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RESEARCH ARTICLE

A Virtual Screening Approach for the Identification of High Affinity Small Molecules Targeting BCR-ABL1 Inhibitors for the Treatment of Chronic Myeloid Leukemia

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Abstract: CML originates due to reciprocal translocation in Philadelphia chromosome leading to the formation of fusion product BCR-ABL which constitutively activates tyrosine kinase signaling pathways eventually leading to abnormal proliferation of granulocytic cells. As a therapeutic strategy, BCR-ABL inhibitors have been clinically approved which terminates its phosphorylation activity and retards cancer progression. However, a number of patients develop resistance to inhibitors which demand for the discovery of new inhibitors. Given the drawbacks of present inhibitors, by high throughput virtual screening approaches, present study pursues to identify high affinity compounds targeting BCR-ABL1 anticipated to have safer pharmacological profiles. Five established BCR-ABL inhibitors formed the query compounds for identification of structurally similar compounds by Tanimoto coefficient based linear fingerprint search with a threshold of 95% against PubChemdatabase. Assisted by MolDock algorithm all compounds were docked against BCR-ABL protein in order to retrieve high affinity compounds. The parents and similars were further tested for their ADMET propertiesand bioactivity. Rebastinib formed higher affinity inhibitor than rest of the four established compound investigated in the study. Interestingly, Rebastinib similar compound with Pubchem ID: 67254402 was also shown to have highest affinity than other similars including the similars of respective five parents. In terms of ADMET properties Pubchem ID: 67254402 had appreciable ADMET profile and bioactivity. However, Rebastinib still stood as the best inhibitor in terms of binding affinity and ADMET properties than Pubchem ID: 67254402. Nevertheless, owing to the similar pharmacological properties with Rebastinib, Pubchem ID: 67254402 can be expected to form potential BCR-ABL inhibitor.

Keywords: Chronic Myeloid Leukemia, BCR-ABL, BCR-ABL1 inhibitors, Virtual screening methods, Kinase, ADMET, Leukemia.

1. INTRODUCTION

Chronic Myeloid Leukemia (CML) incurs major health burden worldwide and in developing countries like India has an estimated 3 lakhs CML patients, with 20,000 registered every year (Cancer Patients Aid Association (CPAA) annual report 2014). CML is a malignant neoplastic growth of hematopietic stem cells predominately granulocytic cells which mature into dysfunctional white blood cells and subsequently occurrence of cancer. The myeloid leukemia is clinically divided into three stages. CML is the first stage and indolence in its treatment leading to the accelerated myeloid leukemia presented as second stage leukemia which further transforms to third stage clinically defined by blastic myeloid leukemia for which no therapeutic interventions are available. *In-vitro* as well as *in-vivo* studies have unveiled that BCR-ABL chimeric protein is carcinogenic and sufficient to transform normal cells into malignant cells [1-4].

In more than 90% of CML patients, Philadelphia chromosome has been seen as a consistent marker originated with reciprocal translocation t(9;22) (q34;q11) between 5' of BCR gene and 3' of ABL gene with subsequent formation of fusion product of 210 KDa protein (Fig. **1**) [5-6]. Studies have shown fusion protein of 190 KDa and 230 KDa to be involved in leukemic cancer which is formed due to the variation in translocation site between bcr and abl1 gene.

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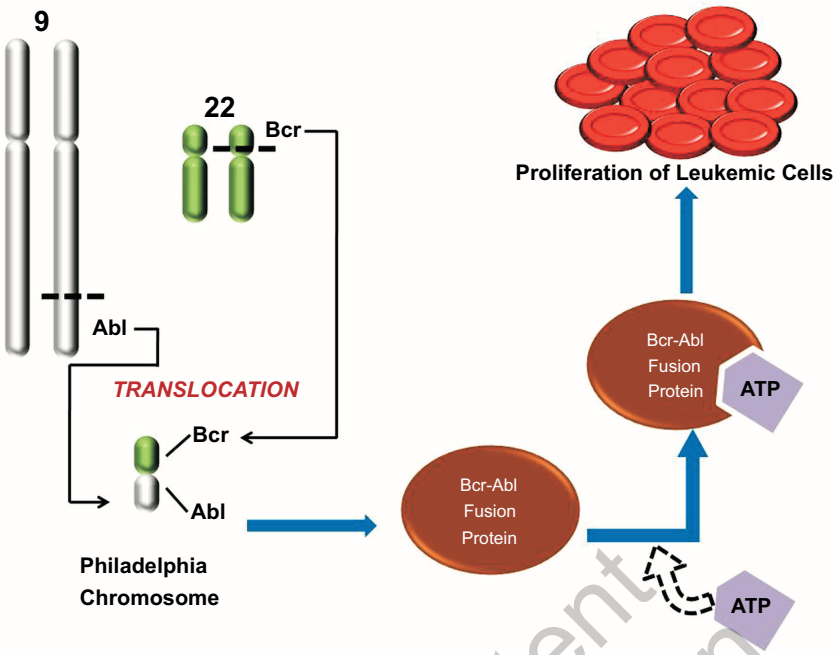

Fig. (1). Reciprocal translocation of genetic material between chromosome 9 and 22 forms short defective chromosome called Philadelphia chromosome. The chromosome contains a fusion gene called BCR-ABL1 translating to hybrid protein which is constitutively active for tyrosine kinase signaling. The uninterrupted signaling directs uncontrolled cell proliferation leading to CML.

mRNA molecules transcribed from the hybrid gene usually contain BCR-ABL junctions, designated as e13a2 and e14a2. Both mRNAs are translated into an 210 kDa and 190kDA oncoprotein [7, 8]. The p210 product is the hallmark of CML, both p190 and the p210 subtype occur in B-lineage ALL.

Bcr promotes dimerization of the oncoprotein in a way that 2 adjacent BCR-ABL molecules phosphorylate their respective proteins kinase activation loops on tyrosine residues [9-10]. The uncontrolled kinase activity of BCR-ABL then takes over the physiological functions of the normal Abl enzyme by interacting with a variety of effector proteins, leading to decreased adherence of leukemia cells to the bone marrow stroma and deregulated cellular proliferation and reduced apoptotic response to mutagenic stimuli [11-13]. Owing to its utmost potential in oncogenic transformations, the tyrosine kinase encoded by the Src-homology 1 (SH1) domain of the Abl component of BCR-ABL is undoubtedly the most crucial component for clinical diagnosis.

ABL, non receptor tyrosine kinase, having nuclear localization signal is human homologous of v-abl proto-onco gene present in the Abelson murine leukemia virus [14]. It regulates cell cycle [15], maintenance of homestasis through integrin signaling, stress responses [16]. Bcr gene present in chromosome 22 encodes a 160Kda protein having serine-threonine kinase activity. It is either in monomer or in dimer form control cellular activities such as regulation of NADPH function in phagocytic cells, reactive oxygen species in neutrophils, cellular differentiation and transformation. The core region of BCR protein carried GTP exchange activity which assists in activation of transcription factor NF-κB [17]. It can also phosphorylate tyrosine residue present in 177th position in Grb-2, to activate Ras protein which subsequently regulates cell growth, differentiation and survival [17].

The main cellular effect elicited by BCR-ABL fusion product is the constitutive activation of tyrosine kinase based signaling pathways. Once tyrosine kinase gets activated, it transfers phosphate from ATP to hydroxyl group of tyrosine of different cellular protein involved in cell regulation, differentiation, growth, apoptosis and make them permanently active to elicit its carcinogenic effect [18,19].

BCR-ABL1 can autophosphorylate and dimerize its tyrosine residue to interact with other proteins through their SH2 domain. *In-vitro* studies by Carpino *et al. 1997* found autophosphorylated BCR-ABL1 binds GAP and elevated its expression level in Ph^+ cells than normal cells [20]. McGlade and co workers in 1993 through *in-vivo* studies in Rat cells documented that the over expression of amino terminal of GAP leads to disorientation of actin cytoskeletal, focal contacts, cell adhesion. Thus, these two different studies demonstrated, interaction between GAP and BCR-ABL1 lead to disturbance in cell cycle, interaction between cells in Ph+ cells which may or may not lead to leukemic condition depending upon cell type and other genetic factors.

Beside genetic factors, McFarland DC *et al. 2016* found that physiological factors like anxiety and depression accelerate the onset of disease in patients having BCR-ABL-Negative Myeloproliferative Neoplasm [21]. The curative therapies available for CML such as Inhibitors, cytokines and Allogeneic stem-cell transplantation impede with the kinase activity of BCR-ABL fusion protein [22, 23].

The cell lines studies in KG1 showed ionization radiations promote the translocation between bcr-ablgene and stimulate its expression which leads to the transformation of normal cells to malignant cells [24].

The discovery of inhibitors against BCR-ABL have revolutionized the treatment of CML as these are more efficient,

effective and specific in their activity. Before the discovery of imatinib, bone marrow or stem cell transplantation and cytokines therapies such as interferon was alternative to treat CML patients. But these were not as effective and efficient as inhibitors are. It has been seen that disease get relapse in 5 to 20% patients treated with bone marrow or stem cell transplant.

It has been reported that the level of synthesis of cytokine or cytokine's transcription regulators decreases in CML, thus resorting its level to normal delaying disease. Schmidt *et al.*1998 reported that mice deficient in Interferon Consensus Sequence Binding Protein (ICSBP), transcription regulator of interferon shows symptoms similar to human CML and treatment with interferon alpha stimulate the expression of ICSBP with disappearance of disease symptoms. Another study showed that treating Ph+ cells with interferon delays the onset of the disease [25, 26]. Thus, cytokines can be alternatives to CML treatment, but cytokines lack specificity for their target.

Imatinib (Gleevec or STI-571) is universally and clinically approved inhibitor used to treat CML patients which block the ATP binding pocket of ABR protein and inhibits its phosphorylation activity [27, 28].

It has been proven effective in patients having resistance to stem cell/bone marrow transplant treatment [29]. The inhibitory effect of imatinib is not uniform in all CML patients partly due to genome mosaicity. In a study by Pavlovsky C *et al. 2008 in* which they treated CML patients with imatanib and evaluated BCR-ABL transcript expression with QRT-PCR at 0, 6, 12, 18 month of treatment have found some patients expressed BCR-ABL in low level, in other group transcript level was at stationary phase and in some patients rise in transcript level has been documented beside treatment [30]. Some of the Ph^+ CML patients become resistant to imatinib treatment due to the occurrence of point mutation (T315I) in the catalytic domain of ABR protein which hinder the binding of imatinib with chimeric fusion protein [31, 32].

Nilotinib is second generation inhibitor approved in USA and Europe as first line treatment in CML patients. It has a potency to treat patients harboring BCR-ABL mutation in imatinib binding site and can revert complete cytogenetic response in 42% patients following 12 months of therapy [33]. The recent research conducted by Castagnetti *et al.* 2016 documented treating CML patients with 400mg of Nilotinib twice a day imposes adverse effects ranging from non-hematological to metabolic such as heart strokes, myocardial infarction, atrial fibrillation, superficial thrombophlebitis, arterial thrombosis, congestive heart failure, stroke, depletion of glucose [34] and another group showed causation of Keratosis Pilaris [35].

Destanib is another inhibitor of BCR-ABL1 fusion protein as well as src-kinase family and it in combination with gemcitabine showed to kill gemcitabine resistant CML [36]. Instead long list of inhibitors to treat CML patient is available, but expedition to find potent, efficient and effective inhibitor with lesser adverse effects is underway. Owing to drawbacks of current inhibitors that confer, the present study pursues to identify high efficacious inhibitors with appreciable affinity, ADMET properties and bioactivity through computation based drug discovery process assisted by high throughput virtual screening strategies.

2. METHODOLOGY

2.1. Selection of Inhibitors

In the present study, established inhibitors were collected from literatures those target BCR-ABL chronic myeloid leukemia. A total of 5 established inhibitors were used for further docking studies to identify the best inhibitor which can block BCR-ABL1 for the treatment of chronic myeloid leukemia.

2.2. Preparation of Protein and Compounds

Complex crystal structure of BCR-ABL1 was retrieved from protein data bank with PDB id: 1OPK [37]. Prepwiz module of Schrodinger suite [38] was used for the preparation of protein. The preprocessing of protein has been performed by assigning the bond orders and hydrogen, creating metals *via* zero order bonds and adding disulphide bonds. Prime module of Schrodinger was used to fill missing side chains and loops. Further, all the water molecules were deleted beyond 5Å from hetero groups. Once the protein structure was preprocessed, H bonds were assigned which were chased by energy minimization by OPLS 2005 force field. For further studies, the final structure obtained was saved in .pdb format. All the ligands were optimized through OPLS 2005 force field algorithm [39] embedded in the LigPrep module of Schrödinger suite, 2013 (Schrodinger. LLC, New York, NY). The ionizations of the ligand were retained at the original state and were further desalted. For docking procedures the structures thus optimized were saved in .sdf format [40].

2.3. Structure Similarity Search

All the 5 compounds formed the query for structure similarity which was supervised by binary finger print based tanimoto similarity equation to retrieve compounds with similarity threshold of 95 % against NCBI's Pubchem compound database.

2.4. Ligand Receptor Docking

Molecular docking program- Molegro Virtual Docker (MVD, 2010.4.0.0) which integrates highly efficient PLP(Piece Wise Linear Potential)and MolDock scoring function [36] gave a reliable docking surface for the molecular docking [41-43].The leads Imatinib (STI571), Nilotinib (AMN107), Dasatinib (BMS-345825), Bosutinib (SKI-606), Ponatinib (AP24534),)) were docked in cavity of BCR-ABL1. Docking parameters were set to 0.20Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. After the docking Energy minimization and hydrogen bonds were optimized. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. Binding affinity and interactions of inhibitor with protein was evaluated on the basis of the sp2-sp2 Torsions, Internal hydrogen bond Interactions and Internal Electrostatic interactions. Nelder Mead Simplex Minimization (using non-grid force field and H bond directionality) [44] was used to minimize the post docked ligand-receptor complex energy.

Table 1. The list of best 5 established drugs docking results.

Ligand	MolDock Score	Rerank Score	Interaction	HBond	MW
25066467	-170.898	-140.094	-200.413	-2.20777	553.587
24826799	-157.334	-132.517	-183.278	-2.48323	532.559
11442891	-153.398	-130.706	-178.601	-2.01418	474.555
447077	-158.981	-128.472	-182.607	-5.00000	443.349
11442891	-149.487	-127.833	-185.023	-4.19468	474.555

Table 2. Top 5 high affinity compounds obtained with reference to Rebastinib from virtual screening methods.

Ligand	MolDock Score	Rerank Score	Interaction	HBond	MW
67254402	-157.001	-128.357	-187.878	-3.85313	497.524
58385477	-167.034	-122.606	-188.777	-2.5000	488.514
10365610	-156.425	-121.108	-183.907	-5.65519	434.423
58990233	-151.816	-120.67	-179.073	-7.22438	442.47
58990233	-139.664	-118.881	-167.053	-2.5000	442.47

2.5. Lipinski Filters Toxicity Screening and Bioactivity Prediction of Compounds

Whole similar retrieved compounds were screened for their drug ability by Lipinski filters. LAZAR toxicity prediction server were used for the toxicity screening. Biological activity score of the ligands was predicted using molinspiration webserver (©MolinspirationCheminformatics, 2014). LC50 was predicted using T.E.S.T. Version 4.1 (2012, U.S. Environmental Protection Agency) software. admetSAR [45] was used to calculate complete ADMET properties.

3. RESULTS

Table **2** shows the affinity scores (Rerank Scores) of the compound against BCR-ABL1. Docking results showed that Rebastinib (Pubchem ID:25066467) had highest affinity against BCR-ABL1 (Table **1**). Therefore, in pursuit to find better affinity compound than Rebastinib, linear fingerprint similarity search was performed considering Rebastinib. As a result, a total of 13 similar compounds were obtained from drug similarity searching. It is interesting to note that none of the virtually screened compounds had better affinity than that of Rebastinib (Table **2**).

In the further study, we pursued to find the rationale behind the better binding affinity of Rebastinib against BCR-ABL. Considering different interactions we observed that the superior affinity of Rebastinib than the best virtually screened compound pubcem id: 67254402 can be attributed to its excellent interaction profile especially in terms of electrostatic and H-bonding interactions. Apparent from the docking profile of compound Rebastinib values of descriptors of external ligand interactions contribute 6.16 folds higher stability than internal ligand interactions. Further, external ligand interactions were stabilized mostly by stearic energy guided by Piece wise linear potentials and Lenard Jones potentials. While in internal ligand interactions, the torsional strain contributes for the stability of the ligand receptor interactions (Table **3**).

Table 3. Affinity (rerank) scores of the best docking compounds.

-	Pubcid:67254402	Rebastinib
Energy overview: Descriptors	Rerank Score	Rerank Score
Total Energy	-128.492	-140.779
External Ligand interactions	-89.07	-84.708
Protein - Ligand interactions	-89.07	-84.708
Steric (by PLP)	-73.728	-70.421
Steric (by LJ12-6)	-25.754	-22.391
Hydrogen bonds	-7.92	-6.896
Internal Ligand interactions	-14.445	-13.665
Torsional strain	10.226	8.366
Steric (by PLP)	-4.6	-2.3

The ADMET profiles (Table **4**) of the Rebastinib along with the best virtually screened compound Pubcid:67254402 revealed that compound Rebastinib was better compound than any other virtually screened compound. While compound Pubcid: 67254402 and Rebastinib and were predicted to bes afe in all respects. In addition, the predicted bioactivity (Table **5**) as well as the LC 50 values of Rebastinib was

Table 4. Predicted LC50 values and bioactivity of compounds.

Lethal Dose Concentration		Bioactivity					
Compounds with best docking profiles	**LC50 (96 hr) mg/L**	**GPCR ligand**	**Ion channel modulator**	**Kinase inhibitor**	**Nuclear Recep-tor ligand**	**Protease inhibitor**	**Enzyme inhibitor**
Rebastinib	0.14	0.03	0.06	-0.1	0.32	-0.2	0.13
PubCid: 67254402	0.18	0.25	-0.11	-0.13	0.12	0.14	0.15

Table 5. ADMET profiles calculated for best docked compound from each dataset by AdmetSAR.

-	**Rebastinib**	**Probability**	**PubCid: 672544028**	**Probability**
-	**Result**		**Result**	
Absorption				
Blood-Brain Barrier	BBB+	0.8252	BBB+	0.8813
Human Intestinal Absorption	HIA+	1	HIA+	0.8396
Caco-2 Permeability	Caco2+	0.8273	Caco2-	0.6107
Distribution & Metabolism				
CYP450 2C9 Substrate	Non-substrate	0.7833	Non-substrate	0.7795
CYP450 2D6 Substrate	Non-substrate	0.8724	Non-substrate	0.6487
CYP450 3A4 Substrate	Non-substrate	0.6852	Non-substrate	0.5
CYP Inhibitory Promiscuity	High CYP Inhibitory Promis-cuity	0.8978	High CYP Inhibitory Promiscuity	0.7136
Excretion & Toxicity				
Human Ether-a-go- go-Related Gene Inhibition	Weak inhibitor	0.8689	Strong inhibitor	0.5379
AMES Toxicity	Non AMES toxic	0.8786	Non-AMES toxic	0.7975
Carcinogens	Non- carcinogens	0.806	Non- carcinogens	0.9648
Acute Oral Toxicity	III	0.5821	III	0.5416

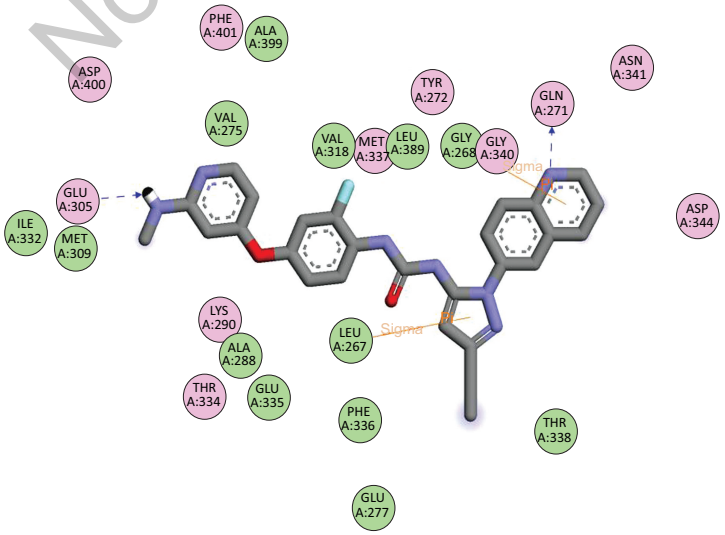

Fig. (2). Interaction profile of Rebastinib. Residues circled in green participate in van der Waals interaction with the ligand while residues in pink forms electrostatic interactions.

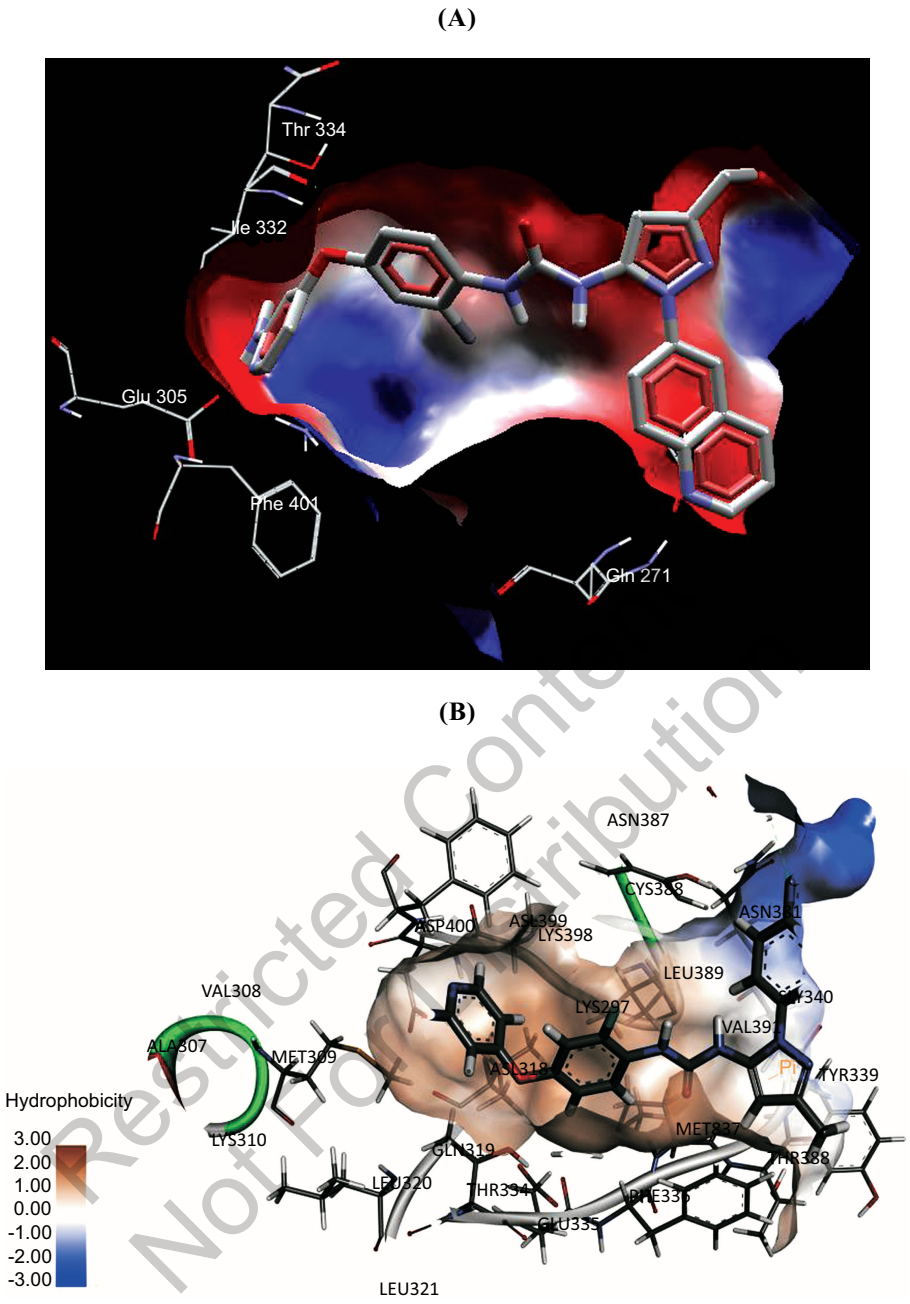

Fig. (3). (**A**) Electrostatic Interactions of Pubcid: Rebastinib in BCR-ABL. (**B**) The binding site of BCR ABL Harboring Rebastinibis shown with Hydrophobic Intensities. The hydrophobic intensities of the binding site ranges from -3.00 (least hydrophobic area - blue shade) to 3.00 (highly hydrophobic area-brownshade). *(The color version of the figure is available in the electronic copy of the article).*

quite appreciable. The LC50 value of Rebastinibat 96 hour interval was predicted to be 2.42 folds superior than virtually screened compound Pubcid: 67254402.

Comprehensively shown in (Fig. **2**), Rebastinib demonstrates vander Waals interactions with Val 275, Ala 399, Val 318, Gly 268 met 309, Ile 332, Met 309, Leu 267, Ala 288, Thr 338 and electrostatic interactions with Asp 400, Phe 401, Tyr 272, Gln 271, Asn 341, Asp 344 andThr 334. Rebastinib is a hydrogen bond donor to electrostatic residue Gln 271 and Glu 305. Electrostatic and hydrophobic interactions of compound Pubcid: 91596862 in the site are shown in Fig. (**3a** and **3b**) respectively.

CONCLUSION

In the recent efforts, meeting conceived by ICON (Indian Cooperative Oncology Network) in 2010, 8115 patients data were presented and 18 centers summoned which reflected an incidence of CML to be invariably high in India. The survival and quality of life of CML patients in India has improved over the years however there still remains a concern in the treatment as patients have known to show serious side effects and at times resistance to drugs like Glivec. In the view of above, in the present study, we found Rebastinib to be highly efficient BCR-ABL inhibitor in the spectrum consisting 82 established drugs. In addition, we also propose

Pubchem ID: 67254402 a similar compound to Rebastinib with appreciable pharmacological profile and potential to target BCL-ABL against kinase activity. In conclusion, we have validated the potential of Rebastinib through in silico studies and also extend the therapeutic potential compound Pubchem ID: 67254402 which according to our study is anticipated to form potential BCR-ABL inhibitor.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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