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-amino crotonate. 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 bioisoster 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 arylpiperazine, 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 Koehler hot stage apparatus and are uncorrected. 1H-NMR spectra were run on a Varian Unity Plus 400 MHz spectrometer. Chemical shift are reported in parts per million (6) 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.
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-e with diketene 5 afforded the corresponding acetocetic esters 6a-e (11-12). The unsymmetrical analogues 9a-j were prepared by modified Hantzsch reaction repoted by Meyer in which 1-methyl-5-nitro-imidazole-2-carboxaldehyde 7 was reacted with 3-oxobutanoic acid esters 6a-e and alkyl 2-aminoethanolate 8a-e (13-15). (Scheme I)

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) were refluxed for 24 hours. The solvent was removed in vacuo and the residue obtained was dissolved in CH₂Cl₂ (50 ml) and washed with water (3 x 25 ml). The organic phase was dried (Na₂SO₄), the solvent was removed, and the residue obtained was purified by silica gel column chromatography using CH₂Cl₂-MeOH (96:4, V/V) as eluent to give 3 as a white solid (3.08 g, 55%).

The assignment of piperazinyl protons was based on the fact that the H-3 and H-4 protons are deshielded by the p-fluorophenyl substituent resulting in their appearance at lower field (δ = 5.15), whereas the H-2 and H-5 piperazinyl protons which are not affected appear at higher field (δ = 2.71).

1H-NMR(CDCCl₃): 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, 3H, OH, exchanges with D₂O), 2.71 (t, J=4.9 Hz, 4H, piperazinyl H-2 and H-6), 2.64 (t, J=5.4 Hz, 2H, CH₂CH₂N).

General procedure for the synthesis of Acetoacetate derivatives 6a-e (procedure A):
Diketene 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 Et₃N (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 acetooctoacetate (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 CH₂Cl₂:MeOH (96:4, V/V) as eluent. The product 6a was isolated as a yellow oil (2.62 g, 85%).

H¹ NMR (CDCl₃): δ 6.82-6.97 (m, 4H, aryl-H), 4.35 (t, J = 5.8 Hz, 2H, COOCH₂), 3.47 (s, 2H, COCH₂COO⁻), 3.09 (t, J = 4.9 Hz, 2H, piperazinyl H-3, H-5), 2.63-2.73 (m, 6H, COOCH₂CH₂ and piperazinyl H-2, H-6), 2.27 (s, 3H, CH₂CO).

IR (film): 1776 (C=O, ester), 1229 (C-F) cm⁻¹.

2-(N,N-Dimethylamino)ethyl acetooctoacetate (6b)

The title compound (6b) was prepared according to procedure A using N,N-dimethylaminoethanolamine (0.89 g, 10mmol), diketene (0.84g, 10mmol) and triethylamine (5 drop). The reaction mixture was purified by distillation in vacuo (bp 98.9°C (3mmHg)) to yield as a colourless liquid (1.46 g, 84.3%).

H¹ NMR (CDCl₃): δ 4.20 (t, J = 5.7 Hz, 2H, COOCH₂), 3.45 (s, 2H, COCH₂COO⁻), 2.55 (t, J = 5.7 Hz, 2H, CH₂NMe₂), 2.23 (s, 9H, CH₂CO and NMe₂).

IR (film): 1745 (C=O, ester), 1726 (C=O, ketone) cm⁻¹.

3-(N,N-Dimethylamino)propionyl acetooctoacetate (6c)

The title compound (6c) was prepared according to procedure A using 3-(N,N-dimethylamino)propionyl (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%).

H¹ NMR (CDCl₃): δ 4.13 (t, J = 5.7 Hz, 2H, COOCH₂), 5.61 (s, 2H, COCH₂COO⁻), 2.47 (t, J = 5.1 Hz, 2H, CH₂NMe₂), 2.11 (s, 9H, CH₂CO and NMe₂) and 1.14 (m, 2H, CH₂).

IR (film): 1751 (C=O, ester), 1719 (C=O, ketone) cm⁻¹.

General procedure for the synthesis of dihydropropionyl derivatives 9a-c (procedure B)

A mixture of the respective acetooctoacetate ester 6a-c (5.0 mmol), 1-methyl-5-nitro-imidazolid-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-3-(1-methyl-5-nitro-2-imidazolyl) 3,5-pyridinedicarboxylate (9a)

H¹ NMR (CDCl₃): δ 8.43 (br s, 1H, NH), 7.91 (s, 1H, imidazole H-4), 5.10 (s, 1H, C₆H₅), 4.42 (t, J = 7 Hz, 2H, COOCH₂), 4.15 (s, 3H, N-CH₃), 4.09 (s, 3H, COOH), 3.76 (s, J = 7 Hz, 2H, CH₂NMe₂), 3.69 (s, 6H, N(CH₃)₂) and 2.39 (s, 6H, C₆H₅CH₂ & C₆H₅CH₃).

IR (KBr): 3335 (NH), 1694 (C=O), 1671 (C=C), 1526 and 1351 cm⁻¹ (NO₂).

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-3-(1-methyl-5-nitro-2-imidazolyl) 3,5-pyridinedicarboxylate (9b)

H¹ NMR (CDCl₃): δ 8.49 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.16 (s, 1H, C₆H₅), 4.42 (t, J = 7 Hz, 2H, COOCH₂), 4.21 (s, 3H, N-CH₃), 4.09 (q, J = 6.8 Hz, 2H, COOH), 3.85 (t, J = 7 Hz, 2H, CH₂NMe₂), 3.67 (s, 6H, N(CH₃)₂), 2.24 (s, 6H, C₆H₅CH₂ & C₆H₅CH₃) and 1.23 (t, J = 6.8 Hz, 3H, CH₃).

IR (KBr): 3415 (NH), 1715 (C=O), 1651 (C=C), 1528 and 1353 cm⁻¹ (NO₂).

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-3-(1-methyl-5-nitro-2-imidazolyl) 3,5-pyridinedicarboxylate (9c)

H¹ NMR (CDCl₃): δ 8.23 (br s, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.13 (s, 1H, C₆H₅), 5.02 (m, 1H, CH(CH₃)₂), 4.39 (t, J = 6.7 Hz, 2H, COOCH₂), 4.22 (s, 3H, N-CH₃), 3.79 (q, J = 6.4 Hz, 2H, CH₂NMe₂), 3.56 (s, 6H, N(CH₃)₂), 2.24 (s, 6H, C₆H₅CH₂ & C₆H₅CH₃), 1.24 and 1.16 (two d, J = 6.5 Hz, 3H each, CH₃CH₂).

IR (KBr): 3375 (NH), 1737 (C=O), 1626 (C=C), 1521 and 1371 cm⁻¹ (NO₂).
3-[2-6-N,N-dimethylamino-propyl]-5-methyl-1,4-dihydro-2,6-dimethyl-4-(1-methyl-5-nitro-2-imidazoyle)-3-pyridinedicarboxylate (9q)

H1 NMR (CDCl3): δ 8.44(br s, 1H, NH), 7.93(s, 1H, imidazole H-4), 5.14(s, 1H, C6H-1), 1.42(t, J=6.8 Hz, 2H, CH2-NMe), 3.65(s, 6H, N(CH3)-3) and 2.22(s, 6H, C2CH3 & C6CH3)
IR(KBr): 3375 (NH), 1734(C=O), 1666(C=C), 1521 and 1351 cm⁻¹ (NO2).
MS: m/z (%) 421(M+), 397(25), 351(100), 224(76), 176(10) and 128(14)

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

H1 NMR (CDCl3): δ 8.98(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 5.17(s, 1H, C6H-1), 4.42(t, J=7 Hz, 2H, CO2CH2), 4.23(s, 3H, N-CH3), 4.09(t, J=7.2 Hz, CO2CH3), 3.86(t, J=6.7 Hz, 2H, CH2NMe2), 3.67(s, 6H, N(CH3)-3) and 2.24(s, 6H, C2CH3 & C6CH3), 1.77(m, 2H, CH2) and 1.23(t, J=7.2 Hz, 3H, CH3)
IR(KBr): 3345 (NH), 1714(C=O), 1655(C=C), 1513 and 1373 cm⁻¹ (NO2).
MS: m/z (%) 435(M+), 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-imidazoyle)-3-pyridinedicarboxylate (9f)

H1 NMR (CDCl3): δ 8.17(br s, 1H, NH), 7.94(s, 1H, imidazole H-4), 5.12(s, 1H, C6H-1), 4.98(m, 1H, CH2CH2NMe2), 4.59(t, J=6.6 Hz, 2H, CO2CH3), 4.22(s, 3H, N-CH3), 3.77(t, J=6.5 Hz, 2H, CH2NMe2), 3.66(s, 6H, N(CH3)-3), 2.24(s, 6H, C2CH3 & C6CH3), 1.80(m, 2H, CH2), 1.25 and 1.17(two d, J=5.2 Hz, 3H each, CH3(CH3))
IR(KBr): 3392 (NH), 1740(C=O), 1619(C=C), 1517 and 1379 cm⁻¹ (NO2).
MS: m/z (%) 435(M+), 379(100), 309(70), 252(93), 156(11) and 128(19)

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% O2) physiological saline solution of the following composition (Mm): NaCl: 137, CaCl2: 1.8, KCl: 2.7, MgSO4: 1.1, NaH2PO4:0.4, NaHCO3:12, 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 (P-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).

**RESULTS AND DISCUSSION**

Nine unsymmetrical analogues of nifedipine were prepared by a procedure reported by Meyer in which 1-methyl-5-nitroimidazol-2-carboxaldehyde 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 longitudinal smooth muscle (GPILSM). Nifedipine was used as reference drug. The results are summarized in Table 1.

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

*a* 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-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


