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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Studies on fused heterocyclic 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazoles: synthesis and biological evaluation

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Abstract In this study, a series of 3,6-disubstituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazoles (**5a–t**) were synthesized by condensing 4-amino-3-mercapto-(4*H*)-1,2,4-triazoles (**4a–c**) with different aromatic or aroyl acids through one-pot reaction. The compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic, and lipid peroxidation actions. Some of the newly synthesized compounds showed very good anti-inflammatory activity with low GI toxicity and reduced lipid peroxidation. These compounds also showed interesting profile of analgesic activity in acetic acid-induced writhing test. The findings of the study indicate that the synthesized compounds have superior GI safety profile along with reduction in lipid peroxidation as compared to that of the standard.

Keywords Fused heterocycles · Triazolothiadiazoles · Anti-inflammatory · Ulcerogenic

Introduction

The chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention in recent years owing to their synthetic and effective medicinal importance. Heterocyclic compounds bearing a symmetrical triazoles or 1,3,4-thiadiazole moiety have been reported to have a broad spectrum of pharmacological activities including potential anti-inflammatory and analgesic activities (Silvia *et al.*, 2006; Kucukguzel *et al.*, 2007; Hafez *et al.*, 2008; Sharma *et al.*, 2008). There are many marketed drugs having the 1,2,4-triazole group, e.g., Triazolam, Alprazolam, Etizolam, and Furacylin (Karegoudar *et al.*, 2008). In addition to these important biological applications, mercapto-1,2,4-triazoles are also of great utility in preparative/synthetic organic chemistry, for example, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds, e.g., thiazolotriazoles, triazolothiadiazoles, triazolothiazines, triazolothiazepines, and triazolothiadiazines. Derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems (triazolothiadiazoles) found to have diverse pharmacological activities including unique anti-inflammatory, anti-edema, and analgesic properties (Mathew *et al.*, 2006, 2009; Karthikeyan *et al.*, 2007; Karegoudar *et al.*, 2008; Prasad *et al.*, 2009). Several triazolothiadiazole derivatives have been prepared from different non-steroidal anti-inflammatory agents and found to possess improved pharmacological profile (Udupi *et al.*, 2004; Metwally *et al.*, 2007; Amir *et al.*, 2007).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications in the world. They are used for the treatment of pain, fever, and inflammation, particularly arthritis (Moore *et al.*, 2006). The most common side effects of the use of NSAIDs are the occurrence of gastrointestinal damage with gastric upset and irritation being the major problems (Cioli *et al.*, 1979; Allison *et al.*, 1992). The search for safer NSAIDs continues with the failure of anticipated "Ideal" anti-inflammatory agents, the coxibs, on long-term usage (Verrico *et al.*, 2003; Wollheim, 2003).

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in a single molecule may lead to compounds with improved pharmacological profile. In this study, prompted

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by these observations, the synthesis and biological evaluation of 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazoles as hybrid molecules including different pharmacophores are aimed at. These compounds were evaluated of their anti-inflammatory, analgesic, ulcerogenic, and lipid peroxidation activities.

Materials and methods

Chemistry

All the solvents were of LR-grade and were obtained from Merck (Mumbai, India), CDH (New Delhi, India), and S.D.fine (Mumbai, India). Melting points were determined in open glass capillary tubes and are uncorrected. Thin layer chromatography (TLC) was performed on Silica gel G (Merck) in the solvent system—Toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v); iodine chamber, and UV lamp were used for visualization of TLC spots. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS) FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on Bruker DPX-300 NMR spectrometer or BRUKER 400 Ultra Shield™ in DMSO-d_6 using tetramethylsilane as an internal standard. The chemical shifts are reported in δ ppm scale. The splitting pattern abbreviations are as follows: s, singlet; br s, broad singlet; t, triplet; m, multiplet. The synthetic pathway is given in Scheme 1. Elemental data for C, H, and N were within ±0.4% of the theoretical values.

Synthesis of the compounds

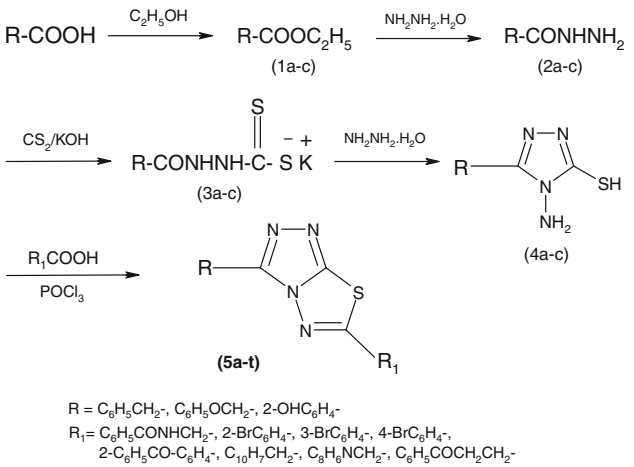
Synthesis of aryl esters (**1a–c**) and their hydrazides (**2a–c**): The aryl esters (**1a–c**) and their hydrazides (**2a–c**) were synthesized according to the literature method (Mathew *et al.*, 2006).

*Synthesis of potassium dithiocarbazinate derivatives (**3a–c**) (Mathew et al., 2006)* *General procedure*: Potassium hydroxide (0.03 mol) was dissolved in absolute ethanol (50 ml). The solution was cooled in ice bath and acid hydrazide (**3a–c**; 0.02 mol) was added with stirring. To this mixture, carbon disulfide (0.025 mol) was added in small portions with constant stirring. The reaction mixture was stirred continuously for 12 h at room temperature. The precipitated potassium dithiocarbazinate was collected by filtration, washed with anhydrous ether, and dried in vacuum. The potassium salt thus obtained was used in the next step without further purification.

*Synthesis of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol (**4a–c**) (Mathew et al., 2006)* *General procedure*: A suspension of potassium dithiocarbazinate derivative (**3a–c**) (0.1 mol), hydrazine hydrate (0.3 mol), and water (5 ml) was heated under reflux for 8 h, hydrogen sulfide was evolved during the reaction and clear solution resulted. Dilution of the reaction mixture with cold water (50 ml) and subsequent acidification with dil. HCl gave a white precipitate. It was filtered, washed with water, and crystallized from aq. ethanol to get TLC pure compound **4a–c**.

4-Amino-5-b-enzyl-4*H*-1,2,4-triazole-3-thiol (**4a**): Yield 76%; m.p. 170°C; FT-IR (KBr) cm^{-1}: 3317 (NH_2), 3068 (CH), 2596 (SH), 1590 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 3.98 (s, 2H, CH_2), 5.65 (s, 2H, NH_2), 7.08–7.31 (m, 5H, phenyl), 13.82 (br s, SH); $C_9H_{10}N_4S$; Calc. N 27.16; Found N 27.20.

4-Amino-5-phenoxymethyl-4*H*-1,2,4-triazole-3-thiol (**4b**): Yield 74%; m.p. 157°C; FT-IR (KBr) cm^{-1}: 3293 (NH_2), 3071 (CH), 2608 (SH), 1605 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 5.11 (s, 2H, OCH_2), 5.72 (s, 2H, NH_2), 7.11–7.36 (m, 5H, phenyl), 13.91 (br s, SH); $C_9H_{10}N_4OS$; Calc. N 25.21; Found N 25.24.



Scheme 1 Protocol for synthesis of the title compounds (**5a–t**)

4-Amino-5-(2-hydroxyphenyl-4*H*-1,2,4-triazole-3-thiol (**4c**): Yield 81%; m.p. 205°C; FT-IR (KBr) cm^{-1}: 3310 (NH_2), 3085 (CH), 2603 (SH), 1598 (C=N); ^1H NMR (DMSO-d_6) δ ppm: 4.98 (s, 1H, OH), 5.58 (s, 2H, NH_2), 7.03–7.37 (m, 5H, phenyl), 13.87 (br s, SH); $C_8H_8N_4OS$; Calc. N 26.91; Found N 26.75.

*3,6-Disubstituted-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazoles (**5a–t**) General procedure*: A mixture of 4-amino-5-benzyl-4H-1,2,4-triazole-3-thiol (**4a–c**) (0.01 mol), aryl/aroyl acid (0.01 mol), and $POCl_3$ (10 ml) was refluxed for 8–12 h. After completion of reaction, the reaction mixture was cooled to room temperature and then poured onto crushed ice. The crude product so obtained was filtered and neutralized with aq. ammonia. It was washed with water and crystallized from DMF–ethanol mixture (1:1) to get pure compound (**5a–t**).

3-Benzyl-6-phenylcarboxamidomethyl-[1,2,4]-triazolo-3,4-*b*][1,3,4]-thiadiazole (**5a**): Yield 67%; m.p. 106–108°C; Rf-value 0.75; FT-IR (KBr) cm^{-1}: 3034 (CH), 1640 (C=O), 1603 (C=N), 1489 (C–N), 721 (C–S-C); ^1H NMR (DMSO-d_6) δ ppm: 3.92 & 4.33 (s, each, 2× CH_2), 7.09–7.78 (m, 10H, Ar H), 8.31 (s, 1H, CONH); $C_{18}H_{15}N_5OS$; Calc. N 21.04; Found N 20.88.

3-Benzyl-6-(2-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5b**): Yield 70%; m.p. 170–172°C; Rf-value 0.66; FT-IR (KBr) cm^{-1}: 3060 (CH), 1636 (C=N), 1489 (C–N), 721 (C–S–C), 545 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 4.31 (s, 2H, CH_2), 7.13–7.73 (m, 9H, Ar–H); $C_{16}H_{11}BrN_4S$; Calc. N 15.09; Found N 15.25.

3-Benzyl-6-(3-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5c**): Yield 61%; m.p. 152–155°C; Rf-value 0.58; FT-IR (KBr) cm^{-1}: 3044 (CH), 1590 (C=N), 1489 (C–N), 716 (C–S–C), 541 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 3.96 (s, 2H, CH_2), 7.17–7.72 (m, 9H, Ar H); $C_{16}H_{11}BrN_4S$; Calc. N 15.09; Found N 15.17.

3-Benzyl-6-(4-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5d**): Yield 68%; m.p. 172–174°C; Rf-value 0.56; FT-IR (KBr) cm^{-1}: 3051 (CH), 1581 (C=N), 1476 (C–N), 721 (C–S–C), 568 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 4.49 (s, 2H, CH_2), 7.20–7.44 (m, 5H, phenyl), 7.88–7.96 (m, 4H, *p*-disubstituted phenyl); $C_{16}H_{11}BrN_4S$; Calc. N 15.09; Found N 15.17.

3-Benzyl-6-(2-benzoylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5e**): Yield 65%; m.p. 112–115°C; Rf-value 0.60; FT-IR (KBr) cm^{-1}: 3034 (CH), 1747 (C=O), 1590 (C=N), 1469 (C–N), 716 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 3.94 (s, 2H, CH_2), 7.13–7.81 (m, 14H, Ar H); $C_{23}H_{16}N_4OS$; Calc. N 14.13; Found N 14.35.

3-Benzyl-6-(1*H*-2-indolylmethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5f**): Yield 65%; m.p. 156–158°C; Rf-value 0.76; FT-IR (KBr) cm^{-1}: 3380 (NH, indole), 3028 (CH), 1608 (C=N), 1490 (C–N), 743 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 4.41 & 4.50 (s, each, 2× CH_2), 6.75–7.47 (complex m, 10H, Ar–H), 8.58 (s, 1H, NH); $C_{19}H_{15}N_5S$; Calc. N 20.47; Found N 20.22.

3-Benzyl-6-(1-naphthylmethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5g**): Yield 62%; m.p. 145–148°C; Rf-value 0.64; FT-IR (KBr) cm^{-1}: 3047 (CH), 1592 (C=N), 1461 (C–N), 718 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 4.44 & 4.98 (s, 2× CH_2), 7.35–8.16 (complex m, 12H, Ar–H); $C_{21}H_{16}N_4$ S; Calc. N 15.72; Found N 16.10.

3-Phenoxymethyl-6-phenylcarboxamidomethyl[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5h**): Yield 54%; m.p. 126–128°C; Rf-value 0.72; FT-IR (KBr) cm^{-1}: 3309 (NH), 3033 (CH), 1697 (C=O), 1583 (C=N), 1474 (C–N), 723 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 4.31 (s, 2H, CH_2), 5.10 (s, 2H, OCH_2), 6.85–7.43 (m, 10H, Ar H), 8.33 (s, 1H, CONH); $C_{18}H_{15}N_5O_2S$; Calc. N 19.40; Found N 19.67.

3-Phenoxymethyl-6-(2-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5i**): Yield 66%; m.p. 80–82°C; Rf-value 0.81; FT-IR (KBr) cm^{-1}: 2990 (CH), 1597 (C=N), 1485 (C–N), 690 (C–S-C), 543 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 5.64 (s, 2H, OCH_2), 7.04 (m, 1H, H-4, *o*-disubstituted phenyl), 7.19 (m, 2H, H-5,6, *o*-disubstituted phenyl), 7.39 (m, 2H, H-2,6, phenyl), 7.68 (m, 2H, H-4, phenyl + H-3, *o*-disubstituted phenyl), 7.98 (m, 2H, H-3,5, phenyl); $C_{16}H_{11}BrN_4OS$; Calc. N 14.47; Found N 14.61.

3-Phenoxymethyl-6-(3-bromophenyl)[1,2,4]triazolo[3,4*b*][1,3,4] thiadiazole (**5j**): Yield 62%; m.p. 110–112°C; Rf-value 0.34; FT-IR (KBr) cm^{-1}: 3028 (CH), 1609 (C=N), 1479 (C–N), 709 (C–S–C), 550 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 5.12 (s, 2H, OCH_2), 6.83–7.46 (m, 9H, Ar H); $C_{16}H_{11}BrN_4OS$; Calc. N 14.47; Found N 14.53.

3-Phenoxymethyl-6-(4-bromophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole (**5k**): Yield 60%; m.p. 82–85°C; Rf-value 0.46; FT-IR (KBr) cm^{-1}: 3037 (CH), 1615 (C=N), 1486 (C–N), 716 (C–S–C), 544 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 5.10 (s, 2H, OCH_2), 6.81–7.49 (m, 9H, Ar H); $C_{16}H_{11}BrN_4OS$; Calc. N 14.47; Found N 14.62.

3-Phenoxymethyl-6-(2-benzoylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5l**): Yield 65%; m.p. 101–103°C; Rf-value 0.63; FT-IR (KBr) cm^{-1}: 3080 (CH), 1747 (C=O), 1655 (C=N), 1466 (C–N), 692 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 5.65 (s, 2H, OCH_2), 7.02–7.85 (complex m, 14H, Ar–H); $C_{23}H_{16}N_4O_2S$; Calc. N 13.58; Found N 13.53.

3-Phenoxymethyl-6-(1*H*-2-Indolylmethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5m**): Yield 70%; m.p. 126–128°C; Rf-value 0.66; FT-IR (KBr) cm^{-1}: 3373 (NH, indole), 3016 (CH), 1614 (C=N), 1456 (C–N), 706 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 4.56 (s, 2H, CH_2), 5.29 (s, 2H, OCH_2), 6.97–7.53 (m, 10H, Ar–H), 8.63 (s, 1H, NH); $C_{19}H_{15}N_5OS$; Calc. N 19.65; Found N 19.38.

3-Phenoxymethyl-6-(1-naphthylmethyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**5n**): Yield 62%; m.p. 172–

175°C; Rf-value 0.73; FT-IR (KBr) cm^{-1}: 3024 (CH), 1598 (C=N), 1450 (C–N), 698 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 4.56 (s, 2H, CH_2), 5.29 (s, 2H, OCH_2), 7.06–7.56 (m, 12H, Ar–H); $C_{21}H_{16}N_4OS$; Calc. N 15.04; Found N 15.37.

3-Phenoxymethyl)-6-(3-oxo-3-phenylpropyl)[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5o**): Yield 58%; m.p. 98–100°C; Rf-value 0.46; FT-IR (KBr) cm^{-1}: 3052 (CH), 1753 (C=O), 1608 (C=N), 1467 (C–N), 701 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 3.08 & 3.85 (s, each, 2× CH_2), 5.14 (s, 2H, OCH_2), 6.40–7.36 (m, 10H, Ar–H); $C_{19}H_{16}N_4O_2S$; Calc. N 15.67; Found N 15.37.

3-(2-Hydroxyphenyl)-6-phenylcarboxamidomethyl-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5p**): Yield 60%; m.p. 136–138°C; Rf-value 0.67; FT-IR (KBr) cm^{-1}: 3652 (OH), 3054 (CH), 1606 (C=N), 1482 (C–N), 707 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 3.08 (s, 2H, CH_2), 4.86 (s, 1H, OH), 6.92–8.13 (m, 9H, Ar H), 8.31 (s, 1H, CONH); $C_{17}H_{13}N_5O_2S$; Calc. N 19.93; Found N 20.20.

3-(2-Hydroxyphenyl)-6-(2-bromophenyl)[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5q**): Yield 64%; m.p. 166–168°C; Rf-value 0.56; FT-IR (KBr) cm^{-1}: 3695 (OH), 3029 (CH), 1598 (C=N), 1489 (C–N), 689 (C–S–C), 571 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 4.95 (s, 1H, OH), 6.94–8.18 (m, 8H, Ar–H); $C_{15}H_9BrN_4OS$; Calc. N 15.01; Found N 15.26.

3-(2-Hydroxyphenyl)-6-(3-bromophenyl)[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5r**): Yield 58%; m.p. 92–95°C; Rf-value 0.78; FT-IR (KBr) cm^{-1}: 3657 (OH), 3005 (CH), 1607 (C=N), 1473 (C–N), 693 (C–S–C), 589 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 4.95 (s, 1H, OH), 6.86–7.95 (m, 8H, Ar–H); $C_{15}H_9BrN_4OS$; Calc. N 15.01; Found N 15.21.

3-(2-Hydroxyphenyl)-6-(4-bromophenyl)[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5s**): Yield 63%; m.p. 183–185°C; Rf-value 0.56; FT-IR (KBr) cm^{-1}: 3690 (OH), 3023 (CH), 1585 (C=N), 1457 (C–N), 688(C–S–C), 572 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 4.94 (s, 1H, OH), 6.83–7.86 (m, 8H, Ar–H); $C_{15}H_9BrN_4OS$; Calc. N 15.01; Found N 15.23.

3-(2-Hydroxyphenyl)-6-(1-naphthyl)[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole (**5t**): Yield 65%; m.p. 202–204°C; Rf-value 0.70; FT-IR (KBr) cm^{-1}: 3660 (OH), 3011 (CH), 1589 (C=N), 1475 (C–N), 701 (C–S–C); ^1H NMR (DMSO-d_6) δ ppm: 4.03 (s, 2H, CH_2), 4.91 (s, 1H, OH), 7.11–8.14 (m, 11H, Ar H); $C_{20}H_{14}N_4OS$; Calc. N 15.63; Found N 15.90.

Biological evaluation

Wistar rats and albino mice of either sex weighing 180–200 and 22–25 g, respectively, were used. The animals were housed in groups of six at room temperature of 25 ± 2°C under 12 h light/12 h dark cycle with free access to food and water ad libitum. The studies were undertaken with prior approval from the Institutional Animal Ethics Committee (IAEC) and utmost care was taken to insure that the animals were treated in the most humane and acceptable manner. Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi, India. Registration number and date of registration of Animal House Facility: 173/CPCSEA, 28, January 2000.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity following carrageenan-induced rat paw edema method (Winter *et al.*, 1962). The rats were randomly divided into groups of six. One group was kept as control, and received only 0.5% carboxymethyl cellulose (CMC) solution and the other groups were treated with test compounds and standard drug (Indomethacin) at a dose level of 20 mg/kg p.o. Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after the administration of the test compounds, and standard drugs. The paw volume was measured by saline displacement shown on screen of digital Plethysmometer (Ugo Basile, Italy) at 2 and 3 h after carrageenan injection. Thus, the edema volume in control group (V_c) and that of in groups treated with test compounds (V_t) was measured and the percentage inhibition of edema was calculated using the formula: Anti-inflammatory activity (% inhibition) = $[(V_c - V_t)/V_c] \times 100$.

Analgesic activity

Analgesic activity was carried out by acetic acid-induced writhing method (Collier *et al.*, 1968) in mice. The compounds, which exhibited good anti-inflammatory activity (>50%), were screened for their analgesic activity. A 1% aqueous acetic acid solution (i.p. injection; 0.1 ml) was used as writhing-inducing agent. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after i.p. administration of test drugs and the reference drug (aspirin) at the dose of 25 mg/kg. All the compounds were injected as CMC suspension (1%). One group was kept as control and received 1% CMC. After 20 min of drug administration, 0.10 ml of 1% acetic acid solution was given to mice intraperitoneally. The severity of writhing response (stretching movements consisting of arching of the back, elongation of body, and extension of hind limbs) was recorded for 20 min after administration of acetic acid solution. The analgesic activity was presented as % protection.

% Analgesic activity = $(n - n'/n) \times 100$, where n and n' are the mean number of writhes of control and test groups, respectively.

Acute ulcerogenesis

Ulcerogenic activity (Cioli *et al.*, 1979) evaluated after p.o. administration of test compounds or indomethacin at the dose of 60 mg/kg. The rats (150–200 g) were divided into different groups consisting of six animals in each group. Control rats received p.o. administration of vehicle (suspension of 1% methyl cellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then killed. The stomach was removed and opened along the greater curvature, washed with distilled water, and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system: 0.5, redness; 1.0, spot ulcers; 1.5, hemorrhagic streaks; 2.0, ulcers > 3 but ≤ 5; 3.0, ulcers > 5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the reported method (Okhawa *et al.*, 1979). After screening for ulcerogenic activity, the gastric mucosa was scrapped with two glass slides, weighed (100 mg), and homogenized in 1.8 ml of 1.15% cold potassium chloride solution. The homogenate was supplemented with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of acetate buffer (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid. The mixture was heated at 95°C for 1 h. After cooling, the reactants were supplemented with 5 ml of the mixture of *n*-butanol:pyridine (15:1 v/v), shaken vigorously for 1 min, and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was taken out and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using extinction coefficient $1.56 \times 10^5 cm^{-1}\ M^{-1}$.

Results and discussion

Synthesis

The preparation of 3,6-disubstituted-[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole derivatives (**5a–t**) (Table 2) is depicted in Scheme 1. The structures of 4-amino-3-substituted-5-mercapto-(4*H*)-1,2,4-triazoles (**4a–c**) were confirmed by IR, ^{1}H NMR spectral data, and microanalysis. The ^{1}H NMR spectra showed a downfield broad singlet at around δ 13.8 attributed to SH group, whereas the NH_2 group appeared as a singlet at around δ 5.6. The absence of signals for NH_2 and SH protons confirmed that the triazoles

Table 1 Biological evaluation data of the title compounds

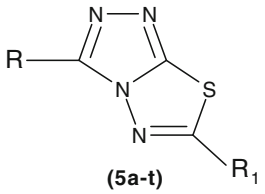
Compound	Anti-inflammatory activity[a] (% inhibition)		Analgesic activity[a] (% protection)	Ulcerogenic activity[a] (severity index)	Lipid peroxidation[a,b]
	After 2 h	After 3 h			
5a	21.23 ± 1.96**	28.03 ± 2.52**			
5e	40.97 ± 2.22	56.26 ± 3.03*	30.43 ± 1.59**	0.91 ± 0.35**	3.26 ± 0.23**
5f	36.73 ± 2.76**	40.98 ± 1.63**			
5h	35.88 ± 2.58**	37.15 ± 2.07**			
5l	54.78 ± 2.56	63.29 ± 2.50	43.48 ± 1.25**	1.25. ± 0.21*	4.42 ± 0.52**
5m	32.06 ± 2.5**	50.53 ± 2.58**	40.22 ± 3.11**	0.83 ± 0.25**	3.15 ± 0.46**
5n	18.47 ± 3.26**	24.63 ± 2.98**			
5o	56.47 ± 1.48	70.91 ± 2.16	65.58 ± 1.72	1.16 ± 0.25**	3.95 ± 0.85**
5p	24.42 ± 1.7**	32.27 ± 2.19**			
5t	28.03 ± 1.97**	35.03 ± 1.43**			
Control	–	–	–	0.00	2.59 ± 0.20**
Indomethacin	50.74 ± 3.86	66.87 ± 1.83		2.25 ± 0.21	7.95 ± 0.65
Acetylsalicylic acid			61.96 ± 1.66		

[a] Values are represented as mean ± SEM. Relative to respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for $n = 6$

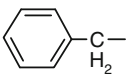
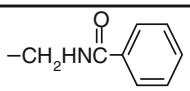
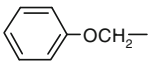
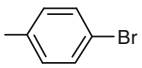
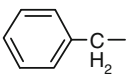
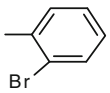
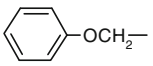
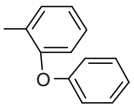
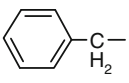
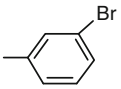
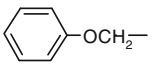
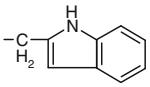
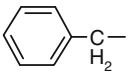
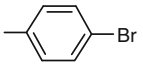
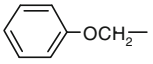
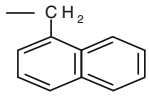
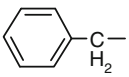
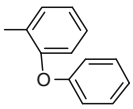
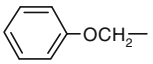
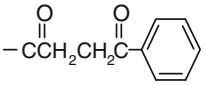
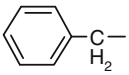
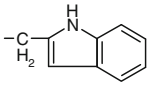
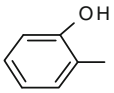
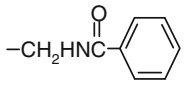
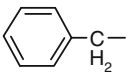
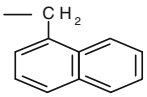
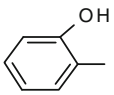
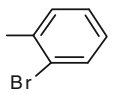
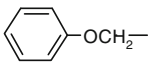
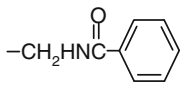
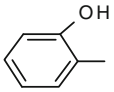
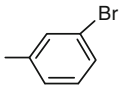
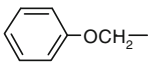
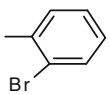
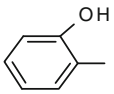
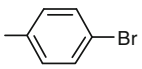
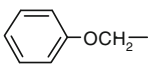
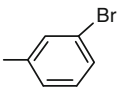
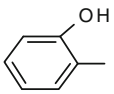
[b] Lipid peroxidation values are presented as nmol MDA content/100 mg tissue

** $P < 0.01$, * $P < 0.05$

Table 2 Structures of the synthesized compounds (**5a–t**)



(5a-t)

No.	R	R_1	No.	R	R_1
5a			5k		
5b			5l		
5c			5m		
5d			5n		
5e			5o		
5f			5p		
5g			5q		
5h			5r		
5i			5s		
5j			5t		

were converted into triazolo–thiadiazoles (**5a–t**) by reacting with the –COOH group of acids.

Biological evaluation

Anti-inflammatory activity

The in vivo anti-inflammatory activity of ten of the synthesized compounds, **5a**, **5e**, **5f**, **5h**, **5l–p**, and **5t**, was evaluated by carrageenan-induced rat paw edema method (Winter *et al.*, 1962). The compounds were tested at 20 mg/kg oral dose and were compared with the standard drug indomethacin at the same oral dose. The tested compounds showed anti-inflammatory activity ranging from 24.63 to 70.91% (Table 1). Compound **5o** presented the highest anti-inflammatory activity (70.91%) better than the standard drug indomethacin (66.87%). Another compound, **5l**, showed better anti-inflammatory activity (63.29%) than that of the standard drug. Two more compounds, **5e** and **5m**, showed significant activity with 56.26 and 50.53% inhibition, respectively. It was observed that the triazolo–thiadiazole derivatives having 3-oxo-3-phenylpropyl group (**5o**)

and 2-benzoylphenyl group (**5l**) at C-6 position possess high activity. Replacement of these groups by 1*H*-2-indolylmethyl (**5m**) resulted a decrease in anti-inflammatory activity. It was also observed that the presence of benzyl (**5a**, **5e**, and **5f**) or 2-hydroxyphenyl moiety (**5p** and **5t**) at C-3 showed moderate anti-inflammatory activity. From the view point of structure–activity relationship, it is clear that the triazolo–thiadiazole derivatives of phenoxy acetic acid were found to be more active than phenyl acetic acid and salicylic acid derivatives. Similar results have been reported by Mathew *et al.* (2006); among the synthesized different series, substituted phenoxy acetic acid series showed highest degree of anti-inflammatory and analgesic activity.

Test compounds **5e**, **5l**, **5m**, and **5o** were exhibited good anti-inflammatory activity (>50%), and were further evaluated for their analgesic, ulcerogenic, and lipid peroxidation actions.

Analgesic activity

The compounds **5e**, **5l**, **5m**, and **5o** evaluated for their analgesic effects using acetic acid-induced writhing method (Collier *et al.*, 1968). The results of analgesic activity (Table 1) indicated that compounds **5o** having 3-oxo-3-phenylpropyl group at C-3 of triazolo–thiadiazole ring showed better activity (65.58%) than that of standard drug acetylsalicylic acid (aspirin) (61.96%). Replacement of this group by 2-benzoylphenyl (**5l**) and 1*H*-2-indolylmethyl (**5m**) resulted a decrease in activity (43.48 and 40.22%, respectively). These findings present an important advantage of compounds **5o** as anti-inflammatory with high analgesic activity better than that of respective standards.

Acute ulcerogenesis

The compounds which were screened for analgesic activity were further tested for their ulcerogenic activity. Compounds **5e**, **5l**, **5m**, and **5o** were tested according to the method reported by Cioli *et al.* (1979). The tested compounds showed low ulcerogenic activity (severity index) ranging from 0.83 to 1.25, whereas the standard drug indomethacin showed high severity index of 2.25. The tested compounds exhibited better GI safety profile than that of standard drug indomethacin (Table 1).

Lipid peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. This process proceeds by free radical chain reaction in which free radicals steal electrons from the lipids in cell membrane and consequently damages the cell. It often affects polyunsaturated fatty acids forming malondialdehyde (MDA). It is evident that compounds showing low ulcerogenic activity also showcase the reduction of MDA content, a byproduct of lipid peroxidation. Therefore, an attempt was made to correlate the low ulcerogenesis of the compounds with that of lipid peroxidation. The lipid peroxidation was measured as nmol of MDA/100 mg of tissue (Okhawa *et al.*, 1979). The compounds, **5e**, **5l**, **5m**, and **5o**, which were screened for ulcerogenic activity, were further tested for their lipid peroxidation action. Indomethacin (standard drugs) produced high lipid peroxidation, 7.95, whereas the control group showed 2.59 nmol/100 mg of tissue (Table 1). It was found that all compounds showing low ulcerogenic activity also reduced lipid peroxidation. Therefore, the study indicated that these fused heterocyclic compounds have inhibited the induction of gastric mucosal lesions, and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

Conclusion

Twenty fused heterocyclic analogues (Table 2) were successfully synthesized. Among the newer derivatives, two compounds, 3-phenoxymethyl-6-(2-benzoylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole **5l** and 3-phenoxymethyl-6-(3-oxo-3-phenylpropyl)[1,2,4]-triazolo-[3,4-*b*][1,3,4]-thiadiazole **5o** emerged as lead compounds. These compounds showed superior GI safety profile along with reduction in lipid peroxidation as compared to that of indomethacin. Biological evaluation results showed that the compounds are promising anti-inflammatory and analgesic agents and could be further modified to develop potential and safer anti-inflammatory and analgesic agents.

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