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3D QSAR Studies on Novel Terphenyls for Selective Inhibition of Cyclooxygenase-2 Enzyme

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Classical non-steroidal antiinflammatory drugs being non selective cyclooxygenase (cyclooxygenase-1 and 2) inhibitors are associated with serious adverse effects while selective cyclooxygenase-2 inhibitors are gastro safer non steroidal antiinflammatory drugs, as it is now well established that antiinflammatory activity of non steroidal antiinflammatory drugs is due to the inhibition of cyclooxygenase-2 enzyme. In view of that we have identified essential biophoric (pharmacophoric) features on novel terphenyls (terphenyl methyl sulphonamides and sulphones) by the molecular modeling studies using APEX-3D expert system for selective inhibition of cyclooxygenase-2 and cyclooxygenase-1 enzyme. In addition to that multiparameter 3 dimensional quantitative structure activity relationship equations have also been generated, described relationships of biological activities with biophoric centers, global property and secondary sites and results can be used for structure optimization of novel terphenyls for selective inhibition of cyclooxygenase-2 enzyme and also for lead generation. As results of this study corroborates with the active sites of cyclooxygenase-2 and cyclooxygenase-1 enzymes, validate this study. Among several biophoric models generated, two models (1 and 2) for inhibition of cyclooxygenase-2 enzyme and other two models (3 and 4) for inhibition of cyclooxygenase-1 enzyme with good statistical values were selected. The purpose of study was to optimize selectivity of novel terphenyls towards inhibition of cyclooxygenase-2 enzyme, in order to that we have predicted the activity of prediction set compounds and described perfect prediction in 80% of the cases, validate the robustness of biophoric models for selective inhibition of cyclooxygenase-2. Different biophoric features and secondary sites for inhibition of cyclooxygenase-2 and cyclooxygenase-1 enzyme give an opportunity to design potent and highly selective cyclooxygenase-2 inhibitors.

Prostaglandins (PGs) are well known to be mediators of inflammation, pain and swelling. Nonsteroidal antiinflammatory drugs (NSAIDs) exert their pharmacological action by inhibiting prostaglandin biosynthesis[1,2]. The pharmacological target of NSAIDs is PG synthase, also known as cyclooxygenase (COX), which catalyses the first committed step in arachidonic acid (AA) metabolism[3,4]. There are two isoforms of COX[5], the COX-1 is a constitutive enzyme present in most tissues and it is responsible for biosynthesis of PGs[6], the other isoform known as COX-2 is an inducible enzyme, which is induced by cytokinins, mitogens and endotoxins in inflammatory cells[7] and is involved in elevated production of PGs during inflammation[8,9]. The enzymatic activity of COX involves two different catalytic activities: cyclooxygenase activity,

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which converts AA to PGG_2 and peroxidase activity that converts PGG_2 to PGH_2. The PGH_2 is further metabolized by specific synthases and isomerases to various prostanoids[1].

NSAIDs act at the cyclooxygenase active site and most of them inhibit both isoforms of COX with little specificity[10,11]. It is now well established that antiinflammatory activity is due to the inhibition of COX-2 while inhibition of COX-1 leads to serious side effects[12]. A variety of heterocycles such as 4,5-diaryl pyrroles[13],1,2-diaryl cyclopentenes[14,15], novel terphenyls[16], 5,6-diaryl thiazolo [3,2-*b*] [1,2,4] triazoles[17], 5,6-diarylimidazo [2.1-*b*] thiazole[18], 1,2–diarylpyrroles[19], 1,2-diaryl imidazole[20], 1,3,4 and 1,2,4–thiadiazole[21], thiazolones and oxazolones[22], alkoxy lactones[23], 3–heteroaryloxy-4-phenyl-2(5H)-furanones[26], methanesulphonylphenyl[25], 4-[5-methyl-3-phenylisoxazole-4-yl]-benzenesulphonamide[26], 3,4-aryloxazolnes[27], pyrazolo [1,5-*a*] pyrimidines[28] have been investigated for COX-2 selective inhibition.

Although selective COX-2 inhibitors belong to different chemical classes but share one common feature viz a diaryl substitution to a central ring (pyrazole, furan, thiophene, spirocyclopentens, and cyclopenten-1-one). The structure activity relationship studies suggest that sulphonamido or sulphonyl methyl group on para position of one of the aryl ring contributes for COX-2 selectivity. COX-2 selective inhibition is based on salient structural differences between COX-1 and COX-2 enzymes as evidenced by their X-ray crystal structures where slight differences have been observed in amino acid composition like an extra side pocket in COX-2 due to the presence of valine at position 523 unlike in COX-1 where this position is occupied by isoleucine[29]. Another difference is presence of a small alcove in COX-2 active site created by different positions of leucine 384-side chain between COX-1 and COX-2[30].

In view of the application of 3 dimensional quantitative structure activity relationship (3D QSAR) study in identification of important structural and physicochemical features responsible for activity and selectivity, recently Christophe *et al.*[31] have reported COMFA studies on randomly chosen molecules from diverse chemical structures while 3D QSAR studies for COX-2 inhibition in 5,6-diarylimidazo [2.1-*b*] thiazole have been reported from our laboratory[32]. However no such studies have been reported for both COX-1 and COX-2 inhibitory activities in the same set of molecules. Such studies coupled with above X-ray crystal structural studies derived information may be very useful not only in improving COX-2 inhibitory activity but also selectivity for COX-2 inhibition. So 3D QSAR studies for COX-1 and COX-2 inhibitory activities have been carried out on novel terphenyls and the studies are described in this paper.

MATERIALS AND METHODS

Selection of compounds and biological activity:

The compounds chosen for the present study were taken from the literature[16]. The structure and biological activity data of the compounds forming the training set (44 compounds for COX-2 inhibition and 19 compounds for COX-1inhibition) for all the molecules with definite IC_{50} values are shown in table 1 and prediction set for COX-2 inhibition by the molecules with no definite IC_{50} values in table 2. The reported biological activity values were converted into -log IC_{50}.

Workstation:

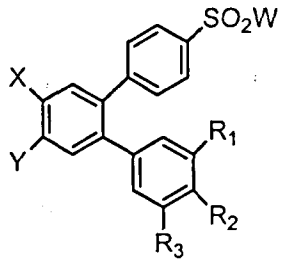
Molecular modelling and 3D QSAR studies were performed on a silicon graphics Indy R 4000 work station employing molecular simulations incorporations (MSI) software (Insight II[33] Discover[34] and Apex-3D[35]).

Molecular modelling and 3D QSAR:

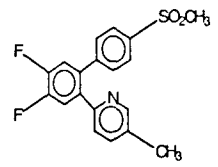
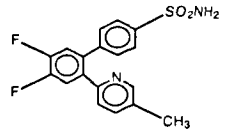
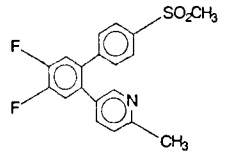
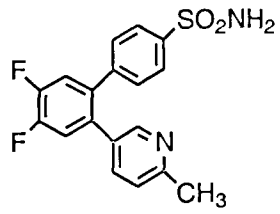
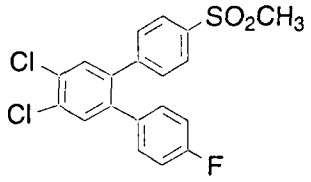
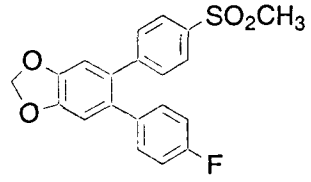
The 3D molecular structures of all the compounds were built using Insight II software and 3D structures were energy minimized using the steepest descent, conjugate gradient, Newton Raphsons algorithms in sequence followed by quasi. Newton Raphson (Va 09a) energy minimization techniques based on CVFF Force fields[36] implemented in the discover module by using 0.001 kcal/mole/Å energy gradient convergence and maximum number of iteration set to 1000 as detailed in our paper[37].

The validity of the above energy minimized techniques *vis a vis* other low energy conformations near global minimum was checked on one of the most active compound 4, which was subjected to molecular dynamics (MD) simulations using CVFF force field. In this procedure the optimised conformations of compound 4 was randomised by setting random velocities and carrying out MD simulations at 0.1 ps at temp of T=1000 K. The obtained average conformation of compound 4 by this calculation was used as starting point for another 5 ps of MD simulations at T=1000 K. The purpose of high temperature was to explore conformational space extensively. An Annealing procedure was subsequently applied to each average conformations obtained in high temperature simulations. The annealing was carried out, as slow cooling down of the structure from 1000 to 300 K. The last step of an annealing procedure was

TABLE 1: *IN VITRO* COX-2 INHIBITION ACTIVITY OF NOVEL TERPHENYLS (TRAINING SET)

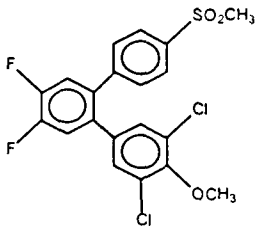
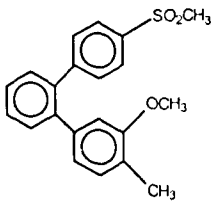
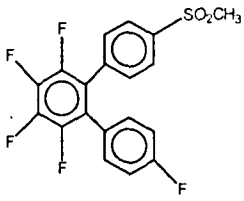
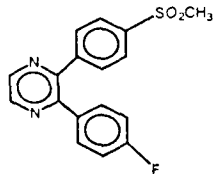
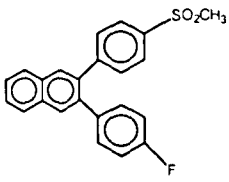


Compound	X	Y	R1	R2	R3	W	COX-1 $-\log IC_{50}$ μM	COX-2 $-\log IC_{50}$ μM
01	F	F	H	F	H	CH_3	——	1.8538
02	F	F	H	F	H	NH_2	-0.7559	2.3979
03	F	F	H	F	Cl	CH_3	——	2.000
04	F	F	H	F	Cl	NH_2	-0.7404	2.6989
05	F	F	H	F	CH_3	CH_3	——	2.3010
06	F	F	H	F	CH_3	NH_2	-0.5682	2.6989
07	F	F	H	OCH_3	F	CH_3	——	1.6778
08	F	F	H	OCH_3	F	NH_2	-1.3522	1.8861
09	F	F	H	OCH_3	Cl	CH_3	——	1.7212
10	F	F	H	OCH_3	Cl	NH_2	-1.2765	1.8861
11	F	F	Cl	OCH_3	Cl	NH_2	——	1.6778
12	F	F	H	OCH_3	CH_3	CH_3	——	1.8861
13	F	F	H	OCH_3	CH_3	NH_2	-1.0374	2.3010
14	F	F	H	OCH_3	OCH_3	CH_3	——	0.4685
15	F	F	H	OCH_3	OCH_3	NH_2	——	1.1871
16	F	F	H	$-OCH_2\ CH_2O-$		CH_3	——	0.4685
17	F	F	H	$-OCH_2\ CH_2O-$		NH_2	-1.2528	1.4946
18	F	F	H	$-OCH_2O-$		CH_3	——	1.9208
19	F	F	H	$-OCH_2\ O-$		NH_2	-0.2304	2.3979
20	F	F	H	CH_3	H	CH_3	——	2.1549
21	F	F	H	CH_3	H	NH_2	-1.2304	2.3979
22	F	F	H	CH_3	Cl	CH_3	——	1.8861
23	F	F	H	CH_3	Cl	NH_2	-1.2175	2.5229
24	F	F	H	CH_3	CH_3	CH_3	——	1.6382
25	F	F	H	CH_3	CH_3	NH_2	-1.1703	2.3010
26	F	F	H	Cl	CH_3	CH_3	——	2.2218
27	F	F	H	Cl	CH_3	NH_2	-0.5911	2.5229
28	F	F	H	$(CH_3)_2N$	Cl	CH_3	——	2.0969
29	F	F	H	$(CH_3)_2N$	Cl	NH_2	0.2291	2.2218

30							—	-1.7185
31							—	0.4815
32							—	0.7696
33							-2.4232	1.8210
34	H	H	H	F	H	CH_3	—	0.5850
35	H	H	Cl	F	H	CH_3	—	0.4436
36	H	H	H	Cl	H	CH_3	—	0.8538
37	H	H	Cl	OCH_3	H	CH_3	—	-1.0569
38	H	H	H	F	H	NH_2	-1.2787	1.2146
39	H	H	Cl	F	H	NH_2	-0.9638	1.7695
40	H	H	H	Cl	H	NH_2	-0.5911	2.2218
41	H	H	F	OCH_3	H	NH_2	-1.1206	1.4814
42	H	H	Cl	OCH_3	H	NH_2	-0.9138	1.7212
43							—	0.6197
44							—	1.880

IC_{50} values were determined for inhibitory activity against COX-1 and COX-2 forms of the human recombinant enzymes.

TABLE 2 : *IN VITRO* COX-2 INHIBITION ACTIVITY OF NOVEL TERPHENYLS (PREDICTION SET)

Compound	Structure	COX-2 $-\log IC_{50}$ μM
01		> - 2.0
02		> - 2.0
03		> - 2.0
04		> - 2.0
05		> - 2.0

IC_{50} values were determined for inhibitory activity against COX-1 and COX-2 forms of the human recombinant enzymes

energy minimization. Using these approach 20 conformations for a given starting geometry which gives a total of 75-150 ps of simulation time was obtained. The total energy of these 20 conformations ranged between 169.93 to 170.12 kcal/mole that was near to the conformational energy (170.12 kcal/mole) obtained from the standard energy minimization procedure described above. Hence the same energy minimized conformations for all molecules were used in the 3D-QSAR-model development.

Automated identification of pharmacophore and 3D QSAR building:

The computational calculations of different physicochemical properties including: atomic charge, π-population, H–donor and acceptor index, HOMO, LUMO, Hydrophobicity, molar refractivity based on atomic contributions[38,39] were carried out on energy minimized structures with MOPAC 6.0 version (MNDO Hamiltonian)[40]. The data was used by APEX-3D programme for automated identification of pharmacophore and 3D QSAR model building[41,42]. The compounds with definite COX-1 and COX-2 inhibitory activities (IC_{50}) of novel terphenyls were classified into following classes (i). Very active (>2.3979), (ii). Active (<2.3979 and >=1.1871) (iii). Moderately active (<1.1871 and >=-1.7185 (iv) Not active (=<-1.7185) for COX-2 inhibition and (i) Very active (>-0.90) (ii) Active (>=-1.3522 and <-0.90) (iii) Less active (<-1.352) for COX-1 inhibitory activity. The 3D QSAR equation were derived by defining COX-1 and COX-2 inhibitory activities ($-\log IC_{50}$) as a dependent variable and biophoric centres properties (π-population, charge, HOMO, LUMO, ACC_01,Don_01,hydrophobicity, refractivity), global properties (total hydrophobicity and total refractivity), secondary sites [(H-acceptor (presence), H-donor (presence), heteroatom (presence), hydrophobic (hydrophobicity), steric (refractivity) and ring (presence)] as independent variables with the occupancy set at 10, site radius at 0.80, sensitivity at 1.00 and randomization value at 100 for COX-2 inhibitory activity and occupancy set at 8, site radius at 0.60, sensitivity at 0.80 and randomization value at 100 for COX-1 inhibitory activity. Quality of each model was estimated from the observed r^2 (coefficient of correlation), RMSA (calculated root mean square error based on all compounds with degree of freedom correction), RMSP (root mean square error based on 'leave one out' with no degree of freedom correction), chance statistics and match parameter as detailed in our earlier paper[37].

RESULT AND DISCUSSION

Among several biophoric models for inhibitory activity of COX-2 enzyme the two models 1 and 2 (fig. 1) were

TABLE 3: 3D-QSAR MODELS DESCRIBING CORRELATION AND STATISTICAL RELIABILITY FOR COX-2 (MODEL 1 AND 2) AND COX-1 (MODEL 3 AND 4) INHIBITORY ACTIVITY

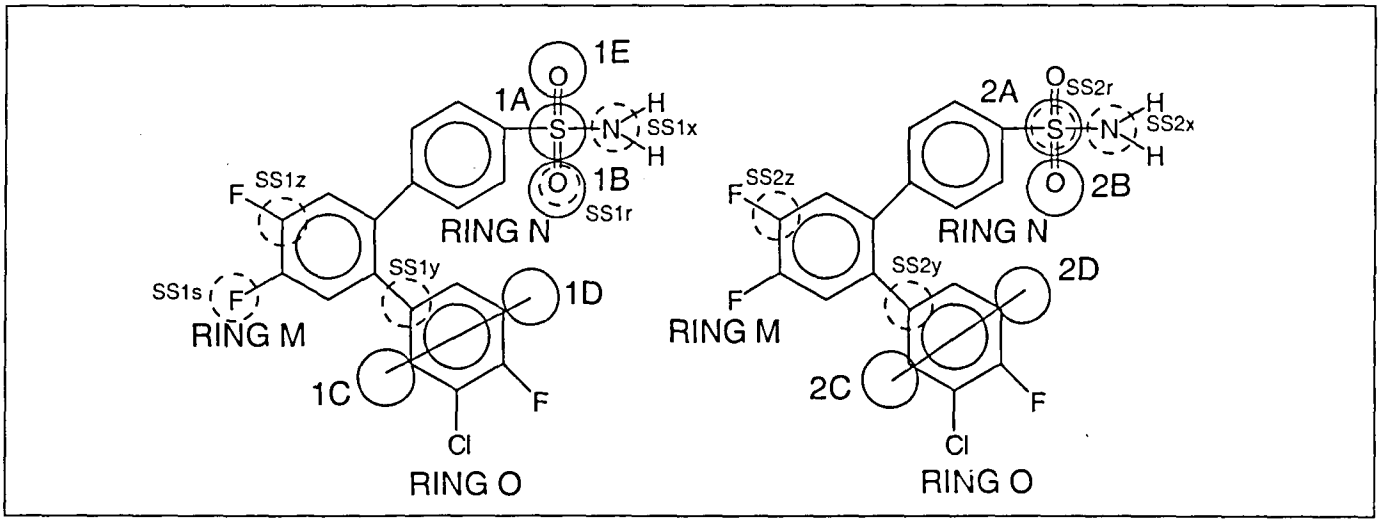
Model	RMSA	RMSP	r^2	Chance	Size	Match	Variable	Compounds
01	0.40	0.54	0.84	0.000	5	0.77	7	44
02	0.42	0.56	0.82	0.000	4	0.66	6	44
03	0.31	0.33	0.72	0.04	3	0.86	3	19
04	0.32	0.35	0.71	0.03	4	0.71	3	19

RMSA: Calculated root mean square error based on all compounds with degree of freedom of correction, RMSP: Calculated root mean square error based on leave one out with no degree of freedom of correction, r^2: Square of correlation coefficient between experimental and approximated activity, Chance: probability of chance correlation, Size: The number of pharmacophoric sites, Match: Quality of match for molecules having common Pharmacophore, Variable: The number of variable in the 3D QSAR model, Compounds: The number of compounds in the 3D QSAR model.

selected based on the criterion, correlation coefficient r^2>0.81, chance=0.0, match value >0.65. These two models described most accurately the distribution of the biophores for COX-2 inhibitory activity. (Table 3)

The models had four common biophoric sites among the five in model 1 and four common biophoric sites in model 2 (fig.1). The four common biophoric sites (A, B, C, D) necessary for COX-2 inhibitory activity corresponding to site 1A and 2A on sulfur of sulphonyl methyl or sulphonamido group, site 1B and 2B on one of the oxygen atom of sulphonyl methyl or sulphonamido group, site 1C, 2C, 1D, 2D are pair of points orthogonal to plane on aryl ring (ring O) in model 1 and 2 respectively. One additional biophoric site in model 1 is on second oxygen atom of sulphonyl methyl or sulphonamido group.

The substrate-enzyme interactions in above two models for selective COX-2 inhibition not only depends on physicochemical properties of biophoric centres corresponding to site 1A (p-population 0.350±0.005, charge heteroatom 1.431±0.012), site 1B (p-population 0.185±0.002, charge heteroatom -0.651±0.001, Don_01 7.445±0.003), site 1C (cycle size 6.0±0.0, p-electron



Model 1 **Model 2**

Fig. 1: Pictorial Representation of Biophoric ◯ and Secondary ◌ sites represented on one of the most active compound 4 for COX-2 inhibitory activity.

TABLE 4: PARAMETER VALUES FOR SECONDARY SITES IN MODEL 1.

Compounds	TH	TR	HD Site SS1x	HYD Site SS1y	REF Site SS1z	DON_01 Site SS1r	REF Site SS1s
01	5.000	90.750	——	0.150	3.750	7.500	0.800
02	4.350	89.950	1.000	0.150	3.750	7.500	0.800
03	5.500	95.550	——	0.150	3.750	7.500	0.800
04	4.850	93.950	1.000	0.150	3.750	7.500	0.800
05	5.450	95.750	——	0.150	3.750	7.500	0.800
06	4.800	94.200	1.000	0.150	3.750	7.500	0.800
07	4.750	97.200	——	0.150	3.750	7.500	0.800
08	4.100	95.600	1.000	0.150	3.750	7.500	0.800
09	5.100	101.800	——	0.150	3.750	7.500	0.800
10	4.500	100.200	1.000	0.150	3.750	7.500	0.800
11	5.000	105.000	1.000	0.150	3.750	7.500	0.800
12	5.550	106.850	——	0.150	3.750	7.500	0.800
13	4.950	105.250	1.000	0.150	3.750	7.500	0.800
14	4.850	108.250	——	0.150	3.750	7.500	0.800
15	4.250	106.650	1.000	0.150	3.750	7.500	0.800
16	4.650	106.300	——	0.150	3.750	7.500	0.800
17	4.050	104.700	1.000	0.150	3.750	7.500	0.800
18	5.050	101.100	——	0.150	3.750	7.500	0.800
19	4.400	99.500	1.000	0.150	3.750	7.500	0.800
20	5.300	95.550	——	0.150	3.750	7.500	0.800
21	4.700	93.950	1.000	0.150	3.750	7.500	0.800
22	5.850	100.350	——	0.150	3.750	7.500	0.800
23	5.200	98.750	1.000	0.150	3.750	7.500	0.800
24	5.800	100.600	——	0.150	3.750	7.500	0.800
25	5.150	99.000	1.000	0.150	3.750	7.500	0.800
26	5.850	100.350	——	0.150	3.750	7.500	0.800
27	5.200	98.750	1.000	0.150	3.750	7.500	0.800
28	5.600	109.750	——	0.150	3.750	7.500	0.800
29	5.000	108.150	1.000	0.150	3.750	7.500	0.800
30	4.150	94.550	——	-0.100	3.750	7.500	0.800
31	3.550	92.950	1.000	-0.100	3.750	7.500	0.800
32	3.700	92.950	——	0.150	3.750	7.500	0.800
33	3.050	91.350	1.000	0.150	3.750	7.500	0.800
34	4.700	90.300	——	0.150	3.450	7.500	——
35	5.200	95.100	——	0.150	3.450	7.500	——
36	5.100	94.900	——	0.150	3.450	7.500	——
37	4.850	101.350	——	0.150	3.450	7.500	——
38	4.100	88.700	——	——	——	6.300	——
39	4.600	93.500	——	——	——	6.300	——
40	4.450	93.300	——	——	——	6.300	——
41	3.800	95.150	——	——	——	6.300	——
42	4.200	99.750	——	——	——	6.300	——
43	5.750	99.900	——	0.150	3.750	7.500	5.400
44	4.400	96.050	——	0.150	3.750	7.500	1.350

(——)– absence of property TH-total hydrophobicity, TR-total refractivity, HD-hydrogen donor, REF-refractivity, DON_01-Electorn donor index

TABLE 5: PARAMETER VALUES FOR SECONDARY SITES IN MODEL 2.

Compounds	TH	TR	REF Site SS2x	HYD Site SS2y	REF Site SS2z	CH Site SS2r
01	1.330	90.750	3.000	0.150	—	1.330
02	4.350	89.150	2.500	0.150	—	1.540
03	5.500	95.550	3.000	0.150	—	1.330
04	4.850	93.950	2.500	0.150	—	1.540
05	5.450	95.750	3.000	0.150	—	1.330
06	4.800	94.200	2.500	0.150	—	1.540
07	4.750	97.200	3.000	0.150	—	1.330
08	4.100	95.600	2.500	0.150	—	1.540
09	5.100	101.800	3.000	0.150	—	1.330
10	4.500	100.200	2.500	0.150	—	1.540
11	5.000	105.000	2.500	0.150	—	1.540
12	5.550	106.850	3.000	0.150	—	1.330
13	4.950	105.250	2.500	0.150	—	1.540
14	4.850	108.250	3.000	0.150	—	1.330
15	4.250	106.650	2.500	0.150	—	1.540
16	4.650	106.300	3.000	0.150	—	1.330
17	4.050	104.700	2.500	0.150	—	1.540
18	5.050	101.100	3.000	0.150	—	1.330
19	4.400	99.500	2.500	0.150	—	1.540
20	5.300	95.550	3.000	0.150	—	1.330
21	4.700	93.950	2.500	0.150	—	1.540
22	5.850	100.350	3.000	0.150	—	1.330
23	5.200	98.750	2.500	0.150	—	1.540
24	5.800	100.600	3.000	0.150	—	1.330
25	5.150	99.000	2.500	0.150	—	1.540
26	5.850	100.350	3.000	0.150	—	1.330
27	5.200	98.750	2.500	0.150	—	1.540
28	5.600	109.750	3.000	0.150	—	1.330
29	5.000	108.150	2.500	0.150	—	1.540
30	4.150	94.550	3.000	-0.100	—	1.330
31	3.550	92.950	2.500	-0.100	—	1.540
32	3.700	92.950	3.000	-0.100	—	1.330
33	3.050	91.350	2.500	0.150	—	1.540
34	4.700	90.300	—	0.150	3.450	1.330
35	5.200	95.100	—	0.150	3.450	1.330
36	5.100	94.900	—	0.150	3.450	1.330
37	4.850	101.350	—	0.150	3.450	1.330
38	4.100	88.700	—	0.150	3.450	1.540
39	4.600	93.500	—	0.150	3.450	1.540
40	4.450	93.300	—	0.150	3.450	1.540
41	3.800	95.150	—	0.150	3.450	1.540
42	4.200	99.750	—	0.150	3.450	1.540
43	5.750	99.900	—	0.150	3.750	1.330
44	4.400	96.050	—	0.150	3.750	1.330

(——)- Absence of property, TH-total hydrophobicity, TR-total refractivity, REF-refractivity, HYD-hydrophobicity, CH-charge.

TABLE 6: EXPERIMENTAL, CALCULATED AND PREDICTED ACTIVITY DATA (-LOG IC_{50} μM) FOR COX-2 INHIBITORY ACTIVITY IN MODEL 1 AND MODEL 2.

Compounds	Experimental	Model 1		Model 2	
		Calculated	Predicted	Calculated	Predicted
01	1.85	1.99	2.01	2.01	2.04
02	2.40	2.56	2.59	2.55	2.58
03	2.00	2.09	2.11	2.08	2.09
04	2.70	2.67	2.66	2.63	2.62
05	2.30	2.05	2.02	2.04	2.01
06	2.70	2.61	2.61	2.58	2.57
07	1.68	1.45	1.43	1.49	1.48
08	1.89	2.02	2.04	2.04	2.05
09	1.72	1.46	1.44	1.48	1.46
10	1.89	2.06	2.08	2.06	2.07
11	1.68	2.17	2.22	2.13	2.18
12	1.89	1.59	1.46	1.51	1.46
13	2.30	2.12	2.10	2.08	2.06
14	0.47	0.92	1.00	0.97	1.06
15	1.19	1.53	1.58	1.54	1.60
16	0.47	0.88	0.95	0.94	1.02
17	1.49	1.49	1.48	1.52	1.52
18	1.92	1.46	1.43	1.48	1.45
19	2.40	2.03	2.00	2.03	2.00
20	2.15	1.95	1.93	1.95	1.93
21	2.40	2.55	2.57	2.53	2.54
22	1.89	2.09	2.11	2.06	2.07
23	2.52	2.66	2.67	2.60	2.61
24	1.64	2.04	2.08	2.01	2.05
25	2.30	2.61	2.64	2.55	2.58
26	2.22	2.09	2.07	2.06	2.04
27	2.52	2.66	2.67	2.60	2.61
28	2.10	1.39	1.26	1.38	1.26
29	2.22	1.99	1.96	1.96	1.92
30	-1.72	-0.92	-0.80	-0.91	0.00
31	0.48	0.80	-1.21	-0.33	-1.24
32	0.77	0.91	0.97	1.03	1.13
33	1.82	1.48	1.34	1.57	1.47
34	0.58	0.29	0.18	0.38	0.31
35	0.44	0.40	0.38	0.45	0.45

36	0.85	0.34	0.16	0.40	0.26
37	-1.06	-0.20	0.15	-0.12	0.20
38	1.21	1.88	2.08	1.94	2.14
39	1.77	1.99	2.05	2.01	2.07
40	2.22	1.89	1.80	1.92	1.85
41	1.48	1.31	1.26	1.39	1.37
42	1.72	1.35	1.23	1.41	1.32
43	0.62	0.62	0.54	0.94	1.02
44	1.08	1.09	1.09	0.26	0.11

Experimental: Experimental activity data in the form of $-\log IC_{50}$ μM, calculated: Activity values calculated according to the 3D QSAR model, Predicted: Activity values predicted using cross validation.

6.0±0.0), site 1D (cycle size 6.0±0.0, π-electron 6.0±0.0), site 1E (π-population 0.184±0.002, charge heteroatom -0.646±0.002, Don_01 7.330±0.050), in model 1 and site 2A (π-population 0.350±0.005, charge heteroatom 1.4131±0.012), site 2B (π-population 0.185±0.002, charge heteroatom -0.652±0.001, Don_01 7.453±0.004), site 2C (cycle size 6.0±0.0, π-electron 6.0±0.0), site 2D (cycle size 6.0±0.0, π-electron 6.0±0.0) in model 2 but also on their spatial arrangements. (mean interatomic distances of five biophores 1A, 1B, 1C, 1D, 1E are 1A-1B (1.587±0.0), 1A-1C (6.141±0.003), 1A-1D (7.790±0.002), 1A-1E (1.597±0.004), 1B-1C (7.161±0.018), 1B-1D (8.163±0.0033), 1B-1E (2.637±0.004), 1C-1D 2.000±0.000), 1C-1E (6.146±0.027), 1D-1E (7.929±0.031) Å in model 1 and four biophores 2A, 2B, 2C, 2D are 2A-2B (1.587±0.0), 2A-2C (6.141±0.003), 2A-2D (7.790±0.002), 2B-2C (6.194±0.034), 2B-2D (8.011±0.036), 2C-2D (2.000±0.000) Å in model 2).

The two-biophoric sites A and B (1A, 2A,and 1B, 2B) are capable of donating electrons. The sulphonyl methyl or sulphonamides group probably interact with His 90,Gln 192 and Arg 513 and oxygen forms a hydrogen bond with His 90.The pair of points orthogonal to plane biophoric sites (1C, 2C and 1D, 2D) probably interact through pi-pi interactions in a hydrophobic cavity of COX-2 enzyme. The additional biophoric site on second atom of oxygen probably links by a hydrogen bond to Arg 513.

In addition to identification of necessary biophoric sites, 3D QSAR multiparameter equations were also developed using these biophores as a template for superimposition. *In vitro* selective inhibition of COX-2 enzyme was correlated with seven (Table 4) and six (Table 5) parameters in model 1 and 2 respectively. The two models had four common parameters in terms of type of property, spatial position and its contributions for COX-2 inhibitory activity. The two common parameters were: 1.total hydrophobicity positive contribution for COX-2 inhibitory activity suggesting that hydrophobic interaction at active site of COX-2 enzyme are favorable as the long narrow channel in COX enzymes is hydrophobic in nature and arachidonic acid is also hydrophobic in nature, in view of that it is an essential requisite for a compound to be hydrophobic and 2.total refractivity negatively contributes for COX-2 inhibitory activity suggesting that overall bulk of molecule is not favorable for COX-2 inhibitory activity as channel is narrow. These two properties are global properties. The third common parameter was secondary site SS1y (6.767±0.001, 7.109±0.020, 1.728±0.001, 1.738±0.001, 7.645±0.018 Å from the biophoric sites 1A, 1B, 1C, 1D, 1E respectively) and SS2y (6.767±0.001, 7.140±0.023, 1.728±0.001, 1.738±0.001 Å from biophoric sites 2A, 2B, 2C, 2D, respectively) being hydrophobicity, in the vicinity of carbon atom of aryl ring (O) through that carbon atom aryl ring (O) attached to central ring (M), positively contribute for COX-2 inhibitory activity suggesting that this site in both the models are favorable for activity and probably involved in binding with Phe 381, Leu 384, Tyr 385, Trp 387, Phe 513 and Ser 530, with contributions from the backbone atoms of Gly 526 and Ala 527. The fourth common parameter was also secondary site SS1z (8.130±0.021, 8.841±0.116, 5.769±0.004, 5.659±0.001, 8.804±0.116 Å from the biophoric sites 1A, 1B, 1C, 1D, 1E, respectively) and SS2z (8.102+0.001, 8.859+0.010, 5.757+0.019, 5.661+0.002 Å from biophoric sites 2A, 2B, 2C, 2D, respectively) is refractivity in the vicinity of carbon atom at para position of ring (M) with respect to ring (O) positively contributes for

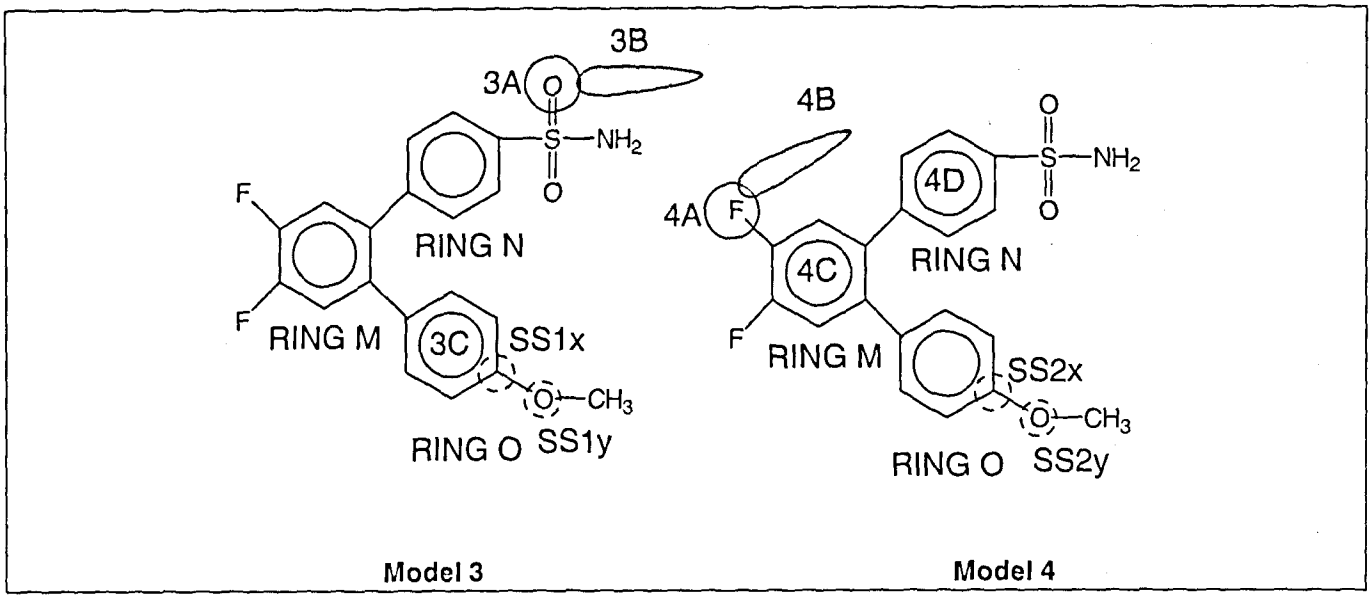


Fig. 2: Pictorial Representation of Biophoric ◯ and Secondary ◌ sites represented on one of the most active compound 30 for COX-1 inhibitory activity.

COX-2 inhibitory activity suggesting that steric interactions at this site are favorable. In addition to this fifth parameter is also common in the two models in terms of its spatial disposition, present on nitrogen atom of sulphonamido group corresponding to secondary site SS1x being H-donor; presence (1.680±0.000, 2.559±0.001, 7.401±0.004, 9.106±0.002, 2.554±0.001 Å from biophoric sites 1A, 1B, 1C, 1D, 1E, respectively) positively contributes for COX-2 inhibition activity suggesting that probably this site is involved in hydrogen bond formation with carbonyl oxygen of the Phe 518 in model 1 and secondary site SS2x being refractivity (1.734±0.010, 2.623±0.018, 7.375±0.006, 9.075±0.006 Å from biophoric sites 2A, 2B, 2C, 2D, respectively) positively contributes for COX-2 inhibition activity suggesting that steric interactions at this site are favorable.

The parameters which were different in the two models corresponding to biophore center SS1r in model 1 (1.680±0.000, 2.559±0.001, 7.401±0.004, 9.106±0.002, 0.0±0.0 Å from the biophoric sites 1A, 1B, 1C, 1D, 1E, respectively) being Don_01on one of the oxygen atom of sulphonyl methyl or sulphonamido group negatively contributes for COX-2 inhibitory activity suggesting that this site is not favorable for nucleophilic reaction with COX-2 enzyme and secondary site SS1s (10.131±0.011, 10.860±0.010, 6.448±0.010, 5.744±0.009, 10.908±0.010 Å from the biophoric sites 1A, 1B, 1C, 1D, 1E, respectively) being refractivity on fluorine atom at meta position (with respect to ring (O) of central ring(M) negatively contributes suggesting that this site is probably not favorable for activity in model 1(Eqn.1) and in model 2 biophore center SS2r (0.0±0.0, 1.590±0.000, 6.140±0.003, 7.783±0.004 Å from

TABLE 7: EXPERIMENTAL AND PREDICTED ACTIVITY VALUES FOR SELECTIVE INHIBITION OF COX-2 ENZYME FOR THE COMPOUNDS OF PREDICTION SET

Compound	Experimental	Predicted	
		Model 1	Model 2
01	> -2.00	-22.11	-6.48
02	> -2.00	-22.41	-6.72
03	> -2.00	-20.69	-7.57
04	> -2.00	-22.62	-6.76
05	> -2.00	1.68	0.73

Experimental: Experimental activity data in the form of –log IC_{50} mM, Predicted: Activity values predicted using cross validation.

biophoric sites 2A, 2B, 2C, 2D, respectively) being charge at sulfur atom positively contributes for activity suggesting that this site is favorable for electrophilic interaction with COX-2 enzyme in model 2. (Christopher *et al.* also proposed that positive charge in the vicinity of sulphonyl methyl increases affinity, Eqn .2).

-log IC_{50}=0.736(±0.128) TH-0.055(±0.013) TR+0.961(±0.154) HD at Site SS1x+8.314(±1.281) HYD at Site SS1y+5.824(±0.832) REF at Site SS1z–19.400 (±2.552) Don_01 at Site SS1r- 0.309(±0.091) REF at Site SS1s+125.966*. n = 44, r = 0.919, $F_{7,36}$ = 27.796 ...Eqn. 1

-log IC_{50}=0.664(±0.134) TH–0.054(±0.013) TR+1.963(±0.642) REF at Site SS2x+8.586(±1.359) HYD at Site SS2y+1.285(±0.509) REF at Site SS2z+8.911(±1.427) Charge at Site SS2r- 15.448.* n = 44, r = 0.906, $F_{6,37}$ = 28.146 Eqn. 2

*TH-total hydrophobicity, TR-total refractivity, HD-hydrogen donor, HYD-hydrophobicity, REF-refractivity.

The statistical results of Eqn.1 and 2 show good correlation coefficient r = 0.919 and 0.906 of high statistical significance >99%. To validate our models we have attempted to predict activity values of compounds of test

TABLE 8: EXPERIMENTAL, CALCULATED AND PREDICTED ACTIVITY DATA (- LOG IC_{50} µM FOR COX-1 INHIBITORY ACTIVITY IN MODEL 3 AND MODEL 4.

Compound	Experimental	Model 3		Model 4	
		Calculated	Predicted	Calculated	Predicted
02	-0.76	-0.91	-0.92	-0.90	-0.91
04	-0.74	-0.67	-0.66	-0.65	-0.64
06	-0.57	-0.69	-0.71	-0.68	-0.69
08	-1.35	-1.03	-0.99	-1.02	-0.97
10	-1.28	-1.03	-1.00	-0.96	-0.91
13	-1.04	-0.86	-0.84	-0.85	-0.82
17	-1.25	-1.29	-1.30	-1.29	-1.30
19	-0.23	-1.12	-1.24	-1.12	-1.28
21	-1.23	-1.44	-1.51	-1.46	-1.54
23	-1.22	-1.20	-1.19	-1.22	-1.22
25	-1.17	-1.22	-1.25	-1.24	-1.27
27	-0.59	-0.50	-0.48	-0.48	-0.45
29	0.23	0.16	-0.09	0.01	-0.18
31	-2.42	-2.23	-1.98	-2.27	-2.09
38	-1.28	-0.84	-0.80	-0.82	-0.77
39	-0.96	-0.79	-0.77	-0.72	-0.68
40	-0.59	-0.86	-0.88	-0.79	-0.82
41	-1.12	-1.00	-0.93	-1.11	-1.11
42	-0.91	-0.98	-0.99	-0.91	-0.91

Experimental: Experimental activity data in the form of –log IC_{50} mM, calculated: Activity values calculated according to the 3D QSAR model, Predicted: Activity values predicted using cross validation.

set, prediction power of both the models were perfect for compounds 1 to 4 but fail to predict activity of compound 5 in the range of reported activity, may be due to presence of naphthalene ring as central ring (M). (Table 7)

Similarly we also identified essential structural and physicochemical properties in terms of common biophoric features, global property and secondary sites for inhibition of COX-1enzyme. Among several biophoric models, two models 3 and 4 (Fig. 2) were selected based on the criterion, correlation coefficient r^2 >0.70, chance <0.005, match value >0.70,described most accurately the distribution of biophores for COX-1 inhibitory activity (Table 3). There were three and four biophoric features in model 3 and 4 respectively. The three biophoric features in model 3 corresponding to site 3A and 3B being oxygen atom and its lone pair of electrons on sulphonamido group, site 3C being charge ring center present on pi orbital of aryl ring (O) attached at position two of central ring and the four biophoric sites in model 4 corresponding to site 4A and 4B on fluorine atom and its lone pair of electrons at Para position (with respect to ring (O)) of ring (M), and these sites corresponded to R_2 substituted group and its lone pair of electrons for compounds 38 and 40 and corresponded to R_1 substituted group and its lone pair of electrons for compounds 41 and 42 as these compounds were not having fluorine atom on central ring.

The substrate-enzyme interactions in above two models for selective COX-1 inhibition depend on physicochemical properties of biophoric centres corresponding to site 3A (Don_01, 7.584±0.034), site 3B (H-site, 1.0±0.0), site 3C (cycle size, 6.0±0.0, π-electron 6.0±0.0) in model 3 and site 4A (Don_01, 8.860±0.123), site 4B (H-site, 1.0±0.0), site 4C (cycle size, 6.0±0.0, π-electron 6.0±0.0), site 4D (cycle size, 6.0±0.0, π-electron 6.0±0.0) in model 4 but also on their spatial arrangements, mean interatomic distances of three biophores 3A, 3B, 3C are 3A-3B (3.0±0.0), 3A-3C (6.960±0.005), 3B-3C (7.934±0.043) for model 3 and four biophores 4A, 4B, 4C, 4D are 4A-4B (3.0±0.0), 4A-4C (6.151±0.066), 4A-4D (2.802±0.029), 4B-4C(8.703±0.074), 4B-4D (5.802±0.029), 4C-4D (4.308±0.037) for model 4.

The biophoric sites 3A, 3B and 3C are all electron rich sites, probably involved in electrostatic interaction with COX-1 enzyme. The lone pair of electrons on oxygen is probably involved in hydrogen bonding with polar Arg at position 120 and charge ring center biophoric site (3C) probably interacts with other amino acids in the channel in model 3 while in model 4, biophoric sites 4A probably involved in electrostatic interactions and 4B probably involved in interaction with Ile 523 but no such interaction involve in COX-2 which has a valine at the corresponding site, site 4C and 4D probably involved in electrostatic interaction in the cavity of COX-1 enzyme.

3D QSAR equation for model 3 (Eqn.3) and 4 (Eqn.4) showed that three parameters corresponding to: 1.global property (total hydrophobicity) positively contributes in both models suggesting that hydrophobicity of molecule is favorable for activity, second parameter being secondary site SS3x (7.582±0.020, 7.552±0.009, 2.838±0.040 Å from biophoric sites 3A, 3B, 3C, respectively) and SS4x (8.810±0.043, 12.646±0.164, 6.680±0.036, 6.907±0.163 Å from biophoric sites 4A, 4B, 4C, 4D, respectively) was H-acceptor (presence) in the vicinity of R_2 substituent (F, Cl, oxygen of OCH_3, $-OCH_2O-$, $-OCH_2CH_2O-$) negatively contributes for the activity suggesting that this site should not be occupied by any atom or group having H-acceptor property, third parameter being secondary site SS3y (7.131±0.004, 7.594±0.005, 1.400±0.003 Å from biophoric sites 3A, 3B and 3C, respectively) and SS4y (8.387±0.005, 11.383±0.005, 5.581±0.005, 5.638±0.005 Å from biophoric sites 4A, 4B, 4C and 4D, respectively) provided on para carbon atom of ring (O) is hydrophobicity negatively contributes for activity suggesting that this hydrophobic site is not favorable for activity.

- $\log IC_{50}$ = 0.481(±0.127) TH-0.752(±0.283) HAP at Site SS3x-5.804(±1.291)HYD at site SS3y- 2.826.# n =19, r= 0.850, $F_{3,15}$ =13.052... Eqn. 3

- $\log IC_{50}$ = 0.490(±0.129) TH-0.585(±0.228) HAP at Site SS4x-5.284(±1.164)HYD at site SS 4y- 2.974#.n =19, r = 0.843,$F_{3,15}$ = 2.25?...Eqn. 4

TH-total hydrophobicity, HAP-hydrogen acceptor presence, HYD-hydrophobicty.

The statistical results of Eqn. 3 and 4 show good correlation coefficient r = 0.850 and 0.843 of high statistical significance >99%. In comparison of biophoric sites of COX-2 and COX-1 activity, it is clear that biophoric sites responsible for inhibition of COX-2 and COX-1 are different; it is true for secondary sites also, except total hydrophobicity which is positive in all biophoric models. It gives useful information for the design of novel COX-2 inhibitors.

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