Research Article

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COMPUTER AIDED DESIGN AND MOLECULAR DOCKING STUDY OF 1-N-SUBSTITUTED-3, 5-DIPHENYL-2-PYRAZOLINE DERIVATIVES AS COX–2 INHIBITORS

M Prasada Rao[1*], P Srinivasa Rao[2], Allam Appa Rao[3] and Manda Rama Narasinga Rao[4]

[1,2]Department of Computer Science & System Engineering, Andhra University – 530003, Visakhapatnam, Andhrapradesh, India
[3]CR Rao advanced institute of Mathematics, Statistics and Computer Science, Central University of Hyderabad – 500046, Andhrapradesh, India
[4]Department of CSE, K L University, Vijayawada – 522303, Andhrapradesh, India

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Abstract: Cyclooxygenase (COX) catalyses the first committed step in the synthesis of prostanoids, a large family of arachidonic acid metabolites and major target of non-steroidal anti-inflammatory drugs (NSAIDs). COX-2 is the inducible isoform, rapidly expressed in several cell types in response to pro-inflammatory molecules. The interaction between the polypeptide and its corresponding receptor is highly selective. Therefore, it is of interest to inhibit COX2 in the context of inflammation. It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. The structure of COX 2 is screened using SP (Standard Precision) method under molecular docking techniques (Computer aided Design) with reference to novel 1-N-substituted-3, 5-diphenyl-2-pyrazoline derivatives. Based on their score and energy few ligands are selected to Induced Fit Docking (IFD) studies and compared with the existing drug molecules. The result showed that the docked ligands maintain favorable interactions with the active site residues of COX-2. All docking studies were performed using the molecular modeling software GLIDE of Schrödinger package.

Keywords: COX-2, Inflammation, Molecular docking, Anti-inflammatory, Drug Discovery.

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 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. Compound selection based on docking calculations alone and or combined with virtual screening has been carried out for various targets.

Cyclooxygenase (COX) is an enzyme that is responsible for formation of essential Biological mediators called prostanoids, including prostaglandins [1], [2], prostacyclin and thromboxanes. All these mediators form prostanoid class of fatty acid derivatives with variety of strong physiological effects, such as regulating the contraction and relaxation of smooth muscle tissue. Three isoforms of the COX enzyme have been characterized such as COX-1, COX-2,[3] and COX-3 which is a splice variant of COX-1[4]. Prostaglandins are produced in the inflamed tissues, and treatment with Non-steroidal anti-inflammatory drugs (NSAIDs), inhibits the production of prostaglandins [5] and down-regulates inflammation-related pathological symptoms such as pain and swelling. During inflammation, COX-1 mRNA, protein and activity levels do not change, but COX-2 levels increase dramatically, and, as a result, prostaglandin production increases. Moreover, when COX-2 specific inhibitors are administered, prostaglandin production and subsequent inflammation are significantly reduced. These data have led to the conclusion that COX-2 is involved in inflammation, whereas COX-1 is not. The COX-2 gene is particularly responsive to mediators of inflammation. Therefore, COX-2 specific inhibitors have been used to attenuate the symptoms of inflammation such as osteoarthritis, rheumatoid arthritis and musculoskeletal pain in patients [6]. NSAIDs, including aspirin, are among the most commonly recommended and prescribed drugs in the world. They have three main effects: analgesic, anti-inflammatory, and antipyretic. Unfortunately, the side effects of these drugs include delayed healing because not only are the inflammatory chemicals reduced, but the body's natural anti-inflammatory chemicals as well [7]. Selective inhibition of this enzyme overcomes the side effects associated with the traditional NSAIDs. 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 [8]. 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.

***Corresponding Author:**
M. Prasad Rao,
Department of Computer Science & System Engineering,
Andhra University-530003, Visakhapatnam,
Andhrapradesh, India

MATERIAL AND METHODS

In this comparative study, the structures were drawn by using ISIS/Draw, a chemical structure drawing program for Windows [9]. By Tsar's easy-to-use chemical spread sheet interface the limits for compounds were observed and converted 2D structures to 3D with physicochemical properties to analyze and promote activity. PYMOL is used to process the images.

Protein-ligand docking: The computational process of searching for a ligand that is able to fit both geometrically and energetically to the binding site of a protein is called molecular docking. It is a key tool in structural biology and computer-aided drug design [10] [11] to process for promising and consistent scoring scheme to evaluate the protein-ligand complex in order to select the best binding conformations. The goal of ligand and protein docking is mainly to predict the major binding mode(s) of a ligand with a protein of known three-dimensional structure [12]. Schrodinger 9.3 is used for molecular docking analysis. Receptor docking is done by Glide [Grid-Based Ligand Docking with Energetics] in Schrodinger suite [13]. Glide is an integrated platform and an efficient approach for searching conformations, orientations and positions of ligand in the receptor site using a series of hierarchical filters under standard precision method- it is a mode for reliably docking tens to hundreds of thousands of ligand with high accuracy,. Which improves the binding affinities by lowering the penalties and accomplished by more extensive sampling and advanced scoring, resulting in even higher enrichment.

Virtual screening: It is a drug designing tool in In-silico analysis. Most widely used for lead identification in drug discovery programs [14]. Due to advancement of technology and rapid growth, the experimental efforts to carry out the biological screening of many compounds are still considerably high and therefore, computer-aided drug design approaches have become attractive alternatives. The protein molecule chosen for the docking studies of is Cyclooxygenase 2 (6COX). The crystal structure of the protein is available in the PDB [15], hence, it has been taken for docking studies.

Induced Fit Docking: Glide docking uses the postulation of a rigid receptor. Although, scaling of van der Waals radii of non-polar atoms, which decrease penalties for close contacts, can be used to model a slight given in the receptor and ligand [16]. This may not be sufficient to treat systems where ligand binding induces substantial conformation changes in the receptor. Schrödinger has developed a procedure for such cases, which uses prime and Glide to perform induced fit docking. It allows the receptor to alter its binding sites so that it more closely conforms to the shape and binding mode of the ligand.

Table.1: List of existing drugs and novel 1-N-substituted-3, 5-diphenyl-2-pyrazoline derivatives.

Compounds	Molecular Formula
Existing drugs	
S58[Native Ligand]	$C_{16}H_{11}BrF_3N_3O_2S$
Dup-697	$C_{17}H_{12}BrFO_2S_2$
Rofecoxib	$C_{17}H_{14}O_4S$
Celecoxib	$C_{17}H_{15}F_3N_3O_2S$
ramifenazone	$C_{14}H_{19}N_3O$
Novel compounds	
Compound 1	$C_{18}H_{17}FN_2O_3S$
Compound 2	$C_{19}H_{17}F_3N_2O_3S$
Compound 3	$C_{25}H_{24}N_2O_4S$
Compound 4	$C_{17}H_{17}N_3O_2S_2$
Compound 5	$C_{17}H_{16}FN_3O_2S_2$
Compound 6	$C_{18}H_{19}N_3O_3S_2$
Compound 7	$C_{24}H_{23}N_3O_3S_2$
Compound 8	$C_{18}H_{18}N_2O_3S$
Compound 9	$C_{19}H_{20}N_2O_3S$
Compound 10	$C_{18}H_{17}ClN_2O_3S$
Compound 11	$C_{19}H_{20}N_2O_4S$
Compound 12	$C_{17}H_{16}ClN_3O_2S_2$
Compound 13	$C_{25}H_{24}N_2O_4S$
Compound 14	$C_{18}H_{16}F_3N_3O_2S_2$
Compound 15	$C_{17}H_{16}FN_3O_2S_2$
Compound 16	$C_{18}H_{19}N_3O_2S_2$
Compound 17	$C_{24}H_{23}N_3O_3S_2$
Compound 18	$C_{24}H_{23}N_3O_3S_2$

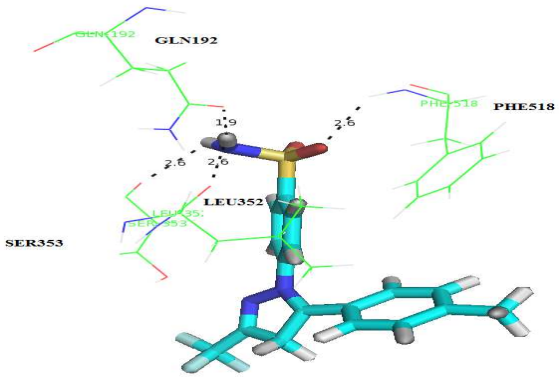
RESULTS AND DISCUSSION

Induced Fit Docking between the target protein 6COX and screened ligands 1-N-substituted-3, 5-diphenyl-2-pyrazoline derivatives were carried out using Glide, and the images were obtained using PYMOL. The following table shows the possible conformations of best ligands and native ligand along with their Docking score and Glide energy.

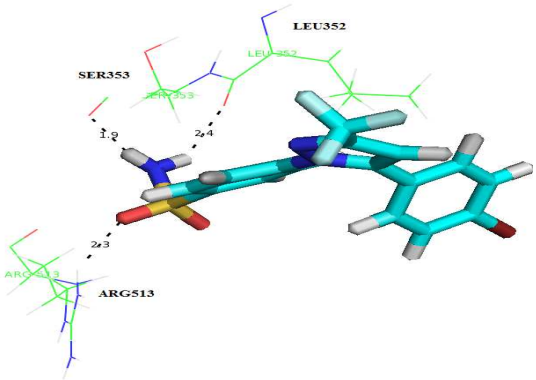
Table 2: Induced Fit Docking Results of Ligands against the Target 6COX

Compound	Interactions	Distance (Å)	Docking Score	Glide energy Kcal/mol
Celecoxib	[N- H…O]Gln192	2.811	-11.45	-59.94
	Phe518 [N- H…O]	3.364	-11.40	-55.85
	[N- H…O]Gln192	2.753	-11.11	-53.22
DuP - 697	His90 [N- H…O]	2.765	-11.60	-61.66
	Tyr 385 [O- H…F]	3.003	-9.98	-55.37
	His90 [N- H…O]	2.679	-9.97	-52.89
Sc-58	Arg153[N – H…O]	3.256	-11.23	-59.85
	Arg120[N – H…F]	2.994	-11.34	-58.07
	His90 [N- H…O]	2.794	-10.9	-59.15
Compound 8	Arg120[N – H…O]	2.849	-11.23	-62.71
	Tyr 355 [N - H…O]	3.011	-10.78	-61.57
	Phe518 [N- H…O]	3.237	-11.01	-58.95
Compound1	Arg120[N – H…O]	2.925	-11.09	-61.13
	Tyr 355 [N - H…O]	3.159	-11.05	-60.34
	His90 [N- H…O]	2.860	-10.74	-59.82
Compound 4	His90 [N- H…O]	2.773	-11.26	-62.79
	Phe518 [N- H…O]	3.148	-9.10	-57.90
	Arg120[N – H…O]	3.156	-11.91	-57.36
Compound 13	Phe518 [N- H…O]	3.030	-10.61	-61.17
	His90 [N- H…O]	2.746	-10.61	-57.97
	Arg120[N – H…O]	2.859	-11.62	-55.15
Compound 9	Arg120[N – H…O]	2.998	-11.49	-63.00
	His90 [N- H…O]	2.676	-11.04	-60.48
	Tyr 355 [O - H…O]	3.146	-11.04	-58.77
Compound 11	Tyr 355 [O - H…O]	2.894	-11.01	-61.72
	His90 [N- H…O]	2.848	-10.80	-60.85
	Arg120[N – H…O]	2.904	-10.91	-58.91
Compound 12	Arg120[N – H…O]	3.167	-11.76	-64.11
	His90 [N- H…O]	2.678	-11.29	-62.65
	Tyr 355 [O - H…O]	2.660	-11.75	-64.02
Compound 3	Arg120[N – H…O]	2.750	-10.70	-64.08
	Tyr 355 [O - H…O]	2.916	-9.32	-60.19
	Arg513[N – H…O]	2.917	-10.28	-59.88

From this analysis, with obtained results from induced fit docking, the results have been summed up as follows: the existing drugs and novel derivatives have the same common interactive residues such as Gln192, Phe518, Tyr385, His90, and Arg120. When comparing the score and energies, the existing drugs and native ligand has high docking score of -11.60 and -61.66 Kcal/mol of Glide energy. Where as in Novel derivatives, along with the existed and native ligand values, it shows greater docking score of -11.76 and the Glide energy of -64.11Kcal/mol in compound 12. Hence, The Generation of an accurate complex structure for a native ligand known to be active but that cannot be docked in an existing rigid structure of the receptor. Rescue of false negatives poorly scored true binders in virtual screening experiments, where instead of screening against a single conformation of the receptor.



A



B

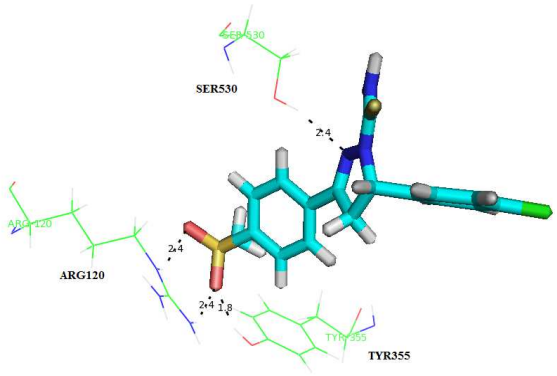


Figure.1: Image showing the Interaction poses of 6COX with ligands A) Celecoxib B) native ligand - S58 C) Compound 12-($C_{17}H_{16}ClN_3O_2S_2$).

CONCLUSION

There is an increasing interest in this study of docking analysis of 6COX protein with the compounds in table 1 have been characterized and further assessed by induced fit docking using GLIDE program in the protein active site region. The Derivatives were found to be interacting with the active site residues ARG120, GLN192, LEU352, SER353, TYR355, HIS90, and PHE518. All the ligands were energy minimized using OPLS force field. After minimization all the ligands were screened using SP [Standard Precision]. Based on the IC_{50} values, docking score and glide energy compounds 1, 3, 4, 5, 8, 9, 11, and 12 are selected for Induced fit docking.

Docking studies showed that the compound 12 has best docking score (-11.76) and Glide energy (-64.11) compared to that of the existing drug Celecoxib which has the docking score (-11.45) and Glide energy (-59.94) and the native ligand S58 which has the docking score (-11.23) and glide energy (-59.85). It also has strong hydrogen bonding interaction with the key residues of ARG120 and TYR355 which is similar to that of native ligand. The study reveals that ligand 12 has a promising inhibiting activity against Cyclooxygenase (COX-2). Further, in vitro and in vivo studies can be done for this compound to emerge the compound as potent anti-inflammatory candidate.

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