Syntheses, Calcium Channel Agonist–Antagonist Modulation Activities, and Voltage-Clamp Studies of Isopropyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylate Racemates and Enantiomers†

Dean Vo,‡ Wandikayi C. Matowe,‡ Manian Ramesh,‡ Nadeem Iqbal,‡ Michael W. Wolowyk,‡,§ Susan E. Howlett,⊥ and Edward E. Knaus*,‡

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8, and Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

Received March 7, 1995®

A novel group of racemic isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylate isomers [(±)-**12**–**14**] were prepared using a modified Hantzsch reaction that involved the condensation of nitroacetone with isopropyl 3-aminocrotonate and 2-, 3-, or 4-pyridinecarboxaldehyde. Determination of their *in vitro* calcium channel-modulating activities using guinea pig ileum longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA) assays showed that the 2-pyridinyl isomer (±)-**12** acted as a *dual cardioselective calcium channel agonist (GPLA)/smooth muscle selective calcium channel antagonist (GPILSM).* In contrast, the 3-pyridinyl [(±)-**13**] and 4-pyridinyl [(±)-**14**] isomers acted as calcium channel agonists on both GPLA and GPILSM. The agonist effect exhibited by (±)-**12** on GPLA was inhibited by nifedipine and partially reversed by addition of extracellular Ca^{2+}. In anesthetized rabbits, the 4-pyridinyl isomer (±)-**14** exhibited a hypertensive effect that was qualitatively similar to that exhibited by the nonselective agonist Bay K 8644 and the 3-pyridinyl isomer (±)-**13**, whereas the 2-pyridinyl isomer (±)-**12** induced a hypotensive effect similar to that of the calcium channel antagonist nifedipine. Similar results were obtained in a spontaneously hypertensive rat model. *In vitro* studies showed that the (+)-2-pyridinyl enantiomer (+)-**12A** exhibited agonist activity on both GPILSM and GPLA, but that the (−)-2-pyridinyl enantiomer (−)-**12B** exhibited agonist activity on GPLA and antagonist activity on GPILSM. Whole-cell voltage-clamp studies using isolated guinea pig ventricular myocytes indicated that (−)-**12B** inhibited the calcium current (I_{Ca}), that (+)-**12A** increased slightly I_{Ca}, and that (±)-**12** inhibited I_{Ca} but the latter inhibition was less than that for (−)-**12B**. (−)-**12B** effectively inhibited I_{Ca} at all membrane potentials examined (−40–50 mV), whereas (+)-**12A** exhibited a weak agonist effect near the peak of the I–V curve. The 2-pyridinyl isomers (enantiomers) **12** represent a novel type of 1,4-dihydropyridine calcium channel modulator that could provide a potentially new approach to drug discovery targeted toward the treatment of congestive heart failure and probes to study the structure–function relationships of calcium channels.

The design of cardioselective 1,4-dihydropyridine (DHP) calcium channel agonists (positive inotropes), that act at the L-type voltage dependent calcium channel, has provided a significant challenge to medicinal chemists.[1–4] A number of 1,4-dihydropyridine calcium channel agonists such as the nitro compounds Bay K 8644 (**1**),[5] PN 202–791 [**2**, (+)-*S*-enantiomer],[6] and LY249933 (**3**, *R,R*-diastereomer),[7] lactone CGP 28392 (**4**),[8] 5-unsubstituted H 160/51 (**5**),[9,10] amide YC 170 (**6**),[11,12] dicarboxylic ester **7**,[13] and benzoylpyrrole FPL 64176 (**8**)[14,15] have been reported (see Figure 1). Unfortunately, calcium channel agonists such as Bay K 8644 also increase calcium entry into vascular smooth muscle, causing vasoconstriction, which precludes their clinical use in treating congestive heart failure (CHF).[16–21]

Although extensive efforts have been undertaken to understand the molecular basis of action and improve the pharmacological profile of calcium channel agonists, to the best of our knowledge, no cardioselective calcium channel stimulant has been reported to date.[22] The future clinical use of calcium channel agonists to treat CHF will therefore be dependent upon separating their vasoconstricting effects from their cardiostimulant properties.[23] The individual enantiomers of racemic 1,4-dihydropyridine calcium channel agonists **1**–**7** often exert diametrically opposite effects or have vastly differing potencies on the same tissue. For example, Bay K 8644 is a racemate consisting of a (−)-*S*-enantiomer that acts as an agonist which is about 10-fold more potent as an activator than its (+)-*R*-antipode is as an antagonist.[24,25] Consequently, the racemate acts as a calcium channel agonist.[5,26] The two enantiomers of PN 202-791 (**2**) show a 100-fold difference in activities favoring the (−)-*R*-antagonist, relative to the (+)-*S*-agonist enantiomer.[24] The *R,R*- and *S,R*-diastereomers of LY249933 (**3**) exhibit calcium agonist and antagonist effects, respectively.[7] Our goal was to discover a dual cardioselective calcium channel agonist/vascular selective smooth muscle calcium channel antagonist *third-generation* DHP drug which would have a suitable therapeutic profile for treating CHF patients. In this report, the synthesis of racemic and chiral 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylates, their *in vitro* calcium channel-modulating activities on guinea pig smooth muscle and heart, their cardiovascular effects on rabbit and spontaneously

† Dedicated to the memory of Professor Michael W. Wolowyk.
‡ University of Alberta.
§ Deceased January 31, 1992.
⊥ Dalhousie University.
® Abstract published in *Advance ACS Abstracts,* June 15, 1995.

0022-2623/95/1838-2851$09.00/0

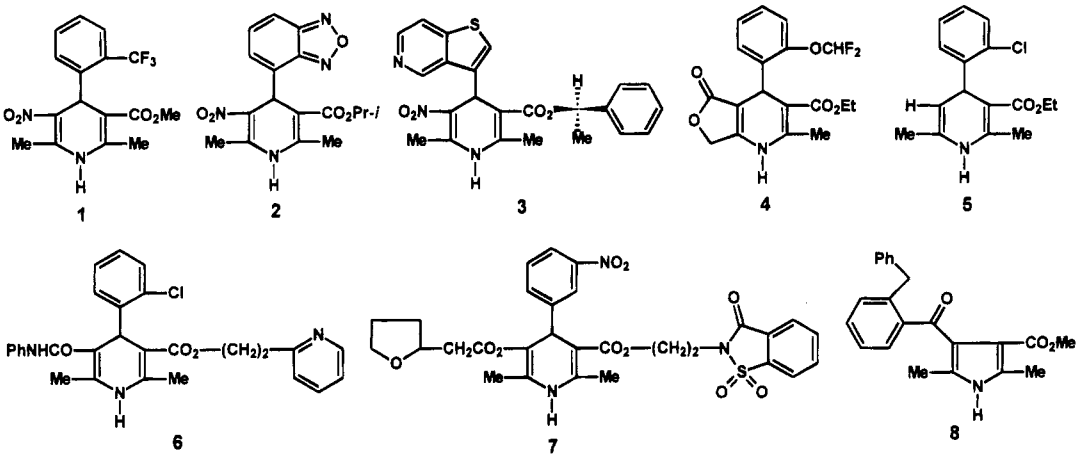
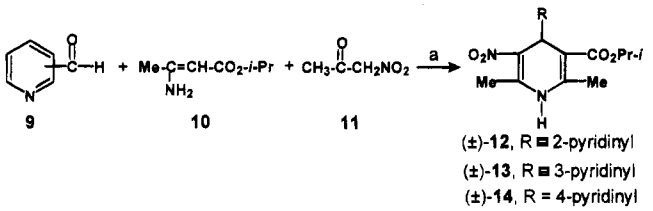


Figure 1. Some representative calcium channel agonists.

Scheme 1[a]



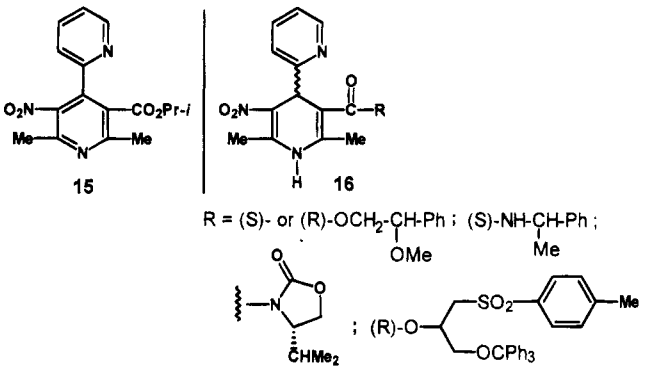
[a] Reagent and conditions: (a) EtOH, reflux, 12 h.

hypertensive rat, and their modulation of L-type voltage sensitive calcium channels in isolated guinea pig ventricular myocytes using the whole-cell voltage-clamp technique are described.

Chemistry

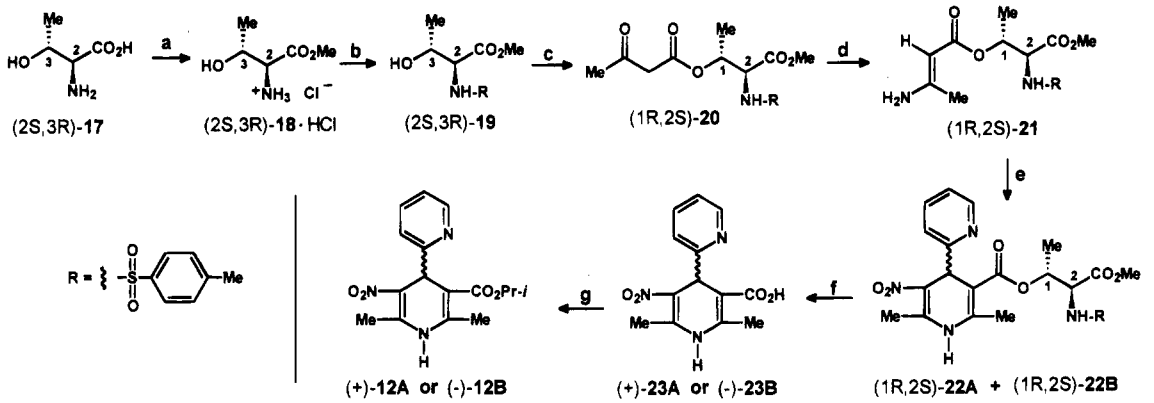
The racemic isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylates **12**–**14** were prepared by a modified Hantzsch reaction. Thus, condensation of nitroacetone **11** with isopropyl 3-aminocrotonate (**10**) and either 2-, 3-, or 4-pyridinecarboxaldehyde (**9**) afforded the title compounds in 64–79% yields as illustrated in Scheme 1. The symmetrical 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-pyridinylpyridine-3,5-dicarboxylate product, resulting from condensation of **9** with **10**, was present as a minor product (<10%) that was readily removed by silica gel column chromatography.

Since the isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylates **12**–**14** are racemic, and stereospecificity of DHPs has been established previously,[5,7,24–26] synthesis and testing of the individual enantiomers of the most promising 4-(2-pyridinyl) compound, **12**, was highly desirable. Attempts to effect the enantiospecific enzymatic hydrolysis of racemic **12** using α-chymotrypsin (type II from bovine pancreas)[27] or lipase (pseudomonas type XIII, E.C. 3.1.1.3)[28] were unsuccessful since no hydrolysis occurred, presumably due to conjugation of the ester moiety with the enamine system. The stereoselective reduction of the parent pyridine compound **15**, using the chiral reducing agent (−)-diisocamphenylchloroborane,[29] was unsuccessful since no reduction occurred. In addition the diastereomers **16** could not be separated by silica gel column chromatography. Attempts to synthesize the enantiomers of **12** using chiral auxiliaries such as (*S*)- or (*R*)-1-amino-2-(dimethylmethoxymethyl)pyrrolidine using modified procedures based on studies reported by Enders *et al.*[30] or D- and L-valine as reported by Iqbal *et al.*[31] for the efficient synthesis of chiral dialkyl 1,4-dihydropyridine-3,5-dicarboxylates afforded racemic **12** (0% ee). Although the two diastereomeric L-menthol esters of **16** could be separated, no reaction occurred upon attempted hydrolysis to the chiral acids (+)-**23A** or (−)-**23B** using Me_3SiI, Me_3SiCl, or NaOH. The use of a chiral esterifying group such as L-threonine, which could be readily removed by a β-elimination reaction using a non-nucleophilic base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), was found to be a suitable derivative for separation of the diastereomeric esters that could be converted to the chiral acids [(+)-**23A** or (−)-**23B**].[32]



The reaction of methyl (2*S*,3*R*)-2-amino-3-hydroxybutanoate hydrochloride [(2*S*,3*R*)-**18**], prepared by esterification of L-threonine [(2*S*,3*R*)-**17**] with thionyl chloride and methanol, with *p*-toluenesulfonyl chloride afforded the 4-tolylsulfamoyl derivative (2*S*,3*R*)-**19** in 72% yield (see Scheme 2). Reaction of the alcohol (2*S*,3*R*)-**19** with diketene, using 4-(dimethylamino)pyridine (DMAP) as catalyst, gave the acetoacetate ester (1*R*,2*S*)-**20** in 98% yield. Elaboration of (1*R*,2*S*)-**20** to the β-aminocrotonate derivative (1*R*,2*S*)-**21** by treatment with ammonia and its subsequent modified Hantzsch condensation with 2-pyridinecarboxaldehyde and nitroacetone afforded a mixture of the 1,4-dihydropyridine diastereomers (1*R*,2*S*)-**22A** (11.8%) and (1*R*,2*S*)-**22B** (10.5%). These two diastereomers, which differ in configuration (*R* or *S*) at the C-4 position of the 1,4-dihydropyridine ring, were separated by silica gel column chromatography. Cleavage of the individual (1*R*,2*S*)-**22A** and (1*R*,2*S*)-**22B** esters using DBU in MeOH at 25 °C afforded the individual (+)-**23A** and (−)-**23B** 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridinyl)pyridine-5-carboxylate enan-

Scheme 2[a]



[a] Reagents: (a) $SOCl_2$, MeOH; (b) K_2CO_3, 4-Me-C_6H_4-SO_2Cl, EtOAc–H_2O; (c) diketene, dry THF, 4-(dimethylamino)pyridine; (d) 4-Me-C_6H_4-SO_3H, dry toluene, NH_3; (e) 2-pyridinecarboxaldehyde, $CH_3COCH_2NO_2$, EtOH; (f) DBU, MeOH, and then HCl; (g) isopropyl bromide, K_2CO_3, DMF.

tiomers, respectively, both in 50% yield. Reaction of (+)-**23A** or (−)-**23B** with isopropyl bromide in the presence of K_2CO_3 in DMF at 25 °C for 16 h afforded the respective (+)-**12A** or (−)-**12B** enantiomer of isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridinyl)pyridine-5-carboxylate, both in 69% yield. The two enantiomers (+)-**12A** and (−)-**12B** each exhibited single resonances for the dihydropyridine C-2 and C-6 methyl resonances upon addition of the ^1H NMR chiral shift reagent (+)-Eu(hfc)$_3$, indicating a very high optical purity (≥96% ee). The absolute configurations of (+)-**12A** and (−)-**12B**, which have not been determined, would require the acquisition of an X-ray crystal structure for one enantiomer for assignment of the absolute configuration.

Results and Discussion

The design of calcium channel agents that are suitable for the treatment of CHF will be dependent upon the separation and/or elimination of their vasoconstricting effect from their positive inotropic cardiostimulant property.[23] The significance of binding with respect to the C-3 and C-5 DHP regions has been noted for differences in the molecular electrostatic potentials between activator and antagonist structures that may constitute a mechanism whereby the receptor distinguishes between activator and antagonist ligands. Accordingly, calcium channel activators were reported to exert a strong negative potential in the region adjacent to their C-3 nitro substituent, whereas antagonists exhibited a positive potential in this region when a C-3 ester group was present.[33] The role of aromatic substituents in the 4-phenyl ring of 1,4-dihydropyridine agonists and antagonists is different.[34] These observations prompted us to investigate analogs of Bay K 8644 (**1**) where the 4-[2-(trifluoromethyl)phenyl] substituent is replaced by C-4 2-pyridinyl, 3-pyridinyl, or 4-pyridinyl substituent. It was anticipated that the position of the pyridinyl nitrogen and its ability to act as an additional electron donor for hydrogen bonding to the calcium channel receptor may offer an approach to modulate calcium channel binding and/or tissue specificity.

The *in vitro* calcium channel-modulating activities of the racemic 2-pyridinyl [(±)-**12**], 3-pyridinyl [(±)-**13**], and 4-pyridinyl [(±)-**14**] isomers were determined using guinea pig ileum longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA). The 2-pyridinyl isomer (±)-**12** acted as a calcium channel antagonist

Figure 2. Response of guinea pig left atrium to (±)-**12** (●, 6.39×10^{-6} M) followed by the cumulative addition of nifedipine (▼). The inhibition by nifedipine was partially reversed by addition of 2.0 mM Ca^{2+} (open arrow).

[$IC_{50} = (4.87 \pm 2.06) \times 10^{-6}$ M, $n = 3$] on GPILSM, whereas the 3-pyridinyl [(±)-**13**; $EC_{50} = 4.8 \times 10^{-5}$ M, $n = 2$] and 4-pyridinyl [(±)-**14**; $EC_{50} = 3.5 \times 10^{-5}$ M, $n = 1$] isomers acted as calcium channel agonists. The 2-pyridinyl [$EC_{50} = (9.67 \pm 1.27) \times 10^{-6}$ M, $n = 3$], 3-pyridinyl [$EC_{50} = (2.85 \pm 0.20) \times 10^{-5}$ M, $n = 3$], and 4-pyridinyl [$EC_{50} = (8.05 \pm 2.14) \times 10^{-6}$ M, $n = 3$] racemates all acted as agonists on GPLA. The EC_{50} value for agonist activity is the molar concentration eliciting 50% of the maximum contractile response produced by the test drug on the tissue (GPILSM or GPLA), as determined graphically from dose–response curves. The agonist activities for the reference drug (±)-Bay K 8644 on GPILSM and GPLA were $(2.30 \pm 0.05) \times 10^{-7}$ and $(7.70 \pm 5.90) \times 10^{-7}$ M ($n = 3$), respectively. The interesting *in vitro* pharmacological profile of (±)-**12**, which included agonist activity on GPLA and antagonist activity on GPILSM, at similar concentrations, warranted further study. It was found that the positive inotropic effect of (±)-**12** on GPLA was inhibited by nifedipine and that addition of extracellular Ca^{2+} partially reversed this inhibition, which confirms the involvement of a L-type calcium channel (see Figure 2).

The *in vivo* hypertensive or hypotensive effect of racemic Bay K 8644, (±)-**12**–**14**, and nifedipine was determined in the anesthetized male rabbit to determine their acute hemodynamic effects and confirm the observed spectrum of pharmacological activities obtained in the *in vitro* studies (see Figure 3). The hypertensive effect of the 4-pyridinyl isomer (±)-**14** was qualitatively similar to that of racemic Bay K 8644 and the 3-pyridinyl isomer (±)-**13** after β-blockade with propranolol (1.0 mg/kg). All three compounds produced transient increases in arterial pressure whose intensity and duration of action were dose dependent. At the concentrations used, the hypertensive effects lasted up to 8 min. In contrast, (±)-**12** exhibited a hypotensive effect indicative of a peripheral vasodilation action which is in

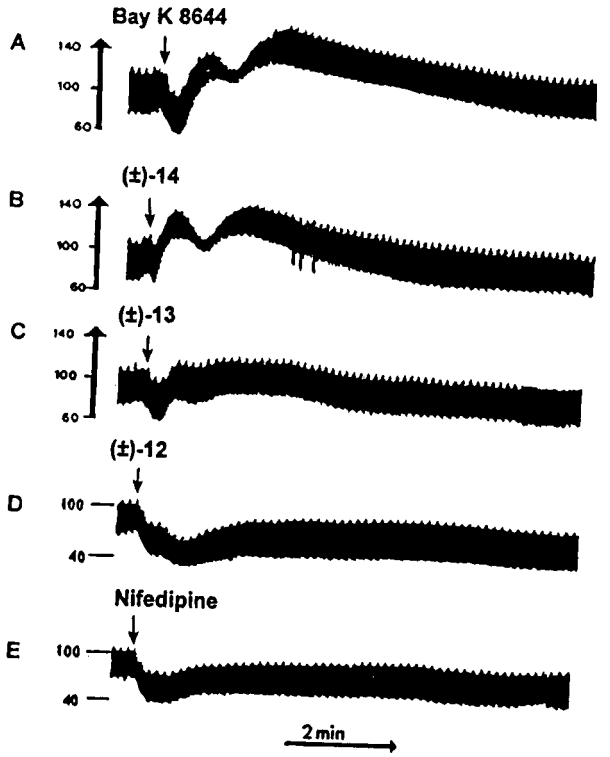


Figure 3. Hypertensive effects of racemic Bay K 8644 (30 μg/kg, panel A), (±)-**14** (300 μg/kg, panel B), and (±)-**13** (300 μg/kg, panel C) and antihypertensive effects of (±)-**12** (30 mg/kg, panel D) and nifedipine (3 mg/kg, panel E) on rabbit aortic blood pressure after the animal was β-blocked with propranolol (1.0 mg/kg). Drugs were administered via a catheter in the jugular vein.

agreement with the effect observed *in vitro* using GPILSM. A single dose of (±)-**12** (30 mg/kg, jugular vein injection) produced an immediate decrease in blood pressure (35 mmHg systolic and 40 mmHg diastolic) which recovered rapidly (*ca.* 30%) within 30 s and then gradually recovered to control levels after *ca.* 6 min. This response was qualitatively similar to the effect produced by the 1,4-dihydropyridine calcium channel antagonist nifedipine. (±)-**12** did not produce any changes in heart rate or EKG pattern even when the animal was not β-blocked (data not shown). Unfortunately, we were not equipped to measure left ventricular pressure development in this study to ascertain that (±)-**12** did increase contractility.

Similar pharmacodynamic responses, to those exhibited in anesthetized rabbit, for Bay K 8644 and (±)-**12**–**14** were observed in the conscious spontaneously hypertensive rat (SHR). Thus, Bay K 8644 was rather toxic causing stress and agitation at lower doses and mortality in two of three rats within 1 h following a 2–3 mg/kg ip dose. Due to this toxicity associated with Bay K 8644 and the observation that (±)-**13** and (±)-**14** exhibited a hypertensive effect like Bay K 8644, further experiments with (±)-**13** and (±)-**14** were not carried out. In contrast, (±)-**12** was nontoxic, and it exhibited a hypotensive effect in the SHR model that was dose dependent, thereby confirming the previous calcium channel antagonist effect observed (see Figure 4).

The promising dual cardioselective calcium agonist/smooth muscle selective calcium channel antagonist activity of (±)-**12** at similar drug concentrations prompted us to evaluate the *in vitro* calcium channel-modulating (GPILSM, GPLA) and chronotropic (GPLA) effects of the

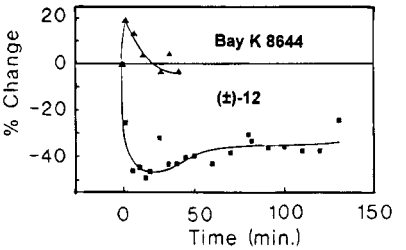


Figure 4. Effect of Bay K 8644 (▲, 3.0 mg/kg ip) and (±)-**12** (■, 30 mg/kg ip) on systolic blood pressure in the spontaneously hypertensive rat (SHR).

individual enantiomers (+)-**12A** and (−)-**12B**. (+)-**12A** enantiomer exhibited *in vitro* agonist activity on both GPILSM (EC_{50} = 5.27 × 10^{-6} M) and GPLA (EC_{50} = 4.58 × 10^{-8} M), whereas the (−)-**12B** enantiomer exhibited antagonist activity on GPILSM (IC_{50} = 2.07 × 10^{-7} M) and agonist activity on GPLA [EC_{50} = (4.58 × 10^{-8})–(4.46 × 10^{-9}) M range] as shown in Figure 5. Thus, (+)-**12A** exhibits a similar agonist profile to (*S*)-(−)-Bay K 8644. The (+)-**12A** enantiomer was a more potent agonist on GPLA at low concentrations since the percent increase in contractile force for (+)-**12A** [(−)-**12B**] were 28 [12], 40 [17], 54 [20], 57 [19], 50 [13], and 41 [10] at drug concentrations of 2.99 × 10^{-10}, 1.49 × 10^{-9}, 4.46 × 10^{-9}, 1.63 × 10^{-8}, 4.58 × 10^{-8}, and 1.64 × 10^{-7} M, respectively. However, at drug concentrations higher than 1.63 × 10^{-6} M, (+)-**12A** continued to increase the contractile force reaching a maximum of 480% at 4.52 x 10^{-5} M, whereas (−)-**12B** became an antagonist decreasing contractility by 34% at 4.52 × 10^{-5} M. (+)-**12A** and (−)-**12B** both began to exhibit negative chronotropic effects on GPLA as the drug concentration was increased. It appears that (−)-**12B** has a superior pharmacological profile (GPILSM antagonist/GPLA agonist), since the differential between effective agonist and antagonist drug concentrations is relatively small. Although, (+)-**12A** is an agonist on GPILSM, it exhibits respectable cardiac agonist activity (28–57% increase in contractile force) in the (1.63 × 10^{-8})–(2.99 × 10^{-10}) M range at which its smooth muscle agonist effect on GPILSM would be expected to be neglible (EC_{50} = 5.27 × 10^{-6} M; see panel A of Figure 5).

The modulation of L-type sensitive calcium channels by (+)-**12A**, (−)-**12B**, and (±)-**12** in isolated guinea pig ventricular myocytes using the whole-cell voltage-clamp technique was investigated, and the results are summarized in Figure 6. The original traces of calcium current (I_{Ca}) elicited by depolarizing steps from a postconditioning potential (V_{PC}) of −40 to 0 or −10 mV in the presence and absence of 10 μM concentrations of (−)-**12B**, (+)-**12A**, and (±)-**12** are shown in panels A–C (left column). Corresponding *I*–*V* curves are shown in panels D–F (right column). Control solution contained 0.001% DMSO as a vehicle control, and all solutions contained 200 μM lidocaine to ensure blockade of Na^+ current. The effect of (−)-**12B** on I_{Ca} initiated by a step to 0 mV from a V_{PC} of −40 mV strongly inhibited I_{Ca} under these conditions (panel A). The effect of (+)-**12A** on the magnitude of I_{Ca} initiated by a step to −10 mV from a V_{PC} of −40 mV (panel B) did not result in blockade of I_{Ca} since it exerted a weak agonist effect under these conditions. The effect of (±)-**12** on original

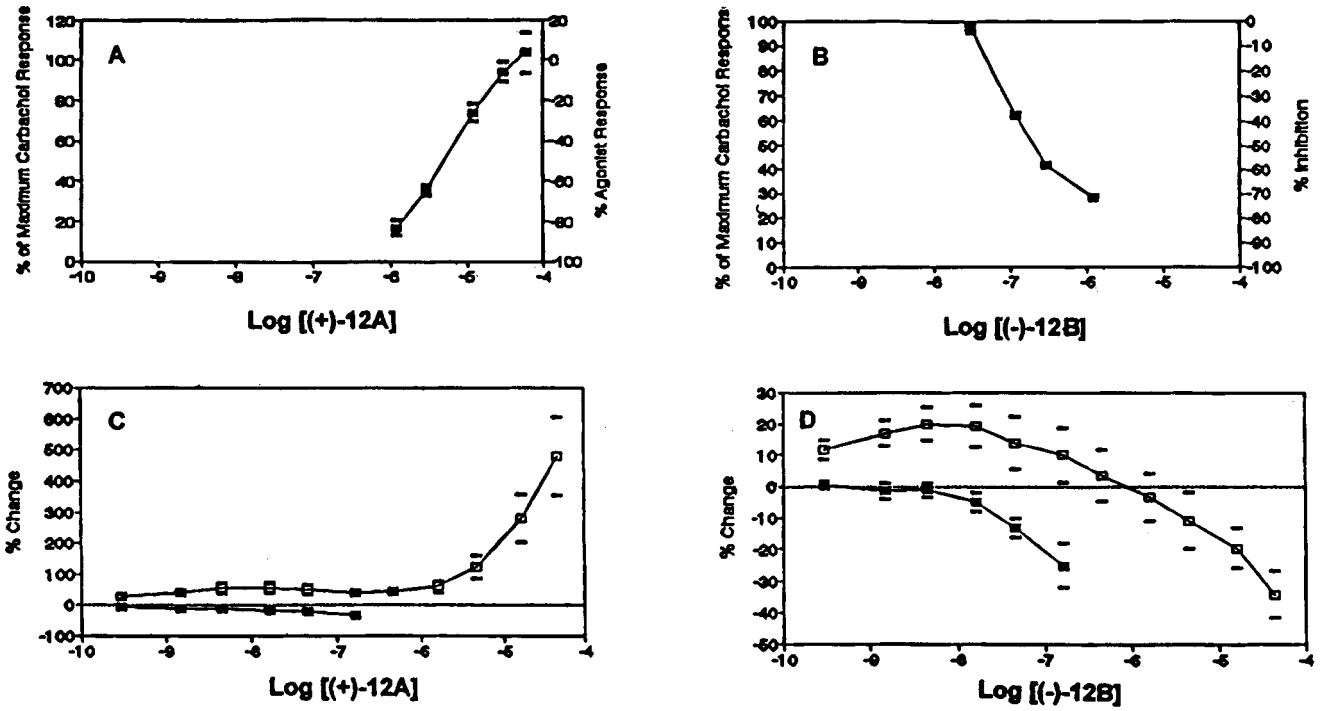


Figure 5. *In vitro* dose–response curves for the agonist effect of (+)-**12A** on GPILSM (panel A), the antagonist effect of (−)-**12B** on GPILSM (panel B), the agonist (increase in contractile force, □) and chronotropic (■) effects of (+)-**12A** on GPLA (panel C), and the inotropic (contractile force, □) and chronotropic (■) effects of (−)-**12B** on GPLA (panel D).

traces of I_{Ca} (panel C) was an inhibition of I_{Ca}, but this effect was less marked than the antagonist effect of (−)-**12B**.

Mean (±SEM) $I-V$ relations for I_{Ca} determined in the presence and absence of 10 μM concentrations of (−)-**12B**, (+)-**12A**, or (±)-**12** are shown in panels D–F (right column). Voltage steps (200 ms) to membrane potentials between −40 and 50 mV were used to determine $I-V$ relations in 2.5 mM external Ca^{2+}. Under control conditions (0.001% DMSO), the threshold for activation of I_{Ca} was near −40 mV and I_{Ca} reached a maximum between −10 and 0 mV (panels D–F). (−)-**12B** effectively inhibited I_{Ca} at all membrane potentials examined (panel D). In contrast, (+)-**12A** did not inhibit I_{Ca} since it exhibited a weak agonist effect near the peak of the $I-V$ curve (panel E). (±)-**12** partially inhibited I_{Ca} at most membrane potentials examined (panel F). Measurements of macrosopic currents have shown that Bay K 8644 is a complicated drug since antagonist and agonist activity can be selected by changes in membrane potential.[35] The enantiomer (−)-**12B**, which inhibits I_{Ca}, exerts an effect similar to (+)-Bay K 8644 whose most prominent effect was an inhibition of calcium channel current, that was more pronounced when currents were measured from depolarized holding potentials.[36] (+)-**12A**, which exerts a modest agonist effect from −40 mV, resembles (−)-Bay K 8644 in this respect.

In a previous study it was found that the orbital on the pyridinyl nitrogen containing the lone electron pair could be viewed as a substituent since a 4-pyridinyl substituent was bioisosteric (calcium channel antagonist activity) with a 4-(nitrophenyl) substituent on a 1,4-dihydropyridine ring system where 2-, 3-, and 4-(nitrophenyl) were bioisosteric to 2-, 3-, and 4-pyridinyl, respectively.[37] The steric effect that an orbital with an electron pair can induce is obviously smaller than that of a substituent attached to a phenyl ring. However, the pyridinyl nitrogen lone electron pair could act as an electron-pair donor involving a hydrogen-bonding interaction to the dihydropyridine calcium channel receptor. In addition, the pyridinyl nitrogen is a determinant of the ring electron density which is higher at the C-3 and C-5 positions (δ^-) than at the C-2, C-4, and C-6 postions (δ^+) due to inductive and resonance effects. The position of the pyridinyl nitrogen atom in compounds **12**–**14** influenced calcium channel modulation since the 3-pyridinyl [(±)-**13**] and 4-pyridinyl [(±)-**14**] isomers exhibited agonist activity on both GPILSM and GPLA, whereas the 2-pyridinyl isomer [(±)-**12**] exhibited dual cardioselective agonist/smooth muscle selective antagonist effects. These differences in calcium channel modulation could be due to a number of possibilities which include differences in the drug–receptor interaction, preferential affinity for or access to the resting (R), open (O), or inactivated (I) states of the receptor, or tissue differences in the α_1-subunit-binding site of the L-type calcium channel.[38]

Summary

The isomeric isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylates represent a novel class of compounds with different calcium channel modulation activities. In particular, the 2-pyridinyl isomer (±)-**12** exhibits a unique profile *in vitro* which includes a *dual cardioselective agonist/smooth muscle selective antagonist activity*. The (+)-**12A** enantiomer exhibited agonist activity on both cardiac and smooth muscle. In contrast, the (−)-**12B** enantiomer acted as a smooth muscle antagonist and cardiac agonist at low drug concentrations or as an antagonist at high drug concentrations. (−)-**12B** appears to have a more clinically acceptable profile since the difference between effective cardioselective agonist and smooth muscle selective antagonist drug concentrations is small. Although (+)-**12A** is an agonist on smooth muscle, the relatively large difference in drug concentration re-

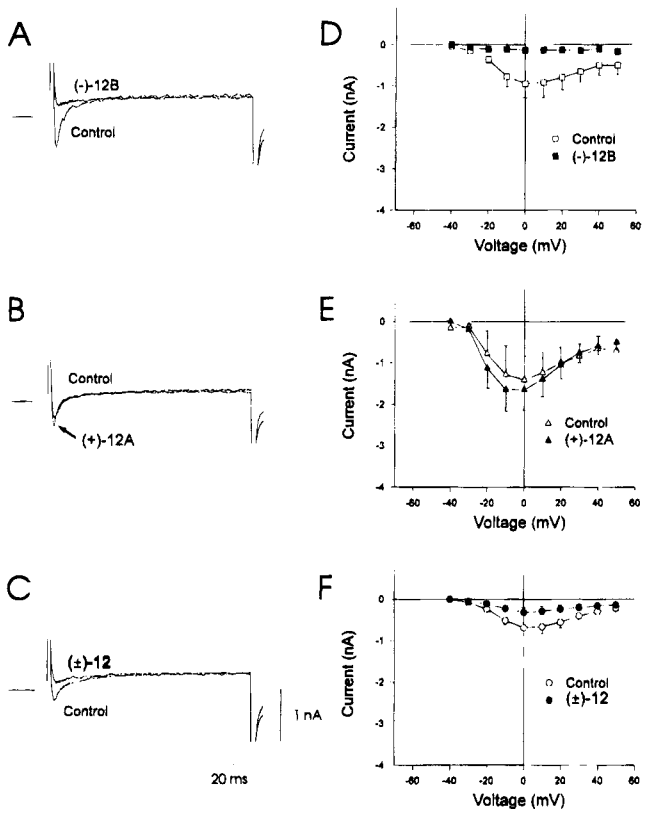


Figure 6. Panels A–C show representative original records of I_{Ca} recorded in the presence and absence of 10 μM concentrations of (−)-**12B** (panel A), (+)-**12A** (panel B), or the racemic (±)-**12** (panel C). Postconditioning potential (V_{PC}) was −40 mV, and I_{Ca} was evoked by 200 ms depolarizing steps to either 0 mV (panels A and C) or −10 mV (panel B). Experiments were conducted in 2.5 mM Ca^{2+} buffer which contained 200 μM lidocaine to inhibit Na^+ currents; control buffer also contained 0.001% DMSO. Line indicates zero current; capacitative transients have been blanked for clarity. I_{Ca} was inhibited by (−)-**12B** (panel A), slightly enhanced by (+)-**12A** (panel B), and inhibited by (±)-**12** (panel C). Panels D–F show mean (±SEM) I–V relationships for I_{Ca}, measured as peak I_{Ca} minus current at 200 ms, in the presence and absence of 10 μM concentrations of (−)-**12B** (panel D), (+)-**12A** (panel E), or (±)-**12** (panel F).

quired for its agonist effect on heart [28–57% increase in contractile force at the (1.63×10^{-8})–(2.99×10^{-10}) M range] suggests its agonist effect on smooth muscle ($EC_{50} = 5.27 \times 10^{-6}$ M) would be minimal at low drug concentrations, resulting in cardioselectivity. The 2-pyridinyl isomers (enantiomers) **12** represent a novel type of 1,4-dihydropyridine calcium channel modulator which could provide a potentially new approach to drug discovery targeted toward the treatment of congestive heart failure. These agents also provide a potential probe to study the structure–function relationship of calcium channels.

Experimental Section

Melting points were determined using a Thomas-Hoover capilliary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (NH, OH, NH_2) was confirmed by the addition of D_2O. Specific rotations were measured on an Optical Activity Ltd. or Perkin-Elmer 241 polarimeter. Silica gel column chromatography was carried out using Merck 7734 (60–200 mesh) silica gel. Microanalyses were within ±0.4% of theoretical values for all elements listed, unless otherwise stated. Isopropyl 3-aminocrotonate, L-threonine, and tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$] were purchased from the Aldrich Chemical Co. Verapamil and lidocaine were obtained from the Sigma Chemical Co. Nitroacetone was prepared according to the reported procedure.[39]

General Method for the Preparation of (±)-Isopropyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-pyridinylpyridine-5-carboxylates (12–14). Nitroacetone (10.4 g, 0.1 mol) in absolute EtOH (5 mL) was added to a solution of the pyridinecarboxaldehyde **9** (10.7 g, 0.1 mol) and isopropyl 3-aminocrotonate (**10**; 14.3 g, 0.1 mol) in dry EtOH (30 mL) with stirring during a period of 10 min. The reaction mixture was heated at reflux for 12 h, cooled to 25 °C, and poured onto crushed ice (1500 g). The resulting gumlike precipitate was extracted with CH_2Cl_2 (16 × 50 mL), the combined CH_2Cl_2 extracts were dried (Na_2SO_4), the solvent was removed *in vacuo*, and the residue obtained was purified by silica gel column chromatography. Elution with hexane–EtOAc (1:1, v/v) yielded the symmetrical diisopropyl ester (<10%) which was discarded. Further elution with hexane–EtOAc (1:9, v/v) afforded the respective title product as bright yellow crystals after recrystallization from hexane–EtOAc (**12**, mp 171–172 °C, 79%; **13**, mp 218 °C, 71%; **14**, mp 172–173 °C, 64%). Anal. ($C_{16}H_{19}N_3O_4$) C, H, N.

Representative spectral data for 13: IR (KBr) 3180 (NH), 1708 (CO_2), 1310, 1515 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.08 and 1.24 (2 d, $J_{CH,Me}$ = 6.5 Hz, 3H each, CH*Me*$_2$), 2.35 (s, 3H, C-6 *Me*), 2.55 (s, 3H, C-2 *Me*), 4.95 (m, 1H, C*H*Me$_2$), 5.35 (s, 1H, H-4), 7.0–7.45 (m, 2H, pyridinyl H-5, N*H*), 7.80 (d, $J_{4,5}$ = 7.5 Hz, 1H, pyridinyl H-4), 8.42 (d, $J_{5,6}$ = 5.2 Hz, 1H, pyridinyl H-6), 8.53 (s, 1H, pyridinyl H-2).

Methyl (2*S*,3*R*)-2-Amino-3-hydroxybutanoate Hydrochloride [(2*S*,3*R*)-18·HCl]. A solution of thionyl chloride (74.9 g, 0.63 mol) in MeOH (100 mL) was cooled to 0 °C, and (2*S*,3*R*)-**17** (L-threonine; 25.0 g, 0.21 mol) was added in aliquots. The reaction was allowed to proceed at 0 °C for 1 h and then at 25 °C for 48 h with stirring. Removal of the solvent *in vacuo* afforded (2*S*,3*R*)-**18** as a viscous oil (35.6 g, 100%) which was used immediately in the subsequent reaction.

Methyl (2*S*,3*R*)-2-(4-Tolylsulfamoyl)-3-hydroxybutanoate [(2*S*,3*R*)-19]. A mixture of (2*S*,3*R*)-**18**·HCl (35.6 g, 0.21 mol) in EtOAc (300 mL) and K_2CO_3 (43.0 g, 0.31 mol) in water (150 mL) was cooled to 0 °C, and *p*-toluenesulfonyl chloride (39.8 g, 0.21 mol) was added in small aliquots with stirring. The reaction was allowed to proceed for 3 h at 5–10 °C and then at 25 °C for 17 h with stirring. The organic layer was separated and washed with water (3 × 40 mL), the organic extract was dried (Na_2SO_4), and the solvent was removed *in vacuo* to give a solid which was recrystallized from EtOAc–ether to yield (2*S*,3*R*)-**19** (43.4 g, 72%): mp 99 °C; $[\alpha]^{23}_D$ = −9° (*c* 3.33, $CHCl_3$ or MeOH); IR (KBr) 1745 (CO_2), 3319 (OH), 3419 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, $J_{CH,Me}$ = 7 Hz, 3H, CH*Me*), 2.42 (s, 3H, -C_6H_4-*Me*), 2.54 (d, $J_{CH,OH}$ = 6 Hz, 1H, O*H*), 3.52 (s, 3H, CO_2*Me*), 3.83 (dd, $J_{CH,CH}$ = 3.0 Hz, $J_{CH,NH}$ = 8.0 Hz, 1H, CH-C*H*-NH), 4.15 (qdd, $J_{CH,Me}$ = 7.0 Hz, $J_{CH,OH}$ = 6.0 Hz, $J_{CH,CH}$ = 3.0 Hz, 1H, H-3), 5.74 (d, $J_{CH,NH}$ = 8.0 Hz, 1H, CH-N*H*), 7.28 (d, J = 8 Hz, 2H, phenyl H-3 and H-5), 7.72 (d, J = 8 Hz, 2H, phenyl H-2 and H-6). Anal. ($C_{12}H_{17}NO_5S$) C, H, N.

(1*R*,2*S*)-2-(4-Tolylsulfamoyl)-2-(methoxycarbonyl)-1-methylethyl Acetoacetate [(1*R*,2*S*)-20]. A solution of freshly distilled diketene (5.85 g, 69 mmol) in dry THF (15 mL) was added slowly to an ice-cold solution of (2*S*,3*R*)-**19** (20.0 g, 69 mmol) and a catalytic amount of 4-(dimethylamino)-pyridine (0.42 g, 3.48 mmol) in dry THF (150 mL) with stirring. The reaction was allowed to proceed at 25 °C for 2 h with stirring, and the solvent was removed *in vacuo* to give a white solid. Recrystallization from EtOAc–ether afforded (1*R*,2*S*)-**20** (25.3 g, 98%): mp 99 °C; $[\alpha]^{23}_D$ = +27.6° (*c* 3.33, $CHCl_3$); IR (KBr) 1745 (CO_2), 1720 (CO), 3263 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37 (d, $J_{CH,Me}$ = 7 Hz, 3H, CH-*Me*), 2.22 (s, 3H, CO*Me*), 2.43 (s, 3H, -C_6H_4-*Me*), 3.42 (s, 2H, COCH_2CO), 3.52 (s, 3H, CO_2*Me*), 4.0 (dd, $J_{CH,CH}$ = 3 Hz, $J_{CH,NH}$ = 8 Hz, 1H, CH-C*H*-NH), 5.36 (qd, $J_{CH,Me}$ = 7 Hz, $J_{CH,CH}$ = 3 Hz, 1H, O-C*H*-Me), 5.58 (d, $J_{CH,NH}$ = 8 Hz, 1H, CH-N*H*), 7.29 (d, J = 8 Hz, 2H, phenyl H-3 and H-5), 7.73 (d, J = 8 Hz, 2H, phenyl H-2 and H-6). Anal. ($C_{16}H_{21}NO_7S$) C, H, N.

(1*R*,2*S*)-2-(4-Tolylsulfamoyl)-2-(methoxycarbonyl)-1-methylethyl β-Aminocrotonate [(1*R*,2*S*)-21]. Ammonia gas was bubbled into a solution of (1*R*,2*S*)-**20** (7.0 g, 18.8 mmol) and *p*-toluenesulfonic acid (50 mg, 0.29 mmol) in dry toluene (100 mL) which was preheated to 130 °C for 20 min. The reaction was allowed to proceed, with azeotropic removal of water using a Dean–Stark apparatus, until ^{1}H NMR spectroscopy indicated the absence of (1*R*,2*S*)-**20** (*ca.* 3–4 h). Removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography using EtOAc–hexane (1:1, v/v) as eluent. Combination of the target fractions yielded (1*R*,2*S*)-**21** as a white solid (3.65 g, 52.5%): mp 112 °C; $[\alpha]^{23}_D$ = +46.8° (*c* 1.81, CH_2Cl_2); IR (KBr) 1743 and 1665 (CO_2), 3310 and 3346 (NH_2), 3458 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (d, $J_{CH,Me}$ = 7.0 Hz, 3H, CH-*Me*), 1.87 (s, 3H, =C-*Me*), 2.40 (s, 3H, -C_6H_4-*Me*), 3.46 (s, 3H, CO_2*Me*), 3.95 (dd, $J_{CH,CH}$ = 3.0 Hz, $J_{CH,NH}$ = 8.0 Hz, 1H, CH-C*H*-NH), 4.38 (s, 1H, C*H*=C-NH_2), 4.72 (br s, 2H, N*H*$_2$), 5.27 (qd, $J_{CH,Me}$ = 7.0 Hz, $J_{CH,CH}$ = 3.0 Hz, 1H, OC*H*-Me), 5.40 (d, $J_{CH,NH}$ = 8.0 Hz, 1H, CH-N*H*SO_2), 7.29 (d, *J* = 8 Hz, 2H, phenyl H-3 and H-5), 7.73 (d, *J* = 8 Hz, 2H, phenyl H-2 and H-6). Anal. ($C_{16}H_{22}N_2O_6S$) C, H; N: calcd, 7.56; found, 7.08.

(1*R*,2*S*)-1-Methyl-2-(4-tolylsulfamoyl)-2-(methoxycarbonyl)ethyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2-pyridinyl)pyridine-5-carboxylate Diastereomers [(1*R*,2*S*)-22A and (1*R*,2*S*)-22B]. A solution of 2-pyridinecarboxaldehyde (1.5 g, 14 mmol), (1*R*,2*S*)-**21** (5.18 g, 14 mmol), and nitroacetone (1.74 g, 16 mmol) in EtOH (100 mL) was stirred at 25 °C for 1 h and then heated at 80 °C for 17 h. Removal of the solvent *in vacuo* gave a foamlike solid that consisted of a mixture of a symmetrical diester product along with the two desired diastereomers (1*R*,2*S*)-**22A** and (1*R*,2*S*)-**22B** in a ratio of 1:1 (^{1}H NMR spectroscopy). This mixture was separated by silica gel column chromatography using EtOAc–hexane (6:4, v/v) as eluent. Upon elution, the undesired symmetrical diester eluted first and was discarded. Further elution gave fractions containing a mixture of the two diastereomers (1*R*,2*S*)-**22A** and (1*R*,2*S*)-**22B** followed by fractions containing pure (1*R*,2*S*)-**22B**. The fractions containing a mixture of (1*R*,2*S*)-**22A** and (1*R*,2*S*)-**22B** were rechromatographed using the same procedure. In this way, after three column purifications, similar fractions were combined and the solvent was removed *in vacuo* to afford (1*R*,2*S*)-**22A** (0.9 g, 11.8%) and (1*R*,2*S*)-**22B** (0.8 g, 10.5%) as foamlike products.

Diastereomer (1*R*,2*S*)-22A: $[\alpha]^{23}_D$ = −61.8° (*c* 2.34, CH_2Cl_2); IR (KBr) 1704 and 1737 (CO_2), 1228 and 1466 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.2 (d, $J_{CH,Me}$ = 7 Hz, 3H, CH-*Me*), 2.24 (s, 3H, C-6 *Me*), 2.39 (s, 3H, -C_6H_4-*Me*), 2.48 (s, 3H, C-2 *Me*), 3.32 (s, 3H, CO_2*Me*), 4.04 (dd, $J_{CH,CH}$ = 3 Hz, $J_{CH,NH}$ = 8 Hz, 1H, CH-C*H*-NH), 5.19 (qd, $J_{CH,Me}$ = 7 Hz, $J_{CH,CH}$ = 3 Hz, 1H, O-C*H*-Me), 5.44 (s, 1H, H-4), 7.22 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3), 7.30 (d, *J* = 8 Hz, 2H, phenyl H-3 and H-5), 7.38 (dd, $J_{4,5}$ = 8 Hz, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5), 7.56 (d, $J_{CH,NH}$ = 8 Hz, 1H, CH-CH-N*H*), 7.68 (ddd, $J_{3,4}$ = 8 Hz, $J_{4,5}$ = 8 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4), 7.80 (d, *J* = 8 Hz, 2H, phenyl H-2 and H-6), 8.08 (br s, 1H, dihydropyridyl N*H*), 8.70 (dd, $J_{5,6}$ = 5 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-6). Anal. ($C_{25}H_{28}N_4O_8S$) C, H, N.

Diastereomer (1*R*,2*S*)-22B: $[\alpha]^{23}_D$ = −117.2° (*c* 2.34, CH_2Cl_2); IR (KBr) 1704 and 1737 (CO_2), 1228 and 1466 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, $J_{CH,Me}$ = 7 Hz, 3H, CH-*Me*), 2.20 (s, 3H, C-6 *Me*), 2.40 (s, 3H, -C_6H_4-*Me*), 2.48 (s, 3H, C-2 *Me*), 3.40 (s, 3H, CO_2*Me*), 3.82 (dd, $J_{CH,NH}$ = 8 Hz, $J_{CH,CH}$ = 3 Hz, 1H, CH-C*H*-NH), 5.33 (qd, $J_{CH,Me}$ = 7 Hz, $J_{CH,CH}$ = 3 Hz, 1H, C*H*-CH-NH), 5.52 (s, 1H, H-4), 6.8 (d, $J_{CH,NH}$ = 8 Hz, 1H, CH-CH-N*H*), 7.27 (m, 3H, phenyl H-3 and H-5, pyridinyl H-5), 7.42 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3), 7.67 (d, *J* = 8 Hz, 2H, phenyl H-2 and H-6), 7.79 (ddd, $J_{3,4}$ = 8 Hz, $J_{4,5}$ = 8 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4), 8.10 (br s, 1H, dihydropyridyl N*H*), 8.64 (dd, $J_{5,6}$ = 5 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-6). Anal. ($C_{25}H_{28}N_4O_8S$) C, H, N.

1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2-pyridinyl)pyridine-5-carboxylic Acid Enantiomers [(+)-23A and (−)-23B]. A solution of either (1*R*,2*S*)-**22A** or (1*R*,2*S*)-**22B** (0.40 g, 0.73 mmol) and DBU (0.33 g, 2.2 mmol) in MeOH (20 mL) was stirred at 25 °C for 3 h. The solvent was removed *in vacuo*, and water (10 mL) was added. The water layer was washed with ether (3 × 50 mL), acidified to pH 1–2 using 0.5 N HCl, and extracted with EtOAc (50 mL) which was discarded. The aqueous layer (pH 1–2) was adjusted to pH 5–6 using 0.1 N NaOH and extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo* to give a viscous oil which was recrystallized from MeOH to afford (+)-**23A** (0.10 g, 50%) or (−)-**23B** (0.10 g, 50%), respectively.

Enantiomer (+)-23A: mp 181 °C; $[\alpha]^{23}_D$ = +24.8° (*c* 1.8, MeOH); IR (KBr) 1228 and 1466 (NO_2), 1671 (CO_2), 2401–3509 (CO_2H) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, C-6 *Me*), 2.56 (s, 3H, C-2 *Me*), 5.46 (s, 1H, H-4), 7.78 (dd, $J_{4,5}$ = 8 Hz, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5), 7.87 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3), 8.38 (ddd, $J_{3,4}$ = 8 Hz, $J_{4,5}$ = 8 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4), 8.73 (dd, $J_{5,6}$ = 5 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-6), 10.34 (br s, 1H, N*H*). Anal. ($C_{13}H_{13}N_3O_4 \cdot {}^3/_4H_2O$) C, N; H: calcd, 5.17; found, 4.76.

Enantiomer (−)-23B: mp 180 °C; $[\alpha]^{23}_D$ = −24.4° (*c* 1.66, MeOH); IR (KBr) 1228 and 1466 (NO_2), 1671 (CO_2), 2401–3509 (CO_2H) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H, C-6 *Me*), 2.54 (s, 3H, C-2 *Me*), 5.44 (s, 1H, H-4), 7.65 (dd, $J_{4,5}$ = 8 Hz, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5), 7.84 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3), 8.34 (ddd, $J_{3,4}$ = 8 Hz, $J_{4,5}$ = 8 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4), 8.70 (dd, $J_{5,6}$ = 5 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-6), 10.21 (br s, 1H, N*H*). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

Isopropyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2-pyridinyl)pyridine-5-carboxylate Enantiomers [(+)-12A and (−)-12B]. A solution of (+)-**23A** or (−)-**23B** (45 mg, 0.163 mmol), isopropyl bromide (22 mg, 0.179 mmol), and K_2CO_3 (24 mg, 0.196 mmol) in DMF (5 mL) was stirred at 25 °C for 16 h. Extraction with EtOAc (3 × 50 mL), washing with water (2 × 20 mL) and then brine (20 mL), drying the organic fraction with Na_2SO_4 (20 g), removal of the solvent *in vacuo*, and recrystallization of the residue obtained afforded the respective product (+)-**12A** (36 mg, 69%) or (−)-**12B** (36 mg, 69%) as a yellow solid.

Enantiomer (+)-12A: mp 198–199 °C; IR (KBr) 1310 and 1474 (NO_2), 1704 (CO_2), 3467 (NH) cm^{-1}; $[\alpha]^{23}_D$ = +282.9° (*c* 0.45, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.09 (d, $J_{CH,Me}$ = 6 Hz, 3H, CHMe*Me'*), 1.23 (d, $J_{CH,Me}$ = 6 Hz, 3H, CH*Me*Me'), 2.26 (s, 3H, C-6 *Me*), 2.45 (s, 3H, C-2 *Me*), 4.96 (septet, $J_{CH,Me}$ = 6 Hz, 1H, C*H*Me_2), 5.6 (s, 1H, H-4), 7.27 (dd, $J_{4,5}$ = 8 Hz, $J_{5,6}$ = 5 Hz, 1H, pyridinyl H-5), 7.63 (d, $J_{3,4}$ = 8 Hz, 1H, pyridinyl H-3), 7.72 (ddd, $J_{4,5}$ = 8 Hz, $J_{3,4}$ = 8 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-4), 8.48 (dd, $J_{5,6}$ = 5 Hz, $J_{4,6}$ = 2 Hz, 1H, pyridinyl H-6), 9.76 (br s, 1H, N*H*). Anal. ($C_{16}H_{19}N_3O_4$) C, H, N.

Enantiomer (−)-12B: mp 197–198 °C; $[\alpha]^{23}_D$ = −286.0° (*c* 0.90, $CHCl_3$); IR (KBr) and ^{1}H NMR spectra for (−)-**12B** were the same as those for (+)-**12A**. Anal. ($C_{16}H_{19}N_3O_4$) C, H, N.

The optical purity of (+)-**12A** and (−)-**12B** was determined by ^{1}H NMR spectrometry. When 10 μL of a solution (100 mg in 1 mL of $CDCl_3$) of the chiral shift reagent (+)-Eu(hfc)$_3$] was added to a solution of *rac*-**12** (5 mg in 0.5 mL of $CDCl_3$), the 1,4-dihydropyridyl C-2 and C-6 methyl resonances at δ 2.45 and 2.26 were each separated into two resonances which appeared at δ 2.47 and 2.49, and 2.28 and 2.30, respectively. Addition of (+)-Eu(hfc)$_3$ (40 μL) to (+)-**12A** or (−)-**12B**, as described above, resulted in retention of single resonances for the C-2 and C-6 methyl resonances (≥96% ee).

***In Vitro* Calcium Channel Antagonist and Agonist Assays.** The calcium channel antagonist activities were determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6 × 10^{-7} M) Ca^{2+} dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure reported previously.[40] The IC_{50} value (±SEM, *n* = 3) was determined graphically from the dose–response curve.

Calcium channel agonist activity of the *test compound* on GPILSM was calculated as the molar concentration required to elicit 50% of the maximal contractile force produced by the test compound, as described above. The EC_{50} value (±SEM, *n* = 3) was determined graphically from the dose–response curve.

Calcium channel agonist activity (positive inotropic effect) was calculated as the percentage increase in contractile force of isolated guinea pig left atrium (GPLA) relative to its basal contractile force in the absence of test compound. The chronotropic effect was calculated as the percentage change in the rate of isolated guinea pig right atrium (GPRA) upon exposure to the test compound, relative to its normal rate that was predetermined in the absence of test compound.

***In Vivo* Determination of Arterial Blood Pressure in a Rabbit Model.** Male rabbits (New Zealand albino) weighing 1.5–3.0 kg were anesthetized with either sodium pentobarbital (30 mg/kg iv via a marginal ear vein or 40 mg/kg ip) or Rompun cocktail [zylazine (8 mg/kg), ketamine (40 mg/kg), acepromazine (0.5 mg/kg)]. Lidocaine (1% xylocaine; Astra) was injected into the neck area, and the trachea was exposed and intubated. Supplemental pentobarbital (8–15 mg/kg) was injected as required during the course of the experiment. The animal was then artificially respirated (Ugo Basile 6025 cat/rabbit ventilator) at a rate of 25–40 breaths/min, with a tidal volume of 10 mL/kg. The jugular vein (left or right) was cannulated and catheterized (18G quik-cath intravascular over-the-needle Teflon catheter with luer plug; Travenol Lab.) which was used for subsequent injection of normal saline and test drugs. The carotid artery (opposite side to catheterized vein) was also cannulated and catheterized for use in measuring arterial pulse pressure with a pressure transducer (Gould P23 ID) attached to a Grass Model 70 polygraph. Heparin (50 units/kg in normal saline) was then infused into the animal via the venous line. The animal was allowed to equilibrate for up to 1 h, depending on stability of blood pressure and ECG, before experiments were initiated. Propranolol (1.0 mg/kg iv) was administered to the animal 10 min before the test drug was administered. A solution of the test drug in water–DMSO (9:1, v/v) was administered via the catheter in the jugular vein.

***In Vivo* Determination of Blood Pressure in a Spontaneously Hypertensive Rat (SHR strain) Model.** Conscious male SHR rats (Charles River Canada Inc.) were restrained in cages and warmed on a heated plate to 28–32 °C. Indirect systolic blood pressure and heart rate of the animals were measured using the tail-cuff method,[41-43] modified to accommodate a semiautomatic pressure-generating system. Pulse and pressure were recorded using a pneumatic pressure transducer (Gould P23 ID) attached to a Grass polygraph. Control blood pressure measurements were made after the animals were warmed sufficiently to produce a discernible pulse in the transducer. At least three measurements were taken, and the average of these was used as a control. A solution of the test drug in water-DMSO (9:1, v/v) was then administered by ip injection, and measurements were recorded at appropriate time intervals. Animals were restrained for the duration of the experiment.

Whole-Cell Voltage-Clamp Studies. 1. Cell isolation: Male guinea pigs (350–400 g; Charles River) were injected with heparin (3.3 IU/g) 30 min prior to anesthesia with sodium pentobarbital (80 mg/kg). The heart was cannulated *in situ* and perfused retrogradely (10–12 mL/min) through the aorta with oxygenated (100% O_2, 36 °C) Ca^{2+}-free solution of the following composition (mM): 120 NaCl, 3.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10 HEPES, 11 glucose, 0.1% fatty acid-free albumin (fraction V; Boehringer Mannheim) (pH 7.4 with NaOH). The heart was removed from the chest, perfused with 50 mL of Ca^{2+}-free solution (7–8 min), and then perfused with Ca^{2+}-free solution supplemented with 40 mg of collagenase A (Boehringer Mannheim), 6 mg of protease XIV (Sigma), and 0.4% fatty acid free albumin (5–8 min). Finally, the ventricles were minced and washed in a high K^+ solution of the following composition (mM): 80 KOH, 50 glutamic acid, 30 KCl, 30 KH_2PO_4, 20 taurine, 10 HEPES, 10 glucose, 3 $MgSO_4$, 0.5 EGTA (pH 7.4 KOH). After a 1–2 h incubation at room temperature, myocytes were placed in a Petri dish in an open perfusion microincubator (Model PDMI-2; Medical Systems Corp.) on the stage of an inverted microscope. The cells were then superfused with the HEPES-buffered solution described above, supplemented with 2.5 mM Ca^{2+}.

2. Experimental methods: Intracellular recordings were made with 18–25 MΩ microelectrodes filled with 2.7 M KCl. Experiments were performed at 35 °C with discontinuous single-electrode voltage clamp (sample rate 10–16 kHz). Voltage-clamp protocols were generated with pClamp software (Axon Instruments Inc.). Current and voltage were recorded with an Axoclamp-2A amplifier (Axon Instruments Inc.), digitized with a Labmaster A/D interface at 125 kHz (TL1-125; Axon Instruments Inc.), and stored on hard disk for subsequent analysis. During voltage clamp, the holding potential was −80 mV. Voltage-clamp test steps were preceded by a 200 ms conditioning step to 0 mV from the holding potential followed by return to a postconditioning potential (V_{PC}) of −40 mV. The voltage-clamp test protocol to measure Ca^{2+} current (I_{Ca}) consisted of a series of 200 ms voltage steps, in 10 mV increments, from −40 to 50 mV. All experiments were conducted in the presence of 200 μM lidocaine to block Na^+ current. The peak amplitude of I_{Ca} was measured as the difference between the peak inward current and the net current after 200 ms. The validity of this measure of I_{Ca} was tested in separate experiments, in which cells were exposed to the Ca^{2+} channel blocker verapamil (2–5 μM); verapamil completely inhibited inward current identified as I_{Ca} in these experiments. Current–voltage (I–V) relationships were constructed by plotting peak amplitude of I_{Ca} as a function of membrane potential. Ionic current recordings were analyzed with pClamp analysis software. Data are presented as means ± SEM. The value of n represents the number of myocytes sampled; each myocyte was exposed to only one drug.

3. Drugs: The test drugs were dissolved in distilled DMSO at a stock concentration of 10 mM. In preliminary experiments, it was determined that this amount of DMSO in the test drug solution (*e.g.*, 0.001%) was sufficient to slightly inhibit I_{Ca}. Therefore, a solution was used which contained 0.001% DMSO as the control, and the effect of each test drug was compared to this control. The effects of 10 μM (+)-**12A**, 10 μM (−)-**12B** and 10 μM (±)-**12** on I_{Ca} were examined. In preliminary experiments it was found that 1 μM drug concentrations had virtually no effect on the magnitude of I_{Ca}. Cells were first exposed to 0.001% DMSO for 10 min and then to one of the three test compounds for 10 min, and finally the drugs were washed out for up to 20 min. It was found that only the effect of (+)-**12A** washed out within this time.

Acknowledgment. We are grateful to the Medical Research Council of Canada (Grant No. MT-8892) for financial support of this research. S.E.H. is a Scholar of the Heart and Stroke Foundation of Canada. The authors would like to acknowledge the excellent technical assistance of C. Mapplebeck, P. Nicholl, and C.-A. McEwen.

References

(1) Langs, D. A.; Triggle, D. J. Conformational Features of Calcium Channel Agonist and Antagonist Analogs of Nifedipine. *Mol. Pharmacol.* **1985**, *27*, 544–548.
(2) Langs, D. A.; Strong, P. D.; Triggle, D. J. Receptor Model for the Molecular Basis of Tissue Selectivity of 1,4-Dihydropyridine Calcium Channel Drugs. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 215–230.
(3) Langs, D. A.; Kwon, Y. W.; Strong, P. D.; Triggle, D. J. Molecular Level Model for the Agonist/Antagonist Selectivity of the 1,4-Dihydropyridine Calcium Channel Receptor. *J. Comput.-Aided Mol. Des.* **1991**, *5*, 95–106.
(4) Mager, P.; Coburn, R. A.; Solo, A. J.; Triggle, D. J.; Rothe, H. QSAR, Diagnostic Statistics and Molecular Modeling of 1,4-Dihydropyridine Calcium Channel Antagonists: A Difficult Road Ahead. *Drug Des Dis.* **1992**, *8*, 273–289.
(5) Schramm, M.; Thomas, G.; Franchowiak, G. Novel Dihydropyridines With Positive Inotropic Action Through Activation of Ca^{2+} Channels. *Nature* **1983**, *303*, 535–537.
(6) Hof, R. P.; Ruegg, U. T.; Hof, A.; Vogel, A. Stereoselectivity at the Calcium Channel: Opposite Action of the Enantiomers of a 1,4-Dihydropyridine. *J. Cardiovasc. Pharmacol.* **1985**, *7*, 689–693.
(7) Holland, D. R.; Wikel, J. H.; Kauffman, R. F.; Smallwood, J. K.; Zimmerman, K. M.; Utterback, B. G.; Turk, J. A.; Steinbert, M. I. LY249933: A Cardioselective 1,4-Dihydropyridine With Positive Inotropic Activity. *J. Cardiovasc. Pharmacol.* **1989**, *14*, 483–491.

(8) Erne, P.; Burgisser, E.; Buhler, F. R.; Duback, B.; Kuhnis, H.; Meier, M.; Rogg, H. Enhancement of Calcium Influx in Human Platelets by CGP 28392, a Novel Dihydropyridine. *Biochem. Biophys. Res. Commun.* **1984**, *118*, 842–847.
(9) Beyer, T.; Gjorstrup, P.; Ravens, U. Comparison of the Cardiac Effects of the Derivative H 160/51 With Those of the 'Ca-agonist' Bay K 8644. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *330* (Suppl.), R34.
(10) Gjorstrup, P.; Harding, H.; Isaksson, R.; Westerlund, C. Enantiomers of the Dihydropyridine Derivative H 160/51 Show Opposite Effects of Stimulation and Inhibition. *Eur. J. Pharmacol.* **1986**, *122*, 357–361.
(11) Takenaka, T.; Maeno, H. A New Vasoconstrictor 1,4-Dihydropyridine Derivative, YC-170. *Jpn. J. Pharmacol.* **1983**, *32* (Suppl.), 139.
(12) Takeda, Y. Single Channel Analysis of Calcium Ion Channel-Agonistic Action of Dihydropyridine Derivatives. Voltage-dependent Effects of YC-170 and Bay K 8644. *Hakkaido Igaku Zasshi* **1993**, *68*, 557–569.
(13) Sunkel, C. E.; Fau de Casa-Juana, M.; Santos, L.; Garcia, A. G.; Artalejo, C. R.; Villarroya, M.; Gonsalez-Morales, M. A.; Lopez, M. G.; Cillero, J.; Alonso, S.; Priego, J. G. Synthesis of 3-[(2,3-dihydro-1,1,3-trioxo-1,2-benzisothiazol-2-yl)alkyl]-1,4-dihydropyridine-3,5-dicarboxylate Derivatives as Calcium Channel Modulators. *J. Med. Chem.* **1992**, *35*, 2407–2414.
(14) Baxter, A. J.; Dixon, J.; Ince, F.; Manners, C. N.; Teague, S. J. Discovery and Synthesis of Methyl 2,5-Dimethyl-4-(2-(phenylmethyl)benzoyl]-1*H*-pyrrole-3-carboxylate (FPL 64176) and Analogues: The First Examples of a New Class of Calcium Channel Activator. *J. Med. Chem.* **1993**, *36*, 2739–2744.
(15) Davis, A. M.; Gensmantel, N. P.; Johansson, E.; Marriott, D. P. The use of the GRID Program in the 3-D QSAR Analysis of a Series of Calcium Channel Agonists. *J. Med. Chem.* **1994**, *37*, 963–972.
(16) Takenaka, T.; Inagaki, O.; Terai, M.; Asano, M.; Kubo, K. New 1,4-Dihydropyridines with Vasoconstrictor Action Through Activation of Ca^{++}-channels. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 699–708.
(17) Gross, R.; Kayser, M.; Schramm, M.; Taniel, R.; Thomas, G. Cardiovascular Effects of the Calcium-agonistic Dihydropyridine Bay K 8644 in Conscious Dogs. *Arch. Int. Pharmacodyn. Ther.* **1985**, *277*, 203–216.
(18) Preuss, K. C.; Cheung, N. L.; Brooks, H. L.; Warltier, D. C. Cardiovascular Effects of the Nifedipine Analog, CGP 28392, in the Conscious Dog. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 949–953.
(19) Preuss, K. C.; Brooks, H. L.; Gross, G. J.; Warltier, D. C. Positive Inotropic Actions of the Calcium Channel Stimulator, Bay K 8644, in Awake, Unsedated Dogs. *Basic Res. Cardiol.* **1985**, *80*, 326–332.
(20) Goldenberg, I. F.; Cohn, J. N. New Inotropic Drugs for Heart Failure. *JAMA, J. Am. Med. Assoc.* **1987**, *258*, 493–496.
(21) Erhardt, P. W. In Search of the Digitalis Replacement. *J. Med. Chem.* **1987**, *30*, 231–237.
(22) For an excellent review, see: Rampe, D.; Kano, J. M. Activators of Voltage-Dependent L-Type Calcium Channels. *Drug Dev. Res.* **1994**, *33*, 344–363.
(23) Bechem, M.; Hebisch, S.; Schramm, M. Ca^{2+} Agonists. New Sensitive Probes for Ca^{2+} Channels. *Trends Pharmacol. Sci.* **1988**, *9*, 257–261.
(24) Wei, X. Y.; Luckowski, E. M.; Rutledge, A.; Su, C. M.; Triggle, D. J. Pharmacologic and Radioligand Binding Analysis of the Actions of 1,4-Dihydropyridine Activator-Antagonist Pairs in Smooth Muscle. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 144–153.
(25) Franckowiak, G.; Bechem, M.; Schramm, M.; Thomas, G. The Optical Isomers of the 1,4-Dihydropyridine Bay K 8644 Show Opposite Effects on Ca Channels. *Eur. J. Pharmacol.* **1985**, *114*, 223–226.
(26) Su, C. M.; Swamy, V. C.; Triggle, D. J. Calcium Channel Activation in Vascular Smooth Muscle by Bay K 8644. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1401–1410.
(27) Lin, Y. Y.; Jones, J. B. Selective Hydrolysis of Dihydrocinnamate Ester Protecting Groups by α-Chymotripsin. Further Studies on the Scope and Limitations of the Method. *J. Org. Chem.* **1973**, *38*, 3575–3582.
(28) Holdgrun, X. K.; Sih, C. J. A Chemoenzymatic Synthesis of Optically-Active Dihydropyridines. *Tetrahedron Lett.* **1991**, *32*, 3465–3468.
(29) Brown, H. C.; Chandrasekharan, J.; Ramachandran, P. V. Chiral Synthesis Via Organoboranes: 14. Selective Reductions. 41. Diisopinocamphenylchloroborane, An Exceptionally Efficient Chiral Reducing Agent. *J. Am. Chem. Soc.* **1988**, *110*, 1539–1546.
(30) Enders, D.; Muller, S.; Demir, A. S. Enantioselective Hantzsch Dihydropyridine Synthesis Via Metalated Chiral Alkyl Acetoacetate Hydrazones. *Tetrahedron Lett.* **1988**, *29*, 6437–6440.
(31) Iqbal, N.; Vo, D.; McEwen, C.-A.; Wolowyk, M. W.; Knaus, E. E. Enantioselective Syntheses and Calcium Channel Modulating Effects of (+)- and (−)-3-Isopropyl 5-(4-Methylphenethyl) 1,4-Dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylates. *Chirality* **1994**, *6*, 515–520.
(32) Goldmann, S.; Stoltefuss, J. 1,4-Dihydropyridines: Effect of Chirality and Conformation on the Calcium Channel Antagonist and Calcium Agonist Activities. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1559–1578 and references cited therein.
(33) Holtje, H.-D.; Marrer, S. A Molecular Graphics Study on Structure-Action Relationships of Calcium Antagonistic and Agonistic 1,4-Dihydropyridines. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 23–30.
(34) Kwon, Y. W.; Franckowiak, G.; Langs, D. A.; Hawthorn, N.; Joslyn, A.; Triggle, D. J. Pharmacologic and Radioligand Binding Analysis of the Actions of 1,4-Dihydropyridine Activators Related to Bay K 8644 in Smooth Muscle, Cardiac Muscle and Neuronal Preparations. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1989**, *339*, 19–30.
(35) Sanguinetti, M. C.; Kass, R. S. Regulation of Cardiac Calcium Channel Current and Contractile Activity by Bay K 8644 is Voltage-Dependent. *J. Mol. Cell Cardiol.* **1984**, *16*, 667–670.
(36) Kass, R. S. Voltage-Dependent Modulation of Cardiac Calcium Channel Current by Optical Isomers of Bay K 8644: Implications for Channel Gating. *Circ. Res.* **1987**, *61* (Suppl. I), 1–5.
(37) Dagnino, L.; Li-Kwong-Ken, M. C.; Wolowyk, M. W.; Wynn, H.; Triggle, C. R.; Knaus, E. E. Synthesis and Calcium Channel Antagonist Activity of Dialkyl 1,4-Dihydro-2,6-dimethyl-4-(pyridinyl)-3,5-pyridinedicarboxylates. *J. Med. Chem.* **1986**, *29*, 2524–2529.
(38) Perez-Reyes, E.; Schneider, T. Calcium Channels: Structure, Function and Classification. *Drug. Dev. Res.* **1994**, *33*, 295–318.
(39) Hurd, C. D.; Nilson, M. E. Aliphatic Nitro Ketones. *J. Org. Chem.* **1955**, *20*, 927–936.
(40) Dagnino, L.; Li-Kwong-Ken, M. C.; Wynn, H.; Wolowyk, M. W.; Triggle, C. R.; Knaus, E. E. Synthesis and Calcium Channel Antagonist Activity of Dialkyl (4-(Dihydropyridyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates. *J. Med. Chem.* **1987**, *30*, 640–646.
(41) Yenn, T.; Pearson, D.; Powell, C.; Kirschner, G. Thermal Stress Elevates the Systolic Blood Pressure of Spontaneously Hypertensive Rats. *Life Sci.* **1978**, *22*, 359–362.
(42) Borg, E.; Viberg, A. Role of Heating in Noninvasive Blood Pressure Measurements in Rats. *Acta Physiol. Scand.* **1980**, *108*, 73–75.
(43) Matsuda, S.; Kurokawa, K.; Higuchi, L.; Imamura, N.; Hakata, H.; Ueda, M. A New Blood Pressure Measuring Apparatus Equipped With a Microcomputer System for Conscious Rats. *J. Pharmacol. Methods* **1987**, *17*, 361–376.

JM950171S