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Synthesis, antiinflammatory evaluation and docking analysis of some novel 1,3,4-oxadiazole derivatives

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In the present study, novel series of 5-{[4-(acetylamino) phenoxy] methyl}-1,3,4-oxadiazole-2-yl-sulfanyl-N-substituted-2-acetamide/2-propanamide/3-propanamide derivatives (**8a** to **8c**, **9a** to **9c** and **10a** to **10c**) have been synthesized. The newly synthesized compounds have been tested for their anti-inflammatory and anti-ulcerogenic activities *in vivo*. Among the present series the compound **8c** is found to be most active against inflammation with inhibition of 65.34% and has been observed to be safe ulcerogenically. It is observed that introduction of an asymmetric centre near the sulfur atom decreases the activity. Molecular docking simulations have been carried out for the compounds. Structures from all the series fit into the active site of cyclooxygenase-2 enzyme with least binding energies and exhibit favorable binding interactions required for the selective inhibition of cyclooxygenase- 2.

Keywords: 1,3,4-Oxadiazole, cyclooxygenase-2, anti-inflammatory, docking, MOE

Nonsteroidal anti-inflammatory drugs (NSAIDs) used for their anti-inflammatory, analgesic and antipyretic therapeutic effects act by cyclooxygenase (COX-1 and COX-2) inhibition. However, their therapeutic benefit is often restricted by some adverse reactions at the gastrointestinal level (gastritis, ulcer, bleeding), focal necrosis and sometimes frantic ulcers[1-3]. Others, like the cardiovascular risk, are still being assessed today[4,5]. It is now well accepted that gastrointestinal side-effects are mainly associated with the inhibition of the cyclooxygenase-1 (COX-1), while cardiovascular side-effects are directly linked with the inhibition of COX-2 possibly by blocking PGI2 biosynthesis while not hindering TXA2 formation[6-9]. The withdrawal from the market of most COX-2 specific inhibitors has proven that they are associated with significantly higher cardiovascular risks[10]. However, the application of COX-2 inhibition in other therapeutic areas including diabetes[11], cancer[12,13], and kidney dysfunction[14] has resulted in renewed interest in selective COX-2 inhibitors. COX-2 remains an important target for the treatment of debilitating diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA).

During the last decade, 686 patent applications have been filed on oxadiazole scaffolds pertaining to drug discovery programmes. From drug discovery perspective, oxadiazole derivatives, such as zibotentan and ataluren are undergoing clinical trials for the treatment of cancer and cystic fibrosis, respectively[15]. Several published reports indicate that incorporation of a five membered heterocyclic moiety having two or more nitrogen atoms in the ring (azoles) such as substituted 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole can significantly reduce the gastric toxicity and increase the anti-inflammatory activity of NSAIDs[16-18]. Several studies also reported that safe NSAIDS can be obtained by derivatization of the carboxylic acid group with heterocyclic, viz. 1,3,4-oxadiazole, 1,2,4-triazole, etc. which converted them less ulcerogenic and with an increased anti-inflammatory activity[19-22]. In addition, several reports have advocated for oxadiazole derivatives as potent anti-inflammatory agents[23,24]. Moreover heterocycles bearing a symmetrical 1,3,4- oxadiazole-2-thione, structure possess promising anti-inflammatory activity.

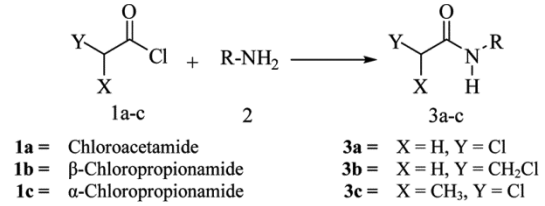
Enol-carboxamide (e.g. meloxicam) represents interesting anti-inflammatory agents bearing the carboxamides have been reported in literature. Noticeably these compounds were reported to inhibit not only COX but also 5-lipoxynase (5-LO) and are regarded as new type of dual inhibitors[25-28].

Keeping these point discussed above and in continuation of our search for novel antiinflammatory agents[29-31], we have decided to combine the two pharmacophore for testing their anti-inflammatory and anti-ulcerogenic activities viz. N-substituted-carboxamides are linked to 1,3,4 oxadiazole pharmacophore through sulfur atom to produce target compounds as a possible antiinflammatory agents. Before stepping into its pharmacological evaluation in silico docking study by using MOE 2009.10 software for its affinity towards the receptor protein cyclooxygenase-2 (prostaglandin synthase-2) was performed.

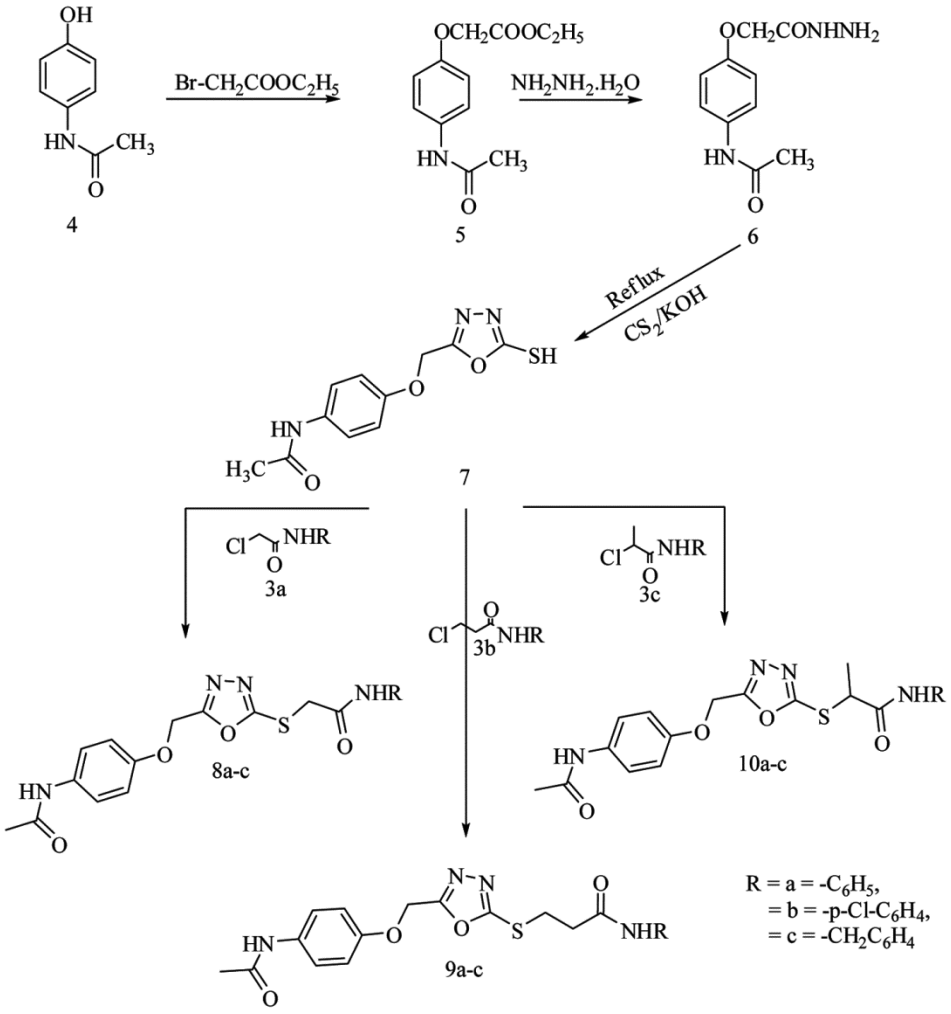
Results and Discussion

Chemistry

The synthetic routes for targeted structures are shown in (Scheme I and Scheme II). Esterification of *N*-(4-hydroxyphenyl) acetamide (**4**) gave Ethyl-2-(4-acetamidophenoxy) acetate (**5**) which when refluxed with hydrazine hydrate gave *N*-[4-(2-hydrazinyl-2-oxoethoxy) phenyl] acetamide (**6**). *N*-{4[(5-sulfanyl-1,3,4-oxadiazole-2-yl)methoxy]phenyl}acetamide (**7**) was obtained by reacting carbon disulfide and hydrazide (**6**) in the alkaline environment and subsequent acidification. Different N-substituted-carboxamides: chloroacetamides (**3a**), β chloropropionamides (**3b**) and α chloropropionamides (**3c**) were obtained by condensation of appropriate



Scheme I



Scheme 2

amines with chloroacetyl chloride, 3-chloropropanoyl chloride and 2-chloropropanoyl chloride respectively. A series of title compounds (**8a-c, 9a-c, 10a-c**) were thereafter prepared by condensation of sodium salts of *N*-{4 [(5-sulfanyl-1,3,4-oxadiazole-2-yl) methoxy]phenyl}acetamide (**7**) with differently substituted chloroacetamides (**3a**), β-chloropropionamides (**3b)** and α-chloropropionamides (**3c**), under *Schotten-Baumann* reaction conditions. TLC was used to check progress and completion of the reaction.

IR absorption bands at 3294 cm^{-1} (N-H), 2363 cm^{-1} (S-H), 1667 cm^{-1} (C=O), 1565 cm^{-1} (C=N) and 1223 cm^{-1} (stretching of oxadiazole ring) confirms the structure of *N*-{4[(5-sulfanyl-1,3,4-oxadiazole-2-yl) methoxy]phenyl}acetamide (**7**). In the presence of base substitution reactions are easily accomplished on –SH group owing to its acidic nature. Formation of 5-{[4-(acetylmino) phenoxy]methyl}-1,3,4 oxadiazole-2-yl-sulfanyl-N-substituted-carboxamides (**8**, **9**, **10**) were confirmed by recording their IR, ^{1}H NMR and mass spectra. IR spectrum of oxadiazole (**8a**) showed absorption at 3096 cm^{-1} due to N-H stretching of amide, band at 3101 cm^{-1} due to aromatic stretching, broad stretching band at 1640 cm^{-1} due to amide carbonyl group (C=O) and absorption band at 1093 cm^{-1} account for stretch of oxadiazole ring.

The ^{1}H NMR spectrum of **8a** showed multiplet in the region of δ, 6.90–7.5 corresponding to aromatic proton, two singlets for two amide protons (CONH) were observed in the region of δ, 10.34 and δ, 10.01 respectively. Similarly singlet of two protons in the region of δ, 3.85 corresponding to $–CH_2$ of acetamide, a singlet at δ, 2.18 corresponding to three protons of the methyl group and a singlet of two methylene proton ($O-CH_2$) in the region of δ, 5.27 were recorded.

The mass spectrum of **8a** showed molecular ion peak at m/z 399.10 $(M+1)^+$ which is in agreement with the molecular formula $C_{19}H_{19}N_4O_4S$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part.

Acute antiinflammatory activity

All the synthesized compounds (**8a-c, 9a-c and 10a-c**) were tested for their anti-inflammatory activity and assayed at the initial dose of 20 mg/kg i.p. As a reference substance in experiment diclofenac sodium was used. None of the compound induces direct signs of toxicity or mortality in the animals subjected to experiment. All the title compounds exhibited anti-inflammatory activity. Aromatic ring substituents, size of these substituents and chain length between S and N (or carbon intermediate) was taken into consideration to draw SAR for maximum antiinflamatory activity. Out of nine tested compounds four compounds (**8a**, **8b**, **8c**, and **9a**) showed a highly significant anti-inflammatory activity. Activity for tested compound was observed in the range of 34.65% to 65.34%. Reference drug diclofenac exhibited 73.26% inhibition (Table I) after 4 hr. The most active compound was **8c** that produced a highly significant inhibition 65.34%. Among the compounds (**8a-c**), compounds bearing benzyl moiety at the terminal nitrogen atom showed 65.34% inhibition. Compounds (**10a-c**) displayed % inhibition in the range of (34.65-44.55%) lower than that of compounds (**8a-c**) (57.42-65.34%) and (**9a-c**) (51.48-54.45%). Above results revealed that

- With compounds bearing unsubstituted phenyl or benzyl group at the terminal nitrogen atom best results was showed by **8c** (64.34%).
- With the compounds bearing chlorosubstituted phenyl group at the terminal nitrogen atom best result was shown by **8b** (57.42%).
- Substitution of phenyl ring with chlorine slightly decreases the activity.
- Introduction of asymmetric centre (**9a-c**) near sulfur atom decreases the activity. It may be hypothesized that the activity may be attributed selectively to one isomer.

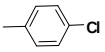
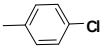
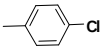
Chronic antiinflamatory activity

The compounds (**8a-c**) which displayed potent acute antiinflammatory activity were further evaluated for chronic antiinflammatory activity by cotton pellet granuloma method. Compound **8a** and **8c** showed 27.02 % and 38.99 % inhibition respectively whereas diclofenac showed 47.44 % inhibition in cotton pellet granuloma method (Table I). Compound **8c** which exhibited highest activity in acute inflammatory activity also exhibited comparable activity to that of standard in chronic antiinflammatory activity.

Acute Ulcerogenic Activity

The tested compounds **8a** and **8c** showed significant decline in ulcerogenic severity index 0.52±0.13 and 0.38±0.09 respectively, whereas the reference drug diclofenac recorded high severity index of 1.95±0.23.

Table I — Anti-inflammatory (acute and chronic) and ulcerogenic activity of the synthesized compounds

Compd	R	Acute Antiinflammatory Activity Paw vol. Mean ± SEM % Inhibition				Chronic Antiinflammatory Activity (**8a** and **8c**)		Ulcerogenic Activity (**8a** and **8c**)
		3h	4h	3h	4h	Net wt. of granul. tissue	% Inhibition	(severity index ± SEM)
STD		0.75±0.16	0.54±0.08	61.92	73.26	22.40 ± 1.43	47.44**	1.95±0.23**
CNT		1.97±0.18	2.02±0.21	–	–	42.62± 2.20	–	–
8a		0.98±0.10	0.75±0.16	50.25	62.87*	31.10 ± 1.80	27.02**	0.52±0.13**
8b		1.25±0.20	0.86±0.17	36.54	57.42*	–	–	–
8c		0.95±0.16	0.70±0.07	51.77	65.34*	26.00 ± 1.67	38.99*	0.38±0.09**
9a		0.92±0.08	0.92±0.03	53.29	54.45*	–	–	–
9b		1.10±0.12	0.98±0.08	44.16	51.48	–	–	–
9c		0.88±0.21	0.95±0.09	55.32	52.97*	–	–	–
10a		1.42±0.26	1.18±0.19	27.91	41.58	–	–	–
10b		1.23±0.34	1.32±0.10	37.56	34.65	–	–	–
10c		1.18±0.30	1.12±0.20	40.10	44.55	–	–	–

**p<0.01*vs* Standard, *p<0.01*vs* Standard (n=6)

Molecular Docking Study

In modern drug designing scenario, the molecular docking is commonly used for the understanding of target-receptor binding interaction and is quite often used to predict the binding orientation of target molecule of the lead with their protein receptor in order to find out the affinity of that target compound. In order to rationalize the biological results of our compounds, an in silico molecular docking studies with the cyclooxygenase-2 enzyme were carried out to determine the best conformation.

All the docked conformations for each compound were analyzed and it was found that the most favorable docking poses with a maximum number of interactions were those which were ranked the highest score based on the minimal binding energy, which was computed as a negative value by the MOE software. Then considering the root mean square deviation (RMSD) value, generally acceptable range of the RMSD when the model is overlapped to the template is 2 Å. For protein ligand docking 2 Angstroms is good, 1 Angstrom and less is great. But this RMSD value may not be considered as the only criteria for evaluation of the model constructed. Some deviations at times can be considered. Based on this discussion, in this present report, the compounds showed the RMSD value range of between 1.7278 to 4.1982 and this result suggests that some of the synthesized compounds showed a good RMSD value (Table II).

The MOE 2009.10 software generated docking score of the all the ligands were summarized in Table II. The most favorable docking poses of the 10 docked conformations for each molecule were analyzed for further investigation of the ligand interactions within the active sites. The wonderful number of interactions with active site residues coupled with favorable binding energy state that these target molecules may serve as an effective replacement agent for the antiinflammatory drugs. These ligands showed a proper binding pattern and anchored tightly inside the active site canyon (Site I) of the protein. The 2D ligand-protein interactions were visualized using the MOE ligand interaction program for all the molecules are shown in Figure 1. The best docking poses of almost all the synthesized compounds were formed a single cluster inside the active site cleft of the receptor. Among these 9 synthesized compounds, the best two molecules are viz. **8a** and **9a** ligands and they showed the best

Table II — Molecular modeling results of synthesized compound with cyclooxygenase-2 (PDB ID: 1CX2)

Compd	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	No. of conf.	Residues interacting with the ligand
8a	−32.3252	3.6623	5.0791	−74.1938	−11.7496	−32.3252	10	Polar, Receptor contact SCA: Cys 47
8b	−27.8487	3.3844	4.3353	−48.8384	−10.2949	−27.8487	10	Polar, Receptor contact SCA: Cys 47
8c	−29.5931	3.3401	−3.0251	−72.4360	−9.4275	−29.5931	10	Receptor contact SCA: Lys 473
9a	−25.0747	3.2582	−3.7748	−81.1957	−10.9601	−25.0747	10	Polar SCA: Gln 461; basic arene-cation: Arg 44; acidic SCD: Asp 125
9b	−34.1501	1.7278	−15.1777	−112.2330	−11.9900	−34.1501	10	Polar SCA: Asn 34
9c	−32.1501	3.4150	−22.9362	−93.9287	−10.6189	−32.1501	10	Polar, Receptor contact SCA: Cys 47
10a	−34.4209	2.9410	3.0597	−85.0496	−10.8177	−34.4209	9	Acidic polar SCA: Glu 465
10b	−28.7784	3.6838	14.2492	−107.3403	−10.8177	−28.7784	10	Polar SCA: Arg 44
10c	−29.1449	4.1982	−1.0483	−92.1654	−11.5530	−29.1449	10	Polar, Receptor contact SCA: Asn 34
Diclof.	−23.4125	3.0930	45.5496	−43.3913	−10.4837	−23.4125	9	Greasy SCA: Ala 156; Greasy BBD: Pro 154

S - The final score, **rmsd_refine-** The root mean square deviation between the pose before refinement and the pose after refinement, **E_conf-** The energy of the conformer, **E_place -** Score from the placement stage, **E_score1-** Score from the rescoring stage(s), **E_refine-** Score from the refinement stage, **No. of conf-** number of conformations generated by ligand, **SCA-** side chain acceptor, **SCD-**side chain donor and **BBD-** Back bone donor.

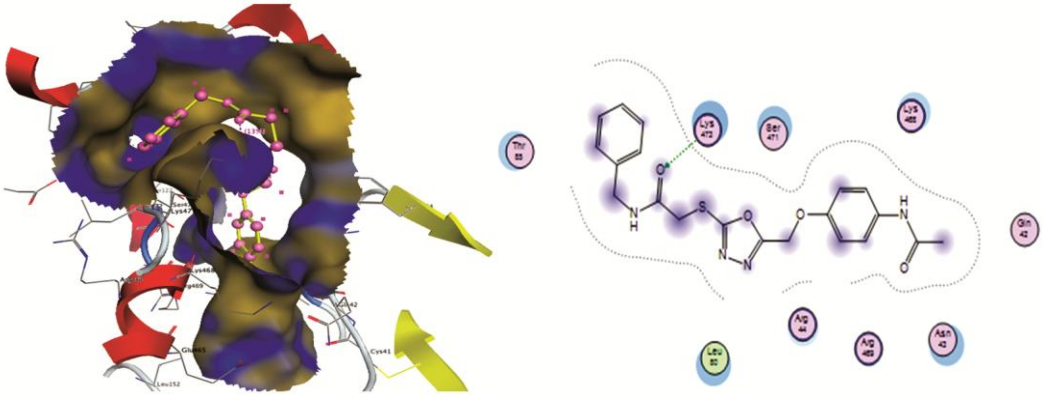


Figure 1 — Ligand Receptor interaction and binding surface of the compound **8c** with PDB ID: 1CX2

interaction within the receptor are shown in Figure 2. The calculated surface analysis pocket for these molecules was shown in Figure 2. The synthesized compounds have a higher binding affinity with the receptors, in the narrow range of binding energy for the protein PDB ID: 1CX2 is in the range of -25.0747 to -34.4209 kcal/mol, which is compared to that of reference drug diclofenac (-23.4125 kcal/mol, Table II).

Docking analysis reveals that the synthesized compounds interacted with receptor through side chain acceptor and side chain donor and arene – cation interaction amino acid binding pocket (Figure 2). The numbers of conformations generated by all the compounds were 10 which indicated that flexibility is an important parameter for the ligand to dock deeply within the binding pocket of cyclooxygenase-2 enzyme. The lowest docking score of the compounds indicates compounds are active at this energy of conformation. Further a careful calculation of surface analysis of the binding pocket of this compounds indicated that all of them were adopted a position in a hydrophobic cage surrounded by the following amino acids residues such as, Gln 461, Arg 44, Asp 125, Lys 473, Cys 47, Asn 34, Glu 465, Ser 49, Tyr 136, Gly 135, Ser 471, Pro 153, Leu

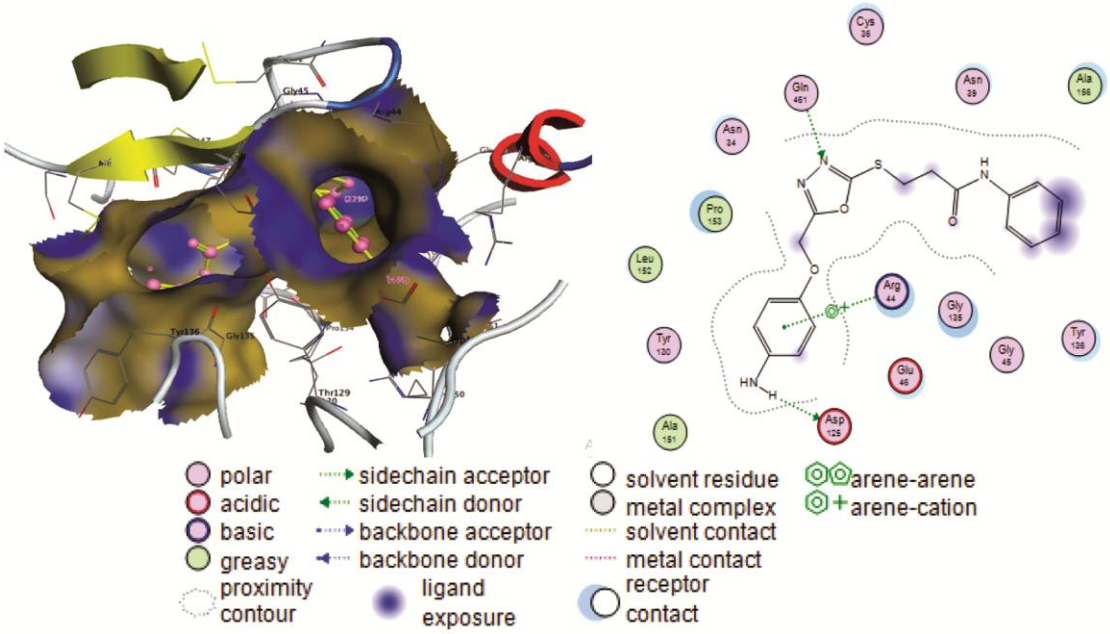


Figure 2 — Ligand Receptor interaction and binding surface of the compound **9a** with PDB ID: 1CX2

152, and etc. and these were approach closely to the ligands for the strong interactions.

Experimental Section

Precoated silica gel plates (Merk, Darmstadt, Germany) were used to check progress of reaction and to check homogeneity of compounds by using solvent system chloroform: methanol (8:2). Visualization was done against UV lamp. Melting points were checked by open capillary tubes and are uncorrected. Shimadzu-8400 FTIR spectrophotometer was used to record IR spectra and Bruker spectrometer was used to record ^{1}H NMR spectra. Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer. Shimadzu LCMS 2010 spectrometer was used for generating mass spectra. The entire chemicals were provided by Sigma-Aldrich.

General procedure for the synthesis of N-substituted carboxamides, 3a-c

Different N-substituted carboxamides were synthesized as per the reported method[32].

Synthesis of ethyl-2-(4-acetoamidophenoxy) acetate, 5

Ethyl-2-(4-acetoamidophenoxy) acetate (**5**) was prepared according to reported method[33]. Yield 79 %, m.p. 90-94 °C.

Synthesis of N-[4-(2-hydrazinyl-2-oxoethoxy) phenyl] acetamide, 6

N-[4-(2-hydrazinyl-2-oxoethoxy) phenyl] acetamide (**6**) was prepared according to reported method[33]. Yield 77 %, m.p. 180-184 °C.

Synthesis of N-{4[(5-sulfanyl-1,3,4-oxadiazole-2-yl)methoxy]phenyl} acetamide, 7

To a solution of N-[4-(2-hydrazinyl-2-oxoethoxy) phenyl] acetamide **6** (0.013 mol) in ethyl alcohol (40 ml), potassium hydroxide (0.013 mol) and carbon disulphide 1.5 ml (0.025 mol) were added dropwise with continuous stirring. The resulting solution was then refluxed until the evolution of hydrogen sulphide gas almost ceased, concentrated, added excess water and acidified with concentrated HCl. The precipitated solid obtained was recrystallized from hot ethanol[34]. Yield 64%, m.p. 220-222 °C. Colour: White crystalline solid, IR (KBr):): 3294 (N-H), 2363 (S-H), 1667 (C=O), 1565 (C=N), 1223 (C-O-C). ^{1}H NMR: δ 10.04 (s, 1H, NH), 6.95-7.52 (m, 4H, Ar-H), 5.28 (s, 2H, O-CH_2), 3.06 (s, 1H, SH), 2.16 (s, 3H, CH_3).

Synthesis of 5-{[4-(acetylmino) phenoxy]methyl}-1,3,4 oxadiazole-2-yl-sulfanyl-N-substituted-2-acetamide (8a-c), 2-propanamide (9a-c) and 3-Propanamide (10a-c)

Metallic sodium (0.038 mol) was added in dry ethanol and solution was cooled. To this

solution *N*-{4[(5-sulfanyl-1,3,4-oxadiazole-2-yl) methoxy] phenyl}acetamide **7** was added with stirring, at about 15 ºC and the resulting solution was filtered. The excess of solvent was removed by heating on water bath and added cold water. To the clear solution thus obtained added N-substituted-chloroacetamides **(3a)** (0.00038 mol) in small portion with stirring. Reaction mixture was then stirred for another 8 h at temperature around 60 to 65 ºC. The solid get precipitated out in between the stirring. The mixture was kept at room temperature for two to three hour. The resultant mixture is then filtered and product 5-{[4-(acetylmino) phenoxy]methyl}-1,3,4 oxadiazole-2-yl-sulfanyl-N-substituted-2-acetamide (**8a-c**) obtained was collected on wattman filter paper, dried and was recrystallized from super dried ethanol. Compounds **9a-c** and **10a-c** were prepared by the same procedure[35].

2-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-Phenylacetamide, 8a: m.p. 194-196^{0}C. Yield: 55%. IR (KBr): 3196 (N-H), 3101 (Ar-H), 1640 (C=O), 1545 (C=N), 1093 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.34 (s, 1H, NH), 10.02 (s, 1H, NH), 6.90-7.51 (m, 9H, Ar-H), 5.27 (s, 2H, O-CH_2), 3.85 (s, 2H, S-CH_2), 2.18 (s, 3H, CH_3); MS: *m/z* $(M+1)^+$ 399.10. Anal. Calcd for $C_{19}H_{19}N_4O_4S$: C 57.27; H 4.55; N 14.06. Found: C 57.25; H 4.52; N 14.02.

2-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl)acetamide, 8b: m.p. 146-148^{0}C. Yield: 43%. IR (KBr): 3233 (N-H), 3057 (Ar-H), 1622 (C=O), 1550 (C=N), 1087 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.34 (s, 1H, NH), 10.01 (s, 1H, NH), 6.92-7.49 (m, 8H, Ar-H), 5.20 (s, 2H, O-CH_2), 3.87 (s, 2H, S-CH_2), 2.16 (s, 3H, CH_3); MS: *m/z* $(M+2)^+$ 434.02. Anal. Calcd for $C_{19}H_{17}N_4O_4ClS$: C 52.72; H 3.96; N 12.94. Found: C 52.69; H 3.95; N 12.90.

2-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-benzyl acetamide, 8c: m.p. 138-140^{0}C. Yield: 60%. IR (KBr): 3229 (N-H), 3047 (Ar-H), 1651 (C=O), 1545 (C=N), 1082 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.21 (s, 1H, NH), 9.38 (s, 1H, NH), 6.92-7.50 (m, 9H, Ar-H), 5.10 (s, 2H, O-CH_2), 4.42 (s, 2H, Ar-CH_2), 3.80 (s, 2H, S-CH_2), 2.10 (s, 3H, CH_3); MS: *m/z* $(M+1)^+$ 412.07. Anal. Calcd for $C_{20}H_{20}N_4O_4S$: C 58.24; H 4.89; N 13.58. Found: C 58.23; H 4.88; N 13.55.

3-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-phenylpropanamide, 9a: m.p.110-112^{0}C. Yield: 56%. IR (KBr): 3343 (N-H), 3043 (Ar-H), 1565 (C=N), 1681 (C=O), 1067 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.01 (s, 1H, NH), 9.50 (s, 1H, NH), 7.08-7.51 (m, 9H, Ar-H), 5.20 (s, 2H, O-CH_2), 3.85-3.89 (t, 2H, S-CH_2, *J* = 6.40 Hz), 2.78-2.82 (t, 2H, $COCH_2$, *J* = 6.40 Hz), 2.44 (s, 3H, CH_3); MS: *m/z* $(M+1)^+$ 413.1. Anal. Calcd for $C_{20}H_{20}N_4O_4S$: C 58.24; H 4.89; N 13.58. Found: C 58.22; H 4.88; N 13.55.

3-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl) propanamide, 9b: m.p. 120-122^{0}C. Yield: 54%. IR (KBr): 3233 (N-H), 3032 (Ar-H), 1622 (C=O), 1574 (C=N), 1065 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 9.98 (s, 1H, NH), 9.50 (s, 1H, NH), 6.78-7.52 (m, 8H, Ar-H), 5.20 (s, 2H, O-CH_2), 3.76-3.80 (t, 2H, S-CH_2, *J* = 6.48 Hz), 2.81-2.83 (t, 2H, $COCH_2$, *J* = 6.48 Hz), 2.1 (s, 3H, CH_3); MS: *m/z* $(M+2)^+$ 448.02. Anal. Calcd for $C_{20}H_{19}N_4O_4ClS$: C 53.75; H 4.29; N 12.54. Found: C 53.73; H 4.28; N 12.52.

3-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-benzyl propanamide, 9c: m.p. 118-120^{0}C. Yield: 52%. IR (KBr): 3289 (N-H), 3031 (Ar-H), 1639 (C=O), 1560 (C=N), 1078 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.20 (s, 1H, NH), 10.01 (s, 1H, NH), 6.88-7.60 (m, 9H, Ar-H), 5.20 (s, 2H O-CH_2), 4.40 (s, 2H, Ar-CH_2), 3.84-3.87 (t, 2H, S-CH_2, *J* = 6.42 Hz), 2.48-2.52 (t, 2H, $COCH_2$, *J* = 6.42 Hz), 2.18 (s, 3H, CH_3); MS: *m/z* $(M+1)^+$ 427.1. Anal. Calcd for $C_{21}H_{22}N_4O_4S$: C 59.14; H 5.20; N 13.14. Found: C 59.11; H 5.18; N 13.13.

2-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-phenyl propanamide, 10a: m.p. 152-154^{0}C. Yield: 58%. IR (KBr): 3260 (N-H), 3128 (Ar-H), 1668 (C=O), 1545 (C=N), 1057 (C-O, stretch of oxadiazole ring) ; ^{1}H NMR: δ 10.12 (s, 1H, NH), 10.01 (s, 1H, NH),7.01-7.68 (m, 9H, Ar-H), 5.50 (s, 2H, O-CH_2), 3.04-3.07 (q, 1H, S-CH, *J* = 7.10 Hz), 2.10 (s, 3H, CH_3), 1.20-1.23 (d, 3H, CH_3, *J* = 7.10 Hz); MS: *m/z* $(M+1)^+$ 413.04. Anal. Calcd for $C_{20}H_{20}N_4O_4S$: C 58.24; H 4.89; N 13.58. Found: C 58.21; H 4.87; N 13.57.

2-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl) propanamide, 10b: m.p. 160-162^{0}C. Yield: 69%. IR (KBr): 3252 (N-H), 3106 (Ar-H), 1650 (C=O), 1540 (C=N), 1062 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.10 (s, 1H, NH), 10.01 (s, 1H, NH),7.18-7.80 (m, 8H, Ar-H), 5.48 (s, 2H, O-CH_2), 3.08-3.11 (q, 1H, S-CH, *J* = 7.20 Hz), 2.12 (s, 3H, CH_3), 1. 22-1.25 (d, 3H, CH_3, *J* = 7.20 Hz); MS: *m/z* $(M+2)^+$ 448.04. Anal. Calcd for $C_{20}H_{19}N_4O_4ClS$: C 53.75; H 4.29; N 12.54. Found: C 53.73; H 4.27; N 12.51.

2-(5-((4-Acetamidophenoxy)methyl)-1,3,4-oxadiazol-2-ylthio)-N-benzyl propanamide, 10c: m.p. 150-152^{0}C. Yield: 62%. IR (KBr): 3266 (N-H), 3032 (Ar-H), 1651 (C=O), 1539 (C=N), 1060 (C-O, stretch of oxadiazole ring); ^{1}H NMR: δ 10.10 (s, 1H, NH), 10.01 (s, 1H, NH),6.80-7.70 (m, 9H, Ar-H), 5.51-5.54 (s, 2H, O-CH_2), 4.42 (s, 2H, Ar-CH_2), 3.06-3.10 (q, 1H, S-CH, *J* = 7.18 Hz), 2.10 (s, 3H, CH_3), 1.20-1.24 (d, 3H, CH_3, *J* = 7.10 Hz); MS: *m/z* $(M+1)^+$ 427.02. Anal. Calcd for $C_{21}H_{22}N_4O_4S$: C 59.14; H 5.20; N 13.14. Found: C 59.12; H 5.18; N 13.11.

Anti-inflammatory Activity

All the designed structures were screened for anti-inflammatory activity by using Winter et al.[36] proposed carrageenan-induced rat paw edema method. All the animals divided into group of six animals in the random fashion. Prior to experiment animals were fasted for 24 h and only free acess to water. 0.5 % CMC solution was administered to control group (Vc) and remaining group animals(Vt) were administered with the test compounds and reference drugs diclofenac (20 mg/kg p.o). 1% carrageenan solution was prepared in saline water and 0.1 ml prepared solution was injected subcataneously in to the right hind paw sub-planar region of each rat, 30 minutes after the administration of test and reference drug. Digital plethysmometer (Orchid life sciences) was used to measure right hind paw volume before and after 3 and 4 h of carrageenan injection. Anti-inflammatory activity

$$(\%\ \text{inhibition}) = [(Vc - Vt)/Vc] \times 100$$

Where (Vc) : edema volume in control group; (Vt): edema volume in test compounds.

Chronic anti-inflammatory Activity

Cotton pellet Granuloma method[37] was used for chronic antiinflammatory model. Grannuloma represents the exudative and proliferative phases of inflammation. 50 mg cotton pellets were implanted subcutaneously into the rats to develop chronic inflammation. A control group was administered a 5% sodium bicarbonate solution. Other group was treated with test and standard drug diclofenac (20 mg/kg). Treatment was sustained for the period of seven days and the granuloma were removed at the end of this period, and weighted to calculate the difference in weight gain. The cotton pellets were dried at 60 °C until constant weight, and then the final weight was estimated by subtraction.

Acute Ulcerogenic Activity

Acute ulcerogenicity was studied as per the the reported method[38].

Molecular Modelling

Molecular modeling study was accomplished via the Molecular Operating Environment (MOE) 2009.10 software package[39]. The preparation of target ligand files were achieved by drawing the molecular geometries and correct 3D structures were ensured and followed by energy optimization at a standard MMFF94 force field level, with a 0.0001 kcal/mol energy gradient convergence criterion[40].

The preparation of receptor was processed through downloading the crystal structure of enzyme cyclooxygenase-2 (prostaglandin synthase-2) complexed with a selective inhibitor, SC-558 (Protein Data Bank ID: 1CX2)[41], (http://www. rcsb.org/pdb). The pdb file was imported to MOE suite where receptor preparation module was used to prepare the protein. All the bound water molecules and hetero atom were removed from the complex by using sequence editor (SEQ) window which is default in MOE 2009.10 programme. Both polar and non-polar hydrogens were added and 3D structure was corrected. The 3-D protonated structure was energy minimized. Since the protein was devoid of associated ligand, so the pocket was identified using the active site finder module of the MOE 2009.10. In order to visualize the binding pocket, alpha spheres were created followed by the generation of dummy atoms on the centers of these spheres. The pockets were found to be deep small canyons lined with the key residues including both hydrophobic and hydrophilic amino acids.

The proposed docking methodology includes, the optimized target ligands were docked with the enzymes cyclooxygenase-2 (PDB ID: 1CX2) using the MOE 2009.10. For docking simulations, the placement was set as triangular matcher, rescoring was set as London dG, the number of retaining was set as 10 and the refinement was set as forcefield on MOE suite to generate 10 poses of each target ligand confirmations. As a result of docking run, the .mdb output files were created with scoring and multiple conformations of each compound. All the docked conformations were analyzed and the best scored pose for each compound was selected for further interaction studies. Besides, the ligand-receptor interaction followed by surface analysis of the selected best pose ligand molecule was generated and viewed for interpretation. Most appropriate docked

ligand target structure was selected on the basis of higher S-score and Root Mean Square Deviation (RMSD) values. The S-score is the value calculated by built-in scoring functions of MOE on the basis of ligand binding affinity with receptor protein after docking. Whereas RMSD value is generally used to compare the docked conformation with the reference conformation or with other docked conformation. The only compounds that have higher S-score and lower RMSD value than its natural substrates can be developed as potential inhibitors[42,43].

Conclusion

The 5-{[4-(acetylamino) phenoxy] methyl}-1,3,4-oxadiazole-2-yl-sulfanyl-N-substituted-2-acetamide/2-propanamide/3- propanamide derivatives were synthesized and were tested for their anti-inflammatory and anti-ulcerogenic activities *in-vivo*. Among the present series the compound **8c** was found to be most active against inflammations with inhibition of 65.34% when compared to diclofenac (73.26%). Introduction of asymmetric centre near to the sulfur atom decreases the activity. Molecular modeling simulations were done for the compounds to identify important binding modes responsible for the inhibition activity of cyclooxygenase-2. Structures from all the series were fit into the active site of cyclooxygenase-2 enzyme with least binding energies and exhibited favorable binding interactions required for the selective inhibition of cyclooxygenase- 2.

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