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Synthesis of Some New 1-Acylthiosemicarbazides and 1,2,4-Triazol-5-Thiones, and Their Analgesic and Anti-Inflammatory Activities

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We synthesized new 1-[3-(2-oxobenzothiazolin-3-yl)propanoyl]-4-substituted-thiosemicarbazides and their corresponding cyclized 3-[2-(2-oxobenzothiazoline-3-yl)ethyl]-4-substituted-1,2,4-triazol-5-thione analogs in which position 4 of the triazole ring was substituted by cyclohexyl, methyl, allyl, phenyl, p-methylphenyl, p-methoxyphenyl, p-chlorophenyl, p-nitrophenyl, benzyl, and phenylethyl to screen their analgesic and anti-inflammatory activities as well as gastric ulceration potential in test animals. None of the compounds, except **5a, 5e,** and **5h,** caused gastric lesions or bleeding. Compound **5g** was found to have higher analgesic and anti-inflammatory activity among the synthesized compounds.

Key Words: 2-Benzothiazolinone, thiosemicarbazide, 1,2,4-triazol-5-thione, analgesic and anti-inflammatory activity.

Introduction

Prostaglandins (PGs), which are produced via degradation of arachidonic acid by cyclooxygenase enzymes (COXs), have various biological effects in the human body. Their main functions are to control a variety of physiological processes and to mediate pain and inflammatory responses. COX activity originates from 2 distinct and independently regulated enzymes, namely COX-1 and COX-2 isoforms. While COX-1 ensures normal production of PGs, which have protecting roles in the gastrointestinal tract, the inducible form, COX-2, was found almost exclusively in inflamed tissues as a canse of inflammatory stimuli. The inhibition of COX-2 isoforms, which results in a decrease in the amount of inflammatory PGs, is the mechanism of action of currently used non-steroidal anti-inflammatory drugs (NSAIDs). There are classical NSAIDs that inhibit both COX-1 and COX-2, to varying extents. NSAIDs are among the most widely used therapeutics, primarily for the treatment of pain and inflammation, including arthritis. The principal side effect associated with

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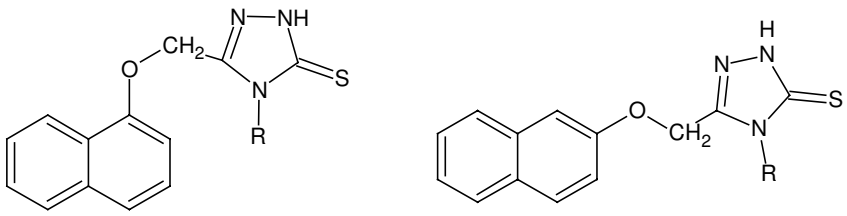
chronic consumption of NSAIDs is significant gastrointestinal irritation and the formation of life-threatening gastrointestinal ulcers, which limits the therapeutic utilization of this type of drug. For this reason, selective COX-2 inhibitors were developed in order to improve the gastrointestinal safety profile of NSAIDs. However, COX-2 inhibitors have recently been linked to an increased risk of adverse cardiovascular events.[1−4] A recent discovery, the third COX isoform (COX-3) enzyme, primary expressed in the brain and the heart, is thought to be the target for acetaminophen.[5,6] Therefore, the development of new compounds with analgesic and anti-inflammatory activities that do not produce the previously mentioned side effects still constitutes a challenge for researchers.

1,2,4-Triazol-5-thione derivatives with antibacterial,[7−9] antidepressant,[10] antiviral,[11] diuretic,[12] and hypoglycemic[13] activities have been previously reported. Meanwhile, many reports suggest that acylthiosemicarbazides and their 1,2,4-triazole analogs may exhibit anti-inflammatory activity.

1-(1-Naphthyloxy)acetyl-4-substituted-3-thiosemicarbazides, 3-(1-naphthyloxy)methyl-4-substituted-1,2,4-triazol-3-thiones, 1-(2-naphthyloxy)acetyl-4-substituted-3-thiosemicarbazides, and 5-(2-naphthyloxy) methyl-4-substituted-1,2,4-triazol-3-thiones reported by Palaska and co-workers showed significant analgesic and anti-inflammatory activity.[14,15] (Figure 1)



R: Methyl, ethyl, allyl, phenyl.

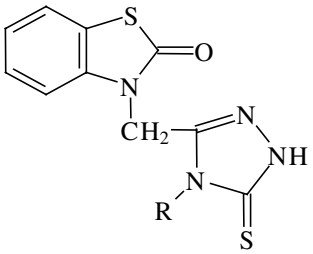
Figure 1. 3-(1-Naphthyloxy)methyl-4-substituted-1,2,4-triazol-3-thiones and 5-(2-naphthyloxy)methyl-4-substituted-1,2,4-triazol-3-thiones derivatives.

In addition, 5-alkoxyphenyl-3-carboxyalkylthio-1,2,4-triazol, 4-(aryl-2/9-substituted indophenazine-6-acetyl)-3-thiosemicarbazide, and their corresponding cyclized 1,2,4-triazol-5-thione derivatives, 3-(1-adamantoyl)-4-substituted-1,2,4-triazol-5-thione, and 5-(3,5-ditert-butyl-4-hydroxyphenyl)-1,2,4-triazol-3-thione derivatives have been shown to exhibit anti-inflammatory and analgesic activity.[16−19]

A large number of 2-oxobenzothiazoline derivatives bearing various substituents at position 3 have been reported to exhibit analgesic and anti-inflammatory activity. Doğruer et al. synthesized 3-(2-benzothiazolinon-3-yl)acetamide derivatives, indicating that these compounds showed potential analgesic and anti-inflammatory activity.[20,21] In our previous work, we also prepared and reported the analgesic activity of 3-(2-oxobenzothiazolin-3-yl)propanamide derivatives and 3-(3-aminopropyl)-2-oxobenzothiazoline hydrochloride derivatives. Some of these compounds have been found to have noteworthy antinociceptive activity.[22]

In a previous study, we reported the synthesis of [(2-oxobenzothiazolin-3-yl)methyl]-4-alkyl/aryl-1,2,4-triazolin-5-thiones and their antinociceptive activity. All of the compounds showed potent analgesic activity in the AcOH induced stretching test. In particular, phenyl and phenylethyl derivatives exhibited

antinociceptive activity 2-fold greater than that of aspirin. Methyl and p-nitrophenyl derivatives were found to be more active than aspirin in the same test.[23] (Figure 2)



R: Methyl, ethyl, allyl, phenyl, p-chlorophenyl, p-methylphenyl, p-nitrophenyl, p-methoxyphenyl, benzyl, phenylethyl.

Figure 2. [(2-Oxobenzothiazolin-3-yl)methyl]-4-alkyl/aryl-1,2,4-triazolin-5-thiones derivatives.

These findings have prompted us to prepare the 1-[3-(2-oxobenzothiazolin-3-yl)propanoyl]-4-substituted-thiosemicarbazides and their corresponding cyclized 3-[2-(2-oxobenzothiazoline-3-yl)ethyl]-4-substituted-1,2,4-triazol-5-thione analogs to explore their analgesic and anti-inflammatory activity.

Experimental

Chemistry

All chemicals and solvents were purchased locally from Merck AG and Aldrich Chemicals. Melting points were determined with an Electrothermal-9200 Digital Melting Point Apparatus and are uncorrected. IR spectra of the compounds were recorded on a Bruker Vector 22 FT-IR spectrophotometer (Opus Spectroscopic Software Version 2.0). ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Bruker Avance DPX-400 NMR spectrometer using tetramethylsilane as the internal standard. All chemical shifts were recorded as δ (ppm). Microanalyses for C, H, and N were performed on a Leco-932 at TÜBİTAK (The Scientific and Technological Research Council of Turkey) Analytical Laboratory, Ankara, Turkey, and they were within the range of $\pm$ 0.4% of the theoretical value. The synthesis of 2-oxobenzothiazoline[24] **(1)** and methyl 3-(2-oxobenzothiazolin-3-yl)propanoate[25] **(2)** was previously reported.

3-(2-Oxobenzothiazoline-3-yl)propanohydrazide (3)

In 30 mL of ethanol were dissolved 0.01 mol methyl 3-(2-oxobenzothiazolin-3-yl)propanoate **(2)** and 0.2 mol 50% hydrazine hydrate. The final mixture was stirred for 30 min at room temperature. The precipitate formed was filtered and washed with water. The solid material was dried and recrystallized from methanol to yield 84%. mp: 171-172 °C. FT-IR (KBr), cm^{-1}: 3322.4 (N-H), 1668.0, 1630.3 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 9.12 (1H, s, -NH-), 7.65 (1H, dd, benzothiazolone H^7), 7.42-7.34 (2H, m, benzothiazolone H^4, H^5), 7.24-7.16 (1H, m, benzothiazolone H^6), 4.17 (2H, d, -NH_2), 4.13 (2H, t, -N-$\underline{CH_2}$-CH_2-), 2.43 (2H, t, -CH_2-$\underline{CH_2}$-CO-). Formula $C_{10}H_{11}N_3O_2S$.

Synthesis of 1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-substituted thiosemicarbazide derivatives (4a-4j)

We refluxed 0.01 mol 3-(2-oxobenzothiazolin-3-yl)propanohydrazide **(3)** and 0.01 mol appropriate substituted isothiocyanate derivatives in methanol for a given period. At the end of this period, the reaction mixture was cooled and the precipitate that formed was filtered, washed with methanol, and then the solid material was dried and recrystallized from appropriate solvents.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-cyclohexylthiosemicarbazide 4a

Recrystallized from ethanol to yield 81.7%. mp: 211°C. Reaction time is 3 h. FT-IR (KBr), cm^{-1}: 3312.4, 3293.8, 3136.3 (N-H), 1690.4, 1657.2 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 9.68 (1H, s, -CO-NH-NH-CS-), 8.92 (1H, s, -CO-NH-NH-CS-), 7.58 (1H, dd, benzothiazolone H^7), 7.33-7.11 (4H, m, benzothiazolone H^4, H^5, H^6, -CS-NH-C_6H_{11}), 4.10 (2H, t, -N-CH_2-CH_2-), 2.50 (2H, t, -CH_2-CH_2-CO-), 1.70- 1.48 (5H, m, cyclohexyl eqv.), 1.24-0.98 (6H, m, cyclohexyl axial). Formula $C_{17}H_{22}N_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-methyl thiosemicarbazide 4b

Recrystallized from ethanol to yield 76.4%. mp: 195 °C. Reaction time is 5 h. FT-IR (KBr), cm^{-1}: 3295.1, 3123.8 (N-H), 1684.0, 1668.8 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 9.84 (1H, s, -CO-NH-NH-CS-), 9.19 (1H, s, -CO-NH-NH-CS-), 7.84 (1H, d, -CS-NH-CH_3, J: 3.97 Hz), 7.67 (1H, dd, benzothiazolone H^7), 7.43-7.37 (2H, m, benzothiazolone H^4, H^5), 7.24-7.20 (1H, m, benzothiazolone H^6), 4.18 (2H, t, -N-CH_2-CH_2-), 2.87 (3H, d, -CS-NH-CH_3, J: 4.25 Hz), 2.56 (2H, t, -CH_2-CH_2-CO-). Formula $C_{12}H_{14}N_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-allylthiosemicarbazide 4c

Recrystallized from methanol to yield 79.4%. mp: 175 °C. Reaction time is 12 h. FT-IR (KBr), cm^{-1} 3297.3 (N-H), 1683.3, 1662.0 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 9.88 (1H, s, -CO-NH-NH-CS-), 9.25 (1H, s, -CO-NH-NH-CS-), 8.04 (1H, t, -CS-NH-CH_2-CH=, J: 5.45 Hz), 7.67 (1H, dd, benzothiazolone H^7), 7.43-7.36 (2H, m, benzothiazolone H^4, H^5), 7.24-7.20 (1H, m, benzothiazolone H^6), 5.85-5.76 (1H, m, -NH-CH_2-CH=CH_2), 5.14-5.09 (1H, dd, -NH-CH_2-CH=CH_2, $H_{AX(trans)}$ J: 17.25 Hz, $H_{AB(gem)}$ J: 1.72 Hz), 5.06-5.03 (1H, dd, -NH-CH_2-CH=CH_2, $H_{BX(cis)}$ J: 10.32 Hz, $H_{AB(gem)}$ J: 1.72 Hz), 4.18 (2H, t, -N-CH_2-CH_2-), 4.10 (2H, t, -NH-CH_2-CH=CH_2, J: 5.29 Hz), 2.57 (2H, t, -CH_2-CH_2-CO-). Formula $C_{14}H_{16}N_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-phenylthiosemicarbazide 4d

Recrystallized from ethanol to yield 75.0%. mp: 175-176 °C. Reaction time is 6 h. FT-IR (KBr), cm^{-1}: 3229.5, 3179.4, 3076.0 (N-H), 1664.2 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 10.08 (1H, s, -CS-NH-C_6H_5), 9.58 (2H, s, -CO-NH-NH-CS-), 7.67 (1H, dd, benzothiazolone H^7), 7.40-7.32 (6H, m, benzothiazolone H^4, H^5, phenyl H^2, H^3, H^5, H^6), 7.23-7.18 (2H, m, benzothiazolone H^6, phenyl H^4), 4.21 (2H, t, -N-CH_2-CH_2-), 2.63 (2H, t, -CH_2-CH_2-CO-). Formula $C_{17}H_{16}N_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-(4-methylphenyl)thiosemicarbazide 4e

Recrystallized from ethanol to yield 68.7%. mp: 176 °C. Reaction time is 15 h. FT-IR (KBr), cm^{-1}: 3270.7, 3175.1 (N-H), 1688.7, 1656.5 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 10.05 (1H, s, -CS-NH-C_6H_4-CH_3(p)), 9.51 (2H, s, -CO-NH-NH-CS-), 7.67 (1H, dd, benzothiazolone H^7), 7.39 (2H, d, benzothiazolone H^4, H^5), 7.27-7.19 (3H, m, benzothiazolone H^6, phenyl H^2, H^6), 7.14 (2H, d, phenyl H^3, H^5), 4.21 (2H, t, -N-CH_2-CH_2-), 2.62 (2H, t, -CH_2-CH_2-CO-), 2.29 (3H, s, -C_6H_4-CH_3(p)). Formula $C_{18}H_{18}N_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-(4-methoxyphenyl)thiosemicarbazide 4f

Recrystallized from ethanol/water to yield 72.0%. mp: 130 °C. Reaction time is 5 h. FT-IR (KBr), cm^{-1}: 3567.5, 3463.8, 3168.1 (N-H), 1684.9 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 10.19 (1H, s, -CS-NH-C_6H_4-OCH_3(p)), 9.60 (2H, d, -CO-NH-NH-CS-), 7.81 (1H, dd, benzothiazolone H^7), 7.56 (2H, d, benzothiazolone H^4, H^5), 7.41-7.35 (3H, m, benzothiazolone H^6, phenyl H^2, H^6), 7.08-7.04 (2H, m, phenyl H^3, H^5), 4.37 (2H, t, -N-CH_2-CH_2-), 3.91 (3H, s, -C_6H_4-OCH_3(p)), 2.77 (2H, t, -CH_2-CH_2-CO-). Formula $C_{18}H_{18}N_4O_3S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-(4-chlorophenyl)thiosemicarbazide 4g

Recrystallized from ethanol to yield 80.2%. mp: 193 °C. Reaction time is 10 min. FT-IR (KBr), cm^{-1}: 3265.7 (N-H), 1664.7 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 10.09 (1H, s, -CS-NH-C_6H_4-Cl(p)), 10.00-9.42 (2H, m, -CO-NH-NH-CS-), 7.67 (1H, dd, benzothiazolone H^7), 7.49-7.38 (6H, m, benzothiazolone H^4, H^5, phenyl H^2, H^3, H^5, H^6), 7.25-7.19 (1H, m, benzothiazolone H^6), 4.21 (2H, t, -N-CH_2-CH_2-), 2.63 (2H, t, -CH_2-CH_2-CO-). Formula $C_{17}H_{15}ClN_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-(4-nitrophenyl)thiosemicarbazide 4h

Recrystallized from ethanol to yield 80.0%. mp: 196-197 °C. Reaction time is 6 h. FT-IR (KBr), cm^{-1}: 3249.4 (N-H), 1656.4 (C=O), 1510.1, 1342.7 (NO_2), ^{1}H-NMR (DMSO-d_6)δ: 10.44-9.85 (3H, m, -NH-), 8.22-8.20 (2H, dd, phenyl H^3, H^5), 7.88-7.86 (2H, dd, phenyl H^2, H^6), 7.66 (1H, dd, benzothiazolone H^7), 7.41-7.36 (2H, m, benzothiazolone H^4, H^5), 7.24-7.19 (1H, m, benzothiazolone H^6), 4.22 (2H, t, -N-CH_2-CH_2-), 2.66 (2H, t, -CH_2-CH_2-CO-). Formula $C_{17}H_{15}N_5O_4S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-benzylthiosemicarbazide 4i

Recrystallized from ethanol to yield 81.0%. mp: 193 °C. Reaction time is 5 h. FT-IR (KBr), cm^{-1}: 3266.9, 3163.9, 3057.2 (N-H), 1701.1, 1651.6 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 9.93 (1H, s, -CO-NH-NH-CS-), 9.34 (1H, s, -CO-NH-NH-CS-), 8.41 (1H, d, -CS-NH-CH_2), 7.67 (1H, dd, benzothiazolone H^7), 7.41-7.19 (8H, m, benzothiazolone H^4, H^5, H^6, phenyl H^2, H^3, H^4, H^5, H^6), 4.74 (2H, d, -N-CH_2-C_6H_5), 4.18 (2H, t, -N-CH_2-CH_2-), 2.57 (2H, t, -CH_2-CH_2-CO-). Formula $C_{18}H_{18}N_4O_2S_2$.

1-[3-(2-Oxobenzothiazolin-3-yl)propanoyl]-4-(2-phenylethyl)thiosemicarbazide 4j

Recrystallized from ethanol to yield 63.0%. mp: 167-168 °C. Reaction time is 14 h. FT-IR (KBr), cm^{-1}: 3402.4, 3156.5 (N-H), 1696.0, 1682.7 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 10.03 (1H, s, -CO-NH-NH-CS-), 9.38

(1H, s, -CO-NH-NH-CS-), 8.12 (1H, s, -CS-NH-CH_2-), 7.83 (1H, dd, benzothiazolone H^7), 7.57-7.33 (8H, m, benzothiazolone H^4, H^5, H^6, phenyl H^2, H^3, H^4, H^5, H^6), 4.35 (2H, t, -N-CH_2-CH_2-), 3.78 (2H, q, -N-CH_2-CH_2-C_6H_5), 2.96 (2H, t, -N-CH_2-CH_2-C_6H_5), 2.73 (2H, t, -CH_2-CH_2-CO-). Formula $C_{19}H_{20}N_4O_2S_2$.

Synthesis of 3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-substituted-1,2,4-triazol-5-thione derivatives (5a-5j)

In 30 mL of methanol was dissolved 0.01 mol appropriate thiosemicarbazide derivative and 0.01 mol sodium hydroxide was added and refluxed for a certain period. At the end of this period, the reaction mixture was cooled and acidified to pH 2 with 5% hydrochloric acid, and then the precipitated product was filtered, washed with water, dried, and recrystallized from appropriate solvent.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-cyclohexyl-1,2,4-triazol-5-thione 5a

Recrystallized from methanol to yield 80.1%. mp: 194-196 °C. Reaction time is 6 h. FT-IR (KBr), cm^{-1}: 3222.7 (N-H), 1646.3 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.52 (1H, s, -NH), 7.68 (1H, dd, benzothiazolone H^7), 7.42-7.36 (2H, m, benzothiazolone H^4, H^5), 7.24-7.20 (1H, m, benzothiazolone H^6), 4.28 (2H, t, -N-CH_2-CH_2-), 3.16 (2H, t, -CH_2-CH_2-C=), 1.80- 1.59 (5H, m, cyclohexyl eqv.), 1.35-1.11 (6H, m, cyclohexyl axial). Formula $C_{17}H_{20}N_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-methyl-1,2,4-triazol-5-thione 5b

Recrystallized from methanol/water to yield 80.1%. mp: 252-253 °C. Reaction time is 2 h. FT-IR (KBr), cm^{-1}: 3232.8 (N-H), 1652.6 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.48 (1H, s, -NH), 7.67 (1H, dd, benzothiazolone H^7), 7.39-7.38 (2H, m, benzothiazolone H^4, H^5), 7.24-7.20 (1H, m, benzothiazolone H^6), 4.29 (2H, t, -N-CH_2-CH_2-), 3.43 (3H, s, -N-CH_3), 3.08 (2H, t, -CH_2-CH_2-C=). Formula $C_{12}H_{12}N_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-allyl-1,2,4-triazol-5-thione 5c

Recrystallized from methanol/water to yield 70.0%. mp: 166 °C. Reaction time is 3 h. FT-IR (KBr), cm^{-1}: 3104.1 (N-H), 1668.7, 1653.8 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.62 (1H, s, -NH), 7.67 (1H, dd, benzothiazolone H^7), 7.38-7.37 (2H, m, benzothiazolone H^4, H^5), 7.23-7.19 (1H, m, benzothiazolone H^6), 5.90-5.83 (1H, m, -N-CH_2-CH=CH_2), 5.19-5.16 (1H, dd, -N-CH_2-CH=CH_2, $H_{AX(cis)}$ J: 10.39 Hz, $H_{AB(gem)}$ J: 1.17 Hz), 5.05-5.01 (1H, dd, -N-CH_2-CH=CH_2 , $H_{BX(trans)}$ J: 17.23 Hz, $H_{AB(gem)}$ J: 1.2 Hz), 4.65 (2H, d, -N-CH_2-CH=CH_2), 4.29 (2H, t, -N-CH_2-CH_2-), 3.01 (2H, t, -CH_2-CH_2-C=). Formula $C_{14}H_{14}N_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-phenyl-1,2,4-triazol-5-thione 5d

Recrystallized from isopropanol to yield 86.1%. mp: 255-256 °C. Reaction time is 5 h. FT-IR (KBr), cm^{-1}: 3090.3 (N-H), 1669.2 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.75 (1H, s, -NH), 7.64 (1H, dd, benzothiazolone H^7), 7.56-7.53 (3H, m, phenyl H^3, H^4, H^5), 7.44-7.42 (2H, m, phenyl H^2, H^6), 7.28 (1H, t, benzothiazolone H^4), 7.19 (1H, t, benzothiazolone H^5), 7.03 (1H, d, benzothiazolone H^6), 4.06 (2H, t, -N-CH_2-CH_2-), 2.82 (2H, t, -CH_2-CH_2-C=). Formula $C_{17}H_{14}N_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-(4-methylphenyl)-1,2,4-triazol-5-thione 5e

Recrystallized from methanol/water to yield 82.5%. mp: 236-237 °C. Reaction time is 3 h. FT-IR (KBr), cm^{-1}: 3344.5 (N-H), 1676.0 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.71 (1H, s, -NH), 7.64 (1H, dd, benzothiazolone H^7), 7.35-7.27 (5H, m, phenyl H^2, H^3, H^5, H^6, benzothiazolone H^4), 7.19 (1H, m, benzothiazolone H^5), 7.03 (1H, d, benzothiazolone H^6), 4.05 (2H, t, -N-$\underline{CH_2}$-CH_2-), 2.81 (2H, t, -CH_2-$\underline{CH_2}$-C=), 2.38 (3H, s, -C_6H_4-$\underline{CH_3}$(p)). Formula $C_{18}H_{16}N_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-(4-methoxyphenyl)-1,2,4-triazol-5-thione 5f

Recrystallized from methanol/water to yield 70.1%. mp: 230-231 °C. Reaction time is 3 h. FT-IR (KBr), cm^{-1}: 3095.5 (N-H), 1671.2 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.86 (1H, s, -NH), 7.80 (1H, dd, benzothiazolone H^7), 7.51-7.43 (3H, m, benzothiazolone H^4, phenyl H^2, H^6), 7.35 (1H, t, benzothiazolone H^5), 7.24-7.20 (3H, m, benzothiazolone H^6, phenyl H^3, H^5), 4.22 (2H, t, -N-$\underline{CH_2}$-CH_2-), 3.98 (3H, s, -N-C_6H_4-O$\underline{CH_3}$(p)), 2.97 (2H, t, -CH_2-$\underline{CH_2}$-C=). Formula $C_{18}H_{16}N_4O_2S_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-(4-chlorophenyl)-1,2,4-triazol-5-thione 5g

Recrystallized from methanol to yield 84.0%. mp: 209-210 °C. Reaction time is 2 h. FT-IR (KBr), cm^{-1}: 3187.6 (N-H), 1634.4 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.77 (1H, s, -NH), 7.63-7.58 (3H, m, benzothiazolone H^7, phenyl H^3, H^5), 7.47-7.44 (2H, m, phenyl H^2, H^6), 7.31-7.27 (1H, m, benzothiazolone H^4), 7.19-7.15 (1H, m, benzothiazolone H^5), 7.08 (1H, d, benzothiazolone H^6), 4.05 (2H, t, -N-$\underline{CH_2}$-CH_2-), 2.82 (2H, t, -CH_2-$\underline{CH_2}$-C=). Formula $C_{17}H_{13}ClN_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-(4-nitrophenyl)-1,2,4-triazol-5-thione 5h

Recrystallized from methanol to yield 65.0%. mp: 263-264 °C. Reaction time is 10 h. FT-IR (KBr), cm^{-1}: 3105.5 (N-H), 1631.8 (C=O), 1522.1, 1344.7 (NO_2), ^{1}H-NMR (DMSO-d_6)δ: 13.77 (1H, s, -NH), 8.26-8.23 (2H, dd, phenyl H^3, H^5), 7.67-7.64 (2H, dd, phenyl H^2, H^6), 7.51 (1H, dd, benzothiazolone H^7), 7.19-7.17 (1H, m, benzothiazolone H^4), 7.08-7.06 (2H, m, benzothiazolone H^5, H^6), 3.99 (2H, t, -N-$\underline{CH_2}$-CH_2-), 2.82 (2H, t, -CH_2-$\underline{CH_2}$-C=). Formula $C_{17}H_{13}N_5O_3S_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-benzyl-1,2,4-triazol-5-thione 5i

Recrystallized from methanol/water to yield 93.6%. mp: 220-221 °C. Reaction time is 3 h. FT-IR (KBr), cm^{-1}: 3193.0 (N-H), 1643.8 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.89 (1H, s, -NH), 7.80 (1H, dd, benzothiazolone H^7), 7.53-7.28 (8H, m, benzothiazolone H^4, H^5, H^6, phenyl H^2, H^3, H^4, H^5, H^6), 5.45 (2H, s, -N-$\underline{CH_2}$-C_6H_5), 4.30 (2H, t, -N-$\underline{CH_2}$-CH_2-), 3.07 (2H, t, -CH_2-$\underline{CH_2}$-C=). Formula $C_{18}H_{16}N_4OS_2$.

3-[2-(2-Oxobenzothiazolin-3-yl)ethyl]-4-(2-phenylethyl)-1,2,4-triazol-5-thione 5j

Recrystallized from butanol to yield 74.2%. mp: 85-87 °C. Reaction time is 4 h. FT-IR (KBr), cm^{-1}: 3390.7 (N-H), 1674.2 (C=O), ^{1}H-NMR (DMSO-d_6)δ: 13.60 (1H, s, -NH), 7.66 (1H, dd, benzothiazolone H^7), 7.38 (1H, m, benzothiazolone H^4), 7.29-7.20 (5H, m, benzothiazolone H^5, phenyl H^2, H^3, H^5, H^6), 7.16-7.14 (2H,

m, benzothiazolone H^6, phenyl H^4), 4.18 (2H, t, -N-$\underline{CH_2}$-CH_2-), 4.11 (2H, t, -N-$\underline{CH_2}$-CH_2-C_6H_5), 2.99 (2H, t, -N-CH_2-$\underline{CH_2}$-C_6H_5), 2.70 (2H, t, -CH_2-$\underline{CH_2}$-C=). Formula $C_{19}H_{18}N_4OS_2$.

Pharmacology

Animals

Male Swiss albino mice (The Animal Breeding Laboratories of Refik Saydam Hıfz-ı Sıhha Institute, Ankara, Turkey), weighing 20-25 g, were used for all experiments. The animals were kept in colony cages (6 mice each), maintained on a standard pellet diet with water ad libitum, and left for 2 days for acclimatization before the experimental session. The food was withdrawn on the day before the experiment, but free access to water was allowed. 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 suspended in a mixture of distilled water and 0.5% sodium carboxymethylcellulose (CMC) and were given orally to the test animals. The animals of the control group received the same experimental handling, except that the drug treatment was replaced with appropriate volumes of only the dosing vehicle. Indomethacin (10 mg/kg) and acetylsalicylic acid (ASA) in 0.5% CMC (100 mg/kg) were used as reference drugs.

p-Benzoquinone-induced writhing test[26]

The mice were injected intraperitoneally with 0.1 mL/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ, Merck, Darmstadt, Germany) solution in distilled water 60 min after the oral administration of test samples. 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 5 min after the PBQ injection. The data represent average values of the total number of writhings observed. The analgesic activity was expressed as the percentage change from writhing controls.

Carrageenan-induced hind paw edema test[27,28]

The test was performed according to the method of Kasahara et al.[28] The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Sixty minutes after the oral administration of the test sample or dosing vehicle, each mouse was injected with a freshly prepared (0.5 mg/25 mL) suspension of carrageenan (Sigma, St. Louis, MO, USA) in physiological saline (154 mM NaCl) into the subplantar tissue of the right hind paw and 25 μL of saline solution was injected into that of the left hind paw as a secondary control. Measurements were obtained and evaluated every 90 min for 360 min following the induction of inflammation, as described above.

Gastric ulceration side effects

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

Statistical analysis of data

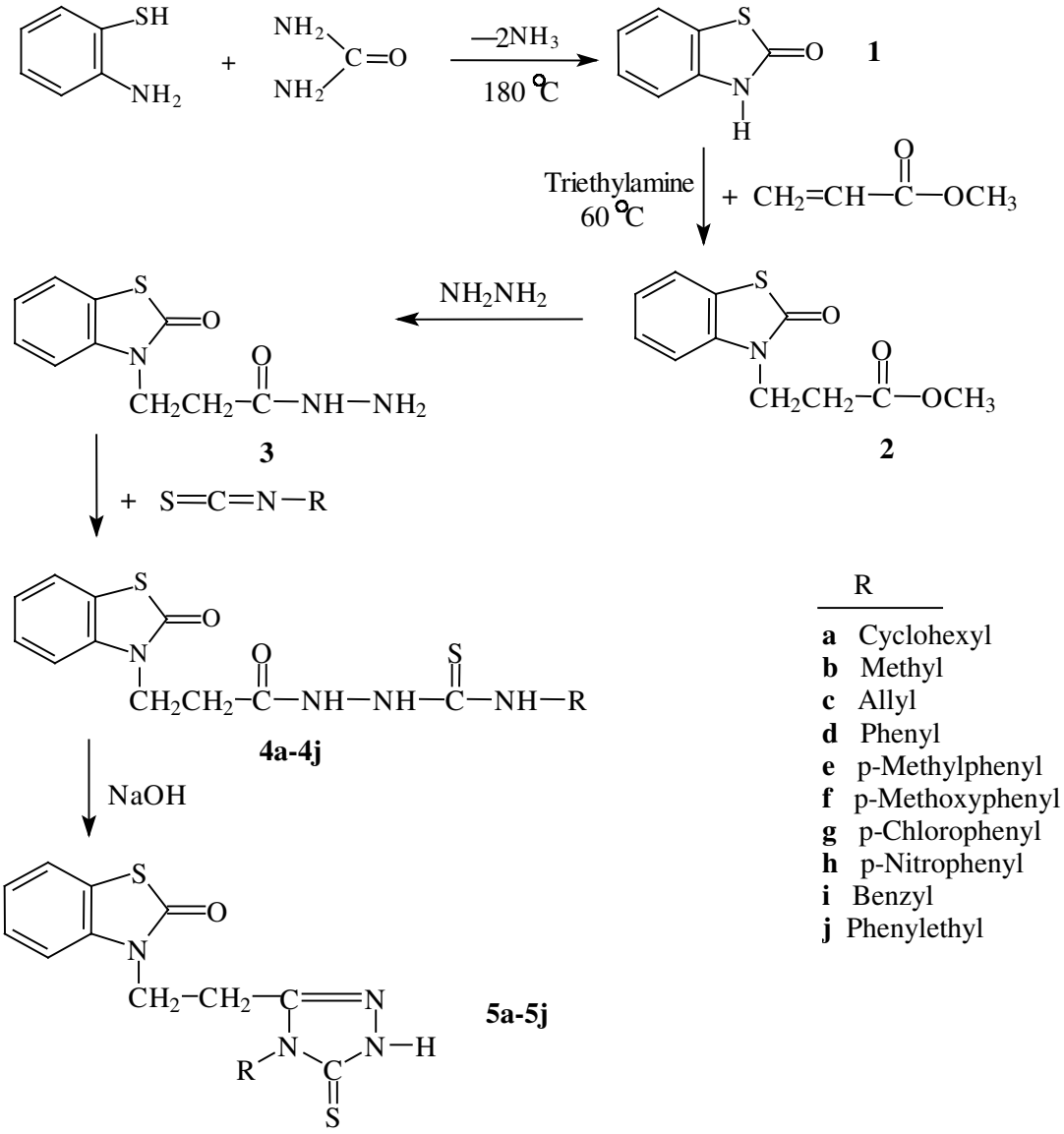
Data obtained from the animal experiments are expressed as the mean standard error (± SEM). Statistical differences between the treatments and the control were tested by ANOVA test.

Results and Discussion

The synthetic pathway leading to the title compounds is given in Scheme. 2(3H)-Oxobenzothiazolin **(1),** the starting material, was synthesized according to the previously published procedure, using 2-aminothiophenol and urea.[24] **1** was reacted with methyl acrylate to obtain methyl [3-(2-oxobenzothiazolin-3-yl)]propanoate **(2)**,[25] which was subsequently reacted with hydrazine hydrate to produce 3-(2-oxobenzothiazolin-3-yl)propanohydrazide **(3)**. The reaction of the hydrazide derivative with isothiocyanate derivatives resulted in the formation of 1-[3-(2-oxobenzothiazolin-3-yl)propanoyl]-4-substituted thiosemicarbazides **(4).** Finally, cyclization of **4** in basic media produced the title compounds, 3-[2-(2-oxobenzothiazolin-3-yl)ethyl]-4-substituted-1,2,4-triazol-5-thione derivatives **(5)**. All the 3-(2-oxobenzothiazolin-3-yl)propanohydrazide **(3)**, 1-[3-(2-oxobenzothiazolin-3-yl)propanoyl]-4-substituted thiosemicarbazides **(4)**, and 3-[2-(2-oxobenzothiazolin-3-yl) ethyl]-4-substituted-1,2,4-triazol-5-thiones derivatives **(5)** synthesized in this study are reported for the first time.

The structures of the compounds have been elucidated by IR, ^{1}H-NMR, and microanalyses. Reaction time, crystallization solvents, melting points, % yields, and spectral data of the compounds are given in the Experimental Part.

The IR spectra of 1-[3-(2-oxobenzothiazolin-3-yl)propanoyl]-4-substituted thiosemicarbazides **(4)** showed N-H stretching bands between 3057 and 3402 cm^{-1}, and 2 C=O stretching bands at 1651-1701 cm^{-1}. 1,2,4-Triazol-5-thione derivatives **(5)** showed N-H stretching bands at 3090-3390 cm^{-1} and C=O stretching bands at 1631-1674 cm^{-1}. In the ^{1}H-NMR spectra, the N-H protons of compounds **4a-j** were observed at 9.42-10.44 (CO-NH), 8.92-10.44 (HN-CS), and 7.11-10.44 (HN-R) ppm. Also observed were aromatic phenyl protons and 2-oxobenzothiazoline groups at 8.22-7.04 ppm, and ethylenic protons at 4.37-4.10 (-N-CH_2-CH_2-) and 2.77-2.50 (-CH_2-CH_2-CO) ppm. We observed N-H protons of compounds **5a-j** at 13.48-13.89 ppm, aromatic protons of phenyl rings and 2-oxobenzothiazoline groups at 8.26-7.01 ppm, and ethylenic protons at 4.30-3.99 (-N-CH_2-CH_2-) and 3.16-2.70 (-CH_2-CH_2-C=) ppm.



Scheme. Synthetic route of the title compounds.

In this preliminary screening study, analgesic and anti-inflammatory activity of the title compounds (**5a-5j**) were assessed by the *p*-benzoquinone-induced writhing test[26] and carrageenan-induced hind paw edema model,[27,28] respectively. As seen in Tables 1 and 2, the overall analgesic and anti-inflammatory activity of the compounds were lower than those of the reference compounds, aspirin and indomethacin, respectively. In the *p*-benzoquinone-induced writhing test, some of the compounds showed significant activity and the highest potency was exhibited by compound **5g**. In addition, compounds **5a** and **5h** were inactive in the same test system. Compound **5g** exhibited the highest anti-inflammatory activity at 100 mg/kg, although it was less pronounced than that of the reference compound, indomethacin, tested at the 10 mg/kg dose. Compounds **5a, 5f,** and **5h** were inactive in the same test. Additionally, none of the compounds, except for **5a, 5e, and 5h**, caused any gastric lesions or bleeding in the stomachs of the tested animals.

Table 1. Effect of the synthesized compounds against *p*-benzoquinone-induced writhing in mice.[a]

Compounds	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration
Control		42.7 ± 3.98		0/6
5a	100	43.3± 3.10	—-	1/6
5b	100	33.1 ± 2.97	22.5	0/6
5c	100	29.2 ± 3.02	31.6**	0/6
5d	100	36.5 ±2.19	14.5	0/6
5e	100	30.5 ± 2.92	28.6**	1/6
5f	100	40.9 ± 2.45	4.2	0/6
5g	100	23.8 ± 2.34	44.3***	0/6
5h	100	43.5 ± 3.96	—-	2/6
5i	100	31.9 ± 2.18	25.3*	0/6
5j	100	34.7 ± 3.08	18.7	0/6
ASA	100	21.3 ± 2.05	50.1***	4/6

(* P < 0.05, ** P < 0.01, *** P <0.001).

[a]Analgesic activity of the compounds and aspirin was tested at the 100 mg/kg dose, as described in the Experimental Part. P < 0.5 was found for all tests in comparison to the control group.

Table 2. Effects of the synthesized compounds against carrageenan-induced paw edema in mice.[a]

Compounds	Dose (mg/kg)	Swelling thickness ($\times 10^{-2}$ mm) ± SEM (inhibition %)			
		90 min	180 min	270 min	360 min
Control		43.4 ± 4.36	49.1 ± 4.02	53.9 ± 3.81	57.8 ± 4.12
5a	100	46.9 ± 4.02	54.2 ± 3.11	58.9 ± 3.98	64.5 ± 4.13
5b	100	40.1 ± 2.18 (7.6)	43.2 ± 2.56 (12.0)	47.9 ± 2.67 (11.1)	50.1 ± 2.88 (13.3)
5c	100	35.8 ± 3.12 (17.5)	38.9 ± 3.45 (20.8)	41.3 ± 3.09 (23.4)	43.9 ± 2.98 (24.0)*
5d	100	42.7 ± 3.11 (1.6)	48.9 ± 3.34 (1.8)	52.3 ± 4.12 (2.9)	55.8 ± 3.98 (3.5)
5e	100	34.9 ± 2.58 (19.6)	40.1 ± 3.45 (18.3)	43.6 ± 2.98 (19.1)	45.8 ± 3.15 (20.8)
5f	100	45.1 ± 2.91	52.6 ± 2.89	57.9 ± 2.11	61.3 ± 3.01
5g	100	33.4 ± 3.03 (23.0)	36.8 ± 2.46 (25.1)*	38.9 ± 2.37 (27.8)**	40.3 ± 2.89 (30.3)**
5h	100	44.7 ± 3.12	51.8 ± 2.98	55.9 ± 3.01	60.1 ± 3.13
5i	100	39.2 ± 3.14 (9.7)	41.2 ± 3.67 (16.1)	45.7 ± 3.12 (15.2)	48.7 ± 3.56 (15.7)
5j	100	41.4 ± 2.98 (4.6)	46.7 ± 3.01 (4.9)	50.4 ± 3.92 (6.5)	53.1 ± 3.26 (8.1)
Indomethacin	10	31.3 ± 2.65 (27.9)*	30.2 ± 2.11 (38.5)**	31.4 ± 2.10 (41.7)***	33.8 ± 2.24 (41.5)***

(* P < 0.05, ** P < 0.01, *** P < 0.001).

[a]Anti-inflammatory activity of the compounds was tested at the 100 mg/kg dose, and anti-inflammatory activity of the reference compound, indomethacin, was tested at the 10 mg/kg dose, as described in the Experimental Part. P < 0.5 was found for all tests in comparison to the control group.

In a previous study, [(2-oxobenzothiazolin-3-yl)methyl]-4-phenyl-1,2,4-triazoline-5-thiones and [(2-oxobenzothiazolin-3-yl)methyl]-4-phenylethyl-1,2,4-triazoline-5-thiones exhibited antinociceptive activity 2-fold greater than that of aspirin, and all of the compounds showed potent activity in the acetic acid-induced-stretching test.[21]

As a result, we may conclude that when the distance between the 1,2,4-triazoline-5-thione ring and the 2-oxobenzothiazoline was increased by 2 carbon atoms, the activity of these compounds decreased and the carbon chain length between the 2-ring system was important for analgesic and anti-inflammatory activity.

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