Research Article

Synthesis and Myorelaxant Activity of Fused 1,4-Dihydropyridines on Isolated Rabbit Gastric Fundus

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT In the present study, 25 novel condensed 1,4-dihydropyridine (DHP) derivatives bearing cyclopentane, cyclohexane, or tetrahydrothiopene ring with a bulky and lipophilic moiety (3-pyridylmethyl) in the ester group were synthesized via a modified Hantzsch reaction, and their calcium channel modulator activities were assayed on isolated rabbit gastric fundus smooth muscle strips. To evaluate the myorelaxant effects of the compounds, the maximum relaxant response (*E*max) and pD_2 values were calculated. The results indicated that all compounds produced concentration-dependent relaxation and the introduction of five- or six-membered rings to the DHP nucleus and 3-pyridiylmethyl moiety to the ester group led to potent calcium antagonists. Drug Dev Res •• : ••–••, 2012.

Key words: condensed 1,4-dihydropyridine; calcium channel modulator; myorelaxant activity

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 [Dolphin, 2006].Although there are different types of calcium channel with distinct physiological roles, L-type channels are typically confined to cell bodies and regulate contractility in muscle cells [Zamponi, 1997].Calcium channel blockers inhibit selectively the calcium influx through cell membranes [Edraki et al., 2009]. 1,4-Dihydropyridines (DHPs), e.g., nifedipine, nicardipine, amlodipine, represent a well-known class of calcium antagonists and are commercially employed for the treatment of cardiovascular diseases, particularly hypertension and angina [Vaghy et al., 1987; Triggle, 2003; Şafak and Şimşek, 2006].

DHPs have attracted interest since their introduction into clinical medicine because of their high potency and selectivity of action [Zhou et al., 2011]. Many modifications have been carried out on the structure of nifedipine, the prototype of DHPs (Fig. 1), in order to enhance calcium modulating effects and lead new active compounds [Gordeev et al., 1998].

Some studies indicated that racemic hexahydroquinolines, indenopyridines, acridines, and furoquinolines, which are the condensed ring systems bearing DHP structure, exhibited calcium antagonist effects [Rose, 1990; Şimşek et al., 2003, 2008; Tu et al., 2004]. At the C-4 phenyl group, with one or more electron-withdrawing substituents at the *ortho* or *meta* position

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Received 22 May 2012; Accepted 29 June 2012

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/ddr.21024

are preferable rather than the other groups such as alkyls or phenyl groups carrying a substituent at the *para*position [Coburn et al., 1988]. The substituents at the C-3 and C-5 positions modulate tissue selectivity and asymmetrical substituents in these positions alter the activity [Miri et al., 2006]. 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 [Leonardi et al., 1998] including nicardipine, barnidipine, and benidipine [Tamazawa et al., 1986; Sohda et al., 1990]. In addition, the compounds possessing a 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, or 2-pyridylethyl moieties at the C-3 and C-5 positions were determined to show strong calcium channel blocking effect [Mehdipour et al., 2007]. It was estimated that the pyridinyl nitrogen free electron pair or charge distribution on the pyridinyl ring could be the important determinants for calcium channel modulatory effects [Vo et al., 1992].

The aim of the present study is to examine the influence of cyclopentane, cyclohexane, or tetrahydrothiophene rings fused to the DHP ring having a bulky moiety (3-pyridylmethyl) in the ester group and two electron-withdrawing substituents attached to the phenyl ring and to investigate the calcium modulator activities of the compounds on isolated rabbit gastric fundus smooth muscle strips.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma-Aldrich and Fluka (Steinheim, 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 (Beaconsfield, UK). Proton Nuclear Magnetic Resonance (^{1}H NMR) and Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) spectra were obtained in dimethylsulfoxide (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. The X-ray crystallographic analysis was carried out on a Nonius Kappa CCD area-detector diffractometer (Philadelphia, PA USA). 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). Purification by column chromatography was carried out on Merck silica gel 60 (0.040–0.063 mm) (Steinheim, Germany).

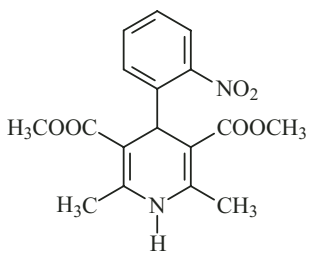


Fig. 1. Nifedipine.

CHEMISTRY

Synthesis

The synthesis of tetrahydrothiophene-3-one-1,1-dioxide and pyridine-3-ylmethyl 3-oxo-butanoate are shown in Figures 2 and 3, respectively.

Tetrahydrothiophen-3-one (0.1 mol), triethylorthoformate (0.1 mol), p-toluensulfonic acid (0.26 mmol), and 2 ml ethanol was stirred for 20 h. The mixture was treated with anhydrous sodium acetate (4 mmol), sodium tungstate dihydrate (0.00085 mmol), and 28 ml water. Twenty-eight milliliters of a 35% solution of hydrogen peroxide in water was added dropwise while keeping the reaction cooled to 30°C. After stirring overnight at room temperature, the resulting product was filtered and washed with water to achieve 3,3-diethoxytetrahydro-thiephene-1,1-dione. The product was stirred in a mixture of HCl and water at 60°C for 2 h. The mixture was extracted with dichloromethane (60 ml). The dichloromethane layer was isolated and dried with magnesium sulfate, filtered, and concentrated. The residue was crystallized from ethanol to provide tetrahydrothiophene-3-one-1,1-dioxide [Altenbach et al., 2004].

3-Pyridylcarbinol (0.02 mol), 2,2,6-trimethyl-4H-1,3-dioxin-4-one (0.02 mol), and 10 ml xylene were stirred in an oil bath at 150°C. After an hour, xylene was removed under diminished pressure, and the residue

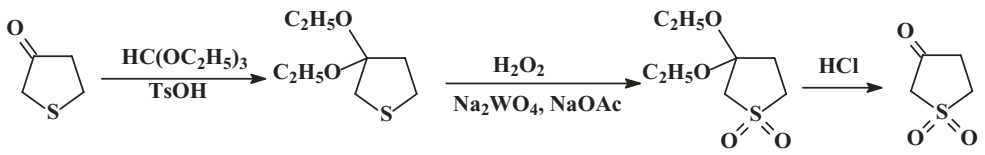


Fig. 2. Synthesis of tetrahydrothiophene-3-one-1,1-dioxide.

was then dissolved in water, extracted with ethyl acetate. The ethyl acetate layer was isolated and dried with magnesium sulfate. Ethyl acetate was removed via a rotary evaporator in order to achieve 3-pyridylmethyl 3-oxobutanoate.

Compounds one to 25 were prepared (Fig. 4) by heating appropriate 1,3-cyclodicarbonyl or tetrahydrothiophene-3-one-1,1-dioxide, the aromatic aldehyde, 3-pyridylmethyl 3-oxo-butanoate and ammonium acetate in methanol, according to the Hantzsch reaction.

3-Pyridylmethyl 2-methyl-4-(2,3-disubstituted phenyl)-5-oxo-4,5,6,7-tetrahydro-1H-cyclopenta [b]pyridine-3-carboxylate (compounds one to five)

1,3-Cyclopentanedione (0.001 mol), 2,3-disubstituted benzaldehyde (0.001 mol), 3-pyridylmethyl 3-oxobutanoate (0.001 mol), and ammonium acetate (0.005 mol) were refluxed for 8 h in 15 ml methanol. After the reaction was completed, the reaction mixture was poured into ice water. The obtained precipitate was filtered and crystallized from appropriate solvents. Some compounds were purified by column chromatography using silica gel as the solid phase and a 7:3 mixture of ethyl acetate: methanol as mobile phase.

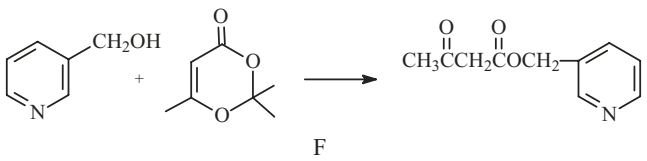


Fig. 3. Synthesis of 3-pyridylmethyl 3-oxo-butanoate.

3-Pyridylmethyl 5-methyl-7-(2,3-disubstituted phenyl)-2,3,4,7-tetrahydrothieno[3,2-b]pyridine-6-carboxylate-1,1-dioxide (compounds six to 10)

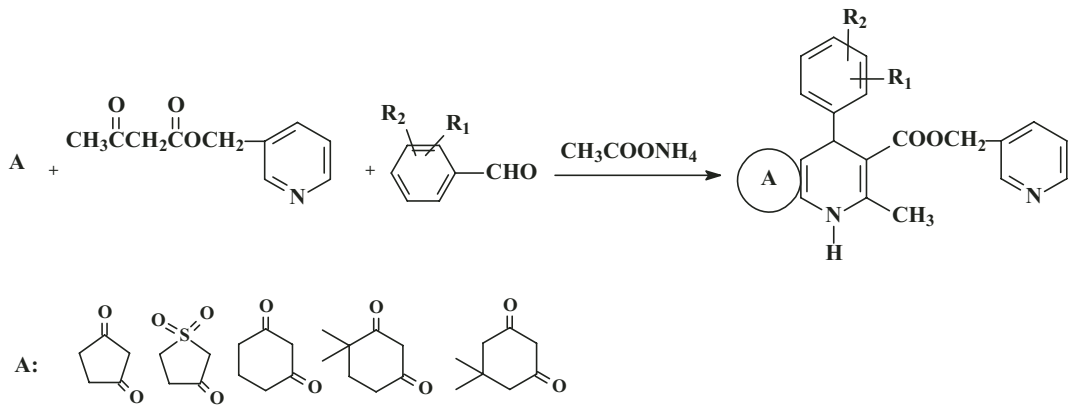
Tetrahydrothiophene-3-on-1,1-dioxide (0.001 mol), 2,3-disubstituted benzaldehyde (0.001 mol), 3-pyridylmethyl 3-oxobutanoate (0.001 mol), and ammonium acetate (0.005 mol) were refluxed for 8 h in 15 ml methanol. The precipitate, which was obtained after cooling the reaction mixture, was crystallized from methanol.

3-Pyridylmethyl (2-methyl/2,6,6-trimethyl/2,7,7-trimethyl)-5-oxo-4-(2,3-disubstituted phenyl)-1, 4,5,6,7,8-hexahydroquinoline-3-carboxylate (compounds 11–25)

0.001 mol appropriate 1,3-cyclohexanedione (1,3-cyclohexanedione, 4,4-dimethyl-1,3-cyclohexane dione, 5,5-dimethyl1,3-cyclohexanedione), 2,3-disubstituted benzaldehyde (0.001 mol), 3-pyridylmethyl 3-oxobutanoate (0.001 mol), and ammonium acetate (0.005 mol) were refluxed for 8 h in 15 ml methanol. The precipitate, which was obtained after cooling the reaction mixture, was filtered and crystallized from appropriate solvents to achieve the target compound.

3-Pyridylmethyl 2-methyl-4-(2,3-dichlorophenyl)-35-oxo-4,5,6,7-tetrahydro-1H-cyclopenta[b]pyridine-3-carboxylate (1)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 2.10–2.23 (2H, m, H-6), 2.32 (3H, s, 2-CH_3), 2.50–2.56 (2H, m, H-7), 4.88 and 5.06 (2H, AB system, J_{AB}: 12.8 Hz, $COOCH_2$), 5.18 (1H, s, H-4), 7.15–7.40 (5H, m, aromatic), 8.30 (1H, s, pyridine H-2), 8.45 (1H, d, J: 3.6 Hz, pyridine H-6), 9.90 (1H, s, NH).



R_1, R_2: 2,3-Cl_2; 2-F, 3-Cl; 2,3-F_2; 2-Cl, 3-CF_3; 2-F, 3-CF_3

Fig. 4. Synthesis of compounds 1 to 25.

^{13}CNMR (δ, DMSO-d_6): 18.8, 23.7, 33.2, 35.9, 62.4, 102.5, 115.8, 123.2, 127.7, 128.0, 129.2, 130.1, 131.3, 131.9, 135.4, 146.9, 147.7, 148.8, 149.0, 163.8, 166.0, 200.4. MS (m/z): 429 $[M]^+$. Anal. calcd. (analysis calculated) for $C_{22}H_{18}Cl_2N_2O_3$: C: 61.55, H: 4.23, N: 6.53. Found: C: 61.42, H: 4.19, N: 6.49.

3-Pyridylmethyl 2-methyl-4-(2-fluoro-3-chlorophenyl)-5-oxo-4,5,6,7-tetrahydro-1H-cyclopenta[b]pyridine-3-carboxylate (2)

Purified via column chromatography. ^{1}HNMR (δ, DMSO-d_6): 2.10–2.20 (2H, m, H-6), 2.30 (3H, s, 2-CH_3), 2.50–2.56 (2H, m, H-7), 4.90 and 5.08 (2H, AB system, J_{AB}: 12.8 Hz, $COOCH_2$), 5.18 (1H, s, H-4), 7.15–7.40 (5H, m, aromatic), 8.32 (1H, s, pyridine H-2), 8.46 (1H, d, pyridine H-6), 9.95 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.8, 23.8, 33.2, 36.0, 62.4, 102.5, 115.8, 123.1, 127.7, 128.0, 129.2, 130.1, 131.4, 131.9, 135.4, 146.8, 147.7, 148.8, 149.0, 163.8, 166.0, 200.3. MS (m/z): 412 $[M]^+$. Anal. calcd. for $C_{22}H_{18}ClFN_2O_3$: C, 64.00; H, 4.39; N, 6.79. Found: C 64.12; H, 4.41; N, 6.88.

3-Pyridylmethyl 2-methyl-4-(2,3-difluorophenyl)-5-oxo-4,5,6,7-tetrahydro-1H-cyclopenta[b]pyridine-3-carboxylate (3)

Crystallized from ethyl acetate. ^{1}HNMR (δ, DMSO-d_6): 2.12–2.24 (2H, m, H-7), 2.30 (3H, s, 2-CH_3), 2.46–2.54 (2H, m, H-6), 4.93 (1H, s, H-4), 4.90 and 5.03 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 6.85–7.40 (5H, m, aromatic), 8.4 (1H, d, J: 1.6 Hz, pyridine H-6), 8.45 [1H, doublet of doublets (dd), J: 4.8/1.6 Hz, pyridine H-2], 9.87 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.8, 30.7, 33.3, 38.8, 62.5, 101.6, 114.5, 123.2, 124.1, 131.8, 135.4, 136.3, 145.5, 147.9, 148.0, 148.9, 149.2, 150.4, 150.6, 164.0, 166.0, 200.5. MS (m/z): 395 $[M-1]^+$. Anal. Calcd. for$C_{22}H_{18}F_2N_2O_3$: C, 66.66; H, 4.58; N, 7.07. Found: C, 66.78; H, 4.63; N, 7.12.

3-Pyridylmethyl 2-methyl-4-(2-chloro-3-trifluoromethylphenyl)-5-oxo-4,5,6,7-tetra-hydro-1H-cyclopenta[b]pyridine-3-carboxylate (4)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 2.12–2.25 (2H, m, H-7), 2.33 (3H, s, 2-CH_3), 2.49–2.57 (2H, m, H-6), 5.28 (1H, s, H-4), 4.89 and 5.04 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 7.18–7.58 (5H, m, aromatic), 8.23 (1H, d, J: 2 Hz, pyridine H-2), 8.43 (1H, dd, J: 4.8/2 Hz, pyridine H-6), 9.93 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.9, 33.2 34.8, 38.8, 62.4, 102.2, 115.6, 121.7, 123.1, 124.5, 125.8, 126.1, 127.2, 129.3, 131.9, 134.8, 135.3, 146.9, 148.2, 148.9, 164.0, 166.0, 200.4. MS (m/z): 462 $[M]^+$. Anal. calcd. for $C_{23}H_{18}ClF_3N_2O_3$: C, 59.68; H, 3.92; N, 6.05. Found: C, 59.71; H, 4.00; N, 6.11.

3-Pyridylmethyl 2-methyl-4-(2-fluoro-3-trifluoromethylphenyl)-5-oxo-4,5,6,7-tetra-hydro-1H-cyclopenta[b]pyridine-3-carboxylate (5)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 2.16–2.25 (2H, m, H-7), 2.34 (3H, s, 2-CH_3), 2.49–2.57 (2H, m, H-6),5.02 (1H, s, H-4), 4.94 and 5.03 (2H, AB system, J_{AB}: 12.8 Hz, $COOCH_2$), 7.20–7.50 (5H, m, aromatic), 8.3 (1H, d, J: 1.6 Hz, pyridine H-6), 8.45 (1H, dd, J: 4.8/1.6 Hz, pyridine H-2), 9.93 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.9, 30.6, 33.3, 39.0, 62.5, 101.4, 115.0, 121.4, 123.2, 124.1, 124.5, 124.7, 124.9, 131.8, 135.0, 135.3, 135.4, 135.6, 148.3, 148.9, 164.2, 165.9, 200.5. MS (m/z): 445 $[M-1]^+$. Anal. calcd. for$C_{23}H_{18}F_4N_2O_3$: C, 61.88; H, 4.06; N, 6.28. Found: C, 62.01; H, 4.17; N, 6.22.

3-Pyridylmethyl 7-(2,3-dichlorophenyl)-5-methyl-2,3,4,7-tetrahydrothieno[3,2-b] pyridine-6-carboxylate-1,1-dioxide (6)

Crystallized from methanol. ^{1}HNMR (δ, DMSO-d_6): 2.12 (1H, doublet of doublet of doublets [ddd], H-3_A), 2.35 (3H, s, 5-CH_3), 2.45 (1H, ddd, H-3_B), 3.04 (1H, ddd, H-2_A), 3.39 (1H, ddd, H-2_B), 4.73 (1H, s, H-7), 4.78 and 4.9 (2H, AB system, J_{AB}: 13.2 Hz, $COOCH_2$), 6.01 (1H, s, NH), 7.05–7.47 (5H, m, aromatic), 8.16 (1H, d, J: 1.6 Hz, pyridine H-2), 8.42 (1H, dd, J: Hz, 4.8/1.6, pyridine H-6). ^{13}CNMR (δ, DMSO-d_6): 20.7, 34.1, 34.7, 51.0, 62.1, 65.5, 81.3, 90.8, 123.5, 127.6, 128.8, 129.4, 130.5, 131.8, 132.9, 135.0, 143.3, 148.8, 149.1, 155.0, 166.4. MS (m/z): 464 $[M]^+$. Anal. calcd. for $C_{21}H_{18}Cl_2N_2O_4S$: C, 54.20; H, 3.90; N, 6.02; S, 6.89. Found: C, 54.13; H, 4.01; N, 6.11; S, 6.93.

3-Pyridylmethyl 7-(2-fluoro-3-chlorophenyl)-5-methyl-2,3,4,7-tetrahydrothieno[3,2-b]pyridine-6-carboxylate-1,1-dioxide (7)

Crystallized from isopropanol. ^{1}HNMR (δ, DMSO-d_6): 2.13 (1H, ddd, H-3_A), 2.33 (3H, s, 5-CH_3), 2.45 (1H, ddd, H-3_B), 3.04 (1H, ddd, H-2_A), 3.42 (1H, ddd, H-2_B), 4.61 (1H, s, H-7), 4.81 and 5.01 (2H, AB system, J_{AB}: 13.6 Hz, $COOCH_2$), 5.94 (1H, s, NH), 7.05–7.40 (5H, m, aromatic), 8.18 (1H, d, J: 1.6 Hz, pyridine H-2), 8.42 (1H, dd, J: 4.8/1.6 Hz, pyridine H-6). ^{13}CNMR (δ, DMSO-d_6): 20.3, 28.4, 30.6, 34.2, 50.5, 61.6, 65.7, 80.7, 89.3, 119.1, 123.1, 124.4, 128.9,

132.2, 132.4, 134.6, 148.6, 153.5, 154.6, 155.9, 165.9. MS (m/z): 447 $[M-1]^+$. Anal. calcd. for $C_{21}H_{18}ClFN_2O_4S$: C, 56.19; H, 4.04; N, 6.24; S, 7.14. Found: C, 56.33; H, 4.14; N, 6.33; S, 7.08.

3-Pyridylmethyl 7-(2,3-difluorophenyl)-5-methyl-2,3,4,7-tetrahydrothieno[3,2-b] pyridine-6-carboxylate-1,1-dioxide (8)

Crystallized from methanol. ^{1}HNMR (δ, DMSO-d_6): 2.14 (1H, ddd, H-3_A), 2.33 (3H, s, 5-CH_3), 2.46 (1H, ddd, H-3_B), 3.04 (1H, ddd, H-2_A), 3.42 (1H, ddd, H-2_B), 4.63 (1H, s, H-7), 4.81 and 5.02 (2H, AB system, J_{AB}: 13.2 Hz, $COOCH_2$), 5.97 (1H, s, NH), 6.99–7.25, (5H, m, aromatic), 8.16 (1H, s, pyridine H-2), 8.41 (1H, dd, J: 4.8/1.6 Hz, pyridine H-6). ^{13}CNMR (δ, DMSO-d_6): 20.8, 28.6, 34.7, 50.9, 62.1, 66.3, 81.2, 89.8, 115.4, 115.6, 123.6, 124.1, 125.7, 133.0, 133.4, 133.5, 135.1, 148.9, 149.1, 155.0, 166.4. MS (m/z): 432 $[M]^+$. Anal. calcd. for$C_{21}H_{18}F_2N_2O_4S$: C, 58.33; H, 4.20; N, 6.48; S, 7.41. Found: C, 58.55; H, 4.29; N, 6.41; S, 7.45.

3-Pyridylmethyl 7-(2-chloro-3-trifluoromethylphenyl)-5-methyl-2,3,4,7-tetrahydro-thieno[3,2-b]pyridine-6-carboxylate-1,1-dioxide (9)

Crystallized from methanol. ^{1}HNMR (δ, DMSO-d_6): 2.14 (1H, ddd, H-3_A), 2.37 (3H, s, 5-CH_3), 2.45 (1H, ddd, H-3_B), 3.06 (1H, ddd, H-2_A), 3.44 (1H, ddd, H-2_B), 4.81 (1H, s, H-7), 4.76 and 4.99 (2H, AB system, J_{AB}: 13.2 Hz, $COOCH_2$), 5.95 (1H, s, NH), 7.06–7.68 (5H, m, aromatic), 8.14 (1H, d, J: 2 Hz, pyridine H-2), 8.39 (1H, dd, J: Hz, 4.8/2.0, pyridine H-6). ^{13}CNMR (δ, DMSO-d_6): 20.2, 32.6, 34.1, 50.6, 61.6, 64.9, 80.8, 89.9, 121.6, 123.0, 124.4, 125.9, 126.3, 126.7, 127.0, 129.4, 132.2, 134.6, 142.9, 148.6, 154.9. 165.8. MS (m/z): 498 $[M]^+$. Anal. calcd. for $C_{22}H_{18}ClF_3N_2O_4S$: C, 52.96; H, 3.64; N, 5.61; S, 6.43. Found: C, 53.17; H, 3.77; N, 5.72; S, 6.50.

3-Pyridylmethyl 7-(2-fluoro-3-trifluoromethylphenyl)-5-methyl-2,3,4,7-tetrahydro-thieno[3,2-b]pyridin-6-carboxylate-1,1-dioxide (10)

Crystallized from ethanol. ^{1}HNMR (δ, DMSO-d_6): 2.16 (1H, ddd, H-3_A), 2.35 (3H, s, 5-CH_3), 2.46 (1H, ddd, H-3_B), 3.06 (1H, ddd, H-2_A), 3.43 (1H, ddd, H-2_B), 4.65 (1H, s, H-7), 4.79 and 5.02 (2H, AB system, J_{AB}: 13.6 Hz, $COOCH_2$), 6.00 (1H, s, NH), 7.11–7.60 (5H, m, aromatic), 8.15 (1H, s, pyridine H-2), 8.41 (1H, dd, J: 4.4/2.0 Hz, pyridine H-6). ^{13}CNMR (δ, DMSO-d_6): 20.3, 27.9, 34.1, 50.5, 61.6, 65.6, 80.7, 88.9, 121.4, 123.0. 123.9, 124.1, 125.1, 132.1, 132.2, 132.4, 134.5, 135.0, 148.4, 148.6, 154.9, 165.8. MS (m/z): 481 $[M-1]^+$. Anal. calcd. for $C_{22}H_{18}F_4N_2O_4S$: C, 54.77; H, 3.76; N, 5.81; S, 6.65. Found: C, 54.91; H, 3.65; N, 5.94; S, 6.71.

3-Pyridylmethyl 2-methyl-4-(2,3-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (11)

Crystallized from ethanol. ^{1}HNMR (δ, DMSO-d_6): 1.70-2.50 (6H, m, H-6,7,8), 2.26 (3H, s, 2-CH_3), 4.98 and 5.06 (2H, AB system, J_{AB}: 12.8 Hz, $COOCH_2$), 5.25 (1H, s, H-4), 7.13–7.47 (5H, m, aromatic), 8.36 (1H, d, J: 2.4 Hz, pyridine H-2), 8.47 (1H, dd, J: 4.0/ 1.6 Hz, pyridine H-6), 9.32 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.8, 22.2, 26.6, 36.3, 37.1, 62.8, 102.8, 111.4, 112.5, 123.7, 127.6, 128.3, 130.3, 131.9, 132.6, 136.0, 146.9, 148.5, 149.6, 152.1, 166.8, 172.7, 194.9. MS (m/z): 442 $[M]^+$. Anal. calcd. for $C_{23}H_{20}Cl_2N_2O_3$: C, 62.31; H, 4.55; N, 6.32. Found: C, 62.55; H, 4.66; N, 6.21.

3-Pyridylmethyl 2-methyl-4-(2-fluoro-3-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (12)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 1.75-2.50 (6H, m, H-6,7,8), 2.27 (3H, s, 2-CH_3), 5.08 (1H, s, H-4), 4.98 and 5.08 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 6.98–7.53 (5H, m, aromatic), 8.40 (1H, d, J: 2.0 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.0/ 2.0 Hz, pyridine H-6), 9.31 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.8, 21.2, 26.5, 31.7, 37.0, 63.0, 102.0, 110.6, 119.7, 123.8, 125.1, 128.3, 129.9, 132.4, 136.1, 137.1, 147.2, 149.4, 152.1, 153.4, 155.9, 166.6, 194.9.MS (m/z): 425 $[M-1]^+$. Anal. calcd. for $C_{23}H_{20}ClFN_2O_3$: C, 64.72; H, 4.72; N, 6.56. Found: C, 65.03; H, 4.91; N, 6.69.

3-Pyridylmethyl 2-methyl-4-(2,3-difluorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (13)

Crystallized from carbon tetrachloride. ^{1}HNMR (δ, DMSO-d_6): 1.71–2.51 (6H, m, H-6,7,8), 2.28 (3H, s, 2-CH_3), 4.99 and 5.08 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.09 (1H, s, H-4), 6.92–7.54 (5H, m, aromatic), 8.40 (1H, d, J: 2.4 Hz, pyridine H-2), 8.49 (1H, dd, J: 5.2/ 2.4 Hz, pyridine H-6), 9.30 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.3, 20.7, 26.0, 30.6, 36.5, 62.5, 101.5, 110.2, 114.5, 123.3, 123.9, 125.4, 132.0, 135.6, 137.5, 146.6, 148.2, 148.9, 149.1, 150.6, 151.6, 166.1, 194.3. MS (m/z): 410 $[M]^+$. Anal. calcd. for

$C_{23}H_{20}F_2N_2O_3$: C, 67.31; H, 4.91; N, 6.83. Found: C, 67.52; H, 5.11; N, 6.67.

3-Pyridylmethyl 2-methyl-4-(2-chloro-3-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carboxylate (14)

Crystallized from methanol. ^{1}HNMR (δ, DMSO-d_6): 1.781–2.51 (6H, m, H-6,7,8), 2.27 (3H, s, 2-CH_3), 4.98 and 5.06 (2H, AB system, J_{AB}: 13.2 Hz, $COOCH_2$), 5.34 (1H, s, H-4), 7.23–7.57 (5H, m, aromatic), 8.33 (1H, d, J: 2.0 Hz, pyridine H-2), 8.45 (1H, dd, J: 4.8/ 2.0 Hz, pyridine H-6), 9.35 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.3, 20.6, 26.3, 32.9, 36.6, 62.3, 102.1, 110.8, 111.8, 121.8, 123.2, 124.5, 125.4, 126.1, 126.9, 129.4, 132.1, 135.4, 146.8, 148.0, 149.0, 151.8, 166.2, 194.4. MS (m/z): 475 $[M-1]^+$. Anal. calcd. for $C_{24}H_{20}ClF_3N_2O_3$: C, 60.45; H, 4.23; N, 5.87. Found: C, 60.61; H, 4.40; N, 6.00.

3-Pyridylmethyl 2-methyl-4-(2-fluoro-3-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carboxylate (15)

Crystallized from cyclohexane. ^{1}HNMR (δ, DMSO-d_6): 1.69–2.50 (6H, m, H-6,7,8), 2.20 (3H, s, 2-CH_3), 4.99 and 5.04 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.14 (1H, s, H-4), 7.18–7.52 (5H, m, aromatic), 8.38 (1H, d, J: 2.0 Hz, pyridine H-2), 8.48 (1H, dd, J: 4.4/ 2.0 Hz, pyridine H-6), 9.34 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.9, 21.2, 26.7, 31.1, 36.9, 63.0, 101.8, 110.6, 111.8, 116.3, 123.7, 124.7, 125.2, 132.5, 135.8, 136.0, 137.2, 147.5, 149.5, 152.3, 154.9, 157.4, 166.6, 194.9. MS (m/z): 460 $[M]^+$. Anal. calcd. for $C_{24}H_{20}F_4N_2O_3$: C, 62.61; H, 4.38; N, 6.08. Found: C, 62.77; H, 4.44; N, 5.87.

3-Pyridylmethyl 2,6,6-trimethyl-4-(2,3-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (16)

Crystallized from ethanol. ^{1}HNMR (δ, DMSO-d_6): 0.79 (3H, s, 6-CH_3), 0.95 (3H, s, 6-CH_3), 1.64–1.76 (2H, m, H-7), 2.24 (3H, s, 2-CH_3), 2.40–2.50 (2H, m, H-8), 4.99 and 5.06 (2H, AB system, J_{AB}: 13.2 Hz, $COOCH_2$), 5.20 (1H, s, H-4), 7.12–7.48 (5H, m, aromatic), 8.37 (1H, d, J: 1.6 Hz, pyridine H-2), 8.47 (1H, dd, J: 4.4/ 1.6 Hz, pyridine H-6), 9.24 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.3, 22.9, 24.1, 24.6, 33.9, 36.1, 62.3, 101.9, 109.2, 123.2, 127.5, 127.7, 129.6, 130.1, 131.3, 132.1, 133.4, 135.6, 146.3, 148.2, 148.9, 149.1, 149.7, 166.3, 199.1. MS (m/z): 470 $[M]^+$. Anal. calcd. for $C_{25}H_{24}Cl_2N_2O_3$: C, 63.70; H, 5.13; N, 5.94. Found: C, 63.88; H, 5.22; N, 6.13.

3-Pyridylmethyl 2,6,6-trimethyl-4-(2-fluoro-3-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylate (17)

Crystallized from benzene–petroleum benzene (40:60). ^{1}HNMR (δ, DMSO-d_6): 0.81 (3H, s, 6-CH_3), 0.95 (3H, s, 6-CH_3), 1.65–1.76 (2H, m, H-7), 2.27 (3H, s, 2-CH_3), 2.40–2.52 (2H, m, H-8), 4.98 and 5.07 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.04 (1H, s, H-4), 6.97–7.55 (5H, m, aromatic), 8.42 (1H, d, J: 1.6 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.8/ 1.6 Hz, pyridine H-6), 9.25 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 22.8, 24.1, 24.7 31.5, 33.9, 62.5, 101.1, 108.4, 119.2, 123.3, 124.6, 127.8, 129.2, 132.0, 135.7, 136.7, 136.9, 146.7, 149.0, 149.8, 152.8, 155.2, 166.2, 199.1. MS (m/z): 453 $[M-1]^+$. Anal. calcd. for $C_{25}H_{24}Cl_2N_2O_3$: C, 66.00; H, 5.32; N, 6.16. Found: C, 65.73; H, 5.21; N, 6.05.

3-Pyridylmethyl 2,6,6-trimethyl-4-(2,3-difluorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (18)

Crystallized from benzene. ^{1}HNMR (δ, DMSO-d_6): 0.81 (3H, s, 6-CH_3), 0.95 (3H, s, 6-CH_3), 1.65–1.74 (2H, m, H-7), 2.27 (3H, s, 2-CH_3), 2.40–2.52 (2H, m, H-8), 4.99 and 5.07 (2H, AB system, J_{AB}: 12.8 Hz, $COOCH_2$), 5.05 (1H, s, H-4), 6.91–7.56 (5H, m, aromatic), 8.41 (1H, d, J: 1.6 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.8/ 1.6 Hz, pyridine H-6), 9.25 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.3, 22.8, 24.1, 24.7, 30.9, 33.9, 39.0, 62.4, 101.2, 108.4, 114.2, 114.4, 123.3, 123.8, 123.9, 125.3, 132.0, 133.1, 135.6, 146.6, 148.9, 149.1, 149.8, 166.2, 199.1. MS (m/z): 438 $[M]^+$. Anal. calcd. for $C_{25}H_{24}F_2N_2O_3$: C, 68.48; H, 5.52; N, 6.39. Found: C, 68.63; H, 5.40; N, 6.56.

3-Pyridylmethyl 2,6,6-trimethyl-4-(2-chloro-3-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexa-hydroquinoline-3-carboxylate (19)

Crystallized from methanol. ^{1}HNMR (δ, DMSO-d_6): 0.79 (3H, s, 6-CH_3), 0.95 (3H, s, 6-CH_3), 1.63–1.74 (2H, m, H-7), 2.25 (3H, s, 2-CH_3), 2.40–2.53 (2H, m, H-8), 4.98 and 5.04 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.29 (1H, s, H-4), 7.25–7.55 (5H, m, aromatic), 8.34 (1H, d, J: 2.0 Hz, pyridine H-2), 8.46 (1H, dd, J: 5.2/ 2.0 Hz, pyridine H-6), 9.30 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 21.6, 22.9, 24.1, 24.6, 33.8, 35.3, 62.3, 101.6, 109.0, 121.8, 123.2, 124.5, 125.3, 126.0, 126.8, 129.4, 132.1, 135.5, 146.8, 148.0, 148.8, 149.1, 150.0, 166.3, 199.1. MS (m/z): 503 $[M-1]^+$. Anal. calcd. for $C_{26}H_{24}ClF_3N_2O_3$: C, 61.85; H, 4.79; N, 5.55. Found: C, 61.61; H, 4.83; N, 5.53.

3-Pyridylmethyl 2,6,6-trimethyl-4-(2-fluoro-3-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (20)

Crystallized from ethyl acetate/benzene. ^{1}HNMR (δ, DMSO-d_6): 0.80 (3H, s, 6-CH_3), 0.95 (3H, s, 6-CH_3), 1.62–1.75 (2H, m, H-7), 2.27 (3H, s, 2-CH_3), 2.38–2.52 (2H, m, H-8), 5.02 (2H, s, $COOCH_2$), 5.09 (1H, s, H-4), 7.16–7.54 (5H, m, aromatic), 8.39 (1H, d, J: 2.0 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.8/ 2.0 Hz, pyridine H-6), 9.29 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 22.8, 24.1, 24.6, 31.2, 33.9, 40.1, 62.5, 100.9, 108.2, 115.6, 121.5, 123.2, 124.1, 124.3, 124.6, 132.0, 135.5, 136.6, 147.0, 149.1, 150.0, 154.4, 157.0, 166.1, 199.1. MS (m/z): 488 $[M]^+$. Anal. calcd. for $C_{26}H_{24}F_4N_2O_3$: C, 63.93; H, 4.95; N, 5.73. Found: C, 63.76; H, 4.86; N, 5.86.

3-Pyridylmethyl 2,7,7-trimethyl-4-(2,3-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (21)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 0.81 (3H, s, 7-CH_3), 0.99 (3H, s, 7-CH_3), 1.91 and 2.14 (2H, AB system, J_{AB}: 16.0 Hz, H-8), 2.26 (3H, s, 2-CH_3), 2.26 and 2.42 (2H, AB system, J_{AB}: 16.8 Hz, H-6), 4.98 and 5.07 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.23 (1H, s, H-4), 7.13–7.47 (5H, m, aromatic), 8.36 (1H, d, J: 2.0 Hz, pyridine H-2), 8.47 (1H, dd, J: 4.4/ 2.0 Hz, pyridine H-6), 9.24 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 26.3, 28.9, 31.9, 35.8, 38.8, 50.1, 62.3, 102.2, 109.7, 123.2, 127.4, 127.8, 129.7, 130.1, 131.4, 132.1, 135.5, 146.6, 147.8, 148.9, 149.1, 149.6, 166.4, 194.0. MS (m/z): 470 $[M]^+$. Anal. calcd. for $C_{25}H_{24}Cl_2N_2O_3$: C, 63.70; H, 5.13; N, 5.94. Found: C, 63.46; H, 5.25; N, 5.88.

3-Pyridylmethyl 2,7,7-trimethyl-4-(2-fluoro-3-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (22)

Crystallized from methanol. ^{1}HNMR (δ, DMSO-d_6): 0.81 (3H, s, 7-CH_3), 0.99 (3H, s, 7-CH_3), 1.92 and 2.14 (2H, AB system, J_{AB}: 16.0 Hz, H-8), 2.28 (3H, s, 2-CH_3), 2.27 and 2.42 (2H, AB system, J_{AB}: 17.2 Hz, H-6), 4.98 and 5.07 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.05 (1H, s, H-4), 6.98–7.54 (5H, m, aromatic), 8.41 (1H, d, J: 2.0 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.0/ 1.4 Hz, pyridine H-6), 9.25 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 26.2, 28.9, 31.2, 32.0, 48.5, 50.0, 62.5, 101.5, 108.9, 119.3, 123.3, 124.5, 127.9, 129.3, 131.9, 135.7, 136.5, 146.8, 149.0, 149.8, 152.8, 155.3, 166.1, 194.0. MS (m/z): 454 $[M]^+$. Anal. calcd. for $C_{25}H_{24}Cl_2N_2O_3$: C, 66.00; H, 5.32; N, 6.16. Found: C, 66.22; H, 5.47; N, 6.22.

3-Pyridylmethyl 2,7,7-trimethyl-4-(2,3-difluorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (23)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 0.81 (3H, s, 7-CH_3), 0.99 (3H, s, 7-CH_3), 1.92 and 2.15 (2H, AB system, J_{AB}: 16.0 Hz, H-8), 2.28 (3H, s, 2-CH_3), 2.26 and 2.42 (2H, AB system, J_{AB}: 16.8 Hz, H-6), 4.99 and 5.08 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.06 (1H, s, H-4), 6.92–7.54 (5H, m, aromatic), 8.0 (1H, d, J: 1.6 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.0/ 1.6 Hz, pyridine H-6), 9.25 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 26.2, 28.9, 31.8, 35.8, 38.9, 50.2, 62.3, 101.5, 108.9, 114.5, 123.3, 125.4, 132.0, 135.6, 137.2, 145.4, 146.8, 147.8, 148.1, 148.9, 149.7, 150.0, 166.3, 194.1. MS (m/z): 437 $[M-1]^+$. Anal. calcd. for $C_{25}H_{24}F_2N_2O_3$: C, 68.48; H, 5.52; N, 6.39. Found: C, 68.22; H, 5.69; N, 6.59.

3-Pyridylmethyl 2,7,7-trimethyl-4-(2-chloro-3-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (24)

Crystallized from ethyl acetate/n-hexane. ^{1}HNMR (δ, DMSO-d_6): 0.82 (3H, s, 7-CH_3), 1.00 (3H, s, 7-CH_3), 1.92 and 2.14 (2H, AB system, J_{AB}: 16.0 Hz, H-8), 2.27 (3H, s, 2-CH_3), 2.27 and 2.43 (2H, AB system, J_{AB}: 17.2 Hz, H-6), 4.98 and 5.06 (2H, AB system, J_{AB}: 13.2 Hz, $COOCH_2$), 5.32 (1H, s, H-4), 7.23–7.56 (5H, m, aromatic), 8.33 (1H, d, J: 1.6 Hz, pyridine H-2), 8.45 (1H, dd, J: 5.2/ 1.6 Hz, pyridine H-6), 9.28 (1H, s, NH). ^{13}CNMR (δ, DMSO-d_6): 18.4, 26.3, 28.9, 31.9, 34.9, 38.8, 50.00, 62.3, 101.9, 109.5, 123.2, 125.5, 126.1, 126.8, 129.4, 132.1, 135.4, 135.5, 136.6, 147.0, 147.7, 148.8, 149.0, 149.9, 166.2, 194.0. MS (m/z): 504 $[M]^+$. Anal. calcd. for $C_{26}H_{24}ClF_3N_2O_3$: C, 61.85; H, 4.79; N, 5.55. Found: C, 62.07; H, 4.66; N, 5.32.

3-Pyridylmethyl 2,7,7-trimethyl-4-(2-fluoro-3-trifluoromethylphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (25)

Crystallized from ethyl acetate. ^{1}HNMR (δ, DMSO-d_6): 0.82 (3H, s, 7-CH_3), 1.00 (3H, s, 7-CH_3), 1.92 and 2.15 (2H, AB system, J_{AB}: 16.0 Hz, H-8), 2.29 (3H, s, 2-CH_3), 2.26 and 2.44 (2H, AB system, J_{AB}: 16.8 Hz, H-6), 4.99 and 5.04 (2H, AB system, J_{AB}: 12.4 Hz, $COOCH_2$), 5.10 (1H, s, H-4), 7.17–7.54 (5H, m, aromatic), 8.39 (1H, d, J: 2.0 Hz, pyridine H-2), 8.49 (1H, dd, J: 4.8/ 1.6 Hz, pyridine H-6), 9.29 (1H, s, NH).^{13}CNMR (δ, DMSO-d_6): 18.4, 22.4, 25.9, 28.4, 31.1, 32.0, 49.9, 62.6, 101.1, 108.7, 116.1, 121.5, 123.3, 124.0, 124.7, 126.9, 131.9., 135.6, 136.2, 147.2, 149.0, 150.0, 154.6, 157.2, 166.1, 194.0. MS (m/z): 487 $[M-1]^+$. Anal. calcd. for $C_{26}H_{24}F_4N_2O_3$: C, 63.93; H, 4.95; N, 5.73. Found: C, 64.11; H, 5.10; N, 5.88.

PHARMACOLOGY

New Zealand white rabbits weighing 2.5–3 kg (Laboratory Animal Production Center, Gazi University, Ankara, Turkey) were used in pharmacological studies. After the rabbits were sacrificed with injection of sodium pentobarbital (30–40 mg/kg, i.v.), the stomach was removed through an abdominal incision. The fundal part of the stomach was then dissected parallel to the longitudinal muscle wall. The obtained approximately 15–20 mm long and 2 mm wide muscle strip was allowed to equilibrate for a period of 60 min in 20-ml organ baths filled with Ca^{2+} free Krebs–Henseleit solution (KHS) of the following composition (mmol/l): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; $MgCl_2$, 0.54; $NaHPO_4$, 0.9; and glucose, 10.04.The pH of the saturated solution was 7.4. During the studies, the solution was aerated with 95% O_2 and 5% CO_2 and temperature was maintained at 37°C by a thermoregulated water circuit. Each strip was connected to a force transducer (FDT 10-A, May IOBS 99, COMMAT İletişim Co., Ankara, Turkey) for the measurement of isometric force, which was continuously displaced and recorded on an online computer via four-channel transducer data acquisition system (MP30B-CE, BIOPAC Systems Inc., Santa Barbara, CA, USA) using software (BSL PRO v 3.6.7, BIOPAC Systems Inc.) that also had the capacity of analyzing the data. After mounting, each strip was allowed to equilibrate with a basal tension of 1 g for 60 min. Ca^{2+} free KHS was replaced with fresh solution every 15 min during this period of time.

In order to eliminate whether the relaxation induced by the test compounds was due to an interaction with cyclooxygenase, adrenergic, or nitric oxide pathways, all experiments were carried out in the presence of indomethacin (COX inhibitor, 10^{-5} M), propranolol (beta adrenergic receptor blocker, 10^{-6} M), and Nω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (the nitric oxide synthase inhibitor, 10^{-4} M). L-NAME, propranolol hydrochloride, and nifedipine were dissolved in distilled water, whereas the compounds and indomethacin were dissolved in DMSO.

The calcium antagonist activities of the compounds were determined by tests performed on isolated rabbit gastric fundus smooth muscle strips. Preparations were placed in high K^+ containing (80 mM) solution. When Ca^{+2} (2.5 mM) was added to the organ bath, a contraction occurred. At the plateau level of contraction, the test compounds ($10^{-8} - 3 \times 10^{-4}$ M) and nifedipine ($10^{-9} - 10^{-5}$ M) were applied. Concentration relaxation for compounds and nifedipine were obtained by adding into the bath in a cumulative manner. Responses of test compounds and nifedipine were expressed as the percentage of the precontraction using Ca^{2+} (2.5 mM). DMSO was also tested in Ca^{2+} (2.5 mM) precontracted rings.

For evaluating the effects of an antagonist, the maximum relaxant response (Emax) and pD_2 (–log EC_{50}) values were computed by using GraphPad Prism 5.0 package program. All data were expressed as mean ± standard error of mean. Statistical comparison between the groups was performed by Mann–Whitney *U*-test and *P*-values less than 0.05 were considered to be statistically significant.

This study was approved by the Ethics Committee of Gazi University, Faculty of Medicine, Ankara, Turkey (GÜET-10.031). All procedures involving animals and their care were conducted in conformity with international laws and policies.

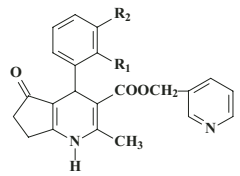
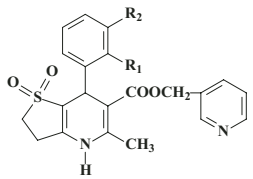
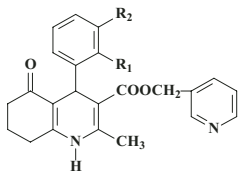
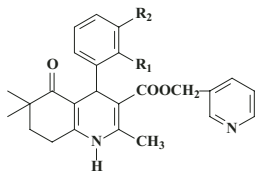
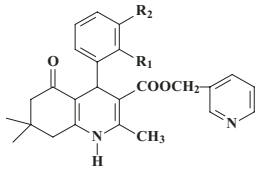
The maximum relaxant effects (*E*max) and the negative logarithm of the concentration for the half-maximal response (pD_2) values of the compounds and

TABLE 1. Maximum Relaxant Responses (Emax) and pD_2 Values of the Compounds and Nifedipine on Isolated Strips of Rabbit Gastric Fundus Smooth Muscle

Compound	Emax	pD_2	Compound	Emax	pD_2
1*	90.59 ± 3.70	5.19 ± 0.14	14	96.92 ± 3.08	5.63 ± 0.16
2	100.00 ± 0.00	5.31 ± 0.18	15	100.00 ± 0.00	5.43 ± 0.08
3	99.62 ± 0.38	5.08 ± 0.12	16	99.09 ± 0.91	5.78 ± 0.13
4	96.70 ± 2.13	4.88 ± 0.11	17	97.49 ± 2.50	5.45 ± 0.10
5	99.68 ± 0.32	4.62 ± 0.12	18	96.13 ± 3.87	5.44 ± 0.11
6*	91.76 ± 2.68	4.27 ± 0.07	19	100.00 ± 0.00	5.52 ± 0.09
7*	77.48 ± 11.97	3.81 ± 0.05	20	90.64 ± 5.42	5.17 ± 0.11
8	98.14 ± 1.17	4.78 ± 0.10	21	96.54 ± 2.90	4.89 ± 0.12
9	90.04 ± 5.20	4.87 ± 0.12	22	100.00 ± 0.00	5.11 ± 0.07
10*	71.73 ± 8.64	4.46 ± 0.17	23	100.00 ± 0.00	5.35 ± 0.15
11	98.40 ± 1.60	6.05 ± 0.09	24*	85.77 ± 5.29	4.72 ± 0.10
12	100.00 ± 0.00	5.81 ± 0.08	25	99.44 ± 0.46	4.89 ± 0.25
13	100.00 ± 0.00	5.88 ± 0.18	Nifedipine	100.00 ± 0.00	8.10 ± 0.03

*$P < 0.05$ different from nifedipine.

TABLE 2. Structural Data of Synthesized Compounds

Compound	R_1	R_2	Melting point (°C)	Empirical formula	M. W.
					
1	Cl	Cl	177	$C_{22}H_{18}Cl_2N_2O_3$	429
2	F	Cl	194	$C_{22}H_{18}ClFN_2O_3$	412
3	F	F	216	$C_{22}H_{18}F_2N_2O_3$	396
4	Cl	CF_3	209	$C_{23}H_{18}ClF_3N_2O_3$	462
5	F	CF_3	210	$C_{23}H_{18}F_4N_2O_3$	446
					
6	Cl	Cl	203	$C_{21}H_{18}Cl_2N_2O_4S$	464
7	F	Cl	184	$C_{21}H_{18}ClFN_2O_4S$	448
8	F	F	233	$C_{21}H_{18}F_2N_2O_4S$	432
9	Cl	CF_3	201	$C_{22}H_{18}ClF_3N_2O_4S$	498
10	F	CF_3	211	$C_{22}H_{18}F_4N_2O_4S$	482
					
11	Cl	Cl	205	$C_{23}H_{20}Cl_2N_2O_3$	442
12	F	Cl	200	$C_{23}H_{20}ClFN_2O_3$	426
13	F	F	204	$C_{23}H_{20}F_2N_2O_3$	410
14	Cl	CF_3	196	$C_{24}H_{20}ClF_3N_2O_3$	476
15	F	CF_3	202	$C_{24}H_{20}F_4N_2O_3$	460
					
16	Cl	Cl	221	$C_{25}H_{24}Cl_2N_2O_3$	470
17	F	Cl	204	$C_{25}H_{24}Cl_2N_2O_3$	454
18	F	F	211	$C_{25}H_{24}F_2N_2O_3$	438
19	Cl	CF_3	192	$C_{26}H_{24}ClF_3N_2O_3$	504
20	F	CF_3	185	$C_{26}H_{24}F_4N_2O_3$	488
					
21	Cl	Cl	230	$C_{25}H_{24}Cl_2N_2O_3$	470
22	F	Cl	167	$C_{25}H_{24}Cl_2N_2O_3$	454
23	F	F	200	$C_{25}H_{24}F_2N_2O_3$	438
24	Cl	CF_3	191	$C_{26}H_{24}ClF_3N_2O_3$	504
25	F	CF_3	162	$C_{26}H_{24}F_4N_2O_3$	488

nifedipine on isolated strips of rabbit gastric fundus smooth muscle are given in Table 1. DMSO, the solvent used to dissolve the test compounds, had no significant relaxant effect.

RESULTS AND DISCUSSION

Structure and properties of the prepared compounds are given in Table 2. Maximum relaxant responses (Emax) and pD_2 values of the compounds and nifedipine on isolated strips of rabbit gastric fundus smooth muscle are given in Table 1. This series of novel condensed 1,4-DHPs were prepared via a modified Hantzsch reaction and the structures elucidated by IR (infrared), ^{1}HNMR, ^{13}CNMR, and mass spectra. Elemental analysis results were within ± 0.4% of theoretical values for all compounds.

In the IR spectra, characteristic N-H, C=O (ester), C=O (ketone), and S=O stretching bonds were observed. In the ^{1}HNMR spectra, each proton of the tetrahydrothiophene ring was observed at 2.12–3.44 ppm separately and as ddd. The methyl and methine protons on the DHP ring were seen as singlets at 2.20–2.37 ppm and 4.65–5.34 ppm, respectively. The protons, which are on phenyl and pyridine rings, were seen at 6.85–8.49 ppm. The N-H signals were observed at either 5.94–6.01 ppm or 9.24–9.95 ppm as singlets. In the ^{13}CNMR spectra, the number of the signals fit 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 DHP ring to the pyridine analog were seen in the spectra of all compounds. Cleavage of the ester group and phenyl ring from the parent molecule was the next most observed fragmentations.

The structure of compound 20 was also confirmed by an X-ray crystal structure analysis (Fig. 5). The 1,4-DHP ring has a shallow boat conformation, while the oxocyclohexene ring has a half-chair conformation and the amine group forms an intermolecular N-H·····.N hydrogen bond with the pyridine ring N atom of a neighboring molecule. The halogen-substituted phenyl ring has a weak intramolecular π–π interaction with the pyridine ring. A detailed description of the structure is presented in Figure 2 [Linden et al., 2011].

The results of the tests performed on with Ca^{2+} (2.5 mM) precontracted isolated rabbit gastric fundus smooth muscle strips indicated that all of compounds and nifedipine had concentration-dependent relaxation responses with the efficacy order: Nifedipine = 2 = 12 = 13 = 15 = 19 = 22 = 23 ≥ 5 = 3 = 25 = 16 ≥ 11 = 8 ≥ 17 ≥ 14 = 4 = 21 = 18 > 6 ≥ 20 = 1 = 9 > 24 > 7 > 10. While compounds 2, 12, 13, 15, 19, 22, and 23 had the

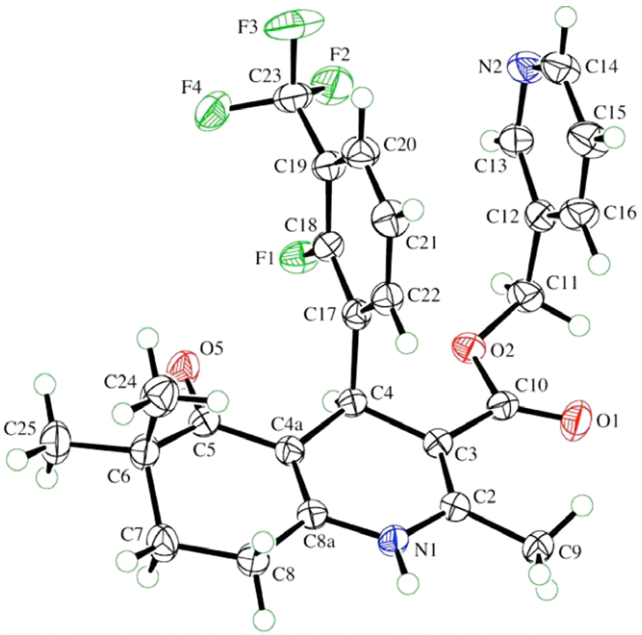


Fig. 5. The molecular structure of compound 20. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

same efficacy as nifedipine, the potency of these compounds was less compared with nifedipine. Compounds with a cyclohexane ring have the highest activities. Although there is no discrete correlation between calcium antagonist activity and the substitution of the phenyl ring, in general, compounds with a 2,3-difluorophenyl substitution showed an increased inhibition of the contraction. The introduction of the sulfur atom into the cyclopentane ring did not enhance the mentioned activity positively. There is no clear difference between the activities of the compounds with respect to the substitution of the cyclohexane ring although compounds with six membered rings fused to the 1,4-DHP nucleus were more active than their five membered ring analogs.

All compounds had lower pD_2 values compared with nifedipine. Introduction of a pyridylmethyl instead of a methyl group in ester function increases activity.

These results showed that the synthesized compounds are potent relaxing agents on isolated rabbit gastric fundus smooth muscle due to blockade of Ca^{2+} channels, similar to that of nifedipine. These results enhance understanding of the structure–activity relationships for these Ca^{2+} channels blockers.

ACKNOWLEDGMENTS

This study was supported by Hacettepe University Scientific Research and Development Office (Project No: 5222).

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