Turkish Journal of Chemistry
http://journals.tubitak.gov.tr/chem/
Research Article

Turk J Chem
(2015) 39: 886 – 896

doi:10.3906/kim-1412-72

Microwave-assisted synthesis of condensed 1,4-dihydropyridines as potential calcium channel modulators

Erdem Kamil ÖZER[1], Miyase Gözde GÜNDÜZ[2,*], Ahmed EL-KHOULY[2], Mehmet Yıldırım SARA[3], Rahime ŞİMŞEK[2], Alper Bektaş İSKİT[3], Osman Cihat ŞAFAK[2]
[1]Department of Pharmacology, Faculty of Medicine, Selçuk University, Konya, Turkey
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey
[3]Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Received: 01.01.2015 • **Accepted/Published Online:** 06.05.2015 • **Printed:** 28.08.2015

Abstract: This study reports the design, synthesis, and calcium channel modulatory activity evaluation of a series of 14 novel fused 1,4-dihydropyridine derivatives. The molecular design of the compounds was based on modifications of nifedipine, which is a calcium channel blocker. The compounds were achieved by one-pot microwave-assisted reaction of 4,4-dimethyl-1,3-cyclohexanedione, 5-chlorosalicylaldehyde/3,5-dichlorosalicylaldehyde, an appropriate alkyl acetoacetate, and ammonium acetate in ethanol according to a modified Hantzsch reaction. The structures of the compounds were confirmed by spectral methods and elemental analysis. To evaluate their relaxant activities, the maximum relaxant response (E_{max}) and pD_2 values of the compounds and nifedipine were determined on isolated rat aorta rings. The obtained results indicated that all compounds produced concentration-dependent relaxation on the rings possibly due to the blockade of calcium channels. The E_{max} values (a measure of efficacy) of five compounds were higher than those of nifedipine.

Key words: 1,4-Dihydropyridine, hexahydroquinoline, synthesis, calcium channel

1. Introduction

Calcium is a ubiquitous second messenger that plays a critical role in numerous biological functions including muscle contraction, neurotransmitter release, and neuronal excitability.[1,2] Calcium entry into the cytosol is mediated by multiple types of calcium channel with distinct physiological roles. Among the high-voltage activated channels, L-type calcium channels are typically confined to cell bodies and regulate contractility in muscle cells.[3,4]

Calcium channel blockers are a class of drugs that inhibit selectively the calcium influx through cell membranes. L-type channels are highly sensitive to 1,4-dihydropyridines (DHPs) such as nifedipine, nicardipine, and amlodipine, which represent a well-known class of calcium antagonists. DHPs are clinically used as treatments for cardiovascular diseases, particularly hypertension and angina.[5,6]

The versatility of the 1,4-DHP scaffold, with its wide range of activity, high potency, and easy chemical accessibility, has made 1,4-DHPs one of the most studied class of drugs since their introduction into clinical medicine. Important chemical modifications have been carried out on the structure of nifedipine, the prototype of DHPs (Figure 1), in order to elucidate the structure–activity relationships, enhance calcium modulating

*Correspondence: miyasegunduz@yahoo.com

effects, and lead to new active compounds.[7,8] The nature and position of C-4-aryl ring substituents optimize activity. Although some modifications have been carried out at the 4-position of the 1,4-DHP ring to replace the phenyl ring with different heteroaromatic rings such as xanthone, indole, and benzofuroxan, a substituted phenyl ring is still preferred because of animal toxicity observed with heteroaromatic rings.[9−12] The analysis among 4-phenyl-1,4-DHP analogues revealed that biological activity depends on the hydrophilic, electronic, and steric properties of the substituents on the phenyl ring.[13] Although electron-withdrawing groups at the ortho- or meta-position of the 4-phenyl ring are important for L-type calcium channel blocking activity, 1,4-DHP derivatives carrying a hydroxyl group at 2-position of the phenyl ring have been demonstrated to block both L- and T-type calcium channels.[9,13,14]

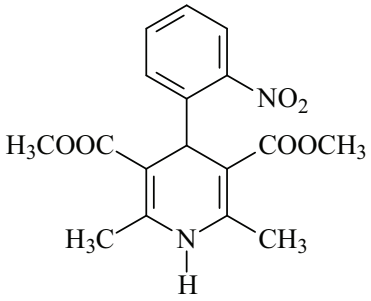


Figure 1. Nifedipine.

Ester functionalities at the C-3 and C-5 position are of utmost importance to modulate activity and tissue selectivity.[15] It has been previously shown that modification of the ester moiety plays the key role in the ability of condensed 1,4-DHPs to block calcium current.[16] It has been also reported that asymmetrical substituents in C-3 and C-5 alter the activity.[15,17] X-ray structural investigations, theoretical calculations, and in vitro analyses of fused 1,4-DHPs (compounds with an immobilized ester group) indicated that at least one ester must be in the cis arrangement to the double bond of DHP to allow for hydrogen bonding to the receptor.[8,18] Among the performed modifications at C-3 and C-5, the introduction of bulky and lipophilic substituents as one of the esterifying groups led to novel, potent calcium antagonists including nicardipine, barnidipine, and benidipine.[19−21]

Fused DHPs like hexahydroquinolines, indenopyridines, and acridines, which could be obtained by introducing the DHP ring into condensed ring systems, were active derivatives exhibiting calcium antagonistic effects.[22−24] It has been previously shown that L-type channel inhibition is sensitive to substitution at the 6-position of the hexahydroquinoline ring.[25]

Microwave (MW) irradiation as an energy source for the activation of chemical reactions has been recently introduced and gained great popularity compared to conventional reactions because of its ability to reduce reaction times, to improve yields, and to simplify the work-up processes.[26,27]

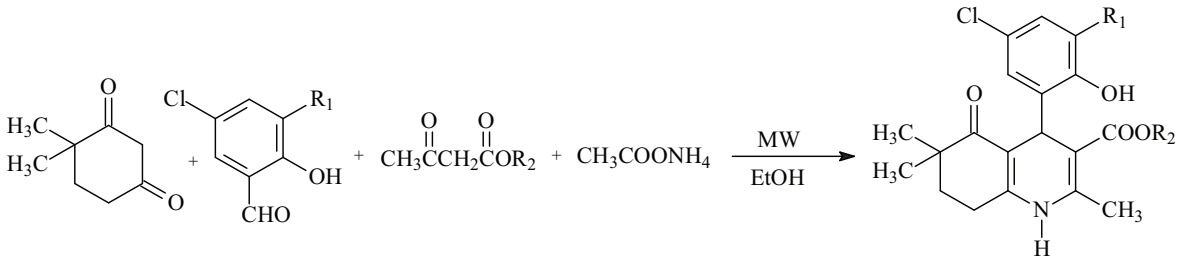
Conventional reactions to obtain 1,4-DHP derivatives were also performed by applying this technique; ethanol was proved to be a much better solvent in terms of yield than the other ones including tetrahydrofuran, acetonitrile, and water.[28−30]

Here, we describe an efficient, rapid, and convenient method with high yields based on MW irradiation for the preparation of 14 novel DHP derivatives in which substituted cyclohexane rings are fused to the DHP ring, and we determine how different ester groups attached to this backbone affect calcium channel block.

2. Results and discussion

2.1. Chemistry

A series of new condensed 1,4-DHP derivatives were obtained via a one-pot modified Hantzsch reaction. In order to prepare the target compounds, 4,4-dimethyl-1,3-cyclohexanedione, 5-chlorosalicylaldehyde/3,5-dichlorosalicylaldehyde, and an appropriate alkyl acetoacetate were heated in the presence of excess ammonium acetate under MW irradiation in ethanol. The synthetic route for the preparation of compounds **1**–**14** is outlined in Figure 2.



R_1: H, Cl

R_2: CH_3, C_2H_5, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $C(CH_3)_3$, $CH_2CH_2OCH_3$, $CH_2C_6H_5$

Figure 2. Synthesis of compounds **1**–**14**.

The Hantzsch reaction is one of the oldest multicomponent reactions, and it proceeds effectively by the dehydrative coupling of an aldehyde, two equivalents of a 1,3-dicarbonyl compound, and ammonia, forming 2,3,5,6-substituted-1,4-DHP.[31] However, long reaction times, unexpected products, or low yields can be obtained, depending on the reaction conditions and the reagents.[32] MW irradiation has recently gained great popularity in conventional reactions as an energy source for the Hantzsch reaction.[33,34]

The heating characteristics of a solvent under MW irradiation conditions are dependent on its dielectric properties. The ability of a solvent to convert electromagnetic energy into heat at a given frequency and temperature is determined by the so-called loss factor tan δ, which is a measure of the amount of MW energy that is lost by dissipation as heat.[35] Ethanol, which is also the most preferred solvent for the synthesis of 1,4-DHPs, with high tan δ value and/or dielectric constant, was classified as an excellent MW-absorbing solvent.[26,27,34]

The appearance of the products was monitored by TLC and the reaction time was determined as 10 min, which is quite short compared to conventional heating.[26]

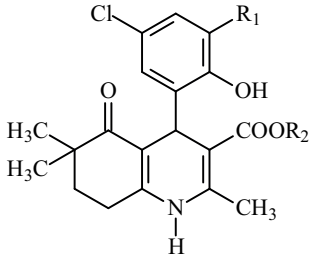
In previous papers, we reported the conventional synthesis of some compounds that have similar structures to compounds **1**–**14** and so it is obvious that this method reduces the solvent use and reaction time.[22,36,37]

The structures and chemical characteristics of the synthesized compounds are given in Table 1.

The structures of the synthesized compounds were elucidated by spectral methods (IR, ^{1}H NMR, and mass spectra) and confirmed by elemental analysis.

In the IR spectra, characteristic N–H, C=O (ester), and C=O (ketone) stretching bonds were observed. In the ^{1}H NMR spectra, the signals of the methyl protons at the 6-position of the hexahydroquinoline ring were observed at 0.88–1.06 ppm separately and as singlets, while the signals of the methylene groups of the same ring were at 1.47–2.70 ppm. The signal of the methine protons of the 1,4-DHP ring was seen as a singlet at 4.36–5.03 ppm. The signals belonging to the aromatic protons of the phenyl ring were observed at 6.69–7.35

Table 1. Structural data of the synthesized compounds.

Compound	R_1	R_2	Melting point (°C)	Empirical formula	Molecular weight
					
1	H	CH_3	230–232	$C_{20}H_{22}ClNO_4$	376
2	H	C_2H_5	248–250	$C_{21}H_{24}ClNO_4$	390
3	H	$CH(CH_3)_2$	245–247	$C_{22}H_{26}ClNO_4$	404
4	H	$CH_2CH(CH_3)_2$	180–182	$C_{23}H_{28}ClNO_4$	418
5	H	$C(CH_3)_3$	228–230	$C_{23}H_{28}ClNO_4$	418
6	H	$CH_2CH_2OCH_3$	218–220	$C_{22}H_{26}ClNO_5$	420
7	H	$CH_2C_6H_5$	196–198	$C_{26}H_{26}ClNO_4$	452
8	Cl	CH_3	280–282	$C_{20}H_{21}Cl_2NO_4$	410
9	Cl	C_2H_5	225–227	$C_{21}H_{23}Cl_2NO_4$	424
10	Cl	$CH(CH_3)_2$	261–263	$C_{22}H_{25}Cl_2NO_4$	438
11	Cl	$CH_2CH(CH_3)_2$	210–212	$C_{23}H_{27}Cl_2NO_4$	452
12	Cl	$C(CH_3)_3$	215–217	$C_{23}H_{27}Cl_2NO_4$	452
13	Cl	$CH_2CH_2OCH_3$	212–214	$C_{22}H_{25}Cl_2NO_5$	454
14	Cl	$CH_2C_6H_5$	220–222	$C_{26}H_{25}Cl_2NO_4$	486

ppm. In the ^{1}H NMR spectra of compounds **1**–**7**, the signals of the protons on the phenyl ring H^3, H^4, and H^6 were observed as a doublet (d), doublet of doublets (dd), and doublet, respectively. After the H atom at 3-position of the aromatic ring was replaced with a Cl atom, the signal of this proton disappeared and the peaks, which belong to H^4 and H^6, were seen as a doublet. The signals of N–H protons of the DHP ring and the O–H protons at the 2-position of the phenyl ring were seen at 8.16–9.94 ppm and 9.67–10.79 ppm as singlets, respectively. The mass spectra of the compounds were recorded via the electron ionization technique. The molecular ion peak (M^+) or the M – 1 peak (due to the aromatization of the DHP ring to the pyridine analogues) was seen in the spectra of all compounds. Cleavage of the ester group and the substituted phenyl ring from the parent molecule was the next most observed fragmentation.

Elemental analysis results were within $\pm 0.4\%$ of the theoretical values for all compounds.

3. Pharmacology

The inhibitory actions of compounds **1**–**14** on calcium channel activity were tested on isolated rat aorta preparations. The maximum relaxant effects (E_{max}) and the negative logarithm of the concentration for the half-maximal inhibitory response values (pD_2) of the compounds and nifedipine on isolated strips of rat aorta smooth muscle are given in Table 2.

Table 2. E_{max} and pD_2 values on precontracted tissues with Ca^{2+} (2.5 mM) and high K^+ of the compounds and nifedipine on rat aorta rings.

Compound	E_{max}	pD_2
1	99.34 ± 0.44	6.48 ± 0.52^b
2	98.24 ± 0.59	6.69 ± 0.35^a
3	96.86 ± 1.08	6.02 ± 0.21^b
4	83.91 ± 3.12^b	5.57 ± 0.23^b
5	95.00 ± 1.86	6.24 ± 0.24^b
6	98.73 ± 0.62	6.04 ± 0.16^b
7	91.45 ± 2.39	6.06 ± 0.13^b
8	89.45 ± 2.78	6.30 ± 0.38^b
9	94.91 ± 1.90	5.96 ± 0.49^b
10	62.57 ± 8.76^b	4.80 ± 0.68^b
11	78.96 ± 4.86^b	5.57 ± 0.21^b
12	88.19 ± 4.17	6.01 ± 0.23^b
13	97.00 ± 1.02	6.24 ± 0.15^b
14	74.28 ± 4.91^b	5.22 ± 0.45^b
Nifedipine	96.08 ± 1.60	7.79 ± 0.07

$^a P < 0.01$, $^b P < 0.001$, compounds **1**–**14** were compared with nifedipine responses (n = 6 for each compound and nifedipine).

The obtained pharmacological results showed that all synthesized compounds are potent relaxing agents on isolated rat aorta smooth muscle due to blockade of calcium channels, similar to that of nifedipine.

The pharmacological analysis of the Ca^{2+} block action of the compounds yielded concentration-dependent responses in the rat aorta rings precontracted with Ca^{2+} (2.5 mM) with the following efficacy order: compound **1** > **6** > **2** > **3** = **13** > nifedipine > **5** = **9** > **7** > **8** > **12** > **4** > **11** > **14** > **10**. E_{max} values (a measure of efficacy) of compounds **1**–**3**, **6**, and **13** were higher than that of nifedipine, while the pD_2 values (a measure of potency) of all compounds were significantly lower than that of nifedipine. E_{max} values of compounds **4**, **10**, **11**, and **14** were significantly less than that of nifedipine, but other compounds were not significantly different from nifedipine.

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.

Given that the main difference between these compounds is their ester groups, this suggests that ester moiety plays a key role in the ability of these compounds to block calcium current. The relaxant effects of the compounds could not be improved by increasing the alkyl chain length of the ester or introducing a ring structure at this locus. The introduction of the second chlorine atom on the phenyl ring did not mediate a significant change in blocking activity.

Lipinski's "rule of five" was also calculated in an attempt to predict the drug likeness of the compounds found to be more active than nifedipine (compounds **1**–**3**, **6**, **13**). The numbers of hydrogen bond acceptors and donors were calculated in LigandScout[38] and cLog p values were calculated by Molinspiration Property Calculation Service (www.molinspiration.com/cgi-bin/properties). All of them adhered to this rule (cLog < 5, MW < 500, number of hydrogen bond donors (HBD) < 5, and number of hydrogen bond acceptors (HBA) < 10) and the results are reported in Table 3.

Table 3. Lipinski parameters of the compounds that were found more active than nifedipine.

Compound	Number of HBA	Number of HBD	cLog p	Molecular mass (Da)
1	3	2	4.24	375.85
2	3	2	4.62	389.87
3	3	2	4.98	403.90
6	4	2	4.04	419.90
13	4	2	4.44	454.35

4. Experimental

4.1. General

All chemicals used in this study were purchased from Aldrich and Fluka (Steinheim, Germany). The reactions were carried out using a Discover Microwave Apparatus (CEM). Thin layer chromatography (TLC) was run on Merck aluminum sheets, Silica gel 60 F254 (Darmstadt, Germany), mobile phase ethyl acetate–hexane (1:1), and ultraviolet (UV) absorbing spots were detected by short-wavelength (254 nm) UV light (Camag UV Cabinet, Wiesloch, Germany). Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and were uncorrected. Infrared spectra (IR) were recorded on a PerkinElmer FT-IR Spectrum BX (Beaconsfield, UK). ^{1}H NMR spectra were obtained in dimethyl sulfoxide (DMSO) solutions on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR Spectrometer (Palo Alto, CA, USA). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). Mass spectra were obtained on an Agilent 5973 Network Mass Selective Detector by electron ionization (Philadelphia, PA, USA). Elemental analyses were performed on a Leco CHNS-932 Elemental Analyzer (Philadelphia, PA, USA).

4.2. Synthesis

The general procedure for the preparation of alkyl 4-(2-hydroxy-5-chlorophenyl/2-hydroxy-3,5-dichlorophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (compounds **1**–**14**) was as follows: a one-pot four-component mixture of 2 mmol 4,4-dimethyl-1,3-cyclohexanedione, 2 mmol 5-chlorosalicylaldehyde or 3,5-dichlorosalicylaldehyde, 2 mmol appropriate alkyl acetoacetate, and 10 mmol ammonium acetate was placed into a 35-mL MW pressure vial and heated under MW irradiation (power 50 W, maximum temperature 120 °C) for 10 min in 5 mL of ethanol. After the reaction was completed, monitored by TLC, the reaction mixture was poured into ice-water; the obtained precipitate was filtered and crystallized from ethanol–water.

4.2.1. Methyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 1):

Yield: 83%. mp 230–232 °C. IR (ν, cm^{-1}): 3310 (N–H), 1720 (C=O, ester), 1635 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.93 (3H; s; 6-CH_3), 1.02 (3H; s; 6-CH_3), 1.57–1.79 (2H; m; H-7), 2.33 (3H; s; 2-CH_3), 2.49–2.54 (2H; m; H-8), 3.33 (3H; s; $COOCH_3$), 4.87 (1H; s; 4-H), 6.69 (1H; d; J 8,4 Hz; Ar-H^3), 6.80 (1H; d; J = 2.4 Hz; Ar-H^6), 6.98 (1H; dd; J = 2.4, 8.4 Hz; Ar-H^4), 9.41 (1H; s; NH), 9.67 (1H; s; OH). MS (m/z): 375 [M]$^+$. Anal. Calcd. for $C_{20}H_{22}ClNO_4$: C, 63.91; H, 5.90; N, 3.73. Found: C, 63.85; H, 5.94; N, 3.75.

4.2.2. Ethyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 2):

Yield: 87%. mp 248–250 °C. IR (ν, cm^{-1}): 3290 (N–H), 1695 (C=O, ester), 1642 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.94 (3H; s; 6-CH_3), 1.02 (3H; s; 6-CH_3), 1.03 (3H; t; J = 7.6 Hz; $COOCH_2C\underline{H}_3$), 1.54–1.80 (2H; m; H-7), 2.33 (3H; s; 2-CH_3), 2.49–2.55 (2H; m; H-8), 3.88 (1H; dq; $COOC\underline{H}_{2A}$-CH_3), 3.93 (1H; dq; $COOC\underline{H}_{2B}$-CH_3), 4.87 (1H; s; 4-H), 6.70 (1H; d; J = 8.4 Hz; Ar-H^3), 6.79 (1H; d; J = 2.8 Hz; Ar-H^6), 6.98 (1H; dd; J = 2.8, 8.4 Hz; Ar-H^4), 9.39 (1H; s; NH), 9.70 (1H; s; OH). MS (m/z): 388 $[M-1]^+$. Anal. Calcd. for $C_{21}H_{24}ClNO_4$: C, 64.69; H, 6.20; N, 3.59. Found: C, 64.60; H, 6.23; N, 3.55.

4.2.3. Isopropyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 3):

Yield: 82%. mp 245–247 °C. IR (ν, cm^{-1}): 3301 (N–H), 1695 (C=O, ester), 1637 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.90 (3H; s; 6-CH_3), 0.92 (3H; s; 6-CH_3), 1.08 (3H; d; J = 6.4 Hz; $COOCHC\underline{H}_3$), 1.17 (3H; d; J = 6.4 Hz; $COOCHC\underline{H}_3$), 1.55–1.70 (2H; m; H-7), 2.21–2.36 (2H; m; H-8), 2.34 (3H; s; 2-CH_3), 3.03 (1H; s; OH), 4.36 (1H; s; 4-H), 4.79–4.85 (1H; m; $COOC\underline{H}(CH_3)_2$), 6.76 (1H; d; J = 8.8 Hz; Ar-H^3), 7.03 (1H; dd; J = 2.8, 8.8 Hz; Ar-H^4), 7.10 (1H; d; J = 2.8 Hz; Ar-H^6), 8.16 (1H; s; NH). MS (m/z): 403 $[M]^+$. Anal. Calcd. for $C_{22}H_{26}ClNO_4$: C, 65.42; H, 6.49; N, 3.47. Found: C, 65.47; H, 6.45; N, 3.50.

4.2.4. Isobutyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 4):

Yield: 85%. mp 180–182 °C. IR (ν, cm^{-1}): 3295 (N–H), 1697 (C=O, ester), 1646 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.71 (3H; d; J = 2.0 Hz;$COOCH_2CHC\underline{H}_3$), 0.72 (3H; d; J = 2.0 Hz; $COOCH_2CHC\underline{H}_3$), 0.95 (3H; s; 6-$CH_3$), 1.03 (3H; s; 6-$CH_3$), 1.46–1.53 (1H; m; $C\underline{H}(CH_3)_2$), 1.56–1.73 (2H; m; H-7), 2.38 (3H; s; 2-CH_3), 2.47–2.58 (2H; m; H-8), 3.60 (1H; dd; J = 10.8/6.0 Hz; $C\underline{H}_{2A}CH(CH_3)_2$), 3.75 (1H; dd; J = 10.8/6.0 Hz; $C\underline{H}_{2B}CH(CH_3)_2$), 4.87 (1H; s; 4-H), 6.70 (1H; d; J = 8.4 Hz; Ar-H^3), 6.78 (1H; d; J = 2.4 Hz; Ar-H^6), 6.98 (1H; dd; J = 8.4, 2.4 Hz; Ar-H^4), 9.49 (1H; s; NH), 9.84 (1H; s; OH). MS (m/z): 416 $[M-1]^+$. Anal. Calcd. for $C_{23}H_{28}ClNO_4$: C, 66.10; H, 6.75; N, 3.35. Found: C, 66.17; H, 6.77; N, 3.31.

4.2.5. Tert-butyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 5):

Yield: 78%. mp 228–230 °C. IR (ν, cm^{-1}): 3245 (N–H), 1702 (C=O, ester), 1655 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.86 (3H; s; 6-CH_3), 1.01 (3H; s; 6-CH_3), 1.21 (9H; s; $COOC(C\underline{H}_3)_3$), 1.52–1.74 (2H; m; H-7), 2.22–2.43 (2H; m; H-8), 2.35 (3H; s; 2-CH_3), 2.83 (1H; s; OH), 4.34 (1H; s; 4-H), 6.93 (1H; d; J = 9.2 Hz; Ar-H^3), 7.91 (1H; dd; J = 2.4, 9.2 Hz; Ar-H^4), 7.98 (1H; d; J = 2.4 Hz; Ar-H^6), 8.31 (1H; s; NH). MS (m/z): 417 $[M]^+$. Anal. Calcd. for $C_{23}H_{28}ClNO_4$: C, 66.10; H, 6.75; N, 3.35. Found: C, 66.03; H, 6.79; N, 3.33.

4.2.6. 2-Methoxyethyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8 hexahydroquinoline-3-carboxylate (Compound 6):

Yield: 78%. mp 218–220 °C. IR (ν, cm^{-1}): 3299 (N–H), 1696 (C=O, ester), 1665 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.94 (3H; s; 6-CH_3), 1.02 (3H; s; 6-CH_3), 1.55–1.72 (2H; m; H-7), 2.33 (3H; s; 2-CH_3), 2.49–2.55 (2H; m; H-8), 3.33 (3H; s; OCH3), 3.30–3.38 (2H; m; C$\underline{H}_2$OCH$_3$), 4.30 (1H; ddd; C$\underline{H}_{2A}$CH$_2$OCH$_3$), 4.34 (1H; ddd; C$\underline{H}_{2B}$CH$_2$OCH$_3$), 4.89 (1H; s; 4-H), 6.72 (1H; d; J = 8.0 Hz; Ar-H^3), 6.97 (1H; d; J = 2.4 Hz; Ar-H^6), 7.05 (1H; dd; J = 2.4, 8.0 Hz; Ar-H^4), 9.43 (1H; s; NH), 9.73 (1H; s; OH). MS (m/z): 419 [M]$^+$. Anal. Calcd. for $C_{22}H_{26}ClNO_5$: C, 62.93; H, 6.24; N, 3.34. Found: C, 62.97; H, 6.25; N, 3.38.

4.2.7. Benzyl 4-(5-chloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 7):

Yield: 80%. mp 196–198 °C. IR (ν, cm^{-1}): 3324 (N–H), 1710 (C=O, ester), 1659 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.94 (3H; s; 6-CH_3), 1.02 (3H; s; 6-CH_3), 1.52–1.81 (2H; m; H-7), 2.35 (3H; s; 2-CH_3), 2.49–2.5 (2H; m; H-8), 5.02, 5.07 (2H; AB system; J_{AB} = 9.2 Hz, COOC$\underline{H}_2$C$_6$H$_5$), 5.03 (1H; s; 4-H), 6.71–7.35 (8H; m; Ar-H) 9.49 (1H; s; NH), 9.82 (1H; s; OH). MS (m/z): 451 [M]$^+$. Anal. Calcd. for $C_{26}H_{26}ClNO_4$: C, 69.10; H, 5.80; N, 3.10. Found: C, 69.03; H, 5.77; N, 3.07.

4.2.8. Methyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 8):

Yield: 80%. mp 280–282 °C. IR (ν, cm^{-1}): 3276 (N–H), 1697 (C=O, ester), 1634 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.99 (3H; s; 6-CH_3), 1.05 (3H; s; 6-CH_3), 1.58–1.75 (2H; m; H-7), 2.40 (3H; s; 2-CH_3), 2.48–2.56 (2H; m; H-8), 3.49 (3H; s; COOCH_3), 4.89 (1H; s; 4-H), 6.71 (1H; d; J = 2.4 Hz; Ar-H^6), 7.29 (1H; d; J = 2.4 Hz; Ar-H^4), 9.70 (1H; s; NH), 10.62 (1H; s; OH). MS (m/z): 409 [M]$^+$. Anal. Calcd. for $C_{20}H_{21}Cl_2NO_4$: C, 58.55; H, 5.16; N, 3.41. Found: C, 58.49; H, 5.18; N, 3.40.

4.2.9. Ethyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 9):

Yield: 76%. mp 225–227 °C. IR (ν, cm^{-1}): 3294 (N–H), 1720 (C=O, ester), 1640 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.94 (3H; s; 6-CH_3), 0.96 (3H; t; J = 7.2 Hz; COOCH$_2$C$\underline{H}_3$), 1.02 (3H; s; 6-CH_3), 1.59–1.73 (2H; m; H-7), 2.36 (3H; s; 2-CH_3), 2.49–2.56 (2H; m; H-8), 3.84 (1H; dq; COOC$\underline{H}_{2A}$-CH_3), 3.91 (1H; dq; COOC$\underline{H}_{2B}$-CH_3), 4.85 (1H; s; 4-H), 6.70 (1H; d; J = 2.8 Hz; Ar-H^6), 6.79 (1H; d; J = 2.8 Hz; Ar-H^4), 9.61 (1H; s; NH), 10.53 (1H; s; OH). MS (m/z): 423 [M]$^+$. Anal. Calcd. for $C_{21}H_{23}Cl_2NO_4$: C, 59.44; H, 5.46; N, 3.30. Found: C, 59.40; H, 5.50; N, 3.33.

4.2.10. Isopropyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 10):

Yield: 77%. mp 261–263 °C. IR (ν, cm^{-1}): 3283 (N–H), 1702 (C=O, ester), 1665 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.79 (3H; d; J = 6 Hz; COOCHC$\underline{H}_3$), 0.95 (3H; s; 6-CH_3), 1.01 (3H; s; 6-CH_3), 1.09 (3H; d; J = 6 Hz; COOCHC$\underline{H}_3$), 1.60–1.79 (2H; m; H-7), 2.38 (3H; s; 2-CH_3), 2.59–2.69 (2H; m; H-8), 4.71–4.80 (1H;

m; COOC$\underline{H}(CH_3)_2$), 4.78 (1H; s; 4-H), 6.69 (1H; d; J = 2.4 Hz; Ar-H^4), 7.25 (1H; d; J = 2.4 Hz; Ar-H^6), 9.94 (1H; s; NH), 10.79 (1H; s; OH). MS (m/z): 437 [M]$^+$. Anal. Calcd. for $C_{22}H_{25}Cl_2NO_4$: C, 60.28; H, 5.75; N, 3.20. Found: C, 60.34; H, 5.70; N, 3.22.

4.2.11. Isobutyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 11):

Yield: 83%. mp 212–214 °C. IR (ν, cm^{-1}): 3288 (N–H), 1696 (C=O, ester), 1643 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.66 (3H; d; J = 7.2 Hz; $COOCH_2CHC\underline{H}_3$), 0.69 (3H; d; J= 7.2 Hz; $COOCH_2CHC\underline{H}_3$), 0.99 (3H; s; 6-$CH_3$), 1.06 (3H; s; 6-$CH_3$), 1.47–1.54 (1H; m; C$\underline{H}(CH_3)_2$), 1.62–1.71 (2H; m; H-7), 2.43 (3H; s; 2-CH_3), 2.50–2.60 (2H; m; H-8), 3.59 (1H; dd; J = 10.4, 6.4 Hz; C$\underline{H}_{2A}CH(CH_3)_2$), 3.77 (1H; dd; J = 10.4, 6.4 Hz; C$\underline{H}_{2B}CH(CH_3)_2$), 4.88 (1H; s; 4-H), 6.73 (1H; d; J = 2.4 Hz; Ar-H^6), 7.28 (1H; d; 2.4 Hz; Ar-H^3), 9.71 (1H; s; NH), 10.75 (1H; s; OH). MS (m/z): 451 [M]$^+$. Anal. Calcd. for $C_{23}H_{27}Cl_2NO_4$: C, 61.07; H, 6.02; N, 3.10. Found: C, 60.59; H, 6.05; N, 3.14.

4.2.12. Tert-butyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 12):

Yield: 85%. mp 215–217 °C. IR (ν, cm^{-1}): 3301 (N–H), 1703 (C=O, ester), 1663 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.95 (3H; s; 6-CH_3), 1.01 (3H; s; 6-CH_3), 1.23 (9H; s; $COOC(C\underline{H}_3)_3$), 1.75–1.83 (2H; m; H-7), 2.54–2.70 (2H; m; H-8), 2.36 (3H; s; 2-CH_3), 4.78 (1H; s; 4-H), 6.71 (1H; d; J = 2.4 Hz; Ar-H^4), 7.08 (1H; d; J = 2.4 Hz; Ar-H^6), 9.93 (1H; s; NH), 10.78 (1H; s; OH). MS (m/z): 451 [M]$^+$. Anal. Calcd. for $C_{23}H_{27}Cl_2NO_4$: C, 61.07; H, 6.02; N, 3.10. Found: C, 61.10; H, 5.99; N, 3.12.

4.2.13. 2-Methoxyethyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 13):

Yield: 70%. mp 212–214 °C. IR (ν, cm^{-1}): 3296 (N–H), 1692 (C=O, ester), 1647 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.98 (3H; s; 6-CH_3), 1.06 (3H; s; 6-CH_3), 1.62–1.81 (2H; m; H-7), 2.40 (3H; s; 2-CH_3), 2.46–2.56 (2H; m; H-8), 3.19 (3H; s; OCH3), 3.25–3.37 (2H; m; C$\underline{H}_2OCH_3$), 3.96 (1H; ddd; C$\underline{H}_{2A}CH_2OCH_3$), 4.05 (1H; ddd; C$\underline{H}_{2B}CH_2OCH_3$), 4.89 (1H; s; 4-H), 6.72 (1H; d; J = 2.4 Hz; Ar-H^4), 7.28 (1H; d; J = 2.4 Hz; Ar-H^6), 9.67 (1H; s; NH), 10.55 (1H; s; OH). MS (m/z): 453 [M]$^+$. Anal. Calcd. for $C_{22}H_{25}Cl_2NO_5$: C, 58.16; H, 5.55; N, 3.08. Found: C, 58.10; H, 5.50; N, 3.10.

4.2.14. Benzyl 4-(3,5-dichloro-2-hydroxyphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 14):

Yield: 88%. mp 220–222 °C. IR (ν, cm^{-1}): 3308 (N–H), 1698 (C=O, ester), 1650 (C=O, ketone). ^{1}H NMR (δ, DMSO-d_6): 0.95 (3H; s; 6-CH_3), 1.06 (3H; s; 6-CH_3), 1.56–1.75 (2H; m; H-7), 2.42 (3H; s; 2-CH_3), 2.47–2.58 (2H; m; H-8), 4.89, 5.14 (1H; AB system; J_{AB} = 13.2 Hz, $COOC\underline{H}_2C_6H_5$), 4.95 (1H; s; 4-H), 6.72–7.32 (7H; m; Ar-H) 9.74 (1H; s; NH), 10.75 (1H; s; OH). MS (m/z): 484 [M – 1]$^+$. Anal. Calcd. for $C_{26}H_{25}Cl_2NO_4$: C, 64.20; H, 5.18; N, 2.88. Found: C, 64.24; H, 5.20; N, 2.90.

4.3. Pharmacological studies

The inhibitory actions of compounds **1**–**14** on calcium channel activity were tested on isolated rat aorta preparations. Male Wistar rats weighing 200–250 g were used. Following the diethyl ether anesthesia, the animals were sacrificed by exsanguination and their thoraces were opened and the thoracic part of the aorta was gently removed. The isolated aorta was cleaned of fat and connective tissues and then 3–5 mm wide rings were obtained. All these preparation procedures were conducted in Krebs–Henseleit solution gassed with carbogen (95% O_2/5% CO_2). The aorta rings were mounted in isolated organ baths containing 50 mL of Ca^{2+}-free Krebs–Henseleit solution (mmol: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.5) and kept at 37 °C and gassed with carbogen. A resting tension of ∼1 g was applied and the muscle contractions were recorded using a force-displacement transducer and digitized data acquisition system (PowerLab/8sp, Adinstruments, Australia). All aorta preparations were allowed to equilibrate in the Ca^{2+}-free Krebs–Henseleit solution for about 45 min with washing out of the tissues every ∼15 min and subsequently high K^+ (80 mM) Krebs–Henseleit solution without Ca^{2+} was applied. The rings were then contracted with 2.5 mM Ca^{2+}. Following the maximal contractile response with Ca^{2+}, data required for the concentration–response curves were obtained by cumulative administration of the drugs under investigation. In order to achieve maximal relaxation at the end of cumulative drug administrations, all rings were treated with 10^{-4} M papaverine. For each drug, 6 trials were conducted, the obtained data were fit into a curve, and EC_{50} values were calculated using GraphPad Prism 5 software (GraphPad, UK). The potencies of the compounds were compared to that of nifedipine. To exclude relaxations that can be induced by mechanisms other than the calcium channels, the cyclooxygenase (COX), adrenergic, and nitregic systems were all blocked by indomethacin (COX inhibitor, 10^{-5} M), guanethidine (an adrenergic nerve blocker, 10^{-6} M), and L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride, the nitric oxide synthase inhibitor, 10^{-4} M), respectively. All test compounds and nifedipine were dissolved in DMSO. The final concentration of DMSO was 0.1% and was found to have no effect on aorta activity.

The data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using GraphPad Prism 5. The differences were considered to be significant when $P < 0.05$.

Acknowledgments

Alper B. İskit has been supported by the Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (EA-TÜBA-GEBİP/2001-2-11).

References

1. Dolphin, A. C. *Brit. J. Pharmacol.* **2006**, *147*, S56–S62.
2. Camerino, D. C.; Desaphy, J. F.; Tricarico, D.; Pierno, S.; Liantonio, A. *Adv. Genet.* **2008**, *64*, 81–145.
3. Zamponi, G. W. *Drug Develop. Res.* **1997**, *42*, 131–143.
4. Carafoli, E. *Biochem. Bioph. Res. Co.* **2004**, *322*, 1097.
5. Triggle, D. J. *Cell. Mol. Neurobiol.* **2003**, *23*, 293–303.
6. Safak, C.; Simsek, R. *Mini-Rev. Med. Chem.* **2006**, *6*, 747–755.
7. Gordeev, M. F.; Patel, D. V.; England, B. P.; Jonnalagadda, S.; Combs, J. D.; Gordon, E. M. *Bioorgan. Med Chem.* **1998**, *6*, 883–889.
8. Edraki, N.; Mehdipour, A. R.; Khoshneviszadeh, M.; Miri, R. *Drug Discov. Today* **2009**, *14*, 1058–1066.

9. Goldmann, S.; Stoltefuss, J. *Angew. Chem. Int. Edit.* **1991**, *30*, 1559–1578.
10. El-Khouly, A.; Gunduz, M. G.; Cengelli, C.; Simsek, R.; Erol, K.; Safak, C.; Ozturk Yildirim, S.; Butcher, R. J. *Drug Research* **2013**, *63*, 579–585.
11. Bisi, A.; Budriesi, R.; Rampa, A.; Fabbri, G.; Chiarini, A.; Valenti, P. *Arzneimittel-Forsch.* **1996**, *46*, 848–851.
12. Ermondi, G.; Visentin, S.; Boschi, D.; Fruttero, R.; Gasco, A. *J. Mol. Struct.* **2000**, *2*, 149–162.
13. Coburn, R. A.; Wierzba, M.; Suto, M. J.; Solo, A. J.; Triggle, A. M.; Triggle, D. J. *J. Med. Chem.* **1988**, *31*, 2103–2107.
14. Bladen, C.; Gadotti, V. M.; Gündüz, M. G.; Berger, N. D.; Şimşek, R.; Şafak, C.; Zamponi, G. W. *Pflug. Arch. Eur. J. Phy.* **2015**, *467*, 1237–1247.
15. Miri, R.; Javidnia, K.; Sarkarzadeh, H.; Hemmateenejad, B. *Bioorgan. Med. Chem.* **2006**, *14*, 4842–4849.
16. Bladen, C.; Gadotti, V. M.; Gündüz, M. G.; Berger, N. D.; Şimşek, R.; Şafak, C.; Zamponi, G. W. *Pflug. Arch. Eur. J. Phy.* **2014**, *466*, 1355–1363.
17. Ioan, P.; Carosati, E.; Micucci, M.; Cruciani, G.; Broccatelli. F.; Zhorov, B. S.; Chiarini, A.; Budriesi, R. *Curr. Med. Chem.* **2011**, *18*, 4901–4922.
18. Linden, A.; Simsek, R.; Gunduz, M.; Safak, C. *Acta Crystallogr. C* **2005**, *61*, O731–O734.
19. Leonardi, A.; Motta, G.; Pennini, R.; Testa, R.; Sironi, G.; Catto, A.; Cerri, A.; Zappa, M.; Bianchi, G.; Nardi, D. *Eur. J. Med. Chem.* **1998**, *33*, 399–420.
20. Tamazawa, K.; Arima, H.; Kojima, T.; Isomura, Y.; Okada M, Fujita S, Furuya, T.; Takenaka, T.; Inagaki, O., Terai, M. *J. Med. Chem.* **1986**, *29*, 2504–2511.
21. Gkogkos, K.; Pavlidis, G.; Karakozoglou, A.; Kopras, A.; Memi, E.; Tsoutsouli, V. *J. Hypertens.* **2006**, *24*, S32.
22. Safak, C.; Gunduz, M. G.; Ilhan, S. O.; Simsek, R.; Isli, F, Yildirim S, Öztürk Fincan; G. S.; Sarıoğlu, Y.; Linden, A. *Drug Develop. Res.* **2012**, *73*, 332–342.
23. Rose, U. *J. Heterocyclic Chem.* **1990**, *27*, 237–242.
24. Tu, S. J.; Miao, C. B.; Fang, F.; Feng, Y. J.; Li, T. J.; Zhuang, Q. Y.; Zhang, X., Zhu, S.; Shi, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1533–1536.
25. Lipkind, G. M.; Fozzard, H. A. *Mol. Pharmacol.* **2003**, *63*, 499–511.
26. Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225–9283.
27. Kappe, C. O. *Angew. Chem. Int. Edit.* **2004**, *43*, 6250–6284.
28. Ladani, N. K.; Mungra, D. C.; Patel, M. P.; Patel, R. G. *Chinese Chem. Lett.* **2011**, *22*, 1407–1410.
29. Bagley, M. C.; Fusillo, V.; Jenkins, R. L.; Lubinu, M. C.; Mason, C. *Beilstein J. Org. Chem.* **2013**, *9*, 1957–1968.
30. Mithlesh, S.; Pareek, P. K.; Kant, R.; Ojha, K. G. *Main Group. Chem.* **2009**, *8*, 323–335.
31. Santos, V. G.; Godoi M. N.; Regiani. T.; Gama, F. H. S.; Coelho, M. B.; de Souza, R. O.; Eberlin, M. N.; Garden, S. *J. Chem-Eur. J.* **2014**, *20*, 12808–12816.
32. Undale, K. A.; Park, Y.; Park, K.; Dagade, D. H.; Pore, D. M. *Synlett.* **2011**, *6*, 791–796.
33. Debache, A.; Ghalem, W.; Boulcina, R.; Belfaitah, A.; Rhouati, S.; Carboni, B. *Tetrahedron Lett.* **2009**, *50*, 5248–5250.
34. Saini, A.; Kumar, S.; Sandhu, J. S. *J. Sci. Ind. Res.* **2008**, *67*, 95–111.
35. Gabriel, C.; Gabriel, S.; Grant, E. H.; Halstead, B. S. J.; Mingos, D. M. P. *Chem. Soc. Rev.* **1998**, *27*, 213–223.
36. Gunduz, M. G.; Safak, C.; Kaygisiz, B.; Kosar, B. C.; Simsek, R.; Erol, K.; Safak, C.; Linden, A. *Arzneimittelforsch.* **2012**, *62*, 167–175.
37. Gunduz, M. G.; Ozturk, G. S.; Vural, I. M.; Simsek, R.; Sarioglu, Y.; Safak, C. *Eur. J. Med. Chem.* **2008**, *43*, 562–568.
38. Wolber, G.; Langer, T. *J. Chem. Inf. Model.* **2005**, *45*, 160–169.