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Synthesis and Antinociceptive Activity of (5-Chloro-2(3*H*)-Benzoxazolon-3-yl) Propanamide Derivatives

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In this study, (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanamide derivatives were synthesized. The chemical structures of the compounds were elucidated by their IR and ^{1}H-NMR spectral data and microanalysis. The compounds were tested for antinociceptive activity by using the tail clip, tail flick, hot plate, and writhing methods. The varying levels of antinociceptive activity of the compounds were compared with those of dipyrone and aspirin. Among these compounds, compound **5e**, **5g**, and **5h** have been found to be significantly more active than the others and the standards in all the tests.

Key words: 5-Chloro-2(3*H*)-benzoxazolone, (5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanamide, Antinociceptive activity

INTRODUCTION

Many currently marketed non-steroidal anti-inflammatory drugs (NSAIDs) are inhibitors of the cyclooxygenase enzyme, which is associated with the metabolism of cellular arachidonic acid. This mechanism is thought to be primarily responsible for the analgesic and anti-inflammatory properties of NSAIDs, through the inhibition of prostaglandin biosynthesis. However, cyclooxygenase inhibition has also been associated with the nephrotoxicity and gastrointestinal side effects, which is characteristic of current NSAIDs (Kalgutkar *et al.*, 2000).

Recently, different lactamic systems such as 2-benzothiazolone (Nakamura *et al.*, 1986; Onkol *et al.*, 2000), 2-benzoxazolone (Palaska, *et al.*, 1993; Unlu *et al.*, 2002), pyridazinone (Takaya *et al.*, 1979; Dal Piaz *et al.*, 1996; Dogruer *et al*, 2000; Dal Piaz, *et al.*, 2003), and oxazolo[4,5-b]pyridinone (Flouzat *et al.*, 1993; Viaud *et al.*, 1995) derivatives bearing an arylpiperazine moiety have been reported to have significant analgesic activity.

Close and co-workers reported on the analgesic activities of 2(3*H*)-benzoxazolones, which have been structurally modified at the positions 3, 5, and 6 in order to screen for their antinociceptive properties (Close *et al.*, 1949).

Renard and Lespagnol synthesized 3-alkanoic acid derivatives of 2(3*H*)-benzoxazolone in which the analgesic effect were found to be more potent than aspirin (Renard *et al.*, 1980; Lespagnol *et al.*, 1967).

2(3*H*)-Benzoxazolone derivatives bearing the 2-pyridylethyl or 4-pyridylethyl substituent at position 3 exhibited significant analgesic and anti-inflammatory activities (Safak *et al.*, 1992).

Dündar and co-workers synthesized some 1-[4-(2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)butanoyl]-4-arylpiperazine derivatives for antinociceptive activity, and concluded that when the distance between the amido nitrogen of piperazine ring and the nitrogen atom located at position 3 of 2(3*H*)-benzoxazolone increased to four carbon atoms, antinociceptive activity was increased as well (Fig. I) (Dündar *et al.*, 2003).

On the other hand, it was reported that 4-(5-chloro-2(3*H*)-benzoxazolon-3-yl)butanoic acid showed a greater analgesic potential than their amide derivatives (Fig. I) (Gülcan *et al.*, 2003).

We previously discovered that the antinociceptive activity of 2(3*H*)-benzoxazolon-3-yl)propanamide and propanoic acid derivatives was more pronounced than the corresponding acetic acid derivatives (Önkol *et al.*, 2001, 2002).

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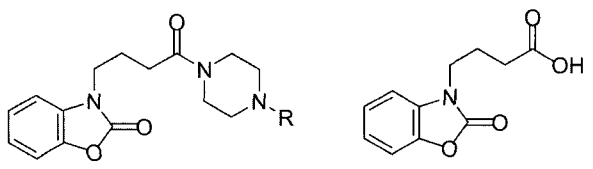
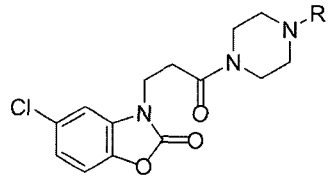


R = phenyl, benzyl, fluorophenyl chlorophenyl, pyridyl

Fig. 1. 4-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)butanoic acid and their amide derivatives



R = phenyl, benzyl, chlorophenyl fluorophenyl, trifluoromethylphenyl, pyridyl, methoxyphenyl, piperonyl

Fig. 2. (5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanamide derivatives

Therefore, in the course of our research on 5-chloro-2(3*H*)-benzoxazolone derivatives with analgesic activity, synthesis of (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanamide derivatives, which have a three carbon side chain at the 3 positions of 5-chloro-2(3*H*)-benzoxazolone was planned (Fig. 2), and antinociceptive activity was investigated.

MATERIALS AND METHODS

Chemicals and all the solvents used in this study were locally purchased from Merck A. G. and Aldrich Chemical. Melting points were determined on Electrothermal-9300 Digital Melting Point Apparatus. The IR spectra of the compounds were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrophotometer. The ^{1}H-NMR spectra of compounds were recorded in DMSO-d_6 on a Bruker 400 MHz NMR spectrometer using tetramethylsilane as the internal standard. All chemical shifts were recorded as δ (ppm). Microanalyses for C, H, and N were performed at TÜBİTAK Analytical Laboratory, Ankara, Turkey, and the results of the microanalyses were within the range of ±0.4% of the theoretical value. Synthesis of (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanenitrile (Zinner *et al.*, 1966), (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanoic acid (Zinner *et al.*, 1966), and (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl chloride (Onkol *et al.*, 2002; Fujii, 1957) (Scheme I) were reported by other authors.

Synthesis of (5-chloro-2(3*H*)-benzoxazolon-3-yl) propanenitrile (2)

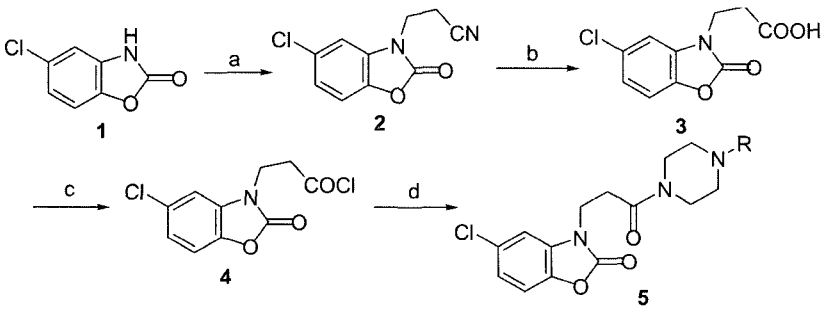
Ten mmole of 5-chloro-2(3H)-benzoxazolone and 12 mmole of triethylamine were dissolved in 500 mL of water, and the mixture was stirred at 50°C. 12 mmole of acrylonitrile was added to the solution. The mixture was heated at 50-60°C for six hours and was stirred at room temperature for 18 h. Then, the solid material that was precipitated was filtered, washed with water until neutral pH, dried, and crystallized from ethanol.

Synthesis of (5-chloro-2(3*H*)-benzoxazolon-3-yl) propanoic acid (3)

Ten mmole of (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanenitrile, in 10 N hydrochloric acid (50 mL), were stirred at room temperature for 2 h and then was refluxed for 4 hours. The reaction mixture was cooled, and the precipitate was collected by filtration, washed with water, dried, and crystallized from water.

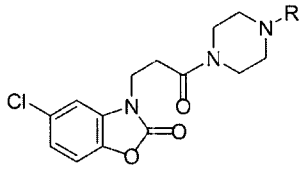
Synthesis of (5-chloro-2(3*H*)-benzoxazolon-3-yl) propanoyl chloride (4)

Ten mmole of (5-chloro-2(3*H*)-benzoxazolon-3-yl)propanoic acid was dispersed in 50 mL of benzene. 20 mmole of thionyl chloride were added to the solution and then refluxed for six hours. The final mixture was stirred one hour at room temperature and then was evaporated to dryness. The acid chloride was left unpurified when it was used in the next step.



Scheme 1. Synthetic route of the title compounds. a: Acrylonitrile, Et_3N, H_2O; b: HCl, reflux; c: $SOCl_2$, benzene, reflux; d: piperazin derivatives, $NaHCO_3$, THF, reflux.

Table I. Mps, crystallization solvents, yield percentages of title compounds **5a-j**



Comp.	R	Crys. Sol.	Mp (°C)	Formula	Mol. Weight	Yield %
5a	phenyl	Ethanol-Water	137-138	$C_{20}H_{20}ClN_3O_3$	385.8	53
5b	benzyl	Ethanol-Water	112-113	$C_{21}H_{22}ClN_3O_3$	399.8	58
5c	4-fluorophenyl	Ethanol	145-146	$C_{20}H_{19}ClFN_3O_3$	403.8	55
5d	2-fluorophenyl	Ethanol-Water	98-99	$C_{20}H_{19}ClFN_3O_3$	403.8	38
5e	4-chlorophenyl	Ethanol-Water	164-166	$C_{20}H_{19}Cl_2N_3O_3$	420.2	52
5f	3-chlorophenyl	Ethanol-Water	119-121	$C_{20}H_{19}Cl_2N_3O_3$	420.2	35
5g	3-trifluoromethylphenyl	Ethanol	149-150	$C_{21}H_{19}ClF_3N_3O_3$	453.8	50
5h	2-pyridyl	Ethanol-Water	141-143	$C_{19}H_{19}ClN_4O_3$	386.8	54
5i	2-methoxyphenyl	Ethanol-Water	132-133	$C_{21}H_{22}ClN_3O_3$	415.8	55
5j	piperonyl	Ethanol	252-255	$C_{22}H_{22}ClN_3O_5.2HCl$	516.8	43

Synthesis of (5-chloro-2(3*H*)-benzoxazolon-3-yl) propanamides (5)

Five mmole of (5-chloro-2(3H)-benzoxazolon-3-yl)propanoyl chloride derivatives, 15 mmole of sodium carbonate, 15 mmole of secondary amine derivatives, and 50 mL of tetrahydrofuran were mixed, refluxed for 3-20 h, and stirred for 18 h at room temperature. Then, the precipitated solid material was filtered and crystallized from corresponding solvents (Table I).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-phenylpiperazine (5a)

IR (KBr) υ_{max} (cm^{-1}): 1786 (C=O, lactam), 1638 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6,) δ (ppm) 7.54 (1H, d, *J*=1.83 Hz, H^4), 7.33 (1H, d, *J*=8.55 Hz, H^7), 7.20 (2H, m, phenyl-$H^{3,5}$), 7.14 (1H, dd, *J*=8.54 Hz; 2.44 Hz, H^6), 6.92 (2H, d, *J*=7.94 Hz, phenyl-$H^{2,6}$), 6.79 (1H, t, *J*=7.33 Hz, phenyl-H^4), 4.03 (2H, t, *J*=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.55 (4H, m, piperazine-$H^{2,6}$), 3.10 (2H, t, *J*=4.88 Hz, piperazine-$H^{3(5)}$), 3.05 (2H, t, *J*=4.89 Hz, piperazine-$H^{5(3)}$), 2.86 (2H, t, *J*=7.02 Hz, $CH_2\underline{CH_2}CO$).

1-[3-(5-chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-benzylpiperazine (5b)

IR (KBr) υ_{max} (cm^{-1}): 1773 (C=O, lactam), 1634 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm) 7.52 (1H, d, *J*=2.44 Hz, H^4), 7.35-7.23 (6H, m, phenyl-H, H^7), 7.15 (1H, dd, *J*=8.54 Hz; 2.44 Hz, H^6), 4.01 (2H, t, *J*=6.72 Hz, $\underline{CH_2}CH_2CO$), 3.45 (2H, s, CH_2-phenyl), 3.42 (2H, t, *J*=4.89 Hz, piperazine-$H^{2(6)}$), 3.39 (2H, t, *J*=4.89 Hz, piperazine-$H^{6(2)}$), 2.79 (2H, t, *J*=7.02 Hz, $CH_2\underline{CH_2}CO$), 2.30 (2H, t, *J*=4.88 Hz, piperazine-$H^{3(5)}$), 2.26 (2H, t, *J*=4.89 Hz, piperazine-$H^{5(3)}$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(4-fluorophenyl)piperazine (5c)

IR (KBr) υ_{max} (cm^{-1}): 1789 (C=O, lactam), 1630 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 7.55 (1H, d, *J*=1.83 Hz, H^4), 7.34 (1H, d, *J*=8.55 Hz, H^7), 7.15(1H, dd, *J*=8.54 Hz; 2.44 Hz, H^6), 7.07-7.03 (2H, m, phenyl-$H^{2,6}$), 6.97-6.94 (2H, m, phenyl-$H^{3,5}$), 4.04 (2H, t, *J*=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.58-3.54 (4H, m, piperazine-$H^{2,6}$), 3.05 (2H, t, *J*=4.88 Hz, piperazine-$H^{3(5)}$), 3.00 (2H, t, *J*=4.89 Hz, piperazine-$H^{5(3)}$), 2.87 (2H, t, *J*=7.02 Hz, $CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(2-flourophenyl)piperazine (5d)

IR (KBr) υ_{max} (cm^{-1}): 1784 (C=O, lactam), 1634 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 7.55 (1H, d, *J*=1.83 Hz, H^4), 7.34(1H, d, *J*=8.55 Hz, H^7), 7.16 7.09 (3H, m, phenyl-$H^{3,4}$, H^6), 7.02-6.97 (2H, m, phenyl-$H^{5,6}$), 4.05 (2H, t, *J*=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.603.56 (4H, m, piperazine-$H^{2,6}$), 2.97 (2H, t, *J*=4.88 Hz, piperazine-$H^{3(5)}$), 2.93 (2H, t, *J*=4.88 Hz, piperazine-$H^{5(3)}$), 2.87 (2H, t, *J*=7.02 Hz, $CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(4-chlorophenyl)piperazine (5e)

IR (KBr) υ_{max} (cm^{-1}): 1764 (C=O, lactam), 1629 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 7.55 (1H, d, *J*=2.45 Hz, H^4), 7.34 (1H, d, *J*=8.55 Hz, H^7), 7.23 (2H, dd, *J*=2.14 Hz; 4.89Hz, phenyl-$H^{3,5}$), 7.15 (1H, dd, *J*=8.54 Hz; 2.44 Hz, H^6), 6.94 (2H, dd, *J*=2.14 Hz; 4.88 Hz, phenyl-$H^{2,6}$), 4.04 (2H, t, *J*=6.72 Hz, $\underline{CH_2}CH_2CO$),

3.57-3.53 (4H, m, piperazine-$H^{2,6}$), 3.12 (2H, t, J=4.88 Hz, piperazine-$H^{3(5)}$), 3.07 (2H, t, J=5.19 Hz, piperazine-$H^{5(3)}$), 2.87 (2H, t, J=6.72 Hz, $CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(3-chlorophenyl)piperazine (5f)

IR (KBr) υ_{max} (cm^{-1}): 1784 (C=O, lactam), 1634 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 7.55 (1H, d, J=1.83 Hz, H^4), 7.34 (1H, d, J=8.55 Hz, H^7), 7.21 (1H, t, J=8.24 Hz, phenyl-H^5), 7.15(1H, dd, J=8.54 Hz; 1.83 Hz, H^6), 6.95 (1H, t, J=2.14 Hz, phenyl-H^2), 6.89 (1H, dd, J=2.44 Hz; 6.11 Hz, phenyl-H^6), 6.80 (1H, dd, J=1.84 Hz, 6.10 Hz, phenyl-H^4), 4.04 (2H, t, J=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.57-3.53 (4H, m, piperazine-$H^{2,6}$), 3.18(2H, t, J=4.89 Hz, piperazine-$H^{3(5)}$), 3.13 (2H, t, J=4.88 Hz, piperazine-$H^{5(3)}$), 2.87 (2H, t, J=7.02 Hz, $CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(3-trifluoromethylphenyl) piperazine (5g)

IR (KBr) υ_{max} (cm^{-1}): 1774 (C=O, lactam), 1645 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 7.55 (1H, d, J=2.44 Hz, H^4), 7.42 (1H, t, J=7.93 Hz, phenyl-H^5), 7.34 (1H, d, J=7.55 Hz, H^7), 7.22 (1H, dd, J=1.83 Hz; 6.71 Hz, phenyl-H^4), 7.17 (1H, s, phenyl-H^2), 7.15 (1H, dd, J=2.44 Hz, 6.10 Hz, phenyl-H^5), 7.09 (1H, d, J=7.93 Hz, phenyl-H^6), 4.04 (2H, t, J=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.59-3.55 (4H, m, piperazin-$H^{2,6}$), 3.23 (2H, t, J=4.88 Hz, piperazine-$H^{3(5)}$), 3.18 (2H, t, J=4.88 Hz, piperazine-$H^{5(3)}$), 2.88 (2H, t, J=7.02 Hz, $CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(2-pyridyl)piperazine (5h)

IR (KBr) υ_{max} (cm^{-1}): 1785 (C=O, lactam), 1642 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 8.11 (1H, dd, J=1.22 Hz; 3.66 Hz, pyridyl-H^6), 7.55 (1H, d, J=1.83 Hz, H^4), 7.53 (1H, dd, J=1.83 Hz; 5.49 Hz, pyridyl-H^4), 7.34 (1H, d, J=8.55 Hz, H^7), 7.16 (1H, dd, J=8,54 Hz; 2,44 Hz, H^6), 6,82 (1H, d, J=9.16 Hz, pyridyl-H^3), 6.65 (1H, dd, J=5.49 Hz; 1.22 Hz, pyridyl-H^5), 4.04 (2H, t, J=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.55-3.52 (4H,m, piperazin-$H^{2,6}$), 3,46-3,44 (4H, m, piperazine-$H^{3,5}$), 2.87 (2H, t, J=7.02 Hz,$CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-(2methoxyphenyl)piperazine (5i)

IR (KBr) υ_{max} (cm^{-1}): 1760 (C=O, lactam), 1642 (C=O, amide), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 7.54 (1H, d, J=2.45 Hz, H^4), 7.33 (1H, d, J=8.55 Hz, H^7), 7.14 (1H, dd, J=8.23; 1.83 Hz, H^6), 6.97-6.91 (2H, m, phenyl-$H^{3,4}$), 6.87-6.82 (2H, m, phenyl-$H^{5,6}$), 4.03 (2H, t, J=7.02 Hz, $\underline{CH_2}CH_2CO$), 3.76 (3H, s, OCH_3), 3.55 (2H, t, J=4.89 Hz, piperazin-$H^{2(6)}$), 3.52 (2H, t, J=4.88 Hz, piperazin-$H^{6(2)}$), 2.89 (2H, t, J=4.89 Hz, piperazine-$H^{3(5)}$), 2.85 (4H, m, piperazine-$H^{5(3)}$, $CH_2\underline{CH_2}CO$).

1-[3-(5-Chloro-2(3*H*)-benzoxazolon-3-yl)propanoyl]-4-piperonylpiperazine (5j)

IR (KBr) υ_{max} (cm^{-1}): 1777 (C=O, lactam), 1635 (C=O, amide), ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 7.53 (1H, d, J=2.11 Hz, H^4), 7.35 (1H, d, J=7.81 Hz, H^7), 7.24 (1H, s, piperonyl-H^2), 7.16- (1H, m, H^6), 6.98 (2H, m, piperonyl-$H^{5,6}$), 6.06 (2H, s, O-CH_2-O), 4.20 (2H, s, N-CH_2-C_6H_3), 4.03 (2H, t, J=4.88 Hz, $\underline{CH_2}CH_2CO$, piperazin-$H^{2(6)}$), 3.69 (2H, t, J=4.89Hz, piperazin-$H^{6(2)}$), 2.85 (6H, m, piperazin-$H^{3,5}$, $CH_2\underline{CH_2}CO$).

Pharmacology

Albino mice weighting 25-30 g were used in the present study. The laboratory temperature was maintained at 20±1°C under a 12 h light-dark schedule. Before the experiment, the mice were allowed 1 week adaptation, and they were used only once. The study was approved by the Ethics Committee of Osmangazi University, Medical School.

The animals were divided into 13 groups. Each group included eight animals. All compounds were dissolved in DMSO/water (1:4) and were given to the animals intraperitoneally (i.p.) at 100 mg/kg doses. The control animals received 0.1 mL DMSO/water i.p. (1:4). The compounds were dissolved in 20% DMSO in order to increase their solubility and absorption and to avoid the possible central activity of pure DMSO. 20% DMSO was also injected into the control animals (Önkol *et al.*, 2001, 2002). In order to compare the activity of aspirin at the same dosage, 100 mg/kg was used as the dosage for the compounds. 100 mg/kg dose is the usual analgesic and anti-inflammatory dose of aspirin.

The tail clip test, tail flick test to radiant heat, hot plate test, and writhing test induced by acetic acid were performed 60 min after the administration of the compounds or vehicle (DMSO for control group).

Tail clip test

This analgesic was based on the method that is described in the literature. A pressure-standardized artery clip was placed placement by turning or biting at the clip within 15 s were used in this test (Biancchi *et al.*, 1954; Dajani *et al.*, 1999).

Tail flick test to radiant heat

This test described by DAmour and Smith (DAmour *et al.*, 1941) was performed with a beam of high-intensity light focused on the dorsal surface of the tail. The response latency between the onset of the radiant heat stimulus and the movement of the tail out of light beam of the apparatus (MAY produced in Turkey) was determined. The light intensity was set to provide a predrug response time of 2-4 s. A cut-off of 15 s was used in order to

prevent damage to the tail.

Hot plate test

A glass cylinder (16 mm in height 16 mm in diameter) was used to keep the mouse on the heated surface of the plate which was kept at a temperature of 55±0.5°C through the use of a thermo-regulated water-circulating pump. The latency period until the mouse licked a foot or jumped was registered by the means of a stopwatch (cut-off time 45 s.) (Eddy *et al.*, 1953; Noble *et al.*, 1994).

The results were expressed as a percentage of the maximal possible effect (% MPE±S.D.), which is defined by the following equation.

$$\%MPE = \frac{(\text{post drug latency-predrug latency})}{(\text{cutoff time-predrug latency})} \times 100$$

Writhing test

The abdominal constrictor test (Koster *et al.*, 1959) was performed by the IP application of 0.6% acetic acid (60 mg/kg), and stretching movements (arching of the back, development of tension in the abdominal muscles, elongation of the body, and extension of forelimbs) were counted over a period of 10 min starting 5 min after the i.p. administration of acetic acid.

All tests were conducted between 9 and 12 a.m., and all results were expressed as the mean±S.D. Statistical comparisons were performed by using the Student's *t*-test.

RESULTS AND DISCUSSION

Chemistry

The synthetic route of the title compounds is illustrated in Scheme 1. Syntheses of the compounds were started by obtaining 5-chloro-2(3*H*)-benzoxazolone (**1**). Synthesis of (5-chloro-2-benzoxazolone-3-yl)propannitrile (**2**), (5-chloro-2-benzoxazolone-3-yl) propanoic acid (**3**), and (5-chloro-2-benzoxazolone-3-yl)propanoyl chloride (**4**) have previously been reported (Onkol *et al.*, 2002; Zinner *et al.*, 1966; Fujii, 1958). The acid chlorides have been reacted with piperazine derivatives in tetrahydrofuran to form the title propanamide derivatives.

The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR spectral data.

Pharmacology

In the testing of biological activity, antinociceptive activities of the compounds were determined by utilizing the tail clip test, tail flick test to radiant heat, hot plate test, and writhing test. Because it has been reported that aspirin-like drugs (Bannwarth *et al.*, 1995), especially dipyrone (Akman *et al.*, 1996; Aguirre-Banuelos *et al.*, 2000), also show a central analgesic effect, the tail clip test to pressure, tail flick to radiant heat, and hot plate test were used to test for central antinociceptive activity. Peripheral activity was evaluated using the acetic acid-induced stretching test (Koster *et al.*, 1959). All compounds had both the central and peripheral antinociceptive activities, and their activities were greater than that of dipyrone which was administered at the same dose. High MPE % values were obtained in three central antinociceptive activities. The results were shown in Table II.

Table II. Antinociceptive activity of compounds **5a-j**

Comp.	Tail clip test % MPE	Tail flick test % MPE	Hot plate test % MPE	AcOH Stretching number
Control	44.3 ± 16.8	31.8 ± 15.4	2.0 ± 0.7	33.5 ± 9.8
5a	57.9 ± 16.9	54.8 ± 16.4*	7.3 ± 1.8	15.2 ± 13.6*
5b	46.0 ± 18.0	76.1 ± 11.9*	14.4 ± 5.3*	10.3 ± 7.6*
5c	88.7 ± 11.2*	35.9 ± 14.5	9.5 ± 2.6*	14.1 ± 8.7*
5d	97.3 ± 7.6*	44.2 ± 16.2	3.3 ± 1.9	21.4 ± 17.2
5e	92.4 ± 5.8*	57.7 ± 13.1*	12.2 ± 2.9*	6.8 ± 4.3*
5f	95.4 ± 13.0*	62.0 ± 15.4*	8.3 ± 2.5*	21.7 ± 10.7
5g	76.5 ± 14.1*	87.5 ± 12.5*	5.4 ± 1.8*	17.8 ± 11.4*
5h	82.4 ± 13.2*	62.9 ± 14.5*	10.9 ± 5.2*	12.8 ± 11.7*
5i	49.0 ± 13.6	18.8 ± 10.5	11.4 ± 2.9*	15.9 ± 12.8*
5j	72.5 ± 12.5*	33.2 ± 12.4	3.4 ± 1.7	38.0 ± 13.7
Dipyrone	68.2 ± 12.4*	40.5 ± 5.0*	4.4 ± 1.6*	13.5 ± 1.6*
Aspirin	65.5 ± 11.2*	47.3 ± 6.5*	8.1 ± 3.5*	18.2 ± 4.3*

*p<0.05; MPE: Maximum possible effect; all values are given as X±SD

According to the results, it is evident that all the compounds exhibited antinociceptive activities in the tail clip test. Compounds **5a**, **5b**, and **5i** have not shown any significant antinociceptive activity in this test, but the others compounds have exhibited higher activities than dipyrone and aspirin. The highest antinociceptive activity in the tail clip test was illustrated by compounds **5d**, **5e**, and **5f**.

In the tail flick test, compounds **5c**, **5d**, **5i**, and **5j** have not shown any significant antinociceptive activity. On the other hand, the other compounds resulted in a significant level of activity when compared with the control group and dipyrone. In this test, compound **5g** is found to show the highest antinociceptive activity.

Compounds **5a**, **5d**, and **5j** were found to not be sufficiently active in the hot plate test. The other compounds have shown higher activity levels than dipyrone and the control group in the same test. In addition, compound **5b** has a two-fold potent antinociceptive activity than dipyrone in the test at the same dose level.

In the writhing test, compounds **5d**, **5f**, and **5j** did not exhibit any significant activity when compared with the

control group and standard compounds. Compounds **5a**, **5c**, and **5i** have shown antinociceptive activity compared to aspirin. Compounds **5b**, **5e**, and **5h** have exhibited the most antinociceptive activity when compared with aspirin and the control group. Therefore, one can say that these compounds have aspirin-like antinociceptive activity.

Compound **5f** showed results of high activity in the tail clip, tail flick, and hot plate test. Therefore, this compound was thought to be centrally active as an analgesic agent.

In addition, compounds **5e**, **5g**, and **5h** have been found active in the all tests when compared with the control group and standard compounds. These results might prompt us to say that these three compounds can possibly be both central acting analgesic agents and peripheral acting analgesic agents.

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