European Journal of Medicinal Chemistry 70 (2013) 579–588

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel 1,2,3-triazole based benzoxazolinones: Their TNF-α based molecular docking with *in-vivo* anti-inflammatory, antinociceptive activities and ulcerogenic risk evaluation

Saqlain Haider [a], M. Sarwar Alam [a,*], Hinna Hamid [a], Syed Shafi [a], Amit Nargotra [c], Priya Mahajan [c], Syed Nazreen [a], Arunasree M. Kalle [d], Chetna Kharbanda [a], Yakub Ali [a], Aftab Alam [b], Amulya K. Panda [b]

[a] Department of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi 110 062, India
[b] Product Development Cell, National Institute of Immunology, New Delhi 110067, India
[c] Discovery Informatics, Indian Institute of Integrative Medicine, Jammu 180001, India
[d] Department of Animal Sciences, University of Hyderabad, Hyderabad 500046, India

ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form
8 October 2013
Accepted 12 October 2013
Available online 22 October 2013

Keywords:
1,2,3-Triazole
Click chemistry
Molecular docking
Benzoxazolinone
Carrageenan cyclooxygenase

ABSTRACT

A library of novel *bis*-heterocycles containing benzoxazolinone based 1,2,3-triazoles has been synthesized using click chemistry approach. The compound **3f** exhibited potent selective COX-2 inhibition of 59.48% in comparison to standard drug celecoxib (66.36% inhibition). The compound **3i** showed significant ($p < 0.001$, 50.95%), TNF-α inhibitory activity as compared to indomethacin ($p < 0.001$, 64.01%). The results of the carrageenan induced hind paw oedema showed that compounds **3a**, **3f**, **3i**, **3o**, and **3e** exhibited potent anti-inflammatory activity in comparison to Indomethacin. The molecular docking studies revealed that **3i** exhibits strong inhibitory effect due to the extra stability of the complex because of an extra π–π bond. The histopathology report showed that none of the compounds caused gastric ulceration.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are still used for the treatment of many rheumatic diseases but they cause many adverse side effects, the most important being the gastric injuries that might later cause gastric ulceration leading to death [1]. Drugs with selective COX-1 vs. COX-2 inhibition mechanisms [2] have of late been used as anti-inflammatory agents.

Benzoxazolinone is a cyclic isostere of the coumarin nucleus. Benzoxazolinone and their derivatives are an important class of biologically active molecules. Their importance is due to their diverse application in the field of pharmaceuticals and in chemical systems. 2-benzoxazolinones have been shown to exhibit analgesic, anti-inflammatory, antineoplastic, anticonvulsant and antimicrobial activities [3–12]. Zoxazolamine is a Benzoxazole analogue and is mainly used as skeletal muscle relaxant [13]. Some benzoxazolinone derivatives have been shown to inhibit iNOS and constitute an important class of non-aminoacid based NOS inhibitors [14]. On the other hand 1,2,3-triazoles and their derivatives have emerged as powerful pharmacophores [15] and have remained at the centre of interest due to their chemotherapeutic value [16]. They are found in many drugs and have been an interesting component in terms of their biological activity [17]. Many 1,2,3-triazoles have been found to possess potent antimicrobial [18,19], analgesic [20], anti-inflammatory, local anaesthetic [21], anticonvulsant [22], antineoplastic [23], antimalarial [24] and antiviral activity [25]. Considering the biological importance of benzoxazolinone and 1,2,3-triazoles as anti-inflammatory and antinociceptive agents, we aim to conjugate these two important ligands under one construct through a methylene linkage. We herein reporting, the design and synthesis of benzoxazolinone and 1,2,3-triazole based unsymmetrical *bis*-heterocycles and their *in vivo* anti-inflammatory and analgesic activities for the first time along with the TNF-α based molecular docking.

* Corresponding author. Tel.: +91 11 26059688x5555; fax: +91 11 26059663.
E-mail addresses: msalam5555@gmail.com, msalam@jamiahamdard.ac.in (M.S. Alam).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.10.032

2. Results and discussion

2.1. Chemistry

Different benzoxazolinone derivatives **1** were synthesized by refluxing substituted *ortho*-aminophenols with 1,1-carbonyl dimidazole in dry THF under nitrogen atmosphere [26,27]. As shown in the Scheme 1, different substituted benzoxazolinone derivatives were refluxed with propargyl bromide in the presence of potassium carbonate in dry acetone for 7–10 h to yield propargylated benzoxazolinone derivatives **2**. The 1,3-diploar cycloaddition between propargylated benzoxazolinone derivatives and substituted aromatic azides under click chemistry conditions produced novel unsymmetrical *bis*-heterocycles **3a**–**3v** in quantitative yields. Different aromatic azides with various substitutions including electron withdrawing and electron donating groups have been used. The propargylation of the different benzoxazolinone derivatives was confirmed by the presence of a signal at δ 4.69–4.73 (s, 2H, CH_2) along with another signal at δ 3.43–3.47 (s, 1H, CH) corresponding to terminal alkyne, in the ^{1}H NMR spectra. The formation of 1,2,3-triazoles was confirmed by the resonance of the proton in the triazollyl ring at a δ 9.0–9.2 as a singlet. The structure was further supported by the ^{13}C NMR spectra, which showed the C-atom signals corresponding to triazole derivatives. MALDI–MS/ESI–MS of all compounds showed [M^+] or [M + K] or [M + Na] or [M + 1].

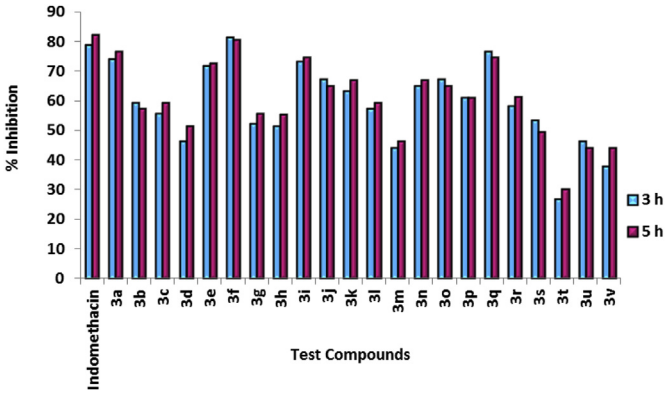
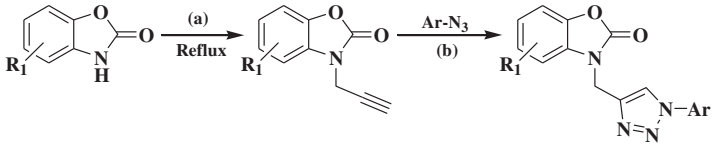


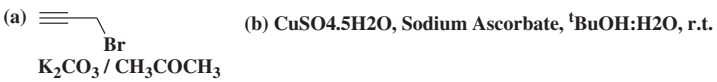
Fig. 1. *In-vivo* anti-inflammatory activity of novel bis-heterocycles.

2.2. In vivo anti-inflammatory activity

All the synthesized compounds have been tested for their *in vivo* anti-inflammatory activity by carrageenan-induced hind paw oedema model. The results obtained indicate that the compound **3f** exhibited potent anti-inflammatory activity with 81.39% and 80.62% inhibition after 3 h and 5 h as compared to indomethacin which showed 79.06% and 82.25% inhibition after 3 h and 5 h respectively (Fig 1). The compound **3a** exhibited 74.00% inhibition



Compound	R1	Ar
3a	6-CH_3	4-C_6H_4Cl
3b	6-CH_3	4-$C_6H_4NO_2$
3c	6-CH_3	3-$C_6H_4NO_2$
3d	6-CH_3	4-$C_6H_4OC_2H_5$
3e	6-CH_3	2-C_6H_4Cl
3f	6-CH_3	4-C_6H_4F
3g	5-CH_3	4-$C_6H_4NO_2$
3h	5-CH_3	3-$C_6H_4NO_2$
3i	5-CH_3	4-C_6H_4Br
3j	5-CH_3	2-C_6H_4Cl
3k	5-CH_3	4-C_6H_4F
3l	5-CH_3	2-C_6H_4F
3m	5-CH_3	-C_5H_4N
3n	5-CH_3	4-C_6H_4Cl
3o	5-Cl	4-C_6H_4Cl
3p	5-Cl	3-$C_6H_4NO_2$
3q	5-Cl	2-C_6H_4F
3r	5-Cl	-C_5H_4N
3s	H	3-$C_6H_4NO_2$
3t	H	4-$C_6H_4OC_2H_5$
3u	H	4-$C_6H_4OCH_3$
3v	H	-C_5H_4N



Scheme 1. Synthesis of novel benzoxazolinone based 1,2,3-triazoles.

at 3 h post-carrageenan and 76.74% inhibition 5 h post-carrageenan administration as compared to indomethacin. Whereas the compounds **3i** and **3e** showed a time-dependent increase in the inhibition of inflammation (73.25% and 71.76% inhibition at 3 h post-carrageenan and 74.80% and 72.86% inhibition at 5 h post-carrageenan respectively). The compound **3o** showed comparable anti-inflammatory activity with 67.44% and 65.11% inhibition after 3 h and 5 h respectively.

The structure activity relationship of the synthesized compounds is analysed on the basis of the nature of the substituents on the benzoxazolinone ring along with the position and nature of substitutions on aryl group attached to the 1,2,3-triazollyl ring. The compounds having substituted benzoxazolinone ring system exhibited more potent anti-inflammatory activity as compared to those having unsubstituted benzoxazolinone ring. The compounds **3t-3v** conferred reduced activity whereas **3s** did not show any significant anti-inflammatory activity. It has been observed that compounds **3a**, **3f**, **3i**, **3k**, **3o** and **3n** having weak electron withdrawing halogen atoms on the aromatic ring attached to the triazollyl ring conferred greater activity in comparison to compounds **3b**, **3c**, **3g**, **3h**, **3p** and **3s** having *para* or *meta* substituted NO_2 group. This is further substantiated by the docking studies where the glide scores of halogen containing compounds are better than that of nitro containing compounds **(**Table 2).Compounds **3d**, **3t** and **3u** containing the electron donating groups (OCH_3, OC_2H_5**)** as well as compounds **3v**, **3m** and **3r** containing pyridyl substitutions showed significant loss in the anti-inflammatory activity.

2.3. COX assay

The compounds exhibiting significant *in vivo* anti-inflammatory activity were evaluated for their anti-inflammatory activity by biochemical selective COX-2 inhibitory assay. Compounds **3a**, **3f**, **3i**, **3o** and **3e** showed significant COX-2 inhibition as compared to the standard drug celecoxib (Fig 2). The COX-2 selectivity of these compounds and their gastric liability may be related to their selective percentage COX-2 inhibition **(**Table 1**).** The compounds **3a** (COX-1 $IC_{50} = 389.2$ μM; COX-2 $IC_{50} = 5.6$ μM; SI = 69.5), **3f** (COX-1 $IC_{50} = 174.72$ μM; COX-2 $IC_{50} = 2.4$ μM; SI = 72.8) and **3i** (COX-1 $IC_{50} = 188.20$ μM; COX-2 $IC_{50} = 2.78$ μM; SI = 67.7) exhibited potent selective COX-2 inhibition as compared to celecoxib (COX-1 $IC_{50} = 25.74$ μM; COX-2 $IC_{50} = 0.32$ μM; SI = 80.43). The COX-1/COX-2 Selective Index (SI value) of the compounds **3a**, **3f** and **3i** shows the selective nature of these compounds towards COX-2 inhibition as compared to celecoxib.

2.4. TNF-α assay

The compounds showing significant *in-vivo* anti-inflammatory activity were further screened for their *in-vitro* TNF-α activity (Fig 3). The compound **3i** showed significant ($p < 0.001$), TNF-α inhibitory activity with 50.95% inhibition as compared to the standard drug indomethacin which exhibited 64.01%, ($p < 0.001$) inhibition.

Table 1
Inhibitory activity of the 1,2,3-triazole based benzoxazolinones.

Compounds	IC_{50}(μM)		Selectivity index (SI) COX-1/COX-2
	(COX-1)	(COX-2)	
3a	389.2	5.6	69.5
3f	174.72	2.4	72.8
3i	188.20	2.78	67.7
3e	207.36	3.6	57.6
3o	111.34	2.7	41.23
3q	138.25	3.1	44.59
Celecoxib	25.74	0.32	80.43

Values are the means ± SEM from three independent experiments using COX assay kits (Cayman Chemicals Inc., Ann Arbor, MI, USA).

Table 2
XP Docking and MMGBSA results of molecules for TNF-α.

Test Compound	XP glide score	MMGBSA energy
Co-crystallized Ligand	−7.67	−65.348
Rolipram	−5.66	−69.445
Indomethacin	−5.39	−55.975
3e	−5.87	−63.048
3o	−5.38	−65.004
3f	−5.33	−55.522
3i	−5.15	−63.353
3a	−4.76	−53.364

2.5. Docking studies on TNF-α

Docking studies of five compounds were carried out on the selected protein target 2AZ5 (TNF-α dimer). There was no apparent difference in the dock scores of these five molecules, which certainly is not the only criterion for predicting the affinity of a ligand with the target protein (Fig 4). But, from the interaction figures of these compounds with the protein, it was observed that the compound **3i** is oriented in the binding site in such a fashion that it favours the possibility of two π–π interactions on the two benzene rings with Tyr59 (chain A of the dimer) and Tyr119 (chain B of the dimer) respectively. There is no H-bonding observed in the interaction and the dock score is also in the range of −5, thereby indicating that these are not very strong inhibitors of the protein. The comparative strong inhibitory effect of the compound **3i** among others could be ascertained due to the extra stability of the complex because of an extra π–π bond. From the interaction figures, it seems that Tyr59, Tyr119 and Tyr151 plays an important role in the binding affinity of the ligand. The compound **3e** exhibited (Table 2) a glide score of −5.87 in comparison to the standard drug indomethacin (−5.39) and the co-crystallized ligand which showed a glide score of −7.67. Hence, these important parameters should be considered while designing inhibitors of this protein.

2.6. Analgesic activity

The compounds showing significant anti-inflammatory activity in comparison to the standard drug indomethacin were further tested for their antinociceptive activity by the writhing test and tail immersion method. The results of the writhing test (Fig 5) indicate that compound **3a** exhibited potent

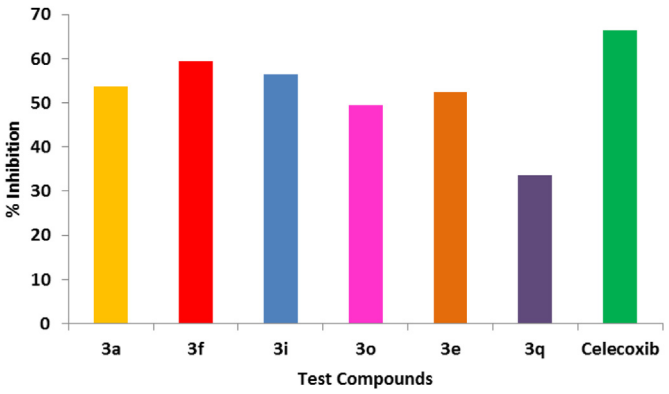


Fig. 2. *In-vitro* COX-2 activity of novel bis-heterocycles.

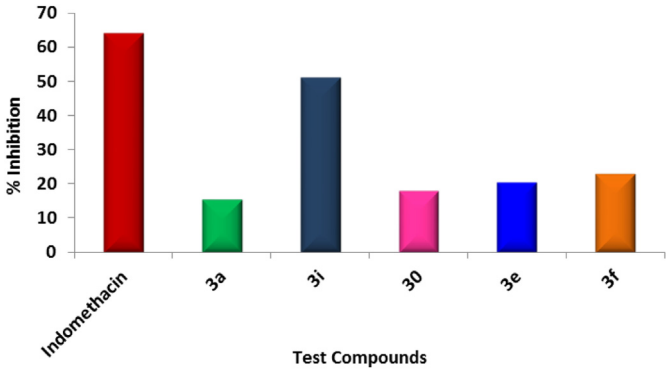


Fig. 3. *In-vitro* TNF-α inhibitory activity of novel bis-heterocycles.

antinociceptive activity with 41.83% inhibition as compared to the standard drug indomethacin which caused 44.69% inhibition. The results of the tail immersion (Table 3) method demonstrate that the compounds **3a** and **3f** ($p < 0.01$) showed significant antinociceptive activity in comparison to the standard drug indomethacin. The antinociceptive activity of the active compounds has been evaluated using both chemical and thermal methods of nociception. These methods are used to detect the central and peripheral mechanisms of analgesia. Acetic acid induced writhing test is used for detecting both central and peripheral analgesia, whereas tail flick test is sensitive to centrally acting analgesia. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE2 and PGF2α and their levels are increased in the peritoneal fluid of the acetic acid induced mice [28]. Thermal induced nociception indicates narcotic involvement [29]. Thermal nociceptive tests are more sensitive to opioid μ receptors and non-thermal tests are to opioid κ receptors [30,31].

2.7. Ulcerogenic studies

The compounds showing potential anti-inflammatory and antinociceptive activity were further tested for their gastric ulceration activity (Table 4 & Fig 6). When compared with Indomethacin,

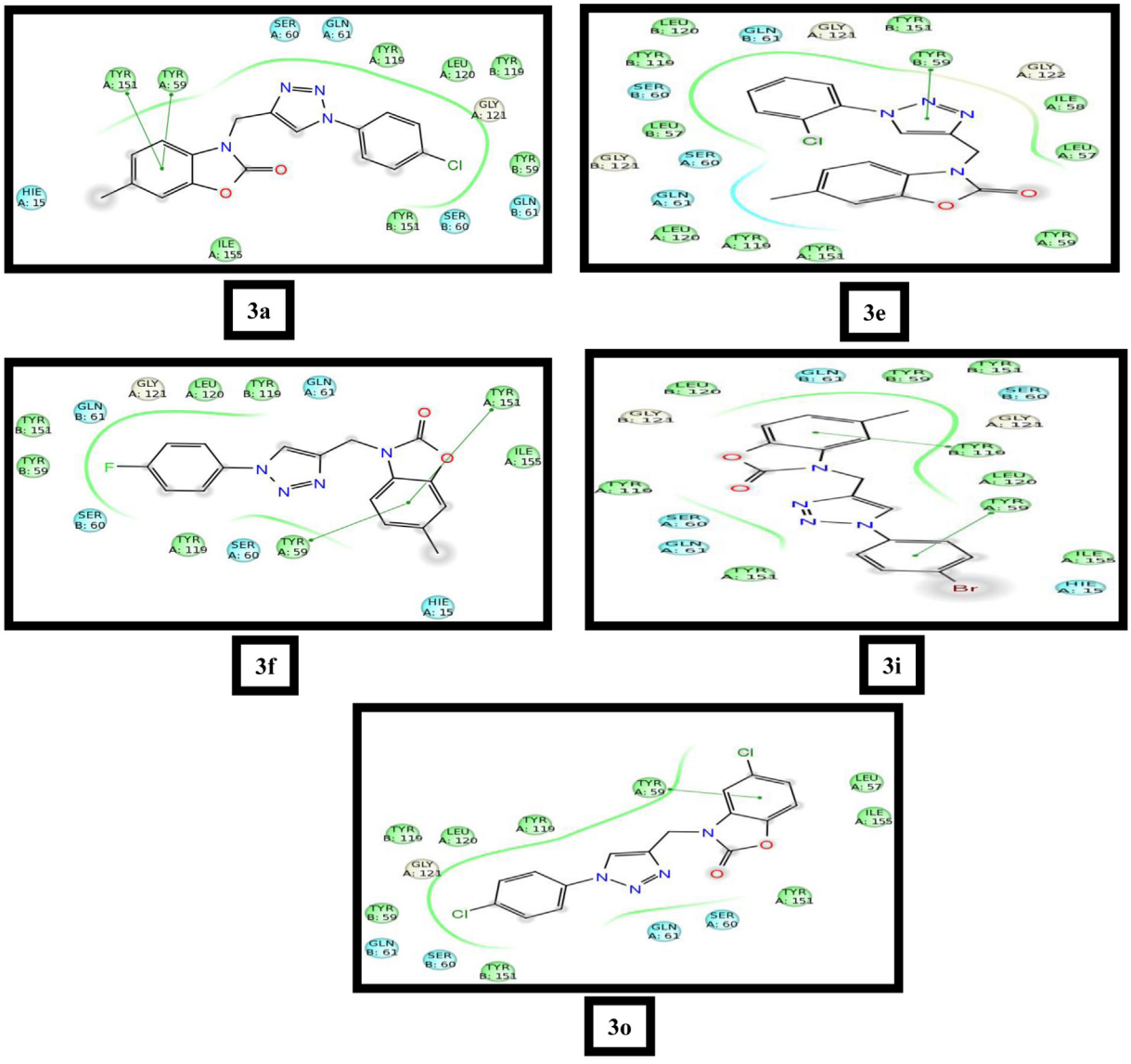


Fig. 4. Docking results of the synthesized compounds onto TNF-α.

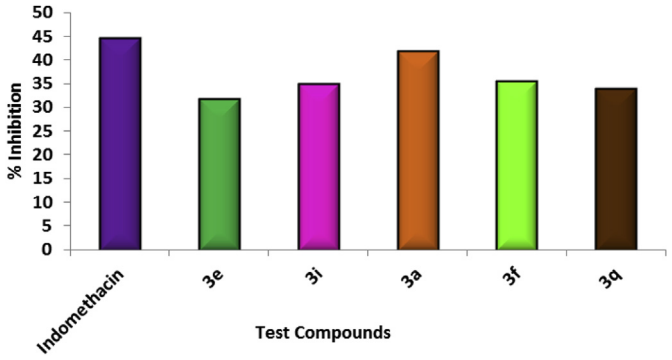


Fig. 5. *In-vivo* antinociceptive activity by Writhing test method.

compounds **3a**, **3e**, **3f**, **3i**, and **3o** did not induce any gastric ulceration and rupture of the gastric mucosal layer.

3. Conclusion

We have synthesized a focussed library of novel *bis*-heterocycles encompassing benzoxazolinone-1,2,3-triazole moieties conjugated through a methylene linkage and evaluated them for their anti-inflammatory, antinociceptive activity and ulcerogenic risk evaluation. The compounds **3e**, **3i**, **3a**, **3f** and **3q** exhibited potent anti-inflammatory and antinociceptive activity without exhibiting any gastric ulceration. The selective COX-2 inhibitory potential of compound **3f** (COX-1/COX-2 SI = 72.8) along with other molecules concludes that these molecules can be considered as potent anti-inflammatory agents as predicted by their COX-1/COX-2 selective index. The results of *in-vivo* TNF-α activity indicated that the compound **3i** has comparable TNF-α inhibition similar to the standard drug Indomethacin. From the molecular docking studies it was found that the compound **3i** is oriented in the binding site in such a manner that it favours the possibility of two π–π interactions on the two benzene rings with Tyr59 (chain A of the dimer) and Tyr119 (chain B of the dimer) respectively.

4. Experimental

4.1. Chemistry

All the chemicals and reagents used in this study were purchased from Merck (India), Spectrochem, and Sigma Aldrich. 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), 1H NMR spectra was 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 70eV (EI Ms-QP 1000EX, Shimadzu, Japan) and 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 from spectral data.

Compound **2** was dissolved in 20 mL of ^{t}Butanol:water (1:1) solvent at ambient temperature. $CuSO_4.5H_2O$ was charged into it and the reaction mixture was stirred for 5 min. Reaction mixture became light blue in colour. Then sodium ascorbate was added to the reaction mixture and stirred for 15 min. The colour of the reaction mixture changed to dark yellow. After 15 min azide was added at once. The reaction mixture was allowed to stir for further 7–10 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. 3-[1-(4-Chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzooxazol-2-one(*3a*)

Yellowish brown crystals; yield 85%; m. p. 180–181 °C; IR (KBr): ν (cm^{-1}) 3178, 3045, 1503, 1441, 1238, 1175, 1047, 825; ^{1}H NMR (300 MHz, DMSO): δ 2.32 (s, 3H), 5.17 (s, 2H), 7.01 (d, 1H, J = 7.5 Hz), 7.19 (d, 2H, J = 8.1 Hz), 7.66 (d, 2H, J = 8.4 Hz), 7.92 (d, 2H, J = 8.7 Hz), 8.92 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.35, 37.48, 109.73, 110.66, 112.46, 122.21, 122.58, 124.67, 128.66, 130.32, 132.62, 133.50, 135.72, 142.53, 143.13, 156.15; ESI–MS: 341$(M + 1)^+$; Anal. Calcd. for $C_{17}H_{13}ClN_4O_2$: C, 59.92; H, 3.85; N, 16.44. Found C, 59.89; H, 3.83; N, 16.42.

4.1.2. 6-Methyl-3-[1-(4-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(*3b*)

Yellow crystals; yield 88%; m. p. 194–195 °C; IR (KBr): ν (cm^{-1}) 3176, 3068, 1759, 1597, 1441, 1242, 1172, 854, 748; ^{1}H NMR (300 MHz, DMSO): δ 2.32 (s, 3H), 5.21 (s, 2H), 7.02 (d, 1H, J = 5.4 Hz), 7.19–7.21 (m, 2H), 8.20 (d, 2H, J = 6.8 Hz), 8.44 (d, 2H, J = 6.8 Hz), 9.12 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 22.34, 36.98, 110.73, 112.64, 113.45, 122.61, 123.68, 125.65, 129.77, 131.52, 133.63, 134.46, 136.75, 143.56, 144.15, 157.25; ESI–MS: 390$(M + K)^+$; Anal. Calcd. for $C_{17}H_{13}N_5O_4$: C, 58.12; H, 3.73; N, 19.93. Found C, 58.15; H, 3.80; N, 19.95.

4.1.3. 6-Methyl-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(*3c*)

Yellow crystals; yield 86%; m. p. 203–204 °C; IR (KBr): ν (cm^{-1}) 3234, 3143, 1756, 1533, 1172, 1045, 809, 737; ^{1}H NMR (300 MHz, DMSO): δ 2.32 (s, 3H), 5.21 (s, 2H), 7.02 (d, 1H, J = 5.9 Hz), 7.18–7.21 (m, 2H), 7.88 (t, 1H, J = 6.1 Hz), 8.32 (d, 1H, J = 6.2 Hz), 8.39 (d, 1H, J = 6.0 Hz), 8.70 (t, 1H, J = 1.5 Hz), 9.14 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 22.40, 37.38, 110.11, 111.32, 123.15 127.13, 128.49, 129.32,

Table 3
In-vivo antinociceptive activity by tail immersion method.

Group	Dose	Basal reaction time (min)	Reaction time (min)		
			30	60	120
Control	2 ml/kg	2.00 ± 0.23	2.22 ± 0.20	2.54 ± 0.05	2.38 ± 0.11
Indomethacin	0.05 mmol/kg	2.08 ± 0.22	3.12 ± 0.11*	3.68 ± 0.16**	3.54 ± 0.15**
3e	–	1.90 ± 0.20	2.18 ± 0.11	2.44 ± 0.18	2.56 ± 0.14
3i	–	2.28 ± 0.14	2.62 ± 0.26	2.74 ± 0.11	2.88 ± 0.09*
3a	–	2.30 ± 0.21	3.08 ± 0.24*	3.22 ± 0.14*	3.34 ± 0.12**
3f	–	2.48 ± 0.20	3.10 ± 0.28*	3.24 ± 0.20**	3.36 ± 0.15**
3q	–	2.44 ± 0.15	2.88 ± 0.13	2.78 ± 0.07	2.64 ± 0.06

Data is analysed by one way ANOVA followed by Dunnett's 't' test and expressed as mean ± SEM from six observations.
$^{***}p < 0.001$, $^{**}p < 0.01$ & $^{*}p < 0.05$.

Table 4
Histopathology report of ulcerogenic activity.

Group	Surface Epith. Damage	Sup. Mucosal damage	Deep mucosal damage	Muscular layer damage
Control	–	–	–	–
Indomethacin	+	+	–	+
3e	–	–	–	–
3i	–	–	–	–
3a	–	–	–	–
3f	–	–	–	–
3q	–	–	–	–

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

132.12, 134.34, 135.46, 136.24, 141.34, 142.34, 155.31; ESI–MS: 351 $(M + 1)^+$; Anal. Calcd. for $C_{17}H_{13}N_5O_4$: C, 58.12; H, 3.73; N, 19.93. Found C, 58.10; H, 3.72; N, 19.90.

*4.1.4. 3-[1-(4-Ethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzooxazol-2-one(**3d**)*

White crystals; yield 85%; m. p. 175–176 °C; IR (KBr): ν (cm^{-1}) 3478, 3147, 2977, 1756, 1615, 1446, 1178, 1048, 804; ^1H NMR (300 MHz, DMSO): δ 1.34 (t, 3H, $J = 6.3$ Hz), 2.32 (s, 3H), 4.08 (q, 2H, $J = 6.6$ Hz), 5.15 (s, 2H), 7.00–7.11 (m, 3H), 7.19 (d, 2H, $J = 6.6$ Hz), 7.75 (d, 2H, $J = 8.4$ Hz), 8.78 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 15.01, 21.35, 37.52, 64.00, 109.75, 110.64, 115.72, 122.17, 122.39, 124.65, 128.69,132.58, 142.54, 142.65, 154.11, 159.05; ESI–MS: 350 $(M)^+$; Anal. Calcd. for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found C, 65.11; H, 5.15; N, 15.97.

*4.1.5. 3-[1-(2-Chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzooxazol-2-one(**3e**)*

Brown crystals; yield 85%; m. p. 173–174 °C; IR (KBr): ν (cm^{-1}) 3542, 3146, 1751, 1678, 1440, 1172, 1061, 832; ^1H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.20 (s, 2H), 7.02 (d, 1H, $J = 8.1$ Hz), 7.20–7.22 (m, 2H), 7.5–7.6 (m, 3H), 7.76 (d, 1H, $J = 7.8$ Hz), 8.68 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.35, 37.36, 109.80, 110.66, 124.64, 126.57, 128.68, 128.68, 128.87, 128.93, 131.00, 132.21, 132.60, 134.79, 141.83, 142.52, 154.10; MALDI–MS: 341 $(M + 1)^+$; Anal. Calcd. for $C_{17}H_{13}ClN_4O_2$: C, 59.92; H, 3.85; N, 16.44. Found C, 59.90; H, 3.83; N, 16.42%.

*4.1.6. 3-[1-(4-Fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzooxazol-2-one(**3f**)*

Brown crystals; yield 93%; m. p. 181–182 °C; IR (KBr): ν (cm^{-1}) 3685, 3132, 1768, 1445, 1236, 1172, 1046, 835; ^1H NMR (300 MHz,

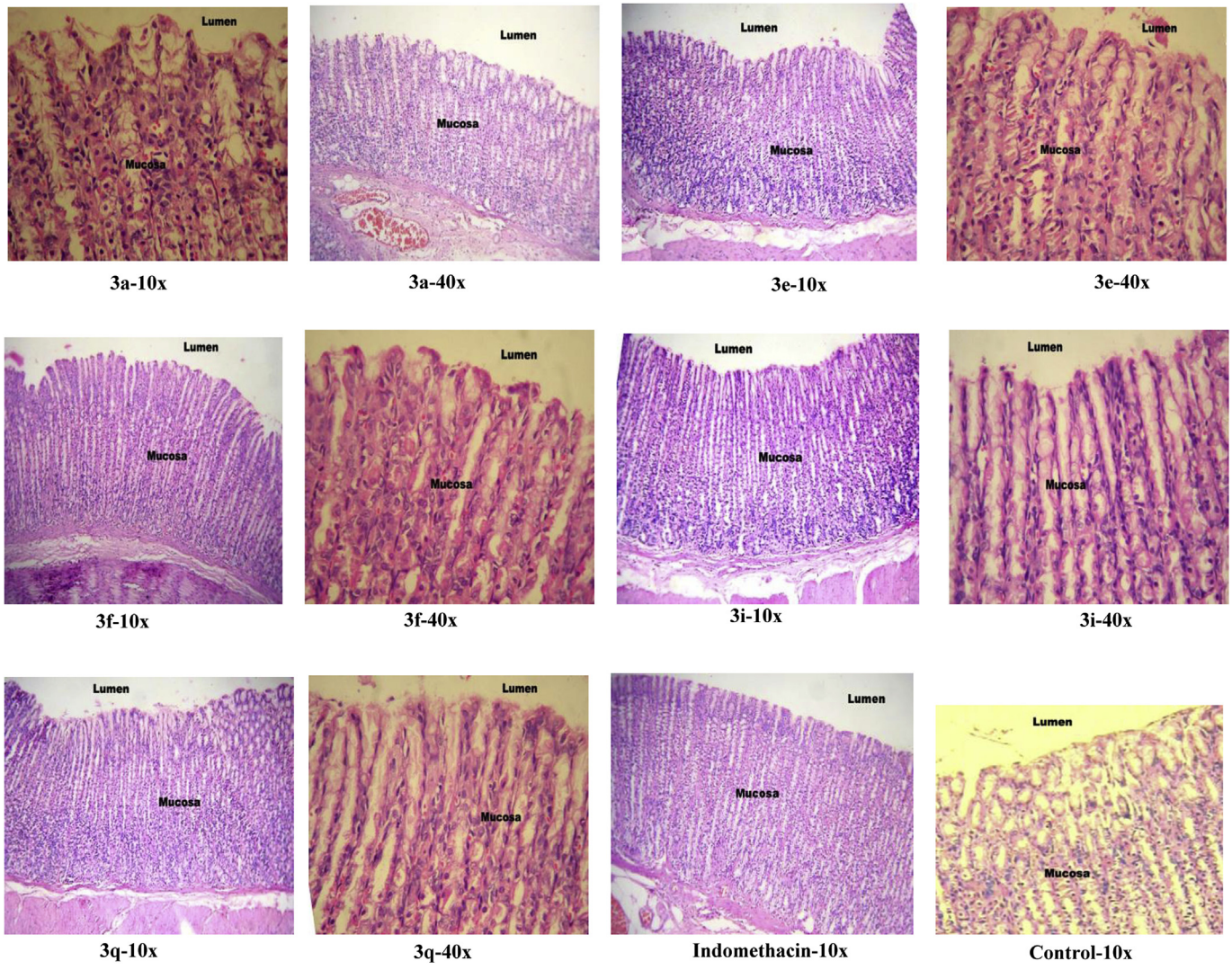


Fig. 6. Haematoxylin and eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.

DMSO): δ 2.32 (s, 3H), 5.17 (s, 2H), 7.02 (d, 1H, $J = 7.8$ Hz), 7.19 (d, 2H, $J = 7.5$ Hz), 7.44 (t, 2H, $J = 8.7$ Hz), 7.90–7.94 (m, 2H), 8.88 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.34, 37.49, 109.73, 110.65, 117.03, 117.34, 122.73, 122.85, 122.97, 124.66, 128.66, 132.61, 133.49, 142.54, 142.98, 154.10, 163.76; ESI–MS: 324 $(M)^+$; Anal. Calcd. for $C_{17}H_{13}FN_4O_2$: C, 62.96; H, 4.04; N, 17.28. Found C, 62.94; H, 4.02; N, 17.26%.

4.1.7. 5-Methyl-3-[1-(4-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3g**)

Brown crystals; yield 90%; m. p. 166–167 °C; IR (KBr): ν (cm^{-1}) 3628, 3067, 1774, 1519,1381, 1246, 1082, 855; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.21 (s, 2H), 6.95 (d, 1H, $J = 8.4$ Hz), 7.17–7.25 (m, 2H), 8.21 (d, 2H, $J = 8.4$ Hz), 8.44 (d, 2H, $J = 8.4$ Hz), 9.12 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.52, 37.41, 109.81, 110.38, 121.22, 122.92, 123.24, 126.03, 131.00, 133.92, 141.15, 143.70, 147.24, 154.24; ESI–MS: 352.1 $(M + 1)^+$; Anal. Calcd. for $C_{17}H_{13}N_5O_4$: C, 58.12; H, 3.73; N, 19.93. Found C, 58.09; H, 3.72; N, 19.91%.

4.1.8. 5-Methyl-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3h**)

Yellow crystals; yield 83%; m. p. 209–210 °C; IR (KBr): ν (cm^{-1}) 3668, 3243, 1776, 1535, 1494, 1250, 1194, 805; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.21 (s, 2H), 6.95 (d, 1H, $J = 8.1$ Hz), 7.17 (s, 1H), 7.24 (d, 1H, $J = 8.1$ Hz), 7.88 (t, 1H, $J = 8.1$ Hz), 8.33 (d, 1H, $J = 8.1$ Hz), 8.41 (d, 1H, $J = 1.2$ Hz), 8.71 (t, 1H, $J = 2.1$ Hz), 9.15 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.51, 37.46, 109.81, 110.37, 115.25, 122.93, 123.23, 123.68, 126.56, 130.99, 132.99, 133.91, 137.50, 140.50, 143.51, 148.96, 154.25; ESI–MS: 352.1 $(M + 1)^+$; Anal. Calcd. for $C_{17}H_{13}N_5O_4$: C, 58.12; H, 3.73; N, 19.93. Found C, 58.08; H, 3.69; N, 19.90%.

4.1.9. 3-[1-(4-Bromo-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one(**3i**)

Yellow crystals; yield 90%; m. p. 215–216 °C; IR (KBr): ν (cm^{-1}) 3135, 3028, 1767, 1625, 1385, 1248, 1183, 1018, 835; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.17 (s, 2H), 6.94 (d, 1H, $J = 7.8$ Hz), 7.16 (s, 1H), 7.23 (d, 1H, $J = 8.1$ Hz), 7.79 (d, 2H, $J = 9.0$ Hz), 7.86 (d, 2H, $J = 9.0$ Hz), 8.94 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.51, 37.43, 109.79, 110.40, 115.08, 122.46, 123.21, 123.68, 131.02, 133.89, 136.16, 140.53, 143.20, 156.45; MALDI–MS: 424 $(M + K)^+$; Anal. Calcd. for $C_{17}H_{13}BrN_4O_2$: C, 53.00; H, 3.40; N, 14.54. Found C, 52.98; H, 3.38; N, 14.51%.

4.1.10. 3-[1-(2-Chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one(**3j**)

Brown crystals; yield 83%; m. p. 141–142 °C; IR (KBr): ν (cm^{-1}) 3271,3035, 1761, 1622, 1435, 1249 1036, 823; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.20 (s, 2H), 6.94 (d, 1H, $J = 7.5$ Hz), 7.16 (s, 1H), 7.23 (d, 1H, $J = 8.1$ Hz), 7.5–7.7 (m, 3H), 7.77 (d, 1H, $J = 7.5$ Hz), 8.71 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.50, 37.33, 109.78, 110.54, 123.17, 126.66, 128.89, 128.96, 131.01, 132.23, 133.83, 134.81, 140.46, 141.80, 154.24; MALDI–MS: 341 $(M + 1)^+$, 363 $(M + Na)^+$; Anal. Calcd. for $C_{17}H_{13}ClN_4O_2$: C, 59.92; H, 3.85; N, 16.44. Found C, 59.89; H, 3.83; N, 16.41%.

4.1.11. 3-[1-(4-Fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one(**3k**)

White crystals; yield 92%; m. p. 177–178 °C; IR (KBr): ν (cm^{-1}) 3154, 3104, 1768, 1623, 1428, 1247, 1104, 843; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.17 (s, 2H), 6.95 (d, 1H, $J = 7.8$ Hz), 7.17 (s, 1H), 7.23 (d, 1H, $J = 8.1$ Hz), 7.42–7.47 (m, 2H), 7.91–7.95 (m, 2H), 8.89 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.51, 37.42, 109.78, 110.40, 117.04, 122.69, 126.66, 122.88, 122.99, 123.20, 131.02, 133.49, 133.90, 140.47, 143.01, 154.25; MALDI–MS: 347 $(M + Na)^+$; Anal. Calcd. for $C_{17}H_{13}FN_4O_2$: C, 62.96; H, 4.04; N, 17.28. Found C, 62.93; H, 4.02; N, 17.26%.

4.1.12. 3-[1-(2-Fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one(**3l**)

Yellow crystals; yield 90%; m. p. 200–201 °C; IR (KBr): ν (cm^{-1}) 3138, 3027, 1769, 1496, 1253, 1192, 1092 829; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.20 (s, 2H), 6.95 (d, 1H, $J = 7.8$ Hz), 7.17 (s, 1H), 7.24 (d, 1H, $J = 8.1$ Hz), 7.63–7.67 (m, 3H), 8.31 (d, 1H, $J = 8.4$ Hz), 9.00 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.52, 37.33, 109.79, 110.43, 122.87, 123.22, 131.02, 133.91, 140.49, 141.81, 143.25, 150.26, 151.92, 152.29, 154.33, 164.50; ESI–MS: 324 $(M)^+$; Anal. Calcd. for $C_{17}H_{13}FN_4O_2$: C, 62.96; H, 4.04; N, 17.28. Found C, 62.95; H, 4.02; N, 17.26%.

4.1.13. 5-Methyl-3-(1-pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzooxazol-2-one(**3m**)

Brown crystals; yield 88%; m. p. 229–230 °C; IR (KBr): ν (cm^{-1}) 3145, 3067, 1772, 1623, 1595, 1240, 1132, 1043, 851; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.20 (s, 2H), 6.94 (d, 1H, $J = 7.8$ Hz), 7.20–7.24 (m, 2H), 7.43 (t, 1H, $J = 7.5$ Hz), 7.53–7.60 (m, 2H), 7.82 (t, 1H, $J = 7.5$ Hz), 8.74 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.50, 37.26, 109.77, 110.45, 117.47, 123.18, 124.99, 125.14, 125.89, 126.05, 131.05, 140.46, 142.40, 152.59, 154.25, 155.91; ESI–MS: 307 $(M)^+$; Anal. Calcd. for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.26; N, 22.79. Found C, 62.51; H, 4.24; N, 22.76%.

4.1.14. 3-[1-(4-Chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one(**3n**)

Yellow crystals; yield 83%; m. p. 205–206 °C; IR (KBr): ν (cm^{-1}) 3135, 30216, 1754, 1495, 1246, 1134, 1088, 895; ^{1}H NMR (300 MHz, DMSO): δ 2.33 (s, 3H), 5.18 (s, 2H), 6.94 (d, 1H, $J = 7.5$ Hz), 7.16–7.25 (m, 2H), 7.66 (d, 2H, $J = 7.8$ Hz), 7.93 (d, 2H, $J = 8.1$ Hz), 8.94 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 21.51, 37.43, 109.78, 110.39, 122.23, 122.53, 123.20, 130.31, 131.02, 133.50, 135.72, 140.48, 143.17, 154.24; ESI–MS: 341 $(M + 1)^+$; Anal. Calcd. for $C_{17}H_{13}ClN_4O_2$: C, 59.92; H, 3.85; N, 16.44. Found C, 59.88; H, 3.82; N, 16.42%.

4.1.15. 5-Chloro-3-[1-(4-chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2one(**3o**)

Yellow crystals; yield 83%; m. p. 225–226 °C; IR (KBr): ν (cm^{-1}) 3206, 3123, 1780, 1610, 1248, 1092, 827; ^{1}H NMR (300 MHz, DMSO): δ 5.22 (s, 2H), 7.2 (dd, 1H, $J = 1.8$ and 8.4 Hz), 7.41 (d, 1H, $J = 8.7$ Hz), 7.53 (d, 1H, $J = 2.1$ Hz), 7.67 (d, 2H, $J = 8.7$ Hz), 7.92 (d, 2H, $J = 8.7$ Hz), 8.94 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.15, 109.87, 111.07, 121.75, 122.11, 128.05, 129.83, 131.98, 133.03, 135.22, 140.73, 142.40, 153.41; ESI–MS: 360 $(M)^+$; Anal. Calcd. for $C_{16}H_{10}Cl_2N_4O_2$: C, 53.21; H, 2.79; N, 15.51. Found C, 53.18; H, 2.76; N, 15.49%.

4.1.16. 5-Chloro-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3p**)

Yellow crystals; yield 80%; m. p. 163–164 °C; IR (KBr): ν (cm^{-1}) 3250, 3182, 1781, 1540, 1484, 1352, 1256, 823; ^{1}H NMR (300 MHz, DMSO): δ 5.25 (s, 2H), 7.21 (dd, 1H, $J = 2.1$ and 8.7 Hz), 7.42 (d, 1H, $J = 8.7$ Hz), 7.53 (d, 1H, $J = 2.1$ Hz), 7.89 (t, 1H, $J = 8.10$ Hz), 8.33–8.41 (m, 2H), 8.70 (t, 1H, $J = 2.1$ Hz), 9.15 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.69, 110.36, 111.60, 122.64, 122.96, 123.71, 126.56, 126.59, 128.57, 132.99, 137.49, 140.14, 143.21, 149.68, 153.91; ESI–MS: 371 $(M)^+$; Anal. Calcd. for $C_{16}H_{10}ClN_5O_4$: C, 51.70; H, 2.71; N, 18.84. Found C, 51.67; H, 2.69; N, 18.82%.

4.1.17. 5-Chloro-3-[1-(2-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3q**)

White crystals; yield 82%; m. p. 159–160 °C; IR (KBr): ν (cm^{-1}) 3152, 3109, 1780, 1618, 1492, 1245, 1112, 1048, 846; ^{1}H NMR

(300 MHz, DMSO): δ 5.24 (s, 2H), 7.20 (d, 1H, $J = 7.5$ Hz), 7.40 (d, 2H, $J = 8.7$ Hz), 7.56–7.82 (m, 4H), 8.75 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.47, 109.94, 111.43, 114.68, 121.66, 123.54, 124.61, 132.22, 135.98, 137.12, 141.53, 143.56, 145.25, 155.35; ESI–MS: 345 $(M + 1)^+$; Anal. Calcd. for $C_{16}H_{10}ClFN_4O_2$: C, 55.75; H, 2.92; N, 16.25. Found C, 55.73; H, 2.89; N,16.23%.

4.1.18. 5-Chloro-3-(1-pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzooxazol-2-one(**3r**)

White crystals; yield 86%; m. p. 196–197 °C; IR (KBr): ν (cm^{-1}) 3066, 3012, 1788, 1611, 1488, 1244, 1096, 807; ^{1}H NMR (300 MHz, DMSO): δ 5.24 (s, 2H), 7.19–7.22 (m, 1H), 7.41 (d, 1H, $J = 8.4$ Hz), 7.55 (s, 1H), 7.61 (dd, 1H, $J = 8.4$ and 5.1 Hz), 8.31 (d, 1H, $J = 8.1$ Hz), 8.69 (s, 1H), 9.01 (s, 1H), 9.12 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.46, 109.77, 110.45, 117.47, 123.79, 124.16, 124.69, 126.05, 131.05, 132.65 140.46, 142.40, 143,62 153.59; ESI–MS: 328 $(M + 1)^+$; Anal. Calcd. for $C_{15}H_{10}ClN_5O_2$: C, 54.97; H, 3.08; N, 21.37. Found C, 54.95; H, 3.07; N, 21.36%.

4.1.19. 3-[1-(3-Nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3s**)

Yellow crystals; yield 86%; m. p. 207–208 °C; IR (KBr): ν (cm^{-1}) 3173, 3090, 1781, 1533, 1439, 1248, 1081, 816; ^{1}H NMR (300 MHz, DMSO): δ 5.24 (s, 2H), 7.12–7.24 (m, 2H), 7.36 (t, 2H, $J = 7.8$ Hz), 7.88 (t, 1H, $J = 8.4$ Hz), 8.30–8.41 (m, 2H), 8.70 (t, 1H, $J = 2.1$ Hz), 9.15 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.66, 109.78, 111.27, 116.55, 123.93, 124.33, 125.68, 127.56, 131.99, 132.66, 136.51, 140.50, 143.51, 148.96, 155.25; ESI–MS: 336 $(M)^+$, 338 $(M + 2)^+$; Anal. Calcd. for $C_{16}H_{11}N_5O_4$: C, 56.98; H, 3.29; N, 20.76. Found C, 56.96; H, 3.27; N, 20.74%.

4.1.20. 3-[1-(4-Ethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3t**)

Yellow crystals; yield 88%; m. p. 175–176 °C; IR (KBr): ν (cm^{-1}) 3172, 3123, 1774, 1521, 1488, 1252, 1050, 802; ^{1}H NMR (300 MHz, DMSO): δ 1.34 (t, 3H, $J = 6.9$ Hz), 4.08 (q, 2H, $J = 6.9$ Hz), 5.18 (s, 2H), 7.08–7.21 (m, 4H), 7.34 (t, 2H, $J = 8.7$ Hz), 7.75 (d, 2H, $J = 9.0$ Hz), 8.78 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 15.01, 37.54, 64.00, 110.15, 115.73, 122.18, 122.43, 122.92, 124.41, 130.19, 131.08, 142.45, 146.61, 148.96, 154.00, 159.05; ESI–MS: 336 $(M)^+$; Anal. Calcd. for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66. Found C, 64.27; H, 4.77; N, 16.64%.

4.1.21. 3-[1-(4-Methoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one(**3u**)

Yellow crystals; yield 88%; m. p. 169–170 °C; IR (KBr): ν (cm^{-1}) 3215, 3137, 1765, 1486, 11241, 1044, 830; ^{1}H NMR (300 MHz, DMSO): δ 3.81 (s, 3H), 5.19 (s, 2H), 7.10–7.21 (m, 4H), 7.35 (t, 2H, $J = 7.8$ Hz), 7.77 (d, 2H, $J = 9.0$ Hz), 8.81 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.53, 56.02, 110.17, 115.32, 122.19, 124.08, 130.34, 132.09, 143.17, 160.11; ESI–MS: 322 $(M)^+$; Anal. Calcd. for $C_{17}H_{14}N_4O_3$: C, 63.25; H, 4.38; N, 17.38. Found C, 63.23; H, 4.35; N, 17.36%.

4.1.22. 3-(1-Pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzooxazol-2-one(**3v**)

Brown crystals; yield 90%; m. p. 179–180 °C; IR (KBr): ν (cm^{-1}) 3182, 3106, 1757, 1584, 1439, 1151, 1022, 811; ^{1}H NMR (300 MHz, DMSO): δ 5.23 (s, 2H), 7.12–7.24 (m, 2H), 7.35 (t, 2H, $J = 6.3$ Hz), 7.62 (d, 1H, $J = 4.5$ Hz), 8.30 (d, 1H, $J = 7.5$ Hz), 8.69 (s, 1H), 8.99 (s, 1H), 9.12 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 37.48, 110.15, 110.19, 122.94, 124.44, 124.99, 128.44, 131.04, 141.78, 142.44, 143.17, 150.26, 153.99; ESI–MS: 294 $(M + 1)^+$; Anal. Calcd. for $C_{15}H_{11}N_5O_2$: C, 61.43; H, 3.78; N, 23.88. Found C, 61.42; H, 3.76; N, 23.85%.

4.2. Pharmacology

4.2.1. 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. Drugs

Indomethacin, Carrageenan, Carboxymethylcellulose, were purchased from Sigma–Aldrich Chemicals Pvt. Limited, Bangalore, India.

4.2.3. Anti-inflammatory activity

The synthesized compounds were tested for their anti-inflammatory activity using carrageenan-induced hind paw oedema method. The rat paw oedema 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 standard drug, indomethacin (0.05 mmol/kg) 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 equimolar dosage of the synthesized compounds as the standard drug, 1 h before the administration of carrageenan. The paw volumes were measured using plethysmometer [33] at interval of 3 h and 5 h.

4.2.4. COX assay

Recombinant human COX-2 has been expressed in insect cell expression system. The enzymes have been purified by using conventional chromatographic techniques. Enzymatic activities of COX-2 was measured according to the method of Copeland [34], 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 PGG2 to PGH2 [35]. Briefly, the assay mixture contained TriseHCl buffer (100 mM, Ph 8.0), haematin (15 mM), EDTA (3 mM), enzyme (100 mg 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 nonenzymatic oxidation observed in the absence of COX-2 was subtracted from the experimental value while calculating the percent inhibition.

4.2.5. Molecular docking studies on TNF-α

All the computational studies were carried out in the Schrodinger suite 2010 molecular modelling software. The 2D structure of the ligands (3a, 3e, 3f, 3i and 3°) was built in the maestro window [36]. The structures were then converted to their respective 3D structures, with various conformers, tautomers and ionization states using the Ligprep and Confgen modules [37–40]. The 3D crystal structure 2AZ5 [41], solved at a resolution of 2.10 A°, was downloaded from Protein Data Bank for carrying out the docking studies. The crystal structure constitutes four chains A,B,C and D. Chains A and B were homologous to chains C and D and hence, chains A and B were used for docking studies, while chains C and D were discarded for computational purpose. The coordinates of TNF-Alpha in complex with this ligand were obtained from protein data bank. The protein was prepared for docking using the protein

preparation wizard. In the pre-process step hydrogen's were added to the protein with assigning bond order, creating disulfide bond and water residues were removed beyond 5 A°from the heteroatom. Further, the interactions of water residues with protein and heteroatom were checked and only those water residues were kept, which were interacting with protein as well as heteroatom. Then the heteroatom was extracted and protein was refined by assigning H-bonds and minimization at OPLS 2005 force field. A grid was generated at active site, identified on the bases of already co-crystallised ligand to the receptor using receptor grid generation module. Docking studies were then carried out with the co-crystallized ligand, in order to confirm the protocol. The conformation of the co-crystallized ligand matched best with the docked conformation using extra precision (XP) docking algorithm of Glide module.

4.2.6. TNF-α assay

Macrophages cells were grown in RPMI 1640 containing 10% FBS, 1 M HEPES, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin was obtained by passage through a stainless mesh. Supernatants of cell culture collected from form macrophages cells seeded with varying concentrations of compounds was assayed for the pro-inflammatory cytokine levels using e-Biosciences ELISA kits. For this, macrophages were pre-incubated with cytochalasin D (2.5 μM/1 × 10^6 cells) for 30 min before the start of the experiment and continued till the end. Cytokine levels were measured according to the protocol suggested by the manufacturer. Cells were cultured in 96-well plates at 2 × 106 cells/mL and cytokines were then measured from the supernatants by ELISA. The assay was performed according to the manufacturer's instruction with multipoint analysis [42]. Briefly, 100 μL of diluted capture antibody was added to each well in a 96 well plate and was allowed to adhere overnight for 4 °C. Plates were washed and then blocked with 1 × PBS supplemented with 10% FBS for 1 h at room temperature. After washing, serial dilutions of the standard and samples were prepared in the plates and were then incubated for 2 h at room temperature. Then, plates were washed and working detector solution (including detector antibody and avidin–horse radish peroxidase reagent) was added into each well. Plates were then sealed and incubated for 1 h at room temperature. After washing, 100 μL of tri-methyl benzidine (TMB) substrate was added into each well. Stop solution (2 N H_2SO_4) was finally added after incubation in the dark for 30 min at room temperature. The absorbance was read at 450 nm. The result was analysed using softmax program and values determined against the standard provided by the manufacturer.

4.2.7. Antinociceptive activity

4.2.7.1. Writhing test. The writhing test in mice was carried out using the method of Koster [43]. The writhes were induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). The standard drug i.e. indomethacin was given orally at a dose 0.05 mmol/kg of 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.

4.2.7.2. Tail immersion method. In the present study analgesia was assessed by employing tail immersion method [44]. Prior to the experiment the animals were screened for the sensitivity test by immersing the tail of the rats gently in hot water maintained at 55 °C [45]. The animals flicking their tail from hot water in 5 s were selected for the study in order to avoid any thermal injury to the tail. The selected rats were then divided into six groups of six rats each. The control group was administered orally with 0.9% of 0.1 mL of saline solution only. The standard drug, i.e. indomethacin and the test compounds given orally at a dose of 0.05 mmol/kg of body weight. After administration of the drugs, the reaction time was measured at 30, 60 and 120 min. The basal reaction time was calculated as the reaction time prior to the drug administration.

4.2.8. Ulcerogenic activity

The test compounds having anti-inflammatory & analgesic activities comparable with the indomethacin were further tested for their ulcerogenic risk evaluation [46]. This was done at three times higher dose in comparison to the dose used for anti-inflammatory activity, i.e. 0.15 mmol/kg body weight of indomethacin and the test compounds were used. Each group had three animals which were later sacrificed. When compared with indomethacin, 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 indomethacin.

Acknowledgements

The authors thank Dr. G.N. Qazi, Vice-Chancellor, Jamia Hamdard for providing necessary facilities to the department of chemistry to carry out this work. One of the authors (SH) is also thankful to CSIR for awarding the senior research fellowship.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.10.032.

References

[1] M.M. Wolfe, D.R. Lichtenstein, G. Singh, N. Engl. J. Med. 340 (1999) 1888.
[2] G. Dannhardt, W. Kiefer, Eur. J. Med. Chem. 36 (2001) 109–126.
[3] L. Jund, J. Corse, A.S. King, H. Bayne, K. Mihrag, Phytochemistry 10 (1971) 2971.
[4] S.L. El-Ansary, E.I. Aly, M.A. Halem, Egypt J. Pharm. Sci. 33 (1992) 379.
[5] J. Sam, J.L. Valentine, J. Pharm. Sci. 58 (1969) 1043–1054.
[6] R.L. Clark, A.A. Pessolano, J. Am. Chem. Soc. 80 (1953) 1662–1664.
[7] R.S. Varma, W.L. Nobles, J. Pharm. Sci. 57 (1968) 39–44.
[8] N. Gökhan, H. Erdoğan, B.C. Tel, R. Demirdamar, Eur. J. Med. Chem. 31 (1996) 625–628.
[9] M.P. Vaccher, D. Lesieur, C. Lespagnol, J.P. Bonte, J. Lamar, M. Beaughard, G. Dureng, Farmaco Ed. Sci. 41 (1986) 257–269.
[10] R.S. Varma, A. Kapoor, Indian J. Chem. 18B (1979) 200–204.
[11] D.D. Erol, H. Erdoğan, N. Yuluğ, Pharm. Belg. 44 (1989) 334–336.
[12] V. Kalcheva, Z. Mincheva, P. Andreeva, Arzneim. Forsch. 40 (1990) 1030–1034.
[13] Hansch, Sammes, Taylor, Compr. Med. Chem. 6 (1989) 964.
[14] Shankaran, K.L. Donnelly, S.K. Shah, J.L. Humes, S.G. Pacholok, S.K. Grant, B.G. Green, M. MacCoss, Bioorg. Med. Chem. Lett. 7 (1997) 2887–2892.
[15] (a) Y. Bourne, H.C. Kolb, Z. Radi, K.B. Sharpless, P. Taylor, P. Marchot, Proc. Natl. Acad. Sci. U S A 101 (2004) 1449–1454.
[16] (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.
[17] (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.
[18] M.D. Chen, S.J. Lu, G.P. Yuag, S.Y. Yang, X.L. Du, Heterocyclic Comm. 6 (2000) 421–426.
[19] E.A. Sherement, R.I. Tomanov, E.V. Trukhin, V.M. Berestovitskaya, Russ. J. Org. Chem. 40 (2004) 594–595.
[20] A. Allais, J. Meier, (Roussel-UCLAF) Ger. Offen., 1969, p. 1815467.

[21] K.M. Banu, A. Dinakar, C. Ananthanarayanan, Indian J. Pharm. Sci. 4 (1999) 202–205.
[22] R. Meier, EP. 199262; eidem, US. 4789680, 1986.
[23] A. Passannanti, P. Diana, P. Barraja, F. Mingoia, A. Lauria, G. Cirrincione, Heterocycles 48 (1998) 1229–1235.
[24] M. Jilino, F.G. Stevens, J. Chem. Soc. Perkin Trans. 1 (1998) 1677–1684.
[25] G.D. Diana, J.J. Nitz, EP. 566199, 1993.
[26] R.J. Nachmann, J. Heterocyclic Chem. 19 (1982) 1545–1547.
[27] A. Bali, R. Ohri, P.K. Deb, Eur. J. Med. Chem. 49 (2012) 397–405.
[28] R. Deraedt, S. Joughney, F. Delevakee, M. Falhour, Eur. J. Pharmacol. 51 (1980) 17–24.
[29] S.E. Besra, R.M. Sharma, A. Gomes, J. Ethnopharmacol. 54 (1996) 1–6.
[30] F. Abbott, S.N. Young, Pharmacol. Biochem. Behav. 31 (1988) 855–860.
[31] S. Furst, K. Gyires, J. Knoll, Drug Res. 4 (1988) 552–557.
[32] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[33] S.H. Ferreira, J. Pharm. Pharmacol. 31 (1979) 648.
[34] R.A. Copeland, J.M. Williams, J. Giannaras, S. Nurnberg, M. Covington, D. Pinto, S. Pick, J.M. Trzaskos, Proc. Natl. Acad. Sci. U S A 91 (23) (1994) 11202–11206.
[35] (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.
[36] Schrödinger, Maestro, Version 9.2, LLC, New York, NY, 2011.
[37] Schrödinger, ConfGen, Version 2.3, LLC, New York, NY, 2011.
[38] Schrödinger, LigPrep, Version 2.5, LLC, New York, NY, 2011.
[39] K.S. Watts, P. Dalal, R.B. Murphy, W. Sherman, R.A. Friesner, J.C. Shelley, J.Chem. Inf. Model. 50 (2010) 534–546.
[40] I. Chen, N. Foloppe, J.Chem. Inf. Model. 50 (2010) 822–839.
[41] M.M. He, A.S. Smith, J.D. Oslob, W.M. Flanagan, A.C. Braisted, A. Whitty, M.T. Cancilla, J. Wang, A.A. Lugovskoy, J.C. Yoburn, A.D. Fung, G. Farrington, J.K. Eldredge, E.S. Day, L.A. Cruz, T.G. Cachero, S.K. Miller, J.E. Friedman, I.C. Choong, B.C. Cunningham, Science 310 (2005) 1022–1025.
[42] F. Malik, J. Singh, K. Anamika, A.S. Krishan, K.S. Naresh, S. Surjeet, K.K. Maharaj, K. Arun, B. Aruna, G.N. Qazi, Life Sci. 80 (2007) 1525–1538.
[43] R. Koster, M. Anderson, E.J. Beer, Fed. Proc. 18 (1959) 412–416.
[44] L.C. Distasi Costa, M. Mendacolli, S.J.L. Kirzawa, M. Games, C.G. Trolin, J. Ethnopharmacol. 24 (1988) 205.
[45] A.K. Agrahari, M. Khaliquzzama, S.K. Panda, J. Curr. Pharm. Res. 1 (2010) 8–11.
[46] S. Haider, S. Nazreen, M.M. Alam, H. Hamid, M.S. Alam, J. Ethnopharmacol. 143 (2012) 236–240.