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Article

Microwave-assisted synthesis and pharmacological screening of some triazolothiadiazole derivatives

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In this study, twenty-two new [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (5a-n, 6a-h) were synthesized under microwave irradiation (MWI). The chemical structures of the compounds were elucidated by their IR, ^{1}H-NMR, LC-MS, and elemental analysis. The compounds were tested for antinociceptive activity by using the tail clip, tail flick, hot plate, and writhing methods in mice. The varying levels of antinociceptive activity of the compounds were compared with those of aspirin. Among these compounds, compound **5g** and **5j** were found to be significantly more active than the other compounds and the standard in the tests. Also, inhibitory effects of the test compounds on COX-1 and COX-2 activities were investigated. DuP-697 for COX-2 and SC-560 for COX-1 were used as reference standards.

Keywords: 2(3*H*)-Benzoxazolone. Triazolothiadiazole. Antinociceptive activity. Microwave-assisted synthesis.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a heterogeneous family of pharmacologically active compounds used to alleviate acute and chronic inflammation, pain, and fever. Their clinical efficacy is closely related to their ability to inhibit both COX-1 and COX-2 isoforms of the enzyme cyclooxygenase (COX) which is also referred to as prostaglandin H2 synthase since it catalyzes the conversion of arachidonic acid to prostaglandin H2 (PGH2) (Dannhardt, Kiefer, 2001).

A large number of N-bridged heterocycles derived from 1,2,4-thiadiazole nucleus are important pharmacological agents and there is a significant amount of research on this class of compounds. 1,2,4-Thiadiazole ring is associated with a wide variety of biological activities named antimicrobial, antimycobacterial, anticonvulsant, antidepressant, antihypertensive, and analgesic agents. Moreover, some 1,2,4-triazoles and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles derived from 4-amino-3-thioxo-1,2,4-triazoles are associated with diverse pharmacological activities such as anti-Alzheimer's (Shiradkar, *et al*., 2007), anti-inflammatory, analgesic (Mathew, Keshavayya, Vaidya, 2006; Sarıgöl *et al*., 2015; Haider *et al*., 2014; Akhter, Hassan, Amir, 2014), antiviral (Kritsanida *et al*., 2002), antifungal and antibacterial (Kumar *et al.,* 2010a; Hussein *et al*., 2011; Aggarwal, Kumar, Dureja, 2011; Mathew *et al*., 2007), antitubercular (Kumar *et al.,* 2010b; Mathew *et al*., 2007), and anticancer (Ibrahim, 2009) activities.

On the other hand, some compounds having small and simple 2(3H)-benzoxazolone ring show a broad spectrum of biological activity such as antimicrobial (Gülkok *et al.,* 2012; Koksal *et al*., 2002), antitubercular (Gulkok *et al*., 2012), antioxidant (Aichaoui *et al*., 2009; Satyendra *et al*., 2011), anticonvulsant (Ucar *et al*., 1998), cytotoxic (Petrov *et al*., 2008), anti-inflammatory (Unlu *et al*., 2003; Dogruer *et al*., 1997), and analgesic (Onkol *et al*., 2002; Gokhan-Kelekci, Koksal, Univar, 2009; Abdelazeem *et al*., 2015) activities.

Design of new drugs can be based on the development of hybrid molecules by linking different pharmacophore fragments in a single structure, which may lead to compounds with interesting biological profiles.

These observations prompted us to synthesize new 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives, which were attached to position-3 of the 2(3*H*)-benzoxazolone ring through a methylene bridge. Also, the structure of the

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synthesized compounds was elucidated by IR, ^{1}H-NMR, LC-MS, and elemental analysis data.

The observed antinociceptive activities in all the tests such as hot-plate, tail-clip, tail flick and acetic acid-induced writhing tests clearly showed that mechanical, thermal and chemical nociceptive pathways had a role on their pharmacological effects (Sharma *et al*., 2012). Hot-plate and tail-clip tests have been reported as a measure of centrally mediated transient pain (Gabra, Sirois, 2003; Wong *et al*., 1994).

MATERIAL AND METHODS

All chemicals and solvents used in this study were purchased from Aldrich, (Germany), Merck (Germany) and Acros (Germany) Chemical. Melting points of the compounds were recorded on an Electrothermal-9200 digital melting points apparatus and were uncorrected. Microwave reactions were carried out in MicroSYNTH Microwave Labstation at 1600 W (2 magnetrons 800Wx2) (Milestone S.R.L. Italy). ^{1}H-NMR and ^{13}C spectras of compounds were recorded in DMSO-d_6 on Bruker 400 MHz NMR spectrometer. Chemical shifts were reported in parts per million relative to internal standard tetramethylsilane. The mass spectra were recorded on a Micromass LCT Premier XE (Waters, Milford, MA, USA) LC–MS spectrometer using an positive electrospray ion source (ESI+). FTIR spectra of the surface layer of grafted membranes were measured with a Perkin-Elmer 400 (USA) ATR attachment (32 scans, wavenumber 4000–650 cm^{-1}) and analyzed using the Spectrum v2.0 software. Elemental analyses were performed on Leco 932 CHNS instrument (St. Joseph, MI, USA) and were within ± 0.4 % of the theoretical values.

Chemistry

2(3*H*)-Benzoxazolone **(1)** (Eren *et al*., 2010), ethyl (2(3*H*)-benzoxazolone-3-yl)acetate **(2)** (Onkol *et al*., 2002), (2(3*H*)-Benzoxazolone-3-yl)acetic acid **(3)** (Onkol *et al*. 2002), 3-[(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone **(4)** (Urlu Cicekli *et al*., 2012) were prepared according to the previously published procedures.

General method

*General method for synthesis of 3-substituted [1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]-2(3H)-benzoxazolone derivatives (**5a-n**) (**6a-h**)*

To a mixture of corresponding 3-(4-pyridyl)-4-amino- 5-mercapto-1,2,4-triazole, **1** (0.01 mol) and the substituted benzoic acid or substituted phenylacetic acid (0.012 mol) in phosphorus oxychloride (5 mL) were heated at 140 ºC under MWI (250 W) for 5 to 15 minutes. The reaction mixture was slowly poured into crushed ice with stirring and neutralized with solid sodium bicarbonate. Solid product was filtered, washed with cold water, dried, and recrystallized from the appropriate solvent.

Spectral data

*3-[(6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl) methyl]-2(3H)-benzoxazolone (**5a**)*

Yield: 74%. mp: 244-245 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 5.61(2H, s, CH_2) ; 7.16 (1H , td, H^6) ; 7.24 (1H , td , H^5) ; 7.35-7.40 (2H, m, H^4, H^7) ; 7.60-7.68 (3H , m , phenyl-$H^{3.4.5}$) ; 7.87-7.89 (2H, m, phenyl-$H^{2.6}$). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 167,47 (C=O), 154.27 (triazolothiadiazole-C), 153,95 (triazolothiadiazole-C), 142.87 (triazolothiadiazole-C), 142.44 (C), 133,51(C), 131.11 (C), 130.20 (2CH), 129.25 (CH), 127.52 (2CH), 124.46 (CH), 123.18(CH), 110.36(CH), 110.25(CH), 40.81 (CH_2). MS ESI(+) m/e 350.0702 (M+H, 100). Anal. calc. for $C_{17}H_{11}N_5O_2S$: C, 58.44; H, 3.17; N, 20.05; S, 9.18. Found C, 57.96; H, 3.37; N, 19.79; S, 9.30.

*3-{[6-(4-fluorophenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5b**)*

Yield: 76%. mp: 255-256 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.60 (2H, s, CH_2),7.16 (1H, t, H^6), 7.36 (1H, t, H^5), 7.34-7.39 (2H, m, H^4, H^7), 7.49 (2H, t, phenyl $H^{2,6}$), 7.94-7.98 (2H, m, phenyl $H^{3,5}$). MS ESI(+) m/e 368.0621 (M+H, 100). Anal. calc. for $C_{17}H_{10}FN_5O_2S$: C, 55.58; H, 2.74; N, 19.06; S, 8.73. Found C, 55.26; H, 2.91; N, 18.67; S, 8.78.

*3-{[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5c**)*

Yield: 73%. mp: 262-263 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.60 (2H, s, CH_2), 7.16 (1H, t, H^6), 7.37 (1H, t, H^5), 7.35-7.39 (2H, m, H^4, H^7), 7.71 (2H, d, phenyl $H^{2,6}$), 7.90 (2H, d, phenyl $H^{3,5}$). MS ESI(+) m/e 384.0329 (M+H, 100). Anal. calc. for $C_{17}H_{10}ClN_5O_2S$: C, 53.20; H, 2.63; N, 18.25; S, 8.35. Found C, 52.92; H, 2.81; N, 18.03; S, 8.43.

*3-{[6-(4-bromophenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5d**)*

Yield: 72%. mp: 266-267 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1760 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.60 (2H, s, CH_2), 7.16 (1H, t, H^6), 7.24 (1H, t, H^5), 7.35-7.39 (2H, m, H^4, H^7), 7.81-7.86 (4H, m, phenyl H). MS ESI(+) m/e 427.9806 (M+H, 100). Anal. calc. for $C_{17}H_{10}BrN_5O_2S$: C, 47.68; H, 2.35; N, 16.35; S, 7.49. Found C, 47.50; H, 2.53; N, 16.20; S, 7.56.

*3-{[6-(4-methoxyphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5e**)*

Yield: 67%. mp: 235-236 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.83 (3H, s, CH_3), 5.55 (2H, s, CH_2), 7.11-7.15 (3H, m, H^6, phenyl $H^{2,6}$), 7.20 (1H, td, H^5), 7.30-7.36 (2H, m, H^4, H^7), 7.79 (2H, d, phenyl $H^{3,5}$). MS ESI(+) m/e 380.0742 (M+H, 100). Anal. calc. for $C_{18}H_{13}N_5O_3S$: C, 56.98; H, 3.45; N, 18.46; S, 8.45. Found C, 57.08; H, 3.50; N, 18.32; S, 8.56.

*3-{[6-(4-tert-butylphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5f**)*

Yield: 59%. mp: 197-198 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1768 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.32 (9H, s, CH_3), 5.60 (2H, s, CH_2), 7.16 (1H, td, H^6), 7.24 (1H, td, H^5), 7.34-7.40 (2H, m, H^4, H^7), 7.64 (2H, d, phenyl $H^{2,6}$), 7.80 (2H, d, phenyl $H^{3,5}$). MS ESI(+) m/e 406.1354 (M+H, 100). Anal. calc. for $C_{21}H_{19}N_5O_2S$: C, 62.21; H, 4.72; N, 17.27; S, 7.91. Found C, 62.39; H, 4.70; N, 17.21; S, 8.07.

*3-{[6-(4-methylphenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5g**)*

Yield: 60%. mp: 247-248 ºC. Recrystallized from DMF-H_2O. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.40 (3H, s, CH_3), 5.59 (2H, s, CH_2), 7.16 (1H, td, H^6), 7.23 (1H, td, H^5), 7.35-7.39 (2H, m, H^4, H^7), 7.43 (2H, d, phenyl $H^{2,6}$), 7.77 (2H, d, phenyl H ^{3,5}). MS ESI(+) m/e 364.0858 (M+H, 100). Anal. calc. for $C_{18}H_{13}N_5O_2S$: C, 59.49; H, 3.61; N, 19.27; S, 8.82. Found C, 59.44;H, 3.80; N, 19.04; S, 8.97.

*3-({6-[4-(trifluoromethyl)phenyl][1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl}methyl)- 2(3H)-benzoxazolone (**5h**)*

Yield: 67%. mp: 307-308 ºC. Recrystallized from DMF-H_2O. FTIR-ATR ν max (cm^{-1}): 1769 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.63 (2H, s, CH_2), 7.17 (1H, t, H^6), 7.25 (1H, t, H^5), 7.36-7.40 (2H, m, H^4, H^7), 8.01 (2H, d, phenyl $H^{2,6}$), 8.11 (2H, d, phenyl H ^{3,5}). MS ESI(+) m/e 418.0584 (M+H, 100). Anal. calc. for $C_{18}H_{10}F_3N_5O_2S$: C,. 51.80; H, 2.42; N, 16.78; S, 7.68. Found C, 51.55; H, 2.59; N, 16.65; S, 7.78.

*3-({6-[4-(methylsulfonyl)phenyl][1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl}methyl)- 2(3H)-benzoxazolone (**5i**)*

Yield: 70%. mp: 302-303 ºC. Recrystallized from DMF-H_2O. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.32 **(**3H, s, SO_2CH_3), 5.63 (2H, s, CH_2), 7.17 (1H, t, H^6), 7.26 (1H, t, H^5),7.38-7.41 (2H, m, H^4, H^7), 8.13-8.18 (4H, m, phenyl H). MS ESI(+) m/e 428.0490 (M+H, 100). Anal. calc. for $C_{18}H_{13}N_5O_4S_2$: C, 50.58; H, 3.07; N, 16.38; S, 15.00. Found C, 50.91; H, 3.18; N, 16.41; S, 14.97.

*3-{[6-(4-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5j**)*

Yield: 66%. mp: 283-284 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1769 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.63 (2H, s, CH_2), 7.17 (1H, t, H^6), 7.26 (1H, t, H^5), 7.37-7.40 (2H, m, H^4, H^7), 8.16 (2H, d, phenyl-$H^{2,6}$), 8.45 (2H, d, phenyl-$H^{3,5}$). MS ESI(+) m/e 395.0558 (M+H, 100). Anal. calc. for $C_{17}H_{10}N_6O_4S$: C, 51.77; H, 2.56; N, 21.31; S, 8.13. Found C, 52.13; H, 2.69; N, 21.31; S, 8.31.

*3-{[6-(4-cyanophenyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5k**)*

Yield: 70%. mp: 272-273 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1770 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.62 (2H, s, CH_2), 7.16 (1H, td, H^6), 7.24 (1H, t, H^5), 7.35-7.39 (2H, m, H^4, H^7),8.05-8.12 (4H, m, phenyl-H). MS ESI(+) m/e 375.0659 (M+H, 100). Anal. calc. for $C_{18}H_{10}N_6O_2S$: C, 57.75; H, 2.69; N, 22.45; S, 8.56. Found C, 57.67; H, 2.79; N, 22.18; S, 8.66.

*3-{[6-(pyridin-4-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5l**)*

Yield: 34%. mp: 282-283 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1757 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.63 (2H, s, CH_2), 7.17 (1H, t, H^6), 7.25 (1H, t, H^5), 7.36-7.40 (2H, m, H^4, H^7), 7.84 (2H, d, pyridin $H^{2,6}$), 8.85 (2H, d, pyridin $H^{3,5}$). MS ESI(+) m/e 351.0664 (M+H, 100). Anal. calc. for $C_{16}H_{10}N_6O_2S$): C, H, N, S calc. 54.85, 2.88, 23.99, 9.15 found 54.43, 3.01, 23.59, 9.17.

*3-{[6-(thiophen-3-yl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5m**)*

Yield: 50%. mp: 233-234 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1769 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5,58(2H, s, CH_2) ; 7,16(1H

, td , H^6) ; 7,24 (1H , td , H^5) ; 7,35-7,40(2H , m ,$H^{7,4}$) ; 7,51-7,52 (1H , m , thienyl-H^4) ; 7,84-7,86(1H , m , thienyl-H^5) ; 8,47-8,48(1H, m, thienyl-H^2). MS ESI(+) m/e 356.0278 (M+H, 100). Anal. calc. for $C_{15}H_9N_5O_2S_2$: C, 50.69; H, 2.55; N, 19.71; S, 18.04. Found C, 53.04; H, 2.81; N, 20.43; S, 18.12.

*3-{[6-(furan-3-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**5n**)*

Yield: 85%. mp: 202-203 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1771 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 5,56(2H , s , CH_2) ; 6,91(1H , d , furan-H^4) ; 7,16(1H , t , H^6) ; 7,24(1H , t , H^5) ; 7,34-7,40(2H , m , $H^{7,4}$) ; 7,98-7,99(1H , m , furan-H^5) ; 8,72(1H , m ,furan-H^2). MS ESI(+) m/e 340.0508 (M+H, 100). Anal. calc. for $C_{15}H_9N_5O_3S$: C, 53.09; H, 2.67; N, 20.64; S, 9.45. Found C, 50.94; H, 2.65; N, 19.63; S, 9.54.

*3-[(6-benzyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl) methyl]-2(3H)-benzoxazolone (**6a**)*

Yield: 49%. mp: 188-189 ºC. Recrystallized from EtOH. FTIR-ATR ν max (cm^{-1}): 1767 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.42 (2H, s, CH_2) , 5.53 (2H, s, CH_2), 7.22-7.14 (2H, m, H^5, H^6), 7.27-7.25 (1H, m, H^4), 7.40-7.31 (6H, m, H^7, phenyl-H). MS ESI(+) m/e 364.0871 (M+H, 100). Anal. calc. for $C_{18}H_{13}N_5O_2S$: C, 59.49; H, 3.61; N, 19.27; S, 8.82. Found C, 58.83; H, 3.79; N, 18.27; S, 8.53.

*3-{[6-(4-fluorobenzyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**6b**)*

Yield: 74%. mp: 194-195 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1765 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.39 (2H, s, CH_2), 5.48 (2H, s, CH_2), 7.22-7.10 (5H, m, H^4, H^5, H^6, phenyl-$H^{2,6}$), 7.37-7.33 (3H, m, H^7, phenyl-$H^{3,5}$). MS ESI(+) m/e 382.0782 (M+H, 100). Anal. calc. for $C_{18}H_{12}FN_5O_2S$: C, 56.69; H, 3.17; N, 18.36; S, 8.41. Found C, 56.69; H, 3.18; N, 18.12; S, 8.45.

*3-{[6-(4-chlorobenzyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**6c**)*

Yield: 72%. mp: 198-199 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1763 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.44 (2H, s, CH_2), 5.52 (2H, s, CH_2), 7.26-7.14 (2H, m, H^5, H^6), 7.26-7.24 (1H, m, H^4), 7.44-7.36 (5H, m, H^7, phenyl-H). MS ESI(+) m/e 398.0481 (M+H, 100). Anal. calc. for $C_{18}H_{12}ClN_5O_2S$: C, 54.34; H, 3.04; N, 17.60; S, 8.06. Found C, 54.22; H, 3.14; N, 17.50; S, 8.02.

*3-{[6-(4-methylbenzyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**6d**)*

Yield: 71%. mp: 178-179 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1767 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.29 (3H, s, CH_3) , 4.36 (2H, s, CH_2),), 5.52 (2H, s, CH_2), 7.27-7.16 (7H, m, H^4, H^5, H^6, phenyl-H), 7.39 (1H, m, H^7). MS ESI(+) m/e 378.1029 (M+H, 100). Anal. calc. for $C_{19}H_{15}N_5O_2S$: C, 60.46; H, 4.01; N, 18.56; S, 8.50. Found C, 60.01; H, 4.17; N, 17.48; S, 8.02.

*3-{[6-(4-methoxybenzyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**6e**)*

Yield: 65%. mp: 145-146 ºC. Recrystallized from EtOH. FTIR-ATR ν max (cm^{-1}): 1768 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.75 (3H, s, OCH_3) , 4.34 (2H, s, CH_2), 5.52 (2H, s, CH_2), 6.92 (2H, d, phenyl-$H^{2,6}$), 7.22-7.14 (2H, m, H^5, H^6), 7.27-7.25 (3H, m, H^4, phenyl- $H^{3,5}$), 7.39 (1H, dd, H^7). MS ESI(+) m/e 394.0979 (M+H, 100). Anal. calc. for $C_{19}H_{15}N_5O_3S$: C, 58.01; H, 3.84; N, 17.80; S, 8.15. Found C, 57.53; H, 4.17; N, 16.70; S, 7.75.

*3-({6-[4-(trifluoromethyl)benzyl][1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl}methyl)- 2(3H)-benzoxazolone (**6f**)*

Yield: 65%. mp: 243-244 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1765 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.56 (2H, s, CH_2) , 5.52 (2H, s, CH_2), 7.20-7.13 (2H, m, H^5, H^6), 7.73 (2H, d, phenyl-$H^{2,6}$), 7.58 (2H, d, phenyl-$H^{3,5}$), 7.25-7.23 (1H, m, H^4), 7.39-7.36 (1H, m, H^7). MS ESI(+) m/e 432.0750 (M+H, 100). Anal. calc. for $C_{19}H_{12}F_3N_5O_2S$): C, 52.90; H, 2.80; N, 16.23; S, 7.43. Found C, 52.57; H, 2.96; N, 16.20; S, 7.48.

*3-{[6-(4-tert-butylbenzyl)[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl]methyl}-2(3H)-benzoxazolone (**6g**)*

Yield: 66%. mp: 154-155 ºC. Recrystallized from EtOH. FTIR-ATR ν max (cm^{-1}): 1765 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.23 (9H, s, CH_3), 4.33 (2H, s, CH_2), 5.49 (2H, s, CH_2), 7.18-7.10 (2H, m, H^5, H^6), 7.24-7.21 (3, m, H^4, phenyl $H^{2,6}$), 7.37-7.33 (3H, m, H^7, phenyl-$H^{3,5}$). MS ESI(+) m/e 420.1494 (M+H, 100). Anal. calc. for $C_{22}H_{21}N_5O_2S$: C, 62.99; H, 5.05; N, 16,69; S, 7.64. Found C, 62.97; H, 4.99; N, 16.53; S, 7.76.

*3-({6-[4-(methylsulfonyl)benzyl][1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl}methyl)- 2(3H)-benzoxazolone (**6h**)*

Yield: 63%. mp: 247-248 ºC. Recrystallized from EtOH-DMF. FTIR-ATR ν max (cm^{-1}): 1765 (C=O). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.23 (3H, s, SO_2CH_3) , 4.58 (2H, s, CH_2), 5.52 (2H, s, CH_2), 7.21-7.13 (2H, m,

H^5, H^6), 7.25-7.23 (1H, m, H^4), 7.39-7.37 (1H, m, H^7), 7.61 (2H, d, phenyl $H^{2,6}$), 7.91(2H, d, phenyl-$H^{3,5}$). MS ESI(+) m/e 442.0644 (M+H, 100). Anal. calc. for $C_{19}H_{15}N_5O_4S_2$: C, 51.69; H, 3.42; N, 15.86; S, 14.53. Found C, 51.76; H, 3.48; N, 15.74; S, 14.35.

Pharmacology

Swiss albino mice weighing 30–35g were used in the present study. Laboratory temperature was maintained at 20 ± 1 °C under conditions of a 12 hour light dark schedule. Before experimentation, mice were allowed 1 week of adaptation. They were used only once. The study was approved by the Local Ethics Committee of Eskisehir Osmangazi University for The Care and Use of Laboratory Animals. The animals were divided into 25 groups. Each group included seven animals. All compounds were dissolved in DMSO / water (1:4) and given to the animals per orally at 100 mg/kg doses. Control animals received orally 0.1 mL DMSO / water (1:4). Tail clip test, tail flick test to radiant heat, hot plate test, and writhing test induced by acetic acid were performed 60 minutes after the administration of the compounds or vehicle (DMSO for control group).

Tail clip test

This analgesic test is based on a method as described by Bianchi and Franceschini (1954), and Dajani *et al*. (1999). A pressure-standardized artery clip was placed approximately 2 cm from the base of tail and only the mice that responded to the clip placement by turning or biting the clip within 15 seconds were used in this test.

Tail flick test to radiant heat

This test described by D'Amour and Smith was done with a beam of high-intensity light focused on the dorsal surface of the tail (D'Amour, Smith, 1941). 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 seconds. A 15 second cut off was used in order to prevent damage to the tail.

Hot plate test

The test was based on the description by Eddy and Leimbach (1953) and Noble, Smadja and Roques (1994). A glass cylinder (16 mm high 16 mm 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 by 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 (cutoff time 45 s). The results were expressed as the percent of the maximal possible effect (% MPE ± SD).

$$\%\text{MPE} = \frac{\text{postdrug latency} - \text{predrug latency}}{\text{cutoff time} - \text{predrug latency}} \times 100$$

Writhing test

Abdominal constrictor test 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 in a period of 10 min starting 5 min after the i.p. administration of acetic acid (Koster, Anderson, De Beer, 1959). All tests were conducted between 9 and 12 a.m.

All results were expressed as mean of ± S.D. Statistical comparison was performed by using Student's t test.

Cyclooxygenase inhibitory assay

Inhibitory effects of the test compounds on COX-1 and COX-2 activities were investigated by using Cayman's Colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI, USA, Catalog No:760111; Lot Number: 043345). DMSO was used as solvent control. SC-560 for COX-1 and DuP-697 for COX-2 were used as reference standards. Each compound was tested at 10μM and all experiments were performed in triplicate.

RESULTS AND DISCUSSION

Chemistry

The synthesis of 3-[(6-substituted phenyl[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone (**5a-n**) and 3-[(6-substitutedbenzyl[1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone (**6a-h**) were accomplished as presented in Figure 1. Synthesis of 2(3*H*)-benzoxazolone **1** (Eren *et al*. 2010), ethyl-(2(3*H*)-benzoxazolone-3-yl)acetate **2** (Onkol *et al*. 2002) , (2(3*H*)-benzoxazolone-3-yl)acetic acid **3** (Onkol *et al*. 2002) and 3-[(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone **4** (Urlu Cicekli *et al*. 2012) was accomplished according to the previously reported procedures. 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone **4** was treated with substituted benzoic acid or phenylacetic acids using $POCl_3$ as cyclizing agent under microwave irradiation

to yield 3-[(6-substitutedphenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone (**5a-n**) and 3-[(6-substitutedbenzyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone (**6a-h**) in good yields (Figure 1). Advantages of microwave irradiation such as high yield, short reaction time, pure product, and easy work up prompted us to synthesize compounds under microwave irradiation.

The structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, LC-MS, and elemental analysis.

IR spectroscopic data of the compound **4** structure showed two characteristic absorption bonds, one of which appearing at 2585 cm^{-1} was attributed to SH and the other at 3200–3300 cm^{-1} was assigned to NH_2. However, both bonds disappeared with the formation of triazolothiadiazole derivatives (**5a-n, 6a-h**).

In the ^{1}H-NMR spectrum of **5a-n, 6a-h**, a singlet that appeared at δ 5.60 ppm was attributed to the methylene protons and **6a–h** exhibited the -CH_2-C_6H_5 signals as a singlet between δ 4.33 and 4.58 ppm.

Biological activity

The antinociceptive activity of the all compounds were evaluated using both chemical and thermal methods of nociception. These methods are used to detect the central and peripheral mechanisms of analgesia. Acetic acid induced writhing test is used for detecting peripheral analgesia, whereas tail flick test, hot plate and tail clip test are sensitive to centrally acting analgesia. In order to have a standard drug for comparison, the compounds were tested at an equimolar oral dose relative to 100 mg/kg aspirin. The percentage inhibition was calculated after 60 min. The effect of synthesized compounds is summarized in Table I.

The tail clip test revealed that the compounds **5i** and **6e** exhibited moderate antinociceptive activity in comparison to the standard drug aspirin.

Compounds **5f, 5g, 5m** and **6c** were more effective than aspirin in the tail flick test. It was observed that triazolothiadiazole derivatives having 4-ter-butylphenyl (**5f**), 4-methylphenyl (**5g**), 3-thienyl (**5m**)

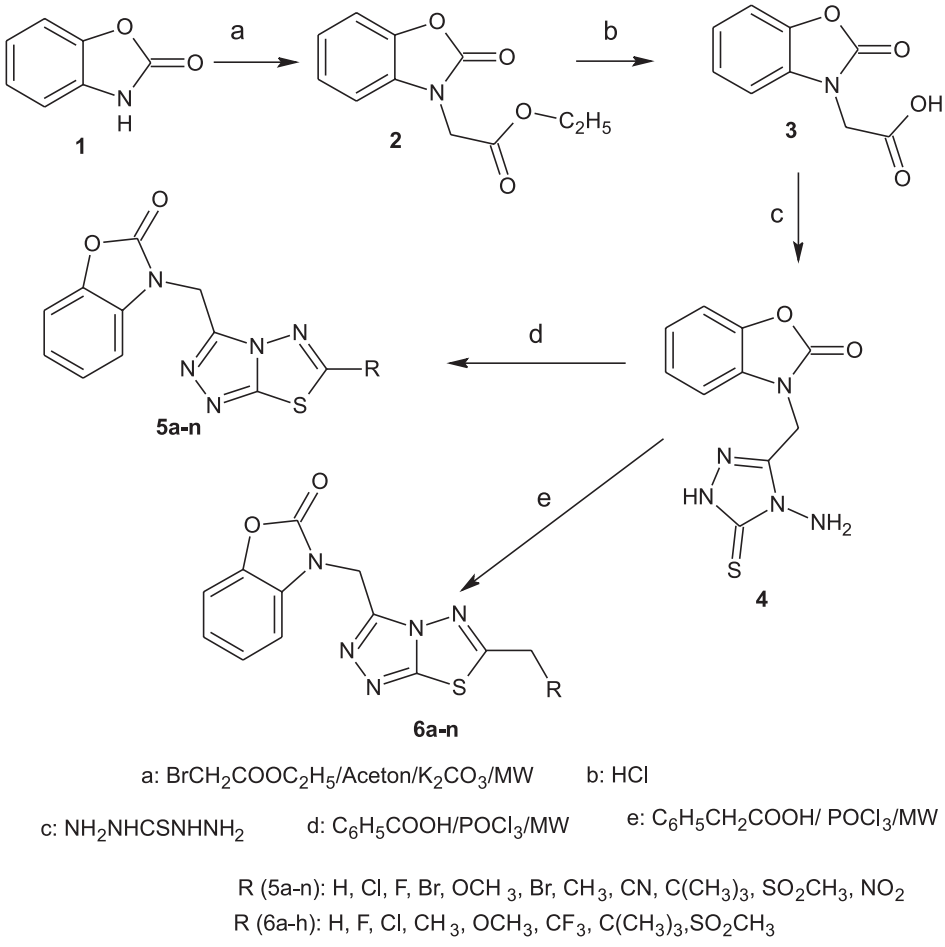

FIGURE 1 - Synthesis of compounds **5a-n** and **6a-h.**

TABLE I - Antinociceptive activity of synthesised compounds

Comp.	R	Tail Clip % MPE	Tail Flick % MPE	Hot Plate % MPE	Writting test Stretching number
Control		4,11±2,72	0	12,41±5,09	37,80±10,03
4		32,61±19,66*	64,25±19,14*	2,14±1,37	19,33±9,10*
5a	phenyl	38,03±13,64*	45,11±18,46*	10,75±6,91	33,83±9,80
5b	4-fluorophenyl	33,05±15,54*	16,85±6,79*	6,25±4,06	8,50±6,59*+
5c	4-chlorophenyl	35,68±15,93*	33,33±21,08*	31,85±14,68*+	20,33±7,36*
5d	4-bromophenyl	3,16±1,77	46,08±20,89*	17,50±6,86	21,83±6,82*
5e	4-methoxyphenyl	28,28±14,68*	34,25±14,58*	5,42±2,87	22,17±7,61*
5f	4-*tert*-butylphenyl	17,56±12,43*	77,69±14,13*+	15,12±8,59	13,83±4,62*+
5g	4-methylphenyl	36,12±20,37*	66,67±21,08*+	24,55±15,40	7,00±3,07*+
5h	4-(trifluoromethyl)phenyl	3,98±2,40	19,00±16,36*	3,84±1,31	43,20±8,76
5i	4-(methylsulfonyl)phenyl	40,77±13,79*	58,50±19,22*	24,77±13,32	13,50±7,67*
5j	4-nitrophenyl	26,30±16,79*	54,17±20,83*	8,17±5,07	6,17±2,91*+
5k	4-cyanophenyl	23,33±16,66*	26,15±17,25*	0	30,67±10,90
5l	pyridin-4-yl	7,25±4,41	36,00±17,29*	18,45±8,56	11,83±4,31*+
5m	thiophen-3-yl	9,21±6,44	68,83±19,78*+	4,83±2,81	31,17±4,85
5n	furan-3-yl	14,34±7,70*	45,32±16,11*	10,93±5,52	6,33±2,32*+
6a	phenyl	0	0	14,83±5,42	27,50±9,28
6b	4-fluorophenyl	33,33±21,05*	44,25±19,64*	0	10,00±5,03*+
6c	4-chlorophenyl	37,65±17,33*	63,94±17,29*+	20,96±13,59	25,33±7,88*
6d	4-methylphenyl	16,67±6,80*	29,73±19,01*	18,07±12,28	6,50±4,23*+
6e	4-methoxyphenyl	41,26±20,04*	0	9,26±3,29	22,50±5,96*
6f	4-(trifluoromethyl)phenyl	5,77±3,89*	21,56±15,85*	6,46±3,34	14,83±6,12*
6g	4-*tert*-butylphenyl	19,34±13,09*	38,35±15,47*	1,55±1,21	23,17±6,98*
6h	4-(methylsulfonyl)phenyl	33,46±16,34*	47,27±21,28*	4,29±2,04	24,67±6,45*
Aspirin		43,67±20,49*	37,39±20,10*	13,36±6,57	22,50±7,97*

(MPE : Maximum possible effect; all the values are given as X ± SD; n:7); *P< 0.05;as compared to control; +: P< 0.05; as compared to aspirin.

and 4-chlorophenyl (**6c**) groups showed good activity with 77.69%, 66.67%, 68.83% and 63.94%, respectively.

The hot plate test is generally used for centrally acting analgesic drugs. In this study, compound **5c** (4-chlorophenyl) was more effective than aspirin and compound **5g** (4-methylphenyl), **5i** (4-methylsulfonylphenyl)**,** and **6c** (4-chlorophenyl) were as effective as aspirin. Additionally, compound **5c** (4-chlorophenyl)**, 5g** (4-methylphenyl)**, 5i** (4-methylsulfonylphenyl) and **6c** (4-chlorophenyl) were more effective than compound **4** in the hot plate test. It was observed that triazolothiadiazole derivatives having 4-chlorophenyl (**5c**), 4-*tert*-butylphenyl (**5f**), 4-methylsulfonylphenyl (**5i**), 4-chlorophenyl (**6c**), and 4-methylphenyl (**6d**) groups also showed good activity.

The acetic acid–induced writhing method was widely used for the evaluation of peripheral antinociceptive activity. This method is considered to be a non–selective antinociceptive model. Compound **5b** (4-fluorophenyl) **, 5f** (4-*tert*-butylphenyl), **5g** (4-methylphenyl), **5j** (4-nitrophenyl), **5l** (pyridin-4-yl), **5n** (furan-3-yl), **6b** (4-fluorobenzyl), and **6d** (4-methylphenyl) exhibited higher antinociceptive activity in writhing test when compared with aspirin and compound **4**.

The effects of compounds on peripheral nervous system were found to be higher than central nervous system. Therefore, few compounds exhibiting significant *in vivo* antinociceptive activity were screened for their *in vitro* COX-1 and COX-2 inhibitory activity (Figure 2) by using colorimertic COX inhibitor screening assay method. SC-560 for COX-1 and DuP-697 for COX-2 were used

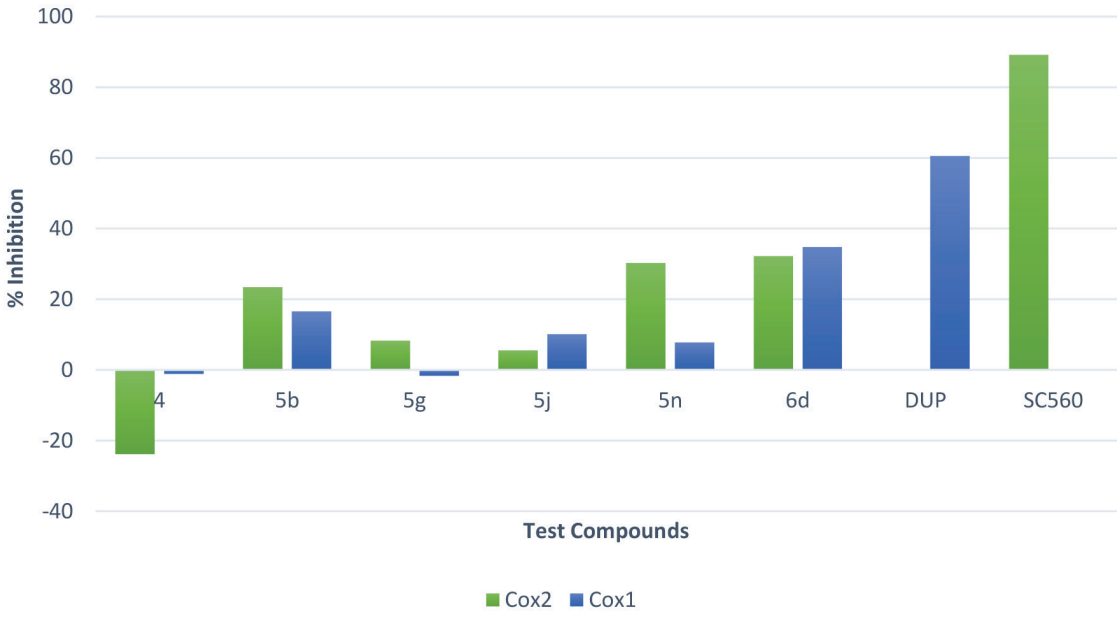

FIGURE 2 - COX-1 and COX-2 Inhibitory activities of test compounds at 10 µM concentration.

as reference standards. Except for compound **6d,** the test compounds showed no significant difference compared to reference standards. The percentage inhibition of *in-vitro* COX inhibition was depicted in **Figure 2**. Compound **6d** showed the highest COX-1 and COX-2 inhibition rate with 34.79% and 32.19%, respectively.

Compound **5i** showed significant activity in the tests which indicates that this compound shows its activity peripherally as well as centrally. Substitution of heteroaromatic groups on compound **4** decreases the antinociceptive activity which is comparable with the reference drugs. Also, the effect of electron withdrawing groups has increased in hot plate, tail flick and tail click tests. On the other hand, compound **5g** could be centrally active whereas compound **5j** might be peripherally active.

It was also noticed that the 3-[(6-substituedphenyl[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone derivatives (**5a-n**) showed higher activity than 3-[(6-substituebenzyl[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone derivatives (**6a-h**) in this study. These results suggest that these compounds could possibly have peripheral antinociceptive activity.

ETHICS STATEMENT

The experimental protocols were approved in accordance with the Guide for the Care and Use of Laboratory Animals at Eskisehir Osmangazi University (Protocol number; 31.03.2011/200).

CONCLUSION

In this study, new series of 3-substituted[1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]-2(3*H*)-benzoxazolone derivatives were synthesized and their antinociceptive activity was determined. Among these compounds, compound **5g** and **5j** have been found to be significantly more active than the other compounds and the standard in the tests. The mechanism of the biological activity needs further investigations.

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