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ORIGINAL ARTICLE

Synthesis, anti-inflammatory and analgesic activity of 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives

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1,2,4-Triazolyl thioacetic acid;
Anti-inflammatory activity;
Analgesic activity

Abstract The title compounds **3a–l** have been synthesized by the reaction of thiocarbohydrazide with substituted phenoxy acetic acid to obtained substituted 1,2,4-triazoles (**1**). Compound **1** was treated with various substituted aromatic aldehydes which results in 4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-2*H*-1,2,4-triazol-3(4*H*)-thiones **(2a–g)**, further **2a–g** is converted to 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid **(3a–l)** derivatives by the reaction with chloroacetic acid. All the newly synthesized compounds were evaluated for in vivo anti-inflammatory and analgesic activities. Among the series 2-[4-(2,4-dichlorobenzylideneamino)-5-(phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid **(3d)**, 2-[4-(4-dichlorobenzylideneamino)-5-(phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid **(3e)**, 2-[4-(2,4-dichlorobenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-4*H*-1,2,4-triazol-3-yl thio]

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acetic acid **(3j)** and 2-[5-[(2,4-dichlorophenoxy)methyl)]-4-(4-chlorobenzylideneamino)-4*H*-1,2,4-triazol-3-yl thio] acetic acid **(3k)** showed significant anti-inflammatory activity with $P < 0.001$ (63.4%, 62.0%, 64.1% and 62.5% edema inhibition, respectively), as compared to the standard drug diclofenac (67.0%) after third hour respectively and also compounds **3j**, **3k** exhibited significant analgesic activity with $P < 0.001$ (55.9% and 54.9% protection, respectively) and less ulcerogenic activity as compared with standard drug aspirin (57.8%).

1. Introduction

Inflammation is a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult. Cyclooxygenase (COX) and 5-lipoxygenase (5-LO) are enzymes which catalyze the rate-limiting steps in the biosynthesis of prostaglandins and leukotrienes from arachidonic acid. A large number of non-steroidal anti-inflammatory drugs (NSAIDs) are available clinically to treat inflammatory disorders. The most important mechanism of anti-inflammatory action of NSAIDs is considered to be primarily by inhibition of prostaglandin synthesis. The principal side effects associated with chronic use of non selective NSAIDs are gastrointestinal irritation, bleeding and formation of life threatening gastrointestinal ulcer. In addition, there is evidence to suggest that leukotriene promotes gastric ulceration, which limits the therapeutic utilization of these drugs (Lombardino, 1985; Mullican et al., 1993; Osiri and Moreland, 1999). Therefore, there is a need to develop new compounds. Literature survey revealed that Schiff bases of 1,2,4-triazoles bearing aryl groups or heterocyclic residues (Olcay et al., 2006) which possess excellent biological activities, viz. antibacterial (Francis et al., 2008), antifungal (Desai et al., 1984), antitubercular (Iraj et al., 2008; Kucukguzel et al., 2008), anticancer (Mahendra et al., 2007; Upadhaya et al., 2004), anti-inflammatory (Fahmy and Soliman, 2001; Latifeh et al., 2006; Yasemin et al., 2007) and anticonvulsant activities (Almarirad, 2004).

In view of these facts and in continuation of our research program on synthesis and pharmacological importance of various heterocyclic Schiff bases (Ronad et al., 2008a, 2008b; Maddi et al., 1992; Hunashal et al., 2010), now we are reporting the synthesis, anti-inflammatory and analgesic activity of 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives. Compounds which showed significant activity in acute anti-inflammatory model were further screened for analgesic and ulcerogenic activity.

2. Experimental

2.1. General considerations

All research chemicals were purchased from Acros organics (NY, USA), Sigma–Aldrich (St. Loius, Missouri, USA), Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Solvents except laboratory reagent grade were dried and purified according to the literature whenever necessary. Purification of title compounds were carried out by flash chromatography from Biotage Isolera ST-1 (Darmstadt, Germany). Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany).

Melting points of synthesized compounds were determined in Thermonik melting point apparatus (Chennai, India) and are uncorrected. The IR spectra were recorded on Thermo Nicolet FTIR-200 Spectrometer (Madison, WI, USA), using KBr pellets. The ^{1}H NMR and ^{13}C NMR were recorded on Bruker AVANCE II 400 (Bruker, Rheinstetten/Karlsruhe, Germany) in $CDCl_3$/DMSO-d_6 as solvent. Chemical shifts are reported in δ ppm units with respect to TMS. The mass spectra were recorded using GCMS-QP 2010 (Shimaduzu, Japan). The anti-inflammatory activity was carried out using digital plethysmometer (Ugo-Basile, Italy). All the animal experiments were approved by Institutional Animal Ethical Committee (IAEC).

2.2. Synthesis

2.2.1. General method for synthesis of 3-(substituted phenoxymethyl)-4-amino-5-mercapto 1,2,4-triazole (*1*)

An intimate mixture of thiocarbohydrazide (1.06 g, 0.01 mol) and substituted phenoxy acetic acid (0.01 mol) was heated in an oil bath at 175–180 °C for 2 h. The fused mass was triturated with hot water, filtered and washed with sodium bicarbonate. The crude product was recrystallized from methanol.

2.2.1.1. 3-(Phenoxymethyl)-4-amino-5-mercapto 1,2,4-triazole (*1a*).

$C_9H_{10}N_4OS$, mp 186–187 °C, Yield is 69.0%.

IR (KBr, cm^{-1}): 3215.4 (NH_2), 3209.4 (NH), 2363.2 (SH), 1628.3, 1589.2 (C═N).

^{1}H NMR (DMSO-d_6, δ ppm): 13.6 (br, 1H, SH), 7.6–6.9 (m, 5H, Ar-H), 5.1 (s, 2H, OCH_2) and 3.3 (s, 2H, NH_2).

MS (ESI) (M^+): m/z 222.0; calcd. 222.2.

2.2.1.2. 3-(2,4-Dichloro phenoxymethyl)-4-amino-5-mercapto 1,2,4-triazole (*1b*).

The compound was further purified by flash chromatography using dimethyl formamide (DMF) and methanol as mobile phase.

$C_9H_8Cl_2N_4OS$, mp 130–132 °C, Yield is 62.6%.

IR (KBr, cm^{-1}): 3457.4 (NH_2), 3241.1 (NH), 1620.6 (C═N), 1282.1 (C═S), 781.5 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6 δppm): 7.8–6.8 (m, 3H, Ar-H and NH), 4.5 (s, 2H, OCH_2), 2.1 (s, 2H, NH_2).

2.2.2. General method for synthesis of 4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-2H-1,2,4-triazole-3(4H)-thione (*2a–g*)

A mixture of *N*′-4-amino-5-(substituted phenoxymethyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (**1**) (0.01 mol) and the corresponding various aromatic aldehydes (0.01 mol) in ethanol (25 ml) was treated with concentrated HCl (0.5 ml) and refluxed for 2 h. The reaction mixture was cooled to room temperature

and the separated crystalline compound is filtered and recrystallized from ethanol to give **2a–g**.

*2.2.2.1. 4-(4-Methylbenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazolo-3-thiol (**2a**).* IR (KBr, cm^{-1}): 3054.3 (Ar-H), 2916.8 (OCH_2), 2755.2 (SH), 1594.0 (HC═N), 1534.5 (C═N).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.5 (br, 1H, SH), 10.2 (s, 1H, HC═N), 7.2–6.9 (m, 9H, Ar-H), 5.2 (s, 2H, OCH_2), 2.4 (s, 3H, CH_3).

MS (ESI) (M + 1): 325.3; calcd. 324.4.

^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 163.4 (triazole-C_3), 146.7 (triazole-C_5), Ar C [158.9 (C_1), 129.3 (C_3 and C_5), 123.3 (C_6), 122.2 (C_4), 115.3 (C_2)], 155.9 (–CH═N), Ar C (benzylidene) [139.8 (C_4), 131.2 (C_3 and C_5), 130.2 (C_2 and C_6), 129.9 (C_1)], 68.9 (OCH_2), 61.2 (OCH_3), 24.5 (CH_3).

*2.2.2.2. 4-(4-Nitrobenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-2H-1,2,4-triazolo-3(4H)-thione (**2b**).* IR (KBr, cm^{-1}): 3050.4 (Ar C–H), 2461.2 (SH), 1592.3 (HC═N), 1545.7 (NO_2), 1531.3 (C═N), 1285.7 (C═S), 825.3 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.8 (s, 1H, SH), 8.9 (s, 1H, HC═N), 7.4–6.8 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 152.8 (triazole-C_3), 176.9 (triazole-C_5), Ar C [152.1 (C_1), 130.3 (C_5), 128.3 (C_3 and C_4), 124.2 (C_6), 116.3 (C_2)], 142.3 (HC═N), Ar C (benzylidene) [139.6 (C_1), 138.6 (C_2 and C_4), 127.6 (C_6), 120.6 (C_3 and C_5)], 64.8 (OCH_2).

MS (ESI): *m*/*z* 424.2; calcd. 424.0.

*2.2.2.3. 4-(4-Methoxyoxybenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-2H-1,2,4-triazolo-3(4H)-thione (**2c**).* IR (KBr, cm^{-1}): 3061.1 (Ar C–H), 2924.3 (OCH_2), 2547.2 (SH), 1606.5 (HC═N), 1537.3 (C═N), 824.5 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.6 (s, 1H, SH), 8.1 (s, 1H, N═CH), 7.7–6.9 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 3.3 (s, 3H, OCH_3).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 154.8 (triazole-C_3), 182.8 (triazole-C_5), Ar C [153.1 (C_1), 131.8 (C_5), 128.7 (C_3 and C_4), 123.3 (C_6), 116.9 (C_2)], 142.9 (HC═N), Ar C (benzylidene) [161.7 (C_4), 130.5 (C_2 and C_6), 125.3 (C_1), 114.7 (C_3 and C_5)], 68.9 (OCH_2), 60.2 (OCH_3).

*2.2.2.4. 4-(2-Hydroxybenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-2H-1,2,4-triazolo-3(4H)-thione (**2d**).* IR (KBr, cm^{-1}): 3093.8 (OH), 3063.3 (Ar C–H), 2926.3 (OCH_2), 2547.8 (SH), 1596.3 (HC═N), 1532.5 (C═N), 826.2 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.6 (br, 1H, SH), 10.2 (s, 1H, HC═N), 8.3 (s,1H, OH), 7.3–6.9 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2).

MS (ESI) (M + 1): 396.2; calcd. 395.2.

*2.2.2.5. 4-(2,4-Dichlorobenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-2H-1,2,4-triazolo-3(4H)-thione (**2e**).* IR (KBr, cm^{-1}): 3055.5 (Ar C–H), 2925.4 (OCH_2), 2554.2 (SH), 1610.4 (HC═N), 1532.7 (C═N), 834.2 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.3 (s, 1H, SH), 8.4 (s, 1H, HC═N), 7.3–6.9 (m, 6H, Ar-H), 5.2 (s, 2H, OCH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 154.4 (triazole-C_3), 180.7 (triazole-C_5), Ar C [152.4 (C_1), 131.3 (C_5), 128.2 (C_3 and C_4), 124.7 (C_6), 116.8 (C_2)], 142.7 (HC═N), Ar C (benzylidene) [129.6 (C_1), 136.4 (C_2 and C_4), 133.0 (C_6), 128.6 (C_3 and C_5)], 68.9 (OCH_2).

MS (ESI): *m*/*z* 448.1; calcd. 448.0.

*2.2.2.6. 4-(4-Chlorobenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-2H-1,2,4-triazolo-3(4H)-thione (**2f**).* IR (KBr, cm^{-1}): 3039.2 (Ar C–H), 2916.4 (OCH_2), 2543.6 (SH), 1608.7 (HC═N), 1533.8 (C═N), 826.7 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.9 (s, 1H, SH), 8.2 (s, 1H, HC═N), 7.3–6.9 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2).

MS (ESI) (M + 1): *m*/*z* 413.7; calcd. 412.0.

*2.2.2.7. 4-(4-Methylbenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-2H-1,2,4-triazolo-3(4H)-thione (**2g**).* IR (KBr, cm^{-1}): 3039.8 (Ar-H), 2927.4 (OCH_2), 2564.6 (SH), 1604.5 (C═N), 825.3 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.3 (br, 1H, SH), 9.2 (s, 1H, HC═N), 7.3–6.8 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 2.5 (s, 3H, CH_3).

*2.2.3. General method for synthesis of 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3a–l**)*

Equimolar proportions of **2a–g** and chloroacetic acid were dissolved in ethanol containing 3–4 drops of pyridine and refluxed for 2–3 h, on pouring the reaction mixture into cold water a solid **3a–l** was separated which was filtered, washed with water and recrystallized from ethanol to give **3a–l**.

*2.2.3.1. 2-[4-(4-Nitrobenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3a**).* IR (KBr, cm^{-1}): 3200.0 (OH), 3068.9 (Ar C–H), 2928.4 (OCH_2), 2374.2 (CH_2), 1715.6 (C═O), 1590.1 (C═N), 1580.4 (NO_2).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.8 (s, 1H, OH), 10.8 (s, 1H, HC═N), 8.2–6.9 (m, 9H, Ar-H), 5.1 (s, 2H, OCH_2), 1.1 (s, 2H, CH_2).

^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 178.0 (C═O), 162.8 (triazole-C_3), 157.9 (triazole-C_5), Ar C [156.1 (C_1), 129.3 (C_3 and C_5), 124.0 (C_4 and C_6), 115.1 (C_2)], 162.8 (HC═N), Ar C (benzylidene) [149.6 (C_1), 138.6 (C_4), 129.6 (C_3 and C_5), 127.6 (C_2 and C_6)], 59.8 (OCH_2), 29.5 (S–CH_2).

MS (ESI): *m*/*z* 413.3; calcd. 413.4.

*2.2.3.2. 2-[4-(4-Methoxybenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3b**).* IR (KBr, cm^{-1}): 3117.6 (OH), 3056.3 (Ar C–H), 2952.6 (OCH_2), 2755.3 (CH_2), 1733.4 (C═O), 1598.5 (C═N).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 13.7 (s, 1H, OH), 10.0 (s, 1H, N═CH), 7.7–6.8 (m, 9H, Ar-H), 5.2 (s, 2H, OCH_2), 3.7 (s, 3H, OCH_3), 1.1 (s, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 169.3 (C═O), 162.0 (triazole-C_3), 146.6 (triazole-C_5), Ar C [157.3 (C_1), 129.1 (C_3 and C_5), 114.6 (C_4), 113.9 (C_2 and C_6)], 162.6 (–CH═N), Ar C (benzylidene) [130.6 (C_4), 130.1 (C_1), 124.4 (C_2 and C_6), 121.3 (C_3 and C_5)], 59.3 (OCH_2), 55.0 (OCH_3), 38.9 (S–CH_2).

*2.2.3.3. 2-[4-(4-Methylybenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3c**).* IR (KBr, cm^{-1}): 3200.2 (OH), 3055.2 (Ar-H), 2916.5 (OCH_2), 2755.2 (CH_2), 1734.0 (C═O), 1594.7 (C═N).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 13.5 (br, 2H, 2-OH), 10.2 (br, 1H, HC═N), 7.5–6.8 (m, 9H, Ar-H), 5.1 (s, 2H, OCH_2), 1.2 (s, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 169.3 (C═O), 157.3 (triazole-C_3), 136.9 (triazole-C_5), Ar C [134.4 (C_1), 129.6 (C_3 and C_5), 115.9 (C_2 and C_6), 117.3 (C_4)], 157.3 (HC═N), Ar C (benzylidene) [159.4 (C_2), 132.1 (C_5), 122.0 (C_1 and C_3), 119.9 (C_4 and C_6)], 59.9 (OCH_2), 39.4 (S–CH_2).

MS (ESI) (M−2): *m*/*z* 382.4; calcd. 384.4.

*2.2.3.4. 2-[4-(2,4-Dichlorobenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3d**).* IR (KBr, cm^{-1}): 3200.0 (OH), 3051.3 (Ar C–H), 2920.4 (OCH_2), 2373.5 (CH_2), 1735.1 (C═O), 1589.5 (C═N).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.9 (s, 1H, OH), 11.0 (s, 1H, HC═N), 7.9–6.9 (m, 9H, Ar-H), 5.2 (s, 2H, OCH_2), 1.2 (s, 2H, CH_2).

*2.2.3.5. 2-[4-(4-Chlorobenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3e**).* IR (KBr, cm^{-1}): 3200.2 (OH), 3050.5 (Ar C–H), 2914.8 (OCH_2), 2373.8 (CH_2), 1716.4 (C═O), 1591.0 (C═N).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 13.8 (s, 1H, OH), 10.5 (s, 1H, HC═N), 7.7–6.9 (m, 9H, Ar-H), 5.2 (s, 2H, OCH_2), 2.5 (br, 2H, CH_2).

*2.2.3.6. 2-[4-(2-Hydroxybenzylideneamino)-5-(phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid (**3f**).* IR (KBr, cm^{-1}): 3098.8 (OH), 3051.2 (Ar C–H), 2915.9 (OCH_2), 2751.6 (CH_2), 1734.9 (C═O), 1595.4 (C═N).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.7 (s, 1H, OH), 10.1 (s, 1H, HC═N), 7.6–6.8 (m, 9H, Ar-H), 5.1 (s, 2H, OCH_2), 2.3 (s, 3H, CH_3), 1.1 (s, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 162.5 (C═O), 162.5 (triazole-C_3), 143.1 (triazole-C_5), Ar C [157.9 (C_1), 129.6 (C_3 and C_5), 121.8 (C_4), 115.1 (C_2 and C_6)], 147.2 (–CH═N), Ar C (benzylidene) [129.7 (C_4), 129.5 (C_2 and C_6), 129.1 (C_1), 128.7 (C_3 and C_5)], 59.8 (OCH_2), 39.4 (S–CH_2), 21.7 (CH_3).

MS (ESI) (M−1): *m*/*z* 381.4; calcd. 382.4.

*2.2.3.7. 2-{5-[(2,4-Dichlorophenoxy)methyl]-4-(4-nitrobenzylideneamino)-4H-1,2,4-triazol-3-yl thio} acetic acid (**3g**).* IR (KBr, cm^{-1}): 3210.2 (OH), 3060.4 (Ar C–H), 2920.7 (OCH_2), 2360.3 (CH_2), 1710.6 (C═O), 1600.3 (C═N), 1545.8 (NO_2), 768.7 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.1 (br, 1H, OH), 10.2 (s, 1H, HC═N), 8.2–6.5 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 2.1 (s, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 173.6 (C═O), 162.8 (triazole-C_3), 146.5 (triazole-C_5), Ar C [152.3 (C_1), 131.8 (C_2), 130.6 (C_3), 128.3 (C_4 and C_5), 117.4 (C_6)], 157.8 (HC═N), Ar C (benzylidene) [149.8 (C_4), 139.6 (C_1), 129.4 (C_2 and C_6), 127.6 (C_3 and C_5)], 62.3 (OCH_2), 33.7 (S–CH_2).

MS (ESI): *m*/*z* 482.2; calcd. 482.4.

*2.2.3.8. 2-{5-[(2,4-Dichlorophenoxy)methyl]-4-(4-methoxybenzylideneamino)-4H-1,2,4-triazol-3-yl thio} acetic acid (**3h**).* IR (KBr, cm^{-1}): 3200.2 (OH), 3060.2 (Ar C–H), 2915.6 (OCH_2), 2350.2 (CH_2), 1725.6 (C═O), 1610.3 (C═N), 872.4 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.2 (s, 1H, OH), 10.1 (s, 1H, HC═N), 7.5–6.6 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 3.7 (s, 3H, OCH_3), 2.5 (s, 2H, CH_2).

MS (ESI): *m*/*z* 467.2 calcd. 467.3

*2.2.3.9. 2-{5-[(2,4-Dichlorophenoxy)methyl]-4-(2-hydroxybenzylideneamino)-4H-1,2,4-triazol-3-yl thio} acetic acid (**3i**).* IR (KBr, cm^{-1}): 3208.6 (OH), 3057.0 (Ar C–H), 2903.4 (OCH_2), 2350.7 (CH_2), 1720.3 (C═O), 1610.3 (C═N), 872.4 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.9 (s, 2H, OH), 10.4 (s, 1H, N═CH), 7.2–6.6 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 2.8 (s, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 173.8 (C═O), 162.7 (triazole-C_3), 147.3 (triazole-C_5), Ar C [152.7 (C_1), 131.3 (C_2), 130.5 (C_3), 128.1 (C_4 and C_5), 116.9 (C_6)], 156.9 (HC═N), Ar C (benzylidene) [159.8 (C_2), 132.8 (C_4), 130.2 (C_6), 118.6 (C_1), 117.3 (C_3 and C_5)], 62.9 (OCH_2), 34.6 (S–CH_2).

MS (ESI) (M−1): *m*/*z* 452.3; calcd. 453.3.

*2.2.3.10. 2-{4-(2,4-Dichlorobenzylideneamino)-5-[(2,4-dichlorophenoxy)methyl]-4H-1,2,4-triazol-3-yl thio} acetic acid (**3j**).* IR (KBr, cm^{-1}): 3110.4 (OH), 3065.6 (Ar C–H), 2930.3 (OCH_2), 2363.6 (CH_2), 1720.3 (C═O), 1610.5 (C═N), 795.3 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.1 (s, 1H, OH), 10.3 (s, 1H, N═CH), 7.2–6.5 (m, 6H, Ar-H), 5.2 (s, 2H, OCH_2), 2.7 (s, 2H, CH_2).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 173.0 (C═O), 162.5 (triazole-C_3), 146.6 (triazole-C_5), Ar C [151.9 (C_1), 131.4 (C_2), 131.7 (C_3), 127.9 (C_4 and C_5), 117.9 (C_6)], 158.2 (HC═N), Ar C (benzylidene) [139.3 (C_4), 136.42(C_2), 132.2 (C_6), 131.3 (C_1), 129.4 (C_3 and C_5)], 60.4 (OCH_2), 34.3 (S–CH_2).

MS (ESI): *m*/*z* 506.4; calcd. 506.3.

*2.2.3.11. 2-{5-[(2,4-Dichlorophenoxy)methyl]-4-(4-chlorobenzylideneamino)-4H-1,2,4-triazol-3-yl thio} acetic acid (**3k**).* IR (KBr, cm^{-1}): 3210.2 (OH), 3060.4 (Ar C–H), 2920.7 (OCH_2), 2360.3 (CH_2), 1712.3 (C═O), 1600.3 (C═N), 856 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.2 (br, 1H, OH), 10.2 (s, 1H, HC═N), 7.6–6.5 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 2.8 (s, 2H, CH_2).

MS (ESI) (M+1): *m*/*z* 472.8; calcd. 471.7.

*2.2.3.12. 2-{5-[(2,4-Dichlorophenoxy)methyl]-4-(4-methylbenzylideneamino)-4H-1,2,4-triazol-3-yl thio} acetic acid (**3l**).* IR (KBr, cm^{-1}): 3200.8 (OH), 3063.0 (Ar C–H), 2914.4 (OCH_2), 2351.3 (CH_2), 1720.3 (C═O), 1613.5 (C═N), 872.4 (C–Cl).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.7 (s, 1H, OH), 10.4 (s, 1H, N═CH), 7.5–6.6 (m, 7H, Ar-H), 5.2 (s, 2H, OCH_2), 2.9 (s, 2H, CH_2), 2.3 (s, 3H, CH_3).

^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 172.8 (C═O), 162.7 (triazole-C_3), 147.3 (triazole-C_5), Ar C [152.2 (C_1), 123.8 (C_2), 131.0 (C_3), 129.0 (C_4 and C_5), 117.2 (C_6)], 157.5 (HC═N), Ar C (benzylidene) [139.9 (C_4), 130.2 (C_2 and C_6), 129.5 (C_1), 117.3 (C_3 and C_5)], 62.3 (OCH_2), 34.8 (S–CH_2), 24.4 (CH_3).

MS (ESI) (M+1): *m*/*z* 452.3; calcd. 451.3.

3. Pharmacological screening

3.1. Animals

Albino mice of either sex weighing between 20 and 25 g were used for acute toxicity and analgesic activity studies. Healthy male albino adult rats weighing between 150 and 230 g were used for anti-inflammatory and ulcerogenic activities. Animal ethical clearance was obtained from Ethics Committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore, India (115/1999/CPCSEA). Animals were procured from K.S. Hegde Medical Academy, Deralakatte, Mangalore, India, and housed individually in polypropylene cages, maintained under standard conditions of alternating 12-h light-and-dark cycles at a constant temperature (25 ± 2 °C and 35–60% relative humidity). Animals were fed with standard rat pellet diet, (Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

3.2. Acute toxicity

The acute toxicity test was carried out according to OECD guidelines (OECD, 2000) to establish the effective dose of the test compounds after obtaining ethical clearance from Ethics Committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore, India. Albino mice of either sex weighing between 20 and 25 g were divided into six groups of 6 animals each. Animals were starved for 24 h with water ad libitum prior to test. On the day of the experiment, animals were administered with different compounds to different groups in an increasing dose of 10, 20, 100, 200, 1000 and 2000 mg/kg body weight orally. The animals were then observed continuously for 3 h for general behavioral, neurological, autonomic profiles and then every 30 min for next 3 h and finally for next 24 h or till death.

From preliminary toxicity studies, it was observed that animals were found to be safe up to a maximum dose of 2000 mg/kg body weight. But there were few changes in the behavioral response like alertness, touch response, and restlessness. Therefore, 1/10th of the maximum tolerated dose, i.e. 200 mg/kg body weight (b.w.) was chosen for the studies.

3.3. In vivo anti-inflammatory activity

In vivo anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema assay model (Winter et al., 1962) for the compounds listed in Table 1. Male albino rats (170–220 g) of either sex were used. The animals were divided randomly into fourteen groups of six each. They were starved overnight with water ad libitum prior to the day of experiment. Control group received 1 ml of 0.5% sodium carboxymethyl cellulose (sodium CMC), standard group received 13.5 mg/kg diclofenac and test groups received 200 mg/kg of synthesized compounds **3a–l** orally. One hour later; a sub plantar injection of 0.05 ml of 1% solution of carrageenan (Sigma) in sterile distilled water was administered to the left hind footpad of each animal. The paw edema volume was measured with a digital plethysmometer at 0, 1, 2, 3, 4, 5 and 6 h after carrageenan injection. Paw edema volume was compared with vehicle control group and percent reduction was calculated as

$$\% \text{ edema inhibition} = 1 - \mathrm{Vt}/\mathrm{Vc} \times 100$$

where, Vt and Vc were edema volume in treated and control groups, respectively.

3.4. Analgesic activity (Koster et al., 1959)

Twenty-four hours prior to actual testing a large number of albino mice of either sex weighing between 20 and 25 g received intraperitoneally 10 mg/kg 0.6% glacial acetic acid. Animals were observed for writhing movements. Only those showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received compounds half an hour prior to glacial acetic acid challenge. Compounds **3d, 3e, 3j** and **3k** were orally administered at a dose of 200 mg/kg as a suspension in 0.5% sodium CMC. Standard drug used was aspirin at a dose of 30 mg/kg as a suspension in 0.5% sodium CMC. Each mouse was then observed for the total number of stretching episodes or writhings for 15 min following glacial acetic acid injection. The mean value for each group was calculated.

3.5. Gastric ulceration (Alich et al., 1983)

Rats of either sex were fasted for 24 h. Test compounds and positive control Aspirin were administered at a dose of 250 mg/kg p.o. in a group of six rats. Similarly the negative controls were treated with 10 ml/kg 0.5% sodium CMC. Four hours after treatment, the rats were sacrificed; the stomach was removed, opened along the lesser curvature and observed for gastric lesions on the mucosa. The lesion index for each group was determined according to a previously reported method, by counting the number of lesions (x) in each of five size classes (y). The classes were defined as $y = 1$ (pinpoint lesion), $y = 2$ (lesions < 1 mm diameter), $y = 3$ (lesions 1–2 mm diameter), $y = 4$ (lesions 2–4 mm diameter) and $y = 5$ (lesions > 4 mm diameter). The lesions index was calculated using the formula $\sum_{i=1}^{5} x_i y_i$.

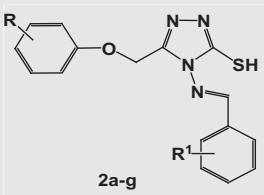
3.6. Statistical analysis

Data were presented as arithmetic mean ± SEM. Statistical analysis was performed by one way variance (ANOVA) followed by Dunnett's test. "p" value of less than 0.05 was considered as statistically significant using PARAMETRIC STASTICS, IBM PC version 1.01 by Londan soft ware INC-1985.

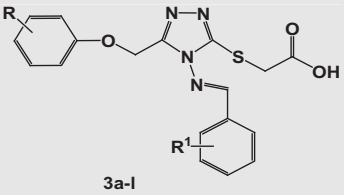
4. Results and discussion

A novel series of 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives **(3a–l)** were synthesized as shown in Scheme 1 by the reaction between thiocarbohydrazide and substituted phenoxy acetic acid, which on fusion will yield 3-(substituted phenoxymethyl)-4-amino-5-mercapto-1,2,4-triazoles (**1**). Compound **1** was treated with various substituted aromatic aldehydes in ethanol medium in presence concentrated hydrochloric acid at 90 °C for 2 h. It resulted in **2a–g**, which converted to compounds **3a–l** by the reaction with chloroacetic acid in absolute ethanol medium in presence of drop of pyridine as catalyst and refluxed for 2–3 h. The purity of the compounds was confirmed by TLC using silica gel G as

Table 1 Characterization data of 4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-2*H*-1,2,4-triazole-3(*4H*)-thione **(2a-g)** and 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-*4H*-1,2,4-triazol-3-yl thio] acetic acid derivatives **(3a-l).**



2a-g



3a-l

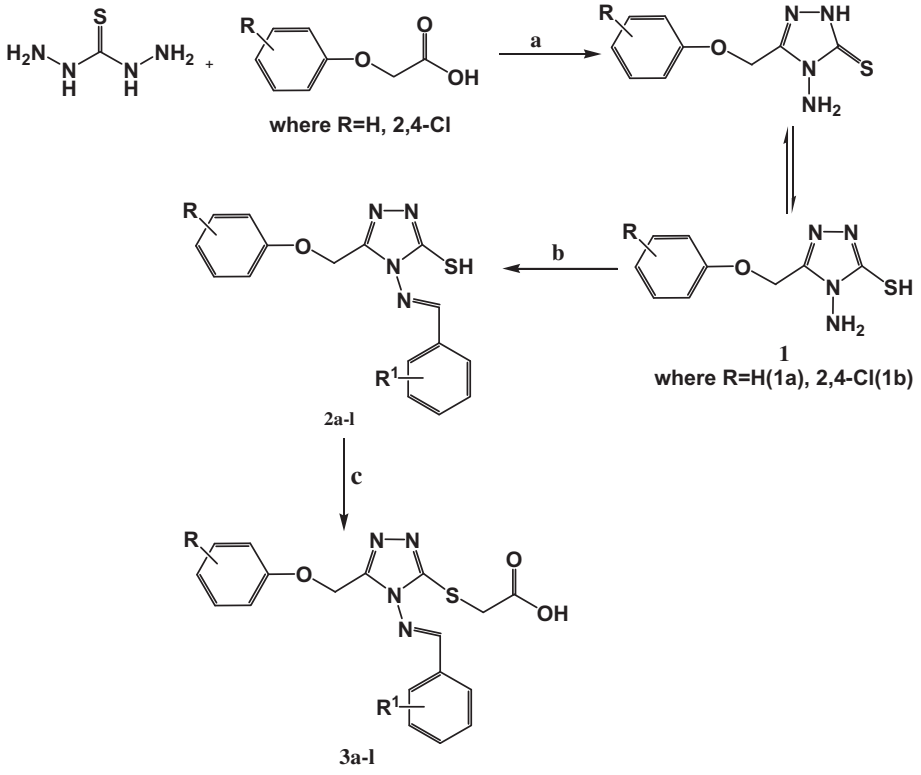
Compd. No.	R	R^1	Yield (%)	Melting Point* (^{0}C)	R_f Value#	Mol. Formula	Analysis (%) Found (Calculated)		
							C	H	N
2a	H	4-CH_3	67.0	202-204	0.36	$C_{17}H_{16}N_4OS$	62.92 (62.94)	4.95 (4.97)	17.29 (17.27)
2b	2,4-Cl	4-NO_2	68.2	186-188	0.42	$C_{16}H_{11}Cl_2N_5O_3S$	45.33 (45.30)	2.65 (2.61)	16.53 (16.51)
2c	2,4-Cl	4-OCH_3	65.0	189-191	0.36	$C_{17}H_{14}Cl_2N_4O_2S$	49.88 (49.89)	3.44 (3.45)	13.67 (13.69)
2d	2,4-Cl	2-OH	65.0	202-204	0.28	$C_{16}H_{12}Cl_2N_4O_2S$	48.63 (48.62)	3.07 (3.06)	14.16 (14.17)
2e	2,4-Cl	2,4-Cl	61.0	180-182	0.24	$C_{16}H_{10}Cl_4N_4OS$	42.85 (42.88)	2.23 (2.25)	12.52 (12.50)
2f	2,4-Cl	4-Cl	66.8	198-200	0.16	$C_{16}H_{11}Cl_3N_4OS$	46.44 (46.45)	2.65 (2.68)	13.53 (13.54)
2g	2,4-Cl	4-CH_3	63.0	170-172	0.22	$C_{17}H_{14}Cl_2N_4OS$	51.91 (51.92)	3.57 (3.59)	14.21 (14.25)
3a	H	4-NO_2	63.0	148-150	0.29	$C_{18}H_{15}N_5O_5S$	52.31 (52.30)	3.65 (3.66)	16.92 (16.94)
3b	H	4-OCH_3	69.0	199-201	0.46	$C_{19}H_{18}N_4O_4S$	57.25 (57.27)	4.53 (4.55)	14.07 (14.06)
3c	H	2- OH	71.1	210-212	0.36	$C_{18}H_{16}N_4O_4S$	56.26 (56.24)	4.23 (4.20)	14.56 (14.57)
3d	H	2,4-Cl	70.0	158-160	0.42	$C_{18}H_{14}Cl_2N_4O_3S$	49.46 (49.44)	3.21 (3.23)	12.83 (12.81)
3e	H	4-Cl	72.2	186-188	0.43	$C_{18}H_{15}ClN_4O_3S$	53.69 (53.67)	3.74 (3.75)	13.94 (13.91)
3f	H	4-CH_3	68.4	175-177	0.33	$C_{19}H_{18}N_4O_3S$	59.68 (59.67)	4.73 (4.74)	14.64 (14.65)
3g	2,4-Cl	4-NO_2	67.0	160-162	0.41	$C_{18}H_{13}Cl_2N_5O_5S$	44.84 (44.83)	2.70 (2.72)	14.56 (14.52)
3h	2,4-Cl	4-OCH_3	67.2	190-192	0.34	$C_{19}H_{16}Cl_2N_4O_4S$	48.85 (48.83)	3.42 (3.45)	11.98 (11.99)
3i	2,4-Cl	2- OH	65.2	182-184	0.45	$C_{18}H_{14}Cl_2N_4O_4S$	47.68 (47.69)	3.13 (3.11)	12.37 (12.36)
3j	2,4-Cl	2,4-Cl	68.4	178-180	0.36	$C_{18}H_{12}Cl_4N_4O_3S$	42.72 (42.71)	2.42 (2.39)	11.05 (11.07)
3k	2,4-Cl	4-Cl	68.5	204-206	0.42	$C_{18}H_{13}Cl_3NO_3S$	45.84 (45.83)	2.80 (2.78)	11.85 (11.88)
3l	2,4-Cl	4-CH_3	70.3	174-176	0.36	$C_{19}H_{16}Cl_2N_4O_3S$	50.59 (50.56)	3.56 (3.57)	12.44 (12.41)

* Recrystallization with ethanol. # Stationary Phase: Silica gel G, Mobile phase: Chloroform : Benzene (1:1), Iodine vapors as visualizing agent.

stationary phase and suitable solvent system as mobile phase and by melting point. Structure of all the newly synthesized compounds is well supported by the spectral data such as IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. The physical data of compounds is tabulated in Table 1.

The IR spectrum of compound **1** showed characteristic absorption bands at 3457.4 cm^{-1} due to NH_2 and the other at 1282.1/2580 cm^{-1} was attributed to C=S/SH. The ^{1}H NMR characteristic signal showed at δ 13.2/7.8 as a singlet due to SH/NH proton derived from tautomeric equilibrium of compound **1** and δ 2.1 due to NH_2 protons.

The IR spectrum of compound **2a–g** showed a characteristic absorption bands at range of 1588.5–1610.4 cm^{-1} and 1531.3–1535.7 cm^{-1} due to the presence of HC=N of Schiff base and C=N of triazole ring, respectively. The ^{1}H NMR spectrum of these compounds displayed a singlet at δ 8.1 to δ 10.4 which accounted HC=N. The ^{13}C NMR data which displayed characteristic signal at δ 142.3 to δ 155.9 due to HC=N. It suggests



Scheme 1 Synthesis of 2-[4-(substituted bezylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives **(3a–l)**. Reagents and conditions: (a) Heat 175–180 °C, oil bath, 2–3 h. (b) Aromatic aldehydes, absolute ethanol, reflux, 2 h. (c) Chloroacetic acid, ethanol, reflux, 2–3 h.

the formation of Schiff base. The structure of compounds was further conformed by evidence for the formation of Schiff base, which was obtained by mass spectral data.

The IR spectrum of compound **3a–l** showed characteristic absorption bands at range of 3098.8–3210.2 cm^{-1} was attributed to OH and 1710.6–1735.1 cm^{-1} accounting for C═O of carboxyl group. The ^{1}H NMR spectrum of these compounds displayed a broad singlet at δ 13.5 to δ 13.9 due to OH of carboxyl group and singlet at δ 1.1 to δ 2.9 due to S–CH_2 protons indicates the formation of S–CH_2–COOH at 5th position of triazole. The ^{13}C NMR data observed characteristic singlet at δ 29.5 to δ 39.4 due to S–CH_2. It reveals the formation of thioacetic acid by the reaction of SH with chloroacetic acid. The structure of compounds was further supported by mass spectral data.

All compounds **3a–l** were subjected for preliminary toxicity studies, according to Organization for Economic Co-operation and Development (OECD) guidelines in mice. Compounds were found to be safe up to 2000 mg/kg body weight (b.w.). Therefore 1/10th of the maximum tolerated dose, i.e., 200 mg/kg b.w. was used as therapeutic dose.

Acute anti-inflammatory activity by carrageenan induced rat paw edema model by Winter et al. Diclofenac was used as reference standard. The anti-inflammatory activity results revealed that the compounds **3d, 3e, 3j** and **3k** exhibited significant anti-inflammatory activity (up to 63.4%, 62.0%, 64.1% and 62.5% edema inhibition, respectively), as compared to the standard drug diclofenac (67.0%) after third hour. Where as compounds **3g–i, 3l** showed good anti-inflammatory activity and among compounds **3a–c**, **3f** showed lesser degree anti-inflammatory activity compared to that of standard drug Diclofenac. The anti-inflammatory activity of these compounds may be attributed to the inhibition of cyclooxygenase enzyme, which plays vital role in the inflammation process. The results indicating the percentage inhibition of inflammation have been shown in Table 2.

Table 2 Anti-inflammatory activity of 2-[4-(substituted bezylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives **(3a–l)**.

Compound	Paw mean volume (±SEM)	Edema inhibition at 3 h (%)
Control	0.443 ± 0.008	–
3a	0.339 ± 0.021	23.5
3b	0.330 ± 0.028	25.5
3c	0.328 ± 0.022	25.9
3d	0.162 ± 0.011**	63.4**
3e	0.168 ± 0.014**	62.0**
3f	0.345 ± 0.023	22.1
3g	0.322 ± 0.020*	27.3*
3h	0.322 ± 0.021*	27.3*
3i	0.316 ± 0.018*	28.6*
3j	0.159 ± 0.013**	64.1**
3k	0.166 ± 0.018**	62.5**
3l	0.320 ± 0.006*	27.8*
Diclofenac	0.146 ± 0.017**	67.0**

Dose of 200 mg/kg b.w., p.o.
* $p \leqslant 0.05$.
** $p \leqslant 0.001$.

Table 3 Analgesic activity of 2-[4-(substituted bezylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives **(3a–l)**.

Compound	No. of writhes in 15 min (mean ± SEM)	(%) Protection
Control	68.6 ± 7.0	–
3d	31.3 ± 3.4	53.9*
3e	31.8 ± 7.6	53.6*
3j	30.2 ± 8.3	55.9**
3k	30.9 ± 3.6	54.9**
Aspirin	29.0 ± 5.6	57.8**

Dose of 200 mg/kg b.w., p.o.
* $p \leqslant 0.05$.
** $p \leqslant 0.001$.

Abdominal construction responses induced by acetic acid is a sensitive procedure to establish efficacy of peripherally-acting analgesics, it may causes increase in the level of PGE_2 and $PGF_{2\alpha}$ by intraperitoneally administration of acetic acid. The analgesic activity was expressed as percentage of protection. Among the series compounds **3j**, **3k** showed significant analgesic activity (up to 55.9%, 54.9% protection, respectively), where as among compounds **3d, 3e** showed good analgesic activity (up to 53.9%, 53.6% protection, respectively), as compared to the standard drug aspirin (57.8%) as illustrated in Table 3.

Among the compounds **3d, 3e, 3j** and **3k**, the compound **3j** showed highest anti-inflammatory and analgesic activities because of two chlorine groups at ortho and para position on both phenoxymethyl ring and imine phenyl ring. Hence, it reveals that the compounds having more than one electron withdrawing group (halogen) on phenoxymethyl ring and imine phenyl ring showed increasing anti-inflammatory and analgesic activities.

The major drawback of non steroidal anti-inflammatory drugs is their gastric side effects. In order to determine the extent of these effects, the compounds **3d, 3e, 3j** and **3k** were further tested for ulcerogenicity (Rainsford, 1975) in rats at 200 mg/kg. All four compounds showed comparable ulcerogenic activity when compared to standard drug aspirin. Among these **3j, 3k** showed less ulcerogenic activity, respectively. Results are shown in Table 4.

5. Conclusion

The present study revealed that the synthesis of the title compounds had satisfactory yields. It was observed that the test compounds **3j** and **3k** containing electron withdrawing moiety (halogen) on phenyl group of Schiff base as well as on phenoxymethyl were found to have significant anti-inflammatory and analgesic activity compared to the standard. Results of these compounds showed less ulcerogenic activity to aspirin. These can be regarded as strong candidates for future investigations.

Table 4 Ulcerogenic activity of 2-[4-(substituted bezylideneamino)-5-(substituted phenoxymethyl)-4*H*-1,2,4-triazol-3-yl thio] acetic acid derivatives **(3a–l)**.

Compound	Ulcer index ± SEM
Control	1.4 ± 0.45
3d	4.7 ± 1.33*
3e	4.5 ± 1.26*
3j	3.9 ± 1.22*
3k	3.6 ± 1.30*
Aspirin	5.3 ± 1.55*

Dose 200 mg/kg p.o.
* $p \leqslant 0.05$.

Conflict of interest statements

The authors have declared no conflict of interest.

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References

Alich, A.A., Welsh, V.J., Wittmess, L.E., 1983. J. Pharm. Sci. 72, 1457–1461.
Almarirad, A., 2004. Bioorg. Med. Chem. Lett. 14, 6057–6059.
Desai, N.C., Shukla, H.K., Thaker, K.A., 1984. J. Indian Chem. Soc. 61, 239–241.
Fahmy, H.H., Soliman, G.A., 2001. Arch. Pharmacol. Res. 24 (3), 180–189.
Francis, G., Cedric, L., Fabrice, P., Damien, C., Patrice, L., Marc Le, B., 2008. Bioorg. Med. Chem. Lett. 18, 1820–1824.
Hunashal, R.D., Ronad, P.M., Satyanarayana, D., Maddi, V.S., Kamadod, M.A., 2010. Int. J. Drug Des. Drug Disc. 1, 107–114.
Iraj, R., Charalabou, C., Panagiotis, Z., Athina, G., Marina, S., Jasmina, G., 2008. Bioorg. Med. Chem. 16, 1160–1161.
Koster, R., Anderson, M., Debeer, E., 1959. Fed. Proc. Fed. Am. Soc. Exp. Biol. 18, 412–413.
Kucukguzel, I., Esra, T., Guniz Kucukguzel, S., Rollas, S., De Clercq, E., 2008. Eur. J. Med. Chem. 43, 381–392.
Latifeh, N., Hamed, S., Khosrou, A., Mohsen, A., Mohammad, H., Ghahremani, R., 2006. Bioorg. Med. Chem. 14, 2507–2517.
Lombardino, G., 1985. Nonsteroidal Antiinflammatory Drugs. Wiley Interscience, John Wiley and Sons, New York, pp. 3–15.
Maddi, V., Raghu, K.S., Rao, M.N.A., 1992. J. Pharm. Sci. 81, 964–966.
Mahendra, R.S., Kiran, K.M., Hanimi, R.G., Tatikonda, S., Chakravarthy, A.K., Dolly, P., 2007. Bioorg. Med. Chem. 15, 3997–4008.
Mullican, M.D., Wilson, M.W., Connor, D.T., Kostlan, C.R., Schrier, D.J., Dyer, D., 1993. J. Med. Chem. 36, 1902–1909.
OCED/OCDC, OECD, October 2000. Guidelines for testing of chemicals. Revised draft guidelines 423, acute oral toxicity class method, Revised document.
Olcay, B., Bahittin, K., Murat, K., 2006. Turk. J. Chem. 30, 29–40.
Osiri, M., Moreland, L.W., 1999. Arthitis Care Res. 12, 351–362.
Rainsford, K.D., 1975. Gut 16, 514–527.
Ronad, P.M., Hunashal, R.D., Satyanarayana, D., Maddi, V.S., 2008a. Arzneim. Forsch. (Drug Res.) 58, 641–646.

Ronad, P.M., Satyanarayana, D., Hunashal, R.D., Maddi, V.S., 2008b. Arch. Pharm. Chem. Life Sci. 341, 696–700.
Upadhaya, R.S., Sinha, N., Jain, S., Kishore, N., Chandra, R., Arora, S., 2004. Bioorg. Med. Chem. 12, 2225–2228.
Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Proc. Soc. Exp. Biol. Med. 111, 544–547.
Yasemin, D., Bilge, C., Esra, K., Fethi, S., Ningur, N., 2007. Turk. J. Chem. 31, 1–13.