Turkish J. Pharm. Sci. 4 (2) 57-70, 2007

SYNTHESIS OF 2-[5,6-DIPHENYL-3(2H)-PYRIDAZINONE-2-YL]ACETAMIDE AND 3-[5,6-DIPHENYL-3(2H)-PYRIDAZINONE-2-YL]PROPANAMIDE DERIVATIVES AS ANALGESIC AND ANTI-INFLAMMATORY AGENTS

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Abstract

*In this study, new sixteen 2-[5,6-diphenyl-3(2H)-pyridazinone-2-yl] acetamide and 3-[5,6-diphenyl-3(2H)-pyridazinone-2-yl] propanamide derivatives were synthesized and their in vivo analgesic and anti-inflammatory activities were tested in mice. Propanamide derivatives were found more potent than acetamide derivatives in terms of anti-inflammatory activity. The compound **8e** which is a propanamide derivative exhibited higher analgesic and anti-inflammatory activities than other synthesized compounds and did not cause any gastric lesions and bleeding in stomachs of tested animals. Inhibitory activity of the active compounds on cyclooxygenase isoforms was also investigated by using in vitro human "whole blood assay" and found that these derivatives did not exert their analgesic and anti-inflammatory activities through COX inhibition and other mechanisms might be involved.*

***Key words**: 5,6-Diphenyl-3(2H)-pyridazinone, analgesic and anti-inflammatory activity, cyclooxygenase inhibition*

Analjezik ve Antienflamatuvar Ajan Olarak 2-[5,6-Difenil-3(2H)-piridazinon-2-il]asetamit ve 3-[5,6-Difenil-3(2H)-piridazinon-2-il]propanamit Türevlerinin Sentezleri

Bu çalışmada, onaltı yeni 2-[5,6-difenil-3(2H)-piridazinon-2-il] asetamit ve 3-[5,6-difenil-3(2H)-piridazinon-2-il] propanamit türevleri sentez edilmiş ve bunların analjezik ve antienflamatuvar etkileri fareler üzerinde test edilmiştir.Propanamit türevleri antienflamatuvar etki bakımından asetamit türevlerinden daha güçlü bulunmuştur. Propanamit türevi olan bileşik 8e sentezi yapılan diğer bileşikler ile karşılaştırıldığında daha yüksek analjezik ve antienflamatuvar etki göstermiş ve test edilen hayvanlarda gastrik lezyon veya kanamaya neden olmamıştır. Aktif bileşiklerin siklooksijenaz izoformları üzerine inhibitör etkileri "in vitro insan tam kan" yöntemiyle araştırılmış ve bu bileşiklerin analjezik ve antienflamatuvar etkilerini COX inhibibisyonu aracılığıyla göstermedikleri, başka mekanızmaların etkili olabileceği bildirilmiştir.

***Anahtar Kelimeler:** 5,6-Difenil-3(2H)-piridazinon, analjezik ve antienflamatuvar aktivite, siklooksijenaz inhibisyonu*

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) have huge therapeutic value in the treatment and management of pain and inflammation. These compounds inhibit the cyclooxygenase enzymes (COX-1 and COX-2), which catalyze the conversion of arachidonic acid to prostaglandins (PGs) and thus prevent the formation of PGs (1-4). COX-1 is constitutive isoform and is found in the gastrointestinal tract, the kidney and platelets, and is believed to be responsible for the maintenance of physiological homeostasis such as gastrointestinal integrity and renal function (4).

On the other hand, COX-2 is induced by many kinds of inflammatory mediators, and plays an important role in the prostaglandin biosynthesis associated with inflammatory responses. Inhibition of both COX-1 and COX-2 by classical NSAIDs leads to a decrease in all PG synthesis, which accounts for the beneficial anti-inflammatory and analgesic effects of NSAIDs as well as the gastrointestinal side effects (4). Several approaches have been proposed to reduce the unwanted side effects of NSAIDs. Many studies have focused on potent and selective COX-2 inhibitors as the next generation of anti-inflammatory agents (4). However, under physiological conditions constitutive expression of COX-2 was discovered in the brain, spinal cord and kidney as well as many organs, suggesting that this isoenzyme may play a more complex physiological role than was expected (5). So, there is still a necessity to synthesize novel, potent COX-2 inhibitors with reduced side effects when compared with COX-2 inhibitors currently in the market.

There are several classes of compounds having selective COX-2 inhibitory activity such as diarylheterocycles, diaryl ethers or thioethers, cis-stilbene derivatives, diaryl ketones. Among them, diarylheterocycles have been extensively studied (6-10). It has been reported that two aryl rings must be found on adjacent position on a central five or six membered heterocyclic ring for selective COX-2 activity (3, 4). In addition, the convertion of carboxylate moiety into amides in currently used NSAIDs such as indomethacin and meclofenamic acid was reported to result in selective COX-2 inhibitors (11,12).

Many research groups as well as our group have been interested in 3(2H)-pyridazinones for the development of potential analgesic and anti-inflammatory activities. Among them, emorfazone is marketed in Japan as an analgesic and anti-inflammatory (Figure 1.) (13, 14). In addition, 3-*O*-substituted benzyl pyridazinone derivatives have been found to exhibit *in vivo* potent anti-inflammatory activity using carrageenan-induced rat paw edema assay through the mechanism involving selective COX-2 inhibition (15).

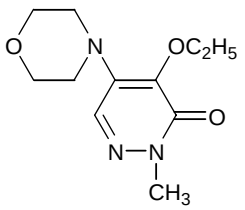


Figure 1. Emorfazone

During our researchs for developing novel non-steroidal anti-inflammatory drugs, we have been interested in several heterocyclic systems, one of which is 3(2H)-pyridazinone ring. We have recently synthesized 3(2H)-pyridazinones having acetamide or propanamide groups at

position 2 of the pyridazinone ring and some derivatives were found to have potent analgesic and anti-inflammatory activities (16-18).

These findings prompted us to prepare 5,6-diphenyl-3(2H)-pyridazinone derivatives carrying acetamide and propanamide groups at the position 2 (Figure 2.) and evaluate their analgesic and anti-inflammatory activities by using *p*-benzoquinone-induced writhing test and carrageenan-induced hind paw edema model, respectively. Inhibitory activity on cyclooxygenase isoforms of the active compounds was also investigated by using *in vitro* human whole blood assay.

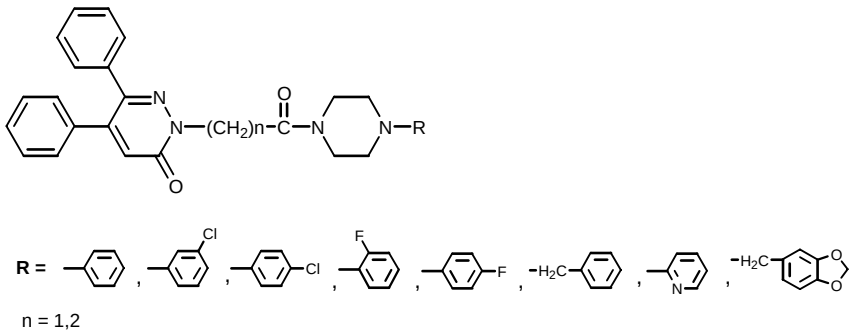


Figure 2. General structure of the title compounds

EXPERIMENTAL

Chemistry

2-Phenylacetophenone (deoxybenzoin) and other all chemicals were purchased from Aldrich Co (Germany). Synthesis of the compounds **1-4** were accomplished according to the previously reported procedures (19-21). Synthesis of intermediary compounds **5-8** were prepared for first time in this study. Melting points were determined with Electrothermal-9300 digital melting point apparatus, and the values are uncorrected. IR spectra of the compounds were recorded on Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrophotometer (KBr, ν, cm^{-1}). The ^{1}H-NMR spectra were recorded on a Jeol 500 MHz-NMR spectrometer using TMS as internal standard in DMSO-d_6 at Institute for Medicinal and Dental Engineering of Tokyo Medicinal and Dental University of Tokyo-Japan. All chemical shifts were recorded as δ (ppm). Elemental analyses were performed with Leco-932 (C, H, N Elemental analyzer, St. Joseph, USA) at Scientific and Technical Research Council of Ankara-Turkey.

Ethyl 2-[5,6-diphenyl-3(2H)-pyridazinone-2-yl]acetate **(5)**

0.025 Mol of 5,6-diphenyl-3(2H)-pyridazinone, 0.0375 mol of ethyl bromoacetate and 0.1 mol of anhydrous potassium carbonate in 25 ml anhydrous DMF were stirred at room temperature for 1 hour. The reaction mixture was poured into ice water. The precipitate was filtered, dried and crystallized from ethanol-water (mp 92-93 °C, yield 90 %).

***2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetic acid* (6)**

0.01 Mol of compound **5** in 100 ml 10 % NaOH was hydrolyzed for 4 hours. After cooling to 5 ^{0}C, the reaction mixture was acidified 20 % HCl. The precipitate was filtered, washed with water to neutral pH and dried and crystallized from isopropanol (mp 218 °C, yield 80 %).

***3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propannitrile* (7)**

0.25 Mol of 5,6-diphenyl-3(2H)-pyridazinone, 0.3 mol of triethylamine, 0.4 mol of acrylonitrile were added in 500 ml of methanol, refluxed for 2 hours. At the end of this time, The reaction mixture was evaporated to dryness and oil residue was crystallized from butanol (mp 98 °C, yield 65 %).

***3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoic acid* (8)**

0.01 Mol of compound **7** in DMF/H_2O/H_2SO_4 (1:1:2) (50 ml) was stirred at room temperature for 2 hours and then refluxed for 4 hours. The reaction mixture was cooled and the precipitate was collected by filtration, washed with water, dried and crystallized from ethanol (mp 165 °C, yield 55 %).

General procedure of 2-[5,6-diphenyl-3(2H)-pyridazinone-2-yl]acetamides or 3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanamides (6a-h ; 8a-h)

0.01 Mol of compound **6** or 0.01 mol compound **8** acid in 40 ml dichloromethane at 0 ^{0}C (ice-bath) was treated with triethylamine (1 ml) and 0.01 mol of ethyl chloroformate. After stirring the reaction mixture at 0 ^{0}C for 15 min, 0.011 mole of an appropriate amine derivative was added to this solution. The final mixture was stirred at 0-25 ^{0}C for 24 h and evaporated to dryness then the product was solidified with ice-cold water and crystallized from appropriate solvent.

1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-phenylpiperazine *(6a)*

Recrystallized from ethanol (yield 48 %, m.p. 183°C). ^{1}H-NMR (DMSO-d_6), δ 7.38-7.12 (m, 12H, 5,6-diphenyl protons, phenyl H^3, H^5); 6.99 (s, 1H, pyridazinone H^4); 6.97 (d, 2H, phenyl H^2, H^6); 6.82 (t, 1H, phenyl H^4); 5.16 (s, 2H, -CH_2CO); 3.71 (t, 2H, piperazine-$H^{2(6)}$); 3.63 (t, 2H, piperazine-$H^{6(2)}$); 3.24 (t, 2H, piperazine-$H^{3(5)}$); 3.15 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1677 (CO ring), 1658 (CO amide).

1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-(3-chlorophenyl)piperazine *(6b)*

Recrystallized from ethanol (yield 44 %, m.p. 215°C). ^{1}H-NMR (DMSO-d_6), δ 7.39-7.12 (m, 11H, 5,6-diphenyl protons, phenyl H^5); 7.02 (s, 1H, pyridazinone H^4); 7.00-6.98 (m,1H, phenyl H^2); 6.92-6.95 (d, 1H, phenyl H^4); 6.81-6.84 (m, 1H, phenyl H^6); 5.16 (s, 2H, -CH_2CO); 3.70 (t, 2H, piperazine-$H^{2(6)}$); 3.62 (t, 2H, piperazine-$H^{6(2)}$); 3.30 (t, 2H, piperazine-$H^{3(5)}$); 3.2 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1677 (CO ring), 1658 (CO amide).

***1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-(4-chlorophenyl)piperazine* (6c)**

Recrystallized from ethanol (yield 44 %, m.p. 177°C). ^{1}H-NMR (DMSO-d_6), δ 7.36-7.12 (m, 12H, 5,6-diphenyl protons, phenyl H^3, H^5); 7.02-6.98 (m, 3H, pyridazinone H4, phenyl H^2, H^6); 5.16 (s, 2H, -CH_2CO); 3.70 (t, 2H, piperazine-$H^{2(6)}$); 3.62 (t, 2H, piperazine-$H^{6(2)}$); 3.25 (t, 2H, piperazine-$H^{3(5)}$); 3.16 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1651 (CO ring, amide).

***1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-(2-fluorophenyl)piperazine* (6d)**

Recrystallized from ethanol (yield 53 %, m.p. 178 °C). ^{1}H-NMR (DMSO-d_6), δ 7.37-7.00 (m, 15H, 5,6-diphenyl protons, pyridazinone H^4, phenyl protons); 5.16 (s, 2H, -CH_2CO); 3.72 (t, 2H, piperazine-$H^{2(6)}$); 3.65 (t, 2H, piperazine-$H^{6(2)}$); 3.10 (t, 2H, piperazine-$H^{3(5)}$)); 3.01 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1661 (CO ring, amide).

***1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-(4-fluorophenyl)piperazine* (6e)**

Recrystallized from ethanol (yield 43 %, m.p. 156 °C). ^{1}H-NMR (DMSO-d_6), δ 7.38-6.99 (m, 15H, 5,6-diphenyl protons, pyridazinone H^4, phenyl protons); 5.16 (s, 2H, -CH_2CO); 3.70

(t, 2H, piperazine-$H^{2(6)}$); 3.62 (t, 2H, piperazine-$H^{6(2)}$); 3.17 (t, 2H, piperazine-$H^{3(5)}$); 3.08 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1661 (CO ring, amide).

1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-benzylpiperazine **(6f)**

Recrystallized from ethanol (yield 41 %, m.p. 179 °C). ^{1}H-NMR (DMSO-d_6), δ 7.35-7.10 (m, 15H, 5,6-diphenyl protons, phenyl protons); 6.97 (s, 1H, pyridazinone H^4); 5.07 (s, 2H, -CH_2CO); 3.53 (t, 2H, piperazine-$H^{2(6)}$); 3.51 (s, 2H, -CH_2-Φ); 3.46 (t, 2H, piperazine-$H^{6(2)}$); 3.43 (t, 2H, piperazine-$H^{3(5)}$); 2.35 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1656 (CO ring, amide).

1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-(2-pyridyl)piperazine **(6g)**

Recrystallized from ethanol (yield 51 %, m.p. 167 °C). ^{1}H-NMR (DMSO-d_6), δ 8.14-8.12 (m, 1H, pyridine H^6); 7.57-7.53 (m, 1H, pyridine H^4); 7.36-7.12 (m, 10H, 5,6-diphenyl protons); 6.99 (s, 1H, pyridazinone-H^4); 6.86 (d, 1H, pyridine H^3); 6.68-6.65 (m, 1H, pyridine H^5); 5.16 (s, 2H,-CH_2CO); 3.67-3.52 (m, 8H, piperazine protons) ppm. IR ν cm^{-1} (KBr): 1657 (CO ring, amide).

1-[2-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]acetyl]-4-piperonylpiperazine **(6h)**

Recrystallized from ethanol (yield 60 %, m.p. 160 °C). ^{1}H-NMR (DMSO-d_6), δ 7.36-7.12 (m, 10H, 5,6-diphenyl protons); 6.98 (s, 1H, pyridazinone H^4); 6.88-6.75 (m, 3H, phenyl protons); 5.99 (s, 2H, -OCH_2O-); 5.08 (s, 2H, -CH_2CO); 3.53 (t, 2H, piperazine-$H^{2(6)}$); 3.46 (t, 2H, piperazine-$H^{6(2)}$); 3.42 (s, 2H, -CH_2-Φ); 2.42 (t, 2H, piperazine-$H^{3(5)}$); 2.34 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1660 (CO ring, amide).

1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-phenylpiperazine **(8a)**

Recrystallized from ethanol (yield 48 %, m.p. 104 °C). ^{1}H-NMR (DMSO-d_6), δ 7.35-7.12 (m, 12H, 5,6-diphenyl protons, phenyl H^3, H^5); 6.96 (s, 1H, pyridazinone H^4); 6.93 (d, 2H, phenyl H^2, H^6); 6.80 (t, 1H, phenyl H^4); 4.39 (t, 2H, -NC$\underline{H_2}$$CH_2$); 3.62-3.55 (m, 4H, piperazine-H^2, H^6); 3.10 (t, 2H, piperazine-$H^{3(5)}$); 3.06 (t, 2H, piperazine-$H^{5(3)}$); 2.95 (t, 2H, -CH_2C$\underline{H_2}$CO) ppm. IR ν cm^{-1} (KBr): 1677 (CO ring), 1635 (CO amide).

1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-(3-chlorophenyl)piperazine **(8b)**

Recrystallized from ethanol (yield 46 %, m.p. 151 °C). ^{1}H-NMR (DMSO-d_6), δ 7.34-7.19 (m, 11H, 5,6-diphenyl protons, phenyl H^5); 6.94 (s, 1H, pyridazinone H^4); 6.92-6.90 (m, 1H, phenyl H^2); 6.86-6.88 (m, 1H, phenyl H^4); 6.79-6.81 (m, 1H, phenyl H^6); 4.38 (t, 2H, -NC$\underline{H_2}$$CH_2$); 3.60-3.55 (m, 4H, piperazine-H^2, H^6); 3.15 (t, 2H, piperazine-$H^{3(5)}$); 3.09 (t, 2H, piperazine-$H^{5(3)}$); 2.93 (t, 2H, -CH_2C$\underline{H_2}$CO) ppm. IR ν cm^{-1} (KBr): 1652 (CO ring), 1640 (CO amide).

1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-(4-chlorophenyl)piperazine **(8c)**

Recrystallized from ethanol (yield 52 %, m.p.159 °C). ^{1}H-NMR (DMSO-d_6), δ 7.34-6.95 (m, 12H, 5,6-diphenyl protons, phenyl H^3, H^5); 6.94 (s, 1H, pyridazinone H^4); 6.92 (d, 2H, phenyl H^2, H^6); 4.38 (t, 2H, -NC$\underline{H_2}$$CH_2$); 3.60-3.55 (m, 4H, piperazine-H^2, H^6); 3.10 (t, 2H, piperazine-$H^{3(5)}$); 3.04 (t, 2H, piperazine-$H^{5(3)}$); 2.95 (t, 2H, -CH_2C$\underline{H_2}$CO) ppm. IR ν cm^{-1} (KBr): 1652 (CO ring, amide).

1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-(2-fluorophenyl)piperazine **(8d)**

Recrystallized from ethanol (yield 47 %, m.p. 113 °C). ^{1}H-NMR (DMSO-d_6), δ 7.34-6.95 (m, 15H, 5,6-diphenyl protons, pyridazinone H^4, phenyl protons); 4.38 (t, 2H, -NC$\underline{H_2}$$CH_2$); 3.62-3.55 (m, 4H, piperazine-H^2, H^6); 2.95-2.91 (m, 6H, piperazine-H^3, H^5, -CH_2C$\underline{H_2}$CO) ppm. IR ν cm^{-1} (KBr): 1665 (CO ring), 1639 (CO amide).

***1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-(4-fluorophenyl)piperazine* (8e)**

Recrystallized from ethanol (yield 53 %, m.p. 120 °C). ^{1}H-NMR (DMSO-d_6), δ 7.34-6.95 (m, 15H, 5,6-diphenyl protons, pyridazinone H^4, phenyl protons); 4.37 (t, 2H, -NC$\underline{H_2}$CH_2); 3.60-3.55 (m, 4H, piperazine-H^2, H^6); 3.03 (t, 2H, piperazine-$H^{3(5)}$); 2.98 (t, 2H, piperazine-$H^{5(3)}$); 2.93 (t, 2H, -CH_2C$\underline{H_2}$CO) ppm. IR ν cm^{-1} (KBr): 1666 (CO ring), 1640 (CO amide).

***1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-benzylpiperazine* (8f)**

Recrystallized from ethanol (yield 25 %, m.p. 149 °C). ^{1}H-NMR (DMSO-d_6), δ 7.35-7.13 (m, 15H, 5,6-diphenyl protons, phenyl protons); 6.93 (s, 1H, pyridazinone H^4); 4.34 (t, 2H, -NC$\underline{H_2}$CH_2); 3.48-3.40 (m, 6H, piperazine-H^2, H^6, -CH_2-Φ); 2.87 (t, 2H, -CH_2C$\underline{H_2}$CO); 2.30 (t, 2H, piperazine-$H^{3(5)}$); 2.27 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1660 (CO ring), 1635 (CO amide).

***1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-(2-pyridyl)piperazine* (8g)**

Recrystallized from ethanol (yield 40 %, m.p. 116 °C). ^{1}H-NMR (DMSO-d_6), δ 8.11-8.10 (m, 1H, pyridine, H^6); 7.55-7.52 (m, 1H, pyridine, H^4); 7.34-7.11 (m, 10H, 5,6-diphenyl protons); 6.94 (s, 1H, pyridazinone H^4); 6.80 (d, 1H, pyridine H^3); 6.66-6.64 (m, 1H, pyridine H^5); 4.38 (t, 2H, -NC$\underline{H_2}$CH_2); 3.55-3.40 (m, 8H, piperazine protons); 2.93 (t, 2H, -CH_2C$\underline{H_2}$CO) ppm. IR ν cm^{-1} (KBr): 1671 (CO ring), 1630 (CO amide).

***1-[3-[5,6-Diphenyl-3(2H)-pyridazinone-2-yl]propanoyl]-4-piperonylpiperazine* (8h)**

Recrystallized from ethanol (yield 43 %, m.p. 149 °C). ^{1}H-NMR (DMSO-d_6), δ 7.34-7.13 (m, 10H, 5,6-diphenyl protons); 6.93 (s, 1H, pyridazinone H^4); 6.83-6.71 (m, 3H, phenyl protons); 5.97 (s, 2H, -OCH_2O-); 4.34 (t, 2H, -NC$\underline{H_2}$CH_2); 3.50-3.45 (m, 4H, piperazine-H^2, H^6); 3.34 (s, 2H, -CH_2-Φ); 2.86 (t, 2H, -CH_2C$\underline{H_2}$CO); 2.27 (t, 2H, piperazine-$H^{3(5)}$); 2.25 (t, 2H, piperazine-$H^{5(3)}$) ppm. IR ν cm^{-1} (KBr): 1661 (CO ring), 1633 (CO amide).

Pharmacology

Male Swiss albino mice (The Animal Breeding Laboratories of Refik Saydam Hıfzısıhha Institute Ankara, Turkey) weighing 20-25 g were used for all experiments. The animals were housed in colony cages (6 mices each), maintained on standart pellet diet and water ad libitum and left for for two days for acclimatization before the experimental sessions. The food was withdrawn on the day before experiment, but allowed free access to water. All experiments were carried out according to the suggested ethical guidelines for the care of laboratory animals.

Preparation of test samples for bioassay

Test samples were suspended in a mixture of distilled water and 0.5% sodium carboxymethylcellulose (CMC) and were given orally to the test animals. The animals of the control group received the same experimental handling except that the drug treatment was replaced with appropriate volumes of the vehicle. Indomethacin (10 mg/kg) or aspirin in 0.5% CMC (100 mg/kg) was used as reference drug.

p-Benzoquinone-induced writhing test (22)

Sixty min after the oral administration of the test samples and aspirin, the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5 % *(vlv) p*-benzoquinone (PBQ, Merck, Darmstadt, Germany) solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contraction (writhing movements) was counted for the next 15 min, starting on the fifth min after the PBQ injection. The data represent an average of the

total number of writhing movements observed. The analgesic activity was expressed as the percentage change compared to writing controls.

$$\text{Analgesic activity (writhing inhibition \%)} = \frac{n-n'}{n} \times 100$$

n = The mean writhing count of control group

n' = The mean writhing counts of test groups

Carrageenan-induced hind paw edema test (23)

Sixty minutes after the oral admistration of either test sample or dosing vehicle, each mouse was injected with freshly prepared (0.5 mg/25 µl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiologycal saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 µl saline solution was injected into that of the left hind paw. Paw edema was measured in every 90 min during 6h after induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods.

Ulcerogenic effect

After *p*-benzoquinone-induced writhing test, the surviving mice were killed under deep ether anesthesia and stomachs were removed. Then, each stomach was opened through great curvature and examined under dissecting microscope for lesions or bleedings.

Statistical analysis

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were tested by ANOVA test and Student-Newman-Keuls post-hoc test. A value of $p < 0.05$ was considered to be significant.

COX-1 and COX-2 assay (24,25)

The percentage inhibition of compounds at a concentration of 10 µM on blood COX-1 and COX-2 activities was calculated with the program Sigma Plot 9.0 (Systat Software, Germany). DuP-697 and SC-560 (Cayman Chemical, Ann Arbor, MI) were used as reference standards for selective inhibition of COX-2 and COX-1, respectively.

COX-1 assay

Fresh blood from healthy volunteers who had not taken NSAIDs in the previous week was collected into vacutainers containing no anticoagulants. Aliquots of 0.5 mL were immediately transferred into tubes containing vehicle or the test compound. Each drug was evaluated at final 10 µM concentration in duplicate determinations. The samples were incubated at 37 °C with gentle shaking for 60 min to allow the blood to clot. At the end of incubation, the reaction was stopped by submerging the tubes in a cold bath and centrifuging at 13 000 rpm for 10 min at 4 °C. Levels of TXB_2 in the serum were determined by using an enzyme immunoassay kit (Amersham, TXB_2 Biotrak Assay, #RPN 220).

COX-2 assay

The blood was collected into heparinized (20 U/mL) tubes and distributed in 0.5 aliquots in tubes containing 10 µg/mL of LPS (Sigma, St. Louis, MO, #L-2630 from E. coli serotype 0111:B4, 100 mg/ml final concentration, diluted in phosphate-buffered saline) together with vehicle or test compound at a final concentration of 10 µM. Each drug was evaluated in duplicate determinations. The samples were incubated in a bath at 37 °C for 24 h; during this

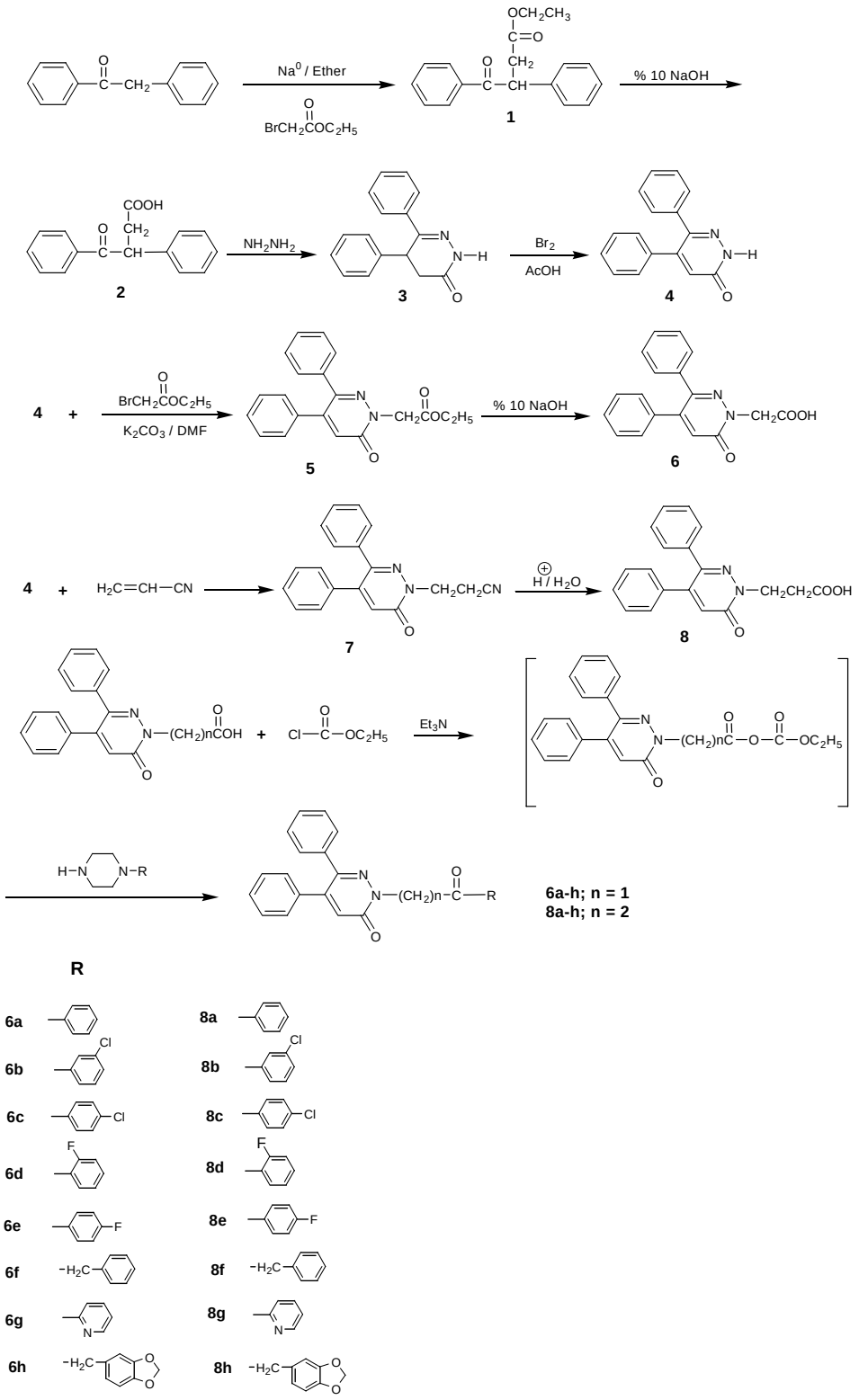
time COX-2 was induced in mononuclear cells. The reaction was stopped by submerging the tubes in a cold bath and centrifuging at 13,000 rpm for 10 min at 4 °C. Levels of PGE_2 in the supernatant were determined by using an enzyme immunoassay kit (Amersham, PGE_2 Biotrak Assay, #RPN 222).

RESULTS AND DISCUSSION

Sixteen new amide derivatives (**6a-h** ; **8a-h**) were prepared by treatment of 2-[5,6-diphenyl-3(2H)-pyridazinone-2-yl]acetic acid or 3-[5,6-diphenyl-3(2H)-pyridazinone-2-yl]propanoic acid with appropriate amine derivatives in the presence of triethylamine and ethyl chloroformate in dichloromethane at room temperature. The structure of the title compounds (**6a-h ; 8a-h**) has been elucidated by their IR, ^{1}H-NMR, and elemental analyses. Synthetic route of the title compounds is illustrated in Scheme 1.

Analgesic and anti-inflammatory activities of the title compounds were determined by *p*-benzoquinone-induced writhing test and carrageenan-induced hind paw edema test respectively (22,23). As seen in Table 1, analgesic activity results indicated that there was no considerable difference between acetamide and propanamide derivatives. Compounds **6b-e, 8a-e** showed higher analgesic activity than aspirin at 100 mg/kg. In the case of acetamide derivatives, while the compound **6a** having phenylpiperazine group caused a small decrease in the analgesic activity, compound **8a** which is the propanamide analog exhibited a little bit higher analgesic activity than aspirin. The highest analgesic activity in the acetamide derivatives was observed with the compound **6c** having a p-chlorophenylpiperazine group. Moreover, this compound did not exhibit any ulcerogenic activity in the tested animals. The compound **8e,** which is the propanamide derivative with p-fluorophenylpiperazine group at the side chain showed the highest analgesic and anti-inflammatory activities in comparison with other derivatives. In addition, this derivative did not cause any gastric lesions and bleeding in stomachs of the tested animals. As seen in Table 1, propanamide derivatives were found more potent than acetamide derivatives in terms of anti-inflammatory activity. Analgesic activity results of the compounds **6b-d, 8a-e** also showed good correlation with their anti-inflammatory activity. The compound **8e** with the highest analgesic activity was found more potent at 100 mg/kg dose than indomethacin at 10 mg/kg dose and it did not cause any gastric lesions and bleeding in stomachs of tested animals. This shows the compounds are less toxic than indomethacin in the gastric irritation, and one can decline to say that their activity is higher than indomethacin since they were administered at different doses. It is worth comparing their activity with indomethacin at the same doses. It is known that an edema produced by carrageenan is a biphasic event and it is reported that the inhibitory effects of agents which act on the first stage of the carrageenan-induced hind paw inflammation are attributable to the inhibition of chemical mediators such as histamine, serotonin and bradykinin (26). On the other hand, the second stage of the edema might be related to the arachidonic acid metobolites since it is inhibited by aspirin, indomethacin and other cyclooxygenase inhibitors (26,27). The compounds **6b-d, 8a-b** exhibited anti-inflammatory activity in the second phase of carrageenan-induced edema (270 and 360 min) indicating that these compounds might exert their activities through the inhibition of cyclooxygenase enzymes. The compounds **8c-e** produced anti-inflammatory activity in both phases of carrageenan-induced edema.

When the chemical structures of the active compounds are taken into consideration, it was observed that m- or p-chloro substitutients or o- or p-fluoro substitutients on the phenyl ring of phenylpiperazine group increased both analgesic and anti-inflammatory activities for two series. However, 4-benzylpiperazine, 2-pyridylpiperazine and 4-piperonylpiperazine groups decreased both analgesic and anti-inflammatory activities for two series.



Scheme 1. Synthetic route of the title compounds

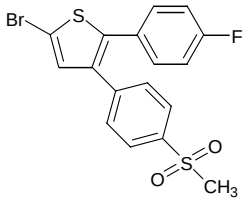
Table 1. Analgesic and Anti-inflammatory Activites of the compounds

Compounds	**Analgesic Activity Number of ± SEM (% inhibition)**	**Anti-inflammatory Activity Swelling thickness (x 10^{-2}mm)± SEM (% inhibition)**				**Gastric Ulcerogenic effect**
		90 min	**180 min**	**270 min**	**360 min**	
Control	55.5±3.20	41.7±4.09	50.3±4.15	57.2±3.71	63.0±4.20	0/6
6a	31.0±3.72 (44.1)***	35.8±4.62 (14.1)	41.0±4.61 (18.5)	44.8±4.79 (21.7)	49.2 ±4.69 (21.9)	2/6
6b	23.0±1.98 (58.6)***	26.52±3.36 (36.4)	32.82±3.82 (34.7)	36.42±3.33 (36.3)**	41.82 ± 3.36 (33.6)**	0/6
6c	19.7±1.69 (64.5)***	34.5±4.94 (17.3)	37.7±5.33 (25)	39.7±3.43 (30.6)*	42.2±3.12 (33.0)**	0/6
6d	20.5±1.71 (63.1)***	31.7±3.49 (23.9)	34.2±3.15 (32)	43.2±5.48 (24.5)	41.3±3.75 (34.4)**	0/6
6e	22.7±1.48 (59.1)***	35.8±5.21 (14.1)	39.2±5.38 (22.1)	42.3±5.55 (26.0)	44.8±5.04 (28.9)	0/6
6f	45.2±4.59 (18.6)	43.8±4.62 -	43.8±4.43 (3.9)	53.5±3.91 (6.5)	58.3±3.34 (7.5)	1/6
6g	43.3±4.26 (21.9)	45.2±4.11 -	49.3±4.08 (1.9)	54.7±3.90 (4.4)	59.7±3.72 (5.2)	0/6
6h	41.8±3.29 (24.7)	35.22±2.13 (15.5)	40.86±2.18 (18.8)	46.43±1.85 (18.8)	51.54±1.91 (18)	0/6
8a	24.0±2.67 (56.8)***	27.44±3.32 (34.2)	32.57±3.60 (35.2)	36.84±3.19 (35.6)**	41.40±3.15 (34.3)**	0/6
8b	22.2±3.29 (60)***	29.73±2.99 (28.7)	35.44±3.50 (29.5)	39.53±2.77 (30.9)**	43.10±2.99 (31.6)**	1/6
8c	17.2±2.19 (69)***	29.3±3.25 (29.7)	31.3±2.49 (37.8)**	35.1±2.38 (38.6)**	39.2±2.43 (37.8)***	0/6
8d	17.8±1.36 (67.9)***	30.0±2.5 (28.1)	32±2.49 (36.4)**	35.8±2.71 (37.4)**	37.2±2.18 (40.9)***	0/6
8e	15.0±1.13 (72.9)***	28.2±4.29 (32.4)	33.5±3.49 (39.3)*	34.5±3.30 (39.7)**	34.2±3.24 (45.7)***	0/6
8f	38.8±4.79 (30.1)*	40.3±3.86 (3.4)	49±5.32 (2.6)	51.5±4.62 (9.9)	50.5±5.19 (19.8)	0/6
8g	35.0±4.12 (36.9)*	33.54±3.66 (19.6)	39.22±4.49 (22.0)	43.32±3.71 (24.3)	47.63±4.07 (23.4)	0/6
8h	34.0±6.65 (38.7)	29.88±3.04 (28.3)	35.94±3.21 (28.5)	41.21±2.72 (27.9)*	46.52±2.64 (26.2)*	0/6
Aspirin	25.3± 2.71 (53.5)***	-	-	-	-	2/6
İndomethacin	-	28.2±3.42 (32.4)*	32.3±3.08 (35.8)**	36.2±2.96 (36.7)**	38.5±2.29 (38.9)***	-

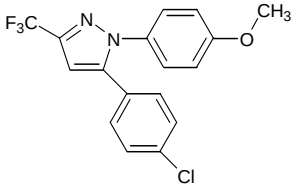
*P< 0.05; **P <0.01; ***P <0.001

Analgesic and anti-inflammatory activity of the compounds were tested at 100 mg/kg doses. Analgesic activity of aspirin was tested at 100 mg/kg and anti-inflammatory activity of indomethacin was tested at 10 mg/kg dose as described in experimental part.

The compounds which were found active in terms of analgesic and anti-inflammatory activities were chosen to investigate their *in vitro* inhibitory activity on COX-1 and COX-2 enzymes (**6b-d, 8a-e),** and tested for their inhibitory potency against to COX-1 and COX-2 in a human whole blood assay. DuP-697 and SC-560 were used as reference standards for selective inhibition of COX-2 and COX-1, respectively (Figure 3.). Since DuP-697 and SC-560 carry diaryl heterocycle structure and they are selective inhibitor of COX-2 and COX-1, respectively, they have been chosen as reference compounds in this study (28,29).



DuP-697



SC-560

Figure 3. The structures of DuP-697 and SC-560

Many COX-2 inhibitors carry vicinal diaryl substituents on a heterocyclic ring. Therefore, we hypothesized that title compounds having the vicinal diaryl substitutents on a central pyridazinone ring might exhibit selective inhibitory activity on COX-2 enzyme, but none of the selected *in vivo* active compounds have resulted considerable inhibition neither in COX-2 nor in COX-1 (Table 2). Although they were found analgesic and anti-inflammatory activities, one can say that these compounds do not exert their analgesic and anti-inflammatory activities through COX inhibition.

Emorfazone which is well known analgesic and anti-inflammatory, having pyridazinone ring like our title compounds, exhibits an interesting pharmacological profile (30,31). Its activity is not originated either from intereaction with prostaglandins system or from affinity to opioid receptors. So, we think that our compounds might have same mechanism of activity like emorfazone. The mechanism that underlines the analgesic and anti-inflammatory activities of the resulting amide derivatives are currently under investigation in our laboratory.

Table 2. COX inhibitory activities of the selected compounds

Compound	**COX-1 inhibition, (%), 10 µM**	**COX-2 inhibition, (%), 10 µM**
6b	7.14	0
6c	30.95	9.48
6d	26.19	15.04
8a	0	14.66
8b	11.90	15.72
8c	0	0
8d	0	19.24
8e	0	0
SC-560	100	-
DUP-697	-	100

SC-560 and DuP-697 were used as reference standards for selective inhibition of COX-1 and COX-2, respectively.

ACKNOWLEDGEMENT

This study is financially supported by Gazi University Research Fund (Project No: 02/2002-11)

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Received: 01.12.2006
Accepted: 20.03.2007