in which k_{app} is the pseudo-first-order rate constant. The bimolecular rate constant (k_3')[48] is equal to k_3/K_I.

Acknowledgment. This work was done while H. L. held a National Research Council-WRAIR Research Fellowship. We thank A. H. Newman, N. D. Brown, and A. D. Wolfe for advice and J. W. Covington for technical assistance. Research was conducted in compliance with the Animal Welfare Act and Federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 85-23, 1985, and the WRAIR protocol.

Registry No. 2, 92549-67-2; 3, 120665-63-6; 4, 120637-92-5; 5, 120637-93-6; 6, 1823-91-2; 7, 22156-51-0; 8, 22156-69-0; 9, 120637-94-7; 10, 120637-95-8; 12, 120637-96-9; 13, 120637-97-0; 14, 120637-98-1; 15, 120637-99-2; 16, 120665-64-7; β-(*N*,*N*-diethylamino)ethyl chloride, 100-35-6; 4-chloronitrobenzene, 100-00-5; cholinesterase, 9001-08-5.

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Organic Phosphorus Compounds. 2. Synthesis and Coronary Vasodilator Activity of (Benzothiazolylbenzyl)phosphonate Derivatives[1]

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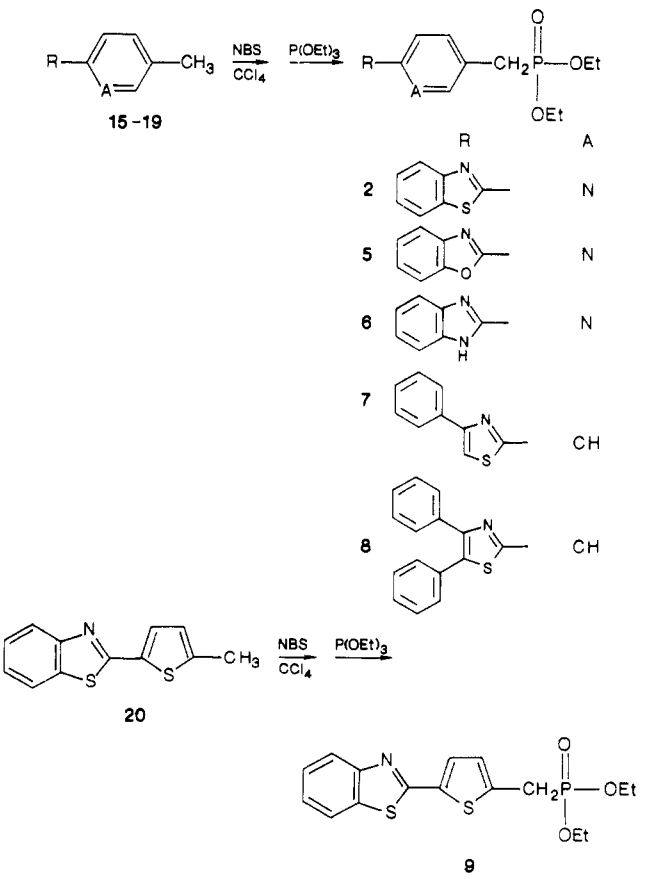
Received July 12, 1988

Structural modification and the coronary vasodilator activity of the calcium antagonist fostedil (KB-944) are described. Elimination of the benzothiazole ring or replacement of the benzothiazole ring of fostedil with other hetero rings leads to a decrease in vasodilator activity. Change of the distance from the aromatic ring to the phosphorus of fostedil causes a decrease in the activity. The present study indicates that the presence of an aromatic ring substituted thiazole ring and the presence of phosphonate at an appropriate distance from the thiazole ring are important for the coronary vasodilator action of fostedil.

Previously, we reported a new calcium antagonist fostedil[1,2] whose structure is totally different from that of conventionally known calcium antagonists. At present, three types of calcium antagonists (nifedipine and its derivatives,[3] diltiazem,[4] and verapamil[5]) are widely used. These agents exert their action by binding to the protein of the voltage-operated calcium channel present on the membrane of smooth muscles. The three calcium antagonists bind to different sites; they have allosteric effects on each other.[6] Fostedil seems to bind to the diltiazem binding site from the results of the binding studies with ^{3}H-labeled calcium antagonists.[7-9] Fostedil inhibited [^{3}H]diltiazem binding to the calcium channel in rat cerebral cortex.[7] Like diltiazem, fostedil promoted the binding of nifedipine through an allosteric effect.[8] It is very interesting that fostedil, whose structure is quite different from that of diltiazem, binds to the diltiazem binding site and has an effect similar to that of diltiazem. For this reason, we have studied which sites are essential for the action of fostedil.

Scheme I



In our previous study,[1] the presence of the phosphonate moiety proved to be important to the action of fostedil. In this paper, three types of structural modification of fostedil and the coronary vasodilator activity are reported (see Figure 1): (1) the benzene ring of fostedil was replaced

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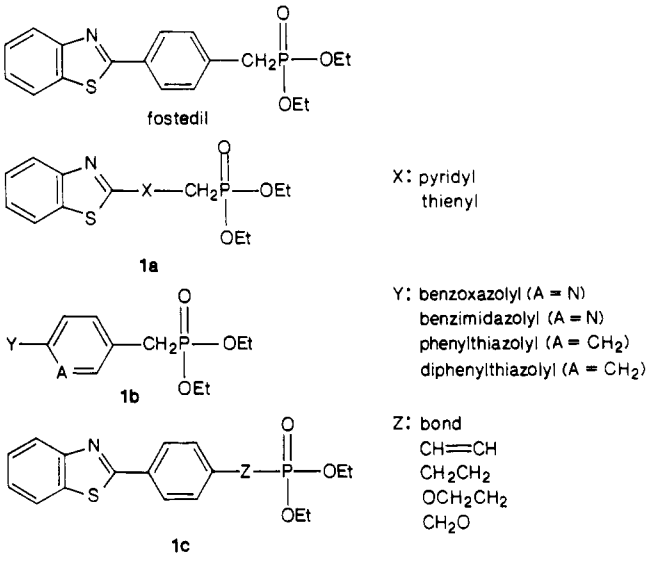
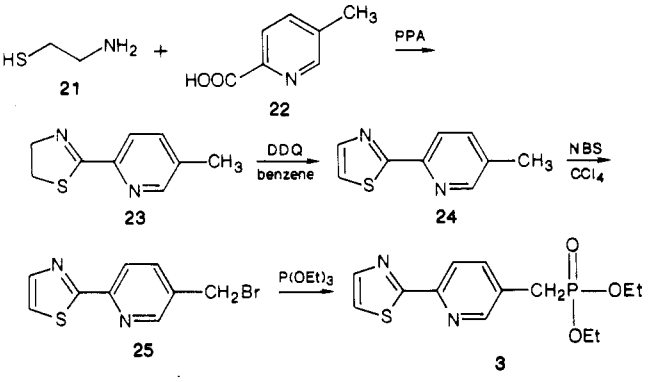


Figure 1.

Scheme II



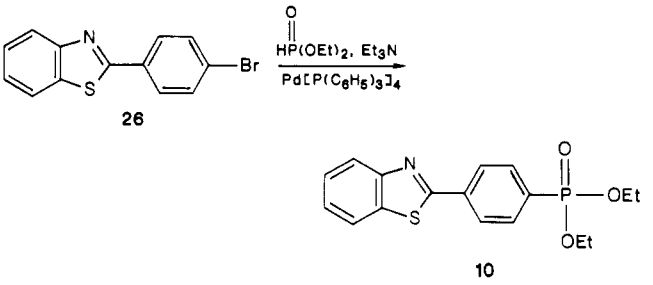
with a pyridine or thiophene ring (**1a**); (2) the benzothiazole ring of fostedil was removed or replaced by another azole ring (**1b**); and (3) the distance from the phosphorus atom to the aromatic ring was changed (**1c**). The coronary vasodilator activity of these compounds was assessed by Langendorff's method.[10]

Chemistry

The general procedure for the replacement of the aromatic rings of fostedil by other hetero rings is illustrated in Scheme I. (Benzothiazolylpicolyl)phosphonate (**2**) and (benzoxazolylpicolyl)phosphonate (**5**) were synthesized according to the method of Yamaguchi et al.[11] That is, benzothiazolylpicoline (**15**) or benzoxazolylpicoline (**16**) were brominated with NBS, followed by condensation with triethyl phosphite. In exactly the same way, the phosphonates **6**, **7**, **8**, and **9** were synthesized from benzimidazolylpicoline[12] (**17**), (phenylthiazolyl)toluene[13] (**18**), (diphenylthiazolyl)toluene[13] (**19**), and benzothiazolylmethylthiophene[14] (**20**), respectively.

(Thiazolylpicolyl)phosphonate (**3**) was synthesized by the method shown in Scheme II. Thiazolinylmethyl-

Scheme III



pyridine (**23**) was obtained by condensation of aminoethanethiol (**21**) and 5-methylpicolinic acid[15] (**22**) in polyphosphoric acid. When compound **23** was oxidized by chloranil, no thiazole **24** was obtained. When compound **23** was oxidized with NBS, some thiazole **24** was obtained although the yield was low (<25%). The use of DDQ as an oxidizing agent resulted in the highest yield of compound **24**. By bromination of compound **24** with NBS and subsequent exposure to triethyl phosphite, compound **3** was obtained.

Schemes III and IV show how the length of the chain between the phosphonate and the aromatic ring was changed. (Benzothiazolylphenyl)phosphonate (**10**) was obtained by condensation of benzothiazolylbromobenzene[16] (**26**) and diethyl phosphite with the use of tetrakis(triphenylphosphine)palladium according to the method reported in the literature.[17] Vinylphosphonate **11** was synthesized by the Wittig reaction between thiazolylbenzaldehyde[16] (**27**) and diphosphonate[18] **28**. Compound **11** proved to be a trans isomer when its NMR spectrum was compared with that reported in the literature.[19] The reduction of compound **11** under Pd/C catalyst afforded ethylphosphonate **12**.

α-Hydroxy phosphonate **29** was synthesized by condensation of benzothiazolylbenzaldehyde (**27**) and diethyl phosphite with CH_3ONa as a catalyst in the absence of solvent. Phosphate **13** was synthesized by [1,2]-sigmatropic rearrangement of α-hydroxy phosphonate **29**. Compound **29** was dissolved in ethanol and then rearranged with EtONa to yield phosphate **13**. Scheme V shows the preparation of phenoxyethylphosphonate. Compound **32** was synthesized from benzothiazolylphenol (**30**)[16] by the usual method; then, it was condensed with triethyl phosphite to yield compound **14**.

Results and Discussion

The coronary vasodilator effect of the compounds was assessed in isolated hearts of guinea pigs according to Langendorff's method.[10] The results are shown in Tables I and II.

Our study to explore the influence of the structure of the benzothiazole ring region on that effect provided the following findings. Comparing the compounds **2**, **3**, and **4** showed clearly that replacing the benzothiazole ring with a thiazole ring or eliminating the benzothiazole ring leads

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Scheme IV

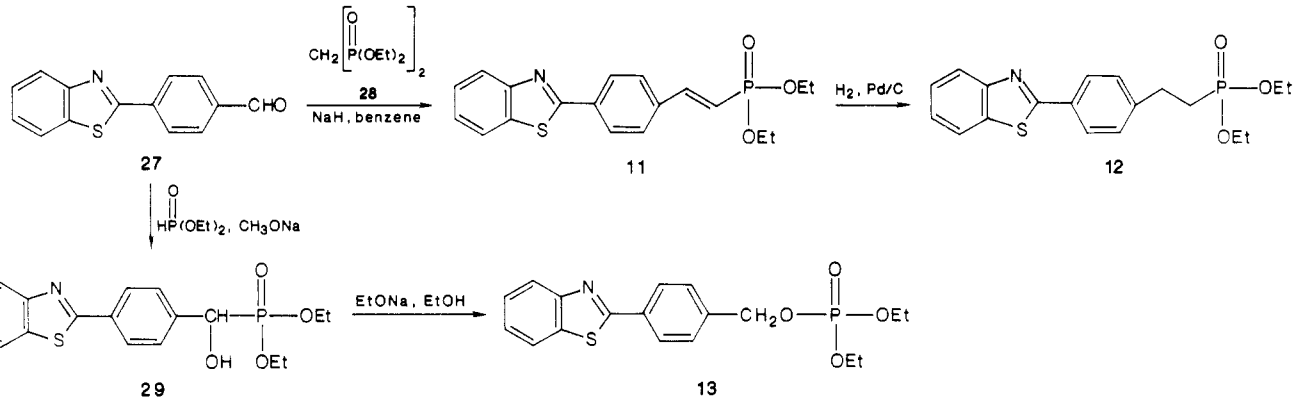
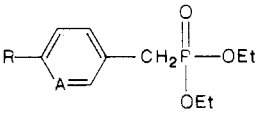
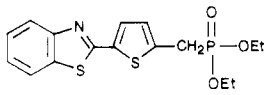


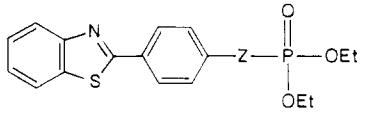
Table I. Effect of Phosphonates on Coronary Flow of Isolated Guinea Pig Heart



compd	R	A	mp, °C	recrystn solvent	max increase in coronary flow, % (10 μg/heart)[a]
2	2-benzothiazolyl	N	96.0–96.5	*n*-hexane	94.0 ± 13.8
3	2-thiazolyl	N	oil (bp 190 °C/1 mmHg)		23.2 ± 12.6
4[b]	H	N	oil		21.4 ± 8.1
5	2-benzoxazolyl	N	90.0–92.5	*n*-hexane	38.7 ± 10.6
6	2-benzimidazolyl	N	169.0–170.0	$CHCl_3$–ether	27.1 ± 3.6
7	4-phenyl-2-thiazolyl	CH	oil		60.3 ± 4.0
8	4,5-diphenyl-2-thiazolyl	CH	oil		23.0 ± 4.8
9			54.0–56.0		51.1 ± 4.0
fostedil	2-benzothiazolyl	CH			79.9 ± 8.6

[a] Langendorff's method in isolated guinea pig heart. See text. 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 results are presented as the mean ± SE for five experiments. [b] Synthesized according to the literature, ref 20.

Table II. Effect of Phosphonates and Phosphate on Coronary Flow of Isolated Guinea Pig Heart



compd	Z	mp, °C	recrystn solvent	max increase in coronary flow, % (10 μg/heart)[a]
10	bond	69.5–70.0	*n*-hexane	27.2 ± 3.2
11	CH=CH (trans)	113.5–115.0	cyclohexane	49.5 ± 15.9
12	CH_2CH_2	oil		40.7 ± 14.9
13	CH_2O	oil		47.7 ± 17.7
14	OCH_2CH_2	93.0–94.0	cyclohexane	27.2 ± 4.0
fostedil				79.9 ± 8.6

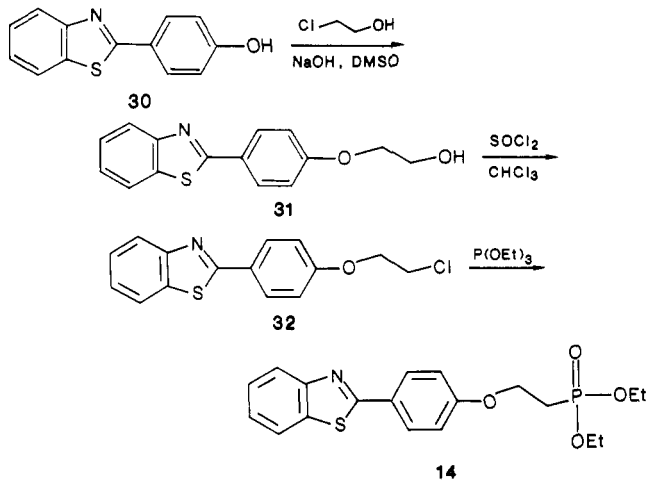
[a] Langendorff's method in isolated guinea pig heart. See text. The test compound dissolved in propylene glycol to a concentration of 100 μg/mL was then infused at a rate of 0.1 mL/min. The results are presented as the mean ± SE for five experiments.

to a substantial decrease in activity. The activity also became less when the benzothiazole ring was replaced with a benzoxazole or benzimidazole ring to yield compound **5** or **6**. In phenylthiazole **7**, which possesses a noncondensed ring, the activity was slightly decreased. Diphenylthiazole **8** showed very low activity.

With respect to the influence of the benzene ring region, compound **2**, which possesses a pyridine ring instead of a benzene ring, exhibited similar efficacy to that of fostedil. In thiophene compound **9** a slight decrease in the activity was observed. From these results it may be assumed that the benzene ring region does not decisively affect the activity of the compounds and that it can be replaced with various aromatic rings without impairing the activity.

In the study of how activity varies when the distance from the benzene ring to phosphorus is changed, the activity of fostedil became substantially smaller when phosphorus was directly linked to the benzene ring after removing a methylene (**10**). Lengthening of one carbon chain resulted in a slight decrease in the activity in both unsaturated linkage (**11**) and saturated linkage (**12**) types. A similar decrease in the activity was noted when oxygen was inserted between the methylene and phosphorus (**13**). When the distance was further lengthened as in compound **14**, the activity became much smaller. These results indicate that the distance from the phosphorus to the benzene ring (which can be taken as the distance from the phosphorus to the benzothiazole ring) could be important

Scheme V



for the activity and that the activity is highest when only one methylene lies between the phosphorus and the benzene ring. In addition, since the activity was almost the same in compounds **11**, **12**, and **13**, the nature of linkage between phosphorus and benzene does not seem to play any significant role in the activity.

In conclusion, the present study indicates that the presence of an aromatic ring substituted thiazole ring and the presence of phosphonate at an appropriate distance from the thiazole ring are important for the coronary vasodilator action of fostedil.

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.

2-(Thiazolin-2-yl)-5-methylpyridine (23). A mixture of 2-aminoethanethiol (4.2 g, 0.05 mol), 5-methylpicolinic acid[15] (6.9 g, 0.05 mol), and 35 g of polyphosphoric acid (PPA) was stirred at 185 °C for 15 min under N_2. After the mixture cooled to 100 °C, it was poured into 250 mL of water. After the PPA was dissolved, the resulting solution was cooled and the pH was adjusted to 10 by the addition of NaOH aqueous solution. The precipitated solid was collected by filtration. Column chromatography of the solid on silica gel with benzene as an eluent gave 3.0 g (34%) of **23** as yellow needles: mp 83.0–84.5 °C.

2-(Thiazol-2-yl)-5-methylpyridine (24). DDQ (5.5 g, 0.024 mol) was added to a solution of **23** (3.6 g, 0.02 mol) in 90 mL of benzene at room temperature. After stirring for 2 h under reflux, the reaction mixture was evaporated under reduced pressure. The residual solid was purified via column chromatography on silica gel, eluting with benzene to give 2.5 g (70%) of **24** as colorless needles: mp 46.0–50.0 °C.

General Procedure. Diethyl [[6-(Thiazol-2-yl)pyridin-3-yl]methyl]phosphonate (3). To a solution of **24** (2.8 g, 0.016 mol) in dry carbon tetrachloride (120 mL) was added *N*-bromosuccinimide (3.2 g, 0.0177 mol) and a catalytic quantity of benzoyl peroxide. The resulting mixture was stirred and heated at reflux for 0.5 h and allowed to cool to room temperature. The precipitated succinimide was filtered off, and the filtrate was evaporated to dryness. The residual solid was recrystallized from *n*-hexane to give 2.3 g (56%) of **25** as pale yellow needles. A mixture of the bromide **25** (1.0 g, 0.0039 mol) and triethyl phosphite was stirred at 90 °C for 40 min under nitrogen gas flow. After being cooled to room temperature, the reaction mixture was chromatographed on silica gel with ether as an eluent to give 0.50 g (40%) of **3** as colorless oil: bp 190 °C/1 mmHg.

Diethyl [[6-(Benzimidazol-2-yl)-3-pyridyl]methyl]phosphonate (6). To a solution of **17**[12] (5.4 g, 0.026 mol) in 300 mL of benzene were added *N*-bromosuccinimide (5.0 g, 0.028 mol) and a catalytic amount of benzoyl peroxide. After being stirred under reflux for 7.5 h, the cooled mixture was washed with water, dried over $MgSO_4$, and evaporated. The residual oil was crystallized from a small quantity of $CHCl_3$ to give 1.6 g of 2-(benzimidazol-2-yl)-5-(bromomethyl)pyridine as a pale yellow powder. This material was used without purification in the next step. A mixture of the above bromide (1.6 g, 0.0055 mol) and triethyl phosphite (8 mL) was stirred and heated at 130 °C for 10 min under an atmosphere of nitrogen. The reaction mixture was allowed to cool to room temperature, and the precipitated solid was collected by filtration and purified via column chromatography using silica gel and eluting with a benzene/EtOAc mixture. The phosphonate **6** was obtained as pale yellow prisms: yield 0.45 g (23%); mp 169–170 °C.

Diethyl [4-(Benzothiazol-2-yl)phenyl]phosphonate (10). To a stirred mixture of bromide **26**[16] (1.2 g, 4.0 mmol), diethyl phosphite (0.6 g, 4.4 mmol), and triethylamine (0.61 mL, 4.4 mmol) in 5 mL of toluene was added tetrakis(triphenylphosphine)palladium (231 mg, 0.2 mmol) at 90 °C. The resultant mixture was stirred for 10 min and was cooled to room temperature. After the addition of ether (50 mL), insoluble material was removed by filtration. Evaporation of the organic layer gave a pale yellow oil, which was chromatographed on silica gel with benzene and ethyl acetate as eluent to give a colorless powder. Recrystallization from *n*-hexane gave 0.84 g (60%) of **10** as a colorless leaflet: mp 69.5–70.0 °C.

Diethyl (*E*)-[4-(Benzothiazol-2-yl)styryl]phosphonate (11). A mixture of diphosphonate **28**[18] (2.2 g, 0.0076 mol), NaH (0.3 g, 0.0063 mol), and benzene (20 mL) was stirred for 0.5 h at room temperature to give an almost clear solution. Aldehyde **27**[16] (1.5 g, 0.0063 mol) was added to the solution, and stirring was continued for a further 0.5 h. The reaction mixture was washed with 20 mL of water, dried over $MgSO_4$, and evaporated. The residue was recrystallized from cyclohexane to give 2.0 g (85%) of **11** as pale yellow plates: mp 113.5–115.0 °C. The H–H coupling constants of the α- (to the phosphorus) and β-hydrogens (J = 15 Hz) and the P–H coupling constants (J = 15 and 22 Hz, respectively) are in accord with a *E* configuration.[19]

Diethyl [4-(Benzothiazol-2-yl)phenethyl]phosphonate (12). A mixture of **11** (1.3 g, 0.0035 mol), 5% Pd/C (0.2 g), and methanol (60 mL) was hydrogenated at atmospheric pressure and room temperature for 24 h. The catalyst was filtered, and the filtrate was evaporated to give **12** (1.3 g, quantitative) as a pale yellow oil: NMR (CCl_4) δ 1.4 (6 H, m), 1.8–2.4 (2 H, m), 2.2–3.3 (2 H, m), 3.9–4.4 (4 H, m), 7.2–8.1 (4 H, m), 7.3–8.2 (4 H, m).

Diethyl [[4-(Benzothiazol-2-yl)phenyl]hydroxymethyl]phosphonate (29). To a stirred mixture of aldehyde **27**[16] (1.5 g, 0.0063 mol) and diethyl phosphite (1.0 g, 0.0072 mol) was added a catalytic amount of CH_3ONa at room temperature. After the reaction mixture was stirred at room temperature for 1 h, the precipitated solid was collected by filtration. Recrystallization from methanol and water gave **29** (2.0 g, 84%) as colorless needles: mp 163.5–165.5 °C.

Diethyl 4-(Benzothiazol-2-yl)benzyl Phosphate (13). EtONa (0.29 g, 4.3 mmol) was added to a solution of phosphonate **29** (0.90 g, 2.4 mmol) in 25 mL of EtOH at 0–5 °C. The ice bath was removed, and the reaction mixture was stirred for 1.5 h. The resulting solution was evaporated in vacuo to give **13** (0.90 g, quantitative) as a pale yellow oil: NMR (CCl_4) δ 1.3 (6 H, t), 3.7–4.3 (4 H, m), 5.0 (2 H, d, J = 9 Hz), 7.2–7.5 (4 H, m), 7.2–8.1 (4 H, m).

2-[4-(Benzothiazol-2-yl)phenoxy]ethanol (31). A mixture of phenol **30**[16] (4.5 g, 0.020 mol), ethylene chlorohydrin (2.1 g, 0.026 mol), and NaOH (0.85 g, 0.021 mol) in 15 mL of DMSO was stirred for 6 h at 140 °C. After being cooled to room temperature, the resulting mixture was poured into water. The aqueous mixture was extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated. The residue was recrystallized from MeOH to give 4.5 g (84%) of **31**: mp 154–157 °C.

2-[4-(2-Chloroethoxy)phenyl]thiazole (32). To a suspension of alcohol **31** (4.0 g, 0.015 mol) in 40 mL of chloroform was added $SOCl_2$ (3.5 g, 0.030 mol) in one portion at room temperature. The resulting solution was heated at reflux for 4 h. The mixture was evaporated to dryness, and the residue was dissolved in water. The aqueous solution was basified with aqueous NaOH solution,

and the precipitated solid was collected by filtration. Recrystallization from cyclohexane gave 4.3 g (100%) of **32**.

Diethyl [2-[4-(Benzothiazol-2-yl)phenoxy]ethyl]-phosphonate (14). A mixture of chloride **32** (2.0 g, 0.0069 mol) and triethyl phosphite (3 mL) was heated at 190–200 °C for 5 h. After the reaction mixture had cooled, the precipitated solid was collected by filtration. The crude phosphonate was chromatographed on a silica gel column by eluting with a benzene/EtOAc mixture to give 1.5 g (56%) of **14**. Recrystallization from cyclohexane yielded **14** as colorless needles: mp 93.0–94.0 °C.

Effect on Coronary Flow in the Isolated Guinea Pig Heart. Male guinea pigs of 400–500-g body weight were killed and exsanguinated 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 methods 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.

Registry No. **2**, 41716-26-1; **3**, 120332-20-9; **4**, 2682-86-2; **5**, 41806-42-2; **6**, 120332-21-0; **7**, 120332-22-1; **8**, 120332-23-2; **9**, 120332-24-3; **10**, 83524-89-4; **11**, 120332-25-4; **12**, 120332-26-5; **13**, 120332-27-6; **14**, 120332-28-7; **15**, 41806-41-1; **16**, 41716-19-2; **17**, 67273-40-9; **18**, 2227-61-4; **19**, 120332-29-8; **20**, 23222-36-8; **21**, 60-23-1; **22**, 4434-13-3; **23**, 120332-30-1; **24**, 120332-31-2; **25**, 120332-32-3; **26**, 19654-19-4; **27**, 2182-80-1; **28**, 1660-94-2; **29**, 120332-33-4; **30**, 6265-55-0; **31**, 84396-09-8; **32**, 84396-10-1; triethyl phosphite, 122-52-1; 2-(benzimidazol-2-yl)-5-(bromomethyl)-pyridine, 120332-34-5; diethyl phosphite, 762-04-9; fostedil, 75889-62-2.

Diethylstilbestrol-Linked Cytotoxic Agents: Synthesis and Binding Affinity for Estrogen Receptors

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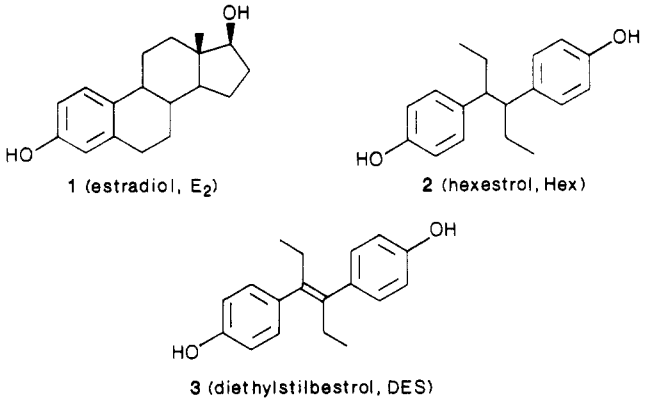
Received August 16, 1988

The syntheses of diethylstilbestrol derivatives with a C_4 side chain at the double bond bearing various functional and potentially alkylating groups (**9–25**, **38–40**, **43**, **44**) as well as the coupling product with daunorubicine (**41**) are described. Derivatives with *free* phenolic groups show easy isomerization to (*Z*)-stilbenes and styrenes, which could be minimized with silyl protecting groups. Estrogen receptor binding is decreased by polar groups such as carboxylic acids (**10**) as well as sterically demanding substituents.

The chemotherapy of cancer in its present form suffers from the fact that, in principle, no difference is made between normal and tumor cells, regardless of whether the drug belongs to the group of alkylants, enzyme inhibitors, or DNA intercalators.[1] A certain degree of selectivity is mainly due to the higher sensitivity of rapidly growing cells to various kinds of toxic compounds. Numerous efforts have been made to increase the selectivity toward cells and to decrease the systemic toxicity. A possibility for selectivity is offered by hormone-dependent tumors, such as certain breast tumors, which selectively concentrate natural and synthetic estrogens.[2] The idea to induce cyto*toxic* effects to hormone-dependent tumor cells by covalent linkage of N-mustard groups to the steroidal skeleton was tested in the late sixties.[3] Since that time many compounds have been synthesized and tested in which various cytotoxic groups were linked to estradiol[4–9] (**1**) (E_2), hexestrols[10–13] (**2**) (HEX), diethylstilbestrols[5,10–14] (**3**) (DES), or the antiestrogen tamoxifen[15] (Chart I).

A prerequisite for specificity of these cytotoxic agents is a sufficient binding of the drug to the estrogen receptor, which allows the selective uptake into the hormone sensitive cells (relative binding affinity compared to E_2 = 100%; RBA). Calculations on the basis of the number of receptors per cell (about 1000–10000) and the possible drug concentration show that the RBA value should be at least 1% of that of E_2.[16] However, chemical modification of estrogens usually produces a dramatic decrease in the binding affinity. Early work on the chemically easy derivatization of the hydroxy groups in E_2 (**1**), HEX (**2**), and

Chart I



DES (**3**) gave products with very low or no RBA, thus establishing the essential function of both hydroxy groups

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0022-2623/89/1832-1532$01.50/0