Synthesis and Smooth Muscle Calcium Channel Antagonist Effects of Alkyl 1,4-Dihydro-2,6-dimethyl-4-(pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylates

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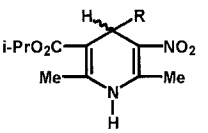
Summary

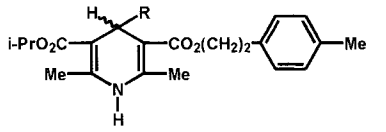
A group of racemic alkyl 1,4-dihydro-2,6-dimethyl-4-(3- or 4-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylates **11a–e** were prepared by using the Hantzsch reaction involving condensation of the Knoevenagel adducts **9a–e** with 1-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1-propen-2-amine (**10**). In contrast, the 4-(2-pyridinyl) analogue **11f** was prepared by thionyl chloride mediated cyclization of the 5-{*N*-(1,1-dimethyl-2-hydroxyethyl)aminocarbonyl} moiety of **16** to the 5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)] ring system (**11f**). *In vitro* calcium channel antagonist activity was determined by using the guinea pig ileum longitudinal smooth muscle (GPILSM) assay. Compared to the reference drug nifedipine ($IC_{50} = 1.43 \times 10^{-8}$ M), the title compounds **11** exhibited weak calcium channel antagonist activity (10^{-5} to 10^{-6} M range). A comparison of compounds **11** having a C-4 3-pyridinyl substituent showed that with respect to the alkyl ester R^2-subsituent, the relative potency order was *i*-Bu (**11c**) ≥ *i*-Pr (**11e**) > Me (**11a**). The point of attachment of the C-4 pyridinyl substituent in the isopropyl ester isomeric series of compounds was a determinant of activity where the potency profile was 4-py (**11d**) ≥ 3-py (**11e**) > 2-py (**11f**). Although less effective, the 4,5-dihydro-4,4-dimethyloxazolin-2-yl moiety acts as a bioisostere of the alkyl ester substituent present in classical 1,4-dihydropyridine calcium channel antagonists. The 4,5-dihydro-4,4-dimethyl-oxaxolin-2-yl ring system is not an effective bioisostere of the 3-nitro group present in 1,4-dihydropyridine calcium channel agonists since isopropyl 1,4-dihydro-2,6-dimethyl-4-(2-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate (**11f**) produced a modest 10% increase in the *in vitro* contractile force of guinea pig left atrium at a concentration of 1.64×10^{-7} M, relative to the reference 3-nitro analogue **1** ($EC_{50} = 9.6 \times 10^{-6}$ M).

Introduction

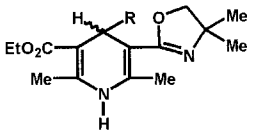
A novel group of (±)-isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(pyridinyl)-5-pyridinecarboxylate isomers (**1–3**) have been described recently. The *rac*-2-pyridinyl isomer **1** showed *dual cardioselective calcium channel agonist/smooth muscle selective calcium channel antagonist effects.* In contrast, *rac*-3-pyridinyl **2** and *rac*-4-pyridinyl **3** isomers exhibited agonist activity on both heart and smooth muscle. The (–)-2-pyridinyl enantiomer (–)-**1** was shown to exhibit *in vitro* cardiac agonist and smooth muscle antagonist activities[1]. It has also been observed that the (+)- and (–)-enantiomers of 3-isopropyl 5-(4-methylphenethyl)-1,4-dihydro-2,6-dimethyl-4-(2-pyridinyl)-3,5-pyridinedicarboxylate **4** both exhibit a dual cardioselective partial calcium channel agonist / smooth muscle selective calcium channel antagonist effect. The relative *in vitro* smooth muscle calcium channel antagonist activities of (–)-**4**:(+)-**4** was 26:1. Hereby (+)-**4** was *a more active in vitro positive inotrope* on heart whereas, (–)-**4** increased contractile force by a maximum of 14%[2]. 1,4-Dihydropyridine compound **5**, containing a 4,5-dihydro-4,4-dimethyloxazolin-2-yl moiety designed to act as a latent carboxylate appendage or carboxylate isostere[3,4], was prepared, but no pharmacological data were reported[5,6]. A dual cardioselective agonist/smooth muscle selective antagonist *third generation* modulator such as (–)-2-pyridinyl **1**, or (+)-2-pyridinyl **4**, would have an ideal therapeutic profile for treating congestive heart failure (CHF) patients. Therefore it was of interest to extend these important structure-activity correlations by replacing the nitro group of the pyridinyl isomers **1–3** and the *para*-tolylphenethyl ester group of **4** by a 4,5-dihydro-4,4-dimethyloxazolin-2-yl moiety. This latter modification would be expected to alter the nature of the drug-receptor interaction and possibly tissue selectivity. We now report the synthesis and *in vitro* calcium channel modulating effects of the alkyl 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate class of compounds (**11**).



1, R = 2-pyridinyl
2, R = 3-pyridinyl
3, R = 4-pyridinyl



4, R = 2-pyridinyl



5, R = 3-nitrophenyl

Figure 1. Structures of nitro (**1–3**), ester (**4**), and oxazolin-2-yl (**5**) compounds.

Chemistry

Alkyl 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylates (**11a–e**) were prepared by a modified Hantzsch reaction (see Scheme 1). Thus, condensation of the respective aldehydes (**6a–c**) with *n*-butylamine led to the imines (**7a–c**), which on reaction with an alkyl acetoacetate (**8a–c**) resulted

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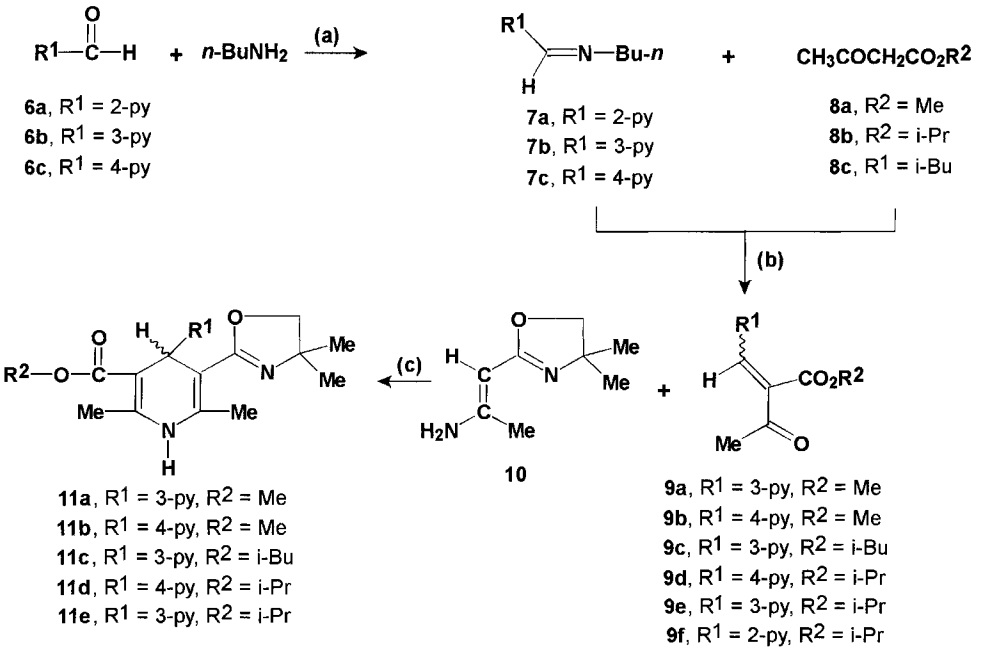
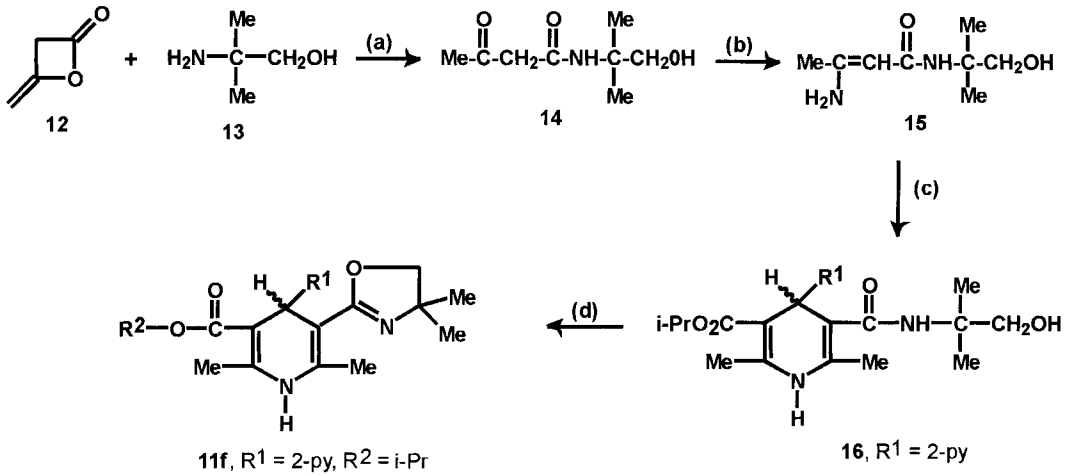


Scheme 1. Reagents and conditions: (a) benzene, reflux, 3–5 h; (b) acetic anhydride, 25 °C, 18–24 h; (c) EtOH, reflux, 18–48 h.



Scheme 2. Reagents and conditions: (a) 4-dimethylaminopyridine (DMAP), THF, 25 °C, 2 h; (b) NH_3, MeOH, 25 °C; (c) isopropyl acetoacetate, 2-pyridinecarboxaldehyde, EtOH, reflux, 48 h; (d) $SOCl_2$, pyridine, dichloromethane, –5 to –10 °C, 1.5 h.

in the target Knoevenagel adducts (**9a–e**) as a mixture of (*E*)- and (*Z*)-isomers which were not separated. Condensation of the 2-alkoxycarbonyl-1-(3- or 4-pyridinyl)-but-1-en-3-ones (**9a–e**) with 1-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1-propen-2-amine (**10**) afforded the respective title compounds (**11a–e**).

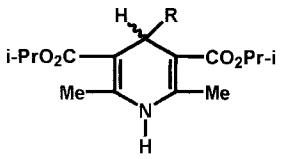
An attempt to prepare 2-isopropoxycarbonyl-1-(2-pyridinyl)-but-1-en-3-one (**9f**) by reaction of *C*-(2-pyridinyl)-*N*-(*n*-butyl)imine (**7a**) with isopropyl acetoacetate (**8b**) in the presence of acetic anhydride produced a complex mixture of products from which **9f** could not be isolated. The target 4-(2-pyridinyl) isomer **11f** was therefore prepared by using an alternate procedure (see Scheme 2). Thus, reaction of diketene (**12**) with 2-amino-2-methyl-1-propanol (**13**) yielded *N*-(1,1-dimethyl-2-hydroxyethyl)acetoacetamide (**14**) which was converted to the 3-aminocrotonamide derivative **15**. The Hantzsch condensation of **15** with 2-pyridinecarboxaldehyde and isopropyl acetoacetate yielded isopropyl 5-{*N*-[1-(1,1-dimethyl-2-hydroxyethyl)aminocarbonyl} 1,4-dihydro-2,6-dimethyl-3-pyridinecarboxylate **16**. The thionyl chloride mediated cyclization of the C-5 substituent present in **16** to an oxazolinyl ring produced the target 4-(2-pyridinyl) isomer **11f**.

Results and Discussion

The design of calcium channel agents suitable for the treatment of CHF depends on the separation and/or removal of their vasoconstrictor effect from their positive inotropic cardiostimulant property[1,7]. Apparent differences in the molecular electrostatic potentials between agonist and antago-

nist 1,4-dihydropyridine (DHP) structures, with respect to the C-3 and C-5 DHP regions, may be a mechanism allowing the receptor to distinguish between activator and antagonist ligands. For example, calcium channel antagonists display a positive potential in this region when a C-3 ester substituent is present, whereas agonists show a strong negative potential in the region adjacent to their C-3 nitro substituent[8]. The point of attachment of a C-4 pyridinyl substituent for compounds **1-3** is a major determinant of calcium channel modulating activity and tissue specificity[1]. These observations prompted us to investigate analogs of the C-4 pyridinyl compounds **1–3** where the nitro group is replaced by a 2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl) moiety. Due to their potential to act as additional electron donors for hydrogen bonding to the calcium channel receptor, they may provide a method to alter calcium channel receptor binding and/or tissue specificity.

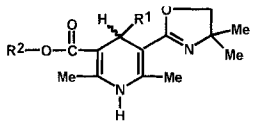
The *in vitro* calcium channel antagonist activities of compounds **11a–f** were determined by using the guinea pig ileum longitudinal smooth muscle (GPILSM). The calcium channel antagonist activities for **11a–f**, determined as the concentration required to produce 50% inhibition of GPILSM contractility,[9] are presented in Table 1. Compounds **11a–f** showed weak calcium channel antagonist activity (10^{-5} to 10^{-6} M range) relative to the reference drug nifedipine ($IC_{50} = 1.43 \times 10^{-8}$ M). Since the differences in antagonist activity were about one log unit the correlations described represent profile differences in potency generally having a low level of significance. A comparison of compounds **11** having a R^1 = 3-pyridyl substituent showed that the relative potency order was *i*-Bu (**11c**) ≥ *i*-Pr (**11e**) > Me (**11a**). The point of attachment of a C-4 3-pyridyl or 4-pyridyl substituent was not a determinant of activity when R^2 = Me (**11a** ≅ **11b**) or R^2 = *i*-Pr (**11d** ≅ **11e**). However, the C-4 2-pyridyl isomer (**11f**) was about 10 times less active than the corresponding 3-pyridyl (**11e**) and 4-pyridyl (**11d**) isomers when R^2 = *i*-Pr. This latter observation was unexpected since previous structure-activity correlations for dialkyl 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-3,5-pyridinedicarboxylates showed the relative potency order for pyridyl analogs was 2-pyridyl > 3-pyridyl > 4-pyridyl[10]. Replacement of the 4,5-dihydro-4,4-dimethyloxazolin-2-yl ring system present in **11d** ($IC_{50} = 2.59 \times 10^{-6}$ M), **11e** ($IC_{50} = 3.27 \times 10^{-6}$ M) and **11f** ($IC_{50} = 2.17 \times 10^{-5}$ M) by a -CO_2Pr-i substituent (**17a**, $IC_{50} = 2.31 \times 10^{-7}$ M; **17b**, $IC_{50} = 2.57 \times 10^{-7}$ M; **17c**, $IC_{50} = 1.25 \times 10^{-7}$ M)[10] reduced calcium channel activity by 11, 13, and 173 times, respectively. The observation that the relative potency order for the isomeric pyridyl analogs **11d–f** was 4-pyridyl (**11d**) ≥ 3-pyridyl (**11e**) > 2-pyridyl (**11f**) suggests that in the design of 1,4-DHP calcium channel antagonists possessing larger C-3 isopropyl ester and C-5 cyclic ring substituents such as the 2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl) ring system, a C-4 3-pyridyl or *meta*-substituted-phenyl substituent should be considered[11]. The results of this study indicate that the 4,5-dihydro-4,4-dimethyloxazolin-2-yl moiety, although less effective, acts as a bioisostere of the alkyl ester substituent present in classical 1,4-DHP calcium channel antagonists.



17a, R = 4-pyridinyl
17b, R = 3-pyridinyl
17c, R = 2-pyridinyl

In an earlier study it was observed that (±)-isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-pyridinyl)-5-carboxylate (**1**) produced an *in vitro* calcium channel agonist effect (positive inotrope) on guinea pig left atria (GPLA) ($EC_{50} = 9.6 \times 10^{-6}$ M, the molar concentration eliciting 50% of the maximum contractile response produced by **1** on GPLA as determined graphically from the dose-response curve)[1]. A similar study with **11f**, where the 3-nitro substituent of **1** was replaced by a 4,5-dihydro-4,4-dimethyloxazolin-2-yl ring system, resulted in a modest 10% increase in the contractile response of GPLA at a concentration of 1.64×10^{-7} M. This observation indicates that the 4,5-dihydro-4,4-dimethyloxa-

Table 1. Physical and calcium channel antagonist activities of alkyl 1,4-dihydro-2,6-dimethyl-4-(pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)-3-pyridinecarboxylates (**11a-f**).



Compd	R^1	R^2	mp °C	Yield %	Formula	Anal.[a]	Calcium channel antagonist act:IC_{50} [M][b]
11a	3-py	Me	140–142	30	$C_{18}H_{23}N_3O_3$	C,H,N	$1.60 \pm 0.06 \times 10^{-5}$
11b	4-py	Me	193–195	57	$C_{19}H_{23}N_3O_3$	C,H,N	$2.78 \pm 0.04 \times 10^{-5}$
11c	3-py	*i*-Bu	181–183	49	$C_{22}H_{29}N_3O_3$	C,H,N	$2.22 \pm 0.03 \times 10^{-6}$
11d	4-py	*i*-Pr	207–208	45	$C_{21}H_{27}N_3O_3$	C,H,N	$2.59 \pm 0.06 \times 10^{-6}$
11e	3-py	*i*-Pr	200–202	51	$C_{21}H_{27}N_3O_3$	C,H,N	$3.27 \pm 0.09 \times 10^{-6}$
11f	2-py	*i*-Pr	187–189	54	$C_{21}H_{27}N_3O_3$	C,H,N[c]	$2.17 \pm 0.05 \times 10^{-5}$
Nifedipine							$1.43 \pm 0.19 \times 10^{-8}$

[a] Microanalytical analyses were within ±0.4% of theoretical values, unless otherwise stated.

[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 curves (n = 3).

[c] Exact mass calcd: 369.2052. Found (HRMS): 369.2046.

zolin-2-yl ring system is not an effective bioisostere of a nitro substituent with respect to calcium channel agonist activity.

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Experimental

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 $[D_2]H_2O$. ^{13}C NMR spectra were obtained by using the J modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and methine carbon resonances appear as negative peaks. Infrared spectra were acquired using a Nicolet 5DX-FT 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. Methyl (**8a**) and isobutyl acetoacetate (**8c**) were purchased from the Aldrich Chemical Co., and isopropyl acetoacetate (**8b**) was purchased from the Lancaster Chemical Co. 1-[2-(4,5-Dihydro-4,4-dimethyloxazolin-2-yl)]-1-propen-2-amine (**10**) was prepared according to the reported procedure[5].

General Method for the Preparation of C-(Pyridinyl)-N-(n-butyl)imines **7a–c**

A mixture of the respective pyridinecarboxaldehyde **6a, 6b** or **6c** (10.7 g, 0.1 mol) and *n*-butylamine (7.3 g, 0.1 mol) in benzene (100 ml) was refluxed for 3–5 h using a Dean-Stark trap to remove water formed in the reaction. Removal of the benzene *in vacuo* and distillation of the product produced the respective imine product which was used immediately in the subsequent reaction; **7a**, bp 65 °C/0.9 mm Hg, 85% yield; **7b**, bp 79–81 °C/5 mm Hg, 87% yield; **7c**, bp 75–80 °C/0.4 mm Hg, 90% yield. The ^{1}H NMR spectral data for **7a**, representative for **7a–c** except for differences in pyridinyl proton chemical shifts, are provided below.

C-(2-Pyridinyl)-N-(n-butyl)imine **7a**

^{1}H NMR ($CHCl_3$-d_1): δ 8.64 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.38 (s, 1H, C*H*=N), 7.98 (d, $J_{3,4}$ = 8.0 Hz, 1H, pyridyl H-3), 7.72 (ddd, $J_{3,4} = J_{4,5}$ = 8.0, $J_{4,6}$ = 1.40 Hz, 1H, pyridyl H-4), 7.23 (d, $J_{4,5}$ = 8.0, $J_{5,6}$ = 5.0, $J_{3,5}$ = 1.6 Hz, 1H, pyridyl H-5), 3.68 (t, *J* = 7.1 Hz, 2H, =NC*H*$_2$CH$_2$CH$_2$Me), 1.72 (quintet, *J* = 7.1 Hz, 2H, CH$_2$C*H*$_2$CH$_2$Me), 1.40 (sextet, *J* = 7.1 Hz, 2H, CH$_2$CH$_2$C*H*$_2$Me), 0.95 (t, *J* = 7.1 Hz, 3H, -CH$_2$*Me*).

General Method for the Preparation of 2-Alkoxycarbonyl-1-(pyridinyl)-but-1-en-3-ones **9a–e**

Acetic anhydride (2 ml) was added to a mixture of the respective *C*-(pyridinyl)-*N*-(*n*-butyl)imine **7b** or **7c** (100 mmol), and alkyl acetoacetate **8a, 8b** or **8c** (100 mmol). The reaction was allowed to proceed at 25 °C with stirring for 18-24 h, at which time the reaction was completed. Addition of water (20 ml), extraction with EtOAc (3 × 50 ml), washing the combined EtOAc extracts with brine solution (30 ml), drying the EtOAc fraction ($MgSO_4$), and removal of the solvent *in vacuo* produced the respective products **9a-e** as an oil purified either by silica gel column chromatography using EtOAc-hexane (1:3, *v/v*) as eluent (**9a–b**) or by distillation (**9c**, bp 80 °C/0.5 mm Hg; **9d**, bp 85–87 °C/1.5 mm Hg; **9e**, bp 80–82 °C/0.7 mm Hg). Products **9a–e**, obtained in 65–85% isolated yield after purification as described above, existed as a mixture of (*E*)- and (*Z*)-isomers (*E*:*Z* ratio = 1:1 to 2:3 as determined from the ^{1}H NMR *Me*CO resonances integrals for the two isomers) were used immediately in the subsequent syntheses of **11a–e**. The ^{1}H NMR spectral data for **9a–e** were qualitatively similar except for differences in R^1 pyridinyl and R^2 alkyl resonances. Representative ^{1}H NMR spectral data for **9a** and **9d** are listed below.

2-Methoxycarbonyl-1-(3-pyridinyl)-but-1-en-3-one **9a**

^{1}H NMR ($CHCl_3$-d_1): δ 8.68 and 8.69 (two s, 1H total, pyridyl H-2), 8.61–8.65 (m, 1H, pyridyl H-6), 7.74–7.80 (m, 1H, pyridyl H-4), 7.63 and 7.67 (two s, 1H total, C*H*=C-CO$_2$Me), 7.34–7.41 (m, 1H, pyridyl H-5), 3.80 and 3.86 (two s, 3H total, CO$_2$*Me*), 2.40 and 2.46 (two s, 3H total, CO*Me*). The ratio of the (*E*):(*Z*) isomers calculated from the integrals for CO*Me* protons at δ 2.40 and 2.46 was 2:3, respectively.

2-Isopropoxycarbonyl-1-(4-pyridinyl)-but-1-ene-3-one **9d**

^{1}H NMR ($CHCl_3$-d_1): δ 8.48 and 8.50 (two d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H total, pyridyl H-2 and H-6), 7.34 and 7.38 (two s, 1H total, C*H*=C-CO$_2$), 7.09 and 7.21 (two d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H total, pyridyl H-3 and H-5), 5.03 (septet, *J* = 6.2 Hz, 1H, C*H*Me$_2$), 2.20 and 2.27 (two s, 3H total, CO*Me*), 1.08 and 1.16 (two d, *J* = 6.2 Hz, 3H each, CH*Me*$_2$). The ratio of the (*E*):(*Z*) isomers calculated from the integrals for CO*Me* protons at δ 2.20 and 2.27 was 1:1, respectively.

General Method for the Preparation of Alkyl 1,4-Dihydro-2,6-dimethyl-4-(pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylates **11a–e**

A solution of 1-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-1-propen-2-amine (**10**, 0.46 g, 2 mmol) and the Knoevenagel adduct (**9a–e**, 2 mmol) in ethanol (30 ml) was refluxed for 18–48 h to complete the reaction. Removal of the solvent *in vacuo* resulted in the respective products **11a–e** that were purified by silica gel column chromatography using EtOAc-hexane as eluent (3:1, *v/v* for **11a–c**; 4:1, *v/v* for **11d–e**) prior to recrystallization from EtOAc-hexane (2:1, *v/v* for **11a–c, 11e**; 3:1, *v/v* for **11d**). The mp and % yield of products **11a–e** are summarized in Table 1.

Methyl 1,4-Dihydro-2,6-dimethyl-4-(3-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate **11a**

IR (KBr): ν = 3197 cm^{-1} (NH), 1679 (CO$_2$).– ^{1}H NMR ($CHCl_3$-d_1): δ 8.55 (s, 1H, pyridyl H-2), 8.36 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 7.63 (d, $J_{4,5}$ = 7.9 Hz, 1H, pyridyl H-4), 7.12–7.16 (m, 1H, pyridyl H-5), 5.93 (s, 1H, N*H*), 5.05 (s, 1H, dihydropyridyl H-4), 3.83 (q, *J* =8.0 Hz, 2H, oxazolinyl H-5), 3.60 (s, 3H, CO$_2$*Me*), 2.22 and 2.35 (two s, 3H each, dihydropyridyl C-2 and C-6 *Me's*), 1.17 and 1.22 (two s, 3H each, oxazolinyl C-4 *Me's*).

Methyl 1,4-Dihydro-2,6-dimethyl-4-(4-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate **11b**

IR (KBr): ν = 3201 cm^{-1} (NH), 1683 (CO$_2$).– ^{1}H NMR ($CHCl_3$-d_1): δ 8.42 (d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-2 and H-6), 7.23 (d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-3 and H-5), 5.84 (s, 1H, N*H*), 5.07 (s, 1H, dihydropyridyl H-4), 3.85 (q, *J* = 8.0 Hz, 2H, oxazolinyl H-5), 3.61 (s, 3H, CO$_2$*Me*), 2.23 and 2.37 (two s, 3H each, dihydropyridyl C-2 and C-6 *Me's*), 1.20 and 1.23 (two s, 3H each, oxazolinyl C-4 *Me's*).

Isobutyl 1,4-Dihydro-2,6-dimethyl-4-(3-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate **11c**

IR (KBr): ν = 3219 cm^{-1} (NH), 1702 (CO$_2$).– ^{1}H NMR ($CHCl_3$-d_1): δ 8.56 (d, $J_{2,4}$ = 2.0 Hz, 1H, pyridyl H-2), 8.35 (dd, $J_{5,6}$ = 5.0, $J_{4,6}$ = 1.5 Hz, 1H, pyridyl H-6), 7.63 (ddd, $J_{4,5}$ = 8.0, $J_{2,4}$ = 2.0, $J_{4,6}$ = 1.5 Hz, 1H, pyridyl H-4), 7.13 (dd, $J_{4,5}$ = 8.0, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 5.56 (s, 1H, N*H*), 5.05 (s, 1H, dihydropyridyl H-4), 3.83 (d, *J* = 6.1 Hz, 2H, Me$_2$CHC*H*$_2$O$_2$C), 3.73 (q, *J* = 8.0 Hz, 2H, oxazolinyl H-5), 2.22 and 2.37 (two s, 3H each, C-2 and C-6 *Me's*), 1.85 (m, 1H, CH$_2$C*H*Me$_2$), 1.19 and 1.21 (two s, 3H each, oxazolinyl C-4 *Me's*), 0.78 and 0.82 (two d, *J* = 6.2 Hz, 3H each, CH$_2$CH*Me*$_2$).

Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(4-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate **11d**

IR (KBr): ν = 3197 cm^{-1} (NH), 1708 (CO$_2$).– ^{1}H NMR ($CHCl_3$-d_1): δ 8.40 (d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-2 and H-6), 7.24 (d, $J_{2,3} = J_{5,6}$ = 5.0 Hz, 2H, pyridyl H-3 and H-5), 6.30 (s, 1H, N*H*), 5.01 (s, 1H, dihydropyridyl H-4), 4.91 (septet, *J* = 6.2 Hz, 1H, C*H*Me$_2$), 3.81 (q, *J* = 8.0 Hz, 2H, oxazolinyl H-5), 2.19 and 2.33 (two s, 3H each, dihydropyridyl C-2 and C-6 *Me's*), 1.02

and 1.22 (two d, J = 6.2 Hz, 3H each, CH*Me*$_2$), 1.17 and 1.20 (two s, 3H each, oxazolinyl C-4 *Me's*).

Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate **11e**

IR (KBr): ν = 3206 cm^{-1} (NH), 1685 (CO_2).– ^{1}H NMR ($CHCl_3$-d_1): δ 8.53 (s, 1H, pyridyl H-2), 8.33 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 7.62–7.68 (m, 1H, pyridyl H-4), 7.13 (dd, $J_{5,6}$ = 5.0, $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-5), 6.17 (s, 1H, N*H*), 4.99 (s, 1H, dihydropyridyl H-4), 4.90 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 3.79 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 2.19 and 2.32 (two s, 3H each, dihydropyridyl C-2 and C-6 *Me's*), 1.10 and 1.21 (two d, J = 6.2 Hz, 3H each, CH*Me*$_2$).).– ^{13}C NMR ($CHCl_3$-d_1): δ 166.89 (CO_2), 162.13 (oxazolinyl C-2), 149.66 and 146.91 (pyridyl C-2 and C-6), 145.65 and 143.20 (dihydropyridyl C-2 and C-6), 138.01 (pyridyl C-3), 135.85 (pyridyl C-4), 122.95 (pyridyl C-5), 101.53 and 101.23 (oxazolinyl O*C*H$_2$ and C-4), 66.84 (dihydropyridyl C-4), 66.70 (dihydropyridyl C-3 and C-5), 39.05 (*C*HMe$_2$), 28.32 and 28.21 (dihydropyridyl C-2 and C-6 *Me's*), 22.09 and 22.71 (oxazolinyl C-4 *Me's*), 19.54 and 18.41 (CH*Me*$_2$).

N-(1,1-Dimethyl-2-hydroxyethyl)acetoacetamide **14**

Diketene (8.40 g, 100 mmol) was added dropwise to a solution of 2-amino-2-methyl-1-propanol (8.90 g, 100 mmol) in THF (30 ml) containing 4-dimethylaminopyridine (100 mg) and the mixture was stirred for 2 h at 25 °C. Removal of the solvent *in vacuo* and purification of the product by silica gel column chromatography using EtOAc-hexane (1:3, *v/v*) as eluent yielded **14** as a pale yellow viscous oil (90% yield) used immediately in the subsequent reaction.

N-(1,1-Dimethyl-2-hydroxyethyl)-3-aminocrotonamide **15**

Ammonia gas was passed through a stirred solution of **14** (7.9 g, 50 mmol) in methanol (50 ml) at 25 °C until TLC indicated that the reaction had gone to completion. Removal of the solvent *in vacuo* led to **15** as a white solid used immediately in the subsequent reaction without further purification.– mp 99–101 °C, 95% yield.– ^{1}H NMR ($CHCl_3$-d_1): δ 6.0–6.4 (br s, 3H, CON*H* and =C-N*H*$_2$), 5.02 (s, 1H, O*H*), 4.31 (s, 1H, =C*H*), 3.56 (s, 2H, C*H*$_2$OH), 1.84 (s, 3H, *Me*-C=CH), 1.28 (s, 6H, NHC*Me*$_2$).

Isopropyl 5-[N-(1-(1,1-Dimethyl-2-hydroxyethyl)aminocarbonyl]] 1,4-Dihydro-2,6-dimethyl-3-pyridinecarboxylate **16**

A mixture of **15** (0.18 g, 1.1 mmol), isopropyl acetoacetate (0.15 g, 1.1 mmol) and 2-pyridinecarboxaldehyde (0.112 g, 1.1 mmol) in ethanol (50 ml) was refluxed for 48 h. Removal of the solvent *in vacuo* and purification of the residue obtained by silica gel column chromatography using EtOAc-hexane (3:1, *v/v*) as eluent yielded **16** (0.12 g, 30%) as a white solid.– mp 170–172 °C.– IR (KBr): ν = 3160–3311 cm^{-1} (NH, OH), 1671 (CONH), 1592 (NH).– ^{1}H NMR ($CHCl_3$-d_1): δ 9.36 (s, 1H, CON*H*), 8.43 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 7.65 (dd, $J_{3,4}$ = $J_{4,5}$ = 7.5 Hz, 1H, pyridyl H-4), 7.07–7.13 (m, 2H, pyridyl H-3 and H-5), 6.32 (t, $J_{CH,OH}$ = 4.2 Hz, 1H, CH$_2$O*H*), 6.13 (s, 1H, dihydropyridyl N*H*), 4.85–5.0 (m, 2H, dihydropyridyl H-4 and C*H*Me$_2$), 3.60 (d, $J_{CH,OH}$ = 4.2 Hz, 2H, C*H*$_2$OH), 2.14 and 2.43 (two s, 3H each, dihydropyridyl C-2 *and C-6 Me's*), 1.35 and 1.43 (two s, 3H each, CONHC*Me*$_2$), 1.01 and 1.20 (two d, $J_{CH,Me}$ = 6.1 Hz, 3H each, CH*Me*$_2$). Anal. Calcd. for $C_{21}H_{29}N_3O_4$.1/2H_2O: C 63.62, H 7.62, N 10.60. Found: C 63.66, H 7.60, N 10.27.

Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(2-pyridinyl)-5-[2-(4,5-dihydro-4,4-dimethyloxazolin-2-yl)]-3-pyridinecarboxylate **11f**

Thionyl chloride (0.18 g, 1 mmol) was added dropwise to a solution of **16** (0.388 g, 1 mmol) in dichloromethane (25 ml) and pyridine (10 ml) while maintaining the reaction temperature in the –5 to –10 °C range. The reaction was allowed to proceed at this temperature for 1.5 h prior to warming to 0 °C and removal of the solvent *in vacuo*. Purification of the product by silica gel column chromatography using EtOAc-hexane (2:1, *v/v*) as eluent and recrystallization from EtOAc-hexane (1:1, *v/v*) yielded **11f** as a white solid (54%).– IR (KBr): ν = 3206 cm^{-1} (NH), 1682 (CO_2).– ^{1}H NMR ($CHCl_3$-d_1): δ 8.46 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.09 (s, 1H, N*H*), 7.46 (dd, $J_{3,4}$ = $J_{4,5}$ =7.5 Hz, 1H, pyridyl H-4), 7.25 (d, $J_{3,4}$ = 7.5 Hz, 1H, pyridyl H-3), 7.05 (dd, $J_{4,5}$ = 7.5, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 5.06 (s, 1H, dihydropyridyl H-4), 4.83 (septet, J = 6.1 Hz, 1H, C*H*Me$_2$), 3.73 (q, J = 8.0 Hz, 2H, oxazolinyl H-5), 2.06 and 2.22 (two s, 3H each, dihydropyridyl C-2 and C-6 *Me's*), 1.06 and 1.18 (two s, 3H each, oxazolinyl C-4 *Me's*), 0.93 and 1.14 (two d, J = 6.1 Hz, 3H each, CH*Me*$_2$).– ^{13}C NMR ($CHCl_3$-d_1): δ 167.40 (CO_2), 165.50 (oxazolinyl C-2), 162.73 (pyridyl C-2), 148.21 (pyridyl C-6), 147.04 and 138.97 (dihydropyridyl C-2 and C-6), 135.08 (pyridyl C-4), 124.41 and 121.25 (pyridyl C-3 and C-5), 100.45 and 99.83 (O*C*H$_2$ and oxazolinyl C-4), 66.43 (dihydropyridyl C-3 and C-5), 66.27 (dihydropyridyl C-4), 44.29 (*C*HMe$_2$), 28.25 (dihydropyridyl C-2 and C-6 *C*H$_3$'s), 22.13 and 21.70 (oxazolinyl C-4 *C*H$_3$'s), 19.16 and 17.90 (CH*Me*$_2$).

In vitro Calcium Channel Antagonist Assay

The calcium channel antagonist activities of compounds **11a–f** 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 described above[9]. The IC_{50} value (± SEM) was determined graphically from the dose-response curve.

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