Quantitative Structure–Activity Relationships of Ca^{2+}-Antagonistic Semotiadil Congeners

Ken-ichi FUJIMURA,* Atsutoshi OTA, and Yoichi KAWASHIMA

Developmental Research Laboratories, Santen Pharmaceutical Co., Ltd., 9–19, Shimoshinjo 3-chome, Higashiyodogawa-ku, Osaka 533, Japan. Received August 10, 1995; accepted November 9, 1995

Structure–Ca^{2+} antagonistic activity relationships of semotiadil (1) congeners having a benzothiazine cyclic system were studied quantitatively by the Hansch–Fujita method. A quadratic dependency of the activity on *C*log*P*, a lipophilic descriptor, of terminal arylalkylamine moieties was suggested. A correlation between the dipole moment component of the 5′-substituted 2-phenylbenzothiazine parts and the potency was also suggested. Additionally, quantitative analysis was successfully shown for the 2-substituted 1 congeners. The results gave information about the mode of binding of 1 with Ca^{2+} receptor.

Key words semotiadil; calcium antagonist; quantitative structure–activity relationships; lipophilicity; dipole moment; free energy

Semotiadil (**1**)[1] (Table 1) fumarate is a new non-dihydropyridine Ca^{2+} antagonist with a 2-phenylbenzothiazine (2-PBT) skeleton. Its activity is vasoselective, potent and long-lasting, with few adverse effects.[1] We have been studying the stereochemistry of **1** to clarify its structure–activity relationships.[2] In this report, we present a study of quantitative structure–activity relationships (QSAR) of **1** and its analogs, using the Hansch–Fujita method.

Results

QSAR on the Arylalkylamino Side Chain Correlations between the Ca^{2+}-antagonistic activities of the compounds **1**, **5**—**7**, **9)** (Table 1) and several physicochemical descriptors of the substituents on the distal aromatic rings were examined. These compounds were selected from those having simple substituents whose descriptor values have been reported.[3] They all have (*R*)-configuration at C-2. These limitations would reduce perturbation of the antagonistic activity by physicochemical factors, and simplify the early stage of the analysis. The descriptors used are typical ones for QSAR analysis and can be categorized into electronic (σ_p, σ_p^0, σ_p^-, σ_p^+, *F*, *R*), steric (*MR*, *Es*, *L*, B_1, B_4) and hydrophobic (π) types.[3] A better correlation ($r=0.775$ (correlation coefficient)) of the activity was found with π than with the others. Similar potency dependence ($r=0.783$) on the retention times in reversed-phase high-performance liquid chromatography was obtained for 6 compounds (**1** (retention time = 5.90 min), **5** (6.61), **6** (5.62), **7** (8.82), **8** (12.13), **9** (8.48)). Since these results suggest the dependence of the activity on hydrophobicity of the substituents at the distal aromatic ring, we examined the QSAR with *C*log*P*, an empirically estimated log*P* value, as a hydrophobic descriptor. This is because *C*log*P* values can easily be calculated for diverse structures with a computer program.[4]

Equation 1 (Eq. 1) was derived for compounds **1**, **4**—**9**, having (*R*)-configuration at C-2 of the benzothiazine skeleton. In this expression, *n* represents the number of data, *s* is the standard deviation, and the figures in parentheses are 95% confidence limits of the corresponding coefficients. The equation is highly significant in terms of the *F* statistic ($F^1_{5(0.001)}=47.18$).

$$pIC_{50} = -1.075(\pm 0.317)C\log P + 11.902(\pm 1.779) \quad (1)$$
$$(n=7,\ r=0.969,\ s=0.223,\ F_{1,5}=75.71)$$

Observation of the activity distribution of the racemic compounds (**2**, **3**, **10**—**17**) revealed a quadratic relation of *C*log*P* against the activity for a given number of methylene units (*m*). The coefficients of the parabolic regression equations of those relations were quite similar to each other, except for the location of $C\log P_{opt}$, which gives the highest calculated activity from the quadratic regression equation. The disparity of $C\log P_{opt}$ was about 0.53, being equal to the lipophilicity of one methylene unit. However, merging the two equations with an indicator variable expressing the difference of *m* was unsuccessful. We then tried to combine the two equations using *C*log*P* calculated for $CH_3N(CH_3)CH_2CH_2$–Z (Table 1), so as to eliminate the contribution of the lipophilicity of this one methylene unit (Eq. 2). In this equation, compound **13** was excluded since its inclusion gave a poor result. The steric effect of the two *ortho* methoxy substituents of the compound, that constrain the phenyl group rotation, may cause this irregularity.

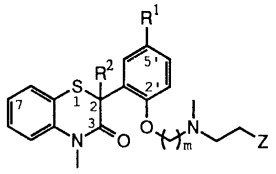
$$pIC_{50} = -0.758(\pm 0.330)(C\log P)^2 + 1.895(\pm 0.925)C\log P + 5.557(\pm 0.580) \quad (2)$$
$$(n=9,\ r=0.943,\ s=0.077,\ F_{2,6}=24.02,\ C\log P_{opt}=1.25)$$

Since Eq. 1 and Eq. 2 cover *C*log*P* ranges of 1.4—3.5 and 0.7—2.1, respectively, both equations were merged into one equation, except for **1** (*C*log*P* values were from $CH_3N(CH_3)CH_2CH_2$–Z). Compound **13** was successfully included in this equation to give Eq. 3. Inclusion of compound **1** in Eq. 3 decreased the statistical significance, albeit there was still a fairly good correlation. Addition of an indicator variable that discriminates the optically active compounds from the racemic ones into the equation was not satisfactory. This may be a bias owing to the set of racemic compounds having smaller ranges of activity and *C*log*P* to **1** than the optically active compounds.

$$pIC_{50} = -0.294(\pm 0.139)(C\log P)^2 + 0.568(\pm 0.560)C\log P + 6.334(\pm 0.494) \quad (3)$$
$$(n=16,\ r=0.957,\ s=0.177,\ F_{2,13}=71.26,\ C\log P_{opt}=0.97)$$

* To whom correspondence should be addressed.

Table 1. Ca^{2+}-Antagonistic Activities of Semotiadil Congeners and Their *C*log *P* Values



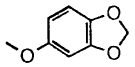
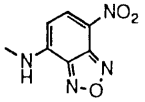
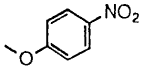
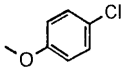
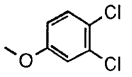
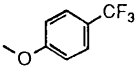
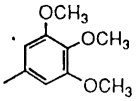
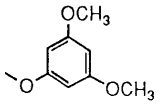
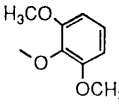
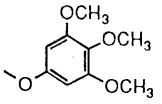
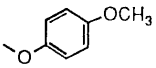
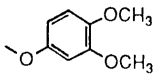
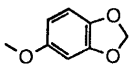
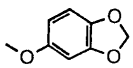
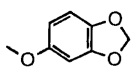
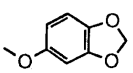
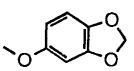
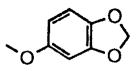
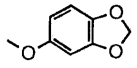
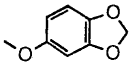
Compd.	*m*	Z	R^1	R^2	Config[a]	π[b]	*C*log *P*[c]	*DIPx*	log *K*	$pIC_{50,obs}$[d]	$pIC_{50,cal}$[e]
1	3		OCH_3	H	(*R*)	−0.05	1.40	0.923	3.70	7.31	6.58
2	3				(*RS*)					6.74	6.58
3	4				(*RS*)					6.77	6.58
4	3		OCH_3	H	(*R*)	—	2.07	0.923	3.70	6.28	6.27
5	3		OCH_3	H	(*R*)	0.00	1.98	0.923	3.70	6.17	6.33
6	3		OCH_3	H	(*R*)	−0.28	2.02	0.923	3.70	6.17	6.31
7	3		OCH_3	H	(*R*)	0.71	2.83	0.923	3.70	5.55	5.60
8	3		OCH_3	H	(*R*)	—	3.47	0.923	3.70	5.00[f]	4.75
9	3		OCH_3	H	(*R*)	0.88	3.11	0.923	3.70	5.00[f]	5.26
10	3		OCH_3	H	(*RS*)	—	0.73	0.923	3.70	6.57	6.60
11	4				(*RS*)					6.46	6.60
12	3		OCH_3	H	(*RS*)	—	2.05	0.923	3.70	6.26	6.29
13	3		OCH_3	H	(*RS*)	—	0.95	0.923	3.70	6.29	6.63
14	3		OCH_3	H	(*RS*)	—	0.89	0.923	3.70	6.64	6.62
15	4				(*RS*)					6.80	6.62
16	4		OCH_3	H	(*RS*)	−0.02	2.07	0.923	3.70	6.28	6.27
17	4		OCH_3	H	(*RS*)	—	1.50	0.923	3.70	6.64	6.55
18	3		CH_3	H	(*RS*)	−0.05	1.40	0.013	3.70	6.44	6.20
19	3		OH	H	(*RS*)	−0.05	1.40	−0.402	3.70	6.00	6.03
20	3		NO_2	H	(*RS*)	−0.05	1.40	−0.589	3.70	5.92	5.95
21	3		Cl	H	(*RS*)	−0.05	1.40	−1.336	3.70	5.59	5.65
22	3		OCH_3	OCH_3	(*RS*)	−0.05	1.40	0.923	3.15	6.60	6.41

Table 1. (continued)

Compd.	m	Z	R^1	R^2	Config[a]	π[b]	$C\log P$[c]	*DIPx*	$\log K$	$pIC_{50.obs}$[d]	$pIC_{50.cal}$[e]
23	3		OCH_3	SCH_3	(*RS*)	−0.05	1.40	0.923	1.51	5.82	5.93
24	3		OCH_3	CH_3	(*RS*)	−0.05	1.40	0.923	0.53	5.68	5.64
25	3		OCH_3	iso-Pr	(*RS*)	−0.05	1.40	0.923	—	<5	—

a) Config; configuration at C-2. *b*) π values of the substituent on the phenoxy group of Z. *c*) $C\log P$ values of $CH_3N(CH_3)CH_2CH_2$–Z. Addition of 3.15 ($m=3$) or 3.68 ($m=4$) gives $C\log P$ of the original structure. *d*) pIC_{50}; negative logarithm of observed IC_{50}. *e*) Calculated pIC_{50} from Eq. 6. *f*) pIC_{50} obtained from the inhibition (**8**; 49.2%, **9**; 47.5%) at 10 μM.

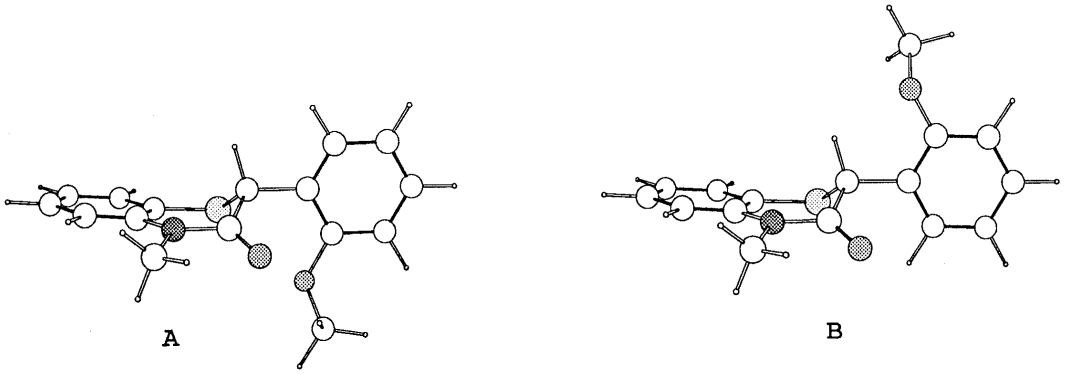


Fig. 1. Two Principal Conformations (A, B) of the 2-PBT Part of **1**

QSAR of the 5′-Substituted 1 Congeners Several compounds (**2**, **18**—**21**) having different functional groups at C-5′ are shown in Table 1. Since their antagonistic activities seem to depend on some electronic features, many kinds of electronic parameters were applied to search for correlations. The parameters included reported ones for the substituents[3] or descriptors on the 2-PBT part calculated by the molecular orbital method[2d] or molecular mechanics method.[2c] Among the contingent descriptors (various Hammett's σ, *F*, *R*, atomic charge, highest occupied molecular orbital (HOMO) or lowest unoccupied molecular orbital (LUMO) energy, atomic polarizability, superdelocalizability, chemical shifts of protons and so forth), the dipole moment vector (*DIP*) was found to have some value as a descriptor. In particular, one component vector (*DIPx*) of the *DIP* was found to give a satisfactory equation (Eq. 4); the *DIPx* vector runs roughly from C-2 to C-7 of the benzothiazine moiety.

$$pIC_{50}=0.535(\pm0.195)DIPx+6.287(\pm0.155) \quad (4)$$
$$(n=5,\ r=0.981,\ s=0.102,\ F_{1,3}=75.95)$$

QSAR of the 2-Substituted 1 Congeners Previously, we reported the qualitative structure–activity relationship of the 2-PBT part of **2** analogs having R^2 (**2**, **22**—**25**) at C-2 (Table 1).[2d] The ratio of molar fractions (Z_A, Z_B) between the two principal conformations A and B (Fig. 1) involving 2-phenyl group rotation, calculated by the modified neglect of diatomic overlap (MNDO) method, was suggested to correlate with Ca^{2+}-antagonistic activity. A greater contribution of B was expected to give higher activity. Quantitative treatment of the relationship could also be

Table 2. Correlation Matrix among the Descriptors in Eq. 6

	$(C\log P)^2$	$C\log P$	*DIPx*	$\log K$
$(C\log P)^2$	1.000			
$C\log P$	0.978	1.000		
DIPx	0.187	0.151	1.000	
$\log K$	0.144	0.117	−0.144	1.000

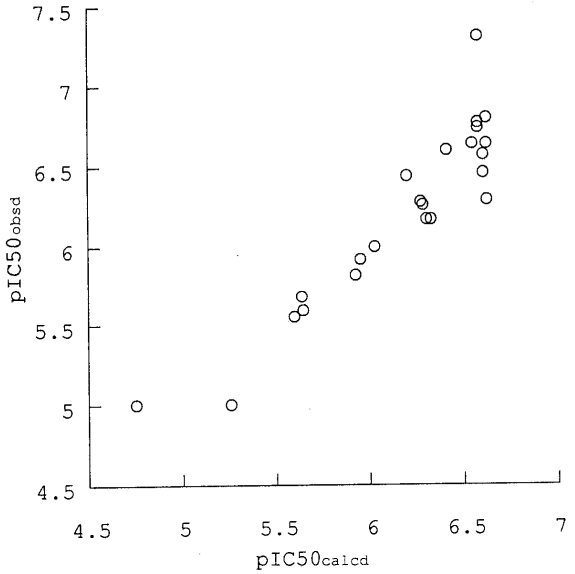


Fig. 2. Relationship between Observed and Calculated (Eq. 6) Ca^{2+}-Antagonistic Activities (pIC_{50}) of Semotiadil Congeners (**1**—**25**)

done by assuming $\log K$ ($=\log(Z_B/Z_A)$) is equivalent to the free energy difference between the two conformations (Eq. 5). Non-activity of compound **25**, which has no B conformation ($Z_B=0$), would be consistent with this.

$$pIC_{50}=0.362(\pm0.198)\log K+5.406(\pm0.507) \quad (5)$$
$$(n=4,\ r=0.984,\ s=0.117,\ F_{1,2}=61.57)$$

QSAR of the Whole Molecules Three kinds of QSAR regression equations (Eqs. 3—5) were obtained for the distal aromatic part and 2- or 5′-substituted 2-PBT part of **1** congeners. These equations were then merged into one equation (Eq. 6) (**2**—**24**). The correlations among the descriptors in Eq. 6 were insignificant except for the one between $C\log P$ and its quadratic term (Table 2). Figure 2 shows the relationship between the observed and calculated activities from Eq.6.

$$pIC_{50}=-0.306(\pm0.128)(C\log P)^2+0.610(\pm0.521)C\log P+0.412(\pm0.130)DIPx+0.296(\pm0.101)\log K+4.847(\pm0.638) \quad (6)$$
$$(n=23,\ r=0.955,\ s=0.171,\ F_{4,18}=46.86,\ C\log P_{opt}=1.00)$$

Discussion

Semotiadil analogs with diverse functional groups on their distal aromatic rings were found to have parabolic lipophilic dependency of their Ca^{2+} antagonistic activities. In particular, $C\log P$ showed a very good correlation with the activities.

The optimum value of $C\log P$ ($C\log P_{opt}$) according to Eq. 3 or Eq. 6 was close to unity. The $C\log P$ value of the corresponding arylalkylamino side chain, that has a methylenedioxyphenoxy (MDP) group, of **1** is 1.40 (Table 1) being close to the $C\log P_{opt}$. Therefore, compound **1** is expected to have potent Ca^{2+} antagonistic activity. There are some other potent compounds with comparable $C\log P$ and activity (Table 1) to **1**. Among those congeners, the MDP group was also reported to be preferable for vasocardioselectivity.[1a)]

In the compound set used to obtain Eqs. 1—3, most have substituted phenoxy groups as the terminal structure Z (Table 1). Compound **4**, however, has a benzofurazanylamino group as Z. Also, a trimethoxyphenyl group was included in **10** or **11**. The binding site of the receptor for those terminal groups, then, might accept more than simply substituted phenoxy groups *via* hydrophobic interaction. Alternatively, the lipophilic nature may play a role in passive permeation to the target receptor. Participation of lipophilicity in the permeation process has been suggested for other Ca^{2+} antagonists.[5)]

The effect on the activity of the methylene units that link the 2-phenyl group and the tertiary amine in the compound set appeared insignificant, because it was not included explicitly in Eq. 2 or Eq. 3. In order to discuss the role of the methylene units for the activity, however, more compounds having different numbers (m) of units will be required.

Dipole properties have an important role in long-range ligand-receptor recognition and subsequent binding. Since the *DIPx* vector runs approximately from C-2 to C-7 of the 2-PBTs, one recognition site for the 2-PBT part of **1** derivatives in the receptor may have a counter dipole

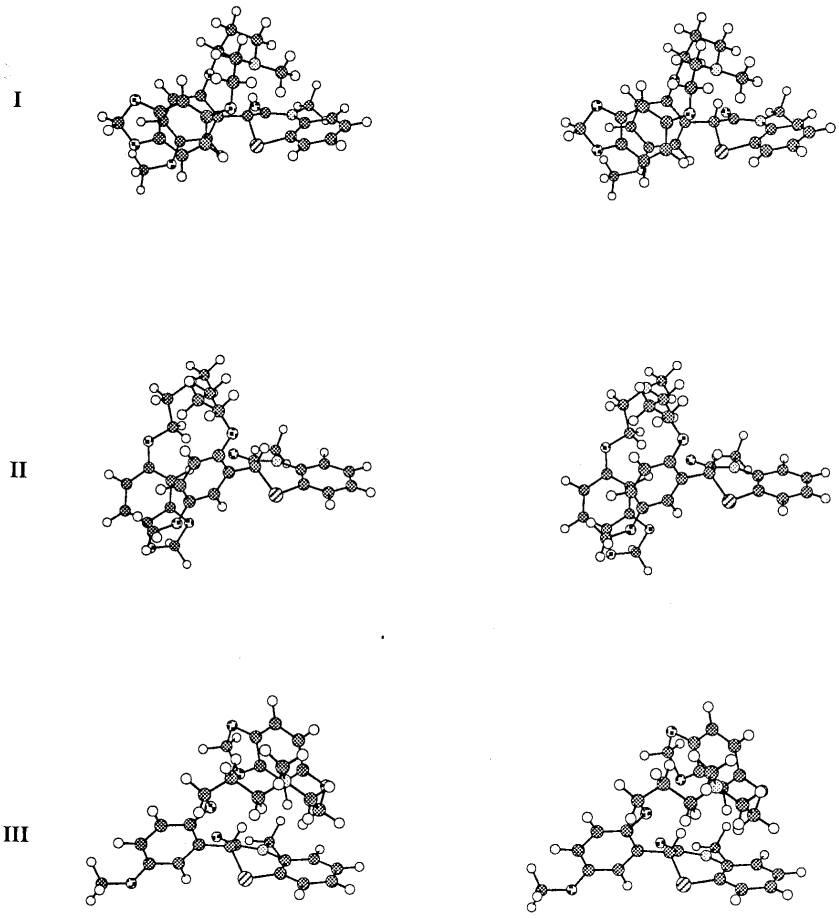


Fig. 3. Stereoview of Three Possible Conformations (I, II, III) of **1**

nature to attract those antagonists electrostatically.

Equation 6 was obtained from Eqs. 3—5 without large perturbation of the coefficients of the original equations. Since the descriptors (*DIPx*, log *K*) in Eq. 4 and Eq. 5 were estimated with 2-phenylbenzothiazine model compounds that lack the long arylalkylamine side chain, these results suggest that the roles of the three physicochemical features in Eq. 6 in bringing about the antagonistic activities are not dependent on one another.

Molecular mechanics calculations, in a previous study, led us to propose three possible conformations (I, II, III) (Fig. 3) of **1** under a constraint of close proximity between the 2-phenyl ring and the MDP group,[2c] that was suggested by NMR spectrometry. Since there is no evidence regarding the active conformation of **1** in the Ca^{2+} receptor, we can consider these conformations as candidates for the active conformation.

Of the 3 possible conformations, II appears to be most acceptable as the active conformation. This is because the 2′-arylalkylamine side chain bypasses the H-2 on the 2-PBT and thus allows the free rotation of the 2-phenyl group around the benzothiazine ring. In contrast, the arylalkylamine of I or III would affect the rotation of the 2-phenyl group. The free rotation of the 2-phenyl group is relevant to the molar fractions, Z_A and Z_B, which afford the descriptor, log *K*, in Eq. 5 and Eq. 6. In addition, the 2-PBT part of II is exposed and its dipole nature (*DIPx*) might be recognized by the receptor.

Experimental

*C*log *P* values were estimated using MacLog *P*.[4] The structures of the model compounds ((*R*)-2-2′-methoxy-5′-R^1-phenylbenzothiazin-3-one) were obtained by optimization with semiempirical (PM3)[6a] and *ab initio* (STO-3G)[6b] molecular orbital methods. Dipole moment of each model compound was estimated for the global minimum structure (conformation B in Fig.1) by a computer program, QSAR+,[7] with the atomic charges assigned by QUANTA.[7] Biological potencies were measured as reported elsewhere with isolated taenia caecum of guinea pig.[1a,b]

Acknowledgment We thank Professor Toshimasa Ishida (Osaka University of Pharmaceutical Science) for his helpful suggestions.

References and Notes

1) *a*)Fujita M., Ito S., Ota A., Kato N., Yamamoto K., Kawashima Y., Yamauchi H., Iwao J., *J. Med. Chem.*, **33**, 1898—1905 (1992); *b*) Nishimura K., Miyawaki N., Yamauchi H., Iso T., *Arzneim.-Forsch./Drug Res.*, **40**, 244—248 (1990); *c*) Miyawaki N., Furuta T., Shigei T., Yamauchi H., Iso T., *J. Cardiovasc. Pharmacol.*, **16**, 769—775 (1990); *d*) Kageyama M., Nishimura K., Takada T., Miyawaki N., Yamauchi H., *ibid.*, **17**, 102—107 (1991); *e*) Miyawaki N., Furuta T., Shigei T., Yamauchi H., Iso T., *Life Sciences*, **48**, 1903—1909 (1991); *f*)Takada T., Miyawaki N., Nishimura K., Nakata K., Matsuno K., Ishida N., Yamauchi H., Iso T., *Arch. Int. Pharmacodyn.*, **309**, 75—87 (1991); *g*) Takada T., Miyawaki N., Kageyama M., Matsuno K., Ishida N., Yamauchi H., Iso T., *J. Cardiovasc. Pharmacol.*, **18**, 855—862 (1991); *h*) Nakayama K., Nozawa Y., Fukuta Y., *ibid.*, **23**, 731—740(1994).

2) *a*)Ota A., Kawashima Y., Ohishi H., Ishida T., *Chem. Pharm. Bull.*, **41**, 1681—1685 (1993); *b*) Ota A., Suhara H., Kawashima Y., *ibid.*, **40**, 833—836 (1992); *c*) Fujimura K., Suhara H., Ota A., Kawashima Y., *ibid.*, **40**, 2901—2904 (1992); *d*) Fujimura K., Fujita M., Suhara H., Kawashima Y., *Bioorg. Med. Chem.*, **2**, 235—242 (1994).

3) Hansch C., Leo A., Unger S. H., Kim K.-H., Nikaitani D., Lien E. J., *J. Med. Chem.*, **16**, 1207—1216 (1973); Hansch C., Rockwell S. D., Jow P. Y. C., Leo A., Steller E. E., *ibid.*, **20**, 304—306 (1977).

4) Leo A., *Chem. Rev.*, **93**, 1281—1306 (1993); BioByte Corp., 201 W. Fourth St., Suite #204, Claremont, California 91711.

5) Mannhold R., Bayer R., Ronsdorf M., Martens L., *Arzenim.-Forsch./Drug Res.*, **37**, 419—424 (1987); Mitani K., Yoshida T., Suzuki T., Koshinaka E., Kato H., Ito Y., Fujita T., *Chem. Pharm. Bull.*, **36**, 776—783 (1988); Mitani K., Sakurai S., Suzuki T., Morikawa K., Koshinaka E., Kato H., Ito Y., Fujita T., *ibid.*, **36**, 4103—4120 (1988).

6) *a*) Modified version of MOPAC (ver. 6) (Quantum Chemistry Program Exchange, No. 455) interfaced with QUANTA.[7]; *b*) Frisch M., Binkley J. S., Schlegel H. B., Raghavachari K., Martin R., Stewart J. J. P., Bobrowicz F., Defrees D., Seeger R., Whiteside R., Fox D., Fluder E., Pople J. A., Gaussian-86 (Revision C), Gaussian, Inc., Carnegie Office Park Building 6, Pittsburgh, PA 15106.

7) Molecular Simulations Inc., 16 New England Executive Park, Burlington, Massachusetts 01803.