Turk J Chem
27 (2003) , 727 – 738.

Synthesis and Analgesic and Anti-Inflammatory Activity of New Pyridazinones

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Received 20.01.2003

A new series of 2-(6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)-acetamides and 3-[6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)-propanamides were synthesized and evaluated in terms of their analgesic and anti-inflammatory activities. All compounds except for **7g** were more potent than aspirin in a *p*-benzoquinone–induced writhing test at 100 mg/kg dose. Compounds **7b**, **7c** and **7e** had the highest anti-inflammatory activity; compound **7e** was the most potent in terms of analgesic and anti-inflammatory activities and had no ulcerogenic side effects.

Key Words: 6-Oxo-3,5-diphenylpyridazine, synthesis, spectral analysis, analgesic, anti-inflammatory.

Introduction

Most currently used nonsteroidal anti-inflammatory drugs (NSAIDs) have limitations for therapeutic use since they cause gastrointestinal and renal side effects that are inseparable from their pharmacological activities. Therefore, the synthesis of new compounds devoid of such side effects has become an important goal for medicinal chemists in recent years. For this purpose, various compounds incorporating a 3(2H)-pyridazinone ring have been synthesized and their pharmacological activities have been reported[1−3]. Recently, it has been reported that a considerable number of 3(2H)-pyridazinone derivatives bear analgesic activity. Among these compounds, emorfazone (4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone) is an analgesic and anti-inflammatory compound marketed as pentoil and nandron[4−6]. Rohet et al. reported that most 4,6-diphenyl-2-[3-(4-arylpiperazin-1-yl)propyl]-3(2H)-pyridazinone derivatives, which were synthesized by inspiration from Trazodone (an antidepressant compound), were more potent than acetaminophen and noramidopyrine in a *p*-benzoquinone-induced writhing test[7]. In addition, Santagati et al. claimed that 2-substituted 4,5-dihalo-3(2H)-pyridazinone derivatives had high analgesic activity[8].

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During our search for new analgesic compounds, we previously synthesized 6-(4-methoxyphenyl)-3(2H)-pyridazinone derivatives carrying acetamide and propanamide moieties at position 2 of the pyridazinone ring and reported that 1-[3-[6-(4-methoxyphenyl)-3(2H)-pyridazinon-2-yl]propanoyl]-4-(4-fluorophenyl) piperazine had the highest significant analgesic activity[9]. We also synthesized 6-substituted-3(2H)-pyridazinones and reported that the 6-[4-(4-fluorophenyl)]piperazine-3(2H)-pyridazinone derivative showed the highest activity as an analgesic agent[10]. Similar studies with benzoxazolinone and benzothiazolinone derivatives in our laboratory also showed that the derivatives carrying alkanoic acid residue on the nitrogen of the rings had significant analgesic and anti-inflammatory activity[11−13].

In this study, based on the above findings, our aim was to synthesize new 4,6-diphenyl-3(2H)-pyridazinones substituted by 4-arylpiperazin-1-yl-carbonylalkyl moieties on the nitrogen atom in the 2 position of the pyridazinone ring and to investigate their analgesic and anti-inflammatory activity (Figure 1).

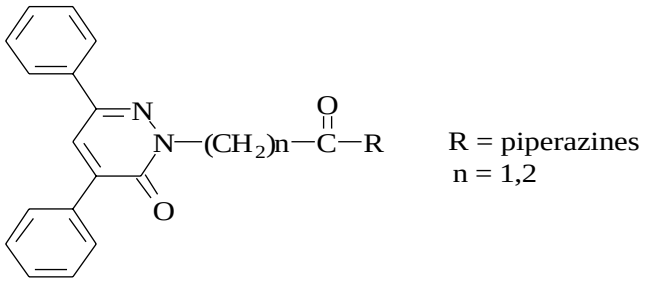


Figure 1.

Experimental

General

All chemicals and solvents were purchased from Aldrich and Merck AG (Germany). Melting points were determined with an Electrothermal-9200 digital melting point apparatus (Electrothermal Engineering, Southend, UK) and are uncorrected. The IR spectra (KBr) of the compounds were recorded on a Bruker Vector 22 IR spectrometer. The ^{1}H-NMR spectra were recorded on a Jeol 500 MHz-NMR spectrometer in DMSO-d_6 using TMS as internal standard. All chemical shifts were recorded as δ (ppm). Elemental analyses were performed with a Leco-932 (C,H,N,S- Elemental analyzer, St. Joseph, USA) in Ankara at the Scientific and Technical Research Council of Turkey (TUBITAK).

3-(6-Oxo-3,5-diphenyl-6H-pyridazin-1-yl)propionitrile (8)

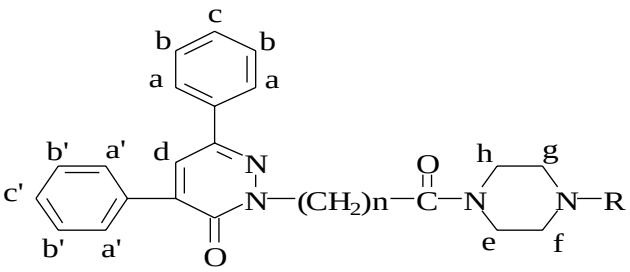
To 500 ml of water were added 0.25 mol of 4,6-diphenyl-3(2H)-pyridazinone, 0.3 mol of triethylamine, 0.3 mole of acrylonitrile, followed by heating at 50-60 °C for 6 h and then stirring at room temperature for 18 h. After this, the solid was collected by filtration, washed with water to neutral pH, dried and crystallized from butanol. Yield (65%); m.p: 111-112 °C. IR (KBr cm^{-1}) 1639 C=O, 2247 CN; ^{1}H-NMR (DMSO-d_6); δ 8.19 (s, 1H, d), 8.06-7.93 (m, 4H, 2a, 2a'), 7.54-7.48 (m, 6H, 2b, 2b', c, c'), 4.48 (t, 2H,-NC$\underline{H}_2$CH$_2$), 3.14 (t, 2H, -CH$_2$C$\underline{H}_2$CN). Anal. ($C_{19}H_{15}N_3O$): C,H,N calcd. 75.73, 5.02, 13.94; found. 75.64, 4.68, 13.58.

3-(6-Oxo-3,5-diphenyl-6H-pyridazin-1yl)propionic acid (9)[18]

First 0.01 mol of 3-[4,6-diphenyl-3(2H)-pyridazinone-2-yl]propanenitrile was refluxed in 50 mL of 10 N hydrochloric acid for 3 h. It was then cooled to 0 °C and the precipitate was filtered and recrystallized from ethanol-water. Yield (83%). ^{1}H-NMR and IR data were identical to literature values.[18,19]

General procedure of 2-(6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)acetamides (7a-g) or 3-[6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)propanamides (9a-g)

First, 0.01 mol of 2-(6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)acetic acid or 0.01 mol 3-(6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)propanoic acid in 40 mL dichloromethane at 0 °C (ice- bath) was treated with triethylamine (1 mL) and 0.01 mole of ethyl chloroformate. After stirring the reaction mixture at 0 °C for 15 min, 0.011 mol of an appropriate amine derivative was added to this solution. The final mixture was stirred at 0-25 °C for 24 h, evaporated to dryness and then treated with either acetone or acetone-hexane mixture. All solid materials thus obtained were dried and crystallized from appropriate solvents.



2-{2-[4-(4-Chloro-phenyl)-piperazin-1-yl]-2-oxo-ethyl}-4,6-diphenyl-2H-pyridazine-3-one (7a)

Crystallized from ethanol; yield (57%); m.p. 158 °C; IR (KBr cm^{-1}) 1646 (C=O ring, amide); ^{1}H-NMR (DMSO-d$_6$); δ 8.18 (s, 1H, d), 7.98-7.94 (m, 4H, 2a, 2a'), 7.52-7.44 (m, 6H, 2b, 2b', c, c'), 7.26 (d, 2H, phenyl 3-H, 5-H), 6.98 (d, 2H, phenyl 2-H, 6-H), 5.21 (s, 2H, -CH$_2$CO), 3.70 (t, 2H, e), 3.60 (t, 2H, h), 3.25 (t, 2H, f), 3.12 (t, 2H, g). Anal. ($C_{28}H_{25}ClN_4O_2$): C,H,N calcd. 69.34, 5.20, 11.55; found. 68.94, 5.01, 11.24.

2-{2-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-2-oxo-ethyl}-4,6-diphenyl-2H-pyridazine-3-one (7b)

Crystallized from ethanol; yield (59%); m.p. 159 °C; IR (KBr cm^{-1}) 1652 (C=O ring, amide);^{1}H-NMR (DMSO-d$_6$); δ 8.19 (s, 1H, d), 8.02-7.94 (m, 4H, 2a, 2a'), 7.54-7.45 (m, 6H, 2b, 2b', c, c'), 7.19-6.98 (m, 4H, phenyl 3-H, 4-H, 5-H, 6-H), 5.21 (s, 2H, -CH$_2$CO), 3.75 (t, 2H, e), 3.65 (t, 2H, h), 3.11 (t, 2H, f), 3.02 (t, 2H, g). Anal. ($C_{28}H_{25}FN_4O_2$): C,H,N calcd. 71.78, 5.38, 11.96; found. 71.74, 5.26, 11.81.

2-{2-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-2-oxo-ethyl}-4,6-diphenyl-2H-pyridazine-3-one (7c)

Crystallized from ethanol; yield (65%); m.p. 148-149 °C; IR (KBr cm^{-1}) 1652 (C=O ring, amide); ^{1}H-NMR (DMSO-d$_6$); δ 8.19 (s, 1H, d), 8.01-7.94 (m, 4H, 2a, 2a'), 7.53-7.45 (m, 6H, 2b, 2b', c, c'), 7.10-6.98 (m, 4H, phenyl 2-H, 3-H, 5-H, 6-H), 5.21 (s, 2H, -CH$_2$CO), 3.72 (t, 2H, e), 3.63 (t, 2H, h), 3.19 (t, 2H, f), 3.09 (t, 2H, g). Anal. ($C_{28}H_{25}FN_4O_2$): C,H,N calcd. 71.78, 5.38, 11.96; found. 71.94, 4.94, 11.89.

2-{2-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-2-oxo-ethyl}-4,6-diphenyl-2H-pyridazine-3-one (7d)

Crystallized from ethanol; yield (51%); m.p. 190-191 °C; IR (KBr cm^{-1}) 1645 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.18 (s, 1H, d), 8.00-7.94 (m, 4H, 2a, 2a'), 7.53-7.44 (m, 6H, 2b, 2b', c, c'), 7.01-6.87 (m, 4H, phenyl 3-H, 4-H, 5-H, 6-H), 5.21 (s, 2H, -CH_2CO), 3.80 (s, 3H, -OCH_3), 3.70 (t, 2H, e), 3.62 (t, 2H, h), 3.04 (t, 2H, f), 2.95 (t, 2H, g). Anal. ($C_{29}H_{28}N_4O_3$): C,H,N calcd. 72.48, 5.87, 11.66; found. 72.16, 5.76, 11.55.

2-[2-Oxo-2-(4-pyridin-2-yl-piperazin-1-yl)-ethyl]-4,6-diphenyl-2H-pyridazine-3-one (7e)

Crystallized from ethanol; yield (54%); m.p. 205 °C; IR (KBr cm^{-1}) 1656 (C=O ring), 1634 (C=O amide); ^{1}H-NMR (DMSO-d_6); δ 8.18 (s, 1H, d), 8.13 (d& d, 1H, pyridine H-6), 8.00-7.94 (m, 4H, 2a, 2a'), 7.58-7.54 (m, 1H, pyridine H-4), 7.52-7.44 (m, 6H, 2b, 2b', c, c'), 6.87 (d, 1H, pyridine H-3), 6.67 (d& d, 1H, pyridine H-5), 5.21 (s, 2H, -CH_2CO), 3.72-3.49 (m, 8H, e, h, f, g). Anal. ($C_{27}H_{25}N_5O_2$): C,H,N calcd. 71.82, 5.58, 15.51; found. 71.56, 4.99, 15.79.

2-[2-(4-Benzyl-piperazin-1-yl)-2-oxo-ethyl]-4,6-diphenyl-2H-pyridazine-3-one (7f)

Crystallized from ethanol; yield (66%); m.p. 160-161 °C; IR (KBr cm^{-1}) 1652 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.16 (s, 1H, d), 7.98-7.93 (m, 4H, 2a, 2a'), 7.52-7.44 (m, 6H, 2b, 2b', c, c'), 7.34-7.24 (m, 5H, phenyl 2-H, 3-H, 4-H, 5-H, 6-H), 5.18 (s, 2H, -CH_2CO), 3.58-3.44 (m, 6H, e, h, -CH_2-), 2.45 (t, 2H, f), 2.36 (t, 2H, g). Anal. ($C_{29}H_{28}N_4O_2$): C,H,N calcd. 74.98, 6.07, 12.06; found. 74.63, 5.61, 12.38.

2-[2-(4-Benzo[1,3]dioxol-5-yl-methyl-piperazin-1-yl)-2-oxo-ethyl]-4,6-diphenyl-2H-pyridazine-3-one (7g)

Crystallized from ethanol; yield (48%); m.p. 143-144 °C; Cryst. sol. Ethanol; IR (KBr cm^{-1}) 1647 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.17 (s, 1H, d), 7.98-7.94 (m, 4H, 2a, 2a'), 7.53-7.45 (m, 6H, 2b, 2b', c, c'), 6.90-6.75 (m, 3H, phenyl protons), 6.00 (s, 2H, -OCH_2O-), 5.18 (s, 2H, -CH_2CO), 3.55 (t, 2H, e), 3.47 (t, 2H, h), 3.44 (s, 2H,-CH_2-), 2.43 (t, 2H, f), 2.34 (t, 2H, g). Anal. ($C_{30}H_{28}N_4O_4$): C,H,N calcd. 70.85, 5.55, 11.02; found. 70.72, 5.22, 11.30.

2-{3-[4-(4-Chloro-phenyl)-piperazin-1-yl]-3-oxo-propyl}-4,6-diphenyl-2H-pyridazine-3-one (9a)

Crystallized from ethanol; yield (58%); m.p. 128 °C; IR (KBr cm^{-1}) 1639 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.13 (s, 1H, d), 7.98-7.93 (m, 4H, 2a, 2a'), 7.50-7.42 (m, 6H, 2b, 2b', c, c'), 7.23 (d, 2H, phenyl 3-H, 5-H), 6.93 (d, 2H, phenyl 2-H, 6-H), 4.46 (t, 2H,-NC$\underline{H}_2$$CH_2$), 3.64-3.57 (m, 4H, e, h), 3.14 (t, 2H, f), 3.07 (t, 2H, g), 2.98 (t, 2H, -CH_2C$\underline{H}_2$CO). Anal. ($C_{29}H_{27}ClN_4O_2$): C,H,N calcd. 69.80, 5.45, 11.23; found. 69.59, 4.97, 11.59.

2-{3-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-3-oxo-propyl}-4,6-diphenyl-2H-pyridazine-3-one (9b)

Crystallized from ethanol; yield (67%); m.p. 104 °C; Cryst. sol. Ethanol; IR (KBr cm^{-1}) 1640 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.14 (s, 1H, d), 8.02-7.93 (m, 4H, 2a, 2a'), 7.52-7.43 (m, 6H, 2b, 2b', c, c'), 7.17-6.97 (m, 4H, phenyl 3-H, 4-H, 5-H, 6-H), 4.46 (t, 2H,-NC$\underline{H}_2$$CH_2$), 3.67-3.60 (m, 4H, e, h), 3.03-2.92 (m, 6H, f, g, -CH_2C$\underline{H}_2$CO). Anal. ($C_{29}H_{27}FN_4O_2$): C,H,N calcd. 72.18, 5.64, 11.61; found. 72.45, 5.99, 11.67.

2-{3-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-3-oxo-propyl}-4,6-diphenyl-2H-pyridazine-3-one (9c)

Crystallized from ethanol; yield (60%); m.p. 113 °C; IR (KBr cm^{-1}) 1641 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.14 (s, 1H, d), 8.00-7.93 (m, 4H, 2a, 2a'), 7.52-7.42 (m, 6H, 2b, 2b', c, c'), 7.08-6.93 (m, 4H, phenyl 2-H, 3-H, 5-H, 6-H), 4.46 (t, 2H,-NC$\underline{H}_2CH_2$), 3.65-3.58 (m, 4H, e, h), 3.10-2.96 (m, 6H, f, g, -$CH_2C\underline{H}_2$CO). Anal. ($C_{29}H_{27}FN_4O_2$): C,H,N calcd. 72.18, 5.64, 11.61; found. 72.17, 5.27, 11.54.

2-{3-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-3-oxo-propyl}-4,6-diphenyl-2H-pyridazine-3-one (9d)

Crystallized from ethanol; yield (65%); m.p. 150 °C; IR (KBr cm^{-1}) 1648 (C=O ring), 1634 (C=O amide); ^{1}H-NMR (DMSO-d_6); δ 8.14 (s, 1H, d), 8.01-7.93 (m, 4H, 2a, 2a'), 7.51-7.43 (m, 6H, 2b, 2b', c, c'), 6.98-6.82 (m, 4H, phenyl 3-H, 4-H, 5-H, 6-H), 4.45 (t, 2H,-NC$\underline{H}_2CH_2$), 3.77 (s, 3H, -OCH_3), 3.63-3.57 (m, 4H, e, h), 2.98 (t, 3H, -$CH_2C\underline{H}_2$CO), 2.93 (t, 2H, f), 2.87 (t, 2H, g). Anal. ($C_{30}H_{30}N_4O_3$): C,H,N calcd. 72.85, 6.11, 11.33; found. 72.97, 5.84, 11.26.

2-[3-Oxo-3-(4-pyridin-2-yl-piperazin-1-yl)-propyl]-4,6-diphenyl-2H-pyridazine-3-one (9e)

Crystallized from ethanol; yield (55%); m.p. 138 °C; IR (KBr cm^{-1}) 1645 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.14 (s, 1H, d), 8.12 (d& d, 1H, pyridine H-6), 8.00-7.94 (m, 4H, 2a, 2a'), 7.57-7.53 (m, 1H, pyridine H-4), 7.52-7.42 (m, 6H, 2b, 2b', c, c'), 6.83 (d, 1H, pyridine H-3), 6.66 (d& d, 1H, pyridine H-5), 4.46, (t, 2H,-NC$\underline{H}_2CH_2$), 3.63-3.42 (m, 8H, e, h, f, g), 3.00 (t, 2H, -$CH_2C\underline{H}_2$CO). Anal. ($C_{28}H_{27}N_5O_2$): C,H,N calcd. 72.24, 5.85, 15.04; found. 71.89, 5.63, 14.81.

2-[3-(4-Benzyl-piperazin-1-yl)-3-oxo-propyl]-4,6-diphenyl-2H-pyridazine-3-one (9f)

Crystallized from ethanol; yield (50%); m.p. 112 °C; IR (KBr cm^{-1}) 1648 (C=O ring), 1634 (C=O amide); ^{1}H-NMR (DMSO-d_6); δ 8.14 (s, 1H, d), 7.98-7.93 (m, 4H, 2a, 2a'), 7.51-7.44 (m, 6H, 2b, 2b', c, c'), 7.33-7.22 (m, 5H, phenyl 2-H, 3-H, 4-H, 5-H, 6-H), 4.44 (t, 2H,-NC$\underline{H}_2CH_2$), 3.48-3.42 (m, 6H, e, h, -CH_2-), 2.91 (t, 2H, -$CH_2C\underline{H}_2$CO), 2.32 (t, 2H, f), 2.27 (t, 2H, g). Anal. ($C_{30}H_{30}N_4O_2$): C,H,N calcd. 75.29, 6.32, 11.71; found. 75.42, 6.12, 11.55.

2-[3-(4-Benzo[1,3]dioxol-5-yl-methyl-piperazin-1-yl)-3-oxo-propyl]-4,6-diphenyl-2H-pyridazine-3-one hydrochloride (9g)

Crystallized from ethanol; yield (55%); m.p. 214 °C; IR (KBr cm^{-1}) 1641 (C=O ring, amide); ^{1}H-NMR (DMSO-d_6); δ 8.18 (s, 1H, d), 8.00-7.93 (m, 4H, 2a, 2a'), 7.52-7.44 (m, 6H, 2b, 2b', c, c'), 6.85-6.72 (m, 3H, phenyl protons), 6.00 (s, 2H, -OCH_2O-), 4.44 (t, 2H, -NC$\underline{H}_2CH_2$), 3.48-3.43 (m, 4H, e, h,), 3.36 (s, 2H,-CH_2-), 2.92 (t, 2H, -$CH_2C\underline{H}_2$CO), 2.32 (t, 2H, f), 2.27 (t, 2H, g). Anal. ($C_{31}H_{30}N_4O_4$): C,H,N calcd. 66.60, 5.59, 10.02; found. 66.29, 5.80, 9.80.

Pharmacology

Male Swiss albino mice (20-25 g) were used. All the animals were left for 2 days in the laboratory for acclimatization before the day of experiment, and on the last day they were given water only. A minimum of 6 animals were used in each group.

PBQ (Merck A.G.), carboxymethyl cellulose sodium salt (CMC Na) (Aldrich), aspirin (Bayer), carrageenan (Sigma), and Evans blue (Sigma), and gauge calipers (Peacock, Ozaki Co., Tokyo) were used.

Analgesic activity

A *p*-benzoquinone (PBQ)-induced writhing test was used[14]. Test samples were given orally at 2 different doses, i.e. 100 mg/kg and 50 mg/kg as a suspension in 0.2 mL of 0.5% CMC Na; aspirin also used as a reference was administered at a 100 mg/kg dose as a suspension in 0.2 mL of 0.5% CMC Na. Sixty minutes after the oral administration of test samples and aspirin, each mouse was intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% (v/v) PBQ solution in distilled water. The control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal constrictions (writhing movements) were counted for the next 15 min, starting 5 min after the PBQ injection. The percentage of analgesic activity was calculated by comparisons with the control group.

Anti-inflammatory activity

Carrageenan-induced edema model

For the determination of the effects on acute inflammation, the carrageenan-induced paw edema model described by Kasahara et al. was employed with modifications[11,15]. Test samples and indomethacin used as a reference were administered orally respectively at doses of 100 mg/kg and 10 mg/kg as a suspension in 0.2 mL of 0.5% CMC Na. Sixty minutes after the oral administration of test samples and reference or dosing vehicle, the subplantar tissue of the right hind paw of each each mouse was injected with a freshly prepared (0.5 mg/25 μL) suspension of carrageenan in physiological saline (154 mM NaCl). For the control, 25 μL saline solution was injected into the left hind paw. Paw edema was measured every 90 min for 6 h after the induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers. Mean values of the treated groups were compared with those of the control group and analyzed using statistical methods.

Acetic acid-induced increase in capillary permeability model

The effects of test samples on the increased vascular permeability induced by acetic acid in mice were determined according to Whittle's method with some modification[16,17]. Test samples and indomethacin used as a reference were administered orally, respectively, at doses of 100 mg/kg and 10 mg/kg as a suspension in 0.2 mL of 0.5% CMC Na. Thirty minutes after the administration the tail of each mouse was injected with 0.1 mL of 4% Evans blue in saline solution (i.v.). Then, 10 min after the i.v. injection of the dye solution, 0.4 mL of 0.5% (v/v) AcOH was injected i.p. After 20 min, the mice were killed by cervical dislocation, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 mL volumetric flasks through glass wool. To each flask was added 0.1 ml of 0.1 N NaOH solution. The flasks were then made up to 10 mL with distilled water. Absorbances of the solutions were measured at 590 nm (Beckman Dual Spectrometer). In control animals, a mixture of distilled water and 0.5% CMC was given orally, and they were treated in the same manner as described above.

Ulcerogenic effect

After the PBQ-induced writhing test, surviving mice were killed under deep ether anesthesia and their stomachs were removed. Then each stomach was opened through the great curvature and examined under a 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 and Student's-Newman-Keuls posthoc tests. $P < 0.05$ was considered significant.

Results and Discussion

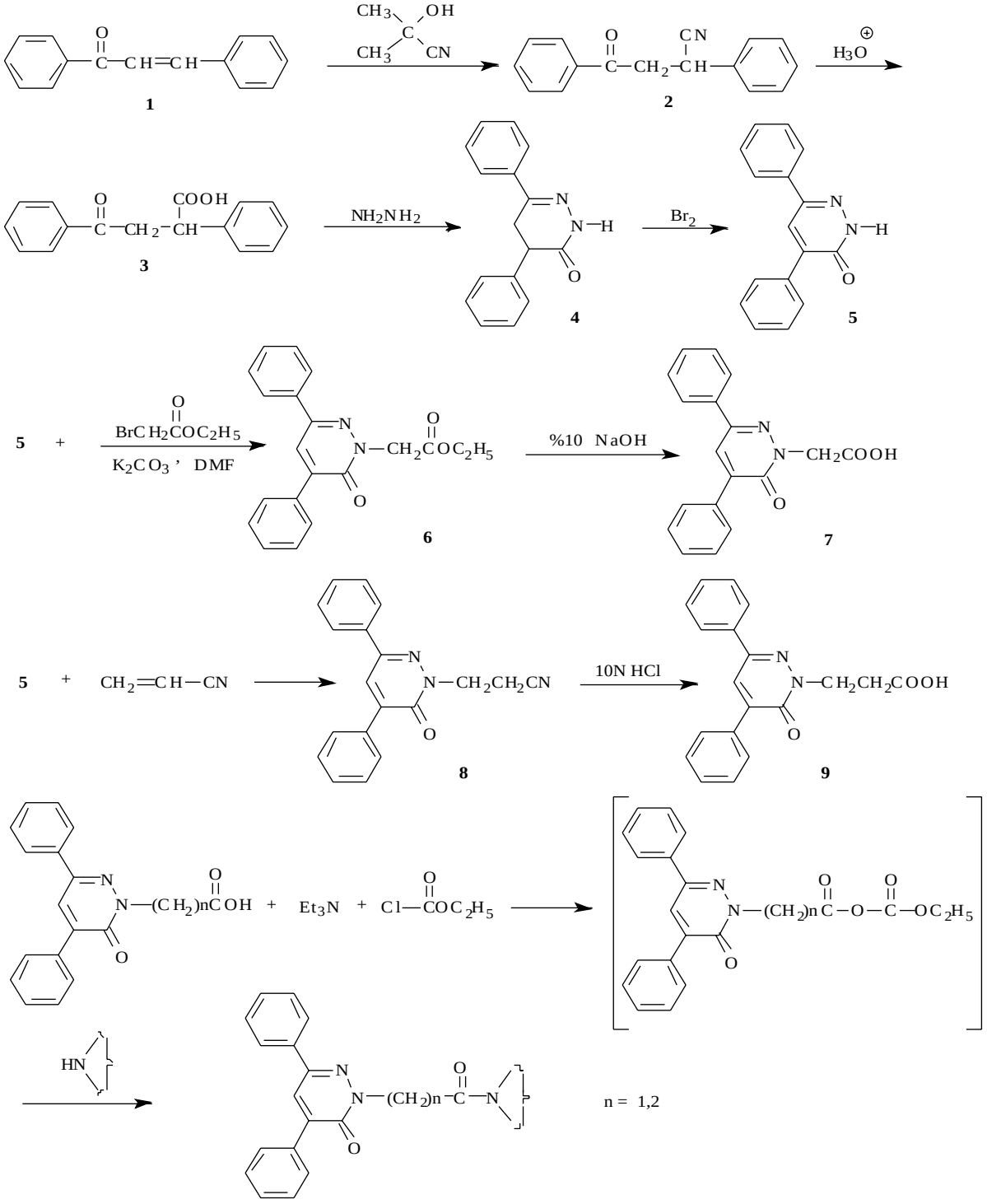
In the initial step of the synthesis, corresponding chalcone (1) was synthesized by the reaction of acetophenone with benzaldehyde and then the γ–ketonitrile derivative (2) was prepared by the reaction of acetone cyanohydrin with the chalcone. The γ–ketonitrile derivative was hydrolyzed by 10 N hydrochloric acid to corresponding 2,4-diphenyl-4-oxobutanoic acid (3). This acid was reacted with hydrazine hydrate to obtain 4,6-diphenyl-4, 5-dihydro-3(2H)-pyridazinone (4). Then dihydropyridazinone derivative was treated with bromine in acetic acid to give 4,6-diphenyl-3(2H)-pyridazinone (5). Ester derivative (6) was synthesized by the reaction of 4,6-diphenyl-3(2H)-pyridazinone with ethyl bromoacetate. Then ester derivative was hydrolyzed by 10% sodium hydroxide and carboxylic acid derivative was obtained (7). The propanenitrile derivative (8) was formed by reacting 4,6-diphenyl-3(2H)-pyridazinone with acrylonitrile. This derivative was then hydrolyzed by 10 N hydrochloric acid to carboxylic acid derivative (9).

The title compounds were synthesized by the reaction of appropriate amine derivatives with the mixed anhydrides that were obtained by the reaction of (6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)-acetic acid and 3-(6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)propanoic acid with ethyl chloroformate in dichloromethane at 0 °C in the presence of triethylamine (Scheme 1). The chemical structures of the compounds synthesized are given in Table 1. The synthesis of compounds **1-7** and **9** was performed as described previously [18,19]. Fourteen title compounds (**7a-g** and **9a-g**) and 3-(6-oxo-3,5-diphenyl-6H-pyridazin-1-yl)propanenitrile **(8)** were synthesized for the first time in this study.

The analgesic activity of the compounds was studied using a p-benzoquinone (PBQ)-induced writhing test. As shown in Table 1, all compounds showed significant analgesic activity at 100 mg/kg dose level in ratios from 55.6 to 82.7%. All compounds except for **7g** were more potent than aspirin in the PBQ–induced writhing test at 100 mg/kg dose. Four compounds (**7, 7c**, **7e** and **9f)** exhibited more than 80% analgesic activity. Among the compounds synthesized, compound **7e** was the most potent in terms of analgesic and anti-inflammatory activity and had no ulcerogenic side effects. As reported in Table 1, significant analgesic activity was also observed at the 50 mg/kg dose level, but in lesser degrees (15.9-50.2%). On the other hand, compounds **7f, 7g**, **9f** and **9g** resulted in an ulcerogenic effect even at a half dose.

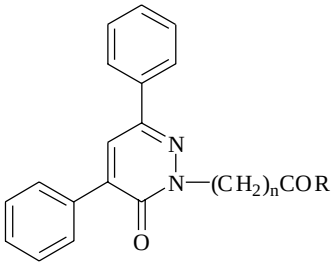
The anti-inflammatory activity of the synthesized compounds was studied using a carrageenan-induced hind paw edema model in mice at 100 mg/kg dose. The results are shown in Table 1. Vinegar et al. claimed that the inhibitory effects of agents that act on the first stage of carrageenan-induced hind paw inflammation are attributable to inhibition of the release of chemical mediators such as histamine and serotonin. They also claimed that the second stage of the hind paw edema may be related to arachidonic acid metabolites since it is inhibited by aspirin and other arachidonate cyclooxygenase inhibitors[20]. All pyridazinone derivatives bearing acetamide moieties (**7a-7g**) showed remarkably potent anti-inflammatory activity, especially 270 min after the drug was administered, but compounds **7b**, **7c** and **7e** had the most potent anti-inflammatory

activity throughout the study. None of these compounds displayed ulcerogenic side effects. These compounds might have effect in both stages of inflammation.



Scheme 1. Synthesis of compounds 7a-g and 9a-g.

Table 1. Analgesic and anti-inflammatory activity of the title compounds.



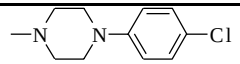
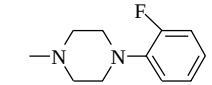
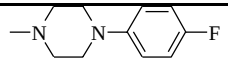
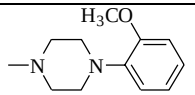
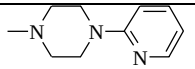
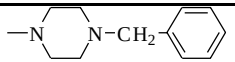
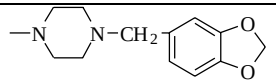
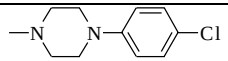
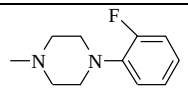
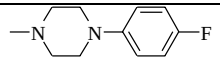
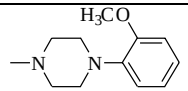
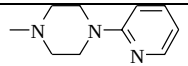
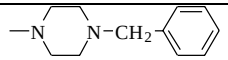
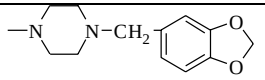
No.	R	n	Analgesic activity at 100 mg/kg			Analgesic activity at 50 mg/kg			Anti-inflammatory activity Inhibition of edema %			
			Number of writhings SEM	Inhibition %	Ratio of ulceration	Number of writhings SEM	Inhibition %	Ratio of ulceration	90 min	180 min	270 min	360 min
7a		1	17.4±1.25	58.9 [a]	0/6	33.8±1.40	15.9[c]	0/6	0	10.9	21.9[a]	22.9[a]
7b		1	10.7±1.20	73.8[a]	0/6	25.7±1.56	36.1[a]	0/6	28.5	28.8 [b]	37.9[a]	40.6[a]
7c		1	8.0±1.18	80.1[a]	0/6	21.7±0.92	46.0[a]	0/6	30.5[c]	33.3[b]	41.6[a]	44.9[a]
7d		1	17.7±1.52	59.3[a]	1/6	28.7±0.95	28.7[a]	0/6	8.2	9.8	21.7[a]	25.3[a]
7e		1	7.3±0.61	82.7[a]	0/6	21.5±1.18	46.5[a]	0/6	38.4[b]	36.3[a]	48.1[a]	46.9[a]
7f		1	13.8±1.64	67.4[a]	0/6	28.0±2.48	30.3[a]	1/6	18.5	18.9	26.2[a]	28.3[a]
7g		1	18.8±0.95	55.6[a]	3/6	30.3±2.47	24.6[a]	3/6	15.3	15.6	29.8[a]	32[a]

Table 1. Contunied.

No.	R	n	Analgesic activity at 100 mg/kg			Analgesic activity at 50 mg/kg			Anti-inflammatory activity Inhibition of edema %			
			Number of writhings SEM	Inhibition %	Ratio of ulceration	Number of writhings SEM	Inhibition %	Ratio of ulceration	90 min	180 min	270 min	360 min
9a		2	9.2±1.22	78.3[a]	0/6	26.7±0.84	33.6[a]	0/6	18.7	21.8	26 [c]	26.5[b]
9b		2	10.3±1.05	76.7[a]	0/6	26.5±1.67	34.1[a]	0/6	15.6	17.7	20.4 [c]	23.6[c]
9c		2	10.5±1.48	75.2[a]	0/6	24.3±1.52	39.6[a]	0/6	13.4	15.6	20.4 [c]	22.6[c]
9d		2	12.5±1.20	70.5[a]	0/6	26.8±2.40	33.3[a]	0/6	23.9	25.2	23.7[c]	21.2[c]
9e		2	9.2±1.08	78.3[a]	0/6	23.5±1.34	41.5[a]	0/6	21.3	20.3	25.9[c]	30.5[a]
9f		2	8.2±0.60	80.6[a]	1/6	21.8±1.74	45.7[a]	2/6	20.1	26.3	38.8[a]	37.6[a]
9g		2	16.4±1.50	61.2[a]	3/6	27.8±1.66	30.8[a]	1/6	11	16.5	20.4[c]	17.4[c]
7	-OH	1	7.5±1.18	82.3[a]	1/6	20.0±1.18	50.2[a]	0/6	31.2	31.4[c]	33.5[a]	38.1[a]
9	-OH	2	8.7±0.63	79.3[a]	0/6	28.2±1.99	29.9[a]	0/6	16.1	22.4	27.1[c]	33.4[a]
Aspirin			18.2±2.14	56.9[a]	0/6							
Indomethacin[d]									35.7 [c]	34.8[b]	40.3[a]	46[a]

[a]P <0.001; [b]P <0.01; [c]P <0.05. [d]Anti-inflammatory activity of indomethacin was tested at 10 mg/kg dose.

In conclusion, there was no difference in terms of the analgesic and anti-inflammatory activity of acetamide and propanamide derivatives. However the more active compounds in terms of anti-inflammatory activity were found in acetamides derivatives in general. When the chemical structures of the active compounds are taken into consideration, it appears that substitutions on the phenyl ring of the phenylpiperazine moiety by *o*- or *p*-fluoro groups or a 2-pyridyl group increased both the analgesic and anti-inflammatory activity of acetamide derivatives markedly. As for the ulcerogenic effects of the synthesized compounds, compounds **7g** and **9g** caused severe damage to the gastric mucosa at 100mg/kg dose. However, the compounds **7b**, **7c** and **7e** possessing the highest anti-inflammatory activity were safe in terms of ulcerogenic effects.

Table 2. Effects of the compounds at 100 mg/kg on the increased vascular permeability induced by acetic acid.

Compound	Evans blue concentration (μg/mL) $\pm$ SEM	Inhibition (%)
Control	7.3 ± 0.75	
7b	4.4 ± 0.29^{b}	40.03
7c	4.2 ± 0.32^{b}	42.3
7e	3.7 ± 0.26^{a}	49.03
9a	5.1 ± 0.55^{c}	30.1
9b	5.3 ± 0.48^{c}	27.4
9c	5.4 ± 0.42^{c}	26.3
9d	5.5 ± 0.73^{c}	24.4
9e	4.9 ± 0.34^{c}	32.9
9f	5.0 ± 0.52^{c}	31.6
7	5.5 ± 0.33^{c}	25.4
9	4.6 ± 0.36^{b}	36.5
Indomethacin	4.1 ± 0.47^{b}	43.8

$^{a}P < 0.001$; $^{b}P < 0.01$; $^{c}P < 0.05$.

In order to confirm the results, compounds possessing inhibitory activity higher than 70% in the PBQ-induced writhing test were studied further using an acetic acid-induced increase in a capillary permeability model and comparable results were obtained (Table 2).

The antinociceptive activity of acids used as starting materials in the preparation of acetamide and propanamide derivatives was generally more potent than that of other compounds except for compounds **7b, 7e** and **7f.**

Acknowledgments

Financial support from the Gazi University Research Fund is gratefully acknowledged.

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