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Synthesis of antipyrine/pyridazinone hybrids and investigation of their in vivo analgesic and anti-inflammatory activities

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Eleven antipyrine/pyridazinone hybrids were synthesized and evaluated for their in vivo analgesic and anti-inflammatory activities by p-benzoquinone-induced writhing test and carrageenan-induced paw edema model, respectively. The test results indicated that compounds **6a, 6c,** and **6d** were equally or more potent analgesic and anti-inflammatory agents than aspirin and indomethacin, respectively. Side effects of the compounds were examined on gastric mucosa. Most of the compounds were found to be non-ulcerogenic under test conditions.

Key Words: Hybrid, antipyrine, pyridazinone, writhing, carrageenan

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are useful for the treatment of inflammation, pain, and fever. The clinical effects of NSAIDs are based on the inhibition of the enzyme cyclooxygenase (COX), which catalyzes the rate-limiting step in the metabolism arachidonic acid to prostaglandin H_2 (PGH_2). PGH_2 is further metabolized to prostanoids, prostaglandins (PGs), and thromboxane A_2 (TxA_2). Various physiological effects of PGs include inflammatory reactions, blood pressure change, platelet aggregation, induction of labor, and

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intensification of pain and fever. Utility of NSAIDs in the treatment of inflammation and pain is often limited by gastrointestinal side effects including ulceration and bleeding.[1–5] Developing safer analgesic and anti-inflammatory compounds with no such side effects has recently been the goal of many researchers.

Antipyrine was the first pyrazolin-5-one derivative used as an analgesic and antipyretic and anti-inflammatory drug. Bioactive antipyrine derivatives have been synthesized and evaluated as potent anti-inflammatory, analgesic, antipyretic, and antimicrobial agents.[6–9] Furthermore, the synthesis of pyridazinone derivatives possessing such diverse pharmacological properties as anti-inflammatory, analgesic, antimicrobial, and antiviral activities has been attracting widespread attention.

In designing new bioactive agents, besides the development of completely new agents, there is another approach involving the synthesis of hybrid molecules. Combination of different pharmacophores each with a different mode of action in the same structure may lead to compounds having more efficiency in biological activity.[10] Diverse pharmacological properties of the compounds containing antipyrine and pyridazinone moieties have prompted us to design and synthesize hybrid molecules incorporating those scaffolds in a single molecule.

To identify new candidates that may be of value in designing new, potent, selective and less toxic analgesic and anti-inflammatory agents, we report herein the synthesis of new antipyrine derivatives incorporating pyridazinone pharmacophore as hybrid molecules possessing analgesic and anti-inflammatory activities. All compounds were also tested for the irritative and ulcerogenic action on the gastric mucosa. Finally, these derivatives were submitted to in silico oral biodisponibility screening to analyze their overall potential to qualify as a drug.

Experimental

The chemicals were purchased from commercial vendors and were used without purification. Thin-layer chromatography (TLC) was performed on Merck 60F254 plates. The reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or charring Dragendorff reagent.[11] Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geaetetechnik, Germany). IR spectra were obtained in-house using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory and only carbonyl stretching frequencies were given. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of the Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). High resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in either ESI (+) or ESI (–) methods, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). Flash chromatography was performed with a Combiflash® Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using hexane-ethyl acetate or dichloromethane-methanol solvent gradients.

General procedures for the synthesis of compounds

Synthesis of 6-substituted-3(2*H*)-pyridazinone derivatives 2a-f

A solution of 0.05 mol of a 6-substituted-3-chloropyridazine **1** derivative in 30 mL of glacial acetic acid was refluxed for 6 h. The acetic acid was removed under reduced pressure, and the residue was dissolved in water and extracted with $CHCl_3$. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The new residue was purified by recrystallization from ethanol.

Synthesis of 6-substitutedphenyl-3(2*H*)-pyridazinone derivatives 3a,b

In an oil bath 0.05 mol (4.6 g) glyoxalic acid and 0.15 mol appropriate acetophenone derivative were heated at 100-105 °C for 2 h. After the reaction mixture cooled down to 40 °C, 20 mL of water and 5 mL of ammonium hydroxide solution (25%) were added until the medium pH became 8. Then the reaction mixture was extracted with $CHCl_2$ (4 × 25 mL). To the aqueous layer was added 0.05 mol hydrazine hydrate (2.5 mL) and the reaction mixture was refluxed for 2 h. After completion of the reaction, the reaction mixture cooled down to room temperature. The resulting white precipitate was filtered and recrystallized from ethanol.

Synthesis of ethyl 6-substituted-3(2*H*)-pyridazinone-2-yl-acetate/propionate derivatives 4a-k

A mixture of required 6-substituted-3(2*H*)pyridazinones **2a-f** or **3a,b** (0.01 mol), ethyl bromoacetate/ethyl bromopropionate (0.02 mol), and potassium carbonate (0.02 mol) in acetone (40 mL) was refluxed for 12 h. After the mixture cooled, the organic salts were filtered off, the solvent evaporated, and the residue was purified by recrystallization with appropriate alcohol to give the esters.

Synthesis of 6-substituted-3(2*H*)-pyridazinone-2-yl-acetic/propionic acid derivatives 5a-k

To 50 mL of HCl (37%) was added 0.01 mol ethyl 6-substituted-3(2*H*)-pyridazinone-2-yl-acetate/propionate derivatives **4a-k** and the reaction mixture was refluxed for 1 h. After completion of hydrolysis, the reaction mixture was transferred into 300 mL of ice-cold water. The formed colorless crystals were collected by filtration.

Synthesis of 6-(4-substituted phenyl)-3(2*H*)-pyridazinone-2-ylacetamide and propionamide derivative 6a-k

To a solution of appropriate carboxylic acid derivative (1 mmol) and aminoantipyrine (1 mmol) in 10 mL of dichloromethane (DCM) were added dimethylaminopyridine (DMAP) (0.2 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) (1.1 mmol) and the resulting solution was stirred overnight at room temperature. The reaction mixture was then quenched with 1 N HCl and extracted with DCM. The organic phase was washed with a 1% $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified by flash column chromatography (Combiflash® Rf) using DCM-MeOH as eluents.

_N_-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1_H_-pyrazol-4-yl)-2-[6-oxo-3-(4-phenylpiperazin-1-yl) pyridazin-1(6_H_)-yl]acetamide 6a

Elution with DCM-MeOH (0%-4%) yielded **6a** as a white solid (yield 62%); mp 198-200 °C; IR (FTIR/FTNIR-ATR): 1709 cm^{-1} (C = O), 1668 cm^{-1} (C = O), 1645 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ 7.97 (1H, s, NH), 7.41-7.45 (2H, m, J = 7.6 Hz, J = 8 Hz, phenyl H-2, 6), 7.35-7.38 (2H, d, J = 7.2 Hz, phenyl H-3, 5), 7.26-7.30 (3H, m, phenyl H-4, phenylpiperazin H-3, 5), 7.16-7.19 (1H, d, J = 10 Hz, pyridazine H-4), 6.91-6.93 (1H, d, J = 10 Hz, pyridazine H-5), 6.89-6.96 (3H, m, phenylpiperazin H-2, 4, 6), 4.83 (2H, s, pyridazine-C$\underline{H}_2$), 3.43-3.48 (4H, m, J = 4.8 Hz, J = 5.2 Hz, piperazine H-2, 6), 3.24-3.27 (4H, m, J = 4.8 Hz, J = 5.2 Hz, piperazine H-3, 5), 3.05 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.67 (3H, s, pyrazole-5-C$\underline{H}_3$). $C_{27}H_{29}N_7O_3$ ESI-MS 500.2418 $[M+H]^+$.

2-[3-[4-(4-Chlorophenyl)piperazin-1-yl]-6-oxopyridazin-1(6_H_)-yl]-_N_-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1_H_-pyrazol-4-yl)acetamide 6b

Elution with DCM-MeOH (0%-4%) yielded **6b** as a white solid (yield 44%); mp 204-205 °C; IR (FTIR/FTNIR-ATR): 1703 cm^{-1} (C = O), 1646 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ: 7.99 (1H, s, NH), 7.27-7.45 (5H, m, phenyl), 7.21 (1H, d, J = 5.2 Hz, pyridazine H-4), 7.16 (2H, d, J = 10.4 Hz, chlorophenyl H-2, 6), 6.92 (2H, d, J = 10.4 Hz, chlorophenyl H-3, 5), 6.86 (1H, d, J = 4.8 Hz, pyridazine H-5), 4.83 (2H, s, pyridazine-C$\underline{H}_2$), 3.43 (4H, t, J = 5.2 Hz, piperazine H-2, 6), 3.21 (4H, m, J = 4.4 Hz, J = 5.6 Hz, piperazine H-3,5), 3.05 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.26 (3H, s, pyrazole-5-C$\underline{H}_3$). $C_{27}H_{28}ClN_7O_3$ ESI-MS 534.2010 $[M+1]^+$ (100%), 536.4231 $[M+1+2]^+$ (35%).

_N_-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1_H_-pyrazol-4-yl)-2-[3-[4-(4-fluorophenyl)piperazin-1-yl]-6-oxopyridazin-1(6_H_)-yl]acetamide 6c

Elution with DCM-MeOH (0%-4%) yielded **6c** as a white solid (yield 63%); mp 174-176 °C; IR (FTIR/FTNIR-ATR): 1711 cm^{-1} (C = O), 1665 cm^{-1} (C = O), 1642 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ: 7.93 (1H, s, NH), 7.42-7.45 (2H, m, J = 7.6 Hz, J = 8 Hz, phenyl H-2, 6), 7.35-7.37 (2H, d, J = 7.2 Hz, phenyl H-3, 5), 7.26-7.29 (1H, m, J = 7.6 Hz, phenyl H-4), 7.16-7.19 (1H, d, J = 10.4 Hz, pyridazine H-4), 6.93-6.95 (1H, d, J = 10.4 Hz, pyridazine H-5), 6.88-6.99 (4H, m, fluorophenyl H-2, 3, 5, 6), 4.83 (2H, s, pyridazine-C$\underline{H}_2$), 3.43-3.45 (4H, m, J = 4.8 Hz, J = 5.6 Hz, piperazine H-2, 6), 3.16-3.18 (4H, m, J = 4.4 Hz, J = 5.2 Hz, piperazine H-3, 5), 3.05 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.26 (3H, s, pyrazole-5-C$\underline{H}_3$). $C_{27}H_{28}FN_7O_3$ ESI-MS 540.2147 $[M+Na]^+$.

_N_-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1_H_-pyrazol-4-yl)-2-[3-[4-(2,3-dimethylphenyl)piperazin-1-yl]-6-oxopyridazin-1(6_H_)-yl]acetamide 6d

Elution with DCM-MeOH (0%-4%) yielded **6d** as a white solid (yield 50%); mp 225-227 °C; IR (FTIR/FTNIR-ATR): 1705 cm^{-1} (C = O), 1660 cm^{-1} (C = O), 1643 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ: 7.82 (1H, s, NH), 7.42-7.45 (2H, m, J = 7.6 Hz, J = 8 Hz, phenyl H-2, 6), 7.36-7.38 (2H, d, J = 7.6 Hz, phenyl H-3, 5), 7.26-7.29 (1H, m, J = 7.2 Hz, phenyl H-4), 7.19-7.21 (1H, d, J = 10 Hz, pyridazine H-4), 7.06-7.10 (2H, m, J = 7.2 Hz, J = 8 Hz, dimethyl phenyl H-5, 6), 6.90-6.93 (2H, m, J = 10.4 Hz, pyridazine H-5, dimethyl phenyl H-4), 4.83 (2H, s, pyridazine-C$\underline{H}_2$), 3.43-3.45 (4H, m, piperazine H-2, 6), 3.06 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.95-2.97 (4H, m,

J = 4 Hz, J = 4.8 Hz, piperazine H-3, 5), 2.27 (3H, s, phenyl-3-C$\underline{H}_3$), 2.27 (3H, s, pyrazole-5-C$\underline{H}_3$), 2.23 (3H, s, phenyl-2-C$\underline{H}_3$). $C_{29}H_{33}N_7O_3$ ESI-MS 528.2740 $[M+H]^+$.

2-[3-(4-Benzylpiperidin-1-yl)-6-oxopyridazin-1(6*H*)-yl]-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide 6e

Elution with DCM-MeOH (0%-4%) yielded **6e** as a white solid (yield 62%); mp 198-200 °C; IR (FTIR/FTNIR-ATR): 1664 cm^{-1} (C = O), 1642 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$) δ: 7.73 (1H, s, NH), 7.20-7.45 (10H, m, phenyl, benzyl), 7.15 (1H, d, J = 9.2 Hz, pyridazine H-4), 6.86 (1H, d, J = 10 Hz, pyridazine H-5), 4.80 (2H, s, pyridazine-C$\underline{H}_2$), 3.82 (2H, d, J = 13.2 Hz, phenyl-C$\underline{H}_2$), 3.05 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.26 (3H, s, pyrazole-5-C$\underline{H}_3$), 2.55-2.72 (4H, m, piperidine H-2, 6), 1.66-1.71 (5H, m, piperidine H-3, 4, 5). $C_{29}H_{32}N_6O_3$ ESI-MS 535.2410 $[M+Na]^+$.

3-[3-[4-(4-Chlorophenyl)piperazin-1-yl]-6-oxopyridazin-1(6*H*)-yl]-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)propanamide 6f

Elution with DCM-MeOH (0%-4%) obtained **6f** as a white solid (yield 51%); mp 185-187 °C; IR (FTIR/FTNIR-ATR): 1681 cm^{-1} (C = O), 1655 cm^{-1} (C = O), 1623 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ: 7.88 (1H, s, NH), 7.39-7.43 (2H, m, J = 7.2 Hz, J = 8.4 Hz, phenyl H-2, 6), 7.34-7.36 (2H, d, J = 7.2 Hz, phenyl H-3, 5), 7.25-7.29 (1H, m, J = 7.2 Hz, phenyl H-4), 7.20-7.22 (2H, d, J = 4.8 Hz, chlorophenyl H-3, 5), 7.12-7.15 (1H, d, J = 9.6 Hz, pyridazine H-4), 6.89-6.92 (1H, d, J = 9.6 Hz, pyridazine H-5), 6.82-6.84 (2H, d, J = 4.8 Hz, chlorophenyl H-2, 6), 4.39-4.42 (2H, t, J = 6.8 Hz, pyridazine-C$\underline{H}_2$-CH_2), 3.36-3.39 (4H, t, J = 5.2 Hz, piperazine H-2, 6), 3.18-3.21 (4H, t, J = 5.2 Hz, piperazine H-3, 5), 3.05 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.83-2.87 (2H, t, J = 6.8 Hz, pyridazine-CH_2-C$\underline{H}_2$), 2.22 (3H, s, pyrazole-5-C$\underline{H}_3$). $C_{28}H_{30}ClN_7O_3$ ESI-MS 548.2176 $[M+H]^+$ (100%), 550.3263 $[M+1+2]^+$ (35%).

***N*-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-[3-[4-(4-fluorophenyl)piperazin-1-yl]-6-oxopyridazin-1(6*H*)-yl]propanamide 6g**

Elution with DCM-MeOH (0%-4%) obtained **6g** as a white solid (yield 42%); mp 206-208 °C; IR (FTIR/FTNIR-ATR): 1662 cm^{-1} (C = O), 1628 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$) δ: 7.83 (1H, s, NH), 7.35-7.43 (4H, m, phenyl H-2, 3, 5, 6), 7.28 (1H, m, phenyl H-4), 7.13-7.15 (1H, d, J = 9.6 Hz, pyridazine H-4), 6.95-6.97 (1H, d, J = 8.8 Hz, pyridazine H-5), 6.86-6.91 (4H, m, fluorophenyl H-2, 3, 5, 6), 4.39-4.43 (2H, t, J = 6.4 Hz, pyridazine-C$\underline{H}_2$-CH_2), 3.38-3.40 (4H, t, J = 5.2 Hz, piperazine H-2, 6), 3.14-3.16 (4H, t, J = 5.2 Hz, piperazine H-3, 5), 3.05 (3H, s, pyrazole-1-C$\underline{H}_3$), 2.84-2.88 (2H, dd, J = 6.8 Hz, J = 7.2 Hz, pyridazine-CH_2-C$\underline{H}_2$), 2.23 (3H, s, pyrazole-5-C$\underline{H}_3$). $C_{28}H_{30}FN_7O_3$ ESI-MS 532.2464 $[M+H]^+$.

3-[3-[4-(3-Chlorophenyl)piperazin-1-yl]-6-oxopyridazin-1(6*H*)-yl]-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)propanamide 6h

Elution with DCM-MeOH (0%-4%) yielded **6h** as a white solid (yield 54%); mp 145-147 °C; IR (FTIR/FTNIR-ATR): 1650 cm^{-1} (C = O), 1620 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$) δ: 7.93 (1H, s, NH), 7.38-7.42 (2H, m, J = 7.2 Hz, J = 7.6 Hz, phenyl H-2, 6), 7.34-7.36 (2H, d, J = 7.2 Hz, phenyl H-3, 5), 7.28 (1H, m, phenyl

H-4), 7.15-7.19 (1H, t, J = 8 Hz, chlorophenyl H-5), 7.12-7.15 (1H, d, J = 10.4 Hz, pyridazine H-4), 6.89-6.92 (1H, d, J = 9.6 Hz, pyridazine H-5), 6.77-6.88 (3H, m, chlorophenyl H-2, 4, 6), 4.38-4.41 (2H, t, J = 6.8 Hz, pyridazine-C$\underline{\text{H}}_2$-CH$_2$), 3.35-3.38 (4H, t, J = 5.6 Hz, piperazine H-2, 6), 3.22-3.25 (4H, t, J = 5.6 Hz, piperazine H-3, 5), 3.05 (3H, s, pyrazole-1-C$\underline{\text{H}}_3$), 2.82-2.85 (2H, dd, J = 6.8 Hz, J = 7.2 Hz, pyridazine-CH$_2$-C$\underline{\text{H}}_2$), 2.22 (3H, s, pyrazole-5-C$\underline{\text{H}}_3$). $C_{28}H_{30}ClN_7O_3$ ESI-MS 548.2174 $[M+H]^+$ (100%), 550.6259 $[M+1+2]^+$ (35%).

3-[3-(4-Benzylpiperidin-1-yl)-6-oxopyridazin-1(6*H*)-yl]-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)propanamide 6i

Elution with DCM-MeOH (0%-4%) yielded **6i** as a white solid (yield 51%); mp 173-175 °C; IR (FTIR/FTNIR-ATR): 1688 cm^{-1} (C = O), 1658 cm^{-1} (C = O), 1623 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ: 7.71 (1H, s, NH), 7.42-7.45 (2H, t, J = 7.6 Hz, phenyl H-2, 6), 7.37 (2H, d, J = 7.6 Hz, phenyl H-3, 5), 7.26-7.30 (3H, m, benzyl H-3, 4, 5), 7.20 (1H, t, J = 7.6 Hz, phenyl H-4), 7.13-7.15 (2H, d, J = 7.6 Hz, benzyl H-2, 6), 7.08-7.11 (1H, d, J = 9.6 Hz, pyridazine H-4), 6.83-6.85 (1H, d, J = 10 Hz, pyridazine H-5), 4.38-4.41 (2H, dd, J = 6.8 Hz, J = 7.2 Hz, pyridazine-C$\underline{\text{H}}_2$-CH$_2$), 3.76-3.79 (2H, d, J = 13.2 Hz, phenyl-C$\underline{\text{H}}_2$), 3.04 (3H, s, pyrazole-1-C$\underline{\text{H}}_3$), 2.85-2.88 (2H, dd, J = 6.8 Hz, J = 7.2 Hz, pyridazine-CH$_2$-C$\underline{\text{H}}_2$), 2.54-2.69 (4H, m, piperidine H-2, 6), 2.23 (3H, s, pyrazole-5-C$\underline{\text{H}}_3$), 1.68-1.77 (5H, m, piperidine H-3, 4, 5). $C_{30}H_{34}N_6O_3$ ESI-MS 527.2777 $[M+H]^+$.

***N*-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-(6-oxo-3-phenylpyridazin-1(6*H*)-yl)propanamide 6j**

Elution with DCM-MeOH (0%-4%) yielded **6j** as a white solid (yield 47%); mp 188-190 °C; IR (FTIR/FTNIR-ATR): 1671 cm^{-1} (C = O), 1644 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$) δ: 8.10 (1H, s, NH), 7.77-7.79 (2H, d, J = 8 Hz, phenylpyridazine H-2, 6), 7.67-7.69 (1H, d, J = 9.2 Hz, pyridazine H-5), 7.32-7.46 (7H, m, phenyl H-2, 3, 5, 6, phenylpyridazine H-3, 4, 5), 7.23-7.26 (1H, m, J = 7.2 Hz, phenyl H-4), 7.02-7.04 (1H, d, J = 9.6 Hz, pyridazine H-4), 4.56-4.60 (2H, t, J = 6.4 Hz, pyridazine-C$\underline{\text{H}}_2$-CH$_2$), 3.01 (3H, s, pyrazole-1-C$\underline{\text{H}}_3$), 2.90-2.93 (2H, t, J = 6.4 Hz, pyridazine-CH$_2$-C$\underline{\text{H}}_2$), 2.10 (3H, s, pyrazole-5-C$\underline{\text{H}}_3$). $C_{24}H_{23}N_5O_3$ ESI-MS 430.1868 $[M+H]^+$.

***N*-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-3-[3-(4-methylphenyl)-6-oxopyridazin-1(6*H*)-yl]propanamide 6k**

Elution with DCM-MeOH (0%-4%) yielded **6k** as a white solid (yield 60%); mp 233-235 °C; IR (FTIR/FTNIR-ATR): 1680 cm^{-1} (C = O), 1663 cm^{-1} (C = O), 1641 cm^{-1} (C = O). ^{1}H-NMR ($CDCl_3$)δ: 8.01 (1H, s, NH), 7.65-7.68 (3H, m, methylphenyl H-2, 6, pyridazine H-5), 7.39-7.42 (2H, m, phenyl H-2, 6), 7.33-7.35 (1H, m, phenyl H-3, 5), 7.22-7.26 (3H, m, methylphenyl H-3, 5, phenyl H-4), 7.00-7.02 (1H, d, J = 10 Hz, pyridazine H-4), 4.56-4.59 (2H, dd, J = 6.8 Hz, J = 7.2 Hz, pyridazine-C$\underline{\text{H}}_2$-CH$_2$), 3.02 (3H, s, pyrazole-1-C$\underline{\text{H}}_3$), 2.90-2.93 (2H, dd, J = 6.8 Hz, J = 7.2 Hz, pyridazine-CH$_2$-C$\underline{\text{H}}_2$), 2.38 (3H, s, phenyl-C$\underline{\text{H}}_3$), 2.13 (3H, s, pyrazole-5-C$\underline{\text{H}}_3$). $C_{25}H_{25}N_5O_3$ ESI-MS 444.2026 $[M+H]^+$.

Pharmacological screening

Animals

Male Swiss albino mice (25-30 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals were kept and housed in a controlled temperature (22 ± 1 °C), humidity (55 ± 10%), and photoperiod (12 h light and 12 h dark, lights on at 0800) room for at least a week before they were used and throughout the experiments. The animals were allowed free access to standard pellet diet (Korkuteli Yem Sanayii T.A.S., Ankara) and tap water. A minimum of 6 animals were caged separately in each group. Throughout the experiments, the animals were treated under the audit of Gazi University's Commission of Animal Ethics according to the suggested international ethical guidelines for the care of laboratory animals (Permission No: 10.094).

Drugs

All chemicals used in this study, including p-Benzoquinone (PBQ) and λ-carrageenan (type I) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Analgesic activity

Analgesic activity was assessed using the PBQ-induced writhing test (visceral pain model) in mice according to Okun et al.[12] Prior to the chemical induction of algesia, all test samples were subcutaneously injected (100 mg/kg body weight) with a 30 min latency time and concomitantly followed by the intraperitoneal injection of 0.1 mL/10 g body weight of PBQ solution in saline (at a dose of 2.5 mg/kg) into mice. In the control groups the animals were given an appropriate volume of dosing vehicle. After PBQ challenge, the mice were housed individually in transparent glass cages for observation. The observers were blinded as to the agents the mice received while assessing the time of onset (approximately 3 min after PBQ injection) and the total count of writhing during the 15 min post-treatment period. The data represent the average of the total number of writhing movements observed. Antinociceptive activity was then expressed as the percentage change from the writhing controls.

Anti-inflammatory activity

Anti-inflammatory activity was assessed by the carrageenan-induced hind paw edema model.[13] The control and treated groups included a minimum of 7 animals, and 30 min after the subcutaneous administration of a test sample (100 mg/kg body weight) or dosing vehicle each mouse was injected subplantarly in the right hind paw with a freshly prepared suspension of carrageenan (0.5 mg/25 μL) in saline. Control injections (25 μL of saline) were administered to paired contralateral left hind paws. Monitoring of carrageenan induced paw edema was maintained in a timebase scale of 90, 180, 270, and 360 min. The difference in hind paw thicknesses was measured by caliber compasses (Ozaki Co., Tokyo, Japan). Mean values of each treated group were compared with the control group and analyzed by statistical methods.

Acute toxicity

Following the end of the anti-inflammatory activity experiments, the mice were observed for 48 h to any possible recordings of morbidity or mortality.[14]

Gastric ulcerogenic effect

Following the end of the analgesic activity experiments, as described elsewhere[15] the animals were sacrificed with an overdose of diethyl ether after 270 min of administration of the compounds for the investigation of ulcerogenicity. Each esophagus was then tied in a knot nearest the cardia by a surgical suture and the stomach was injected duodenally with a formalin solution (1.5 mL of 10%). The distended stomach was immediately tied to the pyloric sphincter using another surgical suture to avoid leakage of the formalin solution. After the removal of the stomachs from the abdominal cavity the outer layers were fixed by the immersion of the same formalin solution. Each stomach was then dissected along the greater curvature, rinsed with tap water to remove the gastric contents and examined under a dissecting microscope (20 $\times$ 6.3) to assess the formation of ulcers. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index.

Statistical analysis

The data were expressed as means $\pm$ SEM. The significance of differences between the treatment and the control group of animals was determined by one-way ANOVA with Bartlett's test following a post hoc Student-Newman-Keuls multiple comparisons test for analgesic activity, and two-way ANOVA following a post hoc Bonferroni test for anti-inflammatory activity. Values of $P < 0.05$ were considered statistically significant.

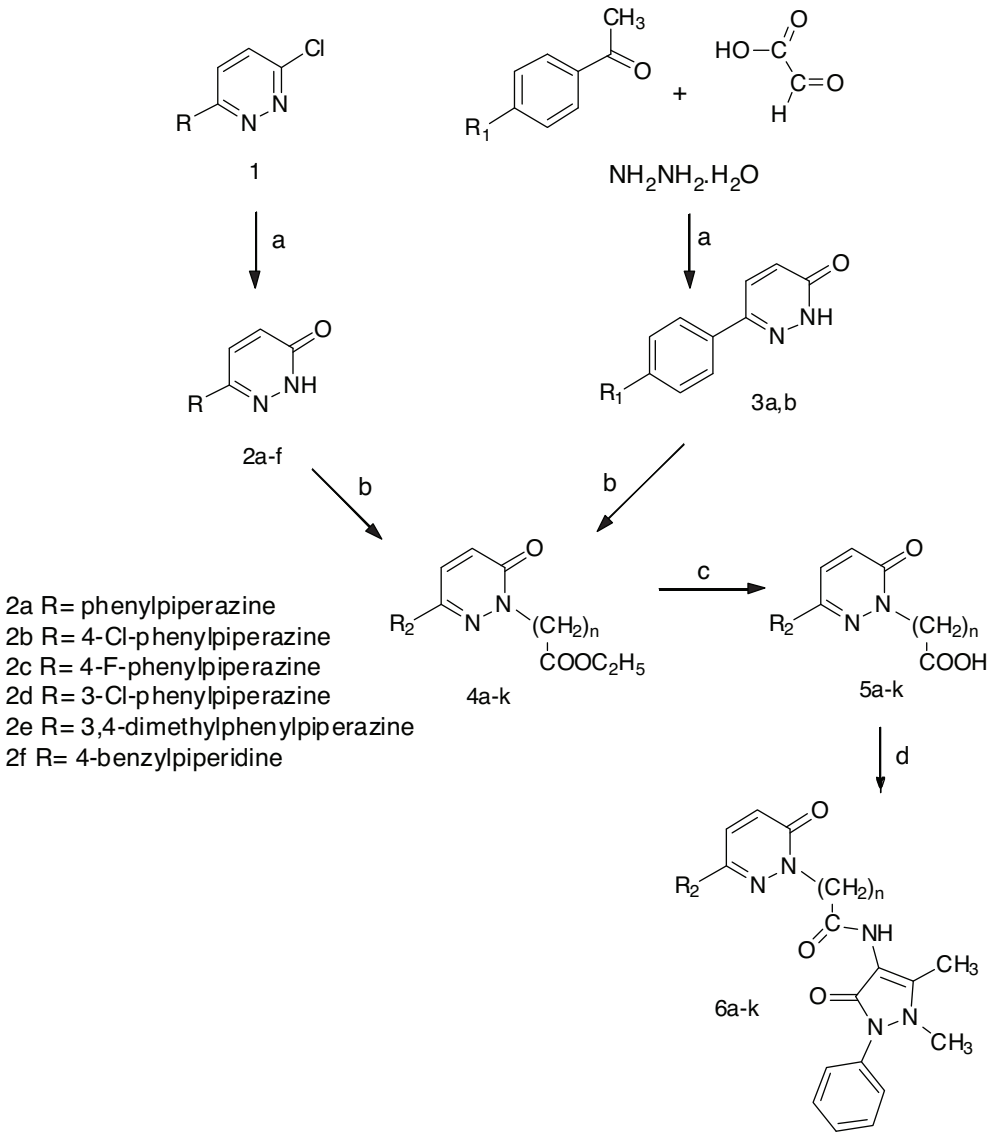
In silico oral biodisponibility

The theoretical study of oral biodisponibility (Lipinski rule-of-five) was performed in the molinspiration online program (http://www.molinspiration.com). Lipinski et al. have described an approach to estimate drug solubility and permeability in the human body. Lipinski's rule-of-five is a method routinely used for identifying leads and drug candidates. The rule-of-five predicts that an active compound shows poor absorption and permeation if it presents more than 5 hydrogen-bond donors (HBD) and 10 hydrogen-bond acceptors (HDA), and molecular weight (MW) and the calculated logP (clogP) are greater than 500 and 5, respectively.[16]

Results and discussion

Chemistry

New 6-(4-substituted piperazin/piperidin-1-yl)-3(2*H*)-pyridazinone-2-acetamide and propionamide derivatives **6a-i** were synthesized according to Scheme 1. Initially, nucleophilic displacement reaction of commercial 3,6-dichloropyridazine with arylpiperazines in ethanol afforded 3-chloro-6-substituted pyridazines **1**. The physical and spectral properties of 3-chloro-6-substitutedpyridazine **1** were in compliance with the literature.[17,18] Therefore, we carried out the next steps of the reaction without any further analysis. The synthesis procedures for derivatives **2** and **3** were reported by us previously.[19–21] Hydrolysis of 3-chloro-6-substituted pyridazines **1**

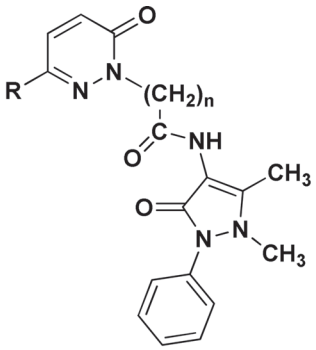


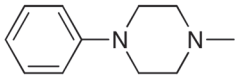
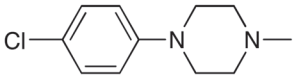
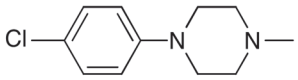
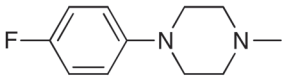
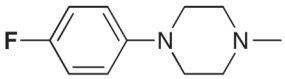
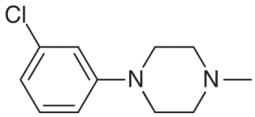
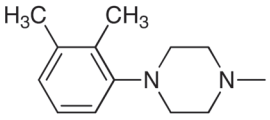
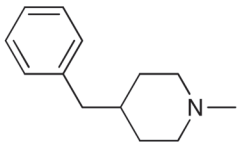
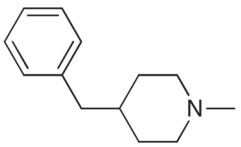
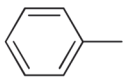
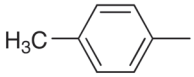
Scheme 1. Synthetic route for antipyrine/pyridazinone hybrids.

was carried out upon heating in glacial acetic acid to obtain 6-substituted-3(2*H*)-pyridazinone **2** derivatives. 6-(4-Substituted phenyl)-3(2*H*)-pyridazinone-2-ylacetamide and propionamide derivatives **6j** and **6k** were synthesized according to Scheme 1. The starting reagents for the preparation of 6-phenyl-3(2*H*)-pyridazinone **3a** and 6-(4-methylphenyl)-3(2*H*)-pyridazinone **3b** were glyoxalic acid, appropriate acetophenone derivative, and hydrazine hydrate. Condensation of these reagents provided an efficient route to **3a** and **3b**. Alkylation of derivatives **2a-f** and **3a,b** was performed using ethyl bromoacetate or ethyl bromopropionate in the presence of potassium carbonate in acetone at reflux temperature and the resulting ester derivatives **4a-k** were prepared in good yields. Hydrolysis of these esters with concentrated HCl 37% resulted in carboxylic acid

5a-k derivatives. By treatment of these carboxylic acid derivatives with aminoantipyrine in the presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) and dimethylaminopyridine (DMAP), which was used as carboxylate activator, the resulting amide derivatives **6a-k** were prepared in good yield. Chemical structures of these compounds were elucidated by their elemental analysis, IR, HRMS, and ^{1}H-NMR spectral data (Table 1).

Table 1. Synthesized compounds for analgesic and anti-inflammatory activity.



Compound	n	R	Compound	n	R
6a	1		6f	2	
6b	1		6g	2	
6c	1		6h	2	
6d	1		6i	2	
6e	1		6j	2	
			6k	2	

Pharmacology

Analgesic activities of the resultant compounds were investigated by *p*-benzoquinone-induced writhing test in mice,[12] which is a well-established method for testing the analgesic activity of compounds and sufficiently sensitive to detect the effect of analgesics that are less active than aspirin.

As seen in Figures 1 and 2 and Table 2, compounds having phenylpiperazine **6a** and 4-(2,3-dimethylphenyl) piperazine **6d** at the 6 position of the pyridazine ring show analgesic activities higher than aspirin, which serves as the reference drug in the assays, although these results do not show statistical significance. Meanwhile, compounds **6c, 6e,** and **6h**, as shown in Table 2, result in activities approximately equipotent to aspirin at the same subcutaneous dose of 100 mg/kg.

Analgesic activities of derivatives **6a-k** seem to be sensitive to electronic effects of the substituent at the sixth position of the pyridazine ring. While the substituted piperazine or piperidine derivatives **6a-i** show potent analgesic activities (except for **6f**), the derivatives possessing phenyl or methylphenyl groups (**6j, 6k)** have diminished analgesic activities.

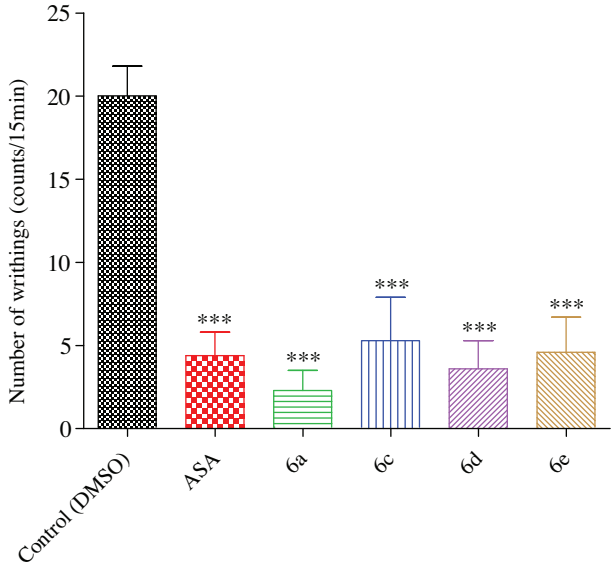


Figure 1. Writhing responses (means ± SEM, n = 6-9) of antipyrine/pyridazinone hybrids (**6a**, **6c**, **6d**, and **6e**) and acetylsalicylic acid (ASA) compared to control *Significantly different from the control (***P < 0.0001).

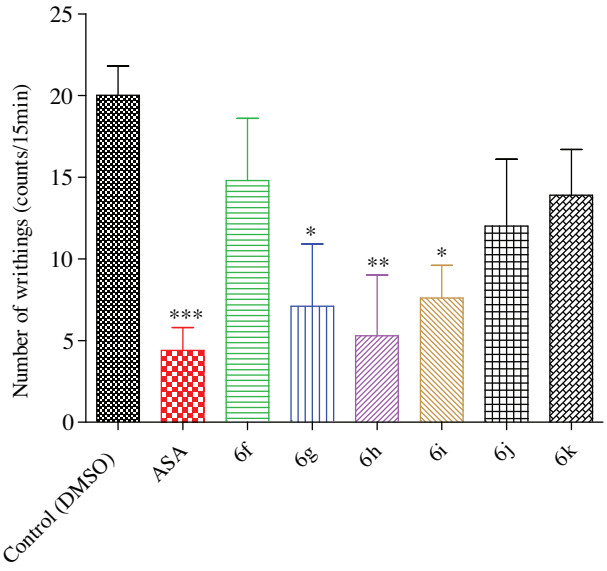


Figure 2. Writhing responses (means ± SEM, n = 6-9) of antipyrine/pyridazinone hybrids (**6f**, **6g**, **6h**, **6i**, **6j**, and **6k**) and acetylsalicylic acid (ASA) compared to control *Significantly different from the control (*P < 0.01, **P < 0.001, ***P < 0.0001).

Recent studies have shown that preparation of amide derivatives of well-known NSAID drugs with free carboxylic acid for developing new analgesic and anti-inflammatory drugs with reduced side effects resulted in compounds with good analgesic and anti-inflammatory activity.[22,23] In our study, to achieve hybrid molecules, pyridazinone was linked to the antipyrine through an acetylenic or propionic amide bond. From this perspective, the acetamides have generally been found more potent than propionamides. In the case of 4-fluorophenylpiperazine and benzylpiperidine derivatives, compounds **6c** and **6e** in which the pyridazinone ring

is incorporated to antipyrine via an acetylenic amide bond showed higher analgesic activity than derivatives **6g** and **6i** possessing propionic amide bonds. Dogruer et al. reported that, in the case of [6-(4-methoxyphenyl)-3(2*H*)-pyridazinone-2-yl]acetamide and propanamide derivatives, the highest analgesic activity was observed with acetamide 4-fluorophenylpiperazine derivative in the amide portion of the compounds.[24]

In vivo systemic anti-inflammatory activities of the compounds were assessed by carrageenan-induced hind paw edema model in mice at 100 mg/kg body weight doses.[25] Carrageenan edema has been used in the development of indomethacin. Carrageenan-induced paw edema is a traditional model to evaluate anti-inflammatory drugs. This model is a non-specific acute inflammation resulting from a complex of diverse mediators.[26] The edema formed is a multi-mediated case divided into 2 phases. The first phase is mediated by release of histamine and serotonin for 1 h followed by the kinin-mediated increased vascular permeability up to 2.5, h whereas the second phase (3 and 4 h after carrageenan injection) is mainly mediated by release of prostaglandins and prostaglandin-associated leukocytes into the site of edema.[27−29] Subcutaneous injection of carrageenan into the rat paw causes inflammation resulting from plasma extravasations, increased tissue water, and plasma protein exudation along with neutrophil extravasations, all due to the metabolism of arachidonic acid.[30,31]

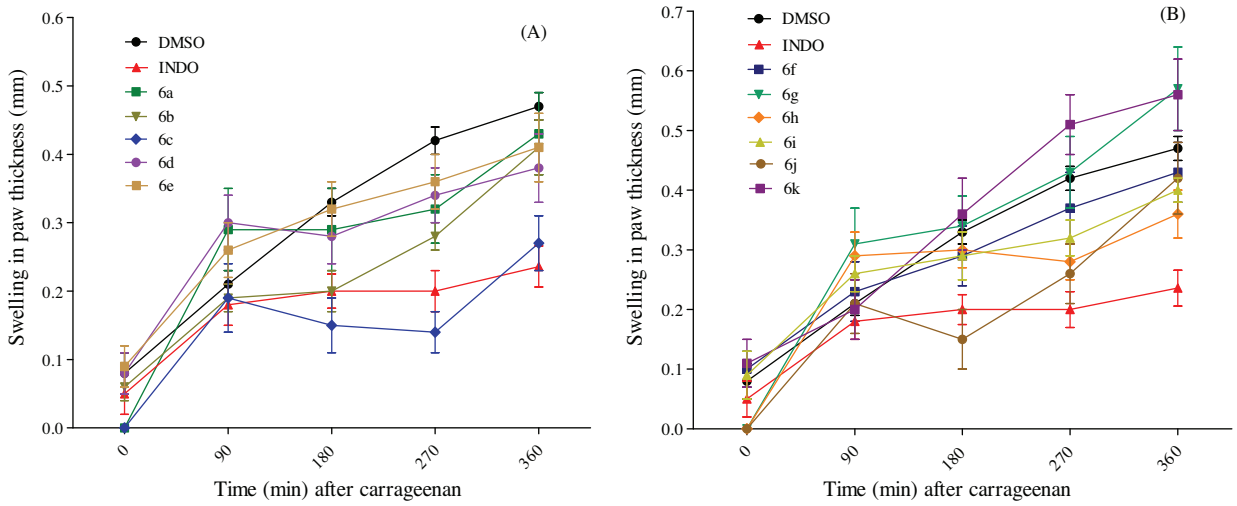


Figure 3. Timebase scale of carrageenan-induced paw edema in 90, 180, 270, and 360 min. Panel A: Control, indomethacin (INDO), **6a**, **6b**, **6c**, **6d**, and **6e**. Panel B: Control, INDO, **6f**, **6g**, **6h**, **6i**, **6j**, and **6k**.

Compound **6c** showed remarkably potent anti-inflammatory activity, especially 270 min after the administration of carrageenan to mice, indicating that cyclooxygenase inhibition may be related to the observed anti-inflammatory activities. Analgesic activity result of the compound also showed good correlation with its anti-inflammatory activity in that compound **6c** strongly inhibited the peripheral pain response in the mice, as the amount of writhing was found to be significantly diminished as compared to the control animals treated with the vehicle. Compounds **6b**, **6h**, and **6j** produced poor anti-inflammatory activity.

When the chemical structures of the active compounds are taken into consideration, *para*-fluoro substitution in the phenyl ring of the phenylpiperazine moiety caused both analgesic and anti-inflammatory activities to increase in antipyrine/pyridazinone hybrids possessing acetylenic amide bond.

Moreover, acute toxicity and gastric ulcerogenic effects of the title compounds were investigated in test animals. As for the ulcerogenic effects of the synthesized compounds, compounds **6a**, **6c**, and **6h** caused weak damage to the gastric mucosa at 100 mg/kg dose. The other compounds had no ulcerogenic side effect (Table 2).

Table 2. Analgesic and anti-inflammatory effects of test compounds on p-benzoquinone (PBQ)-induced abdominal constriction test and carrageenan (CG)-induced hind paw edema model in mice, respectively, and ratio of ulceration.

Test compounds	% Analgesic activity ± SEM[a]	Swelling in paw thickness ($\times 10^{-2}$ mm) ± SEM (% inhibition of edema) 270th minute after CG[b]	Ratio of ulceration[c]
DMSO (Control)	—	41.67 ± 2.37 (n = 19)	0/6
ASA	78.21 ± 7.22 (n = 14)	—	2/6
INDO	—	20.04 ± 1.44 (51.92)*** (n = 16)	1/6
6a	88.33 ± 5.87 (n = 6)	31.82 ± 5.23 (23.64) (n = 11)	1/6
6b	—	27.89 ± 2.30 (33.05)*** (n = 19)	0/6
6c	73.33 ± 13.02 (n = 6)	13.75 ± 3.43 (67.00)*** (n = 12)	1/6
6d	82.14 ± 8.72 (n = 7)	34.09 ± 3.56 (18.18) (n = 11)	0/6
6e	77.14 ± 10.34 (n = 7)	35.79 ± 3.69 (14.11) (n = 19)	0/6
6f	26.00 ± 18.87 (n = 10)	37.22 ± 6.02 (10.67) (n = 9)	0/6
6g	64.29 ± 19.01 (n = 7)	42.92 ± 6.14 (–3.00) (n = 12)	0/6
6h	73.33 ± 18.47 (n = 6)	27.50 ± 2.79 (34.00)*** (n = 12)	1/6
6i	62.22 ± 9.93 (n = 9)	32.00 ± 2.80 (23.20)* (n = 20)	0/6
6j	40.00 ± 20.41 (n = 7)	26.25 ± 4.65 (37.00)** (n = 12)	0/6
6k	30.71 ± 14.03 (n = 7)	50.56 ± 5.43 (–21.33) (n = 9)	0/6

Data obtained from animal experiments were expressed as means ± standard error of mean (SEM); Dimethylsulfoxide: DMSO, Indomethacin: INDO, acetylsalicylic acid: ASA, carrageenan: CG. [a,c] Groups were employed for the PBQ-induced writhing test, [b] Groups were employed for the CG-induced paw edema model. All test drugs were subcutaneously administered to mice, INDO at the dose of 10 mg/kg and the other drugs at the doses of 100 mg/kg (body weight). *Swelling in paw-thickness significantly different from the control (Unpaired t test, *P $<$ 0.05, **P $<$ 0.005, ***P $<$ 0.0005).

In silico oral biodisponibility–molecular modeling approach

The synthesized compounds (**6a**–**k**) were submitted to an in silico evaluation using a molecular modeling approach. Good absorption after oral administration is obligatory for anti-inflammatory and antiplatelet medical use. Therefore, we analyzed these derivatives according to the rule-of-five developed by Lipinski et al. (Table 3).[16] The rule-of-five theoretically characterizes molecular properties for orally active drugs. The rule states that the 90% of orally active drugs present number of hydrogen bond acceptors $\leq$ 10 and donors $\leq$ 5, clogP $\leq$ 5 and molecular weight (MW) $\leq$ 500. Molecules violating more than one of these rules may have problems with bioavailability. The results show that compounds **6a**, **6j**, and **6k** fulfill the Lipinski rule-of-five (Table 3). The rest of the compounds violated only the criterion of molecular weight.

Table 3. Parameters for the Lipinski rule-of-five for synthesized compounds predicted using a molecular modeling approach.

Compound	Theoretical oral biodisponibility (Lipinski rule-of-five)[a)]			
	HBA	HBD	MW	clog$P$
6a	10	1	499.56	2.144
6b	10	1	533.19	2.822
6c	10	1	517.55	2.308
6d	10	1	526.61	2.945
6e	9	1	512.25	3.132
6f	10	1	547.20	3.093
6g	10	1	531.58	2.579
6h	10	1	547.20	3.069
6i	9	1	526.26	3.403
6j	8	1	429.47	2.273
6k	8	1	443.49	2.721

[a] For good theoretical oral biodisponibility – number of hydrogen bond acceptors (HBA) $\leq$ 10 and donors (HBD) $\leq$ 5, clog $P \leq$ 5 and molecular weight $\leq$ 500.

In conclusion, we report herein a simple and convenient route for the synthesis of antipyrine/pyridazinone hybrids for analgesic and anti-inflammatory evaluation. According to the results of in vivo studies conducted in the present study, the analgesic and anti-inflammatory activities of **6c** are comparable to those of known drugs, i.e. aspirin and indomethacin, without inducing any visible gastric damage. Therefore, our initial screening results demonstrate that the presence of certain arylpiperazine substituents at the pyridazine ring in antipyrine/pyridazinone hybrids may contribute to their analgesic and anti-inflammatory activities. Further detailed studies are under way in order to investigate the effect of the synthesized hybrid molecules on chronic inflammatory test models and COX-2 selective inhibitory effects.

Acknowledgment

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