containing the internal standard (*O*-acetophenitidine) was added 300 μL of cold acetonitrile with vigorous mixing to precipitate proteins. Mixing was continued for 30 s, and then 100 mg of sodium chloride was added to each tube, and the tubes were briefly mixed and centrifuged at 10000 rpm for 5 min. For serum, 200 μL of supernatant obtained from centrifugation was evaporated to dryness under nitrogen at 25 °C whereas for brain homogenates all of the supernatant was evaporated to dryness. The residual film was reconstituted in 100 μL of mobile phase and a 75-90-μL aliquot was injected onto the HPLC system.

Chromatographic separations were achieved on a Hypersil ODS analytical column (5 μ, 4.6 × 150 mm) preceded by a guard column filled with 30-40 μm pellicular RP-18 perisorb material. The mobile phase consisted of 30% (v/v) acetonitrile in 40 mM aqueous sodium acetate containing 4 mM sodium lauryl sulfate. The mobile phase flow rate was 2 mL/min and the detector wavelength was set at 260 nm. AzddU and AZT were quantitated in serum and brain homogenates by a previously published method.[22] Serum and brain homogenate samples prepared for prodrug and quaternary salt were processed immediately for HPLC analyses to avoid degradation. All analytical procedures had intra- and interday coefficients of variations less than 15%.

In Vitro Stability Determination in Serum, Brain Homogenate, and Phosphate Buffer (pH 7.4). AzddU-DHP or AZT-DHP (as a 5 or 1 mg/mL standard in DMSO) were added to give an initial concentration of either 50 or 10 μg/mL, respectively, in 3 mL of phosphate buffer (pH 7.4) and freshly collected human serum, mouse serum, or brain homogenate (1:1 g/mL in a physiological phosphate buffer) maintained at 37 °C on a shaking water bath. One-hundred microliter aliquots were withdrawn at time zero and at 0.083, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 10 h after adding the prodrugs. The aliquots were analyzed for the dihydropyridine, quaternary salt, and parent drug moieties as described above.

In Vivo Studies. Female NIH-Swiss mice weighing 25-30 g were administered intravenously with AzddU (50 mg/kg), AZT (50 mg/kg), AzddU-DHP (73.9 mg/kg, equivalent to 50 mg/kg AzddU), or AZT-DHP (72.7 mg/kg, equivalent to 50 mg/kg AZT) dissolved in DMSO (50 mg/mL) through a tail vein over 30 s. Animals were momentarily restrained during dosing and then placed in individual cages and allowed food and water ad libitum. Three mice each were killed at 5, 15, 30, 45, and 60 min and 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h following dosing. Animals were killed by exsanguination via left ventricle heart puncture after anesthetization with diethyl ether. Serum was harvested from blood collected from the heart. The brain was excised, rinsed with normal saline, blotted dry, and weighed. A brain homogenate was prepared in a 1:1 (g/mL) ratio with ice-cold, pH 7.4, isotonic phosphate buffer. Serum and brain samples were processed immediately for the analysis of the prodrug and salt by an ion-pair HPLC method. Parent drug was analyzed separately by an HPLC method.[22]

Data Analysis. In Vitro Studies. The terminal phase concentrations for AzddU-DHP AzddU-QS, AZT-DHP, and AZT-QS were used to determine degradation rate constant (k) and the associated half-life in each media. The degradation rate constant (K) was equal to the slope of the equation obtained from linear regression of the natural log of the concentration versus time values in the terminal phase. The terminal phase concentrations for the quaternary salt species could represent the formation of the salt from the prodrug or degradation of the salt to the parent compound. In the current analysis, it is assumed that the degradation rate of the salt is slower than the formation rate from the dihydropyridine derivatives.

In Vivo Studies. The measured mouse serum and brain concentrations were used to calculate the area under the serum or brain concentration-time curve (AUC) from time zero to infinity. A Lagrange polynomial integration method[32] was used to obtain the AUC to the last observed time point, t_n. The AUC from t_n to infinity was estimated by C_N/k, where C_N equals the observed concentration at t_n and k equals the terminal disposition rate constant. The terminal disposition rate constant (k) was equal to the slope of the equation obtained from linear regression of the natural log of the concentration and time values in the terminal phase.

The relative brain exposure[33] to AzddU and AZT was calculated as $r_e = (AUC)_{pd\rightarrow p}/(AUC)_p$, where $(AUC)_{pd\rightarrow p}$ equals the area under the AzddU or AZT brain concentration-time curve following administration of the prodrug (PD), and $(AUC)_P$ is the same area obtained following administration of the parent (P) compounds AzddU or AZT. r_e values greater than 1 indicate favorable brain delivery of the parent compounds following prodrug administration.

An apparent elimination half-life for AzddU and AZT in brain following prodrug and parent drug administration was calculated as $0.693/k$, where k was determined as described above.

Antiviral Assays. The antiviral activity of the DHP analogues of AZT and AzddU were determined In PBM cells infected with HIV-1 (strain LAV) as described previously.[15] For comparison, AZT and AzddU were included.

Acknowledgment. This work was supported by the U.S. Public Health Service Grant No. AI25899 and AI26055 from the National Institute of Allergy and Infectious Diseases and the Department of Veterans Affairs.

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Organic Phosphorus Compounds. 5. (4-Benzothiazol-2-ylbenzyl)amidophosphonate as Potent Calcium Antagonistic Vasodilators[1]

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Structural modifications of the calcium antagonist fostedil (KB-944) and their coronary vasodilator activity are described. Amidophosphonates **4a-m**, lactam amidophosphonates **7a-l**, and diamide dilactam **10** were prepared, and their coronary vasodilator activity was assessed in dogs. Many compounds exhibited coronary vasodilator activity superior to that of fostedil. Among them, the 2-oxopyrrolidine derivative **7a** was the most effective compound. Its action as a coronary vasodilator was 3 and 2 times more potent than that of fostedil and diltiazem hydrochloride, respectively.

Compounds of general structure 1 have been shown to exhibit coronary vasodilator activity[2,3] by binding to the protein of the voltage-sensitive calcium channel present on the membrane of smooth muscles.[4-6] Among these

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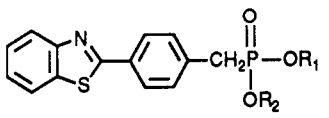


1

fostedil (1a): $R_1 = R_2 = Et$

compounds, fostedil (1a, $R_1 = R_2 = Et$) was found to have superior coronary vasodilator activity.[2] Previous studies have shown that the (diethoxyphosphinyl)methyl moiety plays an important role in the coronary vasodilator action of fostedil.[2,7] Modification of the (diethoxyphosphinyl)-methyl moiety demonstrated that the vasodilator activity was retained in asymmetric dialkyl phosphonates or cyclic phosphonates.[1] As part of a structure–activity study on fostedil, the amidophosphonate derivatives **4a–m**, **7a–l**, and **10** were prepared and evaluated for coronary vasodilator activity.

Chemistry

Preparation of amidophosphonates **4a–m** and lactam amidophosphonates **7a–i** are summarized in Schemes I and II. A phosphonochloridate **2** was synthesized by treating fostedil (**1a**) with thionyl chloride. Chloridate **2** was further reacted with amines **3** in dichloromethane, to give the amidophosphonates **4a–m**. Reaction of phosphonochloridate **2** and soodium salts of lactams **5** in THF gave the lactam amidophosphonates **7a–i**. Treatment of **7a** with Lawesson's reagent[8,9] in toluene gave the thiolactam amidophosphonate **7j**. Thiolactam amide **7k** and **7l** were prepared from phosphonochloridate **2** and the sodium salt of thiolactam **6** in THF.

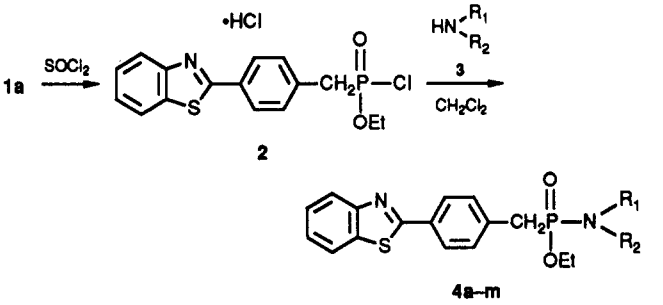
Phosphonic diamide dilactam **10** was synthesized as shown in Scheme III. Hydrolysis of fostedil (**1a**) in 6 N HCl gave the phosphonic acid **8**, which was chlorinated with thionyl chloride. The resulting phosphonic dichloride **9** was reacted with 2 molar equiv of sodium salt of pyrrolidone in THF to give a phosphonic diamide dilactam **10**.

Physicochemical properties of the diethoxyphosphinyl and (ethoxy)(2-oxopyrrolidino)phosphinyl groups were examined briefly, and the results are shown in Table IV. Diethoxyphosphinyl, (ethoxy)(2-oxopyrrolidino)phosphinyl, and ethoxycarbonyl groups are electron-withdrawing group. The introduction of the diethoxyphosphinyl or (ethoxy)(2-oxopyrrolidino)phosphinyl group into **13** increased the solubility in water (3.5 and 7.5 times, respectively). On the other hand, the ethoxycarbonyl group did not increase the solubility in water.

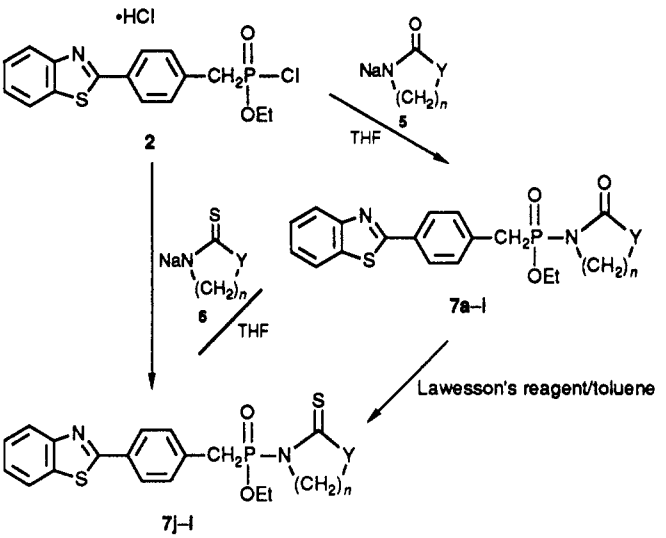
Biological Results and Discussion

The coronary vasodilator activity of the amidophosphonates **4a–m** was assessed in dogs (iv injection). The results are shown in Table I. Except for **4e**, all the amidophosphonates exhibited some degree of coronary

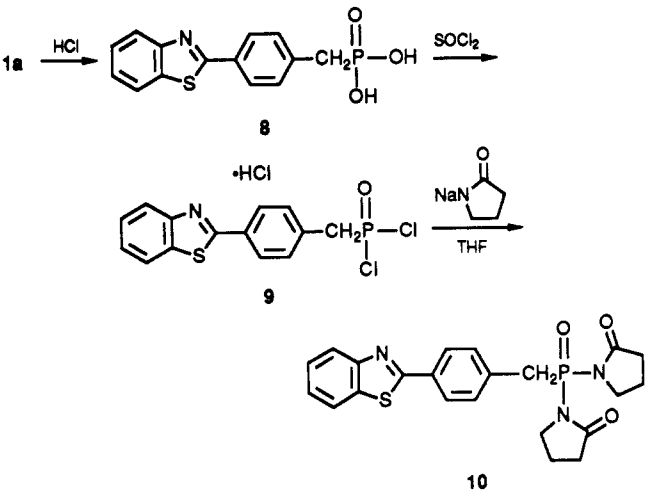
Scheme I



Scheme II



Scheme III



vasodilator activity. In particular, cyclic amidophosphonates had potent activity. Pyrrolidinophosphonate **4g** and piperazinophosphonate **4l** showed higher coronary vasodilator activity than that of fostedil. However, when **4g** or **4l** was orally administered to dogs, no coronary vasodilator effect was observed. To clarify this finding, we examined the stability of **4g** in acidic solution (pH 1.5) and found that **4g** was rapidly hydrolyzed to the corresponding inactive monoester **11**, as shown in Scheme IV. The half-life of acid-catalyzed hydrolysis of **4g** was about 7 min. These results suggested that **4g** may be unstable in the gastrointestinal tract and may be rapidly hydrolyzed to an inactive monoester.

It was believed that the instability of **4g** under acidic conditions might arise from protonation of the basic ni-

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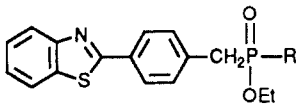
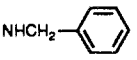
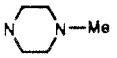
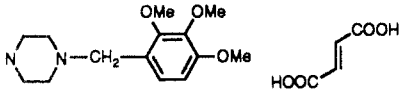
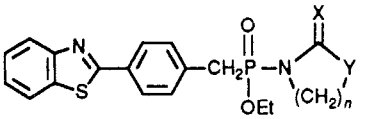
Table I. Effect of Amidophosphonates on Coronary Flow of Anesthetized Dogs



compd no.	R	yield, %	mp, °C	recryst solvent[a]	formula[b]	max increase in coronary flow, % (dog, 0.1 mg/kg, iv)
4a	NHEt	42	149–150	A	$C_{18}H_{21}N_2O_2PS$	31.2[c]
4b	NHiPr	57	166–167	A	$C_{19}H_{23}N_2O_2PS$	73.2[c]
4c		54	157–158	B	$C_{19}H_{21}N_2O_2PS$	41.1[c]
4d	$NHCH_2CH_2Cl$	18	146–147	C	$C_{18}H_{20}C_1N_2O_2PS$	31.2[c]
4e		76	151–152	B	$C_{23}H_{23}N_2O_2PS$	inactive
4f	NEt_2	54	100–101	D	$C_{20}H_{25}N_2O_2PS$	79.7 ± 0.1[d]
4g		78	145–146	E	$C_{20}H_{23}N_2O_2PS$	101.1 ± 13.1[d]
4h		21	146–147	B	$C_{20}H_{21}N_2O_2PS$	80.6[c]
4i		60	133–135	E	$C_{21}H_{25}N_2O_2PS$	81.3 ± 8.2[d]
4j		57	142–143	E	$C_{20}H_{23}N_2O_3PS$	69.0[c]
4k		53	105–107	D	$C_{21}H_{26}N_3O_2PS$	49.3[c]
4l		42	161–162	B	$C_{34}H_{40}N_3O_9PS$	103.5 ± 3.3[d]
4m		30	96–101	E	$C_{22}H_{27}N_2O_2PS$	49.3[c]
fostedil (R = OEt)						82.2 ± 5.0[d]

[a] Solvent of crystallization: A = benzene, B = AcOEt; C = *n*-heptane, D = *n*-hexane, and E = cyclohexane. [b] Elemental analyses for C, H, N were within 0.4% of calculated values. [c] The tested compound was dissolved in 0.9% saline which contained 20% polyethylene glycol 400 (PEG-400) and 20% ethanol and injected into the femoral vein at a dose 0.1 mg/kg. Experiments were carried out in duplicate; typical variation was less than ±15%. [d] The results are presented as the mean ± SE for five experiments.

Table II. Effect of Lactam amidophosphonates on Coronary Flow of Anesthetized Dogs



compd no.	X	Y	*n*	yield, %	mp, °C	recryst solvent[a]	formula[b]	max increase in coronary blood flow, % (dog, 0.1 mg/kg, iv)[c]
7a	O	CH_2	2	88	128–130	A	$C_{20}H_{21}N_2O_3PS$	124.1 ± 9.0
7b	O	CH_2	3	46	121–123	A	$C_{21}H_{23}N_2O_3PS$	101.9 ● 7.4
7c	O	CH_2	4	60	125–127	B	$C_{22}H_{25}N_2O_3PS$	102.8 ± 9.0
7d	O	CH_2	5	30	134–136	A	$C_{23}H_{27}N_2O_3PS$	70.7 ± 9.9
7e	O	O	2	54	134–136	C	$C_{19}H_{19}N_2O_4PS$	141.4 ± 22.2
7f	O	S	2	38	114–116	C	$C_{19}H_{19}N_2O_3PS_2$	155.3 ± 27.0
7g	O	NH	2	10	198–202	D	$C_{19}H_{20}N_3O_3PS$	17.3 ± 5.1
7h	O	NMe	2	9	132–135	C	$C_{20}H_{22}N_3O_3PS$	47.7 ± 10.8
7i	O	CONEt[d]	2	6	172–174	C	$C_{22}H_{24}N_3O_4PS$	51.8 ± 12.9
7j	S	CH_2	2	48	101–103	A	$C_{20}H_{21}N_2O_2PS_2$	64.9 ± 7.4
7k	S	CH_2	3	49	125–127	A	$C_{22}H_{25}N_2O_2PS_2$	24.7 ± 6.6
7l	S	S	2	44	142–144	E	$C_{19}H_{19}N_2O_2PS_3$	51.8 ± 7.4

[a] Solvent of crystallization: A = cyclohexane, B = AcOEt, C = benzene–cyclohexane, D = benzene, E = CH_3CN. [b] Elemental analyses for C, H, N were within 0.4% of calculated values. [c] The tested compound was dissolved in 0.9% saline which contained 20% polyethylene glycol 400 (PEG-400) and 20% ethanol and was injected into the femoral vein at a dose 0.1 mg/kg. The results are presented as the mean ± SE for five experiments. [d] 2,3-Dioxo-4-ethylpiperazino substituent.

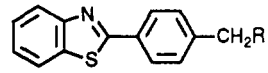
trogen atom of amidophosphonate, which facilitates elimination. To reduce the basisity of the nitrogen atom, we introduced a carbonyl moiety and designed (oxopyrrolidino)phosphonate **7a**. Compound **7a** was quite stable in acidic solution (pH 1.5). Subsequently, lactam amidophosphonates **7b–d** and their related compounds **7e–l** were prepared, and their coronary vasodilator activity was assessed in dogs (iv injection). Table II shows the results. Many of these lactamamides were more active than fostedil. On the basis of these results, the following structure–activity relationship was estimated. From the comparison of **7a**, **7b**, **7c**, and **7d** it is evident that the

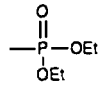
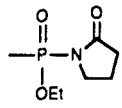
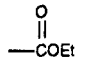
Table III. Pharmacological Profiles of Selected Compounds

compd	coronary blood flow increasing activity: CBF-ID_{75}[a] (dog, iv), μg/kg	coronary blood flow increasing activity (dog, 3 mg/kg, id),[b] Δ%	calcium antagonistic activity: pA_2[c] (guinea pig, tc)	LD_{50}[d] (mouse, po), mg/kg
7a	41.4	104 ± 24	7.53 ± 0.10	2112
7b	55.2	40 ± 21	7.45 ± 0.11	>1000
7c	55.0	69 ± 38	7.46 ± 0.10	>1000
7e	77.3	ND	7.59 ± 0.13	671
7f	50.4	ND	ND	>1000
fostedil	116.0	92 ± 27[e]	7.21 ± 0.08	1186
diltiazem hydrochloride	77.1	52 ± 23	7.30 ± 0.06	508

[a] Dose that increased coronary blood flow by 75%. [b] The tested compound was diluted with 0.5% CMC to a concentration of 10 mg/mL and injected into the duodenum at a dose 3 mg/kg. The results are presented as the mean ± SE for five experiments. [c] The results are presented as the mean ± SE for 10 experiments. See the Experimental Section. [d] A suspension of each compound in 0.5% CMC was orally administered to ddY strain male mice (body weight 18–24 g; five per group) and the mortality of the animals was observed for 7 days. [e] Result at a dose 10 mg/kg.

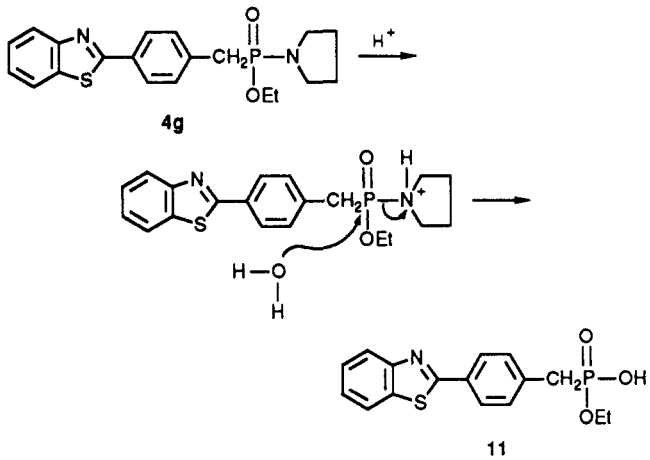
Table IV. Physicochemical Properties of Phosphonates and Related Compounds



no.	R	NMR[a] ($CDCl_3$), δ	solubility in water (μg/mL, 22 °C)	log P[b] (1-octanol/water) (22 °C)
1a		3.2(d)	36	3.5
7a		3.6	75	3.0
12		3.6	13	3.2
13	H	2.4	10	3.7

[a] Chemical shift of benzyl proton. [b] See ref 10.

Scheme IV



activity decreases as the ring size of lactam increases. The coronary vasodilator activity varied with the difference in the heterocycles. Those containing thiazolidinone (**7f**), oxazolidinone (**7e**), and lactam (**7a**) were more active, whereas those having imidazolidinone (**7g**), *N*-methylimidazolidinone (**7h**), and dioxopiperazine (**7i**) were less active. Conversion of the lactams (**7a**, **7b**, and **7f**) to the corresponding thiolactam (**7j**, **7k**, and **7l**) caused substantial loss of activity. Diamide dilactam **10** did not show any coronary vasodilator activity.

Compounds **7a–c**, **7e**, and **7f**, which were more active than fostedil, were subjected to further pharmacologic study. The results are presented in Table III. The lactam amido derivatives **7a–c** and **7f** showed some improvement in calcium antagonistic activity over the dialkyl ester derivative fostedil. Compounds **7a–c** were active when injected into the duodenum of dogs and proved to be stable in the gastrointestinal tract. Among them, the 2-oxopyrrolidine derivative **7a** exhibited particularly potent activity. The coronary vasodilator activity of **7a** was 3 and 2 times higher than that of fostedil and diltiazem hydrochloride, respectively.

In conclusion, the structural modification of the novel calcium antagonist fostedil (KB-944) was investigated and the lactam amidophosphonates **7a–c**, **7e**, and **7f** exhibited coronary vasodilator activity superior to that of fostedil in iv injection. Among them, the 2-oxopyrrolidine derivative **7a** was the most effective, and its coronary vasodilator action is twice as potent as that of the conventional calcium antagonist diltiazem hydrochloride when administered orally.

Experimental Section

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

Ethyl (4-Benzothiazol-2-ylbenzyl)phosphonochloridate Hydrochloride (2). A mixture of diethyl (4-benzothiazol-2-ylbenzyl)phosphonate (**1a**)[2] (3.6 g, 0.01 mol), $SOCl_2$ (25 g), and a catalytic quantity of DMF was heated at reflux for 3 h. Evaporation of the excess $SOCl_2$ yielded phosphonochloridate **2** as a colorless powder (3.9 g, quant).

Ethyl (4-Benzothiazol-2-ylbenzyl)-*N*-isopropylamidophosphonate (4b). To a stirred suspension of **2** (3.9 g, 0.01 mol)

in 50 mL of THF was added isopropylamine (2.4 g, 0.04 mol) dropwise at 0–5 °C. The ice bath was removed, and the reaction mixture was stirred for 2 h. The resulting solution was diluted with water (100 mL) and concentrated to 30 mL under reduced pressure. The resulting solid was collected by filtration. The solid was purified via column chromatography on silica gel with AcOEt as eluent and recrystallized from benzene to give 2.1 g (57%) of **4b** as colorless needles: mp 166.0–167.0 °C; NMR ($CDCl_3$) δ 1.14 (6 H, dd, J = 7 and 2 Hz), 1.28 (3 H, t, J = 7 Hz), 2.04–2.38 (1 H, m), 3.20 (2 H, d, J = 21 Hz), 3.20–3.68 (1 H, m), 3.80–4.24 (2 H, m), 7.24–7.60 (4 H, m), 7.80–8.12 (4 H, m).

Similarly, **4l** was prepared from **2** (3.9 g, 0.01 mol) and (2,3,4-trimethoxybenzyl)piperazine hydrochloride[11] (3.4 g, 0.01 mol): yield 2.9 g (42%); mp 161.0–162.0 °C; NMR ($CDCl_3$) δ 1.30 (3 H, t, J = 7 Hz), 2.70–3.50 (10 H, m), 3.80–4.28 (13 H, m), 6.30 (2 H, s), 6.84 (2 H, dd, J = 20 and 7 Hz), 7.30–7.56 (4 H, m), 7.78–8.10 (4 H, m).

Ethyl (4-Benzothiazol-2-ylbenzyl)(2-oxopyrrolidino)-phosphinate (7a). To a suspension of phosphonochloridate **2** (3.9 g, 0.01 mol) in 30 mL of THF was added 5 g of triethylamine at 0 °C to give a colorless solution. A mixture of 2-pyrrolidone (1.7 g, 0.02 mol) and NaH (50%, 0.46 g, 0.01 mol) in 50 mL of THF was refluxed for 1 h, cooled to room temperature, and added to the above solution. The mixture was stirred for 3 h at room temperature and was evaporated to dryness. The residue was chromatographed on silica gel with AcOEt and MeOH (10:1) as eluent to give a colorless powder. Recrystallization from AcOEt gave 3.1 g (77%) of **7a** as colorless plates: mp 128.0–129.0 °C; NMR (DMSO-d_6) δ 1.26 (3 H, t, J = 7 Hz), 1.76–2.16 (2 H, m), 2.36–2.64 (2 H, m), 3.16–3.80 (2 H, m), 3.62 (2 H, d, J = 21 Hz), 3.88–4.28 (2 H, m), 7.34–7.68 (4 H, m), 7.98–8.24 (4 H, m).

Similarly, **7h** was prepared from **2** (4.3 g, 0.11 mol) and 1-methyl-2-imidazolidinone[12] (1.1 g, 0.11 mol): yield 0.43 g (9%); mp 132.0–135.0 °C; NMR ($CDCl_3$) δ 1.34 (3 H, t, J = 7 Hz), 2.84 (3 H, s), 3.10–3.74 (6 H, m), 3.88–4.28 (2 H, m), 7.82–8.12 (4 H, m).

Ethyl (4-Benzothiazol-2-ylbenzyl)(2-thioxopyrrolidino)-phosphinate (7j). A solution of **7a** (2.0 g, 5 mmol) and 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide (Lawesson's reagent,[8,9] 1.0 g, 2.5 mmol) in 40 mL of toluene was refluxed for **4h**. The reaction mixture was evaporated, and the residual solid was purified via column chromatography on silica gel eluting with AcOEt and recrystallized with n-hexane to give 0.7 g (34%) of **7j** as pale yellow leaflets: mp 101.0–103.0 °C; NMR ($CDCl_3$) δ 1.38 (3 H, t, J = 7 Hz), 1.70–2.14 (2 H, m), 2.92–3.12 (2 H, m), 3.30–4.36 (6 H, m), 7.24–7.56 (4 H, m), 7.80–8.10 (4 H, m).

(4-Benzothiazol-2-ylbenzyl)bis(2-oxopyrrolidino)phosphine Oxide (10). A mixture of phosphonic acid **8**[2] (6.1 g, 0.02 mol), thionyl chloride (60 mL), and a catalytic amount of DMF was refluxed for 5 h. The reaction mixture was evaporated to give phosphonic dichloride **9** as a colorless powder. A mixture of 2-pyrrolidone (5.1 g, 0.06 mol) and NaH (60%, 1.5 g, 0.04 mol) in 250 mL of THF was refluxed for 1 h, cooled to room temperature, and added to the suspension of **9** in 200 mL of THF. After stirring for 5 h at room temperature, the reaction mixture was evaporated and the residue was purified via column chromatography using silica gel and eluting with AcOEt. Recrystallization from AcOEt gave 3.0 g (34%) of **10** as colorless leaflets: mp 180.0–183.0 °C; NMR ($CDCl_3$) δ 1.80–2.24 (4 H, m), 2.36–2.60 (4 H, m), 3.35–3.80 (4 H, m), 3.86 (2 H, d, J = 20 Hz), 7.24–7.56 (4 H, m), 7.80–8.10 (4 H, m).

Effect of the Coronary Arterial Blood Flow in Anesthetized Dogs. Dogs were anesthetized with pentobarbital sodium (35 mg/kg, iv) and thoracotomized at the fifth left intercostal space under artificial respiration. The blood flow of the circumflex branch of the left coronary artery and that of the carotid artery was measured with an electromagnetic flowmeter (Nihon Kohden, MF-26). The tested compounds were dissolved in 0.9% saline which contained 20% polyethylene glycol 400 (PEG-400) and 20% ethanol and were injected into the femoral vein at a dose of 0.1 mg/kg. The coronary flow CBF-ID_{75} (dose that increased coronary flow by 75%) values were calculated from a linear regression analysis of log dose curves.

Calcium Antagonistic Activity. Male guinea pigs, each weighing 350–450 g, were sacrificed, and the test was conducted with isolated tenia coli specimens, each about 2 cm long. The contractile response of each specimen, 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 6×10^{-3} g/mL of K^+, to obtain a dose–response curve for calcium, which was determined with the test compound. On the basis of the difference between the responses, the pA_2 value of the calcium antagonistic activity of the test compound was computed.

Effect of Intraduodenal Administration of Coronary Flow in Dogs. Dogs weighing 12–22 kg were anesthetized with pentobarbital sodium (35 mg/kg, ip), and under supportive respiration, a 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 test compound was diluted with 0.5% CMC to a concentration of 10 mg/mL and injected into the duodenum at a dose of 3 mg/kg.

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(12) Frick, J. G., Jr.; Kottes, B. A.; Reid, J. D. *Text. Res. J.* **1959**, *29*, 314.