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Synthesis and Antinociceptive Activity of 2-[(2-Oxobenzothiazolin-3-yl)methyl]-5-aminoalkyl/aryl-1,3,4-thiadiazole

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Ten 2-[(2-oxobenzothiazolin-3-yl)methyl]-5-aminoalkyl/aryl-1,3,4-thiadiazole derivatives were synthesized. The chemical structures of these compounds were elucidated by their FT-IR and ^{1}H-NMR spectral data, as well as their elemental analyses. The compounds were tested for antinociceptive activity. Among these compounds, 2-[(2-oxobenzothiazolin-3-yl)methyl]-5-aminomethyl-1,3,4-thiadiazole **(5a)** was found to be significantly more active than the others and the standards in all the tests.

Key Words: 2-Oxobenzothiazoline, 1,3,4-thiadiazole, antinociceptive activity.

Introduction

The search for new analgesic compounds devoid of the side effects typical of morphine-like opioid agonists (respiratory depression, constipation, and physical dependence) as well as of the gastrointestinal irritation and kidney damage associated with nonsteroidal anti-inflammatory drugs has attracted considerable attention in recent years.

2-Oxobenzothiazoline derivatives bearing substituents at position 3 have also been reported to exhibit various pharmacological properties, such as antimicrobial[1], antiallergenic[2], diuretic[3], antihistaminic[4], and anticonvulsant[5] activities.

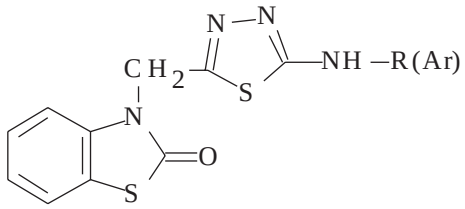
3-Aminoalkyl-2-oxobenzothiazoline derivatives with significant antinociceptive activity were reported in our previous studies[6]. We subsequently synthesized 1-phenyl-2-(2-oxobenzothiazolin-3-yl)ethanones, 1-phenyl-2-(2-oxobenzothiazolin-3-yl)ethanols, and 3-(3-aminopropyl)-2-oxobenzothiazoline and found that some of these compounds possessed noteworthy antinociceptive activity[7,8].

The synthesis of 1,3,4-thiadiazole derivatives, which were investigated for antibacterial[9−11], antifungal[11,12], cardiotonic[13], antitubercular[14,15], and antidepressant[16,17] activities, has been reported previously.

More recently, researchers reported 1,3,4-thiadiazole derivatives that exhibited analgesic and anti-inflammatory activities. 5-Arylamino substituted 3-nicotinoyl/isonicotinoyl-1,3,4-thiadiazol-2(3H)-one, 5-

arylamino-1,3,4-thiadiazol-2(3H)-one, 3-(5-bromo-2-thienyl)-1-phenyl-4-[3-acetyl-5-(N-substitutedacetamido)-2,3-dihydro-1,3,4-thiadiazol-2-yl]-1H-pyrazol, and 2-(2-naphthyloxymethyl)-5-substituted amino-1,3,4-thiadiazole derivatives showed anti-inflammatory and analgesic activities[18−21].

For these reasons, we aimed to incorporate 2-oxobenzothiazoline and 1,3,4-thiadiazole in the same structure. Thus, we synthesized 10 new 2-[(2-oxobenzothiazolin-3-yl)methyl]-5-aminoalkyl/aryl-1,3,4-thiadiazole derivatives (Figure).



Figure

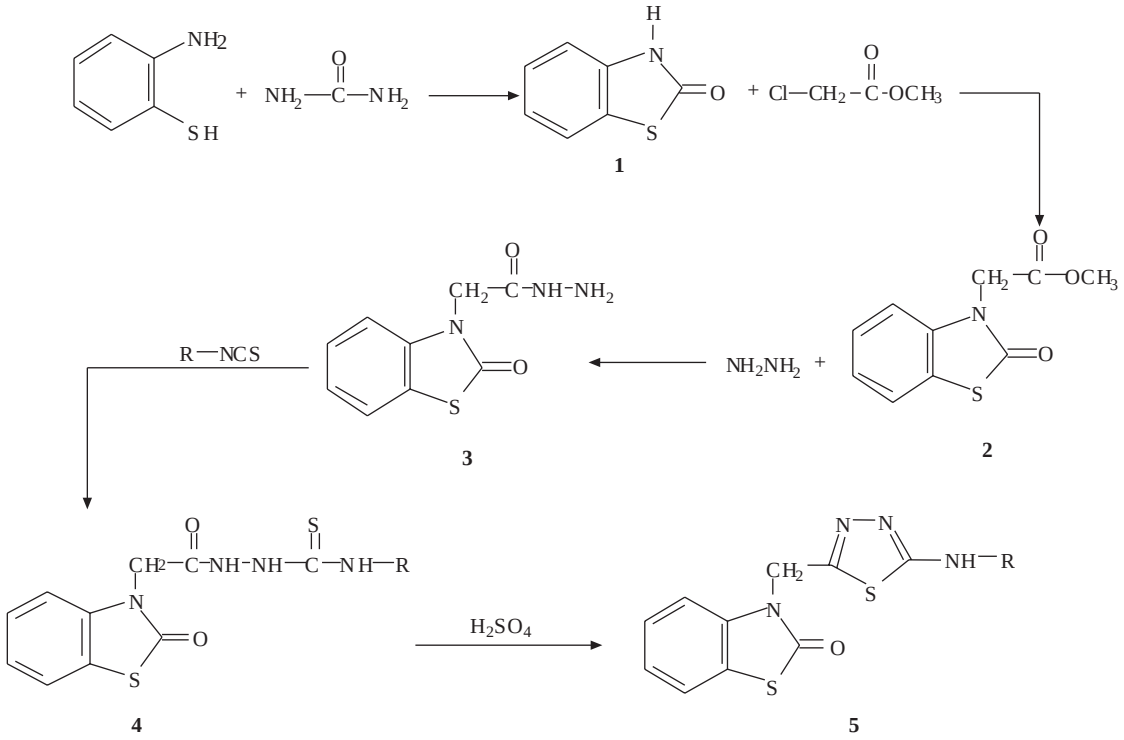
Experimental

All chemicals and solvents used in this study were purchased locally from Merck AG and Aldrich Chemical. Melting points were determined with an Electrothermal-9300 digital melting point apparatus. The IR spectra of the compounds were recorded on a Bruker Vector 22 FT-IR (Opus Spectroscopic Software Version 2.0) spectrophotometer. The ^{1}H-NMR of compounds' spectra was recorded in DMSO-d_6 on a Bruker 400 FT-NMR spectrometer using TMS as the internal standard. All the chemical shifts were recorded as δ (ppm) values. Microanalyses for C, H, N were performed with Leco-932 at the Instrumental Analysis Center of the Scientific and Technical Research Council of Turkey (Ankara, Turkey) and the results were within ± 0.4% of the theoretical values.

Syntheses of 2-oxobenzothiazoline **1**[22], (2-oxobenzothiazolin-3-yl)acetate **2**[23], and (2-oxobenzothiazolin-3-yl)-acetohydrazide **3**[17,23] were previously reported in the literature. We previously reported the synthesis 4-alkyl- and 4-aryl substituted 2-[(2-oxobenzothiazolin-3-yl)acetyl]thiosemicarbazides **4**[24] (Scheme).

Synthesis of 2-[(2-oxobenzothiazolin-3-yl)methyl]-5-alkyl/arylamino-1,3,4-thiadiazol (5a-j)

The appropriate 2-[(2-oxobenzothiazolin-3-yl)acetyl]thiosemicarbazide derivative (2.5 mmol) was added portionwise to H_2SO_4 (5 mL) cooled in an ice bath with constant stirring. After dissolution, the reaction mixture was further agitated for 30 min, poured over crushed ice and neutralized by saturated Na_2CO_3 solution at room temperature. Then the solid material precipitated was filtered, washed with water, dried and crystallized from the corresponding solvents (Table 1). IR and ^{1}H-NMR spectral data of the prepared compounds are given in Table 2.



Scheme. Synthetic route of the title compounds 5a-j.

Pharmacology

Albino mice weighing 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 for adaptation. They were used only once. The study was approved by the Ethics Committee of Osmangazi University Medical School (Eskişehir, Turkey).

The animals were divided into 13 groups. Each group included 8 animals. All compounds were dissolved in DMSO / water (1:4) and given to the animals intraperitonally (i.p.) in 100 mg/kg doses. The control animals received i.p. 0.1 mL of DMSO / water (1:4).

A 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 the control group).

Tail clip test

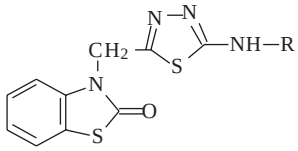
This analgesic activity test was based on a method described in the literature. A pressure-standardized artery clip was placed approximately 2 cm from the base of the tail and only the mice that responded to the clip placement by turning or biting at the clip within 15 s were used in this test[25].

Tail flick test to radiant heat

This test described by D'Amour and Smith[26] 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 the light beam of the apparatus (MAY produced in Turkey) was determined.

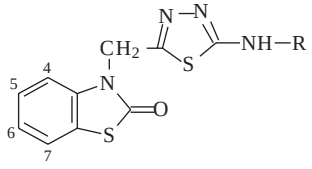
The light intensity was set to provide a predrug response time of 2-4 s. A cut-off of 15 s was used in order to prevent damage to the tail.

Table 1. Synthesized 2-[(2-oxobenzothiazolin-3-yl)methyl]-5-alkyl/arylamino-1,3,4-thidiazol derivatives (5a-j) and their mps, crystallization solvents, yield percentages, and elemental analysis.



Comp.	R	Mp (°C)	Crys. Sol.	Yield (%)	Molecular Formula	Elemental Analysis (Calculated/ Found)
5a	Methyl	176-177	Methanol	55.07	$C_{11}H_{10}N_4OS_2$	C 47.46 / 48.04 H 3.62 / 3.94 N 20.13 / 20.32
5b	Ethyl	218-219	Benzene-Ethanol	73.61	$C_{12}H_{12}N_4OS_2$	C 49.29 / 49.85 H 4.14 / 4.45 N 19.16 / 19.02
5c	Allyl	176-177	Methanol	36.00	$C_{13}H_{12}N_4OS_2$	C 51.30 / 51.37 H 3.97 / 4.02 N 18.41 / 18.45
5d	Cyclohexyl	163-165	Methanol	72.13	$C_{16}H_{18}N_4OS_2$	C 55.47 / 56.00 H 5.24 /5.16 N 16.17 / 16.37
5e	Phenethyl	206-208	Ethanol	32.81	$C_{18}H_{16}N_4OS_2.H_2O$	C 55.94 / 55.52 H 4.69 / 4.23 N 14.50 / 14.36
5f	Phenyl	223-224	Ethanol	30.92	$C_{16}H_{12}N_4OS_2$	C 56.45 /56.75 H 3.55 / 3.73 N 16.46 / 16.57
5g	4-Methylphenyl	199-200	Ethanol	67.04	$C_{17}H_{14}N_4OS_2$	C 57.61 /58.10 H 3.98 /3.82 N 15.81 / 15.97
5h	4-Chlorophenyl	158-159	Benzene-Methanol	59.14	$C_{16}H_{11}ClN_4OS_2$	C 51.26 / 51.69 H 2.95 / 2.48 N 14.95 / 15.11
5i	4-Methoxyphenyl	219-220	Ethanol	31.52	$C_{17}H_{14}N_4O_2S_2$	C 55.12 / 55.45 H 3.81 / 3.79 N 15.12 / 14.81
5j	4-Nitrophenyl	243-244	2-Propanol	55.26	$C_{16}H_{11}N_5O_3S_2$	C 49.86 / 50.38 H 2.88 / 3.64 N 18.17 / 17.58

Table 2. IR and ^{1}H-NMR spectral data of the compounds **5a-j**.



Comp.	IR (cm^{-1})	^{1}H-NMR (δ ppm) (DMSO-d_6)
5a	3302 (NH), 1650 (C=O)	7.61 (2H, m, NH, H^7), 7.30 (2H, m, H^4, H^5), 7.14 (1H, m, H^6), 5.27 (2H, s, CH_2), 2.74 (3H, d, J=4.23 Hz, CH_3)
5b	3372 (NH), 1655 (C=O)	7.67 (1H, t, J= 5.24 Hz, NH), 7.60 (1H, d, J=7.76 Hz, H^7), 7.30(2H, m, H^4, H^5), 7.13 (1H, m, H^6), 5.26 (2H, s, CH_2), 3.13 (2H, m, $\underline{CH_2}CH_3$), 1.03 (3H, t, J=7.17 Hz, CH_3)
5c	3358 (NH), 1651 (C=O)	7.88 (1H, t, J= 5.54 Hz, NH), 7.60 (1H, d, J=7.75 Hz, H^7), 7.31(2H, m, H^4, H^5), 7.13 (1H, m, H^6), 5.76 (1H, m, CH), 5.27 (2H, s, CH_2), 5.06 (2H, m, CH=$\underline{CH_2}$), 3.75 (2H, m, $\underline{CH_2}$-CH=)
5d	3299 (NH), 1643 (C=O)	7.64 (1H, d, J=7.28 Hz, NH), 7.60 (1H, d, J=7.64 Hz, H^7), 7.31(2H, m, H^4, H^5), 7.13 (1H, m, H^6), 5.25(2H, s, CH_2),3.34 (1H, m, cyclohexyl-H^1), 1.81 (2H, m, cyclohexyl-$H^{2e,6e}$), 1.57 (2H, m, cyclohexyl-$H^{3e,5e}$), 1.43 (1H, m, cyclohexyl-H^{4e}), 1.12 (5H, m, cyclohexyl-$H^{2a,3a,4a,5a,6a}$)
5e	3329 (NH), 1652 (C=O)	7.78 (1H, t, J= 5.39 Hz, NH), 7.60 (1H, d, J=7.75 Hz, H^7), 7.30 (2H, m, H^4, H^5), 7.13 (6H, m, H^6, phenyl-H), 5.26 (2H, s, CH_2), 3.36 (2H, m, $\underline{CH_2}CH_2C_6H_5$), 2.74 (2H, m, $CH_2\underline{CH_2}C_6H_5$)
5f	3292 (NH), 1656 (C=O)	10.38 (1H, s, NH), 7.70 (1H, d, J=7.19 Hz, H^7), 7.56 (2H, d, J=7.76 Hz, phenyl-$H^{2,6}$), 7.45 (1H, d, J=7.45 Hz, H^4), 7.40 (1H, m, H^5), 7.32 (2H, t, J= 7.53 Hz, phenyl-$H^{3,5}$), 7.23 (1H, m, H^6), 6.99 (1H, t, J=7.35 Hz, phenyl-H^4), 5.47 (2H, s, CH_2)
5g	3244 (NH), 1667 (C=O)	10.17 (1H, s, NH), 7.60 (1H, d, J=7.22 Hz, H^7), 7.31 (4H, m, H^4, H^5, phenyl-$H^{3,5}$), 7.14 (1H, m, H^6), 7.03 (2H, d, J=8.3 Hz, phenyl-$H^{2,6}$), 5.36 (2H, s, CH_2), 2.15 (3H, s, CH_3)
5h	3244 (NH), 1668 (C=O)	10.42 (1H, s, NH), 7.61 (1H, d, J=7.79 Hz, H^7), 7.51 (2H, d, J= 8.75 Hz, phenyl-$H^{3,5}$), 7.31 (4H, m, H^4, H^5, phenyl-$H^{2,6}$), 7.14 (1H, t, J=7.41 Hz, H^6), 5.39 (2H, s, CH_2).
5i	3182 (NH) 1668 (C=O)	10.02 (1H, s, NH), 7.61(1H, d, J=7.78 Hz, H^7), 7.33 (4H, m, H^4, H^5, phenyl-$H^{2,6}$), 7.15 (1H, m, H^6), 6.82 (2H, m, phenyl-$H^{3,5}$), 5.35 (2H, s, CH_2), 3.64 (3H, s, OCH_3)
5j	3273 (NH), 1644 (C=O)	10.76 (1H, s, NH), 8.15 (2H, d, J= 7.21 Hz, phenyl-$H^{3,5}$), 7.71 (2H, d, J=7.24 Hz, phenyl-$H^{2,6}$), 7.62 (1H, d, J=7.81 Hz, H^7), 7.34 (2H, m, H^4,H^5), 7.16 (1H, m, H^6), 5.44 (2H, s, CH_2)

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 using a thermoregulated water-circulating pump. The latency period until the mouse licked a foot or jumped was registered by a means of a stopwatch (cut-off time 45 s)[27,28].

The results were expressed as a percentage of the maximal possible effect (% MPE ± S.D.)

% MPE = (Postdrug latency-predrug latency) / (cutoff time-predrug latency) x 100

Writhing test

The abdominal constrictor test[29] was performed by the i.p. 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 10 min starting 5 min after the i.p. administration of acetic acid.

All tests were conducted between 9 and 12 a.m. All results were expressed as the mean ± S.D. Statistical comparisons were performed using Students'*t* test.

Results and Discussion

The synthesis pathway leading to the title compounds is given in Scheme 1. 2(3H)-Oxobenzothiazoline **1**, the starting material, was synthesized according to the literature method using 2-aminothiophenol and urea[22]. 2(3H)-Oxobenzothiazoline **1** was reacted with methyl chloroacetate to obtain (2-oxobenzothiazolin-3-yl)acetate **2**[23]. (2-Oxobenzothiazolin-3-yl)acetohydrazide **3** was obtained by the reaction of **2** with hydrazine hydrate[17,23]. The hydrazide thus obtained was reacted with isothiocyanate derivatives to obtain the 4-alkyl and aryl substituted [(2-oxobenzothiazolin-3-yl)acetyl]thiosemicarbazides **4**[10,17,24]. Finally, cyclization of 4-alkyl and aryl substituted 2-[(2-oxobenzothiazolin-3-yl)acetyl]thiosemicarbazides **4** in acidic media gave the title compounds **5**. Syntheses of compounds **5** have been described in the literature[11,12,17]. None of the 2-[(2-oxobenzothiazolin-3-yl)methyl]-5-alkyl/arylamino-1,3,4-thiadiazole derivatives **5** synthesized in this study have been reported previously (Scheme 1).

The structures of the compounds were elucidated by FT-IR, ^{1}H-NMR and microanalyses. Crystallization solvents, melting points, yield %, microanalyses and spectral data of the compounds are given in Tables 1 and 2.

The FT-IR spectra of 1,3,4-thiadiazoles showed N-H stretching bands at 3244-3358 cm^{-1} and 2-oxobenzothiazolines have C=O stretching bands at 1643-1668 cm^{-1}. In the ^{1}H-NMR spectra, all protons were inaccordance with the expected chemical shift and integral values. Methylene and aromatic protons of 2-oxobenzothiazoline-3-methyl groups were seen at 7.61-7.13 ppm and 5.47-5.26 ppm. The N-H protons of compound **5a-e** were observed at 7.88-7.61 ppm, and of compound **5f-j** at 10.17-10.76 ppm.

Antinociceptive activity testing of the synthesized compounds was carried out utilizing tail clip, tail flick, hot plate and writhing methods.

Compounds **5a, 5b** and **5i** were more active than dipyrone, aspirin and the groups in the tail clip test.

Compounds **5a, 5b, 5d, 5e** and **5h** were more potent than others and the standards in tail flick test.

On the other hand, compounds **5j** and **5h** exhibited no activity in the hot plate test, while compounds **5a, 5b** and **5c** showed higher activity than aspirin and **5e, 5f** and **5g** showed higher activity than dipyrone.

In the writhing test compounds **5a** and **5j** were more active than aspirin and dipyrone.

More surprisingly, compound **5b** exhibited lower activity than aspirin in the writhing test. In contrast, compound **5j** showed higher activity than aspirin only in the writing test.

Compound **5a** showed the highest activity in all the tests. This indicates that this compound shows its activity peripherally as well as centrally. On the other hand, compound **5b** could be active centrally whereas compound **5j** might be peripherally active.

In conclusion, further studies are needed to shed light on the mechanisms of action of these compounds.

Table 3. The synthesized compounds of antinociceptive activity.

Compound	Tail clip test % MPE	Tail flick test % MPE	Hot plate test % MPE	AcOH Stretching number
5a	100*	100*	15.00 ± 2.40*	11.00 ± 5.93*
5b	85.69 ± 12.36*	90.19 ± 9.80*	11.40 ± 3.61*	19.00 ± 6.04*
5c	62.94 ± 16.12	46.97 ± 16.34	8.69 ± 3.63*	21.29 ± 9.98*
5d	44.10 ± 16.87	57.56 ± 16.66*	3.88 ± 1.73*	18.38 ± 8.40*
5e	56.50 ± 16.71	56.40 ± 16.37*	6.36 ± 1.67*	23.75 ± 17.62
5f	39.23 ± 16.48	44.75 ± 11.40	6.91 ± 1.26*	23.00 ± 11.65
5g	54.52 ± 15.29	47.73 ± 15.42	7.51 ± 4.55*	22.50 ± 11.74
5h	56.64 ± 16.84	55.19 ± 14.96*	1.18 ± 0.75	32.50 ± 12.27
5i	80.12 ± 13.24*	36.58 ± 14.03	4.35 ± 1.49*	16.13 ± 4.49*
5j	36.38 ± 14.87	47.47 ± 14.08	3.33 ± 2.24	11.63 ± 6.69*
Control	44.31 ± 16.84	31.87 ± 15.39	2.06 ± 0.73	33.57 ± 9.83
Dipyrone	68.29 ± 12.49*	40.57 ± 5.01*	4.42 ± 1.63*	13.50 ± 1.63*
Aspirin	65.50 ± 11.25*	47.33 ± 6.55*	8.14 ± 3.51*	18.24 ± 4.33*

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

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