# SYNTHESIS AND SMOOTH MUSCLE CALCIUM CHANNEL ANTAGONIST EFFECT OF ALKYL, AMINOALKYL 1,4-DIHYDRO-2,6-DIMETHYL-4-NITROIMIDAZOLE-3,5 PYRIDINE DICARBOXYLATES



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## ABSTRACT

The discovery that 1,4-dihydropyridine (DHP) class of calcium channel antagonist inhibits the $Ca^{+2}$ influx represented a major therapeutic advance in the treatment of cardiovascular diseases such as hypertension, angina pectoris and other spastic smooth muscle disorders. A novel class of calcium channel antagonist of flunarizine containing arylpiperazinyl moiety has recently been reported. It was therefore of interest to determine the effect that selected C-3 substituents contained amino alkyl and arylpiperazine, in conjunction with a C-4 1-methyl-5-nitro-2-imidazolyl substituents on calcium channel antagonist activity. The unsymmetrical analogues were prepared by a procedure reported by Meyer in which 1-methyl-5-nitro-imidazol-2-carboxaldehyde was reacted with acetoacetic esters and alkyl 3-aminocrotonate. *In vitro* calcium channel antagonist activities were determined by the use of high $K^+$ contraction of guinea pig ileal longitudinal smooth muscle. All compounds exhibited comparable calcium channel antagonist activity ($IC_{50}$ =$10^{-9}$ to $10^{-11}$ M) against reference drug nifedipine ($IC_{50}$= 2.75±0.36 x $10^{-10}$ M).

**Key words:** $Ca^{+2}$ channel antagonist, Nitroimidazole, DHP, Arylpiperazine

## INTRODUCTION

The L-type class of voltage dependent calcium channels provides an important pathway for entry of $Ca^{+2}$ into vascular and cardiac muscles (1-2). The discovery that 1,4-dihydropyridine (DHP) class of calcium channel antagonist inhibits this $Ca^{+2}$ influx represented a major therapeutic advance in the treatment of cardiovascular diseases such as hypertension, angina pectoris and other spastic smooth muscle disorders (3-5).

The dihydropyridine class of compounds in which nifedipine is the prototype, has been the aim of many structure activity relationship studies. The changes in the substitution pattern at C-3, C-4, and C-5 positions of nifedipine alter activity and tissue selectivity (6-8). A novel class of calcium channel antagonist of Flunarizine containing arylpiperazinyl moiety has recently been reported (9). Previously we reported that 1-methyl-5-nitro-2-imidazolyl is bioisoester of nitrophenyl in nifedipine analogues (10). It was therefore of interest to determine the effect of the selected C-3 substituents contained amino-alkyl and aryl-piperazine, in conjunction with a C-4 1-methyl-5-nitro-imidazolyl substituents on calcium channel antagonist activity.

## MATERIAL AND METHODS

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 shift 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.



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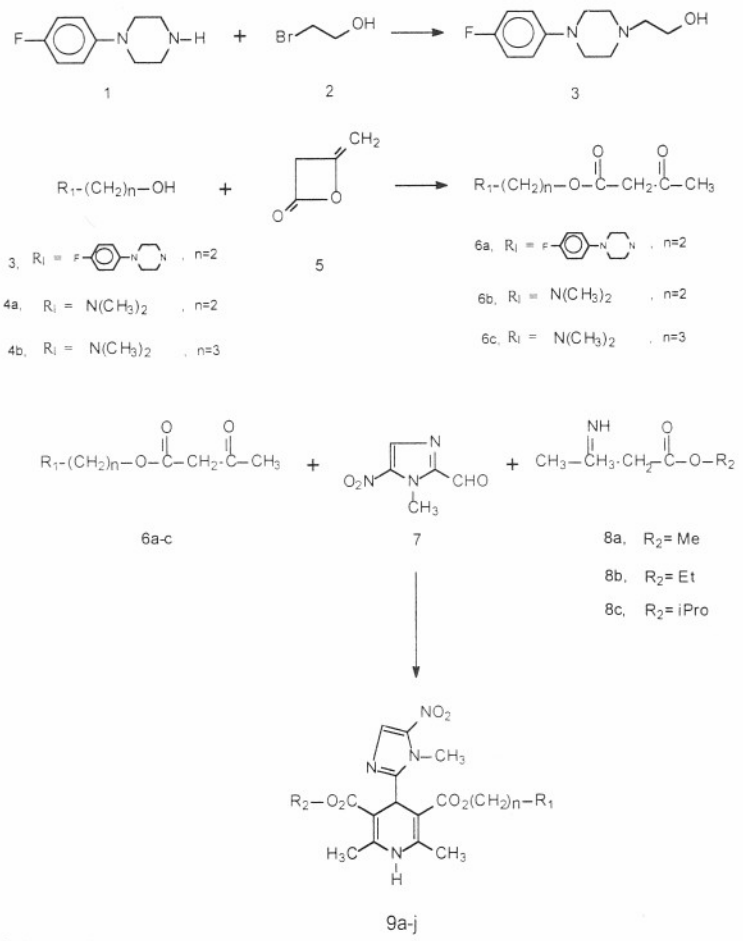


Scheme 1

*Chemistry:* The 2-[4-(*p*-fluorophenyl)piperazine-1-yl]ethanol **3** were obtained from reaction of 1-(*p*-fluorophenyl) piperazine **1** and 2-bromoethanol **2** in presence of triethyl amine as catalyst. Reaction of alcohols **4a-c** with diketene **5** afforded the corresponding acetoacetic esters **6a-c** (11-12). The unsymmetrical analogues **9a-j** were prepared by modified Hantsch reaction reported by Meyer in which 1-methyl-5-nitro-imidazole-2-carboxaldehyde **7** was reacted with 3-oxobutanoic acid esters **6a-c** and alkyl 3-aminocrotonate **8a-c** (13-15). (Scheme **1**)

*2-[4-(p-fluorophenyl) piperazine-1-yl] ethanol (3)*
A solution of 1-(*p*-fluorophenyl)piperazine (4.5 g, 25 mmol),2-bromoethanol (3.17 g, 25 mmol) and triethyl amine (7 ml, 50.2 mmol) in acetone (50 ml) were refluxed for 24 hours. The solvent was removed *in vacuo* and the residue obtained was dissolved in $CH_2Cl_2$ (50 ml) and washed with water (3 x 25 m). The organic phase was dried ($Na_2SO_4$), the solvent was removed, and the residue obtained was purified by silica gel column chromatography using $CH_2Cl_2$-MeOH (96:4,V/V) as eluent to give **3** as a white solid (3.08g, 55%). The assignment of piperazinyl protons was based on the fact that the H-3 and H-4 protons are deshielded by the p-flurophenyl substituent resulting in their appearance at lower field (δ =3.15), whereas the H-2 and H-5 piperazinyl protons which are not affected appear at higher field (δ =2.71).
$^1$H-NMR($CDCl_3$): 6.86-7.01 (m, 4H, aryl-H), 3.68(t, J=4.9 Hz, 4H, piperazinyl H-3 and H-5), 2.87(s, 1H, OH, exchanges with $D_2O$), 2.71 (t, J=4.9 Hz, 4H, piperazinyl H-2 and H-6), 2.64 (t, J=5.4 Hz, 2H, $CH_2CH_2N$).

*General procedure for the synthesis of Acetoacetate derivatives 6a-c (procedure A):*
Diketen **5** (0.84g, 10mmol) was added dropwise with stirring to respective alcohol **3**, **4a or 4b** (10mmol) pre-heated to 50-60 °C in presence of a catalytic amount of $Et_3N$ (5 drop). Diketene was

added at such a rate 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 product was isolated by silica gel column chromatography or distillation *in vacuo*.

*2-[4-(p-Fluorophenyl) piperazine-1-yl] ethyl acetoacetate (6a)*

The method was used similar to that described in procedure A. Reaction of 2-[4-(*p*-fluorophenyl) piperazine-1-yl] ethanol **3** (2.42 g, 10 mmol) and diketene **5** (0.84 g, 10 mmol) and triethylamine (5 drop) gave a product which was isolated by silica gel column chromatography with $CH_2Cl_2$-MeOH (96:4, V/V) as eluent. The product **6a** was isolated as a yellow oil (2.62 g, 85%).

$H^1$ NMR ($CDCl_3$) δ: 6.82-6.97 (m, 4H, aryl-H),4.30(t , J = 5.8 Hz, 2H, $COOCH_2$), 3.47 (s, 2H, $COCH_2COO$) , 3.09 (t,J= 4.9 Hz , 4H, piperazinyl H-3,H-5), 2.63-2.73 (m, 6H, $COOCH_2CH_2$ and piperazinyl H-2 , H-6 ), 2.27 (s,3H,$CH_3CO$).

IR (film): 1776 (C=O, ester), 1229 (C-F) $cm^{-1}$.

*2-(N,N-Dimethylamino)ethyl acetoacetate (6b)*

The title compound (6b) was prepared according to the procedure A by using N,N-dimethylethanolamine (0.89 g, 10mmol), diketene (0.84g, 10mmol) and triethylamine (5 drop).The reaction mixture was purified by distillation *in vacuo* [bp 98-99°C (3mmHg)] to yield as a colourless liquid (1.46 g, 84.3%).

$^1$HNMR ($CDCl_3$): δ 4.20 (t, J=5.7 Hz, 2H,$COOCH_2$), 3.45 (s, 2H, $COCH_2COO$), 2.53 (t, J=5.7 Hz, 2H, $CH_2NMe_2$), 2.23 (s, 9H,$CH_3CO$ and $NMe_2$).

IR (film): 1745 (C=O, ester), 1726(C=O, ketone) $cm^{-1}$.

*3-(N,N-Dimethylamino)propyl acetoacetate (6c)*

The title compound (6c) was prepared according to procedure A using 3-(N,N-dimethylamino)propanol (1.03g, 10mmol), diketene(0.84g, 10mmol) and triethylamine (5 drop). The reaction mixture was purified by distillation *in vacuo* [bp 103-104 °C(3mmHg)] to yield as a colourless liquid (1.36g, 73.6%).

$H^1$ NMR ($CDCl_3$): δ 4.13(t, J=5.7 Hz, 2H, $COOCH_2$), 3.61 (s, 2H, $COCH_2COO$), 2.47(t, J=5.1 Hz, 2H, $CH_2NMe_2$), 2.11 (s, 9H, $CH_3CO$ and $NMe_2$) and 1.14(m, 2H, $CH_2$).

IR (film): 1751(C=O, ester), 1719 (C=O, ketone) $cm^{-1}$.

*General procedure for the synthesis of dihydropyridine derivatives 9a-j (procedure B):*

A mixture of the respective acetoacetate ester **6a-c** (5.0 mmol), 1-methyl-5-nitro-imidazol-2-carboxaldehyde (0.78g, 5mmol) and the respective alkyl 3-aminocrotonate (5.0 mmol) **8a-c** in absolute ethanol (25 ml) was refluxed for 10 h with stirring. After cooling, the precipitated product was filtered off, washed with cold ethanol, and then dried *in vacuo*. Recrystallization from methanol gave **9a-j** (38-61%) as yellow or white crystals.

*3-[2-(N,N-dimethylamino)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9a)*

$H^1$ NMR ($CDCl_3$): δ 8.43(br s, 1H, NH), 7.91(s, 1H, imidazole H-4), 5.10(s, 1H, $C_4$-H), 4.42(t, J=7 Hz, 2H, $CO_2CH_2$), 4.15(s, 3H, N-$CH_3$), 4.09(s, 3H, $CO_2CH_3$), 3.76(t, J=7 Hz, 2H, $CH_2NMe_2$), 3.69(s, 6H, $N(CH_3)_2$) and 2.39(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$)

IR(KBr): 3335 (NH),1694(C=O), 1671(C=C), 1526 and 1351 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 407($M^+$,100), 397(93), 318(18), 224(67), 156(12) and 128(9)

*3-[2-(N,N-dimethylamino)ethyl] 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9b)*

$H^1$ NMR ($CDCl_3$): δ 8.49(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 5.16(s, 1H, $C_4$-H), 4.42(t, J=7.1 Hz, 2H, $CO_2CH_2$), 4.21(s, 3H, N-$CH_3$), 4.09(q, J=6.8 Hz, 2H, $CO_2CH_2$ ), 3.86(t, J=7.1 Hz, 2H, $CH_2NMe_2$), 3.67(s, 6H, $N(CH_3)_2$), 2.24(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$) and 1.23((t, J=6.8 Hz, 3H, $CH_3$)

IR(KBr): 3415 (NH),1715(C=O), 1661(C=C), 1528 and 1353 $cm^{-1}$ ($NO_2$).

MS: m/z (%) 421($M^+$,18), 393(32), 364(100), 238(93), 156(9) and 128(17)

*3-[2-(N,N-dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9c)*

$H^1$ NMR ($CDCl_3$): δ 8.23(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 5.13(s, 1H, $C_4$-H), 5.02(m, 1H, $CH(CH_3)_2$), 4.39(t, J=6.4Hz, 2H, $CO_2CH_2$ )4.22(s, 3H, N-$CH_3$), 3.79(t, J=6.4 Hz, 2H, $CH_2NMe_2$), 3.66(s, 6H, $N(CH_3)_2$), 2.24(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$), 1.24 and 1.16((two d, J=6.5 Hz, 3H each, $CH(CH_3)_2$)

IR(KBr): 3375 (NH),1737(C=O), 1626(C=C), 1521 and 1371 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 435($M^+$,8), 407(16), 379(100), 252(93), 156(11) and 128(19)

*3-[2-(N,N-dimethylamino)propyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9d)*

$H^1$ NMR ($CDCl_3$): δ 8.44(br s, 1H, NH), 7.93(s, 1H, imidazole H-4), 5.14(s, 1H, $C_4$-H), 4.34(t, J=6.8 Hz, 2H, $CO_2CH_2$), 4.19(s, 3H, N-$CH_3$), 4.01(s, 1H, $CO_2CH_3$), 3.71(t, J=6.8 Hz, 2H, $CH_2NMe_2$), 3.66(s, 6H, $N(CH_3)_2$) and 2.22(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$) and 1.83(m, 2H, $CH_2$)

IR(KBr): 3375 (NH),1734(C=O), 1666(C=C), 1521 and 1351 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 421($M^+$,87), 397(25), 351(100), 224(76), 156(10) and 128(14)

*3-[2-(N,N-dimethylamino)propyl] 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9e)*

$H^1$ NMR ($CDCl_3$): δ 8.98(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 5.17(s, 1H, $C_4$-H), 4.42(t, J=7 Hz, 2H, $CO_2CH_2$), 4.22(s, 3H, N-$CH_3$), 4.09(q, 2H, J=7.2 Hz, $CO_2CH_2$), 3.86(t, J=6.7 Hz, 2H, $CH_2NMe_2$), 3.67(s, 6H, $N(CH_3)_2$) and 2.24(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$),1.77(m, 2H, $CH_2$) and 1.23(t, J=7.2 Hz, 3H,$CH_3$)

IR(KBr): 3345 (NH),1741(C=O), 1655(C=C), 1513 and 1375 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 435($M^+$,94), 393(28), 364(100), 238(73), 156(9) and 128(12)

3-[2-(N,N-dimethylamino)propyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9f)

$H^1$ NMR ($CDCl_3$): δ 8.17(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 5.12(s, 1H, $C_4$-H), 4.98(m, 1H, $CH(CH_3)_2$), 4.39(t, J=6.6Hz, 2H, $CO_2CH_2$ )4.22(s, 3H, N-$CH_3$), 3.77(t, J=6.6 Hz, 2H, $CH_2NMe_2$), 3.66(s, 6H, $N(CH_3)_2$), 2.24(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$), 1.89(m, 2H, $CH_2$), 1.25 and 1.17((two d, J=5.2 Hz, 3H each, $CH(CH_3)_2$)

IR(KBr) : 3392 (NH),1740(C=O), 1619(C=C), 1517 and 1379 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 435($M^+$,8), 379(100), 309(9), 252(93), 156(11) and 128(19)

*3-[2-[4-(p-Fluorophenyl) piperazine-1-yl] ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate(9g)*

$H^1$ NMR ($CDCl_3$): δ 8.47(br s, 1H, NH), 7.93(s, 1H, imidazole H-4), 6.81(m, 4H, $C_6H_4$-F), 5.16(s, 1H, $C_4$-H), 4.23(s, 3H, N-$CH_3$), 4.20(t, J=7.1 Hz, 2H, $CO_2CH_2$), 3.66(s, 3H, $CO_2CH_3$), 2.99(br t, J=4.6 Hz, 4H, piperazenyl H-3 & H-5), 2.62(m, 6H, piperazenyl H-2 & H-6 and $CH_2$), 2.25 and 2.39(two s, 3H each, $C_2$-$CH_3$ & $C_6$-$CH_3$)

IR(KBr): 3387 (NH),1736(C=O), 1652(C=C), 1517 and 1379 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 542($M^+$,6), 536(100), 392(41), 265(36), 177(11) and 122(24)

*3-[2-[4-(p-Fluorophenyl)piperazine-1-yl]ethyl] 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate(9h)*

$H^1$ NMR ($CDCl_3$): δ 8.67(br s, 1H, NH), 7.93(s, 1H, imidazole H-4), 6.91(m, 4H, $C_6H_4$-F), 5.16(s, 1H, $C_4$-H), 4.24(s, 3H, N-$CH_3$), 4.17(m, 4H, $CO_2CH_2$), 3.08(br t, J=4.6 Hz, 4H, piperazenyl H-3 & H-5), 2.60(m, 6H, piperazenyl H-2 & H-6 and $CH_2$), 2.24(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$) and 1.22((t, J=6.8 Hz, 3H, $CH_3$)

IR(KBr): 3375 (NH),1716(C=O), 1666(C=C), 1527 and 1371 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 556($M^+$,4), 540(10), 279(79), 206(63), 193(100), 150(46) and 122(30)

*3-[2-[4-(p-Fluorophenyl)piperazine-1-yl]ethyl], 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazolyl)-3,5-pyridinedicarboxylate (9j)*

$H^1$ NMR ($CDCl_3$): δ 8.49(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 6.91(m, 4H, $C_6H_4$-F), 5.14(s, 1H, $C_4$-H), 4.97(m, 1H, $CH(CH_3)_2$), ), 4.26(s, 3H, N-$CH_3$), 4.20(t, J=6.8 Hz, 2H, $CO_2CH_2$ ), 2.99(br t, J=4.3 Hz, 4H, piperazenyl H-3 & H-5), 2.63(m, 6H, piperazenyl H-2 & H-6 and $CH_2$), 2.23(s, 6H, $C_2$-$CH_3$ & $C_6$-$CH_3$), 1.22 and 1.14((two d, J=4.6 Hz, 3H each, $CH(CH_3)_2$)

IR(KBr): 3375 (NH),1717(C=O), 1666(C=C), 1527 and 1371 $cm^{-1}$ ($NO_2$).

MS: m/z(%) 570($M^+$,11), 552(16), 291(100), 259(24), 196(47) and 123(27)

*Pharmacology:*

Male albino guinea pig (body weight 300-450 g) was sacrificed by blow on the head. The intestine was removed above the ileocecal junction longitudinal smooth muscle segments of 2 cm length were mounted under a resting tension of 500 mg. The segments were maintained at 37° C in a 20 ml jacketed organ bath containing oxygenated (100% $O_2$) physiological saline solution of the following composition (Mm): NaCl: 137, $CaCl_2$ 1.8; KCl: 2.7; $MgSO_4$: 1.1; $NaH_2PO_4$:.4; $NaHCO_3$:12; Glucose:5. The muscles were equilibrated for 1 hour with a solution changes every 15 min. The contractions were recorded with a force displacement

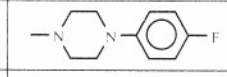
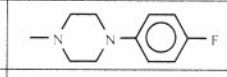
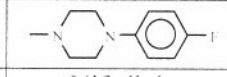
transducer (F-50) on a NARCO physiograph. All compounds were dissolved in DMSO and the same volume of the solvent was used as control. The contractile response was taken as the 100% value for the tonic (slow) component of the response. Test compounds were cumulatively added after the dose response for KCl was determined. Compound-induced relaxation of contracted muscle was expressed as percent of control. The $IC_{50}$ values were graphically determined from the contraction-response curves (16-17).

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

## RESULTS AND DISCUSSION

Nine unsymmetrical analogues of nifedipine were prepared by a procedure reported by Meyer in which, 1-methyl-5-nitro-imidazol-2-carbox-aldehyde was reacted with 3-oxobutanoic acid esters and 3-aminocrotonate. The *in vitro* calcium channel antagonist activities ($IC_{50}$) of compound **9a-j** determined as contraction required producing, 50% relaxation of contracted guinea pig ileal lungitudinal smooth muscle (GPILSM). Nifedipine was used as reference drug. The results are summarized in Table 1.

**Table 1.** Physical properties of compounds 9a-j and their calcium channel antagonist activity in GPILSM

| Comp. No. | $R_1$ | $R_2$ | n | Mp ($^0$C) | Yield (%) | Calcium- channel antagonist activity ($IC_{50}$ ± SEM, n=5) M |
|---|---|---|---|---|---|---|
| 9a | -N(Me)$_2$ | Me | 2 | 303-305 | 53 | $7.15 \pm 1.83 \times 10^{-9}$ * |
| 9b | -N(Me)$_2$ | Et | 2 | 242-244 | 61 | $1.09 \pm 0.72 \times 10^{-9}$ * |
| 9c | -N(Me)$_2$ | iPro | 2 | 239-240 | 58 | $4.61 \pm 1.60 \times 10^{-10}$ |
| 9d | -N(Me)$_2$ | Me | 3 | 253-255 | 55 | $3.15 \pm 0.76 \times 10^{-9}$ * |
| 9e | -N(Me)$_2$ | Et | 3 | 183-185 | 40 | $4.89 \pm 0.72 \times 10^{-10}$ |
| 9f | -N(Me)$_2$ | iPro | 3 | 121-123 | 60 | $7.39 \pm 1.12 \times 10^{-11}$ * |
| 9g |  | Me | 2 | 250-252 | 38 | $2.65 \pm 0.61 \times 10^{-10}$ |
| 9h |  | Et | 2 | 218-220 | 54 | $4.88 \pm 1.34 \times 10^{-11}$ * |
| 9j |  | iPro | 2 | 186-188 | 47 | $2.12 \pm 0.98 \times 10^{-11}$ * |
| | Nifedipine | | | | | $2.57 \pm 0.36 \times 10^{-10}$ |

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

The results for asymmetrical esters possessing one $R_1$ and $R_2$ substituents indicated that increasing the length of methylene chain in $C_3$-ester substituent decreases activity .The relative activity profile for the same $R_1$ and n esters was iPr>Et>Me. Comparison of esters having the same number of methylene (n=2) show that the arylpiperazine compounds were more active than amine derivatives.

Comparison of the activities of compounds **9g-j** with the compounds reported by Shafiee et al. (10) having the same structure without arylpiperazine group reveals that the presence of an arylpiperazine group substituted on C-3 position of the 1,4-dihydropyridine ring increases the smooth muscle relaxant activity. Compound **9j** was the most active compound and its activity was more than the reference drug, nifedipine.

## 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. 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.
3. Luscher, T.F., Cosentino, F. (1998) The classification of calcium antagonists and their selection in treatment of hypertension. Drugs 55: 509-512.
4. Herrera, G.M., Walker, B.R. (1998) Involvement of L-type calcium channels in hypoxic relaxation of vascular smooth muscle. J. Vasc. Res. 35: 265-273.
5. Uneyama, H., Takahara, A., Wakamori, M., Mori, Y., Yoshimoto, R. (1999) Pharmacology of N-type $Ca^{+2}$ channels distributed in cardiovascular system. Int. J. Mol. Med. 3: 455-466.
6. Gaudio, A,C., Korolkovas, A., Takahata, Y. (1994) Quantitative structure activity relationships for 1,4-dihydropyridine calcium channel antagonist. J. Pharm. Sci. 83: 1110-1114.
7. 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.
8. 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.
9. El-Sabban, F., Reid, K.H., Edmonds, H.L. (2000) Comparative effect of flunarizine in two animal models of stroke. Clin. Exp. Pharmacol. Physiol. 27: 107-111.
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. Clemens, R.J. (1993) Dibetene. Chem. Rev. 86: 241-318.
12. 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-pyridinecarboxylates. Arch. Pharm. Med. Chem. 330: 290-294.
13. Hantsch, A. (1882) Ueber die synthese pyridinartiger verbindungen aus acetessigather und aldehydammoniak. Justus Liebigs Ann. Chem. 215: 1-82.
14. Meyer, H., Bossert, F., Wehinger, E., Stoepel, K., Voter, W. (1981) Synthesis and vergleichende pharmakologische untersuchungen Von 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarbon saureestern mit nichidentischer ester funktionen. Arzneim Forsch. Drug Res. 31: 407-409.
16. Shafiee, A., Pirouzzadeh, B., Ghasemian, F., Parang, K. (1992) Synthesis of 2-acetyl-1-methyl-5-nitroimidazole. Heterocyclic Chem. 29: 1021-1023.
17. Rovnyak, G.C., Kimball, S.D., Beyer, B., Cucinotta, G., DiMarco, J.D., Gougoutas, J., Hedberg, A., Malley, M., McCarthy, J.P., Zhang, R., Moreland, S. (1995) Calcium entry blockers and activators: conformational and structural determinations of calcium channel modulators. J. Med. Chem. 38: 119-123.
18. 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.