Diethyl 3,6-Dihydro-2,4-dimethyl-2,6-methano-1,3-benzothiazocine-5,11-dicarboxylates as Calcium Entry Antagonists: New Conformationally Restrained Analogues of Hantzsch 1,4-Dihydropyridines Related to Nitrendipine as Probes for Receptor-Site Conformation

John J. Baldwin, David A. Claremon,* Patricia K. Lumma, David E. McClure,† Seth A. Rosenthal, Raymond J. Winquist, Elizabeth P. Faison, Gregory J. Kaczorowski,‡ Mayme J. Trumble, and Graham M. Smith

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Rahway, New Jersey 07065.
Received August 25, 1986

The pharmacological activity of rigid analogues of 1,4-dihydropyridine calcium entry antagonists **9–16** is demonstrated by dose-dependent inhibition of the calcium contraction in depolarized rat aortic strips and by a [^{3}H]nitrendipine binding assay in using cardiac sarcolemmal membranes. From the results, a model is proposed as the receptor-bound conformation of the dihydropyridine calcium entry antagonists.

Among a heterogenous group of agents known as calcium channel antagonists,[1] which inhibit the influx of calcium ions across cell membranes, the most potent class is the 1,4-dihydropyridine derivative, represented in Figure 1 by nitrendipine. The recent investigations by Fossheim et al.[2] and Seidel et al.[3] strengthened their hypothesis that calcium entry antagonists of the Hantzsch dihydropyridine class have a boat-shaped pyridine ring as the efficacious conformation. In this conformation, the 4-aryl substituent is in a pseudoaxial orientation perpendicularly bisecting the plane of the 1,4-dihydropyridine ring shown as A in Figure 2. NMR investigations[4,5] support this hypothesis for the preferred solution equilibrium conformation as well. These studies have not ruled out the possibility that the receptor may induce a nonpreferred conformation or may preferentially interact with a minor component of the conformational equilibrium shown in Figure 2.

The classical approach to circumvent such arguments around the receptor-bound conformation is through the synthesis and evaluation of rigid analogues. The availability of conformationally restricted molecules that would contain all of the features of Hantzsch 1,4-dihydropyridines has been limited to those that connect the ortho position of the 4-aryl substituent with the 3-carboxylate[3] as represented in Figure 3. While these offer insight about the receptor, they still have the possibility of conformational mobility, especially when bound in the receptor site.

This paper describes the synthesis and biological properties of novel conformationally rigid calcium antagonists having the general structure shown in Figure 4. The biological results, by analogy with 1,4-dihydropyridines, suggest a preferred orientation of the biologically important[1,6] electron-withdrawing substituent on the aryl ring and support a receptor-site conformation most like that of A in Figure 2.

Results

Chemistry. Despite a voluminous amount of chemical and patent literature[6] describing Hantzsch dihydropyridines and their utility as either calcium entry agonists[7] or antagonists,[1] no examples of the intramolecular reaction of nucleophiles with C-2 on the pyridine have been described until recently.[8,9]

The preparation of the racemic sulfur-bridged analogues in Table I was performed as previously described[9a] to give a mixture of diastereomers, which was separated by flash chromatography. The examples with an unsubstituted 4-aryl substituent, **9** and **10**, were prepared as shown in Scheme I. Dihydropyridine **1** was cleanly and selectively oxidized at sulfur with *m*-chloroperbenzoic acid at −78 °C in dichloromethane to sulfoxide **7**. Pummerer rearrangement of the sulfoxide with sodium acetate in refluxing acetic anhydride followed by flash chromatography gave the masked thiol **8**, which was directly cyclized by treatment with potassium ethoxide in ethanol in 90% yield. Benzothiazocines **9** and **10** were assigned their structures

Table I. IC_{50} of Dose-Dependent Inhibition of the Calcium Contraction in Depolarized Rat Aortic Strips and the K_i Values for [^{3}H]Nitrendipine Binding in Cardiac Membranes

drug	rat aorta IC_{50} (95% confidence limit),[a] M	[^{3}H]nitrendipine binding: K_i,[a,b] M
2	1.3×10^{-7} (8.6×10^{-8}, 1.9×10^{-7})	8.0×10^{-8}
3	5.8×10^{-9} (3.9×10^{-9}, 8.7×10^{-9})	1.0×10^{-9}
9	1.2×10^{-6} (7.9×10^{-7}, 1.8×10^{-6})	5.0×10^{-7}
10	2.2×10^{-5} (1.4×10^{-5}, 3.3×10^{-5})	5.0×10^{-6}
11	3.1×10^{-8} (2.1×10^{-8}, 4.6×10^{-8})	3.0×10^{-9}
12	3.4×10^{-6} (2.3×10^{-6}, 5.2×10^{-6})	6.5×10^{-7}
13	3.7×10^{-6} (2.5×10^{-6}, 5.6×10^{-6})	6.0×10^{-6}
14	2.3×10^{-5} (1.5×10^{-5}, 3.5×10^{-5})	2.5×10^{-5}
15	1.4×10^{-5} (9.2×10^{-6}, 2.1×10^{-5})	3.0×10^{-6}
16	$>1.0 \times 10^{-4}$	5.0×10^{-6}
nitrendipine	3.1×10^{-9} (2.1×10^{-9}, 4.6×10^{-9})	2.5×10^{-10}
nifedipine	8.8×10^{-9} (5.8×10^{-9}, 1.3×10^{-8})	1.0×10^{-9}

[a] See Experimental Section for the method of calculation. [b] Standard error of the mean <5%.

† Present address: McNeil Pharmaceutical, Springhouse, PA 19477.

‡ Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

(1) For review, see: Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 775 and references cited therein.
(2) Fossheim, R.; Svarteng, K.; Mostad, A.; Romming, C.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1982**, *25*, 126.
(3) Seidel, W.; Meyer, H.; Born, L.; Kazda, S.; Dompert, W. In *Abstracts, Division of Medicinal Chemistry*; 187th National Meeting of the American Chemical Society, St. Louis, MO, April 8–13, 1984.
(4) Goldman, S.; Geiger, W. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 301.
(5) Berntsson, P.; Carter, R. E. *Acta Pharm. Suec.* **1981**, *18*, 221.
(6) Loev, B.; Goodman, M. M.; Snader, K. M., Tedeschi, R.; Macko, E. *J. Med. Chem.* **1974**, *17*, 956.
(7) Langs, D. A.; Triggle, D. J. *Mol. Pharmacol.* **1985**, *27*, 544 and references cited therein.
(8) Stout, D. M.; Meyers, A. I. *Chem. Rev.* **1982**, *6*, 427.
(9) (a) Claremon, D. A.; Hirshfield, J.; Lumma, P. K.; McClure, D. E.; Springer, J. P. *Synthesis* **1986**, 144. (b) Remy, D. C.; King, S. W.; Cochran, D.; Springer, J. P.; Hirshfield, J. *J. Org. Chem.* **1985**, *50*, 4120. (c) Hartman, G. D.; Halczenko, W.; Cochran, D. W. *Can. J. Chem.* **1986**, *64*, 556. (d) Hartman, G. D.; Halczenko, W.; Phillips, B. T. *J. Org. Chem.* **1986**, *51*, 142. (e) Hartman, G. D.; Halczenko, W.; Phillips, B. T. *J. Org. Chem.* **1985**, *50*, 2423 and 2427.

0022-2623/87/1830-0690$01.50/0

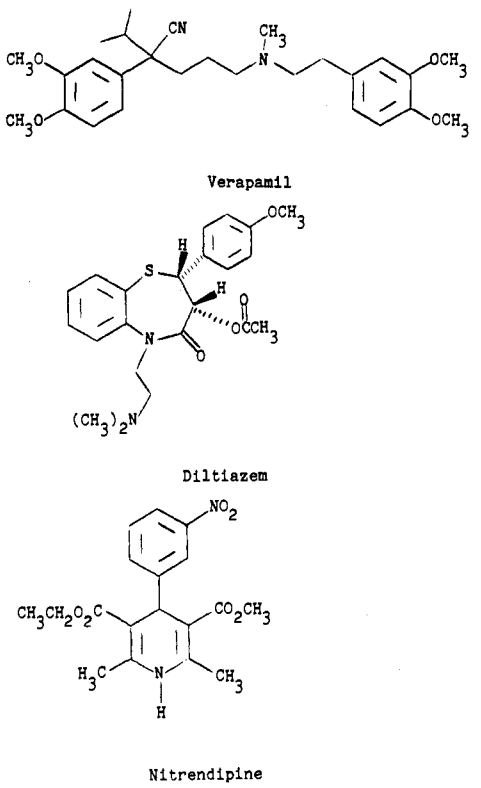


Figure 1. Calcium entry antagonists.

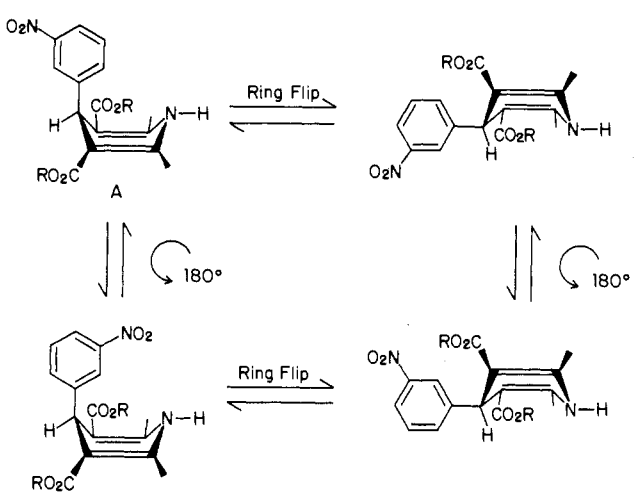


Figure 2. Simplified equilibrium conformation of nitrendipine.

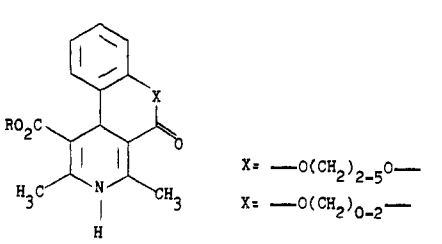


Figure 3. Lactone-bridged 1,4-dihydropyridine calcium antagonists.

on the basis of observation of a *W* coupling present in the ^{1}H NMR spectrum of isomer **10**.[9a] The dihydropyridines **2** and **3** in Table I were prepared by using the standard conditions for Hantzsch condensation.[6] The previously unreported benzothiazocines **15** and **16** were prepared from 2,4-dinitrobenzaldehyde by the sequence described in Scheme II and cyclization of **6** with the previously described procedure.[9a]

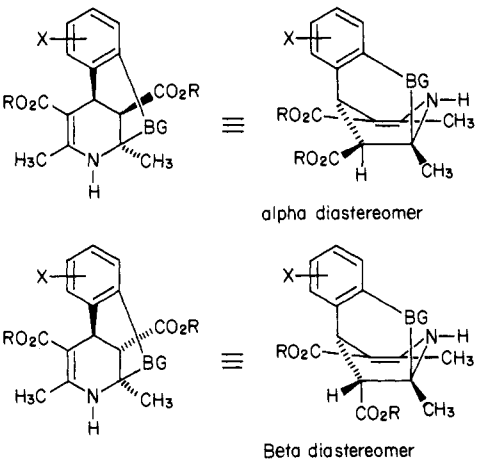


Figure 4. General structures of α and β isomers of bridged dihydropyridines.

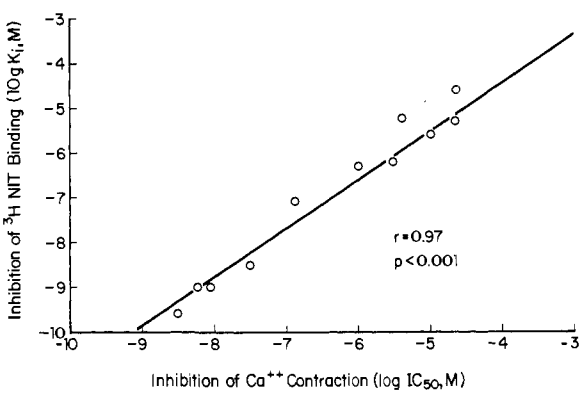


Figure 5. Inhibition of calcium contraction of depolarized aortic strips vs. inhibition of nitrendipine binding in partially purified cardiac sarcolemmal membrane vesicles.

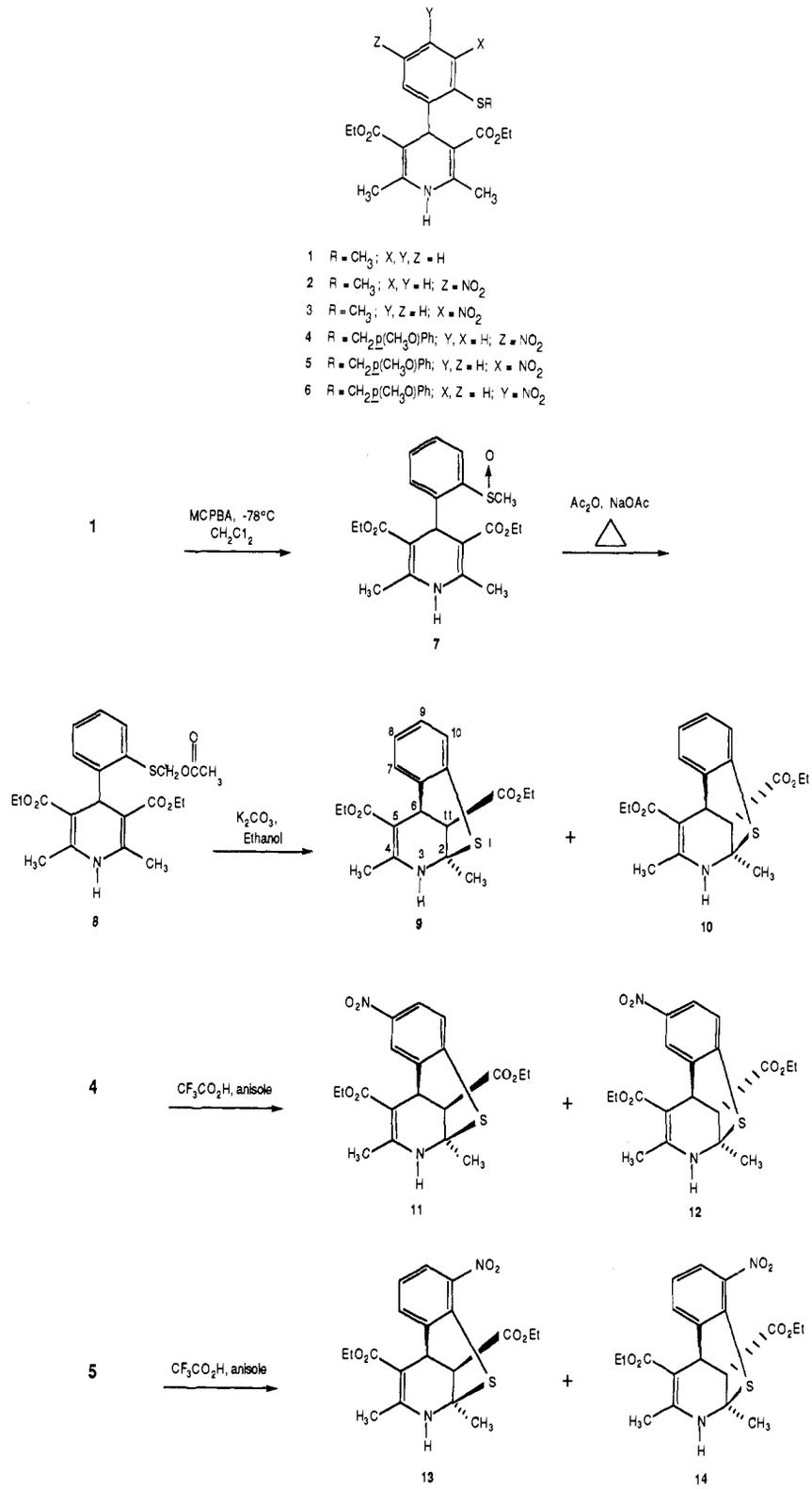
Discussion

The compounds, as summarized in Table I, caused dose-dependent inhibition of the calcium contraction in depolarized rat aortic strips, with IC_{50}'s ranging between 5.8×10^{-9} M for compound **3** and 2.3×10^{-5} M for compound **14**. With compound **16**, concentrations as high as 10^{-4} M did not elicit 50% relaxation and thus precluded calculation of an IC_{50} value. The most potent compound tested, **3** ($IC_{50} = 5.8 \times 10^{-9}$ M), was similar in activity to nifedipine ($IC_{50} = 8.8 \times 10^{-9}$ M) and slightly less active than nitrendipine ($IC_{50} = 3.1 \times 10^{-9}$ M).

The interaction of various conformationally restricted dihydropyridine analogues with the dihydropyridine receptor in cardiac sarcolemmal membranes was investigated by using a [^{3}H]nitrendipine binding assay. It has been shown previously that a number of different dihydropyridine agonists and antagonists interact at a unique receptor in cardiac membranes, which exists in a complex with binding sites for two other structural classes of Ca^{2+} entry blockers.[10] With this methodology, nitrendipine binding can be used as a probe to assess the rank order of potency of various dihydropyridines at this site.[11] In a partially purified cardiac sarcolemmal membrane vesicle preparation used for these experiments, binding of [^{3}H]-nitrendipine is time-dependent and saturable. Analysis of binding data in a Scatchard representation yields a K_d

(10) Garcia, M. L.; King, V. F.; Siegl, P. K. S.; Reuben, J. P.; Kaczorowski, G. J. *J. Biol. Chem.*, in press.
(11) Triggle, D. J.; Janis, R. A. In *New Pharmacological Methods*; Back, N., Spector, S., Eds.; Alan Liss: New York, 1984; p 2.

Scheme I

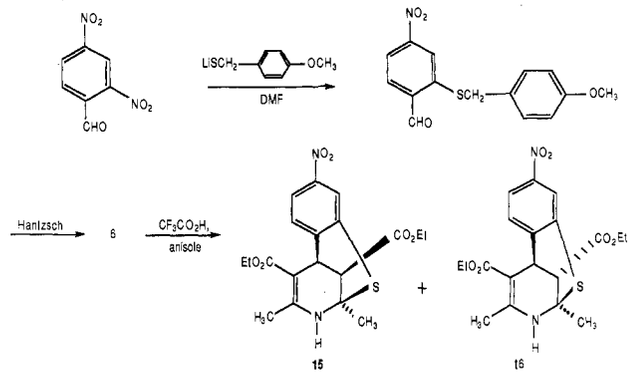


of 0.25 nM and B_{max} of 0.4 pmol/mg protein for this ligand (data not shown). For comparison, nifedipine inhibits nitrendipine binding with a K_i of 1×10^{-9} M (Table I).

A plot of inhibition of the calcium contraction of depolarized aortic strips vs. inhibition of nitrendipine binding in partially purified cardiac sarcolemmal membrane vesicle for the compounds in Table I (excluding **16**) is shown in Figure 5. The activities of these compounds in the pharmacological and binding assays exhibited a highly significant ($p < 0.001$) positive correlation ($r = 0.97$).

To determine the effect of ring formation on receptor affinity, the corresponding unrestricted thioethers **2** and **3** were evaluated for effects on [^{3}H]nitrendipine binding. These compounds were chosen for comparison since they simulate the electronic characteristics of the substituted-aryl rings of bridged compounds **11–14**. In both cases, inhibition of binding was complete and occurred over 2 log units of inhibitor concentration, as would be expected for

Scheme II



a strictly competitive interaction. Calculation of K_i values from I_{50} determinations by the Cheng–Prusoff relation[12] yields values of 8×10^{-8} M and 1×10^{-9} M for **2** and **3**, respectively (Table I). The slightly greater affinity of **3** for the nitrendipine site is in accord with previous observations regarding dihydropyridine Ca^{2+} entry blockers, which suggest that a preferred conformation of the aryl ring is perpendicular and pseudoaxial to the pyridine ring, and the nitro group is synperiplanar with the hydrogen at the 4-position of the pyridine ring. This conformation is more easily attained with the 2,3-disubstitution of the 4-aryl ring in **3** than with the 2,5-disubstitution of **2**.

The receptor preference for the near coplanarity of the 3,5-dicarboalkoxy groups with the 1,4-dihydropyridine ring is apparent from the comparison of the biological potencies of the bridged analogue diastereomers. The C-11 carboalkoxy functionality of the β-diastereomers **9**, **11**, and **13** is nearly coplanar with the pyridine ring, as in the hypothetical receptor conformation in nonrigid dihydropyridines. In addition, the β-ester isomers do not interfere with binding by unfavorable steric interactions with the receptor, as may be the case with the α-ester diastereomers **10**, **12**, and **14**. As demonstrated in Table I, each β-carbethoxy diastereomer (**9**, **11**, or **13**) is a more potent antagonist in the aorta assay and inhibitor of nitrendipine binding than its respective α-diastereomer (**10**, **12**, or **14**).

As is expected by analogy with 4-(*p*-nitrophenyl)dihydropyridines,[1] the two diastereomeric 9-nitrobenzothiazocine bridged analogues, **15** and **16**, show very weak activity as calcium entry antagonists and nitrendipine binding inhibitors. This reinforces the concept that the benzothiazocine class of compounds is interacting in a conformationally similar manner to dihydropyridines at the binding site. The data in Table I also indicates that the 10-nitro-substituted benzothiazocines **13** and **14** are no better than the unsubstituted compounds **9** and **10**. Considering only the β-diastereomers **9** and **13**, the added 10-nitro group of **13** decreased both the binding affinity and the potency in the aorta assay. Thus, the receptor may contain some functionality in proximity to the 9- and 10-positions of the benzothiazocine nucleus that sterically interferes with the nitro substituents. The increased potency of the 8-nitrobenzothiazocine **11** may be the result of the favorable positionings of the β-ester group and the nitro group. The positioning of the nitro group appears more crucial than any electronic interaction of the para sulfur atom, since the [2-(methylthio)-5-nitrophenyl]dihydropyridine **2** is significantly less potent than the regioisomeric (3-nitrophenyl)dihydropyridine **3**. The potency of **11** is weaker than that of **3**, nitrendipine, and nifedipine,

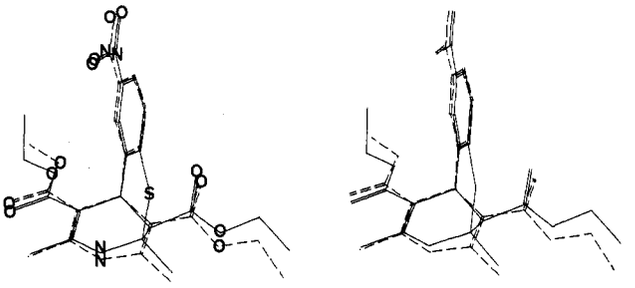


Figure 6. Superimposition of bridged analogue **11** and nitrendipine.

and this may be the result of the small steric effect of the additional C-11 proton in the bridged compound, which is necessarily absent in dihydropyridines.

On the basis of the above results and computer-generated graphics, the model shown in Figure 6 is proposed as the receptor-bound conformation of the dihydropyridine calcium entry antagonist nitrendipine. This structure (in dashed lines) has been superimposed on the bridged analogue **11**. A comparison of classic dihydropyridines with structures having a bridging sulfur atom using Dreiding molecular models and computer graphics demonstrated an exceptionally good overlap of the potential critical binding components. On the basis of the above observations, as well as those of others, it is suggested that the pharmacological activity of dihydropyridine calcium entry antagonists results from a conformation in which the 4-aryl ring is pseudoaxial and perpendicularly bisects the boat-shaped pyridine ring. This model suggests that the aryl nitro substituent is synperiplanar to the C-4 proton, supporting the recent hypotheses of others. We cannot rule out that other conformations of the dihydropyridines may also interact with the receptor, but we have shown by these rigid analogues that the presentation of the pharmacophore as described above is preferred, eliciting superior effects as an in vitro calcium antagonist and in the displacements of radiolabeled substrate from the receptor.

Experimental Section

Chemistry. Melting points are uncorrected and were determined on a Thomas-Hoover Unimelt apparatus. Proton NMR spectra were recorded in the solvent indicated on a Varian EM390 (90 MHz), Varian XL-300 (300 MHz), or Nicolet NT-360 (360 MHz) spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as the internal standard. Elemental analyses for carbon, hydrogen, and nitrogen were determined with a Perkin-Elmer Model 240 elemental analyzer and were within ±0.4% of theory unless otherwise noted. All starting materials were commercially available unless indicated otherwise and were used without further purification. Ethereal solvents were distilled from sodium benzophenone ketyl. Dichloromethane and dimethylformamide were distilled from calcium hydride. Reactions were followed by thin-layer chromatography using E. Merck 0.25-mm silica gel GF254 and visualized with ultraviolet light and/or 7% phosphomolybdic acid in ethanol and then heat.

Hantzsch 1,4-dihydropyridines were prepared by refluxing equimolar amounts of the respective benzaldehyde, ethyl 3-aminocrotonate, and ethyl acetoacetate at 2–5 molar concentrations in isopropyl alcohol for 12 h under argon. The products were isolated by flash chromatography or recrystallization.

Benzothiazocines **11–14** were prepared as previously described.[9a]

Diethyl 2,6-Dimethyl-4-[2-(methylthio)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (1). Compound **1** was prepared by Hantzsch condensation of 2-(methylthio)benzaldehyde[13] in 65% yield after isolation by flash chromatography (silica gel,

(12) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

(13) Holland, G. F.; Back, C. J.; Weissman, A. *J. Med. Chem.* **1963**, *6*, 519.

30% ethyl acetate in hexane) as an oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.20 (t, J = 7.5 Hz, 6 H), 2.21 (s, 6 H), 2.43 (s, 3 H), 4.10 (q, J = 7.5 Hz, 4 H), 5.45 (s, 1 H), 6.01 (br s, 1 H), 6.9–7.4 (m, 4 H).

Diethyl 2,6-Dimethyl-4-[2-(methylthio)-5-nitrophenyl]-1,4-dihydropyridine-3,5-dicarboxylate (2). Compound **2** was prepared by Hantzsch condensation of 2-(methylthio)-5-nitrobenzaldehyde[14] in 45% yield after isolation by crystallization from cold isopropyl alcohol and washing with ether/hexane: mp 177.5–179.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, J = 7.3 Hz, 6 H), 2.33 (s, 6 H), 2.54 (s, 3 H), 4.07 (q, J = 7.3 Hz, 4 H), 5.42 (s, 1 H), 5.74 (br s, 1 H), 7.20 (d, J = 8.8 Hz, 1 H), 7.95 (dd, J = 2.4, 8.8 Hz, 1 H), 8.15 (d, J = 2.4 Hz, 1 H). Anal. ($C_{20}H_{24}N_2O_6S$) C, H, N.

Diethyl 2,6-Dimethyl-4-[2-(methylthio)-3-nitrophenyl]-1,4-dihydropyridine-3,5-dicarboxylate (3). A suspension of sodium methylmercaptide (prepared from 958 mg (20.0 mmol) of 50% sodium hydride and gaseous methylmercaptan in 20 mL of THF at 0 °C) was added to 4.30 g (20 mmol) of methyl 2-chloro-3-nitrobenzoate in 10 mL of THF at 0 °C under argon over 15 min. The cooling bath was removed, and the reaction mixture was stirred for 1.5 h at 25 °C. The mixture was diluted with water and extracted with ethyl acetate. After the organic portion was dried ($MgSO_4$) and concentrated, an oil was obtained, which was dissolved in 40 mL of dichloromethane and cooled to −78 °C under argon. Diisobutylaluminum hydride (50 mL of 1.0 M solution in hexane) was added dropwise and the reaction mixture stirred an additional 3 h at −78 °C. After the reaction was quenched with 3.5 mL of methanol, saturated aqueous sodium potassium tartrate was added and the mixture warmed to 25 °C. The reaction mixture was extracted with ethyl acetate, and the combined organics were dried ($MgSO_4$) and concentrated to a yellow oil. This crude oil was dissolved in 250 mL of dichloromethane and mechanically stirred under argon with 12.0 g of activated MnO_2 for 3 days. After filtration through Celite and concentration, an oil was obtained, which was crystallized from ether to provide 2-(methylthio)-3-nitrobenzaldehyde as a pale yellow solid (3.35 g, 86%): mp 67.5–69 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (s, 3 H), 7.63 (t, J = 7.8 Hz, 1 H), 7.91 (d, J = 7.8 Hz, 1 H), 8.13 (d, J = 7.8 Hz, 1 H), 10.73 (s, 1 H). Anal. ($C_8H_7NO_3S$) C, H, N.

2-(Methylthio)-3-nitrobenzaldehyde (2.00 g, 10.0 mmol) was subjected to Hantzsch condensation in 5 mL of isopropyl alcohol for 12 h to give, after concentration and crystallization of the crude reaction mixture from ether/hexane, a solid, which was recrystallized from dichloromethane/ether/hexane to provide the analytical sample of 3.80 g as a yellow solid: mp 158–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, J = 7.1 Hz, 6 H), 2.28 (s, 6 H), 2.34 (s, 3 H), 4.07 (q, J = 7.1 Hz, 4 H), 5.61 (br s, 1 H), 5.70 (s, 1 H), 7.25–7.34 (m, 2 H), 7.58 (dd, J = 1.7, 7.5 Hz, 1 H). Anal. ($C_{20}H_{24}N_2O_6S$) C, H, N.

Diethyl 2,6-Dimethyl-4-[2-[(*p*-methoxybenzyl)thio]-4-nitrophenyl]-1,4-dihydropyridine-3,5-dicarboxylate (6). A solution of 2,4-dinitrobenzaldehyde (7.45 g, 38.0 mmol) in 15 mL of anhydrous dimethylformamide was stirred at 0 °C under argon and treated dropwise with a solution of 38.0 mmol of lithium (*p*-methoxybenzyl)thiolate (prepared by stirring under argon 300 mg, (38.0 mmol) of lithium hydride and 5.3 mL (38.0 mmol) of *p*-methoxy-α-toluenethiol in 15 mL of anhydrous dimethylformamide at 25 °C for 30 min). After the mixture was stirred at 0 °C for 1 h, the reaction was quenched by addition of water and extracted with ethyl acetate. The organic portion was washed with water and brine, dried ($MgSO_4$), concentrated on a rotary evaporator, and flash chromatographed (silica gel, 10% ethyl acetate in hexane). 2-[(*p*-Methoxybenzyl)thio]-4-nitrobenzaldehyde, 6.50 g (56%), was obtained as a yellow oil contaminated with traces of 2,4-dinitrobenzaldehyde: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (s, 3 H), 4.22 (s, 2 H), 6.89 (d, J = 9.0 Hz, 2 H), 7.28 (d, J = 9.0 Hz, 2 H), 8.01 (d, J = 9.0 Hz, 1 H), 8.14 (d, J = 9.0 Hz, 1 H), 8.34 (s, 1 H), 10.32 (s, 1 H). This aldehyde (1.00 g, 3.3 mmol) was subjected to Hantzsch condensation in 1.5 mL of isopropyl alcohol for 12 h. The crude reaction was concentrated and flash chromatographed (silica gel, dichloromethane) to give 700 mg (39%) of **6** isolated as a foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (t, J = 7.1 Hz, 6 H), 2.27 (s, 6 H), 3.79 (s, 3 H), 4.02–4.10 (m, 4 H), 4.19 (s, 2 H), 5.50 (s, 1 H), 5.86 (br s, 1 H), 6.85 (d, J = 8.8 Hz, 2 H), 7.31 (d, J = 8.8 Hz, 2 H), 7.48 (d, J = 8.3 Hz, 1 H), 7.86 (dd, J = 2.0, 8.3 Hz, 1 H), 8.06 (d, J = 2.0 Hz, 1 H).

Diethyl 3,6-Dihydro-2,4-dimethyl-9-nitro-2,6-methano-2*H*-1,3-benzothiazocine-5,11-dicarboxylate (15 and 16). A suspension of 700 mg (1.33 mmol) of dihydropyridine **6** in 5 mL of dichloromethane was stirred under argon and treated with 1 mL of anisole and 1 mL of trifluoroacetic acid. The mixture was stirred at 25 °C for 45 min and diluted with dichloromethane, washed with water and saturated aqueous sodium bicarbonate, dried ($MgSO_4$), and concentrated to an oil. Flash chromatography (silica gel, 1% acetone in dichloromethane) and crystallization from ether/hexane provided 260 mg (48%) of **15** and 190 mg (35%) of **16**.

15: mp 76 °C dec; t_R = 9.36 min (98:2 hexane/isopropyl alcohol, 2 mL/min; Waters Resolve, 3.9 mm × 15 cm, 5-μm spherical silica); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, J = 7.3 Hz, 3 H), 1.33 (t, J = 7.3 Hz, 3 H), 1.98 (s, 3 H), 2.23 (s, 3 H), 3.03 (d, J = 3.4 Hz, 1 H), 4.00–4.30 (m, 4 H), 4.76 (d, J = 3.4 Hz, 1 H), 7.61 (d, J = 9.3 Hz, 1 H), 7.85 (dd, J = 2.4, 9.3 Hz, 1 H), 7.90 (d, J = 2.4 Hz, 1 H); HRMS calcd for $C_{19}H_{22}N_2O_6S$: 406.1198. Found: 406.1199.

16: mp 117.5–120 °C; t_R = 5.20 min (98:2 hexane/isopropyl alcohol, 2 mL/min; Waters Resolve, 3.9 mm × 15 cm, 5-μm spherical silica); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7.3 Hz, 3 H), 1.33 (t, J = 7.3 Hz, 3 H), 1.94 (s, 3 H), 2.25 (s, 3 H), 2.97 (dd, J = 1.4, 1.5 Hz, 1 H, collapsed to d, J = 1.4 Hz upon irradiation at 4.86 and to d, J = 1.5 Hz upon irradiation at 4.65), 4.19 (m, 4 H), 4.65 (br s, 1 H), 4.86 (br s, 1 H), 7.65 (d, J = 8.3 Hz, 1 H), 7.81 (dd, J = 2.0, 8.3 Hz, 1 H), 7.92 (d, J = 2.0 Hz, 1 H). HRMS calcd for $C_{19}H_{22}N_2O_6S$: 406.1198. Found: 406.1199.

Diethyl 2,6-Dimethyl-4-[2-(methylsulfinyl)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (7). A dichloromethane (320 mL) solution of **1** (17.3 g, 46.1 mmol) was stirred under an argon atmosphere at −78 °C and treated with 9.83 g of *m*-chloroperbenzoic acid over 30 min. This was stirred for an additional 2 h at −78 °C and then diluted with dichloromethane. After the mixture was washed with 5% aqueous sodium bicarbonate and brine, the dried ($MgSO_4$) organic phase was concentrated by rotary evaporation and the crude sulfoxide **7** was obtained (17.14 g, 95%) and used without further purification. An analytical sample of a yellow solid was obtained by recrystallization from methanol: mp 233–235 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.18 (t, J = 7.5 Hz, 6 H), 2.14 (s, 3 H), 2.28 (s, 6 H), 4.00–4.24 (m, 4 H), 5.45 (s, 1 H), 5.91 (br s, 1 H), 7.38 (m, 3 H), 7.99 (m, 1 H). Anal. ($C_{20}H_{25}NO_5S$) C, H, N.

Diethyl 2,6-Dimethyl-4-[2-[(acetoxymethyl)thio]-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (8). Crude **7** (15.0 g, 38.4 mmol) was suspended in 36 mL of acetic anhydride and stirred with 4.92 g (60 mmol) of anhydrous sodium acetate under an argon atmosphere. This mixture was refluxed for 40 min and then the acetic anhydride removed by rotary evaporation. The crude product was flash chromatographed (silica gel, 25% ethyl acetate in hexane) to give 11.7 g (69%) of **8** as an oil, which crystallized on standing. An analytical sample of an off-white solid was obtained by recrystallization from ether/hexane: mp 117–118 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.18 (t, J = 7.5 Hz, 6 H), 2.14 (s, 3 H), 2.28 (s, 6 H), 3.99–4.14 (m, 4 H), 5.39 (s, 2 H), 5.42 (s, 1 H), 5.51 (br s, 1 H), 7.13 (m, 2 H), 7.37 (dd, J = 7.0, 2.0 Hz, 1 H), 7.48 (dd, J = 7.0, 2.0 Hz, 1 H). Anal. ($C_{22}H_{27}NO_6S$) C, H, N.

Diethyl 3,6-Dihydro-2,4-dimethyl-2,6-methano-2*H*-1,3-benzothiazocine-5,11-dicarboxylate (9 and 10). An ethanol (30 mL) solution of sulfide **8** (1.0 g, 2.3 mmol) was degassed with Ar and stirred with 2.20 g (15.9 mmol) of powdered anhydrous potassium carbonate for 3 h. The mixture was filtered, treated with aqueous ammonium chloride, and extracted with dichloromethane. The organic portion was washed with water and brine, dried (Na_2SO_4), concentrated, flash chromatographed (silica gel, 2% acetone in dichloromethane), and crystallized from ether/hexane to give 250 mg (30%) of **9** and 500 mg (60%) of **10**.

9: mp 117–120 °C; R_f 0.30 (silica gel, 2% acetone in dichloromethane); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.03 (t, J = 7.5 Hz, 3 H), 1.33 (t, J = 7.5 Hz, 3 H), 1.96 (s, 3 H), 2.22 (s, 3 H), 2.96 (d, J = 4.0 Hz, 1 H), 3.88–4.16 (m, 2 H), 4.17–4.25 (m, 2 H), 4.63 (br s, 1 H), 4.67 (d, J = 4.0 Hz, 1 H), 6.92–7.06 (m, 3 H), 7.43

(14) Nagata, K. *Chem. Pharm. Bull.* **1969**, *17*, 661.

(dd, J = 7.0, 2.0 Hz, 1 H). Anal. ($C_{19}H_{23}NO_4S$) C, H, N.

10: mp 147–148.5 °C; R_f 0.25 (silica gel, 2% acetone in dichloromethane); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.26 (t, J = 7.5 Hz, 3 H), 1.33 (t, J = 7.5 Hz, 3 H), 1.91 (s, 3 H), 2.23 (s, 3 H), 3.02 (dd, J = 1.5, 1.7 Hz, 1 H, collapses to d, J = 1.7 Hz upon irradiation at 4.77), 4.07–4.24 (m, 4 H), 4.55 (br s, 1 H), 4.77 (br s, 1 H), 6.95–7.05 (m, 3 H), 7.50 (d, J = 7.0 Hz, 1 H). Anal. ($C_{19}H_{23}NO_4S$) C, H, N.

Biological Methods. Inhibition of Ca^{2+} Contraction in Depolarized Rat Aorta. Male Sprague–Dawley rats (250–300-g body weight) were killed, and the thoracic aorta was rapidly removed and placed in physiological salt solution (PSS) of the following composition (in mM): NaCl (130), KCl (4.7), KH_2PO_4 (1.18), $MgSO_4 \cdot 7H_2O$ (1.17), $NaHCO_3$ (14.9), dextrose (11.0), EDTA (0.026), and $CaCl_2$ (1.6). Vessels were mounted on a fine steel wire (32–35 gauge) and cleaned with the aid of a dissecting scope. Two helically cut strips (1 × 10 mm) were isolated from each aorta, beginning from the end most proximal to the aortic arch. Strips were then suspended in jacketed glass tissue baths via 4-0 surgical thread; one end was secured to a glass hook, and the other end was connected to a force displacement transducer. Tissues were equilibrated for 1 h under 2 g of resting force in aerated PSS (95% O_2, 5% CO_2) at 37–39 °C. Strips were washed every 20 min with PSS. Tissue baths and glass hooks were pretreated for a minimum of 20 min with bovine albumin (2.5 g/500 mL of distilled H_2O).

After the equilibration period, the tissues were washed in a depolarizing solution (40 mM KCl; equimolar substitution of K^+ for Na^+) containing 1.6 mM calcium. When a maximum contraction was attained, the tissues were washed in calcium-free PSS containing 1 mM EGTA (buffer A) for 5 min and then washed in O-Ca^{2+} depolarizing solution (40 mM KCl, no EGTA) (buffer B) for 15 min. The tissues were then contracted by the addition of calcium ($CaCl_2$, 2.5 mM) to obtain the control response. Tissues were washed with buffer A (5 min), then with buffer B (15 min). Either a test compound or a vehicle was added to the bath during the last 15 min. Two tissues were always treated with the vehicle, and each compound was tested in at least four strips, never from the same rat.

The sequence of buffer A (5 min), buffer B + drug or vehicle (15 min) was repeated with the drug being added to the bath in a noncumulative fashion in half-log increments with each cycle.

The following formula was used to calculate the percent inhibition for a particular tissue:

$$\% \text{ inhibition} = 100 \times [1 - (\text{response in presence of drug}) / (\text{response to initial 2.5 mM } Ca^{2+} \text{ contraction} \times J)]$$

where

J = (response in presence of vehicle)/(response to initial 2.5 mM Ca^{2+} contraction)

J was calculated in two parallel control strips (n = 2), then averaged (J). IC_{50} values (95% confidence limits) were obtained by using simple linear regression on ARCSIN transformation of percent relaxation vs. log of the concentration.

[^{3}H]Nitrendipine Binding in Cardiac Sarcolemmal Membrane Materials. [^{3}H]Nitrendipine (78 Ci/mmol) was purchased from New England Nuclear. GF/C glass fiber filters were obtained from Whatman Co. All other reagents were of the highest purity available from commercial source.

Preparation of Membrane Vesicles. Partially purified cardiac sarcolemmal membrane vesicles were prepared from fresh porcine left ventricle tissue by the method of Reeves and Sutko.[15] Vesicles were resuspended in 50 mM Tris·HCl, pH 7.4 at a final protein concentration of 20 mg/mL and quick frozen in liquid nitrogen. Vesicles could be stored at −70 °C for up to 6 months without any loss of dihydropyridine binding activity. Although partially purified sarcolemmal vesicles were used in these experiments, it was found that binding data were essentially identical with that obtained with highly purified cardiac sarcolemmal membrane vesicles.

Nitrendipine Binding Assay. Cardiac membranes (100 μg) resuspended in 50 mM Tris·HCl, 10 μM $CaCl_2$, 10 μM $MgCl_2$, pH 7.4 were incubated with 0.25 nM [^{3}H]nitrendipine in the presence or absence of other drugs for 1.5 h at 25 °C. The final volume of the reaction mixture was maintained at 200 μL. At the end of the incubation period, samples were diluted with 4 mL of 10 mM Tris·HCl, pH 7.4 kept at 4 °C and rapidly filtered through GF/C filters, which were subsequently washed twice with the same ice-cold quench buffer. Nonspecific binding was assessed with 1 μM [^{1}H]nitrendipine. For each determination, assays were performed in triplicate and yielded data with a standard error of the mean typically less than 5%. In these experiments, specific ligand binding represented at least 75% of total binding and test compounds were found not to change the nonspecific interaction of [^{3}H]nitrendipine with glass fiber filters. All stock solutions of drugs were made in dimethyl sulfoxide, and the concentration of this solvent was never allowed to exceed 0.5% in the assay medium. I_{50} values for test compounds were determined graphically from inhibitory profiles, and K_i values were calculated according to the Cheng–Prusoff relation.[12]

(15) Reeves, J. P.; Sutko, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 590.