SYNTHESIS AND CALCIUM CHANNEL ANTAGONIST ACTIVITY OF NIFEDIPINE ANALOGUES WITH CHLOROINDOLYL SUBSTITUENT

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ABSTRACT

Various diester analogues of nifedipine in which the ortho nitrophenyl group at position 4 were replaced by 3-chloro-1H-2-indolyl substituent, were synthesized and evaluated as calcium antagonists on guinea-pig ileal smooth muscle. Nifedipine was used as a standard. Compound **6f** was found to be the most active.

Key words: Calcium channel blockers; Dihydropyridine, Nifedipine analogues.

INTRODUCTION

Very soon after the discovery of the cardiovascular properties of 1,4-dihydropyridines, it was found that these substances act by inhibiting the entry of Ca^{2+} into the cells of cardiac and vascular muscle through the voltage-dependent calcium channels (1).

Structurally diverse groups of compounds are known to be effective as calcium antagonists (2). The most potent class of antagonists comprises derivatives of 1,4-dihydropyridine of which the most widely known agent is nifedipine (3). This class of compounds have been the subject of many structure-activity relationship studies (4-6). Previously we reported the effect of C-4 nitroimidazolyl and methylsulfonylimidazolyl substituents in conjunction with various C-3, C-5 diesters on calcium channel antagonist activities (7, 8). This paper describes the synthesis and activity of 1,4-dihydro-2,6-dimethyl- 4 - (3- chloro - 1H– 2-indolyl-) - 3, 5 – pyridine - dicarboxylic acid esters.

MATERIALS AND METHODS

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H -NMR spectra were run on a Varian 400 Unity plus or a Brucker FT-80 spectrometer. Tetramethylsilane was used as an internal standard. Mass spectra were measured with a Finnigan TSQ-70 spectrometer at 70 eV. The IR spectra were obtained using a Nicolet FT-IR Magna 550. All compounds gave satisfactory elemental analyses within ±0.4% of the theoretical values.

Dimethyl1,4-Dihydro-2,6-dimethyl-4-(3-chloro-1H-2-indolyl)-3,5-pyridinedicarboxylate (3a).

A solution of ammonium hydroxide (25%, 0.4 ml) was added to a stirring solution of compound **2** (0.268 g, 1.5 mmol) and methyl 3-oxobutanoate **1** (0.371 g, 3.2 mmol) in absolute ethanol (8 ml). The mixture was protected from light and heated overnight under reflux. After cooling the reaction, ethanol was removed and the residue purified by thin layer chromatography (petroleum ether- ethylacetate; 75:25) to give 0.31 g, (55%) of **3a**, mp 230-232°C (CCl_4/hexane); IR (KBr): ν(cm^{-1}) 3420 (NH), 3340 (NH), 1700 (C=O); ^{1}H-NMR ($CDCl_3$):δ 8.29 (brs, 1H,NH indole), 7.50 (d, J=8Hz, 1H, H_7 indole), 7.24 (d, J=8Hz, 1H, H_4 indole), 7.14 (t, J=8Hz, H_6 indole) 7.09 (t, J=8 Hz, H_5 indole), 5.77 (brs, 1H, NH), 5.33 (s, 1H, H_4-dihydropyridine), 3.66 (s, 6H, 2COOMe), 2.34 (s, 6H, 2Me); ^{13}C($CDCl_3$) δ 167.89 (CO), 145.12(C_8), 138.08 (C_9) 133.64 (C_2, indole), 126.45 (C_3, indole) 122.31 (C6, indole), 119.81 (C_4, indole), 117.71 (C_5, indole), 110.9 (C_7), 100.24 (C_2 and C_6, dihydropyridine), 96.12 (C_3 and C_5 dihydropyridine), 51.24 (OMe), 33.04 (C_4, dihydropyridine), 19.53 (C_2 and C_6, methyl); MS: m/z (%) 374 (M^+, 18), 339 (62), 307 (22), 224 (100), 192 (12), 59 (8). Other

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compounds of the table 1 (**3b-e**) were prepared similarly.

Methyl 2-[(3-Chloro-1H-2-indolyl)methylene]-3-oxobutanoate (4,R_1=methyl, n=0): A mixture of compound **2** (268 mg, 1.5 mmol), methyl 3-oxobutanoate **1** (174 mg, 1.5 mmol), glacial acetic acid (0.12 ml), piperidine (0.04 ml), anhydrous magnesium sulfate (360 mg, 3 mmol) and dry chloroform (20 ml) was refluxed for 2 h. The reaction mixture was filtered and the chloroform was removed. The oily residue was purified by thin layer chromatography (petroleum ether-ethyl acetate; 75:25) to give 375 mg (90%) of **4**, mp 117-118°C (methanol); IR (KBr): ν(cm^{-1}), 1700; ^{1}H-NMR ($CDCl_3$): δ 10.84 (brs, 1H, NH indole), 8.02 (s, 1H, CH=C), 7.63 (m, 1H, arom), 7.25 (m, 3H, arom), 3.91 (s, 3H, CH_3O), 2.58 (s, 3H, CH_3).

3-Methyl,5-isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-chloro-1H-2-indolyl)-3, 5-pyridinedicarboxylate (6b): To a stirring solution of compound **4** (R_1=methyl, n=0, 277 mg, 1 mmol) in absolute ethanol (8 ml), was added isopropyl aminocrotonate **5** (R_2=CH(CH_3)$_2$, 143 mg, 1 mmol). The solution was protected from light and refluxed overnight. After cooling, ethanol was removed and the residue was purified by thin layer chromatography (petroleum ether-ethyl acetate; 75:25) to give 245 mg (61%) of **6b**, mp 209-210°C (CCl_4/hexane). IR (KBr): ν(cm^{-1}), 3400, 3300 (NH), 1670 (CO); ^{1}H-NMR ($CDCl_3$): δ 8.29 (brs, 1H, NH indole), 7.48 (d, J=8Hz, 1H, H_7 indole), 7.24 (d, J=8Hz, 1H, H_4 indole), 7.14 (t, J=8Hz, 1H, H_6 indole), 7.09 (t, J=8Hz, H_5 indole), 5.77 (brs, 1H, NH), 5.33(s, 1H, H_4-dihydropyridine), 4.98[m, 1H, $CHMe_2$], 3.66 (s, 3H, COOMe), 2.34 (s, 6H, $2CH_3$), 1.19 and 1.23 [d,d J=7.5 Hz, 6H, $(CH_3)_2CH$] , ^{13}C($CDCl_3$): δ 167.89 and 166.97 (CO), 145.14 (C_8), 138.26 (C_9), 133.56 (C_2, indole), 126.44 (C_3, indole), 122.27 (C_6, indole), 119.74 (C_4, indole), 117.68 (C_5, indole), 110.83 (C_7), 100.83 (C_2 and C_6, dihydropyridine), 95.85 (C_3 and C_5, dihydropyridine), 67.57 [-CHOCO], 51.14 (OMe), 32.99 (C_4, dihydropyridine), 22.13 and 21.93[-(CH)Me_2], 19.65 (C_2 and C_6-methyl); MS: m/z (%) 402 (M^+, 35), 367 (100), 329 (5), 210 (88).

Other compounds **6a-k** were prepared similarly.

Pharmacology: Male albino guinea-pigs (300-450 g) were killed by a blow on the head. The intestine was removed above the ileocaecal junction and longitudinal smooth muscle segments of 2 cm length were mounted under a resting tension of 0.5 g. The segments were maintained at 37°C in a 20-ml jacketed organ bath containing oxygenated physiological saline solution of the following millimolar composition: NaCl, 137; $CaCl_2$, 1.8; KCl, 2.7; $MgSO_4$, 1.1; NaH_2PO_4, 0.4; $NaHCO_3$, 12 and glucose, 5. The muscles were equilibrated for 1 h with a solution which change was every 15 min. The contractions were recorded with a force displacement transducer (F-50) on a NARCO physiograph. Test agents were prepared as 10^{-2} M stock solutions in ethanol and stored protected from light. Dilutions were made into double distilled water. The contractile response was taken as the 100% value for the tonic (slow) component of the response. The contraction was elicited with 80 mM KCl. Test compounds were cumulatively added after the dose response for KCl was determined. Test compound-induced relaxation of contracted muscle was expressed as percent of control. The IC_{50} values (concentration needed to produce 50% relaxation on contracted ileal smooth muscle) were graphically determined from the concentration-response curves (9,10).

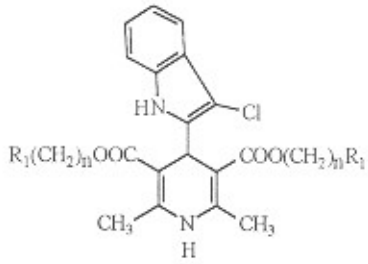
RESULTS AND DISCUSSION

Chemistry: Symmetrical **3a-e** and asymmetrical **6a-k** analogues of nifedipine were synthesized according to scheme 1. The symmetrical analogues **3a-e** were prepared by classical Hantzsch condensation (11) in which 3-chloro-1H-indol-2-carboxaldehyde **2** (12) was reacted with 3-oxobutanoic acid esters **1** (13) and ammonium hydroxide. The asymmetrical analogues **6a-k** were synthesized by a procedure reported previously (14).

Pharmacology: The calcium channel antagonist activities (IC_{50}) of compounds **3a-e** and **6a-k** were determined as the concentration needed to produces 50% relaxation of contracted guinea-pig ileal longitudinal smooth muscle (9). The results are summarized in tables 1 and 2. A comparison of the activities of symmetrical esters **3a-e**, indicate that increasing the size of the ester group increases activity **3c>3a**.

In asymmetrical series of phenylalkyl esters when R_2 is a small substituent (R_2=CH_3) increasing the length of methylene chain

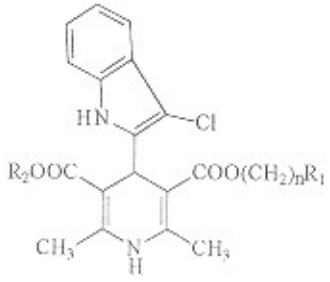
Table 1: Physical properties and calcium channel antagonist activities of symmetrical esters **3a-e**



Compound	R	n	Mp(°C)	Yield (%)	IC_{50}^{a}(M)
3a	CH_3	0	230-232	55	2.43 (0.33) × 10^{-8}
3b	CH_3	1	213-215	44	4.32(0.46) × 10^{-8}
3c	$C(CH_3)_3$	0	241-243	65	6.35 (0.48) × 10^{-9}
3d	C_6H_5	1	165-166	51	8.29 (0.43) × 10^{-9}
3e	C_6H_5	4	120-121	47	1.77 (0.87) × 10^{-7}
nifedipine					2.75 (0.36) × 10^{-10}

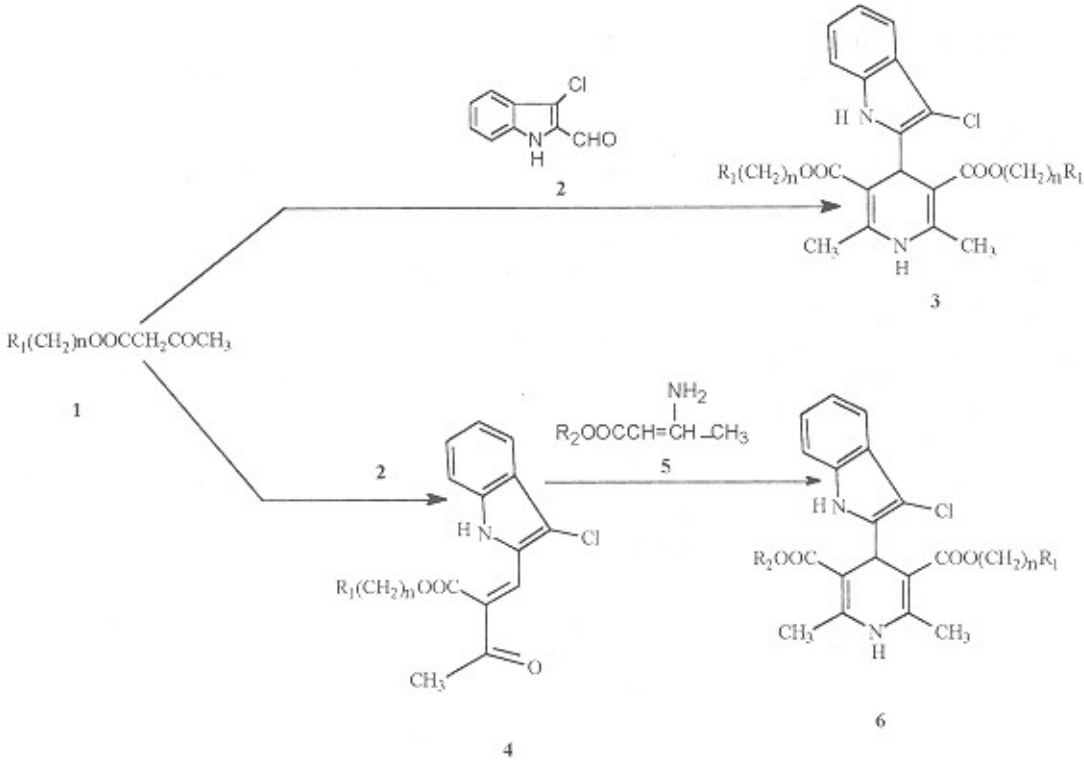
a; n=6, Standard deviation in parentheses.

Table 2: Physical properties and calcium channel antagonist activities of asymmetrical esters **6a-k**



Compound	R_1	n	R_2	Mp(°C)	Yield (%)	IC_{50}^{a}(M)
6a	CH_3	1	CH_3	168-169	45	1.46(0.64) ×10^{-8}
6b	$CH(CH_3)_2$	0	CH_3	209-210	61	2.01 (0.45) ×10^{-8}
6c	$CH(CH_3)_2$	0	C_2H_5	256-257	55	1.17 (0.30) ×10^{-8}
6d	$C(CH_3)_3$	0	CH_3	220-221	57	9.02 (0.50) ×10^{-9}
6e	$C(CH_3)_3$	0	C_2H_5	222-224	52	6.14 (0.41) ×10^{-9}
6f	C_6H_5	1	CH_3	170-171	62	1.13 (0.36) ×10^{-9}
6g	C_6H_5	1	C_2H_5	95-96	54	2.56 (0.35) ×10^{-9}
6h	C_6H_5	2	CH_3	125-126	65	3.51 (0.26) ×10^{-9}
6i	C_6H_5	2	C_2H_5	79-80	55	4.51 (0.56) ×10^{-9}
6j	C_6H_5	4	CH_3	147-148	40	6.72 (0.34) ×10^{-9}
6k	C_6H_5	4	C_2H_5	143-144	46	8.24 (0.30) ×10^{-9}
nifedipine						2.75 (0.36) × 10^{-10}

a; n=6, Standard deviation in parentheses.



Scheme 1

decreased activity (**6j<6h<6f**). Comparison of the effect of phenyl relative to alkyl substituent, shows that phenyl derivatives are more active than alkyl derivatives. Compound 6f (R_1=C_6H_5, R_2=CH_3, n=1) was the most active compound.

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