SYNTHESIS AND SMOOTH MUSCLE CALCIUM CHANNEL ANTAGONIST EFFECTS OF NEW DERIVATIVES OF 1,4-DIHYDROPYRIDINE CONTAINING NITROIMIDAZOLYL SUBSTITUENT

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ABSTRACT

A group of racemic 3-[(2-hydroxyethyl), (2-Methoxyethyl), (2-acetylethyl) or (2-cyanoethyl)], 5-methyl, ethyl or isopropyl-1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylates [XIV-XXV] were prepared by the reaction of 1-methyl-5-nitroimidazol-2-carboxaldehyde [X] with acetoacetic esters [VI-IX] and alkyl 3-aminocrotonate [XI-XIII]. *In vitro* calcium channel antagonist activities of the tested compounds were determined by their effects on contraction of Guinea Pig Ileal Longitudinal Smooth Muscle (GPILSM) which was induced by carbacol (1.67×10^{-7} M). All compounds exhibited calcium channel antagonist activity ($IC_{50}=10^{-12}$ to 10^{-13} M range) comparable to nifedipine as reference drug ($IC_{50}=1.07\pm0.12 \times 10^{-11}$ M).

Keywords: Dihydropyridine; Calcium channels antagonist; Nitroimidazole; GPILSM

INTRODUCTION

The influx of extracellular Ca^{2+} through L-type potential dependent calcium channel is responsible for regulation of many physiological functions including smooth and cardiac muscle contraction (1-4). The discovery that the 1,4-dihydropyridine (Nifedipine, Nimodipine) class of calcium channel antagonists inhibits Ca^{2+} influx represented a major therapeutic advance in treatment of cardiovascular diseases such as hypertension, angina pectoris and other spastic smooth muscle disorders (5-7). Changes in substitution pattern at the C-3, C-4 and C-5 positions of nifedipine alter activity and tissue selectivity (8-9). In the previous article calcium channel antagonist activities of a series of compounds resulting from the replacement of nitrophenyl in Nifedipine analogues with its bioisoster 1-methyl-5-nitroimidazole was described (10). This paper describes the effects of different substituents such as hydroxyethyl, acetylethyl, cyanoethyl or methoxyethyl in the alkyl group of the esteric group at position 3 along with different alkyls in the position 5 in dialkyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl) -3,5-pyridinedicarboxylate on their calcium channel antagonist activities.

MATERIAL AND METHODS

Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H-NMR spectra were run on a Varian Unity Plus 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) relative to TMS as an internal standard. The mass spectra were measured with a Finnigan TSQ-70 spectrometer at 70 ev. The IR spectra were obtained by using a Nicolet 50X-FT spectrophotometer (KBr disks). All spectra were consistent with the assigned structures. Diketene 5 and methyl, ethyl or isopropyl 3-aminocrotonate XI-XIII were purchased from the Aldrich Chemical Co. Reaction of alcohols I-IV with diketene 5 afforded the corresponding acetoacetic esters VI-IX (11-12). The unsymmetrical

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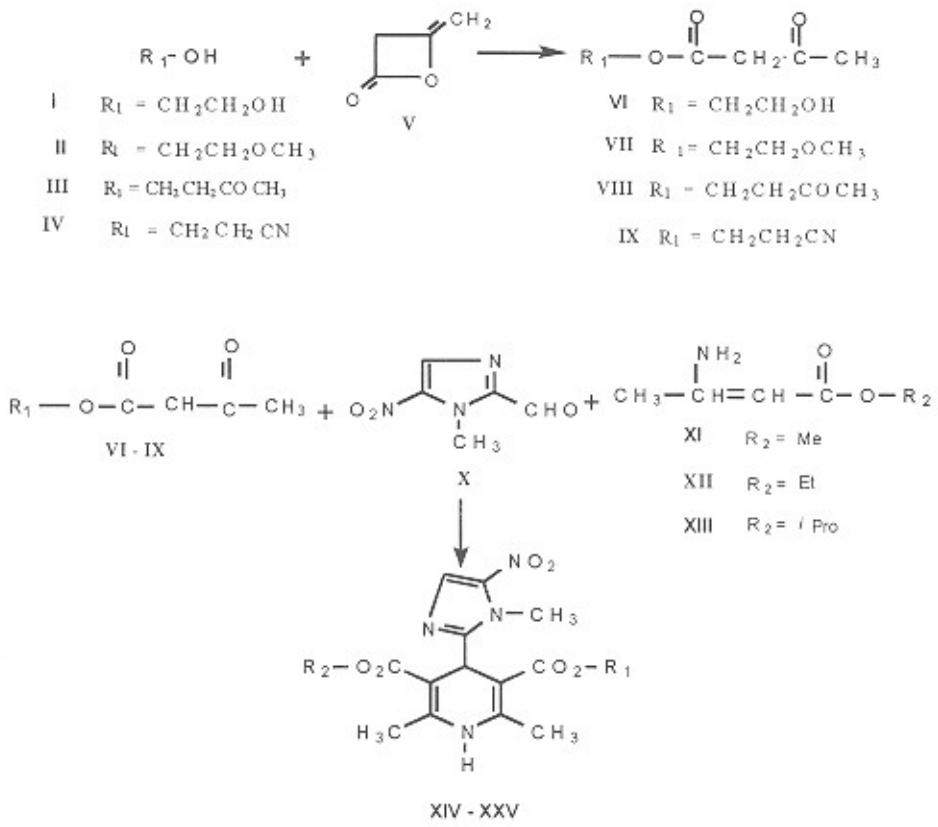


Scheme 1

analogues XIV-XXV were prepared by modified Hantsch reaction reported by Meyer through the reaction of 1-methyl-5-nitro-imidazole-2-carboxaldehyde 10 was reacted with 3-oxobutanoic acid esters VI-IX and alkyl 3-aminocrotonate XI-XIII (13-15). (Scheme 1)

General procedure for the synthesis of acetoacetate derivatives VI-IX (procedure A)

Diketen V (0.84 g, 10 mmol) was added dropwise with stirring to the preheated alcohol I-IV (10 mmol) at 50-60°C in the presence of a catalytic amount of Et_3N (5 drop). Diketene was added in a rate such that 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. The products were isolated by silica gel column chromatography or distillation *in vacue*.

2-Hydroxyethyl acetoacetate (VI)

The reaction of ethylenglycol I (24.04 g, 387 mmol) and diketene V (8.15 g, 97 mmol) and triethylamine (0.25 ml, 4.6 mmol) gave a product which was isolated by silica gel column chromatography using EtOAc-nC_6H_{14} (2:1, V/V) as eluent. The product 6 was isolated as yellow oil (11.9 g, 84%).

^{1}H NMR ($CDCl_3$)δ: 4.24 (t, J=4.6 Hz, 2H, CO_2CH_2), 3.78 (t, J=4.6 Hz, 2H,CH_2OH), 3.49 (s, 2H, $COCH_2CO$), 2.92 (br s, 1H, OH, exchangeable with D_2O), 2.24 (s, 3H, CH_3CO).

IR (KBr) :ν 3074-3706(br, OH), 1760(C=O, ester), 1720 cm^{-1} (C=O, ketone).

2-Methoxyethyl acetoacetate (VII)

The reaction of methoxyethanol II (2.73 g, 35.9 mmol) and diketene V (3.02 g, 35.9 mmol) and triethylamine (0.5 ml, 9.2 mmol) gave a product which was isolated by distillation at reduced pressure (bp 82-84 °C/3 mmHg) as a colorless oil (2.62 g, 84%).

^{1}H NMR ($CDCl_3$): δ 4.26(t, J=4.7 Hz, 2H, CO_2CH_2), 3.78(t, J=4.7 Hz, 2H,CH_2OMe), 3.46 (s,

2H, $COCH_2CO$) , 3.34 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3CO).
IR (KBr):ν 1752(C=O, ester), 1727 cm^{-1} (C=O, ketone).

2-Acethylethyl acetoacetate (VIII)
The reaction of acethylethanol III (2.2 g, 25 mmol) and diketene V (2.1 g, 25 mmol) and triethylamine (0.3 ml, 5.5 mmol) gave a product which was isolated by distillation at reduced pressure (bp 104-106^0 C/ 3 mmHg) as a colorless oil (3.74 g, 87%) .
^{1}H NMR ($CDCl_3$): δ 4.06 (t, J=6.4 Hz, 2H, CO_2CH_2), 3.38 (s, 2H, $COCH_2CO$), 2.45 (t, J=6.4 Hz, 2H, CH_2CO), 2.19 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO).
IR (KBr):ν 1752(C=O, ester), 1727 (C=O, ketone) cm^{-1}.

2-Cyanoethyl acetoacetate (IX)
The reaction of 3-hydroxypropionitril IV (7.11 g, 100 mmol) and diketene V (8.41 g, 100 mmol) and triethylamine (0.5 ml, 9.2 mmol) gave a product which was isolated by silica gel column chromatography with EtOAc-C_6H_{14} (1:1, V/V) as eluent. The product IX was isolated as yellow oil (11.9 g, 84%).
^{1}H NMR ($CDCl_3$): δ 4.26(t, J=4.7 Hz, 2H, CO_2CH_2), 3.78 (t, J= 4.7 Hz , 2H,CH_2OMe), 3.46 (s, 2H, $COCH_2CO$) , 3.34 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3CO).
IR (KBr):ν 1745(C=O, ester), 1724 & 1718 (C=O, ketone) cm^{-1}.

General procedure for the synthesis of dihydropyridine derivatives XIV-XXV (procedure B)
A mixture of the corresponding acetoacetate ester VI-IX (5.0 mmol), 1-methyl-5-nitro-imidazol-2-carboxaldehyde X (0.78 g, 5 mmol) and the respective alkyl 3-aminocrotonate (5.0 mmol XI-XIII) in absolute ethanol (25 ml) was refluxed for 10 hrs with stirring. After cooling, the precipitated product was filtered off, washed with cold ethanol, and then dried *in vacuo*. Recrystallization from methanol gave XIV-XXV (51-81%) as yellow or white crystals.

3-(2-Hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XIV)
^{1}H NMR ($CDCl_3$): δ 8.21 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.18 (s, 1H, C_4-H), 4.22 (t, J=4.6 Hz, 2H, CO_2CH_2), 4.20 (s, 3H, N-CH_3), 3.71 (t, J=4.6 Hz, 2H, CH_2OH), 3.66 (s, 3H, CO_2CH_3), 2.25 (s, 6H, C_2-CH_3 & C_6-CH_3) and 1.58 (br s, 1H, OH exchangable with D_2O).
IR(KBr): ν 3336 (br, NH & OH), 1718(C=O), 1657(C=C), 1541 and 1366 (NO_2) cm^{-1}.
MS: m/z (%) 380 (M^+,43), 321 (14), 291 (40), 259 (100), 210 (41) and 149 (21).

3-(2-Hydroxyethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XV)
^{1}H NMR ($CDCl_3$): δ 8.91 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.18(s, 1H, C_4-H), 4.21 (m, 4H, CO_2CH_2), 4.20 (s, 3H, N-CH_3), 3.81 (t, J=5.1 Hz, 2H, CH_2OH), 3.66 (s, 3H, CO_2CH_3), 2.31 (s, 6H, C_2-CH_3 & C_6-CH_3), 2.19 (brs, 1H, OH exchangable with D_2O) and 1.21 (t, J=6.8 Hz, 3H, CH_3).
IR(KBr): ν 3341(br, NH & OH), 1722 (C=O), 1655 (C=C), 1535 and 1381 (NO_2) cm^{-1}.
MS: m/z (%) 394 (M^+,50), 321 (24), 305 (29), 259 (100), 195 (21) and 150 (16).

3-(2-Hydroxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XVI)
^{1}H NMR ($CDCl_3$): δ 8.51 (br, s, 1H, NH), 7.93 (s, 1H, imidazole H-4), 5.17 (s, 1H, C_4-H), 5.02 (m, 1H, CO_2CHMe_2), 4.23 (s, 3H, N-CH_3), 4.20 (t, J=5.2 Hz, 2H, CO_2CH_2), 3.78 (t, J=5.2 Hz, 2H, CH_2OH), 2.25 (s, 6H, C_2-CH_3 & C_6-CH_3), 1.71 (br s, 1H, OH exchangeable with D_2O) 1.23 and 1.16 (tJ=4.3 Hz, 3H each, $CH(CH_3)_2$).
IR(KBr): ν 3341 (br, NH & OH), 1734 (C=O), 1648 (C=C), 1541 and 1378 (NO_2) cm^{-1}.
MS: m/z (%) 408 (M^+,38), 321 (62), 259 (100), 195 (21), 149 (23) and 106 (16).

3-(2-Methoxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XVII)
^{1}H NMR ($CDCl_3$): δ 8.86 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.17 (s, 1H, C_4-H), 4.21 (s, 3H, N-CH_3), 4.19 (t, J=5.1 Hz, 2H, CO_2CH_2), 3.69 (s, 3H, CO_2CH_3), 3.51 (t, J=5.1 Hz, 2H, CH_2OMe), 3.29 (s, 2H, OCH3), 2.24 and 2.22 (ds, 3H each, C_2-CH_3 & C_6-CH_3).
IR(KBr): ν 3395 (NH), 1731 (C=O), 1658 (C=C), 1561 and 1358 (NO_2) cm^{-1}.
MS: m/z (%) 394 (M^+,27), 335 (12), 291 (31), 259 (100), 210 (25) and 191 (23).

3-(2-Methoxyethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XVIII)
^{1}H NMR ($CDCl_3$): δ 9.00 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.17 (s, 1H, C_4-H), 4.25 (s,

3H, N-CH_3), 4.18 (t, J=4.6 Hz, 2H, CO_2CH_2), 4.04 (t, J=6.2 Hz, 2H, CO_2CH_2), 3.51 (t, J=4.6 Hz, 2H, CH_2OMe), 3.29 (s, 3H, OCH_3), 2.22 (s, 6H, C_2-CH_3 & C_6-CH_3) and 1.24 (t, J=6.2 Hz, 3H, CH_3).
IR (KBr): ν 3417 (NH), 1729 (C=O), 1671 (C=C), 1557 and 1381 (NO_2) cm^{-1}.
MS: m/z (%) 408 (M^+,52), 333 (20), 282 (32), 259 (100), 205 (24) and 196 (28).

3-(2-Methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl- 5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XIX)
^{1}H NMR ($CDCl_3$): δ 8.81 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4-H), 5.11 (m, 1H, CO_2CHMe_2), 4.24 (s, 3H, N-CH_3), 4.20 (t, J=4.6 Hz, 2H, CO_2CH_2), 3.51 (t, J=4.6 Hz, 2H, CH_2OMe), 3.29 (s, 3H, OCH_3), 2.24 and 2.22 (d s, 3H each, C_2-CH_3 & C_6-CH_3), 1.24 and 1.17 (d, J=5.1 Hz, 3H each , $CH(CH_3)_2$).
IR(KBr): ν 3402 (NH), 1721 (C=O), 1659 (C=C), 1549 and 1369 (NO_2) cm^{-1}.
MS: m/z(%) 422 (M^+,24), 331 (25), 296 (14), 259 (100), 233 (15), and 195 (35).

3-(2-Acetylethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XX)
^{1}H NMR ($CDCl_3$): δ 8.91 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4-H), 4.21 (s, 3H, N-CH_3), 4.09 (t, J=6.8 Hz, 2H, CO_2CH_2), 3.76 (s, 3H, CO_2CH_3), 2.42 (t, J=6.8 Hz, 2H, CH_2CO), 2.21 (s, 6H, C_2-CH_3 & C_6-CH_3) and 2.11 (s, 3H, COCH3).
IR(KBr): ν 3378(NH), 1744, 1719 (C=O), 1661 (C=C), 1518 and 1368 (NO_2) cm^{-1}.
MS: m/z (%) 406 (M^+,42), 318 (11), 259 (65), 192 (20) and 85 (100).

3-(2-Acetylethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXI)
^{1}H NMR ($CDCl_3$): δ 8.71 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4-H), 4.23 (s, 3H, N-CH_3), 4.09 (m, 4H, CO_2CH_2), 2.42 (t, J=6.7 Hz, 2H, CH_2CO), 2.22 (s, 6H, C_2-CH_3 & C_6-CH_3), 2.11 (s, 3H, $COCH_3$), and 1.23 (t, J=6.2 Hz, 3H, CH_3).
IR(KBr): ν 3474(NH), 1727, 1719 (C=O), 1657 (C=C), 1551 and 1378 (NO_2) cm^{-1}.
MS: m/z (%) 420 (M^+,19), 308 (18), 259 (71), 177 (22) and 85 (100).

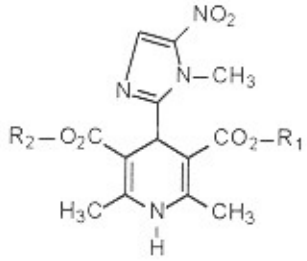
3-(2-Acetylethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl- 5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXII)
^{1}H NMR ($CDCl_3$): δ 8.81 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4-H), 5.11 (m, 1H, CO_2CHMe_2), 4.24 (s, 3H, N-CH_3), 4.20 (t, J=4.6 Hz, 2H, CO_2CH_2), 3.51 (t, J=4.6 Hz, 2H, CH_2OMe), 3.29 (s, 3H, OCH_3), 2.21 (s, 6H, C_2-CH_3 & C_6-CH_3), 1.24 and 1.17 (dd, J=5.1 Hz, 3H each , $CH(CH_3)_2$).
IR(KBr): ν 3464(NH), 1731, 1716 (C=O), 1666 (C=C), 1559 and 1371 (NO_2) cm^{-1}.
MS: m/z (%) 434 (M^+,15), 318 (22), 259 (56), 192 (32), and 85 (100).

3-(2-Cyanoethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXIII)
^{1}H NMR ($CDCl_3$): δ 8.89 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.13 (s, 1H, C_4-H), 4.35 (t, J=6.3 Hz, 2H, CO_2CH_2), 4.21 (s, 3H, N-CH_3), 3.69 (s, 3H, CO_2CH_3), 2.69 (t, J=6.3 Hz, 2H, CH_2CN), 2.33 and 2.29 (ds, 3H each, C_2-CH_3 & C_6-CH_3).
IR(KBr): ν 3341 (NH), 2242 (CN), 1724 (C=O), 1658 (C=C), 1522 and 1378 (NO_2) cm^{-1}.
MS: m/z (%) 389 (M^+,57), 330 (26), 291 (28), 259 (100), 210 (28) and 150 (18).

3-(2-Cyanoethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXIV)
^{1}H NMR ($CDCl_3$): δ 8.97 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C_4-H), 4.31 (t, J=6.2 Hz, 2H, CO_2CH_2), 4.23 (s, 3H, N-CH_3), 4.10 (t, J=6.8 Hz, 2H, CO_2CH_2), 2.68 (t, J=6.2 Hz, 2H, CH_2CN), 3.66 (s, 3H, CO_2CH_3), 2.31 and 2.26 (two s, 3H each, C_2-CH_3 & C_6-CH_3) and 1.24 (t, J=6.8 Hz, 3H, CH_3).
IR(KBr): ν 3418 (NH), 2252 (CN), 1729 (C=O), 1665 (C=C), 1531 and 1369 (NO_2) cm^{-1}.
MS: m/z (%) 403 (M^+,43), 330 (32), 305 (18), 259 (100), 230 (22) and 196 (15).

3-(2-Cyanoethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXV)
^{1}H NMR ($CDCl_3$): δ 8.87 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.14 (s, 1H, C_4-H), 4.98 (m, 1H, CO_2CHMe_2), 4.30 (t, J=6.1 Hz, 2H, CO_2CH_2), 4.25 (s, 3H, N-CH_3), 2.66 (t, J=6.1 Hz, 2H, CH_2CN), 2.26 and 2.23 (two s, 3H each, C_2-CH_3 &

Table 1. Physical properties and calcium channel antagonist activity of compound XIV-XXV



Compound	R_1	R_2	Mp(°C)	Yield (%)	Calcium channel antagonist activity IC_{50}±SEM, n=7
XIV	$(CH_2)_2OH$	Me	226-228	53	$1.29\pm0.59 \times 10^{-12}$*
XV	$(CH_2)_2OH$	Et	223-224	64	$3.55\pm1.03 \times 10^{-12}$
XVI	$(CH_2)_2OH$	isoPro	209-211	61	$2.92\pm0.51 \times 10^{-12}$*
XVII	$(CH_2)_2OCH_3$	Me	212-213	68	$4.20\pm0.79 \times 10^{-12}$*
XVIII	$(CH_2)_2OCH_3$	Et	195-197	51	$7.49\pm1.77 \times 10^{-12}$
XIX	$(CH_2)_2OCH_3$	isoPro	198-200	55	$6.22\pm1.02 \times 10^{-13}$
XX	$(CH_2)_2COCH_3$	Me	174-176	53	$1.48\pm0.27 \times 10^{-12}$*
XXI	$(CH_2)_2COCH_3$	Et	156-158	62	$2.53\pm0.92 \times 10^{-12}$*
XXII	$(CH_2)_2COCH_3$	isoPro	163-164	58	$2.11\pm0.55 \times 10^{-12}$*
XXIII	$(CH_2)_2CN$	Me	225-227	71	$6.86\pm0.33 \times 10^{-13}$*
XXIV	$(CH_2)_2CN$	Et	221-223	66	$2.46\pm0.19 \times 10^{-12}$*
XXV	$(CH_2)_2CN$	isoPro	224-226	81	$1.48\pm0.07 \times 10^{-12}$*
	Nifedipine				$1.07\pm0.12 \times 10^{-11}$

* Single asterisk indicates $P< 0.05$ compared to nifedipine in that experiment using Student's *t*-test.

C_6-CH_3), 1.24 and 1.16 (d, J=5.9 Hz, 3H each , $CH(CH_3)_2$).
IR(KBr): ν 3341 (NH), 2238 (CN), 1734 (C=O), 1659 (C=C), 1521 and 1352 (NO_2) cm^{-1}.
MS: m/z (%) 417 (M^+,43), 331 (32), 296 (14), 259 (100), 230 (22) and 195 (43).

Pharmacology

Male albino guinea pigs (300-450 g) were killed by a blow to the head. The intestine was removed above the ileocecal junction. Smooth muscle segments of about 2 cm length were mounted under a resting tention of 500 mg and were maintained at 37° C in a 20 ml jacketed organ bath containing oxygenated (95%O_2 & 5%CO_2) physiological saline solution of the following millimolar compositions: 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 muscle was equilibrated for 1 hour with a solution changing every 15 min. The contractions were recorded with a forced displacement transducer (FTO3C) on a GRASS physiograph. All compounds were dissolved in DMSO and the same volume of solvent was used as the control. The contractile response was taken as the 100% value for the tonic (slow) component of the response. Test compounds were added by accumulative amounts after the dose response for Carbacol (1.67×10^{-7} M). Test compound -induced relaxation of contracted muscle was expressed as the percent of the control (16-17).
The IC_{50} values were graphically determined from the contraction- response curve.

Statistics

The results obtained were presented as means and evaluated statistically using Student's t-test.

RESULTS AND DISCUSSION

The *in vitro* calcium channel antagonist activities (IC_{50}) of compounds XIV-XXV were determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbacol, 1.67×10^{-7}

M) Ca^{+2} dependent contraction (tonic response) of guinea pig ileal longitudinal smooth muscle (GPILSM), and are presented in Table 1.

These results indicate that compounds XIV-XXV possessing a hydroxy, alkoxy, acetyl or cyano substituent exhibit superior or equipotent calcium channel antagonist activity (10^{-12}-10^{-13} M) relative to the reference drug Nifedipine (IC_{50}= 1.07±0.12 x 10^{-11} M). A comparison of activities of the compounds XIV-XXV with compounds reported by Shafiee et al. (10) having the same structure without hydroxyl (alkoxy, acetyl or cyano) group, reveals that the presence of a hydroxyl (alkoxy, acetyl or cyano) group substituted on C-3 position of the 1,4-dihydropyridine ring increases the smooth muscle relaxant activity.

Also, a comparison of the results of this study with the report of Miri et al. (12), indicated that 1,4-dihydropyridine compounds with hydroxyl (methoxy, acetyl or cyano) substituted on C-3 or C-5 ester position of the ring have more activity than similar compounds with aminoalkyl substitutents.

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REFERENCES

1. Uneyama, H., Uchida, H., Konda, T., Yoshimoto, R., Akaike, N. (1999) Selectivity of dihydropyridines for L-type and sympathetic N-type Ca^{2+} channels. Eur. J. Pharmacol. 373: 93-100.
2. Schleifer, K.J. (1999) Stereoselective characterization of 1,4-dihydropyridine binding site L-type calcium channels in the resting state and the opened/inactivated state. J. Med. Chem. 42: 2204-2211.
3. Morel, N., Buryi, V., Feron, O., gomez, J.P., Christen, M.O., Godfraind, T. (1998) The action of calcium channel blockers on recombinant L-type calcium channel alpha 1-subunite. Br. J. Pharmacol. 125: 1005-1012.
4. Triggle, D.J. (1990) Calcium, calcium channel, and calcium channel antagonists. Can. J. Physio. Pharmacol. 68: 1474-1481.
5. Luscher, T.F., Cosentino, F. (1998) The classification of calcium antagonists and their selection in treatment of hypertention. Drugs 55: 509-512.
6. Lacinova, L., Hofmann, F. (1998) Isradipine interacts with the open state of the L-type calcium channel at high concentrations. Receptors Channels. 5: 153-164.
7. Yusuf, S. (1995) Calcium antagonists in coronary artery disease and hypertension. Circulation 92: 1079-1082.
8. Gaudio, A.C., Korolkovas, A., Takahata, Y. (1995) Quantitative structure activity relationships for 1,4-dihydropyridine calcium channel antagonist. J. Pharm. Sci. 83: 1110-1115.
9. Mager, P.P., Coburn, R.A., Solo, A.J., Triggle, D.J., Rothe, H. (1992) QSAR, Diagnostic statistic and molecular modeling of 1,4-dihydropyridine calcium channel antagonists. Drug. Des. Discov. 8: 273-289.
10. Shafiee, A., Miri, R., Dehpour, A.R., Soleymani, F. (1996) Synthesis and calcium-channel antagonist activity of Nifedipine analogues containing nitroimidazolyl substituent in guinea-pig smooth Ileal muscle. Pharm. Sci. 2: 541-543.
11. Clemenes, R. J. (1993) Diketene, Chem. Rev., 86: 241-318.
12. Miri, R., Dehpour, A.R., Azimi, M., Shafiee, A. (2001) Synthesis and calcium channel antagonist Effect of alkyl, aminoalkyl 1,4-dihydro-2,6-dimethyl-4-nitroimidazole-3,5- pyridine dicarboxylates. Daru 9: 40-45.
13. Shafiee, A., Pirouzzadeh, B., Ghasemian, F., Parang, K. (1992) Synthesis of 2-acetyl-1-methyl-5-nitroimidazole. J. Heterocyclic Chem. 29: 1021-1023.
14. Miri, R., McEwen, C.A., Knaus, E.E. (2000) Synthesis and calcium channel modulating effects of modified Hantzsch nitrooxyalkyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(pyridinyl or 2-trifluoromethylphenyl)-5-pyridinedicarboxylates. Drug Dev. Res. 51: 225-232.
15. Iwanami, M., Shibanuma, T., Fujimoto, M., Kawai, R., Tamazawa, K., Takenaka, T., Takahashi, K., Murakami, M. (1979) Synthesis of new water soluble dihydropyridine vasodilators. Chem. Pharm. Bull. 27: 1426-1440.

16. Triggle, C.R., Swamy, V., Triggle, D.J. (1979) Calcium antagonists and contractile responses in vas deferens and guinea pig ileal smooth muscle. Can. J. Physiol. Pharmacol. 57: 804-818.
17. Miri, R., Howlett, S.E., Knaus, E.E. (1997) Synthesis and calcium channel modulating effects of isopropyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-thienyl-5-pyridine carboxylates. Arch. Pharm. Med. Chem. 330: 290-294