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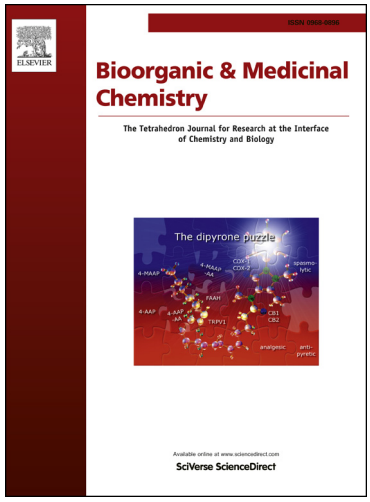

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Synthesis, biological evaluation and docking study of maleimide derivatives bearing benzenesulfonamide as selective COX-2 inhibitors and anti-inflammatory agents

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Abstract

A series of maleimide analogs bearing benzenesulfonamide were synthesized (**4a-r**). The anti-inflammatory activity of synthesized derivatives was evaluated using carageenan induced rat paw edema model. COX-1 and COX-2 potency was evaluated through *invitro*cyclooxygenase assays. The results revealed that, compounds **4a, 4h, 4j, 4k** and **4r** had potent COX-2 percentage inhibition as well as *invivo* anti- inflammatory activity. The potent compound **4j** was docked into the COX-2 active site to determine the probable binding model. The results of *invivo* and *invitro* studies demonstrate that phenyl ring with electron withdrawing groups on maleimide ring would generate more potent anti- inflammatory agents. Thus, these compounds can serve as potential leads for further anti-inflammatory studies.

Keywords: Maleimide, COX-2, Ulcerogenic effects, Docking, Carageenan paw edema.

1. Introduction:

Inflammation is a localized physical condition with heat, swelling, redness and usually pain. It is mediated by the release of proinflammatory mediators (bradykinin, and cytokines), which in turn increases the rate of prostaglandin synthesis.[1, 2] Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the biosynthesis of prostaglandins (PGs) by inhibiting cyclooxygenases (COX). It exists in two isoforms; a constitutive form (COX-1) and an inducible form (COX-2).[3, 4]COX-1 enzyme is responsible for maintenance of gastric integrity and kidney function whereas COX-2 is involved in the inflammation and pain.[5, 6]

Literature revealed that many NSAIDs including aspirin and indomethacin were found to interact with these enzymesnonselectively and inhibit both COX-1 and COX-2.[7]Non-selective inhibitor aspirin inhibits COX-1 more strongly than COX-2[7] and reduces the production of prostaglandin E2 (PG-E_2) and prostacyclin (PG-I_2) which has an adverse ulcerogenic effects.[8]COX-2 selective inhibitors used for treating inflammation and they have been shown a much lower gastrointestinal side effects.[9, 10] However, COX-2 selective inhibitors found to possesses cardiovascular side effects,[11]it gives an idea about the need to discover novel scaffolds with COX-2 inhibitory activity and evaluate their anti-inflammatory effects. Consequently, selective COX-2 inhibitorshave been developed to diminish the risk of unwanted side effects.

So far, various classes of cyclic imides like maleimide, succimimde, phthalimide have been received great attention due to their anti-inflammatory, antihyperlipidemic and antitumor activities.[12-15] Recently, it was reported that *N*-substituted cyclic imide derivatives afforded a new scaffold for small molecule COX-2 inhibitors which should offer opportunities for various kinds of structural development.[16]In addition to this, *N*-substituted cyclic imide derivatives

possess remarkable anti-inflammatory activity through inhibition of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6).

On the other hand, sulfonamide has been consistently rewarded as promising molecule because of its broad spectrum of pharmacological activities like antibacterial[17],carbonic anhydrase inhibitors[18, 19], hypoglycemic[20, 21], antithyroid[22] and antiproton activities.[23-25]Moreover, among the highly marketed COX-2 inhibitors that comprise the sulfonamide moiety, celecoxib and SC-558 (Fig.1) are the major determinant for COX-2 selectivity and *in-vivo* efficacy.[13, 15, 26]Promising anti-inflammatory activity of some maleimide as well as sulfonamide derivatives (Fig.2) prompted us to investigate them further. However, a maleimide ring has not been appearing to be linked with benzenesulfonamide so far. Based on the literature information, we decided to combine maleimide ring and benzenesulfonamide moiety to get new compounds, which is prerequisite for anti-inflammatory activity. Based on the above mentioned fact, in the proposed study, we aim to investigatewhether maleimide analogs bearing benzenesulfonamide shows anti-inflammatory activity and cyclooxygenase inhibitory activity.

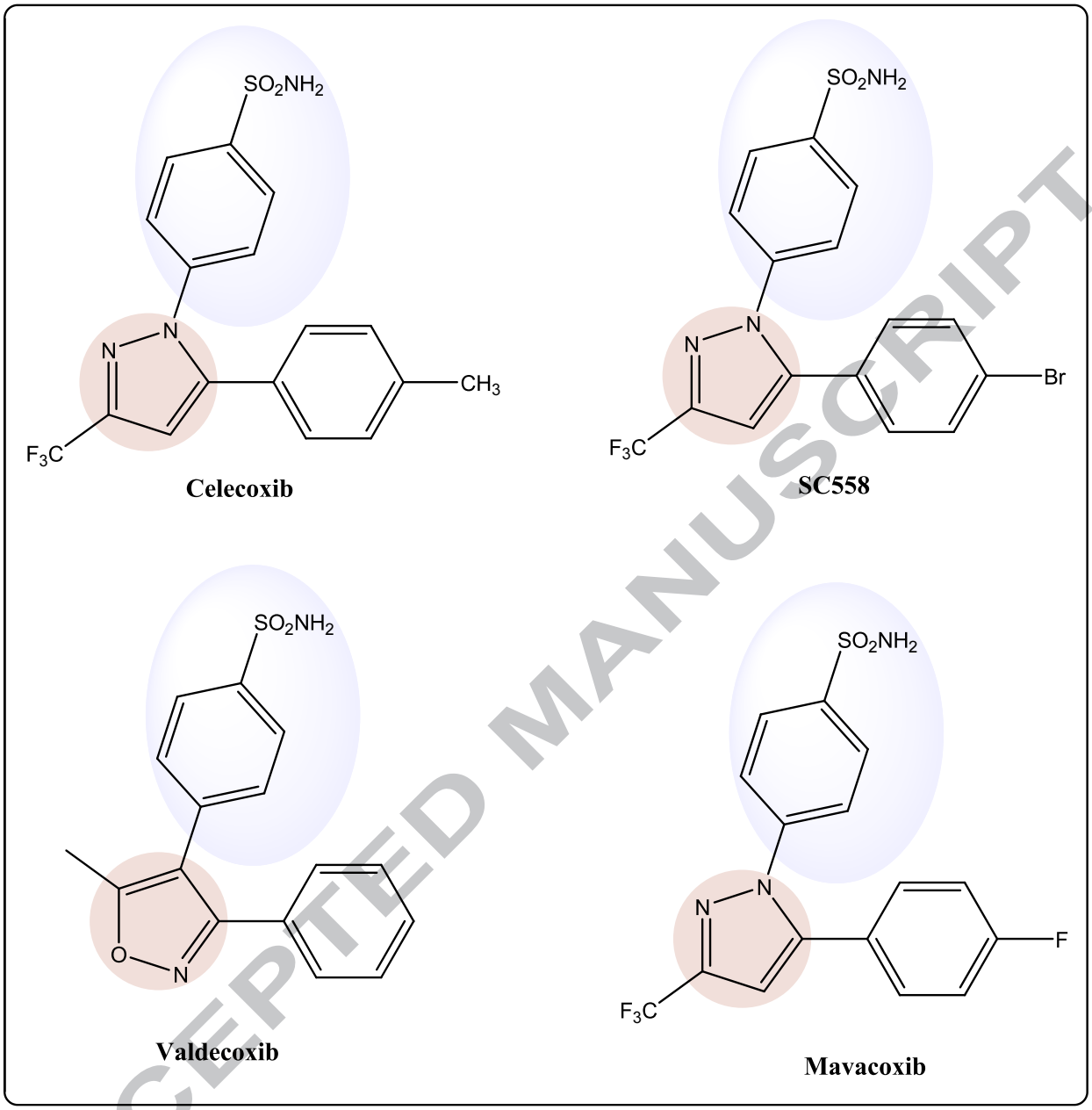

Figure 1. Structures of COX-2 inhibitors containing sulfonamide moiety

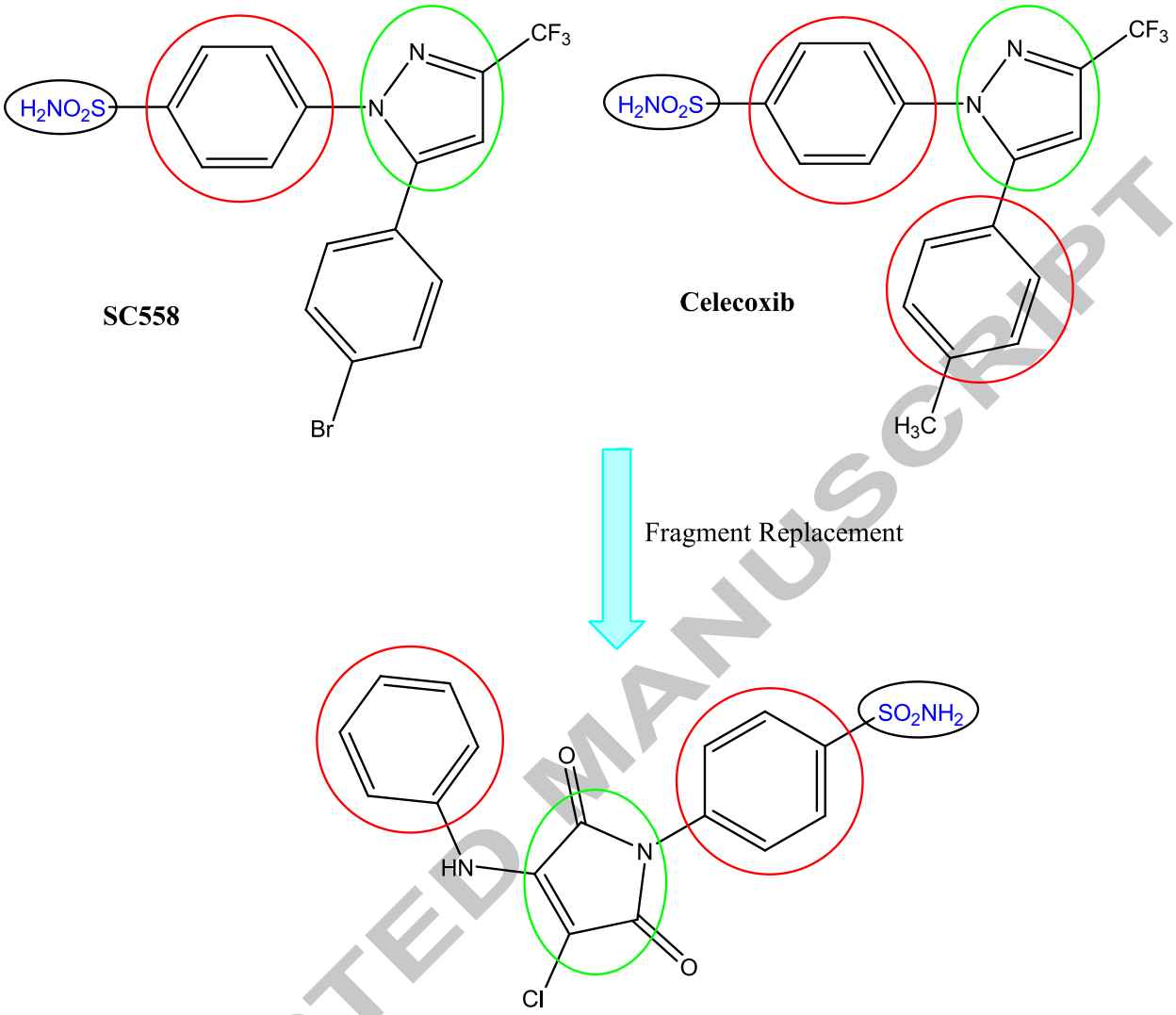

Figure 2 Common features present in COX-2 inhibitors and targeted compounds

2. Results and discussion

2.1 Chemistry

Synthesis of the titled compounds (**4a-r**) has been carried out as being depicted in **scheme 1**. The intermediate 3, 4-dichloromaleimido benzenesulfonamide (**3**) was obtained by reacting 3, 4-dichloromaleic anhydride(**1**) with benzenesulfonamide in glacial acetic acid at 90°C. The IR spectra of dichloromaleimido benzenesulfonamide (**3**) showed the presence of the NH_2 absorption band in 3458 cm^{-1} and SO_2 absorption bands in 1344cm^{-1}, 1158 cm^{-1} region, which

confirms the presence of free SO_2NH_2whereas its ^{1}HNMR revealed the singlet of -NH_2 protons at δ7.36. Furthermore, the substitution of only one chlorine atom of compound **3** by appropriate aryl/alkylamine in dimethyl formamide (DMF) at room temperature probably occurs by a mechanism of addition-elimination, which loses a chlorine atom to form the desired compounds **4a-r**.

The IR spectra of desired derivatives **4a-r** exhibited –NH stretching vibrations in the region of 3364-3200cm^{-1}. The ^{1}HNMR spectrum exhibited singlet signal due to-NH proton of substituted anilineat δ 12.92-8.15 and -NH_2 of -SO_2NH_2 at δ7.40. Most of the synthesized compounds are reported for the first time and gave satisfactory spectroscopic data.All the synthesized compounds were characterized by their physical and spectral data.

2.2 Biological evaluation

2.2.1 *In vitro*cyclooxygenase (COX) inhibition

The ability of thesynthesized compounds(**4a-r**) to inhibit ovine COX-1 and human recombinant COX-2 isozymes was determined using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA).The % inhibition values calculated represents means of two determinations (Table 1). Moreover, the selectivity indices (SI values) defined as COX-2/COX-1 were calculated and compared with that of the standard selective COX-2 inhibitor; celecoxib. We estimated COX inhibition at a concentration of 1μM and eliminated those derivatives that did not have an inhibitory action on either COX-1 or COX-2. Results demonstrate that most of the synthesized compounds exhibited remarkable and selective inhibition of COX-2 and weak inhibition of COX-1. Among these synthesized derivatives, compounds **4a, 4h, 4j, 4k, 4q,** and **4r** showed potent % inhibitory activity. An analysis of cyclooxygenase inhibitory activity of compounds **4a-r** showed the subsequent structure activity

relationship.The activities of compounds may be due to the sulfonamide moieties, which may interact with the active site of the COX-2 enzyme.[27]The unsubstituted 3, 4-dichloromaleimido benzenesulfonamide (**3**) showed poor COX-2 inhibition (7.37%). The substitution on the phenyl ring attached at the fourth position of maleimide ring was a determinant of COX-2 inhibitory potency and selectivity of 3-chloro-4-substitutedmaleimido benzenesulfonamide derivatives(**4a-r**). The results confirmed that the introduction of electronegative groups to *meta* position improves the COX-2 inhibitory activities than *para* position. The results also indicated that replacement of substituted anilines by cyclic amines also affect the COX-2 inhibitory activities.

Table 1 *In vitro* COX-1/COX-2 enzyme inhibition assay of the compounds

Compound No.	% inhibition (1μM)		SI[b]
	COX-1	COX-2	(COX-2/COX-1)
3	12.05	7.373	0.611
4a	0.89	39.63	44.52
4b	9.37	24.42	2.60
4c	6.25	10.14	1.62
4d	0.89	17.05	19.15
4e	0.44	6.45	14.65
4f	0.89	10.6	11.9
4g	1.33	27.65	20.78
4h	0.89	42.40	47.64
4i	7.14	15.67	2.19
4j	0.89	49.31	55.40
4k	0.89	44.20	49.66
4l	4.01	27.19	6.78
4m	0.44	6.912	15.70
4n	4.01	27.19	6.78
4o	5.35	13.82	2.57

4p	1.78	23.04	12.94
4q	8.03	30.41	3.78
4r	0.89	39.63	44.52
Celecoxib	0.45	75.12	166.93

[a]% inhibition values represents means of two determinations using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA). [b]Selectivity index (COX-2/COX-1).

2.2.2 *In vivo*anti-inflammatory activity

Compounds with good COX-2 inhibitory activity were selected for *in-vivo*anti-inflammatory activity and examined by carrageenan-induced rat paw edema method (Table 2).These derivatives exhibited varying degree of anti-inflammatory activity (35% - 80%, 56% - 81% at 3h and 62% - 82% at 5h). Compound **4j**has showed maximum activity at 5h (comparable to that of celecoxib at 5h). Compounds**4h, 4k** and **4r** showed promising activity.

Table 2*In vivo* anti-inflammatory activity against carrageenan-induced rat paw edema of the compounds

Compound	**Dose /kg b.w.**	**% inhibition**		
		1h	**3h**	**5h**
Control[a]	10ml/kg	0	0	0
Celecoxib	20mg	92.17	100	100
4a	20mg	35.37	56.79	62.13
4h	20mg	69.12	70.41	72.14
4j	20mg	79.78	81.01	82.56
4k	20mg	72.96	71.55	77.32
4r	20mg	69.18	71.03	74.91

[a]vehicle (1% Carboxymethyl cellulose (CMC) in water in a volume of 10 mL/kg p.o.)

2.2.3 Ulcerogenic Potential

The ulcerogenic potential of most potent compound**4j**was evaluated and compared to celecoxib (20mg/kg). From the data obtained, Table 3, it has been observed that compound **4j**showeda

lower ulcer index than celecoxib. Compound **4j** showed a good ulcer index (8.3) when compared with that of celecoxib (11.9).

Table 3 Ulcerogenic effect of **4j** in rats (20 mg/kg)

Compound No.	No. of animals	% of incidence divided by 10	Average no. of ulcer	Average severity (Ulcer score)	Ulcer Index
Control[a]	05	0	0	0	0
Celecoxib	05	10	4	1.5	11.9
4j	05	06	1.2	1.1	8.3

[a] control group received 7% gum acacia (suspending vehicle) orally

2.3 Molecular docking study

A molecular docking study helps to understand the ligand-protein interactions and selectivity in detail. Towards this goal, docking studies were carried out on the active site of COX-2 protein, (PDB code: 6COX[28])co-crystal structure of SC-558 as a template, using Glide (V5.5) module in Schrodinger's molecular modeling suite.It was observed that compound **4j**(Figure 3), SC-558 (Figure4) and celecoxib (Figure 5) showed hydrogen bonding interactions, hydrophilic interactions and hydrophobic interactions at the active site of the COX. SC558 and celecoxib showed hydrophobic interactions with Ala^{527}, Val^{103}, Leu^{525}, Leu^{503}, Phe^{523}, Gly^{526}, Phe^{357}, Tyr^{355}, Lys^{358}, Asn^{382}, Gln^{350}, Val^{349}, Tyr^{385}, His^{356}, Gly^{354}, Leu^{352} and less hydrophilic interactions.

It was observed that, one of the O-atom and $-NH_2$ of the SO_2NH_2 moiety of compound **4j**exhibitedhydrogen bonding interaction with Gln^{192}, Ala^{516} and the phenyl ring bearing sulfonamide showed hydrophobic interactions with Leu^{359}, Leu^{352}, Gln^{350}, His^{356}, Tyr^{355}, Met^{522}, Tyr^{504}, Ala^{527}, and Phe^{523}.Since the structure is polar the magnitude of hydrophilic interactions is more as compare to hydrophobic interactions. This may be the reason behind the low glide score

of compound **4j** (glide score -8.80) as compared toSC558 (glide score -10.73) and celecoxib (glide score -10.85)**.**

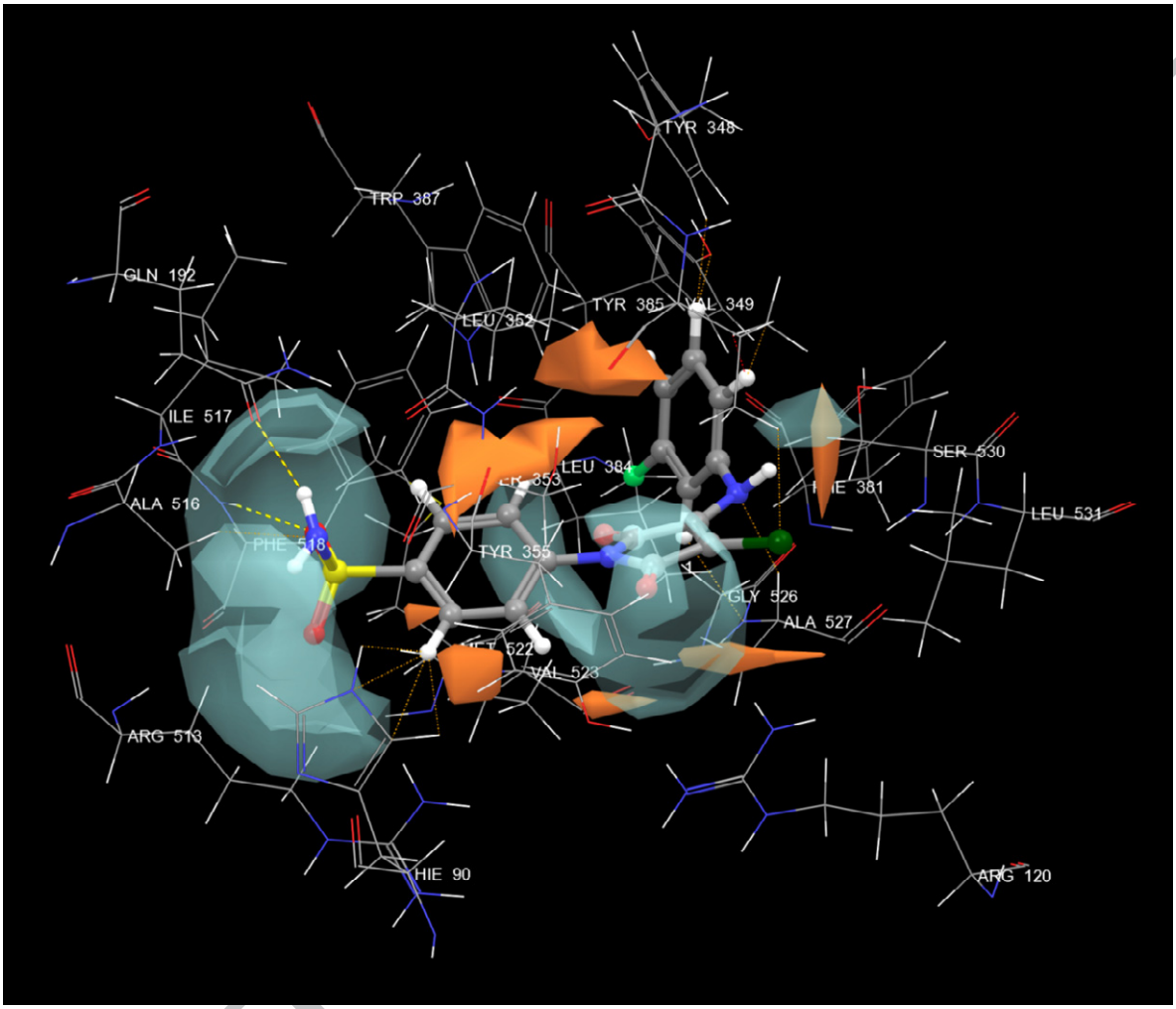

Figure 3. The 3D image of 6COX-**4j** complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite. The hydrogen bonds between one of the O-atoms of SO_2NH_2 with Ala^{516} and -NH_2 of the SO_2NH_2 with Gln^{192} are shown in yellow dotted lines.

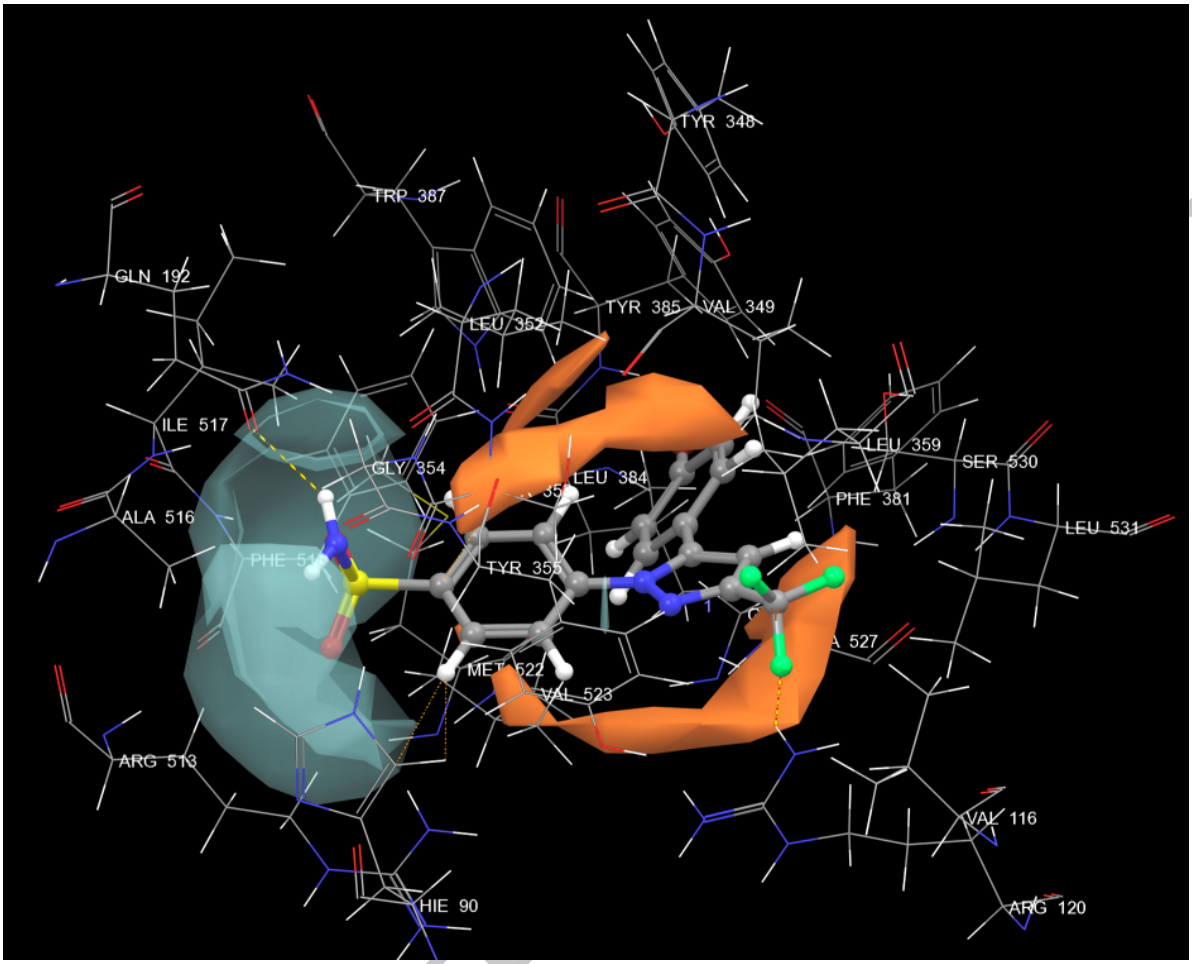

Figure 4. The 3D image of 6COX-SC558 complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite. Hydrogen bond between -NH_2 of the SO_2NH_2 with Gln^{192} is shown in yellow dotted lines.

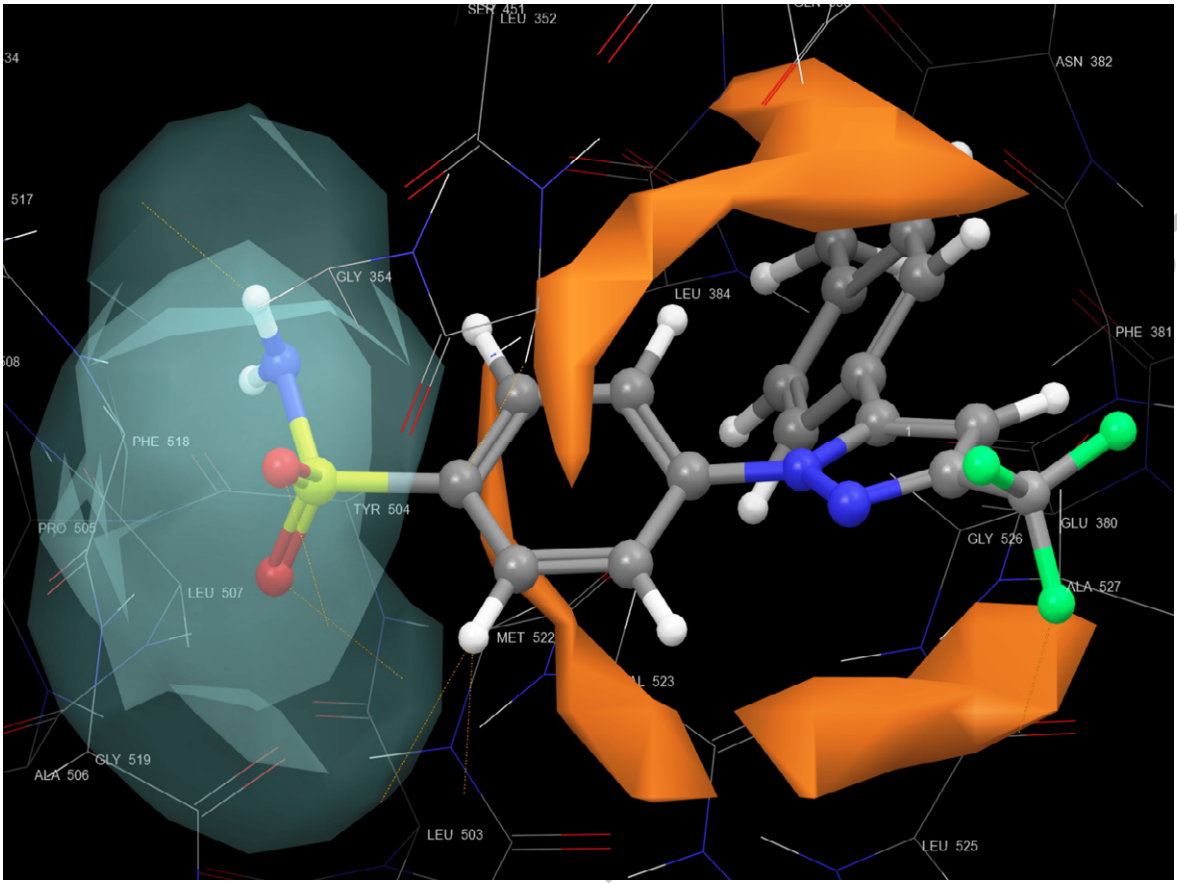

Figure 5. The 3D image of 6COX-Celecoxib complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite.

3 Conclusion

In summary, we have reporteda simple and efficient method for the synthesis of 3-chloro-4-substitutedmaleimido benzenesulfonamide derivatives with excellent yield. All the synthesized compounds have been screened for their *invitro* and selected compounds for*invivo* anti-inflammatory activity. In the newly synthesized compounds, **4h, 4j, 4k and 4r** were found to have moderate to goodanti-inflammatory activity.Moderate i*n vivo* and *in vitro* activity of these compounds may be due to poor hydrophobic interaction with the COX-2 active site. The overall studies inferred that compound **4j** rendered it as a hit molecule for the further development of more potent anti-inflammatory agent. The *in vivo* and *in vitro* studies of these compoundsevidenced that, the electron withdrawing groups enhances the anti- inflammatory

activity, which might be served as new template in the synthesis and development of potent therapeutics.

4. Experimental

4.1 Chemistry

All the chemicals are purchased from Sigma Aldrich, Avera chemicals and solvents are reagent grade and used without purification. The melting points were determined on Analab Melting point apparatus in open capillary tubes and were uncorrected. The IR spectra in KBr were recorded with the Schimadzu IR 48 spectrophotometer and were reported in cm^{-1}. The ^{1}H NMR, ^{13}C spectra (DMSO-d6) were recorded on BrukerAvance II spectrometer. The chemical shifts are expressed in δ values (ppm). The abbreviation s = singlet, d= doublet, and m= multiplet were used throughout. Mass spectra were recorded on Waters Q time of flight MS-ES-1.0 1e4 mass spectrometer. All reactions were followed by TLC (silica gel 60 F_{254} Merck) and visualization was accomplished with UV light (254 or 366 nm).

4.1.1. *General Procedure for the synthesis of 3,4-dichloromaleimido sulfonamide[29] (**4**)*

The sulfonamide intermediate was prepared by the condensation of the sulfanilamide (**2**) and the dichloromaleic anhydride (**1**) in glacial acetic acid solution. The procedure used to prepare the 3,4-dichloromaleimido sulfonamide (**3**) consisted in the addition of 0.1 mole of the sulfanilamide (**2**) compound in 25-30 mL of glacial acetic acid to a solution of 0.1 mole of the dichloromaleic anhydride (**1**) in 50-75 mL of glacial acetic acid at room temperature. The temperature was gradually increased to reflux and the refluxing was continued for 15-30 min. Dilute the cooled reaction mixture with water to obtain the 3,4-dichloromaleimido sulfonamide. Yield (%): 90%; mp (°C): > 300°C; IR (KBr, cm^{-1}):3458(NH_2), 3126(Ar, C-H), 1734(C=O), 1594(Ar C=C), 1344,1158(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 7.92(d, *J* = 8.52Hz, 2H, Ar), 7.53(d, *J* =

8.52, 2H, Ar), 7.36(br s, 2H, NH_2); ^{13}C NMR (100MHz, DMSO-d6) ppm: 161.6 (2; C=O imide), 143.6 (2; =C-Cl), 133.63(SO_2-C), 132.93(=C-N), 126.78, 126.57(4C; Ar); *m/z* 319.2 $[M+H]^+$.

4.1.2. *General procedure for the synthesis of compounds (**4a-r**)*

Compounds **4a-r** were obtained by reaction of 3,4-dichloromaleimido sulfonamide (**3**) (0.009mole), with appropriate amines (0.011mole) in 5mL DMF, which were stirred for overnight at room temperature and then poured onto ice-cold water. The precipitate obtained was filtered off, washed with water and dried. The crude product was purified by column chromatography with 10% methanol in chloroform as an eluent.

4.1.2.1. *4-(3-chloro-2,5-dioxo-4-(phenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide **(4a).*** Yield (%): 90%; mp (°C): 255-257°C; IR (KBr, cm^{-1}):3457(NH), 1757(C=O), 1589(Ar C=C), 1348,1156(SO_2); ^1H NMR (400MHz, DMSO-d6) ppm: 10.01(s, 1H, NH), 7.96(d, *J* = 8.0Hz, 2H, Ar), 7.62(d, *J* = 8.56Hz, 2H, Ar), 7.4(s, 2H, NH_2), 7.39(d, *J* = 8.24Hz, 2H, Ar); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.64(C=O imide), 164.04(C=O imide), 142.65(C-N), 138.33(C-SO_2), 136.48(=C-NH), 134.35(C-NH, Ar), 123.95–128.10(9C; Ar), 92.51(=C-Cl); *m/z* 378.2$[M+H]^+$.

4.1.2.2. *4-(3-chloro-4-(4-chlorophenylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide **(4b).*** Yield (%): 86%; mp (°C): 263-265°C; IR (KBr, cm^{-1}):3452(NH), 1761(C=O), 1591(Ar C=C), 1340, 1152(SO_2); ^1H NMR (400MHz, DMSO-d6) ppm: 8.15(s, 1H, NH), 7.99(d, 2H, *J* = 7.76Hz, 2H, Ar), 7.61(d, *J* = 8.56Hz, 2H, Ar), 7.4(s, 2H, NH_2),7.37(d, *J* = 8.56Hz, 2H, Ar), 7.23(d, *J* = 8.76Hz, 2H, Ar); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.43(C=O imide), 163.92(C=O imide), 143.63(C-N), 138.01(C-SO_2), 135.42(=C-NH), 134.24(C-NH, Ar), 125.21–126.39(8C, Ar), 120.15(C-Cl, Ar), 93.53(=C-Cl); *m/z* 411.96 $[M+H]^+$.

4.1.2.3. *4-(3-chloro-2,5-dioxo-4-(o-tolylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4c).*** Yield (%): 69%; mp (°C): 273-275°C; IR (KBr, cm^{-1}):3412, 3307(NH), 1720(C=O), 1593(Ar C=C), 1389,1170(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.7(s, 1H, NH), 7.96(d, *J* = 8.64, 2H, Ar), 7.63 (d, *J* = 9.08, 2H, Ar), 7.38(s, 2H, NH_2), 7.27(m,4H,Ar), 2.29 (s,3H, CH_3); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.69 (C=O imide), 163.71(C=O imide), 142.56(C-N), 139.96(C-SO_2), 135.16(=C-NH), 134.73 (C-NH, Ar), 130.02(C-CH_3, Ar), 125.64 – 127.32(8C, Ar), 90.52(=C-Cl), 17.30(CH_3); *m/z* 392.3 $[M+H]^+$.

4.1.2.4. *4-(3-chloro-2,5-dioxo-4-(4-nitrophenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4d).*** Yield (%): 80.7%; mp (°C): 295-297°C; IR (KBr, cm^{-1}):3374, 3267(NH), 1741(C=O), 1509(Ar C=C), 1311,1162(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 8.22(s, 1H, NH),7.98(d, *J*= 8.6Hz, 2H, Ar), 7.9(d, *J* = 8.92Hz, 2H, Ar), 7.59(d, *J* = 8.36Hz, 2H, Ar), 7.42(s, 2H, NH_2),6.60(d, *J* = 9.16Hz, 2H, Ar); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.42(C=O imide), 162.96(C=O imide), 158.69(C-NO_2), 143.65(C-N), 138.32(C-SO_2), 133.63(=C-NH), 132.92(C-NH, Ar), 126.13 – 126.79(8C, Ar), 112.27(=C-Cl); *m/z* 421.2$[M+H]^+$.

4.1.2.5. *4-(3-chloro-2,5-dioxo-4-(piperidin-1-yl)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4e).*** Yield (%): 86.3%; mp (°C): 193-195°C; IR (KBr, cm^{-1}):3334(NH), 2936, 2854(C-H),1778(C=O), 1511(Ar, C=C), 1337,1162(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 7.92(d, *J* = 8.64Hz, 2H, Ar), 7.53(d, *J* = 8.6Hz, 2H, Ar), 7.38(s, 2H, NH_2), 3.9(s, 4H, CH_2), 1.69 (s, 6H, CH_2); ^{13}C NMR (100MHz, DMSO-d6) ppm: 164.48(C=O imide), 163.55(C=O imide), 143.66(C-N), 142.62(C-SO_2), 134.34(-C-N, piperidine), 126.22–126.50(4C, Ar), 92.26(=C-Cl), 23.44, 26.33, 49.19 (CH_2, piperidine); *m/z* 370.3$[M+H]^+$.

4.1.2.6. *4-(3-chloro-2,5-dioxo-4-(4-methoxyphenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4f).*** Yield (%): 92%; mp (°C): 252-255°C; IR (KBr, cm^{-1}):3368(NH), 1709(C=O), 1403(Ar C=C), 1329,1162(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.86(s, 1H, NH), 7.96(d, *J* = 8.6Hz, 2H, Ar), 7.61(d, *J* = 8.6Hz,2H, Ar), 7.37(s, 2H, NH_2), 7.18(d, *J* = 8.84Hz, 2H, Ar), 6.93(d, *J* = 8.88Hz, 2H, Ar), 3.78(s, 3H, OCH_3); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.70(C=O imide), 163.94(C=O imide), 157.20(C-OCH_3), 142.57(C-N), 138.86(C-SO_2), 134.40(=C-NH), 132.93(C-NH, Ar), 125.83-129.18(8C, Ar), 90.72(C-Cl), 55.15(OCH_3); *m/z* 406.2$[M+H]^+$.

4.1.2.7. *4-(3-chloro-2,5-dioxo-4-(4-fluorophenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4g).*** Yield (%): 69%; mp (°C): 243-245°C; IR (KBr, cm^{-1}):3454 (NH), 1762(C=O), 1592(Ar C=C), 1341, 1154(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.90(s, 1H, NH), 7.90(d, *J* = 8.88Hz, 2H, Ar), 7.54(d, *J* = 8.6Hz, 2H, Ar), 7.28(s, 2H, NH_2),7.06(d, *J* = 8.6Hz, 2H, Ar); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.50(C=O imide), 163.88(C=O imide), 158.58(C-F, Ar), 142.58(C-N), 138.40(C-SO_2), 134.24(=C-NH), 132.60(C-HN, Ar), 125.99-126.08(8C, Ar), 125.71, 125.90(CH-Ar-F), 114.66 – 114.96(CH-Ar-F), 92.22(=C-Cl); *m/z* 395.20 $[M+H]^+$.

4.1.2.8. *4-(3-chloro-4-(3-chlorophenylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4h).*** Yield (%): 74.51%; mp (°C): 252-255°C; IR (KBr, cm^{-1}):3302 (NH), 1713(C=O), 1410 (Ar C=C), 1332,1162 (SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 8.58(s, 1H, NH), 7.97(d, *J* = 8.64Hz, 2H, Ar), 7.62(d, *J* = 8.6Hz, 2H, Ar), 7.42-7.32(m, 4H, Ar), .25(s, 2H, NH_2);^{13}CNMR(100MHz, DMSO-d6) ppm: 165.50(C=O imide), 163.88(C=O imide), 158.58(C-F, Ar), 142.58(C-N), 138.40(C-SO_2), 134.24(=C-NH), 132.60(C-HN, Ar), 125.99-

126.08(8C, Ar), 125.71, 125.90(CH-Ar-F), 114.66-114.96(CH-Ar-F), 92.22(=C-Cl); *m/z* 411.96 $[M+H]^+$

4.1.2.9. *4-(3-chloro-2,5-dioxo-4-(2-methoxyphenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4i).*** Yield (%): 76%; mp (°C): 280-282°C; IR (KBr, cm^{-1}):3360(NH), 1770,(C=O), 1594(Ar C=C), 1328,1152 (SO_2); ^1H NMR (400MHz, DMSO-d6) ppm: 9.52(s, 1H, NH), 7.98(d, *J* = 8.6Hz, 2H, Ar), 7.62(d, *J* = 8.68Hz, 2H, Ar), 7.39(s, 2H, NH_2), 7.30(t, *J* = 7.44Hz, *J* = 7.56Hz, 1H, Ar), 7.22(d, *J* = 7.72Hz, 1H, Ar), 7.07(d, *J* = 8.0Hz, 2H, Ar), 3.82(s, 3H, OCH_3); ^{13}C NMR(100MHz, DMSO-d6) ppm: 165.58(C=O imide), 163.72(C=O imide), 154.11(C-OCH_3), 142.57(C-N), 139.50(C-SO_2), 134.35(=C-NH), 132.91(C-NH, Ar) 126.09–127.95(8C, Ar), 91.61(=C-Cl), 55.39(OCH_3); *m/z* 406.2$[M+H]^+$.

4.1.2.10. *4-(3-chloro-2,5-dioxo-4-(3-fluorophenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4j).*** Yield (%): 53%, mp (°C): 268-270°C, IR (KBr, cm^{-1}):3260(NH), 1765(C=O), 1589(Ar C=C), 1348, 1154(SO_2); ^1H NMR (400MHz, DMSO-d6) ppm: 9.89(s, 1H, NH), 7.99(d, *J* = 7.6Hz, 2H, Ar) 7.62(d, *J* = 7.7Hz, 2H, Ar), 7.43(m, 4H, Ar), 7.29(s, 2H, NH_2); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.42 (C=O imide), 163.65(C=O imide), 159.14(C-F, Ar), 142.40(C-N), 138.37(C-SO_2), 134.34 (=C-NH, Ar), 132.72(C-NH, Ar), 125.88-126.36(8C, Ar), 125.58, 125.76(CH, Ar-F), 113.96, 114.26(CH, Ar-F), 92.18(=C-Cl); *m/z* 395.20 $[M+H]^+$.

4.1.2.11. *4-(3-chloro-2,5-dioxo-4-(3-methoxyphenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4k).*** Yield (%): 73.68%; mp (°C): 280-282°C; IR (KBr, cm^{-1}):3252(NH), 1761(C=O), 1589(Ar C=C), 1349,1151(SO_2); ^1H NMR (400MHz, DMSO-d6) ppm: 9.53(s, 1H, NH), 7.98(d, *J* = 8.6Hz, 2H, Ar), 7.63(d, *J* = 8.64Hz, 2H, Ar), 7.38(s, 2H, NH_2), 7.31(t, *J* = 7.48Hz, *J* = 7.44Hz, 1H, Ar), 7.23(d, *J* = 7.72Hz, 1H, Ar), 7.07(d, *J* = 8.0Hz, 2H, Ar), 3.81(s, 3H, OCH_3); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.68(C=O imide), 163.80(C=O imide),

154.28(C-OCH_3), 142.61(C-N), 139.56(C-SO_2), 134.30(=C-NH), 132.86(C-NH, Ar), 126.06–127.86(8C, Ar), 91.72(=C-Cl), 55.28(OCH_3); *m/z* 406.2$[M+H]^+$.

4.1.2.12. *4-(3-chloro-4-(2,3-dichlorophenylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4l).*** Yield (%): 90%; mp (°C): 286-288°C; IR (KBr, cm^{-1}):3258(NH), 1762(C=O), 1590(Ar C=C), 1343,1149 (SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.98(s, 1H, NH), 7.96(d, *J* = 8.6Hz, 2H, Ar), 7.64(d, *J* = 8.64Hz, 2H, Ar), 7.42(m, 3H, Ar), 7.3(s, 2H, NH_2); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.61(C=O imide), 163.69(C=O imide), 142.53(C-N), 139.52(C-SO_2), 134.29(=C-NH), 123.8 and 132.64(2C-Cl, Ar), 132.71(C-NH, Ar), 124.93–126.86(7C, Ar), 89.34(=C-Cl); *m/z* 466.70 $[M+H]^+$.

4.1.2.13. *4-(3-chloro-4-(3,5-dimethylphenylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4m).*** Yield (%): 68%, mp (°C): 276-278°C, IR (KBr, cm^{-1}):3278(NH), 1750(C=O), 1589 (Ar C=C), 1351,1142(SO_2), ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.69(s, 1H, NH), 7.96(d, *J* = 8.64Hz, 2H, Ar), 7.63(d, *J* = 9.08Hz, 2H, Ar), 7,59(d, *J* = 8.6Hz, 2H, Ar), 7.53(d, *J* = 8.6Hz, 1H, Ar), 7.37(s, 2H, NH_2), 2.32(s,6H, CH_3); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.11(C=O imide), 162.86(C=O imide), 142.55(C-N), 139.60(C-SO_2),134.36 (=C-NH), 132.72(C-NH, Ar), 130.18 and 130.34 (2C-CH_3,Ar), 120.86 – 126.36(7C, Ar), 94.32(=C-Cl), 21.3(2CH_3); *m/z* 405.10$[M+H]^+$.

4.1.2.14. *4-(3-chloro-2,5-dioxo--4-(4-(trifluoromethyl)phenylamino)- 2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4n).*** Yield (%): 70.7%; mp (°C): 270-272°C; IR (KBr, cm^{-1}):3460 (NH), 1761(C=O), 1589 (Ar C=C), 1339,1140 (SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.96(s, 1H, NH), 7.89(d, *J* = 8.6Hz, 2H, Ar), 7.61(d, *J* = 8.6Hz, 2H, Ar), 7.36(s 2H, NH_2), 7.18(d, *J* = 8.84Hz, 2H, Ar), 6.93(d, *J* = 8.88Hz, 2H, Ar); ^{13}C NMR (100MHz, DMSO-d6) ppm: 164.98 (C=O imide), 163.02 (C=O imide), 142.62(C-N), 139.71(C-SO_2), 134.21(=C-NH), 132.61(C-

NH. Ar), 130.21(C-CF_3), 125.09-126.32 (8C, Ar), 121.68(CF_3), 90.91(=C-Cl); *m/z* 445.21$[M+H]^+$.

4.1.2.15. *4-(3-chloro-4-(2,4-dimethylphenylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4o).*** Yield (%): 57.8%; mp (°C): 280-282°C; IR (KBr, cm^{-1}):3248(NH), 1756(C=O), 1591(Ar C=C), 1348,1137(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.78(s, 1H, NH), 7.97(d, *J* = 8.4Hz, 2H, Ar), 7.69(d, *J* = 8.4Hz, 2H, Ar), 7.59(d, *J* = 7.8Hz, 2H, Ar), 7.54(d, *J* = 7.8Hz, 2H, Ar), 7.31(s, 2H, NH_2), 2.31(s,6H, CH_3); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.18(C=O imide), 163.84(C=O imide), 142.59(C-N), 139.51(C-SO_2), 134.28 (=C-NH), 132.61(C-NH, Ar), 130.21 and 130.41(2C-CH_3, Ar), 120.82–126.39(7C, Ar), 93.18(=C-Cl), 21.36(2CH_3); *m/z* 405.10$[M+H]^+$.

4.1.2.16. *4-(3-(2-aminophenylamino)-4-chloro-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4p).*** Yield (%): 72%, mp (°C): 289-291°C, IR (KBr, cm^{-1}):3223(NH), 1779(C=O), 1589(Ar C=C), 1343,1135(SO_2), ^{1}H NMR (400MHz, DMSO-d6) ppm: 12.92(s, 1H, NH), 8.00(d, *J* = 8.68Hz, 2H, Ar), 7.81(d, *J* = 8.68Hz, 2H, Ar), 7.66(d, *J* = 9.2Hz, 1H, Ar), 7.61(d, *J* = 9.32Hz ,1H, Ar), 7.45(d, *J* = 8.48Hz, 1H, Ar), 7.41(s, 2H, NH_2), 7.29(t, *J* = 8.28Hz, *J* = 8.36Hz, 1H, Ar); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.21(C=O imide), 161.28(C=O imide), 154.80(C-NO_2, Ar), 141.62(C-N), 138.33 (C-SO_2),134.26(=C-NH),132.56(C-NH, Ar), 132.12 (C-Cl, Ar), 125.21–129.63(7C, Ar), 91.26(=C-Cl); *m/z* 455.65$[M+H]^+$.

4.1.2.17. *4-(3-chloro-4-(3,4-dichlorophenylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4q).*** Yield (%): 78%; mp (°C): 278-280°C; IR (KBr, cm^{-1}):3197(NH), 1781(C=O), 1591(Ar C=C), 1352,1139(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 10.01(s, 1H, NH), 7.96(d, *J* = 8.6Hz, 2H, Ar), 7.64(d, *J* = 8.6Hz , 2H, Ar), 7.41(m, 3H, Ar), 7.29(s, 2H, NH_2); ^{13}C NMR (100MHz, DMSO-d6) ppm: 161.31(C=O imide), 159.26(C=O imide), 143.48(C-N),

138.29(C-SO_2), 134.22 (=C-NH), 132.28(C-CH, Ar), 123.19 and 132.18(2C-Cl, Ar),125.89 – 126.68(7C, Ar), 90.03(=C-Cl); *m/z* 466.70 $[M+H]^+$.

4.1.2.18. *4-(3-chloro-2,5-dioxo-4-(2-fluorophenylamino)-2,5-dihydro-1H-pyrrol-1-yl)benzenesulfonamide* ***(4r).*** Yield (%): 53%; mp (°C): 268-270°C; IR (KBr, cm^{-1}):3242(NH), 1762(C=O), 1591(Ar C=C), 1338, 1141(SO_2); ^{1}H NMR (400MHz, DMSO-d6) ppm: 9.83(s, 1H, NH), 7.95(d, *J* = 7.6Hz, 2H, Ar),7.61(d, *J* = 7.7Hz, 2H, Ar), 7.48(m, 4H, Ar), 7.31(s, 2H, NH_2); ^{13}C NMR (100MHz, DMSO-d6) ppm: 165.37(C=O imide), 163.62(C=O imide), 158.34(C-F, Ar), 142.42(C-N), 138.39(C-SO_2), 134.41(=C-NH), 132.61(C-NH, Ar), 125.71–126.18(8C, Ar), 125.71, 125.48(CH, Ar-F), 116.14-115.23(CH, Ar-F), 92.01(=C-Cl); *m/z* 395.20 $[M+H]^+$.

4.2 Pharmacological Evaluation

4.2.1 *Invitro*cyclooxygenase *(COX)*inhibition assay

The ability of the test compounds**4a-r** to inhibit ovine COX-1 and human recombinant COX-2 was determined by using a colorimetric enzyme assay kit. (Catalog No. 760131, Cayman Chemicals Inc., Ann Arbor, MI, USA) according to the manufacturer's assay protocol. Colorimetric Inhibitor Screening Assay measures the peroxidase component of COXs. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.[30] Inhibition of COX activity, by a variety of selective and nonselective inhibitors, showed potencies similar to those observed with other *in vitro* methods. Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reagents, buffer solution (0.1 M Tris-HCl, pH 8), heme and either COX-1 or COX-2 enzyme were mixed with test compounds. These solutions were incubated for a period of 5 min at 25°C, after which colorimetric substrate TMPD and quickly arachidonic acid were added. Shake the solutions for a few seconds and then incubate for

25°C for 2 min. The product of this reaction produces a blue colour that absorbs at 590nm. The intensity of the blue colour was determined by a microplate reader (power wave XS2). The calculations were performed as per kit guidelines.

4.2.2*In vivo* anti-inflammatory activity

4.2.2.1 Animals:

Adult male Wister albino rats (150-200 g) were used to study the anti-inflammatory activity and ulcerogenic liabilities. The animalswere maintained under standard laboratory conditions (light period of 12 h/day and temperature 27± 2°C), with access to food and water. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. All experiments were performed according to the guidelines for the care of laboratory animals. [31]

4.2.2.2 Carageenan-induced hind paw edema method:

Carageenan-induced hind paw edema method[32] was used for evaluating anti-inflammatory activity. Wistar albino rats (150-200g) were fasted for 24h and were divided into the following groups: carageenan control, test compounds, celecoxib as standards, each comprising six animals. The volume of the right hind paw of rat was measured by plethysmometer (UGO Basile, Italy). One group of rats, which served as control was given vehicle (1% Carboxymethyl cellulose (CMC) in water in a volume of 10 mL/kg p.o.) only. Test compounds (20 mg/kg b.w.) and celecoxib (20 mg/kg b.w.) suspended in vehicle (10 mL/ kg) were administered orally to the respective groups. After 30 min of administration of the drug and compounds, carageenan solution in normal salinewas injectedinto the planter surface right hind paw of each animal. The volume of swelling of the right hind paw of each rat was measured after 1, 3, and5h. The mean

increase in the volume of the right hind paw was compared with control and standard. The percentage inhibition of paw edema was calculated as follows,

Reduction of inflammation = [1 - (Vt/Vc) × 100]

Where, Vt is the change in paw volume in the test compound treated group, and Vc, is the changein paw volume in the control group.

4.2.2.3 Ulcerogenic activity

Male albino rats weighing 150-200 g were fasted for 12h prior to drug administration. Water was supplied *adlibitum*. The animals were divided into three equal groups (each of four). The first group received 7% gum acacia (suspending vehicle) orally once a day and was left as a control, whereas the other groups received the reference drugs and test compound**4j** with a dose of 20mg/kg/day orally. The test compounds were administered once a day for three successive days. Animals were sacrificed by diethyl ether 6h after the last dose and the animals were killed by an overdose of ether 6h after the last dose. The stomachs were removed, opened along the greater curvature, and examined for ulceration. The ulcer score was calculated according to the method of Vijaya and Mishra [33]: 0.0- normal (no injury); 0.5- latent injury; 1.0- slight injury (two to three dotted lines); 2.0- severe injury (continuous lined injury or five to six dotted injuries); 3.0- very severe injury (several continuous lined injuries); 4.0- widespread lined injury. The ulcer index was calculated according to the method of Robert et al[34]. The degree of ulcerogenic effect was expressed in terms of:

I. The percentage incidence of ulcers in each group ofanimals divided by 10.

II. The average number of ulcers per stomach.

III. The average severity of ulcers by visual observation.

Finally, the ulcer index was expressed as the summationvalue of the above three items.Results are tabulated in **Table 3**.

4.3 Molecular docking studies

4.3.1 Preparation of Target Protein X-ray Structure

The crystal structure ofCOX-2 in complex with SC-558 inhibitor (PDB code: 6COX, http://www.pdb.org/) was selected as the protein target model in this study. Using Schrodinger software[35] hydrogens were added and enzyme structure was subjected to a refinement protocol in which the constraints on the enzyme were gradually removed.

4.3.2 Ligands Preparation

LigPrep (v2.2) module in the Schrodinger molecular modeling suit was used for ligand preparation, which produced low energy conformations of ligand using OPLS 2005 force field. The bond orders of these ligands were fixed and the ligands 'cleaned' through LigPrep specifying a pH value of 7.0.

4.3.3 Protein-Ligand docking using Glide

Compound **4j** was docked into the active site of COX-2. The best docking conditions which succeeded to retrieve the pose of the co-crystallized ligands were used.Glide provides three different levels of docking precisions viz. High Throughput Virtual Screening, HTVS; Standard precision, SP and Extra precision, XP. We carried out our calculations using XP docking mode as the tool for better refinement.

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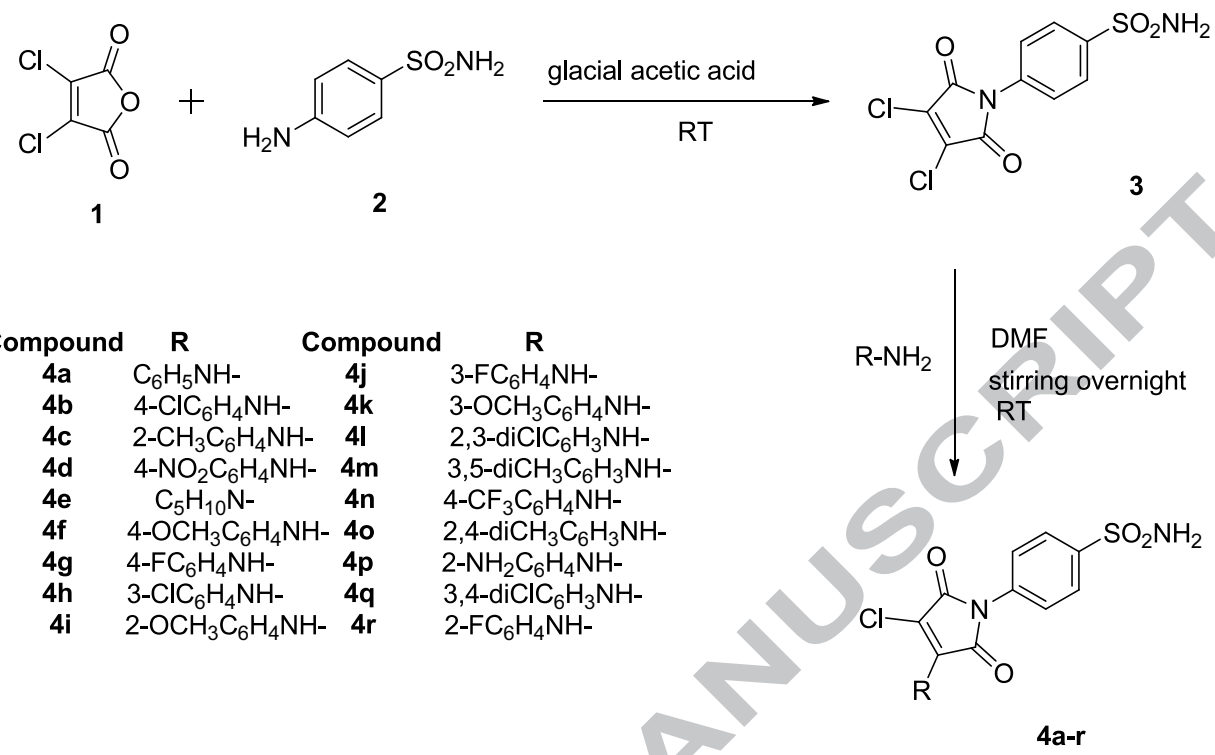

Compound	R	Compound	R
4a	C_6H_5NH-	**4j**	3-FC_6H_4NH-
4b	4-ClC_6H_4NH-	**4k**	3-$OCH_3C_6H_4NH$-
4c	2-$CH_3C_6H_4NH$-	**4l**	2,3-diClC_6H_3NH-
4d	4-$NO_2C_6H_4NH$-	**4m**	3,5-di$CH_3C_6H_3NH$-
4e	$C_5H_{10}N$-	**4n**	4-$CF_3C_6H_4NH$-
4f	4-$OCH_3C_6H_4NH$-	**4o**	2,4-di$CH_3C_6H_3NH$-
4g	4-FC_6H_4NH-	**4p**	2-$NH_2C_6H_4NH$-
4h	3-ClC_6H_4NH-	**4q**	3,4-diClC_6H_3NH-
4i	2-$OCH_3C_6H_4NH$-	**4r**	2-FC_6H_4NH-

Scheme 1

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Table 1 *In vitro* COX-1/COX-2 enzyme inhibition assay of the compounds

Table 2 Effect of **4a, 4h, 4j, 4k,** and **4r** on paw volume in carrageenan induced paw edema in rats

Table 3 Ulcerogenic effect of **4j** in rats (20 mg/kg)

Figure 1 Structures of COX-2 inhibitors containing sulfonamide moiety

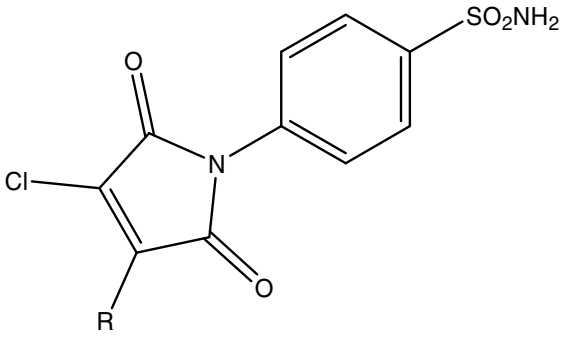
Figure 2 Common features present in COX-2 inhibitors and targeted compounds

Figure 3 The 3D image of 6COX-**4j** complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite. Hydrogen bonds between one of the O-atoms of SO_2NH_2 with Ala^{516} and -NH_2 of the SO_2NH_2 with Gln^{192} are shown in yellow dotted lines.

Figure 4 The 3D image of 6COX-SC558 complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite. Hydrogen bond between -NH_2 of the SO_2NH_2 with Gln^{192} is shown in yellow dotted lines.

Figure 5 The 3D image of 6COX-Celecoxib complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite.

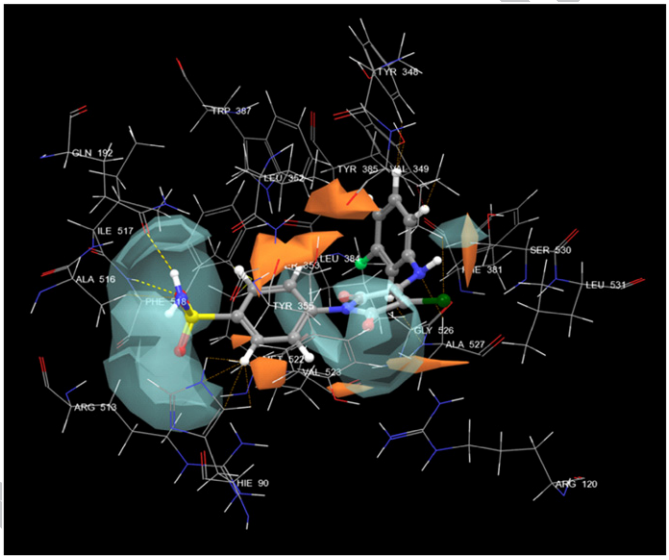
Graphical abstract



4a-r

R= Aliphatic and
Aromatic aniline

Anti inflammatory activity
4j: In vivo= 82.56%
In vitro= 49.31%



The 3D image of 6COX-4j complex constructed using Glide (V5.5) module in Schrodinger's molecular modeling suite.