FABAD J. Pharm. Sci., 35, 1-11, 2010

RESEARCH ARTICLE

Synthesis of (6-Substituted-3(2H)-pyridazinon-2-yl) acetic Acid and (6-Substituted-3(2H)-pyridazinon-2-yl)acetamide Derivatives and Investigation of Their Analgesic and Anti-inflammatory Activities

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Synthesis of (6-Substituted-3(2H)-pyridazinon-2-yl) acetic Acid and (6-Substituted-3(2H)-pyridazinon-2-yl)acetamide Derivatives and Investigation of Their Analgesic and Anti-inflammatory Activities

Summary

*In our previous study we reported that synthesis of ethyl (6-substituted-3(2H)-pyridazinon-2-yl)acetate derivatives which showed potent analgesic and anti-inflammatory activities. In the present paper, as an extension of our earlier work, we described acid **IVa-h** and amide **Va-h** analog of ethyl (6-substituted-3(2H)-pyridazinon-2-y) acetate derivatives. Except for **IVa**, all compounds were synthesized for the first time in this study. The chemical structures of newly synthesized compounds were elucidated by their IR, ^{1}H-NMR spectral data, and elementary analysis. The compounds were screened for their analgesic and anti-inflammatory activities, acute toxicity and ulcerogenic effect. Some compounds showed significant analgesic and anti-inflammatory activities and at the same time, nil ulcerogenic effect. Among the (6-substituted-3(2H)-pyridazinon-2-yl) acetic acid **IV** derivatives the best activity was presented by **IVe** and **IVg** derivatives.*

Key Words: *3(2H)-Pyridazinone, analgesic activity, anti-inflammatory activity.*

(6-Sübstitüe-3(2H)-piridazinon-2-il) asetik Asit ve (6-Sübstitüe-3(2H)-piridazinon-2-il)asetamit Türevlerinin Sentezi ve Analjezik ve Antienflamatuvar Aktivitelerinin Araştırılması

Özet

*Önceki çalışmamızda güçlü analjezik ve antienflamatuvar aktivite gösteren etil (6-sübstitüe-3(2H)-piridazinon-2-il)asetat türevlerinin sentezini rapor ettik. Sunulan bu çalışmada önceki çalışmalarımızın bir genişletilmesi olarak etil (6-sübstitüe-3(2H)-piridazinon-2-il)asetat türevlerinin asit **IVa-IVh** ve amit **Va-Vh** türevlerinin sentezini tanımladık. **IVa** hariç tüm bileşikler, ilk defa bu çalışmada sentezlenmiştir. Yeni sentezlenen bileşiklerin yapısı IR, ^{1}H-NMR ve elementel analiz çalışmaları ile aydınlatılmıştır. Bileşikler analjezik, antieflamatuvar aktivite, akut toksisite ve ülserojenik etki yönünden taranmışlardır. Bazı bileşikler belirgin analjezik antienflamatuvar aktivite gösterirken, ülser yapıcı etki göstermemişlerdir. (6-sübstitüte-3(2H)-piridazinon-2-il) asetik asit **IV** türevleri arasında en iyi aktivite **IVe** ve **IVg** tarafından gösterilmiştir.*

Anahtar Kelimeler: *3(2H)-Piridazinon, analjezik aktivite, antienflamatuvar aktivite.*

Received: 18.01.2011
Revised: 30.11.2011
Accepted: 12.12.2011

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INTRODUCTION

The ability of nonsteroidal anti-inflammatory drugs (NSAIDs) to modulate the pain, inflammation and fever made them one of the most used therapeutical classes in the world (1-4). The utility of nonsteroidal anti-inflammatory drugs in the treatment of inflammation and pain is often limited by gastrointestinal liabilities including ulceration and bleeding. Many papers have been reported on the analgesic anti-inflammatory and antipyretic evaluation of several pyrazolones (5-10). It is known that some pyrazolone derivatives like dipyrone and phenylbutazone possess analgesic and anti-inflammatory activities, but several side effects have limited the clinical use of these drugs (11-15). Pyridazinone derivatives have aroused a great deal of attention due to its structural relationship to pyrazolone derivatives in the point of ring enlargement of pyrazolone to pyridazinone.

A lot of 3(2H)-pyridazinone derivatives have been reported as analgesic and anti-inflammatory agents without gastrointestinal side effects (16-26). This is in agreement with in our experience in the pyridazinone field (27-31). In fact, most of these compounds have been designed as close structural relatives to emorfazone (Figure 1), which is currently

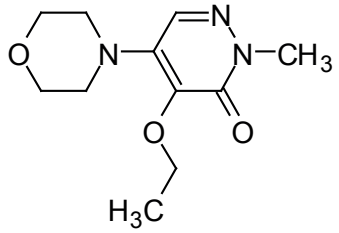


Figure 1. Emorfazone

being marketed in Japan as an analgesic and anti-inflammatory drug (25).

In continuation of our efforts in search of potent anti-inflammatory and analgesic compounds which can be developed as safer anti-inflammatory drugs, we have synthesized seven new (6-substituted-3(2*H*)-pyridazinon-2-yl)acetic acid **IV** derivatives and eight new (6-substituted-3(2*H*)-pyridazinon-2-yl) acetamide **V** derivatives (Scheme 1). Compounds **IV** have been linked to an acetic acid moiety, a characteristic feature of many NSAIDs such as indometacin, sulindac, tolmetin, diclofenac, lonazolac and isofezolac (32-35). Furthermore some of amide compounds have been reported high analgesic and anti-inflammatory activities and a lower ulcerogenic effect in literature (36-40).

MATERIAL and METHODS

Chemistry

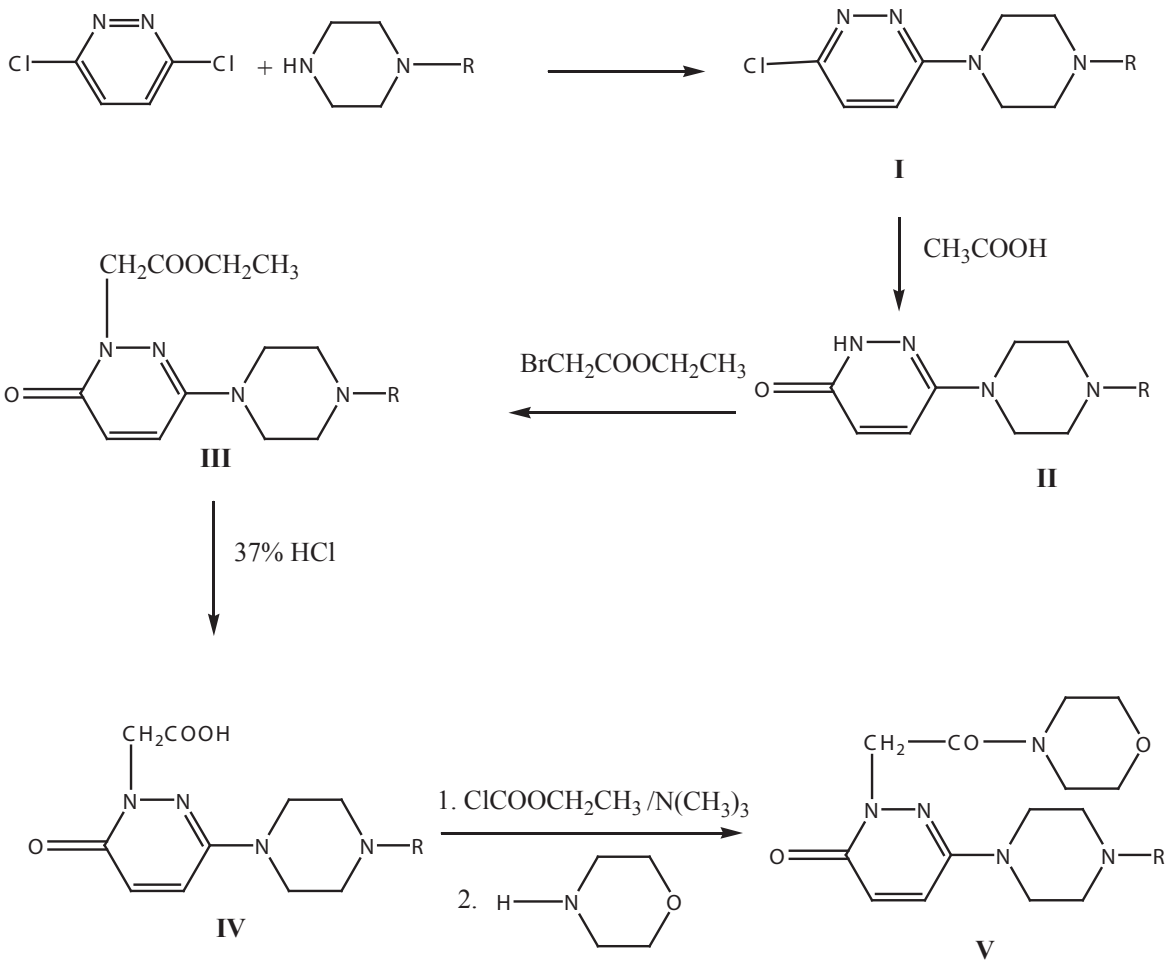
All chemicals were purchased locally from Merck AG and Aldrich Chemical. Melting points of the compounds were determined on Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the values given are uncorrected. The IR spectra of the compounds were recorded on a Bruker Vector 22 IR Spectrophotometer (Bruker Analytische Messtechnik, Karlrure, Germany). The ^{1}H-NMR spectra of the compounds were recorded on a Bruker 400 MHz-NMR Spectrometer (Rheinstetten, Karlrure, Germany) using tetramethylsilane as an internal standard. All the chemical shifts were recorded as δ (ppm). Elemental analyses were performed with Leco-932 (C, H, N, S-Elemental analyzer, St. Joseph, USA) at Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara-Turkey) and within ±0.4% of the theoretical values. 6-Substituted-3-chloropyridazines **I** were synthesized in our laboratory according to the reports in the literature (40, 41).

Synthesis of 6-substituted-3(2*H*)-pyridazinone derivatives **(IIa-h) (**27, 28)

A solution of 0.05 mol of a 6-substituted-3-chloropyridazinone **I** derivative in 30 ml glacial acetic acid was refluxed for 6 h. The acetic acid was removed under reduced pressure, and the residue dissolved in water and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by crystallization from ethanol.

Synthesis of ethyl (6-substituted-3(2*H*)-pyridazinon-2-yl)acetate derivatives **(IIIa-h)** (31)

A mixture of required 6-substituted-3(2H)pyridazinones **II** (0.01 mol), ethyl bromoacetate (0.02 mol) and potassium carbonate (0.02 mol) in acetone (40 ml) was refluxed overnight. After the mixture was cooled, the



Scheme 1. Synthesis of (6-substituted-3(2H)-pyridazinon-2-yl)acetic acid **IV** and (6-substituted-3(2H)-pyridazinon-2-yl)acetamide **V** derivatives

organic salts were filtered off, the solvent evaporated, and the residue was purified by crystallization with appropriate alcohol to give the esters.

Synthesis of (6-substituted-3(2*H*)-pyridazinon-2-yl) acetic acid derivatives **(IVa-h)**

Ethyl (6-substituted-3(2*H*)-pyridazinon-2-yl) acetate **III** derivatives were heated up to reflux temperature in hydrochloric acid (37% w/v) for 10 h. After cooling, the reaction mixture was neutralized with NaOH (10% w/v) and acidified with diluted hydrochloric acid and the precipitate formed was filtered off, washed with water, dried and crystallized from ethanol.

(6-(4-Phenylpiperazin-1-yl)-3(2H)-pyridazinon-2-yl) acetic acid **(IVa)**

Recrystallized from ethanol to yield 53%. m.p.: 122°C. IR (KBr) cm^{-1}: 1743 C=O acid, 1660 C=O lactam, 1588-1515 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.60-11.06 (s, 1H, -COOH) 7.69 (d, 1H, pyridazinone H_5), 7.22 (t, 2H, phenyl H_3+H_5), 7.00 (d, 2H, phenyl H_2+H_6), 6.93 (d, 1H, pyridazinone H_4), 6.85-6.73 (t, 1H, phenyl H_4), 4.75 (s, 2H, -$\underline{CH_2}$COOH), 4.60 (s, 2H, -$\underline{CH_2}$COOH), 3.40-3.33 (m, 4H, piperazine a+a'), 3.26-3.10 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{16}H_{18}N_4O_3$ (314.42): C: 61.13; H: 5.77; N: 17.82 Found: C: 61.37; H: 5.63; N: 17.59.

(6-(4-Benzylpiperazin-1-yl)-3(2H)-pyridazinon-2-yl) acetic acid **(IVb)**
Recrystallized from ethanol to yield 90%. m.p.: 153°C. IR (KBr) cm^{-1}: 1735 C=O acid, 1665 C=O lactam, 1584-1515 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.50-11.04 (s, 1H, -COOH), 7.65 (d, 1H, pyridazinone H_5), 7.35 (m, 2H, phenyl H_2+H_6), 7.18 (m, 3H, phenyl H_3+H_4+H_5), 6.83 (d, 1H, pyridazinone H_4), 4.68 (s, 2H, -$\underline{CH_2}$COOH), 4.42 (s, 2H, -$\underline{CH_2}$COOH), 3.83 (d. 2H, -CH_2-phenyl), 3.38-3.35 (m, 4H, piperazine a+a'), 3.24-3.14 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{17}H_{20}N_4O_3$ (328.15): C: 62.18; H: 6.14; N: 17.06 Found: C: 62.49; H: 6.65; N: 17.41.

(6-(4-(4-Chlorophenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)acetic acid **(IVc)**
Recrystallized from ethanol to yield 50%. m.p.: 236°C. IR (KBr) cm^{-1}: 1750 C=O acid, 1662 C=O lactam, 1585-1495 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.31-11.39 (s, 1H, -COOH), 7.65 (d, 1H, pyridazinone H_5), 7.24 (d, 2H, phenyl H_2+H_6), 7.03 (d, 2H, phenyl H_3+H_5), 6.85 (d, 1H, pyridazinone H_4), 4.74 (s, 2H, -$\underline{CH_2}$COOH), 4.64 (s, 2H, -$\underline{CH_2}$COOH), 3.40-3.34 (m, 4H, piperazine a+a'), 3.25-3.10 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{16}H_{17}ClN_4O_3$ (348.78): C: 55.10; H: 4.91; N: 16.06. Found: C: 55.43; H: 4.61; N: 16.41.

(6-(4-(2-Ethoxyphenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)acetic acid **(IVd)**
Recrystallized from ethanol to yield 53%. m.p.: 237°C. IR (KBr) cm^{-1}: 1758 C=O acid, 1647 C=O lactam, 1597-1505 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.33-11.40 (s, 1H, -COOH), 7.65 (d, 1H, pyridazinone H_5), 7.05-6.70 (m, 5H, phenyl protons+pyridazinone H_4), 4.83 (s, 2H, -$\underline{CH_2}$COOH), 4.55 (s, 2H, -$\underline{CH_2}$COOH), 4.07-3.91 (q, 2H, O$\underline{CH_2}CH_3$), 3.40-3.26 (m, 4H, piperazine a+a'), 3.17-2.94 (m, 4H, piperazine b+b'), 1.45-1.26 (t, 3H, -$OCH_2\underline{CH_3}$). Analysis Calculated for $C_{18}H_{22}N_4O_4$ (358.16): C: 60.32; H: 6.19; N: 15.63. Found: C: 60.70; H: 6.51; N: 15.72.

(6-(4-(2-Fluorophenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)acetic acid **(IVe)**
Recrystallized from ethanol to yield 63%. m.p.: 246°C. IR (KBr) cm^{-1}: 1755 C=O acid, 1656 C=O lactam, 1592-1504 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.34-11.42 (s, 1H, -COOH), 7.69-7.57 (d, 1H, pyridazinone H_5), 7.24-7.04 (m, 4H, phenyl protons) 7.04-6.96 (d, 1H, pyridazinone H_4), 4.80 (s, 2H, -$\underline{CH_2}$COOH), 4.62 (s, 2H, -$\underline{CH_2}$COOH), 3.50-3.37 (m, 4H, piperazine a+a'), 3.15-3.07 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{16}H_{17}FN_4O_3$ (332.13): C: 57.83; H: 5.16; N: 16.86. Found: C: 57.56; H: 5.41; N: 16.82.

(6-(4-(2-piridyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)acetic acid **(IVf)**
Recrystallized from ethanol to yield 78%. m.p.: 221°C. IR (KBr) cm^{-1}: 1756 C=O acid, 1664 C=O lactam, 1587-1500 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.35-11.37 (s, 1H, -COOH), 7.75-7.45 (d, 1H, pyridazinone H_5), 7.25-6.67(m, 5H, aromatic protons+pyridazinone H_4), 4.71 (s, 2H, -$\underline{CH_2}$COOH), 4.61 (s, 2H, -$\underline{CH_2}$COOH), 3.39-3.33 (m, 4H, piperazine a+a'), 3.13-2.96 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{15}H_{17}N_5O_3$ (315.13): C: 57.13; H: 5.43; N: 22.21. Found: C: 57.32; H: 5.09; N: 22.43.

(6-(4-(3-Trifluoromethylphenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)acetic acid **(IVg)**
Recrystallized from ethanol to yield 56%. m.p.: 232°C. IR (KBr) cm^{-1}:1741 C=O acid, 1663 C=O lactam, 1590-1502 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.38-11.40 (s, 1H, -COOH), 7.66 (d, 1H, pyridazinone H_5), 7.30-7.08 (m, 4H, aromatic protons), 6.93 (d, 2H, pyridazinone H_4), 4.75 (s, 2H, -$\underline{CH_2}$COOH), 4.06 (s, 2H, -$\underline{CH_2}$COOH), 3.47-3.18 (m, 4H, piperazine a+a'), 3.29-3.20 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{17}H_{17}F_3N_4O_3$ (382.13): C: 53.40; H: 4.48; N: 14.65. Found: C: 53.16; H: 4.48; N: 14.32.

(6-(4-(2,3-Xylyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)acetic acid **(IVh)**
Recrystallized from ethanol to yield 88%. m.p.: 245°C. IR (KBr) cm^{-1}: 1742 C=O acid, 1663 C=O lactam, 1589-1510 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 14.30-11.32 (s, 1H, -COOH), 7.64 (d, 1H, pyridazinone H_5), 7.14-6.85(m, 4H, aromatic protons+ pyridazinone H_4), 4.85 (s, 2H, -$\underline{CH_2}$COOH), 4.60 (s, 2H, -$\underline{CH_2}$COOH), 3.55-3.36 (m, 4H, piperazine a+a'), 3.03-2.73 (m, 4H, piperazine b+b'), 2.13-2.06 (s, 6H, xylyl methyl protons). Analysis Calculated for $C_{18}H_{22}N_4O_3$ (342.17): C: 63.14; H: 6.48; N: 16.56. Found: C: 63.23; H: 6.56; N: 16.33.

Synthesis of (6-Substituted-3(2H)-pyridazinon-2-yl) acetamide derivatives **(Va-h)**

To the solution of **(**6-substituted-3(2H)-pyridazinon-2-yl)acetic acid (**IV**) derivatives (1.3 mmol) were added triethylamine (4 mmol) and ethyl chloroformate (1.5 mmol), followed by stirring at 0°C for 30 min. After the addition of morpholine (1.6 mmol), the mixture was stirred for an additional 1 h at 0°C. Then, the reaction mixture was warmed to room temperature, and kept stirring overnight. After the solvent was evaporated under reduce pressure, acetone was added, and filtered. To precipitate the product, 20 mL of water was added to the residue. The precipitate was filtered, dried, washed with boiling ether, dried, and crystallized from isopropyl alcohol.

(6-(4-Phenylpiperazin-1-yl)-3(2H)-pyridazinon-2-yl) morpholinoacetamide **(Va)**

Recrystallized from ethanol to yield 56%. m.p.: 215°C. IR (KBr) cm^{-1}: 1681 C=O amide, 1652 C=O lactam, 1590-1510 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.60 (d, 1H, pyridazinone H_5), 7.46-6.90 (m, 5H, aromatic protons), 6.75 (d, 1H, pyridazinone H_4), 4.79 (s, 2H, -$\underline{CH_2}$CO), 4.78 (s, 2H, -$\underline{CH_2}$CO), 3.60-3.41 (m, 8H, morpholine protons), 3.40-3.34 (m, 4H, piperazine a+a'), 3.32-3.26 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{20}H_{25}N_5O_3$ (383.4): C: 62.65; H: 6.57; N: 18.26. Found: C: 62.99; H: 6.32; N: 18.70.

(6-(4-Benzylpiperazin-1-yl)-3(2H)-pyridazinon-2-yl) morpholinoacetamide **(Vb)**

Recrystallized from isopropyl alcohol to yield 62%. m.p.: 211°C. IR (KBr) cm^{-1}: 1679 C=O amide, 1653 C=O lactam, 1588-1505 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.59 (d, 1H, pyridazinone H_5), 7.43-6.91 (m, 5H, aromatic protons), 6.80 (d, 1H, pyridazinone H_4), 4.78 (s, 2H, -$\underline{CH_2}$CO), 4.76 (s, 2H, -$\underline{CH_2}$CO), 3.83 (d. 2H, CH_2-phenyl), 3.60-3.40 (m, 8H, morpholine protons), 3.38-3.33 (m, 4H, piperazine a+a'), 3.30-3.26 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{21}H_{27}N_5O_3$ (397.27): C: 63.46; H: 6.85; N: 17.62. Found: C: 63.67; H: 6.54; N: 17.98.

(6-(4-(4-Chlorophenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)morpholinoacetamide **(Vc)**

Recrystallized from isopropyl alcohol to yield 52%. m.p.: 222°C. IR (KBr) cm^{-1}: 1682 C=O amide, 1653 C=O lactam, 1593-1512 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.65-7.59 (d, 1H, pyridazinone H_5), 7.47-6.93 (m, 4H, aromatic protons), 6.87-6.80 (d, 1H, pyridazinone H_4), 4.79 (s, 2H, -$\underline{CH_2}$CO), 4.77 (s, 2H, -$\underline{CH_2}$CO), 3.61-3.41 (m, 8H, morpholine protons), 3.37-3.33 (m, 4H, piperazine a+a'), 3.31-3.27 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{20}H_{24}ClN_5O_3$ (417.88): C: 57.48; H: 5.79; N: 16.76. Found: C: 57.72; H: 5.92; N: 16.57.

(6-(4-(2-Ethoxyphenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)morpholinoacetamide **(Vd)**

Recrystallized from isopropyl alcohol to yield 61%. m.p.: 229°C. IR (KBr) cm^{-1}: 1680 C=O amide, 1651 C=O lactam, 1590-1510 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.64 (d, 1H, pyridazinone H_5), 7.50-6.95 (m, 4H, aromatic protons), 6.83 (d, 1H, pyridazinone H_4), 4.80 (s, 2H, -$\underline{CH_2}$CO), 4.76 (s, 2H, -$\underline{CH_2}$CO), 4.05-3.94 (q, 2H, O$\underline{CH_2}$$CH_3$), 3.59-3.40 (m, 8H, morpholine protons), 3.36-3.33 (m, 4H, piperazine a+a'), 3.31-3.26 (m, 4H, piperazine b+b') 1.47-1.37 (t, 3H, -O$CH_2$$\underline{CH_3}$). Analysis Calculated for $C_{22}H_{29}N_5O_4$ (427.22): C: 61.81; H: 6.84 N: 16.38. Found: C: 61.67; H: 6.59; N: 16.11.

(6-(4-(2-Fluorophenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)morpholinoacetamide **(Ve)**

Recrystallized from isopropyl alcohol to yield 59%. m.p.: 219°C. IR (KBr) cm^{-1}: 1683 C=O amide, 1653 C=O lactam, 1595-1515 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.63 (d, 1H, pyridazinone H_5), 7.48-6.94 (m, 4H, aromatic protons), 6.89-6.81 (d, 1H, pyridazinone H_4), 4.80 (s, 2H, -$\underline{CH_2}$CO), 4.78 (s, 2H, -$\underline{CH_2}$CO), 3.62-3.43 (m, 8H, morpholine protons), 3.42-3.35 (m, 4H, piperazine a+a'), 3.34-3.25 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{20}H_{24}FN_5O_3$ (401.43): C: 59.84; H: 6.03; N: 17.45. Found: C: 59.54; H: 6.31; N: 17.81.

(6-(4-(2-Pyridyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)morpholinoacetamide **(Vf)**

Recrystallized from isopropyl alcohol to yield 47%. m.p.: 241°C. IR (KBr) cm^{-1}: 1685 C=O amide, 1656 C=O lactam, 1595-1515 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.73 (d, 1H, pyridazinone H_5), 7.65-6.70

(m, 5H, aromatic protons+pyridazinone H_4), 4.74 (s, 2H, -CH_2CO), 4.65(s, 2H, -CH_2CO), 3.61-3.42 (m, 8H, morpholine protons), 3.39-3.36 (m, 4H, piperazine a+a'), 3.33-3.28 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{19}H_{24}N_6O_3$ (384.19) C: 59.36; H: 6.29; N: 21.86. Found: C: 59.54; H: 6.35; N: 21.65.

(6-(4-(3-Trifluoromethylphenyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)morpholinoacetamide **(Vg)**
Recrystallized from isopropyl alcohol to yield 62%. m.p.: 229°C. IR (KBr) cm^{-1}: 1682 C=O amide, 1652 C=O lactam, 1590-1510 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.73-7.68 (d, 1H, pyridazinone H_5), 7.63-6.88 (m, 4H, aromatic protons), 6.85-6.78 (d, 1H, pyridazinone H_4), 4.74 (s, 2H, -CH_2CO), 4.65 (s, 2H, -CH_2CO), 3.42-3.35 (m, 8H, morpholine protons), 3.37-3.34 (m, 4H, piperazine a+a'), 3.32-3.28 (m, 4H, piperazine b+b'). Analysis Calculated for $C_{21}H_{24}F_3N_5O_3$ (441.42) C: 55.87; H: 5.36 N: 15.51. Found: C: 56.12; H: 5.67; N: 15.90.

(6-(4-(2,3-Xylyl)piperazin-1-yl)-3(2H)-pyridazinon-2-yl)morpholinoacetamide **(Vh)**
Recrystallized from isopropyl alcohol to yield 76%. m.p.: 213°C. IR (KBr) cm^{-1}: 1680 C=O amide, 1651 C=O lactam, 1590-1510 C=N, ^{1}H-NMR (DMSO-d_6) ppm, 7.63 (d, 1H, pyridazinone H_5), 7.58-6.87 (m, 3H, aromatic protons), 6.85-6.78 (d, 1H, pyridazinone H_4), 4.73(s, 2H, -CH_2CO), 4.64 (s, 2H, -CH_2CO), 3.41-3.34 (m, 8H, morpholine protons), 3.34-3.32 (m, 4H, piperazine a+a'), 3.29-3.26 (m, 4H, piperazine b+b'), 2.14-2.08 (s, 6H, xylyl CH_3 protons). Analysis Calculated for $C_{22}H_{29}N_5O_3$ (411.5) C: 64.21; H: 7.10; N: 17.02. Found: C: 63.96; H: 7.43; N: 17.31.

PHARMACOLOGICAL ACTIVITY

Animals

Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals were kept in colony cages (6 mice each), maintained on a standard pellet diet with water ad libitum, and left for two days for acclimatization before the experimental session. The food was withdrawn one day before the experiment, but allowed free access to water. Six animals were used in each group. Throughout the experiments, the animals were treated under the audit of the Gazi University Commission of Animal Ethics according to the suggested international ethical guidelines for the care of laboratory animals (Permission No: 62-12904)

Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethylcellulose (CMC). The control group animals received the same experimental handling as those of the test group except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indometacin (CAS 53-86-1) (10 mg/kg) or acetylsalicylic acid (CAS 50-78-2; ASA) (100 mg/kg) in 0.5% CMC was used as reference drug.

p-Benzoquinone-induced abdominal constriction test in mice ***(42)***

60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10g body weight of 2.5% (v/v) p-benzoquinone (PBQ; Merck) solution in distilled H_2O. The control group animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent the average of the total number of writhing observed. The antinociceptive activity was expressed as writhing percentage of that of the control group. 100 mg/kg ASA was used as the reference.

Carrageenan-induced hind paw edema ***(43)***

The method of Kasahara et al. (1985) was used with modifications in measuring periods. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). The mean values of the treated groups were compared with the mean values of the control group and analyzed using statistical methods. 60 min after the oral administration of 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 physiological saline (154 nmol/l NaCl) into subplantar tissue of the right hind paw. As the control, 25 µl saline solutions were injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after the induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). The mean values of the treated groups were compared with the mean values of the control group and analyzed using statistical methods. Indometacin (10 mg/kg) was used as reference drug.

Acute toxicity

Animals employed in the carrageen-induced paw edema experiment were observed during 24 h and mortality was recorded, if happens, for each group at the end of observation period.

Gastric-ulcerogenic effect

After the analgesic activity experiment, mice were euthanized under deep ether anesthesia and their stomachs were removed. Then, the abdomen of each mouse was opened through the great curvature and examined under dissecting microscope for lesions or bleedings.

Statistical analysis of data

Data obtained from the animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Students-Newman-Keels post-hoc tests. $p<0.05$ was considered to be significant [* $p<0.05$; ** $p<0.01$; *** $p<0.001$].

RESULTS AND DISCUSSION

Chemistry

Reaction of 3,6-dichloropyridazine with equimolar amount of the requisite secondary amine afforded 3-chloro-6-substituted pyridazine **I** in 68-89% yields. The physical and spectral properties of 3-chloro-6-substituted-pyridazine **I** was in accordance with the literature (41, 42). Therefore, we carried out the next steps of the reaction without any further analysis. Hydrolysis of compound **I** by acetic acid was performed in good yields to give 6-substituted-3(2H)-pyridazinone **II** derivatives. Ethyl (6-substituted-3(2*H*)-pyridazinon-2-yl)acetate **III** derivatives were obtained by the reaction of **II** with ethyl bromoacetate in the presence of K_2CO_3 in acetone. (6-Substituted-3(2*H*)-pyridazinon-2-yl)acetic acid derivatives **IV** were prepared by the hydrolysis of ethyl (6-substituted-3(2*H*)-pyridazinon-2-yl)acetate **III** derivatives in the presence of 37% hydrochloric acid. Finally, the acid **IV** derivatives were treated with ethyl chloroformate and morpholine, respectively in the presence of triethylamine in dry dichloromethane and at the end of this reaction (6-substituted-3(2*H*)-pyridazinon-2-yl)acetamide **V** derivatives were synthesized. The synthesis method of II and **III** derivatives were reported in our previous study (27-31). Except for **IVa** all of the **IV** and **V** derivatives were synthesized for the first time in this study. All the targets compounds were identified by spectroscopic data and confirmed by elemental analysis.

Analgesic and Anti-inflammatory Activity

Synthesized (6-substituted-3(2H)-pyridazinon-2-yl) acetic acid **IV** and (6-substituted-3(2H)-pyridazinon-2-yl)acetamide **V** derivatives were tested for analgesic activity by the phenylbenzoquinone-induced writhing test (43) (PBQ test), and for anti-inflammatory activity using the carragenan-induced paw edema method (44). The animals tolerated the tests well and no animals died during the experiments. All the pyridazinone derivatives were also evaluated for acute toxicity and gastric ulcerogenic effect tests. The obtained results are reported in Table 1 and Table 2.

Among the (6-substituted-3(2H)-pyridazinon-2-yl) acetic acid **IV** derivatives the best anti-inflammatory activity was presented by (6-(4-(2-fluorophenyl) piperazin-1-yl)-3(2*H*)-pyridazinon-2-yl)acetic acid **IVe** and (6-(4-(3-trifluorophenyl)piperazin-1-yl)-3(2*H*)-pyridazinon-2-yl)acetic acid **IVg** derivatives. Furthermore, compounds **IVg** showed higher analgesic activity as compared to the ASA. (Table 1)

(6-(4-(4-Fluorophenyl)piperazin-1-yl)-3(2H)-

Table 1. Effect of (6-substituted-3(2H)-pyridazinon-2-yl)acetic acid **IV** against carrageenan-induced paw edema and p-benzoquinone-induced writhing tests in mice

Compound	Dose, mg/kg, Per os	Anti-inflammatory Activity Thickness of Edema± SEM (Inhibition%)				Analgesic Activity Number of Stretching (Inhibition%)	Gastric Ulcerogenic Effect
		90 min	180 min	270 min	360 min		
Control		39.7±3.21	45.8±3.94	55.7±4.02	61.4±4.19	48.4±3.72	0/6
IVa	100	37.8±2.64 (4.8)	43.5±2.96 (5.0)	50.1±3.11 (10.1)	57.4±3.23 (6.5)	39.2±3.02 (19.0)	06
IVb	100	40.1±2.12	39.2±2.41 (14.4)	40.2±2.92 (27.8)*	42.3±3.05 (31.1)**	41.5±2.76 (14.2)	0/6
IVc	100	35.5±3.02 (10.3)	39.0±2.64 (14.8)	45.1±2.92 (19.0)	50.2±3.41 (18.2)	35.1 ±4.12 (27.5)*	0/6
IVd	100	40.0±3.27 (14.3)	42.5±3.36 (22.4)	45.2±4.19 (28.8)*	47.8±4.19 (29.9)*	37.6±3.14 (21.6)	0/6
IVe	100	34.7±3.62 (25.7)	38.2±3.82 (30.3)	42.3±3.91 (33.4)**	45.2±3.90 (33.7)**	27.3±3.29 (43.6)***	0/6
IVf	100	37.5±2.9 (16.7)	41.2±3.2 (20.5)	52.5±3.2 (12.5)	57.5±3.1 (15.4)	33.1±1.84 (31.6)**	0/6
IVg	100	36.3±4.25 (22.3)	40.7±4.12 (25.7)	42.7±3.48 (32.7)**	43.5±3.66 (34.2)**	20.8±1.30 (53.6)***	0/6
IVh	100	41.3±3.23 (11.6)	44.7±3.32 (18.4)	41.7±3.12 (24.5)	48.5±3.23 (23.5)	30.7±2.38 (31.5)**	0/6
Indometacin	10	30.7±1.92 (22.6)	33.1±2.04 (27.7)*	35.9±3.11 (35.5)**	39.8±2.96 (35.2)**		4/6
ASA	100					24.1.±1.72 (50.2)***	5/6

*p<0.05, **: p<0.01, ***: p<0.001 significant from the control value

pyridazinon-2-yl)morpholinoacetamide **Ve** and (6-(4-(3-trifluorophenyl)piperazin-1-yl]-3(2H)-pyridazinon-2-yl)morpholinoacetamide **Vg** were found to be more active than ASA (Table 2). Similarly, the anti-inflammatory activity of **Ve** and **Vg** were found close to that of indometacin in carrageenan-induced paw edema test (Table 2). A moderate activity was generally shown by all the remaining compounds although they were tested at a dosage much higher than indometacin. As a result, it can be said that substituted fluorophenylpiperazine and 3-trifluorophenylpiperazine substitutents on the pyridazinone ring could be critical for analgesic and anti-inflammatory activity. This is also in agreement with our previous study (27-31).

In addition, it is well known that most of anti-inflammatory drugs provide an ulcerogenic activity. In the present experiment, ASA and indometacin used as reference showed marked ulcerogenic effect. Thus, this study revealed that **IVg**, **Ve** and **Vg** possessed anti-inflammatory activity as well as indometacin and more potent analgesic activity than ASA and did not induce any gastric lesions or death the observation period. Most of non-steroidal anti-inflammatory drugs are acidic and generally referred to as drugs similar to ASA. On the other hand, a few chemically basic compounds such as benzydamine HCl, tiaramide HCl and mepirizole have pharmacological properties in common with ASA-like drugs. One of the most interesting characteristics of (6-substituted-3(2H)-pyridazinon-2-yl)acetic acid **IV** and (6-substituted-3(2H)-pyridazinon-2-yl) acetamide **V** derivatives is their basic nature, which differentiates them from the classical acidic nonsteroidal anti-inflammatory agents. In conclusion, these compounds appear to provide

Table 2. Effect of (6-substituted-3(2H)-pyridazinon-2-yl)acetamide V derivatives against carrageenan-induced paw edema and p-benzoquinone-induced writhing tests in mice

Compound	Dose, mg/kg, Per os	Anti-inflammatory Activity Thickness of Edema± SEM (Inhibition%)				Analgesic Activity Number of Stretching (Inhibition%)	Gastric Ulcerogenic Effect
		90 min	180 min	270 min	360 min		
Control		45.0±4.5	51.8±4.8	60.0±4.7	68.0±5.0	44.3±3.9	0/6
Va	100	45.0±4.4	50.0±4.8 (3.5)	53.7±4.9 (10.5)	49.3±2.6 (27.5)**	29.5±2.5 (33.4)***	06
Vb	100	42.2±3.5 (6.2)	46.8±3.7 (9.7)	51.8±3.4 (13.7)	57.0±3.9 (16.2)	31.7±2.2 (28.4)**	0/6
Vc	100	41.3±3.0 (8.2)	45.7±3.0 (11.7)	43.3±3.9 (27.8)**	47.3±4.5 (30.4)**	29.3 ±2.2 (33.9)**	0/6
Vd	100	42.2±3.5 (6.2)	46.8±3.7 (9.7)	51.8±3.4 (13.7)	57.0±3.9 (16.2)	31.7±2.2 (28.4)**	0/6
Ve	100	34.5±3.0 (23.3)	38.3±2.9 (26.1)	41.2±2.5 (31.3)*	44.2±2.5 (35.0)**	13.8±1.5 (68.8)***	0/6
Vf	100	33.5±3.2 (26.6)	37.8±2.7 (27.0)	41.8±1.7 (30.3)*	46.5±2.1 (31.6)**	29.3 ±2.8 (33.9)***	0/6
Vg	100	41.8±3.8 (8.2)	46.8±3.9 (9.7)	52.3±4.2 (12.8)	56.7±3.4 (36.6)***	16.3±1.3 (63.2)***	0/6
Vh	100	42.3±3.5 (6.0)	47.5±4.0 (8.3)	52.2±3.7 (12.8)	55.7±3.4 (20.5)	29.8±3.2 (32.7)**	0/6
Indometacin	10	32.7±3.1 (27.3)	35.3±2.8 (31.9)*	37.8±2.4 (37.0)**	41.2±1.8 (39.4)***		4/6
ASA	100					21.8±2.1 (50.8)***	5/6

*p<0.05, **: p<0.01, ***: p<0.001 significant from the control value

a starting point for the design and development of the novel and more active nonsteroidal anti-inflammatory agents.

ACKNOWLEDGEMENTS

This study was supported financially with the grant from Research Foundation of Gazi University (EF 02/2011-26).

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