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Studies on Novel 7-Acyl-5-chloro-2-oxo-3*H*-benzoxazole Derivatives as Potential Analgesic and Anti-Inflammatory Agents

In this study, a series of (7-acyl-5-chloro-2-oxo-3*H*-benzoxazol-3-yl)alkanoic acid derivatives were synthesized and evaluated for their analgesic and anti-inflammatory activities by using the *p*-benzoquinone-induced writhing test and the carrageenan hind paw edema model, respectively. Acetic acid-induced peritoneal capillary permeability test and serotonin-induced hind paw edema test were also employed for the most active compounds. The test results indicated that (7-acyl-2-oxo-3*H*-benzoxazol-3-yl)alkanoic acids (Compounds **6 a–c, 8 a–c, 10 a–c**) were equally or more potent analgesic and anti-inflammatory agents than aspirin and indomethacin respectively. The compounds **8 a** and **8 c**, but not **8 b** which have the longest carbon chain on alkanoic acid moiety did not induce gastric lesion in mice.

Keywords: 7-Acyl-2-oxo-3*H*-benzoxazoles; Alkanoic acids; Propionitriles; QSAR; Analgesic and Anti-inflammatory Activity

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Introduction

Although there are many nonsteroidal anti-inflammatory drugs (NSAIDs) on the market, a considerable amount of research focusses on these drugs due to the serious side effects of NSAIDs, i.e. gastrointestinal irritation and kidney damage. Therefore, investigations on new anti-inflammatory agents are still a challenge. Recently, in efforts to synthesize anti-inflammatory drugs without or with minimal side effect, 2-oxo-3*H*-benzoxazoles have emerged as a promising group of substances.

Since the pioneering discovery of the hypnotic properties of 2-oxo-3*H*-benzoxazole over the last twenty-five years, the 2-oxo-3*H*-benzoxazole ring has become an important building block in medicinal chemistry and led to the discovery of a number of derivatives endowed with antispasmodic, antitubercular, antibacterial, antimicrobial, antifungal, and normolipemic effects [1-7]. Anti-inflammatory, analgesic, and antipyretic activity have been described in 2-oxo-3*H*-benzoxazole and its 6-acyl derivatives. Studies on analgesic activity of 2-oxo-3*H*-benzoxazole was first reported by Close et al. [8] showing that substitution of alkyl, allyl, and acetyl groups at position 3 and alkyl and halogen groups at position 6 of 2-oxo-3*H*-benzoxazole **(I)** derivatives possess equal or higher analgesic activity than aspirin.

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As already demonstrated in 1973, 6-acyl-2-oxo-3*H*-benzoxazole derivatives (**II**, **III**) prepared by Bonte et al. [9] showed higher analgesic activity than that of aspirin.

Renard et al. [10] showed that the analgesic and anti-inflammatory activity of (6-acyl-2-oxo-3*H*-benzoxazol-3-yl)alkanoic acid was higher than those of aspirin and ketoprofen. Following this study, a numbers of (6-acyl-2-oxo-3*H*-benzoxazol-3-yl)alkanoic acid and their ester and amide derivatives were synthesized and their high analgesic and anti-inflammatory activities were demonstrated [11–16].

Although many reports on 2-oxo-3*H*-benzoxazoles involved the synthesis and biological activity of 3-, 5-, and 6-acyl-2-oxo-3*H*-benzoxazole derivatives, there is no published report on the synthesis and biological activity of 7-acyl-5-chloro-2-oxo-3*H*-benzoxazoles and the other 7-acyl derivatives of 2-oxo-3*H*-benzoxazole in the literature. Therefore, we aimed to synthesize 7-benzoyl derivatives of 5-chloro-2-oxo-3*H*-benzoxazole and their alkanoic acid derivatives which possess a better structural analogy with well known anti-inflammatory drugs, such as indomethacin, than 6-acyl-2-oxo-3*H*-benzoxazole derivatives.

Results and discussion

Chemistry

The acylation of the 2-oxo-3*H*-benzoxazole ring was investigated by various aromatic electrophilic substitution

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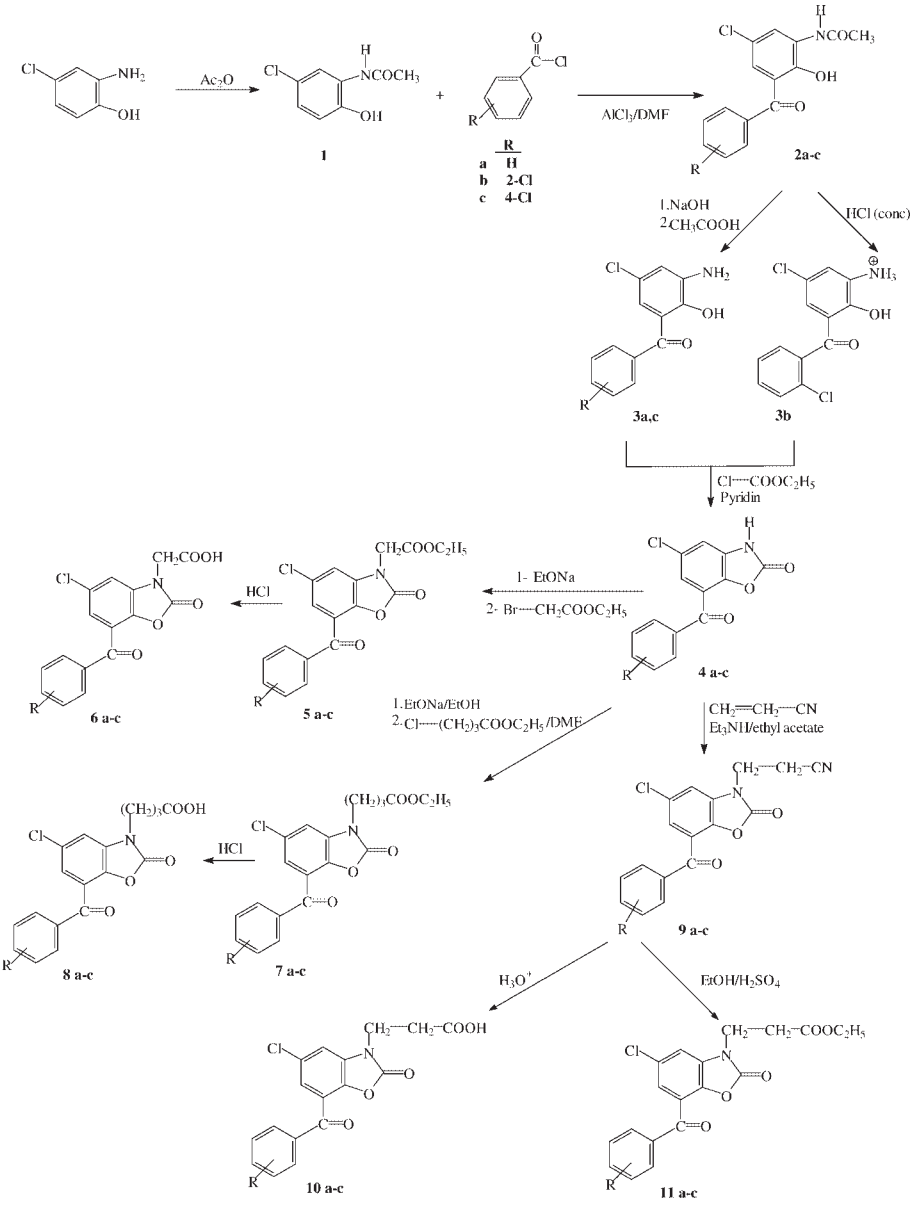
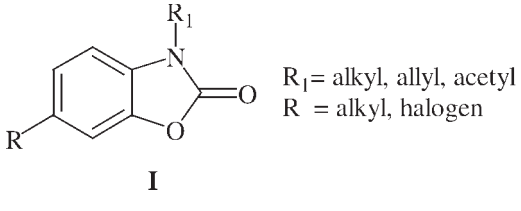


Scheme 1. Synthetic route leading to the compounds 10 a–c and 11 a–c.

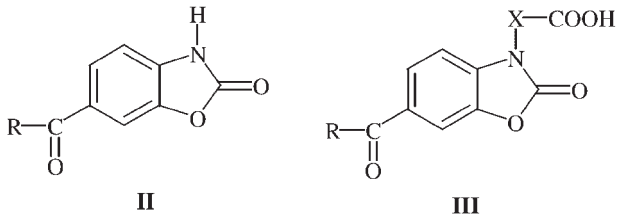
conditions. Acyl derivatives of 2-oxo-3*H*-benzoxazoles were obtained in good yields with aliphatic, aromatic, and arylaliphatic carboxylic acids in the presence of polyphosphoric acid (PPA) [9]. Additionally, C-acylation of 2-oxo-3*H*-benzoxazole under various Friedel-Crafts reaction conditions using carboxylic acids in PPA or acid halides or anhydrides in the presence of $AlCl_3$-DMF or $AlCl_3$-CS_2 was published in the literature [17]. The regioselectivity of the C-acylation of 2-oxo-3*H*-benzoxazole was also reported; the electrophilic aromatic substitution always led to 6-acyl derivatives and the structures of the acyl derivatives were elucidated by ^{1}H-NMR, IR, X-ray diffraction, and theoretical calculations [17–19]. 5-Acyl-



I

Figure 1. Derivative **I**.

2-oxo-3*H*-benzoxazole derivatives were synthesized by the C-acylation of 2-(N-acetylamino)phenol at position 4 without any isomer and by-product [20] and the subsequent cyclization of this acyl derivative to 2-oxo-3*H*-



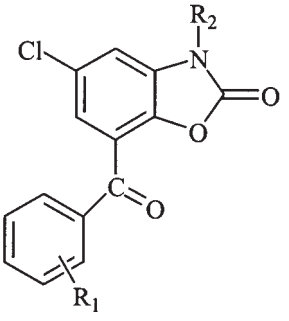
II III

X = CH_2, CH_2CH_2, CHMe
R = C_6H_5, (2-Cl)C_6H_4, (4-Cl)C_6H_4, (4-OCH_3)C_6H_4

Figure 2. Derivatives **II** and **III**.

benzoxazole ring [17]. Based on these reports, the impossibility of preparing 7-acyl-2-oxo-3*H*-benzoxazole derivatives using 2-oxo-3*H*-benzoxazole as a starting material prompted us to synthesize 7-benzoyl-2-oxo-3*H*-benzoxazole by using 2-oxo-3*H*-benzoxazole precursor, such as 2-acetylamino-4-chlorophenol in which the amino group was protected by acetyl group (Scheme 1). Since the phenolic hydroxy group has a better electron donating effect than the acetyl amino group and the position 4 is closed by the chlorine substituent, 2-acetylamino-5-chloro-6-benzoylphenol derivatives (compounds **2 a–c**) were synthesized in good yields and

Table 1. Yields, melting points, and crystallization solvents of the synthesized compounds.



Compound	R_1	R_2	Yield %	MP. °C	Crystallization Solvent
4 a	H	H	74.00	183-84	Benzene
4 b	2-Cl	H	64.00	169–70	Toluene
4 c	4-Cl	H	71.00	233–34	Toluene
5 a	H	$CH_2COOC_2H_5$	78.20	128–29	Ethanol
5 b	2-Cl	$CH_2COOC_2H_5$	57.33	119–20	Ethanol/Water
5 c	4-Cl	$CH_2COOC_2H_5$	77.30	137–39	Ethanol/Water
6 a	H	CH_2COOH	62.80	209–12	Benzene
6 b	2-Cl	CH_2COOH	61.32	190–91	Toluene
6 c	4-Cl	CH_2COOH	55.30	224–26	Toluene
7 a	H	$(CH_2)_3COOC_2H_5$	82.50	106–07	Ethanol
7 b	2-Cl	$(CH_2)_3COOC_2H_5$	78.10	104–05	1-Propanol
7 c	4-Cl	$(CH_2)_3COOC_2H_5$	76.38	185–86	1-Propanol
8 a	H	$(CH_2)_3COOH$	67.30	213–15	Acetonitrile
8 b	2-Cl	$(CH_2)_3COOH$	48.00	142–43	Toluene
8 c	4-Cl	$(CH_2)_3COOH$	48.00	214–19	Toluene
9 a	H	$(CH_2)_2CN$	65.70	182–83	Ethanol
9 b	2-Cl	$(CH_2)_2CN$	72.00	134–35	Ethanol
9 c	4-Cl	$(CH_2)_2CN$	62.80	213–14	Ethanol
10 a	H	$(CH_2)_2COOH$	63.90	168–70	Benzene
10 b	2-Cl	$(CH_2)_2COOH$	66.00	167–69	Toluene
10 c	4-Cl	$(CH_2)_2COOH$	43.60	214–15	Toluene
11 a	H	$(CH_2)_2COOC_2H_5$	51.20	84–85	n-Hexane
11 b	2-Cl	$(CH_2)_2COOC_2H_5$	51.20	112–13	n-Hexane
11 c	4-Cl	$(CH_2)_2COOC_2H_5$	55.80	132–33	n-Hexane

without any isomer by the reaction of 2-acetylamino-4-chlorophenol (**1**) with benzoylchloride derivatives in the presence of $AlCl_3$-DMF prepared by a method published previously [21]. Subsequently, these intermediates were hydrolyzed to aromatic amine derivatives (compounds **3 a–c**) in concentrated HCl or sodium hydroxide solution. 2-oxo-3*H*-benzoxazoles can be prepared by cyclization of 2-aminophenol derivatives as a source of carbonyl group such as urea [22], N,N′-carbonyldiimidazole [23], or ethyl chloroformate [24] in different reaction conditions.

In this study, 7-benzoyl-5-chloro-2-oxo-3*H*-benzoxazole derivatives (**4 a–c**) were obtained in good yields by the cyclization of **3 a–c** using ethyl chloroformate in pyridine under mild conditions. The sodium salts of compound **4 a–c** were reacted with ethyl 2-bromoacetate and ethyl 4-chlorobutanoate to prepare compound **5 a–c** and **7 a–c**, respectively. These ester derivatives were hydrolyzed to corresponding free acids (compound **6 a–c** and **8 a–c**) in conc. HCl.

Instead of nucleophilic substitution reaction, Michael-type addition of compound **4 a–c** to acrylonitrile was applied to prepare propionitrile derivatives (**9 a–c**). Finally, these propionitrile derivatives were converted to corresponding acids (**10 a–c**) and ethyl ester derivatives (**11 a–c**) by treatment with aqueous acid and absolute ethanol in the presence of acid catalyst, respectively.

The structure and the other properties of the title compounds are given in Table 1.

Pharmacology

Analgesic activities of the resulting compounds were investigated by *p*-benzoquinone-induced writhing test [25] which is a well established method of testing the analgesic activity of compounds and sufficiently sensitive to detect the effect of analgesics which are less active than aspirin. However, the test can not distinguish between narcotic and nonnarcotic drugs and may even give a positive result for compounds which are not regarded as clinically useful analgesics [26–28]. Therefore, we also applied the acetic acid-induced peritoneal capillary permeability test in mice which is a relatively reliable way of testing nonnarcotic analgesics [29] for those carboxylic acids that are more active compounds than the other derivatives in the *p*-benzoquinone-induced writhing test.

Anti-inflammatory activities of the compounds were assessed by utilizing carrageenan-induced hind paw edema model [30]. Since the carrageenan edema has been used in the development of indomethacin, many researchers adapted this procedure for screening potential anti-inflammatory compounds. It is well known that an edema produced by carrageenan is a biphasic event and it was 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 [31, 32]. On the other hand, the second stage of the edema might be related to the arachidonic acid metabolites, since inhibition occurs by aspirin, indomethacin, and other cyclooxygenase inhibitors [31, 32]. Therefore, in addition to edema measurements every 90 min during 360 min by the carrageenan-induced hind paw edema test, serotonin-induced edema test [30] was also applied to explore the stage of inflammation on which the compounds exhibited their activity. Moreover, acute toxicity and gastric ulcerogenic effects of the title compounds were investigated in test animals.

Among the synthesized compounds, anti-inflammatory and analgesic activity of nonsubtituted derivatives on position 3 (**4 a–c**) were found weaker than those bearing cyanoalkyl, ester, or acid residues on this position at 100 mg/kg dose.

A comparable analgesic activity to aspirin was observed for 7-acyl-2-oxo-3*H*-benzoxazole derivatives (**9 a–c**) bearing a cyanoethyl group on position 3. It is noteworthy that chlorine (2-Cl or 4-Cl) substitution on the acyl group did not induce any remarkable change in the activity of these derivatives.

At 100 mg/kg dose level, the highest anti-inflammatory and analgesic activity was observed, as expected, by the derivatives bearing alkanoic acid residue at position 3 (**6 a–c**, **8 a–c**, **10 a–c**) (Table 2). Therefore, these compounds were further tested in higher (200 mg/kg) and lower (50 mg/kg) dose levels (Table 3). Moreover, inhibitory effects on serotonin-induced hind paw edema and acetic acid-induced capillary permeability (modified Whittle test) are shown in Table 4.

Among the nonsubstituted benzoyl derivatives (**6 a**, **8 a**, **10 a**) higher analgesic and anti-inflammatory activities were observed in connection with a longer chain length in alkanoic acid residue which also coincided with the Whittle test results. Interestingly, in spite of the gastric lesion inducing effect of **6 a**, propanoic acid (**10 a**) and butanoic acid (**8 a**) derivatives were found free of such a side effect. However, aspirin which is known to induce gastric ulceration was also found free of gastric lesion effect at a dose of 100 mg/kg under our test conditions.

2-Chlorobenzoyl derivatives (**6 b**, **8 b**, **10 b**) showed the highest inhibitory activity in all analgesic and anti-inflammatory tests employed in the present study. It is noteworthy that the incidence of gastric damage increased significantly for these derivatives. Although the gastric lesion incidence was established for these derivatives, compound **10 b** showed a smaller gastric lesion-induc-

Table 2. Effects of compounds on carrageenan-induced hind paw edema model and PBQ-induced writhings.

Compound	Anti-inflammatory Activity Thickness of the edema ± SEM (Inhibition, %)				Analgesic Activity Number of stretching (Inhibition, %)	Gastric-ulcerogenic Effect
	90 min	180 min	270 min	360 min		
Control	37.5 ± 2.74	51.3 ± 2.88	60.7 ± 2.64	60.3 ± 2.55	37 ± 4.89	0/6
4a	33 ± 4.1 (12.0)	46.3 ± 3.02 (9.7)	55.2 ± 2.4 (9.1)	54 ± 4.25 (10.4)	24.2 ± 1.54 (34.6)*	0/6
4b	31.8 ± 2.15 (15.2)	44.7 ± 2.99 (12.9)	51 ± 3.62 (15.9)	50.5 ± 2.54 (16.3)*	20.7 ± 2.74 (44.2)*	1/6
4c	31.2 ± 3.89 (16.8)	43.3 ± 2.64 (15.6)	50.3 ± 3.58 (17.1)*	48.3 ± 2.25 (19.9)**	20.2 ± 1.58 (45.4)**	0/6
5a	32 ± 3.52 (14.7)	41.3 ± 3.20 (19.6)	47.0 ± 2.88 (22.5)	44.6 ± 1.94 (25.9)*	19.2 ± 1.96 (48.1)**	0/6
5b	30.6 ± 2.67 (18.4)	40.2 ± 2.4 (21.6)	42.9 ± 1.8 (29.3)*	39.4 ± 1.65 (34.6)**	14.7 ± 1.28 (60.3)*	0/6
5c	30.6 ± 2.9 (18.4)	38.7 ± 2.81 (24.5)	42 ± 1.85 (30.8)*	40.1 ± 1.97 (33.5)**	24.2 ± 1.54 (34.6)*	0/6
6a	30.8 ± 3.7 (17.9)	42.8 ± 3.18 (16.6)	47 ± 2.75 (22.6)**	42.5 ± 2.5 (29.5)***	15.58 ± 1.21 (58.1)**	2/6
6b	26 ± 1.88 (30.7)**	35.3 ± 2.26 (31.2)**	26.5 ± 3.29 (56.3)***	26.2 ± 3.29 (56.6)***	9.14 ± 1.22 (75.3)***	1/6
6c	32.2 ± 2.27 (14.1)	41.5 ± 3.18 (19.1)*	50.2 ± 3.18 (17.3)*	49.5 ± 3.52 (17.9)*	23.4 ± 1.94 (36.6)*	0/6
7a	34 ± 2.06 (9.3)	45.6 ± 1.95 (11.2)	49.7 ± 1.63 (18.1)	48.3 ± 1.74 (19.9)	18.7 ± 2.12 (49.5)**	0/6
7b	33.6 ± 2.5 (10.5)	45.8 ± 1.94 (10.8)	46.1 ± 2.46 (24.1)	45.3 ± 2.12 (24.9)*	16.3 ± 1.78 (55.9)**	1/6
7c	34 ± 2.54 (9.3)	44 ± 2.1 (14.3)	43.5 ± 1.25 (28.3)*	43.5 ± 1.5 (27.8)*	19.8 ± 1.01 (46.5)**	0/6
8a	28.3 ± 3.37 (24.5)	38.3 ± 3.65 (25.3)*	35.8 ± 3.54 (41.0)***	34.2 ± 3.34 (43.3)***	11.83 ± 0.76 (68.0)***	0/6
8b	26.8 ± 2.92 (28.5)*	36.8 ± 3.24 (28.3)**	31.7 ± 4.04 (47.8)***	30.7 ± 2.04 (49.1)***	8.42 ± 1.51 (77.2)***	2/6
8c	29 ± 3.44 (22.7)	37.5 ± 3.24 (26.9)**	40 ± 3.2 (34.1)***	35.3 ± 3.31 (41.5)***	12.6 ± 1.24 (65.9)***	0/6
9a	33.7 ± 2.41 (10.0)	43.9 ± 1.65 (14.3)	44.9 ± 1.25 (25.9)*	42.1 ± 1.57 (30.2)**	14 ± 1.63 (62.2)**	2/6
9b	32.6 ± 4.18 (12.9)	42.1 ± 2.82 (18.0)	45.8 ± 2.68 (24.6)	41.7 ± 1.6 (30.8)**	13.2 ± 0.7 (64.3)***	0/6
9c	30.3 ± 2.14 (19.1)	44.6 ± 2.46 (13.1)	47 ± 2.52 (22.5)	43.1 ± 1.85 (28.5)*	13.3 ± 1.17 (64.1)***	0/6
10a	30.3 ± 1.99 (19.2)	40.8 ± 3.04 (20.5)*	40.7 ± 3.77 (32.9)**	41 ± 2.56 (32.0)***	13.8 ± 2.14 (62.8)**	0/6
10b	25 ± 2.38 (33.3)**	29 ± 2.89 (43.5)***	20.8 ± 2.02 (65.7)***	25.5 ± 1.98 (57.7)***	7.17 ± 1.06 (80.6)***	1/6

Table 2. (continued).

Compound	Anti-inflammatory Activity Thickness of the edema ± SEM (Inhibition, %)				Analgesic Activity Number of stretching (Inhibition, %)	Gastric-ulcerogenic Effect
	90 min	180 min	270 min	360 min		
10 c	30.7 ± 3.89 (18.1)	41.3 ± 3.43 (19.5)*	45.5 ± 2.83 (25.0)**	47.3 ± 4.09 (21.6)*	18.6 ± 2.06 (49.7)**	0/6
11 a	33.4 ± 2.55 (11.0)	44.8 ± 2.41 (12.7)	51.2 ± 2.45 (15.6)	51 ± 2.02 (15.4)	20.2 ± 2.09 (45.4)	0/6
11 b	36.3 ± 3.66 (3.2)	43.5 ± 2.81 (15.3)	47.6 ± 2.11 (21.5)	50.6 ± 1.6 (16.2)	26.2 ± 1.99 (29.2)	0/6
11 c	30.1 ± 4.21 (19.8)	41 ± 3.64 (20.2)	43.8 ± 2.85 (25.8)	44 ± 2.3 (27.0)*	13.3 ± 1.26 (64.1)***	1/6
Indomethacin	26.3 ± 2.33 (29.8)*	26.3 ± 2.78 (48.7)***	25.3 ± 3.28 (58.3)***	29.5 ± 3.25 (51.1)***	–	2/6
Aspirin					13 ± 1.79 (64.9)***	0/6

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table 3. Carrageenan-induced hind paw edema model test results.

Compound	Dose. mg/kg. Per os	Inhibition of swelling (%)				Analgesic Activity % inhibition of writhings	Gastric-ulcerogenic Effect
		90 min	180 min	270 min	360 min		
6 a	50	12.4	11.8	11.8	17.6	27.5**	1/6
	100	17.9	16.6	22.6**	29.5***	58.1**	2/6
	200	32.8	35.9	34.3	33.5**	62.3***	3/6
6 b	50	16.2	17.6	18.4	22.1	38.7***	0/6
	100	30.7**	31.2**	56.3***	56.6***	75.3***	1/6
	200	33.1*	38.2***	42.7***	50.2***	76.9***	3/6
6 c	50	12.9	11.2	13.7	14.9	15.0	0/6
	100	14.1	19.1*	17.3*	17.9*	36.6*	0/6
	200	16.8	24.9*	20.8***	22.1**	46.3***	0/6
8 a	50	12.9	12.5	12.2	18.3	35.6***	0/6
	100	24.5	25.3*	41.0***	43.3***	68.0***	0/6
	200	41.5**	38.5***	40.9***	44.7***	70.4***	0/6
8 b	50	11.2	14.4	16.9	19.6	40.5***	0/6
	100	28.5*	28.3**	47.8***	49.1***	77.2***	2/6
	200	44.2**	44.1***	43.6***	46.3***	75.0***	3/6
8 c	50	12.2	11.2	15.2	16.8	32.2**	0/6
	100	22.7	26.9**	34.1***	41.4***	65.9***	0/6
	200	37.5**	38.2***	41.4***	40.2***	70.6***	0/6

Table 3. (continued).

Compound	Dose. mg/kg. Per os	Inhibition of swelling (%)				Analgesic Activity % inhibition of writhings	Gastric-ulcerogenic Effect
		90 min	180 min	270 min	360 min		
10 a	50	12.2	12.9	13.7	17.9	26.4**	0/6
	100	19.2	20.5*	32.9**	32.0***	62.8**	0/6
	200	27.4*	25.1**	30.8***	35.8***	64.8***	0/6
10 b	50	10.5	17.8	20.3	26.6	45.1***	0/6
	100	33.3**	43.5***	65.7***	57.7***	80.6***	1/6
	200	35.2*	42.8***	50.6***	48.7***	78.8***	2/6
10 c	50	11.7	12.9	15.6	15.9	22.9*	0/6
	100	18.1	19.5*	25.0**	21.6*	49.7**	0/6
	200	24.1	22.2	27.2	34.2**	58.3***	0/6
Indomethacin	10	29.8*	48.7***	58.3***	51.1***	–	2/6
ASA	100					64.9***	0/6
ASA	200					69.4***	2/6
ASA	50					31.3**	0/6

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table 4. Serotonin-induced paw edema model and acetic acid-induced capillary permeability test results.

Com-pound	Dose. mg/kg. Per os	Swelling in thickness (× 10^{-2} mm) ± SEM (% inhibition)						Dye leakage ± SEM (% inhibition)
		0 min	6 min	12 min	18 min	24 min	30 min	
Control		4.0 ± 0.4	11.2 ± 1.7	19.7 ± 1.2	30.2 ± 2.6	35.7 ± 3.1	40.2 ± 2.7	8.37 ± 0.43
6 a	100	4.3 ± 0.3	12.7 ± 1.3	17.7 ± 1.1 (10.2)	26.2 ± 2.1 (13.2)	31.2 ± 2.1 (12.6)	33.3 ± 2.6 (17.2)	6.60 ± 0.44 (21.9)*
6 b	100	3.7 ± 0.3 (8.3)	11.7 ± 1.2	17.0 ± 1.9 (13.7)	24.3 ± 1.5 (19.5)	28.3 ± 0.9 (20.7)*	30.5 ± 2.5 (24.1)*	4.73 ± 0.31 (43.4)***
6 c	100	3.5 ± 0.4 (12.5)	17.7 ± 1.9	25.2 ± 2.3	25.3 ± 2.8 (16.2)	29.2 ± 2.0 (18.2)	35.0 ± 2.1 (12.9)	7.06 ± 0.72 (15.7)
8 a	100	3.5 ± 0.4 (12.5)	10.7 ± 2.0 (4.5)	17.2 ± 1.9 (12.7)	25.3 ± 2.7 (16.2)	29.3 ± 2.1 (17.9)	33.8 ± 2.6 (15.9)	5.70 ± 0.41 (31.9)***
8 b	100	4.0 ± 0.51	1.8 ± 1.2	17.5 ± 1.2 (11.2)	25.7 ± 1.9 (14.9)	31.7 ± 1.3 (11.2)	34.5 ± 1.3 (14.2)	5.19 ± 0.38 (38.0)***
8 c	100	4.2 ± 0.5	12.7 ± 1.11	8.3 ± 1.9 (7.1)	26.0 ± 1.6 (13.9)	31.2 ± 2.7 (12.6)	33.2 ± 2.6 (17.4)	5.53 ± 0.34 (33.9)***
10 a	100	3.8 ± 0.5 (4.3)	12.5 ± 1.9	16.5 ± 1.7 (16.2)	25.5 ± 2.2 (15.6)	29.3 ± 2.5 (17.9)	35.3 ± 2.5 (12.2)	6.10 ± 0.29 (27.1)***
10 b	100	3.7 ± 0.5 (8.3)	11.8 ± 1.2	15.3 ± 1.5 (22.3)*	22.8 ± 3.7 (24.5)	27.5 ± 1.6 (22.9)*	31.5 ± 1.8 (21.6)*	4.45 ± 0.32 (46.8)***
10 c	100	4.5 ± 0.6	12.8 ± 1.2	17.7 ± 2.4 (10.2)	27.8 ± 1.9 (7.9)	30.5 ± 2.3 (14.6)	33.5 ± 2.4 (16.7)	6.02 ± 0.23 (28.0)***
Indo-methacin	10	4.0 ± 0.4	11.7 ± 1.2	15.2 ± 1.3 (22.8)*	22.2 ± 0.9 (26.5)*	24.8 ± 1.2 (30.5)**	26.2 ± 1.8 (34.8)**	4.34 ± 0.27 (48.1)***

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

ing effect than compounds **6 b** and **8 b**. In addition, especially among the synthesized compounds, the highest and significant activity against serotonin-induced edema was shown by **6 b** and **10 b**.

A less remarkable analgesic and anti-inflammatory activity was observed for 4-chlorobenzoyl derivatives (**6 c**, **8 c** and **10 c**) which also correlated with the results of peritoneal capillary permeability test, although, even the highest doses of these derivatives did not induce any apparent gastric toxicity. The activity of these compounds increased considerably upon increasing alkanoic acid chain length. In fact, this kind of conclusion may be drawn for the afore-mentioned other derivatives as well. If a comparison would be made between the butanoic acid derivatives, **8 b** caused gastric damage while the other two compounds **8 a** and **8 c** did not.

In general, the following conclusions may be drawn:

1. All of the compounds exhibited their analgesic and anti-inflammatory activity at the second stage of the inflammation according to the test results. Higher analgesic and anti-inflammatory activity of 2-chlorobenzoyl derivatives (**6 b**, **8 b**, and **10 b**) may be attributed to the effect of chlorine substituent that forces the phenyl ring to take a position in a different plane than the 2-oxo-3*H*-benzoxazole ring causing a structure that resembles indomethacin derivatives. This effect of the chlorine substituent might explain the activity observed with these compounds.

2. It should be noticed that the analgesic and anti-inflammatory activities of alkanoic acid derivatives bearing benzoyl and 4-chlorobenzoyl substituents (**6 a**, **6 c**, **8 a**, **8 c**, **10 a**, and **10 c**) were enhanced, apparently by elongating the alkanoic acid chain while especially propanoic acid (**10 a** and **10 c**) and butanoic acid derivatives (**8 a** and **8 c**) were devoid of any gastric lesion.

According to the results of in vivo studies conducted in the present study, the analgesic and anti-inflammatory activities of **8 a** and **8 c** were comparable to that of known drugs, i.e. aspirin and indomethacin, without inducing any visible gastric damage. Further detailed studies are under way in order to investigate the effect of the synthesized alkanoic acid derivatives on chronic inflammatory test models, as well as antipyretic effect, and COX-2 selective inhibitory effects.

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Experimental

Chemistry

All chemicals were obtained from Aldrich (Germany) or Merck Chemical Co. (Darmstadt, Germany); they were stored over a 4Å-molecular sieve and used as received. 2-Acetylamino-4-chlorophenol was prepared according to the method reported earlier [21]. Melting points were determined with Thomas Hoover Capillary Melting Points Apparatus (Arthur H. Thomas Co., Philadelphia, PA, USA) and the values given are uncorrected. IR spectra were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer (KBr, υ, cm^{-1}) (Bruker Analytic GmbH, Ettlingen, Germany). Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker 400 FT-NMR spectrometer (Bruker) Spectra were calibrated using tetramethylsilane (TMS) (δ = 0.00). All chemical shifts were reported as δ (ppm) values. Elemental analyses were performed with Leco-932 (C,H,N,S-O-Elemental Analyzer) at Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara, Turkey) and were within the range of ±0.4 % of theoretical value.

2-Acetylamino-6-benzoyl-4-chlorophenol derivatives (**2 a–c**)

N,N-dimethylformamide (DMF) (0.22 mol) was added dropwise to anhydrous aluminium chloride (0.8 mol) at 0–10 °C, stirring constantly and protecting the reaction mixture from atmospheric moisture. After addition was completed, the mixture was stirred for further 10 minutes. The appropriate benzoyl chloride derivative (0.14 mol) was added portionwise to this mixture over 15 minutes. After addition of 2-acetylamino-4-chlorophenol (0.1 mol), the mixture was heated for six hours at 120 °C, cooled and poured into 1 L ice-water containing 10 mL concentrated hydrochloric acid. The product precipitated, it was filtered and dissolved in 1 L aqueous sodium hydroxide solution (0.2 N). The solution was filtered and acidified with concentrated hydrochloric acid. The precipitate was filtered, washed with water to neutral pH, dried, and crystallized from the appropriate solvent.

2-Amino-6-benzoyl-4-chlorophenol derivatives (**3 a**, **3 c**)

2-Acetylamino-6-benzoyl-4-chlorophenol derivative (0.1 mol) was added to 300 mL of 40 % sodium hydroxide solution and 200 mL ethanol. This solution was refluxed for 7 h, evaporated under vacuum to remove ethanol, and cooled. After adding 500 mL water, the solution was filtered to remove insoluble material and acidified with acetic acid to pH = 5.5. The product precipitated was filtered, washed with water to neutral pH, dried, and crystallized from appropriate solvent.

2-Amino-6-benzoyl-4-chlorophenol hydrochloride (**3 b**)

The mixture of 2-acetylamino-4-chloro-6-(2-chlorobenzoyl)-phenol (0.1 mol) and 250 mL concentrated hydrochloric acid was refluxed for 3 h, and then diluted with 500 mL water. The insoluble material was removed by hot filtration. The solution was allowed to cool and crystals separated from the solution were collected by suction filtration.

7-Benzoyl-5-chloro-2-oxo-3H-benzoxazole derivatives (**4 a–c**)

Ethyl chloroformate (0.2 mol) was added dropwise to the solution of 2-amino-6-benzoyl-4-chlorophenol derivative (0.1 mol) in pyridine while stirring and cooling in an ice bath. After stirring for further 30 minutes, the solution was heated under a condenser at 100 °C in an oil bath for 2.5–5 h. After cooling to room temperature, the solution was poured into 500 mL ice-water and acidified with 150 mL concentrated hydrochloric acid. The

precipitate was collected by suction filtration, washed with water, dried, and crystallized from the appropriate solvent.

Ethyl 2-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)acetate Derivatives (**5 a–c**)

7-Benzoyl-5-chloro-2-oxo-3*H*-benzoxazole derivative (10 mmol) was dissolved in an ethanolic solution of sodium ethoxide prepared by the reaction of Na° (10 mmol) with 10 mL ethanol. After addition of ethyl bromoacetate (11 mmol), the solution was refluxed for 3–8 h and cooled to room temperature. To precipitate the product, 50 mL water was added to this solution. The precipitate was filtered, dried, and crystallized from the appropriate solvent.

Ethyl 4-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoate Derivatives (**7 a–c**)

7-Benzoyl-5-chloro-2-oxo-3*H*-benzoxazole derivative (10 mmol) was treated with sodium ethoxide (10 mmol) in 20 mL ethanol. After evaporation to dryness under vacuum, the residue was dissolved in 30 mL N,N-dimethylformamide and then ethyl 4-chlorobutanoate (11 mmol) was added, heated for 12–20 h. at 80–100 °C in an oil-bath, cooled to room temperature, poured into 50 mL ice-water. The precipitate was collected by suction filtration, dried and crystallized from the appropriate solvent.

2-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)acetic acid (**6 a–c**) *and 4-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid* (**8 a–c**) *Derivatives*

The mixture of ethyl (7-benzoyl-5-chloro-2-oxo-3*H*-benzoxazol-3-yl)acetate (3 mmol) or ethyl 4-(7-benzoyl-5-chloro-2-oxo-3*H*-benzoxazol-3-yl)butanoate (3 mmol) and 30 mL hydrochloric acid (conc.) was refluxed for 3–5 h. and cooled to room temperature. The product precipitated, it was filtered off and dissolved in 50 mL aqueous sodium bicarbonate (5 %). The solution was filtered and acidified with hydrochloric acid (2 N). The precipitate was filtered, washed with water, dried, and crystallized from the appropriate solvent.

3-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)propionitrile Derivatives (**9 a–c**)

The solution of 7-benzoyl-5-chloro-2-oxo-3*H*-benzoxazole (10 mmol), acrylonitrile (15 mmol), and triethylamine (50 mmol) in 40 mL ethyl acetate was refluxed for 10–15 h, and cooled. The precipitated product was filtered, dried, and crystallized from the appropriate solvents.

3-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)propanoic acid Derivatives (**10 a–c**)

3-(7-Benzoyl-5-chloro-2-oxo-3*H*-benzoxazol-3-yl)propionitrile (3 mmol) was refluxed in the mixture of N,N-dimethylformamide (20 mL), sulphuric acid (10 mL), and water (10 mL) for 10 h, left overnight without refluxing and was then refluxed for additional 8 h. After cooling to room temperature, the product precipitated, it was filtered and dissolved in 50 mL aqueous sodium bicarbonate solution (5 %). The solution was filtered to remove insoluble material and acidified with hydrochloric acid (conc.). The precipitate that formed was filtered, dried, and crystallized from the appropriate solvent.

Ethyl 3-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)propanoate Derivatives (**11 a–c**)

The solution of 3-(7-benzoyl-5-chloro-2-oxo-3*H*-benzoxazol-3-yl)propionitrile derivative (3 mmol) in a previously prepared and cooled mixture of ethanol (20 mL) and sulphuric acid (10 mL) was refluxed for 1 h, then cooled to room temperature. Water was added to this solution until the precipitation was complete. The precipitate was filtered, washed with water, dried, and crystallized from appropriate solvents.

2-Acetylamino-6-benzoyl-4-chlorophenol (**2 a**)

^{1}H-NMR ($CDCl_3$): δ 12.7 (s, 1 H, -OH), 8.76 (d, 1 H, J = 2.5, H^5), 8.02 (s, 1 H, -NH-CO), 7.79–7.61 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.36 (d, 1 H, J = 2.5, H^3) and 2.35 (s, 3 H, -CO-CH_3). IR ν_{max} cm^{-1} (KBr): 3308 (N-H), 3145 (O-H), 1676 (C=O, amide) 1625 (C=O, ketone), 1608, 1598 (C=C, aromatic). Anal.: $C_{15}H_{12}ClNO_3$.

2-Acetylamino-4-chloro-6-(2-chlorobenzoyl)phenol (**2 b**)

^{1}H-NMR (DMSO-d_6): δ 11.44 (s, 1 H, -OH), 9.57 (s, 1 H, -NH-CO), 8.13 (d, 1 H, J = 2.43, H^5), 7.54–7.41 (m, 4 H, $H^{3'}$-$H^{6'}$), 6.84 (d, 1 H, J = 2.59, H^3), 2.06 (s, 3 H, CO-CH_3). IR ν_{max} cm^{-1} (KBr): 3347 (N-H), 3196 (O-H), 1691 (C=O, amide), 1642 (C=O, ketone), 1593 (C=C, aromatic). Anal.: $C_{15}H_{11}Cl_2NO_3$.

2-Acetylamino-4-chloro-6-(4-chlorobenzoyl)phenol (**2 c**)

^{1}H-NMR (DMSO-d_6): δ 12.43 (s, 1 H, OH), 8.73 (d, 1 H, J = 2.35, H^5), 7.92 (s, 1 H, -NH-CO), 7.67–7.53 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.26 (d, 1 H, J = 2.49, H3), 2.28 (s, 3 H, CO-CH_3). IR ν_{max} cm^{-1} (KBr): 3269 (N-H), 3125 (O-H), 1672 (C=O, amide), 1625 (C=O, ketone), 1607 (C=C, aromatic). Anal.: $C_{15}H_{11}Cl_2NO_3$.

2-Amino-6-benzoyl-4-chlorophenol (**3 a**)

^{1}H-NMR (DMSO-d_6) δ: 10.6 (s, 1 H, OH), 7.72–7.54 (m, 5 H, benzoyl $H^{2'}$-$H^{6'}$), 6.88 (d, 1 H, J = 2.52, H^5), 6.54 (d, 1 H, J = 2.52, H^3), 5.4 (s, 2 H, NH_2). IR ν_{max} cm^{-1} (KBr): 3441 (NH_2 asymmetric), 3332 (NH_2 symmetric), 3079 (OH), 1621 (C=O, ketone), 1596, 1574 (C=C, aromatic). Anal.: $C_{13}H_{10}ClNO_2$.

2-Amino-4-chloro-6-(2-chlorobenzoyl)phenol hydrochloride (**3 b**)

^{1}H-NMR (DMSO-d_6) δ: 7.45–7.28 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.27–6.95 (broad, 3 H, -$^+NH_3$), 6.92 (d, 1 H, J = 2.49, H^5), 6.23 (d, 1 H, J = 2.49, H^3). IR ν_{max} cm^{-1} (KBr): 3150–3090 (OH), 2793–2537 ($^+NH_3$), 1632 (C=O, ketone), 1613, 1588 (C=C, aromatic). Anal.: $C_{13}H_{10}Cl_3NO_2$.

2-Amino-4-chloro-6-(4-chlorobenzoyl)phenol (**3 c**)

^{1}H-NMR ($CDCl_3$): δ 12.00 (s, 1 H, OH), 7.66–7.52 (m, 4 H, $H^{2'}$-$H^{6'}$), 6.90 (s, 2 H, H^5-H^3), 4.12 (s, 2 H, -NH_2). IR ν_{max} cm^{-1} (KBr): 3423 (NH_2 asymmetric), 3310 (NH_2 symmetric), 3175 (OH), 1620 (C=O, ketone), 1597 (C=C, aromatic). Anal.: $C_{13}H_9Cl_2NO_2$.

7-Benzoyl-5-chloro-2-oxo-3H-benzoxazole (**4 a**)

^{1}H-NMR ($CDCl_3$): δ 8.71 (s, 1 H, -NH-), 8.04–7.71 (m, 5 H, $H^{2'}$-$H^{6'}$) 7.58 (d, 1 H, J = 2.05, H^6), 7.44 (d, 1 H, J = 2.05, H^4). IR ν_{max} cm^{-1} (KBr): 3318 (N-H, lactam), 1780 (C=O, lactam), 1663 (C=O, ketone), 1608 (C=C, aromatic). Anal.: $C_{14}H_8ClNO_3$.

5-Chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazole (**4 b**)

^{1}H-NMR ($CDCl_3$): δ 8.98 (s, 1 H, -NH-), 7.43 (d, 1 H, J = 1.95, H^6), 7.42–7.30 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.19 (d, 1 H, J = 2.07, H^4). IR ν_{max} cm^{-1} (KBr): 3176 (N-H, lactam), 1775 (C=O, lactam), 1658 (C=O, ketone), 1600, 1587 (C=C, aromatic). Anal.: $C_{14}H_7Cl_2NO_3$.

5-Chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazole (**4 c**)

^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 11.78 (s, 1 H, -NH-), 7.70–7.42 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.18 (d, 1 H, J = 2.09, H^6), 7.15 (d, 1 H, J = 2.12, H^4). IR ν_{max} cm^{-1} (KBr): 3193 (N-H, lactam), 1780 (C=O, lactam), 1666 (C=O, ketone), 1609, 1589 (C=C, aromatic). Anal.: $C_{14}H_7Cl_2NO_3$.

Ethyl 2-(7-benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)acetate (**5 a**)

^{1}H-NMR ($CDCl_3$): δ 8.02–7.70 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.60 (d, 1 H, J = 2.02, H^6), 7.23 (d, 1 H, J = 2.02, H^4), 4.76 (s, 2 H, N-CH_2-CO), 4.49 (q, 2 H, J = 7,14, O-*CH_2*-CH_3), 1.52 (t, 3 H, J = 7,14, O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 2992 (C-H, aliphatic), 1789 (C=O, lactam), 1732 (C=O, ester), 1659 (C=O, ketone), 1614, 1597 (C=C, aromatic). Anal.: $C_{18}H_{14}ClNO_5$.

Ethyl 2-[5-chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]acetate (**5 b**)

^{1}H-NMR (DMSO-d_6): δ 7.81 (d, 1 H, J = 2.1, H^6), 7.55–7.42 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.28 (d, 1 H, J = 2.1, H^4), 4.70 (s, 2 H, -N-CH_2-CO), 4.13 (q, 2 H, J = 7,10, -O-*CH_2*-CH_3), 1.15 (t, 3 H, J = 7,10, -O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3070, 3059 (C-H, aromatic), 2987, 2937 (C-H, aliphatic), 1793 (C=O, lactam), 1741 (C=O, ester), 1659 (C=O, ketone), 1612, 1590 (C=C, aromatic). Anal.: $C_{18}H_{13}Cl_2NO_5$.

Ethyl 2-[5-chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]acetate (**5 c**)

^{1}H-NMR ($CDCl_3$): δ 7.70–7.41 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.32 (d, 1 H, J = 2.0, H^6), 7.00 (d, 1 H, J = 2.0, H^4), 4.49 (s, 2 H, N-*CH_2*-CO), 4.22 (q, 2 H, J = 7,15, O-*CH_2*-CH_3), 1.24 (t, 3 H, J = 7,15, O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3067, 3062 (C-H, aromatic), 2998, 2980 (C-H, aliphatic), 1782 (C=O, lactam), 1742 (C=O, ester), 1664 (C=O, ketone), 1600, 1586 (C=C, aromatic). Anal.: $C_{18}H_{13}Cl_2NO_5$.

2-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)acetic acid (**6 a**)

^{1}H-NMR ($CDCl_3$): δ 7.87–7.57 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.44 (d, 1 H, J = 2.01, H^6), 7.11 (d, 1 H, J = 2.03, H^4), 4.65 (s, 2 H, N-*CH_2*-CO). IR ν_{max} cm^{-1} (KBr): 3300–2500 (O-H, carboxylic acid), 1744 (C=O, lactam), 1734 (C=O, carboxylic acid), 1663 (C=O, ketone), 1608, 1594 (C=C, aromatic). Anal.: $C_{16}H_{10}ClNO_5$.

2-[5-Chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]acetic acid (**6 b**)

^{1}H-NMR (DMSO-d_6): δ 7.81 (d, 1 H, J = 2.1, H^6), 7.55–7.42 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.27 (d, 1 H, J = 2.1, H^4), 4.58 (s, 2 H, N-CH_2-CO). IR ν_{max} cm^{-1} (KBr): 3300–2500 (O-H, carboxylic acid), 3098 (C-H, aromatic), 2987, 2956 (C-H, aliphatic), 1773 (C=O, lactam), 1748 (C=O, carboxylic acid), 1676 (C=O, ketone), 1613, 1500 (C=C, aromatic). Anal.: $C_{16}H_9Cl_2NO_5$.

2-[5-Chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]acetic acid (**6 c**)

^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 7.89–7.61 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.47 (d, 1 H, J = 1.98, H^6), 7.42 (d, 1 H, J = 1.98, H^4), 4.70 (s, 2 H, N-*CH_2*-CO). IR ν_{max} cm^{-1} (KBr): 3300–2700 (O-H, carboxylic acid), 3100, 3075 (C-H, aromatic), 2980, 2939 (C-H, aliphatic), 1763 (C=O, lactam), 1758 (C=O, carboxylic acid), 1666 (C=O, ketone), 1609, 1586 (C=C, aromatic). Anal.: $C_{16}H_9Cl_2NO_5$.

Ethyl 4-(7-benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoate (**7 a**)

^{1}H-NMR ($CDCl_3$): δ 7.75–7.45 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.31 (d, 1 H, J = 2.04, H^6), 7.19 (d, 1 H, J = 2.06, H^4), 4.12 (q, 2 H, J = 7,15, O-*CH_2*-CH_3), 3.86 (t, 2 H, N-*CH_2*-CH_2), 2.38 (t, 2 H, -CH_2-*CH_2*-CO), 2.07 (m, 2 H, CH_2-*CH_2*-CH_2), 1.23 (t, 3 H, J = 7,15, -O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3110 (C-H, aromatic), 2984, 2810 (C-H, aliphatic), 1790 (C=O, lactam), 1729 (C=O, ester), 1652 (C=O, ketone), 1611, 1500 (C=C, aromatic). Anal.: $C_{20}H_{18}ClNO_5$.

Ethyl 4-[5-chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]butanoate (**7 b**)

^{1}H-NMR ($CDCl_3$): δ 7.42 (d, 1 H, J = 2.07, H^6), 7.39–7.28 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.15 (d, 1 H, J = 2.08, H^4), 4.06 (q, 2 H, J = 7,13, -O-*CH_2*-CH_3), 3.79 (t, 2 H, N-*CH_2*-CH_2), 2.31 (t, 2 H, CH_2-*CH_2*-CO), 1.99 (m, 2 H, CH_2-*CH_2*-CH_2), 1.17 (t, 3 H, J = 7,13, -O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3078 (C-H, aromatic), 2981, 2920, 2890 (C-H, aliphatic), 1786 (C=O, lactam), 1724 (C=O, ester), 1670 (C=O, ketone), 1604, 1589 (C=C, aromatic). Anal.: $C_{20}H_{17}Cl_2NO_5$.

Ethyl 4-[5-chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]butanoate (**7 c**)

^{1}H-NMR ($CDCl_3$): δ 7.68–7.40 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.29 (d, 1 H, J = 2.03, H^6), 7.17 (d, 1 H, J = 2.03, H^4), 4.07 (q, 2 H, J = 7,15, O-*CH_2*-CH_3), 3.85 (t, 2 H, N-*CH_2*-CH_2), 2.37 (t, 2 H, -CH_2-*CH_2*-CO), 2.04 (m, 2 H, -CH_2-*CH_2*-CH_2), 1.20 (t, 3 H, J = 7,15, O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3100 (C-H, aromatic), 2985, 2936 (C-H, aliphatic), 1782 (C=O, lactam), 1729 (C=O, ester), 1647 (C=O, ketone), 1609, 1587 (C=C, aromatic). Anal.: $C_{20}H_{17}Cl_2NO_5$.

4-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)butanoic acid (**8 a**)

^{1}H-NMR (DMSO-d_6): δ 11.81 (s, 1 H, -COOH), 7.69–7.43 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.61 (d, 1 H, J = 2.13, H^6), 7.18 (d, 1 H, J = 2.07, H^4), 3.73 (t, 2 H, N-*CH_2*-CH_2), 2.35 (t, 2 H, CH_2-*CH_2*-CO), 1.79 (m, 2 H, CH_2-*CH_2*-CH_2). IR ν_{max} cm^{-1} (KBr): 3300–2400 (O-H, carboxylic acid), 3110 (C-H, aromatic), 2928 (C-H, aliphatic), 1786 (C=O, lactam), 1731 (C=O, carboxylic acid), 1652 (C=O, ketone), 1610, 1597 (C=C, aromatic). Anal.: $C_{18}H_{14}ClNO_5$.

4-[5-Chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]butanoic acid (**8 b**)

^{1}H-NMR ($CDCl_3$): δ 7.41 (d, 1 H, J = 2.06, H^6), 7.40–7.28 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.13 (d, 1 H, J = 2.06, H^4), 3.82 (t, 2 H, N-*CH_2*-CH_2-), 2.42 (t, 2 H, CH_2-*CH_2*-CO), 2.02 (m, 2 H, CH_2-*CH_2*-CH_2). IR ν_{max} cm^{-1} (KBr): 3300–2750 (O-H, carboxylic acid), 3031 (C-H, aromatic), 2968, 2953 (C-H, aliphatic), 1782 (C=O, lactam), 1733 (C=O, carboxylic acid), 1664 (C=O, ketone), 1608, 1589 (C=C, aromatic). Anal.: $C_{18}H_{13}Cl_2NO_5$.

4-[5-Chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]-butanoic acid (**8 c**)

^{1}H-NMR (DMSO-d_6): δ 11.77 (s, 1 H, -COOH), 7.55-7.26 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.29 (d, 1 H, J = 2.1, H^6), 7.04 (d, 1 H, J = 2.1, H^4), 3.66 (t, 2 H, N-*CH_2*-CH_2-), 2.15 (t, 2 H, CH_2-*CH_2*-CO), 1.76 (m, 2 H, CH_2-*CH_2*-CH_2). IR ν_{max} cm^{-1} (KBr): 3300–2500 (O-H, carboxylic acid), 3058 (C-H, aromatic), 2950 (C-H, aliphatic), 1790 (C=O, lactam), 1703 (C=O, carboxylic acid), 1651 (C=O, ketone), 1607, 1589 (C=C, aromatic). Anal.: $C_{18}H_{13}Cl_2NO_5$.

3-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)propionitrile (**9 a**)

^{1}H-NMR (DMSO-d_6): δ 7.68 (d, 1 H, J = 2.08, H^6), 7.62–7.37 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.15 (d, 1 H, J = 2.08, H^4), 3.97 (t, 2 H, N-*CH_2*-CH_2), 2.81 (t, 2 H, -CH_2-*CH_2*-CN). IR ν_{max} cm^{-1} (KBr): 3062 (C-H, aromatic), 2958, 2937 (C-H, aliphatic), 2250 (CN), 1783 (C=O, lactam), 1651 (C=O, ketone), 1609 (C=C, aromatic). Anal.: $C_{17}H_{11}ClN_2O_3$.

3-[5-Chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]propionitrile (**9 b**)

^{1}H-NMR (DMSO-d_6): δ 7.88 (d, 1 H, J = 2.1, H^6), 7.55–7.43 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.27 (d, 1 H, J = 2.1, H^4), 4.09 (t, 2 H, N-*CH_2*-CH_2),

2.93 (t, 2 H, CH_2-*CH_2*-CN). IR ν_{max} cm^{-1} (KBr): 3099, 3054 (C-H, aromatic), 2970, 2937 (C-H, aliphatic), 2250 (CN), 1787 (C=O, lactam), 1677 (C=O, ketone), 1608, 1591 (C=C, aromatic). Anal.: $C_{17}H_{10}Cl_2N_2O_3$.

3-[5-Chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]propionitrile (**9 c**)

^{1}H-NMR (DMSO-d_6): δ 8.06–7.78 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.81 (d, 1 H, J = 2.03, H^6), 7.66 (d, 1 H, J = 2.03, H^4), 4.51 (t, 2 H, N-*CH_2*-CH_2), 3.23 (t, 2 H, CH_2-*CH_2*-CN). IR ν_{max} cm^{-1} (KBr): 3085, 3072 (C-H, aromatic), 2983, 2968, 2937 (C-H, aliphatic), 2250 (CN), 1771 (C=O, lactam), 1660 (C=O, ketone), 1608, 1584 (C=C, aromatic). Anal.: $C_{17}H_{10}Cl_2N_2O_3$.

3-(7-Benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)propanoic acid (**10 a**)

^{1}H-NMR (DMSO-d_6): δ 12.17 (s, 1 H, -COOH), 7.60–7.34 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.58 (d, 1 H, J = 2.11, H^6), 7.10 (d, 1 H, J = 2.11, H^4) 3.82 (t, 2 H, N-*CH_2*CH_2), 2.52 (t, 2 H, -CH_2*CH_2*-CO). IR ν_{max} cm^{-1} (KBr): 3300-2500 (O-H, carboxylic acid), 2926 (C-H, aliphatic), 1789 (C=O, lactam), 1698 (C=O, carboxylic acid), 1648 (C=O, ketone), 1612, 1598 (C=C, aromatic). Anal.: $C_{17}H_{12}ClNO_5$.

3-[5-Chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]propanoic acid (**10 b**)

^{1}H-NMR ($CDCl_3$): δ 7.42 (d, 1 H, J = 2, H^6), 7.4–7.30 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.21 (d, 1 H, J = 2, H^4), 4.02 (t, 2 H, N-*CH_2*-CH_2), 2.81 (t, 2 H, N-CH_2-*CH_2*). IR ν_{max} cm^{-1} (KBr): 3390–3100 (O-H, carboxylic acid), 3086 (C-H, aromatic), 2979, 2950 (C-H, aliphatic), 1767 (C=O, lactam), 1745 (C=O, carboxylic acid), 1678 (C=O, ketone), 1607, 1592 (C=C, aromatic). Anal.: $C_{17}H_{11}Cl_2NO_5$.

3-[5-Chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]propanoic acid (**10 c**)

^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 12.02 (s, 1 H, COOH), 7.58–7.30 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.37 (d, 1 H, J = 2, H^6), 7.08 (d, 1 H, J = 2, H^4), 3.92 (t, 2 H, N-*CH_2*-CH_2), 2.55 (t, 2 H, CH_2-*CH_2*-CO). IR ν_{max} cm^{-1} (KBr): 3300–2500 (O-H, carboxylic acid), 3071 (C-H, aromatic), 2930 (C-H, aliphatic), 1786 (C=O, lactam), 1706 (C=O, carboxylic acid), 1653 (C=O, ketone), 1610, 1588 (C=C, aromatic). Anal.: $C_{17}H_{11}Cl_2NO_5$.

Ethyl 3-(7-benzoyl-5-chloro-2-oxo-3H-benzoxazol-3-yl)propanoate (**11 a**)

^{1}H-NMR (DMSO-d_6): δ 7.61–7.37 (m, 5 H, $H^{2'}$-$H^{6'}$), 7.58 (d, 1 H, J = 2, H^6), 7.11 (d, 1 H, J = 2, H^4), 3.88 (m, 4 H, N-*CH_2*-CH_2, O-*CH_2*-CH_3), 2.59 (t, 2 H, -CH_2-*CH_2*-CO), 0.92 (t, 3 H, J = 7,13, -O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3062, 3060 (C-H, aromatic), 2997, 2910 (C-H, aliphatic), 1780 (C=O, lactam), 1719 (C=O, carboxylic acid), 1651 (C=O, ketone), 1608, 1597 (C=C, aromatic). Anal.: $C_{19}H_{16}ClNO_5$.

Ethyl 3-[5-chloro-7-(2-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]propanoate (**11 b**)

^{1}H-NMR ($CDCl_3$): δ 7.41 (d, 1 H, J = 2.08, H^6), 7.40–7.30 (m, 4 H, $H^{3'}$-$H^{6'}$), 7.22 (d, 1 H, J = 2.08, H^4), 4.07 (m, 4 H, O*CH_2*CH_3, N-*CH_2*-CH_2), 2.73 (t, 2 H, N-CH_2-*CH_2*), 1.15 (t, 3 H, J = 7,14, -OCH_2*CH_3*). IR ν_{max} cm^{-1} (KBr): 3060, 3058 (C-H, aromatic), 2985, 2980, 2974, 2937 (C-H, aliphatic), 1778 (C=O, lactam), 1743 (C=O, ester), 1678 (C=O, ketone), 1604, 1590 (C=C, aromatic). Anal.: $C_{19}H_{15}Cl_2NO_5$.

Ethyl 3-[5-chloro-7-(4-chlorobenzoyl)-2-oxo-3H-benzoxazol-3-yl]propanoate (**11 c**)

^{1}H-NMR ($CDCl_3$): δ 7.68–7.40 (m, 4 H, $H^{2'}$-$H^{6'}$), 7.29 (d, 1 H, J = 2, H^6), 7.26 (d, 1 H, J = 2, H^4), 4.10 (m, 4 H, O-*CH_2*-CH_3, N-*CH_2*-CH_2), 2.77 (t, 2 H, -CH_2-*CH_2*-CO), 1.19 (t, 3 H, J = 7,15, -O-CH_2-*CH_3*). IR ν_{max} cm^{-1} (KBr): 3095 (C-H, aromatic), 2991 (C-H, aliphatic), 1787 (C=O, lactam), 1728 (C=O, ester), 1647 (C=O, ketone), 1608, 1588 (C=C, aromatic). Anal.: $C_{19}H_{15}Cl_2NO_5$.

Pharmacology

Animals

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 kept in colony cages (6 mice each), maintained on standard pellet diet, 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 the 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 were given orally to test animals after suspending them in a mixture of distilled H_2O and 0.5 % sodium carboxymethyl cellulose (CMC). The animals of the control group received the same experimental handling, 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 [25]

60 Minutes 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) solution in distilled H_2O (PBQ; Merck, Darmstadt, Germany). 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 writhes observed. The analgesic activity was expressed as the percentage change from writhing controls.

Carrageenan-induced hind paw edema test [30]

The method of Kasahara et al.[30] was applied. 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 mouse was injected with freshly prepared (0.5 mg/25 µL) suspension of carrageenan (Sigma, St. Louis, Mo, 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 every 90 min during 360 min as described above.

Serotonin-induced edema test

The method of Kasahara et al. [30] was used. Briefly, 60 min after the oral administration of test sample or dosing vehicle each mouse was injected with serotonin (serotonin creatinin sulfate, Merck, Art.7768) in Tyrode's solution (0.5 µg/5 µL) into sub-

plantar tissue of the right hind paw and 5 µL of Tyrode's solution into that of the left as secondary control. The difference in footpad thickness between the right and left foot was measured every 6 min during 30 min with a pair of dial thickness gauge callipers (Ozaki).

Acetic acid-induced peritoneal capillary permeability test

The influence of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method with some modifications [33]. Each test sample was administered orally to a group of 10 mice in 0.2 mL per 20 g body weight. 30 min after the administration each mouse was injected with 0.1 mL of 4 % Evans blue (Sigma) in saline solution (i.v.) in the tail. Then, 10 min after the i.v. injection of the dye solution, 0.4 mL of 0.5 % (v/v) AcOH was injected ip. After 20 min, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 mL volumetric flasks through glass wool. Each flask was filled up to 10 mL with distilled water, 0.1 of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer; Beckmann, Beckmann Instruments Inc., Fullerton, California, USA). In the control animals a mixture of distilled water and 0.5 % CMC was given orally, otherwise they were treated in the same manner as described above.

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-ulcerogenic effect

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

Statistical analysis of data

Data obtained from the 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.

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