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COMPUTATIONAL MOLECULAR DOCKING OF CPT-11 LIGAND (IRINOTECAN) TO THE ACTIVE SITE OF ACETYLCHOLINE ESTERASE RECEPTOR USING AUTODOCK 3.0 SOFTWARE: A REVIEW ON COMPUTER AIDED DRUG DESIGN

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Abstract

Drugs are small molecules specifically designed to bind, interact, and regulate the activity of biological receptors. Receptors are proteins that bind and interact with other molecules to perform the numerous functions required for the maintenance of life. They include an immense collection of cell- surface receptors, enzymes, and other functional proteins. The role of drugs is to correct the functioning of the receptors to remedy the resulting medical condition. The process of drug discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. The methodology of Computer-Assisted Drug Design (CADD) is to identify highly potent and specific drugs using only computational methods and structural information on the target protein. Three-dimensional molecular structure is one of the foundations of structure-based drug design. Computational molecular docking is a research technique for predicting whether one molecule will bind to another, usually a protein. Protein-ligand docking is done by modeling the interaction between protein and ligand, if the geometry of the pair is complementary and involves favorable biochemical interactions, the ligand will potentially bind the protein in vitro or in vivo. This study involves molecular docking of Acetylcholine esterase receptor with Carbonyloxycamptothecin (CPT-11) ligand which is a potential inhibitor.

Keywords: *Computer aided drug design (cadd), docking, ligand, receptor, acetylcholine esterase cpt-11, autodock.*

1.1 Introduction

Computer-assisted drug design (CADD) also called represents recent applications of computer as tools as drug design process. In this method attempts are made to find a ligand or putative drug that will intereact favourably with a receptor that represents the target site. Binding of ligand to the receptor may include hydrophobic, electrostatic, and hydrogen-bonding interactions. In addition, solvation energies of the ligand and receptor site also are important because partial to complete desolvation must occur prior to binding. This approach to CADD optimizes the fit of a ligand in a receptor site. However, optimum fit in a target site does not guarantee that the desired activity of the drug will be enhanced or that undesired side effects will be diminished. Moreover, this approach does not consider the pharmacokinetics of the drug. The approach used in CADD is dependent upon the amount of information that is available about the ligand and receptor. The ligand-based approach is applicable when the structure of the receptor site is unknown, but when a series of compounds have been identified that exert the activity of interest. To be used most effectively, one should have structurally similar compounds with high activity, with no activity, and with a range of intermediate activities. In recognition site mapping, an attempt is made to identify a pharmacophore, which is a template derived from the structures of these compounds. It is represented as a collection of functional groups in three-dimensional space that is complementary to the geometry of the receptor site. In applying this approach, conformational analysis will be required, the extent of which will be dependent on the flexibility of the compounds under investigation.

One strategy is to find the lowest energy conformers of the most rigid compounds and superimpose them. Conformational searching on the more flexible compounds is then done while applying distance constraints derived from the structures of the more rigid compounds. Ultimately, all of the structures are superimposed to generate the pharmacophore. This template may then be used to develop new compounds with functional groups in the desired positions. In applying this strategy, one must recognize that one is assuming that it is the minimum energy conformers that will bind most favorably in the receptor site. The receptor-based approach to CADD applies when a reliable model of the receptor site is available, as from X-ray diffraction, NMR, or homology modeling. With the availability of the receptor site, the problem is to design ligands that will interact favorably at the site, which is a docking problem. The techniques most often used to refine drugs are combinatorial chemistry and structure based design. Combinatorial chemistry is a synthetic tool that enables chemists to rapidly generate thousands of lead compound derivatives for testing. A scaffold is employed that contains a portion of the ligand that remains constant. Subsite groups are potential sites for derivatization. These subsites are then reacted with combinatorial libraries to generate a multitude of derivative structures, each with different substituent groups. By carefully selecting libraries based upon the study of the active site, we can target the derivatization process towards optimizing ligand receptor interaction.

1.2 Molecular Docking

Three-dimensional molecular structure is one of the foundations of structure-based drug design. Often, data are available for the shape of a protein and a drug separately, but not for the two together. *Docking* is the process by which two molecules fit together in 3D space. In addition to docking, the atomic affinity grids can be visualized. This can help, for example, to guide organic synthetic chemists design better binders. Consider an active site on a usually large receptor molecule, and a ligand molecule, which could be small or large. The general question is how snugly the ligand fits into the active site. Quality of fit has a geometric and a chemical component. The geometric component measures how well the surface shapes complement each other as a hand in glove. The chemical component measures how well the secondary forces between ligand and receptor atoms hold the two together. In the simplest version of the problem, both ligand and receptor are assumed to be rigid bodies, and the objective is to find a best alignment of the surfaces describing their boundaries. Docking functions are believed to be the essential component of docking algorithms. The approach to study both molecular mechanics and statistical potentials are applied. It is found that the statistical potential evaluated is less effective than the Assisted Model Building with Energy Refinement (AMBER). Molecular mechanics function provide an accurate description of the docking process when the exact experimental coordinates are used.

1.2 Acetyl Choline Esterase

Acetylcholinesterase is one of the most efficient enzymes known, whose primary function is believed to be termination of the action of the neurotransmitter acetylcholine (ACh).

A great leap forward in the understanding of the catalytic mechanism, and mode of action of inhibitors, came in 1991 with the determination of the three dimensional structure of dimeric *T. californica* AChE [8]. The active site was found to be located 20Å from the enzyme surface at the bottom of a narrow gorge, lined with 14 aromatic residues, which may be important in guiding the substrate to the active site. There was no discernible 'anionic' site, the quaternary nitrogen of choline binds chiefly through interactions with the pi electrons of the residue Trp-84. The structures of AChE with the bound inhibitors; decamethonium, tetrahydroaminoacridine (Tacrine) and edrophonium [9] and 1,5-*bis*(4-allyldimethylammoniumphenyl) pentan-3- one dibromide have been determined. These show that ligands binding at the peripheral site also do so by interaction with pi electron in this instance with the residue Trp-279.

1.3 Irinotecan (CPT-11)

Irinotecan (CPT-11) is an anticancer drug that occasionally produces acute cholinergic side effects. Preliminary findings suggest that these are mediated through the inhibition of acetyl cholinesterase (AChE). The active site of AChE is present at the bottom of a gorge that is lined with hydrophobic amino acid residues [9]. Hence, hydrophobic molecules are attracted into the gorge where catalysis occurs. Because CPT-11 has a very hydrophobic planar aromatic ring structure, the drug would be expected to localize within the active site gorge. This suggests a direct interaction of the drug or its metabolites with acetylcholinesterase (AChE). Kinetic studies indicated that CPT-11 was primarily responsible for AChE inhibition with the 4-piperidinopieridine moiety, the major determinant in the loss of enzyme activity.

2.1 Methodology

2.1.1 Retrieval of receptor complex from protein data bank (PDB)

Receptor complex was downloaded from PDB and active site of the domain was found using CAST p. (Computer Atlas of Surface Topography of proteins)

2.1.2 Separation of receptor and ligand

Receptor and ligand are separated from the complex using DS View Pro Software. The separated receptor and ligand are saved as separate files.

2.1.3 Docking

AUTODOCK is a script driven program to dock flexible ligands to a crystallographic protein structure. Ligand (cpt-11) will be docked to the active site of the enzyme acetylcholoine esterase using autodocking. Auto grid has been used to calculate affinity grid values around the target molecule. This is done by specifying program path name and opening the gpf file. Then autogrid is launched for run. When Autogrid execution got over, auto dock is started by selecting the filename of receptor and also choosing the ligand. After this Genetic Algorithm Parameters and Docking Run Parameters is set. Ligand file is saved with dpf extension (docking parameter file). Once the runs are completed, the software will begin analysis phase and records details of the process.

2.1.4 Analysation of autodocking

If there is more than one molecule with conformations in the viewer, then it will be shown by histogram. The similarity of docked structures is measured by computing the root-mean-square- deviation(RMSD) between the coordinates of the atoms. The docking results consist of the PDBQ of the Cartesian coordinates of the atoms in the docked molecule, along with the state variables that describe the docked conformation and position. This docked structures will be visualized as spheres. This represents each docked conformation by a sphere. A sphere is placed at the average position of the coordinates of all the atoms in each conformation. A clear overview of the distribution of the docked results are taken by changing the radii of the spheres, their color and their smoothness.

3.1. Results

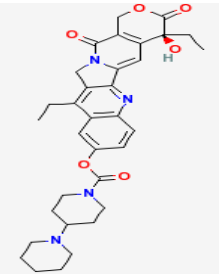

Figure 1: Ligand Structure

Table 1: Properties computed from structure

Molecular weight	566.678 g/mol
Molecular formula	$C_{33}H_{38}N_4O_6$
Hydrogen Bond Donor count	1
Hydrogen Bond Donor count	8
Rotable bond Count	5

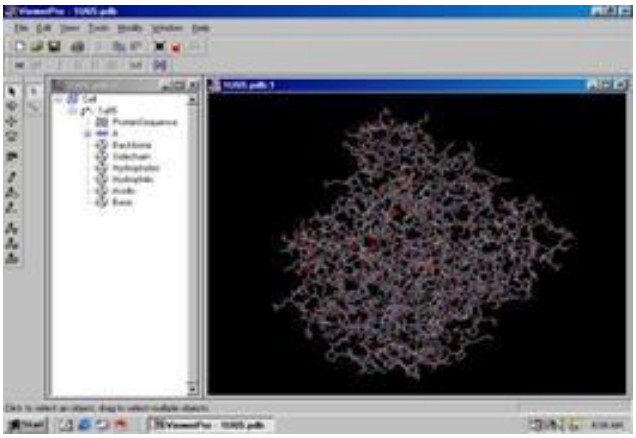

Figure 2: Acetylcholine esterase receptor structure using dsview prosofware

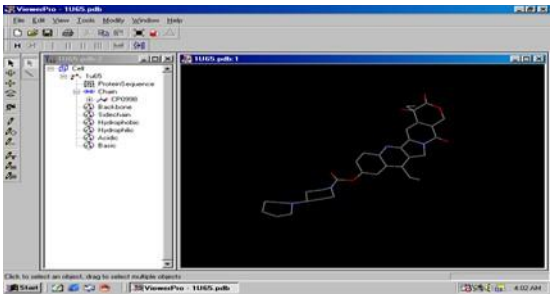

Figure 3: CPT-11 ligand structure (PDB id: 1U65) using DS view pro software

Figure 4: VAST (vector alignment structure tool)

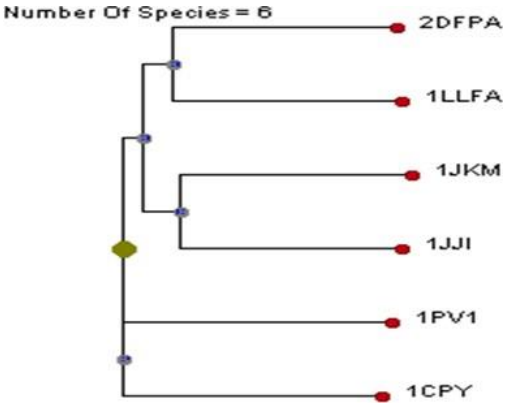

Figure 5: Phylogram

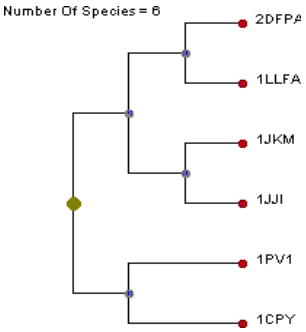

Figure 6: Cladogram

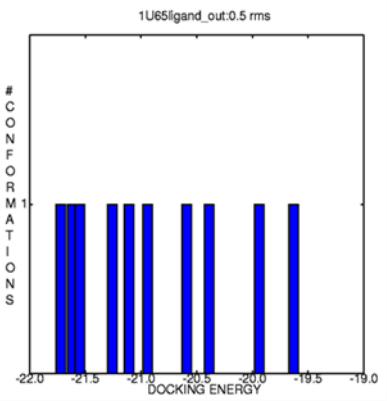

Figure 7: Histogram

Table 2: Clustering histogram

Cluster Rank	Lowest Docked Energy	Run	Mean Docked Energy
1	-21.72	8	-21.72
2	-21.62	1	-21.62
3	-21.55	9	-21.55
4	-21.26	3	-21.26
5	-21.11	5	-21.11
6	-20.94	10	-20.94
7	-20.59	4	-20.59
8	-20.39	6	-20.39
9	-19.94	2	-19.94
10	-19.63	7	-19.63

Table 3: RMSD table

Rank	Sub Rank	Run	Docked Energy	Cluster RMS D	Ref RMS D
1	1	8	-21.72	0	1.88
2	1	1	-21.62	0	1.64 Ac
3	1	9	-21.55	0	2.59 com
4	1	3	-21.26	0	2.16 fr
5	1	5	-21.11	0	2.02 wa
6	1	10	-20.94	0	1.75 Au

3.2 Discussion

The structural details of acetylcholine esterase (receptor) and CPT- 11(ligand) complex had been retrieved from Protein Data Bank (PDB id:1U65). Similarities for structural neighbours of the complex had done using Vector Alignment Structure Tool (VAST). From phylogram six species of protein were found to be more related with

4.1 Conclusion

Acetylcholine esterase receptor-ligand complex (pdb code: 1U65) was retrieved from PDB. The receptor-ligand complex was seperated using DS view Pro software. Autodocking of the ligand with receptor is done using autodock 3.0 software. The cpt- 11 ligand was bound successfully to the active site of the acetylcholine esterase receptor with lowest docked energy. Analysis for the similarity of the docked structures was done with root mean square deviation (RMSD).Insilco docking of CPT- 11 ligand to the active site of acetylcholine esterase receptor was successfully done and ten different conformations are found. Predicting how molecules dock each other will have a great significance in computer assisted drug design. Insilico docking can be made useful in designing drugs for various diseases. This technique will help to identify different conformations of ligands in the binding pocket of a protein and predict the affinity between the ligand and the protein by calculating least docked energy. The main step in drug discovery is the identification of flexible ligand structures with a reasonable ADMET (absorption, distribution, metabolism, excretion, toxicity).This will enable to ease the drug development process. The behaviour of ligands in the binding pockets of target proteins can be explained by molecular docking. Hence this technique is widely used in the discovery of lead compounds that can be developed into effective drug in less time and cost.

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