chloride, 101333-64-6; *trans*-1,2,3,4-tetrahydro-5,8-dimethoxy-3-[(3,3-dicyclohexylpropyl)amino]-2-naphthalenol hydrochloride, 101333-60-2; *trans*-1,2,3,4-tetrahydro-6,7-dimethoxy-3-[(2-(9*H*-fluoren-9-yl)ethyl)amino]-2-naphthalenol hydrochloride, 101333-74-8; *trans*-1,2,3,4-tetrahydro-6,7-dimethoxy-3-[(3,3-diphenylpropyl)amino]-2-naphthalenol oxalate, 101333-79-3; 1,2,3,4-tetrahydro-6,7-dimethoxy-3-[(3,3-diphenylpropyl)amino]naphthalene, 106359-35-7; 3-(benzothiazolyl)propyl chloride, 65655-72-3; potassium phthalimide, 1074-82-4; 2-[2-(benzothiazolyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione, 106359-43-7; 3-(2-benzothiazoyl)propanamine, 51124-73-3; 6,7-dimethoxy-2-tetralone, 2472-13-1; 3-(3-propylamine)indole, 6245-89-2; 3,3-dicyclohexylpropylamine, 101333-99-7; 2-(9-9*H*-fluorenyl)ethanamine, 21745-79-9.

Synthesis and Biological Activity of Novel Calcium Channel Blockers: 2,5-Dihydro-4-methyl-2-phenyl-1,5-benzothiazepine-3-carboxylic Acid Esters and 2,5-Dihydro-4-methyl-2-phenyl-1,5-benzodiazepine-3-carboxylic Acid Esters

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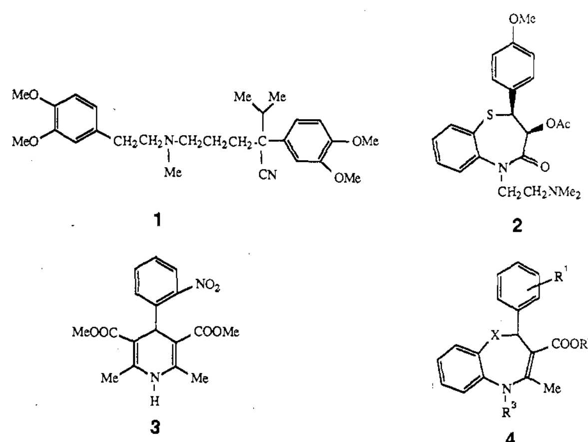
2,5-Dihydro-4-methyl-2-phenyl-1,5-benzothiazepine-3-carboxylic acid esters, based on the structures of dihydropyridines and diltiazem, were synthesized from *o*-aminothiophenol and 2-(phenylmethylene)-3-oxobutanoic acid esters. Biological evaluation in the potassium-depolarized rabbit aorta suggests that these compounds are calcium channel blockers. The in vitro activity was further confirmed by electrophysiological techniques. Structure–activity studies for the aromatic substitution showed that the 2-nitro derivative was the most potent (IC_{50} = 0.3 μM) compound in vitro while the ethyl ester was slightly better than the corresponding methyl ester. Replacement of sulfur with nitrogen atom provided 2,5-dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzodiazepine-3-carboxylic acid ethyl ester, which was only slightly less active than the corresponding benzothiazepine. Derivatization of the nitrogen in 2,5-dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester with a (dimethylamino)ethyl group (present in diltiazem) provided 2,5-dihydro-5-[(dimethylamino)ethyl]-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester, which was found to be equipotent to diltiazem in vitro. Radioligand binding studies using [^{3}H]nitrendipine and [^{3}H]diltiazem showed that the compound with the free nitrogen binds competitively into the dihydropyridine binding site while the molecule in which the nitrogen is alkylated with a (dimethylamino)ethyl group interacts competitively with both diltiazem and dihydropyridine binding sites. Our results therefore show that 2,5-dihydro-4-methyl-2-phenyl-1,5-benzothiazepine-3-carboxylic ester is a good starting point for designing dihydropyridine as well as diltiazem mimics.

Calcium channel blockers have been proven to be clinically useful agents in treating various cardiovascular disorders.[1] The drugs that are most prescribed in this area are, verapamil (**1**), diltiazem (**2**), and nifedipine (**3**). Because of their potency and their selectivity for the vascular smooth muscle, there has been tremendous activity in the synthesis of new dihydropyridine analogues. Efforts in the area of diltiazem like molecules are relatively few. Verapamil, which possesses a variety of pharmacological properties,[2] has seen very little activity.[3] Considering the diverse pharmacology of verapamil, one is inclined to design novel molecules based on the structures of diltiazem (**2**) and nifedipine (**3**); however, such an endeavor is extremely complicated due to the dissimilar nature of the structures of these compounds. In the present paper we describe our approach for designing novel calcium channel blocking agents, e.g., benzothiazepines and benzodiazepines (**4**).

The structures of compound **4** (X = S) is derived from both diltiazem and a dihydropyridine type calcium channel blocker. With the reasonable assumption that only half of the dihydropyridine molecule is necessary for binding into the receptor site,[4] molecules such as **4** (X = S, R^3 = H) were expected to be dihydropyridine mimics. We had also hoped that derivatization of the nitrogen atom in **4** with a (dimethylamino)ethyl group (found in diltiazem structure) might provide us with compounds (**4**, X = S, R^3 = $CH_2CH_2NMe_2$) that would display diltiazem-like activity. In order to determine if sulfur is necessary for calcium channel blocking activity, we evaluated the corresponding benzodiazepine analogue (**4**, X = NH, R^3 = H).



(1) Mannhold, R. *Drugs Today* **1984**, *20*, 69 and references therein.
(2) Atwal, K. S.; O'Reilly, B. C.; Ruby, E. P.; Turk, C. F.; Aberg, G.; Asaad, M. M.; Bergey, J. L.; Moreland, S.; Powell, J. R. preceding paper in this issue.
(3) For a most recent reference on compounds related to verapamil, see for example: Gualtieri, F.; Teodori, E.; Bulluci, C.; Pesce, E.; Piacenza, G. *J. Med. Chem.* **1985**, *28*, 1621.
(4) Dihydropyridine type calcium channel blockers having ester on only one side of the molecule have been described; see for example: (a) U.S. Patent 3910917, 1975. (b) Jpn. Kokai JP 81,173, 1985. (c) German Offen. 3234684 A1, 1984.

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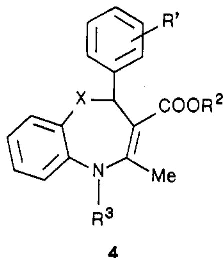
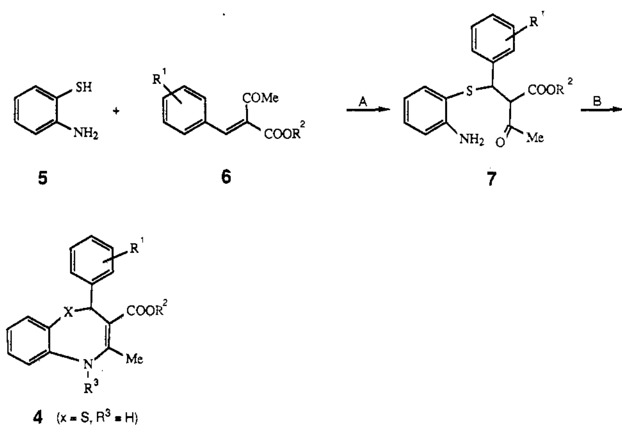
Table I. Physical Properties and Biological Activity of Benzothiazepines and Benzodiazepines



no.	X	R^1	R^2	R^3	analysis	mp,[a] °C	% yield	calcium blocking activity (*n* = 4): IC_{50}, μM
8	S	3-NO_2	Me	H	$C_{18}H_{16}N_2O_4S$ (C, H, N, S)	139..5–141.5	46	1.2
9	S	2-NO_2	Me	H	$C_{18}H_{16}N_2O_4S$ (C, H, N, S)	166–172 dec	64	0.3
10	S	2-CF_3	Me	H	$C_{19}H_{16}F_3NO_2S$ (C, H, N, F, S)	165–166.5	38	12.0
11	S	4-NO_2	Me	H	$C_{18}H_{16}N_2O_4S$ (C, H, N, S)	149–151	40	3
12	S	H	Me	H	$C_{18}H_{17}NO_2S$ (C, H, N, S)	154–156	35	1.7
13	S	4-OMe	Me	H	$C_{19}H_{19}NO_3S$ (C, H, N, S)	107–109	24	22.0
14	S	3-NO_2	Et	H	$C_{19}H_{18}N_2O_4S$ (C, H, N, S)	151–152	30	0.4
15	S	3-NO_2	*i*-Pr	H	$C_{20}H_{20}N_2O_4S$ (C, H, N, S)	130–132	31	0.5
16	SO_2	H	Me	H	$C_{18}H_{17}NO_4S$ (C, H, N, S)	223–225 (CH_2Cl_2–ether)	30	10.0
17	S	3-NO_2	Me	$CH_2CH_2NMe_2$	$C_{22}H_{25}N_3O_4S(COOH)_2$ (C, H, N, S)	141–145 (isopropyl alcohol)	11	1.1
18	NH	3-NO_2	Et	H	$C_{19}H_{19}N_3O_4$ (C, H, N)	147.5–150	44	1.6
							nifedipine	0.001
							diltiazem	1.0

[a] All compounds were crystallized from dichloromethane–isopropyl ether unless otherwise mentioned.

Scheme I[a]



[a] Key: (A) DMF, room temperature; (B) 60–65 °C.

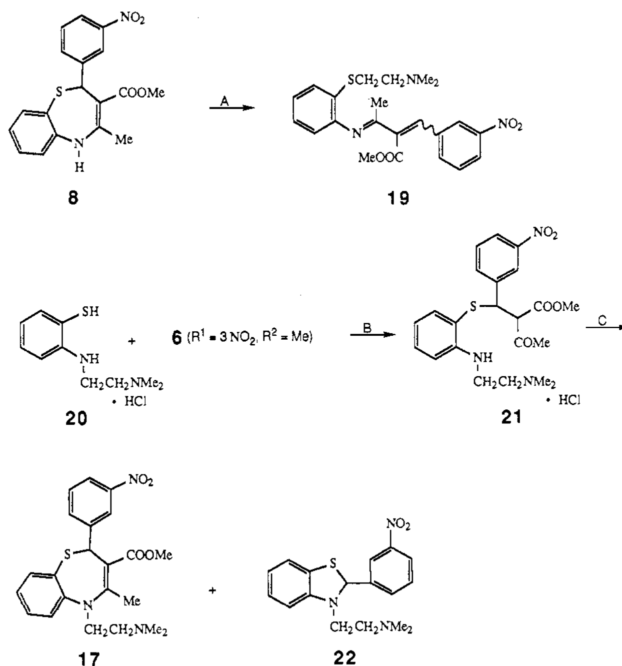
Chemistry

The synthesis of the unsubstituted benzothiazepines (4, X = S, R^3 = H) was accomplished in a straightforward manner. When the o-aminothiophenol (5) was treated with the benzylidene (6)[5] in dimethylformamide, a quantitative yield of the Michael-addition product (7) was obtained. On warming, the intermediate 7 was converted to the desired product (4, X = S, R^3 = H) in good yield (Scheme I). The analogues prepared (8–15) are listed in Table I along with their physical properties and biological activity. The oxidation of compound 8 to the sulfone (16) was conveniently carried out by treatment with *m*-chloroperoxybenzoic acid in dichloromethane. To our surprise, none of the intermediate sulfoxide was isolated.

In order to prepare compound 17, we attempted direct alkylation of the benzothiazepine 8 with (dimethylamino)ethyl chloride (Scheme II). Only the cleavage product (19) was obtained under a variety of reaction conditions. We were therefore forced to synthesize the alkylated *o*-aminothiophenol (20).[6] Using *o*-[[(di-

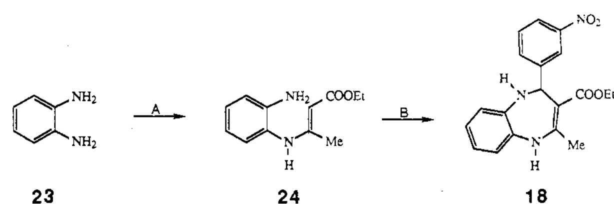
(5) The benzylidene was prepared in high yield from the corresponding benzaldehyde and acetoacetic ester by standard Knoevenagel condensation.

Scheme II[a]



[a] Key: (A) $ClCH_2CH_2NMe_2$, K_2CO_3, DMF; (B) DMF, room temperature; (C) dioxane, DMF, 120 °C.

Scheme III[a]



[a] Key: (A) Ethyl acetoacetate, benzene heat; 3-nitrobenzaldehyde, ethanol, room temperature.

methylamino)ethyl]amino]thiophenol (**20**), the formation of the Michael addition product (**21**) was uneventful; however, the cyclization to the desired product (**17**) required forcing conditions. The yield of this step was considerably depressed due to the formation of the side product (**22**).

The synthesis of the benzodiazepine analogue (**18**) was carried out from *o*-phenylenediamine (**23**) in two steps (Scheme III). On heating *o*-phenylenediamine (**23**) with ethyl acetoacetate, 3-[(2-aminophenyl)amino]-2-butenoic acid ethyl ester (**24**) was obtained in a reasonable yield. Treatment of **24** with 3-nitrobenzaldehyde provided 2,5-dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzodiazepine-3-carboxylic acid ethyl ester (**18**) in good overall yield.

Pharmacology

In order to identify compounds that block the voltage-sensitive calcium channel, we used rabbit aorta tissue depolarized with potassium. The IC_{50} values, reported for calcium channel blocking activity, were calculated from the logit transformation of the concentration response data. The vasorelaxant activity recorded in this test, according to our experience, is fairly reliable for determining the potency of calcium channel blockers.[7] Methods similar to ours are routinely employed to compare the activity of calcium channel blockers in a variety of smooth muscle preparations.[8] In selected cases the activity recorded in this test was further confirmed by radioligand binding studies and electrophysiological experiments. The protocols employed are detailed in the Experimental Section. The IC_{50} values for in vitro activity of various analogues along with nifedipine and diltiazem are listed in Table I.

One of the first compounds prepared, 2,5-dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**8**), was found to relax the potassium-contracted rabbit aorta with an IC_{50} value of 1.2 μM (Table I). Because we were dealing with a potential dihydropyridine mimic, we decided to look at few more aromatic substitutions. The substituents were selected on the basis of some known drugs of the dihydropyridine class.[9] The best compound, according to our in vitro test, was obtained when the phenyl ring carried a nitro group in the ortho position (**9**). This is the same substitution as found in the clinically useful calcium channel blocker nifedipine (**3**). 2,5-Dihydro-4-methyl-2-(2-nitrophenyl)-1,5-benzothiazepine-3-carboxylic ester methyl ester (**9**) is at least 2 orders of magnitude less active than nifedipine (Table I). As expected from the dihydropyridine literature,[10] the substitution in the para position by electron releasing group (**13**) led to a considerable drop in the smooth muscle relaxing activity. The effect of electron withdrawing group (**11**) in the same position was minimal.

In order to investigate the importance of the size of the ester group, we prepared two additional analogues (**14**, **15**). As shown in Table I, the ethyl ester (**14**) showed some improvement over the methyl ester (**8**) while the activity of the isopropyl ester (**15**) was very similar to that of the ethyl ester (**14**). The aromatic substitution selected for evaluating the effect of the ester group was the 3-nitro group. This group is found in a variety of dihydropyridine type calcium channel blockers.[9] The effect of the ester group on compounds substituted in the ortho position of the phenyl ring may not be the same.[11] The substitution in different positions of the aromatic ring can result in subtle changes in the conformation of the molecule. These changes are often reflected in the activity profile of the compound. This is especially true in the area of dihydropyridine type calcium channel blockers and studies along these lines have recently been reported in the literature.[12]

The oxidation of sulfur in **8** to the sulfone (**16**) led to some drop in the smooth muscle relaxing activity. In dihydropyridines, the presence of a hydrogen on the nitrogen atom is considered essential for calcium channel blocking activity;[13] however, in the present situation we were delightfully surprised that alkylation of the nitrogen with (dimethylamino)ethyl group (**17**) had essentially no effect on the in vitro calcium channel blocking activity. The activity of this compound is very similar to that of diltiazem (Table I).[14] This indicated that molecules such as **17** might now be binding into the diltiazem receptor site. The replacement of sulfur with nitrogen atom provided the benzodiazepine **18**, which was slightly less active than the corresponding benzothiazepine **8** in the in vitro test.

Although we were quite convinced that potassium depolarized rabbit aorta is a reliable method for identifying calcium channel blockers, we decided to do some secondary testing to confirm this activity. We tested some of these compounds (e.g., **8**) for blockade of the slow-response action potential in the cardiac tissue. 2,5-Dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**8**) produced a selective reduction in the amplitude of the calcium-dependent slow-response action potential (IC_{50} = 0.14 μM). It did not alter the normal action potential parameters associated with Na^+ or K^+ conductances, even at a concentration nearly 30 times higher than the IC_{50} for slow-response blockade. This confirmed our results from in vitro experiments that compounds described in this paper are selective blockers of the voltage-sensitive calcium channel.

From the experiments discussed so far, it is clear that benzothiazepines and benzodiazepines (**4**) are selective calcium channel blockers. These experiments, however, did not distinguish whether these compounds are acting as diltiazem or dihydropyridine mimics. As pointed out earlier, the molecules discussed in this paper are modeled after the structures of diltiazem and dihydropyridines. In order to find out whether these compounds were binding into the dihydropyridine or diltiazem receptor site, we carried out radioligand binding studies.

Specific recognition sites for dihydropyridines and diltiazem have been demonstrated in cardiac muscle.[15,16] The

(6) Fujii, K. *Yakugaku Zasshi* **1957**, *77*, 347.
(7) Brittain, R. J.; Moreland, S. *Physiologist* **1985**, *24*, 325.
(8) Yousif, F. B.; Triggle, D. J. *Can. J. Physiol. Pharmacol.* **1986**, *64*, 273 and references therein.
(9) For a most recent reference, see: Meguru, K.; Aizawa, M.; Sohda, T.; Kawamatsu, Y.; Nagaoka, A. *Chem. Pharm. Bull.* **1985**, *33*, 3787.
(10) Fossheim, R.; Svarteng, K.; Mostad, A.; Romming, C.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1982**, *25*, 126 and references cited therein.
(11) Atwal, K. S.; Hedberg, A.; Moreland, S., manuscript in preparation.
(12) Mahmoudian, M.; Richards, G. *J. Chem. Soc., Chem. Commun.* **1985**, 739. Fossheim, R. *Acta Chem. Scand., Ser. B* **1986**, *39*, 785.
(13) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 775.
(14) It is interesting to note that diltiazem has a methoxy group in the para position of the aromatic ring. Benzothiazepine analogue 17 has a nitro group in the meta position. Regardless of this apparent difference in the substitution pattern, the two compounds are equipotent in the in vitro test.
(15) Bolger, G. T.; Gengo, P. J.; Luchowsky, E. M.; Seigel, H.; Triggle, D. J.; Janis, R. A. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1604.
(16) (a) Balwierczak, J. L.; Schwartz, A. *Eur. J. Pharmacol.* **1985**, *116*, 193. (b) Siegel, P. K. S.; Garcia, M. L.; Kaczorowski, G. L. *Pharmacologist* **1985**, *27*, 161.

binding characteristics reported follow simple Michaelis–Menten kinetics with saturability and reversibility and show classical competition within the dihydropyridine class of calcium channel blockers. Diltiazem has been demonstrated to interfere with dihydropyridine binding via allosteric interaction.[17] In order to demonstrate a direct competition for diltiazem or dihydropyridine binding sites, we decided to look at the binding of [^{3}H]nitrendipine and [^{3}H]diltiazem. The compounds selected for radioligand binding studies were 2,5-dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**8**) and 2,5-dihydro-5-[(dimethylamino)ethyl]-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**17**). The receptor binding properties of these molecules were studied with regard to inhibition of specific binding of [^{3}H]nitrendipine and [^{3}H]diltiazem in guinea pig myocardium. The details of the binding experiments are given in the Experimental Section. In order to conserve space, concentration–inhibition curves are not shown here.

Both the unalkylated compound **8** and the alkylated derivative **17** inhibited the specific binding of [^{3}H]nitrendipine to the same extent as did 2 μM of nifedipine. Affinity values (K_d) calculated from the observed IC_{50} values were 360 ± 87 and 488 ± 150 nM, respectively. Slope factors of 1.4 ± 0.26 and 2.3 ± 0.5 might indicate that the drug–receptor interaction is influenced by positive cooperativity. Considering the structural similarity of benzothiazepines (**8**, **17**) to diltiazem this could possibly result from simultaneous binding to the diltiazem site. The occupancy of the diltiazem receptor site by specific ligands has been demonstrated to influence the binding of dihydropyridine ligands to their receptor.[18]

The specific binding of [^{3}H]diltiazem was completely inhibited by 2,5-dihydro-5-[(dimethylamino)ethyl]-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**17**) with an affinity of 505 ± 106 nM. Slope factor analysis indicated classical competitive interaction with a Hill coefficient of 1.0 ± 0.01.[19] The interaction of the unalkylated benzothiazepine **8** with [^{3}H]diltiazem receptor could not be studied accurately due to an apparent increase in nonspecific radioligand binding at high concentration.

From the radioligand binding studies it is clear that the unalkylated benzothiazepine **8** interact competitively with the dihydropyridine binding site. Derivatization of 2,5-dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**8**) with a (dimethylamino)ethyl group led to 2,5-dihydro-5-[(dimethylamino)ethyl]-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**17**), which interacts competitively with diltiazem and dihydropyridine binding sites. This is not unexpected as this compound shares structural features with both diltiazem and dihydropyridines. It is therefore possible, at least in principle, that one can derive calcium channel blockers of both diltiazem and dihydropyridine types from a common precursor.

Although most of the compounds described in this paper relaxed the vascular smooth muscle in vitro, none of them showed any meaningful antihypertensive activity when given (up to 50 mg/kg po) to spontaneously hypertensive rats. The reasons for this apparent lack of antihypertensive activity are not clear at the present time. Nifedipine (**3**) shows antihypertensive activity (~25% decrease in blood pressure) at a much lower dose (16 mg/kg po). Diltiazem (**2**) shows only marginal antihypertensive activity (10% decrease in blood pressure) at the highest dose (60 mg/kg po) tested and was without effect at a lower dose (20 mg/kg).

We have shown that benzothiazepines and benzodiazepines (**4**) are selective blockers of the voltage-sensitive calcium channel. These molecules serve as dihydropyridine mimics when the nitrogen atom carries a hydrogen. The most potent compound, 2,5-dihydro-4-methyl-2-(2-nitrophenyl)-1,5-benzothiazepine-3-carboxylic acid methyl ester (**9**), was nearly 2 orders of magnitude less active than nifedipine in the in vitro test (Table I). The benzothiazepine analogue **17**, where the nitrogen is substituted with the (dimethylamino)ethyl group, showed in vitro calcium channel blocking activity that is very similar to that of diltiazem. This molecule displaced [^{3}H]dilitiazem and [^{3}H]nitrendipine from their receptor sites. Our results demonstrate that suitable manipulations of molecules such as **4** can provide compounds that would serve as dihydropyridines or diltiazem mimics.

Experimental Section

Chemistry. All melting points were taken on a capillary melting point apparatus and are uncorrected. The infrared spectra were recorded with a Perkin-Elmer 983 spectrophotometer in KBr pellet. ^{1}H NMR spectra were measured on JEOL GX-400 and FX-270 spectrometers with Me_4Si as an internal standard. Flash chromatography was run with Whatman LPS-1 silica gel. Spectral data of only key intermediates and final compounds are included. Microanalyses of all crystalline compounds was in line with the structures assigned.

Preparation of 2,5-Dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic Acid Methyl Ester (8). A suspension of the benzylidene **6**[5] (R^1 = 3-nitro, R^2 = Me) (2.0 g, 8.0 mmol) in dry dimethylformamide (7.0 mL) was treated with *o*-aminothiophenol (**5**) (1.1 g of 90%, 8.8 mmol). The reaction mixture was allowed to stir at room temperature for 1 h and then heated at 60–65 °C for 24 h. It was allowed to cool down to ambient temperature and was diluted with ether. The resulting solution was washed with water and brine and was dried over anhydrous magnesium sulfate. Evaporation of the solvent provided a yellow foam, which was crystallized from ether–hexanes to yield a yellow solid (1.85 g, 65%). Recrystallization from dichloromethane–isopropyl ether provided yellow needles (1.3 g, 45.7%): mp 139.5–141.5 °C; NMR (acetone-d_6) δ 2.64 (s, 3 H), 3.56 (s, 3 H), 6.07 (s, 1 H), 6.69 (dt, J = 6.9 and 1.1 Hz, 1 H), 6.82 (d, J = 8.0 Hz, 1 H), 7.02 (dt, J = 7.4 and 1.5 Hz, 1 H), 7.09 (d, J = 6.9 Hz, 1 H), 7.33 (t, J = 7.9 Hz, 1 H), 7.57 (d, J = 8.4 Hz, 1 H), 7.83 (d, J = 7.91 Hz, 1 H), 7.88 (s, 1 H), 8.20 (br s, 1 H); IR (KBr) 1696, 1626 cm^{-1}.

Compounds **9**–**15** were prepared in a similar manner.

Preparation of 2,5-Dihydro-5,5-dioxo-4-methyl-2-phenyl-1,5-benzothiazepine-3-carboxylic Acid Methyl Ester (16). The solution of compound **8** (1.0 g, 3.21 mmol) in dry dichloromethane (20 mL) was treated with solid sodium bicarbonate (588 mg, 7.0 mmol) and to the resulting suspension was added *m*-chloroperbenzoic acid (1.41 g of 85%, 7.0 mmol) in small portions at 0 °C. After the addition was over, the reaction mixture was allowed to warm up to room temperature and stirred for 5 more h. It was then diluted with ethyl acetate (100 mL) and washed with 10% sodium bisulfite, 1 N sodium hydroxide, and brine. After the mixture was dried over anhydrous magnesium sulfate, the solvent was stripped off to give a yellow solid. It was triturated with ether and filtered off to provide an off-white solid. It was purified by flash chromatography (7% EtOAc in CH_2Cl_2) and the colorless solid obtained was crystallized from dichloromethane–isopropyl ether to yield pure product (322 mg, 29.3%): mp,

(17) (a) Ehlert, F. J.; Roeske, W. R.; Itoga, E.; Yamamura, H. I. *Life Sci.* **1982**, *104*, 937. (b) Ferry, D. R.; Glossmann, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *321*, 80. (c) Gould, R. J.; Murphy, K. M. M.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3656. (d) Murphy, K. M. M.; Snyder, S. H. *Eur. J. Pharmacol.* **1982**, *77*, 201.
(18) Boles, R. G.; Yamamura, H. I.; Schoemaker, H.; Roeske, W. R. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 333.
(19) Hill, A. V. *J. Physiol.* **1910**, *40*, 190.

223–225 °C dec; NMR (acetone-d_6) δ 2.68 (s, 3 H), 3.65 (s, 3 H), 5.98 (s, 1 H), 6.96 (t, J = 7.9 Hz, 1 H), 7.09 (m, 3 H), 7.22 (m, 2 H), 7.36 (m, 2 H), 7.45 (d, J = 7.9 Hz, 1 H); IR (KBr) 1699, 1626 cm^{-1}.

Preparation of 2,5-Dihydro-5-[(dimethylamino)ethyl]-4-methyl-2-(3-nitrophenyl)-1,5-benzothiazepine-3-carboxylic Acid Methyl Ester Oxalate (17). The solution of *o*-[[(dimethylamino)ethyl]amino]thiophenol (**20**) (2.95 g, 12.6 mmol) in acetonitrile (15 mL) was treated with methanolic hydrochloric acid (4 mL of 3.5 N solution). After about 30 min, the solvent was evaporated to provide a yellow foam. After subjecting it to high vacuum for 30 min, it was dissolved in acetonitrile (15 mL) and treated ith benzylidene **6** (R^1 = 3-NO_3, R^2 = Me) (1.74 g, 6.98 mmol). The resulting suspension was stirred at room temperature for 12 h whereby a yellow solution resulted. The solvent was removed under reduced pressure and the foamy residue (4.59 g) was heated in dioxane/dimethylformamide (37 mL, 4:1) at 120 °C (oil bath temperature) for 24 h. The red reaction mixture was cooled to ambient temperature and dioxane was removed under vacuum. The residue was taken up in ethyl acetate and washed with 1 N sodium hydroxide, water (5 × 20 mL), and brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated to give a dark oil. It was purified by flash chromatography (CH_2Cl_2/MeOH/AcOH, 18:1:1) to provide a yellow oil (810 mg). This material was dissolved in isopropyl alcohol and treated with oxalic acid (180 mg, 2 mmol). A yellow precipitate was formed. It was filtered off and dissolved in hot ethanol. The resulting solution was allowed to cool gradually. The impurity running lower on TLC (**22**) precipitated out. It was filtered off and the mother liquor was concentrated to give a yellow solid. The free amine was regenerated by partition between chloroform and 1 N sodium hydroxide. It was purified by preparative TLC (CH_2Cl_2/MeOH/AcOH, 20:1:1, run twice) to provide a yellow oil. This was taken up in isopropyl alcohol (5 mL) and treated with oxalic acid. The yellow precipitate formed was filtered off and recrystallized from isopropyl alcohol to yield the desired product **17**: mp 141.5–145 °C; NMR ($CDCl_3$) δ 2.0 (s, 3 H), 2.90 (s, 6 H), 3.30 (br m, 2 H), 3.64 (s, 3 H), 3.70 (m, 2 H), 6.47 (d, J = 8.0 Hz, 1 H), 6.75 (t, J = 8.15 Hz, 1 H), 6.82 (s, 1 H), 7.07–7.03 (m, 2 H), 7.83 (t, J = 7.9 Hz, 1 H), 7.61 (d, J = 8.1 Hz, 1 H), 8.17 (s, 1 H), 8.26 (d, J = 8.0 Hz, 1 H); IR (KBr) 1721, 1629 cm^{-1}.

Preparation of 2,5-Dihydro-4-methyl-2-(3-nitrophenyl)-1,5-benzodiazepine-3-carboxylic Acid Ethyl Ester (18). (a) Preparation of 3-[(2-Aminophenyl)amino]-2-butenoic Acid Ethyl Ester (24). The solution of *o*-phenylenediamine (**23**) (16.2 g, 15.0 mmol) and ethyl acetoacetate (19.57, 15.0 mmol) in benzene (125 mL) and acetic acid (0.6 mL) was heated at reflux temperature for 2 h with a water separator. The reaction mixture was allowed to cool down to room temperature and the solvent was evaporated. The residue was crystallized from isopropyl ether–hexanes to provide a dull white solid (**24**) (19.5 g, 57.6%): mp 73–81 °C; NMR ($CDCl_3$) δ 1.28 (t, J = 6.8 Hz, 3 H), 1.80 (s, 3 H), 3.8 (br s, 2 H), 4.15 (q, J = 6.9 Hz, 2 H), 4.72 (s, 1 H), 6.71 (m, 2 H), 6.97 (d, J = 7.4 Hz, 1 H), 7.1 (t, J = 7.4 Hz, 1 H), 9.7 (br s, 1 H); IR (KBr) 3475, 1648, 1620 cm^{-1}.

(b) The solution of 3-[(2-aminophenyl)amino]-2-butenoic acid ethyl ester (**23**) (5.0 g, 22.7 mmol) in absolute ethanol (30 mL) was treated with 3-nitrobenzaldehyde (3.43 g, 22.7 mmol) and acetic acid (0.25 mL). The reaction mixture was allowed to stir under argon at room temperature for 24 h. The solvent was evaporated and the residue was purified by flash chromatography (35%) ethyl acetate in hexanes). The product was crystallized from dichloromethane–isopropyl ether to give an orange solid (**18**) (3.51 g, 43.7%): mp 147.5–150 °C; NMR (acetone-d_6) δ 1.1 (t, J = 7.4 Hz, 3 H), 2.56 (s, 3 H), 4.03 (m, 2 H), 5.83 (d, J = 6.3 Hz, 1 H), 5.94 (d, J = 5.8 Hz, 1 H), 6.57 (m, 3 H), 7.78 (br s, 1 H), 6.85 (m, 1 H), 7.41 (t, J = 7.9 Hz, 1 H), 7.63 (d, J = 7.4 Hz, 1 H), 7.90 (dd, J = 7.4 and 1.6 Hz, 1 H), 8.09 (s, 1 H); IR (KBr) 1670 cm^{-1}.

Pharmacology. (a) Calcium Blockade IC_{50}. The experimental protocol is very similar to the one described in the literature.[7,8] Male New Zealand rabbits weighing 1.8–2.4 kg were sacrificed by an injection of sodium pentobarbital (50 mg/kg) in the marginal ear vein. The thoracic aorta was removed and placed in a petri dish containing Krebs (PSS) solution at room temperature. Excess fat and tissue were removed and rings of approximately 3 mm were cut and opened to yield circumferential strips approximately 3 mm wide and 1 cm long. Strips were then mounted in 10-mL jacketed organ muscle chambers under a 4-g preload, which was applied and maintained during a minimum 1-h equilibration period. Bath temperature was maintained at 37 °C and PSS was gassed with 95% O_2 + 5% CO_2 to yield a pH of 7.35. The strips were stimulated by replacing the normal Krebs solution with a high K^+ (100 mM KCl) Krebs in which NaCl was reduced by an equimolar amount. After attainment of a steady plateau tension, strips were exposed to increasing concentrations of various agents and relaxant responses recorded and normalized with respect to initial recorded tensions. IC_{50} values were determined by a logit transformation of the concentration–response curves.

(b) Slow-Response Action Potentials. The method used for recording slow-response action potentials has been described previously.[20] Canine Purkinje fibers were superfused with oxygenated Tyrode's solution with 15 μM atropine sulfate (Sigma) for approximately 30 min, at which time they were superfused with Tris-buffer solution for the remainder of the experiment. The composition of the superfusate was as follows (mM): 140.1, tetraethylammonium (TEA) chloride; 8.0, $CaCl_2$; 2.7, KCl; 0.5, $MgCl_2$; 5.0, Tris (Trizma 7.7); 15 μM, atropine sulfate. The solution was aerated with 100% O_2 and maintained at 35 ± 0.5 °C. The fiber was placed between the tips of the stimulating electrode and electrically stimulated with 60–90-V pulses of 30-ms duration at a rate of 0.2 Hz. Intracellular action potentials were recorded with glass microelectrodes (15–40 meg ohm) filled with 3 M KCl and coupled to high-impedance, negative-capacitance electrometers (WPI Instruments). Ag/Ag–Cl half cells were used as reference electrodes.

The amplitude of the Ca^{2+}-dependent, slow-response potential as produced under these experimental conditions was used as an index of the slow inward current and drug-induced changes were taken as a measure of Ca^{2+}-blockade in cardiac tissue.

(c) Radioligand Binding. Tissue Preparation. Male guinea pigs were sacrificed by placement in a saturated CO_2 atmosphere until respiration was inhibited. The hearts were excised and immediately placed in ice-cold Tris-isosaline (50 nM Tris in 0.154 M NaCl, pH 7.4) containing 0.1 mM EGTA. The tissue was finely minced with scissors and homogenized with a Polytron (Brinkman PT 35, setting 7 for 15 s). The homogenate was centrifuged at 1000g for 20 min (4 °C) and the resulting pallet was washed four times in 10 mL of ice-cold Tris-isosaline (EGTA free, containing 1 mM $CaCl_2$) per gram of tissue weight.

[^{3}H]Nitrendipine Receptor Binding Assay. Receptor binding using [^{3}H]nitrendipine was carried out largely according to the technique described by Bolger et al.[15] One hundred microliters of the ice-cold membrane solution (100–175 μg of protein) was added to 0.06–1.0 nM of 5-methyl[^{3}H]nitrendipine from New England Nuclear (NET 741, 70–90 Ci/mmol) and increasing concentrations of test compound in a final volume of 0.25 mL of Tris-isosaline containing 0.2% bovine serum albumin (BSA) and 2.5% ascorbic acid.

The binding reaction was carried out in new disposable polypropylene tubes (Sarstedt #55.538), which were incubated in a water bath at 25 °C, starting immediately after administration of the membrane suspension. After the desired time of incubation, (equilibrium established after 25 min of incubation), the samples were diluted 40-fold by addition of 10 mL of ice-cold Tris-isosaline and rapidly filtered under vacuum through glass fiber filter disks (Schleicher and Schuell ZE 22, #30) with a Millipore Sampling Manifold apparatus. The filters were washed with two volumes of 5 mL of ice-cold Tris-isosaline, dried under vacuum, and soaked in 10 mL of Aquasol scintillation fluid (New England Nuclear). Each sample was counted for 5 min in a Packard TriCarb 4640 scintillation counter. Specific binding of [^{3}H]nitrendipine was determined as the binding detectable in the absence ("total binding") minus that in the presence of 2 μM unlabled nitrendipine ("nonspecific binding"). Specific binding routinely amounted to 75–80% of total binding. The amount of radioligand

(20) Bergey, J. L.; Reiser, J.; Wiggins, J. R.; Freeman, A. R. *Eur. J. Pharmacol.* **1981**, *71*, 315 and references cited therein.

never exceeded 5% of total amount of radioligand added in each assay.

For analysis of the binding characteristics of nonlabled test compounds, binding assays were performed with 14 concentrations of the competing molecule, each logarithmic decade subdivided into three increments and each concentration step assayed in duplicate. This protocol allows for a total concentration range of more than four logarithmic decades to be covered in each separate concentration–effect curve (i.e., the highest concentration = 2×10^4 times the lowest concentration studied) and, thus, provides a high-resolution sequence of data points.

Graphic analysis of the data was performed by a computer-aided nonlinear regression, least-squares curve-fitting procedure. Assuming simple Michaelis–Menten kinetics of the interaction between radioligand and competing molecule on any hypothesized number of coexpressed specific receptor types, the computation procedure fits the sigmoidal concentration–effect curve, defined by the law of mass action, to the untransformed data. The analytical procedure allows for evaluation of affinity of the competing molecule for one or more subtypes of receptors which the radioligand nonselectively recognizes as specific binding sites. Calculation of the dissociation constant (K_d) from the observed IC_{50} value (concentration of test compound that causes 50% inhibition of specific binding of the radioligand) was performed by using the formula

$$K_d = IC_{50}(1 + [RL]/K_{RL})$$

where [RL] is the concentration of radioligand and K_{RL} is the affinity of the radioligand for its specific receptors.

The test compounds were dissolved in 95% ethanol at a concentration of 10^{-3} M and diluted 1000-fold in Tris-isosaline containing 0.2% BSA and 2.5% ascorbic acid. The final concentration of ethanol in the incubation cocktail was thus less than 0.02%. This concentration of ethanol has been demonstrated to be without detrimental effect on receptor integrity in the plasma membrane.[15] For a valid calculation of specific binding, the total binding assay (absence of nifedipine) contained the same concentration of ethanol as the nonspecific binding assay.

[^{3}H]Diltiazem Receptor Binding Assay. [^{3}H]Diltiazem binding was performed as described by Ehlert et al.[17a] One hundred microliters of the ice-cold membrane solution (400–600 mg of protein) was added to 0.02–1.0 μM of *d-cis-*[*methyl-*^{3}H]-diltiazem from New England Nuclear (TRK 789, Amersham Corp., 128 Ci/mmol, or NET 847, New England Nuclear, 77 Ci/mmol) and various concentrations of competing molecules in a final volume of 0.25 mL of Tris-isosaline containing 0.2% bovine serum albumin (BSA) and 2.5% ascorbic acid.

The binding reaction was performed at 25 °C as indicated for nitrendipine receptor system. Specific binding of diltiazem was determined as the binding detectable in the absence ("total binding") minus that in the presence of 100 μM unlabled *d-cis-*diltiazem ("nonspecific binding"). Specific binding routinely amounted to 60–75% of the total binding. The amount of radioligand never exceeded 5% of the total amount of radioligand added in each assay.

The evaluation of the affinity of the test compound for the specific diltiazem binding site was performed as indicated for the nitrendipine receptor system. Whenever insoluble in aqueous solutions, the test compounds were dissolved in 95% ethanol at a concentration of 10^{-3} M and diluted 1000-fold in Tris-isosaline containing 0.2% BSA and 2.5% ascorbic acid.

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Registry No. **5**, 137-07-5; **6** (R^1 = 3-NO_2, R^2 = Me), 39562-17-9; **6** (R^1 = 2-NO_2, R^2 = Me), 39562-27-1; **6** (R^1 = 2-CF_3, R^2 = Me), 39561-94-9; **6** (R^1 = 4-NO_2, R^2 = Me), 40641-47-2; **6** (R^1 = H, R^2 = Me), 15768-07-7; **6** (R^1 = 4-OMe, R^2 = Me), 106521-81-7; **6** (R^1 = 3-NO_2, R^2 = Et), 39562-16-8; **6** (R^1 = 3-NO_2, R^2 = Pr-*i*), 39562-25-9; **8**, 106521-82-8; **9**, 106521-83-9; **10**, 106521-84-0; **11**, 106521-85-1; **12**, 106521-86-2; **13**, 106521-87-3; **14**, 106521-88-4; **15**, 106521-89-5; **16**, 106521-90-8; **17**, 106521-91-9; **18**, 106521-92-0; **20**, 106521-93-1; **22**, 106521-94-2; **23**, 95-54-5; **24**, 79923-70-9; 3-nitrobenzaldehyde, 99-61-6; ethyl acetoacetate, 141-97-9.

Synthesis and Calcium Channel Antagonist Activity of Dialkyl 4-(Dihydropyridinyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates

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The sodium borohydride reduction of 3,5-disubstituted 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)pyridines **2** and **5** in the presence of methyl, phenyl, or *tert*-butyl chloroformate afforded the respective 4-(dihydropyridinyl)-1,4-dihydropyridines **4** and **6** in good yield. Products **4** comprised a mixture of the 1,2- and 1,6-dihydropyridinyl regioisomers **4a** and **4b** where **4a** was always the predominant regioisomer. Compounds possessing a 4-[dihydro-1-(phenoxycarbonyl)-3-pyridinyl] substituent, such as **26**, were also a mixture of two regioisomers **26a** and **26b**, and each regioisomer existed as a mixture of two rotamers in Me_2SO-d_6 at 25 °C (**26a′**, **26a″**, and **26b′**, **26b″**) due to restricted rotation about the nitrogen-to-carbonyl carbamate bond. The calcium antagonist activities for **4** and **6** were determined by using the muscarinic receptor-mediated Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle. The relative order of activities for the 4-(dihydropyridinyl) analogues was 4-(dihydro-3-pyridinyl) > 4-(dihydro-4-pyridinyl). Increasing the size of the C-3(5) alkyl ester substituents increased activity. Compounds having nonidentical ester substituents were more active than those having identical ester substituents. Replacement of the C-3 and/or C-5 ester substituents by a cyano substituent(s) decreased activity significantly. An approximate 1:1 correlation between the IC_{50} value for inhibition of [^{3}H]nitrendipine binding and inhibition of the tonic component of the muscarinic-induced contractile response was observed. The test results suggest that a 4-(dihydropyridinyl) substituent is bioisosteric with a 4-(nitrophenyl) substituent on a 1,4-dihydropyridine ring where *m*- and *p*-nitrophenyl are bioisosteric with the 4-[1,2(1,6)-dihydro-3-pyridinyl] **4** and 4-(1,2-dihydro-4-pyridinyl) **6** isomers, respectively.

The utility of 4-aryl-1,4-dihydropyridines as therapeutic agents in cardiovascular disorders[1,2] has stimulated studies to investigate the geometrical requirements at the dihydropyridine binding site.[3-6] Changes in the substitution pattern at the C-3, C-4, and C-5 positions of the first-

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(1) Van Houtte, P. M.; Cohen, R. A. *Am. J. Cardiol.* **1983**, *52*, 99A.
(2) *Calcium Modulators*; Godfraind, T., Albertini, A., Paoletti, R., Eds.; Elsevier Biomedical: New York, 1982.

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