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Synthesis, Analgesic, and Anti-Inflammatory Activities of [6-(3,5-Dimethyl-4-Chloropyrazole-1-yl)-3(2*H*)-Pyridazinon-2-yl]Acetamides

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A series of structurally diverse amide derivatives of [6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetic acid were prepared and tested for their *in vivo* analgesic and anti-inflammatory activity by using *p*-benzoquinone-induced writhing test and carrageenan-induced hind paw edema model, respectively. The analgesic and anti-inflammatory activity of the compounds, **7c**, **7d** and **7k** were found to be equipotent to aspirin (as an analgesic) and indometacin (as an anti-inflammatory drug), respectively. The other amide derivatives generally resulted in lower activity on comparision with reference compounds.

Key words: Pyridazinone, Pyrazole, Analgesic, Anti-Inflammatory, Writhing, Carrageenan

INTRODUCTION

The majority of currently known non-steroidal anti-inflammatory and analgesic drugs (NSAIDs), i.e., aspirin and ibuprofen, mainly act peripherally by blocking the production of prostaglandins through inhibition of cyclooxygenase (COX) enzymes, COX-1 and COX-2, to varying extents (Meade *et al.*, 1993). These drugs tend to produce side effects such as gastrointestinal ulceration and suppression of renal function due to inhibition of the constitutive COX-1, which is responsible for the production of prostaglandins, responsible for gastroprotection and vascular homeostasis (Brooks *et al.*, 1999; Clinch *et al.*, 1983; Patrono *et al.*, 1987). Therefore, the main trend nowadays in pain therapy focuses on improved nonsteroidal analgesics which are effective as an analgesic but devoid of the side effects which are inherent to traditional NSAIDs.

In terms of this aspect, many studies have been focussed on 3(2*H*)-pyridazinones, which are characterized to possess good analgesic and anti-inflammatory activities and also very low ulcerogenicity (Rubat *et al.*, 1988, 1992; Rohet *et al.*, 1996; Coudert *et al.*, 2000; Dogruer *et al.*, 2000).

Among the various pyridazinone derivatives, 4-ethoxy-2-methyl-5-morpholino-3(2*H*)-pyridazinone (emorfazone) is currently being marketed in Japan as an analgesic and anti-inflammatory drug (Takaya *et al.*, 1979). Moreover, it has been reported that 4-amino-2-methyl-6-phenyl-5-vinyl-3(2*H*)-pyridazinone was seven-fold more potent than emorfazone (Dal Piaz *et al.*, 1996) in bringing about analgesic and anti-inflammatory response.

Additionally, Santagati's group synthesized 2-substituted 4,5-dihalo-3(2*H*)-pyridazinone derivatives with high analgesic activity and with no ulcerogenic side effects (Santagati *et al.*, 1985). Subsequently, 2-substituted 4,5-functionalized 6-phenyl-3(2*H*)-pyridazinone derivatives have also been reported to bear potent analgesic activity with negligible general side effects as those of currently used NSAIDs (Pieretti *et al.*, 1999). In the meantime, 3-*O*-substituted benzyl pyridazinone derivatives were recently shown to exhibit *in vitro* potent anti-inflammatory activity by using carrageenan-induced rat paw edema assay (Chintakunta *et al.*, 2002).

As a continuation of our work for the development of improved NSAIDs; which are effective but devoid of the well-known side-effects associated with the obligatory use

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of NSAIDs, we also got interested in 3(2*H*)-pyridazinones (Dogruer *et al.*, 2000; Gokçe *et al.*, 2001, 2004; Ökçelik *et al.*, 2003; Banoglu *et al.*, 2004), which resulted in good analgesic and anti-inflammatory activities.

In the previously reported studies, we found that the heterocyclic ring substitutions at six position, and the presence of acetamide side chain that is linked to the lactam nitrogen of pyridazinone ring at two position of pyridazinone ring improved the analgesic and anti-inflammatory activity along with nil or very low ulcerogenicity (Fig. 1).

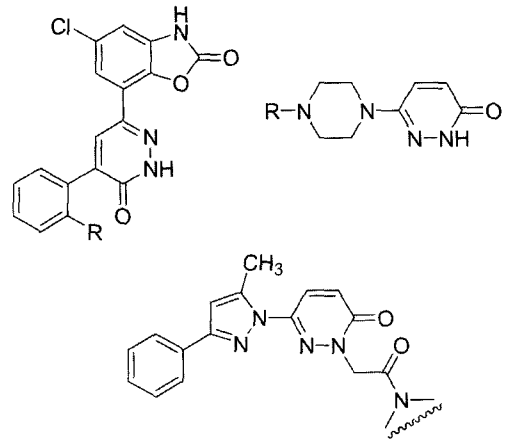


Fig. 1. Some of the 3(2*H*)-pyridazinone derivatives with analgesic and anti-inflammatory activity

In the present study, we have synthesized the structurally diverse amide derivatives of [6-(3,5-dimethyl-4-chloro-1-pyrazolyl)-3(2H)-pyridazinone]acetic acid and investigated the ability of the resulting amide derivatives (Fig. 2) as analgesic and anti-inflammatory compounds. Herein, we describe the methodology employed for the synthesis of the derivatives and their resulting *in vivo* activities.

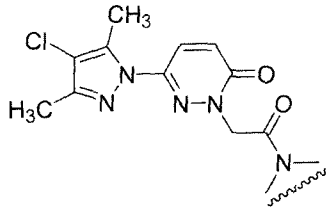


Fig. 2. General structure of synthesized amide derivatives

MATERIALS AND METHODS

3,6-dichloropyridazine, hydrazine hydrate, acetylacetone, ethyl bromoacetate, sulfuryl chloride, triethylamine, and amine derivatives were obtained from Aldrich, Deisenhofen (Germany) and Merck, Darmstadt (Germany). 3-Chloro-6-hydrazinopyridazine (Druey *et al.*, 1954), 3-chloro-6-(3,5-dimethylpyrazol-1-yl)pyridazine, and 3-chloro-6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)pyridazine (Szilagyi *et al.*, 1979) were synthesized according to previously published procedures. All other chemicals were obtained from local commercial sources. IR spectra were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer (KBr, υ, cm^{-1}) (Bruker Spectrospin, Wissembourg Cedex, France). ^{1}H-NMR spectra were recorded on a VARIAN Mercury 400 FT-NMR spectrometer by using TMS as an internal standard in DMSO-d_6 or $CDCl_3$ at the NMR facilty of Faculty of Pharmacy, Ankara University. The elemental analyses for C, H, N were performed at Scientific and Technical Council of Turkey, Instrumental Analysis Center (Ankara, Turkey) and was within ± 0.4% of the therotical values.

Synthesis of 6-(3,5-dimethylpyrazole-1-yl)-3-chloropyridazine (2)

A mixture of 3-chloro-6-hydrazinopyridazine (0.01 mol) and acetylacetone (0.01 mol) in 30 mL ethanol was heated to reflux for 4 h. After cooling, the separated crystals were filtered off, washed with ice-cold ethanol, dried and recrystallized from ethanol to obtain a yield of 71%. The product had a m.p. of 115°C. ^{1}H-NMR ($CDCl_3$) δ 8.06 (d, 1H, pyridazinone-H5); 7.44 (d, 1H, pyridazinone-H4); 5.96 (s, 1H, pyrazole-H4); 2.62 (s, 3H, pyrazole-3-CH_3); 2.18 (s, 3H, pyrazol-5-CH_3) ppm. IR ν_{max} cm^{-1} (KBr): 3055, 2985, 2930, 1576, 1425, 1085, 791. Anal. C, H, N ($C_9H_9ClN_4$).

Synthesis of 6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3-chloropyridazine (3)

To a mixture of **2** (0.01 mol) in 100 mL of ether, sulphuryl chloride (0.02 mol) was added dropwise at 0 °C under stirring and then the mixture was stirred at 0°C for 1 h, set aside at room temperature for 1 h and heated to reflux for 2 h. After cooling, the seperated crystals were filtered, washed with water, dried and recrystallized from methanol to obtain a yield of 74%. The product had a m.p. of 141-142°C. ^{1}H-NMR (DMSO-d_6) δ 8.15 (d, 1H, pyridazinone-H5); 8.05 (d, 1H, pyridazinone-H4); 2.60 (s, 3H, pyrazole-3-CH_3); 2.23 (s, 3H, pyrazol-5-CH_3) ppm. IR ν_{max} cm^{-1} (KBr): 3091, 2930, 1572, 1047, 849. Anal. C, H, N ($C_9H_8Cl_2N_4$).

Synthesis of 6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone (4)

A solution of **3** (0.01 mol) and sodium acetate (0.013 mol) in 20 mL of glacial acetic acid was refluxed for 5 h. After cooling, it was poured into ice-water (50 mL) and the precipitate formed was filtered off, washed with water, dried and recrystallized from ethanol to obtain a yield of 92%. The product had a m.p. of 287-288°C. ^{1}H-NMR (DMSO-d_6) δ 13.0 (s, 1H, NH); 7.83 (d, 1H, pyridazinone-

H5); 7.04 (d, 1H, pyridazinone-H4); 2.40 (s, 3H, pyrazole-3-CH_3); 2.18 (s, 3H, pyrazol-5-CH_3) ppm. IR ν_{max} cm^{-1} (KBr): 3082, 2969, 1693, 1243, 857. Anal. C, H, N ($C_9H_9ClN_4O$).

Synthesis of ethyl [6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetate (5)

To the solution of **4** (0.01 mol) and potassium carbonate (0.04 mol) in 40 mL of dimethylformamide was added ethyl bromoacetate (0.015 mol), and stirred at room temperature for 90 min. The reaction mixture was then poured into ice-water and the precipitate formed was filtered off, washed with water, dried and recrystallized from methanol to obtain a yield of 91%. The product had a m.p. of 138-139°C. ^{1}H-NMR (DMSO-d_6) δ 7.92 (d, 1H, pyridazinone-H5); 7.18 (d, 1H, pyridazinone-H4); 4.86 (s, 2H, N-C$\underline{H}_2$-CO-); 4.14 (q, 2H, -O-C$\underline{H}_2$-CH_3); 2.39 (s, 3H, pyrazole-3-CH_3); 2.19 (s, 3H, pyrazole-5-CH_3); 1.18 (t, 3H, -O-CH_2-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3012, 2968, 1738, 1673, 1598, 1228, 846. Anal. C, H, N ($C_{13}H_{15}ClN_4O_3$).

Synthesis of 2-[6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetic acid (6)

Compound **5** (0.01 mol) was heated up to reflux temperature in concentrated HCl for 3 h. After cooling, the reaction mixture was treated with $NaHCO_3$ (% 10 w/v) and the precipitate formed was filtered off, washed with water, dried and recrystallized from ethanol to obtain a yield of 85.6%. The product had a m.p. of 225-226°C. ^{1}H-NMR (DMSO-d_6) δ 13.2 (s, 1H, COOH); δ 7.90 (d, 1H, pyridazinone-H5); 7.16 (d, 1H, pyridazinone-H4); 4.76 (s, 2H, N-C$\underline{H}_2$-CO-); 2.40 (s, 3H, pyrazole-3-CH_3); 2.19 (s, 3H, pyrazole-5-CH_3) ppm. IR ν_{max} cm^{-1} (KBr): 3022, 2975, 1735, 1668. Anal. C, H, N ($C_{11}H_{11}ClN_4O_3$).

General procedure for the amidation of [6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetic acid (7a-l)

0.01 Mol of 2-[6-(4-chloro-3,5-dimethylpyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetic acid in 40 mL dichloromethane at 0°C (ice-bath) was treated with triethylamine (0.015 mol) and 0.01 mol of ethyl chloroformate. After stirring the reaction mixture at 0°C for further 20 min, 0.011 mol of appropriate amine derivative (0.013 mol) was added, and the final mixture was stirred at room temperature for overnight. After evaporation to dryness, the product was solidified with ice-cold water and crystallized from the appropriate solvent.

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetyl]-4-phenylpiperazine (7a)

Recrystallized from methanol (yield 85.4%, m.p. 164°C). ^{1}H-NMR (DMSO-d_6), δ 7.89 (d, 1H, pyridazinone-H5); 7.23 (t, 2H, phenyl-H3, H5); 7.16 (d, 1H, pyridazinone-H4); 6.97 (d, 2H, phenyl-H2, H6); 6.81 (t, 1H, phenyl-H4); 5.05 (s, 2H, N-C$\underline{H}_2$-CO-); 3.66 (t, 2H, piperazine-H2(6)); 3.60 (t, 2H, piperazine-H6(2)); 3.21 (t, 2H, piperazine-H3(5)); 3.12 (t, 2H, piperazine-H5(3)); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.20 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3054, 2922, 1660, 1596. Anal. C, H, N ($C_{21}H_{23}ClN_6O_2$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-(4-fluorophenyl)piperazine (7b)

Recrystallized from ethanol (yield 64.8%, m.p. 204-205 °C). ^{1}H-NMR (DMSO-d_6), δ 7.89 (d, 1H, pyridazinone-H5); 7.16 (d, 1H, pyridazinone-H4); 7.09-6.97 (m, 4H, phenyl-H2, H3, H5, H6); 5.05 (s, 2H, N-C$\underline{H}_2$-CO-); 3.66 (t, 2H, piperazine-H2(6)); 3.59 (t, 2H, piperazine-H6(2)); 3.14 (t, 2H, piperazine-H3(5)); 3.05 (t, 2H, piperazine-H5(3)); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.21 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 2997, 2923, 1662, 1595. Anal. C, H, N ($C_{21}H_{22}ClFN_6O_2$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-(2-fluorophenyl)piperazine (7c)

Recrystallized from methanol (yield 60%, m.p. 171-172 °C). ^{1}H-NMR (DMSO-d_6), δ 7.89 (d, 1H, pyridazinone-H5); 7.16 (d, 1H, pyridazinone-H4); 7.16-6.99 (m, 4H, phenyl-H3, H4, H5, H6); 5.05 (s, 2H, N-C$\underline{H}_2$-CO-); 3.68 (t, 2H, piperazine-H2(6)); 3.62 (t, 2H, piperazine-H6(2)); 3.06 (t, 2H, piperazine-H3(5)); 2.98 (t, 2H, piperazine-H5(3)); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.21 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 2997, 2922, 1668, 1596. Anal. C, H, N ($C_{21}H_{22}ClFN_6O_2$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-[(3-trifluoromethyl)phenyl] piperazine (7d)

Recrystallized from ethanol-water (yield 74.2%, m.p. 153-154°C). ^{1}H-NMR (DMSO-d_6), δ 7.88 (d, 1H, pyridazinone-H5); 7.42 (t, 1H, phenyl-H5); 7.24 (d, 1H, phenyl-H4); 7.19 (s, 1H, phenyl-H2); 7.15 (d, 1H, pyridazinone-H4); 7.08 (d, 1H, phenyl-H6); 5.05 (s, 2H, N-C$\underline{H}_2$-CO-); 3.66 (t, 2H, piperazine-H2(6)); 3.29 (t, 2H, piperazine-H6(2)); 3.23 (t, 2H, piperazine-H3(5)); 3.09 (t, 2H, piperazine-H5(3)); 2.39 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.19 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 2993, 2928, 1666, 1595. Anal. C, H, N ($C_{22}H_{22}ClF_3N_6O_2$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-(3-chlorophenyl)piperazine (7e)

Recrystallized from methanol (yield 54%, m.p. 136°C). ^{1}H-NMR (DMSO-d_6), δ 7.89 (d, 1H, pyridazinone-H5); 7.22 (t,

1H, phenyl-H5); 7.16 (d, 1H, pyridazinone-H4); 6.98 (s, 1H, phenyl-H2); 6.92 (d, 1H, phenyl-H4); 6.81 (d, 1H, phenyl-H6); 5.05 (s, 2H, N-C$\underline{H}_2$-CO-); 3.65 (t, 2H, piperazine-H2(6)); 3.58 (t, 2H, piperazine-H6(2)); 3.28 (t, 2H, piperazine-H3(5)); 3.17 (t, 2H, piperazine-H5(3)); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.20 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3025, 2965, 1677, 1592. Anal. C, H, N ($C_{21}H_{22}Cl_2N_6O_2$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-(4-chlorophenyl)piperazine (7f)

Recrystallized from methanol (yield 80.5%, m.p. 176-177 °C). ^{1}H-NMR (DMSO-d_6), δ 7.89 (d, 1H, pyridazinone-H5); 7.25 (d, 2H, phenyl-H3, H5); 7.16 (d, 1H, pyridazinone-H4); 6.98 (d, 2H, phenyl-H2, H6); 5.05 (s, 2H, N-C$\underline{H}_2$-CO-); 3.65 (t, 2H, piperazine-H2(6)); 3.59 (t, 2H, piperazine-H6 (2)); 3.22 (t, 2H, piperazine-H3(5)); 3.12 (t, 2H, piperazine-H5(3)); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.20 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 2996, 2923, 1662, 1594. Anal. C, H, N ($C_{21}H_{22}Cl_2N_6O_2$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-piperonylpiperazine (7g)

Recrystallized from ethanol-water (yield 88.8%, m.p. 160 °C). ^{1}H-NMR (DMSO-d_6), δ 7.87 (d, 1H, pyridazinone-H5); 7.14 (d, 1H, pyridazinone-H4); 6.86 (d, 1H, piperonyl-H7); 6.83 (s, 1H, piperonyl-H4); 6.75 (d, 1H, piperonyl-H6); 5.98 (s, 2H, -O-CH_2-O-); 4.97 (s, 2H, pyridazine-*N*-C$\underline{H}_2$-CO-); 3.49 (t, 2H, piperazine-H2(6)); 3.43 (m, 4H, piperazine-H6(2)), -N-C$\underline{H}_2$-piperonyl); 2.38 (m, 5H, piperazine-H3(5) and pyrazole-3-C$\underline{H}_3$); 2.30 (t, 2H, piperazine-H5(3)); 2.20 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 2989, 2943, 1667, 1599. Anal. C, H, N ($C_{23}H_{25}ClN_6O_4$).

1-[2-[6-(3,5-Dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetyl]-4-(2-pyridyl)piperazine (7h)

Recrystallized from methanol (yield 84%, m.p. 181°C). ^{1}H-NMR (DMSO-d_6), 8.12 (m, 1H, pyridine-H6); 7.88 (d, 1H, pyridazinone-H5); 7.54 (m, 1H, pyridine-H4); 7.15 (d, 1H, pyridazinone-H4); 6.85 (d, 1H, pyridine-H3); 6.66 (m, 1H, pyridine-H5); 5.04 (s, 2H, N-C$\underline{H}_2$-CO-); 3.48-3.60 (m, 8H, piperazine-H2, H3, H5, H6); 2.39 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.19 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3000, 2924, 1669, 1596. Anal. C, H, N ($C_{20}H_{22}ClN_7O_2$).

***N*-Morpholino-[6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetamide (7i)**

Recrystallized from methanol (yield 84.3%, m.p. 191-192 °C). ^{1}H-NMR (DMSO-d_6), δ 7.89 (d, 1H, pyridazinone-H5); 7.16 (d, 1H, pyridazinone-H4); 5.0 (s, 2H, N-C$\underline{H}_2$-CO); 3.63 (t, 2H, morpholine–H3(5)); 3.57 (t, 2H, morpholine–H5(3)); 3.51 (t, 2H, morpholine–H2(6)); 3.43 (t, 2H, morpholine-H6(2)); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.21 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 2987, 2965, 1660, 1594. Anal. C, H, N ($C_{15}H_{18}ClN_5O_3$).

***N*-(3-Pyridyl)-2-[6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetamide (7j)**

Recrystallized from methanol (yield 52.4%, m.p. 262°C). ^{1}H-NMR (DMSO-d_6), δ 10.56 (s, 1H, NH); 8.71 (d, 1H, pyridine-H2); 8.27 (m, 1H, pyridine-H6); 7.99 (m, 1H, pyridine-H5); 7.92 (d, 1H, pyridazinone-H5); 7.34 (m, 1H, pyridine-H4); 7.18 (d, 1H, pyridazinone-H4); 4.92 (s, 2H, N-C$\underline{H}_2$-CO-); 2.40 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.20 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3264, 3122, 3003, 1701, 1657, 1583. Anal. C, H, N ($C_{16}H_{15}ClN_6O_2$).

***N*-Octyl-2-[6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetamide (7k)**

Recrystallized from methanol (yield 79.9%, m.p. 145-146 °C). ^{1}H-NMR (DMSO-d_6), δ 8.1 (t, 1H, NH); 7.87 (d, 1H, pyridazinone-H5); 7.12 (d, 1H, pyridazinone-H4); 4.62 (s, 2H, N-C$\underline{H}_2$-CO-); 3.04 (q, 2H, NH-C$\underline{H}_2$-CH_2); 2.38 (s, 3H, pyrazol-3-CH_3); 2.19 (s, 3H, pyrazole-5-CH_3); 1.36 (m, 2H, -NH-CH_2-C$\underline{H}_2$-); 1.21 (s, 10H, CH_2-(C$\underline{H}_2$)$_5$-CH_3); 0.83 (t, 3H, -CH_2-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3320, 3282, 2922, 2851, 1690, 1659,1595. Anal. C, H, N ($C_{19}H_{28}ClN_5O_2$).

***N*-(2-Phenethyl)-2-[6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetamide (7l)**

Recrystallized from methanol (yield 85.4%, m.p. 188°C). ^{1}H-NMR (DMSO-d_6), δ 8.27 (t, 1H, NH); 7.9 (d, 1H, pyridazinone-H5); 7.27 (m, 2H, phenyl-H2, H6); 7.19 (m, 3H, phenyl-H3, H4, H5); 7.15 (d, 1H, pyridazinone-H4); 4.64 (s, 2H, N-C$\underline{H}_2$-CO-); 3.29 (q, 2H, NH-C$\underline{H}_2$-CH_2); 2.71 (t, 2H, CH_2-C$\underline{H}_2$-phenyl); 2.38 (s, 3H, pyrazole-3-C$\underline{H}_3$); 2.21 (s, 3H, pyrazole-5-C$\underline{H}_3$) ppm. IR ν_{max} cm^{-1} (KBr): 3277, 3088, 2947, 1683,1670, 1648,1599. Anal. C, H, N ($C_{19}H_{20}ClN_5O_2$).

Pharmacology

Male Swiss albino mice weighing 20-25 g from the animal breeding Laboratories of the Refik Saydam Hifzisihha Institute of Ankara, Turkey were used for all experiments. Mice were kept in groups of six in a temperature-controlled room. The allocation of animals to different groups was randomized and the experiments were carried out under blind conditions. For each of the compounds tested, for references (aspirin and indometacin), and for controls, a group of animals comprising of 6 mice were used. The animals were housed in colony cages (6 mice per cage), maintained on a standard pellet diet with water given ad-lib and left for two days for acclimatization before the experimental sessions. The food was withheld the day before the experiment, but animals were allowed to have free access to water. All experiments were carried out

according to the suggested ethical guidelines for the care of laboratory animals.

Preparation of test samples for bioassay

Test samples, suspended in a mixture of distilled water and 0.5% sodium carboxymethyl cellulose (CMC), were given orally to the animals. Control animals received the same experimental handling as the test groups with the exception that the drug treatment was replaced with an appropriate volume of the dosing vehicle. Either indometacin (10 mg/kg) or acetyl salicylic acid (100 mg/kg) in 0.5% CMC was used as the reference drug.

p-Benzoquinone-induced writhing test (Okun *et al.*, 1963)

After 60 minutes of oral administration of test samples, the mice were intraperitoneally injected with 2.5% (v/v) *p*-benzoquinone solution in distilled water (0.1 mL/10 g bodyweight). Control animals received an appropriate volume of dosing vehicle. The mice were housed individually for observation and from the start of the 5th min after *p*-benzoquinone injection, the total number of abdominal contractions (writhing movements) was counted for a period of 15 min. The data represent an average of the total number of writhing movements observed. The analgesic activity was expressed as the percentage change compared to writhing controls.

Carrageenan-induced hind paw edema test

For the Carrageenan-induced hind paw edema test, the method of Kasahara (Kasahara *et al.*, 1985) was followed. The difference in footpad thickness between the right and left foot was measured using a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated versus control groups were compared and analyzed using statistical methods. After 60 min of oral administration of test sample or dosing vehicle, a freshly prepared (0.5 mg/25 μL) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) was injected into the subplantar tissue of the right hind paw of each mouse. A saline solution (25 μL) was injected into the left paw as a secondary control. Measurements were performed and evaluated as described above for every 90 min during a 360 min period.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed for 24 h and the mortality rate was recorded for each group at the end of the observation period.

Gastric-lesions inducing effect

Eights hours after the analgesic activity experiment, mice under deep ether anesthesia were killed and their stomachs were removed. The abdomen of each mouse was opened through great curvature and examined for lesions or bleedings using a dissecting microscope.

Statistical analysis of data

Data obtained from animal experiments were expressed as the mean standard error (± SEM). Statistical differences between treatment and control groups were determined by using the ANOVA test. Data with $p < 0.05$ value were considered to be significant.

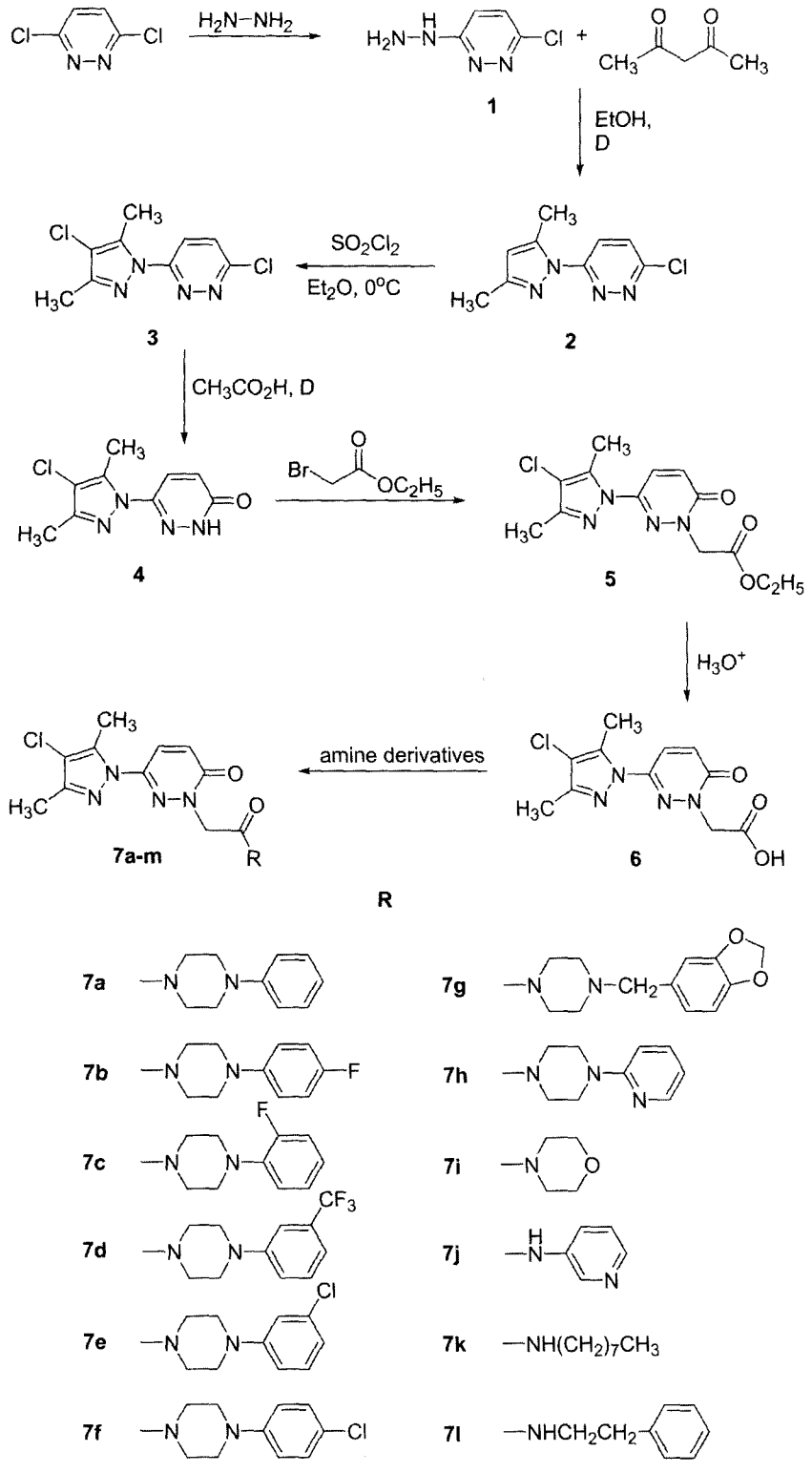
RESULTS AND DISCUSSION

Chemistry

The title amide derivatives were prepared by treatment of [6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetic acid with the appropriate amine derivatives in the presence of triethylamine and ethyl chloroformate which was used as the carboxylate activator. The structure of all the compounds was established by NMR and elemental analyses. The preparation of the resulting amide derivatives **7a-l** are outlined in Scheme 1. Commercially available 3,6-dichloropyridazine was used as the starting material, and 6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinone (**4**) was prepared by adapted procedures according to the previously published methods (Druey *et al.*, 1954; Szilagyi *et al.*, 1979). Alkylation of **4** with ethyl bromoacetate generated ethyl [6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetate (**5**). Acid-catalyzed hydolysis of the ester linkage in **5** afforded [6-(3,5-dimethyl-4-chloro-pyrazol-1-yl)-3(2*H*)-pyridazinon-2-yl]acetic acid (**6**). Amidation of **6** with appropriate secondary and tertiary amines in the presence of ethyl chloroformate in dichloromethane at room temperature, resulted in the synthesis of amide derivatives **7a-l** with quantitative yields (52-88%).

Pharmacology

Analgesic activity of the synthesized amide derivatives (**7a-l**) was evaluated in mice *via* a screening procedure by using the *p*-benzoquinone-induced writhing test (Okun *et al.*, 1963). The results shown in Table I indicate that the amide derivatives having 4-(2-fluorophenyl)piperazine (**7c**) and 4-(3-trifluoromethylphenyl)piperazine (**7d**) were approximately equipotent to aspirin, while the compound having *N*-octyl substituent **7k** at the amide portion was bit more potent than aspirin at the same oral dose of 100 mg/kg. Meanwhile, all other amide derivatives resulted in less analgesic activity than that of aspirin which served as control in the assays. Interestingly, analgesic activity of flurorophenylpiperazine derivatives **7b** and **7c** was sensitive to the fluoro group positioning, and while the



Scheme 1. Synthetic pathways of [6-(3,5-dimethyl-4-chloro-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetamide derivatives

derivative possessing a fluorine atom at the para-position of phenyl ring **7b** had lower analgesic activity, the 2-fluorophenyl derivative **7c** showed potent analgesic activity, which was in good correlation with our previously published results with [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetamides, where the 5-methyl of pyrazole ring was replaced with a phenyl substituent (Banoglu *et al.*, 2004). Introduction of a chloro substituent

at para or meta position of phenyl ring **7e** and **7f** had detrimental effect on analgesic activity. In addition, incorporation of a terminal phenyl ring in the alkyl amide derivative also generated poor analgesic activity, as illustrated with 2-phenethyl amide analog **7l**. In addition, aromatic secondary amide derivative, including *N*-(3-pyridyl) side chain **7j** led to a poor analgesic activity. The replacement of piperazine by a morpholine **7i** strongly diminished or even abolished analgesic activity.

Analgesic activity results of the compounds were also in good correlation with their anti-inflammatory activities, tested by using the carrageenan-induced hind paw edema model (Kasahara *et al.*, 1985). As can be seen from Table I, the same amide derivatives **7c**, **7d**, and **7k** showed similar activity, as analgesics exhibited (at 100 mg/kg) potent anti-inflammatory activity as indometacin. The *N*-octyl derivative **7k** especially showed the highest anti-inflammatory activity comparable to indometacin.

It is known that an edema produced by carrageenan is a biphasic event and it is reported that the inhibitory effects of agents which act on the first stage of the carrageenan-induced hind paw inflammation are attributable to the inhibition of the chemical mediators such as histamine, serotonin and bradykinin (Vinegar *et al.*, 1969, 1987). On the other hand, the second stage of the edema might be related to the arachidonic acid metabolites, since it is inhibited by aspirin, indometacin and other cyclooxygenase inhibitors (Vinegar *et al.*, 1987). The tested compounds, **7c**, **7d**, and **7k** exhibited consi derable anti-inflammatory activity both in the first and second phases of edema and the activity did show a gradual increase in the second phase of the edema, indicating that these compounds might exert their anti-inflammatory activities through the mechanisms that involve the inhibition of chemical mediators such as histamine and serotonin and also presumably the COX isoforms. In addition, none of the active compounds neither showed acute toxicity nor gastric lesions in the stomach of mice utilized in the *in vivo* assays, as shown in Table I.

Table I. Percentage analgesic and anti-inflammatory activity of the synthesized compounds

Compound	Analgesic Activity[a] Inhibition of writhing, %	Anti-inflammatory activity[a] Inhibition of edema, %				Gastric Ulcerogenic Effect
		90 min	180 min	270 min	360 min	
7a	27.1	24.5	27.3	26.6	28.5	0/6
7b	22.5	-	3.2	6.2	11.1	0/6
7c	46.7	27.8	32.9	35.9	42.2	0/6
7d	52.4	20.9	26.7	31.5	37.5	0/6
7e	20.6	17.6	16.7	16.2	17.0	0/6
7f	13.3	-	-	-	4.1	0/6
7g	16.7	-	3.7	7.2	8.1	1/6
7h	33.7	6.4	15.9	23.1	29.7	0/6
7i	14.1	-	1.9	2.9	4.7	1/6
7j	25.5	28.2	29.7	28.8	29.1	0/6
7k	54.3	23.1	32.9	39.8	45.9	0/6
7l	24.9	25.5	26.5	25.5	26.6	0/6
Aspirin	52.9	-	-	-	-	3/6
Indometacin	-	42.7	46.5	51.0	53.9	-

[a]Analgesic and anti-inflammatory activity of the compounds were tested at 100 mg/kg doses. Analgesic acitivity of aspirin was tested at 100 mg/kg and anti-inflammatory activity of indometacin was tested at 10 mg/kg dose as described in Experimental Part. $P<0.05$ was found for all testing as in comparison with control group.

Some recent studies for developing safer analgesic and anti-inflammatory drugs which inhibit COX-2 enzyme have concentrated on the preparation of the amide derivatives of well-established NSAID templates such as indometacin (Kalgutkar *et al.*, 2000) and meclofenamic acid (Kalgutkar *et al.*, 2002). The similar group of researchers (Kalgutkar *et al.*, 2000, 2002) observed that neutralization of the NSAIDs accomplished by preparing amide derivatives resulted in compounds that selectively inhibited COX-2 but not COX-1 and produced compounds with good analgesic and anti-inflammatory activity and with no gastric side effects in animal models. Based on this approach, in our previous work dealing with [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetamides (Banoglu *et al.*, 2004), we found that certain amide derivatives including 4-fluorophenylpiperazine, 4-phenylpiperazine, 4-(2-pyridyl) piperazine, 4-methoxyphenyl and the *N*-octyl in the amide portion showed superior analgesic and anti-inflammatory activity similar to the reference compounds. Dogruer *et al.* also 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 4-fluorophenylpiperazine derivative at the amide portion of the compounds (Dogruer *et al.*, 2000). Other published works from different laboratories also indicated that the presence of fluorophenylpiperazine and trifluoromethylphenylpiperazine moiety at the side chain of the pyridazinone ring have positive influence on their analgesic activity (Rubat *et al.*, 1989, 1992; Gokçe *et al.*, 2001; Moreau *et al.*, 1996; Rohet *et al.*, 1996).

Thus, it appeared that certain amide derivatives of [6-(3,5-dimethylpyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetic acid show analgesic and anti-inflammatory activity, and the presence of fluroroarylpiperazine and alkylamine substituents in the amide portion might contribute to their activity. These type of compounds along with our previously published results (Banoglu *et al.*, 2004) might lead to further studies for developing better candidates with potent analgesic and anti-inflammatory activities.

REFERENCES

Banoglu, E., Akoglu, Ç., Ünlü, S., Küpeli, E., Yesilada, E., and Sahin, M. F., Amide derivatives of [6-(5-Methyl-3-phenyl-pyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetic acids as potential analgesic and anti-inflammatory compounds. *Arch. Pharm.*, 337, 7-14 (2004).

Brooks, P., Emery, P., Evans, J. F., Fener, H., Hawkey, C. J., Patrono, C., Smolen, J., Breedveld, F., Day, R., Dougados, M., Ehrich, E. W., Gijon-Banos, J., Kvien, T. K., Van Rijswijk, M. H., Warner, T., and Zeidler, H., Interpreting the clinical significance of the differential inhibition of cyclooxygenase-1 and cyclooxygenase-2. *Rheumatology*, 38, 779-788 (1999).

Chintakunta, V. K., Akella, V., Vedula, M. S., Mamnoor, P. K., Mishra, P., Casturi, S. R., Vangoori, A., and Rajagopalan, R., 3-*O*-substituted benzyl pyridazinone derivatives as COX inhibitors. *Eur. J. Med. Chem.*, 37, 339-347 (2002).

Clinch, D., Banerjee, A. K., Ostick, G., and Levy, D. W., Non-steroidal anti-inflammatory drugs and gastrointestinal adverse effects. *J. R. Coll. Physicians Lond.*, 17, 228-230 (1983).

Coudert, P., Rubat, C., Rohet, F., Leal, F., Fialip, J., and Couquelet, J., Synthesis of new pyridazinones substituted by 4-arylpiperazine-1-yl-carbonyl alkyl moieties and their analgesic properties in mice. *Pharm. Pharmacol. Commun.*, 6, 387-396 (2000).

Dal Piaz, V., Giovannoni, M. P., Ciciani, G., Barlocco, D., Giardina, G., Petrone, G., and Clarke, G. D., 4,5-Functionalized 6-Phenyl-3(2*H*)-pyridazinones: Synthesis and Evaluation of Antinociceptive Activity. *Eur. J. Med. Chem.*, 31, 65-70 (1996).

Dogruer, D. S., Sahin, M. F., Ünlü, S., and Shigeru, I., Studies on some 3(2*H*)-pyridazinone derivatives with antinociceptive activity. *Arch. Pharm.*, 333, 79-86 (2000).

Druey, J., Meier, Kd., and Eichenberger, K., Heilmittelchemiseke Studies in der heterocyclischen Reihe I. *Helv. Chim. Acta*, 37, 121-131 (1954).

Gökçe, M., Dogruer, D., and Þahin, M. F., Synthesis and antinociceptive activity of 6-substituted-3-pyridazinone derivatives. *Farmaco*, 56, 233-237 (2001).

Gökçe, M., Sahin, M. F., Küpeli, E., and Yesilada, E., Synthesis and evaluation of the analgesic and anti-inflammatory activity of new 3(2*H*)-pyridazinone derivatives. *Arzneim. Forsch./ Drug Res.*, 54, 396-401 (2004).

Kalgutkar, A. S., Marnett, A. B., Crews, B. C., Remel, R. P., and Marnett, L. J., Ester and amide derivatives of the nonsteroidal antiinflammatory drug, indometacin, as selective cyclooxygenase-2 inhibitors. *J. Med. Chem.*, 43, 2860-2870 (2000).

Kalgutkar, A. S., Rowlinson, S. W., Crews, B. C., and Marnett, L. J., Amide derivatives of meclofenamic acid as selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.*, 12, 521-524 (2002).

Kasahara, Y., Hikino, H., Tsurufuji, S., Watanabe, M., and Ohuchi, K., Antiinflammations Actions of Ephedrines in Acute Inflammations. *Planta Med.*, 51, 325-331 (1985).

Meade, E. A., Smith, W. L., and DeWitt, D. L., Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by acetyl salicylic acid and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.*, 268, 6610-6614 (1993).

Moreau, S., Coudert, P., Rubat, C., Albuisson, E., and Couquelet, J., Synthesis and peripherally acting analgesic 3-arylpiperazinyl-5-benzyl-pyridazines. *Arzneim. Forsch./Drug Res.*, 46, 800-805 (1996).

Okçelik, B., Ünlü, S., Banoglu, E., Küpeli, E., Yesilada, E., and Sahin, M. F., Investigations of new pyridazinone derivatives for the synthesis of potent analgesic and anti-inflammatory compounds with cyclooxygenase inhibitory activity. *Arch. Pharm.*, 336, 406-412 (2003).

Okun, R., Liddon, S. C., and Lasagnal, L., The effects of aggregation, electric shock, and adrenergic blocking drugs on inhibition of the "writhing syndrome". *J. Pharmacol. Exp. Ther.*, 139, 107-114 (1963).

Patrono, C. and Dunn, M. J., The clinical significance of inhibition of renal prostaglandin synthesis. *Kidney Int.*, 32, 1-12 (1987).

Pieretti, S., Dal Piaz, V., Matucci, R., Giovannoni, M. P., and Galli, A., Antinociceptive activity of a 3(2*H*)-pyridazinone derivative in mice. *Life Sci.*, 65, 1381-1394 (1999).

Rohet, F., Rubat, C., Coudert, P., Albuisson, E., and Couquelet, J., Synthesis and trazodone-like analgesic activity of 4-phenyl-6-aryl-2-[3-(4-arylpiperazin-1-yl)propyl]pyridazin-3-ones. *Chem. Pharm. Bull.*, 44, 980-986 (1996).

Rubat, C., Coudert, P., Couquelet, J., Bastide, P., and Bastide, J., Synthesis and analgesic effect of *N*-substituted 5-arylidene-6-methyl-3(4*H*)-pyridazinones. *Chem. Pharm. Bull.*, 36,1558-1561 (1988).

Rubat, C., Coudert, P., Tronche, P., Bastide, J., Bastide, P., and Privat, A. M., Synthesis and Pharmacological Evaluation of *N*-Substituted 4,6-Diaryl-3-pyridazinones as Analgesic, Anti-inflammatory and Antipyretic Agents. *Chem. Pharm. Bull.*, 37, 2832-2835 (1989).

Rubat, C., Coudert, P., Albuisson, E., Bastide, J., Couquelet, J., and Tronche, P., Synthesis of Mannich bases of arylidene-pyridazinones as analgesic agents. *J. Pharm. Sci.*, 81, 1084-1087 (1992).

Santagati, N. A., Duro, F., Caruso, A., Trombadore, S., and Amico-Roxas, M., Synthesis and Pharmacological Study of A Series of 3(2*H*)-pyridazinones as Analgesic and Anti-inflammatory Agents. *Farmaco*, 40, 921-929 (1985).

Szilagyi, G., Kasztreiner, E., Tardos, L., Jaszlits, L., Kosa, E., Cseh, G., Tolnay, P., and Kovacs-Szabo, I., Studies in the field of pyridazine compounds, III (1). Hypotensive 3-(1-Pyrazolyl)-pyridazine derivatives. *Eur. J. Med. Chem.*, 14, 439-445 (1979).

Takaya, M., Sato, M., Terashima, K., and Tanizawa, H., A new nonsteroidal analgesic-antiinflammatory agent. Synthesis and activity of 4-ethoxy-2-methyl-5-morpholino-3(2*H*)-

pyridazinone and related compounds. *J. Med. Chem.*, 22, 53-58 (1979).

Vinegar, R., Schreiber, W., and Hugo, R., Biphasic development of carrageenin edema in rats. *J. Pharmacol. Exp. Ther.*, 166, 96-103 (1969).

Vinegar, R., Truax, J. F., Selph, J. L., Johnston, P. R., Venable, A. L., and McKenzie, K. K., Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Fed. Proc.*, 46, 118-126 (1987).