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Benzylpiperazine Derivatives. X.[1] Syntheses and Structure–Antiulcer Activity Relationship of 1-Benzyl-4-piperazineacetic Acid Esters

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A series of ester derivatives of 1-benzyl-4-piperazineacetic acid was synthesized and evaluated as antiulcer agents. Quantitative structure–activity relationships (QSAR) analyses by using the ALS (adaptive least-squares) method were performed in each step to decrease the synthetic efforts. The QSAR for the esters is much the same as that for the previously examined amide derivatives. The antiulcer activity of these compounds was considered to be based on the cytoprotective activity. The most active and the least toxic compounds, **5n** and **5y**, were selected for further study.

Keywords——benzylpiperazine derivative; 1-piperazineacetate; quantitative structure–activity relationship; ALS method; antiulcer activity

In the past few years there has been increasing interest in gastrointestinal mucosal defensive factors against damaging agents, and in compounds which stimulate the defensive mechanisms. In the course of our study to search for antiulcer agents with cytoprotective activity, we found that some derivatives of 1-benzyl-4-piperazineacetamides (**1**) meet the requirements.[2] Quantitative structure–activity relationship (QSAR) study of these derivatives revealed that 1-(2,3,4- or 3,4,5-trimethoxybenzyl)-4-piperazineacetamides with small NRR' groups are preferable.[1] If the smallness itself is the condition of potent antiulcer activity, then the NRR' groups are replaceable with other small groups. With this thought in mind, we prepared some ester analogs of **1** and examined their antiulcer activity. This paper deals with not only syntheses and antiulcer activity of the esters (**5**) but also QSAR analyses of these derivatives by using the adaptive least-squares (ALS)[3] method.

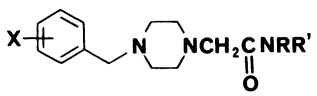


Chart 1

1

Chemistry

The compounds for biological testing were prepared by two general methods as shown in Chart 2. In method A, a 1-benzylpiperazine derivative (**2**)[4] was condensed with a chloroacetic acid ester (**3**). Almost all of **3** are known compounds,[5] and they were prepared from chloroacetyl chloride and alcohol or phenol, then purified by distillation. Esters of 1-piperazinepropionic acid were obtained from 4-methoxyphenyl acrylate (**4**)[6] and **2**. In method B, compound **6** was alkylated with alkyl halide in *N*,*N*-dimethylformamide (DMF) in the presence of potassium carbonate. Compound **6** was prepared by hydrolysis of **5b** with hydrochloric acid. Compounds **5** prepared were converted to acid addition salts and purified by recrystallization.

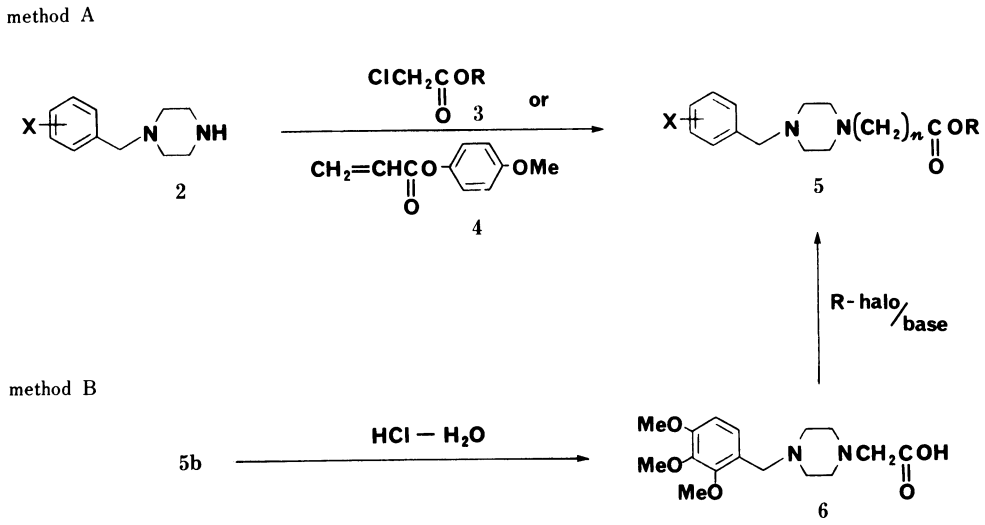


Chart 2

Results and Discussion

A facile indomethacin-induced gastric ulcer model in rats at a dose of 200 mg/kg was used as a first screening method, and a test compound which showed statistically significant activity ($p<0.05$) compared to the control was judged to be active. Oral toxicity (LD_{50}) was determined in mice for the active derivatives.

First, in order to examine the effects of variation in the ester moiety on the antiulcer activity, eleven alkyl and aralkyl esters (**5a**—**k**) were synthesized and tested. Table I shows that bulky ester residues reduce the activity, as expected. Therefore, the bulkiness of the OR group was evaluated in terms of van der Waals volume (V_w)[7] and ALS analysis was performed to obtain Eq. 1:

$$Y = -2.977V_w + 2.426 \quad (1)$$

$$n=11,\ R_s=1.000,\ n_{mis}=0$$

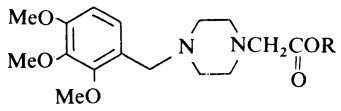
where Y is the discriminant score for the classification of activity ratings, n represents the number of compounds used to derive the equation, n_{mis} is the number misclassified, and R_s is the Spearman rank correlation coefficient.[8] Equation 1 classified the compounds perfectly, and this equation is quite similar to that reported for the amide derivatives previously.[1] Among these derivatives, the phenyl ester (**5i**) is the most active and the least toxic (see Tables I and VI), so the substituted phenyl ester derivatives (**5l**—**q**) were synthesized and tested. The ALS analysis of these derivatives (**5l**—**q**) gave Eq. 2:

$$Y = -4.591\sigma - 0.459 \quad (2)$$

$$n=6,\ R_s=1.000,\ n_{mis}=0$$

where σ is the electronic substituent constant of Hammett[9] and the σ_P value was used for the *ortho* substituent. In this case, V_w was not necessary, because the difference of bulkiness was not so large in these substituents. Equation 2 suggests that a substituent with negative sigma value is preferable for the activity. This may suggest that the electron-withdrawing substituent makes the ester bond electronically unstable to hydrolysis, and hydrolysis prior to absorption decreases the antiulcer activity (because compound **6** is inactive).

TABLE I. Aralkyl 1-(2,3,4-Trimethoxybenzyl)-4-piperazineacetate Salts (**5a—k**)



No.	R	Yield (Method)	mp (°C) (Recryst. solvent)	Formula[a]	Analysis (%) Calcd (Found) C	H	N	Antiulcer activity[b]	Toxicity LD_{50} (mg/kg *p.o.*)
5a	CH_3	47 (A)	145—147 (EtOH)	$C_{17}H_{26}N_2O_5 \cdot 2MA$	52.63 (52.52)	6.01 (5.87)	4.91 (4.85)	+	3120
5b	CH_2CH_3	54 (A)	190—194 (dec.) (iso-PrOH)	$C_{18}H_{28}N_2O_5 \cdot 2HCl \cdot H_2O$	48.76 (48.53)	7.27 (7.18)	6.32 (6.49)	+	2670
5c	$(CH_2)_2CH_3$	36 (B)	136—138 (EtOH)	$C_{19}H_{30}N_2O_5 \cdot 2MA$	54.18 (54.22)	6.40 (6.50)	4.68 (4.72)	+	3930
5d	$(CH_2)_3CH_3$	61 (A)	135—137 (AcOEt–EtOH)	$C_{20}H_{32}N_2O_5 \cdot 2MA$	54.90 (54.91)	6.58 (6.58)	4.57 (4.53)	+	4000
5e	$(CH_2)_4CH_3$	21 (B)	141—142 (EtOH)	$C_{21}H_{34}N_2O_5 \cdot 2MA \cdot H_2O$	54.03 (53.85)	6.57 (6.57)	4.35 (4.09)	—	
5f	$(CH_2)_5CH_3$	64 (B)	136—138 (EtOH)	$C_{22}H_{36}N_2O_5 \cdot 2MA$	56.24 (56.41)	6.92 (6.92)	4.37 (4.41)	—	
5g	$(CH_2)_6CH_3$	43 (B)	140—143 (EtOH)	$C_{23}H_{38}N_2O_5 \cdot 2MA$	56.87 (56.66)	7.08 (7.23)	4.28 (4.35)	—	
5h	$CH_2(CH_3)_2$	43 (A)	132—135 (AcOEt–EtOH)	$C_{19}H_{30}N_2O_5 \cdot 2MA$	54.18 (53.98)	6.40 (6.22)	4.68 (4.79)	+	2120
5i	C_6H_5	20 (A)	211—214 (dec.) (EtOH)	$C_{22}H_{28}N_2O_5 \cdot 2HCl \cdot H_2O$	53.77 (54.02)	6.56 (6.47)	5.70 (5.75)	+	5660
5j	$CH_2C_6H_5$	67 (A)	175—177 (CH_3CN)	$C_{23}H_{30}N_2O_5 \cdot 2HCl$	56.67 (56.67)	6.62 (6.72)	5.75 (5.81)	—	
5k	$(CH_2)_2C_6H_5$	41 (A)	134—137 (CH_3CN)	$C_{24}H_{32}N_2O_5 \cdot 2.5MA$	56.82 (56.70)	5.89 (5.83)	3.90 (3.97)	—	

a) MA stands for maleic acid. *b*) Indomethacin-induced gastric ulcer (200 mg/kg). Statistically significant activity ($p<0.05$) is assessed on the following scale: +, active; −, inactive.

Next, the effects of the substituent X on the benzyl moiety were examined. The substituents were selected from those of the amide series[2] and 8 compounds (**5r—y**) were prepared. A substituent which diminishes the activity in the amide series, also reduces the activity in the ester derivatives. Therefore, the parameter set used in the QSAR of amide series[1] was employed in this series, and Eq. 3 was obtained.

$$Y = 2.028B_{1-3} + 0.897B_{1-4} - 4.163 \quad (3)$$

$$n = 9,\ R_s = 1.000,\ n_{mis} = 0$$

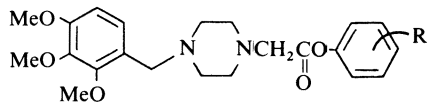
where B_{1-3} and B_{1-4} are Verloop's B_1 value[10] of the substituents at the 3 and 4 positions, respectively. A substituent which has a large B_1 value is favorable for antiulcer activity. When the substituent is forced to be in the in-plane conformation, B_2 value was used instead of B_1 value as previously. The 3,4-methylenedioxy group is regarded as an in-plane conformation of the methoxy group and 1.90 was assigned for both the B_{1-3} and B_{1-4} values.

Next, all 1-benzyl-4-piperazineacetic acid esters (**5a—y**) were analyzed to give Eq. 4. The σ values were assigned as 0 except for substituted phenyl esters.

$$Y = -2.625V_w + 1.796B_{1-3} + 1.624B_{1-4} - 4.086\sigma - 3.241 \quad (4)$$

$$n = 25,\ R_s = 1.000,\ n_{mis} = 0$$

TABLE II. Substituted Phenyl 1-(2,3,4-Trimethoxybenzyl)-4-piperazineacetate Salts (**5l—q**) Prepared by Method A



No.	R	Yield	mp (°C) (Recryst. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	Antiulcer activity[a)]	Toxicity LD_{50} (mg/kg *p.o.*)
5l	4-CH_3	22	218—220 (dec.) (EtOH)	$C_{23}H_{30}N_2O_5 \cdot 2HCl$	56.68 (56.51)	6.62 (6.61)	5.75 (5.92)	+	2670
5m	4-Cl	18	208—212 (dec.) (AcOEt–MeOH)	$C_{22}H_{27}ClN_2O_5 \cdot 2HCl$	52.03 (52.08)	5.76 (5.76)	5.52 (5.59)	−	
5n	4-OCH_3	46	227—231 (dec.) (MeOH)	$C_{23}H_{30}N_2O_6 \cdot 2HCl$	54.88 (54.82)	6.41 (6.49)	5.56 (5.58)	+	4200
5o	3-OCH_3	17	212—215 (dec.) (MeOH)	$C_{23}H_{30}N_2O_6 \cdot 2HCl$	54.88 (54.79)	6.41 (6.41)	5.56 (5.58)	−	
5p	2-OCH_3	20	210—215 (dec.) (EtOH)	$C_{23}H_{30}N_2O_6 \cdot 2HCl$	54.88 (54.79)	6.41 (6.45)	5.56 (5.54)	+	2670
5q	4-OCH_2CH_3	12	223—227 (dec.) (MeOH)	$C_{24}H_{32}N_2O_6 \cdot 2HCl$	55.71 (55.60)	6.62 (6.66)	5.41 (5.46)	+	2670

a) See footnote *b* of Table I.

Two 1-benzyl-4-piperazinepropionic acid esters (**5z** and **5aa**) were synthesized to investigate the effect of the number of methylene groups between piperazine and carbonyl on the antiulcer activity. These compounds were inactive against indomethacin-induced gastric ulcer, so an attempt to study longer methylene derivatives was terminated. All compounds including these two were subjected to analysis using a dummy variable N, which indicates the number of methylene groups, and the following equation was obtained.

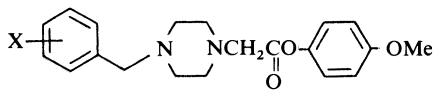
$$Y = -2.624V_w + 1.779B_{1-3} + 1.649B_{1-4} - 4.109\sigma - 1.621N - 1.562 \quad (5)$$

$$n = 27,\ R_s = 1.000,\ n_{mis} = 0$$

It is perhaps questionable although not entirely unreasonable to use a dummy variable (N) for only two compounds. In order to confirm the validity of the ALS results, the leave-one-out technique was applied. The predictive results based on Eqs. 4 and 5 classified 100% of the compounds correctly. The parameters and results of ALS recognition and prediction are summarized in Table IV and the correlation matrix of the parameters used in Eq. 5 is shown in Table V. Equation 5 shows ① a bulky ester moiety is disadvantageous, except for a phenyl moiety without an electron-attracting group, ② a smaller number of methylene groups is favorable, ③ a substituent on the benzyl moiety which has a large B_1 value (or B_2 value when the substituent is forced to be in the in-plane conformation) is favorable for antiulcer activity.

From the standpoint of potent antiulcer activity and low toxicity, five compounds (**5c**, **5d**, **5i**, **5n** and **5y**) were found to be preferable. Their ED_{50} values in various ulcer models are summarized in Table VI along with those of reference compounds. Table VI shows that **5n** and **5y** possess superior antiulcer activity to the reference compounds. Their toxicity is acceptable, so we selected these two compounds as candidates for the development of antiulcer drugs. The antiulcer effect of this novel series of compounds seems to be due to the cytoprotective activity, because no acid or pepsin antisecretory activities were observed. The precise mechanism(s) of cytoprotection remains to be clarified.

TABLE III. 4-Methoxyphenyl 1-Benzyl-4-piperazineacetate Salts (**5r**—**aa**) Prepared by Method A



No.	X	Yield	mp (°C) (Recryst. solvent)	Formula[a]	Analysis (%) Calcd (Found) C	H	N	Antiulcer activity[b]	Toxicity LD_{50} (mg/kg *p.o.*)
5r	H	23	225—230 (dec.) (MeOH)	$C_{20}H_{24}N_2O_3 \cdot 2HCl$	58.12 (58.13)	6.34 (6.45)	6.78 (6.82)	−	
5s	4-Me	27	223—231 (dec.) (EtOH)	$C_{21}H_{26}N_2O_3 \cdot 2HCl$	59.02 (58.75)	6.60 (6.66)	6.55 (6.75)	−	
5t	4-Cl	27	230—244 (dec.) (MeOH)	$C_{20}H_{23}ClN_2O_3 \cdot 2HCl$	53.65 (53.76)	5.63 (5.56)	6.26 (6.29)	−	
5u	4-OMe	50	226—232 (dec.) (EtOH)	$C_{21}H_{26}N_2O_4 \cdot 2HCl$	56.89 (56.64)	6.37 (6.40)	6.32 (6.36)	−	
5v	3,4-Cl_2	43	233—243 (dec.) (MeOH)	$C_{20}H_{22}Cl_2N_2O_3 \cdot 2HCl$	49.81 (49.83)	5.02 (5.19)	5.81 (5.84)	+	2520
5w	3,4-$(OMe)_2$	30	145—151 (dec.) (EtOH)	$C_{22}H_{28}N_2O_5 \cdot HCl \cdot 0.1H_2O$	60.23 (60.37)	6.71 (7.00)	6.39 (6.58)	−	
5x	3,4-OCH_2O	46	198—201 (dec.) (EtOH)	$C_{21}H_{24}N_2O_5 \cdot HCl$	59.93 (59.77)	5.99 (5.89)	6.66 (6.75)	+	2000
5y	3,4,5-$(OMe)_3$	73	163—167 (CH_3CN–H_2O)	$C_{23}H_{30}N_2O_6 \cdot 2FA \cdot H_2O$	54.70 (54.76)	5.92 (5.93)	4.12 (4.14)	+	4800
5z[c]	2,3,4-$(OMe)_3$	53	209—214 (dec.) (MeOH)	$C_{24}H_{32}N_2O_6 \cdot 2HCl$	55.71 (55.90)	6.62 (6.75)	5.41 (5.50)	−	
5aa[d]	3,4,5-$(OMe)_3$	32	211—213 (dec.) (MeOH)	$C_{24}H_{32}N_2O_6 \cdot 2HCl \cdot 0.2H_2O$	55.32 (55.31)	6.65 (6.63)	5.38 (5.39)	−	

a) FA stands for fumaric acid. *b*) See footnote *b* of Table I. *c*) Piperazinepropionate analog of **5n**. *d*) Piperazinepropionate analog of **5y**.

Several structures satisfying the above criteria could be designed. However, Eq. 5 predicts only whether the compound is active, but not whether a compound is more potent than **5n** and **5y**. On the other hand, Hansch–Fujita analysis of these five compounds gave Eq. 6.

$$\log 1/C = 1.964(\pm 0.495)V_w + 2.630(\pm 0.443) \quad (6)$$

$$n = 5,\ r = 0.990,\ s = 0.06,\ F = 159.14$$

where C is the value calculated from ED_{50} (indomethacin-induced ulcer) in Table VI on a molar basis, r is the correlation coefficient, s is the standard deviation and F is the F-ratio between the variance of calculated and observed activities. Equation 6 suggested that the larger the V_w value of the OR group, the more potent the antiulcer activity. Although this result seems to be opposite to the ALS results, this discrepancy might have occurred because of the small range of the activity in the regression analysis. In other words, some parabolic type of correlation might exist between the activity and the bulkiness, which was not estimated by a rough analysis such as ALS. However, the above results suggest that even if a compound is predicted to be active, it need not be synthesized and tested when the OR group is smaller than that of **5n**. Equation 5 predicts that the 4-propyloxyphenyl ester of **6** is inactive, though it is larger than **5n** by only two methylene units. Therefore, we thought it pertinent to the termination of further analog synthesis from the standpoints of availability of raw materials and synthetic ease.

TABLE IV. Parameters and Results of ALS Recognition and Prediction

No.	$N^{a)}$	$V_W{}^{b)}$	$\sigma^{c)}$	$B_{1-3}{}^{d)}$	$B_{1-4}{}^{d)}$	Results		
						Obsd.[e)]	Recog.[f)]	Pred.[g)]
5a	1	0.304	0	1.35	1.90	1	1	1
5b	1	0.458	0	1.35	1.90	1	1	1
5c	1	0.612	0	1.35	1.90	1	1	1
5d	1	0.766	0	1.35	1.90	1	1	1
5e	1	0.920	0	1.35	1.90	0	0	0
5f	1	1.074	0	1.35	1.90	0	0	0
5g	1	1.228	0	1.35	1.90	0	0	0
5h	1	0.607	0	1.35	1.90	1	1	1
5i	1	0.844	0	1.35	1.90	1	1	1
5j	1	0.998	0	1.35	1.90	0	0	0
5k	1	1.152	0	1.35	1.90	0	0	0
5l	1	0.998	−0.17	1.35	1.90	1	1	1
5m	1	1.009	0.23	1.35	1.90	0	0	0
5n	1	1.079	−0.27	1.35	1.90	1	1	1
5o	1	1.079	0.12	1.35	1.90	0	0	0
5p	1	1.079	−0.27	1.35	1.90	1	1	1
5q	1	1.233	−0.24	1.35	1.90	1	1	1
5r	1	1.079	−0.27	1.00	1.00	0	0	0
5s	1	1.079	−0.27	1.00	1.52	0	0	0
5t	1	1.079	−0.27	1.00	1.80	0	0	0
5u	1	1.079	−0.27	1.00	1.35	0	0	0
5v	1	1.079	−0.27	1.80	1.80	1	1	1
5w	1	1.079	−0.27	1.35	1.35	0	0	0
5x	1	1.079	−0.27	1.90	1.90	1	1	1
5y	1	1.079	−0.27	1.90	1.35	1	1	1
5z	2	1.079	−0.27	1.35	1.90	0	0	0
5aa	2	1.079	−0.27	1.90	1.35	0	0	0

a) Number of methylenes between piperazine and carboxyl. *b*) Van der Waals volume of OR group. *c*) Hammett sigma value of the substituent on the phenyl ester. *d*) See text. *e*) See footnote *b* of Table I, active, 1; inactive, 0. *f*) Equation 5. *g*) Leave-one-out test based on Eq. 5.

TABLE V. Correlation Matrix of the Parameters Used in Eq. 5

	N	V_W	σ	B_{1-3}	B_{1-4}
N	1.000				
V_W	0.135	1.000			
σ	−0.275	−0.427	1.000		
B_{1-3}	0.291	0.051	−0.104	1.000	
B_{1-4}	−0.157	−0.261	0.530	0.074	1.000

The QSAR results of the esters are much the same as those of the amide series reported previously, so 54 compounds (27 amides and 27 esters) were subjected to the ALS analysis, and Eqs. 7 and 8 were obtained.

$$Y = -2.643V_w + 1.875B_{1-3} + 1.313B_{1-4} - 3.458\sigma - 0.585N - 1.963 \quad (7)$$

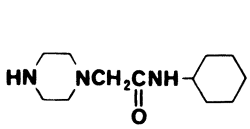
$n = 54$, $R_s = 0.922$, $n_{mis} = 2$

$t = 17.17$, $p < 0.001$

TABLE VI. Comparative Pharmacological Effects of **5c**, **5d**, **5i**, **5n**, **5y** and Reference Compounds

Compound	Antiulcer activity ED_{50} (mg/kg, *p.o.*)			Antisecretory activity ED_{50} (mg/kg, *p.o.*)	
	Indomethacin	Ethanol	Stress	Acid	Pepsin
5c	94	NT	NT	NT	NT
5d	38	30	245	NT	NT
5i	28	81	NT	NT	NT
5n	10	27	88	>300	NT
5y	11	30	123	>300	>300
Cetraxate hydrochloride	361	112	>1000	>300	>300
Sucralfate	216	150	NT	>300	NT
Teprenone	90	55	140	>300	NT

NT: not tested.



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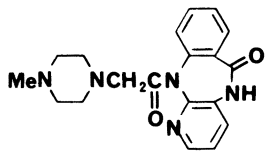

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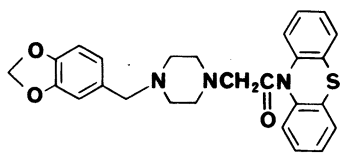

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Chart 3

$$Y = -2.621V_w + 1.668B_{1-3} + 1.576B_{1-4} - 3.545\sigma - 0.539N - 0.040D - 2.184 \quad (8)$$

$n = 54$, $R_s = 0.883$, $n_{mis} = 3$

$t = 13.56$, $p < 0.001$

where t is Student's t-value[8] calculated as $t = R_s\,[(n-2)/(1-R_s^2)]^{1/2}$ and p is the level of significance. A dummy variable (D) for distinction between the amides and the esters was not necessary as judged from the contribution index ($C.I._D = 0.020$).[3] Thus, it was confirmed that the QSAR for both the amides and the esters can be expressed in one model (Eq. 7).

Several 1-piperazineacetamides, including esaprazole (**7**), pirenzepine (**8**) and fenoverine (**9**), have been used clinically as antiulcer agents. These compounds show antisecretory activity. It is impossible to deny that experienced medicinal chemists would easily be able to design **1** as antiulcer agents from the structures of existing drugs, but it would not be readily predictable that not only some amides (**1**) but also some esters (**5**) of 1-benzyl-4-piperazine-acetic acid show antiulcer activity based on cytoprotection. The structural resemblance does not necessarily imply similarity of the mechanisms of antiulcer activity. This study shows that the extrapolative application of the QSAR results of the amide series (**1**) brought forth a new lead compound (alkyl ester) and the antiulcer activity was enhanced efficiently by substituent modifications aided by the QSAR.

Experimental

Melting points were determined on 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 $60F_{254}$ (Merck) thin layer chromatography (TLC) plates were used for TLC.

Typical Procedures of Method A: 4-Methoxyphenyl 1-(3,4,5-Trimethoxybenzyl)-4-piperazineacetate Difumarate Hydrate (5y)——A mixture of 1-(3,4,5-trimethoxybenzyl)piperazine dihydrochloride (50 g), 4-methoxyphenyl chloroacetate (33 g), $NaHCO_3$ (49 g) and CH_3CN (700 ml) was stirred at 60 °C for 3 h. The reaction mixture was filtered and the filtrate was concentrated. The residue and fumaric acid (41.g) were dissolved in hot H_2O (200 ml)–CH_3CN (50 ml). The mixture was allowed to cool and the precipitated crystals were separated and recrystallized from H_2O–CH_3CN to give **5y** (73.5 g). mp 163—167 °C. *Anal.* Calcd for $C_{23}H_{30}N_2O_6 \cdot 2C_4H_4O_4 \cdot H_2O$: C, 54.70; H, 5.92; N, 4.12. Found: C, 54.76; H, 5.93; N, 4.14.

1-(2,3,4-Trimethoxybenzyl)-4-piperazineacetic Acid Dihydrochloride (6)——A mixture of **5b** (8.5 g) and 3 N HCl was refluxed for 3 h. The solvent was evaporated off, and the residue was recrystallized from EtOH–H_2O to give **6** (4 g). mp 225—227 °C (dec.). *Anal.* Calcd for $C_{16}H_{24}N_2O_5 \cdot 2HCl$: C, 48.37; H, 6.60; N, 7.05. Found: C, 48.23; H, 6.72; N, 7.17.

Typical Procedures of Method B: Heptyl 1-(2,3,4-Trimethoxybenzyl)-4-piperazineacetate Dimaleate (5g)——A mixture of **6** (6.77 g), heptyl bromide (3.22 g), K_2CO_3 (8.29 g) and DMF (40 ml) was stirred at 70 °C for 4 h. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with saline and dried over $MgSO_4$. After removal of the solvent, the residue was diluted with EtOH (15 ml) and maleic acid (2.79 g) in EtOH was added to it. The precipitated solid was collected and recrystallized from EtOH to give **5g** (3.35 g). mp. 140—143 °C. *Anal.* Calcd for $C_{23}H_{38}N_2O_5 \cdot 2C_4H_4O_4$: C, 56.87; H, 7.08; N, 4.28. Found: C, 56.66; H, 7.23; N, 4.35.

Antiulcer Activity; Indomethacin-Induced Gastric Ulcer[11]——Male Sprague-Dawley (SD) rats (weighing 180—220 g, 8 weeks of age, 16 rats per group) were fasted for 24 h, and a 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. Five hours after the administration of indomethacin, the rats were sacrificed under ether anesthesia and the stomachs were removed. The stomachs were 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 rat 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 by using Student's *t*-test.

The ED_{50} value was calculated from the dose–response curve which was drawn based on the ratio of the ulcer index of the drug-treated group to the mean ulcer index of the control group.

Ethanol-Induced Gastric Ulcer——Male SD rats (weighing 180—220 g, 8 weeks of age, 16 rats per group) were fasted for 24 h, and the test compound in the form of a solution in distilled water or a suspension in 1% aqueous gum arabic was administered orally. After 30 min, ethanol (99.5%, 1 ml) was orally administered to the rats in the same manner as described by Robert *et al.*[12] One hour after the administration of ethanol, the rats were sacrificed and the ulcer index as well as ED_{50} value were determined as described in indomethacin-induced gastric ulcer.

Water-Immersion Stress-Induced Gastric Ulcer——Male SD rats (weighing 180—220 g, 8 weeks of age, 16 rats per group) were fasted for 24 h, and the test compound (dissolved in distilled water or suspended in 1% aqueous gum arabic) was administered orally. After 15 min, the rats were immersed vertically to the height of the xiphoid process in a water bath at 23 °C within a stress cage in the same manner as described by Takagi *et al.*[13] After 17 h, the rats were sacrificed and the ulcer index as well as ED_{50} value were determined in the same manner as described in the case of indomethacin-induced gastric ulcer.

Grastric Antisecretory Activity——Male SD rats (weighing 180—200 g, 8 weeks of age, 8 rats per group) were fasted for 48 h. A small midline incision was performed, and the pylorus was ligated under ether anesthesia.[14] The test compound (dissolved in distilled water or suspended in 1% aqueous gum arabic) was administered intraduodenally to the rats immediately after the ligation. Four hours later, the abdomen was closed, the stomach was removed, and the volume of accumulated gastric juice therein was measured. The acid concentration was measured by titration against 0.05 N NaOH solution.

The peptic activity was determined according to Anson and Mirsky.[15]

Acute Toxicity——A test compound was orally administered to male ddY mice (weighing 18—22 g, 4 weeks of age, 5 mice per group) fasted overnight, in the form of a solution in distilled water or a suspension in 1% aqueous gum arabic. Thereafter, the death of mice was observed for 7 d. From the number of dead mice, the LD_{50} value was determined by using Weil's method.[16]

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