12·CF_3CO_2H, 125780-80-5; 13·CF_3CO_2H, 125780-82-7; 14·CF_3CO_2H, 125780-96-3; **15**, 125780-84-9; **16**, 125780-85-0; **17**, 125780-86-1; 4-phenyl-1-piperazineacetic acid, 119378-70-0; 4-morpholineacetic acid, 3235-69-6; nicotinic acid, 59-67-6; retinoic acid, 302-79-4; *N*-Boc-L-phenylalanine, 13734-34-4; *N*-Boc-L-tyrosine, 3978-80-1; *N*-Boc-L-isoleucine, 13139-16-7; *N,N'*-di-Boc-L-lysine, 2483-46-7; *N*-Boc-L-glutamic acid, 1-*tert*-butyl ester, 24277-39-2; *N*-Boc-*O*-*tert*-butyl-L-serine, 13734-38-8.

Dihydropyrimidine Calcium Channel Blockers: 2-Heterosubstituted 4-Aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic Acid Esters as Potent Mimics of Dihydropyridines

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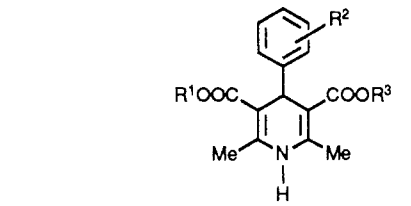
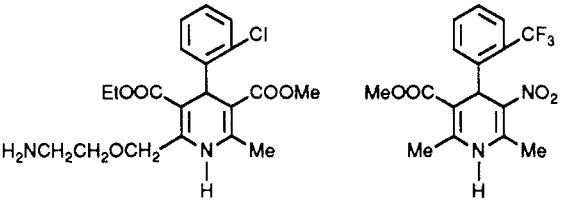
2-Heterosubstituted-4-aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic acid esters **8**, which lack the potential C_s symmetry of dihydropyridine calcium channel blockers, were prepared and evaluated for biological activity. Biological assays using potassium-depolarized rabbit aorta and radioligand binding techniques showed that some of these compounds are potent mimics of dihydropyridine calcium channel blockers. The combination of a branched ester (e.g. isopropyl, *sec*-butyl) and an alkylthio group (e.g. SMe) was found to be optimal for biological activity. When compared directly with similarly substituted 2-heteroalkyldihydropyridines **9**, dihydropyrimidines **8** were found to be 30-fold less active. The solid-state structure of dihydropyrimidine analogue **8g** shows that these compounds can adopt a molecular conformation which is similar to the reported conformation of dihydropyridine calcium channel blockers.

Dihydropyridines are the largest and most studied class of organic calcium channel blockers.[1] In addition to their proven clinical utility in cardiovascular medicine, dihydropyridines are employed extensively as biological tools for the study of voltage-activated calcium channel structure and function.[2] The structures of some of these compounds are shown in formulae **1–5**. Biological activity appears to depend on an axial aromatic ring, the plane of which bisects the dihydropyridine ring in a boat conformation.[3] When the substituents at C2/C6 and the esters at C3/C5 are equivalent, the molecule possesses C_s symmetry and is nonchiral. Unsymmetrical modification of these substituents generates a chiral center at C4. In certain cases (e.g. **6** and **7**), individual isomers possess opposite actions on the calcium channel; one showing blocking activity and the other demonstrating activating activity.[4] Additional modification of both the ester (e.g. **1–4**) and alkyl substituents (e.g. **5**) can affect duration of action in vivo and is claimed to alter tissue selectivity.[5,6]

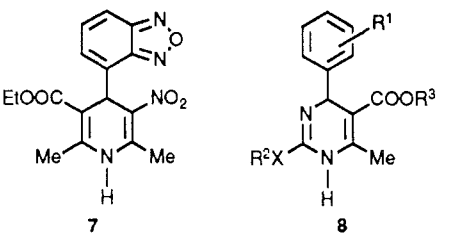
The study of inherently unsymmetrical molecules facilitates obtaining information about structural requirements important to biological activity and the investigation of the effects of absolute stereochemistry. It also affords an opportunity to expand existing structure–activity relationships and, potentially, to discover additional structural modifications consistent with improved biological activity. We have recently demonstrated that the potential C_s symmetry of dihydropyridine calcium channel blockers



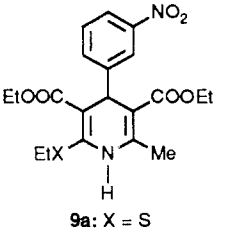
1, nifedipine: R^1 = Me, R^2 = 2 - NO_2, R^3 = Me
2, nitrendipine: R^1 = Me, R^2 = 3 - NO_2, R^3 = Et
3, nimodipine: R^1 = CH_2CH_2OMe, R^2 = 3 - NO_2, R^3 = iPr
4, nicardipine: R^1 = $CH_2CH_2N(Me)Bn$, R^3 = 3 - NO_2, R^3 = Et



5, amlodipine **6**, Bay K - 8644



7 **8**



9a: X = S
b: X = O

(1) Godfraind, T.; Miller, R.; Wibo, M. *Pharmacol. Rev.* **1986**, *38*, 321.
(2) Bellemann, P. *Innovative Approaches In Drug Research*; Elsevier: Amsterdam, 1986; p 23–46.
(3) (a) Fossheim, R.; Svarteng, K.; Mostad, A.; Romming, C.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1982**, *25*, 126. (b) Fossheim, R. *J. Med. Chem.* **1986**, *29*, 305.
(4) (a) Franckowiak, G.; Bechem, M.; Schramm, M.; Thomas, G. *Eur. J. Pharmacol.* **1985**, *114*, 223. (b) Hof, R. P.; Ruegg, U. T.; Hof, A.; Vogel, A. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 689. (c) Kongsamut, S.; Kamp, T. J., Miller, R. J.; Sanguinetti, M. C. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 141. (d) Gjorstrup, P.; Harding, H.; Isaksson, R.; Westerlund, C. *Eur. J. Pharmacol.* **1986**, *122*, 357.
(5) (a) Arrowsmith, J. E.; Campbell, S. F.; Cross, P. E.; Stubbs, J. K.; Burgess, R. A.; Gardiner, D. G.; Blackburn, K. J. *J. Med. Chem.* **1986**, *29*, 1696. (b) Meguro, K.; Aizawa, M.; Sohda, T.; Kawamatsu, Y.; Nagaoka, A. *Chem. Pharm. Bull.* **1985**, *33*, 3787.
(6) Langs, D. A.; Triggle, D. J. *Mol. Pharmacol.* **1985**, *27*, 544.

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Table I. Physical Properties and Method of Preparation of 2-Hetero-1,4-dihydropyrimidines and 2-Hetero-1,4-dihydropyridines

compd	analysis	mp, °C	% yield	method
8a	$C_{15}H_{17}N_3O_4S$ (C, H, N, S)	91.5–93 (ether–hexanes)	79	B
8b	$C_{15}H_{17}N_3O_4S$·HCl (C, H, N, S, Cl)	188–190 (2-propanol)	55	A
8c	$C_{16}H_{17}F_3N_2O_2S$·HCl (C, H, N, F, S)	175–177 (2-propanol)	43	A
8d	$C_{15}H_{16}Cl_2N_2O_2S$·HCl (C, H, N, Cl, S)	199–201 (2-propanol)	58	A
8e	$C_{17}H_{19}N_3O_4S$ (C, H, N, S)	91–93 (isopropyl ether-hexanes)	76	B
8f	$C_{19}H_{25}N_3O_4S$ (C, H, N, S)	103–105 (isopropyl ether)	75	B
8g	$C_{21}H_{21}N_3O_4S$ (C, H, N, S)	129–130 (isopropyl ether)	72	B
8h	$C_{24}H_{28}N_4O_4S$·$(COOH)_2$ (C, H, N, S)	148–151 (2-propanol)	20	B
8i	$C_{19}H_{26}N_4O_4S$·2HCl (C, H, N, S, Cl)	195–197 (CH_3CN)	44	B
8j	$C_{14}H_{15}N_3O_4S$·HCl (C, H, N, S, Cl)	221–223 dec (CH_3CN)	47	A
8k	$C_{16}H_{19}N_3O_4S$·HCl (C, H, N, S, Cl)	208–210 dec (CH_3CN)	47	A
8l	$C_{17}H_{21}N_3O_4S$·HCl (C, H, N, S, Cl)	215–217 (CH_3CN)	40	A
8m	$C_{16}H_{18}Cl_2N_2O_2S$·HCl (C, H, N, S, Cl)	172–174 (CH_2Cl_2)	49	A
8n	$C_{23}H_{26}N_4O_4S$·2$(COOH)_2$ (C, H, N, S)	108–110 (CH_3CN)	20	A
8o	$C_{23}H_{26}N_4O_4S$·$(COOH)_2$ (C, H, N, S)	135–138 (2-propanol)	16	A
8p	$C_{24}H_{26}F_3N_3O_2S$·$(COOH)_2$ (C, H, F, N, S)	116–121 dec (2-propanol)	33	A
8q	$C_{15}H_{17}N_3O_5$ (C, H, N)	103–105 (ether-hexanes)	72	Exp
8r	$C_{16}H_{20}N_4O_4$·HCl (C, H, N, Cl)	229–230 (2-propanol)	25	Exp
9a	$C_{20}H_{24}N_2O_6S$ (C, H, N, S)	91–93 (ether)	45	Exp
9b	$C_{20}H_{24}N_2O_7$ (C, H, N)	103–104 (isopropyl ether)	7	Exp

is not a prerequisite for potency in vitro.[7] Incorporation of the vinologous urethane functionality into a benzothiazepine results in compounds that possess calcium channel blocking activity.[7] Similarly, suitably substituted 2-alkyl-1,4-dihydropyrimidines are reported to block calcium channels.[8]

Below we describe the synthesis and calcium channel blocking activity of 2-heterosubstituted 1,4-dihydropyrimidines 8. Our decision to pursue dihydropyrimidines containing a 2-hetero group was predicated on the ability to more easily study the effects of modifications in this portion of the molecule compared to the previously reported 2-alkyl system.[8] We also report on 2-hetero-1,4-dihydropyridines,[9] which allow us to examine separately the effects on biological activity associated with incorporation of heteroatom substitution at C2/C6. Finally, we describe X-ray crystallographic data demonstrating that dihydropyrimidines adopt a conformation similar to that believed important for biological activity of dihydropyridines.

Chemistry

S-Alkylpyrimidines 8 (X = S) in Table I were prepared by one of two methods. In method A, commercially available 2-methyl-2-thiopseudourea sulfate (**10**; X = S, R^2 = Me)[10] was condensed with α-benzylidene-β-keto ester **11**[11] in the presence of sodium acetate (Scheme I). This method is most suitable for the preparation of analogues in which the alkylthio group is constant. Alternate alkylthiopseudoureas required for this method are obtained by alkylation of thiourea.

Method B involved the base-catalyzed alkylation of 1,2,3,4-tetrahydro-6-methyl-4-(3-nitrophenyl)-2-thioxo-5-pyrimidinecarboxylic acid ethyl esters (**15**; Scheme I). This process is particularly useful for the preparation of thio-

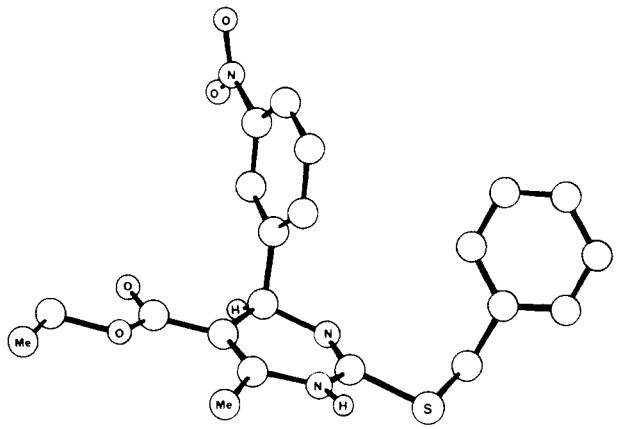


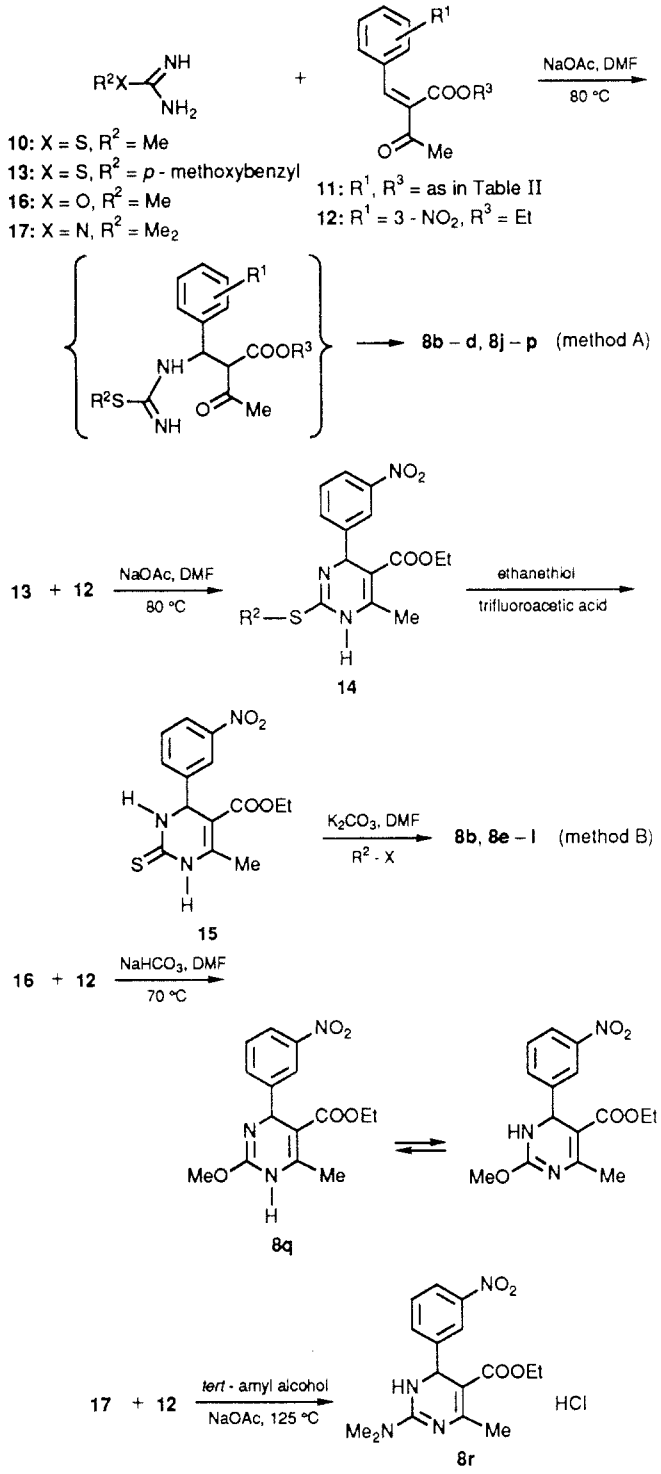
Figure 1. The solid-state structure of 1,4-dihydro-6-methyl-4-(3-nitrophenyl)-2-[(phenylmethyl)thio]-5-pyrimidinecarboxylic acid ethyl ester (**8g**).

alkyl analogues because **15** is employed as a common intermediate. Although **15** could be prepared via the Biginelli condensation,[12] we have worked out a more convenient alternate procedure.[13] Accordingly, protected thiourea **13** was allowed to react with α-benzylidene-β-keto ester **12** and the resulting 1,4-dihydropyrimidine **14**, obtained in excellent yield, was deprotected with trifluoroacetic acid and ethanethiol. Using this methodology, we obtained pyrimidinethione **15** in high overall yield. The 2-(alkylthio)-1,4-dihydropyrimidines **8a** and **8e–i** prepared by this method are listed in Table II. By ^{1}H NMR, (alkylthio)pyrimidines **8a–p** exist predominantly in the 1,4-dihydro form in solution. The most diagnostic signal was due to the pyrimidine C-4 proton, which appears as a singlet for the 1,4-dihydro isomer and as a doublet for the 3,4-dihydro isomer.

2-Methoxypyrimidine **8q** was conveniently generated in 72% yield by the condensation of *O*-methylisourea hydrogen sulfate (**16**)[10] with α-benzylidene-β-keto ester **12**. Aza analogue **8r** was prepared from 1,1-dimethylguanidine sulfate (**17**)[10] and **12** in an analogous manner. Whereas 2-methoxy derivative **8q** exists in solution as a mixture of 1,4- and 3,4-dihydro isomers, the aza analogue **8r** exists

(7) Atwal, K. S.; Bergey, J. L.; Hedberg, A.; Moreland, S. *J. Med. Chem.* **1987**, *30*, 635.
(8) (a) German Offen. 3234684 A1, 1984 (*Chem. Abstr.* **1984**, *101*, 55110*v*) (b) Jpn. Kokai JP 60/81, 173, 1985 (*Chem. Abstr.* **1985**, *103*, 178274*a*).
(9) 2-Alkylthiodihydropyridines have been disclosed to possess calcium channel blocking activity; EP 125-803-A assigned to Fisons PLC (*Chem. Abstr.* **1985**, **102**, 203874*k*).
(10) 2-Methyl-2-thiopseudourea sulfate, *O*-methylisourea hydrogen sulfate, and 1,1-dimethylguanidine sulfate were purchased from Aldrich Chemical Co.
(11) α-Benzylidene-β-keto ester **11** was readily prepared from the corresponding benzaldehyde and acetoacetic ester by standard Knoevenagel condensation.
(12) (a) Brown, D. J. *The Pyrimidines*; Wiley: New York, 1962, 440. (b) *Ibid.* **1970**, Suppl. I, 326.
(13) Atwal, K. S.; O'Reilly, B. C. Gougoutas, J. Z.; Malley, M. F. *Heterocycles* **1987**, *26*, 1189.

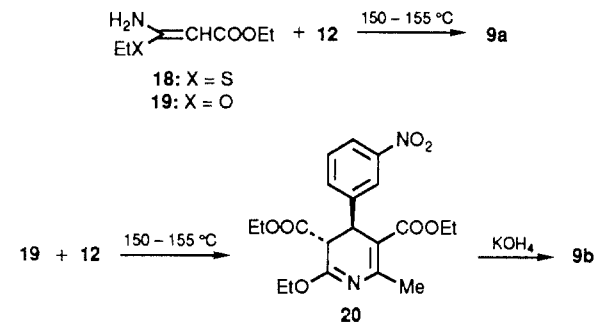
Scheme I



predominantly in the 3,4-dihydro form, by NMR analysis.

We prepared both 2-(ethylthio)- and 2-ethoxydihydropyridines **9a** and **9b** for comparison to analogous dihydropyrimidines. Preparation of **9a** was accomplished simply via condensation of 3-amino-3-(ethylthio)-2-propenoic acid ethyl ester (**18**) with α-benzylidene-β-keto ester **12** (Scheme II). The reaction involving 3-amino-3-ethoxy-2-propenoic acid ethyl ester (**19**)[14] and **12** under similar conditions led to *trans*-3,4-dihydropyridine **20**,[15] which was equilibrated to a mixture **20** and **9b** under basic

Scheme II



conditions. The desired product **9b** was separated from the mixture by chromatography.

In order to correlate the structure of 1,4-dihydropyrimidines **8** with dihydropyridine calcium channel blockers, we determined the solid-state structure of **8b** by X-ray crystallography.[16] As shown in Figure 1, the solid-state conformation of this compound is very similar to that reported for dihydropyridine calcium channel blockers.[3] The dihydropyrimidine ring is in a boat conformation with the phenyl ring in a pseudoaxial position. The plane of the aromatic ring is nearly bisecting the dihydropyrimidine ring and the 3-nitro group is oriented synperiplanar to the C4 methine of the dihydropyrimidine ring. Therefore, in spite of the differences in ring structure and substituents, the dihydropyrimidines closely mimic the conformational features of biologically active dihydropyridines.

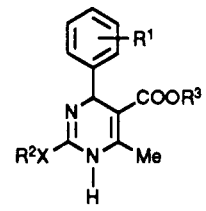
Biological Results and Discussion

Vasorelaxant potency was determined by comparison of the IC_{50} values (concentration necessary for 50% relaxation) obtained from concentration–effect curves with strips of K^+-depolarized rabbit thoracic aortae. Relaxation of the K^+-depolarized strips is predictive of calcium channel blocking activity and we have routinely employed this method to assess rank order of potency.[7] The activity of the compounds prepared is summarized in Table II. For structure–activity studies, we chose the aromatic substituents that are commonly employed in dihydropyridines.[5] 3-Nitro derivative **8a** relaxed the K^+-depolarized aorta with an IC_{50} value of 130 nM. Although 2-nitro analogue **8b** (IC_{50} = 300 nM) is slightly less potent than the corre-

(14) Glickman, S. A.; Cope, A. C. *J. Am. Chem. Soc.* **1945**, *67*, 1017.
(15) The stereochemistry assigned was proved by X-ray crystallography.[16]

(16) For **8g**: a = 10.661 (6) Å, b = 10.771 (6) Å, c = 10.653 (4) Å, α = 93.96 (4)°, β = 111.04 (3)°, γ = 62.53 (4)°, V = 1006 (2) Å^3, D_{obs} = 1.35 g cm^{-3} (D_{calc} = 1.36 for $C_{21}H_{21}N_3O_4S$, Z = 2), space group P$\bar{1}$. For **20**: a = 11.96 (1) Å, b = 12.24 (1) Å, c = 8.049 (3) Å, α = 75.48 (5)°, β = 83.38 (7)°, γ = 112.05 (7)°, V = 1028 (2) Å^3, D_{obs} = 1.29 g cm^{-3} (D_{calc} = 1.31 for $C_{20}H_{24}N_2O_7$, Z = 2), space group P$\bar{1}$. A total of 2745 reflections were measured for **8g** (2114 for **20**) on a Syntex $P2_1$ diffractometer at 23 °C with the θ–2θ variable-scan technique (λ = 1.5418 Å) and were corrected only for Lorentz–polarization factors. The structures were solved by direct methods and refined by full-matrix least-squares analysis on the basis of "observed" reflections with $I > 3\sigma(I)$ (2022 for **8g**, 1345 for **20**). Although most hydrogen positions were evident on difference maps, they were introduced in idealized positions and their scattering was taken into account in the later stages of refinement. The least squares weights, $w = \sigma^{-2}(F_o)$ were calculated with the assumption that $\sigma^2(I) = e^2 + (pI)^2$ where e is a statistical counting error and p = 0.04. The refinements, assuming anisotropic motion for all non-hydrogen atoms, converged at R = 0.048 and R_w = 0.059 for **8g** (for **20**, R = 0.066 and R_w = 0.080). The final difference maps contained no significant features. Tables of atomic coordinates, bond distances and angles, and thermal parameters are included as supplementary material.
(17) Prous, J.; Blancafort, P.; Castaner, J.; Serradell, M. N.; Mealy, N. *Drugs Future* **1981**, VI, 427.

Table II. The IC_{50} Values for Vasorelaxant Activity and K_d Values for Inhibition of [^{3}H]Nitrendipine Binding



compd	X	R^1	R^2	R^3	IC_{50}, nM (95% confidence limit)	K_d, nM (n = 4)
8a	S	3-NO_2	Me	Et	130 (58–280)	23 ± 3
8b	S	2-NO_2	Me	Et	300 (190–470)	
8c	S	2-CF_3	Me	Et	36 (25–54)	35 ± 2
8d	S	2,3-Cl_2	Me	Et	16 (12–20)	
8e	S	3-NO_2	$CH_2CH{=}CH_2$	Et	300 (190–480)	
8f	S	3-NO_2	$CH_2(CH_2)_3CH_3$	Et	180 (200–390)	
8g	S	3-NO_2	$CH_2C_6H_5$	Et	190 (79–420)	
8h	S	3-NO_2	$CH_2CH_2N(Me)Bn$	Et	170 (100–270)	
8i	S	3-NO_2	$CH_2CH_2NMe_2$	iPr	1200 (800–1800)	
8j	S	3-NO_2	Me	Me	280 (200–390)	120 ± 8
8k	S	3-NO_2	Me	iPr	7 (3–15)	12 ± 3
8l	S	3-NO_2	Me	sBu	9 (6–12)	2.6 ± 0.18
8m	S	2,3-Cl_2	Me	iPr	6 (4–10)	
8n	S	3-NO_2	Me	$CH_2CH_2N(Me)Bn$	30 (25–36)	
8o	S	2-NO_2	Me	$CH_2CH_2N(Me)Bn$	1900 (1200–3100)	
8p	S	2-CF_3	Me	$CH_2CH_2N(Me)Bn$	>100000	
8q	O	3-NO_2	Me	Et	710 (240–2100)	105 ± 5
8r	N	3-NO_2	Me_2	Et	6100 (4400–8500)	3400 ± 190
9a					4 (2–7)	
9b					26 (13–53)	
3 (nitrendipine)					1 (0.2–3)	0.26 ± 0.015

sponding 3-nitro derivative **8a**, both the 2-trifluoromethyl (**8c**, IC_{50} = 36 nM) and the 2,3-dichloro (**8d**, IC_{50} = 16 nM) analogue are more potent than **8a**. The reference agent nitrendipine (**3**, IC_{50} = 1 nM) was found to be 10–100-fold more potent than **8a–d** in this test.

Neither the length (**8e**, **8f**) or the size (**8g**) of the alkylthio group had a significant effect on calcium channel blocking activity in vitro. The effect on potency from introduction of a basic nitrogen into the alkylthio group is more complex and depends on the nature of the substituents on the nitrogen atom. Whereas [(benzylmethylamino)ethyl]thio analogue **8h** has potency (IC_{50} = 170 nM) similar to methylthio analogue **8a**, [(dimethylamino)ethyl]thio derivative **8i** (IC_{50} = 1200 nM) is considerably less potent than the corresponding the methylthio analogue **8k** (IC_{50} = 7 nM).

Variation in the size of ester group is most critical for optimization of potency. Isopropyl ester **8k** (IC_{50} = 7 nM) was nearly 20-fold more potent than ethyl ester **8a** (IC_{50} = 130 nM), which, in turn, is similar in potency to methyl ester **8j** (IC_{50} = 280 nM). The observed increase in potency in going from methyl/ethyl to isopropyl ester was independent of the nature of the aromatic substitution (compare **8d** vs **8m** and **8a** vs **8k**). However, the magnitude of the effect was found to be dependent on the position of the aromatic substituent. The 3-nitrophenyl compound shows a more pronounced increase in potency in going from ethyl to isopropyl ester (**8a** vs **8k**) than the 2,3-dichlorophenyl analogue (**8d** vs **8m**). Therefore, there appears to be an interdependency between the aromatic substituent and the alkyl group of the ester. Increasing the size of the ester group to *sec*-butyl (**8l**) was without further effect on potency.

Incorporation of a basic amine into the ester side chain showed results which were dependent on the position of the aryl substituent. Whereas 3-nitro derivative **8n** displayed good potency in vitro (IC_{50} = 30 nM), its 2-nitro analogue **8o** was considerably less potent (IC_{50} = 1900 nM). In order to determine if this loss in potency is a general property of the 2-substituted phenyl compounds, we prepared the 2-trifluoromethyl analogue **8p** and found very poor activity (IC_{50} = >100 000 nM). The result further demonstrates that the effects of aryl substituent and ester modification are interdependent.

The effect on potency of the heteroatom was studied by preparing both the oxa (**8q**) and the aza (**8r**) analogues. Dimethylamino derivative **8r** was selected to keep the double bond in the endocyclic position. As shown in Table II, the calcium channel blocking activity followed the order thio (**8a**) > oxo (**8q**) > aza (**8r**). It is difficult to comment on whether the loss in potency in going from sulfur to nitrogen is due to the nature of the heteroatom or to a possible difference in the potency between 1,4- and 3,4-dihydropyrimidine isomers. Although the alkylthio compounds **8a–p** exist predominantly in the 1,4-dihydro form, the nitrogen analogue **8r** exists almost exclusively in the 3,4-dihydro form. It is conceivable that **8a** expresses better activity than **8q** and **8r** because the alkylthio group at C2 of **8a** more closely resembles the alkyl groups at C2 and C6 of dihydropyridines (**1–5**).

We studied a selected number of dihydropyrimidines (Table II) for inhibition of the specific binding of [^{3}H]-nitrendipine in purified plasma membranes from guinea pig ventricular myocardium.[18] The affinity values (K_d),

(18) Characterization of the radioligand binding by analysis of receptor saturability yielded an apparent affinity (K_d) of 0.48 ± 0.03 nM and a density of a single class of membrane receptors (B_{max}) of 220.3 ± 10.4 fmol/mg of protein (n = 18; data not shown). These values are in good agreement with previously published data from studies of [^{3}H]nitrendipine binding in guinea pig myocardial membranes.[19]

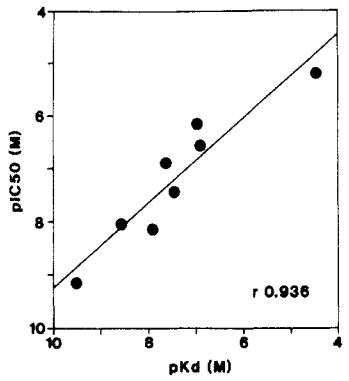


Figure 2. Correlation between IC_{50} values for vasorelaxant activity and K_d values for displacement of [^{3}H]nitrendipine binding of **8a,c,j–l,q,r** and **3**.

calculated from IC_{50} values, are listed in Table II. When the IC_{50} values for relaxation of depolarized rabbit aorta were plotted against the corresponding K_d values, a statistically significant ($p = 0.001$) positive correlation ($r = 0.94$) was observed (Figure 2). This demonstrates that the rank order of potency of 1,4-dihydropyrimidines **8** predicted by the K^+-depolarized rabbit aorta test agrees with the relative affinity of these compounds for the dihydropyridine receptor. The most potent inhibitor **8l** (K_d = 2.6 nM) in radioligand binding was 1 order or magnitude less potent than the dihydropyridine nitrendipine (**3**, K_d = 0.26 nM). This indicates that the dihydropyrimidines, as a class, possess lower affinity for the receptor than closely related dihydropyridines.

To separate the effects of dihydropyrimidine ring and heterosubstitution at C2, we compared dihydropyrimidines **8a** and **8q** with similarly substituted dihydropyridines **9a** and **9b**, respectively. As found in dihydropyrimidines **8**, the 2-(ethylthio)dihydropyridine **9a** (IC_{50} = 4 nM) is more potent than the corresponding ethoxy analogue **9b** (IC_{50} = 26 nM). Both **9a** and **9b**, though less potent than the reference agent nitrendipine (IC_{50} = 1 nM), are about 30-fold more potent than the corresponding dihydropyrimidines **8a** (IC_{50} = 130 nM) and **8q** (IC_{50} = 710 nM), respectively. Our combined results show that introduction of a heteroatom at C2 and substitution of a dihydropyrimidine for a dihydropyridine ring leads to compounds with lower potency. However, as shown by the activity of compounds **8k–m**, some of this loss can be recovered by modification of the ester group.

Regardless of their potency in vitro, none of the dihydropyrimidines **8** or 2-heterosubstituted dihydropyridines **9** showed antihypertensive activity when given to spontaneously hypertensive rats at 135 μmol/kg po. Nitrendipine given at the same dose expressed potent antihypertensive activity. The reason for the lack of antihypertensive activity in dihydropyrimidines **8** or dihydropyridines **9** is unknown.

In conclusion, 1,4-dihydropyrimidines **8** are potent calcium channel blockers which bind to the dihydropyridine receptor. When compared directly with similarly substituted 2-hetero-1,4-dihydropyridines, these compounds are about 30-fold less active. X-ray crystallographic analysis of dihydropyrimidine analogue **8g** shows that the molecule possesses a conformation similar to that observed for dihydropyridine calcium channel blockers in spite of the lack of potential C_s symmetry. Therefore, dihydropyrimidines **8** should assume a receptor-bound conformation similar to that of dihydropyridines. The level of potency displayed by some dihydropyrimidine analogues (e.g. **8k–m**) clearly indicates that C_s symmetry is not required for biological activity in vitro.

(19) (a) Fawzi, A. B.; McNeill, J. H. *J. Pharmacol.* **1984**, *104*, 357. (b) Schwartz, J.; Velly, J. *Br. J. Pharmacol.* **1985**, *84*, 511.

Experimental Section

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The infrared spectra were recorded with a Perkin-Elmer 983 spectrophotometer in KBr pellets. ^{1}H NMR spectra were measured on JOEL-GX-400 and FX-270 spectrometers using Me_4Si as internal standard. Flash chromatography was run with Whatman LPS-1 silica gel. Spectra data of only key intermediates and final compounds are included. Microanalyses of all crystalline compounds were in agreement (within 0.4% of theoretical value) with the structures assigned.

Method A. Illustrated for 4-(2,3-Dichlorophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinecarboxylic Acid Ethyl Ester, Monohydrochloride (8d). A suspension of 2-[(2,3-dichlorophenyl)methylene]-3-oxobutanoic acid ethyl ester (3.0 g, 10.45 mmol),[11] 2-methyl-2-thiopseudourea sulfate (2.9 g, 10.45 mmol),[10] and sodium acetate (1.8 g, 21.9 mmol) in dimethylformamide (12 mL) was heated at 65 °C overnight. The reaction was cooled to room temperature, diluted with ethyl acetate, and filtered. The filtrate was washed with water, sodium bicarbonate, and brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was purified by flash chromatography (5% ethyl acetate in dichloromethane) to yield a colorless foam. It was converted to its hydrochloride salt and crystallized from 2-propanol to yield 4-(2,3-dichlorophenyl)-1,4-dihydro-6-methyl-2-(methylthio)-5-pyrimidinecarboxylic acid ethyl ester monohydrochloride (**8d**; 2.38 g) as a colorless solid: NMR ($CDCl_3$ + DMSO-d_6) δ 7.50 (m, 1 H), 7.30 (m, 4 H), 6.14 (s, 1 H), 4.07 (q, J = 7.4 Hz, 2 H), 2.76 (s, 3 H), 1.14 (t, J = 7.4 Hz, 3 H); IR (KBr) 1657, 1606, 1511, and 1451 cm^{-1}.

Method B: Illustrated for 1,4-Dihydro-6-methyl-2-(methylthio)-4-(3-nitrophenyl)-5-pyrimidinecarboxylic Acid Ethyl Ester (8a). A suspension of 1,2,3,4-tetrahydro-6-methyl-4-(3-nitrophenyl)-2-thioxo-5-pyrimidinecarboxylic acid ethyl ester (**15**; 1.0 g, 3.1 mmol)[13] in acetone (10 mL) was treated with finely ground potassium carbonate (1.0 g, 7.25 mmol) and methyl iodide (220 μL, 3.5 mmol). The reaction was allowed to stir at room temperature overnight and was diluted with ethyl acetate. It was filtered and the filtrate was washed with water and brine and dried over magnesium sulfate. The solvent was evaporated and the residue was crystallized from ether–hexanes to provide 1,4-dihydro-6-methyl-2-(methylthio)-4-(3-nitrophenyl)-5-pyrimidinecarboxylic acid ethyl ester (**8a**) as a colorless solid (820 mg): NMR ($CDCl_3$) δ 8.16 (s, 1 H), 8.09 (d, J = 7.9 Hz, 1 H), 7.65 (d, J = 7.4 Hz, 1 H), 7.45 (t, J = 7.9 Hz, 1 H), 6.40 (br s, 1 H), 5.80 (s, 1 H), 4.13 (q, J = 6.85 Hz, 2 H), 2.40 (s, 3 H), 1.2 (t, J = 6.85 Hz, 3 H); IR (KBr) 1655, 1530, and 1483 cm^{-1}.

Preparation of 1,4-Dihydro-2-methoxy-6-methyl-4-(3-nitrophenyl)-5-pyrimidinecarboxylic Acid Ethyl Ester (8q). A suspension of 2-[(3-nitrophenyl)methylene]-3-oxobutanoic acid ethyl ester (**12**; 1.72 g, 10.0 mmol),[11] *O*-methylisourea hydrogen sulfate (**16**; 2.62 g, 10.0 mmol),[10] and sodium bicarbonate (4.2 g, 50.0 mmol) in dimethylformamide was heated at 65 °C overnight. The reaction was cooled to room temperature, diluted with ether, and filtered. The filtrate was washed (water and brine), dried (magnesium sulfate), and evaporated. The residue was crystallized from ether–hexanes to yield 1,4-dihydro-2-methoxy-6-methyl-(3-nitrophenyl)-5-pyrimidinecarboxylic acid ethyl ester **8q** (2.3 g): NMR ($CDCl_3$) δ 8.16, 8.11 (s, 1 H), 8.07 (dd, J = 8.0 and 1.0 Hz, 1 H), 7.7 (d, J = 7.4 Hz, 1 H), 7.4 (t, J = 8.0 Hz, 1 H), 6.5, 6.0 (s, 1 H), 5.7, 5.55 (s and d, respectively, J = 2.7 Hz, 1 H), 4.1 (dq, J = 6.9 and 1.0 Hz, 2 H), 3.85, 3.7 (s, 3 H), 2.4, 2.3 (s, 3 H), 1.2 (t, J = 6.9 Hz, 3 H); IR (KBr) 1691, 1647, and 1529 cm^{-1}.

Preparation of 2-(Dimethylamino)-3,4-dihydro-6-methyl-4-(3-nitrophenyl)-5-pyrimidinecarboxylic Acid Ethyl Ester, Hydrochloride (8r). A suspension of 2-[(3-nitrophenyl)methylene]-3-oxobutanoic acid ethyl ester (**12**; 1.31 g, 5.0 mmol),[11] 1,1-dimethylguanidine sulfate (**17**; 680 mg, 5.5 mmol),[10] and sodium acetate (820 mg, 10.0 mmol) in *tert*-amyl alcohol (10 mL) was heated at 125 °C for 24 h. The reaction was cooled to

room temperature and diluted with ethyl acetate. The resulting suspension was extracted with 2 N hydrochloric acid and the combined aqueous extracts were basified with 2 N sodium hydroxide and extracted with dichloromethane. The residue, after evaporation of the solvent, was converted to its hydrochloric acid salt and crystallized from 2-propanol to yield a colorless solid **8r** (412 mg): NMR ($CDCl_3$) δ 8.3 (s, 1 H), 8.1 (d, J = 7.9 Hz, 1 H), 7.8 (d, J = 7.4 Hz, 1 H), 7.5 (t, 7.9 Hz, 1 H), 5.8 (d, J = 6.2 Hz, 1 H), 4.1 (m, 2 H), 3.3 (s, 6 H), 2.7 (s, 3 H), 1.2 (t, J = 7.4 Hz, 3 H); IR (KBr) 1713, 1665, and 1530 cm^{-1}.

Preparation of 2-(Ethylthio)-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic Acid Diethyl Ester (9a). (a) 3-Amino-3-(ethylthio)-2-propenoic Acid Ethyl Ester (18). A solution of ethyl cyanoacetate (28.28 g, 0.25 mol) and ethanethiol (15.5 g, 0.25 mole) in 15 mL of ether was cooled to 0 °C and treated with hydrochloric acid gas until the weight increase was 9.2 g (0.25 mol). Upon standing at 0–5 °C for 24 h, crystallization occurred. The product was collected and washed with ether to give a colorless solid (35.5 g, 67%), mp 117–119 °C. A cold solution of sodium bicarbonate (15.3 g, 0.18 mol) in 150 mL of water was treated in portions with the above material (19.0 g, 0.09 mol). Ether was added, the mixture was stirred for 10 min, and the layers were separated. After the aqueous fraction was extracted with fresh ether (2×), the combined organic fractions were washed with water, dried (anhydrous $MgSO_4$), and concentrated in vacuo to give 3-amino-3-(ethylthio)-2-propenoic acid ethyl ester (18; 15.9 g) as a viscous oil.

(b) 2-(Ethylthio)-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic Acid Diethyl Ester (9a). A mixture of 3-amino-3-(ethylthio)-2-propenoic acid ethyl ester (18; 5.0 g, 0.028 mol) and 2-[(3-nitrophenyl)methylene]-3-oxobutanoic acid ethyl ester (**12**; 7.5 g, 0.028 mol)[11] was heated neat in an oil bath (155–160 °C) for 1 h. This material was cooled to 40 °C dissolved in 75 mL of ether and cooled to room temperature to give **9a** (5.3 g) as a light yellow solid: NMR ($CDCl_3$) δ 8.13 (s, 1 H), 8.01 (d, J = 7.2 Hz, 1 H), 7.63 (d, J = 7.2 Hz, 1 H), 7.38 (t, J = 7.2 Hz, 1 H), 6.15 (s, 1 H), 5.19 (s, 1 H), 3.95–4.25 (m, 4 H), 2.80–3.05 (m, 2 H), 2.42 (s, 3 H), 1.35 (t, J = 7.0 Hz, 3 H), 1.25, 1.26 (2 t, J = 7.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$) 166.6, 165.9, 148.8, 147.9, 144.8, 144.2, 133.9, 128.5, 122.5, 121.0, 104.0, 103.4, 59.9, 40.2, 25.6, 18.9, 13.9; IR (KBr) 1700, 1668, 1529, 1477, 1351, 1283, 1199, 1095 cm^{-1}.

Preparation of 2-Ethoxy-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic Acid Diethyl Ester (9b). (a) 2-Ethoxy-3,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic Acid Diethyl Ester (20). A mixture of 3-amino-3-ethoxy-2-propenoic acid ethyl ester (19; 13.7 g, 0.08 mol)[14] and 2-[(3-nitrophenyl)methylene]-3-oxobutanoic acid ethyl ester (**12**; 22.7 g, 0.08 mol)[11] was heated in an oil bath at 150–155 °C (internal temperature 125 °C) under vacuum for 2 h. After cooling and standing overnight, the solids that formed were triturated with isopropyl ether. The solid was filtered and recrystallized twice from isopropyl ether to give **20** (6.7 g, 21%): mp 80–81 °C; NMR ($CDCl_3$) δ 8.1 (m, 1 H), 8.0 (s, 1 H), 7.45 (m, 2 H), 4.65 (d, J = 1.5 Hz, 1 H), 4.5–4.25 (m, 2 H), 4.2 (q, J = 7 Hz, 2 H), 4.1 (q, J = 7 Hz, 2 H), 3.3 (d, J = 1.5 Hz, 1 H), 2.5 (s, 3 H), 1.25 (t, J = 7 Hz, 6 H), 1.15 (t, J = 7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) 167.4, 166.5, 162.1, 156.5, 148.37, 142.39, 133.17, 129.4, 122.0, 109.14, 63.16, 61.73, 61.1, 59.9, 49.39, 41.3, 22.2, 13.67; IR (KBr) 1741, 1698, 1577, 1534, 1189 cm^{-1}.

(b) 2-Ethoxy-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic Acid Diethyl Ester (9b). A stirred solution of 2-ethoxy-3,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid diethyl ester (**20**; 5.0 g, 0.012 mole) in ethanol (50 mL) at room temperature was treated gradually with a solution of potassium hydroxide (0.8 g, 0.012 mol) in ethanol (10 mL). After 1.5 h, thin-layer chromatography (ethyl acetate–hexane 1:1) indicated a mixture of starting material and product in approximately equal amounts. Solvent was evaporated and the residue was dissolved in chloroform. The solution was washed with water (2×), dried ($MgSO_4$), and evaporated to give an oil (5 g). Flash chromatography on silica gel and elution with ethyl acetate–hexane (1:3) gave predominantly **9b** (1.8 g). Crystallization from isopropyl ether–hexane and then from isopropyl ether gave pure **9b** (0.56 g, 10%): NMR ($CDCl_3$) δ 8.16 (s, 1 H), 8.02 (d, J = 7.2 Hz, 1 H), 7.68 (d, J = 7.2 Hz, 1 H), 7.39 (t, J = 7.2 Hz, 1 H), 5.96 (s, 1 H), 5.15 (s, 1 H), 4.25–4.50 (m, 2 H), 3.95–4.25 (m, 4 H), 2.38 (s, 3 H), 1.37 (t, J = 7.0 Hz, 3 H), 1.24, 1.28 (2 t, J = 7.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$) 166.7, 165.6, 156.7, 149.5, 145.0, 134.0, 128.5, 122.4, 121.0, 103.6, 89, 70.2, 59.8, 40.4, 18.5, 14.9, 13.9; IR (KBr) 1703, 1647, 1530, 1466, 1350, 1211 cm^{-1}.

Pharmacology. (a) Vasorelaxant Potency. The experimental protocol is very similar to the one previously described in the literature.[7] The compounds were dissolved in 95% ethanol and added to the muscle chambers in a volume such that the concentration of ethanol in the chambers was always less than 0.01%. IC_{50} values were determined with a quadratic fit to the logit transformation of the concentration response curves.

(b) Radioligand Binding. Receptor binding using [^{3}H]nitrendipine was carried out according to the method described previously.[7]

Registry No. **8a**, 125734-74-9; **8b**, 125734-75-0; **8b** free base, 125734-76-1; **8c**, 125734-77-2; **8c** free base, 125734-78-3; **8d**, 125734-79-4; **8d** free base, 125734-80-7; **8e**, 106720-52-9; **8f**, 125734-81-8; **8g**, 125734-82-9; **8h**, 125762-76-7; **8h** free base, 125734-83-0; **8i**, 125734-84-1; **8i** free base, 125734-85-2; **8j**, 125734-86-3; **8j** free base, 125734-87-4; **8k**, 125734-88-5; **8k** free base, 125734-89-6; **8l**, 116680-00-3; **8l** free base, 125734-90-9; **8m**, 125734-91-0; **8m** free base, 125734-92-1; **8n**, 125762-77-8; **8n** free base, 125734-93-2; **8o**, 125734-95-4; **8o** free base, 125734-94-3; **9p**, 125734-97-6; **9p** free base, 125734-96-5; **8q**, 125734-98-7; **8r**, 125734-99-8; **8r** free base, 125735-00-4; **9a**, 125735-01-5; **9b**, 125735-02-6; **10**, 867-44-7; **11** (R^1 = 2-NO_2, R^3 = Et), 67593-37-7; **11** (R^1 = 2-CF_3, R^3 = Et), 39561-91-6; **11** (R^1 = 2,3-Cl_2, R^3 = Et), 94739-24-9; **11** (R^1 = 3-NO_2, R^3 = Me), 39562-17-9; **11** (R^1 = 3-NO_2, R^3 = *i*-Pr, 39562-25-9; **11** (R^1 = 3-NO_2, R^3 = *s*-Bu, 116679-99-3; **11** (R^1 = 2,3-Cl_2, R^3 = *i*-Pr), 103295-97-2; **11** (R^1 = 3-NO_2, R^3 = $CH_2CH_2N(Me)Bn$), 54527-98-9; **11** (R^1 = 2-NO_2, R^3 = $CH_2CH_2N(Me)Bn$), 98050-60-3; **11** (R^1 = 3-CF_3, R^3 = $CH_2CH_2N(Me)Bn$), 106720-63-2; **12**, 39562-16-8; **15**, 125735-03-7; **16**, 52328-05-9; **17**, 1186-46-5; **18**, 53371-88-3; **19**, 39632-87-6; **20**, 125735-04-8; $Br(CH_2)_2N(Me)CH_2Ph$, 53977-06-3; $Br(CH_2)_2NMe_2$, 5459-68-7; ethyl cyanoacetate, 105-56-6.

Supplementary Material Available: Tables listing atomic coordinates, bond distances and angles, and thermal parameters for **8g** and **20** (15 pages). Ordering information is given on any current masthead page.