Calcium Channel Blocking and Positive Inotropic Activities of Ethyl 5-Cyano-1,4-dihydro-6-methyl-2-[(phenylsulfonyl)methyl]-4-aryl-3-pyridinecarboxylate and Analogues. Synthesis and Structure–Activity Relationships

Ila Sircar,*,† Eva K. Gregor,† K. R. Anderson,† Stephen J. Haleen,‡ Yu-Hsin Shih,‡ Ronald E. Weishaar,‡,§ Robert P. Steffen,‡,∥ Thomas A. Pugsley,‡ and M. D. Taylor†

Departments of Chemistry and Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received April 2, 1990*

The synthesis and pharmacological evaluation of a series of 2-[(arylsulfonyl)methyl]-4-aryl-5-cyano-1,4-dihydropyridine-3-carboxylic acid esters and analogues are described. These compounds possess a unique profile namely, calcium channel blocking and positive inotropic activities in vitro. Compound **54** was selected as the best compound in the series and was studied in detail. The synthesis and biological profiles of enantiomers of **54** are also reported. The data indicate that although the calcium channel blocking property of **54** is stereospecific the positive inotropic activity is not. Examples of 3- and 6-cyano and other closely related 1,4-dihydropyridine derivatives are described and evaluated for comparison and were found to be devoid of dual activities mentioned above.

1,4-Dihydropyridines have been widely explored as cardiovascular agents in recent years. Nifedipine has been approved for clinical use as an antianginal agent and represents the prototype 1,4-dihydropyridine (DHP) structure found useful in both antianginal and antihypertensive therapy. Studies using animal models have shown that calcium channel blockers (CCB) such as nifedipine and the nondihydropyridines diltiazem and verapamil also produce cardioprotection when administered either acutely or chronically.[1] These agents reduce vascular resistance by blocking calcium entry through calcium slow channel, but also decrease ventricular contractility by the same mechanism. Although felodipine and several other DHP derivatives have been reported to have enhanced vascular selectivity,[2,3] calcium channel blockers have a limited role in the treatment of heart failure.[4-7] Conversely, the calcium channel stimulant BAY K 8644 increases ventricular contractility by stimulating calcium entry into cardiac muscle.[8,9] However, it also increases calcium entry into vascular smooth muscle, causing vasoconstriction, thereby limiting the role of these agents in heart failure patients.[10]

To date, no calcium channel stimulant has been reported to be cardioselective. Great efforts have been undertaken to understand the molecular basis of action of these agents and to improve their pharmacological profile.[11a-d] Our goal was to discover DHPs which would retain calcium channel blocking activity and, in addition, would possess cardiotonic activity to compensate for negative inotropic effects associated with CCB drugs. Such dual activities in a single agent might offer some novel and attractive research tools. In addition, these second generation DHPs might possess a suitable therapeutic profile for treating congestive heart failure (CHF) patients. In this report, a series of 2-[(arylsulfonyl)methyl]-1,4-dihydropyridines (I, Chart I) that possess the dual activities mentioned above are described.

Chemistry

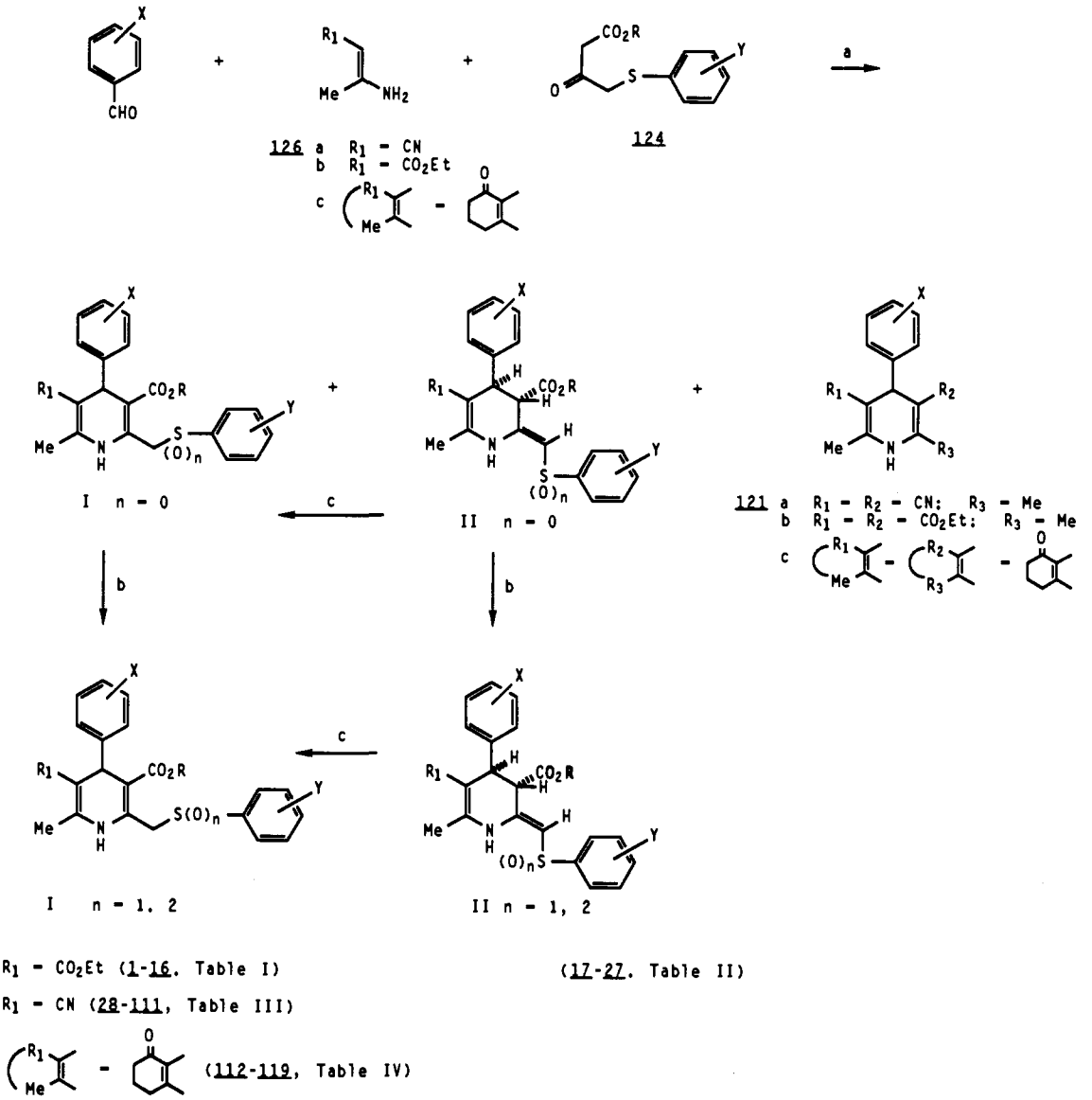
The synthesis of the title compounds described in Tables I–IV was accomplished by using a classical three-component Hantzsch reaction with suitably substituted benzaldehyde, enamine, and the requisite β-keto ester as starting material.[12] As shown in Scheme I, this procedure yields both DHP (I, $n = 0$, endo isomer) and tetrahydropyridine (THP) (II, $n = 0$, exo isomer) in varying amounts[13,14] in addition to the symmetrical DHP (**121a–c**). These compounds were separated via extensive chromatography to give the desired DHP I in 30%–40% yields. It is worthwhile to note that in the cases of (a) 2-difluoromethoxy benzaldehyde and (b) cyclohexyl carboxaldehyde only the exo isomer products were isolated by chromatography. Structures of I and II were confirmed by analysis and spectral data. The ^{1}H NMR spectra of the exo isomers differed from related 1,4-DHPs by the presence of three one-proton singlets at δ 5.25, 3.54, and 4.23

† Department of Chemistry.
‡ Department of Pharmacology.
§ Present address: Coromed, Inc., Rensselaer Technology Park, 185 Jordan Park, Troy, NY 12180.
∥ Present address: Burroughs Wellcome Company, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

(1) Naylor, W. G.; Thoras, J. *Cardiovasc. Surg.* **1982**, *84*, 897.
(2) (a) Timmis, A. D.; Smyth, P.; Kenney, J. F.; Cambell, S.; Jewitt, D. E. *Br. Heart J.* **1984**, *52*, 314. (b) Timmis, A.; Jewitt, D. E. *Drugs* **1985**, *29* (Suppl. 2), 66.
(3) (a) Timmis, A. D.; Cambell, S.; Monaghan, M. J.; Walker, L.; Jewitt, D. E. *Br. Heart J.* **1984**, *51*, 441. (b) Singh, B. N.; Baky, S.; Nademanee, K. *Am. J. Cardiol.* **1985**, *55*, 214.
(4) Baughman, K. L. *Am. J. Med.* **1986**, *80*, 46.
(5) Colucci, W. S. *Am. J. Cardiol.* **1987**, *59*, 528.
(6) Colucci, W. S.; Fifer, M. A.; Lorell, V. H.; Wynne, J. *Am. J. Med.* **1985**, *78*, 9.
(7) Packer, M.; Kessler, P. D.; Lee, W. H. *Circulation* **1987**, *75* (Suppl. V), 56.
(8) (a) Schramm, M.; Towart, R.; Lamp, B.; Thomas, G. J. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 493. (b) Leonetti, G.; Gradnik, R.; Terzoli, R.; Fruscio, M.; Rupoli, L.; Zanchelti, A. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 392.
(9) (a) Preuss, K. C.; Gross, G. J.; Brooks, H. L.; Warltier, D. C. *Life Sciences* **1985**, *37*, 1271. (b) Schramm, M.; Thomas, G.; Toward, R.; Franckowiak, G. *Nature* **1983**, *303*, 535.
(10) (a) Janis, R. A.; Silver, P. J.; Triggle, D. J. *Adv. Drug Res.* **1987**, *16*, 309. (b) Porzig, H. *J. Cardiol. Pharmacol.* **1989**, *14* (Suppl. 3), S15. (c) Triggle, D. J.; Janes, R. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 347.
(11) (a) Arrowsmith, J. A.; Campbell, S. F.; Cross, P. E.; Stubb, J. K.; Burges, R. A.; Gardiner, D. G.; Blackburn, K. J. *J. Med. Chem.* **1986**, *29*, 1696. (b) Kimura, Y.; Fukui, H.; Tanaka, M.; Okamoto, M.; Morino, A.; Miura, A.; Kimura, K.; Enamoto, H. *Arzneim. Forsch.* **1986**, *36*, 1329. (c) Baldwin, J. A.; Clameron, A.; Lumma, P. K.; McClure, D. E.; Rosenthal, S. A.; Winquist, R. J.; Farson, E. P.; Kaczorowsky, G. J.; Trumble, M. J.; Smith, G. M. *J. Med. Chem.* **1987**, *30*, 690. (d) For a review on pharmacological activities, see: F. R. Buhler, Ed. *J. Cardiovasc. Pharmacol.* **1984**, *6* (Suppl. 7), S929–S1113.
(12) Sausins, A.; Duburs, G. *Heterocycles* **1988**, *27*, 269.
(13) Taylor, M. D.; Badger, E. W.; Steffen, R. P.; Haleen, S. J.; Pugsley, T. A.; Shih, Y. H.; Weishaar, R. E. *J. Med. Chem.* **1988**, *31*, 1659.
(14) Frigerio, M.; Zaliani, A.; Riva, C.; Palmisano, G.; Pilati, T.; Gandolfi, C. A. *Tetrahedron Lett.* **1988**, *29* (48), 6335.

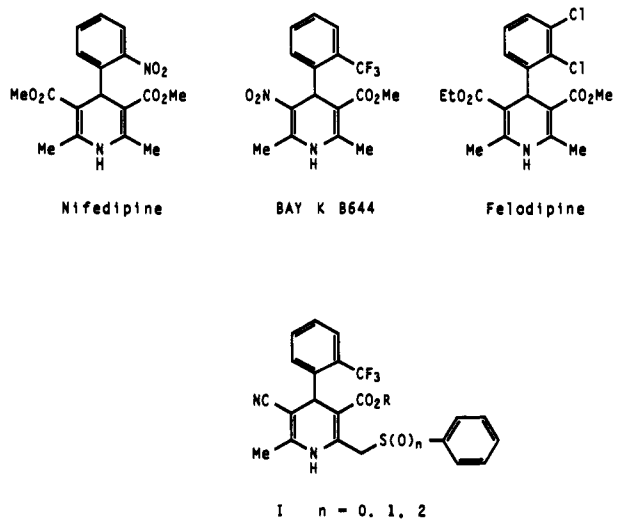
0022-2623/91/1834-2248$02.50/0

Scheme I[a]



[a] (a) EtOH; (b) *m*-CPBA, CH_2Cl_2; (c) NaOEt, EtOH.

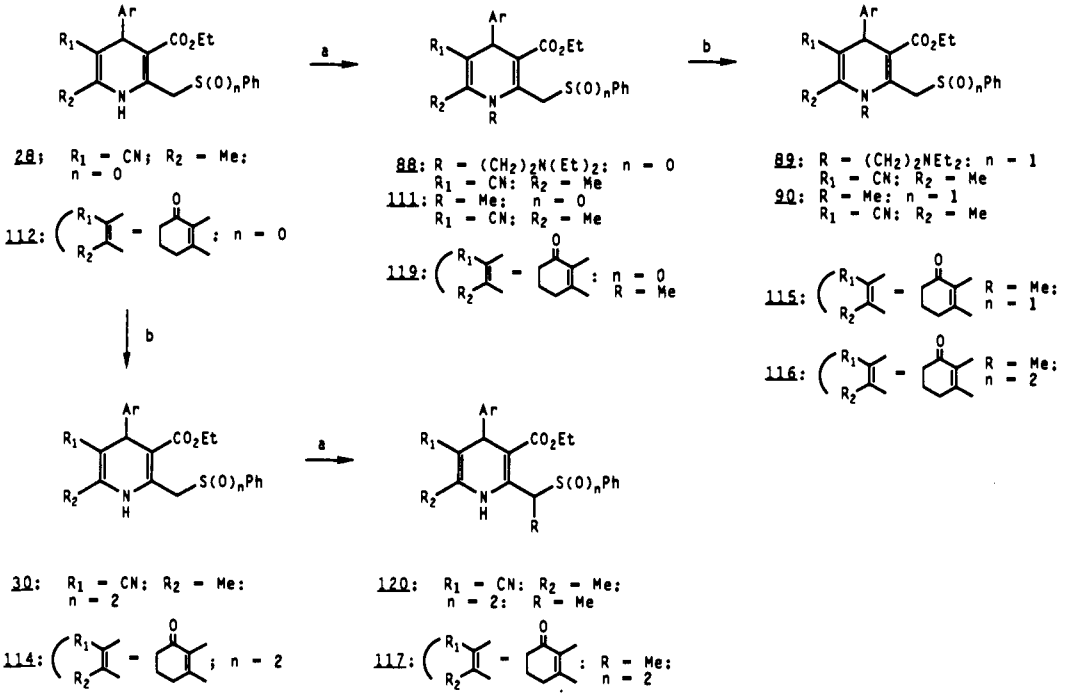
Chart I



(for **19**). IR spectrum also indicated a strong carbonyl absorption at 1739 cm^{-1} versus 1705 cm^{-1} for 1,4-DHPs. Lack of demonstrable coupling between the vicinal C-3 and C-4 protons indicates an orthogonal torsional angle for C-3,4 protons. Therefore, a trans configuration was assigned because an orthogonal torsional angle is not accessible to the cis isomer. Only a single geometrical isomer possessing a *Z* exocyclic double bond,[13,14] with S–Ar and NH bonds mutually parallel, was isolated except for the bicyclic derivative in which case both *Z* and *E* isomers (**118a** and **118b**) were obtained (see Experimental Section for details).

Both endo and exo isomers (I and II, respectively) were oxidized with mCPBA to give corresponding sulfoxides (as diastereomeric mixtures) and sulfones (I and II, $n = 1, 2$). The oxidation products were always contaminated with each other and separated by chromatography. In some cases the two diastereomeric sulfoxides were also separated (for example, compounds **8a/8b**, **10a/10b**, **29a/29b**, and **32a/32b**). The exo isomers (II) were converted to the corresponding endo isomers (I) by base treatment (NaOEt/EtOH). These exo–endo tautomerizations were carried out either at the sulfide or at the sulfoxide/sulfone stage. Several modifications of the initial three-part Hantzsch reaction were attempted to improve the yields of DHP without much success. An improved and efficient procedure to prepare DHP I was eventually discovered

Scheme II[a]



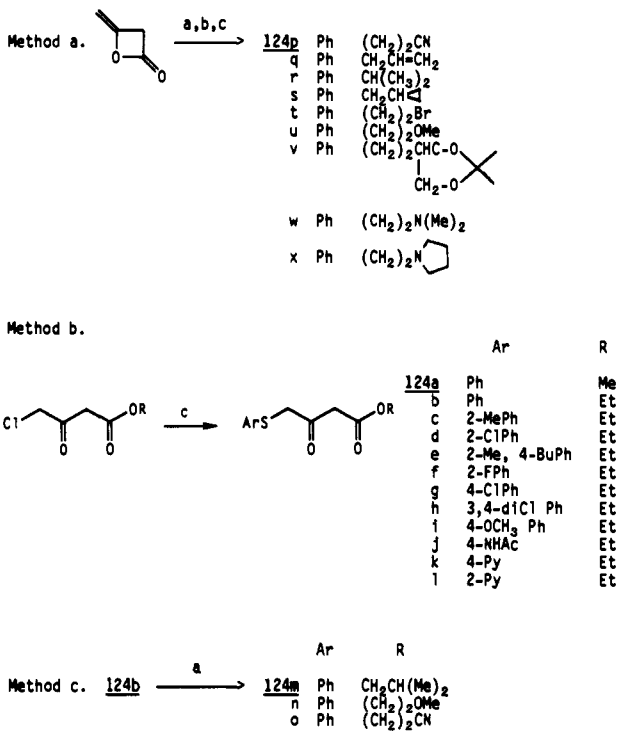
[a] (a) NaH, RX, DMF; (b) *m*-CPBA, CH_2Cl_2.

starting from DHP **121d** via bromination followed by displacement of bromine with requisite thiols (Scheme VI).[15] Alkylation of DHP sulfide (**28** and **112**) in the presence of NaH/DMF afforded N-alkylated products (**88**, **111**, and **119**) which were oxidized to the corresponding sulfoxides (**89**, **90**, and **115**) and sulfone (**116**). When the alkylation was carried out on the DHP sulfone (**30** and **114**) C-alkylated products (**120** and **117**) instead of N-alkylated products were obtained as mixture of diastereomers (Scheme II). The structure of **120** and **117** were proven unambiguously from the ^1H NMR spectra (see Experimental Section for details).

Since modification of the 3-ester function of 1,4-DHPs has been reported to improve the vasodilator activity[10] several similar variations of the 3-ester function of DHP **28** were undertaken. Synthesis of the requisite β-keto esters **124a–x** are shown in Scheme III. The most practical method (method a) involved a three-step synthesis starting from diketene. Diketene was reacted with a variety of alcohols to produce desired ketoester derivatives[16] which were converted to 4-bromo esters with bromine in $CHCl_3$. Displacement of bromine with the requisite thiol in the presence of base afforded desired compounds. Method b involved utilization of commercially available ethyl (or methyl) 4-chloroacetoacetate[17] instead of the corresponding bromoacetate as described in method a. Method c involved an ester exchange method[18] on compounds derived from method a.

Considerable effort was spent on the synthesis of DHP containing aminoalkyl esters at 3-position. These compounds (**108–110**) were targeted for improving the aqueous solubility of these agents. Direct synthesis using the

Scheme III[a]



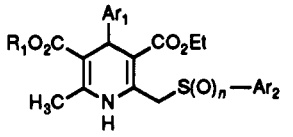
[a] (a) ROH, Δ; (b) Br_2, $CHCl_3$, 0 °C; (c) ArSH, Et_3N, THF.

conventional three-part Hantzsch reaction was unsuccessful, leading to primarily the symmetrical dihydropyridine **121a**. Ester exchange procedure on DHP ethyl ester with dialkylamino ethanol was not successful either (Scheme IV). Compound **108** was obtained in 50% yield from the reaction of DHP bromoethyl ester **106** with piperidine. These compounds were, however, best prepared from the carboxylic acid **101** by using mixed anhydride method (Scheme IV).

The synthesis of the corresponding 3-cyano DHP derivatives (**122a–c**, $n = 0, 1, 2$) is illustrated in the Scheme

(15) Sircar, I.; Anderson, K.; Bonadies, L. *Tetrahedron Lett.* **1988**, *29* (52), 6835.
(16) Lawesson, S. O.; Gronwali, S.; Sandberg, R. *Org. Syn.* V, 155.
(17) Bodalski, R.; Pietrusiewicz, K. M.; Monkiewicz, J.; Koszuk, J. *Tetrahedron Lett.* **1980**, *21*, 2287.
(18) Iwanani, M.; Shibanuma, T.; Fujimoto, M.; Kawai, R.; Tamazawa, K.; Takenara, T.; Takahasi, K.; Murakami, M. *Chem. Pharm. Bull.* **1979**, *27*, 1426.

Table I. 4-Aryl-1,4-dihydropyridine 3,5-Diester Derivatives[a]



compd	Ar_1	R_1	n	Ar_2	mp, °C[b] (crystn solvent)	mol formula[c]	receptor affinity data inhibition of [^{3}H]nitrendipine binding: IC_{50}, nM[e]
1	2-$CF_3C_6H_4$	Et	0	Ph	129–130 (A)	$C_{26}H_{26}F_3NO_4S$	1.6
2	2-$CF_3C_6H_4$	Et	1	Ph	142–143	$C_{26}H_{26}F_3NO_5S$	27
3	2-$CF_3C_6H_4$	Et	2	Ph	194–195	$C_{26}H_{26}F_3NO_6S$	NT
4	2-$CF_3C_6H_4$	Me	0	2-ClC_6H_4	123–125 (A)	$C_{25}H_{23}ClF_3NO_4S$	36
5	2-$CF_3C_6H_4$	Me	1	2-ClC_6H_4	155–157 (B)	$C_{25}H_{23}ClF_3NO_5S$	NT
6	2-$CF_3C_6H_4$	Me	2	2-ClC_6H_4	152–153 (A)	$C_{25}H_{23}ClF_3NO_6S$	NT
7	2-$CF_3C_6H_4$	Et	0	4-$OMeC_6H_4$	oil	$C_{27}H_{28}F_3NO_5S$	NT
8a	2-$CF_3C_6H_4$	Et	1	4-$OMeC_6H_4$	190–191	$C_{27}H_{28}F_3NO_6S$	21
8b	2-$CF_3C_6H_4$	Et	1	4-$OMeC_6H_4$	159–160	$C_{27}H_{28}F_3NO_6S$	18
9	2-$CF_3C_6H_4$	Et	0	2-MeC_6H_4	117–119 (A)	$C_{27}H_{28}F_3NO_4S$	49
10a	2-$CF_3C_6H_4$	Et	1	2-MeC_6H_4	143–147 (A)	$C_{27}H_{28}F_3NO_5S$	160
10b	2-$CF_3C_6H_4$	Et	1	2-MeC_6H_4	140–144 (A)	$C_{27}H_{28}F_3NO_6S$	170
11	2-$CF_3C_6H_4$	Et	0	4-Py	146–148 (D)	$C_{25}H_{25}F_3N_2O_4S$	32.5
12	2-$CF_3C_6H_4$	Et	1	4-Py	161–163 (C)	$C_{25}H_{25}F_3N_2O_5S$	37.4
13	2-$CF_3C_6H_4$	Et	0	2-Py	118–119	$C_{25}H_{25}F_3N_2O_4S$	51
14	2-$CF_3C_6H_4$	Et	1	2-Py	182–183 dec	$C_{25}H_{25}F_3N_2O_5S$	10
15	3-Py	Et	0	Ph	116–118	$C_{24}H_{26}N_2O_4S$	523
16	3-Py	Et	1	Ph	172–173	$C_{24}H_{26}N_2O_5S$	>1000

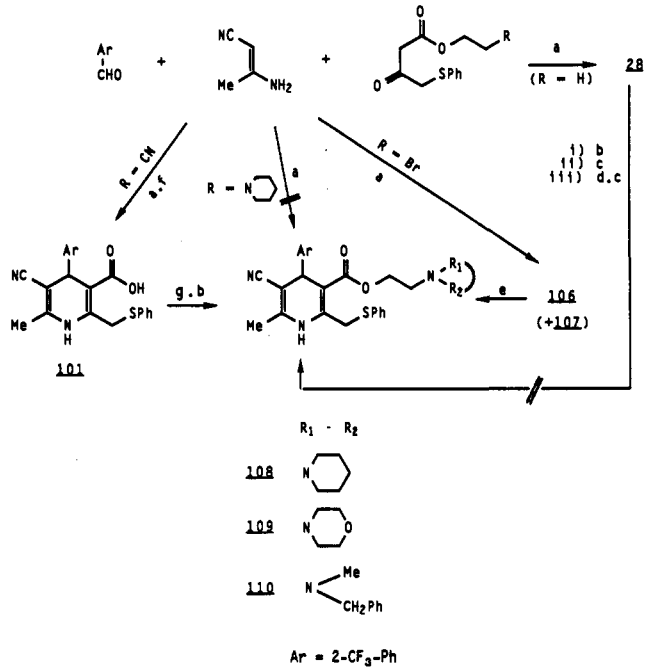
[a] Yields of compounds were not optimized. Compounds (n = 0) were obtained in 30–45% yields. Compounds (n = 1, 2) were obtained in 70–85% yields. [b] A, EtOAc/isopropyl ether; B, isopropyl ether: C, EtOAc; D, EtOAc/MeOH. All other compounds were purified via chromatography. [c] Compounds were analyzed within 0.4% theory. NT = not tested. [e] Index of DHP receptor affinity. In order to obtain an initial estimate of the binding affinity of the test compounds, each compound was tested at 1000 nM. If a compound elicited >40% inhibition of ligand ([^{3}H]nitrendipine) at 1000 nM, a four to seven point curve with unlabeled compound used was carried out. The IC_{50} values are in general means of two separate experiments each carried out in triplicate and varied by less than 25%. IC_{50} values > 1000 nM means the compound produced less than 40% inhibition of control binding at a concentration of 1000 nM.

V. The requisite β-keto nitrile (**125**) was prepared via the base-catalyzed ring opening of 5-[(phenylthio)methyl]-isoxazole.[19a] This, in turn, was obtained from 5-methyl-isoxazole via bromination[19b] followed by treatment with benzenethiol. The keto nitrile **125** was utilized in the conventional three-part Hantzsch synthesis to give the phenyl sulfide derivative (**122a**; n = 0) which was subsequently oxidized to the corresponding sulfoxide and sulfone (**122b,c**; n = 1, 2).

Scheme VI outlines the synthesis of the corresponding 6-cyano 3,5-diester DHP derivatives (**123a–c**; n = 0, 1, 2). This was achieved from the corresponding 6-cyano-2-methyl compound (**121e**)[20] via bromination (PBB/$CHCl_3$) followed by reaction with 4-pyridinethiol to give **123a** according to the methodology described earlier. The sulfide was oxidized as usual to give the desired sulfoxide and sulfone, respectively (**123b,c**, n = 1, 2).

Since the 5-cyano-2-(arylsulfonyl)-DHP derivatives are racemic, and stereospecificity of DHPs has been established previously[21] isolation and testing of individual enantiomers of these DHPs was highly desirable. Resolution was attempted on the DHP sulfone **54** without much success. Resolution was accomplished on the key intermediate, 2,6-dimethyl-1,4-dihydropyridine-3-carboxylic acid (**121f**; R_2 = CO_2H, R_1 = CN, R_3 = Me), by using (−)-cinchonidine/(+)-cinchonine for salt pair formation following the procedure of Murakami et al.[22] to give individual isomers. Optical purity of the individual enantiomers **121f** were assessed on the corresponding ethyl esters (**121d**) by using a chiral shift reagent Eu(facam)$_3$ (see

Scheme IV[a]



[a] (a) EtOH, Δ; (b) $HO(CH_2)_2NR_1R_2$, Δ; (c) $HO(CH_2)_2NR_1R_2$, Na, toluene; (d) NaH, DMF, $ClCH_2OEt$; (e) (R_1-R_2) NH, CH_3CN; (f) LiOH, EtOH; (g) CDI, THF.

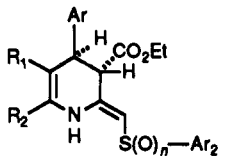
(19) (a) Sircar, I.; Mukherji, P. C. *J. Org. Chem.* **1977**, *42*, 3744. (b) Deshong, P.; Lowmaster, N. E.; Oswaldo, B. *J. Org. Chem.* **1983**, *48*, 1149.

(20) Sato, Y. US 4,145,432. March 20, 1979; *Chem. Abstr.* **1977**, *86*, 189726f.

(21) (a) Hof, R. P.; Ruegg, A.; Vogel, A. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 689. (b) Franckowiak, G.; Bechem, M.; Schramm, M.; Thomas, G. *Eur. J. Pharmacol.* **1985**, *114*, 223.

(22) Shibanuma, T.; Iwanani, M.; Okuda, K.; Takenara, T.; Murakami, M. *Chem. Pharm. Bull.* **1980**, *29*, 2809.

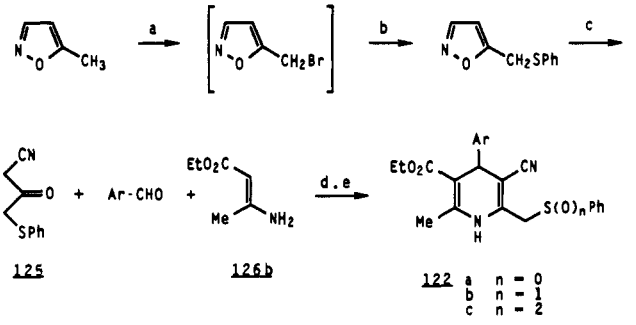
Table II. 4-Aryl-1,2,3,4-tetrahydropyridine Derivatives[a,d]



compd	Ar	n	R_1	R_2	Ar_2	mp, °C[b] (crystn solvent)	mol formula[c]
17	2-$CF_3C_6H_4$	0	CO_2Et	Me	Ph	103–104	$C_{26}H_{28}F_3NO_4S$
18	3-$NO_2C_6H_4$	0	CN	Me	Ph	152–154	$C_{23}H_{21}N_3O_4S$
19	2-$CF_3C_6H_4$	0	CN	Me	Ph	146–148 (B)	$C_{24}H_{21}F_3N_2O_2S$
20	2-$CF_3C_6H_4$	0	CO_2Et	Me	2-MeC_6H_4	100–102 (A)	$C_{27}H_{28}F_3NO_4S$
21	2-$CF_3C_6H_4$	0	CO_2Me	Me	2-ClC_6H_4	138–141 (A)	$C_{25}H_{23}ClF_3NO_4S$
22	2-$CF_3C_6H_4$	1	CO_2Me	Me	2-ClC_6H_4	153–154 (B)	$C_{25}H_{23}ClF_3NO_5S$
23	2-$CF_3C_6H_4$	0	CO_2Et	Me	4-Py	153–154 (C)	$C_{25}H_{25}F_3N_2O_4S$
24	2-$CF_3C_6H_4$	1	CO_2Et	Me	4-Py	118–120 (C)	$C_{25}H_{25}F_3N_2O_5S$
25	2-$OCHF_2C_6H_4$	0	CN	Me	Ph	152–155 (B)	$C_{24}H_{22}F_2N_2O_3S$
26	2-$OCHF_2C_6H_4$	1	CN	Me	Ph	165–167	$C_{24}H_{22}F_2N_2O_4S$
27		0	CN	Me	Ph	foam	$C_{23}H_{26}N_2O_2S$

[a] Yields of compounds were not optimized. Compounds (n = 0) were obtained in 5–15% yields except for **25** and **27** which are obtained in 40% yields. Compounds (n = 1, 2) were obtained in 70–85% yields. [b] A, EtOAc/isopropyl ether; B, isopropyl ether; C, EtOAc. All other compounds were purified via chromatography. [c] Compounds were analyzed within 0.4% theory. [d] These compounds had poor receptor affinities (IC_{50} >5000 nM).

Scheme V[a]



[a] (a) *N*-bromosuccinimide, CCl_4; (b) PhSh, Et_3N; (c) KOH, dioxan/H_2O; (d) EtOH, Δ; (e) *m*-CPBA, $CHCl_3$.

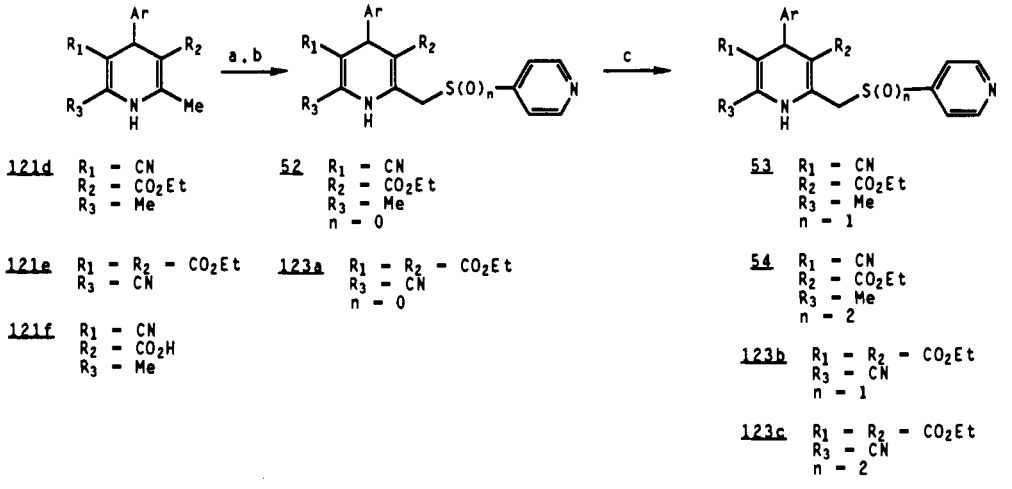
Experimental Section for details). These were converted to the desired compounds (+) isomer of **54** (**54a**) and (–) isomer of **54** (**54b**) via methodology developed earlier (Scheme VI). The high optical purity of **54a** and **54b** were also assessed from HPLC chromatogram obtained by using a Pirkle naphthylalanine column. Baseline separation was achieved with racemic (±)-**54**, whereas single peaks were seen with each of the individual enantiomers (**54a** and **54b**).

Biological Results and Discussion

The compounds were evaluated for both vascular and cardiac activity by using tissues from several species. Possible involvement of the calcium channel in the pharmacologic responses to compounds in Tables I–IV were evaluated from DHP receptor binding experiments followed by functional assays in both rat isolated cardiac and rabbit vascular muscle. The in vitro cardiac effects were examined in two species, namely, rat and guinea pig. Most of the data were generated from the 5-nitrile 3-ester series, which exhibited the most interesting pharmacological responses.

Dihydropyridine Receptor Binding Studies. The affinities of these agents for the DHP receptor were established from the binding assays by following the method preciously described by Ehlert et al.[23] and Gould et al.[24]

Scheme VI[a]



[a] (a) Pyridinium bromide perbromide, $CHCl_3$; (b) 4-Mercaptopyridine, NaH; (c) *m*-CPBA, $CHCl_3$.

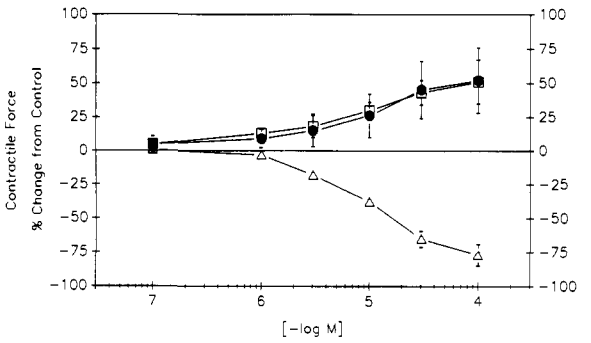


Figure 1. Inotropic responses of compounds **53**, **54**, and **2** in isolated guinea pig left atrial muscle. Each point represents the mean ±SE of three experiments. Symbols are as follows: -●-**53**; -□-**54**; -△-**2**.

using rat brain cortex (RBC1). The DHP receptor binding potency of the 5-nitrile DHP series varied significantly and IC_{50} values ranged from 69 to 1000 nM (Table III). The sulfones were the most potent and the activity decreased in the order $PhSO_2$ > PhS > PhSO. For example, compare **30** vs **28** vs **29**, **33** vs **31** vs **32**, and **54** vs **52** vs **53**, respectively. Modification of the 4-aryl moiety was limited to several 2- and/or 3-substituted phenyl (for example, Cl, NO_2, CF_3, $OCHF_2$, 2,3-$DiCl_2$) that are reported to enhance the DHP binding activities.[10] Replacing the phenyl ring with 2-furoyl improved binding to some extent (compare **71** and **65**) while cyclohexyl (**73**) and 2-pyridyl (**59**) replacements provided inactive compounds which is in agreement with the general trend of the structure–activity relationships (SAR) in the 1,4-DHP series.

From the limited number of compounds studied the following trend emerged regarding substitution effect on the aryl ring of the 2-position side chain. A para substitution produced more potent compounds, whereas an ortho substitution seems to be detrimental. For example, compare **47**, **49**, **75**, and **77** (IC_{50} range 69–109 nM) and **36**, **38**, **41**, **43**, **44**, and **46** (IC_{50} range >1000 nM) with **28** and **30** (IC_{50}s = 450 and 120 nM). An alkyl substitution at the DHP nitrogen significantly reduced the activity (compare **88** vs **28**). Variation of the alkyl group at the 3-ester moiety did not affect the receptor binding affinity. DHP, wherein the CN and CO_2Et moieties were interchanged, showed an enhancement in binding [IC_{50} = 130 nM (**122c**) and 85 nM (**122b**)]. In the diester series the aryl sulfides, sulfoxides, and sulfones all retained excellent receptor affinity (Table I). Moderate receptor binding affinities were observed with 6-cyano DHP derivatives (IC_{50} values for **123b,c** are 171 nM and 121 nM, respectively). The phenyl sulfide analogue **1** was the most potent compound in this series with an IC_{50} of 1.6 nM. The corresponding exocyclic double bond isomer analogues (Table II) and the bicyclic DHP derivatives (Table IV) were inactive (IC_{50} > 5000 mM).

Cardiac Activity in Isolated Guinea Pig Left Atrial Muscle. Prototype compounds were evaluated for inotropic activity in isolated guinea pig left atria.[25] As a class the DHP I series covers a broad range of positive, biphasic, and negative inotropic activity as demonstrated in Figure 1 (parts A–C). In the 5-cyano 3-ester series, positive inotropic activity was absent in the thioethers, most potent in the sulfoxides, and less potent in the sulfones. In general, the diester derivatives produced biphasic or strictly

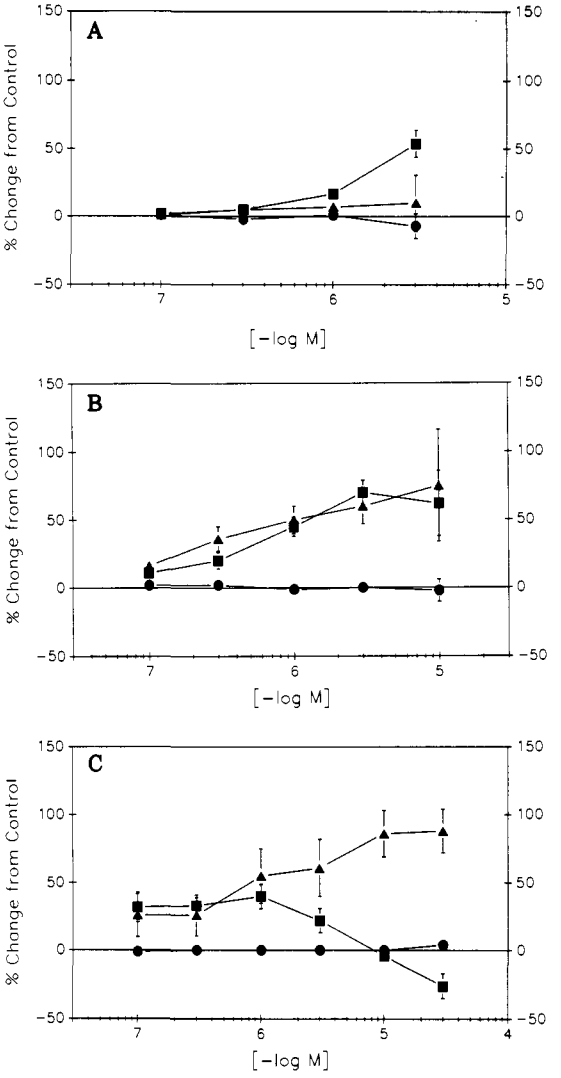


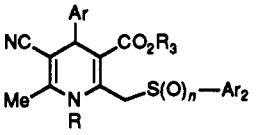
Figure 2. Profile of compounds **53** (A), **54** (B), and **2** (C) in isolated rat hearts. Each point represents the average of two separate experiments except for **54** (average of six experiments): LV + dP/dt_{max}, -■-; CF, -▲-; HR, -●-.

negative inotropic effects. The profile of these agents can be correlated with their respective receptor binding affinities. Agents that were very effective receptor ligands produced negative inotropic effects, and agents that demonstrated strictly positive inotropic effects had decreased receptor affinity.

Cardiovascular Activity in Isolated Perfused Rat Heart. Prototype compounds from the 5-cyano 3-ester series were screened in isolated rat hearts perfused by the Langerndorff method at a constant aortic pressure for inotropic, chronotropic, and coronary vascular activities. Left ventricular inotropic activity was measured from the first derivative of left intraventricular pressure (LV +dP/dt_{max}), chronotropic activity from the electrocardiogram (HR), and vascular activity from changes in coronary arterial flow (CF).[26] A typical profile of a 3-ester 5-nitrile sulfoxide **53** and sulfone **54** are shown in Figure 2, parts A and B, respectively. In the case of **54** (and analogues) an increase in LV +dP/dt_{max} was associated with an increase in CF. (A profile being present in the same molecule is unique for DHP.) The vascular activity of these agents were ascribed to their effects on the calcium channel. The 3,5-diester sulfoxide **2** on the other hand showed a profile

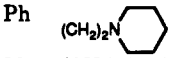
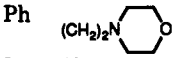
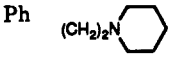
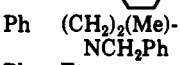
(23) Ehlert, F. J.; Itoga, E.; Roeske, W. R.; Yamamura, H. I. *Biochem. Biophys. Res. Comm.* **1982**, *104*, 937.
(24) Gould, R. J.; Murphy, K. M. M.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3657.
(25) Weishaar, R. E.; Quade, M. M.; Pugsley, T. A.; Shih, Y. H.; Taylor, D. G. *J. Mol. Cell. Cardiol.* **1988**, *20*, 897.
(26) Haleen, S. J.; Steffen, R. P.; Hamilton, H. W. *Life Sci.* **1987**, *40*, 555.

Table III. 4-Aryl-1,4-dihydropyridine-5-cyano-3-carboxylic Acid Derivatives[a]



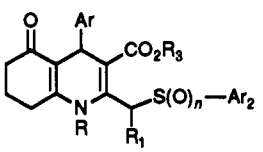
compd	Ar	R	*n*	Ar_2	R_3	mp, °C[b] (crystn solvent)	mol formula[c]	receptor affinity data inhibition of [^{3}H]nitrendipine binding: IC_{50}, nM[e]
28	2-$CF_3C_6H_4$	H	0	Ph	Et	122–123 (A)	$C_{24}H_{21}F_3N_2O_2S$	450
29a	2-$CF_3C_6H_4$	H	1	Ph	Et	183–185 (A)	$C_{24}H_{21}F_3N_2O_3S$	1600
29b	2-$CF_3C_6H_4$	H	1	Ph	Et	120–122	$C_{24}H_{21}F_3N_2O_3S$	2800
30	2-$CF_3C_6H_4$	H	2	Ph	Et	210–211 (B)	$C_{24}H_{21}F_3N_2O_4S$	120
31	3-$NO_2C_6H_4$	H	0	Ph	Et	121–122	$C_{23}H_{21}N_3O_4S$	870
32a	3-$NO_2C_6H_4$	H	1	Ph	Et	194–195	$C_{23}H_{21}N_3O_5S$	>1000
32b	3-$NO_2C_6H_4$	H	1	Ph	Et	169–170	$C_{23}H_{21}N_3O_5S$	>1000
33	3-$NO_2C_6H_4$	H	2	Ph	Et	175–176 dec	$C_{23}H_{21}N_3O_6S$	300
34	2,3-$Cl_2C_6H_4$	H	0	4-Py	Et	197–198	$C_{22}H_{19}Cl_2N_3O_2S$	1400
35	2,3-$Cl_2C_6H_4$	H	2	4-Py	Et	207–208 dec	$C_{22}H_{19}Cl_2N_3O_4S$	434
36	2-$CF_3C_6H_4$	H	0	2-MeC_6H_4	Et	146–148 (A)	$C_{25}H_{23}F_3N_2O_2S$	>1000
37	2-$CF_3C_6H_4$	H	1	2-MeC_6H_4	Et	176–180 (A)	$C_{25}H_{23}F_3N_2O_3S$	>1000
38	2-$CF_3C_6H_4$	H	2	2-MeC_6H_4	Et	223–229 (A)	$C_{25}H_{23}F_3N_2O_4S$	>1000
39	2-$CF_3C_6H_4$	H	0	2-Me-4-BuC_6H_4	Et	126–128 (B)	$C_{29}H_{31}F_3N_2O_2S$	>1000
40	2-$CF_3C_6H_4$	H	2	2-Me-4-BuC_6H_4	Et	155–159	$C_{29}H_{31}F_3N_2O_4S$	>1000
41	2-$CF_3C_6H_4$	H	0	2-ClC_6H_4	Et	172–173	$C_{24}H_{20}ClF_3N_2O_2S$	>1000
42	2-$CF_3C_6H_4$	H	1	2-ClC_6H_4	Et	174–184	$C_{24}H_{20}ClF_3N_2O_3S$	>1000
43	2-$CF_3C_6H_4$	H	2	2-ClC_6H_4	Et	222–223 dec	$C_{24}H_{20}ClF_3N_2O_4S$	>500
44	2-$CF_3C_6H_4$	H	0	2-FC_6H_4	Et	121–122	$C_{24}H_{20}F_4N_2O_2S$	267
45	2-$CF_3C_6H_4$	H	1	2-FC_6H_4	Et	186–187	$C_{24}H_{20}F_4N_2O_3S$	>1000
46	2-$CF_3C_6H_4$	H	2	2-FC_6H_4	Et	212–213 dec	$C_{24}H_{20}F_4N_2O_4S$	>1000
47	2-$CF_3C_6H_4$	H	0	4-ClC_6H_4	Et	107–109 (E)	$C_{24}H_{20}ClF_3N_2O_2S$	100
48	2-$CF_3C_6H_4$	H	1	4-ClC_6H_4	Et	207–210 (B)	$C_{24}H_{20}ClF_3N_2O_3S$	>1000
49	2-$CF_3C_6H_4$	H	2	4-ClC_6H_4	Et	foam	$C_{24}H_{20}ClF_3N_2O_4S$	91
50	2-$CF_3C_6H_4$	H	0	3,4-$Cl_2C_6H_4$	Et	foam	$C_{24}H_{19}Cl_2F_3N_2O_2S$	69
51	2-$CF_3C_6H_4$	H	1	3,4-$Cl_2C_6H_4$	Et	173–176 (D)	$C_{24}H_{19}Cl_2F_3N_2O_3S$	681
52	2-$CF_3C_6H_4$	H	0	4-Py	Et	229–230	$C_{23}H_{20}F_3N_3O_2S$	1000
53	2-$CF_3C_6H_4$	H	1	4-Py	Et	202–203 dec	$C_{23}H_{20}F_3N_3O_3S$	1100
54	2-$CF_3C_6H_4$	H	2	4-Py	Et	219–220 dec	$C_{23}H_{20}F_3N_3O_4S$	271
55	2-$CF_3C_6H_4$	H	0	2-Py	Et	179–180 dec	$C_{23}H_{20}F_3N_3O_2S$	>1000
56	2-$CF_3C_6H_4$	H	1	2-Py	Et	155–174 dec	$C_{23}H_{20}F_3N_3O_3S$	1900
57	2-Py	H	0	Ph	Et	164–167 (D)	$C_{22}H_{21}N_3O_2S$	>1000
58	2-Py	H	1	Ph	Et	167–170 (F)	$C_{22}H_{21}N_3O_3S$	>1000
59	2-Py	H	2	Ph	Et	196–198 (F)	$C_{22}H_{21}N_3O_4S$	>1000
60	2-Py-[N->0]	H	2	Ph	Et	233–235 (F)	$C_{22}H_{21}N_3O_5S$	>1000
61	3-Py	H	0	Ph	Et	242–243	$C_{22}H_{21}N_3O_2S$	NT
62	3-Py	H	2	Ph	Et	201–202 dec	$C_{22}H_{21}N_3O_4S$	>1000
63	2-furoyl	H	0	Ph	Et	109–110 dec	$C_{21}H_{20}N_2O_3S$	1354
64	2-furoyl	H	1	Ph	Et	182–183	$C_{21}H_{20}N_2O_4S$	>1000
65	2-furoyl	H	2	Ph	Et	137–138	$C_{21}H_{20}N_2O_5S$	254
66	2-ClC_6H_4	H	0	Ph	Et	162–163	$C_{23}H_{21}ClN_2O_2S$	NT
67	2-ClC_6H_4	H	1	Ph	Et	198–202	$C_{23}H_{21}ClN_2O_3S$	>1000
68	2-ClC_6H_4	H	2	Ph	Et	214–215	$C_{23}H_{21}ClN_2O_4S$	720
69	Ph	H	0	Ph	Et	168–170 (B)	$C_{23}H_{22}N_2O_2S$	>1000
70	Ph	H	1	Ph	Et	185–188 (A)	$C_{23}H_{22}N_2O_3S$	>1000
71	Ph	H	2	Ph	Et	218–220 (A)	$C_{23}H_{22}N_2O_4S$	480
72		H	1	Ph	Et	144–146 (A)	$C_{23}H_{28}N_2O_3S \cdot 0.2EtOAc$	>1000
73		H	2	Ph	Et	150–152 (A)	$C_{23}H_{28}N_2O_4S$	>1000
74	2-$OCHF_2C_6H_4$	H	1	Ph	Et	122–124 (A)	$C_{24}H_{22}F_2N_2O_4S$	>1000
75	2-$CF_3C_6H_4$	H	0	4-$OCH_3C_6H_4$	Et	108–109 (E)	$C_{25}H_{23}F_3N_2O_3S$	71
76	2-$CF_3C_6H_4$	H	1	4-$OCH_3C_6H_4$	Et	153–156 (A)	$C_{25}H_{23}F_3N_2O_4S$	1200
77	2-$CF_3C_6H_4$	H	2	4-$OCH_3C_6H_4$	Et	208–210	$C_{25}H_{23}F_3N_2O_5S$	109
78	2-$CF_3C_6H_4$	H	0	4-NHAc	Et	187–189	$C_{26}H_{24}F_3N_3O_3S$	205
79	2-$CF_3C_6H_4$	H	1	4-NHAc	Et	228–230 (A)	$C_{26}H_{24}F_3N_3O_4S$	>1000
80	2-$CF_3C_6H_4$	H	2	4-NH_2	Et	237–240 (D)	$C_{24}H_{22}F_3N_3O_4S$	1000
81	2-ClC_6H_4	H	0	4-Py	Et	205–207	$C_{22}H_{20}ClN_3O_2S$	366
82	2-ClC_6H_4	H	2	4-Py	Et	214–215 dec	$C_{22}H_{20}ClN_3O_4S$	713
83	2-MeC_6H_4	H	0	4-Py	Et	188–191	$C_{23}H_{23}N_3O_2S$	NT
84	2-MeC_6H_4	H	2	4-Py	Et	217–218	$C_{23}H_{23}N_3O_4S$	1410
85	2-Py	H	0	4-Py	Et	198–199	$C_{21}H_{20}N_4O_2S$	>1000
86	2-Py	H	1	4-Py	Et	170–171 dec	$C_{21}H_{20}N_4O_3S$	>1000
87	2-Py	H	2	4-Py	Et	196–197 dec	$C_{21}H_{20}N_4O_4S$	>1000

Table III (Continued)

compd	Ar	R	n	Ar_2	R_3	mp, °C[b] (crystn solvent)	mol formula[c]	receptor affinity data inhibition of [^{3}H]nitrendipine binding: IC_{50}, nM[e]
88	2-$CF_3C_6H_4$	$(CH_2)_2N(Et)_2$	0	Ph	Et	74–75	$C_{30}H_{34}F_3N_3O_2S$	1000
89	2-$CF_3C_6H_4$	$(CH_2)_2N(Et)_2$	1	Ph	Et	128–131	$C_{30}H_{34}F_3N_3O_3S$	>1000
90	2-$CF_3C_6H_4$	Me	1	Ph	Et	109–113	$C_{25}H_{23}F_3N_2O_3S$	>1000
91	2-$CF_3C_6H_4$	H	0	Ph	$CH_2CH{=}CH_2$	113–114	$C_{25}H_{21}F_3N_2O_2S$	580
92	2-$CF_3C_6H_4$	H	2	Ph	$CH_2CH{=}CH_2$	187–189	$C_{25}H_{21}F_3N_2O_4S$	580
93	2-$CF_3C_6H_4$	H	0	Ph	$CH(Me)_2$	123–125 (B)	$C_{25}H_{23}F_3N_2O_2S$	250
94	2-$CF_3C_6H_4$	H	1	Ph	$CH(Me)_2$	189–191 (A)	$C_{25}H_{23}F_3N_2O_3S$	500
95	2-$CF_3C_6H_4$	H	2	Ph	$CH(Me)_2$	205–207 (A)	$C_{25}H_{23}F_3N_2O_4S$	390
96	2-$CF_3C_6H_4$	H	0	Ph	Me	117–118 (B)	$C_{23}H_{19}F_3N_2O_2S$	1000
97	2-$CF_3C_6H_4$	H	1	Ph	Me	207–210	$C_{23}H_{19}F_3N_2O_3S$	>1000
98	2-$CF_3C_6H_4$	H	2	Ph	$CH_2CH(OH)$-$CH_2(OH)$	102–103	$C_{25}H_{23}F_3N_2O_6S$	>1000
99	2-$CF_3C_6H_4$	H	1	Ph	$(CH_2)_2OH$	172–173	$C_{24}H_{21}F_3N_2O_4S$	1000
100	2-$CF_3C_6H_4$	H	0	Ph	CH_2CH_2CN	158–160	$C_{25}H_{20}F_3N_3O_2S$	1000
101	2-$CF_3C_6H_4$	H	0	Ph	H	204–206 (D)	$C_{22}H_{17}F_3N_2O_2S$	>1000
102	2-$CF_3C_6H_4$	H	1	Ph	H	209–211	$C_{22}H_{17}F_3N_2O_3 \cdot 0.4EtOAc$	>1000
103	2-$CF_3C_6H_4$	H	1	Ph		171–172	$C_{29}H_{30}F_3N_3O_3S$	>1000
104	2-$CF_3C_6H_4$	H	1	Ph	$(CH_2)_2(Me)$-NCH_2Ph	158–159	$C_{32}H_{30}F_3N_3O_3S$	>1000
105	2-$CF_3C_6H_4$	H	1	Ph		204–205	$C_{28}H_{28}F_3N_3O_4S$	>1000
106[d]	2-$CF_3C_6H_4$	H	0	Ph	$(CH_2)_2Br$	viscous oil	$C_{24}H_{20}BrF_3N_2O_2S$	NT
107	2-$CF_3C_6H_4$	H	0	Ph	$(CH_2)_2OH$	110–111 (A)	$C_{24}H_{21}F_3N_2O_3S$	NT
108	2-$CF_3C_6H_4$	H	0	Ph		viscous oil	$C_{29}H_{30}F_3N_3O_2S$	NT
109	2-$CF_3C_6H_4$	H	0	Ph		viscous oil	$C_{28}H_{28}F_3N_3O_3S$	NT
110	2-$CF_3C_6H_4$	H	0	Ph	$(CH_2)_2(Me)$-NCH_2Ph	viscous oil	$C_{32}H_{30}F_3N_3O_2S$	NT
111[d]	2-$CF_3C_6H_4$	Me	0	Ph	Et	viscous oil	$C_{25}H_{23}F_3N_2O_2S$	NT

[a] Yields of compounds were not optimized. Compounds (n = 0) were obtained in 30–45% yields. Compounds (n = 1, 2) were obtained in 70–85% yields. [b] A, EtOAc/isopropyl ether; B, isopropyl ether; C, EtOAc; D, EtOAc/MeOH; E, Et_2O/hexane; F, MeOH. All other compounds were purified via chromatography. [c] Compounds were analyzed within 0.4% theory except for 110. [d] Not analyzed. NT = not tested. [e] Index of DHP receptor affinity. See Table I for details.

Table IV. 4-Aryl-1,4,5,6,7,8-hexahydro-5-oxo-3-quinolinecarboxylic Acid Derivatives[a,f]



compd	Ar	R_1	R	n	Ar_2	R_3	mp, °C[b] (crystn solvent)	mol formula[c]
112	2-$CF_3C_6H_4$	H	H	0	Ph	Et	180–181 (A)	$C_{26}H_{24}F_3NO_3S$
113	2-$CF_3C_6H_4$	H	H	1	Ph	Et	223–224 (F)	$C_{26}H_{24}F_3NO_4S$
114[e]	2-$CF_3C_6H_4$	H	H	2	Ph	Et	235–236	$C_{26}H_{24}F_3NO_5S$
115[e]	2-$CF_3C_6H_4$	H	Me	1	Ph	Et	177–178	$C_{27}H_{26}F_3NO_4S$
116[e]	2-$CF_3C_6H_4$	H	Me	2	Ph	Et	213–214	$C_{27}H_{26}F_3NO_5S$
117[e]	2-$CF_3C_6H_4$	Me	H	2	Ph	Et	182–192	$C_{27}H_{26}F_3NO_5S$
118a[d]	2-$CF_3C_6H_4$	H	H	0	Ph	Et	178–179 (C)	$C_{26}H_{24}F_3NO_3S$
118b[d]	2-$CF_3C_6H_4$	H	H	0	Ph	Et	204–205 (C)	$C_{26}H_{24}F_3NO_3S$
119[e]	2-$CF_3C_6H_4$	H	Me	0	Ph	Et	129–130	$C_{27}H_{26}F_3NO_3S$

[a] Yields of compounds were not optimized. Compounds (n = 0) were obtained in 30–45% yields. Compounds (n = 1, 2) were obtained in 70–85% yields. [b] A, EtOAc/isopropyl ether; B, isopropyl ether; C, EtOAc; D, EtOAc/MeOH; E, Et_2O/hexane; F, MeOH. [c] Compounds were analyzed within 0.4% theory. [d] Exocyclic isomers. [e] All compounds were purified via chromatography. [f] These compounds had poor receptor affinities (IC_{50} >5000 nM).

of a typical calcium channel blocker (Figure 2C). Compound **2** showed a decrease in LV $+dP/dt_{max}$ associated with some increase in CF and practically no change in HR. Similar profiles were demonstrated with 3-cyano-DHP sulfone **122c** [ED_{25} (CF) = 0.102 ± 0.020 μM] and 6-cyano sulfoxide **123b** [EC_{25} (CF) = 0.045 ± 0.020 μM], respectively. The relative ratio of decrease in LV $+dP/dt_{max}$ and increase in CF depends on (i) cardioselectivity and (ii) potency of compounds as calcium channel blockers. Table V lists CF and LV $+dP/dt_{max}$ (EC_{25}) data for some prototype compounds. As seen from the table, the effect on LV $+dP/dt_{max}$ was maximum with bicyclic DHP sulfones **116** and **117**, although these agents were inactive in DHP receptor binding assay.

The best profile was demonstrated by 2-(arylsulfonyl)-methyl DHP derivatives and compound **54** was selected for in-depth studies. The individual isomers, **54A** and **54B**, were evaluated for receptor affinity, coronary vasodilation

Table V. Cardiovascular Activity in Isolated Rat Heart Model

compd	CVIH: EC_{25}, $\mu M^{a,b}$ LV $+dP/dt_{max}$ (n)	CF (n)
29a	0.12 ± 0.04	NA
29b	0.17 ± 0.03	1.3 ± 0.34
30	0.11 ± 0.08	0.07 ± 0.01
32a	2.5 ± 1.4	NA
32b	3.0 ± 1.1	NA
33	0.52 ± 0.27	0.51 ± 0.16
37	0.13 ± 0.08	0.80 ± 0.02
38	0.32 ± 0.12	0.47 ± 0.43
42	0.20 ± 0.01	0.26 ± 0.06
43	0.36 ± 0.23	0.35 ± 0.06
45	0.93 ± 0.08	0.36
46	0.39 ± 0.11	0.09 ± 0.04
49	NA	0.55 ± 0.06
53	1.2 ± 0.2 (4)	NA (4)
54	0.47 ± 0.11 (6)	0.29 ± 0.08 (6)
67	0.91 ± 0.26	0.54 ± 0.04
71	0.96 ± 0.27	NA
73	1.09 ± 0.37 (4)	0.52 ± 0.35 (4)
76	NA	0.30
77	NA	0.30
78	NA	0.26 ± 0.02
80	0.84 ± 0.087	0.94 ± 0.22
86	NA	NA
87	NA	NA
89	0.33 ± 0.08	0.50 ± 0.27
92	0.30 ± 0.06	<0.10
94	NA	0.24 ± 0.08
95	NA (3)	<0.10 (3)
97	0.26 ± 0.16	1.48 ± 0.46
103	NA	NA
104	0.12 ± 0.02	0.42 ± 0.26
116	<0.1	0.18 ± 0.05
117	<0.1	1.40 ± 0.07

[a] A minimum of two hearts were used for each compound tested. Effects on heart rate were negligible in all cases. [b] Values are concentration producing a 25% increase in LV $+dP/dt_{max}$ and CF and represented as mean ± SE. n = number of hearts evaluated. NA = not active, <25% increase was observed.

Table VI. Effects of 54 and the Enantiomers of 54 on [^{3}H]Nitrendipine Binding, Coronary Resistance, and Ventricular Contractility

compd	[^{3}H]nitrendipine binding: IC_{50}, μM^a	coronary resistance:[b] EC_{25}, μM	LV $+dP/dt_{max}$: EC_{25}, μM^b
54	0.271 ± 0.005	0.284 ± 0.069	0.535 ± 0.140
54A	0.152 ± 0.003	0.128 ± 0.049	0.701 ± 0.377
54B	>1000	1.622 ± 0.472	0.888 ± 0.306

[a] [^{3}H]Nitrendipine binding was determined in rat brain cortical membranes. IC_{50} values represent the mean three separate experiments. [b] Effects on coronary vasodilator and ventricular contractility activity were determined in isolated rat hearts. EC_{25} values represent the mean ± SEM of four to six hearts.

and positive inotropic activity. Table VI shows that the calcium channel binding and coronary vasodilatory activity are stereospecific but not the positive inotropic activity. The (+) enantiomer **54A** was twice as potent as the racemic mixture **54** in the receptor binding assay and showed a slightly greater vasodilator potency. In contrast, the positive inotropic activity was not stereospecific, with the (+) and (−) enantiomers (**54A** and **54B**) having comparable potency.

That the inotropic activity of these agents was not due to calcium channel stimulation was suggested from a comparison with BAY K 8644 in this model. BAY K 8644 showed a profound increase in LV $+dP/dt_{max}$ associated with a decrease in CF which is typical of a calcium channel agonist (Figure 3A). Veratridine (Na channel stimulant) on the other hand produced increases in dP/dt_{max} associated with no change in CF in this model (Figure 3B).

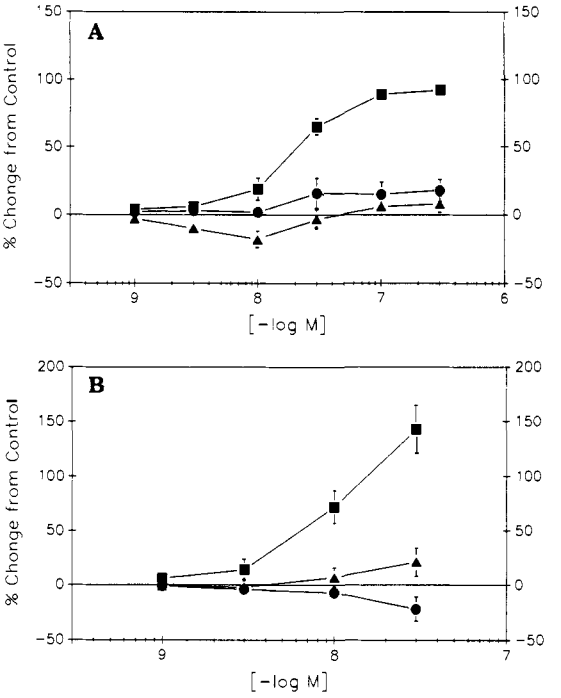


Figure 3. Profile of reference agents BAY K8644 (A) and veratridine (B) in isolated rat hearts. Each point represents the mean ±SE of six determinations: LV + dP/dt_{max}, -■-; CF, -▲-; HR, -●-.

The inotropic response to **54** was reversed by the sodium channel blocker tetrodotoxin (TTX) and blocked by the sodium–calcium exchange inhibitor dichlorobenzamil (DCB). The effects of TTX and DCB on the inotropic response to the sodium channel stimulant veratridine were comparable to **54**. These results published earlier[27] support an involvement of the sodium channel in the inotropic response to **54**.

Isolated Aortic Ring Studies. Two protocols were conducted to determine the vascular responses of calcium channel blocking and/or stimulating effects of a few prototypes of sulfones and the reference agents nifedipine and BAY K 8644. To determine calcium channel blocking activity, a protocol similar to that previously described by Kazda et al.[28] was used. In this procedure aortic rings were contracted with either 50 mM KCl or 1.0 μM norepinephrine (NE), after which they were exposed to increasing concentrations of the test agent. Selective relaxation of KCl-induced contractions vs norepinephrine-induced contractions were taken to indicate calcium channel blocking activity.[29] Table VII shows that such selectivity was observed with 2-(arylsulfonyl)methyl DHPs, and also the reference calcium channel blocker nifedipine. In contrast, the reference vasodilator nitroprusside nonselectively relaxed both the KCl- and norepinephrine-induced contractions.

To determine the calcium channel stimulator activity, a protocol previously described by Schramm et al.[30] was used in which selective contractile activity of agents on

(27) Haleen, S. J.; Steffen, R. P.; Sircar, I.; Major, T. C.; Taylor, M. D.; Pugsley, T. A.; Weishaar, R. E. *J. Pharmacol. Exp. Ther.* **1989**, *250* (1), 22.
(28) Kazda, S.; Garthoff, B.; Meyer, H.; Schlobmann, K.; Stoepel, K.; Towart, R.; Vater, W.; Wehinger, E. *Arzneim. Forsch.* **1980**, *30*, 2144.
(29) Weishaar, R. E.; Quade, M. M.; Schenden, J. A.; Kaplan, H. R. *J. Pharm. Exp. Ther.* **1983**, *227*, 767.
(30) Schramm, M.; Thomas, G.; Towart, R.; Franckowiak, G. *Arzneim. Forsch. Drug. Res.* **1983**, *33* (II), 1268.

Table VII. The Vasorelaxant Effects of Increasing Concentrations of **54** and Analogues, Nifedipine, and Nitroprusside on Rabbit Aortic Rings Previously Contracted with either 1.0 μM Norepinephrine or 50 mM KCl[a]

drug	54[b]		30		93		80		71		nifedipine[b]		nitroprusside	
conc, M	KCl	NE	KCl	NE	KCl	NE	KCl	NE	KCl	NE	KCl	NE	KCl	NE
10^{-10}	-	-									-3.1 ± 1.5	-1.0 ± 0.8	-	-
10^{-9}	-4.8 ± 0.3	-0.9 ± 0.3									-1.7 ± 1.8	-1.2 ± 1.7	-5.9 ± 1.0	-0.003 ± 0.
10^{-8}	-8.6 ± 1.3	-1.4 ± 0.4									24.7 ± 3.0	-1.1 ± 1.2	-4.8 ± 3.9	0.7 ± 0.
10^{-7}	-9.1 ± 1.9	-1.8 ± 0.7	4	5	0	-1	2	2	4	0	65.8 ± 4.1	1.0 ± 0.7	5.6 ± 10.2	7.0 ± 2.7
10^{-6}	6.2 ± 1.3	-2.3 ± 0.7	24	7	2	-2	5	2	1	1	89.4 ± 5.7	3.5 ± 1.2	22.0 ± 16.6	22.8 ± 6.7
10^{-5}	53.8 ± 2.9	0.7 ± 0.4	67	13	30	8	38	2	26	0	92.6 ± 6.6	6.1 ± 2.2	40.4 ± 22.7	39.8 ± 10.
10^{-4}	88.9 ± 2.1	4.2 ± 1.2	80	23	74	9	69	5	54	6	-	-	52.1 ± 26.5	55.8 ± 15.

[a] Data represents the mean of four aortic rings from separate rabbits. [b] Mean ± SE from six separate determinations.

Table VIII. The Vasoconstrictor Effects of **54**, Bay K 8644, and Norepinephrine Using Nondepolarized and Partially Depolarized Isolated Rings from Rabbits[a]

drug	54		BAY K 8644		norepinephrine	
conc, M	depolarized	nondepolarized	depolarized	nondepolarized	depolarized	nondepolarized
10^{-9}	3.3 ± 0.6	0.0 ± 0.0	3.2 ± 0.8	0.0 ± 0.0	7.8 ± 1.3	0.0 ± 0.0
3×10^{-9}	-	-	10.2 ± 2.0	0.0 ± 0.0	18.2 ± 2.3	0.0 ± 0.0
10^{-8}	4.5 ± 1.1	0.0 ± 0.0	32.4 ± 6.3	0.0 ± 0.0	46.2 ± 2.3	22.2 ± 2.6
3×10^{-8}	-	-	59.2 ± 9.2	1.5 ± 0.7	63.4 ± 2.8	49.1 ± 5.8
10^{-7}	5.0 ± 1.2	0.0 ± 0.0	82.1 ± 10.1	10.0 ± 2.1	84.4 ± 2.9	80.2 ± 5.8
10^{-6}	2.4 ± 1.7	-	93.2 ± 9.6	15.9 ± 2.6	106.3 ± 2.9	112.6 ± 7.
10^{-5}	0.0 ± 0.0	0.0 ± 0.0	-	-	137.2 ± 2.4	151.3 ± 3.
10^{-4}	0.0 ± 0.0	0.0 ± 0.0	-	-	-	-

[a] Data represents the mean ± SE from six separate determinations.

partially depolarized vs nondepolarized aortic rings were used to indicate functional calcium channel stimulating activities. Table VIII summarizes results obtained for **54**, BAY K 8644, and the α-receptor agonist norepinephrine. Norepinephrine produced a constrictor response in both preparations while BAY K 8644 selectively contracted partially depolarized aortic rings, indicating functional calcium channel stimulating activity. Compound **54** failed to contract either preparation, indicating a lack of functional calcium channel stimulatory activity.

Conclusion. In summary, DHP I series represents a class of compounds with a wide range of vascular and cardiac activity. In particular, the 2-(arylsulfonyl)methyl DHP series represented by **54** exhibits an unique profile in vitro relative to the calcium channel stimulator BAY K 8644 and the calcium channel blocker nifedipine by increasing left ventricular contractility and relaxing vascular smooth muscle at comparable concentrations. The vascular relaxant activity of **54** appears to be mediated through a blocking effect on the calcium slow channel. This was supported by the binding studies with [^{3}H]nitrendipine, and data obtained from functional studies with rabbit aortic rings and the isolated perfused rat heart. Additionally, it was also shown that both the binding activity and the vasorelaxant activity of **54** resides in the (+) isomer,[10c] which is consistent with the well-known stereoselectivity of other DHPs.[21] In contrast, the positive inotropic activity of **54** in nonstereospecific and appears to result from a stimulant effect on the sodium channel.[27] The inotropic response to this series of DHP is also shown to be species dependent.[27] At the present time the basis for this variation is not understood, but appears to exist with other agents known to stimulate sodium channel.[31] These compounds, therefore, represent a novel class of DHP, which possesses both calcium channel blocking and sodium channel stimulating activity. Such dual actions represent a potentially new mechanistic approach to drug discovery targeted toward the treatment of hypertension and congestive heart failure. In addition, these agents also provide a potential probe to study structure–function relationships between ion channels.[32]

Experimental Section

Chemistry. Microanalyses were within ±0.4% of the calculated values for the specified elements, unless indicated otherwise. Melting points were determined on a Thomas-Hoover apparatus and are uncorrrected. NMR spectra were obtained in $CDCl_3$ solution on a Varian XL-200, and IR spectra, reported in cm^{-1}, were recorded on a Nicolet FTIR spectrophotometer with KBr disks. Mass spectra were recorded on a VG 7070 E/HR mass spectrometer with an 11/250 data system. Silica gel 60 PF_{354} plates were used for thin-layer chromatography, and spots were visualized with UV light or iodine vapor. HPLC was performed on a Pirkle-D naphthylamine column [250 × 4.6 mm, from Regis] connected to a LKB Model 2150 pump equipped with a Rheodyne 7125 injector, a Perkin Elmer LC-95 variable-wavelength detector, and a Hewlett-Packard 3390 integrator. Retention times are given for an eluent flow rate of 1.0 mL/min.

3-Amino-2-cyclohexen-1-one was prepared according to the literature procedure.[33] The following examples represent prototype cases of the synthesis of the title compounds.

Ethyl 5-Cyano-1,4-dihydro-6-methyl-2-[(phenylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylate (28, Table III) and Ethyl 5-Cyano-1,2,3,4-tetrahydro-6-methyl-2-[(phenylthio)methylene]-4-[(2-trifluoromethyl)phenyl]-3-pyridinecarboxylate (19, Table II). A solution of 2-(trifluoromethyl)benzaldehyde (7.3 g, 4.2 mmol), 3-aminocrotononitrile (**126a**; 3.5 g, 4.2 mmol), and ethyl 4-(phenylthio)-acetoacetate (**124b**; 10 g, 4.2 mmol) in ethanol (250 mL) was heated under reflux for 24 h. Ethanol was distilled under reduced pressure, the residue was triturated with ether and filtered to remove the symmetrical DHP **121a**. The filtrate was evaporated to dryness and chromatographed (SiO_2, ether/pentane, 2:1) to obtain the desired compound (**28**, 6.5 g) which was recrystallized from EtOAc/isopropyl ether to give analytically pure material (5.3 g, 28%): mp 122–123 °C; ^{1}H NMR δ 4.85 (s, 1 H, H-4), 4.78 (d, J = 12.6 Hz, 1 H), 4.8 (d, J = 12.6 Hz, 1 H) (CH_2S); IR (KBr) 2205 (CN) and 1705 (CO_2Et) cm^{-1}.

The second compound (0.6 g) that was obtained from the column was recrystallized from isopropyl ether to give 0.4 g (2%) of analytically pure **19**: ^{1}H NMR δ 5.25 (s, 1 H, *H*-4), 4.23 (s, 1

(31) Haleen, S. J.; Steffen, R. P.; Weishaar, R. E. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 1460.

(32) Abbott, A. *Trends Pharm. Sci.* **1988**, *9* (4), 111.

(33) Zymalkowski, F.; Rimek, J. *Naturwissenschaften* **1960**, *47*, 83; *Chem. Abstr.* **1960**, *54*, 15385g.

H, C=C*H*S), 3.54 (s, 1 H, *H*-3); IR (KBr) 2205 (CN) and 1739 (CO_2Et) cm^{-1}.

Diethyl 1,4-Dihydro-2-methyl-6-[(phenylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (1, Table I) and Diethyl 1,2,3,4-Tetrahydro-6-methyl-2-[(phenylthio)methylene]-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (17, Table II). A mixture of ethyl 4-(phenylthio)acetoacetate (**124b**; 12 g, 39.68 mmol), 2-(trifluoromethyl)benzaldehyde (6.9 g, 39.68 mmol), and ethyl 3-aminocrotonate (**126b**, 5.1 g, 39.68 mmol) in ethanol (120 mL) was heated at reflux for 18 h. Ethanol was evaporated, and the residue was chromatographed (SiO_2, ether/hexane, 3:2) to obtain compounds 1 and 17, respectively. Compound 1 was purified via recrystallization from EtOAc/isopropyl ether (2.33 g, 11%): mp 129–130 °C; ^1H NMR δ 4.85 (s, 1 H, H-4), 4.25 (d, 1 H, J = 13.2 Hz), 4.73 (d, H, J = 13.2 Hz) (CH_2S); IR (KBr) 1703 (CO_2Et) cm^{-1}.

Compound 17 was purified via recrystallization from hexane/isopropyl ether (0.3 g, 1.5%): mp 103–104 °C; ^1H NMR δ 5.65 (s, 1 H, H-4), 4.32 (s, 1 H, C=C*H*), 4.15 (s, 1 H, H-3); IR (KBr) 1739 (CO_2Et) cm^{-1}.

By following similar methodology and substituting 3-amino-2-cyclohexen-1-one[33] in place of ethyl 3-aminocrotonate compounds **112** (14%), **118a** (11%), and **118b** (1%) were obtained.

112: mp 176–177 °C; IR (KBr) 1697 cm^{-1} (CO_2Et); ^1H NMR δ 5.82 (s, 1 H, C4-H), 4.95 (d, J = 13 Hz), 4.25 (d, J = 13 Hz) CH_2SPh; MS 489 (M + 1). **118a**: mp 204–205 °C; IR (KBr) 1729 cm^{-1} (CO_2Et); ^1H NMR δ 5.21 (s, 1 H, C4-H); 5.05 (s, 1 H, C=C*H*SPh), 3.52 (s, 1 H, C*H*CO_2Et); MS 489 (M + 1). **118b**: mp 178–179 °C; IR (KBr) 1737 cm^{-1} (CO_2Et); ^1H NMR δ 5.20 (s, 1 H, C4-H), 5.06 (s, 1 H, C=C*H*SPh), 3.52 (s, 1 H, $CHCO_2Et$); MS 489 (M + 1).

Ethyl 5-Cyano-1,4-dihydro-6-methyl-2-[(phenylsulfinyl)methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylate (29a and 29b). *m*-CPBA (0.43 g, 2 mmol) was added to a solution of **28** (0.96 g, 2 mmol) in CH_2Cl_2 (50 mL) at 0 °C with stirring. After stirring for 2 h at 0 °C, the reaction mixture was washed with saturated K_2CO_3 solution followed by water, dried ($MgSO_4$), and stripped to give a semisolid mass. This was purified via chromatography (SiO_2 70 g, EtOAc) to give three compounds. The first compound eluted from the column was recrystallized from isopropyl ether to give 100 mg of the sulfone **30**: mp 210 °C. ^1H NMR δ 4.85 (d, J = 13.2 Hz, 1 H), 5.14 (d, J = 13.2 Hz, 1 H) (CH_2SO_2), 5.07 (s, 1 H, H-4); mass spectrum indicate M + 1 (491) mass ion. Isomer A (sulfoxide **29b**, 0.25 g): mp 120–122 °C; ^1H NMR (δ) 4.43 (d, J = 13.1 Hz, 1 H), 4.95 (d, 1 H, J = 13.1 Hz, CH_2SO); mass spectrum 475 (M + 1). Isomer B (sulfoxide **29a**, 0.30 g): mp 183–185 °C; ^1H NMR 4.43 (d, J = 13.2 Hz, 1 H) and 4.95 (d, 1 H, J = 13.2 Hz, CH_2SO), 5.07 (s, 1 H, H-4); mass spectrum 475 (M + 1) ion.

Ethyl 5-Cyano-1,4-dihydro-6-methyl-2-[(phenylsulfonyl)methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylate (30). *m*-CPBA (0.4 g, 2 mmol) was added to a solution of **28** (0.5 g, 1 mmol) in CH_2Cl_2 (20 mL) at room temperature. After stirring for 4 h, the reaction mixture was washed successively with saturated $NaHCO_3$ and water, dried ($MgSO_4$), and stripped to give a solid. This was purified via chromatography (SiO_2, isopropyl ether) to give the desired sulfone, (0.3 g, 60%), mp 210–211 °C. Following the same general procedure as described above the tetrahydropyridine sulfides were oxidized to the corresponding sulfoxides and sulfones.

Ethyl 5-Cyano-1,4-[2-(difluoromethoxy)phenyl]-1,2,3,4-tetrahydro-6-methyl-2-[(phenylsulfinyl)methylene]-3-pyridinecarboxylate (26). **25** (1.1 g, 2.4 mmol) was oxidized with 0.5 g of mCPBA in CH_2Cl_2 (50 mL) to give 0.76 g (70%) of sulfinyl derivative **26**, mp 165–167 °C.

Conversion of 26 to 74. To a solution of 0.5 g of **26** in EtOH (20 mL) was added a catalytic amount of $NaOCH_3$, and the solution was stirred overnight at room temperature. EtOH was evaporated, and the residue was treated with water and filtered. The solid was washed with ether and air-dried to give 0.4 g (80%) of the corresponding 1,4-dihydropyridine isomer **74**, mp 122–124 °C.

Ethyl 5-Cyano-1,4-dihydro-2-methyl-6-[(phenylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylate (122a). A solution of Et_3N (16.5 mL, 0.15 mol) in THF (50 mL) was added dropwise to an ice-cold solution of a mixture of 5-bromomethyl isoxazole (19 g, 0.12 mol prepared from 5-methylisoxazole[19]) and benzenethiol (12 mL, 0.12 mol) in THF (300 mL) with stirring. The mixture was allowed to warm up to room temperature followed by refluxing for 2 h to complete the reaction. The reaction mixture was stripped under vacuum, the residue was diluted with water, and the organic matter was extracted with EtOAc. The EtOAc layer was washed with brine, dried ($MgSO_4$), stripped, and purified via chromatography (SiO_2, hexane/EtOAc 3:1) to give 14 g of the desired product, 5-(phenylthio)methylisoxazole. This material (5 g) was dissolved in a mixture of dioxane (10 mL) and aqueous KOH (1.6 g in 20 mL of water) and the solution stirred for 1 h. It was acidified with ice cold 6 N HCl and extracted with CH_2Cl_2. The organic layer was washed with brine, dried, and stripped to yield 3 g (60%) of the β-ketonitrile **125** as light orange oil which was used as is: ^1H NMR δ 7.5–7.1 (m, 5 *H*, aromatic), 4.25 (s, 2 *H*, CH_2S), and 3.68 (s, 2 *H*, CH_2CN).

A mixture of the above nitrile (2.5 g, 13 mmol), 2-(trifluoromethyl)benzaldehyde (2.3 g, 13 mmol), and ethyl 3-aminocrotonate (1.7 g, 13 mmol) in EtOH (50 mL) was heated under reflux for 18 h. Usual work-up procedure followed by chromatography (SiO_2, hexane/EtOAc 1:1) gave 2 g (34%) of the title compound, mp 144–145 °C. The structure was proven from the microanalysis and spectral data: ^1H NMR (DMSO-d_6) δ 9.40 (s, 1 H, N*H*), 7.66–7.22 (m, 9 H, aromatic), 4.86 (s, 1 H, C4-*H*), 3.86–3.76 (m, 4 H, CH_2S and $CO_2CH_2CH_3$), 2.31 (s, 3 H, CH_3) and 0.91 (t, J = 7.22 Hz, $CO_2CH_2CH_3$); IR (KBr) 2206 (CN) and 1700 (CO_2Et) cm^{-1}. Anal. ($C_{24}H_{21}F_3N_2SO_2$) C, H, N.

***m*-CPBA Oxidation of 122a.** **122a** (1.85 g, 4 mmol) was oxidized with 1.6 g (8 mmol) of *m*-CPBA in $CHCl_3$ (100 mL) to give both the sulfoxide **122b** (0.3 g, 15%) and sulfone **122c** (1.2 g, 63%).

122b: mp 168–170 °C; ^1H NMR (DMSO-d_6) δ 9.32 (s, 1 H, N*H*), 8.05–7.15 (m, 9 H, aromatic), 5.25 (s, 1 H, C_4-H), 4.85 (d, J = 13.1 Hz, 1 H), 4.35 (d, J = 13.1 Hz, 1 H), 3.82 (q, J = 7.26 Hz, 2 H, $CO_2CH_2CH_3$), 2.25 (s, 3 H, CH_3), and 0.89 (t complex, J = 7.22 Hz, 3 *H*, $CO_2CH_2CH_3$). Anal. ($C_{24}H_{21}F_3N_2O_3S$) C, H, N.

122c (white foam): ^1H NMR δ 9.42 (s, 1 H, N*H*), 7.79–7.41 (m, 9 H, aromatic), 4.86 (s, 1 H, C4-*H*), 4.33 (d, J = 12.1 Hz, 1 H), 4.22 (d, J = 12.1 Hz, 1 H), 3.85 (q, J = 7.05 Hz, 2 H, $CO_2CH_2CH_3$), 2.28 (s, 3 H, CH_3), and 0.86 (t, J = 7.22 Hz, 3 *H*, $CO_2CH_2CH_3$. Anal. ($C_{24}H_{21}F_3N_2O_4S$) C, H, N.

Ethyl 5-Cyano-1-[2-(diethylamino)ethyl]-1,4-dihydro-6-methyl-2-[(phenylthio)methyl]-4-[2-(trifluoromethyl)-phenyl]-3-pyridinecarboxylate (88, Table III). To a suspension of NaH (60%, 0.43 g, 10.9 mmol) in DMF (4 mL) was added a solution of **28** (2.5 g, 5.45 mmol) in DMF (15 mL) under N_2. The mixture was stirred at room temperature for 1 h to complete the reaction. A solution of 2-(diethylamino)ethyl bromide hydrobromide (1.42 g, 5.45 mmol) in DMF (5 mL) was added with stirring at room temperature. Stirring was continued for 16 h at room temperature followed by heating at 60 °C for 4 h to complete the reaction. DMF was distilled under reduced pressure, the residue was treated with water, and the organic material was extracted with ether. The extract was dried, stripped, and chromatographed (SiO_2, Et_2O) to yield a solid (**88**, 1.2 g, 40%), mp 74–75 °C. This was converted to the hydrochloride salt by treatment with EtOH/HCl, mp 219–221 °C, which was subsequently oxidized to the corresponding sulfoxide **89**.

Ethyl 1,4,5,6,7,8-Hexahydro-1-methyl-5-oxo-2-[(phenylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3-quinolinecarboxylate (119). Compound **119** was prepared from **112** with use of NaH/CH_3I by following similar procedure described for **88**, mp 129–130 °C. ^1H NMR indicate a singlet at 3.38 ppm (N-Me).

Ethyl 1,4,5,6,7,8-Hexahydro-5-oxo-2-[(phenylsulfonyl)methyl]-4-[2-(trifluoromethyl)phenyl]-3-quinolinecarboxylate (114). Compound **112** was oxidized with *m*-CPBA to give the sulfone **114**: mp 235–236 °C; ^1H NMR δ 5.65 (s, 1 H, C4-*H*), 4.98 (s, 2 H, CH_2SO_2Ph).

Ethyl 1,4,5,6,7,8-Hexahydro-5-oxo-2-[(1-phenylsulfonyl)-ethyl]-4-[2-(trifluoromethyl)]phenyl]-3-quinolinecarboxylate (117). Compound **117** was prepared (60%, 3.8 vs 1.0 diastereomers) from **114** in a fashion similar to **88** with use of NaH/CH_3Cl/DMF: mp 182–192 °C (mixture of diastereoisomers); ^1H NMR (DMSO-d_6) δ 8.68 (s) and 8.58 (s) N*H*; 6.02 (q, J = 4.5 Hz)

and 5.79 (q, J = 4.5 Hz) CHCH$_3$; 5.26 (s) and 5.36 (s) C4-H; 3.75–3.86 (2 q, superimposed CO$_2$CH_2CH$_3$), 1.68 (d, J = 7.5 Hz) and 1.71 (d, J = 7.5 Hz) CHCH_3; 0.90–1.09 (2 t, superimposed).

Similar methylation of compound **30** (0.8 g) produced a diastereomeric mixture (**120**, 0.8 g), which when crystallized from EtOAc yielded 0.3 g (40%) of one diastereomer: mp 239–240 °C; ^{1}H NMR (DMSO-d_6) δ 6.27 (q, J = 4.5 Hz, 1 H, CHCH$_3$), 4.90 (s, 1 H, C4-H), 3.78 (q, J = 6.5 Hz, CO$_2$CH_2CH$_3$), 1.55 (d, J = 7.5 Hz, 3 H, CHCH_3), 0.71 (t, J = 6.5 Hz, 3 H, CO$_2$CH$_2$CH_3). Anal. (C$_{24}$H$_{22}$F$_3$N$_3$O$_4$S) C, H, N.

Synthesis of β-Keto Ester 124. Method a. Ethyl 3-Oxo-4-(4-pyridinylthio)butanoate (124k). Ethyl 4-chloroacetoacetate (12.4 g, 75 mmol) was added to an ice-cold solution of a mixture of 4-mercaptopyridine (8.3 g, 75 mmol) and Et$_3$N (7.6 g, 75 mmol) with stirring. Immediate precipitation was observed and the reaction mixture was stirred for an additional 4 h. It was filtered, and the filtrate was evaported under high vacuum. The residue was treated with water and the organic material was extracted with EtOAc. The extract was dried, evaporated, and filtered through a small bed of silica (CH$_2$Cl$_2$/MeOH, 100:1) to yield a yellow oil (15 g) which was used as is. Anal. (C$_{11}$H$_{13}$NO$_3$S) C, H, N.

Method b. 2-Cyanoethyl 3-Oxo-4-(phenylthio)butanoate (124p). A mixture of ethyl 4-(phenylthio)acetoacetate (**124b**; 50 g) and 3-hydroxy propionitrile (50 g) was heated at 120 °C for 48 h. TLC still showed the presence of some unreacted starting material. The reaction mixture was diluted with ether, and the ether solution was washed with water to remove the 3-hydroxypropionitrile, dried, stripped, and chromatographed (SiO$_2$, isopropyl ether) to yield 15 g of the cyanoethyl ester. Anal. Calcd for C$_{13}$H$_{13}$NO$_3$S: C, 59.30; H, 4.97; N, 5.31. Found: C, 58.99; H, 5.27; N, 5.31.

Method c. 1-Methylethyl 3-Oxo-4-(phenylthio)butanoate (124r). To a mixture of 2-propanol (60 g, 1 mol) containing NaOAc (0.1 g) at 80 °C was added diketene (84 g, 1 mol) dropwise. The temperature was maintained throughout the addition. The mixture was stirred at room temperature for 16 h and distilled to give the desired product (110 g, 68%), bp 64 °C (4 mmHg). Anal. (C$_{13}$H$_{16}$O$_3$S) C, H, N.

A solution of Br$_2$ (7 mL, 22 g, 0.13 M) in CHCl$_3$ (25 mL) was added dropwise with stirring to a solution of the above ester (20 g, 0.13 M) in CHCl$_3$ (80 mL) at 0 °C, and the reaction mixture was stirred for 16 h at 23 °C. Air was bubbled for 2 h and CHCl$_3$ was distilled under reduced pressure to yield an yellow oil (27 g, 87%). This was used as is without further purification (^{1}H NMR δ 4.25 (s, 2 H, CH_2Br)). This was converted to **124r** by following the same procedure as described in method a: ^{1}H NMR δ 6.75–7.73 (m, 5 H, aromatic), 3.72 (s, 2 H, SCH_2), 4.70 (q, 1 H, J = 9.2 Hz, CH(CH$_3$)$_2$), 3.44 (s, 2 H, COCH_2CO), 0.95 (d, 6 H, J = 9.2 Hz, CH(CH$_3$)$_2$).

2-(Dimethylamino)ethyl 3-Oxo-4-(phenylthio)butanoate (124w). By replacing 2-propanol with 2-(dimethylamino)ethanol in the above procedure the corresponding 2-(dimethylamino)ethyl acetoacetate was obtained in 81% yield. This was converted to the corresponding HBr salt for subsequent reactions, mp 90–91 °C. Anal. Calcd for C$_8$H$_{15}$NO$_3$·HBr: C, 37.81; H, 6.35; N, 5.51. Found: C, 37.50; H, 6.36; N, 5.82.

A solution of Br$_2$ (25 mL) in CHCl$_3$ (50 mL) was added with stirring to a solution of the above ester (70 g, 0.5 mol) in CHCl$_3$ (400 mL) at 0 °C over a 3-h period and the mixture was stirred overnight at room temperature. Air was passed through the solution for 20 min, and the solution was concentrated in vacuo to yield an oil (108 g, 66%). The bromine was subsequently displaced with thiophenol by following a slight modification of method a. A solution of the bromide–HBr salt (56 g, 0.1 mol) in DMF (50 mL) was added with stirring to a solution of preformed anion of thiophenol (24.5 g, 0.22 mol and 13.3 g, 0.33 mol 60% NaH) in DMF (250 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. Upon usual workup the crude product was purified via chromatography (SiO$_2$, EtOAc/THF, 1:1) to yield an oil (16 g, 52%) which was used as is. Anal. Calcd for C$_{14}$H$_{19}$NO$_3$S: C, 59.76; H, 6.82; N, 4.97. Found: C, 60.22; H, 6.60; N, 4.52.

2-(4-Morpholinyl)ethyl 5-Cyano-1,4-dihydro-6-methyl-2-[(phenylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylate (109). A mixture of 2-(trifluoromethyl)-benzaldehyde (1.5 g, 8.2 mmol), 3-aminocrotononitrile (0.7 g, 8.2 mmol) and 2-bromoethyl 4-(phenylthio)acetoacetate (2.6 g, 8.2 mmol) in EtOH (15 mL) was heated at reflux for 16 h. EtOH was stripped, and the residue was chromatographed (SiO$_2$, isopropyl ether). The first fraction, which was the desired DHP **106**, was obtained as a gum (0.4 g). Mass spectrum showed molecular ion 537. The second fraction, the corresponding hydroxyethyl ester DHP **107**, was isolated as a solid (0.8 g). Mass spectrum indicate molecular ion of 474. A mixture of **106** (9.0 g, 15 mmol) and morpholine (4.5 g, 60 mmol) in CH$_3$CN (75 mL) was heated at reflux for 2 h. The solution was stripped, and the residue was taken up in EtOAc and filtered. The solution was concentrated to a small volume and chromatographed (SiO$_2$, EtOAc) to yield a viscous gum ^{1}H NMR of which was consistent with the desired product. This was used as is for oxidation.

5-Cyano-1,4-dihydropyridine-6-methyl-2-[(phenylthio)-methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylic Acid (101). A mixture **100** (3.7 g, 7.12 mmol) and LiOH (0.4 g, 7.84 mmol) in EtOH (40 mL) was stirred at room temperature for 16 h. EtOH was distilled, the residue was dissolved in water, and the solution was adjusted to pH 5 and filtered. The residue was washed with ether and dried to give 3.0 g of the product, mp 206–208 °C.

2-[Methyl(phenylmethyl)amino]ethyl 5-Cyano-1,4-dihydro-6-methyl-2-[(phenylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3-pyridinecarboxylate (110). A mixture of **101** (1.15 g, 2.7 mmol) and 1,1-carbonyldiimidazole (CDI) (0.9 g, 5.4 mmol) in THF (8 mL) was stirred at 23 °C for 1 h. THF was distilled, 2-(benzylmethylamino)ethanol in excess (2 mL) was added, and the solution was heated at 120 °C for 0.5 h. The solution was stripped under high vacuum, and the residue was chromatographed (ether) to yield the desired product as a viscous gum (**110**, 1.4 g).

Resolution of the Acid 121f (R_1 = CN, R_3 = Me, R_2 = CO$_2$H). A mixture of 9.66 g of **121f** and 8.82 g of (−)-cinchonidine was dissolved in EtOH (300 mL) by heating. The solution was concentrated to 100 mL and allowed to stand at 23 °C overnight. The solid was filtered, and the residue was washed with a small volume of cold EtOH followed by ether, and air-dried to give 9.0 g of the salt, mp 144–145 °C dec. This was recrystallized from EtOH (120 mL) to give 7.7 g of solid, mp 155–157 °C. The salt was treated with 0.5 N HCl (250 mL) and stirred for 0.5 h, and the precipitate was filtered. The residue washed with cold water followed by ether and air-dried to give the (+) acid (**121f**; 4 g, 83%): mp 185–186 °C dec; $[\alpha]^{23}_D$ (+) 212° (c = 0.326, acetone). Anal. Calcd for C$_{16}$H$_{13}$F$_3$N$_2$O$_2$: C, 59.88; H, 4.03; N, 8.69. Found: C, 59.70; H, 4.33; N, 8.55.

The mother liquor from the original crystallization was evaporated to dryness and the residue was treated with 1 N HCl to generate the acid. A mixture of 5.1 g of this acid and 4.8 g of (+)-cinchonine was dissolved in EtOH (200 mL) by heating, and the solution was concentrated to 100 mL and allowed to stand overnight at 23 °C. The solid was filtered, and the residue was washed with cold EtOH followed by ether to give 7.8 g of the salt, mp 217–222 °C. This was recrystallized twice from EtOH (80 mL) to give 6.2 g of the salt, mp 237–238 °C. This was treated with 0.5 N HCl to generate the (−) acid (**121f**; 3.2 g, 66%): mp 177–178 °C; $[\alpha]^{23}_D$ (−) 218° (c = 0.337, acetone). Anal. Calcd for C$_{16}$H$_{13}$F$_3$N$_2$O$_2$: C, 59.58; H, 4.03; N, 8.69. Found: C, 59.49; H, 3.96; N, 8.55.

Conversion of the Acid (+)-121f to the Ethyl Ester (+)-121d. A solution of **121f** (4.2 g, 13 mmol) and CDI (5.2 g, 32 mmol) in dry THF (40 mL) was stirred at 23 °C for 4 h. THF was replaced with EtOH (50 mL), and the solution was refluxed for 17 h. EtOH was distilled under vacuo, and the residue was taken up in CHCl$_3$. The CHCl$_3$ layer was washed several times with brine, dried, and stripped to yield a foam: $[\alpha]^{23}_D$ +206 (c = 0.30, acetone); ^{1}H NMR δ 8.00–7.25 (m, 4 H, aromatic), 7.15 (s, 1 H, NH), 5.15 (s, 1 H, C4-H), 4.03 (q, J = 7.26 Hz, 2 H, CO$_2$CH_3), 2.41 (s, 3 H, CH$_3$), 2.07 (s, 3 H, CH$_3$), and 1.07 (t, J = 7.22 Hz). Anal. (C$_{18}$H$_{17}$F$_3$N$_2$O$_2$) C, H, N.

The (−)-**121d** was prepared by following procedure similar to (+)-**121d**: $[\alpha]^{25}_D$ (−) 230.4° (c = 0.51, acetone); ^{1}H NMR δ 8.00–7.25 (m, 4 H, aromatic), 7.12 (s, 1 H, NH), 5.18 (s, 1 H, C4-H), 4.11 (q, 2 H, CO$_2$CH_2CH$_3$), 2.40 (s, 3 H, CH_3), 2.10 (s, 3 H, CH$_3$) and 1.07 (t, J = 7.22 Hz). Anal. (C$_{18}$H$_{17}$F$_3$N$_2$O$_2$) C, H, N.

The ^1H NMR spectra of (+)-, (−)-, and (±)-**121d** in the presence of the chiral shift reagent Eu(facam)$_3$ were determined. The methine proton at the C_4 position of (±)-**121d** was observed at 4.87 ppm and 4.93 ppm in $CDCl_3$ containing (83 mg) of the shift reagent. This solution was spiked with pure (+)- and (−)-**121d**. The signal at 4.87 ppm increased in intensity when the (+) isomer was added and the signal at higher frequency responded to the addition of the (−) isomer, thus uniquely identifying each component. Additionally, (+)- and (−)-**121d** each exhibited only a single peak at the expected position. These studies confirmed optical purity of (+)- and (−)-**121d**.

The individual isomers (+)- and (−)-**121d** were converted to **54A** and **54B** by following methodology similar to compound **123a** and **123c**, respectively. (+)-**52** (60%): mp 174–176 °C; $[\alpha]^{23}_D$ (+) 121.6° (c = 0.55, $CHCl_3$). Anal. Calcd ($C_{23}H_{20}F_3N_3O_2S$) C, H, N. (−)-**52** (65%): mp 174–175 °C; $[\alpha]^{23}_D$ (−) 116.0° (c = 0.58, $CHCl_3$). Anal. Calcd ($C_{23}H_{20}F_3N_3O_2S$) C, H, N. **54A** (35%): white foam; $[\alpha]^{23}_D$ (+) 161° (c, 0.33, $CHCl_3$); HPLC (hexane/2-propanol 95:5) retention time 36.57 min. Anal. Calcd ($C_{23}H_{20}F_3N_3O_4S \cdot 0.8\ H_2O$) C, H, N. **54B** (37%): white foam; $[\alpha]^{23}_D$ (−) 147° (c = 0.33, $CHCl_3$); HPLC (hexane/2-isopropanol 95:5) retention time 41.16 min. Anal. Calcd ($C_{23}H_{20}F_3N_3O_4S \cdot 0.7\ H_2O$) C, H, N.

Diethyl 2-Cyano-1,4-dihydro-6-[(4-pyridylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (123a). Pyridinium bromide perbromide (1 g, 31.2 mmol) was added to a solution of **121e** (1.14 g, 3.0 mmol) in $CHCl_3$ (15 mL) containing pyridine (0.24 mL) at −10 °C. The reaction mixture was stirred at that temperature for 1 h. Chloroform was distilled and the residue chromatographed with isopropyl ether to give 1.6 g of the bromide as a pale yellow foam. This was dissolved in THF (15 mL) and added to a solution of the anion [prepared from 4-mercaptopyridine (0.36 g) and NaH (60%, 1.37 g) in DMF (10 mL)]. The reaction mixture was stirred overnight at 23 °C. DMF was distilled, the residue was poured into water, and the dark gummy material was extracted with EtAC. EtOAc was evaporated and the residue chromatographed (EtAC/isopropyl ether 1:1) to yield 0.70 g (50%) of the desired material, mp 180–181 °C (red melt). Anal. ($C_{25}H_{22}F_3N_3O_4S$) C, H, N. Sulfone **123c** (foam): ^1H NMR δ 6.71 (s, 1 H, NH), 5.63 (s, 1 H, C4-H), 5.19 (d, J = 13 Hz, 1 H), 4.74 (d, J = 13.1 Hz, 1 H). Anal. ($C_{25}H_{22}F_3N_3O_6S$) C, H, N. Sulfoxide **123b** (foam): Anal. ($C_{25}H_{22}F_3N_3O_5S \cdot H_2O$) C, H, N.

Biological Methods. The procedures used to measure specific binding of [^{3}H]nitrendipine to calcium channels has been previously described by Taylor et al.[13] and Ehlert and co-workers.[23] Guinea pig left atrial contractile function and hemodynamic responses in the isolated Langendorff-perfused rat heart was evaluated by using methods described by Weishaar et al.[25] and Haleen and co-workers,[26] respectively. The ability of various compounds to block potassium-induced contractions in isolated rabbit aortic rings was examined by using the method of Kazda,[28] as modified by Weishaar et al.[29] The effect of various compounds on partially depolarized vascular muscle was studied by using the protocol previously described by Schramm and co-workers.[30]

Acknowledgment. We thank Dr. Forrest A. MacKellar and his associates for spectral determinations and microanalyses. We also thank Dr. S. Priebe for performing HPLC analysis on racemic and the pure enantiomers of **54**.

Synthesis and Biological Evaluation of Substituted Benzenesulfonamides as Novel Potent Membrane-Bound Phospholipase A_2 Inhibitors

Hitoshi Oinuma,*[,†,‡] Tadanobu Takamura,[†] Takashi Hasegawa,[†] Ken-Ichi Nomoto,[†] Toshihiko Naitoh,[†] Yoshiharu Daiku,[†] Sachiyuki Hamano,[†] Hiroshi Kakisawa,*[,‡] and Norio Minami[†]

Departments of Chemistry and Pharmacology, Tsukuba Research Laboratories, Eisai Co., Ltd., 5-1-3, Tokodai, Tsukuba, Ibaraki, 300-26, Japan, and Department of Chemistry, University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki, 305, Japan.
Received December 27, 1990

A novel series of 4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]benzenesulfonamides has been prepared and evaluated as membrane-bound phospholipase A_2 inhibitors. A structure–activity relationship study indicated that the optimum potency was realized with the *N*-(phenylalkyl)piperidine derivatives **3** and **4**. These compounds inhibited the liberation of arachidonic acid from the rabbit heart membrane fraction with IC_{30} values of 0.028 and 0.009 μM, respectively. Several compounds (**3**, **4**, and **28**), which proved to be potent inhibitors in vitro, significantly reduced the size of myocardial infarction in coronary occluded rats by iv administrations prior to the ligation. *N*-(1-Benzyl-4-piperidinyl)-4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]-benzenesulfonamide (**3**, ER-3826), which showed the protective in vivo effects at doses higher than 0.3 mg/kg iv, was finally chosen as a leading candidate.

Phospholipase A_2 (PLA_2) is an enzyme that catalyses the hydrolysis of the fatty acid ester bond at the 2-position of membrane phospholipids to produce two potent inflammatory mediators, e.g., arachidonic acid (AA) and lysophospholipids.[1-3] This enzyme is usually classified into two species, extracellular PLA_2 and intracellular PLA_2. The former PLA_2 is found in the venoms of bees and snakes and mammalian pancreatic secretions and is well characterized both mechanistically and with regard to their primary sequence.[4] The ability to inhibit the extracellular PLA_2 has been the focus of several laboratories for the potential discovery of antiinflammatory agents.[1,5] In contrast, little is known about intracellular PLA_2', thereby the pharmacological consequences caused by the inhibition of this enzyme are not well-understood.[6]

† Eisai Co., Ltd.
‡ University of Tsukuba.

(1) Lapetina, E. G. *Annu. Rep. Med. Chem.* 1984, *19*, 213.
(2) Needleman, P.; Turk, J.; Jakschik, B. A.; Morrison, A. R.; Lefkowith, J. B. *Annu. Rev. Biochem.* 1986, *55*, 69.
(3) (a) Hanahan, D. J. *Annu. Rev. Biochem.* 1986, *55*, 483. (b) Snyder, F. *Annu. Rep. Med. Chem.* 1983, *17*, 314.
(4) (a) Slotboom, A. J.; Verheij, H. M.; DeHaas, G. H. *Posopholipids New Biochemistry*; Hawthrone and Ansell, Eds.; Elsevier: Amsterdam, 1982; Vol. 4, p 359. (b) Volwerk, J. J.; DeHaas, G. H. *Lipid-Protein Interactions*; Jost, P. C., Griffith, O. H., Eds.; John Wiley and Sons: New York, 1982; p 69. (c) Velheij, H. M.; Slotboom, A. J.; DeHaas, G. H. *Rev. Physiol. Biochem. Pharmacol.* 1981, *91*, 91. (d) Heinriksson, R. L.; Sakman, T. P.; Randolph, A. *Frontiers in Protein Chemistry*; Liu, T. Y., Mamiya, C., Yasunobu, K., Eds.; Elsevier, North Holland Biomedical Press: Amsterdam, 1980; p 297.

0022-2623/91/1834-2260$02.50/0