3400 (-NH_2), 3175 (-NH), 1625 cm^{-1}; ^1H NMR (300 MHz) (DMSO-d_6) δ 1.53 (m, 1 H, 7-H), 1.96 (m, 4 H, H_{6a}, and 7-H, and β-CH_2) 2.34 (m, 2 H, γ-CH_2) 2.75–3.90 (m, 7 H, 6-H_2, 8-H_2, 10-H_2, and H_{10a}), 4.39 (m, 1 H, α-CH), 6.09 (s, 2 H, C-2 NH_2), 6.47 (s, 1 H, 8-NH), 6.98 (d, 2 H, 3′ and 5′-CH), 7.77 (d, 2 H, 2′ and 6′-CH), 8.26 (d, 1 H, amide NH) 9.85 (br s, 1 H, COOH of glutamate). Anal. ($C_{22}H_{26}N_6O_6 \cdot 1H_2O$) C, H, N.

Acknowledgment. This work was supported by a grant (no. CH-332) (A.G.) from the American Cancer Society and grant (no. CA 10914) (R.L.K.) from the National Cancer Institute. The authors also thank Dr. F. T. Lin, Department of Chemistry, University of Pittsburgh for the high resolution ^1H NMR and ^{13}C NMR spectra.

Registry No. (6a*R*,10a*S*)-L-**5**, 130985-82-9; (6a*S*,10a*R*)-L-**5**, 131064-26-1; **6**, 130985-83-0; **7**, 1004-38-2; **8**, 39514-19-7; **9**, 130985-84-1; **11**, 130985-85-2; **12**, 130985-86-3; **13**, 131010-65-6; *cis*-(±)-**19**, 130985-88-5; *cis*-(±)-**20**, 130985-89-6; *cis*-(±)-**21**, 130985-90-9; (6a*R*,10a*S*)-L-**22**, 131100-23-7; (6a4S,10a*R*)-L-**22**, 131010-66-7; TS, 9031-61-2; 4-FC_6H_4COOBU-*t*, 58656-98-7; H-Glu(OEt)-OEt·HCl, 1118-89-4.

Novel Benzamides as Selective and Potent Gastrokinetic Agents. 2.[1] Synthesis and Structure–Activity Relationships of 4-Amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide Citrate (AS-4370) and Related Compounds

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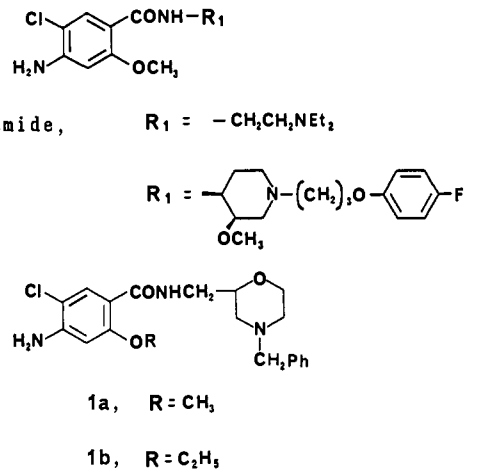
The title compounds (**19–55**) with a 4-substituted 2-(aminomethyl)morpholine group were prepared and evaluated for the gastrokinetic activity by determining their effect on gastric emptying of phenol red semisolid meal in rats. Introduction of chloro, fluoro, and trifluoromethyl groups to the benzyl group of the parent compounds **1a** and **1b** enhanced the activity. Among compounds tested, 4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide (**23b**) showed the most potent gastric emptying activity (effects on phenol red semisolid meal in rats and mice, and on resin pellets solid meal in rats). The gastrokinetic activity of **23b** citrate (AS-4370) compared very favorably with that of cisapride and was higher than that of metoclopramide. In contrast to metoclopramide and cisapride, AS-4370 was free from dopamine D_2 receptor antagonistic activity in both in vitro ([^3H]spiperone binding) and in vivo (apomorphine-induced emesis in dogs) tests.

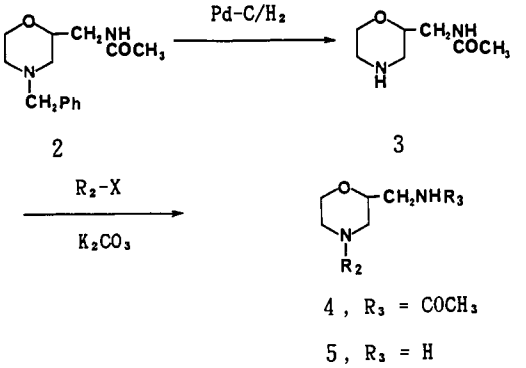
Metoclopramide as a stimulant of the upper gastrointestinal motility is limited in clinical use because of unfavorable side effects such as extrapyramidal symptoms arising from its dopamine D_2 receptor antagonistic property.[2] Substituted benzamides, which are structurally related to metoclopramide having a potent gastric prokinetic activity with minimized side effects, have recently been reported (Chart I).[3-9]

In a previous paper,[1] we reported that a series of benzamide derivatives appending a new amine moiety, 2-(aminomethyl)-4-benzylmorpholine, had a potent gastrokinetic activity with lack of the dopamine D_2 receptor antagonistic activity. In particular, 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide (**1a**) and its ethoxy analogue (**1b**) were of much interest to us, because their gastrokinetic activity was much higher than that of metoclopramide. In the present study, we focused on the introduction of various substituents to

Chart I



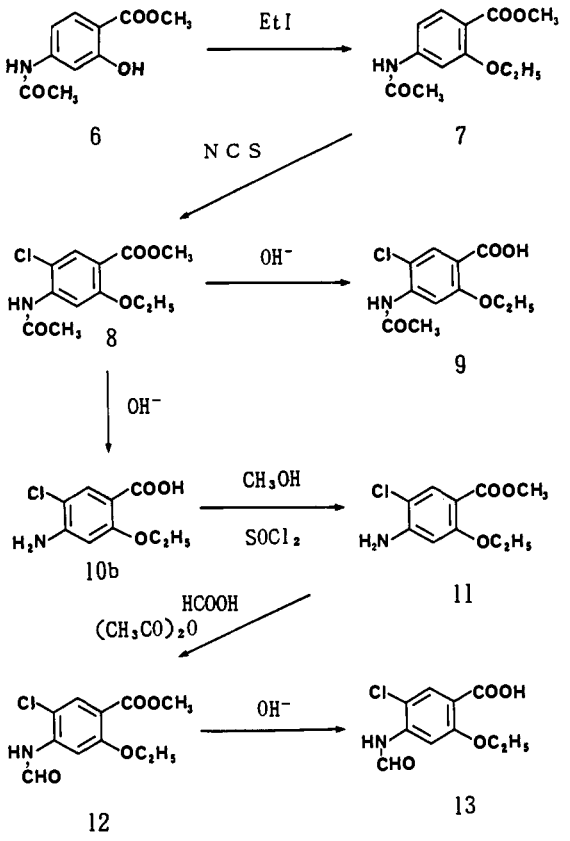
Scheme I



(1) Part 1 of this series: Kato, S.; Morie, T.; Hino, K.; Kon, T.; Naruto, S.; Yoshida, N.; Karasawa, T.; Matsumoto, J. *J. Med. Chem.* **1990**, *33*, 1406.
(2) McRitchie, B.; McClelland, C. M.; Cooper, S. M.; Turner, D. H.; Sanger, G. J. In *Mechanisms of Gastrointestinal Motility and Secretion*; Bennett, A., Velo, G. P., Eds.; Plenum Press: New York, 1984; p 287.
(3) Schuurkes, J. A. J.; Van Neuten, J. M.; Van Daele, G. H. P.; Reyntjens, A. J.; Janssen, P. A. J. *J. Pharmacol. Exp. Ther.* **1985**, *234*, 775.
(4) Van Daele, G. H. P.; De Bruyn, M. F. L.; Sommen, F. M.; Janssen, M.; Van Nueten, J. M.; Schuurkes, J. A. J.; Niemegeers, C. J. E.; Leysen, J. E. *Drug Dev. Res.* **1986**, *8*, 225.
(5) Alphin, R. S.; Proakis, A. G.; Leonard, C. A.; Smith, W. L.; Dannenburg, W. N.; Kinnier, W. J.; Johnson, D. N.; Sancilio, L. F.; Ward, J. W. *Dig. Dis. Sci.* **1986**, *31*, 524.
(6) *Drugs Future* **1987**, *12*, 1009.
(7) Dunbar, A. W.; McClelland, C. M.; Sanger, G. J. *Br. J. Pharmacol.* **1986**, *88*, 319.
(8) Sanger, G. J. *Br. J. Pharmacol.* **1987**, *91*, 77.
(9) Sanger, G. J.; King, F. D. *Drug. Des. Del.* **1988**, *3*, 273.

0022-2623/91/1834-0616$02.50/0

Scheme II



the benzyl groups of **1a** and **1b** and modification of the benzyl group itself with the aim of finding more potent analogues. As a result of screening tests, 4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]-methyl]benzamide (**23b**) was found to be essentially equipotent to cisapride in gastrokinetic activity. The present paper describes the synthesis and structure–activity relationships (SARs) of 2-alkoxy-4-amino-5-chloro-*N*-[(4-substituted-2-morpholinyl)methyl]benzamides (**19–51**) and their related compounds (**52–55**).

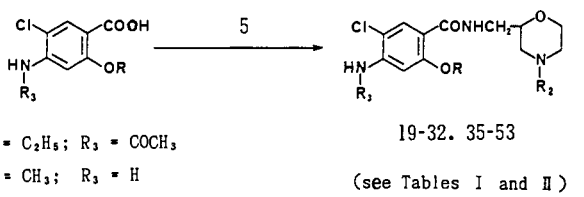
Chemistry

The requisite amines, 2-(aminomethyl)-4-substituted morpholines (**5**) were prepared by the method depicted in Scheme I. Thus, hydrogenation of 2-[(acetylamino)-methyl]-4-benzylmorpholine[1] (**2**) in the presence of palladium-on-carbon gave 2-[(acetylamino)methyl]morpholine (**3**), which was then treated with an appropriate alkylating agent to give 4-substituted 2-[(acetylamino)methyl]-morpholines (**4**). Acid hydrolysis of the acetylamino group of **4** produced the desired amines, 4-substituted 2-(aminomethyl)morpholines (**5**).

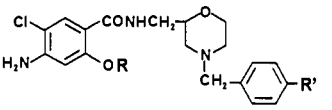
Benzoic acids **9**, **10b**, and **13** were prepared as follows (Scheme II). Methyl 4-(acetylamino)-2-hydroxybenzoate (**6**)[10] was converted to 2-ethoxy derivative **7** with ethyl iodide, and then chlorination of **7** with *N*-chlorosuccinimide (NCS) followed by alkaline hydrolysis of **8** gave *N*-acetylbenzoic acid (**9**). Compound **10b** was obtained by alkaline hydrolysis of the *N*-acetyl and ester groups of **8**. The reaction of **10b** with thionyl chloride in methyl alcohol gave methyl ester **11**, which was treated with formic acid and acetic anhydride to give *N*-formyl compound **12**. Alkaline hydrolysis of **12** gave the *N*-formylbenzoic acid (**13**).

(10) Debat, J. Fr. M. 6,052; *Chem. Abstr.* **1970**, *72*, 43687*h*.

Scheme III. Methods A, B, F, and G

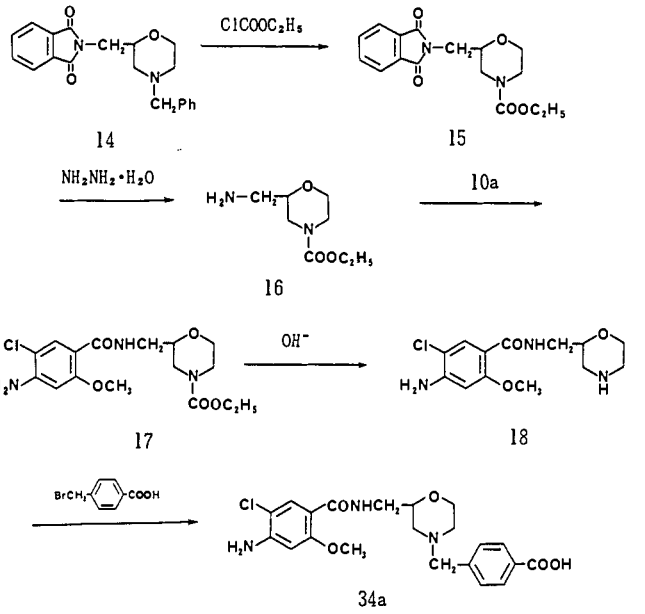


9. R = C_2H_5; R_3 = $COCH_3$
10a, R = CH_3; R_3 = H
10b. R = C_2H_5; R_3 = H
13. R = C_2H_5; R_3 = CHO



32a. R = CH_3; R' = NO_2
33a, R = CH_3; R' = NH_2
35b. R = C_2H_5; R' = $COOCH_3$
34b. R = C_2H_5; R' = COOH

Scheme IV. Method D

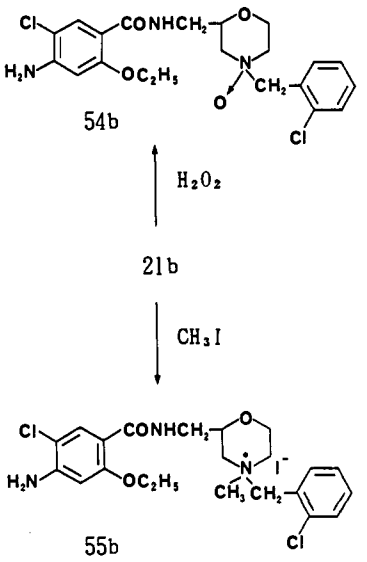


Benzamide derivatives **19–53**, except **33a**, **34a**, and **34b**, were synthesized by the reaction of 2-alkoxy-4-amino-5-chlorobenzoic acid (**10a**[11] and **10b**) and their derivatives (**9** and **13**) with **5** (Scheme III, methods A, B, F, and G). The 4-aminobenzyl derivative **33a** was prepared by hydrogenation of the corresponding nitro compound **32a** in the presence of Raney nickel (method C). The 4-carboxybenzyl analogue **34b** was derived from 4-methoxycarbonyl compound **35b** by acidic hydrolysis (method E).

4-Amino-*N*-[[(4-carboxybenzyl)-2-morpholinyl]-methyl]-5-chloro-2-methoxybenzamide (**34a**) was synthesized according to Scheme IV. Thus, *N*-[(4-benzyl-2-morpholinyl)methyl]phthalimide (**14**)[1] was converted to carbamate **15** by the reaction with ethyl chloroformate. Compound **15** was treated with hydrazine monohydrate to give intermediate amine **16**, of which condensation with **10a** afforded 4-(ethoxycarbonyl)morpholine derivative **17**. Alkaline hydrolysis of **17**, followed by the reaction of **18** with 4-(bromomethyl)benzoic acid, gave carboxylic acid **34a** (method D).

(11) Murakami, M.; Inukai, N.; Koda, A.; Nakano, K. *Chem. Pharm. Bull.* **1971**, *19*, 1696.

Scheme V. Methods H and I



N-Oxide **54b** and quaternary salt **55b** were obtained by oxidation of **21b** with hydrogen peroxide and the reaction of **21b** with methyl iodide, respectively (Scheme V, methods H and I).

Pharmacological Results and Discussion

All compounds **19a–55b** were evaluated for gastrokinetic activity by determining their effects on the gastric emptying rates of phenol red semisolid meal through the stomach at an oral dose of 2.0 mg/kg in rats. The biological results are shown in Tables I and II. Included for comparison are the activities of metoclopramide, cisapride, **1a**, and **1b**.[1] The series of benzamide derivatives, commonly having a 2-alkoxy-4-amino-5-chlorobenzoyl group, are divided into the following two series: series **a**, benzamides with a 2-methoxy group (analogues of **1a**) and series **b**, benzamides with a 2-ethoxy group (analogues of **1b**).

The SARs associated with substituents on the benzyl group of **1a** and **1b** were firstly examined. Although the substituents did not so influence activity as expected, a number of compounds were more potent than metoclopramide, and several were more potent than cisapride as well as the parent compound **1b**. Introduction of a substituent, such as chloro, fluoro, and trifluoromethyl groups, generally enhanced the activity, although there are a few differences between series **a** and **b**. In series **a** (the 2-methoxy derivatives), introduction of a halogeno group (**19a**, **22a**, and **23a**), except a bromo group, at the para position of **1a**, resulted in retention of the activity, whereas *m*- and *o*-fluoro substituents (**24a** and **25a**) slightly decreased the activity compared with that of **23a**. The *m*-trifluoromethyl analogue **27a** was more potent than its para isomer **26a** and was superior to **1a**. Variation of the para substituent caused an increase in activity in the order OCH_3 (**28a**) = CH_3 (**30a**) < Br (**22a**) ≤ NH_2 (**33a**) = CO_2H (**34a**) < NO_2 (**32a**) = CF_3 (**26a**) ≪ Cl (**19a**) ≤ F (**23a**) = H (**1a**) ≪ CN (**36a**). Thus, in general, compounds having an electron-donating group showed a poorer activity, whereas those having an electron-withdrawing group showed a higher activity. Introduction of a cyano group into the benzyl group of **1a** (giving **36a–38a**) caused a remarkable increase in activity; the *p*-cyano substitution (**36a**), in particular, exhibited the most potent activity. Compound **44a** with fluorine atoms at all positions on the phenyl ring, being more lipophilic than **1a**, was equipotent to **1a**.

On the basis of the result from series **a**, an electron-withdrawing group, such as fluoro, chloro, cyano, trifluoromethyl, and carboxy groups, was selected as a substituent to be introduced into the benzyl group of **1b**. Most compounds of series **b** (the 2-ethoxy derivatives) were, in general, more potent than the counterparts of series **a**. Chloro (**19b–21b**) and fluoro (**23b–25b**) substitutions at the ortho, meta, and para positions of **1b** caused an enhancement in activity compared with that of **1b**. Cyano substitution (**36b–38b**), however, reduced the activity in contrast to the case in series **a**. Para substitution was the most favorable for enhancing the activity as in series **a**. *m*-Trifluoromethyl substitution (**27b**) improved the activity. Introduction of a methoxycarbonyl group into the para position of **1b** (giving **35b**) kept the same level of activity as that of **1b**, whereas a carboxy group (**34b**) caused a complete loss of activity. Para substitution increased activity in the order COOH (**34b**) ≪ CN (**36b**) < $COOCH_3$ (**35b**) ≤ H (**1b**) < F (**23b**) ≤ Cl (**19b**). As a result, introduction of fluoro, chloro, and trifluoromethyl groups into the benzyl group caused an increase in activity; a similar trend had been observed in series **a**.

Further introduction of chloro (**39a**) and methyl groups (**31a**) into the phenyl rings of **19a** and **30a**, respectively, led to a significant decrease in activity (Table I). Introduction of a nitro group (**40b**) into **19b**, and a fluoro group (**42b** and **43b**) into **24b** decreased the activity; however, compound **41b**, having a 2,4-difluoro group, was much more potent than **1b**. The substitution by fluorine atoms at all positions on the phenyl ring (giving **44b**) caused a considerable decrease in activity. These findings indicate that an electronic factor of the substituent on the benzyl group, rather than a steric factor, influences the in vivo gastric emptying activity of the benzamides (**19a–44b**).

SARs of the methylene moiety on the benzyl groups of **1a** and **1b** were then examined. The substitution of an additional methyl group at **1a** (yielding **45a**) slightly enhanced the activity, whereas the methyl substitution at **1b**, **23a**, and **19b** (giving **45b**, **46a**, and **47b**, respectively) resulted in a decrease in activity. Furthermore, replacement of the methylene moiety by ethylene (**49a**), *n*-propylene (**50b**), and *n*-butylene (**51b**) groups caused a decrease in activity. Addition of a bulkier and more lipophilic group, such as a phenyl group, to the methylene group of **1a** (giving **48a**) remarkably decreased activity.

Effects of the substituent on the 4-amino group of **23b** and on the morpholinyl nitrogen of **21b** were examined. Acetyl derivative **52b** showed an increased activity. Formyl derivative **53b**, however, was much less potent than **52b**. Morpholinyl *N*-oxide analogue **54b** was essentially equipotent to **21b**, whereas quaternary salt analogue **55b** was less potent.

In the acute toxicity test in mice (Table I), the substituted benzyl derivatives showed a slight or moderate toxicity with the exception of **37a**. Compounds **45a** and **45b**, having a methyl group on the methylene moiety of **1**, were more toxic than their corresponding parent compounds, **1a** and **1b**.

On the basis of the potent gastric emptying activity and the weak acute toxicity, eight compounds (**19b**, **23b**, **27b**, **36a**, **41b**, **52b**, **1a**, and **1b**) were selected for further biological assays involving the gastric emptying activity of phenol red semisolid meal in rats and mice. The gastric emptying activity of resin pellets solid meal in rats was also tested, because a different mechanism is reported between assays with semisolid meal and solid meal.[12,13] Moreover,

(12) Kelly, K. A. *Am. J. Physiol.* 1980, *239*, G71.

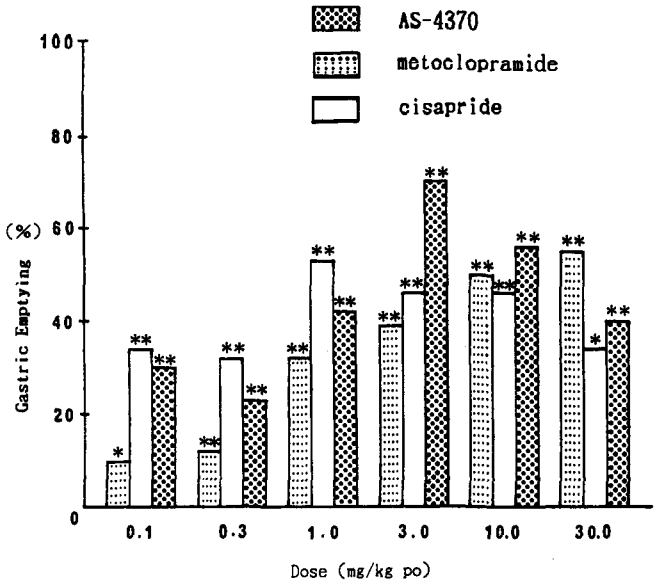


Figure 1. Effects of AS-4370, metoclopramide, and cisapride on gastric emptying of phenol red semisolid meal in rats. Gastric emptying is expressed as the enhancing percentage based on comparison with the mean value for control groups (0.5% tragacanth). The asterisks indicate a statistically significant difference from the control group: *, $p < 0.05$; **, $p < 0.01$ (Duncan's multiple range test).

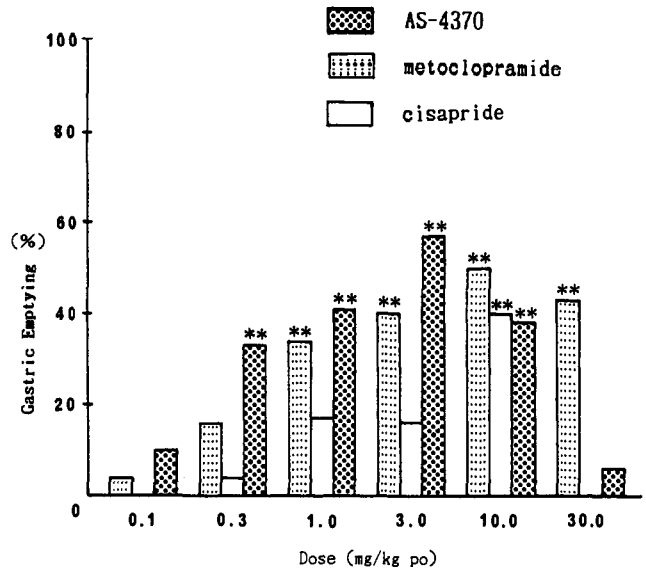


Figure 2. Effects of AS-4370, metoclopramide, and cisapride on gastric emptying of phenol red semisolid meal in mice. Gastric emptying is expressed as the enhancing percentage based on comparison with the mean value for control groups (0.5% tragacanth). The asterisks indicate a statistically significant difference from the control group: **, $p < 0.01$ (Duncan's multiple range test).

the activity for dopamine D_2 receptor antagonism in vitro was determined with the [^{3}H]spiperone binding test. The results are presented in Table III. In the gastric emptying activity of semisolid meal in rats, compounds **19b**, **23b**, **27b**, and **52b** were more potent than others at oral doses of 0.2, 0.5, and 2.0 mg/kg, whereas, in the activity in mice, **23b**, **41b**, **52b**, and **1a** were more potent. In the gastric emptying activity of solid meal in rats, **19b**, **23b**, **41b**, and **1b** were more potent than others. Furthermore, in the [^{3}H]spiperone binding test at the concentration of 10^{-6} M, none of the selected compounds showed a dopamine D_2 binding affinity.

Compound **23b**, on the whole, was found to possess the most favorable activity profile of gastric emptying enhancement without the dopamine D_2 receptor antagonistic activity. Compound **23b** (AS-4370 as its citrate) was therefore selected and tested for dose–response on the gastrokinetic activity, for suppression of apomorphine-induced emesis in dogs, and by receptor binding assay with rat brain synaptic membranes. As shown in Figures 1–3, the activity of AS-4370 compared very favorably with that of cisapride and was much higher than that of metoclopramide. On the other hand, AS-4370 and cisapride were completely free from dopamine D_2 antagonistic activity as measured by the blockade of apomorphine-induced emesis at oral doses of 1.0 and 10 mg/kg, respectively, in sharp contrast to metoclopramide showing the ED_{50} value of 0.45 mg/kg (Table IV). In the radioligand binding assay (Table V), AS-4370 showed no affinities at the concentration of 10^{-4} M for dopamine D_2, serotonin S; and S_2, and adrenaline α_1 and α_2 binding sites in rat brain synaptic membranes; on the contrary, metoclopramide had a high dopamine D_2 binding affinity with the IC_{50} of 0.63 μM, and cisapride also showed affinities for dopamine D_2 and serotonin S_2 binding sites with the IC_{50}'s of 0.39 and 0.0098 μM, respectively.

In conclusion, AS-4370 (citrate of **23b**) was found to possess a potent gastrokinetic activity and to be free from

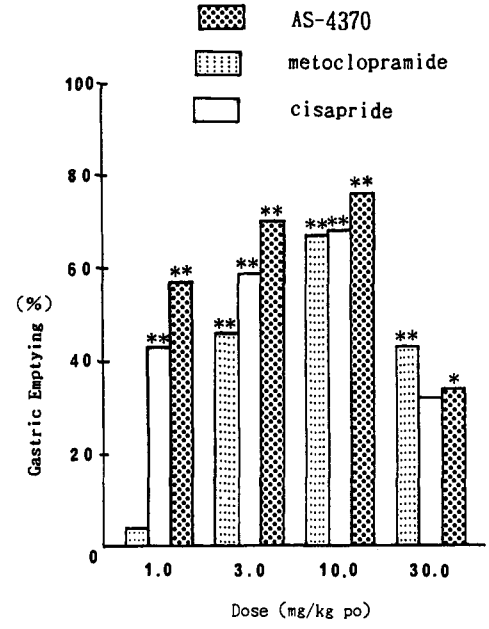


Figure 3. Effects of AS-4370, metoclopramide, and cisapride on gastric emptying of resin pellets solid meal in rats. Gastric emptying is expressed as the enhancing percentage based on comparison with the mean value for control groups (0.5% tragacanth). The asterisks indicate a statistically significant difference from the control group: *, $p < 0.05$; **, $p < 0.01$ (Duncan's multiple range test).

dopamine D_2 receptor antagonistic activity in both in vitro and in vivo tests. Therefore, AS-4370 was selected as a promising candidate for a selective gastrokinetic agent and, after further biological evaluation,[14] is now undergoing a clinical study.

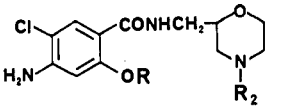
Experimental Section

Chemistry. All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR

(13) Decktor, D. L.; Pendleton, R. G.; Elnitsky, A. T.; Jenkins, A. M.; McDowell, A. P. *Eur. J. Pharmacol.* 1988, *147*, 313.

(14) Yoshida, N.; Omoya, H.; Oka, M.; Furukawa, K.; Ito, T.; Karasawa, T. *Arch. Int. Pharmacodyn. Theor.* 1989, *300*, 51.

Table I. Physicochemical and Pharmacological Data for 2-Alkoxy-4-amino-5-chloro-*N*-[(4-substituted-2-morpholinyl)methyl]benzamides (19–51)



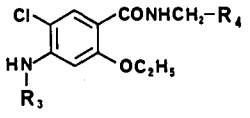
no.	R	R_2[a]	mp, °C	recryst solvent[b]	method[c]	% yield[d]	formula[e]	% GE[f] (2.0 mg/kg po)	acute toxicity[g] (1.0 g/kg po)
19a	CH_3	4-$ClC_6H_4CH_2$	175–181	EA	A	79	$C_{20}H_{23}Cl_2N_3O_3 \cdot 2.0C_4H_4O_4$[h]	36**	++
19b	C_2H_5	4-$ClC_6H_4CH_2$	150–151	EA	B	65	$C_{21}H_{25}Cl_2N_3O_3$	63**	+
20b	C_2H_5	3-$ClC_6H_4CH_2$	155–158	EA	B	41	$C_{21}H_{25}Cl_2N_3O_3 \cdot HCl \cdot 0.5H_2O$	58**	++
21b	C_2H_5	2-$ClC_6H_4CH_2$	181–183	EA	B	35	$C_{21}H_{25}Cl_2N_3O_3 \cdot HCl \cdot 0.5H_2O$	51**	+
22a	CH_3	4-$BrC_6H_4CH_2$	103–108	EA	A	41	$C_{20}H_{23}BrClN_3O_3 \cdot C_4H_4O_4$[h]	15	
23a	CH_3	4-$FC_6H_4CH_2$	172–175	EA	A	75	$C_{20}H_{23}ClFN_3O_3 \cdot 0.25H_2O$	38**	
23b[i]	C_2H_5	4-$FC_6H_4CH_2$	160–163	EA	B	77	$C_{21}H_{25}ClFN_3O_3 \cdot 1.25HCl \cdot 1.75H_2O$	61**	++
24a	CH_3	3-$FC_6H_4CH_2$	185–188	EA	A	78	$C_{20}H_{23}ClFN_3O_3 \cdot 2.0C_4H_4O_4$[h]	30*	
24b	C_2H_5	3-$FC_6H_4CH_2$	183–184	IA	B	80	$C_{21}H_{25}ClFN_3O_3 \cdot C_4H_4O_4$[h]	54**	
25a	CH_3	2-$FC_6H_4CH_2$	138–141	EA	A	67	$C_{20}H_{23}ClFN_3O_3 \cdot 0.5C_4H_4O_4$[h]	28*	
25b	C_2H_5	2-$FC_6H_4CH_2$	175–178	IA	B	75	$C_{21}H_{25}ClFN_3O_3 \cdot 2.0C_4H_4O_4$[h]	60**	
26a	CH_3	4-$CF_3C_6H_4CH_2$	150–157	EA	A	95	$C_{21}H_{23}ClF_3N_3O_3 \cdot 1.5C_4H_4O_4$[h]	23*	
27a	CH_3	3-$CF_3C_6H_4CH_2$	96–99	EA	A	81	$C_{21}H_{23}ClF_3N_3O_3 \cdot 1.5C_4H_4O_4$[h] $\cdot 0.5H_2O$	43**	++
27b	C_2H_5	3-$CF_3C_6H_4CH_2$	146–149	EA	B	68	$C_{22}H_{25}ClF_3N_3O_3$	58**	+
28a	CH_3	4-$CH_3OC_6H_4CH_2$	61–64	EA	A	92	$C_{21}H_{26}ClN_3O_4$	10	
29a	CH_3	3-$CH_3OC_6H_4CH_2$	154–156	EA	A	68	$C_{21}H_{26}ClN_3O_4 \cdot C_4H_4O_4$[h] $\cdot 0.25H_2O$	10	
30a	CH_3	4-$CH_3C_6H_4CH_2$	79–82	EA	A	89	$C_{21}H_{26}ClN_3O_3$	11	
31a	CH_3	2,4,6-$(CH_3)_3C_6H_2CH_2$	192–194	EA	A	90	$C_{23}H_{30}ClN_3O_3 \cdot 1.5C_4H_4O_4$[h]	5	
32a	CH_3	4-$NO_2C_6H_4CH_2$	97–99	MA	A	68	$C_{20}H_{23}ClN_4O_5$	22	
33a	CH_3	4-$NH_2C_6H_4CH_2$	143–145	AC-W	C	90	$C_{20}H_{25}ClN_4O_3 \cdot 0.5C_4H_4O_4$[h] $\cdot 0.5H_2O$	17	
34a	CH_3	4-$(CO_2H)C_6H_4CH_2$	240–242	EA	D	64	$C_{21}H_{24}ClN_3O_5 \cdot HCl \cdot H_2O$	18	
34b	C_2H_5	4-$(CO_2H)C_6H_4CH_2$	182–184	MA	E	93	$C_{22}H_{26}ClN_3O_5 \cdot 0.75H_2O$	13	
35b	C_2H_5	4-$(CO_2CH_3)C_6H_4CH_2$[19]	173–176	EA	B	50	$C_{23}H_{28}ClN_3O_5 \cdot HCl \cdot 0.5H_2O$	52**	
36a	CH_3	4-$CNC_6H_4CH_2$	163–167	EA	A	75	$C_{21}H_{23}ClN_4O_3 \cdot C_4H_4O_4$[h]	49**	+
36b	C_2H_5	4-$CNC_6H_4CH_2$	170–173	MA	B	53	$C_{22}H_{25}ClN_4O_3$	46**	+
37a	CH_3	3-$CNC_6H_4CH_2$	168–172	EA	A	60	$C_{21}H_{23}ClN_4O_3 \cdot C_2H_2O_4$[j] $\cdot 0.8H_2O$	42**	+++
37b	C_2H_5	3-$CNC_6H_4CH_2$	206–207	EA-W	B	72	$C_{22}H_{25}ClN_4O_3 \cdot C_2H_2O_4$[j]	33**	
38a	CH_3	2-$CNC_6H_4CH_2$	162–165	IA	A	54	$C_{21}H_{23}ClN_4O_3$	43**	+
38b	C_2H_5	2-$CNC_6H_4CH_2$	154–158	IA	B	72	$C_{22}H_{25}ClN_4O_3 \cdot 0.3H_2O$	29*	
39a	CH_3	3,4-$Cl_2C_6H_3CH_2$	89–91	EA	A	59	$C_{20}H_{22}Cl_3N_3O_3 \cdot 0.2H_2O$	16	
40b	C_2H_5	2-NO_2-4-$ClC_6H_3CH_2$	202–205	CH	B	30	$C_{21}H_{24}Cl_2N_4O_5 \cdot 0.1CHCl_3$[k]	47**	
41b	C_2H_5	2,4-$F_2C_6H_3CH_2$	156–157	EA	B	63	$C_{21}H_{24}ClF_2N_3O_3$	76**	
42b	C_2H_5	3,4-$F_2C_6H_3CH_2$	168–170	EA	B	46	$C_{21}H_{24}ClF_2N_3O_3$	46**	
43b	C_2H_5	3,5-$F_2C_6H_3CH_2$	143–145	EA	B	65	$C_{21}H_{24}ClF_2N_3O_3 \cdot 0.25H_2O$	52**	
44a	CH_3	$C_6F_5CH_2$	160–163	T-H	A	76	$C_{20}H_{19}ClF_5N_3O_3$	39**	+
44b	C_2H_5	$C_6F_5CH_2$	156–158	AC-W	B	79	$C_{21}H_{21}ClF_5N_3O_3$	23**	
45a	CH_3	$C_6H_5CH(CH_3)$	171–175	EA	A	90	$C_{21}H_{26}ClN_3O_3 \cdot C_4H_4O_4$[h] $\cdot 0.5H_2O$	42**	+++
45b	C_2H_5	$C_6H_5CH(CH_3)$	135–137	EA-DE	B	72	$C_{22}H_{28}ClN_3O_3 \cdot C_2H_2O_4$[j] $\cdot 0.75H_2O$	44**	+++

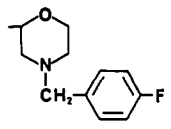
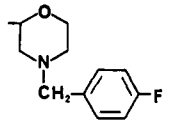
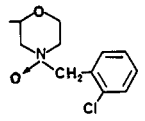
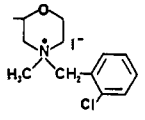
Table I (Continued)

no.	R	R_2[a]	mp, °C	recryst solvent[b]	method[c]	% yield[d]	formula[e]	% GE[f] (2.0 mg/kg po)	acute toxicity[g] (1.0 g/kg po)
46a	CH_3	4-$FC_6H_5CH(CH_3)$[20]	228–231	EA	A	40	$C_{21}H_{25}ClFN_3O_3 \cdot C_2H_2O_4{}^j \cdot 0.25H_2O$	13	
47b	C_2H_5	4-$ClC_6H_5CH(CH_3)$[21]	131–135	IA	B	59	$C_{22}H_{27}Cl_2N_3O_3$	33*	
48a	CH_3	$(C_6H_5)_2CH$	184–186	EA	A	91	$C_{26}H_{28}ClN_3O_3 \cdot 0.25H_2O$	15	
49a	CH_3	$C_6H_5(CH_2)_2$	175–176	IA	A	73	$C_{21}H_{26}ClN_2O_3 \cdot 0.3H_2O$	29*	
50b	C_2H_5	$C_6H_5(CH_2)_3$[22]	138–141	EA	B	74	$C_{23}H_{30}ClN_3O_3 \cdot C_2H_2O_4{}^j \cdot 1.75H_2O$	18*	
51b	C_2H_5	$C_6H_5(CH_2)_4$[23]	168–174	EA	B	71	$C_{24}H_{32}ClN_3O_3 \cdot C_2H_2O_4{}^j \cdot 1.5H_2O$	47**	
metoclopramide								21*	
cisapride								47**	
1a	CH_3	CH_2Ph						39**	++
1b	C_2H_5	CH_2Ph						54**	+

[a] Alkylating agents R_2X are obtained commercially or synthesized via referred literature procedures. [b] Abbreviations for the solvents used are as follows: EA = ethyl alcohol, IA = isopropyl alcohol, MA = methyl alcohol, AC = acetone, W = water, CH = chloroform, T = toluene, H = hexane, DE = diethyl ether. [c] Capital letters refer to the procedures described in the Experimental Section. [d] Yields are based on 2-[(acetylamino)methyl]-4-benzylmorpholine (**2**) except for **33a**, **34a**, and **34b**. [e] All compounds were analyzed for C, H, N, and halogen; analytical results were within ±0.4% of theoretical values. [f] GE = gastric emptying of phenol red semisolid meal in rats. Gastric emptying is expressed as the enhancing percentage based on comparison with the mean value for control groups (0.5% tragacanth). The control values for the gastric emptying with standard error is 50–60%. [g] The symbols show the following meanings: +, slight toxicity (number of dead mouse/number of mouse used: 0–2/10); ++, moderate toxicity (3–6/10); +++, potent toxicity (7–10/10). [h] Fumaric acid. [i] Free base of **23b**: mp 150–151 °C. Anal. ($C_{21}H_{25}ClFN_3O_3$) C, H, N, Cl, F. [j] Oxalic acid. [k] ^1H NMR indicates the presence of crystallization solvent. The asterisks indicate a statistically significant difference from the control group; *, $p < 0.05$; **, $p < 0.01$ (Duncan's multiple range test).

Table II. Physicochemical and Pharmacological Data for 2-Ethoxy-*N*-[(2-morpholinyl)methyl]benzamide Derivatives (**52–55**)



no.	R_3	R_4	mp, °C	recryst solvent[a]	method[b]	% yield[c]	formula[d]	% GE[e] (2.0 mg/kg po)
52b	CH_3CO		161–163	AC	F	51	$C_{23}H_{27}ClFN_3O_4$	74**
53b	HCO		194–197	AC–W	G	40	$C_{22}H_{25}ClFN_3O_4 \cdot 1.75C_4H_4O_4{}^f$	33**
54b	H		154–157	IA–DI	H	14	$C_{21}H_{25}Cl_2N_3O_4 \cdot 1.25H_2O$	52**
55b	H		184–188	MA	I	50	$C_{22}H_{28}Cl_2IN_3O_3 \cdot 0.5H_2O$	34**

[a] See footnote *b* in Table I. DI = diisopropyl ether. [b–f] See footnotes *c–f* and *h*, respectively, in Table I. The asterisks indicate a statistically significant difference from the control group; *, $p < 0.05$; **, $p < 0.01$ (Duncan's multiple range test).

spectra were recorded on a Hitachi 260-10 spectrometer with KBr disks, and electron-impact mass spectra (EIMS) were recorded on a JEOL JMS D-300 or a Hitachi RMU-6L spectrometer. ^1H NMR spectra were taken at 200 MHz with a Varian GEMINI-200 spectrometer. Chemical shifts are expressed as δ (ppm) values with tetramethylsilane as an internal standard. Organic extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. Elemental analyses are given only by symbols of the elements, and analytical results were within ±0.4% of the theoretical values. Merck Kieselgel 60 was used for column chromatography.

4-Substituted 2-(Aminomethyl)morpholines (5). A Typical Procedure. 2-(Aminomethyl)-4-[3-(trifluoromethyl)benzyl]morpholine [5; R_2 = 3-(trifluoromethyl)benzyl]. A solution of 2-[(acetylamino)methyl]-4-benzylmorpholine (**2**;[1] 10.0 g, 0.040 mol) in ethyl alcohol (EtOH, 100 mL) and acetic acid (AcOH, 3 mL) was hydrogenated over 10% palladium-on-carbon (0.5 g) at 60 °C. After the calculated amount of the hydrogen was absorbed, the catalyst was removed by filtration. The filtrate was concentrated to dryness to give ca. 7 g of crude [2-(acetylamino)methyl]morpholine (**3**, AcOH salt) as an oil. A mixture of **3** (7 g), 3-(trifluoromethyl)benzyl chloride (9.1 g, 0.047 mol), potassium carbonate (56 g, 0.41 mol), potassium iodide (1 g), and methyl ethyl ketone (100 mL) was heated to reflux for 17 h and then cooled to room temperature. The insoluble materials were removed by filtration, and the filtrate was concentrated to dryness.

Table III. Gastric Emptying for the Selected 2-Alkoxy-N-[(2-morpholinyl)methyl]benzamides

compd	% phenol red semisolid meal[a] rat (mg/kg po) 0.2	0.5	2.0	mouse (mg/kg po) 0.5	1.0	% resin pellets solid meal in rats[a] (2.0 mg/kg po)
19b	37**	50**	63**	0	21	65**
23b	44**	46**	61**	44**	37**	79**[b]
27b	32**	37**	58**	4	21*	22
36a	0	21*	49**	15	44**	36*
41b	0	42*	76**	83**	89**	76**
52b	33**	22	74**	75**	82**	0
1a[c]	NT[d]	18	39**	41**	59**	16
1b[c]	NT	34**	54**	35*	23	58**

[a] See footnote *f* in Table I. The control values for the gastric emptying of resin pellets solid meal with standard error is 40–50%. [b] The value for the free base of **23b**. [c] See ref 1. [d] NT, not tested. The asterisks indicate a statistically significant difference from the control group; *, $p < 0.05$; **, $p < 0.01$ (Duncan's multiple range test).

Table IV. Anti-Apomorphine Effect of AS-4370, Metoclopramide, and Cisapride in Dogs

	dose, mg/kg po	N[a]	vomiting frequency	ED_{50}, mg/kg (95% CL)[b]
saline		8	5.9 ± 0.7	
AS-4370 (**23b** citrate)	1.0	8	6.4 ± 1.6	>10
	10.0	8	6.3 ± 0.8	
saline		6	9.0 ± 1.3	
metoclopramide	0.2	3	7.7 ± 1.2	0.45
	0.5	3	4.0 ± 0.6*	(0.21–0.94)
	1.0	3	1.3 ± 0.3**	
	2.0	3	0.3 ± 0.3**	
saline		8	5.9 ± 0.7	
cisapride	1.0	2	6.0	>1.0

[a] N, number of dogs. [b] ED_{50} and 95% confidential limits (CL) values were obtained by probit analysis. The asterisks indicate a statistically significant difference from the control group; *, $p < 0.05$; **, $p < 0.01$ (Student's *t* test).

Table V. ^{3}H-Labeled Ligand Binding Profile of AS-4370, Metoclopramide, and Cisapride in Rat Brain Synaptic Membranes

Binding site	IC_{50},[a] μM AS-4370	metoclopramide	cisapride
dopamine D_2	>100	0.63	0.39
serotonin S_1	>100	7.8	4.47
serotonin S_2	>100	13.2	0.0098
adrenaline α_1	>100	32.8	0.11
adrenaline α_2	>10	7.7	>10

[a] The IC_{50} values were obtained by log–logit regression analysis.

The residue was diluted with water and extracted with $CHCl_3$. The extract was washed successively with water and brine and then dried. The solvent was evaporated to give a solid, which was recrystallized from toluene to afford 12.5 g (98%) of 2-[(acetylamino)methyl]-4-[3-(trifluoromethyl)benzyl]morpholine [4; R_2 = 3-(trifluoromethyl)benzyl]: mp 94–95 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (3 H, s, $NCOCH_3$), 3.55 (2 H, s, 3-$CF_3C_6H_4CH_2$), 5.83 (1 H, br s, NH), 7.40–7.62 (4 H, m, arom H); IR 3300 (NH), 1650 ($COCH_3$) cm^{-1}. Anal. ($C_{15}H_{19}F_3N_2O_2$) C, H, N, F.

A solution of 4 (3.2 g, 0.010 mol) in 10% HCl (60 mL) was heated to reflux for 4 h and allowed to cool. The reaction mixture was basified with 10% NaOH and then extracted with $CHCl_3$. The extract was washed successively with water and brine and dried. The solvent was evaporated to give 2.6 g (94%) of 2-(aminomethyl)-4-[3-(trifluoromethyl)benzyl]morpholine [5; R_2 = 3-(trifluoromethyl)benzyl] as an oil: EIMS *m/z* 274 (M^+).

Methyl 4-(Acetylamino)-2-ethoxybenzoate (7). A mixture of methyl 4-(acetylamino)-2-hydroxybenzoate (6;[10] 20.8 g, 0.10 mol), ethyl iodide (23.4 g, 0.15 mol), potassium carbonate (41.7 g, 0.30 mol), and *N*,*N*-dimethylformamide (DMF, 60 mL) was vigorously stirred at 50 °C for 6 h. The reaction mixture was poured into ice–water, and the resulting precipitates were collected by filtration, washed with water, and recrystallized from toluene–hexane to afford 22.8 g (97%) of 7: mp 151–153 °C; IR 3240 (NH), 1690, 1660 ($COOCH_3$, $NCOCH_3$) cm^{-1}. Anal. ($C_{12}H_{15}NO_4$) C, H, N.

Methyl 4-(Acetylamino)-5-chloro-2-ethoxybenzoate (8). A mixture of 7 (53.0 g, 0.22 mol), *N*-chlorosuccinimide (31.4 g, 0.24 mol), and DMF (150 mL) was stirred at 70 °C for 2 h. The reaction mixture was poured into ice–water, and the resulting precipitates were collected by filtration, washed with water, and recrystallized from methyl alcohol (MeOH) to afford 58.8 g (97%) of 8: mp 142–145 °C; IR 3250 (NH), 1690, 1660 ($COOCH_3$, $NCOCH_3$) cm^{-1}. Anal. ($C_{12}H_{14}ClNO_4$) C, H, N, Cl.

4-(Acetylamino)-5-chloro-2-ethoxybenzoic Acid (9). A mixture of 8 (54.4 g, 0.20 mol), NaOH (8.8 g, 0.22 mol), H_2O (150 mL), and MeOH (80 mL) was stirred at 60 °C for 1 h and then cooled to room temperature. After removal of the MeOH, the aqueous solution was acidified with concentrated HCl. The resulting precipitates were collected by filtration, washed with water, and recrystallized from EtOH to afford 37.7 g (73%) of 9: mp 169–171 °C; ^{1}H NMR ($(CH_3)_2SO$-d_6) δ 1.32 (3 H, t, *J* = 7 Hz, OCH_2CH_3), 2.16 (3 H, s, $COCH_3$), 4.06 (2 H, q, *J* = 7 Hz, OCH_2CH_3), 7.72 (1 H, s, arom H), 7.77 (1 H, s, arom H), 9.55 (1 H, s, NH), 12.70 (1 H, br s, COOH); EIMS *m/z* 257 (M^+); IR 3360 (NH), 1725, 1665 (COOH, $NCOCH_3$) cm^{-1}. Anal. ($C_{11}H_{12}ClNO_4$) C, H, N, Cl.

4-Amino-5-chloro-2-ethoxybenzoic Acid (10b). A mixture of 8 (10.0 g, 0.037 mol), NaOH (4.4 g, 0.11 mol), H_2O (20 mL), and EtOH (10 mL) was heated to reflux for 5 h and then cooled to ca. 0 °C. The reaction mixture was acidified with concentrated HCl. The resulting precipitates were collected by filtration, washed with water, and recrystallized from MeOH to afford 7.9 g (99%) of **10b**: mp 167–168 °C; ^{1}H NMR ($(CH_3)_2SO$-d_6) δ 1.32 (3 H, t, *J* = 7 Hz), OCH_2CH_3), 3.99 (2 H, q, *J* = 7 Hz, OCH_2CH_3), 6.02 (2 H, s, NH_2), 6.45 (1 H, s, arom H), 7.58 (1 H, s, arom H), 11.82 (1 H, br s, COOH); EIMS *m/z* 215 (M^+); IR 3500, 3330 (NH_2), 1700 (COOH) cm^{-1}. Anal. ($C_9H_{10}ClNO_3$) C, H, N, Cl.

Methyl 4-Amino-5-chloro-2-ethoxybenzoate (11). To a stirred suspension of **10b** (12.0 g, 0.056 mol) in MeOH (150 mL) was added dropwise thionyl chloride (15 mL, 0.21 mol) during a period of 0.5 h at 0 °C. The mixture was stirred at the same temperature for 1 h and then at room temperature for 3 h. After removal of the solvent, water was added to the residue. The insoluble materials were collected by filtration, washed with water, and recrystallized from isopropyl alcohol (*i*-PrOH)–diisopropyl ether (*i*-Pr_2O) to give 11.7 g (91%) of 11: mp 116–117 °C; IR 3440, 3340 (NH_2), 1680 ($COOCH_3$) cm^{-1}. Anal. ($C_{10}H_{12}ClNO_3$) C, H, N, Cl.

Methyl 5-Chloro-2-ethoxy-4-(formylamino)benzoate (12). Formic acid (39.3 g, 0.85 mol) was added dropwise to acetic anhydride (22.7 g, 0.22 mol) at room temperature. The mixture was stirred at 50 °C for 1 h, and 11 (10.0 g, 0.044 mol) was added to the solution. The mixture was stirred at 50 °C for 2.5 h and was poured into ice–water. The resulting precipitates were collected by filtration, washed with water, and recrystallized from *i*-PrOH–*i*-Pr_2O to afford 10.5 g (94%) of 12: mp 132–134 °C; IR 3320 (NH), 1715, 1690 (CHO, $COOCH_3$) cm^{-1}. Anal. ($C_{11}H_{12}ClNO_4$) C, H, N, Cl.

5-Chloro-2-ethoxy-4-(formylamino)benzoic Acid (13). In a manner similar to that described for **9**, compound **12** (5.0 g, 0.021 mol) was treated with a solution of NaOH (0.85 g, 0.021 mol) in H_2O (20 mL) and EtOH (30 mL) and then worked up, giving 3.9 g (82%) of 13: mp 184–188 °C (EtOH–diethyl ether); ^{1}H NMR

($(CH_3)_2SO$-d_6) δ 1.32 (3 H, t, J = 7 Hz, OCH_2CH_3), 3.97 (2 H, q, J = 7 Hz, OCH_2CH_3), 7.74 (1 H, s, arom H), 8.11 (1 H, s, arom H), 8.43 (1 H, s, NHCHO), 10.08 (1 H, s, CHO), 12.58 (1 H, br s, COOH); EIMS m/z 243 (M^+); IR 3450, 3350 (NH_2), 1690 (CHO, COOH) cm^{-1}. Anal. ($C_{10}H_{10}ClNO_4$) C, H, N, Cl.

2-Alkoxy-4-amino-5-chloro-*N*-[(4-substituted-2-morpholinyl)methyl]benzamides and Their Related Compounds (19–55, Tables I and II). General Procedures.

Method A. To a solution of an appropriate amine **5** (9.5 mmol) in CH_2Cl_2 (50 mL) were added 4-amino-5-chloro-2-methoxybenzoic acid (**10a**;[11] 11 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (11 mmol). The mixture was stirred at room temperature for 4 h and then washed successively with water, 10% NaOH, and brine. The mixture was dried and concentrated to dryness. The resulting residue was chromatographed on silica gel with $CHCl_3$–MeOH (9:1) to give the crude product, which was purified by crystallization from the solvent given in Table I or converted to the fumarate or oxalate in the usual manner, followed by recrystallization.

Method B. 4-Amino-5-chloro-2-ethoxybenzoic acid (**10b**) was used as a starting material, instead of **10a** in method A.

Method C. 4-Amino-*N*-[[4-(4-aminobenzyl)-2-morpholinyl]methyl]-5-chloro-2-methoxybenzamide (33a). A solution of **32a** (2.5 g, 0.0058 mol) in MeOH (100 mL) was hydrogenated over 5% palladium-on-carbon (0.5 g) at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated to dryness to give 2.1 g (90%) of **33a** as an oil, which was converted to the fumarate in the usual manner.

Method D. 4-Amino-*N*-[[4-(4-carboxybenzyl)-2-morpholinyl]methyl]-5-chloro-2-methoxybenzamide (34a). To a stirred solution of *N*-[(4-benzyl-2-morpholinyl)methyl]-phthalimide (14;[1] 30.0 g, 0.089 mol) in toluene (200 mL) was added dropwise ethyl chloroformate (19.4 g, 0.18 mol) at 60 °C. The mixture was heated to reflux for 1 h and then cooled to room temperature. The reaction mixture was washed successively with water and brine and dried. The solvent was evaporated to give a solid, which was recrystallized from toluene–hexane to give 27.8 g (98%) of *N*-[[4-(ethoxycarbonyl)-2-morpholinyl]methyl]-phthalimide (**15**): mp 117–118 °C; IR 1770, 1710, 1700 ($C_6H_4(CO)_2N$, $NCO_2C_2H_5$) cm^{-1}. Anal. ($C_{16}H_{18}N_2O_5$) C, H, N.

A mixture of **15** (10.0 g, 0.031 mol), 85% hydrazine monohydrate (2.9 g, 0.049 mol), and EtOH (10 mL) was heated to reflux for 10 min and then cooled to room temperature. The reaction mixture was diluted with $CHCl_3$ (200 mL). The insoluble materials were removed by filtration, and the filtrate was washed with a small amount of water and then brine and dried. The solvent was evaporated to give 4.8 g of crude 2-(aminomethyl)-4-(ethoxycarbonyl)morpholine (**16**) as an oil. To a solution of crude **16** (4.8 g) in CH_2Cl_2 (100 mL) were added **10a** (5.0 g, 0.025 mol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (5.2 g, 0.027 mol) at room temperature. The resulting mixture was stirred at room temperature for 4 h, then washed successively with water, 10% NaOH and brine, dried, and concentrated to dryness. The residue was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (9:1) to give 7.5 g (64%) of 4-amino-5-chloro-*N*-[[4-(ethoxycarbonyl)-2-morpholinyl]methyl]-2-methoxybenzamide (**17**) as an oil. This oil was converted to the oxalate in the usual manner: mp 145–151 °C (EtOH–diethyl ether); EIMS m/z 371 (M^+). Anal. ($C_{16}H_{22}ClN_3O_5 \cdot 0.5C_2H_2O_4$) C, H, N, Cl. A mixture of **17** (6.1 g, 0.016 mol), KOH (10.1 g, 0.18 mol), and *i*-PrOH (60 mL) was heated to reflux for 3 h. The reaction mixture was concentrated to dryness. The residue was diluted with water and extracted with $CHCl_3$. The extract was washed successively with water and brine and dried. The solvent was evaporated to give a solid, which was recrystallized from *i*-PrOH to afford 1.4 g (28%) of **18**: mp 161–162 °C. Anal. ($C_{13}H_{18}ClN_3O_3$) C, H, N, Cl.

A mixture of **18** (3.8 g, 0.013 mol), 4-(bromomethyl)benzoic acid (2.7 g, 0.013 mol), potassium carbonate (17.7 g, 0.13 mol), potassium iodide (1 g), methyl ethyl ketone (80 mL), and DMF (20 mL) was heated to reflux for 21 h. The solvent was evaporated, and the residue was dissolved in water and then washed with $CHCl_3$. The aqueous layer was acidified with concentrated HCl. The resulting precipitates were collected by filtration, washed with water, and recrystallized from EtOH to give 3.5 g (64%) of **34a**.

Method E. 4-Amino-*N*-[[4-(4-carboxybenzyl)-2-morpholinyl]methyl]-5-chloro-2-ethoxybenzamide (34b). A mixture of **35b** (2.6 g, 0.0051 mol), NaOH (0.63 g, 0.016 mol), water (200 mL), and EtOH (80 mL) was heated to reflux for 2 h and then cooled to room temperature. The EtOH was evaporated, and the aqueous solution was acidified with concentrated HCl. The resulting precipitates were collected by filtration, washed with water, and recrystallized from MeOH to afford 2.2 g (93%) of **34b**.

Method F. 4-(Acetylamino)-5-chloro-2-ethoxybenzoic acid (**9**) was used as a starting material, instead of **10a** in method A.

Method G. 5-Chloro-2-ethoxy-4-(formylamino)benzoic acid (**13**) was used as a starting material, instead of **10a** in method A.

Method H. 4-Amino-5-chloro-*N*-[[4-(2-chlorobenzyl)-2-morpholinyl]methyl]-2-ethoxybenzamide *N*-Oxide (54b). To a stirred solution of the free base of **21b** (4.0 g, 0.0091 mol) in MeOH (150 mL) was added 30% hydrogen peroxide (H_2O_2, 1.2 g, 0.011 mol). After the mixture was heated to reflux for 8 h, an additional 30% H_2O_2 (1.2 g) was added, and the reaction mixture was heated to reflux for an additional 24 h. The solvent was evaporated, and $CHCl_3$ and water were added to the residue. The mixture was stirred for 0.5 h at room temperature. The insoluble materials were collected by filtration and recrystallized from *i*-PrOH-*i*-Pr_2O to give 0.6 g (14%) of **54b**.

Method I. 2-[(4-Amino-5-chloro-2-ethoxybenzoyl)aminomethyl]-4-(2-chlorobenzyl)-4-methylmorpholinium Iodide (55b). To a solution of the free base of **21b** (2.4 g, 0.0055 mol) in MeOH (100 mL) was added methyl iodide (4.0 g, 0.028 mol). The mixture was stirred at room temperature for 24 h. A proper amount of charcoal was added to the reaction mixture. The mixture was heated for 10 min and filtered to remove the charcoal, and the filtrate was concentrated to about 10 mL. The resulting precipitates were collected by filtration and recrystallized from MeOH to afford 1.6 g (50%) of **55b**.

Reference Compounds. Cisapride was prepared according to the literature.[4] Metoclopramide was purchased from Sigma Chemical Co.

Pharmacology. Gastric emptying of semisolid meal and solid meal and effect on apomorphine-induced emesis were tested by the methods reported in the previous paper.[1] The number of animals used was five for each dose of the control, metoclopramide, and cisapride, and four for each dose of the other test compounds.

Acute Toxicity. Male ddY mice, weighing 18–25 g, were used in groups of 10 animals each. The test compounds, dissolved or suspended in a 0.5% tragacanth solution, were administered at an oral dose of 1.0 g/kg to the animals. The mortality was observed for 7 days after the administration.

Radioligand Binding Assay. The test compounds at the concentrations of 0.01–1000 μM were tested in binding assays using rat brain synaptic membranes for competition with the following ligands at their respective binding sites: dopamine D_2, [^{3}H]-spiperone in the striatum;[15] serotonin S_1, [^{3}H]serotonin in the frontal cortex;[16] serotonin S_2, [^{3}H]spiperone in the frontal cortex;[17] adrenaline α_1, [^{3}H]WB-4101 in the whole brain;[18] and adrenaline α_2, [^{3}H]clonidine in the whole brain.[18] Each assay was started by an addition of tissue preparations (10 mg wet tissue) and terminated by rapid filtration through Whatman GF/B glass-fiber filters under the reduced pressure. The filters were washed two or three times with 5 mL of ice-cold buffer and transferred to scintillation vials that contained 1 mL of Soluene-350. After 1 h of incubation at 25 °C, the solubilized filters were shaken

(15) Creese, I.; Schneider, R.; Snyder, S. H. *Eur. J. Pharmacol.* **1977**, *46*, 377.
(16) Peroutka, S. J.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 687.
(17) Creese, I.; Snyder, S. H. *Eur. J. Pharmacol.* **1978**, *49*, 201.
(18) U'Prichard, D. C.; Greenberg, D. A.; Snyder, S. H. *Mol. Pharmacol.* **1977**, *13*, 454.
(19) Davies, W.; Perkin, jun., W. H. *J. Chem. Soc.* **1922**, 2202.
(20) Protiva, M.; Navak, L.; Sedivy, Z. *Collect. Czech. Chem. Commun.* **1962**, *27*, 2102.
(21) Kuchar, M.; Brunova, B.; Rejholec, V.; Roubal, Z. *Collect. Czech. Chem. Commun.* **1976**, *41*, 633.
(22) Huston, R. C.; Sager, D. D. *J. Am. Chem. Soc.* **1926**, *48*, 1955.
(23) Baddeley, G.; Williamson, R. *J. Chem. Soc.* **1956**, 4647.

vigorously with 10 mL of toluene scintillator, and the radioactivity in the filters was counted with a Packard Tris-Carb scintillation counter (B-2450). IC_{50} values of the test compounds (the concentrations causing 50% inhibition of ^3H-labeled ligand specific binding) were determined by probit analysis.

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Registry No. **1a**, 112885-23-1; **1b**, 112885-33-3; **2**, 112913-96-9; **3**·AcOH, 131322-23-1; **4** (R_2 = 3-$CF_3C_6H_4CH_2$), 112887-04-4; **5** (R_2 = 3-$CF_3C_6H_4CH_2$), 131322-24-2; **6**, 4093-28-1; **7**, 59-06-3; **8**, 4235-43-2; **9**, 112914-09-7; **10a**, 7206-70-4; **10b**, 108282-38-8; **11**, 112914-03-1; **12**, 131322-25-3; **13**, 131322-26-4; **14**, 110859-48-8; **15**, 112887-44-2; **16**, 112914-11-1; **17**, 112885-36-6; **18**, 112914-02-0;)19a, 131322-27-5; **19a**·$2C_4H_4O_4$, 131322-36-6; **19b**, 112886-52-9; **20b**, 131322-28-6; **20b**·HCl, 112886-53-0; **21b**, 112886-55-2; **21b**·HCl, 112886-54-1; **22a**, 112885-14-0; **22a**·$C_4H_4O_4$, 112885-15-1; **23a**, 112885-17-3; **23b**, 112885-41-3; **23b**·1.25HCl, 131322-37-7; **24a**, 112885-18-4; **24a**·$2C_4H_4O_4$, 112885-19-5; **24b**, 112886-56-3; **24b**·$C_4H_4O_4$, 112886-57-4; **25a**, 112885-20-8; **25a**·$0.5C_4H_4O_4$, 112885-21-9; **25b**, 112886-58-5; **25b**·$2C_4H_4O_4$, 112886-59-6; **26a**, 112885-05-9; **26a**·$1.5C_4H_4O_4$, 112885-06-0; **27a**, 112885-07-1; **27a**·$1.5C_4H_4O_4$, 112885-08-2; **27b**, 112886-49-4; **28a**, 112885-02-6; **29a**, 112885-03-7; **20a**·$C_4H_4O_4$, 112885-04-8; **30a**, 112885-11-7; **31a**, 112885-09-3; **31a**·$1.5C_4H_4O_4$, 112885-10-6; **32a**, 112884-98-7; **33a**, 112884-97-6; **33a**·$0.5C_4H_4O_4$, 131322-38-8; **34a**, 131322-29-7; **34a**·HCl, 131322-39-9; **34b**, 131322-35-5; **35b**, 131322-30-0; **35b**, 131322-30-0; **35b**·HCl, 131322-40-2; **36a**, 112885-32-2; **36a**·$C_4H_4O_4$, 112885-35-5; **36b**, 112886-47-2; **37a**, 112884-99-8; **37a**·$C_2H_2O_4$, 112885-00-4; **37b**, 112886-45-0; **37b**·$C_2H_2O_4$, 112886-46-1; **38a**, 112885-01-5; **38b**, 112886-48-3; **39a**, 112885-13-9; **40b**, 112886-44-9; **41b**, 131322-31-1; **42b**, 131322-32-2; **43b**, 131322-33-3; **44a**, 112885-12-8; **44b**, 112886-50-7; **45a**, 112884-94-3; **45a**·$C_4H_4O_4$, 112884-95-4; **45b**, 112886-38-1; **45b**·$C_2H_2O_4$, 112886-39-2; **46a**, 112884-92-1; **46a**·$C_2H_2O_4$, 112884-93-2; **47b**, 112886-37-0; **48a**, 112913-72-1; **49a**, 112884-96-5; **50b**, 112886-42-7; **50b**·$C_2H_2O_4$, 112886-43-8; **51b**, 112886-40-5; **51b**·$C_2H_2O_4$, 112886-41-6; **52b**, 112885-34-4; **53b**, 131322-34-4; **53b**·$1.75C_4H_4O_4$, 131322-41-3; **54b**, 112914-14-4; **55b**, 112914-15-5.

Structure-Activity Relationship of Quinazolinedione Inhibitors of Calcium-Independent Phosphodiesterase

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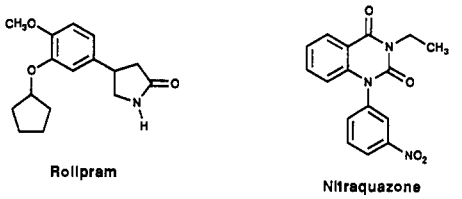
A series of quinazolinediones and azaquinazolinediones is described which possess potent inhibitory activity toward the calcium-independent phosphodiesterase enzyme (CaIPDE). In vivo testing showed that this in vitro activity translates to animal models predictive of chronic diseases such as depression and inflammation. These results support the hypothesis that inhibition of CaIPDE may lead to useful activity in such chronic diseases.

The phosphodiesterase (PDE) enzymes have been classified into four groups based on selective inhibition by a number of structurally diverse compounds.[1] We have studied the calcium-independent, low K_m, cAMP-selective PDE (CaIPDE) which is selectively inhibited by the antidepressant drug rolipram[2] (Chart I) as a target for novel antidepressant agents,[3] and in this context, we sought new structures to complement our work. Our attention was thus attracted to a report[4] describing the drug nitraquazone, coded TVX 2706, which was characterized as a phosphodiesterase (PDE) inhibitor with antiinflammatory activity in vivo. Despite its marked structural dissimilarity to rolipram, nitraquazone proved to be both a selective CaIPDE inhibitor and a potent binder to the [^3H]rolipram binding site.[5] Our attempts to understand the structural basis for the inhibition of CaIPDE by both nitraquazone and rolipram led us to synthesize an exceptionally potent CaIPDE inhibitor, CP-77,059 (**3e**). The discovery of **3e** and its biological characterization are chronicled in this paper.

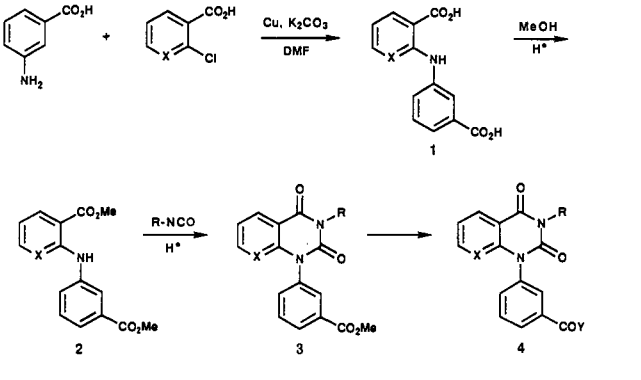
Chart I



Chemistry

The synthetic methodology developed for the analogues of nitraquazone (Table III) is shown in Scheme I; it is based on literature precedent for the preparation of the quinazolinedione ring system by the reaction of an anthranilate derivative with isocyanate under acid catalysis.[6]

Scheme I. Preparation of Quinazolinediones and Pyridopyrimidinediones



(1) Reeves, M. L.; Leigh, B. K.; England, P. J. *Biochem. J.* 1987, *241*, 535.
(2) Eckmann, F.; Fichte, K.; Meya, U.; Sastre-y-Hernandez, M. *Curr. Ther. Res.* 1988, *43*, 291.
(3) Koe, B. K.; Lebel, L. A.; Nielsen, J. A.; Russo, L. L.; Saccomano, N. A.; Vinick, F. J.; Williams, I. H. *Drug. Dev. Res.*, in press.
(4) Glaser, T.; Traber, J. *Agents Actions* 1984, *15*, 341.
(5) Russo, L. L.; Lebel, L. A.; Koe, B. K. *Soc. Neurosci. Abstr.* 1987, *13*, 903.

0022-2623/91/1834-0624$02.50/0