

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/264582360>

# ChemInform Abstract: Synthesis of Novel 2-Mercapto Benzothiazole and 1,2,3-Triazole Based Bis-Heterocycles: Their Antiinflammatory and Antinociceptive Activities.

ARTICLE in CHEMINFORM · JULY 2012

Impact Factor: 0.74 · DOI: 10.1002/chin.201231133

CITATIONS

3

READS

14

8 AUTHORS, INCLUDING:



**Mohammad Mahboob Alam**

Albaha University, Albaha, Saudi Arabia

16 PUBLICATIONS 93 CITATIONS

SEE PROFILE



**Naveen Mulakayala**

University of Hyderabad

35 PUBLICATIONS 300 CITATIONS

SEE PROFILE



**Arunasree Mk**

University of Hyderabad

39 PUBLICATIONS 608 CITATIONS

SEE PROFILE



**Reddanna Pallu**

University of Hyderabad

226 PUBLICATIONS 3,138 CITATIONS

SEE PROFILE



## Original article

Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based *bis*-heterocycles: Their anti-inflammatory and anti-nociceptive activities

Syed Shafi<sup>a,\*</sup>, Mohammad Mahboob Alam<sup>a</sup>, Naveen Mulakayala<sup>b</sup>, Chaitanya Mulakayala<sup>c</sup>,  
G. Vanaja<sup>d</sup>, Arunasree M. Kalle<sup>d</sup>, Reddanna Pallu<sup>d</sup>, M.S. Alam<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India

<sup>b</sup> Institute of Life Science, University of Hyderabad Campus, Hyderabad 500046, India

<sup>c</sup> Department of Biochemistry, Sri Krishnadevaraya University, Anantapur 515003, India

<sup>d</sup> Department of Animal Sciences, University of Hyderabad, Hyderabad 500046, India

## ARTICLE INFO

## Article history:

Received 17 September 2011

Received in revised form

6 January 2012

Accepted 16 January 2012

Available online 24 January 2012

## Keywords:

Benzothiazole

1,2,3-Triazole

Click chemistry

*bis*-Heterocycles

Anti-inflammatory activity

COX

## ABSTRACT

A focused library of novel *bis*-heterocycles encompassing 2-mercapto benzothiazole and 1,2,3-triazoles were synthesized using click chemistry approach. The synthesized compounds have been tested for their anti-inflammatory activity by using biochemical cyclooxygenase (COX) activity assays and carrageenan-induced hind paw edema. Among the tested compounds, compound **4d** demonstrated a potent selective COX-2 inhibition with COX-2/COX-1 ratio of 0.44. Results from carrageenan-induced hind paw edema showed that compounds **4a**, **4d**, **4e** and **4f** possess significant anti-inflammatory activity as compared to the standard drug Ibuprofen. The compounds showing significant activity were further subjected to anti-nociceptive activity by writhing test. These four compounds have shown comparable activity with the standard Ibuprofen. Further ulcerogenic studies shows that none of these compounds causing gastric ulceration.

© 2012 Elsevier Masson SAS. All rights reserved.

## 1. Introduction

Benzothiazoles are bicyclic ring system with multiple applications. Benzothiazole ring is a part of several marine and terrestrial natural compounds that are having useful biological activities [1]. Heterocycles containing thiazole fraction are present in many natural products, such as bleomycin, epothilone B, lyngbyabellin and dolastatin 10 [2]. In the 1950s, a number of 2-aminobenzothiazoles were intensively studied as central muscle relaxants after the discovery of Riluzole [3]. Riluzole (6-trifluoromethoxy-2-benzothiazolamine, PK-26124, RP-25279, Rilutek) was found to interfere with glutamate neurotransmission in biochemical, electrophysiological and behavioral experiments. Since then benzothiazole derivatives have been studied extensively and found to have diverse chemical reactivity and broad spectrum of biological activity. A large number of therapeutic agents were

synthesized with the help of Benzothiazole nucleus. These compounds have special significance due to their remarkable pharmacological potentialities.

Although thiazoles have been known from long time [4–6], their varied biological features are still of great scientific interest. Benzothiazoles shows broad spectrum of biological activities including antitumor activity, especially phenyl-substituted benzothiazoles [7–9] while condensed pyrimidobenzothiazoles and benzothiazoloquinazolines exert antiviral activity [10]. Some of the *bis*-substituted amidinobenzothiazoles have been found to be potential anti-HIV agents [11] while some of the substituted 6-nitro- and 6-aminobenzothiazoles [12] are reported to exhibit potential antimicrobial activity.

The studies of structure–activity relationship of benzothiazoles interestingly reveal that change of the structure of substituent group at C-2 position commonly results the change of its bioactivity [13]. The new class of 2-substituted aminobenzothiazoles has shown a wide range of biological activities including antibacterial, antitumor, anti-tubercular, carbonic anhydrase inhibitory activities [14]. Significant anti-inflammatory activity is displayed by some new 2-(4'-butyl-3'-5'-dimethylpyrazol-1-yl)-6-substituted benzothiazoles and 4-butyl-1-(6'-substituted-2'-benzothiazolyl)-3-

\* Corresponding authors. Tel.: +91 9717927759/9891171278; fax: +91 011 26059663.

E-mail addresses: [shafirrl@gmail.com](mailto:shafirrl@gmail.com) (S. Shafi), [msalam@jamiahamdard.ac.in](mailto:msalam@jamiahamdard.ac.in) (M.S. Alam).

methylpyrazol-5-ones [15]. 2-Substituted 5- or 6-Benzo thiazole-acetic acids and their derivatives have been demonstrated to be nonsteroidal anti-inflammatory agents [16]. Some of the benzo-thiazole derivatives have been reported to selectively inhibit human Cyclooxygenase-2-enzyme (COX-2) [17].

Similarly 1,2,3-triazoles and their derivatives have attracted considerable attention for the past few decades due to their chemotherapeutic value [18]. They have been considered as an interesting component in terms of biological activity and found in many drugs [19]. Many 1,2,3-triazoles are found to be potent antimicrobial [20,21], analgesic [22], anti-inflammatory, local anesthetic [23], anticonvulsant [24], anti-neoplastic [25], anti-malarial [26] and antiviral agents [27]. Thus 1,2,3-triazoles have emerged as powerful pharmacophores on their own right [28].

Several combinations of *bis*-heterocycles bearing benzothiazole as a main constituent have been synthesized in order to obtain the COX-2 selectivity. Some of them are selectively inhibiting COX-2 while most of them inhibiting both COX-1 and COX-2. But majority of the NSAIDs so far developed to heal the inflammation are causing adverse effects like gastric ulcer [29], kidney damage [30] and some of the NSAIDs also cause hepatotoxicity [31]. Even with selective coxibs that inhibit only COX-2 unexpected cardiovascular adverse effects were observed [32]. Thus there remains a compelling need for effective NSAIDs with an improved safety profile.

In view of the biological importance of benzothiazoles and 1,2,3-triazoles as anti-inflammatory and anti-nociceptive agents, we aim to conjugate these two important ligands under one construct through a sulfur linkage. Hitherto for the first time, we report the synthesis of benzothiazoles and 1,2,3-triazole based *bis*-heterocycles and their anti-inflammatory and analgesic activities.

## 2. Results and discussion

### 2.1. Chemistry

From the *in silico* data it is revealed that the *bis*-heterocyclic compounds encompassing 2-mercaptobenzthiazole and 1,2,3-triazole moieties showing significant binding potential towards COX enzyme. The designer molecules were synthesized and evaluated for their anti-inflammatory and analgesic activities. As illustrated in Scheme 1 novel *bis*-heterocycles were synthesized by the cycloaddition reaction of 2-(prop-2-ynylthio)benzo[d]thiazole, **2** with various azides. Towards the synthesis of compound **2**, 2-mercaptobenzthiazole was refluxed with propargyl bromide in dry THF. Various substituted aromatic/sugar azides **3** were reacted with propargylated 2-mercaptobenzthiazole, **2** under click chemistry reaction conditions to obtain the novel *bis*-heterocycles **4a–4m** in quantitative yields. Aromatic azides with varying substitutions including electron withdrawing and electron donating groups were reacted with compound **2**.

To improve the hydrophilicity of the resultant compounds; galactosyl, glyceryl and pyridyl azides were synthesized and

reacted with propargylated 2-mercapto benzothiazole (**2**) in order to obtain the novel *bis*-heterocycles with improved hydrophilicity compared to the aromatic azide derived *bis*-heterocycles (Scheme 2). A focused library of fifteen compounds was synthesized (Table 1) and all the synthesized *bis*-hetero cycles were screened for their anti-inflammatory activity.

All the products were characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, IR, MALDI-MS/ESI-MS. In the  $^1\text{H}$  NMR spectra, the formation of triazoles was confirmed by the resonance of H-C(5) of the triazole ring in the aromatic region. The structure was further supported by the  $^{13}\text{C}$  NMR spectra, which showed the C-atom signals corresponding to triazole derivatives. MALDI-MS/ESI-MS of all compounds showed  $[\text{M} + \text{Na}]$  or  $[\text{M} + \text{Na}]$  or  $[\text{M} + 1]$ .

### 2.2. COX assays

All the above synthesized compounds were evaluated for their anti-inflammatory activity by biochemical COX (COX-1 & COX-2) inhibitory assay. The ratio of  $\text{IC}_{50}$  of COX-2 to  $\text{IC}_{50}$  of COX-1 (COX-2/COX-1) would suggest the selectivity of the compound and hence its gastric liability. The COX-2/COX-1 ratio of the above compounds showed that compound **4d** is a selective COX-2 inhibitor with a ratio of 0.44 and 2-fold selective when compared to COX-1 (Table 2).

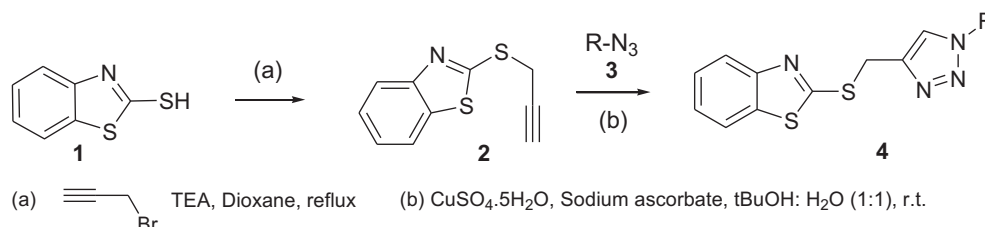
### 2.3. In vivo anti-inflammatory activity

Further the above synthesized compounds were tested for their *in vivo* anti-inflammatory activity by carrageenan-induced hind paw edema model. The results (Table 3 & Fig. 1) indicated that the compounds **4a**, **4d**, **4e** and **4f** possessed good anti-inflammatory activity. The anti-inflammatory activity profile of compound **4d** (77.83% inhibition at 3 h post-carrageenan and 81.13% inhibition at 5 h post-carrageenan) was better than that of standard NSAID, Ibuprofen (69.50% inhibition at 3 h as well as 71.69% inhibition at 5 h) and showed a time-dependent increase in the inhibition of inflammation.

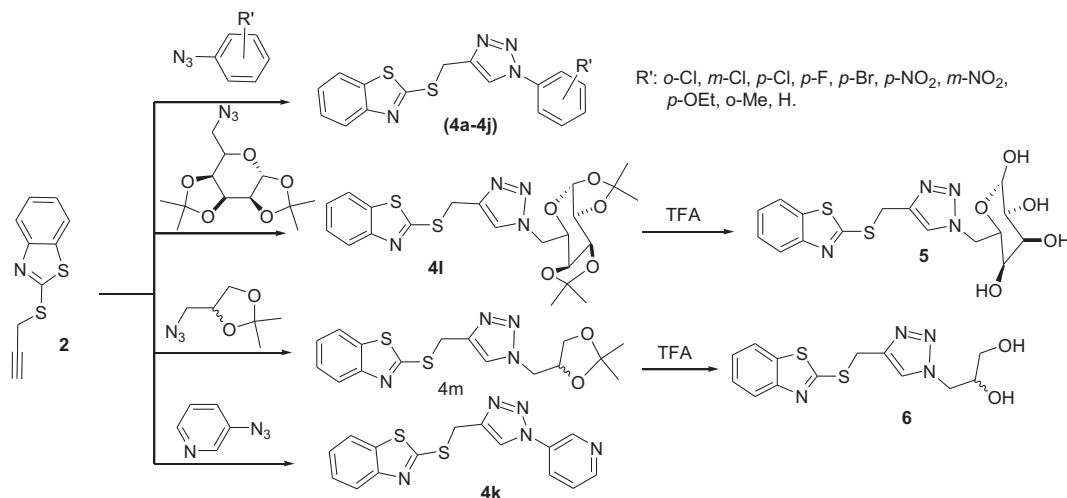
Whereas compounds **4a**, **4e** and **4f** showed the opposite profile with a time-dependent decrease in the inhibition of inflammation (83.30, 75.00, 75.00% inhibition at 3 h post-carrageenan and 78.11, 65.47, 59.24% inhibition at 5 h post-carrageenan respectively). Compounds **4k** and **4l** showed moderate anti-inflammatory effect comparable to Ibuprofen, at 3 h hour intervals (64.00 and 59.66%). Compounds **4c** and **4h** showed weak anti-inflammatory activities and compound **4g** exerted no activity.

Further, the anti-inflammatory efficacy of the synthesized compounds was analyzed based on the structure–activity relationships considering the three structural components: the nature of the group attached to 1,2,3-triazolyl ring, nature of the substituents (functional group) and the position of substitutions on aromatic ring.

First, the influence of the nature of the group attached to triazolyl ring was easily observed as aryl substitution on triazolyl ring (**4a**, **4d**,



Scheme 1. Synthetic approach for novel *bis*-heterocycles.



**Scheme 2.** Click chemistry approach for novel bis-heterocycles.

**4e** and **4f**) showed superior activity over the galactosyl, glyceryl and pyridyl substitutions. Regarding the substitution on the aromatic ring, electron withdrawing groups conferred the greater activity over the electron donating groups. The derivatives with the electron withdrawing groups i.e. –Cl (**4a**), –F (**4d**), –Br (**4e**), –NO<sub>2</sub> (**4f**) substitutions showed significantly higher activities compared to –CH<sub>3</sub>, –OEt substitutions. Compounds with fluoro and chloro substitutions confer significantly higher activity at 3 h as well as at 5 h.

Regarding the position of the substituent on the aromatic ring, halogens substituted at para position has mostly shown significant activity. When chlorine was *ortho* substituted, it exerts significant activity compared to the *para* substitution. When –NO<sub>2</sub> group was substituted at *para* position, it conferred greater activity than it was substituted at *meta* position (significantly lost the activity).

## 2.4. Molecular docking studies

### 2.4.1. In silico modeling of human COX-2

In order to further demonstrate that the anti-inflammatory efficacy of the compounds synthesized is attributed to the inhibition of COX-2, *in silico* docking studies have been carried out. Considering the limited sequence identity between human Cox-2 and the templates used for its modeling, an objective validation gives results suggestive of reliable models. Human COX-2 model construction starts with PSI-BLAST analysis, which was used to select best template. Human COX-2 sequence was directly compared to amino acid residue sequences that possess structures experimentally resolved and deposited in the Protein Data Bank [33]. Human COX-2 presented 87% of identity with 1PXX, being chosen as a template. Alignments among sequences and structures were carried by using Clustal-W [34]. After alignment analysis, atomic coordinated were transferred to human COX-2 primary structure for the construction of 3D model using Modeller 9v6 (Fig. 2).

Procheck summary of human COX-2 showed that 100% amino acid residues were in most favorable regions, additionally allowed regions and generously allowed regions with 0.0% residues in the disallowed regions. Structural differences between crystal structure 1PXX and predicted three dimensional structure of human COX-2 were calculated by superimposing both structures. The RMSD values between the crystal structure 1PXX and homology model human COX-2 calculated for C $\alpha$  traces and main chain atom were 1.25 Å. The RMSD values and small variability among experimental structures template and the structure modeled reflect the presence of strong restraints in most regions and emphasize a similar folding

patterns. Furthermore, the lower score acquired for PROSA –7.68 indicating the high quality of the model.

### 2.4.2. Docking studies on human COX-2

Docking analysis was performed on novel benzothiazole derivatives to identify key amino acid residues involved in making interactions with the human COX-2 model structure. All inhibitors were docked successfully in the human COX-2 homology model, showing good docking scores. The docking program AutoDock computes binding energy, RMSD and inhibition constant (*K<sub>i</sub>*) with respect to the docked inhibitors. The AutoDock computed binding energy; RMSD and *K<sub>i</sub>* values along with H-bond interacting residues are presented in Table 4.

The binding 3D conformations of COX-2 with bis-heterocycles are displayed in Fig. 2. It was observed that the interaction of bis-heterocycles with COX-2 occurred in a distinct site, formed by Tyr<sup>334</sup>, Val<sup>335</sup>, Leu<sup>338</sup>, Leu<sup>370</sup>, Ser<sup>448</sup>, Met<sup>508</sup>, Gly<sup>512</sup>, and Ser<sup>516</sup>. All these residues are functionally important and are found to be conserved. Among all the benzothiazole derivatives **4e** has given the best predicted binding free energy (–12.54 kcal/mol) onto human COX-2. The docked pose of the most potent molecule, **4e** obtained using AutoDock4 software is represented in Fig. 2. **4e** showed strong hydrogen bonding interactions, hydrophobic interactions and van der Waals interactions with Tyr<sup>334</sup>, Val<sup>335</sup>, Leu<sup>338</sup>, Leu<sup>370</sup>, Gly<sup>422</sup>, Ser<sup>448</sup>, Phe<sup>449</sup>, Asn<sup>450</sup>, Glu<sup>451</sup>, Glu<sup>465</sup>, Glu<sup>470</sup>, Glu<sup>478</sup>, Met<sup>508</sup>, Val<sup>509</sup>, Glu<sup>510</sup>, Gly<sup>512</sup>, Ala<sup>513</sup>, Pro<sup>514</sup>, and Ser<sup>516</sup>. In summary, the benzothiazole derivatives studied are potent inhibitors targeting human COX-2. We observed that the homology model of human COX-2 assisted molecular docking analysis of benzothiazole derivatives.

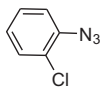
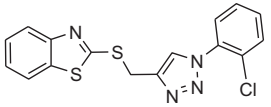
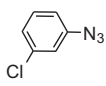
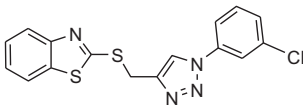
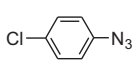
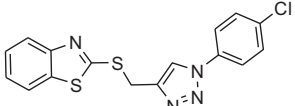
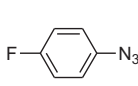
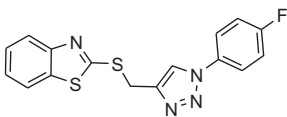
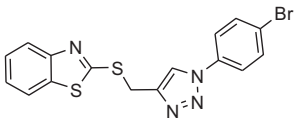
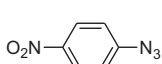
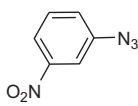
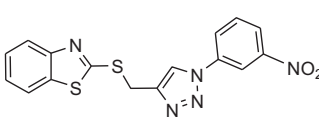
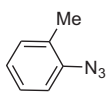
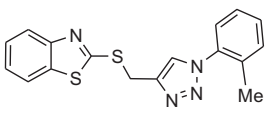
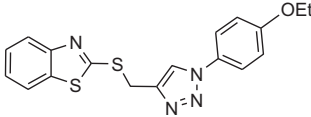
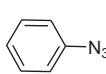
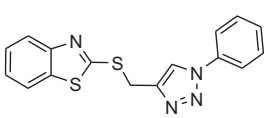
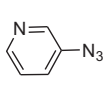
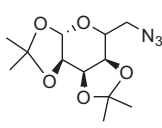
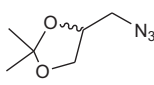
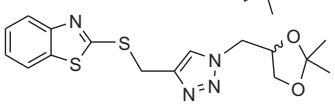
## 2.5. Analgesic activity

The results of analgesic activity are summarized in Fig. 3. Among tested compounds, compounds **4d** and **4e** are showing better activity compared to standard drug Ibuprofen, while compounds **4a** and **4f** are showing comparable analgesic activity with reference to standard drug. All data's were analyzed by one-way ANOVA test followed by Dunnett's test.

## 2.6. Ulcerogenic studies

The compounds showing potential anti-inflammatory activity were further carried out for ulcerogenic studies. When compared with Ibuprofen, compounds **4a**, **4d**, **4e** and **4f** did not cause any

**Table 1**  
Novel *bis*-heterocycles.

Sr. No.	Azide ( <b>3</b> )	<i>bis</i> -Heterocycles ( <b>4</b> )	Reaction time (h)	Yields <sup>a</sup> (%)
a			8	95
b			7	93
c			7	89
d			8	95
e			6	97
f			8	93
g			7	92
h			8	88
i			7	94
j			8	92
k			8	95
l			9	90
m			9	87

<sup>a</sup> Isolated yields.

**Table 2**  
COX-2/COX-1 ratio of the benzothiazole derivatives.

Sr. No.	Compound	COX-2/COX-1
1	<b>4a</b>	0
2	<b>4c</b>	14.90136
3	<b>4d</b>	0.440456
4	<b>4e</b>	0
5	<b>4f</b>	0
6	<b>4g</b>	5.212629
7	<b>4k</b>	0
8	<b>4l</b>	1.532059
9	<b>4m</b>	0
10	Indo methacin	7.164179
11	Celecoxib	0.0028

gastric ulceration and disruption of gastric epithelial cells at the below mentioned oral doses. Stomach wall of Ibuprofen treated group at low power (10×) photomicrograph showed damage of the mucosa and the sub mucosa. Stomach wall of the same section at high power (40×) photomicrograph showing desquamated epithelial cells in the lumen whereas in tested compounds (**4a**, **4d**, **4e** and **4f**) treated animals, no surface epithelial damage and submucosal damage was seen. Stomach wall of **4a**, **4d**, **4e** and **4f** treated animals showed no damage of any layer. The results are shown in Table 5 and Fig. 4.

### 3. Conclusion

In the present study a focussed library of 2-mercaptobenzothiazole derived bis-heterocycles encompassing benzothiazole-1,2,3-triazole moieties conjugated through a sulphur linkage are synthesized and evaluated for their anti-inflammatory activity. From the structure–activity relationship aromatic ring attached to triazolyl moiety are showing potential activity when compared to aliphatic/alicyclic rings. Electron withdrawing groups when substituted on aromatic ring mostly at the *para* position are exhibiting potential anti-inflammatory activity (compounds **4a**, **4d**, **4e** and **4f**) compared to the standard drug ibuprofen without causing any ulceration. The COX inhibitory potential of compound **4d** and the ulcerogenic studies further concludes that these kinds of molecules can be considered as potent anti-inflammatory agents. Further chemical and biological studies are under way.

**Table 3**  
*In vivo* anti-inflammatory activity of novel bis-heterocycles.

Sr. No.	Compound	Change in paw edema volume (ml) after drug treatment		Antiinflammatory activity % inhibition	
		3 h	5 h	3 h	5 h
1	<b>4a</b>	<b>0.10 ± 0.051***</b>	<b>0.11 ± 0.016***</b>	<b>83.30</b>	<b>78.11</b>
2	<b>4b</b>	0.39 ± 0.048	0.32 ± 0.042	35.00	39.60
3	<b>4c</b>	0.30 ± 0.051 <sup>ns</sup>	0.23 ± 0.055*	50.00	43.39
4	<b>4d</b>	<b>0.13 ± 0.033**</b>	<b>0.10 ± 0.044***</b>	<b>77.83</b>	<b>81.13</b>
5	<b>4e</b>	<b>0.15 ± 0.042**</b>	<b>0.21 ± 0.030*</b>	<b>75.00</b>	<b>59.24</b>
6	<b>4f</b>	<b>0.15 ± 0.042**</b>	<b>0.18 ± 0.040*</b>	<b>75.00</b>	<b>65.47</b>
7	<b>4g</b>	0.53 ± 0.180 <sup>ns</sup>	0.41 ± 0.107 <sup>ns</sup>	11.66	21.50
8	<b>4h</b>	0.31 ± 0.079 <sup>ns</sup>	0.30 ± 0.051 <sup>ns</sup>	47.30	43.39
9	<b>4i</b>	0.37 ± 0.045 <sup>ns</sup>	0.35 ± 0.05 <sup>ns</sup>	38.33	33.96
10	<b>4j</b>	0.32 ± 0.045 <sup>ns</sup>	0.36 ± 0.045 <sup>ns</sup>	46.66	32.07
11	<b>4k</b>	0.21 ± 0.030*	0.23 ± 0.055*	64.00	56.03
12	<b>4l</b>	0.24 ± 0.060 <sup>ns</sup>	0.23 ± 0.098 <sup>ns</sup>	59.66	56.60
13	<b>5</b>	0.22 ± 0.051 <sup>ns</sup>	0.21 ± 0.046 <sup>ns</sup>	63.33	60.37
14	<b>4m</b>	0.28 ± 0.042 <sup>ns</sup>	0.27 ± 0.042 <sup>ns</sup>	53.33	49.05
15	<b>6</b>	0.26 ± 0.042 <sup>ns</sup>	0.25 ± 0.046 <sup>ns</sup>	56.66	52.83
16	Ibuprofen	<b>0.18 ± 0.054**</b>	<b>0.15 ± 0.056**</b>	<b>69.50</b>	<b>71.69</b>
17	Control	0.60 ± 0.089	0.53 ± 0.071	—	—

Data is analyzed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from six observations; \*\*\* indicates  $P < 0.001$ , \*\* indicates  $P < 0.01$  & \* indicates  $P < 0.05$ .

## 4. Experimental

### 4.1. Chemistry

All commercial chemicals used as starting materials and reagents in this study were purchased from Merck (India), Spectrochem, and Sigma Aldrich which were of reagent grade. All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan); IR spectra were recorded as potassium bromide pellets on a Perkin Elmer 1650 spectrophotometer (USA),  $^1\text{H}$  NMR spectra were determined on a Bruker (200, 300 and 400 MHz) spectrometer and chemical shifts were expressed as ppm against TMS as internal reference. Mass spectra were recorded on MALDI-AB4800 and 70 eV (EI Ms-QP 1000EX, Shimadzu, Japan), Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06e 0.20 mm). All compounds prepared in this paper are new and confirmed with spectral data.

Compound **2** was dissolved in 20 mL of Butanol:water (1:1) solvent at ambient temperature. Then was charged  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and the reaction mixture was stirred for 5 min. Reaction mixture was light blue in colour. Then sodium ascorbate was added at once to the reaction mixture and allowed to stir for 15 min. Reaction mixture colour was changed to dark yellow. After 15 min azide was added at once. The reaction mixture was allowed to stir for further 8 h at ambient temperature. After the completion of the reaction, monitored by TLC, reaction mixture was quenched with water and extracted with ethyl acetate. Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to obtain the final product.

#### 4.1.1. 2-((1-(2-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (**4a**)

M.P. 104–105 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3074, 2920, 2851, 2372, 1496, 1461, 1381, 1231, 1075, 1039, 994, 753, 719;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 4.79 (s, 2H), 7.30 (t, 1H,  $J = 7.20$  Hz), 7.39–7.44 (m, 3H), 7.52–7.54 (m, 1H), 7.59–7.61 (m, 1H), 7.76 (d, 1H,  $J = 8.00$  Hz), 7.89 (d, 1H,  $J = 8.00$  Hz), 8.09 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.68, 121.10, 121.50, 124.41, 125.13, 126.07, 127.68, 127.87, 128.40, 130.67, 130.72, 134.77, 135.50, 143.74, 152.99, 165.58; MALDI-MS: 359 ( $\text{M}^+ + 1$ ), 381 ( $\text{M}^+ + \text{Na}$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{S}_2$ : C, 53.55; H, 3.09; Cl, 9.88; N, 15.61; S, 17.87%, Found: C, 53.51; H, 3.13; Cl, 9.82; N, 15.67; S, 17.82%.



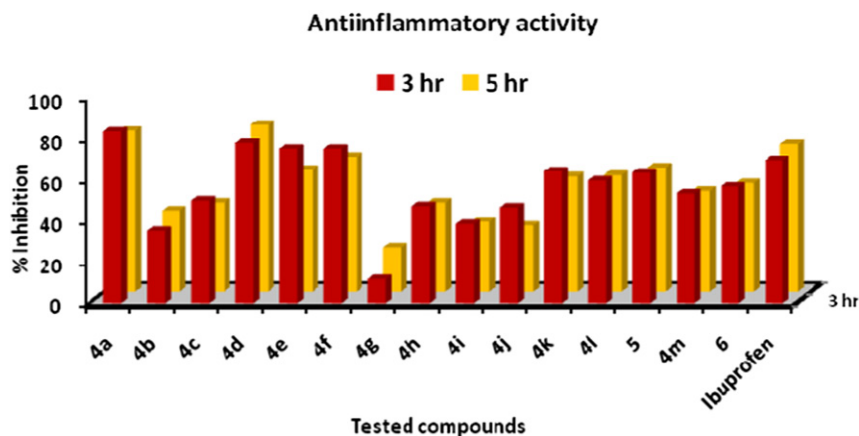


Fig. 1. *In vivo* anti-inflammatory activity of novel bis-heterocycles.

**4.1.2. 2-((1-(3-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4b)**

M.P. 102–104 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3114, 3078, 1592, 1455, 1426, 1252, 1048, 790, 751, 680;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 4.70 (s, 2H), 7.25 (t, 1H,  $J = 8.00$  Hz), 7.31–7.39 (m, 3H), 7.50–7.52 (dd, 1H,  $J = 7.20$  and 1.6 Hz), 7.66 (s, 1H), 7.70 (d, 1H,  $J = 8.00$  Hz), 7.85 (d, 1H,  $J = 8.00$  Hz), 7.99 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.63, 121.06, 121.44, 124.37, 125.10, 126.03, 127.61, 127.83, 128.33, 130.66, 134.69, 135.44, 143.66, 152.92, 165.52; MALDI-MS: 359 ( $\text{M}^++1$ ), 381 ( $\text{M}^++\text{Na}$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{S}_2$ : C, 53.55; H, 3.09; Cl, 9.88; N, 15.61; S, 17.87%, Found: C, 53.49; H, 3.16; Cl, 9.79; N, 15.57; S, 17.92%.

**4.1.3. 2-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4c)**

M.P. 150–152 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3136, 3062, 3041, 2441, 1501, 1462, 1427, 1229, 1094, 1050, 999, 831, 746, 722;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  (ppm): 4.77 (s, 2H), 7.32 (t, 1H,  $J = 8.00$  Hz), 7.43–7.48 (m, 3H), 7.62–7.64 (dd, 2H,  $J = 7.0$  and 1.5 Hz), 7.70 (d, 1H,  $J = 8.00$  Hz), 7.91 (d, 1H,  $J = 8.5$  Hz), 8.05 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.57, 121.20, 121.48, 121.73, 124.51, 126.18, 129.91, 134.60, 135.42, 135.51, 152.98, 165.69; MALDI-MS: 359 ( $\text{M}^++1$ ), 381 ( $\text{M}^++\text{Na}$ ), 397 ( $\text{M}^++\text{K}$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{ClN}_4\text{S}_2$ : C, 53.55; H, 3.09; Cl, 9.88; N, 15.61; S, 17.87%, Found: C, 53.47; H, 3.01; Cl, 10.91; N, 15.66; S, 17.93%.

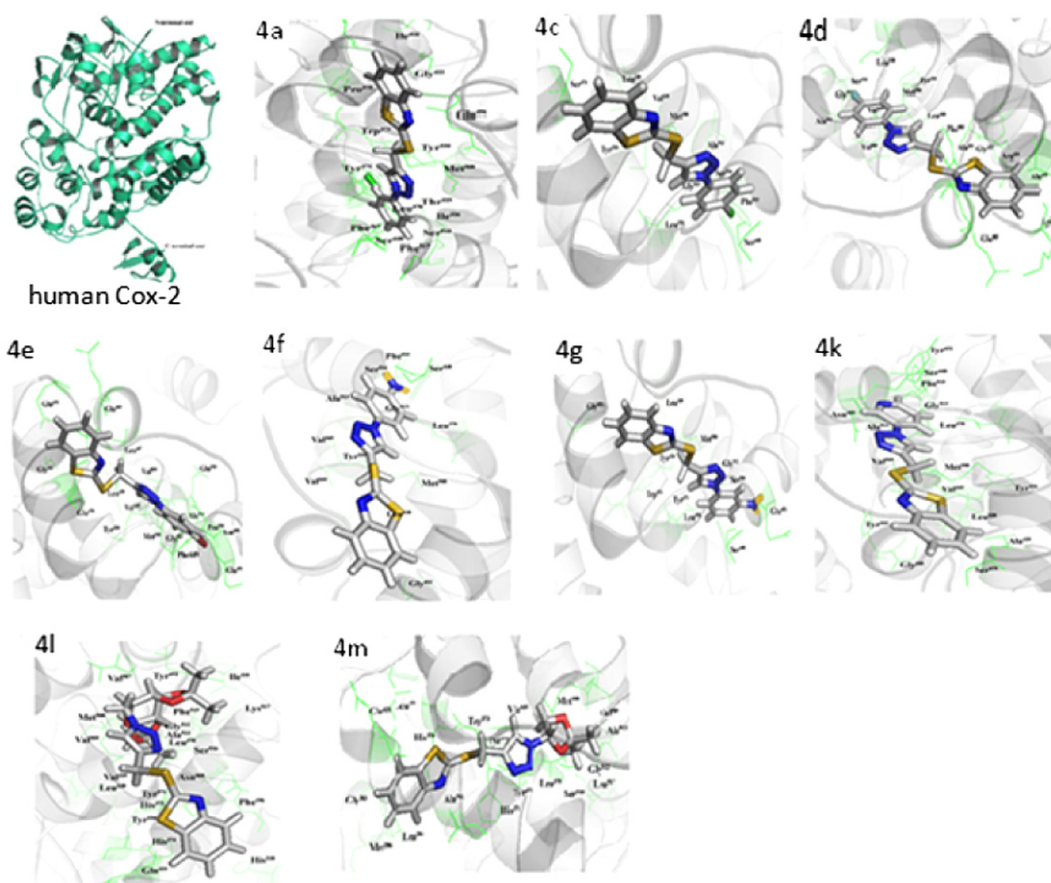


Fig. 2. Docking results of bis-heterocycles onto human COX-2.

**Table 4**  
Docking results of bis-heterocycles onto human COX-2.

Compound	Binding energy (Kcal/mol)	Inhibition constant ( $K_i$ ) ( $\mu$ m)	RMSD ( $\text{\AA}$ )
<b>4a</b>	−11.86	2.56	1.65
<b>4c</b>	−10.93	5.56	1.12
<b>4d</b>	−11.52	1.25	1.09
<b>4e</b>	−12.54	0.36	0.56
<b>4f</b>	−10.65	5.12	1.59
<b>4g</b>	−11.23	6.96	1.38
<b>4k</b>	−11.09	1.99	1.16
<b>4l</b>	−12.38	0.97	0.69
<b>4m</b>	−12.25	1.06	0.93

**4.1.4. 2-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4d)**

M.P. 126–128 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3135, 3074, 2928, 1779, 1514, 1463, 1427, 1231, 1048, 998, 834, 747;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  (ppm): 4.77 (s, 2H), 7.19 (t, 2H,  $J = 8.5$  Hz), 7.32 (t, 1H,  $J = 7.5$  Hz), 7.44 (t, 1H,  $J = 7.5$  Hz), 7.64–7.67 (m, 2H), 7.77 (d, 1H,  $J = 8.0$  Hz), 7.91 (d, 1H,  $J = 8.5$  Hz), 8.02 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.45, 116.41, 116.72, 121.08, 121.28, 121.35, 122.37, 122.48, 124.38, 126.06, 133.37, 135.37, 144.80, 152.83, 163.94, 165.60; MALDI-MS: 365 ( $\text{M}^+ + \text{Na}$ ), 381 ( $\text{M}^+ + \text{K}$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{FN}_4\text{S}_2$ : C, 56.12; H, 3.24; F, 5.55; N, 16.36; S, 18.73%; Found: C, 56.08; H, 3.19; N, 16.27; S, 18.65%.

**4.1.5. 2-((1-(4-Bromophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4e)**

M.P. 148–150 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3126, 3058, 2394, 1496, 1458, 1426, 1050, 999, 821, 746, 722;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 200 MHz)  $\delta$  (ppm): 4.77 (s, 2H), 7.32 (t, 1H,  $J = 7.42$  Hz), 7.45 (t, 1H,  $J = 7.13$  Hz), 7.55–7.61 (m, 4H), 7.77 (d, 1H,  $J = 7.44$  Hz), 7.92 (d, 1H,  $J = 7.85$  Hz), 8.06 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.56, 121.20, 121.49, 121.96, 122.48, 124.52, 126.19, 132.88, 135.54, 135.90, 152.98, 165.69; MALDI-MS: 403 ( $\text{M}^+$ ), 405 ( $\text{M}^+ + 2$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{BrN}_4\text{S}_2$ : C, 47.65; H, 2.75; Br, 19.81; N, 13.89; S, 15.90%; Found: C, 47.57; H, 2.69; Br, 19.89; N, 13.91; S, 16.02%.

**4.1.6. 2-((1-(4-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4f)**

M.P. 178–180 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3106, 3030, 2381, 1596, 1523, 1474, 1407, 1333, 1009, 1050, 852;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 200 MHz)  $\delta$  (ppm): 4.79 (s, 2H), 7.33 (t, 1H,  $J = 7.86$  Hz), 7.46 (t, 1H,  $J = 8.48$  Hz), 7.78 (d, 1H,  $J = 7.16$  Hz), 7.90–7.94 (m, 3H), 8.20 (s, 1H), 8.35–8.41 (dd, 2H,  $J = 9.13$  and 2.0 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.28, 120.48, 121.05, 121.22, 121.41, 124.58, 125.48, 126.22, 135.49, 141.00, 145.97, 147.16, 152.85, 165.44; MALDI-MS: 392 ( $\text{M}^+ + \text{Na}$ ), 408

( $\text{M}^+ + \text{K}$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{N}_5\text{O}_2\text{S}_2$ : C, 52.02; H, 3.00; N, 18.96; S, 17.36; Found: C, 52.11; H, 3.05; N, 18.92; S, 17.42%.

**4.1.7. 2-((1-(3-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4g)**

M.P. 153–155 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3121, 3030, 2438, 1534, 1454, 1426, 1343, 1002, 758;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  (ppm): 4.79 (s, 2H), 7.32 (t, 1H,  $J = 7.20$  Hz), 7.45 (t, 1H,  $J = 7.20$  Hz), 7.69–7.78 (m, 2H), 7.92 (d, 1H,  $J = 7.80$  Hz), 8.12 (d, 1H,  $J = 8.10$  Hz), 8.20 (s, 1H), 8.28 (d, 1H,  $J = 8.10$  Hz), 8.55 (d, 1H,  $J = 1.8$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  27.42, 115.36, 121.11, 121.22, 121.53, 123.24, 124.59, 126.00, 126.25, 130.95, 135.55, 137.67, 145.87, 148.93, 152.96, 165.46; MALDI-MS: 369 ( $\text{M}^+$ ), 370 ( $\text{M}^+ + 1$ ), 392 ( $\text{M}^+ + \text{Na}$ ); Anal. Calcd. for  $\text{C}_{16}\text{H}_{11}\text{N}_5\text{O}_2\text{S}_2$ : C, 52.02; H, 3.00; N, 18.96; S, 17.36; Found: C, 52.08; H, 3.03; N, 19.03; S, 17.33%.

**4.1.8. 2-((1-(2-Methylphenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4h)**

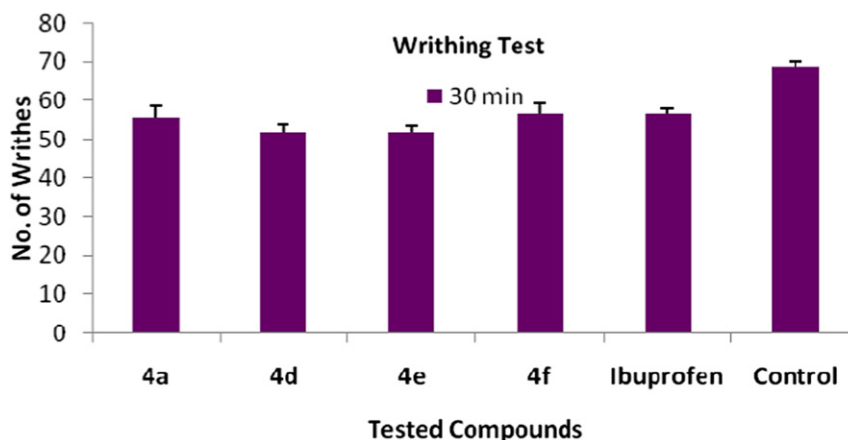
M.P. 103–105 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3244, 3115, 2920, 2517, 1544, 1455, 1426, 1324, 1263, 1048, 760;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  (ppm): 2.13 (s, 3H), 4.78 (s, 2H), 7.26–7.46 (m, 6H), 7.76 (d, 1H,  $J = 8.10$  Hz), 7.81 (s, 1H), 7.88 (d, 1H,  $J = 8.10$  Hz); ESI-MS: 339 ( $\text{M}^+ + 1$ ), 361 ( $\text{M}^+ + \text{Na}$ ); Anal. Calcd. for  $\text{C}_{17}\text{H}_{14}\text{N}_4\text{S}_2$ : C, 60.33; H, 4.17; N, 16.55; S, 18.95%; Found: C, 60.36; H, 4.25; N, 16.52; S, 18.97%.

**4.1.9. 2-((1-(4-Ethoxyphenyl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4i)**

M.P. 110–112 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3107, 3030, 2922, 2382, 1515, 1457, 1426, 1305, 1248, 1051, 996, 825, 752;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 1.43 (t, 3H,  $J = 7.20$  Hz), 4.06 (q, 2H,  $J = 7.20$  Hz), 4.77 (s, 2H), 6.97 (dd, 2H,  $J = 7.20$  & 2.00 Hz), 7.31 (t, 1H,  $J = 8.00$  Hz), 7.43 (t, 1H,  $J = 7.60$  Hz), 7.55 (dd, 2H,  $J = 7.20$  and 2.00 Hz), 7.76 (d, 1H,  $J = 7.60$  Hz), 7.91 (d, 1H,  $J = 8.00$  Hz), 7.97 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  14.68, 27.72, 63.88, 115.20, 121.13, 121.26, 121.47, 122.19, 124.40, 126.09, 130.22, 135.50, 144.42, 153.01, 159.22, 165.83; MALDI-MS: 369 ( $\text{M}^+ + 1$ ), 391 ( $\text{M}^+ + \text{Na}$ ); Anal. Calcd. for  $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}_2\text{S}_2$ : C, 58.67; H, 4.38; N, 15.21; S, 17.40%; Found: C, 58.62; H, 4.45; N, 15.29; S, 17.52%.

**4.1.10. 2-((1-Phenyl-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (4j)**

M.P. 98–100 °C; IR (KBr) ( $\text{cm}^{-1}$ ): 3114, 3067, 1592, 1499, 1419, 1378, 1238, 1046, 996, 754, 684;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 4.77 (s, 2H), 7.31 (t, 1H,  $J = 7.80$  Hz), 7.38–7.51 (m, 4H), 7.67 (d, 2H,  $J = 7.20$  Hz), 7.76 (d, 1H,  $J = 7.80$  Hz), 7.9 (d, 1H,  $J = 8.10$  Hz), 8.06 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  27.68, 120.60, 121.17, 121.50,



**Fig. 3.** Analgesic activity of bis-heterocycles by writhing test.



**Table 5**  
Histopathology report of ulcerogenic activity.

Group	Surface epith. damage	Sub. mucosal damage	Deep mucosal damage	Muscular layer damage
<b>4a</b>	—	—	—	—
<b>4d</b>	—	—	—	—
<b>4e</b>	—	—	—	—
<b>4f</b>	—	—	—	—
<b>Control</b>	—	—	—	—
<b>Ibuprofen</b>	+++	+++	—	—

—, No damage; +++, indicates high degree of damage.

124.46, 126.15, 128.82, 129.73, 135.54, 136.96, 144.80, 153.03, 165.78; ESI-MS: 325 ( $M^+ + 1$ ), 347 ( $M^+ + Na$ ), 363 ( $M^+ + K$ ); Anal. Calcd. for  $C_{16}H_{12}N_5S_2$ : C, 59.23; H, 3.73; N, 17.27; S, 19.77%, Found: C, 59.29; H, 3.66; N, 17.31; S, 19.82%.

#### 4.1.11. 2-((1-(Pyridine-3-yl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (**4k**)

M.P. 136–138 °C; IR (KBr) ( $cm^{-1}$ ): 3131, 3058, 2920, 2437, 1584, 1498, 1419, 1233, 1009, 806, 755, 703;  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  (ppm): 4.71 (s, 2H), 7.19–7.2 (m, 3H), 7.35–7.48 (m, 2H), 7.69 (d, 1H,  $J = 7.60$  Hz), 7.84 (d, 1H,  $J = 7.60$  Hz), 8.03 (d, 1H,  $J = 8.00$  Hz), 8.07 (s, 1H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz):  $\delta$  27.46, 121.21, 121.49, 124.55, 126.22, 128.08, 135.53, 145.51, 150.26, 152.94, 165.55; MALDI-MS: 348 ( $M^+ + Na$ ), 364 ( $M^+ + K$ ); Anal. Calcd. for  $C_{15}H_{11}N_5S_2$ : C, 55.36; H, 3.41; N, 21.52; S, 19.71%, Found: C, 55.29; H, 3.46; N, 21.61; S, 19.77%.

#### 4.1.12. Table entry (**4l**)

M.P. 123–125 °C; IR (KBr) ( $cm^{-1}$ ): 2978, 2926, 2455, 1455, 1425, 1372, 1258, 1079, 1005, 801, 755;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  (ppm): 1.23 (s, 3H), 1.28 (s, 3H), 1.30 (s, 3H), 1.45 (s, 3H), 4.12 (d, 2H,

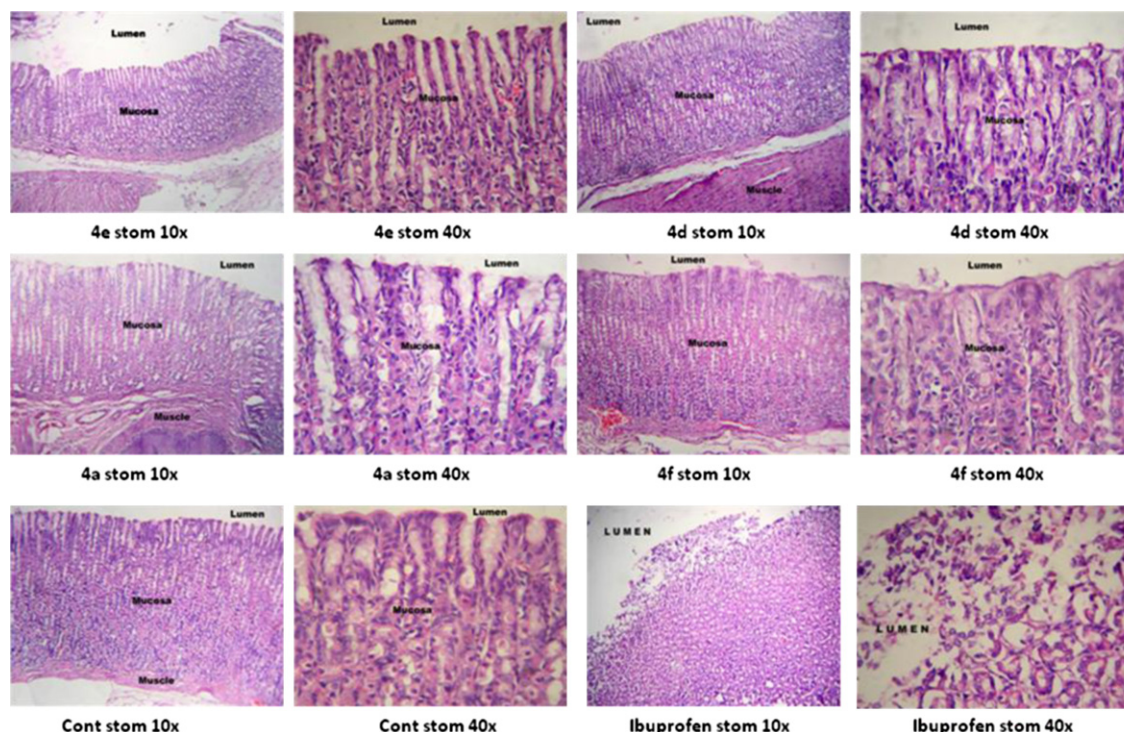
$J = 7.20$  Hz), 4.27–4.29 (m, 1H), 4.37–4.44 (m, 1H), 4.55–4.60 (m, 2H), 4.69 (s, 2H), 5.43 (d, 1H,  $J = 8.0$  Hz), 7.30 (t, 1H,  $J = 7.8$  Hz), 7.42 (t, 1H,  $J = 7.5$  Hz), 7.75 (d, 1H,  $J = 8.1$  Hz), 7.81 (s, 1H), 7.90 (d, 1H,  $J = 8.1$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz):  $\delta$  24.24, 24.76, 25.75, 25.89, 27.81, 50.49, 67.09, 70.23, 70.66, 71.04, 96.13, 108.96, 109.79, 121.01, 121.53, 124.09, 124.25, 125.96, 135.45, 143.47, 153.06, 165.86; MALDI-MS: 491 ( $M^+ + 1$ ), 513 ( $M^+ + Na$ ), 529 ( $M^+ + K$ ); Anal. Calcd. for  $C_{22}H_{26}N_4O_5S_2$ : 53.86; H, 5.34; N, 11.42, S, 13.07%, Found: C, 53.77; H, 5.38; N, 11.39; S, 13.12%.

#### 4.1.13. 2-(1-((2,2-Dimethyl-1,3-dioxalan-4-yl)-1H-1,2,3-triazol-4-yl)methylthio)benzo[d]thiazole (**4m**)

Semisolid; IR ( $CHCl_3$ ) ( $cm^{-1}$ ): 3090, 2935, 2363, 1426, 1233, 1098, 988, 931, 793, 758;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  (ppm): 1.31 (s, 3H), 1.40 (s, 3H), 3.56–3.61 (m, 1H), 3.92–4.03 (m, 1H), 4.25–4.35 (m, 2H), 4.39–4.47 (m, 1H), 4.61 (d, 2H,  $J = 3.0$  Hz), 7.22 (t, 1H,  $J = 7.8$  Hz), 7.35 (t, 1H,  $J = 8.1$  Hz), 7.66–7.74 (m, 2H), 7.81 (d, 1H,  $J = 8.1$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz):  $\delta$  23.80, 24.88, 27.60, 52.17, 65.86, 73.50, 110.70, 121.04, 121.40, 124.32, 126.01, 135.42, 143.87, 152.95, 165.81; MALDI-MS: 463 ( $M^+ + 1$ ), 385 ( $M^+ + Na$ ); Anal. Calcd. for  $C_{16}H_{18}N_4O_2S_2$ : C, 53.02; H, 5.01; N, 15.46; S, 17.69%, Found: C, 53.07; H, 4.95; N, 15.41; S, 17.65%.

#### 4.1.14. 3-(4-((Benzo[d]thiazol-2-ylthio)methyl)-1H-1,2,3-triazol-1-yl)propane-1,2-diol (**6**)

(Gly-OH): semisolid; IR ( $CHCl_3$ ) ( $cm^{-1}$ ): 3303, 3298, 3039, 2913, 1396, 1266, 1109, 972, 922, 793, 758;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  (ppm): 3.33–3.41 (m, 2H), 3.77–3.80 (m, 1H), 4.27–4.33 (m, 1H), 4.36–4.41 (m, 1H), 4.75 (s, 2H), 7.34 (t, 1H,  $J = 6.0$  Hz), 7.46 (t, 1H,  $J = 6.0$  Hz), 7.78 (d, 1H,  $J = 6.0$  Hz), 7.83 (s, 1H), 7.93 (d, 1H,  $J = 6.0$  Hz); MALDI-MS: 323 ( $M^+ + 1$ ), 345 ( $M^+ + Na$ ) Anal. Calcd. for



**Fig. 4.** Histopathology report of ulcerogenic activity. As illustrated in above figure, the high and low power micrograph of the Specimen. **4e, 4d, 4a, 4f, Control** Stom 10 $\times$ . Low power photomicrograph of stomach wall from corresponding **4e, 4d, 4a, 4f, Control**, group animal showing intact mucosal layer of the stomach. (HE  $\times$  100). **4e, 4d, 4a, 4f, Control** Stom 40 $\times$ . High power photomicrograph of stomach wall from **4e, 4d, 4a, 4f, Control** group animal showing intact surface lining epithelium and superficial glands in the stomach mucosa. (HE  $\times$  400). The stomach wall from Ibuprofen group animal at 10 $\times$  showing superficial ulceration of the mucosa. Desquamated epithelial cells are seen in the lumen and at 40 $\times$  showing the desquamated epithelial cells in the lumen. The control group animal showing intact mucosal layer of the stomach and at 40 $\times$  showing intact surface lining epithelium and superficial glands in the stomach mucosa.

C<sub>13</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: C, 48.43; H, 4.38; N, 17.38; S, 19.89%, Found: C, 48.51; H, 4.35; N, 17.41; S, 19.96%.

4.1.15. (2*S*,3*S*,4*S*,5*R*)-6-((4-(Benzo[d]thiazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-tetrahydro-2*H*-pyran-2,3,4,5-tetraol (**5**) semisolid; IR (KBr) (cm<sup>-1</sup>): 2978, 2926, 2455, 1455, 1425, 1372, 1258, 1079, 1005, 801, 755; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  (ppm): 4.12 (d, 2H, *J* = 7.20 Hz), 4.27–4.29 (m, 1H), 4.37–4.44 (m, 1H), 4.55–4.60 (m, 2H), 4.74 (s, 2H), 5.38 (d, 1H, *J* = 8.1 Hz), 7.33 (t, 1H, *J* = 7.8 Hz), 7.42 (t, 1H, *J* = 7.8 Hz), 7.74 (d, 1H, *J* = 8.1 Hz), 7.83 (s, 1H), 7.89 (d, 1H, *J* = 8.1 Hz); MALDI-MS: 411 (M<sup>+</sup>+1), 433 (M<sup>+</sup>+Na), 449 (M<sup>+</sup>+K); Anal. Calcd. for C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>O<sub>5</sub>S<sub>2</sub>: C, 46.82; H, 4.42; N, 13.65; S, 15.62%, Found: C, 46.77; H, 4.37; N, 13.61; S, 15.69%.

## 4.2. Pharmacology

### 4.2.1. Assays for cyclooxygenase 1 and 2

COX-1 has been isolated from Ram seminal vesicles. Recombinant human COX-2 has been expressed in insect cell expression system. These enzymes have been purified by employing conventional chromatographic techniques. Enzymatic activities of COX-1 and COX-2 were measured according to the method of Copeland et al. (1994) [35], with slight modifications using a chromogenic assay based on the oxidation of N,N,N,N-tetra methyl-p-phenylene diamine (TMPD) during the reduction of PGG<sub>2</sub> to PGH<sub>2</sub> (Egan et al., Pagels et al., 1983) [36]. Briefly, the assay mixture contained Tris–HCl buffer (100 mM, Ph 8.0), hematin (15  $\mu$ M), EDTA (3  $\mu$ M), enzyme (100  $\mu$ g COX-1 or COX-2) and the test compound. The mixture was pre-incubated at 25 °C for 1 min and then the reaction was initiated by the addition of arachidonic acid and TMPD, in total volume of 1 ml. The enzyme activity was determined by estimating the velocity of TMPD oxidation for the first 25 s of the reaction by following the increase in absorbance at 603 nm. A low rate of non-enzymatic oxidation observed in the absence of COX-1 and COX-2 was subtracted from the experimental value while calculating the percent inhibition.

### 4.2.2. In vivo anti-inflammatory activity

4.2.2.1. *Animals*. Albino Wistar rats of either sex (150–200 g) were obtained from Central Animal House, Hamdard University, New Delhi. 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 (registration number 173-CPCSEA).

4.2.2.2. *Drugs*. Ibuprofen, Indomethacin, Celecoxib, (Ranbaxy Pvt. Ltd) and Carrageenan (Merck co.) were used in the biological assays.

4.2.2.3. *Anti-inflammatory assay*. The synthesized compounds were assessed for their anti-inflammatory activity using carrageenan-induced hind paw edema method. The rat 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 (Winter et al., 1962) [37]. The standard drug Ibuprofen (10 mg/kg/p.o) was given orally as a positive control. The control group was administered orally with 0.9% of 0.1 ml of saline solution only. The test groups were administered orally with the synthesized compounds at the equimolar dosage of the standard drug, 1 h before the administration of carrageenan. The paw volumes were measured using plethysmometer (Ferreira, 1979) [38] at interval of 3 h and 5 h.

4.2.2.4. *Statistical analysis*. Results are expressed as the mean  $\pm$  SEM, and different groups were compared using one way analysis of variance (ANOVA) followed by Tukey Kramer test for multiple comparisons.

### 4.2.3. In silico inhibitor and enzyme interactions

4.2.3.1. *Molecular modeling*. Primary sequence of human Cyclooxygenase (COX-2) was obtained from NCBI gene bank with accession number (gi: 181254). PSI-BLAST [39] was used for template data mining. Mouse Diclofenac bound COX-2 PDB: 1PXX [40], which shows 87% identity, was chosen as template. The COX-2 three-dimensional model was constructed by using crystal atomic coordinates of free 1Pxx at a resolution of 2.9 Å. Fifty models were constructed by using Modeller 9v.6 [41] where protein tertiary structure models were chosen for their fulfillment of spatial restraints, taking into account loops energy minimization conducted by default parameters [42]. Predicted human COX-2 model evaluation, i.e geometry, stereochemistry, and energy distributions in the models was performed using PROSA to analyze packing and solvent exposure characteristics and PROCHECK for additional analysis of stereochemical quality [43,44]. In addition, RMSD was calculated by overlap of C $\alpha$  traces and backbones onto the template crystal structure through SPDB viewer [45]. The protein structures were visualized and analyzed on Delano Scientific's PYMOL <http://pymol.sourceforge.net/>.

4.2.3.2. *Molecular docking*. AutoDock 4.0 program [46] was used for docking calculations combined with Lamarckian genetic algorithm (LGA) to search for the preferable conformation. The dimensions of the box were set to 60  $\times$  60  $\times$  60 with grid spacing of 0.375 Å. During the docking experiments, the receptor was kept rigid and ligand was set flexible. The maximum number of energy evaluations was set to 2.5  $\times$  10<sup>7</sup>, and the rest of docking parameters were used the default values. 50 independent docking runs were performed to find the preferable conformation. The docking results were clustered according to the root mean square deviation (RMSD) of 2.0 Å. The structures with the relative lower binding free energy and the most cluster members were chosen, best docking conformation.

4.2.3.3. *In silico modeling of human COX-2*. Considering the limited sequence identity between human COX-2 and the templates used for its modeling, an objective validation gives results suggestive of reliable models. Human COX-2 model construction starts with PSI-BLAST analysis, which was used to select best template. Human COX-2 sequence was directly compared to amino acid residue sequences that possess structures experimentally resolved and deposited in the Protein Data Bank [33]. Human COX-2 presented 87% of identity with 1PXX, being chosen as a template. Alignments among sequences and structures were carried by using Clustal-W [34]. After alignment analysis, atomic coordinated were transferred to human COX-2 primary structure for the construction of 3D model using Modeller 9v.6 (Fig. 2).

Procheck summary of human COX-2 showed that 100% amino acid residues were in most favorable regions, additionally allowed regions and generously allowed regions with 0.0% residues in the disallowed regions. Structural differences between crystal structure 1PXX and predicted three dimensional structure of human COX-2 were calculated by superimposing both structures. The RMSD values between the crystal structure 1PXX and homology model human COX-2 calculated for C $\alpha$  traces and main chain atom were 1.25 Å. The RMSD values and small variability among experimental structures template and the structure modeled reflect the presence of strong restraints in most regions and emphasize a similar folding patterns. Furthermore, the lower score acquired for PROSA –7.68 indicating the high quality of the model.

#### 4.2.4. Antinociceptive activity

**4.2.4.1. Writhing test.** The writhing test in mice was carried out using the method of (Koster et al., 1959) [47]. The writhes were induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). The standard drug i.e. Ibuprofen was given orally at a dose of 10 mg/kg body weight. The test compounds were administered orally at an equimolar dosage to groups of six animals each, 30 min before chemical stimulus. The numbers of muscular contractions were counted over a period of 20 min after acetic acid injection. The data represents the total number of writhes observed during 20 min and is expressed as writhing numbers.

#### 4.2.5. Ulcerogenic activity

The test compounds having anti-inflammatory & analgesic activities comparable with the standard drugs were further tested for their ulcerogenic activity. The ulcerogenic activity was done at three times higher dose in comparison to the dose used for anti-inflammatory activity, i.e. Dose of 30 mg/kg body weight of standard drug Ibuprofen and equimolar dosage of test compounds were used. Each group had three animals which were later sacrificed. When compared with Ibuprofen, these compounds did not cause any gastric ulceration and disruption of gastric epithelial cells at the above mentioned oral dose. Hence gastric tolerance towards the test compounds was better than that of Ibuprofen indicating that carboxylic group present in Ibuprofen is responsible for ulceration.

#### Acknowledgements

The authors thank Dr. G. N. Qazi, Vice-Chancellor Jamia Hamdard for providing necessary facilities. The authors are also thankful to Hamdard National Foundation (HNF) for providing Hamdard centenary research fellowship to SS and Hamdard National fellowships to MA. CM thanks CSIR for awarding fellowship.

#### References

- [1] L.L. Bozec, C.J. Moody, Aust. J. Chem. 62 (2009) 639.
- [2] C.L. Lee, Y. Lam, S. Lee, Tetrahedron Lett. 42 (2001) 109.
- [3] M. Bryson, B. Fulton, P. Benfield, Drugs 52 (1996) 549.
- [4] M. Lacova, J. Chovancova, O. Hyblova, S. Varkonda, Chem. Pap. 45 (1991) 411.
- [5] I. Chulak, V. Sutorius, V. Sekerka, Chem. Pap. 44 (1990) 131.
- [6] T. Papenfuhs, Ger. Offen. De. 3 (1987) 528.
- [7] (a) T.D. Bradshaw, M.C. Bibby, J.A. Double, I. Fichtner, P.A. Cooper, M.C. Alley, S. Donohue, S.F. Stinson, J.E. Tomaszewski, E.A. Sausville, M.F.G. Stevens, Mol. Cancer Ther. 1 (2002) 239–246; (b) T.D. Bradshaw, M.S. Chua, H.L. Browne, V. Trapani, E.A. Sausville, M.F.G. Stevens, Brit. J. Cancer 86 (2002) 1348–1354.
- [8] (a) I. Hutchinson, S.A. Jennings, B.R. Vishnu vajjala, A.D. Westwell, M.F.G. Stevens, J. Med. Chem. 45 (2002) 744–747; (b) I. Hutchinson, M.S. Chua, H.L. Browne, V. Trapani, T.D. Bradshaw, A.D. Westwell, M.F.G. Stevens, J. Med. Chem. 44 (2001) 1446–1455.
- [9] (a) E. Kashiyama, I. Hutchinson, M.S. Chua, F. Sherman, Stinson, R. Lawrence, J. Med. Chem. 42 (1999) 4172–4184; (b) V. Benetau, T. Besson, J. Guillard, S. Leonce, B. Pfeiffer, Eur. J. Med. Chem. 34 (1999) 1053–1060.
- [10] M.A. El-Sherbeny, Arzneim-Forsch 50 (2000) 843–847.
- [11] L. Racane, V. Tralic-Kulenovic, L. Fiser-Jakic, D.W. Boykin, G. Karminski-Zamola, Heterocycles 55 (2001) 2085–2098.
- [12] Mahmood-ul-Hasan, Z.H. Chohan, C.T. Supuran, Main Group Met. Chem. 25 (2002) 291–296.
- [13] Akhilesh Gupta, Swati Rawat, J. Curr. Pharm. Res. 3 (2010) 13–23.
- [14] (a) Sanjay K. Yadav, S.M. Malipatil, S.K. Yadav, Int. J. Drug Discov. Herbal Res. 1 (2011) 42–43; (b) Priyanka, N.K. Sharma, K.K. Jha, Int. J. Curr. Pharm. Res. 2 (2010) 01–06; (c) P.S. Yadav, Devprakash, G.P. Senthilkumar, Int. J. Pharm. Sci. Drug Res. 3 (2011) 01–07; (d) P. Venkatesh, S.N. Pandeya, Int. J. Chem. Tech. Res. 1 (2009) 1354–1358; (e) Akhilesh Gupta, Swati Rawat, J. Curr. Pharm. Res. 3 (2010) 13–23; (f) P. Gajdos, P. Magdolen, P. Zahradnik, P. Foltinová, Molecules 14 (2009) 5382–5388; (g) V.A. Jagtap, E. Jayachandran, G.M. Sreenivasa, B.S. Sathe, J. Pharm. Res. 4 (2011) 1359–1360; (h) M.R. Shiradkar, K.K. Murahari, H.R. Gangadasu, T. Suresh, C.A. Kalyan, D. Panchal, R. Kaur, B. Prashant, G. Jyoti, M. Vinod, M. Raut, Bioorg. Med. Chem. Lett. 15 (2007) 3997–4008.
- [15] S.P. Singh, R.K. Vaid, Indian J. Chem. 25B (1986) 288.
- [16] J. Wada, T. Suzuki, M. Iwasaki, H. Miyamatsu, S. Ueno, M. Shimizu, J. Med. Chem. 16 (1973) 930–934.
- [17] E. Aki-Sener, K.K. Bingol, T. Paramashivappa, P. Phani Kumar, P.V. Subba Rao, A. Srinivasa Rao, Bioorg. Med. Chem. Lett. 13 (2003) 657–660.
- [18] (a) Y.S. Sanghvi, B.K. Bhattacharya, G.D. Kini, S.S. Matsumoto, S.B. Larson, W.B. Jolley, R.K. Robins, G.R. Revankar, J. Med. Chem. 33 (1990) 336–344; (b) H. Wamhoff, in: A.R. Katritzky, C.W. Rees (Eds.), Comprehensive Heterocyclic Chemistry, vol. 5, Pergamon, Oxford, 1984, p. 669; (c) M. Journet, D. Cai, J.J. Kowal, R.D. Larsen, Tetrahedron Lett. 42 (2001) 9117–9118.
- [19] (a) D.R. Buckle, C.J.M. Rockell, J. Chem. Soc. Perkin Trans. (1982) 627–630; (b) D.R. Buckle, D.J. Outred, C.J.M. Rockell, H. Smith, B.A. Spicer, J. Med. Chem. 26 (1983) 251–254; (c) D.R. Buckle, C.J.M. Rockell, H. Smith, B.A. Spicer, J. Med. Chem. 29 (1986) 2262–2267; (d) R. Alvarez, S. Velázquez, A. San Felix, S. Aquaro, E.D. Clercq, C.F. Perno, A. Karlsson, J. Balzarini, M.J. Camarasa, J. Med. Chem. 37 (1994) 4185–4194; (e) M.J. Genin, D.A. Allwine, D.J. Anderson, M.R. Barbachyn, D.E. Emmert, S.A. Garmon, D.R. Graber, K.C. Grega, J.B. Hester, D.K. Hutchinson, J. Morris, R.J. Reischer, C.W. Ford, G.E. Zurenko, J.C. Hamel, R.D. Schaadt, D. Stapert, B.H. Yagi, J. Med. Chem. 43 (2000) 953–970.
- [20] M.D. Chen, S.J. Lu, G.P. Yuag, S.Y. Yang, X.L. Du, Heterocyclic Comm. 6 (2000) 421–426.
- [21] E.A. Sherement, R.I. Tomanov, E.V. Trukhin, V.M. Berestovitskaya, Russ. J. Org. Chem. 40 (2004) 594–595.
- [22] A. Allais, J. Meier, (Roussel–UCLAF) Ger. Offen. (1969) 1815467.
- [23] K.M. Banu, A. Dinakar, C. Ananthanarayanan, Indian J. Pharm. Sci. 4 (1999) 202–205.
- [24] R. Meier, EP. 199262; eidem, US. 4789680, 1986.
- [25] A. Passannanti, P. Diana, P. Baraja, F. Mingoia, A. Lauria, G. Cirrincione, Heterocycles 48 (1998) 1229–1235.
- [26] M. Jilino, F.G. Stevens, J. Chem. Soc. Perkin Trans. 1 (1998) 1677–1684.
- [27] G.D. Diana, J.J. Nitz, EP. 566199, 1993.
- [28] (a) Y. Bourne, H.C. Kolb, Z. Radi, K.B. Sharpless, P. Taylor, P. Marchot, Proc. Natl. Acad. Sci. USA. 101 (2004) 1449–1454; (b) W.G. Lewis, L.G. Green, F. Grynszpan, Z. Radi, P.R. Carlier, P. Taylor, M.G. Finn, K.B. Sharpless, Angew. Chem. Int. Ed. 41 (2002) 1053–1057.
- [29] I.A. Alsarra, M.O. Ahmed, F.K. Alanazi, K.E.H. ElTahir, M.A. Alsheikh, H.S. Neau, Int. J. Med. Sci. 7 (2010) 232–239.
- [30] J.G. Ruiz, D.T. Lowenthal, Geriatr. Nephrol. Urol. 7 (1997) 51–57.
- [31] H.H. Tan, W.M.C. Ong, S.H. Lai, W.C. Chow, Singapore Med. J. 48 (2007) 582–585.
- [32] (a) L.G. Howes, Ther. Clin. Risk Manag. 5 (2007) 831–845; (b) J.A. Mitchell, T.D. Warner, Nat. Rev. Drug Discov. 5 (2006) 75–85; (c) J.J. Zhang, E.L. Ding, Y.Q. Song, JAMA 296 (2006) 1619–1632; (d) V. Dhikav, S. Singh, K.S. Anand, J. Indian Acad. Clin. Med. 3 (2002) 332–338.
- [33] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, Protein Data Bank, Nucleic Acids Res. 28 (2000) 235–242.
- [34] J.D. Thompson, D.G. Higgins, T.J. Gibson, W. Clustal, Nucleic Acids Res. 22 (1994) 4673–4680.
- [35] R.A. Copeland, J.M. Williams, J. Giannaras, S. Nurnberg, M. Covington, D. Pinto, S. Pick, J.M. Trzaskos, Proc. Natl. Acad. Sci. USA. 91 (23) (1994) 11202–11206.
- [36] (a) W.R. Pagels, R.J. Sachs, L.J. Marnett, D.L. Dewitt, J.S. Day, W.L. Smith, J. Biol. Chem. 258 (1983) 6517–6523; (b) R.W. Egan, J. Paxton Jr., F.A. Kuehl, J. Biol. Chem. 251 (1976) 7329–7335.
- [37] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. for Exp. Biol. Med. 111 (1962) 544–547.
- [38] S.H. Ferreira, J. Pharm. Pharmacol. 31 (1979) 648.
- [39] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, J. Mol. Biol. 215 (1990) 403–410.
- [40] S.W. Rowlinson, J.R. Keifer, J.J. Prusakiewicz, J.L. Pawlitz, K.R. Kozak, A.S. Kalgutkar, W.C. Stallings, R.G. Kurumballi, L.J. Marnett, J. Biol. Chem. 278 (2003) 45763–45769.
- [41] N. Eswar, B. Webb, M.A. Marti-Renom, M.S. Madhusudhan, D. Eramian, M.Y. Shen, U. Pieper, A. Sali, Curr. Protoc. Protein Sci. (2007) (Chapter 2), Unit 29.
- [42] K. Arnold, L. Bordoli, J. Kopp, T. Schwede, Bioinform. (Oxford, England) 22 (2006) 195–201.
- [43] M. Wiederstein, M.J. Sippl, Nucleic Acids Res. 35 (2007) W407–W410.
- [44] R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, J. Appl. Crystallogr. 26 (1993) 283–291.
- [45] K. Sumathi, P. Ananthalakshmi, M.N. Roshan, K. Sekar, Nucleic Acids Res. 34 (2006) 128–132.
- [46] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, J. Comput. Chem. 19 (1998) 1639–1662.
- [47] R. Koster, M. Anderson, E.J. Beer, Fed. Proceeds 18 (1959) 412–416.