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RESEARCH ARTICLE

Synthesis, Anti-inflammatory, Antimicrobial Potential and Molecular Docking Studies of 4,5-Disubstituted-1,2,4-Triazole Thioacetate Derivatives

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Abstract: ***Background*:** In the present study synthesis and biological assessment of nine new ethyl [(4,5-disubstituted- 4H-1,2,4-triazol-3-yl)sulfanyl]acetate derivatives 2(a-i) is performed.

***Methods*:** The title compounds were characterized by their analytical and spectral data. All the synthesized compounds were screened for their *in vivo* anti-inflammatory activity using carrageenan-induced rat paw oedema method and *in vitro* antimicrobial activity. All the compounds exhibited good anti-inflammatory activity; especially compound 2h produced the maximum effect *i.e.*, 62.5 % comparable to that of standard, diclofenac. The antimicrobial screening results indicated that some of the newly synthesized compounds showed good antibacterial activity, especially against Escherichia coli.

***Results*:** All the synthesized thioacetate derivatives of triazoles were also studied for their interactions with the enzymes COX-I and COX-II, two important targets of inflammation pathway, through docking analysis. All the compounds showed good binding affinities with both the enzymes with a maximum value of -8.1 for 2e kcal/mol against COX-I.

***Conclusion*:** Docking analysis predicted that our compounds reduce inflammation nonselectively by inhibiting both COX-I and COX-II of inflammatory pathway just like other nonselactive NSAIDS.

Keywords: 1,2,4-triazole thioacetate derivatives, biological evaluation, molecular docking, COX inhibitor, *in vivo, in vitro,* nonslactive NSAIDS

1. INTRODUCTION

Despite the accessibility to wide array of compounds, there is a substantial number of diseases for which either there is no offered drug therapy or existing drugs are unable to cure the disease completely. So, there is always a liberty for the progress by developing new drugs with a wide therapeutic window and fewer side effects [1]. Amid many doable strategies for improving the therapeutic effectiveness of numerous drugs, one of the most imperative is the synthesis of new derivatives with tailored structure. Inflammation is a series of pathological changes coupled with local vascular reaction and cellular response of the living tissue to an injury insufficient to kill the tissue. A large number of non-steroidal anti-inflammatory drugs (NSAIDs) are available clinically to take care of inflammatory disorders. Long-term use of NSAIDs results in gastrointestinal ulceration and bleeding and platelet dysfunction [2]. Moreover, there are evidence to suggest that free –COOH present in NSAIDS is responsible for GI irritation [3]. So, there is a need to develop new compounds with better anti-inflammatory potential and lesser side effects. Among the nitrogen-containing heterocycles, the mercapto-substituted 1,2,4-triazole ring systems have been well investigated and a large number of biological activities have been reported for many of their derivatives [4]. There is enough literature available highlighting the importance of 1,2,4-triazole nucleus in the development of novel compounds with antiphlogistic [5-7], antitumor [8, 9],

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antifungal [10, 11], antitubercular action [12-14], analgesic [15], anticonvulsant [16, 17] and antibacterial action [18-22]. Triazole containing ring system is present in many therapeutically interesting drug candidates. Many of them are approved drugs like alprazolam [23] or vibunazole [24]. Letrozole, vorozole, and anastrozole are used for cancer as non-steroidal drugs [25]. Triazoles are also used for the synthesis of very effective antifungal agents such as fluconazole, voriconazole, and itraconazole [26, 27]. Furthermore, the literature survey showed that 1,2,4-triazole-3-acetate bearing aryl groups or heteroaryl residues [28] have shown prominent anti-inflammatory activity.

Provoked by these annotations and in the persistence of our hunt for better therapeutic agents, the present study was designed to synthesize ethyl [(4,5-disubstituted-4H-1,2,4-triazol-3-yl) sul fanyl]acetate derivatives and screening them for anti-inflammatory and antimicrobial potential. These newly synthesized compounds have the distinctive feature of having esterified hydroxyalkyl chain to minimize the effects of free carboxylic group. Further to investigate the mechanism, these compounds were docked against both COX-1 and COX-2, the major targets involved in the inflammation pathway.

2. EXPERIMENTAL

2.1. Chemistry

Solvents and chemicals from Merck and Sigma Aldrich were used without further purification. All the compounds synthesized were purified by recrystallization in suitable solvent. Elemental analyses (C, H, N) were in agreement with the proposed structures and within ±0.5% of the theoretical values. Monitoring of the reactions to check the purity of the final products was done using thin layer chromatography with silica gel-precoated aluminum sheets (60 F254; Merck Schuchardt, Darmstadt, Germany) and visualization with ultraviolet light at 365 nm and 254 nm. Characterization of synthesized compounds was done by spectrophotometric analysis *i.e*., FT-IR (Thermoscientific NICOLET IS10 Spectrophotometer), 1H NMR and 13C NMR spectroscopy (Bruker AM-300 spectrophotometer) using DMSO as solvent (Figs. **1**-**6**).

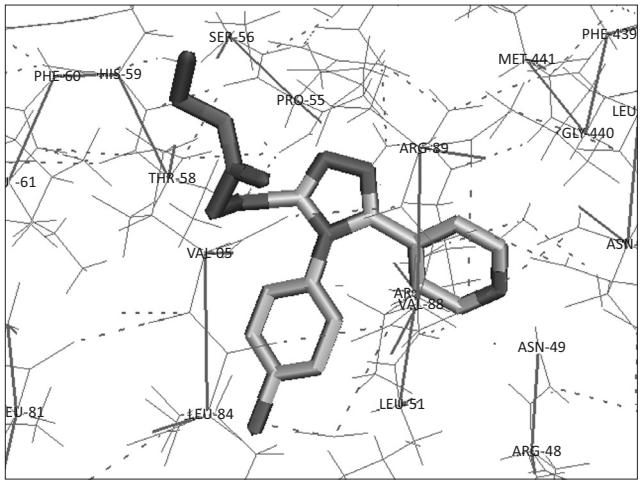


Fig. (1). Binding interactions of compound 2a with COX-I. Picture from Discovery studio visualizer showing Ethyl {[4-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate binding interactions with the binding site of COX-I.

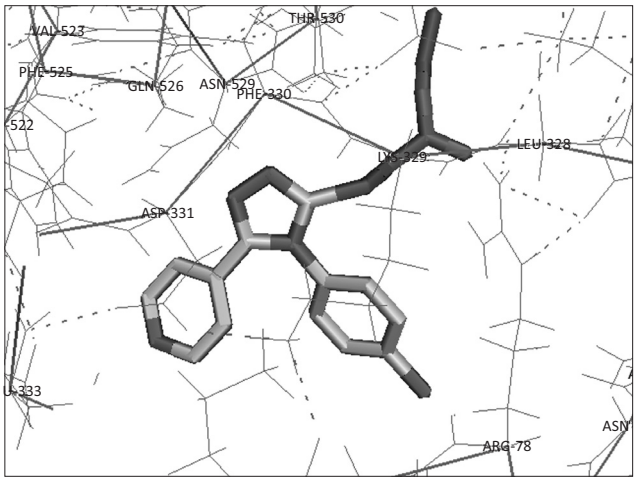


Fig. (2). Binding interactions of compound 2a with COX-II. Picture from Discovery studio visualizer showing Ethyl {[4-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate binding interactions with the binding site of COX-II.

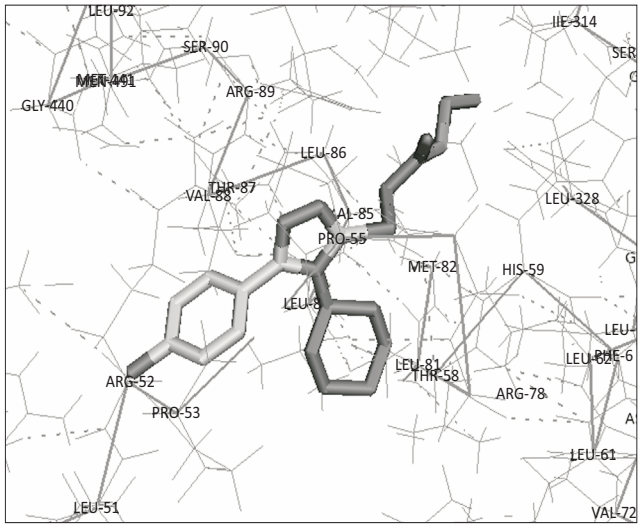


Fig. (3). Binding interactions of compound 2e with COX-I. Picture from Discovery studio visualizer showing Ethyl {[4-cyclohexyl-5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate binding interactions with the binding site of COX-I.

2.1.1. Synthesis of 4,5-disubstituted-4H-1,2,4-triazole-3-thiols 1(a-i)

Equimolar mixture of carboxylic acid hydrazide and isothiocynate (0.03 mol) was refluxed with stirring for 6-8 hours. After completion, the reaction mixture was cooled and the corresponding thiosemicarbazide was separated and dissolved in 4N NaOH solution. The solution was refluxed for 4-5 hours and after completion, it was filtered and the filterate acidified to pH of 4-5. The solid separated was filtered and recrystallized from ethanol.

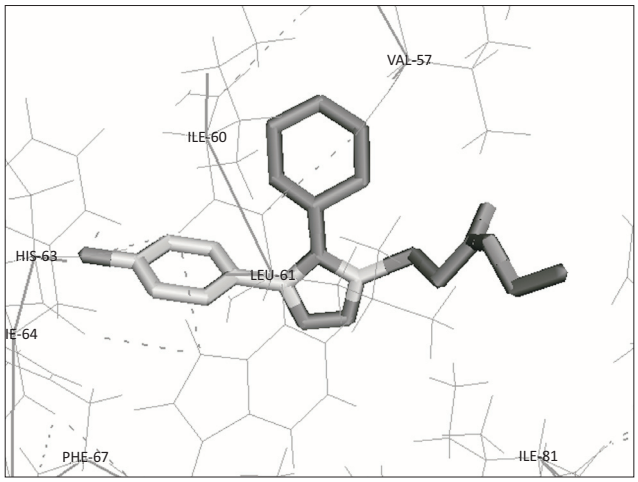


Fig. (4). Binding interactions of compound 2e with COX-II. Picture from Discovery studio visualizer showing Ethyl {[4-cyclohexyl-5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate binding interactions with the binding site of COX-II.

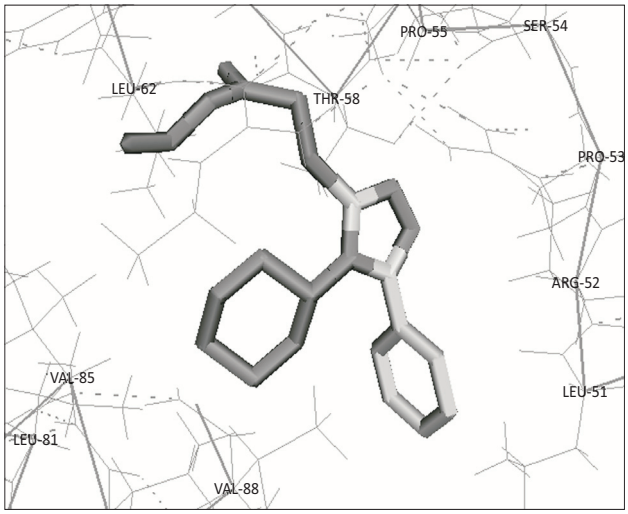


Fig. (5). Binding interactions of compound 2g with COX-I. Picture from Discovery studio visualizer showing Ethyl [(4-cyclohexyl-5-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetate binding interactions with the binding site of COX-I.

2.1.1.1. 4-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (1a)

Yield 71.7%, M.p.: 240-241°C, Rf 0.85 (ethyl acetate: petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.52-8.01 (m, 6H, ArH), 8.65 (d, 2H, ArH), 11.65 (bs, 1H, NH/SH); IR cm^{-1}: 3171 (NH), 1559 (C=N), 1289 (C=S).

2.1.1.2. 4-ethyl-5-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol (1b)

Yield 69.7%, M.p.: 147-148°C, Rf 0.83 (ethyl acetate: petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.32 (t, J = 7.0 Hz, 3H, CH_3), 2.3 (s, 3H, Ar-CH_3), 4.15 (q, J = 7.1 Hz, 2H, CH2), 7.34-7.48 (m, 4H, ArH), 11.75 (bs, 1H, NH/SH); IR cm^{-1}: 3199 (NH), 1579 (C=N), 1404 (C=C), 1277 (C=S).

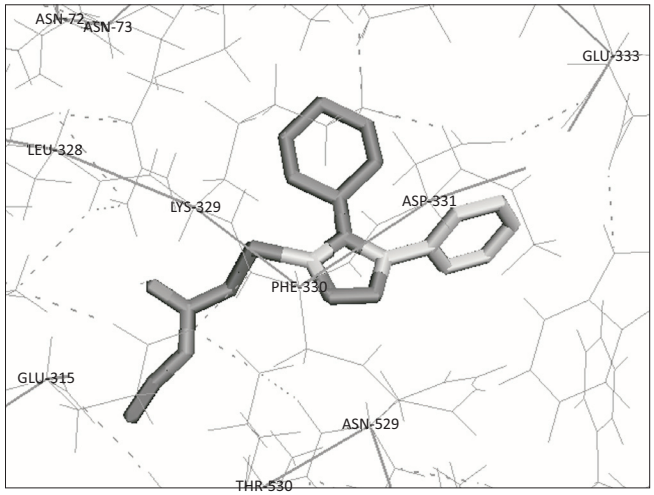


Fig. (6). Binding interactions of compound 2g with COX-II. Picture from Discovery studio visualizer showing Ethyl [(4-cyclohexyl-5-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetate binding interactions with the binding site of COX-II.

2.1.1.3. 4-(4-fluorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (1c)

Yield 74.2%, M.p.: 267-269°C, Rf 0.78 (ethyl acetate : petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.52-8.56 (m, 6H, ArH), 8.65 (d, 2H, ArH), 11.60 (bs, 1H, NH/SH); IR cm^{-1}: 3212 (NH), 1588 (C=N), 1397 (C=C), 1289 (C=S).

2.1.1.4. 4-hexyl-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazole-3-thiol (1d)

Yield 74.4%, M.p.: 191-192°C, Rf 0.86 (ethyl acetate: petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm:0.85 (t, 3H, CH_3), 1.55-1.71 (m, 6H, hexyl CH_2), 1.85 (m, 2H, hexyl CH2, 3.78 (s, 9H, OCH_3), 4.16 (t, 2H, NH-CH_2), 6.98 (s, 2H, ArH), 11.54 (bs, 1H, NH/SH); IR cm^{-1}: 3219 (NH), 1602 (C=N), 1421 (C=C), 1296 (C=S).

2.1.1.5. 4-cyclohexyl-5-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol (1e)

Yield 70.1%, M.p.: 178-180°C, Rf 0.88 (ethyl acetate: petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.28-1.75 (m, 10H, cyclohexyl CH_2), 2.31 (s, 3H, Ar-CH_3), 4.45 (m, 1H, cyclohexyl CH), 7.31-7.40 (m, 4H, ArH), 11.63 (bs, 1H, NH/SH); IR cm^{-1}: 3221 (NH), 1581 (C=N), 1409 (C=C), 1279 (C=S).

2.1.1.6. 4-hexyl-5-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol (1f)

Yield 66.34%, M.p.: 90-91°C, Rf 0.83 (ethyl acetate: petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm): 0.86 (t, 3H, CH_3), 1.52-1.70 (m, 6H, hexyl CH_2), 1.79 (m, 2H, hexyl CH_2), 2.31 (s, 3H, Ar-CH_3), 4.14 (t, 2H, NH-CH_2), 7.36-7.42 (m, 4H, ArH), 11.65 (bs, 1H, NH/SH); IR cm^{-1} : 3239 (NH), 1596 (C=N), 1265 (C=S).

2.1.1.7. 4-cyclohexyl-5-phenyl-4H-1,2,4-triazole-3-thiol (1g)

Yield 70.3%, M.p.: 198-200°C, Rf 0.77 (ethyl acetate: petroleum ether 2:1); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.28-

1.81 (m, 10H, cyclohexyl CH_2), 4.38 (m, 1H, cyclohexyl CH), 7.32-7.38 (m, 5H, ArH), 11.69 (bs, 1H, NH/SH); IR cm^{-1} : 3207 (NH), 1583 (C=N), 1418 (C=C), 1294 (C=S).

2.1.1.8. 5-benzyl-4-cyclohexyl-4H-1,2,4-triazole-3-thiol (1h)

Yield 68.1%, M.p.: 156-157°C, Rf 0.79 (ethyl acetate: petroleum ether 2:1); ^1H NMR (DMSO-d_6) δ (ppm): 1.28-1.81 (m, 10H, cyclohexyl CH_2), 3.72 (s, 2H, CH_2), 4.38 (m, 1H, cyclohexyl CH), 7.32-7.40 (m, 5H, ArH), 11.83 (bs, 1H, NH/SH); IR cm^{-1}: 3193 (NH), 1611 (C=N), 1414 (C=C), 1288 (C=S).

2.1.1.9. 4-hexyl-5-phenyl-4H-1,2,4-triazole-3-thiol (1i)

Yield 71.7%, M.p.: 109-110°C, Rf 0.91 (ethyl acetate: petroleum ether 2:1); ^1H NMR (DMSO-d_6) δ (ppm): 0.80 (t, 3H, J = 6.5Hz, CH_3), 1.20-1.69 (m, 6H, hexyl CH_2), 1.81 (m, 2H, hexyl CH2), 4.10 (t, 2H, N-CH_2), 7.42-7.54 (m, 5H, ArH), 11.70 (bs, 1H, NH/SH); IR cm^{-1} : 3251 (NH), 1589 (C=N), 1414 (C=C), 1275 (C=S).

2.1.2. Formats: Synthesis of Ethyl [(4,5-disubstituted-4H-1,2,4-triazol-3-yl)sulfanyl]acetate Derivatives 2(a-i)

To a solution of 4,5-disubstitutedl-4H-1,2,4-triazole-3-thiol derivatives (0.003 mol) in absolute ethanol (20 ml), 0.003 moles of KOH were added and refluxed for 30 minutes. After cooling 0.003 moles of ethyl chloroacetate were added and reaction mixture was refluxed further for 8-10 hours. The progress of reaction was monitored by TLC (Petroleum ether: Ethyl acetate 1:2). After completion of reaction, the solvent was removed under reduced pressure to give the solid product. The crude product was recrystallized from ethanol: water (1:1).

2.1.2.1. Ethyl {[4-(4-chlorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (2a)

Yield: 72.6%, M.p.: 141-142°C. Analysis for $C_{17}H_{15}ClN_4O_2S$ (374.84) Calculated: C 54.47%, H 4.03, N 14.95%; Found C 54.51%, H 4.01, N 14.89%. ^1H NMR (DMSO-d6) δ (ppm): 1.19 (t, 3H, J = 7.1 Hz, CH_3), 4.01 (s, 2H, SCH_2), 4.13 (q, 2H, J = 7.2 Hz, OCH_2), 7.73-7.92 (m, 6H, ArH), 8.67 (d, 2H, ArH); ^{13}C NMR (DMSO-d6) δ (ppm): 171.8 (C=O), 152.8 (triazole-C_5), 145.3 (triazole-C_3), Ar C (pyridine) [128.6 (C1), 122.3 (C_2 and C_6), 151.5 (C_3 and C_5)] Ar C [116.4 (C_1), 128.4 (C_2 and C_6), 117.1 (C_3 and C_5), 161.4 (C_4)], 63.8 (OCH_2), 32.1(SCH_2), 14.2 (CH_3). FTIR cm^{-1}: 1708 (C=O ester), 1609 (C=N), 1199 (C-O).

2.1.2.2. Ethyl {[4-ethyl-5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (2b)

Yield: 70.5%, M.p.: 125-126°C. Analysis for $C_{15}H_{19}N_3O_2S$ (305.40) Calculated: C 58.99%, H 6.27, N 13.76%; Found C 58.78%, H 6.13, N 13.57% ^1H NMR (DMSO-d6) δ (ppm): 1.17 (t, 3H, J = 7.0 Hz, CH_3), 1.81 (t, 3H, J = 6.9 Hz, CH_3.), 2.39 (s, 3H, $ArCH_3$), 3.68 (s, 2H, SCH_2), 4.01 (q, 2H, J = 7.1 Hz, CH_2), 4.11 (q, 2H, J = 7.0 Hz, N-CH_2), 7.34-7.42 (m, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.8 (C=O), 155.8 (triazole-C_5), 144.3 (triazole-C_3), Ar C [118.7 (C1), 129.6 (C_2 and C_6), 118.3 (C_3 and C_5), 161.4 (C_4)], 64.1 (OCH_2), 46.7 (N-CH_2), 32.1(SCH_2), 23.4 (Ar-CH_3), 15.8 (CH_3), 14.2 (CH_3). FTIR cm^{-1}: 1713 (C=O ester), 1170 (C-O) 1611 (C=N).

2.1.2.3. Ethyl {[4-(4-fluorophenyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (2c)

Yield: 76.7%, M.p.: 138-140°C. Analysis for $C_{17}H_{15}FN_4O_2S$, Calculated: C 56.97%, H 4.22, N 15.63%. Found C 56.91%, H 4.17, N 15.64%. ^1H NMR (DMSO-d6) δ (ppm): 1.3 (t, 3H, J = 7.2 Hz, CH_3), 4.11 (s, 2H, SCH_2.), 4.20 (q, 2H, J = 7.4 Hz,CH_2), 7.23-7.35 (m, 6H, ArH), 8.50 (d, 2H, ArH); 13C NMR (DMSO-d_6) δ (ppm): 173.1 (C=O), 156.9 (triazole-C_5), 144.1 (triazole-C_3), Ar C (pyridine) [125.1 (C1), 125.7 (C_2 and C_6), 154.9 (C_3 and C_5)] Ar C [117.2 (C_1), 123.8 (C_2 and C_6), 118.3 (C_3 and C_5), 163.3 (C4)], 61.6 (OCH_2), 32.0 (SCH_2), 14.2 (CH_3). IR cm^{-1}: 1729 (C=O ester), 1157 (C-O) 1598 (C=N).

2.1.2.4. Ethyl{[4-hexyl-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazole-3-yl]sulfanyl}acetate (2d)

Yield: 67.7%, M.p.: 117-118°C. Analysis for $C_{21}H_{31}N_3O_5S$ (437.55) Calculated: C 57.64%, H 7.14, N 9.6%; Found C 57.60%, H 7.13, N 9.6%. 1H NMR (DMSO-d6) δ (ppm): 0.86 (t, 3H, J = 7.1 Hz, CH_3), 1.16 (t, J = 7.2 Hz, 3H, CH_3.), 1.28-1.32 (m, 6H, hexylCH_2), 1.91 (m, 2H, CH_2), 3.61 (s, 6H, OCH_3), 3.73 (s, 2H, SCH_2), 3.85 (s, 3H, OCH_3), 3.91 (t, 2H, J = 7.0 Hz, NCH2), 4.15 (q, 2H, J = 7.2Hz, OCH_2), 6.9 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.9 (C=O), 155.3 (triazole-C_5), 144.8 (triazole-C_3), Ar C [118.8 (C1), 129.9 (C_2 and C_6), 118.6 (C_3 and C5), 161.4 (C_4)], $ArOCH_3$ [58.4 (C_3-C_5), 61.8 (C1)], 32.1 (SCH_2), 64.1 (OCH_2), Hexyl C [49.3 (C_1), 27.43, 26.8, 31.6, 23.7, 14.6], 14.27 (CH_3); FTIR cm^{-1}: 1723 (C=O ester), 1077 (C-O) 1587 (C=N).

2.1.2.5. Ethyl {[4-cyclohexyl-5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (2e)

Yield: 71.8%, M.p.: 128-129°C. Analysis for $C_{19}H_{25}N_3O_2S$ (359.49) Calculated: C 63.48%, H 7.01, N 11.69%; Found C 63.49%, H 7.01, N 11.63%.^1H NMR (DMSO-d_6) δ (ppm): 1.23 (t, 3H, J = 7.0 Hz, CH_3), 1.28-1.75 (m, 10H, cyclohexyl CH_2), 2.38 (s, 3H, $ArCH_3$), 3.67 (s, 2H, SCH_2), 4.04 (q, 2H, J = 7.1 Hz, CH_2), 4.13 (m, 1H, cyclohexyl CH), 7.35-7.54 (m, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.0 (C=O), 155.2 (triazole-C_5), 144.5 (triazole-C_3), Ar C [118.7 (C1), 129.5 (C_2 and C_6), 118.6 (C_3 and C_5), 161.6 (C4)], 64.1 (OCH_2), Cyclohexyl C [58.4 (C1), 31.4, 27.4, 26.9], 23.4 ($ArCH_3$), 32.1 (SCH_2), 14.2 (CH3). FTIR cm^{-1}: 1730 (C=O ester), 1064 (C-O) 1653 (C=N).

2.1.2.6. Ethyl {[4-hexyl-5-(4-methylphenyl)-4H-1,2,4-triazole-3-yl]sulfanyl}acetate (2f)

Yield: 70.1%, M.p.: 130-131°C. Analysis for $C_{19}H_{27}N_3O_2S$: (361.50) Calculated: C 66.13%, H 7.535%, N 11.62%; Found C 66.09%, H 7.48, N 11.60%. ^1H NMR (DMSO-d6) δ (ppm): 0.87 (t, 3H, J= 7.2 Hz, hexyl CH3), 1.19 (t, 3H, J = 7.3 Hz, CH_3), 1.29-1.34 (m, 6H, hexyl CH_2), 1.83 (m, 2H, hexyl CH_2), 2.26 (s, 3H, $ArCH_3$), 3.77 (s, 2H, SCH_2), 4.13 (q, 2H, J = 7.4 Hz, OCH_2), 4.18 (t, 2H, J= 7.2 Hz, NCH_2), 7.71-7.82 (m, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.5 (C=O), 155.8 (triazole-C_5), 144.4 (triazole-C_3), Ar C [118.7 (C1), 129.7 (C_2 and C_6), 118.6 (C_3 and C_5), 161.4 (C_4)], Ar C [118.8 (C1), 129.7 (C_2 and C_6), 118.5 (C_3 and C_5), 161.4 (C4)], 63.4 (OCH_2), Hexyl C [49.3 (C1), 27.4, 26.8, 31.6, 23.7, 14.6] 23.41 (Ar-CH_3), 32.1 (SCH_2), 14.27 (CH_3) IR cm^{-1}: 1731 (C=O ester), 1086 (C-O) 1597 (C=N).

2.1.2.7. Ethyl [(4-cyclohexyl-5-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetate (2g)

Yield: 72.1%, M.p.: 136-137°C. Analysis for $C_{18}H_{23}N_3O_2S$ (345.46) Calculated: C 62.58%, H 6.71, N 12.16%: Found C 62.55%, H 6.69, N 12.13%; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15 (t, 3H, J = 7.0 Hz, CH_3), 1.37-1.52 (m, 6H, cyclohexyl H), 1.96 (m, 4H, cyclohexyl H), 3.95 (s, 2H, SCH_2), 4.01 (q, 2H, J = 7.2 Hz, OCH_2), 4.65 (m, 1H, CH-N), 7.77-7.90 (m, 5H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.6 (C=O), 155.8 (triazole-C_5), 144.3 (triazole-C_3), Ar C [118.7 (C1), 129.76 (C_2 and C_6), 118.6 (C_3 and C_5), 161.4 (C4)], 64.2 (OCH_2), cyclohexyl C [58.43 (C_1), 31.45, 27.43, 26.98], 14.27 (CH_3); FTIR cm^{-1}: 1738 (C=O ester), 1083 (C-O), 1643 (C= N).

2.1.2.8. Ethyl [(5-benzyl-4-cyclohexyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetate (2h)

Yield: 69.8%, M.p.: 136-137°C. Analysis for $C_{19}H_{25}N_3O_2S$ (359.49) Calculated: C 63.48%, H 7.01, N 11.69% Found: C 63.44%, H 7.02, N 11.67%. ^{1}H NMR (DMSO-d6) δ (ppm): 1.07 (t, 3H, J = 7.4 Hz, CH_3), 1.36-1.41 (m, 10H, cyclohexyl H.), 2.63 (s, 2H, $ArCH_2$), 3.86 (s, 2H, SCH_2), 4.01 (q, 2H, J = 7.5 Hz, OCH_2), 4.10 (m, 1H, CH-N), 7.2-7.3 (m, 5H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.8 (C=O), 155.8 (triazole-C_5), 144.3 (triazole-C_3), Ar C [141.7 (C1), 129.7 (C_2 and C_6), 128.6 (C_3 and C_5), 129.4 (C4)], 64.1 (OCH_2), cyclohexyl C [58.43 (C1), 31.45, 27.43, 26.98], 32.47 ($ArCH_2$), 32.2 (SCH_2), 14.27 (CH_3). FTIR cm^{-1}: 1741 (C=O ester), 1146 (C-O) 1619 (C=N).

2.1.2.9. Ethyl [(4-hexyl-5-phenyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetate (2i)

Yield: 73.3%, M.p.: 106-107°C. Analysis for $C_{18}H_{25}N_3O_2S$, (347.48) Calculated: C 62.22%, H 7.25, N 12.09%; Found: C 62.19%, H 7.24, N 12.05%. ^{1}H NMR (DMSO-d_6) δ (ppm): 0.86 (t, 3H, J = 7.1 Hz, hexyl CH_3), 1.23 (t, 3H, J = 7.2 Hz, CH_3), 1.25-1.39 (m, 6H, hexyl CH_2), 1.83 (m, 2H, hexyl CH_2), 3.85 (s, 2H, SCH_2), 4.13 (q, 2H, J = 7.1 Hz, NCH_2), 4.22 (t, 2H, J= 7.2 Hz, OCH_2), 7.47-7.71 (m, 5H, ArH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.8 (C=O), 155.6 (triazole-C_5), 144.3 (triazole-C_3), Ar C [118.7 (C1), 129.7 (C_2 and C_6), 118.6 (C_3 and C_5), 161.4 (C_4)], 63.5 (OCH2), Hexyl C [49.37 (C_1), 27.4, 26.8, 31.6, 23.7, 14.6], 32.1 (SCH_2), 14.27 (CH_3). FTIR cm^{-1}: 1738 (C=O ester), 1122 (C-O) 1586 (C=N).

2.2. Pharmacological Screening

2.2.1. In vivo Anti-inflammatory Activity

The anti-inflammatory potential was assessed using carrageenan-induced rat paw oedema method. Sprague Dawley rats (140-180g) were divided into eleven groups each group having 5 animals. Animals were starved overnight with water *ad libitum* prior to the day of the experiment. Group 1 taken as negative control group & group 11 was taken as positive control which received diclofenac sodium 10 mg/kg, group 2 to 10 received synthesized compounds 2a to 2i 30mg/kg respectively. The initial volume of right hind paw of Sprague Dawley rat was determined using plethysometer without administration of drug. The drug was administered intraperitoneally in 0.5% aqueous carboxymethyl cellulose suspension. After 30 min of drug administration, car rageenan (0.1 ml, 1% w/v solution in normal saline) as phlogistic agent was injected into the plantar surface of the right hind paw of every animal. The volume of the right hind paw was determined before phlogistic agent injection and then after 1 hour, 2 hours, 3 hours and 4 hours. The mean difference in the volume of the right hind paw of rats was compared with control and standard. Percent inhibition was calculated by;

% inhibition = (Vc-Vt / Vc) x100

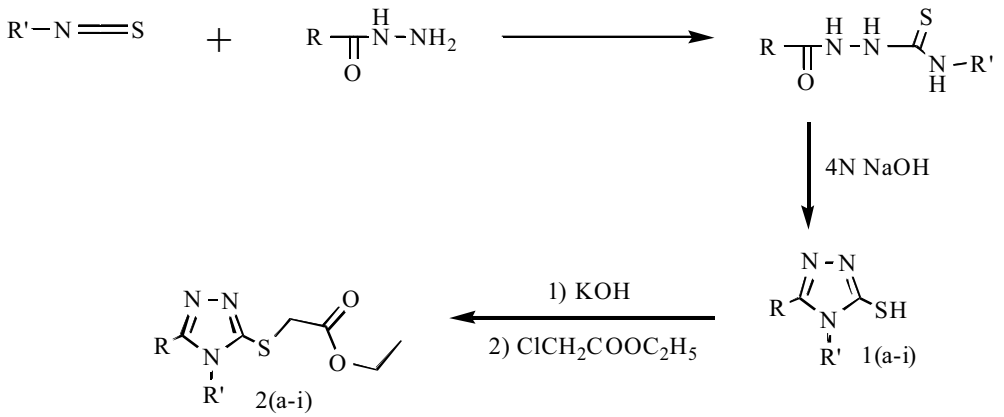
Where Vc is the mean relative change in paw oedema volume in control group and Vt is mean relative change in paw edema volume in test group [29].

2.2.2. Antimicrobial Assay

The antibacterial and antifungal activity of synthesized triazole esters 2(a-i) was evaluated by agar well diffusion method [30, 31]. Three bacterial strains namely *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 6538) and two fungal strains, *Candida glabrata* (ATCC 62934) and *Candida albicans* (ATCC 60387) were used. Ciprofloxacin was taken as positive control for antibacterial activity and Nystatin was used as positive control for antifungal activity. DMSO was taken as a negative control in both antibacterial and antifungal assays. Nutrient agar/ Sabouraud dextrose agar and nutrient broth were sterilized in the autoclave at 121°C for 15 minutes. Sterile agar plates were prepared by pouring 25mL sterile nutrient agar into each plate and kept in an incubator at 37°C for 24 hours. The fresh bacterial/fungal strains were prepared in sterile nutrient broth and incubated at 37°C for 24hrs. Lawn of each bacterial/fungal stain was made on the agar plates and wells were made using sterile borer (6mm). With the help of micropipette 50µg/ml of the synthesized compounds, ciprofloxacin and DMSO were poured into the individual well. The concentration of test compounds and positive control was 10mg/ml and 100µg/ml respectively. Finally the plates were incubated for 24 hours at 37°C in case of bacteria and at 28°C for four days in antifungal activity. The zones of inhibition were measured accordingly.

2.3. Docking Studies

ChemSketch 12.0 was used for drawing nine different ligands. Different derivatives of main nucleus ethyl (4,5-disubstituted-4H-1,2,4-triazol-3-ylsulfanyl)acetate were designed by substituting different groups at 4 and 5 positions of triazole. Uniprot (www.uniprot.org) [32] was used for obtaining the sequences of COX-1 (M1-L599: (ID No. P23219)). Three dimensional (3D) homologous sequences of structures present in PDB were obtained by submitting sequences to NCBI given protein specific iteration (PSI)-BLAST [33].Structure having PDB ID: 1CQE (The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1 resolved at 3.1 Å) [34] with 93% sequence similarity with human COX-1. This three-dimensional structure was used as a model/template for COX-1 homology modelling. Structural sequencing between the COX-I and its template was done by a web server expresso.



	R	R'
1a, 2a	4-C_5H_4N	4-ClC_6H_4
1b, 2b	4-$CH_3C_6H_4$	C_2H_5
1c, 2c	4-C_5H_4N	4-FC_6H_4
1d, 2d	3,4,5-$OCH_3C_6H_2$	C_6H_{13}
1e, 2e	4-$CH_3C_6H_4$	C_6H_{11}
1f, 2f	4-$CH_3C_6H_4$	C_6H_{13}
1g, 2g	C_6H_5	C_6H_{11}
1h, 2h	$C_6H_5CH_2$	C_6H_{11}
1i, 2i	C_6H_5	C_6H_{13}

Scheme 1. Synthesis of 4,5-disubstituted-1,2,4-triazole-3-thioacetate derivatives

(http://www.tcoffee.org/Projects/expresso/) [35]. Homology modeling of COX-1 was done by the use of MODELLER. Hundred models were prepared which were divided into different groups depending upon RMSD among the residues in their structures by the use of NMRCLUST. Typical models were obtained from each group on the basis of their potential energies and scores given by MODELLER, ERRAT, Ramachandran plot, Qmean, PROCHECK and PROSA. Similarly, the structure of COX-2 with PDB ID: 5F1A (The Crystal Structure of Salicylate Bound to Human Cyclooxygenase-2 resolved at 2.38 Å) [36] was used for additional analyzation.

The re-docking procedure is used to validate docking protocols [37].

For validation of docking protocols, we used the COX-II crystallographic structure with a PDB ID: 5F1A, docking protocols were performed into the active site of COX-II and pose were compared with the original crystallographic structure. Re-docked ligand binds to pCOX-II with similar binding mode and interaction compared to the original crystallographic structure.

Protein-Ligand docking was carried out using Auto dockvina [38], Open Babel GUI [39], Discovery Studio 4.1 Visualizer and Pymol were utilized for preparing and conversion of protein molecules by removing the ligand and water. The results obtained were shown in the form of binding affinity.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The purpose of the present work was the synthesis, spectral analysis and screening of antimicrobial and anti-inflammatory activities of a series of Ethyl [(4,5-disubstituted-4H-1,2,4-triazol-3-yl)sulfanyl]acetate derivatives (2a-i). The target compounds were synthesized according to steps outlined in Scheme **1**. In the first step different carboxylic acid hydrazides were condensed with respective isothiocyanates to yield thiosemicarbazides which were cyclized in alkaline media to the corresponding 4, 5-disubstituted-4H-1, 2, 4-triazole-3-thiols 1(a-i). The triazoles were finally reacted with KOH and ethyl chloroacetate to yield the target thioacetates 2(a-i). Physical data of target triazole derivatives is presented in Table **1**.

All the synthesized compounds are stable solids and dissolved in DMSO at room temperature. Each compound yielded a single spot in a different solvent system establishing the purity of synthesized compounds. The triazoles (1a-i) are known compounds and characterized by comparing their melting points and spectral data with the reported values [40]. FTIR data showed strong peak of C=N in each compound. The strong peak of C=S was also observed in each case indicating the presence of thione form as the predominant form. In the 1H NMR spectra of triazoles downfield signal of tautomeric proton NH/SH was observed confirming the formation of products.

The final compounds 2(a-i) were characterized by FTIR, 1H NMR, 13C NMR and elemental analysis data. FTIR spectral data of ester derivatives 2(a-i) showed a strong peak of C=O (ester) in each compound in the range 1708-1738 cm-1. Strong peak of C=N was also observed in each compound in the range 1609-1619 cm -1. Also appearance of C-O peak indicated the formation of ester derivatives. 1H NMR data confirmed the formation of ethyl [(4,5-disubstituted-4H-1,2,4-triazol-3-yl)sulfanyl]acetate derivatives. Triplet of CH3 protons was observed at 1.3 ppm in each compound. The quartet of CH2 protons of ester moiety appeared at higher chemical shift in the range of 4.01 ppm to 4.13 ppm which was deshielded due to the electronegative effect of adjacent oxygen atom. Another evidence for the formation of sulfanyl acetate derivatives was the appearance of singlet of S-CH2 in all the newly synthesized compounds in the range of 3.68 ppm to 4.13 ppm due to adjacent electronegative sulphur. In the 13C NMR spectra the most downfield peak of ester carbonyl confirmed the formation of ester derivatives. The two carbons of triazole moiety resonated in the range 144.1-155.8 ppm. Also, methylene protons of ester moiety attached to oxygen were observed at 61.1-64.1 ppm. Elemental analysis data further confirmed the formation of thioacetate derivatives of triazoles.

3.2. Pharmacology

3.2.1. In vivo Anti-inflammatory Activity

The acute anti-inflammatory activity of synthesized target compounds 2(a-i) was determined *in vivo* using the carrageenan-induced paw edema method in rats. The compounds were tested at dose level of 30 mg/kg. The volume of right hind paw of rats was measured before carrageenen injection and then after 1, 2, 3 and 4 hours. The mean difference in the volume of the right hind paw of rats was compared with control and standard. The results for the anti-inflammatory activity of compounds (at 30 mg/kg) and the potent anti-inflammatory drug indomethacin (at 10 mg/kg) are listed in Table **2**. The best activity was shown by compounds 2h which produced strong inhibition of carrageenan-induced paw edema of 62.5% at 30 mg/kg dose. The compound 2h produced inhibition quite comparable with that of standard (Diclofenac), which was 46.6 % inhibition after 1 hour, 56.25 % inhibition after 2 hours, 57.8% inhibition after 3 hours and 62.5% inhibition after 4 hours while standard drug produced 26.49% inhibition after 1 hour, 39.50% inhibition after 2 hours, 57.37% inhibition after 3 hours and 74.07% after 4 hours. Compounds 2a, 2b, 2c and 2e also showed good activities *i.e*., 51.23%, 50.0%, 56.23% and 56.25% respectively after 4 hours. Compound 2g and 2f were moderately active while 2d and 2i showed weak anti-inflammatory activity. All the compounds were also docked against COX-I and COX-II to find their potential interactions with these two targets of inflammation pathway. All the compounds showed good binding affinities with both COX-I and COX-II especially with COX-I. Compound 2h having maximum anti-inflammatory activity also exhibited high binding affinity of -6.5 and -7.7 kcal/mol with COX-II and COX-I respectively. Compound 2c showed maximum binding affinity with COX-II *i.e*. -6.7 kcal/mol while with compound 2g has highest binding affinity of -8.0 with COX-I. The structure-activity relationship of the tested triazole deriva¬tives 2(a-i) revealed that hexyl-substituted triazoles 2e and 2h were the most active compounds with maximum inhibition of 56.25 and 62.50% respectively. Also 4-halosubstituted triazoles 2a and 2c exhibited good activities while cyclohexyl containing triazoles 2d, 2f and 2i showed weak activity. In general, it can be inferred from the results that hexyl substitution is the most beneficial in terms of anti-inflammatory activity of triazole thioacetate derivatives.

3.2.2. Antimicrobial Assay

All newly synthesized ester derivatives were also explored for their antibacterial and antifungal potential. Agar well diffusion method was used to determine the antimicrobial activity of the synthesized compounds. *Staphylococus aureus* (ATCC 6538), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC 10145), were selected bacterial stains while antifungal stains were *Candida albicans*

Table 1. Physical data of compounds 2(a-i).

Comp	Molecular Formula	MW Calculated (gm/mol)	M.P (°C)	Physical State	Color	% Yield	Rf Value
2a	C17H15ClN4O2S	374.84	141	Powder	White	72.6	0.52
2b	C15H19N3O2S	305.40	125	Powder	White	70.1	0.38
2c	C17H15FN4O2S	358.39	138	Powder	White	76.7	0.41
2d	C21H31N3O5S	437.55	117	Crystalline Powder	White	67.4	0.28
2e	C19H25N3O2S	359.49	128	Crystalline Powder	White	71.8	0.33
2f	C19H27N3O5S	361.50	130	Crystalline Powder	White	69.6	0.55
2g	C18H23N3O2S	345.46	136	Powder	White	72.1	0.25
2h	C19H25N3O2S	359.49	125	Powder	White	69.8	0.28
2i	C18H25N3O2S	347.48	106	Crystalline powder	White	73.3	0.43

Table 2. Anti-inflammatory activity of synthesized compounds 2(a-i).

Compounds	Normal Paw Volume	Mean Paw Volume ± SEM (ml) and % Inhibition				
		Time after Carrageenan Injection				
		0 hr	1 hr	2 hr	3 hr	4 hr
Control	0.251±0.008	0.381±0.006	0.402±0.015	0.413±0.009	0.441±0.011	0.413±0.013
Standard	0.212±0.013	0.33±0.015	0.323±0.021 (26.49%)	0.310±0.011 (39.50%)	0.293±0.019 (57.37%)	0.254±0.016 (74.07%)
2a	0.203±0.017	0.35±0.0037	0.327±0.005 (17.88%)	0.304±0.004 (37.65%)	0.292±0.006 (53.16%)	0.282±0.005 (51.23%)
2b	0.31±0.005	0.44±0.009	0.44±0.011 (13.3%)	0.41±0.013 (37.5%)	0.40±0.008 (52.63%)	0.39±0.007 (50.0%)
2c	0.222±0.011	0.34±0.03	0.326±0.029 (31.12%)	0.321±0.028 (38.89%)	0.313±0.03 (52.10%)	0.293±0.026 (56.17%)
2d	0.26±0.012	0.39±0.008	0.38±0.011 (20.0%)	0.38±0.007 (25.0%)	0.39±0.013 (31.58%)	0.38±0.009 (20.0%)
2e	0.28±0.013	0.41±0.016	0.39±0.011 (26.67%)	0.37±0.009 (43.75%)	0.37±0.012 (52.63%)	0.35±0.008 (56.25%)
2f	0.30±0.006	0.45±0.015	0.46±0.011 (-6.67%)	0.44±0.009 (12.5%)	0.43±0.01 (31.57%)	0.39±0.0011 (43.75%)
2g	0.201±0.011	0.38±0.027	0.344±0.018 (5.29%)	0.353±0.021 (6.17%)	0.322±0.011 (36.31%)	0.301±0.017 (38.27%)
2h	0.29±0.018	0.39±0.014	0.37±0.009 (46.6%)	0.36±0.007 (56.25%)	0.37±0.012 (57.8%)	0.35±0.015 (62.5%)
2i	0.25±0.008	0.40±0.011	0.40±0.01 (0.0%)	0.39±0.008 (12.5%)	0.39±0.012 (26.3%)	0.38±0.009 (18.75%)

Table 3. Antimicrobial activity of synthesized compounds 2(a-i).

Compounds	Antimicrobial Activity of Synthesized Compounds (Zone of Inhibition mm)				
	Bacterial Stains			Fungal Stains	
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida glabrata*	*Candida albicans*
2a	16.2	7.2	-------	7.7	6.8
2b	18.4	7.9	-------	8.4	----
2c	17.4	8.3	-------	11.3	7.1
2d	16.6	7.6	-------	----	7.9
2e	15.2	8.0	-------	9.4	---
2f	15.3	8.1	-------	7.9	---
2g	16.3	8.3	-------	----	---
2h	13.1	7.5	-------	7.3	11.2
2i	14.2	7.7	-------	8.2	9.6
Ciprofloxacin	23.7	24.0	23.9	-------	-------
Nystatin	------	-------	-------	23.2	24.1

(ATCC 60387) and *Candida glabrata* (ATCC 62934). Ciprofloxacin was used as a reference for antibacterial assay whereas; Nystatin was used as a reference for antifungal assay.

All the synthesized ester derivatives showed moderate to good antibacterial potential against gram negative bacterial species and didn't show any remarkable potential against gram positive species. The results of antimicrobial activity are given in Table **3**. Compounds 2b, 2d, 2f and 2i having alkyl group at 4 position showed good activity against *Escherichia coli* with the zone of inhibition (mm) 18.4, 16.6, 15.3 and 14.2 respectively. Compound 2b with ethyl group at 4 position exhibited highest activity, substituting position 4 with higher alkanes like hexanes showed a slight decrease in activity. Substitution of phenyl or substituted phenyl at position 4 also showed good activity against *Escherichia coli.* All the compounds exhibited weak activity against *Pseudmonas aeruginosa*. Presence of benzyl ring at position 5 in compound 2h showed decreased activity against both Escherichia coli and Pseudmonas aeruginosa with zone of inhibition 13.1 mm and 7.5 mm respectively. None of the compounds showed activity against *Staphylococcus aureus*. Against fungal stains all the compounds 2(a-i) showed very unpredictable results. By substitution at 4 and 5 position no general trend has been shown regarding antifungal activity. Compounds 2a, 2c, 2h and 2i showed weak potential against both *Candida glabarata* and *Candida albicans*. Compound 2e and 2f only showed mild activity against *Candida glabarata* whereas compound 2d showed slight potential against *Candida albicans*. Rest of the compounds did not show any potential against both fungal stains.

3.3. Docking Analysis

Computational ligand-target binding approach was employed in analyzing the complexes of synthesized compounds with target proteins (COX-I and COX-II) in order to interpret the structural basis of target protein specificity. Compounds were docked into the active site of COX-1 surrounding by Val85, Thr 58, Arg 89, Val 85, Asn 49, Phe 68, Ser 56, Met 441, Phe 439, Leu 84, Gly 440, His 59, Phe 60 and with COX-2 residues Phe 330, Asp 331, Lys 329, Leu 328, Arg 89, Asn 49, Thr 53, Pro 52, Gly 323, Val 85, Arg322, Val523, His 58, Glu 493. Leu534. Results of docking of compounds 2 (a-i) in the form of binding affinities (kcal/mol) are given in Table **4**. Docking results showed that all the compounds 2 (a-i) bind to both COX-I and COX-II at their active site successfully. Compound 2e showed the maximum binding affinity against COX-II, found to be -7.9Kcal/mol. While compound 2e also showed the maximum binding affinity against COX-I, which was -8.1Kcal/mol. The binding affinities of newly synthesized ligands against COX-II and COX-I were better than Diclofenac, which are -7.5 and -7.6 Kcal/mol respectively. In general, the triazole derivatives show higher binding affinity for COX-I as compared to COX-II. However, the overall anti-inflammatory effect of our synthesized compounds is supposed to be due to nonselective inhibition of both COX-I and COX-II.

The newly synthesizes derivatives interact with COX-II and COX-I at the binding site in a slightly different manner as compared to diclofenac. Diclofenac is relatively a simple molecule and interacts with the COX-II mainly by pi-alkyl interactions involving residues mainly leucine and valine. Moreover, the carbonyl carbon of diclofenac showed conventional hydrogen bonding with residues like tyrosine. On the other hand, newly synthesized triazoles derivatives are relatively complex structures, thereby enhancing the binding interactions like pi-sulfur interactions, hydrophobic interactions and hydrogen bonding, van der Waals interactions with different amino acid residues such as cystine, proline and asparagine respectively compared to diclofenac as shown in Fig. (**7**).

Similarly with diclofenac interact with the COX-I mainly by pi-anion interaction and hydrogen bonding with glutamic acid and asparagine respectively. Whereas triazole derivatives showed hydrogen bonding, pi-pi interaction and pi-sigma interaction with various amino acids like leucine, histidine and lysine as shown in the Fig. (**8**).

Table 4. Binding affinities of compounds 2 (a-i) with COX-I and COX-II.

Protein Target	Ligand	Binding Affinity (kcal/mol)
COX-II	2a	-7.5
	2b	-6.9
	2c	-7.7
	2d	-6.0
	2e	-7.9
	2f	-6.4
	2g	-7.6
	2h	-7.7
	2i	-6.3
	Diclofenac	-7.5
Protein Target	**Ligand**	**Binding Affinity (kca/mol)**
COX-I	2a	-7.9
	2b	-7.2
	2c	-7.9
	2d	-6.9
	2e	-8.1
	2f	-7.1
	2g	-8.0
	2h	-7.7
	2i	-7.0
	Diclofenac	-7.6

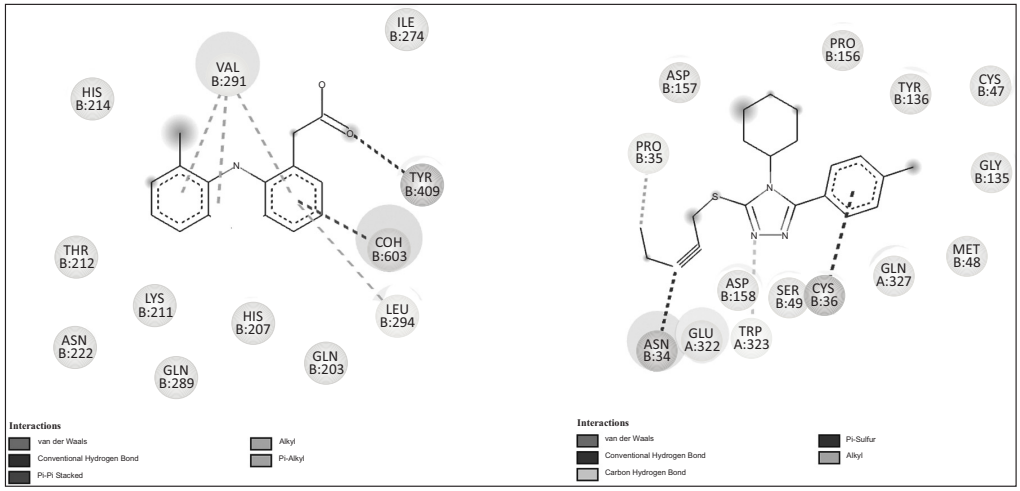


Fig. (7). Comparison of binding modes of Diclofenac (left hand side) and proposed compound 2e (right hand side) with COX-II. (*The color version of the figure is available in the electronic copy of the article*).

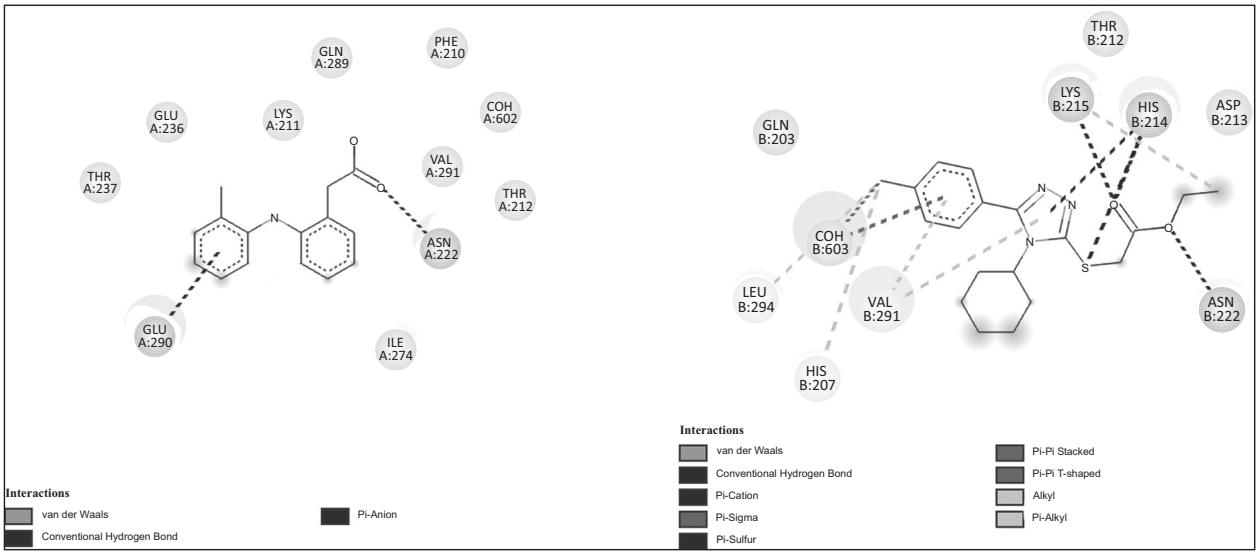


Fig. (8). Comparison of binding modes of Diclofenac (left hand side) and proposed compound 2e (right hand side) with COX-I. (*The color version of the figure is available in the electronic copy of the article*).

CONCLUSION

This study reports the synthesis of nine thioacetae derivatives of substituted 1,2, 4 triazoles. In the first step, the 5-substituted-1,2,4 triazole-3-thiols **1 a-i** were synthesized from corresponding hydrazides which were treated with ethyl chloroacetate to get the corresponding triazol-3-yl(sulfanyl)acetate derivatives **2 a-i**. All the newly synthesized acetate derivatives were screened for their anti-inflammatory, antibacterial and antifungal potential. The best activity was shown by compound **2h** which produced strong inhibition of carrageenan-induced paw edema of 62.5% at 30 mg/kg dose. 2g and 2f also exhibited moderate anti-inflammatory effect. None of the derivatives showed prominent antibacterial or antifungal potential. Further the inhibitory potential of thioacetates was further investigated by docking analysis against COX-I and COX-II enzymes of inflammation pathway using autodock vina. All the compounds showed good binding affinities with both COX-I and COX-II explaining their nonselective mode of inhibition.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for this study was obtained from Research Ethics Committee-Riphah Institute of Pharmaceutical Sciences, Pakistan.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All animal procedures were conducted in accordance with the animals ethical guidelines by National Institute of Health (NIH) have been followed.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available at https://drive.google.com/drive/folders/1F51nPVu_FHGozYygELOuvH9oArke1eNs

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

[1] Hansch, C.; Sammes, P.G.; Taylor, J.B. Comprehensive medicinal chemistry: The rational design, mechanistic study & therapeutic applications of chemical compounds. Pergamon Pr: **1989**; Vol. 5.

[2] (a) Rang, H.; Dale, M.; Ritter, J.; Flower, R.; Henderson, G., Antidepressant drugs. Rang and Dale's pharmacology. 7th ed. Edinburgh: Elsevier/Churchill Livingstone **2012**, 564-83; (b) Helms, R. A.; Quan, D.J. Textbook of therapeutics: Drug and disease management. Lippincott Williams & Wilkins: **2006**.

[3] Mahdi, M.F.; Alsaad, H.N. Design, synthesis and hydrolytic behavior of mutual prodrugs of NSAIDs with gabapentin using glycol spacers. *Pharmaceuticals,* **2012**, *5*(10), 1080-1091.

[4] Küçükgüzel, Ş.G.; Çıkla-Süzgün, P. Recent advances bioactive 1, 2, 4-triazole-3-thiones. *Eur. J. Med. Chem.,* **2015**, *97*, 830-870.

[5] Kumar, H.; Javed, S.A.; Khan, S.A.; Amir, M. 1, 3, 4-Oxadiazole/thiadiazole and 1, 2, 4-triazole derivatives of biphenyl-4-yloxy acetic acid: Synthesis and preliminary evaluation of biological properties. *Eur. J. Med. Chem*., **2008**, *43*(12), 2688-2698.

[6] El Shehry, M.; Abu-Hashem, A.; El-Telbani, E. Synthesis of 3-((2, 4-dichlorophenoxy) methyl)-1, 2, 4-triazolo (thiadiazoles and thiadiazines) as anti-inflammatory and molluscicidal agents. *Eur. J. Med. Chem.,* **2010**, *45*(5), 1906-1911.

[7] Schenone, S.; Brullo, C.; Bruno, O.; Bondavalli, F.; Ranise, A.; Filippelli, W.; Rinaldi, B.; Capuano, A.; Falcone, G. New 1, 3, 4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities. *Bioorg. Med. Chem.,* **2006**, *14*(6), 1698-1705.

[8] Duran, A.; Dogan, H.; Rollas, S. Synthesis and preliminary anticancer activity of new 1, 4-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5H-1, 2, 4-triazoline-5-thiones. Il Farmaco, **2002**, *57*(7), 559-564.

[9] Kumar, D.; Kumar, N.M.; Chang, K.-H.; Shah, K. Synthesis and anticancer activity of 5-(3-indolyl)-1, 3, 4-thiadiazoles. *Eur. J. Med. Chem*., **2010**, *45*(10), 4664-4668.

[10] Collin, X.; Sauleau, A.; Coulon, J. 1, 2, 4-Triazolo mercapto and aminonitriles as potent antifungal agents. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(15), 2601-2605.

[11] Wei, Q.-L.; Zhang, S.-S.; Gao, J.; Li, W.-h.; Xu, L.-Z.; Yu, Z.-G., Synthesis and QSAR studies of novel triazole compounds containing thioamide as potential antifungal agents. *Bioorg. Med. Chem.,* **2006**, *14*(21), 7146-7153.

[12] Klimešová, V.; Zahajska, L.; Waisser, K.; Kaustová, J.; Möllmann, U. Synthesis and antimycobacterial activity of 1, 2, 4-triazole 3-benzylsulfanyl derivatives. *Il Farmaco,* **2004**, *59*(4), 279-288.

[13] Gadad, A.K.; Noolvi, M.N.; Karpoormath, R.V. Synthesis and antitubercular activity of a series of 2-sulfonamido/trifluoromethyl-6-substituted imidazo [2, 1-b]-1, 3, 4-thiadiazole derivatives. *Bioorg. Med. Chem.,* **2004**, *12*(21), 5651-5659.

[14] Shiradkar, M.R.; Murahari, K.K.; Gangadasu, H.R.; Suresh, T.; Kalyan, C.A.; Panchal, D.; Kaur, R.; Burange, P.; Ghogare, J.; Mokale, V. Synthesis of new S-derivatives of clubbed triazolyl thiazole as anti-*Mycobacterium tuberculosis* agents. *Bioorg. Med. Chem.,* **2007**, *15*(12), 3997-4008.

[15] Turan-Zitouni, G.; Kaplancikli, Z.; Erol, K.; Kilic, F. Synthesis and analgesic activity of some triazoles and triazolothiadiazines. *Il Farmaco,* **1999**, *54*(4), 218-223.

[16] Dogan, H.N.; Duran, A.; Rollas, S.; Sener, G.; Uysal, M.K.; Gülen, D. Synthesis of new 2, 5-disubstituted-1, 3, 4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. *Bioorg. Med. Chem*., **2002**, *10*(9), 2893-2898.

[17] Almasirad, A.; Tabatabai, S.A.; Faizi, M.; Kebriaeezadeh, A.; Mehrabi, N.; Dalvandi, A.; Shafiee, A. Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1, 3, 4-oxadiazoles and 1, 2, 4-triazoles. *Bioorg. Med. Chem. Lett.,* **2004**, *14*(24), 6057-6059.

[18] Ulusoy, N.; Gürsoy, A.; Ötük, G. Synthesis and antimicrobial activity of some 1, 2, 4-triazole-3-mercaptoacetic acid derivatives. *Il Farmaco*, **2001**, *56*(12), 947-952.

[19] Gülerman, N.; Doğan, H.; Rollas, S.; Johansson, C.; Celik, C. Synthesis and structure elucidation of some new thioether derivatives of 1, 2, 4-triazoline-3-thiones and their antimicrobial activities. *Il Farmaco,* **2001**, *56*(12), 953-958.

[20] Padmavathi, V.; Reddy, G.S.; Padmaja, A.; Kondaiah, P. Synthesis, antimicrobial and cytotoxic activities of 1, 3, 4-oxadiazoles, 1, 3, 4-thiadiazoles and 1, 2, 4-triazoles. *Eur. J. Med. Chem.,* **2009**, *44*(5), 2106-2112.

[21] Demirbas, A.; Sahin, D.; Demirbas, N.; Karaoglu, S.A. Synthesis of some new 1, 3, 4-thiadiazol-2-ylmethyl-1, 2, 4-triazole derivatives and investigation of their antimicrobial activities. *Eur. J. Med. Chem.,* **2009**, *44*(7), 2896-2903.

[22] Shiradkar, M.R.; Murahari, K.K.; Gangadasu, H.R.; Suresh, T.; Kalyan, C.A.; Panchal, D.; Kaur, R.; Burange, P.; Ghogare, J.; Mokale, V. Synthesis of new S-derivatives of clubbed triazolyl thiazole as anti-Mycobacterium tuberculosis agents. *Bioorg. Med. Chem.,* **2007**, *15*(12), 3997-4008.

[23] Pick, C.G. Antinociceptive interaction between alprazolam and opioids. *Brain Res Bullet.,* **1997**, *42*(3), 239-243.

[24] Holmwood, G.; Buechel, K.; Plempel, M.; Haller, J. In Pat. RFN DE 3018865, 1981, Chem. Abstr, **1982**; p 62979s.

[25] Clemons, M.; Coleman, R.E.; Verma, S. Aromatase inhibitors in the adjuvant setting: Bringing the gold to a standard? *Cancer Treat Rev.,* **2004**, *30*(4), 325-332.

[26] Bailey, E.M.; Krakovsky, D.J.; Rybak, M.J. The triazole antifungal agents: A review of itraconazole and fluconazole. *Pharmacotherapy,* **1990**, *10*(2), 146-153.

[27] McGinnis, M.R.; Pasarell, L.; Sutton, D.A.; Fothergill, A.W.; Cooper, C.R.; Rinaldi, M.G. *In vitro* evaluation of voriconazole against some clinically important fungi. *Antimicrob. Agents Chemother.,* **1997**, *41*(8), 1832-1834.

[28] Abdel-Megeed, A.M.; Abdel-Rahman, H.M.; Alkaramany, G.-E.S.; El-Gendy, M.A. Design, synthesis and molecular modeling study of acylated 1,2,4-triazole-3-acetates with potential anti-inflammatory activity. *Eur. J. Med. Chem.,* **2009**, *44*(1), 117-123.

[29] Rahman, M.A.; Akter, N.; Rashid, H.; Ahmed, N.U.; Uddin, N.; Islam, M.S. Analgesic and anti-inflammatory effect of whole Ageratum conyzoides and *Emilia sonchifolia* alcoholic extracts in animal models. *Afr. J. Pharm. Pharmacol.,* **2012**, *6*(20), 1469-1476.

[30] Valgas, C.; Souza, S.M.d.; Smânia, E.F.; Smânia Jr,A. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.,* **2007**, *38*(2), 369-380.

[31] Sen, A.; Batra, A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. *Int. J. Curr. Pharm. Res.,* **2012**, *4*(2), 67-73.

[32] Apweiler, R.; Bairoch, A.; Wu, C.H.; Barker, W.C.; Boeckmann, B.; Ferro, S.; Gasteiger, E.; Huang, H.; Lopez, R.; Magrane, M.; Martin, M.J.; Natale, D.A.; O'Donovan, C.; Redaschi, N.; Yeh, L.S. "UniProt: The universal protein knowledgebase." *Nucleic Acids Res.,* **2004**, *32*(suppl_1), D115-D119.

[33] Altschul, S.F.; Koonin, E.V. Iterated profile searches with PSI-BLAST-a tool for discovery in protein databases. *Trends Biochem. Sci.,* **1998**, *23*(11), 444-447.

[34] Picot, D.; Loll, P.J.; Garavito, R.M. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature*, **1994**, *367*, 243-249

[35] Armougom, F.; Moretti, S.; Poirot, O.; Audic, S.; Dumas, P.; Schaeli, B.; Keduas, V.; Notredame, C. Expresso: Automatic incorporation of structural information in multiple sequence alignments using 3D-Coffee. *Nucleic Acids Res.,* **2006**, *34*(suppl_2), W604-W608.

[36] Lucido, M.J.; Orlando, B.J.; Vecchio, A.J.; Malkowski, M.G. Crystal structure of aspirin-acetylated human cyclooxygenase-2: Insight into the formation of products with reversed stereochemistry. *Biochemistry,* **2016**, *55*(8), 1226-38.

[37] Lakehal, S.; Ferkous, F.; Kraim, K.; Attoui Yahia, O.; Saihi, Y. Molecular docking study on xanthone derivatives toward alpha-glucosidase. *Res. J. Pharm., Biol. Chem. Sci.,* **2016**, *7*(3), 1739-1750.

[38] Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.,* **2010**, *31*(2), 455-461.

[39] O'Boyle, N.M.; Banck, M.; James, C.A.; Morley, C.; Vandermeersch, T.; Hutchison, G.R. Open Babel: An open chemical toolbox. *J. Cheminformatics,* **2011**, *3*(1), 1.

[40] Akthar, H. Synthesis & characterization of potentially bioactive nucleosides bearing different heterocyclic moieties. Quaid-e-Azam University Islamabad, **2006**.