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Virtual Screening and Molecular Docking Analysis of Zap-70 Kinase Inhibitors

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Molecular docking is normally used technique for considering the drug - receptor interaction in drug design. The purpose of protein docking is to obtain a representation for the bound complex from the coordinates of the unbound constituent molecules. This method calculates a huge number of docked conformations with simple functions which measures the surface complementarities. In this study, the main objective was to evaluate the ability of ligand binding affinity of zap-70 kinase with Staurosporine. The 3D structure of zap-70 kinase –ligand complexes were used for this comparative study. It revealed that few compounds extracted from chemical libraries represented better energy values (kcal/mol) and orientation than the co-crystallized ligand Staurosporine.

Keywords: Virtual Screening, Zap-70 Kinase, Molecular Docking, Staurosporine

INTRODUCTION

Molecular docking approaches are commonly used in modern drug design process to understand the drug–receptor interactions. The majority of biological processes are well-known through protein-ligand interactions. The three-dimensional structure of the protein-ligand composite could be served as a considerable source of understanding the way of proteins interact with one another and perform biological functions. Thus, knowing the detailed structure of protein-ligand and its complexes in atomic level is one of the significant issues in biological sciences. However, in the databank of proteins where in most of the docking studies, conformational changes occur on ligand binding. This may occupy small side chain rotations to increase interactions with the ligand. Molecular Docking and Virtual Screening based studies on molecular level have become an integral part of many modern structure-based drug discovery efforts. Hence, knowledge of the protein and ligand interactions with the specific drugs may provide a significant insight into the binding interactions and relativeness of the drug.

Taking into consideration of protein in this current study Zeta-chain-Associated Protein Kinase 70 (ZAP-70)[1],[2] is a member in the protein-tyrosine Kinase family and comprised of two src homology 2 (SH2) domains connected by a central inter domain linking region[3]. It was found to be necessary and has a critical role in the initiation of T-cell signaling such as for human T cell function and recommend that the CD4+ and CD8+ T cells depend on different intracellular signaling pathways to support their development or survival [4].

Activation: ZAP-70 activates two proteins: LAT (linker of activation in T cells) and SLP-76. LAT is an adaptor protein that performs signal transduction in a lipid-raft mediated function [5].

It is phosphorylated by activated ZAP-70 to create docking sites for other SH2 domains, including phospholipase C-γ (PLC-γ) and SLP-76 the activation of PLC-γ initiates the exhaustedly studied pathway, beginning with cleavage of PIP_2 and the release of calcium, ultimately activating transcription factors NFκB and NFAT. SLP-76 plays a crucial role in activating the MAP Kinase signaling pathway used to transcribe the AP-1 transcription factor. LAT, in conjunction with Grb2 (another adaptor protein) allows SLP-76 to activate SOS, a GEF involved in the pathway, which stimulates the small G-protein Ras to ultimately up-regulate the transcription factor Elk to produce Fos, half of the AP-1 transcription factor SLP-76 and LAT also work to activate another GEF, Vav, which also initiates a MAP Kinase cascade to produce Jun, the other half of the AP-1 transcription factor New research also shows ZAP-70 has a role in apoptosis. TCR-mediated apoptosis [6] [7] takes place via the ζ-chain that ZAP-70 associates with. Although the exact mechanism is unknown, ZAP-70 deficient T cells [8][9] did not express FasL and undergo apoptosis and manly in cancer therapeutics [10].

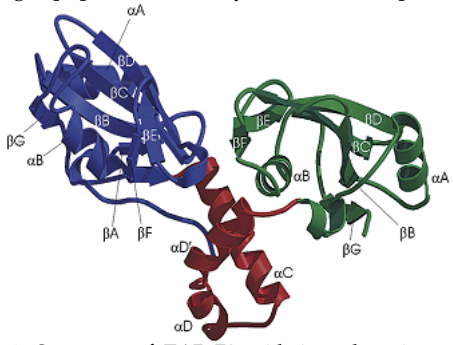

Figure 1: Structure of ZAP-70 with interdomain region in red, ZAP-N in blue, and ZAP-C in green.

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MATERIALS AND METHODS

In this comparative study, the structures were drawn by using ISIS/Draw, a chemical structure drawing program for Windows [11]. By using Tsar's easy-to-use chemical spreadsheet interface the limits for Staurosporine was observed and converted 2D structures to 3D with physicochemical properties to analyze and promote activity.

Virtual screening: It is an Insilco tool for drug designing and widely used for lead identification in drug discovery programs [12]. 7 structure hits of zap-70 kinase were found from PDB [13]. Out of which 1u59 with ligand STAUROSPORINE is selected for docking studies haviing resolution 2.3 angstroms and for searching and filtering of chemical structures screening was done with 3 types of databases namely ZINC, Enhanced NCI database, Pubchem compounds [14].

Protein-ligand docking: Molecular Docking is the process in which two molecules fit together in 3D space. It is a key tool in structural biology and computer-aided drug design [15] [16]. The goal of ligand and protein docking is mainly to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure [17]. For the molecular docking analysis the Molegro Virtual Docker was used [18]. It is an integrated platform for predicting protein -ligand interactions and it handles all aspects of the process, from preparing the molecules to determining the potential binding site of the target protein and predicting the binding mode of the ligand. It offers high-quality docking based on a novel optimization technique combined with a user interface experience focusing on usability and productivity [19]. It based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm.

RESULTS AND DISCUSSION

Virtual screening has become a vital part of contemporary drug research. A range of computational tools are being developed and refined to effectively employ fast screening methods to yield potent hits. In this study, the following properties X-Log p, Rotatable bonds, Hydrogen bond donors and Hydrogen bond acceptors, Mol.Wt range when given in different Databases the following [Table 1] commercial ligands are retrieved.

Table: 1. Complete list of ligands from databases

Data Bases	No. of ligands
Zinc Data base	388
NCI DB2	33
Pubchem	2859

Now, all these ligands are docked with the original protein 1U59 and this score is compared to original Ligand Docked score [Table 2]. The process of docking using Molegro Virtual Docking was given in methods of Docking.

All the commercial available ligands should have a docking score less than -184.422. Only such type of ligands is selected and compared. This is to attain stability between protein-ligand interaction. From this, screening for available chemical structures was done with different databases and tabulated the top 5 ligands with its scores in Table 3, 4, 5 respectively.

Table: 2. Original Ligand docked score

Name	Ligand	Mol Dock Score(kcal/mol)	Re-rank	RMSD (ang)	H-Bond
00STU	STU-100[A]	-184.422	-142.273	0.455704	-2.85214

Table: 3. Top 5 Ranks of Zinc Data Base

Name	Ligand	Mol.Docking Score	Rerank	H-Bond
[00]ZINC	ZINC 195340	-161.863	-118.016	-2.97284
[01]NSC	NSC 359493	-159.05	-100.476	-0.262417
[00]ZINC	ZINC 19534016	-159.048	-118.428	-2.51824
[00]NSC	NSC 359493	-158.621	-59.4881	-1.9084
[02]NSC	NSC 359493	-157.024	-113.619	-2.32031

Table: 4. Top 5 Ranks of NCI Data Base

NAME	LIGAND	MOL.DOCK SCORE	RERANK SCORE	HYDROGEN BOND
[00]NSC	NSC 359493	-167.694	-113.943	-2.13781
[00]NSC	NSC 164079	-157.509	-125.376	-4.90799
[00]NSC	NSC 359493	-156.369	-113.132	-1.80982
[03]NSC	NSC 359493	-155.99	-100.596	-1.71866
[01]NSC	NSC 359493	-154.747	-108.875	-2.92411

Table: 5. Top 5 Ranks of Pub Chem Compounds

LIGAND NAME	SCORE	RERANK SCORE	HYDROGEN BOND
3830593	-196.634	-154.126	-6.32831
3830593	-185.776	-109.912	-9.19335
16525141	-184.586	-102.815	-5
22986078	-182.279	-135.979	-9.01293
16525141	-182.597	-118.111	-8.8157

Out of these ligands ZINC003830593 (bis [3-(2aminoethyl)-1H–indol-5yl] benzene-1, 3-dicarboxylate of Pubchem compound has the score -196.634 kcalmol and hence ZINC003830593 can be considered as the best Ligand.

Figure 2: Image showing original Ligand interacting residues within the active site regions of 1u59.

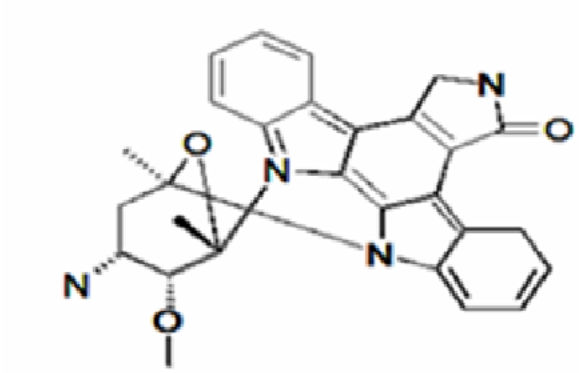
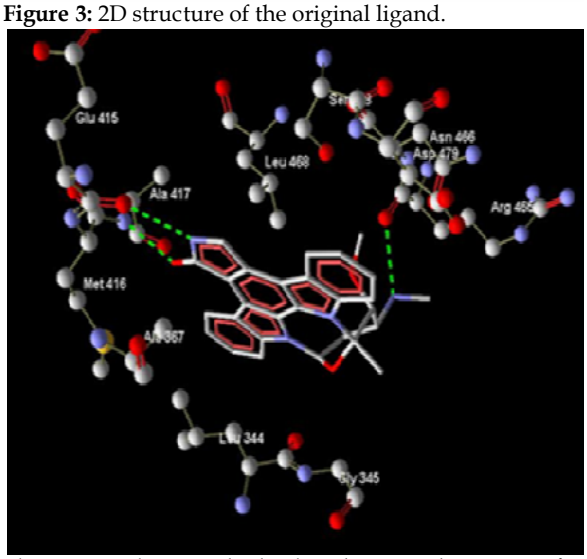


Figure 3: 2D structure of the original ligand.



The Original Ligand displayed a Dock Score of -184.422Kcal/mol. The number of H-bonds formed by the original Ligand with active site residues of 1u59 is 2; Arg465, Ala417.Original Ligand showed 2 interactions with O of Arg465 and N of Ala417.

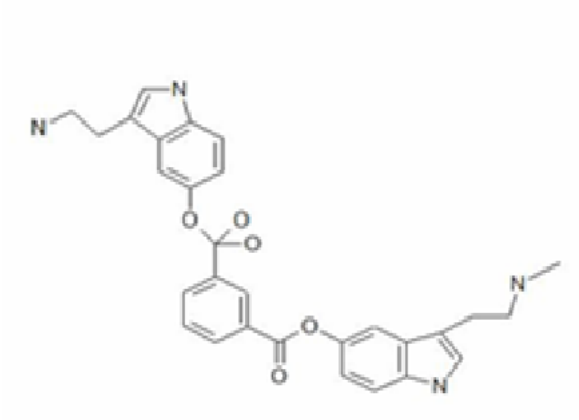


Figure 4: 2-D structure of 3830593 (docked Ligand)

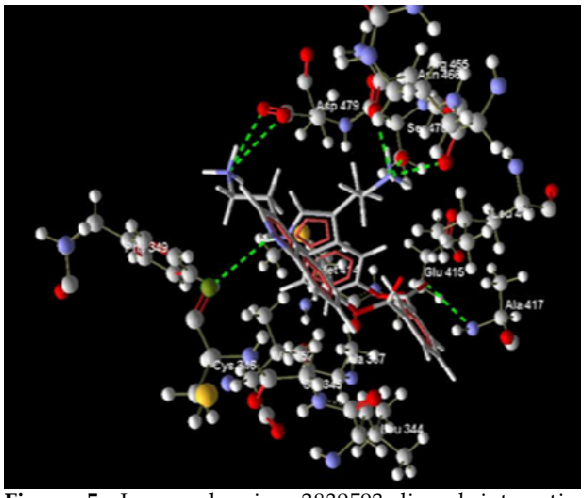


Figure 5: Image showing 3830593 ligand interacting residues within the active site region of 1u59

The Ligand displayed in [Figure 4] a Dock Score of -196.634 Kcal/mol. Six H-Bonds formed by the Ligand with the active residues of 1u59 ie., Ala417, Cys346, Arg465, Ser478, Asp479, Asp479 respectively. This Ligand showed 6 interactions with N of Ala417, O of Cys346, O of Arg465, OG of Ser478, OD1 & OD2 of Asp479.

CONCLUSION

In this study of docking analysis of 1u59 protein with the original ligand Staurosporine and its similar compounds from database of compounds, the final refined model was further assessed by Molegro Virtual Docker program and the validation of software was performed by drawing a graph between computational value and the experimental value. The correlation was found to be 0.98. Hence different inhibitors were docked in the protein active site region.

The databases such as ZINC, NCBI and PubChem were screened based on physico-chemical properties of ZAP-70 kinase inhibitors. Dock runs resulted in binding energy scores that range from -146.083 of NCBI Database to -196.634Kcal/mol of Pubchem Database. A few inhibitors were designated in order to attain affinity higher than the most active molecule and these inhibitors were docked in the active site region of the protein. For instance, the computationally reported most active molecule from ZINC library screening study was ZINC003830593 (-196.634Kcal/mol). The dock score of this molecule is higher than the original ligand Staurosporine (-184.422Kcal/mol).

Finally, the results showed that the model was stable and reliable. This work displays the importance of computational studies using the Molegro Virtual Docker software is screening new ligands that have energy values better than the experimental compounds.

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