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Computer Aided Drug Design and Molecular Docking Studies on a Series of 1, 5 Dihydro Benzothiazepines as New Class of Cox-2 Inhibitors

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Conflicts of Interest: Nil

Abstract

Molecular docking study was performed on a series of 20 new 1,5-dihydro benzothiazepines BTP1-BTP20 as Cyclooxygenase inhibitors. The docking technique was applied to dock a set of representative compounds within the active site region of 3n8v using pyrx virtual screening tool and evaluation of these molecules is done by using the pymol and chimera software. For these compounds, the binding affinity (kcal/mol) was determined. The docking score of BTP11 is -10.7 kcal/mol and is compared with the standard drug nimesulide and it's docking score is -7.4 kcal/mol. Based on the validations and hydrogen bond interactions made by R substituents were considered for evaluation. The results avail to understand the type of interactions that occur between designed ligands with 3n8v binding site region and explain the importance of R substitution on 1,5 dihydro benzothiazepine basic nucleus.

Keywords:

Docking, 1,5-dihydro benzothiazepine, cyclooxygenase, pyrx virtual screening tool.

Introduction:

Drug discovery and development is an interdisciplinary, expensive and time consuming process. Scientific technology advancements during the past two decades have changed the approach of the pharmaceutical research to generate novel bioactive molecules. Advances in computational techniques and in parallel hardware support made easy evaluation of molecules, and in particular structure-based drug design method, to speed up new target selection through the identification of hits to the optimization of lead compounds in the drug discovery process. Genomics, proteomics, bioinformatics and chemoinformatics have gained immense popularity and have become an integral part of the industrial and academic research, directing drug design and discovery. Virtual screening emerged as an important tool in our quest to access novel drug like compounds 1-3. Rational drug design can be done in two ways: ligand-based or structure-based. With the availability of the 3D structure of a biological target, it is feasible to use a structure-based approach to evaluate and predict the binding mode of a ligand within the active site of the receptor with docking methods 4-8. Now it is a popular technique used for increasing the speed of drug designing process. This was made possible by the availability of many protein structures which helped in developing tools to understand the structure function relationships, automated docking and virtual screening. Furthermore, when no 3D structural information about target proteins with their receptor site is available ligand-based design is applied 9-12. The ligand-based approach starts with a group of ligands binding to the same receptor with the same mechanism. Today four

different strategies based on the prior knowledge of the targets 3D structure and the ligands binding to it are predominant. Cox it is also called as prostaglandin endoperoxide synthase and it plays an important role in the biosynthesis of prostaglandins, the mediators of pain and inflammation and which are derivatives of eicosanoids and plays pivotal role in counteracting Zollinger- ellison syndrome.

Materials and Methods:

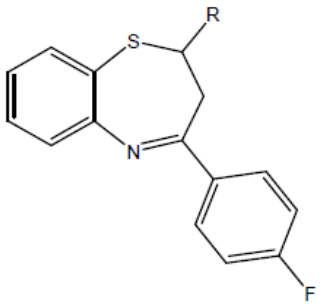
Software Methodology:

In the present molecular docking study, software PYRX virtual screening tool along with Graphical User Interface (GUI), pyrx tools was utilized to generate grid, calculate dock score and evaluate conformers. PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process - from data preparation to job submission and analysis of the results. While it is true that there is no magic button in the drug discovery process, PyRx includes docking wizard with easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design. PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for Rational Drug Design. Please visits http://pyrx.sourceforge.net to learn more about PyRx.

Molecular Modeling:

A set of 20 new 1,5-Dihydro benzothiazepines BTP1-BTP20 listed in table 1, were designed and modeled based on the compounds synthesized and reported earlier . In the present study, para isomers have been constructed and subjected for molecular docking experiments. However, certain chemical rules are utilized to prevent unreasonable structures during molecular design. For instance, structures that include heteroatoms bonded to each other (e.g. O-O, N-N and N-O etc.) and eliminating too many heteroatoms bonded to the same carbon atom. Also, certain fragments attached to an aromatic ring possess toxicity.

General structure of 1,5-dihydro benzothiazepine



LIGAND CODE	R Group substituent	BINDING AFFINITY (KCAL/MOLE)	No of H-bonds/H-bondinteracting residues
BTP1	4-MeC6H5	-9.9	Interacted with non-bonded atom
BTP2	4-FC6H5	-9.6	Interacted with non-bonded atom
BTP3	4-ClC6H5	-9.9	Interacted with non-bonded atom
BTP4	2-clC6H5	-9.2	Interacted with non-bonded atom
BTP5	2,4-FC6H4	-9.6	Interacted with non-bonded atom
BTP6	2,4-ClC6H4	-9.5	Interacted with non-bonded atom
BTP7	**2-Cl-4-NO2C6H4**	**-10.4**	**2/PRO-538(1),ASN375(1)**
BTP8	**3-NO2C6H5**	**-10.2**	**2/ASN-375(1),ARG-374(1)**
BTP9	4-NO2C6H5	-10	Interacted with non-bonded atom
BTP10	3-OHC6H5	-9.4	Interacted with non-bonded atom
BTP11	**3-NO2-4-MeC6H4**	**-10.7**	**3/ARG-376(1),ARG374(2)**
BTP12	3,4,5-Tri-OMeC6H4	-9.1	Interacted with non-bonded atom
BTP13	**4-diMe-amino-C6H4**	**-10.4**	**2/LEU-224(1),SER-143(1)**
BTP14	2-bromo furyl-C4H4	-9.3	Interacted with non-bonded atom
BTP15	4-dimethylamino-C6H4	-9.5	Interacted with non-bonded atom
BTP16	**3-OMe-4-OH-C6H4**	**-9.6**	**2/SER143(2)**
BTP17	**-1-PYRIDINYL**	**-9.2**	**1/LEU-224(1)**
BTP18	-2-PYRIDINYL	-9.5	Interacted with non-bonded atom
BTP19	-3-PYRIDINYL	-9.3	Interacted with non-bonded atom
BTP20	-1-THIOPHENYL	-8.8	Interacted with non-bonded atom

Ligand Preparation:

The structures of 1,5 Dihydro benzothiazepines BTP1-BTP20 were drawn using MOL-EDITOR website and that files are saved in the form of sdf file format. finally subjected to energy minimization using virtual screening tool. The minimization was executed until the root mean square gradient value reached a value smaller than 0.001kcal/mol. Such energy minimized structures are considered for docking in the pyrx virtual screening tool.

Protein Selection:

The selection of protein for docking studies is based upon several factors i.e. structure should be determined by X-ray diffraction, and resolution should be between 2.0-2.5A^{o}, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure. However, we considered ramachandran plot statistics as the important filter for protein selection that none of the residues present in disallowed regions.

The protein that should be selected should meet requirements of docking studies and it should be downloaded in the form of pdb format.

Following are few points to keep in mind while selecting a protein structure from PDB

1. Resolution must be minimum possible (This will ensure the better quality of protein structure.)

2. Domain completeness. Examine your PDB structure and confirm the under study domain full structure availability. Partial domain will lead to false interpretations.

3. Variant /Mutations. According to your case study look for whether your structure is a wild type or a mutant/variant. In case of a mutant structure requirement you may have to introduce required mutations manually and model them.

4. Side Chain Completeness (is of secondary importance). Structures determined through old techniques might have (Not always) missing side chains due to flaw in tech or manual error. Right 3D confirmation of Side chains is critical in small ligand binding thus ensure their completeness. As a possible solution you may look for latest structure availability of the same.

5. Ligand /Crystalline Water / Co factor presence. To get out the right docking result removal (As per case study) of these elements form PDB file is important.

Protein Preparation:

All COX X-ray crystal structures were obtained from the brookhaven protein Data Bank (http://www.rcsb.org/pdb). Subsequent to screening for the above specific standards the resultant protein target (PDB Code: 3n8v) was selected and prepared for molecular docking simulation in such a way that all heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.) were removed.

Software Method Validation:

Software method validation was performed in PYRX VIRTUAL SCREENING TOOL using Protein Data Bank (PDB) protein 3n8v. The x-ray crystal structure of 3n8v complex with co-crystallized ligand was recovered from PDB. The bio active co-crystallized bound ligand was docked with in the active site region of 3n8v. The resolution of 3n8v is 3.05A0 and R value free is 0.235 and R value work is 0.203 indicating that the parameters for docking simulation are good in reproducing X-ray crystal structure.

Molecular Docking:

In the present investigation, we make use of a docking algorithm called molecular docking. Molecular docking is based on a new hybrid search algorithm, called guided differential evolution. The guided differential evolution algorithm combines the differential evolution optimization technique with a cavity prediction algorithm. We used pyrx virtual screening tool because it showed higher docking accuracy than other stages of the docking products (MVD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%) in the market 20, 21. coordinates in either sdf or PDB format. Non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Molecular docking was performed using Molecular docking engine of pyrx software. The binding site was defined as a spherical region which encompasses all protein atoms within 15.0

Ao of bound crystallographic ligand atom. Default settings were used for all the calculations. Docking was performed using a grid resolution.

Note: The Greater Is the Negative Binding Affinity the Greater Is The Activity Of The Molecule Towards The Particular Protein

Results & Discussions:

Ligand-Protein Inverse Docking (LPID) approach has been used as a useful tool in facilitating drug design. In this approach, docking single or multiple small molecules in single or multiple conformations to a receptor site is attempted to find putative ligands. A number of flexible docking algorithms have been introduced. These include multiple-conformer shape matching, genetic algorithm, evolutionary programming, simulated annealing, fragment-based docking, and other novel algorithms. Testing results have shown that these algorithms are capable of finding ligands and binding conformations at a receptor site close to experimentally determined structures. Because of their capability in identifying potential ligands and binding conformations, these algorithms are expected to be equally applicable to an inverse-docking process for finding multiple putative protein targets to which a small molecule can bind or weakly bind. This may be applied to the identification of unknown and secondary therapeutic targets of drugs, drug leads, natural products and other ligands. LPID approach is now applied to the database of 20 compounds in the present study for finding 'best fit' (hit identification) against COX-1. The compound with least binding energy against target protein is considered for further study. By this means, it is possible to understand how the compounds interact with the target protein. The results emerging out of this study can be used to identify the binding properties of compounds synthesized in the present study.

The ligand-protein inverse docking simulation technique was performed using with 20 designed Benzothiazpines BTP1-BTP25 basic benzothiazepine moiety reported to be having cyclooxygenase inhibitory activity. Docking simulations with 3n8v bound ligand BTP resulted in a binding affinity docking score of -10.7 kcal/mol showed hydrogen bond interactions with in the active binding site region Docking studies on experimental compounds (Table 1) showed that most of the compounds are involved in hydrogen bonding with residues **ARG-376,ARG374** the binding site region of 3n8v. Therefore, although other H-bond interactions exist, these hydrogen bonds are relevant for the binding activities of benzothaizepes to be highly selective and potent cyclo oxygenase inhibitors. Moreover, from the data given in (Table 1), it appears that the residues btp11 represent most significant residue for binding diverse range of compounds. The important residue that participates in H-bond interactions was recognized by our studies on experimental compounds. Therefore, this approach appears to be useful in predicting key interacting ligand binding residue. Hence interaction with **ARG-376, ARG374** which is common interacting residue among all the compounds with stable binding conformations as seen in case of compounds such as BTP7, BTP8, BTP11 and BTP13 (Fig 1) with binding affinity score i.e. least binding scores -10.7, -10.4, -10.2 and -10.4 kcal/mol respectively (Fig 1)

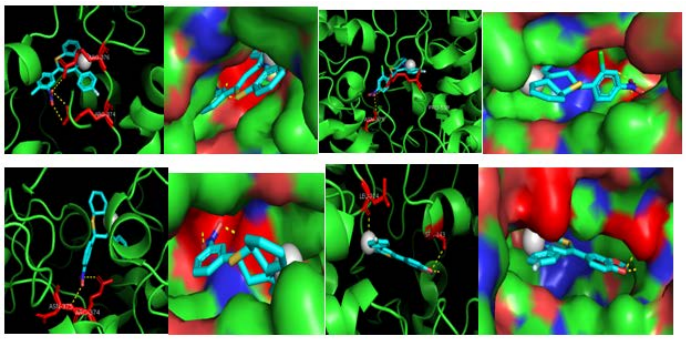


Fig 1: Shows binding mode and hydrogen bond interactions of most stable ligands (Yellow, Pink) in the binding site region of cox-1. The side chains of the residues are shown in stick model. Red ribbon represents the secondary structure of the protein.

Conclusions:

In this study the ligand-protein molecular docking simulation was used to preliminarily investigate and to confirm the potential molecular target for the designed ligands BTP1-BTP20. The analysis of the best docked ligands against selected target revealed the binding mode of compounds involved in this study and confirm the role as cyclooxygenase inhibitors. Binding energies of the drug–enzyme (receptor) interactions are important to describe how fit the drug binds to the target macromolecule. The residues participated in the hydrogen bond formation within the active binding site region revealed the importance of these residues towards the observed binding energy with respect to the hit identified against cyclooxygenase target protein. The obtained hypothesis could be the remarkable starting point to develop some new leads as potential cyclooxygenase inhibitors with enhance the affinity as well as intrinsic activity. The results of this work indicate efficient computational tools are capable of identify potential ligands such as BTP7,BTP8,BTP11 and BTP13, even though their biological profile has not known. The utilization of computational tools in the drug discovery and development can be used to save time and reduce the bench work of a medicinal chemist.

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