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Original article

Condensed 1,4-dihydropyridines with various esters and their calcium channel antagonist activities

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ABSTRACT

New alkyl 2,6,6-(2,7,7)-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates and 9-(3-chloro-2-fluoro-5-trifluoromethylphenyl)-6,6(7,7)-dimethyl-6,7-dihydrofuro[3,4-*b*]quinoline-1,8-diones have been synthesised and their calcium antagonistic activities on isolated rabbit sigmoid colon have been investigated and compared with Nifedipine. The investigation examined the influence of ester groups in the 3-position of the HHQ ring and the 2-methoxyethyl analogs were found to be the most active derivatives.

1. Introduction

According to the WHO (World Health Organization) reports, 17.5 million people worldwide died from cardiovascular disease in 2005 in the World. This represents 30% of all global deaths. Of these deaths, 7.6 million were due to heart attacks and 5.7 million were due to stroke. Cardiovascular diseases include disorders of the heart and blood vessels, hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure.

Calcium channel antagonists inhibit muscle contraction by blocking the influx of Ca^{2+} through calcium channels and are used as antianginal and antihypertensive drugs [1–11]. 1,4-Dihydropyridine (DHP) derivatives are the most studied group and Nifedipine is the prototype of calcium channel antagonists. Nifedipine (Fig. 1) was synthesised in 1882 and introduced to therapy 100 years later [12,13].

Today, calcium entry blockers are divided into two structural groups the 1,4-DHPs, represented by Nifedipine, and nondihydropyridines (non-DHP, represented by verapamil and diltiazem) (Fig. 2).

Many active compounds have been synthesised by making modifications on the Nifedipine molecule. One of these modifications is to replace the ester function by the various acyl groups such as amide, nitro, nitrile, ketone. It is well known that, in the unsymmetrical diacyl series, when one of the acyl groups is a methyl or ethyl ester, the activity increases due to lipophilic properties. In addition, in order to fix one carbonyl group in an antiperiplanar position, the 1,4-DHP structure has been annelated. Thus condensed 1,4-DHP derivatives have been obtained and these analogs showed calcium antagonistic activity [14–28].

According to structure–activity relationships of Nifedipine analogs the C-2 and C-6 substituents and the C-3 and C-5 esters must be the same. Although asymmetrical derivatives also exert calcium channel blocker effects, the biological activity depends on the presence of an axial aromatic ring substituent at the 4-position of the 1,4-DHP. The C-3 and C-5 substituents restrict the orientation of the aromatic ring such that its plane bisects the DHP. The DHP ring also adopts a flattened boat conformation. Calcium channels are like a pore having one α_1 primary subunit and α_2-δ, β and γ subunits. 1,4-DHP derivatives have their binding location on the α_1 subunit. These drugs are used as an antihypertensive and antianginal agents by effecting the transportation of Ca^{2+} through the L type calcium channels [10,29].

Abbreviations: WHO, World Health Organization; DHP, dihydropyridine; HHQ, hexahydroquinoline; eNOS, endothelial nitric oxide synthase; COX, cyclooxygenase; L-NAME, *N*ω-nitro-L-arginine methyl ester; TEA, tetraethylammonium; DMSO, dimethylsulfoxide.

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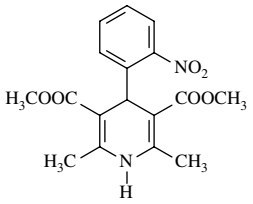


Fig. 1. Nifedipine.

Voltage-gated calcium channels are integral membrane proteins that open via electrical depolarization of the plasma membrane and mediate the entry of calcium ions into the cell. Calcium channels are found in every excitable cell, including neurons, myocytes and pancreatic β 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 intracellular events such as contraction, secretion, synaptic transmission and gene expression [1].

The cytosolic calcium concentration regulates many cellular functions. The influx of extracellular Ca^{2+} is a prerequisite for many cellular functions including cell proliferation and motility. In gastrointestinal smooth muscle, the upstroke of action potential is principally mediated by Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels and is responsible for initiation of contraction. Calcium influx through voltage-gated channels contributes to this rise in cytosolic calcium. Because of their central role in the excitation–contraction coupling for cardiac and smooth muscle, voltage-sensitive calcium channels have become a target for the drug therapy of cardiovascular and gastrointestinal diseases [6,30].

The aim of this work was to synthesise the compounds having a 1,4-DHP ring in a condensed system with different ester moieties in the C-3 position and also including furoquinoline derivatives as a metabolite of this group of compounds and to screen calcium channel blocker activity. In this paper the influence of the different ester groups in the C-3 position of the condensed 1,4-DHP ring was evaluated. Synthesised compounds were tested as a racemate. The cardiovascular activities of the synthesised compounds were assayed on isolated rabbit sigmoid colon by using Nifedipine as standard.

2. Results and discussion

2.1. Chemistry

The hexahydroquinoline (HHQ) derivatives (**4a**–**n**) were prepared by a modification of the Hantzsch synthesis [31]. Thus, 4,4-dimethyl (or 5,5-dimethyl)-1,3-cyclohexanedione was treated with the 2-fluoro-3-chloro-5-trifluoromethyl benzaldehyde to yield the benzylidine derivatives which, when condensed with alkyl acetoacetate, yielded the corresponding HHQ derivatives (Scheme 1).

The furoquinoline derivatives (**5a**,**b**) were obtained by using the procedure described by Şimşek and coworkers [10,23] In this procedure, the appropriate HHQ derivative reacted in the presence of pyridinium bromide perbromide (Scheme 2).

The reaction of the HHQ derivative and pyridinium bromide perbromide results in allylic bromination and forms 2-bromomethylhexahydroquinoline derivatives. This intermediate could not be isolable. Then intermolecular cyclisation of 2-bromomethyl derivatives gives lactone analogs.

The structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, COSY, DEPT, mass spectra and elemental analyses. In the IR spectra, characteristic N–H and C═O stretching bonds were seen. In the ^{1}H NMR spectra, 6,6-dimethyl- or 7,7-dimethyl protons of the HHQ and furoquinoline ring were seen at 0.80–0.90 ppm as separate singlets. The chemical shifts of the aromatic, 2-methyl, methylene, methine, and ester protons of the compounds have expected values. The N–H signals were seen at 9.30–10.00 ppm. The ^{13}C NMR spectra of the compounds displayed the appropriate number of resonances that exactly fitted the number of carbon atoms. The DEPT spectra of one compound are in accordance with its structure. The DEPT spectra of compound **4m**, displayed three methine, two methylene and six methyl peaks. In the COSY spectrum of the compound **4m**, 2,3,5-trisubstituted aryl ring protons resonate at 7.41–7.42 and 7.76–7.77 ppm.

The mass spectra of the compounds were recorded using the electrospray ionisation technique. Molecular ion peaks were seen in the spectra of all compounds. The base peak forms by cleavage of the aryl ring from the parent molecule. In further fragmentation, the ion is formed by the cleavage of the ester group. Aromatisation of the DHP ring to the pyridine analogue was also realised. These findings are in accordance with the literature [22–24]. The elemental analysis results are also consistent with the postulated structures. The X-ray crystal structure analysis[1] of compound **4f** confirmed the structure of this compound (Fig. 3).

In this structure, intermolecular hydrogen bonds between the amine group and the oxo O atom of a neighbouring molecule link the molecules into infinite one-dimensional chains.

2.2. Pharmacology

The maximum relaxant effects (E_{max}) and pD_2 values of the compounds and Nifedipine on isolated strips of rabbit sigmoid colon circular smooth muscle are given in Table 1. Nifedipine and several compounds produced concentration-dependent relaxation in rabbit sigmoid colon circular smooth muscle strips, such that Nifedipine>. **4k** ≥ **4d** ≥ **4h** > **4b** = **4i** ≥ **4a**. Compounds **4c**, **4e**–**g**,**j**,**l**,**n**, **5a**,**b** displayed inhibitory responses, which were not statistically significant from control inhibition produced by DMSO.

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 [32].

The Ca^{2+} channel types described for smooth muscle consist of the L- and T-types, with slow and fast inactivation characteristics, respectively [31]. L-type Ca^{2+} channels are inhibited by Mg^{2+} and by DHPs such as Nifedipine, nimodipine and isradipine [32]. The function of L-type Ca^{2+} channels can be regulated both by guanosine 5′-triphosphate(GTP)-binding proteins and by phosphorylation, the latter often having a negative effect on smooth muscle [32–35].

Given 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 selective and vasodilating properties may arise from an ability to stimulate nitric oxide release from vascular endothelial cells [36]. Such an action would add to the vasodilation produced by directing the blockade of the calcium channels in vascular smooth muscle cells. Endothelial nitric oxide synthase (eNOS) converts substrate L-arginine to nitric oxide, which diffuses to the vascular smooth muscle cells and promotes vascular relaxation [37–39]. The nitric oxide releasing actions of the 1,4-DHPs appear to be a class action, to be exerted at therapeutic

[1] CCDC-677108 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via, www.ccdc.cam.ac.uk/data_request/cif.

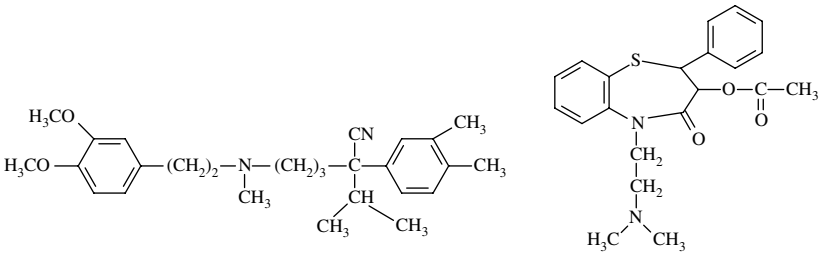


Fig. 2. Verapamil and Diltiazem.

concentrations and to involve the stimulation of calcium influx into endothelial cells through a non-L-type process [40–42].

To investigate whether relaxation induced by the test compounds was due to interaction with the cyclooxygenase (COX), adrenergic system or nitric oxide pathways tissues, were pretreated with indomethacin (COX inhibitor), guanethidine (an adrenergic nerve blocker) or *N*ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor), respectively. Pretreatment of the strips with indomethacin, guanethidine and L-NAME did not significantly alter the relaxant responses to the compounds indicating that cyclooxygenase, adrenergic and nitric oxide (NO) pathways do not play a role in relaxations evoked by these substances.

Compound **5b** did not induce relaxation responses in the presence of tetraethylammonium (TEA) but induced relaxation responses (%49.19 ± 6.83) in the absence of TEA. Similarly, compound **4a**-induced relaxation responses in the absence of TEA (%36.87 ± 4.52) were higher than in the presence of TEA (%17.19 ± 3.15). These results suggest that compounds **5b** and **4a** had a potency for relaxing isolated rabbit sigmoid colon circular smooth muscle, due to the activation of K^+ channels. The absence of TEA did not alter the relaxation responses induced by Nifedipine and the other compounds. These results suggest that the tested compounds, other than **5b** and **4a**, have a potency for relaxing isolated rabbit sigmoid colon circular smooth muscle, a result of the possible blockade of Ca^{2+} channels. To ascertain the ability of these compounds to block Ca^{2+} channels further investigation is needed.

The *in vitro* hepatic microsomal biotransformation of compound **4a** gives its lactone derivatives. This phenomenon was verified by HPLC. The retention times of the HHQ (**4a**) and the furoquinoline derivatives (**5a**) were 10.68 and 16.69 min, respectively. This study gives evidence that HHQ derivatives converted to their less active lactone derivatives during *in vitro* hepatic microsomal biotransformation. These findings are in accordance with the literature.

The relaxant effects of the compounds were expressed as percentage of the precontraction using Ca^{+2}. To evaluate the effects of the compounds, the maximum response (E_m) and pD_2 values [the negative logarithm of the concentration for the half-maximal response (EC_{50})] were calculated, as predicted from the *Scatchard* equation for drug–receptor interaction. Agonist pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. All data are expressed as mean ± standard error. Statistical comparison between groups was performed using general linear models by Scheffe's *F*-test and *P* values of less than 0.05 were considered to be statistically significant.

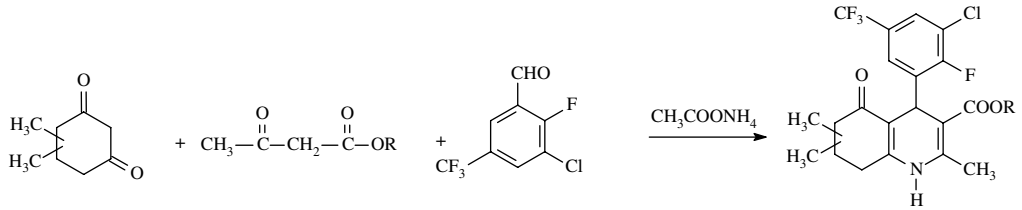
3. Conclusions

In this study, new condensed 1,4-DHP derivatives have been synthesised by the reaction of substituted cyclohexanedione derivatives, various alkyl acetoacetate esters and trisubstituted aromatic aldehydes according to modified *Hantzsch* synthesis. In pharmacological screening tests, the obtained results showed that condensed 1,4-DHP derivatives exerted calcium channel blocking effects. The inclusion of two or three electron-withdrawing substituents on aromatic ring does not change the mentioned activity. The investigation examined the influence of ester groups in the 3-position of the HHQ ring and the 2-methoxyethyl analogs were found to be the most active derivatives. The metabolite of the HHQ derivative (compound **4a**), which has a furoquinoline structure, (compound **5a**) was found less active.

4. Experimental protocol

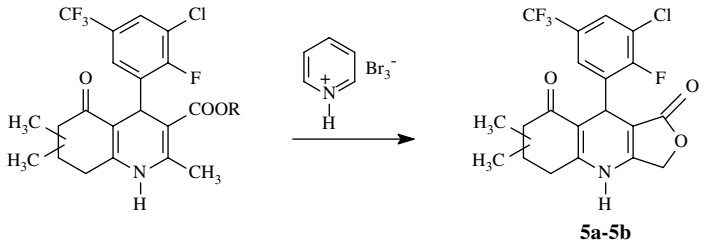
4.1. Chemistry

Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and uncorrected values. Infrared spectra were recorded on a Perkin Elmer FT-IR Systems Spectrum BX. ^{1}H NMR and ^{13}C NMR spectra were obtained for DMSO solutions on a Varian Mercury 400, 400 MHz High Performance Dijital FT-NMR Spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane, spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were obtained on a Waters 2996 Photoiodide array dedector. Microanalysis was obtained on a Leco CHNS-932



R: -CH_3, -C_2H_5, -$CH_2CH{=}CH_2$, -$CH_2CH_2OCH_3$, -$CH_2CH(CH_3)_2$, -$C(CH_3)_3$, -$CH_2C_6H_5$

Scheme 1. Synthesis of hexahydroquinoline derivatives.



Scheme 2. Synthesis of furoquinoline derivatives.

Elemental Analyzer and the results were within ±0.4% of the theoretical values.

*4.1.1. Alkyl 2,6,6-(2,7,7)-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (**4a**–**n**)*

The ester derivatives were prepared by the reaction of an equimolar amount of alkyl acetoacetate, 4,4-(5,5)-dimethyl-1,3-cyclohexanedione, and 2-fluoro-3-chloro-5-trifluoromethyl benzaldehyde with ammonium acetate in methanol and crystallised from methanol–water.

*4.1.1.1. Methyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4a**).* Yield 61%. M.p. 197 °C. IR 3288, 1709, 1652, 860. ^{1}H NMR δ 0.81 (3H; s; 6-CH_3), 0.96 (3H; s; 6-CH_3), 1.67–1.74 (4H; m; H 7-8), 2.27 (3H; s; 2-CH_3), 3.49 (3H; s; OCH_3), 5.08 (1H; s; H-4), 7.40–7.42 (1H; d; Ar-H), 7.80–7.82 (1H; d; Ar H), 9.30 (1H; s; NH). ^{13}C NMR δ 18.6, 24.6, 24.9, 33.5, 39.4, 39.6, 51.0, 101.3, 108.0, 121.0, 125.3, 126.0, 127.7, 147.0, 151.0, 158.7, 167.3, 199.7. Mass (*m*/*z*) 446, 413, 248, 163, 136, 101, 88, 73, 59. Anal. calcd. $C_{21}H_{20}ClF_4NO_3$ (C, H, N).

*4.1.1.2. Ethyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4b**).* Yield 46%. M.p. 210 °C′. IR 3285, 1702, 1651, 861. ^{1}H NMR δ 0.82 (3H; s; 6-CH_3), 0.97 (3H; s; 6-CH_3), 1.02 (3H; t; CH_2CH_3), 1.64-1. (4H; m; H 7–8), 2.27 (3H; s; 2-CH_3), 3.90 (2H; q; CH_2CH_3), 5.06 (1H; s; H-4), 7.30–7.44 (1H; d; Ar-H), 7.79–7.90 (1H; d; Ar H), 9.16 (1H; s; NH). ^{13}C NMR δ 14.1, 23.3, 24.9, 33.6, 39.5, 39.6, 101.2, 107.9, 121.0, 121.2, 122.3, 124.9, 125.9, 126.2, 126.8, 127.6, 147.0, 151.0, 158.7, 166.6, 199.7. Mass (*m*/*z*) 460, 381, 363, 364, 152, 150, 136, 73, 70, 59. Anal. calcd. $C_{22}H_{22}ClF_4NO_3$ (C, H, N).

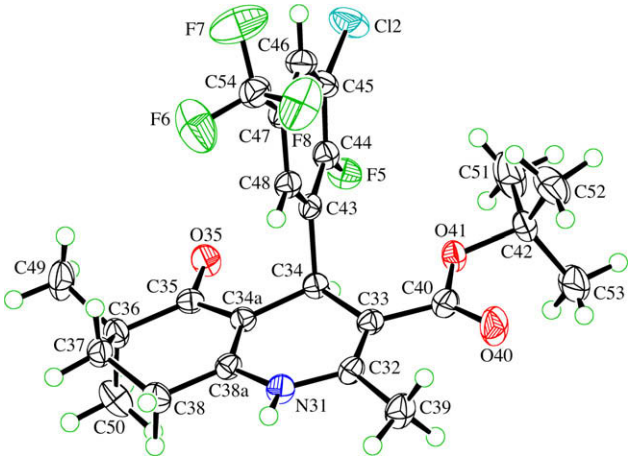


Fig. 3. X-ray diagram of compound **4f**.

*4.1.1.3. Allyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4c**).* Yield 29%. M.p. 194 °C. IR 3213, 1709, 1652, 860. ^{1}H NMR δ 0.80 (3H; s; 6-CH_3), 0.95 (3H; s; 6-CH_3), 1.65–1.72 (4H; m; H 7–8), 2.27 (3H; s; 2-CH_3), 4.40–4.44 (2H; d; OCH_2), 5.03 (1H; s; H-4), 5.10 (2H; d; CH=CH_2), 5.80 (1H; m; CH=CH_2), 7.40–7.42 (1H; d; Ar-H), 7.76–7.78 (1H; d; Ar H), 9.28 (1H; s; NH). ^{13}C NMR δ 18.2, 22.7, 24.0, 24.4, 33.1, 33.8, 63.8, 100.3, 107.4, 117.3, 120.7, 121.6, 124.3, 125.3, 126.4, 132.6, 137.4, 146.9, 150.4, 155.7, 158.2, 165.7 204.0. Mass (*m*/*z*) 472, 235, 229, 158, 141. Anal. calcd. $C_{23}H_{22}ClF_4NO_3$ (C, H, N).

*4.1.1.4. 2-Methoxyethyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethyl phenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4d**).* Yield 23%. M.p. 183 °C. IR 3186, 1711, 1652, 862. ^{1}H NMR δ 0.82 (3H; s; 6-CH_3), 0.97 (3H; s; 6-CH_3), 1.65–1.76 (4H; m; H 7–8), 2.28 (3H; s; 2-CH_3), 3.19 (2H; t; CH_2-OCH_3), 3.42 (3H; s; OCH_3), 4.40–4.44 (2H; t; OCH_2), 5.08 (1H; s; H-4), 7.45–7.59 (1H; d; Ar-H), 7.73–7.80 (1H; d; Ar H), 9.41 (1H; s; NH). ^{13}C NMR δ 18.7, 23.3, 23.5, 24.8, 33.6, 39.3, 40.1, 58.3, 62.7, 70.1, 100.9, 109.0, 121.1, 121.3, 122.2, 125.0, 125.2, 126.9, 127.7, 150.7, 158.9, 166.6, 199.7. Mass (*m*/*z*) 490, 414, 271, 136, 101, 59. Anal. calcd. $C_{23}H_{24}ClF_4NO_4$ (C, H, N).

*4.1.1.5. Isobutyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4e**).* Yield 39%. M.p. 179 °C. IR 3086, 1703, 1652, 860. ^{1}H NMR δ 0.69–0.70 (3H; d; CH-CH_3), 0.77–0.79 (3H; d; CH-CH_3), 0.81 (3H; s; 6-CH_3), 0.96 (3H; s; 6-CH_3), 1.66–1.78 (4H; m; H 7-8), 2.28 (3H; s; 2-CH_3), 2.29 (3H; s; 2-CH_3), 3.35 (2H; d; OCH_2), 3.63–3.77 (1H; m; CH_2-CH), 5.08 (1H; s; H-4), 7.43–7.45 (1H; d; Ar-H), 7.81–7.83 (1H; d; Ar H), 9.32 (1H; s; NH). ^{13}C NMR δ 18.8, 19.2, 23.3, 24.5, 25.0, 27.6, 33.6, 39.3, 39.6, 69.9,

Table 1
Maximum relaxant responses (E_{max}) and pD_2 values of the compounds **4a**–**n**, **5a**,**b** and Nifedipine on isolated strips of rabbit sigmoid colon circular smooth muscle. Relaxation responses are expressed as a percentage of the relaxations evoked by papaverine (10^{-4} M). The negative logarithm of the concentration for the half-maximal response (pD_2) and E_{max} values represents mean value ± S.E.M. (*$p < 0.05$, compared with control responses) ($n = 6$).

Compound	E_{max}	pD_2
4a*	17.19 ± 3.15	4.94 ± 0.21
4b*	22.97 ± 7.59	5.06 ± 0.22
4c	No activity	No activity
4d*	43.67 ± 5.01	4.89 ± 0.14
4e	No activity	No activity
4f	No activity	No activity
4g	No activity	No activity
4h*	32.22 ± 4.90	4.84 ± 0.07
4i*	22.83 ± 4.88	4.74 ± 0.12
4j	No activity	No activity
4k*	44.99 ± 4.07	5.11 ± 0.06
4l	No activity	No activity
4m	No activity	No activity
4n	No activity	No activity
5a	No activity	No activity
5b	No activity	No activity
Nifedipine	94.02 ± 3.58	6.92 ± 0.05

101.0, 107.9, 121.1, 121.3, 122.2, 125.8, 125.9, 126.8, 147.3, 150.9, 158,7 166.7, 199.7. Mass (*m*/*z*) 488, 441, 413, 148, 136, 104, 101, 88, 73, 59. Anal. calcd. $C_{24}H_{26}ClF_4NO_3$ (C, H, N).

*4.1.1.6. Terbutyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4f**).* Yield 23%. M.p. 227 °C. IR 3286, 1702, 1652, 862. ^{1}H NMR δ 0.80 (3 H; s; 6-CH_3), 0.94 (3H; s; 6-CH_3), 1.25 (9H; s; $C(CH_3)_3$), 1.65–1.69 (4H; m; H7-8), 2.21 (3H; s; 2-CH_3), 4.99 (1H; s; H-4), 7.41–7.43 (1H; d; Ar-H), 7.79–7.81 (1H; d; Ar H), 9.15 (1H; s; NH). ^{13}C NMR δ 18.8, 23.5, 24.8, 25.3, 28.3, 28.3, 28.3, 34.5, 34.6, 39.5, 79.8, 102.8, 107.6, 121.3, 121.5, 125.9, 127.3, 138.2, 146.1, 151.3, 156.6, 159.15, 166.4, 199.9. Mass (*m*/*z*) 488, 413, 148, 136, 101, 88, 73, 59. Anal. calcd. $C_{24}H_{26}ClF_4NO_3$ (C, H, N).

*4.1.1.7. Benzyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4g**).* Yield 21%. M.p. 198 °C. IR 3291, 1708, 1653, 860. ^{1}H NMR δ 0.80 (3H; s; 6-CH_3), 0.95 (3H; s; 6-CH_3), 1.63–1.73 (4H; m; H 7-8), 2.30 (3H; s; 2-CH_3), 4.92 (2H; s; OCH_2), 5.11 (1H; s; H-4), 7.14–7.81 (7H; m; Ar-H), 9.31 (1H; s; NH). ^{13}C NMR δ 18.9, 23.3, 24.5, 24.9, 34.3, 39.3, 40.0, 40.2, 40.4, 40.6, 65.4, 100.8, 108.0, 121.1, 121.3, 122.1, 125.7, 126.8, 128.1, 128.6, 136.8, 137.9, 138.1, 147.6, 150.87, 166.5, 199.7. Mass (*m*/*z*) 522, 441, 413, 150, 136, 101, 90. Anal. calcd. $C_{27}H_{24}ClF_4NO_3$ (C, H, N).

*4.1.1.8. Methyl 2,7,7-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4h**).* Yield 39%. M.p. 199 °C. IR 3285, 1709, 1651, 861. ^{1}H NMR δ 0.80 (3H; s; 7-CH_3), 1.01 (3H; s; 7-CH_3), 2.26–2.29 (2H; s; H-6), 2.27 (3H; s; 2-CH_3), 2.45–2.51 (4H; m; H 7-8), 3.49 (3H; s; OCH_3), 3.87–4.00 (2H; s; H-8), 5.10 (1H; s; H-4), 7.36–7.43 (1H; d; Ar-H), 7.80–7.81 (1H; d; Ar H), 9.27 (1H; s; NH). ^{13}C NMR δ 18.7, 26.1, 29.5, 32.5, 32.8, 40.4, 50.3, 51.1 101.8, 108.7, 121.1, 121.3, 122.2, 124.9, 125.4, 126.0, 138.2, 147.0 158.4, 167.1, 194.5. Mass (*m*/*z*) 446, 413, 248, 148, 136, 104, 101, 70, 59. Anal. calcd. $C_{21}H_{20}ClF_4NO_3$ (C, H, N).

*4.1.1.9. Ethyl 2,7,7-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4i**).* Yield 51%. M.p. 209 °C. IR 3297, 1709, 1651, 862. ^{1}H NMR δ 0.81 (3H; s; 7-CH_3), 1.01 (3H; s; 7-CH_3), 1.05–1.08 (3H; t; CH_2CH_3), 2.26–2.29 (2H; s; H-6), 2.30 (3H; s; 2-CH_3), 3.87–4.00 (2H; s; H-8), 3.87–4.00 (2H; q; CH_2-CH_3), 5.10 (1H; s; H-4), 7.43–7.44 (1H; d; Ar-H), 7.79–7.81 (1H; d; Ar H), 9.26 (1H; s; NH). ^{13}C NMR δ 14.1, 18.5, 18.7, 26.2, 29.5, 32.6, 33.0, 49.0, 50.3, 59.6, 101.7, 108.6, 122.2, 124.9, 125.7, 126.8, 127.6, 147.1, 150.9, 158.5, 166.5, 194.5. Mass (*m*/*z*) 460, 441, 413, 262, 136, 104, 101, 73, 59. Anal. calcd. $C_{22}H_{22}ClF_4NO_3$ (C, H, N).

*4.1.1.10. Allyl 2,7,7-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4j**).* Yield 28%. M.p. 172 °C. IR 3282, 1688, 1616, 862. ^{1}H NMR δ 0.80 (3H; s; 7777-CH_3), 1.00 (3H; s; 7-CH_3), 2.19 (2H; s; H-6), 2.48–2.50 (2H; s; H-8), 2.30 (3H; s; 2-CH_3), 4.38–4.49 (2H; d; OC*H*2), 5.04 (1H; s; H-4), 5.12 (2H; d; CH=CH_2), 5.74 (1H; m; C*H*=CH_2), 7.43–7.44 (1H; d; Ar-H), 7.75–7.76 (1H; d; Ar-H), 9.29 (1H; s; NH). ^{13}C NMR δ 18.8, 26.2, 29.5, 32.5, 33.1, 39.8, 40.1, 50.3, 64.4, 101.4, 108.6, 117.6, 121.2, 124.9, 125.8, 126.0, 126.9, 137.8, 147.5, 150.8, 158.6, 166.2, 194. Mass (*m*/*z*) 472, 413, 163, 136, 101, 73, 59. Anal. calcd. $C_{23}H_{22}ClF_4NO_3$ (C, H, N).

*4.1.1.11. 2-Methoxyethyl 2,7,7-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4k**).* Yield 38%. M.p. 92 °C. IR 3287, 1686, 1653, 861. ^{1}H NMR δ 0.81 (3H; s; 7-CH_3), 1.01 (3H; s; 7-CH_3), 0.97 (3H; s; 6-CH_3), 1.92–2.20 (2H; dd; H-6), 2.30 (3H; s; 2-CH_3), 3.18 (2H; t; CH_2-OCH_3), 3.32–3.37 (2H; s; H-8), 3.43 (3H; s; OCH_3), 3.93–4.08 (2H, t, CH_2-CH_2), 5.05 (1H; s; H-4), 7.20–7.75 (1H; d; Ar-H), 7.80–8.20 (1H; d; ArH), 9.40 (1H; S; NH). ^{13}C NMR δ 18.8, 26.2, 29.5, 32.0, 32.6, 49.0, 50.3, 58.3, 62.8, 70.1, 101.4, 108.7, 119.5, 121.3, 122.2, 126.2, 126.9, 127.6, 147.5, 150.8, 158.6, 166.6, 194.5. Mass (*m*/*z*) 490, 441, 414, 413, 136, 101, 73, 59. Anal. calcd. $C_{23}H_{24}ClF_4NO_4$ (C, H, N).

*4.1.1.12. Isobutyl 2,7,7-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4l**).* Yield 43%. M.p. 180 °C. IR 3314, 1708, 1650, 862. ^{1}H NMR δ 0.69 (3H; d; CH-CH_3), 0.79 (3H; d; CH-CH_3), 0.82 (3H; s; 7-CH_3), 1.01 (3H; s; 7-CH_3), 1.76–2.26 (2H; m; H-8), 2.31 (3H; s; 2-CH_3), 2.44–2.52 (2H; m; H-6), 3.36 (2H; s; OCH_2), 3.64–3.79 (1H; m; CH_2-C*H*), 5.11 (1H; s; H-4), 7.44–7.46 (1H; d; Ar-H), 7.81–7.83 (1H; d; Ar-H), 9.32 (1H; s; NH). ^{13}C NMR δ 18.9, 19.1, 19.2, 26.2, 27.5, 29.5, 32.6, 33.0, 40.6, 50.3, 69.9, 101.6, 108.6, 121.2, 121.4, 122.2, 124.9, 125.8, 125.9, 126.8, 147.4, 150.8, 166.7, 194.5. Mass (*m*/*z*) 488, 441, 414, 290, 163, 136, 101, 88, 73, 59. Anal. calcd. $C_{24}H_{26}ClF_4NO_3$ (C, H, N).

*4.1.1.13. Terbutyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4m**).* Yield 51%. M.p. 183 °C. IR 3286, 1702, 1652, 860. ^{1}H NMR 0.77 (3H; s; 7-CH_3), 0.96 (3H; s; 7-CH_3), 1.24 (9H; s; $C(CH_3)_3$), 1.88–2.41 (2H; dd; H-8), 2.22 (3H; s; 2-CH_3), 2.26–2.47 (2H; dd; H-6), 5.02 (1H; s; H-4), 7.41–7.42 (1H; d; Ar-H), 7.76–7.77 (1H; d; Ar-H), 9.16 (1H; s; NH). ^{13}C NMR δ 18.8, 26.5, 28.3, 28.3, 28.3, 29.6, 32.7, 33.8, 40.6, 50.6, 79.8, 103.3, 108.3, 121.5, 122.4, 125.1, 125.6, 126.0, 127.2, 146.2, 151.2, 158.9, 166.3, 194.7. DEPT-135 18.8 (CH_3), 26.5(CH_3), 28.3(CH_3), 28.3(CH_3), 28.3(CH_3), 29.6 (CH_3), 32.7, 33.8 (CH), 40.6 (CH_2), 50.6 (CH_2), 126.0 (CH), 127.2 (CH). Mass (*m*/*z*) 488, 413, 148, 136, 101, 88, 73, 59. Anal. calcd. $C_{24}H_{26}ClF_4NO_3$ (C, H, N).

*4.1.1.14. Benzyl 2,6,6-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**4n**).* Yield 36%. M.p. 161 °C. IR 3291, 1708, 1653, 860. ^{1}H NMR δ 0.74 (3H; s; 7-CH_3), 0.99 (3H; s; 7-CH_3), 1.79–2.26 (4H; m; H 6-8), 2.31 (3H; s; 2-CH_3), 4.92–4.97 (2H, s, OCH_2), 5.13 (1H; s; H-4), 7.09–7.74 (7H; m; Ar-H), 9.31 (1H; s; NH). ^{13}C NMR δ 18.9, 26.2, 29.5, 32.5, 33.0, 40.4, 40.6, 50.3, 65.4, 101.3, 108.7, 121.2, 121.4, 124.8, 125.8, 126.0, 126.8, 128.2, 128.6, 136.7, 137.8, 137.9, 147.8, 150.8, 158.6, 166.5, 194.5. Mass (*m*/*z*) 522, 441, 413, 148, 136, 101, 90, 59. Anal. calcd. $C_{27}H_{24}ClF_4NO_3$ (C, H, N).

*4.1.2. 9-(3-Chloro-2-fluoro-5-trifluoromethylphenyl)-6,6(7,7)-dimethyl-6,7-dihydrofuro [3,4-b]quinoline-1,8-diones (**5a**,**b**)*

The mixture of 0.532 mmol methyl 2,6,6-(2,7,7)-trimethyl-4-(2-fluoro-3-chloro-5-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate and 0.6 mmol pyridinium bromide perbromide in chloroform was stirred in ice-water for 1 h. Then, the mixture was heated under reflux for 4 h. The precipitate was crystallised from methanol.

*4.1.2.1. 9-(3-Chloro-2-fluoro-5-trifluoromethylphenyl)-7,7-dimethyl-6,7-dihydrofuro [3,4-b]quinoline-1,8-dione (**5a**).* Yield 11%. M.p. >300 °C. IR 3194, 3084, 1722, 1671, 861. ^{1}H NMR δ 0.84 (3H; s; 7-CH_3), 0.94 (3H; s; 7-CH_3), 1.72–1.80 (2H; t; H-6), 2.50–2.55 (2H; t; H-5), 4.86 (2H; s; H-3), 4.97 (1H; s; H-9), 7.42–7.44 (1H; d; Ar-H), 7.82–7.84 (1H; d; Ar-H), 10.17 (1H; s; NH). ^{13}C NMR δ 24.4, 24.7, 24.9, 29.9, 34.5, 39.5, 66.1, 100.5, 108.9, 119.6, 119.7, 121.1, 121.3, 122.4, 125.1, 153.7, 157.5, 158.9, 171.7, 200.3. Mass (*m*/*z*) 522, 452, 441, 413, 153, 136, 101, 88, 70, 59. Anal. calcd. $C_{20}H_{16}ClF_4NO_3$ (C, H, N).

*4.1.2.2. 9-(3-Chloro-2-fluoro-5-trifluoromethylphenyl)-6,6-dimethyl-6,7-dihydrofuro [3,4-b]quinoline-1,8-dione (**5b**).* Yield 13%. M.p. >300 °C. IR 3197, 1725 1666 1491, 862. ^{1}H NMR δ 0.93 (3H; s; 6-CH_3), 1.03 (3H; s; 6-CH_3), 2.01–2.24 (2H; t; H-5), 2.38–2.56 (2H; t; H-7),

4.87 (2H; s; H-3), 4.99 (1H; s; H-9), 7.45–7.47 (1H; d; Ar-H), 7.88–7.90 (1H; d; Ar-H), 10.25 (1H; s; NH). ^{13}C NMR δ 26.7, 29.4, 29.6, 32.8, 39.58, 50.6, 66.2, 100.9, 109.7, 121.3, 121.5, 122.3, 125.0, 125.9, 127.8, 136.2, 152.3, 157.8, 171.7, 195.2. Mass (*m*/*z*) 522, 452, 441, 413, 153, 136, 101, 88, 70, 59. Anal. calcd. $C_{20}H_{16}ClF_4NO_3$ (C, H, N).

4.2. Pharmacology

The calcium channel blocker effect of compounds **4a**–**n** and **5a**,**b** was tested on isolated rabbit sigmoid colon. New Zealand white rabbits, weighing 2.5–3 kg were used in this study. At the time of study, rabbits were sacrificed with i.v. injection of sodium pentobarbital (30–40 mg/kg, i.v.), followed by removal of the sigmoid colon through abdominal incision. The fundal part of the sigmoid colon was then dissected parallel to the circular muscle. The strips (*n*: 4–8 from each rabbits) were mounted in tissue baths (20 ml) containing Ca^{2+}-free Krebs–Henseleit solution at 37 °C and gassed with % $95O_2$ and % $5CO_2$. 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 a four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA) 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 eliminate whether relaxation induced by the test compounds was due to an interaction with cyclooxygenase, adrenergic, potassium channel or nitric oxide pathways, all experiments were done in the presence of indomethacin (COX inhibitor, 10^{-5} M), guanethidine (an adrenergic nerve blocker, 10^{-6} M), tetraethylammonium (TEA) (Ca^{2+}-activated potassium channels (KCa) blocker, 10^{-4} M) and *N*ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M).

To determine whether a Ca^{2+} antagonistic activity plays a role in the relaxation induced by the 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} was added to the organ bath in a cumulative manner (2.5, 5, 10 mM), a contraction developed. At the plateau level of contraction, the compounds (10^{-8}–10^{-4} M), Nifedipine (10^{-9}–10^{-4} M) and DMSO (10^{-9}–10^{-4} M) were applied. The concentration–relaxation for the compounds, Nifedipine and DMSO were added into the bath in a cumulative manner [43]. At the end of the experimental procedure the papaverine (10^{-4} M)-induced relaxation response also was obtained.

The relaxant effects of the compound, Nifedipine and DMSO were expressed as a percentage of the relaxations evoked by papaverin (10^{-4} M).

In another set of experiments to test effects of compounds on the potassium channels, the same experimental protocol was applied in the absence of tetraethylammonium (TEA) (Ca^{2+}-activated potassium channels (KCa) blocker).

The pharmacological results are given in Table 1.

4.3. Biotransformation studies

The *in vitro* microsomal metabolism studies of compound **4a** were performed in rat microsomes which were prepared according to the calcium chloride precipitation technique. Then *in vitro* microsomal oxidation was used to convert the HHQ derivative to the furoquinoline analogs. This metabolism was also proven by HPLC data.

The characteristics of the HPLC system are given below:

HPLC pump (Gilson 302, Villiers le Bel, France), injection valve (Rheodyne 7125, Rohnert Park, CA, USA), UV detector (Hewlett Packard HP 1050 Variable Wavelength Dedector, USA), HPLC integrator (C-R8A Chromatopac, Shimadzu, Kyoto, Japan), reverse phase column (Waters, m-Bondapak C18 column, USA). Mobile phase: 50:50 (acetonitrile:water v/v), flow velocity: 1 ml/min, maximum absorption: 254 nm.

4.3.1. Method of microsomal preparations [44,45]

The animals were sacrificed by cervical dislocation and the liver was removed, cut into small pieces and homogenized with sucrose solution (0.25 mol/l) to make a 25% v/v homogenate. The homogenate was first centrifuged at 10,500 rpm at 4 °C for 30 min to remove cell debris, intact cells, nuclei and mitochondria. The supernatant containing the microsomal and soluble cell fractions was carefully decanted and its volume measured. Calcium chloride solution (80 mmol/l) was added to the supernatant (1 ml for 9 ml supernatant) and the mixture was then centrifuged at 15,500 rpm for 15 min. For the washing step, the pellet containing microsomes was resuspended in KCl solution (0.15 mol/l), equal volume with the first supernatant. Finally, the washed microsomes were prepared by centrifuging this homogenate at 15,500 rpm for 15 min. The pellet (washed microsomes) was resuspended in phosphate buffer (0.2 mol/l, pH 7.4) and the final suspension contained 1 g original liver per 1 ml. This was used freshly or stored at −80 °C until required.

The production of NADPH was effected by incubation of cofactors. Substrates and microsomal preparations were incubated in a water bath for 30 min. Metabolic extracts were injected into a reversed HPLC system.

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