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ORIGINAL RESEARCH

Synthesis and cardiovascular activity of difluro-substituted hexahydroquinoline

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Abstract Twenty-two new methyl (ethyl) 2,6,6- or 2,7,7-trimethyl-5-oxo-4-(disubstituted phenyl)-1,4,5,6,7,8-hexahydro-quinoline-3-carbohydrates and 12 new N-N-diethyl 2,6,6 or 2,7,7-trimethyl-5-oxo-4-(disubstituted phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives are synthesized and their activity on calcium channel antagonists was observed. Synthesis was done by the Hantzsch reaction. Calcium antagonistic activity was determined by tests performed on isolated rat ileum and thoracic arteries.

Introduction

It is generally observed that calcium ions regulate enzymatic reactions, activate excitable cells, and induce the contraction of muscles, which is a well known process. The influx of Ca^{2+} is blocked by calcium channel antagonists and are used as antianginal and antihypertensive drugs (Godfraind *et al.*, 1986; Fleckenstein, 1972; Hof *et al.*, 1985; Janis and Triggle, 1984; Nayler, 1988; Wehinger and Gross, 1986). Though several compounds act as calcium channel blockers, 1,4-dihydropyridines (DHPs) have been the most popular group in this area. The introduction of 1,4-DHPs as calcium channel antagonists ensures new approaches for cardiovascular therapy. Nifedipine is the prototype drug in this group. Many nifedipine-like compounds have been synthesized by making various modifications to the nifedipine molecules to yield compounds that can display antagonistic and agonistic behaviors. They have similar structure requirements but interact with different regions of the same receptor (Franckowiak *et al.*, 1985; Gorlitzer and Schmidt,

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1991; Hof *et al.*, 1985; Schramm *et al.*, 1983). For example, some modifications of the 1,4-DHP ring yield compounds that exhibit pharmacological actions opposite to those of nifedipine. Active compounds have also been obtained by the introduction of the 1,4-DHP moiety into condensed systems such as hexahydroquinoline, acridine, and furoquiodine (Altas *et al.*, 1999; Kismetli *et al.*, 2004; Pastor *et al.*, 1994; Safak *et al.*, 1993; 1995; 1997; Sausins and Duburs, 1988a; 1988b; Simsek *et al.*, 2000; 2001a; 2001b). In these compounds the orientation of the carbonyl group of the ester substituent at the 5-position in the 1,4-DHP ring of nifedipine has been fixed. We hypothesize that the orientation of this ester group is an important requirement for the compound to bind to a receptor.

Active calcium channel antagonists with a 1,4-DHP moiety have an aromatic ring in the 4-position of the dihydropyridine ring that tends to flatten the boat conformation of the DHP ring, while the aromatic ring is restricted to an orientation essentially perpendicular to that of the DHP ring. It is known that the introduction of fluorine atoms as substituents on the aromatic ring is restricted to an orientation essentially perpendicular to that of the DHP ring. It is also well known that the introduction of fluorine atoms as substituents on the aromatic ring increases the biological activity and decreases the toxicity of these compounds. It has been demonstrated that fluorinated 1,4-DHPs resemble the intro analogue in terms of electronic structure and lipophilicity (Yagupolskii *et al.*, 2001; 1996). In this study we synthesized a series of hexahydroquinoline derivatives with two fluoro substituents on the aromatic phenyl ring and have investigated their calcium modulatory activity with a view to determine the contribution of fluorine atoms to the activity.

Materials and methods

Chemistry

All chemicals used in this study were purchased from S.D. Fine and Alderich (Stenhenri, Germany). Elemental analysis was determined using Carlo Erba model 1108 (Rodano, Italy). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) was carried out using a Bruckers advanced DXP 200 spectrophotometer. Mass spectroscopy was done on fast atom bombardment (FAB) mass spectra Geol-SX-102/TA-6000 model using organon/xenon (6 kV, 10 mA) as the FAB gas. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer.

*Synthesis of methyl(ethyl) 2,6,6- or 2,7,7-trimethyl-5-oxo-4-(disubstituted phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (**1–20**)*

A mixture of methyl (ethyl) aminocrotonate (1 mmol) 4,4(5,5)-dimethyl-1, 3-cyclohexanedione (1 mmol) and the appropriate aromatic aldehyde (1 mmol) in 20 mL methanol was refluxed for 4 h. The solvent was evaporated and the residue was recrystallized from ethanol.

*Methyl 2,6,6-trimethyl-5-oxo-4-(2,6-diflourophenyl) 1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**3**)*

m.p. 270°C; infrared (IR) (υ cm^{-1}), 3270, 1700, 1580, 690;^{1}H NMR δ(ppm) 0.80 (3H, s , 2-CH_3), 100(3H, s, 6-CH_3), 1.60(2H, t, H-7), 2.30(3H, s, 2-CH_3), 2.45(2H, t, H-8), 3.55(3H, s, $COOCH_3$), 5.20 (1H, S_7H_4), 6.80–7.20(3H, m-Ar-H), 9.05(1H, S, NH); ^{13}C NMR-(ppm), 203, 167, 162, 151, 150, 132, 110, 100, 50, 43, 35, 26, 24, 20, 17; ,mass (m/e) : 361(M^+), 248(100%); anal: C, H, N ($C_{20}H_{21}F_2NO_3$)

*Ethyl 2,6,6-trimethyl-5-oxo-4-(2,5-diflourophenyl) 1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**7**)*

m.p. 240°C; IR (υ cm^{-1}), 3258, 1715, 1600, 750 ;^{1}H NMR δ(ppm) 0.80(3H, s, 6-CH_3), 0.95(3H, s, 6CH_3), 1.15(3u, t, CH_2CH_3), 1.70 (2H, t, H-7), 2.20(3H, s, 2-CH_3), 2.40(2H, t, H-8), 3.90(3H, q CH_2CH_3), 5.0(1H, s, H-4), 7.20–8.00(3H, m-Ar-H), 9.10 (1H, s, NH); Mass (m/e) : 374(M^+), 262(100%); anal: C, H, N ($C_{21}H_{23}F_2NO_3$)

*Methyl 2,7,7-trimethyl-5-oxo-4-(2,6-diflourophenyl)1,4, 5,6,7,8-hexahydroquinoline-3-carboxylate (**13**)*

m.p. 230°C; ^{1}H NMR δ(ppm) 0.85(3H, s, 7-CH_3), 1.00(3H, s, 7-CH_3), 2.00(2H, s, H-6), 2.25(3H, s, 2-CH_3), 2.40(2H, s, H-8), 3.55(3H, s, $COOCH_3$), 5.04(1H, s, H-4), 6.70–7.30(3H, m-Ar-H), 9.00(1H, s, NH); ^{13}C-NMR δ(ppm), 195, 168, 163, 154, 150, 141, 136, 117, 102, 51, 41, 39, 32 ; IRυ an(1/2) - 3270, 1720, 1605, 780; mass (m/e) : 361(M^+), 248(100%); anal: C, H, N ($C_{20}H_{21}F_2NO_3$)

*Synthesis of N,N-diethyl-2,6,6- or 2,7,7-trimethyl-5-oxo-4-(disubstituted phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (**21–30**)*

N-N-diethyl aminoacetamide (1 mmole) was added to a mixture of 4,4(5,5)-dimethyl-1,3-cyclohexanedione (1 mmol), the appropriate aromatic aldehyde (0.001 mol) and 1 mL ammonia in 20 mL methanol. The reaction was refluxed with stirring for 4–5 h, after which the reaction was completed. After cooling, the resultant precipitate was filtered and crystallized from ethanol.

*N-N-diethyl-2,6,6-trimethyl-5-oxo-4-(2,3-diflourophenyl)-1,4,5, 6,7,8-hexahydroquinoline-3-carboxamide (**25**)*

m.p. 232°C; IR (υ cm^{-1}), 3190, 1610, 785; ^{1}H NMR δ(ppm) 0.50(6H, t, CH_2CH_3), 0.80(3H, s, 6CH_3), 0.90(3H, s, 6-CH_3), 1.60(3H, s, 2CH_3), 2.25(4H, m, H-7, H-8), 3.15(4H, q, CH_2CH_3), 4.90(1H, s, H-4), 6.20(1H, s, NH), 6.85–7.30(3H, m-Ar-H); ^{13}C NMR-(ppm), 204, 170, 163, 160, 152, 149, 132, 120, 111, 104, 103, 50, 40, 35, 32, 25, 24, 16, 14; mass (m/e) : 402(M^+), 330(100%); anal: C, H, N ($C_{23}H_{25}F_2N_2O_2$)

*N-N-diethyl-2,7,7-trimethyl-5-oxo-4-(2,3-diflourophenyl)-1,4,5, 6,7,8-hexahydroquinoline-3-carboxamide (**30**)*

m.p. 95°C; IR (υ cm^{-1}), 3290, 1680, 1601, 775; ^{1}H NMR δ(ppm) 0.50(6H, t, CH_2CH_3), 0.85(3H, s, 7-CH_3), 0.95(3H, s, s, 7-CH_3), 2.80(2H, s, H-6), 2.30(3H, s, 2-CH_3), 2.45(2H, s, H-8), 3.25(4H, q, CH_2CH_3), 5.00(1H, s, H-4), 6.90–7.35(3H, m-Ar-H), 9.00(1H, s, NH); ^{13}C NMR-(ppm), 203, 170, 160, 158, 149, 132, 120, 111, 104, 103, 52, 42, 38, 32, 25, 24, 16, 14; mass (m/e) : 402(M^+), 330(100%); anal: C, H, N ($C_{23}H_{28}F_2N_2O_2$)

Pharmacology

The calcium antagonistic activities of the compounds were determined by tests performed on isolated rat ileum and rat thoracic artery. All procedures involving animals and their care was conducted in conformity with international laws and policies.

Studies on isolated rat ileum (Kazda *et al*., 1980)

Albino rats of either sex weighing 150–200 g were used for this study. The animals used in the test were fasted overnight. After the animals were sacrificed by cervical dislocation, the ileum (10–15 cm) terminal portion was immediately revoked and the 5.8 cm segmented proximal to the ileocaecal junction discarded. Segments 1.5–2 cm long were mounted vertically in a 10 mL organ bath containing Tyrode solution with the following composition (mmol/L): NaCl 136.87, KCl 2.68, $CaCl_2$ 1.80, $MgSO_4$ 0.81, NaH_2PO_4 4.06, $NaHCO_3$ 11.9, and glucose 5.55. The bath contents were maintained at 37°C and aerated by 95% O_2 and 5% CO_2. A tension of 2g was applied and isometric recording was done by using an isometric isotransductor ($FDT_{10\text{-}A}$) May TDA 95 transducer data acquisition system. The preparations were allowed to equilibrate for 60 min with regular washes every 15 min to check for calcium antagonistic effects. Contractions were induced with barium chloride (3×10^{-3} mol/L, bath concentration). After washing out, this process was repeated. After addition of the test substances dissolved in dimethyl sulfoxide at different concentrations (10^{-6} mol/L) and 5 min exposure time the amplitude of the contractions were induced. Only one compound was included in each preparation.

Studies of rat thoracic artery (Rose and Drager, 1992)

We sacrificed 30 animals in isolated rat ileum experiments. Rat thoracic artery preparations were also obtained from the same animals. Rings (3 mm) were suspended in organ baths of 10 mL capacity which contained tyrode solutions. The bath contents were maintained at 37°C and aerated by 95% O_2 and 5% CO_2. A tension of 2g was applied. The preparation were allowed to equilibrate for 60 min

with regular washes every 15 min in order to check for antagonistic effects contractions were induced with 67 mmol/L potassium chloride. After washing out, this process was repeated until the amplitude of the contractions became constant. Investigations of the substances were performed using the single-dose techniques. Potassium chloride contractions were induced after the addition of the test substances and 10 min exposure time. During the administration of the individual substances the preparation was washed until the initial situation had been re-established and the potassium chloride concentration was induced. The isometric transducer ($FDT_{10\text{-}A}$) May TDA 95 transducer data acquisition system was used.

Results and discussion

The spectroscopic data of some active compounds are given in "Synthesis of methyl(ethyl) 2,6,6- or 2,7,7-trimethyl-5-oxo-4-(disubstituted phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (**1–20**)." The in vitro calcium antagonistic activities of the compounds and the reference drug nicradipine were determined by tests performed on isolated rat ileum and rat thoracic artery respectively. The results are given in Table 1 and 2.

The hexahydroquinoline was prepared by the Hantzsch reaction (Hantzsch, 1882). The reaction of the appropriate difluoro benzaldehyde derivative with alkyl amino crotonate and 4,4- or 5,5-dimethyl-1,3-cyclohexane diol in methanol yielded the corresponding hexahydroquinoline compound. The amide derivative was also synthesized by the reaction of aromatic benzaldehyde *N-N*-diethyl amino acetoacetamide and appropriate 1,3-cyclohexanedione in the presence of ammonia (Scheme 1).

Table 1 The relaxant effects of the studied compounds and nicardipine (10^{-5} mol/l) on isolated rat ileum precontracted with $BaCl_2$ ($4–10^{-3}$ mol/L)

Compound	Inhibition (%)[a]	Compound	Inhibition (%)[a]	Compound	Inhibition (%)[a]
1	54.0 ± 17.0	**11**	45.3 ± 06.5	**21**	45.9 ± 12.0
2	64.0 ± 06.6	**12**	49.6 ± 07.5	**22**	55.9 ± 16.0
3	79.4 ± 14.0	**13**	74.6 ± 07.1	**23**	26.5 ± 11.5
4	53.0 ± 07.0	**14**	46.6 ± 20.6	**24**	59.5 ± 10.0
5	75.0 ± 07.1	**15**	40.4 ± 16.0	**25**	54.2 ± 06.8
6	60.4 ± 04.9	**16**	47.4 ± 10.0	**26**	49.6 ± 14.7
7	86.8 ± 04.2	**17**	31.2 ± 11.0	**27**	40.0 ± 12.7
8	74.0 ± 04.1	**18**	48.4 ± 11.0	**28**	36.5 ± 08.5
9	47.2 ± 13.9	**19**	31.0 ± 09.1	**29**	45.4 ± 12.4
10	70.6 ± 05.0	**20**	45.5 ± 05.7	**30**	51.3 ± 08.4
Nicardipine	70.0 ± 05.3				

[a] Percentage inhibition ± standard deviation (SD) ($n = 6$)

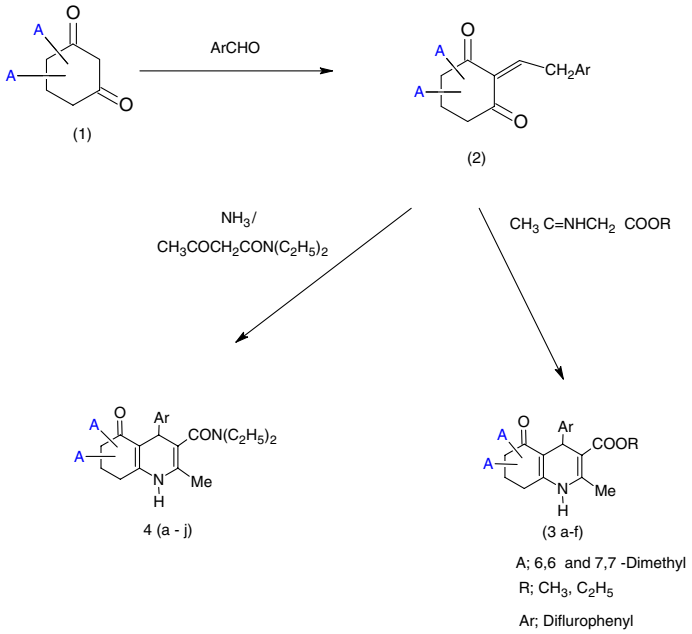
Difference ($p < 0.05$) from the values obtained with compounds

Table 2 Relaxant effects of the studied compounds and nicardipine (10^{-5} mol/L) on rat thoracic artery precontracted with KCl (67 mmol/L)

Compound	Inhibition (%)[a]	Compound	Inhibition (%)[a]	Compound	Inhibition (%)[a]
1	33.3 ± 14.6	**6**	11.7 ± 6.6	**22**	10.8 ± 4.7
2	27.9 ± 9.9	**7**	21.4 ± 11.5	**24**	7.2 ± 4.6
3	23.6 ± 6.8	**8**	14.3 ± 9.0	**25**	37.0 ± 16.5
4	24.5 ± 12.8	**9**	30.3 ± 9.1	**30**	31.5 ± 16.4
5	15.0 ± 8.8	**10**	6.8 ± 3.4	Nicardipine	15.9 ± 7.9

[a] Percentage inhibition ± SD ($n = 6$)

Difference ($p < 0.05$) from the values obtained with the compounds



Scheme 1

The structures of the compounds were elucidated by IR, ^{1}H, and ^{13}C-NMR spectroscopy, mass spectroscopy, and elemental analysis.

The elemental analysis results were within 0.4% of theoretical values and consistent with the postulated structures. The IR spectra displayed the characteristic N–H and C=O stretching bands. The 6-CH_3 and 7-CH_3 protons of the hexahydroquindine moiety resonate at 0.80–1.00 ppm as separate singlets in the ^{1}H NMR spectra. The chemical shifts of the aromatic, 2-methylmethylene, and methane protons of the compounds had expected values. The N–H signals occurred at 1.00–9.20 ppm. The ^{13}C NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of carbon atoms.

On isolated rat ileum strips precontracted with barium chloride (4.10^{-3} mmol/L), compounds **3**, **5**, **7**, **8**, **10**, and **13** are more potent as calcium antagonists than nicardipine at the 10^{-5} mol/L concentration. The most potent compound is **7** (87.8%), whereas nicardipine exhibited 69.6% inhibition at this concentration. A comparison of the 6,6- and 7,7-dimethyl substituted series of the dimethyl hexahydroquinoline compounds shows that 6,6-dimethyl derivatives have more potent activity than the 7,7-dimethyl analogues (Linden *et al*., 2005). In the 6,6-dimethyl series (**1**–**10**), all of the compounds increased the inhibition of muscle contraction by more than 50%. No meaningful pattern of activity difference could be found between the methyl and ethyl esters. The position of the fluoro substituents on the phenyl ring also apparently influences the activity. The compounds which possess a 4-(2,6-difluorophenyl) moiety are generally more active than the others. The 15 compounds (**1**–**10**, **13**, **22**, **24**, **25**, and **30**) that possessed calcium antagonistic activity at a level higher than 50% in isolated rat ileum were investigated further in the rat thoracic artery studies. In thoracic artery preparations preconcentrated with barium chloride (67 mmol/L), compounds **6**, **10**, **22**, **24,** and **25** are less active than nicardipine at the 10^{-5} mol/L concentration. Compound **25** was the most active substance of this series while compounds **5** and **8** were equipotent to nicardipine. These results showed that the methylester derivatives are generally more active than the corresponding ethylester compounds. These findings are in accordance with the results that were obtained by using rat ileum. All findings are in agreement with the structure–activity relationships of these derivatives (Simsek *et al*., 2003). In addition, the replacement of the amide group by an ester function in the 3-position of the hexahydroquinoline moiety does not result in a positive contribution to the activity of the compound.

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