

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

RAMIN MIRI\*, HOSSEIN NIKNAHAD\*\*, AFSANEH VAZIN\*\*, ALI AZARPIRA\*,  
ABBAS SHAFIEE\*\*\*

\*Department of Medicinal Chemistry, Faculty of Pharmacy, \*\*Department of Pharmacology & Toxicology, Faculty of Pharmacy, Shiraz University of Medical Science, Shiraz, \*\*\*Department of Chemistry, Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran

### ABSTRACT

A group of racemic 3-[(2-hydroxyethyl), (2-Methoxyethyl), (2-acetyethyl) 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 ( $1.67 \times 10^{-7}$  M). All compounds exhibited calcium channel antagonist activity ( $IC_{50}=10^{-12}$  to  $10^{-13}$  M range) comparable to nifedipine as reference drug ( $IC_{50}=1.07 \pm 0.12 \times 10^{-11}$  M).

**Keywords:** Dihydropyridine; Calcium channels antagonist; Nitroimidazole; GPILSM

### INTRODUCTION

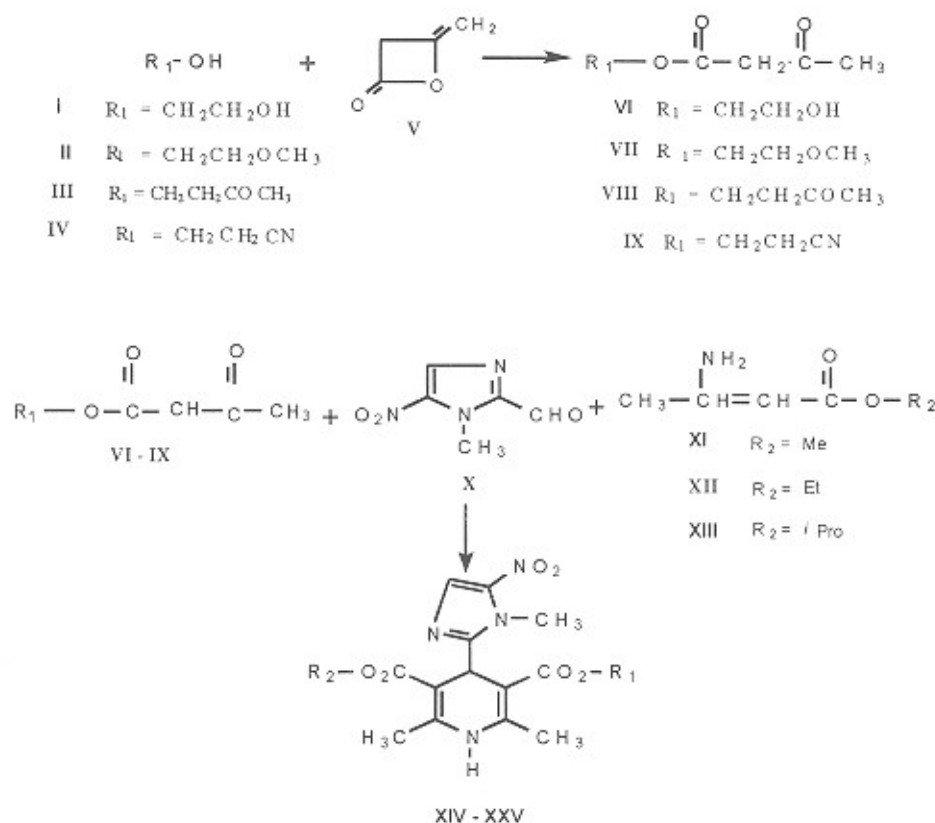
The influx of extracellular  $Ca^{2+}$  through L-type potential dependent calcium channel is responsible for regulation of many physiological functions including smooth and cardiac muscle contraction (1-4). The discovery that the 1,4-dihydropyridine (Nifedipine, Nimodipine) class of calcium channel 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, acetyethyl, 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.  $^1H$ -NMR spectra were run on a Varian Unity Plus 400 MHz spectrometer. Chemical shifts are reported in parts per million ( $\delta$ ) 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



Scheme 1

analogues XIV-XXV were prepared by modified Hantsch reaction reported by Meyer through the reaction of 1-methyl-5-nitroimidazole-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<sub>3</sub>N (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 vacuo*.

*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<sub>6</sub>H<sub>14</sub> (2:1, V/V) as eluent. The product 6 was isolated as yellow oil (11.9 g, 84%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>)δ: 4.24 (t, J=4.6 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.78 (t, J=4.6 Hz, 2H, CH<sub>2</sub>OH), 3.49 (s, 2H, COCH<sub>2</sub>CO), 2.92 (br s, 1H, OH, exchangeable with D<sub>2</sub>O), 2.24 (s, 3H, CH<sub>3</sub>CO).

IR (KBr): ν 3074-3706(br, OH), 1760(C=O, ester), 1720 cm<sup>-1</sup> (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%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.26(t, J=4.7 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.78(t, J=4.7 Hz, 2H, CH<sub>2</sub>OMe), 3.46 (s,

2H, COCH<sub>2</sub>CO), 3.34 (s, 3H, OCH<sub>3</sub>), 2.23 (s, 3H, CH<sub>3</sub>CO).

IR (KBr):  $\nu$  1752 (C=O, ester), 1727 cm<sup>-1</sup> (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°C/3 mmHg) as a colorless oil (3.74 g, 87%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  4.06 (t, J=6.4 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.38 (s, 2H, COCH<sub>2</sub>CO), 2.45 (t, J=6.4 Hz, 2H, CH<sub>2</sub>CO), 2.19 (s, 3H, CH<sub>3</sub>CO), 2.03 (s, 3H, CH<sub>3</sub>CO).

IR (KBr):  $\nu$  1752 (C=O, ester), 1727 (C=O, ketone) cm<sup>-1</sup>.

#### 2-Cyanoethyl acetoacetate (IX)

The reaction of 3-hydroxypropionitrile 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<sub>6</sub>H<sub>14</sub> (1:1, V/V) as eluent. The product IX was isolated as yellow oil (11.9 g, 84%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  4.26 (t, J=4.7 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.78 (t, J=4.7 Hz, 2H, CH<sub>2</sub>OMe), 3.46 (s, 2H, COCH<sub>2</sub>CO), 3.34 (s, 3H, OCH<sub>3</sub>), 2.23 (s, 3H, CH<sub>3</sub>CO).

IR (KBr):  $\nu$  1745 (C=O, ester), 1724 & 1718 (C=O, ketone) cm<sup>-1</sup>.

#### 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)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.21 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.18 (s, 1H, C<sub>4</sub>-H), 4.22 (t, J=4.6 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 4.20 (s, 3H, N-CH<sub>3</sub>), 3.71 (t, J=4.6 Hz, 2H, CH<sub>2</sub>OH), 3.66 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 2.25 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>) and 1.58 (br s, 1H, OH exchangeable with D<sub>2</sub>O).

IR (KBr):  $\nu$  3336 (br, NH & OH), 1718 (C=O), 1657 (C=C), 1541 and 1366 (NO<sub>2</sub>) cm<sup>-1</sup>.

MS: m/z (%) 380 (M<sup>+</sup>, 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)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.91 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.18 (s, 1H, C<sub>4</sub>-H), 4.21 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>), 4.20 (s, 3H, N-CH<sub>3</sub>), 3.81 (t, J=5.1 Hz, 2H, CH<sub>2</sub>OH), 3.66 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 2.31 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>), 2.19 (brs, 1H, OH exchangeable with D<sub>2</sub>O) and 1.21 (t, J=6.8 Hz, 3H, CH<sub>3</sub>).

IR (KBr):  $\nu$  3341 (br, NH & OH), 1722 (C=O), 1655 (C=C), 1535 and 1381 (NO<sub>2</sub>) cm<sup>-1</sup>.

MS: m/z (%) 394 (M<sup>+</sup>, 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)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.51 (br s, 1H, NH), 7.93 (s, 1H, imidazole H-4), 5.17 (s, 1H, C<sub>4</sub>-H), 5.02 (m, 1H, CO<sub>2</sub>CHMe<sub>2</sub>), 4.23 (s, 3H, N-CH<sub>3</sub>), 4.20 (t, J=5.2 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.78 (t, J=5.2 Hz, 2H, CH<sub>2</sub>OH), 2.25 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>), 1.71 (br s, 1H, OH exchangeable with D<sub>2</sub>O) 1.23 and 1.16 (t, J=4.3 Hz, 3H each, CH(CH<sub>3</sub>)<sub>2</sub>).

IR (KBr):  $\nu$  3341 (br, NH & OH), 1734 (C=O), 1648 (C=C), 1541 and 1378 (NO<sub>2</sub>) cm<sup>-1</sup>.

MS: m/z (%) 408 (M<sup>+</sup>, 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)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.86 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.17 (s, 1H, C<sub>4</sub>-H), 4.21 (s, 3H, N-CH<sub>3</sub>), 4.19 (t, J=5.1 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.69 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.51 (t, J=5.1 Hz, 2H, CH<sub>2</sub>OMe), 3.29 (s, 2H, OCH<sub>3</sub>), 2.24 and 2.22 (ds, 3H each, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>).

IR (KBr):  $\nu$  3395 (NH), 1731 (C=O), 1658 (C=C), 1561 and 1358 (NO<sub>2</sub>) cm<sup>-1</sup>.

MS: m/z (%) 394 (M<sup>+</sup>, 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)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  9.00 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.17 (s, 1H, C<sub>4</sub>-H), 4.25 (s,

3H, N-CH<sub>3</sub>), 4.18 (t, J=4.6 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 4.04 (t, J=6.2 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.51 (t, J=4.6 Hz, 2H, CH<sub>2</sub>OMe), 3.29 (s, 3H, OCH<sub>3</sub>), 2.22 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>) and 1.24 (t, J=6.2 Hz, 3H, CH<sub>3</sub>). IR (KBr):  $\nu$  3417 (NH), 1729 (C=O), 1671 (C=C), 1557 and 1381 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 408 (M<sup>+</sup>, 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)*

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.81 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C<sub>4</sub>-H), 5.11 (m, 1H, CO<sub>2</sub>CHMe<sub>2</sub>), 4.24 (s, 3H, N-CH<sub>3</sub>), 4.20 (t, J=4.6 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.51 (t, J=4.6 Hz, 2H, CH<sub>2</sub>OMe), 3.29 (s, 3H, OCH<sub>3</sub>), 2.24 and 2.22 (d s, 3H each, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>), 1.24 and 1.17 (d, J=5.1 Hz, 3H each, CH(CH<sub>3</sub>)<sub>2</sub>). IR(KBr):  $\nu$  3402 (NH), 1721 (C=O), 1659 (C=C), 1549 and 1369 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 422 (M<sup>+</sup>, 24), 331 (25), 296 (14), 259 (100), 233 (15), and 195 (35).

*3-(2-Acetyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XX)*

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.91 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C<sub>4</sub>-H), 4.21 (s, 3H, N-CH<sub>3</sub>), 4.09 (t, J=6.8 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.76 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 2.42 (t, J=6.8 Hz, 2H, CH<sub>2</sub>CO), 2.21 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>) and 2.11 (s, 3H, COCH<sub>3</sub>). IR(KBr):  $\nu$  3378(NH), 1744, 1719 (C=O), 1661 (C=C), 1518 and 1368 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 406 (M<sup>+</sup>, 42), 318 (11), 259 (65), 192 (20) and 85 (100).

*3-(2-Acetyethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXI)*

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.71 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C<sub>4</sub>-H), 4.23 (s, 3H, N-CH<sub>3</sub>), 4.09 (m, 4H, CO<sub>2</sub>CH<sub>2</sub>), 2.42 (t, J=6.7 Hz, 2H, CH<sub>2</sub>CO), 2.22 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>), 2.11 (s, 3H, COCH<sub>3</sub>), and 1.23 (t, J=6.2 Hz, 3H, CH<sub>3</sub>). IR(KBr):  $\nu$  3474(NH), 1727, 1719 (C=O), 1657 (C=C), 1551 and 1378 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 420 (M<sup>+</sup>, 19), 308 (18), 259 (71), 177 (22) and 85 (100).

*3-(2-Acetyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (XXII)*

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.81 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C<sub>4</sub>-H), 5.11 (m, 1H, CO<sub>2</sub>CHMe<sub>2</sub>), 4.24 (s, 3H, N-CH<sub>3</sub>), 4.20 (t, J=4.6 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 3.51 (t, J=4.6 Hz, 2H, CH<sub>2</sub>OMe), 3.29 (s, 3H, OCH<sub>3</sub>), 2.21 (s, 6H, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>), 1.24 and 1.17 (dd, J=5.1 Hz, 3H each, CH(CH<sub>3</sub>)<sub>2</sub>). IR(KBr):  $\nu$  3464(NH), 1731, 1716 (C=O), 1666 (C=C), 1559 and 1371 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 434 (M<sup>+</sup>, 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)*

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.89 (br s, 1H, NH), 7.95 (s, 1H, imidazole H-4), 5.13 (s, 1H, C<sub>4</sub>-H), 4.35 (t, J=6.3 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 4.21 (s, 3H, N-CH<sub>3</sub>), 3.69 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 2.69 (t, J=6.3 Hz, 2H, CH<sub>2</sub>CN), 2.33 and 2.29 (ds, 3H each, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>). IR(KBr):  $\nu$  3341 (NH), 2242 (CN), 1724 (C=O), 1658 (C=C), 1522 and 1378 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 389 (M<sup>+</sup>, 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)*

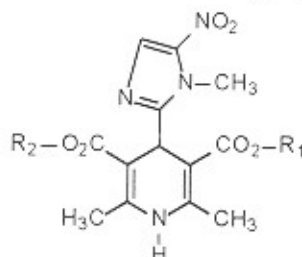
<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.97 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.15 (s, 1H, C<sub>4</sub>-H), 4.31 (t, J=6.2 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 4.23 (s, 3H, N-CH<sub>3</sub>), 4.10 (t, J=6.8 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 2.68 (t, J=6.2 Hz, 2H, CH<sub>2</sub>CN), 3.66 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 2.31 and 2.26 (two s, 3H each, C<sub>2</sub>-CH<sub>3</sub> & C<sub>6</sub>-CH<sub>3</sub>) and 1.24 (t, J=6.8 Hz, 3H, CH<sub>3</sub>).

IR(KBr):  $\nu$  3418 (NH), 2252 (CN), 1729 (C=O), 1665 (C=C), 1531 and 1369 (NO<sub>2</sub>) cm<sup>-1</sup>. MS: m/z (%) 403 (M<sup>+</sup>, 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)*

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.87 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.14 (s, 1H, C<sub>4</sub>-H), 4.98 (m, 1H, CO<sub>2</sub>CHMe<sub>2</sub>), 4.30 (t, J=6.1 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>), 4.25 (s, 3H, N-CH<sub>3</sub>), 2.66 (t, J=6.1 Hz, 2H, CH<sub>2</sub>CN), 2.26 and 2.23 (two s, 3H each, C<sub>2</sub>-CH<sub>3</sub> &

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



Compound	R <sub>1</sub>	R <sub>2</sub>	Mp(°C)	Yield (%)	Calcium channel antagonist activity IC <sub>50</sub> ±SEM, n=7
XIV	(CH <sub>2</sub> ) <sub>2</sub> OH	Me	226-228	53	1.29±0.59 × 10 <sup>-12</sup> *
XV	(CH <sub>2</sub> ) <sub>2</sub> OH	Et	223-224	64	3.55±1.03 × 10 <sup>-12</sup>
XVI	(CH <sub>2</sub> ) <sub>2</sub> OH	isoPro	209-211	61	2.92±0.51 × 10 <sup>-12</sup> *
XVII	(CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>	Me	212-213	68	4.20±0.79 × 10 <sup>-12</sup> *
XVIII	(CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>	Et	195-197	51	7.49±1.77 × 10 <sup>-12</sup>
XIX	(CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>	isoPro	198-200	55	6.22±1.02 × 10 <sup>-13</sup>
XX	(CH <sub>2</sub> ) <sub>2</sub> COCH <sub>3</sub>	Me	174-176	53	1.48±0.27 × 10 <sup>-12</sup> *
XXI	(CH <sub>2</sub> ) <sub>2</sub> COCH <sub>3</sub>	Et	156-158	62	2.53±0.92 × 10 <sup>-12</sup> *
XXII	(CH <sub>2</sub> ) <sub>2</sub> COCH <sub>3</sub>	isoPro	163-164	58	2.11±0.55 × 10 <sup>-12</sup> *
XXIII	(CH <sub>2</sub> ) <sub>2</sub> CN	Me	225-227	71	6.86±0.33 × 10 <sup>-13</sup> *
XXIV	(CH <sub>2</sub> ) <sub>2</sub> CN	Et	221-223	66	2.46±0.19 × 10 <sup>-12</sup> *
XXV	(CH <sub>2</sub> ) <sub>2</sub> CN	isoPro	224-226	81	1.48±0.07 × 10 <sup>-12</sup> *
	Nifedipine				1.07±0.12 × 10 <sup>-11</sup>

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

C<sub>6</sub>-CH<sub>3</sub>), 1.24 and 1.16 (d,  $J=5.9$  Hz, 3H each, CH(CH<sub>3</sub>)<sub>2</sub>).

IR(KBr):  $\nu$  3341 (NH), 2238 (CN), 1734 (C=O), 1659 (C=C), 1521 and 1352 (NO<sub>2</sub>) cm<sup>-1</sup>.

MS:  $m/z$  (%) 417 (M<sup>+</sup>, 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 tension of 500 mg and were maintained at 37° C in a 20 ml jacketed organ bath containing oxygenated (95% O<sub>2</sub> & 5% CO<sub>2</sub>) physiological saline solution of the following millimolar compositions: NaCl, 137; CaCl<sub>2</sub>, 1.8; KCl, 2.7; MgSO<sub>4</sub>, 1.1; NaH<sub>2</sub>PO<sub>4</sub>, 0.4; NaHCO<sub>3</sub>, 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 ( $1.67 \times 10^{-7}$  M). Test compound-induced relaxation of contracted muscle was expressed as the percent of the control (16-17).

The IC<sub>50</sub> 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<sub>50</sub>) 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,  $1.67 \times 10^{-7}$



M)  $\text{Ca}^{2+}$  dependent contraction (tonic response) of guinea pig ileal longitudinal smooth muscle (GPIISM), 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 ( $\text{IC}_{50} = 1.07 \pm 0.12 \times 10^{-11}$  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 substituents.

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