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Amide Derivatives of [6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic Acids as Potential Analgesic and Anti-Inflammatory Compounds

In this study, amide derivatives of [6-(5-methyl-3-phenyl-pyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid were synthesized and tested for their *in vivo* analgesic and anti-inflammatory activity by using the *p*-benzoquinone-induced writhing test and carrageenan-induced hind paw edema model, respectively. The analgesic and anti-inflammatory activity of the compounds **6a**, **6d**, **6e**, **6g**, **6h** and **6m** were more potent than that of aspirin as an analgesic and indomethacin as an anti-inflammatory drug, respectively. The other derivatives generally resulted in comparable activity to reference compounds. Inhibitor activity of the active compounds on cyclooxygenase isoforms was also investigated by using *in vitro* human whole blood assay and found that these derivatives did not exert their analgesic and anti-inflammatory activities through COX inhibition and other mechanisms might be involved.

Keywords: Pyridazinone; Analgesic; Anti-Inflammatory; COX-1; COX-2

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Introduction

Most currently used nonsteroidal anti-inflammatory drugs (NSAIDs) have limitations for their therapeutic use since they cause gastrointestinal and renal side effects which are inseparable from their pharmacological activities [1–3]. However, the discovery of the two distinct cyclooxygenase (COX) isoforms, namely cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), made it possible to separate the pharmacological effects from the side effects of NSAIDs since COX-1 is expressed constitutively in most cells and tissues and responsible for the synthesis of PGs that are important for gastric protection and vascular homeostasis while COX-2 is mostly expressed as a result of mitogenic and inflammatory stimuli [2]. Many studies indicated that inhibition of COX-2 but not COX-1 resulted compounds that lacked the gastrointestinal and renal side effects of currently used NSAIDs [1–4]. Celecoxib [5] and rofecoxib [6] have been marketed as the first drugs that selectively inhibit COX-2 (Figure 1).

The structure-activity studies indicated that the spatial orientation of the two aromatic rings of this tricyclic inhibitor drug class was critical, that is, two aryl rings must reside on adjacent positions (1,2-disubstitution pattern) about the central 5 or 6-membered carbocyclic or heterocyclic ring for selective COX-2 inhibition [7, 8]. However, a few reports also indicated that 1,3-diarylsubstitution about an annulated pyrazole ring was also an effective pattern for selective COX-2 inhibition (Figure 1) [9, 10].

In addition, many studies also focused on 3(2H)-pyridazinone derivatives for developing potent and safer NSAIDs without gastric side effects [11–13]. Various compounds incorporating 3(2H)-pyridazinone nucleus have been synthesized and their analgesic and anti-inflammatory activity have been reported. Among these compounds, 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (emorfazone) is currently being marketed in Japan as an analgesic and anti-inflammatory drug [14]. Moreover, 3-*O*-substituted benzyl pyridazinone derivatives were synthesized and shown that these compounds demonstrated *in vitro* selective COX-2 inhibitory activity and also potent anti-inflammatory activity using carrageenan-induced rat paw edema assay [11]. Additionally, 2-substituted 4,5-functionalized 6-phenyl-3(2H)-pyridazinone derivatives [12] have also been reported to bear potent analgesic activity lacking the general side effects of currently used NSAIDs. Meantime, many authors reported that pyridazinone derivatives bearing an arylpiperazine moiety at the side chain on the lactam nitrogen of the ring had significant analgesic activity and stressed the significance of this structural feature [15, 16]. Moreover, they claimed that these derivatives exhibited better analgesic activity if they bear a car-

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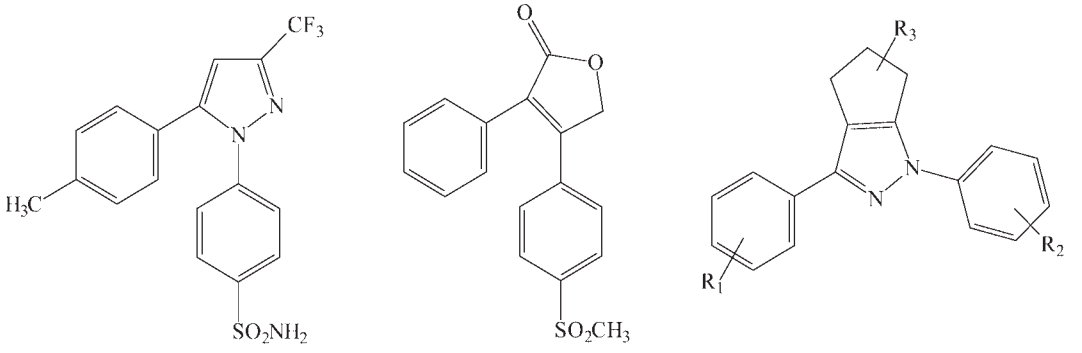


Celecoxib **Rofecoxib** **1,3-diarylpyrazoles**

Figure 1. Structures of COX-2 inhibitors.

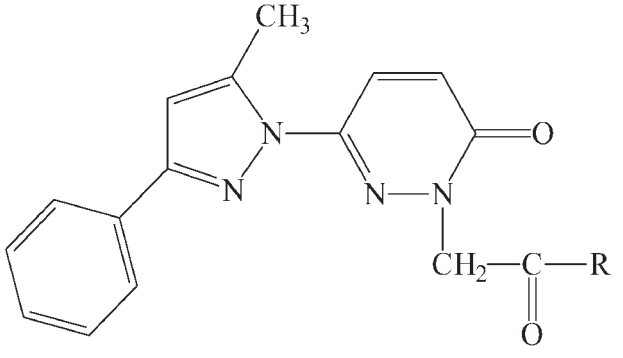


Figure 2. General structure of the synthesized compounds.

bon chain between the nitrogen atom of the lactam and the amine component of the side chain. Dogruer et al. subsequently synthesized [6-(4-methoxyphenyl)-3(2H)-pyridazinone-2-yl]acetamide and propanamide derivatives and reported that these compounds showed potential analgesic activity [13]. Meantime, some studies for developing COX-2 inhibitors have concentrated on the preparation of the amide derivatives of currently used NSAIDs such as indomethacin [17] and meclofenamic acid [18] and found that neutralization of these NSAIDs by preparing amide derivatives resulted compounds that selectively inhibited COX-2 but not COX-1.

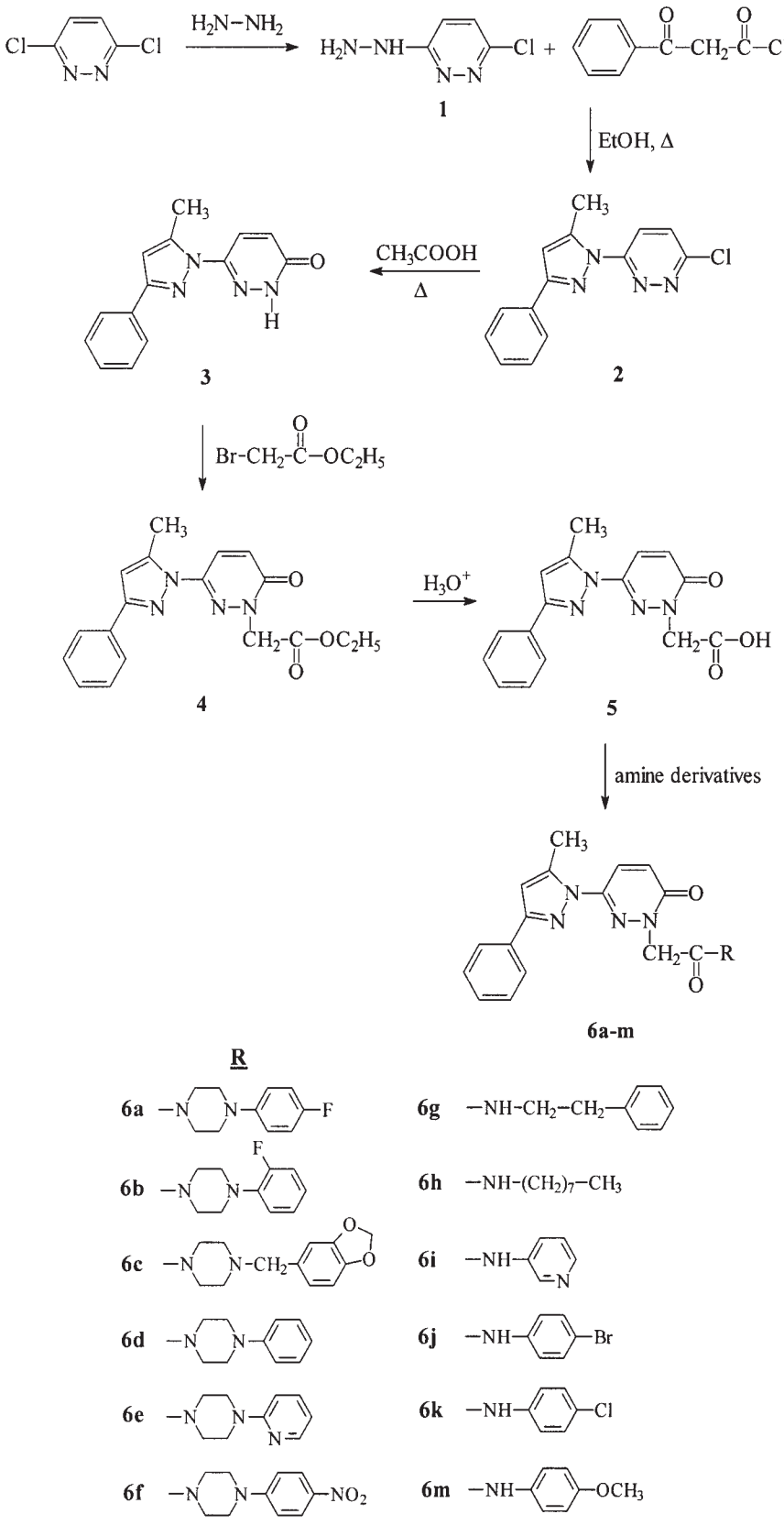
These findings stimulated us to search for new compounds with 1,3-diaryl substitution pattern about a central pyrazole ring. Based on the information that 3(2H)-pyridazinone derivatives bear potent analgesic and anti-inflammatory activities [11–16], we have chosen the pyridazinone ring as one of the aryl substituents about the central pyrazole ring. We also aimed to explore the presence of the acetamide side chain that is linked to the lactam nitrogen of pyridazinone ring to determine the contribution of this structural feature to the analgesic and anti-inflammatory activity. Therefore, we synthesized amide derivatives of [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid (Figure 2) to test their *in vivo* analgesic and anti-inflammatory activities and also their *in vitro* inhibitory activity on COX-1 and COX-2 enzymes.

Results and discussion

Chemistry

The title amide derivatives were synthesized by the reaction of appropriate amine derivatives with [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid in the presence of *N*,*N*-bis(2-oxo-3-oxazolidinyl)-phosphorodiamidic chloride (BOP-Cl) [19] as the carboxylate activator. Analytical data for structure elucidation were given in Experimental Part. The preparation of the resulting amide derivatives **6 a**–**m** were outlined in Scheme 1.

In the synthesis of the amide derivatives (**6 a**–**m**), commercially available 3,6-dichloropyridazine was used as the starting material, and 6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone (**3**) were prepared by adapted procedures according to the previously published methods [20, 21]. **3** was then treated with ethyl bromoacetate to prepare the ethyl [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetate (**4**) which was subsequently acid-hydrolysed to obtain [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid (**5**). By treatment of **5** with appropriate amines in the presence of BOP-Cl in dichloromethane at room temperature, resulting amide derivatives (**6 a**–**m**) were prepared in moderate to good yield (52–70 %).



Scheme 1. Synthetic pathways of [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide derivatives.

Pharmacology

Analgesic and anti-inflammatory activities of the synthesized amide derivatives (**6 a**–**m**) and intermediate 6-(5-methyl-3-phenylpyrazole-1-yl)-3-chloropyridazine (**2**), 6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone (**3**), ester (**4**) and acid (**5**) derivatives were assessed by *p*-benzoquinone-induced writhing test [22] and carrageenan-induced hind paw edema model [23], respectively. As seen in Table 1, analgesic and anti-inflammatory activity of the compounds **6 a**–**m** were comparable to the reference compounds aspirin and indomethacin, respectively. Analgesic activity results indicated that compounds having 4-fluorophenylpiperazine (**6 a**), phenylpiperazine (**6 d**), 2-pyridylpiperazine (**6 e**), octyl (**6h**) and 4-methoxyphenyl (**6 m**) substituents at the amide portion of the synthesized compounds showed superior analgesic activity to that of aspirin in the *p*-benzoquinone-induced writhing test. These results correlated well with the previous results indicating that the presence of arylpiperazine moiety at the side chain of the pyridazinone ring increases the analgesic activity [15, 16]. Dogruer et al. reported that in [6-(4-methoxyphenyl)-3(2H)-pyridazinone-2-yl]acetamide and propanamide derivatives, the highest analgesic activity was observed with the 4-fluorophenylpiperazine derivative at the amide portion of the compounds [13]. In their work 2-pyridylpiperazine amide derivative also showed potential analgesic activity. Kalgutkar et al. also reported that *N*-octyl and *N*-4-methoxyphenylamide derivatives of indomethacin were potent selective inhibitors of COX-2 [17]. However, other secondary amide derivatives including 2-aminopyridine (**6i**), 4-bromophenyl (**6j**) and 4-chlorophenyl (**6k**) substituents caused a decrease in the analgesic activity. In addition, 6-(5-methyl-3-phenylpyrazole-1yl)-3-chloropyridazine (**2**), 6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone (**3**), ethyl [6-(5-methyl-3-phenyl-pyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetate (**4**) and [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid (**5**) derivatives were also tested for their analgesic activity and found that they exhibited moderate analgesic activities which is comparable to aspirin but lower than some of the corresponding amide derivatives. These results indicated that certain amide derivatives contributed to the higher analgesic activity of the compounds.

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

Compound	Analgesic activity[#] Inhibition of writhing (%)	Antiinflammatory activity[#] Inhibition of edema (%) 90 min	180 min	270 min	360 min
6 a	62.5	23.7	17.8	26.6	31.4
6 b	41.1	10.3	8.3	17.3	16.6
6 c	17.3	8.4	6.7	11.7	10.5
6 d	66.9	28.1	21.9	24.3	28.5
6 e	65.6	29.2	27.4	31.3	37.1
6 f	44.9	6.6	9.1	12.3	15.6
6 g	54.1	18.7	17.2	27.3	26.9
6 h	71.1	35.6	34.1	39.6	37.6
6 i	38.5	16.5	11.8	14.9	15
6 j	44.9	16.0	12.4	17.3	16.1
6 k	44.9	14.7	10.3	10.7	13.4
6 m	64.6	22.4	19.3	20.4	22.9
2	50.9	19.1	13.4	15.1	15.3
3	44.4	10.5	7.7	12.3	10.9
4	55.6	24.2	21.5	22.2	22.1
5	50.4	10.3	6.3	14.9	16.6
Aspirin	56.8	–	–	–	–
Indomethacin	–	38.0	33.7	34.9	36.3

[#] 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 indomethacin 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.

Table 2. COX inhibitory activity of the selected compounds.

Compound	COX-1 inhibition, (%) 10 μM	COX-2 inhibition, (%) 10 μM
6 a	0	14
6 d	0	3
6 e	1.5	14
6 g	0	16
6 h	1.4	8
6 m	1.6	5

Inhibitor activity of the selected compounds at 10-μM concentrations on COX-1 and COX-2 were tested by using *in vitro* human whole blood assay as described in Experimental Part. Indomethacin (COX-1, IC_{50} = 0.19 μM and COX-2, IC_{50} = 0.22 μM) and celexocib (COX-1, IC_{50} = 12.2 μM and COX-2, IC_{50} = 1.1 μM) were used as nonselective COX inhibitor and selective COX-2 inhibitor references in the assays.

Analgesic activity results of the compounds also showed good correlation with their anti-inflammatory activities tested by using the carrageenan-induced hind paw edema model. As seen in Table 1, the same amide derivatives (**6 a**, **6 d**, **6 e**, **6 h** and **6 m**) exhibited at 100 mg/kg as potent anti-inflammatory activity as indomethacin. The *N*-octylamide derivative (**6 h**) especially showed high anti-inflammatory activity that was comparable to indomethacin. 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 [24]. On the other hand, the second stage of the edema might be related to the arachidonic acid metabolites since it is inhibited by aspirin, indomethacin and other cyclooxygenase inhibitors [24]. As seen in Table 1, the tested compounds exhibited considerable anti-inflammatory activity both in the first and second phases of edema and the activity did not show a significant 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 compounds except **6 f** (in one of the 6 animals caused gastric lesions) and **6 j** (in 2 of the 6 animals caused gastric lesions) showed gastric lesions in the stomach of mice utilized in the *in vivo* assays. Based on these results, compounds **6 a**, **6 d**, **6 e**, **6 g**, **6 h**, and **6 m** were considered to bear potent analgesic and anti-inflammatory activity at 100 mg/kg doses in mice, and selected for further studies to investigate their COX inhibitory activities by using *in vitro* human whole blood assay [25] to elucidate if the COX inhibition is present relating to their analgesic and anti-inflammatory activities.

Although we hypothesized that the selected compounds would have inhibitory activity on COX-enzymes to shut down prostaglandin synthesis to exert their analgesic and anti-inflammatory activities, this was not the case and none of the selected *in vivo* active compounds have resulted considerable inhibition at 10 μM neither in COX-2 nor in COX-1 (Table 2). Therefore, we concluded that these [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide derivatives do not exert their analgesic and anti-inflammatory activities through COX inhibition and other mechanisms might be involved. The mechanism that underlie the analgesic and anti-inflammatory activities of the resulting amide derivatives are currently under investigation in our laboratory.

Experimental

3,6-dichloropyridazine, hydrazine hydrate, benzoylacetone, ethyl bromoacetate, triethylamine, *N,N*-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride and amine derivatives were obtained from Aldrich (Germany) and Merck (Germany). 3-Chloro-6-hydrazinopyridazine was synthesized according to a previously published procedure (mp 136–137 °C; compared to literature mp 137–138 °C [20]). All other chemicals were obtained from commercial sources. IR spectra were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer (KBr, υ, cm^{-1}). ^{1}H-NMR spectra were recorded on a Bruker 400 FT-NMR spectrometer using TMS as an internal standard in DMSO-d_6. LC-MS analysis of the compounds was performed on ThermoQuest Finnigan AQA Mass Spectrometry equipped with ThermoQuest Liquid Chromatograph.

Synthesis of 6-(5-methyl-3-phenylpyrazole-1-yl)-3-chloropyridazine (**2**)

A mixture of 3-chloro-6-hydrazinopyridazine (0.01 mol) and benzoylacetone (0.01 mol) in 30 mL ethanol was heated to reflux for 5 h. After cooling, the separated crystals are filtered off, washed with ice-cold ethanol, dried and recrystallized from ethanol to yield 71.2 %. mp 150 °C. LC-MS (+ESI) m/z 271.5 ($M^{+\bullet}$ + 1) – ^{1}H-NMR ($CDCl_3$): δ 7.83 (d, 1 H); 7.45 (d, 1 H); 7.38–7.08 (m, 5 H); 6.28 (s, 1 H); 2.3 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3064, 2627, 1570, 763, 697.

Synthesis of 6-(5-methyl-3-phenylpyrazol-1yl)-3(2H)-pyridazinone (**3**)

A solution of **2** (0.01 mol) in 20 mL of glacial acetic acid was refluxed for 18 h. After cooling, it was poured into ice-water (50 mL) and the precipitate formed was filtered off, washed with water and dried and recrystallized from ethanol (yield 96 %). mp 210 °C. LC-MS (+ESI) m/z 253 ($M^{+\bullet}$ + 1). – ^{1}H-NMR ($CDCl_3$): δ 12.7 (s, 1 H); 7.75 (d, 1 H); 7.45–7.27 (m, 5 H); 7.06 (d, 1 H); 6.5 (s, 1 H); 2.27 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3063, 2948, 1679, 1602, 762.

Synthesis of ethyl [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetate (**4**)

To the solution of **3** (0.01 mol) and potassium carbonate (0.04 mol) in 40 mL dimethylformamide was added ethylbromoacetate (0.015 mol) and stirred at room temperature for 90 min. The reaction mixture was then poured into ice-water and the precipitate formed filtered off, washed with water, dried and recrystallized from ethanol to yield 85 %, mp 88 °C. LC-MS (-ESI) m/z 337 ($M^{+\bullet}$ – 1). – ^{1}H-NMR ($CDCl_3$): δ 7.53 (d, 1 H); 7.34–7.14 (m, 5 H); 6.92 (d, 1 H); 6.23 (s, 1 H); 4.5 (s, 2 H); 4.1 (q, 2 H); 2.29 (s, 3 H); 1.17 (t, 3 H). – IR ν_{max} cm^{-1} (KBr): 3058, 2999, 2958, 1758, 1671, 1597, 1204, 853, 762, 695.

Synthesis of 2-[6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid (**5**)

Compound 4 (0.01 mol) was heated up to reflux temperature in NaOH (10 % w/v) for 90 min. After cooling, the reaction mixture was acidified with concentrated HCl and the precipitate formed was filtered off, washed with water, dried and recrystallized from ethanol-water to yield 96 %, mp 170–171 °C. LC-MS (+ESI) m/z 311 ($M^{+\bullet}$ + 1). ^{1}H-NMR ($CDCl_3$): δ 7.36 (d, 1 H); 7.29–7.19 (m, 5 H); 6.92 (d, 1 H); 6.24 (s, 1 H); 5.8–4.7 (w, 1 H); 4.6 (s, 2 H); 2.3 (s, 3 H). IR ν_{max} cm^{-1} (KBr): 3005, 2873, 2568, 2477, 1732, 1648, 1572, 1248, 848, 763, 695.

General Procedure for the amidation of [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid (**6 a**–**m**)

A reaction mixture containing [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetic acid (0.01 mol) and bis-(2-oxazolidinyl)phosphinic chloride (0.01 mol) in 10 mL anhydrous CH_2Cl_2 was treated with Et_3N (0.01 mol) and allowed to stir at room temperature for 30 min. The mixture was then treated with appropriate amine derivatives and continued to stir at room temperature overnight. After evaporation to dr8yness, the residue was solidified by stirring in ice-cold water, and the precipitate formed was recrystallized from the appropriate solvent.

1-[2-[6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetyl]-4-(4-fluorophecyl)piperazine (**6 a**)

Recrystallized from 2-propanol (yield 52,6 %, mp 152 °C). LC-MS (-ESI) m/z 471 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 7.45 (d, 1 H); 7.32–7.21 (m, 5 H); 6.98–6.86 (m, 3 H); 6.85–6.77 (m, 2 H); 6.23 (s, 1 H); 4.68 (s, 2 H); 3.68 (t, 2 H); 3.45 (t, 2 H); 3.01 (m, 4 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3055, 2923, 1668, 1591, 1236, 760, 695.

1-[2-[6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetyl]-4-(2-fluorophenyl)piperazine (**6 b**)

Recrystallized from 2-propanol (yield 51 %, mp 121 °C). LC-MS (-ESI) m/z 471 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 7.46 (d, 1 H); 7.36–7.1 (m, 5 H); 7.1–6.78 (m, 5 H); 6.23 (s, 1 H); 4.68 (s, 2 H); 3.7 (t, 2 H); 3.46 (t, 2 H); 3.01 (m, 4 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3052, 2929, 1669, 1595, 1234, 757, 696.

1-[2-[6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetyl]-4-(4-piperonyl)piperazine (**6 c**)

Recrystallized from methanol (yield 58 %, mp 99 °C). LC-MS (+ESI) m/z 513 ($M^{+\bullet}$ + 1) – ^{1}H-NMR ($CDCl_3$): δ 7.41 (d, 1 H); 7.34–7.13 (m, 5 H); 6.88 (d, 1 H); 6.77 (s, 1 H); 6.66 (d, 2 H); 6.22 (s, 1 H); 5.87 (s, 2 H); 4.62 (s, 2 H); 3.51 (t, 2 H); 3.36 (s, 2 H); 3.27 (t, 2 H); 3.01 (m, 4 H); 2.27 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3024, 2960, 2882, 1671, 1645, 1586, 1033, 764.

1-[2-[6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetyl]-4-phenylpiperazine (**6 d**)

Recrystallized from 2-propanol (yield 70 %, mp 161 °C). LC-MS (-ESI) m/z 453 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 7.47 (d, 1 H); 7.35–7.12 (m, 7 H); 6.98–6.88 (m, 4 H); 6.23 (s, 1 H); 4.68 (s, 2 H); 3.68 (t, 2 H); 3.45 (t, 2 H); 3.1 (m, 4 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 2995, 2905, 2816, 1671, 1665, 1595, 1230, 757, 693.

1-[2-[6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetyl]-4-(2-pyridyl)piperazine (**6 e**)

Recrystallized from methanol (yield 64 %, mp 171 °C). LC-MS (+ESI) m/z 456 ($M^{+\bullet}$ + 1) – ^{1}H-NMR (DMSO-d_6): δ 8.28 (m, 1 H), 7.86 (d, 1 H); 7.8–7.66 (m, 1 H); 7.61–7.42 (m, 5 H); 7.26 (d, 1 H); 7.03 (d, 1 H); 6.92 (m, 1 H); 6.69 (s, 1 H); 4.93 (s, 2 H); 3.69 (m, 8 H); 2.45 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3032, 2982, 2912, 2843,1662, 1593, 1233, 758.

1-[2-[6-(5-Methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetyl]-4-(4-nitrophenyl)piperazine (**6 f**)

Recrystallized from methanol (yield 57 % mp 216 °C). LC-MS (-ESI) m/z 498 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 8.1 (d, 2 H), 7.45 (d, 1 H); 7.35–7.13 (m, 5 H); 6.88 (d, 1 H); 6.75 (d, 2 H); 6.24 (s, 1 H); 4.68 (s, 2 H); 3.7 (t, 2 H); 3.52 (t, 2 H); 3.39 (m, 4 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3054, 2986, 2909,1668, 1595, 1236, 767.

N-Phenylethyl-2-[6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide (**6 g**)

Recrystallized from ethanol (yield 63 %, mp 130 °C). LC-MS (-ESI) m/z 412 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 7.63 (d, 1 H); 7.27 (m, 3 H); 7.17 (m, 5 H); 7.02 (m, 2 H); 6.9 (d, 1 H); 6.23 (s, 1 H); 5.76 (t, 1 H); 4.33 (s, 2 H); 3.37 (q, 2 H); 2.67 (t, 2 H); 2.3 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3286, 3084, 3027, 2926, 1683, 1655, 1599, 757, 695.

N-Octyl-2-[6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide (**6 h**)

Recrystallized from ethanol-water (yield 57.8 %, mp 105 °C). LC-MS (-ESI) m/z 420 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 7.61 (d, 1 H); 7.4–7.1 (m, 5 H); 6.95 (d, 1 H); 6.23 (s, 1 H); 5.9 (t, 1 H); 4.33 (s, 2 H); 3.1 (q, 2 H); 2.29 (s, 3 H); 1.36 (m, 2 H); 1.27–1.03 (m, 10 H); 0.8 (t, 3 H). – IR ν_{max} cm^{-1} (KBr): 3320, 2932, 2851, 1677, 1653, 1603, 760.

N-(3-Pyridyl)-2-[6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide (**6 i**)

Recrystallized from toluene (yield 61 %, mp 173 °C). LC-MS (+ESI) m/z 387 ($M^{+\bullet}$ + 1) – ^{1}H-NMR ($CDCl_3$): δ 9.2 (s, 1 H); 8.61 (s, 1 H); 8.25 (m, 1 H); 8.16 (m, 1 H); 7.65 (d, 1 H); 7.34–7.21 (m, 6 H); 6.97 (d, 1 H); 6.24 (s, 1 H); 4.65 (s, 2 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3587, 3071, 1701, 1674, 1587, 760.

N-(4-Bromophenyl)-2-[6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide (**6 j**)

Recrystallized from ethanol (yield 70 %, mp 199 °C). LC-MS (+ESI) m/z 465 ($M^{+\bullet}$ + 1) – ^{1}H-NMR ($CDCl_3$): δ 8.45 (s, 1 H); 7.65 (d, 1 H); 7.39–7.13 (m, 9 H); 6.96 (d, 1 H); 6.23 (s, 1 H); 4.66 (s, 2 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3278, 3054, 1684, 1666, 1588, 759.

N-(4-Chlorophenyl)-2-[6-(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide (**6 k**)

Recrystallized from methanol (yield 64 %, mp 200 °C). LC-MS (-ESI) m/z 418.5 ($M^{+\bullet}$ – 1) – ^{1}H-NMR ($CDCl_3$): δ 8.38 (s, 1 H);

7.66 (d, 1 H); 7.4–7.1 (m, 9 H); 6.98 (d, 1 H); 6.24 (s, 1 H); 4.64 (s, 2 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3284, 3100, 3047, 1713, 1665, 1589, 759.

N-(4-Methoxyphenyl)-2-[(5-methyl-3-phenylpyrazole-1-yl)-3(2H)-pyridazinone-2-yl]acetamide (**6 m**)

Recrystallized from ethanol (yield 57 %, mp 172 °C). LC-MS (-ESI) m/z 414 ($M^{+\bullet} - 1$) – ^{1}H-NMR ($CDCl_3$): δ 8.02 (s, 1 H); 7.65 (d, 1 H); 7.38–7.13 (m, 7 H); 6.96 (d, 1 H); 6.73 (d, 2 H); 6.24 (s, 1 H); 4.63 (s, 2 H); 3.7 (s, 3 H); 2.29 (s, 3 H). – IR ν_{max} cm^{-1} (KBr): 3282, 3136, 3084, 1703, 1663, 1591, 766.

Pharmacology

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

Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or aspirin (100 mg/kg) in 0.5 % CMC was used as reference drug.

p-Benzoquinone-induced writhing test [22]

60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5 % (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control 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 analgesic activity was expressed as the percentage change from writhing controls.

Carrageenan-induced hind paw edema test

The method of Kasahara et al. [23] was used. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. 60 min after the oral administration of test sample or dosing vehicle, each mice was injected with freshly prepared (0.5 mg/25 µL) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw and 25 µL of saline solution into that of the left as secondary control. Measurements were done and evaluated as described above in every 90 min during 360 min.

Acute toxicity

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

Gastric-side effects

After 8 h the analgesic activity experiments, mice were killed under deep ether anesthesia and 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 animal experiments were expressed as the mean standard error (±SEM). Statistical differences between the treatments and the control were tested by ANOVA test. Data with $p < 0.05$ value was considered to be significant.

Effect of compounds on human whole blood COX-1 and COX-2 activities

The human whole blood assay, originally developed by Patrignani et al [25], is considered to be the more biologically relevant way to assess the inhibition of the cyclooxygenase isoenzymes, COX-1 and COX-2, by a test compound [2]. In this assay, platelets stimulated by calcium ionophore are believed to be the main source of COX-1, whereas monocytes stimulated with LPS are thought to be the source of COX-2. COX-1 activity is determined by the production of thromboxane B_2 (TXB_2), while COX-2 activity is determined by the production of prostaglandin E_2 (PGE_2).

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