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Computer-Automated Structure Evaluation of Gastric Antiulcer Compounds: Study of Cytoprotective and Antisecretory Imidazo[1,2-*a*]pyridines and -Pyrazines

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SUMMARY

A quantitative structure-activity relationship study of a set of antiulcer compounds has been performed using the computer-automated structure evaluation methodology. Computer-automated structure evaluation identified cyanomethyl and hydroxymethyl functionalities, substituted in the 3-position of imidazo[1,2-*a*]pyridine and -Pyrazine, as descriptors relevant to antisecretory activity. The phenoxy group at the 8-position and the methyl group at the 2-position were shown to be sterically involved in the activity. A parabolic relationship was observed between the antisecretory activity and the logarithm of the partition coefficient of the compounds. Thus, hydrophobicity is found to be a necessary criterion for the inhibition of acid secretion. An attempt has been made to provide a rationale for designing a more potent antiulcer agent in this series of congeneric compounds.

Intensive research has shown over the years that gastric ulcers generally result from the combined action of gastric acid secretions and the weakening of mucosal resistance. Consequently, most of the modern strategies for designing effective drugs against ulcers have been directed at controlling these two factors. Examples of compounds that possess antisecretory activity are H_2 histamine receptor antagonists, anticholinergics, and antacids (1, 2). The prostaglandin analogues, on the other hand, are known to act as gastrointestinal cytoprotectants (3, 4). Other cases, like that of compounds inhibiting the M1 muscarinic receptor, are also known (5). However, the H_2 histamine receptor antagonists, such as cimetidine and ranitidine, remain the most widely studied class of antiulcer compounds (6). These compounds inhibit the binding of histamine to the H_2 receptor site and prevent the initiation of formation of acid by histamine (1).

Most of the known active antiulcer compounds are either antisecretory or cytoprotective. Very few compounds are known to possess dual activity. However, Kaminski *et al.* (7, 8) have recently synthesized a new class of compounds, the substituted imidazo[1,2-*a*]pyridines and -pyrazines, that are neither histamine (H_2) receptor antagonists nor prostaglandin analogues yet exhibit both gastric antisecretory and cytoprotective activity. Enough information for a conclusive elucidation of the mechanism of action of these compounds is not yet available. Nevertheless, some experimental evidence has been found to suggest that the antisecretory effect of 3-(cyanomethyl)2-methyl-8-phenylmethoxyimidazo[1,2-*a*]pyridine involves the parietal cells (7). These cells apparently are involved in the inhibition of the enzyme H^+/K^+ ATPase, which is responsible for the transport of protons into the gastric region (9). Thus, these compounds are probably similar to the H^+/K^+ ATPase inhibitors like Omeprazole (10, 11), which has been shown recently to be a potent antiulcer drug. The mechanism of cytoprotection is not well understood either. One of the proposed hypotheses relates the activity to the release of gastric mucous and an increase in its thickness, which forms a protective barrier between the gastric mucosa and acid (12, 13).

The present study has been carried out to bring forth a quantitative relationship between the activity of the compounds and their structure, in order to lay the basis for designing even more potent antiulcer drugs. Not many QSAR studies have been performed on antiulcer compounds, despite decades of research in this field. Discriminant analysis was one of the methods that had been used by Ogino *et al.* (14) to study some of the antiulcer drugs. A *de novo* method (15) that considers the interaction effects of the substituents as a function of their position has also been propounded to explain the antiulcer activity of some pyridyl oxime ethers. Perhaps the most significant theoretical advance was made by Bustard and Martin (16), who used the results of an extended Huckel theory program and conformational analysis to propose that the drugs

[1] Unpublished observations.

ABBREVIATIONS: QSAR, quantitative structure-activity relationship; CASE, computer-automated structure evaluation; Me, methyl; Ar, aromatic; Ph, phenyl; Bu, butyl; Py, pryidine; Et, ethyl.

must fulfill a necessary interatomic distance criterion in order to have antiulcer activity. The Bustard and Martin model was especially significant because its validity was proved on several classes of anti-peptic ulcer compounds. Nevertheless, the scarcity of QSAR studies of antiulcer compounds has turned our attention to the study of this database. The results presented below were very interesting to us and led us to postulate a hypothesis about the chemical modifications needed to improve the efficacy of these compounds. Nevertheless, they were not sufficiently specific to provide us with the basis needed to postulate a general mechanism of action.

Experimental Data and Methodology

The data (7, 8) for our study were obtained from the biological testing of the compounds in two animal models. For the measurement of the gastric antisecretory activity, adult mongrel dogs with surgically prepared Heidenhain pouches were used. In two different sets of experiments, the compounds were first administered intravenously at a 5 mg/kg dose and, in some cases, a 2 mg/kg dose. The reduction in acid output compared with the non-drug-treated (control) case was measured. The activity was then described as the percentage of inhibition of acid secretion. A different model was used for testing for cytoprotection activity. The compounds were administered orally to rats at a dose of 1–30 mg/kg, before absolute ethanol was given 30 min later. After 1 hr, the effect of the compounds against ethanol-induced lesions was determined. In this case the activity was expressed in terms of the effective dose required (mg/kg) to effect 50% of cytoprotection activity.

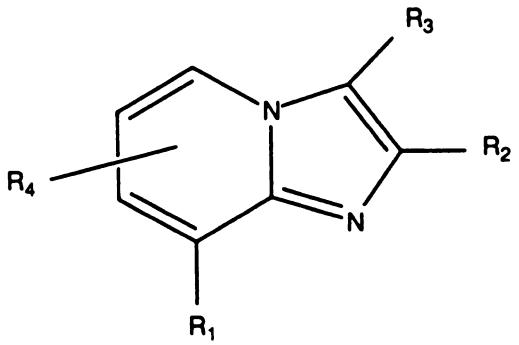
Our QSAR study of the antiulcer compounds was carried out using the CASE methodology (17–21). The method consists of tabulating the fragments formed by breaking the molecules into smaller structural subunits. These are labeled as active or inactive, depending on the activity of the originating molecule. A statistical analysis is performed and any significant discrepancy from a random distribution of the fragments between active and inactive molecules is taken as an indication that the fragment is relevant to the activity. Subsequently, the fragments are processed through a multivariate regression analysis for a quantitative representation of the structure-activity relationship.

In this paper, we have addressed the antisecretion activity of these compounds. We shall deal with their cytoprotection activity in a forthcoming publication.

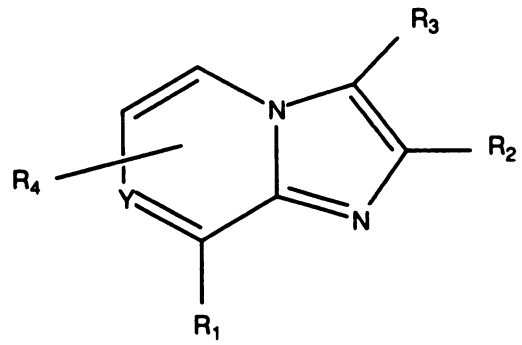
Results and Discussion

The database consisted of experimental results for the antisecretion activity of a series of imidazo[1,2-*a*]pyridine- and -pyrazine substituted compounds. The general structure of the model compound and the antisecretory activities are provided in Fig. 1 and Table 1, respectively.

The antisecretion activity of the compounds had been determined by administration of dosages of either 5 or 2 mg/kg, as described above. Because it is not possible to combine results from tests performed under different conditions (the dose-response relationship was found to be different for different compounds), we selected the set containing the maximum number of data for our learning set, i.e., the data set tested at the 5 mg/kg dose (64 compounds). It should also be noted that the dosage has been reported as the actual weight (in mg) of the compound and not as a molar quantity. Thus, there is a variation in the amount of compound used for testing of the response in each case. However, the problem is circumvented to a certain extent by the fact that the molecular weights of the compounds do not vary significantly from each other, because they share the same basic skeletal structure. The dosage varies from 1.401



Imidazo [1,2-a] Pyridine



Imidazo [1,2-a] Pyrazine (Y=N)

Fig. 1. General structures of imidazo[1,2-*a*]pyridine and -pyrazine antiulcer agents.

$\times 10^{-5}$ to 2.100×10^{-5} mol. The compounds tested at the 2 mg/kg dose, which were not included in the database, were used at a later stage to evaluate the predictive ability of the resultant QSAR.

In our analysis, we classified the compounds with activities below 40% inhibition of acid secretion as inactive, those between 40 and 49% as marginal, and the ones above 50% as active. The cutoffs were selected so as to divide the database as equally as possible among active and inactive compounds. This is necessary for the program to work reliably. The rather high value of the cutoff is also meant to bring forth the chemical characteristics of the molecules that are considered to be potent enough to warrant further study. The database of 64 compounds was then submitted to the CASE program after the mathematical transformation of the raw activity values yielded 25 active, 1 marginal, and 38 inactive molecules. The CASE analysis generated 14 activating fragments and 9 inactivating fragments. In the resulting multivariate regression analysis, the program selected nine variables, including both log*P* (partition coefficient between octanol and water) and $(\log P)^2$. The following QSAR equation was obtained:

$$\% \text{ of inhibition} = -58.5 + 73.21 \log P - 13.52 (\log P)^2 + 51.66 n_{\mathrm{I}} F_{\mathrm{I}} + 45.66 n_{\mathrm{II}} F_{\mathrm{II}} - 6.85 n_{\mathrm{III}} F_{\mathrm{III}} - 9.80 n_{\mathrm{IV}} F_{\mathrm{IV}} + 9.74 n_{\mathrm{V}} F_{\mathrm{V}} + 48.75 n_{\mathrm{VI}} F_{\mathrm{VI}} + 20.18 n_{\mathrm{VII}} F_{\mathrm{VII}}$$

$$n = 64,\ r^2 = 0.887,\ s = 11.7,\ F(9,54,0.05) = 47.38,$$

TABLE 1

Imidazo[1,2-a]pyridine and -pyrazine experimental and calculated antisecretory activity

Compound	Substitution			Antisecretory activity			
	R_1	R_2	R_3	Inhibition[a]	Experimental[b]	Calculated	logP
				%			
1	$PhCH_2O$	CH_3	H	61	++++	–	3.16
2	$PhCH_2O$	CH_3	CH_2CN	99	++++	++++	2.39
3	$PhCH_2O$	$(CH_3)_2CH$	CH_2CN	96	++++	++++	3.21
4	2-$FPhCH_2O$	CH_3	CH_2CN	97	++++	++++	2.32
5	4-$ClPhCH_2O$	CH_3	CH_2CN	95	++++	++++	2.89
6	3-CF_3PhCH_2O	CH_3	CH_2CN	30	–	–	2.51
7	4-*t*-$BuPhCH_2O$	CH_3	CH_2CN	52	++	++++	3.94
8	$PhCH_2CH_2O$	CH_3	CH_2CN	95	++++	++++	2.80
9	2-$PyCH_2O$	CH_3	CH_2CN	29	–	+	0.81
10	3-$PyCH_2O$	CH_3	CH_2CN	8	–	+	0.81
11	4-$PyCH_2O$	CH_3	CH_2CN	36	–	+	0.81
12	2-Thienyl-CH_2O	CH_3	CH_2CN	99	++++	++++	2.42
13	$PhCH_2CH_2$	CH_3	CH_2CN	96	++++	++++	3.35
14	H	CH_3	CH_2CN (R_4 = 6-$PhCH_2CH$)	0	–	–	3.35
15	4-$FPhCH_2O$	CH_3	CH_2CN	95	++++	++++	2.32
16	4-CF_3PhCH_2O	CH_3	CH_2CN	59	++	++++	2.51
17	4-$CNPhCH_2O$	CH_3	CH_2CN	71	++++	+++	1.22
18	246-Me_3-$PhCH_2O$	CH_3	CH_2CN	86	++++	++++	3.36
19	OH	CH_3	CH_2CN	0	–	–	0.19
20	3-Thienyl-CH_2O	CH_3	CH_2CN	97	++++	++++	2.42
21	1-Naphthyl-CH_2O	CH_3	CH_2CN	78	++++	++++	3.60
22	$PhCH_2CH_2CH_2O$	CH_3	CH_3CN	80	++++	++++	3.21
23	$PhCHCH_3$	CH_3	CH_2CN	97	++++	++++	3.35
24	$PhCH_2NH$	CH_3	CH_2CN	98	++++	++++	1.95
25	$PhCH_2S$	CH_3	CH_2CN	89	++++	++++	3.25
26	$PhCH_2SO$	CH_3	CH_2CN	26	–	–	0.20
27	$PhCH_2SO_2$	CH_3	CH_2CN	6	–	++	0.98
28	$PhCH_2O$	CH_3	CO_2Et	0	–	–	3.11
29	$PhCH_2O$	CH_3	CO_2H	4	–	–	2.29
30	$PhCH_2O$	CH_3	CN	4	–	–	1.99
31	$PhCH_2O$	CH_3	CH_2CH_2CN	20	–	+	2.80
32	$PhCH_2O$	CH_3	$C(CH_3)_2CN$	0	–	–	3.21
33	$PhCH_2O$	CH_3	CH_2OH	80	++++	++++	2.46
34	$PhCH_2O$	CH_3	CH_2OCH_3	29	–	+	2.86
35	$PhCH_2O$	CH_3	$CH_2OCH_2CH_3$	35	–	–	3.27
36	$PhCH_2O$	CH_3	CH_2SCH_3	8	–	–	3.97
37	$PhCH_2O$	CH_3	$CH_2SCH_2CH_3$	0	–	–	4.38
38	$PhCH_2O$	CH_3	CH_2SOCH_3	18	–	–	0.97
39	$PhCH_2O$	CH_3	$CH_2SO_2CH_3$	0	–	–	1.75
40	$PhCH_2O$	CH_3	Cl	37	–	–	3.36
41	$PhCH_2O$	CH_3	Br	63	+++	–	3.45
42	$PhCH_2O$	CH_3	$CH_2CO_2CH_3$	16	–	–	3.11
43	$PhCH_2O$	CH_3	CH_2CO_2H	24	–	+	2.71
44	$PhCH_2O$	CH_3	$CH_2CSNHCH_3$	0	–	–	2.94
45	$PhCH_2O$	CH_3	$CH_2CSN(CH_3)$	0	–	–	3.35
46	$PhCH_2O$	CH_3	$CH_2C(NH)NH_2$	0	–	–	1.88
47	$PhCH_2O$	CH_3	$CH_2(O_2CCH_3)$	87	++++	++++	3.11
48	$PhCH_2O$	CH_3	$CH_2(n\text{-}C_4H_9CO_2)$	96	++++	++++	4.33
49	$PhCH_2O$	CH_3	CH_2O_2C-3-Py	88	++++	++++	3.00
50	$PhCH_2O$	CH_3	$CH_2O_2CN(Me)_2$	23	–	–	2.20
51	$PhCH_2O$	H	$CH_2CH_2CH_3$	0	–	–	3.97
52	$PhCH_2O$	CF_3	CH_2CN	0	–	–	2.18
53	$PhCH_2O$	CH_3	CH_2CN (R_4 = 5-CH_3)	0	–	+	2.80
54	$PhCH_2O$	*t*-C_4H_9	NH_2	2	–	–	3.08
55	CH_2OPh	CH_3	NH_2	34	–	–	1.94
56	$PhCH_2CH_2$	CH_3	NH_2 (Y = N)	18	–	–	1.23
57	$PhCH_2O$	CH_3	CH_2NH_2	49	+	–	2.26
58	$PhCH_2CH_2$	CH_3	CH_3CONH	3	–	–	2.99
59	$PhCH_2O$	CH_3	$CH_3NHCONH$	27	–	–	1.18
60	$PhCH_2O$	CH_3	CH_3CH_2NH	70	++++	+	2.67
61	$PhCH_2CH_2$	CH_3	CH_3CH_2NH	11	–	–	3.63
62	$PhCH_2O$	CH_3	$PhCH_2NH$	0	–	–	4.06
63	$PhCH_2O$	CH_3	CH_3CH_2NH (Y = N)	0	–	–	0.95
64	$PhCH_2O$	CH_3	$N(n\text{-}C_3H_7)_2$	0	–	–	4.30
65	$PhCH_2O$	CH_3	NH_2	97[c]			1.94
66	$PhCH_2O$	H	NH_2	81[c]			1.54
67	$PhCH_2O$	CH_3CH_2	NH_2	72[c]			2.27
68	$PhCH_2CH_2$	CH_3	OH	0[c]			3.32
69	$PhCH_2CH_2$	CH_3	CH_3CO_2	6[c]			3.75

[a] Dose is 5 mg/kg, except where mentioned otherwise.
[b] ++++, extremely active; +++, very active; ++, active; +, marginal; –, inactive.
[c] 2 mg/kg dose.

sensitivity = 0.8462, and specificity = 0.9737, where n_i represents the number of times a fragment F_i appears in a molecule.

The relationship reproduced correctly the activity of 22 active and 30 inactive compounds of the 25 active and 38 inactive molecules in the database, thus accounting for 88% of the database. Two active compounds were falsely predicted as inactive and 1 was found to be marginal. Of the remaining 8 inactive compounds, 1 was calculated to be active and 7 marginally active.

One of the very interesting outcomes of the QSAR equation is the prominent dependence of activity on the partition coefficient. We call this interesting because, to our knowledge, the activity of most of the antiulcer drugs known today has not been found to correlate with their hydrophobicity. What is even more interesting is the fact that $(\log P)^2$ was also selected as a variable, thereby implying a parabolic relationship with the activity. In only one previous instance, the flavonoid compounds (22), has the CASE methodology identified log*P* as a relevant descriptor. However, in the present case the experimental log*P* values are not available and, hence, the observed dependence on the partition coefficient should be treated with caution, because the log*P* values were obtained by a theoretical method.[1] The calculated log*P* values are shown in Tables 1 and 2.

Examples of parabolic relationships between activity and drug action have been reported before, e.g., by Hansch and Clayton (23), who presented arguments that such a relationship represented a more realistic nonequilibrium situation in living cells than the simple linear relationship described by the log*P* term, which holds true for equilibrium conditions. A significant result of this relationship is that it allows for the determination of an optimum value of the partition coefficient at which the activity of the drug is maximum. This $\log P_{opt}$ is such that a drug with this partition coefficient would have the optimum solubility characteristics to reach the active site. We can calculate this optimum value from our QSAR equation

$$d(\text{Activity})/d(\log P) = 73.21 - 2 \times 13.52 \times \log P_{opt} = 0$$

Hence, $\log P_{opt} = 2.705$.

However, this dependence on log*P*, coupled with the high negative regression constant, indicates that the inhibitory activity of a compound would be at best only 40% in the absence of any of the QSAR fragments in the compound, even with an optimal log*P*. One may, thus, hypothesize that a suitable value of log*P* is a necessary but not sufficient condition for activity. Indeed, the correlation between activity and the log*P* term alone was found to be poor. This means that a compound will not be a good inhibitor if it only possesses a correct log*P* value. It will probably reach the active site in a short time frame and in relatively high concentration but will be ineffective if significant fragments are not present in the compound. On the other hand, if the log*P* of a compound is not within the required range, the molecule will not be expected to reach the active site in sufficient concentration to be active, even if it contains the correct chemical functionalities.

The structures of the significant fragments, along with their probabilities and distribution, are shown in Fig 2. As can be seen, fragments **I, II,** and **VI** confer the highest potency to the molecules that contain them. Of these, fragment **I** was found to be the most prevalent within the database and carries the highest probability of being related to activity. The structure of the fragment suggests that it is the cyanomethyl substituent at the 3-position that is acting as the biophore in conjunction with the rest of the backbone of the molecule.

Fragments **III** and **IV** are inactivating fragments. It can be inferred from an inspection of these two fragments that substitution at the *m*-position in the 8-phenoxy group and a sterically crowded alkyl substituent (longer than methyl) at the 2-position in imidazo[1,2-*a*]pyridine lead to a reduction in the activity. Both these observations suggest that the substituents at the 2-position and the substituents on the phenoxy group at the 8-position affect antisecretory activity due to steric factors. Two of the compounds in the database, **54** and **67**, that were omitted from the CASE analysis support this conclusion. Compound **67** differs from **65** in that it has an ethyl substituent instead of a methyl group at the 2-position. As a result, its activity drops from 99% inhibition to 72%. On the other hand, compound **66** tells us that a methyl group is required for optimum activity. When the —CH_3 substituent was replaced with a —H in that compound, its activity dropped to 81%. Hence, the presence of a methyl substitution at the 2-position leads to optimum activity.

The rest of the fragments in the QSAR equation, i.e., activating fragments **II, V, VI,** and **VII**, are observed as single occurrences. Thus, one would tend to ignore them because they lack statistical support. Actually, ignoring the 4 molecules that contain these activating fragments and the inactive molecule that contains the single occurrent fragment **III**, the whole database can be well correlated by the following equation, containing only three descriptors:

$$\%\ \text{of inhibition} = -52.41 + 66.00\log P - 11.85(\log P)^2 + 48.96 n_i F_I$$

$n = 59$, $r^2 = 0.800$, $s = 13.95$, and $F(3,55,0.05) = 73.5$.

However, a close inspection of the single occurrence terms yields some very interesting conclusions. Indeed, all these questionable activating fragments contain either an ester or an alcohol functionality. The fragment —CO—O— itself was not selected as an activating fragment because it also exists in three inactive molecules. There seems to exist a difference, though, in the nature of the ester group in the different compounds. The database contains a total of 6 compounds possessing an ester functionality at the 3-position, namely compounds **28**, **42**, and **47–50** (see Fig. 3). These 6 compounds differ from each other in the nature of the substituents at the 3-position. The —CO—O— fragment in these compounds is connected to the aromatic imidazo[1,2-*a*]pyridine either through the O— end or through the C— end of the carboxylic group (R′ —CO—O—CH_2—Ar and R—O—CO—CH_2—Ar). Table 3 lists the corresponding hydrolytic products of the two types of esters.

Type A was observed in 4 cases, of which 3 were active. Type B was true for 2 cases, both of which were inactive. This led to the suspicion that the difference in activity could be due to the different nature of the ester linkage to the aromatic system. Indeed, it is possible that the hydrolytic products hold the key to the activity. This looks even more reasonable when one considers the fact that these compounds are injected into a medium in the stomach that is highly acidic.

If the nature of the hydrolysis products is important, we would expect the alcoholic moiety, linked to the aromatic heterocyclic group, to be active, whereas the moiety that includes the carboxylic group would be inactive. We find support

TABLE 2

Test data set of imidazo [1,2-a]pyridines and -pyrazines

Compounds were tested at the 2 mg/kg dosage.

Compound	Substitution				Antisecretory activity[a]				logP	Probability
					Experimental		Calculated			
	R_1	R_2	R_3	R_4/Y	5 mg/kg	2 mg/kg				
1	$PhCH_2O$	CH_3	H		+++	+++	++++	W	3.16	32.9
2	$PhCH_2O$	CH_3	CH_2CN		++++	++++	++++		2.39	94.9
5	4-$ClPhCH_2O$	CH_3	CH_2CN		++++	++	++++		2.89	99.7
13	$PhCH_2CH_2$	CH_3	CH_2CN		++++	++++	++++		3.35	90.6
16	4-CF_3PhCH_2O	CH_3	CH_2CN		++	+	++++		2.51	99.3
18	2,4,6-Me_3-$C_6H_2CH_2O$	CH_3	CH_2CN		++++	−	++++		3.36	98.0
23	$PhCHCH_3$	CH_3	CH_2CN		++++	++++	++++		3.35	90.6
24	$PhCH_2NH$	CH_3	CH_2CN		++++	++++	++++		1.95	90.6
33	$PhCH_2O$	CH_3	CH_2OH		++++	++++	++++		2.46	32.9
47	$PhCH_2O$	CH_3	$CH_2(O_2CCH_3)$		++++	++	++++		3.11	49.9
60	$PhCH_2O$	CH_3	CH_3CH_2NH		++++	−	+		2.67	32.9
65	$PhCH_2O$	CH_3	NH_2			++++	−		1.94	27.0
66	$PhCH_2O$	H	NH_2			++++	++++	W	1.54	27.0
68	$PhCH_2CH_2$	CH_3	HO			−	+	W	3.32	0.0
69	$PhCH_2CH_2$	CH_3	CH_3CO_2			−	+		3.75	0.0
70	$PhCH_2O$	H	CH_2CN			++++	++++	W	1.99	51.8
71	$PhCH_2O$	CH_2CH_3	CH_2CN			++++	++++		2.80	80.3
72	3,4-$Cl_2C_6H_4CH_2O$	CH_3	CH_2CN			++++	++++		3.39	98.6
73	H	CH_3	CH_2CN	R_4 = 7-$PhCH_2CH_2$		−	++		3.35	58.0
74	4-$MeOPhCH_2O$	CH_3	CH_2CN			+	++++		2.25	99.7
75	$PhCH_2NMe$	CH_3	CH_2CN			+	++++	W	2.36	76.2
76	$PhOCH_2$	CH_3	CH_3			+	++++		3.65	0.0
77	CH_2SOPh	CH_3	CH_3			−	−		1.46	25.0
78	$PhCH_2O$	CH_3	CH(Me)CN			−	+	W	2.80	27.0
79	$PhCH_2O$	CH_3	CH_2CSNH_2		+++	−	+	W	2.53	27.0
80	$PhCH_2O$	CH_3	$CH_2O_2CC_4H_9$-*t*			+		W	4.33	42.9
81	$PhCH_2O$	CH_3	CH_2CH_3			−	−		3.97	27.0
82	$PhCH_2O$	CH_2CH_3	CH_3			++++	−		3.97	27.0
83	$PhCH_2O$	CH_3	CH_3	R_4 = 5-CH_3		−	−		4.02	27.0
84	$PhCH_2O$	CH_3	CH_3	R_4 = 6-CH_3		+	−		4.02	27.0
85	$PhCH_2O$	CH_3	CH_3	$Y = CH_3C$		++++	−		4.02	27.0
86	$PhCH_2O$	CH_3	CH_2CN	$Y = CH_3C$		++++	++		3.00	50.9
87	$PhCH_2O$	*i*-C_3H_7	NH_2			−	+		2.76	32.9
88	$PhCH_2O$	*n*-C_4H_9	NH_2			+	−		3.17	27.0
89	2-$FC_6H_4CH_2O$	CH_3	NH_2			++++	−		1.88	0.0
90	4-$FC_6H_4CH_2O$	CH_3	NH_2			++++	−		1.88	66.0
91	4-$CF_3C_6H_4CH_2O$	CH_3	NH_2			−	−		2.15	66.0
92	2-$PyCH_2O$	CH_3	NH_2			−	−		0.46	11.0
93	2-$ThienlCH_2O$	CH_3	NH_2			++++	−		1.64	0.0
94	3-$ThienlCH_2O$	CH_3	NH_2			++++	−		1.64	0.0
95	$PhCH_2NH$	CH_3	NH_2			++++	−		1.49	0.0
96	$PhCH_2S$	CH_3	NH_2			−	−		2.64	0.0
97	$PhCH_2CH_2O$	CH_3	NH_2			−	−		2.35	0.0
98	PhO	CH_3	NH_2			+	−		2.32	0.0
99	$PhCH_2CH_2$	CH_3	NH_2			++++	−		2.87	0.0
100	H	CH_3	NH_2	$Y = PhCH_2CH_2C$		−	−		2.87	0.0
101	H	CH_3	NH_2	R_4 = 6-$PhCH_2CH_2$		−	−		2.87	0.0
102	$PhCH_2O$	CH_3	NH_2	R_4 = 6-CH_3		+	−		2.35	27.0
103	$PhCH_2O$	CH_3	NH_2	$Y = CH_3C$		++	−		2.35	27.0
104	$PhCH_2O$	CH_3	NH_2	Y = N		+++	−		−0.21	8.5
105	$PhCH_2O$	CH_3	$CH_2CH_2NH_2$			−	+	W	2.76	27.0
106	$PhCH_2O$	CH_3	HCONH			−	−	W	1.64	27.0
107	$PhCH_2O$	CH_3	CH_3CONH			−	−		2.04	27.0
108	$PhCH_2O$	CH_3	$PhCH_2O_2CNH$			++++		W	4.31	32.9
109	$PhCH_2O$	CH_3	$(CH_3)_2NCHN$			−	+	W	2.81	5.7
110	$PhCH_2O$	CH_3	$(CH_3)_2N$			−	+		2.76	11.0
111	$PhCH_2O$	CH_3	CH_3O			−	+	W	2.81	27.0
112	$PhCH_2CH_2$	CH_3	CH_3O			−	+	W	3.73	0.0
113	$PhCH_2CH_2$	CH_3	CH_3S			−	+	W	4.42	0.0

[a] ++++, extremely active; +++, very active; ++, active; +, marginal; −, inactive.
W indicates the presence of unknown functionalities.

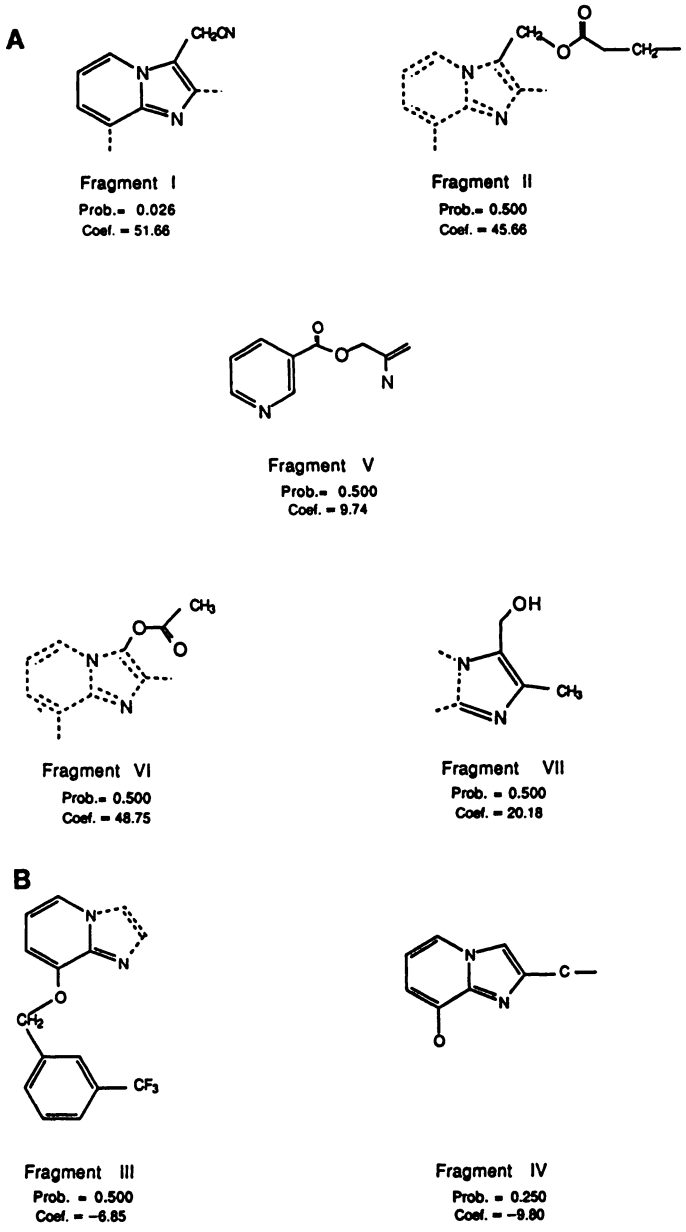


Fig. 2. QSAR activating (A) and deactivating (B) fragments. *Prob.*, probability, Coef., QSAR coefficient.

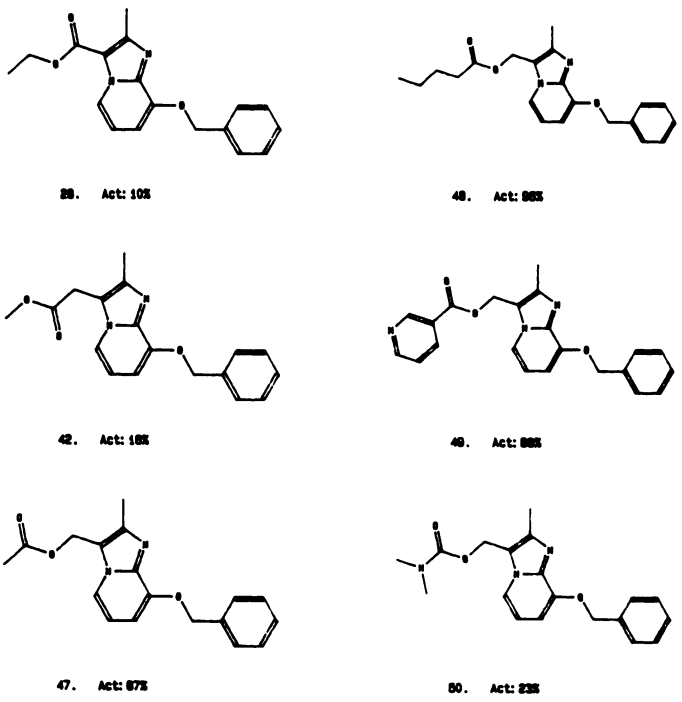


Fig. 3. Antiulcer compounds containing ester substitution. *Act.*, activity.

TABLE 3

Esters and their hydrolytic products

	Ester	Hydrolytic products
Type A	R′-CO-O-CH_2-Ar	R′-COOH + HO-CH_2-Ar
Type B	R-O-CO-CH_2-Ar	R-OH + HOOC-CH_2-Ar

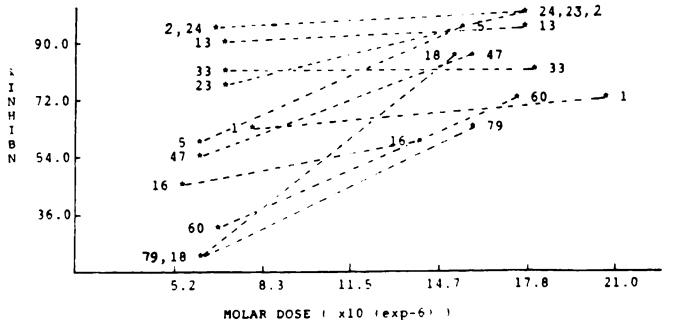


Fig. 4. Correlation of antisecretion activities of imidazo[1,2-*a*]pyridine analogues at two different molar dosage levels. – – –, Change in activity for each compound. *Inhib.*, inhibition.

for this hypothesis by examining other compounds in the database that have a —OH or a —COOH group at the 3-position. There are 2 compounds, **29** and **43**, that have a terminal —COOH at the 3-position. Both of these compounds are inactive. On the other hand, compound **33** has a terminal —OH group at the 3-position and is found to be active. The only exception to this hypothesis is compound **50**, which is found to be inactive, although it belongs to the type A category. However, this compound contains a $(CH_3)_2N—CO—O—CH_2$ fragment. The presence of the NMe_2—group next to the carbonyl group increases the electron density at the carbon atom, which, as a result, will only undergo slow hydrolysis. This may result in negligible formation of the aromatic alcohol. Hence, compound **50** fails to exhibit any significant antiulcer action.

There was yet another alcohol in the database that was not included in our analysis, compound **68**. This molecule differs from the other members of the group mentioned above by two structural features. It possesses a $—CH_2—CH_2—$ group at the 8-position instead of a $—O—CH_2—$ group. However, this does not seem to produce much difference, because it also occurs in several other compounds. The second difference is that the alcohol functionality is attached directly to the aromatic skeleton at the 3-position, instead of being of the benzylic type. This seems to have a large impact on the antisecretory activity, because the activity drops to just 30% from 96%. This shows the importance of the benzylic type of linkage at the 3-position. This was also found to be true in the case of fragment **I**. Finally, when the Ar-CH_2-CN is replaced by Ar-CN (compounds **2** and **30**), a complete loss of activity was observed.

There are 5 molecules for which large differences exist between the predicted and the actual activity. They are compounds **1**, **41**, **60**, **7**, and **16**. The first three are the active

TABLE 4
Molar dose and antisecretion activity of a few imidazo[1,2-*a*] pyridine analogues

Compound	Molecular weight	2 mg/kg		5 mg/kg	
		Dose	Activity	Dose	Activity
		mol × 10^6	% of inhibition	mol × 10^6	% of inhibition
1	311.33	6.4	21	16.1	62
2	319.41	6.3	22	15.7	86
3	281.36	7.1	32	17.8	70
4	345.33	5.8	44	14.5	59
5	310.36	6.4	54	16.1	87
6	311.77	6.4	58	16.0	95
7	238.29	8.4	61	21.0	70
8	275.36	7.3	78	18.2	97
9	268.32	7.5	80	18.6	80
10	275.36	7.3	92	18.2	96
11	277.33	7.2	95	18.0	99
12	276.34	7.2	96	18.1	98

compounds that were falsely predicted to be either inactive or marginal. CASE does not provide any clue to help us understand the incorrect predictions. However, the last two cases can possibly be explained by the following consideration. Both compounds **7** and **16** contain a substituent at the *para*-position of the phenyl group. There are 4 more compounds in the database that are similar to the two mentioned above and contain different substituents at the same *p*-position; they are compounds **2, 5, 15**, and **17**. They contain —H, —Cl, —F, and —CN substitutions, respectively. For these 6 compounds, we find that the activities are roughly in the order H > F = Cl > CN > CF_3 > $C(Me)_3$, which may indicate that as the size of the substituent at the *p*-position of the phenyl group increases, the activity decreases. This may indicate that the phenyl group of the phenoxy substituent at the 8-position of the imidazo[1,2-*a*]pyridine offers an optimum fit to the enzyme for the enzyme-substrate binding. Any substitution in this phenyl ring then leads to a decrease in the effectiveness of the binding to the enzyme.

The compounds for which the activity was determined by administration of a dose of 2 mg/kg were now submitted as a test data set. The results are provided in Table 2. The predictions for the activities of these 39 compounds were found to be rather poor. There are two possible explanations that can account for the lack of agreement. One is that the R_3 substituent, which was the most important descriptor in the database, was totally different in this test set. This could explain why some of the active compounds in the test set were calculated to be inactive. The major reason, though, might well be that the test set consisted of data obtained at a different dose from that of the training set. This obviously creates a great deal of ambiguity. Indeed, we find that the 5 mg/kg experimental data are poor predictors of the 2 mg/kg experimental data. This is shown by the fact that a plot of the 12 compounds for which the activity at both 2 and 5 mg/kg doses had been reported yielded a poor correlation (r^2) coefficient of only 0.45. This can also be seen from the data in Fig. 4, where the percentage of inhibition was plotted against the molar dose for these 12 compounds (data are provided in Table 4). As can be seen, considerable crossing exists among the plotted lines. Although all the compounds exhibited higher activity when going from 2 mg/kg to 5 mg/kg doses, the relative increment in each case was noticeably different. Consequently, the validity of the test set is questionable, because a separate analysis based on the 2 mg/kg dosage data might yield considerably different results than those in the 5 mg/kg data set. This stresses the need to determine the activity under better conditions, in order to generate data that can be rationalized. Indeed, it is not possible to provide quantitative predictions for experiments carried out with varying concentrations of the compounds.

Conclusion

The analysis of the antisecretion activity of a series of imidazo[1,2-*a*]pyridines and -pyrazines has yielded some interesting and useful results. The importance of hydrophobicity for optimal antiulcer action was established as a necessary criterion, and an optimum log*P* value was determined. The phenoxy group at the 8-position of imidazo[1,2-*a*]pyridine and the methyl group at its 2-position were shown to be involved in the enzyme-substrate binding. The relationship between activity and substitutions at the 3-position was also demonstrated. Although the cyanomethyl group contributes more than the alcohol group to the activity, it has also been found to undergo extensive metabolism and eventually leads to liver toxicity (24). It is, therefore, suggested that a drug might be designed with a structure similar to the backbone of imidazo[1,2-*a*]pyridine. Suitable alterations should be made so as to have a log*P* value of about 2.7 and a —CH_2OH substitution at the 3-position. This may produce not only an antiulcer drug with high activity but also one devoid of liver toxicity. Finally, the observed poor correlation between the responses obtained at the two different doses underscores the need to test potential drugs in a more controlled way, in order to yield chemically useful information. It is hoped that dosages will be expressed in mol equivalents, so that more significant results could be obtained by QSAR studies.

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