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Synthesis of hexahydroquinoline derivatives possessing calcium antagonistic activity

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Nifedipine is the prototype of dihydropyridine (DHP) calcium channel blockers [1–4], which have been used as antianginal and antihypertensive drugs. Many active compounds have been synthesised by modifying the nifedipine molecule. For example, annelated 1,4-DHP have been obtained by the introduction of the 1,4-DHP moiety to condensed systems [5–11]. 1,4-DHP derivatives may act as calcium antagonists or agonists. For example, Bay K 8644, which is a calcium channel activator, is a DHP derivative and has a structure similar to that of nifedipine.

Agonists and antagonists compounds have similar structural requirements and interact with different regions of the same receptor [12–13]. The discovery of various subtypes of Ca^{2+} channels with different tissue distribution makes it possible to enhance the tissue selectivity of DHP compounds. The aim of this work was to synthesise compounds having a 1,4-DHP ring in a condensed system and to screen their calcium channel blocking activities.

2,6,6-Trimethyl-3-carboxyalkyl-4-(mono and/or disubstituted) phenyl-5-oxo-1,4-hexahydroquinoline derivatives were prepared by a modification of the Hantzsch synthesis [14]. In order to synthesise the compounds 4,4-dimethyl-1,3-cyclohexanedione (**I**) was treated with the appropriate aldehyde (**II**) to yield the benzylidene derivatives which when condensed with the methyl or ethyl acetoacetate (**III**) yielded the corresponding hexahydroquinoline derivative (Scheme).

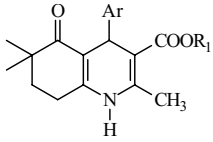
The synthesised compounds are listed in Table 1, their structures have been elucidated by IR, ^{1}H NMR, ^{13}C NMR, and mass spectra.

In studies on rat ileum, compound **7** has been found the most active. It was even more active than nicardipine. Among the methyl ester derivatives carrying a tolyl group, the most active isomer was the ortho substituted derivative, while among the methyl ester derivatives with an anisyl group, the activity order was found to be ortho>meta>para. Among the ethyl esters with a tolyl substituent, the meta isomer has been found more active than the ortho and para isomers. The same order of activity was seen with the ethyl esters having an anisyl substituent.

In preliminary studies on lamb carotid artery, compound **8** has been found to be the most active in this series. In addition, compounds **4** and **8** were more active than nicardipine, while compounds **1** and **7** are as active as nicardipine. The compounds carrying a 4-substituted phenyl group in 4-position of the hexahydroquinoline ring have not been found active. No difference could be found between methyl and ethyl esters, although the ethyl ester derivative having a piperonyl group in its molecule shows promising activity on rat ileum. This compound has, however, not been found active in the lamb carotid artery.

The results of these studies fit in respect to relaxant activity. The obtained results are in accordance with the structure-activity relationships of 1,4-DHP derivatives with calcium antagonistic activity. Thus, it is known that the compounds having a phenyl ring with electron withdrawing substituents are active analogs.

Table 1: Empirical formula, molecular weight, yield (%), melting point and analysis of the hexahydroquinoline derivatives



Compd.	R_1	Ar	Emprical formula	M.w.	Yield (%)	M.p. (°C)
1	CH_3	2-Methylphenyl	$C_{21}H_{25}NO_3$	339	90	238
2	C_2H_5	2-Methylphenyl	$C_{22}H_{27}NO_3$	353	88	176
3	CH_3	3-Methylphenyl	$C_{21}H_{25}NO_3$	339	79	207
4	C_2H_5	3-Methylphenyl	$C_{22}H_{27}NO_3$	353	67	185
5	CH_3	4-Methylphenyl	$C_{21}H_{25}NO_3$	339	81	230
6	C_2H_5	4-Methylphenyl	$C_{22}H_{27}NO_3$	353	76	168
7	CH_3	2-Methoxyphenyl	$C_{21}H_{25}NO_4$	355	77	246
8	C_2H_5	2-Methoxyphenyl	$C_{22}H_{27}NO_4$	369	83	223
9	CH_3	3-Methoxyphenyl	$C_{21}H_{25}NO_4$	355	89	225
10	C_2H_5	3-Methoxyphenyl	$C_{22}H_{27}NO_4$	369	88	171
11	CH_3	4-Methoxyphenyl	$C_{21}H_{25}NO_4$	355	84	242
12	C_2H_5	4-Methoxyphenyl	$C_{22}H_{27}NO_4$	369	80	151
13	CH_3	3,4-Methylene-dioxyphenyl	$C_{21}H_{23}NO_5$	369	83	251
14	C_2H_5	3,4-Methylene-dioxyphenyl	$C_{22}H_{25}NO_5$	383	85	168

Experimental

1. Chemistry

All chemicals used in this study were purchased Aldrich (Steinheim, Germany), and Fluka (Buchs, Switzerland).

Melting point: Thomas Hoover Capillary Apparatus (Philadelphia, PA, USA); the values are uncorrected. UV spectra: Shimadzu UV-160A UV-Visible Spectrophotometer. IR spectra: Perkin Elmer FT-IR Spectrometer 1720 X (Beaconsfield, UK) (KBr disc) (γ, cm^{-1}). ^{1}H NMR spectra: Bruker GMBH DPX-400 MHz Digital FT NMR and H^1 AMX 600 MHz FT NMR spectrophotometer (Karlsruhe, Germany) (DMSO-d_6; tetramethylsilane as internal

Scheme

I **II** **III** **1-14**

R: CH_3, C_2H_5

Table 2: Relaxant effects of the compounds and nicardipine on barium chloride (10^{-5} M) contraction in isolated rat ileum and lamb carotid artery (10^{-5}M (% ± SE) (n = 8)

Compound	isolated rat ileum % Inhibition	lamb carotid artery % Inhibition
1	60.53 ± 11.39	17.52 ± 5.64
2	0	–
3	0	–
4	68.84 ± 7.49	23.87 ± 3.43
5	0	–
6	46.00 ± 37.28	–
7	85.60 ± 4.04	17.03 ± 3.13
8	69.19 ± 43.59	26.21 ± 3.87
9	77.29 ± 10.47	11.37 ± 1.56
10	74.60 ± 2.39	9.44 ± 1.53
11	0	–
12	31.47 ± 6.15	–
13	0	–
14	71.60 ± 9.77	4.33 ± 0.59
Nicardipine	82.08 ± 1.88	17.95 ± 4.45

standard). ^{13}C NMR Spectra: ^{13}C AMX 150 MHz Ft NMR spectrophotometer. Chemical shift values are given as ppm. Mass spectra: Hewlett Packard Series II Plus 5890 GAS Cromatograph-Hewlett Packard 5972 Series Mass Selective Detector (Philadelphia, USA). Elemental analysis: Leco 932 CHNS-O Elemental Analyser (Philadelphia, USA) (TÜBİTAK-Ankara, Turkey). The results of elemental analysis were in an acceptable range.
In mass spectra of the compounds were recorded by the electron impact technic. Molecular ion peaks were seen in most of the compounds. The base peak forms by cleavage of aryl ring from parent molecule. In further fragmentation, the ions formed by the rupture of cyclohexene ring and acylium ions formed by the cleavage of ester function.

1.1. 2,6,6-Trimethyl-3-alkyloxycarbonyl-4-aryl-5-oxo-1,4,5,6,7,8-hexahydroquinolines (**1**–**14**)

The mixture of 0.001 mol 4,4-dimethyl-1,3-cyclohexanedione (I), 0.001 mol methyl (ethyl) acetoacetate (II) and 0.001 mol substituted benzaldehyde (III) with 1 ml ammonium in 25 ml methanol was refluxed for 4 h. Then, the solution was poured into ice-water. The precipitate was filtered, dried and crystallized from appropriate solvents.

1.2. 2,6,6-Trimethyl-3-carbethoxy-4-(4-methylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline (**6**)

M.p. 168 °C. IR (cm^{-1}) 3294, 3075, 2929, 1704, 1648, 1602, 697, 760; ^{1}H NMR (δ, ppm) 0.90 (3 H; s; 6-CH_3), 1.00 (3 H; s; 6-CH_3), 1.15 (2 H; t; H^7), 2.30 (3 H; s; 2-CH_3), 2.35 (3 H; s; Ar-CH_3), 2.70 (2 H; t; H^8), 3.60 (3 H; s; $COOCH_3$), 4.90 (1 H; s; H^4), 6.90–7.20 (5 H; m; aromatic), 9.00 (1 H; s; NH); ^{13}C NMR (δ, ppm) 13.2, 18.1, 22.2, 25.0, 25.0, 37.0, 47.2, 53.5, 59.8, 60.1, 108.8, 117.2, 128.3, 128.8, 129.5, 130.0, 134.4, 136.9, 141.2, 144.0, 164.2, 203.6; Mass (m/z) 323, 280, 264, 248, 235, 192, 152, 139, 102, 77
Spectroscopic values are given for only one compound, since those of their analogues are similar.

2. Pharmacology

The calcium antagonistic activities of the compounds were determined by the tests performed on isolated rabbit ileum and lamb carotid artery (Table 2).
Procedures involving animals and their care were conducted in conformity with international laws and policies.

2.1. Studies on the isolated rabbit ileum [**15**]

Albino rats of either sex, weighing (150–200 g) were used in the present study. The albino rats were purchased from Laboratory Animal Production Centre in Department of Pharmacology, School of Medicine, Osman Gazi University, Eskisehir, Turkey. They were housed under a 12 h light-dark cycle with room temperature maintained between 20–22 °C and food and water available ad libitum. Animals entered the test fasted over night. After animals were sacrificed by cervical dislocation, the ileum (10–15 cm terminal portion) was immediately removed, discarding the 5–8 cm segment proximal to the ileocaecal junction. Segments 1.5–2 cm long were mounted vertically in a 10 ml organ bath containing Tyrode solution of the following composition (mM): NaCl: 136.87; KCl: 2.68; $CaCl_2$: 1.80; $MgSO_4$: 0.81; NaH_2PO_4: 4.16; $NaHCO_3$: 11.9; Glucose: 5.55. The bath contents were maintained at 37 °C and aerated by 95% O_2 and 5% CO_2. A tension of 2 g was applied and isometric recording was done by using an isometric transducer (FDT_{10}-A) MAY TDA95 Transducer Data Acquisition System (Commat Ltd. Pharmacology and Physiology Instruments, Ankara, Turkey). The preparations were allowed to equilibrate for 60 min, regular washes every 15 min. In order to check for antagonistic effects, contractions were induced with barium chloride (3×10^{-3} mol/l, bath concentration). After throughout washing out, this process was repeated until the amplitude of the contraction became constant. Investigations of the substances were performed using the single dose technique. Barium chloride contractions were induced after addition of the test substances dissolved in dimethylsulphoxide at different concentrations (10^{-6}, 10^{-5}, 10^{-4} M) and 5 min exposure time. Only one compound was tested in each preparation.

2.2. Studies on lamb carotid artery [**12**]

Sheep (Ovis aries) carotid artery preparations were stemmed from the local slaughter-house. The aorts taken from lambs were cut in spirals and 0.3 cm long strips were fastened in a organ bath of 10 ml capacity which contains tyrode solution in a gas of % 95 O_2 and % 5 CO_2 and a tension of 2 g was applied. The preparations were allowed to equilibrate for 60 min, regular washes every 15 min. In order to check for antagonistic effects, contractions were induced with 67 mmol/l potassium chloride. After thorough washing out, this process was repeated until the amplitude of the contraction become constant. Investigations of the substances were performed using the single dose technique. Potassium chloride contractions were induced after addition of the test substance and ten minute exposure time. During the administration of the individual substances, the preparation was washed until the initial situation had been reestablished and the potassium chloride contractions were induced. The contractions were enrolled by 96 Transducer Data Acquisition System.

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