Organic Phosphorus Compounds. 1. 4-(Benzothiazol-2-yl)benzylphosphonate as Potent Calcium Antagonistic Vasodilator

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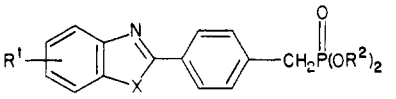
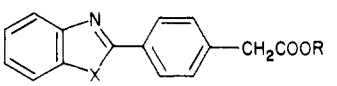
A series of 4-(benzothiazol-2-yl)benzylphosphonic acid dialkyl ester derivatives were synthesized and evaluated for coronary vasodilatory activity by Langendorff's method in the isolated guinea pig heart. Many of the phosphonic acid dialkyl esters exhibited vasodilatory activity and calcium antagonism comparable with those of diltiazem hydrochloride, whereas phosphonic acid **1b** and its nonphosphonated precursor **7a** were inactive. These results indicate the necessity of the diethoxyphosphinyl moiety for vasodilatory activity. Substitution of the benzothiazole ring with a variety of substituents did not significantly enhance the activity of the unsubstituted compound. Compound **10b** (KB-944) was chosen for detailed pharmacological evaluation.

In recent years many compounds have been shown to exhibit calcium antagonism[1-4] through their ability to interfere with calcium flux across cellular membranes. Verapamil,[5] nifedipine,[6] and diltiazem[7] are calcium channel blockers that are widely used clinically to treat angina pectoris, hypertension, and cardiac arrhythmias. Calcium antagonists have also been increasingly important in clinical applications because of their ability to regulate calcium activity and hence influence a variety of cellular functions. This regulation of calcium makes these agents potentially beneficial as therapy for atherosclerosis,[8] platelet aggregation,[9] myocardial infarction,[10] and asthma.[11]

In the course of our effort to find a new class of antiinflammatory agents,[12-14] we synthesized and evaluated a number of benzimidazole-, benzothiazole-, and benzoxazole-substituted benzylphosphonic acid derivatives (**1a** and **1b**) that can be regarded as bioisosteres of phenylacetic acid derivatives **2a**. The phosphonic acid group has been shown to be biologically equivalent to the carboxylic acid group in some cases.[15,16] However, although phenylacetic acid derivatives **2a** are potent antiinflammatory agents,[17,18] phosphonic acid derivatives **1a** and **1b** have not exhibited antiinflammatory activities in our standard assay.[19] On the contrary, some of the intermediate dialkyl phosphonates **1c** showed marked coronary vasodilatory activity along with calcium antagonism.[20] Compound **1c** is a completely new structure having a calcium antagonistic action. These findings prompted us to develop a new class of calcium antagonistic vasodilators from the benzothiazole-substituted phosphonate series. We have synthesized a variety of derivatives whose structural modifications include variation of the alkyl group of the phosphonate and the introduction of various substituents on the benzothiazole ring. Compounds were evaluated for vasodilatory activity in isolated guinea pig hearts (Langendorff's method)[21] and selected agents were evaluated for calcium antagonistic and hypotensive activities. This paper reports the syntheses and cardiovascular activities of these benzothiazole-substituted benzylphosphonates **1c**.



1a, X = O, NH; R^1 = H; R^2 = H
b, X = S; R^1 = H; R^2 = H
c, X = S; R^1 = H, Me, MeO, Cl, HO, AcO, AcNH, NH_2; R^2 = alkyl



2a, X = S, O, NH; R = H
b, X = S; R = Et

Chemistry

Phosphonic acid diesters were prepared by condensing bromomethyl compounds **8** with trialkyl phosphites **9** via the Michaelis–Arbuzov reaction (Scheme I).[22] The intermediate bromomethyl compounds **8** were synthesized through three routes. The unsubstituted compound (**8**, R^1 = H) was obtained by bromination of the condensation product (**7**, R^1 = H) made from *o*-aminothiophenol **11** and *p*-toluic acid in polyphosphoric acid (PPA) (method A). The substituted compounds **8** were derived from the corresponding aniline derivatives **3**, which were condensed with *p*-toluoyl chloride (**4**) in dry pyridine to give the amides **5**. These amides were converted to the thioamides **6** with P_2S_5 or Lawesson's reagent[23,24] in toluene, and ox-

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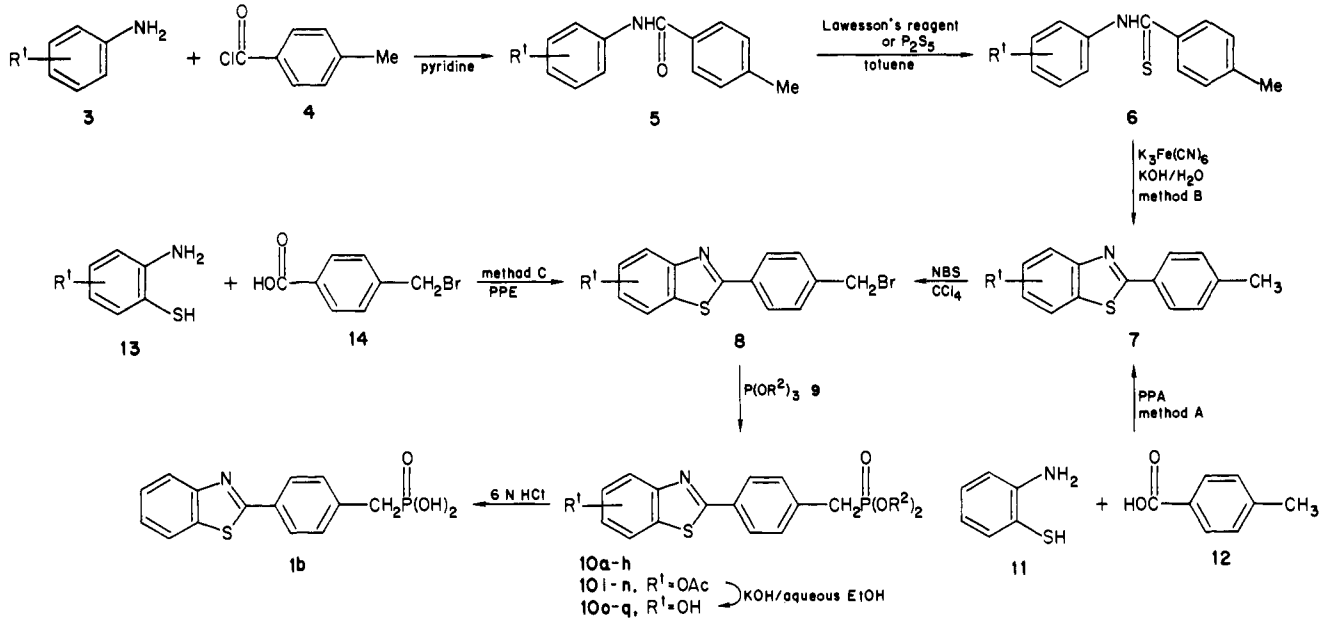
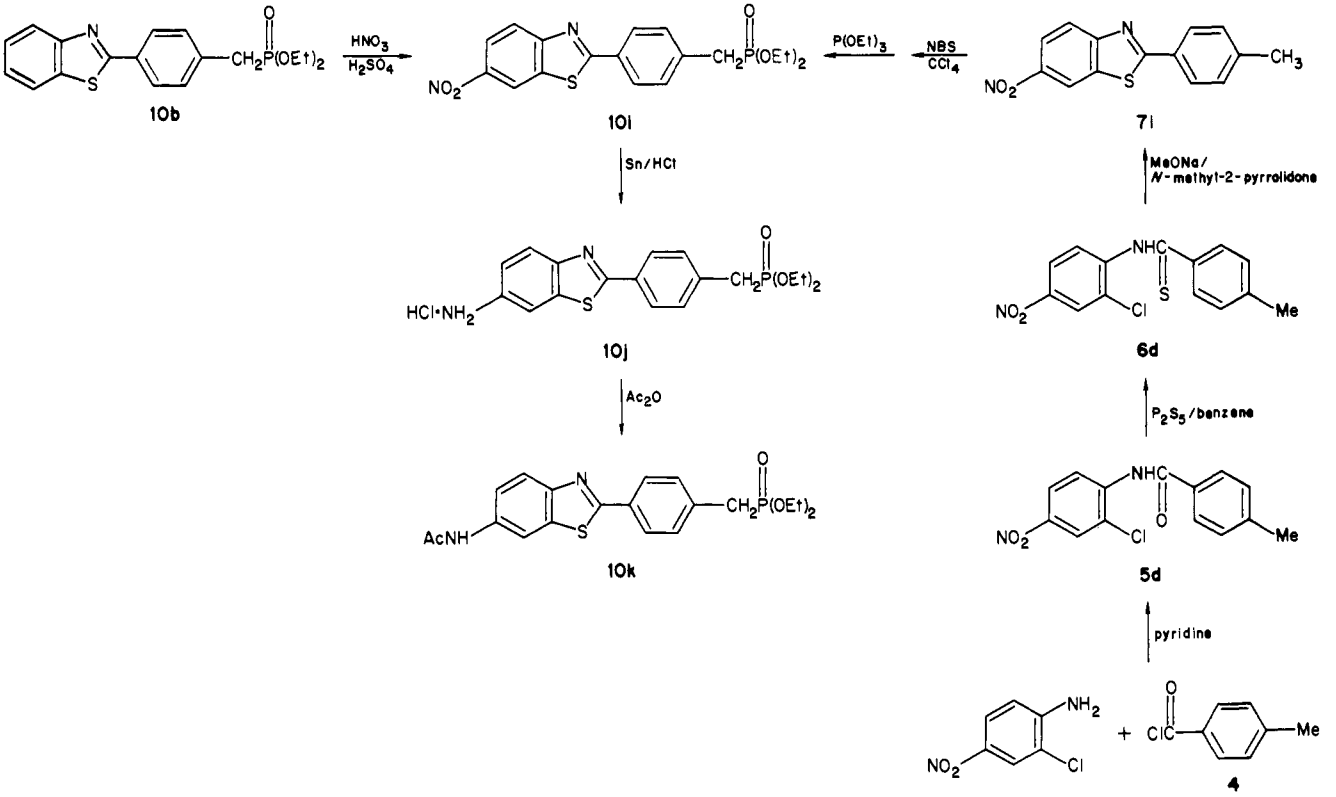
Scheme I



Scheme II



idative ring formation of the thioamides with $K_3Fe(CN)_6$ in the presence of aqueous KOH solution gave the corresponding benzothiazoles **7**.

The 6-chloro-substituted benzothiazole **7k** was brominated with NBS to give **8k** (method B). However, bromination of the methoxy-substituted compounds (**7b–d**, R^1 = MeO) with NBS gave not only the desired **8** but also a benzothiazole ring-brominated compound. Therefore, methoxy- or methyl-substituted benzothiazole derivatives **8** were synthesized by the condensation of methoxy- or methylaminothiophenol **13** and 4-(bromomethyl)benzoic acid (**14**) in PPE (polyphosphate ester) (method C).[25] The acetoxy-substituted derivatives **8** were prepared from the methoxy-substituted compounds (**8**, R^1 = MeO) through hydrolysis with concentrated aqueous HBr to give the phenol (**8**, R^1 = OH), followed by acetylation with acetic anhydride to yield the desired compounds (**8**, R^1 = AcO). The Michaelis–Arbuzov reaction of **8** with trialkyl phosphite **9** was carried out under a nitrogen gas flow without solvent to produce the phosphonate **10** in a good yield.

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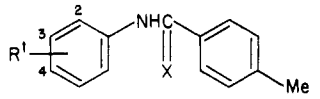
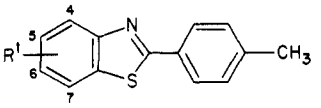
Table I. Substituted Anilides



compd	R^1	X	mp, °C	yield, %	recryst solvent	formula
5a	3-OMe	O	118.3–120.0	70	benzene	$C_{15}H_{15}NO_2$
5b	4-OMe	O	168.0–169.0	quant	benzene	$C_{15}H_{15}NO_2$
5c	4-Cl	O	215.5–216.0	64	benzene	$C_{14}H_{12}ClNO$
5d	2-Cl, 4-NO_2	O	161.0–163.0	79	benzene–cyclohexane	$C_{14}H_{11}ClN_2O_3$
6a	3-OMe	S	73.0–74.0	92[a]	cyclohexane	$C_{15}H_{15}NOS$
6b	4-OMe	S	145.0–146.5	85[a]	benzene	$C_{15}H_{15}NOS$
6c	4-Cl	S	195.0–197.0	67[b]	benzene	$C_{14}H_{12}ClNOS$
6d	2-Cl, 4-NO_2	S	158.0–159.0	39[b]	benzene	$C_{14}H_{11}ClN_2O_2S$

[a] Prepared from the corresponding amide with Lawesson's reagent. [b] Prepared from the corresponding amide with P_2S_5.

Table II. 2-*p*-Tolylbenzothiazole Derivatives



compd	R^1	mp, °C	yield, %	recryst solvent	method of prepn	formula
7a	H	84.0–85.0	76	*n*-hexane	A[b]	$C_{14}H_{11}NS$
7b	5-OMe	99.0–100.0	34	*n*-hexane	B[a]	$C_{15}H_{13}NOS$
7c	6-OMe	112.0–114.0	quant	*n*-hexane	B	$C_{15}H_{13}NOS$
7d	7-OMe	126.0–127.5	46	*n*-hexane	B	$C_{15}H_{13}NOS$
7e	5-OH	255.0–256.0	84	*i*-PrOH	D[c]	$C_{14}H_{11}NOS$
7f	6-OH	245.0–246.0	87	CH_3CN	D	$C_{14}H_{11}NOS$
7g	7-OH	239.0–240.0	96	CH_3CN	D	$C_{14}H_{11}NOS$
7h	5-OAc	128.5–129.5	80	cyclohexane	E[d]	$C_{16}H_{13}NO_2S$
7i	6-OAc	161.0–162.0	64	cyclohexane	E	$C_{16}H_{13}NO_2S$
7j	7-OAc	121.0–122.0	quant	cyclohexane	E	$C_{16}H_{13}NO_2S$
7k	6-Cl	161.0–162.0	70	*n*-hexane	B	$C_{14}H_{10}ClNS$
7l	6-NO_2	201.0–202.5	82	benzene	F[a]	$C_{14}H_{10}N_2O_2S$

[a] See text and Experimental Section. [b] Prepared from the condensation of *o*-aminothiophenol and *p*-toluic acid with PPA. See ref 26. [c] Prepared from the hydrolysis of methoxysubstituted compounds. [d] Prepared from the acetylation of hydroxysubstituted compounds.

The benzylphosphonic acid derivative **1b** was obtained from the phosphonate **10b** by hydrolysis with 6 N HCl. The acetoxy groups of **10l–n** were selectively hydrolyzed with KOH in an aqueous EtOH solution to give the phenol derivatives **10o–q** (Scheme I).

Nitration of **10b** with HNO_3 in concentrated H_2SO_4 gave the 6-nitro-substituted compound **10i** (Scheme II). The position of this nitro group was confirmed by comparison of the product of this reaction with that which was synthesized by an alternative route. Since the oxidative ring formation of **6** (R^1 = 4-NO_2) into the nitrobenzothiazole derivative **7l** as shown in Scheme I failed, the latter was obtained by the cyclization of **6d** in the presence of sodium methoxide as shown in Scheme II. Bromination of **7l** with NBS, followed by condensation with $P(OEt)_3$, produced **10i**, the IR spectrum and melting point of which were identical with those of the nitrated compound obtained from **10b**.

Reduction of **10i** with Sn powder in concentrated HCl gave the aniline derivative **10j**, which was acylated with acetic anhydride to yield the acetanilide **10k**.

Results and Discussion

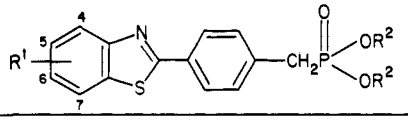
All of the examined phosphonic acid dialkyl esters exhibited excellent coronary vasodilatory activity. The potency of diethyl derivative **10b** was superior to that of reference compounds papaverine hydrochloride or diltiazem hydrochloride,[7] while dimethyl ester **10a** was less potent. Alkyl esters higher than ethyl also showed decreased activity and the phosphonic acid **1b** was inactive. The presence of the electron-donating (**10f**, **10g**, **10o–q**), electron-accepting (**10h**, **10i**, **10l–n**), hydrophilic (**10i**, **10j**, **10l–q**), or hydrophobic groups (**10f**, **10h**) on the benzothiazole ring lowered the activity in comparison to that of the parent compound. These results suggest that the binding site for the benzothiazole ring is sterically limited, and hence, that the projection of a substituent on this ring causes a reduction of the activity. In order to evaluate the role of the dialkyloxyphosphinyl moiety ($P(O)(OR)_2$), the vasodilatory activity of the phosphonate **10b** was compared with that of the precursor **7a** and the corresponding carboxylic acid ethyl ester **2b**[17] (Table III). Since **2b** and **7a** were inactive, the dialkoxyphosphinyl moiety appears to play an important role in imparting the coronary vasodilatory activity to this series of compounds.

Within this series, the diethyl ester **10b** was selected for pharmacological and toxicological evaluation. The results of these studies along with those of diltiazem are shown in Table IV. When **10b** was administered orally to dogs, blood flow through the circumflex branch of the left coronary artery increased and heart rate decreased. A decrease of systemic blood pressure was also observed upon oral administration of **10b**. These coronary vasodilatory and hypotensive activities of **10b** were nearly equipotent to those of diltiazem hydrochloride. On the other hand, the substance was less toxic than diltiazem hydrochloride, as demonstrated in Table IV. Interestingly, in isolated guinea pig tenia coli, **10b** exhibited calcium antagonistic activity comparable to that of diltiazem hydrochloride. Therefore, the coronary vasodilatory and hypotensive activities of this agent may be due to this calcium antagonistic property.[20] These results suggest that the phosphonate **10b**, coded KB-944, is a promising coronary vasodilatory agent, and it is now undergoing clinical trial.

Experimental Section

Melting points were taken on a capillary melting point apparatus (Yamato MR-21) and were uncorrected. The structures of

Table III. Chemical and Pharmacological Data of 4-Benzothiazol-2-ylbenzylphosphonic Acid Derivatives



compd	R^1	R^2	mp, °C	yield, %	recryst solvent	dose, μg/heart	max increase in coronary flow,[a] %
1b	H	H	256.0–258.0	quant	*i*-PrOH	10	inactive
10a	H	Me	130.0–131.0	57	*n*-hexane	10	34.8 ± 8.1
10b	H	Et	96.0–97.0	92	*n*-hexane	10	79.9 ± 8.6[b]
10c	H	*n*-Pr	108.0–109.0	93	*n*-hexane	10	62.4 ± 11.7
10d	H	*i*-Pr	121.0–122.0	75	*n*-hexane	10	75.6 ± 12.4
10e	H	*n*-Bu	106.0–107.0	81	*n*-hexane	10	37.3 ± 6.9
10f	6-Me	Et	101.0–103.0	82	cyclohexane	10	31.3 ± 7.7
10g	6-OMe	Et	127.0–128.0	88	cyclohexane	10	20.1 ± 4.5
10h	6-Cl	Et	146.0–147.0	73	cyclohexane	10	54.6 ± 5.6
10i	6-NO_2	Et	145.0–147.0	87	chclohexane	10	inactive
10j	6-NH_2·HCl	Et	200 dec	75	MeOH–ether	10	50.0 ± 12.7
10k	6-NHAc	Et	197.0–200.0	70	MeOH	10	48.7 ± 17.4
10l	5-OAc	Et	116.0–117.0	82	cyclohexane	10	23.2 ± 2.3
10m	6-OAc	Et	124.0–125.0	79	cyclohexane	10	56.7 ± 14.4
10n	7-OAc	Et	99.0–101.0	77	cyclohexane	10	71.7 ± 8.8
10o	5-OH	Et	137.0–138.0	75	*i*-PrOH	10	49.0 ± 10.6
10p	6-OH	Et	202.0–203.0	83	EtOAc	10	63.3 ± 7.6
10q	7-OH	Et	196.0–197.0	63	benzene	10	20.6 ± 6.6
7a						10	inactive
2b						10	inactive
papaverine hydrochloride						10	55.8 ± 4.7
diltiazem hydrochloride						10	63.9 ± 8.9

[a] Langendorff's method in isolated guinea pig heart. See ref 21. The results are presented as the mean ± SE for five experiments. [b] $p < 0.05$ when compared with the reference compound papaverine hydrochloride.

Table IV. Pharmacological Profile of **10b** and Diltiazem

	coronary flow increase and hypotensive action (dogs, intraduodenal administration, 10 mg/kg)[a]					
compd	time after administration, min	increase in blood flow through circumflex branch of left coronary artery, %	change in mean blood pressure, mmHg	change in heart rate, beats/min	pA_2[b] (mean ± standard error)	mouse LD_{50},[c] mg/kg, ip
10b	5	28.2 ± 15.1	−13.4 ± 3.0	−6.9 ± 2.9	6.92 ± 0.28	962
	30	65.2 ± 16.0	−9.7 ± 3.3	−18.3 ± 7.2		
	60	87.5 ± 16.1	−12.0 ± 3.0	−20.8 ± 6.7		
	120	91.8 ± 26.6	−13.3 ± 4.7	−18.7 ± 6.0		
diltiazem hydrochloride	5	6.5 ± 4.1	−2.7 ± 2.6	3.2 ± 1.1	6.38 ± 0.12	177
	30	85.5 ± 21.1	−10.7 ± 5.1	−19.7 ± 4.0		
	60	89.4 ± 23.3	−13.8 ± 7.1	−29.2 ± 4.7		
	120	74.6 ± 23.5	−14.5 ± 7.2	−27.2 ± 4.9		

[a] The results are presented as the mean ± SE for six experiments. [b] Calcium antagonistic activity. See text. [c] A suspension of each compound in 0.5% CMC was administered and the number of dead animals within 72 h were counted, the LD_{50} values being calculated by the method of Weil.[30]

all compounds were supported by their IR (Shimazu IR-440) and 60- and 100-MHz ^1H NMR (Hitachi R-24A and Nihon Denshi PS-100) spectra. All compounds were analyzed for C, H, and N, and the results were within 0.4% of the calculated theoretical values. No attempt was made to maximize the yields.

2-[4-(Bromomethyl)phenyl]benzothiazole (8a). General Brominating Procedure. To a solution of 45 g (0.20 mol) of 2-(4-methylphenyl)benzothiazole (**7a**) in 1000 mL of dry carbon tetrachloride were added 35.6 g (0.20 mol) of *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide. The mixture was refluxed for 12 h and then allowed to cool to room temperature. The precipitated succinimide was filtered, and the filtrate was evaporated to dryness in vacuo to give 55.0 g of crude crystals. Recrystallization from cyclohexane gave 41.0 g (67.4%) of purified **8a** as colorless flakes: mp 134.0–135.0 °C.

Diethyl 4-Benzothiazol-2-ylbenzylphosphonate (10b). General Arbuzov Procedure. A mixture of 6.08 g (0.02 mol) of **8a** and 10 mL (ca. 0.058 mol) of triethyl phosphite was heated at 130–160 °C for 15 min under a slow nitrogen gas flow. The reaction mixture was allowed to cool to room temperature, and the resulting solid was recrystallized from *n*-hexane to give 12.0 g (92%) of **10b** as colorless plates: mp 96.0–97.0 °C.

4-Methyl-3′-methoxybenzanilide (5a). A mixture of *p*-toluic acid (28 g, 0.20 mol), $SOCl_2$ (80 g, 0.67 mol), and a catalytic amount of DMF was refluxed for 1.5 h. The resulting solution was evaporated under reduced pressure to give an oil, which was then dissolved in THF (40 mL) and added, over a 30 min-period, to a stirred solution of *m*-anisidine (25 g, 0.02 mol) in dry pyridine (250 mL) at 0–5 °C. After addition, stirring was continued at room temperature for 1 h, before the reaction mixture was poured into ice–water (2.5 L). The precipitated solid was collected and recrystallized from benzene to yield 36.8 g (76%) of **5a** as colorless needles: mp 118.0–120.0 °C.

4-Methyl-3′-methoxythiobenzanilide (6a). To a solution of **5a** (12.0 g, 0.05 mol) in toluene (60 mL) was added 12.0 g (0.03 mol) of 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide) (Lawesson's reagent).[23,24] After stirring for 1 h under reflux, the reaction mixture was evaporated under reduced pressure. The residual oil was chromatographed on silica gel (Merck Kieselgel 60, 230–400 mesh, 300 g; eluted with benzene), and the purified material was recrystallized from cyclohexane to yield 11.8 g (92%) of **6a** as yellow needles: mp 73.0–74.0 °C.

2-(4-Methylphenyl)-5-methoxybenzothiazole (7b). Method B. To a suspension of **6a** (11.8 g, 0.04 mol) in 1000 mL of H_2O was added $K_3Fe(CN)_6$ (30.2 g, 0.092 mol) and KOH (10.3 g, 0.184 mol), and the reaction mixture was stirred at room temperature for 3 h. The resulting suspension was filtered and the filtrate was washed with H_2O (500 mL). After drying and solvent removal, the resulting mixture of isomers was separated with column chromatography (silica gel) by eluting with benzene. Recrystallization of the two isolated compounds from *n*-hexane yielded 4.0 g (34%) of 2-(4-methylphenyl)-5-methoxybenzothiazole (**7b**; mp 99.0–100.0 °C) and 5.4 g (46%) of 2-(4-methylphenyl)-7-methoxybenzothiazole (**7d**; mp 126.0–127.5 °C).

2-(4-Methylphenyl)-5-hydroxybenzothiazole (7e). A suspension of **7b** (3.3 g, 0.0129 mol) in 100 mL of concentrated HBr was refluxed for 6 h. The reaction mixture was cooled to room temperature and poured into 200 g of ice–water, and the pH of the solution was adjusted to 5 by the addition of concentrated aqueous Na_2CO_3 solution. The resulting solid was collected and recrystallized from *i*-PrOH to yield 2.2 g (71%) of **7e**: mp 255.0–256.0 °C.

2-(4-Methylphenyl)-5-acetoxybenzothiazole (7h). A solution of **7e** (3.1 g, 0.013 mol) in Ac_2O (50 g, 0.49 mol) was refluxed for 3 h. The solution was then evaporated under reduced pressure to give a solid, which was washed with 10% aqueous Na_2CO_3 solution followed by H_2O. Recrystallization of the solid from cyclohexane yielded 2.9 g (80%) of **7h** as colorless needles: mp 128.5–129.5 °C.

Diethyl 4-(5-Hydroxybenzothiazol-2-yl)benzylphosphonate (10o). To a solution of 0.40 g (0.95 mmol) of **10l** in 10 mL of EtOH was added 0.19 g (2.9 mmol) of KOH in 2 mL of H_2O. The mixture was refluxed for 30 min and then evaporated under reduced pressure. The residue was dissolved in 10 mL of H_2O and the insoluble material was removed by filtration. The aqueous solution was acidified with 10% aqueous HCl and the precipitated solid was collected by filtration. Recrystallization of the solid from *i*-PrOH yielded 0.27 g (75%) of **10o** as pale yellow leaflets: mp 137.0–138.0 °C.

2-[4-(Bromomethyl)phenyl]-6-methylbenzothiazole (8c). Method C. A mixture of 2-amino-5-methylbenzenethiol[27] (6.95 g, 0.05 mol), 4-(bromomethyl)benzoic acid[28] (10.8 g, 0.05 mol), and PPE[25] (150 g) was heated at 82 °C for 5 h under a nitrogen gas flow. The mixture was poured into water and the solution was extracted with chloroform. The organic layer was washed with water and dried over anhydrous sodium sulfate and the chloroform was evaporated under reduced pressure to give a brown residue. Column chromatography of the residue on silica gel (300 g) with chloroform as an eluent gave a pale brown solid. Recrystallization of the solid from ethyl acetate yielded 3.6 g (18%) of **8c** as pale yellow needles: mp 164.0–166.0 °C.

2-[4-(Bromomethyl)phenyl]-6-methoxybenzothiazole (8d). A mixture of 2-amino-5-methoxybenzenethiol[27] (7.75 g, 0.05 mol), 4-bromomethylbenzoic acid[28] (10.8 g, 0.05 mol), and PPE[25] (150 g) was heated at 86 °C for 2 h under a nitrogen gas flow. The mixture was poured into water and extracted with chloroform. The organic layer was then separated, washed with water, and dried over anhydrous sodium sulfate. Removal of the chloroform under reduced pressure gave a brown residue, which was chromatographed on silica gel (300 g) with chloroform as an eluent to give a pale yellow solid. Recrystallization of the solid from ethyl acetate gave 1.47 g (8.8%) of **8d** as yellow plates: mp 162.0–164.0 °C.

4-Methyl-2′-chloro-4′-nitrobenzanilide (5d). *p*-Toluoyl chloride (25 g, 0.16 mol) was added dropwise with cooling to a stirred solution of 2-chloro-4-nitroaniline (27.6 g, 0.16 mol) in pyridine. After addition, the mixture was heated at 100 °C for 30 min and then cooled to room temperature. Water was added and the precipitate was collected and washed with water. Recrystallization of the solid from benzene–cyclohexane gave 36.9 g (79%) of **5d** as pale yellow needles: mp 161.0–163.0 °C.

4-Methyl-2′-chloro-4′-nitrothiobenzanilide (6d). Phosphorus pentasulfide (20.1 g, 90 mmol) was added to a solution of **5d** (17.5 g, 60 mmol) in benzene (250 mL) and the mixture was refluxed for 20 h. After the mixture was cooled, the insoluble materials were removed by filtration and then washed with benzene. The combined benzene solution was evaporated to dryness, and the residual brown solid was chromatographed on silica gel (300 g) with benzene as an eluent to afford 7.3 g (39%) of **6d** as yellow prisms after recrystallization from benzene: mp 158.0–159.0 °C.

2-(4-Methylphenyl)-6-nitrobenzothiazole (7l). To a solution of 6.15 g (0.02 mol) of **6d** in *N*-methyl-2-pyrrolidone (100 mL) was added 1.62 g (0.03 mol) of sodium methoxide, and the mixture was heated at 110 °C for 3.5 h. The cooled reaction mixture was poured into water and the resulting precipitate was collected and dried. Recrystallization from benzene yielded 4.35 g (82%) of **7l** as yellow needles: mp 201.0–202.5 °C.

Diethyl 4-(6-Nitrobenzothiazol-2-yl)benzylphosphonate (10i). Nitric acid (d = 1.42, 1.5 mL, 0.022 mol) was added to a stirred solution of **10b** (6.0 g, 0.017 mol) in concentrated H_2SO_4 (25 mL) at 0–5 °C over a 10-min period and the mixture was stirred for 1 h at 0–10 °C. The solution was poured into ice–water (50 mL) and the aqueous solution was then extracted with benzene (50 mL × 3). The extracts were dried ($MgSO_4$) and evaporated to give an oil, which was recrystallized from cyclohexane to afford 5.9 g (87%) of **10i**: mp 145.0–147.0 °C.

Diethyl 4-(6-Aminobenzothiazol-2-yl)benzylphosphonate Hydrochloride (10j). Tin powder (1.2 g, 0.01 mol) was added to a solution of **10i** (2.5 g, 6.1 mmol) in concentrated HCl (25 mL) at a rate such that the temperature of the reaction mixture did not exceed 30 °C. Stirring of the mixture was continued for 1 h at room temperture. The resulting suspension was added at 10–20 °C to ice–water (100 mL) with stirring. The mixture was stirred for 0.5 h and the pH then adjusted to 10 by the addition of 1 N NaOH. The resulting suspension was extracted with ethyl acetate (200 mL × 3). The extracts were dried ($MgSO_4$) and evaporated to give a solid 1.9 g (82%), which was dissolved in $CHCl_3$ (30 mL). The solution was saturated with HCl gas and the precipitated solid was collected by filtration. Recrystallization of the solid from MeOH–Et_2O yielded 1.7 g (68%) of **10j** as colorless prisms: mp 200 °C dec.

Diethyl 4-[6-(Acetylamino)benzothiazol-2-yl]benzylphosphonate (10k). A solution of **10j** (1 g, 2.7 mmol) in Ac_2O (4.0 g, 39 mmol) was stirred at 120 °C for 20 min. The mixture was cooled to room temperature, and the precipitated solid was collected and washed with 10% aqueous Na_2CO_3 solution and water. Recrystallization of the solid from MeOH gave 0.80 g (70%) of **10k** as colorless prisms: mp 197.0–200 °C.

4-Benzothiazol-2-ylbenzylphosphonic Acid (1b). A suspension of **10b** (12.5 g, 0.0346 mol) in 6 N HCl (100 mL) was refluxed for 6 h. The resulting solution was cooled and the precipitated solid was collected and washed with water to give a yellow crystalline powder (9.8 g, quantitative). Recrystallization of the powder from *i*-PrOH gave **1b** as colorless needles: mp 256.0–258.0 °C.

Effect on Coronary Flow in the Isolated Guinea Pig Heart. Male guinea pigs of 400–500 g body weight were slaughtered and promptly thoracotomized. After cannulation of the ascending aorta, the heart was enucleated. The isolated heart was then perfused with Krebs–Henseleit fluid, which was oxygenated with a gaseous mixture of 95% O_2 and 5% CO_2, at 34 ± 1 °C under a perfusion pressure of 60 cm of H_2O by the method of Langendorff. The test compound dissolved in propylene glycol to a concentration of 100 μg/mL was then infused at a rate of 0.1 mL/min. The coronary flow was measured with a square-wave electromagnetic flow meter (Nihon Kohden, MF-26) with an extracorporal probe (Nihon Kohden, **FE**) set at the top of the cannula and recorded with a multipurpose polygraph (Nihon Kohden, RM-85). The coronary flows before and after infusion were measured, and the percentage gain in coronary flow was obtained. The results are shown in Table IV. For reference, the corresponding data on diltiazem hydrochloride are also shown.

Calcium Antagonistic Activity. Male guinea pigs, each weighing 350–450 g, were slaughtered, and the test was conducted with isolated tenia coli specimens, each about 2 cm long. The contractile response of each tenia coli, suspended in aerated Locke solution in a Magnus chamber at 25 °C, was recorded through an isotonic transducer. Calcium was cumulatively added (from 0.1 to 100 mM) to the decalcified tenia coli, in the presence of

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6×10^{-3} g/mL of K^+, to obtain a dose–response curve for calcium, which was determined in the presence of the test compound. On the basis of difference between the responses, the pA_2 value of the calcium antagonistic activity of the test compound was computed.[29] The results are shown in Table IV.

Effect of Intraduodenal Administration on Coronary Flow and Blood Pressure in Dogs. Dogs weighing 12–22 kg were anesthetized with pentobarbital sodium (35 mg/kg, ip), and under supportive respiration, at left thoracotomy was performed at the fourth intercostal space. The pericardium was incised to expose the heart and to facilitate measurement of the blood flow through the circumflex branch of the left coronary artery by means of an electromagnetic flowmeter (Nihon Kohden Co. Ltd., MF-26). The blood pressure was measured via a cannula inserted into the carotid artery connected to a pressure transducer (Nihon Kohden Co. Ltd., MPU-0.5), while the heart rate was calculated from the electrocardiogram. The abdomen was then sutured, leaving the end of the cannula outside of the body, and the test compound was administered. The test compound was diluted with 0.5% CMC to a concentration of 10 mg/mL and administered at a dose of 10 mg/kg.

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Agents for the Treatment of Brain Edema. 2. [(2,3,9,9a-Tetrahydro-3-oxo-9a-substituted-1*H*-fluoren-7-yl)oxy]alkanoic Acids and Some of Their Analogues

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Our initial paper discussed brain edema resulting from traumatic head injury and the need for specific and effective agents to treat the disorder and disclosed a novel approach for the discovery of a drug of this kind. The current study describes the synthesis of a series of [(2,3,9,9a-tetrahydro-3-oxo-9a-substituted-1*H*-fluoren-7-yl)oxy]alkanoic acids and their analogues. These compounds were evaluated in an in vitro cerebrocortical tissue slice assay for their relative potencies in inhibiting $K^+ + HCO_3^-$ induced swelling. Structural modification at a number of sites in the "lead" compound revealed that significant biological activity was inherent only within a very narrow range of structural types. The observation that nearly all the biological activity resided in one of the two enantiomers demonstrated the marked stereospecificity of the most active compounds. One of the most potent compounds, (*R*)-(+)-[(5,6-dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1*H*-fluoren-7-yl)oxy]acetic acid ((+)-**5c**), exhibited a dose–response relationship in the in vivo acceleration/deceleration brain edema assay, and the data from the two highest doses were statistically significant. Electron microscopic examination demonstrated that the perivascular astroglial swelling that arises from this procedure is abolished in the animals treated with (+)-**5c**. This compound is currently being evaluated for its clinical efficacy and safety in the treatment of traumatic head injury.

Our first report[1] on the design and development of agents for treating brain injury emphasized the high incidence of this disorder, the present lack of effective, specific therapeutic agents to treat this problem, and other potential medical applications for a drug of this type. The rationale for a novel approach to drug therapy for this disorder was delineated. The concept was based on the sequence of events believed to follow traumatic insult to the brain: edema (including cellular swelling or cellular edema), ischemia, hypoxia, neuronal death, and necrosis, which sometimes leads to irreversible coma and death.[1]

Prevention of the cellular edema was viewed as a logical place to focus therapeutic intervention. Gray matter was considered to be the major site of clinical importance, with the astrocyte being the specific cell type involved.[2-4] The swelling was shown to result from a chloride plus cation transport into astrocytes accompanied by an osmotic equivalent of water.[3,4] The investigation was facilitated by the development of in vitro assays using cat cerebrocortical tissue slices in which inhibition of chloride transport and/or inhibition of swelling, all or a significant part of which is due to astrocytes, could be readily measured.[1,3]

Our initial studies involved an investigation of a variety of loop diuretics such as ethacrynic acid and (indanyloxy)acetic acids, which were known to owe at least part of their saliuretic activity to the inhibition of Cl^--transport in Henle's loop. The examination of several series of (aryloxy)alkanoic acids that had been designed as salidiuretic agents, including their nonsalidiuretic members, was instituted. It soon became obvious that the effects of these compounds on Cl^- transport in the kidney and the brain did not always run parallel. Of greatest interest were those compounds that exhibited marked Cl^--transport inhibitory activity in brain slices but displayed little or no effect in the kidney. Some (indanyloxy)alkanoic acids possessing these properties were described in our first paper[1] along with a discussion of the structural features that appeared to be responsible for the separation of effects.

Subsequent to the observation of specific anti brain edema activity in certain (indanyloxy)alkanoic acids, it was

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