

## Research Article



Erdem Kamil Ozer\*, Miyase Gozde Gunduz, Ahmed El-Khouly, Yildirim Sara, Rahime Simsek, Alper Bektas Iskit and Cihat Safak

# Synthesis of fused 1,4-dihydropyridines as potential calcium channel blockers

## Potansiyel kalsiyum kanal blokerleri olarak kaynaşmış 1,4-dihidropiridin türevlerinin sentezi

DOI 10.1515/tjb-2016-0247

Received November 23, 2016; accepted March 28, 2017; previously published online June 12, 2017

## Abstract

**Objective:** The aim of this study was to synthesize ten 1,4-dihydropyridine (DHP) derivatives in which substituted cyclohexane rings were fused to the DHP ring and to determine how different ester groups and the benzoyl substituent introduced in 4-phenyl ring affected their calcium channel blocking activity.

**Methods:** A microwave-assisted one-pot method was applied for the synthesis of compound 1–5 according to a modified Hantzsch reaction. The benzoyl moiety was introduced in the 4-phenyl ring of these dihydropyridines by refluxing with benzoyl chloride in acetone in the presence of anhydrous potassium carbonate. Synthesized products were characterized by elemental analysis, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectroscopy. The inhibitory actions of compounds 1–10 on calcium channel blocking activity were tested on isolated rat aorta preparations.

**Results:** The obtained pharmacological results showed that although all compounds are potent relaxing agents on isolated rat aorta smooth muscle, introduction of a benzoyloxy substituent on the phenyl ring (compound 6–10) decreased the relaxant effect of these compounds.

**Conclusion:** The reported 1,4-DHP derivatives have calcium channel blocking activity on rat aorta smooth muscle.

**Keywords:** 1,4-Dihydropyridine; Calcium channel; Hexahydroquinoline; Isolated aortic rings; Relaxant effects.

## Özet

**Amaç:** Bu çalışmanın amacı, sübstitüe sikloheksan halkasının 1,4-dihidropiridin (DHP) halkasına kaynaştırıldığı on DHP türevi sentezlemek ve farklı ester grupları ile 4-fenil halkasına eklenen benzoil sübstitüentinin kalsiyum kanal bloke edici aktiviteyi ne kadar etkilediğini belirlemektir.

**Yöntem:** 1-5 no'lu bileşiklerin sentezi için mikrodalga yardımıyla tek basamak olarak modifiye Hantzsch reaksiyonu uygulanmıştır. Benzoil grubu, bu dihidropiridinlerin 4-fenil halkasına, susuz potasyum karbonat varlığında aseton içinde benzoil klorür ile kaynatılarak sübstitüe edilmiştir. Sentezlenmiş bileşikler, eleman analizi, IR, <sup>1</sup>H-NMR ve <sup>13</sup>C-NMR spektroskopisi ile karakterize edilmiştir. 1-10 no'lu bileşiklerin kalsiyum kanalı inhibe edici etkileri izole sıçan aort preparatları üzerinde test edilmiştir.

**Sonuç:** Elde edilen farmakolojik sonuçlar, tüm bileşiklerin izole sıçan aort düz kasında güçlü bir gevşetici ajan olmasına rağmen, fenil halkasına (bileşik 6-10) bir benzoiloksi sübstitüentinin katılmasının, bu bileşiklerin gevşetici etkisini azalttığını göstermiştir.

**Tartışma:** Bildirilen 1,4-DHP türevleri, sıçan aortu düz kası üzerinde kalsiyum kanal bloke edici aktiviteye sahiptir.

**Anahtar Kelimeler:** 1,4-Dihidropiridin; Kalsiyum kanalı; Hekzahidrokinolin; izole aort halkaları; Gevşetici etkiler.

\*Corresponding author: Erdem Kamil Ozer, Department of Pharmacology, Faculty of Medicine, Selcuk University, 42250, Selcuklu, Konya, Turkey, Phone: +90 332 2243839, e-mail: drekfarma@hotmail.com

**Miyase Gozde Gunduz, Ahmed El-Khouly, Rahime Simsek and Cihat Safak:** Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey  
**Yildirim Sara and Alper Bektas Iskit:** Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey.  
<http://orcid.org/0000-0002-7258-8902> (A.B. Iskit)

## Introduction

Calcium channels play a critical role both in the normal biological functions and also in various pathological processes that occur in neuronal, muscle and neurosecretory

cells [1, 2]. Although several types of calcium channels have been identified; L-type channels are typically confined to cell bodies and are responsible for regulating contractility in muscle cells [3, 4].

Calcium channel blockers are the class of drugs that inhibit selectively the calcium movement through voltage sensitive calcium channels [5, 6]. 1,4-dihydropyridines (DHPs) are an important class of L-type calcium channel blockers that are used to treat cardiovascular diseases such as hypertension and angina [7, 8]. Their principal target in the cardiovascular system is thought to be the Cav1.2 L-type calcium channel isoform [9].

The versatility of the 1,4-DHP scaffold, with its wide range of therapeutic benefits and high potency, 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 and enhance calcium modulating effects [6, 10].

Fused DHPs such as hexahydroquinolines, indenopyridines and acridines, which could be obtained by introducing the DHP ring into condensed ring systems, were active derivatives exhibiting calcium antagonistic effects [11–13]. It has been previously showed that L-type channel inhibition is sensitive to the substitution at the six-position of the hexahydroquinoline ring [14].

The nature and position of C-4-aryl ring substituents optimize activity. Although rather simple as well as more complex modifications such as introducing xanthone, indole and benzofuroxan into 4-position of the 1,4-DHP nucleus were carried out; previous studies have shown that the preferred substituent at the C-4 position of DHPs is a phenyl ring because of animal toxicity observed with heteroaromatic rings [9, 15–17]. The analysis among 4-phenyl-1,4-DHP analogs revealed that biological activity depends on the hydrophilic, electronic and steric properties of the substituents on the phenyl ring [18].

In addition, ester functionalities at C-3 and C-5 position are of utmost importance to modulate activity and

tissue selectivity [19]. It has been reported that asymmetrical substituents in C-3 and C-5 alter the activity [20]. X-ray structural investigations, theoretical calculations of fused 1,4-DHPs 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 [9].

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 nifedipine, barnidipine and benidipine [21–23].

The aim of this study is to evaluate the relaxant effect of ten DHP derivatives in which substituted cyclohexane rings are fused to the DHP ring and determine how different ester groups and the addition of the second ester moiety affect the calcium channel block.

## Materials and methods

### Experimental chemistry

All chemicals used in this study were purchased from Aldrich and Fluka (Steinheim, Germany). Some reactions were carried out in Discover Microwave Apparatus (CEM). Thin layer chromatography (TLC) was run on Merck aluminium sheets, Silica gel 60 F<sub>254</sub> (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 were recorded on a Perkin Elmer FT-IR Spectrum BX (USA). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were obtained in dimethylsulphoxide (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. 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).

### Synthesis

Synthesis of compound **1–10** has been described previously [24] but briefly: Compound **1–5** were achieved

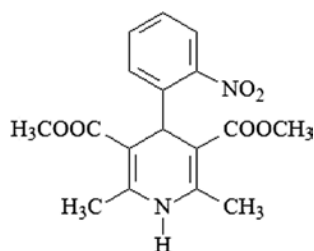


Figure 1: Structure of nifedipine.

by the reaction of 4,4-dimethyl-1,3-cyclohexanedione, 5-nitrosalicylaldehyde, appropriate alkyl acetoacetate and ammonium acetate under microwave irradiation. In order to determine the effect of the second ester group; the benzoyl moiety was introduced in the 4-phenyl ring of these dihydropyridines by refluxing with benzoyl chloride in acetone in the presence of anhydrous potassium carbonate (compound 6–10).

### General procedure for the preparation of alkyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (Compound 1–5)

One-pot four component mixture of 0.002 mol 4,4-dimethyl-1,3-cyclohexanedione, 0.002 mol 5-nitrosalicylaldehyde, 0.002 mol appropriate alkyl acetoacetate and 0.01 mol ammonium acetate was filled into 35 mL-microwave pressure vial and heated under microwave irradiation (power 50 W, maximum temperature 120°C) for 10 min in 5 mL methanol. After the reaction was completed, monitored by TLC, the reaction mixture was poured into ice-water, the obtained precipitate was filtered and crystallized from methanol-water.

### General procedure for the preparation of alkyl 4-(2-(benzoyloxy)-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylates (Compound 6–10)

0.001 mol synthesized 1,4-dihydropyridine derivative (Compound 1–5), 0.0015 mol benzoyl chloride and 2 g anhydrous potassium carbonate were refluxed in 15 mL acetone for 4 h. The resulting slurry was filtered out and the solvent was removed using a rotary evaporator. The obtained sticky residue was crystallized from ethanol-water to achieve the target compound.

The synthetic route used to prepare the compounds has been outlined in Figure 2.

### Methyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 1)

Yield: 85%. m.p. 200–202°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.91 (3H; s; 6-CH<sub>3</sub>), 1.01 (3H; s; 6-CH<sub>3</sub>), 1.58–1.76 (2H; m; H-7), 2.30 (3H; s; 2-CH<sub>3</sub>), 2.49–2.55 (2H; m; H-8), 3.32 (1H; s; OH), 3.48 (3H; s; COOCH<sub>3</sub>), 4.99 (1H; s; 4-H), 6.69 (1H; d; J: 8.4 Hz; Ar-H<sup>3</sup>), 6.80 (1H; d; J: 2.8 Hz; Ar-H<sup>6</sup>), 6.98 (1H; dd; J: 2.4/8.4 Hz; Ar-H<sup>4</sup>), 9.42 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>):

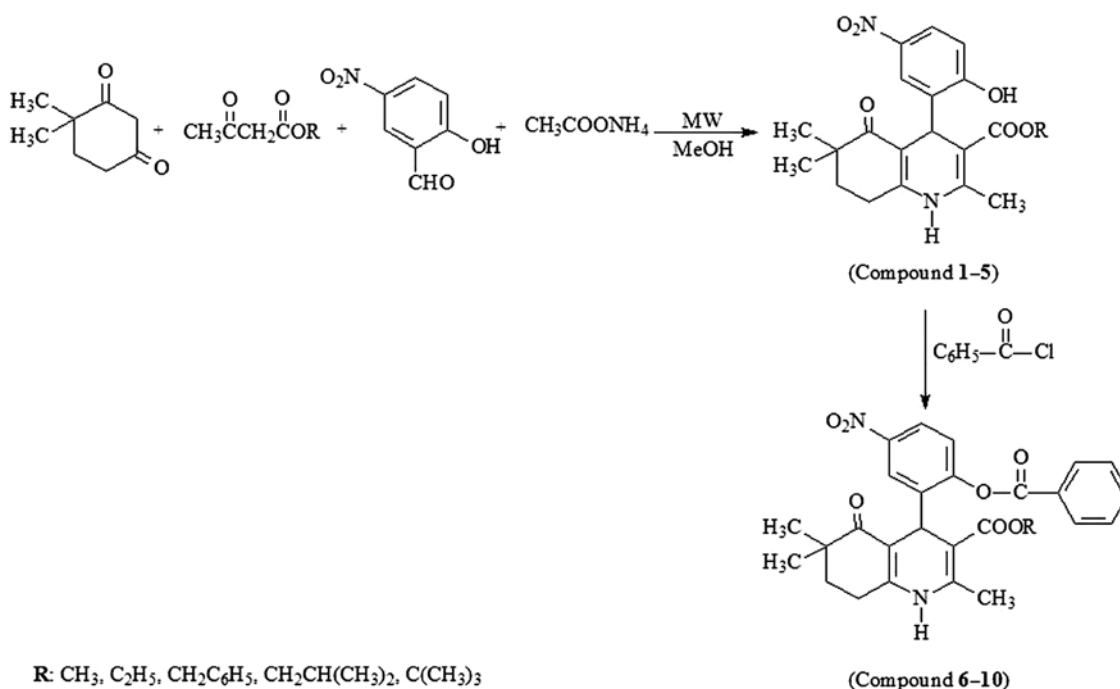


Figure 2: Synthesis of the compound 1–10.

18.1, 22.9, 24.1, 24.9, 31.4, 38.8, 40.0, 50.3, 104.8, 110.7, 122.4, 125.0, 127.6, 129.7, 136.2, 142.2, 144.0, 149.3, 167.4, 199.5. MS (m/z): 386 [M]<sup>+</sup>. Anal. Calcd. for C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>O<sub>6</sub>: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.14; H, 5.76; N, 7.28.

**Ethyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 2)**

Yield: 82%. m.p. 208–210°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.89 (3H; s; 6-CH<sub>3</sub>), 1.00 (3H; s; 6-CH<sub>3</sub>), 1.11 (3H; t; J: 7.2 Hz; COOCH<sub>2</sub>CH<sub>3</sub>), 1.55–1.76 (2H; m; H-7), 2.30 (3H; s; 2-CH<sub>3</sub>), 2.48–2.54 (2H; m; H-8), 3.93 (1H; dq; COOCH<sub>2A</sub>-CH<sub>3</sub>), 4.09 (1H; dq; COOCH<sub>2B</sub>-CH<sub>3</sub>), 4.97 (1H; s; 4-H), 6.84 (1H; d; J: 9.2 Hz; Ar-H<sup>3</sup>), 7.81 (1H; d; J: 2.8 Hz; Ar-H<sup>6</sup>), 6.98 (1H; dd; J: 2.8/9.2 Hz; Ar-H<sup>4</sup>), 9.36 (1H; s; NH), 10.91 (1H; s; OH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 13.8, 18.1, 23.0, 24.1, 24.9, 31.4, 34.0, 40.0, 58.8, 105.1, 110.8, 124.8, 125.0, 125.8, 126.1, 130.3, 143.9, 147.0, 149.2, 167.0, 199.5. MS (m/z): 399 [M-1]<sup>+</sup>. Anal. Calcd. for C<sub>21</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub>: C, 62.99; H, 6.04; N, 7.00. Found: C, 62.95; H, 6.01; N, 6.98.

**Benzyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 3)**

Yield: 80%. m.p. 185–187°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.90 (3H; s; 6-CH<sub>3</sub>), 1.00 (3H; s; 6-CH<sub>3</sub>), 1.58–1.77 (2H; m; H-7), 2.33 (3H; s; 2-CH<sub>3</sub>), 2.49–2.5 (2H; m; H-8), 3.33 (1H; s; OH), 4.90, 5.02 (2H; AB system; J<sub>AB</sub> = 12.8 Hz, COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.00 (1H; s; 4-H), 6.84–7.91 (8H; m; Ar-H) 9.47 (1H; s; NH), 9.82 (1H; s; OH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.3, 23.0, 24.1, 24.9, 31.4, 33.9, 40.0, 64.5, 104.7, 110.9, 124.9, 125.0, 125.6, 125.7, 126.1, 126.2, 127.5, 127.6, 128.1, 130.3, 132.7, 136.4, 146.7, 149.2, 166.7, 199.5. MS (m/z): 462 [M]<sup>+</sup>. Anal. Calcd. for C<sub>26</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub>: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.56; H, 5.65; N, 6.10.

**Isobutyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 4)**

Yield: 78%. m.p. 170–171°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.71 (3H; d; J: 6.8 Hz; COOCH<sub>2</sub>CHCH<sub>3</sub>), 0.73 (3H; d; J: 6.8 Hz; COOCH<sub>2</sub>CHCH<sub>3</sub>), 0.92 (3H; s; 6-CH<sub>3</sub>), 1.02 (3H; s; 6-CH<sub>3</sub>), 1.46–1.53 (1H; m; CH(CH<sub>3</sub>)<sub>2</sub>), 1.59–1.78 (2H; m; H-7), 2.34 (3H; s; 2-CH<sub>3</sub>), 2.48–2.56 (2H; m; H-8), 3.61 (1H; dd; J: 10.8/6.4 Hz; CH<sub>2A</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 3.73 (1H; dd; J: 10.8/6.4 Hz; CH<sub>2B</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 4.96 (1H; s; 4-H), 6.85 (1H; d; J: 8.8 Hz; Ar-H<sup>3</sup>), 7.80 (1H; d;

J: 2.4 Hz; Ar-H<sup>6</sup>), 7.89 (1H; dd; J: 8.8/2.4 Hz; Ar-H<sup>4</sup>), 9.48 (1H; s; NH), 11.02 (1H; s; OH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.4, 18.9, 19.2, 22.9, 24.1, 25.1, 27.2, 34.0, 36.2, 40.0, 69.2, 102.6, 109.1, 125.1, 126.6, 127.2, 127.5, 131.5, 135.2, 145.5, 149.6, 166.9, 199.5. MS (m/z): 428 [M]<sup>+</sup>. Anal. Calcd. for C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.40; H, 6.61; N, 6.58.

**Tert-butyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 5)**

Yield: 82%. m.p. 198–200°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.86 (3H; s; 6-CH<sub>3</sub>), 1.01 (3H; s; 6-CH<sub>3</sub>), 1.21 (9H; s; COOC(CH<sub>3</sub>)<sub>3</sub>), 1.52–1.74 (2H; m; H-7), 2.22–2.43 (2H; m; H-8), 2.35 (3H; s; 2-CH<sub>3</sub>), 2.83 (1H; s; OH), 4.34 (1H; s; 4-H), 6.93 (1H; d; J: 9.2 Hz; Ar-H<sup>3</sup>), 7.91 (1H; dd; J: 2.4/9.2 Hz; Ar-H<sup>4</sup>), 7.98 (1H; d; J: 2.4 Hz; Ar-H<sup>6</sup>), 8.31 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.0, 23.0, 24.1, 25.0, 27.7, 27.9, 28.1, 32.0, 33.9, 40.0, 78.8, 107.0, 110.2, 124.8, 125.8, 126.2, 127.5, 130.3, 132.7, 146.4, 149.5, 166.7, 199.4. MS (m/z): 428 [M]<sup>+</sup>. Anal. Calcd. for C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.42; H, 6.55; N, 6.50.

**Methyl 4-(2-(benzoyloxy)-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 6)**

Yield: 65%. m.p. 230–232°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.83 (3H; s; 6-CH<sub>3</sub>), 0.94 (3H; s; 6-CH<sub>3</sub>), 1.55–1.75 (2H; m; H-7), 2.20 (3H; s; 2-CH<sub>3</sub>), 2.49–2.56 (2H; m; H-8), 3.35 (3H; s; COOCH<sub>3</sub>), 5.22 (1H; s; 4-H), 7.43–8.25 (8H, m, Ar-H), 9.01 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.3, 22.8, 24.1, 25.1, 34.0, 35.9, 40.0, 50.6, 102.5, 109.0, 124.8, 125.1, 125.7, 126.5, 127.2, 127.4, 127.6, 131.5, 132.8, 133.6, 138.9, 145.0, 145.4, 149.8, 167.4, 199.5, 200.5. MS (m/z): 489 [M-1]<sup>+</sup>. Anal. Calcd. for C<sub>27</sub>H<sub>26</sub>N<sub>2</sub>O<sub>7</sub>: C, 66.11; H, 5.34; N, 5.71. Found: C, 66.06; H, 5.33; N, 5.73.

**Ethyl 4-(2-(benzoyloxy)-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 7)**

Yield: 68%. m.p. 236–238°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.83 (3H; s; 6-CH<sub>3</sub>), 0.92 (3H; s; 6-CH<sub>3</sub>), 0.99 (3H; t; J: 7.2 Hz; COOCH<sub>2</sub>CH<sub>3</sub>), 1.58–1.70 (2H; m; H-7), 2.05–2.37 (2H; m; H-8), 2.30 (3H; s; 2-CH<sub>3</sub>), 3.86 (1H; dq; COOCH<sub>2A</sub>-CH<sub>3</sub>), 3.89 (1H; dq; COOCH<sub>2B</sub>-CH<sub>3</sub>), 5.17 (1H; s; 4-H), 7.42–8.19 (8H, m, Ar-H), 8.86 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 14.1, 18.3,



22.9, 24.1, 25.0, 34.1, 36.2, 40.0, 59.0, 102.9, 108.9, 125.10, 125.15, 125.7, 126.6, 127.2, 127.3, 127.6, 131.5, 132.7, 136.9, 138.2, 145.0, 145.1, 149.8, 166.9, 199.5, 200.4. MS (m/z): 504 [M]<sup>+</sup>. Anal. Calcd. for C<sub>28</sub>H<sub>28</sub>N<sub>2</sub>O<sub>7</sub>: C, 66.66; H, 5.59; N, 5.55. Found: C, 66.70; H, 5.61; N, 5.55.

**Benzyl 4-(2-(benzoyloxy)-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 8)**

Yield: 57%. m.p. 182–183°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.81 (3H; s; 6-CH<sub>3</sub>), 0.91 (3H; s; 6-CH<sub>3</sub>), 1.60–1.71 (2H; m; H-7), 2.07–2.38 (2H; m; H-8), 2.16 (3H; s; 2-CH<sub>3</sub>), 4.92, 4.95 (2H; AB system; J<sub>AB</sub> = 12.4 Hz, COOCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.16 (1H; s; 4-H), 7.05–8.13 (13H; m; Ar-H), 8.89 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.8, 23.4, 24.6, 25.5, 34.5, 36.7, 40.6, 65.2, 102.9, 109.6, 125.6, 125.7, 126.1, 127.1, 127.7, 127.9, 128.11, 128.15, 128.2, 128.6, 132.1, 133.2, 135.1, 136.9, 137.1, 142.2, 143.1, 145.5, 146.4, 150.2, 167.1, 200.0, 200.9. MS (m/z): 565 [M–1]<sup>+</sup>. Anal. Calcd. for C<sub>33</sub>H<sub>30</sub>N<sub>2</sub>O<sub>7</sub>: C, 69.95; H, 5.34; N, 4.94. Found: C, 69.94; H, 5.33; N, 4.96.

**Isobutyl 4-(2-(benzoyloxy)-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 9)**

Yield: 68%. m.p. 225–227°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.65 (3H; d; J: 6.8 Hz; COOCH<sub>2</sub>CHCH<sub>3</sub>), 0.74 (3H; d; J: 6.8 Hz; COOCH<sub>2</sub>CHCH<sub>3</sub>), 0.81 (3H; s; 6-CH<sub>3</sub>), 0.90 (3H; s; 6-CH<sub>3</sub>), 1.58–1.67 (2H; m; H-7), 1.68–1.73 (1H; m; CH(CH<sub>3</sub>)<sub>2</sub>), 2.02–2.38 (2H; m; H-8), 2.13 (3H; s; 2-CH<sub>3</sub>), 3.62 (1H; dd; J: 10.8/6.4 Hz; CH<sub>2A</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 3.68 (1H; dd; J: 10.8/6.4 Hz; CH<sub>2B</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 5.14 (1H; s; 4-H), 7.38–8.17 (8H; m; Ar-H), 8.81 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.3, 18.5, 19.1, 23.0, 24.2, 25.1, 26.9, 34.5, 38.2, 41.1, 69.5, 101.9, 110.2, 125.5, 126.8, 127.2, 127.9, 128.5, 131.5, 133.5, 135.2, 136.4, 138.2, 139.3, 140.8, 145.5, 149.6, 166.9, 199.5, 200.4. MS (m/z): 532 [M]<sup>+</sup>. Anal. Calcd. for C<sub>30</sub>H<sub>32</sub>N<sub>2</sub>O<sub>7</sub>: C, 67.66; H, 6.06; N, 5.26. Found: C, 67.68; H, 6.07; N, 5.29.

**Tert-butyl 4-(2-(benzoyloxy)-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound 10)**

Yield: 70%. m.p. 235–237°C. <sup>1</sup>H-NMR (δ, DMSO-d<sub>6</sub>): 0.83 (3H; s; 6-CH<sub>3</sub>), 0.90 (3H; s; 6-CH<sub>3</sub>), 1.26 (9H; s; COOC(CH<sub>3</sub>)<sub>3</sub>), 1.57–1.65 (2H; m; H-7), 1.86–2.31 (2H; m; H-8), 2.07 (3H; s; 2-CH<sub>3</sub>), 5.05 (1H; s; 4-H), 7.39–8.21 (8H; m; Ar-H), 8.61 (1H; s; NH). <sup>13</sup>C-NMR (δ, DMSO-d<sub>6</sub>): 18.2, 23.5, 24.1, 25.1, 27.4,

27.8, 28.0, 34.1, 36.7, 40.0, 78.7, 102.1, 108.7, 125.0, 125.2, 125.7, 126.7, 127.2, 127.5, 127.9, 128.2, 131.5, 132.7, 135.5, 136.1, 143.8, 145.3, 166.4, 199.3, 200.4. MS (m/z): 532 [M]<sup>+</sup>. Anal. Calcd. for C<sub>30</sub>H<sub>32</sub>N<sub>2</sub>O<sub>7</sub>: C, 67.66; H, 6.06; N, 5.26. Found: C, 66.70; H, 6.03; N, 5.22. Spectral data of selected compounds are provided as supplementary material.

## Pharmacology

The inhibitory actions of compounds **1–10** on calcium channel activity were tested on isolated rat aorta preparations according to the previous study [25]. Male Wistar albino rats weighing 200–250 g were used. Following the diethyl ether anesthesia, animals were sacrificed by exsanguination and their thoraces were opened and the thoracic part of the aorta was gently removed. Isolated aorta was cleaned of the 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<sub>2</sub>/5% CO<sub>2</sub>). Aorta rings were mounted in isolated organ baths containing 50 mL Ca<sup>2+</sup>-free Krebs-Henseleit solution (mmol: NaCl 118, KCl 4.7, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 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 force-displacement transducer and digitized data acquisition system (PowerLab/8sp, Adinstruments, Australia). All aorta preparations were allowed to equilibrate in the Ca<sup>2+</sup>-free Krebs-Henseleit solution for about 45 min while washing out the tissues every ~15 min and subsequently high K<sup>+</sup> (80 mM) Krebs-Henseleit solution without Ca<sup>2+</sup> was applied. The rings were then contracted with 2.5 mM Ca<sup>2+</sup>. Following the maximal contractile response with Ca<sup>2+</sup>, data required for the concentration-response curves were obtained by cumulative administration of drugs under investigation. In order to achieve maximal relaxation at the end of cumulative drug administrations, all rings were treated with 10<sup>–4</sup> M papaverine. For each drug, six trials were conducted, the obtained data was fit into a curve and EC<sub>50</sub>, pD<sub>2</sub> and E<sub>max</sub> 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 the mechanisms other than the Ca<sup>2+</sup> channels, cyclooxygenase (COX), adrenergic and nitregic systems were all blocked by indomethacin (COX inhibitor, 10<sup>–5</sup> M), guanethidine (an adrenergic nerve blocker, 10<sup>–6</sup> M) and L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride, the nitric oxide synthase inhibitor, 10<sup>–4</sup> M), respectively. All test compounds and nifedipine were dissolved

in DMSO. The final concentration of DMSO was 0.1% and found to have no effect on aorta activity.

This study was approved by the Ethics Committee of Hacettepe University, Faculty of Medicine, Ankara, Turkey (Approval Number: 2013/47-04). All procedures involving animals and their care were conducted in conformity with international laws and policies.

## Statistical analysis

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

## Results and discussion

### Chemistry

A microwave-assisted one-pot method was applied for the synthesis of compound **1–5**. These compounds were achieved by the reaction of 4,4-dimethyl-1,3-cyclohexanedione, 5-nitrosalicylaldehyde, alkyl acetoacetate and ammonium acetate under microwave irradiation in methanol, according to a modified Hantzsch reaction. The benzoyl moiety was introduced in the 4-phenyl ring of these dihydropyridines by refluxing with benzoyl chloride in acetone in the presence of anhydrous potassium carbonate (compound **6–10**) [24]. Structures and chemical characteristics of the synthesized compounds are given in Table 1.

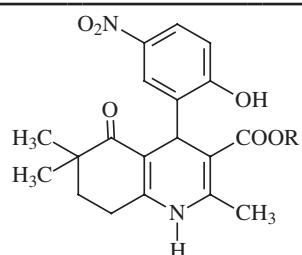
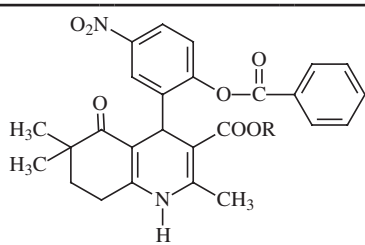
The appearance of the products was monitored by TLC and the reaction time was determined for compound **1–5** as 10 min, which is quite a short time compared to conventional heating [26].

We reported the conventional synthesis of some compounds, which have similar structures to compound **1–5** in previous papers, so it is obvious that this microwave-assisted method reduces the solvent use and reaction time [11, 27, 28].

The structures of the compounds were confirmed by spectral methods  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and mass spectra and elemental analysis.

In the  $^1\text{H-NMR}$  spectra, the protons of the methyl substituents at the six-position of the hexahydroquinoline ring were observed at 0.81–1.02 ppm separately and as singlets. The methylene groups of the same ring were at 1.52–2.56 ppm. The N-H protons of the DHP ring were seen

**Table 1:** Structural data of the synthesized compounds.

| Compound   | R                                     | Melting point (°C) | Empirical formula                                | Molecular weight |
|--|---------------------------------------|--------------------|--|------------------|
|  |                                       |                    |  |                  |
| <b>1</b>   | $\text{CH}_3$                         | 200–202            | $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_6$ | 386              |
| <b>2</b>   | $\text{C}_2\text{H}_5$                | 208–210            | $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_6$ | 400              |
| <b>3</b>   | $\text{CH}_2\text{C}_6\text{H}_5$     | 185–187            | $\text{C}_{26}\text{H}_{26}\text{N}_2\text{O}_6$ | 462              |
| <b>4</b>   | $\text{CH}_2\text{CH}(\text{CH}_3)_2$ | 170–171            | $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_6$ | 428              |
| <b>5</b>   | $\text{C}(\text{CH}_3)_3$             | 198–200            | $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_6$ | 428              |
|  |                                       |                    |  |                  |
| <b>6</b>   | $\text{CH}_3$                         | 230–232            | $\text{C}_{27}\text{H}_{26}\text{N}_2\text{O}_7$ | 490              |
| <b>7</b>   | $\text{C}_2\text{H}_5$                | 236–238            | $\text{C}_{28}\text{H}_{28}\text{N}_2\text{O}_7$ | 504              |
| <b>8</b>   | $\text{CH}_2\text{C}_6\text{H}_5$     | 182–183            | $\text{C}_{33}\text{H}_{30}\text{N}_2\text{O}_7$ | 566              |
| <b>9</b>   | $\text{CH}_2\text{CH}(\text{CH}_3)_2$ | 225–227            | $\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_7$ | 532              |
| <b>10</b>  | $\text{C}(\text{CH}_3)_3$             | 235–237            | $\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_7$ | 532              |

at 8.31–9.82 ppm and the signal of O-H proton at the two-position of the phenyl ring disappeared after the introduction of the benzoyl moiety as the second ester group.

In the  $^{13}\text{C-NMR}$  spectra the number of the signals fitted exactly the number of carbon atoms.

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 dihydropyridine ring were seen in the spectra of all compounds. Cleavage of the ester groups and substituted phenyl rings from the parent molecule are the next most observed fragmentations.

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

### Pharmacology

The inhibitory actions of compounds **1–10** on calcium channel activity were tested on isolated rat aorta preparations. The maximum relaxant effects ( $E_{\text{max}}$ ) 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. The pharmacological analysis of  $Ca^{2+}$  block action of all compounds yielded concentration-dependent responses in the rat aorta rings precontracted with  $Ca^{2+}$  (2.5 mM). While  $E_{max}$  values (a measure of efficacy) of compound **2**, **3** and **4** were higher, the  $pD_2$  values (a measure of potency) of all compounds were found to be lower than that of 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.

**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   | $E_{max}$          | $pD_2$            |
|------------|--------------------|-------------------|
| <b>1</b>   | $95.92 \pm 1.87$   | $6.20 \pm 0.14^a$ |
| <b>2</b>   | $97.65 \pm 1.89$   | $5.94 \pm 0.13^a$ |
| <b>3</b>   | $97.64 \pm 0.75$   | $7.24 \pm 0.23$   |
| <b>4</b>   | $97.38 \pm 2.30$   | $6.94 \pm 0.37$   |
| <b>5</b>   | $95.74 \pm 1.58$   | $7.16 \pm 0.49$   |
| <b>6</b>   | $94.50 \pm 1.92$   | $6.27 \pm 0.48^a$ |
| <b>7</b>   | $76.93 \pm 8.31^a$ | $5.57 \pm 0.83^a$ |
| <b>8</b>   | $77.45 \pm 6.15^a$ | $5.44 \pm 0.39^a$ |
| <b>9</b>   | $68.09 \pm 4.50^a$ | $4.91 \pm 0.28^a$ |
| <b>10</b>  | $67.26 \pm 0.87^a$ | $6.25 \pm 0.08^a$ |
| Nifedipine | $96.81 \pm 0.93$   | $7.74 \pm 0.04$   |

<sup>a</sup> $p < 0.001$ , compounds **1–10** were compared with nifedipine responses ( $n = 6$  for each compounds and nifedipine).

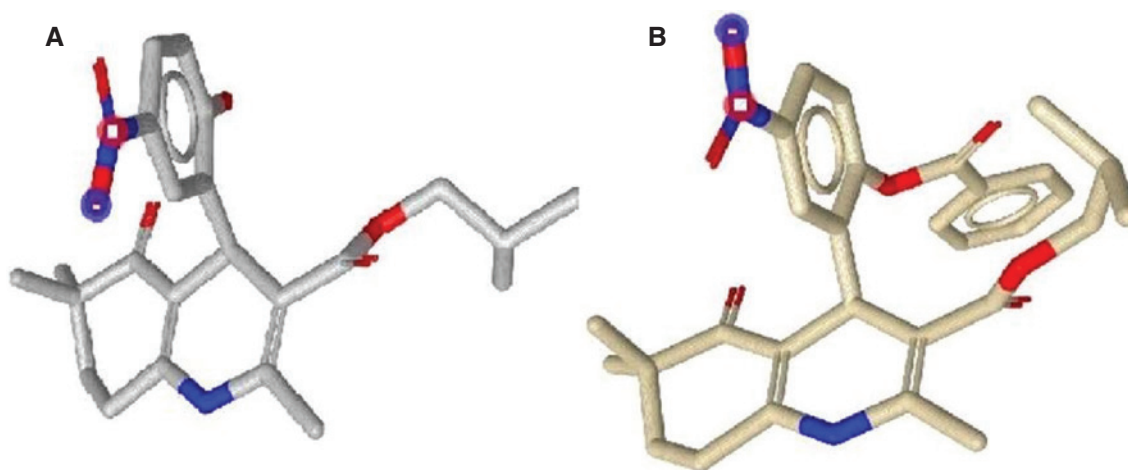
Given that the main difference between compound **1–5** and compound **6–10** is the second ester group (benzyloxy substituent on the phenyl ring); this suggests that adding a bulky ring to the phenyl ring dramatically increases the size of the molecules (as shown in Figure 3) and may have a negative effect on the ability of these compounds to block calcium channel.

Although electron-withdrawing groups at the ortho or meta-position of the 4-phenyl ring are important for L-type calcium channel blocking activity [9], the present study demonstrated that hydroxyl group at two-position of the phenyl ring also played a key role in the ability of these compounds to block calcium channels.

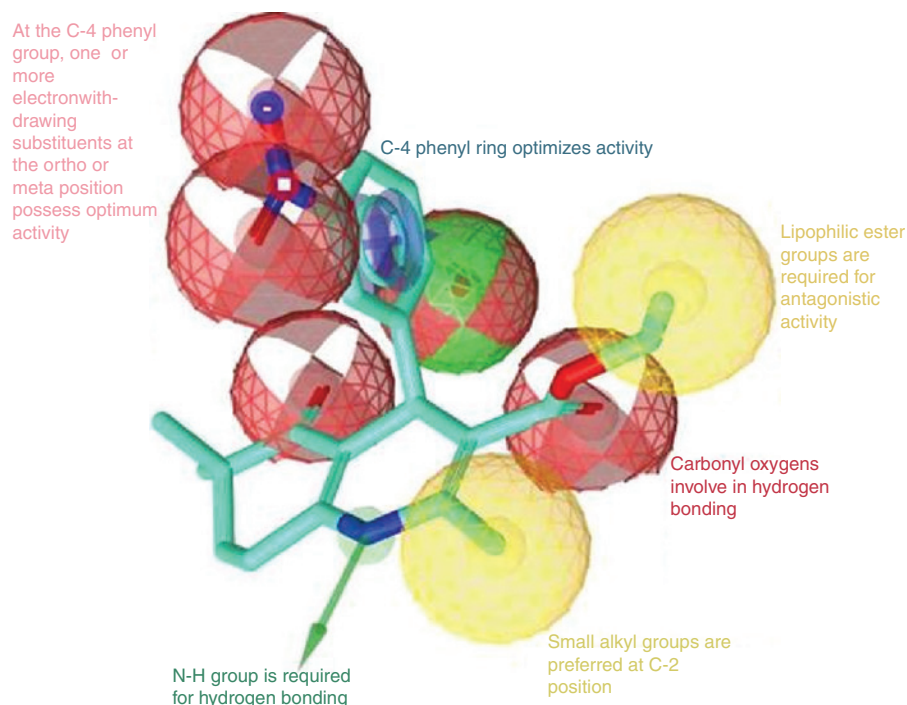
Two methyl groups at six-position of the hexahydroquinoline ring are present in all compounds thus they could not be the critical components for the relaxant effect. In compound **1–5** series, increasing the side chain of the ester group from methyl to ethyl and isobutyl, or introducing a ring structure (compound **3**) at this position mediated a slight increase in relaxant activity.

A pharmacophore model was generated for compound **2**, which was found to be more efficient than nifedipine and proved to block L-type calcium channel effectively [24]. Color-coded pharmacophore features are represented as follows: hydrophobic feature (yellow sphere), electron donor group (red vector), hydrogen bonding domain feature (green vector) and aromatic ring (blue circle) [29]. As demonstrated in Figure 4; the generated ligand-based pharmacophore model features have been found consistent with the reported structure-activity relationships for 1,4-DHP derivatives [6, 20, 30].

In summary, a series of condensed 1,4-DHPs as calcium channel blockers was reported in the study. The obtained pharmacological results showed that although



**Figure 3:** Geometrically optimized and energy minimized conformations of compound **4** (A) and compound **9** (B).



**Figure 4:** Structure-activity relationships for 1,4-DHPs binding to L-type calcium channels.

all compounds are potent relaxing agents on isolated rat aorta smooth muscle, introduction of a benzyloxy substituent on the phenyl ring (compound **6–10**) decreased the relaxant effect of these compounds.

**Acknowledgements:** Alper B. Iskit has been supported by the Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (EA-TUBA-GEIP/2001-2-11).

**Conflict of interest statement:** The authors have no conflict of interest.

## References

- Dolphin AC. A short history of voltage-gated calcium channels. *Br J Pharmacol* 2006;147:S56–62.
- Camerino DC, Desaphy JF, Tricarico D, Pierno S, Liantonio A. Therapeutic approaches to ion channel diseases. *Adv Genet* 2008;64:81–145.
- Carafoli E. Special issue: calcium signaling and disease – Preface. *Biochem Bioph Res Co* 2004;322:1097.
- Zamponi GW. Antagonist binding sites of voltage-dependent calcium channels. *Drug Develop Res* 1997;42:131–43.
- Schleifer KJ. Stereoselective characterization of the 1,4-dihydropyridine binding site at L-type calcium channels in the resting state and the opened inactivated state. *J Med Chem* 1999;42:2204–11.
- Edraki N, Mehdipour AR, Khoshneviszadeh M, Miri R. Dihydropyridines: evaluation of their current and future pharmacological applications. *Drug Discov Today* 2009;14:1058–66.
- Triggle DJ. 1,4-dihydropyridine calcium channel ligands: Selectivity of action. The roles of pharmacokinetics, state-dependent interactions, channel isoforms, and other factors. *Drug Develop Res* 2003;58:5–17.
- Safak C, Simsek R. Fused 1,4-dihydropyridines as potential calcium modulatory compounds. *Mini-Rev Med Chem* 2006;6:747–55.
- Goldmann S, Stoltefuss J. 1,4-Dihydropyridines – effects of chirality and conformation on the calcium-antagonist and calcium agonist activities. *Angew Chem Int Edit* 1991;30:1559–78.
- Gordeev MF, Patel DV, England BP, Jonnalagadda S, Combs JD, Gordon EM. Combinatorial synthesis and screening of a chemical library of 1,4-dihydropyridine calcium channel blockers. *Bioorgan Med Chem* 1998;6:883–9.
- Safak C, Gunduz MG, Ilhan SO, Simsek R, Isli F, Yildirim S, et al. Synthesis and myorelaxant activity of fused 1,4-dihydropyridines on isolated rabbit gastric fundus. *Drug Develop Res* 2012;73:332–42.
- Rose U. 5-Oxo-1,4-Dihydroindenopyridines – calcium modulators with partial calcium agonistic activity. *J Heterocyclic Chem* 1990;27:237–42.



13. Tu SJ, Miao CB, Fang F, Feng YJ, Li TJ, Zhuang QY, et al. New potential calcium channel modulators: design and synthesis of compounds containing two pyridine, pyrimidine, pyridone, quinoline and acridine units under microwave irradiation. *Bioorg Med Chem Lett* 2004;14:1533–6.
14. Lipkind GM, Fozzard HA. Molecular modeling of interactions of dihydropyridines and phenylalkylamines with the inner pore of the L-type  $\text{Ca}^{2+}$  channel. *Mol Pharmacol* 2003;63:499–511.
15. El-Khouly A, Gunduz MG, Cengelli C, Simsek R, Erol K, Safak C, et al. Microwave-assisted synthesis and spasmolytic activity of 4-indolylhexahydroquinoline derivatives. *Drug research* 2013;63:579–85.
16. Bisi A, Budriesi R, Rampa A, Fabbri G, Chiarini A, Valenti P. Synthesis and pharmacological profile of some chloroxanthone-1,4-dihydropyridine derivatives. *Arzneimittel-Forsch* 1996;46:848–51.
17. Ermondi G, Visentin S, Boschi D, Fruttero R, Gasco A. Structural investigation of  $\text{Ca}^{2+}$  antagonists benzofurazanyl and benzofuroxanyl-1,4-dihydropyridines. *J Mol Struct* 2000;523:149–62.
18. Coburn RA, Wierzba M, Suto MJ, Solo AJ, Triggles AM, Triggles DJ. 1,4-Dihydropyridine antagonist activities at the calcium-channel – a quantitative structure activity relationship approach. *J Med Chem* 1988;31:2103–7.
19. Miri R, Javidnia K, Sarkarzadeh H, Hemmateenejad B. Synthesis, study of 3D structures, and pharmacological activities of lipophilic nitroimidazolyl-1,4-dihydropyridines as calcium channel antagonist. *Bioorgan Med Chem* 2006;14:4842–9.
20. Ioan P, Carosati E, Micucci M, Cruciani G, Broccatelli F, Zhorov BS, et al. 1,4-Dihydropyridine scaffold in medicinal chemistry, the story so far and perspectives (Part 1): action in ion channels and GPCRs. *Curr Med Chem* 2011;18:4901–22.
21. Leonardi A, Motta G, Pennini R, Testa R, Sironi G, Catto A, et al. Asymmetric N-(3,3-diphenylpropyl)aminoalkyl esters of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acids with antihypertensive activity. *Eur J Med Chem* 1998;33:399–420.
22. Tamazawa K, Arima H, Kojima T, Isomura Y, Okada M, Fujita S, et al. Stereoselectivity of a potent calcium-antagonist, 1-benzyl-3-pyrrolidinyl methyl 2,6-dimethyl-4-(Meta-Nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. *J Med Chem* 1986;29:2504–11.
23. Gkogkos K, Pavlidis G, Karakozoglou A, Kopras A, Memi E, Tsoutsouli V. Barnidipine: assessment of safety and efficacy: 1 year experience. *J Hypertens* 2006;24:S32.
24. Bladen C, Gunduz MG, Simsek R, Safak C, Zamponi GW. Synthesis and evaluation of 1,4-dihydropyridine derivatives with calcium channel blocking activity. *Pflugers Archiv* 2014;466:1355–63.
25. Ozer EK, Gunduz MG, El-Khouly A, Sara MY, Simsek R, Iskit AB, et al. Microwave-assisted synthesis of condensed 1,4-dihydropyridines as potential calcium channel modulators. *Turk J Chem* 2015;39:886–96.
26. Lidstrom P, Tierney J, Wathey B, Westman J. Microwave assisted organic synthesis – a review. *Tetrahedron* 2001;57:9225–83.
27. Gunduz MG, Safak C, Kaygisiz B, Kosar BC, Simsek R, Erol K, et al. Synthesis of cyclopentapyridine and thienopyridine derivatives as potential calcium channel modulators. *Arzneimittelforsch* 2012;62:167–75.
28. Gunduz MG, Ozturk GS, Vural IM, Simsek R, Sarioglu Y, Safak C. Evaluation of myorelaxant activity of 7-substituted hexahydroquinoline derivatives in isolated rabbit gastric fundus. *Eur J Med Chem* 2008;43:562–8.
29. Wolber G, Langer T. LigandScout: 3-d pharmacophores derived from protein-bound Ligands and their use as virtual screening filters. *J Chem Inf Model* 2005;45:160–9.
30. Carosati E, Ioan P, Micucci M, Broccatelli F, Cruciani G, Zhorov BS, et al. 1,4-Dihydropyridine scaffold in medicinal chemistry, the story so far and perspectives (Part 2): action in other targets and antitargets. *Curr Med Chem* 2012;19:4306–23.

**Supplementary Material:** The online version of this article (DOI: 10.1515/tjb-2016-0247) offers supplementary material, available to authorized users.