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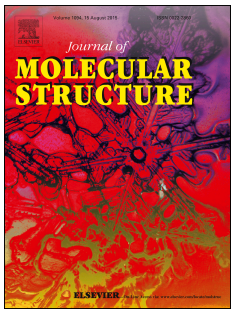

Focused library design and synthesis of 2-mercapto benzothiazole linked 1,2,4-oxadiazoles as COX-2/5-LOX inhibitors

Satayanarayana Yatam, Rambabu Gundla, Surender Singh Jadav, Narayana reddy Pedavenkatagari, Jithendra Chimakurthy, Namratha Rani B, Thyagaraju Kedam

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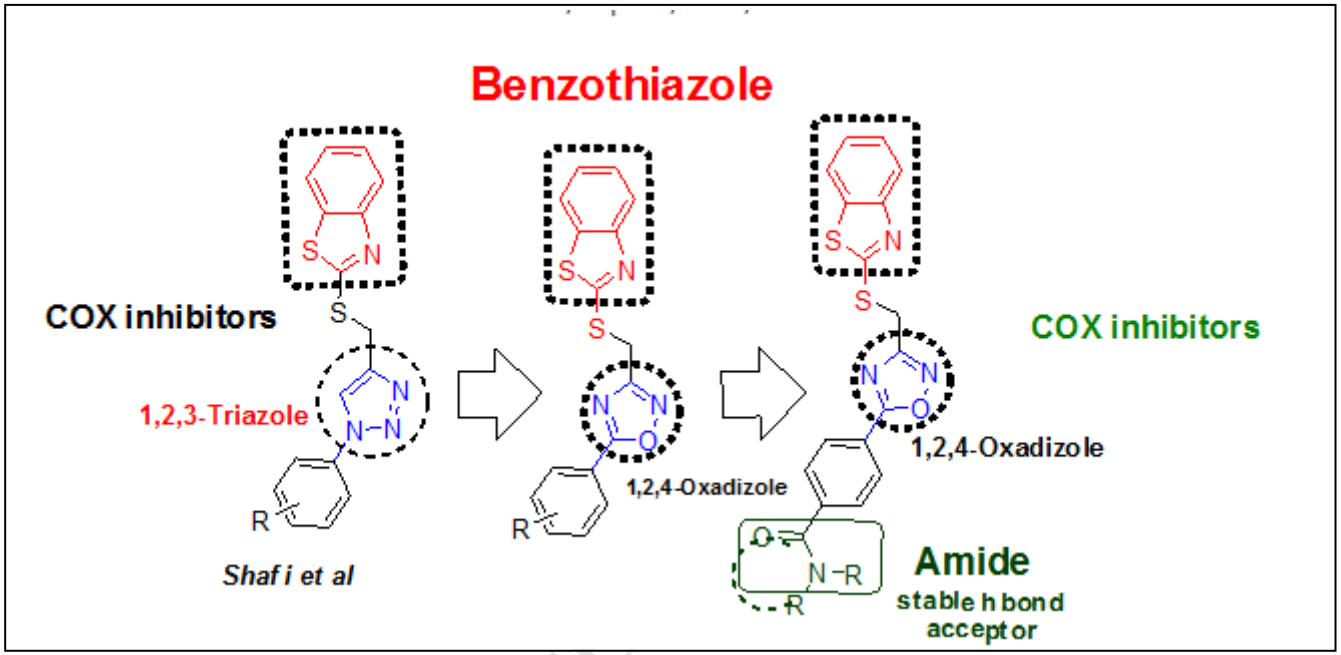

Design approach of 2-mercapto benzothiazole linked 1,2,4 oxadiazoles

Focused Library Design and Synthesis of 2-Mercapto Benzothiazole linked 1,2,4-Oxadiazoles as COX-2/5-LOX Inhibitors

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Abstract

Mercapto benzothiazole linked 1,2,4-oxadiazole derivatives were designed (**4a-u**) as new anti-inflammatory agents using bioisosteric approach and docking studies. The docking results clearly indicated that the compounds **4a-u** shown good docking interaction towards COX-2 enzyme. *In silico* drug-like properties were also calculated for compounds (**4a-u**) and exhibited significant H-bond acceptor ratio. All compounds were synthesized and biologically evaluated using *in vitro* COX-1, COX-2 and 5-LOX assays. Compound **4k** and **4q** (IC_{50} = 6.8 µM and IC_{50} = 5.0 µM) found to be potent, selective COX-2 inhibitors and display better anti-inflammatory activity than standard Ibuprofen. Compound **4l** and **4e** found to be potent inhibitors against 5-LOX (IC_{50} = 5.1 µM and IC_{50} = 5.5 µM). The *in vivo* anti-inflammatory activity studies shown that the compounds **4q** and **4k** effectively reducing the paw edema volume at 3h and 5h than standard drug Ibuprofen. The DPPH radical scavenging activity provided anti-oxidant activity of compound **4e** (IC_{50} = 25.6 µM) than reference standard Ascorbic acid.

Keywords

1,2,4-oxadiazole, Design, Docking, COX-2, 5-LOX, Anti-inflammatory, Anti-oxidant

Abbreviations

NSAID = Non Steroidal Anti-Inflammatory Drugs

COX = Cyclooxyegenase

LOX = Lypooxygenase

T3P = Propylphosphonic anhydride

BZT = Benzthiazole

1. Introduction

Inflammation is a multi-factorial process is progressed due to collective response of cytokines and immune cells with respect to injury and infection. These cells create an environment of either pro or antitumor progression [1] chronic inflammation can lead to the development of cancer [2]. The reactive oxygen free radicals like hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot}$) and peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$) are the part of oxidative stress and responsible for causing chronic diseases [3]. Cyclooxygenases (COXs) COX-1 and COX-2 isoforms catalyze critical step in the oxidation of arachidonic acid (AA) to the prostanoids. COX-1 is a constitutively expressed enzyme and COX-2 is induced in the presence of inflammatory stimulus. Pharmacological inhibition of COX provides relief from the symptoms of inflammation and pain and hence inhibition of COX activity is targeted for anti-inflammatory activity in several diseases [4]. Arachidonic acid is also get metabolized through lipoxygenase (LOX) pathway, leading to the production of leukotrienes (LTs), which are potent inflammatory mediators. Lipoxygenases (LOXs) are non-heme iron containing dioxygenases that initiate the synthesis of oxylipins to produce hydroperoxy fatty acids which in turn are converted to different compounds.

Non Steroidal Anti-Inflammatory Drugs (NSAIDs) are the most popular and well recognized drugs for the treatment against inflammatory diseases [5]. These drugs prevent the metabolism of arachidonic acid to prostaglandins (PGs) by binding and inhibiting COX enzymes and hence exert their therapeutic effects [6]. NSAIDs are effective as anti-inflammatory agents but they fail to address the oxidative stress component of inflammation. Hence there is a need for the development of anti-oxidant based anti-inflammatory agents. The conventional NSAIDs have gastric side effects, while COX-2 inhibitors (COXIBs), beside preventing side effect of NSAIDs associated with inhibition of COX-1, lead to upregulation of arachidonic acid metabolism by the 5-LOX enzyme [6, 7]. Simultaneous inhibition of both enzymatic pathways (COX-1/COX-2 and 5-LOX enzymes) would possibly exhibit enhanced anti-inflammatory potency along with reduced GI tract damage and other inflammatory side effects [8-10].

Among various heterocycles, benzothiazole skeleton has attracted much attention in heterocyclic chemistry [11]. Additionally, in the field of medicinal and pharmaceutical chemistry these derivatives have considerable attention due to their wide range of applications in biological and pharmacological activity such as anti-cancer, anti-microbial, anti-tubercular, anti-HIV and anti-inflammatory agents [12-18]. The drug candidates like

Lubeluzole, Zopolrestat, Ethoxazolamide and Bentaluron with benzothiazole skeleton played significant role in medicinal chemistry. Similarly substituted 1,2,4-oxadiazole possess antimicrobial activity [19], anticonvulsant activity [20], and anti-inflammatory activity [21]. Known commercially available anti-inflammatory drugs, including Celecoxib, Rofecoxib, Valdecoxib and Cemicoxib were developed using bioisosteric approach [22]. A successful classical bioisosteres with potent and selective COX inhibitors were identified from anti-inflammatory database (**Figure 1**) [22-27]. Based on the above facts, we have designed a library of 2-mercapto benzothiazole linked 1,2,4-oxadiazoles (**4a-u**) using bioisosteric replacement of 2-mercapto benzothiazole linked 1,2,3-triazole shown in **Figure 2** and subjected for docking studies [18]. The new set of twenty one benzthiazole compounds was synthesized and performed *in vitro* COX and LOX enzymes inhibitory activity. The *in vivo* anti-inflammatory profile of highly active COX-2 inhibitors was carried out. Additionally, these compounds were also screened for anti-oxidant activity.

2. Experimental Section

The molecular docking experiments were carried out using Schrodinger's LLC installed on Dell work station (16GB-RAM). All chemicals were purchased from Lancaster (Alfa Aesar, Johnson Matthey Co, Ward Hill, MA, USA), Sigma-Aldrich (St Louis, MO, USA) and Spectrochem Pvt Ltd (Mumbai, India). The progress of reaction was monitored by TLC. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of pet ether and ethyl acetate (EtOAc) as the eluent, visualization on TLC was achieved by UV light. ^{1}H NMR spectra were recorded on Avance (300 MHz); Bruker, Fallanden, Switzerland instruments. Chemical shifts values were reported in ppm, downfield from internal TMS standard. Spectral patterns were designated as s, singlet; d, doublet; dd, double doublet; t, triplet; td, triplet of doublet; bs, broad singlet; m, multiplet. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage of 3.98 kV and ESI mode positive ion trap detector. IR spectra were recorded on a FT-IR spectrometer (Shimadzu FT-IR 8300 spectrophotometer) and only major peaks are reported in cm^{-1}. Melting points were measured in open capillary tubes and results were uncorrected. Linoleic Acid, TMPD (N,N,N′,N′-tetra methyl p-phenylenediamine), hematin and Tween 20 were purchased from Sigma, Arachidonic acid purchased from Nu-check Prep, Inc (MN, USA) and Dimethyl sulfoxide (DMSO) of HPLC grade, DPPH (2,2-diphenyl-1-picryl hydrazyl). All solutions were prepared in deionised distilled water. All other reagents were of standard quality and commercially available. All the synthesized compounds were characterized by their physical

and spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy). Two compounds from the series were also determined by the 2D NMR techniques.

2.1 Molecular docking protocol

The X-ray three-dimensional structures of human cyclooxyegenase –II along with reference ligand mefenamic acid (PDB: 5IKR) and active site of flurbiprofen with prostaglandin H2 synthase-1 (PDB: 1CQE) were retrieved from protein data bank. Prepared and energy was minimized by protein preparation wizard, receptor grid was generated employing active site of Mefanamic acid and Flurbiprofen respectively. The extra precision mode Glide 5.0 docking was employed for the present protein ligand interactions. The ligplot^{+} was utilized for getting 2D interaction diagrams.

2.2 Chemistry

2.2.1 Synthesis of 2-(benzo[*d*]thiazol-2-ylthio)acetonitrile (2)

Chloroacetonitrile (1.91 mL, 30.12 mmol) was added to a stirred solution of benzo[*d*]thiazole-2-thiol **1** (5.00 g, 30.12 mmol) and K_2CO_3 (6.20 g, 45.14 mmol) in DMF (50 mL) and allowed the reaction mixture to stir at rt for 1 h under nitrogen. Reaction was monitored by TLC (R_f = 0.50, petether:EtOAc = 1:1), after completion of reaction, 200 mL of H_2O was added to the reaction mixture and the separated solid was collected by filtration and dried to obtain **2** (5.90 g, 95%) as an off white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.95–7.93 (m, 1H), 7.80–7.78 (m, 1H), 7.48–7.44 (m, 1H), 7.38–7.33 (m, 1H), 4.20 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.53, 152.53, 135.70, 126.46, 125.09, 122.17, 121.29, 115.71, 18.02 ppm. LC-MS (APCI + ESI) *m/z*: 207 [M + H].

2.2.2 Synthesis of 2-(benzo[*d*]thiazol-2-ylthio)-N'-hydroxyacetimidamide (3)

Et_3N (3.49 mL, 24.25 mmol) was added to a stirred solution of 2-(benzo[*d*]thiazol-2-ylthio)acetonitrile **2** (1.00 g, 4.85 mmol) and hydroxylamine hydrochloride (0.68 g,9.70 mmol) in EtOH (15 mL) and the reaction mixture was allowed to stir at reflux temperature for 2 h under nitrogen. Reaction was monitored by TLC (R_f = 0.20, petether:EtOAc = 1:1), after completion of reaction, cooled the reaction mixture and concentrated the reaction mixture under reduced pressure. The crude residue was dissolved in water and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain **3** (1.05 g, 90%) as a light yellow solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.31 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.47 (t, J = 7.2 Hz, 1H), 7.37 (t, J = 7.2 Hz, 1H), 5.70 (bs, 2H), 4.01 (s, 2H). ^{13}C NMR

(100 MHz, $CDCl_3$) δ: 166.64, 152.49, 148.60, 134.71, 126.33, 124.46, 121.73, 121.08, 33.12. LC-MS (APCI + ESI) *m/z*: 240 .0 [M + H].

2.2.3 General procedure for the synthesis of compounds (4a -4i)

50% T_3P in EtOAc (1.045 mmol) was added drop wise to a solution of 2-(benzo[*d*]thiazol-2-ylthio)-N'-hydroxyacetimidamide **3** (0.418 mmol), aromatic acid (0.418 mmol) and Et_3N (1.25 mmol) in DMF (3.0 mL) and allowed the reaction mixture to stir at 140 °C for 2 h under nitrogen. Reaction was monitored by TLC (petether: EtOAc = 8:2), after completion of reaction, cooled the reaction mixture and diluted with ice cold H_2O and extracted with MTBE (2 × 10 mL). The combined organic layer was washed with saturated $NaHCO_3$ (10 mL) followed by H_2O (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo and the residue was purified by column chromatography over silica gel (100–200 mesh) with eluent petether–EtOAC followed by CH_3OH washings to obtain compound **(4).** Data of the representative compounds are as follows.

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(4-nitrophenyl)-1,2,4-oxadiazole (4a).

Yield:60%; yellow solid; mp: 120-122°C; TLC R_f = 0.35 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1513, 1424, 1367, 1337, 1000, 855, 719; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.38 (dd, *J* = 6.9, 2.1 Hz, 2H), 8.31 (dd, *J* = 7.2, 2.1 Hz, 2H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.47–7.41 (m,1H), 7.35–7.30 (m, 1H), 4.80 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.28, 168.32, 163.82, 152.89, 150.34, 135.60, 129.29, 129.11, 126.25, 124.72, 121.96, 121.13, 27.48. LC-MS (APCI + ESI) *m/z*: 371 [M + H].

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (4b).

Yield:57%; off white solid; mp: 80-82 °C ; TLC R_f = 0.50 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1501, 1413, 1327, 1129, 1064, 854, 748; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.24 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.79–7.75 (m, 3H), 7.45–7.41 (m, 1H), 7.33–7.29 (m, 1H), 4.78 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.99, 168.01, 163.98, 152.98, 135.58, 128.58, 126.20, 126.17, 126.13, 124.65, 121.94, 121.10, 27.56. LC-MS (APCI + ESI) *m/z*: 394 [M + H].

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(2-chlorophenyl)-1,2,4-oxadiazole (4c).

Yield:30%; white solid; mp: 88 -90°C ; TLC R_f = 0.40 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1422, 1366, 1006, 869, 753, 723; ^{1}H-NMR (300 MHz, $CDCl_3$)δ: 8.12 (s, 1H), 8.00 (d, *J* = 7.5 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 7.49–7.41 (m, 2H), 7.36–7.30 (m, 1H), 4.77 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.92, 167.41, 164.18, 152.95, 135.59, 133.90, 133.35, 132.01, 131.48, 127.10, 126.18, 124.61, 123.21, 121.94, 121.10, 27.67. LCMS (ESI+APCI): *m/z* = 359.7 $[M + H]^+$.

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(3-chlorophenyl)-1,2,4-oxadiazole (4d).

Yield:50%; White solid; mp: 95-97 °C ; TLC R_f = 0.40 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1425, 1351, 997, 890, 755, 741; ^{1}H-NMR (300 MHz, $CDCl_3$)δ: 8.06 (d, *J* = 6.6 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.57–7.29 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 175.14, 167.83, 164.05, 152.92, 135.58, 135.33, 133.04, 130.48, 128.21, 126.25, 126.21, 125.42, 124.64, 121.95, 121.11, 27.59. LCMS (ESI+APCI): *m*/*z* = 359.6 [M + H]$^+$.

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(4-chlorophenyl)-1,2,4-oxadiazole (4e).

Yield:55%; White solid; mp:90-92°C; TLC R_f = 0.40 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1427, 1356, 1088, 836, 759, 745; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.45–7.41 (m, 1H), 7.33–7.30 (m, 1H), 4.76 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.46, 167.78, 164.11, 152.95, 139.50, 135.59, 129.55, 129.49, 124.63, 121.95, 121.10, 27.62. LC-MS (APCI + ESI) *m/z*: 360 [M + H].

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(2-fluorophenyl)-1,2,4-oxadiazole (4f).

Yield:30%; White solid; mp:103-105°C; TLC R_f = 0.45 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1424, 1350, 1075, 1002, 752; ^{1}H-NMR (300 MHz, $CDCl_3$)δ: 8.14 – 8.08 (m, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.62–7.55 (m, 1H), 7.46–7.41 (m, 1H), 7.34–7.23 (m, 3H), 4.79 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.33, 173.29, 167.44, 164.18, 162.03, 159.44, 152.94, 135.57, 134.90, 134.82, 130.91, 126.18, 124.74, 124.70, 124.60, 121.94, 121.09, 117.27, 117.06, 112.50, 112.39, 27.63. LCMS (ESI+APCI): *m*/*z* = 343.7 [M + H]$^+$.

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (4g).

Yield:60%; White solid; mp: 107-109°C; TLC R_f = 0.45 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1428, 1359, 1233, 996, 844, 754; ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.15 – 8.11 (m, 2H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.45–7.41 (m, 1H), 7.33–7.30 (m, 1H), 7.22–7.18 (m, 2H), 4.75 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 1754.44, 167.69, 166.86, 164.33, 164.16, 152.94, 135.57, 130.74, 130.65, 126.19, 124.62, 121.94, 121.10, 120.26, 116.64, 116.42, 27.62. LCMS (ESI+APCI): *m*/*z* = 344 [M + H]$^+$.

methyl 4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)benzoate (4h).

Yield:60%; White solid; mp:113-115°C; TLC R_f = 0.30 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1721, 1410, 1367, 1272, 1093, 869, 749; ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.21–8.16 (m, 4H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.45–7.41 (m, 1H), 7.34–7.30 (m, 1H), 4.78 (s, 2H), 3.96 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.44, 167.93, 165.94,

164.05, 152.91, 135.57, 133.93, 130.25, 128.19, 127.45, 126.20, 124.64, 121.94, 121.11, 52.57, 27.58. LCMS (ESI+APCI): *m*/*z* = 384.1 $[M + H]^+$.

3-((benzo[*d*]thiazol-2-ylthio)methyl)-5-(pyridin-2-yl)-1,2,4-oxadiazole (4i).

Yield:40%; Brown solid; mp:117-119°C; TLC R_f = 0.20 (petether:EtOAc = 8:2); IR (KBr): υ (cm^{-1}) 1423, 1361, 1307, 1002, 759; ^{1}H-NMR (400 MHz, $CDCl_3$)*δ*: 8.82 (d, *J* = 4.4 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 7.92–7.88 (m, 2H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.52–7.49 (m, 1H), 7.42 (t, *J* = 7.2 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 4.81 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 174.94, 167.98, 164.11, 152.90, 150.70, 143.28, 37.34, 135.55, 126.86, 126.16, 124.59, 124.29, 121.92, 121.08, 27.58. LCMS (ESI+APCI): *m*/*z* = 327 $[M + H]^+$.

2.2.4 Synthesis of 4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)benzoic acid (4j)

Lithium hydroxide monohydrate (0.22 g, 5.22 mmol) was added to a stirred solution of methyl 4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)benzoate **4h** (1.00 g, 2.61 mmol) in CH_3OH:THF:H_2O (20mL: 20 mL: 10 mL) and allowed the reaction mixture to stir at rt for 18h. Reaction was monitored by TLC (R_f = 0.10, petether:EtOAc = 3:7), after completion of reaction, concentrated the reaction mixture in vacuo and crude residue was dissolve in H_2O (20 mL). The aqueous layer was acidified to pH 2 using the 2 M aq.HCl and the separated solid was collected by filtration and dried to obtain **4j** (0.87g, 90%) as a white solid. mp:140-142°C; IR (KBr): υ (cm^{-1}) 2983, 1686, 1427, 1292, 1097, 871, 718; ^{1}H-NMR (300 MHz, DMSO-d_6)*δ*: 8.21 (d, *J* = 8.4 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.51–7.45 (m, 1H), 7.41–7.36 (m, 1H), 4.90 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 174.77, 168.01, 166.28, 164.54, 152.32, 134.85, 134.72, 130.23, 128.09, 126.45, 126.41, 124.73, 121.89, 121.33, 27.07. LCMS (ESI+APCI): *m*/*z* = 370.0 $[M + H]^+$.

2.2.5 General procedure for synthesis of compounds (4k-4u).

HATU (0.324 mmol) and Et_3N (0.54 mmol) were added to a stirred solution of 4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)benzoic acid **4j** (0.27 mmol) in DMF (3.0 mL) and allowed the reaction mixture to stir at rt for 10 mins, than added the Amine (0.297 mmol) and allowed the reaction mixture to stir at rt for 2 h under nitrogen. Reaction was monitored by TLC (petether:EtOAc = 1:1), after completion of reaction diluted the reaction mixture with H_2O and allowed the reaction mixture to stir at rt for 30 min and the separated solid was collected by filtration and dried to obtain (**4k-4u**). Data of the representative compounds are as follows.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)benzamide (4k).

Yield:80%; White solid; mp:125-127°C; TLC R_f = 0.15 (petether:EtOAc = 3:7); IR (KBr): υ (cm^{-1}) 3172, 1653, 1616, 1571, 1424, 1393, 1003, 871, 753; ^{1}H-NMR (400 MHz, DMSO-d_6)δ: 8.17–8.15 (m, 3H), 8.08 – 8.03 (m, 3H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.59 (bs, 1H), 7.50–7.46 (m, 1H), 7.41 – 7.37 (m, 1H), 4.89 (s, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ:174.94, 167.94, 166.74, 164.56, 152.35, 138.34, 134.89, 128.52, 127.82, 126.43, 125.14, 121.89, 121.35, 27.09. LCMS (ESI+APCI): *m*/*z* = 369 $[M + H]^+$.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)-N-methoxy-N-methylbenzamide (4l).

Yield:85%; White solid; mp:80-82°C; TLC R_f = 0.45 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1648, 1420, 1363, 985, 853, 749; ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.16 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 4.78 (s, 2H), 3.53 (s, 3H), 3.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.59, 168.52, 167.82, 164.13, 152.90, 138.29, 135.54, 128.90, 127.86, 126.19, 125.40, 124.62, 121.90, 121.10, 61.28, 33.39, 27.61. LCMS (ESI+APCI): *m*/*z* = 413.1 $[M + H]^+$.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)-N-(tert-butyl)benzamide (4m).

Yield:80%; White solid; mp:50-53°C; TLC R_f = 0.50 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 2973, 1637, 1539, 1461, 1362, 1008, 861, 756; ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.16 (d, *J* = 8.8 Hz, 2H), 7.93–7.91 (m, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.78–7.76 (m, 1H), 7.45–7.42 (m, 1H), 7.34–7.30 (m, 1H), 6.08 (bs, 1H), 4.78 (s, 2H), 1.49 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.51, 167.83, 165.59, 164.08, 152.91, 139.84, 135.56, 128.33, 127.53, 126.20, 125.91, 124.63, 121.92, 121.10, 52.05, 28.80, 27.60. LCMS (ESI+APCI): *m*/*z* = 424.8 $[M + H]^+$.

(4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)phenyl)(piperidin-1-yl)methanone (4n).

Yield:83%; White solid; mp:65-67°C; TLC R_f = 0.25 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 2929, 1646, 1427, 1364, 996, 854, 755; ^{1}H-NMR (400 MHz, DMSO-d_6)δ: 8.13 (d, *J* = 8.4 Hz, 2H), 8.05 –8.03 (m, 1H), 7.89–7.86 (m, 1H), 7.59 (8.4 Hz, 2H), 7.50–7.46 (m, 1H), 7.41–7.36 (m, 1H), 4.89 (s, 2H), 3.59 (bs, 2H), 3.23 (bs, 2H), 161–1.45 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.63, 168.78, 167.79, 164.14, 152.19, 140.89, 135.55, 128.39, 127.55, 126.19, 124.62, 124.52, 121.91, 121.10, 48.69, 43.18, 27.62, 26.53, 25.56, 24.47. LCMS (ESI+APCI): *m*/*z* = 436.7 $[M + H]^+$.

(4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)phenyl)(4,4-difluoropiperidin-1-yl)methanone(4o).

Yield:89%; White solid; mp:70-72°C; TLC R_f = 0.30 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1636, 1450, 1359, 1133, 1099, 854, 745; ^{1}H-NMR (300 MHz, $CDCl_3$)δ: 8.10 (d, *J* = 6.9 Hz, 2H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.77 (d, *J* = 7.2 Hz, 1H), 7.56 (d, *J* = 6.6 Hz, 2H), 7.44–7.41 (m, 1H), 7.34–7.32 (m, 1H), 4.78 (s, 2H), 3.89 (bs, 2H), 3.52 (bs, 2H), 1.99 (bs, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.37, 169.12, 167.89, 164.07, 152.92, 139.50, 135.57, 128.57, 127.64, 126.21, 125.23, 124.65, 121.93, 121.24, 121.11, 44.41, 39.20, 34.44, 27.59. LCMS (ESI+APCI): *m*/*z* = 472.7 $[M + H]^+$.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)-N-(tetrahydro-2H-pyran-4-yl)benzamide (4p).

Yield:35%; mp:75-77°C; TLC R_f = 0.20 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1631, 1539, 1431, 996, 866, 756; ^{1}H-NMR (300 MHz, DMSO-d_6)δ: 8.58 (d, *J* = 7.5 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 2H), 8.07–8.04 (m, 3H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.51–7.45 (m, 1H), 7.41–7.36 (m, 1H), 4.90 (s, 2H), 4.07–3.97 (m, 1H), 3.90–3.86 (m, 2H), 3.42–3.38 (m, 2H), 1.79–1.74 (m, 2H), 1.65–1.51 (m, 2H). LCMS (ESI+APCI): *m*/*z* = 452.7 $[M + H]^+$.

(4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)phenyl)(morpholino)methanone (4q).

Yield:80%; White solid; mp:72-74°C; TLC R_f = 0.35 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1631, 1498, 1363, 1111, 837, 728; ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.18 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.45–7.41 (m, 1H), 7.34–7.26 (m, 1H), 4.78 (s, 2H), 3.79 –3.63 (m, 6H), 3.41 (bs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.43, 168.96, 167.86, 164.09, 152.90, 139.59, 135.55, 128.51, 127.87, 126.21, 125.02, 124.65, 121.92, 121.11, 66.80, 48.50, 42.50, 27.59. LCMS (ESI+APCI): *m*/*z* = 438.7 $[M + H]^+$.

(4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)phenyl)(thiomorpholino)methanone (4r).

Yield:80%; White solid; mp:68-70°C; TLC R_f = 0.40 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1634, 1430, 1359, 994, 850, 755; ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.18 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.77 (*J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.45–7.42 (m, 1H), 7.34–7.30 (m, 1H), 4.77 (s, 2H), 4.06 – 4.04 (m, 2H), 3.64 (bs, 2H), 2.75–2.56 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.44, 169.24, 167.86, 164.08, 152.92, 141.10, 135.57, 128.57, 127.52, 126.20, 124.92, 124.64, 121.93, 121.11, 50.04, 44.64, 28.05, 27.60. LCMS (ESI+APCI): *m*/*z* = 454.7 $[M + H]^+$.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4oxadiazol-5-yl)-N-(1-methylpiperidin-4-yl)benzamide (4s).

Yield:75%; White solid; mp:80-82°C; TLC R_f = 0.30 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1630, 1544, 1427, 1360, 994, 862, 751; ^{1}H-NMR (400 MHz, DMSO-d_6)δ: 8.51 (d, *J* = 7.6 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 2H), 8.05–8.03 (m, 3H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.41–7.37 (m, 1H), 4.89 (s, 2H), 3.77–3.70 (m, 1H), 2.78–2.73 (m, 2H), 2.15 (s, 3H), 1.96–1.91 (m, 2H), 1.77–1.75 (m, 2H), 1.60–1.56 (m, 2H).LCMS (ESI+APCI): *m*/*z* = 465.8 $[M + H]^+$.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)-N-(4-fluorophenyl)benzamide (4t).

Yield:90%; White solid; mp:110-112°C; TLC R_f = 0.45 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1651, 1510, 1427, 1215, 998, 831, 756; ^{1}H-NMR (400 MHz, DMSO-d_6)δ: 10.55 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 2H), 8.16 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.82 – 7.78 (m, 2H), 7.50–7.46 (m, 1H), 7.41–7.39 (m, 1H), 7.37–7.19 (m, 2H), 4.91 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.87, 168.80, 164.56, 164.25, 159.64, 157.24, 152.36, 138.75, 135.15, 134.89, 128.74, 127.93, 126.44, 125.41, 124.73, 122.33, 122.25, 121.90, 121.35, 115.33, 115.11, 27.09. LCMS (ESI+APCI): *m*/*z* = 462.7 $[M + H]^+$.

4-(3-((benzo[*d*]thiazol-2-ylthio)methyl)-1,2,4-oxadiazol-5-yl)-N-(4-chlorophenyl)benzamide (4u).

Yield:88%; White solid; mp:114-116°C; TLC R_f = 0.40 (petether:EtOAc = 1:1); IR (KBr): υ (cm^{-1}) 1654, 1523, 1427, 1359, 999, 824, 757, 725; ^{1}H-NMR (400 MHz, DMSO-d_6)δ: 10.63 (bs, 1H), 8.24 (d, *J* = 8.0 Hz, 2H), 8.16 (d, *J* = 8.0 Hz, 2H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.48–7.39 (m, 4H), 4.91 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.58, 168.02, 164.57, 164.47, 152.36, 138.65, 137.81, 134.89, 128.82, 128.55, 127.95, 127.60, 126.47, 125.48, 124.75, 121.95, 121.36, 27.08. LCMS (ESI+APCI): *m*/*z* = 478.6 $[M + H]^+$.

2.3 Biological screening

2.3.1 *In vitro* 5-LOX assay

Lipoxygenase was isolated from Soyabean (Glycine max.) seedlings and assay was carried out according to the method [28] with slight modifications. The assay mixture contains 100mM phosphate buffer pH 6.5, enzyme source (100µg), Linoleic Acid (80 mM), test compound in a total volume of 2 mL. Lox activity was measured by monitoring the increase in absorbance at 234 nm over a period of time in spectrophotometer. The reaction product is a

hydroperoxide of the corresponding PUFA, which has characteristic absorption maxima at 234 nm.

2.3.2 *In vitro* COX-1 and COX-2 Assay

COX-1enzyme was isolated from ram seminal vesicles as per previously described method [29]. The enzyme COX-2 was isolated according to the reported method [10] with slight modifications. Enzymatic activities of COX-1 and COX-2 were measured as per preliminary process outlined [30], with slight modifications using a chromogenic assay based on the oxidation of N,N,N′,N′-tetra-methyl-p-phenyl diamine (TMPD) during the reduction of prostaglandin G2 (PGG2) to prostaglandin H2 (PGH2). The reaction mixture contained Tris-HCl buffer (100 mM, pH 8.0), hematin (15 μM), EDTA (3 μM), enzyme (100 μg) and test compound. The mixture was pre incubated at 25ºC for 15 min and then the reaction was initiated by the addition of Arachidonic acid and TMPD in total volume of 2 mL. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation followed by the increase in absorbance at 610 nm.

2.3.3 DPPH (2, 2-diphenyl-1-picrayl hydrazyl) Antioxidant assay

The DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging activity was conducted as per established procedure [31]. The basic step of assay include, an antioxidant compound is to react with DPPH results and later it converts DPPH in to α,α-diphenyl,-β-picryl hydrazine. Test compounds were prepared in various concentrations (20, 40, 60, 80 and 100 μM) using methanol. Further, 1 mL of each test solution with predefined test concentrations (20, 40, 60, 80 and 100 μM) were transferred in to individual test tubes. 4 mL of 0.1 mM DPPH solution (prepared in methanol) was added to individual test tubes, resulting solution were subjected to vigorous shaking for a few minutes and kept in dark chamber to incubate for about 20 min at room temperature. A DPPH blank solution was formulated using methanol to correct baseline. The fluctuations of absorbance associated with all samples were estimated at 517 nm using a double beam UV-visible spectrophotometer. Decreasing in percentage of absorbance was measured for each concentration, and narrowing of DPPH in sample was determined against blank. The radical scavenging activity was indicated with the percentage inhibition and was predicted using reported formula: Radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$ (%). Where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the test compound. The ascorbic acid used as standard drug for the present antioxidant assay [31].

2.3.4 *In vivo* anti-inflammatory Activity

Albino wistar rats of either sex (150-200g) were obtained from our animal facility. The animals were kept in cages at the room temperature and fed with food and water ad libitum. Fourteen hours before the start of the experiment the animals were sent to lab and fed only with water ad libitum. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee. Rats were randomly divided into different groups, each consisting of six animals, of which group I was kept as control giving only distilled water. group II was standard which received Ibuprofen (10mg/kg) as the reference standard for comparison while other groups received test compounds at equimolar dosage of the standard drug. Half an hour after the oral administration of the test compounds, the rat hind paw edema was induced by subcutaneous injection of 0.1 mL of 1% freshly prepared saline solution of carrageenan into the right hind paw of rats [32]. The volume of paw edema was measured at interval of 3h and 5h, using Plethysmometer. After administration of carrageenan the left hind paw served as a reference non inflamed paw for comparison. The average percent increase in paw volume with time was calculated and compared against the control group.

3. Results and discussion

3.1 Rationale for design of COX-2 Inhibitors

The diverse focused library of twenty one 2-mercapto menzothiazole linked 1,2,4-oxadiazole derivatives (**4a-u**) with similar chemicophysical properties were designed using bioisosteric replacement and docking studies. Mono substituted compounds with different functional like halogens, nitro, acid and ester were designed (**4a–4g, 4h and 4j)** and subsequently, the phenyl ring was substituted with its classical isostere pyridyl ring (**4i**). Later the acid functional group was converted into amide (**4k**) and substituted amides (**4l** and **4m)**. To get the structural diversity, amide was replaced with piperdine and converted as cyclic tertiary amine (**4n**) and was substituted with halogenated piperdine (**4o**). To know pharmacophoric effect of morpholine (**4q**) and thiomorpholine (**4r**) they were added to the library. Keeping secondary amine as constraint, six membered tetrahydropyran (**4p**) and *N*-methyl piperidine (**4s**) were designed. Similarly, the secondary amine was replaced with fluoro aniline (**4t**) and chloro aniline (**4u**) to get other ring equivalent with diversified ligands (**Figure 3**).

To know the binding pose and interaction profile of compounds **4a-4u** with both COX-1 and COX-2 enzymes (PDB: 1CQE & 5IKR), they were studied using glide based docking methods [33, 34]. Compounds **4a-u** exhibited elevated binding energies (**Table S1**) when compared with earlier 1,2,3-triazole analogs (**Table S2**) with similar binding

orientation. It has been observed that, all the ligands (**4a-u**) were accommodated in active site of both monomeric enzymes and a systematic interaction analysis revealed a new fact. The orientations of binding of ligands at active sites were found in unusual approach. The *N*4-nitrogen position and binding orientation of 1,2,4-oxadiazole ring of ligands at active site of COX-2 displayed a vital h-bond, π-π, π-cation interactions and even an inverted orientation of ligands were displayed similar binding of 1,2,4-oxadiazole ring. The binding of mercapto benzothiazoles at acid site of COX-2 revealed that *N*4-nitrogen of 1,2,4-oxadiazole ring exhibited a side chain hydrogen bond with Arg120 and π-π interactions with Tyr355. The phenyl ring of benzothiazole displayed a π-π interaction with Tyr115, and carbonyl oxygen of amide bond found to be participating side chain hydrogen bond with Ser530. In case of mercapto benzothiazoles at Flurbiprofen site (COX-1), *N*4-nitrogen of 1,2,4-oxadiazole ring turns in to opposite, resulted in formation of side chain hydrogen bond with Tyr355. Similarly a very less chance of making side chain hydrogen bond with Ser530 and mutation of Tyr115 with Leu115 might be a hypothetical valid root cause for loosing selectivity to COX-2 enzyme. At last, as explained above, three distinct types of mercapto benzothiazole interactions with active site of COX-2 might be a valid hypothetical phenomenon for imparting selectivity. The *in silico* COX-2 enzyme interaction of highest active (**4q**) ligand was provided in **Figure 4**. *In silico* ADMET properties of compounds (**4a-u**) along with reported 1,2,3-tirazole (**Table S3**) were calculated [18]. It was observed that compounds **4a-i** were displayed poor HBA property in both cases whereas compounds (**4h, 4j-u**) showed higher HBA property indicated that the significance and essential role of amide bond as reported in peptidomimitc approaches (**Figure 2 and Table 1**) [35]. Based on these rational studies, we have selected a library of 21 compounds for synthesis and biological evolution using *in vitro* COX-1 and COX-2 enzyme assay. The *in vitr*o active compounds were tested against *in vivo* anti-inflammatory activity. In addition to above compounds (**4a-u**) were also screened using 5-LOX enzyme assay and DPPH free radical assay.

3.2 Synthesis

The target 2-mercapto benzothiazole linked 1,2,4-oxadiazole derivatives **4a-i** were synthesized as shown in **Scheme 1**. Benzo[*d*]thiazole-2-thiol **1** on alkylation with chloroacetonitrile gave **2**, which on treatment with hydroxylamine hydrochloride gave 2-(benzo[d]thiazol-2-ylthio)-N'-hydroxyacetimidamide **3**. Treatment of **3** with different substituted benzoic acids in presence of 50% T_3P in EtOAc and triethylamine (Et_3N) in DMF solvent at 140 ^oC gave the desired target compounds **4a-i** [36]. Electron-withdrawing and electron-donating groups on benzoic acid adducts were all well tolerated and the desired

products were obtained in moderate-to-good yields. The 2D HSQC spectral characterization of compound 4a and 4e has also been conducted and the obtained values were observed in line with existing NMR interpretation.

The target 2-mercapto benzothiazole linked 1,2,4-oxadiazole derivatives **4j-u** were synthesized as shown in **Scheme 2**. The acid derivative **4j** was synthesized by hydrolysis of **4h** using lithium hydroxide monohydrate as a base in a mixture of CH_3OH, THF and water**.** The compound **4j** was treated with various substituted amines in presence of HATU and Et_3N as base in DMF solvent at room temperature for 2 hours. Under these conditions, the expected benzothiazolothiomethyl-oxadiazole-amide derivatives were obtained in moderate-to good yields. (**4k-u, Scheme 2**).

First, we studied the reactions with aliphatic amines including primary, secondary, tertiary amines afforded the corresponding amide derivatives obtained in high yields (**4k-m**). We then turned our attention of the use of cyclic secondary amines in the amide bond formation reaction. In this context, we chose **4j** as substrate to couple with various cyclic amines including, piperidine, 4,4-difluoropiperidine hydrochloride, 4-aminotetrahydropyran, morpholine, thiomorpholine, N-methylpiperidine. It is worthy of note that all the cyclic amines well tolerated under the present reaction conditions, provided the amide derivatives in very good yields (**4n-s**), we also tested the reactions of halo substituted (fluoro and chloro) aromatic anilines with acid derivative **4j** under standard reaction conditions. To our delight, the benzothiazolothiomethyl-oxadiazole- amide derivatives were obtained in 90%, and 88 % respectively.

In the first step the base deprotonates the carboxylic acid and the resulting carboxylate attacks the T3P to provide the activated carboxylic acid intermediate. The hydroxyl group of amidoxime 3 attacks the now activated carboxylic acid derived intermediate to provide the O-acylated amidoxime. This intermediate will undergoes the intra molecular cyclodehydration at higher temperature and will give the oxadiazole [37-40]. The possible mechanism of the formation of oxadiazole is represented in the **Scheme 3**.

3.3 Biological screening

3.3.1 *In vitro* COX-1 and COX-2 assay

The target compounds **4a-4u** were evaluated for their ability to inhibit COX-1, COX-2 and LOX according to reported methods [28, 30]. Initially, the compounds were tested at 10 µM and their effects on COX-1, COX-2 and LOX inhibition are shown in **Table 2**. The study was further extended to examine the concentration–activity responses at different concentrations to determine the IC_{50} values for COX-1, COX-2 and LOX of compounds exhibiting potent

COX and LOX inhibition. Indomethacin (COX-1 inhibitor) and Celecoxib (selective COX-2 inhibitor) were used as positive controls in the study (**Table 2**). For calculation of the IC_{50} (µM) values, compounds were tested at five different concentrations (0.1–10 µM). The IC_{50} value of Celecoxib on COX-2 was found 0.038µM, indicating that Celecoxib is a selective COX-2 inhibitor. A few compounds exhibited moderate to strong inhibitory effects on COX-1 and COX-2 activity (> 60%). Compounds (**4c, 4d, 4e, 4g, 4k, 4l, 4p, 4q** and **4u**) were found to be most potent against COX-2 (>50% inhibition) at 10 µM concentration.

In detail, halogenated either *ortho*, *meta* or *para* chloro substituted (**4c, 4d** and **4e**) or *para* fluoro substituted compounds (**4g**), were exhibited COX-2 inhibition between 51-57%. The para substituted carboxylic group (**4j**), methyl ester (**4h**) and ring equivalent substituted compound **4i** were found to be not in favor for both enzymes. The replacement of carboxylic acid with amide group (primary amine - **4k** and secondary amine - **4l**), compound **4k** exhibited a potent COX-2 inhibition than compound **4l**; presence of free amine in compound **4k** favored COX-2 enzyme inhibition when compared to tertiary amine of compound **4l**. The presence of *tert*-butyl amine, cyclic amine piperdine and halogenated piperdine makes compound **4m**, **4n** and **4o** inactive. The presence of six membered tetrahydropyran ring of compound (**4p**) was imparted equivalent inhibition as earlier. The compound **4q** with morpholine ring displayed maximum potency towards COX-2 inhibition among series. Whereas, its ring equivalent compound **4r** substituted with thiomorpholine become less potent. The methyl piperdine consisted compound (**4s**) found to be inactive. The enlargement of amide with *para* fluoro aniline (**4t**) not affected COX-2 enzyme. In final presence of *para* chloro aniline (**4u**) showed better inhibition of COX-2 enzyme. Simultaneously screening of the above series against COX-1 enzyme provided inhibition between 29.88 – 49.50%. The obtained results were tabulated and provided in **Table 2**. Compounds **4q** exhibited the highest COX-2 inhibition (IC_{50} = 5.0 µM) followed by **4k** (IC_{50} = 6.8 µM) (**Figure 5**). The presence of morpholine might be effective for COX-2 inhibition when compared with amide. The significant COX-2 inhibition by some of the halogenated compounds was noticed, however, they are less active than compound **4q**.

3.3.2 5-LOX assay

The 5-LOX screening results provided potent to moderate enzyme inhibition. Among all compounds **4c**, **4d**, **4e**, **4k**, **4l**, **4p**, **4q** ad **4u** showed 56.97%, 50.99%, 61.94%, 71.55%, 67.15%, 56.74%, 61.66% and 57.87% inhibition of LOX respectively. Compound **4l** exhibited the highest LOX inhibition (IC_{50} = 5.1µM). Compounds **4c**, **4d**, **4e**, **4g**, **4k**, **4l**, **4p**, **4q**, **4u** showed both COX-2 and LOX inhibition activity (**Table 2** & **Figure 5**).

3.3.3 *In vivo* anti-inflammatory activity

Based on inhibitory potency results obtained in *in vitro* COX-2 selectivity, compounds (**4c**, **4d**, **4e**, **4k**, **4l**, **4p**, **4q**, **4u**) were selected for *in vivo* anti-inflammatory activity using the standard carrageenan-induced rat paw edema assay. Each test compound was dosed orally (10mg/kg/1body weight) 30 minutes prior to induction of inflammation by carrageenan injection. Ibuprofen was used as a reference standard and the anti-inflammatory activity was calculated at intervals 3h and 5h after injection and is presented in **Table 3 & Figure 6**. The results indicated that the compounds **4c**, **4d**, **4e**, **4k**, **4l**, **4q** and **4u**, possessed good anti-inflammatory activity. The anti-inflammatory activity profile of compounds **4k**, **4q** (75.97 % inhibition at 3h post-carrageenan and 69.73% inhibition at 5h post-carrageenan, 80.62% inhibition at 3h post-carrageenan and 81.23% inhibition at 5h post-carrageenan) was better than that of standard NSAID, Ibuprofen (72.48% inhibition at 3h as well as 61.30% inhibition at 5h) and showed a time-dependent decrease in the inhibition of inflammation. Whereas, compounds **4c**, **4d**, **4e** and **4u** showed the opposite profile with a time dependent increase in the inhibition of inflammation.

3.3.4 DPPH radical antioxidant activity

Evaluation of DPPH radical scavenging activity is a rapid and convenient method of screening the antioxidant activities. Hence, we carried out our experiments to explore the free radical scavenging ability of synthesized oxadiazole derivatives using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). Samples at different concentration were prepared and tested for DPPH scavenging activity. The half inhibition concentration (IC_{50}) for all the derivatives including the reference antioxidant Ascorbic acid was calculated and showed in **Table 4**. Compounds **4e**, **4k** have shown potent DPPH radical scavenging activity (IC_{50} = 25.6 μM and IC_{50} = 48.9 μM) when compared with standard Ascorbic acid.

4. Conclusion

A new 1,2,4-oxadiazole analogs fused with mercapto benzothiazoles designed (**4a-u**) via bioisosteric approach based on anti-inflammatory 1,2,3 triazole. The docking results clearly indicated the selectivity of compounds **4a-u** towards COX-2 enzyme. The essential pharmacophoric interactions of active compounds with active site residues of COX-2 include Ser530, Arg120 and Tyr115 provided the selectivity. All the compounds synthesized and screened against *in vitro* COX-1, COX-2and 5-LOX enzymes along with *in vivo* anti-inflammatory and DPPH radical scavenging activity. Two selective COX-2 inhibitors **4q** and **4k** (IC_{50} = 5.0 μM and IC_{50} = 6.8 μM) were found to be most predominant anti-inflammatory

activity than standard drug Ibuprofen in *in vivo* studies. Whereas, two compounds **4l** and **4e** found to be inhibiting lypoxygenase enzyme at lower micomolar concentrations (IC_{50} = 5.1 µM and IC_{50} = 5.5 µM). Later to confirm *in vitro* studies, *in vivo* anti-inflammatory activity was carried out and found to be matching with earlier.

Acknowledgements

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Table 1. ADMET properties of compounds 4a-i

SNO	Mol wt	HBD	HBA	logP	#rotor	Rule Of Five
4a	370.4	0	5.5	3.207	4	0
4b	393.401	0	4.5	5	3	1
4c	359.847	0	4.5	4.434	3	0
4d	359.847	0	4.5	4.49	3	0
4e	359.847	0	4.5	4.462	3	0
4f	343.393	0	4.5	4.156	3	0
4g	343.393	0	4.5	4.192	3	0
4h	383.439	0	6.5	3.572	4	0
4i	326.39	0	5.5	3.061	3	0
4j	369.412	1	6.5	3.382	4	0
4k	368.427	2	7	2.57	4	0
4l	412.48	0	8.7	3.056	5	0
4m	424.535	1	7	4.321	5	0
4n	436.546	0	7.5	4.357	4	0
4o	472.527	0	7.5	4.769	4	0
4p	452.545	1	8.7	4.067	5	0
4q	438.518	0	9.2	3.225	4	0
4r	454.579	0	8	4.285	4	0
4s	465.587	1	9	3.91	5	0
4t	462.515	1	7	5.138	5	1
4u	478.97	1	7	5.421	5	1

Table 2. *Invitro* COX-1, COX-2 and 5-LOX ASSAY

S.No	Test Compounds	% of COX and LOX Inhibition at 10 µM			COX and LOX inhibition (IC_{50} µM)		
		COX-1	COX-2	LOX	COX-1	COX-2	LOX
1	4a	39.16	24.69	36.47	ND	ND	ND
2	4b	25.3	31.07	34.35	ND	ND	ND
3	4c	45.92	56.97	56.97	ND	7.8	8.7
4	4d	38.12	51.99	50.99	ND	8.8	8.0
5	4e	35.32	57.34	61.94	ND	6.9	5.5
6	4f	32.52	19.06	19.06	ND	ND	ND
7	4g	45.69	57.47	40.45	ND	8.5	ND
8	4h	40.95	41.31	43.21	ND	ND	ND
9	4i	35.9	39.47	16.18	ND	ND	ND
10	4j	48.15	15.46	24.77	ND	ND	ND
11	4k	49.93	62.53	71.55	ND	6.8	6.4
12	4l	38.35	50.12	67.15	ND	7.6	5.1
13	4m	42.43	14.88	14.88	ND	ND	ND
14	4n	36.78	46.03	46.03	ND	ND	ND
15	4o	25.54	24.65	24.65	ND	ND	ND
16	4p	36.77	56.74	56.74	ND	6.9	6.2
17	4q	29.98	62.66	61.66	ND	5.0	5.8
18	4r	40.08	47.9	47.90	ND	ND	ND
19	4s	33.68	30.64	30.64	ND	ND	ND
20	4t	35.45	32.01	32.01	ND	ND	ND
21	4u	29.88	57.87	57.87	ND	7.1	5.6
22	Indomethacin	95.4	83.2	ND	0.25	0.36	ND
23	Celecoxib	50.6	93.4	ND	3.2	0.038	ND

ND: Not Determined (Not showing any activity at maximum concentration)

Table 3.*Invivo* anti-inflammatory activity of active compounds

S.No	Compound	Change in Paw edema volume (mL) after drug treatment		Anti-inflammatory activity % of inhibition	
		3h	**5h**	**3h**	**5h**
1.	Control	0.86±0.173	0.87±0.140	--	--
2.	4c	0.33±0.124	0.28±0.064	61.24	68.20
3.	4d	0.48±0.091	0.37±0.065	44.57	57.09
4.	4e	0.58±0.090	0.32±0.044	32.95	63.22
5.	4k	0.21±0.55	0.26±0.205	75.97	69.73
6.	4l	0.28±0.069	0.38±0.040	67.44	56.32
7.	4q	0.17±0.078	0.16±0.086	80.62	81.23
8.	4u	0.39±0.035	0.39±0.050	55.04	55.56
9.	Ibuprofen	0.24±0.135	0.34±0.145	72.48	61.30

Table 4. DPPH Radical Antioxidant activity

S.No	Test Compounds	% of Inhibition at 100µM	IC_{50} (µM)
1	4a	19.25	ND
2	4b	44.78	ND
3	4c	55.65	65.8
4	4d	26.09	ND
5	4e	88.39	25.6
6	4f	34.65	ND
7	4g	45.52	ND
8	4h	36.27	ND
9	4i	16.25	ND
10	4j	51.74	70.2
11	4k	79.73	48.9
12	4l	52.33	81
13	4m	32.31	ND
14	4n	24.25	ND
15	4o	15.80	ND
16	4p	12.09	ND
17	4q	16.94	ND
18	4r	25.74	ND
19	4s	35.77	ND
20	4t	17.35	ND
21	4u	15.55	ND
22	Ascorbic acid	91.23	40.7

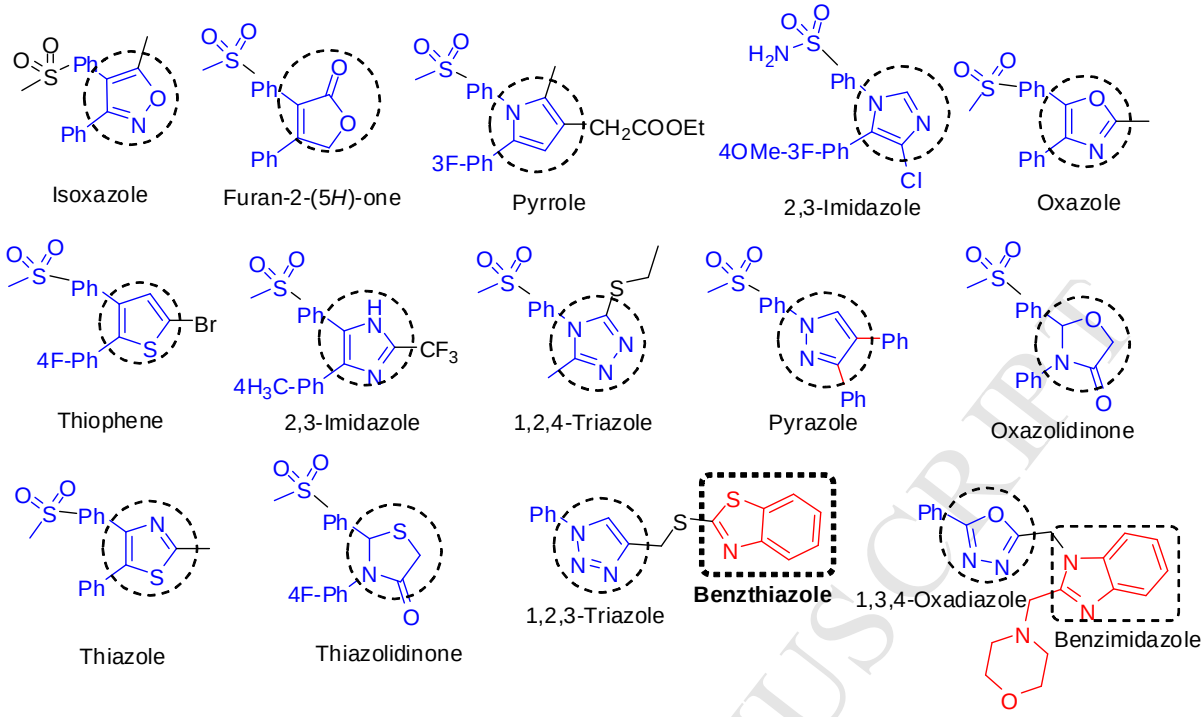


Figure 1: Bioisostere based design approach

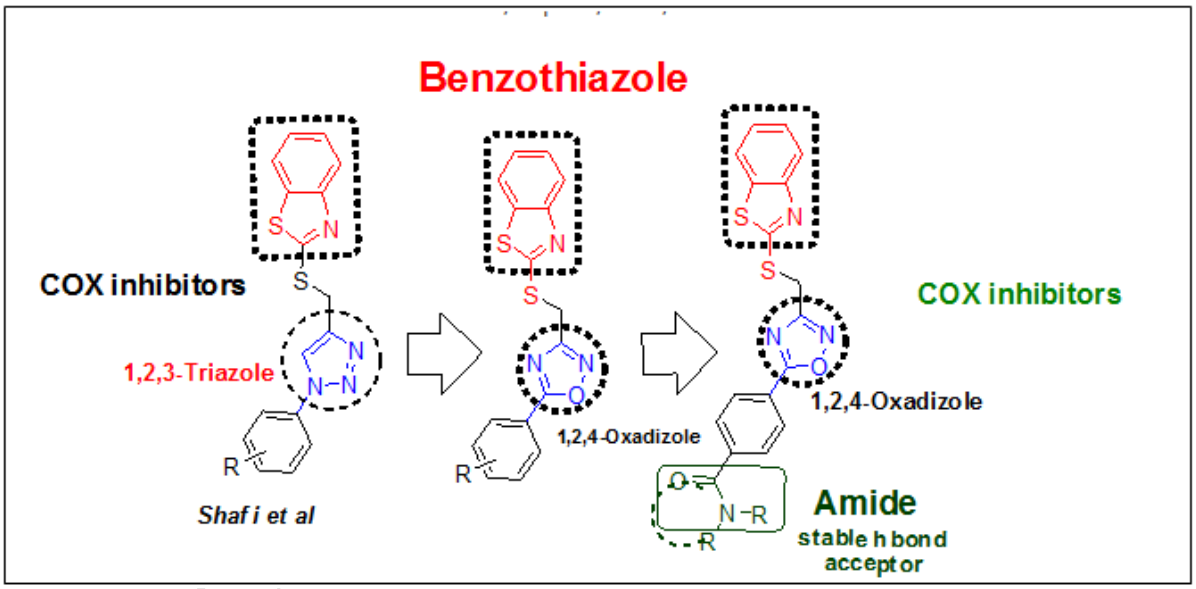


Figure 2: 2-mercapto benzothiazole linked 1,2 4 oxadiazoles design approach

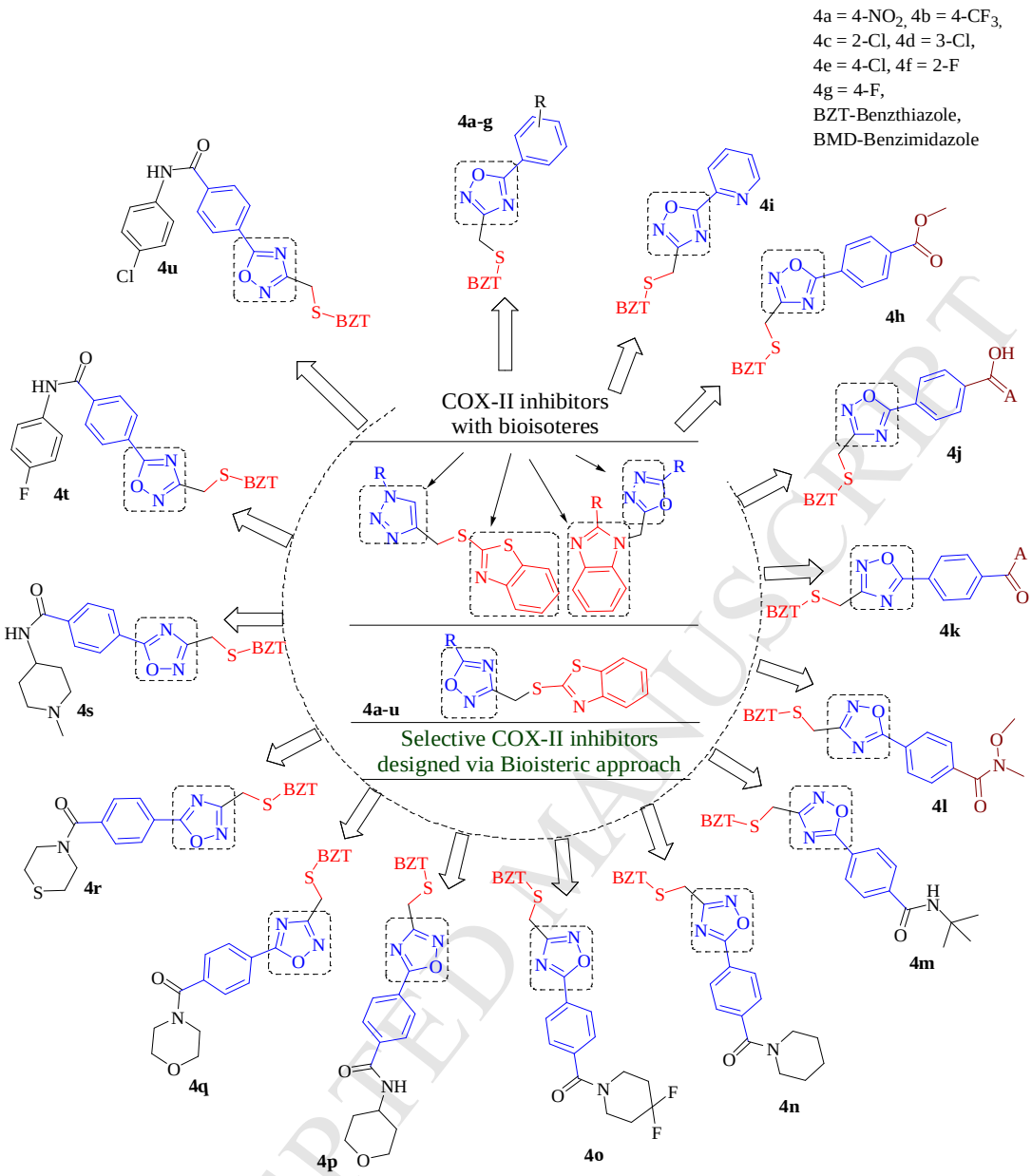


Figure 3: A overview of final designed focused library

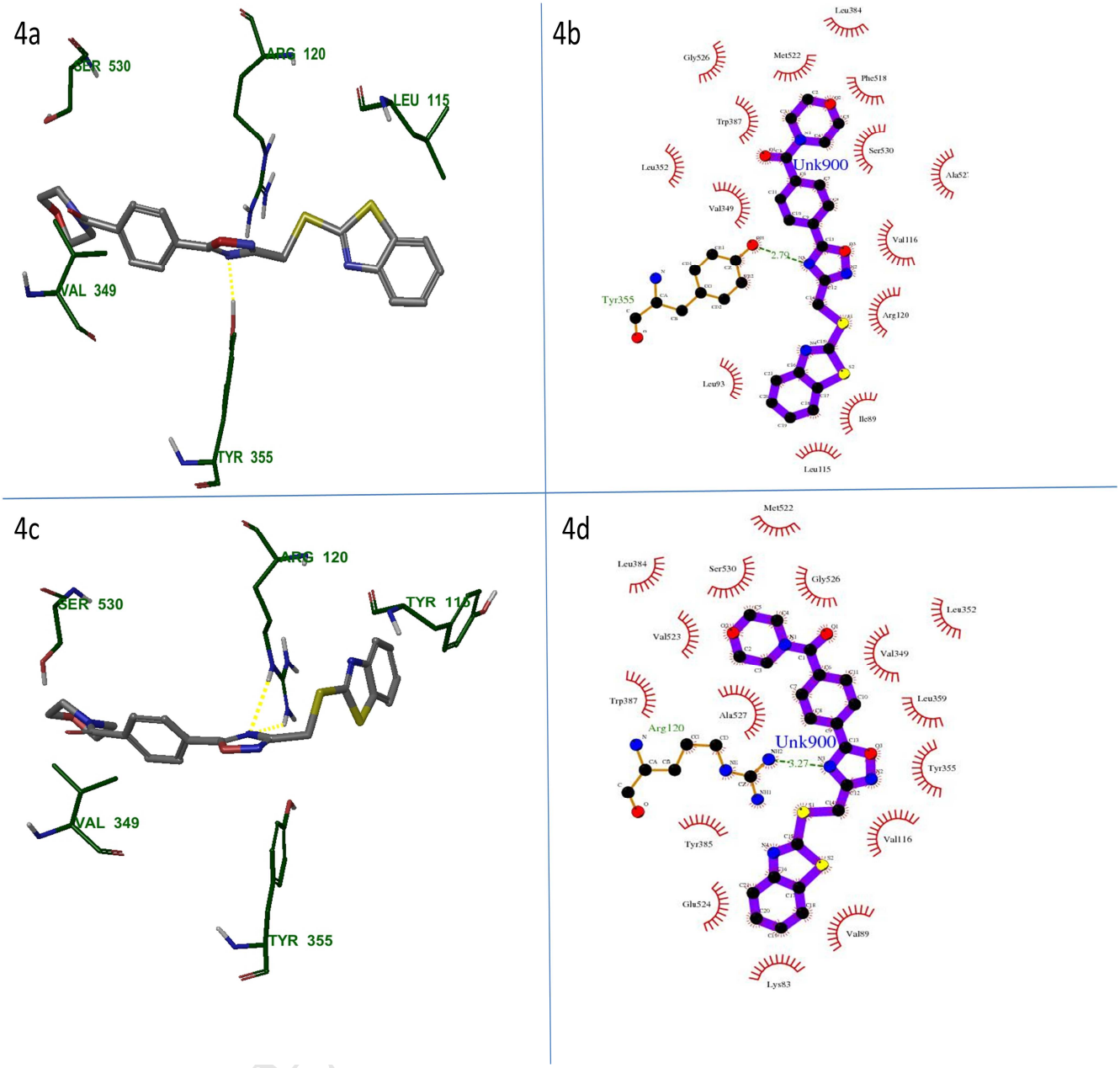
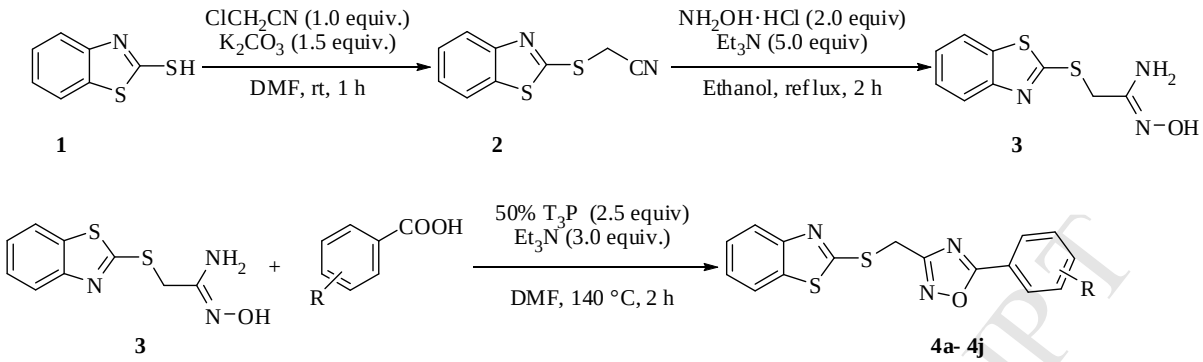
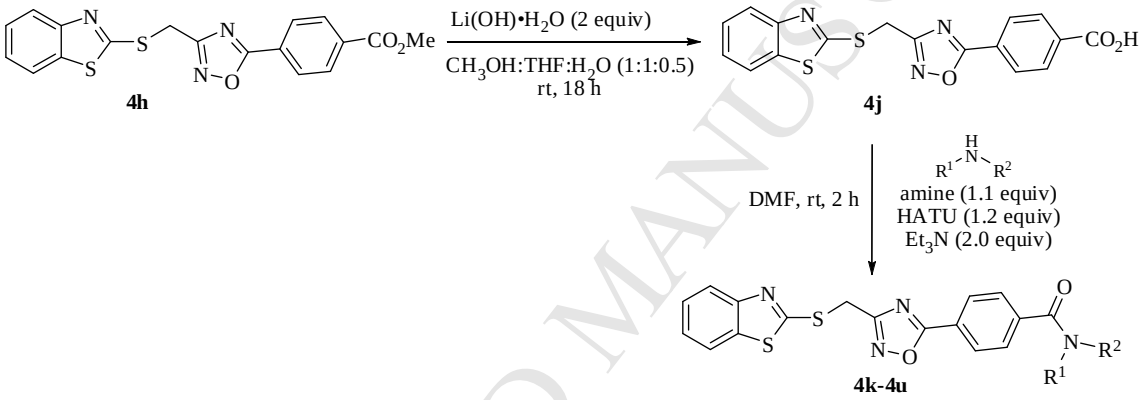


Figure 4a) Compound 4q at COX-I site, 4b) 2D-interaction diagram of compound 4q; 4c) Compound 4q at COX-II active site & 4d) 2D-interaction diagram of compound 4q

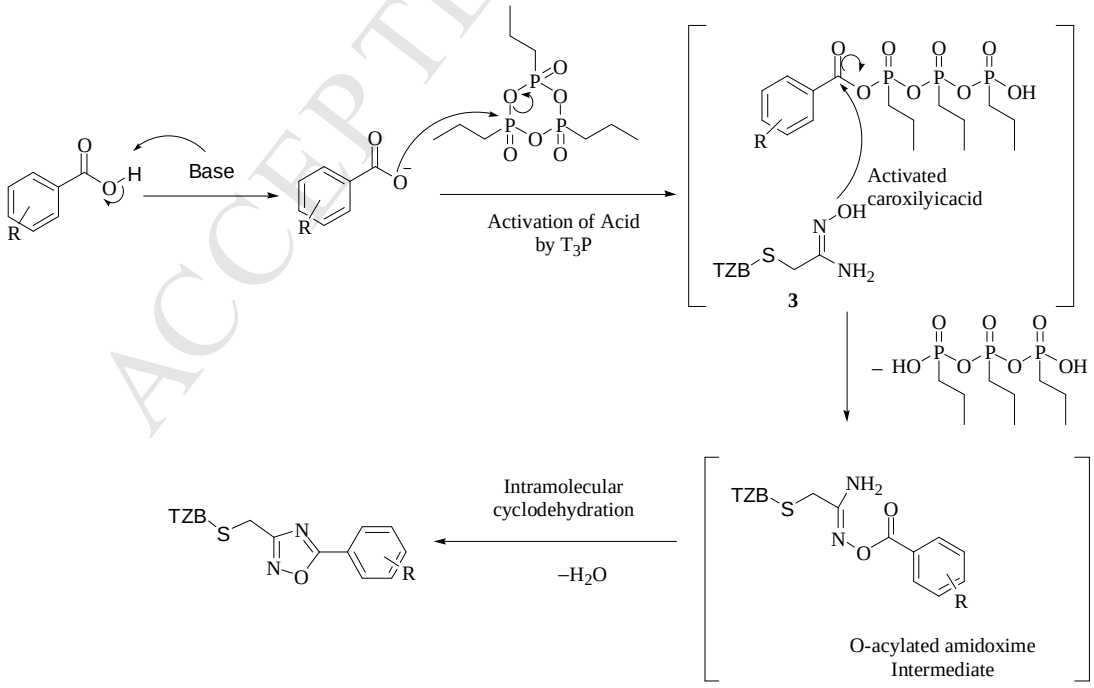
Scheme 1: Synthetic route for synthesis of compound **4a** to **4i**



Scheme 2: Synthetic route for synthesis of compound **4j** to **4u**



Scheme 3. General reaction mechanism for oxadiazole analogs



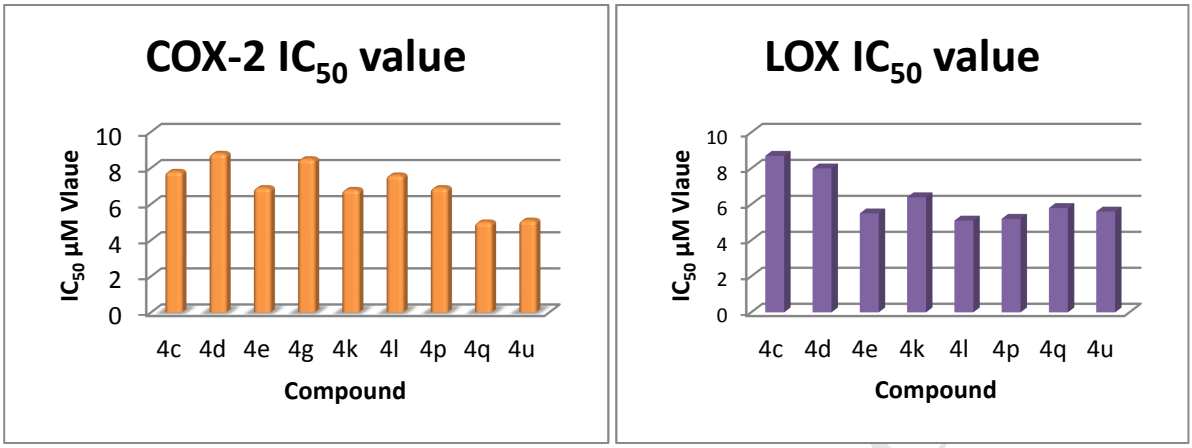


Figure 5: IC_{50} values of COX-2 and **5**-LOX

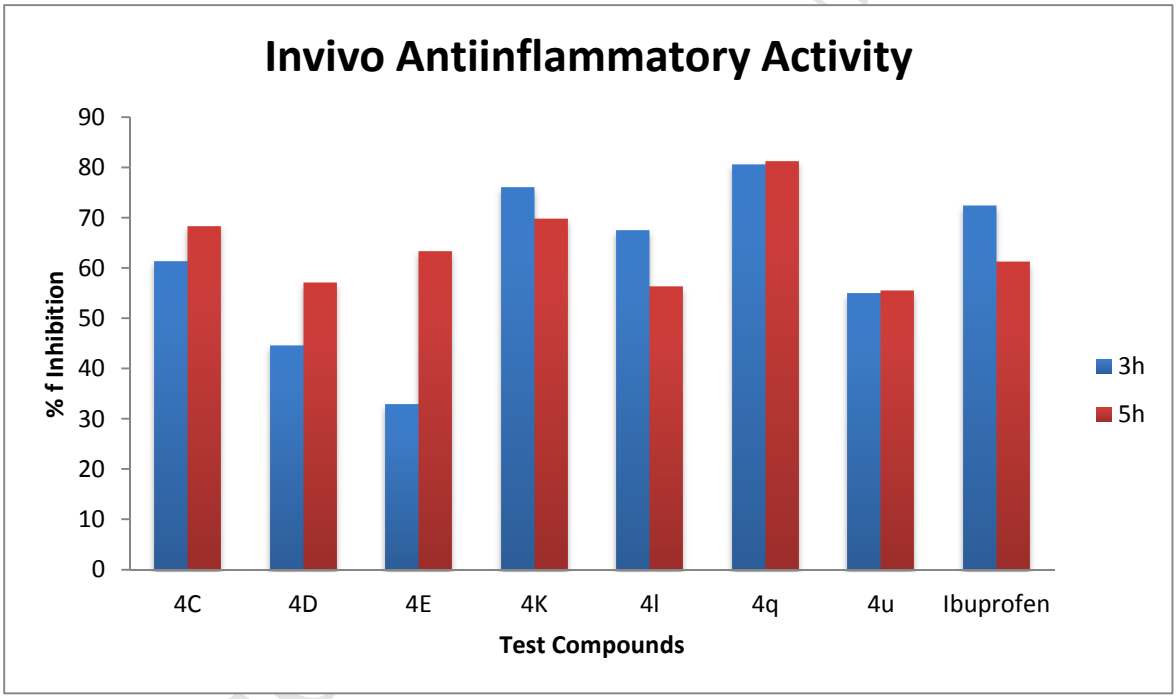


Figure 6: *In vivo* Anti inflammatory Activity of highly active compounds

- Focused library design and docking studies
- 1,2,4-oxadiazole linked mercapto benzothiazole derivatives
- Synthesis and biological screening
- Selective COX-2 and potent 5-LOX inhibitors