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Quantitative Structure–Activity Relationships for Calmodulin Inhibitors

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Using the discriminant analysis method, we completely distinguished 24 calmodulin inhibitors in three groups, as classified by Zimmer *et al.* The resultant discriminant functions distinguished the three groups in terms of positive potential surface area on the side chain, as well as the total and neutral surface areas on the ring in the inhibitor molecules.

Group assignment of additional calmodulin inhibitors from other sources was then estimated according to the discriminant functions. The relationship between structure and inhibitory potency on calmodulin-activated phosphodiesterase for group I inhibitors, together with those estimated, was studied using the adaptive least squares method with several parameters dependent on molecular conformations. A "best conformer" was selected for each inhibitor on the basis of quantitative structure–activity relationship (QSAR). The results of QSAR analysis of group I inhibitors showed that hydrophobicity was important for the ring moiety but not for the side chain. The negative potential surface area of the side chain is necessary for activity. It is desirable for the nitrogen atom in the side chain, which is considered the center of the negative potential area, to be located far from the ring moiety. Thus, the ring moiety and side chain may possibly play different roles in interactions with the receptor system.

Keywords QSAR; conformation analysis; calmodulin inhibitor; MNDO; discriminant analysis; adaptive least squares; conformation-dependent parameter

Introduction

Calmodulin is a highly conserved intracellular calcium binding protein. One of its important molecular functions is to activate more than ten different enzymes, such as phosphodiesterase (PDE) and myosin light chain kinase (MLCK), in a calcium-dependent manner.[1,2] Several hydrophobic areas have been found on calmodulin. They are exposed to its surface following calcium binding,[3,4] and many compounds known as calmodulin inhibitors[5] bind to them.

Zimmer *et al.*[6] have proposed a new criterion for the classification of calmodulin inhibitors according to the manner in which they inhibit the activation of PDE and MLCK by calmodulin. They listed 36 calmodulin inhibitors in four inhibitor groups (groups I—IV) according to the following criterion. The compounds of groups I and II competitively inhibit the activation of PDE and MLCK. Those of group I inhibit the activation of PDE and MLCK at the same concentrations, while those of group II inhibit the activation of PDE at a concentration 5—10 times less than the concentration for the activation of MLCK. The compounds of group III noncompetitively inhibit the activation of these enzymes. Group IV is composed only of those compounds which inhibit the activation of PDE at high concentrations with apparent K_i values above 10 μM and do not affect the activation of MLCK up to a concentration of 200 μM. All the inhibitors are listed in Table I.

Zimmer *et al.* reported that the inhibitors in groups I, II and III possess three different binding sites. Having an interest in the classification of calmodulin inhibitors acting on different binding sites, we first characterized the three inhibitor groups by discriminant analysis. Twenty-nine additional inhibitors[5,7] with inhibitory activity (K_i) on PDE less than 10 μM were collected and distinguished on the basis of resultant discriminant functions. Elucidation was made of the quantitative structure–activity relationship (QSAR) of 31 group I inhibitors, including those estimated. The additional compounds are also shown in Table I.

TABLE I. Calmodulin Inhibitors from Zimmer's Paper[a] and Other Sources[b,c]

Group I[a]		
1 Trifluoperazine	**2** Lidoflazine	**3** Cinnarizine
4 Aprindine	**5** Flunarizine	**6** S811705
Group II[a]		
7 W-7	**8** Calmidazolium	**9** Prenylamine
10 (+)Fendiline	**11** TH1091	**12** TH1090
13 TH1274	**14** TH1011	**15** TH1257
16 S847445	**17** TH1231	**18** S728011
19 S813071	**20** S808314	**21** TMB-8
Group III[a]		
22 Felodipine	**23** Nitrendipine	**24** Nimodipine
Additional inhibitors[b,c]		
25 Clozapine	**26** Desipramine	**27** Thioridazine
28 Pimozide	**29** Chlorprothixene	**30** Chlorpromazine
31 Benperidol	**32** Haloperidol	**33** 1-Chloropromazine
34 Promazine	**35** 3-Chloropromazine	
36 4-Chloropromazine	**37** 7-Hydroxychlorpromazine	
38 8-Hydroxychlorpromazine	**39** 7,8-Dihydroxychlorpromazine	
40 8-Hydroxypromazine	**41** Thiomethylpromazine	
42 Trifluopromazine	**43** Didesmethylchlorpromazine	
44 Desmethylchlorpromazine	**45** Chlorproethazine	
46 Prochlorperazine	**47** Desmethyltrifluopromazine	
48 2-Chloro-10-[2-(dimethylamino)ethyl]phenothiazine		
49 2-Chloro-10-[4-(dimethylamino)butyl]phenothiazine		
50 Penfluridol	**51** Quinacrine	
52 2-Chloroimipramine	**53** r-6033	

a) Ref. 6. *b*) Ref. 5 for **25**—**32**. *c*) Ref. 7 for **33**—**53**.

Method

Compounds and Activity The inhibitors were cited from Zimmer *et al.*[6] and Weiss *et al.*[5,7] All were found to inhibit the activation of PDE by calmodulin. The potency values reported by Zimmer *et al.* were described as K_i, while those by Weiss *et al.* were recorded in different units (IC_{50}). Moreover, potency values from the different sources were not always comparable. In this study, the IC_{50} values of the inhibitors by Weiss *et al.* were linearly transformed to approximate K_i values based on potency data for trifluoperazine included in both sources. Due to the low reliability of the transformed activity, it was thought appropriate to rate potency by activity. Thus, we assigned these compounds to three activity classes according to observed or transformed K_i values (μM), as follows: class 1 (low potency), $5.00 < K_i$; class 2 (intermediate potency), $2.00 < K_i \leq 5.00$; class 3 (high potency), $K_i \leq 2.00$.

Conformation Analysis Since a molecular mechanics method such as MM2 was not applicable to all the inhibitors in Table I, we used

MNDO[8] (modified neglect of diatomic differential overlap, QCPE 353), a semi-empirical molecular orbital method, for conformation analysis. MNDO calculation was performed on a Sony computer NWS-830 and a Kobe Steel transputer KTR-B08.

The initial structure of each compound for conformation analysis was constructed using "MOLDA & GRIMM",[9] a molecular modeling program for micro computer (NEC-9801). Substructure "$-(CH_2)_n-$" was constructed as an extended, energetically low form. For some molecules, the structures were found in the Cambridge Structural Database System (CCD).[10] These were used as the initial structures.

Because the ring moieties were relatively rigid, conformation analysis was mainly carried out on the dihedral angles of bonds in the chain substructures. The number of conformational variables generally ranged from 2 to 5. We defined conformational variables with three dihedral angle sets according to the type of bond: 60°, −60°, and 180° for a single bond, 0° and 180° for a double bond, and −30°, 30°, and 90° for a single bond connecting to a benzene ring. Structural energy was first calculated for each conformer with fixed dihedral angles. Then, structures with the lowest energy and those with somewhat higher energy (within 2 kcal/mol from the lowest energy) for each inhibitor were optimized. The optimization was carried out on bond lengths, bond angles, and dihedral angles.

Parameters for Discriminant Analysis and QSAR Analysis **A) Geometrical Parameters** From the character of the structural patterns of the inhibitors, each conformer structure was considered to be composed of the ring and the chain substructures shown in Fig. 1. We also defined the atom connecting the ring and chain as "Z".

The geometrical and steric features of the molecular structures were parameterized. N_A stands for the number of atoms between Z and the nearest nitrogen atom in the chain. S_r[total] represents the total area of solvent accessible surface (SAS)[11] for a ring. 1.5 Å was used as the radius of a solvent molecule. N_{OH} represents the number of –OH in a ring. *DM* is 1 for the presence of Cl, CF_3, or SCH_3 in the ring. Otherwise it is 0.

B) Electronic Parameters We assigned numbers to the atoms in a ring as shown in Fig. 1. The numbering starts from a benzene ring with Cl, CF_3, or SCH_3 groups, and if such a ring is not included, it starts from a benzene ring without an OH group.

As electronic parameters, Q_Z expresses the atomic charge of atom Z, and Q_1, Q_2,... show the atomic charges of atoms 1, 2, *etc.*

We previously studied the features of SAS of molecules which were divided according to the level of electrostatic potential (EP, kcal/mol).[12] SAS divided by the EP level was called an electrostatic potential surface (EP surface) in this study. S, S_r, and S_c were used to describe the EP surface area for the whole molecule, ring, and chain, respectively. The area of the EP surface in the range of $-3 \leq EP < +3$ of the whole molecule ($S[-3 \leq EP < +3]$) is considered a reflection of the molecule's hydrophobicity.

EP surface areas were calculated by a self-written program[12] based on atomic charges estimated by MNDO and the radius of the solvent molecule, 1.5Å.

Discriminant Analysis The Rao method,[13,14] generally used for discriminant analysis of several groups, was used.

ALS81 Adaptive least squares (ALS),[15-17] a nonparametric pattern classifier, was devised to formulate QSAR in a single mathematical equation regardless of the number of activity classes and to categorize multidimensional structural patterns into multiple ordered classes by the equation. The equation (discriminant function) has a linear form generated by an error-correcting feedback adaptation procedure. In this study, the 1981 version (ALS81)[17] was used, and the correction term (C_i) for a misclassified compound i at the tth iteration is given as

$$C_i(t) = 0.1/[\delta_i(t) + 0.45]^2 + 0.1$$

where

$$\delta_i(t) = |L_i(t) - b_k|$$

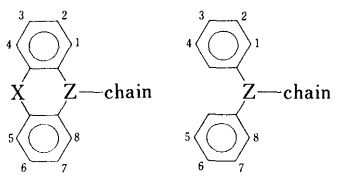


Fig. 1. Model Structures and Numbering in the Ring for Inhibitor Molecules

In this equation, $L_i(t)$ is the value of the discriminant function for compound i, and b_k is the cutting point [nearer to $L_i(t)$] of the observed class for the compound. ALS iteration was performed a maximum of 20 times. The best discriminant function was selected according to Spearman's rank correlation coefficient and apparent variance of error detailed in ref. 16.

The results of ALS calculation were confirmed by the leave-one-out prediction.[18]

Determination of the Best Conformer Set and Discriminant Function First, all conformer combinations were generated. A combination contains the conformers which come from every compound. The correlation of structural parameters with inhibitory potency was then studied by ALS analysis for each conformer combination. The conformer set which showed the highest correlation was selected as the best conformer set for inhibitors. The discriminant function with the highest correlation was finally adopted.

The conformations taken by the best conformers can be considered closely associated with the inhibitory potency of the inhibitors.

Results and Discussion

Structures for Discriminant Analysis and QSAR Analysis Because most of the parameters used for the discriminant analysis of groups I, II, and III were those based on a three-dimensional molecular structure, a standard conformer had to be selected for each compound.

First, we sought the coordinates of inhibitor molecules from CCD. The structures observed by X-ray crystal analysis were used as the standard when available. For inhibitors whose structures could not be found in CCD, we chose the most stable conformers estimated by MNDO. (see "Conformation Analysis" under Methods).

In searching through the CCD, the coordinates of six inhibitors were found: **2**,[19] **3**,[20] **25**,[21] **27**,[22] **28**,[23] and **30**.[24] The structure of compound **5** was constructed by modifying the structure of **3**, since there was little difference between the two. Two compounds, 'W-7' and 'calmidazolium', were omitted in the following discriminant analysis. MNDO could not be applied to molecules with atom group "$-SO_2-$" such as W-7, and calmidazolium had too large a molecular structure to be calculated by MNDO.

In QSAR analysis, since the activity of flexible compounds, such as those we handled here, was considered closely associated with the spatial location of the component atoms, we calculated some parameters dependent on steric molecular structures. Conformation analysis was carried out using MNDO to construct the most stable and semistable conformers, called "candidate conformers," for each inhibitor. Parameters were calculated for each conformer.

Discriminant Analysis of Zimmer's Inhibitors and Group Estimation of Additional Compounds Discriminant analysis was carried out for groups I, II, and III to clarify the structural characters of the inhibitor groups. Although there are only three compounds in group III, the discriminant analysis of three different groups was necessary for classifying the additional compounds. The parameters presented in the section on "Parameters for Discriminant Analysis and QSAR Analysis" were investigated in order to formulate the discriminant functions.

Discriminant analysis produced a set of discriminant functions which provided complete discrimination of the 22 inhibitors in the three groups. The discriminant functions are as follows:

$$Y(\mathrm{I}) = -4.451 S_c[+3 \leq EP] + 43.755 S_r[\text{total}] + 4.180 S_r[-3 \leq EP < +3] - 90.360 \quad (1)$$

$$Y(\mathrm{II}) = -6.045S_c[+3 \leq EP] + 44.837S_r[\mathrm{total}] + 12.755S_r[-3 \leq EP < +3] - 111.654 \quad (2)$$

$$Y(\mathrm{III}) = -1.696S_c[+3 \leq EP] + 28.057S_r[\mathrm{total}] + 3.030S_r[-3 \leq EP < +3] - 39.850 \quad (3)$$

$n=22$; $n_{mis}=0$; maharanobis $D^2=1.76\times10^2$

Here, n is the number of compounds; n_{mis} is the number of misclassified compounds; S_r[total] shows the total area of SAS for the ring moiety.

The discriminant functions distinguish three inhibitor groups as follows: a) group III has a larger SAS area with positive potential in the chain and smaller total area in the ring than groups I and II; b) group II shows a smaller SAS area with positive potential and a larger hydrophobic area in the ring than groups I and III. The discriminant result of the inhibitors in the three groups is shown in Table II.

Twenty-nine additional inhibitors[5,7] with K_i values on PDE of less than 10 μM were classified. Application of the discriminant functions to group recognition of the additional inhibitors showed that 25 compounds could be classified into group I, three into group III, and one into group II. The results are listed in Table III.

ALS Analysis for All Group I Inhibitors Including the additional inhibitors classified by discriminant analysis, the assignment of compounds in the three groups was 31 in group I, 14 in group II, and 6 in group III. Thus, only the inhibitors in group I were subjected to QSAR analysis. This was the only group to maintain sufficient compounds for QSAR analysis. Table IV shows the structures of the 31 inhibitors in group I. Inhibition of calmodulin-activated PDE was analyzed for correlation with the structural features of the inhibitors.

QSAR analysis of the three activity ratings was performed using ALS81, the method usually used to formulate QSAR for structure/activity rating data.

Using ALS, the best discriminant function with satisfactory Spearman's rank correlation coefficients, both in recognition and leave-one-out prediction, was derived from all combinations of conformers.

$$L = 0.714S_c[EP<-3] + 2.901Q_Z - 3.546Q_1 + 1.922N_A \quad [CI=0.646]\ [CI=0.595]\ [CI=0.237]\ [CI=0.899]$$
$$+ 1.191DM - 1.344N_{OH} - 4.181 \quad [CI=1.107]\ [CI=0.601] \quad (4)$$

$n=31$; $n_{mis}=2(0)$; $r_s=0.953$ ($p<0.001$)

leave-one-out prediction:

$n=31$; $n_{mis}=6(0)$; $r_s=0.843$ ($p<0.001$)

Here, CI represents the contribution index; the figure in parentheses after n_{mis} is the number of compounds misclassified by two grades; r_s is Spearman's rank correlation coefficient. The high r_s value shown above proved that the discriminant function had good discriminative ability. Prediction by the leave-one-out technique[18] also confirmed the high reliability ($p<0.001$) of the subset of parameters in this equation. The results of recognition and prediction are listed in Table V. The best conformers selected for the inhibitors with more than one candidate conformer were **3**(A), **5**(A), **6**(B), **25**(A), **27**(A), **28**(A), **29**(B), **46**(B), and **50**(C). The values of the six parameters calculated

TABLE II. Results of the Discriminant Analysis of Zimmer's Group I, II, and III Inhibitors

No.[a]	S_c [$+3 \leq EP$]	S_r [total]	S_r [$-3 \leq EP < +3$]	PLDF[b]	Discrimination
Group I					
1	3.69	4.41	0.94	0.999	I
2	3.44	4.48	0.89	0.999	I
3	0.00	3.77	0.05	0.999	I
4	3.73	4.04	2.41	0.896	I
5	0.00	4.08	1.02	0.999	I
6	0.37	4.46	1.85	0.767	I
Group II					
9	2.34	3.89	3.56	0.999	II
10	0.01	3.90	3.58	1.000	II
11	1.65	3.89	3.62	0.999	II
12	0.00	3.89	3.66	1.000	II
13	1.34	3.89	3.59	0.999	II
14	2.22	4.48	3.02	0.997	II
15	0.94	4.48	2.86	0.998	II
16	1.98	4.47	3.13	0.999	II
17	1.95	4.50	3.14	0.999	II
18	1.11	4.47	2.98	0.999	II
19	2.09	4.88	3.90	1.000	II
20	1.81	4.49	3.47	0.999	II
21	0.27	3.66	2.53	0.981	II
Group III					
22	2.87	2.96	0.98	0.999	III
23	2.81	2.87	1.12	0.999	III
24	3.02	2.87	1.12	1.000	III

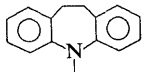
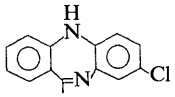
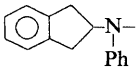
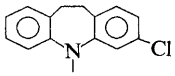
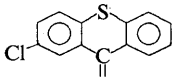
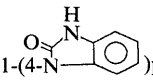
a) For the compound number, see Table I. b) PLDF: probability associated with the largest discriminant function.

TABLE III. Classification of the Additional Inhibitors by Eqs. 1, 2, and 3

No.[a]	S_c [$+3 \leq EP$]	S_r [total]	S_r [$-3 \leq EP < +3$]	PLDF[b]	Discrimination
25	2.72	4.06	1.68	0.998	I
26	2.69	4.06	0.00	0.997	I
27	3.22	4.48	0.16	0.999	I
28	2.74	4.09	2.43	0.598	I
29	2.57	4.16	2.22	0.864	I
30	2.77	4.16	0.00	0.999	I
31	1.96	2.50	1.70	1.000	III
32	1.39	2.52	1.83	1.000	III
33	2.76	4.11	0.00	0.998	I
34	2.78	3.84	0.00	0.895	I
35	2.77	4.14	0.00	0.999	I
36	2.75	4.06	0.00	0.996	I
37	2.77	4.28	0.04	0.999	I
38	2.76	4.27	0.03	0.999	I
39	2.77	4.41	0.15	0.999	I
40	2.77	3.98	0.00	0.987	I
41	2.76	4.44	0.17	0.999	I
42	2.77	4.37	0.28	0.999	I
43	2.25	4.13	0.07	0.999	I
44	2.57	4.13	0.06	0.999	I
45	3.32	4.14	0.00	0.995	I
46	3.65	4.14	0.39	0.992	I
47	2.57	4.37	0.51	0.999	I
48	2.50	4.13	0.04	0.999	I
49	3.01	4.13	0.07	0.998	I
50	2.86	4.09	2.41	0.682	I
51	2.05	4.42	2.33	0.550	II
52	2.94	4.36	0.34	0.999	I
53	3.72	2.53	1.09	1.000	III

a) For the compound number, see Table I. b) PLDF: probability associated with the largest discriminant function.

TABLE IV. Structures of 31 Inhibitors Used in QSAR Analysis

No.[a]	Ring	Chain
5	4-F-Ph$_2$CH-	-NYN-CH_2-CH=CH-Ph
3	Ph$_2$CH-	-NYN-CH_2-CH=CH-Ph
2	Ph$_2$CH-	-$(CH_2)_3$-NYN-CH_2-CONH-(2,6-diMe)Ph
26		-$(CH_2)_3$-NHMe
34	PZ-	-$(CH_2)_3$-N(Me)$_2$
39	2-Cl,7,8-diOH-PZ-	-$(CH_2)_3$-N(Me)$_2$
25		-NYN-Me
40	8-OH-PZ-	-$(CH_2)_3$-N(Me)$_2$
33	1-Cl-PZ-	-$(CH_2)_3$-N(Me)$_2$
37	2-Cl,7-OH-PZ-	-$(CH_2)_3$-N(Me)$_2$
4		-$(CH_2)_3$-N(Et)$_2$
48	2-Cl-PZ-	-$(CH_2)_2$-N(Me)$_2$
44	2-Cl-PZ-	-$(CH_2)_3$-NHMe
52		-$(CH_2)_3$-N(Me)$_2$
41	2-SCH_3-PZ-	-$(CH_2)_3$-N(Me)$_2$
30	2-Cl-PZ-	-$(CH_2)_3$-N(Me)$_2$
43	2-Cl-PZ-	-$(CH_2)_3$-NH_2
38	2-Cl,8-OH-PZ-	-$(CH_2)_3$-N(Me)$_2$
42	2-CF_3-PZ-	-$(CH_2)_3$-N(Me)$_2$
45	2-Cl-PZ-	-$(CH_2)_3$-N(Et)$_2$
47	2-CF_3-PZ-	-$(CH_2)_3$-NHMe
6	4-Cl-PH$_2$CH-	-$(CH_2)_2$-NH-$(CH_2)_3$-S-Ph
36	4-Cl-PZ-	-$(CH_2)_3$-N(Me)$_2$
1	2-CF_3-PZ-	-$(CH_2)_3$-N(Et)$_2$
35	3-Cl-PZ-	-$(CH_2)_3$-N(Me)$_2$
46	2-Cl-PZ-	-$(CH_2)_3$-NYN-Me
49	2-Cl-PZ-	-$(CH_2)_4$-N(Me)$_2$
27	2-SCH_3-PZ-	-$(CH_2)_2$-2-(1-Me)piperidine
29		=CH-$(CH_2)_2$-N(Me)$_2$
28	4-F-Ph$_2$CH-	)piperidine
50	4-F-Ph$_2$CH-	-$(CH_2)_3$-1-[4-OH,4-(3-CF_3,4-Cl)Ph]piperidine

a) For the compound number, see Table I; all compounds are in order of K_i values for PDE.

-NYN-:  . PZ-: 10-phenothiazinyl.

TABLE V. Observed and Estimated Activity Ratings for the 31 Inhibitors in Group I

No.[a]	Obsd.[b]	Recog.[c]	Pred.[d]
5[e]	1	1	1
3	1	1	1
2	1	1	2
26	1	1	1
34	1	1	1
39	1	1	1
25	1	1	2
40	1	1	1
33	1	1	1
37	1	1	2
4	2	2	1
48	2	2	2
44	2	2	2
52	2	2	2
41	2	2	2
30	2	2	2
43	2	2	2
38	2	1	1
42	2	2	2
45	2	2	2
47	2	2	2
6	3	3	3
36	3	3	3
1	3	3	3
35	3	3	3
46	3	2	2
49	3	3	3
27	3	3	3
29	3	3	3
28	3	3	3
50	3	3	3

a) For the compound number, see Table I; all compounds are in order of K_i values for PDE. b) Rating 1: 5 μM < K_i; rating 2: 2 < $K_i \leq 5$; rating 3: $K_i \leq 2$. c) Calculated using Eq. 4. d) Using leave-one-out technique. e) All compounds are in order of K_i values.

from all the conformers, along with the inhibitory potency, are listed in Table VI.

From the signs of the coefficients in the equation, it can be concluded that the following contribute to the enhancement of inhibitory activity: a) negative potential surface area in the chain, b) a positive charged atom connecting the ring and the chain, c) Cl, CF_3, and SCH_3 substituent groups in the ring, and d) more atoms between the ring and the nitrogen atom in the chain, within the range of 0 to 4 atoms. The OH group in the ring and the higher charge of atom 1 in the ring weaken activity. Item c) implies that the hydrophobicity of the ring is important to inhibition.

The squared cross-correlation matrix for the parameters included in Eq. 4 given in Table VII indicates no serious statistical problem in the derivation of Eq. 4.

Conclusions

The structural features of three inhibitor groups defined by Zimmer *et al.* were found by discriminant analysis. The parameters forming the discriminant functions characterize the three groups in terms of positive potential SAS area in the chain, total SAS area in the ring, and hydrophobic area in the ring.

The results of QSAR analysis of group I inhibitors show that hydrophobicity is important for the ring but not for the chain. The negative potential SAS of the chain is required for activity. The nitrogen atom in the chain is located near the center of the negative potential region. This implies that the ring and chain may perform important roles in interactions, hydrophobic and hydrogen bonding, with calmodulin, respectively.

Using conformation-dependent parameters in the QSAR calculation, we made a simultaneous selection of the best set of conformers and the best subset of structural parameters. By this technique, we successfully studied the QSAR of calmodulin inhibitors with different rings and flexible chains. This technique may contribute to an ex-

TABLE VI. Values of Inhibitory Potency and Six Parameters Used in Eq. 4

Compd.[a]	Conf.[b]	K_i (PDE)	S_c [$EP<-3$] (Å^2/100)	Q_Z	Q_1	N_{OH}	N_A	DM
Class 1								
5	A	30.00[c]	3.600	−0.010	−0.013	0.000	0.000	0.000
	B		3.728	0.222	0.000	0.000	0.000	0.000
3	A	14.00	3.630	0.058	−0.015	0.000	0.000	0.000
	B		3.687	0.208	−0.015	0.000	0.000	0.000
2	[d]	12.00	0.740	−0.091	0.103	0.000	3.000	0.000
26	[d]	10.00	0.000	−0.049	0.120	0.000	3.000	0.000
34	[d]	8.80	0.000	−0.336	−0.107	0.000	3.000	0.000
39	[d]	6.56	0.000	−0.332	−0.086	2.000	3.000	1.000
25	A	6.40	0.510	0.307	−0.018	0.000	0.000	1.000
	B		1.189	0.276	−0.008	0.000	0.000	1.000
40	[d]	6.24	0.000	−0.334	−0.117	1.000	3.000	0.000
33	[d]	5.92	0.000	−0.331	−0.189	0.000	3.000	1.000
37	[d]	5.44	0.000	−0.333	−0.089	1.000	3.000	1.000
Class 2								
4	[d]	5.00	0.000	0.061	0.061	0.000	3.000	0.000
48	[d]	4.80	0.000	−0.334	−0.090	0.000	2.000	1.000
44	[d]	3.60	0.000	−0.333	−0.090	0.000	3.000	1.000
52	[d]	3.36	0.000	−0.349	−0.096	0.000	3.000	1.000
41	[d]	3.36	0.000	−0.334	−0.080	0.000	3.000	1.000
30	[d]	3.36	0.000	−0.334	−0.092	0.000	3.000	1.000
43	[d]	3.04	0.000	−0.333	−0.091	0.000	3.000	1.000
38	[d]	2.96	0.000	−0.332	−0.089	1.000	3.000	1.000
42	[d]	2.24	0.000	−0.333	−0.047	0.000	3.000	1.000
45	[d]	2.24	0.000	−0.333	−0.092	0.000	3.000	1.000
47	[d]	2.08	0.000	−0.333	−0.046	0.000	3.000	1.000
Class 3								
6	A	2.00	0.420	0.064	−0.044	0.000	2.000	1.000
	B		0.279	0.064	−0.040	0.000	2.000	1.000
36	[d]	2.00	0.000	−0.334	−0.100	0.000	3.000	1.000
1	[d]	2.00	0.000	0.158	−0.036	0.000	3.000	1.000
35	[d]	1.92	0.000	−0.334	−0.106	0.000	3.000	1.000
46	A	1.76	0.000	−0.330	−0.039	0.000	3.000	1.000
	B		0.000	−0.330	−0.004	0.000	3.000	1.000
49	[d]	1.76	0.000	−0.334	−0.098	0.000	4.000	1.000
27	A	1.44	0.000	−0.106	0.077	0.000	3.000	1.000
	B		0.000	−0.305	0.003	0.000	3.000	1.000
29	A	1.28	0.000	0.037	−0.018	0.000	3.000	1.000
	B		0.000	−0.038	−0.019	0.000	3.000	1.000
28	A	0.56	0.970	0.154	−0.091	0.000	3.000	0.000
	B		0.756	0.069	−0.019	0.000	3.000	0.000
	C		0.755	0.070	−0.018	0.000	3.000	0.000
50	A	0.24	1.120	0.154	−0.088	0.000	3.000	0.000
	B		1.238	0.011	−0.063	0.000	3.000	0.000
	C		1.247	0.070	−0.020	0.000	3.000	0.000

a) For the compound number, see Table I. *b*) Candidate conformers. *c*) All compounds are in order of K_i values. *d*) Compound with only one conformer.

TABLE VII. Cross-Correlation Matrix of Parameters in Eq. 4

	S_c [$EP<-3$]	Q_Z	Q_1	N_{OH}	N_A	DM
S_c [$EP<-3$]	1.000					
Q_Z	0.576	1.000				
Q_1	0.233	0.479	1.000			
N_{OH}	−0.161	−0.285	−0.242	1.000		
N_A	−0.562	−0.431	−0.208	0.114	1.000	
DM	−0.787	−0.586	−0.230	0.135	0.120	1.000

tension of the field of QSAR studies.

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