZ 2u differs only by a factor of eleven compared with the tertiary amine 2h (16). These findings indicate electrostatic forces rather than hydrogen bonding interactions as important features to stabilize the ammonium group at the binding site.

The carbonyl oxygens of BTZs and BZs induce strong negative potentials (Fig. 5). Interestingly, also the pyrrole rings of PBTZ and B6Z derivatives produce similar electrostatic fields, although their intensity is weaker. Besides the common

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Fig. 1. X-ray structure of diltiazem hydrochloride (code CEYHUJ01) extracted from Cambridge Structural Database (13,14).