Synthesis of Alkyl 6-Methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates for Evaluation as Calcium Channel Antagonists

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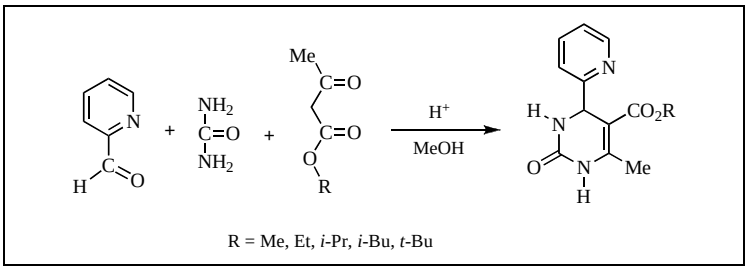

R = Me, Et, *i*-Pr, *i*-Bu, *t*-Bu

The Bigenelli acid catalyzed condensation of 2-pyridylcarboxaldehyde (**1**), urea (**2**) and an alkyl acetoacetate (**3**) afforded the respective alkyl (Me, Et, *i*-Pr, *i*-Bu, *t*-Bu) 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates (**4a-e**). The most potent calcium channel antagonist ethyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylate (**4b**, IC_{50} = 1.67 x 10^{-5} M) was a much weaker calcium channel antagonist than the reference drug nifedipine (Adalat®, IC_{50} = 1.40 x 10^{-8} M) on guinea pig ileal longitudinal smooth muscle (GPILSM). The alkyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates did not show any inotropic effect on heart since no increase, or decrease, in the contractile force of guinea pig left atrium was observed. These structure-activity studies show that the alkyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates (**4a-e**) are partial bioisosteres of nifedipine with respect to calcium channel antagonist activity on guinea pig ileal longitudinal smooth muscle (GPILSM).

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INTRODUCTION

Discovery of the first-generation calcium channel antagonist dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (nifedipine, Adalat®), that is used to treat hypertension [1], prompted extensive investigations to determine the geometrical requirements of the 1,4-dihydropyridine calcium channel binding site [2-5]. Structure-activity correlations acquired in these studies indicated that the collective combination of the 1,4-dihydropyridine C-3, C-4 and C-5 substituents modulated activity, tissue selectivity, and the conformation of the 1,4-dihydripyridine ring system [6]. In a previous study, calcium channel antagonist results suggested that a 4-(pyridyl) substituent is bioisosteric with a 4-(nitrophenyl) substituent on a 1,4-dihydropyridine ring system where *ortho*-, *meta*-, and *para*-nitrophenyl are bioisosteric with 2-pyridyl, 3-pyridyl, and 4-pyridyl, respectively [7]. This observed potency profile is consistent with this postulate since it is well-documented that the potency sequence for substituted-phenyl derivatives is generally *ortho* > *meta* > *para* [8]. The pyridyl nitrogen atom has an orbital with a lone electron pair. When this orbital is viewed as a substituent, the 2-pyridyl, 3-pyridyl and 4-pyridyl ring systems may be bioisosteric with a phenyl ring with respective *ortho*, *meta* and *para* substituents. In this regard, the steric effect that an orbital with an electron pair is able to induce is clearly much smaller than that of a substituent attached to a phenyl ring.

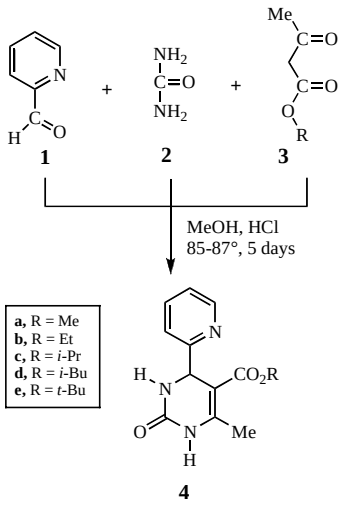
A number of N^3-substituted-3,4-dihydropyrimidines with vasodilative and antihypertensive activity [9], alkyl 3-alkoxycarbonyl-4-aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylates that are inactive *in vivo* due to metabolism to the 3-NH metabolite [10], and alkyl 3-carbamoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl-5-pyrimidinecarboxylates which are orally active and metabolically stable [11], have been reported. These 1,4-dihydropyrimidines, considered to be mimics of 1,4-dihydropyridines, can adopt a conformation similar to 1,4-dihydropyridines [12], they bind to the 1,4-dihydropyridine calcium channel receptor but with lower affinity [12], and the dihydropyrimidine ring is more stable to oxidation than the 1,4-dihydropyridine ring [10].

As part of our ongoing program to develop structure-activity relationships for heterocyclic mimics of 1,4-dihydropyridines with calcium channel modulating effects, and for use as probes to study the structure-function relationships of calcium channels, we now describe the synthesis and calcium channel modulating effects for a group of alkyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates (**4a-e**).

RESULTS AND DISCUSSION

A group of alkyl (Me, Et, *i*-Pr, *i*-Bu, *t*-Bu) 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates (**4a-e**) were prepared using a classical Biginelli reaction as illustrated in Scheme 1. Thus, the hydrochloric acid catalyzed condensation of 2-pyridinecarboxaldehyde (**1**), urea (**2**) and an alkyl acetoacetate (**3**) in MeOH at 85-87° for 5 days afforded the respective products (4a-e) in 20-41% yield. Numerous improved procedures have been described using Lewis acids, ionic liquids and microwave irradiation to increase the yield and decrease the reaction time of the Biginelli reaction for products possessing aryl moieties at the C-4 position [13]. Although, the synthesis of methyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (**4a**) in 89% yield with a reaction time of 3 hours at 25° using a bismuth triflate catalyzed Bigenelli reaction [14], and ethyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (**4b**) in 83% yield with a reaction time of 13 minutes using a microwave irradiation enhanced indium triflate catalyzed Bigenelli reaction [15] have been described, no biological studies were reported for compounds **4a** and **4b**.

Scheme I



1 **2** **3** **4**

The biological objectives of this study were to determine i) whether a 2-pyridyl substituent is a bioisostere of an *ortho*-substituted-phenyl substituent, and ii) if the alkyl 6-methyl-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylate moiety is a mimetic of the dialkyl 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate moiety, present in traditional calcium channel antagonists such as nifedipine with respect to calcium channel modulation effects of the target compounds **4a-e**.

In vitro calcium channel modulation studies showed that the alkyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates (**4a-e**) exhibited a weak calcium channel antagonist effect [19-68% decrease in the tonic contractile response in guinea pig ileal longitudinal muscle (GPILSM) induced by the muscarinic agonist carbachol (1.6×10^{-7} M)]. Accordingly, the percentage (%) reduction in contractile force at the highest test compound concentration of 2.99×10^{-5} M employed was as follows: **4a** (19%), **4b** (68%), **4c** (45%), **4d** (36%) and **4e** (39%). The most potent calcium channel antagonist ethyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (**4b**, $IC_{50} = 1.67 \pm 0.16 \times 10^{-5}$ M) was a much weaker calcium channel antagonist than the reference drug nifedipine (Adalat®, $IC_{50} = 1.40 \pm 0.19 \times 10^{-8}$ M). In vitro calcium channel modulation studies on guinea pig left atrium (heart) (GPLA) showed that the alkyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-ones (**4a-e**) did not decrease (negative inotropic or calcium channel antagonist effect), or increase (positive inotropic or calcium channel agonist effect), the contractile force of GPLA at the maximum test compound concentration of 4.46×10^{-5} M that was used. These structure-activity studies on GPLLSM show that the alkyl 6-methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-2*H*-pyrimidine-2-one-5-carboxylates (**4a-e**) are partial bioisosteres of nifedipine with respect to calcium antagonist activity on GPILSM.

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 (NH) was confirmed by the addition of deuteriowater (D_2O). Silica gel column chromatography was carried out using Silicyle (70-230 mesh) silica gel. Isopropyl acetoacetate was purchased from the Lancaster Chemical Co. All other reagents were purchased from the Aldrich Chemical Co. Elemental analyses were performed for C, H and N (Micro-Analytical Service Laboratory, Department of Chemistry, University of Alberta). In vitro calcium channel antagonist and agonist activities were determined using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

General Procedure for the Synthesis of Alkyl 6-Methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-ones (**4a-e**). Urea (**2**) (2.4 g, 40 mmoles) was added to a solution of 2-pyridinecarboxaldehyde (**1**) (6.97 g, 40 mmoles) and an alkyl acetoacetate (**3**, R = Me, Et, *i*-Pr, *i*-Bu, or *t*-Bu)) (40 mmoles) in methanol (40 ml) with stirring. Concentrated hydrochloric acid (4 drops of 37%, w/v) was added, the reaction was allowed to proceed at 85-87° for 5 days with stirring, the reaction mixture was cooled to 25°, and stirring was continued at 25° for 1 hour. Removal of volatile components *in vacuo* gave an off-white residue that was washed with cold water to remove urea and other water soluble impurities. Recrystallization of this material from EtOH (98%, v/v) afforded the respective product (**4a,** R = Me; **4b**, R = Et; **4c**, R = *i*-Pr; **4d**, R = *i*-Bu, or **4e**, R = *t*-Bu). The physical and spectral data for compounds **4a-e** are listed below.

Compound **4a** was obtained as a white solid in 20% yield, mp 236-238° (the mp and microanalytical data for **4a** was not

previously reported, Lit. [14]); ^{1}H nmr (deuteriodimethylsulfoxide): δ 2.21 (s, 3H, C-6 C*H*$_3$), 3.51 (s, 3H, CO_2CH_3), 5.12 (s, 1H, pyrimidone H-4), 7.22-7.27 (m, 2H, pyridyl H-3, H-5), 7.62 (s, 1H, N^3-*H*), 7.74 (dd, *J* = 7.0, 7.0 Hz, 1H, pyridyl H-4), 8.50 (d, *J* = 4.0 Hz, 1H, pyridyl H-6), 9.15 (s, 1H, N^1-*H*). *Anal*. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.21; H, 5.41; N, 16.86.

Ethyl 6-Methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (4b). Compound **4b** was obtained as a white solid in 41% yield, mp 228-230° (Lit. [15] mp 212-214°); ^{1}H nmr (deuteriodimethylsulfoxide): δ 1.07 (t, *J* = 7.0 Hz, 3H, CH_2CH_3), 2.22 (s, 3H, C-6 C*H*$_3$), 3.95 (q, 2H, CH_2CH_3), 5.20 (s, 1H, pyrimidone H-4), 7.21-7.27 (m, 2H, pyridyl H-3, H-5), 7.61 (s, 1H, N^3-*H*), 7.74 (dd, *J* = 7.0, 7.0 Hz, 1H, pyridyl H-4), 8.51 (d, *J* = 4.0 Hz, 1H, pyridyl H-6), 9.31 (s, 1H, N^1-*H*). *Anal*. Calcd. for $C_{13}H_{15}N_3O_3$: C, 59.31; H, 5.83; N, 15.96. Found: C, 59.43; H, 5.80; N, 15.91.

Isopropyl 6-Methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (4c). Compound **4c** was obtained as a white solid in 24% yield, mp 210-212°; ^{1}H nmr (deuteriodimethylsulfoxide): δ 0.96 and 1.14 [two d, *J* = 6.0 Hz, 3H each, $CH(CH_3)_2$], 2.21 (s, 3H, C-6 C*H*$_3$), 4.79 [septet, *J* = 6.0 Hz, 1H, $CH(CH_3)_2$], 5.18 (s, 1H, pyrimidone H-4), 7.23-7.28 (m, 2H, pyridyl H-3, H-5), 7.57 (s, 1H, N^3-*H*), 7.74 (dd, *J* = 7.0, 7.0 Hz, 1H, pyridyl H-4), 8.49 (d, *J* = 4.0 Hz, 1H, pyridyl H-6), 9.08 (s, 1H, N^1-*H*). *Anal*. Calcd. for $C_{14}H_{17}N_3O_3$: C, 60.64; H, 6.26; N, 15.15. Found: C, 60.85; H, 6.47; N, 15.17.

Isobutyl 6-Methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (4d). Compound **4d** was obtained as a white solid in 23% yield, mp 204-206°; ^{1}H nmr (deuteriodimethylsulfoxide): δ 0.72 and 0.74 [two d, *J* = 6.0 Hz, 3H each, $CH_2CH(CH_3)_2$], 1.69-1.77 [m, 1H, $CH_2CH(CH_3)_2$], 2.24 (s, 3H, C-6 C*H*$_3$), 3.64-3.78 [m, 2H, $CH_2CH(CH_3)_2$], 5.21 (s, 1H, pyrimidone H-4), 7.21-7.27 (m, 2H, pyridyl H-3, H-5), 7.64 (s, 1H, N^3-*H*), 7.74 (dd, *J* = 7.0, 7.0 Hz, 1H, pyridyl H-4), 8.49 (d, *J* = 4.0 Hz, 1H, pyridyl H-6), 9.15 (s, 1H, N^1-*H*). *Anal*. Calcd. for $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.19; H, 6.42; N, 14.36.

***Tert*-Butyl 6-Methyl-4-(2-pyridyl)-1,2,3,4-tetrahydro-(2*H*)-pyrimidine-2-one (4e)**. Compound **4e** was obtained as a white solid in 20% yield, mp 210-212°; ^{1}H nmr (deuteriodimethylsulfoxide): δ 1.27 (s, 9H, *tert*-butyl hydrogens), 2.18 (s, 3H, C-6 C*H*$_3$), 5.14 (s, 1H, pyrimidone H-4), 7.20-7.27 (m, 2H, pyridyl H-3, H-5), 7.53 (s, 1H, N^3-*H*), 7.74 (dd, *J* = 7.0, 7.0 Hz, 1H, pyridyl H-4), 8.50 (d, *J* = 4.0 Hz, 1H, pyridyl H-6), 9.96 (s, 1H, N^1-*H*). *Anal*. Calcd. for $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 61.99; H, 6.31; N, 14.78.

***In Vitro* Calcium Channel Antagonist and Agonist Assays.** Smooth muscle calcium channel antagonist activity was determined as the molar (M) concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 0.167 μM) Ca^{+2}-dependent contraction (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure previously reported [16]. The IC_{50} value (± SEM, n=3) was determined graphically from the dose-response curve (for compound **4b**), or the % reduction in contractile force at the highest test compound concentration used when an IC_{50} value could not be obtained (compounds **4a** and **4c-e**).

The cardiac calcium channel agonist effect was determined as the percentage increase (positive inotropic or calcium channel agonist effect), or the percentage decrease (negative inotropic or calcium channel antagonist effect), in contractile force of isolated guinea pig left atrium (GPLA) relative to its basal contractile force in the absence of test compound. Compounds **4a-e** did not increase, or decrease, the contractile force of GPLA in this assay.

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