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Studies on Chromene based 2, 6-disubstituted-Thiazolo [3,2-B] [1,2,4] Triazole derivatives: Synthesis and Biological Evaluation

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ABSTRACT

In this study, a series of novel chromene based 2,6-disubstituted-thiazolo[3,2-b] [1,2,4] triazole derivatives **(7a-g)** were synthesized by condensing 5-substituted-1,2,4-traizole-thione **(6a-g)** using poly phosphoric acid through one pot reaction. The structure of new compounds was supported by ^{1}H NMR, ^{13}C NMR and MS data. The synthesized compounds were evaluated using writhing assays for analgesic and carrageenan-induced rat paw edema method for anti-inflammatory activities respectively. Some of the newly synthesized compounds **7c, 7f** and **7g** showed very good anti-inflammatory activity with 90.83%, 85.81% and 88.40% protection respectively along with low GI toxicity as compared to standard drug ibuprofen while compounds **7a, 7b, 7d** and **7f** showed highest analgesic activity with 52.54%, 54.02%, 56.76% and 52.45% protection among them compound **7d** showed better protection than standard drug ibuprofen.

Keywords: thiazolo-triazoles, Chromene, Anti-inflammatory, Analgesic

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INTRODUCTION

Chromene appeared as an important structural pharmacophore in various natural compounds and reported having useful medicinal properties such as antitumor [1], antivascular [2], antimicrobial [3], antioxidant [4], TNF-α inhibitor [5], antifungal [6], anticoagulant, antispasmolytic, estrogenic [7], antiviral [8], anti-helminthic, anticancer [9], anti-HIV [10], antitubercular [11], anti-inflammatory [12], herbicidal, analgesic and anticonvulsant [13] activity. Chromene derivatives can be capable of interacting with cellular targets which leads to their anti-inflammatory and analgesic activity. Inflammation is the initial response of the immune system to infection, irritation or foreign substance [14]. The chromene pharmacophore has COX-2 selective inhibitions in non-steroidal anti-inflammatory drugs (NSAIDs) which provide higher potency, efficacy, and selectivity [15-16]. The effectiveness of this useful moiety in treatment of inflammation and other problems stimulated the development of some more potent and important compounds. The structure activity relationship studies suggested that substitution in the chromene increases the ability of the molecule to prevent inflammation [17]. Condensed nucleus systems of 1,2,4-triazole and 1,3,4-thiadiazole (thiazolo-triazoles) found to have diverse pharmacological activities including anti-inflammatory, anti-edema, and analgesic properties [18-22]. Based on the above mentioned studies, the present work aims to design a variety of ligands using various chromene based 2,6-disubstituted-thiazolo[3,2-b][1,2,4]triazole derivatives with the hope to get better and safer anti-inflammatory and analgesic agents. The title compounds were synthesized as per synthetic protocol mentioned in scheme 1.

MATERIALS AND METHODS

Chemistry

LR-grade solvents used were obtained from Merck (Mumbai, India), CDH (New Delhi, India), and S.D.fine (Mumbai, India). Open tube capillary method adopted for determining melting point and was uncorrected. Thin layer chromatography (TLC) was performed on Silica gel G (Merck) in the solvent system, Benzene: Acetone (8:2, v/v) and Toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v); UV lamp and iodine chamber were used for visualization of TLC spots. ^{1}H NMR and ^{13}C NMR were recorded in DMSO-∂_6 or $CDCl_3$ on Bruker 300 MHz instrument using TMS as internal standard. Mass spectra presented as m/z, were recorded on LC/MS of Perkin-Elmer, LABINDIA and Applied Biosystem model no. API 3000.

Experimental Protocol

Synthesis of 7-hydroxy-4-methyl-2H-chromen-2-one **(1)**

Mixture of 4gm of powdered resorcinol in 5ml of ethyl aceto acetate was prepared in a conical flask and cooled 15 ml of conc. H_2SO_4 were added dropwise into it, keeping in view the temperature does not rise above 10˚C [20]. After Stirring for 10 minutes, the mixture was poured into 50 ml ice cold water, filtered, washed and dried. The compound was found pure on TLC examination (B: A, 8:2).

Synthesis of ethyl 2-(4-methyl-2-oxo-4a,8a-dihydro-2H-chromen-7-yloxy)acetate **(2)**

An equimolar mixture of compound **1** (2.0g, 1.12 mmol), ethyl acetoacetate (1.86g, 1.12 mmol) and anhydrous potassium carbonate (2.30gm, 1.68 mmol) in dry acetone (50ml) was refluxed on a water bath for 12 hrs. The solid was filtered and the excess of solvent was removed under reduced pressure. The product was filtered, dried and recrystallized. The compound was found pure on TLC examination (B: A, 8:2).

Synthesis of 2-(4-methyl-2-oxo-4a,8a-dihydro-2H-chromen-7-yloxy)acetohydrazide **(3)**

A mixture of **2** (0.01 mol), abs. ethanol (50ml) and hydrazine hydrate (0.015 mol; 99%) was heated under reflux on water bath for 16 hrs. The alcohol was reduced to half of its volume and cooled. The product separated **3** was filtered and washed with small portions of cold alcohol first and then with cold water, repeatedly and dried. The product was purified by recrystallization from suitable solvents. The compound was found pure on TLC examination (B: A, 9:1 and 8:2).

Synthesis of 2-(2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetyl)hydrazinecarbothioamide (**4**)

To a solution of corresponding hydrazide **3** (0.02 mol) in 50 ml of ethanol was added a solution of KSCN (0.03 mol) and 3 ml of HCl with constant stirring. The mixture was evaporated to dryness on a steam bath and heated for an additional 1 h with another 50 ml of ethanol. The resulting solid mixture was treated with distilled water. The crude thiosemicarbazides **4** were used as such for next step.

Synthesis of 7-((5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one **(5)**

To a solution of corresponding thiosemicarbazides **4** (0.01 mol) in 15 ml of ethanol was added a solution of 10.0% NaOH (20 ml) and the reaction mixture was refluxed immediately for 5-6 h upto reaction was completed. The mixture was cooled and acidified with dilute HCl at pH 5-6. The resulting solid was filtered, washed with distill water and dried. The crude compound was recrystallized from ethanol.

Yield: 72%; m.p. 164-166°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 3.0 (s, 1H, C-S-H), 2.42 (d, 3H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 1158.2, 159.3, 160.8, 154.3, 160.2, 111.9, 152.27, 104.0, 112.5, 111.0, 125.8, 111.0, 71.6, 19.4; ESI MS (m/z): 289.1 (M+); Anal. calcd. for C13H11N3O3S: C, 53.97; H, 3.83; N, 14.52; O, 16.59; S, 11.08 %. Found: C, 53.82; H, 3.87; N, 14.55; O, 16.72; S, 11.09 %.

General procedure for the synthesis of 5-substituted-1,2,4-traizole-thione derivatives **(6a-g)**

To 0.01 mol of 7-((5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one **5**, 0.01 mol of 2-bromo-1-substituted-ethanone were added in the presence of 0.015 mol of KOH in absolute ethanol and refluxed for 10 h. The reaction mixture was cooled and poured onto crushed ice. The resulting solids **6** were filtered, dried and recrystallized from mixture of ethanol and dimethyl formamide (DMF).

4-methyl-7-((5-(2-oxo-2-phenylethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2H-chromen-2-one (6a)

Yield: 72%; m.p. 196-198°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.56-7.94 (m, 5H, benzene), 6.97-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 6.39 (s, 2H, O-CH2), 4.97 (s, 2H, S-CH2), 3.42 (d, 3H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 160.2, 159.3, 158.8, 154.2, 152.7, 111.9, 111.0, 107.43, 135.13, 127.22, 21.4; ESI MS (m/z): 407.1 (M+); Anal. calcd. for C21H17N3O4S: C, 61.90; H, 4.21; N, 10.31; O, 15.71; S, 7.87%. Found: C 61.82, H 4.31, N 10.25, O, 15.76; S 7.86 %.

7-((5-(2-(4-(dimethylamino)phenyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one (6b)

Yield: 74%; m.p. 188-190°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 6.87-7.76 (m, 4H, benzene), 6.97-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 5.45 (s, 2H, S-CH2), 3.06 (d, 6H, CH3), 2.42 (s, 3H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 194.1, 160.8, 160.2, 159.3, 158.8, 155.5, 154.3, 152.7,129.7, 125.8, 124.9, 112.5, 111.9, 111.7, 111.0, 104.0,107.43, 38.2, 135.13, 127.22, 71.6, 41.3, 41.3, 21.4, 19.4; ESI MS (m/z): 450.1 (M+); Anal. calcd. for C23H22N4O4S: C, 61.32; H, 4.92; N, 12.44; O, 14.21; S, 7.12 %. Found: C, 61.40; H, 4.84; N, 12.40; O, 14.28; S, 7.09 %.

7-((5-(2-(4-methoxyphenyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one (6c)

Yield: 80%; m.p. 204-206°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.10-7.83 (m, 4H, benzene), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 4.92 (s, 2H, S-CH2), 3.83 (d, 6H, CH3), 2.42 (s, 3H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 158.8, 159.3, 160.8, 154.3, 160.2, 165.0, 111.9, 152.7, 127.7, 104.0, 114.2, 129.8, 111.0, 125.8, 129.8, 38.2, 194.1, 71.6, 55.8, 19.6; ESI MS (m/z): 437.1 (M+); Anal. calcd. for C22H19N3O5S: C, 60.40; H, 4.38; N, 9.61; O, 18.29; S, 7.33%. Found: C, 60.46; H, 4.34; N, 9.67; O, 18.32; S, 7.26%.

4-methyl-7-((5-(2-oxo-2-(1H-pyrrolo[2,3-b]pyridin-2-yl)ethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2H-chromen-2-one (6d)

Yield: 72%; m.p. 186-188°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.36-8.43 (m, 3H, pyridine), 6.83 (s, 1H, pyrrole), 6.95-7.73 (m, 4H, coumarine), 5.16 (s, 2H, O-CH2), 5.0 (s, 1H, NH , 2-pyrrole), 4.50 (s, 2H, S-CH2), 2.45 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 158.8,159.3,155.7, 139.1, 109.1, 121.9, 160.8, 154.3, 160.2, 142.4, 128.3, 111.9, 152.7, 104.0, 115.6, 112.5, 111.0, 125.8, 35.2, 178.0, 71.6, 19.4; ESI MS (m/z): 447.1 (M+); Anal. calcd. for C22H17N5O4S: C, 59.05; H, 3.83; N, 15.65; O, 14.30; S, 7.17 %. Found: C, 59.11; H, 3.80; N, 15.68; O, 14.20; S, 7.21 %.

7-((5-(2-(imidazo[1,2-a]pyridin-3-yl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one (6e)

Yield: 74%; m.p. 196-198°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.48 (s, 1H, imidazo-pyridine) 6.86-8.48 (m, 4H, imidazo-pyridine), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 4.50 (s, 2H, S-CH2), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 158.8, 152.2, 159.3, 135.9, 109.1, 129.1, 160.8, 154.3, 160.2, 128.4, 114.2, 111.9, 152.7, 104.0, 114.6, 126.2, 112.5, 111.0, 125.8, 35.7, 188.0, 71.6, 19.4; ESI MS (m/z): 447.1 (M+); Anal. calcd. for C22H17N5O4S: C, 59.05; H, 3.83; N, 15.65; O,

14.30; S, 7.17%. Found: C, 59.15; H, 3.77; N, 15.68; O, 14.20; S, 7.20 %.

4-methyl-7-((5-(2-oxo-2-(pyridin-4-yl)ethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2H-chromen-2-one(6f)

Yield: 76%; m.p. 200-202°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.92-8.82 (m, 4H, pyridine), 6.95-7.73 (m, 4H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 4.77 (s, 2H, S-CH2), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 158.8, 159.3, 160.8, 154.3, 160.2, 150.9, 152.7, 142.3, 104.0, 119.9, 112.5, 111.9, 111.0, 125.8, 38.2, 194.1, 71.6, 19.4; ESI MS (m/z): 408.1 (M+); Anal. calcd. for C20H16N4O4S: C, 58.81; H, 3.95; N, 13.72; O, 15.67; S, 7.85 %. Found: C, 58.90; H, 3.90; N, 13.75; O, 15.62; S, 7.83 %.

4-methyl-7-((5-(2-morpholino-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2H-chromen-2-one (6g)

Yield: 80%; m.p. 187-189°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 4.29 (s, 2H, S-CH2), 3.65 (d, 4H, oxazine), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 158.8, 159.3, 160.8, 154.3, 160.2, 150.9, 152.7, 142.3, 104.0, 119.9, 112.5, 111.9, 111.0, 125.8, 36.7, 168.2, 71.6, 66.2, 160.2, 47.3, 19.4; ESI MS (m/z): 416.1 (M+); Anal. calcd. for C19H20N4O5S: C, 54.80; H, 4.84; N, 13.45; O, 19.21; S, 7.70 %. Found: C, 54.86; H, 4.87; N, 13.38; O, 19.25; S, 7.64 %.

General procedure for the synthesis of 4-methyl-7-((6-substituted-thiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-2H-chromen-2-one **(7a-g)**

To 0.01 mol of 4-methyl-7-((5-(2-oxo-2-substituted-ethylthio)-4H-1,2,4-triazol-3-yl)methoxy)-2H-chromen-2-one **(6a-g)** , approx. 5ml of poly phosphoric acid (PPA) were added and refluxed at 125°C for 12 h. The reaction mixture was cooled and poured onto crushed ice and neutralized by adding $NaHCO_3$. The resulting solid **(7a-g)** was filtered, dried and recrystallized from a mixture of ethanol and dimethyl formamide (DMF).

4-methyl-7-((6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-2H-chromen-2-one (7a)

Yield: 82%; m.p. 184-186°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.96 (s, 1H, thiazole), 7.41-8.27 (m, 5H, benzene), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 3.42 (d, 3H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 147.9, 117.9, 148.3, 126.7, 160.8, 154.3, 160.2, 130.1, 111.9, 152.7, 104.0, 127.5, 112.5, 111.0, 125.8, 129.2, 128.7, 72.0, 19.4, ; ESI MS (m/z): 389.1 (M+); Anal. calcd. for C21H15N3O3S: C, 64.77; H, 3.88; N, 10.79; O, 12.33; S, 8.23 %. Found: C, 64.80; H, 3.82; N, 10.82; O, 12.38; S, 8.18 %.

7-((6-(4-(dimethylamino)phenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one (7b)

Yield: 68%; m.p. 178-180°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.41 (s, 1H, thiazole), 6.82-7.61 (m, 4H, benzene), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 2.42 (s, 2H, S-CH2), 3.06 (d, 6H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 147.9, 117.9, 148.3, 126.7, 160.8, 154.3, 160.2,155.3, 111.9, 111.9, 152.7, 104.0, 127.5, 112.7, 128.4, 112.5, 111.0, 112.7, 125.8, 72.0, 41.3, 19.4; ESI MS (m/z): 432.1 (M+); Anal. calcd. for C23H20N4O3S: C, 63.87; H, 4.66; N, 12.95; O, 11.10; S, 7.41 %. Found: C, 63.85; H, 4.72; N, 12.86; O, 11.13; S, 7.33 %.

7-((6-(4-methoxyphenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one (7c)

Yield: 72%; m.p. 189-191°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.96 (s, 1H, thiazole), 7.05-7.55 (m, 4H, benzene), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 2.43 (s, 2H, S-CH2), 3.83 (s, 3H, CH3); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 147.9, 117.9, 148.3, 126.7, 160.8, 154.3, 160.6, 122.4, 155.3, 111.9, 152.7, 104.0,114.8, 128.5, 112.5, 114.8, 111.0, 128.5, 125.8, 72.0, 55.8, 19.4; ESI MS (m/z): 419.1 (M+); Anal. calcd. for C22H17N3O4S: C, 63.00; H, 4.09; N, 10.02; O, 15.26; S, 7.64 %. Found: C, 62.90; H, 4.06; N, 10.12; O, 15.30; S, 7.61 %.

7-((6-(1H-pyrrolo[2,3-b]pyridin-2-yl)thiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one (7d)

Yield: 75%; m.p. 198-200°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 8.13 (s, 1H, thiazole), 7.36-8.51 (m, 3H, pyridine), 6.40 (s, 1H, pyrrole), 6.95-7.73 (m, 4H, coumarine), 5.16 (s, 2H, O-CH2), 5.0 (s, 1H, NH , 2-pyrrole), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 147.9, 120.1, 148.3, 148.6, 143.2, 139.4, 99.3, 119.6, 160.8, 154.3, 160.2, 128.3, 111.9, 104.0, 115.6, 112.5, 111.0, 125.8, 72.0, 19.4; ESI MS (m/z): 429.1 (M+); Anal. calcd. for C22H15N5O3S: C, 61.53; H, 3.52; N, 16.31; O, 11.18; S, 7.47 %. Found: C, 61.58; H, 3.56; N, 16.28; O, 11.20; S, 7.39 %.

7-((6-(imidazo[1,2-a]pyridin-3-yl)thiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one (7e)

Yield: 76%; m.p. 180-182°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 8.13 (s, 1H, thiazole), 7.48 (s, 1H, imidazo-pyridine) 6.86-8.48 (m, 4H, imidazo-pyridine), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 147.9, 120.1, 145.1, 148.3, 143.2, 127.9, 119.9, 160.8, 154.3, 160.2, 127.5, 114.2, 111.9, 152.7, 104.0, 114.6, 126.2,112.5, 111.0, 125.8, 72.0, 19.4; ESI MS (m/z): 429.1 (M+); Anal. calcd. for C22H15N5O3S: C, 61.53; H, 3.52; N, 16.31; O, 11.18; S, 7.47 %. Found: C, 61.53; H, 3.52; N, 16.31; O, 11.18; S, 7.47 %.

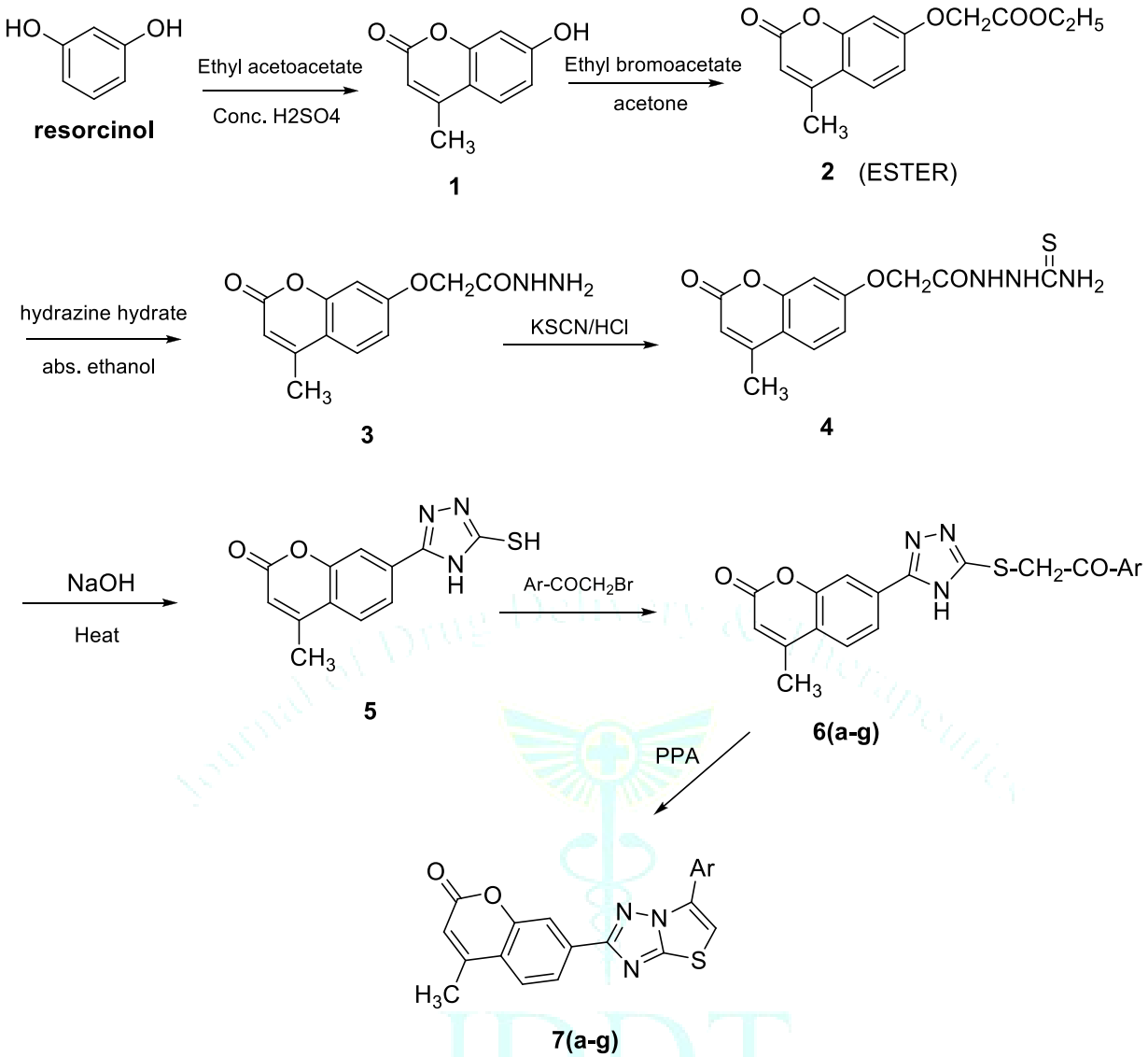
4-methyl-7-((6-(pyridin-4-yl)thiazolo[3,2-b][1,2,4]triazol-2-yl)methoxy)-2H-chromen-2-one (7f)

Yield: 82%; m.p. 174-176°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 7.96 (s, 1H, thiazole), 7.99-8.75 (m, 4H, pyridine), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm): 147.9, 122.5, 148.3, 114.2, 160.8, 154.3, 160.2, 149.8, 140.3, 111.9, 152.7, 104.0, 121.3, ,112.5, 111.0, 125.8, 72.0, 19.4; ESI MS (m/z): 408.1 (M+); Anal. calcd. for C20H14N4O3S: C, 61.53; H, 3.61; N, 14.35; O, 12.29; S, 8.21 %. Found: C, 61.60; H, 3.65; N, 14.30; O, 12.31; S, 8.13 %.

4-methyl-7-((6-morpholinothiazolo [3,2-b][1,2,4]triazol-2-yl)methoxy)-2H-chromen-2-one (7g)

Yield: 70%; m.p. 156-158°C; ^{1}H NMR (300 MHz, DMSO-d6, δ in ppm): 6.31 (s, 1H, thiazole), 6.95-7.73 (m, 3H, coumarine), 6.23 (t, 1H, coumarine), 5.16 (s, 2H, O-CH2), 3.57-3.65 (d, 4H, oxazine), 2.42 (d, 3H, CH3-coumarine); ^{13}C NMR (75 MHz, CDCl3, δ in ppm):): 147.9, 118.6, 148.3, 143.0, 160.8, 154.3, 66.3, 160.2, 48.4, 111.9, 152.7, 104.0,112.5, 111.0, 125.8, 72.0, 19.4; ESI MS (m/z): 398.1 (M+); Anal. calcd. for C19H18N4O4S: C, 57.27; H, 4.55; N, 14.06; O, 16.06; S, 8.05 %. Found: C, 57.32; H, 4.58; N, 14.02; O, 16.07; S, 8.00 %.

Scheme-1: Synthetic protocol of the title compounds (7a-g).



Ar= -C_6H_5 , -$C_6H_4N(CH_3)_2$, -$C_6H_4OCH_3$, -$C_7H_5N_2$, -$C_7H_5N_2$, -C_5H_4N , -NC_4H_8O

Biological Evaluations

Wistar rats and albino mice of either sex weighing 180–200 and 25–30 g, respectively, were used. The animals obtained from Animal House Facility, Adarsh Vijendra Institute of Pharmaceutical Sciences, Saharanpur (U.P), India and were housed in groups of six at room temperature of 25 ±2°C under 12 h light/12 h dark cycle with free access to food and water ad libitum. The studies were undertaken with prior approval from the Institutional Animal Ethics Committee (IAEC) registration No. 1487/PO/a/11/CPCSEA and utmost care was taken to insure that the animals were treated in the most humane and acceptable manner.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity following carrageenan-induced rat paw edema method [23]. The rats were randomly divided into groups of six. One group was kept as control, and received only 0.5% carboxymethyl cellulose (CMC) solution and the other groups were treated with test compounds and standard drug (Ibuprofen) at a dose level of 20 mg/kg p.o. Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after the administration of the test compounds, and standard drugs. The paw volume was measured by saline displacement shown on screen of digital Plethysmometer (Ugo Basile, Italy) at 3 and 4 h after carrageenan injection. Thus, the edema volume in control group (Vc) and that of in groups treated with test compounds (Vt) was measured and the percentage inhibition of edema was calculated using the formula: Anti-inflammatory activity (% inhibition) = [(Vc-Vt)/Vc] x 100.

Analgesic activity

Analgesic activity was carried out by acetic acid-induced writhing method [24] in albino mice. A 1% aqueous acetic acid solution (i.p. injection; 0.1 ml) was used as writhing-inducing agent. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 minutes. Screening of analgesic activity was performed after i.p. administration of test drugs and the reference drug (ibuprofen) at the dose of 20 mg/kg. All the compounds were injected as CMC suspension (1%). One group was kept as control and received 1% CMC. After 20 min of drug

administration, 0.10 ml of 1% acetic acid solution was given to mice intraperitoneally. The severity of stretching movements consisting of arching of the back, elongation of body, and extension of hind limbs was recorded for 20 min after administration of acetic acid solution. The analgesic activity was presented as % protection. % Analgesic activity = (n-n' /n) x 100, where n and n' are the mean number of writhes of control and test groups, respectively.

RESULTS AND DISCUSSION

Chemistry

7-hydroxy-4-methyl-2H-chromen-2-one **(1)** synthesized through Pechman condensation of resorcinol with ethyl acetoacetate which on reflux with anhydrous potassium carbonate in dry acetone resulted in synthesis of ethyl 2-(4-methyl-2-oxo-4a,8a-dihydro-2H-chromen-7-yloxy)acetate **(2).** Compounds 2-(2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetyl) hydrazine carbothioamide **(4)** was synthesized by reflux using carbon disulfide and 2-(4-methyl-2-oxo-4a,8a-dihydro-2H-chromen-7-yloxy)acetohydrazide **(3)** which were synthesized by refluxing with hydrazine hydrate in absolute alcohol [18]. Compound 7-((5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one **(5)** was synthesized by cyclization of 2-(2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetyl)hydrazinecarbothioamide **(4)** in presence of ethanol and NaOH. A series of novel 5-substituted-1,2,4-traizole-thione derivatives **(6a-g)** were synthesized by refluxing 7-((5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one **(5)** with different substituted bromoethanone using potassium hydroxide in ethanol. Further, a series of title compounds **(7a-g)** were synthesized by refluxing 5-substituted-1,2,4-traizole-thione derivatives **(6a-g)** with PPA followed by neutralization by $NaHCO_3$. ^1H NMR, ^{13}C NMR and mass spectra were used for the structural characterization of the prepared compounds.

Anti-inflammatory activity

Fourteen compounds of the Scheme I were tested for their anti-inflammatory activity (Table 1). The compounds showed anti-inflammatory activity ranging from 47.67% to 90.83% inhibition at 4 hr of carrageenan injection, which was compared to standard drug ibuprofen (93.57%). Among the screened compounds only three compounds **7c, 7f** and **7g** showed highest anti-inflammatory activity with 90.83%, 85.81% and 88.40% protection respectively. Rests of the compounds were moderate in their action.

Table 1:- Anti-inflammatory activity (Percent inhibition of edema)

Compd. no.	Body Wt. (gm) ±SEM	Initial Paw Vol. ±SEM	Paw Volume After ±SEM		Difference After ±SEM		% Inhibition ±SEM After	
			3 hr	4 hr	3 hr	4 hr	3 hr	4 hr
Control	150.00 ±2.23	1.58 ±0.04	2.37 ±0.03	2.37 ±0.03	0.71 ±0.03	0.77 ±0.03		
Ibuprofen	150.00 ±1.82	1.70 ±0.06	1.87 ±0.01	1.82 ±0.02	0.12 ±0.02	0.05 ±0.02	**82.27 ± 3.23**	**93.57 ±2.72**
6a	148.33 ±1.05	1.45 ±0.02	1.98 ±0.02	1.88 ±0.02	0.49 ±0.03	0.40 ±0.02	29.63 ±2.05	47.67 ±3.66
6b	145.00 ±1.82	1.43 ±0.03	1.83 ±0.07	1.73 ±0.06	0.39 ±0.05	0.30 ±0.04	48.50 ±5.25**	60.40 ±4.77
6c	150.00 ±1.29	1.37 ±0.03	1.80 ±0.02	1.72 ±0.03	0.43 ±0.02	0.34 ±0.06	39.32 ±3.07	56.24 ±4.17
6d	146.66 ±1.66	1.47 ±0.02	1.82 ±0.03	1.72 ±0.03	0.34 ±0.02	0.25 ±0.01	50.93 ±4.14	66.74 ±3.13
6e	146.66 ±1.66	1.41 ±0.02	1.80 ±0.01	1.68 ±0.01	0.37 ±0.02	0.26 ±0.03	46.17 ±3.90**	66.13 ±4.03
6f	147.50 ±1.11	1.42 ±0.02	1.88 ±0.02	1.83 ±0.01	0.45 ±0.00	0.40 ±0.02	35.26 ±2.12	53.34 ±3.20
6g	145.00 ±2.23	1.3 ±0.06	1.71± 0.05	1.64± 0.05	0.35± 0.05	0.27 ±0.05	49.97 ±4.29**	63.89 ±4.14
7a	160.50 ±2.35	1.43 ±0.03	1.78 ±0.04	1.62 ±0.03	0.31 ±0.02	0.24 ±0.01	42.41 ±2.97	68.08 ±3.28
7b	162.50 ±1.70	1.53 ±0.03	1.75 ±0.02	1.68 ±0.04	0.22 ±0.01	0.11 ±0.04	69.71 ±2.15	81.27 ±3.11
7c	145.83 ±1.53	1.53 ±0.03	1.69 ±0.02	1.59 ±0.02	0.14 ±0.02	0.07 ±0.02	80.44 ±3.17	90.83 ±5.02
7d	153.33 ±1.66	1.38 ±0.04	1.78 ±0.04	1.76 ±0.04	0.40 ±0.02	0.37 ±0.01	43.74 ±1.29*	50.74 ±1.82
7e	158.33 ±1.10	1.53 ±0.01	2.04 ±0.02	1.94 ±0.03	0.51 ±0.01	0.41 ±0.02	31.93 ±4.16	47.08 ±4.46
7f	148.33 ±1.66	1.48 ±0.02	1.67 ±0.02	1.61 ±0.01	0.15 ±0.01	0.11 ±0.06	78.74 ±1.29*	85.81 ±5.13
7g	150.00 ±1.82	1.59 ±0.01	1.89 ±0.01	1.71 ±0.01	0.29 ±0.02	0.10 ±0.03	60.23 ±6.92	88.40 ±5.73

Each value represents the mean ± SEM (n = 6), n = number of mice
Dose = 20 mg/kg; *p < 0.05, **p < 0.01, ***p < 0.001 (compared to standard).
Data was analyzed by ANOVA's bonferron's multiple tests.

Analgesic activity

All fourteen compounds of the Scheme I showed analgesic activity (Table 2) ranging from 26.90% to 56.76% protection, which was compared to that of standard drug ibuprofen (56.20%). Among the screened compounds only four compounds 7a, 7b, 7d and 7f showed highest analgesic activity with 52.54%, 54.02%, 56.76% and 52.45% protection. Compound **7d** showed better protection than standard drug ibuprofen. Four compounds, 6c, 7c, 7e and 7g showed good activity with 47.80, 47.25, 41.89 and 49.05% inhibition respectively. Rests of the compounds were moderate in their action.

Table 2:- Analgesic activity

Compd. no.	Body wt. (gm) ±SEM	No. of writhing (15 min)±SEM	(%) Protection ±SEM
Control	26.66±1.05	48.33±0.21	0.00±0.00
Ibuprofen	25.83±1.53	21.16±0.30	56.20±0.59
6a	25.83±0.83	35.33±0.61	26.90±1.11
6b	26.66±1.05	30.66±0.61	36.21±1.25
6c	31.33±0.33	25.17±0.50	47.80±0.11**
6d	28.33±1.05	30.16±0.30	37.58±0.73
6e	26.33±1.05	31.50±0.34	35.51±0.80
6f	27.50±1.11	28.83±0.30	40.33±0.76*
6g	28.33±1.05	32.00±0.36	33.79±0.68
7a	26.66±1.66	23.66±0.33	52.54±0.78**
7b	27.55±0.89	22.78±0.12	54.02±0.14***
7c	31.02±1.12	25±0.40	47.25±0.80*
7d	26.66±1.66	21.66±0.42	56.76±0.72***
7e	28.87±0.84	28.09±0.68	41.89±0.67*
7f	27.50±1.11	22.98±0.30	52.45±0.38**
7g	26.66±1.66	24.62±0.23	49.05±0.60*

Each value represents the mean ± SEM (n = 6), n = number of mice
Dose = 20 mg/kg; *p < 0.05, **p < 0.01, ***p < 0.001 (compared to standard).
Data was analyzed by ANOVA's bonferron's multiple tests.

CONCLUSION

Novel chromen based hybrids of thiazolo[3,2-b] [1,2,4]triazole were designed, synthesized and characterized by different spectroscopic techniques. The prepared compounds retain good-to-moderate anti-inflammatory and analgesic activity. The methoxy group containg compound 7c was found to have most prominent anti-inflammatory agent while pyrrolo-pyridine containing compound 7d was the most potent analgesic agent even showed better protection than standard drug ibuprofen. Furthermore, molecular docking studies may be carried out to identify their possible binding mode within the catalytic domain of TNF-α, Cox-1 and Cox-2.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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