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Structure-Based Virtual Screening of Eugenol Against CTNNB1, Ep300, and CD44

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Running title: Structure-Based Virtual Screening of Eugenol

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ABSTRACT

Eugenol is a natural component obtained mainly from cloves. It is proven that eugenol has an anticancer and anti-oxidant properties in many cancers. Eugenol can also inhibit the expression of β-catenin in breast and lung cancer. CTNNB1 is a gene that encodes β-catenin which is the intracellular scaffold protein and a crucial component in the Wnt signaling pathway and responsible for carcinogenesis of various types of cancer. EP300/p300 is found to be a nuclear transcriptional regulator of Wnt signaling and CD44 is a β-catenin target gene. Structure-based virtual screening has been used in the early stage of drug discovery to discover small ligand molecules. The study aims to predict the binding mode of eugenol as a ligand with known three-dimensional structure of CTNNB1, EP300, and CD44. AutoDock Vina was utilized to perform molecular docking analysis of the ligand-receptor complexes. Ligand-binding affinity was predicted by computing negative Gibbs free energy (-ΔG) using AutoDock Vina scoring function (Kcal/mol). This study revealed the significant binding energy between eugenol-CTNNB1, eugenol-EP300, eugenol-CD44, and the standard erlotinib-CTNNB1, erlotinib-EP300, and erlotinib-CD44 complexes.

***Key words*:** Eugenol, β-catenin, Wnt signaling, Molecular docking, AutoDock Vina

1. INTRODUCTION

Cancer is a chief cause of death globally. Though there is an advancements in treatment strategies, patients suffer from poor prognoses. To fulfill this, there are plenty of natural resources available for cancer chemotherapy. There has been well documented that flavonoids, terpenoids, phenolic acids, stilbenes, phenylpropanoids, etc shows the anticancer properties. Among them a phenylpropanoid, eugenol a promising candidate against leukemia, melanomas, gliomas, osteosarcomas, breast, and lung cancer, etc. (Padhy et al., 2022). Wnt signaling pathway involved in embryonic development, and tissue homeostasis. The genetic change in the Wnt signaling gives rise to many human cancers (Zhong & Virshup, 2020). The activated *Wnt*-signaling allows the accumulation of *β-catenin* in the nucleus and further act as a promoter to initiate transcription of several oncogenes responsible for carcinogenesis (Li et al., 2007). EP300/p300 is a multifunctional gene that interact with transcription factors and act as transcriptional coactivator and manifest acetylation of protein/histones (Warner et al., 2016). In Wnt signaling p300 and *β-catenin* are the transcriptional activators and the phosphoprotein where it binds through phosphorylation (Ma et al., 2012). CD44 is a transmembrane glycoprotein and is the main receptor of hyaluronic acid, an extracellular matrix molecule. By its overexpression, hyaluronic acid results in tumerigenesis and metastasis of certain human cancers (Agrawal 2021). All these genes/proteins provide prognostic information for several cancer types which are mediated by Wnt signaling and serves as the promising targets for cancer.

Computational methods are emerged as a commonly used approach in almost all fields of health science research. Among those, structure-based virtual screening (molecular docking) has been used intensively in the field of computer assisted drug discovery (CADD) (Zhu et al., 2022). In the present study, PyMol molecular graphics system was used for the analysis where it allows AutoDock Vina for the study. This platform needs ligand and receptor pdb files in pdbqt format for the screening. Binding site of the proteins are defined by the rectangular boxes which are displayed in the PyMol window. After ligand, protein preparation and active site prediction, molecular docking analysis was carried out (Seelinger and Groot, 2010). The proteins, CTNNB1, EP300, and CD44 were subjected for virtual screening against the ligand, eugenol using AutoDock Vina and their binding affinities were compared to standard erlotinib.

2. METHODS

2.1. Softwares and Tools Used

Open Babel, SPDBV v 4.1.0, Auto Dock Vina, MGL v 1.5.6, Ligplot+ v 1.4.5, PyMol v 1.7.4.5, PubChem, RCSB PDB (Research Collaboratory for Structural Bioinformatics, Protein Data Bank), PDBsum, Discovery studio.

2.2. Ligand Preparation

The 2D structure of compound eugenol (PubChem ID: 3314) and the standard drug erlotinib (PubChem ID:176870) were obtained by PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Open Babel, an online server was used to convert obtained SD files to 3D structures (Álvarez-Carretero et al., 2018; Lin et al., 2022) and saved in pdb format. SPDBV v 4.1.0 was used for the energy minimization. All the water molecules were removed, non-polar hydrogen and Gasteiger charges were computed to the ligand and torsion degrees of freedom were allocated using MGL v 1.5.6 tool and saved in pdbqt format for further analysis.

2.3. Receptor Preparation

X-ray crystallographic structure of CTNNB1 (PDB ID: 7AFW, Resolution: 1.81Å), EP300 (PDB ID: 6V8N, Resolution: 2.3Å), and CD44 (PDB ID: 4PZ3, Resolution: 1.08Å) were retrieved from RCSB PDB (https://www.rcsb.org/). These PDB files were prepared by removing heteroatoms and subjected to MGL v 1.5.6 tool to remove the water molecules, and to add non-polar hydrogen, and Kollman charges and Gasteiger charges were computed for the protein structures and saved in pdbqt format for further studies (Kandagalla et al., 2017).

2.4. Prediction of Active Site

PDBsum online tool (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) was utilized to identify the amino acid residues associated with the protein structures. These residues are used to find out the possible active binding sites of proteins in MGL tool. MGL is a grid-based method helps to determine the potential binding pockets purely based on the 3D structure of the protein. With the help of this tool, the protein 3D coordinates were grid centered to get the efficient binding score the grid box dimension was set to cover the hot-spots along with active pocket amino acid residues with a spacing of 1 Å for X, Y, Z co-ordinates to minimize the run time.

2.4. Molecular Docking Analysis

AutoDock Vina was utilized to perform molecular docking analysis of the ligand-receptor complex. To attain more realistic outcome, the analysis was performed with exhaustiveness 10. Ligand-binding affinity was predicted by computing negative Gibbs free energy (-ΔG) using AutoDock Vina scoring function (Kcal/mol) (Trott and Olson, 2010). The molecular docking was carried out by proper commands resulting in the generation of log files. LigPlot+ v 1.4.5 and PyMol v 1.7.4.5 tools were utilized to procure the 2D and 3D graphical representations (Conformation) of the ligand-protein interaction.

3. RESULTS

3.1. The active site prediction

The grid box of CTNNB1 coordinates were assigned at the values X=40, Y=40, Z=52 and the grid center were set at X=60.517, Y= -26.317, Z= 11.749. EP300 coordinates were set at X=40, Y=40, Z=40 and the grid center were set at the values X=36.102, Y= 14.275, Z= -22.688. CD44 coordinates were assigned at X=30, Y=30, Z=30 and the grid center were set at X=10.038, Y= 2.351, Z= 13.051.

The active pocket amino acids involved in binding of CTNNB1, CD44, and EP300 are found to be Lys288, Asn287, Ser250, Thr289, Asn290, Asp249, Phe293, Phe253, Asn151, Gly152, Ile26, Arg150, Thr27, Asn25, His35, Cys77, Glu37, Arg78, Trp1466, Leu1463, Leu1398, Ser1400, Tyr1414, Arg1462, Pro1458, Asp1399, Ile1457, Pro1440, Gln1455, His1451, Asp1444, Asn149, Val148, His1402, Lys1456, Arg1410, Thr1411, Tyr1467, Pro1439, Cys1438, and Asp1399.

3.2. Molecular docking analysis

Standard erlotinib has -6.0 kcal/ mol binding affinity with two hydrogen bonds against CTNNB1 at a distance of 3.16Å, and 3.21Å (Fig-1), -7.9 kcal/ mol binding affinity with one hydrogen bond against EP300 at a distance of 2.91Å (Fig-2), and it has -6.4 kcal/ mol binding affinity with three hydrogen bonds against CD44 at a bond distance of 3.31Å (Fig-3, Table-1). The hydrophobic interaction between the ligand eugenol and the protein CTNNB1 was created by the amino acid residues, Phe253, Lys288, Thr289, Asn290, Phe293. The amino acids, Leu1398, Cys1438, Pro1439, Pro1440, Lys1456, Pro1458, Leu1463, Tyr1467 are responsible to create hydrophobic bond between eugenol and the protein EP300. Similarly, the hydrophobic bond between eugenol and the protein CD44 was created by the amino acid residues such as Asn25, His35, Asn149, Arg150.

A) B)

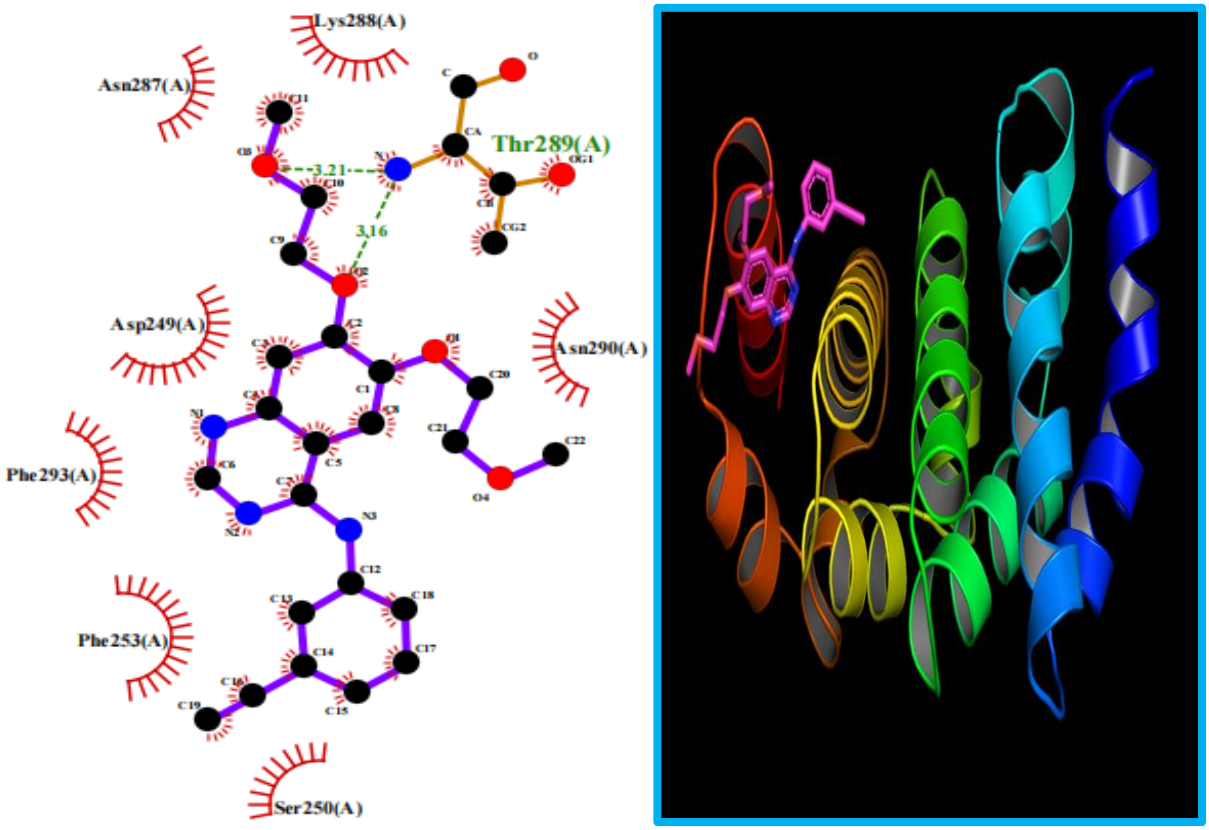

Fig-1: Molecular docking analysis of standard erlotinib against the protein CTNNB1. A) The 2D erlotinib-CTNNB1 docked complex B) The 3D docked complex;The ligand is highlighted in pink colour

A) B)

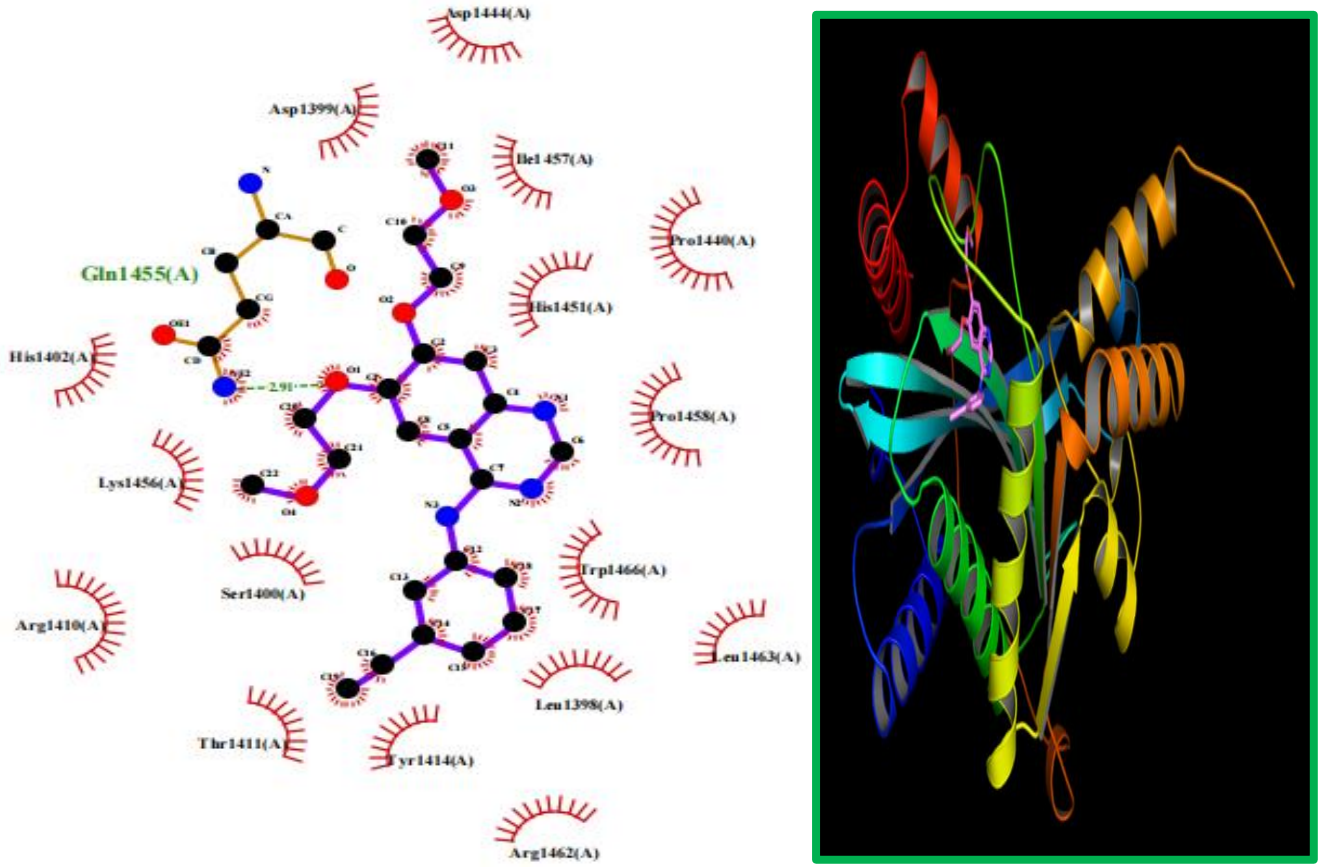

Fig-2: Molecular docking analysis of standard erlotinib against the protein EP300. A) The 2D erlotinib-EP300 docked complex B) The 3D docked complex;The ligand is highlighted in pink colour

A) B)

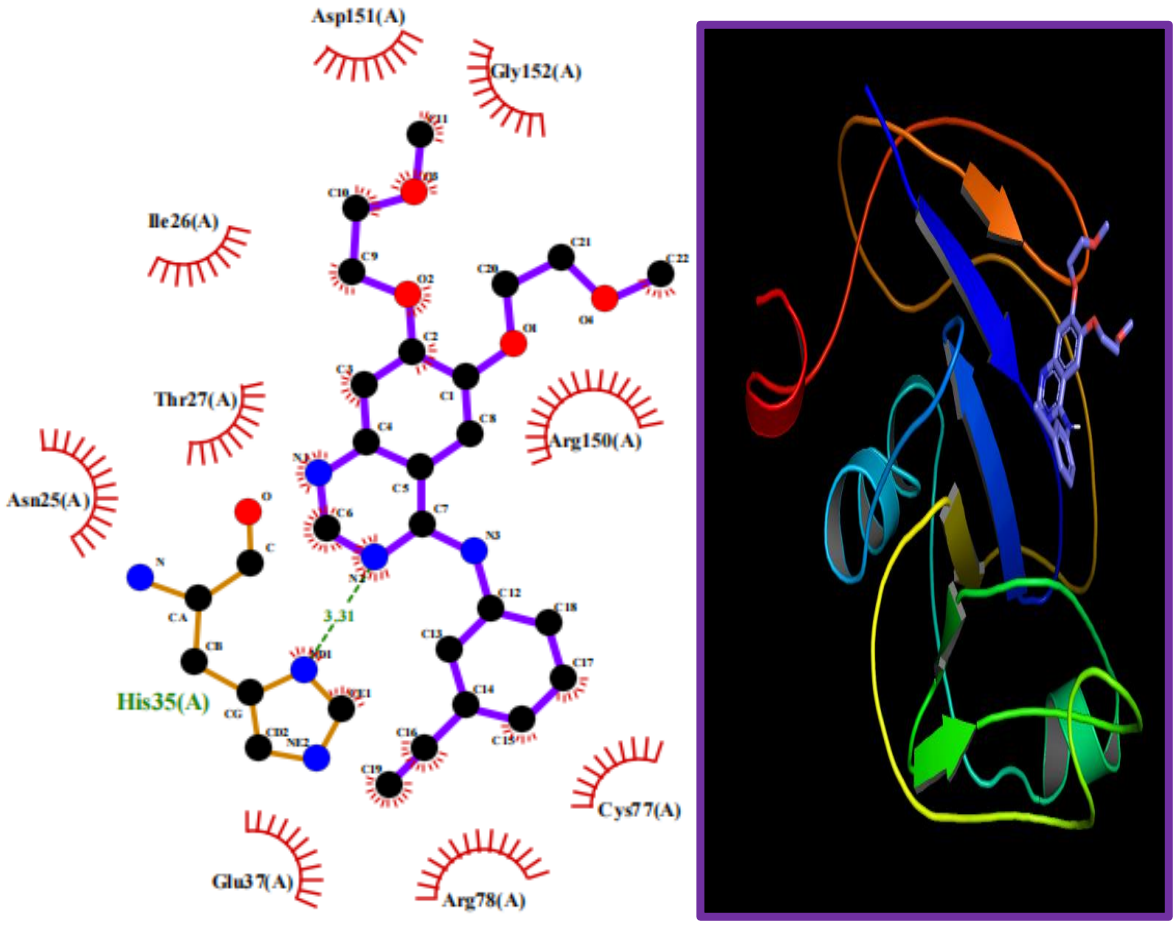

Fig-3: Molecular docking analysis of standard erlotinib against the protein CD44. A) The 2D erlotinib-CD44 docked complex B) The 3D docked complex;The ligand is highlighted in purple colour

Table-1: Molecular docking analysis of standard erlotinib against the proteins

Ligand	Protein	PDB ID	Binding affinity (Kcal/mol)	No. of H bonds	Amino acid residues	Distance (Å)
Erlotinib	CTNNB1	7AFW	-6.0	2	Thr289	3.16 3.21
Erlotinib	EP300	6V8N	-7.9	1	Gln1455	2.91
Erlotinib	CD44	4PZ3	-6.4	1	His35	3.31

Eugenol has the binding affinity of -4.7 kcal/ mol with four hydrogen bonds against CTNNB1 at a bond distance of 3.05Å (Fig-4), -6.0 kcal/ mol with one hydrogen bond against EP300 at abond distance of 2.71Å, 2.85Å, 3.14Å, and 3.17Å (Fig-5), and -5.2 kcal/ mol with one hydrogen bond against CD44 at abond distance of 3.11Å, and 2.74Å (Fig-6, Table-2). The amino acid residues Asp249, Ser250, Phe253, Asn287, Lys288, Asn290, Phe293 are responsible for hydrophobic interaction between erlotinib and CTNNB1. the amino acid residues Leu1398, Asp1399, Ser1400, His1402, Arg1410, Thr1411, Tyr1414, Pro1440, His1451, Lys1456, Ile1457, Pro1458, Arg1462, Trp1466 are associated with the hydrophobic interaction between erlotinib and EP300 and the amino acid residues Asn25, Ile26, Thr27, Glu37, Cys77, Arg78, Arg150, Asp151, Gly152 are involved in hydrophobic interaction between ligand erlotinib and the protein CD44.

A) **B)**

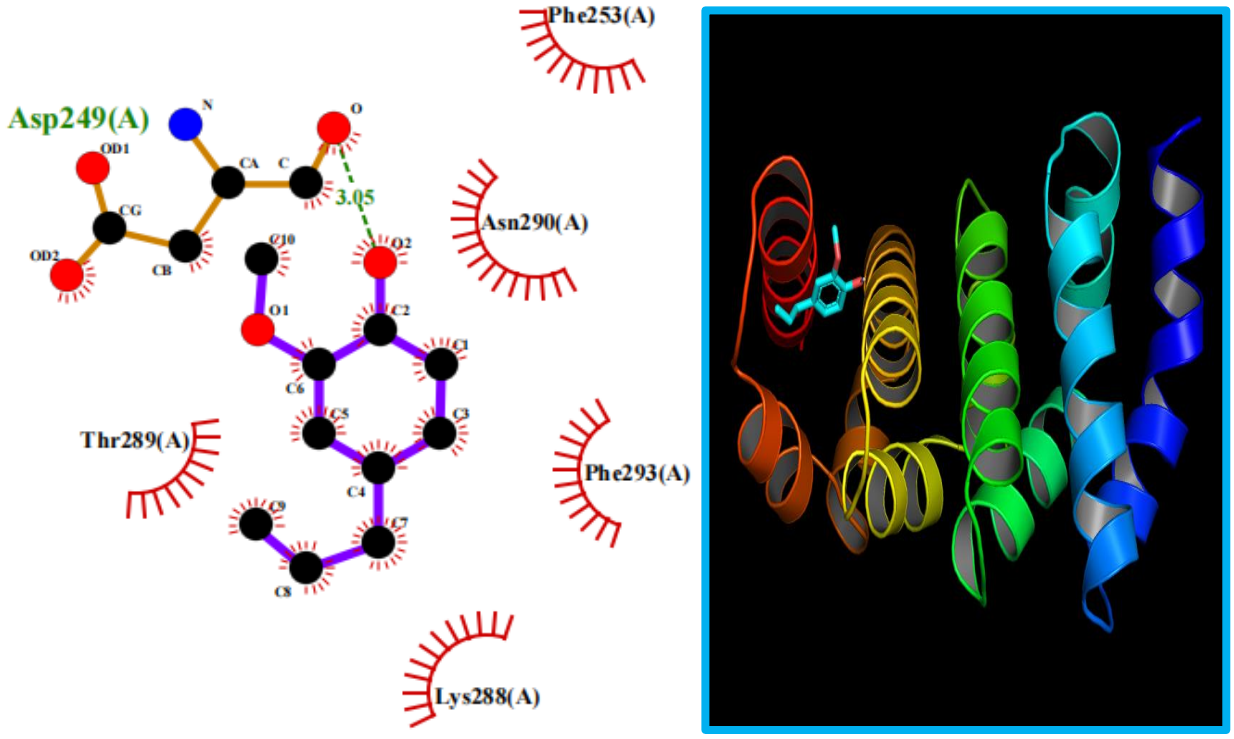

Fig-4: Molecular docking analysis of ligand eugenol against the protein CTNNB1. A) The 2D eugenol-CTNNB1 docked complex B) The 3D docked complex;The ligand is highlighted in blue colour

A) B)

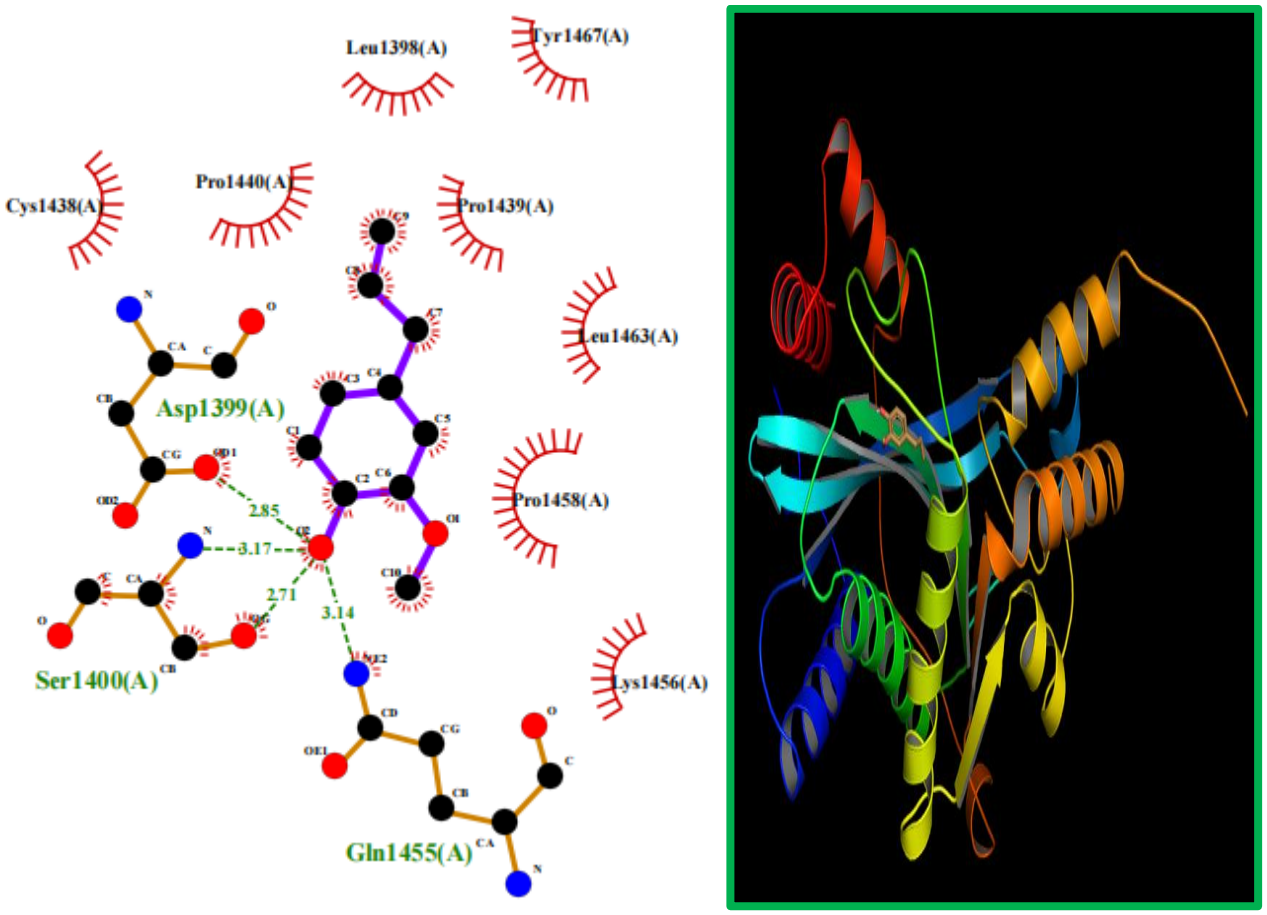

Fig-5: Molecular docking analysis of ligand eugenol against the protein EP300. A) The 2D eugenol-EP300 docked complex B) The 3D docked complex;The ligand is highlighted in brown colour

A) B)

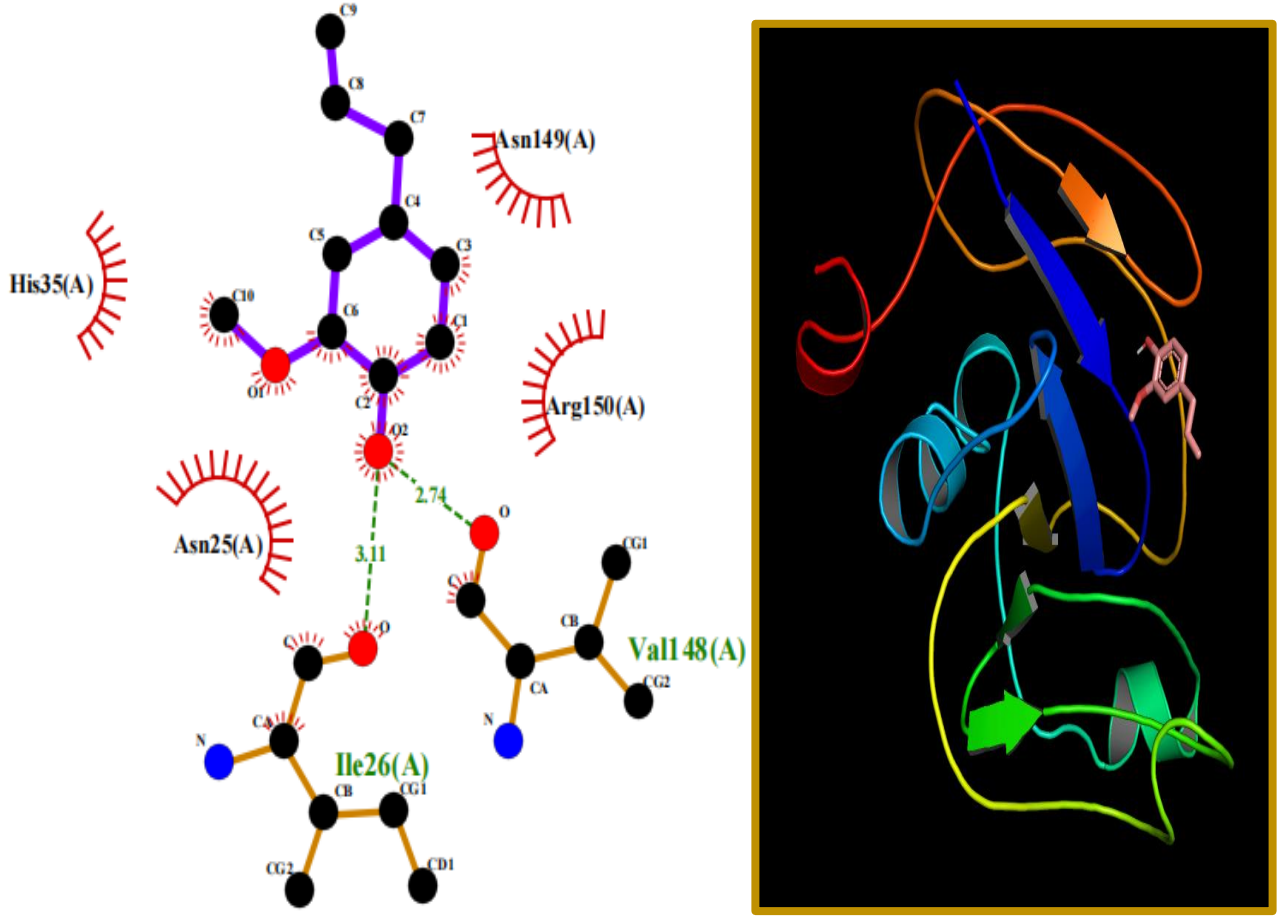

Fig-6: Molecular docking analysis of ligand eugenol against the protein CD44. A) The 2D eugenol-CD44 docked complex B) The 3D docked complex;The ligand is highlighted in brown colour

Table-2: Molecular docking analysis of eugenol against the proteins

Ligand	Protein	PDB ID	Binding affinity (Kcal/mol)	No. of H bonds	Amino acid residues	Distance (Å)
Eugenol	CTNNB1	7AFW	-4.7	1	Asp249	3.05
Eugenol	EP300	6V8N	-6.0	4	Asp1399, Ser1400, Gln1455	2.71, 2.85, 3.14, 3.17
Eugenol	CD44	4PZ3	-5.2	2	Ile26, Val148	3.11, 2.74

The analysis suggests that the compound eugenol shows a good binding affinity with all the targets compared to standard erlotinib.

4. CONCLUSION

Among the three receptors EP300 and CD44 has exhibited significant affinity with more hydrogen bond than compare to standard erlotinib. EP300 and CD44 are found to be the proteins potentially involved in causing various types of cancer including bladder cancer, breast cancer, non-small cell lung cancer, colorectal cancer, pancreatic cancer, etc. The study reveals that these proteins are found to be a novel therapeutic targets in cancer. The knockdown of these proteins may be results in tumor growth inhibition, and may prevents the metastasis. The ligand-protein interaction will be further validated using *in vitro* studies.

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