A mixture of 8.1 g of **5**, 150 mL of 3 N HCl, and 80 mL of *n*-BuOH was refluxed for 3 h. It was concentrated in vacuo to give a yellow solid, which was recrystallized from EtOH to a white solid (5.3 g, 83%), mp 230–231 °C. Anal. ($C_{11}H_{13}NO$·HCl) C, H, N.

2-Acetyl-7-chloro-8-[(1,1,2,2-tetrafluoroethyl)thio]-1,2,3,4-tetrahydroisoquinoline (7). A glass pressure bomb was charged with 4.5 g (1.86 mmol) of 2-acetyl-7-chloro-8-mercapto-1,2,3,4-tetrahydroisoquinoline, 1.56 g (3.72 mmol) of Triton B, and 35 mL of dry DMF. The solution was magnetically stirred while it was flushed twice with tetrafluoroethylene to 20 psi, charged at 20 psi, and sealed. The pressure bottle was recharged each hour until the pressure stabilized at 20 psi and was then stirred for 16 h. The reaction mixture was poured into 80 mL of cold H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, 5% Na_2CO_3, and then with H_2O, dried (K_2CO_3), and concentrated. The residual oil was crystallized from CCl_4–hexane and then from toluene–hexane to give 4.7 g (74%) of **7**: mp 79–94 °C; TLC (silica, 40% CH_2Cl_2–ether) single spot.

7-Chloro-8-[(1,1,2,2-tetrafluoroethyl)thio]-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8). A mixture of 2.2 g (6.4 mmol) of **7** and 25 mL of concentrated HCl was refluxed at 135–140 °C for 5 h. The reaction mixture was concentrated in vacuo, and the residue was recrystallized from EtOH–ether with decolorizing carbon to give 1.8 g (83%) of **8**, mp 234 °C. Anal. ($C_{11}H_{10}ClF_4N_3$·HCl) C, H, N.

7,8-Dichloro-3,4-dihydroisoquinoline (9). To a stirred solution of 62.6 g (0.233 mol) of Fremy's salt in 1-L of 5% Na_2CO_3 was added portionwise 21.9 g (0.092 mol) of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline hydrochloride.[11] The reaction mixture was stirred at 25 °C for 24 h. It was extracted with 3 × 250 mL of CH_2Cl_2, which was washed with a saturated salt solution, dried, and concentrated. The orange residual oil was crystallized upon standing and recrystallized twice from EtOAc to give 9.6 g (52%) of **9**, mp 55–57 °C.

7,8-Dichloro-1-methyl-1,2,3,4-tetrahydroisoquinoline (10, R = Methyl). To flame-dried magnesium turnings (3.7 g, 0.15 g-atom) was added 40 mL of anhydrous Et_2O. A solution of 24.8 g (0.175 mol) of methyl iodide in 125 mL of ether was added dropwise at a rate sufficient to maintain a gentle reflux. After the addition was completed, the Grignard solution was cooled to 25 °C, and a solution of 10.54 g (0.053 mol) of **9** in 40 mL of dry toluene was added dropwise. It was refluxed for 2 h. The reaction mixture was decomposed **cautiously** by adding 90 mL of H_2O and was filtered through Celite, and the residue was washed with EtOAc. The combined organic–EtOAc filtrates were washed with a saturated salt solution, dried, and concentrated. The residual oil was purified by Kughelrohr distillation to give 8.6 g (75%) of **10** (R = methyl).

Biological Test Procedures. Sections of ileum, proximal to Peyer's patch, are resected from male, albino, Hartley strain, guinea pigs (400–600 g) and placed in 5-mL tissue baths containing modified Tyrode's solution (37.5 °C) of the following composition (mM): NaCl, 137; KCl, 3.4; $CaCl_2$, 1.3; $MgCl_2$, 0.10; NaH_2PO_4, 11.9; atropine, 0.000738; pyrilamine, 0.00249; glucose, 5. In experiments using carbachol and histamine as agonists, atropine and pyrilamine, respectively, are omitted from the Tyrode's solutions. One end of the tissue is fixed to a glass tissue holder and the other is connected to a Grass force-displacement transducer, and the tissue is placed under a tension of 500 mg. Isometric tissue contractions are recorded on a six-channel polygraph. Baths are constantly aerated with 95% O_2–5% CO_2. After a 20-min "stabilization" period, a concentration of the appropriate agonist that provides a contraction height of 60–80% of the maximum obtainable to that agonist (as determined from full sequential concentration–response curves in separate experiments) is added to the tissue bath, and the response is recorded. The procedure is repeated until reproducible responses are obtained. For most agonists, two applications in rapid succession, followed 15 min later by a third, is sufficient to establish reproducibility. Experimental tissues are incubated with the concentration of the test compound indicated in the tables for 15 min. Experimental and control tissues are subjected to five bath changes during the incubation interval. Changes in bath fluid during the incubation period are helpful in ensuring the reproducibility of tissue responses to the agonist. Control tissues are incubated with test compound vehicle (if any). The same concentration of the agonist is reapplied in the presence of the test compounds, and the response is registered and compared with controls. Percent inhibition produced by the test compound is calculated by subtracting the mean percentage change in control tissue from the mean percentage change in tissues exposed to the test compound. Additional compounds are then evaluated as long as the tissue remains reproducibly responsive to the agonist. Six tissues obtained from six animals are used simultaneously—three controls and three experimental. Partially purified guinea pig SRS-A was prepared and purified as described.[17] Compound **1** was used as a reference for each compound tested.

(17) D. M. Engineer, V. Niederhauser, P. J. Piper, and P. Sirois, *Br. J. Pharmacol.*, **62**, 61 (1978); H. R. Morris, G. W. Taylor, P. J. Piper, P. Sirois, and J. R. Tipping, *FEBS Lett.*, 87, 203 (1978); M. O'Donnel, K. Falcone, and R. D. Krell, *Prostaglandins*, submitted.

Synthesis and Spasmolytic Activities of 2-(1,2-Benzisoxazol-3-yl)-3-[[ω-(dialkylamino)alkoxy]phenyl]acrylonitriles[1]

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Several 2-(1,2-benzisoxazol-3-yl)-3-[[ω-(dialkylamino)alkoxy]phenyl]acrylonitrile derivatives were synthesized and screened for potential spasmolytic activity. The effect of structural variation of these molecules on biological activities was systematically examined. Among these compounds, (*Z*)-2-(1,2-benzisoxazol-3-yl)-3-[2-(2-piperidinoethoxy)-phenyl]acrylonitrile (**1d**), (*Z*)-2-(1,2-benzisoxazol-3-yl)-3-[2-(2-morpholinoethoxy)phenyl]acrylonitrile (**1f**), and their analogues (**3c,d**) having a methoxy substituent at C_5 of the benzoisoxazole ring showed potent antispasmodic activities in the in vitro and in vivo studies.

It has been suggested that an agent showing inhibitory action on the release of acetylcholine from the vagus nerve has antispasmodic potency comparable to antimuscarinic agents on the gastrointestinal system in experimental animals, and such an agent may be attractive for clinical use as a spasmolytic.[2] We have tried to find a novel type of spasmolytic agent having potent antispasmodic activity

(1) Part 8 in the series "Studies on 3-Substituted 1,2-Benzisoxazole Derivatives". For Part 7, see H. Uno and M. Kurokawa, *Chem. Pharm. Bull.*, **30**, 333 (1982).

(2) I. Takayanagi, K. Nakazo, and Y. Kizawa, *Jpn. J. Pharmacol.*, **30**, 647 (1980).

0022-2623/82/1825-1240$01.25/0

Scheme I

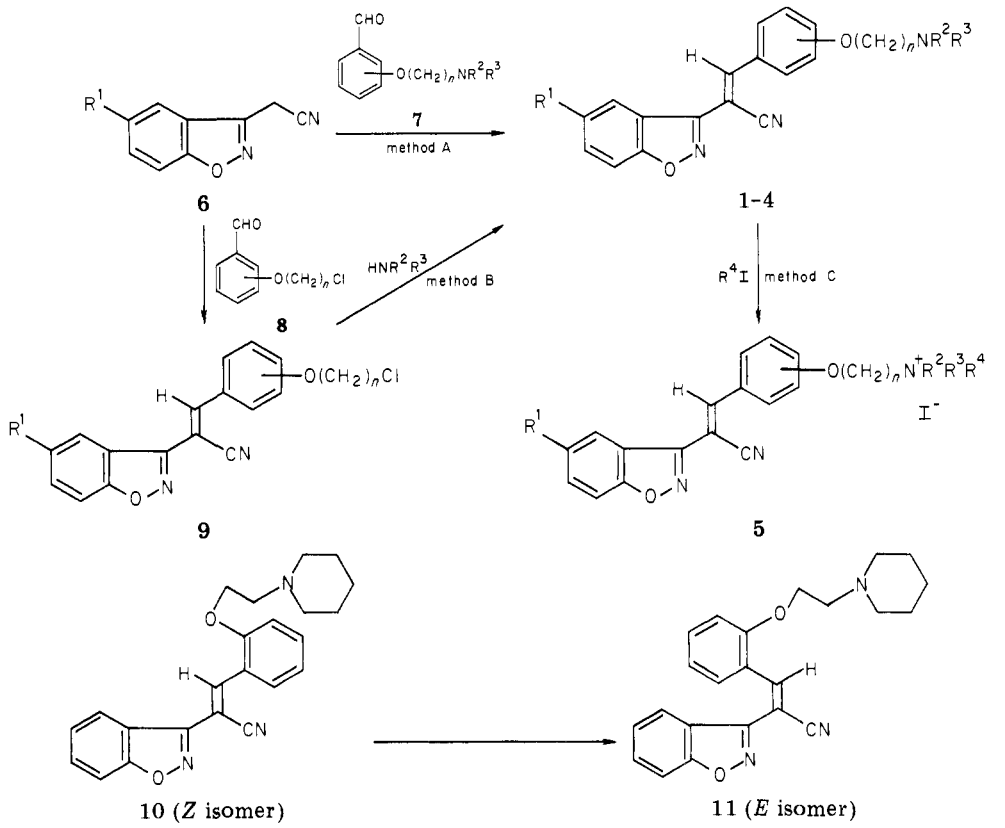
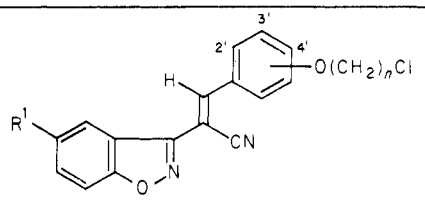


Table I. (Z)-2-(1,2-Benzisoxazol-3-yl)-3-[(ω-chloroalkoxy)phenyl]acrylonitriles



no.	R^1	position	n	yield, %	mp, °C	crystn solvent	formula[a]
9a	H	2'	2	77	168-170	benzene	$C_{18}H_{13}ClN_2O_2$
9b	H	3'	3	45	98-100	ether-hexane	$C_{19}H_{15}ClN_2O_2$
9c	H	4'	3	69	130-131	benzene	$C_{19}H_{15}ClN_2O_2$
9d	Cl	2'	2	85	144-146	toluene-ether	$C_{18}H_{12}Cl_2N_2O_2$
9e	Cl	2'	3	72	118-120	toluene-ether	$C_{19}H_{14}Cl_2N_2O_2$
9f	Cl	4'	2	77	181-183	toluene	$C_{18}H_{12}Cl_2N_2O_2$
9g	OCH_3	2'	2	76	129-130	toluene-ether	$C_{19}H_{15}ClN_2O_3$
9h	OCH_3	4'	2	81	161-163	toluene-ether	$C_{19}H_{15}ClN_2O_3$
9i	OCH_3	2'	3	75	73-75	toluene-hexane	$C_{20}H_{17}ClN_2O_3$
9j	OH	2'	2	72	183-185	ethyl acetate-toluene	$C_{18}H_{13}ClN_2O_3$

[a] Analyses for C, H, Cl, and N were within ±0.4% of the theoretical values.

without an antimuscarinic effect. During the course of such a search, (Z)-2-(1,2-benzisoxazol-3-yl)-3-[2-[2-(dimethylamino)ethoxy]phenyl]acrylonitrile (**1a**) showed a marked suppressive activity on the response of isolated ileum from guinea pig to transmural electrical stimulation and a little inhibitory effect on the response to acetylcholine. This paper deals with the synthesis of several derivatives of **1a** and the result of their primary pharmacological evaluation.

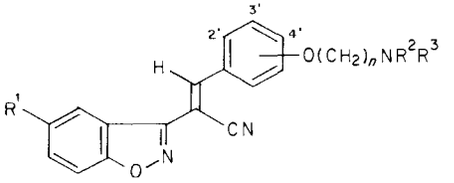
Chemistry. The title compounds (**1–5**) were prepared by the methods shown in Scheme I. The Knoevenagel condensation of 1,2-benzisoxazole-3-acetonitriles[3] (**6**) with appropriate ω-(dialkylamino)alkoxybenzaldehydes (**7**) gave the target compounds (**1–4**) (method A). On the other hand, condensation of **6** with ω-chloro aldehydes (**8**) afforded the ω-chloro derivatives (**9**) shown in Table I. Amination of **9** with appropriate secondary amines gave **1–4** (method B). These condensations gave predominantly the products (**1–4**, **9**) having the Z configuration,[4] as in the case of the reaction of phenylacetonitrile with benzaldehyde.[5] Stereochemical assignment was confirmed as follows. A photoirradiation of a free base of **1d** (**10**, Z isomer) afforded an E isomer (**11**). Absorption spectra of **11** showing hypsochromic shifts in comparison with those

(3) H. Uno, M. Kurokawa, K. Natsuka, Y. Yamato, and H. Nishimura, *Chem. Pharm. Bull.*, **24**, 632 (1976).

(4) J. E. Blackwood, C. L. Gladys, K. L. Loening, A. E. Petrarca, and J. E. Rush, *J. Am. Chem. Soc.*, **90**, 509 (1968).

(5) J. F. Codington and E. Mosettig, *J. Org. Chem.*, 17, 1027 (1952).

Table II. Chemical and Pharmacological Data of (*Z*)-2-(1,2-Benzisoxazol-3-yl)-3-[[ω-(dialkylamino)alkoxy]phenyl]acrylonitriles



no.	R^1	position	n	NR^2R^3	yield, % (synth method)	mp, °C	crystn solvent[a]	formula[b]	anti-TMS:[c] ID_{50}, mM	charcoal meal test:[d] % inhibn
1a	H	2′	2	$N(CH_3)_2$	74 (A)	218-220	M	$C_{20}H_{19}N_3O_2 \cdot HCl \cdot 0.33H_2O$	1.4×10^{-4}	37
1b	H	2′	2	$N(C_2H_5)_2$	43 (A)	170-174	A-H	$C_{22}H_{23}N_3O_2 \cdot HCl$	3.0×10^{-4}	20
1c	H	2′	2	c-NC_4H_8	37 (A)	225-230	M-E	$C_{22}H_{21}N_3O_2 \cdot HCl$	7.1×10^{-5}	22
1d	H	2′	2	c-NC_5H_{10}	60 (B)	197-199	M-A	$C_{23}H_{23}N_3O_2 \cdot HCl \cdot 0.33H_2O$	2.4×10^{-4}	45[e]
1e	H	2′	2	c-NC_6H_{12}	30 (B)	159-161	IP	$C_{24}H_{25}N_3O_2 \cdot HBr$	6.8×10^{-5}	27
1f	H	2′	2	c-$N(CH_2CH_2)_2O$	70 (A)	193-195	M	$C_{22}H_{21}N_3O_3 \cdot HCl \cdot 0.75H_2O$	1.3×10^{-5}	55[e]
1g	H	2′	2	c-$N(CH_2CH_2)_2N$-CH_3	30 (B)	245-255	M	$C_{23}H_{24}N_4O_2 \cdot 2HCl$	1.2×10^{-3}	
1h	H	2′	2	c-$N(CH_2CH_2)_2N$-C_6H_5	14 (B)	165-167	A	$C_{28}H_{26}N_4O_2$	1.1×10^{-2}	
1i	H	2′	2	c-$N(CH_2CH_2)_2N$-$CH_2C_6H_5$	24 (B)	213-222	A	$C_{29}H_{28}N_4O_2 \cdot 2HCl$	4.7×10^{-3}	
1j	H	2′	3	$N(CH_3)_2$	69 (A)	238-240	M	$C_{21}H_{21}N_3O_2 \cdot HCl$	2.6×10^{-4}	9
1k	H	2′	3	c-NC_5H_{10}	56 (B)	232-241	MS	$C_{24}H_{25}N_3O_2 \cdot HCl$	9.9×10^{-4}	
1l	H	2′	3	c-NC_6H_{12}	23 (B)	199-202	A	$C_{25}H_{27}N_3O_2 \cdot HCl$	4.3×10^{-4}	21
1m	H	2′	3	c-$N(CH_2CH_2)_2O$	58 (B)	230-237	MS	$C_{23}H_{23}N_3O_3 \cdot HCl$	2.1×10^{-5}	46
1n	H	2′	4	c-$N(CH_2CH_2)_2O$	28 (B)	189-191	A	$C_{24}H_{25}N_3O_3 \cdot HCl$	6.6×10^{-4}	22
1o	H	2′	4	c-NC_5H_{10}	11 (B)	200-202	A	$C_{25}H_{27}N_3O_2 \cdot HCl \cdot 0.33H_2O$	1.2×10^{-3}	
1p	H	3′	2	$N(C_2H_5)_2$	68 (A)	198-200	M	$C_{22}H_{23}N_3O_2 \cdot HCl$	3.0×10^{-3}	
1q	H	3′	2	c-NC_5H_{10}	25 (B)	238-247	MS	$C_{23}H_{23}N_3O_2 \cdot HCl$	1.6×10^{-3}	
1r	H	3′	2	c-$N(CH_2CH_2)_2O$	23 (B)	237-248	MS	$C_{22}H_{21}N_3O_3 \cdot HCl$	5.1×10^{-4}	32
1s	H	3′	3	c-NC_5H_{10}	51 (B)	198-200	M-A	$C_{24}H_{25}N_3O_2 \cdot HCl \cdot 0.5H_2O$	1.6×10^{-3}	
1t	H	3′	3	c-$N(CH_2CH_2)_2O$	44 (B)	195-198	M-E	$C_{23}H_{23}N_3O_3 \cdot HCl$	1.4×10^{-3}	
1u	H	4′	2	$N(CH_3)_2$	60 (A)	228-230	M	$C_{20}H_{19}N_3O_2 \cdot HCl$	1.2×10^{-2}	
1v	H	4′	2	$N(C_2H_5)_2$	87 (A)	208-210	M-A	$C_{22}H_{23}N_3O_2 \cdot HCl$	4.3×10^{-3}	
1w	H	4′	2	c-NC_5H_{10}	52 (B)	235-240	A-C	$C_{23}H_{23}N_3O_2 \cdot HCl$	3.2×10^{-3}	
1x	H	4′	2	c-$N(CH_2CH_2)_2O$	57 (B)	242-247	M-C	$C_{22}H_{21}N_3O_3 \cdot HCl$	1.8×10^{-2}	
1y	H	4′	3	c-NC_5H_{10}	28 (B)	226-228	M	$C_{24}H_{25}N_3O_2 \cdot HCl \cdot 0.5H_2O$	1.3×10^{-3}	19
1z	H	4′	3	c-$N(CH_2CH_2)_2O$	16 (B)	243-245	M	$C_{23}H_{23}N_3O_3 \cdot HCl$	2.8×10^{-3}	
2a	Cl	2′	2	$N(CH_3)_2$	74 (A)	193-195[f] 212-214	M	$C_{20}H_{18}ClN_3O_2 \cdot HCl$	2.5×10^{-4}	11
2b	Cl	2′	2	$N(C_2H_5)_2$	71 (A)	203-205	A	$C_{22}H_{22}ClN_3O_2 \cdot HCl$	3.2×10^{-4}	
2c	Cl	2′	2	c-NC_4H_8	38 (A)	210-213	M-E	$C_{22}H_{20}ClN_3O_2 \cdot HCl$	1.7×10^{-4}	19
2d	Cl	2′	2	c-NC_5H_{10}	32 (B)	197-199	M-E	$C_{23}H_{22}ClN_3O_2 \cdot HCl \cdot 0.5H_2O$	1.0×10^{-5}	37
2e	Cl	2′	2	c-$N(CH_2CH_2)_2O$	31 (B)	124-126	M-A	$C_{22}H_{20}ClN_3O_3 \cdot HCl \cdot 0.5H_2O$[g]	4.4×10^{-6}	11
2f	Cl	2′	3	$N(CH_3)_2$	64 (A)	200-202	M	$C_{21}H_{20}ClN_3O_2 \cdot HCl$	4.1×10^{-4}	
2g	Cl	2′	3	c-NC_5H_{10}	24 (B)	213-215	A	$C_{24}H_{24}ClN_3O_2 \cdot HCl \cdot 0.33H_2O$	6.5×10^{-4}	
2h	Cl	2′	3	c-$N(CH_2CH_2)_2O$	40 (B)	218-220	A	$C_{23}H_{22}ClN_3O_3 \cdot HCl$	6.3×10^{-4}	
2i	Cl	3′	2	$N(C_2H_5)_2$	78 (A)	193-195	A-E	$C_{22}H_{22}ClN_3O_2 \cdot HCl$	1.9×10^{-3}	
2j	Cl	4′	2	$N(C_2H_5)_2$	80 (A)	212-214	A	$C_{22}H_{22}ClN_3O_2 \cdot HCl$	7.4×10^{-4}	
2k	Cl	4′	2	c-NC_5H_{10}	50 (B)	223-225	M	$C_{23}H_{22}ClN_3O_2 \cdot HCl \cdot 0.33H_2O$	2.9×10^{-3}	
2l	Cl	4′	2	c-$N(CH_2CH_2)_2O$	28 (B)	235-240	M	$C_{22}H_{20}ClN_3O_3 \cdot HCl$	1.7×10^{-2}	
3a	OCH_3	2′	2	$N(C_2H_5)_2$	75 (A)	200-202	A-E	$C_{23}H_{25}N_3O_3 \cdot HCl$	3.0×10^{-4}	
3b	OCH_3	2′	2	c-NC_4H_8	52 (A)	205-207	A-E	$C_{23}H_{23}N_3O_3 \cdot HCl \cdot 0.5H_2O$	6.7×10^{-6}	33
3c	OCH_3	2′	2	c-NC_5H_{10}	56 (B)	215-220	A	$C_{24}H_{25}N_3O_3 \cdot HCl \cdot 0.33H_2O$	1.7×10^{-4}	51
3d	OCH_3	2′	2	c-$N(CH_2CH_2)_2O$	40 (B)	210-215	M	$C_{23}H_{23}N_3O_4 \cdot HCl$	1.7×10^{-6}	48
3e	OCH_3	2′	3	c-NC_5H_{10}	53 (B)	212-214	A-E	$C_{25}H_{27}N_3O_3 \cdot HCl \cdot H_2O$	3.2×10^{-4}	

3f	OCH_3	2′	3	c-$N(CH_2CH_2)_2O$	50 (B)	214–216	M–E	$C_{24}H_{25}N_3O_4 \cdot HCl \cdot 0.5H_2O$	3.4×10^{-5}
3g	OCH_3	3′	2	$N(C_2H_5)_2$	43 (A)	186–189	A–E	$C_{23}H_{25}N_3O_3 \cdot HCl \cdot 0.33H_2O$	2.5×10^{-3}
3h	OCH_3	4′	2	$N(C_2H_5)_2$	70 (A)	171–173	A–E	$C_{23}H_{25}N_3O_3 \cdot HCl$	3.0×10^{-3}
3i	OCH_3	4′	2	c-NC_5H_{10}	56 (B)	216–218	M–E	$C_{24}H_{25}N_3O_3 \cdot HCl$	5.5×10^{-4}
3j	OCH_3	4′	2	c-$N(CH_2CH_2)_2O$	40 (B)	210–215	M–E	$C_{23}H_{23}N_3O_4 \cdot HCl$	1.0×10^{-2}
4a	OH	2′	2	$N(C_2H_5)_2$	55 (A)	215–220	M	$C_{22}H_{23}N_3O_3 \cdot HCl$	2.4×10^{-4}
4b	OH	2′	2	c-NC_5H_{10}	40 (B)	230–232	A	$C_{23}H_{23}N_3O_3 \cdot HCl$	6.6×10^{-6}
4c	OH	2′	2	c-$N(CH_2CH_2)_2O$	63 (B)	247–250	M–E	$C_{22}H_{21}N_3O_4 \cdot HCl$	1.4×10^{-6}
5a	H	2′	2	$N(C_2H_5)_2 \cdot CH_3I$	47 (C)	223–226	M	$C_{23}H_{26}IN_3O_2$	3.2×10^{-3}
5b	H	2′	2	c-$NC_5H_{10} \cdot CH_3I$	52 (C)	240–249	M	$C_{24}H_{26}IN_3O_2$	2.1×10^{-3}
5c	H	2′	2	c-$N(CH_2CH_2)_2O \cdot CH_3I$	36 (C)	237–240	MS	$C_{23}H_{24}IN_3O_3$	6.6×10^{-4}
atropine sulfate									5.0×10^{-5}
N-butylscopolamine bromide									1.0×10^{-3}
papaverine hydrochloride									1.9×10^{-2}
morphine hydrochloride									9.3×10^{-5}

3f									12
3g									27
3h									19
3i									1
3j									64^e
4a									14
4b									39
4c									4
5a									
5b									
5c									
atropine sulfate									
N-butylscopolamine bromide									
papaverine hydrochloride									
morphine hydrochloride									

[a] A = EtOH; C = cyclohexane; E = ether; H = hexane; IP = *i*-PrOH; M = MeOH; MS = methylcellosolve. [b] Analyses for C, H, N, and, where present, halogen were within ±0.4% of the theoretical values, unless otherwise noted. Salts obtained as hydrates showed the presence of water in their IR and ^1H NMR spectra even after drying at 80–90 °C for 6–8 h under reduced pressure. [c] Concentration required for 50% inhibition of the response induced by transmural electrical stimulation (see Experimental Section). [d] Percent inhibition of the charcoal meal transfer in mice (100 mg/kg, po, unless otherwise noted (see Experimental Section). [e] Percent inhibition of the charcoal meal transfer in mice (30 mg/kg, po). [f] Double mp. [g] Cl: calcd, 15.57; found, 15.10.

of **10** indicated that two aromatic rings of **10** had a *trans*-stilbene-like configuration. Two isomers were also distinguished by ^1H NMR spectra in which vinyl proton singlets of **10** and **11** appeared at δ 8.62 and 6.82 in $CDCl_3$, respectively. Quaternary ammonium salts (**5**) were obtained by the usual method (method C).

Pharmacological Results and Discussion

Spasmolytic activities of the compounds were examined by the inhibitory effect on the response of isolated ileum from guinea pig to transmural electrical stimulation (anti-TMS activity)[6] and by the charcoal meal test in mice.[7] From the results of anti-TMS activity shown in Table II, it seems that the activities of the compounds were mainly affected by three factors of the side chain:[8] the kind of terminal amino substituents, the substituted position on the phenyl ring, and side-chain length. The introduction of a piperazinyl moiety to **1** and the quaternization of the terminal nitrogen atom of a parent compound resulted in a marked decrease in activity. On the contrary, morpholino derivatives (**1f**,**m**) showed a marked increase in activity. Activities of the compounds having cyclic imino terminations to the side chain (**1c**–**e**) were similar to that of **1a**. Structure–activity relationships between the substituted position of the side chain on the phenyl ring and the length of the side chain were systematically examined on the compounds whose terminal side-chain substituents were morpholino or piperidino. When the results of **1f**,**m**,**n**,**r**,t,**x**,**z** are compared for the morpholino terminal or the results of **1d**,**k**,**o**,**q**,**s**,**w**,**y** are compared for the piperidino terminal, it seems that the order of increasing potency of the substituted position on the phenyl ring was as follows: 4′ < 3′ < 2′. In regards to the length (the number of *n* in **1**) of the side chain substituted at the 2′-position of the phenyl ring, the order of increasing potency of these compounds was as follows: $n = 4 \leq n = 3 < n = 2$. Thus, the optimum side chain for anti-TMS activity was suggested to be 2-(2-morpholinoethoxy). Of these compounds, **1f** showed marked activity. The introduction of a substituent, such as OCH_3 and OH, to the benzisoxazole ring at R^1 resulted in marked increased activity, as shown in **3b**, **3d**, **4b**, and **4c**. As to a steric requirement for anti-TMS activity, it is interesting to note that the *Z* isomer (**10**, $ID_{50} = 6.7 \times 10^{-5}$ mM) was about 300 times as active as the *E* isomer (**11**, $ID_{50} = 2.0 \times 10^{-2}$ mM).

The compounds exhibiting a remarkable anti-TMS activity were administered orally; many of them also inhibited the transfer of charcoal meal in the gastrointestinal tract of mice, as shown in Table II. However, **2e** and **4c** were less active or inactive in the test in mice, whereas they showed potent activities by the anti-TMS method.

Concerning several compounds showing potent spasmolytic activities in the in vitro and in vivo studies, their antimuscarinic activity was examined by the inhibitory effect on the response of isolated ileum from guinea pig to acetylcholine (anti-ACh activity). As shown in Table III, these compounds blocked the muscle response induced by acetylcholine chloride (1.1×10^{-4} mM) only at a high concentration of 10^{-2}–10^{-3} mM, and their activities were weak or negligible in comparison with those of atropine sulfate or *N*-butylscopolamine bromide. The dose–response curve of acetylcholine in the presence of these compounds was shifted to the right with a reduction in the maxima.[9]

(6) W. D. M. Paton, *Br. J. Pharmacol.*, **12**, 119 (1957).
(7) A. F. Green, *Br. J. Pharmacol.*, **14**, 26 (1959).
(8) The intermediates (**9**) were inactive in these biological tests.

Table III

compd	anti-ACh: ID_{50},[a] mM
1a	1.6×10^{-2}
1d	8.7×10^{-3}
1f	2.2×10^{-2}
1m	1.2×10^{-2}
3c	2.5×10^{-2}
3d	3.2×10^{-2}
atropine sulfate	7.2×10^{-6}
N-butylscopolamine bromide	7.5×10^{-5}
morphine hydrochloride	5.3×10^{-2}

[a] Concentration required to produce 50% inhibition of the response induced by acetylcholine (1.1×10^{-4} mM) (see Experimental Section).

Judging from the results described above, compounds **1d**, **1f**, **3c**, and **3d** were selected for further pharmacological evaluations. Further studies[9] revealed that these compounds had a marked suppressive effect on spontaneous movement of the gastrointestinal tracts and lacked both musculotropic and competitive antimuscarinic actions. The mode of action studies suggested that **1d** specifically inhibited acetylcholine release from the vagus nerve.[9] It was also noted that their spasmolytic activities were not antagonized by naloxone.[9] The LD_{50} values of **1d** and **1f** were 2.29 (2.04–2.56) and 2.64 (1.78–3.89) mmol/kg (male mice, po), respectively. Thus, compounds **1d**, **1f**, **3c**, and **3d** might be of interest as a new type of antispasmodic drug.

Experimental Section

Chemistry. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^1H NMR spectra were taken in $CDCl_3$ or Me_2SO-d_6 solution (Me_4Si internal standard) with a Varian HA-100 or EM-360 spectrometer. IR spectra were taken in KBr disks with a Hitachi 260-10 spectrometer. Mass spectra were obtained on a Hitachi RMU-6 spectrometer. UV spectra were recorded with a Shimazu MPS 5000 spectrophotometer. For purity tests, TLC was performed on silica gel 60 F_{254} plates (Merck) with $CHCl_3$ or 5–20% MeOH–$CHCl_3$ as a developing solvent. All compounds were analyzed for C, H, N, and, where present, halogen atom, and analytical results were within ±0.4% of the theoretical values unless otherwise noted. Organic extracts were dried over Na_2SO_4.

(*Z*)-2-(1,2-Benzisoxazol-3-yl)-3-[2-[2-(dimethylamino)ethoxy]phenyl]acrylonitrile Hydrochloride (1a). Method A. A mixture of 1 g (0.0063 mol) of **6** (R^1 = H), 1.25 g (0.0065 mol) of 2-[2-(dimethylamino)ethoxy]benzaldehyde, 2 mL (0.033 mol) of glacial AcOH, 0.5 g (0.0065 mol) of $AcONH_4$, and 50 mL of toluene was refluxed for 16 h in a Dean–Stark apparatus. After being cooled, the reaction mixture was washed with 50 mL of H_2O and dried. The organic layer was made acidic (pH <1) by addition of ethanolic HCl. The resulting precipitate was collected and recrystallized from MeOH to give **1a** (1.7 g, 74%): mp 218–220 °C; IR 3400 (OH), 3480 (OH), 2210 (CN) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.53 (s, 1 H, vinyl H), 3.9 (s, 0.6–0.7 H, OH, exchangeable with D_2O), 2.51 (s, 6 H, NMe_2); MS, *m/z* 333 ($C_{20}H_{19}N_3O_2$). Anal. ($C_{20}H_{19}N_3O_2$·HCl·0.33H_2O) C, H, Cl, N.

(*Z*)-2-(1,2-Benzisoxazol-3-yl)-3-[2-(2-chloroethoxy)phenyl]acrylonitrile (9a). A mixture of 6 g (0.038 mol) of **6** (R^1 = H), 7.1 g (0.039 mol) of 2-(2-chloroethoxy)benzaldehyde, 8 mL (0.13 mol) of glacial AcOH, 3 g (0.039 mol) of $AcONH_4$, and 300 mL of benzene was refluxed for 18 h in a Dean–Stark apparatus. The reaction mixture was washed with hot H_2O, dried, and concentrated. The solid residue was washed with 100 mL of ether to give **9a** (10 g, 77%), mp 166–169 °C. An analytical sample was recrystallized from benzene: mp 168–170 °C; IR 2210 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.65 (s, 1 H, vinyl H). Anal. ($C_{18}H_{13}ClN_2O_2$) C, H, Cl, N.

(9) I. Takayanagi and T. Kadokawa, unpublished results.

(*Z*)-2-(1,2-Benzisoxazol-3-yl)-3-[2-(2-piperidinoethoxy)phenyl]acrylonitrile Hydrochloride (1d). Method B. A mixture of 8.58 g (0.026 mol) of **9a**, 12 mL (0.122 mol) of piperidine, 1 g (0.006 mol) of KI, and 300 mL of toluene was refluxed for 24 h. After being cooled, the reaction mixture was washed with H_2O and dried. The organic layer was made acidic (pH <1) by addition of ethanolic HCl. The resulting precipitate was collected and recrystallized from a mixture of MeOH–EtOH to give **1d** (6.57 g, 60%): mp 197–199 °C; IR 3300 (OH), 2210 (CN) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.60 (s, 1 H, vinyl H), 3.0 (br s, 0.6–0.7 H, OH, exchangeable with D_2O); MS, *m/z* 373 ($C_{23}H_{23}N_3O_2$). Anal. ($C_{23}H_{23}N_3O_2$·HCl·0.33H_2O) C, H, Cl, N.

(*Z*)-2-(1,2-Benzisoxazol-3-yl)-3-[2-(2-piperidinoethoxy)phenyl]acrylonitrile Methiodide (5b). Method C. A mixture of 0.97 g (0.0026 mol) of **10**, 7.3 g (0.0514 mol) of CH_3I, and 100 mL of MeOH was refluxed for 5 h. After being cooled, the resulting crystals were collected and recrystallized from MeOH to give **5b** (0.7 g, 52%), mp 240–249 °C. Anal. ($C_{24}H_{26}IN_3O_2$) C, H, I, N.

(*E*)-2-(1,2-Benzisoxazol-3-yl)-3-[2-(2-piperidinoethoxy)phenyl]acrylonitrile (11). A solution of 3 g (0.008 mol) of **10** (a free base of **1d**) in 170 mL of $CHCl_3$ in a flask attached with a reflux condenser was irradiated by a 375-W tungsten lamp (Toshiba) for 3 days. $CHCl_3$ was vacuum distilled, and the residue was recrystallized from EtOH to recover 2.6 g of **10**. The mother liquor showing two spots on TLC (10% MeOH–$CHCl_3$, R_f 0.47 of **10**, and R_f 0.26 of **11**, in a ratio of about 1:1) was concentrated in vacuo to give a residue (376 mg), which was purified by preparative TLC on glass plates coated with a 2-mm layer of silica gel 60F_{254} (Merck), with 10% MeOH–$CHCl_3$ as a developing solvent. The extract obtained from the bands showing a lower R_f value was recrystallized from EtOH–ether to give 0.15 g of **11**. **10**: mp 113–115 °C (from EtOH); IR 2210 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.62 (s, 1 H, vinyl H); UV λ_{max} (EtOH) 354 nm (log ε 4.10), 306 (4.15), 240 sh (3.99); λ_{min} 330 (4.03), 267 (3.68). Anal. ($C_{23}H_{23}N_3O_2$) C, H, N. **11**: mp 208–209 °C; IR 2220 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.82 (s, 1 H, vinyl H); UV λ_{max} (EtOH) 286 nm (log ε 3.86), 230 sh (4.06); λ_{min} 260 (3.28). Anal. ($C_{23}H_{23}N_3O_2$) C, H, N.

Pharmacology. Isolated Guinea Pig Ileum Preparations. From male, Harley strain, guinea pigs, weighing 300–350 g, sections of the ileum about 3 cm in length were prepared and mounted in physiological solution kept at 35 °C and oxygenated with 95% O_2–5% CO_2, and responses of the ileum were recorded isotonically with an initial tension of 1 g. The physiological solution used had the following composition (mM): NaCl, 137; KCl, 2.7; $MgCl_2$, 1.0; $CaCl_2$, 1.80; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; glucose, 5.55. The ileum was stimulated electrically by the method of Paton.[6] The electrode was made of platinum, and the intraluminal electrode was used as the anode. Rectangular pulses of 0.1-ms duration were used at a frequency of 5 Hz and at a voltage sufficient to give a maximal response (20–30 V). Test compounds were applied 20 min before the stimulation. In some experiments, responses of the ileum to acetylcholine (1.1×10^{-4} mM) were examined. In this case, test compounds were applied 2 min before adding the agonist. Each compound was tested at three concentrations. On the basis of the percent inhibition (mean value of four experiments) at each concentration, ID_{50} values, i.e., the concentration required for 50% inhibition of the responses induced by transmural electrical stimulation and acetylcholine, were determined by the usual graphic method.

Charcoal Meal Test. We examined the charcoal meal transfer in the gastrointestinal tract in mice using the modified method of Green et al[7]. Male ddN strain mice, weighing 20–25 g, were fasted for 18 h. Test compounds were administered orally; 0.2 mL of charcoal meal (5% charcoal in 10% tragacanth solution) was given orally 20 min after the test compound treatment, and the animal was sacrificed 30 min later. The length traversed by the charcoal was expressed as a percent of the total length of the small intestine (from pylorus to caecum). The mean value of the percentage of test-compound-treated group (seven mice) was compared to that of the vehicle-treated group (seven mice), and the percent change induced by compound administration is listed in Table II.

Acute Toxicity. Male ddN strain mice, weighing 18–21 g, were used. Groups of ten mice received food and water ad libitum. The test compounds were administered orally. The LD_{50} was calculated according to the method of Litchfield and Wilcoxon[10] on the 7th day after administration.

(10) J. T. Lichtfield, Jr., and F. W. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

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Notes

A Consideration for Structure–Taste Correlations of Perillartines Using Pattern-Recognition Techniques

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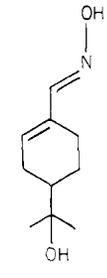
The relationships between molecular structure and taste quality (sweet or bitter) or several perillartine derivatives were investigated using pattern-recognition techniques. For the classification of these compounds into two classes (sweet or bitter), a significant discriminant function was developed by the use of linear learning machine. All the compounds were assigned correctly to their observed taste classes by the function involving three parameters (one hydrophobic and two steric). In addition, the K–L transformation technique was used for examination of classification results.

Recently, Iwamura[1] has performed a quantitative analysis of the structure–taste relationships of 49 perillartine derivatives reported by Acton and co-workers.[2] In his paper, Iwamura used the taste potencies of the compounds as dependent variables in the regression analysis with physicochemical parameters and STERIMOL parameters[3] and suggested the commonness between sweet and bitter receptor from the results. However, his analysis was based on only relative taste potencies of sweetness and bitterness, and he did not give precise consideration to the absolute taste potencies of them.

According to Acton's paper, only half of the 49 compounds give more than 50% bitter or sweet taste potencies for total taste potencies. Moreover, there are several compounds that give far less than 50% potencies even for the sum of bitterness and sweetness. Therefore, it does not seem appropriate to apply the result of the regression analysis based on the data set containing those compounds which give mainly other tastes to the discussion of structure–activity relationships in bitter and sweet tastes. In other words, for those compounds that give more than 50% of other taste potencies, the structure–activity relationships in bitter and sweet tastes could not be clearly determined.

Acton has also presented the quantitative structural features for bitter and sweet compounds. In this case, some compounds that do not give bitter or sweet taste as their main taste were studied. For example, compound I gives 4% sweetness and 18% bitterness for total taste potency; therefore, bitterness or sweetness could not be superior to other tastes. However, Acton categorized it to be a bitter compound based on a ratio of sweetness vs. bitterness of 0.222.



I

To avoid this ambiguity, we have selected those compounds that give more than 50% bitterness or sweetness for total taste potencies as typical examples, and we studied them from the standpoint of discrimination between sweetness and bitterness by using various pattern-recognition techniques.

Data Set and Preprocessing. Pattern-recognition methods are powerful techniques for the investigation of structure–activity relations (SAR).[4–7] In chemical applications, pattern-recognition techniques are generally implemented in three successive procedures: preprocessing; feature selection, by which significant parameters for classification are selected; and development of the clas-

(1) H. Iwamura, *J. Med. Chem.*, **23**, 308 (1980).
(2) E. M. Acton and H. Stone, *Science*, **193**, 584 (1976).
(3) A. Verloop, W. Hoogenstraaten, and J. Tipker, in "Drug Design", Vol. 7, E. J. Ariëns, Ed., Academic Press, New York, 1976, p 165.
(4) G. L. Kirschner and B. R. Kowalski, in "Drug Design" Vol. 8, E. J. Ariëns, Ed., Academic Press, New York, 1977, p 73.
(5) Y. Takahashi, Y. Miyashita, H. Abe, S. Sasaki, Y. Yotsui, and M. Sano, *Anal. Chim. Acta*, **122**, 241 (1980).
(6) Y. Miyashita, Y. Takahashi, Y. Yotsui, H. Abe, and S. Sasaki, Proceeding of 7th International CODATA Conference, No. 41, Pergamon Press, Oxford, 1981, p 37.
(7) Y. Miyashita, T. Seki, Y. Takahashi, S. Daiba, Y. Tanaka, Y. Yotsui, H. Abe, and S. Sasaki, *Anal. Chim. Acta*, **133**, 603 (1981).

0022-2623/82/1825-1245$01.25/0