Med Chem Res (2013) 22:3316–3328
DOI 10.1007/s00044-012-0329-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and pharmacological evaluation of *N*-(6-chlorobenzo[*d*]thiazol-2-yl)hydrazine carboxamide derivatives of benzothiazole

Sadaf J. Gilani · Suroor A. Khan

Received: 21 June 2012 / Accepted: 6 November 2012 / Published online: 21 November 2012

Abstract A series of 6-substituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole (**6a–g**) and 1,3,4-oxadiazole (**7a–g**, **8**) derivatives of benzothiazole were synthesized in satisfactory yield and pharmacologically evaluated for their anti-inflammatory, analgesic, ulcerogenic, and lipid peroxidation activities by known experimental models. All the synthesized compounds were in good agreement with elemental and spectral data. Some of the synthesized compounds have significant anti-inflammatory and analgesic activities. Ulcerogenic and irritative action on the gastrointestinal mucosa, in comparison with standard are low.

Keywords Triazolo-thiadiazole · 1,3,4-Oxadiazole · Anti-inflammatory · Analgesic · Ulcerogenic · Lipid peroxidation

Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are the drugs with analgesic, anti-pyretic, and anti-inflammatory effects. The anti-inflammatory activity of the NSAIDs is mediated chiefly through inhibition of biosynthesis of prostaglandins. Prostaglandins are derived from arachidonic acid, which originates from cell membrane phospholipids through the action of phospholipase A_2. The metabolism of arachidonic acid to prostaglandins and leukotrienes is catalyzed by cyclo-oxygenase and 5-lipoxygenase pathways, respectively (Tripathi, 2003; Brunton *et al.*, 2006).

Two related, but unique isoforms of cyclo-oxygenase, designated COX-1 and COX-2 have been demonstrated in mammalian cells (Gilani *et al.*, 2011a; Kumar *et al.*, 2008). COX-1 is constitutively expressed and generates PGs believed to be involved in GI mucosal protection (Habeeb *et al.*, 2001), whereas at the sites of inflammation throughout the body, COX-2 is induced to generate PGs, believed to mediate inflammation and pain. The anti-inflammatory effects of non selective NSAIDs therefore seen to be mediated via inhibition of COX-2 (Almansa *et al.*, 2003), whereas deleterious effects in GI tract such as gastroduodenal ulceration, occurs as a result of inhibition of COX-1.

Traditional NSAIDs such as aspirin, diclofenac, flurbiprofen, and ibuprofen are non selective; however, they show greater selectivity for COX-1 than COX-2 (Jackson and Hawkey, 1999; DeWitt, 1999; Mitchell *et al.*, 1993). Therefore chronic use of NSAIDs may elicit appreciable GI irritation, bleeding, and ulceration. The discovery of COX-2 provided the rationale for the development of drugs devoid of GI disorders while retaining clinical efficacy as anti-inflammatory agents. But the recent reports showed that selective COX-2 inhibitors (coxibs) could lead to adverse cardiovascular effects (Dogne *et al.*, 2005). Therefore, development of novel compounds having anti-inflammatory and analgesic activity with improved safety profile is still a necessity.

Despite numerous attempts to develop new structural prototype in the search for more effective NSAIDs,

S. J. Gilani (✉)
Department of Pharmaceutical Chemistry, KIET School of Pharmacy, Ghaziabad 201206, Uttar Pradesh, India
e-mail: gilanisadaf@gmail.com

S. J. Gilani · S. A. Khan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi 110062, India

benzothiazole still remain as one of the most versatile class of compounds against inflammation and therefore, are useful substructures for further molecular exploration. Benzothiazole's literature is enriched with progressive findings of the moiety in respect of anti-inflammatory (Paramashivappa *et al.*, 2003) and analgesic (Rana *et al.*, 2007) activities. In addition, triazolo-thiadiazoles nucleus constitutes the active part of several biologically active compounds, including anti-bacterial (Gilani *et al.*, 2011b), anti-fungal (Gilani *et al.*, 2011c), anti-tumor (Ibrahim, 2009), anti-inflammatory (Mathew *et al.*, 2006), analgesic (Amir *et al.*, 2007) and so on. Moreover, 1,3,4-oxadiazole also reported significant anti-microbial (Padmavathi *et al.*, 2009), anti-inflammatory (Amir and Shikha, 2004), and analgesic activities (Gilani *et al.*, 2010). Apart from above 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., thiazolo-triazoles, triazolo-thiadiazoles, triazolo-thiazines, triazolo-thiazepines, and triazolo-thiadiazines (Husain and Naseer, 2011). A triazolo-thiadiazole system may be viewed as a cyclic analog of two very important components-thiosemicarbazide (Khalil, 2007) and biguanide (Mathew *et al.*, 2007), which often display diverse biological activities. Inspired by these observations a composite system was investigated, which combine these two biolabile components in a ring together to give a compact and planar structure, and screened for their biological activities. The prime objective for the current study is to develop novel derivatives of benzothiazole and finally screen them against anti-inflammatory analgesic, ulcerogenic, and lipid peroxidation activities.

Therefore, in continuation of our interest in the synthesis of heterocycles containing benzothiazole moiety it was planned to synthesize hybrid compounds incorporating benzothiazole moiety with triazolo-thiadiazole and oxadiazole ring systems through different linkages. This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. The substitution pattern of triazolo-thiadiazole and oxadiazole rings was carefully selected so as to confer different electronic environment to the molecules. Hence, to discover new and useful agents for treatment of aforementioned diseases, we have replaced the hydrazide group of *N*-(6-chlorobenzo[*d*]thiazol-2-yl)hydrazine carboxamide with additional heterocycles, which have been found to possess an interesting profile of anti-inflammatory and analgesic activity with significant reduction in their ulcerogenic risks in the stomach.

Materials and methods

Chemistry

All the chemicals were purchased from Merck Chemical Company, S.D. Fine (India) and Qualigens (India). Melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FTIR spectrometer (ν_{max} in cm^{-1}), and ^1H NMR spectra were recorded in DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR) spectrometer using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are expressed in parts per million (ppm); coupling constants (*J*) are reported in hertz and refer to apparent peak multiplicities, which may not necessarily be true coupling constants. Mass spectra were recorded using Jeol SR-102 (FAB) mass spectrometer. The homogeneity of the compounds was checked by thin layer chromatography (TLC) on silica gel G (Merck) coated plates using toluene:ethylacetate:formic acid (5:4:1) as solvent system. Iodine chamber and UV lamp were used for the visualization of TLC spots. Spectral data (^1H NMR, IR and mass) of the synthesized compounds were in full agreement with the proposed structures. Elemental data were performed on Perkin Elmer models 240 CHN analyzer and found to be within ±0.4 % of the theoretical values.

*Synthesis of 6-chloro-1,3-benzothiazole-2-amines (**1**)*

A mixture of aniline (0.01 mol) and potassium thiocyanate (0.01 mol) in glacial acetic acid (g.a.a., 10 %) was cooled and stirred. To this solution bromine (0.01 mol) was added dropwise at such a rate as to keep the temperature below 10 °C throughout the addition. Stirring was continued for an additional 3 h and the separated hydrochloride salt was filtered, washed with acetic acid, and dried. It was dissolved in hot water and neutralized with aqueous ammonia solution (25 %), filtered, washed with water and dried, recrystallized with benzene.

Yield 81 %; m.p. 208–210 °C; Rf-value 0.70; IR (KBr) ν cm^{-1}: 817 (C–Cl), 1570 (C=N), 3480 (NH); ^1H NMR (DMSO-d_6) δ (ppm): 6.12 (s, 2H, NH_2), 6.61–6.64 (m, $J = 9$ Hz, 3H, Ar–H).

*Synthesis of 1-(6-chloro-1,3-benzothiazol-2-yl) urea (**2**)*

To the solution of sodium cyanate in minimum quantity of water, glacial acetic acid (5 mL) was added. This solution was heated with 2-amino-6-chloro-benzothiazole (**1**, 0.01 mol) in alcohol till the contents of mixture become turbid and volume remained half of the original volume. The contents were added to ice cool water. The solid obtained was filtered off and dried.

Yield 76 %; m.p. 230–232 °C; Rf-value 0.76; IR (KBr) ν cm^{-1}: 830 (C–Cl), 1560 (C=N), 1628 (C=O), 3310 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 6.34 (s, 2H, NH_2), 6.68–6.70 (m, $J = 6$ Hz, 3H, Ar–H), 8.10 (s, 1H, NHC=O).

*Synthesis of N-(6-chlorobenzo[d]thiazol-2-yl)hydrazine carboxamide (**3**)*

To the warm hydrazine hydrate solution of compound **2** in alcohol, conc. NaOH was added and refluxed for 6 h. Reaction mixture was poured into crushed ice and solid obtained was filtered off and dried. The solid collected out was recrystallized from suitable solvent.

Yield 82 %; m.p. 241–243 °C; Rf-value 0.71; IR (KBr) ν cm^{-1}: 657 (C–S–C), 817 (C–Cl), 1588 (C=N), 1660 (C=O), 3300 (NH); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28 (s, 1H, $NHNH_2$), 7.70–7.74 (m, $J = 12$ Hz, 3H, Ar–H), 9.12 (s, 1H, NHC=O).

*Synthesis of potassium dithiocarbazinate (**4**)*

Potassium hydroxide (0.03 mol) was dissolved in absolute ethanol (50 mL). The solution was cooled in ice bath and acid hydrazide (**3**; 0.02 mol) was added with stirring. To this carbon disulfide (0.025 mol) was added in small portions with constant stirring. The reaction mixture was agitated continuously for 12 h at room temperature. The precipitated potassium dithiocarbazinate was collected by filtration, washed with anhydrous ether (100 mL) and dried in vacuum. The potassium salt thus obtained was in quantitative yield and was used in the next step without further purification.

*Synthesis of 4-amino-5-(6-chlorobenzo[d]thiazol-2-ylamino)4H-1,2,4-triazole-3-thiol (**5**)*

A suspension of potassium dithiocarbazinate (**4**; 0.02 mol) in water (50 mL) and hydrazine hydrate (99 %, 0.04 mol) was refluxed for 18–20 h with occasional shaking. The color of the reaction mixture changed to green, with evolution of hydrogen sulfide gas. A homogenous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water (20 mL). On acidification with acetic acid, the required triazole was precipitated out. It was filtered, washed thoroughly with cold water, dried, and recrystallized from ethanol.

Yield 74 %; m.p. 198–200 °C; Rf-value 0.68; IR (KBr) ν cm^{-1}: 635 (C–S–C), 817 (C–Cl), 1528 (C=N), 2518 (SH), 3338 (NH_2); ^{1}H NMR (DMSO-d_6) δ (ppm): 5.15 (s, 2H, NH_2), 7.28–7.32 (m, $J = 12$ Hz, 3H, Ar–H), 9.29 (s, 1H, NH), 13.18 (s,1H, SH).

*Synthesis of 6-chloro-N-(6-substituted[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-3-yl) benzo[d]thiazol-2-amine (**6a–g**) general procedure*

An equimolar mixture of 4-amino-5-(6-chlorobenzo[*d*]thiazol-2-ylamino)-4*H*-1,2,4-triazole-3-thiol (**5,** 0.10 mol) and aromatic acids in phosphorus oxychloride (10 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, treated with dilute sodium hydroxide solution and washed thoroughly with cold water. The compound so obtained was dried and recrystallized from ethanol.

*6-Chloro-N-(6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzo[d]thiazol-2-amine (**6a**): Yield 74 %; m.p. 238–240 °C; Rf-value 0.70; IR (KBr) ν cm^{-1}* 610 (C–S–C benzothiazole), 674 (C–S–C triazolo-thiadiazole), 837 (C–Cl), 1269 (N–N=C triazolo-thiadiazole), 1416 (C–N benzothiazole), 1518 (C=C aromatic), 3084 (C–H aromatic), 3314 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.41–7.44 (m, $J = 9$ Hz, 8H, Ar–H), 8.06 (s,1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 128.7 (C-4′), 129.2 (C-3′ & C-5′), 129.8 (C-6, benzothiazole), 130.9 (C-2′ & C-6′), 132.3 (C-1a, benzothiazole), 133.5 (C-1′), 143.3 (C-6, triazolo-thiadiazole), 151.3 (C-4a, benzothiazole), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 174.5 (C-2, benzothiazole); MS: *m/z* 384 (M^+), 385 (M^++1), 386 (M^++2); Anal. Calc. for $C_{16}H_9ClN_6S_2$ (384.87): C 49.93, H 2.36, N 21.84, S 16.66, Cl 9.21. Found: C 49.96, H 2.40, N 21.88, S 16.69, Cl 19.24.

*6-Chloro-N-(6-(2-chlorophenyl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl)benzo[d]thiazol-2-amine (**6b**): Yield 71 %; m.p.254–256 °C; Rf-value 0.60; IR (KBr) ν cm^{-1}* 616 (C–S–C benzothiazole), 688 (C–S–C triazolo-thiadiazole), 821 (C–Cl), 1274 (N–N=C triazolo-thiadiazole), 1422 (C–N benzothiazole), 1524 (C=C aromatic), 3114 (C–H aromatic), 3318 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.36–7.40 (m, $J = 12$ Hz, 7H, Ar–H), 8.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 127.3 (C-5′), 128.9 (C-6′), 129.3 (C-3′), 129.8 (C-6, benzothiazole), 130.1 (C-4′), 132.2 (C-2′), 132.3 (C-1a, benzothiazole), 136.9 (C-1′), 151.3 (C-4a, benzothiazole), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 174.1 (C-6, triazolo-thiadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 419 (M^+), 417 (M^+-2); Anal. Calc. for $C_{16}H_8Cl_2N_6S_2$ (419.31): C 45.83, H 1.92, N 20.04, S 15.29, Cl 16.91. Found: C 45.86, H 1.94, N 20.07, S 15.31, Cl 16.94.

*6-Chloro-N-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzo[d]thiazol-2-amine (**6c**)* Yield 68 %; m.p. 248–250 °C; Rf-value 0.90; IR (KBr) ν cm^{-1}: 614 (C–S–C benzothiazole), 686 (C–S–C triazolo-thiadiazole), 822 (C–Cl), 1275 (N–N=C triazolo-thiadiazole), 1424 (C–N benzothiazole), 1526 (C=C aromatic), 3112 (C–H aromatic), 3317 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.40–7.44 (m, $J = 12$ Hz, 6H, Ar–H), 8.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3(C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 128.8 (C-6′), 129.8 (C-6, benzothiazole), 130.2 (C-4′), 130.3 (C-2′), 130.7 (C-3′), 132.9 (C-5′), 132.3 (C-1a, benzothiazole), 138.3 (C-1′), 151.3 (C-4a, benzothiazole), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 174.1 (C-6, triazolo-thiadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 453 (M^+), 451(M^+-2), 455 (M^++2); Anal. Calc. for $C_{16}H_{17}Cl_3N_6S_2$ (453.76): C 42.35, H 1.55, N 18.52, S 14.13, Cl 23.44. Found: C 42.37, H 1.58, N 18.56, S 14.15, Cl 23.47.

*6-Chloro-N-(6-o-tolyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzo[d]thiazol-2-amine (**6d**)* Yield 71 %; m.p. 282–284 °C; Rf-value 0.80; IR (KBr) ν cm^{-1}: 618 (C–S–C benzothiazole), 691 (C–S–C triazolo-thiadiazole), 829 (C–Cl), 1281 (N–N=C triazolo-thiadiazole), 1427 (C–N benzothiazole), 1531 (C=C aromatic), 3119 (C–H aromatic), 3319 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.34 (s, 3H, CH_3), 7.35–7.37 (m, $J = 6$ Hz, 7H, Ar–H), 8.07 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.7 (C-1″), 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 126.2 (C-5′), 127.4 (C-6′), 128.6 (C-4′), 129.5 (C-3′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 136.9 (C-2′), 137.2 (C-1′), 151.3 (C-4a, benzothiazole), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 174.1 (C-6, triazolo-thiadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 398 (M^+), 400 (M^++2); Anal. Calc. for $C_{16}H_{17}Cl_3N_6S_2$ (398.89): C 51.19, H 2.78, N 21.07, S 16.08, Cl 8.89. Found: C 51.21, H 2.82, N 21.10, S 16.12, Cl 8.92.

*2-(3-(6-Chlorobenzo[d]thiazol-2-ylamino)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)phenyl acetate (**6e**)* Yield 66 %; m.p. 286–288 °C; Rf-value 0.70; IR (KBr) ν cm^{-1}: 626 (C–S–C benzothiazole), 684 (C–S–C triazolo thiadiazole), 817 (C–Cl), 1267 (N–N=C triazolo-thiadiazole), 1436 (C–N benzothiazole), 1527 (C=C aromatic), 3106 (C–H aromatic), 3324 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.51 (s, 3H, $OCOCH_3$), 7.24–7.28 (m, $J = 12$ Hz, 7H, Ar–H), 8.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.3 (C-1″), 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 123.2 (C-3′), 125.8 (C-5, benzothiazole), 126.0 (C-5′), 127.9 (C-6′), 129.1 (C-4′), 129.4 (C-1′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 151.1 (C-2′), 151.3 (C-4a, benzothiazole), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 169.0 (C-2″), 174.1 (C-6, triazolo-thiadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 442 (M^+), 444 (M^++2); Anal. Calc. for $C_{18}H_{11}ClN_6O_2S_2$ (442.90): C 48.81, H 2.50, N 18.97, S 14.48, Cl 8.00. Found: C 48.84, H 2.52, N 18.99, S 14.51, Cl 8.04.

*6-Chloro-N-(6-pheoxy-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzo[d]thiazol-2-amine (**6f**)* Yield 76 %; m.p. 218–220 °C; Rf-value 0.60; IR (KBr) ν cm^{-1}: 626 (C–S–C benzothiazole), 692 (C–S–C triazolo-thiadiazole), 836 (C–Cl), 1274 (N–N=C triazolo-thiadiazole), 1434 (C–N benzothiazole), 1532 (C=C aromatic), 3123 (C–H aromatic), 3318 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.29–7.31 (m, $J = 6$ Hz, 8H, Ar–H), 8.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 122.7 (C-6′), 124.6 (C-4′), 125.8 (C-5, benzothiazole), 129.7(C-3′ & C-5′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 151.3 (C-4a, benzothiazole), 155.2 (C-1′), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 174.1 (C-6, triazolo-thiadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 400 (M^+), 401 (M^++1), 403 (M^++3); Anal. Calc. for $C_{16}H_9ClN_6OS_2$ (400.87): C 47.94, H 2.26, N 20.96, S 16.00, Cl 8.84. Found: C 47.97, H 2.30, N 20.98, S 16.04, Cl 8.86.

*6-Chloro-N-(6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzo[d]thiazol-2-amine (**6g**)* Yield 63 %; m.p. 116–118 °C; Rf-value 0.70; IR (KBr) ν cm^{-1}: 621 (C–S–C benzothiazole), 689 (C–S–C triazolo-thiadiazole), 830 (C–Cl), 1280 (N–N=C triazolo-thiadiazole), 1369 (NO_2), 1429 (C–N benzothiazole), 1527 (C=C aromatic), 3118 (C–H aromatic), 3320 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.39–7.41 (m, $J = 6$ Hz, 7H, Ar–H), 8.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 124.4 (C-5′), 125.8 (C-5, benzothiazole), 128.4 (C-6′), 129.8 (C-6, benzothiazole), 132.3(C-1a, benzothiazole), 139.6 (C-1′), 143.3 (C-6, triazolo-thiadiazole) 147.9 (C-4′), 151.3 (C-4a, benzothiazole), 157.2 (C-4, triazolo-thiadiazole), 167.6 (C-3, triazole), 174.5 (C-2, benzothiazole); MS: *m/z* 429 (M^+), 428 (M^+-1), 430 (M^++1); Anal. Calc. for $C_{16}H_{17}Cl_3N_6S_2$ (429.86): C 48.80, H 2.49, N 18.97, S 14.48, Cl 8.25. Found: C 48.84, H 2.52, N 18.99, S 14.51, Cl 8.27.

*Synthesis of N-(6-chlorobenzo[d]thiazol-2-yl)-5-substituted phenyl-1,3,4-oxadiazol-2-amine (**7a–g**) general procedure:*

Compound 3 (0.001 mol) and appropriate aromatic acid (0.001 mol) was dissolved in phosphorus oxychloride and refluxed for 20 h. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus

separated out was filtered, washed with water, dried, and recrystallized from ethanol.

*N-(6-Chlorobenzo[d]thiazol-2-yl)-5-phenyl-1,3,4-oxadiazol-2-amine (**7a**)* Yield 67 %; m.p. 262–264 °C; Rf-value 0.8; IR (KBr) ν cm^{-1}: 622 (C–S–C benzothiazole), 1454 (C–N benzothiazole), 1488 (C–O–C oxadiazole), 1518 (C=C aromatic), 3318 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.24–7.28 (m, $J = 12$ Hz, 8H, Ar–H), 8.10 (s,1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 126.1 (C-1′), 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 127.5 (C-2′-& 6′), 129.2 (C-3′& 5′), 128.7 (C-4′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 151.3 (C-4a, benzothiazole), 160.3 (C-5, oxadiazole), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole);

MS: *m/z* 328 (M^+), 330 (M^++2); Anal. Calc. for $C_{15}H_9ClN_4OS$ (328.78): C 54.80, H 2.76, N 17.04, S 9.75, Cl 10.78. Found: C 54.82, H 2.78, N 17.08, S 9.77, Cl 10.81.

*N-(6-Chlorobenzo[d]thiazol-2-yl)-5-(2-chlorophenyl)-1,3,4-oxadiazol-2-amine (**7b**)* Yield 64 %; m.p. 234–236 °C; Rf-value 0.60; IR (KBr) ν cm^{-1}: 624 (C–S–C benzothiazole), 821 (C–Cl), 1456 (C–N benzothiazole), 1491 (C–O–C oxadiazole), 1512 (C=C aromatic), 3317 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.22–7.26 (m, $J = 12$ Hz, 7H, Ar–H), 8.14 (s,1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 127.3 (C-5′), 128.9 (C-6′), 129.3 (C-3′), 129.8 (C-6, benzothiazole), 130.1 (C-4′), 132.2 (C-2′),132.3 (C-1a, benzothiazole), 136.9 (C-1′), 151.3 (C-4a, benzothiazole), 164.5 (C-5, oxadiazole), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole);MS: *m/z* 363 (M^+), 361 (M^+-2); Anal. Calc. for $C_{15}H_8Cl_2N_4OS$ (363.22): C 49.60, H 2.22, N 15.42, S 8.83, Cl 19.52. Found: C 49.64, H 2.24, N 15.45, S 8.85, Cl 19.54.

*N-(6-Chlorobenzo[d]thiazol-2-yl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-amine (**7c**)* Yield 66 %; m.p. 257–259 °C; Rf-value 0.70; IR (KBr) ν cm^{-1}: 628 (C–S–C benzothiazole), 826 (C–Cl), 1461 (C–N benzothiazole), 1497 (C–O-C oxadiazole), 1516 (C=C aromatic), 3321 (N–H). ^{1}H NMR (DMSO-d_6) δ (ppm): 7.26–7.30 (m, $J = 12$ Hz, 6H, Ar–H), 8.20 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm):118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 127.4 (C-5′), 129.8 (C-6, benzothiazole), 130.3 (C-6′), 130.9(C-3′), 130.7, 132.3 (C-1a, benzothiazole), 133.6 (C-2′), 135.0 (C-1′), 135.7 (C-4′), 151.3 (C-4a, benzothiazole), 164.5 (C-5, oxadiazole), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 397 (M^+), 395 (M^+-2), 399 (M^++2); Anal. Calc. for $C_{15}H_7Cl_3N_4OS$ (397.67): C 45.30, H 1.77, N 14.09, S 8.06, Cl 26.75. Found: C 45.32, H 1.79, N 14.11, S 8.08, Cl 26.77.

*N-(6-Chlorobenzo[d]thiazol-2-yl)-5-o-tolyl-1,3,4-oxadiazol-2-amine (**7d**)* Yield 65 %; m.p. 204–206 °C; Rf-value 0.80; IR (KBr) ν cm^{-1}: 632 (C–S–C benzothiazole), 812 (C–Cl), 1467 (C–N benzothiazole), 1496 (C–O–C oxadiazole), 1514 (C=C aromatic), 3331 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.21(s, 3H, CH_3), 7.64–7.68 (m, $J = 12$ Hz, 7H, Ar–H), 8.12 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 18.7 (C-2″), 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 126.2(C-5′), 127.4 (C-6′), 128.6 (C-4′), 129.5 (C-3′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 136.9 (C-2′), 137.2 (1′), 151.3 (C-4a, benzothiazole), 164.5 (C-5, oxadiazole), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 342 (M^+), 344 (M^++2); Anal. Calc. for $C_{16}H_{11}ClN_4OS$ (342.80): C 56.04, H 3.21, N 16.32, S 9.33, Cl 10.34. Found: C 56.06, H 3.23, N 16.34, S 9.35, Cl 10.37.

*2-(5-(6-Chlorobenzo[d]thiazol-2-ylamino)-1,3,4-oxadiazol-2-yl)phenyl acetate (**7e**)* Yield 64 %; m.p. 244–246 °C; Rf-value 0.70; IR (KBr) ν cm^{-1}: 638 (C–S–C benzothiazole), 824 (C–Cl), 1468 (C–N benzothiazole), 1489 (C–O–C oxadiazole), 1517 (C=C aromatic), 3336 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.59 (s, 3H, $OCOCH_3$), 7.24–7.28 (m, $J = 12$ Hz, 7H, Ar–H),8.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.3 (C-1″), 117.8 (C-1′), 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 123.2 (C-3′), 125.8 (C-5, benzothiazole), 126.0 (C-5′), 129.1 (C-4′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 137.1 (6′), 151.1 (C-2′), 151.3 (C-4a, benzothiazole), 164.5 (C-5, oxadiazole), 169.0 (C-2″), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 386 (M^+), 388 (M^++2); Anal. Calc. for $C_{17}H_{11}ClN_4O_3S$ (386.81): C 52.81, H 2.84, N 14.46, S 8.27, Cl 9.17. Found: C 52.79, H 2.87, N 14.48, S 8.29, Cl 19.20.

*N-(6-Chlorobenzo[d]thiazol-2-yl)-5-phenoxy-1,3,4-oxadiazol-2-amine (**7f**)* Yield 73 %; m.p. 264–266 °C; Rf-value 0.80; IR (KBr) ν cm^{-1}: 634 (C–S–C benzothiazole), 824 (C–Cl), 1464 (C–N benzothiazole), 1492 (C–O–C oxadiazole), 1518 (C=C aromatic), 3326 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.25–7.28 (m, $J = 9$ Hz, 8H, Ar–H), 8.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 122.7 (C-2′& 6′), 124.6 (C-4′), 125.8 (C-5, benzothiazole), 129.7 (C-3′& 5′), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 151.3 (C-4a, benzothiazole), 155.2 (C-1′), 158.7 (C-5, oxadiazole), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 344 (M^+), 345 (M^++1), 346 (M^++2); Anal. Calc. for $C_{15}H_9ClN_4O_2S$ (344.78): C 52.22,

H 2.61, N 16.23, S 9.27, Cl 10.28. Found: C 52.25, H 2.63, N 16.25, S 9.30, Cl 10.30.

*N-(6-Chlorobenzo[d]thiazol-2-yl)-5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine (**7g**)* Yield 67 %; m.p. 223–225 °C; Rf-value 0.60; IR (KBr) ν cm^{-1}: 637 (C–S–C benzothiazole), 828 (C–Cl), 1378 (NO_2), 1468 (C–N benzothiazole), 1496 (C–O–C oxadiazole), 1521 (C=C aromatic), 3336 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.21–7.24 (m, J = 9 Hz, 7H, Ar–H), 8.16 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 128.8 (3′& 5′), 125.8 (C-5, benzothiazole), 129.8 (C-6, benzothiazole), 130.9 (C-2′& 6′), 132.2 (C-1′),147.9 (4′), 151.3 (C-4a, benzothiazole), 164.5 (C-5, oxadiazole), 169.3 (C-2, oxadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 373 (M^+), 374 (M^++1), 375 (M^++2); Anal. Calc. for $C_{15}H_8ClN_5O_3S$ (373.77): C 48.23, H 2.18, N 18.76, S 8.60, Cl 9.49. Found: C 48.20, H 2.16, N 18.74, S 8.58, Cl 9.51.

*Synthesis of 5-(6-chlorobenzo[d]thiazol-2-ylamino)-1,3,4-oxadiazole-2-thiol (**8**)*

A mixture of **3** (0.005 mol), KOH (0.005 mol) and carbon disulfide (5 mL) in ethanol (50 mL) was refluxed on a steam bath for 12 h. The solution was then concentrated, cooled, and acidified with dilute HCl. The solid mass that separated out was filtered, washed with ethanol, dried, and recrystallized from ethanol.

Yield 74 %; m.p. 192–194 °C; Rf-value 0.80; IR (KBr) ν cm^{-1}: 614 (C–S–C benzothiazole), 826 (C–Cl), 1421 (C–N), 1505 (C–O–C oxadiazole), 1514 (C=C), 1614 (C=O), 2516 (SH), 3316 (N–H); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.17–7.19 (m, J = 6 Hz, 3H, Ar–H), 8.05 (s, 1H, NH), 13.11 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 118.3 (C-4, benzothiazole), 121.2 (C-7, benzothiazole), 125.8 (C-5, benzothiazole), 129.8 (C-6, benzothiazole), 132.3 (C-1a, benzothiazole), 151.3 (C-4a, benzothiazole), 169.3 (C-5, oxadiazole), 171.6 (C-2, oxadiazole), 174.5 (C-2, benzothiazole); MS: *m/z* 284 (M^+), 283 (M^+-1), 285 (M^++1). Anal. Calc. for $C_9H_5ClN_4OS_2$ (284.75): C 37.96, H 1.77, N 19.68, S 22.52, Cl 12.45. Found: C 37.98, H 1.79, N 19.71, S 22.56, Cl 12.47.

Pharmacological evaluation

Adult Wistar strain rats of either sex, weighing 150–200 g were used for anti-inflammatory, ulcerogenic, and lipid peroxidation activities, whereas swiss albino mice weighing 25–30 g were used for analgesic activity. The animals were allowed food and water ad libitum except during the experiments. They were housed in a room at 25 ± 2 °C, and 50 ± 5 % relative humidity with 12 h light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiments. The experimental protocol was approved by the animal ethics committee of Hamdard University. All the test compounds and the reference drug were administered orally suspended in 0.5 % carboxymethyl cellulose (CMC) solution.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenan induced hind paw edema method (Winter *et al.*, 1962). The animals were randomly allocated into groups of six animals each and were fasted for 24 h before the experiment with free access to water. Control group received only 0.5 % carboxymethyl cellulose solution. Standard drug ibuprofen was administered orally at a dose of 70 mg/kg. The test compounds were administered orally at an equimolar oral dose relative to 70 mg/kg ibuprofen. Into the sub plantar region of the right hind paw of each rat, 0.1 mL of 1 % carrageenan solution in saline was injected subcutaneously, 1 h after the administration of the test compounds and standard drug. The right hind paw volume was measured before and after 3 and 4 h of carrageenan treatment by means of a plethysmometer. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\text{Percent edema inhibition} = (V_c - V_t / V_c) \times 100$$

where, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.

Analgesic activity

Analgesic activity was evaluated by tail immersion method (Adeyemi *et al.*, 2004). Swiss albino mice allocated into different groups consisting of six animals in each, of either sex, weighing 25–30 g were used for the experiment. Analgesic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 70 mg/kg ibuprofen. Test compounds and standard drugs were administered orally as suspension in carboxymethyl cellulose solution in water (0.5 % w/v). The analgesic activity was assessed before and after 4 h interval of the administration of test compounds and standard drugs. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5 °C. The time in seconds for tail withdrawal from the water was taken as the reaction time with a cut-off time of immersion, set at 10 s for both control as well as treated groups of animals.

Acute ulcerogenicity

Acute ulcerogenicity was determined according to the standard method (Cioli *et al.*, 1979). The animals were allocated into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 210 mg/kg ibuprofen. Control group received only 0.5 % carboxymethyl cellulose solution. 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 sacrificed. 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 method given in literature (Ohkawa *et al.*, 1979). After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 mL of 1.15 % ice cold KCl solution. The homogenate was supplemented with 0.2 mL of 8.1 % sodium dodecyl sulfate (SDS), 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8 % thiobarbituric acid (TBA). The mixture was heated at 95 °C for 60 min. After cooling the reactants were supplemented with 5 mL of the mixture of *n*-butanol and pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4,000 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 1.56×10^5 cm^{-1} M^{-1}.

Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150–200 g. Animals were divided into three groups, six rats in each. Group 1 was kept as control and received only vehicle (0.5 % w/v solution of carboxymethyl cellulose in water), rest of the groups received test compounds, at an equimolar oral dose relative to 210 mg/kg ibuprofen in 0.5 % w/v solution of carboxymethyl cellulose in water once in a day for 15 days. After the treatment (15 days) blood was obtained from all groups of rats by puncturing the retro-orbital plexus. Blood samples were allowed to clot for 45 min at room temperature and serum was separated by centrifugation at 2,500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of liver function

Liver functions such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assessed by a reported method (Reitman and Frankel, 1957). The alkaline phosphatase, total protein, and total albumin were measured according to the reported procedures (King and Armstrong, 1934). All data are recorded in Table 1.

Histopathological studies of liver

The histopathological studies were carried out by a reported method (Luna, 1968). The rats were killed under light ether anesthesia after 24 h of the last dosage, the livers were removed and washed with normal saline and stored in formalin solution. Sections of 5–6 mm in thickness were cut, stained with hematoxylin and eosin and then studied under a light microscope (Figs. 1, 2, 3, 4).

Statistical analysis of data

All the statistical analyses were carried out using the software SigmaStat 4.0 using ANOVA followed by dunnett's multiple comparison tests and the results are expressed in mean ± SEM.

Results and discussion

Synthesis

N-(6-Chlorobenzo[*d*]thiazol-2-yl) hydrazine carboxamide (**3**) have been synthesized through a multi step reaction as depicted in Scheme 1. The 6-chloro-1,3-benzothiazole-2-amine (**1**) was synthesized by reacting 4-chloroaniline with potassium thiocyanate in a satisfactory yield. In FT-IR spectrum bands at 3,480 and 1,570 cm^{-1} confirms the presence of NH_2 and C=N stretching vibrations, respectively. The ^1H NMR spectrum showed a singlet at δ 6.12 ppm due to NH_2 protons (D_2O exchangeable).

The benzothiazole-2-yl urea (**2**) were prepared by treating 6-chloro-1,3-benzothiazole-2-amine (**1**) with sodium cyanate in the presence of glacial acetic acid. The benzothiazole-2-yl-urea (**2**) was then refluxed with hydrazine hydrate to yield the product *N*-(6-chlorobenzo[*d*]thiazol-2-yl)hydrazine carboxamide (**3**) as outlined in Scheme 1. The infrared spectra showed characteristic absorption bands at 3,300 cm^{-1} due to NH_2 function beside the C=O at

Table 1 Effect of selected compounds on serum enzymes, total proteins, and total albumin

Compounds	SGOT (units/mL)[a]	SGPT (units/mL)[a]	Alkaline phosphatase[a]	Total protein (g/dL)[a]	Total albumin (g/dL)[a]
Control	143.71 ± 1.10	32.46 ± 0.76	12.17 ± 0.16	1.72 ± 0.02	1.63 ± 0.02
Ibuprofen	140.14 ± 1.12	34.26 ± 0.72	11.67 ± 0.18	1.74 ± 0.06	1.71 ± 0.01
6f	131.18 ± 2.12**	28.33 ± 0.60**	11.09 ± 0.11	1.79 ± 0.17	1.74 ± 0.22
7c	138.42 ± 2.11**	30.74 ± 0.54	12.11 ± 0.14	1.75 ± 0.10	1.70 ± 0.11

[a] Relative to control and data were analyzed by ANOVA followed by Dunnett's multiple comparison test, for $n = 6$

** $P < 0.01$

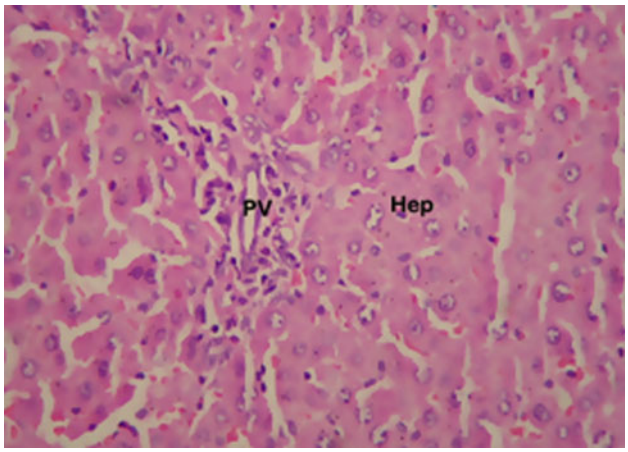


Fig. 1 Control; section of liver showing portal triad structures and normal hepatic parenchyma. *PV* portal vein, *Hep* hepatocyte (×100)

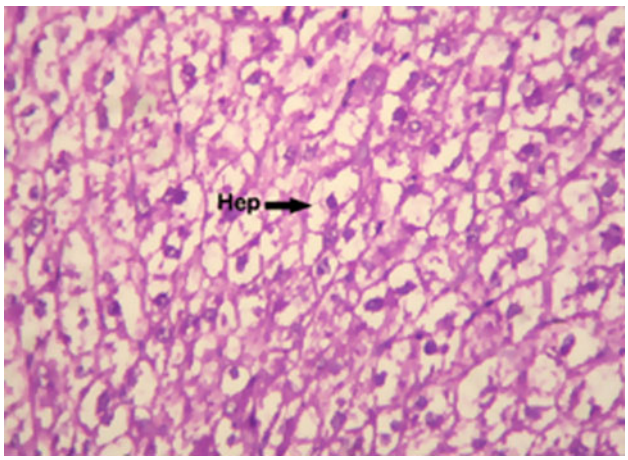


Fig. 2 Ibuprofen; section of liver showing hepatocytes with clearing of cytoplasm and compression of sinusoidal spaces. *Hep* hepatocyte (×100)

1,660 cm^{-1}, respectively. Similarly, the ^1H NMR spectra of the synthesized compounds showed singlet characteristic peaks at δ 9.12 cm^{-1} and the other at δ 7.28 cm^{-1} due to NHC=O (D_2O exchangeable) and $NHNH_2$ (D_2O exchangeable) proton, respectively.

The acid hydrazide (**3**) was allowed to react with carbon disulfide in the presence of potassium hydroxide in ethanol

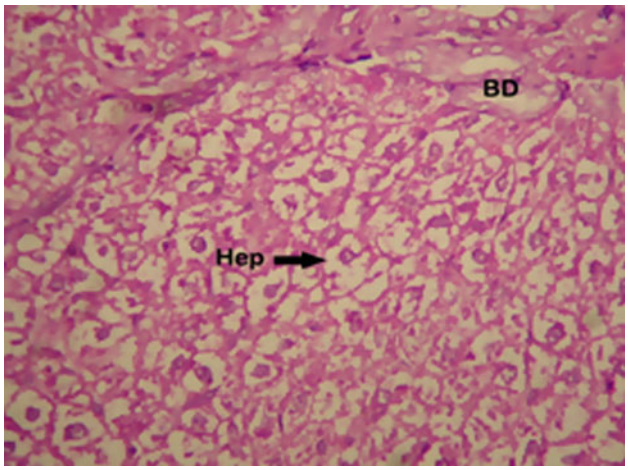


Fig. 3 Compound **6f**; section of liver showing hepatocytes with clearing of cytoplasm and compression of sinusoidal spaces. *BD* bile duct, *Hep* hepatocyte (×100)

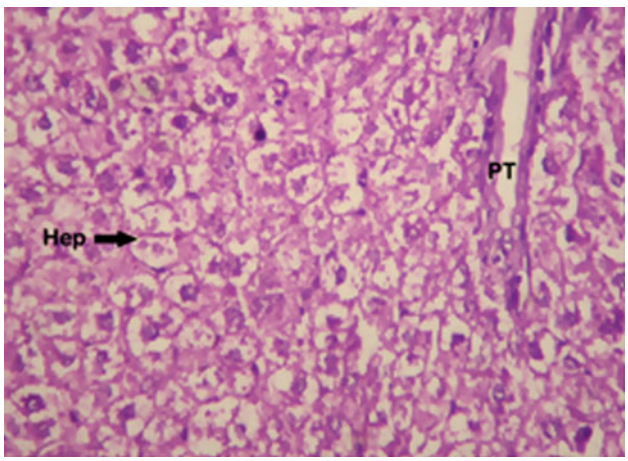
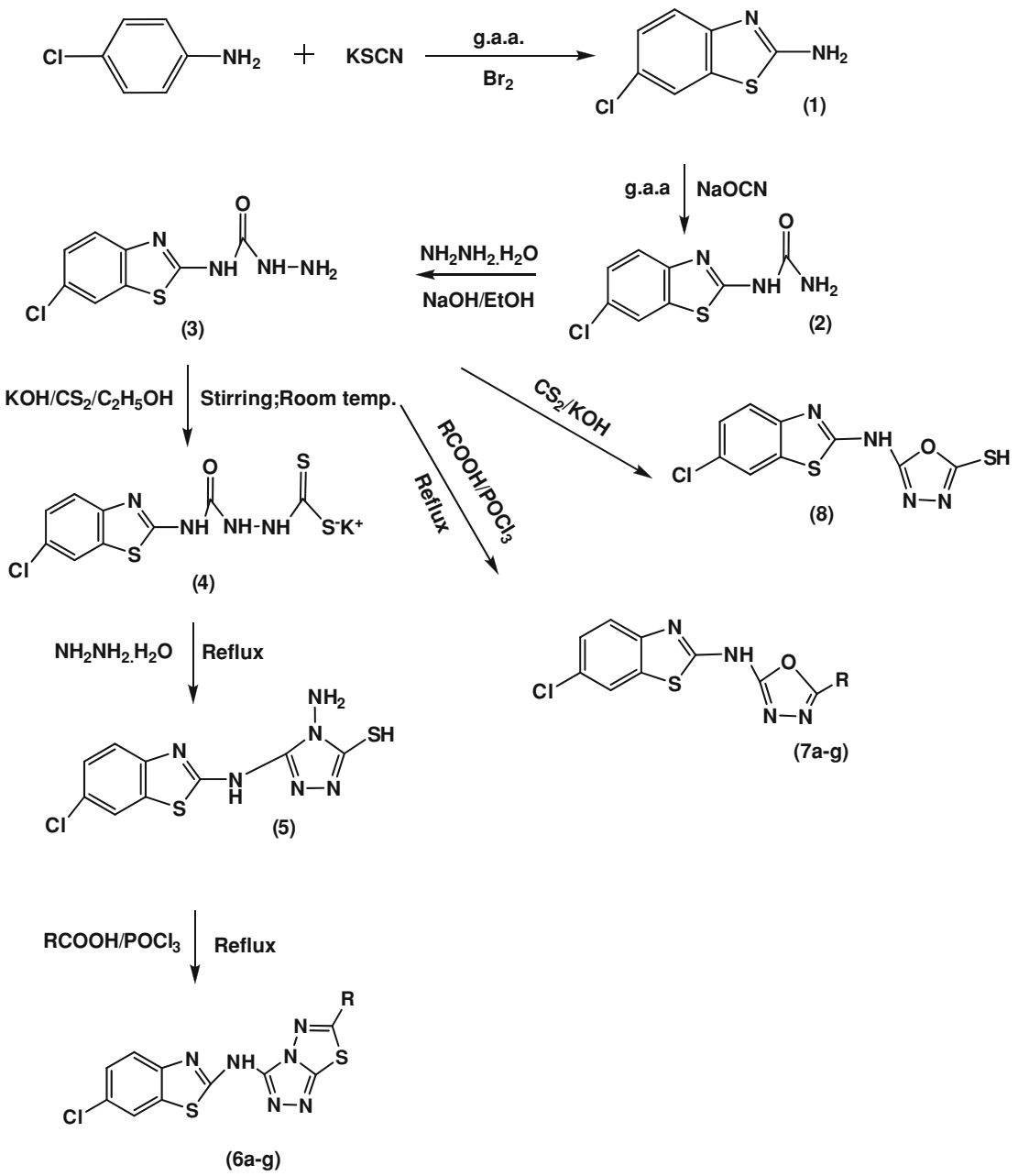


Fig. 4 Compound **7c**; section of liver showing hepatocytes with clearing of cytoplasm and compression of sinusoidal spaces. *PT* portal triad, *Hep* hepatocyte (×100)

to afford the corresponding intermediate potassium dithiocarbazinate (**4**). This salt underwent ring closure with an excess of 99 % hydrazine hydrate to give 4-amino-5-(6-chlorobenzo[*d*]thiazol-2-ylamino)-4*H*-1,2,4-triazole-3-thiol (**5**) in good yield. The resultant triazole (**5**) was



6,7 R : [**a** = C_6H_5-, **b** = 2-ClC_6H_4-, **c** = 2,4-ClC_6H_3-, **d** = 2-$CH_3C_6H_4$-, **e** = 2-$OCOCH_3C_6H_4$-, **f** = OC_6H_5-, **g** = 4-$NO_2C_6H_4$-]

Scheme 1 Synthetic route for the title compounds

further converted to 6-chloro-*N*-(6-substituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzo[*d*]thiazol-2-amine (**6a–g**) through one-pot reaction by condensation with appropriate aromatic acids in the presence of phosphorus oxychloride, as outlined in Scheme 1. Phosphorus oxychloride was necessary for this condensation, which activate the carbonyl group of aromatic acids and increases its electrophilicity to enhance the addition of triazole to it. The structure of 4-amino-5-(6-chlorobenzo[*d*]thiazol-2-ylamino)-4*H*-1,2,4-triazole-3-thiol (**5**) was confirmed by FT-IR, ^{1}H NMR spectral data and microanalysis. The infrared spectra showed two characteristic absorption bands, one of which appearing at 2,518 cm^{-1}, was attributed to SH and the other at 3,338 cm^{-1}, was assigned to NH_2, which were disappeared by the formation of the triazolo-thiadiazoles. The ^{1}H NMR spectra showed a downfield D_2O exchangeable singlet at δ 13.18, attributed to the SH group, while the NH_2 group appeared as a singlet

at δ 5.15. The absence of signals due to NH_2 and SH protons confirmed that the triazole was converted into triazolo-thiadiazoles (**6a–g**) by reacting with group of aromatic acids.

5-(6-Chlorobenzo[*d*]thiazol-2-ylamino)-1,3,4-oxadiazole-2-thiol (**8**) was prepared by reacting hydrazide (**3**) with carbon disulfide in alkaline medium and *N*-(6-chlorobenzo[*d*]thiazol-2-yl)-5-substituted phenyl-1,3,4-oxadiazol-2-amine (**7a–g**) were prepared by treatment of hydrazide (**3**) with appropriate aromatic acids in the presence of phosphorus oxychloride (Scheme 1). IR spectra of the compound **8** showed band at 1,505 cm^{-1} due to C–O–C stretching vibration of oxadiazole and at 2,516 cm^{-1} corresponding to SH group, whereas compounds **7a–g** also showed bands at 1,488 cm^{-1} due to C–O–C stretching vibration of oxadiazole but lacks SH stretching vibration. The structure was further supported by its ^{1}H NMR spectrum which showed a singlet of SH proton at δ 13.11 for compound **8**.

The elemental analysis and molecular ion peaks of compounds **6a–g**, **7a–g** & **8** were consistent with the assigned structure. The numbering pattern on the basic nucleus is assigned in Scheme 2.

Pharmacological evaluation

Anti-inflammatory activity

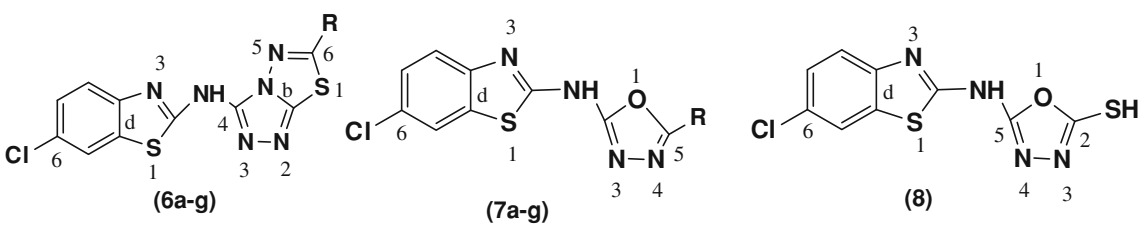
The anti-inflammatory activity of the synthesized compounds **6a–g**, **7a–g** & **8** were evaluated by the carrageenan induced paw edema method (Winter *et al.*, 1962). The compounds were tested at an equimolar oral dose relative to 70 mg/kg ibuprofen. The percentage inhibition was calculated both after 3 and 4 h, and since it was found to be more after 4 h, this was made the basis of discussion. The tested compounds showed anti-inflammatory activity ranging from 20.62 ± 2.93 to 89.67 ± 2.58 %, whereas the standard drug ibuprofen showed 82.69 ± 1.65 % inhibition, after 4 h (Table 2).

The anti-inflammatory activity of triazolo-thiadiazole derivatives were in the range of 20.62 ± 2.93 to 89.67 ± 2.58 %. It was observed that the triazolo-thiadiazole derivatives having 2,4-dichlorophenyl (**6c**), methyl phenyl (**6d**), and phenoxy group (**6f**) at sixth position possess highest activity (89.60 ± 1.90, 88.81 ± 2.34 and 89.67 ± 2.58 %, respectively) comparable to that of ibuprofen (82.69 ± 1.65 %). Furthermore, it was observed that the presence of 2-chlorophenyl moiety (**6b**) at C-6 showed decrease of activity (50.78 ± 4.70 %) and replacement of this group by acetyl phenyl group (**6e**) resulted in sharp decrease of anti-inflammatory activity (20.62 ± 2.93 %). Compounds **6a** and **6g** showed moderate activity (57.93 ± 4.15 and 66.66 ± 2.74 %).

The anti-inflammatory activity of 1,3,4-oxadiazole derivatives were found between 32.53 ± 3.56 and 88.81 ± 1.73 %. The highest activity (88.81 ± 1.73 %) was found in the oxadiazole derivative **7f** having phenoxy group at second position. When this group was replaced by methyl and 4-nitro phenyl group (**7d** and **7g**) the activity was found to be decreased (37.69 ± 5.23 and 32.53 ± 3.56 %). It was observed that compounds having phenyl (**7a**) and 2,4-dichlorophenyl group (**7c**) at second position showed equivalent or better activity (88.27 ± 1.73 and 87.14 ± 1.09 %) than the standard drug. When the 2-substituted phenyl group was replaced by 2-mercapto group (**8**) the activity was found to be good (69.76 ± 4.55 %). Rest of the oxadiazole derivatives showed moderate activity.

Analgesic activity

The compounds **6c**, **6d**, **6f**, **7a**, **7c**, **7f**, and **8** which showed comparable or equal anti-inflammatory activity to that of standard reference drug, were further tested for their analgesic activity at an equimolar oral dose relative to 70 mg/kg ibuprofen (Table 3). The compounds showed analgesic activity ranging from 52.00 ± 2.21 to 79.03 ± 1.62 %, whereas the standard drug ibuprofen 73.50 ± 1.10 % inhibition. The highest analgesic activity (79.03 ± 1.62 %) was shown by oxadiazole derivative **7c**, which also has significant anti-inflammatory activity (87.14 ± 1.09 %). It was noted that compound **6f**, a triazolo-thiadiazole derivative showing the highest anti-inflammatory activity, also exhibited significant analgesic activity (75.00 ± 1.42 %). The remaining compounds showed reduced analgesic activity. These compounds were further screened for their acute ulcerogenic activity. The compounds were tested at an equimolar oral dose relative to 210 mg/kg ibuprofen.



Scheme 2 Numbering pattern on basic nucleus

Table 2 Anti-inflammatory activity of the synthesized compounds

Compounds	Paw volume			% Inhibition ± SEM[a]		Potency
	0 h (Basal)	After 3 h	After 4 h	After 3 h	After 4 h	
6a	0.34 ± 0.017	0.56 ± 0.017	0.51 ± 0.018	48.33 ± 5.42*	57.93 ± 4.15**	0.70
6b	0.36 ± 0.011	0.61 ± 0.018	0.57 ± 0.014	41.66 ± 3.57*	50.78 ± 4.70*	0.61
6c	0.40 ± 0.012	0.48 ± 0.029	0.44 ± 0.016	80.00 ± 1.82	89.60 ± 1.90	1.08
6d	0.41 ± 0.010	0.50 ± 0.019	0.45 ± 0.029	77.5 ± 1.70*	88.81 ± 2.34	1.07
6e	0.28 ± 0.014	0.76 ± 0.015	0.61 ± 0.017	10.00 ± 2.23	20.62 ± 2.93*	0.24
6f	0.43 ± 0.015	0.50 ± 0.011	0.47 ± 0.024	78.33 ± 3.07	89.67 ± 2.58	1.08
6g	0.38 ± 0.009	0.57 ± 0.019	0.45 ± 0.022	53.33 ± 3.07*	66.66 ± 2.74*	0.80
7a	0.44 ± 0.013	0.53 ± 0.022	0.49 ± 0.015	78.83 ± 3.06	88.27 ± 1.73	1.06
7b	0.35 ± 0.011	0.56 ± 0.013	0.53 ± 0.021	53.00 ± 5.59	53.17 ± 2.584*	0.64
7c	0.38 ± 0.015	0.46 ± 0.024	0.43 ± 0.016	80.00 ± 1.82	87.14 ± 1.09	1.05
7d	0.33 ± 0.021	0.65 ± 0.019	0.60 ± 0.014	23.91 ± 8.18*	37.69 ± 5.23*	0.45
7e	0.44 ± 0.012	0.71 ± 0.016	0.67 ± 0.017	31.00 ± 4.96*	50.78 ± 7.44*	0.61
7f	0.44 ± 0.016	0.55 ± 0.024	0.50 ± 0.019	74.16 ± 3.00**	88.81 ± 2.34	1.07
7g	0.36 ± 0.014	0.68 ± 0.019	0.64 ± 0.017	19.16 ± 3.74*	32.53 ± 3.56*	0.39
8	0.44 ± 0.010	0.59 ± 0.015	0.56 ± 0.015	61.66 ± 4.01*	69.76 ± 4.55*	0.84
Ibuprofen	0.36 ± 0.016	0.45 ± 0.012	0.29 ± 0.018	78.16 ± 1.08	82.69 ± 1.65	1.00
Control	0.35 ± 0.012	0.75 ± 0.014	0.77 ± 0.013	–	–	–

[a] Relative to their respective standard and data were analyzed by ANOVA followed by dunnett's multiple comparison test for $n = 6$; dose = 70 mg/kg

* $P < 0.01$

** $P < 0.05$

Acute ulcerogenicity

The tested compounds showed significant reduction in ulcerogenic activity ranging from 0.666 ± 0.166 to 2.166 ± 0.357, whereas standard drug ibuprofen showed high severity index of 2.315 ± 0.18. The maximum reduction in ulcerogenic activity (0.666 ± 0.166 and 0.917 ± 0.22) was found in compounds **6f** and **7c**, respectively. The rest of the compounds also showed better GI safety profile as compared to ibuprofen, as illustrated in Table 3. Thus, the results showed that substitution of the hydrazide group by the bioisosteric groups triazolo-thiadiazole and 1,3,4-oxadiazole, has resulted in significant anti-inflammatory and analgesic activities along with reduced ulcerogenic potential.

Lipid peroxidation

It has been reported that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation (Pohle *et al.*, 2001). Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for their effect on lipid peroxidation. Lipid peroxidation is measured as nanomoles of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. Ibuprofen exhibited maximum tissue lipid peroxidation 7.51 ± 0.16, whereas control group showed 3.26 ± 0.01. It was found that all the cyclized derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 3). Thus, these studies showed that the synthesized 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.

Hepatotoxic and histopathological studies of liver

The compounds **6f** and **7c**, derivatives of triazolo-thiadiazole and 1,3,4-oxadiazole, respectively, showing potent anti-inflammatory and analgesic activities with reduced ulcerogenicity and lipid peroxidation, were further studied for their hepatotoxic effect in comparison with standard ibuprofen drugs. Both compounds were studied for their effect on biochemical parameters (Reitman and Frankel, 1957; King and Armstrong, 1934) (serum enzymes, total proteins and total albumin), and liver histopathological (Luna, 1968) testing were also carried out. As shown in Table 1 activities of liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, total protein,

Table 3 Analgesic, ulcerogenic and lipid peroxidation activities of selected compounds

Compounds	Analgesic activity[a]				Ulcerogenic activity (severity index ± SEM)[b]	Nanomoles of MDA content ± SEM/100 mg tissue[b]
	Pre-treatment/normal 0 h (s) (normal)	Post-treatment/ after 4 h (s)	% Inhibition	Potency		
6c	1.26 ± 0.047	2.21 ± 0.054	75.39 ± 1.24	0.92	0.925 ± 0.325*	5.74 ± 0.14*
6d	1.60 ± 0.033	2.83 ± 0.035	76.87 ± 2.01	0.93	1.583 ± 0.436*	7.44 ± 0.12*
6f	1.24 ± 0.046	2.17 ± 0.045	75.00 ± 1.42	0.91	0.666 ± 0.166**	3.04 ± 0.31**
7a	0.90 ± 0.036	1.45 ± 0.032	61.11 ± 2.73*	0.74	1.667 ± 0.13*	4.47 ± 0.19*
7c	0.24 ± 0.055	2.22 ± 0.048	79.03 ± 1.62	0.96	0.917 ± 0.22*	3.29 ± 0.15**
7f	1.25 ± 0.062	1.90 ± 0.032	52.00 ± 2.21*	0.63	1.083 ± 0.327*	9.10 ± 0.14*
8	1.30 ± 0.031	2.24 ± 0.047	72.30 ± 2.10**	0.88	2.166 ± 0.357*	9.04 ± 0.24*
Ibuprofen	1.43 ± 0.009	2.60 ± 0.01	73.50 ± 1.10	1.00	2.315 ± 0.18	7.51 ± 0.16
Control	–	–	–	–	0.000 ± 0.00	3.26 ± 0.01

[a] Relative to normal and data was analyzed by paired student's *t* test for $n = 6$; dose = 70 mg/kg; ** $P < 0.0001$, * $P < 0.005$

[b] Relative to their respective standard and data were analyzed by ANOVA followed by dunnett's multiple comparision test for $n = 6$; dose = 210 mg/kg; ** $P < 0.05$, * $P < 0.01$

and total albumin were almost identical with control values, whereas ibuprofen showed slight difference with control values. The studies of the liver samples do not show any significant pathological changes in comparison to control group (Figs. 1, 2, 3, 4). No hepatocyte necrosis or degeneration was seen in any of the samples. The hepatocytes are swollen and compression of sinusoidal spaces is seen. Ibuprofen also showed hepatocytes with clearing of cytoplasm and compression of sinusoidal spaces.

Conclusion

In summary, fifteen fused heterocyclic analogs of various triazolo-thiadiazole and 1,3,4-oxadiazole derivatives of benzothiazole were successfully synthesized. Among these the 6-chloro-*N*-(6-pheoxy-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzo[*d*]thiazol-2-amine (**6f**) and *N*-(6-chlorobenzo[*d*]thiazol-2-yl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-amine (**7c**) emerged as lead compounds. These compounds showed maximum reduction of severity index along with minimum lipid peroxidation, with no hepatocyte necrosis or degeneration. The compounds are promising anti-inflammatory and analgesic agents and could be further modified to develop potential and safer anti-inflammatory and analgesic agents. Therefore, it was concluded that triazolo-thiadiazole and 1,3,4-oxadiazole derivatives of benzothiazole might afford a safer alternative for the treatment of inflammatory disease, pain, and hepatotoxicity caused by available marketed drugs.

Acknowledgments The authors are thankful to the Head of the Department, Pharmaceutical Chemistry for providing laboratory facilities, Central Drug Research Institute (CDRI) for spectral analysis of the compounds. Authors are also thankful in-charge, animal house, Jamia Hamdard, for providing Wistar rats and Dr. A. Mukherjee, M.D., Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, for carrying out histopathological studies.

Conflict of interest None.

References

Adeyemi OO, Okpo SO, Okpaka O (2004) The analgesic effect of the methanolic extract of *Acanthus montanus*. J Ethnopharmacol 90:45–48

Almansa C, Alfon J, de Arriba AF, Cavalcanti FL, Escamilla I, Gomez LA, Miralles A, Soliva R, Bartroli J, Carceller E, Merlos M, Garcia-Rafanell J (2003) Synthesis and structure–activity relationship of a new series of COX-2 selective inhibitors: 1,5-diarylimidazoles. J Med Chem 46:3463–3475

Amir M, Shikha K (2004) Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino)phenyl]acetic acid derivatives. Eur J Med Chem 39:535–545

Amir M, Kumar H, Javed SA (2007) Synthesis and pharmacological evaluation of condensed heterocyclic 6-substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole derivatives of naproxen. Bioorg Med Chem Lett 17:4504–4508

Brunton LL, Lazo JS, Parker KL (2006) The pharmacological basis of therapeutics. McGraw-Hill, New York, p 671

Cioli V, Putzolu S, Rossi V, Scorza Barcellona P, Corradino C (1979) The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats. Toxicol Appl Pharmacol 50:283–289

DeWitt DL (1999) Cox-2-selective inhibitors: the new super aspirins. Mol Pharmacol 55:625–631

Dogne JM, Supuran CT, Pratico D (2005) Adverse cardiovascular effects of the coxibs. J Med Chem 48:2251–2257

Gilani SJ, Khan SA, Siddiqui N (2010) Synthesis and pharmacological evaluation of condensed heterocyclic 6-substituted 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid. Bioorg Med Chem Lett 20:4762–4765

Gilani SJ, Khan SA, Alam O, Siddiqui N (2011a) Synthesis and in vitro antimicrobial evaluation of condensed heterocyclic

6-substituted 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid. Acta Pol Pharm 68:205–211

Gilani SJ, Khan SA, Alam O, Singh V, Arora A (2011b) Thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole derivatives of isonicotinic acid hydrazide: synthesis and their biological evaluation. J Serb Chem Soc 76:1057–1067

Gilani SJ, Khan SA, Siddiqui N, Verma SP, Mullick P, Alam O (2011c) Synthesis and in vitro antimicrobial activity of novel *N*-(6-chlorobenzo[*d*]thiazol-2-yl) hydrazine carboxamide derivatives of benzothiazole class. J Enzyme Inhib Med Chem 26:332–340

Habeeb AG, Praveen Rao PN, Knaus EE (2001) Design and synthesis of 4,5-diphenyl-4-isoxazolines: novel inhibitors of cyclooxygenase-2 with analgesic and antiinflammatory activity. J Med Chem 44:2921–2927

Husain A, Naseer MA (2011) Studies on fused heterocyclic 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazoles: synthesis and biological evaluation. Med Chem Res 20:47–54

Ibrahim DA (2009) Synthesis and biological evaluation of 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives as a novel class of potential anti-tumor agents. Eur J Med Chem 44:2776–2781

Jackson LM, Hawkey CJ (1999) Gastrointestinal effects of COX-2 inhibitors. Expert Opin Investig Drugs 8:963–971

Khalil NS (2007) *N*-and *S*-alpha-l-arabinopyranosyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles. First synthesis and biological evaluation. Eur J Med Chem 42:1193–1199

King EJ, Armstrong AR (1934) A convenient method for determining serum and bile phosphatase activity. Can Med Assoc J 31:376–381

Kumar H, Javed SA, Khan SA, Amir M (2008) 1,3,4-Oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxy acetic acid: synthesis and preliminary evaluation of biological properties. Eur J Med Chem 43:2688–2698

Luna GL (1968) Manual of histological staining methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York, p 567

Mathew V, Keshavayya J, Vaidya VP (2006) Heterocyclic system containing bridgehead nitrogen atom: synthesis and pharmacological activities of some substituted 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles. Eur J Med Chem 41:1048–1058

Mathew V, Keshavayya J, Vaidya VP (2007) Synthesis, characterization and pharmacological activities of 3,6-disubstituted-1,2,4-triazolo [3,4-*b*]-1,3,4-thiadiazoles and their dihydro analogues. Eur J Chem 4:320–342

Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR (1993) Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 90:11693–11697

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Padmavathi V, Nagendra Mohan AV, Thriveni P, Shazia A (2009) Synthesis and bioassay of a new class of heterocycles pyrrolyl oxadiazole/thiadiazoles/triazoles. Eur J Med Chem 44:2313–2321

Paramashivappa R, Phani Kumar P, Subba Rao PV, Srinivasa Rao A (2003) Design, synthesis and biological evaluation of benzimidazole/benzothiazole and benzoxazole derivatives as cyclooxygenase inhibitors. Bioorg Med Chem Lett 13:657–660

Pohle T, Brzozowski T, Becker JC, Van der Voort IR, Markmann A, Konturek SJ, Moniczewski A, Domschke W, Konturek JW (2001) Role of reactive oxygen metabolites in aspirin-induced gastric damage in humans: gastroprotection by vitamin C. Aliment Pharmacol Ther 15:677–687

Rana A, Siddiqui N, Khan SA (2007) Benzothiazoles: a new profile of biological activities. Ind J Pharm Sc 69:10–17

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–62

Tripathi KD (2003) Essentials of medical pharmacology. Jaypee Brothers, New Delhi, p 173

Winter CA, Risley EA, Nuss GW (1962) Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 111:544–547