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Computer-aided drug design of optimal ratio selective inhibition of COX-1 and COX-2

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The selective inhibition of both COX-1 and COX-2 by NSAIDS results in beneficial as well as harmful effects. To reduce the side effects, a natural phytochemical having coumarin moiety has been selected for optimal ratio inhibition of COX-1 and COX-2. Based on docking studies and information obtained from interaction analysis, structure-based virtual screening has been performed by designing 150 analogues with structural diversity. The compound **58** has been found to be a potent selective inhibitor for both COX-1 and COX-2 with the optimal ratio. The docking studies reveal that these features show good interaction with amino acid His43, Gln42, Lys468, His119 and Thr119 and also show further π-alkyl hydrophobic interaction with Lys 468, π-sulfur interaction with His94, His96 in the binding site of COX-1 and COX-2. Toxicity and drug likeness have been estimated by using OSIRIS molecular property explorer tool. These compounds have been found to be free from toxicity risk, exhibit better positive drug likeness and drug score compared to reference compound with favorable values of ClogP, solubility, molecular weight and TPSA.

Keywords: Anti-inflammatory, cyclooxygenase, NSAIDs, molecular docking, toxicity

Cyclooxygenase is the key enzyme in arachidonic acid metabolism[1-3]. It exists in two isoforms, COX-1 and COX-2 (Figure 1). The COX-1 is constitutively expressed enzyme with a house-keeping role in regulating many normal biological processes *viz*., stomach lining where prostaglandins help a protective role. It prevents the stomach mucosa from being wrinkled by its own acid[4,5] and induction of labor pains. The COX-2 is an inducible form which is expressed only after an inflammatory stimulus releases metabolites which are used to induce inflammation and pain[6-8]. The classical NSAIDs can inhibit both COX-1 and COX-2 which results in beneficial as well as harmful effects[9]. On the other hand, COX-1 selective inhibitors yield to toxicity and associated side effects (ulcers, prolonged bleeding time, kidney problems)[10]. The COX-2 selective inhibitors inhibit the production of prostaglandin, a hormone that dilates tubules associated with cardiac, renal and hepatic systems. Therefore, these selective drugs become cardio-toxic, nephro-toxic and hepato-toxic[11-13]. At present, the majority of the NSAIDs available in the market are not considered as safe drugs to treat inflammation. This prompted us to

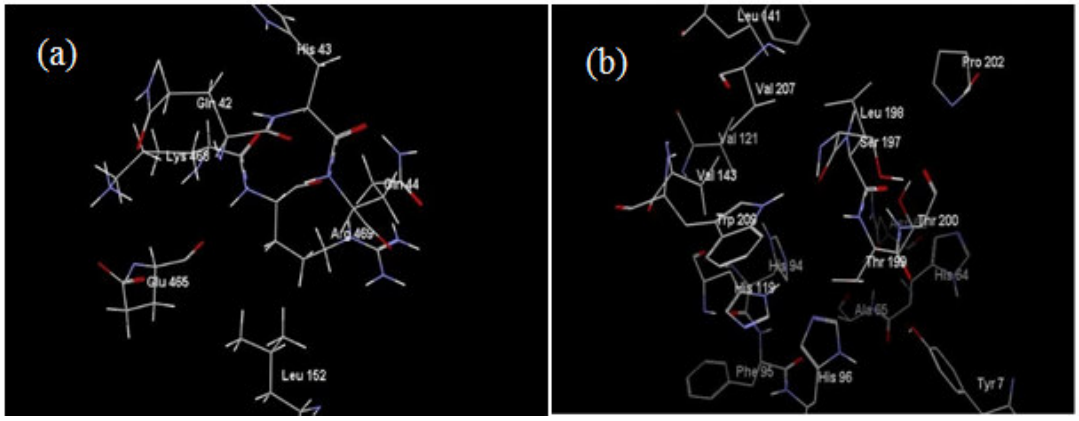


Figure 1 — (a) Active site amino acids of COX-1 (b) Active site amino acids of COX-2

develop safe NSAIDS which inhibit both COX-1 and COX-2 with optimal selectivity ratio with fewer side effects. Phytochemicals are the natural sources of biologically active lead compounds including anti-inflammatory agents. In this project, a lead compound having coumarin moiety was identified for optimization by molecular modeling studies. Here we designed 150 compounds based on molecular modeling studies [Genetic Optimization of Ligand Docking (GOLD) software] with the main skeleton of coumarin scaffold having best fitness. If score is more then the drug fits in the best mode of the active site of protein with higher number of interactions.

Results and Discussion

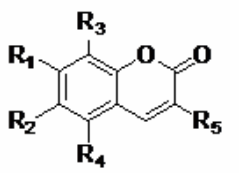
Version 2.0 of the Genetic Optimization for Ligand Docking (GOLD) docking program was evaluated in the present study. The GOLD program uses a genetic algorithm to explore the full range of ligand flexibility and the rotational flexibility of selected receptor hydrogens. The 3D structures of selected proteins for COX-1 (1AEX, 2EIJ, 2QPM, 1CQE) and COX-2 (2Y69, 3AG2, 3NT1, and 1OQ5), were downloaded from protein data bank with resolution in the range of 1.5 to 3.2 Å (Figure 1). Among all the mentioned proteins, 1CQE and 1OQ5 were screened on the basis of the fitness score. The fitness function that was executed in GOLD involved basically of H-bonding, complex energy, and ligand internal energy terms.

In the present investigation, a set of **150** compounds were used for docking into the active site of COX-1 and COX-2 by maintained RMSD 1.5 Å. Based on fitness score among all of these compounds, only **67** compounds (Table I) were found to have good Gold score value. The fitness score of these compounds is shown in Table II. Among these compounds **9**, **38**, **55**, **56**, **57** and **58** were observed to have reasonably good Gold fitness value against COX-1 and COX-2. The results of docking study reveal that Gold scores range from 65.38 to 32.43 and 60.29 to 13.17 for COX-1 and COX-2 respectively. The compounds 58 and 57 are ranked first and second top-rank among all compounds according to docking GOLD score. The compound 9 came up with the third rank on the basis of GOLD score against COX-1 and COX-2. The analysis of docking study reveals that the groups methyl sulfonamyl pyrazine and phenyl ring at adjacent positions (the compounds from 46 to 59) enhance the inhibitory activity at COX-1 and COX-2. Further, only **45** compounds were found to be free from toxicity risk. The structure of these compounds are shown in Table I. The predicted toxicity risk and drug-like properties of these compounds are shown in Table III.

Out of these 45 compounds **30**, **31**, **36**, **38**, **55**, **58**, **59**, **63**, **64**, **67** and **68** exhibit positive drug-likeness, and better drug score than the reference (Celecoxib) compound. The compounds **58** and **57** and **9** are the most potent towards the inhibition of COX-1 and COX-2 although compound **9** is discarded because it shows negative drug-likeness score, poor solubility, and low drug score than the reference compound. The compound **58** and **57** exhibit better positive drug-likeness, drug score than the reference compound with favorable values for ClogP, solubility, molecular weight and TPSA. To reduce the side effects caused by selective inhibition of COX-1, and COX-2 the designed coumarin derivatives may inhibit both COX-1 and COX-2 in optimal ratio. Therefore, based on the knowledge of the risk of side effect, gold score and active site requirement the compound **58** and **57** from the highest scoring function were selected. The compound **58** (64.32 with COX-1, 60.29 with COX-2) and **57** inhibit the COX-1 and COX-2 with 1.1:1.0 and 2:1 ratio respectively.

Molegro molecular viewer and Discovery Studio Visualizer are used to identify binding modes of these compounds. The shape of the compounds **57**, **58** were observed complementary to the shape of the binding pockets of COX-1 and COX-2. Surface representation of compound **57** and **58** at the active site of COX-1 and COX-2 are shown in Figure 2. In the binding mode of compound **57** with COX-1 [Figure 2, (a)] the coumarin unit took the position in the narrow cavity, the oxygen atom at the position one shows hydrogen bonds with Gln42 (H-bond distance 3.309 Å) and Lys468 (H-bond distance 3.5139 Å). The carbonyl group of coumarin shows hydrogen bond with Lys468 (H-bond distance 3.028 Å). The nitrogen atoms of 1,4-pyrazine ring form hydrogen bonds with Met472 (H-bond distance 3.101 Å) and His43 (H-bond distance 3.13 Å). The sulfonamyl group is surrounded by Arg471 (H-bond distance 3.15 Å) and Gly471 (H-bond distance 3.15 Å). The coumarin ring and 1,4-pyrazine show π-alkyl hydrophobic interactions with Lys468 (π-alkyl hydrophobic bond distance 5.20, 4.35 and 4.32 Å). In the binding mode of compound **58** with COX-1 [Figure 3, (b)], the oxygen atom of coumarin shows hydrogen bonds with Gln42 (H-bond

Table I — Designed compounds



S.No.	Compd	R_1	R_2	R_3	R_4	R_5
1	**1**	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H
2	**2**	$-SCH_3$	$-SCH_3$	$-OCH_3$	$-OCH_3$	-H
3	**7**	$-SCH_3$	$-SCH_3$	$-SCH_3$	$-CH_3$	-H
4	**8**	$-SCH_3$	$-SCH_3$	$-SCH_3$	$-S-CH_3$	-H
5	**9**	$-SCH_3$	$-SCH_3$	-SPh	$-CH_3$	-H
6	**12**	$-OCH_3$	-H	$-OCH_3$	$-OCH_3$	-H
7	**13**	-H	-H	$-OCH_3$	$-OCH_3$	-H
8	**14**	$-OCH_3$	$-OCH_3$	-H	-H	-H
9	**15**	-COOH	-OH	-H	-H	-H
10	**17**	-COOH	$-SCH_3$	-H	-H	-H
11	**18**	-COOH	$-SCH_3$	$-OCH_3$	-H	-H
12	**19**	-COOH	$-SCH_3$	$-OCH_3$	$-OCH_3$	-H
13	**20**	-COOH	$-SCH_3$	$-SCH_3$	-H	-H
14	**22**	$-CH_3$	-OH	-OH	$-CH_3$	-H
15	**23**	$-CH_3$	-OH	-OH	$-CH_3$	$-CH_3$
16	**24**	$-CH_3$	$-CH_3$	-OH	-OH	$-CH_3$
17	**25**	$-CH_3$	$-CH_3$	-OH	-OH	-OH
18	**27**	$-CH_3$	$-CH_3$	-OH	-OH	$-OCH_3$
19	**28**	$-CH_3$	-OH	-OH	-OH	$-CH_3$
20	**29**	-H	-Ph	$-OCH_3$	$-OCH_3$	-H
21	**30**	-OH	-OH	-OH	$-CH_3$	$-CH_3$
22	**31**	-OH	-OH	-OH	-OH	$-CH_2CH_3$
23	**32**	-OH	-OH	-OH	$-OCH_3$	$-CH_2CH_3$
24	**34**	-OH	$-OCH_3$	-OH	$-OCH_3$	$-CH_2CH_3$
25	**36**	-OH	$-SCH_3$	-OH	$-SCH_3$,	$-CH_2CH_3$
26	**37**	-OH	$-SCH_3$	-SH	$-SCH_3$	$-CH_2CH_3$
27	**38**	$-SCH_3$	$-SCH_3$	SCH_3	$-SCH_3$	$-CH_2CH_3$
28	**39**	-H	-Ph	$-OCH_3$	$-OCH_3$	-H
29	**40**	-H	-Ph	$-OCH_3$	$-OCH_3$	-H
30	**41**	-H	-Ph	$-OCH_3$	$-OCH_3$	$-N(CH_3)_2$
31	**42**	-H	$-4-SO_2HC_6H_4$	$-OCH_3$	$-OCH_3$	$-N(CH_3)_2$
32	**55**	$-C_4H_4N_2$	-Ph	$-OCH_3$	-OH	$-CH_3$
34	**56**	$-4-SO_2HC_4H_4N_2$,	-Ph	$-OCH_3$	-OH	$-CH_3$
35	**57**	$-4-SO_2HC_4H_4N_2$,	-Ph	-H	-H	$-CH_3$
36	**58**	$-4-SO_2CH_3C_4H_4N_2$,	-Ph	-H	-H	$-CH_3$
37	**59**	$-4-SO_2CH_3C_4H_4N_2$,	-Ph	-H	-H	$-N(CH_3)_2$
38	**60**	$-C_4H_4N_2$	$-OCH_3$	-H	-H	$-N(CH_3)_2$
39	**63**	$-C_4H_4N_2$	-H	$-3-SO_2HC_6H_4$	-H	$-N(CH_3)_2$
40	**64**	$-C_4H_4N_2$	$-3-SO_2HC_6H_4$	-H	-H	$-N(CH_3)_2$
41	**65**	-H	-H	$-C_4H_4N_2$	-Ph	$-N(CH_3)_2$
12	**66**	-H	$-4-SO_2HC_6H_4$	$-OCH_3$	$-OCH_3$	$-N(CH_3)_2$.
43	**67**	-OH	$-4-SO_2HC_6H_4$	$-OCH_3$	$-OCH_3$	$-N(CH_3)_2$.
44	**68**	-H	$-4-SO_2HC_6H_4$	-OH	-OH	$-N(CH_3)_2$

Table II — Gold fitness scores and hydrogen bonds formed with amino acid residues of COX-1 and COX-2

Compd	Gold fitness Score COX-1	Residues of COX-1 involved in HB interactions	Gold fitness score COX-2	Residues of COX-2 involved in HB interactions
1	47.24	Lys546	31.27	Gln92, Gln92, Thr199, Asn62
2	49.77	Gly44, His43	38.66	Gln92, Thr199, Asn62
3	42.02	His43	30.63	Thr199, Asn62 Asn67
4	47.96	Gln42	39.59	Gln92, Thr199, Asn62
5	50.80	Gln42	45.65	Thr200, Thr200, Asn62, Asn67, Ala65
6	44.17	Gln42, Gln42	34.73	Asn62, Asn67
7	49.21	-	36.29	Asn67, Ala65, Asn62, Asn62
8	57.75	-	45.21	-
9	65.38	Gln42, Gln42	55.5	Gln42
10	50.05	Gln44	44.51	-
11	51.84	Gln42, Lys468	47.02	-
12	47.60	Tyr130,Arg469, Arg469, Arg469	34.75	Gln92, Thr199
13	54.12	-	34.79	Thr199, Thr199
14	53.27	Gln44	36.20	Thr199, Thr199, Thr199
15	36.12	Gln44, Gln44,Arg83	33.39	Thr199, Thr199, Thr199, Tyr7, Asn62
16	41.32	Gln42, Gln44, His43	33.02	
17	43.54	Gln44, Gln42	38.21	Thr199,Thr199,His119
18	56.16	Gln44, Gln42	39.32	Gln92,Gln92
19	44.51	Gln44, Gln42, His43	32.85	Thr199,Gln92,Gln92Ala65,Asn62
20	47.68	Gln42	35.95	Thr119,Gln92,Asn62, Ala65
21	33.34	Thr62	29.63	Thr199, Thr200
22	37.96	-	32.23	Thr199,Thr199,Thr199
23	38.07	Gln44, Gln44	31.03	Gln92,Gln92,Asn62
24	39.17	Gln42, Gln42, Lys468	32.21	Thr199,Thr199,Gln92,Gln92
25	37.01	Gln44	36.10	Asn62,Thr199,Asn62
26	37.49	Gln44, Gln44	32.07	Lys546,Lys546
27	39.87	Gln44, Gln44, Gln42	34.21	Thr199,Gln92, Gln92, Asn62
28	40.12	Gln44, Gln44, Gln42, Lys468	31.76	Thr199, Thr199, Thr199,Gln92,Gln92
29	37.09	Gln44, Gln44,Arg83	28.78	Thr199,Thr199,Thr199,Thr200,Asn62,Gln92
30	38.50	Gln42, Lys468, His43	31.45	Thr199,Thr199, Thr199,Gln92, Gln92
31	40.50	Gln42, Lys468	29.99	Thr199,Thr199,Thr199, Gln92,Gln92
32	44.21	Gln42, Gln42, His43	32.49	Thr199,Thr199,Thr199,Thr200, Gln92,Gln92
33	41.98	Gln44, Gln44, Gln44, Gln42	33.26	Gln42,Lys468
34	44.78	Gln42, Gln42, Lys468	31.83	Thr199,Thr199,Thr199, Gln92,Asn62,Asn62
35	48.95	Gln44, Gln42, Lys468	38.86	Thr199,Thr199,Thr200, Gln92,Gln92,Asn62
36	51.13	Gln42, Gln42, Lys468	42.97	Thr199,Thr199,Thr199,Thr200
37	56.13	Gln42, Lys468	45.97	Asn62, Asn62
38	58.72	Gln44	52.37	Asn67, Asn62
39	51.72	Gln44,Lys468	43.94	Thr199,Thr200, Thr200,Gln92
40	51.32	Gln44, Gln42	44.29	Thr199,Thr200, Thr200,Gln92
41	32.43	Gln44, Gln42, Gln42, Lys468	13.17	Thr199, Thr199,Gln92
43	52.23	Gln44, Gln44, Gln44, Gln42	43.32	Thr199, Thr199,Gln92
44	52.41	Gln44, Gln465, Gln461,Cys41	42.47	Thr200, His119
45	55.75	Gln44	26.90	Thr199,Thr200,Asn62
46	52.39	Gln44, Lys473	45.81	Thr199,His119, Asn62
47	38.90	-	28.90	Thr199,His119, Asn62
49	58.58	Gln44, Gln44, His43	50.64	Thr199,His119, Asn62
50	57.01	Gln42, Gln42, Gln44, Lys468,Arg83	49.04	Thr199,His119, Asn62
51	59.32	Gln42, Arg83	55.46	Thr199,His119

(*Contd.*)

Table II — Gold fitness scores and hydrogen bonds formed with amino acid residues of COX-1 and COX-2 (*Contd.*)

Compd	Compd	Compd	Compd	Compd
52	55.71	Gln471, Arg83	55.70	Asn62, Asn62
53	59.15	Gln44, Gln44	46.39	Thr199,Thr199,Thr199,His119,His94,His96
54	63.16	Gln44, Gln44, His43,Thr62	51.60	Asn62
55	59.96	Lys468, Lys468	47.68	Thr119,Asn62,Gln92
56	61.71	Gln44, Gln44, Gln42, Lys468	51.67	Thr200,Thr200,Thr199,Asn62, His94,His119
57	65.25	Lys468, Gln42,His43,Met472, Arg83,Gly471	53.12	Asn62,Thr200
58	64.32	Arg83, Lys468	60.29	His119,His96,Thr199,Thr199,Thr199
59	49.72	Arg83, His43,Met472,Lys468	52.47	Thr199,Thr199,Thr199His119,His96
60	35.24	Gln44,Lys468	33.51	Thr199,Tyr7,His96,Thr199
61	44.79	Gln44	37.52	Glu92,Glu69, Glu69, Glu69,Tyr7, Asn244,His96,Ala65
62	41.82	Gln42,His43,His43,Thr62	35.65	Asn62, Asn62, Ala65, Ser197
63	47.80	Gln44,Lys473,Lys468	42.35	Thr119, His94,Asn62, Tyr7, Phe95
64	50.50	Gln44,His43,Tyr64	30.40	Ser197
65	37.56	Gln44	33.51	Thr119,Thr119,His94,His119,Asn67,His96,Asn67
66	38.58	Gln44, Gln44,Gln44,Gln44	23.10	Thr199,Thr200, Asn62, Asn62, Ser97
67	37.31	Gln44, Gln44,Gln42,Gln42, Lys468,Gly461	20.40	Thr200,Asn62,Asn62
68	38.26	Gln44,Lys468	19.26	Thr200, Thr199

Table III — Toxicity and drug-relevant properties prediction for optimized compounds

Compd	Mu	Tu	Ir	Re	cLogp	Solubility	M.weight	TPSA	Drug-likeness	Drug score
1	G	G	G	G	1.22	-2.44	266.0	62.22	-3.17	0.48
2	G	G	G	G	2.31	-4.1	298.0	95.36	-7.81	0.42
7	G	G	G	G	3.27	-5.26	298.0	102.2	-2.97	0.33
8	G	G	G	G	3.41	-5.26	330.0	127.5	-2.99	0.30
9	G	G	G	G	4.75	-6.7	392.0	127.5	-4.25	0.20
12	G	G	G	G	1.29	-2.42	236.0	53.99	-3.13	0.49
13	G	G	G	G	1.36	-2.41	206.0	44.76	-4.2	0.48
14	G	G	G	G	3.02	-3.38	202.0	26.3	-3.09	0.44
15	G	G	G	G	0.64	-2.09	206.0	83.83	-5.47	0.48
17	G	G	G	G	1.46	-3.23	236.0	88.9	-3.72	0.46
18	G	G	G	G	1.39	-3.25	266.0	98.1	-2.65	0.47
19	G	G	G	G	1.32	-3.27	296.0	107.3	-2.28	0.48
20	G	G	G	G	1.94	-4.08	282.0	114.2	-2.8	0.43
22	G	G	G	G	1.49	-2.47	206.0	66.76	-4.76	0.47
23	G	G	G	G	1.93	-2.59	220.0	60.76	-1.83	0.50
24	G	G	G	G	1.93	-2.59	220.0	60.76	-0.34	0.65
25	G	G	G	G	0.99	-2.4	222.0	86.99	-0.35	0.68
27	G	G	G	G	1.42	-2.53	236.0	75.89	-0.36	0.66
28	G	G	G	G	1.24	-1.95	222.0	81.99	-0.34	0.67
29	G	G	G	G	0.3	-1.76	224.0	107.2	-0.35	0.68
30	G	G	G	G	0.83	-1.62	238.0	96.22	0.16	0.70
31	G	G	G	G	1.01	-1.5	238.0	107.2	0.95	0.82
32	G	G	G	G	1.28	-1.89	252.0	86.22	1.08	0.82
34	G	G	G	G	1.56	2.01	266.0	85.22	1.08	0.81
35	G	G	G	G	2.11	-3.04	282.0	101.2	1.36	0.79
36	G	G	G	G	2.65	-3.7	298.0	117.3	1.22	0.71
37	G	G	G	G	2.94	-5.27	314.0	135.9	-1.06	0.40
38	G	G	G	G	4.3	-3.16	358.0	127.5	1.17	0.42
39	G	G	G	G	3.02	-4.49	282.0	44.76	-2.98	0.39

(*Contd.*)

Table III — Toxicity and drug-relevant properties prediction for optimized compounds (*Contd.*)

Compd	Compd	Compd	Compd	Compd	Compd	Compd	Compd	Compd	Compd	Compd
40	G	G	G	G	3.46	-4.62	296.0	44.76	-0.07	0.52
41	G	G	G	G	2.74	-4.05	325.0	48.0	2.68	0.75
42	G	G	G	G	1.64	-4.08	389.0	82.14	2.87	0.73
55	G	G	G	G	1.98	-4.28	424.0	115.6	0.53	0.55
56	G	G	G	G	2.93	4.49	360.0	81.54	0.16	0.54
57	G	G	G	G	2.39	-4.8	378.0	86.22	0.41	0.56
58	G	G	G	G	2.44	-4.95	392.0	94.6	1.12	0.57
59	G	G	G	G	1.72	-4.39	421.0	97.84	3.39	0.69
60	G	G	G	G	0.9	-2.14	297.0	64.55	3.60	0.91
63	G	G	G	G	.158	-4.23	402.0	89.46	2.98	0.71
64	G	G	G	G	1.58	-4.23	407.0	89.46	2.71	0.70
66	G	G	G	G	1.69	-4.08	389.0	82.14	2.87	0.73
67	G	G	G	G	1.34	-3.79	405.0	102.3	2.87	0.75
68	G	G	G	G	1.41	-3.45	361.0	104.1	2.8	0.81
Celecoxib	G	G	G	G	2.59	-4.17	381.0	86.36	-8.11	0.37

G = Green (No toxic); R = Red (toxic); Mu = Mutagenic; Tu =Tumorigenic; Ir = Irritant; Re = Reproductive

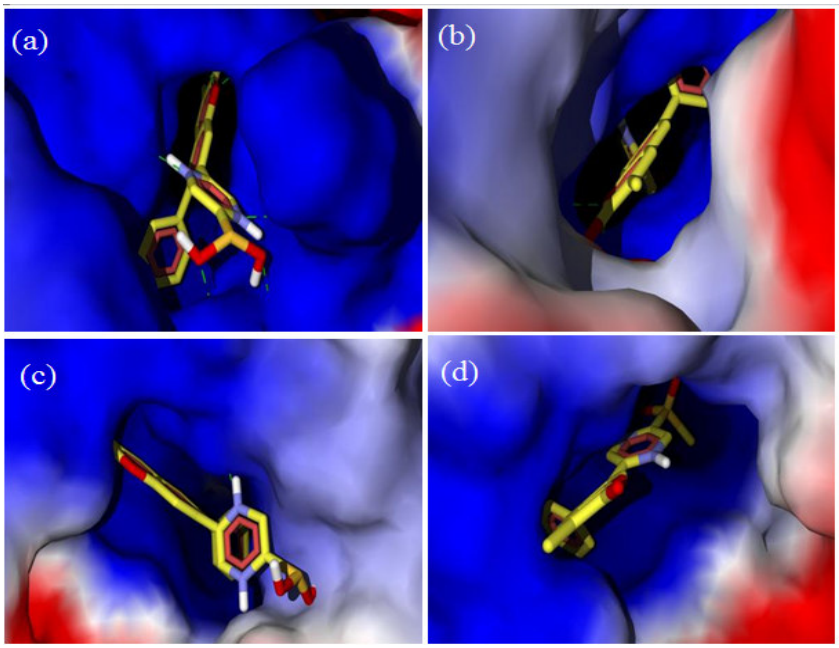


Figure 2 — (a) Surface representation of compound **57** with COX-1 (b) Surface representation of compound **57** with COX-2 (c) Surface representation of compound **57** with COX-1 (d) Surface representation of compound **57** with COX-2

distance 3.3399 Å) and Lys468 (H-bond distance 3.4125 Å). The carbonyl group of coumarin shows hydrogen bond with Lys468 (H-bond distance 3.0086 Å). The nitrogen atoms of 1,4-pyrazine ring form hydrogen bonds with Met472 (H-bond distance 3.099 Å) and His43 (H-bond distance 3.099 Å). The sulfonamyl group forms hydrogen bond with Lys 473 (H-bond distance 2.855 Å). The coumarin ring and 1,4-pyrazine show π-alkyl hydrophobic interaction with Lys468 (π-alkyl hydrophobic bond distance 4.18, 4.38 and 5.08 Å).

The binding mode of compound **57** with COX-2 [Figure 4, (a)] shows that nitrogen atom of pyrazine forms a hydrogen bond with Thr200 (H-bond distance 3.00 Å). The carbonyl group of coumarin forms hydrogen bond with Asn62 (H-bond distance 3.433 Å), the methyl group present on coumarin moiety shows π-alkyl hydrophobic interaction with amino acid His96, His94, and Ala65. The phenyl ring present on coumarin moiety shows hydrophobic interactions with amino acids Val143, Val121, and Lue198. The binding mode of compound **58** with

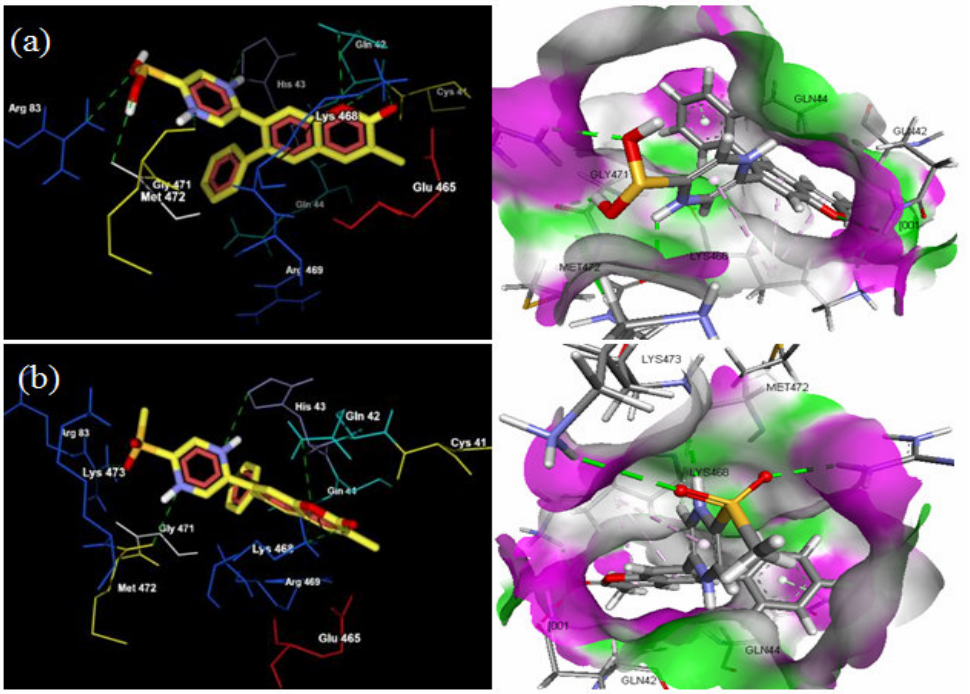


Figure 3 — (a) Binding mode of compound **57** with COX-1 (b) Binding mode of compound **58** with COX-1

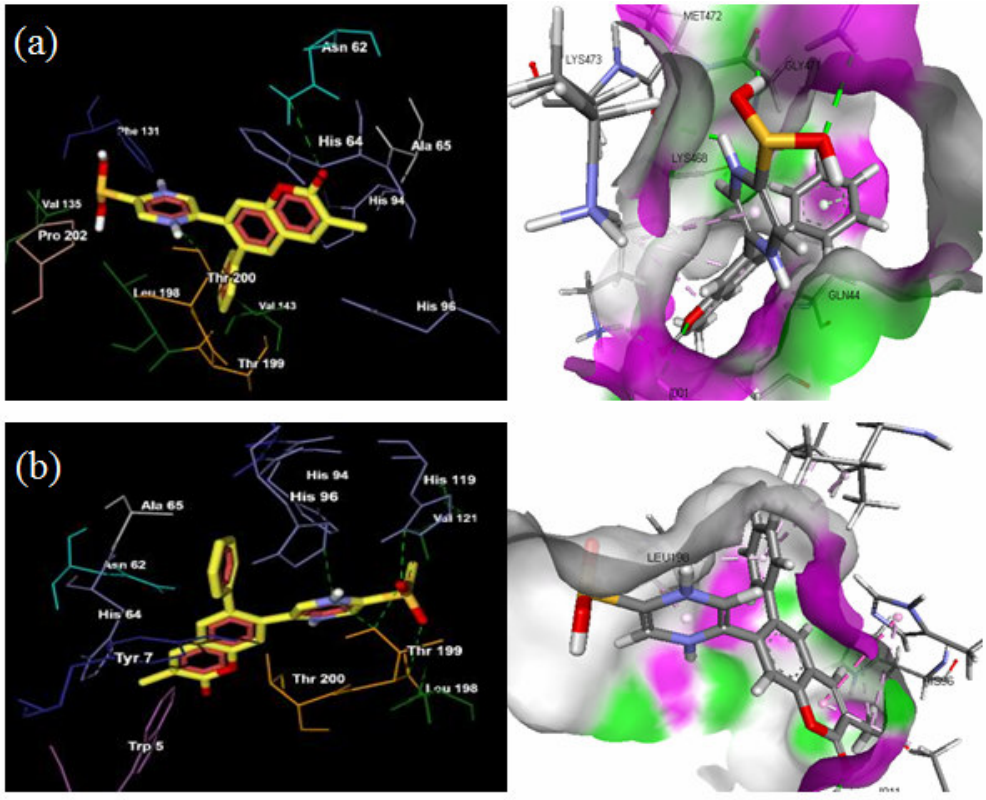


Figure 4 — (a) Binding mode of compound **57** with COX-2 (b) Binding mode of compound **58** with COX-2

COX-2 [Figure 4, (b)] shows oxygen atoms of methyl sulfonyl group form three hydrogen bond interactions with amino acids Thr199 (H-bond distance 2.526Å), His119, (H-bond distance 2.526 Å), three π-sulfur interactions with amino acids His94 (π-sulfur distance 4.93 Å), His96 (π-sulfur distance 5.65 Å) and Trp205(π-sulfur distance 4.94 Å). The coumarin ring and the methyl group present on coumarin form hydrophobic interactions with amino acids Trp5, His64.

Materials and Methods

Protein

In the current study, four different PDB structures for COX-1 (2AEX, 2EIJ, 2QPM and1CQE), and the same number for COX-2 (2Y69, 3AG2, 3NT1 and 1OQ5) were chosen form Protein Data Bank[14]. Swiss Protein Data Base Viewer (SPDBV) 3.7 software[15] was used for the analysis of active site of protein containing specific amino acids. This software was also used to predict possible and appropriate binding site regions between ligand and protein.

Drug design

The compounds are designed based on the combinatorial method by considering coumarin scaffold. The energy minimization has been carried out on all the compounds as per the prescribed guidelines of the Hyper-Chem software[16]. These compounds were subsequently used for molecular docking study using the software, GOLD and Discovery Studio Visualizer.

Active site analysis

SPDBV software was used to find out specific amino acids at the active site of protein. It was also used to predict possible binding site regions between compound and enzyme. Ligand explorer of PDB was used to understand any other possible interactions.

Docking software

GOLD 2.0

GOLD 2.0 version docking program was used in the present study for evaluating the docking results[15]. The fitness function that was implemented in GOLD consisted basically of H-bonding, complexing energy, and compound internal energy expressions. The GOLD Score was calculated by importance, the position using the list of atom numbers and holding all the other default parameters. In the docking calculations with GOLD, all atoms, and their associated residues within 10 Å of the compound were used to define the active site. The information about the compound hydrogen-bonding interactions and conformation was encoded into the corresponding genetic algorithms (GA) of GOLD (Gold Score, ChemScore), ASP and Chem PLP using the default GA parameters. The mechanism of the compounds placement is based on fitting points. The program adds fitting points to hydrogen bonding groups on the protein and compound and also maps acceptor points in the compound, on donor points in the protein and *vice versa* by default. The docking poses are ranked based on a molecular mechanics–like scoring function. In GOLD software, the Gold score or fitness score can be evaluated from the equation mentioned below.

$$\text{Fitness} = S(\text{hb\_ext}) + 1.375 * S(\text{vdw\_ext}) + S(\text{hb\_int}) + 1.000 * S(\text{vdw\_int})$$

The expression S (hb_ext) reveals the protein – ligand hydrogen bond scores whereas S (vdw_ext) indicates the protein ligand van der Waals scores. S (hb_int) is the contribution to the fitness and it is based on the occurrence of intra-molecular hydrogen bonds within the ligand. S (vdw_int) represents its contribution which occurs due to intra-molecular strain present within the ligand.

In the present study, with respect to GOLD software protein–compound interactions were selected within the range of 0.5–5 Å, whereas active site radius was 10 Å. In order to obtain quality and docking accuracy, search settings were studied in this investigation using RMSD value 1.5 Å.

Toxicity

Toxicity and drug likeness were estimated by using OSIRIS molecular property explorer tool[17,18]. The toxicity risk assessment is mandatory to avoid the unhelpful compounds for further processing of the drug development. The mutagenic, tumorigenic, irritant and reproductive toxicity risks were measured for the designed docking compounds. The undesired effects of the compounds are displayed in red, while green color indicates the desired effects of the compound.

The ClogP is a partition coefficient between *n*-octanol and water. It plays a crucial role in governing passive membrane partitioning, influencing permeability opposite to its effect on solubility. Most of the drugs available in the market have clogP value > 5.

Molecular weight is a very important aspect of drug action. If molecular weight increases, crowding increases which effects on abortion of drug action. Keeping lower molecular weight (> 450) is an essential factor in the drug design process.

The solubility property of a drug affects absorption and distribution characteristics in aqueous solutions. OSIRIS tool is used to identify low solubility behavior of drug compounds. The value is not more than -4. The drug-likeness is the sum of the score values of the fragments present in the molecule. The positive value in drug-likeness indicates that the tested molecule mainly contains fragments which are frequently present in marketed drugs. Drug score value indicates the overall potential of a compound to be a drug candidate.

Conclusion

The aim of this study is to understand the salient structural features of selective COX-1 and COX-2 inhibitors. The results of docking studies have revealed that the compounds which contain methyl sulfonamyl pyrazine and phenyl ring at the adjacent position are found to show the best fitness against COX-1 and COX-2. The compound **58** and **57** interact with COX-1 and COX-2 with 1.1:1.0 and, 2:1 ratio respectively. The molecular property explorer tool was used to predict the mutagenic, tumorigenic, irritant, reproductive risk, and drug-relevant properties of the compound. Finally, it is expected that these results will contribute to the development of newer NSAIDS molecules with fewer side effects.

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