COMPARATIVE STUDY OF MOLECULAR INTERACTIONS OF VINBLASTINE AND IMATINIB WITH BCR-ABL FUSION PROTEIN IN-SILICO FOR THE SELECTIVE ANTI-CML ACTIVITY OF VINBLASTINE

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Abstract

Aim: To study the comparative molecular interactions of Vinblastine and Imatinib with the active sites of Breakpoint Cluster Region (BCR)-Abelson (ABL) fusion protein using molecular docking analysis for the selective anti-Chronic myeloid leukemia (CML) activity.
Materials and Methods: In this study, the binding affinity and the type of molecular interaction between the Vinblastine and the active site amino acid residues of BCR-ABL fusion protein was studied in comparison with known BCR-ABL inhibitor Imatinib. The sample size was calculated by keeping pretest G power 80%. The sample size per group is 10 (N=10) and total sample size is 20. The protein structure of BCR-ABL fusion protein was collected from the protein data bank (PDB) website and the ligand structures were collected from the NCBI-PUBCHEM website. The binding energy (kcal/mol) was calculated using Autodock Vina Software. The non-covalent protein ligand interactions were detected using protein–ligand interaction profiler (PLIP) webserver. **Results:** The mean binding affinity of Imatinib (-10.36 kcal/mol) was significantly ($p=0.000$, $p<0.001$, 2-tailed t-test) higher than Vinblastine (-8.72 kcal/mol) towards the active sites of BCR-ABL fusion protein.
Conclusion: Though the binding affinity of Vinblastine was significantly less compared to Imatinib, Vinblastine can make strong hydrogen bonds and hydrophobic interactions with the aminoacid residues at the active sites of BCR-ABL fusion protein. It suggests that, Vinblastine may bind selectively to the cells of CML and inhibit their proliferation and can act as a novel Anti-CML agent.

Keywords: Vinblastine, Imatinib, BCR-ABL fusion protein, Novel Anti-CML agent, Molecular docking, Autodock Vina software.

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1. Introduction

Nowadays, the major cause of death and disease all over the world is Cancer. Nearly ten millions of people are diagnosed with cancer each year around the world, with more than half of those diagnosed dying from it (Johnston et al. 2021). Chronic Myeloid Leukemia (CML) is a hematological condition that is characterized by the malignant growth of bone marrow stem cells which is caused by a reciprocal translocation between chromosomes 9 and 22, resulting in the Breakpoint Cluster Region (BCR)-Abelson (ABL) fusion gene, which produces a 210-kd protein with tyrosine kinase activity ((Jabbour and Kantarjian 2020)). Protein kinases can get mutated, trapped in the "on" position, and induce uncontrolled cell growth, which is a prerequisite for cancer development (Arora and Scholar 2005). The discovery is crucial because researchers revealed that abnormal activation of a number of receptor tyrosine kinases (RTKs) and their ligands can lead to uncontrolled tumor cell proliferation. As a result, the BCR-ABL fusion protein becomes a molecular target for CML progression inhibition. Novel anti-CML agents are chemicals that bind with the BCR-ABL fusion protein and decrease its tyrosine kinase activity. Molecular Docking defines the atomic-level interaction between a small molecule and a protein, allowing us to analyze small molecule behavior in target protein binding sites and highlight critical biochemical processes (Meacham and Morrison 2013). Molecular Docking studies are critical in the development of novel anti-CML agents with higher selectivity (Cui et al. 2020). In the current study, the binding affinity of Vinblastine towards the prospective target site of CML, the BCR ABL fusion protein, was investigated, which aids in the in silico validation of Vinblastine as a novel anti-CML agent.

Total number of articles published related to this topic in recent years are 23 in Pubmed, 2,810 in google scholar and 527 in science direct. In the pathogenesis of human cancers preventions, some cancer therapeutic drugs are being used. Among many of them, Imatinib is one such drug that selectively targets CML cells and inhibits cancer progression (Soverini et al. 2008). It specifically inhibits tyrosine kinase, especially BCR-ABL fusion protein in CML and c-kit in Gastrointestinal stromal tumors (GIST) (Balachandran and DeMatteo 2014). Imatinib not only reduces this tumor but also other cancerous tumors. Imatinib is being marketed with the brand names Gleevec and Glivec. This tyrosine kinase inhibitor is also nicknamed as the magical bullet (Iqbal and Iqbal 2014). Vinblastine is a plant based alkaloid that is used to treat various types of human cancers. It has been used to treat Hodgkin's lymphoma, lung cancer, bladder cancer, brain tumor, melanoma, and testicular cancer (Gu and Liang 2022; Zhao et al. 2022). It mainly inhibits the microtubule assembly during mitosis, spiral aggregates are coiled by inducing self-association of tubulin and arrest the cell division (Chatterjee 2003; Deer 2009).

Our institution is passionate about high quality evidence based research and has excelled in various domains (Vickram et al. 2022; Bharathiraja et al. 2022; Kale et al. 2022; Sumathy et al. 2022; Thanigaivel et al. 2022; Ram et al. 2022; Jothi et al. 2022; Anupong et al. 2022; Yaashikaa, Keerthana Devi, and Senthil Kumar 2022; Palanisamy et al. 2022).Though the anti-cancer activity of Vinblastine was well reported, the selective inhibition of CML by Vinblastine has not been reported. The authors are expertised in the field of molecular docking using Autodock Vina Software. In the current study, The *in-silico* molecular interaction between Vinblastine and BCR-ABL fusion protein was studied in comparison with a known BCR-ABL inhibitor Imatinib for the selective anti-CML activity of Vinblastine.

2. Materials and Methods

Saveetha School of Engineering's simulation lab was used for this research. This study has no ethical implications. Two groups are involved in this research. Binding affinity of Imatinib (N=10) towards BCR-ABL fusion protein as positive control group and Binding affinity of Vinblastine (N=10) towards BCR-ABL fusion protein as study group. The sample size was calculated using previous study results, with an alpha error-threshold of 0.05, pretest G power of 80%, and a 95% Confidence Interval (CI) (Rajendran et al. 2016). As a result, the overall sample size was calculated as 20.

Preparation of Protein: The structure of the BCR-ABL (PDB ID 5MO4) protein has been obtained at a resolution of 2.17Å from the PDB (Protein Data Bank) database. The protein's structure is saved in PDB format. The water molecules in the protein structure are changed into PDBQT format during conversion. The water molecules in the protein structure are eliminated during conversion and replaced with polar hydrogen atoms. Finally, kollman charges are incorporated into the protein structure, and the PDBQT structure is saved (Rajendran et al. 2016; Makala et al. 2021).

Preparation of ligands: The determined Ligand structures in this study are Vinblastine and Imatinib (Pubchem CID 13342 and 5291, respectively)

which have been obtained from the PUBCHEM database. The structures of obtained ligands are in SDF format. The SDF format can be converted into PDB format using PYMOL software. The PDB format of the ligand structures are converted into PDBQT format by using the tools of Autodock (1.5.6) (Rajendran et al. 2016; Makala et al. 2021).

Molecular docking using autodock vina software: To carry on the process of molecular docking in silico, Autodock Vina is an open source program that is used. This software program was designed and implemented by Dr.Oleg Trott in the molecular graphics lab at the Scripps Research Institute to predict the scores of the binding energy for the interactions of the targeted protein-ligand. The binding site of the residues of amino acids of the defined protein have been determined and applied for the autodock vina software. For analyzing the binding mode, the docked conformations that had the higher score of fitness have been taken. Thereafter, the autodock vina program has been run, and the binding energies have been observed, saved and visualized (Rajendran et al. 2016; Makala et al. 2021).

Protein–ligand interaction profiler (PLIP) analysis: The output PDB files obtained from autodock analysis were uploaded into the PLIP web server for the identification and visualization of non-covalent protein ligand interactions in the 3D structures (Salentin et al. 2015).

Statistical Analysis

IBM-SPSS (28.0.0) was used for the comparison of binding affinities for the BCR-ABL fusion protein with Vinblastine and BCR-ABL fusion protein with Imatinib. As the variables were independent to each other, to compare the binding affinities of different inhibitors an independent t-test was used.

3. Results

In this research work, the binding energy of the protein-ligand complex was estimated by the molecular docking analysis using Autodock Vina Software. Table 1 shows the binding affinities of Imatinib towards the active site of BCR-ABL fusion protein. The compound Imatinib binds with the active sites of BCR-ABL protein with higher affinity of -10.6 kcal/mol (mean of -10.36 kcal/mol). Table 3 and Fig. 1 show the interaction of Imatinib with amino acid residues and nature of interaction at the active site of BCR-ABL fusion protein. The Imatinib interacts with active binding site amino acid residues LEU267, ALA288, ILE334, PHE336, TYR345, LEU389, and PHE401 through Hydrophobic interactions, and with the residues MET337 and THR338 via hydrogen interactions. The type of non covalent interactions of Imatinib with active site amino acid resiues were visually shown in Fig. 4, obtained from PLIP server.

Table 2 shows the binding affinities of Vinblastine towards the active sites of BCR-ABL fusion protein measured using autodock vina software. The Vinblastine shows a higher binding affinity of -8.8 kcal/mol (mean of -8.72 kcal/mol) towards the target BCR-ABL fusion protein. Table 4, Fig. 2 and Fig. 4 shows the different types of interactions of Vinblastine with the amino acid residues at the active sites of BCR-ABL fusion protein. Vinblastine interacts with the amino acid residues namely ASN133 and TYR361 via hydrophobic interactions and ASN133, ASN240, and ASN316 through hydrogen bonding.

The statistical significance of the results were analyzed by performing an independent sample t-test using IBM SPSS software. Table 5 and Table 6 demonstrated the grouping of samples for the analysis of independent sample t-test using IBM SPSS software. Fig. 5 and Table 5 show that, there is a statistically significant difference ($p=0.000$, $p<0.001$, 2-tailed t-test) between the binding affinities of Vinblastine and Imatinib towards BCR-ABL fusion protein. Table 6 shows the mean difference, significance, std error difference of the binding energies of the BCR-ABL fusion protein with Vinblastine and Imatinib.

4. Discussion

With the help of targeted therapy, the creation of substantial host cell toxicity could be reduced or eliminated which is one of the many challenges in the field of cytotoxic chemotherapy (Danchev, Nikolova, and Momekov 2008; Gu and Liang 2022). In patients with CML and other malignancies caused by Imatinib-specific aberrations, the molecularly targeted anticancer medicine called Imatinib has shown promising results in them (Melge et al. 2019); (Guilhot 2004). The molecular docking is a promising tool widely used in the discovery of novel Anti-CML agents with high selectivity index (Cui et al. 2020). In the current study, the probability of selective anti-CML activity of Vinblastine was tested in comparison with a standard BCR-ABL inhibitor Imatinib in silico using Autodock Vina software by molecular docking analysis. The results show that Vinblastine can interact with BCR-ABL protein with a higher affinity of -8.8 kcal/mol. The active binding site residues of Vinblastine namely ASN133, and TYR361 interact via hydrophobic interactions and ASN133, ASN240, and ASN316 interact via hydrogen bonds.

The binding affinity of Vinblastine was significantly ($p=0.000$, $p<0.001$, 2-tailed t-test) lesser when compared to Imatinib. But, the results says that still it can make effective interaction with the active site amino acid residues of BCR-ABL fusion protein via both hydrogen and hydrophobic interactions. Already it has been reported that the Vinblastine has anti-cancer attributes towards various types of human cancers (Gu and Liang 2022; Zhao et al. 2022). The current study results show that it can effectively interact with amino acid residues at the active sites of the BCR-ABL fusion protein. Hence, Vinblastine may selectively binds to the CML cells and inhibit their proliferation.

The current study was limited to in-silico molecular simulation studies between the drug molecule and the target protein. Further validation is required through *in vitro* and *in vivo* studies in cell line and animal models, respectively.

5. Conclusion

In this study, the in silico probability of selective anti-CML activity of Vinblastine was tested. The results suggest that Vinblastine can make both hydrogen and hydrophobic interactions with the amino acid residues at the active site of BCR-ABL fusion protein. This demonstrates Vinblastine may inhibit the proliferation of CML cells by selectively binding at the active sites of BCR-ABL fusion protein. But, in vitro and in vivo studies are also required for further validation to confirm the Vinblastine as a novel anti-CML agent.

Declarations

Conflict of interest

In this manuscript, there are no conflicts of interest.

Author contribution

Author KVSM was involved in data collection, data analysis and manuscript writing. Author MRCR was involved in conceptualisation, data validation and critical review of manuscript.

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Tables and Figures

Table 1. The binding affinities of Imatinib with the active sites of BCR-ABL protein analyzed through Autodock Vina Software.

S. No.	Binding affinity(kcal/mol)
1	-10.5
2	-10.4
3	-10.2
4	-9.9
5	-10.5
6	-10.5
7	-10.2
8	-10.6
9	-10.2
10	-10.6

Table 2. The binding affinities of Vinblastine with the active sites of BCR-ABL fusion protein analyzed through Autodock Vina Software.

S. No.	Binding affinity (kcal/mol)
1	-8.7
2	-8.7
3	-8.7
4	-8.7
5	-8.7
6	-8.7

7	-8.8
8	-8.8
9	-8.7
10	-8.7

Table 3. Interaction of Imatinib with the amino acids at the active sites of BCR-ABL fusion protein. The active binding site residues of Imatinib namely LEU267, ALA288, ILE334, PHE336, TYR345, LEU389, PHE401 forms hydrophobic interaction. The residue namely MET337, THR338 forms Hydrogen interactions.

S. No.	Compound name	Residue	Amino acid	Distance	Nature of Interactions
1	**Imatinib**	267	LEU	3.01	Hydrophobic
		267	LEU	3.48	Hydrophobic
		288	ALA	3.67	Hydrophobic
		334	ILE	3.85	Hydrophobic
		336	PHE	3.58	Hydrophobic
		345	TYR	3.67	Hydrophobic
		345	TYR	3.79	Hydrophobic
		389	LEU	3.92	Hydrophobic
		389	LEU	3.35	Hydrophobic
		401	PHE	3.90	Hydrophobic
		337	MET	2.66	Hydrogen
		338	THR	2.08	Hydrogen

Table 4. Interaction of Vinblastine with BCR ABL fusion protein. The active binding site residues of Vinblastine namely ASN133, TYR361 forms hydrophobic interactions. The residues namely ASN133, ASN240, ASN316 form hydrogen interactions.

S. No.	Compound name	Residue	Amino acid	Distance	Nature of Interactions

1	**VINBLASTINE**	133	ASN	3.45	Hydrophobic
		361	TYR	3.87	Hydrophobic
		133	ASN	2.91	Hydrogen
		240	ASN	3.11	Hydrogen
		316	ASN	3.97	Hydrogen

Table 5. The group statistics data of binding affinities of Vinblastine and Imatinib performed through Independent sample t-test using IBM SPSS software.

Group	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
Vinblastine	10	-8.72	.04216	.01333
Imatinib	10	-10.36	.22706	.07180

Table 6. Independent sample t-test in predicting the significance, mean difference, std error difference of binding affinities of BCR-ABL fusion protein with different inhibitors.

Energy	**Levene's Test for Equality of Variances**		**t-test for Equality of Means**						
	F	**Sig.**	**t**	**df**	**Significance (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**95% Conf. Interval Lower**	**95% Conf. Interval Upper**
Equal Variances assumed	18.829	.000	22.457	18	.000	1.64000	.07303	1.48657	1.79343
Equal Variances not assumed			22.457	9.620	.000	1.64000	.07303	1.47640	1.80360

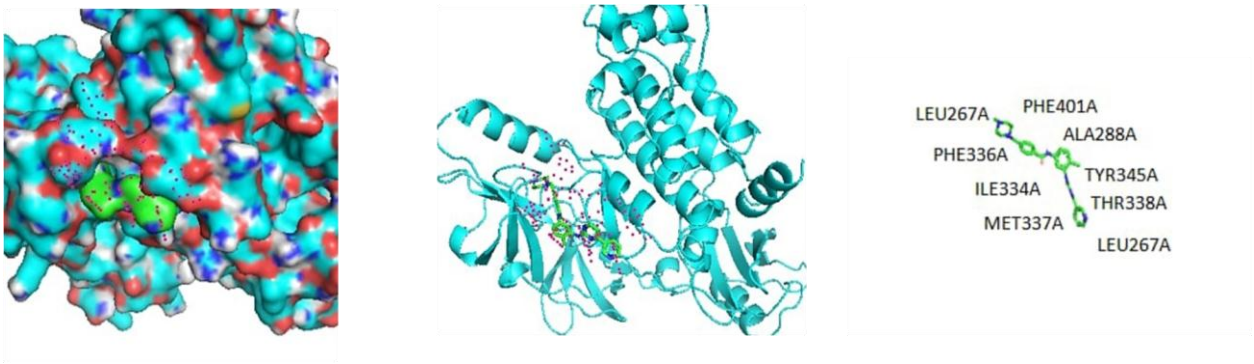

Fig. 1. Interaction analysis of Imatinib in the active site pocket of BCR ABL fusion protein. The structure of BCR ABL and Imatinib are represented in green and blue sticks. Amino acid residues of BCR ABL fusion protein namely LEU267, PHE336, ILE334, MET337, PHE401, ALA288, TYR345, THR338, LEU267 interact with Imatinib.

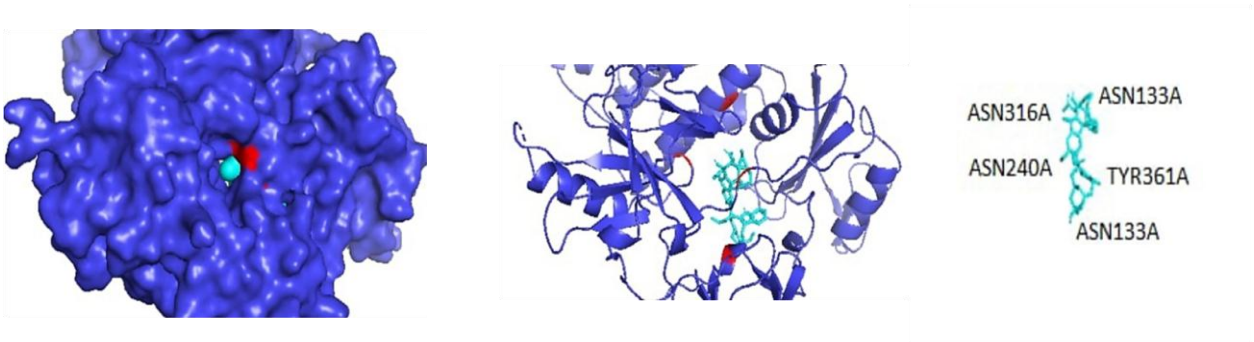

Fig. 2. Interaction analysis of Vinblastine in the active pocket site of BCR ABL fusion protein. The structure of BCR ABL and Vinblastine are represented in red and blue sticks. Amino acid residues of BCR ABL protein namely ASN316, ASN240, ASN133, TYR361, ASN133 interact with Vinblastine.

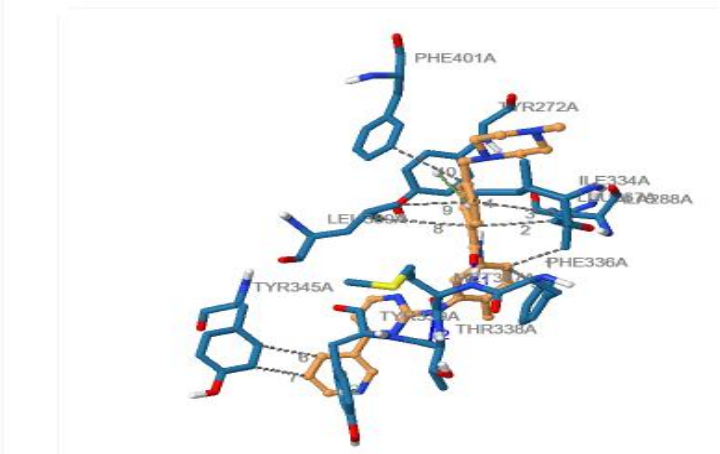

Fig. 3. Interaction chains of Imatinib with BCR ABL fusion protein with residues in which blue color represents the protein, orange represents the ligand, gray color represents water, yellow color represents charge center, white color represents aromatic ring center, pink color represents metal ion, …. Represents hydrophobic interaction, — represents hydrogen bond, …. Represents pi-stacking (perpendicular).

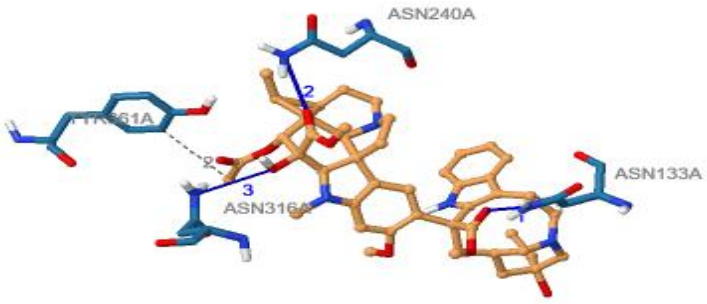

Fig. 4. Interaction chains of Vinblastine with BCR ABL fusion protein with residues in which blue color represents protein, orange represents ligand, gray color represents water, yellow represents charge center, white color represents aromatic ring center, pink represents metal ion, …. Represents hydrophobic interactions, — represents hydrogen bond.

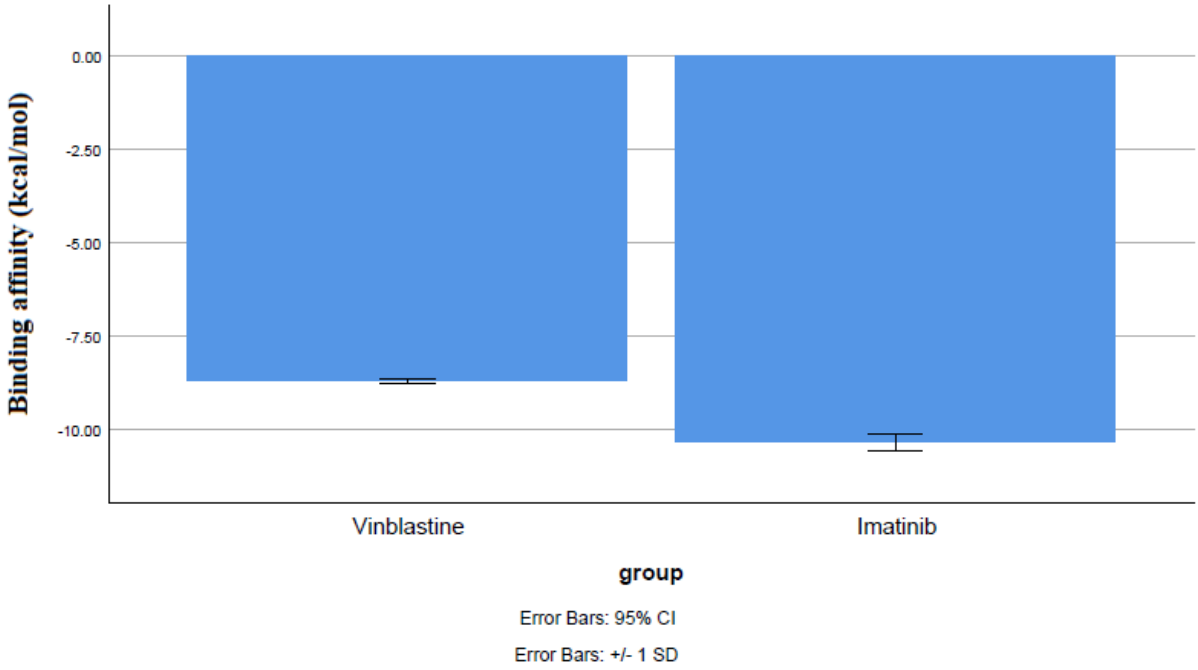

Fig. 5. The binding energies of Vinblastine and Imatinib towards the active site of BCR-ABL fusion protein. The X axis represents the screening of inhibitors and the Y axis represents binding energy of the targeted molecules. Results were represented as mean +/- 1SD and the error bars with 95% CI.