A New Class of Calcium Antagonists. 2.[1] Synthesis and Biological Activity of 11-[[4-[4-(4-Fluorophenyl)-1-piperazinyl]butyryl]amino]-6,11-dihydrodibenzo[b,e]-thiepin Maleate and Related Compounds

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A series of [(ω-aminoalkanoyl)amino]-6,11-dihydrodibenzo[b,e]thiepins and -5*H*-dibenzo[a,d]cycloheptenes and related compounds were synthesized and evaluated for calcium antagonistic activity by calcium-induced constriction of potassium-depolarized rat aorta. Semiempirical molecular orbital calculations of the dibenzotricyclic systems indicated that calcium antagonistic activity increased with a decrease of the angle between the planes of the two phenyl rings. AM1 net charge calculations showed that a neutral or positive charge distribution in the bridge portion was necessary for activity. 11-[[4-[4-(4-Fluorophenyl)-1-piperazinyl]butyryl]amino]-6,11-dihydrodibenzo[b,e]thiepin maleate (16, AJ-2615) showed a more gradual and longer lasting antihypertensive effect than diltiazem and nifedipine in spontaneously hypertensive rats (SHR) administered orally. Compound 16 also possessed antianginal effects in methacholine-induced ST elevation and vasopressin-induced ST depression tests in rats. The alteration of the dibenzotricyclic system of 16 to 5*H*-dibenzo[a,d]cycloheptene (19, 5-[[4-[4-(4-fluorophenyl)-1-piperazinyl]-butyryl]amino]-5*H*-dibenzo[a,d]cycloheptene) resulted in selectivity for cardiac tissue over vascular tissue, thereby conferring antianginal activity without an effect on blood pressure. Antianginal potencies of 16 and 19 were equal to or somewhat more potent than those of diltiazem.

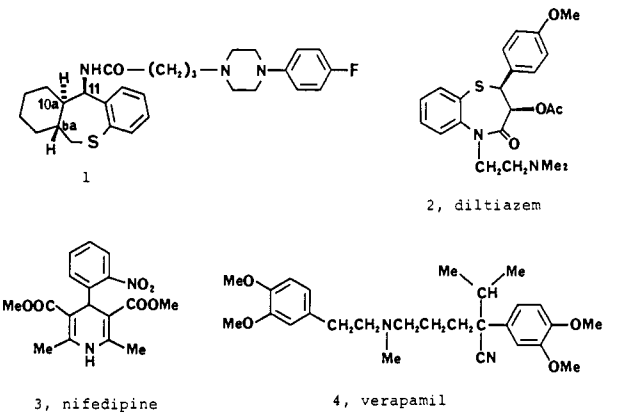
In a previous paper,[1] we reported the synthesis and biological activity of a structurally new class of calcium antagonists, 11-[(ω-aminoalkanoyl)amino]-6,6a,7,8,9,10,10a,11-octahydrodibenzo[b,e]thiepins. Among them, the most potent was compound 1 (pA_2 8.16), which was superior to diltiazem **2** (pA_2 7,42) in calcium antagonistic activity. In that study, the relative configuration of three chiral centers (C6a, C10a, and C11), hence the spatial structure of the tricyclic system of 1 (Chart I), was found to be important to the activity. Furthermore, the chemical structure of the bridge portion of the tricyclic system was also crucial to the activity. These findings prompted us to extend the work to a new series of (ω-aminoalkanoyl)amino derivatives where a variety of dibenzotricyclic systems were substituted for the tricyclic system of 1.

This paper[2] describes the synthesis of the (ω-aminoalkanoyl)amino dibenzotricyclic derivatives **16-48** and their structure-activity relationships (SARs) for calcium antagonistic activity associated with variations of the tricyclic system (R_1), the substituent (R_2) on the piperazine ring, and the methylene chain length (*n*). Antihypertensive and antianginal evaluations of selected members, particuarly **16** (AJ-2615), representing a new class of calcium antagonist, are also discussed.

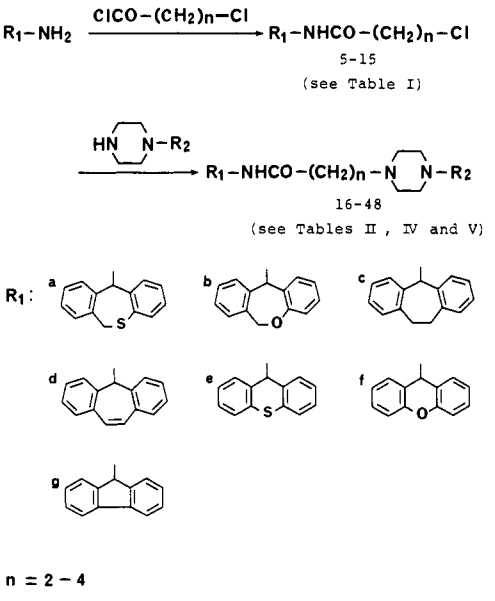
Chemistry

A synthetic route to the designed (ω-aminoalkanoyl)-amino derivatives **16-48** is given in Scheme I. The requisite primary amines and piperazines were synthesized according to the reported methods or purchased (see Experimental Section). Treatment of the amines with ω-chloroalkanoyl chloride in refluxing toluene gave the corresponding (ω-chloroalkanoyl)amino derivatives **5-15**, of which physical properties are summarized in Table I. Replacement reaction of the terminal chlorine atom of **5-15** with an appropriate piperazine was sluggish and needed heating in toluene for over 10 h. However, in the presence of excess sodium iodide, the reaction proceeded smoothly to completion in 1 h at 100 °C in dimethylformamide, giving (ω-aminoalkanoyl)amino derivatives **16-48** in good to moderate yields (Tables II, IV, and V). The structures of all compounds thus prepared were confirmed by proton nuclear magnetic resonance (^{1}H NMR) spectra and elemental analysis.

Chart I



Scheme I



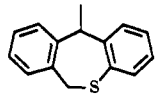
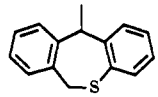
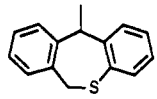
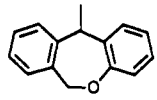
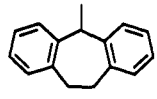
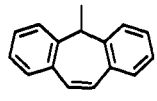
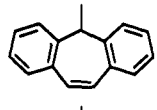
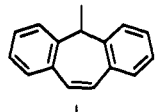
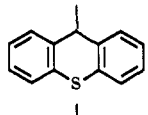
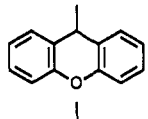
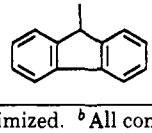
(1) Paper 1: Kurokawa, M.; Sato, F.; Hatano, N.; Honda, Y.; Uno, H. *J. Med. Chem.*, in press.

(2) This work was presented in part at The Japanease-United States Congress of Pharmaceutical Science, Honolulu, HI, Dec 1987, Abstr S 173, and at the 10th Symposium on Medicinal Chemistry, Kyoto, Japan, Nov 1989, Abstr, p 17.

0022-2623/91/1834-0927$02.50/0

Table I. (ω-Chloroalkanoyl)amino Derivatives

$R_1NHCO(CH_2)_nCl$

compd	R_1	n	yield,[a] %	mp, °C	recryst solvent	formula[b]
5		2	73	177–178	EtOH	$C_{17}H_{16}NOSCl$
6		3	78	189–190	EtOH	$C_{18}H_{18}NOSCl$
7		4	80	155–156	EtOH	$C_{19}H_{20}NOSCl$
8		3	80	169–171	toluene	$C_{18}H_{18}NO_2Cl$
9		3	79	220–222	toluene	$C_{19}H_{20}NOCl$
10		2	67	220–223	EtOH/hexane	$C_{18}H_{16}NOCl$
11		3	82	220–223	toluene	$C_{19}H_{18}NOCl$
12		4	72	221–222	$CHCl_3$	$C_{20}H_{20}NOCl$
13		3	84	150–153	toluene	$C_{17}H_{16}NOSCl$
14		3	83	160–163	toluene	$C_{17}H_{16}NO_2Cl$
15		3	85	193–195	toluene	$C_{17}H_{16}NOCl$

[a] Yields were not optimized. [b] All compounds were analyzed for C, H, N, Cl, and, where present, S; analytical results are within ±0.4% of the theoretical values.

Biological Results and Discussion

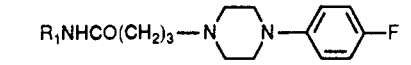
In vitro calcium antagonistic activity of compounds 16–48 was assessed against calcium-induced constriction of potassium-depolarized rat aorta.[3] The results are summarized in Table II, IV, and V. Data for diltiazem (2), nifedipine (3), and verapamil (4) are included for comparison in Table II.

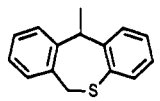
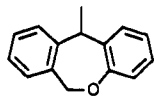
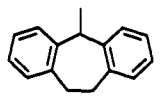
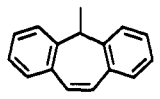
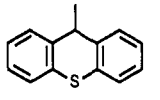
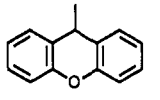
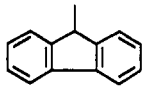
Unsaturation of the cyclohexyl ring of 6,6a,7,8,9,10,10a,11-octahydrodibenzo[*b*,*e*]thiepin 1, providing the 6,11-dihydrodibenzo[*b*,*e*]thiepin derivative 16, considerably increased the activity (Table II). In order to know their most stable conformations, semiempirical molecular orbital calculations (using the AM1 method[4]) of the tricyclic systems of 1 and 16 were performed. Figure 1, where a common sequence of atoms included in the right-hand benzene ring and the sulfur atom were superimposed, shows structural features of both tricyclic systems. Overall spatial structures resemble each other; thus the left-hand benzene ring of 16 occupies a region similar to that of the chair-shaped cyclohexyl ring of 1. This implies that the receptor favors an aromatic interaction in this region.

Therefore, variation of tricyclic systems with two benzene rings (16–22) and their effects on the calcium antagonistic activity were then examined (Table II). Replacements of the sulfur atom in 16 by a bioisosteric oxygen atom (17) and a methylene group (18) resulted both in a decrease in activity. Interestingly, unsaturation of the carbon bond of the bridge portion (18, CH_2—CH_2 → 19, CH=CH) led to an increase in activity. The dibenzotricyclic systems containing a seven-membered central ring were superior in activity to the corresponding six- and five-membered congeners (16 vs 20, 17 vs 21, and 18 vs 22). The computer-generated, most stable structures of dibenzotricyclic systems 16–22 were examined. It is worth noting that the calcium antagonistic activity increased with a decrease in the angle between the two planes of the benzene rings of the dibenzotricyclic systems, except for the 6,11-dihydrodibenz[*b*,*e*]oxepin analogue (17) (Table

(3) Godfrand, T.; Kaba, A. *Br. J. Pharmacol.* **1969**, *36*, 549; *Arch. Int. Pharmacol.* **1972**, *196*, 35.
(4) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

Table II. Compounds 16–22 and Effects of the Dibenzotricyclic Systems (R_1) on Calcium Antagonistic Activity



compd	R_1	yield,[a] %	mp, °C	recryst solvent	formula[b]	calcium antagonistic activity (n = 4): pA_2[c]
16		75	149–150	EtOH	$C_{28}H_{30}N_3OSF \cdot C_4H_4O_4$[d]	8.71 ± 0.08
17		66	149–153	EtOH	$C_{28}H_{30}N_3O_2F$	7.48 ± 0.11
18		42	170–172	EtOH/ether	$C_{29}H_{32}N_3OF \cdot C_4H_4O_4$[d]	8.03 ± 0.06
19		77	164–167	EtOH/ether	$C_{29}H_{30}N_3OF \cdot C_4H_4O_4$[d]	8.98 ± 0.08
20		43	146–149	MeOH	$C_{27}H_{28}N_3OSF$	7.98 ± 0.07
21		51	170–172	AcOEt	$C_{27}H_{28}N_3O_2F$	7.16 ± 0.25
22		66	207–209	$CHCl_3$/AcOEt	$C_{27}H_{28}N_3OF$	7.16 ± 0.09
diltiazem						7.42 ± 0.05
verapamil						8.16 ± 0.15
nifedipine						9.83 ± 0.08

[a] Yields were not optimized. [b] All compounds were analyzed for C, H, N, and, where present, S and F; analytical results are within ±0.4% of the theoretical values. [c] Negative logarithm of the molar concentration of the test compounds that cause a shift of factor 2 toward higher concentration in the calcium concentration–response curve. See text and the Experimental Section for details. [d] Maleate.

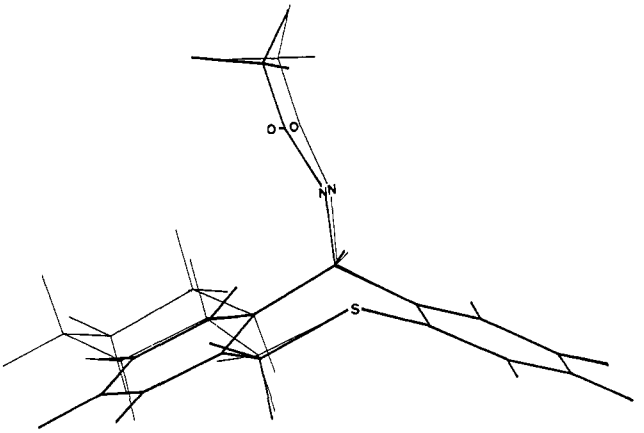


Figure 1. Superimposition of 1 (light line) and 16 (heavy line) by the program COMPAR.[26] The right-hand benzene rings, carbon (C-11), and sulfur were subjected to a least-squares fit. The acetamide group is included, for clarity, as a representative side chain.

III). It has been reported that the inhibitory activity of some dibenzotricyclic antidepressants against the uptake of norepinephrine by rabbit aortic strips and the uptake into rat cerebral cortex slices increases as the angle between the two benzene ring planes decreases;[5] compounds having a seven- (or eight-) membered central ring were the most potent inhibitors. This observation is consistent with our results. Thus, structural requirements of our dibenzotricyclic calcium antagonists for the receptor appear similar to those of the dibenzotricyclic antidepressants reported. The oxygen-bridged dibenzotricyclic systems (**17**, 126.93° and **21**, 150.89°), whose angles were slightly larger than those of the corresponding sulfur-bridged analogues (**16**, 123.64° and **20**, 144.78°), showed however a considerably reduced activity compared to **16** and **20**, respectively. This fact may be due to a difference of charge density around the bridge portion. Therefore the electrostatic potentials on the Connolly's solvent accessible surface[6] of the di-

Table III. Angles between the Planes of the Two Benzene Rings in the Dibenzotricyclic Derivatives and the Calcium Antagonistic Activities

compd[a]	angle, deg	calcium antagonistic activity (n = 4): pA_2[b]
19	119.37	8.98 ± 0.08
16	123.64	8.71 ± 0.08
18	126.41	8.03 ± 0.06
17	126.93	7.48 ± 0.11
20	144.78	7.98 ± 0.07
21	150.89	7.16 ± 0.25
22	178.77	7.16 ± 0.09

[a] Arranged in ascending order of degree of the angle. [b] See footnote c in Table II.

(5) Wells, J. N.; Shirodkar, A. V.; Knevel, A. M. *J. Med. Chem.* **1966**, *9*, 195. Salama, A. I.; Insalaco, J. R.; Maxwell, R. A. *J. Pharmacol. Exp. Ther.* **1971**, *178*, 474.

(6) Connolly, M. L. QCPE Program No. 429, version 2.

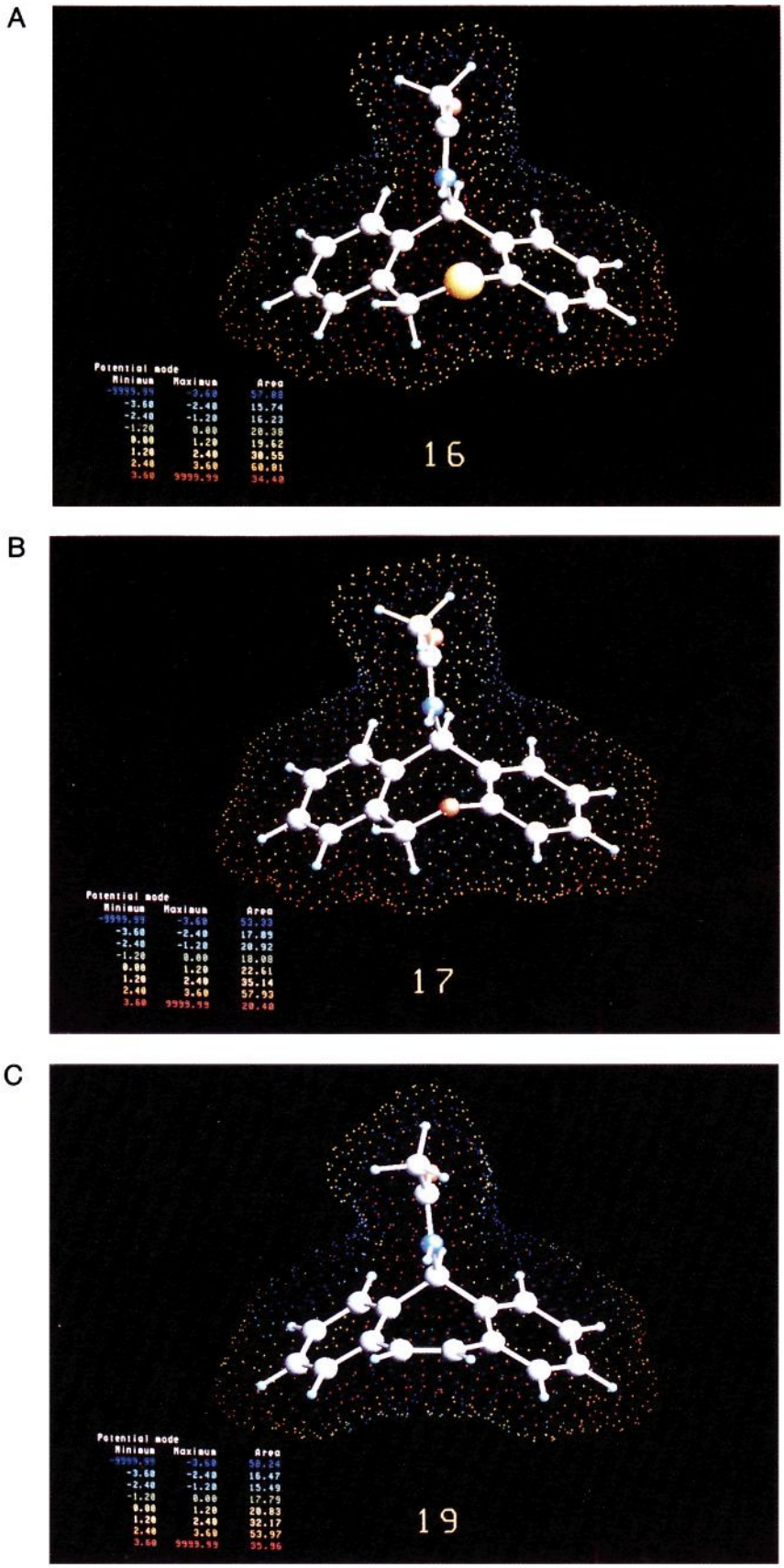
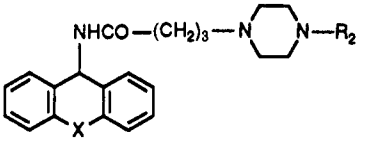


Figure 2. Electrostatic potentials at surfaces of the tricyclic systems **16** (A), **17** (B), and **19** (C). The potential values (V) for contour levels (kcal/mol) and the corresponding molecular surface areas (Å^2) are also represented. Contour levels: dark blue, $V < -3.60$ kcal/mol; blue, $-3.60 \leq V < -2.40$ kcal/mol; light blue, $-2.40 \leq V < -1.20$ kcal/mol; green, $-1.20 \leq V < 0.00$ kcal/mol; light green, $0.00 \leq V < 1.20$ kcal/mol; yellow, $1.20 \leq V < 2.40$ kcal/mol; orange, $2.40 \leq 3.60$ kcal/mol; red, $V \geq 3.60$ kcal/mol. The acetamide group is included, for clarity, as a representative side chain.

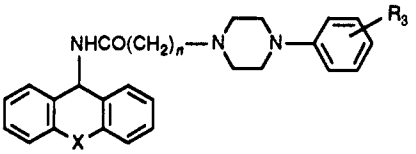
Table IV. Compounds 23–35 and Effects of Substituents (R_2) on the Piperazine Nitrogen on Calcium Antagonistic Activity



compd	R_2	X	yield,[a] %	mp, °C	recryst solvent	formula[b]	calcium antagonistic activity (n = 4): pA_2[c]
23	Ph	CH_2S	60	142–143	EtOH/ether	$C_{28}H_{31}N_3OS·C_4H_4O_4$[d]	8.76 ± 0.03
24	CH_2Ph	CH_2S	55	190–191	EtOH	$C_{29}H_{33}N_3OS·2C_4H_4O_4$[d]$·0.75H_2O$	8.54 ± 0.06
25	$(CH_2)_3Ph$	CH_2S	62	178–180	EtOH	$C_{31}H_{37}N_3OS·2C_4H_4O_4$[d]	7.91 ± 0.18
26	$CH_2CH=CHPh$	CH_2S	66	183–184	EtOH	$C_{31}H_{35}N_3OS·2C_4H_4O_4$[d]$·0.75H_2O$	8.12 ± 0.05
27	CH_2COPh	CH_2S	32	156–157	EtOH	$C_{30}H_{33}N_3O_2S·C_4H_4O_4$[d]$·0.5H_2O$	7.81 ± 0.17
28	COPh	CH_2S	51	232–234	MeOH/$CHCl_3$	$C_{29}H_{31}N_3O_2S·2C_4H_4O_4$[d]	7.44 ± 0.08
29	COOPh	CH_2S	29	110–112	EtOH/ether	$C_{29}H_{31}N_3O_3S·C_6H_8O_7$[e]$·0.5H_2O$	7.88 ± 0.17
30	SO_3Ph	CH_2S	55	95–100	EtOH/ether	$C_{28}H_{31}N_3O_4S_2$	7.98 ± 0.08
31	Ph	CH=CH	72	156–161	EtOH/ether	$C_{29}H_{31}N_3O·C_4H_4O_4$[d]	8.43 ± 0.06
32	CH_2Ph	CH=CH	65	175–178	MeOH/ether	$C_{30}H_{33}N_3O·2HCl·1.5H_2O$	8.22 ± 0.08
33	$(CH_2)_2Ph$	CH=CH	81	204–205	$CHCl_3$/hexane	$C_{31}H_{35}N_3O$	8.05 ± 0.22
34	$CH_2CH=CHPh$	CH=CH	58	204–206	EtOH	$C_{32}H_{35}N_3O·2C_4H_4O_4$[d]$·0.5H_2O$	7.51 ± 0.08
35	COPh	CH=CH	61	246–250	EtOH/ether	$C_{30}H_{31}N_3O_2·C_2H_2O_4$[f]	7.68 ± 0.07

[a–c] See footnotes a–c in Table II. [d] Maleate. [e] Citrate. [f] Oxalate.

Table V. Compounds 36–48 and Effects of Substituents (R_3) on the Phenyl Ring and Length (n) of the Methylene Chain on Calcium Antagonistic Activity



compd	n	X	R_3	yield,[a] %	mp, °C	recryst solvent	formula[b]	calcium antagonistic activity (n = 4): pA_2[c]
36	3	CH_2S	2-F	62	151–152	MeOH/ether	$C_{28}H_{30}N_3OSF$	8.49 ± 0.09
37	3	CH_2S	4-Cl	38	216–217	AcOEt	$C_{28}H_{30}N_3OSCl·0.25H_2O$	8.55 ± 0.04
38	3	CH_2S	4-Me	36	186–187	$CHCl_3$/hexane	$C_{29}H_{33}N_3OS·0.25H_2O$	8.48 ± 0.04
39	3	CH_2S	4-NO_2	49	275–277	$CHCl_3$	$C_{28}H_{30}N_4O_3S$	8.10 ± 0.17
40	3	CH_2S	4-OMe	78	193–195	$CHCl_3$/hexane	$C_{29}H_{33}N_3O_2S·0.25H_2O$	8.47 ± 0.12
41	3	CH=CH	2-F	67	197–198	$CHCl_3$/hexane	$C_{29}H_{30}N_3OF$	8.46 ± 0.10
42	3	CH=CH	4-CF_3	53	171–172	MeOH/ether	$C_{30}H_{30}N_3OF_3$	8.68 ± 0.08
43	3	CH=CH	3-OMe	67	135–138	MeOH	$C_{30}H_{33}N_3O_2$	8.27 ± 0.18
44	3	CH=CH	3,4-OMe	38	130–132	MeOH	$C_{31}H_{35}N_3O_3·0.5H_2O$	8.30 ± 0.18
45	2	CH_2S	4-F	52	172–175	EtOH	$C_{27}H_{28}N_3OSF·C_4H_4O_4$[d]	8.55 ± 0.11
46	4	CH_2S	4-F	70	194–195	EtOH	$C_{29}H_{32}N_3OSF·C_4H_4O_4$[d]	8.46 ± 0.08
47	2	CH=CH	4-F	55	118–120	$CHCl_3$	$C_{28}H_{28}N_3OF·C_4H_4O_4$[d]$·H_2O$	8.56 ± 0.13
48	4	CH=CH	4-F	68	195–196	EtOH	$C_{30}H_{32}N_3OF·C_4H_4O_4$[d]$·0.5H_2O$	8.35 ± 0.06

[a–c] See footnotes a–c in Table II. [d] Maleate.

benzotricyclic systems of **16**, **17**, and **19** were calculated with use of AM1 net charge. The surfaces of electrostatic potential (V) of these three molecules are shown in Figure 2, which reveals that the positive potential regions (orange, $+2.40 \le V < +3.60$ kcal/mol or red, $V \ge +3.60$ kcal/mol) appear around the surface of the bridge portion of **16** and **19** which show potent activity. On the other hand, the surface at the corresponding region for **17** has the negative potential (green, $-1.20 \le V < 0.00$ kcal/mol and light blue, $-2.40 \le V < -1.20$ kcal/mol). The difference of the electrostatic potential values are due to that of the charge densities of the oxygen atom of **17**, the sulfur atom of **16**, and the olefinic carbon of **19**, which all constitute seven-membered ring systems.

Thus, this opposite electronic feature may be responsible for the difference of calcium antagonistic activities between the oxygen-containing congener and other compounds having high activities; accordingly, a positive charge distribution around the bridge portion, as well as the conformation of two benzene rings, appears to be a crucial factor for the activity.

Optimization of the side chains in a series of 6,11-dihydrodibenzo[b,e]thiepin and 5H-dibenzo[a,d]cycloheptene derivatives (R_1; **a** and **d**, respectively, in Scheme I) was then studied, including the following variations: (1) R_2, a substituent on the piperazine nitrogen, (2) R_3, a substituent on the phenyl ring of the phenylpiperazine moiety, (3) n, length of the methylene chain.

(1) The unsubstituted phenyl derivatives **23** and **31** (R_2 = Ph) showed potent activity (Table IV). However, insertion of one or two methylene units (**24**, **25**, **32**, and **33**) or a functional group (**26–30**, **34**, and **35**) between the phenyl and the piperazinyl rings of **23** or **31** caused a decrease in activity.

(2) A 4-fluoro group as R_3 was optimal (**16** and **19**); thus other substituents (**36–44**) studied did not improve the activity (Table V).

(3) Fixing the substituent R_2 to 4-fluorophenyl, we ex-

Table VI. Preventive Effects of 16, 19, and Diltiazem on Methacholine-Induced ST Elevation in Anesthetized Rats

compd	dose, mg/kg, iv	ST elevation (ΔmV ± SE[a]) without test compound	with test compound maximum (0–90 min)
saline control[b]		+0.224 ± 0.040	+0.227 ± 0.052
16	0.1	+0.198 ± 0.022	+0.140 ± 0.017
	0.3	+0.203 ± 0.024	+0.093 ± 0.015[c]
	1.0	+0.241 ± 0.019	+0.088 ± 0.014[c]
19	0.1	+0.165 ± 0.019	+0.120 ± 0.023
	0.3	+0.188 ± 0.022	+0.042 ± 0.019[c]
	1.0	+0.224 ± 0.026	+0.083 ± 0.012[c]
diltiazem	0.1	+0.227 ± 0.024	+0.148 ± 0.032
	0.3	+0.156 ± 0.020	+0.059 ± 0.020[c]
	1.0	+0.223 ± 0.027	+0.091 ± 0.023[c]

[a] Results are presented as the mean ± SE from five experiments. [b] 5% ethanol in saline. [c] Significantly different from respective control ($p < 0.01$).

Table VII. Preventive Effects of 16, 19, and Diltiazem on Vasopressin-Induced ST Depression in Rats

compd	dose, mg/kg, po	preventive effect on ST depression:[a] time after administration, h 1	2	3	7
16	3	+[b]	–[c]		
	10	+	+	+	–
	30	+			+
16	3	+	+	–	
	10	+		+	–
	30				+
diltiazem	3	–			
	10	+	+	–	
	30	+		+	–

[a] The results were determined from 10–20 experiments. [b] (+) differ from control values with $p < 0.05$. [c] (–) no significance.

amined an effect of the methylene chain length (n) on the activity (Table V). Shortening (n = 2, 45 and 47) and lengthening (n = 4, 46 and 48) of the chain length reduced activity; hence, the optimal length (n) was 3 (16 and 19).

As the result of the SAR studies described above, the optimum side chain for both 6,11-dihydrodibenzo[b,e]-thiepin and 5H-dibenzo[a,d]cycloheptene nuclei was a [4-[4-(4-fluorophenyl)-1-piperazinyl]butyryl]amino group. This is consistent with our previous results[1] from the series of 6,6a,7,8,9,10,10a,11-octahydrodibenzo[b,e]thiepin derivatives.

Among the compounds prepared in the present study, 16 and 19 were selected for the following pharmacological evaluations. As shown in Figure 3, the antihypertensive effect of 16 (30 mg/kg, po) in spontaneously hypertensive rats (SHR) appeared more gradually and lasted much longer than those of the reference drugs, diltiazem and nifedipine. Compound 19, which possessed the most potent calcium antagonistic activity in this series, did not exhibit the antihypertensive effect at the same dose as 16. Compound 16 also showed preventive effects on methacholine-induced ST elevation (Table VI) and vasopressin-induced ST depression (Table VII), both useful indexes for evaluating the antianginal effects of calcium antagonists.[7] The potencies of 16 were equal to or somewhat higher than those of diltiazem. Interestingly, despite less antihypertensive activity, compound 19 showed the same antianginal potencies as 16. Compound 19 thus is a calcium antagonist with a selective efficacy on cardiac tissue over smooth muscle tissue. The reason why the alteration of the bridge portion in the dibenzotricyclic system (16, CH_2S → 19, CH=CH) causes the cardiac selectivity is now not fully understood. Compound 16 was also evaluated for its ability to inhibit ^{45}Ca influx in rat aorta and the results are given in Table VIII; the potencies are expressed in terms of an IC_{50} value (nM). A good correlation was observed between the potencies of 16, diltiazem, and nifedipine on inhibiting Ca^{2+} influx and potassium-induced contraction in rat aorta. This result implies that the calcium antagonistic activity of 16 is caused by the inhibition of calcium influx into the cell.

Table VIII. Inhibitory Effects of Compound 16 on Calcium Influx and Contraction Induced by High Potassium in Isolated Rat Aorta

compd	inhibitory effect: calcium influx: IC_{50}, nM	contraction: IC_{50}, nM
16	16	21
diltiazem	352	203
nifedipine	4.0	2.0

In conclusion, we showed that the angle between the planes of two benzene rings as well as the electronic potential of the bridge portion in the dibenzotricyclic system are crucial to the calcium antagonistic activity of the new compounds. The SAR studies of the side chain of the dibenzotricyclic systems revealed the [4-[4-(4-fluorophenyl)-1-piperazinyl]butyryl]amino group to be optimal. Compound 16 had a more gradual and longer lasting antihypertensive effect in SHR than diltiazem and nifedipin, and also was found to possess antianginal effects in rats, with a potency equal to or somewhat higher than that of diltiazem. Compound 19, producing the antianginal effect without an effect on blood pressure at the same dose, was found to be a calcium antagonist with a good selectivity for cardiac tissue over vascular tissue. Therefore 16 and 19 appear to be promising candidates as cardiovascular agents. The biological properties of 16 (11-[[4-[4-(4-fluorophenyl)-1-piperazinyl]butyryl]amino]-6,11-dihydrodibenzo[b,e]thiepin maleate) have been briefly reviewed.[8] Compound 16 is now under clinical evaluation.

Experimental Section

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained on a Varian XL-300 spectrometer with tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a Hitachi 260-10 grating infrared spectrophotometer. Column chromatography was carried out on Merck silica gel 60.

The following known compounds were prepared according to the cited literature: 11-amino-6,11-dihydrodibenzo[b,e]thiepin;[9] 11-amino-6,11-dihydrodibenz[b,e]oxepin, 5-amino-10,11-dihydro-5H-dibenzo[a,d]cycloheptene, and 5-amino-5H-dibenzo[a,d]cycloheptene;[10] 10-aminothioxanthene and 10-aminoxanthene;[11] 1-(3-phenylpropyl)piperazine;[12] 1-phenacylpiperazine;[13] 1-benzoylpiperazine;[14] 1-piperazinecarboxylic acid phenyl ester;[15]

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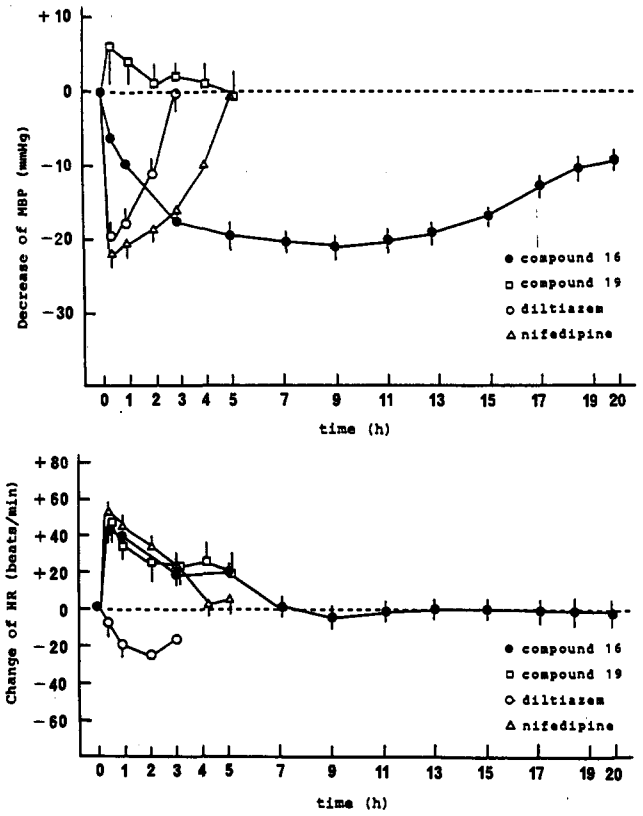

Figure 3. Effects of orally administered compounds **16** and **19** (30 mg/kg, n = 9), diltiazem (50 mg/kg, n = 5), and nifedipine (3 mg/kg, n = 6) on mean blood pressure (MBP, upper) and heart rate (HR, lower) in spontaneously hypertensive rats. Each point indicates the mean value and vertical lines represent the standard error of the mean.

1-piperazinesulfonic acid phenyl ester;[16] 1-(2-phenylethyl)-piperazine;[17] 1-(3-phenyl-2-propenyl)piperazine;[18] 1-(2-fluoro-, 4-methyl-, 4-methoxy-, 4-trifluoromethyl- and 3,4-dimethoxyphenyl)piperazines.[19] 9-Aminofluorene and 1-(4-fluorobenzyl-, 4-chlorophenyl, and 4-nitrophenyl)piperazines were purchased from Aldrich Chemical Co., Inc.

(ω-Chloroalkanoyl)amino Derivatives (5–15, Table I). General Procedure. A mixture of 11-amino-6,11-dihydrodibenzo[*b*,*e*]thiepin (3.7 g, 16 mmol) and 4-chlorobutyryl chloride (2.3 g, 16 mmol) in toluene (70 mL) was refluxed for 6 h. After cooling to ambient temperature, the solid was collected by filtration, washed with toluene, and air-dried to afford 4.5 g of the crude product. This was recrystallized from ethanol to give **6** (4.2 g, 78%): mp 189–190 °C; ^{1}H NMR ($CDCl_3$) δ 2.09 (2 H, m), 2.39 (2 H, t, J = 6 Hz), 3.56 (2 H, t, J = 6.5 Hz), 4.22 (2 H, s), 6.32 (1 H, d, J = 9 Hz), 7.09–7.38 (9 H, m). Anal. ($C_{18}H_{16}NOSCl$) C, H, N, S, Cl.

(ω-Aminoalkanoyl)amino Derivatives (16–48; Tables II, IV, and V). General Procedure. A mixture of **6** (2.5 g, 7.5 mmol), 1-(4-fluorophenyl)piperazine (2.7 g, 15 mmol), and sodium iodide (3.0 g, 20 mmol) in dimethylformamide (50 mL) was stirred at 80 °C for 3 h. The reaction mixture was poured into water and extracted with chloroform. The extract was successively washed with water and brine and dried over anhydrous magnesium sulfate. The solvent was evaporated to leave a dark oil, which was chromatographed on silica gel, using chloroform as an eluent, to give the solid. This was recrystallized from chloroform–hexane to afford the free base of **16** (2.7 g, 75%): mp 194–194.5 °C; IR (KBr) 1695 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.86 (2 H, m), 2.24–2.52 (6 H, m), 3.02 (4 H, t, J = 6 Hz), 4.10 (1 H, d, J = 16 Hz), 4.31 (1 H, d, J = 16 Hz), 6.29 (1 H, d, J = 8 Hz), 6.80–7.45 (12 H, m), 7.62 (1 H, d, J = 8 Hz). A solution of the free base of **16** in ethanol was treated with maleic acid, and the resulting precipitate was filtered off and recrystallized from ethanol to give **16** as colorless crystals, mp 149–150 °C. Anal. ($C_{28}H_{30}N_3OSF\cdot C_4H_4O_4$) C, H, N, F, S.

Conformational Analysis and Molecular Graphics. AM1 calculations[4] with geometry optimization in MOPAC[20] for the acetamido derivatives of compounds **1** and **16–22** were carried out. The keyword "PARASOK" was used for 6,6a,7,8,9,10,10a,11-octahydrodibenzo[*b*,*e*]thiepin, 6,11-dihydrodibenzo[*b*,*e*]thiepin, and thioxanthene derivatives. Molecular modeling and graphics including the calculation of electrostatic potential were performed by using commercially available programs.[21] Initial atomic coordinates for the calculation of the molecular orbital of the tricyclic systems were determined as follows: data from X-ray crystallographic literature (10,11-dihydro-5*H*-[22] and 5*H*-dibenzo[*a*,*d*]cycloheptenes,[23] and thioxanethene[24]); replacement of the C-11 methylene group in 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene by sulfur and oxygen atoms (6,11-dihydrodibenzo[*b*,*e*]thiepin and -oxepin). 6,6a,7,8,9,10,10a,11-Octahydrodibenzo[*b*,*e*]thiepin, xanthene, fluorene, and an acetamide group were constructed by use of standard bond lengths and angles.

The solvent-accessible surface for each structure was calculated by using program MS[6] with the solvent radius of 1.5 Å. The value of electrostatic potential V at a point x on a molecular surface for a system charge Q_i of atom i in a medium dielectric constant ϵ is given by the classical formula

$$V = \sum_i (Q_i/\epsilon R_i)$$

where R_i is a distance between atom i and point x; a distance-dependent dielectric constant,[27] $\epsilon = R_i$ was used. Net atomic charges obtained from AM1 calculation were used to represent Q_i. The contribution was calculated of each atom within 15 Å of the electrostatic potential point x. The surfaces of electrostatic potential are colored correspondingly to eight contour levels of potential in Figure 2.

Calcium-Induced Contraction of Depolarized Arterial Smooth Muscle. Std-Wistar rats were killed by a blow on the head. The thoracic aorta was removed and cut into spinal strips 3–4 mm in width and about 30 mm in length. The preparation was mounted under 1.0-g tension in a 10-mL organ containing Krebs–bicarbonate solution aerated with 95% O_2 and 5% CO_2 at 37 °C. Isometric contractions were recorded on a flat recorder with force displacement transducer. The normal Krebs–bicarbonate solution was composed of (mmol): NaCl, 112.0; $CaCl_2\cdot 2H_2O$, 1.25; KCl, 5.0; $MgSO_4\cdot 7H_2O$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.0; glucose, 11.5. After the vascular preparations were eqilibrated in the normal Krebs–bicarbonate solution, the solution was replaced with the calcium-free, potassium-rich Krebs–bicarbonate solution (excluding $CaCl_2$ and replacing NaCl with equimolar KCl) to cause a depolarization and then $CaCl_2$ was cumulatively added to make the Ca^{2+} concentration–response curve. After the test compounds were incubated in the solution for 5 min, the same experiment was carried out. The pA_2 value was calculated according to the method of van Rossum.[25] The

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pA_2 value is defined as the negative logarithm of the molar concentration of the test compound that will cause a shift of a factor of 2 toward higher concentration in the Ca^{2+} concentration-response curve.

Blood Pressure and Heart Rate in Spontaneously Hypertensive Rats. Experiments were carried out in spontaneously hypertensive male rats (Okamoto strain), which were at least 16 weeks old. An arterial catheter (Clay Adams, PE-50) was implanted into an abdominal aorta via a femoral artery under ether anesthesia. Conscious blood pressure was measured directly by the arterial catherter that was connected to a pressure transducer (Nihon Koden, TR-200T). Heart rate was measured by a pulse rate tachometer (Nihon Koden, AT-601G).

Methacholine-Induced ST Elevation in Rats. Male Sprague-Dawley rats, weighing about 500 g, were anesthetized with sodium pentobarbital (60 mg/kg ip). For bolus injection of methacholine into the ostia of the left and right coronary arteries, an arterial cannula was introduced through the exposed right carotid artery down closely to the aortic valve. Methacholine solutions (8 μg/kg) were administered ia into the aorta by means of microsyringes before, 0.5 min and every 5 min (until abolish of methacholine-induced ST elevation) after test drug administration. Test compound solutions were intravenously administered into the femoral vein. Recording of ECG, sensitivity of ECG, and amplitude of ST segment were achieved by the same method as used with the vasopressin-induced ST depression assay. Values are represented as mean ± SE. The difference of paired mean values was analyzed by the Student's *t*-test and judged to be significant when *p* values were less than 0.05.

Vasopressin-Induced ST Depression in Rats. Male rats of the Donryu strain, weighing 150–220 g, were anesthetized with sodium pentobarbital (60 mg/kg ip). The test compounds suspended in 0.5% tragacanth solution were administered orally 60 min before intravenous administration of vasopressin (0.2 IU/kg). After 60 min, using the method of Hiramatsu et al.,[7a] myocardial hypoxia was produced with vasopressin, which was administered through a cannula in the femoral vein. The electrocardiogram (ECG) of the standard limb lead II was recorded. ST segment depression after vasopressin was measured, as previously described by Hatano et al.[7b] The amplitude of the ST segment was measured at intervals of 30 s for 5 min after administration of vasopressin in each rat.

Calcium Influx in Rat Aorta. Thoracic aorta was cut open longitudinally and a segment weighing about 5–10 mg was used for the experiments. The artery strips were incubated for 5 min in ^{45}Ca (0.8 μCi of ^{45}Ca/mL) containing physiological solution (mM: NaCl 136.9, KCl 5.4, $MgSO_4$ 1.0, $CaCl_2$ 1.5, $NaHCO_3$ 23.8, glucose 5.5). Test compounds were added 30 min prior to and during the ^{45}Ca incubation period. After incubation with ^{45}Ca, the strips were washed for 60 min at 4 °C in the La-substituted solution containing 73.8 mM $LaCl_3$, 5.5 mM glucose and 24 mM tris(hydroxymethyl)aminomethane. After the La-wash period, tissues were placed in scintillation vials and dissolved in soluene solution at 40 °C. The scintillation mixture was added to toluene scintillation solution (mM: dimethyl-POPOP 0.25, PPO 22.6, acetic acid 143), and the radioactivity was counted in a liquid scintillation counter (Packard Model 1460 CD). The results of each determination were convered to the apparent tissue content of

$$^{45}Ca\ (\text{mmol/kg of wet wt}) = \frac{\text{dpm in muscle}}{\text{wet wt (kg)}} \times \frac{\text{nmol of Ca/L of medium}}{\text{dpm/L of medium}}$$

IC_{50} values were estimated from concentration–effect curves.

High Potassium Induced Contraction in Rat Aorta. Muscle tension was recorded isometrically with a force-displacement transducer (Nihon Koden TB-611T). Isosmotic 80.0 mmol K-induced contractions in rat aorta were used for standard K-induced contractions. The test compounds were added 30 min before measurement. The concentrations of the test compounds required to induce a 50% inhibition of contraction (IC_{50}) were calculated from the cumulative concentration-inhibition curves.

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Modeling Alcohol Metabolism with the DARC/CALPHI System

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We present our general system for QSAR search, CALPHI (Computer-Aided Law by Hyperstructure Investigation) set up in the context of the DARC structural language. We use it to construct global, fragmentary, and topological models of the capacity of alcohols to undergo glucuronidation. The DARC/PELCO model, more precisely and more significantly, explains 98% of the total variance with only three parameters, while treating the whole set of primary, secondary, and tertiary alcohols, whereas the best previously reported treatment restricted to primary alcohols, explains only 90% of the variance with two parameters. It provides an explicit and more precise interpretation of alcohol metabolism. The PELCO methodology is extended to evaluate the prediction reliability of both global and fragmentary models. PELCO leads to more predictions when comparison is made at the same level of reliability.

Introduction

It is generally agreed that aliphatic alcohols are metabolized and eliminated from the body by two main pathways, namely by in vivo oxidation to aldehydes, acids, ketones, and carbon dioxide or by conjugation with glucuronic acid to give highly water soluble glucoronides. It is worthwhile determining the factors which influence the path of elimination and to predict that chosen for a particular alcohol, for example in the field of detoxication studies. Thirty alcohols have been tested for their in vivo glucuronic acid conjugation capacity in rabbits.[1] Hansch et al.[2] correlated this activity with the partition coefficient, log *P*, and steric hindrance, E_s, but in two separate correlations for only some of the compounds. Furthermore, while these relationships allow one to evaluate the relative

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