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Il Farmaco 55 (2000) 665–668

IL FARMACO

Synthesis and calcium antagonistic activity of 2,6,6-trimethyl-3-carbomethoxy(ethoxy)-4-aryl-1,4,5,6,7,8-hexahydroquinoline derivatives

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Received 7 December 1999; accepted 31 July 2000

Abstract

Twelve new 2,6,6-trimethyl-3-carbomethoxy(ethoxy)-4-aryl-1,4,5,6,7,8-hexahydroquinoline derivatives have been prepared. Their structures were confirmed by IR, ^{1}H NMR, mass and elemental analysis. The calcium antagonistic activity of these compounds was tested in rat aortic rings precontracted with 30 mM K^+. The compounds **IVa**, **IVc**, **IVe**, **IVf**, **IVh**–**l** induced concentration dependent relaxation response in precontracted aortic rings. The concentrations that cause 50% relaxation of K^+-contraction were also calculated for the compounds **IVe**, **IVf**, **IVj**. According to pharmacological results, compound **IVl** exert the most activity and compound **IVc** has been found to be least active in this series. The methyl ester derivatives carrying mono halogensubstitutent in the phenyl ring, the activity order is F > Br > Cl. Replacement of the substituted phenyl ring with the pyridine ring increases the activity.

Keywords: Calcium antagonistic activity; Hexahydroquinoline; 1,4-Dihydropyridine; Pharmacology; Spectra

1. Introduction

Dihydropyridine calcium entry blockers have been widely explored as cardiovascular agents. Nifedipine is the prototype of 1,4-dihydropyridine (1,4-DHP) derivatives and has been approved as a clinical agent and used in antianginal and antihypertensive therapy [1,2]. DHP calcium channel antagonists with high selectivity, that exert a minimal inotropic effect, are effective for the treatment of vasospastic disorders and hypertension [3]. Many attempts were made to increase the activity and decrease the toxicity of the nifedipine molecule. Traditional antagonistic dihydropyridines all have ester groups in both the 3- and 5- positions [4]. However, some results indicate that only one ester moiety is sufficient for the mentioned activity. So the 1,4-DHP structure has been introduced in condensed systems such as quinoline and acridine [5–9]. The aim of this study was to fix carbonyl groups in an antiperiplanar position by anellation at the 1,4-DHP structure and introduce the 1,4-DHP moiety into condensed systems.

2. Experimental

Melting points: Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA); the values are uncorrected. IR spectra: Perkin–Elmer FT-IR spectrometer 1720 X (Beaconsfield, UK) (KBr disk) (γ, cm^{-1}). ^{1}H NMR spectra: Bruker GMBH DPX-400 MHz Digital FT NMR spectrophotometer (Karlsruhe, Germany) (DMSO-d_6; tetramethylsilane as internal standard). Chemical shift values are given as ppm. Mass spectra: Hewlett Packard series II Plus 5890 GAS chromatograph, Hewlett Packard 5972 series mass selective detector (Philadelphia, USA). Elemental analysis: Leco 932 CHNS-O elemental analyzer (Philadelphia, USA) (Tübitak, Ankara, Turkey).

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0014-827X/00/$ - see front matter
PII: S0014-827X(00)00086-0

2.1. 2,6,6-Trimethyl-3-carbomethoxy(carbethoxy)-4-(4-substituted phenyl)-1,4,5,6,7,8-hexahydroquinolines (IVa–l)

The mixture of 0.001 mol methyl (ethyl) acetoacetate (**I**), 0.001 mol 4,4-dimethyl-1,3-cyclohexanedione (**II**), 0.001 mol 4-substituted benzaldehyde (**III**) and 1 ml ammonia solution (25%) was refluxed in methanol for 4 h. At the end of this period, the solution was concentrated under diminished pressure. The precipitate formed was filtered and washed with water. The compound was crystallized from alcohol. The melting points of the compounds are given in Table 1.

2.2. Pharmacology [10]

The rats (150–200 g, male) were killed by bleeding, and the thoracic aorta was isolated and cut into rings of 2–3 mm length. Aortic rings were suspended between two stainless-steel hooks in a 20 ml organ bath filled with Krebs–Hanseleit solution. The composition of the Krebs–Henseleit solution was (in mM): NaCl, 95; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.6. The solution was maintained at 37°C and gassed with a mixture of 95%O_2–5%CO_2. The aortic rings were equilibrated for 90 min under a resting tension of 2 g before the experimental procedure. The changes in tension were recorded with an isometric force displacement transducer connected to a transducer data acquisition system (MAY 95, Turkey) on an IBM-compatible personal computer.

Aortic rings were contracted with 30 mM K^+ (70–80% of its maximum contraction), and after the plateau tension was established, the relaxation response to cumulative concentrations of nifedipine (10^{-9}–10^{-6} M) and the compounds (10^{-8}–10^{-5} M) were determined. Only one compound was tested in each preparation. Nifedipine and all the compounds were dissolved in DMSO. The final concentration of DMSO in the bath did not exceed 0.5% and did not alter the contractility of aortic rings. The relaxation response is expressed as the percentage of 30 mM K^+-induced contraction in rat aortic rings. Data are given as mean $\pm$ SEM. Statistical comparisons were made by analysis of variance (ANOVA) followed by the Bonferroni test and significance was considered at the $P < 0.05$ level.

3. Results and discussion

To synthesize the proposed compounds, a modified Hantzsch synthesis was used which has been reported in the literature. The reaction is a one pot reaction. To do this aromatic aldehyde (**III**) was refluxed with methyl (ethyl) acetoacetate (**I**) and 4,4-dimethylcyclohexanedione (**II**) in the presence of concentrated ammonia in methanol for 4 h (Fig. 1).

In this reaction, it is possible that methyl (ethyl) aminocrotonate can be used instead of methyl (ethyl) acetoacetate. Therefore aminocrotonate was tried but higher yields were obtained with methyl (ethyl) acetoacetate. The reaction of 4,4-dimethylcyclohexanedione with aromatic aldehyde gives 2-arylidene-1,3-cyclohexa-

Table 1
Melting points and analysis of the compounds **IVa–l**

Comp.	R_1	R_2	Empirical formula	Yield (%)	M.p. (°C)
IVa	CH_3	4-bromophenyl	$C_{20}H_{22}BrNO_3$	78.7	259
IVb	C_2H_5	4-bromophenyl	$C_{21}H_{24}BrNO_3$	81.3	191
IVc	CH_3	4-chlorophenyl	$C_{20}H_{22}ClNO_3$	82.3	251
IVd	C_2H_5	4-chlorophenyl	$C_{21}H_{24}ClNO_3$	84.1	185
IVe	CH_3	4-fluorophenyl	$C_{20}H_{22}FNO_3$	76.3	254
IVf	C_2H_5	4-fluorophenyl	$C_{21}H_{24}FNO_3$	77.9	185
IVg	CH_3	4-trifluoromethylphenyl	$C_{21}H_{22}F_3NO_3$	66.6	238
IVh	C_2H_5	4-trifluoromethylphenyl	$C_{22}H_{24}F_3NO_3$	70.1	194
IVi	CH_3	4-nitrophenyl	$C_{20}H_{22}N_2O_5$	78.8	231
IVj	C_2H_5	4-nitrophenyl	$C_{21}H_{24}N_2O_5$	79.7	205
IVk	CH_3	4-pyridyl	$C_{19}H_{22}N_2O_3$	59.2	174
IVl	C_2H_5	4-pyridyl	$C_{20}H_{24}N_2O_3$	60.4	220

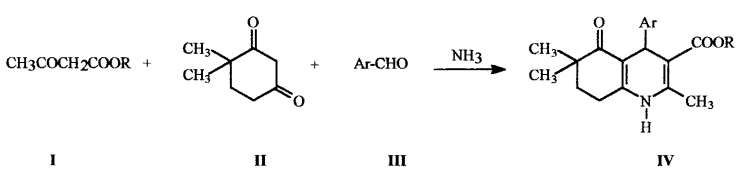

Fig. 1. Synthesis of compounds. R, CH_3, C_2H_5; Ar, 4-substituted phenyl and 4-pyridyl.

Table 2
Spectroscopic data of the compounds [a]

Comp.	^{1}H NMR (ppm)	Mass (EI, 40 eV)	IR
IVa	0.90 (s; 3H; 6-CH_3), 1.00 (s; 3H; 6-CH_3), 1.70 (t; 2H; 7-CH_2), 2.20 (s; 3H; 2-CH_3), 2.50 (t; 2H; 8-CH_2), 3.50 (s; 3H; $COOCH_3$), 4.90 (s; 1H; 4-CH), 7.00–7.40 (m; 4H; aromatic protons), 9.10 (s; 1H; NH)	405, 403, 374, 372, 346, 248 (%100), 232, 204, 139, 76, 41	3300, 2959, 1704, 1651, 1600, 1274, 1197, 814
IVb	0.90 (s; 3H; 6-CH_3), 1.00 (s; 3H; 6-CH_3), 1.10 (t; 3H; CH_2CH_3), 1.70 (t; 2H; 7-CH_2), 2.20 (s; 3H;2-CH_3), 2.50 (t; 2H; 8-CH_2), 4.00 (s; 2H; $COOCH_2$), 4.80 (s; 1H; 4-CH), 7.00–7.50 (m; 4H;aromatic protons), 9.10 (s; 1H; NH)	419, 417, 372, 360 (100%), 344, 315, 280, 252, 219, 181, 152, 137, 75, 41	3300, 2930, 1739, 1703, 1273, 1196, 835

[a] For compounds **IVc–l** ^{1}H NMR and IR values are not given, because these values are more or less the same as those of compounds **IVa**,**b**.

nedione (Knoevenagel reaction). Arylidene derivative and methyl(ethyl) acetoacetate react in a Michael addition to give 2,6,6-trimethyl-3-carbomethoxy (carbethoxy) - 4 - (4 - substituted phenyl) - 1,4,5,6,7,8 - hexahydroquinolines.

The purity of the compounds was checked by TLC. The structures of the compounds were in agreement with their IR, ^{1}H NMR, mass spectra and microanalysis data (Tables 1 and 2).

In IR spectra, the presence of γ (NH) and γ (C=O) indicated that these compounds have structure **IV**. In ^{1}H NMR spectra, methyl protons of six positions of the hexahydroquinoline ring were seen at appropriate chemical shift values. NH protons are seen at about δ 9.00 ppm. The other protons in structures have expected chemical shift and integral values. The mass spectra of the compounds show that the fragments contain ions corresponding to M, $M-Ar$, $M-H$, $M-OCH_3$($M-OC_2H_5$), M–aryl fragmentation. Our compounds give spectra where the most important peak corresponds to the loss from the molecular anion of the alcohol moiety of the ester groups, and these findings are in accordance with analogs [11]. The results of microanalysis also support the postulated structures.

The quantitative structure–activity relationships show that the phenyl ring might be in the C^4 position of the 1,4-DHP ring. Replacement of phenyl ring with the pyridyl group gives active compounds with respect to calcium antagonistic activity. The position of the pyridinyl nitrogen free electron pair, and/or charge distribution in the pyridinyl ring, may be important determinants of calcium channel agonist–antagonist modulation effects. Accordingly, it was envisaged that incorporation of a nitro group at the *meta*- or *para*-position of a C^4-phenyl ring on 1,4-DHP would be capable of electrostatic binding to the α_1-subunit binding site of the L-type calcium channel receptor [3].

In addition, substitution of the phenyl ring with electron-withdrawing groups increases calcium channel blocking activity. An electron-withdrawing substituent at 2 or 3 position in the phenyl ring increases the calcium channel activity but the compounds which contain an electron-withdrawing substituent at the 4 position in the phenyl ring also have calcium antagonistic activity. Therefore we synthesized the compounds containing the substituent at this position. Similarly electron withdrawing substituents on the phenyl ring such as trifluoromethyl exhibit comparable activities to a nitro group [12].

Quantitative structure–activity relationships of 4-aryl substituted-1,4-DHP derivatives have been established by Rodenkirchen et al. [13]. Recently, however, 1,4-DHPs with different ester groups at C^3 and C^5 have been gaining clinical prominence, since these asymmetrically substituted derivatives often have superior pharmacological activities compared to the corresponding symmetrical derivatives [14]. The compounds synthesized in this study possess unsymmetrical ester groups at C^3 and C^5. Thus C^4 of the compounds have an asymmetric center but isomers of these compounds

Table 3
The relaxation response elicited by 10^{-6} M and 10^{-5} M concentrations of the compounds (**IVa**, **IVc**, **IVe**, **IVf**, **IVh–l**) and nifedipine in rat aortic rings [a]

Comp.	% Reversal of K^+-induced contraction	
	10^{-6} M	10^{-5} M
IVa	15.95 ± 4.89*	36.34 ± 9.04
IVc	13.32 ± 2.12*	31.48 ± 3.80
IVe	17.23 ± 3.54*	50.57 ± 10.01
IVf	19.31 ± 4.18*	77.45 ± 3.88
IVh	16.26 ± 3.98*	39.54 ± 6.51
IVi	18.81 ± 6.33*	47.63 ± 11.32
IVj	19.77 ± 3.43*	59.21 ± 2.95
IVk	15.06 ± 0.97*	29.02 ± 3.02
IVl	20.45 ± 1.74*	49.24 ± 6.36
Nifedipine	97.91 ± 2.09	

[a] The data are expressed as percentages of the reversal of 30 mM K^+-induced contraction and given as mean $\pm$ SEM ($n = 4$). * Significantly different from 10^{-6} M nifedipine response $P < 0.05$.

Table 4
The concentrations of the compounds that cause 50% reversal of 30 mM K^+-induced contraction in rat aortic rings [a]

Comp.	Concentration (M)
IVe	$5.60 \pm 2.00 \times 10^{-6}$*
IVf	$4.18 \pm 0.6 \times 10^{-6}$*
IVj	$6.53 \pm 0.70 \times 10^{-6}$*
Nifedipine	$1.30 \pm 0.20 \times 10^{-8}$

[a] The data are given as mean ± SEM ($n = 4$). * Significantly different from nifedipine $P < 0.05$.

could not be separated due to technical shortage. So the effect of isomerism on the mentioned activity could not be determined.

In the present study the calcium antagonistic activity of these compounds was determined by the reversal of 30 mM K^+ induced contraction in rat aortic rings. The compounds **IVa**, **IVc**, **IVe**, **IVf**, **IVh**–**l** (10^{-8}–10^{-5} M) induced concentration dependent relaxation response in precontracted aortic rings (Table 3). However, the maximum relaxation could not be determined because of the solubility problem at concentrations higher than 10^{-5} M. On the other hand, nifedipine-induced relaxation response was obtained at a lower concentration range (10^{-9}–10^{-6} M) than the test compounds. K^+ induced contraction was reversed almost totally ($97.91 \pm 2.09\%$) by 10^{-6} M nifedipine while less than 25% relaxation was elicited by the compounds at this concentration. The percentage relaxations obtained by 10^{-6} M and 10^{-5} M concentrations of the test compounds are given in Table 3. Moreover, the concentrations that cause 50% relaxation of K^+ contraction were also calculated for the compounds **IVe**, **IVf**, **IVj** (Table 4). These concentrations were significantly higher than that of nifedipine, and were not different from each other. On the other hand, 50% reversal of K^+ contraction could not be determined for the compounds **IVa**, **IVc**, **IVh**, **IVi**, **IVl**, **IVk** because of the solubility problem mentioned above. In conclusion, according to pharmacological results, compound **IVl** has been found to be the most active and compound **IVc** has been found to be the least active in this series. For the methyl ester derivatives monohalosubstituted in the phenyl ring, activity order is F > Br > Cl. Replacement of the substituted phenyl ring with a pyridine ring increases activity.

Acknowledgements

This work was partially supported by Hacettepe University Research Fund.

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