Monatsh Chem
DOI 10.1007/s00706-015-1557-x

ORIGINAL PAPER

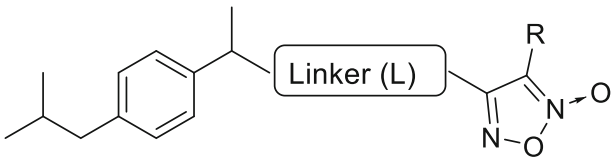
Synthesis, characterization, and biological evaluation of furoxan coupled ibuprofen derivatives as anti-inflammatory agents

Mohd Amir[1] · **Mohd Wasim Akhter**[1] · **Ozair Alam**[1]

Received: 4 May 2015 / Accepted: 9 August 2015

Abstract A series of furoxan-based nitric oxide releasing ibuprofen derivatives were synthesized and tested for their anti-inflammatory, analgesic, ulcerogenic, lipid peroxidation, and hepatotoxic properties. The compounds exhibited more protection than ibuprofen with regard to gastric toxicity. Among the tested compounds 4-[2-[2-(4-isobutylphenyl) propanamido]ethoxycarbonyl]-3-methylfuroxan and 4-[2-[2-(4-isobutylphenyl)propanoyl]hydrazinecarbonyl]-3-phenyl-furoxan emerged as most active anti-inflammatory agents with reduced gastrotoxicity. The results showed that incorporation of NO donating group caused a moderate increase in anti-inflammatory activity with a marked decrease in gastric ulcerations compared to their parent drug ibuprofen. A molecular docking study of all the compounds was also performed to provide the binding modes of COX-1 enzyme. Among all the titledcompounds, 4-[2-[2-(4-isobutylphenyl)propanamido]ethoxycarbonyl]-3-methyl-furoxan was found to be most potent and have high docking score showing favorable orientation within the COX-1 binding site.

Graphical abstract



Keywords Furoxan · Ibuprofen · Nitric oxide donors · Anti-inflammatory · Gastric toxicity · Hepatotoxic

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation [1]. The worldwide NSAIDs market for both occasional and chronic users has been conservatively estimated at over 60 million people, and certain NSAIDs (aspirin, naproxen, ibuprofen) are among the most popular over-the-counter medications [2, 3]. Chronic NSAIDs therapy effectively reduces the symptoms of many painful arthritic syndromes, but invites adverse gastrointestinal (GI) complications ranging from stomach irritation to life threatening GI ulceration and bleeding [4, 5]. At the tissue level, the most common clinical manifestation of NSAIDs-related GI damage is a combination of gastroduodenal erosions and ulcerations often called NSAIDs-induced gastropathy [6], affecting at least 25 % of chronic NSAIDs patients. NSAIDs exert their anti-inflammatory effect mainly through inhibition of cyclooxygenase (COXs), key enzymes in prostaglandin (PG) biosynthesis from arachidonic acid [7, 8]. There are at least two mammalian COX

Electronic supplementary material The online version of this article (doi:10.1007/s00706-015-1557-x) contains supplementary material, which is available to authorized users.

✉ Mohd Amir
mamir_s2003@yahoo.co.in

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110 062, India

Published online: 25 August 2015

isoforms, COX-1 and COX-2 [9, 10]. COX-1 is the constitutive isoform of COX, which performs a housekeeping function to synthesize prostaglandins, which regulate normal cell activity. Thus, the nonselective inhibition of both cyclooxygenase isoenzymes (COX-1 and COX-2) by traditional ulcerogenic NSAIDs clearly illustrated the clinical need for a new generation of non ulcerogenic selective COX-2 inhibitors [11, 12]. This was a turning point which stimulated pharmaceutical companies to seek selective COX-2 inhibitors that would be free from unwanted gastrointestinal toxicity. This effort led to the discovery and development of a number of selective COX-2 inhibitors, viz., celecoxib, rofecoxib, valdecoxib, parecoxib, and etoricoxib [13]. But careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effects [14]. Therefore, selective COX-2 inhibitors may not be the proper strategy to overcome the gastric toxicity of conventional NSAIDs due to its adverse cardiovascular effects. One of the most important strategies used to overcome NSAIDs side effects is to design nitric oxide (NO) donating NSAIDs (NO-NSAIDs) which are capable of generating gastroprotective agent NO [15, 16]. Hybrid molecules comprised of NSAID and nitric oxide (NO) donor moieties are devoid of adverse cardiovascular effects of selective COX-2 inhibitors, and also decreases GI toxicity observed on long-term use of traditional NSAIDs [17]. It has been reported that an increased generation of endothelial NO or release of NO from a nitric oxide donor drugs produces beneficial effects such as reduction of blood pressure and prevention of atherosclerosis [18]. At nanomolar concentrations, NO reversibly activates soluble guanylate cyclase (sGC) by 400 folds, catalyzing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP) [19]. Elevation of cGMP relaxes smooth muscles in blood vessels, inhibits platelet aggregation and blocks the adhesion of white cells to blood vessels walls [20]. Besides these cardiovascular effects, NO is also recognized as a critical mediator of gastrointestinal mucosal defense. NO is able to protect gastric mucosa by a number of mechanisms including promotion of mucus secretion, increased mucosal blood flow resulting in enhanced mucosal resistance to ulceration. NO also prevents adherence of neutrophils to the gastric vascular endothelium [21]. NO also increases the ability of mucous cells to undergo healing and repair of the existing ulcers [22].

It has been reported that furoxans (1,2,5-oxadiazole-2-oxides) are biologically active compounds that are capable of releasing nitric oxide in the presence of thiol cofactors [23]. Compared to other NO donating agents, furoxans possess favorable pharmacological properties, since they frequently release NO slowly resulting in a longer duration of action. The absence of tolerance is also an important property of furoxan moiety [24]. Thiol induced release of NO from furoxan moiety involves attack by a thiolate anion at C-3 and/or C-4 of the furoxan ring followed by ring opening and subsequent release of NO [25]. Encouraged by these observations and in continuation of our ongoing research program [26–29] to discover new and useful agents for treatment of anti-inflammatory disease, we report herein the synthesis and pharmacological profile of furoxan derivatives of ibuprofen as potential NO donor drugs (Fig. 1).

Results and discussion

Chemistry

Substituted furoxans were synthesized from crotonaldehyde and cinnamaldehyde [30–34] (Fig. 2). Compound **8** was synthesized by treating 2-(4-isobutylphenyl)propanoyl chloride (**7**) with ethylenediamine to form an intermediate *N*-(2-aminoethyl)-2-(4-isobutylphenyl)propanamide which on further treatment with 4-(chlorocarbonyl)-3-methylfuroxan (**6a**) resulted in the formation of 4-[2-[2-(4-isobutylphenyl)propanamido]ethylcarbamoyl]-3-methylfuroxan (**9**). The compound **7** was treated with ethylene glycol to form an intermediate 2-hydroxyethyl 2-(4-isobutylphenyl)-propionate (**10**) which on treatment with **6a** resulted in the formation of 4-[2-[2-(4-isobutylphenyl)propanoyloxy]ethoxycarbonyl]-3-methylfuroxan (**11**). The ethyl ester of ibuprofen (**12**) on treatment with 2-aminoethanol afforded

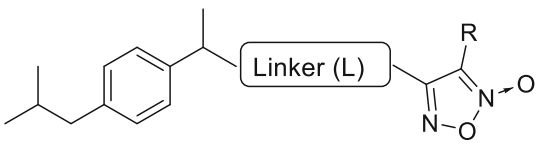


Fig. 1 General structure of hybrid models

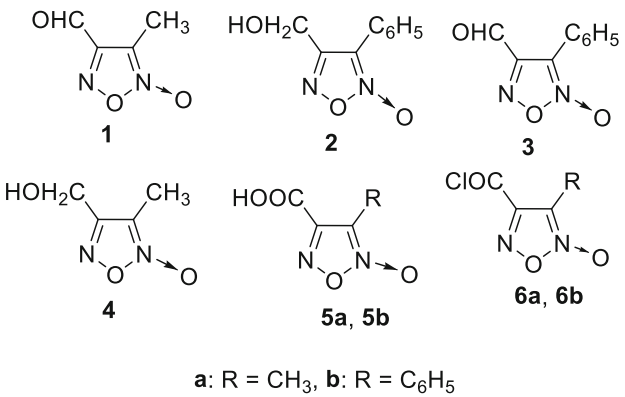
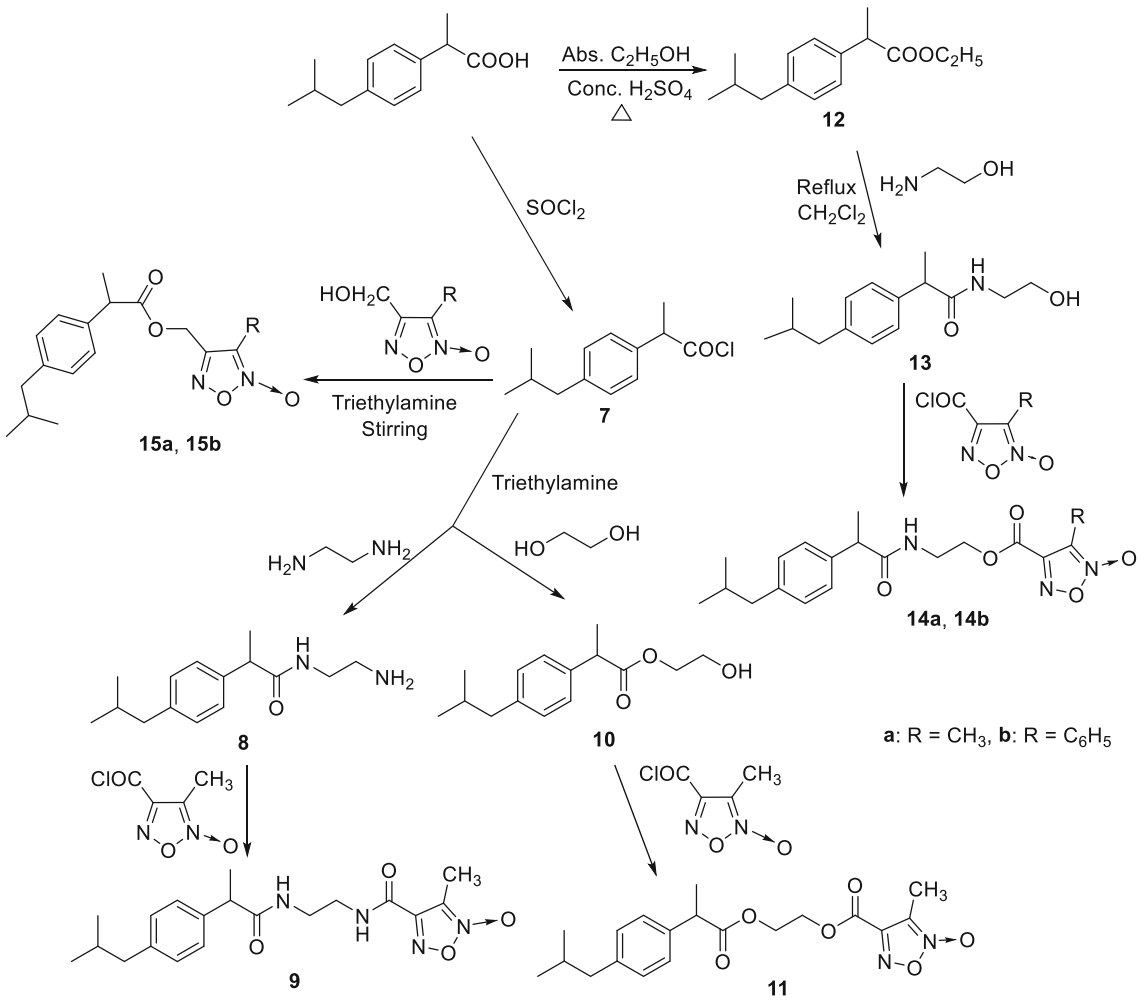


Fig. 2 Different substituted furoxans

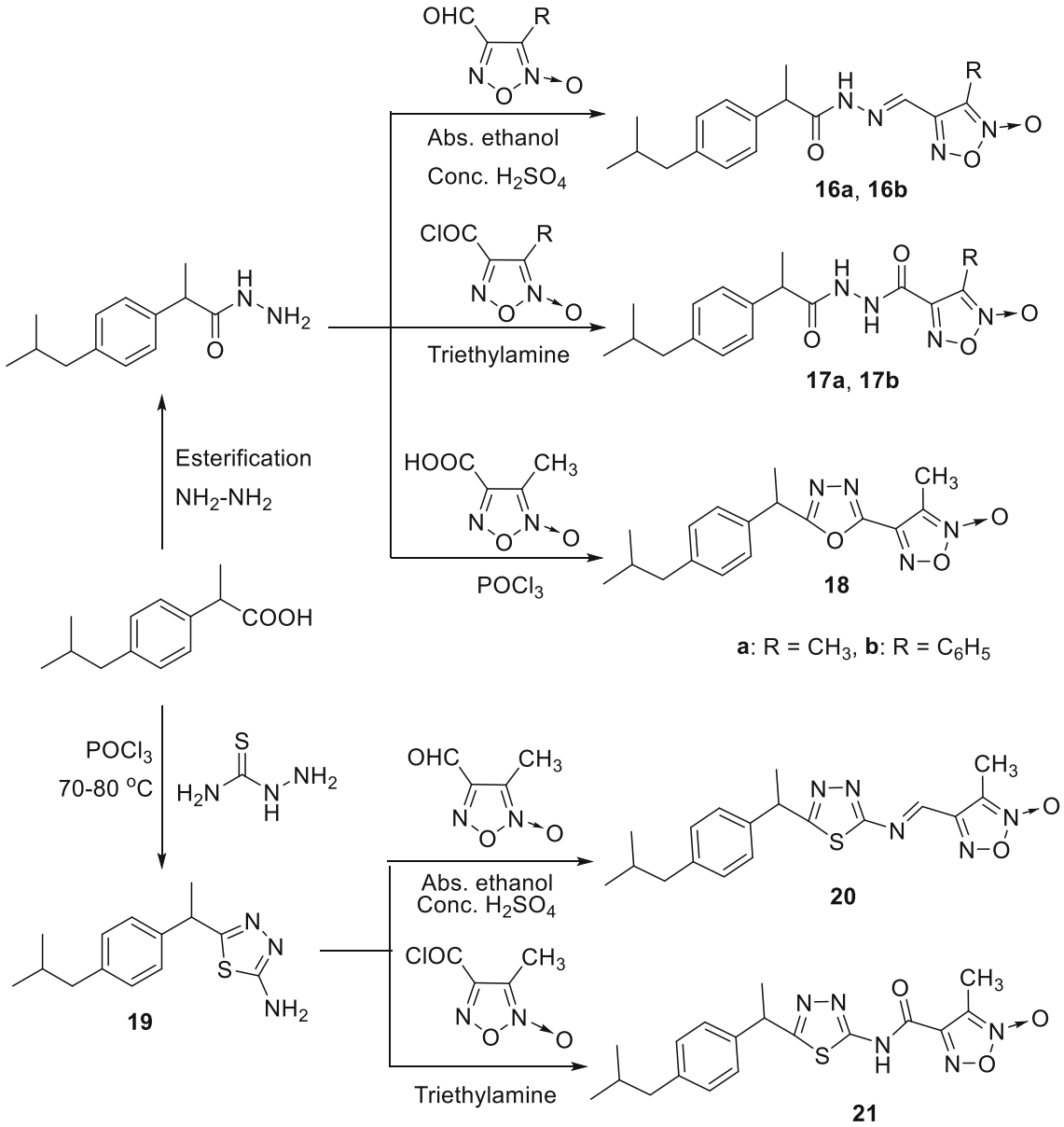
the intermediate *N*-(2-hydroxyethyl)-2-(4-isobutylphenyl)-propanamide (**13**). The final compounds 4-[2-[2-(4-isobutylphenyl)propanamido]ethoxycarbonyl]-3-substituted furoxan (**14a**, **14b**) were prepared by treating intermediate **13** with **6a** and **6b** in the presence of anhydrous dichloromethane and triethyl amine. The compounds 4-[[2-(4-isobutylphenyl)propanoyloxy]methyl]-3-substituted-furoxan (**15a**, **15b**) were prepared by reacting intermediate **7** with 4-(hydroxymethyl)-3-substituted furoxan. Synthesis of these compounds is outlined in Scheme 1.

The compounds 4-[[2-[2-(4-isobutylphenyl)propanoyl]-hydrazono]methyl]-3-substituted-furoxan (**16a**, **16b**) were prepared by reacting 2-(4-isobutylphenyl)propionic acid hydrazide [30] with intermediate **6a** and **6b** in the presence of concentrated sulfuric acid. The compounds 4-[2-[2-(4-isobutylphenyl)propanoyl]hydrazinecarbonyl]-3-substituted-furoxan (**17a**, **17b**) were synthesized by treating the hydrazide with intermediate **6a**, **6b** in presence of anhydrous dichloromethane. The compound 4-[5-[1-(4-isobutylphenyl)ethyl]-1,3,4-oxadiazol-2-yl]-3-methyl-furoxan (**18**) was synthesized by refluxing hydrazide with 4-carboxy-3-methylfuroxan in phosphorus oxychloride. The intermediate compound 5-[1-(4-isobutylphenyl)ethyl]-1,3,4-thiadiazol-2-amine (**19**) was synthesized by refluxing the thiosemicarbazide and 2-(4-isobutylphenyl)propanoic acid with an excess of phosphorus oxychloride [31]. The final compound 4-[[5-[1-(4-isobutylphenyl)ethyl]-1,3,4-thiadiazol-2-ylimino]methyl]-3-methylfuroxan (**20**) was synthesized by refluxing intermediate **19** and 4-formyl-3-methylfuroxan for 8 h. The compound 4-[5-[1-(4-isobutylphenyl)ethyl]-1,3,4-thiadiazol-2-ylcarbamoyl]-3-methylfuroxan (**21**) was prepared by treating **19** with 4-(chlorocarbonyl)-3-methylfuroxan. Synthesis of these compounds is outlined in Scheme 2. All the compounds showed characteristic peaks at appropriate δ values in ^{1}H NMR and ^{13}C NMR spectral data.

Scheme 1



Scheme 2



Nitric oxide release

The NO releasing properties of the synthesized furoxan derivatives of ibuprofen **9**, **11**, **14a**, **14b**, **15a**, **15b**, **16a**, **16b**, **17a**, **17b**, **18**, **20**, and **21** were assessed in vitro in both phosphate buffer of pH 7.4 and 0.1 N HCl of pH 1 by using Griess reagent. The reaction was carried out in the presence of L-cysteine as a source of the SH group. It was found that a reduced thiol group from endogenous L-cysteine could mediate the release of NO from furoxan derivatives. The amount of NO released from the tested compounds was measured relative to NO released from standard sodium nitrite solution and calculated as amount of NO released (mol/mol) [32] and listed in Table 1.

The results of measurement of NO release revealed that the tested compounds could only release NO at pH 7.4. Linker groups which attach furoxan with ibuprofen had significant effect on NO releasing capacity of compounds. The compound **14a** having an amide–ester linkage and a methyl group in the furoxan ring and compound **17b** having an amide–amide linkage and a phenyl group in the furoxan ring showed maximum nitric oxide releasing capacity. The compound **21** having amide linkage and a thiadiazole ring showed minimum nitric oxide releasing

Table 1 The amount of NO released from tested compounds in phosphate buffer (pH = 7.4)

Compound	Amount of NO released/mol mol^{-1} ± SEM	Compound	Amount of NO released/mol mol^{-1} ± SEM
9	0.48 ± 0.036	**16b**	0.39 ± 0.034
11	0.48 ± 0.108	**17a**	0.45 ± 0.060
14a	0.64 ± 0.042	**17b**	0.63 ± 0.050
14b	0.59 ± 0.030	**18**	0.39 ± 0.041
15a	0.58 ± 0.083	**20**	0.42 ± 0.033
15b	0.50 ± 0.177	**21**	0.27 ± 0.047
16a	0.41 ± 0.061		

capacity. Other compound of the series having an amide-Schiff base, 1,3,4-oxadiazole, 1,3,4-thiadiazole/Schiff base spacers showed moderate nitric oxide releasing capacity. On the other hand the tested compounds, were not able to release NO at pH 1, which suggest that NO donating furoxan derivatives of ibuprofen are weakly hydrolysed in the gastric lumen and this confirmed that the suggested gastro protective action of NO is mediated systemically [32].

Anti-inflammatory activity

All the synthesized compounds were screened for their anti-inflammatory activity by carrageenan induced rat paw edema method of Winter et al. The results revealed that compounds **14a**, **14b**, and **17b** exhibited excellent anti-inflammatory activity (86.10, 83.39, and 82.03 %, respectively), at an equimolar oral dose relative to 70 mg/kg ibuprofen which showed 74.40 % inhibition after 4 h. Compounds **11**, **15a**, and **15b** showed anti-inflammatory activity comparable to standard drug ibuprofen i.e. 74.07, 73.82, and 71.35 %, respectively. Compounds **16a**, **16b**, and **17a** also showed good anti-inflammatory activity, whereas compounds **9**, **18**, **20**, and **21** showed lesser degree of activity. It was interesting to note that although compound **17a** and **17b** have similar amide–amide linkage but there is difference in their anti-inflammatory activity. The high activity of compound **17b** having a phenyl group could be a consequence of their inhibitory effects on COX-2 along with the COX-1 enzyme. The poor activity of compound **18** (58.81 %) and **20** (58.13 %) may be due to the absence of carbonyl group which is required for binding with COX-1 enzyme. Thus it was concluded that furoxan derivatives of ibuprofen having amide-ester linkage (**14a**, **14b**) or ester linkage (**15a**, **15b**) or amide–amide linkage (**17b**) showed very good activity. These results indicated that the incorporation of NO donating furoxan moieties to ibuprofen has not only retained but showed enhanced anti-inflammatory activity (Table 1).

Analgesic activity

The compounds that showed anti-inflammatory activity higher than 65 % were further tested for their analgesic activity at an equimolar oral dose related to 70 mg/kg ibuprofen. Compounds **11**, **14a**, **14b**, **15a**, **15b**, **16a**, **16b**, **17a**, and **17b** showed analgesic activity ranging from 48.86 to 75.87 % whereas standard drug ibuprofen showed 73.45 % inhibition. It was noted that compound **14a** having amide–ester linkage with a methyl group at furoxan ring showing highest anti-inflammatory activity also exhibited highest analgesic activity (75.87 %), whereas in compound **14b** when methyl group was replaced by a phenyl group there was slight decrease in analgesic activity (70.95 %). The compound **17b** having an amide–amide linkage and a phenyl group at furoxan ring also showed good analgesic activity (72.12 %). Compound **16b** having an amide–schiff base linkage showed poor analgesic activity (48.86 %). The remaining compound showed moderate to good analgesic activity (Table 2).

Ulcerogenic activity

The compounds **11**, **14a**, **14b**, **15a**, **15b**, **17a**, and **17b** which showed significant anti-inflammatory and good analgesic activity were further tested for their acute ulcerogenicity. The compounds were tested at an equimolar oral dose relative to 210 mg/kg ibuprofen. The maximum reduction in ulcerogenic activity (0.166 ± 0.10) was found in compound **14a** having an ester amide linkage and a methyl group at the furoxan ring. Compound **17b** having an amide–amide linkage and a phenyl group at furoxan ring also showed reduced ulcerogenic potential (0.250 ± 0.11). Compounds **15a** and **11** having an ester and ester–ester linkage, respectively, showed ulcerogenicity equal to standard drug ibuprofen (0.666 ± 0.10) which may be due to in vivo hydrolysis of ester into carboxylic group resulting in topical irritating action. Rest of the tested compounds also showed better GI safety profile as compared to ibuprofen (Table 3). Thus, the results indicated that nitric oxide released from the ibuprofen–furoxan derivatives have a significant effect on decreased gastric toxicity (Fig. 4). The decreased severity index may be explained by the release of NO that increased mucosal blood flow resulting in enhanced mucosal resistance to ulceration and/or an enhanced ability of the NO donating

Table 2 Anti-inflammatory activity of furoxan derivatives using carrageenan-induced paw edema in rats

Compound	Anti-inflammatory activity % inhibition ± SEM[#]		Compound	Anti-inflammatory activity % inhibition ± SEM[#]	
	After 3 h	After 4 h		After 3 h	After 4 h
9	61.48 ± 0.86	64.74 ± 0.93[a]	**16a**	65.12 ± 1.10	67.80 ± 1.13[b]
11	68.59 ± 0.98	71.35 ± 1.35[c]	**16b**	63.30 ± 0.88	66.27 ± 0.88[a]
14a	81.48 ± 0.79	86.10 ± 0.67[a]	**17a**	64.96 ± 0.84	67.63 ± 0.61[a]
14b	79.83 ± 0.61	83.39 ± 0.86[a]	**17b**	77.52 ± 0.91	82.03 ± 0.90[a]
15a	70.91 ± 0.82	74.07 ± 0.97	**18**	55.21 ± 0.83	58.81 ± 0.82[a]
15b	70.66 ± 0.66	73.82 ± 0.70	**20**	55.04 ± 0.87	58.13 ± 1.16[a]
Ibuprofen	71.90 ± 0.66	74.40 ± 0.61	**21**	58.84 ± 0.98	61.52 ± 0.89[a]

[#] Relative to standard and data were analyzed by Student's *t* test for $n = 6$

[a] $p < 0.0001$

[b] $p < 0.001$

[d] $p < 0.5$

Table 3 Analgesic, ulcerogenic and lipid peroxidation activities of selected compounds

Compound	Analgesic activity			Ulcerogenic activity (severity index ± SEM)	nmol MDA content ± SEM/100 mg tissue
	Pre-treatment/normal 0 h (s)	Post-treatment/after 4 h (s)	% Inhibition		
11	1.72 ± 0.01	2.86 ± 0.02	67.62 ± 1.52[c]	0.666 ± 0.10	6.40 ± 0.09[b]
14a	1.69 ± 0.00	2.98 ± 0.01	75.87 ± 0.99[d]	0.166 ± 0.10[c]	3.67 ± 0.03[a]
14b	1.51 ± 0.01	2.59 ± 0.02	70.95 ± 0.75[c]	0.333 ± 0.10[c]	4.87 ± 0.05[a]
15a	1.77 ± 0.01	2.91 ± 0.02	63.80 ± 0.96[a]	0.666 ± 0.10	5.07 ± 0.05[a]
15b	1.64 ± 0.00	2.76 ± 0.03	68.71 ± 0.86[c]	0.583 ± 0.08	5.71 ± 0.11[a]
16a	1.60 ± 0.01	2.46 ± 0.01	53.24 ± 1.29[a]	–	–
16b	1.58 ± 0.01	2.34 ± 0.01	48.86 ± 1.17[a]	–	–
17a	1.52 ± 0.01	2.56 ± 0.01	68.27 ± 1.57[c]	0.500 ± 0.00[d]	5.29 ± 0.09[a]
17b	1.47 ± 0.00	2.54 ± 0.01	72.12 ± 0.61[d]	0.250 ± 0.11[c]	3.70 ± 0.07[a]
Control	–	–	–	0.000 ± 0.00	3.41 ± 0.08
Ibuprofen	1.60 ± 0.01	2.78 ± 0.01	73.45 ± 0.76	0.666 ± 0.10	6.95 ± 0.06

[#] Relative to standard and data were analyzed by Student's *t* test for $n = 6$

[a] $p < 0.0001$

[b] $p < 0.001$

[c] $p < 0.05$

[d] $p < 0.5$

furoxan derivatives to cross the gastric mucosal layer prior to the subsequent release of NO.

Lipid peroxidation activity

All the compounds screened for ulcerogenic activity were also analyzed for their lipid peroxidation. The lipid peroxidation was measured as nanomoles of malondialdehyde (MDA/100 mg) of gastric mucosa tissue. Ibuprofen exhibited high lipid peroxidation 6.95 ± 0.06 whereas control group showed 3.41 ± 0.081. It was found that all the furoxan derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation. The compound **14a** having amide–ester linkage and compound **17b** having amide–amide linkage showed maximum reduction 3.67 ± 0.03 and 3.70 ± 0.07, respectively, whereas compound **11** having ester–ester linkage showed minimum reduction 6.40 ± 0.09 nmol MDA/100 mg tissue. Thus, these studies showed that hybrid molecules have inhibited the induction of gastric mucosal lesion, and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa and due to the release of nitric oxide from furoxan derivatives of ibuprofen (Table 3).

Hepatotoxic and histopathological studies

The compounds **14a** and **17b**, furoxan derivatives of ibuprofen showing potent anti-inflammatory and analgesic activities with reduced ulcerogenicity and lipid peroxidation, were further studied for their hepatotoxic effect. Both compounds were studied for their effect on biochemical parameters (serum enzyme, total protein, and total albumin). Liver histopathological testing of these compounds was also carried out. As shown in Table 4, activities of liver enzyme SGOT, SGPT, alkaline phosphatase, total protein and total albumin where less than the standard drug ibuprofen. The histopathological studies of the liver samples do not show any significant pathological changes in comparison to standard drug ibuprofen (Fig. 3). No hepatocyte necrosis or degeneration was seen in any of the samples.

In silico ADME/molecular docking

Oral bioavailability is considered to play an important role for the development of bioactive molecules as therapeutic agents. Most of the compounds have more than 80 % human oral absorption. From all these pharmacokinetic parameters (Table 5), it is concluded that none of the compounds violated Lipinski's and Jorgensen's parameters, making them potentially promising drug candidates for the treatment of inflammation.

All the compounds are well fitted in the active sites of COX-I. The dG binding energy of compounds were calculated by Prime MM-GBSA, Maestro 10.1 and found in the range of 170.0 to −383.15 kJ/mol for COX-I. The docking scores and binding energy related with intermolecular interactions of the titled compounds and co-crystals ligand with the active site of COX-1 is summarized in Table 6.

Among all the titled compounds studied for COX-1, compound **14a** was found to be most potent and have high docking score. The compound **14a** also assumes favourable orientation within the COX-1 binding site. The binding mode of compound **14a** is exactly same as the co-crystal ligand (IBPA: 701) which was represented in 2D ligand interaction diagram (Supplementary Material). The docked pose of the compound **14a** showed the hydrogen bond interaction with amino acid ARG 120 (O….HN, 2.04 Å) and TYR 355 (O….HO, 2.13 Å). The ARG 83 (O….HN, 1.57 Å) makes an additional hydrogen bond with the oxygen atom of side chain of oxadiazole ring (Fig. 4). Interestingly **14a** also forms a π–π interaction between oxadiazole ring and same residue of ARG 120 (oxadiazole…C = 0. 3.97 Å) whereas co-crystal ligand IBPA: 701 forms three hydrogen bonds with ARG 120 (O…HN, 1.72 Å; O^-…HN, 1.70 Å) and TYR 355 (O…HO, 1.63 Å) (Fig. 4). The isobutyl group also occupied hydrophobic side-pocket of COX-1 surrounded by the residues LEU 352, MET 522, LEU 384, and PRO 86 diagram (Supplementary Material).

Further docked pose of compounds **17b** and **18** against COX-1 were also represented in Fig. 4. The oxygen atom of carbonyl group of compound **17b** formed strong hydrogen bonds with ARG 120 and ARG 83 approximately at distances of 1.86 Å (O…HN) and 1.90 Å (O^-…HO), respectively, as shown in Fig. 4. Furthermore, two π–π stacking and one π-cation with ARG 120 (phenyl…=C; oxadiazole…=C and N^+….=C) were also observed at distances of 4.49, 3.85, and 4.21 Å, respectively (Supplementary Material). It was observed that the carbonyl group in the molecule is important for hydrogen bond interaction with amino acids of COX-1, but there are compounds without having carbonyl group also formed hydrogen bond such as compound **18** and its docked pose was shown in Fig. 4. The compound **18** showed the hydrogen bond interaction with amino acid ARG 120 (N….HN, 1.72 Å) and TYR 355 (O….HO, 1.82 Å). Interestingly compound also formed a π-cation interaction between oxadiazole ring and same residue of ARG 120 (oxadiazole N^+…C = 4.40 Å) and π–π stacking with TYR 355 (phenyl…C = 5.31 Å). The superimposed structure of

Table 4 Effect of compounds **14a** and **17b** on serum enzymes, total proteins, and total albumin

Compound	SGOT/units $cm^{-3\#}$	SGPT/units $cm^{-3\#}$	Alkaline phosphatase[#]	Total protein g/100 $cm^{3\#}$	Total albumin g/100 $cm^{3\#}$
Control	149.76 ± 0.81	31.54 ± 1.46	32.50 ± 0.45	1.69 ± 0.012	1.59 ± 0.017
Ibuprofen	155.91 ± 1.49	44.84 ± 1.17	35.06 ± 0.47	1.88 ± 0.011	1.70 ± 0.014
14a	143.99 ± 1.36[a]	33.53 ± 1.20[a]	27.17 ± 0.51[a]	1.82 ± 0.021[c]	1.68 ± 0.018[d]
17b	146.03 ± 1.43[a]	25.99 ± 0.64[a]	23.14 ± 0.32[a]	1.69 ± 0.031[b]	1.57 ± 0.020[b]

[#] Relative to standard and data were analyzed by Student's *t* test for $n = 6$

[a] $p < 0.0001$

[b] $p < 0.001$

[c] $p < 0.05$

[d] $p < 0.5$

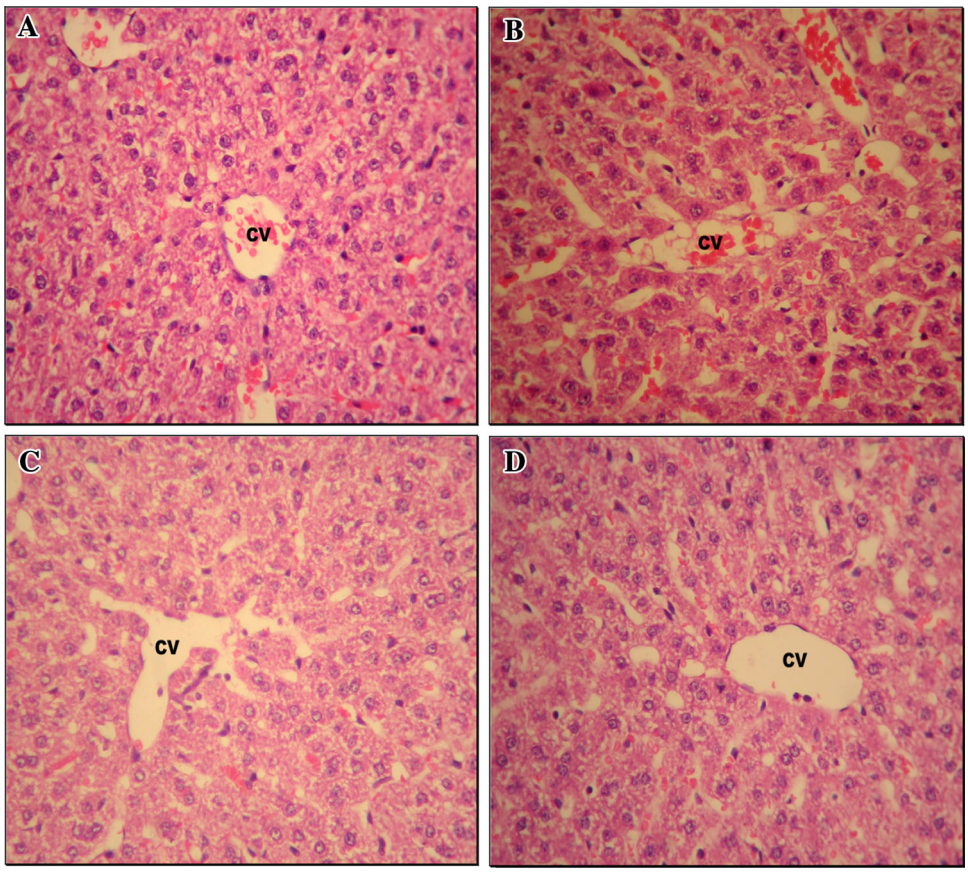


Fig. 3 **a** Control: section of liver showing normal arrangement of hepatocytes in the centrizonal area. *CV* Centrizonal area (×400). **b** Ibuprofen: section of liver showing evident centrizonal sinusoidal dilatation (×400). **c** Compound **14a**: section of liver showing mild centrizonal sinusoidal dilatation (×400). **d** Compound **17b**: section of liver showing mild centrizonal sinusoidal dilatation (×400)

compounds **14a** and **17b** with co-crystal ligand IBPA: 107 were also shown in Fig. 5. The binding orientations of title superimposed structures are same as co-crystal IBPA: 107.

Conclusions

Thirteen furoxan derivatives were synthesized by attaching furoxan moieties into the molecule of ibuprofen by replacing its carboxylic acid with various linker groups i.e., amide–amide, amide-ester, ester–ester, ester, amide–Schiff base, 1,3,4-oxadiazole/1,3,4-thiadiazole, and amide/Schiff base linkages. All the synthesized compounds were evaluated for their anti-inflammatory and nitric oxide releasing properties. Compounds **11**, **14a**, **14b**, **15a**, **15b**, **17a**, and **17b** showing high anti-inflammatory and analgesic activity were also tested for their ulcerogenic potential and lipid peroxidation. It was noted that compounds **14a** and **17b** showed maximum anti-inflammatory and analgesic activity. These compounds exhibited reduced gastric ulcerogenicity when compared with ibuprofen. It is assumable that masking the free carboxylic group of ibuprofen may have reduced its topical irritant action. Furthermore, pronounced gastroprotective activity of these compounds might attribute to the release of NO that promoted mucus secretion and increased mucosal blood flow resulting in enhance mucosal resistance to ulceration. Thus, the use of hybrid molecules containing NO releasing furoxan moieties looks as promising approach to improve the safety profile of NSAIDs. Furthermore, molecular docking study was performed to provide the binding patterns of the compound **14a**, **17b**, and **18** into the binding sites of COX-1 (PDB code: 1EQG) enzymes. The study showed that **14a** has favorable orientation within the COX-1 enzyme binding site and have a high docking score. In view of these studies, the compound **14a** could be a subject of further investigations for searching potential new anti-inflammatory molecules.

Experimental

All chemicals for synthesis were supplied by Merck (Darmstadt, Germany) and S.D. Fine Chemicals (Delhi, India). The melting points of newly synthesized compounds were determined in one-end open capillary tubes on a Hicon melting-point apparatus. IR spectra (KBr) were recorded on a Jasco FT-IR spectrometer. ^{1}H NMR spectra were measured on Bruker 300 MHz and Bruker Avance 400 MHz instruments. ^{13}C NMR spectra were measured on

Table 5 ADME parameters of all the synthesized compounds

Comp.	MW[a]	Dipole	SASA[b]	PSA[c]	Donor[d] HB	Accpt[e] HB	LogPo/w[f]	logS[g]	PCaco[h]	Human oral absorption	% Human oral absorption	Rule of five[i]	Rule of three[j]
Range	<500	1–12.5	300–1000	7–200	≤5	≤10	<5	−6.5 to 0.5	>500 great <25 poor	1 Low 2 Med 3 High	>80 % high <25 % poor	≤1	≤1
9	374.439	9.608	741.805	128.259	2	8	2.073	−4.831	116.284	3	76.053	0	0
11	376.408	6.624	624.96	114.897	0	7	2.721	−3.093	509.364	3	91.328	0	0
14a	375.424	8.264	741.407	125.559	1	7.5	2.384	−5.018	123.165	3	78.319	0	0
14b	437.494	8.141	842.857	125.188	1	7.5	3.756	−6.718	115.286	1	85.842	0	1
15a	318.372	6.332	602.183	88.594	0	5	3.156	−4.034	674.298	3	96.057	0	0
15b	380.443	6.076	691.731	87.201	0	5	4.561	−5.731	794.885	3	100	0	1
16a	330.386	11.429	673.476	105.965	1	5.5	3.15	−5.375	214.052	3	87.1	0	0
16b	392.457	11.647	682.197	104.373	1	5.5	4.114	−5.53	243.494	3	93.748	0	0
17a	346.385	7.045	681.058	131.725	0.5	6.5	2.612	−5.217	103.781	3	78.327	0	0
17b	408.456	6.828	770.283	129.863	0.5	6.5	4.023	−6.83	135.748	1	88.67	0	1
18	328.37	6.178	641.547	92.835	0	5.5	2.89	−4.771	310.573	3	88.473	0	0
20	371.456	6.555	708.566	96.908	0	6	3.416	−5.495	199.751	3	88.121	0	0
21	387.456	5.325	711.183	122.081	1	7.5	2.623	−5.513	94.749	3	77.68	0	0

[a] Molecular weight of the molecule

[b] Total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius

[c] Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms

[d] Estimated number of hydrogen bonds that would be donated by the compound to water molecules in an aqueous solution

[e] Estimated number of hydrogen bonds that would be accepted by the compound from water molecules in an aqueous solution

[f] Predicted octanol/water partition coefficient

[g] Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid

[h] Predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells are a model for the gut-blood barrier

[i] Lipinski's violations

[j] Jorgensen's violations

Bruker Avance-400 instrument (100 MHz) with complete proton decoupling. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Elemental analyses (C, H, and N) were undertaken with a CHNS Vario EL III (Elementar Analysen Systeme GmbH, Germany) and the results are within ±0.4 % of theoretical values. The reactions were monitored by silica gel-GF coated aluminum plates and visualized by iodine vapors and ultraviolet light as visualizing agents. Mass (MS) spectral data were recorded on a Jeol SR-102 (FAB) spectrometer. Removal of solvents was carried out at reduced pressure using a rotary evaporator. Compounds **1** [33], **2** [34], **3** [35], **4** [36], **5a**, **5b** [37] and **6a**, **6b** [37] were synthesized according to methods reported in literature. Intermediate compounds **8**, **10**, and **13** were synthesized according to literature method by slightly modifying reaction conditions [38].

4-[2-[2-(4-Isobutylphenyl)propanamido]ethylcarbamoyl]-3-methylfuroxan (**9**, $C_{19}H_{26}N_4O_4$)

Ethylene diamine (0.017 mol) and 50 cm^3 glacial acetic acid were slowly mixed together resulting in the formation of a white solid which dissolved gradually at room temperature to form a colorless solution. 2-(4-Isobutylphenyl)propionyl chloride (**7**, 0.01 mol) was then added drop wise to this solution with continuous stirring at room temperature. The mixture was stirred for 2 h, then diluted with water and the pH was adjusted to 12 with NaOH solution. The solution was filtered to remove some white precipitate and the filtrate was extracted with CH_2Cl_2. The organic layer was separated and washed with saturated NaCl solution and water. It was dried and distilled under reduced pressure to afford the intermediate *N*-(2-aminoethyl)-2-(4-isobutylphenyl)propanamide (**8**) which was used immediately for the next step of the

reaction. A solution of 4-(chlorocarbonyl)-3-methylfuroxan (**6a**, 0.01 mol) in 5 cm^3 anhydrous THF in the presence of 0.5 cm^3 triethylamine was added drop wise at room temperature to a stirred solution of intermediate **8** in 20 cm^3 CH_2Cl_2. The stirring was continued for 6 h and reaction mixture was then poured into ice cold water. The organic layer was separated, washed with water, dried and distilled under reduced pressure. The product thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **9**. Yield 25 %; m.p.: 98–100 °C; IR (KBr): $\bar{\nu} = 3299, 3272$ (NH), 1676, 1656 (C=O), 1593 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (d, 6H, $J = 6.3$ Hz, $(CH_3)_2CH$), 1.45 (d, 3H, $J = 7.2$ Hz, CH_3-CH), 1.86 (m, 1H, $(CH_3)_2CH$-CH_2), 2.35 (s, 3H, CH_3), 2.41 (d, 2H, $J = 7.2$ Hz, $(CH_3)_2CH$-CH_2), 3.26 (t, 2H, $J = 5.1$ Hz, CH_2), 3.47 (t, 2H, $J = 5.1$ Hz, CH_2), 3.56 (q, 1H, $J = 7.2$ Hz, CH_3CH), 5.83 (bs, 1H, CH_3-CH-CONH), 7.05–7.22 (m, 4H, ArH), 7.64 (bs, 1H, CONHfuroxan) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 176.12$, 157.82, 150.63, 141.01, 138.11, 129.71, 127.27, 112.36, 46.68 (CH), 44.96 (CH_2), 40.34 (CH_2), 39.17 (CH_2), 30.18 (CH), 22.41 ((CH_3)$_2$), 18.41 (CH_3), 8.48 (CH_3) ppm; MS: $m/z = 375$ (M^++1).

Table 6 Docking scores and binding energy of compounds at the active sites of COX-1

Compound	COX-I (PDB ID: 1EQG)	
	XP Docking scores	dG Binding energy (kJ/mol)
9	−9.864	−74.537
11	−10.683	−79.256
14a	−11.634	−76.938
14b	−11.055	−76.692
15a	−8.700	−78.105
15b	−10.336	−91.515
16a	−7.012	−79.904
16b	−8.316	−68.081
17a	−9.364	−40.601
17b	−8.891	−52.857
18	−9.467	−79.256
20	−8.510	−66.343
21	−8.579	−71.044
IBPA:701	−9.868	−103.143

4-[2-[2-(4-Isobutylphenyl)propanoyloxy]ethoxycarbonyl]-3-methylfuroxan (**11**, $C_{19}H_{24}N_2O_6$)

Ethylene glycol (0.015 mol) and 20 cm^3 dry CH_2Cl_2 were slowly mixed together at room temperature. 2-(4-Isobutylphenyl)propionyl chloride (**7**, 0.01 mol) in 5 cm^3 dry CH_2Cl_2 was added drop wise to this mixture with continuous stirring in ice cold condition. The stirring was continued for 5 h and progress of the reaction was monitored by TLC using 30 % ethyl acetate-hexane solvent system. After completion, it was washed twice

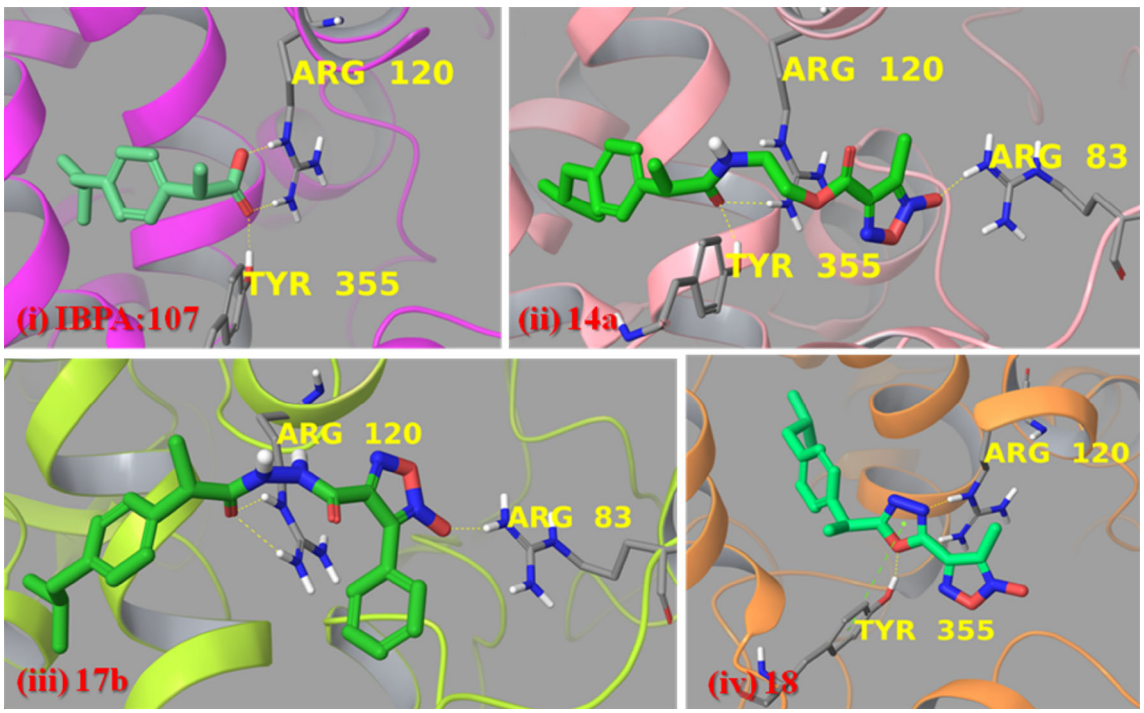


Fig. 4 Docked pose of compounds (*i*) co-crystal IBPA: 701, (*ii*) **14a**, (*iii*) **17b**, and (*iv*) **18** represented as tube in the binding site of COX-I showing hydrogen bond interaction (*yellow dash lines*) with ARG 120, TYR 355 and ARG 83. The amino acids are represented as thin tube

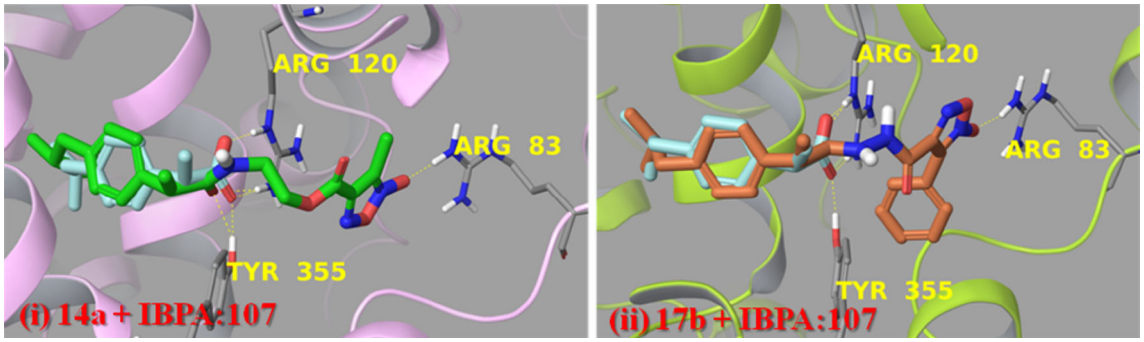


Fig. 5 Superimpose docked 3D image and binding orientation of compound (*i*) **14a** (*green color*) with co-crystal ligand (IBPA: 701, *turquoise color*) (*ii*) **17b** (*orange color*) with co-crystal ligand (IBPA:701, *turquoise color*) in COX-I site

with water, dried, and distilled under reduced pressure to afford the intermediate 2-hydroxyethyl 2-(4-isobutylphenyl)propionate (**10**) which was used immediately for next step of the reaction. A solution of **6a** (0.01 mol) in 5 cm^3 anhydrous THF in the presence of 0.5 cm^3 triethylamine was added drop wise at room temperature to a stirred solution intermediate **10** in 30 cm^3 dry CH_2Cl_2. The stirring was continued for 6 h and the reaction mixture was poured into ice cold water. The organic layer was separated, washed twice with water, dried, and distilled under reduced pressure. The pale yellow residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **11** as a pale yellow semisolid material. Yield 30 %; IR (KBr): $\bar{\nu}$ = 1755, 1748 (C=O), 1610 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.88 (d, 6H, J = 6.6 Hz, $(CH_3)_2CH$), 1.47 (d, 3H, J = 7.2 Hz, CH_3-CH), 1.83 (m, 1H, $(CH_3)_2CH$-CH_2), 2.25 (s, 3H, CH_3), 2.42 (d, 2H, J = 7.2 Hz, $(CH_3)_2CH$-CH_2), 3.73 (t, 2H, J = 5.4 Hz, CH_2), 4.41 (t, 2H, J = 5.4 Hz, CH_2), 4.54 (q, 1H, J = 7.2 Hz, CH_3CH), 7.02–7.21 (m, 4H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 175.10, 163.22, 152.63, 140.11, 137.21, 129.73, 126.21, 110.30, 56.62 (CH_2), 54.96 (CH_2), 41.54 (CH), 40.36 (CH_2), 29.98 (CH), 22.38 (($CH_3)_2$), 17.31 (CH_3), 8.70 (CH_3) ppm; MS: m/z = 378 (M^++1).

4-[2-[2-(4-Isobutylphenyl)propanamido]ethoxycarbonyl]-3-methylfuroxan (**14a**, $C_{19}H_{25}N_3O_5$)

Ethyl 2-(4-isobutylphenyl)propionate (**12**) was added to ethanolamine (0.02 mol) in 30 cm^3 CH_2Cl_2 with stirring. The mixture was refluxed for 8 h, cooled at room temperature and the solvent was removed by distillation at reduced pressure. Pale brown oil was obtained which became a sticky solid on cooling in the refrigerator. Subsequent grinding of the sticky solid with acetone and diethyl ether gave a white solid which was the intermediate *N*-(2-hydroxyethyl)-2-(4-isobutylphenyl)propanamide (**13**). The compound thus obtained was recrystallized with petroleum ether. A solution of **6a** (0.01 mol) in 5 cm^3 anhydrous THF was added drop wise to a stirred solution of intermediate **13** in 30 cm^3 dry CH_2Cl_2 in the presence of triethylamine (0.01 mol). The stirring was continued for 5 h, after which the reaction mixture was poured into ice cold water. The organic layer was separated, washed with water, dried and distilled under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **14a**. Yield 32 %; m.p.: 94–96 °C; IR (KBr): $\bar{\nu}$ = 3308 (NH), 1755 (C=O_{ester}), 1655 (C=O_{amide}), 1604 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.87 (d, 6H, J = 6.6 Hz, $(CH_3)_2CH$), 1.48 (d, 3H, J = 7.2 Hz, CH_3-CH), 1.82 (m, 1H, $(CH_3)_2CH$-CH_2), 2.32 (s, 3H, CH_3), 2.43 (d 2H, J = 7.2 Hz $(CH_3)_2CH$-CH_2), 3.52 (t, 2H, J = 5.1 Hz, CH_2), 3.61 (q, 1H, J = 7.2 Hz, CH_3CH), 4.44 (t, 2H, J = 5.1 Hz, CH_2), 5.69 (bs, 1H, CH_3-CH-CONH), 7.06–7.20 (m, 4H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 174.96, 157.90, 148.82, 140.97, 138.09, 129.68, 127.32, 112.22, 65.30 (OCH_2), 46.67 ($NHCH_2$), 44.80 (CH), 38.28 (CH_2), 30.18 (CH), 22.35 (($CH_3)_2$), 18.56 (CH_3), 8.51 (CH_3) ppm; MS: m/z = 376 (M^++1).

4-[2-[2-(4-Isobutylphenyl)propanamido)ethoxy]carbonyl]-3-phenylfuroxan (**14b**, $C_{24}H_{27}N_3O_5$)

It was prepared by following the procedure of compound **14a** by taking the starting material 4-(chlorocarbonyl)-3-methyl furoxan (**6b**). The residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **14b**. Yield 28 %; m.p.: 56 °C; IR (KBr): $\bar{\nu}$ = 3318 (NH), 1758 (C=O_{ester}), 1650 (C=O_{amide}), 1601 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.85 (d, 6H, J = 6.6 Hz, $(CH_3)_2CH$), 1.50 (d, 3H, J = 7.2 Hz, CH_3CH), 1.79 (m, 1H, $(CH_3)_2CH$-CH_2), 2.40 (d, 2H, J = 7.2 Hz, $(CH_3)_2CH$-CH_2), 3.52–3.61 (3H, q of CH_3CH and t of CH_2), 4.34 (t, 2H, J = 5.4 Hz, CH_2), 5.72 (bs, 1H, CH_3-CH-CONH), 7.03–7.15 (m, 4H, ArH), 7.40–7.60 (m, 5H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 174.89, 169.57, 140.81, 138.20, 133.17, 130.10,

129.72, 129.65, 129.63, 128.40, 127.39, 63.36 (OCH_2), 46.70 (NCH_2), 45.11 (CH), 39.36 (CH_2), 30.14 (CH), 22.38 (($CH_3)_2$), 18.23 (CH_3) ppm; MS: *m/z* = 438 (M^++1).

4-[[2-(4-Isobutylphenyl)propanoyloxy]methyl]-3-methylfuroxan (**15a**, $C_{17}H_{22}N_2O_4$)

4-(Hydroxymethyl)-3-methylfuroxan (0.01 mol) was dissolved in 20 cm^3 dry toluene and 0.1 cm^3 triethylamine was added to it. To this solution, 2-(4-isobutylphenyl)propionyl chloride (**7**) in 5 cm^3 dry toluene was added drop wise with continuous stirring. The reaction mixture was further stirred for 4 h and then poured into ice cold water. The organic layer was separated, washed with water, dried, and distilled under reduced pressure. The crude residue, thus, obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **15a** as a pale yellow oily material. Yield 52 %; IR (KBr): $\bar{\nu}$ = 1743 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.81 (d, 6H, *J* = 6.3 Hz, (CH_3)$_2$CH), 1.42 (d, 3H, *J* = 7.2 Hz, CH_3-CH), 1.76 (m, 1H, (CH_3)$_2$C*H*-CH_2), 2.10 (s, 3H, CH_3), 2.35 (d, 2H, *J* = 7.2 Hz, (CH_3)$_2$-CH-CH_2), 3.64 (q, 1H, *J* = 7.2 Hz, CH_3CH), 4.51 (s, 2H, OCH_2), 7.01–7.52 (m, 4H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 172.96, 149.52, 142.17, 139.18, 133.57, 128.61, 126.43, 54.47 (OCH_2), 40.80 (CH), 38.20 (CH_2), 29.06 (CH), 21.39 (($CH_3)_2$), 15.47 (CH_3), 8.37 (CH_3) ppm; MS: *m/z* = 319 (M^++1).

4-[[2-(4-Isobutylphenyl)propanoyloxy]methyl]-3-phenylfuroxan (**15b**, $C_{22}H_{24}N_2O_4$)

It was prepared by following the procedure of compound **15a** by taking the starting material 4-(chlorocarbonyl)-3-phenyl furoxan (**6b**). The crude residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **15b** as a pale yellow solid product. Yield 43 %; m.p.: 48 °C; IR (KBr): $\bar{\nu}$ = 1750 (C=O), 1601 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.80 (d, 6H, *J* = 6.6 Hz, (CH_3)$_2$CH), 1.41 (d, 3H, *J* = 7.2 Hz, CH_3-CH), 1.75 (m, 1H, (CH_3)$_2$C*H*-CH_2), 2.34 (d, 2H, *J* = 7.2 Hz, (CH_3)$_2$CH-CH_2), 3.63 (q, 1H, *J* = 7.2 Hz, CH_3CH), 4.49 (s, 2H, OCH_2), 6.99–7.49 (m, 9H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 173.78, 140. 87, 137.02, 136.74, 131.94, 131.24, 129.58, 129.42, 129.29, 127.62, 127.30, 127.16, 54.51 (OCH_2), 45.05 (CH), 32.69 (CH_2), 30.20 (CH), 22.41 (($CH_3)_2$), 18.14 (CH_3) ppm; MS: *m/z* = 381 (M^++1).

4-[[2-[2-(4-Isobutylphenyl)propanoyl]hydrazono]methyl]-3-methylfuroxan (**16a**, $C_{17}H_{22}N_4O_3$)

2-(4-Isobutylphenyl)propionic acid hydrazide (0.01 mol) and 4-formyl-3-methylfuroxan (0.01 mol) was dissolved in 30 cm^3 ethanol. The reaction mixture was heated until clear solution was obtained and few drops of conc. sulfuric acid were added to it. The solution was refluxed for 6-8 h. Progress of the reaction was monitored by TLC using a mixture of ethyl acetate and *n*-hexane (2:3). After completion, reaction mixture was poured into ice cold water. The crude residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **16a** as a solid product. Yield 52 %; m.p.: 70–72 °C; IR (KBr): $\bar{\nu}$ = 3201 (NH), 1682 (C=O), 1594 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.81 (d, 6H, *J* = 6.6 Hz, (CH_3)$_2$CH), 1.46 (d, 3H, *J* = 7.2 Hz, (CH_3)$_2$CH-CH_2), 3.64 (q, 1H, *J* = 7.2 Hz, CH_3CH), 6.99–7.12 (m, 4H, ArH), 8.22 (s, 1H, N=CH), 10.53 (bs, 1H, CONH) ppm; ^{13}C NMR ($CDCl_3$): δ = 175.59, 140.54, 140.10, 139.62, 134.09, 130.23, 127.02, 111.34, 44.37 (CH), 43.58 (CH_2), 31.28 (CH), 22.76 (($CH_3)_2$), 18.12 (CH_3), 8.35 (CH_3) ppm; MS: *m/z* = 331 (M^++1).

4-[[2-[2-(4-Isobutylphenyl)propanoyl]hydrazono]methyl]-3-phenylfuroxan (**16b**, $C_{22}H_{24}N_4O_3$)

It was prepared by following the procedure of compound **16a** by taking the starting material 4-formyl-3-phenylfuroxan. The crude residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **16b** as a pale yellow oily material. Yield 38 %; IR (KBr): $\bar{\nu}$ = 3213 (NH), 1692 (C=O), 1583 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.90 (d, 6H, *J* = 6.3 Hz, (CH_3)$_2$CH), 1.51 (d, 3H, *J* = 7.2 Hz, CH_3-CH), 1.85 (m, 1H, (CH_3)$_2$C*H*-CH_2), 2.45 (d, 2H, *J* = 7.2 Hz, (CH_3)$_2$CH-CH_2), 3.71 (q, 1H, *J* = 7.2 Hz, CH_3CH), 7.12–761 (m, 9H, ArH), 8.01 (s, 1H, N=CH), 10.01 (bs, 1H, CONH) ppm; ^{13}C NMR ($CDCl_3$): δ = 179.91, 140.86, 137.07, 133.81, 131.95, 131.01, 130.67, 129.45, 128.97, 127.62, 127.08, 127.38, 45.05 (CH), 44.08 (CH_2), 30.20 (CH), 22.41 (($CH_3)_2$), 18.18 (CH_3) ppm; MS: *m/z* = 393 (M^++1).

4-[2-[2-(4-Isobutylphenyl)propanoyl]hydrazinecarbonyl]-3-methylfuroxan (**17a**, $C_{17}H_{22}N_4O_4$)

2-(4-Isobutylphenyl)propionic acid hydrazide (0.01 mol)) was dissolved in 20 cm^3 dry toluene and triethylamine (0.01 mol) was added to it. Intermediate compound **6a** in 5 cm^3 dry toluene was added to this solution drop wise with continuous stirring. The reaction mixture was further stirred for 3 h at room temperature and then was poured into ice cold water. The organic layer was separated, washed with water, dried, and distilled under reduced pressure. The residue, thus, obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **17a**. Yield: 64 %; m.p.: 86–88 °C; IR (KBr): $\bar{\nu}$ = 3237, 3231 (NH), 1703, 1680 (C=O), 1612 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 0.91 (d, 6H, *J* = 6.6 Hz, (CH_3)$_2$CH), 1.56 (d, 3H, *J* = 7.2 Hz, CH_3-CH), 1.81 (m, 1H, (CH_3)$_2$C*H*-CH_2), 2.35 (s, 3H, CH_3), 2.45 (d, 2H, *J* = 7.2 Hz, (CH_3)$_2$CH-CH_2), 3.67 (q, 1H,

$J = 7.2$ Hz, CH_3CH), 7.09–7.26 (m, 4H, ArH), 7.85 (bs, 1H, CONH), 8.71 (bs, 1H, CONH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 173.52$, 162.41, 147.45, 141.23, 133.37, 129.93, 128.30, 111.23, 41.53 (CH), 39.76 (CH_2), 29.26 (CH), 21.27 (($CH_3)_2$), 16.75 (CH_3), 8.21 (CH_3) ppm; MS: $m/z = 347$ (M^++1).

4-[2-[2-(4-Isobutylphenyl)propanoyl]hydrazinecarbonyl]-3-phenylfuroxan (**17b**, $C_{22}H_{24}N_4O_4$)

It was prepared by following the procedure of compound **17a** by taking the starting material 4-(chlorocarbonyl)-3-phenylfuroxan (**6b**). The residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **17b**. Yield 64 %; m.p.: 93–95 °C; IR (KBr): $\bar{\nu} = 3232$, 3231 (NH), 1687, 1682 (C=O), 1597 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 0.88$ (d, 6H, $J = 6.6$ Hz, ($CH_3)_2$CH), 1.51 (d, 3H, $J = 7.2$ Hz, CH_3-CH), 1.83 (m, 1H, $(CH_3)_2CH$-CH_2), 2.43 (d, 2H, $J = 7.2$ Hz, $(CH_3)_2$CH-CH_2), 3.75 (q, 1H, $J = 7.2$ Hz, CH_3CH), 7.10–8.10 (m, 9H, ArH), 9.16 (bs, 1H, CONH), 9.41 (bs, 1H, CONH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 174.78$, 164.78, 149.38, 142.33, 138.37, 133.25, 131.23, 129.32, 129.04, 128.82, 127.96, 127.23, 45.25 (CH), 44.53 (CH_2), 29.62 (CH), 21.41 (($CH_3)_2$), 16.18 (CH_3) ppm; MS: $m/z = 409$ (M^++1).

4-[5-[1-(4-Isobutylphenyl)ethyl]-1,3,4-oxadiazol-2-yl]-3-methylfuroxan (**18**, $C_{17}H_{20}N_4O_3$)

2-(4-Isobutylphenyl)propionic acid hydrazide (0.01 mol) was dissolved in 10 cm^3 phosphorus oxychloride and to it was added 4-carboxy-3-methylfuroxan (**5a**, 0.01 mol). The reaction mixture was refluxed for 5 h, cooled to room temperature, and poured on to crushed ice. The solution was neutralized with sodium bicarbonate (10 %), an oily compound was obtained which was separated, washed with water and dried. It was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **18** as an oily pale yellow material. Yield 38 %; IR (KBr): $\bar{\nu} = 1595$ (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 0.80$ (d, 6H, $J = 6.6$ Hz, ($CH_3)_2$CH), 1.54 (d, 3H, $J = 7.2$ Hz, CH_3-CH), 1.82 (m, 1H, $(CH_3)_2CH$-CH_2), 2.36 (d, 2H, $J = 7.2$ Hz, $(CH_3)_2$CH-CH_2), 2.44 (s, 3H, CH_3), 4.37 (q, 1H, $J = 7.2$ Hz, CH_3CH), 7.04–7.18 (m, 4H, ArH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 170.64$, 155.31, 144.71, 141.53, 136.37, 129.81, 127.05, 111.17, 44.99 (CH), 37.09 (CH_2), 30.19 (CH), 22.36 (($CH_3)_2$), 19.40 (CH_3), 8.77 (CH_3) ppm; MS: $m/z = 329$ (M^++1).

4-[5-[1-(4-Isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-ylimino]methyl]-3-methylfuroxan (**20**, $C_{18}H_{21}N_5O_2S$)

An equimolar amount of 5-[1-(4-isobutylphenyl)ethyl]-1,3,4-thiadiazol-2-amine (**19**, 0.01 mol) and 4-formyl-3-methylfuroxan (**1**) was dissolved in 25 cm^3 ethanol. The reaction mixture was heated until clear solution was obtained. Few drops of conc. sulfuric acid were added to the solution and it was refluxed for 8 h. The reaction mixture was cooled at room temperature and poured into crushed ice. An oily compound was obtained which was separated, washed with water and dried. It was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **20** as an oily light yellow material. Yield 46 %; IR (KBr): $\bar{\nu} = 1612$ (C=N), 789 (C-S-C) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 0.88$ (d, 6H, $J = 6.6$ Hz, ($CH_3)_2$CH), 1.49 (d, 3H, $J = 7.2$ Hz, CH_3-CH), 1.81 (m, 1H, $(CH_3)_2CH$-CH_2), 2.42 (s, 3H, CH_3), 2.44 (d, 2H, $J = 7.2$ Hz, $(CH_3)_2$CH-CH_2), 4.51 (q, 1H, $J = 7.2$ Hz, CH_3CH), 7.07–7.26 (m, 5H, 4 ArH and N=CH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 170.22$, 167.34, 154.56, 147.42, 139.25, 137.65, 128.78, 127.31, 111.43, 45.23 (CH), 38.54 (CH_2), 29.37 (CH), 22.56 (($CH_3)_2$), 20.34 (CH_3), 8.78 (CH_3) ppm; MS: $m/z = 372$ (M^++1).

4-[5-[1-(4-Isobutylphenyl)ethyl)-1,3,4-thiadiazol-2-yl]carbamoyl]-3-methylfuroxan (**21**, $C_{18}H_{21}N_5O_3S$)

Intermediate compound **19** (0.01 mol) was dissolved in 25 cm^3 dry toluene and triethylamine (0.01 mol) was added to it. Compound **6a** in 5 cm^3 dry toluene was added to the solution drop wise with continuous stirring. The reaction mixture was further stirred for 3 h and progress of the reaction was monitored by TLC using TEF (5:4:1) solvent system. After completion, it was poured into ice cold water. The oily compound thus obtained was separated, washed with water, and dried. It was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound **21** as an oily light yellow material. Yield 45 %; IR (KBr): $\bar{\nu} = 3022$ (NH), 1701 (C=O), 1600 (C=N), 794 (C-S-C) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 0.88$ (d, 6H, $J =$ Hz, ($CH_3)_2$CH), 1.56 (d, 3H, $J = 7.2$ Hz, CH_3-CH), 1.80 (m, 1H, $(CH_3)_2CH$-CH_2), 2.38 (s, 3H, CH_3), 2.45 (d, 2H, $J = 7.2$ Hz, $(CH_3)_2$CH-CH_2), 4.50 (q, 1H, $J = 7.2$ Hz, CH_3CH), 7.00–7.37 (m, 4H, ArH), 12.54 (bs, 1H, CONH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 172.12$, 168.13, 158.91, 147.82, 139.12, 137.23, 128.57, 126.31, 111.21, 44.83 (CH), 38.65 (CH_2), 29.32 (CH), 22.05 (($CH_3)_2$), 20.11 (CH_3), 8.01 (CH_3) ppm; MS: $m/z = 388$ (M^++1).

Measurement of nitric oxide release

To a solution of furoxan derivatives of ibuprofen (20 mm^3) in dimethyl sulfoxide (DMSO) was added to 2 cm^3 of 1:1 (v/v) mixture of 50 mM phosphate buffer (pH 7.4) with MeOH, containing 5×10^{-4} M L-cysteine. The final concentration of drug was 10^{-4} M. After 1 h at 37 °C, 1 cm^3 of the reaction mixture was treated with 250 mm^3 of Griess reagent [4 g sulfanilamide, 0.2 g naphthylene diamine dihydrochloride, 10 cm^3 85 % phosphoric acid, in distilled

water (final volume 100 cm^3)]. After 10 min at room temperature the absorbance was measured at 540 nm. Sodium nitrite standard solutions (10–80 nmol/cm^3) were used to construct the calibration curve. The same procedure was repeated using different solutions of the test compounds under the same conditions using 0.1 N HCl of pH 1 instead of phosphate buffer of pH 7.4. The results were expressed as the percentage of NO released relative to a theoretical maximum release of 1 mol NO/mol of test compounds [39].

Pharmacology

The synthesized compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation, hepatotoxic and histopathological properties. The Wister rats and albino mice used in the present study were housed and kept in accordance with the Hamdard University Animal Care Unit, which applies the guidelines and rules laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the test compounds and standard drug were administered in the form of solution (0.5 % w/v carboxymethyl cellulose as a vehicle) by an oral route. Each group consisted of six animals. All the animals were procured from the CPCSEA and maintained in colony cages at 25 ± 2 °C, relative humidity of 45–55 %, under a 12 h light and dark cycle and were fed a standard animal feed. All the animals were climatised for a week before use. The anti-inflammatory activity of the test compounds were compared with the control. The analgesic, ulcerogenic, and lipid peroxidation activities were compared with the standard drug ibuprofen. Data were analyzed by student's t test for $n = 6$.

Anti-inflammatory activity

Anti-inflammatory activity was carried out by the carrageenan induced paw edema test in Wistar albino rats by Winter et al. method [40]. The standard drug, ibuprofen and test compounds were given orally (70 mg/kg body weight) as a suspension using 0.5 % w/v carboxymethyl cellulose as a vehicle. One hour later foot paw edema was induced by injecting 0.1 cm^3 of 1 % carrageenan subcutaneously into the planter portion of the right hind paw of each rat. Initial paw volume was measured immediately by mercury plethysmometer. The paw volume was again measured after the time interval of 3 and 4 h. The percentage inhibition of inflammation was calculated for the standard drug and other test compounds and comparison was made. The percentage inhibition of inflammation was calculated according to the formula

$$\% \text{ anti-inflammatory activity} = 100 \times (1 - V_t/V_c)$$

where V_t and V_c are the volume edema in test compounds and control groups, respectively.

Analgesic activity

Analgesic activity was evaluated by tail immersion method [41] using Swiss albino mice (25–30 g) of either sex selected by random sampling technique. The standard drug, ibuprofen and test compounds were administered orally (70 mg/kg body weight) as a suspension using 0.5 % w/v carboxymethyl cellulose as a vehicle. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5 °C. The time in seconds for tail withdrawal from the water was taken as the reaction time with a cut of time of immersion, set at 10 s for both control as well as treated groups of animals. The reaction time was measured before and after 4 h interval of the administration of test compounds and standard drugs.

Acute ulcerogenicity

Acute ulcerogenesis test was performed according to Cioli et al. [42] using Wistar rats (180–200 g) of either sex. The animals were divided into various groups each group consisting of six rats. All the rats were fasted for 24 h with free access to water. The control groups of animals were administered, 0.5 % CMC solution intraperitoneally. One group was administered with standard drug ibuprofen orally in a dose of 210 mg/kg once a daily for 3 days. The remaining group of animals was administered with test compounds through the same route. The animals were immediately fed and kept for 17 h after dose administration. After 17 h they were killed and dissected for the estimation of ulcerogenic activity. The stomach was dissected out and washed with running water and opened along the greater curvature and carefully observed with magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streak, 2.0: ulcers >3 but ≤5, 3.0: ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. [43]. After the evaluation of stomach for ulcers the gastric mucosa of glandular portion was scrapped, weighed (100 mg) and homogenized in pestle and mortar and homogenate was prepared in 1.8 cm^3 of ice cold 1.15 % KCl solution. The

homogenate was supplemented with 0.2 cm^3 of 8.1 % sodium dodecyl sulfate (SDS), 1.5 cm^3 of acetate buffer, and 1.5 cm^3 of 0.8 % thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 60 min on boiling water bath then extracted with a mixture of *n*-butanol : pyridine (15:1, v/v; 5 cm^3) by shaking vigorously for 1 min and kept in ice for 2 min. Organic layer of reaction mixture were centrifuged at 3000 rpm for 10 min and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue.

Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150–200 g. The animals were divided into three groups of six rats each. Group I was kept as control and received only vehicle (0.5 % w/v solution of CMC in water), while group II and III received compound **14a** and **17b,** respectively, in 0.5 % w/v solution of CMC in water for 15 days. After the treatment (15 days) blood was obtained from all the groups of rats by puncturing the retro-orbital plexus. Blood samples were allowed to clot for 45 min at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters. Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by a reported method [44]. The alkaline phosphatase, total protein and total albumin were measured according to reported procedures [45, 46]. Histopathological studies were also carried out by reported method [47]. The rats were killed under light ether anesthesia after 24 h of the last dosage; the liver was removed and washed with normal saline, and stored in formalin solution. Sections of 5–6 microns thickness were cut, stained with haematoxylin and eosin, and then studied under an electron microscope.

In silico ADME/molecular docking

It was noticed that too many compounds were terminated in clinical development because of unsatisfactory pharmacokinetics profile. It became necessary that chemists needed to address this pharmacokinetic parameter during optimization of compound. To check the criteria of synthesized compounds for desirable pharmacokinetic properties, a QikProp study for prediction of ADME properties of the derivatives was performed using Schrodinger Maestro 10.1, running on Linux 64 operating system. Binding energy estimation can be used to calculate ligand binding energies and ligand strain energies for a set of ligand and a single receptor (COX-1). The ligands and the receptors were prepared by using LigPrep and Protein preparation wizard.

To predict good anti-inflammatory activity on a basis of structure, a molecular docking studies of all the compounds were carried out by taking X-ray crystal structure data to establish the interaction of the synthetic compounds with COX-1 (PDB code: 1EQG) Molecular docking studies mainly involve selection and preparation of appropriate protein, grid generation, ligand preparation followed by docking and its analysis. The docking score, binding free energy, and hydrogen bonds and π–π interaction formed with the surrounding amino acids are used to predict their binding affinities and proper alignment of these compounds at the active site of the COX-1 enzyme.

Acknowledgments The authors are grateful to University Grants Commission (UGC) New Delhi for financial support F. No. 36-1-08/2008(SR). Authors are also thankful Central Instrumentation Facility (CIF) Faculty of Pharmacy for spectral analysis of compounds and to Dr. Ambrish Kumar Tiwari, In-charge animal house, Jamia Hamdard, for providing animals. The authors are also thankful to Dr. A. Mukharjee, MD, Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, for carrying out histopathological studies.

References

1. Singh G, Triadafilopoulos G (1999) J Rheumatol Suppl 56:18
2. Garner A (1992) Scand J Gastroenterol Suppl 193:83
3. Lichtenstein DR, Syngal S, Wolfe MM (1995) Arthritis Rheum 38:5
4. Cash JM, Klippel JH (1994) New Eng J Med 330:1368
5. Davies NM, Wallace JL (1997) J Gastroenterol 32:127
6. Wolfe MM, Lichtenstein DR, Singh G (1999) New Eng J Med 340:1888
7. Dannhardt G, Kiefer W (2001) Eur J Med Chem 36:109
8. Talley JJ (1999) Prog Med Chem 36:201
9. Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL (1991) Proc Natl Acad Sci USA 88:2692
10. Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR (1999) J Biol Chem 266:12866
11. Mitchell T, Warner T (1999) Br J Pharmacol 128:1121
12. Warner T, Giuliano F, Vojnovic I, Bukasa A, Mitchell J, Vane J (1999) Proc Natl Acad Sci USA 96:7563
13. Flower RJ (2003) Nat Rev Drug Discov 2:179
14. Dogne JM, Supuran CT, Pratico D (2005) J Med Chem 48:2251
15. Velázquez CA, Praveen Rao PN, Citro ML, Keefer LK, Knaus EE (2007) Bioorg Med Chem 15:4767
16. Bhandari SV, Parikh JK, Bothara KG, Chitre TS, Lokwani DK, Devale TL, Modhave NS, Pawar VS, Panda S (2010) J Enzyme Inhib Med Chem 25:520
17. Wallace JL, Soldato PD (2003) Fundam Clin Pharmacol 17:11
18. Vallance P (2003) Fundam Clin Pharmacol 17:1
19. Ignarro LJ (1996) Kidney Int Suppl 55:S2
20. Russwurm M, Koesling D (2002) Mol Cell Biochem 230:159
21. Wallace JL, Granger DN (1996) FASEB J 10:731
22. Iwata F1, Leung FW (1995) Am J Physol 268:G153
23. Nirode WF, Luis JM, Wicker JF, Wachter NM (2006) Bioorg Med Chem Lett 16:2299

24. Bohn H, Brendel J, Martorana PA, Schonafinger K (1995) Br J Pharmacol 114:1605
25. Velázquez C, Rao PNP, McDonald R, Knaus EE (2005) Bioorg Med Chem 13:2749
26. Amir M, Somakala K, Ali S (2013) Min Rev Med Chem 13:2082
27. Amir M, Saifullah K, Akhter W (2011) J Enzyme Inhib Med Chem 26:141
28. Amir M, Kumar H, Khan SA (2008) Bioorg Med Chem Lett 18:918
29. Amir A, Kumar H, Javed SA (2008) Eur J Med Chem 43:2056
30. Amir M, Kumar S (2007) Acta Pharm 57:31
31. Mathew B, Suresh AJ, Mathew GW, Shankar S, Kumar SS (2011) Int J Chem Tech Res 3:364
32. Lolli ML, Cena C, Medana C, Lazzarato L, Morini G, Coruzzi G, Manarini S, Fruttero R, Gasco A (2001) J Med Chem 44:3463
33. Fruttero R, Ferrarotti B, Serafino A, Stilo AD, Gasco A (1989) J Heterocycl Chem 26:1345
34. Young KG, Jucheon K, Seung LH, Hyung KJ, Kwang JH (2009) Bull Korean Chem Soc 30:459
35. Collins JC, Hess WW (1988) Org Synth 6:644
36. Stilo DA, Visentin S, Cena C, Gasco AM, Ermondi G, Gasco A (1998) J Med Chem 41:5393
37. Harding KE, Masy LM, Dig KF (1975) J Org Chem 40:1664
38. Mu LI, Feng S, Go ML (2000) Chem Pharm Bull 48:808
39. Ferioli R, Folco GC, Ferretti C, Gasco AM, Medana C, Fruttero R, Civelli M, Gasco A (1995) Br J Pharmacol 114:816
40. Winter CA, Risley EA, Nus GN (1962) Proc Soc Exp Biol 111:544
41. Adeyemi OO, Okpo SO, Okpaka O (2004) J Ethnopharmacol 90:45
42. Cioli V, Putzolu S, Rossi V, Barcellona SP, Corradino C (1979) Toxicol Appl Pharmacol 50:283
43. Ohkawa H, Ohishi N, Yagi K (1979) Anal Biochem 95:351
44. Reitman S, Frankel S (1957) Am J Clinical Pathol 28:56
45. King EJ, Armstrong AR (1934) Can Med Assoc J 31:376
46. Varley H (1988) Practical clinical biochemistry, 1st edn. CBS Publishers and Distributors, New Delhi, p 236
47. Luna LG (1968) Manual of histological staining methods of the armed forces institute of pathology, 3rd edn. Mc Graw-Hill, New York, p 567