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ORIGINAL ARTICLE

2nd Heterocyclic Update

Synthesis and pharmacological evaluation of 3-diphenylmethyl-6-substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles: A condensed bridgehead nitrogen heterocyclic system

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Abstract A series of 3-diphenylmethyl-6-substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole derivatives (**4a**–**j** and **5a**–**d**) were synthesized by condensation of 4-amino-5-diphenylmethyl-4*H*-1,2,4-triazole-3-thiol with various substituted aromatic acids and aryl/alkyl-isothiocyanates. The structures of synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic studies. These compounds were tested *in vivo* for their anti-inflammatory activity. The compounds which showed activity comparable to the standard drug ibuprofen were screened for their analgesic, ulcerogenic, lipid peroxidation and hepatotoxic effects. Compounds 6-(4-chlorophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole (**4a**) and 6-(2,4-dichlorophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole(**4c**) emerged as the most active compounds of the series and were moderately more potent than the standard drug ibuprofen.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat sign and symptoms of inflammation, particularly arthritic pain. NSAIDs exert their anti-inflammatory effects mainly through inhibition of cyclooxygenase (COX) enzymes, thus preventing prostaglandin biosynthesis from arachidonic acid which is also responsible for their main undesirable side effects (Warner et al., 1999). The chronic use of non selective NSAIDs results in gastrointestinal irritation, bleeding and formation of life threatening gastrointestinal ulcers (Allison et al.,

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1992). Later on, selective NSAID agents (coxibs) were developed as new generation drugs, free from GI toxicity (Tally et al., 2000), but unfortunately coxibs were found to have adverse cardiovascular effects (Dogne et al., 2005). Therefore the search for novel compounds having anti-inflammatory and analgesic activity with improved safety profile is still a necessity.

Heterocyclic compounds bearing symmetrical 1,2,4-triazole or 1,3,4-thiadiazole moieties have been reported possessing a broad spectrum of pharmacological properties including potential anti-inflammatory and analgesic activities (Schenone et al., 2006; Tozkoparan et al., 2007). Moreover, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance (Swamy et al., 2006; Karthikeyan et al., 2007; Karegouder et al., 2008). For example derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems (triazolothiadiazole) were found to have diverse pharmacological properties (Mathew et al., 2006, 2009). Furthermore, literature survey revealed that modification of carboxyl function of various aryl alkanoic acids resulted in increased anti-inflammatory activity with reduced ulcerogenic effects (Kalgutkar et al., 2000; Kucukguzel et al., 2007). Our earlier studies (Amir and Kumar, 2004, 2005) have shown that certain compounds bearing 1,2,4-triazole and 1,3,4-thiadiazole nuclei possess significant anti-inflammatory activities with reduced GI toxicity. Furthermore, several triazolothiadiazole derivatives have been prepared from different nonsteroidal anti-inflammatory agents and were found to possess improved pharmacological profiles (Metwally et al., 2007). Encouraged by these observations and in continuation of our research program on the synthesis of heterocyclic compounds of arylalkanoic acids (Amir et al., 2007, 2008), we report herein the synthesis of some new triazolothiadiazole derivatives of diphenylacetic acid. The synthesized compounds have been found to possess an interesting profile of anti-inflammatory and analgesic activities with significant reduction in the ulcerogenic effect.

2. Experimental

2.1. Chemistry

The entire chemical reagents which are used in the study are procured from E. Merck (Germany) and S.D. Fine Chemicals (India). The completion of reaction is monitored by thin layer chromatography (TLC) using chloroform–methanol (9:1) as the solvent system. The products were purified by recrystallisation with absolute ethanol and purity of the compounds was checked by thin layer chromatography (TLC) using silica gel G plates (Merck). The spot was developed in iodine chamber or viewed under UV lamp. Melting points were determined in an open capillary using melting point apparatus and are uncorrected. The proton magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker 300 MHz instrument in DMSO d_6 using tetramethylsilane as an internal standard. The infrared spectra of compounds were recorded in KBr on a Bio-Rad FTIR spectrophotometer. Diphenyl acetic acid hydrazide (**1**) was prepared by the procedure given in the literature (Metwally et al., 2007).

2.2. Synthesis of potassium dithiocarbazinate (*2*)

To a solution of diphenyl acetic acid hydrazide **1** (0.02 mol) in absolute ethanol (50 mL) and KOH (0.03 mol), 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 was thus obtained in quantitative yield and was used in the next step without further purification.

2.3. Synthesis of 4-amino-5-diphenylmethyl-4H-1,2,4-triazole-3-thiol (*3*)

A solution of potassium dithiocarbazinate (**2**) (0.02 mol) and hydrazine hydrate (99%, 0.04 mol) in water (10 mL) was refluxed for 15 h with occasional shaking. The color of the reaction mixture turned green as the evolution of H_2S gas ceased. The reaction mixture was cooled, diluted with water (20 mL) and acidified with acetic acid. The precipitate obtained was filtered, washed, dried and recrystallized from ethanol.

Yield: 71%, m.p.: 198 °C. IR (KBr, cm^{-1}): 3293 (NH), 2890 (CH), 2532 (SH), 1610 (C═N); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.42 (s, 1H, NH_2), 5.68 (s, 1H, CH), 7.15–7.26 (m, 10H, ArH), 13.62 (s, 1H, SH); MS (*m*/*z*): 282 (M^+). Anal. Calcd for $C_{15}H_{14}N_4S$: C, 63.80; H, 5.00; N, 19.84; Found: C, 63.61; H, 5.12; N, 19.71%.

2.4. General procedure for synthesis of 3-diphenylmethyl-6-(substituted)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (*4a–j*)

An equimolar mixture of 4-amino-5-diphenylmethyl-4*H*-1,2,4-triazole-3-thiol (**3**) (0.01 mol) and aromatic acids (0.01 mol) in phosphorus oxychloride (10 mL) was refluxed for 3–5 h. The reaction mixtures were cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixtures were allowed to stand overnight and the solids separated out were filtered, treated with dilute sodium hydroxide solution and washed thoroughly with cold water. The compound so obtained was dried and recrystallized with ethanol.

2.4.1. 6-(4-Chlorophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (*4a*)

Yield: 74%; m.p.: 238 °C. IR (KBr, cm^{-1}): 2912 (CH), 1622 (C═N), 698 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.96 (s, 1H, CH), 7.16–7.37 (m, 10H, ArH), 7.40 (d, 2H, J = 8.4 Hz, ArH), 7.66 (d, 2H, J = 8.4 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.18 (CH), 127.31 ($2CH_{arom}$), 127.89 (CH_{arom}), 128.32 ($2CH_{arom}$), 128.62 ($4CH_{arom}$), 128.81 ($4CH_{arom}$), 129.74 ($2CH_{arom}$), 131.36 (CH_{arom}), 139.0 ($2CH_{arom}$), 139.11 (CH_{arom}), 149.12 (CH_{arom}), 165.26 (CH_{arom}); MS (*m*/*z*): 402 (M^+), 404 ($M^+ + 2$). Anal. Calcd for $C_{22}H_{15}ClN_4S$: C, 65.58; H, 3.75; N, 13.91; Found: C, 65.39; H, 3.58; N, 13.98%.

2.4.2. 6-(2-Chlorophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (*4b*)

Yield: 69%; m.p.: 145 °C. IR (KBr, cm^{-1}): 2943 (CH), 1631 (C═N), 701 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.93 (s,

1H, CH), 7.21–7.41 (m, 10H, ArH), 7.47 (d, 2H, J = 7.8 Hz, ArH), 7.64 (d, 2H, J = 8.1 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.28 (CH), 126.34 ($2CH_{arom}$), 127.79 (CH_{arom}), 128.39 ($2CH_{arom}$), 129.12 ($4CH_{arom}$), 129.53 ($4CH_{arom}$), 129.94 ($2CH_{arom}$), 132.30 (CH_{arom}), 139.56 ($2CH_{arom}$), 139.91 (CH_{arom}), 148.93 (CH_{arom}), 164.16 (CH_{arom}); MS (m/z): 402 (M^+), 404 ($M^+ + 2$). Anal. Calcd for $C_{22}H_{15}ClN_4S$: C, 65.58; H, 3.75; N, 13.91; Found: C, 65.34; H, 3.53; N, 13.73%.

*2.4.3. 6-(2,4-Dichlorophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4c**)*

Yield: 68%; m.p.: 172 °C. IR (KBr, cm^{-1}): 2945 (CH), 1653 (C═N), 708 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 6.03 (s, 1H, CH), 7.27–7.42 (m, 10H, ArH), 7.55 (d, 1H, J = 1.2 Hz, ArH,), 7.80 (d, 2H, J = 6.3 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.38 (CH), 126.31 ($2CH_{arom}$), 127.59 (CH_{arom}), 128.33 ($4CH_{arom}$), 128.92 ($4CH_{arom}$), 129.50 ($2CH_{arom}$), 130.53 (CH_{arom}), 132.90 (CH_{arom}), 136.26 (CH_{arom}), 137.92 (CH_{arom}), 145.32 ($2CH_{arom}$), 149.67 (CH_{arom}), 165.26 (CH_{arom}); MS (m/z): 436 (M^+), 438 ($M^+ + 2$). Anal. Calcd for $C_{22}H_{14}Cl_2N_4S$: C, 60.42; H, 3.23; N, 12.81; Found: C, 60.17; H, 3.29; N, 12.61%.

*2.4.4. 3-Diphenylmethyl-6-(2-methylphenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4d**)*

Yield: 85%, m.p.: 141 °C. IR (KBr, cm^{-1}): 2964 (CH), 1653 (C═N), 723 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.38 (s, 3H, CH_3), 6.05 (s, 1H, CH), 7.25–7.45 (m, 14H, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.48 (CH_3), 48.38 (CH), 126.63 ($3CH_{arom}$), 127.39 (CH_{arom}), 128.13 (CH_{arom}), 128.69 ($4CH_{arom}$), 128.80 ($4CH_{arom}$), 129.95 (CH_{arom}), 131.80 (CH_{arom}), 132.09 ($2CH_{arom}$), 137.92 (CH_{arom}), 138.76 ($2CH_{arom}$), 166.67 (CH_{arom}); MS (m/z): 382 (M^+). Anal. Calcd for $C_{23}H_{18}N_4S$: C, 72.22; H, 4.74; N, 14.65; Found: C, 72.01; H, 4.54; N, 14.48%.

*2.4.5. 6-(4-Aminophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4e**)*

Yield: 76%, m.p.: 153 °C. IR (KBr, cm^{-1}): 2925 (CH), 1628 (C═N), 713 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.54 (s, 1H, CH), 6.23 (s, 2H, NH_2), 6.67 (d, 2H, J = 6 Hz, ArH), 7.29–7.47 (m, 10H, ArH), 7.56 (d, 2H, J = 6.3 Hz, ArH), ^{13}C NMR ($CDCl_3$) δ (ppm): 48.31 (CH), 122.21 ($2CH_{arom}$), 125.36 ($2CH_{arom}$), 128.32 ($2CH_{arom}$), 129.43 ($4CH_{arom}$), 129.98 ($4CH_{arom}$), 135.20 (CH_{arom}), 135.87 ($2CH_{arom}$), 137.90 (CH_{arom}), 145.27 (CH_{arom}), 149.43 (CH_{arom}), 165.16 (CH_{arom}); MS (m/z): 383 (M^+). Anal. Calcd for $C_{22}H_{17}N_5S$: C, 68.91; H, 4.47; N, 18.26; Found: C, 68.59; H, 4.27; N, 18.02%.

*2.4.6. 3-Diphenylmethyl-6-(4-nitrophenyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4f**)*

Yield: 79%; m.p.: 198 °C. IR (KBr, cm^{-1}): 2958 (CH), 1646 (C═N), 697 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 6.05 (s, 1H, CH), 7.26–7.43 (m, 10H, ArH), 7.99 (d, 2H, J = 6 Hz, ArH), 8.34 (d, 2H, J = 6.3 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.31 (CH), 125.21 ($2CH_{arom}$), 126.32 ($2CH_{arom}$), 128.10 ($2CH_{arom}$), 129.23 ($4CH_{arom}$), 129.79 ($4CH_{arom}$), 134.21 (CH_{arom}), 135.29 ($2CH_{arom}$), 138.92 (CH_{arom}), 145.17 (CH_{arom}), 149.23 (CH_{arom}), 164.36 (CH_{arom}); MS (m/z): 413 (M^+). Anal. Calcd for $C_{22}H_{15}N_5O_2S$: C, 63.91; H, 3.66; N, 16.94; Found: C, 63.75; H, 3.35; N, 16.73%.

*2.4.7. 6-(2-Bromophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4g**)*

Yield: 65%; m.p.: 152 °C. IR (KBr, cm^{-1}): 2956 (CH), 1637 (C═N), 721 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.91 (s, 1H, CH), 7.23–7.39 (m, 10H, ArH), 7.41 (d, 2H, J = 7.2 Hz, ArH), 7.66 (d, 2H, J = 7.2 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.43 (CH), 127.46 ($2CH_{arom}$), 128.43 ($4CH_{arom}$), 128.92 ($4CH_{arom}$), 129.86 ($2CH_{arom}$), 130.74 ($2CH_{arom}$), 133.36 (CH_{arom}), 137.43 ($2CH_{arom}$), 138.93 (CH_{arom}), 139.38 (CH_{arom}), 146.90 (CH_{arom}), 167.26 (CH_{arom}); MS (m/z): 448 (M^+), 450 ($M^+ + 2$). Anal. Calcd. for $C_{22}H_{15}BrN_4S$: C, 59.07; H, 3.38; N, 12.52; Found: C, 59.19; H, 3.11; N, 12.32%.

*2.4.8. 6-(4-Bromophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4h**)*

Yield: 87%; m.p.: 248 °C. IR (KBr, cm^{-1}): 2943 (CH), 1632 (C═N), 698 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 6.01 (s, 1H, CH), 7.27–7.39 (m, 10H, ArH), 7.41 (d, 2H, J = 8.1 Hz, ArH), 7.62 (d, 2H, J = 8.4 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.12 (CH), 127.21 ($2CH_{arom}$), 128.73 ($4CH_{arom}$), 128.99 ($4CH_{arom}$), 130.86 ($2CH_{arom}$), 131.74 ($2CH_{arom}$), 133.76 (CH_{arom}), 137.78 ($2CH_{arom}$), 139.71 (CH_{arom}), 140.35 (CH_{arom}), 145.99 (CH_{arom}), 164.26 (CH_{arom}); MS (m/z): 448 (M^+), 450 ($M^+ + 2$). Anal. Calcd for $C_{22}H_{15}BrN_4S$: C, 59.07; H, 3.38; N, 12.58; Found: C, 58.89; H, 3.12; N, 12.33%.

*2.4.9. 6-(4-Bromo-2-chlorophenyl)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4i**)*

Yield: 78%, m.p.: 169 °C. IR (KBr, cm^{-1}): 2935 (CH), 1623 (C═N), 723 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 6.03 (s, 1H, CH), 7.25–7.42 (m, 10H, ArH), 7.55 (dd, 1H, J = 1.2 Hz, 5.1 Hz, ArH), 7.70–7.74 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.26 (CH), 126.86 (CH_{arom}), 126.90 (CH_{arom}), 127.42 ($2CH_{arom}$), 128.73 ($4CH_{arom}$), 128.84 ($4CH_{arom}$), 130.99 (CH_{arom}), 131.92 (CH_{arom}), 133.70 (CH_{arom}), 133.84 ($2CH_{arom}$), 139.04 (CH_{arom}), 148.87 (CH_{arom}), 154.33 (CH_{arom}), 162.32 (CH_{arom}); MS (m/z): 482 (M^+), 484 ($M^+ + 2$). Anal. Calcd for $C_{22}H_{14}BrClN_4S$: C, 54.84; H, 2.93; N, 11.63; Found: C, 54.67, H, 2.70; N, 11.45%.

*2.4.10. 6-[(2,4-Dichlorophenoxy)methyl]-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**4j**)*

Yield: 73%; m.p.: 176 °C. IR (KBr, cm^{-1}): 2975 (CH), 1624 (C═N), 718 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 5.26 (s, 2H, OCH_2), 5.95 (s, 1H, CH), 6.84 (d, 1H, J = 6.6 Hz, ArH), 7.16 (d, 1H, J = 6.3 Hz, ArH), 7.27–7.36 (m, 10H, ArH), 7.41 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$) δ (ppm): 48.35 (CH), 73.20 (CH), 123.61 (CH_{arom}), 126.23 (CH_{arom}), 126.96 ($2CH_{arom}$), 127.34 (CH_{arom}), 128.31 (CH_{arom}), 129.14 ($4CH_{arom}$), 129.56 ($4CH_{arom}$), 136.58 (CH_{arom}), 137.98 ($2CH_{arom}$), 139.53 (CH_{arom}), 141.35 (CH_{arom}), 145.21 (CH_{arom}), 163.11 (CH_{arom}); MS (m/z): 466 (M^+), 468 ($M^+ + 2$). Anal. Calcd for $C_{23}H_{16}Cl_2N_4OS$: C, 59.11; H, 3.45; N, 11.99; Found: C, 59.21, H, 3.17; N, 11.76%.

2.5. General procedure for synthesis of 3-diphenylmethyl-6-(substituted amino)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (**5a–d**)

An equimolar mixture of 4-amino-5-diphenylmethyl-4*H*-1,2,4-triazole-3-thiol (**3**) (0.01 mol) and aryl/alkyl isothiocyanates (0.01 mol) in DMF (20 mL) was refluxed for 12–16 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, and washed thoroughly with cold water. The compound so obtained was dried and recrystallized with ethanol.

2.5.1. 3-Diphenylmethyl-6-phenylamino-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazol (**5a**)

Yield: 80%; m.p.: 153 °C. IR (KBr, cm^{-1}): 3215 (NH), 2963 (CH), 1624 (C═N), 706 (C─S─C); ^{1}H NMR (DMSO d_6) δ (ppm): 5.97 (s, 1H, CH), 7.27–7.56 (m, 15H, ArH), 12.97 (bs, 1H, NH); ^{13}C NMR (DMSO d_6), δ (ppm): 48.31 (CH), 126.64 (2CH_{arom}), 127.14 (CH_{arom}), 128.31 (2CH_{arom}), 128.94 (4CH_{arom}), 129.12 (4CH_{arom}), 133.24 (2CH_{arom}), 135.46 (2CH_{arom}), 139.19 (CH_{arom}), 141.75 (CH_{arom}), 145.71 (CH_{arom}), 162.34 (CH_{arom}); MS (*m*/*z*): 383 (M^+). Anal. Calcd for $C_{22}H_{17}N_5S$: C, 68.91; H, 4.47; N, 18.26; Found: C, 68.79, H, 4.19; N, 18.02%.

2.5.2. 6-(4-Chlorophenylamino)-3-diphenylmethyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazol (**5b**)

Yield: 80%; m.p.: 212 °C. IR (KBr, cm^{-1}): 3230 (NH), 2948 (CH), 1627 (C═N), 709 (C─S─C); ^{1}H NMR (DMSO d_6) δ (ppm): 6.02 (s, 1H, CH), 7.14 (d, 2H, J = 6.66 Hz, ArH), 7.29 (d, 2H, J = 6.9 Hz, ArH), 7.39–7.47 (m, 10H, ArH), 12.86 (bs, 1H, NH); ^{13}C NMR (DMSO d_6) δ (ppm): 48.27 (CH), 126.31 (2CH_{arom}), 127.54 (CH_{arom}), 128.10 (2CH_{arom}), 128.72 (4CH_{arom}), 128.95 (4CH_{arom}), 129.79 (2CH_{arom}), 132.31 (CH_{arom}), 138.98 (2CH_{arom}), 139.54 (CH_{arom}), 148.76 (CH_{arom}), 163.20(CH_{arom}); MS (*m*/*z*): 417 (M^+), 419 ($M^+ + 2$). Anal. Calcd for $C_{22}H_{16}ClN_5S$: C, 63.23; H, 3.86; N, 16.76; Found: C, 68.01, H, 3.67; N, 16.51%.

2.5.3. 3-Diphenylmethyl-6-(4-methylphenylamino)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (**5c**)

Yield: 76%; m.p.: 223 °C. IR (KBr, cm^{-1}): 3210 (NH), 2958 (CH), 1624 (C═N), 716 (C─S─C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.31 (s, 3H, CH_3), 5.67 (s, 1H, CH), 6.85 (d, 1H, J = 6.9 Hz, ArH), 7.12 (d, 1H, J = 6.3 Hz, ArH), 7.25–7.32 (m, 12H, ArH), 11.24 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.32 (CH), 48.39 (CH), 126.54 (2CH_{arom}), 126.97 (CH_{arom}), 127.89 (2CH_{arom}), 128.32 (4CH_{arom}), 128.43 (4CH_{arom}), 130.23 (2CH_{arom}), 132.45 (CH_{arom}), 139.08 (2CH_{arom}), 139.69 (CH_{arom}), 146.59 (CH_{arom}), 162.24 (CH_{arom}); MS (*m*/*z*): 397 (M^+). Anal. Calcd for $C_{23}H_{19}N_5S$: C, 69.50; H, 4.82; N, 17.62; Found: C, 69.39, H, 5.02; N, 17.49%.

2.5.4. 3-Diphenylmethyl-6-propylamino-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazol (**5d**)

Yield: 70%; m.p.: 170 °C. IR (KBr, cm^{-1}): 3205 (NH), 2958 (CH), 1654 (C═N), 714 (C─S─C); ^{1}H NMR (DMSO d_6) δ (ppm): 0.90 (t, 3H, CH_3), 1.56–1.61 (m, 2H, CH_2), 3.31 (t, 2H, N-CH_2), 5.71 (s, 1H, CH), 7.27–7.33 (m, 10H, ArH), 13.18 (bs, 1H, NH); ^{13}C NMR (DMSO d_6) δ (ppm): 20.32 (CH), 29.67 (CH), 48.39 (CH), 57.34 (CH), 126.37 (2CH_{arom}), 128.56 (4CH_{arom}), 128.93 (4CH_{arom}), 134.32 (2CH_{arom}), 139.21 (CH_{arom}), 145.19 (CH_{arom}), 163.37 (CH_{arom}); MS (*m*/*z*): 349 (M^+). Anal. Calcd for $C_{19}H_{19}N_5S$: C, 65.30; H, 5.48; N, 20.04; Found: C, 65.02, H, 5.32; N, 19.91%.

3. Biological activity

The synthesized compounds were evaluated for anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities. Wistar rats and albino mice used in the present study were housed and kept in accordance with the Hamdard university animal care unit, which applies the guidelines and rules laid down by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of social justice and empowerment, Government of India. All the test compounds and standard drug were administered in the form of solution (0.5% w/v carboxymethyl cellulose as a vehicle) by oral route. Each group consists of six animals. Anti-inflammatory activity, hepatotoxic and histopathological studies of the test compounds were compared with the control. Analgesic, ulcerogenic and lipid peroxidation activity were compared with the standard drug *i.e.*, ibuprofen. Data were analyzed by student's t test for $n = 6$.

3.1. Anti-inflammatory activity

Anti-inflammatory activity was carried out by the carrageenan induced paw edema test in Wistar albino rats by Winter et al. (1962) method. The standard drug, ibuprofen and test compounds were given orally (70 mg/kg body weight) as a suspension using 0.5% w/v carboxymethyl cellulose as a vehicle. One hour later foot paw edema was induced by injecting 0.1 mL of 1% carrageenan subcutaneously into the planter portion of the right hind paw of each rat. Initial paw volume was measured immediately by mercury plethysmometer. The paw volume was again measured after the time interval of 3 and 4 h. The percentage inhibition of inflammation was calculated for the standard drug and other test compounds and comparison was made. The percentage inhibition of inflammation was calculated according to the formula, % anti-inflammatory activity = $100 \times (1 - Vt/Vc)$ where, Vt and Vc are the volume edema in test compounds and control groups respectively.

3.2. Analgesic activity

Analgesic activity was evaluated by the tail immersion method (Adeyemi et al., 2004) using Swiss albino mice (25–30 g) of either sex selected by the random sampling technique. The standard drug, ibuprofen and test compounds were administered orally (70 mg/kg body weight) as a suspension using 0.5% w/v carboxymethyl cellulose as a vehicle. 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 of time of immersion, set at 10 s for both control as well as treated groups of animals. The reaction time was

measured before and after 4 h interval of the administration of test compounds and standard drugs.

3.3. Acute ulcerogenicity

Acute ulcerogenesis test was performed according to Cioli et al. (Cioli et al., 1979) using Wistar rats (180–200 g) of either sex. The animals were divided into various groups, each group consisting of 6 rats. All the rats were fasted for 24 h with free access to water. The control groups of animals were administered, 0.5% CMC solution intraperitoneally. One group was administered with standard drug ibuprofen orally in a dose of 210 mg/kg once daily for three days. The remaining group of animals was administered with test compounds through the same route. The animals were immediately fed and kept for 17 h after dose administration. After 17 h they were killed and dissected for the estimation of ulcerogenic activity. The stomach was dissected out and washed with running water and opened along the greater curvature and carefully observed with 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 streak, 2.0: ulcers >3 but $\leqslant 5$, 3.0: ulcers >5. The mean score of each treated group minus the mean score of the control group was regarded as severity index of gastric mucosal damage.

3.4. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. (Ohkawa et al., 1979). After the evaluation of the stomach for ulcers the gastric mucosa of glandular portion was scrapped, weighed (100 mg) and homogenized in pestle and mortar and homogenate was prepared in 1.8 mL of ice cold 1.15% KCl solution. The homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of acetate buffer and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 60 min on boiling water bath then extracted with a mixture of n-butanol: pyridine (15:1, v/v; 5 mL) by shaking vigorously for 1 min and kept in ice for 2 min. Organic layer of reaction mixture was centrifuged at 3000 rpm for 10 min and absorbance was measured at 532 nm on a UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue.

3.5. Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150–200 g. The animals were divided into three groups of six rats each. Group I was kept as control and received only vehicle (0.5% w/v solution of CMC in water), while group II and III received compound **4a** and **4c** respectively, in 0.5% w/v solution of CMC in water for 15 days. After the treatment (15 days) blood was obtained from all the 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 2500 rpm for 15 min and analyzed for various biochemical parameters. Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) was estimated by a reported method (Reitman and Frankel, 1957). The alkaline phosphatase, total protein and total albumin were measured according to reported procedures (King and Armstrong, 1934; Varley, 1988). Histopathological studies were also carried out by the reported method (Luna, 1968). The rats were sacrificed under light ether anesthesia after 24 h of the last dosage; the liver was removed and washed with normal saline, and stored in formalin solution. Sections of 5–6 microns thickness were cut, stained with hematoxylin and eosin, and then studied under an electron microscope.

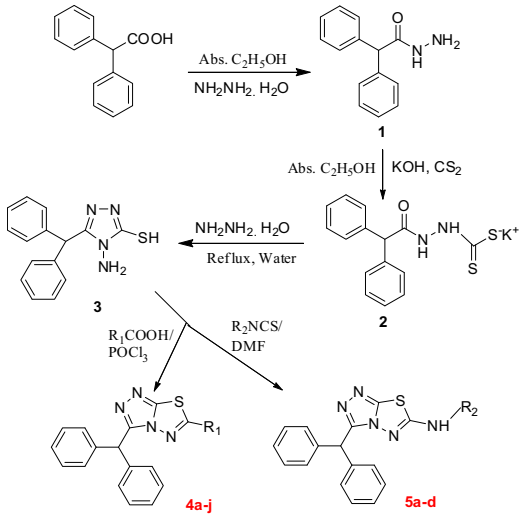
3.6. Statistical analysis

Data are expressed as mean ± S.E.M., Student's *t*-test was applied to determine the significance of the difference between the standard group and rats/mice treated with the test compounds. The difference in results was considered significant when $P < 0.01$.

4. Results and discussion

4.1. Chemistry

The title compounds triazolo-thiadiazole derivatives (**4a**–**j** and **5a**–**d**) were synthesized as outlined in Scheme 1. The intermediate product 4-amino-5-(diphenylmethyl)-*4H*-1,2,4-triazole-3-thiol (**3**) was prepared following the earlier reported procedure (Reid and Heindel, 1976). Condensation of **3** with substituted aromatic acids in the presence of phosphorous oxychloride afforded the corresponding 3-diphenylmethyl-6-(substituted)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (**4a**–**j**), whereas reaction of **3** with aryl/alkyl isothiocyanates in the presence of DMF provided 3-diphenylmethyl-6-(substitutedamino)-1,2,4-triazolo[3,4-*b*]-1,3,4 thiadiazoles (**5a**–**d**). The analytical and spectral data of all the synthesized compounds were in full agreement with the proposed structures. ^{1}H



R$_1$ = **a**: 4-Cl-C_6H_4; **b**: 2-Cl-C_6H_4; **c**: 2,4-$(Cl)_2$-C_6H_3; **d**: 2-CH_3-C_6H_4; **e**: 4-NH_2-C_6H_4; **f**: 4-NO_2-C_6H_4; **g**: 2-Br-C_6H_4; **h**: 4-Br-C_6H_4; **i**: 2-Cl-4-Br-C_6H_3; **j**: 2,4-$(Cl)_2$-C_6H_3-OCH_2;

R$_2$ = **a**: C_6H_5; **b**: 4-Cl-C_6H_4; **c**: 4-CH_3-C_6H_4; **d**: C_3H_7

Scheme 1 Synthetic protocol of the title compounds.

NMR spectra of intermediate compound **3** showed a down field broad singlet at δ 13.62 whereas the NH_2 group appeared as a singlet at δ 5.42 which are D_2O exchangeable. The absence of signals for NH_2 and SH protons confirms that the triazole has been converted into triazolo-thiadiazole derivatives (**4a–j** and **5a–d**). The elemental analysis results were within ±0.4% of the theoretical values.

4.2. Pharmacology

The anti-inflammatory activity of the synthesized compounds **4a–j** and **5a–d** was evaluated by the carrageenan induced paw edema method of Winter et al. The compounds were tested at an equimolar oral dose relative to 70 mg/kg of ibuprofen. The percentage inhibition was calculated 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 33.46% to 80.13% (Table 1), whereas standard drug ibuprofen showed 73.23% inhibition after 4 h. The anti-inflammatory activity of triazolo-thiadiazole derivatives of **4a–j** series was in the range of 46.72–80.13%. It was observed that the triazolo-thiadiazole derivatives having the 4-chlorophenyl group (**4a**) at the 6th position showed the highest activity (80.13%) more than the standard drug ibuprofen (73.23%). Furthermore it was noted that the presence of the 2-chlorophenyl group (**4b**) and the 2,4-dichlorophenyl group (**4c**) at the 6th position of the triazolo-thiadiazole ring resulted in a slight decrease of anti-inflammatory activity (74.39% and 76.12% respectively) but still it was found to be more than ibuprofen (73.23%). It was observed that triazolothiadiazole derivatives having 2-methylphenyl (**4d**), 2-bromophenyl (**4g**), 4-bromophenyl (**4h**) and 2,4-dichlorophenoxy methyl (**4j**) groups also showed good activity viz, 63.65%, 62.67%, 67.47% and 69.45% respectively. Other compounds of the series showed moderate to poor activity. The anti-inflammatory activity of 1,2,4-triazolo-thiadiazole derivatives of **5a–d** series was found in the range of 33.46–70.36%. The compound having the 4-chlorophenyl amino group showed high anti-inflammatory activity (70.36%) comparable to standard drug ibuprofen (73.23%). Replacement of this with phenyl amino (**5a**) and the 4-methylphenyl amino group (**5c**) resulted in a slight decrease of activity (61.26% and 63.03% respectively). The compound having the isopropyl amino group (**5d**) showed poor activity (33.46%). Thus it was found that the presence of 4-chlorophenyl, 2-chlorophenyl 2,4-dichlorophenyl and 4-chlorophenyl amino groups at the 6th position of the triazolo-thiadiazole ring resulted in high anti-inflammatory activity.

All these compounds were further tested for their analgesic activity at the same oral dose as used for the anti-inflammatory activity. The compounds showed analgesic activity ranging from 40.52% to 79.14% inhibition, whereas standard drug showed 73.89% (Table 2). The compounds having 4-chlorophenyl (**4a**), 2-chlorophenyl (**4b**) and 2-methylphenyl (**4d**) groups at the 6th position of the triazolo-thiadiazole ring showed high analgesic activities (74.35%, 79.14% and 74.16% respectively) in comparison to standard drug ibuprofen (73.89%). Replacement of these groups by 2-bromophenyl (**4g**), 2,4-dicholorophenoxy methyl (**4j**) and 4-chlorophenyl amino (**5b**) groups resulted in a slight decrease of activity (62.41%, 68.22% and 65.18% respectively). Rest of the compounds showed very low analgesic activities. In general, the presence of 4-chlorophenyl, 2-chlorophenyl, 2-methylphenyl groups at the 6th position of the triazolo-thiadiazole ring resulted in high analgesic activities.

The compounds that exhibited anti-inflammatory and analgesic activity higher than 60% were further tested for their acute ulcerogenic activity. Compounds **4a–d**, **4g**, **4j** and **5a–c** were tested at an equimolar oral dose related to 210 mg/kg ibuprofen. The tested compounds showed low ulcerogenic activity ranging from 0.166 ± 0.10 to 0.750 ± 0.17, compared to standard drug ibuprofen showing high severity index of 0.500 ± 0.00. The maximum reduction in ulcerogenic risk (0.166 ± 0.10) was found in compound **4c** having the 2,4-dichlorophenyl group at the 6th position of the triazolo-thiadiazole ring. The compounds **4a** and **4b** showing high anti-inflammatory and analgesic activities and compound **5c** having moderate anti-inflammatory and analgesic activities also showed reduction in severity index (0.250 ± 0.11 and 0.333 ± 0.10 respectively). The compounds **4d**, **4g**, **4j** and **5a–b** showed slightly higher ulcerogenic activities in comparison to standard drug ibuprofen (Table 2).

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 lipid in the cell

Table 1 Anti-inflammatory activity of compounds **4a–j** and **5a–d**.

Compound	Anti-inflammatory activity inhibition ± SEM[*]		Compound	Anti-inflammatory activity % inhibition ± SEM[#]	
	After 3 h	After 4 h		After 3 h	After 4 h
4a	77.88 ± 0.91	80.13 ± 0.91[b]	**4h**	64.03 ± 0.88	67.47 ± 0.78[b]
4b	71.18 ± 0.88	74.39 ± 1.15[d]	**4i**	54.05 ± 0.39	58.81 ± 0.82[a]
4c	72.54 ± 0.66	76.13 ± 0.87[c]	**4j**	67.05 ± 0.43	69.45 ± 0.75[c]
4d	58.47 ± 0.89	63.65 ± 0.94[a]	**5a**	57.09 ± 0.54	61.26 ± 0.73[a]
4e	48.21 ± 1.04	50.91 ± 1.05[a]	**5b**	66.26 ± 0.84	70.36 ± 0.65[b]
4f	45.39 ± 1.37	46.72 ± 1.36[a]	**5c**	57.59 ± 0.83	63.03 ± 0.78[a]
4g	58.94 ± 0.60	62.67 ± 1.23[a]	**5d**	28.96 ± 1.09	33.46 ± 0.72[a]
Ibuprofen	70.15 ± 0.80	73.23 ± 1.01	–	–	–

[#] Relative to standard and data were analyzed by student's t test for $n = 6$.
[a] $p < 0.0001$.
[b] $p < 0.001$.
[c] $p < 0.05$.
[d] $p < 0.5$.

Table 2 Analgesic activity of compounds **4a–j** and **5a–d**. Ulcerogenic and lipid peroxidation activities of selected compounds.

Compound	Analgesic activity[#]			Ulcerogenic activity (severity index ± SEM)[†]	nmol MDA content ± SEM/100 mg tissue[‡]
	Pre-treatment/ normal 0 h (s)	Post-treatment/ after 4 h (s)	% Inhibition		
4a	1.51 ± 0.02	2.64 ± 0.07	74.35 ± 1.46[d]	0.250 ± 0.11[*]	5.39 ± 0.13[*]
4b	1.63 ± 0.02	2.93 ± 0.22	79.14 ± 1.82[c]	0.333 ± 0.10[**]	5.83 ± 0.16[***]
4c	2.05 ± 0.01	3.40 ± 0.03	65.67 ± 1.18	0.166 ± 0.10[**]	3.75 ± 0.08[*]
4d	1.27 ± 0.01	2.22 ± 0.02	74.16 ± 0.96[a]	0.666 ± 0.10[**]	5.60 ± 0.14[**]
4e	1.63 ± 0.01	2.52 ± 0.04	54.23 ± 1.98[a]	–	–
4f	1.51 ± 0.01	2.39 ± 0.00	57.85 ± 1.03[a]	–	–
4g	1.36 ± 0.01	2.20 ± 0.01	62.41 ± 1.31[b]	0.750 ± 0.17[**]	5.35 ± 0.11*
4h	1.76 ± 0.03	2.65 ± 0.01	50.99 ± 1.50[a]	–	–
4i	1.59 ± 0.02	2.47 ± 0.01	55.04 ± 1.17[a]	–	–
4j	1.91 ± 0.01	3.21 ± 0.01	68.22 ± 0.85[c]	0.583 ± 0.08[**]	5.10 ± 0.19*
5a	1.74 ± 0.15	2.70 ± 0.01	55.70 ± 1.09[a]	0.750 ± 0.17[**]	5.98 ± 0.19[****]
5b	1.49 ± 0.01	2.47 ± 0.01	65.18 ± 1.12[b]	0.750 ± 0.11[**]	5.32 ± 0.17*
5c	1.38 ± 0.01	2.16 ± 0.01	56.33 ± 1.55[a]	0.250 ± 0.11[**]	4.48 ± 0.22*
5d	1.60 ± 0.00	2.24 ± 0.01	40.52 ± 0.58[a]	–	–
Control	–	–	–	0.000 ± 0.00	3.31 ± 0.06
Ibuprofen	1.36 ± 0.00	2.37 ± 0.01	73.89 ± 1.35	0.500 ± 0.00	6.60 ± 0.11

Relative to standard and data were analyzed by student's t test for $n = 6$.
[#] [a]$p < 0.0001$, [b]$p < 0.001$, [c]$p < 0.01$, [d]$p < 0.05$, [e]$p < 0.5$.
[†] [*]$p < 0.05$, [**]$p < 0.5$.
[‡] [*]$p < 0.0001$, [**]$p < 0.001$, [***]$p < 0.01$, [****]$p < 0.05$.

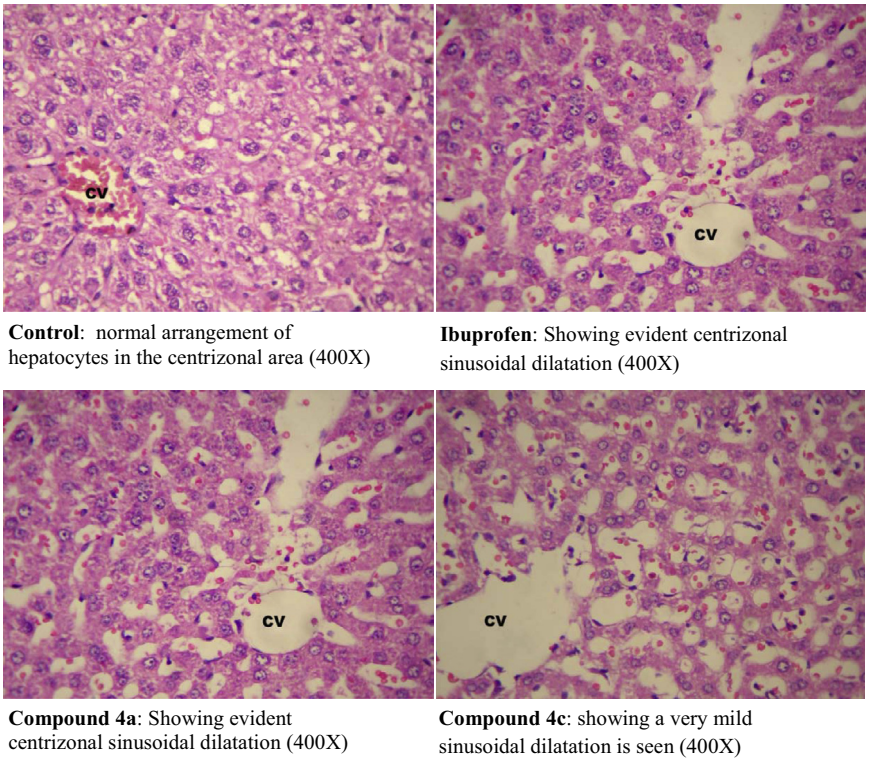
membrane and consequently damage the cell. It most often affects polyunsaturated fatty acids forming malondialdehyde (MDA). The colorimetric reaction of thiobarbituric acid (TBA) with MDA, a secondary product of lipid peroxidation (LPO) has been widely adopted as a sensitive assay method for measuring LPO in animal tissues. It is used as an index of the extent to which LPO has progressed. Since the assay procedure estimates the amount of TBA reactive substances e.g., MDA, it is also referred to as TBARS (Thiobarbituric Acid Reactive Substance) test. It has been reported that the compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a by-product of lipid peroxidation (Phole 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 lipid peroxidation. The lipid peroxidation was measured as nanomoles of malondialdehyde (MDA)/100 mg of gastric mucosa tissue. Ibuprofen exhibited high lipid peroxidation 6.60 ± 0.11, whereas the control group showed 3.31 ± 0.06. It was found that all the triazolo-thiadiazole derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 2). Thus these studies showed that the synthesized compounds have inhibited the induction of gastric mucosal ulcer and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

The compounds **4a** and **4c**, triazolo-thiadiazole derivatives of diphenylacetic acid showing potent anti-inflammatory and analgesic activities with reduced ulcerogenicity and lipid peroxidation, were further studied for their hepatotoxic effect. Both compounds were studied for their effect on biochemical parameters (serum enzyme, total protein and total albumin). Liver histopathological testing of these compounds was also carried out. As shown in Table 3, activities of liver enzyme SGOT, SGPT, alkaline phosphatase, total protein and total albumin were almost identical with ibuprofen value, except for compound **4c** whose SGOT and SGPT level was found to be reduced. The histopathological studies of the liver sample of standard drug ibuprofen showed evident centrizonal sinusoidal dilation in comparison to control. The compound **4a** when tested for their histopathological studies showed centrizonal sinusoidal dilation equivalent to ibuprofen whereas compound **4c** showed very mild sinusoidal dilation (Fig. 1).

Table 3 Effect of compounds **4a** and **4c** on serum enzymes, total proteins and total albumin.

Compound	SGOT units/ml[#]	SGPT units/ml[#]	Alkaline phosphatase[#]	Total protein g/dl[#]	Total albumin g/dl[#]
Control	161.04 ± 2.29	51.96 ± 0.82	45.62 ± 0.72	1.83 ± 0.00	1.74 ± 0.01
Ibuprofen	165.57 ± 2.32[e]	53.14 ± 1.53	47.72 ± 1.30[e]	1.90 ± 0.01[c]	1.68 ± 0.01[d]
4a	173.71 ± 3.13[c]	55.39 ± 1.18[d]	51.51 ± 0.80[b]	2.01 ± 0.02[a]	1.90 ± 0.02[a]
4c	135.71 ± 2.80[e]	41.44 ± 1.38[a]	43.63 ± 1.53[e]	1.78 ± 0.01[c]	1.63 ± 0.01[b]

[#] Relative to control and data were analyzed by student's t test for $n = 6$.
[a] $p < 0.0001$.
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.05$.
[e] $p < 0.5$.



Control: normal arrangement of hepatocytes in the centrizonal area (400X)

Ibuprofen: Showing evident centrizonal sinusoidal dilatation (400X)

Compound 4a: Showing evident centrizonal sinusoidal dilatation (400X)

Compound 4c: showing a very mild sinusoidal dilatation is seen (400X)

Figure 1 Histopthalogical studies of the liver.

This indicates the safety of compound **4c** with respect to standard drug.

5. Conclusion

In summary, various triazolo-thiadiazole derivatives of diphenyl acetic acid were synthesized and screened for anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities. It was observed that three cyclized compounds **4a**, **4b** and **4c** were found to have anti-inflammatory properties more than or comparable to their standard reference drug ibuprofen. When these compounds were subjected to analgesic activity by the tail immersion method in mice, they exhibited moderate to good activity. These compounds were also tested for ulcerogenic activity and lipid peroxidation, and showed superior GI safety profile along with reduction in lipid peroxidation as compared with standard drug ibuprofen. From these studies, compounds **4a**, 6-(4-chlorophenyl)-3-(diphenylmethyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole and **4c**, 6-(2, 4-dichlorophenyl)-3-(diphenylmethyl)-1,2,4-triazolo[3,4-*b*]-1,3, 4-thiadiazole have emerged as the lead compounds, which showed the most prominent and consistent activity with maximum reduction in gastrointestinal toxicity and minimum lipid peroxidation. Thus the series provided new opportunities for possible modification of pharmacophoric requirements and future exploitation.

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