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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and biological evaluation of 1,3,4-oxadiazoles as promising anti-inflammatory agents

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Abstract A series of 1,3,4-oxadiazole derivatives were designed, synthesized and evaluated for radical scavenging and anti-inflammatory properties. Molecular docking simulation studies onto the proteins cyclooxygenase-1 (PDB: 1CQE) and cyclooxygenase-2 (PDB: 3LN1) to visualize the probable binding affinity towards anti-inflammatory importance and in silico studies, towards their appreciable ADME & probable toxicity property were screened. The best-ranked molecules; *N*-((5-substituted-1,3,4-oxadiazol-2-yl)methyl) benzo[*d*]thiazol-2-amine **(5a–5j)** were synthesized from 2-(benzo[*d*]thiazol-2-ylamino)acetohydrazide (4) on reaction with aryl/heteroaryl/aliphatic carboxylic acid derivatives via acid catalyzed dehydrative cyclization. *N*-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl) benzo[*d*]thiazol-2-amine (**5k**) was synthesized by base catalyzed condensation of hydrazide derivative **4** and with carbon disulfide. The newly synthesized compounds were characterized and established on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass studies. The 1,3,4-oxadiazoles were evaluated for in vitro antioxidant property by 2,2′-diphenyl-1-picryl hydrazyl radical scavenging assay method and in vivo anti-inflammatory activity by carrageenan induced paw edema method. The radical scavenging activity indicated that the 1,3,4-oxadiazoles at 25 µM test concentration exhibited significant radical scavenging property ranging from 32.0 to 87.3 % in comparison to 76.0 % radical scavenging activity obtained for the reference drug, ascorbic acid. The results of the in vivo anti-inflammatory activity highlighted that the 1,3,4-oxadiazoles at 25 mg Kg^{-1} test dose exhibited significant edema inhibition with a mean value ranging from 23.6 to 82.3 % in comparison to 48.3 % edema inhibition obtained for the reference drug, indomethacin. The compound **5h** with mean edema inhibition value of 82.3 % and potent among the series was further evaluated for in vitro COX inhibition and was found to more selective towards COX-2. The acute ulcerogenic evaluation of compound **5h** indicated it to be safe at the dose of 50 mg Kg^{-1}.

Keywords 1,3,4-Oxadiazole · Anti-inflammatory · Benzothiazole · Radical scavenging · Ulcerogenic evaluation

Introduction

Prostaglandins (PGs), thromboxanes and leukotrienes are classified under derivatives of eicosanoid, which are biosynthesized by several enzymatic steps in the arachidonate cascade from arachidonic acid and essential fatty acid. PGs have been involved in multiple biological functions, responsible of numerous physiological processes (Nathan 2002). The prostaglandin H synthase (PGHS) enzyme is an integral membrane protein and is the dedicated step in the conversion of arachidonic acid to PGG_2. PGG_2 is further reduced to PGH_2 catalyzed by peroxidase activity of PGHS. PGHS is sequentially the limiting step in the synthesis of

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other PGs, as PGH_2 is consequently converted to prostanoids, thromboxane A_2 by thromboxane synthases and other PGs including PGE_2 by prostaglandin synthases (Williams et al. 2002). PGHS is primarily located in the endoplasmic reticulum, PGHS is bi functional in nature, the cyclooxygenase (COX) activity which converts the arachidonic acid to PGG_2 and the peroxidase activity responsible for the conviction of PGG_2 to PGH_2. The PGs have significant role in tissue injury to exude inflammatory signals. Inflammationis a protective and defense mechanism to an injury caused by factors like physical trauma, microbial infections, injurious stimuli or even a chemical burn. Equally, the PGs have considerable role in the physiology involved in release of renin and aggregation of platelet, and have been implicated in cardiovascular disorders (Gund and Shen 1977).

The COX activity of the PGHS is targeted by the non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are first line agents for the management of osteoarthritis, arthritis and other clinical condition associated with pain and inflammation (Talley et al. 2000). NSAIDs are widely prescribed, easily accessible as over the counter drugs and are also commonly used as self-medication for the management of any pain and inflammation related clinical disorders. NSAIDs act by inhibiting the enzyme prostaglandin endoperoxidase; COX, thus suppressing prostaglandin biosynthesis.(Warner et al. 1999). Two isoforms of PGHS have been identified and characterized, PGHS-1 (COX-1), and PGHS-2 (COX-2).The physiological importance of both the PGHS isoenzyme are extensively investigated, both PGHS-1 and PGHS-2 differ for regulation, expression and distribution (Dannhardt and Kiefer 2001). PGHS-1 isoenzyme is involved in cell–cell signaling, regulation of the mucus formation and thromboxane production, maintaining tissue homeostasis and provides cytoprotection in the gastrointestinal (GI) tract. The PGHS-2 isoenzyme expression occurs in a limited number of cell types, mediates inflammatory signals and is regulated by specific stimulatory signals indicative that PGHS-2 positive role in prostanoid biosynthesis involved in mitogenesis and inflammation. Prostaglandin biosynthesis has be eventually targeted in the management of inflammatory conditions (Habeeb et al. 2001). Most of the clinical available NSAIDs are non-selective COX inhibitor or exhibit greater selectivity for COX-1 than COX-2 (Kujubu et al. 1991; Smith et al. 1998). The long term and chronic usage of these non-selective NSAIDs has led to the precipitation of secondary clinical condition like dyspepsia, gastroduodenal ulcers, gastritis bleeding and nephrotoxicity (Sondhi et al. 2007).

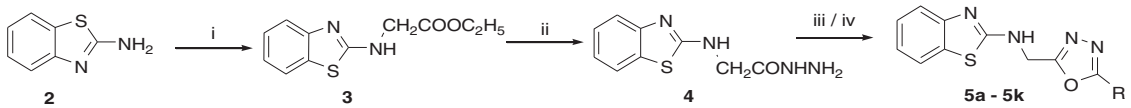
At the same time, clinical studies have indicated significant correlation between reactive oxygen species (ROS) and generation of free radicals during the expression of inflammatory responses (Galanakis et al. 2004). Increased production of the ROS produces a state of oxidative stress and this oxidative stress is also an equally important component for GI ulceration. The ROS have substantial role in the biosynthesis of PGs and in the COX and lipoxygenase mediated conversion of arachidonic acid into proinflammatory intermediates. Signifying that, a molecule which has free radical scavenging potential is more likely to possess anti-inflammatory activity too. The management of ROS with antioxidant abolishes the production of series of proinflammatory cytokinins (El-Gazzar et al. 2009). Thus highlighting that, the management of inflammatory condition could be benefited from the use of drugs that combine antioxidant and anti-inflammatory activities. Tolfenamic acid is an evident example under commercially available NSAIDs with dual antioxidant and anti-inflammatory activities. In view of the above facts, anti-inflammatory investigations are always associated with preliminary antioxidant evaluation for the tested compounds (Sakat et al. 2010).

Literature studies highlight that 1,3,4-oxadiazole have been widely explored for its anti-inflammatory property (Kucukguzel et al. 2007; Singh et al. 2013; Ravindra et al. 2006; Amir et al. 2007; Burbuliene et al. 2004; Kadi et al. 2007). 1,3,4-oxadiazole moiety provides a scaffold on which pharmacophores can be arranged to afford potent and selective drugs. 1,3,4-oxadiazole being a toxophoric moiety (N-C-O), highlighting its importance as a potential biodynamic molecule (Somani and Bhanushali 2011), has been widely explored and reported for its biological activities like anticonvulsant (Almasirad et al. 2004), antidepressant (Zarghi et al. 2005), anticancer (Jin et al. 2006; Padmavathi et al. 2009), analgesic (Kumar et al. 2008), anti-HIV (El-Emam et al. 2004) antibacterial (Naveena et al. 2010; Sahin et al. 2002), antifungal (Liu et al. 2008), antimycobacterial (Ali and Shaharyar 2007; Kumar et al. 2010), anthelmintic (Bharathi et al. 2010), hypoglycemic (Shingalapur et al. 2010) and antiangiogenic (Kumar et al. 2009) properties. The pursuit for better therapeutic agents with clinical advantage has always been the core of pharmaceutical research. In view of the above facts and in continuation of our study on 1,3,4-oxadiazoles (Vishwanathan et al. 2016), we report here the synthesis of a small library of 1,3,4-oxadiazoles and their anti-inflammatory efficacy.

Results and discussion

Chemistry

Synthesis of the intermediates and the target compounds **5a–5k** were accomplished according to the steps depicted in Scheme 1. The starting material benzo[*d*]thiazol-2-amine **(2)** was synthesized as per the reported method (Giorgioni



Scheme 1 Schematic representation for the synthesis of oxadiazole derivatives

et al. 2005) by condensation of aniline with potassium thiocyanate to obtain 1-phenyl thiourea and cyclized in the presence of bromine. The 2-amino benzothiazole derivative **2** was condensed with ethyl 2-chloroacetate in the presence of anhydrous potassium carbonate to yield ethyl-2-(benzo[*d*]thiazol-2-yl amino) acetate **(3)**.The nucleophilic addition of the ester derivative **3** with hydrazine monohydrate in ethanol gave 2-(benzo[*d*]thiazol-2-ylamino)acetohydrazide **(4)**. The hydrazide derivative **4** on condensation with appropriate heteroaryl/aryl/aliphatic carboxylic acids in the presence of phosphorous oxychloride to undergo dehydrative cyclization and to yield *N*-((5-substituted-1,3,4-oxadiazol-2-yl)methyl) benzo[*d*]thiazol-2-amine **(5a–5j)**. The acyl hydrazide derivative **4** on condensation with carbon disulphide and potassium hydroxide yielded *N*-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl) benzo[*d*]thiazol-2-amine **(5k)** (Bhandari et al. 2008).

In the ^{1}H NMR spectrum of the acyl hydrazide derivative, 2-(benzo[*d*]thiazol-2-yl amino) acetohydrazide **(4)** two isolated singlets at δ 11.73 and 4.08 ppm can be attributed to the NH and NH_2 protons of the hydrazide function respectively and the multiplet between δ 7.03 to 7.64 ppm was assigned to the four protons of the benzothiazole moiety. The singlet at δ 5.58 ppm was assigned to the amino proton and the methylene protons between the amino group and the carbohydrazide function resonated at δ 3.25 ppm as a singlet.

In the proton NMR spectrum of *N*-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)benzo[*d*]thiazol-2-amine **(5a)**, the signals at δ 3.33 and 5.78 ppm values can be assigned to methylene protons and amino proton at position C2 of the benzothiazole moiety respectively. The multiplet at δ 7.02–7.59 ppm in the proton NMR spectrum of compound **5a** accounts for nine aromatic protons of benzothiazole and phenyl moiety out of the twelve protons. The absence of hydrazide protons at δ 11.73 and 4.08 ppm in the proton NMR spectrum confirms for the oxadiazole ring formation. In the ^{13}C NMR spectrum of compound **5a**, the chemical signal δ 174.7 and 167.5 ppm were assigned to the C_5 and C_2 positions of 1,3,4-oxadiazole moiety respectively. The chemical signals at δ 154.4 and 38.4 ppm were attributed to the C_2 position of the benzothiazole moiety and the methylene bridge, respectively. The mass spectrum characterization of the compound **5a** exhibits an ion peak at 309 *m*/*z* which can be designated as the M^{+1} ion peak. The ion peak at 159 and 149 *m*/*z* can be attributed to the 2-phenyl-5-methyl-1,3,4-oxadiazole and 2-aminomethyl benzothiazole fragments respectively.

In the proton NMR spectrum of *N*-[((5-(2-amino)phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo[*d*]thiazol-2-amine **(5d),** the signal at δ 4.34 ppm was assigned to the amino proton of the 2-aminophenyl moiety substitution at position C_5 of the oxadiazole nucleus. The eight aromatic protons of benzothiazole and 2-aminophenyl moiety have resonated at δ 7.23–8.02 ppm as multiplet. The signals at δ 3.29 and 5.81 ppm can be assigned to methylene protons and amino proton respectively. The mass spectrum characterization of the compound **5d** exhibits an ion peak at 324 *m*/*z* which can be designated as the M^{+1} ion peak. The ion peak at 174 *m*/*z* can be attributed to the 2-(2-aminophenyl)-5-methyl-1,3,4-oxadiazole fragment.

In the proton NMR spectrum of *N*-[((5-(2-hydroxy)phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo[*d*]thiazol-2-amine **(5f)**, the singlet at δ 10.29 ppm was assigned to the hydroxy group proton of the 2-hydroxyphenyl moiety at position C_5 of the oxadiazole. The eight aromatic protons of benzothiazole and 2-hydroxyphenyl moiety have resonated at δ 7.12–7.71 ppm as multiplet. The signals at δ 3.34 and 5.69 ppm can be assigned to methylene protons and amino proton respectively. The ion peak at 324 *m*/*z* can be designated as the M^{+} molecular ion peak and a major ion peaks at 175 and 149 *m*/*z*, corresponding to the 2-phenol-1,3,4-oxadiazole and 2-aminobenzothiazole fragments respectively.

The IR spectrum characterization of compound 5-[(benzo[*d*]thiazol-2-ylamino)methyl]-1,3,4-oxadiazole-2-thiol **(5k)** indicated by a shift of absorption band from 1721 to 1230 cm^{-1}, corresponding to the carbonyl group stretching. This can be correlated to the ring stretching of oxadiazole moiety. In the proton NMR spectrum of compound **5k**, the mercapto proton has resonated as a singlet at δ 12.83 ppm and the multiplet at δ 7.54–7.38 ppm value can be attributed to the aromatic protons of benzothiazole

Table 1 The in silico molecular properties of the designed compounds

Comp	% ABS[a]	TPSA[b]	n-ROTB[b]	n-HBA[b]	n-HBD[b]	*mi* logP[b]	Molecular weight	n violations[b]	Log S[c]	Drug likeness[c]	Drug score[c]
5a	87	63.84	4	5	1	3.17	308.37	0	−4.98	2.98	0.65
5b	71	109.67	5	8	1	3.08	353.36	0	−5.44	−4.66	0.32
5c	55	155.49	6	11	1	3.02	398.36	1	−5.9	−7.86	0.28
5d	78	89.87	4	6	3	2.61	323.38	0	−5.06	0.23	0.53
5e	78	89.87	4	6	3	2.25	323.38	0	−5.06	1.44	0.61
5f	80	84.07	4	6	2	2.91	324.37	0	−4.68	2.5	0.68
5g	80	84.07	4	6	2	2.69	324.37	0	−4.68	1.49	0.64
5h	83	76.73	4	6	1	2.1	309.35	0	−4.18	3.19	0.77
5i	87	63.84	3	5	1	1.25	232.27	0	−2.83	2.45	0.88
5j	87	63.84	3	5	1	0.79	246.29	0	−2.46	2.42	0.89
5k	87	63.84	3	5	1	1.77	264.33	0	−3.71	0.17	0.66

[a] Data calculated as per the equation 1

[b] Molecular property as obtained from Molinspiration online property calculation toolkit

[c] Molecular property as obtained from OSIRIS property explorer software

moiety. The methylene protons and amino proton have resonated at δ 3.36 and 5.85 ppm respectively. All the other newly synthesized compounds were also characterized similarly and were found in agreement with the proposed structures.

In silico ADME results

All the compounds had appropriate values towards Lipinski parameters for biological efficacy with zero to one Lipinski violation. The in silico results indicated that the compounds agree to the Lipinski rules of the five and theoretically, the designed compounds were presenting a positive oral bioavailability. The compounds with a percentage of absorption (% ABS) values ranging between 55 and 87 % highlighting a moderate to high predicted oral availability. The molecular weight of the designed compounds was within the range of 232–398 D. The lipophilicity score of the designed compounds were encouraging as the lipophilicity data suggested that the compounds were optimally lipophilic in nature ranging from 0.79 to 3.17.The topological polar surface area (TPSA) values of the designed compounds were less than 140 Å (except compound **5c**), a TPSA value less than 140 Å indicates a good permeability of the molecule in the plasma membrane. The designed molecules exhibited a positive value in the drug score calculation and the values ranged from 0.28 to 0.89. The positive drug scores highlight that the designed compounds have potential to be considered as a new drug candidate, and the drug score highlights that the screened compounds have predominant pharmacophoric groups, which are often found in the drug molecules. The ADMET results were encouraging, and calculated % ABS, TPSA, and Lipinski parameters of the designed compounds are presented in Table 1.

Molecular docking results

The molecular modelling technique was used to explore, predict and understand the protein/enzyme interactions with our designed 1,3,4-oxadiazole library and also to visualize the probable binding. The docking study was performed using the SYBYL-X 2.1. Wherein, the designed 2,5-disubstituted-1,3,4-oxadiazoles were docked into the active site of the selected enzymes. The crystal structure of the enzyme along with its co-crystallized ligand was downloaded from Brookhaven Protein Data Bank (www.rcsb.org) and was used for docking studies. The docking results were validated by aligning the designed ligands with the co-crystallized reference standards for the respective enzymes. The reference standards employed were drawn and subjected for the docking protocol along with the designed series of oxadiazoles onto to the binding site to visualize the binding affinity and the strength of binding to ascertain the docking process and validate the same.

Molecular docking studies on COX-I enzyme (1CQE)

The docking study onto the active site of the enzyme COX-1 (PDB: 1CQE) of the designed 2,5-disubstituted 1,3,4-oxadiazoles was encouraging. The co-crystallized ligand, flurbiprofen in the B chain of the protein showed a docking score of 6.5498 Kcal M^{-1} highlighting the hydrophobic interactions with the surrounding amino acid residues Leu352, Trp387, Val349, Ser530 and Ala527, resulting in its stabilization, and Vanderwall's interaction with Tyr355, Ser353, and Arg120 residues, and strongly contributing to its inhibitory effect. The carboxylic acid group as carboxylate ion exhibited hydrogen bond interaction with the residue Ser353 at the distance of 2.392 Å. The docking

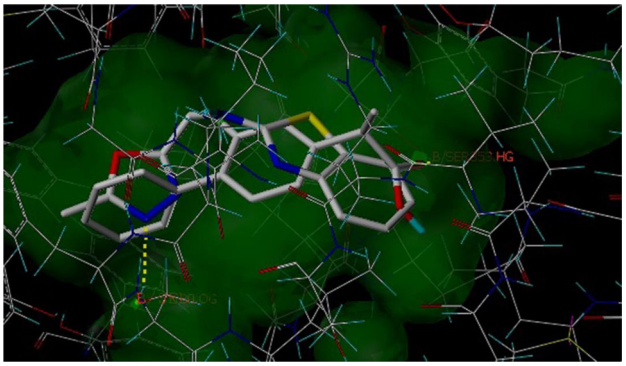


Figure 1 Molecular docking interaction of compound **5j** along with the reference standard flurbiprofen interacting with 1CQE

study revealed that the designed 1,3,4-oxadiazole derivatives were exhibiting a considerable affinity towards 1CQE, as the compounds **5a–5k** exhibited a promising docking score, ranging from 2.54 to 6.61 Kcal $M^{-1.}$ Compound **5j** (methyl) showed a docking score of 6.6107 Kcal M^{-1}, highest among the series and compound **5c** (3,5-dinitrophenyl) with the lowest docking score (2.5377 Kcal M^{-1}). The compound **5j** (methyl) exhibited a good docking score of 6.6107 Kcal M^{-1}, higher than that of the co-crystallized ligand flurbiprofen indicating a better interaction with the protein COX-1 and highlighting its ability to inhibit the same and produce a significant anti-inflammatory action (Fig. 1). The compound **5j** was well aligned in the hydrophobic core of the 1CQE. The nitrogen at position 3 of the 1,3,4-oxadiazole moiety of compound **5j** showed hydrogen bond interaction with Ser530 residue at a distance of 2.182 Å. Compound **5j** exhibited a crash score of −0.7027 Kcal M^{-1}, indicating the ease of penetration of the designed ligand into the binding site of the target enzyme, resulting in better interaction with the amino acid residues. The hydrogen bond interaction between the nitrogen at position 4 of the 1,3,4-oxadiazole with Ser530 residue was the major interaction exhibited by the designed1,3,4-oxadiazoles. Among the series, some of the designed compounds exhibited a higher crash score highlighting that the ligand were not able to stabilize in the binding site of the target enzyme; 1CQE, and indicating an inappropriate penetration. Table 2 represents the molecular docking results of designed compounds; **5a–5k** along with their crash score and polar score (Fig. 2).

Molecular docking studies on COX-2 enzyme (3LN1)

The docking results of our designed 1,3,4-oxadiazoles onto the active site of the enzyme COX-2 (3LN1) was considerable. The 3LN1 protein, representing a typical cylindrical core of 23 Å between Ser516, Ser339, and Asp510 accounting the hydrophobic interaction. The ligand, celecoxib exhibited a docking score of 9.6857 Kcal M^{-1}, exhibiting the hydrophobic interactions with the surrounding amino acid residues Ser339, Gly512, Leu338, and Val509, strongly contributed to the stabilization. The Tyr371, Trp373, and Val335 residues were involved in Vanderwall's interaction with the alkyl/halo alkyl chain present in the celecoxib. The hydrogen bonding interaction of the amino group protons of the celecoxib was with the residues Leu338 and Gln178 at a distance of 2.109 and 2.689 Å, respectively. The sulfonyl oxygen of the ligand celecoxib, showed hydrogen bonding interaction with Arg499 and Phe504 residues at a distance of 2.246 and 2.495 Å, respectively.

The docking study was promising and the result indicated that our designed 1,3,4-oxadiazole molecules exhibited a considerable affinity towards 3LN1. The 1,3,4-oxadiazoles **5a–5k** exhibited docking score in the range of 4.99–7.79 Kcal $M^{-1.}$ Compound **5g** (4-hydroxyphenyl) exhibited a docking score of 7.79 Kcal M^{-1}, highest among the series and compound **5k** (mercapto) exhibited the lowest docking score (4.99 Kcal M^{-1}). The hydrogen bond interaction between nitrogen at position C4 of the 1,3,4-oxadiazole and Ser516 residue was the major interaction exhibited by designed1,3,4-oxadiazoles. Docking result indicated that the compounds showed a lower crash score for 3LN1 to that of 1CQE. Table 2 represents the molecular docking results of designed compounds; **5a–5k** along with their crash score and polar score (Fig. 2).

The docking studies towards anti-inflammatory efficacy highlighted that the compounds have a significant affinity to inhibit COX-2 (3LN1) enzyme than the COX-1 enzyme, with a higher docking score in comparison, the docking results highlight that the compounds are exhibiting higher selectivity towards COX-2 than COX-1. Moreover, most of the designed oxadiazoles were exhibiting a higher crash score for COX-1 than that of COX-2 indicating the difficulties in penetration and inhibiting the COX-1 enzyme. This higher selectivity towards COX-2 is advantageous in preventing secondary clinical conditions like gastroduodenal ulcers, gastritis bleeding etc., associated with the inhibition of COX-1.

Docking validation

The reference standards employed were drawn and subjected to the docking protocol along with the designed series of 1,3,4-oxadiazoles. In order to visualize the binding affinity and strength to ascertain the docking process and validate the same. The docking pose was compared with the co-crystallized binding pose of flurbiprofen and celecoxib for 1CQE and 3LN1 protein targets, respectively. The validation of the docking process indicated by the root mean square deviation (RMSD) value and poses for

Table 2 Molecular docking results of the compounds **5a–5k** onto the target enzymes

Sl. No.	Compound	Docking score towards 1CQE[a]			Docking score towards 3LN1[a]		
		Total score	Crash score	Polar score	Total score	Crash score	Polar score
1.	**5a**	3.8538	−4.816	0.491	6.6834	−1.4289	0.1711
2.	**5b**	3.9529	−4.9012	0.0444	6.7892	−1.3142	0.8868
3.	**5c**	2.5377	−7.3806	1.1621	5.4541	−4.39	1.9631
4.	**5d**	6.2515	−3.9454	1.9183	7.1129	−1.504	1.0318
5.	**5e**	5.0101	−5.7075	0.0085	7.4284	−1.546	0.7364
6.	**5f**	4.2473	−5.2355	0.0046	7.2264	−1.3352	1.2273
7.	**5g**	4.7278	−4.7015	1.0538	7.7898	−1.2447	2.4121
8.	**5h**	5.0651	−3.2302	0.3336	7.5655	−0.8683	0.8615
9.	**5i**	4.9779	−0.8156	1.0589	5.4017	−0.3331	1.0396
10.	**5j**	6.6107	−0.7027	1.0664	5.9914	−0.5833	1.0067
11.	**5k**	4.0659	−1.7477	0.8714	4.9993	−0.8089	0.2921
12.	Flurbiprofen	6.5498	−0.5917	0.1284			
13.	Celecoxib				9.6857	−0.5373	2.1673

[a] Data expressed in Kcal M^{-1}as obtained from the docking utility of SYBYL 2.1 software

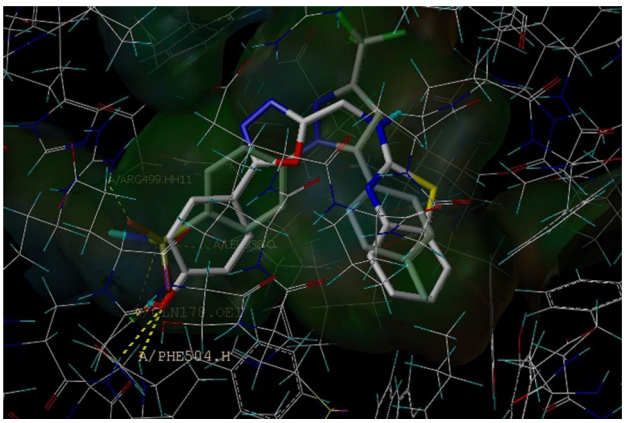


Figure 2 Molecular docking interaction of compound **5g** along with the reference standard celecoxib interacting with 3LN1

the co-crystalized and docked ligand were 0.95 and 0.59 Å for flurbiprofen and celecoxib, respectively. The RSMD values are affirmative that the overall docking study is corroborated, as the tolerance limit for RMSD value is 1.5 Å. Hence, the docking results are acceptable and indicative that the docking protocol is validated.

In vitro radical scavenging evaluation

The 1,3,4-oxadiazole derivatives, **5a–5k** were evaluated for their free radical scavenging activity at 25 μM strength by DPPH radical assay method using ascorbic acid as standard. The compounds **5a–5k** exhibited significant scavenging activity ranging from 32.0 to 87.3 % in comparison to 76.0 % antioxidant activity obtained for the reference drug, ascorbic acid. The analysis of the compounds antioxidant activity indicate that the compounds **5f** (2-hydroxyphenyl), **5e** (4-aminophenyl), **5g** (4-hydroxyphenyl), **5d** (2-aminophenyl) and **5h** (pyridin-3-yl) exhibited better radical scavenging activity, higher than that of the reference standard ascorbic acid employed.

The structure–activity relationship that can be drawn from radical scavenging results, highlight that compounds with electron donating group substitution on the phenyl moiety were exhibiting a higher radical scavenging property than that of other tested 1,3,4-oxadiazoles. The better radical scavenging efficacy of these derivatives; **5d**, **5e**, **5f**, and **5g** may be attributed to the possible stable resonance hybrid formation with loss of the proton and thus neutralizing the supra-radical. The substantial antioxidant activity of the compound **5h** (pyridin-3-yl) with a radical scavenging property of 81.0 % may be attributed to the *N*-oxide formation. Whilst, **5b** (2-nitrophenyl) and **5c** (3,5-dinitrophenyl) derivatives were exhibiting a radical savaging efficacy 38.6 and 36.6 % respectively, the comparatively lower radical savaging property may be indicated to the electron-withdrawing property of the nitro function. The radical scavenging activity highlighted the importance of electron donating group substitution at the *ortho/para* position of the phenyl moiety at position C_5 of the 1,3,4-oxadizole molecule. The statistical analysis of the compounds **5a–5k** for its antioxidant property by repeated measures Dunnett's ANOVA method indicate that the tested compounds exhibited significance value of $P < 0.001$ highlighting the confidence interval of 99.9 % with respect to the reference standard ascorbic acid. The radical savaging data of the test compounds **5a–5k** at 25 μM concentrations were statistically analyzed using the repeated measures ANOVA with Dunnett's test and the data are expressed as the mean ± SEM in Table 3.

In vivo anti-inflammatory activity

The newly synthesized 1,3,4-oxadiazole derivatives **5a–5k** were evaluated for in vivo anti-inflammatory activity at a dose of 25 mg Kg^{-1} by carrageenan induced paw edema method. Indomethacin at 25 mg Kg^{-1} was used as reference standard and CMC as the control. The result of the anti-inflammatory screening at the end of four hours after the administration of carrageenan were promising, the tested compounds exhibited substantial anti-inflammatory activity with an edema inhibition ranging from 23.55 to 82.27 % in comparison to 48.26 % edema inhibition exhibited by the reference standard indomethacin employed.

A correlation between the structure and anti-inflammatory activity data indicates that the compounds with aryl substitutions at position C_2 of the 1,3,4-oxadiazole moiety **(5a–5h)** were exhibiting higher edema inhibition than that of 1,3,4-oxadiazole derivatives with aliphatic/thiol substitution at C_2 position **(5i–5k)**. The compound **5h** with pyridin-3-yl substitution at position C_2 of the 1,3,4-oxadiazole moiety was found to be the most potent, with edema inhibition value of 82.27 % in comparison to the other tested 1,3,4-oxadiazole derivatives with edema inhibition value ranging from 23.55 to 47.67 % and higher than that of the reference standard indomethacin employed (48.26 %). While compound **5j** (methyl) exhibited the least anti-inflammatory efficacy with an edema inhibition value of only 23.55 %. Interestingly compounds with nitro substitution, compound **5b** (2-nitro phenyl) and **5c** (3,5-dinitro phenyl) were exhibiting substantial anti-inflammatory efficacy with edema inhibition of 44.19 and 47.67 % respectively, similar to that of compound **5a** and **5d–5g** (phenyl/phenyl substituted derivatives), contradicting to their radical scavenging efficacy. The percentage edema inhibition values of tested compounds **5a–5k** at 25 mg Kg^{-1} strength at the end of four hours and the mean difference in paw

Table 3 The radical scavenging efficacy of the compounds **5a–5k**

Sl No.	Compounds	Percentage free radical scavenging activity[a,b]			
		Trial 1	Trial 2	Trial 3	Mean ± SEM
1.	**5a**	55.19	54.47	55.74	55.13 ± 0.37***
2.	**5b**	36.68	35.67	37.46	36.60 ± 0.52***
3.	**5c**	38.71	37.73	39.46	38.63 ± 0.50***
4.	**5d**	83.63	83.37	83.84	83.61 ± 0.14***
5.	**5e**	86.00	85.78	86.18	85.99 ± 0.12***
6.	**5f**	87.36	87.16	87.51	87.34 ± 0.10***
7.	**5g**	85.78	85.55	85.95	85.76 ± 0.12***
8.	**5h**	81.04	80.73	81.27	81.01 ± 0.16***
9.	**5i**	35.33	34.29	36.12	35.25 ± 0.53***
10.	**5j**	32.05	30.96	32.89	31.97 ± 0.56***
11.	**5k**	43.79	42.89	44.48	43.72 ± 0.46***
12.	Ascorbic acid	76.07	75.69	76.37	76.04 ± 0.20

[a] Results are expressed as the mean values from three parallel experiments ±SEM

[b] Data was analyzed by Dunnet's test compared with reference drug ascorbic acid. $n = 3$; (***) equals $P < 0.001$

Table 4 The percentage edema inhibition values of tested compounds **5a–5k**

Sl no.	Compounds	Mean difference in paw volume ± SEM[a,b]				Edema inhibition (%)
		0.5 h	1.0 h	2.0 h	4.0 h	
1.	Control	0.35 ± 0.01	0.59 ± 0.02	0.66 ± 0.02	0.57 ± 0.03	
2.	**5a**	0.35 ± 0.02	0.52 ± 0.02***††	0.53 ± 0.02***††	0.35 ± 0.02***	39.83
3.	**5b**	0.33 ± 0.01	0.48 ± 0.01***	0.48 ± 0.01***	0.32 ± 0.02***	44.19
4.	**5c**	0.33 ± 0.01	0.44 ± 0.01***	0.45 ± 0.00***	0.30 ± 0.01***	47.67
5.	**5d**	0.35 ± 0.00	0.48 ± 0.00***	0.49 ± 0.01***	0.33 ± 0.01***	42.73
6.	**5e**	0.34 ± 0.01	0.48 ± 0.01***	0.48 ± 0.01***	0.33 ± 0.01***	43.31
7.	**5f**	0.35 ± 0.01	0.48 ± 0.01***	0.52 ± 0.01***†	0.35 ± 0.02***	39.24
8.	**5g**	0.35 ± 0.01	0.48 ± 0.01***	0.50 ± 0.02***	0.35 ± 0.01***	38.37
9.	**5h**	0.31 ± 0.02	0.43 ± 0.01***†	0.40 ± 0.01***††	0.10 ± 0.01***†††	82.27
10.	**5i**	0.34 ± 0.01	0.52 ± 0.01***	0.51 ± 0.01***	0.42 ± 0.01***†††	26.45
11.	**5j**	0.35 ± 0.01	0.53 ± 0.01**†	0.53 ± 0.01***††	0.44 ± 0.01***†††	23.55
12.	**5k**	0.34 ± 0.01	0.49 ± 0.01***	0.47 ± 0.01***	0.35 ± 0.01***	38.37
13.	Indomethacin	0.31 ± 0.01	0.48 ± 0.01***	0.46 ± 0.01***	0.30 ± 0.01***	48.26

[a] Data was analyzed by Dunnet's test compared with the negative control. $n = 3$; (***) equals $P < 0.001$, (**) equals $P < 0.01$, (*) equals $P < 0.05$

[b] Data was analyzed by Dunnet's test compared with reference drug indomethacin $n = 3$; (†††) equals $P < 0.001$, (††) equals $P < 0.01$, (†) equals $P < 0.05$

Table 5 The mean percentage values of compound **5h** for in vitro COX-1 and COX-2 enzymes inhibition at the three different test strength along with its IC_{50} values

Compound	Mean percentage COX-1 inhibition ± SEM[a]			Mean IC_{50} value ± SEM	Mean percentage COX-2 inhibition ± SEM[a]			Mean IC_{50} value ± SEM
	6.25 μM	12.50 μM	25.00 μM		6.25 μM	12.50 μM	25.00 μM	
5h	10.19 ± 0.41	42.32 ± 0.34	64.50 ± 0.44	16.73 ± 0.11	45.09 ± 0.48	68.77 ± 0.51	86.64 ± 0.38	7.19 ± 0.10

[a] Results are expressed as the mean values from three parallel experiments ± SEM

volume ± SEM at regular time intervals are presented in Table 4.

While, compound **5d** (2-amino phenyl), **5e** (4-amino phenyl), **5f** (2-hydroxy phenyl) and **5g** (4-hydroxy phenyl) substitution which were exhibiting significant radical scavenging property exhibited moderate anti-inflammatory activity with edema inhibition value ranging from 38.37 to 43.31 % similar to compound **5a** (2-phenyl derivative) with edema inhibition value of 39.83 %, indicating that the amino and hydroxyl functional group substitution at the *ortho/para* position is not considerably contributing toward the anti-inflammatory efficacy.

The statistical analysis of the edema inhibition data at the end of 30 min highlighted that the compounds **5a–5k** did not exhibit a significant reduction in mean edema volume with respect to both control and reference drug indomethacin. At the end of 1 h, compound **5a–5i**, **5k**, and indomethacin exhibited a significance value of $P < 0.001$ highlighting the confidence interval of 99.9 % and compound **5j** (methyl) exhibited a significance value of $P < 0.01$ indicating the confidence interval of only 90.0 % with respect to control. Compound **5h** (pyridin-3-yl) exhibited a significance value of $P < 0.05$ highlighting the confidence interval of only 95.0 % with respect to reference standard indomethacin. All the 1,3,4-oxadiazoles **5a–5k** and indomethacin exhibited a significance value of $P < 0.001$ highlighting the confidence interval of 99.9 % with respect to control at the end of the second and fourth hour, respectively.

It is most obvious that carrageenan induced edema in animal models involves expressing COX-2, wherein COX-2 mRNA and COX-2 protein are induced (Willoughby et al. 2001). Literature study on anti-inflammatory activity of compound 7-hydroxy-3,4-dihydrocadalin (HDC) evaluated for its in vitro COX-1, COX-2 inhibition assay and correlation to its in vivo carrageenan-induced edema inhibition screening highlights that the compound HDC inhibited COX-1 to a higher degree than COX-2, and the carrageenan induced edema in rat paw was impervious (Segura et al. 2000). In view of the same and affirmative about our compounds to inhibit COX-2 enzyme, the compound **5h** potent among the series was also evaluated for in vitro COX inhibition towards its selectivity and potential to inhibit the target proteins COX-1 and COX-2.

In vitro COX inhibition assay

The results of the in vitro COX enzymes inhibition were encouraging and was determined using a COX inhibitor screening assay kit. The test compound **5h** exhibited a substantial inhibition of COX-2 and a moderate inhibition of COX-1. The result highlighted the ability of our test compound **5h** to inhibit the enzyme COX-2 from catalyzing the conversion of arachidonic acid to PGH_2. The enzyme inhibition data indicated that compound **5h** exhibited a higher IC_{50} value for COX-1 than to that of COX-2. The study also indicated that the compound **5h** exhibited a concentration dependent inhibition of the target enzyme. Compound **5h** at the lowest test concentration (6.25 μM) exhibited a mean enzyme inhibition values of 10.2 and 45.1 % for COX-1 and COX-2 respectively. Signifying that compound **5h** has significant affinity to inhibit COX-2 than that of COX-1. The in vitro COX inhibition activity of the test compound **5h** for both COX-1 and COX-2 at the three different test concentrations by enzymes immunoassay (EIA) are expressed as the mean ± SEM, along with the IC_{50} values obtained by regression analysis are presented in Table 5.

In vivo acute ulcerogenic activity

Acute ulcerogenicity of the test compound **5h** was evaluated after a single dose oral administration to 24 h fasting rats. The results indicated that the compound **5h** to be moderately safe at the tested strength of 50 mg Kg^{-1} with a score of 1+ indicting the compound to produce redness (hyperemia) in 66.7 % of the tested animals ($n = 6$).

Conclusions

A new class of oxadiazole derivatives were designed, efficiently synthesized, characterized and evaluated for radical scavenging property and anti-inflammatory efficacy. The result of the study highlights, that the designed compounds have optimal physicochemical parameters to be considered as the potential candidate for drug development with optimal values towards the ADMET screening. The molecular docking results were encouraging as the compounds

exhibited substantial score towards enzyme inhibition. The compounds exhibited a higher affinity to inhibit COX-2 enzyme than COX-1, which is beneficial in minimizing nephrotoxicity and gastrointestinal damages. The radical scavenging evaluation was considerable, as the tested compounds exhibited significant scavenging property, and compounds **5d**, **5e**, **5f**, **5g**, and **5h** showed >80 % antioxidant activity with respect to the reference standard ascorbic acid employed. All the tested oxadiazoles showed promising anti-inflammatory activity. The mean edema inhibition data indicated that the compounds with aryl substitution are significantly active. Compound **5h** has exhibited a significantly higher edema inhibition in comparison to the reference standard indomethacin employed. The in vitro COX enzyme inhibition assay of compound **5h** was considerable. Compound **5h** exhibited a higher affinity to inhibit COX-2 than that of COX-1. The acute ulcerogenic study indicated that compound **5h** to be moderately safe. The newly synthesized compound **5h** may be a possible hit as therapeutic agents. It can be concluded that this class of compounds certainly holds great promise towards good active leads in medicinal chemistry.

Material and methods

Molecular docking studies

The SYBYL-X 2.1 docking program was employed in the molecular docking studies. The docking algorithm was to achieve imminent information regarding the binding mode and affinity of the designed compounds and explicate the impact of structural differences in the selected protein towards their anti-inflammatory activity. Crystallographic data of the selected enzyme with its co-crystallized ligand were downloaded from Brookhaven Protein Data Bank (www.rcsb.org) and employed in the docking studies. The x-ray crystal structure of protein enzymes was defined and established; the hydrophobic active site and hydrogen bond interaction amino acid residuals were distinctly understood. Using the protein preparation module in SYBYL-X 2.1 program, the target protein was prepared, and bond orders were assigned, water and other residues were removed, hydrogen atoms were added and the protein model was charged with AMBER7 FF99. The designed oxadiazoles and the reference standards; flurbiprofen (flurbiprofen available in the market as a COX-1 inhibitor) and celecoxib (celecoxib available in the market as a COX-2 inhibitor) as ligands were drawn in ChemDraw and converted into *.sdf format. The reference standards; flurbiprofen and celecoxib were also docked along with oxadiazole molecules to validate the docking protocol for the COX-1 and COX-2 enzymes, respectively. The 2D molecules were converted to 3D using the ligand preparation utility of SYBYL-X 2.1 application and by applying molecular mechanics force fields (MMFF94s), the ligands were energy minimized and charged using Gasteiger-Marsili method. The docking simulation highlights the basic receptor-ligand interactions and the strength of the ligand binding onto to the core. The docking of the optimized 3D-structures of the molecules was performed within 10 Å radius to appreciate and to optimize the most probable binding pose of each ligand. A series of hierarchical searches is performed by the docking algorithm to realize the possible ligand affinity within the binding site of the enzyme core. The docked poses of geometrically optimized ligands are ranked with total scores, highlighting the affinity of the ligand towards the enzyme core, and also visualizing the most optimal binding pose of each ligand. The essential parameters in terms of hydrogen bonds and hydrophobic interactions, which in-turn governs binding disparities for the each ligand in the series is understood inthe form of score functions, the score of the docked molecules indicate the affinity of the ligands towards the enzyme core.

In order to visualize the importance of possible anti-inflammatory efficacy of the molecules, the compounds were docked onto COX-1(PDB ID: 1CQE) and COX-2 (PDB ID: 3LN1). Crystallographic data of the 1CQE and 3LN1 were used for docking studies since the data on these have been well established and reported stimulation studies to understand the anti-inflammatory efficacy (Eleftheriou et al. 2012). The protein structure of 1CQE and 3LN1 is well recognized with both hydrophobic active site and the proteins were determined at 3.1 and 2.4 Å resolution, respectively. The bound conformations of co-crystallized ligands, flurbiprofen and celecoxib were used as controls in order to define the active site in COX-1 and COX-2, respectively. The compounds were docked along with the co-crystallized ligands, celecoxib for COX-2 enzyme and flurbiprofen in the case of a COX-1 enzyme, respectively and the interactions like hydrogen bonding were visualized.

In silico ADME studies

Poor ADME properties are one of the primary reasons for rejection of drugs candidates in clinical trials, highlighting the importance of screening for drug likeliness and ADME properties of a molecule towards drug development. Oral bioavailability plays a considerable role in the development of bioactive compounds as medicinal agents, which is essential for rational drug design. According to Lipinski rule of five, molecules that breach more than one of Lipinski rules may have complications with bioavailability. The Lipinski rule of five establishes some structural parameters in relevance to the oral bioavailability profile of the molecules and is extensively adapted in designing compounds

(Zhao et al. 2002). The in silico studies were performed for Lipinski parameters, as well as for the TPSA, % ABS and the drug-likeness and drug score were included for the designed 1,3,4-oxadiazoles in order to verify that these compounds exhibit an optimal theoretical oral bioavailability. The drug-likeness and drug score were calculated using the OSIRIS property explorer software and the lipophilicity and TPSA were calculated using Molinspiration online property calculation toolkit (http://www.molinspiration.com). TPSA was also calculated using Molinspiration online property calculation toolkit according to the fragment-based method reported. Polar surface area, together with lipophilicity is an important property of a molecule which indicates the ease of transport across biological membranes. A high TPSA value indicates poor absorption of a drug and, in turn, highlights low bioavailability. The % ABS was estimated according to the Eq. 1.

$$\% \text{ ABS} = 109 - (0.345 \times \text{TPSA}) \quad (1)$$

Eq. 1: Calculation of percentage of absorption

Experimental section

Molecular docking simulations were performed using SYBYL-X 2.1, chemical structures and synthetic scheme was drawn using ChemDraw and statistical analyses were performed using GraphPad Prism 5.0 on the windows-7 professional operating system. The synthetic routes for the preparation of target molecules were planned through retrosynthetic approach. Most facile synthetic routes were selected based on the percentage yield of molecules of each reaction step. Percentage yield of some reaction steps was improved by careful selection of starting materials and catalyst system. While designing the synthetic routes, at most priority was given for high yielding reproducible reaction steps rather than concentrating on novel synthetic routes and complex chemistry. All the reaction steps even if reported in published literature were selected based on our in-house laboratory facilities. Handling and disposal of all reagents and catalysts to be used in the reaction steps were studied thoroughly from the corresponding Material Safety Data Sheets (MSDS).

All the chemicals and solvents used in this study were purchased from Sigma-Aldrich Chemical Co., Spectrochem Ltd., and Sd fine chemicals of LR grade, the solvents were purified by distillation prior to use. All commercially available reagents procured were used without further purification. TLC was used to assess the progress/completion of reactions and the purity of the synthesized compounds using aluminum backed sheets of silica gel 60 GF254 (Merck), ethyl acetate and hexane (4:1) as a solvent system and iodine vapors as the visualizing agent. Melting points were determined in open glass capillaries and are uncorrected. The IR spectra were recorded using Shimadzu FTIR-8400 instrument by KBr disc pellet technique/liquid sampling using NaCl cells and only noteworthy absorption levels (cm^{-1}) are listed. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AC-400 MHz FT NMR spectrophotometer at 400 MHz ^{1}H (100 MHz ^{13}C) with deuteriated dimethyl sulfoxide (DMSO-d6) as solvent and tetra methyl silane (TMS) as internal standard (chemical shifts in δ, ppm). The splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *q*: quartet; *m*: multiplet. The LCMS were recorded using Shimadzu LCMS-2010A instrument by ESI. The elementary analysis was recorded using Thermo Finnigan FLASH EA 1112 CHNS analyzer and all the compounds gave satisfactory elemental analysis.

Synthesis of benzo[*d*]thiazol-2-amine (2)

The mixture of aniline (25 mL, 0.25 M) and potassium thiocyanate (25 g, 0.25 M) in glacial acetic acid (50 mL) was cooled to a temperature less than 10 °C. To this solution bromine (40 mL, 0.25 M) was added dropwise with stirring while maintaining the temperature less than 10 °C throughout the addition. The mixture was further stirred for an additional 3 h, and the separated hydrochloride salt was filtered, washed with acetic acid and dried. Later, the salt was dissolved in hot water and neutralized with ammonia solution to precipitate the free amine derivative **2**. The product was filtered, washed with water and dried, recrystallized using rectified spirit to obtain solid product **2**.Yield: 87 %; M.P: 125–127 °C; R_f: 0.54; IR (KBr): ṽ3467 (NH_2), 3059 (C=C), 1599 (C=N), 736 (C-S-C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6): δ 6. 9–7.5 (*m*, 4H, Ar–H), 5.5 (*s*, 2H; NH_2) ppm.

Synthesis of ethyl-2-(benzo[d]thiazol-2-yl amino) acetate (3)

A mixture of amine derivative **2** (3 g, 0.02 M), ethyl 2-chloroacetate (6 mL, 0.05 M) and activated anhydrous potassium carbonate (4 g) in dry acetone (30 mL) were heated to reflux for 9 h. The progress of the reaction was monitored by TLC. On completion, the mixture was filtered and the filtrate was concentrated and poured into the crushed ice to obtain the solid ester derivative **3**. The product was recrystallized using rectified spirit as the solvent.Yield: 69 %; M.P: 136–138 °C; R_f: 0.62 (ethyl acetate and hexane, 4:1); IR (KBr): ṽ3454 (NH_2), 3030 (C=C), 1737 (C=O), 1633 (C=N), 760 (C–S–C)cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6): δ 7.2–7.6 (m, 4H, Ar–H), 5.6 (s, 1H, NH), 4.2 (q, 2H, $COOCH_2$), 3.4 (d, 2H, CH_2COO), 1.3 (t, 3H, CH_3) ppm.

Synthesis of 2-(benzo[d]thiazol-2-yl amino) acetohydrazide (4)

The mixture of ester derivative **3** (5 g, 0.02 M) and hydrazine monohydrate (10 mL, 0.2 M) in ethanol (30 mL) were heated to reflux for 16 h. The progress of the reaction was monitored by TLC. On completion, the mixture was allowed to cool in refrigerator overnight to precipitate the acyl hydrazide derivative **3**. The product was recrystallized using rectified spirit as the solvent. Yield: 56 %; M.P: 189–191 °C; R_f: 0.74(ethyl acetate and hexane, 4:1); IR (KBr): ṽ3398, 3450 (NH_2, NH), 3045 (C=C), 1721 (C=O), 1627 (C=N), 752 (C–S–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6): δ 11.7 (s, 1H, CONH), 7.6–7.0 (m, 4H, Ar–H), 5.6 (s, 1H, NH), 4.1 (s, 2H, NH_2), 3.3 (s, 2H, CH_2) ppm.

General procedure for the synthesis of N-((5-substituted-1,3,4-oxadiazol-2-yl)methyl) benzo[d]thiazol-2-amine (5a–5j)

To the solution of acyl hydrazide **4**(2.2 g, 0.01 M) and carboxylic acid (0.01 M) in ethanol (25 mL), phosphorous oxychloride (0.01 M) was added slowly in an exhaustion chamber and heated to reflux for 8–14 h. The progress of the reaction was monitored by TLC. On completion, the reaction mixture was cooled to room temperature and crushed ice was added to obtain oxadiazole derivative **(5a–5j)**. The product was recrystallized using rectified spirit as the solvent.

N-*((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)benzo[*d*]thiazol-2-amine (**5a**)*

Yield: 68 %; M.P: 215–216 °C; R_f: 0.60 (ethyl acetate and hexane, 4:1); IR (KBr): ṽ3242 (NH), 3026 (C=C), 1662 (C=N), 1271 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ7.6–7.0 (m, 9H, Ar–H), 5.8 (s, 1H, NH), 3.3 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 174.7 (1C, C5-oxadizole), 167.5 (1C, C2-oxadizole), 154.4 (1C, C2-benzothiazole), 133.8 (1C, C9-benzothiazole), 131.7 (1C, C1-phenyl), 130.2 (1C, C8-benzothiazole), 130.1 (1C, C5-benzothiazole), 129.3 (1C, C6-benzothiazole), 128.6 (1C, C4-phenyl), 128.5 (1C, C7-benzothiazole), 127.1 (1C, C4-benzothiazole), 122.5 (1C, C2-phenyl), 121.5 (1C, C6-phenyl), 120.1 (1C, C5-phenyl), 119.6 (1C, C3-phenyl), 38.4 (1C, NH$\underline{CH_2}$); MS *m/z* (%): 309 [M^{+1}] (85); anal. calcd. for $C_{16}H_{12}N_4OS$: C, 62.32; H, 3.92; N, 18.17; S, 10.40. Found: C, 62.24; H, 3.89; N, 18.19; S, 10.22.

N-*[(5-(2-nitro phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo [*d*]thiazol-2-amine (**5b**)*

Yield: 74 %; M.P: 237–239 °C; R_f: 0.72 (ethyl acetate and hexane, 4:1); IR (KBr): ṽ3263 (NH), 3016 (C=C), 1653 (C=N), 1264 (C–O–C), 1526 (NO, NO_2) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 7.2–6.8 (m, 8H, Ar–H), 5.8 (s, 1H,NH), 3.4 (s, 2H,CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 168.3 (1C, C5-oxadizole), 162.5 (1C, C2-oxadizole), 151.3 (1C, C2-benzothiazole), 148.4 (1C, C2-nitrophenyl), 146.4 (1C, C9-benzothiazole), 142.5 (1C, C1-nitrophenyl), 139.1 (1C, C8-benzothiazole), 133.2 (1C, C4-nitrophenyl), 132.1 (1C, C5-benzothiazole), 129.3 (1C, C6-benzothiazole), 128.6 (1C, C7-benzothiazole), 127.1 (1C, C4-benzothiazole), 126.4 (1C, C6-phenyl), 124.2 (1C, C3-phenyl), 121.9 (1C, C5-phenyl), 38.3 (1C, NH$\underline{CH_2}$); MS *m/z* (%): 354 [M^{+1}] (80); anal. calcd. for $C_{16}H_{11}N_5O_3S$: C, 54.38; H, 3.14; N, 19.82; S, 9.07. Found: C, 54.39; H, 3.13; N, 19.81; S, 9.11.

N-*[(5-(3,5-dinitro phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo[*d*]thiazol-2-amine (**5c**)*

Yield: 81 %; M.P: 255–256 °C; R_f: 0.68 (ethyl acetate and hexane; 4:1); IR (KBr): ṽ3253 (NH), 3019 (C=C), 1663 (C=N), 1256 (C–O–C), 1519, 1522 (NO, NO_2) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 7.6–6.4 (m, 7H, Ar–H), 5.8 (s, 1H, NH), 3.4 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 172.2 (1C, C5-oxadizole), 165.3 (1C, C2-oxadizole), 155.3 (1C, C2-benzothiazole),148.7 (2C, C3,5-dinitrophenyl), 146.3 (1C, C9-benzothiazole), 139.4 (1C, C1-dinitrophenyl), 131.3 (1C, C4-dinitrophenyl), 128.9 (1C, C8-benzothiazole), 128.3 (1C, C5-benzothiazole), 127.1 (1C, C6-benzothiazole), 124.7 (1C, C7-benzothiazole), 123.2 (1C, C4-benzothiazole), 121.8 (2C, C2,6-dinitrophenyl), 39.1 (1C, NH$\underline{CH_2}$); MS *m/z* (%): 398 [M^+] (84); anal. calcd. for $C_{16}H_{10}N_6O_5S$: C, 48.24; H, 2.49; N, 21.10; S, 8.05. Found: C, 48.18; H, 2.49; N, 21.11; S, 8.11.

N-*[(5-(2-amino phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo [*d*]thiazol-2-amine (**5d**)*

Yield: 62 %; M.P: 187–189 °C; R_f: 0.85 (ethyl acetate and hexane; 4:1); IR (KBr): ṽ3318, 3251 (NH, NH_2), 3024 (C=C), 1659 (C=N), 1276 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 8.0–7.2 (m, 8H, Ar–H), 5.8 (s, 1H, NH), 4.3 (s, 2H, NH_2), 3.3 (s,2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 171.3 (1C, C5-oxadizole), 164.3 (1C, C2-oxadizole), 154.1 (1C, C2-benzothiazole),147.6 (1C, C2-aminophenyl), 145.9 (1C, C9-benzothiazole), 144.5 (1C, C1-aminophenyl), 139.1 (1C, C4-aminophenyl), 137.8 (1C, C8-benzothiazole), 134.1 (1C, C5-benzothiazole), 131.8 (1C, C6-benzothiazole), 128.7 (1C, C7-benzothiazole), 127.1 (1C, C4-benzothiazole), 124.4 (1C, C6-aminophenyl), 123.2 (1C, C3-aminophenyl), 119.8 (1C, C5-aminophenyl), 38.7 (1C, NH$\underline{CH_2}$); MS *m/z* (%): 324 [M^{+1}] (70); anal. calcd. for $C_{16}H_{13}N_5OS$: C, 59.43; H,

4.05; N, 21.66; S, 9.92. Found: C, 59.41; H, 4.01; N, 21.67; S 9.97.

N-*[(5-(4-amino phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo [*d*]thiazol-2-amine (**5e**)*

Yield: 68 %; M.P: 178–179 °C; R_f: 0.78(ethyl acetate and hexane; 4:1); IR(KBr): ṽ3324, 3249 (NH, NH_2), 3021 (C=C), 1612 (C=N), 1273 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 8.9–7.8 (m, 8H, Ar-H), 5.7 (s, 1H, NH), 4.7 (s, 2H, NH_2), 3.8 (s, 2H; CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 167.2 (1C, C5-oxadizole), 164.1 (1C, C2-oxadizole), 154.8 (1C, C2-benzothiazole), 149.1 (1C, C4-aminophenyl), 147.2 (1C, C9-benzothiazole), 139.6 (1C, C1-aminophenyl), 138.5 (1C, C8-benzothiazole), 133.7 (1C, C5-benzothiazole), 131.2 (1C, C6-benzothiazole), 128.4 (1C, C7-benzothiazole), 127.3 (1C, C4-benzothiazole), 124.2 (1C, C2-aminophenyl), 121.8 (1C, C6-aminophenyl), 121.7 (1C, C3-aminophenyl), 119.3 (1C, C5-aminophenyl), 38.3 (1C, NH<u>CH_2</u>); MS *m/z* (%): 323 [M^+] (40); anal. calcd. for $C_{16}H_{13}N_5OS$: C, 59.43; H, 4.05; N, 21.66; S, 9.92. Found: C, 59.46; H, 4.07; N, 21.71; S 10.07.

N-*[((5-(2-hydroxy)phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo[*d*]thiazol-2-amine (**5f**)*

Yield: 47 %; M.P: 213–214 °C; R_f: 0.65(ethyl acetate and hexane; 4:1); IR (KBr): ṽ3321 (OH), 3245 (NH), 3023 (C=C), 1663 (C=N), 1334 (C–O) 1278 (C–O–C)cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 10.3 (s, 1H, OH), 7.7–7.1 (m, 8H, Ar-H), 5.7 (s, 1H, NH), 3.3 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 169.1 (1C, C5-oxadizole), 164.6 (1C, C2-oxadizole), 156.3 (1C, C2-benzothiazole), 148.9 (1C, C2-phenol), 147.1 (1C, C9-benzothiazole), 141.6 (1C, C1-phenol), 138.3(1C, C4-phenol), 134.1(1C, C8-benzothiazole), 131.6 (1C, C5-benzothiazole), 127.3 (1C, C6-benzothiazole), 126.8 (1C, C7-benzothiazole), 124.9 (1C, C4-benzothiazole), 124.3 (1C, C6-phenol), 123.8 (1C, C3-phenol), 119.5 (1C, C5-phenol), 38.2 (1C, NH<u>CH_2</u>); MS *m/z* (%): 324 [M^+] (100); anal. calcd. for $C_{16}H_{12}N_4O_2S$: C, 59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 59.23; H, 3.71; N, 17.22; S, 9.76.

N-*[((5-(4-hydroxy)phenyl) 1,3,4-oxadiazol-2-yl) methyl] benzo[*d*]thiazol-2-amine (**5g**)*

Yield: 53 %; M.P: 192–193 °C; R_f: 0.75 (ethyl acetate and hexane, 4:1); IR (KBr): ṽ3343 (OH), 3239 (NH), 3027 (C=C), 1661 (C=N), 1331 (C–O) 1272 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 10.6 (s, 1H, OH), 8.6–7.7 (m, 8H, Ar–H), 5.8 (s, 1H, NH), 3.3 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 171.4 (1C, C5-oxadizole), 166.2 (1C, C2-oxadizole), 157.1 (1C, C2-benzothiazole), 149.4 (1C, C4-phenol), 147.8 (1C, C9-benzothiazole), 142.7 (1C, C1-phenol), 139.4 (1C, C8-benzothiazole), 134.7 (1C, C5-benzothiazole), 133.1 (1C, C6-benzothiazole), 129.6 (1C, C7-benzothiazole), 127.9 (1C, C4-benzothiazole), 126.4 (1C, C2-phenol), 124.2 (1C, C6-phenol), 123.8 (1C, C3-phenol), 123.3 (1C, C5-phenol), 37.4 (1C, NH<u>CH_2</u>); MS *m/z* (%): 324 [M^+] (60); anal. calcd. for $C_{16}H_{12}N_4O_2S$: C,59.25; H, 3.73; N, 17.27; S, 9.89.Found:C, 59.31; H, 3.79; N, 17.32; S, 9.94.

N-*((5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)methyl)benzo[*d*] thiazol-2-amine (**5h**)*

Yield: 69 %; M.P: 246–247 °C; R_f: 0.72 (ethyl acetate and hexane; 4:1); IR(KBr): ṽ3245, 3215 (NH), 3023 (C=C), 1663 (C=N), 1278 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 7.8–7.4 (m, 8H, Ar–H), 5.7 (s, 1H, NH), 3.3 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 169.1(1C, C5-oxadizole), 164.6 (1C, C2-oxadizole), 147.5 (1C, C2-benzothiazole), 147.3 (1C, C6-pyridin-3-yl), 146.9 (1C, C4-pyridin-3-yl), 143.6 (1C, C1-pyridin-3-yl), 141.4 (1C, C9-benzothiazole), 138.3 (1C, C8-benzothiazole), 133.5 (1C, C5-benzothiazole), 131.3 (1C, C6-benzothiazole), 124.1 (1C, C7-benzothiazole), 123.7 (1C, C2-pyridin-3-yl), 123.4 (1C, C3-pyridin-3-yl), 121.1 (1C, C4-benzothiazole), 40.4 (1C, NH<u>CH_2</u>); MS *m/z* (%): 309 [M^+] (100); anal. calcd. for $C_{15}H_{11}N_5OS$: C, 58.24; H, 3.58; N, 22.64; S, 10.37. Found:C, 58.32; H, 3.56; N, 22.61; S, 10.22.

N-*[(1,3,4-oxadiazol-2-yl) methyl] benzo[*d*]thiazol-2-amine (**5i**)*

Yield: 94 %; M.P: 204–206 °C; R_f: 0.65(ethyl acetate and hexane; 4:1); IR(KBr): ṽ3248 (NH), 3028 (C=C), 1661 (C=N), 1274 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 8.6 (s, 1H, C_5-oxadiazole),7.9–7.4 (m, 4H, Ar–H), 5.6 (s, 1H, NH), 3.2 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 167.6(1C, C2-oxadizole), 163.6 (1C, C5-oxadizole), 148.7 (1C, C2-benzothiazole), 147.3 (1C, C9-benzothiazole), 146.9 (1C, C8-benzothiazole), 139.7 (1C, C5-benzothiazole), 133.4 (1C, C6-benzothiazole), 131.3 (1C, C7-benzothiazole), 124.1 (1C, C4-benzothiazole), 39.3 (1C, NH<u>CH_2</u>); MS *m/z* (%): 233 [M^{+1}] (100); anal. calcd. for $C_{10}H_8N_4OS$: C, 51.71; H, 3.47; N, 24.12; S, 13.81. Found: C, 51.72; H, 3.49; N, 24.15; S, 13.86.

N-*[((5-methyl) 1,3,4-oxadiazol-2-yl) methyl] benzo[*d*] thiazol-2-amine (**5j**)*

Yield: 87 %; M.P: 213–214 °C; R_f: 0.76(ethyl acetate and hexane; 4:1); IR(KBr): ṽ3245 (NH), 3023 (C=C), 1663

(C=N), 1278 (C–O–C)cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 8.8–7.8 (m, 4H), 5.8 (s, 1H, NH), 3.3 (s, 2H, CH_2), 1.2 (s, 3H, CH_3); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 168.4 (1C, C2-oxadizole), 164.2 (1C, C5-oxadizole), 147.5 (1C, C2-benzothiazole), 146.8 (1C, C9-benzothiazole), 145.4 (1C, C8-benzothiazole), 139.1 (1C, C5-benzothiazole), 134.2 (1C, C6-benzothiazole), 133.6 (1C, C7-benzothiazole), 124.6 (1C, C4-benzothiazole), 40.1 (1C, NH$\underline{CH_2}$), 12.6 (1C, CH_3); MS *m/z* (%): 246 [M^+] (100); anal. calcd. for $C_{11}H_{10}N_4OS$: C, 53.64; H, 4.09; N, 22.75; S, 13.02. Found: C, 53.62; H, 4.06; N, 22.76; S, 12.87.

*Synthesis of N-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl) benzo[d]thiazol-2-amine (**5k**)*

To a solution of the acyl hydrazide4 (2.22 g, 0.01 M), dissolved in ethanol (25 mL), equimolar quantities of carbon disulphide (0.01 M) and potassium hydroxides (0.01 M) were added to the solution. The contents were heated to reflux for 7 h. The progress of the reaction was monitored by TLC. On completion, the mixture was neutralized with dilute HCl to obtain the solid product **5k**. The product separated out was filtered and recrystallized using rectified spirit as the solvent.Yield: 72 %; M.P: 217–219 °C; R_f: 0.76 (ethyl acetate and hexane; 4:1); IR(KBr): ṽ3238 (NH), 3087 (C=C), 2587 (SH), 1620 (C=N), 1230 (C–O–C) cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6, ppm): δ 12.8 (s, 1H, SH), 7.5–7.4 (m, 4H, Ar–H), 5.9 (s, 1H, NH), 3.4 (s, 2H, CH_2); ^{13}CNMR (400 MHz, DMSO-d_6, ppm): δ 179.7 (1C, C5-oxadizole), 169.2 (1C, C5-oxadizole), 156.9 (1C, C2-benzothiazole), 147.3 (1C, C9-benzothiazole), 143.8 (1C, C8-benzothiazole), 126.7 (1C, C5-benzothiazole), 125.7 (1C, C6-benzothiazole), 122.4 (1C, C7-benzothiazole), 121.4 (1C, C4-benzothiazole), 42.7 (1C, NH$\underline{CH_2}$); MS *m/z* (%): 264 [M^+] (40); anal. calcd. for $C_{10}H_8N_4OS_2$: C, 45.44; H,3.05; N, 21.20; S, 24.26. Found: C, 45.42; H, 3.03; N, 21.23; S, 24.18.

In vitro radical scavenging activity

The in vitro radical scavenging activity of the newly synthesized 1,3,4-oxadiazoles were screened by DPPH radical scavenging assay method (Ma et al. 2013).To the 2 mL solutions of synthesized compounds (25 µM), 2 mL DPPH solution (25 µM) was added into the test tube. The solution was incubated at 37 °C for 30 min and the absorbance of each solution was measured at 517 nm against the reagent blank solution. The ascorbic acid (25 µM) was used as the reference standard. The experimental values obtained for DPPH radical scavenging assays were statistically analyzed. Equation 2 presents the formula for the calculation of the percent free radical scavenging activity.

$$\% \text{ Radical Scavenging} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100 \quad (2)$$

Eq. 2: Calculation of percentage radical scavenging activity

In vivo anti-inflammatory activity

All the animal experiments adapted in the study were reviewed and approved by the Institutional Animal Ethics Committee (Ref No. 059/2010). The experimental procedures and protocols were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Govt. of India. The animals were obtained from the JSS Medical College, Mysuru, India, and were maintained in colony cages at 25 ± 2 °C, relative humidity of 45–55 %, under a 12 h light and dark cycle; they were fed with standard animal feed. All the animals were acclimatized for a week before use. Animals were deprived of food 12 h prior to the experiment and only water was allowed *ad libitum*. Acute toxicity studies were performed to estimate the main study dose value of the synthesized compounds **5a–5k** as per the organization for economic co-operation and development (OECD) guidelines (TG 420 and 425).

The in vivo anti-inflammatory activity of the newly synthesized 1,3,4-oxadiazoles were evaluated by carrageenan-induced paw edema test method (Kucukguzel et al. 2007) using groups of Albino rats of *Wistar* strain of either sex, weighing 100–120 g each and the animals were randomly divided into groups of six. The first group received only 0.5 % carboxymethyl cellulose (CMC) solution and served as the untreated control. The second group received 25 mg Kg^{-1} indomethacin orally and served as the positive control. The other groups received the test compounds suspended in 0.5 % CMC and given to the rats orally at a dose of 25 mg Kg^{-1}. After 1 h of the administration of the test compounds and the standard drug, the animals were injected with 0.1 mL of carrageenan (1 % solution in normal saline) in the subplantar region of the right hind. The right hind paw volume (mL) was measured before and after 0.5, 1, 2 and 4 h of carrageenan treatment by using mercury displacement technique with the help of a digital plethysmograph (7141 UGO Basile, Italy). The reduction in edema volume of the carrageenan-treated paw at regular time intervals of the test group were compared with the negative control and the reference standard indomethacin and the data were statistically analyzed using the repeated measures ANOVA with Dunnett's test. Equation 3 presents the formula for the calculation of the percent

anti-inflammatory activity.

$$\% \text{ Inhibition} = \left(\frac{a-x}{a}\right) \times 100 \quad (3)$$

Eq. 3: Calculation of percentage inhibition of edema

Where '*a*' is the mean increase in edema in the control group of animals and '*x*' is the mean increase in edema in the test group at regular interval of time. The mean percent inhibition of edema by the reference standard indomethacin and the test compounds at 25 mg Kg^{-1} concentrations were compared with the negative control using the repeated measures ANOVA with Dunnet's test.

In vitro COX inhibition assay

The enzyme inhibitory activity of the oxadiazole derivative **5h** on both COX-1 (ovine) and COX-2 (human recombinant) was measured calorimetrically by enzymes immunoassay (EIA) kit using COX inhibitor screening assay purchased from Cayman Chemicals (Cayman Chemical Co., Ann Arbor, MI, USA). The test compound **5h** was screened at 6.25, 12.5 and 25.0 µM strength for the inhibition studies and procedure following manufacturer's protocol. The absorbance at 415 nm was read by using a microtitre plate reader (Automated Microplate Reader BIOTEK; ELX800). In briefly, heme and COX (COX-1/2) enzyme were added to tubes containing COX reaction buffer. The mixture was vortex mixed and exposed to DMSO (negative control) or the test compound 5h in DMSO for 5 min at 37 °C. Later, arachidonic acid (20 µM) was added and further incubated for 5 min. This was followed by the addition of hydrochloric acid (1 M) to stop the COX reaction and followed by chemical reduction with stannous chloride. The COX activity is proportionate to the amount of COX derived PGH_2. The PGH_2 is reduced by stannous chloride in the final step to produce $PGF_{2\alpha}$ in the reaction tube. The so generated $PGF_{2\alpha}$ is measured by the EIA kit.

Acute ulcerogenic activity

The acute ulcerogenicity for the test compound 5h was determined according to the reported method (Cioli et al. 1979). The test animals were Albino rats of *Wistar* strain of either sex, weighing 100–120 g each and the animals were deprived of food 24 h prior to the experiment and only water was allowed *ad libitum*. The test group consisting of six animals, were administered the compound 5h orally as a single dose of 50 mg Kg^{-1}. The control group received only 0.5 % (m/V) carboxymethyl cellulose solution. After 1 h post the treatment, the animals were sacrificed by an overdose of chloroform. The animals were dissected along the greater curvature and stomach was removed and washed gently in normal saline. The mucosal damage was examined by means of a magnifying glass. Mucosal damage was assessed for each stomach according to the following scoring system: 1+ redness (petechiae); 2+ hemorrhagic streaks (hemorrhage with moderate erosion) and 3+ ulcers (hemorrhage with extensive and severe lesions).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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