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# The Biological Activity of Cyclic Bis(bibenzyls): a Rational Approach

György M. Keseru[a,b]* and Mihály Nógrádi[a]

[a]*Department of Chemical Information Technology, Technical University of Budapest, Szt. Gellért tér 4. H-1111, Budapest, Hungary*

[b]*Research Group for Alkaloid Chemistry of the Hungarian Academy of Sciences, POB 91, H-1521, Budapest, Hungary*

**Abstract**—The biological activities reported for marchantin A (**1**), a natural cyclic bis(bibenzyl), were studied in comparison with cepharanthine (**2**), a therapeutically useful bisbenzylisoquinoline alkaloid. Based on the examination of steric, electrostatic, and hydrophobic similarity, as well as on the comparison of biological activities, the similar therapeutic properties of **1** and **2** can be attributed to binding on a common receptor. The wide range of activity of **1** can be interpreted by a mechanism of action based on a calcium binding.

## Introduction

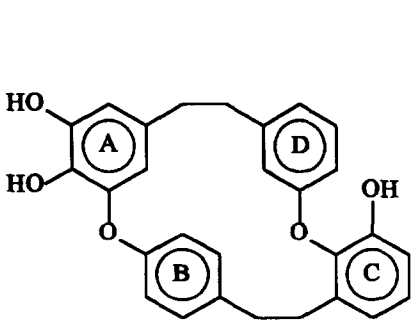
Extensive use of liverwort extracts in oriental medicine prompted the isolation of pure components.[1,2] In addition to terpenoids, phenolics, and lipids, numerous bis(bibenzyls) have been isolated from various liverwort species.[3] Cyclic bis(bibenzyls) belong to a new class of natural products having two diarylether, or a diarylether and a biphenyl bond in their macrocyclic skeleton. Systematic studies on the chemistry of bis(bibenzyls) were initiated by the wide range of their biological activity. Cytotoxic (antitumor and antileukemia) antibacterial, antifungal, 5-lipoxygenase, and calmodulin inhibitor activity have been reported,[4] however, the mechanism of action is still unclear.[5] Biological studies have mainly concentrated on marchantins, compounds constituting the most populous skeletal type of cyclic bis(bibenzyls). Marchantin A (**1**) has been found to be the most effective compound in preliminary pharmacological trials (Fig. 1).

Marchantins show close similarity to some macrocyclic bisbenzylisoquinoline alkaloids which are also characterized by two diarylether moieties joined by ethano bridges. This analogy was suggested by Asakawa *et al.* as an explanation of the muscle-relaxant activity of **1**,[6] however, no systematic study has been reported yet. We provide quantitative evidence in this paper for the analogy mentioned above and give a tentative explanation for the biological activity of marchantins.

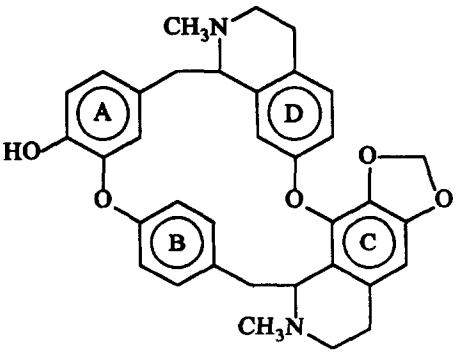
## Results and Discussion

### *Conformational analysis*

Bisbenzylisoquinoline alkaloids represent the largest group of compounds among the isoquinoline alkaloids, with more than 350 compounds belonging to this class. Tubocurarine, thalicarpine, tetrandine, and cepharanthine are typical members of this family having interesting pharmacological properties.[7] Our work was started by searching for a biologically active bisbenzylisoquinoline alkaloid having a constitution similar to that of **1**. This led us to cepharathine (**2**), because in both **1** and **2**, the pattern of aromatic-carbon




**1**




**2**

**Figure 1.** Constitutions of marchantin A (**1**) and cepharanthine (**2**).

atoms forming the macrocyclic rings, as shown in Figure 1, is the same: A(*meta*)–B(*para*)–C(*ortho*)–D(*meta*). First the minimum energy conformers of **1** and **2** were found.

Conformational search of cyclic molecules is a challenging field of computational chemistry. A number of techniques based on torsional angle variation has been developed, including a highly efficient Monte Carlo energy minimization method, termed Monte Carlo multiple minimum search (MCMM), which was devised by Chang *et al.*[8,9] The generation of conformers in MCMM was solved by the 'ringmaker' approach.[10] This method proceeds by defining a 'closure bond' at which the macrocyclic ring will be temporarily opened. A random variation of the remaining torsional angles is followed by the reclosure of the ring resulting in conformations which are subjected to energy minimization. Goodman and Still recently introduced a new procedure termed systematic unbounded multiple minimum search (SUMM).[11] In the SUMM algorithm, the random generation of the torsional angles was replaced by the selection from a fixed set of torsional angles appropriate for a systematic search. SUMM was found to be superior to MCMM and, therefore, this more sophisticated multidimensional method was chosen for the conformational search of **1** and **2**.

Conformational analysis of **1** and **2** was started from their X-ray structures.[6] More than 10 and 16 million conformers were generated and 364 and 312 enantiomeric conformations were stored, respectively. Rejections were based on failure of ring closure, unfavorable van der Waals interactions and the energy window. Distributions of unique conformations of **1** and **2** are shown in Figure 2. Lowest energy conformations (Fig. 3) had $E_1 = 144.0$ kJ mol$^{-1}$ for **1** and $E_2 = 261.2$ kJ mol$^{-1}$ for **2**.

Minimum energy conformations of **1** and **2** were compared to the corresponding X-ray structures based on the positions of all atoms involved in the macrocyclic ring. The average deviations of atoms were found to be 0.448 and 0.348 Å (rms values of 0.743 and 0.686 Å), respectively. In addition to the minimum energy conformations other low energy conformers may play an

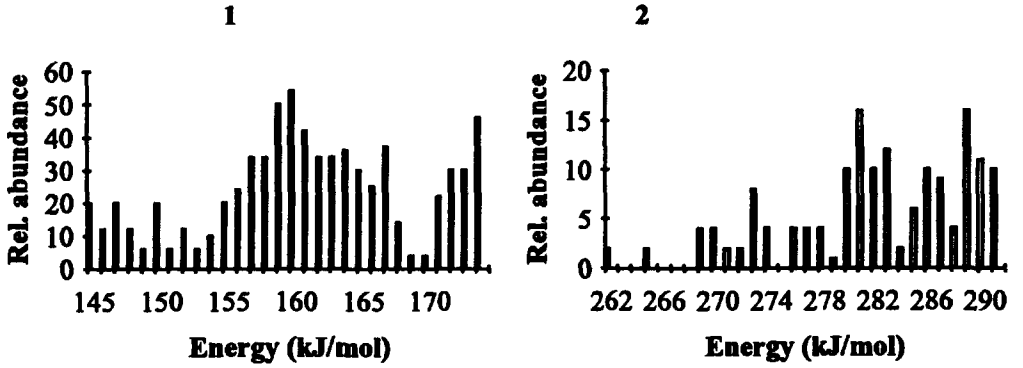



**Figure 2.** Distribution of conformations for **1** and **2** obtained by SUMM calculation.

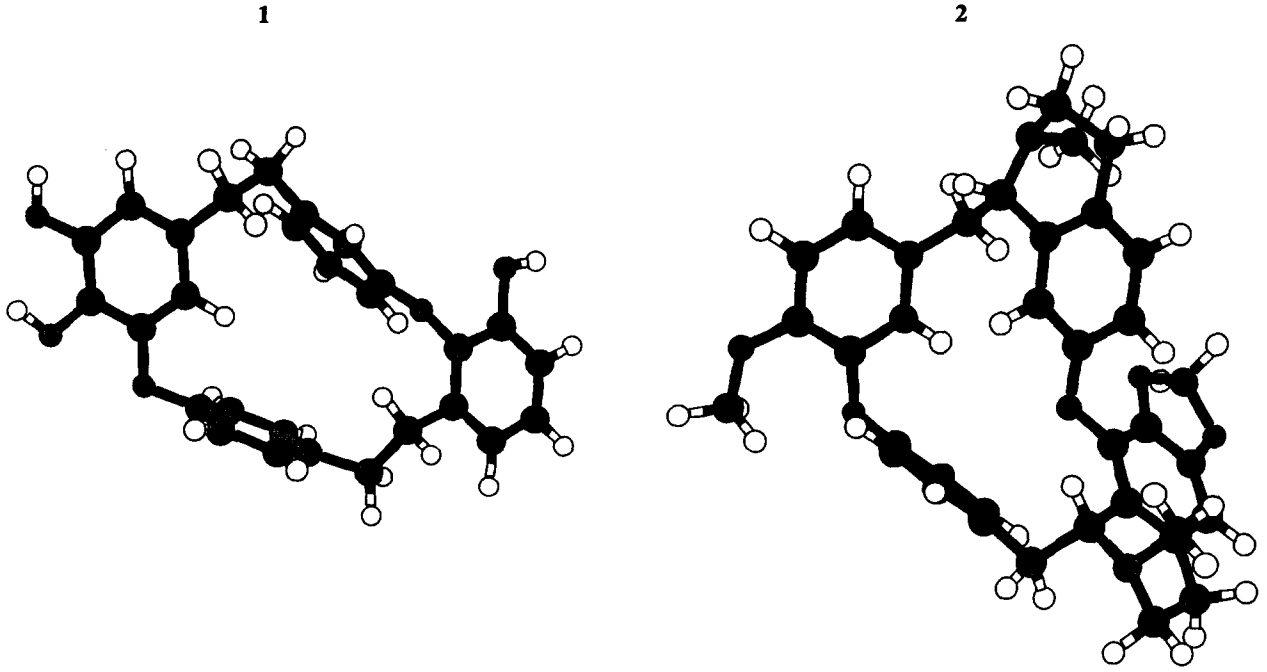



**Figure 3.** Minimum energy conformations of **1** and **2**.

equally important role in the recognition process. Therefore, low energy conformers of **1** and **2** (up to $\Delta E = 5$ kJ mol$^{-1}$ from the corresponding global minimum) were further analyzed. Minimum energy conformations of **1** and **2** were compared to the corresponding sets of multiple conformations which was obtained by the SUMM method. Compare–Conformer,[12] a computer program for the rapid comparison of con-formers allows us to select a set of characteristic torsional angles around the macrocyclic ring which were used in the pairwise comparing algorithm. Employing tolerances of 15 and 30 degrees, 14 and 29, conformers of **1** (38 and 78%, of a total of 37) were located. Similarly, the torsional anglematching of **2** resulted in 59 and 92 (51 and 79%) out of the total of 116 low energy conformers fitted in the range of 15 and 30 degrees, respectively. These results suggest that there is no significant difference between the conformations of the macrocyclic ring in the low energy regions and, therefore, the minimum energy conformers represents all the steric effects which may be important in molecular recognition.

According to the key-lock hypothesis of Fisher,[13] biological activity of an agent can be explained by molecular recognition between the active agent and the biomolecule responsible for implementing the effect. In addition to steric complementarity, electrostatics play a dominant role in molecular recognition.[14] In most cases, when the structure of the biomolecule is unknown, its activity can only be predicted by an indirect method. In this approach several active compounds assumed to bind to the same active site are compared. Molecular similarity can be defined on the basis of complementarity as the combination of at least three types of interactions: steric, electrostatic, hydrophobic.[15] This combination can also be used as the criteria of similarity.

The site of action affected by cyclic bis(bibenzyls) has not been established and therefore the evaluation of molecular similarity was carried out by using the indirect method in order to find out whether there is an analogy in the mechanism of action of **1** and **2**.

*Steric similarity*

The minimum energy conformations of **1** and **2** were superimposed (Fig. 4) and the positions of all heavy atoms were compared. The average deviation of atoms was found to be 0.264 Å corresponding to an rms value of 0.295 Å. The minimum energy structures were very similar for the two molecules and superposition of rings A, B and D yielded excellent fit (rms values 0.096, 0.105, and 0.112 Å, respectively), while overlap of the C rings was less close (rms = 0.387 Å).

Steric volumes were measured by the calculated total area on the van der Waals surfaces of **1** and **2** (Table 1). Areas of polar van der Waals surfaces were found to be nearly identical for two molecules. The cavity sizes of the macrocyclic rings of **1** and **2** were also compared and the centroid–centroid distances between the benzene rings were collected in Table 2. These distances indicate that the holes on the van der Waals surfaces of macrocycles **1** and **2** are of very similar volume that can play an important role in complexation.

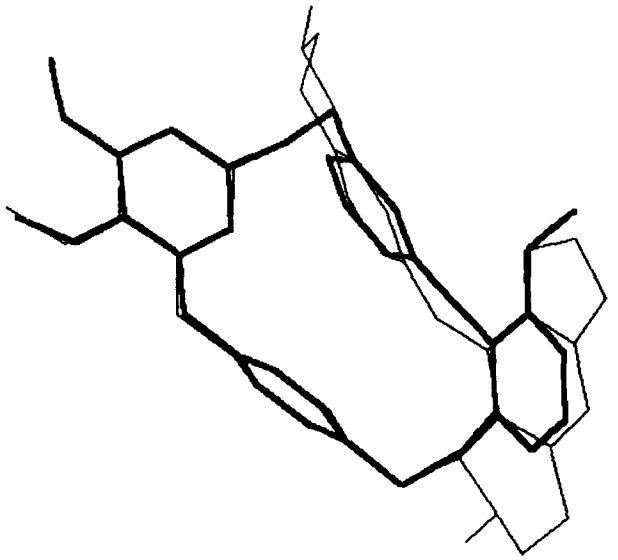


**Figure 4.** Superposition of the minimum energy structures of **1** (thick line) and **2**

**Table 1.** Van der Waals surfaces of **1** and **2** in Å$^2$

| | **1** | **2** |
|---|---|---|
| nonpolar saturated | 315.9 | 380.9 |
| nonpolar unsaturated | 115.6 | 89.4 |
| polar | 37.6 | 38.2 |
| total | 469.1 | 508.5 |

**Table 2.** Centroid–centroid distances in Å between benzene rings in compounds **1** and **2** (in parentheses)

| | A | B | C | D |
|---|---|---|---|---|
| A | - | | | |
| B | 4.96 (4.98) | - | | |
| C | 8.81 (8.74) | 4.91 (5.20) | - | |
| D | 4.45 (4.37) | 4.33 (4.27) | 4.99 (5.10) | - |

*Electrostatic similarity*

In contrast to the steric interactions, quantification of electrostatic similarity is problematic. One of the conceivable ways suggested by Politzer *et al.* is the comparison of molecular electrostatic potential (MEP) maps on the van der Waals surface of molecules.[16] We used this method to study the electrostatic similarity between **1** and **2**. Molecular electrostatic isopotential maps (–15.0 kcal mol$^{-1}$) were calculated (Fig. 5). Comparison between the MEPs of **1** and **2** suggests similar electrostatic properties for the two molecules required for fulfilling the condition of electrostatic complementarity with the binding site. Strongly negative regions were mainly concentrated around the oxygen atoms of the diarylether bonds and the oxy-substituents

of the benzene rings. Slightly negative regions can be attributed to the electronic structure of aromatic rings. The only significant difference is the negative potential around the isoquinoline nitrogens in **2** suggesting the existence of two additional hydrogen bonds in the recognition process. The configuration and substitution of these nitrogen atoms, however, is not a prerequisite of the biological activity of **2**.[7]

*Hydrophobic similarity*

Hydrophobic similarity between **1** and **2** was examined on a quantitative basis solved by the calculation of the water solvation area using the Macromodel computational package. The calculated values are given in Table 3. The total solvation area of **2** was found to be significantly larger than that for **1**. The difference (67 Å$^2$) can be attributed to solvation of the nonpolar regions, however, the solvation areas of the more important polar regions were very close. The calculated hydration enthalpies (1.7 and 2.4 kcal mol$^{-1}$ for **1** and **2**, respectively) were also in fair agreement.

**Table 3.** Water solvation surfaces of **1** and **2** in Å$^2$

| | 1 | 2 |
|---|---|---|
| nonpolar | 640 | 709 |
| polar | 38 | 36 |
| total | 678 | 745 |

In medicinal chemistry hydrophobic interactions are usually modeled by the partition coefficient of the ligands between water and *n*-octanol which is closely parallel to hydrophobicity. Molecular octanol–water partition coefficients of **1** and **2** were calculated using the atomic contributions determined by Crippen *et al.*[17] Partition coefficients ( log*P* = 6.96 and 5.94 for **1** and **2**, respectively) are in fair agreement and suggest both compounds to be strongly lyphophylic.

*Analysis of biological data*

The similarity between **1** and **2** can be also discussed at the level of their biological activity. Based on earlier works, four main therapeutic indications can be attributed to **1**, i.e. antibacterial, antitumor and antileukemia, 5-lipoxygenase, and calmodulin inhibitory activity.

The antibacterial activity of **1** has been shown to be slightly higher against Gram-positive organisms than against Gram-negative ones, but the latter (e.g. *Pasteurella multocida* and *Neisseria meningitis*) were also sensitive.[18] In comparison, **2** was also active against Gram-positive *Staphylococcus aureus*[19] and Gram-negative *N. meningitis*.[20]

Tuberculostatic activity of **1** was anticipated by a similar activity[21] of **2** and was tested against the classical human $H37_{RV}$, *Mycobacterium tuberculosis* Kochii, *M. avium*, and *M. noccardia* SN. Compound **1** was found to be active (MIC: 5, 20, and 10 mg mL$^{-1}$, respectively), demonstrating a new aspect of similarity.

*In vitro* cytotoxic activity of **1** was reported by Asakawa *et al.*[22] against KB-cells and P-388 murine leukemia. Antitumor and antileukemia activity of **2** has been widely investigated and the compound has been found to be active against Meth-A,[23] MM2,[24] and K3 cells,[25] as well as human leukemia,[26] and L1210 murine leukemia cells.[27]

5-Lipoxygenase inhibitory activities manifested by **1** and **2** has also been shown to be similar. Arachidonic acid metabolism was inhibited by both compounds. Compound **1** was active against $LTB_3$ and 5-HETE[5] while the formation of $LTB_4$ was inhibited by **2**.[28] The calmodulin inhibitory activity of **1** may also be compared to the inhibition of calcium uptake mediated by **2**.[4,29]

*Proposed mechanism of action*

Biological activity of some bisbenzylisoquinolines, such as tubocurarine, cepharathine and their synthetic analogues has been widely studied,[30] however, rel-

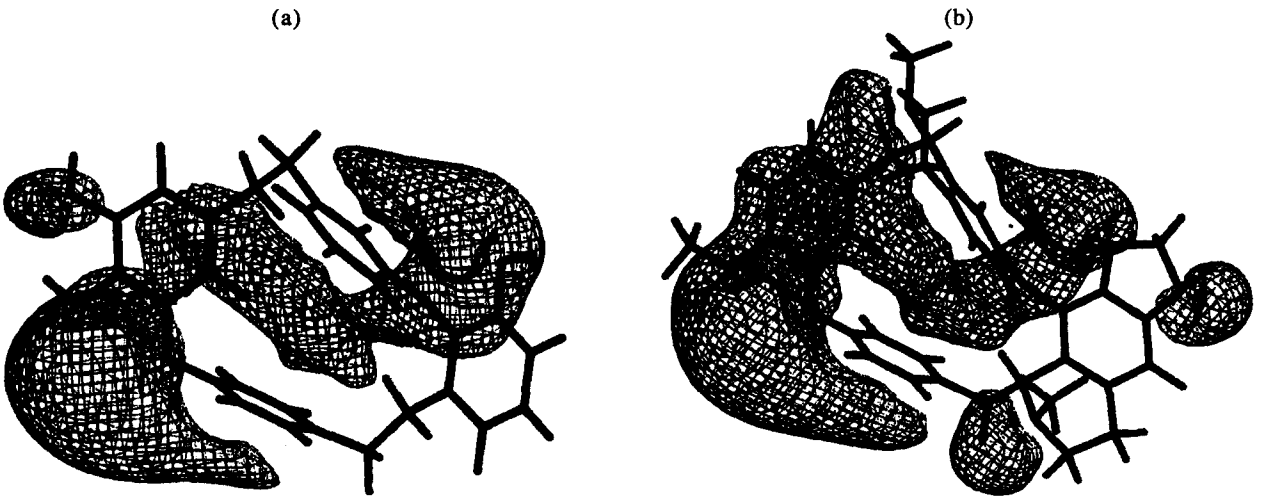



**Figure 5.** Comparison between –15 kcal mol$^{-1}$ isopotential MEPs of **1** (a) and **2** (b).

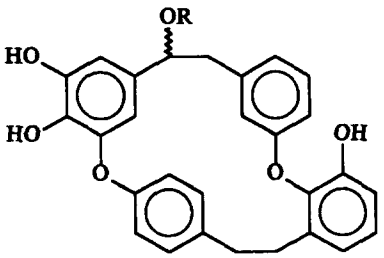
atively few structure–activity studies have been presented. The first paper concerning this topic was published by Kupchan and Altland in 1973.[7] Analysis of active and inactive analogues of tetrandine, oxyacanthine and tubocurarine led to the following findings: (i) monomeric benzylisoquinolines show no activity, (ii) methylation of the isoquinoline *N*-atom has no effect on activity, and (iii) configuration at C1 is unimportant. From a systematic study on the antitumor effects of bisbenzylisoquinoline alkaloids some further conclusions were drawn by Kuroda *et al.*[31]: (iv) the presence of two or more diarylether bonds is a condition for activity, (v) the position of the diphenylether linkages is strongly related to the activity, (vi) methylation of the hydroxy group at ring A has no effect on activity. In summary, the combination of findings (i), (iv) and (v) suggests that it is the constitution and configuration of the macrocyclic ring (i.e. skeletal effect) which have a strong influence on the biological activity. On the basis of points (ii) and (vi) substituent effects seem to be less important, however the contribution of slightly electron-donating aromatic substituents (OH or OMe) to the activity was reported.[32]

The relative importance of the skeletal and substituent effects can also be demonstrated on bis(bibenzyls). The calmodulin inhibitory activities of **1**, marchantin D (MD), E (ME), riccardin A (RA), perrotettin A (PA) and E (PE) (Fig. 6) are collected in Table 4. A comparison of the activities of **1**, MD, and ME suggests that the presence and nature of the nucleophilic atom at the ethano bridge has only a moderate effect. Decreased activity of RA having only one diarylether bond in the macrocyclic ring points to the existence of a skeletal effect. The increased inhibitory concentrations of PE, a linear bis(bibenzyl), and PA, a monomeric stilbene, also demonstrate the influence of the macrocyclic ring on the calmodulin inhibitory activity.

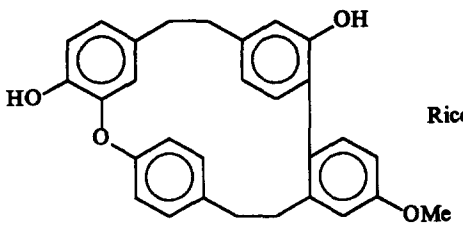
**Table 4.** Calmodulin inhibitory activity of bibenzyl derivatives

| Compound | $ID_{50}$ (μg $mL^{-1}$) |
|---|---|
| **1** | 1.85 |
| MD | 6.0 |
| ME | 7.0 |
| RA | 20.0 |
| PE | 90 |
| PE | 95 |

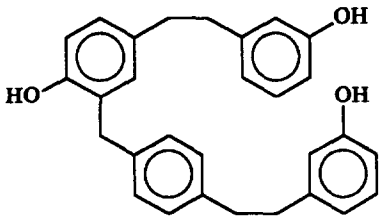
As can be seen in the previous section, marchantin A has been found to be effective in various therapeutic areas. Compounds possessing such a wide range of



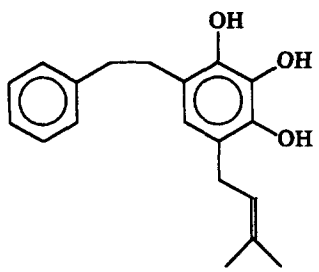

R = H Marchantin D (MD)
R = Me Marchantin E (ME)




Riccardin A (RA)




Perrotettin E (PE)




Perrotettin A (PA)

**Figure 6.** Chemical structure of some bibenzyl derivatives having calmodulin inhibitory activity.

activities are expected to exert their effect by acting on a basic biochemical pathway as an agonist or antagonist. Ionophor activity of **1** was first mentioned by Asakawa *et al.*[33] but no systematic complexation studies have been reported. Owing to the characteristic skeletal effect on its calmodulin inhibitory activity and the extreme importance of metal ion complexation in biological systems, we investigated the ionophor properties of **1**. The calmodulin inhibitor activity of **1** suggested its complexation with $Ca^{2+}$. This was tested by ion selective membrane techniques.[34] PVC electrode membranes containing **1** were prepared and potentiometric selectivity coefficients were determined by the separate solution method. Selectivity coefficients demonstrated that the calcium complex of **1** is more stable than the corresponding cadmium and zinc complexes ($\lg k_{Cd\text{-}Ca} = -0.93$ and $\lg k_{Zn\text{-}Ca} = -1.17$). The preferred formation of the Fe(II) complex ($\lg k_{Fe\text{-}Ca} = +4.72$) can be interpreted by the chelation property of the ortho dihydroxy moiety. In the case of alkali metal ions, the sodium complex was found to be more stable than the corresponding potassium complex. Our results suggest that the calcium complex of **1** is relatively stable and its formation might be preferred in biological systems. Thus, the biological activity of **1** seems to be due to interference with the calcium-signal through complexation of intra- or extracellular calcium. The observed calmodulin inhibitory[4] and the closely related skeletal muscle relaxant[6] activities are in accordance with this mechanism of action.

It is known that phosphorylation effected by a calmodulin activated protein kinase leads to a modification and, therefore, altered function of several enzymes.[35] The regulatory function of these enzymes on cell shape and proliferation has been proposed to be essential in the molecular biology of cancer,[36] presumably, through inducing the degradation of microtubules. Therefore, it seems likely that interference with this enzymatic mechanism is responsible for the cytotoxic activity of **1**, a potent calmodulin inhibitor.

Similarity between **1** and **2** was examined by computational methods. Based on the examination of steric, electrostatic, and hydrophobic similarity, as well as on the comparison of biological activities, similar therapeutic properties of **1** and **2** can be attributed to the binding on a common receptor.

## Experimental

### *Computational methodology*

All molecular mechanics calculations were performed on Silicon Graphics Personal IRIS (4D/35) and Challenge workstations. Calculations on the conformational space of **1** and **2** were carried out using the MacroModel 4.0 computational chemistry program package.[37] The MM2* force field available in MacroModel was applied which is different from the authentic MM2 force field[38] only in that it employs the point-charge Coulomb electrostatic equation. The electrostatic treatment of **1** and **2** was based on our positive experience of reproducing crystal structures of organic molecules by using calculations *in vacuo* with attenuated electrostatics. Therefore, a distance-dependent dielectric constant was employed which was further attenuated by a factor of 10. The conformational space available for **1** and **2** was searched using a particularly efficient systematic unbounded multiple minimum search technique (SUMM)[11] available only in MacroModel. Structures generated during the conformational search were minimized to yield unique conformers within an energy window of 30 kJ $mol^{-1}$ above the global minimum. TNCG truncated Newton conjugate gradient technique[39] (max. iteration 150, convergence criteria in gradient 0.01) was used. MEP profiles were calculated for the minimum energy conformations of **1** and **2** using AM1 electrostatic charges in the SPARTAN 3.1 computational package.[40]

### *Tuberculostatic activity*

Tuberculostatic activity of **1** was tested against *Mycobacterium tuberculosis* Kochii, *M. avium*, and *M. noccardia* SN. MIC values were determined by the twofold bouillon dilution method.[41] Cell cultures preincubated for two weeks were used for the preparation of bacterial suspension which was inoculated to bouillon test tubes containing various concentrations of **1**. The test tubes were incubated at 37 °C for 15 days, and the MIC was defined as the lowest concentrations of **1** at which visible bacterial growth was inhibited.

### *Complexation studies*[42]

Complexation properties of **1** were examined by the determination of potentiometric selectivity coefficients, which were measured by the separate solution method in 0.1 M solutions using Ag|AgCl|1 M KCl|0.1 M KCl reference electrode. Electrode membranes were prepared by the method of Craggs *et al.*[43] using 67.5 mg of plastisizer [bis(2-ethylhexyl)sebacate], 33.5 mg of PVC powder and 0.5 mg of **1** for each membrane.

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