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Computer–Aided Design of Selective COX–2 Inhibitors: Molecular Docking of Structurally Diverse Cyclooxygenase–2 Inhibitors using FlexX

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Computer–Aided Design of Selective COX–2 Inhibitors: Molecular Docking of Structurally Diverse Cyclooxygenase–2 Inhibitors using FlexX [#]

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Abstract

Motivation. Three–dimensional structures of pharmacologically important macromolecules offer a route to the discovery of new drugs. Understanding the macromolecule–ligand interactions and validation of method used for docking and virtual screening of chemical databases is crucial step in structure–based design. We carried out molecular docking for a set of eighty two structurally diverse COX–1/COX–2 inhibitors including traditional NSAIDs and the recently developed coxibs such as celecoxib, rofecoxib, valdecoxib and etoricoxib using FlexX method to find out how good this method differentiate between the active and inactive compounds.

Method. FlexX is one of the fast flexible docking method that uses an incremental construction algorithm to place ligands into an active site. The scoring function (empirical binding free energy) of the FlexX used to estimate the free binding energy of the protein–ligand complex is called F_score.

Results. Reproducibility of the experimental conformations of the bound ligands such as SC–558, indomethacin, and flurbiprofen indicates the better performance of FlexX method. Good correlation between the standard FlexX score (F_score) and the COX–2 inhibitory activity (pIC_{50}) was observed. Simple linear regression analysis provided the correlation coefficient values of 0.731 and 0.670 for two classes of COX–2 inhibitors.

Conclusions. Flexible docking of eighty two structurally diverse COX–2 inhibitors has been successfully carried out. Some false positives and false negatives were observed but considering the limitations of the available docking programs, the results are encouraging. The in depth analysis of the resulted COX–2–ligand complexes may improve our knowledge in understanding the binding interactions in detail. Thus, this study will be useful for the design of novel COX–2 inhibitors based on docking and the resulted bioactive conformations of the ligands will be useful in building structure–based 3–D QSAR model.

Keywords. FlexX; cyclooxygenase–2; docking; structure–based drug design; NSAIDs.

1 INTRODUCTION

The process of structure–based design started with the detailed analysis of binding site of the target protein, preferably in its complex form with a ligand. The knowledge of binding site helps to design novel drug candidates with better potency. Another approach that uses the structural

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information deals with the protein–based virtual screening of chemical databases wherein prior to biological screening, the potent compounds are computationally figured out from a large chemical library. Docking methods have the added advantage compared to 2–D similarity and 3–D pharmacophore search methods because it makes use of 3–D receptor structure in a quantitative way. Compound selection based on docking calculations alone and or combined with virtual screening has been carried out for various targets such as thrombin [1], thymidylate synthase [2], dihydrofolate reductase [3], HIV protease [4], PTP1B [5], and human carbonic anhydrase [6]. Such study led to the identification of novel compounds with the potency between 1–100μM.

COX–2 is one of the well–known targets for the anti–inflammatory therapy. Selective inhibition of this enzyme overcomes the side effects associated with the traditional NSAIDs. The reported 3–D QSAR models [7–10] are mainly focused to a particular class of compounds and such models may not be useful to predict structurally diverse compounds. Stewart *et al.* [11] have reported a novel lead, phenothiazine for the inhibition COX–2 enzyme using combined 3–D database searching and combinatorial chemistry methodologies. The availability of several crystal structures of complexes of COX–2 with the inhibitors provides the possibility to apply structure–based design techniques for the development of specific and potent inhibitors. Therefore, we thought of exploiting the structure–based approach to design novel COX–2 inhibitors by docking studies combined with visualization of active site–ligand interactions.

2 MATERIALS AND METHODS

Table 1. Selected residues within 4 Å distance from SC–558

S.No.	Aminoacid	Number
1	Arg	120
2	Leu	352
3	Ala	527
4	Gly	526
5	Val	523
6	Val	349
7	Leu	359
8	His	90
9	Tyr	355
10	Ser	353
11	Arg	513
12	Phe	518
13	Ile	517
14	Ala	516
15	Gln	192
16	Tyr	385
17	Trp	387

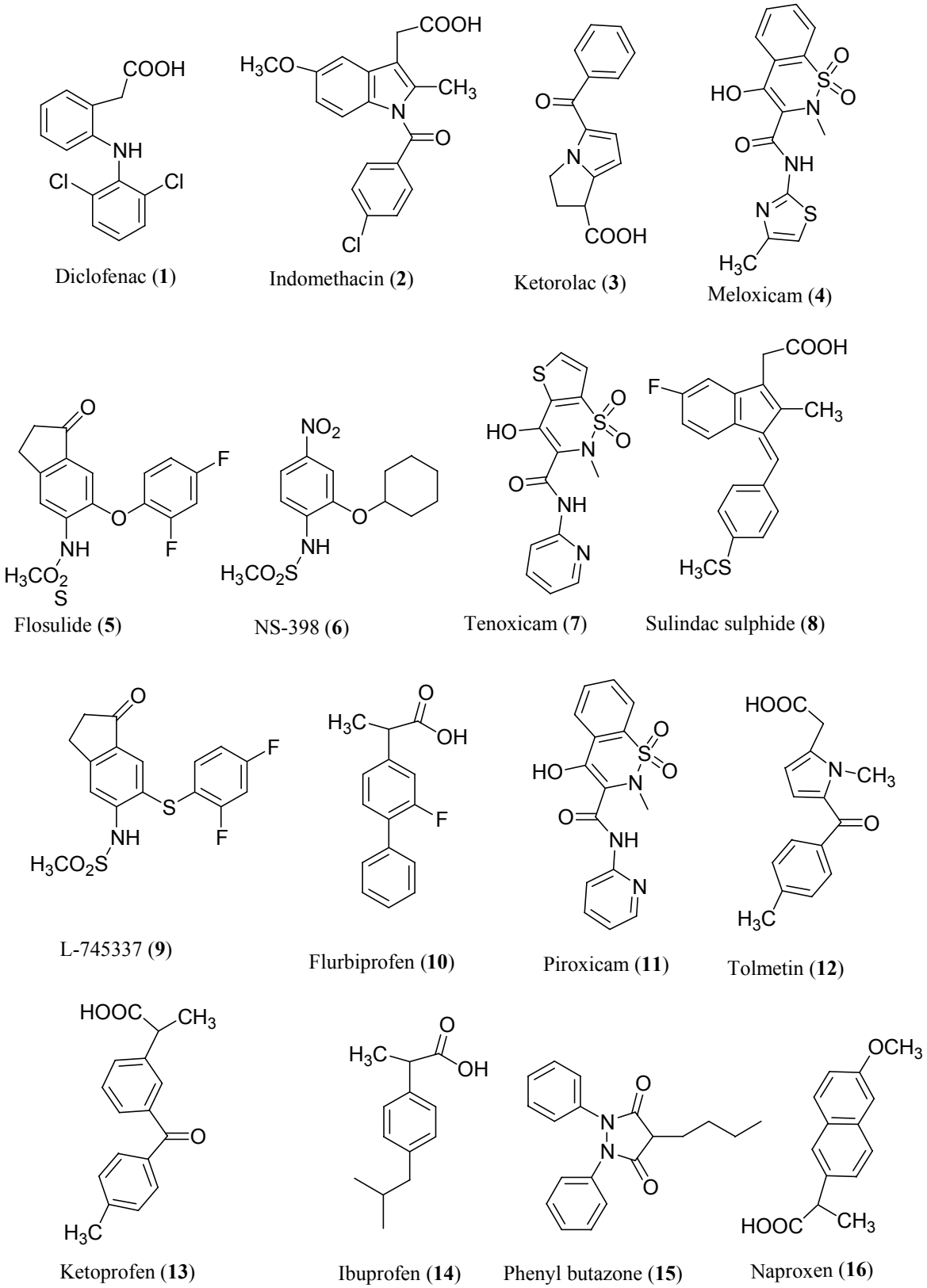
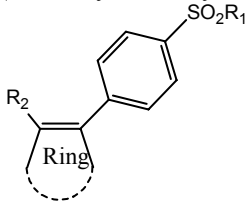


Figure 1. Structures of compounds (including NSAIDs) selected for docking studies.

Table 2. Structures of compounds (1,2–diaryl heterocyclic class) selected for docking studies



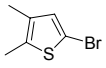
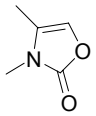
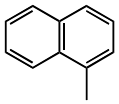
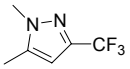
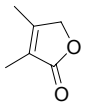
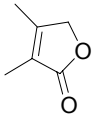
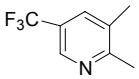
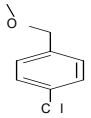
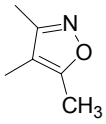
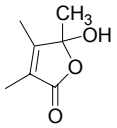
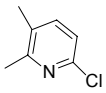
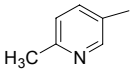
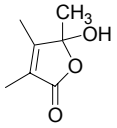
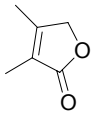
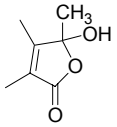
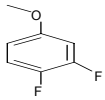
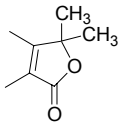
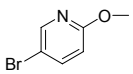
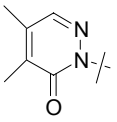
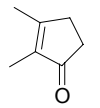
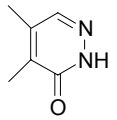
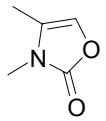
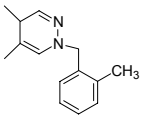
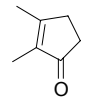
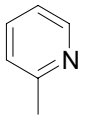
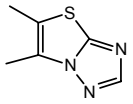
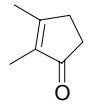
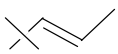
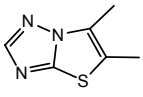
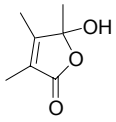
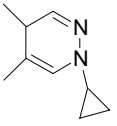
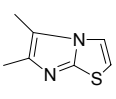
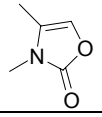
No	Ring	R_1	R_2	No	Ring	R_1	R_2
17		CH_3	4–F–C_6H_4	**33**		NH_2	
18		NH_2	4–CH_3–C_6H_4	**34**		CH_3	
19		CH_3	C_6H_5	**35**		CH_3	
20		NH_2	C_6H_5	**36**		CH_3	4–OH– C_6H_4
21		CH_3		**37**		CH_3	
22[a]		SCH_3	4–OCH_3– C_6H_4	**38**		CH_3	
23		CH_3		**39**		CH_3	C_6H_5
24		CH_3	4–F–C_6H_4	**40**		CH_3	C_6H_5
25		NH_2	3,4–di–Cl–C_6H_3	**41**		CH_3	C_6H_5
26		CH_3		**42**		CH_3	3,4–di–F– C_6H_3
27		CH_3		**43**		CH_3	C_6H_5
28		CH_3	3,5–di–F–C_6H_3	**44**		CH_3	C_6H_5
29		CH_3	3,4–di–F–C_6H_3	**45**		NH_2	4–COOH– C_6H_4

Table 2. (Continued)

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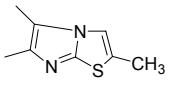
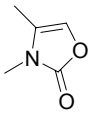
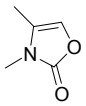
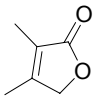
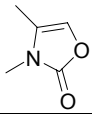
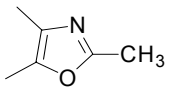
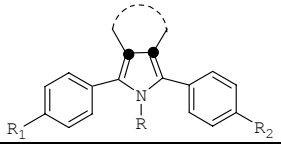
30		CH_3	3,4–di–F–C_6H_3	**46**		NH_2	3–COOH– C_6H_4
31		NH_2	4–CF_3–C_6H_4	**47**		CH_3	C_6H_5
32		NH_2	*c*–C_6H_{11}	**48**[b]		NH_2	*c*–C_6H_{11}

[a] SO_2R_1 replaced by SCH_3
[b] 3–H of the sulphonyl phenyl ring is substituted by F

Table 3. Structures of 1,3–diaryl heterocyclic compounds



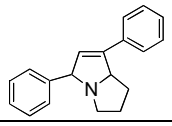
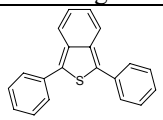
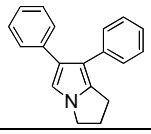
No	Ring	R	R_1	R_2	No	Ring	R	R_1	R_2
49		H	H	H	**66**		H	F	F
50		H	H	H	**67**		H	H	H
51		CH_3	H	H	**68**		H	H	H
52		NH_2	H	H	**69**		H	H	H
53		NHMs	H	H	**70**		H	F	F
54		CH_2COOH	H	H	**71**		H	F	F
55		H	F	F	**72**		H	F	CH_3SO_2
56		H	CH_3S	CH_3S	**73**		H	CH_3SO_2	CH_3SO_2
57		H	CH_3	CH_3	**74**		H	H	H
58		H	OCH_3	OCH_3	**75**		H	F	F
59		H	Cl	Cl	**76**		H	H	H
60		H	F	Imidazol–1–yl	**77**		H	4–F 3–NHAc	4–F 3–NHAc
61		H	Imidazol–1–yl	Imidazol–1–yl	**78**		H	4–F 3–$NHCH_3$	4–F 3–$NHCH_3$
62		H	H	H	**79**[a]		H	–	–
63		H	H	H	**80**				

Table 3. (Continued)

No	Ring	R	R_1	R_2	No	Ring
64		CH_3	H	H	**81**	
65		H	H	H	**82**	

[a] replacement of phenyl with pyridine

Table 4. Compounds assayed by human whole blood method, COX–2 potency and FlexX docking scores

Entry	Compound	IC_{50} (μM) COX–2	pIC_{50}	COX–2 FlexX Score
Class 1 Highly potent molecules (IC_{50}≤1.0μM)				
1	Diclofenac (**1**)	0.05	7.30	–25.80
2	Indomethacin (**2**)	0.46	6.34	–20.50
3	Ketorolac (**3**)	0.86	6.06	–28.70
4	Meloxicam (**4**)	0.7	6.12	–20.20
5	Flosulide (**5**)	0.7	6.12	–21.60
6	NS–398 (**6**)	0.47	6.33	–10.60
7	Dup–697 (**17**)	0.06	7.22	–20.30
8	Celecoxib (**18**)	1.0	6.00	–24.10
9	Rofecoxib (**19**)	0.5	6.30	–21.60
10	Valdecoxib (**20**)	0.89	6.05	–24.87
11	Etoricoxib (**21**)	1.0	6.00	–14.68
12	**22**	0.19	6.72	–22.92
13	**23**	0.03	7.52	–14.50
14	**24**	0.08	7.10	–28.54
15	**25**	0.40	6.40	–28.10
16	**60**	0.08	7.10	–12.20
17	**75**	0.12	6.92	–12.88
Class II Moderately potent molecules (IC_{50}>1–30μM)				
18	**26**	9.08	5.01	–13.90
19	**27**	5.2	5.28	–9.88
20	**28**	13.4	4.87	–14.14
21	**29**	2.2	5.66	–17.56
22	**30**	17.5	4.76	–13.25
23	Tenoxicam (**7**)	14.22	4.85	–19.67
24	Sulidac sulphide (**8**)	10.43	4.98	–11.77
25	L–745337 (**9**)	9.7	5.01	–13.58
26	**31**	2.0	5.70	–28.00
27	**32**	18.9	4.72	–16.20
28	**33**	4.7	5.33	–22.90
29	Flurbiprofen (**10**)	6.46	5.19	–25.70
30	Piroxicam (**11**)	8.99	5.05	–20.00
31	Tolmetin (**12**)	7.09	5.15	–19.10
32	Ketoprofen (**13**)	1.08	5.97	–26.80
Class III Inactive molecules ((IC_{50}>30μM)				
33	Ibuprofen (**14**)	>30	4.52	–8.84
34	Phenylbutazone (**15**)	>30	4.52	–12.15
35	Naproxen (**16**)	73.74	4.13	–19.65
36	**34**	>33	4.48	–16.01
37	**35**	>33	4.48	–23.65
38	**36**	>33	4.48	–15.47
39	**37**	>33	4.48	–13.20
40	**38**	>33	4.48	–11.97

Table 4. (Continued)

Entry	Compound	IC_{50} (μM) COX–2	pIC_{50}	COX–2 FlexX Score
41	**39**	>33	4.48	–6.85
42	**40**	>33	4.48	–15.29
43	**41**	>33	4.48	–13.41
44	**42**	>30	4.48	–15.62
45	**43**	>30	4.48	–13.53
46	**44**	>33	4.48	–15.20
47	**45**	59%[a]	4.00	–31.00
48	**46**	100	4.00	–32.00
49	**47**	Inactive	–	–23.25
50	JTE–522 (**48**)	>33	4.48	–19.68

[a] at 100μM

Table 5. Compounds assayed by mouse macrophage method, COX–2 potency and FlexX docking scores

Entry	Compound	IC_{50} (nM) COX–2	pIC_{50}	COX–2 FlexX Score
51	**49**	1.5	8.82	–27.20
52	**50**	3.3	8.48	–24.80
53	**51**	>1000	6.00	–21.40
54	**52**	1.8	8.74	–20.30
55	**53**	500	6.30	–12.10
56	**54**	>100	7.00	–18.20
57	**55**	1.7	8.77	–27.20
58	**56**	500	6.30	–11.20
59	**57**	16.7	7.78	–12.00
60	**58**	21.3	7.67	–16.40
61	**59**	5.0	8.30	–21.00
62	**60**	42.0	7.38	–17.20
63	**61**	>100	7.00	–20.30
64	**62**	3.1	8.51	–27.50
65	**63**	14.5	7.84	–22.10
66	**64**	>1000	6.00	–5.90
67	**65**	0.7	9.15	–27.50
68	**66**	2.9	8.54	–26.00
69	**67**	>100	7.00	–11.10
70	**68**	>100	7.00	–16.70
71	**69**	2.6	8.58	–15.70
72	**71**	4.5	8.35	–18.80
73	**72**	700	6.15	–15.10
74	**73**	>10000	5.00	–14.50
75	**74**	1.6	8.79	–11.50
76	**76**	35.6	7.45	–14.50
77	**77**	>100	7.00	–30.70
78	**78**	>100	7.00	–19.60
79	**79**	>100	7.00	–17.80
80	**80**	50.0	7.30	–25.90
81	**81**	10.9	7.96	–16.20
82	**82**	28.7	7.54	–13.50

2.1 Ligand Preparation

All the molecular modeling and docking studies were performed on a Silicon Graphics Octane 2 workstations using Sybyl6.8 [12]. Eighty two compounds (Figure 1, Tables 2 and 3) were selected based on structural diversity and wide range of biological activity [13–24]. Major COX–2 inhibitory

data was obtained from the human whole blood method [13] developed by Merck Frost Center. Known ligands were extracted from the PDB file and converted into SYBYL mol2 format. Hydrogens were added and atom and bond types were corrected. Other molecules were sketched and subjected to systematic conformational search to find out the possible lowest energy conformation. The charges were calculated using Gasteiger–Hückel method. The ligands were energy minimized using the Tripos force field. Compounds **49–82** used for CoMFA model [10] were submitted for docking. The compounds having carboxylic acids were treated as carboxylate and the formal charges were supplied.

2.2 Receptor File (rdf) Preparation

The coordinates of cyclooxygenase–2 enzyme crystal structure (1CX2) were retrieved from the PDB. The residues (Table 1) within 4 Å distance from SC–558 [25] were selected manually. After the selection, a radius of 2.5 Å was specified in order to extent the binding site.

2.3 Molecular Docking

2.3.1 Details of FlexX

The physicochemical model behind FlexX [26] can be divided into three parts: the conformational space of the ligand, the model of protein–ligand interactions, and the scoring function. To each acyclic single bond, a set of low–energy torsion angles is assigned using the MIMUMBA torsion angle database. Generated conformations are only tested for intramolecular clashes, and there is no conformational energy term in the scoring function. The scoring function [27] of FlexX is the function developed by Böhm for the de novo design program LUDI with some minor changes.

$$\Delta G = \Delta G_0 + \Delta g_{rot} \times N_{rot} \quad (1)$$

$$+ \Delta G_{hb} \sum f(\Delta R, \Delta\alpha) \quad \text{neutral H–bonds} \quad (2)$$

$$+ \Delta G_{io} \sum f(\Delta R, \Delta\alpha) \quad \text{ionic interactions} \quad (3)$$

$$+ \Delta G_{aro} \sum f(\Delta R \Delta\alpha) \quad \text{aromatic interactions} \quad (4)$$

$$+ \Delta G_{lipo} \sum f(\Delta R) \quad \text{lipohilic contributions} \quad (5)$$

The scoring function can be divided into three parts. The first part (1) consists of a fixed term ΔG_0 and a term $\Delta g_{rot} \times N_{rot}$ taking into account the loss of entropy during ligand binding. The second part (2–4) contains the contributions for matched interaction groups like hydrogen bonds, salt bridges and charged hydrogen bonds and aromatic interactions. Each of these terms consists of a fixed contribution per interaction multiplied by a penalty function $f(\Delta R, \Delta\alpha)$. The penalty functions are piecewise linear functions scaling the contribution of an interaction with respect to its geometry. The third part (5) rates the atom–atom contacts between protein and ligand such as hydrophobic

contacts and forbiddingly close contacts (clashes). The second and third parts of the scoring function are called match score and contact score, respectively.

The selected compounds were docked into the COX–2 active site using the default FlexX parameter settings. The results of top ranked scoring conformation were analyzed and used in the correlation of COX–2 inhibitory activity.

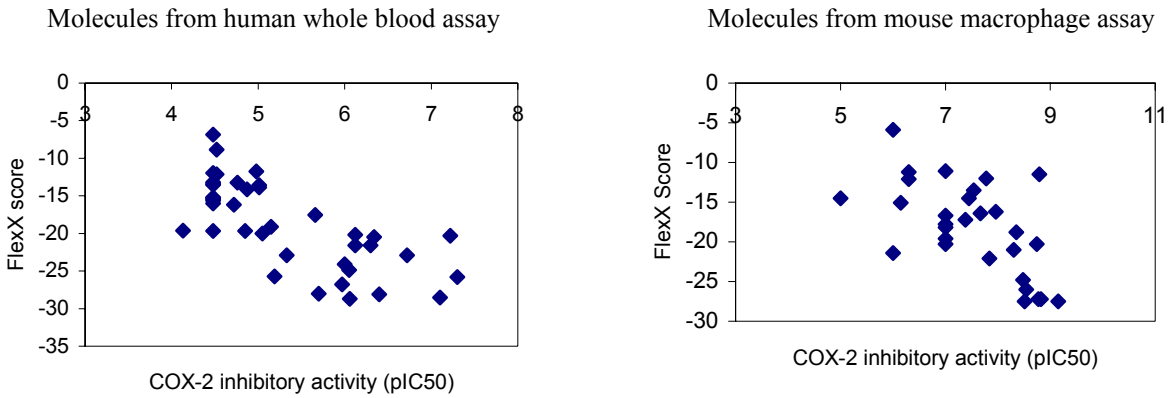


Figure 2. FlexX score *vs* COX–2 Inhibitory Activity (pIC_{50}).

Table 6. The rms deviation of known ligands

S. No.	Ligand	PDB	Enzyme	RMSD (Å)
1	SC–558	1CX2	COX–2	1.062
2	Flurbiprofen	3PGH	COX–2	1.524
3[a]	Indomethacin	4COX	COX–2	0.799

[a] Subcore pocket (only 4Å distance residues from SC–558) was used

3 RESULTS AND DISCUSSION

Reproducibility of the experimental conformations of the bound ligands such as SC–558, indomethacin and flurbiprofen was observed from docking (Figures 8, 4, and 3). As previously observed by Plount–Price *et al.* [28] we found that FlexX docks the sulphonyl amino group of SC–558 in a way that can make hydrogen bonding with Arg513 and His90. However in the crystal structure complex (1CX2.pdb) bad N–N contact was observed between sulphonyl amino group and nitrogen of His90. The carboxylate group of active NSAIDs was oriented towards the guanidine group of Arg120 (Figures 3–7). The top scoring docked conformation was selected and the non–hydrogen atoms were aligned to the experimental conformation of the ligand. The low rms deviations (Table 6) between the theoretical and experimental conformations observed for the ligands studied indicated the better performance of FlexX method.

3.1 Compounds Tested by Human Whole Blood Assay

The resulted FlexX scores and the COX–2 inhibitory activities were shown in Tables 4 and 5. We found more negative scores (Table 4) for the potent molecules, indicating the better binding of the ligands into the active site. Molecules NS–398 (**6**), etoricoxib (**21**), **23**, **60** and **75** were found to be false negatives. AutoDock [29] method could successfully dock NS–398 into the active site of human COX–2. However, the docking scores were found to be less negative in case of moderately potent and inactive molecules. We observed some false positives (**35**, **45**, **46**, and **47**) from the set of inactive molecules. FlexX assigned good scores to these inhibitors as these compounds form more than one hydrogen bond with the protein. These observations are in confirmatory with the virtual screening studies reported by Martin *et al.* [30]. The resulted FlexX docking score was correlated with the COX–2 inhibitory property (Figure 6). The linear regression analysis was performed for the molecules that are assayed by human whole blood method. After removing the false positives and false negatives the following regression equation was obtained for 40 molecules: COX–2 inhibitory activity (pIC_{50}) = 3.181 – (0.114 × FlexX score). The r^2 value was found to be 0.534 and R–value was 0.731 with the standard error of 0.619.

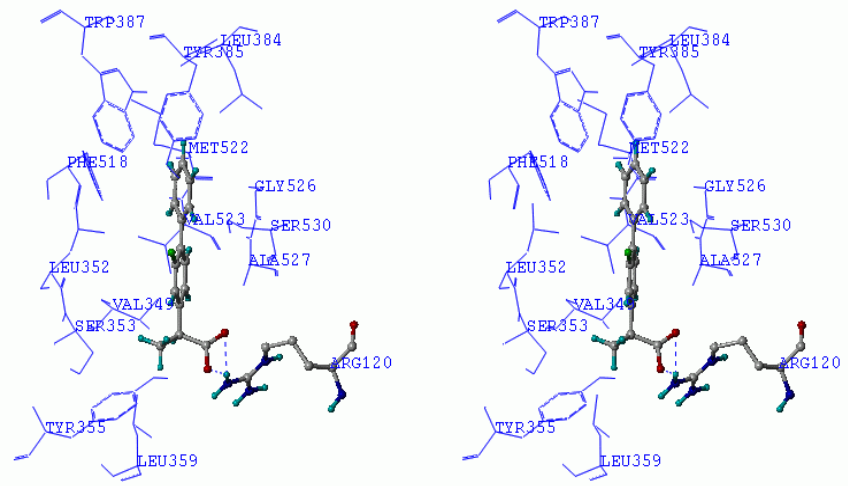


Figure 3. Binding of *S*–Flurbiprofen into the active site of COX–2 (stereoview).

3.1.1 COX–2–Inhibitor Interactions

Visualization of docked inhibitors in COX–2 enzyme reveals that the carboxylate group of NSAIDs is located in a favorable position to interact with the guanidinium group of Arg120 and OH of Tyr 355. Similar kind of orientations was observed from docking of NSAIDs using modeled human COX–2 enzyme by Autodock method by Pouplana *et al.* [29]. A bifurgated hydrogen bond between the carboxylate group of flurbiprofen and Arg120 was observed. Dock4.0 [31] docked flurbiprofen in similar orientation in the COX–2 active site. In case of ketorolac, ketoprofen and diclofenac, two hydrogen bonds were observed between the carboxylate oxygen atoms and OH,

guanidinium NH of Tyr355, Arg 120 respectively. The hydrogen bonding distances observed were 3.02 Å (C=O…H–O–Tyr355) and 2.61Å (C–O…H–N–Arg120) in ketorolac–COX–2 complex. The phenyl ring of ketorolac was surrounded by the aromatic residues Phe518 and Trp387. The other part was oriented in the hydrophobic cleft formed by Val349, Leu531 and Ala527. The hydrogen bonding distances observed were 3.00 Å (C–O…H–O–Tyr355) and 2.91Å (C=O…H–N–Arg120) in ketoprofen–COX–2 complex. The methyl group of ketoprofen was oriented in a way to interact with hydrobhobic residues such as Leu531 and Ala527.

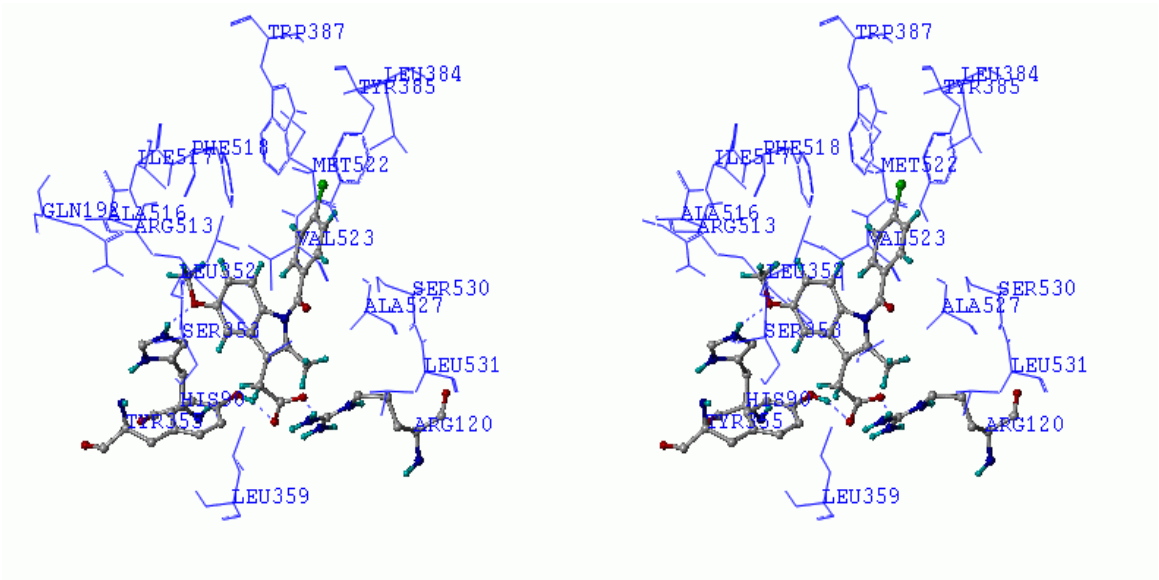


Figure 4. Binding of indomethacin into the active site of COX–2 (stereoview).

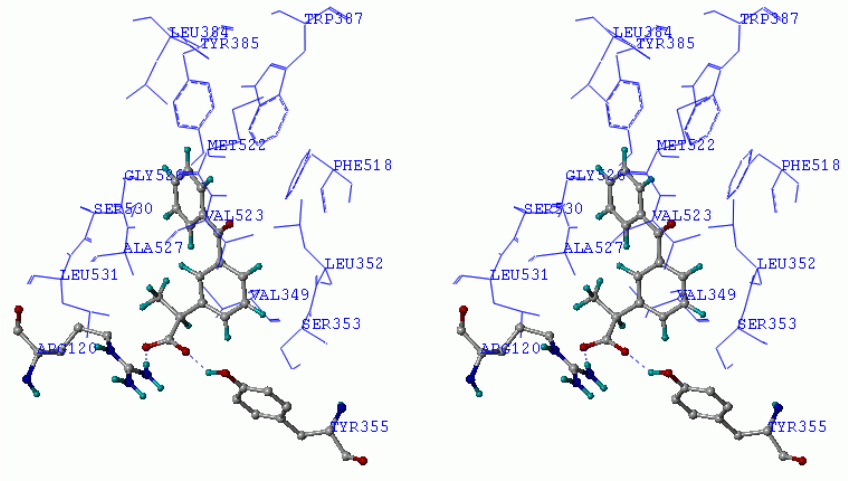


Figure 5. Binding of *S*–ketoprofen into the active site of COX–2 (stereoview).

One of the phenyl rings was oriented in a way to make hydrophobic interaction with Leu352 and Ala527 and Val523. Another phenyl ring was surrounded by the aromatic residues Phe381 and Trp387. However, central carbonyl group of both ketorolac and ketoprofen was not involved in any

hydrogen bonding interactions with enzyme.

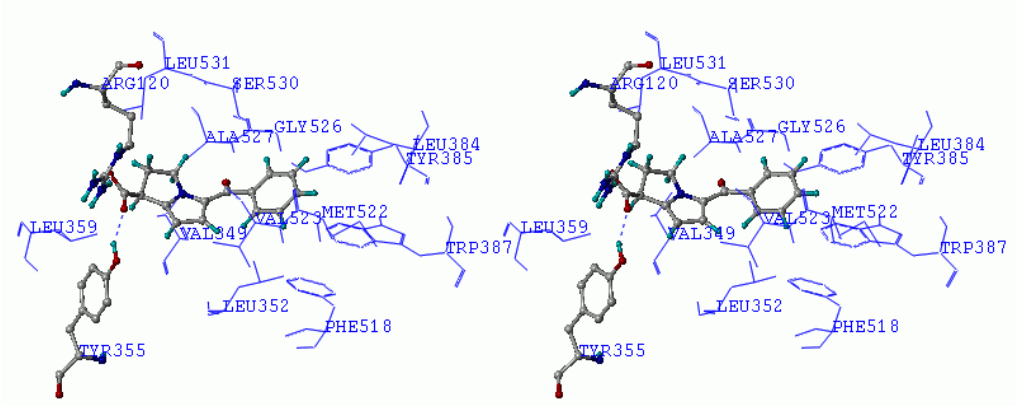


Figure 6. Binding of Ketorolac into the active site of COX–2 (Stereoview).

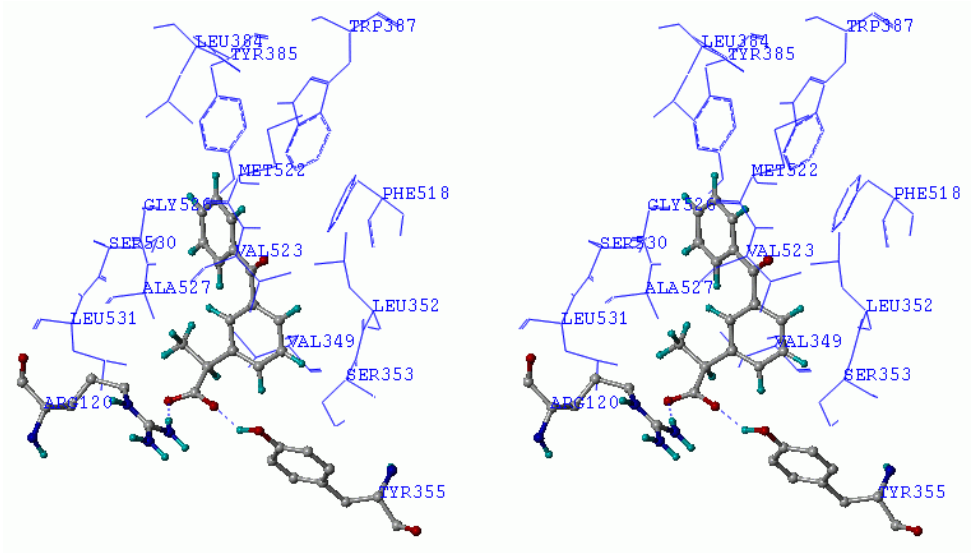


Figure 7. Binding of Diclofenac into the active site of COX–2 (stereoview).

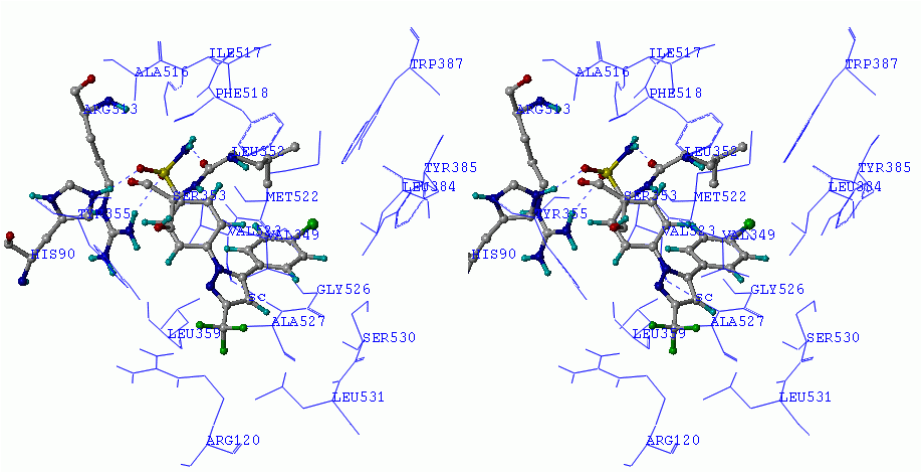


Figure 8. Binding of SC–558 into the active site of COX–2 (stereoview).

The conformation of diclofenac docked by FlexX method was quite different from the experimental conformation (1PXX.pdb). Molecule **49**, a 1,3–diaryl analogue did not produce any hydrogen bonding interaction with the enzyme. Thus, the major interaction of this compound with enzyme is mainly hydrophobic. One of the phenyl rings was surrounded by the aromatic residues Tyr386, Trp387 and Phe518 of COX–2 active site.

3.2 Compounds Tested By *In vitro* Mouse Macrophage Assay

Another class of compounds selected for the docking studies is the non–sulphonyl analogues (Table 3). After removing the false positives (**77**, **80**) and false negative (**74**) the following regression equation was obtained for 29 molecules of 1,3–diaryl isoindole (the binding orientation of the most active molecule **49** is shown Figure 9): COX–2 inhibitory activity (pIC_{50}) = 5.233 – (0.119×FlexX score). The r^2 value was found to be 0.449 and R–value was 0.670 with the standard error of 0.775. The calculated correlation coefficient values indicate a good correlation between the FlexX score and COX–2 inhibitory activity.

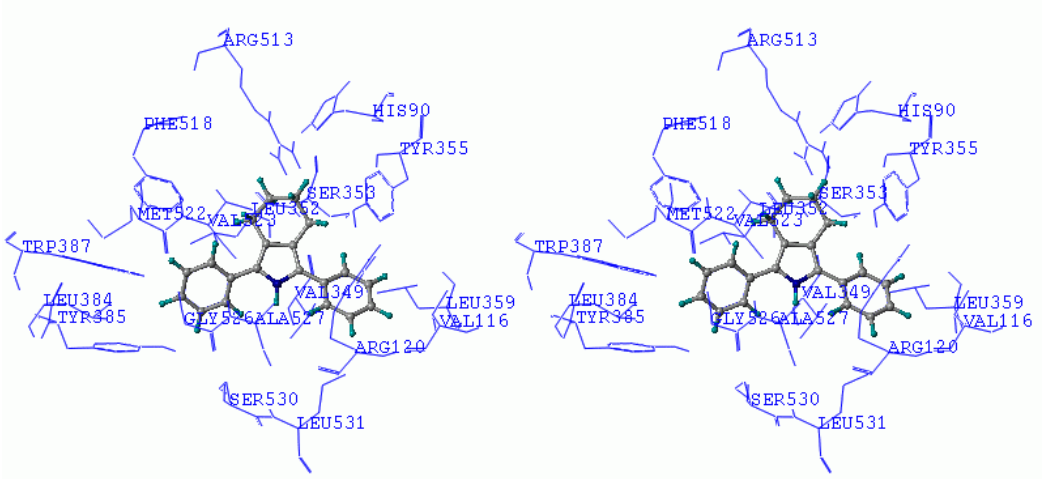


Figure 9. Binding of 1,3–diaryl isoindole derivative (**49**) into the active site of COX–2 (stereoview).

In general, from this exercise it was observed that the more negative is the score (more negative with respect to the value of –20) higher is the affinity of the ligands. Scores with more positive value compared to –20 was observed for moderate to low affinity ligands. Apart from this it was found that some compounds produced good scores and correlation with the pIC_{50} but were out of the actual active site and therefore before going for the selection of compounds for synthesis one should also visually monitor the interaction with the active site amino acid residues. Very recent report [32] showed that F_ score is better than other scoring functions such as PMF, D–score, G–score and chemscore tried in the virtual screening of COX–2 inhibitors. Thus FlexX method is one of the best docking methods that can be used in designing novel COX–2 inhibitors.

4 CONCLUSIONS

We have successfully carried out flexible docking for eighty two structurally diverse COX–2 inhibitors. The obtained FlexX docking score was correlated with the biological activities. Some false positives and false negatives were observed but considering the limitations of the available docking program, the results are encouraging. The in depth analysis of the resulted COX–2–ligand complexes may improve our knowledge in understanding the binding interactions in detail. Thus this study will be useful for the design of novel COX–2 inhibitors based on docking and the resulted bioactive conformations of ligands will be useful in building structure–based 3–D QSAR model.

Acknowledgment

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