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Pharmacophore modelling of structurally unusual diltiazem mimics at L-type calcium channels

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Summary

The purpose of this theoretical study was to investigate the molecular features of some structurally unusual calcium antagonists with experimentally proved affinity to the diltiazem-binding site at L-type calcium channels. Therefore, sterical and electronic characteristics of *cis-/trans*-diclofurime, the verapamil-like derivatives McN-5691 and McN-6186 as well as the natural products papaverine, laudanosine, antioquine and tetrandrine were compared with the pharmacophoric requirements detected for classical diltiazem-like derivatives. This yielded a common pharmacophore model for all of these compounds. Based on this model, one single negative molecular electrostatic potential induced by the free electron pairs of the oxime oxygen of *trans*-diclofurime was detected that might be responsible for the stronger effects compared to the cis isomer. Furthermore, the dual diltiazem- and verapamil-like features of McN-5691 (and McN-6186) as well as the distinct pharmacophoric assignment of the laudanosine enantiomers may be interpreted on a molecular level. Finally, the crucial partial structure of the bis-benzylisoquinoline derivatives antioquine and tetrandrine being responsible for the calcium antagonistic effects could be revealed by superposition on the most active benzothiazepinone derivative 8-methoxydiltiazem. The results obtained for these unusual diltiazem mimics are discussed taking into consideration earlier findings for classical diltiazem-like derivatives.

Abbreviations: BTZ, benzothiazepine; DHP, 1,4-dihydropyridine; MEP, molecular electrostatic potential; PAA, phenylalkylamine; VGCC, voltage-gated calcium channel.

Introduction

Diltiazem, as the most prominent representative of the so-called calcium entry blockers from benzothiazepine (BTZ) type, is therapeutically used for the treatment of cardiovascular diseases like hypertension or angina pectoris. Its pharmacological function is the stabilization of the inactivated-closed channel mode of transmembrane L-type voltage-gated calcium channels (VGCCs) [1] that control the passive flux of Ca^{2+} into the cytoplasm and thereby regulate the essential intracellular calcium balance [2].

Structural variations of the thiazepine ring of diltiazem led to sulphur-free 1-benzazepin-2-ones, pyrrolo[2,1-*d*][1,5]benzothiazepines, pyrrolo[2,1-*c*][1,4]benzothiazines and carbocyclic benzobicyclo[2.2.2]octyl amines that all exert almost identical effects compared to the BTZ template. Several studies [3–6] considering such BTZ-like derivatives yielded a pharmacophore model with three crucial pharmacophoric characteristics, (i) two aromatic ring systems at a distance of about 6.7 Å, (ii) a basic side chain with $pK_a \approx 8$, and (iii) a 4′-methoxy moiety. Our investigations [7] provided two additional pharmacophoric elements, a strong negative molecular electrostatic potential at the 4-position (carbonyl oxygen) and electron-rich features coupled with a hydrophobic environment in the position equivalent to the sulphur atom of BTZ derivatives. Moreover, molecular dynamics simulations could demonstrate the stabilizing

effect of substituents at the 3-position on the bioactive 'M' twist-boat conformation of the heptagonal ring of 1-benzazepin-2-ones [7].

On the other hand, there is a multitude of further non-BTZ-like derivatives where competitive binding to the BTZ site has been experimentally proved. Besides the naturally occurring benzylisoquinolines [8] (represented by papaverine) or bisbenzylisoquinolines [9–11] (e.g., antioquine and tetrandrine), especially some synthetic compounds like *trans*-diclofurime [12, 13] or McN-5691 [14] have to be mentioned in this respect.

The aim of the present study was to investigate these unusual diltiazem mimics in order to compare their molecular features with the postulated pharmacophoric requirements of typical BTZ-like derivatives.

Methods

Ligands

The X-ray crystal structures of papaverine (**1**, code MVERIQ01), papaverine hydrochloride (code PAPAVC), coclaurine (**2**, code CLARBH), three further 1,2,3,4-tetrahydroisoquinoline derivatives (codes BIMROE, HIGENB and MXPHIQ01) and diacetylantioquine (**3**, code SENCET) were extracted from the Cambridge Structural Database [15, 16]. Tetrandrine (**4**) was constructed according to the published data of its X-ray structure [17] (Figure 2). To eliminate short atom-atom contacts and conformational distortions produced by intermolecular interactions in the crystal lattice, all X-ray structures were geometry optimized using 100 iterations of the steepest descent algorithm.

For the construction of laudanosine (**5**), *cis*-diclofurime (**6**), *trans*-diclofurime (**7**), McN-6186 (**8**), and McN-5691 (**9**), the BUILDER module of the SYBYL software package [18] was employed (Figure 2).

Conformational analysis

Applying the SEARCH option within SYBYL a systematic conformational search for low-energy conformations was performed for compounds **1**, **2**, **5**, **6** and **7**. Since experimental results provide evidence that the binding site of diltiazem is buried within the lipophilic membrane [1], these calculations were carried out in vacuum. On the other hand, it was proven that a positively charged nitrogen is essential for BTZ-like calcium antagonistic activity [19, 20]. The chosen environment (gas phase), however, is not appropriate for the simulation of charged molecules [21]. Therefore, the conformational search was carried out applying the protonated form of all ligands in order to consider the sterical bulk of the nitrogen-linked hydrogen atom, but without consideration of charges.

To avoid exclusion of potential conformations in this early stage, the general and 1-4 vdW-factors were scaled down to 75%. In addition, 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 [22] the remaining conformers were grouped into common families belonging to identical local minima. Afterwards, the lowest energy member of each family was energy minimized applying the conjugate gradients algorithm until a maximum convergence criterion of 0.05 kcal/(mol Å) was reached. Geometry of the force field optimized structures was controlled via comparison with the AM1 [23] optimized conformations using the best force field geometry as input for the semi-empirical approach. In order to investigate the highly flexible derivative **9** (11 rotatable bonds), molecular dynamics simulation (mds) was performed for 100 ps within the TRIPOS force field [18]. Use of a simulated annealing protocol with an initial temperature of 600 K and a target temperature of 0 K yielded an ensemble of 50 frozen conformations. Subsequently, each conformation was geometry optimized by use of the conjugate gradients algorithm until a maximum convergence criterion of 0.05 kcal/(mol Å) was reached.

Superimposition method

To compare the geometries of the putative bioactive conformations a rigid least-squares superimposition was carried out. Therefore, three crucial fitting regions were chosen consisting of (i) ring A, (ii) ring B including the oxygen atom of the 4′-methoxy group, and (iii) the amine function.

Molecular Electrostatic Potentials (MEPs)

In order to investigate molecular electrostatic potentials (MEPs) the SPARTAN software [24] was employed to derive ESP partial charges from wave functions of restricted Hartree–Fock (RHF) ab initio calculations applying the 6-31G* basis set.

Hardware

All computations were carried out on SGI Indigo^2 R10000, SGI O2 R10000 and SGI Indy R4600 workstations.

Results and discussion

Diclofurime

In the early 1980s *trans*-diclofurime (**7**) has been claimed to be a calcium antagonist but only in 1987 Spedding confirmed this hypothesis by functional in vitro and in vivo tests [12]. At the same time Mir [13] characterized **7** as a potent displacer of [^{3}H]diltiazem binding (IC_{50} = 15 nM relative to 55 nM for diltiazem).

In order to elucidate the bioactive conformation of **7** the classical BTZ derivative diltiazem – in its postulated bioactive conformation – was used as a template. Considering the critical 4′-methoxy substituent of the dichloromethoxyphenyl ring of diclofurime, this moiety was superimposed over ring B of diltiazem (see Figure 1). In this superposition the distance between the aromatic rings of diclofurime is much smaller (∼4.7 Å) than found for classical BTZ-like derivatives (6.7 Å). The results of conformational analyses indicate that, independent of the investigated isomer, the diethylammonium group may occupy identical energetically favourable positions above and below the ring systems. In agreement with the postulated importance of the electrostatic interactions exerted by the sulphur atom of diltiazem, the furan oxygen in a favoured *E*-configuration relative to the oxime group induces a comparable negative MEP in the equivalent region (Figure 3).

Since **6** and **7** can produce almost identical spatial arrangements, one reasonable explanation for the stronger effects of the trans isomer might be the negative MEP generated by the oxime oxygen, which may substitute for the potential attributable to the carbonyl oxygen of diltiazem (Figure 3).

*McN-6186 (**8**) and McN-5691 (**9**)*

The structural formulae of **8** and **9** resemble the classical phenylalkylamine (PAA) calcium channel blocker verapamil (**10**, see Figure 2), but both compounds have been identified experimentally as competitive inhibitors of [^{3}H]diltiazem binding [14, 25]. However, also an atypical feature was observed in regard to their influence on the binding of 1,4-dihydropyridines (DHPs, e.g. nifedipine). Usually, BTZs act as positive allosteric modulators of DHP binding [1]. In contrast, **9** causes negative allosteric modulation of DHP binding, this being a typical verapamil-like characteristic [1]. Therefore, compound **9** may be assumed as a hybrid derivative with PAA and BTZ features. While the arylalkylamine partial structure of both molecules is quite similar, one striking difference is the phenethynyl-substituted phenyl ring of **8** and **9** (compare Figure 2). Thus, it is likely that BTZ-like effects of **9** can be attributed to this unique structural element, whereas the residual part of the molecule should be responsible for PAA-like behaviour. In an attempt to confirm this assumption, a conformational analysis of the highly flexible derivative **9** (11 rotatable bonds) was carried out via molecular dynamics simulation.

The geometry-optimized conformers yielded by this procedure were superimposed onto the most rigid spiro-linked benzobicyclo[2.2.2]octyl amine derivative which is illustrated in Figure 1. This demonstrated that the dihedral angle between rings A and B (≈ −20°) and the distance between the ring centroids (McN-5691 6.9 Å vs. template 6.5 Å) are almost identical. The conformer fulfilling the pharmacophoric requirements most accurately is only 2.6 kcal/mol less favourable compared with the global minimum conformer. Superimposition of this conformer onto the rigid template shows a good fit of aromatic rings (A and B), amine functions and the 4′-methoxy groups (Figure 4) resulting in an rms value of 0.53 Å.

In this arrangement, the ammonium-linked dimethoxyphenethyl moiety of McN-5691 is placed almost parallel to ring A at a distance of 4.3 Å, but its flexibility observed in mds would also allow occupation of various other positions. At this point, it is worth mentioning that there are known 1-benzazepin-2-ones with quite similar amino-linked phenylbutyl moieties being almost as potent as the classical dimethylammonium substituted derivatives [3].

*Benzylisoquinoline derivatives papaverine (**1**) and laudanosine (**5**)*

Although the effects of all investigated benzylisoquinolines are weaker and not as selective in relation to classical BTZ-like derivatives, specific binding of such structures to VGCCs has been observed [26]. Therefore, **1** and its tetrahydro congener **5** (Figure 2) were examined in order to identify potential calcium

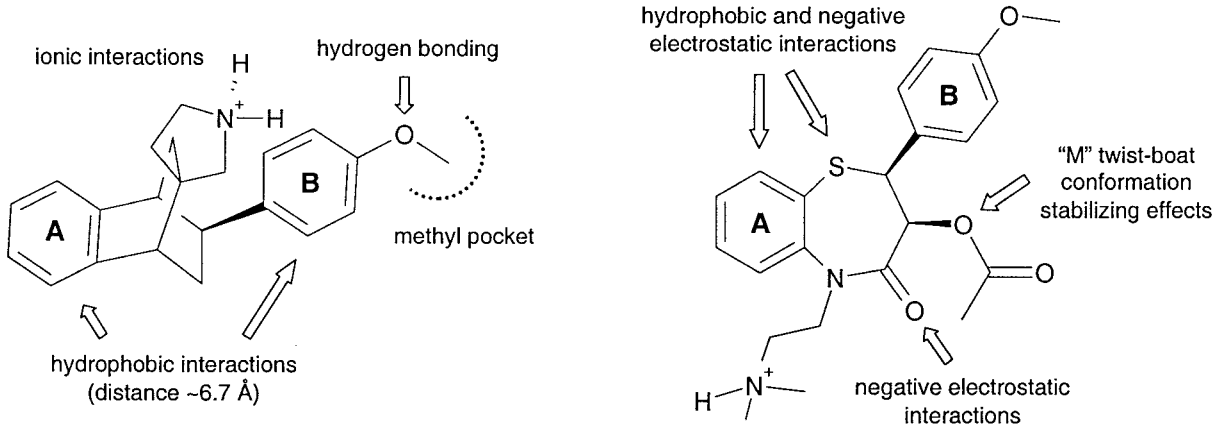

Figure 1. Spiro-linked benzobicyclo[2.2.2]octyl amine derivative representing minimum requirements for specific BTZ binding (left). Structural formula of diltiazem with indicated additional molecular features favourable for binding and calcium antagonistic effects (right).

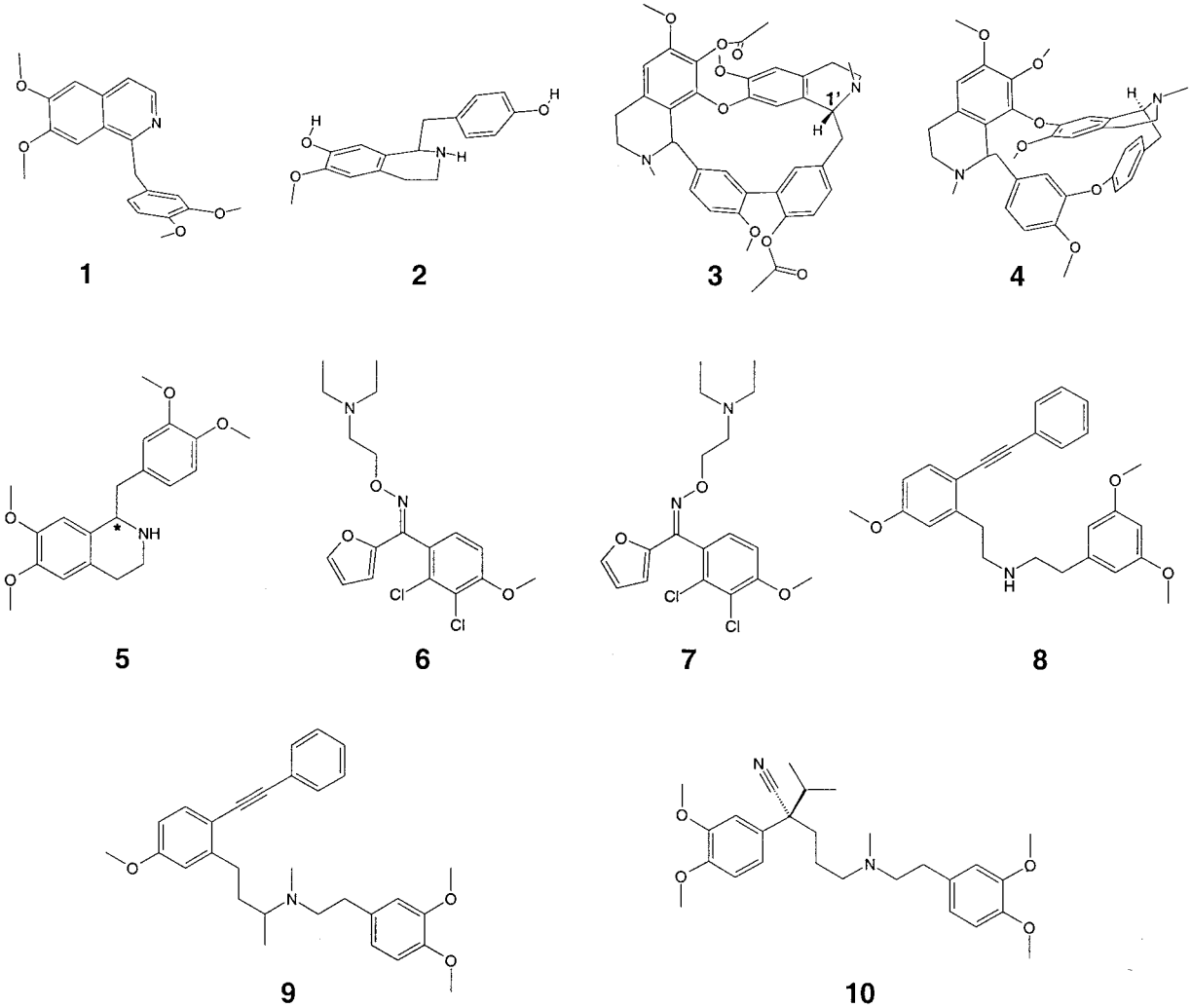

Figure 2. Displayed are the X-ray crystal structures of papaverine (**1**), coclaurine (**2**), diacetylantioquine (**3**), tetrandrine (**4**) and the structural formulae of (R/S-)laudanosine (**5**), *cis*-diclofurime (**6**), *trans*-diclofurime (**7**), McN-6186 (**8**), McN-5691 (**9**), and the calcium entry blocker from PAA-type (S-)verapamil (**10**).

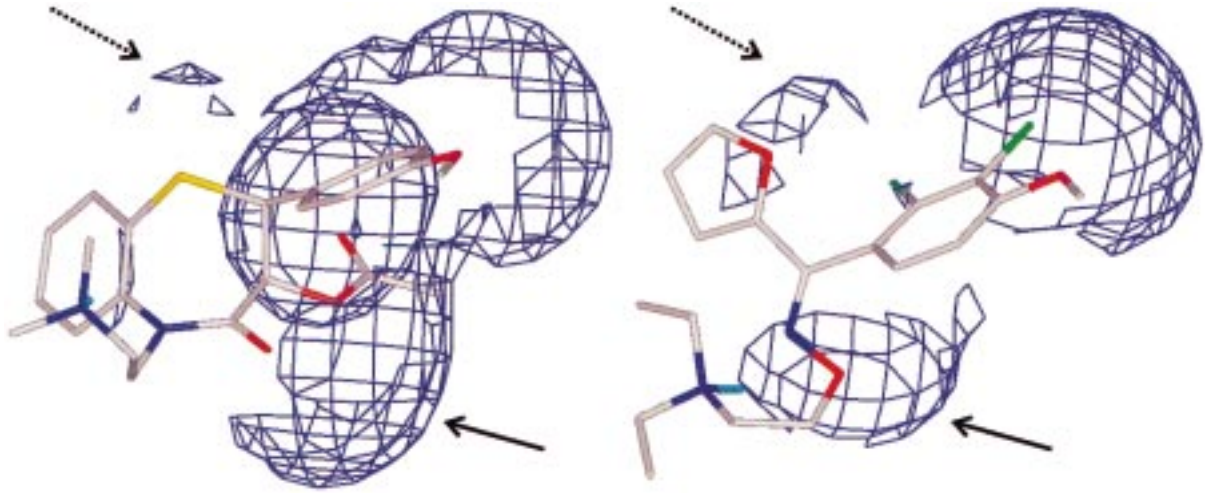

Figure 3. The grid fields represent negative molecular electrostatic potentials (MEPs) of diltiazem (left) and the global minimum conformation of *trans*-diclofurime (right) contoured at a level of -0.8 kcal/mol. Dotted arrows highlight the MEPs indicating a favoured *E*-configuration of the furan oxygen relative to the oxime group. The solid arrows depict equivalent MEPs that could explain the stronger effects of *trans*-diclofurime compared to the cis isomer. For clarity, only the nitrogen-linked hydrogens are displayed (colour code: carbon grey, chlorine green, nitrogen blue, oxygen red, sulphur yellow, and hydrogen cyan).

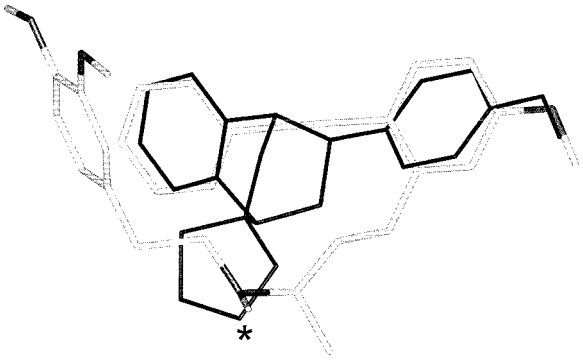

Figure 4. Superimposition of an energetically favourable conformation of McN-5691 (**9**) onto a rigid spiro-linked benzobicyclo[2.2.2]octyl amine BTZ mimic (dark, see Figure 1) yielding an rms value of 0.53 Å. The nitrogen atom of the template is indicated with an asterisk ($*$).

channel modulating conformations. Full rotation of the aryl linking methylene bonds yielded several low energy conformers of **1** orientating the aromatic rings very similar as found in BTZ-like derivatives. However, due to the flat aromatic isoquinoline ring system, the amine function cannot be positioned above the ring plane as usually observed for diltiazem-like derivatives.

For the accurate construction of the partially saturated isoquinoline heterocyclic ring system of **5**, the X-ray crystal structures of four tetrahydroisoquinoline derivatives (e.g., coclaurine (**2**) and higenamine) from the Cambridge Structural Database [15, 16] were used. Different from **1**, the structural formula of **5** contains one stereogenic center. Interestingly, our analyses indicate that both isomers are able to satisfy the essential pharmacophoric requirements. But while for (S-)**5** the tetrahydroisoquinoline ring can be superimposed over ring A of 8-methoxydiltiazem (rms $= 0.97$ Å), the R-enantiomer has to be flipped around to fit the pharmacophore in a head-to-tail orientation (rms $= 0.95$ Å). In this arrangement the dimethoxybenzyl moiety of (R-)**5** matches ring A, whereas the dimethoxytetrahydroisoquinoline mimics ring B of 8-methoxydiltiazem (Figure 5). Both alignments performed with energetically accessible conformers (2.9 kcal/mol over the global minimum conformation) yield optimal positions of the methoxy groups not depending on the investigated enantiomer.

However, also in case of the tetrahydroisoquinoline derivatives the protonated amine functions are fixed near the plane of the isoquinoline ring, suggesting that far-reaching electrostatic interactions rather than hydrogen bonding interactions are involved in receptor binding for these BTZ-site ligands. Unfortunately, the differences of pK_a values for **1** (pK_a 6.4) and **5** ($pK_a \approx 8.0$) may not be used to clarify the influence of the protonation state in relation to calcium antagonistic activity, because only functional experimental data are available [8, 27]. Thus, additional effects of **1** (e.g. cyclic AMP phosphodiesterase inhibition) could not

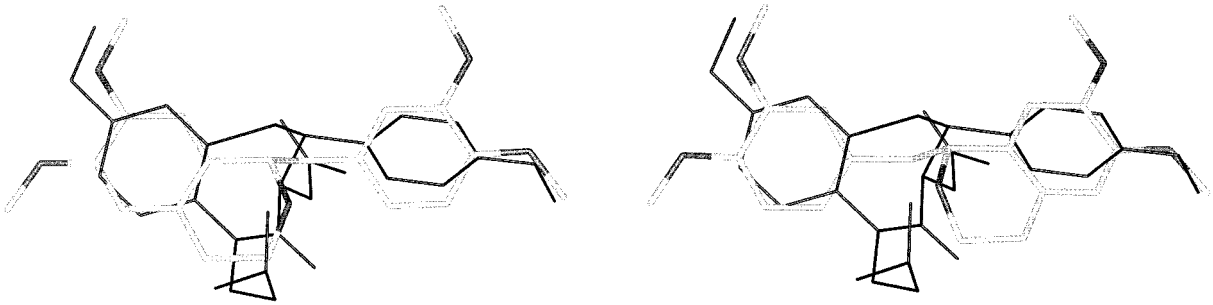

Figure 5. Superimposition of the S- (left) and the R-isomer (right) of laudanosine (**5**) onto the most active BTZ derivative 8-methoxydiltiazem (dark). The rms values are 0.97 Å and 0.95 Å, respectively.

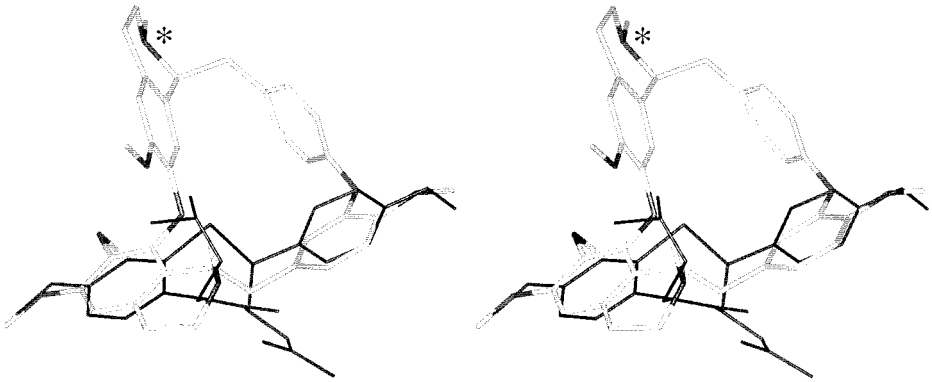

Figure 6. Stereoplot of the superimposed derivatives tetrandrine (**4**, grey) and 8-methoxydiltiazem (dark) yielding an rms value of 0.87 Å. The amine function of **4** with increased basicity is indicated with an asterisk (∗).

completely be excluded. Nevertheless, it is interesting to note that the weaker base (**1**) was more potent than **5** (IC_{50} 2.7 μM vs. 17.2 μM), possibly due to better penetration into the cytoplasm.

*Bis-benzylisoquinoline derivatives antioquine (**3**) and tetrandrine (**4**)*

Calcium antagonism of the natural products **4**, extracted from the Chinese medicinal herb *Radix stephaniae tetrandrae*, and of **3**, the main alkaloid of *Pseudoxandra sclerocarpa*, is well established [10, 11]. Furthermore, there are results showing direct interaction of **4** at the BTZ binding site leading to the BTZ-typical stimulation of DHP binding [9]. Based on the above-described findings for benzylisoquinoline derivatives this is not surprising, since both alkaloids are composed of two almost identical benzylisoquinoline moieties. Differences exist in monomer linkage (biphenyl vs. diaryl ether), aromatic substitution pattern (methoxy vs. hydroxy group), and most strikingly in the configuration of C1′ leading to different relative orientations of the monomers (see Figure 2).

In order to determine the benzylisoquinoline moiety which is responsible for the channel modulating effect, a superimposition was accomplished using the most active BTZ derivative 8-methoxydiltiazem as a template. This revealed that in contradiction to earlier findings [28] not the benzylisoquinoline with the more basic amine function, which is highlighted in Figure 6 [17], but the opposite moiety of **4** complies with the pharmacophoric criteria. The superimposition (rms = 0.87 Å) shows similar positions of the aromatic rings, the essential 4′-methoxy substituent and a second methoxy group that increases the effects of 8-methoxydiltiazem by more than one order of magnitude compared to the unsubstituted diltiazem (Figure 6). In addition, both amine functions are pointing into the same direction although the alicyclic amine of **4** is fixed close to the plane of the isoquinoline ring.

This assignment is supported by the fact that only in this arrangement common positions for the methoxy groups of **4** and the template are found. Moreover, only the superimposed benzylisoquinoline moiety is identically arranged for **3** and **4**. Consequently, it is likely that similarly (and not differently) posi-

tioned moieties of these bis-benzylisoquinoline derivatives are responsible for the calcium antagonistic effects. The decreased basicity of the involved pharmacophoric amine function compared to the second amine group of tetrandrine may not refute this assignment (compare derivative **1**). Of course, a $pK_a \approx 8$ induces an optimal equilibrium between cationic and neutral form at physiological conditions (pH = 7.4). On the other hand, for derivatives with lower basicity the equilibrium is shifted in the direction of the neutral form, which probably can preferentially gain access to the membrane buried binding site. In contrast, derivatives with increased basicity are almost quantitatively protonated and may hardly penetrate into the membrane.

Conclusions

Summarizing the findings, for all investigated molecules low energy conformations could be detected that are in general agreement with the pharmacophore model of classical BTZ-like derivatives. However, the results obtained for **6** and **7** indicate that the postulated essential distance of about 6.7 Å between the aromatic rings (A and B) is not absolutely crucial.

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