

Research Article

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

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

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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, ¹H-NMR and ¹³C-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 benzyloxy 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 siklohekzan 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, ¹H-NMR ve ¹³C-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 benzyloksi sübstitüentinin katılması, 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.

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

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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 benzofuran 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 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 were recorded on a Perkin Elmer FT-IR Spectrum BX (USA). ¹H-NMR and ¹³C-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

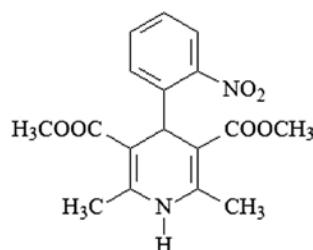


Figure 1: Structure of nifedipine.

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. $^1\text{H-NMR}$ (δ , DMSO- d_6): 0.91 (3H; s; 6- CH_3), 1.01 (3H; s; 6- CH_3), 1.58–1.76 (2H; m; H-7), 2.30 (3H; s; 2- CH_3), 2.49–2.55 (2H; m; H-8), 3.32 (1H; s; OH), 3.48 (3H; s; COOCH_3), 4.99 (1H; s; 4-H), 6.69 (1H; d; J: 8.4 Hz; Ar-H³), 6.80 (1H; d; J: 2.8 Hz; Ar-H⁶), 6.98 (1H; dd; J: 2.4/8.4 Hz; Ar-H⁴), 9.42 (1H; s; NH). $^{13}\text{C-NMR}$ (δ , DMSO- d_6):

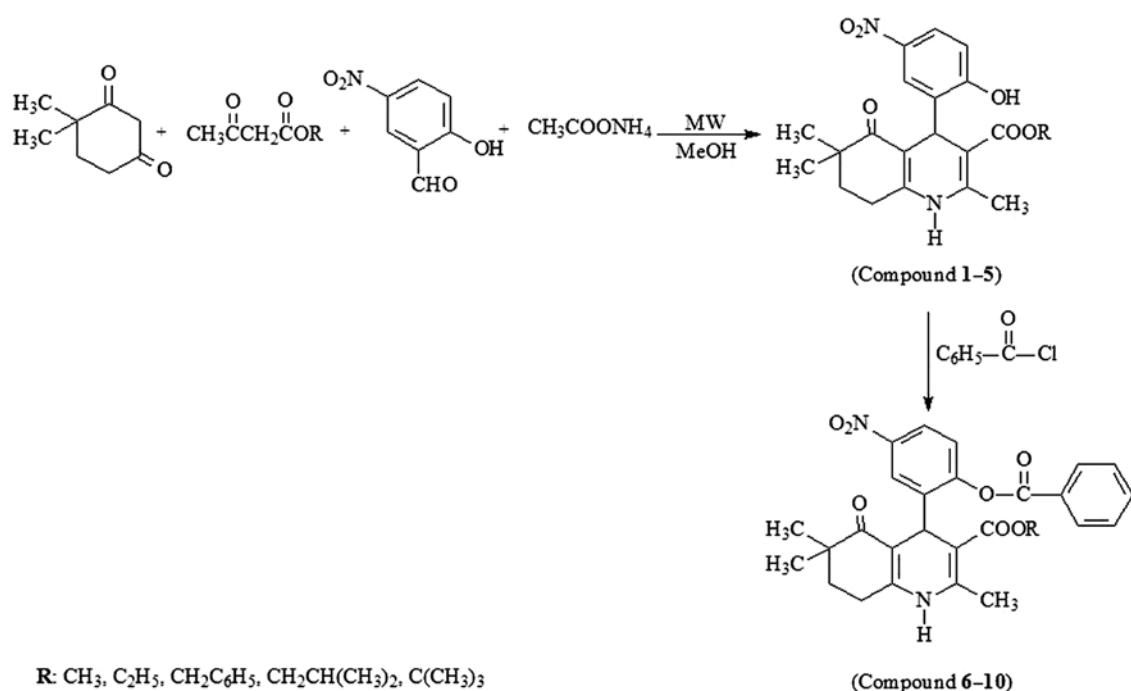


Figure 2: Synthesis of the compound **1-10**.

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]⁺. Anal. Calcd. for C₂₀H₂₂N₂O₆: 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. ¹H-NMR (δ , DMSO-d₆): 0.89 (3H; s; 6-CH₃), 1.00 (3H; s; 6-CH₃), 1.11 (3H; t; J: 7.2 Hz; COOCH₂CH₃), 1.55–1.76 (2H; m; H-7), 2.30 (3H; s; 2-CH₃), 2.48–2.54 (2H; m; H-8), 3.93 (1H; dq; COOCH_{2A}-CH₃), 4.09 (1H; dq; COOCH_{2B}-CH₃), 4.97 (1H; s; 4-H), 6.84 (1H; d; J: 9.2 Hz; Ar-H³), 7.81 (1H; d; J: 2.8 Hz; Ar-H⁶), 6.98 (1H; dd; J: 2.8/9.2 Hz; Ar-H⁴), 9.36 (1H; s; NH), 10.91 (1H; s; OH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₂₁H₂₄N₂O₆: 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. ¹H-NMR (δ , DMSO-d₆): 0.90 (3H; s; 6-CH₃), 1.00 (3H; s; 6-CH₃), 1.58–1.77 (2H; m; H-7), 2.33 (3H; s; 2-CH₃), 2.49–2.5 (2H; m; H-8), 3.33 (1H; s; OH), 4.90, 5.02 (2H; AB system; J_{AB}=12.8 Hz, COOCH₂C₆H₅), 5.00 (1H; s; 4-H), 6.84–7.91 (8H; m; Ar-H) 9.47 (1H; s; NH), 9.82 (1H; s; OH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₂₆H₂₆N₂O₆: 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. ¹H-NMR (δ , DMSO-d₆): 0.71 (3H; d; J: 6.8 Hz; COOCH₂CHCH₃), 0.73 (3H; d; J: 6.8 Hz; COOCH₂CHCH₃), 0.92 (3H; s; 6-CH₃), 1.02 (3H; s; 6-CH₃), 1.46–1.53 (1H; m; CH(CH₃)₂), 1.59–1.78 (2H; m; H-7), 2.34 (3H; s; 2-CH₃), 2.48–2.56 (2H; m; H-8), 3.61 (1H; dd; J: 10.8/6.4 Hz; CH_{2A}CH(CH₃)₂), 3.73 (1H; dd; J: 10.8/6.4 Hz; CH_{2B}CH(CH₃)₂), 4.96 (1H; s; 4-H), 6.85 (1H; d; J: 8.8 Hz; Ar-H³), 7.80 (1H; d;

J: 2.4 Hz; Ar-H⁶), 7.89 (1H; dd; J: 8.8/2.4 Hz; Ar-H⁴), 9.48 (1H; s; NH), 11.02 (1H; s; OH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₂₃H₂₈N₂O₆: 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. ¹H-NMR (δ , DMSO-d₆): 0.86 (3H; s; 6-CH₃), 1.01 (3H; s; 6-CH₃), 1.21 (9H; s; COOC(CH₃)₃), 1.52–1.74 (2H; m; H-7), 2.22–2.43 (2H; m; H-8), 2.35 (3H; s; 2-CH₃), 2.83 (1H; s; OH), 4.34 (1H; s; 4-H), 6.93 (1H; d; J: 9.2 Hz; Ar-H³), 7.91 (1H; dd; J: 2.4/9.2 Hz; Ar-H⁴), 7.98 (1H; d; J: 2.4 Hz; Ar-H⁶), 8.31 (1H; s; NH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₂₃H₂₈N₂O₆: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.42; H, 6.55; N, 6.50.

Methyl 4-(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. ¹H-NMR (δ , DMSO-d₆): 0.83 (3H; s; 6-CH₃), 0.94 (3H; s; 6-CH₃), 1.55–1.75 (2H; m; H-7), 2.20 (3H; s; 2-CH₃), 2.49–2.56 (2H; m; H-8), 3.35 (3H; s; COOCH₃), 5.22 (1H; s; 4-H), 7.43–8.25 (8H; m; Ar-H), 9.01 (1H; s; NH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₂₇H₂₆N₂O₇: C, 66.11; H, 5.34; N, 5.71. Found: C, 66.06; H, 5.33; N, 5.73.

Ethyl 4-(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. ¹H-NMR (δ , DMSO-d₆): 0.83 (3H; s; 6-CH₃), 0.92 (3H; s; 6-CH₃), 0.99 (3H; t; J: 7.2 Hz; COOCH₂CH₃), 1.58–1.70 (2H; m; H-7), 2.05–2.37 (2H; m; H-8), 2.30 (3H; s; 2-CH₃), 3.86 (1H; dq; COOCH_{2A}-CH₃), 3.89 (1H; dq; COOCH_{2B}-CH₃), 5.17 (1H; s; 4-H), 7.42–8.19 (8H; m; Ar-H), 8.86 (1H; s; NH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₂₈H₂₈N₂O₇; C, 66.66; H, 5.59; N, 5.55. Found: C, 66.70; H, 5.61; N, 5.55.

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]⁺. Anal. Calcd. for C₃₀H₃₂N₂O₇; 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.

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. ¹H-NMR (δ , DMSO-d₆): 0.81 (3H; s; 6-CH₃), 0.91 (3H; s; 6-CH₃), 1.60–1.71 (2H; m; H-7), 2.07–2.38 (2H; m; H-8), 2.16 (3H; s; 2-CH₃), 4.92, 4.95 (2H; AB system; J_{AB} = 12.4 Hz, COOCH₂C₆H₅), 5.16 (1H; s; 4-H), 7.05–8.13 (13H; m; Ar-H), 8.89 (1H; s; NH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₃₃H₃₀N₂O₇; 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. ¹H-NMR (δ , DMSO-d₆): 0.65 (3H; d; J: 6.8 Hz; COOCH₂CHCH₃), 0.74 (3H; d; J: 6.8 Hz; COOCH₂CHCH₃), 0.81 (3H; s; 6-CH₃), 0.90 (3H; s; 6-CH₃), 1.58–1.67 (2H; m; H-7), 1.68–1.73 (1H; m; CH(CH₃)₂), 2.02–2.38 (2H; m; H-8), 2.13 (3H; s; 2-CH₃), 3.62 (1H; dd; J: 10.8/6.4 Hz; CH_{2A}CH(CH₃)₂), 3.68 (1H; dd; J: 10.8/6.4 Hz; CH_{2B}CH(CH₃)₂), 5.14 (1H; s; 4-H), 7.38–8.17 (8H; m; Ar-H), 8.81 (1H; s; NH). ¹³C-NMR (δ , DMSO-d₆): 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]⁺. Anal. Calcd. for C₃₀H₃₂N₂O₇; 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. ¹H-NMR (δ , DMSO-d₆): 0.83 (3H; s; 6-CH₃), 0.90 (3H; s; 6-CH₃), 1.26 (9H; s; COOC(CH₃)₃), 1.57–1.65 (2H; m; H-7), 1.86–2.31 (2H; m; H-8), 2.07 (3H; s; 2-CH₃), 5.05 (1H; s; 4-H), 7.39–8.21 (8H; m; Ar-H), 8.61 (1H; s; NH). ¹³C-NMR (δ , DMSO-d₆): 18.2, 23.5, 24.1, 25.1, 27.4,

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 preperation procedures were conducted in Krebs-Henseleit solution gassed with carbogen (95% O₂/5% CO₂). Aorta rings were mounted in isolated organ baths containing 50 mL Ca²⁺-free Krebs-Henseleit solution (mmol: NaCl 118, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 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 preperations were allowed to equilibrate in the Ca²⁺-free Krebs-Henseleit solution for about 45 min while washing out the tissues every ~15 min and subsequently high K⁺ (80 mM) Krebs-Henseleit solution without Ca²⁺ was applied. The rings were then contracted with 2.5 mM Ca²⁺. Following the maximal contractile response with Ca²⁺, 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 10⁻⁴ M papaverine. For each drug, six trials were conducted, the obtained data was fit into a curve and EC₅₀, pD₂ and E_{max} 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 Ca²⁺ channels, 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 (N^ω-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 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-cyclohexanone, 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
1	CH_3	200–202	$\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_6$	386
2	C_2H_5	208–210	$\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_6$	400
3	$\text{CH}_2\text{C}_6\text{H}_5$	185–187	$\text{C}_{26}\text{H}_{26}\text{N}_2\text{O}_6$	462
4	$\text{CH}_2\text{CH}(\text{CH}_3)_2$	170–171	$\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_6$	428
5	$\text{C}(\text{CH}_3)_3$	198–200	$\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_6$	428
6	CH_3	230–232	$\text{C}_{27}\text{H}_{26}\text{N}_2\text{O}_7$	490
7	C_2H_5	236–238	$\text{C}_{28}\text{H}_{28}\text{N}_2\text{O}_7$	504
8	$\text{CH}_2\text{C}_6\text{H}_5$	182–183	$\text{C}_{33}\text{H}_{30}\text{N}_2\text{O}_7$	566
9	$\text{CH}_2\text{CH}(\text{CH}_3)_2$	225–227	$\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_7$	532
10	$\text{C}(\text{CH}_3)_3$	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 $\text{M}-1$ 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_{\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
1	95.92 ± 1.87	6.20 ± 0.14^a
2	97.65 ± 1.89	5.94 ± 0.13^a
3	97.64 ± 0.75	7.24 ± 0.23
4	97.38 ± 2.30	6.94 ± 0.37
5	95.74 ± 1.58	7.16 ± 0.49
6	94.50 ± 1.92	6.27 ± 0.48^a
7	76.93 ± 8.31^a	5.57 ± 0.83^a
8	77.45 ± 6.15^a	5.44 ± 0.39^a
9	68.09 ± 4.50^a	4.91 ± 0.28^a
10	67.26 ± 0.87^a	6.25 ± 0.08^a
Nifedipine	96.81 ± 0.93	7.74 ± 0.04

^a $p < 0.001$, 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 (benzoyloxy 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

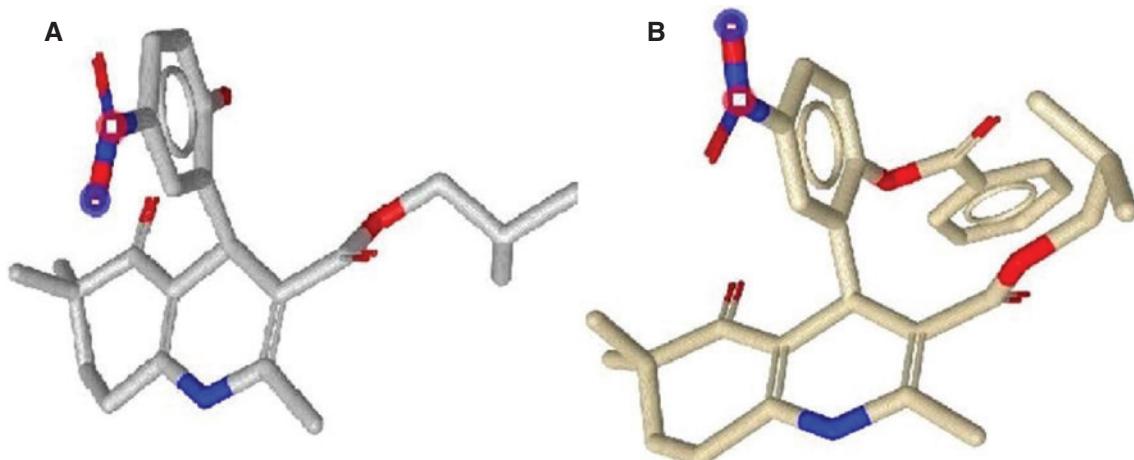


Figure 3: Geometrically optimized and energy minimized conformations of compound **4** (A) and compound **9** (B).

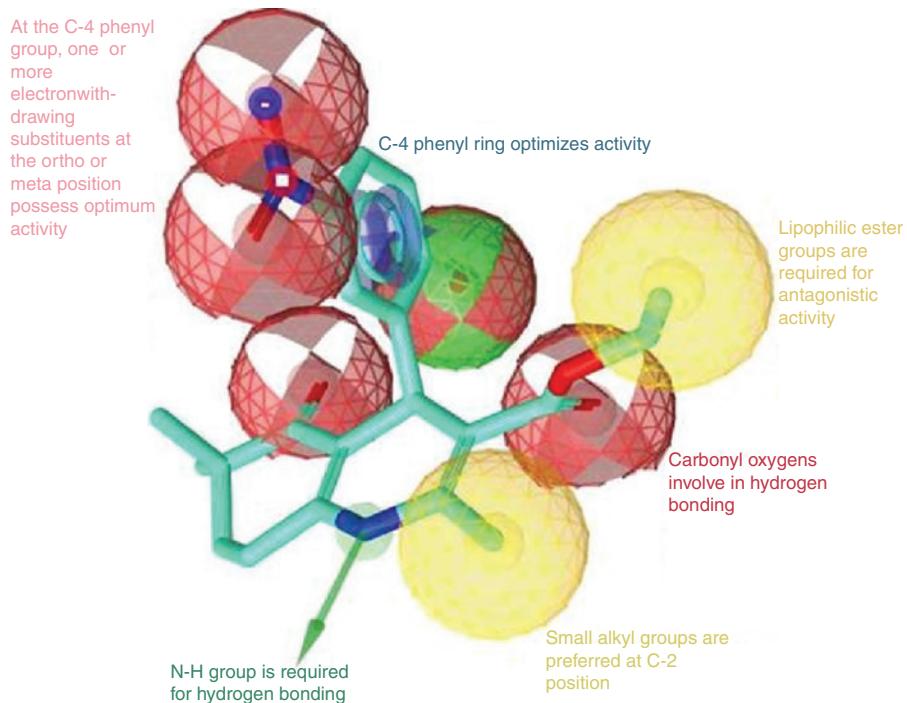


Figure 4: Structure-activity relationships for 1,4-DHPs binding to L-type calcium channels.

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.

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