Full Paper

Synthesis and Calcium Modulatory Activity of 3-Alkyloxycarbonyl-4-(disubstituted)aryl-5-oxo-1,4,5,6,7,8-hexahydroquinoline Derivatives

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In this study, twelve hexahydroquinoline derivatives which are condensed analogs of the 1,4-DHP molecule were synthesized and evaluated for their calcium-antagonistic activity. The results indicated that all compounds and nifedipine produced concentration-dependent relaxation in rabbit gastric fundus smooth muscle strips. The relaxant effects of the compounds on the tissues were expressed as percentage of the precontraction using Ca^{2+}. The maximum response (E_{max}) and pD_2 values (the negative logarithm of the concentration for the half-maximal response (EC_{50})) were calculated. It is generally believed that introduction of a second electron-withdrawing substituent into the phenyl ring increases the mentioned activity. Methyl-2,7,7-trimethyl-4-(2-nitro-5-chlorophenyl)-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate **2a** has been found to be the most active compound in this serie.

Keywords: Calcium antagonist activity / 1,4-DHP / Gastric fundus / Hexahydroquinoline / Nifedipine

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Introduction

Voltage-gated calcium channels are integral membrane proteins that open via electrical depolarization of the cell membrane and mediate the entry of calcium ions into the cell. Calcium channels are expressed in every excitable cell, including neurons, myocytes, and pancreatic ß-cells and form the most efficient molecular link between membrane depolarization and intracellular biochemical signaling. Ca^{2+} entering the cell through voltage-gated Ca^{2+} channels serves as the second messenger of electrical signaling, initiating cellular events such as contraction, secretion, synaptic transmission, and gene expression [1].

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Abbreviations: Dihydropyridine (DHP); nitric oxide (NO); cyclooxygenase (COX); N_w-nitro-L-arginine methyl ester (L-NAME)

The cytosolic calcium concentration regulates many cellular functions. In striated and smooth muscle, increasing of the intracellular calcium triggers the onset of contraction. Calcium influx through voltage-gated channels contributes to this rise in cytosolic calcium. Voltage-sensitive calcium channels have become a target for the drug therapy of cardiovascular and gastrointestinal diseases [2].

Calcium channel antagonists inhibit muscle contraction by blocking the influx of Ca^{2+} through calcium channels and are used as anti-anginal and antihypertensive drugs [3–11]. 1,4-Dihydropyridine (DHP) derivatives, of which nifedipine (Fig. 1) is the prototype, are the most studied calcium channel antagonists.

The derivatives having a 1,4-DHP structure have attracted attention and many active compounds have been obtained by making modifications on the nifedipine molecule. One of these modifications is to replace the ester function by various acyl groups such as amide, nitro, nitril, or ketone [8, 12–18]. In ester derivatives, when one of the R groups is methyl or ethyl ester, the

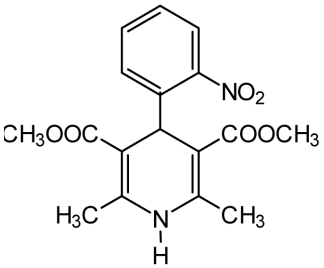


Figure 1. Formula of nifedipine.

activity increases due to the lipophilic properties [19, 20]. In addition, to fix one carbonyl group in an antiperiplanar position, 1,4-DHP structure could be annelated. Thus, condensed 1,4-DHP derivatives have been obtained and these analogs showed calcium antagonistic activity [21–30].

The derivatives carrying a phenyl group with two electron-withdrawing substituents also showed calcium-modulatory activity [14, 31–33]. Many studies have shown that the compounds having a dichlorophenyl moiety in their structure have been more active than nicardipine on isolated rat ileum and lamb carotid artery [18].

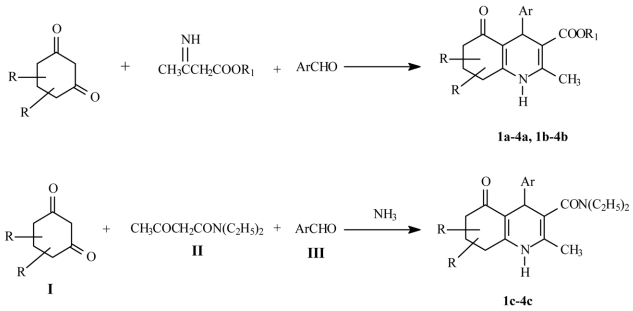
The object of this study is to show the contribution of two different electron-withdrawing substituents in the phenyl ring with respect to the calcium-modulatory activity. Therefore, the compounds having nitro and chlorine substitution in four different positions of the hexahydroquinoline ring were synthesized and evaluated for their calcium antagonistic activity in the rabbit gastric fundus smooth muscle strips.

Results and discussion

Chemistry

In this study, the hexahydroquinoline derivatives were obtained by modified Hantzsch synthesis [34]. The ester derivatives **1a–4a, 1b–4b** were synthesized by the reaction of 4,4- or (5,5)-dimethyl-1,3-cyclohexanedione, appropriate aromatic aldehyde and methyl (or ethyl) aminocrotonoate. In order to synthesize amide derivatives **1c–4c**, 4,4- or (5,5)-dimethyl-1,3-cyclohexanedione and appropriate aromatic aldehyde derivatives were reacted with *N,N*-diethylacetoacetamide (Scheme 1).

Compound **2a** and **4a** are mentioned in the literature, but there without chemical and pharmacological information. Therefore, compounds **2a** and **4a** were also synthesized. The structures of the compounds were elucidated by IR, ^{1}H-NMR, mass spectra, and elemental analysis. In the IR spectra, N-H, C=O (ester), and C=O (amide) stretching bands (only for compounds **1c–4c**) were seen at the expected values. In the ^{1}H-NMR spectra six or seven methyl protons of the hexahydroquinoline ring were seen at about 0.90–1.00 ppm as separate singlets. The peaks belonging to the aromatic protons and the protons of the alkyl groups were seen at the expected chemical shift values. The N-H signal of the compounds was found in their spectra except for compounds **1c** and **2c**. The mass spectra of the compounds was recorded using the electron impact technique. Molecular ion peaks were seen for all compounds. In further fragmentation, the peaks were forming by cleavage of the aryl ring from the parent molecule. Aromatization of the DHP ring to the pyridine analog was also realized (Scheme 2). These findings are in accordance with the literature [25]. In addition, the structure of the compounds was confirmed by elemental analysis.



1a–4a, 1b–4b

I **II** **III** **1c-4c**

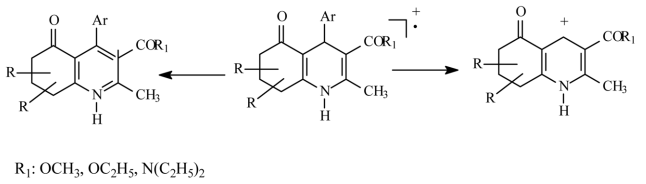
Compound	R	R_1	Ar
1a	6,6-dimethyl	CH_3	5-chloro-2-nitrophenyl
2a	7,7-dimethyl	CH_3	5-chloro-2-nitrophenyl
3a	6,6-dimethyl	CH_3	4-chloro-3-nitrophenyl
4a	7,7-dimethyl	CH_3	4-chloro-3-nitrophenyl
1b	6,6-dimethyl	C_2H_5	5-chloro-2-nitrophenyl
2b	7,7-dimethyl	C_2H_5	5-chloro-2-nitrophenyl
3b	6,6-dimethyl	C_2H_5	4-chloro-3-nitrophenyl
4b	7,7-dimethyl	C_2H_5	4-chloro-3-nitrophenyl
1c	6,6-dimethyl	-	5-chloro-2-nitrophenyl
2c	7,7-dimethyl	-	5-chloro-2-nitrophenyl
3c	6,6-dimethyl	-	4-chloro-3-nitrophenyl
4c	7,7-dimethyl	-	4-chloro-3-nitrophenyl

Scheme 1. Synthesis of the presented compounds.

Pharmacology

The maximum relaxant effects (E_{max}) and pD_2 values of the compounds and nifedipine on isolated strips of rabbit gastric fundus smooth muscle are given in Table 1.

The results indicate that all compounds produced concentration-dependent relaxation in rabbit gastric fundus smooth muscle strips. Compounds and nifedipine



Scheme 2. Mass fragmentation of the presented compounds.

Table 1. Maximum relaxant responses (E_{max}) and pD_2 values and molecular structures of the compounds and nifedipine on isolated strips of rabbit gastric fundus smooth muscle.

Compound	pD_2	E_{max}
1a	4.69 ± 0.48	83.21 ± 8.63
2a	5.10 ± 0.08	99.94 ± 0.06
3a	4.35 ± 0.19	80,82 ± 1.16
4a	6.87 ± 0.83	96.76 ± 3.24
1b	4.88 ± 0.50	88,21 ± 2.09
2b	5.66 ± 0.31	95.35 ± 4.65
3b	5.94 ± 0.41	84.19 ± 5.03
4b	5.92 ± 0.42	82.12 ± 6.45
1c	3.96 ± 0.14	87.33 ± 7.00
2c	4.30 ± 0.34	67.28 ± 6.20
3c	6.25 ± 0.33	98.40 ± 1.60
4c	5.10 ± 0.29	67.55 ± 7.88
Nifedipine	7.94 ± 0.25	99.30 ± 0.70

Relaxation is expressed as a percentage of the precontraction induced by Ca^{2+} (2.5 mM). The negative logarithm of the concentration for the half-maximal response (pD_2) and E_{max} values represent mean value ± S. E.

* $p < 0.05$, compared with vehicle responses (n = 6).

exerted concentration-dependent relaxation responses precontracted with Ca^{2+} (2.5 mM) in the gastric fundus smooth muscle strips with the efficacy order: **2a** = nifedipine > **3c** ≥ **4a** ≥ **2b** > **1b** ≥ **1c** ≥ **3b** ≥ **1a** ≥ **4b** ≥ **3a** > **4c** = **2c**. While the efficacy of the compounds **2a, 4a**, and **3c** have been found the same as nifedipine, potency of compounds **2a** and **3c** have been found less than nifedipine (Fig. 2). There is no difference between ester and amide derivatives with respect to the mentioned activity.

The main site of action of the Ca^{2+}-channel blockers is believed to be the voltage-dependent channels, where inhibition of the influx of extracellular Ca^{2+} results in uncoupling of excitation and contraction. Calcium-channel blockers may not only directly decrease the concentration of cytoplasmic Ca^{2+}, but also cause a decrease in Ca^{2+} release from intracellular stores [35].

The Ca^{2+}-channel types described for smooth muscle consist of the L- and T-types, with slow and fast inactivation characteristics, respectively [36]. L-Type Ca^{2+} channels are inhibited by Mg^{2+} and by dihydropyridines such as nifedipine, nimodipine, and isradipine [37]. The function of L-type Ca^{2+} channels can be regulated both by guanosine 5′-triphosphate (GTP)-binding proteins and by

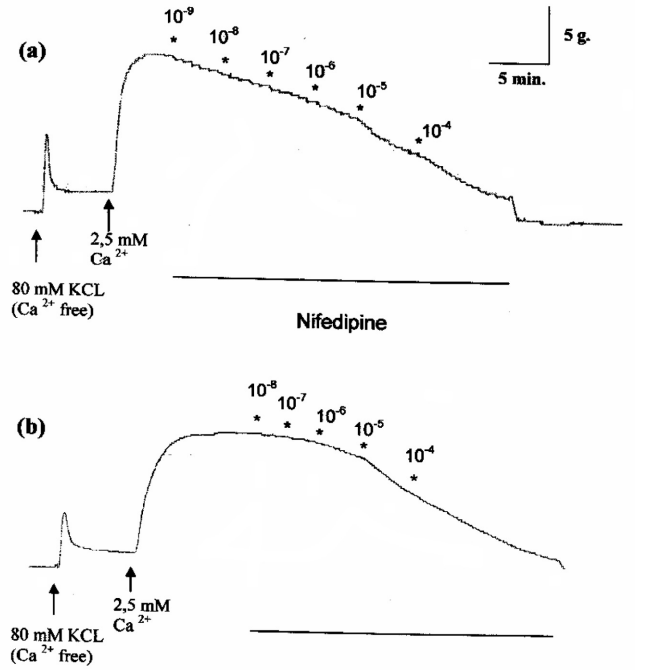


Figure 2. Typical traces showing the relaxant effects of nifedipine (10^{-9} M to 10^{-4} M) (Fig. 2a) and compound **2a** (10^{-8} M to 10^{-4} M) (Fig. 2b) on the precontracted with Ca^{2+} (2.5 mM) in the gastric fundus smooth muscle strips.

phosphorylation, the latter often having a negative effect on smooth muscle [35, 38].

Considering the overall powerful selectivity of action of the clinically available 1,4-DHPs for the L-type channel, some evidence suggests that a component of their vascular selectivity and vasodilating properties may arise from an ability to stimulate nitric oxide (NO) release from vascular endothelial cells [39]. Such an action would add to the vasodilation produced by direct blockade of the calcium channels in vascular smooth muscle cells. Endothelial NO synthase (eNOS) converts substrate L-arginine to NO, which diffuses to the vascular smooth muscle cells and promotes vascular relaxation [40–42]. NO releasing actions of the 1,4-DHPs appear to be a class action, to be exerted at therapeutic concentrations and to involve the stimulation of calcium influx into endothelial cells through a non-L-type process [43–45].

To investigate whether relaxation induced by the test compounds was due to interaction with the cyclooxygenase (COX), adrenergic system, or NO pathways, tissues were pretreated with indomethacin (COX inhibitor), propranolol (beta-adrenergic receptor blocker), or N_w-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the NO synthase inhibitor), respectively. Pretreatment of the strips with indomethacin, propranolol, and L-NAME did not significantly alter the relaxant responses to the com-

pounds indicating that COX, adrenergic, and NO pathways do not play a role in relaxations evoked by these substances.

Our results showed that these compounds had potency for relaxing isolated rabbit gastric fundus smooth muscle, possibly due to the blockade of Ca^{2+} channels, similar to the action of nifedipine.

The authors have declared no conflict of interest.

Experimental

Chemistry

All chemicals used in this study were purchased from Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland). Melting points are determined by a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA); the values are uncorrected. UV spectra: Shimadzu UV-160A UV-visible spectrophotometer (Shimadzu Co., Kyoto, Japan). IR spectra: Perkin Elmer FT-IR spectrophotometer 1720 X (Perkin Elmer, Beaconsfield, UK) (KBr disc) (γ, cm^{-1}). ^{1}H-NMR spectra: Bruker DPX-400 MHz digital FT NMR spectrophotometer (Bruker, 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 cromatograph Hewlett Packard 5972 series mass selective detector (Hewlett Packard, Philadelphia, PA, USA). Thin layer chromatography (TLC) was run on precoated (0.2 mm) silica gel Merck 254 + 366 (E. Merck, Darmstadt, Germany) and short wave UV light (254 nm) was used to detect the UV absorbing spots. Elemental analysis was carried out an a Leco 932 CHNS. Elemental analyzer (Leco, Philadelphia, PA, USA) (TUBITAK, Ankara, Turkey). The elemental analysis results were within 0.4% of theoretical values.

Drugs

*N*ω-nitro-L-arginine-methylester (L-NAME) hydrochloride, indomethacin, propranolol hydrochloride, and nifedipine were supplied by Sigma (Sigma, Taufkirchen, Germany). L-NAME, propranolol hydrochloride, and nifedipine were dissolved in distilled water. Compounds and indomethacin were dissolved in DMSO. DMSO was supplied by Sigma.

General procedure for methyl (and ethyl) 2,6,6-(2,7,7)-trimethyl-4-disubstituted-aryl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates 1a–4a, 1b–4b

Methyl (or ethyl) aminocrotonate (0.001 mol, 0.001 mol), 4,4- (or 5,5)-dimethyl-1,3-cyclohexanedione and 0.001 mol aromatic aldehyde was refluxed in 20 mL methanol for 4 h. The resulting precipitate was filtered and crystallized from methanol.

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

Mp.169°C. Yield 68%. IR (cm^{-1}) 3280, 1700. ^{1}H-NMR δ 0.90 (s, 3H, 6-CH_3), 1.10 (s, 3H, 6-CH_3), 1.60–1.70 (m, 4H, H-7,8), 2.45 (s, 3H, 2-CH_3), 3.35 (s, 3H, $COOCH_3$), 5.25 (s, 1H, H-4), 6.95 (d, 1H, *J* = 2.4 Hz, Ar H-6), 7.20 (dd, 1H, *J* = 8.9, 2.4 Hz, Ar H-4), 7.80 (d, 1H, *J* = 8.9 Hz, Ar H-3), 9.00 (s, 1H, NH). MS: (m/z) 406, 405, 404, 403, 248 (100), 57. Analysis for $C_{20}H_{21}ClN_2O_5$: calculated C: 59.34, H: 5.23, N: 6.92; found C: 59.77 , H: 5.38, N: 6.85.

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

Mp. 208°C. Yield 74%. IR (cm^{-1}) 3282, 1701, 1611, 741. ^{1}H-NMR d 0.80 (s, 3H, 7-C*H*$_3$), 0.95 (s, 3H, 7-CH_3), 1.90–2.60 (m, 7H, 2-CH_3, H-6,8), 3.40 (s, 3H, $COOCH_3$), 5.55 (s, 1H, H-4), 6.90 (d, 1H, *J* = 2.3 Hz, Ar H-6), 7.15 (dd, 1H, *J* = 8.8, 2.3 Hz, Ar H-4), 7.75 (d, 1H, *J* = 8.8 Hz, Ar H-3), 9.25 (s, 1H, NH). MS: (m/z) 406, 405, 404, 403, 387, 356, 301, 248 (100), 232, 192, 55. Analysis for $C_{20}H_{21}ClN_2O_5$: calculated C: 59.34, H: 5.23, N: 6.92; found C: 59.24, H: 5.66, N: 6.99.

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

Mp. 223°C. Yield 69%. IR (cm^{-1}) 3250, 1700. ^{1}H-NMR δ 0.85 (s, 3H, 6-CH_3), 0.95 (s, 3H, 6-CH_3), 1.75 (t, 2H, H-7), 2.30 (s, 3H, 2-CH_3), 2.50 (t, 2H, H-8) 3.50 (s, 3H, $COOCH_3$), 4.95 (s, 1H, H-4), 7.45 (dd, 1H, *J* = 2.0, 7.3 Hz, Ar H-6), 7.60 (d, 1H, *J* = 7.3 Hz, Ar H-5), 7.80 (d, 1H, *J* = 2.0 Hz, Ar H-2), 9.30 (s, 1H, NH). MS: (m/z) 407, 406, 405, 404 403, 248 (100), 76, 53. Analysis for $C_{20}H_{21}ClN_2O_5$: calculated C: 59.34, H: 5.23, N: 6.92; found C: 59.17, H: 4.99, N: 6.96.

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

Mp. 211°C. Yield 77%. IR (cm^{-1}) 3240, 1700. ^{1}H-NMR d 0.90 (s, 3H, 7-CH_3), 1.00 (s, 3H, 7-CH_3), 1.90-2.60 (m, 7H, 2-CH_3, H-6,8), 3.50 (s, 3H, $COOCH_3$), 5.00 (s, 1H, H-4), 7.50 (dd, 1H, *J* = 2.1, 7.4 Hz, Ar H-6), 7.65 (d, 1H, *J* = 7.4 Hz, Ar H-5), 7.80 (d, 1H, *J* = 2.1 Hz, Ar H-2), 9.30 (s, 1H, NH). MS: (m/z) 406, 405, 404, 403, 248 (100), 55. Analysis for $C_{20}H_{21}ClN_2O_5$: calculated C: 59.34, H: 5.23, N: 6.92; found C: 59.72, H: 5.49, N: 6.43.

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

Mp. 107°C. Yield 66%. IR (cm^{-1}) 3250, 1705. ^{1}H-NMR δ 0.90 (s, 3H, 6-CH_3), 1.10 (s, 3H, 6-CH_3), 1.20 (t, 3H, CH_2CH_3), 2.00–2.60 (m, 7H, 2-CH_3, H-7,8), 4.00 (q, 2H, CH_2CH_3), 4.90 (s, 1H, H-4), 6.95 (d, 1H, *J* = 2.4 Hz, Ar H-6), 7.20 (dd, 1H, *J* = 8.9, 2.4 Hz, Ar H-4), 7.80 (d, 1H, *J* = 8.9 Hz, Ar H-3), 9.20 (s, 1H, NH). MS (m/z) 420, 419, 418, 417, 262 (100), 152. Analysis for $C_{21}H_{23}ClN_2O_5$: calculated C: 60.22, H: 5.53, N: 6.69; found C: 60.62, H: 5.38, N: 6.38.

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

Mp. 154°C. Yield 81%. IR (cm^{-1}) 3240, 1700. ^{1}H-NMR d 0.80 (s, 3H, 7-CH_3), 0.95 (s, 3H, 7-CH_3), 1.00 (t, 3H, CH_2CH_3), 1.85-2.50 (m, 7H, 2-CH_3, H-6,8), 3.90 (q, 2H, CH_2CH_3), 5.60 (s, 1H, H-4), 6.95 (d, 1H, *J* = 2.4 Hz, Ar H-6), 7.20 (dd, 1H, *J* = 8.9, 2.4 Hz, Ar H-4), 7.80 (d, 1H, *J* = 8.9 Hz, Ar H-3), 9.20 (s, 1H, NH). MS (m/z) 421, 420, 419, 418, 350, 262 (100), 152, 58. Analysis for $C_{21}H_{23}ClN_2O_5$: calculated C: 60.22, H: 5.53, N: 6.69; found C: 59.87, H: 5.20, N: 6.70.

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

Mp. 220°C. Yield 71%. IR (cm^{-1}) 3290, 1695. ^{1}H-NMR d 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, H-7),

2.30 (m, 5H, 2-CH_3, H-8), 4.00 (q, 2H, CH_2CH_3), 4.90 (s, 1H, H-4), 7.50 (dd, 1H, *J* = 2.1, 7.4 Hz, Ar H-6), 7.65 (d, 1H, *J* = 7.4 Hz, Ar H-5), 7.80 (d, 1H, *J* = 2.1 Hz, Ar H-2), 9.25 (s, 1H, NH). MS (m/z) 421, 420, 418,417, 404, 350, 262 (100), 58. Analysis for $C_{21}H_{23}ClN_2O_5$: calculated C: 60.22, H: 5.53, N: 6.69; found C: 59.89, H: 5.49, N: 6.64.

Ethyl 2,7,7-trimethyl-4-(3-nitro-4-chlorophenyl)-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxylate ***4b***

Mp. 141°C. Yield 63%. IR (cm^{-1}) 3300, 1690. ^{1}H-NMR d 1.00 (s, 3H, 7-CH_3), 1.05 (s, 3H, 7-CH_3), 1.15 (t, 3H, CH_2CH_3), 2.25 (s, 3H, 2-CH_3), 1.95–2.65 (m, 4H, H-6,8), 4.05 (q, 2H, CH_2CH_3), 5.85 (s, 1H, H-4), 7.45 (dd, 1H, *J* = 2.0, 7.3 Hz, Ar H-6), 7.60 (d, 1H, *J* = 7.3 Hz, Ar H-5), 9.05 (s, 1H, N*H*). MS (m/z) 419, 418, 417, 262 (100), 57. Analysis for $C_{21}H_{23}ClN_2O_5$: calculated C: 60.22, H: 5.53, N: 6.69; found C: 59.98, H: 5.41, N: 6.89.

General procedure for *N,N*-diethyl-2,6,6 -(and 2,7,7)-trimethyl-4-aryl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamides 1c–4c

The mixture of 0.001 mol of 4,4-dimethyl-1,3-cyclohexanedione (I), 0.001 mol of aromatic aldehyde derivative (II), and 0.001 mol of *N,N*-diethylacetoacetamide (III) with 1 mL ammonia in 25 mL methanol was refluxed for 4 h. The solution was poured into ice water. The precipitate was filtered, dried, and crystallized from ethanol.

N,N-Diethyl-2,6,6-trimethyl-4-(2-nitro-5-chlorophenyl)-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***1c***

Mp. 139°C. Yield 56%. IR (cm^{-1}) 3200, 1690, 1590. ^{1}H-NMR d 0.95 (s, 3H, 6-CH_3), 1.00 (s, 3H, 6-CH_3), 1.05 (t, 6H, NCH_2CH_3), 1,50–2.20 (m, 4H, H-7,8), 2.50 (s, 3H, 2-CH_3), 3.10 (s, 4H, NCH_2CH_3), 4.60 (s, 1H, H-4), 7.00 (d, 1H, *J* = 2.3 Hz, Ar H-6), 7.20 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar H-4), 7.80 (d, 1H, *J* = 8.7 Hz, Ar H-3). MS (m/z) 446, 445, 444, 312, 289 (100), 261, 83, 55. Analysis for $C_{23}H_{28}ClN_3O_4$: calculated C: 61.95, H: 6.33, N: 9.42; found C: 61.56, H: 6.75, N: 9.46.

N,N-Diethyl-2,7,7-trimethyl-4-(2-nitro-5-chlorophenyl)-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***2c***

Mp. 198°C. Yield 58%. IR (cm^{-1}) 3220, 1680, 1600. ^{1}H-NMR δ 0.90 (s, 3H, 7-CH_3), 0.95 (s, 3H, 7-CH_3), 1.05 (t, 6H, NCH_2CH_3), 1.90 (s, 2H, H-8), 2.45 (s, 3H, 2-CH_3), 2.80 (s, 2H, H-6), 3.20 (q, 4H, NCH_2CH_3), 4.60 (s, 1H, H-4), 7.00 (d, 1H, *J* = 2.3 Hz, Ar H-6), 7.20 (dd, 1H, *J* = 8.7, 2.3 Hz, Ar H-4), 7.80 (d, 1H, *J* = 8.7 Hz, Ar H-3). MS (m/z) 446, 445, 397, 289 (100), 264, 246, 97, 69, 41. Analysis for $C_{23}H_{28}ClN_3O_4$: calculated C: 61.95, H: 6.33, N: 9.42; found C: 61.73, H: 6.62, N: 9.75.

N,N-Diethyl-2,6,6-trimethyl-4-(3-nitro-4-chlorophenyl)-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***3c***

Mp. 139°C. Yield 66%. IR (cm^{-1}) 3220, 1680, 1600. ^{1}H-NMR δ 0.90 (t, 6H, NCH_2CH_3), 0.95 (s, 3H, 6-CH_3), 1.00 (s, 3H, 6-CH_3), 1.70 (s, 3H, 2-CH_3), 1,80–2,50 (m, 4H, H-7, 8), 3.10 (q, 4H, NCH_2CH_3), 4.70 (s, 1H, H-4), 7.60 (dd, 1H, *J* = 2.0, 7.3 Hz, Ar H-6), 7.70 (d, 1H, *J* = 7.3 Hz, Ar H-5), 7.80 (d, 1H, *J* = 2.0 Hz, Ar H-2), 8.75 (s, 1H, NH). MS (m/z) 446, 445, 350, 289 (100), 51. Analysis for $C_{23}H_{28}ClN_3O_4$: calculated C: 61.95, H: 6.33, N: 9.42; found C: 61.88, H: 6.55, N: 9.03.

N,N-Diethyl-2,7,7-trimethyl-4-(3-nitro-4-chlorophenyl)-5-oxo-l,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***4c***

Mp. 137°C. Yield 70%. IR (cm^{-1}) 3210, 1690, 1600. ^{1}H-NMR δ 0.90 (t, 6H, NCH_2CH_3), 0.95 (s, 3H, 6-CH_3), 1.00 (s, 3H, 6-CH_3), 1.70 (s, 3H, 2-CH_3), 1,90 (s, 2H, H-8), 2.50 (s, 2H, H-6), 3.10 (q, 4H, NCH_2CH_3), 4.60 (s, 1H, H-4), 7.50 (dd, 1H, *J* = 2.0, 7.3 Hz, Ar H-6), 7.65 (d, 1H, *J* = 7.3 Hz, Ar H-5), 7.75 (d, 1H, *J* = 2.0 Hz, Ar H-2), 8.80 (s, 1H, NH). MS (m/z) 446, 445, 423, 372, 352, 289 (100), 265, 193, 51. Analysis for $C_{23}H_{28}ClN_3O_4$: calculated C: 61.95, H: 6.33, N: 9.42; found C: 61.69, H: 6.12, N: 9.02.

Pharmacology

New Zealand white rabbits, weighing 2.5–3 kg were used in this study. Experiments were performed in agreement with the ethical standards in the Helsinki declaration and this study was approved by Gazi University Ethics Committee for animals. Procedures involving animals and their care were conducted in conformity with international laws and policies. At time of study, rabbits were sacrificed with injection of sodium pentobarbital (30–40 mg/kg, i.v.), followed by removal of the stomach through abdominal incision.

Organ chamber experiments

The fundal part of the stomach was then dissected parallel to the longitudinal muscle wall. One muscle strip approximately 15–20 mm long and 2 mm wide was obtained, and allowed to equilibrate for a period of 60 min in 20 mL organ bath filled with Ca^{2+}-free Krebs'–Henseleit solution (Ca^{2+}-free KHS). The composition of the Ca^{2+}-free KHS used was as follows (in mmol/L): NaCl 118; KCl 4.7; $NaHCO_3$ 25; $MgCl_2$ 0.54; $NaHPO_4$ 0.9; glucose 10.04. The solution was gassed with 95% O_2 and 5% CO_2 during the study and temperature was maintained at 37°C by a thermoregulated water circuit. The pH of the saturated solution was 7.4. Each strip was connected to a force transducer (FDT 10-A, May IOBS 99, COMMAT Iletisim Co., Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA, USA) using software (BSL PRO v 3.6.7, BIOPAC Systems Inc.) which also had the capacity to analyze the data. After mounting, each strip was allowed to equilibrate with a basal tension of 1 g for 60 min. Ca^{2+}-free KHS was replaced with fresh solution every 15 min during this time period.

To determine whether a calcium-antagonistic activity plays a role in the relaxation induced by compounds, nifedipine and dimethylsulfoxide (DMSO), the following procedure was applied. Preparations were placed in a Ca^{2+}-free, high K^+-containing (80 mM) solution. When Ca^{2+} (2.5 mM) was added to the organ bath, a contraction developed. At the plateau level of contraction, compounds (10^{-8} to 3×10^{-4} M), nifedipine (10^{-9} to 3×10^{-6} M), and DMSO (10^{-9} to 3×10^{-4} M) were applied. Concentration-relaxation for compounds, nifedipine and DMSO were obtained by adding them into the bath in a cumulative manner [46].

The following antagonists and inhibitors were used: *N*ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M), indomethacin (COX inhibitor, 10^{-5} M), propranolol (beta-adrenergic receptor blocker, 10^{-6} M).

The relaxant effects of the compounds, nifedipine and DMSO were expressed as percentage of the precontraction using Ca^{2+} (2.5 mM).

Data analysis

The relaxant effects of the compounds on the tissues, precontracted with Ca^{2+} (2.5 mM), were expressed as percentage of the precontraction using Ca^{2+} (2.5 mM). To evaluate the effects of an agonist, the maximum response (E_{max}), the concentration for a half-maximal response (EC_{50}) and pD_2 values were calculated from the concentration-response curve (CRC) obtained in each experiment, as predicted from the Scatchard equation for drug-receptor interaction, where:

$$\text{Response/concentration} = -1/EC_{50} \times \text{response} + \text{maximum response}/EC_{50}$$

The pD_2 values (apparent agonist affinity constants) were expressed as the negative logarithm of the EC_{50}. While E_{max} is the parameter for efficacy, pD_2 is the parameter for potency. All data are expressed as mean ± standard error. Statistical comparison between groups were performed using general linear models by Scheffe's F-test and P values of less than 0.05 were considered to be statistically significant.

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