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Quantitative Structure-Activity Relationships in MAO-Inhibitory 2-Phenylcyclopropylamines: Insights into the Topography of MAO-A and MAO-B

Gun Il Kang and Suk Kil Hong

College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea

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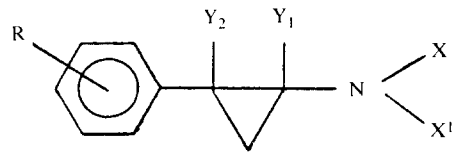
Abstract□Ten (*E*)-and (*Z*)-isomers of 2-phenylcyclopropylamine (PCA), 1-Me-PCA, 2-Me-PCA, N-Me-PCA, and N, N-diMe-PCA and fifteen *o*⁻, *m*⁻, *p*⁻ isomers of (*E*)-PCA with substituents of Me, Cl, F, OMe, OH were synthesized in this laboratory and tested for the inhibition of rat brain mitochondrial MAO-A and MAO-B. The effects of substituents, their positions, and stereochemistry on the inhibition were assessed for the compounds with substituents at cyclopropyl and amino groups and QSAR analyses were performed using the potency data of ring-substituted compounds. The best correlated QSAR equations are as follows: $pI_{50} = 0.804\ \pi^2 - 0.834\ Blo - 1.069\ Blm + 0.334\ Lp - 1.709\ HDp + 7.897$ ($r = 0.945$, $s = 0.211$, $F = 16.691$, $p = 0.000$) for the inhibition of MAO-A; $pI_{50} = 1.815\ \pi - 0.825\ \pi^2 - 1.203\ R + 0.900\ Es^2 + 0.869\ Es^3 + 0.796\ Es^4 - 0.992\ HDp + 0.562\ HAo + 3.893$ ($r = 0.982$, $s = 0.178$, $F = 23.351$, $p = 0.000$) for the inhibition of MAO-B. Based on the potency difference between stereoisomers of cyclopropylamine-modified compounds and on QSAR results, it is proposed that the active sites of MAO-A are composed of one deep hydrophobic cavity near para position, two hydrophobic cavities interacting with Me group, a hydrophobic area accomodating phenyl and cyclopropyl backbone, steric boundaries, a hydrogen-acceptor site near para position, and an amino group binding site and that in addition to the same two hydrophobic cavities, hydrophobic area, steric boundaries, hydrogen-acceptor site, and amino group binding site, another steric boundary near para position and a hydrogen donating site near ortho position constitute active sites of MAO-B.

Keywords□2-Phenylcyclopropylamines, MAO, QSAR, topography.

Since Johnston[1] reported a multiple nature of monoamine oxidase (MAO), many works have been done aiming at synthesizing selective inhibitors of MAO. This came from fact that selective inhibition of MAO-A or -B could ensure better therapeutic outcomes by selectively elevating brain levels of specific biogenic amines whether responsible for mood or Parkinsonism thus reducing side effects due to the change of levels of other biogenic amines.[2] Induction to high selectivity with an enhanced potency has been achieved by modifying known structures in two classes of MAO inhibitors, amphetamines and propynylamines. Ring-modified analogs of amphetamines such as 4-methoxy,[3] 4-dimentylamino-2-methyl,[4] 4-amino-2-fluoro,[5] and 4-amino-2-chloroamphetamines[5] were found to be much more potent and selective for MAO-A than the parent amphetamine. Among propynylamines, clorgyline[1] and deprenyl[6] are known to be highly selective inhibitors of MAO-A and MAO-B, respectively.

Stereoisomeric 2-phenylcyclopropylamines (PCA) belonging to the class of cyclopropylamine inhibitors was first synthesized by Burger and Yost[7] and later a series of analogs and derivatives of 2-phenylcyclopropylamines were prepared by Kaiser *et al.*[8] with a follow-up report regarding their MAO-inhibitory properties by Zirkle *et al*[9]. Among the compounds, a racemate of (*E*)-2-phenylcyclopropylamine (tranylcypromine) is an antidepressant clinically available and the compound is known to be nonselective towards multiple forms of MAO[2]. With regard to cyclopropylamines, modifications were also done for N-phenacylcyclopropylamine[10] and N-phenoxyethylcyclopropylamine[11, 12] and aryl substitution of the latter compound with 2-chloro or 2-iodo groups was found to produce a marked shift towards the inhibition of MAO-A. Since any such a work had not been done for 2-phenylcyclopropylamines, stereoisomers of PCA and 1-methyl-PCA[13] and aromatic nitro analogs of PCA[14] were synthesized in this laboratory and tested for the inhibition of



X, X^1 = H, CH_3
Y_1, Y_2 = H, CH_3
R = H, CH_3, Cl, F, OCH_3, OH

Fig. 1. 2-Phenylcyclopropylamines.

MAO-A and -B at *in vitro* level. The results showed that while stereoisomeric effects on the potency were noticeable such modified compound did not possess notable selectivity. Thereafter, (*E*)-and (*Z*)-isomers of 2-methyl-PCA and N-substituted PCA[15] as well as aromatic methyl and chloro analogs of PCA[16] were tested only to find similar results.

Nevertheless, the MAO-inhibitory data of 2-phenylcyclopropylamines so far accumulated in this laboratory appeared valuable, if more aromatic analogs could be added, in establishing structure-activity relationships of the inhibition of MAO by the compounds. From the studies, it is expected not only to derive structural requirements to achieve an enhanced inhibition with high selectivity towards the multiple forms of enzyme but also to obtain information on active sites of MAO interacting with 2-phenylcyclopropylamine inhibitors and on topological differences between two forms of the enzyme.

A few works have appeared in literatures regarding modes of interaction between 2-phenylcyclopropylamines and MAO. Zirkle *et al.*[9] proposed from the structure-activity data of stereoisomers of PCA and its 1-methyl analog that the compounds would attach to the enzyme with C-2, C-3, and phenyl ring on the same plane and with amino group at a different position. In order to provide an explanation for observed inactivity of 2-phenylcyclobutylamine, Belleau and Moran[17] hypothesized that 2-phenylcyclopropylamines would interact with the enzyme through the electron-rich cyclopropyl ring positioning N, C-1, C-2, and phenyl ring at the same enzyme surface. Since these classical works are only based on comparisons of a few structural data and the ones not taken into account recently advanced concepts of multiple nature of MAO and of suicidal inhibition mechanism of MAO by 2-phenylcyclopropylamines,[18] the approach taken in this work introducing these new concepts is to be considered new in QSAR of MAO inhibitiors.

EXPERIMENTAL METHODS

Instrumentation

The melting points reported in this paper were determined using a sybron thermolyne (Olympus, Tokyo) and are uncorrected. NMR data are the ones obtained with a Varian EM-360A 60 MHz spectrometer using TMS as standard. A Hewlett Packard model HP 5985B GC/MS system was used to collect all direct probe MS data and electron ionization voltage was 70 eV. Gas chromatography was performed on a Hitachi model 163 gas chromatograph equipped with a hydrogen flame detector. A glass column (2.0 m × 3 mm i.d.) packed with 3% OV-17 on 80/100 mesh Chromosorb W (HP) was used with carrier gas (nitrogen) at a flow rate of 50 ml/min. The operating conditions are shown in each table.

2-Phenylcyclopropylamines and their derivatives with substituents at cyclopropyl and amino group (compounds, 1-10)

Synthesis of compounds, (*E*)-2-phenylcyclopropylamine (**1**, (*E*)-PCA), (*Z*)-2-phenylcyclopropylamine (**2**, (*Z*)-PCA), (*E*)-1-methyl-2-phenylcyclopropylamine (**3**, (*E*)-1-Me-PCA), and (*Z*)-1-methyl-2-phenylcyclopropylamine (**4**, (*Z*)-1-Me-PCA) were previously reported[13]. Compounds, (*E*)-2-methyl-2-phenylcyclopropylamine (**5**, (*E*)-2-Me-PCA), (*Z*)-2-methyl-2-phenylcyclopropylamine (**6**, (*Z*)-2-Me-PCA), (*E*)-N-methyl-2-phenylcyclopropylamine (**7**, (*E*)-N-Me-PCA), (*Z*)-N-methyl-2-phenylcyclopropylamine (**8**, (*Z*)-N-Me-PCA), (*E*)-N,N-dimethyl-2-phenylcyclopropylamine (**9**, (*E*)-N,N-diME-PCA), and (*Z*)-N,N-dimethyl-2-phenylcyclopropylamine (**10**, (*Z*)-N,N-diMe-PCA) were synthesized by Myung Hee Choi[15] in this laboratory. 2-Methyl analogs (**5**, **6**) were prepared, *via* condensation of α-methylstyrene with ethyl diazoacetate, following essentially the same procedures as described for the synthesis of **1** and **2**. (*E*)-and (*Z*)-isomers of N-methyl-PCA (**7** and **8**) were synthesized from (*E*)-PCA and (*Z*)-PCA, respectively by the method of Kaiser *et al.*[19] and compound, **9**, treating (*E*)-PCA with HCHO-HCO_2H[20] or HCHO-$NaBH_4$[21]. The compound, **10** was synthesized by reacting (*Z*)-PCA with HCHO-NaH_2PO_3[22].

Melting points of the compounds are recorded in Table I with chromatographic properties which are intended to show isomeric purity of the compounds. NMR (solvent, chemical shifts, δ) and MS (m/z, rel. abund.) for compounds, **5-10** are reported as follows: **5** NMR ($CDCl_3$/DMSO-d_6) 1.53-1.0 (m,

Table I. Chemical and *in vitro* MAO-inhibitory activity data of 2-phenylcyclopropylamines and their derivatives with substituents at cyclopropyl and amino groups

No.	Compound[1]	mp, °C[2]	t_R, min (R_f)[3]	IC_{50} (μM)	
				MAO-A	MAO-B
1	(*E*)-PCA HCl	151-155	(0.31)	0.29	0.078
2	(*Z*)-PCA HCl	164-165	(0.48)	0.23	0.044
3	(*E*)-1-Me-PCA HCl	192-193	(0.53)	0.062	0.063
4	(*Z*)-1-Me-PCA HCl	189-190	(0.73)	2.3	0.47
5	(*E*)-2-Me-PCA HCl(*)	160-163	3.2	72	20
6	(*Z*)-2-Me-PCA HCl(*)	176-177	2.9	720	33
7	(*E*)-N-Me-PCA HCl	111-112	3.45	0.065	0.035
8	(*Z*)-N-Me-PCA HCl $C_2H_2O_4$(*)	115-117	3.2	0.096	0.025
9	(*E*)-N,N-diMe-PCA HCl	185-187	2.95	0.45	0.77
10	(*Z*)-N,N-diMe-PCA $C_2H_2O_4$(*)	–	2.7	43	8.4

[1] a. Abbreviations: See the text. b. (*) New compounds synthesized in this laboratory. c. Structures were positively identified by IR, NMR, and MS. Molecular ions were observed by MS.

[2] a. Lit. values are: **1**, 153.5-156.5[7]; **2**, 164-166[7]; **3**, 198-199[8]; **4**, 193-194[8]; **7**, 112-113[19]; **9**, 187-189.[7]. b. Clearly defined mp was not obtainable for **10**.

[3] a. GC and TLC were performed in a form of free base to determined general and isomeric purity and the compounds were found to be pure. b. TLC was done on Kieselgel 60G with the solvents, $CHCl_3$/EtOAc/Et_3N (17:2:1) for **1** and **2** and EtOAc/MeOH/NH_4OH (17:2:1) for **3** and 4. c. GC conditions are: column temp., 100-250°C, programmed at a rate of 20°C /min; injector temp., 250°C.

2H, cyclopropyl H), 1.70 (s, 3H, CH_3), 3.0-2.45 (m, 1H, cyclopropyl H), 7.3 (s, 5H, arom. H), 9.07 (bs, 3H, NH_3^+) MS 147 (4,M), 146 (4), 132 (base peak), 115 (45), 103 (14), 91 (17); **6** NMR ($CDCl_3$/ DMSO-d_6) 1.48-0.82 (m, 5H, CH_3 & cyclopropyl H), 2.88-2.12 (m, 1H, cyclopropyl H), 7.42 (s, 5H, arom. H), 8.25 (bs, 3H, NH_3^+) MS 147 (4, M), 132 (base peak), 115 (45), 103 (13), 91 (14), 77 (24); **7** NMR ($CDCl_3$) 1.5-1.0 (m, 1H, cyclopropyl H), 2.13-1.6 (m, 1H, cyclopropyl H), 3.1-2.13 (m centered at 2.82, 5H, CH_3 & cylcopropyl H), 7.3 (s, 5H, arom. H), 9.97 (bs, 2H, NH_2^+); **8** NMR ($CDCl_3$/DMSO-d_6) 1.77-0.83 (m, H, cyclopropyl H), 3.17-1.87 (m, 5H, CH_3 & cyclopropyl H). 7.33 (s, 5H, arom. H), 9.78 (s, 3H, NH_2^+ & CO_2H). MS 147 (base peak, M), 146 (96), 132 (77), 115 (65), 91 (53), 70 (72); **9** NMR (D_2O) 2.27-1.47 (m, 2H, cyclopropyl H), 3.67-2.33 (m, 8H, $2CH_3$ & cyclopropyl H), 7.7 (s, 5H, arom. H); **10** NMR (D_2O) 0.85-1.05 (m, 2H, cyclopropyl H), 3.25-2.35 (m, 8H, $2CH_3$ as separate two singlets & cyclopropyl H), 7.45 (s, 5H, arom. H) MS 161 (26, M), 160 (27), 146 (19), 117 (16), 115 (20), 91 (33), 70 (99), 42 (base peak).

(E)-2-Phenylcyclopropylamines with substituents at phenyl ring (compounds, 11-25)

Compounds, (*E*)-2-(*o*-methylphenyl) cyclopropylamine (**11**, (*E*)-*o*-Me-PCA), (*E*)-*m*--Me-PCA (**12**), (*E*)-*p*-Me-PCA (**13**), (*E*)-2-(*o*-chlorophenyl) cyclopropylamine (**14**, (*E*)-*o*-Cl-PCA), (*E*)-*m*-Cl-PCA (**15**), and (*E*)-*p*-Cl-PCA (**16**) had been synthesized by Won Park[16] in this laboratory and the other compounds, (*E*)-2-(*o*-fluorophenyl) cyclopropylamine (**17**, (*E*)-*o*-F-PCA), (*E*)-*m*-F-PCA (**18**), (*E*)-*p*-F-PCA (**19**) and (*E*)-2-(*o*-methoxyphenyl) cyclopropylamine (**20**, (*E*)-*o*-OMe-PCA), (*E*)-*m*-OMe-PCA (**21**), (*E*)-*p*-OMe-PCA (**22**), (*E*)-2-*o*-hydroxyphenyl) cyclopropylamine (**23**, (*E*)-*o*-OH-PCA), (*E*)-*m*-OH-PCA (**24**), and (*E*)-*p*-OH-PCA (**25**) were newly synthesized for the present QSAR study. The compounds, **11-19** and **22** were prepared *via* the condensation of ring-substituted styrenes with ethyl diazoacetate according to the method described by Kaiser *et al.*[8] The intermediates, ethyl 2-(substituted-phenyl) cyclopropanecarboxylates were distilled and (*E*)/(*Z*) ratio of the product was determined by GC. Selective hydrolysis of the esters gave each isomer of 2-(substituted-phenyl)cyclopropanecarboxylic acid and the isomeric purity of the acids was determined by GC following derivatization with diazomethane for which conditions are the same as those reported in Table II for ring-substituted PCA analogs. The retention times (min) of (*E*)/(*Z*)-isomers are shown with the numbers of final compounds as follows:

Table II. Chemical and *in vitro* MAO-inhibitory activity data of (*E*)-2-phenylcyclopropylamines with substituents at phenyl ring

No.	Compound[1)]	mp, °C[2)]	t_R, min[3)]	IC_{50} (μM)	
				MAO-A	MAO-B
11	(*E*)-*o*-Me-PCA HCl(*)	170-172	2.75	0.069	0.076
12	(*E*)-*m*-Me-PCA HCl(*)	168-170	2.9	0.39	0.15
13	(*E*)-*p*-Me-PCA HCl(*)	172-173	2.85	0.098	0.10
14	(*E*)-*o*-Cl-PCA HCl(*)	165-168	3.35	0.46	0.56
15	(*E*)-*m*-Cl-PCA HCl(*)	174-177	3.65	0.55	0.63
16	(*E*)-*p*-Cl-PCA HCl	195-198	3.65	0.027	0.044
17	(*E*)-*o*-F-PCA HCl(*)	121	1.6	0.36	0.14
18	(*E*)-*m*-F-PCA HCl(*)	178	1.7	0.32	0.12
19	(*E*)-*p*-F-PCA HCl(*)	190-192	1.7	0.057	0.025
20	(*E*)-*o*-OMe-PCA HCl	192-193	2.9	0.32	0.074
21	(*E*)-*m*-OMe-PCA HCl	154-155	3.2	0.59	0.074
22	(*E*)-*p*-OMe-PCA HCl	178-180	3.1	0.042	0.068
23	(*E*)-*o*-OH-PCA HCl(*)	196-199	2.4	0.49	0.17
24	(*E*)-*m*-OH-PCA $C_2H_2O_4$(*)	174	2.7	0.36	0.70
25	(*E*)-*p*-OH-PCA HCl(*)	208-209	3.0	3.16	10

1) a. Abbreviations: See the text. b. (*) New compounds synthesized in this laboratory. c. Structures were positively identified by IR, NMR, and MS. Molecular ions were observed by MS.

2) a. Lit. values are: **16**, 195-198[8)]; **20**, 193-194.5[23)]; **21**, 155-156[23)]; **22**, 180-182[23)].

3) a. Determined in a form of free based except for **23**, **24**, and **25** t_R values of which were measured following derivatization with heptafluorobutyric anhydride and the compounds were found to be pure. c. GC conditions are: column temp., 150- 260°C , programmed at a rate of 20°C /min; injector temp., 260°C.

11, 4.05/3.75; **12**, 4.0/3.75; **13**, 3.7/3.4; **14**, 4.6 /4.35; **15**, 4.4/4.1; **16**, 4.75/4.55; **17**, 3.0/2.65; **18**, 3.0/2.65; **19**, 3.0/2.65; **22**, 4.4/4.2. Curtius reaction of (*E*)-carboxylic acids yielded (*E*)-2-phenylcyclopropylamines, **11-19** and **22**. Compounds, **20** and **21** were synthesized according to the procedures described by Arvidsson *et al.*[23)] using (*E*)-cinnamic acids as the starting material. Compounds, **23-25** were obtained by BBr_3 demethylation of **20-22**, respectively and resulting products were purified using ion-exchange chromatography (Amberlite CG-120) followed by flash chromatography.

Melting points and retention times of the compounds, **11-25** are recorded in Table II. MS (m/z, rel. abund.) are reported as follows: **11** 147 (49, M), 132 (base peak), 115 (40), 105 (13), 91 (21), 77 (9); **12** 147 (base peak, M), 146 (86), 132 (95), 115 (57), 105 (22), 91 (48); **13** 147 (50, M), 146 (45), 132 (base peak), 115 (39), 105 (12), 91 (19); **14** 167 (5, M), 166 (13), 132 (base peak), 115 (20), 103 (12), 77 (17); **15** 169 (22), 168 (30), 167 (65, M), 166 (65), 149 (29), 132 (base peak), 115 (40); **16** 169 (13), 168 (19), 167 (39, M), 166 (48), 149 (23), 132 (base peak); **17** 151 (base peak, M), 150 (93), 133 (52), 130 (58), 122 (12), 109 (14) ; **18** 151 (base peak, M), 150 (92), 133 (62), 130 (35), 122 (12), 109 (13); **19** 151 (96, M), 150 (base peak), 133 (61), 130 (39), 122 (15), 109 (19); **20** 163 (8, M), 162 (18), 146 (25), 132 (base peak), 121 (24), 103 (24), 91 (59), 77 (37); **21** 163 (base peak, M), 162 (72), 146 (46), 132 (73), 115 (31), 103 (43), 91 (52), 77 (47); **22** 163 (78, M), 162 (80), 146 (42), 132 (base peak), 121 (89), 103 (33), 91 (50), 77 (45); **23** 149 (10, M), 131 (base peak), 120 (6), 107 (20), 91 (23), 77 (33), 65 (15), 56 (18); **24** 149 (72, M), 132 (70), 120 (17), 103 (49), 91 (28), 77 (39), 65 (22), 56 (59); **25** 149 (85, M), 148 (95), 132 (base peak), 120 (28), 107 (59), 91 (28), 77 (49), 65 (28), 56 (49).

Inhibition of rat brain mitochondrial MAO-A and -B

Activity of MAO-A was measured referring to Sjoerdsma *et al.*[24)] described for the metabolism of serotonin and to UV method of Udenfriend *et al.*[25)] which was applied to the determination of serotonin.

Activity of MAO-B was measured using benzylamine as a substrate according to Tabor *et al.*[26] with modifications. Optimum conditions for the measurement of IC_{50} values were described previously[13, 27].

QSAR parameters and correlation analyses

In addition to IC_{50} values, following parameters were used. π, π^2, F, R, MR, HA, and HD are the Norrington's corrected values and taken from a table of Unger.[28] σ and Es were taken from the tables made by Tute[29] and Chu.[30] Verloop's steric parameters were taken from Verloop *et al.*[31] Rekker's hydrophobicity fragment constants, f were taken from a tabulation of Martin.[32] Important parameter values used to derive the equations in the study are shown in Table V.

Multiple regression analyses were done using IBM PC/AT with a SPSS/PC+ program (SPSS inc., Chicago, U.S.A.). The correlation coefficient (r), standard error of coefficient (s), F test value (F), and level of significance (p) are given. Inclusion of parameters was judged by application of the F test.

RESULTS AND DISCUSSION

Structure-activity relationships of 2-phenylcyclopropylamines

IC_{50} values for the inhibition of MAO-A and -B by the compounds substituted at cyclopropyl and amino groups are shown in Table I and comparisons were made so as to see their differences in terms of potency, selectivity, and stereoisomerism (Table III).

Parent 2-phenylcyclopropylamines were found not to differ much in the potency between (*E*)- and (*Z*)-isomers and it was the same also for their N-methyl derivatives which were a few times more potent than the parent compounds. Cyclopropyl 1-methyl substitution resulted in comparable potency to that of parent compound for (*E*)-isomer whereas (*Z*)-1-Me-PCA was found to be ten times less potent than its parent (*Z*)-PCA. On the other hand, it was found that compounds with 2-methyl substituent were all much less potent than the parent and 1-methyl derivatives. N, N-Dimethyl substitution reduced potencies, in particular, for (*Z*)-isomer, which was a very different feature from N-monomethyl substitution.

Despite the marked differences in potency depending on the substituent positions on cyclopropyl and amino groups, present results are the ones not to be interpreted by the terms of direct structure-affinity relations because the potency of suicide inhibitors are determined by the combining effects of the compounds on affinity and inactivation rate[33]. However, the potency difference between two stereoisomers is thought more closely to represent difference in binding modes though part of the potency is always a reflection of the binding affinity of the compounds to MAO. Therefore, the present results that two isomers produced comparable potencies in PCA and its N-Me derivatives and that in other compounds studied, (*Z*)-isomers were

Table III. Potency ratio, selectivity and stereoisomeric effect of 2-phenylcyclopropylamines with substituents at cyclopropyl and amino groups

Compound[1]	Potency ratio[2]		Selectivity[3] (MAO-A/MAO-B)	Isomeric effect (*E*/*Z*)[3]	
	MAO-A	MAO-B		MAO-A	MAO-B
(*E*)-PCA	1.0	1.0	1/3.7	1/1.3	1/1.8
(*Z*)-PCA	1.3	1.8	1/5.2		
(*E*)-1-Me-PCA	4.7	1.2	1/1.0	37.1/1	7.5/1
(*Z*)-1-Me-PCA	0.13	0.17	1/5.0		
(*E*)-2-Me-PCA	0.004	0.004	1/3.6	10.1/1	2.0/1
(*Z*)-2-Me-PCA	0.0004	0.002	1/21.8		
(*E*)-N-Me-PCA	4.5	2.2	1/1.9	1.5/1	1/1.4
(*Z*)-N-Me-PCA	3.0	3.1	1/3.8		
(*E*)-N,N-diMe-PCA	0.6	0.1	1.7/1	85.7/1	11.1/1
(*Z*)-N,N-diMe-PCA	0.007	0.009	1/5.1		

[1] For the abbreviation, see the text.
[2] Potency relative to that of (*E*)-PCA (tranylcypromine) which is assigned a value of 1.0.
[3] Expressed in potency ratios.

Table IV. Potency ratio and selectivity of (*E*)-2-phenylcyclopropylamines with substituents at phenyl ring

Compound[1]	Potency ratio[2]		Selectivity[3] (MAO-A/MAO-B)
	MAO-A	MAO-B	
(*E*)-PCA	1.0	1.0	1/3.7
(*E*)-*o*-Me-PCA	4.2	1.0	1.1/1
(*E*)-*m*-Me-PCA	0.7	0.5	1/2.5
(*E*)-*p*-Me-PCA	3.0	0.8	1.1/1
(*E*)-*o*-Cl-PCA	0.6	1.4	1/8.3
(*E*)-*m*-Cl-PCA	0.5	1.2	1/8.7
(*E*)-*p*-Cl-PCA	10.7	1.8	1.6/1
(*E*)-*o*-F-PCA	0.8	0.5	1/2.5
(*E*)-*m*-F-PCA	0.9	0.7	1/2.8
(*E*)-*p*-F-PCA	5.1	3.2	1/2.3
(*E*)-*o*-OMe-PCA	0.9	1.1	1/4.3
(*E*)-*m*-OMe-PCA	0.5	1.1	1/8.0
(*E*)-*p*-OMe-PCA	6.9	1.1	1.6/1
(*E*)-*o*-OH-PCA	0.6	0.5	1/2.9
(*E*)-*m*-OH-PCA	0.8	0.05	4.7/1
(*E*)-*p*-OH-PCA	0.09	0.008	3.2/1

[1] For the abbreviation, see the text.
[2] Potency relative to that of (*E*)-PCA (tranylcypromine).
[3] Expressed in potency ratios.

generally much less potent than (*E*)-isomers appear useful to derive a conceptual active site model of MAO. Present results also show values indicating potency differences in the inhibition of between MAO-A and MAO-B depending on substituents, their positions, and stereochemistry but the difference was not so pronounced as to usefully reflect two different active sites of MAO-A and MAO-B.

The IC_{50} values of ring-substituted 2-phenylcyclopropylamines are shown in Table II. Potency comparisons were made for the inhibition of MAO-A and -B by the compounds and selectivity for the inhibition of multiple forms were calculated (Table IV). Compounds with stronger potency than the parent (*E*)-PCA were found in all substituent compounds except in those of hydroxyl group and *p*-substituent compounds were generally more potent than other positional analogs also except for hydroxy compounds among which *p*-hydroxy analog was the least potent. One notable compound was *p*-chloro analog which was ten times as potent as (*E*)-PCA in the inhibition of MAO-A and the potency was comparable to that of parent compound in the inhibition of MAO-B.

In comparison with (*E*)-PCA, notable shift of selectivity was not observed in all substitutions but with two exceptions of *m*-and *p*-hydroxy analogs of (*E*)-PCA, which were found to be ten times more selective towards MAO-A than the parent (*E*)-PCA. A similar result was reported by Baker *et al.*[34] in their recent metabolic works of tranylcypromine that *p*-hydroxy metabolite is less potent than (*E*)-PCA and more selective in the inhibition of MAO-A than MAO-B. A *p*-fluoro analog of (*E*)-PCA also appeared in a literature by Coutts *et al.*[35] in which it was reported to be 10 times more potent than tranylcypromine with no notable selectivity for the inhibition of multiple forms of MAO.

Examining the effect of substituents and substitution positions on the potency, it was not possible, at least without mathematical analyses, to describe, with a generalization, the inhibition potency in relation to physicochemical properties. At the first hand, the compounds of an electron-donating substituent such as methyl group were not different from those of electron-withdrawing ones such as chloro and fluoro groups. While present results are from *in vitro* ones which would represent more direct interaction of the compounds with the enzyme, similar observation has been reported by Kaiser and Setler[36] using *in vivo* inhibition data of the substituent compounds of chloro, trifluoromethyl, and methoxyl groups. Therefore, it was planned in this study to introduce more mathematical method of quantitative structure-activity relationships for the purpose of deriving general rules governing inhibition of MAO in terms of physicochemical parameters of the substituents and in turn to obtain information on the modes of interaction and finally on the topography of multiple forms of MAO.

Quantitative structure-activity relationships of ring-substituted 2-phenylcyclopropylamines.

The QSAR analysis was performed using the inhibition data of MAO-A and -B by the compounds, **1** and **11-25** (n = 16) and physicochemical parameters of the substituents (Table V). The QSAR equations are separately derived for the inhibition of MAO-A and -B, so that differing nature of the inhibition and active sites of the multiple forms could be looked into.

A. Inhibition of MAO-A

Analysis was done first to see whether the potency could be correlated with single parameter of π to find absence of any correlation (eq. 1). Subsequently, steric effect at meta position was linearly combined with π and a significant correlation (r = 0.624)

Table V. Important QSAR parameters used to derive equations in the study

Substituents	π	fp	Es^2	Es^3	Es^4	Blo	Blm	Lp	σ_2	R
H	0.00	0.193	1.24	1.24	1.24	1.00	1.00	2.06	0.00	0.00
o-Me	0.84	0.193	0.00	1.24	1.24	1.52	1.00	2.06	−0.07	−0.12
m-Me	0.52	0.193	1.24	0.00	1.24	1.00	1.52	2.06	−0.17	−0.05
p-Me	0.60	0.702	1.24	1.24	0.00	1.00	3.00	3.00	−0.07	−0.14
o-Cl	0.76	0.193	0.27	1.24	1.24	1.80	1.00	2.06	0.37	−0.14
m-Cl	0.77	0.193	1.24	0.27	1.24	1.00	1.80	2.06	0.23	−0.06
p-Cl	0.73	0.930	1.24	1.24	0.27	1.00	1.00	3.52	0.37	−0.16
o-F	0.00	0.193	0.78	1.24	1.24	1.35	1.00	2.06	0.34	−0.29
m-F	0.22	0.193	1.24	0.78	1.24	1.00	1.35	2.06	0.06	−0.12
p-F	0.15	0.425	1.24	1.24	0.78	1.00	1.00	2.65	0.34	−0.34
o-OMe	−0.33	0.193	0.69	1.24	1.24	1.35	1.00	2.06	0.12	−0.43
m-OMe	0.12	0.193	1.24	0.69	1.24	1.00	1.35	2.06	−0.27	−0.17
p-OMe	−0.03	0.240	1.24	1.24	0.69	1.00	1.00	3.98	0.12	−0.50
o-OH	−0.41	0.193	0.69	1.24	1.24	1.35	1.00	2.06	0.12	−0.56
m-OH	−0.50	0.193	1.24	0.69	1.24	1.00	1.35	2.06	−0.36	−0.22
p-OH	−0.61	−0.370	1.24	1.24	0.69	1.00	1.00	2.74	0.12	−0.64

1) For the source of parameter values, see the text.
2) HAo values are 0.00 except 1.00 for *o*-OMe and *o*-OH substituents.
3) HDP values are 0.00 except for *p*-OH whic is 1.00

was found (eq. 2).

$$pI_{50} = 0.494\,\pi + 6.526 \quad (1)$$
$$r = 0.454\ s = 0.487\ F = 3.628\ p = 0.078$$

$$pI_{50} = 0.603\,\pi - 0.935\ Blm + 7.580 \quad (2)$$
$$r = 0.624\ s = 0.443\ F = 4.155\ p = 0.040$$

$$pI_{50} = 0.526\,\pi - 0.536\ Blm + 0.409\ Lp + 6.151 \quad (3)$$
$$r = 0.760\ s = 0.393\ F = 5.481\ p = 0.013$$

$$pI_{50} = 0.632\,\pi - 0.514\ Blo - 0.899\ Blm + 0.273\ Lp + 7.466 \quad (4)$$
$$r = 0.776\ s = 0.388\ F = 4.169\ p = 0.027$$

$$pI_{50} = 0.242\,\pi - 0.530\ Blm + 0.497\ Lp - 1.243\ HDp + 6.060 \quad (5)$$
$$r = 0.922\ s = 0.238\ F = 15.609\ p = 0.000$$

$$pI_{50} = 0.349\,\pi - 0.526\ Blo - 0.991\ Blm + 0.358\ Lp - 1.246\ HDp + 0.740 \quad (6)$$
$$r = 0.936\ s = 0.228\ F = 14.105\ p = 0.000$$

Equation 3 was derived by including a steric effect at para position and among the parameters such as B1p, B3p, and Lp, the highest correlation was achieved with Lp. A slight improvement in the correlation was noted when steric effect on ortho position, B1o was included (eq. 4). Hydrogen-donating and acceptor groups were considered and it was found that hydrogen-donating group at the para position is very significantly related to the activity as shown by marked improvement of correlation from r = 0.760 (eq. 3) to r = 0.992 (eq. 5), indicating possible involvment of hydrogen-bonding in such interactions. From eq. 5, eq. 6 was derived by including B1o and again the correlation was improved with a statistical significance. The eq. 6 denotes that inhibition potency of MAO-A by the series of compounds can be explained with 88% of the varience by such physicochemical parameters as π, B1o, B1m, Lp and HDp and that while Lp and π positively contribute to the potency, substituents at ortho and meta positions as well as hydrogen donating groups at para position are not favorable to the potency.

$$pI_{50} = 0.361\,\pi - 0.058\ \sigma_1 - 0.535\ Blo - 0.884\ Blm + 0.354\ Lp - 1.255\ HDp + 7.403 \quad (7)$$
$$r = 0.936\ s = 0.240\ F = 10.614\ p = 0.001$$

$$pI_{50} = 0.355\,\pi - 0.060\ \sigma_2 - 0.050\ Blo - 0.906\ Blm + 0.366\ Lp - 1.239\ HDp + 7.369 \quad (8)$$
$$r = 0.936\ s = 0.240\ F = 10.624\ p = 0.001$$

$$pI_{50} = 0.513\ \pi - 0.095\ F - 0.561\ R - 0.717\ Blo - 0.948\ Blm + 0.262\ Lp - 1.345\ HDp + 7.777 \quad (9)$$
$$r = 0.939\ s = 0.247\ F = 8.594\ p = 0.004$$

$$pI_{50} = 0.804\ \pi^2 - 0.834\ Blo - 1.069\ Blm + 0.334\ Lp - 1.709\ HDp + 7.897 \quad (10)$$
$$r = 0.945\ s = 0.211\ F = 16.691\ p = 0.000$$

Importance of electronic effects in potency was examined and it was found that σ_1 and σ_2 as well as both F and R terms are not contributing factors as shown in eq. 7-9. The best correlated equation ($r = 0.945$) was obtained with π^2 instead of π of eq. 6 (eq. 10).

The nature of interactions of *p*-substituents with the active sites of MAO-A was further examined by deriving eq. 11-14. Correlation with single Lp was found with $r = 0.592$ and a positive slope (eq. 11). When fragment hydrophobic parameter, fp was included with Lp, much better correlation was achieved with $r = 0.844$ (eq. 12) than that ($r = 0.727$) obtained from using π and Lp (eq. 13). Besides, high correlation could be achieved with the use of one parameter, fp with $r = 0.798$ (eq. 14). These all might suggest that the longer the substituents at para position, more tight binding through hydrophobic interaction could be expected between *p*-substituent and near region of the active sites.

$$pI_{50} = 0.513\ Lp + 5.377 \quad (11)$$
$$r = 0.592\ s = 0.440\ F = 7.548\ p = 0.016$$

$$pI_{50} = 0.458\ \pi + 0.492\ Lp + 5.348 \quad (12)$$
$$r = 0.726\ s = 0.390\ F = 7.233\ p = 0.008$$

$$pI_{50} = 1.288\ fp + 0.265\ Lp + 5.653 \quad (13)$$
$$r = 0.844\ s = 0.304\ F = 16.106\ p = 0.000$$

$$pI_{50} = 1.542\ fp + 6.227 \quad (14)$$
$$r = 0.798\ s = 0.329\ F = 24.515\ p = 0.000$$

B. Inhibition of MAO-B

A significant correlation of single parameter, π with the potency of the inhibition of MAO-B was obtained with $r = 0.622$ (eq. 15), differing from the result of the inhibition of MAO-A where dependence of the potency on single π was not observed. When steric effect at meta position was included with π, the correlation was slightly but statistically significantly improved with $r = 0.654$ (eq. 16). Steric effect at the para position was also significantly correlated with the potency, resulting in over-all correlation of $r = 0.686$ (eq. 17). Equation 18 is the one showing dependence of the potency on the combined π, Es^2, Es^3, and Es^4. For the analysis of MAO-B data, Taft's steric parameters were used because they gave higher correlation than other steric parameters such as MR or Verloop's steric parameters which were used for the inhibition of MAO-A.

$$pI_{50} = 0.820\ \pi + 6.742 \quad (15)$$
$$r = 0.622\ s = 0.517\ F = 8.853\ p = 0.010$$

$$pI_{50} = 0.878\ \pi + 0.329\ Es^3 + 6.402 \quad (16)$$
$$r = 0.654\ s = 0.519\ F = 4.865\ p = 0.027$$

$$pI_{50} = 0.958\ \pi + 0.486\ Es^3 + 0.370\ Es^4 + 5.858 \quad (17)$$
$$r = 0.686\ s = 0.519\ F = 3.561\ p = 0.047$$

$$PI_{50} = 1.379\ \pi + 0.963\ Es^2 + 1.212\ Es^3 + 1081\ Es^4 + 3.373 \quad (18)$$
$$r = 0.767\ s = 0.479\ F = 3.927\ p = 0.032$$

Among the hydrogen bonding properties of the substituents, HDp, when linearly combined with π, yielded better correlation of $r = 0.848$ than π alone (eq. 19). Inclusion of HDp with the parameters used in eq. 18 gave highly-correlated equation, eq. 20 with $r = 0.910$. Differing from the result of MAO-A, depdendence of the potency of hydrogen accepting properties at the ortho position (HAo) was also noted. Thus, improvement of the correlation was found when HAo was included with π to obtain $r = 0.713$ (eq. 21). Slight improvement was also observed when HAo was included with the parameters in eq. 19 and the resultant correlation coefficient was $r = 0.861$ (eq. 22). Equation 23 is the best correlated equation consisting of π, steric parameters at all three positions, and hydrogen bonding properties of HDp and HAo. When Verloop's steric parameters were used instead of Taft's parameters, eq. 24 was derived with $r = 0.903$.

$$pI_{50} = 0.456\ \pi - 1.631\ HDp + 6.909 \quad (19)$$
$$r = 0.848\ s = 0.364\ F = 16.580\ p = 0.000$$

$$pI_{50} = 0.849\ \pi + 0.645\ Es^2 + 0.908\ Es^3 + 0.544\ Es^4 - 1.509\ HDp + 4.726 \quad (20)$$
$$r = 0.910\ s = 0.324\ F = 9.664\ p = 0.001$$

$$pI_{50} = 1.045\ \pi + 0.725\ HAo + 6.612 \quad (21)$$
$$r = 0.713\ s = 0.481\ F = 6.723\ p = 0.010$$

$$pI_{50} = 0.599\ \pi - 1.465\ HDp + 0.431\ HAo + 6.831 \quad (22)$$
$$r = 0.861\ s = 0.363\ F = 11.490\ p = 0.001$$

$$pI_{50} = 1.132\ \pi + 1.093\ Es^2 + 1.112\ Es^3 + 0.800\ Es^4 - 1.102\ HDp + 0.694\ HAo + 3.619 \quad (23)$$
$$r = 0.946\ s = 0.267\ F = 12.780\ p = 0.001$$

$$pI_{50} = 0.944\ \pi - 0.947\ Blo - 1.108\ Blm - 0.502\ Blp - 1.370\ HDp + 0.509\ HAo + 9.679 \quad (24)$$
$$r = 0.903\ s = 0.355\ F = 6.605\ p = 0.007$$

It was examined whether electronic effects are determining factors for the inhibition of MAO-B and σ_2 among various electronic parameters was found to yield an improved correlation compared to π alone (eq. 25). Since any electronic effect did not influence potency of the inhibition of MAO-A, more precise analyses were done as in eq. 26 and 27. When σ_2 was combined with π and HDp, the correlation coefficient was r = 0.907 (eq. 26) although two terms, π and HDp gave r = 0.848 (eq. 19). Further inclusion of σ_2 with parameters used in eq. 24 gave marked improvement in the correlation from r = 0.903 to r = 0.952 (eq. 27), strongly suggesting that electron deficiency at the ortho position would positively contribute to the potency. While σ_1 effect was not observed in the inhibition of MAO-B, slightly better equation could be derived by including R with the the parameters in eq. 23 as in eq. 28 which might suggest that electron donating resonance effects of the substituents act favorably to the potency. When π^2 was considered, the best correlated equation was obtained with r = 0.982 (eq. 29).

$$pI_{50} = 0.753\ \pi + 0.645\ \sigma_2 + 6.704 \quad (25)$$
$$r = 0.661\ s = 0.515\ F = 5.309\ p = 0.024$$

$$pI_{50} = 0.324\ \pi + 0.948\ \sigma_2 - 1.784\ HDp + 6.868 \quad (26)$$
$$r = 0.907\ s = 0.301\ F = 18.471\ p = 0.000$$

$$pI_{50} = 0.926\ \pi + 1.066\ \sigma_2 - 1.504\ Blo - 1.221\ Blm - 1.074\ Blp - 1.422\ HDp + 0.456\ HAo + 11.035 \quad (27)$$
$$r = 0.952\ s = 0.269\ F = 10.937\ p = 0.002$$

$$pI_{50} = 1.703\ \pi - 1.506\ R + 1.316\ Es^2 + 1.134\ Es^3 + 1.088\ Es^4 - 1.403\ HDp + 0.482\ HAo + 2.689 \quad (28)$$
$$r = 0.970\ s = 0.212\ F = 18.370\ p = 0.000$$

$$pI_{50} = 1.815\ \pi - 0.825\ \pi^2 - 1.203\ R + 0.900\ Es^2 + 0.869\ Es^3 + 0.796\ Es^4 - 0.992\ HDp + 0.562\ HAo + 3.893 \quad (29)$$
$$r = 0.982\ s = 0.178\ F = 23.351\ p = 0.000$$

C. Discussion of the QSAR results

It is not known whether the environment of active sites binding with 2-phenylcyclopropylamines would be the same as that with other inhibitors or substrates but since it is generally accepted concepts that, though specific mode of interaction might be different, they would occupy common sites containing flavin moiety where substrate are oxidized, present results could be discussed in comparison with previous results of inhibitors and substrates.

The importance of hydrophobic interaction of 2-phenylcyclopropylamines with both MAO-A and -B was noted in the present study, indicating that there exist hydrophobic regions in PCA compounds-binding active sites of MAO. In accordance with our results, in a study of propynylamine inhibitors using *in vitro* potency data, Martin *et al.*[37] found positive effect of hydrophobicity on potency. Similarly, *in vitro* potency of propynylamines could be correlated with one partition coefficient by Williams and Lawson[38] proposing that lipid penetration into the MAO might be determining factor in the inhibition of MAO by the compounds. However, a previous study by Fujita[39] using *in vivo* potency data of ten 2-phenylcyclopropylamines with substituents at meta and para positions showed negative dependence of the potency on π (eq. 30). In this case, the negative dependence was attributable to the increased extraction with a high partition. In a report by Johnson[40] which described an absence of the π contribution to the *in vivo* potency of a series of N-isopropylarylhydrazide inhibitors, it was proposed that the absence might be due to the balance of the hydrophobic effects on between penetration into the brain and binding to the enzyme.

$$Los\ 1/c = -0.746\ \pi + 1.858\ \sigma_2 + 0.502\ Es^3 + 5.180 \quad (30)$$

The assumption that more hydrophobic compounds might be better selective towards the inhibition of MAO-A which is known to be in more hydrodrophobic environment than MAO-B has been argued by Williams[41] with propynylamines which showed absence of such relationships. In the present study, high correlation of one π with potency was found in the inhibition of MAO-B but not in MAO-A. It is however of interest that inhibition of MAO-A and -B was positively correlated with π when analyzed including with all other parameters. While the best correlation was achieved with π^2 term for MAO-A, parabolic relationship with respect to hydrophobicity was observed in the inhibition of MAO-B. The positive dependence of the potency in th-

e inhibition of MAO-A on π^2 term might be explained in terms of the ease of access to the active sites of MAO-A embedded in lipophilic membrane and the possible involvment of hydrophobic bonding. On the other hand, in the inhibition of MAO-B which is assumed to be outside of the membrane,[42] optimum π might be needed for binding to the active sites where a fair portion of the hydrophilic environment is also present.

In the present study, the potency was found to be dependent on steric effects at all ring positions. Quite consistently with eq. 30 of Fujita[39] obtained by deleting ortho substituents, negative dependence of potency on ortho and meta substituents was observed in the inhibition of MAO-A and -B. The most typical result of the present study is that positive steric effect elicited by the para substituent for MAO-A is favorable to the potency, contrary to the negative dependence in the inhibition of MAO-B. While simple active site boundary could be set up to account for the negative contribution of the substituent for MAO-B, more precise analyses were done in an attempt to rationalize nature of the interaction between active site of MAO-A and steric bulk leading to the enhanced potency. For the inhibition of MAO-A, better correlation was always found with length term than with bulk term, presumably implying that the longer the substituent at the para position the binding affinity would be greater. The binding force was characterized to be hydrophobic because much better correlation was found with fragment hydrophobic parameter than with π which is a measure of the hydrophobic nature of the molecule as a whole. As will be depicted in the proposed topography, a deep cavity-like hydrophobic environment where interactions occur with *p*-substituent could be assumed to account for the result.

In the present study, when hydrogen-donating property of the para substituent was considered, marked improvement of the correlation was observed in both the inhibition of MAO-A and -B but with negative coefficient, which might indicate involvement of hydrogen bonding in the binding of para substituent to near hydrogen-accepting region of the active sites. It appears evident that such an interaction would cause a distortion of the geometry of the compounds otherwise fit with the active sites. One differing feature of MAO-A from MAO-B was that hydrogen accepting group at ortho position positively contributed to the potency in the inhibition of MAO-B but not to MAO-A. This might suggest presence of hydrogen-donating region near ortho position of the compounds in their binding geometry. This fact of the presence of hydrogen-donating region in active sites of MAO-B might be thought in relation to the fact that while *m* and *p*-tyramine are common substrates for MAO-A and MAO-B, o-tyramine is specific for MAO-B.[43]

Electronic effects of the substituent were found not to affect potency in the inhibition of MAO-A but dependence of σ_2 on the potency was noted in the inhibition of MAO-B, indicating electron deficiency on the ortho position of the aromatic ring favorably contributes to the potency. Similar dependency on σ_2 was reported by Fujita[39] as shown in eq 30 with proposal of the involvement of charge-transfer interaction between aryl-ring and the enzyme. The dependency on σ_2 however does not appear to be contradictory with the positive contribution of hydrogen-accepting group at ortho position because such σ_2 effect will increase electron density at the ortho position through the charge transfer enabling for the electronegative atom connected to it to have high electron density resulting in its tight binding to hydrogen donating region of active sites.

It was found in our previous metabolic studies of tranylcypromine that the drug undergoes metabolism through the pathways forming *p*-hydroxy species and two QSAR equations were derived, using the data of Zirkle *et al.*,[9] by grouping the compounds into two, with and without *p*-substituents.[27] The derived equations showed dependence of potency on σ_1 with higher potency by greater electron-withdrawing effect. Such dependence of potency on σ_1 was frequently referred to in relation to the electron density at the side chain amino groups as in N-isopropylarylhydrazide[40] and propynylamines.[37] The present result of extensive QSAR analysis with aryl-substituted 2-phenylcyclopropylamines was found to be consistent with that of Fujita[39] in that any σ_1 dependence was not noted. Whether σ_1 dependence is related to inactivation rate influenced by stabilization of the radicals by electron-withdrawing groups or by increase of non-protonated amino species which is assumed to be the species interacting with MAO, σ_1 factor was found not to be related to the potency in the present study. In this work, dependence of the potency on an electronic effect, R was noted in the inhibition of MAO-B and in this case electron-donating resonance effect was positive contribution factor. It might be however safe to abstain from giving any explanations on this electronic factor in terms of interactions between drug and MAO-B and we should be satisfied with the result of the best correlated QSAR equation.

Topography of MAO-A and MAO-B

The potency of suicide inhibitors such as 2-phenylcyclopropylamines is determined by two, affinity of the compounds to the enzyme and inactivation rate of the enzyme by the compounds.[33] Cyclopropylamines would exhibit different potencies depending on substituent and its position, which affect the affinity and inactivation rate of the enzyme by the compounds. In fact, 1-methyl substitution of cyclopropylbenzylamine caused a change of both affinity and inactivation rate.[44] Therefore, in order to propose conceptual models of active site of MAO, stereoisomeric potency differences were taken into account based on the assumption that (*E*)- and (*Z*)-isomers would inhibit the MAO in the similar rate and only are different in terms of their affinity to the enzyme. Any differentiation between MAO-A and -B was not made when potency data of the compounds with substituents at cyclopropyl and amino groups were used and it was assumed that better geometrical fit with the active sites would induce higher potency when two stereoisomers are compared. For the purpose, the structure of (*E*)-2-phenylcyclopropylamines in the active sites was constructed modelling (*E*)-(+)-PCA based on the fact that more potent (+)-isomer of (*E*)-PCA is (*1S, 2R*) in its absolute configuration[45] and (*Z*)-isomers were depicted with (*1S, 2S*) configuration.

Ring-substituents of (*E*)-2-phenylcyclopropylamines are to influence overall potency by changing affinity through hydrophobic, electronic, and steric effects and inactivation rate mainly by electronic effect. Because the correlations of the potency could be derived mainly with hydrophobic and steric effects of the substituents as well as their hydrogen bonding properties in this QSAR analysis of using ring-subst ituted 2-phenylcyclopropylamines, major portion of the active sites was able to be drawn by referring to the result of QSAR analysis.

Fig. 2 shows thus constructed topography of MAO-A and -B. Right half of the active sites was constructed with the results of potency data depending upon the stereochemistry of the compounds with substituents at cyclopropylamine portions and the same two hydrophobic cavities and a receptor boundary at connecting upper side as well as hydrophobic area interacting with phenyl, C-1, and C-2 backbone and an amino group binding site were proposed for MAO-A, and MAO-B. The hydrophobic cavities and an active site boundary were also found to account for the potency difference among all cyclopropyl-modified compounds. These are shown in Fig. 3a through 3i.

Fig. 3a and b are to show similar potency between (*E*)-and (*Z*)-isomers of PCA. The partial loss of the fit at the phenyl ring of (*Z*)-isomer might be compensated by the right fit of C-1, C-2 backbone in one of the hydrophobic cavities. Fig. 3c shows good fit of (*E*)-1-Me-PCA additionally through hydrophobic binding of 1-methyl group to the cavity which might account for the higher potency produced by the compound than by (*E*)-PCA. On the other hand, proper fit of (*Z*)-1-Me-PCA which is much less potent than (*E*)-isomer could not be demonstrated with the same active sites because of steric boundary as shown in Fig. 3d. Fig. 3e shows difficulty of the active site to accomodate (*Z*)-2-Me-PCA and similar distortion from the fit can be demonstrated with (*E*)-2-Me-PCA, both of which are all much less potent than either PCA or 1-Me-PCA. Fig. 3f and 3g are to demonstrate good fits of (*E*)-and (*Z*)-isomers of N-Me-PCA which are equipotent each other and comparable in their potency to (*E*)-1-Me-PCA. N-Methyl group of (*E*)-isomer could participate in the binding through hydrophobic or van der Waals forces to hydrophobic cavity. The (*Z*)-isomer can be thought to bind through cyclopropyl backbone and N-methyl group to the cavity and hydrophobic area, respectively. Both isomers of N,N-diMe-PCA in active sites of MAO are shown in Fig. 3h and 3i and it is

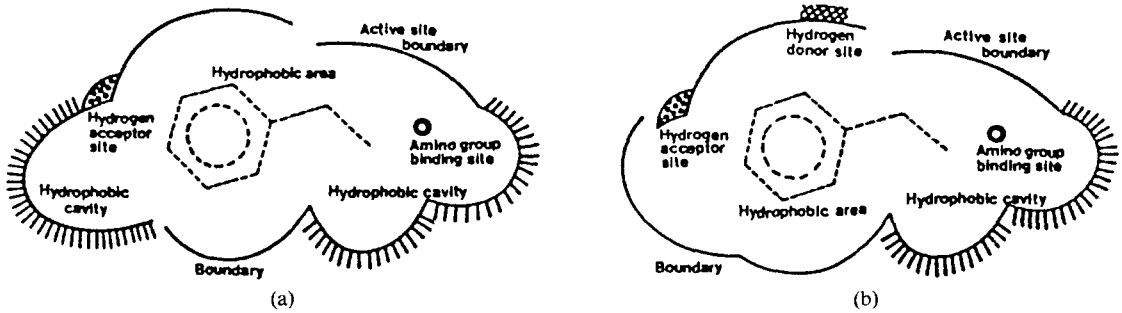


Fig. 2. Proposed topography of (a) MAO-A and (b) MAO-B.

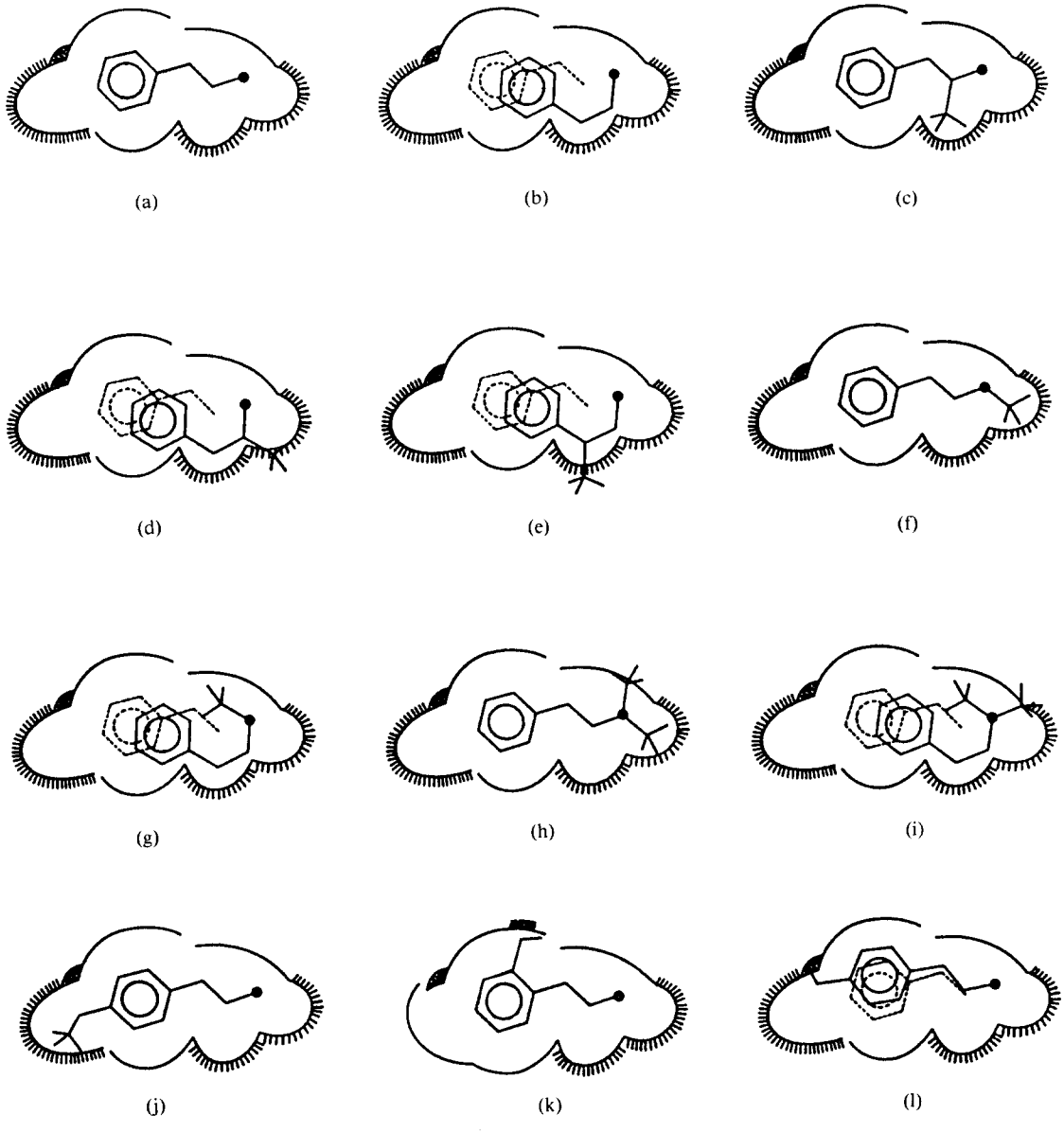


Fig. 3. 2-Phenylcyclopropylamines in active site of MAO.

Fig. 3a through 3i are to demonstrate (a) (*E*)-PCA, (b) (*Z*)-PCA, (c) (*E*)-1-Me-PCA, (d) (*Z*)-1-Me-PCA, (e) (*Z*)-2-Me-PCA, (f) (*E*)-N-Me-PCA, (g) (*Z*)-N-Me-PCA, (h) (*E*)-N, N-diMe-PCA, and (i) (*Z*)-N, N-diMe-PCA in MAO-A. Similar figures could be drawn with MAO-B. Fig. 3j is (*E*)-*p*-OME-PCA in MAO-A; 3k, (*E*)-*o*-OH-PCA in MAO-B; and 31, (*E*)- *p*-OH-PCA in MAO-A.

evident that the compounds all would experience steric hindrance to rest in the active sites with the proper fits. Higher potency of the (*E*)-isomer than (*Z*)-isomer, though less potent than (*E*)-PCA or (*E*)-N-Me-PCA, might be explained by the fit of one N-Me group in the cavity in spite of the fact that some distortion from the fit of the phenyl group should be endured when two N-methyl groups are drawn inside the active site boundary. In case of (*Z*)-N, N-diMe-PCA, it would not be possible to draw the molecule inside the active site boundary without serious disturbance of the fit of essential parts of the

molecule, even amino group.

Left half sides of the topography of active sites of MAO-A and -B were constructed so as to accomodate the results from QSAR analysis (Fig. 2) and the sides are places to express differing nature of the active sites of two forms. Two boundaries at the regions of ortho and meta substituents are the same for the two forms and the same hydrogen acceptor site were proposed on the surface connecting phenyl, C-1, C-2, and amino group binding sites. In active sites of MAO-A, a deep hydrophobic cavity was constructed at the region of para substituents of the compounds and in stead, simple active site boundary was proposed for MAO-B at the same region. In order to account for increased potency with hydrogen accepting group at ortho position, a hydrogen donor site was drawn near ortho substituents for MAO-B. Although the potency of the individual compounds is the one to be calculated according to the equations separately derived for the inhibition of MAO-A and -B by substituting appropriate values for the parameters depending upon positional substituents, demonstrations were made to account for potency differencies elicited by some typical compounds (Fig. 3j, k, and l). Fig. 3j is to demonstrate a good fit of (*E*)-*p*--OMe-PCA with MAO-A which is 6.9 times as potent as (*E*)-PCA in the inhibition of MAO-A and comparably potent in the inhibition of MAO-B. Similar demonstrating figure can be drawn for *p*-Cl analog and the same steric fits could be also possible for methoxy and chloro compounds when substituting MAO-B for MAO-A. Their higher potencies observed for the inhibition of MAO-A than for MAO-B might be explained by additional binding of the substituents in the hydrophobic cavity near para position of active sites of MAO-A. Fig. 3k is to show the higher preference of (*E*)-*o*-OH-PCA for MAO-B while two other hydroxy isomers are more selective towards MAO-A, where additional hydrogen bonding could be noted for the binding of ortho isomer to MAO-B. The anomalous low potency of (*E*)-*p*-OH-PCA compared to other para compounds might be partly due to the distortion of the binding geometry caused by strong hydrogen bonding of *p*-hydroxy group to hydrogen acceptor sites of MAO-A and -B (Fig. 3l).

In order to examine applicability of the QSAR equations, eq. 10 for MAO-A and eq. 29 for MAO-B to the previously-synthesized two nitro analogs of (*E*)-PCA,[14] the calculated values were compared with those of observed ones and it was found that while inhibition potency for MAO-A and -B by *m*-nitro analog and that for MAO-B by *p*-nitro analog are relatively closely predicted by the equations, measured low potency for the inhibition of MAO-A by *p*-nitro analog was far deviated from the calculated one with pI_{50} of 7.18. The reason for this deviation might be thought in relation that serious distortion of the fit could be caused by unacceptibility of the bulky nitro group into the narrow hydrophobic cavity due to the electronic repulsion between nitro group and hydrogen acceptor site of the active sites consisting of carboxylate anion-like amino acid residues. In order to obtain additional proof for the proposed topography of MAO-A and -B and to find highly potent and selective inhibitors on the basis of present results, several new compounds characteristic of their long and hydrophobic para substituents and with the potency and selectivity predicted by QSAR equations derived in this study were considered to synthesize and the works towards the synthesis and test of *p*-bromo, *p*-iodo, and *p*-phenyl analogs of (*E*)-PCA as several of such compounds are in progress.

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