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Synthesis of New 4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanamide Derivatives and Their Analgesic and Anti-Inflammatory Properties

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In this preliminary screening study for developing potent analgesic and anti-inflammatory compounds, eight new 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide derivatives were synthesized and screened for their analgesic and anti-inflammatory activities as well as gastric ulceration potential in tested animals. Their structures were elucidated by their IR, ^{1}H-NMR and elemental analysis. All of the compounds exhibited high analgesic and anti-inflammatory activities. None of the compounds except compound **2g** caused gastric lesions or bleeding in tested animals.

Key Words: 2-Oxo-3H-benzoxazole, butanamide, analgesic, anti-inflammatory, COX-1, COX-2.

Introduction

2-Oxo-3H-benzoxazole derivatives exhibit a broad range of biological properties[1−4] including analgesic and anti-inflammatory activity[5−10]. Among them, especially 3-substituted-2-oxo-3H-benzoxazoles are known to exhibit analgesic and anti-inflammatory properties[8,11]. It has also been reported that mannich bases of 6-acyl-2-oxo-3H-benzoxazoles resulted in compounds with potent analgesic activity[8]. Additional studies with some 3-aminoalkyl-2-oxo-3H-benzoxazole derivatives also demonstrated potent analgesic and anti-inflammatory activity, and showed that these compounds exerted their in vivo activity by inhibiting the synthesis of prostaglandin E_2[7].

For some time, our research group has been interested in studying the 2-oxo-3H-benzoxazoles for developing analgesic and anti-inflammatory compounds, and we have shown that some (2-oxo-3H-benzoxazol-3-yl)acetic acid and propanonic acid derivatives, and also some (2-oxo-3H-benzoxazol-3-yl)propanamides had potent analgesic and anti-inflammatory activity[12−15]. It was also demonstrated that the 6-acyl function

attached to the benzene portion of 2-oxo-3H-benzoxazole ring was favorable for analgesic activity in these derivatives, and that (6-acyl-2-oxo-3H-benzoxazol-3-yl)alkanoic acids possessed potent analgesic and anti-inflammatory activity with reduced gastric toxicity[16]. In general, most of the research on this class of compounds included substitutions on positions 3 and 6 of the 2-oxo-3H-benzoxazole nucleus. As a result, 2-oxo-3H-benzoxazoles bearing N-alkyl, N-acyl, N-diaminoalkyl and 6-acyl substituents were reported to have higher analgesic and anti-inflammatory activity[8,9,16,17].

Therefore, these observations prompted us to prepare the amide derivatives of (5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid (Figure 1) as potential analgesic and anti-inflammatory compounds.

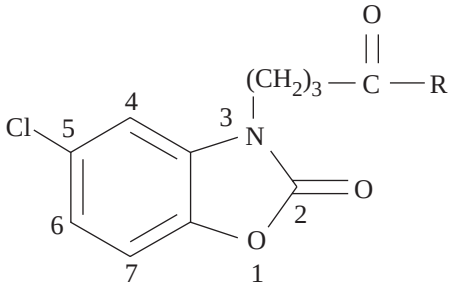


Figure 1.

Experimental

All chemicals were purchased from Merck Co. and Aldrich Co. by local vendors. COX Inhibitor Screening Assay Kits (No: 560131) including recombinant ovine COX-1 and recombinant human COX-2 were obtained from Cayman Chemical (France). 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). Melting points were determined on a electrothermal melting point apparatus and were uncorrected. 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. All chemical shifts were reported as δ (ppm) values. Elemental analyses were performed with 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).

Ethyl [4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)]butanoate

An equimolar amount of ethyl 4-chlorobutanoate was added to a solution of 0.001 mol sodium salt of 5-chloro-2-oxo-3H-benzoxazole in 30 mL DMF. The mixture was heated at 80 °C for 8h, then cooled to room temperature and poured into ice-water. The precipitate formed was filtered off and recrystallized from ethanol-water to yield 85%. mp: 42-43 °C. FT-IR (KBr) cm^{-1} 1778 (C=O ring), 1724 (C=O ester). ^{1}H-NMR (DMSO-d_6) δ 1.41 (t, 3H, -O-CH_2-C$\underline{H}_3$), 2.23 (m, 2H, -CH_2-C$\underline{H}_2$-CH_2-), 2.56 (t, 2H, -CH_2-C$\underline{H}_2$-COO-), 4.00 (t, 2H, -N-C$\underline{H}_2$-CH_2-), 4.2 (q, 2H, -O-C$\underline{H}_2$-CH_3), 7.11-7.34 (m, 3H, 2-oxo-3H-benzoxazole-H^4, H^6, H^7). Anal. ($C_{13}H_{14}ClNO_4$): C,H,N calc. 55.04, 4.97, 4.94; found. 54.83, 5.16, 4.87.

4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid (1)

0.01 Mol ethyl [4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)]butanoate was dispersed in 30 mL concentrated HCl (37%) and refluxed for 30 min. The mixture was cooled and filtered and the precipitate was crystallized

from water to yield 80% . mp: 150 °C. FT-IR (KBr) cm^{-1}: 1758 (C=O lactam), 1700 (C=O acid). ^{1}H-NMR (DMSO-d_6) δ 2.13 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.51 (t, 2H, -CH_2-C<u>H</u>$_2$-COOH), 3.92 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 6.96-7.22 (m, 3H, 2-oxo-3H-benzoxazole-H^4, H^6, H^7). Anal. ($C_{11}H_{10}ClNO_4$): C, H, N calc. 51.68, 3.94, 5.48; found. 51.92, 3.54, 5.38.

4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanamide derivatives

0.012 Mol of thionyl chloride was added to the solution of 0.01 mol 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid in dichloromethane. The solution was refluxed for 5 h and then cooled to 0 °C. The dichloromethane solution of 0.01 mol of triethylamine and 0.02 mol appropriate amine derivative was added dropwise to the solution. After the reaction was complete, dichloromethane was evaporated to dryness, acetone was added to the residue and the precipitate formed (triethylamine hydrochloride) was filtered off. Acetone was evaporated and the residue was recrystallized from appropriate solvents.

N-(2-Pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2a)

Recrystallized from ethanol to yield 60%. Mp: 102 °C. FT-IR (KBr) cm^{-1}: 1759 (C=O ring), 1683 (C=O amide), 3241 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.35 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.65 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 4.15 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 7.2-8.4 (m, 7H, Ar-H), 8.65 (s, 1H, NH). Anal. ($C_{16}H_{14}ClN_3O_3$): C, H, N calc. 57.93, 4.25, 12.67. found. 58.30, 3.89, 12.51.

N-(5-Chloro-2-pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2b)

Recrystallized from methanol to yield 56%. mp: 117 °C. FT-IR (KBr) cm^{-1}: 1792 (C=O lactam), 1667 (C=O amide), 3283 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.30 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.74 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 4.15 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 7.07-8.5 (m, 6H, Ar-H), 8.60 (s, 1H, NH). Anal. ($C_{16}H_{13}Cl_2N_3O_3$): C, H, N calc. 52.48, 3.58, 11.47 found. 52.08, 3.83, 11.42.

N-(2,6-Dichloro-3-pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2c) Recrystallized from 1-propanol to yield 51%. mp: 131 °C. FT-IR (KBr) cm^{-1}: 1766 (C=O lactam), 1672 (C=O amide), 3277 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.30 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.75 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 4.15 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 7.1-8.7 (m, 5H, Ar-H), 8.93 (s, 1H, NH). Anal. ($C_{16}H_{12}Cl_3N_3O_3$): C, H, N calc. 47.97, 3.02, 10.49 found. 48.37, 3.28, 10.51.

N-(3-Methyl-2-pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2d)

Recrystallized from ethanol to yield 49%. mp: 109 °C. FT-IR (KBr) cm^{-1}: 1772 (C=O lactam), 1670 (C=O amide), 3253 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.2 (s, 3H, -C<u>H</u>$_3$), 2.30 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.80 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 4.10 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 7.1-8.4 (m, 6H, Ar-H), 8.75 (s, 1H, NH). Anal. ($C_{17}H_{16}ClN_3O_3$): C, H, N calc. 59.05, 4.66, 12.15 found. 59.12, 4.27, 12.10.

N-(6-Methyl-2-pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2e)

Recrystallized from ethanol-water (2:1) to yield 58%. mp: 110 °C. FT-IR (KBr) cm^{-1}: 1762 (C=O lactam), 1672 (C=O amide), 3275 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.35 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.43 (s, 3H, -C<u>H</u>$_3$), 2.61 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 4.07 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 6.9-8.2 (m, 6H, Ar-H), 8.20 (s, 1H, NH). Anal. ($C_{17}H_{16}ClN_3O_3$): C, H, N calc. 59.06, 4.66, 12.15 found. 58.65, 5.05, 11.86.

N-(4-Methyl-2-pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2f)

Recrystallized from ethanol to yield 50%. mp: 113 °C. FT-IR (KBr) cm^{-1}: 1765 (C=O lactam), 1681 (C=O amide), 3241 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.2 (s, 3H, -C<u>H</u>$_3$), 2.27 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.65 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 4.07 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 6.9-8.3 (m, 6H, Ar-H), 8.91 (s, 1H, NH). Anal.

($C_{17}H_{16}ClN_3O_3$): C, H, N calc. 59.05, 4.66, 12.15 found. 59.44, 4.26, 12.20.

N-(4,6-Dimethyl-2-pyridyl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2g)

Recrystallized from ethanol to yield 56%. m.p: 124 °C. FT-IR (KBr) cm^{-1}: 1771 (C=O lactam), 1652 (C=O amide), 3515 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.05 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.23 (s, 3H, -CH_3), 2.38 (s, 3H, -CH_3), 2.45 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 3.82 (t, 2H, t, -N-C<u>H</u>$_2$-CH_2-), 6.9-7.7 (m, 5H, Ar-H), 9.50 (s, 1H, NH). Anal. ($C_{18}H_{18}ClN_3O_3$): C, H, N calc. 60.09, 5.04, 11.68 found. 60.41, 5.03, 11.74.

N-(Thiazol-2-yl)-4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide (2h)

Recrystallized from ethanol to yield 62%. mp: 147 °C. FT-IR (KBr) cm^{-1}: 1758 (C=O lactam), 1683 (C=O amide), 3496 (N-H). ^{1}H-NMR (DMSO-d_6) δ 2.20 (m, 2H, -CH_2-C<u>H</u>$_2$-CH_2-), 2.60 (t, 2H, -CH_2-C<u>H</u>$_2$-C=O), 3.95 (t, 2H, -N-C<u>H</u>$_2$-CH_2-), 7.10-7.6 (m, 5H, Ar-H), 8.10 (s, 1H, NH). Anal. ($C_{14}H_{12}ClN_3O_3S$): C, H, N calc. 49.78, 3.58, 12.44 found. 50.18, 3.62, 12.55.

Pharmacology

Male Swiss albino mice (The Animal Breeding Laboratories of the Refik Saydam Hıfzısıhha Institute, Ankara, Turkey) weighing 20-25 g were used for all in vivo experiments. The animals were housed in colony cages (six mice each), maintained on a standard pellet diet and water ad libitum and left for 2 days for acclimatization before the experimental sessions. The food was withdrawn on the day before the experiment, but they 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 were given orally to the test animals after suspending the samples in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those in 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 a reference drug.

p-Benzoquinone-induced writhing test[18]

Sixty minutes after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. The control animals received an appropriate volume of the 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 5 min after the PBQ injection. The data represent the average of the total number of writhing movements observed. Analgesic activity was expressed as the percentage change from the writhing controls.

Carrageenan-induced hind paw edema test

The method of Kasahara et al.[19] 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). The mean values of the treated groups were compared with those of the control group and analyzed using statistical methods.

Sixty minutes after the oral administration of the test sample or dosing vehicle, each mouse was injected with a freshly prepared (0.5 mg/25μl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) into the sub-plantar tissue of the right hind paw and 25 μL of saline solution into that of the left as a secondary control. Measurements were obtained and evaluated as described above every 90 min for 360 min.

Acute toxicity

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

Gastric side effects

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

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. Data with $P < 0.05$ values were considered significant.

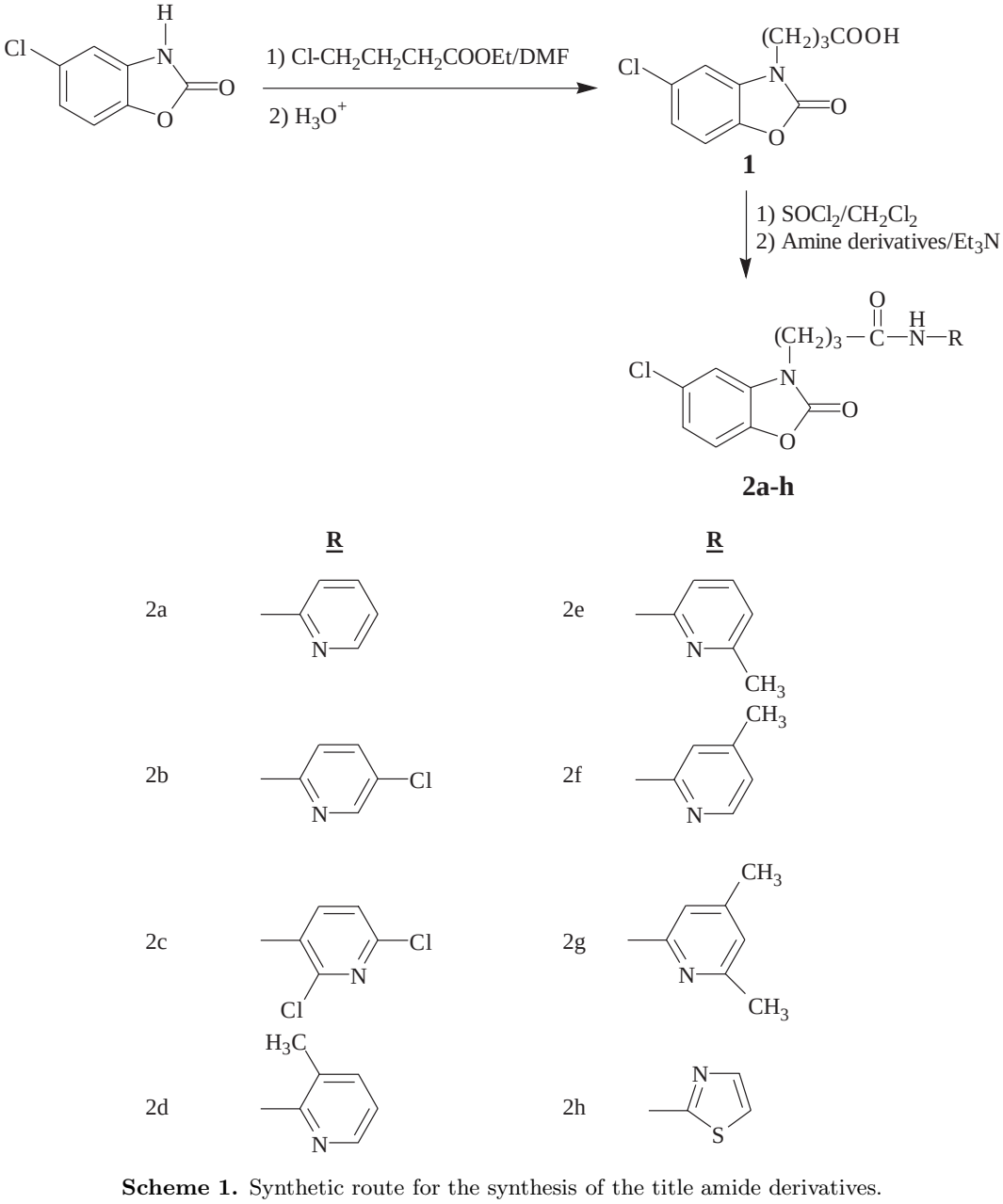
COX inhibitor screening assay

The in vitro inhibitory activity of the compounds on COX-1 and COX-2 was assayed by the use of the COX Inhibitor Screening Assay Kit including recombinant ovine COX-1 and recombinant human COX-2 isoforms (Cayman Chemical No: 560131) according to the enzyme immunoassay (EIA) protocol recommended by the supplier. Preliminary screening of both title compounds and references (non-selective COX inhibitor indomethacin[20] and selective COX-2 inhibitor DFU[21]) was performed at 10 μM concentration to determine the percent inhibition on COX-1 and COX-2 isoforms. Each drug was evaluated at 10 μM concentration in duplicate determinations. In short, the samples were incubated with recombinant COX-1 or COX-2 at 37 °C with gentle shaking for 10 min, and the reaction was then stopped by the addition of 1M HCl and submerging the tubes in a cold bath. Levels of PGE_2 in the supernatants were determined by EIA.

Results and Discussion

In the synthesis of resulting amide derivatives (**2a-h**), commercially available 5-chloro-2-oxo-3H-benzoxazole was used as the starting material. Ethyl 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoate was prepared by the reaction of the sodium salt of 5-chloro-2-oxo-3H-benzoxazole with ethyl 4-chlorobutanoate in DMF, and subsequent acid hydrolysis of this compound gave its free acid (**1**). 4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid was treated with thionyl chloride to prepare the corresponding acid chloride, which was then reacted without subsequent purification with appropriate amine derivatives to obtain resulting novel amide derivatives (**2a-h**). The preparation of the title amide derivatives is outlined in Scheme 1.

In this first screening study, the analgesic activity of the eight synthesized amide derivatives was determined by a *p*-benzoquinone-induced writhing test[18]. As seen in Table 1, the title amide derivatives **2a-h** resulted in compounds with high analgesic activities. In particular, amide derivatives having pyridine groups with methyl substitutions (compounds **2d-g**) exhibited analgesic activity higher than aspirin in the *p*-benzoquinone-induced writhing test. Meanwhile, chlorine substitutions on the pyridine part (**2b** and **2c**) were not well tolerated and resulted in lower analgesic activity. In addition, the presence of unsubstituted pyridine (**2a**) and thiazole (**2h**) substituents in the amide portion also caused lower analgesic activity than aspirin, indicating that alkyl substitution(s) on the pyridine part of the molecule was favored for analgesic activity.



Scheme 1. Synthetic route for the synthesis of the title amide derivatives.

The anti-inflammatory activity of the title amide derivatives was assessed a carrageenan-induced hind paw edema model[19]. Since the carrageenan edema has been used in the development of indomethacin, many researchers have adapted this procedure for screening potential anti-inflammatory compounds. It is known that an edema produced by carrageenan is a biphasic event, and it is reported that the inhibitory effects of agents that 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[22,23]. 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 (COX) inhibitors[22,23]. The anti-inflammatory activity of the synthesized compounds also demonstrated a parallel result with their corresponding analgesic activities in that compounds **2d-g** demonstrated the highest, activity comparable to that of indomethacin. As seen in Table 2, all compounds exhibited considerable anti-inflammatory activity during the last phase of carrageenan-induced edema, indicating that these compounds might exert their activities through the inhibition of COX enzymes, therefore preventing the formation of inflammatory prostaglandins from arachidonic acid. In addition, since the microscopic examination of the stomachs of tested animals showed no gastric lesions or bleeding with any compound except **2g** (in one of the six animals small gastric lesions were observed), this also encouraged us to test the COX inhibitory activities of the title amide derivatives.

Table 1. Analgesic activity of the synthesized compounds.

Compound	Number of Writhings ± SEM	Analgesic Activity[a] Inhibition of Writhing, %	Ratio of Gastric Lesions
Control	38.2 ± 4.29	-	0/6
2a	18.2 ± 1.54	52.3[b]	0/6
2b	19.0 ± 2.27	50.3[b]	0/6
2c	22.3 ± 1.71	41.6[b]	0/6
2d	14.3 ± 1.15	62.6[c]	0/6
2e	10.7 ± 1.20	71.9[c]	0/6
2f	14.2 ± 1.60	62.8[c]	0/6
2g	15.0 ± 1.29	60.7[c]	1/6
2h	22.2 ± 1.71	41.9[b]	0/6
Aspirin	16.3 ± 2.26	57.3[b]	2/6

[a] Analgesic activities of the compounds and aspirin were tested at 100 mg/kg dose as described in the Experimental part. [b] $P < 0.01$ [c] $P < 0.001$.

Based on these results, compounds **2a** and **d-g** were considered to have potent analgesic and anti-inflammatory activity at 100 mg/kg doses, and were selected for further studies to investigate their inhibitor activities on COX enzymes by in vitro COX inhibitor screening assay. As known, cyclooxygenases exist in two isoforms, one constitutive (COX-1), which is involved in many physiological processes including gastric cytoprotection; and the other inducible (COX-2), which appears to play a major role in the production of prostaglandins associated with the inflammatory process[24]. After the discovery that the most currently used non-steroidal anti-inflammatory drugs (NSAIDs) exhibit their therapeutic and side effects through the non-selective inhibition of COX enzymes (COX-1 and COX-2), research on developing selective COX-2 inhibitors has gained a lot of attention[25,26]. In addition, the differential tissue distribution of COX-1 and

COX-2 provides a rationale for the development of selective COX-2 inhibitors as anti-inflammatory and analgesic agents that lack the GI liabilities exhibited by currently marketed NSAIDs.

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 resulted in considerable inhibition at 10 μM neither in COX-2 nor in COX-1 (Table 3). Therefore, we conclude that these 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide derivatives do not exert their analgesic or anti-inflammatory activities through COX inhibition and that other mechanisms might be involved. The mechanism that underlies the analgesic and anti-inflammatory activities of the resulting amide derivatives are currently under investigation in our laboratory.

Table 2. Anti-inflammatory activity of the synthesized compounds.

Compound	Anti-inflammatory activity[a] Thickness of Edema ± SD (Inhibition %)			
	90 min	180 min	270 min	360 min
2a	33.2 ± 2.73 (12.2)	36.7 ± 2.33 (21.6)	34.5 ± 1.20 (40.3)[b]	37.2 ± 2.47 (44.6)[c]
2b	33.5 ± 1.71 (11.4)	38.3 ± 1.65 (18.2)	38.5 ± 1.71 (33.4)[b]	38.0 ± 1.67 (43.5)[c]
2c	31.3 ± 2.45 (17.2)	36.8 ± 2.32 (21.4)	38.8 ± 3.61 (32.9)[d]	38.0 ± 2.92 (43.5)[c]
2d	34.7 ± 1.59 (8.2)	37.3 ± 2.01 (20.3)	40.2 ± 0.95 (30.4)[b]	32.7 ± 1.45 (51.3)[c]
2e	34.7 ± 2.86 (8.2)	34.5 ± 1.99 (26.3)	34.7 ± 2.40 (39.9)[b]	27.7 ± 1.43 (58.8)[c]
2f	33.0 ± 3.28 (12.7)	33.5 ± 2.26 (28.4)[d]	34.2 ± 2.36 (40.8)[b]	32.8 ± 1.4 (51.2)[c]
2g	31.0 ± 2.34 (17.9)	31.7 ± 2.63 (32.3)[d]	37.0 ± 1.86 (35.9)[b]	34.5 ± 2.55 (48.7)[c]
2h	36.5 ± 2.59 (3.4)	40.2 ± 2.98 (14.1	41.0 ± 2.76 (29.1)[d]	43.8 ± 4.39 (34.8)[b]
Indomethacin	26.5 ± 2.06 (29.9)[d]	27.3 ± 1.91 (41.7)[b]	27.2 ± 2.76 (52.9)[c]	29.0 ± 1.07 (56.8)[c]
Control	37.8 ± 3.57	46.8 ± 5.39	57.8 ± 5.15	67.2 ± 3.69

[a]Anti-inflammatory activities of the compounds and indomethacin were tested at 100 mg/kg dose and 10 mg/kg dose, respectively, as described in the Experimental part. [b] $P < 0.01$ [c] $P < 0.001$ [d] $P < 0.05$.

Table 3. Inhibitory activity of selected active compounds on COX-1 and COX-2.

Compound	COX-1 inhibition, %	COX-2 inhibition, %
2a	6.5	8.7
2d	11	15
2e	20	35
2f	9.8	0
2g	26	34.5
Indomethacin	69	78
DFU	0	86

Inhibitor activity of the selected compounds at 10 μM concentration on COX-1 and COX-2 were tested using the COX inhibitor screening assay as described in the Experimental part. Indomethacin and DFU at 10 μM were used as nonselective COX inhibitor and selective COX-2 inhibitor references, respectively, in the assays.

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