Synthesis, Rotamer Orientation, and Calcium Channel Modulation Activities of Alkyl and 2-Phenethyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates[†]

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A group of racemic alkyl and 2-phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates (**13a**–**q**) was prepared using a modified Hantzsch reaction that involved the condensation of a 3- or 6-substituted-2-pyridinecarboxaldehyde (**7a**–**j**) with an alkyl or 2-phenethyl 3-aminocrotonate (**11a**–**d**) and nitroacetone (**12**). Nuclear Overhauser (NOE) studies indicated there is a significant rotamer fraction in solution where the pyridyl nitrogen is oriented above the 1,4-dihydropyridine ring, irrespective of whether a substituent is located at the 3- or 6-position. A potential H-bonding interaction between the pyridyl nitrogen free electron pair and the suitably positioned 1,4-dihydropyridine N*H* moiety may stablize this rotamer orientation. In vitro calcium channel antagonist and agonist activities were determined using guinea pig ileum longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA) assays, respectively. Compounds having an *i*-Pr ester substituent acted as dual cardioselective calcium channel agonists (GPLA)/smooth muscle-selective calcium channel antagonists (GPILSM), except for the C-4 3-nitro-2-pyridyl compound which exhibited an antagonist effect on both GPLA and GPILSM. In contrast, the compounds with a phenethyl ester group, which exhibited antagonist activity ($IC_{50} = 10^{-5}$–10^{-7} M range) on GPILSM, were devoid of cardiac agonist activity on GPLA. Structure–activity relationships showing the effect of a substituent (Me, CF_3, Cl, NO_2, Ph) at the 3- or 6-position of a C-4 2-pyridyl moiety and a variety of ester substituents (Me, Et, *i*-Pr, PhCH₂CH₂-) upon calcium channel modulation are described. Compounds possessing a 3- or 6-substituted-2-pyridyl moiety, in conjuction with an *i*-Pr ester substituent, are novel 1,4-dihydropyridine calcium channel modulators that offer a new drug design approach directed to the treatment of congestive heart failure and may also be useful as probes to study the structure–function relationships of calcium channels.

The structure–activity relationships for Hantzsch type 1,4-dihydropyridines, with respect to calcium channel antagonist–agonist modulation, have presented a significant challenge.[1–14] The calcium ion channel is an important drug design target since it possesses specific drug binding sites for both antagonist and agonist ligands that are modulated by the closed or open conformational state of the channel. Different states of the channel have different affinities and/or access for drugs, and drugs may exhibit both quantitative and qualitative differences in structure–activity relationships, including stereoselectivity between channel states.[14] Accordingly, ion channels can be viewed as multiple drug binding receptors that typically have 4–8 discrete binding sites which may be individually linked to each other and to the gating and permeation machinery of the ion channel by complex allosteric interactions.[15]

1,4-Dihydropyridines of the nifedipine class [dialkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-aryl)-3,5-pyridinedicarboxylates] are flexible molecules (**1a**), in which the C-4 aryl moiety and the C-3/C-5 ester substituents can rotate and the conformation of the 1,4-dihydropyridine ring can change (Figure 1). The 1,4-dihydropyridine ring exists in a flat-boat conformation with the C-4 aryl moiety in a pseudoaxial position and orthogonal to the plane of the 1,4-dihydropyridine ring. In addition, two rotamers may exist if the C-4 aryl ring is substituted at its ortho or meta position (X ≠ H). The X-substituent either could then be on the same side as the C-4H as in **1b** [synperiplanar (sp) to the C-4H or distal to the 1,4-dihydropyridine ring] or, following rotation of the phenyl ring, could be oriented above the 1,4-dihydropyridine ring as in **1c** [antiperiplanar (ap) to the C-4H or proximal to the 1,4-dihydropyridine ring].[16] Although the rotation barrier about the C(4)–C(phenyl) central C–C single bond is small, molecular mechanics calculations for Hantzsch 1,4-dihydropyridines show the rotational barrier separating the distal (sp) and proximal (ap) rotamers is higher for a C-4 phenyl ring having an ortho-substituent relative to analogues having a meta- or para-substituent.[17] Ab initio STO-3G calculations similarly showed that an *o*-phenyl substituent also favors the sp rotamer orientation, although the energy difference or rotational barrier between the sp and ap rotamers is not sufficiently large to exclude the ap rotamer from participation in binding to the calcium channel receptor.[18] The observation that the fraction of the sp rotamer in solution showed a positive correlation with vasorelaxant activity and
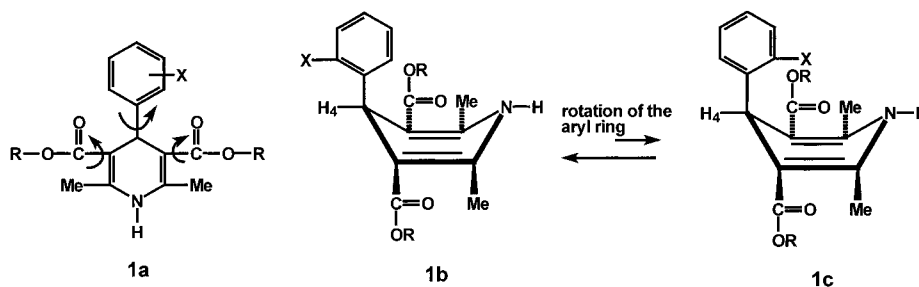

Figure 1. Rotation of the C-4 aryl moiety and the C-3/C-5 ester substituents (**1a**) to give the two rotamers [synperiplanar (distal) **1b** and antiperiplanar (proximal) **1c**].
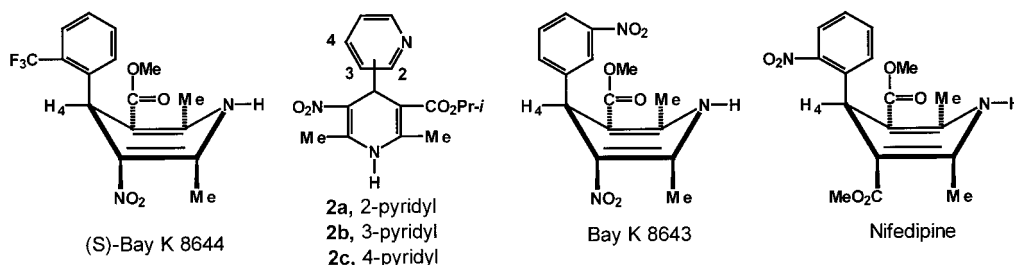

Figure 2. Structures of (S)-Bay K 8644, the isomeric isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(pyridyl)-5-pyridinecarboxylates **2a**−**c**, Bay K 8643, and nifedipine.

receptor binding affinity suggests the sp rotamer of nonrigid unsymmetrically substituted 4-phenyl-1,4-dihydropyridine calcium antagonists is the receptor-bound conformation.[19]
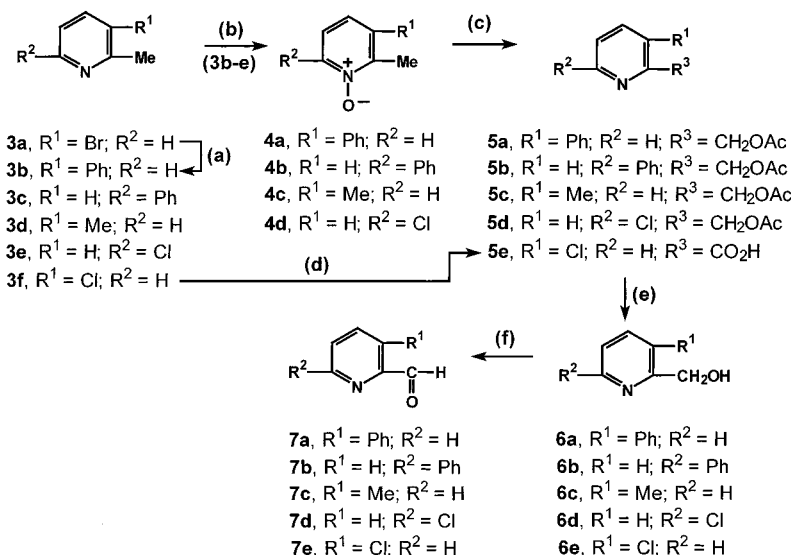
A normal vs capsized DHP boat model was recently proposed to explain structural and conformational requirements for modulation of calcium channel action where a left-hand-side alkoxy (cis) carbonyl interaction is required for maximal DHP receptor affinity, and the effect on channel action is determined by the orientation of the 4-aryl moiety. Thus enantiomers having an up-oriented pseudoaxial aryl group (normal DHP boat) elicit antagonist activity, while enantiomers having a down-oriented pseudoaxial aryl group (capsized DHP boat) exhibit agonist activity.[20] However, the issue of antagonism or agonism is dependent not only on the structure and stereochemistry of the 1,4-DHP but also upon the state of the calcium channel due to its membrane potential.[15] This latter phenomenon is clearly illustrated by the potent Ca^{2+} agonist (S)-Bay K 8644 (see Figure 2) which acts as an agonist at polarized membrane potentials and as an antagonist at depolarized membrane levels, respectively.[21] Although the interaction of calcium antagonists with Ca^{2+} has received little attention, a recent study employing nicardipine showed the predominant species to be a 2:1 drug:Ca^{2+} sandwich complex that involved coordination of Ca^{2+} to oxygen of the *m*-nitrophenyl substituent and the carbonyl moiety of the C-3 ester substituent. Based on this result, it was suggested that the Ca^{2+}-bound form of DHP drugs may constitute their biological active species in the nonpolar milieu of a lipid bilayer.[22]

A novel third-generation class of isomeric 1,4-dihydro-2,6-dimethyl-3-nitro-4-(pyridyl)-5-pyridinecarboxylates (**2a**−**c**; see Figure 2) with different calcium channel modulation activities was recently discovered.[23] The 2-pyridyl isomer (±)-**2a** acted as a dual cardioselective calcium channel agonist/smooth muscle-selective calcium channel antagonist. On the other hand, 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, whereas the (−)-2-pyridyl enantiomer (−)-**2a** exhibited cardiac agonist and smooth muscle antagonist actions. It was therefore of interest to extend these structure−activity data by replacing the isopropyl moiety of **2a** by a 2-phenethyl substituent and/or introducing a 3- or 6-substituent (CH_3, CF_3, Cl, NO_2, C_6H_5) into the 2-pyridyl ring since this novel type of 1,4-DHP calcium channel modulator could provide a potentially new approach directed toward the treatment of congestive heart failure (CHF) and for use as probes to study the structure−function relationships of calcium channels. We now report the synthesis of alkyl and 2-phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates (**13a**−**q**), their rotameric orientation, and their in vitro calcium channel modulating actions on guinea pig smooth muscle and left atrium.

Chemistry

The 3- and 6-substituted-2-pyridinecarboxaldehydes **7a**−**e**, required for the modified Hantzsch 1,4-dihydropyridine reaction, were prepared using a four-step synthetic sequence as illustrated in Scheme 1. Thus, oxidation of the 3- or 6-substituted-2-methylpyridines **3b**−**e** with H_2O_2 in AcOH yielded the corresponding *N*-oxide derivatives **4a**−**d** in 55−89% yields. Reaction of **4a**−**d** with acetic anhydride afforded the respective 2-(acetoxymethyl)-3(or 6)-substituted-pyridines **5a**−**d** in 75−81% yields. Hydrolysis of the acetate derivatives **5a**−**d** with either 1 N NaOH or K_2CO_3/MeOH afforded the corresponding 2-(hydroxymethyl)-3(or 6)-substituted-pyridines **6a**−**d** in 51−86% yields. In contrast, 3-chloro-2-(hydroxymethyl)pyridine (**6e**) was prepared by oxidation of 3-chloro-2-methylpyridine (**3f**) with $KMnO_4$ to yield 3-chloro-2-pyridinecarboxylic acid (**5e**; 45%) which on treatment with $ClCO_2Et$ to form the mixed anhydride and reduction with $NaBH_4$ gave **6e** (67%). Oxidation

Scheme 1[a]

3a, R^1 = Br; R^2 = H
3b, R^1 = Ph; R^2 = H
3c, R^1 = H; R^2 = Ph
3d, R^1 = Me; R^2 = H
3e, R^1 = H; R^2 = Cl
3f, R^1 = Cl; R^2 = H

4a, R^1 = Ph; R^2 = H
4b, R^1 = H; R^2 = Ph
4c, R^1 = Me; R^2 = H
4d, R^1 = H; R^2 = Cl

5a, R^1 = Ph; R^2 = H; R^3 = CH$_2$OAc
5b, R^1 = H; R^2 = Ph; R^3 = CH$_2$OAc
5c, R^1 = Me; R^2 = H; R^3 = CH$_2$OAc
5d, R^1 = H; R^2 = Cl; R^3 = CH$_2$OAc
5e, R^1 = Cl; R^2 = H; R^3 = CO$_2$H

7a, R^1 = Ph; R^2 = H
7b, R^1 = H; R^2 = Ph
7c, R^1 = Me; R^2 = H
7d, R^1 = H; R^2 = Cl
7e, R^1 = Cl; R^2 = H

6a, R^1 = Ph; R^2 = H
6b, R^1 = H; R^2 = Ph
6c, R^1 = Me; R^2 = H
6d, R^1 = H; R^2 = Cl
6e, R^1 = Cl; R^2 = H

[a] Reagents and conditions: (a) benzene, C$_6$H$_5$B(OH)$_2$ in EtOH, 2 M aqueous Na$_2$CO$_3$, Pd[P(Ph)$_3$]$_4$, reflux, 24 h; (b) AcOH, 35% H$_2$O$_2$, 70−80 °C, 12 h; (c) Ac$_2$O, reflux, 1 h; (d) KMnO$_4$, water, reflux, 22 h; (e) 1 N NaOH, 25 °C, 1.5 h (products **6a−c**), K$_2$CO$_3$, MeOH, 25 °C, 1.5 h (product **6d**), or ClCO$_2$Et, THF, Et$_3$N, 5 °C, 30 min and then NaBH$_4$, H$_2$O, 10−15 °C, 4 h (product **6e**); (f) dicyclohexylcarbodiimide, DMF, H$_3$PO$_4$, 25 °C, 1.5 h (products **7a−c,e**) or MnO$_2$, CHCl$_3$, 25 °C, 24 h (product **7d**).

Scheme 2[a]
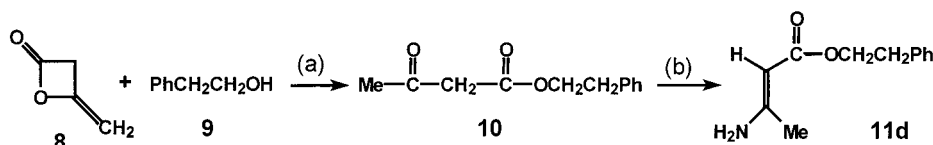
[a] Reagents and conditions: (a) Et$_3$N catalyst, 80 °C, 1 h; (b) NH$_3$, MeOH, 25 °C, 6 h.

of the 2-(hydroxymethyl)-3(or 6)-substituted-pyridines **6a−e** using either dicyclohexylcarbodiimide (DCC) or MnO$_2$ yielded the respective 3(or 6)-substituted-2-pyridinecarboxaldehydes **7a−e** (36−52%).

The Et$_3$N-catalyzed reaction of 2-phenylethanol (**9**) with diketene (**8**) afforded 2-phenethyl acetoacetate (**10**; 73%) which was elaborated to 2-phenethyl 3-aminocrotonate (**11d**; 90%) on treatment with NH$_3$ in MeOH (see Scheme 2).

The racemic alkyl or 2-phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates **13a−q** were prepared by a modified Hantzsch reaction. Accordingly, condensation of the respective aldehyde (**7a−j**) with the respective alkyl or 2-phenethyl 3-aminocrotonate (**11a−d**) and nitroacetone (**12**) afforded the title compounds (**13a−q**) in 24−71% yields as illustrated in Scheme 3 and summarized in Table 1.
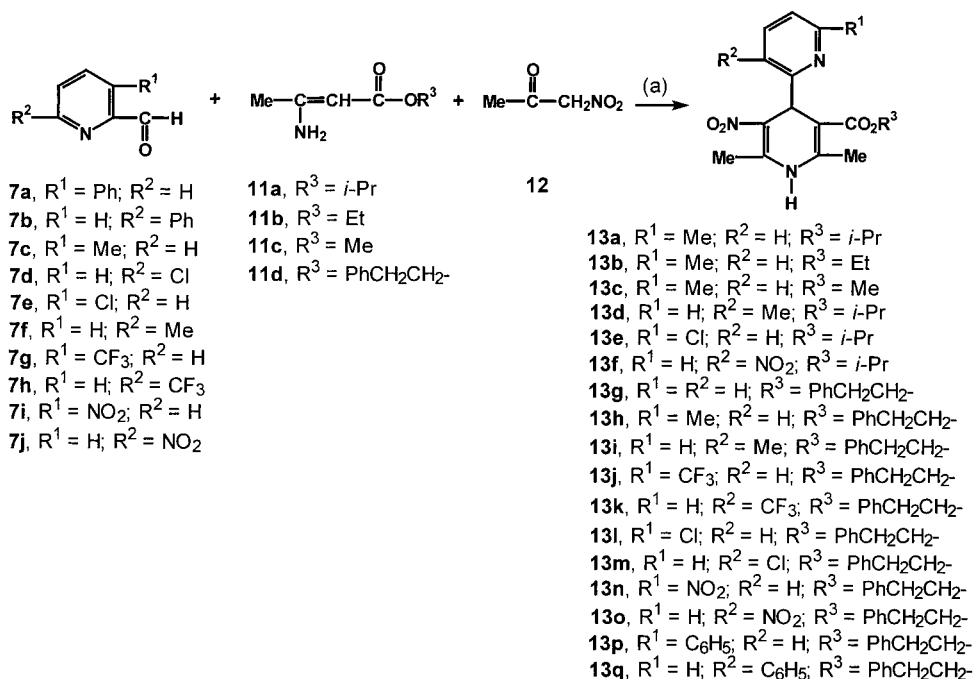
Results and Discussion

The development of calcium channel modulators that are useful for treating CHF will be dependent upon the separation and/or elimination of their vasoconstrictor effect from their cardiostimulant-positive inotropic property.[24] Differences in the molecular electrostatic potentials between agonist and antagonist structures, with respect to binding of the C-3 and C-5 DHP regions, have been observed, which may provide a mechanism by which the receptor differentiates between agonist and antagonist ligands. In this regard, calcium channel agonists have been shown to possess a strong negative potential in the region of the C-3 nitro substituent, while

antagonists showed a positive potential in this region.[25] In addition, the effect of aromatic substituents on the C-4 phenyl ring of 1,4-dihydropyridine agonists and antagonists is also different.[26] These observations, in conjunction with the dual cardioselective agonist/smooth muscle-selective antagonist calcium channel-modulating effects exhibited by isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (**2a**),[23] prompted us to study analogues of **2a** where the isopropyl substituent is replaced by other alkyls or a 2-phenethyl substituent and a substituent is introduced at the 3- or 6-position of the C-4 2-pyridyl moiety.

The nature (electronic properties, steric size) and position (3 or 6) of the substituent on the 2-pyridyl ring system was expected to be a determinant of the electronic charge distribution at the pyridyl ring carbons, the global conformation of the molecule due to nonbonded interactions between the C-3, C-4, and C-5 substituents, and the rotameric orientation and/or preference (sp/ap) of the substituted-2-pyridyl moiety. These factors may provide an approach to optimize calcium channel binding, calcium channel modulation, and/or tissue specificity.

It has been suggested that molar refraction (MR) values are a crude, but useful, measure of substituent bulk (size) which have been used in some quantitative structure−activity relationship (QSAR) studies.[27] A series of 1,4-dihydropyridines **13a−q** were prepared wherein the 3- or 6-substituent on the C-4 2-pyridyl moiety possesses a broad range of MR values (H, 1.03; CF$_3$, 5.02; CH$_3$, 5.65; Cl, 6.03; NO$_2$, 7.36; C$_6$H$_5$-, 25.36).[27] The van der Waals radi for H, Cl, and CH$_3$ are 1.2, 1.8,

Scheme 3[a]

7a, R[1] = Ph; R[2] = H
7b, R[1] = H; R[2] = Ph
7c, R[1] = Me; R[2] = H
7d, R[1] = H; R[2] = Cl
7e, R[1] = Cl; R[2] = H
7f, R[1] = H; R[2] = Me
7g, R[1] = CF_3; R[2] = H
7h, R[1] = H; R[2] = CF_3
7i, R[1] = NO_2; R[2] = H
7j, R[1] = H; R[2] = NO_2

11a, R[3] = *i*-Pr
11b, R[3] = Et
11c, R[3] = Me
11d, R[3] = PhCH_2CH_2-

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13a, R[1] = Me; R[2] = H; R[3] = *i*-Pr
13b, R[1] = Me; R[2] = H; R[3] = Et
13c, R[1] = Me; R[2] = H; R[3] = Me
13d, R[1] = H; R[2] = Me; R[3] = *i*-Pr
13e, R[1] = Cl; R[2] = H; R[3] = *i*-Pr
13f, R[1] = H; R[2] = NO_2; R[3] = *i*-Pr
13g, R[1] = R[2] = H; R[3] = PhCH_2CH_2-
13h, R[1] = Me; R[2] = H; R[3] = PhCH_2CH_2-
13i, R[1] = H; R[2] = Me; R[3] = PhCH_2CH_2-
13j, R[1] = CF_3; R[2] = H; R[3] = PhCH_2CH_2-
13k, R[1] = H; R[2] = CF_3; R[3] = PhCH_2CH_2-
13l, R[1] = Cl; R[2] = H; R[3] = PhCH_2CH_2-
13m, R[1] = H; R[2] = Cl; R[3] = PhCH_2CH_2-
13n, R[1] = NO_2; R[2] = H; R[3] = PhCH_2CH_2-
13o, R[1] = H; R[2] = NO_2; R[3] = PhCH_2CH_2-
13p, R[1] = C_6H_5; R[2] = H; R[3] = PhCH_2CH_2-
13q, R[1] = H; R[2] = C_6H_5; R[3] = PhCH_2CH_2-

[a] Reagents and conditions: (a) EtOH, 1 h at 25 °C and then 16 h at reflux (products **13a–e,g,h,m**) or Me_2CHOH, 9 h at 40 °C and then 13 h at 25 °C (products **13f,i–l,n–q**).

and 2.0 Å, respectively. Examination of the ^{1}H NMR spectra for **13a–q** indicated that the 1,4-DHP H-4 proton appeared in the δ 5.32–6.49 range (CDCl_3). The H-4 proton was always more shielded (higher field) when a C-6 pyridyl R[1]-substituent was present (δ 5.32–5.66 range) as compared to the same C-3 pyridyl R[2]-substituent (δ 5.74–6.49 range). Low-temperature ^{1}H NMR studies (300 MHz) of **13a–q** at −50 °C in CDCl_3 did not give rise to any dual resonances. Although this absence of dual resonances indicates the possibility that a single rotamer and/or preference for the C-4 2-pyridyl moiety exists in solution, it is equally plausible that the rotation barrier is too small to stop rotation at −50 °C or that there is a thermodynamic preference for one rotamer regardless of the magnitude of the energy barrier to rotation. However, Goldmann and Geiger[28] reported that the two *o*-methyl resonances for dimethyl 1,4-dihydro-2,6-dimethyl-4-(2,4,6-trimethylphenyl)-3,5-pyridinedicarboxylate appear as a broad singlet in CDCl_3 at 25 °C, coalescence occurred at −18 °C (ΔG(−18 °C) = 51.1 kJ/mol), and two separate resonances were observed at −50 °C. ^{1}H NMR difference nuclear Overhauser enhancement (NOE) studies were performed for **13g** and the three pairs of isomers **13a** and **13d**, **13h** and **13i**, and **13p** and **13q** to acquire information pertaining to the rotamer orientation of the C-4 2-pyridyl moiety. The percent NOE enhancements (DMSO-d_6, 22 °C) clearly show that a significant rotamer fraction is present in which the pyridyl nitrogen atom is oriented above the 1,4-DHP ring in all these cases irrespective of whether the substituent (H, Me, Ph) is located at the C-3 or C-6 position of the 2-pyridyl moiety (see Figure 3).

Similar NOE enhancement studies (CDCl_3, 22 °C) were performed for **13a**, **13h** (see Figure 4) since the H-bonding potential between the compound **13** and bulk solvent differs significantly between CDCl_3 and DMSO. In contrast to the NOE studies using DMSO-d_6 as

solvent, compounds **13a**,**h** showed NOE enhancements from the 1,4-DHP N*H* proton to the C-6 *Me* substituent on the pyridyl ring of 1.6% and 3.0%, respectively. However, a NOE from the N*H* hydrogen to the pyridyl H-3 hydrogen of **13a** or **13h** was not observed (CDCl_3, 22 °C), which suggests a rotamer in which the pyridyl H-3 hydrogen is oriented above the 1,4-DHP moiety and the pyridyl nitrogen atom is sp to the 1,4-DHP H-4 hydrogen either is not present or is a less predominant rotamer in solution. Rovnyak et al.[29] have elegantly determined the fraction of ap rotamer by measurement of NOEs from the N*H* hydrogen to an *o*-phenyl hydrogen (interatomic distances of 3.2–3.5 Å) for Hantzsch 1,4-DHPs. Variable temperature ^{1}H NMR spectroscopy is a useful method to study H-bonding interactions. Lowering temperature may stop N*H* exchange (gives rise to a sharp, or coupled, N*H* resonance) which can enhance H-bonding that results in a deshielding (downfield shift) of the N*H* proton. Conversely, increasing temperature may disrupt H-bonding resulting in a more rapid rate of N*H* exchange (gives rise to a broader resonance) that results in an upfield shift (shielding effect) for the N*H* proton due to disruption of H-bonding.[30] Accordingly, it was also observed (^{1}H NMR spectra) that the chemical shift of the N*H* proton in **13a**,**h** in CDCl_3 was highly temperature-dependent. For example, the N*H* proton for **13a** appeared at δ 10.28 (sharp singlet, 10 °C), 9.66 (sharp singlet, 22 °C), and 7.67 (broad singlet, 61 °C). Similarly, **13h** showed N*H* resonances at δ 10.13 (sharp singlet, 10 °C), 9.37 (sharp singlet, 22 °C), and 8.11 (broad singlet, 50 °C). All other resonances for **13a** or **13h** showed minor changes in chemical shift positions of less than δ 0.09, irrespective of temperature. These N*H* chemical shift dependence data indicate that the N*H* group must be H-bonded (sharp N*H*, more deshielded) at 10 and 22 °C and that H-bonding is disrupted (broad N*H*, more shielded) upon heating to 61 °C (**13a**) or 50 °C (**13h**).
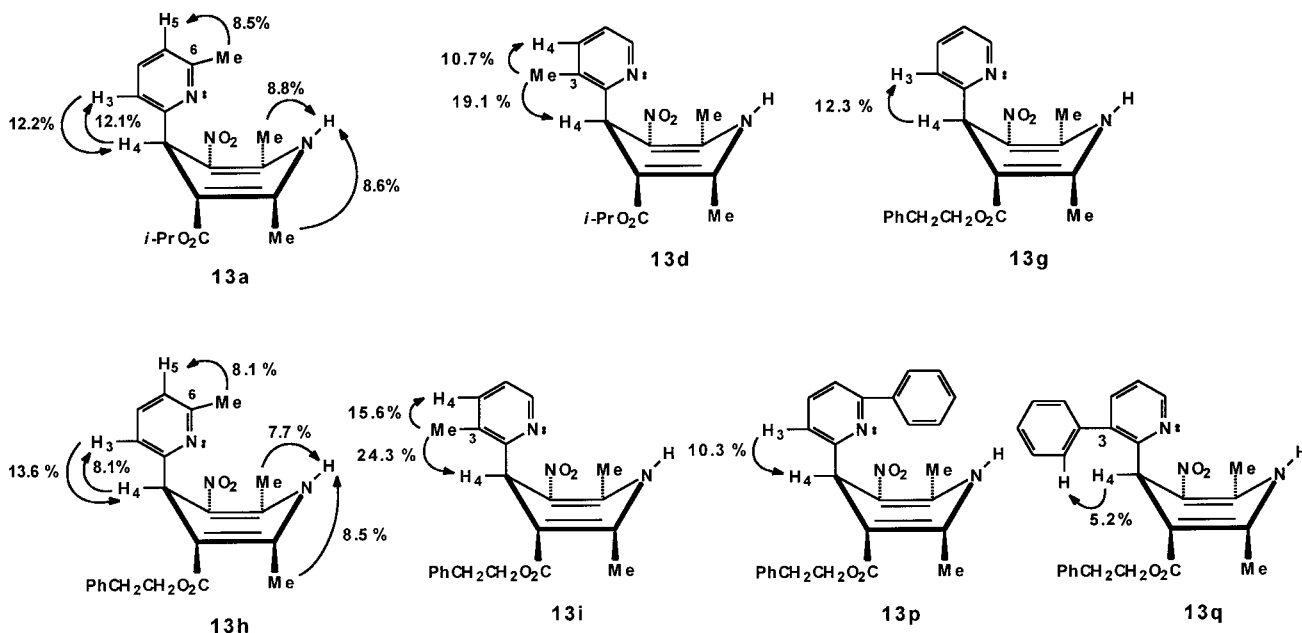
Figure 3. Some NOE determinations to study the rotameric orientation of the 4-(2-pyridyl) moiety for the 1,4-dihydropyridine compounds **13a,d,g−i,p,q** in DMSO-d_6 at 22 °C.
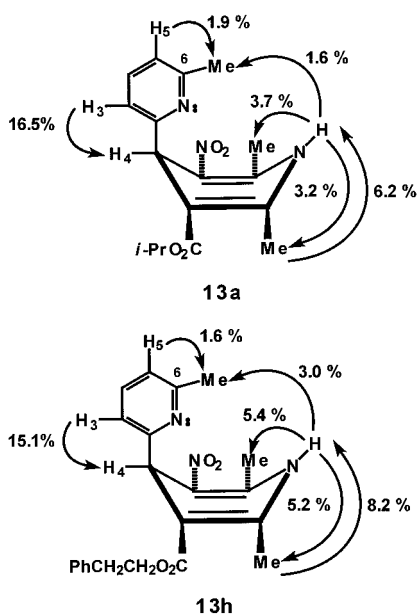

Figure 4. Some NOE determinations to study the rotameric orientation of the 4-(6-methyl-2-pyridyl) moiety for the 1,4-dihydropyridine compounds **13a,h** in CDCl₃ at 22 °C.

It has been reported, using a group of dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-halogenophenyl)-3,5-pyridinedicarboxylates, that the fraction of sp rotamer (f_s) increased with increasing van der Waals radius of the halogen substituent (F, 0.69; Cl, 0.84; Br, 0.92; I, 0.95) in solution.[19] The calcium channel antagonist nifedipine[29] and agonist Bay K 8644[6] both exist as sp rotamers. In contrast, the 4-(3-nitrophenyl) moiety present in the agonist Bay K 8643 has the unexpected ap rotamer orientation (X-ray structure), which places the *m*-nitro substituent on the phenyl ring directly above the 1,4-DHP ring (see structures in Figure 2).[8] The restricted 2-(trifluoromethyl)phenyl ring sp orientation of Bay K 8644 in the solid state is primarily due to steric contacts between the CF₃ fluorine atoms and the carbonyl ester and nitro oxygen atoms which would

occur in the ap orientation.[6] Accordingly, the ap rotamer for Bay K 8643 may be stablized by an electronic attraction between the nitro group on the phenyl ring and the 1,4-DHP moiety. In methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(aryl)-5-pyridinecarboxylates such as Bay K 8644 and Bay K 8643, the structure is stablized by a H-bond (2.94 ± 0.04 Å) between the N*H* and the 3-nitro oxygen atom which is cis to the C(2)=C(3) double bond.[8] The amine group of Bay K 8644 is significantly more acidic, due to electron delocalization, and is more capable of forming a stronger H-bond than the related antagonists having ester substituents at the 3- and 5-positions of the 1,4-DHP ring.[6] It was therefore of interest to investigate the conformation of **13a**, which exhibited the best calcium channel agonist activity on guinea pig left atrium, to gain further information pertaining to the H-bonding effect observed for the N*H* hydrogen in CDCl₃ at 22 °C and the rotamer orientation of the pyridyl ring system. Weaver et al.[31] have shown that AM1 semiempircal molecular orbital conformational analyses of 1,4-DHP calcium channel modulators are best suited to the modeling of DHP geometry.[31] Some interatomic distances for the AM1-minimized structure of **13a** are shown in Figure 5. The distance between the amine and pyridyl nitrogen atoms (3.47 Å) is shorter than that between the amine nitrogen and nitro oxygen atom that is cis to the C(2)=C(3) double bond (4.18 Å) or the carbonyl oxygen atom (4.49 Å). These data suggest there may be a possibility for the amine N*H* hydrogen atom to H-bond to the pyridyl nitrogen free electon pair. A H-bond of this type would position the N*H* hydrogen closer to the pyridyl nitrogen free electron pair where the donor N*H* distance is less than 3.2 Å and the angle made by covalent bonds to the donor and acceptor atoms is less than 120°. Although the preferred geometry of the amine nitrogen is trigonal with the N*H* hydrogen projected away from the DHP ring, the formation of a H-bond between the N*H* and pyridyl nitrogen free electron pair may compensate for the decrease in energy resulting from a change in the
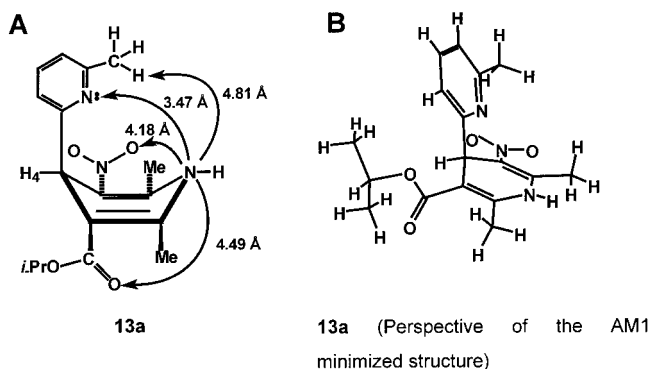
Figure 5. Some interatomic distances for the AM1-minimized structure of isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(6-methyl-2-pyridyl)-5-pyridinecarboxylate (**13a**) (panel A) and perspective of the AM1-minimized structure for **13a** (panel B).

N*H* orientation. Accordingly, a H-bond between the pyridyl nitrogen free electron pair and the 1,4-DHP N*H*, which would place the pyridyl nitrogen atom above the 1,4-DHP ring, would remove the requirement for a high-energy rotational barrier that would be necessary to exclusively, or preferentially, favor this rotameric orientation. It is also possible that an electronic attraction between the pyridyl ring and the 1,4-DHP moiety would increase the fraction of this rotamer population. The interatomic distance between the amine nitrogen and pyridyl *CH₃* of 4.81 Å is consistent with the NOE effect (1.6%) shown in Figure 4. For the purpose of comparison, the distance between the amine nitrogen and H-3 on the pyridyl ring, when **13a** is minimized (AM1) with the pyridyl nitrogen sp to H-4, was 3.26 Å. The observation that no NOE effect was observed from N*H* to the pyridyl H-3 in **13a** also suggests that **13a** exists preferentially as the ap rotamer shown in Figure 4. The nitro group of **13a** in the AM1-minimized structure is in the plane of the C(2)=C(3) bond to which it is attached since the C(2)=C(3)−N−O (*cis*-oxygen) and C(2)=C(3)−N−O (*trans*-oxygen) torsion angles are −2.9° and 176.2°, respectively. AM1 geometry optimizations for compounds **13a,d,g−i,p,q** (see ap rotamer orientation shown in Figure 3) were performed for both the preferred ap rotamer (pyridyl N anti to H-4) and the less favored sp rotamer (pyridyl N syn to H-4). In all cases, the preferential ap rotamer orientation showed a thermodynamic preference, viz.: **13a** (6-Me), 3.00 kcal; **13d** (3-Me), 7.47 kcal; **13g** (3-H), 3.54 kcal; **13h** (6-Me), 3.59 kcal; **13i** (3-Me), 7.92 kcal; **13p** (6-phenyl), 2.96 kcal; and **13q** (3-phenyl), 6.37 kcal. The observation that the energy differences between the two rotamer orientations were greater for **13i** (3-Me) and **13q** (3-phenyl) is attributed to the larger steric interactions between the 1,4-dihydropyridine C-3, C-4, and C-5 substituents. Thermodynamic preferences ≥ 3 kcal are considered to be significant with respect to rotamer orientation. The results of these studies do not indicate whether the N*H* is H-bonded to the *cis*-oxygen atom of the 3-nitro substituent or the pyridyl nitrogen free electron pair. An X-ray structure for **13a** will be required to distinguish between these two potential alternatives.

The in vitro calcium channel antagonist- and agonist-modulating activities of racemic compounds **13a−q** were determined using guinea pig ileum longitudinal smooth muscle (GPILSM) and guinea pig left atrium (GPLA),

respectively. The calcium channel antagonist activities of **13a−q** determined as the concentration required to produce 50% inhibition of GPILSM contractility are presented in Table 1. Compounds **13a−q** exhibited weaker antagonist activity (IC$_{50}$ = 10^{-5}−10^{-7} M range) than the reference drug nifedipine (IC$_{50}$ = 1.40×10^{-8} M). The R³-ester substituent was a determinant of antagonist activity for compounds possessing a C-4 6-methyl-2-pyridyl moiety where the potency order was Me (**13c**) > PhCH₂CH₂ (**13h**) > Et (**13b**) and *i*-Pr (**13a**). A similar activity profile was observed for compounds possessing a C-4 6-chloro-2-pyridyl moiety [PhCH₂CH₂- (**13l**) > *i*-Pr (**13e**)]. In contrast, for compounds having a C-4 3-nitro-2-pyridyl moiety, the *i*-Pr ester (**13f**) was more active than the PhCH₂CH₂- ester (**13o**). In the isopropyl ester group of compounds, the nature of the R¹-substituent at the 6-position [Me (**13a**) ≈ Cl (**13e**)], or the R²-substituent at the 3-position [Me (**13d**) ≈ NO₂ (**13f**)], of the 2-pyridyl moiety was not a determinant of antagonist activity. In the phenethyl ester group of compounds **13g−q**, incorporation of a substituent (Me, CF₃, Cl, NO₂, Ph) at either the 3- or 6-position of the C-4 2-pyridyl moiety decreased antagonist activity (IC$_{50}$ = 1.51×10^{-6} to >5.96×10^{-5} M range) relative to the unsubstituted 2-pyridyl compound **13g** (IC$_{50}$ = 6.39×10^{-7} M). In this latter series of compounds **13h−q**, the nature of the R¹-substituent at the 6-position of the 2-pyridyl ring (**13h,j,l,n,p**) had a small effect on antagonist activity (IC$_{50}$ = 1.41×10^{-6} to 9.98×10^{-6} M range). In contrast, the effect of a R²-substituent at the 3-position of the 2-pyridyl ring (**13i,k,m,o,q**) produced a larger effect on antagonist activity (IC$_{50}$ = 3.35×10^{-6} to >5.96×10^{-5} M range) where the relative potency order was Ph ≥ Cl and Me > NO₂ > CF₃. The effect which the position of the substituent on the C-4 2-pyridyl moiety (6-R¹ versus 3-R²) had upon antagonist activity was variable where R² > R¹ [Me (**13d**) > Me (**13a**); Me (**13i**) > Me (**13h**); Ph (**13q** > Ph (**13p**)], but in other cases R¹ > R² [CF₃ (**13j**) > CF₃ (**13k**); Cl (**13l**) > Cl (**13m**); NO₂ (**13n**) > NO₂ (**13o**)]. These isomeric differences in antagonist activities for the ortho-like-R²-substituent that is sp to the 1,4-DHP H-4 versus the meta-like-R¹-substituent that is ap to the 1,4-DHP H-4 (see Figure 2) could be due to a number of possibilities which include differences in the drug−receptor interaction or preferential affinity or access to the α₁-subunit binding site of the L-type calcium channel.[14]

In vitro calcium channel agonist (positive inotropic) activities were determined as the molar concentration eliciting 50% (EC$_{50}$) of the maximum contractile response produced by the racemic test drug on GPLA, as determined from the dose−response curve. In this assay, the reference drugs (±)-Bay K 8644 and (±)-2-pyridyl **2a** showed agonist EC$_{50}$ values of 7.7×10^{-7} and 9.67×10^{-6} M, respectively.[23] A comparison of the C-4 6-methyl-2-pyridyl compounds **13a−c** showed the R³-ester substituent was a determinant of GPLA agonist activity where the activity profile was *i*-Pr (**13a**, EC$_{50}$ = 1.81×10^{-6} M) > Et (**13b**, EC$_{50}$ = 1.23×10^{-5} M) > Me (**13c**, 10% increase in contractile force at 4.50×10^{-5} M). In the R³-*i*-Pr ester series, the 6-methyl-2-pyridyl compound **13a** (EC$_{50}$ = 1.81×10^{-6} M) was a more potent agonist than the 3-methyl-2-pyridyl isomer **13d** (EC$_{50}$ = 9.97×10^{-6} M). These latter results, in

Table 1. Physical and Calcium Channel Antagonist Activities of Alkyl or 2-Phenethyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates **13a–q**
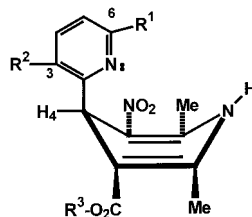
compd	R^1	R^2	R^3	cryst. solvent	mp (°C)	yield (%)	formula[a]	calcium channel antagonist act: IC_{50}[b]	calcium channel agonist act: EC_{50}[c]
13a	Me	H	*i*-Pr	EtOAc–hexane	189–190	53	$C_{17}H_{21}N_3O_4$	$2.51 \pm 0.35 \times 10^{-5}$ (5)	$1.81 \pm 0.92 \times 10^{-6}$ (3)
13b	Me	H	Et	EtOAc–hexane	170–171	33	$C_{16}H_{19}N_3O_4$	$1.72 \pm 0.12 \times 10^{-5}$ (3)	$1.23 \pm 0.58 \times 10^{-5}$ (3)
13c	Me	H	Me	EtOAc–hexane	199–200	43	$C_{16}H_{19}N_3O_4$	$2.29 \pm 0.10 \times 10^{-6}$ (3)	weak agonist[d]
13d	H	Me	*i*-Pr	EtOAc–hexane	240–241	29	$C_{17}H_{21}N_3O_4$	$2.82 \pm 0.66 \times 10^{-6}$ (3)	$9.97 \pm 1.87 \times 10^{-6}$ (3)
13e	Cl	H	*i*-Pr	EtOAc–hexane	173–175	71	$C_{16}H_{18}ClN_3O_4$	$1.67 \pm 0.57 \times 10^{-5}$ (3)	$5.96 \pm 2.13 \times 10^{-6}$ (3)
13f	H	NO_2	*i*-Pr	EtOAc–hexane	205–206	27	$C_{16}H_{18}N_4O_6$	$3.77 \pm 1.86 \times 10^{-6}$ (3)	weak antagonist
13g	H	H	PhCH$_2$CH$_2$-	EtOAc–hexane	184–185	24	$C_{21}H_{21}N_3O_4$	$6.39 \pm 2.63 \times 10^{-7}$ (3)	
13h	Me	H	PhCH$_2$CH$_2$-	EtOAc-ether	193–194	73	$C_{22}H_{23}N_3O_4$	$9.98 \pm 0.56 \times 10^{-6}$ (3)	
13i	H	Me	PhCH$_2$CH$_2$-	EtOAc	233–235	35	$C_{22}H_{23}N_3O_4$	$5.43 \pm 0.51 \times 10^{-6}$ (3)	
13j	CF_3	H	PhCH$_2$CH$_2$-	EtOAc–hexane	134–135	37	$C_{22}H_{20}F_3N_3O_4$	$1.41 \pm 0.24 \times 10^{-6}$ (3)	
13k	H	CF_3	PhCH$_2$CH$_2$-	EtOAc	207–209	33	$C_{22}H_{20}F_3N_3O_4$	$>5.98 \times 10^{-5}$ (3)	
13l	Cl	H	PhCH$_2$CH$_2$-	EtOAc–hexane	167–168	53	$C_{21}H_{20}ClN_3O_4$	$1.51 \pm 0.06 \times 10^{-6}$ (3)	
13m	H	Cl	PhCH$_2$CH$_2$-	EtOAc–hexane	209–211	48	$C_{21}H_{20}ClN_3O_4$	$5.17 \pm 0.76 \times 10^{-6}$ (3)	
13n	NO_2	H	PhCH$_2$CH$_2$-	EtOAc	171–172	28	$C_{21}H_{20}N_4O_6$	$4.74 \pm 0.26 \times 10^{-6}$ (3)	
13o	H	NO_2	PhCH$_2$CH$_2$-	EtOAc	205–206	30	$C_{21}H_{20}N_4O_6$[a]	$7.77 \pm 0.61 \times 10^{-5}$ (3)	
13p	C_6H_5-	H	PhCH$_2$CH$_2$-	CHCl$_3$-hexane	80–81	45	$C_{27}H_{25}N_3O_4$	$8.38 \pm 0.73 \times 10^{-6}$ (3)	
13q	H	C_6H_5-	PhCH$_2$CH$_2$-	EtOAc–hexane	207–209	44	$C_{27}H_{25}N_3O_4$	$3.35 \pm 0.08 \times 10^{-6}$ (3)	
nifedipine								$1.40 \pm 0.19 \times 10^{-8}$ (18)	
2a[e]								$4.87 \pm 2.06 \times 10^{-6}$ (3)	$9.67 \pm 1.27 \times 10^{-6}$ (3)

[a] Microanalytical analyses were within ±0.4% of theoretical values, except for compound **13o**: N, calcd, 13.20; found, 12.77. [b] The molar concentration of antagonist test compound causing a 50% decrease in the slow component or tonic contractile response (IC$_{50}$ ± SEM) in guinea pig ileal longitudinal smooth muscle by the muscarinic agonist carbachol (1.6×10^{-7} M) was determined graphically from the dose–response curve. The number of experiments is shown in parentheses. [c] The molar concentration of the agonist test compound causing a 50% increase of the maximum contractile response (EC$_{50}$ ± SEM) on guinea pig left atrium was determined graphically from the dose–response curves. The number of experiments is shown in parentheses. [d] A 10% increase in contractile force was observed at 4.50×10^{-5} M. [e] Data for racemic **2a** taken from Vo et al.[23]

conjunction with the observation that the 6-chloro-2-pyridyl **13e** (EC$_{50}$ = 5.96×10^{-6} M) exhibited a positive inotropic agonist effect, whereas the 3-nitro-2-pyridyl **13f** exhibited a negative inotropic effect on GPLA, suggest that a substituent at the 6-position of the 2-pyridyl moiety provides superior cardiac agonist activity compared to compounds having a 3-substituted-2-pyridyl moiety. A group of R^3-phenethyl ester compounds (**13g–q**), having Me, CF$_3$, Cl, NO$_2$, and Ph substituents at either the 3- or 6-position of the C-4 2-pyridyl moiety, was investigated as cardiac agonists in an attempt to increase the dual cardiac agonist/smooth muscle potency profile since the C-4 2-pyridyl compound **13g** was the most potent antagonist on GPILSM (IC$_{50}$ = 6.39×10^{-7} M) among the group of compounds **13a–q**. However, all of the R^3-phenethyl esters **13h–q**, including the unsubstituted 2-pyridyl compound **13g**, were devoid of cardiac agonist activity on GPLA. The most potent agonist on GPLA **13a**, having a C-4 6-methyl-2-pyridyl moiety and an isopropyl ester group, was 2–3-fold less active than (±)-Bay K 8644.

Summary

The alkyl and 2-phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates **13a–q** constitute a novel group of compounds with different calcium channel modulation activities. Nuclear Overhauser enhancement (NOE) studies indicate a significant rotamer fraction exists in solution where the pyridyl nitrogen atom is oriented above the 1,4-DHP ring, irrespective of whether a substituent is located at the 3- or 6-pyridyl position. This rotamer orientation may possibly be the result of a H-bonding interaction between the pyridyl nitrogen free electron pair and the suitably positioned 1,4-DHP N*H* moiety. The absence of a second rotamer in which the pyridyl nitrogen atom is sp to the DHP H-4 hydrogen and the pyridyl H-3 hydrogen is oriented above the 1,4-DHP ring has not been proven. However, the failure to observe a NOE effect from the N*H* hydrogen to the pyridyl H-3 hydrogen indicates this second rotamer is certainly less predominant, if present in solution. The orientation of the 6-substituted-2-pyridyl group differs from that of an ortho-substituted-phenyl ring in Hantzsch 1,4-DHP calcium channel antagonists since the rotamer where the *o*-phenyl ring substituent is sp to the 1,4-DHP H-4 is generally thermodynamically more preferential.[19] Isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(6-methyl-2-pyridyl)-5-pyridinecarboxylate (**13a**), a novel type of 1,4-DHP calcium channel modulator, possesses the most favorable activities and function profile (GPILSM antagonist, IC$_{50}$ = 2.51×10^{-5} M; GPLA agonist, EC$_{50}$ = 1.81×10^{-6} M). Compounds such as **13a** offer a potentially new approach in drug design directed toward the treatment of congestive heart failure, and it could be a useful probe to investigate the structure–function relationship of calcium channels.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded and nuclear Overhauser enhancement (NOE) experiments were performed using a Bruker AM-300 spectrometer (300 MHz). The assignment of exchangeable protons (N*H*, O*H*) was confirmed by the addition of D_2O. The NOE studies were performed under steady-state conditions using the Bruker NOE DIFF.AU software program (signal:noise ratio of 136 for a single pulse). DMSO-d_6 was dried using molecular sieves (type 3A, 1.6-mm pellets) and degassed by passage of dry argon gas at 22 °C just prior to use. $CDCl_3$ was similarly dried, treated with neutral alumina to remove acidic impurities, and degassed likewise just prior to use. Molecular tumbling time was not altered. Infrared spectra were acquired using a Nicolet IR-500 Series II spectrometer. 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. 2,3-Lutidine (**3d**), 6-chloro-2-methylpyridine (**3e**), 6-methyl-2-pyridinecarboxaldehyde (**7f**), and alkyl 3-aminocrotonates (**11a−c**) were purchased from the Aldrich Chemical Co. 3-Bromo-2-methylpyridine (**3a**),[32] 2-methyl-3-phenyl-pyridine (**3c**),[33] 3-chloro-2-methylpyridine (**3f**),[32] 3-(trifluoromethyl)-2-pyridinecarboxaldehyde (**7g**),[34] 6-(trifluoromethyl)-2-pyridinecarboxaldehyde (**7h**),[34] 3-nitro-2-pyridinecarboxaldehyde (**7i**),[34] 6-nitro-2-pyridinecarboxaldehyde (**7j**),[34] and nitroacetone (**12**)[35] were prepared according to the reported procedures. The semiempirical AM1-minimized structure of **13a** was determined using the HyperChem molecular visualization and simulation program, Release 4 (Hypercube Inc., Waterloo, Canada).

2-Methyl-3-phenylpyridine (3b). A solution of phenylboronic acid (9.90 g, 27 mmol) in EtOH (18 mL), aqueous Na_2CO_3 (36 mL of 2 M), and Pd[P(Ph)$_3$]$_4$ (1.26 g, 10.8 mmol) were added to a solution of 3-bromo-2-methylpyridine (**3a**; 1.55 g, 9.0 mmol), prepared from 3-amino-2-methylpyridine,[36] in benzene (90 mL) with stirring using a modified procedure.[37] The heterogeneous mixture obtained was purged with nitrogen and heated at reflux with stirring for 24 h. After cooling to 25 °C, the organic layer was separated and the aqueous solution was extracted with ether (2 × 100 mL). The combined organic solutions were dried (MgSO$_4$), the solvent was removed in vacuo, and the residue was distilled to yield **3b** as an oil (1.07 g, 70%): bp 75−77 °C/1 mmHg (lit.[38] bp 67−68 °C/0.5 mmHg); ^{1}H NMR (CDCl$_3$) δ 2.51 (s, 3H, C*H*$_3$), 7.16−7.56 (m, 7H, phenyl hydrogens, pyridyl H-4 and H-5), 8.50 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 2.0 Hz, 1H, pyridyl H-6).

General Method for the Preparation of 2-Methyl-1-oxido-3(or 6)-substituted-pyridines 4a−d. Hydrogen peroxide (1 mL of 35%, w/v, 10 mmol) was added to a solution of the pyridine compound (**3b, 3c, 3d,** or **3e**; 10 mmol) in glacial AcOH (6 mL), and the mixture was heated at 70−80 °C with stirring for 3 h according to the method of Ochiai.[39] An additional aliquot of 35% (w/v) H_2O_2 (1 mL) was added, and the reaction was allowed to proceed for 9 h at 70−80 °C. The volume of the reaction mixture was reduced in vacuo, water (2 mL) was added, the mixture was concentrated in vacuo, and the residue was made alkaline using dry Na_2CO_3. Chloroform (5 mL) was added, this mixture was allowed to stand at 25 °C for 5 min, and the insoluble Na_2CO_3 and NaOAc were removed by filtration. Drying the filtrate (Na$_2$SO$_4$) and removal of the solvent in vacuo gave the target product **4a** (85%), **4b** (60%), **4c** (89%), or **4d** (55%) as oils that were used in subsequent reactions for the preparation of products **5a−d**, respectively. Representative ^{1}H NMR spectral data for compounds **4a,4d** are provided since the spectral data are qualitatively similar.

2-Methyl-1-oxido-3-phenylpyridine (4a): ^{1}H NMR (CDCl$_3$) δ 2.49 (s, 3H, C*H*$_3$), 7.18−7.50 (m, 7H, phenyl hydrogens, H-3, H-4), 8.32 (d, $J_{5,6}$ = 4.5 Hz, 1H, H-6).

6-Chloro-2-methyl-1-oxidopyridine (4d): ^{1}H NMR (CDCl$_3$) δ 2.46 (s, 3H, C*H*$_3$), 7.03 (dd, $J_{3,4}$ = $J_{4,5}$ = 8.0 Hz, 1H, H-4), 7.15 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 7.31 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-5).

General Method for the Preparation of 2-(Acetoxymethyl)-3(or 6)-substituted-pyridines 5a−d. A solution of the 2-methyl-1-oxido-3(or 6)-substituted-pyridine (**4a, 4b, 4c** or **4d**; 6 mmol) in acetic anhydride (4.32 g, 42 mmol, 4 mL) was refluxed for 1 h using a modified literature procedure.[34] Ethanol (3 mL) was added to the reaction mixture, and the reaction was allowed to proceed at reflux for 10 min. The reaction mixture was cooled in an ice−water bath, poured onto water (10 mL), and neutralized with 10% aqueous NaHCO$_3$. Extraction with ether (2 × 25 mL), washing the extract with brine solution (10 mL), drying the organic fraction (Na$_2$SO$_4$), and removal of the solvent in vacuo gave a residue. Purification of the residue by silica gel column chromatography using EtOAc−hexane (30:70, v/v) as eluent afforded the respective product **5a** (80%), **5b** (75%), **5c** (81%), or **5d** (77%) as an oil which was subsequently used for the preparation of **6a−d**. Representative spectral data (IR, ^{1}H NMR) for compounds **5b,5c** are provided since the spectral data are qualitatively similar.

2-(Acetoxymethyl)-6-phenylpyridine (5b): IR (neat) 1753 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.20 (s, 3H, C*H*$_3$), 5.32 (s, 2H, C*H*$_2$), 7.30−8.02 (m, 8H, phenyl and pyridyl hydrogens).

2-(Acetoxymethyl)-3-methylpyridine (5c): IR (neat) 1737 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.16 (s, 3H, COC*H*$_3$), 2.34 (s, 3H, C-3 C*H*$_3$), 5.11 (s, 2H, C*H*$_2$), 7.14 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.5 Hz, 1H, H-5), 7.46 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 8.40 (d, $J_{5,6}$ = 4.5 Hz, 1H, H-6).

3-Chloro-2-pyridinecarboxylic Acid (5e). Potassium permanganate (1.58 g, 10 mmol) was added to a mixture of 3-chloro-2-methylpyridine (**3f**; 1.27 g, 10 mmol) and water (45 mL) according to the method of Ashimori et al.,[34] and the reaction mixture was refluxed for 1.5 h. An additional aliquot of KMnO$_4$ (1.58 g, 10 mmol) was added, and the reaction was allowed to proceed for 20 h at reflux temperature prior to cooling to 25 °C. The reaction mixture was filtered to remove the precipitated MnO$_2$, and the volume of the filtrate was reduced to 50% of its original volume in vacuo. Acidification of the clear solution obtained with 35% (w/v) HCl to pH 3, extraction with EtOAc (2 × 50 mL), drying the EtOAc extracts (Na$_2$SO$_4$), and removal of the solvent in vacuo afforded **5e** (706 mg, 45%): mp 120−124 °C; IR (KBr) 2491−2857 (CO$_2$H), 1712 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.54 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.5 Hz, 1H, H-5), 8.04 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 8.52 (d, $J_{5,6}$ = 4.5 Hz, 1H, H-6). The product **5e** was used in a subsequent reaction for the preparation of compound **6e**.

General Method for the Preparation of 2-(Hydroxymethyl)-3(or 6)-substituted-pyridines 6a−d. A mixture of a 2-(acetoxymethyl)-3(or 6)-substituted-pyridine (**5a, 5b,** or **5c**; 5 mmol), 1 N NaOH (6 mL), and MeOH (12 mL) was stirred at 25 °C for 1.5 h according to a literature method,[34] and the reaction mixture was poured onto water (30 mL). Extraction with EtOAc (2 × 50 mL), washing the EtOAc extract with brine (10 mL), drying the EtOAc fraction (Na$_2$SO$_4$), and removal of the solvent in vacuo gave a residue. Purification of the residue by silica gel column chromatography using EtOAc−hexane (40:60, v/v) as eluent afforded the respective product **6a** (86%), **6b** (55%), or **6c** (81%) as oils which were subsequently used for the preparation of the respective products **7a−c**. Product **6d** was prepared (51%) using a similar procedure by treatment of **5d** with K$_2$CO$_3$ in MeOH, in the place of 1 N NaOH used for the preparation of **6a−c** as described above. Representative ^{1}H NMR spectral data for compounds **6a,d** are provided since the spectral data for **6a−d** are qualitatively similar.

2-(Hydroxymethyl)-3-phenypyridine (6a): ^{1}H NMR (CDCl$_3$) δ 4.60 (s, 1H, O*H*), 4.68 (s, 2H, C*H*$_2$), 7.28−7.50 (m, 6H, phenyl hydrogens, H-5), 7.62 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.0 Hz, 1H, H-4), 8.60 (d, $J_{5,6}$ = 4.5 Hz, $J_{4,6}$ = 2.0 Hz, 1H, H-6).

6-Chloro-2-(hydroxymethyl)pyridine (6d): ^{1}H NMR (CDCl$_3$) δ 3.60 (s, 1H, O*H*), 4.74 (s, 2H, C*H*$_2$), 7.24 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 7.29 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-5), 7.64 (dd, $J_{3,4}$ = 8.0 Hz, $J_{4,5}$ = 8.0 Hz, 1H, H-4).

3-Chloro-2-(hydroxymethyl)pyridine (6e). A solution of ethyl chloroformate (0.54 g, 5 nmol) in THF (2 mL) was added

to a solution of 3-chloro-2-pyridinecarboxylic acid (**5e**; 0.79 g, 5 mmol) and Et$_3$N (0.5 g, 5 mmol) in THF (8 mL) at 5 °C during 30 min with stirring, according to the procedure of Ishizumi et al.,[40] and then the reaction was allowed to proceed for 30 min at 5 °C with stirring. The white precipitate (Et$_3$N$^+$Cl$^-$) was filtered and washed with THF (5 mL). The combined filtrate and THF wash solution were added slowly during 30 min to a solution of NaBH$_4$ (0.473 g, 12.5 mmol) in water (4 mL) at 10−15 °C with external cooling. A rapid evolution of gas was observed after addition of the latter solutions was completed. The reaction mixture was stirred for 4 h at 25 °C prior to acidification with 10% (w/v) HCl which separated into two layers. The aqueous solution was extracted with ether (2 × 30 mL), and the combined ether extracts and organic THF layer were washed consecutively with 10% (w/v) NaOH and water. Drying the organic fraction (Na$_2$SO$_4$) and removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using EtOAc−hexane (30:70, v/v) as eluent to afford **6e** as an oil (0.48 g, 67%); IR (neat) 3172 (OH) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.04 (s, 1H, O*H*), 4.78 (s, 2H, C*H$_2$*), 7.20 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.5 Hz, 1H, H-5), 7.66 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 8.44 (d, $J_{5,6}$ = 4.5 Hz, 1H, H-6).

General Method for the Preparation of 3(or 6)-Substituted-2-pyridinecarboxaldehydes 7a−c,e. A solution of anhydrous H$_3$PO$_4$ in DMSO (1.5 mL of 1.0 M) was added to a solution of the 3- or 6-substituted-2-(hydroxymethyl)-pyridine (**6a**, **6b**, **6c**, or **6e**; 3 mmol) and *N,N*-dicyclohexyl-carbodiimide (1.86 g, 9 mmol) in DMSO (7 mL), and the reaction was allowed to proceed with stirring at 25 °C for 1.5 h. The precipitated dicyclohexylurea was filtered, the filtered solid was washed with ether (15 mL) and water (15 mL), and the aqueous wash was extracted with ether (2 × 30 mL). The combined organic solutions were washed with brine (10 mL), the organic fraction was dried (Na$_2$SO$_4$), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using ether−hexane (40:60, v/v) as eluent to afford the respective product **7a** (36%), **7b** (40%), **7c** (52%), or **7e** (47%) as an oil. Representative spectral data (IR, ^{1}H NMR) for compounds **7b,c** are provided since the spectral data for **7a−c,e** are qualitatively similar.

6-Phenyl-2-pyridinecarboxaldehyde (7b): IR (neat) 1704 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.50−8.12 (m, 8H, phenyl hydrogens, H-3, H-4, H-5), 10.20 (s, 1H, C*H*O).

3-Methyl-2-pyridinecarboxaldehyde (7c): IR (neat) 1712 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.68 (s, 3H, C*H$_3$*), 7.36 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.5 Hz, 1H, H-5), 7.62 (d, $J_{4,5}$ = 8.0 Hz, 1H, H-4), 8.62 (d, $J_{5,6}$ = 4.5 Hz, 1H, H-6), 10.16 (s, 1H, C*H*O).

6-Chloro-2-pyridinecarboxaldehyde (7d). MnO$_2$ (16 g, 184 mmol) was added in eight equal aliquots to a solution of 6-chloro-2-(hydroxymethyl)pyridine (**6d**; 2.8 g, 20 mmol) in chloroform (100 mL), the resulting suspension was stirred at 25 °C for 24 h, and the reaction mixture was filtered through a thoroughly packed Celite pad. Removal of the solvent in vacuo gave a residue which was purified by silica gel column chromatography using EtOAc−hexane (1:3, v/v) as eluent to afford **7d** as an oil (1.46 g, 52%) which was used immediately for the synthesis of **13e,l**.

2-Phenethyl Acetoacetate (10). Diketene (**8**; 0.84 g, 10 mmol) was added dropwise with stirring to 2-phenylethanol (**9**; 1.22 g, 10 mmol) preheated to 50−60 °C in the presence of a catalytic amount of Et$_3$N (5 drops). Diketene was added at a rate such the temperature of the reaction mixture did not exceed 80 °C, and then the reaction was allowed to proceed for 1 h at 80 °C. Distillation of the mixture afforded **10** as an oil which was used immediately in the subsequent reaction (bp 230 °C/1 mmHg; 1.5 g, 73%): IR (neat) 1745 (CO$_2$), 1720 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.21 (s, 3H, C*H$_3$*), 2.99 (t, J = 7.0 Hz, 2H, C*H$_2$*Ph), 3.44 (s, 2H, COC*H$_2$*CO$_2$), 4.38 (t, J = 7.0 Hz, 2H, OC*H$_2$*), 7.22−7.36 (m, 5H, phenyl hydrogens).

2-Phenethyl 3-Aminocrotonate (11d). Ammonia gas was bubbled slowly into a solution of 2-phenethyl acetoacetate (**10**; 1.5 g) in MeOH (10 mL) at 25 °C with stirring for 6 h, the solvent was removed in vacuo, and the residue was distilled to yield **11d** as an oil (bp 285 °C/1 mmHg; 1.35 g, 90%): IR

(neat) 3451 (NH$_2$), 1663 (C=O), 1620 (C=C) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.90 (s, 3H, C*H$_3$*), 2.96 (t, J = 7.0 Hz, 2H, C*H$_2$*Ph), 4.28 (t, J = 7.0 Hz, 2H, OC*H$_2$*), 4.33 (s, 1H, =C*H*), 7.20−7.36 (m, 5H, phenyl hydrogens), 7.80 (br s, 2H, N*H$_2$*).

General Method for the Preparation of Alkyl or 2-Phenethyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(3- or 6-substituted-2-pyridyl)-5-pyridinecarboxylates 13a−q. A mixture of the respective aldehyde (**7a−j**; 1 mmol), either an alkyl 3-aminocrotonate (**11a**, **11b**, or **11c**; 1 mmol) or 2-phenethyl 3-aminocrotonate (**11d**; 1 mmol), and nitroacetone (**12**; 103 mg, 1 mmol) in 2-propanol (5 mL) was stirred at 40 °C for 9 h and then at 25 °C for 13 h (products **13f,i−l,n−q**). For products **13a−e,g,h,m**, equimolar quantities of **7**, **11**, and **12** were used but the reaction was allowed to proceed for 1 h at 25 °C and then for 16 h at reflux using EtOH as solvent. Removal of the solvent in vacuo gave a residue which was purified by silica gel column chromatography using EtOAc−hexane (70:30, v/v) for compounds **13a−f,j,n,p** or EtOAc−hexane (90:10, v/v) for products **13g−i,k−m,o,q** to afford the respective product **13a−q**. The recrystallization solvent, mp, and % yield for products **13a−q** are listed in Table 1. The spectral data (IR, ^{1}H NMR) for representative products **13a,d,g−j,m,p,q** are listed below.

Isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(6-methyl-2-pyridyl)-5-pyridinecarboxylate (13a): IR (KBr) 3057 and 3180 (NH), 1704 (CO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6, 22 °C) δ 1.15 and 1.24 (two d, $J_{CH,Me}$ = 6.0 Hz, 3H each, CH*Me$_2$*), 2.27 (s, 3H, C-6 *Me*), 2.37 (s, 3H, pyridyl *Me*), 2.52 (s, 3H, C-2 *Me*), 4.93 (septet, $J_{CH,Me}$ = 6.0 Hz, 1H, C*H*Me$_2$), 5.35 (s, 1H, H-4), 7.04 (two coincidental d, $J_{3,4}$ and $J_{4,5}$ = 7.5 Hz, 2H total, pyridyl H-3 and H-5), 7.49 (dd, $J_{3,4}$ = $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4), 9.51 (sharp s, 1H, N*H*); ^{1}H NMR (CDCl$_3$, 22 °C) δ 1.09 and 1.23 (two d, $J_{CH,Me}$ = 6.0 Hz, 3H each, CH*Me$_2$*), 2.27 (s, 3H, C-6 *Me*), 2.49 (s, 3H, C-2 *Me*), 2.52 (s, 3H, pyridyl *Me*), 4.94 (septet, $J_{CH,Me}$ = 6.0 Hz, 1H, C*H*Me$_2$), 5.66 (s, 1H, H-4), 7.05 (d, $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-5), 7.42 (d, $J_{3,4}$ = 7.5 Hz, 1H, pyridyl H-3), 7.57 (dd, $J_{3,4}$ = $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4), 9.66 (sharp s, 1H, N*H*). Acquisition of ^{1}H NMR spectra for **13a** in CDCl$_3$ at 10 °C (sharp singlet for N*H* at δ 10.28) and at 61 °C (broad singlet for N*H* at δ 7.67) showed that the N*H* resonance is temperature-dependent, while all other resonances showed minor changes in chemical shift positions of less than δ 0.09. Anal. (C$_{17}$H$_{21}$N$_3$O$_4$) C, H, N.

Isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3-methyl-2-pyridyl)-5-pyridinecarboxylate (13d): IR (KBr) 3057 and 3180 (NH), 1704 (CO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6, 22 °C) δ 1.05 and 1.16 (two d, J = 6.0 Hz, 3H each, CH*Me$_2$*), 2.27 (s, 3H, C-6 *Me*), 2.47 (s, 3H, C-2 *Me*), 2.62 (s, 3H, pyridyl *Me*), 4.89 (septet, J = 6.0 Hz, 1H, C*H*Me$_2$), 5.48 (s, 1H, H-4), 7.07 (dd, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.0 Hz, 1H, pyridyl H-5), 7.46 (d, $J_{4,5}$ = 9.5 Hz, 1H, pyridyl H-4), 8.27 (d, $J_{5,6}$ = 6.0 Hz, 1H, pyridyl H-6), 9.50 (sharp s, 1H, N*H*). Anal. (C$_{17}$H$_{21}$N$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridyl)-5-pyridinecarboxylate (13g): IR (KBr) 3057 and 3180 (NH), 1704 (CO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.18 (s, 3H, C-6 *Me*), 2.43 (s, 3H, C-2 *Me*), 2.82−3.02 (m, 2H, C*H$_2$*Ph), 4.20−4.32 (m, 2H, C*H$_2$*CO$_2$), 5.28 (s, 1H, H-4), 7.02 (d, $J_{3,4}$ = 7.5 Hz, 1H, pyridyl H-3), 7.10 (dd, $J_{4,5}$ = 7.5 Hz, $J_{5,6}$ = 4.5 Hz, 1H, pyridyl H-5), 7.20−7.30 (m, 5H, phenyl hydrogens), 7.63 (dd, $J_{4,5}$ = $J_{3,4}$ = 7.5 Hz, 1H, pyridyl H-4), 8.48 (d, $J_{5,6}$ = 4.5 Hz, 1H, pyridyl H-6), 9.82 (sharp s, 1H, N*H*). Anal. (C$_{21}$H$_{21}$N$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(6-methyl-2-pyridyl)-5-pyridinecarboxylate (13h): IR (KBr) 3180 (NH), 1696 (CO$_2$), 1450, 1294 (NO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6, 22 °C) δ 2.17 (s, 3H, C-6 *Me*), 2.34 (s, 3H, pyridyl *Me*), 2.49 (s, 3H, C-2 *Me*), 2.83−3.01 (m, 2H, C*H$_2$*Ph), 4.25 (t, J = 7.0 Hz, 2H, OC*H$_2$*), 5.32 (s, 1H, H-4), 6.82 (d, $J_{3,4}$ = 8.0 Hz, 1H, pyridyl H-3), 7.00 (d, $J_{4,5}$ = 8.0 Hz, 1H, pyridyl H-5), 7.18−7.34 (m, 5H, phenyl hydrogens), 7.45 (dd, $J_{3,4}$ = $J_{4,5}$ = 8.0 Hz, 1H, pyridyl H-4), 9.54 (sharp s, 1H, N*H*); ^{1}H NMR (CDCl$_3$, 22 °C) δ 2.23 (s, 3H, C-6 *Me*), 2.49 (s, 3H, C-2 *Me*), 2.53 (s, 3H, pyridyl *Me*), 2.83−3.02 (m, 2H, C*H$_2$*Ph), 4.19−4.27 and 4.31−4.42 (two m, 1H each, OC*H$_2$*), 5.62 (s, 1H, H-4), 7.0 (d, $J_{4,5}$ =

8.0 Hz, 1H, pyridyl H-5), 7.13 (d, $J_{3,4}$ = 8.0 Hz, 1H, pyridyl H-3), 7.15−7.36 (m, 5H, phenyl hydrogens), 7.47 (dd, $J_{3,4}$ = $J_{4,5}$ = 8.0 Hz, 1H, pyridyl H-4), 9.37 (sharp s, 1H, NH). Acquisition of ^{1}H NMR spectra for **13h** in CDCl$_3$ at 10 °C (sharp singlet for NH at δ 10.13) and at 50 °C (broad singlet for NH at δ 8.11) showed that the NH resonance is temperature-dependent, while all other resonances showed minor changes in chemical shift positions of less than δ 0.09. Anal. (C$_{22}$H$_{23}$N$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3-methyl-2-pyridyl)-5-pyridinecarboxylate (13i): IR (KBr) 3325 (NH), 1696 (CO$_2$), 1515, 1498, 1392 (NO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6, 22 °C) δ 2.19 (s, 3H, C-6 Me), 2.46 (s, 3H, C-2 Me), 2.53 (s, 3H, pyridyl Me), 2.74−2.91 (m, 2H, CH_2Ph), 4.12−4.32 (m, 2H, OCH_2), 5.46 (s, 1H, H-4), 7.07 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 7.12−7.33 (m, 5H, phenyl hydrogens), 7.42 (d, $J_{4,5}$ = 8.0 Hz, 1H, pyridyl H-4), 8.29 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 9.47 (sharp s, 1H, NH). Anal. (C$_{22}$H$_{23}$N$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-[6-(trifluoromethyl)-2-pyridyl]-5-pyridinecarboxylate (13j): IR (KBr) 3303 (NH), 1704 (CO$_2$), 1515, 1310 (NO$_2$) cm^{-1}; ^{1}H NMR [CDCl$_3$ (not dried with molecular sieves or treated with neutral alumina), 22 °C] δ 2.29 (s, 3H, C-6 Me), 2.55 (s, 3H, C-2 Me), 2.86−3.02 (m, 2H, CH_2Ph), 4.24−4.41 (m, 2H, OCH_2), 5.48 (s, 1H, H-4), 6.15 (s, 1H, NH), 7.14−7.34 (m, 6H, phenyl hydrogens, pyridyl H-5), 7.41 (d, $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-3), 7.57 (dd, $J_{3,4}$ = $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4). Anal. (C$_{22}$H$_{20}$F$_3$N$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3-chloro-2-pyridyl)-5-pyridinecarboxylate (13m): IR (KBr) 3264 (NH), 1704 (CO$_2$), 1507, 1315 (NO$_2$) cm^{-1}; ^{1}H NMR [CDCl$_3$ (not dried with molecular sieves or treated with neutral alumina), 22 °C] δ 2.25 (s, 3H, C-6 Me), 2.49 (s, 3H, C-2 Me), 2.82−3.0 (m, 2H, CH_2Ph), 4.26−4.36 (m, 2H, OCH_2), 5.99 (s, 1H, H-4), 6.04 (s, 1H, NH), 7.07−7.26 (m, 6H, pyridyl H-5 and phenyl hydrogens), 7.30 (dd, $J_{4,5}$ = 7.5 Hz, $J_{4,6}$ = 1.5 Hz, 1H, pyridyl H-4), 8.32 (dd, $J_{5,6}$ = 5.0 Hz, $J_{4,6}$ = 1.5 Hz, 1H, pyridyl H-6). Anal. (C$_{21}$H$_{20}$ClN$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(6-phenyl-2-pyridyl)-5-pyridinecarboxylate (13p): IR (KBr) 3320 (NH), 1687 (CO$_2$), 1591, 1450, 1318 (NO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H, C-6 Me), 2.56 (s, 3H, C-2 Me), 2.86−3.02 (m, 2H, CH_2Ph), 4.35 (t, J = 7.0 Hz, 2H, OCH_2), 5.36 (s, 1H, H-4), 7.02 (d, $J_{3,4}$ = 7.5 Hz, 1H, pyridyl H-3), 7.15−7.30 (m, 5H, aryl hydrogens of CH$_2$CH$_2$$Ph$), 7.35−7.50 (m, 3H, m- and p-aryl hydrogens of 6-$phenyl$-2-pyridyl moiety), 7.70 (dd, $J_{3,4}$ = $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4), 7.80 (d, $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-5), 8.02 (dd, J_{ortho} = 7.5 Hz, J_{meta} = 1.5 Hz, 2H, o-phenyl hydrogens of 6-$phenyl$-2-pyridyl moiety), 9.80 (sharp s, 1H, NH). Anal. (C$_{27}$H$_{25}$N$_3$O$_4$) C, H, N.

2-Phenethyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(3-phenyl-2-pyridyl)-5-pyridinecarboxylate (13q): IR (KBr) 3271 (NH), 1704 (CO$_2$), 1590, 1458, 1340 (NO$_2$) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.01 (s, 3H, C-6 Me), 2.40 (s, 3H, C-2 Me), 2.50−2.72 (m, 2H, CH_2Ph), 3.76−3.84 and 4.08−4.20 (two m, 1H each, OCH_2), 5.88 (s, 1H, H-4), 7.04−7.50 (m, 10H, CH$_2$CH$_2$$Ph$, m- and p-hydrogens of 3-$phenyl$-2-pyridyl moiety, pyridyl H-4 and H-5), 7.65 (d, J_{ortho} = 7.5 Hz, 2H, o-$phenyl$ hydrogens of 3-$phenyl$-2-pyridyl moiety), 8.44 (d, $J_{5,6}$ = 4.5 Hz, 1H, pyridyl H-6), 9.50 (sharp s, 1H, NH). Anal. (C$_{27}$H$_{25}$N$_3$O$_4$) C, H, N.

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.[41] The IC$_{50}$ value ($\pm$SEM, n = 3−18) was determined graphically from the dose−response curve.

Calcium channel agonist activity (positive inotropic response) 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.[23]

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