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Synthesis and analgesic activities of some new 5-chloro-2(3H)-benzoxazolone derivatives

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Abstract

Affordable and practical synthesis methods in drug development have always been very attractive. Herein, microwave assisted synthesis was utilized to prepare piperazine substituted 5-chloro-2(3H)-benzoxazolone derivatives in 5 minutes. Structural characterization of these 5-chloro-2(3H)-benzoxazolone derivatives was achieved by IR, NMR, ESI-MS and elemental analysis. Since these types of compounds have been shown to have anti-inflammatory and analgesic activities there biological activities were also examined. Indomethacin (INDO) and acetylsalicylic acid (ASA) were used as reference. Carrageenan-induced hind paw edema in mice test was used to study anti-inflammatory activity. Compound **1** (100 mg / kg dose) showed the longest anti-inflammatory activity among the title compounds synthesized. For the analgesic activities, both hot-plate and tail-flick tests were employed. Compound **3** was found to have the highest activity in the hot-plate test whereas in the tail-flick test, compounds **1** and **2** showed higher anti-nociceptive activity.

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Introduction

Even though nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used pain relievers in the world and find a broad spectrum of uses in the treatment of diseases, they possess serious side effects which may cause gastroduodenal ulcers, and gastrointestinal bleeding (1, 2). Therefore, new analgesic drugs with minimal side effects are desired.

Many NSAIDs available on the market are nonselective COX inhibitors (do not show selectivity between COX-1 and COX-2 enzymes). The adverse effects of NSAIDs are thought to be due to the inhibition of COX-1 enzyme (3). Therefore, a molecule which will selectively and specifically inhibit COX-2 enzyme will be a safer and more effective drug (4). As a result, new molecules showing selective COX inhibition is preferred for development of anti-inflammatory drugs.

2(3H)-Benzoxazolone derivatives are considered ideal scaffolds for synthesis of drug candidates (5). They have been of interest in medicinal chemistry since they are readily available, affordable, susceptible to chemical modifications and most importantly exhibit wide range of biological activities. Their pharmacological activities include antibacterial, antifungal, analgesics-antiinflammatory, anti-nociceptive, antiulcer, anticancer, and anti-HIV (6-18).

Benzoxazolinones bearing arylpiperazine substitutents have been of interest since they could affect central nervous system (6, 19). All these findings led us to synthesize three new 3-arylpiperazine-5-chloro benzoxazolone derivatives via the Mannich reaction, and their analgesic and anti-inflammatory activities were studied.

Materials and Methods

Chemical Methods

All chemicals and reagents were obtained from Sigma Aldrich Chemical Co. or Riedel Chemical Co. and were used without further purification. Melting point of the compounds was recorded on the Mettler Toledo FP 900 Thermo System Digital melting point apparatus and the values are uncorrected. The FT-IR spectra of the compounds were recorded on a Perkin Elmer Spectrum 100 spectrophotometer with attenuated total reflection (ATR) (in wave numbers) in cm^{-1}. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded on a Mercury Varian 400 MHz NMR Spectrometer using deuterated chloroform ($CDCl_3$) as solvent. Chemical shifts (δ) values were reported in parts per million (ppm). Elemental analyses (C, H, N) were performed on Leco CHNS 932 analyzer. Mass spectra were taken on a Micromass ZQ MS Spectrometer with electron ionization (ESI). The purity of the compounds was assessed by TLC on silica gel GF 254 (DC-Alufplien-Kieselgel, Germany). Microwave irradiation was carried out with a microwave reactor (MicroSYNTH, Milestone, Italy).

General procedures for the synthesis of piperazine derivatives under reflux condition (Method A)

Synthesis was carried out according to the previously published procedure (20). 15 mmol of 5-chloro-2-benzoxazolinone and 15 mmol of piperazine derivative were dissolved in 10 ml of methanol followed by addition of 20 mmol formalin (35% w/v). The reaction mixture was then refluxed in a water bath for 1 hour. The mixture was poured onto crushed ice and the resulting precipitate was filtered off, washed with cold methanol, dried and purified by recrystallization using ethanol as a solvent.

General procedures for the synthesis of piperazine derivatives under microwave condition (Method B)

15 mmol of 5-chloro-2-benzoxazolone and 15 mmol of piperazine derivative were dissolved in 10 ml of methanol followed by addition of 20 mmol formalin (35% w/v). The reaction mixture was irradiated (100 W, 65 °C) for 5 min in a microwave reactor. The mixture was poured onto crushed ice and the resulting precipitate was filtered off, washed with cold methanol, dried and purified by recrystallization using ethanol as a solvent.

5-Chloro-3-{[4-(2-fluorophenyl)]piperazino-1-yl}methyl-2-benzoxazolone (1)

White solid (method A: 31, method B: 46, yield %); mp 153.5 °C. IR (cm^{-1}) 2809-3020 (C-H), 1765 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$), d (ppm): 7.2-6.9 (m, 7 H, Ar-C *H*), 4.7 (s, 2 H, C H_2), 3.1 (t, 4 H, *J* = 4.8 Hz, pip-C H_2 H^2, H^6), 2.9 (t, 4 H, *J* = 4.8 Hz, pip-C H_2 H^3, H^5); ^{13}C NMR (100 MHz, $CDCl_3$) d156.9, 155.0, 154.5, 141.0, 139.8, 139.7, 132.8, 129.4, 124.5, 124.4, 122.8, 122.7, 122.6, 119.08, 119.05, 116.2, 116.0, 110.8, 109.9 (Ar-*C*), 64.8 (CH_2), 50.6, 50.3 (pip-*C*). MS: m / z: 193.16 (100%), 233.15 (52%), 247.16 (50%), respectively. Anal. Calc. for $C_{18}H_{17}ClFN_3O_2$ C, 59.76; H, 4.74; N, 11.61; Found C, 59.61; H, 4.65; N, 11.90.

5-Chloro-3-{[4-(2-methoxyphenyl)]piperazino-1-yl}methyl-2-benzoxazolone (2)

White solid (method A: 66, method B: 63, yield %); mp 161.5 °C. IR (cm^{-1}) 2799-3025 (C-H), 1778 (C=O). ^{1}H NMR (400 MHz,$CDCl_3$), d (ppm): 7.2-6.8 (m, 7H, Ar-C *H*), 4.7 (s, 2 H, C H_2), 3.8 (s, 3 H, OC H_3), 3.1 (t, 4 H, *J* = 4.8 Hz, pip-C H_2 H^2, H^6), 2.9 (t, 4 H, *J* = 4.8 Hz, pip-C H_2 H^3, H^5); ^{13}C NMR (100 MHz, $CDCl_3$) d 155.1, 152.2, 141.0, 133.0, 129.3, 124.5, 123.2, 122.5, 120.9, 118.3, 110.8, 109.9 (Ar-*C*), 64.8 (CH_2), 55.3 (OCH_3), 50.7, 50.4 (pip-*C*). MS: m / z 205.19, (100%), 244.17 (90%), 190.16 (83%), respectively. Anal. Calc. for $C_{19}H_{20}ClN_3O_3$ C, 61.04; H, 5.39; N, 11.24; Found C, 60.68; H, 5.36; N, 11.41 %.

5-Chloro-3-{[4-(2-pyrimidine)]piperazino-1-yl}methyl-2-benzoxazolone (3)

White solid (method A: 56, method B: 68, yield %); mp 173.4 °C. IR (cm^{-1}) 2860-2941 (C-H), 1774 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$), d (ppm): 8.28 (d, 2 H, *J* = 4.8 Hz, pyr-C *H* H^8, H^{10}), 7.2-7.0 (m, 3 H, Ar-C*H*), 6.47 (t, 1 H, *J* = 4.7 Hz, pyr-C *H* H^9), 4.7 (s, 2 H, CH_2), 3.85 (t, 4 H, *J* = 5.2 Hz, pip-C H_2 H^2, H^6), 2.75 (t, 4 H, *J* = 5.2 Hz, pip-C H_2 H^3, H^5); ^{13}C NMR (100 MHz, $CDCl_3$) d 161.4, 157.7, 154.9, 141.0, 132.6, 129.4, 122.6, 110.8, 110.1, 109.9 (Ar-*C*), 64.9 (CH_2), 50.4, 43.4 (pip-*C*). MS: m / z: 177.15 (100%), 148.11 (72%), 122.04 (68%), respectively. Anal. Calc. for $C_{16}H_{16}ClN_5O_2$ C, 55.58; H, 4.66; N, 20.25; Found C, 55.32; H, 4.55; N, 20.49 %.

Biological Methods

For the anti-inflammatory activity test, 42 Swiss albino mice in either sex were randomly divided into 6 groups. For analgesic activity tests another 6 groups of mice were chosen. The animals were weighed (27.2 ± 3.1 g) and thereafter fasted overnight before the experiments, with free access to water.

Anti-inflammatory activity

Carrageenan-induced hind paw edema model

On the day of experiment, all the drugs were suspended in freshly prepared 0.5% carboxymethyl cellulose (CMC). Indomethacin (INDO) and aspirin (ASA) were used as reference compounds. The vehicle, INDO (50 mg/kg), ASA (100 mg/kg), and test compounds (100 mg/kg for each) were administered by oral gavage in a volume of 0.1 ml. One hour later 2% carrageenan (0.01 ml) were injected into the right hind paws of mice in order to induce acute inflammation and the paw volume, which shows edema, was measured using a mechanical caliper just before, and 2 , 4 and 6 hour after the administration of the drug. The thickness of the paws was measured by a dial thickness gauge (0.01-1 mm, Ozaki Co., Japan) immediately before (T0) the injection, and after that at 2, 4 and 6 hours (Tt). The edema was calculated as the increase in thickness (mm) of the paw after treatment subtracted from

the starting paw volume (ΔT=Tt-T0). For each animal, edema inhibition was expressed as the percentage of their control as shown in the Equation 1 below:

Anti-inflammatory Activity (%) = [(Control Δ T-Test Δ T) / Control Δ T] × 100 Eq. (1)

Gastric ulceration studies

After the carrageenan-induced paw edema test, animals were decapitated following exposure to CO_2. Stomachs of mice were then isolated and screened for any lesions in their gastric mucosa under a dissecting microscope. The results were compared with those of a group of mice used as the control group, which were given only 0.5% CMC. Gastric mucosal lesions were scored according to their number and size in a scale from 0 to 7 points (25). This study was approved by Hacettepe University Ethics Committee (No: 2015:38-1).

Analgesic activity

Tail-flick method

An automated tail-flick apparatus (Model TF 0703, 8 V/50 W, Commat Ltd, Ankara) that elicits a flick reflex when a radiant heat is applied on a selected spot on the tail was used. Each mouse was gently held and an automatic timer recorded the tail-flick latency until the mouse flicks its tail away from the source of the light. The latency was recorded with a sensitivity of 0.01 sec. The heat stimulus was set to provide a pre-drug tail-flick latency time of 6–8 sec. The maximum tail-flick latency of 15 sec was used to avert tissue damage to the tail.

Hot-plate method

The hot-plate latency was assessed by placing the mouse on a metal surface maintained at 50 ± 0.5 °C (Model 9601 Analgesic Hot Plate, Commat Ltd, Ankara). The mouse was observed closely by using two external mirrors showing each way during the measurement period. The licking of the paws or the jump response was evaluated as the end point. As soon as the hot-plate latency was recorded, the mouse was removed from the plate. The maximum latency was 15 sec. to avoid tissue damage.

Evaluation of the test results

After the baseline readings (*T*0) were taken, the mice were treated with 0.5% CMC (0.1 ml) as a vehicle, oxycodone (OX; 100 mg/kg) or ASA (100 mg/kg) as reference drugs, or the test compounds (100 mg/kg for each). Response latencies (*T*1) were measured 2 hours after the drug application.

The anti-nociceptive effects of the drugs were described as the fraction of the difference between the cut-off time (*T*2) and the baseline measurement (*T*1) (27) and calculated as the Maximum Possible Effect (MPE) using the Equation 2 below:

MPE (%) = (*T*1 – *T*0) / (*T*2 – *T*0) × 100 Eq. (2)

The maximum value is 100 %, which equals the cut-off time (*T*2: 15 s).

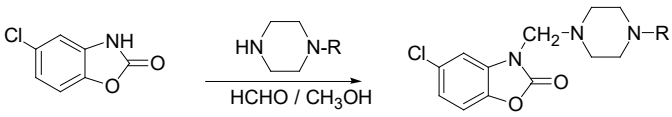
Statistics

Repeated measurements on the same group were presented as Mean ± SE of the mean. Statistical significance was determined using One-sample *t* test for column statistics using the GraphPad Software (Prism-5). $P<0.05$ was considered as significantly different.

Results and Discussions

Chemistry

5-chloro-2(3H)-benzoxazolone was reacted with piperazine derivatives via Mannich reaction to form 3-substituted-5-chloro-2(3H)-benzoxazolone compounds according to the **Fig. 1**. Two different methods were used in the synthesis of these compounds; conventional method as previously reported in the literature (20) and microwave assisted heating method which is a much faster and a convenient synthesis technique. The structures of these compounds obtained from both methods were characterized by IR, ^1H and ^{13}C NMR, ESI-MS and elemental analysis.



R= 2-fluorophenyl (**1**), 2-methoxyphenyl (**2**), 2-pyrimidyl (**3**)

Figure 1. Synthesis of title compounds.

Spectral data of the compounds obtained by both synthesis methods match to the proposed structures. The absence of absorption band at 3100-3400 cm^{-1} in the IR spectra shows the disappearance of N-H band of the 5-chloro-2(3H)-benzoxazolone ring and piperazine derivative upon addition reaction. As expected at 1769 – 1780 cm^{-1}, the lactam (C=O) stretching band was observed. ^1H NMR spectra for all compounds show methylene protons between two nitrogen atoms as a singlet at 4.7 ppm. The piperazine protons (H_6 and H_2) and (H_3 and H_5) were seen as triplets at 3.1 and 2.9 ppm respectively for compounds 1 and 2. On the other hand, the chemical shift of the piperazine protons were observed at 3.8 and 2.7 ppm for compound 3, due to the presence of the aromatic pyrimidyl ring. Additionally, for compound 2, there is a peak at 3.8 ppm due to the presence of OCH_3 protons. Further investigations of ^1H-NMR spectra revealed the aromatic protons as multiplets between 6.8 to 7.2 ppm as expected for compounds 1 and 2. Additionally, for compound 3, the aromatic protons due to pyrimidine ring were observed at 8.3 ppm and 6.4 ppm. The ESI-MS analysis of the compounds do not show the [M]+ molecular ion peaks but reveal [M-167. 99]+ fragment as base peak indicating the loss of 5-chloro-2(3H)-benzoxazolone core structure from the target molecules.

Table 1. Percent inhibition of carrageenan-induced paw edema by compounds 1-3 and the mean ulceration scores of these compounds

Swelling in thickness (mm) ± SEM (inhibition %)					Mean Score of Ulceration
Compound No	control	2 hours	4 hours	6 hours	
vehicle	1.802 ± 0.054	2.282 ± 0.085	2.347 ± 0.08	2.2375 ± 0.074	0.42 ± 0.2
1	1.750 ± 0.028	2.025 ± 0.027 * (42.71 %)	2.025 ± 0.033 ** (49.54 %)	1.990 ± 0.038 (44.82%)	* 2.28 ± 0.52
2	1.735 ± 0.026	2.120 ± 0.033 * (19.79 %)	2.110 ± 0.054 * (31.19 %)	2.125 ± 0.07 (10.34 %)	* 1.71 ± 0.18
3	1.745 ± 0.022	2.150 ± 0.044 * (15.25 %)	2.108 ± 0.041 * (33.39 %)	2.165 ± 0.058 (3.44 %)	* 1.71 ± 0.42
INDO (50 mg/kg)	1.736 ± 0.025	2.090 ± 0.012 * (26.25 %)	2.050 ± 0.041 ** (42.38 %)	2.092 ± 0.064 (18.16 %)	** 4.10 ± 0.58
ASA (100 mg/kg)	1.733 ± 0.041	2.081 ± 0.057 * (27.5 %)	2.211 ± 0.074 * (12.29 %)	2.193 ± 0.066	* 2.57 ± 0.57

INDO: indomethacin; ASA: Acetylsalicylic acid ; * The group is significantly different from vehicle group; ** The group is significantly different from ASA group

Pharmacology

The in vivo anti-inflammatory activity of the compounds was tested in the carrageenan-induced hind paw edema model in mice (21). Analgesic potency of the compounds was examined by using tail-flick and hot-plate tests (22, 23). Compared to the carboxymethyl cellulose (CMC) vehicle group, each compound showed marked inhibition of carrageenan-induced hind paw edema which is a measure of anti-inflammatory activity as given in the Table.

In this study, indomethacin (INDO) and acetylsalicylic acid (ASA) were used as reference drugs for non-selective COX inhibitors. As INDO is considerably known to be more potent anti-inflammatory drug compared to ASA, it was used at a lower concentration than ASA. Two hours after the mice were treated with 50 mg/kg of INDO (n= 9) and 100 mg/kg of ASA (n=6), similar inhibitory response on paw edema was observed. Each of the title compounds (100 mg/kg dose) produced a significant inhibition of carrageenan-induced paw edema after 2 hours as reference drugs did. The inhibitory effect of NSAIDs, such as INDO, is usually weak in the first phase (1-2 hours), in contrast with their strong inhibition in the second phase (3-4 hours) (24). All the compounds' effect lasted at least 4 hours similar to INDO, whereas ASA weakly lost its anti-inflammatory effect in the second phase. Compound 1 was the longest-acting derivative among the title compounds synthesized. Its effect for the relief of edema lasted even in the 6th hour after the treatment. Other two compounds, 2 and 3, also showed strong anti-inflammatory effects with better profiles than that obtained with ASA, even if these effects did not reach to the significance level.

The NSAIDs are associated with a significant incidence of gastritis and/or peptic ulceration. Therefore we also investigated the compounds for gastric ulceration as well. None of the new compounds led to gastric ulceration according to the scale given (25). The mice treated with title compounds and ASA showed a few petechial hemorrhages or alterations of villous, however the ulcerogenic effect was much weaker than INDO. Compounds 1 (100 mg/kg) and 3 (100 mg/kg) had similar gastric side effects compared with ASA (100 mg/kg), whereas 2 (100 mg/kg) showed relatively lower ulcerogenic effects, although it did not reach to significance level as seen from the **Table 1**.

Anti-inflammatory activity of the compounds synthesized was in parallel with their corresponding analgesic activities. All of the com pounds elicited a significant anti-nociceptive activity compared to the CMC group in both tail-flick and hot-plate models as shown in the **Fig. 2**.

Maximum possible effect (MPE) values of the new compounds were in the range of 26% and 42% at 100 mg/kg dose in the tail-flick test. Compound 1 and 2 exhibited better maximum possible MPE (40% and 42%, respectively) than ASA did (23%), whereas 3 was similar to it (26%). However, they did not show analgesic activity as strong as oxycodone (OX) (74%).

Similar results have also been obtained with the hot-plate test. All of the compounds were found effective in the treatment for heat-induced superficial pain. OX, the opioid receptor agonist, was the most effective agent on the nociception and none of the compounds' analgesic activities, including ASA, reached to the analgesic activity of OX. It is known that tail-

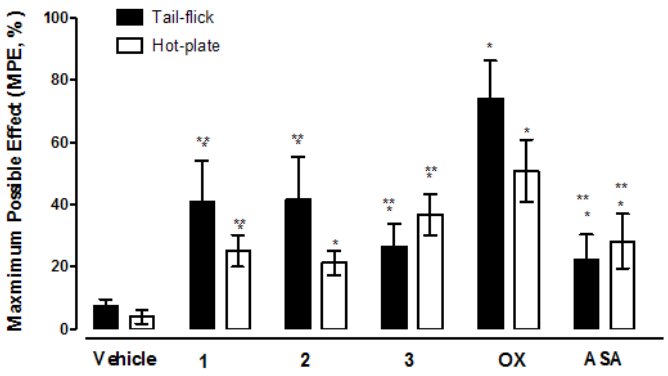


Figure 2. Anti-nociceptive effects of vehicle, OX (100 mg/kg), ASA (100 mg/kg) and compounds 1-3 (100 mg/kg) in mice measured with tail-flick (n=7-9) and hot-plate methods (n=7-8). * Indicates the group is significantly different from the vehicle group, ** indicates the group is significantly different from the OX group.

flick test shows a higher sensitivity of the spinally mediated reflex responses. In the tail-flick test the response latency increases more easily to the cut-off latency than the hot-plate test (26). A similar result was obtained with reference drug OX (100 mg/kg) under same experimental conditions. In the tail-flick test, OX response exceeded cut-off latency in 4/7 mice, whereas it did not pass this limit in the hot-plate test at the same dose. These results were consistent with the literature and proved the accuracy of the tests performed. When the results of both tests were evaluated together, it can be said that compounds 1 and 2 had better analgesic profile in the tail-flick test, and 3 in the hot-plate test.

Conclusion

In conclusion, all compounds revealed promising antiinflammatory activities. Compound **1** with 2-fluorophenyl piperazine substituent had the longest activity where it still showed activity even 6 hours after the treatment. Antinociceptive activities of compounds **1** and **2** where there is a substituent on the 2- position of the phenyl ring of piperazine mioety were significant with 40% MPE values as observed from the tail-flick test. Compound **3**, with pyrimdylpiperazine, showed good analgesic activity with about 35% MPE. Based on these results, synthesis of similar compounds with different piperazine substituents are planned to evaluate the structure activity relationships of these compounds.

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Conflict of interest statement

The authors confirm that this article content has no conflict of interest.

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