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Anti-nociceptive and anti-inflammatory activity of some (2-benzoxazolone-3-yl and 2-benzothiazolone-3-yl)acetic acid derivatives

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Abstract

Sixteen (2-benzothiazolone-3-yl and 2-benzoxazolone-3-yl)acetic acid derivatives **5** have been tested for anti-nociceptive and anti-inflammatory activity in this study. 4-[2-(6-Benzoyl-2-benzoxazolone-3-yl)acetyl]morpholine (**5c**), 4-{2-[6-(2-chloro-benzoyl)-2-benzoxazolone-3-yl]acetyl}morpholine (**5d**), 1-[2-(5-chloro-2-benzoxazolone-3-yl)acetyl]pyrrolidine (**5f**), methyl (6-methyl-2-benzoxazolone-3-yl)acetate (**5k**) and *N,N*-diethyl-2-(2-benzothiazolone-3-yl)acetamide (**5m**) have shown more potent anti-nociceptive activity than others. Among these compounds, **5c**, **5d** and **5m** have exhibited good anti-inflammatory activity, with **5f**, and to a lesser extent, the other molecules displaying some toxic potential.

Keywords: Anti-nociceptive activity; Anti-inflammatory activity; Benzoxazolinone derivatives; Benzothiazolinone derivatives

1. Introduction

It is widely accepted that there are two classes of compounds which are used in clinical analgesia: (a) peripheral analgesic, which can be divided into two subclasses namely cyclooxygenase inhibitors (NSAIDs) and peripheral opioids — dipyrone can be added to this group; (b) central opioids. However, each class has disadvantages. NSAIDs frequently cause gastrointestinal disorders, while opiates lead to tolerance, physical dependency and addiction. The aim of current analgesic research is to develop new NSAIDs without such side effects.

In this respect, many benzoxazolinone [1–4] and oxazolopyridine [5,6] derivatives have been synthesized and claimed to have significant analgesic activity. In addition, benzothiazolinone derivatives have also been reported as potent analgesic agents [7,8]. In 1995, Ferreira et al. screened the anti-nociceptive activity of 6-benzoylbenzothiazolone and concluded that it might release an endogenous, opioid-like substance from the adrenal glands to exert the anti-nociceptive activity [9]. The compound was claimed to have little or no anti-inflammatory activity. On the other hand, one of the benzothiazolinone derivatives, 4-[(5-chloro-2-oxo-3-benzothiazolinyl)acetyl]-1-piperazinylethanol hydrochloride (Tiaramide HCl, Fig. 1) is a well-known analgesic and anti-inflammatory agent. It was also proven to have antihistaminic activity with low incidence of mild side effects [10,11].

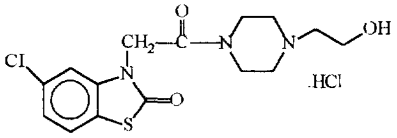

Fig. 1. Tiaramide HCl.

These conflicting findings have urged us to synthesize some benzothiazolinone and benzoxazolinone derivatives and test their anti-nociceptive and anti-inflammatory activities.

1.1. Chemistry

The syntheses of the title compounds have been realized as illustrated in Scheme 1. Eight of the compounds (**5a–h**) have previously been reported by our group [12]. Compounds **5m–p** have also been synthesized by other authors [13–15]. Only compounds **5i–5l** are new. Syntheses of **1** were carried out by the reaction of *o*-aminophenol derivatives

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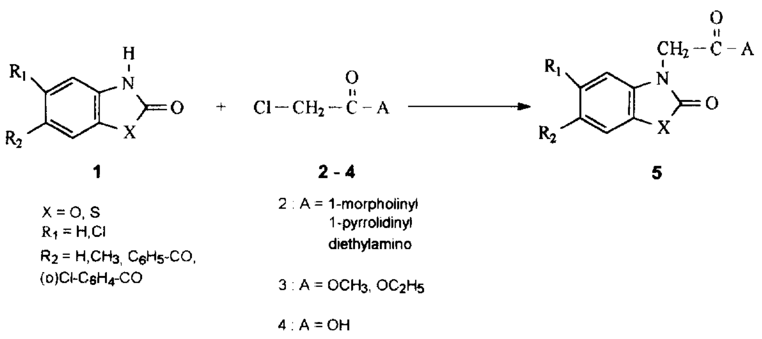


Scheme 1. Syntheses of the title compounds.

and *o*-aminothiophenol with urea, except chlorzoxazone which was commercially available. The physical and spectral properties of benzothiazolone, 6-methylbenzoxazolone and benzoxazolone were in accordance with the data in the literature [16,17]; therefore, they have been used in the next steps of synthesis without any further analysis. The benzazoles synthesized were reacted with chloroacetamide derivatives **2**, chloroacetic acid esters **3** and chloroacetic acid **4** in the presence of sodium ethoxide to form the final compounds **5** (Scheme 1 and Table 1).

2. Experimental

2.1. Chemistry

Melting points of the compounds were determined with an Electrothermal melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded on a Perkin-Elmer 1330 spectrometer. The ^{1}H NMR spectra were recorded on a Bruker 200 FT-NMR spectrometer. Chemical shifts are reported in parts per million (δ) downfield from TMS as internal standard. DMSO-d_6 and trifluoroacetic acid were used as solvents.

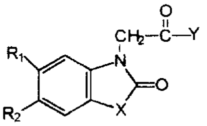
Microanalyses for C, H, N and S were performed by TÜBITAK Analytical Laboratory, Ankara, Turkey and were within the range of ±0.4% of the theoretical value.

Benzazole esters **5k** and **5o** [15], benzazole acids **5l** and **5p** [15], and 6-acyl-2-benzoxazolinones [4] were prepared in our laboratory as already described.

2.1.1. 2-(2-Benzoxazolinone/benzothiazolinone-3-yl)acetamide derivatives

Metallic sodium (1 g) was dissolved in 20 ml absolute alcohol and 0.01 mol of 2-benzoxazolinone or its derivative was added to this solution. The chloroacetamide derivative, prepared with 0.01 mol of amine (morpholine or pyrrolidine or diethylamine) and chloroacetyl chloride, was dissolved in

Table 1
The compounds synthesized, their m.p., crystallization solvent, percentage yield and formula



Compound	R¹	R²	X	Y	Cryst. solvent	M.p. (°C)	Yield (%)	Formula
5a	H	H	O	4-morpholinyl	EtOH	195	69.00	$C_{13}H_{14}N_2O_4$
5b	Cl	H	O	4-morpholinyl	EtOH–acetone	214	72.00	$C_{13}H_{13}ClN_2O_4$
5c	H	C_6H_5CO	O	4-morpholinyl	acetone	204	81.00	$C_{20}H_{18}N_2O_5$
5d	H	(*o*)ClC_6H_4CO	O	4-morpholinyl	EtOH	170	83.00	$C_{20}H_{17}ClN_2O_5$
5e	H	H	O	1-pyrrolidinyl	EtOH	147	63.00	$C_{13}H_{14}N_2O_3$
5f	Cl	H	O	1-pyrrolidinyl	EtOH–water	157	68.00	$C_{13}H_{13}ClN_2O_3$
5g	H	(*o*)ClC_6H_4CO	O	1-pyrrolidinyl	EtOH	139	71.00	$C_{20}H_{17}ClN_2O_4$
5h	H	C_6H_5CO	O	1-pyrrolidinyl	acetone	190	67.00	$C_{20}H_{18}N_2O_4$
5i	H	CH_3	O	diethylamino	water	98	38.16	$C_{14}H_{18}N_2O_3$
5j	H	CH_3	O	4-morpholinyl	EtOH	165	45.28	$C_{14}H_{16}N_2O_4$
5k	H	CH_3	O	OCH_3	EtOH–water	124	61.59	$C_{11}H_{11}NO_4$
5l	H	CH3	O	OH	EtOH–water	213	53.14	$C_{10}H_9NO_4$
5m	H	H	S	diethylamino	water	105	38.27	$C_{13}H_{16}N_2O_2S$
5n	H	H	S	4-morpholinyl	EtOH	243	64.02	$C_{13}H_{14}N_2O_3S$
5o	H	H	S	OC_2H_5	EtOH–water	94	42.19	$C_{11}H_{11}NO_3S$
5p	H	H	S	OH	EtOH–water	172	56.81	$C_9H_7NO_3S$

20 ml ethanol and poured into the former solution. The mixture, thus obtained, was refluxed with stirring for 4 h. The reaction mixture was evaporated to dryness at the end of this period. The residue was washed with 100 ml of cold water, and filtered. The solid material was crystallized from the appropriate solvent listed in Table 1.

2.1.2. Spectral data of the compounds

The IR spectra of the benzoxazolinone derivatives exhibited the following characteristic bands (cm^{-1}): heterocyclic carbonyl: 1750–1785; amide carbonyl: 1640–1680; ketone carbonyl: 1630. In the benzothiazolinone derivatives the band corresponding to the ring carbonyl fluctuated between 1640 and 1720 cm^{-1}.

The ^{1}H NMR spectra do not exhibit any peculiar features, thus only the spectrum of compound **5i** is indicated as an example: δ 7–8 (m, 4H, aromatic H); 4.75 (s, 2H, N–CH_2–CO–); 3.3 (m, 4H, N–$\mathit{CH_2}$–CH_3); 2.33 (s, 3H, Ar–CH_3); 1.2 (t, 3H, CH_2–$\mathit{CH_3}$); 1.02 (t, 3H, CH_2–$\mathit{CH_3}$).

2.2. Pharmacology

2.2.1. Materials

Acetic acid (Merck A.G.), carrageenan (Sigma), aspirin (Bayer), carboxymethylcellulose sodium salt (CMC-Na) (Aldrich) and gauge calipers (Ozaki Co.) were used.

Albino mice of both sexes (25.1 ± 5 g) which are locally bred were employed. All the animals were left for 2 days in the laboratory before the day of experiment and the last day they were given water only.

2.2.2. Methods

(a) Anti-nociceptive activity

A modified Koster test was used [18]. There were 5 mice in each group. Aspirin was used as a reference. There was also a control group. Test compounds and aspirin were administered orally at 100 mg/kg dose as a suspension in 0.2 ml of 0.5% CMC-Na. Control animals received the same volume of vehicle. One hour after the drug administration, every mouse was treated with an aqueous acetic acid solution (3% wt./vol.) injected intraperitoneally at a 300 mg/kg dose. Five minutes later, writhing movements were counted for a period of 10 min. The mean writhing counts for each group were used in the anti-nociceptive activity calculation employing the following equation.

Antinociceptive activity (writhing inhibition %)

$$= \frac{n-n'}{n} \times 100$$

n = the mean stretching count of control group
n' = the mean stretching counts of test groups

According to this calculation maximum anti-nociceptive activity could be 100% whereas it equals 0% in controls.

The differences between the control and test groups were found statistically significant by the Mann–Whitney U test.

(b) Anti-inflammatory activity

The Carrageenan-induced hind paw edema model was employed for anti-inflammatory testing, according to a modification of the method reported by Kasahara et al. [19]. There were 7 mice in each group.

Test samples and aspirin were given orally at 100 mg/kg dose, and 60 min after the drug administration each mouse was injected with 25 μl of freshly prepared carrageenan solution (0.5 mg/ml) in physiological saline (154 mM NaCl) into the subplantar tissue of the right hind paw. The same volume of saline solution was injected into the left hind paw as the internal control. The differences in foot pad thickness between the right and left foot were measured every hour with a pair of dial thickness gauge calipers. Statistical differences between the treatments and the control were tested by a two-tailed Student's t-test.

(c) Evaluation of toxicological effects in mice

Five mice in each group were given orally 100 mg/kg of each test compound as a suspension in 0.2 ml of 0.5% aqueous CMC-Na solution at 24 h intervals for 3 subsequent days, while the control group was given the same volume of vehicle. Each group was observed for mortality and behavioral responses at regular intervals for up to 7 days. The body growth during the period of observation, and the spleen, and the liver weight at the 7th day were measured as an index of toxicity. These values are illustrated in Table 4 and the significance of the differences was tested by a two-tailed Student's t-test.

3. Results and discussion

The anti-nociceptive activity of the compounds is given in Table 2. Table 3 illustrates the anti-inflammatory activity of the title compounds.

Table 2
Anti-nociceptive activity of the compounds

Compounds	Anti-nociceptive activity (%)
Aspirin	68.6 ± 9.1 [a]
5a	54.0 ± 11.7 [a]
5b	38.3 ± 13.5
5c	75.3 ± 2.9 [a]
5d	72.3 ± 9.0 [a]
5e	39.3 ± 14.1
5f	64.9 ± 13.6 [a]
5g	25.5 ± 15.6
5h	21.7 ± 12.9
5i	56.7 ± 14.2 [a]
5j	50.5 ± 8.0
5k	73.8 ± 14.0 [a]
5l	49.5 ± 5.7
5m	71.8 ± 4.4 [a]
5n	54.0 ± 13.3 [a]
5o	48.9 ± 2.2
5p	58.5 ± 11.8 [a]

Data obtained from the animal experiments were expressed as the mean % inhibition of writhings ± standard error (± SEM).

[a] $p < 0.05$ (in comparison with control group).

Table 3
Anti-inflammatory activity of compounds **5c**, **5d**, **5f**, **5k** and **5m**: swelling thickness ($\times 10^{-2}$ mm) ± SEM

Compound	Hours						
	1	2	3	4	5	6	7
Control	40.6 ± 3.3	45.1 ± 4.4	51.7 ± 3.1	58.7 ± 3.6	51.7 ± 2.4	50.4 ± 3.3	44.6 ± 2.9
5c	27.1 ± 4.3 ^{a}	31.8 ± 3.7 ^{a}	31.8 ± 4.0 ^{b}	31.8 ± 6.4 ^{b}	29.4 ± 5.2 ^{b}	32.3 ± 4.4 ^{b}	26.2 ± 5.6 ^{a}
5d	30.6 ± 1.5 ^{a}	31.7 ± 3.7 ^{a}	38.4 ± 4.5 ^{a}	36.8 ± 4.5 ^{b}	34.0 ± 3.1 ^{c}	36.6 ± 2.6 ^{b}	31.4 ± 3.0 ^{b}
5f	44.3 ± 2.6	52.4 ± 4.0	54.0 ± 6.1	49.7 ± 4.7 ^{a}	42.0 ± 3.7 ^{a}	40.3 ± 2.9 ^{a}	35.0 ± 2.7 ^{a}
5k	50.3 ± 5.8	66.6 ± 7.9	64.1 ± 6.3	54.7 ± 4.9	47.4 ± 4.3	43.4 ± 3.7	38.1 ± 2.3
5m	40.8 ± 3.9	45.1 ± 4.9	49.6 ± 3.2	43.6 ± 2.3 ^{b}	35.8 ± 1.5 ^{c}	33.3 ± 2.8 ^{b}	31.1 ± 3.2 ^{b}
Aspirin	31.4 ± 5.3	39.4 ± 7.5	34.3 ± 5.0 ^{a}	35.6 ± 4.2 ^{b}	34.3 ± 5.3 ^{a}	36.6 ± 5.6 ^{a}	29.8 ± 5.7 ^{a}

Data obtained from the animal experiments were expressed as the mean swelling thickness ± standard error (± SEM).

[a] $p < 0.05$.

[b] $p < 0.01$.

[c] $p < 0.001$.

During the 7 day observation period for toxicological activity, all animals survived and we did not observe any depression in animals treated with the test compounds, in fact they were in a rather active mode. The differences in body weights of all animals between the initial and last day of observation period were evaluated. In autopsy after 7 days, changes in weight and appearance for any lesion of the spleen and the liver were examined. The results are shown in Table 4.

Inflammation is a complicated process which is involved in many chemical mediators and cell types, but the paw edema method is most frequently used as the test model for anti-inflammatory activity. The writhing test was chosen since most of the NSAIDs exhibit peripheral non-opioid analgesic properties.

As shown in Table 2, compounds **5a, 5c, 5d, 5f, 5i, 5k, 5m, 5n** and **5p** exhibited significant anti-nociceptive activity in comparison with the control group. The five compounds **5c, 5d, 5f, 5k** and **5m**, which showed more than 60% anti-nociceptive activity, have been tested for their anti-inflammatory activity. Compounds **5c**, **5d** and **5m** showed higher anti-inflammatory activity than **5f** and **5k**. The highest anti-inflammatory activity was observed for compounds **5c** and **5d**, whereas the activity of **5m** became pronounced 3 h after the administration. Vinegar et al. claimed that the inhibitory effects of agents which act on the first phase of the carrageenan-induced hind paw inflammation are attributable to the inhibition of release of chemical mediators such as histamine and serotonin [20]. On the other hand, the second phase of the hind paw edema may be related to arachidonic acid metabolites since it is inhibited by aspirin and other arachidonate cyclooxygenase inhibitors. According to the data in Table 3, **5c** and **5d** might exert their anti-inflammatory activity in both phases, while **5f** and **5m** are active only in the second phase.

Concerning the toxicological aspects of these compounds, **5f** caused marked losses in weight of the body, spleen and liver of the experimental animals which might be interpreted as a toxic effect. Statistically significant weight loss in the spleen and liver was found for **5c** and **5d**, respectively, however these weight changes might not be interpreted as a toxic effect due to the small value of the standard error. Compounds **5k** and **5m** caused significant changes in body weight, but not in spleen and liver weights of the experimental animals during the 7 day observation period.

When the chemical structures of the active compounds are taken into consideration, it is noteworthy that both **5c** and **5d** have a benzoyl moiety at position 6 and a morpholino moiety on the side chain. The only structural difference between the two compounds is that **5d** carries a chloro substituent at the *ortho* position of the benzoyl moiety. The other compounds, **5g** and **5h**, which bear a benzoyl moiety but lack the mor-

Table 4
The effect of the active compounds on the weights of the body, spleen and liver of the mice used in the toxicity test

Test material	Mean differences in body growth ^{a} (g) ± SEM	Mean organ growth (g) ± SEM	
		Spleen	Liver
Control	3.73 ± 0.57	0.19 ± 0.02	1.45 ± 0.08
5c	1.73 ± 1.28	0.11 ± 0.01 ^{b}	1.04 ± 0.16
5d	3.23 ± 1.02	0.18 ± 0.02	1.17 ± 0.07 ^{b}
5f	1.28 ± 0.33 ^{c}	0.09 ± 0.008 ^{c}	0.86 ± 0.02 ^{c}
5k	1.84 ± 0.44 ^{b}	0.13 ± 0.02	1.19 ± 0.67
5m	0.63 ± 0.99 ^{b}	0.14 ± 0.03	1.21 ± 0.11

[a] Values represent the mean difference in body weights between the first and the last days of the observation period.

[b] $p < 0.01$.

[c] $p < 0.001$.

pholino group at the acetyl side chain, showed no anti-nociceptive activity. Thus, one could say that these two groups exert a significant contribution to the activities when they are present together in the same molecule. On the other hand, the *ortho* chloro group might seem to reduce the toxicological effect. In order to better define the biological effects of these structural features there is a need to synthesize new derivatives which carry benzoyl and a substituted benzoyl moiety at position 6 of the benzoxazolinone and benzothiazolinone rings and a different amine portion on the acyl side chain.

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