



## Prediction of Ponatinib as an Effective Drug for Chronic Myelogenous Leukemia

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### ABSTRACT

Chronic myelogenous leukemia (CML) is caused by oncogene Bcr-Abl tyrosine kinase. CML occurs mainly in adults. At present various inhibitors like Ponatinib, Bosulif, Synribo, Kyprolis, Urosolic acid, Boswellic acid, Hydroxy urea, 3-amino propane sulphonic acid, Imatinib, Dasatinib and Nilotinib have been prescribed by the haematologists and physicians for controlling the CML. To find out effective drug against CML, docking is frequently used. Hence docking plays an important role in the rational identification of effective drug for CML. In the present study, Ponatinib was identified as the most effective drug molecule through molecular docking study.

**Keywords:** Bcr-Abl kinase, CML, Molecular docking, Ponatinib.

### INTRODUCTION

Chronic leukemia is characterized by the excessive build up of relatively mature, but still abnormal, white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal, resulting in many abnormal white blood cells. Chronic myelogenous leukemia (CML) occurs mainly in adults. The development of chronic leukemia occurs in four different phases. The first phase is marked by an enlarged presence of lymph nodes. This happens owing to the spurt in the count of lymphocytes. The risk level at this stage is intermediate because at this stage of prognosis, the cancer is yet to spread and affect other physical organs. The second stage is marked by the enlarged presence of spleen as well as liver; in addition to that of the lymph nodes. If not both the organs, at least one of them apart from lymph nodes is affected at the second stage. There is a massive swing in the growth of lymphocytes. The risk level is still moderate. The next stage or the third stage is marked by the development of anemia. The aforementioned organs are still likely to remain swollen. In general two or more organs are likely to be affected at the third stage of cancerous development. The fourth and last phase is marked by a drastic fall in the rate of blood platelets. In the last stage the cancerous development can also affect lungs besides the ones already affected. Anemia is likely to be more acute. Both the third as well as the last phase are more risky from the viewpoint of the patient.<sup>1</sup>

In more than 90% cases CML is caused by chromosomal abnormality resulting in the formation of a so-called Philadelphia chromosome. This abnormality was discovered by Janet Rowley in 1972 and is due to fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at

chromosome 22, resulting in the chimeric oncogene Bcr-Abl and a constitutively active Bcr-Abl tyrosine kinase that has been implicated in the pathogenesis of CML.

At present various inhibitors like Ponatinib, Bosulif, Synribo, Kyprolis, Urosolic acid, Boswellic acid, Hydroxy urea, 3-amino propane sulphonic acid, Imatinib, Dasatinib and Nilotinib have been prescribed by the haematologists and physicians for controlling the CML. Docking is frequently used to predict the binding orientation of small ligand molecule (drug). Hence docking plays an important role in the rational identification of effective drug for CML.

### MATERIALS AND METHODS

Literature pertaining to Bcr-Abl & Bcl-xL and drugs Ponatinib, Bosulif, Synribo, Kyprolis, Urosolic acid, Boswellic acid, Hydroxy urea, 3-amino propane sulphonic acid, Imatinib, Dasatinib and Nilotinib were retrieved from [PubMed](http://pubmed.ncbi.nlm.nih.gov/) database, [www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed). Structures of Bcr-Abl & Bcl-xL protein were retrieved from PDB databases ([www.rcsb.org/](http://www.rcsb.org/)). The molecular structures of drugs were retrieved from Chemspider database ([www.chemspider.com/](http://www.chemspider.com/)). Rasmol tool was used for molecular visualization ([www.rasmol.org/](http://www.rasmol.org/)). Swiss Pdb Viewer ([www.swissmodel.expasy.org/](http://www.swissmodel.expasy.org/)) was used for the conversion of 2D structure into 3D chemical structure. VaxiJen v2.0 is used for antigenicity prediction ([www.ddg-pharmfac.net/vaxijen/](http://www.ddg-pharmfac.net/vaxijen/)). TMHMM v0.2 is used to predict transmembrane helices in proteins ([www.cbs.dtu.dk](http://www.cbs.dtu.dk)). The docking tool Hex was used for molecular docking ([www.hex.loria.fr/](http://www.hex.loria.fr/)). Binding sites, precision rate, 3-D structure of the Bcr-Abl protein molecule and amino acid residues lining the active binding site were drawn through Q-site finder ([www.modelling.leeds.ac.uk/qsitefinder/](http://www.modelling.leeds.ac.uk/qsitefinder/)).

## RESULTS AND DISCUSSION

### Blood Cancer Causing Receptors

The Bcr-Abl receptor tyrosine kinase, formed by 163 amino acid residues, is a protein molecule involved in human leukemias. The Bcl-xL protein is over expressed in solid tumors and renders malignant tumor cells resistant to anticancer therapeutics. It is a polymer protein formed by a single peptide molecule comprising 181 amino acid residues.

### Antigenicity of the Receptor Molecules

The antigenicity of the above two molecules was analyzed using Vaxijen and TMHMM tool. The antigenicity of the receptor Bcr-Abl was 0.7103 which was greater than the threshold value of 0.5 for tumorigenic antigen and the antigen molecule was exomembrane in topology. The other molecule Bcl-xL, even though exomembrane in topology, showed antigenicity score less than that (0.4543) of the threshold value of 0.5 indicating its non antigenic property (Figures 1 and 2). Therefore Bcr-Abl receptor molecule alone is an effective disease producing antigenic protein molecule.

### Binding Sites on the Receptor Molecules

Identification of active sites is crucial in the process of drug discovery. The 3-D structure of Bcr-Abl was analyzed to identify binding sites and design drugs which can fit into them. Bcr-Abl tyrosine kinase enzyme are target for the drugs curing, leukemia. There were 10 possible ligand binding sites on the surface of the receptor molecules, tyrosine kinase enzyme. Each drug binds to the active site of the enzymes and blocks the activity of the leukemia causing enzyme. An important factor in drug design is the strength of binding between the active site of the enzyme and the inhibitor drug molecules. Usually, an enzyme molecule has only one active site, and the active site fits only with one specific type of substrate (Figure 3).

### Drug Molecules for Blood Cancer

Eleven important, U.S. Food and Drug Administration (FDA) approved drugs against human leukemia such as 3-amino propane sulphonic acid, Hydroxy urea, Urosoic acid, Boswellic acid, Synribo, Kyprolis, Bosulif, Ponatinib (Figure 4), Imatinib, Dasatinib and Nilotinib have been selected as ligand molecules for the present study.

### Molecular Docking

Out of two receptors involved in blood cancer, only Bcr-Abl alone was identified as the potential antigen based on the antigenicity score and the Bcl-xL was non antigen due to its antigenicity score less than the threshold value. Therefore it is probable to consider Bcr-Abl receptor molecule as the only potential blood cancer causing receptor. In this investigation Bcr-Abl enzyme receptor molecule alone was considered for molecular docking.

Enzymes play an important role in disease making. Enzymes are the functional proteins, which is controlled by small molecules called ligands. These small molecules

act as switches to turn on or off of the Bcr-Abl enzyme receptor to function. To perform docking, software packages of various kinds have been used. One such software is Hex in which 11 drug molecules were docked with the receptor molecule, Bcr-Abl, causing blood cancer (Table 1).

From the docking study between Bcr-Abl and 11 anticancer drug molecules, the energy value obtained was ranging from -53.06 to -278.53 (Table 1). Ponatinib showed a high e-negative value -278.53 (Figure 5) which was higher than that of the other 10 drug molecules. The precession rate of the docking also indicated the effectiveness of the drug against blood cancer. In the docking 100% binding was observed through Q-site finder tool between Ponatinib and Bcr-Abl at the site no-2 (Figure 6) indicating Ponatinib as an effective drug against blood cancer. From this study it is concluded that binding site no-2 is considered as major active binding site in the Bcr-Abl receptor molecule. Synribo showed 68% of binding on the same binding site no-2 (Figure 6) showing -202.93 as the e-value. In the same binding site drug molecules, other than Ponatinib and Synribo, such as Boswellic acid and Bosulif also tried to bind, but the binding was only 5.1% but not 100% (Figure 6). Boswellic acid and Bosulif showed the e-values as -248.89 and -169.43 respectively when docked with the Bcr-Abl (Table 1). From the study these two molecules not qualified to act as effective drug molecules since they have no complete fitting with the active binding site of the Bcr-Abl receptor molecule.

Urosolic acid showed a binding with site no.1 having 68.1% precision rate showing e-value -180.48. Even though the binding was 68.1%, the e-value obtained was comparatively lesser than that of most of the ligand molecules and this drug also cannot be considered as an effective drug molecule against Bcr-Abl molecule (Figure 7). The drug molecule, 3-amino propane sulphonic acid showed 33.3% binding with the 8<sup>th</sup> binding site (Figure 8) of the receptor molecule Bcr-Abl with very low e-value of -79.58 indicating its ineffective nature of the drug molecule (Table 1).

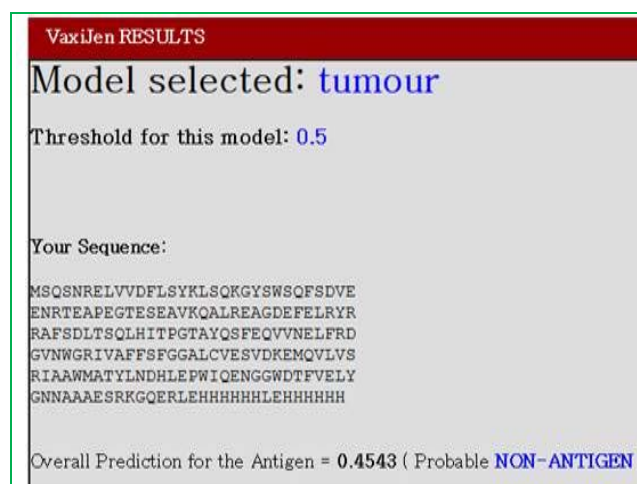
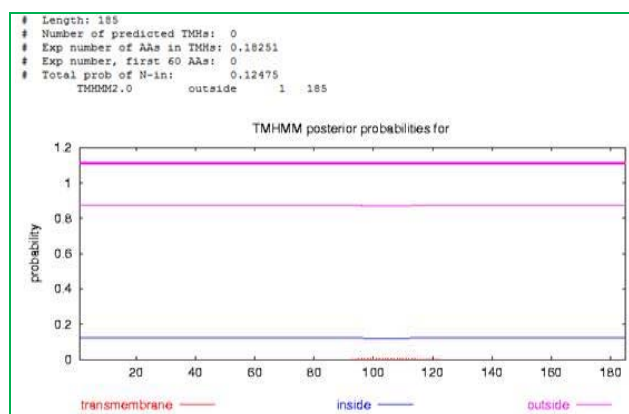
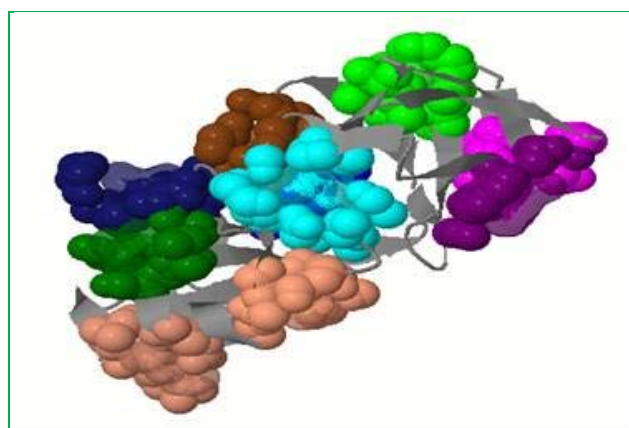


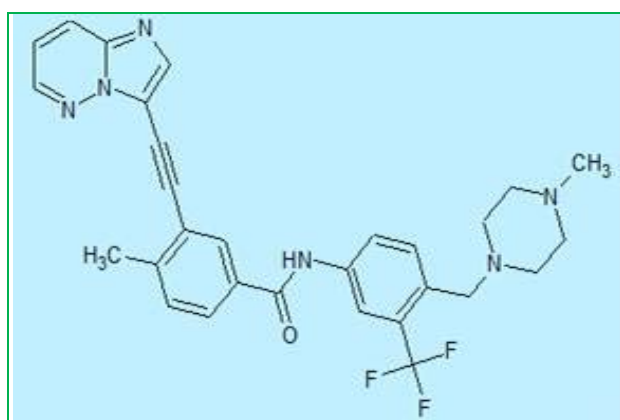
Figure 1: Antigenicity prediction of Bcl-xL



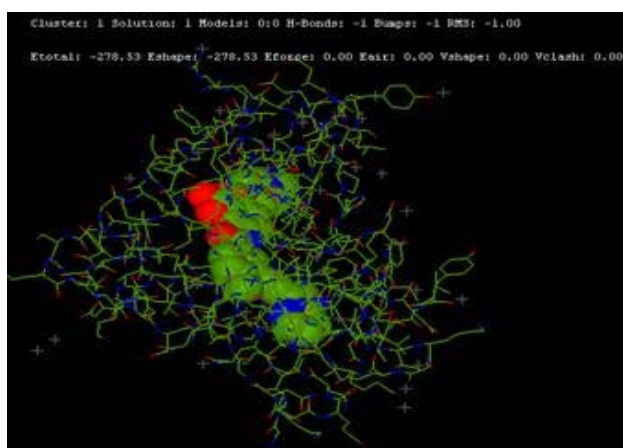
**Figure 2:** Prediction of exomembrane position of Bcl-XL



**Figure 3:** Ligand binding sites on Bcr-Abl



**Figure 4:** Molecular structure of Ponatinib



**Figure 5:** Molecular docking of Ponatinib with Bcr-Abl

The remaining drug molecules such as Dasatinib, Nilotinib, Imatinib, kyprolis and Hydroxy urea showed non binding nature with any of the 10 binding sites of the receptor molecule Bcr-Abl having e-values in the docking as -169.19, -142.57, -140.87, -250.20 and -53.06 indicating their non functional nature of drug molecules against blood cancer (Table 1; Figure 9). Among the eleven drug molecules hydroxy urea which was classically and even currently prescribed for the blood cancer by the most of the physicians, showed a least e-negative value and the precession rate was also 0% on all the 10 sites of the receptor indicating its non effective nature. In the present investigation it has been concluded that Ponatinib is the most suitable effective drug molecule against Bcr-Abl causing blood cancer.

Targeted therapy, which first became available in the late 1990s, has had a significant impact in the treatment of some types of cancer, and is currently a very active research area. This constitutes the use of agents specific for the deregulated proteins of cancer cells. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains. Targeted therapy can also involve small peptides which can bind to cell surface receptors or affected extra cellular matrix surrounding the tumor.<sup>2</sup>

Based on the U.S. Food and Drug Administration (hematology/oncology) approved 23 new cancer medicines in 2012. In the present investigation eleven important drugs such as Ponatinib, Bosulif, Synribo, Kyprolis, Urosolic acid, Boswellic acid, Hydroxy urea, 3-amino propanesulphonic acid, Imatinib, Dasatinib and Nilotinib against human leukemia have been selected for the study. Physicians have prescribed various drugs during various period of time. Recently, Ponatinib was identified as drug (ligand) molecule for the blood cancer receptor. Such drugs have revolutionized treatment of this disease and allow most patients to have a good quality of life when compared to the former chemotherapy drugs.

### Drugs

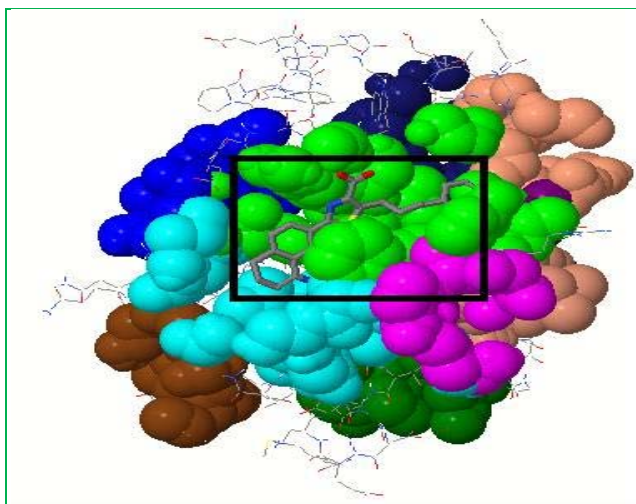
CML is now largely treated with targeted drugs called tyrosine kinase inhibitors, such as imatinib, dasatinib, nilotinib and bosulif. Whilst capable of producing significantly improved responses compared with the action of imatinib, neither dasatinib nor nilotinib could overcome drug resistance caused by one particular mutation found to occur in the structure of Bcr-Abl.<sup>3</sup> In the present study, imatinib, dasatinib, nilotinib and bosulif showed a very poor molecular interaction between the above drugs and the receptor molecule, which indicates poor potentiality of the above drugs.

Kyprolis was approved by the FDA for use in patients with multiple myeloma on 20<sup>th</sup> July 2012 and Onyx launched the drug in U.S. on 1<sup>st</sup> August 2012. The cost of the drug was \$10,000 per 28-day cycle, making it the most expensive FDA-approved drug for multiple myeloma.<sup>4</sup> In the present study, the molecular docking results showed only 0% interaction between the drug Kyprolis and the

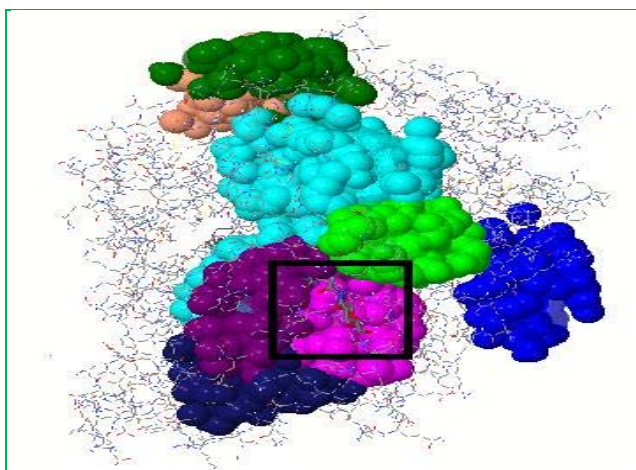


receptor Bcr-Abl indicating very poor effect of the drug over the disease.

Twenty-nine patients with malignant glioma were administered high doses of boswellic acids extract in tablet form (3 x 1200 mg/day) for a period of seven days. Although no effects on tumour proliferation were observed, peritumoral brain edema was reduced by 33%. In addition, neurological symptoms were markedly improved.<sup>5</sup> In the present study also molecular binding between boswellic acids and the Bcr-Abl was very least (5.1%) and thus the inhibition of the Bcr-Abl was also very poor.



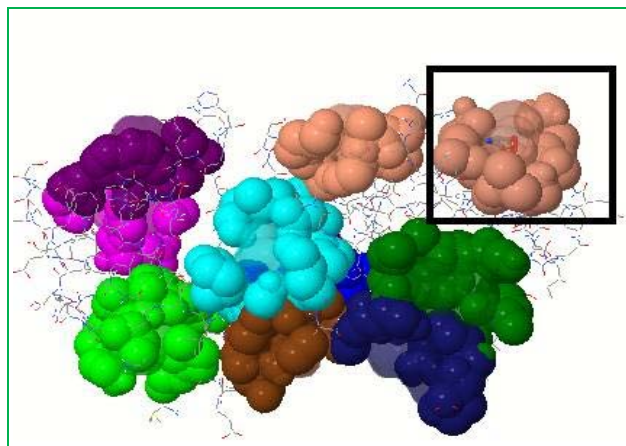
**Figure 6:** Binding of Ponatinib, Bosulif, Synribo and Boswellic acid drug with the major common active 2<sup>nd</sup> site (green site 2) of Bcr-Abl. (Note the binding of the smaller drug molecule on the receptor active site no.2)



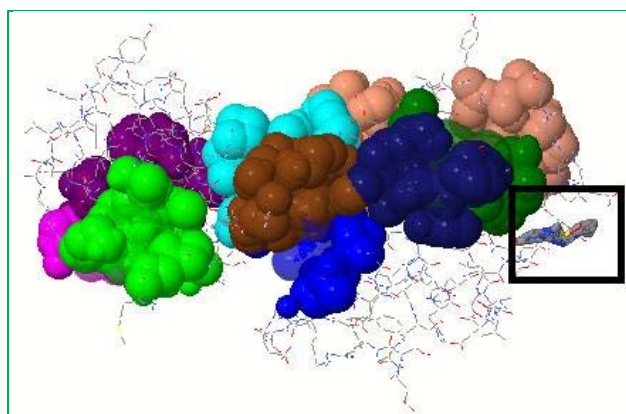
**Figure 7:** Binding of Ursolic acid drug with the major active site (site-1) of Bcr-Abl. (Note the binding of the smaller drug molecule on the receptor active site no.1).

Ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as antitumor agents.<sup>6</sup> Ursolic acid, a weak aromatase inhibitor, has potential use as a cardioprotective compound. Thus, overall results indicate that ursolic acid inhibits various carcinogens. These actions of ursolic acid may mediate its antitumorigenic effects.<sup>7</sup> Overall, these results suggest

that ursolic acid is involved in the prevention and treatment of multiple myeloma and other cancers.<sup>8</sup> In the present study also, the molecular binding between the ursolic acid and the Bcr-Abl was higher (68.1%) than that of other drug molecules and therefore this molecule is also having some potentiality in inhibiting the blood cancer.



**Figure 8:** Binding of 3-amino propane sulphonic acid drug with the major active site (site 8) of Bcr-Abl. (Note the binding of the smaller drug molecule on the receptor active site no.8)



**Figure 9:** Docking of Kyprolis, Hydroxy urea, Imatinib, Dasatinib and Nilotinib drugs with the Bcr-Abl receptor molecule. (Note the non binding nature of the smaller drug molecule on the receptor)

Hydroxyurea is a chemical antineoplastic agent that produces bone marrow suppression, megaloblastosis, and antitumor effects in animals and man in a variety of solid tumors and leukemia. Early clinical studies with hydroxyurea demonstrated bone marrow depression, megaloblastosis in the marrow, and objective improvements in a variety of solid tumors and acute and chronic leukemia. In the adult patients with chronic myelogenous leukemia treated with hydroxyurea, there was a prompt fall in leukocyte counts, decrease in splenomegaly, disappearance of blast cells from marrow and peripheral blood, and a rise in hemoglobin level. Hydroxyurea is as effective as busulfan and may be utilized as primary therapy of chronic myelogenous leukemia.<sup>9</sup> In the present study, the molecular interaction

between hydroxyurea and the leukemia causing Bcr-Abl showed only 0% precision and thus the inhibition of the hydroxyurea on blood cancer is also supposed to be nil.

The drug, Ponatinib, gained FDA approval in December 2012 for treatment of patients with resistant or intolerant CML. Just as with second generation TK inhibitors, early approval is being sought to extend the use of Ponatinib to newly diagnosed CML also. Ponatinib is an FDA approved oral drug candidate developed by ARIAD Pharmaceuticals for the treatment of chronic myeloid leukemia (CML) and Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). It is a multi-targeted tyrosine-kinase inhibitor. Some forms of CML, those that have the Bcr-Abl mutation, are resistant to current therapies such as imatinib. Ponatinib has been designed

to be effective against these types of tumors. In a trial conducted with CML patients in Dec 2012 good results were reported. At the 2010 annual meeting of the American Society of Hematology, ARIAD announced from a Phase I study of ponatinib in patients with resistant and refractory chronic myeloid leukemia (CML) and Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). The study demonstrated that in chronic-phase CML patients treated with ponatinib, 66 percent of patients in the trial achieved a major cytogenetic response, including 100 percent of patients who also had a Bcr-Abl mutation. Ponatinib was designed using ARIAD's computational and structure-based drug design platform to inhibit the enzymatic activity of Bcr-Abl with very high potency and broad specificity.<sup>3</sup>

**Table 1:** Molecular docking of drug (ligands) molecules with blood cancer causing receptor molecule Bcr-Abl

Ligand molecules	Molecular Formula	Mass	E-values	Ligand binding site on Bcr/Abl receptor	Bcr/Abl Precision rate
Ponatinib	C29H27F3N6O	532.559509 Da	-278.53	Predicted site 2	100
Bosulif	C26H31Cl2N5O4	548.461426 Da	-248.89	Predicted site 2	5.1
Synribo	C29H39NO9	545.621277 Da	-202.93	Predicted site 2	68
Kyprolis	C40H57N5O7	719.909912 Da	-250.20	Nil	0
Urosolic acid	C30H48O3	456.700287 Da	-180.48	Predicted site 1	68.1
Boswellic acid	C30H48O3	456.7003 Da	-169.43	Predicted site 2	5.1
Hydroxy urea	CH4N2O2	76.054703 Da	-53.06	Nil	0
3-amino propane sulphonic acid	C3H9NO3S	139.173492 Da	-79.58	Predicted site 8	33.3
Imatinib	C29H31N7O	493.602692 Da	-140.87	Nil	0
Dasatinib	C22H26ClN7O2S	488.005493 Da	-169.19	Nil	0
Nilotinib	C28H22F3N7O	529.183777 Da	-142.57	Nil	0

<sup>10</sup> is of the opinion that nilotinib and dasatinib work for 40-50 percent of the CML patients. Preclinical experiments indicated ponatinib acts against Bcr-Abl and all known mutant forms of the protein. Ponatinib also induced high response rates among the broader group of CML patients. An investigational drug in a phase I clinical trial reported in the current edition of The New England Journal of Medicine, out of 12 patients in the trial with chronic phase CML had a complete hematologic response (absence of CML cells in the blood) after treatment with ponatinib. Eleven had a major reduction in CML cells in the bone marrow and nine achieved a complete cytogenetic response.<sup>10</sup> Reported that Ponatinib is a promising new treatment for patients who have run out of options and its activity against a variety of mutations and in patients with no known mutations suggests a broad range of efficacy for this drug. The U.S. Food and Drug Administration in October also accepted a new drug application by ARIAD Pharmaceuticals for accelerated review of ponatinib for patients with CML. In the present investigation also the drug ponatinib showed a 100% molecular interaction between the drug, ponatinib and

the receptor Bcr-Abl indicating a very high potentiality of inhibiting the blood cancer receptor, Bcr-Abl.

The protein-ligand interaction plays an important role in determining the suitable drug for the treatment. The energy value obtained through docking is used as a criterion for the selection of drugs which involves the identification of lead molecules. The lead molecule is one with maximum interaction showing high negative e-value and higher percentage of precession rate at the binding site of receptor molecules. Thus docking helps in evolving the suitable drug for blood cancer. In the present investigation out of 11 drugs docked against Bcr-Abl receptor, ponatinib showed a high negative e-value (-278.53) and 100% (precision) succession rate.

## CONCLUSION

Bcr-Abl and Bcl-xL tyrosine-kinases are the two major enzymes responsible for the blood cancer leukemia. Bcr-Abl alone was found to be antigenic whereas Bcl-xL was non antigenic. Therefore only Bcr-Abl enzyme alone was taken into consideration for the study. To identify the effective and potential drug molecule for blood cancer, commonly used eleven drug molecules were selected for

the study. Molecular docking between the receptor molecule (Bcr-Abl) and the ligand (drug) molecules was carried out through which the precision rate of the binding and the energy value involved in the docking were determined. Most of the drug molecules recognized the binding site-2 of the Bcr-Abl molecule as the major active binding site in molecular docking. Among the various drugs used, ponatinib with the receptor molecule Bcr-Abl showed 100% binding and highest e-negative value (-278.53) indicating that the ponatinib is the effective and potential drug molecule for curing blood cancer.

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