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Research Article

In-Silico Molecular Docking of Potential Natural Phytochemicals And Known Drugs Against Colorectal Cancer Marker (BRAF V600E) For The Novel Therapeutics

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ABSTRACT

Cancer is the world's second leading noncommunicable cause of death, after cardiovascular disease. A total of 277 distinct cancer types have been identified by scientists. Based on the most recent estimates on the global burden of cancer, the World Health Organization (WHO) reports that colorectal cancer is among the top three cancer types in terms of incidence and death. In terms of new records, colorectal cancer accounts for roughly 1.8 million cases, or 10.2% of all new cases. Additionally, colorectal cancer is the second most common disease in terms of mortality, coming in at about 881,000 cases, or 9.2%, after lung cancer. In this investigation, molecular docking is used to evaluate the correct binding mechanism for BRAF- V600E marker by known drugs and potential phytochemicals as a promising drug target. Total 26 molecules including 5 phytochemicals were tested in the current investigation, to identify a possible blocking agent against BRAF V600E receptor. To evaluate the potential molecule, modern bioinformatics approaches such as molecular docking, virtual prediction were used. The compound Irinotecan were used as the reference drug for this study, the binding affinity of Irinotecan were observed as -9.6 kcal/mol. the compounds Axitinib (-9.5 kcal/mol), Pazopanib (-9.3 kcal/mol), Regorafenib (-9.3 kcal/mol) and Cabozantinib (-9.9) and the phytochemicals Procyanidin (-9.7 kcal/mol) and Silymarin (-9.3 kcal/mol) also shows same affinity like reference drug. Hence it can be further evaluated for the potential drug targets and can be tested for potential anticancer medicine in In-Vivo and Mouse model.

INTRODUCTION

Cancer, as an increasing concern, is the second greatest noncommunicable illness of death globally, after only cardiovascular disease. Scientists have found over 277 distinct forms of cancer. (Sumit *et al.,* 2018). Colorectal Cancer one and two million new cases being diagnosed each year, colorectal cancer (CRC) is one of the most prevalent cancers in the world (Marmot *et al.,* 2019). The World Health Organization (WHO) reports a new instance, colorectal cancer accounts for roughly 1.8 million cases, or 10.2% of all new cases (Rathod *et al.,* 2021). Additionally, colorectal cancer is the second most common disease in terms of mortality, coming in at about 881,000 cases, or 9.2%, after lung cancer. (Marmol *et al.,* 2019) It is the third most frequent type of cancer in men and the second most common type of cancer in women (Zhou *et al.,* 2019). Colorectal cancer is a complicated illness with numerous molecular pathways. The B-rapidly accelerated fibrosarcoma (BRAF) gene mutation is one of the most prevalent in CRC, including a valine to glutamic acid substitution at codon 600 (V600E) (Bond *et al.,* 2014). The BRAF mutation is most closely related with the serrated neoplastic route, which occurs when a serrated type polyp, either a sessile serrated adenoma (SSA) or a conventional serrated adenoma (TSA), develops specified molecular abnormalities that lead to cancer (Rajagopalan *et al.,*2004). The BRAF oncogene is a key player in the MAP kinase pathway, and an activating V600E mutation is found in 15% of sporadic colorectal cancer cases (Garnett *et al.,* 2004). The presence of a BRAF mutation in colorectal cancer can be associated with an aggressive phenotype and is a critical prognostic biomarker for poor outcome, particularly in late-stage illness (Jones *et al.*, 2017). However, over the past few decades, the use of drugs made chemically has not significantly increased the overall survival rate. As a result, innovative chemoprevention techniques and new tactics are required to enhance the effectiveness of current cancer therapies (Zhang X *et al.,* 2019).

Current cancer therapy via phytochemicals a novel approach in which a large number of phytochemicals, or naturally occurring plant molecules, are important sources for new medications and are also used to treat cancer. Taxol analogues, vinca alkaloids like vincristine and vinblastine, and analogues of podophyllotoxin are a few typical examples (Roy *et al.,* 2019). These phytochemicals are selective in their actions, acting only on tumor cells and not on normal cells. Carcinogenesis is a

complicated process involving several signaling pathways. Because of their pleiotropic effects on target events in many ways, phytochemicals are considered promising candidates for anticancer drug development (Iqbal *et al.,* 2017). The development of promising candidates (those that can prevent or reduce the proliferation of cancer cells without causing negative effects) from these phytochemicals is now underway (Choudhari *et al.,* 2020). Many phytochemicals and their derivatives have been identified as potential anticancer therapy candidates. which is also verified by dry lab Computer Aided Drug Discovery (CADD) for their potential Drug targets, in which molecular docking and pathway analysis is the best way to predict the protein responsible for the proliferation of cancer cell (Isyaku *et al*., 2020).

MATERIAL AND METHODS

Computer Configurations:

All molecular docking tests were conducted using HP Intel(R) Core (TM) i3-7100U CPU @ 2.40GHz 2.40 GHz processor, 64-bit Operating System, and x64-based processor running Windows 10. Utilizing Auto Dock Vina 1.1.2 and Auto Dock Tools 1.5.7, the preparation of the ligands and receptor were completed, and the docking process were carried out (Umar *et al.,* 2019).

Receptor Structure Retrieval:

The x-ray structure of BRAF-V600E kinase (receptor) in complex with vemurafenib (PLX4032) (PDB-code: 3OG7) were obtained from the database (www.rcsb.org).

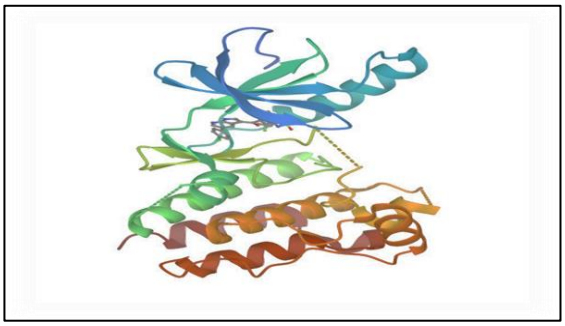

Figure 1: B-Raf Kinase V600E oncogenic mutant in complex with PLX4032, SOURCES: RCSB PDB Database (https://www.rcsb.org)

X-ray crystallographic characteristics of the BRAF V600E protein are mentioned in below in Table 1.

Table 1: Properties of BRAF V600E protein:

Protein PDB Id	Sequence length	Resolution	R- value free	Method	Chains
3OG7	289	2.45 Å	0.258	X-Ray diffraction	A & B

Protein Preparation:

V600E-BRAF was imported into Auto Dock tool, and the PDBQT file was created by preparing the receptor molecule for docking. For preparation, the water molecules are deleted first. Then the ligand molecule was also deleted from the protein chain. Other amino acids that was discovered to have missing side chains was

also repaired (Wang *et al*., 2018). Then the PDB receptor file is provided polar hydrogen atoms and Kollman charges in order to prepare the receptor protein for Docking and the protein file was saved in PDBQT format.

Ligand Structure Retrieval:

The ligands were selected by screening ligand libraries. The known structures of these selected ligands were collected from the PubChem database. The Pubchem ID of these ligands are illustrated in table 2. The ligands were collected from PubChem database in structure-data file (sdf) format and converted into PDBQT format by using Open Bable GUI software (Nisha *et al.,* 2016).

Ligand Structure Preparation:

The ligands were prepared using Auto Dock 1.5.7. For the preparation, the ligands were selected, their torsion roots were detected and number of torsions for each ligand was set (Umar et al., 2023). These prepared ligands then saved into PDBQT format.

Molecular Docking by AutoDock Vina 1.1.2

To choose the most potential inhibitors (ligands) against the receptor protein, active site-specific molecular docking was done using the AutoDock Vina 1.1.2 and AutoDock tools 1.5.7 programmes. Docking were performed using prepared receptor and ligands, comprising twenty known drugs, five phytochemicals and one reference molecule. The compound Irinotecan were used as the reference molecule for this study (Halder *et al.,* 2020). The amino acids defining the receptor's active site were covered by a grid box having setting of center axis of X -1.59, Y -1.28, and Z -6.21 and grid dimension of 62*62*62 for specific docking (Wang *et al.,* 2018).

RESULT

Validation of Protein (BRAF V600E):

For the validation of protein, ProCheck, a web-based tool was used to obtain its Ramachandran plot. The plot shows the most amino acids residues were found to be in the favored region. 91% of the residues were present in the favorable region as shown in the plot depicted in the figure 2.

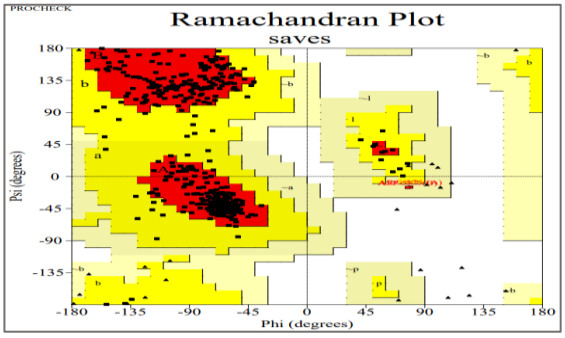

Figure 2: Ramachandran plot of receptor BRAF V600E

Molecular Docking

Using the method of the docking application, the PDBQT file of the ligands and reference molecule were docked against BRAF V600E to determine the

binding confirmations and binding affinities. The binding affinities of each ligand are mentioned in the table given below.

Table 2: Inhibitory molecules and their binding energy score (kcal/mol)

S.NO.	Inhibitory molecules	PubChem ID	Binding energy score (kcal/mol)
1	Vemurafenib	42611257	-11.2
2	Axitinib	6450551	-9.5
3	Vandetanib	3081361	-9.1
4	Lapatinib	208908	-10.2
5	Gefitinib	123631	-8.7
6	Sunitinib	5329102	-7.7
7	Sorafenib	216239	-9
8	Erlotinib	176870	-8.3
9	Afatinib	10184653	-8.9
10	Pazopanib	11525740	-9.3
11	Cabozantinib	25102847	-9.9
12	Dabrafenib	44462760	-9
13	Estramustine	259331	-8.6
14	Regorafenib	11167602	-9.3
15	Trametinib	11707110	-11.1
16	Alitretinoin	449171	-8.6
17	Fluoxymesterone	6446	-8.6
18	Nilotinib	644241	-11.8
19	Olaparib	23725625	-10.2
20	Dasatinib	3062316	-9
21	Luteolin	5280445	-8.9
22	Procyanidin	107876	-9.7
23	Silymarin	5213	-9.3
24	vinblastine	241903	-7.2
25	Panaxadiol	73599	-8.9
26	Irinotecan (Reference molecule)	60838	-9.6

The compound Irinotecan were used as the reference drug for this study, the binding affinity of irinotecan were observed as -9.6. With this reference, the compounds Axitinib, Pazopanib, Regorafenib and Cabozantinib and the phytochemicals Procyanidin and Silymarin can be further investigated for the drug like properties.

The docking interactions between irinotecan, as a reference inhibitor, and the receptor indicate one hydrogen bond interaction with ASN A: 580 residue, one Pi-Pi interaction with PHE A: 583 residue and two Pi-Alkyl/Alkyl interaction with TRY A: 538 and LEU A: 618 residues.

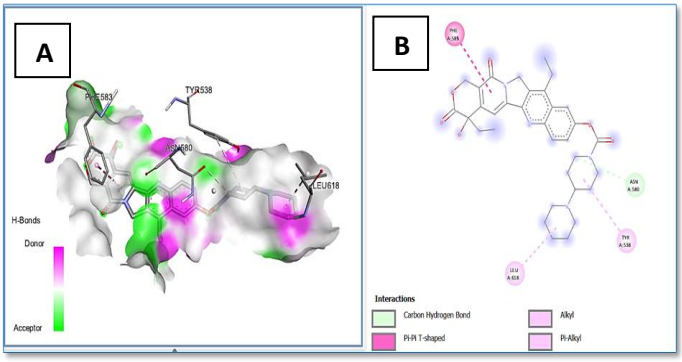

Figure 3: Molecular interaction of Irinotecan with BRAF V600E receptor.
A) 3D representation of docking interaction
B) 2D representation of molecular interaction

The docking interactions between Axitinib and the receptor molecule shows one Pi-Cation bond with LYS A: 483 residue, one Pi-Sigma interaction with residue VAL A: 471, one Pi-Pi interaction with PHE A: 583 and three Pi-Alkyl interactions with ILE A: 527, LEU A: 505 and ALA A: 481 residues.

CONCLUSION

BRAF- V600E is a common oncogenic protein kinase that can be inhibited to protect humans against cancer. In this work, molecular docking is used to evaluate the correct binding mechanism for V600E-BRAF of known drugs and potential phytochemicals. All of the tested ligands were able to block the receptor by completely occupying the active section of the target (receptor). Total 20 compounds and 5 phytochemicals were tested in the current investigation to identify a possible

ligand against BRAF V600E. To find the potential molecule, modern bioinformatics approaches such as molecular docking, virtual prediction were used. The results of molecular docking revealed that the selected known drugs and phytochemicals showing binding affinities between -7.2 and -11.8 kcal/mol, which is near to reference drug. The compound Irinotecan were used as the reference drug for this study, the binding affinity of Irinotecan were observed as -9.6 kcal/mol. With this reference, the compounds Axitinib (-9.5 kcal/mol), Pazopanib (-9.3 kcal/mol), Regorafenib (-9.3 kcal/mol) and Cabozantinib (-9.9) and the phytochemicals Procyanidin (-9.7 kcal/mol) and Silymarin (-9.3 kcal/mol) also has same affinity like reference molecule, hence it can be further investigated for the drug like properties in In-vitro culture for potential drug targets against BRAF receptor.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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