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Investigations of New Pyridazinone Derivatives for the Synthesis of Potent Analgesic and Anti-Inflammatory Compounds with Cyclooxygenase Inhibitory Activity

In this study we describe the synthesis of two novel 4-phenyl- and 4-(2-chlorophenyl)-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-3(2H)-pyridazinone derivatives (compounds **8 a** and **b**) and their testing as inhibitors of cyclooxygenases (COX-1 and COX-2). Both compounds inhibited COX-1 (by 59 % and 61 % for compounds **8** a and **8 b** respectively) and COX-2 (by 37 % and 28 % for compounds **8 a** and **8 b** respectively) at a concentration of 10 μM. Furthermore, we tested the analgesic and anti-inflammatory activities of the synthesized compounds *in vivo* by using the *p*-benzoquinone-induced writhing test and the carrageenan-induced hind paw edema model, respectively. Compounds **8 a** and **b** showed potent analgesic and anti-inflammatory activities without causing gastric lesions in the tested animals.

Keywords: 2-Oxo-3H-benzoxazole; Pyridazinone; Analgesic; Anti-inflammatory; COX-1; COX-2

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Introduction

Cyclooxygenases (COX-1 and COX-2) catalyze the committed step in prostaglandin (PG) synthesis and are of particular interest because they are the major targets of nonsteroidal anti-inflammatory drugs (NSAIDs) [1–3]. Inhibition of PGs by NSAIDs acutely reduces inflammation, pain and fever. The COX-2 isoform is responsible for the production of PGs which mediate the inflammatory response. Therefore, the development of new compounds which selectively inhibit COX-2, without modifying the physiological levels of constitutive COX-1, has emerged as a growing research area for generation of new anti-inflammatory drugs lacking the side-effects of traditional NSAIDs [4–7]. However, COX-2 is also involved in delayed ulcer healing, renal physiology and female reproduction processes indicating that the functions of COX-1 and COX-2 might be more complex then originally thought [8].

The majority of NSAIDs currently used in the therapy of inflammatory conditions belong to the chemical class of arylacetic and arylpropionic acids. Their side effects result from inhibition of the constitutive COX-1 isoform which is responsible for the production of prostaglandins (PGs) important for gastroprotection and vascular homeostasis [3, 8]. The newly developed selective COX-2 inhibitors have the common structural properties of two aromatic moieties attached to adjacent atoms (1,2-diarylsubstituted) in a bridging carbocyclic or heterocyclic five-membered ring [2, 4–6] but do not show the side effects inherent to traditional inhibitors. Some 1,3-diarylpyrazoles fused to a cycloalkane have also been reported to function as selective COX-2 inhibitors (**1** in Figure 1) [9, 10]. The most effective inhibitor, derivative (2), had an IC_{50} of 0.64 μM against COX-2 and >10 μM against COX-1.

Our research group has been interested for some time in studying the effect of substituting selected aromatic rings in current NSAIDs with alternative heteroaromatic moieties such as 2-oxo-3H-benzoxazole, 2-oxo-3H-benzothiazole and 3(2H)-pyridazinone [11–15]. We are particularly interested in whether the biological activity of the derivatives can be preserved by isosteric replacement of a key aromatic ring. With this purpose and based on the fact that heteroaryl acetic acid derivatives bear potential analgesic and anti-inflammatory activities, we have previously studied the 2-oxo-3H-benzoxazole alkanoic acid derivatives and found that 6- or 7-acyl-2-oxo-3H-benzoxazole alkanoic acids had potent analgesic and anti-inflammatory activities [16, 17]. We then proceeded to investigate the 3(2H)-pyridazinones and derivatives carrying acetamide and propanamide moieties at position 2 of the pyridazinone ring and found that these compounds also showed good analgesic and anti-inflammatory properties [14, 15]. Furthermore, many pyridazinone derivatives have been reported to function as novel potent analgesic and anti-inflammatory agents

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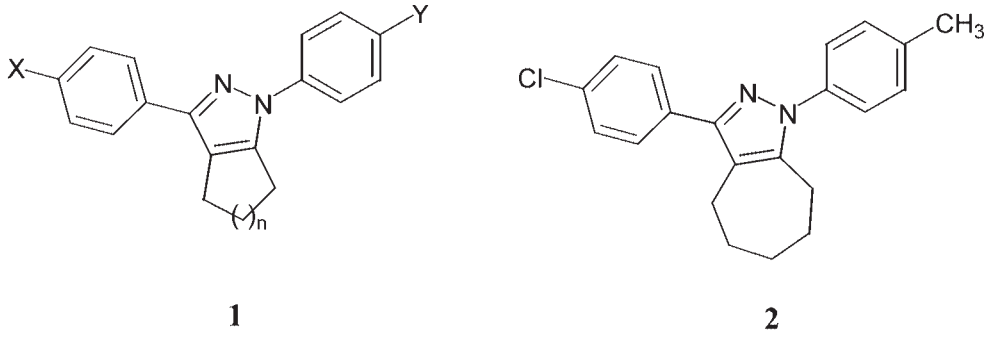

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Figure 1. 1,3-Diarylpyrazoles as selective COX-2 inhibitors.

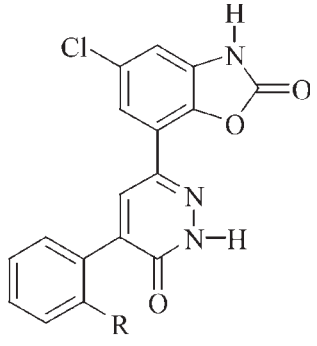

Figure 2. The general structure of the compounds synthesized.

and some have been shown to selectively inhibit COX-2 function [18–20].

These studies prompted us to search for novel lead compounds for use as selective COX-2 inhibitors. In the present study, we describe the biological consequences of incorporation of a 2-oxo-3H-benzoxazole ring as one of the aryl substituents and the effect of a 1,3-diarylsubstitution pattern around the pyridazinone ring (Figure 2) on the in vitro and in vivo activity of the resulting derivatives. Since 2-oxo-3H-benzoxazoles and 3(2H)-pyridazinones have good analgesic and anti-inflammatory properties and the 1,3-diaryl/heteroaryl structures might also be important for these activities, we have combined 2-oxo-3H-benzoxazole and 3(2H)-pyridazinone rings in the same structure and investigated the ability of the resulting derivates to inhibit cyclooxygenase. Here we describe the methodology we employed to the synthesis of the derivatives and their resulting *in vivo* and *in vitro* biological activity.

Results and discussion

Chemistry

The synthetic methodology used in the synthesis of compounds **8 a** and **b** is shown in Scheme 1.

For the synthesis of compounds **8a** and **8 b**, commercially available 2-amino-4-chlorophenol was first converted to 2-acetylamino-4-chlorophenol to maintain the further acylation of this compound at position 6. Acylation of 2-acetylamino-4-chlorophenol, using Friedel-Crafts acylation conditions, allowed us to obtain the acetyl group in the appropriate position to synthesize 7-acetyl-5-chloro-2-oxo-3H-benzoxazole (**3**). Treatment of **3** with the appropriate benzaldehyde derivative, under base-catalysed reaction conditions, resulted in compounds **4 a**–**b**. Synthesis of the corresponding 4-oxobutyronitrile (**5 a**–**b**) and 4-oxobutyric acid (**6 a**–**b**) derivatives was achieved by treatment of **4 a**–**b** with potassium cyanide followed by acid-catalyzed hydrolysis. The 4,5-dihydropyridazinone derivatives (**7 a**–**b**) were obtained by treatment of **6 a**–**b** with hydrazine hydrate, followed by subsequent oxidization to produce 4-phenyl- and 4-(2-chlorophenyl)-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-3(2H)-pyridazinone derivatives (**8 a**–**b**). Synthesis of the intermedite compounds **2**–**7** have not been previously reported in the literature.

Pharmacology

The inhibitory activity of compounds **8a** and **8b** on COX-1 and COX-2 was assayed using the COX Inhibitor Screening Assay Kit (Cayman No: 560131) according to the protocol recommended by the supplier. Preliminary screening of both title compounds (**8a** and **b**) and references (indomethacin and DFU) was performed at a final concentration of 10 μM to determine the percent inhibition of the COX-1 and COX-2 isoforms. The inhibitor activity of indomethacin, at a 10 μM final concentration in the test system, deviated from previously published reports [21, 22]. However, the results in our assay were basically reproducible and the average of typical sets of data are represented here. Previously published literature reports on the inhibitory activity of indomethacin indicate that depending on the *in vitro* assay used, the COX-2/COX-1 ratio can vary from 1.31 to 107.1 [3, 4]. Differences in the published results obtained for indomethacin in

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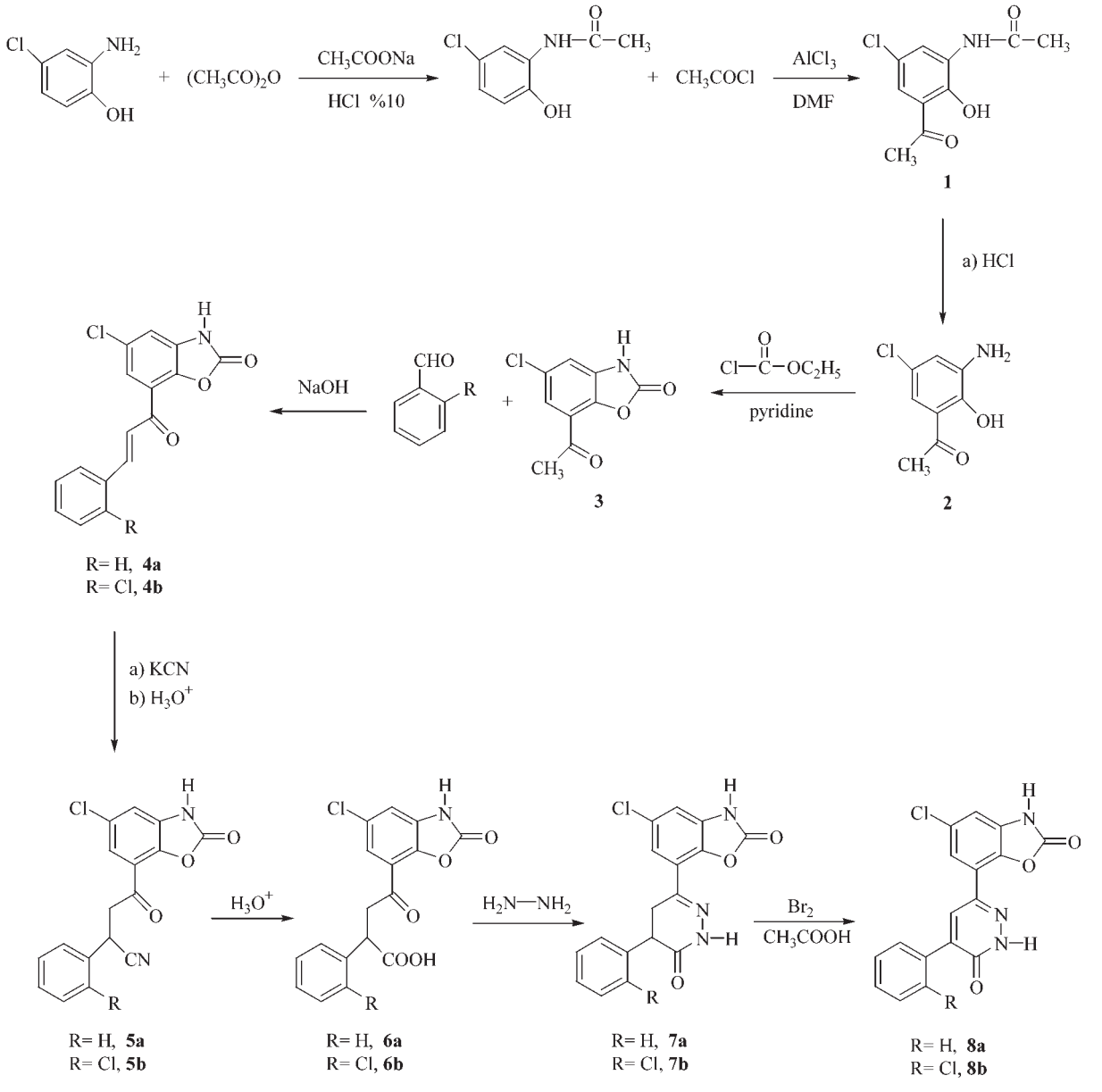


Scheme 1. Synthetic route for the synthesis of compounds **8a–b**.

various test systems illustrate how the determined inhibitory values can vary for a single compound. Variability between assays was reported to be the consequence of many factors including incubation time, the use of exogenous or endogenous substrate, use of whole cells, microsomes or recombinant enzymes, and the presence or absence of plasma proteins in the medium [3]. Therefore, our results from the *in vitro* screening assay utilized serve only as a guide to the relative selectivity of different compounds in the same assay system.

The percent inhibition of COX-1 and COX-2 by indomethacin was determined to be 69 % and 78 %, respectively suggesting that indomethacin was functioning as a non-selective inhibitor as previously reported [16]. DFU inhibited COX-2 (86 %) but did not have any inhibitory effect on COX-1 in the assay system also in accordance with a previously published report [23]. Compounds **8a** and **b** showed inhibitor activity against the COX-1 and COX-2 enzymes but the selectivity was either not very pronounced or only slightly selective for the

Table 1. Anti-inflammatory and analgesic activities of compounds **8 a** and **8 b**.

Compound	**Anti-inflammatory activity** Thickness of Edema ± SD (***Inhibition %***)				**Analgesic activity** Number of writhings ± SD (***Inhibition %***)	**Ratio of gastric lesions**
	90 min	**180 min**	**270 min**	**360 min**		
8 a	31 ± 2.29 (***17.9***)	33.5 ± 1.61 (***28.4***)*	35 ± 2.11 (***39.4***)**	33.8 ± 1.47 (***49.7***)***	11.3 ± 1.52 (***70.4***)***	0/6
8 b	30.3 ± 2.01 (***19.8***)	33.8 ± 1.35 (***27.7***)*	35 ± 2.24 (***39.4***)**	31.2 ± 2.81 (***53.6***)***	17 ± 2.96 (***55.5***)**	0/6
Indomethacin	26.5 ± 2.06 (***29.9***)*	27.3 ± 1.91 (***41.7***)**	27.2 ± 2.76 (***52.9***)***	29 ± 1.07 (***56.8***)***	–	0/6
Aspirin	–	–	–	–	16.3 ± 2.26 (***57.3***)**	2/6
Control	37.8 ± 3.57	46.8 ± 5.39	57.8 ± 5.15	67.2 ± 3.69	38.2 ± 4.29	0/8

The analgesic and anti-inflammatory activity of compounds **8 a** and **b** were tested at 100 mg/kg doses. The analgesic activity of aspirin was tested at a 100 mg/kg dose and the anti-inflammatory activity of indomethacin was tested at a 10 mg/kg dose as described in the Experimental section. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

COX-1 isoform. The inhibitory activity of compounds **8 a** and **8 b** against COX-1 was 59 % and 61 %, respectively, while the inhibitory activity against COX-2 was 37 % and 28 %, respectively.

Compounds **8 a** and **b** were also tested, at a single dose of 100 mg/kg in mice for their analgesic and anti-inflammatory activities using the *p*-benzoquinone-induced writhing test [24] and carrageenan-induced hind paw edema model [25], respectively. As shown in Table 1, compounds **8 a** and **8 b** showed equal or higher analgesic activity than that of aspirin at a 100 mg/kg dose. Compound **8 a** had the highest analgesic activity (70.4 %). Additionally, compounds **8 a** and **8 b** at 100 mg/kg dose showed a reasonable anti-inflammatory activity, but the overall activity was lower than that observed with indomethacin at a 10 mg/kg dose. It is known that edema produced by carrageenan is a biphasic event and that the inhibitory effects of agents which act during the first stage of the carrageenan-induced hind paw inflammation are attributable to inhibition of chemical mediators such as histamine, serotonin and bradykinin. The second stage of the edema might be related to arachidonic acid metabolites since it is inhibited by aspirin, indomethacin and other cyclooxygenase inhibitors [26, 27]. As shown in Table 1, compounds **8 a** and **b** exhibited considerable anti-inflammatory activity in the second phase of carrageenan-induced edema (270 and 360 min). This supports our *in vitro* COX inhibitory activity results indicating that these compounds may also exert their activities *in vivo* through the inhibition of COX enzymes, thereby preventing the formation of inflammatory prostaglandins from arachidonic acid.

In conclusion, the 4-phenyl-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-3(2H)-pyridazinone derivatives represent a promising new diarylheterocyclic structure for cyclooxygenase inhibition and may provide the structural basis for the development of compounds with better analgesic and anti-inflammatory activity capable of selective COX-2 inhibition. Further analyses with derivatives of these compounds, including those containing a 1,2-disubstitution pattern around the pyridazinone ring, are currently ongoing research in our laboratory. This may allow the development of compounds with greater selectivity in inhibition of COX-1 and COX-2 activity.

Experimental

Chemical methods

2-Amino-4-chlorophenol, sodium acetate, acetic anhydride, aluminium chloride, acetyl chloride, benzaldehyde, 2-chlorobenzaldehyde, and ethyl chloroformate were purchased from Merck Co. (Germany). The starting compounds 2-acetylamino-4-chlorophenol and 2-amino-6-acetyl-4-chlorophenol were

synthesized according to previously reported procedures [28]. 2-Acetylamino-6-acetyl-4-chlorophenol was synthesized according to the previously reported procedure with modifications [29]. All other chemicals were obtained from commercial sources. COX Inhibitor Screening Assay Kits (No: 560131), including recombinant ovine COX-1 and recombinant human COX-2, were purchased from Cayman Chemical (France). The selective COX-2 inhibitor reference compound DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone) was obtained from Merck Research Laboratories (USA). 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 or $CDCl_3$. All chemical shifts were reported as δ (ppm) values. Elemental analyses were performed with a Leco-932 (C,H,N,S-O-Elemental analyzer) at the Instrumental Analysis Center of the Scientific and Technical Research Council of Turkey (Ankara, Turkey), and were within the range of 0.4 %.

2-Acetylamino-6-acetyl-4-chlorophenol (**1**)

A reaction mixture of N,N-dimethylformamide (0.22 mol) and aluminium chloride (0.8 mol), obtained in an ice-bath, was treated with acetyl chloride (0.2 mol) and stirred for 10 min. The mixture was then treated with 2-acetylamino-4-chlorophenol, heated to 120 °C and left to incubate for 2 h. The reaction mixture was poured into 1 L ice-water and 10 mL concentrated HCl was added. The formed precipitate was filtered off and recrystallized from 2-propanol to yield 96 % of **1**. ^{1}H-NMR (DMSO-d_6): δ = 12.67 (s, 1 H, O*H*); 9.56 (s, 1 H, N*H*-CO); 8.29 (d, $^4J_{5,3}$ = 2.2 Hz, 1 H, *H*-5); 7.71 (d, $^4J_{3,5}$ = 2,5 Hz, 1 H, *H*-3); 2.68 (s, 3 H, CH_3-CO); 2.14 (s, 3 H, CH_3-CO-NH); IR (KBr) cm^{-1}: ν_{max} 3265 (NH), 1670 (C=O, amide), 1641 (C=O, ketone). Anal. ($C_{10}H_{10}ClNO_3$) C, H, N.

7-Acetyl-5-chloro-2-oxo-3H-benzoxazole (**3**)

2-Amino-6-acetyl-4-chlorophenol (0.1 mol) was dissolved in 100 mL pyridine and cooled to 0 °C. Ethylchloroformate (0.2 mol) was added dropwise and the mixture was stirred at this temperature for 10 min. The reaction mixture was then incubated at 100 °C for 4 h, cooled, poured into iced-water and acidified with 115 mL concentrated HCl. The precipitate formed was filtered off and recrystallized from toluene to yield 62.8 % of **3**. ^{1}H-NMR ($CDCl_3$-DMSO-d_6): δ = 11.74 (s, 1 H, N*H*); 7.42 (d, $^4J_{6,4}$ = 2.17 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.13 (d, $^4J_{4,6}$ = 2.15 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); 2.63 (s, 3 H, CO-CH_3); IR (KBr) cm^{-1}: ν_{max} 3312–2890 (NH, lactam), 3094 (CH, aromatic), 1831 (C=O, lactam), 1658 (C=O, ketone), 1623 (C=C, aromatic). Anal. ($C_9H_6ClNO_3$) C, H, N.

3-Phenyl- and 3-(2-chlorophenyl)-1-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-2-propen-1-one (**4 a**–**b**)

A reaction mixture of 7-acetyl-5-chloro-2-oxo-3H-benzoxazole (0.01 mol) in 100 mL distilled water containing NaOH (0.025 mol) was treated with a solution of benzaldehyde (0.01 mol) or 2-chlorobenzaldehyde (0.01 mol) in 100 mL ethanol and allowed to stir at room temperature for 8 h. It was then poured into 1 L ice-water and neutralized with HCl (10 %, w/v). The formed precipitates were filtered off and recrystallized from ethanol to yield 97.4 % of **4 a** or 96.4 % of **4 b**.

Compound **4 a** ^{1}H-NMR (DMSO-d_6): δ = 11.83 (s, 1 H, N*H*); 7.60 (m, 2 H, ph*H*-2,*H*-6); 7.57 (d, ^3J = 15.49 Hz, 1 H, Ph-C*H*=); 7.49 (d, 1 H, ^3J = 15.74 Hz, =C*H*-CO); 7.39 (d, $^4J_{6,4}$ = 1.93 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.26 (m, 3 H, ph*H*-3,*H*-4,*H*-5); 7.17 (d, $^4J_{4,6}$ = 1.89 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); IR (KBr) cm^{-1}: ν_{max} 3406–3140 (NH, lactam), 3062 (CH, aromatic), 1779 (C=O, lactam), 1669 (C=O, ketone), 1594 (C=C, aromatic). Anal. ($C_{16}H_{10}ClNO_3$) C, H, N.

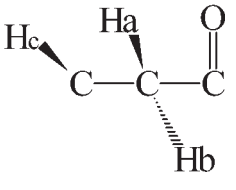
Compound **4 b** ^{1}H-NMR ($CDCl_3$-DMSO-d_6): δ = 12.2 (s, 1 H, N*H*); 8.25 (dd, $^3J_{3,4}$ = 7.06 Hz, $^4J_{3,5}$ = 2.31 Hz, 1 H, ph*H*-3); 8.23 (d, ^3J = 15.71 Hz, 1 H, Ph-C*H*=); 7.99 (d, ^3J = 15.63 Hz, 1 H, =C*H*-CO); 7.86 (d, $^4J_{6,4}$ = 2.14 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.79 (dd, $^3J_{6,5}$ = 7.57 Hz, $^4J_{6,4}$ = 1.92 Hz, 1 H, ph*H*-6); 7.70 (m, 2 H, ph*H*-4,*H*-5); 7.61 (d, $^4J_{4,6}$ = 2.12 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); IR (KBr) cm^{-1}: ν_{max} 3442–3096 (NH, lactam), 3076 (CH, aromatic), 1828–1791 (C=O, lactam), 1653 (C=O, ketone). Anal. ($C_{16}H_9Cl_2NO_3$) C, H, N.

2-Phenyl- and 2-(2-chlorophenyl)-4-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-4-oxobutyronitrile (**5 a**–**b**)

A reaction mixture of 3-phenyl- or 3-(2-chlorophenyl)-1-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-2-propene-1-one (0.03 mol) and potassium cyanide (0.075 mol) in 250 mL methanol containing 25 g glycerin was refluxed for 2.5 h. The reaction mixture was then poured into ice-water containing 5 mL concentrated HCl and the precipitate formed filtered off, washed with water and recrystallized from methanol-water or ethanol to yield 92.5 % of **5 a** or 98.33 % of **5 b,** respectively.

Compound **5 a** ^{1}H-NMR (DMSO-d_6): δ = 11.91 (s, 1 H, N*H*); 7.33 (d, $^4J_{6,4}$ = 2.14 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.28 (m, 2 H, ph*H*-2,*H*-6); 7.21 (m, 2 H, ph*H*-3,*H*-5); 7.19 (d, $^4J_{4,6}$ = 2.05 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); 7.13 (m, 1 H, ph*H*-4); 4.42 (dd, $^3J_{cb}$ = 8.99 Hz, $^3J_{ca}$ = 5.11 Hz, 1 H, *H*c); 3.67 (dd, $^3J_{ba}$ = 18.6 Hz, $^3J_{bc}$ = 9.04 Hz, 1 H, H_b); 3.47 (dd, $^3J_{ab}$ = 18.6 Hz, $^3J_{ac}$ = 5.14 Hz, 1 H, H_a); IR (KBr) cm^{-1}: ν_{max} 3279 (NH, lactam), 3060 (CH, aromatic), 2947 (CH, aliphatic), 2250 (CN), 1818–1782 (C=O, lactam), 1677 (C=O, ketone), 1618 (C=C, aromatic). Anal. ($C_{17}H_{11}ClN_2O_3$) C, H, N.

Compound **5 b** ^{1}H-NMR (DMSO-d_6): δ = 12.2 (s, 1 H, N*H*); 7.78 (m, 1 H, ph*H*-3); 7.64 (m, 1 H, ph*H*-4); 7.63 (d, $^4J_{6,4}$ = 2.25 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.51 (m, 3 H, 2-oxo-3H-benzoxazole*H*-4, ph*H*-5,*H*-6); 4.93 (dd, $^3J_{cb}$ = 8.63 Hz, $^3J_{ca}$ = 5.31 Hz, 1 H, *H*c); 4.10 (dd, $^3J_{ba}$ = 18.73 Hz, $^3J_{bc}$ = 8.7 Hz, 1 H, H_b); 3.85 (dd, $^3J_{ab}$ = 18.75 Hz, $^3J_{ac}$ = 5.32 Hz, 1 H, H_a); IR (KBr) cm^{-1}: ν_{max} 3423–3098 (NH, lactam), 3097,3062 (C-H, aromatic), 2974–2929 (CH, aliphatic), 2192 (CN), 1819–1782 (C=O, lactam), 1688 (C=O, ketone), 1627 (C=C, aromatic). Anal. ($C_{17}H_{10}Cl_2N_2O_3$) C, H, N.



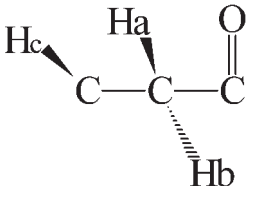
2-Phenyl- and 2-(2-chlorophenyl)-4-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-4-oxobutanoic acid (**6 a**–**b**)

A reaction mixture of 2-phenyl- or 2-(2-chlorophenyl)-4-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-4-oxobutyronitrile (0.02 mol) in 150 mL water-sulphuric acid-DMF (45:45:60) was refluxed for 4.5 h, cooled, and poured into 250 mL ice-water. The precipitate was filtered off, washed with water and recrystallized from acetonitrile to yield 58.17 % of **6 a** or 51.3 % of **6 b**.

Compound **6 a** ^{1}H-NMR (DMSO-d_6): δ = 12.6–11.5 (wide, 2 H, N*H*, COO*H*); 7.28 (d, $^4J_{6,4}$ = 2.17 Hz, 1 H, 2-oxo-3H-benzoxa-

zole*H*-6); 7.16 (d, $^4J_{4,6}$ = 2.15 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); 7.12 (m, 4H, ph*H*-2,*H*-3,*H*-5,*H*-6); 7.06 (m, 1 H, ph*H*-4); 3.90 (dd, $^3J_{cb}$ = 10.25 Hz, $^3J_{ca}$ = 4.06 Hz, 1 H, H_c); 3.60 (dd, $^3J_{ba}$ = 18.60 Hz, $^3J_{bc}$ = 10.31 Hz, 1 H, H_b); 3.12 (dd, $^3J_{ab}$ = 18.64 Hz, $^3J_{ac}$ = 4.12 Hz, 1 H, H_a); IR (KBr) cm^{-1}: ν_{max} 3191 (NH, lactam), 1776–1763 (C=O, lactam), 1698 (C=O, COOH), 1680 (C=O, ketone), 1626 (C=C, aromatic). Anal. ($C_{17}H_{12}ClNO_5$) C, H, N.

Compound **6 b** ^{1}H-NMR ($CDCl_3$-DMSO-d_6): δ = 11.80 (wide, 2 H, N*H*, COO*H*); 7.44 (d, $^4J_{6,4}$ = 2.19 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.36 (dd, 1 H, ph*H*-3); 7.31 (dd, 1 H, ph*H*-6), 7.21 (m, 2 H, ph*H*-4,*H*-5); 7.17 (d, $^4J_{4,6}$ = 2.16 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); 4.63 (dd, $^3J_{cb}$ = 9.95 Hz, $^3J_{ca}$ = 3.93 Hz, 1 H, H_c); 3.80 (dd, $^3J_{ba}$ = 18.73 Hz, $^3J_{bc}$ = 9.96 Hz, 1 H, H_b); 3.19 (dd, $^3J_{ab}$ = 18.72, $^3J_{ac}$ = 3.97, 1 H, H_a). IR (KBr) cm^{-1}: ν_{max} 3163 (NH, lactam), 3062 (CH, aromatic), 1775 (C=O, lactam), 1702 (C=O, COOH), 1680 (C=O, ketone),1622 (C=C, aromatic). Anal. ($C_{17}H_{11}Cl_2NO_5$) C, H, N.

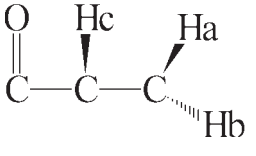


4-Phenyl- and 4-(2-chlorophenyl)-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-4,5-dihydro-3(2H)-pyridazinone (**7 a**–**b**)

2-Phenyl- or 2-(2-chlorophenyl)-4-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-4-oxobutanoic acid (0.005 mol) was dissolved in hot ethanol and then hydrazine hydrate (0.0055 mol) was added. The reaction mixture was refluxed for 4.5 h. After cooling, the mixture was poured into ice-water. The precipitate formed was filtered off and recrystallized from ethanol to yield 47.6 % of **7 a** or 57.44 % of **7 b**.

Compound **7 a** ^{1}H-NMR (DMSO-d_6): δ = 11.76 (s, 1 H, 2-oxo-3H-benzoxazoleN*H*); 11.30 (s, 1 H, pyridazinoneN*H*); 7.30 (d, $^4J_{6,4}$ = 2.09 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.21 (m, 5 H, ph*H*-2,*H*-3,*H*-4,*H*-5,*H*-6); 7.07 (d, $^4J_{4,6}$ = 2.09 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); 3.80 (dd, $^3J_{cb}$ = 10.13 Hz, $^3J_{ca}$ = 7.2 Hz, 1 H, H_c); 3.29 (dd, $^3J_{ab}$ = 17.25 Hz, $^3J_{ac}$ = 7.22 Hz, 1 H, H_a); 3.18 (m, H_b, and DMSO-d_6). IR (KBr) cm^{-1}: ν_{max} 3250–3114 (NH, 2-oxo-3H-benzoxazole, pyridazinone), 3025 (CH, aromatic), 1782–1751 (C=O, 2-oxo-3H-benzoxazole), 1677 (C=O, pyridazinone). Anal. ($C_{17}H_{12}ClN_3O_3$) C, H, N.

Compound **7 b** ^{1}H-NMR (DMSO-d_6): δ = 12.19 (wide, 1 H, 2-oxo-3H-benzoxazoleN*H*); 11.44 (s, 1 H, pyridazinoneN*H*); 7.50 (m, 1 H, ph*H*-3); 7.41 (d, $^4J_{6,4}$ = 2.16 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.36 (m, 3 H, ph*H*-4,*H*-5,*H*-6); 7.16 (d, $^4J_{4,6}$ = 2.13 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4); 4.27 (dd, $^3J_{cb}$ = 12.87 Hz, $^3J_{ca}$ = 7.48 Hz, 1 H, *H*c); 3.39 (dd, $^3J_{ab}$ = 17.15 Hz, $^3J_{ac}$ = 7.48 Hz, 1 H, H_a); 3.28 (dd, $^3J_{ba}$ = 17.10 Hz, $^3J_{bc}$ = 12.89 Hz, 1 H, H_b). IR (KBr) cm^{-1}: ν_{max} 3207–3117 (NH, 2-oxo-3H-benzoxazole, pyridazinone), 1822–1775 (C=O, 2-oxo-3H-benzoxazole), 1694 (C=O, pyridazinone). Anal. ($C_{17}H_{11}Cl_2N_3O_3$) C, H, N.



4-Phenyl- and 4-(2-chlorophenyl)-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-3(2H)-pyridazinone (**8 a**–**b**)

A reaction mixture of 4-phenyl- or 4-(2-chlorophenyl)-6-(5-chloro-2-oxo-3H-benzoxazol-7-yl)-4,5-dihydro-3(2H)-pyridazinone (0.005 mol) in 15 mL acetic acid was treated with bromine (0.0055 mol in 5 mL acetic acid) by drop wise addition over a period of 1.5 h. The mixture was stirred for an additional half hour and then poured into ice-water. The precipitate formed was filtered off and recrystallized from dimethylsulfoxide to yield 44.19 % of **8 a** or 51.8 % of **8 b**.

Compound **8 a** ^{1}H-NMR (DMSO-d_6): δ = 13.61 (s, 1 H, pyridazinoneN*H*); 11.99 (s, 1 H, 2-oxo-3H-benzoxazoleN*H*); 8.12 (s, 1 H, pyridazinone*H*-5); 7.94 (m, 2 H, ph*H*-2,*H*-6); 7.61 (d, $^4J_{6,4}$ = 2.06 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.51 (m, 3 H, ph*H*-3,*H*-4,*H*-5); 7.24 (d, $^4J_{4,6}$ = 2.08 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4). IR (KBr) cm^{-1}: ν_{max} 3192-3151 (NH, 2-oxo-3H-benzoxazole, pyridazinone), 3059 (CH, aromatic), 1767 (C=O, 2-oxo-3H-benzoxazole), 1649 (C=O, pyridazinone). Anal. ($C_{17}H_{10}ClN_3O_3$) C, H, N.

Compound **8 b** ^{1}H-NMR (DMSO-d_6): δ = 13.69 (s, 1 H, pyridazinoneN*H*); 12.04 (s, 1 H, 2-oxo-3H-benzoxazoleN*H*); 8.02 (s, 1 H, pyridazinone*H*-5); 7.61 (m, 1H, ph*H*-3); 7.58 (d, $^4J_{6,4}$ = 2.06 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-6); 7.49 (m, 3 H, ph*H*-4,*H*-5,*H*-6); 7.24 (d, $^4J_{4,6}$ = 2.03 Hz, 1 H, 2-oxo-3H-benzoxazole*H*-4). IR (KBr) cm^{-1}: ν_{max} 3203–3140 (NH, 2-oxo-3H-benzoxazole, pyridazinone), 1770 (C=O, 2-oxo-3H-benzoxazole), 1646 (C=O, pyridazinone). Anal. ($C_{17}H_9Cl_2N_3O_3$) C, H, N.

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. 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 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 H_2O 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 volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or aspirin (100 mg/kg) in 0.5 % CMC was used as the reference drug.

p-Benzoquinone-induced writhing test [22]

60 Minutes after 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 body weight). Control animals received an appropriate volume of dosing vehicle. The mice were housed individually for observation and starting the 5th min after *p*-benzoquinone injection, the total number of abdominal contractions (writhing movements) was counted for a 15 min period. 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 *et al.* [23] was used. The difference in footpad thick-

ness 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. 60 min after oral administration of test sample or dosing vehicle, each mouse was injected with a freshly prepared (0.5 mg/25 µl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) into the subplantar tissue of the right hind paw. A saline solution (25 µl) was injected into the left paw as a secondary control. Measurements were performed and evaluated as described above 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 was determined using the ANOVA test. Data with $p < 0.05$ value was considered to be significant.

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