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Conformational Study of 2-Phenylbenzothiazine Part of SA2995, a Ca^{2+} Antagonist Having a Benzothiazine Skeleton, and Structure–Activity Relationships

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Abstract—Nuclear magnetic resonance (NMR) studies were carried out for the title compound ((±)-3,4-dihydro-2-[5-methoxy-2-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxy)phenoxy]ethyl]amino]propoxy]phenyl]-4-methyl-3-oxo-2*H*-1,4-benzothiazine) (**1a**; R = H) and its 2-substituted analogs (**1b**; R = OCH_3, **1c**; R = SCH_3, **1d**; R = CH_3, **1e**; R = *i*-C_3H_7) which had Ca^{2+} antagonistic activities. Conformational analysis using the model compounds of the 2-phenylbenzothiazine (2-PBT) part of 1 by semiempirical molecular orbital method agreed with the NMR behavior. Two local minimum conformations, having different rotational angles (θ1) of the 2-phenyl ring, were suggested for biologically active **1a–1d**. The molar fractional ratios, including the conformations within a particular θ1 range that contained each global minimum conformation, were found to correlate well with the activities. In the same θ1 range, any stable conformation was not indicated for non-active compound **1e**. From these results, the active conformation for the 2-PBT part of **1** was suggested to be similar to the global minimum conformation indicated for the most potent **1a**.

Introduction

Calcium antagonists affect Ca^{2+} channels of several tissues such as vascular smooth muscle, cardiac muscle and the conducting system of the heart, in different potency. The difference in potency of the antagonists for the tissues, caused by various structural features, could result in tissue selectivity of those antagonists, and define their usefulness in clinical applications.

SA2995 ((±)-3,4-dihydro-2-[5-methoxy-2-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxy) phenoxy] ethyl] amino] propoxy]-phenyl]-4-methyl-3-oxo-2*H*-1,4-benzothiazine)[1,2] (**1a**) has a novel benzothiazine skeleton and has been shown to have potent Ca^{2+} antagonistic activity. The fumarate (semotiadil fumarate; SD-3211)[3,4] of (*R*)-(+)-isomer (**2a**) of **1a** was found to be a vasoselective Ca^{2+} antagonist and to have as potent and long-lasting effects anti-hypertensive and anti-angina pectoris, as well as less unfavorable effects such as bradycardia, atrioventricular block and reflex tachycardia. On the other hand, the (*S*)-(-)-optical antipode[1,2] of **2a** showed only one-tenth to one-seventh Ca^{2+} antagonistic activity of **2a**.

We have been studying the stereochemistry of **2a** in order to elucidate the structure–activity relationships based on the widely accepted idea that a certain conformation of a drug plays a decisive role in bringing about a particular biological *in vivo* or *in vitro* response. Spectrometric and theoretical studies suggested an equatorial 2-phenyl orientation for the 1,4-benzothiazine ring[5] and close proximity between the 2-phenylbenzothiazine (2-PBT) part and the distal methylenedioxyphenyl(MDP) group.[6,7]

In this study, we will represent the analysis of **1a–1e** in dimethylsulfoxide-d_6 (DMSO-d_6) by nuclear magnetic resonance (NMR) spectrometry and the conformational analysis by the molecular orbital (MO) method to find the structure–activity relationships between the conformations and their potencies.

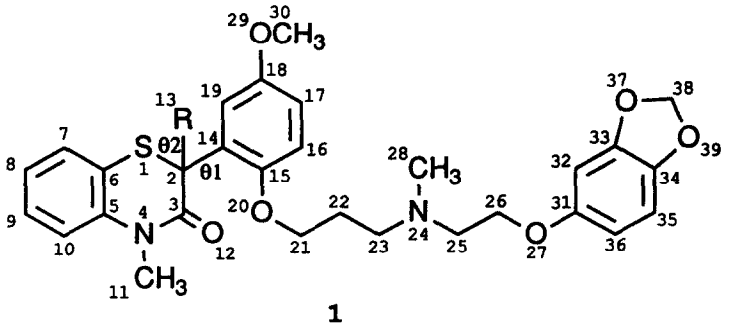
Results and Discussion

NMR spectrometry of 1a–1e

All the protons of **1a–1e** were assigned by the ^1H–^1H COSY technique at 25 °C and are shown in Table 1. The most remarkable difference of chemical shifts in H-19s, and moderate differences in H-8s, H-9s and H-10s, were found among the compounds but other chemical shifts (except for H-13s) were quite similar.

For **2a**, NOE was found between H-13 and H-36, and/or H-13 and H-32 in aqueous solution,[7] however in DMSO-d_6, similar NOE was not observed between any proton of the 2-PBT part or the MDP group of **1a–1e** in a NOESY measurement (mixing time; 0.8 s) that showed H-13···H-21 NOE. This suggests that chemical shifts divergence of H-19s would be attributable to the 2-PBT part of these compounds.

The effects of the 2-substituents of **1a–1e** through bonds would be inappropriate to explain those chemical shift differences. This is because the differences among H-19 (**1a**) (6.32 ppm), H-19 (**1c**) (7.33 ppm) and H-19 (**1d**) (6.93 ppm) were not the ones expected from those of the *ortho* protons in toluene (7.15 ppm), benzylmethylsulfide (7.2–7.29 ppm) and ethylbenzene (7.2 ppm). Additionally, similar chemical shifts of the *ortho* protons in



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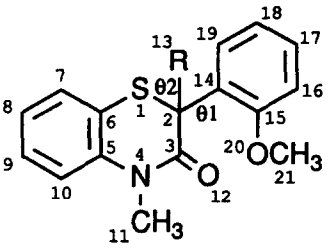

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Figure 1. Structural formulae of **1**, **2** (*R*-configuration) and **3** showing atomic numbering. θ1; C(15)–C(14)–C(2)–C(3), θ2; R(13)–C(2)–C(3) (see Ref. 8), **a**; R = H, **b**; R = OCH_3, **c**; R = SCH_3, **d**; R = CH_3, **e**; R = *i*-C_3H_7.

Table 1. ^{1}NMR Chemical shifts (δ from TMS) of **1a–1e**[a]

	Chemical Shift (ppm)				
position	**1a**	**1b**	**1c**	**1d**	**1e**
7	7.25	7.34	7.27	7.31	7.36
8	7.33	7.06	7.00	7.13	6.87
9	7.02	7.28	7.23	6.91	7.06
10	7.35	7.27	7.15	7.05	6.92
11	3.48	3.45	3.42	3.39	3.35
13	4.97	3.10	1.90	1.68	0.84,1.08,2.75
16	6.92	6.98	6.93	6.78	6.73
17	6.78	6.92	6.85	6.64	6.59
19	6.32	6.94	7.33	6.93	7.03
21	4.00	3.92	3.91	3.88	3.83
22	1.92	1.81	1.84	1.93	1.94
23	2.73	2.64	2.69	2.82	2.84
25	2.86	2.75	2.75	2.93	2.99
26	4.01	3.93	3.94	4.07	4.12
28	2.38	2.28	2.29	2.44	2.49
30	3.53	3.72	3.69	3.57	3.54
32	6.59	6.56	6.57	6.61	6.64
35	6.75	6.76	6.77	6.76	6.79
36	6.32	6.29	6.30	6.35	6.39
38	5.93	5.94	5.94	5.94	5.94

a; All compounds were measured as fumarate salts.

benzylmethylsulfide, benzylmercaptan (7.28 ppm) and benzylalcohol (7.2–7.3 ppm) were incongruent with H-19s of **1b** (6.94 ppm) and **1c**. These results suggested the conformational difference of the 2-PBT part among **1a–1e**. Consequently, we studied the conformation of them by a molecular orbital method.

Conformational analysis by MO

MO calculations were done for respective model compounds **3a–3e** of **1a–1e**, with rotating bonds θ1 (C(15)–C(14)–C(2)–C(3)) and θ2(R(13)–C(2)–C(3))[8] (Figure 1) in a step-like manner (10–30 °).The 2-phenyl group orientation for the 1,4-benzothiazine ring was made equatorial according to the reported study.[9]

The potential energy curve of **3a** with respect to θ1 is shown in Figure 2. Compound **3a** could have two local minimum conformations at θ1 = 70 ° (**3a–A**) and θ1 = 250 ° (**3a–B**). The 2'-methoxy group attached to the 2-phenyl ring is downward in conformation **3a–A** and upward in **3a–B** in Figure 3. Conformation **3a–B** was more stable about 5 kcal/mol than **3a–A**, and the potential curve around **3a–B** was spread more gently than that of **3a–A**. These results suggest conformation **3a–B** is more preferable than **3a–A** in terms of energy. Molecular mechanics (MM2) and other MO (PM3) calculations also gave similar results.[6] In fact, conformational resemblance of the 2-PBT part of **2a** to **3a–B** was found in **2a** mandelate by X-ray crystallography.[9]

For **3b–3e**, the potential energy surfaces were surveyed with θ1 and θ2, and are shown as contour maps in Figure 4. Four local minimum conformations were found for **3b** at about (θ1, θ2) = (80 °, 320 °) (**3b–A**), (300 °, 310 °) (**3b–B**), (210 °, 150 °) (**3b–C**) and (60 °,150 °) (**3b–D**). The potential energies of **3b–C** and **3b–D** were higher (~12kcal/mol) than those of **3b–A** or **3b–B**. The energy wells that involve the former two conformations (**3b–A**, **3b–B**) were shallower and more widely spread than those of the latter (**3b–C**, **3b–D**), thus suggesting predominant contributions of **3b–A** and **3b–B** in all the conformations of **3b**. These conformations (**3b–A**, **3b–B**) could be distinguished only with the rotational angle θ1, whose θ1 values were close to those of **3a–A** (θ1 = 70 °) and **3a–B** (θ1 = 250 °), respectively. For compound **3c**, the situation with local minimum conformations was similar to **3b**, which was expected from their structural resemblance; its two favorable conformations were at (θ1, θ2) = (90 °, 310 °) (**3c–A**) and (300 °, 310 °) (**3c–B**), the others obviously unstable. Compound **3d** gave essentially two local minimum conformations, at about (θ1, θ2) = (80 °, 50 °), (80 °, 170 °) and (80 °, 290 °) (**3d–A**), and (θ1, θ2) = (300 °, 20 °), (300 °, 140 °) and (300 °, 260 °) (**3d–B**). These two conformations were also differentiated with only θ1, and were comparable to each other in energy. The contour map of **3e** is obviously different from those of **3b–3d** as we were only able to find one stable conformation at about (θ1, θ2) = (150 °, 60 °). This is probably due to the sterically crowded 2-*i*-C_3H_7 substituent.

The conformational analysis with the contour maps described above reveals a more important role of the rotational angle θ1 than of θ2 in finding more stable conformations. Therefore, in order to compare the contribution of these conformations of **3b–3e** with the stable conformations of **3a** directly, the potential energy curves are shown as functions only of θ1(Figure 5); each point on these curves was the energy minimum with θ2. With the exception of the potential energy curve of **3e**, which had a global minimum conformation (**3e–A**) at θ1 = 150 ° and is clearly distinguishable, the remaining appear comparatively similar to others. Within these similar potential curves one difference stands out, this difference being the location of the global minimum conformations; namely θ1 = 250 ° for **3a–B**, and θ1 = 300 ° for **3b–B**,

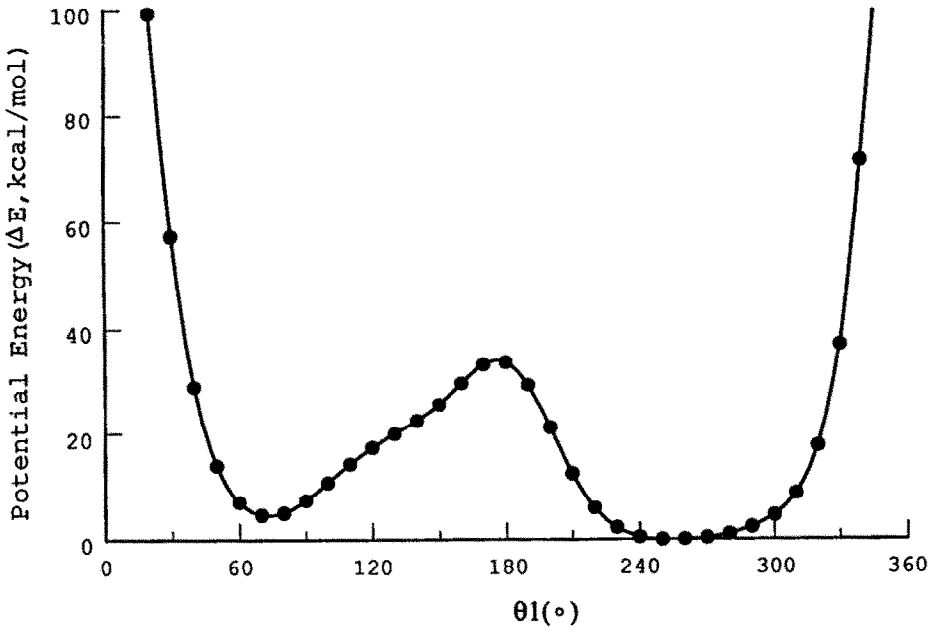

Figure 2. Potential energy curve of the 2-phenyl ring rotation in **3a**.

3c–B and **3d–B**. These could cause the chemical shift difference of H-19s among the parent compounds (Table 1). While H-19 (**3a–B**) is located close (~3.0 Å) to the amido carbonyl group of the 2-PBT part and appears under its magnetic shielding region, other H-19s were a little more distant (~3.8 Å) and out of that shielding region (Figure 6).

In consequence, the potential energy curves obtained with the model compounds would be a good reflection of the circumstances around the 2-PBT part of their parent compounds.

Structure–activity relationships

Consideration of the NMR behavior of **1a–1e** with the potential energy curves of the model compounds (**3b–3d**) suggests the participation of their global minimum conformation **Bs** as the most stable structures in solution. We therefore considered that **Bs** might be relevant to the Ca^{2+} antagonistic activity.

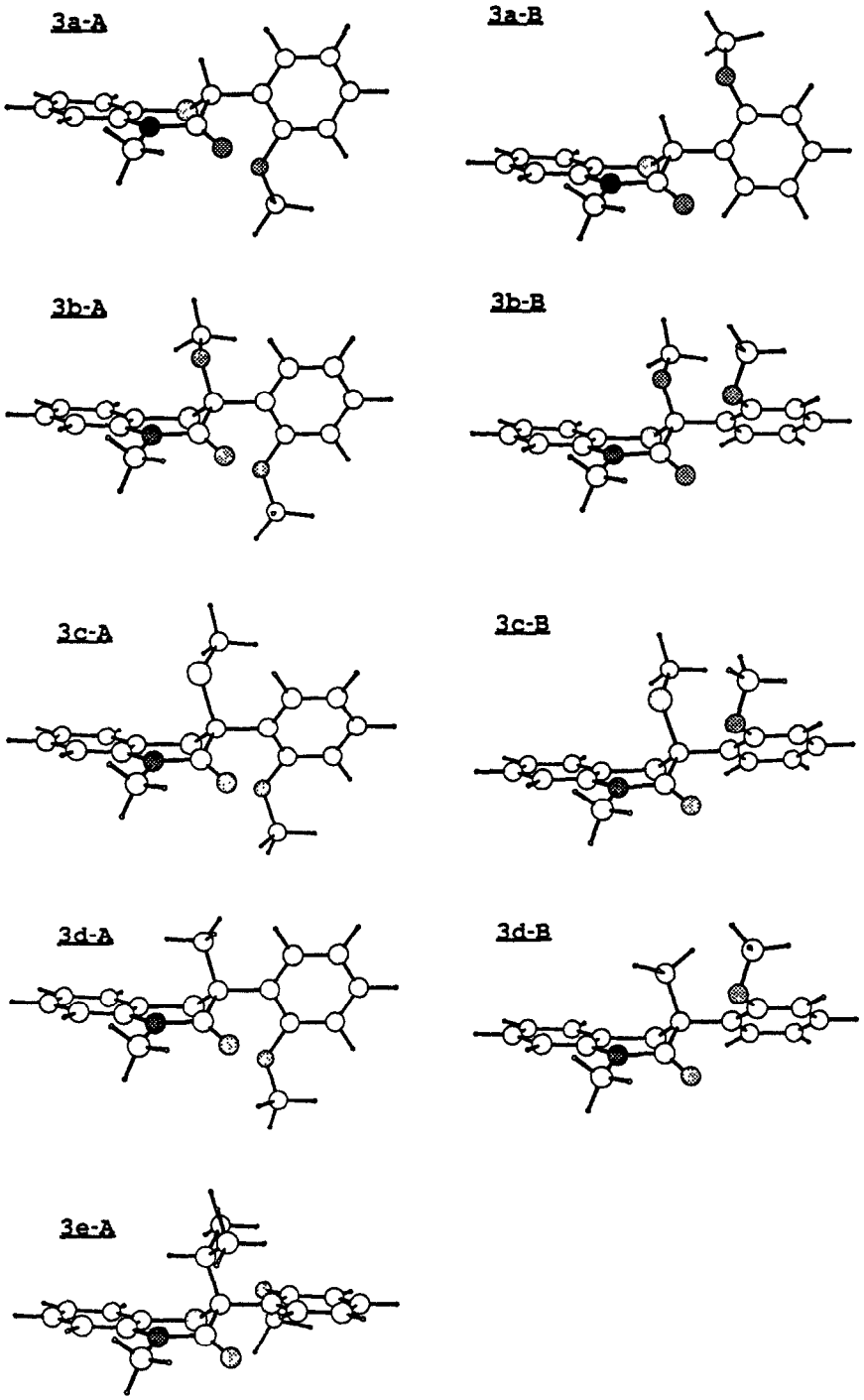

Figure 3. Stable local minimum structures of **3a–3e**. All **B** conformations and **3e–A** were global minimum in energy. **3a–A**; θ1 = 70 °, **3a–B**; θ1 = 250 °, **3b–A**; (θ1, θ2) = (80 °, 320 °), **3b–B**;(θ1, θ2) = (300 °, 310 °), **3c–A**;(θ1, θ2) = (90 °, 310 °), **3c–B**; (θ1, θ2) = (300 °, 310 °), **3d–A**; (θ1, θ2) = (80 °, 50 °), **3d–B**; (θ1, θ2) = (300 °, 20 °), **3e–A**; (θ1, θ2) = (150 °, 60 °).

Inclusion of the amine side chain of **1** terminating with the MDP moiety into a structure–activity study would be desirable since close proximity between the MDP group and the 2-PBT part of **2a** in aqueous solution was suggested by NMR,[7] however, that will bring about a complicated and ambiguous situation on the side chain conformation. This time, then, the structure–activity relationship was examined in the light of the conformational behaviors of the 2-PBT part, assuming the contribution of the amine side chains on the activities could be similar and thus they could be cancelled out among **1a–1e**.

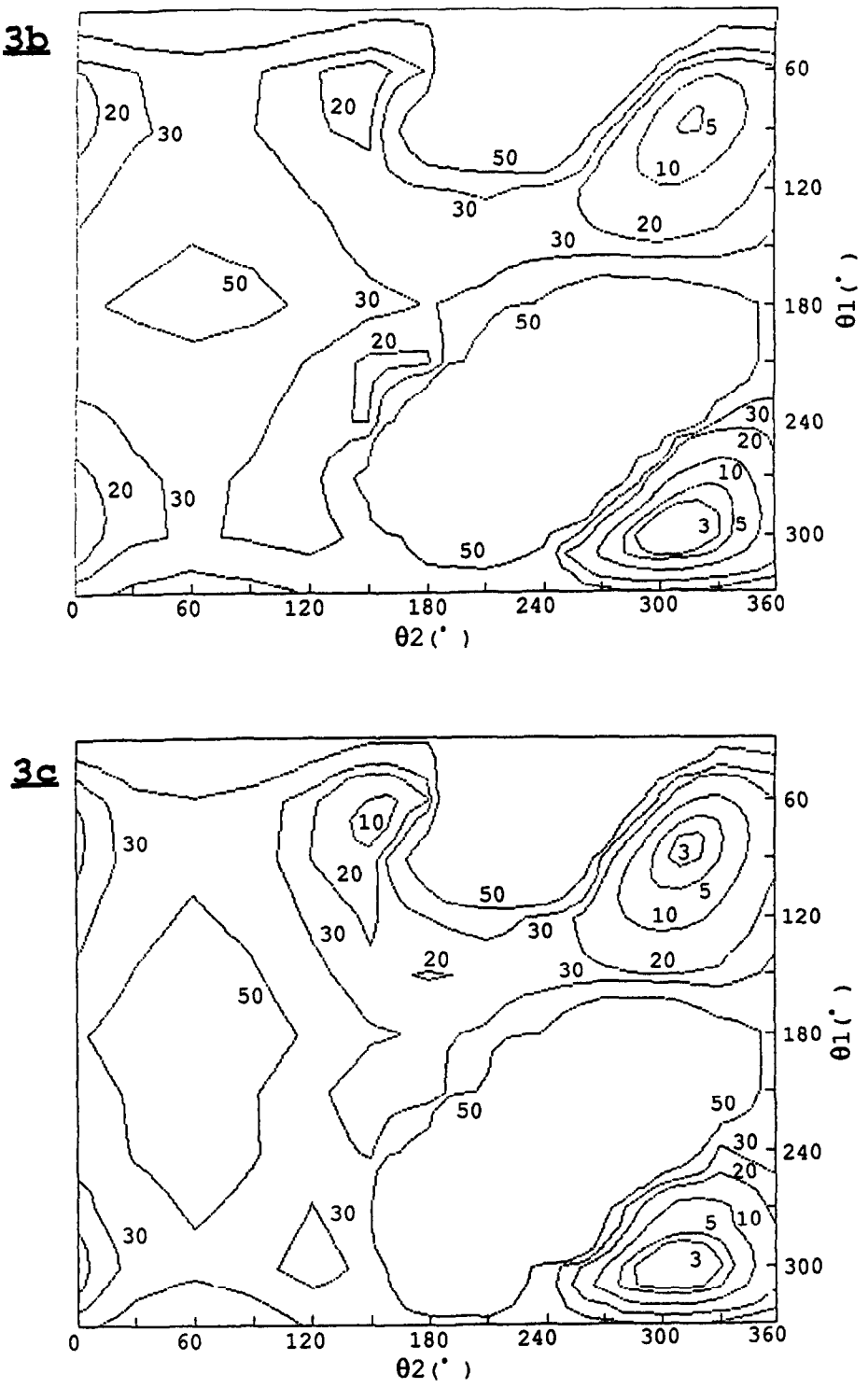

Figure 4.

Figure 4. *Continued.*

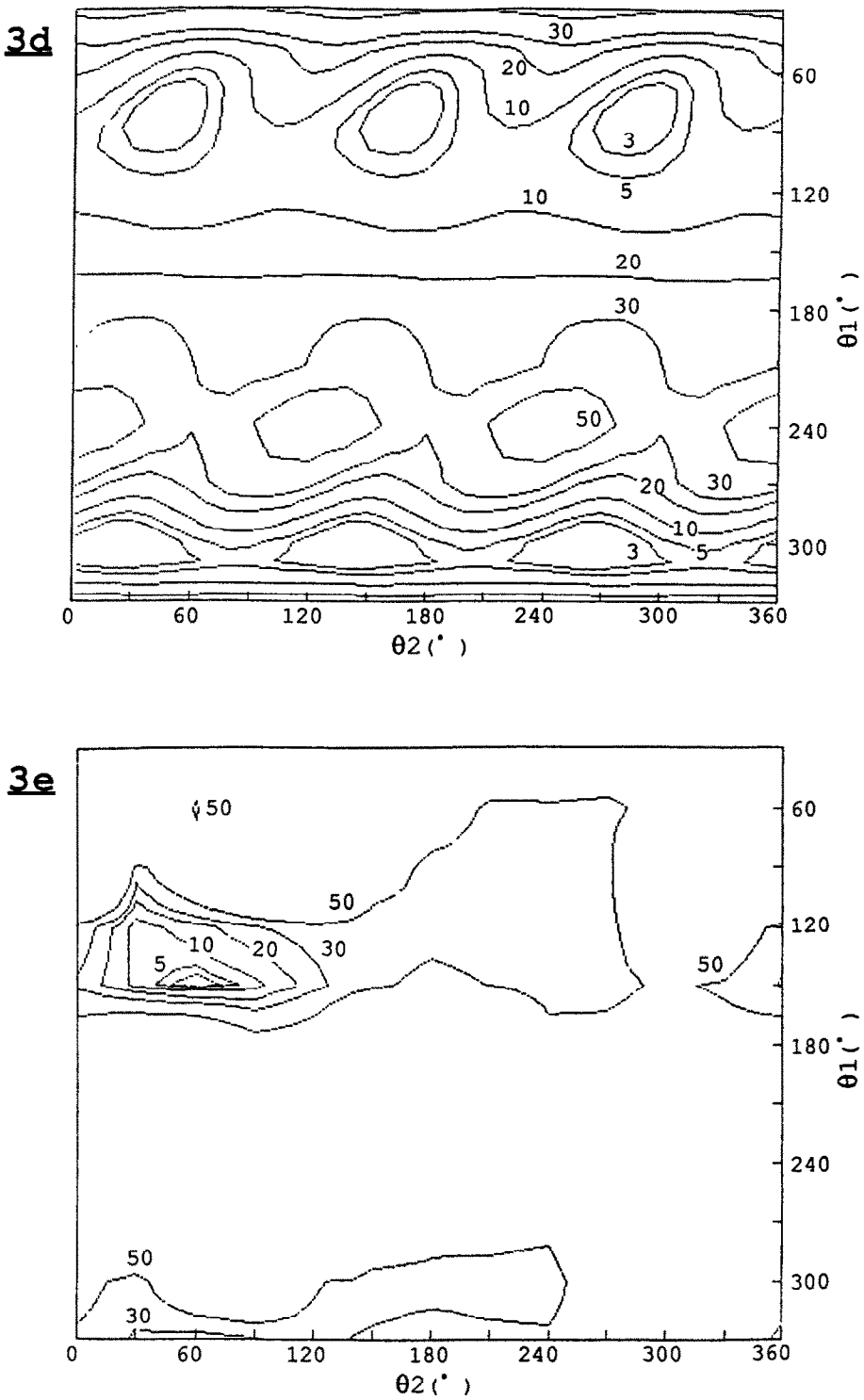

Figure 4. Contour maps of potential energy surface in **3b–3e**. Values in the maps mean relative potential energies for each global minimum structure.

Table 2 shows the energy differences ($\Delta\Delta E_{BA} = \Delta E_B - \Delta E_A$) of conformations **B** and **A** for differing potencies. Conformation **A** can render exclusive perturbation to the contribution of **B**. The potencies were found to increase with the decrease of $\Delta\Delta E_{BA}$, implying the involvement of conformation **B** for the activity but this correlation is not necessarily enough, because the values $\Delta\Delta E_{BA}$ of **1a** and **1b** were the same.

Accordingly, all the contibutions around the global minimum conformation were taken into account. The molar fractional ratio of each compound of this range,

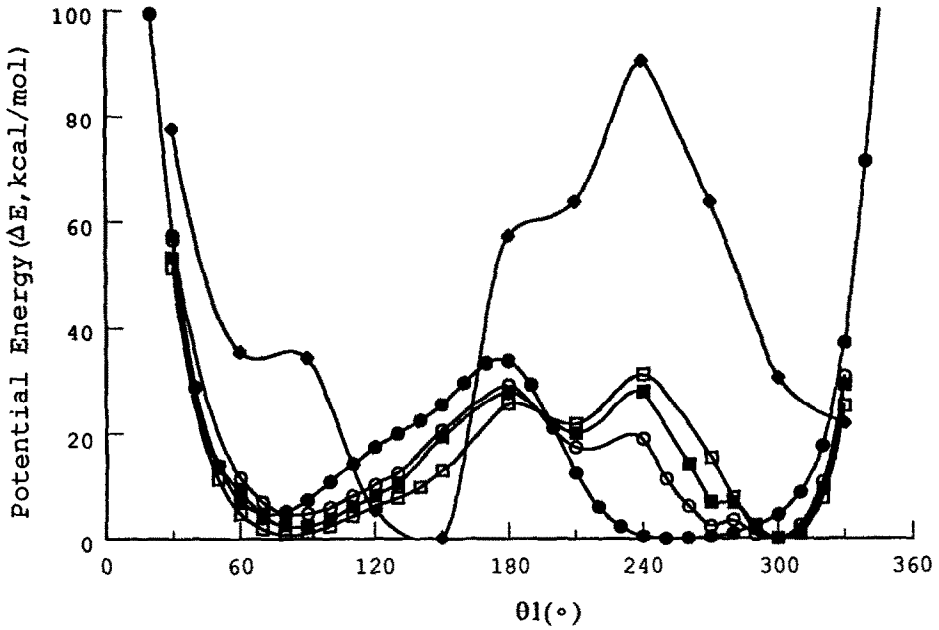

Figure 5. Potential energy curves of 2-phenyl ring rotation in **3a–3e**. ●; **3a**, ○, **3a**, ■; **3c**, □; **3d**, ◆; **3e**

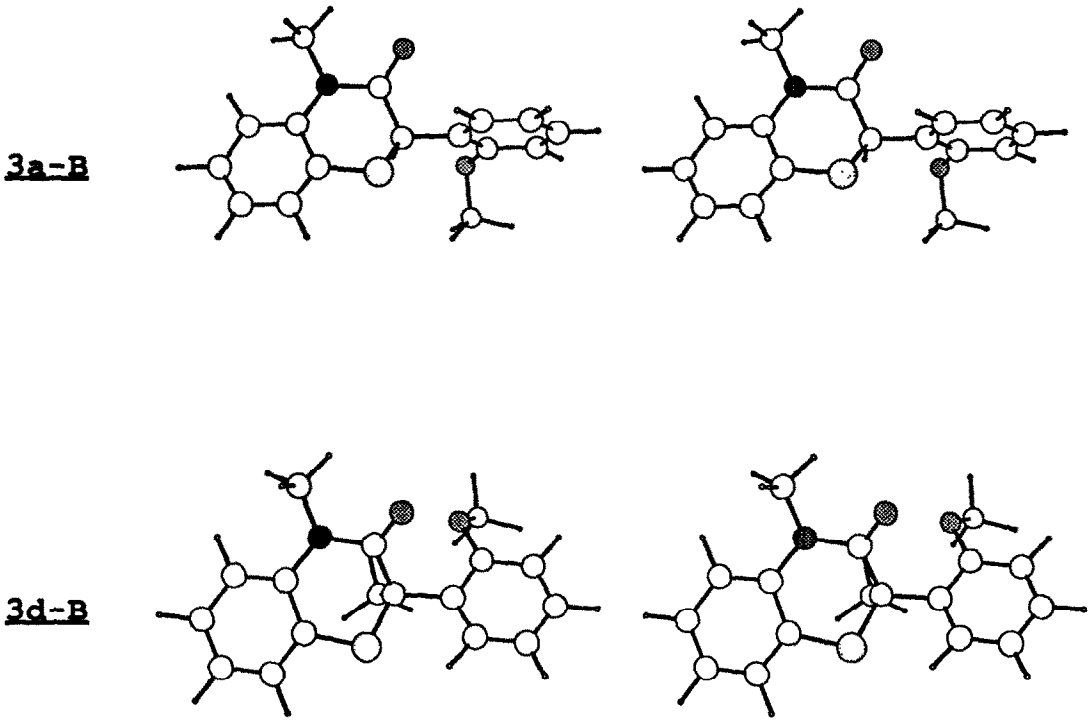

Figure 6. Stereoviews of **3a–B** and **3d–B**.

Table 2. Ca^{2+} Antagonistic activities of **1a–1e** and their calculated properties

	R	Activity	Energy Difference	Molar Fraction(%)	
		IC_{50}(μM)[a]	$\Delta\Delta E_{BA}$(kcal/mol)[b]	Z_A[c]	Z_B[c]
1a	H	0.18±0.01	-4.7	0.02	99.98
1b	OCH_3	0.25±0.06	-4.7	0.07	99.93
1c	SCH_3	1.5±0.4	-2.4	2.99	97.01
1d	CH_3	2.1±0.2	-0.7	22.98	77.02
1e	i-C_3H_7	>10	-	100.00	0.00

a; mean ± S.E. (n=4–10).
b; $\Delta\Delta E_{BA}=\Delta E_B-\Delta E_A$.
c; Z_A and Z_B refer to all the contributions of molar fractions around conformation A and B, respectively.

including conformation **B**, was estimated from the Boltzmann equation ($z = \int \exp(-\Delta E/RT)\, d\theta$). The results are given in Table 2 and show a good correlation of the potencies with the molar ratios for all the compounds. This suggests the importance of the conformation in this θ1 range, as well as, the global minimum conformation itself to bring about the activity.

If we accept the active conformation to have a similar structure among compounds when they are incorporated into a Ca^{2+} receptor, then conformation **3a–B** would be most appropriate as the active conformation of the 2-PBT part of **2a**. This is because **3a–B** would be the global minimum structure of the most potent mother compound **1a**, and no stable conformation was indicated for non-active **1e** in the corresponding energy well. Besides, the other medium potent compounds contributed their molar fractions to the extensive θ1 range of **3a–B** in an activity dependent manner from slightly distant global minimum conformations.

In a protic solution, the distal MDP group of 2a was found to locate adjacent to the 2-PBT part by van der Waals interaction between the two aromatic cycles.[6,7] Since the MDP group was essential for the potent activity (data not shown), rotational preference of the 2-phenyl ring on θ1, and hence, it controls the relative position of the MDP group. This may cause the difference in potency, provided the adjacency brings about no significant change in the conformational properties obtained with the model compounds.

Experimental

^{1}H-NMR spectra were recorded on a JEOL GSX400 spectrometer using tetramethylsilane as an internal standard in DMSO-d_6.

Semiempirical molecular orbital method calculations were performed with a modified version of MNDO.[10] Model structures **3a–3e**, for eliminating ambiguity of the aminoalkyl chain conformations of **1a–1e**, were subjected to all the calculations.

Biological activities were measured by the reported method[2,3] with isolated taenia caecum of guinea pig.

Conclusion

Conformational differences of the 2-PBT part of **1a–1e** were suggested by NMR experiments. MO calculations with the model compounds indicated that biologically active compounds (**1a–1d**) had two stable conformations, respectively, while the non-active one (**1e**) had only one stable conformation. An energy profile analysis of these conformations revealed a correlation of the antagonistic activities with molar fractional ratios in a particular θ1 range which include each global minimum conformation. These results suggest that the active conformation of the 2-PBT part of **1a–1e** was similar to the global minimum conformation that was indicated for the most potent **1a**.

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8. Torsional angles θ2 were defined as follows, **b**; C(13)–O(13)–C(2)–S(1), **c**; C(13)–S(13)–C(2)–S(1), **d**; C(13)H–C(13)–C(2)–S(1), **e**; C(13)H–C(13)–C(2)–S(1).

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