extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated to dryness. The residue was redissolved in chloroform and stirred over solid sodium carbonate for 15 min. The mixture was filtered, and concentration of the resulting solution gave compound **8** as a white, crystalline solid (234 mg, 66%): mp 105-106 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, CH_3), 1.2-1.8 (m, 30 H, methylene envelope), 2.23 (t, 2 H, J = 7 Hz, CH_2CO), 3.43 (m, 2 H, $CONHCH_2$), 3.58 (m, 2 H, CH_2OH), 3.78 (m, 1 H, $CHOH$), 5.93 (br, 1 H, NH); MS, m/z 357 (M^+, 10%), 326 (M - CH_2OH, 55), 297 (M - CH_2OH - CHO, 21), 284 ($CH_3(CH_2)_{16}CONH_3^+$, 28), 267 ($CH_3(CH_2)_{16}CO^+$, 22), 133 (100), 115 (61); exact mass 357.3249, calcd for $C_{21}H_{43}NO_3$ 357.3243. GC analysis of the TMS ether derivative indicated a purity of 94%.

***N*-(2,3-Dihydroxypropyl)-8-(nonylthio)octanamide (9).** 9-Thiastearic acid and (±)-3-amino-1,2-propanediol were condensed as described above to give compound **9**: mp 91-93 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, CH_3), 1.2-1.8 (m, 24 H, methylene envelope), 2.25 (t, 2 H, J = 7 Hz, CH_2CO), 2.53 (t, 4 H, J = 7 Hz, CH_2SCH_2), 3.45 (m, 2 H, $CONHCH_2$), 3.56 (m, 2 H, CH_2OH), 3.78 m, 1 H, $CHOH$), 5.94 (br, 1 H, NH); MS, m/z 376 (M + H, 2%), 357 (M - H_2O, 13), 344 (M - CH_2OH, 12), 285 ($CH_3(CH_2)_8S(CH_2)_7CO^+$, 35), 248 (45), 216 (100), 133 (73), 92 (68); exact mass (M - H_2O) 357.2705, calcd for $C_{20}H_{39}NO_2S$ 357.2701. GC analysis of the TMS ether derivative indicated a purity of 85%; the principal contaminant was the acid **3**.

***N*-(2,3-Dihydroxypropyl)-9-(octylthio)nonanamide (10).** 10-Thiastearic acid and (±)-3-amino-1,2-propanediol were condensed as described above to give compound **10**: mp 89-91 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, CH_3), 1.1-1.8 (m, 24 H, methylene envelope), 2.24 (t, 2 H, J = 7 Hz, CH_2CO), 2.53 (t, 4 H, J = 7 Hz, CH_2SCH_2) 3.43 (m, 2 H, $CONHCH_2$), 3.56 (m, 2 H, CH_2OH), 3.78 (m, 1 H, $CHOH$), 5.99 (br, 1 H, NH); MS, m/z 376 (M + H, 3%), 357 (M - H_2O, 32), 344 (M - CH_2OH, 20), 285 ($CH_3(CH_2)_8S(CH_2)_7CO^+$, 75), 262 (71), 230 (50), 146 (68), 133 (94), 92 (100); exact mass (M - H_2O) 357.2708, calcd for $C_{20}H_{39}NO_2S$ 357.2701. GC analysis of the TMS ether derivative indicated a purity of 94%.

Acknowledgment. This work was supported by grants from the National Institutes of Health (AI24146 and GM31801).

Registry No. **2**, 114692-26-1; **3**, 106689-24-1; **4**, 105099-89-6; **5**, 114692-27-2; **6**, 106689-25-2; **7**, 106689-26-3; **8**, 7336-25-6; **9**, 114692-28-3; **10**, 114692-29-4; stearic acid, 57-11-4; linoleic acid, 60-33-3; γ-linolenic acid, 506-26-3; dihydrosterculic acid, 5711-28-4; oleic acid, 112-80-1.

2-(2-Aryl-2-oxoethylidene)-1,2,3,4-tetrahydropyridines. Novel Isomers of 1,4-Dihydropyridine Calcium Channel Blockers[1]

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The title compounds are novel double bond isomers of 1,4-dihydropyridine-type calcium channel blockers (CCB). These derivatives were prepared by using the Hantzsch dihydropyridine synthesis. The assignment of structure was based on spectroscopic data and a regiochemically unambiguous synthesis. Several of the analogues inhibited [^{3}H]nitrendipine binding with IC_{50} values as low as 25 nM. By comparison, nifedipine, a clinically useful 1,4-dihydropyridine CCB, inhibits [^{3}H]nitrendipine binding with an IC_{50} of 1.6 nM. In the Langendorff rat heart preparation, treatment with the more potent derivatives produced marked dose-related increases in coronary flow with little or no effect on heart rate or contractility, except at the highest concentrations tested. The selectivity for vascular versus cardiac effects was similar to that of nifedipine, i.e. the concentration producing vasodilation was approximately 2 orders of magnitude lower than the concentration eliciting cardiodepression. These novel isomers extend the structure–activity relationships for calcium channel blockers into a series closely related to the 1,4-dihydropyridines.

Verapamil, diltiazem, and nifedipine are the best known examples of calcium channel blockers, compounds that inhibit cellular calcium influx through the voltage-dependent calcium channel. Each of these three compounds represents a distinctly different chemical class and interacts with a discrete binding site linked to the calcium channel.[2,3] Nifedipine, **1**, is the prototype for the 1,4-dihydropyridine class of calcium channel blockers. A large number of 1,4-dihydropyridine analogues have been reported allowing delineation of well-defined structure–activity relationships (SAR). Among the requirements for potent receptor binding and calcium channel blockade are (a) N-1 hydrogen, (b) an ester at C-3 or C-5, preferably both, (c) an aryl group at C-4, optimally substituted with an ortho or meta electron-withdrawing group, (d) small alkyl groups at C-2 and C-6, and (e) a dihydropyridine ring (rather than pyridine or tetrahydropyridine).[4-8] Studies involving X-ray crystallographic analysis[9] and rigid analogues[10] suggest that the conformation of the 1,4-dihydropyridine ring is a shallow boat with the 4-aryl group oriented perpendicular to and bisecting the plane of the dihydropyridine ring.

Recently, relatively small structural modifications have been shown to produce 1,4-dihydropyridine derivatives that activate calcium channels. Bay k 8644, **2**, is the first

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(1) This work was presented in part at the 195th National Meeting of the American Chemical Society, New Orleans, LA, August 30–September 4, 1987. See: *Abstracts of Papers*; American Chemical Society: Washington, DC, 1987; MEDI 83.
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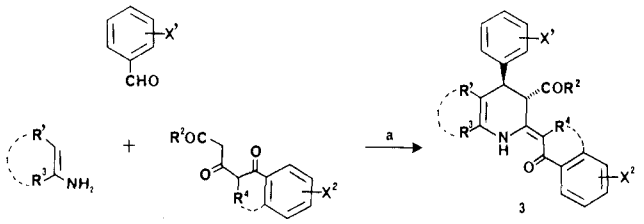
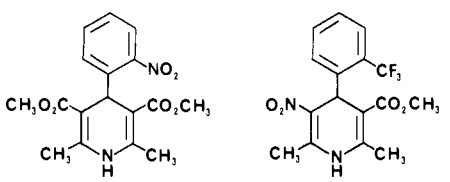
Scheme I[a]



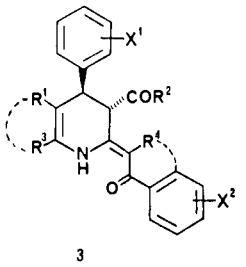
[a] (a) EtOH, reflux, 12–24 h.

example of a 1,4-dihydropyridine calcium channel activator.[11,12] The ability to produce both receptor agonists and antagonists from the same chemical class is an additional incentive for further investigation of the 1,4-dihydropyridines.

In this work, we investigated modifications at the C-2 position of the dihydropyridine ring. SAR at this position are less well defined than for 4-aryl and 3,5-ester modifications. One of our first objectives was to introduce a polar functionality, specifically a carbonyl, into the C-2 side chain. While we were successful, the products isolated were not 1,4-dihydropyridines as expected, but rather isomers containing an exocyclic double bond in conjugation with the sidechain carbonyl.[13] These novel isomers, represented generically as **3**, provided an opportunity to extend the SAR for calcium channel blockers to a series closely related to the 1,4-dihydropyridines. Few 2-alkylidene-1,2,3,4-tetrahydropyridine derivatives have been reported. Two examples are some tricyclic derivatives in which the exocyclic double bond isomer predominated 3:1 over the corresponding 3,4-dihydropyridine[14] and a series containing an exocyclic double bond substituted with two nitrile groups.[15]



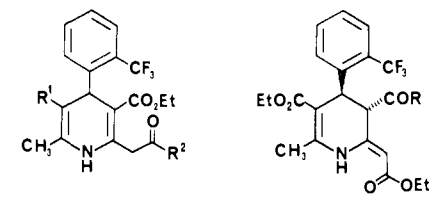
1 2



3

Chemistry

The target compounds, **3**, were prepared by employing the Hantzsch dihydropyridine synthesis[16] using three reactants: aldehyde, aminocrotonate, and diketo ester, as shown in Scheme I. The starting diketo esters, when not commercially available, were obtained by benzoylation of the dilithio dienolate of ethyl acetoacetate.[17] Isolated yields for the Hantzsch reaction were poor, ranging from 3 to 24%. The low yield was due in part to the difficult purification, which required tedious chromatographic separation of the product from complex product mixtures followed by recrystallization. A number of other products could be isolated from the reaction mixtures, including starting materials, the intermediate benzylidenes, dimeric side products, and symmetrical 1,4-dihydropyridines resulting from benzaldehyde and 2 equiv of aminocrotonate.[18] In an attempt to improve the yield, the intermediate enamine and benzylidene were prepared for examples **3g** and **3h**, and a modified Hantzsch reaction was employed. However, overall yields were not significantly improved. Despite the poor yields, the one-step Hantzsch procedure was suitable for the preparation of a variety of analogues in amounts sufficient for biological evaluation. In each case, the exocyclic double bond isomer **3** and not the endocyclic isomer, e.g. **4**, was isolated, except for the 5-cyano analogues **3k**, for which both endo- and exocyclic bond isomers were isolated, and **5**, which was obtained only as the 1,4-dihydropyridine isomer.



4 R¹ = COOEt, R² = Ph
5 R¹ = CN, R² = Ph
15 R¹ = COOEt, R² = OEt
16 R¹ = CN, R² = OEt

6 R = Ph
14 R = OEt

That the products were isomeric with the corresponding 1,4-dihydropyridines was confirmed by microanalyses and mass spectral analyses. The structure assignment was based principally on proton NMR spectral data. The NMR spectra of the isomers differed from related 1,4-dihydropyridines by a substantial downfield shift of the proton on the pyridine nitrogen (δ 12.1) and the presence of three one-proton singlets at δ 5.9, 5.1, and 3.4 (for **3a**). This ensemble of signals, except for the C-4 proton, is inconsistent with the structure of the corresponding 1,4-dihydropyridine isomers. Nuclear Overhauser enhancement (NOE) difference spectra were used to assign the three singlets to protons on the exocyclic double bond, C-4, and C-3, respectively. Since the vicinal C-3 and C-4 protons do not exhibit coupling, an orthogonal torsional angle for C-3,4 protons is indicated. Therefore, a trans configuration was assigned because an orthogonal torsional angle is not accessible to the cis isomer.[19] The exocyclic double bond was assigned the *Z* geometry based on (a) the chemical shift of the N-1 proton, which is consistent with an intramolecular hydrogen bond, and (b) observation of a NOE between C-3H and the olefinic proton.

Although the observations cited above are consistent with the structure of **3**, similar stereochemical arguments based on the spectral data could be developed for regioisomers such as **6**. Therefore, a regiochemically unambiguous synthesis was employed to confirm the assignment.

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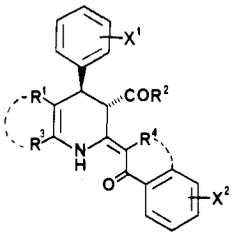
(19) This was determined by examination of Dreiding molecular models and supported by computer molecular modeling studies. The minimum energy conformation determined by modeling had a C3H-C4H torsional angle of 83°.

Table I. 2-(2-Aryl-2-oxoethylidene)-1,2,3,4-tetrahydropyridines and Related Compounds



compd no.	R^1	R^2	R^3	R^4	X^1	X^2	method[a] (% yield)	mp, °C	formula	analysis
3a	COOEt	OEt	Me	H	2-CF_3	H	A (8) B (27)	111–112	$C_{27}H_{26}F_3NO_5$	C, H, N, F
3b	COOEt	OEt	Me	H	2-CF_3	4-aza	A (3)	128–130	$C_{26}H_{25}F_3N_2O_5$	C, H, N, F
3c	COOEt	OEt	Me	H	2-CF_3	4-Cl	A (8)	129–130	$C_{27}H_{25}ClF_3NO_5$	C, H, N, F
3d	COOEt	OEt	Me	H	2-CF_3	4-F	A (13)	111–112	$C_{27}H_{25}F_4NO_5$	C, H, N, F
3e	COOEt	OEt	Me	H	2-CF_3	4-Me	A (4)	85–87	$C_{28}H_{28}F_3NO_5$	C, H, N, F
3f	COOEt	OEt	Me	H	2-CF_3	4-NMe_2	A (8) B (30)	158–159	$C_{29}H_{31}F_3N_2O_5$	C, H, N, F
3g	NO_2	OEt	Me	H	2-CF_3	H	C (22)	133–135	$C_{24}H_{21}F_3N_2O_5$	C, H, N
3h	NO_2	Ph	Me	H	2-CF_3	H	C (4)	84–86	$C_{28}H_{21}F_3N_2O_4$	C, H, N
3i	COOEt	Ph	Me	H	2-CF_3	H	A (6)	187–189	$C_{31}H_{26}F_3NO_4$	C, H, N, F
3j	$CO(CH_2)_2$	OEt	CH_2	H	2-CF_3	H	A (6)	205–206	$C_{27}H_{24}F_3NO_4$	C, H, N, F
3k[b]	CN	OEt	Me	$(CH_2)_2$	2-CF_3	H	A (24)	193–194	$C_{27}H_{23}F_3N_2O_3$	C, H, N, F
3l	COOEt	OEt	Me	$(CH_2)_2$	2-CF_3	H	A (3)	glass	$C_{29}H_{28}F_3NO_5$	C, H, N, F
3m	COOEt	OEt	Me	H	2-CF_3	2-Cl	A (4)	glass	$C_{27}H_{25}ClF_3NO_5$	C, H, N, Cl[c]
3n	COOEt	OEt	Me	H	H	H	B (16)	93–94	$C_{26}H_{27}NO_5$	C, H, N
3o	COOEt	OEt	Me	H	2,3-Cl_2	H	A (6)	135–136	$C_{26}H_{25}Cl_2NO_5$	C, H, N, Cl
3p	COOEt	OEt	Me	H	2-NO_2	H	A (8)	137	$C_{26}H_{26}N_2O_7$	C, H, N
5[d]	CN	OEt	Me	H	2-CF_3	H	A (3)	143–145	$C_{25}H_{21}F_3N_2O_3$	C, H, N, F
14[e]							A (26)[f]	98–99	$C_{23}H_{26}F_3NO_6$	C, H, N
15[e]							A (11)[f]	glass	$C_{23}H_{26}F_3NO_6$	C, H, N
16[e]							A (23)	144–146	$C_{21}H_{21}F_3N_2O_4$	C, H, N

[a] Method A: three component Hantzsch reaction. Method B: three step synthesis using isoxazole intermediate. Method C: two component Hantzsch reaction. See text and the Experimental Section for details. [b] The 1,4-dihydropyridine isomer was also isolated. [c] Calcd: N, 2.61; Cl, 6.62. Found: N, 2.18; Cl, 7.05. [d] The 1,4-dihydropyridine isomer. [e] See text for structures. [f] Compounds 14 and 15 were isolated from the same reaction; combined yield was 37%.

This synthesis, shown in Scheme II, involved the use of a masked β-amino enone, in the form of the ethyl 5-phenylisoxazolylacetates **7** and **8**. The regiochemistry of this synthesis is known because **7** contains only one active methylene group compared to two for the ethyl benzoylacetoacetates used in the Hanztsch synthesis.

Preparation of **7** from ethyl nitropropionate and phenylacetylene by using Mukaiyama's procedure[20] (Scheme II, path a) gave only a 5% yield. The predominate product was ethyl acrylate produced by elimination of nitrous acid from ethyl 3-nitropropanoate. The regiochemistry of the cycloaddition was assigned by analogy to the reaction of the homologue ethyl 4-nitrobutanoate.[21] The isoxazoles were obtained in better yield by reaction of the diketo ester with hydroxylamine (Scheme II, path b). For **7**, the product obtained by this method was identical with that produced by cycloaddition. No isomeric products were observed. Addition of the lithium enolates of **7** or **8** to the benzylideneacetoacetate esters **9** and **10** produced the adducts **11–13** as mixtures of diastereomers. Treatment of the mixtures with molybdenum hexacarbonyl produced **3a**, **3f**, and **3n** as single products in good yield, presumably via the intermediate amino enones. The compounds obtained were identical with those obtained by the Hantzsch synthesis, and despite the increased number of steps, the overall yield was higher.

The related triester derivative, prepared by the Hantzsch synthesis, was isolated as both exocyclic, **14**, and endocyclic, **15**, double bond isomers in a ratio of 7:3, respectively. Treatment of **15** with camphorsulfonic acid in xylene rapidly caused isomerization to **14**. The reverse reaction, starting from pure **14**, resulted in an equilibrium mixture containing only a trace of **15**. In this series also, the 5-cyano analogue was isolated only as the 1,4-dihydropyridine isomer **16**.

Attempts to isomerize **3a** to the corresponding 1,4-dihydropyridine under similar conditions were unsuccessful. None of the 1,4-dihydropyridine derivative was obtained upon treatment of **3a** with camphorsulfonic acid in refluxing xylenes. Treatment with sodium ethoxide in ethanol caused destruction of the starting material.

Biological Results and Discussion

The analogues **3a–p** were evaluated for dihydropyridine receptor affinity in rat brain tissue and for cardiovascular activity in the isolated perfused rat heart. These results are summarized in Table II. Like nifedipine, most of the compounds inhibited binding of [^{3}H]nitrendipine to rat brain membranes. IC_{50} values ranged from 25 nM to >1000 nM, compared to 1.6 nM for nifedipine. No clear SAR were discernable for dihydropyridine receptor affinity in this series, although some similarities to the 1,4-dihydropyridine series were observed. Effect of substitution on the 4-phenyl group was similar to that observed in the 1,4-dihydropyridine series, in that compounds with ortho substituents retained the highest affinity (**3b**, **3o**, **3p**) but the unsubstituted derivative (**3n**) was less potent. Substitution at the 3- or 5-position of the 1,4-dihydropyridine ring with groups other than ester generally reduced receptor affinity (**3g–k**). Phenyl or 4-substituted phenyl groups in the side chain retained good to moderate receptor affinity (examples **3a–f**). However, a 2-substituent (**3m**)

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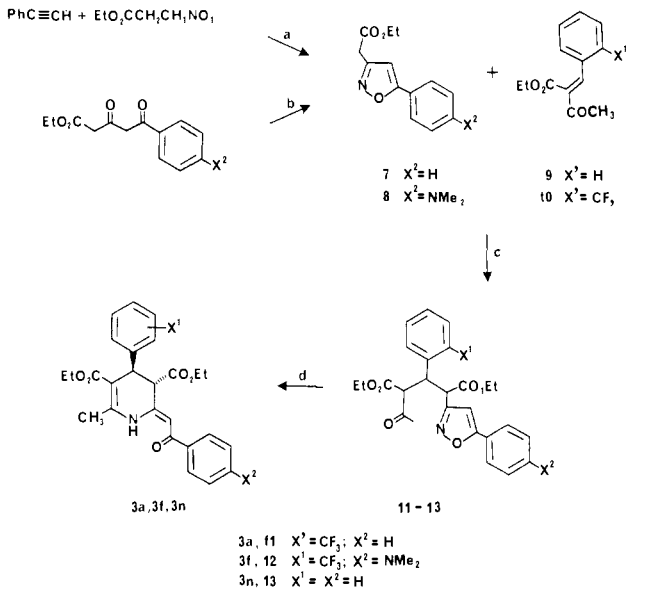
Scheme II[a]



[a] (a) Et_3N, $POCl_3$, $CHCl_3$, reflux, 18 h; (b) NH_2OH·HCl, C_5H_5N, reflux, 1 h; (c) LDA; (d) $Mo(CO)_6$, CH_3CN, H_2O, reflux, 23 h.

Table II. Receptor Affinity and Cardiovascular Activity

	isolated rat heart		
compd no.	coronary flow:[a] EC_{25}, nM[b]	LV dP/dt:[a] EC_{25}, nM[c]	inhibition of [^{3}H]nitrendipine binding: IC_{50} ± SE, nM[d] (*N*)
3a	<100	>3000	296.9 ± 18.2 (3)
3b	30	(>300)	25.5 ± 7.4 (3)
3c	<100	<100	162.5 ± 13.8 (3)
3d	300	>300	116.7 ± 22.3 (3)
3e	130	>300	413.7 ± 27.2 (3)
3f	200	>300	31.7 ± 2.8 (2)
3g	100	>300	335.8 ± 51.7 (3)
3h	NT[e]	NT	>1000 (2)
3i	380	1200	591.4 ± 67.3 (3)
3j	1400	1500	>1000 (2)
3k	1000	>1000	>1000 (2)
3l	NT	NT	>1000 (2)
3m	NT	NT	>1000 (2)
3n	200	500	912.8 ± 82.8 (2)
3o	200	>300	27.7 ± 6.7 (3)
3p	100	(2200)	31.1 ± 3.8 (3)
5	300	3000	1021 (980, 1063) (2)
14	<100	>300	346.9 ± 38.5 (3)
15	NT	NT	20.0 (18, 22) (2)
16	NT	NT	149.6 (129, 170) (2)
nifedipine, 1	10	(300)	1.65 ± .23 (3)

[a] Isolated, perfused rat heart preparation. A minimum of two hearts were used for each compound tested. Effects on heart rate were negligible in all cases except nifedipine. [b] Concentration producing a 25% increase in coronary flow. [c] Concentration producing a 25% increase (decrease) in LV dP/dt. [d] Index of dihydropyridine receptor affinity; IC_{50} for inhibition of [^{3}H]nitrendipine binding in rat brain. For active compounds tested less than three times, the range of determined values is given. [e] Not tested.

resulted in loss of receptor affinity. The derivatives containing additional rings (**3j–l**) were inactive.

To estimate the effect of moving the double bond from the 2,3-position to the exocyclic position, one can compare the relative activities of the triesters **14** and **15**. These derivatives were the only example of an active compound for which both isomers were isolated and both showed significant receptor affinity. The 1,4-dihydropyridine isomer **15** (IC_{50} = 20 nM) was 17 times more potent than the 2-oxoethylidene isomer **14** (IC_{50} = 347 nM), suggesting that the conformational effects of isomerization result in a substantial loss of receptor affinity. In the other case where both isomers were isolated (**3k**), the receptor affinities of both compounds were too low for comparison. The cyano derivative **5** was obtained only as the 1,4-dihydropyridine isomer and exhibited weak receptor affinity (IC_{50} = 1021 nM). This lower activity is consistent with other reports in which 3(5)-cyano-1,4-dihydropyridines are significantly less active than the corresponding diester derivatives.[8,22]

Table III. Inhibition of Potassium-Depolarized Rabbit Aorta[a]

compd	IC_{50} ± SE, μM (*N*)	compd	IC_{50} ± SE, μM (*N*)
3b	3.1 ± 0.8 (4)	**3p**	17 ± 1.7 (4)
3d	150 ± 82 (3)	nifedipine, 1	0.2 ± 0.2 (4)
3f	2.7 ± 0.5 (4)		

[a] None of the compounds tested significantly inhibited norepinephrine-induced contractions up to 10^{-4} M (*N* = 2).

In the isolated perfused rat heart, coronary flow increased dose dependently, whereas contractility and heart rate were affected significantly only at high concentrations. Calcium channel blocking activity was confirmed by observation of selective inhibition of potassium-induced contraction of vascular smooth muscle by several of the more potent derivatives (Table III). None of the compounds tested inhibited norepinephrine-induced contractions. Such selectivity is characteristic of calcium channel blockade.[23] In addition, the selectivity for vascular versus cardiac activity in the isolated rat heart is similar for both nifedipine and **3b**, for example. Approximately a 2 log unit separation in potency between coronary and cardiac activity was observed. Although a qualitative correlation exists between receptor affinity and increase in coronary flow, a precise correlation is difficult with this model due to variability in base-line flow rates among individual hearts. Other factors that may obscure the correlation include tissue differences among receptors (receptor affinity was determined in brain tissue) and/or differential accessibility of the compounds to receptors in various tissues. A further consideration is the relative activities of the individual enantiomers, which were not determined in our case. Nonetheless, relative binding affinity is predictive of vasodilator activity. For example, nifedipine, which has an IC_{50} for the dihydropyridine receptor of 1.6 nM, produced a 25% increase in flow at 10 nM. A similar ratio of IC_{50} to EC_{25} was observed for most of the derivatives evaluated. Those analogues that had IC_{50}'s greater than 1 μM were only weakly active or inactive as vasodilators. A 5-nitro group, as in **2**, has been associated with calcium channel stimulation. In this series, however, **3g** retained moderate binding affinity, and its profile in the isolated heart was consistent with calcium channel blockade rather than stimulation.

The stability of the isomers relative to the corresponding 1,4-dihydropyridines is surprising given the large number and diversity of known 1,4-dihydropyridines. In this series, relief of steric congestion of the 3,5-diester and 4-aryl substituents and the stabilization due to conjugation of the double bond with the side-chain carbonyl combine to favor the exocyclic isomer. Support for the influence of steric factors includes the observation that the 5-cyano analogues **3k** and **5** were isolated as the 1,4-dihydropyridine isomers, presumably due to the less sterically demanding cyano group. In the triester example, the reduced basicity of the ester carbonyl relative to the benzoyl

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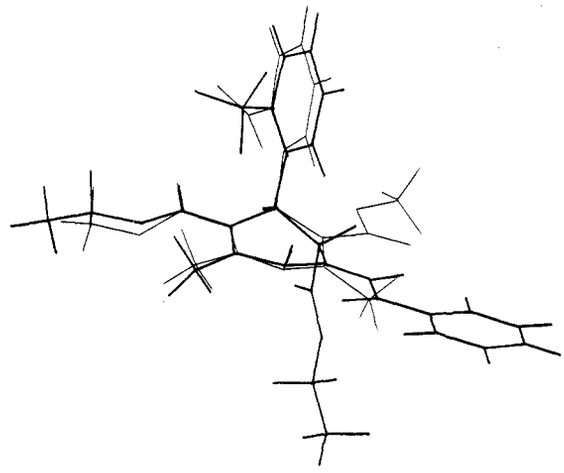

Figure 1. Superposition of **3a** (bold lines) with nifedipine (1, light lines).

carbonyl, which would affect the strength of the intramolecular hydrogen bond, and/or a lesser degree of conjugation may explain the equilibrium observed.

Molecular modeling of **3a** results in the minimized conformation illustrated in Figure 1, which is shown superposed on **1**. The 3-ester and 4-aryl substituents are found in a trans pseudodiaxial orientation. Overlap of **3a** and **1** is good for the dihydropyridine ring and all substituents except the 3-ester. This group extends below the plane of the dihydropyridine ring and thus may not participate in binding to the receptor accounting for the reduced potency, at least in part.

In summary, the title compounds are stable positional double bond isomers of the 1,4-dihydropyridine calcium channel blockers. Many of the analogues exhibited significant affinity for the dihydropyridine receptor. Calcium channel blocking activity was confirmed by the ability of these agents to selectively inhibit potassium-induced contraction of vascular smooth muscle and to produce vasodilation in the isolated rat heart. The potency of the most active derivatives is approximately one-fifteenth that of nifedipine for both receptor affinity and vasodilation.

Experimental Section

Chemistry. Microanalyses were within ±0.4% of the calculated values for the specified elements, unless indicated and were performed by the Parke-Davis Analytical Chemistry Section. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. 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 FT IR spectrometer 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. Flash chromatography refers to the method of Still et al.[24] Preparative MPLC employed Michel–Miller columns packed with 230–400 mesh silica gel. Organic extracts were dried over $MgSO_4$. Dry THF was obtained by distillation from sodium and benzophenone.

The following is a representative example of the synthesis of the title compounds.

(2*Z*,3α,4β)-(±)-Diethyl 1,2,3,4-Tetrahydro-6-methyl-2-(2-oxo-2-phenylethylidene)-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (3a). Via Hantzsch Synthesis. A solution of ethyl β,δ-dioxobenzenepentanoate (2.88 g, 12.3 mmol), 2-(trifluoromethyl)benzaldehyde (2.14 g, 12.3 mmol), and ethyl 3-amino-2-butenoate (1.6 g, 12.3 mmol) in 50 mL of ethanol was heated at reflux for 18 h and then concentrated to a yellow oil. This oil was then separated on a 51 × 450 mm Michel–Miller double-taper high-performance medium-pressure liquid chromatography (MPLC) system, eluting with dichloromethane/ethyl acetate (98/2) and collecting 20-mL fractions. The product appeared in fractions 26–35 after the solvent front. These fractions were combined and evaporated to a yellow oil, which was recrystallized from 2,2,4-trimethylpentane to yield 0.48 g (8%): mp 111–112 °C; IR 3000, 1740, 1692, 1643 cm^{-1}; NMR δ 11.90 (s, 1 H), 7.87–7.11 (m 9 H), 5.89 (s, 1 H), 5.06 (s, 1 H), 4.22 (d of q, J = 7.1, 2.56 Hz, 2 H), 4.03 (q, J = 7.26 Hz, 2 H), 3.42 (s, 1 H), 2.61 (s, 3 H), 1.27 (t, J = 7.1 Hz, 3 H), 1.09 (t, J = 7.2 Hz, 3 H); MS, m/z (intensity) 501 (7) [MI], 456 (4.4), 428 (88.4), 400 (3.9), 382 (11.1), 356 (10), 105 (100), 77 (41.1).

3a via Isoxazole. The isoxazole adduct 11 (2.5 g, 4.8 mmol) was dissolved in 20 mL of acetonitrile and 2 mL of water. Molybdenum hexacarbonyl (0.66 g, 2.5 mmol) was added, and the mixture was heated to reflux. The mixture quickly became very dark and opaque. After 20 h, a small amount of starting material remained, so more molybdenum hexacarbonyl (200 mg) was added and reflux continued for 3 h. The mixture was filtered through a pad of Celite, which was washed with fresh acetonitrile. The filtrate and washings were concentrated to 3.5 g of a brown oil, which was purified by flash chromatography (200 g silica gel, 20 to 30% ether in hexane) to give 2.1 g (88%) of a light yellow solid identical with **3a** obtained through the Hantzsch synthesis.

Diethyl 2-Acetyl-4-(5-phenyl-3-isoxazolyl)-3-[2-(trifluoromethyl)phenyl]pentanedioate (11). Lithium diisopropylamide was prepared in 10 mL dry THF containing a few crystals of 2,2′-dipyridyl as indicator at 0 °C from 1.1 g (11 mmol) of diisopropylamine and 4.8 mL of 2.1 M (10 mmol) n-butyllithium. The deep red solution was cooled to −78 °C under N_2, and 2.3 g (10 mmol) of **7** was added dropwise as a solution in 4 mL of THF. After 20 min, a solution containing 2.9 g (10 mmol) of ethyl 3-oxo-2-[[2-(trifluoromethyl)phenyl]methylene]butanoate, **10**, in 2 mL of THF was added dropwise. The color discharged with the first few drops. Upon completion of the addition, the solution was allowed to warm to room temperature and then quenched with saturated aqueous ammonium chloride and extracted with ether. The ether solution was dried ($MgSO_4$) and concentrated to yield 5.1 g of a yellow gum, which was purified on a 200-g flash chromatography column, eluting with 20–30% ether in pentane to yield 3.1 g (61%) of **11** and 1.5 g of a mixture of the starting materials. Anal. ($C_{27}H_{26}F_3NO_5$) C, H, N.

Ethyl 5-Phenyl-3-isoxazoleacetate (7). To 100 mL of pyridine was added 4.38 g (63 mmol) of hydroxylamine hydrochloride and 14.5 g (62 mmol) of ethyl β,δ-dioxobenzenepentanoate. The mixture was stirred at room temperature for 20 min and then heated to reflux. After 1 h the solution was concentrated in vacuo to yield a dark oil, which was partitioned between ether and 1 N HCl. The aqueous solution was extracted twice more with ether. The organic extracts were combined, dried, and concentrated to 13.5 g of a dark oil. Flash chromatography (120 g of silica gel, 10% ethyl acetate in pentane) produced the product as a pale yellow oil, 8.5 g (59%). The oil was pure by TLC; however, it could be crystallized from 10:1 isooctane/ether to give 6.9 g of white needles: mp 51.5–53 °C; NMR δ 7.78–7.75 (m, 2 H), 7.47–7.41 (m, 3 H), 6.60 (s, 1 H), 4.23 (q, J = 6.9 Hz, 2 H), 3.77 (s, 1 H), 1.29 (t, J = 6.9 Hz, 3 H). Anal. ($C_{13}H_{13}NO_3$) C, H, N.

7 via Cycloaddition.[20] All reagents were freshly distilled. In 30 mL of chloroform were combined 4.5 g (31 mmol) of ethyl 3-nitropropanoate, 3.1 g (31 mmol) of phenylacetylene, and 15 mL of triethylamine. Phosphorous oxychloride (4.9 g, 32 mmol) was diluted with 10 mL of chloroform and added slowly. The solution became orange and warmed slightly. An ice bath was applied, and addition was continued. The mixture became very dark about 15 min following the addition. The solution was warmed to reflux and heated overnight, cooled, poured into 100 mL of ice/bicarbonate solution, and extracted with chloroform. The extract was dried and concentrated to yield a brown residue, which was purified by flash chromatography to yield 0.36 g of **7** (5.1%) identical by NMR with the material obtained above.

(2*Z*,3α,4β)-(±)-Diethyl 2-(2-Ethoxy-2-oxoethylidene)-1,2,3,4-tetrahydro-6-methyl-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (14). Diethyl 2-(2-Ethoxy-2-oxoethyl)-1,4-dihydro-6-methyl-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (15). A solution containing 4.0 g (20 mmol) of diethyl 3-amino-2-pentene-1,5-dioate and 5.7

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g (20 mmol) of ethyl 3-oxo-2-[[2-(trifluoromethyl)phenyl]-methylene]butanoate in 100 mL of absolute ethanol was heated at reflux under nitrogen for 36 h. The yellow solution was cooled and concentrated to yield a bright yellow glass (9.5 g). Analytical HPLC showed this material to be a complex mixture of at least 15 components. A portion of the mixture (7.05 g) was separated by medium-pressure liquid chromatography with a 51 × 450 mm Michel-Miller column containing 200–430-mesh silica gel and eluting with 5% ethyl acetate in methanol; 20-mL fractions were collected. Fractions 1–44 contained 2.21 g (31.4%) of an equimolar mixture of the starting benzylidene isomers. Fractions 45–60 upon concentration produced 1.0 g (14.2%) of **14**, which was crystallized from pentane (80% recovery in one crop) to give a white crystalline solid: mp 98–99 °C; IR 2988, 1744, 1692, 1665, 1655, 1610 cm^{-1}; NMR δ 9.93 (br s, 1 H), 7.67–7.04 (m, 4 H), 4.98 (s, 1 H), 4.70 (s, 1 H), 4.07 (overlapping q, 6 H), 3.24 (s, 1 H), 2.52 (s, 3 H), 1.27–1.18 (overlapping triplets, 6 H), 1.05 (t, J = 7.2 Hz, 3 H); MS, m/z (intensity) 469 (15.2), 424 (10.1), 397 (20.0), 396 (100.0), 350 (31.9), 322 (16.0), 304 (21.2). Fractions 61–104 contained 0.94 g of a mixture of unidentified products. Fractions 105–145 contained 0.45 g (6.4%) of diethyl 3-aminopentanedioate. Fractions 176–190 contained 0.09 g (1.3%) of diethyl 1,4-dihydro-2,6-dimethyl-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate. Fractions 210–285 contained 0.18 g of unidentified products. Fractions 286–365 contained 0.79 g (11.2%) of **15** as a yellow glass: NMR δ 7.61–7.22 (m, 4 H), 6.72 (s, 1 H), 5.60 (s, 1 H), 4.23–3.94 (overlapping q, 6 H), 3.92 (d, J = 14.5 Hz, 1 H), 3.78 (d, J = 14.5 Hz, 1 H), 2.30 (s, 3 H), 1.60–1.11 (overlapping t, 9 H). The column was then stripped with ethyl acetate, and the eluate was concentrated to yield 0.36 g of a yellow glass. This material was combined with mixed fractions obtained above and rechromatographed by using a similar system. In this way an additional 0.8 g (11.3%) of **14** was obtained. Total yield of **14** was 25.5%. Total mass recovery from the first column was 6.02 g (85%).

14 by Isomerization of 15. To 100 mL of xylenes was added 0.72 g of **15** and 0.1 g of camphorsulfonic acid. The mixture was heated at reflux for 1 h, during which the starting material was consumed and a new spot coeluting with **14**, prepared independently, was formed. No other products were detected by TLC. The mixture was concentrated to a crystallizing oil. Crystallization and then recrystallization from pentane gave 0.34 g (47%) of a white solid identical with **14**.

Ethyl 5-Cyano-3-(ethoxycarbonyl)-1,4-dihydro-6-methyl-4-[2-(trifluoromethyl)phenyl]-2-pyridineacetate (16). To 200 mL of absolute ethanol was added 20 g (0.1 mol) of diethyl acetonedicarboxylate, 17.4 g (0.1 mol) of 2-(trifluoromethyl)-benzaldehyde, and 8.2 g (0.1 mol) of 3-aminocrotonitrile. The solution was heated at reflux for 48 h and then concentrated. Crystallization of the residue from ether/isooctane (2:1) gave 23.2 g (55%) of crude material. Recrystallization from 2-propanol produced an off-white solid, 9.6 g (23%): mp 144–146 °C; NMR δ 7.65–7.54 (m, 3 H), 7.37–7.33 (m, 1 H), 7.08 (br, 1 H), 5.13 (s, 1 H), 4.22 (q, J = 7.2 Hz, 2 H), 4.06 (d, J = 17.5 Hz, 1 H), 3.96 (q, J = 7.1 Hz, 2 H), 3.68 (d, J = 17.5 Hz, 1 H), 2.08 (s, 3 H), 1.30 (t, J = 7.2 Hz, 3 H), 0.96 (t, J = 7.1 Hz, 3 H).

Biological Testing. [^{3}H]Nitrendipine Binding Assay. Male Long–Evans rats (160–225 g) were obtained from Blue Spruce Laboratories, Altamont, NY. [^{3}H]Nitrendipine (specific activity 71.6 Ci/mmol) was obtained from New England Nuclear. The preparation of tissue and binding of [^{3}H]nitrendipine to receptor sites associated with slow calcium channels of rat cerebral cortex were performed essentially as described by Ehlert et al.[25] and Gould et al.[26] Briefly, aliquots (200 L) of the tissue homogenate were suspended in 50 mM Tris·HCl (pH 7.7, 25 °C), and the suspension was incubated with [^{3}H]nitrendipine (final concentration 0.1–0.22 nM) in a total volume of 2 mL in the presence or absence of test drugs for 90 min at 25 °C in the dark with constant shaking. Binding was terminated by rapid vacuum filtration (Cell Harvester Brandel, M-48R) over Whatman GF/B glass fiber filters with two 4-mL rinses of cold (4 °C) buffer. The filter containing the membrane-bound [^{3}H]nitrendipine was placed in a vial containing 8 mL of Ready-Solve MP (Beckman), and after the mixture was allowed to stand overnight, the radioactive content was determined by liquid scintillation spectrometry. Nonspecific binding was measured in incubations containing 10^{-6} M nifedipine. Specific binding was the difference between total and nonspecific binding.

Langendorff Rat Heart Preparation. Compounds were evaluated for effects on contractility, coronary flow, and heart rate. Male rats (400–600 g) were anesthetized with sodium pentobarbital (50 mg/kg, ip) and heparinized (2000 units, ip) to prevent blood clotting. Hearts were rapidly excised, and the ascending aorta was fitted to a perfusion cannula and secured with a ligature. The coronary arteries were perfused with a modified Krebs Henseleit bicarbonate buffer initially at a rate of about 15 mL/min for 2–3 min, after which they were perfused at a constant 70 mmHg pressure at 37 °C. Perfusion pressure was maintained constant, drug delivery was controlled by a microcomputer servo mechanism, and the hearts were allowed to beat spontaneously. The electrocardiogram (ECG) was recorded with use of two platinum electrodes positioned at the base and apex of the left ventricle. Heart rate was determined from the ECG. Left intraventricular pressure was measured with a Millar Instruments Micro-Tip pressure transducer (4F) positioned via the left atria through the mitral valve. The maximum first derivative of intraventricular pressure (dP/dt_{max}) was used as an index of contractility. Coronary sinus effluent was used to measure coronary flow. Data were digitized and averaged with a microcomputer (Buxco) data analyzer. Effects of the test agents on contractility, coronary flow, and heart rate were calculated as percent change from control and are reported as the concentration that produced a 25% change from control (EC_{25}) determined graphically from the results in a minimum of two hearts.[27]

Inhibition of Potassium-Induced Contraction of Rabbit Aorta. Compounds were tested by employing the procedure outlined previously.[28] Two rings were used in parallel. Results are summarized in Table III.

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Registry No. **3a**, 114978-18-6; **3b**, 114978-19-7; **3c**, 114957-59-4; **3d**, 114957-60-7; **3e**, 114957-61-8; **3f**, 114957-62-9; **3g**, 114957-63-0; **3h**, 114957-64-1; **3i**, 114957-65-2; **3j**, 114957-66-3; **3k**, 114957-67-4; **3l**, 114957-68-5; **3m**, 114957-69-6; **3n**, 114957-70-9; **3o**, 114957-71-0; **3p**, 114957-72-1; **5**, 114957-73-2; **7**, 3929-70-2; **8**, 114957-74-3; **9**, 15802-63-8; **10**, 114957-75-4; **11**, 114957-76-5; **12**, 114978-33-5; **13**, 114957-77-6; **14**, 115015-34-4; **15**, 114957-78-7; **16**, 114978-34-6; *p*-$ClC_6H_4COCH_2COCH_2CO_2Et$, 114957-79-8; *p*-$FC_6H_4COCH_2COCH_2CO_2Et$, 114957-80-1; *p*-$MeC_6H_4COCH_2COCH_2CO_2Et$, 114957-81-2; *p*-$Me_2NC_6H_4COCH_2COCH_2CO_2Et$, 114957-82-3; $(PhCOCH_2)_2CO$, 1467-40-9; o-$ClC_6H_4COCH_2COCH_2CO_2Et$, 114957-83-4; ethyl 3-nitropropanoate, 3590-37-2; phenylacetylene, 536-74-3; ethyl 3-amino-2-butenoate, 7318-00-5; 3-aminocyclohex-2-enone, 5220-49-5; 3-aminocrotonitrile, 1118-61-2; 2-(trifluoromethyl)-benzaldehyde, 447-61-0; 2,3-dichlorobenzaldehyde, 6334-18-5; ethyl β,δ-dioxobenzenepentanoate, 86969-12-2; ethyl β,δ-dioxo-4-pyridinepentanoate, 114957-84-5; ethyl 1,β-dioxo-1,2,3,4-tetrahydronaphthalene-2-propionate, 114957-85-6; diethyl 3-amino-2-pentene-1,5-dioate, 54889-50-8; ethyl 3-oxo-2-[[2-(trifluoromethyl)phenyl]methylene]butanoate, 39561-91-6; diethyl 1,4-dihydro-2,6-dimethyl-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate, 23191-75-5; diethyl acetonedicarboxylate, 105-50-0; 2-nitrobenzaldehyde, 552-89-6; ethyl acrylate, 140-88-5.

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