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RESEARCH ARTICLE

BIOINFORMATICS

VIRTUAL SCREENING AND MOLECULAR DOCKING ANALYSIS FOR PREDICTING THE POTENTIAL CYCLOOXYGENASE-2 INHIBITING DRUGS IN THE TREATMENT OF CANCER

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ABSTRACT

Cyclooxygenase-2 is being treated as one of the chief anti-cancer targets for colorectal, lung, breast, prostate and head/neck cancer. The focus of this study is to discover new ligand molecules, which can be used as a potential drug against Cyclooxygenase-2. The structure of Cyclooxygenase-2 of *Homo sapiens* was modeled using "MODELLER". The FDA approved and experimental level drugs are available in DrugBank3.0 database was screened against Cyclooxygenase-2 using the virtual screening facility offered by PYR-X0.8 software. Molecular docking studies were performed using AutoDock Wizard and the results were analyzed critically with the help of AutoDock tools 1.4.5. Virtual Screening and Molecular Docking Analysis revealed four molecules. Namely, N-cyclopropyl-4-methyl-3-[1-(2-methylphenyl) phthalazin-6-yl]benzamide, 6-Fluoro-2-(2'-Fluoro-1,1'-Biphenyl-4-Yl)-3-Methylquinoline-4-Carboxylic Acid, Eletriptan and Tamibarotene.

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KEYWORDS

Cyclooxygenase-2; Anti-cancer drugs; molecular modeling and docking; virtual screening.

1. INTRODUCTION

Apoptosis is an evolutionary and conserved programme mode of cell death that is critical for the maintenance of tissue homeostasis. Also, apoptosis contributes to the cytotoxic effects of standard genotoxic chemotherapy and radiotherapy. Apoptosis signaling has been tightly regulated by two main Apoptosis pathways, which are termed as 'extrinsic' and 'intrinsic'. They involve cell surface death receptors or the mitochondria and the endoplasmic reticulum [1, 2]. Both the pathways lead to the activation of specialized proteases; the caspases that cleave diverse cellular substrates, thereby fostering death execution. However, Apoptosis signaling pathways are disrupted or impaired in tumor cells resulting in Apoptosis resistance, which is one of the common traits that tumor cells acquire during malignant transformation [3].Unfortunately, the same cellular changes that allow the tumor cells to survive micro-environmental stress during tumorigenesis can cause cross-resistance to Apoptosis induction by genotoxic therapies [4, 5, 6]. Therefore, the current research concentrates on the identification of novel agents that induce cell death in tumor cells with resistance to Apoptosis induced by chemotherapy and radiotherapy or that enhance the efficacy of genotoxic therapies in tumor cells with Apoptosis resistance in order to improve the outcome of the treatment [1, 2, 7].
Cyclooxygenase-2 performs a vital role in prostaglandin biosynthesis, inflammatory cells and in the central nervous system. Prostaglandin synthesis, which is present in these sites, has a key role in the development of inflammation and hyperalgesia [8]. COX-2 is constitutively over expressed in many human pre-malignant, malignant and metastatic epithelial tumors. Some examples include colorectal **9**, lung [10], breast, prostate [11, 12], mammary tumors [13], thyroid [14] and ovarian cancer [15, 16]. Up-regulated expression of COX-2 is an early event during carcinogenesis and is mostly associated with poor prognosis as it promotes tumor cell proliferation, angiogenesis, invasion and metastasis [17, 18, 19].

The over-expression of COX-2 in RIE cells has been shown to increase the proto-oncogene Bcl-2 and lead to inhibition of Apoptosis. The inhibition of Apoptosis, a process of cell death, appears to be a key pathway in the survival of cancer cells. Experimental models have been able to reverse this inhibition of Apoptosis by using sulindac sulfide, a nonspecific COX inhibitor. This could result in increased cancer cell death and sensitization of cancer cells to chemotherapy agents [12].

The previous studies clearly demonstrate that COX-2 involves carcinogen activation, Apoptosis inhibition, tumor invasion and angiogenesis promotion. Hence, it is reasonable to say that COX-2 inhibitors can offer an important and powerful target for cancer prevention and treatment. Nowadays, many clinical trials are done using COX-2 inhibitors in the prevention and treatment of cancer [20, 21, 22]. Therefore, Cyclooxygenase-2 was fixed as a potential target in this study. A number of molecular docking analyses were performed throughout this study in order to list out the effective inhibitors against Cyclooxygenase-2.

2. MATERIAL AND METHODS

2.1 Sequence analysis

The protein sequences used in this project were isolated from Universal Protein Resource [23]. The templates used for homology modeling were obtained by running BLAST against the Protein Data Bank **(PDB)** [24]. On the basis of these hits given by BLAST, the required template PDB structures were downloaded from the protein data bank [25] and global alignment was then performed between the COX-2 sequence and the selected template. The identities between templates were retrieved in terms of the score provided by ClustalW [26].

2.2 Homology Modeling and structural analysis

The structure was modeled using effective and comparative molecular modeling software named MODELLER [27]. Modeled structures were then validated with the help of DOPE scores [28] defined by MODELLER. Later, these structures were analyzed with the help of PROCHECK [29]. All the macromolecules and ligands were viewed and analyzed with the help of two molecular viewers namely Chimera [30] and VMD [31].

2.3 Inhibitor selection and Molecular properties analysis

In order to list out effective inhibitors against Cyclooxygenase-2, modeled Cyclooxygenase-2 structures were subjected to virtual screening against all the FDA approved and experimental drugs available in Drug Bank 3.0 [32]. Drug-likeness of the compounds was evaluated on the basis of Lipinski rule of 5 [33] based on the data available in Drug Bank 3.0 Database.

2.4 Virtual screening & Molecular Docking studies

Virtual screening is a computational technique used in drug discovery research. It involves the rapid Computational assessment of large libraries of chemical structures in order to identify those structures which are most likely to bind to a drug target. PyRx is a virtual screening software for Computational Drug Discovery (CDD), which can be used to screen libraries of compounds against potential drug targets [34]. It uses a large body of already established open source software such as AutoDock 4 [35] and AutoDock Vina. These two are used as Docking software. Python was used as a programming/scripting language. Open Babel was used for importing SDF files, removing salts and energy minimization. Finally, selected FDA approved and experimental category drugs, utilized for the purpose of virtual screening, were energy minimized using the steepest decent method [36] with MMFF94 force field [37].

3. RESULTS AND DISCUSSION

3.1 Molecular Modeling of Cyclooxygenase-2:

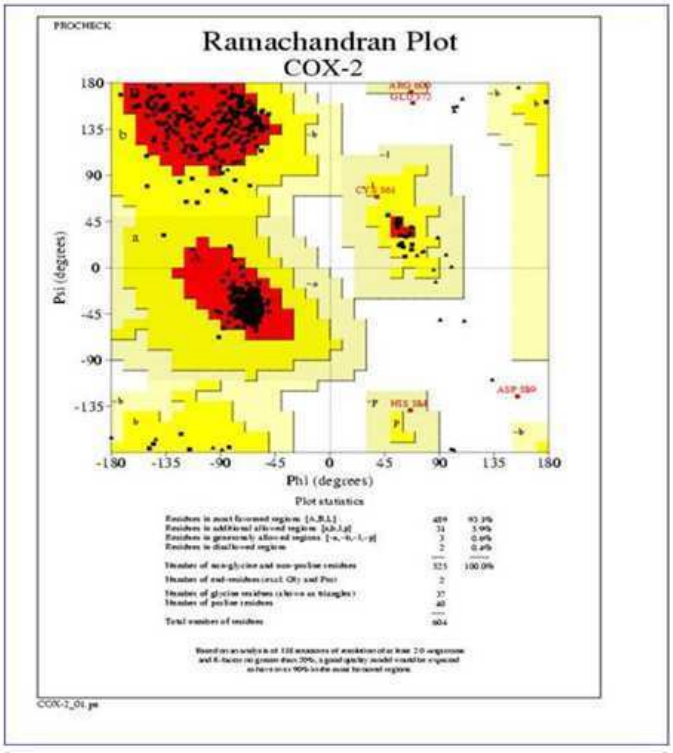
The sequence of Cyclooxygenase-2 was retrieved from Universal Protein Resource (UniProt) and its corresponding sequence id was **P35354.** It consists of 604 amino acids. This sequence was subjected to similarity search against Protein Data Bank, using the BLAST tool offered by NCBI. Later, the templates were selected on the basis of structural hits and its alignment pattern against the query sequence. The selected templates were as follows: chain A of 1PXX [38], chain A of 1DDX [39] and chain P of to 2OYE [40]. Templates and their identity with the Cyclooxygenase-2 sequence are defined in Table 1.

Table 1.
Templates used in Molecular modeling

S.NO	Template(PDB)	Chain	Length	Identity score with cox-2 seq
1	1PXX	A	604	86.00%
2	1DDX	A	552	88.00%
3	2OYE	P	600	59.00%

The advanced modeling tutorial package offered in MODELLER was utilized for comparative molecular modeling. Initially, Cyclooxygenase-2 sequence was converted into MODELLER input file format (.ali). Multiple sequence alignment was done using salign.py script and align_2d.py scripts and the molecular modeling was done using model-multi.py scripts. Among them, the best modeled structure was chosen with the help of a DOPE (Discrete optimized protein energy) score. The DOPE score belonging to the best modeled structure was -69897.460938. The stereo-chemistry qualities of the structures were validated with PROCHECK structural validation tool. PROCHECK results clearly indicated the higher fidelity of modeled Cyclooxygenase-2 structure (Fig.1).

Figure 1
PROCHECK structure validation-plot



3.2 Virtual-screening

Energy minimization with universal force field [41] was done to the modeled Cyclooxygenase-2 structure using Steepest Decent method. FDA approved and experimental drugs from DrugBank3.0 were downloaded and were brought to their most stable configuration. These compounds were converted into the input-file format, namely PDBQT. During this process, drugs that had not been properly minimized and those not supported for conversion were eliminated from the list. The modeled structure was fixed as a potential target for virtual screening. Finally, virtual-screening studies were performed for all the converted drug components against modeled Cyclooxygenase-2 structure, using Vina Wizard available in PYRX-0.8 software. In the end, efficiency of all the ligands was analyzed using binding energy value predicted by PYRX-0.8 software. Binding energy is nothing but the sum of the intermolecular energy and the torsional free-energy penalty, with a more negative binding energy representing a stronger inhibition. Virtual-screening results are given in the supplementary Tables A1 and A2.

Initially, compounds described as a 'potential drug' against Cyclooxygenase-2 by UniProt Knowledgebase database were retrieved from DrugBank 3.0. And energy minimization with MMFF94 force field was done to ligand structures using Steepest Decent algorithm. Later, compounds were subject to virtual screening and the results are described in table 2. The selected compounds and their Drugbank3.0 IDs are as follows: Acetaminophen (DB00316), Aspirin (DB00945), Balsalazide (DB01014), Bromfenac (DB00963), Carprofen (DB00821), Celecoxib (DB00482), Ciclopirox (DB01188), Diclofenac (DB00586), Diflunisal (DB00861), Epoprostenol (DB01240), Etodolac (DB00749), Etoricoxib (DB01628), Fenoprofen (DB00573), Flurbiprofen (DB00712), gamma-Homolinolenic acid (DB00154), Ibuprofen (DB01050), Icosapent (DB00159), Indomethacin (DB00328), Ketoprofen (DB01009), Ketorolac (DB00465), Lenalidomide (DB00480), Lumiracoxib (DB01283), Meclofenamic acid (DB00939), Mefenamic acid (DB00784), Meloxicam (DB00814), Mesalazine (DB00244), Nabumetone (DB00461), Naproxen (DB00788), Oxaprozin (DB00991), Phenylbutazone (DB00812), Rofecoxib (DB00533), Salicyclic acid (DB00936), Salsalate (DB01399), Sulindac (DB00605), Suprofen (DB00870), Tenoxicam (DB00469), Thalidomide (DB01041), Tiaprofenic acid (DB01600), Tolmetin (DB00500) and Valdecoxib (DB00580),

TABLE 2
virtual screening of drugs currently used in medication.

S.No	Ligand	Target	Binding Energy	Molecular weight
1	DB00991	COX-2	-8.5	293.31
2	DB00482	COX-2	-8.4	381.37
3	DB01399	COX-2	-8.4	258.22
4	DB00605	COX-2	-8.3	356.41
5	DB00861	COX-2	-8.3	250.19
6	DB00939	COX-2	-8.3	296.14
7	DB00465	COX-2	-8.2	255.26

8	DB00328	COX-2	-8.1	357.78
9	DB00500	COX-2	-8	257.28
10	DB01600	COX-2	-8	260.3
11	DB00480	COX-2	-7.9	259.26
12	DB01628	COX-2	-7.9	358.84
13	DB00580	COX-2	-7.8	314.35
14	DB00784	COX-2	-7.8	241.28
15	DB01041	COX-2	-7.8	258.22
16	DB00586	COX-2	-7.7	296.14
17	DB00814	COX-2	-7.7	351.4
18	DB01240	COX-2	-7.7	352.46
19	DB00788	COX-2	-7.6	230.25
20	DB01014	COX-2	-7.6	357.31
21	DB01283	COX-2	-7.6	293.72
22	DB00533	COX-2	-7.5	314.35
23	DB00870	COX-2	-7.5	260.3
24	DB01009	COX-2	-7.5	254.28
25	DB00469	COX-2	-7.4	337.37
26	DB00812	COX-2	-7.4	308.37
27	DB00821	COX-2	-7.3	273.71
28	DB00963	COX-2	-7.3	334.16
29	DB00749	COX-2	-7.1	287.35
30	DB01050	COX-2	-7.1	206.28
31	DB00461	COX-2	-7	228.28
32	DB00712	COX-2	-6.9	244.26
33	DB00573	COX-2	-6.6	242.26
34	DB00316	COX-2	-6.5	151.16
35	DB01188	COX-2	-6.5	207.26
36	DB00936	COX-2	-6.4	138.12
37	DB00945	COX-2	-6.4	180.15
38	DB00154	COX-2	-6.3	306.48
39	DB00244	COX-2	-6.3	153.13
40	DB00159	COX-2	-6	302.45

Among these 40 compounds, Celecoxib (DB00482) and Oxaprozin (DB00991) showed significant binding energy when compared to all other compounds, these drugs are already proposed as selective Cyclooxygenase-2 inhibitors [42, 43]. Hence, compounds exhibiting more binding energy than Celecoxib and Oxaprozin will be focused in the forthcoming analysis. Most of the drugs specific to Cyclooxygenase-2 were reported to cause a higher risk of heart attack and stroke [44]. Hence, the importance of searching new drugs against COX-2 becomes obvious.

Totally, 1480 FDA approved drugs were retrieved in the form of structural data files from the DrugBank 3.0. Among them, compounds which lacked proper coordinates were eliminated; finally 1333 compounds were subject

to virtual-screening. Likewise, 4116 compounds were selected from 5211 experimental drugs.

These 5211 experimental drugs were then filtered on the basis of physio-chemical property analysis. Small molecules having a molecular weight of more than 500 Daltons were not considered. Though a number of anti-bacterial and anti-fungal drugs showed a higher binding energy against Cyclooxygenase-2, yet, because of their higher molecular weight, they were neglected. Compounds that were directly related to the central nervous system (CNS) and steroidal drugs were also rejected, due to their high impact on other metabolic processes.

Through a detailed analysis of the characteristics of each ligand used in virtual screening, compounds having low molecular weight with better binding energy were selected from each category (approved and experimental) and considered for further study.

3.3 Molecular Docking studies:

In conclusion, selected compounds were subject to molecular docking analysis using AutoDock Module, which is available in PYRX-0.8 software. In the AutoDock Module, molecular docking was performed using Genetic algorithm parameters with a maximum of 25,00,000 energy evaluations. Later, results were analyzed with the help of Autodock tools 1.4.5. The interactions between the ligand and the target are given in figures 2 & 3. The amino acids interact with drugs are exhibiting remarkably enhanced binding affinities with Cyclooxygenase-2. The higher affinity of these small molecules is presumably attributed to the formation of hydrogen bonds. The hydrogen bond between drugs and Cyclooxygenase-2 are highlighted as green color beads (Fig.2 and fig.3).

Nearly 100 small molecules were treated in molecular docking studies, and from among them, the best compounds are listed in Table 2. Especially, Celecoxib and Oxaprozin performs a better role when compared to other small molecules, the inhibition rate of these two compounds being highly affordable, This is obvious from the binding energy and hydrogen bonds formed between Celecoxib, Oxaprozin and the enzyme. On the other hand, there were a few compounds which exhibited a stronger inhibition when compared to the above mentioned drugs. Eletriptan (DB00216) and Tamibarotene **(**DB04942**)** among the approved category and N-cyclopropyl-4-methyl-3-[1-(2-methylphenyl), phthalazin-6-yl] benzamide (DB07307) in the experimental category showed maximum inhibition than all the other compounds. Even the supporting information associated with DB07307 in the DrugBank database stated its possible role in Apoptosis. 6-Fluoro-2-(2'-Fluoro-1,1'-Biphenyl-4-Yl)-3-Methylquinoline-4-Carboxylic acid (DB03523) from the experimental category also exhibited considerable inhibition. The interaction between the ligand and target are highlighted in figure 2, in which positions and structures of drug molecules are represented as a surface model, and the amino acids interacting with drugs are shown as a wire form model (Fig2 and 3). The predicted binding energy is listed in table 3.

Inhibitatory constant represents the concentration of a drug that is required for 50% inhibition of activity of the target. The lower Inhibitatory constant is more promising one for the inhibitors.

In AutoDock Inhibitatory constant is calculate using following formula:

Ki=exp((deltaG*1000.)/(Rcal*TK)

where **deltaG** is docking energy, **Rcal** is 1.98719 and **TK** is 298.15

The ligand efficiency is defined as the calculated pKi divided by the number of heavy atoms in the ligand. An affordable ligand must possess the ligand efficiency in negative. refRMS is rms difference between current conformation coordinates and current reference structure. By default the input ligand is used as the reference.

Figure 2
Interaction between drugs and Cyclooxygenase-2 (FDA-Approved drug)

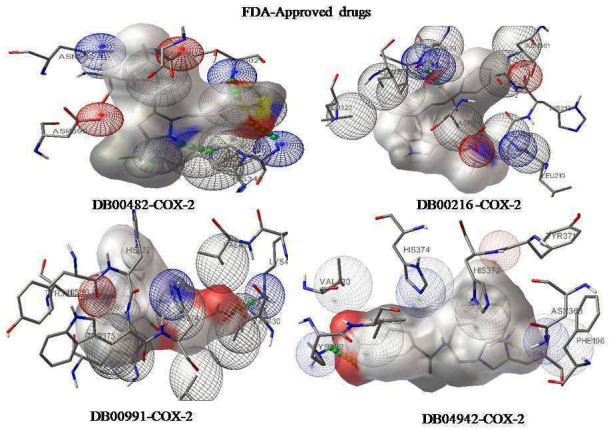


Figure 3
Interaction between drugs and Cyclooxygenase-2 (Experimental category drugs)

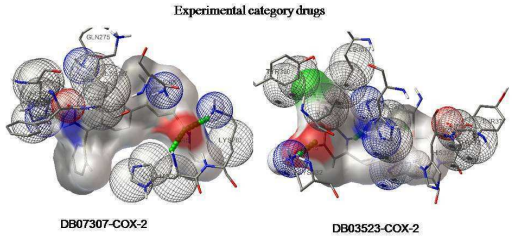


Table 3
Molecular Docking Results

S.No	Drugs	Inhibitory constant	Binding Energy	Ligand Efficiency	Ref RMS	Hydrogen Bond	Molecular Weight (daltons)
			FDA-Approved drugs				
1	DB00482	8.41	-6.92	-0.27	33.73	LYS 82 THR 603 HIS 342	381.37
2	DB00991	3.6	-7.43	-0.34	37.34	LYS 432	293.31
3	DB00216	6.5	-7.08	-0.26	47.18	VAL 524	382.51
4	DB04942	1.25	-8.05	-0.31	38.05	LYS 432	351.43
			Experimental drugs				
1	DB07307	2.37	-7.67	-0.26	46.12	HIS 200 LYS201	393.48
2	DB03523	1.47	-7.96	-0.28	40.97	GLN 189 LYS 432	375.36

3.4 Drug-likeness of selected compounds

The drug-likeness of the compounds was verified by a detailed analysis of the properties of drugs, chiefly properties defined in Lipinski's Rule of 5. They were as follows: Not more than 5 hydrogen bond donors; not more than 10 hydrogen bond acceptors; and molecular weight should not be greater than 500 Daltons. The structure of the finally selected drugs is given in figure 4A and 4B. The Drug-likeness of selected compounds is given in Table 4.

Figure 4 A &4B
structure of the finally selected drugs

Figure 4 A

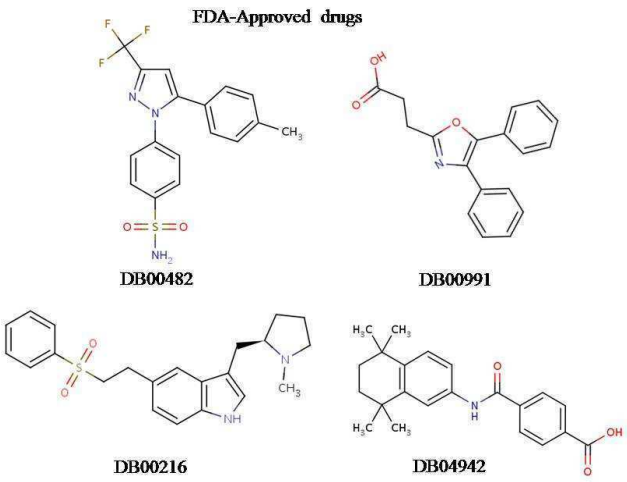


Figure 4 B

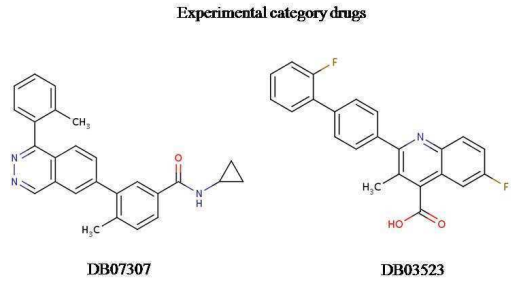


Table 4
Drug-likeness of finally selected compounds

S.No	Drugs	Log P	H-bond Acceptor	H-bond Donor	Molecular Weight (dalton)
FDA-Approved drugs					
1	DB00482	3.99	3	1	381.37
2	DB00991	3.46	3	1	293.31
3	DB00216	3.9	3	1	382.51
4	DB04942	4.99	3	2	351.43
Experimental drugs					
1	DB07307	4.77	3	1	393.48
2	DB03523	5.05	3	1	375.36

4. CONCLUSION

Virtual-screening is an emerging approach and is extensively used to reduce cost, and time in drug discovery. The approach utilized in this study was successful in searching small molecules that could act as a potential drug against Cyclooxygenase- 2 using Molecular Docking studies, compounds were screened on the basis of inhibitory constant, ligand efficiency, lowest binding energy with considerable hydrogen bonds. Hydrogen bonding plays an important role in the structure and function of biological molecules, mainly for inhibition in a complex. Hence, the compounds N-cyclopropyl-4-methyl-3-[1-(2-methylphenyl)phthalazin-6-yl]benzamide, 6-Fluoro-2-(2'-Fluoro-1,1'-Biphenyl-4-Yl)-3-Methylquinoline-4-Carboxylic Acid , Eletriptan and Tamibarotene are strongly recommended for further clinical trials owing to their high potential to act against Cyclooxygenase-2 in the treatment of cancer.

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