Synthesis and Calcium Channel-Modulating Effects of Alkyl (or Cycloalkyl) 1,4-Dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridinecarboxylate Racemates and Enantiomers†

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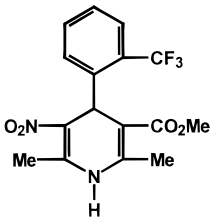
A group of racemic alkyl (or cycloalkyl) 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-, 3-, or 4-pyridyl)-5-pyridinecarboxylate isomers (**6**−**14**) were prepared using a modified Hantzsch reaction that involved the condensation of nitroacetone with an alkyl (or cycloalkyl) 3-aminocrotonate and 2-, 3-, or 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-pyridyl isomers acted as dual cardioselective calcium channel agonists (GPLA)/smooth muscle selective calcium channel antagonists (GPILSM). In contrast, the 3-pyridyl and 4-pyridyl isomers acted as calcium channel agonists on both GPLA and GPILSM. In the C-4 2-pyridyl group of compounds, the size of the C-5 alkyl (or cycloalkyl) ester substituent was a determinant of GPILSM antagonist activity where the relative activity profile was cyclopentyl and cyclohexyl > *t*-Bu, *i*-Bu, and Et > $MeOCH_2CH_2$ > Me. The point of attachment of the C-4 pyridyl substituent was a determinant of GPLA agonist activity where the potency order was generally 4- and 3-pyridyl > 2-pyridyl. (+)-Cyclohexyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate [(+)-**14a**] was a less potent calcium antagonist ($IC_{50} = 5.27 \times 10^{-6}$ M) than the (−)-enantiomer ($IC_{50} = 7.48 \times 10^{-8}$ M) on GPILSM. In the GPLA assay, (+)-**14a** exhibited a much more potent agonist effect ($EC_{50} = 8.45 \times 10^{-6}$ M) relative to the marginal agonist effect produced by (−)-**14a**. The C-4 2-pyridyl compounds (enantiomers) constitute a novel type of 1,4-dihydropyridine calcium channel modulator that could provide a new drug design concept directed toward the treatment of congestive heart failure, and for use as probes to study the structure−function relationships of calcium channels.

The development of cardioselective Hantzsch 1,4-dihydropyridine (DHP) L-type voltage-sensitive calcium channel agonist positive inotropes has provided a significant challenge in drug design.[1−4] Accordingly, the prototype 1,4-DHP calcium channel agonist Bay K 8644 (**1**)[5] simultaneously increases calcium entry into vascular smooth muscle, inducing vasoconstriction, that precludes it clinical use in treating congestive heart failure (CHF).[6−8] Although there have been extensive efforts to understand the molecular basis of action[9,10] and structure−activity relationships,[11] the development of a cardioselective calcium channel stimulant, to the best of our knowledge, has not been reported.[12] The clinical utility of calcium channel agonists to treat CHF is therefore dependent upon separating their vasoconstrictor effects from their positive inotropic cardiostimulant properties.[13]

Recently, we discovered a novel *third generation* class of isomeric isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridinecarboxylates (**2a**−**c**) with different calcium channel modulation activities.[14] The 2-pyridyl isomer [(±)-**2a**)] acted as a *dual cardioselective calcium channel agonist/smooth muscle selective calcium channel antagonist.* In contrast, the 3-pyridyl [(±)-**2b**] and 4-pyridyl [(±)-**2c**] isomers acted as calcium channel agonists on both cardiac and smooth muscle. The (+)-2-pyridyl enantiomer (+)-**2a** exhibited agonist activity on both cardiac and smooth muscle, but the (−)-2-pyridyl enantiomer (−)-**2a** exhibited cardiac agonist and smooth muscle antagonist effects. It was therefore of interest to extend these structure−activity data by replacing the isopropyl moiety of **2a**−**c** by other alkyl and cycloalkyl substituents since this novel type of 1,4-DHP calcium channel modulator could provide a potentially new approach targeted toward the treatment of CHF, and for use as probes to study the structure−function relationships of calcium channels. We now describe the synthesis of alkyl (or cylcoalkyl) 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridinecarboxylates (**6**−**14**) and their in vitro calcium channel-modulating activities on guinea pig smooth muscle and left atrium.



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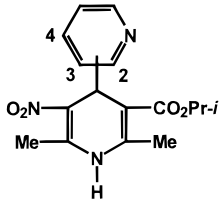

2a, 2-pyridyl
2b, 3-pyridyl
2c, 4-pyridyl

† Dedicated to the memory of Professor Michael W. Wolowyk.
‡ Deceased January 31, 1992.

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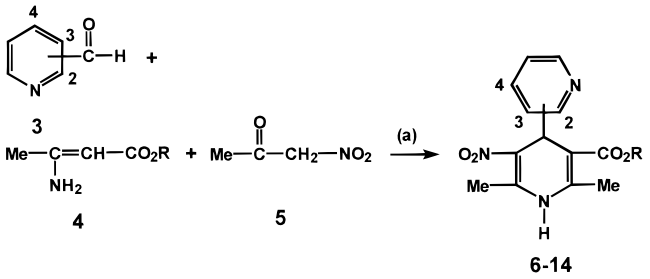
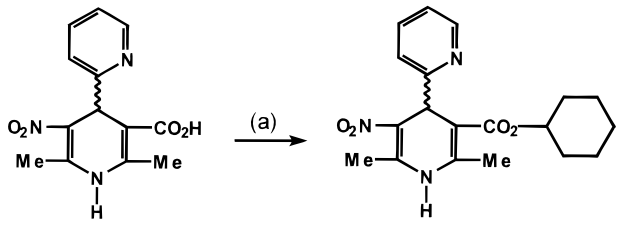
Scheme 1[a]



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

Scheme 2[a]



(+)-15 or (-)-15 (+)-14a or (-)-14a

[a] Reagents and conditions: (a) cyclohexyl bromide, K_2CO_3, DMF, 60 °C, 24 h.

Chemistry

The racemic alkyl (or cycloalkyl) 1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridinecaboxylates **6**−**14** were prepared by a modified Hantzsch reaction. Thus, condensation of nitroacetone (**5**) with an alkyl (or cycloalkyl) 3-aminocrotonate (**4**) and either 2-, 3-, or 4-pyridinecarboxaldehyde (**3**) in absolute EtOH at reflux for 12 h afforded the title compounds in 8−81% yields as illustrated in Scheme 1. The symmetrical 3,5-dialkyl (or dicycloalkyl) 1,4-dihydro-2,6-dimethyl-4-pyridyl-3,5-pyridinedicarboxylate product, resulting from the condensation of **3** with 2 equiv of **4**, was present as a minor product (<10%) that was readily removed by silica gel column chromatography. Nitroacetone (**5**) must be added slowly at 25 °C during a period of 10 min to a solution of the alkyl (or cycloalkyl) 3-aminocrotonate (**4**) and the pyridinecaboxaldehyde (**3**) in absolute EtOH to prevent the formation of the 1,4-dihydro-2,6-dimethyl-3,5-dinitro-4-pyridylpyridine.

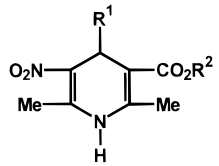
Since cyclohexyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (**14a**), which exhibited an interesting calcium channel-modulating profile, is racemic, and stereospecificity with respect to calcium channel modulation by the isopropyl ester **2a** was established previously,[14] synthesis and pharmacological evaluation of the individual enantiomers (+)-**14a** and (−)-**14a** was highly desirable. Accordingly, reaction of (+)-**15** or (−)-**15** synthesized previously[14] with cyclohexyl bromide in the presence of K_2CO_3 in DMF at 60 °C for 24 h afforded the respective (+)-**14a** or (−)-**14a** enantiomer, both in 12% yield (see Scheme 2). A similar alkylation reaction of (+)-**15**, using cyclohexyl 4-methylbenzenesulfonate in place of cyclohexyl bromide, gave a superior yield of (+)-**14a** (21%). However, this latter reaction was less attractive since a trace amount of *p*-tosyl chloride, which is difficult to remove completely from cyclohexyl 4-methylbenzenesulfonate, caused the reaction to turn black within a period of seconds. The two enantiomers (+)-**14a** and (−)-**14a** each exhibited single resonances for the dihydropyridine C-2 and C-6 methyl resonances upon addition of the ^{1}H NMR chiral shift reagent (+)-Eu(hfc)$_3$, indicating a very high optical purity (≥ 96% ee). The absolute configuration of (+)-**14a** and (−)-**14a**, which have not been determined, would require the acquisition of a X-ray crystal structure for one enantiomer for assignment of the absolute configuration.

Results and Discussion

The design of tissue selective calcium channel agents that are useful for the treatment of CHF dictates that their undesirable vasoconstrictor effect be eliminated and/or separated from the desired positive inotropic stimulant property.[12] Differences in the molecular electrostatic potentials between agonist and antagonist structures, with respect to binding at the C-3 and C-5 regions, may provide a mechanism by which the receptor distinguishes between agonist and antagonist ligands. In this respect, antagonists show a positive potential in this region when a C-3 ester group is present, whereas calcium channel activators were reported to exert a strong negative potential in the region adjacent to the C-3 nitro substituent.[15] In addition, the role of aromatic substituents on the 4-phenyl ring of 1,4-DHP agonists and antagonists are different.[16] It was therefore of interest to evaluate additional analogues of Bay K 8644 (**1**) by replacing the 4-[2-(trifluoromethyl)phenyl] moiety with C-4 2-pyridyl, 3-pyridyl, and 4-pyridyl substituents. The position of the pyridyl nitrogen, and its ability to act as an additional electron donor for hydrogen bonding to the calcium channel receptor, offers a design concept to modulate calcium channel-binding and/or tissue specificity.[14]

The in vitro calcium channel-modulating activities of the racemic alkyl (or cycloalkyl) 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-, 3-, or 4-pyridyl)-5-pyridinecarboxylates (**6**−**8**, **10**, **13**−**14**) were determined using guinea pig ileum longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA) (see Table 1). The point of attachment of the C-4 pyridyl substituent was a determinant of agonist/antagonist activity on GPILSM. For example, all of the 4-(2-pyridyl) compounds (**6a**−**8a**, **10a**, **13a**−**14a**) exhibited a calcium channel antagonist effect on GPILSM ($IC_{50} = 2.12 \times 10^{-4}$ to 4.50×10^{-7} M range) relative to the reference drug nifedipine ($IC_{50} = 1.40 \times 10^{-8}$ M). In this C-4 2-pyridyl series of compounds, the size of the C-5 alkyl (or cycloalkyl) ester substituent was a determinant of antagonist activity where the relative activity profile was cyclopentyl (**13a**) and cyclohexyl (**14a**) > *t*-Bu (**11a**), *i*-Bu (**10a**) and Et (**7a**) > $MeOCH_2CH_2$ (**8a**) > Me (**6a**). These structure−activity relationships are consistent with those previously observed for dialkyl 1,4-dihydro-2,6-dimethyl-4-(2-pyridyl)-3,5-pyridinedicarboxylates where increasing the size of the alkyl ester substituents enhanced antagonist potency.[17] In contrast, the corresponding C-4 3-pyridyl (**6b**, $EC_{50} = (6.72 \pm 1.1) \times 10^{-5}$ M, $n = 3$; **7b**, $EC_{50} = 5.30 \times 10^{-6}$ M, $n = 2$; **10b**, $EC_{50} = 8.20 \times 10^{-9}$ M, $n = 1$) and C-4 4-pyridyl (**6c**, $EC_{50} = (2.67 \pm 0.7) \times 10^{-5}$ M, $n = 3$; **7c**, $EC_{50} = 7.00 \times 10^{-6}$ M, $n = 2$; **8c**, $EC_{50} = 3.85 \times 10^{-5}$ M, $n = 1$; **10c**, $EC_{50} = 2.8 \times 10^{-6}$ M, $n = 2$) isomers exhibited agonist activity on GPILSM like (±)-Bay K 8644 ($EC_{50} = (2.30 \pm 0.05) \times 10^{-7}$ M, *n*

Table 1. Physical and Calcium Channel Modulation Data for Alkyl (or Cycloalkyl) 1,4-Dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridinecarboxylates **6**−**14**



6-14

no.	R^1	R^2	mp, °C	yield, %	calcium channel antagonist act: IC_{50}, M[a]	calcium channel agonist act.: EC_{50}, M[b]
6a	2-pyridyl	Me	192−194	15	$(2.12 \pm 0.70) \times 10^{-4}$	no effect
6b	3-pyridyl	Me	199−201	30		$(7.00 \pm 1.40) \times 10^{-5}$ (4)
6c	4-pyridyl	Me	208−210	8		$(4.85 \pm 0.30) \times 10^{-5}$ (3)
7a	2-pyridyl	Et	190−191	64	$(5.00 \pm 1.97) \times 10^{-6}$	$(2.28 \pm 0.13) \times 10^{-5}$ (3)
7b	3-pyridyl	Et	238−239	71		$(8.84 \pm 1.16) \times 10^{-6}$ (3)
7c	4-pyridyl	Et	173−174	68		$(6.46 \pm 1.21) \times 10^{-6}$ (3)
8a	2-pyridyl	$MeOCH_2CH_2$	178−179	81	$(9.47 \pm 2.76) \times 10^{-5}$	$(9.70 \pm 4.34) \times 10^{-6}$ (3)
8b	3-pyridyl	$MeOCH_2CH_2$	155−156	79		$(4.94 \pm 4.93) \times 10^{-5}$ (3)
8c	4-pyridyl	$MeOCH_2CH_2$	142−143	79		$(3.77 \pm 1.83) \times 10^{-5}$ (3)
9a	2-pyridyl	$Me(CH_2)_7$	167−169	30	not tested	not tested
10a	2-pyridyl	*i*-Bu	176−177	75	$(2.61 \pm 0.06) \times 10^{-6}$	$(9.10 \pm 2.85) \times 10^{-6}$ (4)
10b	3-pyridyl	*i*-Bu	148−149	72		$(1.74 \pm 1.15) \times 10^{-6}$ (3)
10c	4-pyridyl	*i*-Bu	153−154	71		$(8.51 \pm 2.02) \times 10^{-6}$ (3)
11a	2-pyridyl	*t*-Bu	215−216	71	$(1.93 \pm 1.06) \times 10^{-6}$	$(1.81 \pm 0.97) \times 10^{-3}$ (3)
11b	3-pyridyl	*t*-Bu	204−205	69		$(1.46 \pm 0.56) \times 10^{-5}$ (3)
11c	4-pyridyl	*t*-Bu	196	80		$(2.82 \pm 1.42) \times 10^{-6}$ (3)
12a	2-pyridyl	cyclobutyl	189−191	35	not tested	not tested
13a	2-pyridyl	cyclopentyl	195−197	37	$(4.50 \pm 0.48) \times 10^{-7}$	$(1.98 \pm 0.53) \times 10^{-5}$ (3)
13b	3-pyridyl	cyclopentyl	195−197	21		$(3.28 \pm 1.64) \times 10^{-6}$ (3)
13c	4-pyridyl	cyclopentyl	166−168	41		$(4.25 \pm 2.61) \times 10^{-6}$ (3)
14a	2-pyridyl	cyclohexyl	186−188	21	$(8.42 \pm 0.63) \times 10^{-7}$	$(3.70 \pm 2.62) \times 10^{-6}$ (3)
(+)-14a	2-pyridyl	cyclohexyl	188	12	$(5.27 \pm 0.26) \times 10^{-6}$	$(8.45 \pm 1.55) \times 10^{-6}$ (3)
(−)-14a	2-pyridyl	cyclohexyl	186	12	$(7.48 \pm 0.03) \times 10^{-8}$	weak agonist[c]
14b	3-pyridyl	cyclohexyl	165−167	27		$(2.76 \pm 0.21) \times 10^{-6}$ (3)
14c	4-pyridyl	cyclohexyl	209−211	28		$(4.94 \pm 1.69) \times 10^{-6}$ (3)
nifedipine					$(1.40 \pm 0.19) \times 10^{-8}$	
Bay K 8644						$(7.70 \pm 5.90) \times 10^{-7}$ (3)
2a[d]	2-pyridyl	*i*-Pr			$(4.87 \pm 2.06) \times 10^{-6}$	$(9.67 \pm 1.27) \times 10^{-6}$ (3)
2b[d]	3-pyridyl	*i*-Pr				$(2.85 \pm 0.20) \times 10^{-5}$ (3)
2c[d]	4-pyridyl	*i*-Pr				$(8.05 \pm 2.14) \times 10^{-6}$ (3)

[a] The molar concentration of the antagonist test compound causing a 50% decrease in the slow component, or tonic contractile response ($IC_{50} \pm$ SEM, $n = 3$), in guinea pig ileal longitudinal smooth muscle by the muscarinic agonist carbachol (1.6×10^{-7} M) was determined graphically from the dose−response curves. [b]The molar concentration of the agonist test compound causing a 50% increase of the maximum contractile response ($EC_{50} \pm$ SEM) on guinea pig left atrium was determined graphically from the dose response curves. The number of experiments is shown in brackets. [c] The contractile force of GPLA was increased by 3.9, 9.9, and 5.3% at a test compound concentration of 4.57×10^{-7}, 1.63×10^{-6}, and 4.54×10^{-6} M, respectively. [d] Data for the racemates **2a**, **2b**, and **2c** taken from Vo et al.[14]

= 3). 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 the dose−response curves.

Although the 4-(2-pyridyl)-5-(methoxycarbonyl)-1,4-dihydropyridine (**6a**) exhibited no agonist effect on GPLA, all of the remaining racemic alkyl (or cycloalkyl) 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-, 3-, or 4-pyridyl)-5-pyridinecarboxylates evaluated (**6**−**8**, **10**−**11**, **13**−**14**) produced a calcium channel agonist effect on GPLA ($EC_{50} = 1.81 \times 10^{-3}$ to 1.74×10^{-6} M range) relative to the reference drug Bay K 8644 ($EC_{50} = 7.70 \times 10^{-7}$ M). The point of attachment of the C-4 pyridyl substituent was a determinant of GPLA agonist activity where the 4-pyridyl and 3-pyridyl ring system provided in most instances more potent activity in each isomeric series of compounds [R^2 = Me, **6c** ≥ **6b** ≫ **6a** (no effect); R^2 = Et, **7c** ≥ **7b** > **7a**; $R^2 = MeOCH_2CH_2$, **8a** > **8c** ≈ **8b**; R^2 = *i*-Bu, **10b** > **10c** ≈ **10a**; R^2 = *t*-Bu, **11c** > **11b** > **11a**; R^2 = cyclopentyl, **13b** ≈ **13c** > **13a**; R^2 = cyclohexyl, **14b** ≈ **14a** ≈ **14c**]. Replacement of the methyl ester by an ethyl ester enhanced GPLA agonist activity, but the differences in potency between Et, *i*-Bu, cyclopentyl, and cyclohexyl ester substituents were generally small within each of the 2-, 3-, and 4-pyridyl isomeric groups of compounds. The greatest difference in potency was observed for the *tert*-butyl ester isomers (**11a**−**c**) where the activity profile was 4-pyridyl > 3-pyridyl > 2-pyridyl.

The promising dual cardioselective calcium agonist/smooth muscle selective calcium antagonist activity exhibited by (±)-**14** at reasonably similar drug concentrations prompted us to evaluate the in vitro calcium channel-modulating (GPILSM, GPLA) effects of the individual enantiomers (+)-**14a** and (−)-**14a**. The (+)-**14a** ($IC_{50} = 5.27 \times 10^{-6}$ M) enantiomer was a less potent antagonist on GPILSM than (−)-**14a** ($IC_{50} = 7.48 \times 10^{-8}$ M). In the GPLA assay, (+)-**14a** exhibited significantly more potent agonist activity ($EC_{50} = 8.45 \times 10^{-6}$ M) relative to the (−)-**14a** enantiomer which showed marginal agonist activity with a maximum increase in cardiac contractile force of 9.9% observed at a 1.63×10^{-6} M test compound concentration. The in vitro calcium channel-modulating effects induced by the cyclohexyl enantiomers (+)-**14a** and (−)-**14a** are quali-

tatively and semiquantitively similar to the corresponding isopropyl enantiomers (+)-**2a** and (−)-**2a** reported previously,[14] with one single difference. Accordingly, the (+)-**2a** enantiomer induced an agonist effect on GPILSM (EC_{50} = 5.27 × 10^{-6} M) in the absence of carbachol, but it antagonized carbachol-induced muscarinic receptor-mediated Ca^{2+} dependent tonic contraction (IC_{50} = 3.45 × 10^{-6} M, data not previously described). In contrast, (+)-**14a** did not induce an agonist effect on GPILSM in the absence of carbachol but, like (+)-**2a**, (+)-**14a** antagonized the carbachol-induced response (IC_{50} = 5.27 × 10^{-6} M). The reason for this difference between the agonist effect induced by (+)-**2a**, but not by (+)-**14a**, in the absence of carbachol on GPILSM is not known. Although one could speculate that this difference with respect to (+)-**2a** is due to a preferential affinity for or access to the open (O) state of the calcium channel receptor in GPILSM, it is equally plausible that the agonist effect induced by (+)-**2a** on GPILSM in the absence of carbachol is due to a different mechanism of action not involving calcium channel modulation.

It was previously proposed that the orbital on the pyridyl nitrogen containing a lone electron pair could be envisioned as a substituent since a C-4 pyridyl substituent was bioisosteric (calcium channel antagonist) with a C-4 nitrophenyl moiety on a 1,4-DHP ring system where 2-, 3-, and 4-nitrophenyl were bioisosteric to 2-, 3-, and 4-pyridyl, respectively.[17] Although the steric effect associated with an orbital and its electron pair is smaller than that of a substituent attached to a phenyl ring, the pyridyl nitrogen lone electron pair has the potential to act as an electron-pair donor involving a hydrogen-bonding interaction with the dihydropyridine calcium channel receptor. Furthermore, the pyridyl nitrogen influences ring electron density that is greater at the C-3 and C-5 ring positions (δ^-) than at the C-2, C-4, and C-6 positions (δ^+) based on inductive and resonance effects. The pyridyl nitrogen position in compounds **6**−**8**, **10**−**11**, **13**−**14** affected calcium channel modulation since the 3-pyridyl and 4-pyridyl isomers provided agonist activity on both GPILSM and GPLA. In contrast, the 2-pyridyl isomers induced dual cardioselective agonist/smooth muscle selective antagonist effects. There are a number of possibilities, that could be responsible for these differences in calcium channel modulation, such as 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 L-type calcium channel-binding site.[18]

Summary

The isomeric alkyl (or cycloalkyl) 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-, 3-, or 4-pyridyl)-5-pyridinecarboxylates constitutes a novel class of compounds with different calcium channel-modulating effects. Accordingly, the racemic C-4 2-pyridyl isomers, with the exception of the methyl ester **6a**, exert a unique *dual cardioselective agonist/smooth muscle selective antagonist profile in vitro.* On the other hand, the C-4 3-pyridyl and 4-pyridyl isomers act as nonselective agonists on both GPLA and GPILSM. Thus, the 2-pyridyl isomers provide a novel type of 1,4-DHP calcium channel modulator which offers a potentially new approach in drug design directed toward the treatment of congestive heart failure. This class of compounds are also useful probes to investigate the structure−function relationship of calcium channels. The (+)-enantiomer of cyclohexyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate [(+)-**14a**] possesses the most favorable activities and function profile (GPILSM antagonist, IC_{50} = 5.27 × 10^{-6} M; GPLA agonist, EC_{50} = 8.45 × 10^{-6} M) that would be desirable for the potential treatment of congestive heart failure.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (N*H*) was confirmed by the addition of D_2O. Infrared (IR) spectra were acquired on a Nicolet 5DX-FT spectrometer. All spectral data were in agreement with the assigned structures. Specific rotations were measured on a Perkin-Elmer 241 polarimeter. Microanalyses were within ±0.40% of theoretical values for C, H, and N, unless otherwise stated. Silica gel column chromatography was carried out using Merck 7734 (60−200 mesh) silica gel. Methyl 3-aminocrotonate, ethyl 3-aminocrotonate and tris[3-(heptafluoropropyl)hydroxymethylene-(+)-camphorato]uropium(III) [Eu(hfc)$_3$] were purchased from the Aldrich Chemical Co. Other alkyl or cycloalkyl (methoxyethyl, isobutyl, *tert*-butyl, *n*-octyl, cyclobutyl, cyclopentyl, cyclohexyl) 3-aminocrotonates (**4**) were prepared by passage of anhydrous ammonia gas through a solution of the alkyl or cycloalkyl acetoacetate in absolute ethanol according to the procedure of Joslyn et al.[19] Nitroacetone was prepared according to the literature procedure.[20]

General Method for the Preparation of Alkyl (or Cycloalkyl) 1,4-Dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridinecaboxylates (6−14). Nitroacetone (10.4 g, 0.1 mol) in absolute EtOH (5 mL) was added to a solution of either 2-, 3-, or 4-pyridinecarboxaldehyde **3** (10.7 g, 0.1 mol) and an alkyl (or cycloalkyl) 3-aminocrotonate (**4b**, 0.1 mol) in dry EtOH (10 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 crude product was isolated either by filtration of the solid precipitate or extraction with CH_2Cl_2 (3 × 100 mL). When the product was extracted, the combined CH_2Cl_2 extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. Purification of the product by silica gel column chromatography using hexanes−EtOAc (1:1, v/v) as eluent yielded the symmetrical dialkyl or dicycloalkyl ester (<10%) which was discarded. Further elution with hexanes−EtOAc (1:9, v/v) afforded the respective product as bright yellow crystals after recrystallization from hexanes−EtOAc. The percent yield and melting points for products **6**−**14** is shown in Table 1. Representative spectral data (IR, ^{1}H NMR) for products **7a**, **8b**, **10c**, **11a**, **12a**, **13a**, and **14a** are listed below.

Ethyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (7a): IR (KBr) 3180 (NH), 1698 (CO_2), 1524, 1310 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, J = 8 Hz, CH_2CH_3), 2.25 and 2.60 (two s, 3H each, C-6 CH_3, C-2 CH_3), 4.10 (q, J = 7 Hz, 2H, CH_2CH_3), 5.35 (s, 1H, H-4), 7.00−7.85 (m, 3H, pyridyl H-3, H-4, H-5), 8.45 (d, $J_{5,6}$ = 5.2 Hz, 1H, pyridyl H-6), 9.40 (s, 1H, N*H*). Anal. ($C_{15}H_{17}N_3O_4$) C, H, N.

2-Methoxyethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3-pyridyl)-5-pyridinecarboxylate (8b): IR (KBr) 3180 (NH), 1704 (CO_2), 1515, 1310 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.35 and 2.55 (two s, 3H each, C-6 CH_3, C-2 CH_3), 3.35 (s, 3H, OCH_3), 5.45 (s, 1H, H-4), 7.15−7.35 (m, 1H, pyridyl H-5), 7.65−8.05 (m, 1H, pyridyl H-4), 8.25−8.60 (m, 3H, pyridyl H-2 and H-6, N*H*). Anal. ($C_{16}H_{19}N_3O_5$) C, H, N.

Isobutyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(4-pyridyl)-5-pyridinecarboxylate (10c): IR (KBr) 3180 (NH), 1706

(CO_2), 1515, 1310 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 and 0.90 (two d, $J = 7$ Hz, 3H each, $CHMe_2$), 1.65−2.1 (m, 1H, $CHMe_2$), 2.40 and 2.60 (two s, 3H each, C-6 CH_3, C-2 CH_3), 3.85 (d, $J = 6$ Hz, 2H, CH_2CHMe_2), 5.45 (s, 1H, H-4), 7.30 (d, $J_{2,3} = J_{5,6} = 5.2$ Hz, 2H, pyridyl H-3 and H-5), 7.65−8.05 (m, 1H, pyridyl H-4), 8.25−8.60 (m, 3H, pyridyl H-2 and H-6, N*H*). Anal. ($C_{17}H_{21}N_3O_4$) C, H, N.

***tert*-Butyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (11a)**: IR (KBr) 3181 (NH), 1680 (CO_2), 1507, 1302 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.35 (s, 9H, CMe_3), 2.20 and 2.50 (two s, 3H each, C-6 CH_3, C-2 CH_3), 5.30 (s, 1H, H-4), 7.0−7.4 (m, 2H, pyridyl H-3 and H-5), 7.70 (dd, $J_{3,4} = J_{4,5} = 8$ Hz, 1H, pyridyl H-4), 8.40 (d, $J_{5,6} = 5.2$ Hz, 1H, pyridyl H-6), 9.35 (s, 1H, N*H*). Anal. ($C_{17}H_{21}N_3O_4$) C, H, N.

Cyclobutyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (12a): IR (KBr) 3000 (NH), 1696 (CO_2), 1520, 1310 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55−2.12 [m, 6H, $(CH_2)_3$], 2.25 and 2.45 (two s, 3H each, C-6 CH_3, C-2 CH_3), 4.88−5.0 (m, 1H, CO_2CH), 5.65 (s, 1H, H-4), 7.26−7.36 (m, 1H, pyridyl H-5), 7.69 (d, $J_{3,4} = 7.5$ Hz, 1H, pyridyl H-3), 7.76 (dd, $J_{3,4} = J_{4,5} = 7.5$ Hz, 1H, pyridyl H-4), 8.51 (d, $J_{5,6} = 5.0$ Hz, 1H, pyridyl H-6), 10.23 (br s, 1H, N*H*). Anal. ($C_{17}H_{18}N_3O_4$) C, H, N.

Cyclopentyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (13a): IR (KBr) 3057 (NH), 1696 (CO_2), 1515, 1315 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43−1.88 [m, 8H, $(CH_2)_4$], 2.25 and 2.45 (two s, 3H each, C-6 CH_3, C-2 CH_3), 5.08−5.20 (m, 1H, CO_2CH), 5.59 (s, 1H, N*H*), 7.26−7.34 (m, 1H, pyridyl H-5), 7.64 (d, $J_{3,4} = 7.5$ Hz, 1H, pyridyl H-3), 7.73 (dd, $J_{3,4} = J_{4,5} = 7.5$ Hz, 1H, pyridyl H-4), 8.50 (d, $J_{5,6} = 5.0$ Hz, 1H, pyridyl H-6), 9.79 (br s, 1H, N*H*). Anal. ($C_{17}H_{19}N_3O_4$) C, H, N.

Cyclohexyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate racemate (14a): IR (KBr) 3074 (NH), 1687 (CO_2), 1518, 1320 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22−1.90 [m, 10H, $(CH_2)_5$], 2.26 and 2.47 (two s, 3H each, C-6 CH_3, C-2 CH_3), 4.66−4.82 (m, 1H, CO_2CH), 5.65 (s, 1H, H-4), 7.28−7.34 (m, 1H, pyridyl H-5), 7.68 (d, $J_{3,4} = 7.5$ Hz, 1H, pyridyl H-3), 7.73 (dd, $J_{3,4} = J_{4,5} = 7.5$ Hz, 1H, pyridyl H-4), 8.51 (d, $J_{5,6} = 4.8$ Hz, 1H, pyridyl H-6), 10.0 (br s, 1H, N*H*). Anal. ($C_{19}H_{23}N_3O_4$) C, H, N.

Cyclohexyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate Enantiomers [(+)−(14a) and (−)-14a]. A solution of either (+)-**15** or **(−)-15** (0.20 g, 0.72 mmol), cyclohexyl bromide (0.17 g, 1.08 mmol), and K_2CO_3 (93 mg, 0.72 mmol) in DMF (50 mL) was heated at 60 °C for 24 h. Removal of the excess DMF in vacuo, addition of water (20 mL), extraction with EtOAc (4 × 50 mL), drying the EtOAc extract (Na_2SO_4), and removal of the EtOAc in vacuo gave a residue which was separated by silica gel column chromatography using EtOAc−hexane (1:1, v/v) as eluent. Similar eluent fractions were combined, the solvent was removed in vacuo, and the residue was recrystallized from EtOAc−hexane to afford the respective product (+)-**14a** (35 mg, 12%) or (−)-**14a** (35 mg, 12%) as a yellow solid. Unreacted (+)-**15** or (−)-**15** (50 mg) was also recovered.

Enantiomer (+)-14a: mp 188 °C; $[\alpha]^{23}_D = +146.7°$ (*c* 0.37, $CHCl_3$); IR and ^1H NMR spectra of (+)-**14a** were identical with those reported for *rac*-**14** described above.

Enantiomer (−)-14a: mp 186 °C; $[\alpha]^{23}_D = -146.7°$ (*c* 0.37, $CHCl_3$); IR and ^1H NMR spectra of (−)-**14a** were identical with those reported for *rac*-**14** described above.

The optical purity of (+)-**14a** and (−)-**14a** was determined by ^1H NMR. When 40 μL of a solution (50 mg in 1 mL of $CDCl_3$) of the chiral shift reagent (+)-Eu(hfc)$_3$ was added to a solution of *rac*-**14a** (5 mg in 0.5 mL of $CDCl_3$), the 1,4-dihydropyridine C-2 and C-6 methyl resonances at δ 2.47 and 2.26 were each separated into two resonances which appeared at δ 2.52 and 2.58, and 2.40 and 2.46, respectively. Addition of (+)-Eu(hfc)$_3$ (40 μL) to (+)-**14a** or (−)-**14a**, 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 GPILSM using the procedure reported.[21] 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 GPLA relative to its basal contractile force in the absence of test compound.

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