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Benzylpiperazine Derivatives. IX.[1] Structure–Antiulcer Activity Studies of 1-(Aminocarbonylalkyl)-4-benzylpiperazine Derivatives by the Adaptive Least-Squares Method

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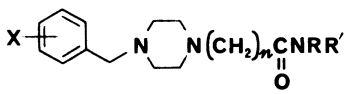
Quantitative structure–antiulcer activity relationships of 1-(aminocarbonylalkyl)-4-benzylpiperazine derivatives (I) were analyzed by using the adaptive least-squares (ALS) technique. Discriminant functions show that (1) a bulky amide moiety is disadvantageous, (2) a small number of methylene groups between carbonyl and piperazine is favorable, (3) a substituent which has a large B_1 value (or B_2 value when the substituent is forced to be in the in-plane conformation) with low lipophilicity at the 3 and/or 4 position of the benzyl moiety is favorable for antiulcer activity with low acute toxicity.

Keywords——quantitative structure–activity relationship; adaptive least-squares technique; prediction; benzylpiperazine; 1-piperazineacetamide; antiulcer activity; cytoprotective activity

In the course of our search for novel antiulcer agents, we have synthesized a series of 1-(aminocarbonylalkyl)-4-benzylpiperazine derivatives (I) and tested them for antiulcer activity against indomethacin-induced gastric ulcer in rats.[1] Among them, we have selected 1-(pyrrolidinocarbonylmethyl)-4-(2,3,4- or 3,4,5-trimethoxybenzyl)piperazine difumarate (Id and It) for further study.

In order to study the structural requirements for antiulcer activity, quantitative structure–activity relationship (QSAR) analyses were performed. The adaptive least-squares technique of Moriguchi *et al.* (ALS 81)[2] was used, because the compounds were classified into two discrete groups, "active" and "inactive."



I

Results and Discussion

Recognition

In the first attempt, the effects of variation in the amide moiety on antiulcer activity (compounds Ia—i) were subjected to analysis. Qualitative study suggested that bulky amide residues diminish the activity.[1] Therefore, the bulkiness of the NRR′ moiety was evaluated in terms of Van der Waals volume (V_w),[3] and ALS analysis was performed to obtain Eq. 1.

$$Y = -2.474V_w + 2.293 \quad (1)$$

$$n=9,\ R_s=0.800,\ n_{mis}=1,\quad t=3.52,\ p<0.01$$

where Y is the discriminant score for the classification of activity ratings, n represents the number of compounds used to derive the equation, n_{mis} is the number misclassified, R_s

is the Spearman rank correlation coefficient,[4] t is Student's t-value[4] calculated by $t=R_s[(n-2)/(1-R_s^2)]^{1/2}$ and p is the level of significance. Equation 1 showed that a bulky group was disadvantageous for the activity, as suggested by the qualitative study.

Next, the effects of the substituent X on the benzyl moiety were subjected to analysis (compounds Ij—u and Id). Qualitative study suggested that substitution at both the 3 and 4 positions is requisite for the activity.[1] This situation was formulated in Eq. 2 using a dummy variable (D) which indicates the presence of 3,4-disubstitution ($D=1$) or not ($D=0$) [method A].

$$Y=1.500D-0.462 \tag{2}$$

$$n=13,\ R_s=0.822,\ n_{mis}=1,\quad t=4.17,\ p<0.001$$

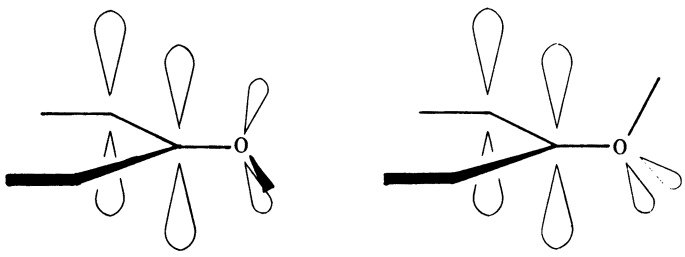
Equation 2 successfully classified the compounds, so 3,4-disubstitution seems to have some significance. However, the reliability of the equation seems marginal, for careful inspection of the structure–activity data reveals that out of the 13 compounds only four have 3,4-disubstitution and three of them are "active." Moreover, it is not acceptable physicochemically that 3,4-disubstitution is important no matter what groups are involved.

Among the compounds which have various substitution patterns of methoxy groups, only two are "active": the 2,3,4- (Id) and 3,4,5-trimethoxy (It) derivatives. It is known that the O–CH_3 bond of an unhindered methoxy group is thought to be in, or nearly in, the plane of the ring (Fig. 1a) due to the conjugation of the nonbonding electrons with the π-electrons of the aromatic ring. On the other hand, the middle methoxy group of the 2,3,4- and 3,4,5-trimethoxyphenyl moiety is sandwiched between two *ortho* substituents and forced out of conjugation with the ring, having O–CH_3 bonds perpendicular to the plane of the aromatic ring (Fig. 1b).[5] With these conformational points in mind, classification of these 13 compounds using Verloop's steric parameter (B_1)[6] gave a satisfactory result (Eq. 3). The middle methoxyl group is perpendicular in conformation to the other methoxy groups, so the B_2 value, minimum width perpendicular to B_1, was used instead of B_1 for the group in the calculation [method B].

$$Y=2.139B_{1-3}+0.873B_{1-4}-3.839 \tag{3}$$

$$n=13,\ R_s=1.000,\ n_{mis}=0$$

where B_{1-3} and B_{1-4} are the B_1 values of the substituents at the 3 and 4 positions, respectively. Equation 3 indicates that the substituent with larger B_1 (minimum width) value is favorable for the antiulcer activity. Thus, bulky substituents at the 3 and 4 positions play some role in the activity.



(a) planar conformation (conjugated)

(b) out of plane conformation (non-conjugated)

Fig. 1

The rotational barrier of the C_{ar}–O bond of substituted anisoles was reported about 3—6 kcal/mol,[7] so it should be relatively easy to rotate the bond. Therefore, the methoxy group may lie out of the plane conformation at the active site to form a complex, and in that case, B_1 can be regarded as the minimum width in the direction perpendicular to the plane of the aromatic ring. If the conformation of the trimethoxyphenyl moiety can not be changed from that stated above because of the steric nature, minimum width should be B_1 for the middle methoxy group and B_2 for the di-*ortho* methoxy groups. This parameterization [method C] gave the same equation as Eq. 3.

Next, all the compounds (Ia—aa) were subjected to analysis using a dummy variable N, which indicates the number of methylene groups between carbonyl and piperazine, to obtain the following equations.

method A

$$Y = -2.678V_w + 1.397D - 0.381N + 1.677 \quad (4)$$

$$n=27,\ R_s=0.822,\ n_{mis}=2,\quad t=7.21,\ p<0.001$$

method B

$$Y = -2.653V_w + 1.601B_{1-3} + 1.598B_{1-4} - 0.421N - 2.029 \quad (5)$$

$$n=27,\ R_s=0.918,\ n_{mis}=1,\quad t=11.57,\ p<0.001$$

method C

$$Y = -2.631V_w + 1.960B_{1-3} + 1.449B_{1-4} - 0.482N - 2.166 \quad (6)$$

$$n=27,\ R_s=0.918,\ n_{mis}=1,\quad t=11.57,\ p<0.001$$

Equations 4—6 show almost the same degree of classification ability. This means that the data set used does not contain sufficient information to distinguish which equation is the best. This is probably because the number of "active" compounds is only 8. Equations 4—6 show that (1) a bulky amide moiety is disadvantageous, and (2) a small number of methylene groups is favorable, but the exact effects of the substituents on the benzyl moiety are still controversial. The results of recognition using Eqs. 4—6 are summarized in Table I. The correlation matrix of parameters used in Eq. 6 is shown in Table II, indicating low colinearity of the variables.

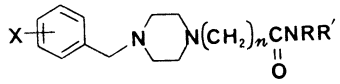
Prediction

Previously, 1-benzyl-4-(pyrrolidinocarbonylmethyl)piperazine was selected as the basic structure of antiulcer agents taking into consideration the acute toxicity, which seems to increase along with the lipophilicity of the substituent X on the benzyl moiety.[1] The next problem is to elucidate the role of the substituent X on the benzyl moiety to the antiulcer activity.

The leave-one-out method, which is an evaluation method based on the predictive power of the equation, was applied to find the best discriminant function. However, the results based on Eqs. 4—6 were almost the same (Table III). It needs additional information to determine the effects of the substituent X.

Therefore, in order to distinguish which discriminant function is the best, three compounds (Ibb; X=3,4-$(OH)_2$, Icc; X=3,5-$(OMe)_2$-4-OH and Idd; X=3,4-OCH_2O) were designed, synthesized and tested. According to method A (Eq. 4), all these have 3,4-disubstitution ($D=1$) and are predicted to be "active." The B_{1-3} and B_{1-4} values of all these compounds are 1.35 based on method B and they are predicted to be "inactive." No steric constraint allows the methoxy group out-of-plane conformation in Icc. Method C (Eq. 6) predicts that only Idd is "active," because the methylenedioxy group is regarded as an in-

TABLE I. Antiulcer Activities and Parameters of 1-(Aminocarbonylalkyl)-4-benzylpiperazine Derivatives



No.	X	R	R′	n	V_w[a]	D[b]	B_{1-3}[c]	B_{1-4}[c]	Results of recognition			
									Obsd.[d]	Eq. 4.	Eq. 5.	Eq. 6.
Ia	2,3,4-$(OMe)_3$	H	H	1	0.177	1	1.35	1.90	1	1	1	1
Ib	2,3,4-$(OMe)_3$	Et	Et	1	0.809	1	1.35	1.90	1	1	1	1
Ic	2,3,4-$(OMe)_3$	Pr	Pr	1	1.117	1	1.35	1.90	0	0	0	0
Id	2,3,4-$(OMe)_3$	$-(CH_2)_4-$		1	0.705	1	1.35	1.90	1	1	1	1
Ie	2,3,4-$(OMe)_3$	$-(CH_2)_5-$		1	0.859	1	1.35	1.90	0	0	1	1
If	2,3,4-$(OMe)_3$	H	cyclo-Hex	1	1.005	1	1.35	1.90	0	0	0	0
Ig	2,3,4-$(OMe)_3$	H	Ph	1	0.879	1	1.35	1.90	1	0	1	1
Ih	2,3,4-$(OMe)_3$	H	CH_2Ph	1	1.033	1	1.35	1.90	0	0	0	0
Ii	2,3,4-$(OMe)_3$	Me	Ph	1	1.041	1	1.35	1.90	0	0	0	0
Ij	H	$-(CH_2)_4-$		1	0.705	0	1.00	1.00	0	0	0	0
Ik	4-Me	$-(CH_2)_4-$		1	0.705	0	1.00	1.52	0	0	0	0
Il	4-Cl	$-(CH_2)_4-$		1	0.705	0	1.00	1.80	0	0	0	0
Im	4-OMe	$-(CH_2)_4-$		1	0.705	0	1.00	1.35	0	0	0	0
In	3,4-Cl_2	$-(CH_2)_4-$		1	0.705	1	1.80	1.80	1	1	1	1
Io	2,4-Cl_2	$-(CH_2)_4-$		1	0.705	0	1.00	1.80	0	0	0	0
Ip	2-OMe	$-(CH_2)_4-$		1	0.705	0	1.00	1.00	0	0	0	0
Iq	3-OMe	$-(CH_2)_4-$		1	0.705	0	1.35	1.00	0	0	0	0
Ir	2,4-$(OMe)_2$	$-(CH_2)_4-$		1	0.705	0	1.00	1.35	0	0	0	0
Is	3,4-$(OMe)_2$	$-(CH_2)_4-$		1	0.705	1	1.35	1.35	0	1	0	0
It	3,4,5-$(OMe)_3$	$-(CH_2)_4-$		1	0.705	1	1.90	1.35	1	1	1	1
Iu	2,4,6-$(OMe)_3$	$-(CH_2)_4-$		1	0.705	0	1.00	1.35	0	0	0	0
Iv	2,3,4-$(OMe)_3$	$-(CH_2)_4-$		2	0.705	1	1.35	1.90	1	1	1	1
Iw	2,3,4-$(OMe)_3$	$-(CH_2)_4-$		3	0.705	1	1.35	1.90	0	0	0	0
Ix	2,3,4-$(OMe)_3$	$-(CH_2)_4-$		4	0.705	1	1.35	1.90	0	0	0	0
Iy	3,4,5-$(OMe)_3$	$-(CH_2)_4-$		2	0.705	1	1.90	1.35	1	1	1	1
Iz	3,4,5-$(OMe)_3$	$-(CH_2)_4-$		3	0.705	1	1.90	1.35	0	0	0	0
Iaa	3,4,5-$(OMe)_3$	$-(CH_2)_4-$		4	0.705	1	1.90	1.35	0	0	0	0

a) Van der Waals volume of the NRR′ group, calculated from ref. 3. *b*) Presence of 3,4-disubstitution, $D=1$. *c*) See text (method C). *d*) Active, 1; inactive, 0.

TABLE II. Correlation Matrix of Variables Used in Eq. 6

	V_w	B_{1-3}	B_{1-4}	N
V_w	1.000			
B_{1-3}	0.005	1.000		
B_{1-4}	0.253	0.044	1.000	
N	−0.137	0.457	0.020	1.000

plane conformation of the methoxy group (B_{1-3} and/or $B_{1-4}=1.9$). The experimental results coincide with the prediction based on method C.

These results suggest that method C (Eq. 6) is the most reliable. So, a substituent which has a large B_1 value (or B_2 value when the substituent is forced to be in the in-plane conformation) with low lipophilicity is favorable to exhibit antiulcer activity with low acute toxicity. Although there may be several substituents and/or substituent combinations which satisfy the above criteria, we concluded that the selection of Id and It as candidate antiulcer

TABLE III. Results of the ALS Prediction

No.	Obsd.	Prediction Eq. 4.	Eq. 5.	Eq. 6.	No.	Obsd.	Prediction Eq. 4.	Eq. 5.	Eq. 6.
Ia	1	1	1	1	Ip	0	0	0	0
Ib	1	1	1	1	Iq	0	0	0	0
Ic	0	0	0	0	Ir	0	0	0	0
Id	1	1	1	1	Is	0	1	0	0
Ie	0	1	1	1	It	1	1	1	1
If	0	0	0	0	Iu	0	0	0	0
Ig	1	0	0	0	Iv	1	1	1	1
Ih	0	0	0	0	Iw	0	0	0	0
Ii	0	0	0	0	Ix	0	0	0	0
Ij	0	0	0	0	Iy	1	1	1	1
Ik	0	0	0	0	Iz	0	0	0	0
Il	0	0	0	0	Iaa	0	0	0	0
Im	0	0	0	0	Ibb[a]	0	1	0	0
In	1	1	1	1	Icc[a]	0	1	0	0
Io	0	0	0	0	Idd[a]	1	1	0	1

a) See text.

drugs is not a misjudgement from the standpoints of availability of raw materials and synthetic ease. The antiulcer effect of this novel series of compound is likely due to the cytoprotective activity because no antisecretory activity was observed.[1] The exact mechanism(s) of cytoprotection remains to be clarified.

This study shows that QSAR studies are useful to establish the structural requirements for activity and the QSAR results can be used as a rational basis for decision making, reducing the need for troublesome and costly analogue syntheses.

This study also shows that even the leave-one-out method can not distinguish the best discriminant function in the two group case, the additional compounds were needed not only to compensate for the information deficiency but also to confirm the validity of the discriminant function.

Experimental

Melting points were determined on a Yamato capillary melting point apparatus, model MP-21, and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Hitachi R-24B NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Silica gel 60 F_{254} (Merck) TLC plates were used for thin layer chromatography (TLC). For column chromatography, Silica gel 60 (Merck) was used.

1-(3,4-Dihydroxybenzyl)-4-(pyrrolidinocarbonylmethyl)piperazine Dimaleate (Ibb)——1-(Pyrrolidinocarbonylmethyl)piperazine[8] (2.07 g) and 3,4-dihydroxybenzaldehyde (2.96 g) were melted in an oil bath at 120 °C and formic acid (1.13 ml) was added dropwise. The mixture was stirred for 50 min under heat, and then allowed to cool to room temperature. The mixture was diluted with EtOH (10 ml), and maleic acid (3.48 g) in EtOH (15 ml) was added. The precipitated solid was collected and recrystallized from MeOH to give Ibb (2.54 g). mp 162—164 °C. *Anal.* Calcd for $C_{17}H_{25}N_3O_3 \cdot 2C_4H_4O_4$: C, 54.44; H, 6.03; N, 7.62. Found: C, 54.34; H, 6.25; N, 7.69.

1-(3,5-Dimethoxy-4-hydroxybenzyl)-4-(pyrrolidinocarbonylmethyl)piperazine Dimaleate (Icc)——Icc was obtained in the same manner as described for Ibb. mp 185—187 °C. *Anal.* Calcd for $C_{19}H_{29}N_3O_4 \cdot 2C_4H_4O_4$: C, 54.45; H, 6.26; N, 7.06. Found: C, 54.59; H, 6.31; N, 7.06.

1-(3,4-Methylenedioxybenzyl)-4-(pyrrolidinocarbonylmethyl)piperazine Dimaleate (Idd)——Idd was obtained in the same manner as described for Ibb. mp 190—192 °C. *Anal.* Calcd for $C_{18}H_{25}N_3O_3 \cdot 2C_4H_4O_4$: C, 55.41; H, 5.90; N, 7.46. Found: C, 55.23; H, 6.05; N, 7.54.

Antiulcer Activity; Indomethacin-Induced Gastric Ulcer[9]——Male Sprague-Dawley (SD) rats (weighing 180—220 g, 8 weeks age, 16 rats per group) were fasted for 24 h, and the test compound (200 mg/kg, dissolved in distilled water or suspended in 1% aqueous gum arabic) or vehicle was administered orally. After 15 min, indomethacin

(30 mg/kg, dissolved in 3% aqueous Na_2CO_3 solution, s.c.) was administered. After 5 h, the rats were sacrificed under ether anesthesia and the stomach was removed. Each stomach was inflated with 1% formalin (12 ml), and placed in 1% formalin for 15 min to fix the outer layer of the stomach. After opening the stomach along the greater curvature, the length (mm) of each ulcer was measured for each rat. The sum of the length of ulcers in each rats was used as the ulcer index. The statistical significance of the difference between the mean ulcer index of the drug-treated group and that of the control group was calculated using Student's *t* test.

References and Notes

1) H. Ohtaka, K. Yoshida, K. Suzuki, K. Shimohara, S. Tajima and K. Ito, *Chem. Pharm. Bull.*, **36**, 3948 (1988).
2) I. Moriguchi, K. Komatsu and Y. Matsushita, *J. Med. Chem.*, **23**, 20 (1980); I. Moriguchi and K. Komatsu, Abstracts of Papers, 8th Symposium on Structure–Activity Relationships, Tokyo, Oct. 1981, p. 55.
3) I. Moriguchi, Y. Kanada and K. Komatsu, *Chem. Pharm. Bull.*, **24**, 1799 (1976).
4) A. L. Delaunois (ed.), "Biostatistics in Pharmacology," Vol. 2, Pergamon Press, Ltd., Oxford, 1973, pp. 943—953.
5) A. Makriyannis and J. J. Knittel, *Tetrahedron Lett.*, **1979**, 2753.
6) A. Verloop, W. Hoogenstraaten and J. Tipker, "Drug Design," Vol. 7, ed. by E. J. Ariens, Academic Press, New York, 1976, pp. 165—207.
7) O. Hofer, *Tetrahedron Lett.*, **1975**, 3415.
8) Y. Ito, H. Kato, N. Ogawa, S. Kurata, T. Yamagishi and E. Ecchuu, Japan Kokai Patent 58-216170 [*Chem. Abstr.*, **100**, 209888*y* (1984)].
9) S. Okabe, K. Takeuchi, H. Kunimi, M. Kanno and M. Kawashima, *Dig. Dis. Sci.*, **28**, 1034 (1983).