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ORIGINAL ARTICLE

DEVELOPMENT OF SMALL MOLECULAR INHIBITOR AGAINST BCR-ABL PROTEIN COMPLEX INVOLVED IN CHRONIC MYELOGENOUS LEUKEMIA THROUGH DOCKING STUDIES.

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Abstract:

Chronic Myelogenous Leukemia (CML) is caused due to the transformation of hematopoietic stem cells and the cardinal reason of abnormality of CML is (9;22)(q34;q11) translocation. This was primarily recognized as an abnormal chromosome and thereafter named as "Philadelphia Chromosome". This translocation results in the Bcr-Abl fusion gene and activation of protein tyrosine kinase (PTK). Mainly, malignant transformation of Bcr-Abl is dependent on the protein tyrosine kinase (PTK) activity. Normally, c-Abl1 is involved in cellular processes and is rigorously controlled in order to prevent oncogenic activity but this activity of c-Abl1 protein is negatively regulated by its SH3 domain, and deletion of the SH3 domain results in transformation of Abl1 into an oncogene. Hence, the drug which we designed may inhibit activity of the protein tyrosine kinase, and has c-docker energy and carcinogenicity of -25.123, 0.000 respectively which can ultimately help in normal regulation of the protein. In this work, systematic target identification, ligand modeling and docking studies were performed, which were validated by previously presented research studies and the drug candidate we designed binds to Bcr-Abl Oligomeric protein domain at different regions and may eventually inhibit the activity of Protein tyrosine kinase (PTK).

KEY WORDS:

CML (Chronic Myelogenous Leukemia), Bcr-Abl, Docking, C-Docker,

1. REVIEW OF LITERATURE

Over 90% of cases of CML 4 (1) and 10–25% of cases of ALL (2) are characterized by a reciprocal translocation between chromosomes 9 and 22. As a result, a BCR-ABL hybrid gene is formed on the derivative Ph chromosome. [1] Imatinib is the only first-line TKI currently available for the treatment of CML; however, intolerance and resistance remain significant clinical challenges. The approved second-line treatment options for CML are dasatinib, nilotinib or escalated-dose imatinib. [11]

2. INTRODUCTION

Initially, Chronic Myelogenous Leukemia (CML) was diagnosed in 18th century and became an archetype in research among malignant disorders. During course of time, a substantial progress in understanding the biology of the disease has been made; along with advancement of therapeutics and ramification of drug alternatives have taken place depending on its mechanism of action (MOA) and side effects. Commonly, drug alternatives open for CML are; Imatinib, Nilotinib, Dasatinib. All these drugs including the drug candidate we designed targets Bcr-Abl oligomeric protein domain which is the target protein of CML. Bcr-Abl oncogene, generally responsible for wide range of leukemia in human and the

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prime reason of malignancy is Oligomerization of Bcr and Abl protein . In this project, 3D structure of Bcr-Abl oligomeric protein, the target protein, was downloaded from PDB and operations such as cleaning geometry, optimization and energy minimization were performed. The chemical compound that we have predicted as Bcr-Abl inhibitor was taken from fish oil on which ligand modeling was performed and chemical libraries were formed using "ChemSketch" tool. C-Docker tool of Accelrys Discovery Studio v2.5 was used for docking lead compound with the target protein. C-Docker runs a random initial ligand placement and full CHARMm forcefield based docking.

ADMET and Toxicity prediction were done using ADMET Descriptor and TOPKAT prediction tool of Discovery Studio v2.5. Likewise, throughout the project, we used numerous databases and tools such as Clustal W, SDSC workbench, KEGG, Drug Bank, Gene Cards, ChemSpider, SOPMA etc for various operations such as multiple sequence alignment, Validation of target, chemical structure search, primary, secondary and tertiary analysis of Bcr-Abl (target protein).

3 .METHODOLOGY

3.1 Identification of Target Protein

The target protein (Bcr-Abl) was identified from the information in KEGG and GENECARDS databases and the sequence as well as the structure was obtained from PDB. (Figure 1(A)).

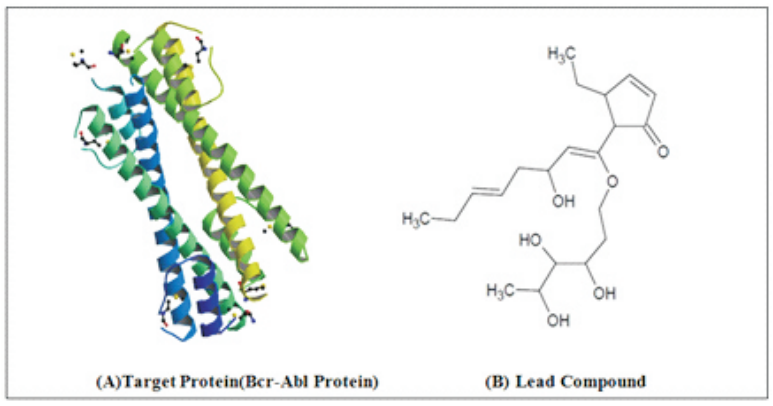

Figure 1: (A) Target protein (BCR-ABL), (B) Lead Compound

3.2 Sequence and Phylogenetic Analysis of Target Protein

Sequence analysis of target protein was done using BLAST against thirteen model organisms ranging from plants to animals. Similarly, phylogenetic analysis of the target protein was done using different tools such as Clustalw, Texshade, Boxshade and SDSC workbench.

3.3 Primary, Secondary and Tertiary Structure Analysis and Active Site Prediction of Target Protein

To gain a better insight into the target protein structure, we analyzed its primary, secondary and tertiary structures with the help of tools like HHpred, HNN and SOPMA. Degree of disorder in target protein was measured using GlobPlot; a protein disorder prediction tool. Active site of target was by was identified by ProSite tool.

3.4 Minimization of Target and Screening of Ligands

Accelyrs Discovery Studio v2.5 was used to figure out the minimized energy value after cleaning and optimizing the target molecule. Ligand modeling was performed in Chemsketch and it was used to form a library of chemical compounds. In the primary screening, chemical library was screened for ligands that had a threshold weight of 500 Da.

3.5 ADMET, Toxicity and Carcinogenicity Prediction of Ligands

For secondary screening, all ligands were passed through ADMET/TOPKAT and were filtered to discard the toxic and carcinogenic compounds. The parameters included for ADMET and TOPKAT were 'aqueous solubility', 'Blood brain barrier penetration (BBB)', 'hepatotoxicity', 'human intestinal absorption (HIA)', 'plasma protein binding' and a broad range of toxicity measures were applied on the set of ligands. Hence, carcinogenicity, absorption, distribution, metabolism, excretion and toxicity values were predicted for all the ligands using ADMET and TOPKAT.

3.6 Optimization and Energy Minimization of Lead Compounds

Optimization and Energy Minimization steps were perfomed on the Ligands with the help of energy minimization tools of Accelyrs Discovery Studio v2.5.

3.7 Docking Lead Compounds with Target

Steps of flexible docking were run using C-Docker's 'ligand placement' and 'CHARMm force field' to obtain the best C-Docker energy values. The molecules with the optimum C-Docker energy values were filtered to choose the best results and this was selected as a potent drug candidate as shown in Figure 1(B).

4 .RESULTS AND DISCUSSION

4.1 Sequence Analysis of Target Protein

To check the sequence similarity of target protein with proteins from other organisms ,twelve sequences taken from different model organisms including *Pan troglodytes, Mus musculus, Bos bovis, Salmonella enterica, Streptococcus intermedius, Bos taurus, Trichinella spiralis, Pediculus humanus corporis, Danio rerio, Oryza sativa japonica, Zea mays* and *Arabidopsis thaliana* to have multiple sequence alignment. In the sequence analysis, *Pan troglodytes* (99%), *Mus musculus* (96%), *Danio rerio* (86%), *Trichinella spiralis* (75%) showed highest sequence similarity amongst all the others. Alignment file from Clustal W was used to obtain phylogenetic tree with closest evolutionary relationship for target protein from which it was evident that *Mus musculus*, *Pan troglodytes*, and *Danio rerios* were the closest relatives (Figure 2). Along with Clustal W, many different tools such as Texshade, Boxshade and SDSC workbench, for validating phylogenetic result of ClustalW.

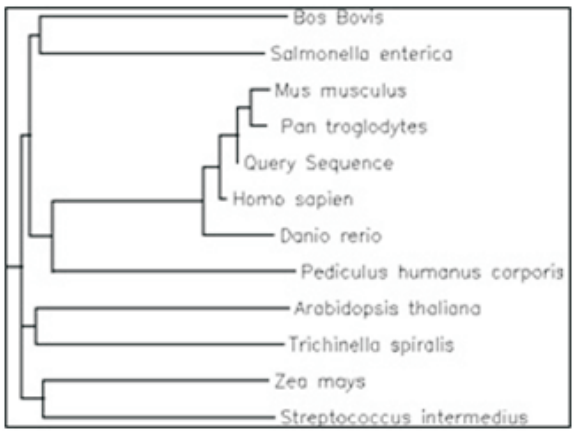

Figure 2: Rooted Phylogenetic trees (generated by Phylip).

4.2 Primary, Secondary and Tertiary Structure Analysis of Target Protein

Bcr-Abl (Target Protein) has eight chains (A,B,C,D,E,F,G,H) which are represented by one sequence unique entity of 72 residues each, as shown in Figure 1.(A), out of which 50(69.44%), 5 (6.94%), 2(2.78%), 15(20.83%) amino acids formed alpha helix, extended strand, beta turn and random coils respectively. The secondary and tertiary structure predictions were taken from HNN, CPH model, HHpred

and SOPMA tools. Hence, it can be assumed that major portion of Bcr-Abl protein comprises alpha helix but other secondary structure elements were rarely found. Figure 3 displays the results of secondary structure prediction from SOPMA which illustrates the above mentioned features for Bcr-Abl.

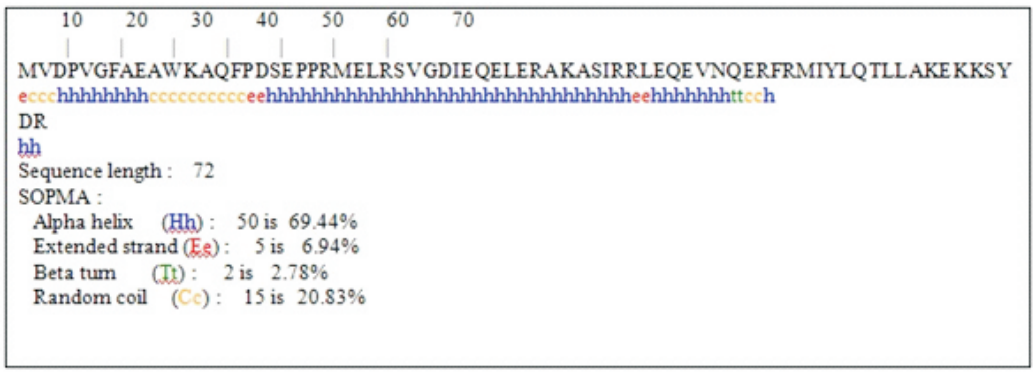

Figure 3: Secondary structure prediction of Bcr-Abl using SOPMA

4.3 Ligand Screening and Energy Minimization of Target Protein

A chemical library of forty seven ligands was formed by Chemsketch tool without changing their scaffold. All the ligands and target protein were minimized by Minimization tool of Accelyrs Discovery Studio v.25. Minimization internal energy of lead compound was 67.62401 Kcal/mol and its structure is shown in Figure1(B). Pharmacophores were identified on set of ligands to know their molecular properties .General molecular properties of lead compound is summarized in Table 1 and absence of aromatic ring minimizes the chances of carcinogenicity of the lead compound. This, also satisfies four conditions of "lipinski rule of five" (weight= 382.491 Dalton).

Molecular Properties Of Lead Compound	
ALogP	1.774
Molecular Weight	382.491
No. Of Hydrogen Acceptors	6
No. Of Hydrogen Donors	4
No. Of Rotatable Bonds	12
No. Of Rings	1
No. Of Aromatic Rings	0
Molecular Fractional Polar Surface Area	0.265

Table 1: General molecular properties of lead compound

4.4 ADMET and Toxicity Prediction of Lead Compound

The ADMET Discriptor protocol of Accelyrs Discovery Studio v2.5 computes and analyzes Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of chemical compounds. Ligands with significant results are summarized in Table 2. If we compare results, 'Noname30' has the highest ADMET absorption level amongst all the molecules (ADMET absorption level= 1) that made it non absorber whereas other three gave comparably good results, such as 'noname28' (BBB LEVEL= 3, ADMET Absorption=0, ADMET solubility= -1.071). Along with this some undesired results like ADMET CYP2D6 and ADMET PPB Level (all four ligands) with zero value was also obtained

Sr. No.	Name Of Ligand	ADMET BBB level	ADMET Aborption level	ADMET solubility level	ADMET Hepatotoxicity	ADMET CYP2D6	ADMET PPB Level
1.	Noname 12	3	0	-1.414	1	0	0
2.	Noname 13	3	0	-1673	1	0	0
3.	Noname 28	3	0	-1.071	1	0	0
4.	Noname 30	4	1	-o.42	0	0	0

Table 2: ADMET of ligands using ADMET Descriptor

4.5 TOPKAT Prediction of Lead Compound

TOPKAT was utilized to predict attributes such as Carcinogenicity, Developmental Toxicity Potential (DTP), Rat Oral LD50 (1/Moles), Probability of SEV, Probability of Biodegradability. With these attributes, four ligands with desirable values as summarized in Table 3 were selected. 'Noname12', 'Noname13', 'Noname28' and 'Noname30' produced good results among forty seven with zero Carcinogenicity and Developmental Toxicity. After discern observation of all the values of the four ligands, 'Noname28' was found be suitable ligand for docking.

Sr. No.	Name of Ligand	Carcinogenicity	Developmental Toxicity Potential (DTP	Rat Oral LD50(1/ Moles)	Probability of SEV	Probability of Biodegradability
1.	Noname12	0.000	0.000	2.189	1.000	0.000
2.	Noname13	0.000	0.000	3.031	1.000	0.000
3.	Noname28	0.000	0.000	2.790	0.000	0.000
4.	Noname30	0.000	0.000	-1.650	0.000	0.000

Table 3: TOPKAT results of ligands

4.6 Docking Ligand with Target Protein

The C-docker and interaction energies of all four ligands are summarized in Table 4 which further validates the aforementioned observation. It is evident that 'Noname28' had the best C-docker and Interaction energies (-21.3655 and 46.0696 respectively).

Name of Ligand	C-Docker Energy	Interaction Energy	Pose No.
Noname12	22.4627	42.4723	10
Noname13	-17.1706	47.5767	10
Noname28	-21.3655	46.0696	10
Noname30	- 72.4766	47.0932	10

Table 4: C-Docker and Interaction energies of ligands docked with the target protein

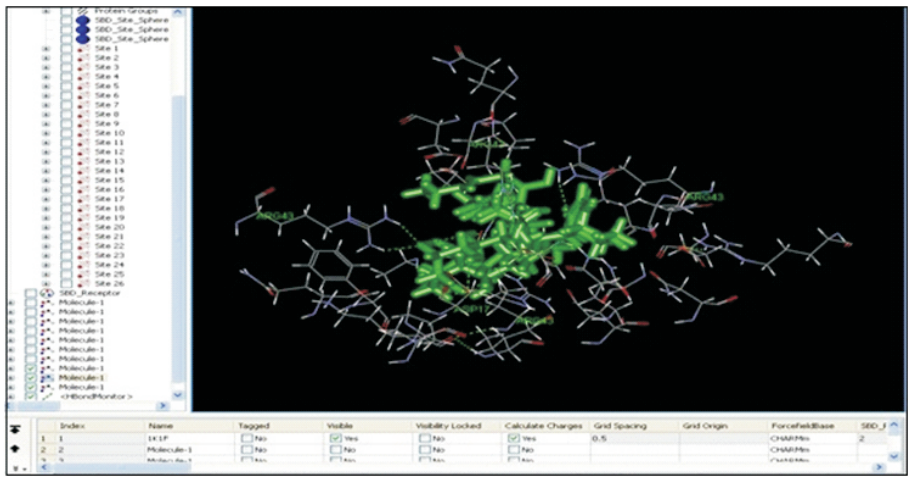

Figure 4: Illustration of Lead Compound and target protein interaction

The intermolecular bonding between lead compound and target protein clearly indicate that it forms four bonds with ARG43 and a single bond with ASP17 (Figure 4). More research is required on pattern studies.

4.7 DISCUSSION

The ligand we finally selected as a lead compound has molecular properties such as molecular weight of 382.491 Da, four hydrogen bond donors, six hydrogen bond acceptors, AlogP (1.774), twelve rotatable bonds that follow four out of five Lipinski's rule of five. Therefore, the selected lead compound has good molecular properties that could possibly become an ideal drug candidate. In addition, the molecule also satisfies few other criteria for ideal drug candidates, such as good ADMET and zero Toxicity values. In ADMET descriptor, it has an absorption level of zero and optimal aqueous solubility level (-1.071). Hence, it can be easily absorbed and optimally soluble in an aqueous medium. Whereas, TOPKAT toxicity prediction got partially favorable results such as zero carcinogenicity, toxicity developmental potential and biodegradability values. Molecules with zero carcinogenicity and toxicity characteristics plays vital role to affect oncogenic activity negatively.

5. CONCLUSION

To conclude, the molecule finally chosen as the lead compound has most of the required features for becoming a drug candidate. However, unfavourable properties (12 rotatable bonds, 0 ADMET CYP2D6 and ADMET PPB level) has to be modified and further studies have to be conducted to obtain an ideal drug candidate that can inhibit or control CML.

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