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Synthesis of some new 1,2,4-triazoles and 1,3,4-oxadiazoles as a safer anti-inflammatory and analgesic agents

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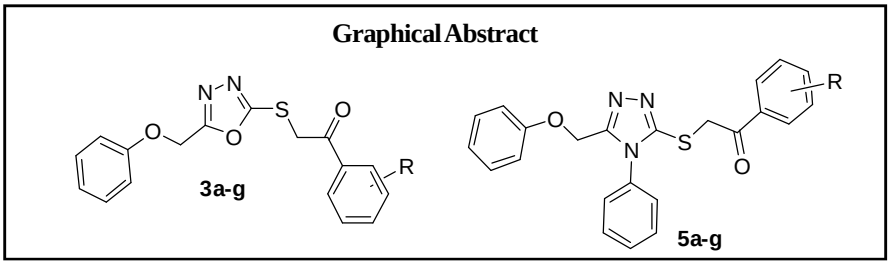
ABSTRACT

A series of 1,2,4-triazole and 1,3,4-oxadiazole derivatives of phenoxyacetic acid were synthesized in order to obtain compounds with promising anti-inflammatory, analgesic activity and lower ulcerogenic potential. All compounds were evaluated for their anti-inflammatory activity by the carrageenan induced rat paw edema test method. The compounds possessing potent anti-inflammatory activity were further tested for their analgesic, ulcerogenic and lipid peroxidation activity. Out of all tested compounds, **3c** and **5d** showed anti-inflammatory and analgesic activity more than the standard drug ibuprofen. These compounds also showed reduced ulcerogenic potential and lipid peroxidation.

KEYWORDS: Triazole, oxadiazole, anti-inflammatory, analgesic, ulcerogenic, lipidperoxidation, hepatotoxic, histopathological

Graphical Abstract



1. INTRODUCTION:

Nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin are among the most widely prescribed drugs worldwide. Through their anti-inflammatory, antipyretic and analgesic activities, they represent a choice treatment in various inflammatory diseases. Chronic NSAID therapy effectively reduces the symptoms of many painful arthritic syndromes, but invites adverse gastrointestinal (GI) complications ranging from stomach irritation to life threatening GI ulceration and bleeding. Long term NSAID therapy makes NSAID induced GI toxicity a substantial patient risk, and a costly healthcare and societal burden in terms of associated hospitalization and morbidity.[1] NSAIDs exert their anti-inflammatory effect mainly through inhibition of cyclooxygenases (COXs), key enzymes in prostaglandin (PG) biosynthesis from arachidonic acid.[2-4] There are at least two mammalian COX isoforms, COX-1 and COX-2.[5] COX-1 is the constitutive isoform, which performs a housekeeping function to synthesize prostaglandins, which regulate normal cell activity. The second isoform COX-2, in contrast, is induced in inflammatory cells in response to pro-inflammatory stimuli such as cytokines, tumor promoting agents, and bacterial endotoxins. The prostaglandins (PGs) produced by COX-2 play a major role in inflammatory reactions and are responsible for characteristic inflammatory symptoms.[6] These findings stimulated the development of selective COX-2 inhibitors (coxibs), as a new generation of NSAIDs, free from GI toxicity.[7,8] But careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effects.[9] Therefore the development of novel compounds having anti-inflammatory-analgesic activity with an improved safety profile is still a necessity. Studies have shown that derivatisation of the carboxylate function of arylalkonic acids resulted in retained anti-inflammatory activity and reduced ulcerogenic potential.[10-13] Furthermore it has been reported in literature that substituted azole derivatives such as 1,3,4-oxadiazole[14,15] and 1,2,4 triazole[16] possess significant anti-inflammatory activity. In view these observations and in continuation of our research program on the

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synthesis of five membered heterocyclic compounds,[17-19] we report herein the synthesis of some new azole derivatives of phenoxy acetic acid. The synthesized compounds have been found to possess an interesting profile of anti-inflammatory and analgesic activity, with significant reduction in their ulcerogenic effect.

EXPERIMENTAL

1. Instrumentation and chemicals

The melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. Elemental analysis (C, H, N, S) was performed on the CHNS Elimentar (Analysen systime, GmbH) Germany Vario EL III. FTIR spectra were recorded as KBr pellets on a Jasco FT/IR 410 spectrometer and frequency was expressed in cm^{-1}. ^1H NMR spectra were measured on Bruker Avance-400 instrument (400 MHz) and ^{13}C NMR spectra were measured on Bruker Avance-400 instrument (100MHz) with complete proton decoupling. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS); coupling constants (J) are reported in Hertz, and refer to apparent peak multiplicities, and may not necessarily be true coupling constants. Mass spectra were measured on a Jeol SR-102 (FAB) mass spectrometer. Unless otherwise specified, all reactions were carried out in oven-dried glassware, and commercially available starting materials were used without further purification.

2. Chemistry

2.1. Synthesis of 2-phenoxyacetohydrazide (1)

To a solution of ethylphenoxy acetate (0.01 mol) in absolute ethanol (50 mL), hydrazine hydrate (0.02 mol) was added. The resultant mixture was refluxed for 18 h, then concentrated, cooled to room temperature and poured into crushed ice. The solid mass obtained was filtered, dried and recrystallized from ethanol. IR (KBr, cm^{-1}): 1545 (C=N), 1687 (C=O), 3314 (NH); ^1H NMR (400 MHz, DMSO d_6, δ): 4.32 (s, 2H, NH_2), 4.47 (s, 2H, OCH_2), 6.93-7.31 (m, 5H, ArH), 9.32 (bs, 1H, CONH).

2.2. Synthesis of 5-(phenoxymethyl)-1,3,4-oxadiazole-2-thiol (2)

A mixture of compound **1** (0.05 mol), potassium hydroxide (0.05 mol) and carbon disulphide (0.05 mol) in ethanol (50mL) was refluxed for 10 h. The solution was then concentrated, cooled and acidified with dilute hydrochloric acid. The solid that separated out was filtered and recrystallized from ethanol. IR (KBr, cm^{-1}): 1172 (C-O-C), 1585 (C=N), 2542 (SH); ^1H NMR (400 MHz, DMSO d_6, δ): 5.06 (s, 2H, OCH_2), 6.96-7.36 (m, 5H, ArH), 11.07 (bs, 1H, SH).

2.3. Synthesis of 5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (4)

A mixture of compound **1** (0.05 mol), aryl isothiocyanate (0.05 mol) and ethanol (50 mL) was refluxed for 5 h. Then NaOH solution (4N, 5 mL) was added to the above mixture which resulted into the formation of clear solution. The reaction mixture was further reflux for 8 hrs on water bath. The solution was then concentrated, cooled and acidified with dilute hydrochloric acid. The solid that separated out was filtered and recrystallized from ethanol. IR (KBr, cm^{-1}): 1558 (C=N), 2583 (SH); ^1H NMR (400 MHz, DMSO d_6, δ): 4.89 (s, 2H, OCH_2), 6.79-7.58 (m, 10H, ArH) 11.81 (bs, 1H, SH).

2.4. General method for synthesis of 1-(substitutedphenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)-1-ethanone (3a-g)

A mixture of compound **2** (0.003 mol) and potassium carbonate (0.004 mol) was dissolved in DMF (25 mL) at 0°C and stirred for 10 min. Substituted phenacyl bromide[20] (0.003 mol) was added to it slowly while stirring and the reaction mixture was further stirred for 4 h. It was poured into ice cold water; the precipitate thus formed was filtered, dried and recrystallized from ethanol.

2-(5-(Phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)-1-phenylethanone 3a. IR (KBr, cm^{-1}): 724 (C-S-C), 1175 (C-O-C), 1598 (C=N), 1653 (C=O); ^1H NMR (400 MHz, DMSO d_6, δ): 4.41 (s, 2H, SCH_2), 5.29 (s, 2H, OCH_2), 6.91-7.81 (m, 10H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 190.29 (C=O) 167.38 (C_{oxad}), 166.54 (C_{oxad}), 157.32 (C_{arom}), 146.47 (C_{arom}), 140.62 (C_{arom}), 134.30 (C_{arom}), 129.98 ($2C_{arom}$),129.61 ($2C_{arom}$), 122.20 ($2C_{arom}$), 115.82 ($2C_{arom}$), 59.76 (OCH_2), 41.38 (SCH_2); Mass (m/z): 327 (M^+) .

1-(4-Chlorophenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)ethanone 3b. IR (KBr, cm^{-1}): 729 (C-S-C), 1183 (C-O-C), 1590 (C=N), 1642 (C=O); ^1H NMR (400 MHz, $CDCl_3$, δ): 4.62 (s, 2H, SCH_2), 5.39 (s, 2H, OCH_2), 7.12-8.11 (m, 9H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 191.49 (C=O),165.68 (C_{oxad}),163.56 (C_{oxad}), 157.47 (C_{arom}), 145.44 (C_{arom}), 141.12 (C_{arom}), 132.32 (C_{arom}), 129.67 ($2C_{arom}$), 128.66 ($2C_{arom}$), 122.26 ($2C_{arom}$), 114.86 ($2C_{arom}$), 59.71 (OCH_2), 41.36 (SCH_2); Mass (m/z): 363 (M^++2), 361 (M^+).

1-(4-Fluorophenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)ethanone 3c. IR (KBr, cm^{-1}): 719 (C-S-C), 1182 (C-O-C), 1596 (C=N), 1663 (C=O); ^1H NMR (400 MHz, DMSO d_6, δ): 4.54 (s, 2H, SCH_2), 5.32 (s, 2H, OCH_2), 6.91-7.89 (m, 7H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 190.34 (C=O), 167.69 (C_{arom}), 165.42 (C_{oxad}), 163.66 (C_{oxad}), 157.45 (C_{arom}), 131.38 (C_{arom}), 131.28 ($2C_{arom}$), 129.76 ($2C_{arom}$), 122.29 (C_{arom}), 116.48 ($2C_{arom}$), 114.85 ($2C_{arom}$), 59.71 (OCH_2), 41.36 (SCH_2); Mass (m/z): 345 (M^+).

1-(4-Bromophenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)ethanone 3d. IR (KBr, cm^{-1}): 716 (C-S-C), 1176 (C-O-C), 1603

(C=N), 1651 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.43 (s, 2H, SCH_2), 5.53 (s, 2H, OCH_2), 7.19-8.11 (m, 9H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 187.49 (C=O), 165.20 (C_{oxad}), 163.51 (C_{oxad}), 157.78 (C_{arom}), 144.49 (C_{arom}), 138.30 (C_{arom}), 132.38 (C_{arom}), 129.17 ($2C_{arom}$), 127.64 ($2C_{arom}$), 123.21 ($2C_{arom}$), 115.86 ($2C_{arom}$), 57.61 (OCH_2), 40.36 (SCH_2); Mass (m/z): 408 (M^++2), 406 (M^+).

2-(5-(Phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)-1-p-tolylethanone 3e. IR (KBr, cm^{-1}): 712 (C-S-C), 1154 (C-O-C), 1583 (C=N), 1644 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 2.44 (s, 3H, CH_3), 4.93 (s, 2H, SCH_2), 5.23 (s, 2H, OCH_2), 6.99-8.01 (m, 9H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 191.49 (C=O), 165.68 (C_{oxad}), 163.56 (C_{oxad}), 157.47 (C_{arom}), 145.44 (C_{arom}), 136.10 (C_{arom}), 132.32 (C_{arom}), 129.67 ($2C_{arom}$),128.66 ($2C_{arom}$), 122.26 ($2C_{arom}$), 114.86 ($2C_{arom}$), 59.71 (OCH_2), 41.36 (SCH_2), 21.82 (CH_3); Mass (m/z): 341 (M^+).

1-(4-Nitrophenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)ethanone 3f. IR (KBr, cm^{-1}): 719 (C-S-C), 1178 (C-O-C), 1592 (C=N), 1664 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.83 (s, 2H, SCH_2), 5.13 (s, 2H, OCH_2), 6.90-7.91 (m, 9H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 179.49 (C=O), 166.63 (C_{oxad}), 165.51 (C_{oxad}), 156.98 (C_{arom}), 147.41 (C_{arom}), 141.60 (C_{arom}), 133.76 (C_{arom}), 129.79 ($2C_{arom}$), 125.63 ($2C_{arom}$), 121.26 ($2C_{arom}$), 113.82 ($2C_{arom}$), 59.76 (OCH_2), 41.54 (SCH_2); Mass (m/z): 372 (M^+).

1-(2-Nitrophenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)ethanone 3g. IR (KBr, cm^{-1}): 709 (C-S-C), 1170 (C-O-C), 1594 (C=N), 1665 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.90 (s, 2H, SCH_2), 5.53 (s, 2H, OCH_2), 7.19-8.11 (m, 9H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 181.41 (C=O), 166.62 (C_{oxad}), 164.50 (C_{oxad}),156.91 (C_{arom}), 148.42 (C_{arom}), 139.18 (C_{arom}), 133.74 (C_{arom}), 129.39 ($2C_{arom}$), 124.61 ($2C_{arom}$), 121.43 ($2C_{arom}$), 113.40 ($2C_{arom}$), 59.71 (OCH_2), 42.04 (SCH_2); Mass (m/z): 372 (M^+).

2.5.General method for synthesis of 1-(substitutedphenyl)-2-(5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-1-ethanone (5a-g)

A mixture of compound **4** (0.003 mol) and potassium carbonate (0.004 mol) was dissolved in DMF (25 mL) at 0^{o}C and stirred for 10 min. Substituted phenacyl bromide[20] (0.003 mol) was added to it slowly while stirring and the reaction mixture was further stirred for 4 h. It was poured into ice cold water, the precipitate thus formed was filtered, dried and recrystallized from ethanol.

2-(5-(Phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-1-phenylethanone 5a. IR (KBr, cm^{-1}): 712 (C-S-C), 1567 (C=N), 1666 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.80 (s, 2H, SCH_2), 5.34 (s, 2H, OCH_2), 7.09-8.18 (m, 15H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 193.55 (C=O), 164.92 (C_{arom}), 162.59 (C_{triaz}), 157.48 (C_{triaz}), 154.25 (C_{arom}), 152.53 (C_{arom}), 138.10 (C_{arom}), 132.12 (C_{arom}), 131.41 ($2C_{arom}$), 130.02 ($2C_{arom}$), 129.17 ($2C_{arom}$), 128.55 ($2C_{arom}$), 126.95 ($2C_{arom}$), 121.39 (C_{arom}), 114.12 ($2C_{arom}$), 59.28 (OCH_2), 41.87 (SCH_2); Mass (m/z): 402 (M^+).

1-(4-Chlorophenyl)-2-(5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)ethanone 5b. IR (KBr, cm^{-1}): 709 (C-S-C), 1578 (C=N), 1670 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.81 (s, 2H, SCH_2), 5.15 (s, 2H, OCH_2), 7.72-7.91 (m, 14H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 194.01 (C=O), 160.34 (C_{arom}), 158.51 (C_{triaz}), 156.23 (C_{triaz}), 153.16 (C_{arom}), 132.39 (C_{arom}), 131.76 ($2C_{arom}$), 130.21 ($2C_{arom}$), 129.13 ($2C_{arom}$), 128.43 ($2C_{arom}$), 126.22 ($3C_{arom}$), 123.11 (C_{arom}), 121.43 (C_{arom}), 114.21 ($2C_{arom}$), 59.01 (OCH_2), 41.16 (SCH_2); Mass (m/z): 438 (M^++2), 436 (M^+).

1-(4-Fluorophenyl)-2-(5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)ethanone 5c. IR (KBr, cm^{-1}): 719 (C-S-C), 1565 (C=N), 1662 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.83 (s, 2H, SCH_2), 5.05 (s, 2H, OCH_2), 7.86-8.10 (m, 14H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 191.55 (C=O), 167.51 (C_{arom}), 164.96 (C_{arom}), 162.59 (C_{triaz}), 157.48 (C_{triaz}), 152.58 (C_{arom}), 132.55 (C_{arom}), 131.40 ($2C_{arom}$), 130.36 ($2C_{arom}$), 129.90 ($2C_{arom}$), 129.55 ($2C_{arom}$), 126.94 (C_{arom}), 121.79 (C_{arom}), 115.95 ($2C_{arom}$), 114.86 ($2C_{arom}$), 59.78 (OCH_2), 41.00 (SCH_2); Mass (m/z,): 420 (M^+) .

1-(4-Bromophenyl)-2-(5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)ethanone 5d. IR (KBr, cm^{-1}): 710 (C-S-C), 1576 (C=N), 1680 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.83 (s, 2H, SCH_2), 5.05 (s, 2H, OCH_2), 7.75-7.97 (m, 14H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 193.52 (C=O), 161.34 (C_{arom}), 159.59 (C_{triaz}), 157.43 (C_{triaz}), 153.58 (C_{arom}), 132.36 (C_{arom}), 131.34 ($2C_{arom}$), 130.56 ($2C_{arom}$), 129.97 ($2C_{arom}$), 129.43 ($2C_{arom}$), 126.94 ($3C_{arom}$), 123.36 (C_{arom}), 121.76 (C_{arom}), 114.23 ($2C_{arom}$), 59.32 (OCH_2), 41.56 (SCH_2); Mass (m/z): 483 (M^++2), 481 (M^+).

2-(5-(Phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-1-p-tolylethanone 5e. IR (KBr, cm^{-1}): 718 (C-S-C), 1583 (C=N), 1672 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 2.42 (s, 3H, CH_3), 4.96 (s, 2H, SCH_2), 5.05 (s, 2H, OCH_2), 6.86-8.01 (m, 14H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 192.66 (C=O), 157.54 (C_{arom}), 152.85 (C_{triaz}), 152.00 (C_{triaz}), 145.06 (C_{arom}), 132.68 (C_{arom}), 130.28 (C_{arom}), 129.88 ($2C_{arom}$), 129.55 ($5C_{arom}$), 128.69 ($2C_{arom}$), 127.00 ($2C_{arom}$), 121.75 ($2C_{arom}$), 114.89 (C_{arom}), 59.83 (OCH_2), 41.45 (SCH_2), 21.78 (CH_3); Mass (m/z): 416 (M^+).

1-(4-Nitrophenyl)-2-(5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)ethanone 5f. IR (KBr, cm^{-1}): 717 (C-S-C), 1576 (C=N), 1679 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.63 (s, 2H, SCH_2), 5.15 (s, 2H, OCH_2), 7.82-8.00 (m, 14H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 194.04 (C=O), 162.53 (C_{arom}), 161.23 (C_{triaz}), 156.32 (C_{triaz}), 154.59 (C_{arom}), 151.50 (C_{arom}), 132.33 ($2C_{arom}$), 131.73 (C_{arom}), 131.11 ($2C_{arom}$), 129.23 ($2C_{arom}$), 128.11 ($2C_{arom}$), 126.91 (C_{arom}), 121.71 (C_{arom}), 115.97 ($2C_{arom}$), 114.20 ($2C_{arom}$), 59.29 (OCH_2), 41.23 (SCH_2); Mass (m/z): 447 (M^+).

1-(2-Nitrophenyl)-2-(5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)ethanone 5g. IR (KBr, cm^{-1}):, 714 (C-S-C), 1588 (C=N), 1673 (C=O); ^{1}H NMR (400 MHz, DMSO d_6, δ): 4.63 (s, 2H, SCH_2), 5.15 (s, 2H, OCH_2), 7.82-8.00 (m, 14H, ArH); ^{13}C NMR (100 MHz, DMSO d_6, δ): 194.23 (C=O), 161.53 (C_{arom}), 160.59 (C_{triaz}), 156.31 (C_{triaz}), 154.51 (C_{arom}), 152.58 (C_{arom}), 132.45 (C_{arom}), 131.36 ($2C_{arom}$), 130.06 ($2C_{arom}$), 129.83 ($2C_{arom}$), 129.32 ($2C_{arom}$), 126.90 (C_{arom}), 122.79 (C_{arom}), 116.91 ($2C_{arom}$), 114.26 ($2C_{arom}$), 59.21 (OCH_2), 41.10 (SCH_2); Mass (m/z): 447 (M^+).

3. Biological activities

3.1. Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory, analgesic activity, ulcerogenic potential and lipid peroxidation using wistar rats and albino mice. Anti-inflammatory activity was performed by the method for Winter *et al*.[21] on the group of six animals in each. Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub plantar region of the right hind paw of each rat, 1 h after the administration of test compounds and standard drug ibuprofen (70 mg/kg, p.o). One group was kept as control, received only 0.5% carboxymethyl cellulose solution. The right hind paw volume was measured before and after 4 h of carrageenan treatment by means of a plethysmometer. The percentage anti-inflammatory activity was calculated according to the following formula.

Anti-inflammatory activity, % $= (1-V_t/V_c) \times 100$

Where, V_t = Mean paw volume in rats tested with test compounds,

V_c = Mean increase in paw volume in control group of rats.

3.2. Analgesic activity

Analgesic activity was evaluated by tail immersion method[22] using Swiss albino mice (25-30 gm) of either sex selected by 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 ± 0.5ºC. The time in second for tail withdrawal from the water was taken as the reaction time with a cut of time of immersion, set at 10 seconds 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

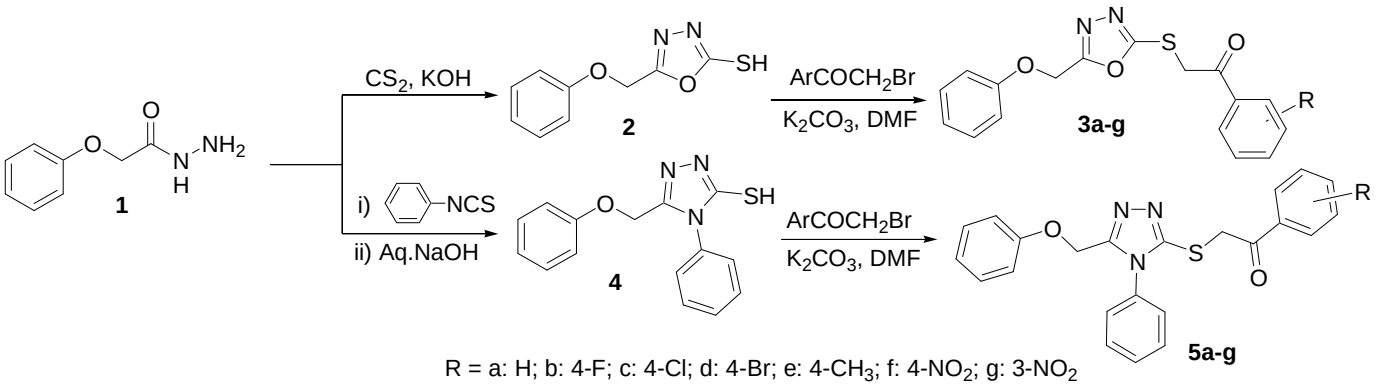
Acute ulcerogenesis test was performed according to the method of Cioli *et al*.[23] using wistar rats (180-200 gm) of either sex. The animals were divided into various group, each group consisting of 6 rats. All the rats were fasted for 24 h with free access to water. The control group of animals were administered, 0.5% CMC solution intraperitoneally. One group was administered with standard drug ibuprofen orally at 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 =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.

3.4. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa *et al*.[24]. After the evaluation of 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 95ºC for 60 minutes on boiling water bath then extracted with a mixture of *n*-butanol: pyridine (15:1, v/v; 5 mL) by shaking vigorously for 1 minute and kept in ice for 2 minutes. Organic layer of reaction mixture were centrifuged at 3000 rpm for 10 minutes and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nmol malondialdehyde/100 mg tissue.

3.5. Hepatotoxic studies

The study was carried out on wistar rats of either sex weighing 150-200gm. 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



Scheme1

solution of CMC in water), while group II and III received compound **3c** and **5d** 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 minutes and analyzed for various biochemical parameters.

Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by a reported method[25]. The alkaline phosphatase, total protein and total albumin were measured according to reported procedures[26,27]. All the data are recorded in **Table 3**.

3.6. Histopathological studies

Histopathological studies were carried out by reported method[28]. The rats were sacrificed under light ether anesthesia after 24 hr 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 haematoxylin and eosin, and then studied under an electron microscope (**Fig. 1-4**).

4. RESULTS AND DISCUSSION

4.1. Chemistry

Synthesis of titled compounds **3a-g** and **5a-g** were carried out by the reactions of 5-(phenoxymethyl)-1,3,4-oxadiazole-2-thiol **(2)** or 5-(phenoxymethyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol **(4)** with substituted phenacyl bromide in presence of anhydrous potassium carbonate in DMF (**Scheme 1**). The structures of the oxadiazoles and triazoles were confirmed by elemental analysis and spectral data. The ^{1}H NMR spectra of both oxadiazole **2** and triazole **4** showed a D_2O exchangeable broad singlet at δ 11.07 and 11.81 due to the presence SH proton. In the oxadiazole (**3a-g**) and triazole (**5a-g**) derivatives the broad singlet of SH proton disappeared instead a singlet of SCH_2 protons was obtained at δ4.41-4.93 and at δ 4.61-4.93 confirming the formation of compounds.

4.2. Biological activities

4.2.1. Antiinflammatory activity

The anti-inflammatory activity of the synthesized compounds **3a-g** and **5a-g** was evaluated by carrageenan induced paw edema method. 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 49.03% to 77.20% (**Table 2**), whereas standard drug ibuprofen showed 72.90% inhibition after 4 hr. The anti-inflammatory activity of triazole derivatives **3a-g** was in the range of 53.33% to 77.20% whereas in the oxadiazole derivatives, it was found to be in the range of 49.03% to 75.05%. It was also observed that the triazole derivatives having 4-chlorophenyl (**3b**) 4-flourophenyl (**3c**), and oxadiazole derivatives having 4-bromophenyl group (**5d**) showed higher activity than standard drug. Replacement of these by 4-methylphenyl, 4-nitrophenyl groups resulted in decrease of anti-inflammatory activity. In general, it was found that triazole derivatives were more active than oxadiazole derivatives.

4.2.2. Analgesic activity

Compounds showing more than 60% anti-inflammatory activity were further tested for their analgesic activity at the same oral dose. The compound showed analgesic activities ranging from 51.46% to 83.40% inhibition, where as standard drug showed 73.54% inhibition (**Table 2**). The triazole having 4-chlorophenyl (**3b**), 4-flourophenyl (**3c**) and oxadiazole having 4-bromophenyl groups (**5d**) showed high analgesic activities (78.79%, 83.40% and 75.51% respectively) in comparison to standard drug ibuprofen (73.54%). Thus it was observed that compounds showing high anti-inflammatory activity also showed high analgesic activity.

Table 1. Elemental analysis and physical constants of the synthesized compounds 3a-g and 5 a-g

Compd.	R	R_f Value	Yield (%)	M.P. (°C)	Molecular Formula	Found (cald.) (%)		
						C	H	N
3a	H	0.73	75	90-92	$C_{17}H_{14}N_2O_3S$ (326.37)	62.72(62.56)	4.15 (4.32)	8.34 (8.58)
3b	4-Cl	0.67	74	116-118	$C_{17}H_{13}ClN_2O_3S$ (360.81)	56.32 (56.59)	3.42 (3.63)	7.57(7.76)
3c	4-F	0.63	74	100-102	$C_{17}H_{13}FN_2O_3S$ (344.36)	59.05 (59.29)	3.58 (3.81)	7.95 (8.13)
3d	4-Br	0.70	63	98-100	$C_{17}H_{13}BrN_2O_3S$ (405.27)	50.14 (50.38)	3.01 (3.23)	6.60 (6.91)
3e	4-CH_3	0.76	68	112-114	$C_{18}H_{16}N_2O_3S$ (340.40)	63.37 (63.51)	4.91 (4.74)	8.01 (8.23)
3f	4-NO_2	0.62	79	108-110	$C_{17}H_{13}N_3O_5S$ (371.37)	54.73 (54.98)	3.23 (3.53)	11.03 (11.31)
3g	3-NO_2	0.66	82	78-80	$C_{17}H_{13}N_3O_5S$ (371.37)	54.75 (54.98)	3.27 (3.53)	11.08 (11.31)
5a	H	0.65	74	88-90	$C_{23}H_{19}N_3O_2S$ (401.48)	68.59 (68.81)	4.54 (4.77)	10.23 (10.47)
5b	4-Cl	0.70	75	142-144	$C_{23}H_{18}ClN_3O_2S$ (435.08)	63.08 (63.37)	4.05 (4.16)	9.48 (9.64)
5c	4-F	0.68	69	270-272	$C_{23}H_{18}FN_3O_2S$ (419.17)	65.99 (65.86)	4.11 (4.33)	10.23 (10.02)
5d	4-Br	0.67	63	121-123	$C_{23}H_{18}BrN_3O_2S$ (480.38)	57.24 (57.51)	3.59 (3.78)	8.54 (8.75)
5e	4-CH_3	0.73	65	104-106	$C_{24}H_{21}N_3O_2S$ (415.51)	69.07 (69.37)	5.22 (5.09)	9.85 (10.11)
5f	4-NO_2	0.71	67	218-220	$C_{23}H_{18}N_4O_4S$ (446.48)	61.62 (61.87)	3.89 (4.06)	12.33 (12.55)
5g	3-NO_2	0.72	72	236-238	$C_{23}H_{18}N_4O_4S$ (446.48)	61.67 (61.87)	3.87(4.06)	12.45 (12.55)

Table 2. Anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation effects of the synthesized compounds 3a-g and 5a-g

Compd.	Anti-inflammatory inhibition ± SEM#		% Analgesia Mean ± SEM	Ulcerogenic Activity (Severity index ± SEM)	nmol MDA content ± SEM / 100 mg tissue
	After 3 h	After 4 h			
Control	-	-	-	0.000 ± 0.000	3.41 ± 0.081
Ibuprofen	69.83 ± 0.76	72.90 ± 0.66	73.54 ± 0.64	0.583 ± 0.083	6.95 ± 0.067
3a	63.92 ± 0.71	67.53 ± 0.77[b]	67.46 ± 1.14[b]	0.750 ± 0.170[d]	5.48 ± 0.104[a]
3b	72.36 ± 0.60	74.40 ± 0.77[d]	78.79 ± 1.10[b]	0.250 ± 0.111[c]	4.51 ± 0.111[a]
3c	73.84 ± 0.62	77.20 ± 0.72[b]	83.40 ± 1.38[a]	0.166 ± 0.105[c]	3.60 ± 0.046[a]
3d	66.03 ± 0.83	69.03 ± 0.94[c]	61.85 ± 0.75[a]	-	-
3e	58.86 ± 0.71	63.05 ± 0.63[a]	57.64 ± 0.82[a]	-	-
3f	56.33 ± 0.54	61.29 ± 0.88[a]	51.46 ± 0.78[a]	-	-
3g	49.58 ± 0.76	53.33 ± 1.12[a]	-	-	-
5a	59.49 ± 0.76	63.22 ± 0.86[a]	61.87 ± 1.46[a]	-	-
5b	65.40 ± 0.62	69.67 ± 0.98[c]	72.68 ± 1.47	0.750 ± 0.111[d]	6.45 ± 0.117[c]
5c	68.77 ± 0.84	70.32 ± 0.57[c]	70.74 ± 0.89[c]	0.583 ± 0.083	5.71 ± 0.098[a]
5d	71.51 ± 0.54	75.05 ± 0.43[c]	75.51 ± 0.82[d]	0.250 ± 0.111[c]	3.86 ± 0.090[a]
5e	60.34 ± 0.63	63.23 ± 0.80[a]	64.49 ± 1.05[a]	-	-
5f	55.06 ± 0.84	58.92 ± 0.52[a]	-	-	-
5g	48.31 ± 0.89	49.03 ± 0.72[a]	-	-	-

#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

4.2.3. Acute ulcerogenic activity

The compounds that exhibited more than 65% anti-inflammatory and analgesic activity were further tested for their acute ulcerogenic activities. Compounds **3a**, **3b**, **3c**, **5b**, **5c** and **5d** were tested at an equimolar oral dose relative to 210 mg/kg ibuprofen. The tested compounds showed ulcerogenic activities ranging from 0.166 ± 0.105 to 0.750 ± 0.170 compared to standard drug ibuprofen which showed a high severity index of 0.583 ± 0.083. The maximum reduction in ulcerogenic potential (0.166 ± 0.105) was found in the compound **3c**, a triazole derivative having 4-flourophenyl group. The other compound showing good anti-inflammatory and analgesic activities also showed reduction in severity index. The compound **3a** and **5b** showed slightly higher ulcerogenic activities in comparison to standard drug ibuprofen (**Table 2**).

4.2.4. Lipid peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. This process proceeds by free radical chain reaction in which free radicals steal electrons from the lipid in the cell membrane and consequently damages 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. 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 MDA content, a byproduct of lipid peroxidation[29] . 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 MDA/100 mg of gastric mucosa tissue. Ibuprofen exhibited high lipid peroxidation 6.95 ± 0.067, whereas control group showed 3.41 ± 0.08. It was found that all the compound 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.

4.2.5. Hepatotoxic studies

Triazole derivative **3c** and oxadiazole derivative **5d** 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 showed decrease in their concentration as compared to standard drug ibuprofen, except for compound **5d** whose alkaline phosphate and SGPT level was found to be more. The histopathological studies of the liver sample of standard drug ibuprofen showed evident centrizonal sinusoidal dilation in comparison to control. Whereas compound **3c** and **5d** treated liver samples do not show any significant pathological changes in comparison to control group (**Fig. 1-4**) thus indicating their safety profile with respect to standard drug.

Table 3. Enzyme and protein estimation of selected compounds 3c and 5d

Treatment	Alkaline Phosphatase ± SEM[#]	SGOT ± SEM[#]	SGPT ± SEM[#]	Total Protein (g/100ml) ± SEM[#]	Albumin (g/100ml) ± SEM[#]	Globulin (g/100ml) ± SEM[#]
Control	32.50 ± 0.45	149.76 ± 0.81	31.54 ± 1.46	1.69 ± 0.012	1.59 ± 0.017	0.09 ± 0.007
Ibuprofen	35.06 ± 0.47	155.91 ± 1.49	44.84 ± 1.17	1.88 ± 0.011	1.70 ± 0.014	0.17 ± 0.004
3c	32.11 ± 0.43[b]	143.61 ± 1.31	36.30 ± 1.33[b]	1.76 ± 0.010[a]	1.66 ± 0.013[d]	0.10 ± 0.012[b]
5d	38.01 ± 0.36[b]	148.83 ± 1.23[c]	51.38 ± 0.77[b]	1.66 ± 0.019[a]	1.53 ± 0.015[a]	0.13 ± 0.016[c]

[#]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

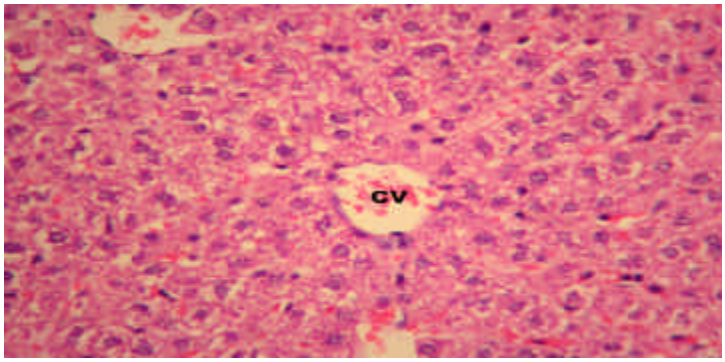


Fig.1. Control: Normal arrangement of hepatocytes in the centrizonal area (400X)

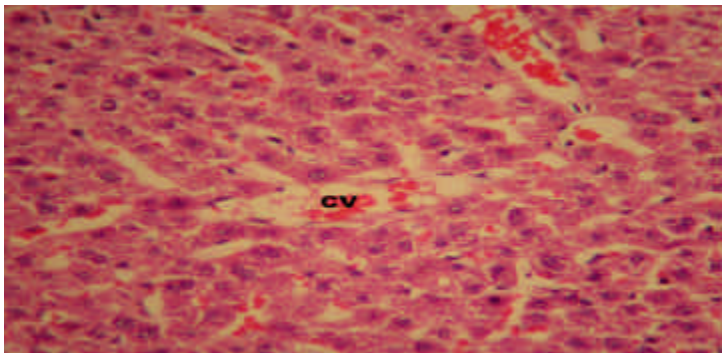


Fig.2. Ibuprofen: Showing evident centrizonal sinusoidal dilatation (400X)

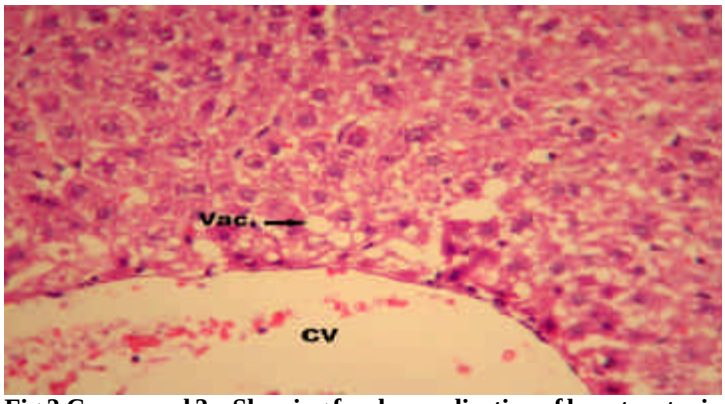


Fig.3.Compound 3c: Showing focal vacuolization of hepatocytes in the centrizonal area. No sinusoidal dilatation is seen

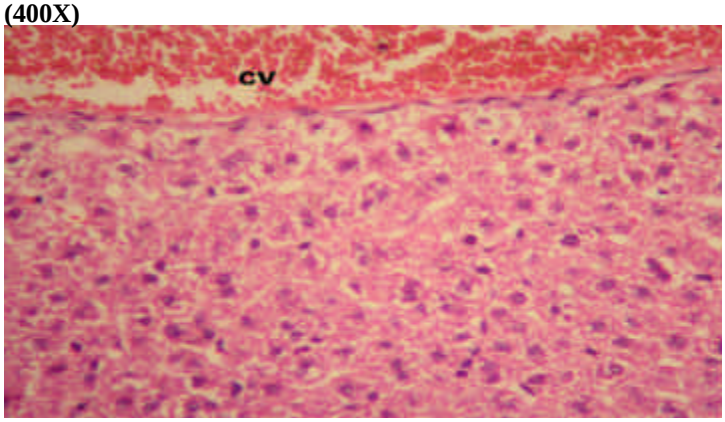


Fig.4. Compound 5d: No sinusoidal dilatation is seen.

Fig. 1-4: Histopthalogical studies of liver

CONCLUSIONS

Various 1,2,4-triazole and 1,3,4-oxadiazole derivatives of phenoxyacetic acid were synthesized and screened for anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities. It was observed that three compounds **3b**, **3c** and **5d** were found to have anti-inflammatory and analgesic activities more than the standard reference drug ibuprofen. These compounds were also tested for their 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 **3c**, 1-(4-fluorophenyl)-2-(5-(phenoxymethyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)ethanone and **5d**, 1-(4-bromophenyl)-2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylthio)ethanone emerged as the lead compounds, without any hepatotoxicity**.** Thus the series provided new opportunities for possible modification of pharmacophoric requirements and future exploitation.

Declaration of interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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