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Synthesis and Calcium Channel Antagonist Activities of New Derivatives of Dialkyl 1,4-Dihydro-2,6-dimethyl-4-(5-phenylisoxazol-3-yl)pyridine-3,5-dicarboxylates

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The new analogues of nifedipine, in which 2-nitrophenyl group at position 4 is replaced by phenylisoxazolyl substituent, were synthesized. The symmetrical dialkyl 1,4-dihydro-2,6-dimethyl-4-(5-phenylisoxazol-3-yl)pyridine-3,5-dicarboxylates were prepared by classical Hantzsch condensation, and the asymmetrical analogues were synthesized using a procedure reported by Dagnino that involved the condensation of alkyl acetoacetate with alkyl 3-aminocrotonate and 5-phenylisoxazole-3-carboxaldehyde. The structure of all compounds was confirmed by IR, ^{1}H NMR and Mass spectra. *In vitro* calcium channel antagonist activities were evaluated as calcium channel antagonists using the high K^+ concentration of guinea-pig ileum longitudinal smooth muscle (GPILSM) assay. These compounds exhibited moderate calcium antagonist activity ($IC_{50} = 10^{-7}$ to 10^{-5} M range) relative to the reference drug nifedipine ($IC_{50} = 1.10 \pm 0.40 \times 10^{-8}$ M).

Keywords*:* Dihydropyridine, Ca^{2+} channels antagonist, Phenylisoxazole

INTRODUCTION

1,4-Dihydropyridine calcium channel antagonists are an important class of drugs which induce relaxation of vascular smooth muscle, preferentially in arteries, and display a negative inotropic effect on isolated cardiac muscle via binding to a high affinity binding site in 2-type voltage-dependent Ca^{2+} channel [1,2]. In therapy, this class of drugs has been used in general medical practice world wide for the treatment of hypertension & vasospastic angina for over 2 decades [3-5].

QSAR studies indicated that the potency of DHPs was dependent upon lipophilicity, an electronic term and separated terms for each position on the aromatic ring [6]. Changes in the substitution pattern at the C_3, C_4, C_5 position of nifedipine alter activity and tissue selectivity [7,8].

Natale *et al*. found that lipophilic 4-isoxazolyl-1,4-dihydropyridines that have an aryl group on isoxazole ring exhibit high binding affinity [9]. They proposed a model for DHP binding and found a highly lipophilic pocket in the receptor's active site. Examination of their model indicated that aryl substituent could be properly oriented to interact with the lipophilic pocket of their hypothesized channel. Other studies suggested that C_4 heterocycle substituents gave active compounds as calcium channel antagonists [10-12].

In previous studies, we reported that a C_4 phenylimidazolyl susbtituent gave very active compounds as calcium channel antagonist [13,14]. As a part of our ongoing program to design novel calcium channel antagonist [15-20], it was of our interest to determine the effects of C-3 & C-5 different alkyl

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substituents, in conjugation with C-4 phenylisoxazolyl substituents, on calcium channel antagonist activity. We now report the synthesis, calcium channel antagonist of dialkyl 1,4-dihydro-2,6-dimethyl-4-(5-phenylisoxazol-3-yl)pyridine-3,5-dicarboxylates.

EXPERIMENTAL PART

Chemistry

Melting points were determined with a Reichert-Jung hot-stage microscope and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker 80 MHz spectrometer in chloroform-d_1 or DMSO-d_6 and tetramethylsilane (TMS) was used as an internal standard. Mass spectra were recorded with a Finnigan Mat TSQ-70 spectrometer. Infrared spectra were acquired on a Nicolet Magna 550-FT spectrometer. Silica gel HT-254 (E. Merck) was used for thin layer chromatography.

Synthesis of 5-phenylisoxazole-3-carboxaldehyde (5). Manganese dioxide (25 g) was added to a stirred solution of 5-phenyl-3-hydroxymethylisoxazole (**4**, 4 g, 23 mmol) [21,22] in chloroform (100 ml). The mixture was refluxed overnight and filtered. The filtrate was evaporated and the residue was crystallized from methanol to give 2.6 g (64%) of compound **5**; m.p.: 59-61 °C [literature 21 m.p.: 59-61 °C].

General procedure for the synthesis of symmetrical esters of dialkyl 1,4-dihydro-2,6-dimethyl-4-(5-phenyl isoxazol-3-yl)pyridine-3,5-dicarboxylates (6a-g). A solution of ammonium hydroxide (25%, 0.5 ml) was added to a stirring solution of compound **5** (173 mg, 1 mmol) and corresponding alkyl-3-oxobutanoate (2 mmol) in methanol (5 ml). The mixture was protected from light and refluxed under argon overnight. After cooling the precipitate was filtered and crystallized from methanol to give pure compounds **6**.

Dimethyl 1,4-dihydro-2,6-dimethyl-4-(5-phenylisoxazol-3-yl) pyridine-3,5-dicarboxylate (6a). Yield: 42%, m.p.: 237-239 °C; IR (KBr): ν (cm^{-1}) 3268 (NH), 1681 (CO); ^{1}H NMR ($CDCl_3$): ppm 7.71 (m, 2H, aromatic), 7.41 (m, 3H, aromatic), 6.46 (s, 1H, H_4-isoxazole), 5.28 (s, 1H, H_4), 3.72 (s, 6H, 2×O-CH_3), 2.29 ppm (s, 6H, 2×CH_3); MS: m/z (%) 368 (M^+, 9), 353 (16), 337 (25), 309 (34), 277 (40), 224 (100), 192 (17), 156 (14), 105 (33), 77 (21). Anal. Calcd. For $C_{20}H_{20}N_2O_5$: C, 65.21; H, 5.47; N, 7.60. Found: C, 65.48; H, 5.22; N, 7.38.

The physical data and calcium channel modulation data for compounds **6a-6g** are given in Table 1.

General procedure for the synthesis of asymmetrical esters of dialkyl 1,4-dihydro-2,6-dimethyl-4-(5-phenyl isoxazol-3-yl)pyridine-3,5-dicarboxylates (7a-j). A mixture of compounds **5** (173 mg, 1 mmol), corresponding acetoacetate ester (1 mmol) and the respective alkyl 3-aminocrotonate (1 mmol) in absolute ethanol (10 ml) was refluxed for 10 h. After cooling the precipitate was filtered off and washed with cold ethanol. Recrystallization from methanol gave **7a-j** as yellow or white crystals.

Ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(5-phenyl isoxazol-3-yl)pyridine-3,5-dicarboxylate (7a). Yield: 68%, m.p.: 198-199 °C; ^{1}H NMR ($CDCl_3$): ppm 7.72 (m, 2H, aromatic), 7.42 (m, 3H, aromatic), 6.49 (s, 1H, H_4 isoxazole), 5.28 (s, 1H, H_4), 4.19 (q, 2H, O-CH_2), 3.72 (s, 3H, CH_3), 2.29 (s, 6H, 2×CH_3), 1.28 ppm (t, 3H, CH_3). Anal. Calcd. For $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.73; H, 5.65; N, 7.52.

The physical data and calcium channel modulation data for compounds **7a-7j** are given in Table 2.

Pharmacology

Investigations on isolated ileum of guinea pigs. Male guinea pigs, weighing 300-400 g, were killed by a blow on the head. The animals were deprived from food 18 h before sacrifice but had free access to water. The non-terminal part of the ileum was removed and cut into segments of 10-15 mm length. Each ileal segment was suspended in organ bath and connected to an isometric transducer (K30, Hugo Sachs Electronic, Germany). The organ bath contained 20 ml physiological solution of the following composition (in mM): NaCl 119, KCl 2.7, $CaCl_2$ 2, $MgCl_2$ 0.88, NaH_2PO_4 0.36, $NaHCO_3$ 12, glucose 5.5. The physiologic salt solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and its temperature was held at 37 °C. The fluid of the organ bath was changed every 15 min. A resting tension of 0.5 g was applied to the ileal segments and they were allowed to equilibrate for 1 h. Contractions of the ileal segments were recorded, using an amplifier (Plugsys, Hugo Sachs Electronic, Germany) and a recorder (Graphtec, model WR3320).

In order to study the effects of synthesized dihydropyridines on KCl-induced contraction of ileum, at the

Table 1. Physical Data and Calcium Channel Modulation Data for Compounds **6a-6g**

Comp.	R_1	Yield (%)	m.p. (°C)	NMR	IC_{50} ±SEM(n) M
6a	Me	42	237-239	See experimental	$9.61\pm1.1\times10^{-6}$(4)
6b	Et	63	151-153	7.71 (m, 2H, aromatic), 7.41 (m, 3H, aromatic), 7.00 (bs, 1H, NH), 6.51 (s, 1H, H-C_4 isoxazole), 5.27 (s, 1H , H-C_4 dihydropyridine), 4.18 (q, 4H, 2×CH_2), 2.28 (s, 6H, 2×CH_3), 1.27 ppm (t, 6H, 2×CH_3).	$8.05\pm0.98\times10^{-7}$(5)
6c	n-Pr	61	134-136	7.7 (m, 2H, aromatic), 7.41 (m, 3H, aromatic), 6.79 (bs, 1H, NH), 6.50 (s, 1H, H-C_4 isoxazole), 5.29 (s, 1H, H-C_4 dihydropyridine), 4.09 (t, 4H, 2×O-CH_2), 2.30 (s, 6H, 2×CH_3), 1.62 ppm (m, 4H, 2×CH_2), 0.93 ppm (t, 6H, 2×CH_3).	$3.62\pm0.90\times10^{-6}$(5)
6d	iso-Pr	41	185-187	7.71 (m, 2H, aromatic), 7.42 (m, 3H, aromatic), 6.51 (s, 1H, H-C_4 isoxazole), 5.25 (s, 1H , H-C_4 dihydropyridine), 5.06 (m, 2H, 2×O-CH), 2.28 (s, 6H, 2×CH_3), 1.28-1.21 (2d, J = 6.4 Hz, 12H, 4×CH_3).	$5.54\pm1.34\times10^{-6}$(4)
6e	n-Bu	43	114-116	7.71 (m, 2H, aromatic), 7.42 (m, 3H, aromatic), 7.08 (bs, 1H, NH), 6.51 (s, 1H, H-C_4 isoxazole), 5.28 (s, 1H, H-C_4 dihydropyridine), 4.13 (t, 4H, 2×OCH_2), 2.28 (s, 6H, 2×CH_3), 1.80-0.8 ppm (m, 14H, CH_2, CH_3).	$1.42\pm2.30\times10^{-5}$(4)
6f	iso-Bu	19	161-163	7.7 (m, 2H, aromatic), 7.41 (m, 3H, aromatic), 6.47 (s, 1H, H-C_4 isoxazole), 6.41 (bs, 1H, NH), 5.32 (s, 1H, H-C_4 dihydropyridine), 3.92 (d, 4H, 2×CH_2), 2.34 (s, 6H, 2×CH_3), 1.95 (m, 2H, 2×CH), 0.93 ppm (d, J = 6.4 Hz, 12H, 4×CH_3).	$4.12\pm0.44\times10^{-6}$(4)
6g	t-Bu	43	214-215	7.72 (m, 2H, aromatic), 7.41 (m, 3H, aromatic), 6.49 (s, 1H, H-C_4 isoxazole), 6.10 (bs, 1H, NH), 5.19 (s, 1H, H-C_4 dihydropyridine), 2.82 (s, 6H, 2 ×CH_3), 1.47 (s, 18H, 6×CH_3).	$1.75\pm0.96\times10^{-5}$(6)
Nif.					$1.10\pm0.4\times10^{-8}$(5)

first step, several contractions with KCl (40 mM) were made. No significant differences between KCl-induced contractions were considered as stability of tissue and thereafter the main experiments were started. At this step, KCl (40 mM)-induced contraction was recorded again and the peak of the first phase (phasic contraction) was considered as a control. Then, tissues were pre-incubated with one certain concentration of each compound (for 15 min.) and the effect of KCl (40 mM) was assessed once again. Each segment was treated with only one compound. From concentration-response curves, the pIC_{50} ($-\log IC_{50}$) value of each compound was calculated. Nifedipine was used as reference compound.

The results are presented as mean ± S.E.M. and evaluated statistically using Student's *t*-test. *P* values less than 0.05 were considered significant (Tables 1-2).

RESULTS AND DISCUSSION

The synthesis of 1,4-dihydropyridine derivatives **6** and **7** was achieved following the steps outlined in Scheme 1.

Table 2. Physical Data and Calcium Channel Modulation Data for Compounds **7a-7j**

Comp.	R_1	R_2	Yield (%)	m.p. (°C)	NMR	IC_{50} ±SEM(n) M
7a	Me	Et	68	198-199	See experimental	$7.89\pm1.09\times10^{-6}$(6)
7b	Me	n-Pr	18	152-153	7.75 (m, 2H, aromatic), 7.43 (m, 3H, aromatic), 7.35 (bs, 1H, NH), 6.48 (s, 1H, H-C_4 isoxazole), 5.27 (s, 1H, H-C_4 dihydropyridine), 4.09 (t, J = 7.2 Hz , 2H, O-CH_2), 3.72 (s, 3H, CH_3O), 2.29 (s, 6H, 2× CH_3), 1.69 (m, 2H, CH_2), 0.93 ppm (t, J = 7.2 Hz, 3H, CH_3).	8.88±0.77×10- (4)
7c	Me	iso-Pr	21	203-206	7.21 (m, 2H, aromatic), 7.43 (m, 3H, aromatic), 6.68 (bs, 1H, NH), 6.47 (s, 1H, H-C_4 isoxazole), 5.27 (s, 1H, H-C_4 dihydropyridine), 4.73 (m, 1H, OCH), 3.73 (s, 3H, O-CH_3), 2.30 (s, 6H, 2×CH_3), 1.27, 1.23 ppm (2d, J = 6.4 Hz, 6H, CH_3).	$5.99\pm0.775\times10^{-6}$(5)
7d	Me	t-Bu	20	159-161	7.67 (m, 2H, aromatic), 7.37 (m, 3H, aromatic), 6.48 (s, 1H, H-C_4 isoxazole), 5.21 (s, 1H, H-C_4 dihydropyridine), 3.71 (s, 3H, O-CH_3), 2.28 (s, 6H, 2× CH_3), 1.47 ppm (s, 9H, 3×CH_3).	$6.55\pm1.2\times10^{-6}$(5)
7e	Me	Cyclohexyl	25	136-138	7.71 (m, 2H, aromatic), 7.42 (m, 3H, aromatic), 6.64 (bs, 1H, NH), 6.49 (s, 1H, H-C_4 isoxazole), 5.26 (s, 1H, H-C_4 dihydropyridine), 4.85 (m, 1H, O-CH), 3.72 (s, 3H, CH_3), 2.30 (s, 6H, 2×CH_3), 2.1-1.1 (m, 10H, cyclohexyl).	$8.62\pm1.35\times10^{-6}$(6)
7f	Me	Cyclohexylmethyl	41	180-182	7.71 (m, 2H, aromatic), 7.32 (m, 3H, aromatic), 6.45 (s, 1H, H-C_4 isoxazole), 6.20 (bs, 1H, NH), 5.25 (s, 1H, H-C_4 dihydropyridine), 3.98 (m, 2H, OCH_2), 3.73 (s, 3H, OCH_3), 2.33 (s, 6H, 2×CH_3), 1.97-1.08 ppm (m, 11H, Cyclohexyl).	$1.60\pm0.45\times10^{-5}$(6)
7g	Me	Phenethyl	18	165-168	7.68 (m, 2H, aromatic), 7.29 (m, 8H, aromatic), 6.84 (bs, 1H, NH), 6.28 (s, 1H, H-C_4 isoxazole), 5.24 (s, 1H, H-C_4 dihydropyridine), 4.36 (t, J = 6.4 Hz, 2H, O-CH_2), 3.27 (s, 3H, O-CH_3), 2.59 (t, J = 6.4Hz , 2H, CH_2), 2.31 (s, 6H, 2×CH_3).	$6.87\pm1.53\times10^{-6}$(6)
7h	Et	n-Pr	55	143-144	7.71 (m, 2H, aromatic), 7.42 (m, 3H, aromatic), 6.47 (bs, 2H, NH, H-C_4 isoxazole), 5.27 (s, 1H, H-C_4 dihydropyridine), 4.05 (m, 4H, 2×O-CH_2), 2.31 (s, 6H, 2×CH_3), 1.69 (m, 2H, CH_2), 1.28 (t, J = 6.4 Hz, 3H, CH_3), 0.93 ppm (t, J = 6.4 Hz, 3H, CH_3).	$7.14\pm1.06\times10^{-6}$(6)

Table 2. Continued

7i	Et	iso-Pro	58	165-168	7.75 (m, 2H, aromatic), 7.45(m, 3H, aromatic), 7.08 (bs, 1H, NH), 6.53 (s, 1H, H-C_4 isoxazole), 5.25 (s, 1H, H-C_4 dihydropyridine), 5.80 (m, 1H, O-CH), 4.17 (q, J = 7.2 Hz, 2H , O-CH_2), 2.28 (s, 6H, 2×CH_3), 1.27 (t, J = 7.2 Hz, 3H, CH_3), 1.20, 1.33 ppm (2d, J = 4 Hz, 6H, 2×CH_3).	$9.86\pm3.01\times10^{-6}$(7)
7j	Et	Cyclohexyl	21	99-102	7.71 (m, 2H, aromatic), 7.41 (m, 3H, aromatic), 7.00 (bs, 1H, NH), 6.52 (s, 1H, H-C_4 isoxazole), 5.28 (s, 1H, H-C_4 dihydropyridine), 4.38 (m, 1H, O-CH), 4.17 (q, J = 7.2 Hz, 2H , O-CH_2), 2.28 (s, 6H, 2×CH_3), 2.11-1.1 ppm (m, 13H, cyclohexyl, CH_3).	$2.08\pm1.06\times10^{-5}$(7)

Reaction of alcohol **1** with 2,2,6-trimethyl-4H-1,3-dioxine-4-one **2** afforded the corresponding acetoacetic esters **3** (76-92% yield) [23]. Manganese(IV) oxide oxidation of 5-phenyl-3-hydroxymethylisoxazole **4** in chloroform afforded the desired aldehyde **5** in 64% yield [21,22].

Symmetrical dihydropyridine analogs **6** were prepared by classical Hantzsch condensation in which the aldehyde **5** was reacted with the acetoacetic ester **3** and ammonium hydroxide in ethanol [24]. Since dihydropyridines may undergo oxidation during synthesis [25,26], the preparation, collection and purification of the product were carried out in the absence of oxidizing materials and in darkness. The asymmetrical analogs **7** were synthesized by a modified Hantzsch reaction, using a procedure reported by Dagnino *et al*. [27].

The *in vitro* calcium channel antagonist activities (IC_{50}) of compounds **6** and **7** were determined as the molar concentration of the test compounds required to produce 50% inhibition of the high K^+ concentration of guinea pig ileal longitudinal smooth muscle (GPILSM), and are presented in Tables 1 and 2. These results indicate that compounds **6** and **7** exhibit less potent calcium channel antagonist activity (10^{-5}-10^{-7} M) relative to the reference drug Nifedipine ($IC_{50} = 1.10 \pm 0.40 \times 10^{-8}$ M).

Though previous studies indicated that 5-substituted-3-phenylisoxazol-4-yl-1,4-dihydropyridines are potent CCBs [9], nevertheless 5-phenylisoxazol-3-yl-1,4-dihydropyridine derivatives under the present study are 10 times less active than Nifedipine. This may be due to steric interference at calcium channel receptor.

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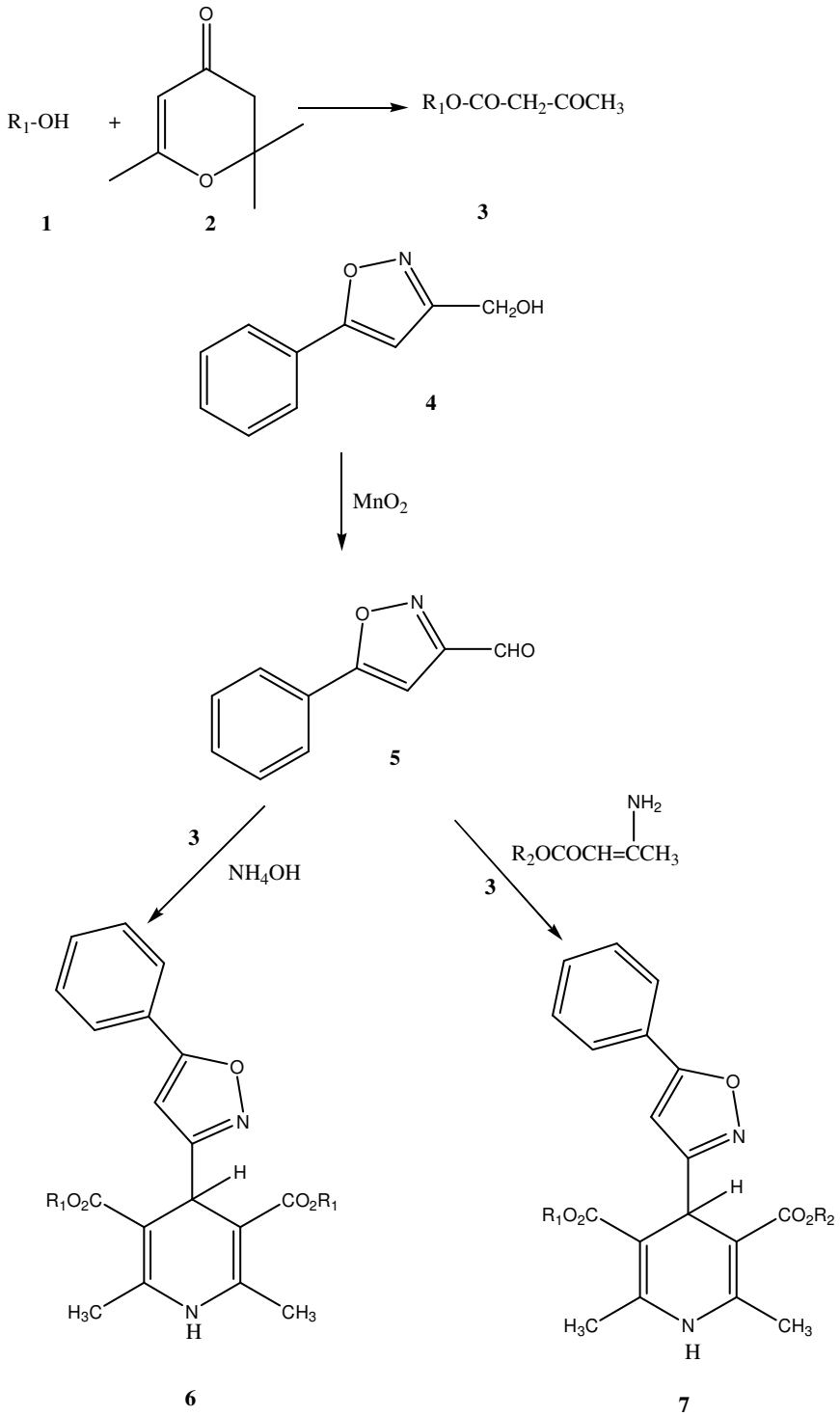

Scheme 1. Synthesis of symmetical and unsymmetical analogues.

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