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

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Abstract

Aim: To study the comparative molecular interactions of Carvacrol 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 the current study, the binding affinity and the type of molecular interaction between the Carvacrol 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.
Results: The mean binding affinity of Imatinib was -10.22 kcal/mol and Carvacrol was -7.46 kcal/mol towards the active sites of BCR-ABL fusion protein. There was a significant ($p=<0.001$, $p<0.001$, 2-tailed t-test) difference between the mean binding affinities of Carvacrol and Imatinib towards the active sites of BCR-ABL fusion protein. Carvacrol interacts with active site amino acid residues via both hydrogen and hydrophobic interactions. **Conclusion:** Though the binding affinity of Carvacrol was significantly less than Imatinib, Carvacrol may bind selectively to the active sites of BCR-ABL fusion protein. Hence, Carvacrol may attach specifically to the CML cells and inhibit their proliferation and could be used as a novel anti-CML agent to treat CML.

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

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1. Introduction

Chronic myeloid leukemia (CML) is a type of cancer characterized by production of an increased number of white blood cells in bone marrow (Jabbour and Kantarjian 2020). It is estimated that 9,110 people were diagnosed with CML in the year 2021. Among them 1,220 patients were dead ("Key Statistics for Chronic Myeloid Leukemia" n.d.) (Eden and Coviello 2022). Hence, it is necessary to develop novel Anti-CML agents to cure CML (Brogi et al. 2020). Before going to the wet lab experiments, the In-Silico simulation studies would help us to get the possible interaction of the drug candidates with the molecular targets of CML (Cui et al. 2020). The major target for CML is a tyrosine kinase, Breakpoint Cluster Region (BCR)-Abelson (ABL) fusion protein. The inhibitors of BCR-ABL fusion protein can act as novel anti-CML agents (Minciacchi, Kumar, and Krause 2021). The higher affinity towards the target site indicates the greater selectivity of the test compound. The greater selectivity of the test compound ensures minimal toxicity (Mencher and Wang 2005). The molecular docking studies have great importance in the discovery of novel anti-CML agents. In the present study, the molecular interactions of imatinib and carvacrol with active site amino acid residues of BCR-ABL fusion protein were studied in-silico using autodock vina software for the evaluation of Carvacrol as a novel anti-CML agent.

Total number of articles published related to this topic are 9 in Pubmed, 2,590 in google scholar and 162 in science direct. Imatinib mesylate is a known tyrosine kinase inhibitor that has been used to target BCR-ABL fusion protein to treat CML patients (Soverini et al. 2008). Imatinib binds to the kinase's ATP-binding site, preventing it from phosphorylating its substrates and activating growth-promoting signal transduction pathways. Imatinib efficiently inhibits tumor cell growth by targeting the kinases bcr-abl in CML and c-kit i-n GIST. (Bunce and Descartes 2000). The phenylaminopyrimidine derivative designed to inhibit the CML-specific tyrosine kinase bcr-abl has also been demonstrated to target c-abl and Arg (abl-related gene) kinases (Ertmer et al. 2007). These kinases are engaged in so many key pathways that influence cell growth and survival, their abnormal or constitutive activation activates a slew of signal transduction pathways eventually leading to uncontrolled cell proliferation and cancer. Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a phenol monoterpene that is the primary phytochemical in the essential oil of fragrant Lamiaceae plant species. Carvacrol is known to exert anti-inflammatory, anticancer, antioxidant, and cytotoxic action (Ultee et al. 2000). Carvacrol has been shown to inhibit the proliferation of lung, breast, and colon cancer cell lines (Koparal, Tansu Koparal, and Zeytinoğlu 2003).

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).It has been reported that Carvacrol has antiproliferative activity against a variety of cancer cells. But, the selective inhibition of CML by Carvacrol 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 Carvacrol and BCR-ABL fusion protein was studied in comparison with a known BCR-ABL inhibitor Imatinib for the selective anti-CML activity of Carvacrol.

2. Materials and Methods

This study was conducted in a Simulation lab, Saveetha School of Engineering. There is no Ethical concern for this study. There are two groups involved, Group 1 is binding affinity of Imatinib (N=10 Positive control group) with BCR-ABL Fusion Protein and Group 2 (N=10 Study group) is binding affinity of Carvacrol with BCR-ABL Fusion Protein. The sample size calculation was done using previous study results by keeping alpha error-threshold by 0.05, pretest power 80% and 95% Confidence Interval (CI) (Rajendran et al. 2016). Therefore the total sample size was 20.

Preparation of protein: The structure of the BCR-ABL protein has been obtained from PDB (Protein Data Bank) online website at the resolution 2.17Å. The structure of the protein is in PDB format using autodock tools. The PDB format of the protein structure is converted into PDBQT format. During conversion the water molecules in the protein structure get removed and added with the polar hydrogen atoms. Finally kollman charges are included in the protein structure and the resultant in PDBQT structure is stored (Rajendran et al. 2016).

Preparation of ligand: Ligand structure of Carvacrol and Imatinib (Pubchem CID 10364 and 5291, respectively) have been obtained from PubChem online website. The structure of the ligand is in SDF format. Using PYMOL software, The SDF format was converted into PDB format. The PDB format of the ligand structures are converted into PDBQT format by using an autodock tool (Rajendran et al. 2016).

Molecular docking: The process of molecular docking is done by using autodock vina software, Autodock vina is an open source program for doing molecular docking in silico. It was designed in the molecular Graphics lab at The Scripps Research institute this was done to predict the scores of the binding energy for the interactions of the targeted protein and ligand. The sites of binding of the residues of amino acids of the defined protein have been determined and applied for the autodock vina software. The docked conformations that had the higher score of fitness have been taken for analyzing the binding mode. Finally, the autodock vina programme was run and binding energies were observed, recorded and displayed (Rajendran et al. 2016).

Statistical Analysis

The comparison of binding affinities for the BCR-ABL fusion protein with Imatinib and BCR-ABL fusion protein with Carvacrol was done in IBM-SPSS (27.0.1) software. Since the variables were independent of each other, an independent t-test was used to compare the mean binding affinities of different inhibitors.

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 inhibitor towards the active sites of BCR-ABL fusion protein. The compound Imatinib binds with the active site amino acid residues of BCR-ABL fusion protein with higher affinity of -10.7 kcal/mol (Mean of -10.22 kcal/mol). Table 3 and Fig. 1 shows the type of interaction between the amino acid residues and the Imatinib. The Imatinib interacts with amino acid residues via both hydrogen bonding and hydrophobic interaction. The active site amino acid residues that interacts with Imatinib are TRP118, ASN240, and VAL247 via hydrophobic interactions and LEU395, PRO242, PRO315, LEU321, SER94, and VAL244 via hydrogen interaction.

Table 2 shows the binding affinities of Carvacrol towards the active sites of BCR-ABL fusion protein. The compound Carvacrol binds with the active site amino acid residues of BCR-ABL fusion protein with higher affinity of -7.5 kcal/mol (Mean of -7.46 kcal/mol). Table 4 and Fig. 2 shows the type of interaction between the amino acid residues and the Carvacrol. The Carvacrol interacts with amino acid residues via hydrogen bonding and hydrophobic interaction. The active site amino acid residues that interacts with Carvacrol are via ILE334, VAL275, TYR272, TYR272, LEU267, ILE334, PHE336, TYR339, TYR349, and LEU389 hydrophobic interactions and MET337 and THR338 via hydrogen interaction.

Table 5, Fig. 3, and table 6 show the group statistical analysis based on unpaired sample t-test performed using IBM SPSS software. The results show that there was a significant ($p=<0.001$, $p<0.001$, 2-tailed t-test) difference between the mean binding affinities of Carvacrol and Imatinib.

4. Discussion

Since the CML is highly fatal and treatment options are limited, there is an urgent need for the development of novel anti-CML agents for treating CML patients (Jabbour and Kantarjian 2020). The molecular docking studies serve as a promising tool in the field of drug discovery for developing highly selective and specific novel anti-CML agents (Cui et al. 2020). In the current study, we have studied the comparative interaction of Carvacrol in comparison with known BCR-ABL inhibitor Imatinib. The results show that the carvacrol binds with amino acid residues at the active site of BCR-ABL fusion protein with higher affinity of -10.5 kcal/mol (Mean of -9.37 kcal/mol). Generally, The binding affinity of the molecule is based on the effective hydrophobic and hydrogen interactions present between the protein and ligand (Danchev, Nikolova, and Momekov 2008). The Carvacrol interacts with the active site amino acid residues of BCR-ABL fusion protein via both hydrophobic and hydrogen interactions.

The mean binding affinity of carvacrol was significantly ($p=<0.001$, $p<0.001$, 2-tailed t-test) lesser than Imatinib but still Carvacrol make effective interaction with the active site amino acid residues of BCR-ABL fusion protein Also, It has been already reported that Carvacrol has anti-proliferative activity against various types of cancer cells (Koparal, Tansu Koparal, and Zeytinoğlu 2003). Hence, Carvacrol may selectively bind to the active sites of BCR-ABL fusion protein and inhibit the proliferation of CML.

The limitation of the present study is that it is entirely In-silico simulation work which will provide only the details of possible interactions between the target molecule and the test compounds. To validate these In-silico interactions further experimentation is required through *in vitro* and *in vivo* methods.

5. Conclusion

The In-silico studies performed in this study show that Carvacrol can interact with the major drug target site of CML, BCR-ABL fusion protein.

Since the Carvacrol has anti-proliferative activity and it is evident from the current study that it may selectively bind to BCR-ABL fusion protein, it may act as novel anti-CML agent for treating CML. But, further *in vitro* and *in vivo* studies are needed to evaluate the anti-CML activity of Carvacrol.

Declaration

Conflict of Interests

No conflict of interests in this manuscript

Author Contribution

Author KM was involved in methodology creation, simulation, data collection, data analysis, manuscript writing. Author MRCR was involved in conceptualization, guidelines 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	-10.6
2	-10.4
3	-10.2
4	-10.2

5	-10.1
6	-10.7
7	-10.3
8	-10.5
9	-9
10	-10.2

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

S. No.	**Binding affinity(kcal/mol)**
1	-7.5
2	-7.5
3	-7.5
4	-7.5
5	-7.5
6	-7.5
7	-7.5
8	-7.5
9	-7.1
10	-7.5

Table 3. Interaction between Imatinib and bcr-abl. The imatinib active binding site residues, TRP118, ASN240, VAL247, form hydrophobic interactions. The residues respectively LEU395, PRO242,PRO315,LEU321,SER94,VAL244 form hydrogen interaction.

S. No.	**Compound Name**	**Residue**	**Amino acid**	**Distance**	**Nature Of Interaction**
1	**Imatinib**	118	TRP	3.68	Hydrophobic
		240	ASN	3.27	Hydrophobic
		247	VAL	3.76	Hydrophobic
		315	PRO	3.35	Hydrogen

		321	LEU	3.49	Hydrogen
		395	LEU	3.58	Hydrogen
		395	LEU	3.36	Hydrogen
		94	SER	3.34	Hydrogen
		241	LYS	3.65	Hydrogen
		242	PRO	3.49	Hydrogen
		244	VAL	3.71	Hydrogen
		319	GLN	3.31	Hydrogen

Table 4. Interaction of Carvacrol with bcr-abl, The carvacrol active binding site residues,respectively ILE334, VAL275, TYR272, TYR272, LEU267, ILE334, PHE336, TYR339, TYR349, LEU389, forms hydrophobic interactions.The residues respectively MET337, THR338 forms hydrogen interactions.

S. No.	Compound Name	Residue	Amino Acid	Distance	Nature Of Interactions
1	**Carvacrol**	267	LEU	3.69	Hydrophobic
		272	TYR	3.68	Hydrophobic
		275	VAL	3.60	Hydrophobic
		275	VAL	3.67	Hydrophobic
		288	ALA	3.57	Hydrophobic
		334	ILE	3.74	Hydrophobic
		336	PHE	3.73	Hydrophobic
		389	LEU	3.76	Hydrophobic
		389	LEU	3.42	Hydrophobic
		401	PHE	3.51	Hydrophobic
		401	PHE	3.48	Hydrophobic
		335	GLU	3.68	Hydrogen

		337	MET	2.88	Hydrogen
		272	TYR	4.99	π-Stacking

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

Group	**N**	**Mean**	**Std.Deviation**	**Std. Error Mean**
Imatinib	10	-10.22	.471	.149
Carvacrol	10	-7.46	.126	.040

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

Independent Samples Test									
	Levene's test for equality of variances		**t-test for Equality of means**						
Energy	**F**	**sig.**	**t**	**df**	**Significance (2-tailed)**	**Mean difference**	**std.error difference**	**lower**	**upper**
Equal variances assumed	2.986	.101	-17.899	18	<.001	-2.760	.154	-3.084	-2.436
Equal variances not assumed			-17.899	10.292	<.001	-2.760	.154	-3.102	-2.418

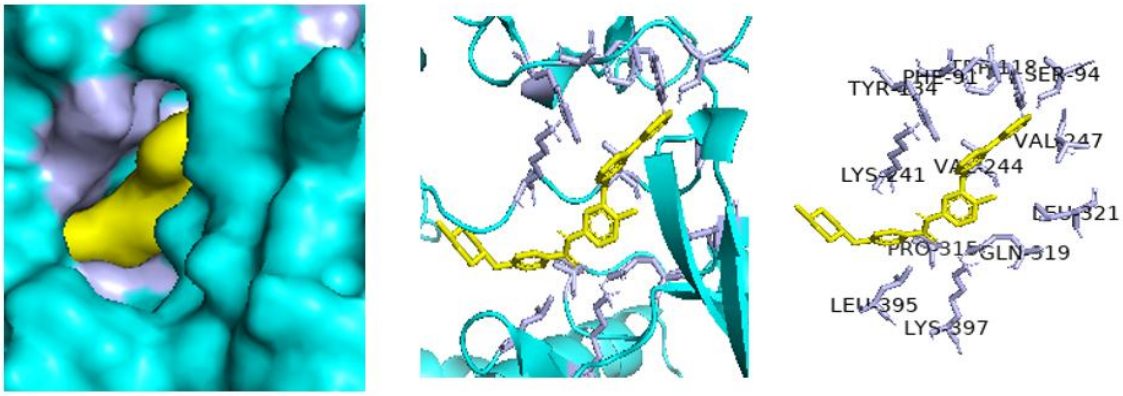

Fig. 1. Interaction analysis of Imatinib in the active pocket site of BCR-ABL fusion protein. The structure of BCR-ABL and Imatinib are represented in grey and yellow sticks . Amino acid residues of BCR ABL protein namely TRP118, ASN240, VAL247, LEU395, PRO242, PRO315, LEU321, SER94, and VAL244 interact with Imatinib.

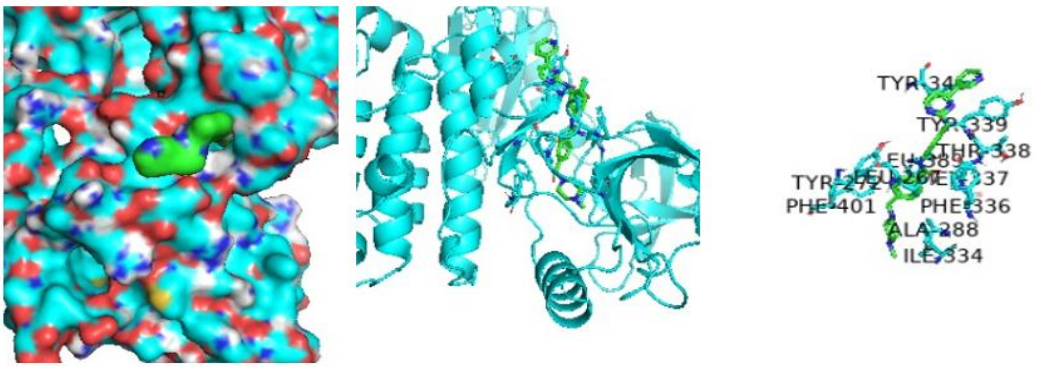

Fig. 2. Interaction analysis of Carvacrol in the active pocket site of BCR-ABL fusion protein. The structure of BCR-ABL and Carvacrol are represented in green and blue sticks. Amino acid residues of BCR-ABL protein namely TRY34, TRY339, THR338, PHE336, ALA288, ILE334, PHE401,TRY272 interact with Carvacrol.

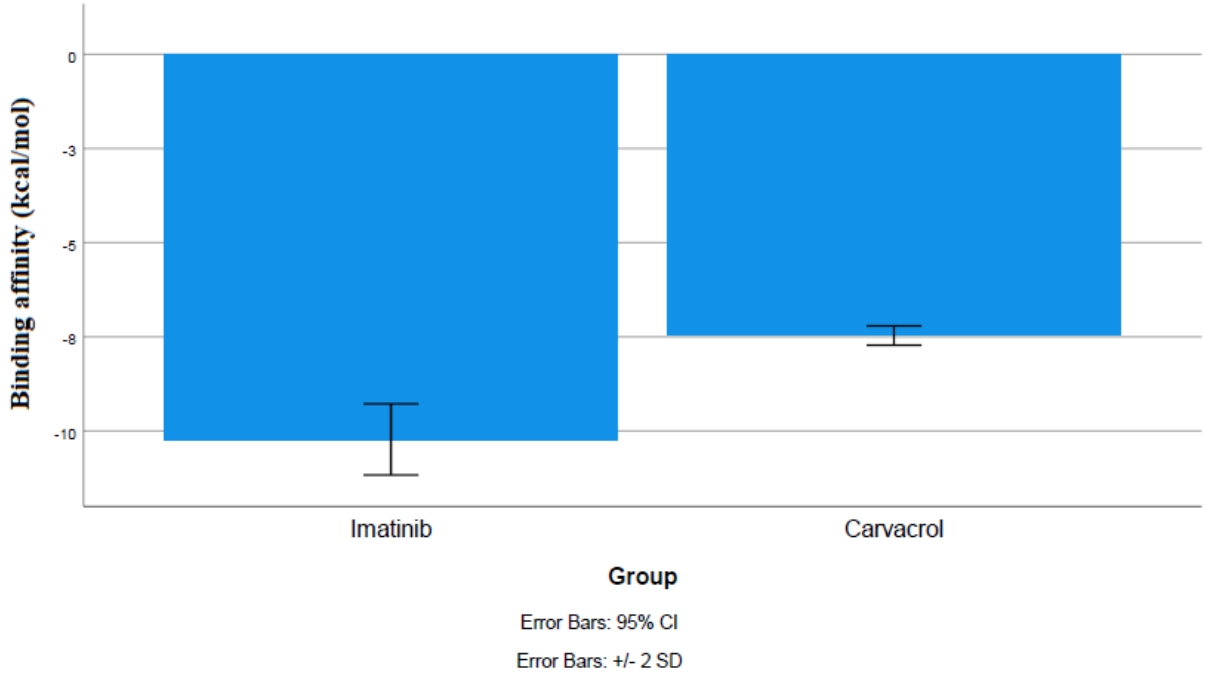

Fig. 3. The binding energies of Imatinib and Carvacrol towards the active sites of BCR-ABL fusion protein. The X axis represents the screening of inhibitors and the Y axis represents binding energy of the targeted molecules. Results were represented as Mean ± 1SD and the error bars with 95% CI.