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Structural Studies on Docking Selective COX-2 Inhibitors

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ABSTRACT

An attempt was made to study the interacting COX-2 active site residues with selective COX-2 inhibitors and evaluated the importance of scoring functions by performing docking studies on selective analogs. Four selective COX-2 (Valdecoxib, Celecoxib, Rofecoxib and Etoricoxib) inhibitors were selected for study to correlate the associated non-bonded interactions with receptor and the binding energy. The importance of various substituents on the analogs in relation with the geometry and orientation of the molecule binding to the active site residues studied. Two programs, X-Score scoring function program for predicting protein-ligand interactions and Fast Dock, protein-ligand docking with PMF scoring function were employed. Of all the conformers generated using CAChe software, the best conformer with lowest possible energy subjected to Fast Dock docking method, to find the effective dock score and possible orientation of analogs within active site space. And of all the selective COX-2 inhibitors and Celecoxib analogs studied, analog 12a has shown relatively high dock score of about -172.267 kcal/mol while the score for Celecoxib is -140.018 kcal/mol. Spatial orientation of ligands and the hydrophobic interactions have led to the high binding energy of Celecoxib and high dock score for analog 12a, as the sulfonamide and substituted pyrazole moieties acting as potential binding sites for residue-ligand interactions.

KEYWORDS: Potential of Mean Force (PMF), prostaglandin synthase-2, Lipinski's Rule of 5, Dock Score

INTRODUCTION

The non-steroidal anti-inflammatory drugs (NSAIDs) have been among the most widely used drugs for the treatment of pain and inflammation. NSAIDs develop their mode of action by blocking the Cyclooxygenase (COX) enzyme and thus the biosynthesis of PGs (Prostaglandins) [1]. Two isoforms of the COX enzyme have been characterized: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [2].

Prostaglandin H2 synthase is a key enzyme in the biosynthesis of PGs mediating inflammation and other important physiological processes. COX-1, described as a "housekeeping" enzyme, is expressed in the gastrointestinal tract, kidneys and platelets. Under the influence of COX-1, prostaglandins maintain the integrity of the gastric mucosa, mediate normal platelet function and regulate renal blood flow [3]. The isoenzyme COX-2 is primarily associated with inflammation. Cytokines and growth factors increase the expression of COX-2, mainly at inflammatory sites, producing prostaglandins that mediate inflammation, pain and fever [3]. Ddiscovery of the COX-2 isoenzyme led to the theory that COX-2 selective inhibition would provide the potent anti-inflammatory and analgesic effects of traditional NSAIDs without influencing COX-1 [4]. The COX molecule consists of three independent folding units: an epidermal growth factor-like domain, a membrane binding site, and an enzymatic domain [5]. The active COX site is a hydrophobic channel with a series of amino acids. Aspirin binds irreversibly to serine 580 by acetylation, whereas most other NSAIDs bind sterically and reversibly to Tyrosine 385 or Arginine 120, blocking the COX action of the enzyme [6-7]. There are spectral and biochemical data suggesting that a tyrosine group(s) is involved in the cyclooxygenase reaction catalyzed by prostaglandin endoperoxide (PGH) synthase [8].

COX-2 specific inhibitors retain some platelet thromboxane A_2 inhibitory properties, but their antiplatelet potency is far less than that of traditional NSAIDs [9]. Celecoxib, the first highly selective COX-2 inhibitor approved by US FDA is indicated against osteoarthritis and rheumatoid arthritis [10]. NSAIDs administration in animal models resulted in inhibition of angiogenesis and proliferation, induction of apoptosis and prevention of metastasis. In clinical setting, NSAIDs and selective COX-2 inhibitors have the capacity to prevent the development of colorectal adenomas [11].

Herein, we describe our structural studies on selective COX-2 inhibitors and Celecoxib analogues to determine the related potency of the compounds against COX-2 active site by employing protein-ligand docking

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using CAChe software. In our work, CAChe docking analysis routine with PMF (Potentials of Mean Force) scoring function and X-score were utilized in the study because of the importance of such scoring functions in protein ligand interactions.

MATERIALS AND METHODS

X-ray crystallographic 3-dimensional structure of mouse Cyclooxygenase, 1CX2 atomic coordinates was taken from protein data bank (PDB) [12] to study protein-ligand interactions. 1CX2 is a Cyclooxygenase-2 (prostaglandin synthase-2 or COX-2) enzyme with EC Number: 1.14.99.1 classified under Oxidoreductase class of enzymes, complexed with a selective inhibitor S58 with 4 chains, with 3.0 A° resolution and 0.216 R-value respectively. Computational analysis was carried out on chain A of 1CX2. The four COX-2 inhibitor molecules viz. Celecoxib, Valdecoxib, Rofecoxib and Etoricoxib were selected to study the associated physico-chemical parameters and protein-ligand interactions. All Celecoxib analogs selected for the study were taken from Bioorganic & Medicinal Chemistry Letters 14 (2004) 95-98. A total of about 27 celecoxib analogs from **11a** to **11n** and **12a** to **12n** were selected from the article, where the analogs were tested in the in vitro canine whole blood COX inhibition assays [13]. The series of analogs can be selectively divided into $–CF_3$ and $–CHF_2$ containing analog series. X-score scoring function was employed to predict the binding energy for active site residue-ligand interactions and docking studies computed for all analogs using CAChe 6.1.1 Work System Pro that predicted interactions in terms of Dock score.

Chemical drawing software ISIS Draw [14], scoring function program X-Score [15] and chemical analysis and Docking software, CAChe Work System Pro 6.1.1 [16] were chosen for analysis. 2-dimensional Celecoxib structure was drawn using ISIS Draw and the drug-like properties were identified based on Lipinski's rule [17] of 5 evaluated from logp.com [18] and three dimensional molecules are drawn using CAChe 6.1.1 workspace where the valence, hybridization and geometry were refined as per the standards.

Table-1: Lipinski's data (calculated from www.logp.com) for selective COX-2 inhibitors and Celecoxib analogs. Molecule No. represented as given in reference article (Bioorg. Med. Chem. Lett. 2004;14:95-98)

S.No	Molecule No.	Log p	FormulaWt.	HBA	HBD	Lipinski Number
1.	11a	1.74	398	9	1	4
2.	11b	2.96	385	8	0	4
3.	11c	3.04	401	8	0	4
4.	11d	2.65	446	7	0	4
5.	11e	3.15	397	8	0	4
6.	11f	3.99	413	8	0	4
7.	11g	3.65	411	8	0	4
8.	11h	3.15	410	9	0	4
9.	11i	3.43	411	8	0	4
10.	11j	3.05	415	9	0	4
11.	11k	3.42	431	9	0	4
12.	11l	3.62	476	8	0	4
13.	11m	3.12	415	9	0	4
14.	12a	1.93	349	6	0	4
15.	12b	2.04	367	7	0	4
16.	12c	2.47	383	7	0	4
17.	12d	2.22	428	6	0	4
18.	12e	2.29	379	7	0	4
19.	12f	3.17	395	7	0	4
20.	12g	2.63	393	7	0	4
21.	12h	2.21	397	8	0	4
22.	12i	2.88	413	8	0	4
23.	12j	2.24	397	8	0	4
24.	12k	2.74	363	6	0	4
25.	12l	3.32	377	6	0	4
26.	12m	3.61	405	7	0	4
27.	12n	2.95	391	7	0	4
28.	Celecoxib	3.87	381	7	1	4
29.	Valdecoxib	3.09	314	5	1	4
30.	Rofecoxib	2.69	314	4	0	4
31.	Etoricoxib	3.24	358	5	0	4

Table-2: Average X-Score and binding energy of selective COX-2 inhibitors.

S. No.	COX-2 inhibitor	HP[a] Score	HM[b] Score	HS[c] Score	Average Score	Binding Energy (kcal/mol)
1.	Celecoxib	6.53	7.27	6.47	6.76	-9.22
2.	Valdecoxib	6.52	7.18	6.48	6.73	-9.18
3.	Rofecoxib	6.16	6.81	5.83	6.27	-8.55
4.	Etoricoxib	5.96	6.36	5.84	6.05	-8.25

[a, b, c represents Hydrophobic Pair, Hydrophobic Match and Hydrophobic Surface.]

Table-1 represents the Lipinski data for the set of selected molecules. From the table it is evident that on an average, all molecules have a logp value in the range from 1.74 to 3.99 with a reasonable Hydrogen Bond Donor and Acceptor values. The four COX-2 inhibitors are subjected to X-Score, scoring function program run under Linux and the predicted binding energy along with HS, HM and HP scores, for each molecule, are listed in Table-2. X-Score computes a binding score for a given protein-ligand complex structure based on the three empirical scoring functions viz. HPScore (Hydophobic Pair), HMScore (Hydrophobic Match) and HSScore (Hydrophobic Surface) respectively. The interaction energy between protein and ligand is computed as the binding affinities and by steric and electrostatic interactions. However, the energy computed is only an approximation to the enthalpy change in the binding process. Apart from generating scores, the program resulted in generating ranks based on high score and high binding energy.

Celecoxib was subjected to conformational analysis using CAChe conformational search procedure by selecting 'standard procedure' method using 'conformations of a long chain' property. Six dihedral angles resulted when a dihedral angle geometry search carried out for single, ionic and coordinate bonds between -180° and +180° in 2 steps along with a double bond search in 1 step. The search performed by including terminal groups.

Sequence Search type calculation carried out with Conjugate Gradient optimization method using CAChe MM3 Augmented force field. The convergence criterion is set to 0.001 kcal/mol with a maximum of 300 iterations. Van der Waals cut-off distance is 9.0 A° while electrostatic interactions were defined using MM2/MM3 bond dipoles. Energy terms like Bond Stretch, Bond Angle, Dihedral Angle etc. were also considered for evaluating conformational energy of all the conformers.

A total of about 27 conformers resulted with conformational energy ranging from -53.03 to -90.85 kcal/mol and the Potential Energy map of the run for Celecoxib showing conformers with best ranks are shown in Figure-1 and Table-3. The best lowest possible energy conformer of Celecoxib and Valdecoxib, Rofecoxib and Etoricoxib were subjected to protein-ligand docking by CAChe Fast Dock Genetic Algorithm method with a fast, simplified potential of mean force (PMF[19]) scoring function. The potential of mean force is a knowledge-based approach that extracts pairwise atomic potentials from structure information of known protein-ligand complexes contained in the Protein Data Bank. PMF has been demonstrated to show a significant correlation between experimental binding affinities and computed score for diverse protein-ligand complexes [20]. The ligand and active site are grouped together for docking. The active site selected within 3.5 A° distance from the ligand where the ligand is kept flexible with rigid active site. Docking of individual molecules carried out by using grids with 0.375 A° as Amber [21] van der Waal's grid spacing potentials and employed PMF scoring function.

Table-3: Celecoxib conformers ranked based on lowest possible conformational energy displaying the conformer **20** as the lowest possible energy conformer.

S. No.	Conformer Rank No.	Conformer Energy (kcal/mol)
1.	20	53.030235
2.	4	53.050537
3.	26	53.056374
4.	17	53.536777
5.	14	53.740494
6.	1	54.056671
7.	7	59.594818

Table-4: Computed Dock score values of Celecoxib analogs with experimental IC_{50} (μM) values showing the analog **12a** with high dock score.

Molecule No.	Dock Score (kcal/mol)	COX-2 inhibition (IC50 (μM))
CF_3 analogs		
11a	-97.808	0.41
11b	-141.272	0.24
11c	-126.320	0.25
11d	-120.805	0.45
11e	-84.517	0.044
11f	-37.781	0.069
11g	-139.492	0.13
11h	-98.621	0.12
11i	-74.308	0.27
11j	-99.400	0.31
11k	-90.776	0.30
11l	-94.850	1.50
11m	-136.206	0.06
CHF_2 analogs		
12a	**-172.267**	0.31
12b	-135.275	0.30
12c	-134.673	0.12
12d	-132.776	0.29
12e	-160.034	**0.064**
12f	-121.971	0.12
12g	-171.563	0.17
12h	-153.315	0.05
12i	-130.606	0.33
12j	-163.392	0.39
12k	-171.573	0.18
12l	-141.308	0.40
12m	319.918	2.00
12n	-147.320	8.10
Celecoxib	-140.018	0.63

A local search method carried out for a maximum of 500 iterations at a rate of 0.01. The PMF scoring function and Genetic Algorithm procedure employed a population size of 100 and crossover rate of 0.5, with a maximum of 1000 generations, 7 elitism ratio, and 0.5 mutation rate. The convergence criteria are set at 1.0 kcal. Similar docking studies computed for all analogs using PMF scoring function. The experimental values along with dock scores are given in Table-4.

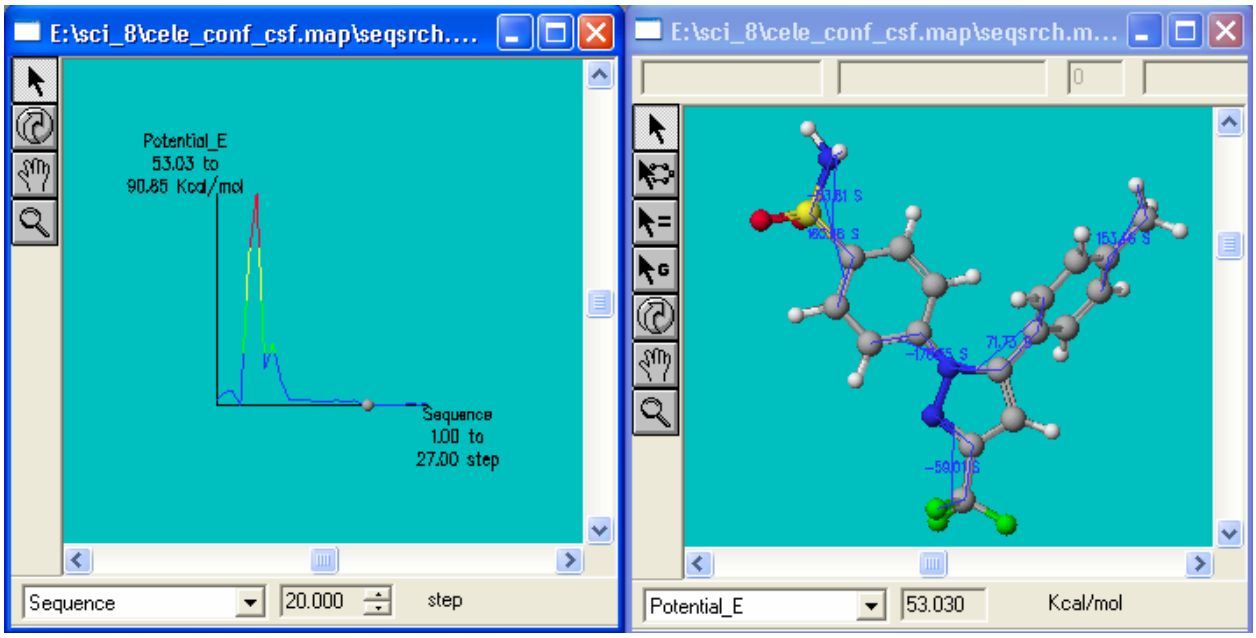

Figure-1: Potential Energy Map of Celecoxib conformational search showing 27 possible conformers scanned from -180° to +180° search space. The lowest possible energy conformer is also shown.

RESULTS AND DISCUSSION

In order to perform docking studies, the inhibitor-bound in the crystal was removed. Water molecules were kept as such as they have null-effect in the studies. S58 ligand with surrounding active site residues within 3.5 A°, hydrogen bonding interactions and the spatial orientation in binding pocket is given in Figure-2. The interacting residues surrounding the ligand within 3.5 A° distance are His90, Arg120, Gln192, Val349, Leu352, Ser353, Tyr355, Leu359, Tyr385, Arg513, Ala516, Phe518, Val523 and Ala527 respectively. The Procheck statistics revealed that about 24 residues are located in disallowed regions whereas the conserved active site residues are located in core and allowed regions.

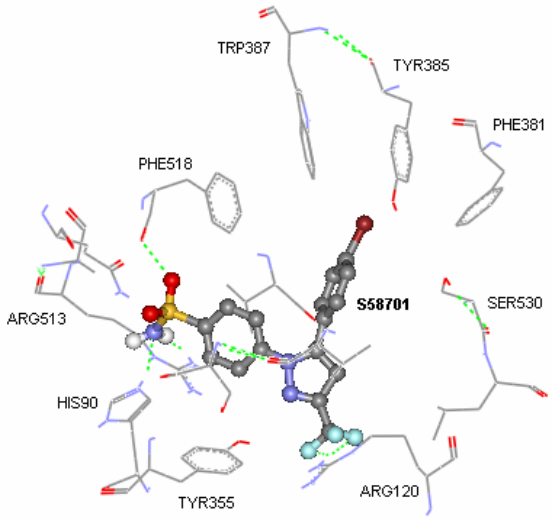

Figure-2: Spatial orientation of S58 crystal ligand within 3.5 A° active site residues. Hydrogens are removed for clarity and hydrogen bonds are shown in green dotted lines.

The selected COX-2 inhibitors and Celecoxib analogs were superimposed onto substituted pyrazole ring of S58 ligand. Of the four selective COX-2 inhibitors subjected to X-Score, Celecoxib has shown high score than others (in terms of HS, HM & HP scores) with greater binding affinity towards receptor surface. The results from Table-2 suggest that the orientation of Celecoxib is more favored for residue-ligand non-bonded interactions. Therefore, Celecoxib analogs were chosen for further investigation. The conformational search procedure carried out for Celecoxib with six dihedral angles resulted in conformer **20** representing the lowest possible energy of 53.030 kcal/mole as given in Figure-1.

Of the dock scores reported for -CF_3 analogs, a high dock score is represented by molecule **11b** with -141.272 kcal/mole and the scores ranged from -37.781 to -141.272 kcal/mole. Whereas, -CHF_2 analogs ranged from -121.971 to -172.267 kcal/mole. The highest dock score, -172.267 kcal/mol predicted for analog **12a**. Figure-3 represents the orientation and comparison of 12a with crystal ligand S58 which suggests the better orientation of interacting groups of ligand within active site space. The -CF_3/-CHF_2 groups lie in the pocket formed by Met113, Val116, Arg120, Val349, Tyr355, Leu359 and Leu531 representing a hydrophobic pocket. While the phenyl ring lies in the cavity formed by Phe381, Leu384, Tyr385, Trp387, Phe518 and Ser530 and the sulfonamide moiety lie in the pocket formed by His90, Gln192, Ser353, Arg513, Ala516, Phe518 and Val523 respectively. Major hydrogen bonding by **12a** observed with sulfonamide moiety forming a 2.80 A° hydrogen bond with Gln192 (S=O….O=C) and 3.10 A° with Leu352 (S=O….O=C) and 1.69 A° between fluorine of –CHF_2 moiety and Arg120 (C-F….H-N). Comparatively, S58 crystal ligand exhibited five hydrogen bond interactions, one forming between S=O of sulfonamide moiety and O=C of Phe518 (3.06 A°), SO2-N….H-N of

His90 (2.27 A°) and between SO2-N….H-N of Arg513 (2.19 A°) and the remaining two are between two fluorines of -CF_3 group with H-N of Arg120 (2.44 and 2.32 A°) respectively.

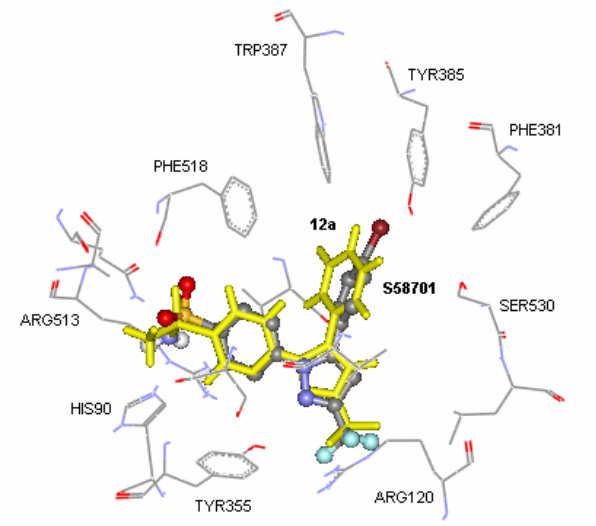

Figure-3: Spatial orientation and superimposition of active analog **12a** over S58 crystal ligand within 3.5 A° active site residues. **12a** molecule is shown in yellow color

All the Celecoxib analogs subjected to Fast Dock method of docking into active site space had shown varied dock scores. (Table-4) This is because the analogs are represented in 2 groups viz., one with -CF_3 group and the other with -CHF_2 group. It has been identified experimentally that -CHF_2 analogs had shown better IC_{50} values against COX-2 enzyme and even though there is not much difference in the activity values between these 2 groups, the AUC values of -CHF_2 analogs made evident that they possess the better choice of regulating COX-2 enzyme under in vivo conditions. Similar evidence can also be drawn from the dock scores resulted from interacting residues of COX-2 with analogs.

A very low dock score predicted for experimentally active analogs from –CF_3 series, analog **11e** and **11f** with -84.517 and -37.781 kcal/mol respectively. The low predicted values are due to the orientation of 4-OMe and 4-SMe substitution on phenyl ring extending into the hydrophobic binding pocket resulting in non-interactions with hydroxyl group of Tyr385. The biologically active analog of –CHF_2 series, **12e** with 4-OMe substitution on phenyl ring with experimental activity of 0.064 µM has a predicted dock value of -160.034 kcal/mol.

Apart from experimental activity, a high predicted dock score next to analog **12a** are **12k** and **12g**. **12k**, a 4-Me substituted phenyl analog and **12g**, a 3-Me, 4-OMe substituted analog has a predicted dock score of -171.573 and -171.563 kcal/mol respectively. The reason can be due to the substituted hydrophobic group on phenyl ring leading to hydrophobic interactions.

Even though the hydrogen-bond interactions are less than that observed for crystal S58 ligand, **12a** exhibited relatively high dock score and binding energy. The reason can be attributed to the spatial orientation of ligand **12a** within the binding pocket and being hydrophobic interactions predominated. Interestingly, the dock score for the lowest possible energy celecoxib conformer is less than the analog **12a**. Even experimental AUC values suggest that **12a** is the better molecule to inhibit COX-2 under in vivo conditions.

The data generated for binding affinity of ligands and docking studies resulted in good correlation with experimental studies. The work identifies the orientation, geometry & pharmacophoric groups of celecoxib analogs as more important parameters in defining non-bonded interactions of ligands with receptor active site residues for selective COX-2 inhibition.

CONCLUSION

Experimental analyses have shown that **12a** is able to inhibit COX-2 to a greater extent under in vivo conditions. Our docking studies confirm that the main interaction of COX-2 inhibitors with enzyme are Hydrogen bond and Hydrophobic interactions with the binding pockets made by sulfonamide moiety and substituted pyrazole groups. These results indicate that the sulfonamide and substituted pyrazole moiety act as potential binding sites for the design of highly selective and potent COX-2 inhibitors. Further, 3D-QSAR investigations are in progress to examine possible descriptors influence on various classes of COX-2 inhibitors.

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