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Analogue-based design, synthesis and docking of non-steroidal anti-inflammatory agents. Part 2: Methyl sulfanyl/methyl sulfonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones

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ABSTRACT

A series of methyl sulfanyl/methyl sufonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-one were designed using analogue-based design, scaffold hopping and shape similarity matching. The designed compounds were synthesized in 2–3 steps with simple chemistry and screened by ovine cyclooxygenases (COXs) inhibitory assay and carrageenan-induced rat paw edema assay. Among the screened compounds, two compounds exhibited 100% cyclooxygenase-2 (COX-2) inhibitory potency without showing cyclooxygenase-1 (COX-1) inhibition at 20 μM. The compounds also showed promising in vivo anti-inflammatory potential. A structure–activity relationship within the dataset was established by correlating the effect of aromatic ring substituent constants, structural variables and physico-chemical descriptors with in vivo anti-inflammatory activity. Molecular docking studies were also performed on the title compounds to study the binding interactions to COX-2 active site residues. The experimentally determined COX-2 inhibitory activity was found moderately correlating with binding modes predicted for compounds by Glide XP dock scoring function. The 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-one pharmacophore reported herein should be a new lead for further development of novel non-steroidal anti-inflammatory agents.

1. Introduction

Quinazolinone scaffold is a privileged structure for design and development of novel non-steroidal anti-inflammatory drugs (NSAIDs).[1] Natural and synthetic quinazolinone derivatives have been extensively studied for its anti-inflammatory potential through different pharmacological mechanism and they are well documented in the literature.[2–7] Especially, the quinazolinone based natural products; rutaecarpine[8,9] and tryptanthrin[10,11] have demonstrated significant anti-inflammatory potential through interfering prostaglandin pathway and cyclooxygenase (COX) inhibition. Rutaecarpine inhibited COX-2 and COX-1 dependent phases of PGD2 generation in BMMC in a concentration-dependent manner with an IC_{50} of 0.28 and 8.7 μM, respectively.[9] Tryptanthrin inhibited COX-2 in lipopolysaccharide stimulated Mono Mac 6 cells assay with an IC_{50} of 64 nM.[11]

COX enzyme plays a crucial role in production of various prostanoids involved in physiological and pathological functions especially inflammation.[12,13] COX exists in two distinct forms as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[14] COX-1 is designated as housekeeping enzyme because of its physiological and homeostatic functions, whereas COX-2 is expressed in pathological condition and triggers inflammatory signals, thus designated as an inducible enzyme. Both of these enzymes share more than sixty percent of homology with respect to their structure.[15] However, there is a small side pocket access in COX-2 because of presence of valine 523, which is absent in COX-1 due to bulkier isoleucine 523.[15] This single amino acid difference between COX-1 and COX-2 at position 523 made a huge impact in designing selective COX-2 inhibitors that can spare the gastric side effects associated with non-selective non-steroidal anti-inflammatory agents (NSAIDs).[15,16] The structure based inhibitors design on COX-2 enzyme identified a methyl sulfonyl or a sulphonamide group as the best chemical feature in accessing the side pocket and thus selectively inhibits COX-2.[17] As a result of this discovery, a wide variety of compounds having either a methyl sulfonyl or a sulphonamide substitution were synthesized, biologically evaluated and reported for COX-2 specific inhibition.[17–22]

In our continuing effort towards the identification of gastric sparring NSAIDs, we have established the quantitative structure–activity relationship (QSAR) of various central monocyclic ring (furanone[23] and pyranone[24,25]), bicyclic scaffold (benzopyran-4-one[25,26]), fused heterocycles (fused pyrazole[27–29] and thiazolotriazole[30]) and straight chain molecules[31] for selective COX-2 inhibition. In our previous communication, we also demonstrated the anti-inflammatory

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potential of a series of rationally designed 2, 3 diaryl quinazolinones acting through COX-2 inhibition.[1] In this Letter, we report our recent study, analogue-based design, scaffold hopping, shape matching, structure–activity relationship and docking studies of methyl sulfanyl and methyl sulfonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones as potential non-steroidal anti-inflammatory agents.

2. Result and discussion

2.1. Analogue-based pharmacophore design

The novel quinazolinone based pharmacophore for selective COX-2 inhibition was elucidated by analogue-based design that uses two natural products (rutaecarpine and tryptanthrin) and two well known synthetic selective COX-2 inhibitors (celecoxib and rofecoxib) shown in Figure 1. To build a complete pharmacophore, the common structural feature 'quinazolinone' present in both natural compounds was chosen as central template. The 'diaryl' system, which is common in celecoxib[18] and rofecoxib[19] was considered for side arm attachment to central quinazolinone core. Further, the secondary biophore feature 'methyl sulfonyl' group, which is known for COX-2 selectivity was introduced at *para* position of either of the side arm-aryl rings of quinazolinone. Finally, the central template was hopped with its dihydro analog to derive target compounds. Six compounds of methyl sulfanyl and six compounds of methyl sulfonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-one analogues were designed for anti-inflammatory activity evaluation based on the present pharmacophore. The designed compounds were then subjected to PHASE[32,33] (Schrodinger software, NY, USA) shape query with a selective COX-2 inhibitor (SC-558) bound in complex with cycloxygenase-2 enzyme (pdb code: 1CX2). The high shape similarity index (SSI >6.5) was obtained for all the designed compounds, which showed good shape and surface complementarily of the compounds to COX-2 enzyme binding pocket. All the designed compounds were also computationally evaluated for their pharmacokinetic and drug likeness property using molinspiration[1] web-portal and shown in Table 1.

2.2. Chemistry

The target compounds, 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-one analogues were synthesized by either two or three steps, according to methyl sulfanyl or methyl sulfonyl substitution pattern and is shown in Scheme 1. First, a series of (4-methylsulfanyl-benzylidene)-phenyl-amine derivatives **3a–f** were prepared by condensing various substituted anilines **1** with 4-thiomethyl benzaldehyde **2** in ethanol medium with few drops of glacial acetic acid at 78 °C. Further, Oxone[34,35] mediated oxidation reaction of these compounds provided (4-methyl sulfonyl-benzylidene)-phenyl-amine **3g–l** in moderate to good yields. Then these intermediate azomethines **3a–l** and an equimolar mixture of isatoic anhydride **4** reacted readily under microwave irradiation, without solvent for less than 3 min, which afforded substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones **5a–l** with yields of 70–80%. The proposed structures of intermediate azomethines and final compounds were confirmed by elemental analysis, IR, ESI-MS,

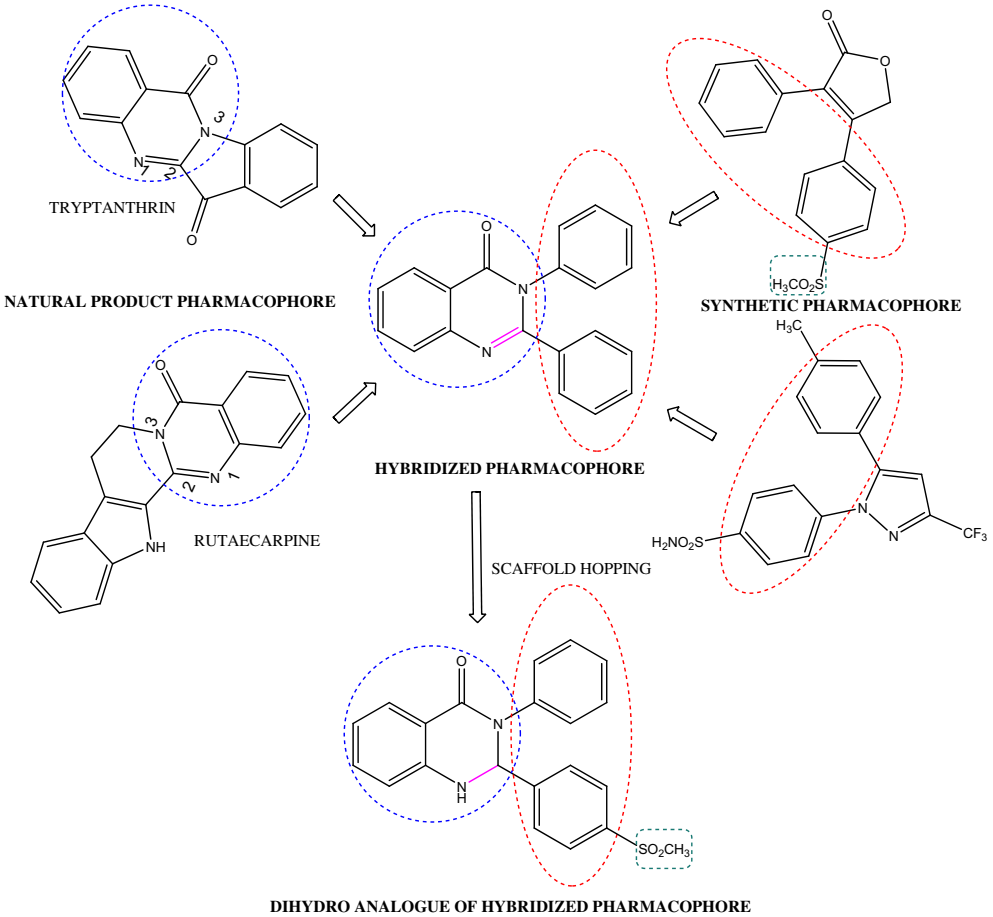
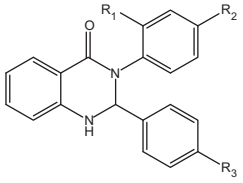
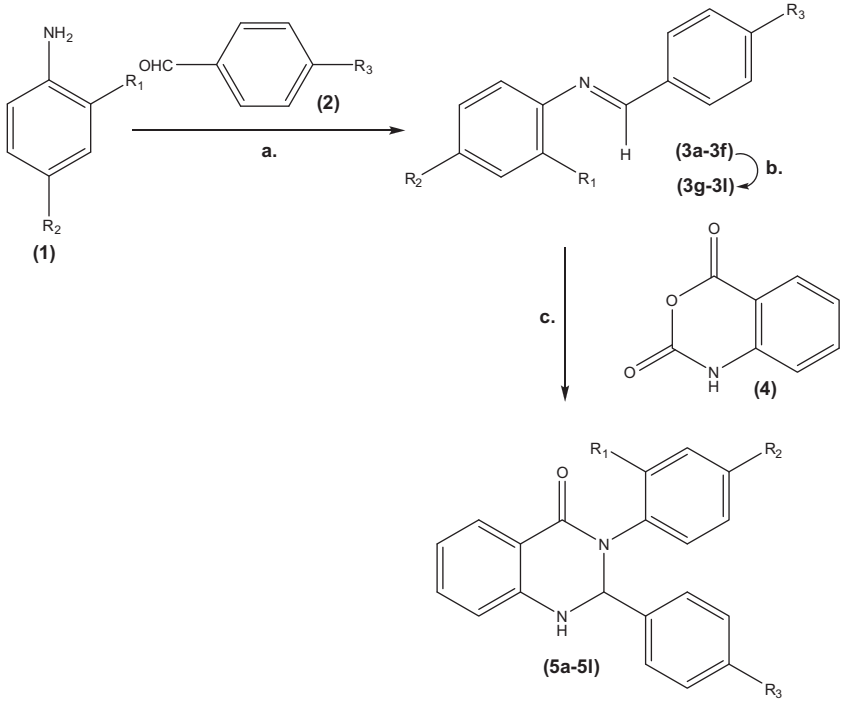


Figure 1. Design of novel anti-inflammatory pharmacophore template.

Table 1
Rationally designed substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones and their physicochemical properties



Compound	Substitution			Physico-chemical properties					
	R_1	R_2	R_3	ClogP	CMR	TPSA	Molecular volume	Number of rotatable bonds	Molecular weight
5a	H	H	SCH_3	1.8368	10.5044	32.34	315.750	3	345.467
5b	H	OCH_3	SCH_3	2.9028	11.1213	41.57	341.295	4	375.493
5c	H	Cl	SCH_3	3.6968	10.9958	32.34	329.285	3	379.912
5d	H	F	SCH_3	3.1268	10.5199	32.34	320.681	3	363.457
5e	F	H	SCH_3	2.9568	10.5199	32.34	320.681	3	363.457
5f	H	CH_3	SCH_3	3.4828	10.9682	32.34	332.311	3	359.494
5g	H	H	SO_2CH_3	−0.3632	10.5706	66.48	329.053	3	377.465
5h	H	OCH_3	SO_2CH_3	0.7028	11.1875	75.71	354.598	4	407.491
5i	H	Cl	SO_2CH_3	1.4968	11.0620	66.48	342.588	3	411.910
5j	H	F	SO_2CH_3	0.9268	10.5861	66.48	333.984	3	395.455
5k	F	H	SO_2CH_3	0.7568	10.5861	66.48	333.984	3	395.455
5l	H	CH_3	SO_2CH_3	1.2828	11.0344	66.48	345.614	3	391.492



Scheme 1. Reagents and conditions: (a) EtOH, CH_3COOH, reflux; (b) Oxone®, THF/MeOH, rt; (c) neat MW, 2–4 min.

and ^1H NMR spectra and presented in experimental section. ^1H NMR spectra showed the signal attributable to CH_3 of methyl sulfanyl in the region of 2.82 ppm. The signals appeared at 8.34–8.40 ppm attributable to benzylidinimino proton. The aromatic ring protons showed the chemical shifts of multiplet signals. For the title compounds, the conversion of methyl sulfanyl ($-SCH_3$) to methyl sulfonyl ($-SO_2CH_3$) was detected by the δ shift from 2.47 to 3.28 ppm for CH_3 protons in the NMR spectrum. Other aromatic protons appeared as multiplets in d 6.1–8.2 ppm. Further the mass spectrum [FAB-MS] showed a molecular ion peak (M+) for all the compounds with different intensities and the base peaks for most of the compounds. All the synthesized compounds were also characterized by elemental analysis and its experimental values in agreement with the calculated values.

2.3. Cyclooxygenases inhibition

The ability of methyl sulfanyl and methyl sulfonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones and reference drug celecoxib to inhibit the COX-1 and COX-2 isozymes was evaluated using colorimetric COX (ovine) inhibitor screening assay and the results obtained are listed in Table 2. The colorimetric COX (ovine) inhibitor screening assay measures the peroxidase component of COX. The peroxidase activity is assayed colorimetrically by

Table 2
COX-1, COX-2 inhibitory activities and in vivo anti-inflammatory activity of substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones

Compound	Percentage inhibition			Percentage edema inhibition			
	COX-1 (20 μM)	COX-2 (20 μM)	Selectivity	90 Min	180 Min	270 Min	360 Min
5a	—	—	—	30 ± 1.68*	34 ± 1.15*	37 ± 1.58*	39 ± 1.26*
5b	—	—	—	33 ± 1.94**	35 ± 0.24*	38 ± 1.12*	40 ± 1.63*
5c	5	91	0.055	38 ± 1.82*	41 ± 1.45***	46 ± 0.52*	47 ± 1.22*
5d	—	—	—	32 ± 1.61*	39 ± 1.08*	42 ± 0.48*	44 ± 1.64**
5e	—	—	—	33 ± 0.25*	37 ± 1.44*	39 ± 0.27***	41 ± 1.62*
5f	—	—	—	30 ± 0.26**	34 ± 0.28*	38 ± 0.88*	39 ± 0.74*
5g	49	66	0.742	31 ± 1.68*	35 ± 1.29*	38 ± 0.48*	40 ± 1.86*
5h	41	71	0.577	39 ± 1.94**	43 ± 0.74*	47 ± 1.52**	49 ± 1.76**
5i	NI	100	>0.001	37 ± 1.18*	42 ± 1.42**	46 ± 0.73**	51 ± 1.89*
5j	NI	100	>0.001	38 ± 1.55*	43 ± 1.48*	45 ± 1.22*	48 ± 1.62*
5k	15	54	0.278	36 ± 0.48*	39 ± 0.29*	42 ± 0.48*	44 ± 1.64*
5l	NI	91	>0.01	34 ± 1.32**	35 ± 1.24*	39 ± 0.23**	40 ± 1.04*
Celecoxib	3	94	0.032	—	—	—	—
Indomethacin	—	—	—	41 ± 0.48*	58 ± 1.26**	49 ± 0.28*	13 ± 0.24*

Significance levels *p <0.05, **p <0.01 and ***p <0.001 as compared with the respective control. All the results were expressed as means ± SEM (standard error of estimate).

monitoring the appearance of oxidized N,N,N′,N′-tetramethyl-*p*-phenylene diamine (TMPD) at 590 nm.

In regard to the COX-1 enzyme inhibition of target compounds, the methyl sulfanyl/methyl sulphonyl substituted analogues **5g** and **5h** were identified as weak COX-1 inhibitors as they have shown 49% and 41% inhibition at 20 μM respectively. Among all the screened compounds, **5i, 5j** and **5l** were proved to be practically inactive for COX-1 as they have shown (0%) no inhibition at 20 μM. In COX-2 enzyme inhibition assay, **5c, 5i, 5j** and **5l** showed higher COX-2 inhibitory activity with a range of 88–100% maximum. The COX-1 and COX-2 inhibitory activity results indicate that overall the methyl sulfonyl substitution on the target compounds increase the COX-2 selectivity in slightly varying magnitude depending up on the presence of other aromatic ring substitution. The moderate to better level of COX-2 selectivity was observed with the compounds, when substituted with electron withdrawing groups at *ortho* and *para* position of the aromatic rings.

2.4. In vivo anti-inflammatory activity

All the synthesized compounds were screened for in vivo anti-inflammatory activities at 50 mg per kg body weight dose level with carrageenan-induced rat paw edema assay using indomethacin as standard drug.[1] The edema induced by subplantar injection of carrageenan was observed more prominent in the group treated with carboxymethyl cellulose sodium (CMC-Na:Vehicle), where in case of indomethacin treated group exerted 58.16% anti-inflammatory effect after 180 min. Among the tested 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-one analogues **5c, 5h, 5i** and **5j** exhibited more than 45% edema inhibition and decreased the difference in paw thickness comparable to that of a group received standard anti-inflammatory drug indomethacin (p <0.05). The anti-inflammatory effects of compounds **5h** and **5i** were 49 ± 1.76* and 51 ± 1.89*% respectively and were comparable to the effect observed with indomethacin at 10 mg/kg dose. All test compounds of the series, the anti-inflammatory activity was retained up to six hours unlike indomethacin, in which a decrease in the activity was observed after 4 h. The efficacy of test compounds was comparable to that of indomethacin, but with a longer duration of action. As stated in our previous communication, this phenomenon may partly be due to the low systemic bioavailability of the test compounds following oral dosing, due to efficient first-pass metabolism and some degree of intestinal metabolism.[1]

Among the most active compounds, **5c** is the only compound with methyl sulfanyl at R_3 substitution position and rest of the compounds have methyl sulfonyl at R_3. As expected, 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones **5i** and **5j** substituted with methyl sulfonyl at R_3 (ie., 2 phenyl ring of dihydro-1*H*-quinazolin-4-ones) along with halogen substitution (Cl and F) at R_2 (ie., *para* position of N-aryl ring of dihydro-1*H*-quinazolin-4-ones) possessed most promising anti-inflammatory activity. Surprisingly, **5h** also showed good anti-inflammatory activity with an electron releasing group (–OCH_3) substitution at R_2 position and the reason for the compound's enhanced potency is not immediately apparent. The results of the anti-inflammatory activity studies clearly indicate that 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones exhibit comparable potency to Indomethacin. Mostly, the anti-inflammatory activity pattern was observed similar to that of our previously reported compound class 2,3-diaryl-quinazolin-4-ones.

2.5. Structure–activity relationship analysis

The in vivo anti-inflammatory activity data expressed in edema inhibition percentage was used for the present QSAR modelling. For the purpose of QSAR study, the percentage edema inhibition was converted into logarithm units. The converted anti-inflammatory activity was considered as dependent variable and the calculated aromatic substituent constants, physicochemical properties, structural variables, as independent variables for developing statistically significant relationships to explore the structure-activity requirements among these compounds.

Initially, a correlation matrix was built between dependant variable and independent variables, and they are graphically represented in Figure 2. All molecular descriptors and substituent constants were shown less correlation (r <0.5) with anti-inflammatory activity except two variables, R_2 (f) that is, inductive/field effect at R_2 position (r = 0.769), and molecular weight (r = 0.769). Interestingly, one of the 2D-molecular descriptors, topological polar surface area (TPSA) was found moderately correlated (r = 0.458) with anti-inflammatory activity. TPSA has been a widely used molecular descriptor in the study of drug transport properties and recently gained greater attention in QSAR modelling. TPSA, is the sum of the contributions of the molecular surface area of polar atoms such as oxygen, nitrogen and hydrogens. The positive correlation (r = 0.445) of an indicator variable $IR_3(SO_2CH_3)$ shows the methyl sulfonyl substitution in the title compounds favours the anti-inflammatory activity than methyl sulfanyl substitution. The statistically reliable QSAR models are discussed below:

Model 1. log (AA) = 0.205 (±0.052) R_2 f + 0.002 (±0.001) MW − 0.926 (±0.198)

n = 12, r = 0.919, r^2 = 0.845, r^2_{adj} = 0.811, s = 0.03, F = 24.612, p = 0.000,

DW = 2.564.

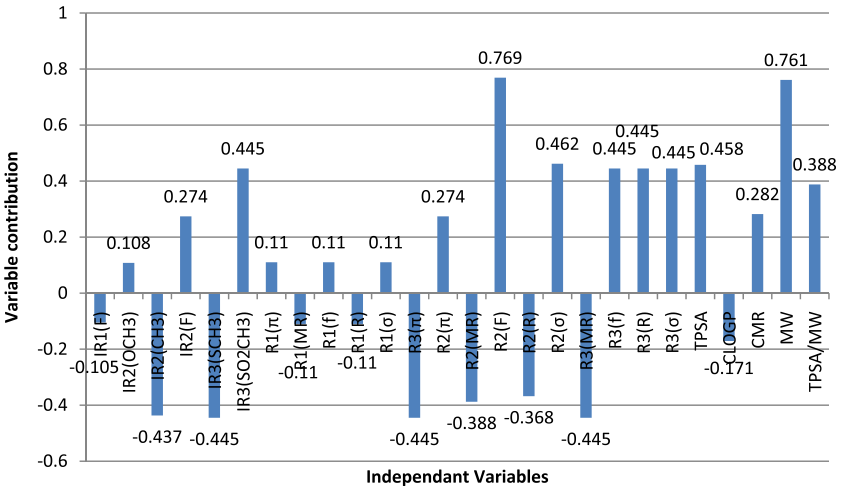


Figure 2. Graphical representation Hansch type structure–activity relationship; contribution of physicochemical constants, properties and structural variables to invivo anti-inflammatory activity.

The above QSAR model is a biparametric equation modelled with all 12 compounds. Compound **5b** (studentized residual = −3.093) was identified as an outlier while deriving the model 1. The substituent constant, R_2 (*f*) and MW together explains 84.5% variance in anti-inflammatory activity. R_2 (*f*) is the field effect/inductive effect at R_2 position of the title compounds. MW is the molecular weight of the compounds.

Model 2. log (AA) = 0.225 (±0.038) R_2 *f* + 0.002 (±0.000) MW − 0.861 (±0.144)

$n = 11$, $r = 0.962$, $r^2 = 0.925$, $r^2_{adj} = 0.906$, $s = 0.02$, $F = 49.084$, $p = 0.000$,

DW = 2.287.

Model 2 is developed for 11 compounds after eliminating compound **5b** as outlier, the reason for the outlying behaviour of this compound is not immediately apparent. However, 5b is the only compound with methoxy substitution at R_2 position in the methyl sulfonyl series. Model 2 explains 92.5% variance in anti-inflammatory activity. R_2 (*f*) is the field effect/inductive effect at R_2 position and molecular weight of the compounds show positive coefficient and thus have favourable effect towards anti-inflammatory activity. The 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones with either an electron-withdrawing or electron-donating groups on one of the phenyl rings (N-aryl) were found highly active. Thus, it can be easily understood that the electronic effect plays a major role in determining the anti-inflammatory activity. The structure–activity models discussed above provide more specific analysis and information about the electronic effects on this ring, showing that *para* position field effect/inductive effect are important for enhanced activity.

2.6. Cycooxygenase-2 binding interaction

Figures 3 and 4 reports the binding mode of the 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones with methyl sulfanyl or methyl sulfonyl group in the R_3 position respectively as compared to SC-558 complexed with COX-2. In the most favourable orientation found

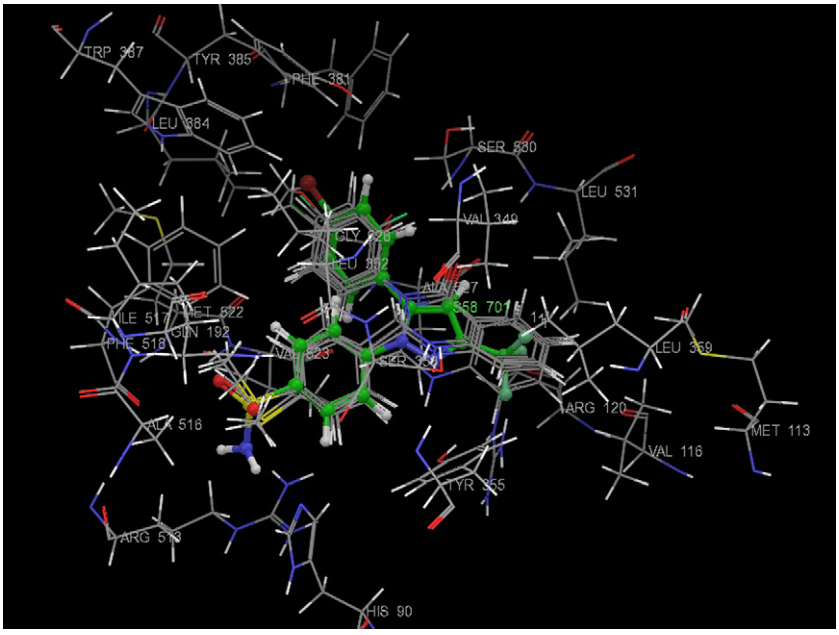


Figure 3. Docking poses of methyl sulfanyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones (**5a**–**f**) with SC-558 in active site of COX-2 Enzyme.

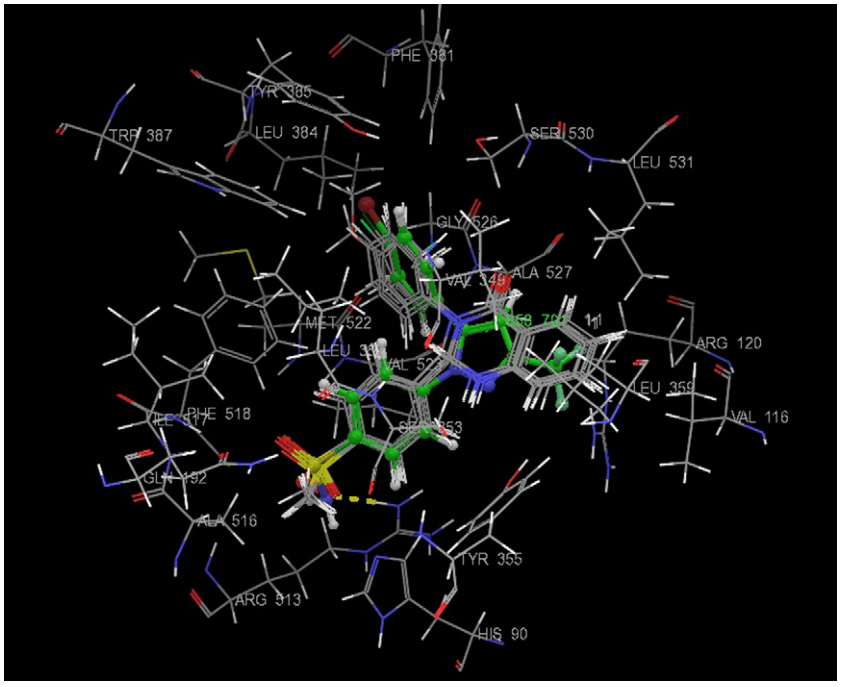


Figure 4. Docking poses of methyl sulfonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones (**5g**–**h**) with SC-558 in active site of COX-2 Enzyme.

by the GLIDE XP docking program, the methyl sulfanyl/methyl sulfonyl moiety is projected towards the selectivity pocket and the vicinal unsubstituted or substituted phenyl ring projected towards the hydrophobic pocket very much similar to the binding mode of SC-558. Though, it is surprising when compared to the docking results of 2,3-diaryl-3*H*-quinazolin-4-ones[1] where only five compounds attained a favourable disposition matching SC-558, it should be noted that the 'benzo ring' of quinazolinone ring is unsubstituted in 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones. 4-Fluoro substitution in the phenyl group oriented towards the hydrophobic pocket appears to have positive influence in the COX-2 binding affinity as indicated by their high Glide score. The docking scores of the compounds are in moderate agreement with the experimental potency determined against COX-2 enzyme. The top scoring ligand **5j** exhibit 100% percentage inhibition at 20 μM concentration and the docking pose of the compound in the active site of COX-2 enzyme is shown by Figure 4. It can also be appreciated from Figure 4 that the methyl sulfonyl group of compound **5j** positioned into hydrophilic cavity forms hydrogen bonding with Arg 513 and similar interaction is seen in all other compounds with methyl sulfonyl group possessing 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones.

3. Conclusion

In this Letter, we described the rational design of non-steroidal anti-inflammatory agents through analogue-based, scaffold hopping and shape similarity search. Further, the designed compounds were synthesized and biologically evaluated for in vitro and in vivo anti-inflammatory activities. The influence of dihydro quinazolinone as central core and different small substituents on the phenyl moieties of sidearm ring features was investigated. The structural optimization of the starting compound quinazolinones led to this new series with better cycloxygenase inhibitory activities and anti-inflammatory potencies. In whole, two compounds **5i** and **5j** were exhibited 100% COX-2 inhibitory potency without showing COX-1 inhibition at 20 μM and promising in vivo anti-inflammatory potential. The quantitative structure–activity model discussed here give more specific analysis and information about the electronic effects on the aromatic ring, showing that *para* position field effect/inductive effect are important for enhanced activity. Docking studies of these compounds with cyclooxygenase-2 enzyme showed that these molecules are in good agreement with the binding mode of inbound reference ligand SC-558. Thus, these compounds seems to be the best candidate for being further evaluation for its pharmacokinetic profile and for its in vivo ulcerogenic activity and toxicity model to validate the safe anti-inflammatory potential.

4. Experimental

4.1. Materials

The starting materials were procured from Merck and Sigma Aldrich or prepared using known procedures. All commercially available solvents and reagents were used without further purification. Column chromatographic separations were carried out by gradient elution with hexane–ethyl acetate mixture, unless otherwise mentioned and silica gel (60–120 mesh) used. TLC was performed on E-Merck pre-coated 60 F254 plates and the spots were rendered visible by exposing to UV light and iodine vapour. Melting points were recorded using an electrothermal melting point apparatus and are uncorrected. IR spectra were recorded using Shimadzu FTIR or Jasco FT/IR-470 PLUS. NMR spectral study was carried out using Brucker DRX 400. The FAB mass spectra were recorded on JEOL SX 102/DA-6000. Microanalytical data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyser. Elemental analyses observed for all the newly synthesized compounds were within the limits of accuracy (±0.3%). Topological polar surface area (TPSA) and molecular volume were calculated using the web-based program Molinspiration.

4.2. Synthesis

4.2.1. Synthesis for the synthesis of (4-methylsulfanyl-benzylidene)-phenyl-amine (3a–f)

Equimolar mixture of various substituted aniline (0.01 mol) and 4-thiomethyl benzaldehyde (0.01 mol), was refluxed in ethanol

with few drops of glacial acetic acid upto 10 h. The solvent was removed and the crude product thus obtained was crystallized in ethanol.

4.2.1.1. (4-Fluoro-phenyl)-(4-methylsulfanyl-benzylidene)-amine (3a). Yield: 81%; mp 94–95 °C; R_f = 0.41 mobile phase hexane–ethyl acetate (6:4); ^{1}H NMR ($CDCl_3$): δ 2.54 (s, 3H, SCH_3), 7.08 (m, 2H), 7.20 (m, 2H), 7.31 (d, *J* = 9 Hz, 2H) 7.79 (*J* = 9 Hz, 2H), 8.38 (s, 1H); ESI-MS *m/z* [M+H]$^+$: 246; Elemental Anal. Calcd. for $C_{14}H_{12}FNS$: C, 68.54; H, 4.93; N, 5.71. Found: C, 68.56; H, 4.93; N, 5.72.

4.2.1.2. Synthesis for the synthesis of (4-methylsulfonyl-benzylidene)-phenyl-amine (3g–l). A solution of Oxone in H_2O (6 ml) and methanol (4 ml) was added dropwise to a solution of 4-thiomethyl compounds (0.015 mol) in THF (10 ml) at 25 °C with stirring. The reaction was allowed to proceed for 2–3 h prior to addition of H_2O (10 ml) and extraction with CH_2Cl_2 (4 × 30 ml). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography.

4.2.1.3. Synthesis of substituted 2,3-diaryl-2,3-dihydro-quinazolin-4-ones (5a–l). A mixture of isatoic anhydride (0.01 mol) and substituted azomethines (**3a–l**) were transferred in to a clean 50 ml vial. The reaction mixture was then microwave irradiated for 2–4 min. After cooling to room temperature, the reaction mixture was extracted with dichloromethane (15 ml) and filtered to remove all the unreacted and insoluble materials. The clear extract was concentrated and cooled to obtain appropriate 2,3-diaryl-2,3-dihydro-quinazolin-4-ones.

4.2.1.4. 2-(4-Methylsulfanyl-phenyl)-3-phenyl-2,3-dihydro-1*H*-quinazolin-4-one (5a). Yield, 76%; mp 203–205 °C; R_f = t0.54 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3299 (NH stretch); 1641 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 2.49 (s, 3H, S-CH_3), 4.75 (s, 1H), 6.20 (s, 1H), 6.55(d, *J* = 8 Hz, 1H), 6.88–7.35 (m, 11H), 8.14 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 347; Elemental Anal. Calcd. for $C_{21}H_{18}N_2OS$: C, 72.80; H, 5.24; N, 8.09. Found: C, 72.82; H, 5.29; N, 8.07.

4.2.1.5. 3-(4-Methoxy-phenyl)-2-(4-methylsulfanyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5b). Yield: 71%; mp 245–247 °C; R_f = 0.38 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3301 (NH stretch); 1639 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 2.49 (s, 3H, SCH_3), 3.76 (s, OCH_3), 4.70 (s, 1H), 6.05 (s, 1H), 6.61 (d, *J* = 8 Hz, 1H), 6.84–7.38 (m, 10H), 8.06 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 377; Elemental Anal. Calcd. for $C_{22}H_{20}N_2O_2S$: C, 70.19; H, 5.35; N, 7.44. Found: C, 70.24; H, 5.38; N, 7.46.

4.2.1.6. 3-(4-Chloro-phenyl)-2-(4-methylsulfanyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5c). Yield: 78%; mp 232–234 °C; R_f = 0.42 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3303 (NH stretch); 1643 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H, SCH_3), 4.76 (s, 1H), 6.08 (s, 1H), 6.65 (d, *J* = 8 Hz, 1H), 6.90–7.44 (m, 10H), 8.12 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 381; Elemental Anal. Calcd. for $C_{21}H_{17}ClN_2OS$: C, 66.22; H, 4.50; N, 7.35. Found: C, 66.28; H, 4.48; N, 7.38.

4.2.1.7. 3-(4-Fluoro-phenyl)-2-(4-methylsulfanyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5d). Yield: 69%; mp 241–243 °C; R_f = 0.41 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3298 (NH stretch); 1640 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 2.48 (s, 3H, SCH_3), 4.70 (s, 1H), 6.05 (s, 1H), 6.57 (d, *J* = 8 Hz, 1H), 6.89–7.41 (m, 10H), 8.14 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 365; Elemental Anal. Calcd. for $C_{21}H_{17}FN_2OS$: C, 69.21; H, 4.70; N, 7.69. Found: C, 69.25; H, 4.76; N, 7.73.

4.2.1.8. 3-(2-Fluoro-phenyl)-2-(4-methylsulfanyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5e). Yield: 60%; mp 246–248 °C; R_f = 0.41 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3298 (NH stretch), 1640 (C=O stretch), ^{1}H NMR ($CDCl_3$): δ 2.47 (s, 3H, SCH_3), 4.71 (s, 1H), 6.04 (s, 1H), 6.57 (d, *J* = 8 Hz, 1H), 6.88–7.40 (m, 10H), 8.14 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 365; Elemental Anal. Calcd. for $C_{21}H_{17}FN_2OS$: C, 69.21; H, 4.70; N, 7.69. Found: C, 69.26; H, 4.62; N, 7.64.

4.2.1.9. 2-(4-Methylsulfanyl-phenyl)-3-*p*-tolyl-2,3-dihydro-1*H*-quinazolin-4-one (5f). Yield: 81%; mp 220–222 °C; R_f = 0.45 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3302 (NH stretch); 1642 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 2.49 (s, 3H, SCH_3), 2.78 (s, 3H), 4.76 (s, 1H), 6.10 (s, 1H), 6.76 (d, *J* = 8.1 Hz, 1H), 7.02–7.50 (m, 10H), 8.21 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 362; Elemental Anal. Calcd. for $C_{22}H_{20}N_2OS$: C, 73.30; H, 5.59; N, 7.77. Found: C, 73.41; H, 5.63; N, 7.81.

4.2.1.10. 2-(4-Methanesulfonyl-phenyl)-3-phenyl-2,3-dihydro-1*H*-quinazolin-4-one (5g). Yield: 81%; mp 179–181 °C; R_f = 0.41 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3295 (NH stretch); 1641 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 3.29 (s, 3H, SO_2CH_3), 4.75 (s, 1H), 6.11 (s, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.98–7.41 (m, 11H), 8.16 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 379; Elemental Anal. Calcd. for $C_{21}H_{18}N_2O_3S$: C, 66.65; H, 4.79; N, 7.40. Found: C, 66.60; H, 4.82; N, 7.42.

4.2.1.11. 2-(4-Methanesulfonyl-phenyl)-3-(4-methoxy-phenyl)-2,3-dihydro-1H-quinazolin-4-one (5h). Yield: 75%; mp 219–221 °C; R_f = 0.39 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3294 (NH stretch), 1636 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 3.29 (s, 3H, SO_2CH_3), 3.71 (s, 3H), 4.70 (s, 1H), 6.10 (s, 1H), 6.75 (d, *J = 8.1Hz,* 1H), 6.88–7.40 (m, 10H), 8.13 (d, *J = 7.9Hz,* 1H); ESI-MS *m/z* [M+H]$^+$: 409; Elemental Anal. Calcd. for $C_{22}H_{20}N_2O_4S$: C, 64.69; H, 4.94 (4.81); N, 6.86 (6.82). Found: C, 64.74, H, 4.81; N, 6.86 6.82.

4.2.1.12. 3-(4-Chloro-phenyl)-2-(4-methanesulfonyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5i). Yield: 71%; mp 192–194 °C; R_f = 0.44 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3294 (NH stretch); 1636 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 3.29 (s, 3H, SO_2CH_3), 4.76 (s, 1H), 6.19 (s, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.94–7.42 (m, 10H), 8.16 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 413; Elemental Anal. Calcd. for $C_{21}H_{17}ClN_2O_3S$: C, 61.09; H, 4.15; N, 6.78. Found: C, 61.88; H, 4.20; N, 6.86.

4.2.1.13. 3-(4-Fluoro-phenyl)-2-(4-methanesulfonyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5j). Yield: 70%; mp 229–231 °C; R_f = 0.41 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3298 (NH stretch); 1636 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 3.28 (s, 3H, SO_2CH_3), 4.74 (s, 1H), 6.16 (s, 1H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.91–7.38 (m, 10H), 8.14 (d, *J* = 7.9 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 397; Elemental Anal. Calcd for $C_{21}H_{17}FN_2O_3S$: C, 63.62; H, 4.32; N, 7.07. Found: C, 63.71; H, 4.40; N, 7.12.

4.2.1.14. 3-(2-Fluoro-phenyl)-2-(4-methanesulfonyl-phenyl)-2,3-dihydro-1*H*-quinazolin-4-one (5k). Yield: 72%; mp 225–227 °C; R_f = 0.40 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3302 (NH stretch); 1638 (C=O stretch); ^{1}H NMR ($CDCl_3$): δ 3.28 (s, 3H, SO_2CH_3), 4.75 (s, 1H), 6.18 (s, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.89–7.37 (m, 10H), 8.14 (d, *J* = 8 Hz, 1H); ESI-MS *m/z* [M+H]$^+$: 397; Elemental Anal. Calcd. for $C_{21}H_{17}FN_2O_3S$: C, 63.62; H, 4.32; N, 7.07. Found: C, 63.65; H, 4.28; N, 7.15.

4.2.1.15. 2-(4-Methylsulfonyl-phenyl)-3-*p*-tolyl-2,3-dihydro-1*H*-quinazolin-4-one (5l). Yield: 80%; mp 183–185 °C; R_f = 0.46 mobile phase hexane–ethyl acetate (6:4); IR (KBr, cm^{-1}): 3299 (NH stretch); 1640 (C=O stretch); 1512; ^1H NMR ($CDCl_3$): δ 3.28 (s, 3H, SO_2CH_3), 4.75 (s, 1H), 6.18 (s, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.89–7.37 (m, 10H), 8.14 (d, J = 8 Hz, 1H); ESI-MS m/z $[M+H]^+$: 393; Elemental Anal. Calcd. for $C_{22}H_{20}N_2O_3S$: C, 67.33 (67.39); H, 5.14 (5.12); N, 7.14 (7.08). Found: C, 67.39; H, 5.12; N, 7.08.

4.3. Pharmacological evaluation

4.3.1. COX-1 and COX-2 inhibition assay

The in vitro ability of the synthesized compounds and the reference drug celecoxib to inhibit the COX-1 and COX-2 isozymes was carried out using cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 760111) supplied by Caymen chemicals, USA. The calculations were performed as per the kit guidelines.[36,37]

4.3.2. Animals

Animals were assigned into different groups randomly and each group consists of six animals. The animals were kept in appropriate cages at temperature controlled (25 ± 2 °C) rooms, under a 12 h light and dark cycle and they were fed standard rodent pellet. All the animals were acclimatized for a week before the experiment. Standard ethical guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed.

4.3.3. Carrageenan-induced rat paw edema assay

The test compounds and the standard drugs were administered orally in the form of a suspension in 1% or 0.5% carboxy methylcellulose sodium (CMC-Na). Anti-inflammatory activity was determined using carrageenan induced foot paw edema assay method in rats using indomethacin 10 mg/kg as standard drug.[38] The test compounds were administered orally at a dose of 50 mg/kg. Edemas were produced by injecting 0.1 ml of a solution of carrageenan in the hind paw. Paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 90, 180, 270 and 360 min after carrageenan injection. The percentage of edema inhibition was calculated using the following formula.[1]

$$\text{Percentage of edema inhibition} = 100[1 - (A - X)/(B - Y)]$$

Where, X is the mean paw volume of rats before the administration of carrageenan and test compounds or reference drugs; A and B is the mean paw volume of rats after the administration of carrageenan in the test group and control group, respectively; Y is the mean paw volume of rats before the administration of carrageenan in the control group.

4.3.4. Statistical calculations

Statistical significance of anti-inflammatory activity of the compounds on rat paw edema model was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A significance level of p <0.05 was considered as acceptable in all cases. All the results were expressed as means ± SEM (standard error of estimate).

4.4. Physico-chemical constants, structural properties and correlation

Hansch 2D-QSAR analysis was performed using two types of predictor descriptors, physicochemical constants and indicator variables. An indicator variable was assigned for any structural variations between the compounds as I with a relevant subscript of the specific chemical feature. I was assigned a value of 1 if a particular chemical feature is present and to 0 if the same is absent. Physicochemical substituent constants—hydrophobic (π), molar refractivity (MR), hammet electronic constant (σ), resonance effect (R) and field/inductive effect (f) were taken from the literature.[39] In addition, some physico–chemical properties like Calculated logarithm of partition co-efficient, (C log P), calculated molar refractivity (CMR), molecular weight (MW) and toplogical polar surface area were calculated by Chemoffice 2008 software package.[40,41] A correlation matrix was constructed to correlate the biological activity with the various physicochemical and structural predictor variables. QSAR models were built using the regression analysis module of systat version 11.[42] The correlation matrix was used to correlate the biological activity with the various predictor variables. Descriptors with inter correlation above r >0.5 are not considered while deriving QSAR models. The predictor variables with p value greater than 0.05 were eliminated whilst deriving the QSAR models in order to assure their statistical reliability. The QSAR models were evaluated by using the statistical parameters viz., correlation coefficient (r) or coefficient of determination (r^2), adjusted r^2 (r^2_{adj}), standard error of estimate (s), Fischer F-value and student_s t-distribution. The figures within the parentheses following the coefficient terms are the standard error of the regression terms and the constants.

4.5. Molecular docking

Docking studies of the compounds were performed using crystal structure of COX-2 enzyme (PDB ID: 1CX2) obtained from the RCSB Protein Data Bank, which in-houses the selective COX-2 inhibitor SC-558 in its active site.[1,43] All the docking simulations were carried out using the program GLIDE (Grid-based Ligand Docking with Energetics) module version 4.5, Schrödinger, LLC, New York, NY, 2007 (Schrodinger Inc.).[44,45] The protein residue was prepared for grid generation by running the protein preparation wizard of Maestro. The impref minimizations were run until the average root mean square deviation (rmsd) of the non-hydrogen atoms reached 0.3 Å. Grid files were generated using the co-crystallized ligand at the centre of the two boxes. The size of the binding box was set at 20 Å in order to explore a large region of the protein. The three-dimensional structures of the compounds were constructed using the Maestro interface. The initial geometry of the structures was optimized using the OPLS-2005 force field performing 1000 steps of conjugate gradient minimization. The compounds were subjected to flexible docking using the pre-computed grid files. For each compound the 100 top-scored poses were saved and analyzed and only the best scoring poses were selected for the study.

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