Targeting BRAF^{V600E} in the Treatment of CRC

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled "Targeting $BRAF^{V600E}$ in the Treatment of CRC" submitted by Mashwiyat Samrin Roja (19346046) of Summer, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October, 2023.

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Ethics Statement

The project does not involve any clinical trial or human participants and no animals were used or harmed.

Abstract

The increasing prevalence of $BRAF^{V600E}$ mutated cancers has led to the search and development of targeted therapies against BRAF mutation. Multiple cancers, including colorectal cancer (CRC), have been linked to this mutation. While the currently available CRC drugs have shown initial promise, long-term use of these drugs could lead to the emergence of resistant CRC cells; therefore, drug repurposing provides a powerful strategy to increase the existing drug pool. This docking-based study's aim was to find the possible compounds that could inhibit the BRAF protein and treat $BRAF^{V600E}$ mutated CRC. Three classes of drugs anti-hypertensive, anti-cholesterol and anti-diabetic drugs were explored. Molecular docking, followed by superimposition, analyzing protein-ligand interactions and comparison of their pharmacokinetic properties were done. The anti-hypertensive drug, Verapamil showed promising results as it demonstrated good binding affinity and interaction with the target protein. However, further *in vitro* studies and biological assays should be performed to elucidate Verapamil's efficacy.

Keywords: $BRAF^{V600E}$; molecular docking; superimposition; ADME.

Dedication

Dedicated to my faculty members, family and friends.

Acknowledgement

First of all, I would like to express my gratitude to Allah (SWT), the Almighty, for His endless blessings, mercy, and kindness. All glory to Him that He has given me the tremendous amount of patience, strength, courage, knowledge, wisdom, and hope that I need to complete this project.

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List of Acronyms

CRC	Colorectal cancer
HR	Hazard ratio
CEA	Carcino-embryonic antigen
CA	Carbohydrate Antigen
TPS	Tissue Polypeptides specific antigen
MAPK	Mitogen activated protein kinase
BRAF	v-raf murine sarcoma viral oncogene homolog B1
ERK	Extracellular signal-regulated kinase
GTP	Guanosine-5'-triphosphate
EGF	Epidermal growth factor
FDA	Food and Drug Administration
ADME	Absorption, distribution, metabolism, and excretion
ADP	Adenosine diphosphate
KRAS	Kirsten rat sarcoma virus
GDP	Guanosine diphosphate
TAG-72	Tumor associated glycoprotein-72
IGF	Insulin like growth factor

QPlogBB	brain/blood partition coefficient
GRB2	Growth factor receptor-bound protein 2
mCRC	Metastatic colorectal cancer
dMMR	Deficient mismatch repair
WT	Wild-type
MSI-H	High microsatellite instability
CSV	Comma-separated values file
%HOA	Percentage of oral absorption
IC50	Half-maximal inhibitory concentration
CNS	Central nervous system
BBB	Blood brain barrier

Chapter 1

Introduction

Every year a total of one to two million new cases of colorectal cancer (CRC) are reported, resulting in being one of the most prevalent diseases in the world. With almost 700,000 deaths annually, CRC has been classified as the third most frequently occurring cancer globally and ranked as the fourth leading cause of death from cancer. Data suggests that CRC is more prevalent in male than in female. In accordance to the hazard ratio (HR) findings HR 0.78, 95% confidence interval (CI) 0.77-0.80, female patients had a significantly higher life expectancy than male patients (He et al., 2022). Moreover, a significant proportion of people with colorectal cancer die, with approximately one in three of all deaths connected with neoplasm (7.1% of male and 7.9% of females). Mainly, CRC starts in the large intestine, more specifically about 41% of all colorectal cancers occur in the proximal colon, 22% occurs in distal colon and 28% in the rectum. However, the site of origin can vary depending on age and gender. Surprisingly, it is more common in developed countries than the least developed countries. Environmental and genetic factors can also contribute to the pathogenesis of colon cancer (Thanikachalam & Khan, 2019). In addition, possible reasons could be predisposition to carcinogenesis, high calorie and fat diet, low physical activity, obesity, anxiety, high blood pressure and a sedentary lifestyle (Świderska et al., 2014). Although the overall relative rate of five-year survival for persons with colon cancer is 63%, if the cancer is detected early on, the survival rate increases to 91%. Again, if the cancer has progressed to adjacent organs or tissues and local lymph nodes, the five-year relative rate of survival is 72% and if the cancer has moved to distant portions of the body, the rate is 13%. Additionally, five years survival rate for rectal cancer is 68% (*Colorectal Cancer: Statistics | Cancer.Net*, 2022).

In terms of colorectal cancer, there are two main categories - GI carcinoid tumors and colorectal adenocarcinoma. Apart from these there are few uncommon types of colorectal cancers, which include familial adenomatous polyposis, primary colorectal lymphomas, colorectal squamous cell carcinoma, gastrointestinal stromal tumors, colon and rectal melanomas, colon and rectal leiomyosarcomas (Ahmed, 2020; Luo et al., 2019). Recently, oncological diagnostics is giving high importance for early detection of neoplasms in an asymptomatic or pre-cancerous stage. In patients who have severe symptoms and a poor prognosis, early diagnosis of CRC is extremely important and this is due to the fact that severe intestinal perforation may occur for CRC due to obstructive ileus. According to estimations, more than 50% of patients would develop colonic polyps, with a higher risk of CRC in 6% of them. Early Detection of any disease is imperative. Screening examinations used for the diagnosis of CRC include the following:

a) Invasive examination is per rectum examination which is one of the simplest methods to recognize CRC along with the case history. It is possible to recognize 30% of CRCs and about 70% of rectal cancers during this examination.

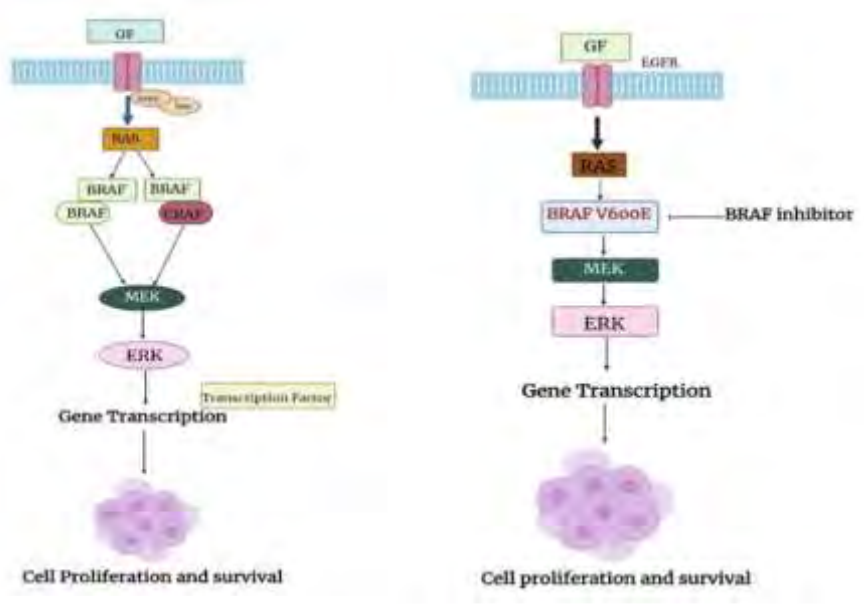
b) Endoscopy is the most common and effective approach of CRC diagnosis includes colonoscopy, sigmoidoscopy and imaging tests.

c) Non- Invasive Examination includes fecal occult blood test.

d) Non-enzymatic tumor markers include TPS (Tissue polypeptide specific antigen), CA 19-9 (Carbohydrate antigen), TAG-72 (Tumor associated glycolprotein-72), CEA (Carcino-embryonic antigen) (Hultcrantz, 2021; Sarandria & Sarandria, 2022; Świderska et al., 2014).

1.1 BRAF in Colorectal Cancer

The BRAF gene, a key component of cell signaling pathways, has gained substantial attention in the field of oncology due to its involvement in a variety of malignancies. Genetic mutations within the BRAF gene, particularly the $BRAF^{V600E}$ mutation, have emerged as critical drivers of tumorigenesis and disease progression. This study covers the multifaceted landscape of the BRAF mutation, exploring its role in cancer development and its significance in specific cancer types such as colorectal cancer (CRC). BRAF (v-raf murine sarcoma viral oncogene homolog B1) is known as serine/threonine protein kinase, a proto oncogene responsible for cell growing as well as maintaining the signaling cascade known as MAP/ERK pathway which mainly drives the cell proliferation, differentiation, migration, survival, and angiogenesis (Dain Md Opo et al., 2022; Guo et al., 2020). BRAF plays a major role in this pathway by participating in cell division through phosphorylation where it binds to the Ras-GTP and produces ADP, phosphorylated protein (Cope et al., 2018). The EGFR receptor is activated when the EGF (Epidermal Growth Factor) (Figure 1a) binds with the cytoplasmic serine, which in turns activates KRAS to release the GDP in the presence of two adaptor protein Growth Factor Receptor-bound protein 2 (Grb2) and son of sevenless (Sos) (Watanabe et al., 2000). This KRAS then binds to the BRAF and activates the MEK kinase which subsequently phosphorylates and activates ERK. Finally, through ERK phosphorylation, variety substrates are produced which include the several transcription factors which regulate the differentiation, cellular proliferation, cell survival and apoptosis (Caputo et al., 2019; Dain Md Opo et al., 2022). A BRAF mutation at position V600E accounts for 80% of the change, and a mutation at location V600K contributes for 10% to 20%. Deregulation of this pathway caused by $BRAF^{V600E}$ mutation (Figure 1b) could also lead to uncontrolled cell proliferation, migration, angiogenesis, and metastasis in CRC.



(a) (b)

Figure 1a: BRAF pathway in wild type. *Figure 1b: BRAF pathway in BRAF^{V600E} mutant cell.*

A valine amino acid change at position 600 causes around 96% of all BRAF mutations, which primarily resemble regulatory phosphorylation and as compared to the wild-type (WT) BRAF, it increases BRAF activity by around ten times. It is interesting to note that during CRC growth and development, BRAF mutated mCRC tumors arise as a distinctive biological entity that exhibits both clinical and molecular heterogeneity. In particular, 8–10% of mCRC have BRAF mutations and more than 90% of these mutations cause missense changes in codon 600 that result in the aminoacidic substitution of valine for glutamic acid (V600E) (Caputo et al., 2019; Mauri et al., 2021). However, 2.2% of patients with metastatic colorectal cancer have non-V600E mutations, which primarily affect codons at positions 594 and 596. Each of these individuals represents a distinct group with respect to age, metastatic sites, histology, gender, MSI (mass spectrometry imaging) status and prognosis. In contrast to the BRAF^{V600E} mutant subgroup, they are also more usually found in males and younger people. They are also

frequently connected to low to medium grade histological tumors on the left side of the colon, however peritoneal involvement in late disease states is very uncommon. In addition, BRAF non-V600E-mutated CRCs exhibit radically altered molecular behavior that can be brought on by immediate RAS mutations, and dMMR is only infrequently present. This shows that, at least in a few cases, rare BRAF mutations may provide cancer cells with a lesser proliferative advantage than other variants with a poor prognostic impact. Surprisingly, BRAF 594 and 596 mutations can phosphorylate ERK roughly two times more than BRAF-WT, but only when endogenous CRAF is present. This discovery contributed to the understanding of the BRAF-CRAF dimerization process (Caputo et al., 2019; Mauri et al., 2021).

1.2 Current Treatment Strategies

Targeted therapy is the most effective strategy with lesser side effects to treat the BRAF positive mutated patients. In addition to receiving targeted therapy the BRAF positive mutated patients also received chemotherapy or immunotherapy. Apart from these the current treatment strategies for $BRAF^{V600E}$ mutant CRC include Doublet Cytotoxic Combination Plus Biological Agents, BRAF-Targeted Combinations, Immune Checkpoint Inhibitors in $BRAF^{V600E}$ Mutant MSI-H mCRC, Triplet Cytotoxic Combination Plus Biological Agent (Dain Md Opo et al., 2022; Mauri et al., 2021). Treatment for BRAF mutations frequently involves combining two medications (combination therapy) or three medications (triple therapy), both of which are now undergoing clinical trials. However, some FDA approved drugs used to treat $BRAF^{V600E}$ mutant CRC include Bevacizumab, Vemurafenib, Encorafenib, Panitumumab, Cetuximab, Tucatinib with Trastuzumab, Dabrafenib (Tafinlar, Novartis) with Trametinib (Mekinist, Novartis), Encorafenib is also approved, in combination with Cetuximab (*Drugs Approved for Colon and Rectal Cancer - NCI*,2023; *FDA Approves Encorafenib for Colorectal Cancer - NCI*,2020;

FDA Grants Accelerated Approval to Tucatinib with Trastuzumab for Colorectal Cancer | FDA,2023; Patel et al., 2020).

1.3 Selection of Drugs

In this computational study, three classes of drugs have been used which include Anti-cholesterol, anti-diabetic and anti-hypertensive. The relationship between these conditions and CRC has been studied due to their potential impact on cancer risk and progression.

1.3.1 Anti-Hypertensive Drugs

Hypertension and CRC: Hypertension (high blood pressure) has been linked to an increased risk of various cancers, including CRC. Chronic inflammation, oxidative stress, and alterations in blood vessel health associated with hypertension could contribute to cancer development. Additionally, there is a positive overall correlation between hypertension and colorectal cancer risk, with a greater risk of 11% for those who have hypertension. Mainly, hypertension increases the chances of CRC by blocking the cell apoptosis and cause abnormal cell growth result in malignant cell. The renin-angiotensin system, which is linked to hypertension, also contributes to the development of tumors, primarily through angiogenesis. As a result, drugs that inhibit the renin-angiotensin system might also have anti-tumor effects (Ozawa et al., 2019; Seretis et al., 2019; Xuan et al., 2021).

Angiotensin System: ARBs (angiotensin II receptor blockers) and ACE (angiotensin-converting enzyme) inhibitors are two examples of anti-hypertensive medications that not only lower blood pressure but also have an impact on the signaling pathways implicated in the development of cancer. They may have potential anti-cancer effects by influencing angiogenesis, cell growth, and inflammation (Ahmad et al., 2023; Ozawa et al., 2019).

1.3.2 Anti-Cholesterol Drugs

Cholesterol and CRC: An increased risk of CRC has been linked to elevated cholesterol levels. Cholesterol is involved in various cellular processes, including membrane structure, and its dysregulation could influence cancer cell growth and metastasis. On the other hand, association between cholesterol and CRC can also be seen though cholesterol is essential for cell membrane formation but changes in serum cholesterol levels can influence the development of cancer. By increasing the production of cholesterol-based bile acids, the increased serum cholesterol levels may cause CRC. Therefore, statin therapy lowers serum cholesterol which may act as a preventative measure against the development of CRC (Han & Kim, 2021; Li et al., 2021; Mamtani et al., 2016; Ozawa et al., 2019).

Statins: Statins are a class of drugs used to lower cholesterol levels. They have gained attention for their potential anti-cancer effects, including inhibition of cell proliferation, modulation of inflammation, and interference with tumor signaling pathways (Han & Kim, 2021; Li et al., 2021; Mengual et al., 2022).

1.3.3 Anti-Diabetic Drugs

Diabetes and CRC: CRC risk is higher in individuals who have type-2 diabetes mellitus. Hyperinsulinemia, insulin resistance, and chronic inflammation associated with diabetes could contribute to cancer development and progression. There is an association between diabetes and CRC as it may influence neoplastic process through numerous factors, such as hyperglycemia, hyperinsulinemia (either exogenous because of injected insulin or insulin secretagogues, or endogenous because of insulin resistance) or chronic inflammation in people with diabetes. Mainly, both proliferation and apoptosis axes, that are mainly regulated by insulin and insulin-like growth factor (IGF), may exert a profound effect on carcinogenesis.

The most evident changes in diabetes individuals are decreased sensitivity to insulin with compensating hyperinsulinemia and increased levels of IGF-1, which may then promote cell proliferation in the colon, liver, breast, pancreas, prostate, ovary, and other organs. By reducing the amount of IGF-binding protein 1, which raises the concentration and bioavailability of total circulation IGF-1, high levels of insulin can additionally be linked to the development of cancer. Insulin resistance is common in type 2 diabetics, despite the possibility of adequate glycemic control while taking large dosages of insulin. Theoretically, type 2 diabetics who need insulin may have a higher risk of CRC. However, inactivity, obesity, and smoking are risk factors that are similar for both diabetes and CRC. So, the increasing obesity epidemic is expected to result in an increase in the prevalence of diabetes globally, which could lead to the emergence of new CRC cases (Chu et al., 2023; Deng et al., 2012).

Metformin: Metformin, a commonly used anti-diabetic drug, has garnered interest for its potential anti-cancer effects. It is thought to impact cancer cells by affecting energy metabolism, cell cycle regulation, and reducing insulin levels (Chu et al., 2023; Hua et al., 2023; Samuel et al., 2019).

Novel drug discovery is extremely challenging, difficult, and time consuming. As such, drug repurposing offers a more cost-effective method by which, the time and cost associated with the drug development process can be reduced and faster the clinical translation. In this research study, the reason of choosing these three classes drug is as they are interlinked with CRC.

1.4 *In silico* Method

In order to fully understand biological systems and relationships, computational biology utilizes data analysis, mathematical modeling, and computational simulations. By using this technique, the molecular interaction between ligand and target can be understood. Through

molecular docking studies, the binding site structure is revealed, including whether it contains cavities, electrostatic characteristics, such as charge distribution, clefts and sub-pockets. In recent years this method has been extensively used to study several diseases and biological mechanism. Making the most of these enormous data sets has been incredibly beneficial for life science research. Through this advanced technique it is simpler to find ligands with the qualities required for successful binding with the target receptor and the ensuing accomplishment of intended pharmacological and therapeutic effects with careful molecular interaction studies (Muthiah et al., 2021; Pamplona et al., 2023; Soliman & Nafie, 2023; Zhu et al., 2018). Thus, computational biology was used in the study in order to understand the mutated BRAF structure, binding pockets and how these can affect its functions. The drug-interaction with the mutated BRAF was also explored.

1.5 Rationale

This study aimed to explore three classes of FDA-approved drugs (antihypertensive, statins and anti-diabetic) in order to repurpose them for CRC. The rationale behind this comes from the strong association between hypertension, high cholesterol levels and diabetes with CRC. The study tried to identify potential small molecules that could be used to treat CRC with optimized side-effects. The interaction with target protein (mutated $BRAF^{V600E}$) and their pharmacokinetic properties were also explored to propose a candidate that could show effect against CRC. Considering the increased mortality and morbidity of CRC, and the development of drug resistance, this study aimed to use drug repurposing to propose new potent candidates from approved synthetic drugs (Dain Md Opo et al., 2022; Opo et al., 2021; Proietti et al., 2020; Tanda et al., 2020). This study provides evidence about the proposed candidate which can further undergo biological assay, *in vitro*, and *in vivo* experiments.

Chapter 2

Methodology

The study uses *in silico* techniques to propose suitable candidates for treating CRC. Screening drug library through molecular docking was performed, followed by assessing protein-ligand interactions.

AutoDock Vina, AutoDock Tools, PyMOL, Discovery Studio, Open Babel, QikProp tool, Schrodinger's Software were used for this *in silico* study. Several online databases, including PubChem and the RCSB-PDB (Protein Data Bank), have been used concurrently.

The following sections describe the methodologies used for this study.

2.1 Protein Preparation

The Protein Data Bank (https://www.rcsb.org/) was used to obtain the protein's (B-raf kinase V600E) 3D crystal structure (PDB ID: 3OG7) with a resolution of 2.45Å in PDB format. Protein curation was then done using PyMOL where the ligand N-(3-{[5-(4-chlorophenyl)-1H-pyrrolo [2,3-b] pyridin-3-yl] carbonyl}-2,4-difluorophenyl) propane-1-sulfonamide (ID:032) and water molecules were removed. The final protein was prepared for docking by AutoDock Vina through grid generation, and through this grid generation the active binding site of the protein was identified.

2.2 Ligand Preparation

The 3D crystal structures of approximately 120 ligands were downloaded from PubChem.com in SDF format. Then, through Open Babel, drugs in the SDF format were converted into the PDB format and the PDB files were converted to pdbqt file through AutoDock Vina. The

reference drug (Vemurafenib) and the other 120 drugs from different classes (anti-hypertensive, anti-cholesterol and anti-diabetic drugs) were prepared for docking.

2.3 Protein-Ligand Docking Analysis

AutoDock Vina and AutoDock tools were employed to determine the binding affinity between the ligands and the target protein and the binding affinity values were analyzed. The reference drug's binding affinity was -8.5 kcal/mol which was later compared with the other 120 ligands.

2.4 Superimposition

This method is performed to identify if the ligand is binding in the same binding pocket within the same cavity in comparison to the reference drug. Ligands that superimposed or showed overlap in the binding pocket of mutated BRAF with the reference drug were chosen for further analysis (Liu et al., 2022; Talevi, 2018; Wang et al., 2020).The superimposition results were analyzed using PyMOL, where the curated protein was taken along with the reference drug. This indicated that the ligand had the same binding pocket as the reference drug in the target protein.

2.5 Interaction Between Protein and Ligand

To see the interaction between protein and ligand, Discovery Studio was used. By this, the amino acid sequence, distance, category and types of bonds between protein and ligand were identified.

2.6 Pharmacokinetic Parameter Analysis

It is vital to identify the pharmacokinetic properties of chosen ligands and for this, QikProp (Schrodinger's software) was used in the study in order to evaluate ligands' ADME (Absorption, Distribution, Metabolism and Excretion) properties. Using this software, the

physicochemical properties of drugs, such as number of hydrogen bond donors and acceptors, brain/blood partition coefficient (QPlogBB), their molecular weight, intestinal permeability (QPPCaco2), percentage of oral absorption (HOA), partition coefficient, Central Nervous System (CNS) activity, number of rotatable bonds, molecular volume, renal permeability (QPPMDCK), coefficient of binding to human serum albumin (QPlogKhSa) can be predicted (Dain Md Opo et al., 2022; Kabir et al., 2021).

Chapter 3

Result

3.1 Molecular Docking Results

For the purpose of drug repurposing, molecular docking was used to assess the binding affinity of the protein and its ligand (Salmaso & Moro, 2018). As the reference drug's binding affinity was -8.5 kcal/mol, it was considered the cut off value; the better the docking scores of the drugs were from this cut off value, the better is the binding to the target protein. Along with the reference drug's binding affinity, drugs with better binding affinity values than the cut off value are shown in Table 1.

Table 1: Docking results of reference drug and ligands with higher binding affinity than the reference drug.

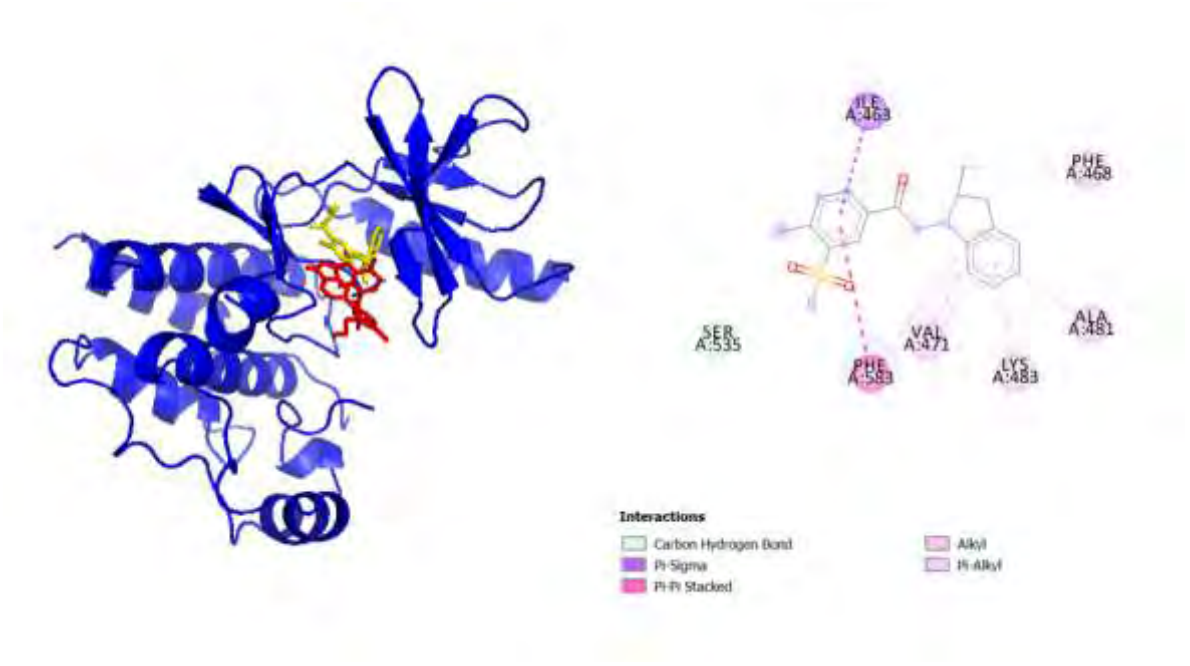
Serial No.	Drug Name	Binding affinity (kcal/mol)
01	Vemurafenib (Reference drug)	-8.5
02	Fimasartan (Anti-hypertensive)	-9.1
03	Valsartan (Anti-hypertensive)	-9.9
04	Olmesartan (Anti-hypertensive)	-9.8
05	Irbesartan (Anti-hypertensive)	-9.4
06	Eprosartan (Anti-hypertensive)	-9.4
07	Candesartan (Anti-hypertensive)	-9.6
08	Azilsartan (Anti-hypertensive)	-11.7
09	Losartan (Anti-hypertensive)	-8.9
10	Ramipril (Anti-hypertensive)	-9.3
11	Lisinopril (Anti-hypertensive)	-9.2
12	Enalapril (Anti-hypertensive)	-9.3
13	Telmisartan (Anti-hypertensive)	-10.1
14	Quinapril (Anti-hypertensive)	-8.7
15	Moexipril (Anti-hypertensive)	-9.6

16	Trandolapril (Anti-hypertensive)	-10.2
17	Benazepril (Anti-hypertensive)	-8.6
18	Terazosin (Anti-hypertensive)	-9.5
19	Prazosin (Anti-hypertensive)	-8.6
20	Phenoxybenzamine (Anti-hypertensive)	-8.9
21	Phentolamine (Anti-hypertensive)	-9.2
22	Reserpine (Anti-hypertensive)	-12
23	Nadolol (Anti-hypertensive)	-9.1
24	Metoprolol (Anti-hypertensive)	-9
25	Labetalol (Anti-hypertensive)	-9.5
26	Betaxolol (Anti-hypertensive)	-9
27	Cilnidipine (Anti-hypertensive)	-10.5
28	Barnidipine (Anti-hypertensive)	-9
29	Triamterene (Anti-hypertensive)	-8.8
30	Metolazone (Anti-hypertensive)	-8.8
31	Chlortalidone (Anti-hypertensive)	-8.7
32	Indapamide (Anti-hypertensive)	-9.1
33	Bendroflumethiazide (Anti-hypertensive)	-10
34	Furosemide (Anti-hypertensive)	-9.1
35	Bumetanide (Anti-hypertensive)	-9.2
36	Spironolactone (Anti-hypertensive)	-9.6
37	Eplerenone (Anti-hypertensive)	-8.9
38	Indoramin (Anti-hypertensive)	-9.8
39	Doxazosin (Anti-hypertensive)	-10.7
40	Verapamil (Anti-hypertensive)	-9.6
41	Lercanidipine (Anti-hypertensive)	-9.8
42	Linagliptin (Anti-diabetic)	-10.4
43	Sitagliptin (Anti-diabetic)	-9.1
44	Empagliflozin (Anti-diabetic)	-10.3
45	Canagliflozin (Anti-diabetic)	-10.7
46	Dapagliflozin (Anti-diabetic)	-10.8

47	Acarbose (Anti-diabetic)	-10.2
48	Repaglinide (Anti-diabetic)	-9
49	Nateglinide (Anti-diabetic)	-9.2
50	Gliquidone (Anti-diabetic)	-8.8
51	Glimepiride (Anti-diabetic)	-9.5
52	Simvastatin (Anti-cholesterol)	-9
53	Pravastatin (Anti-cholesterol)	-9.3
54	Pitavastatin (Anti-cholesterol)	-12
55	Lovastatin (Anti-cholesterol)	-9.7
56	Fluvastatin (Anti-cholesterol)	-11.4
57	Atorvastatin (Anti-cholesterol)	-10.2

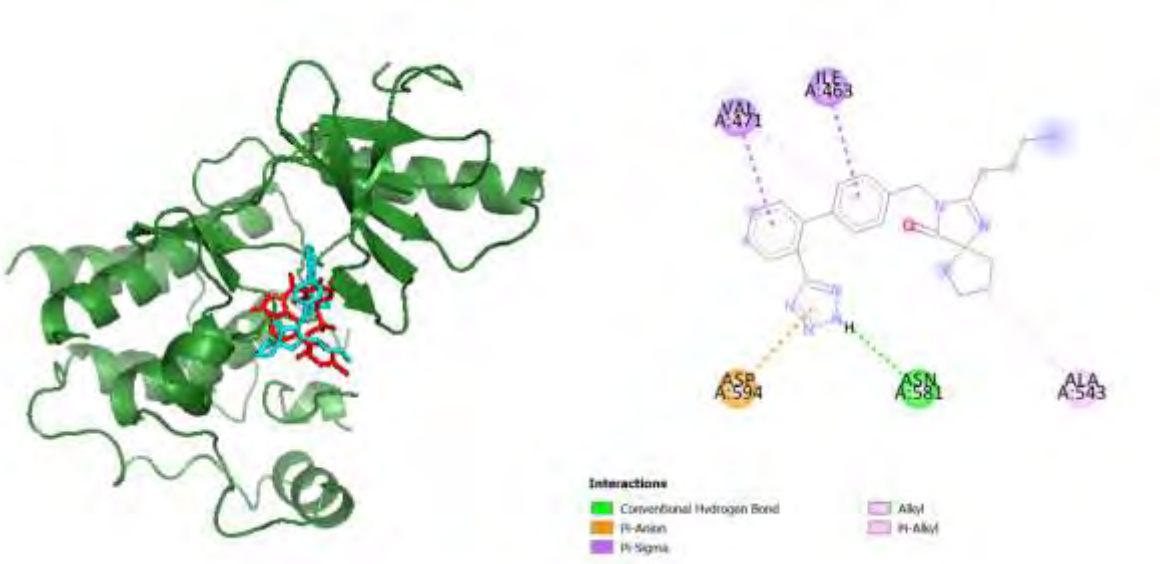
3.2 Superimposition

The selected fifty-six candidates were superimposed on the reference drug to check if the reference and intended candidates were overlapping on each other in the same binding pocket. This was done to determine the binding mechanism. Figure (2-7) shows some of the superimposed drugs (Indapamide, Irbesartan, Pitavastatin, Repaglinide, Valsartan and Verapamil). The figures show that these superimposed drugs are overlapping with the reference drug (Vemurafenib) at a common position in the well-defined binding pocket.



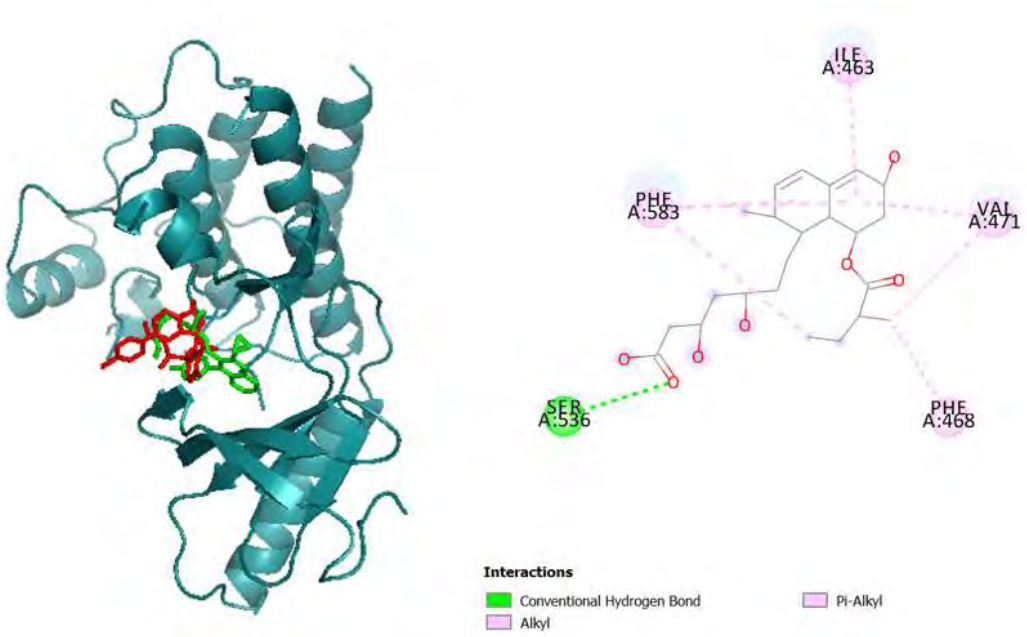
(a) **(b)**

Figure 2:(a) Superimposed binding mode of Vemurafenib (red) and Indapamide (yellow) with BRAF^{V600E} & (b) 2D diagram of BRAF^{V600E}-Indapamide interaction.



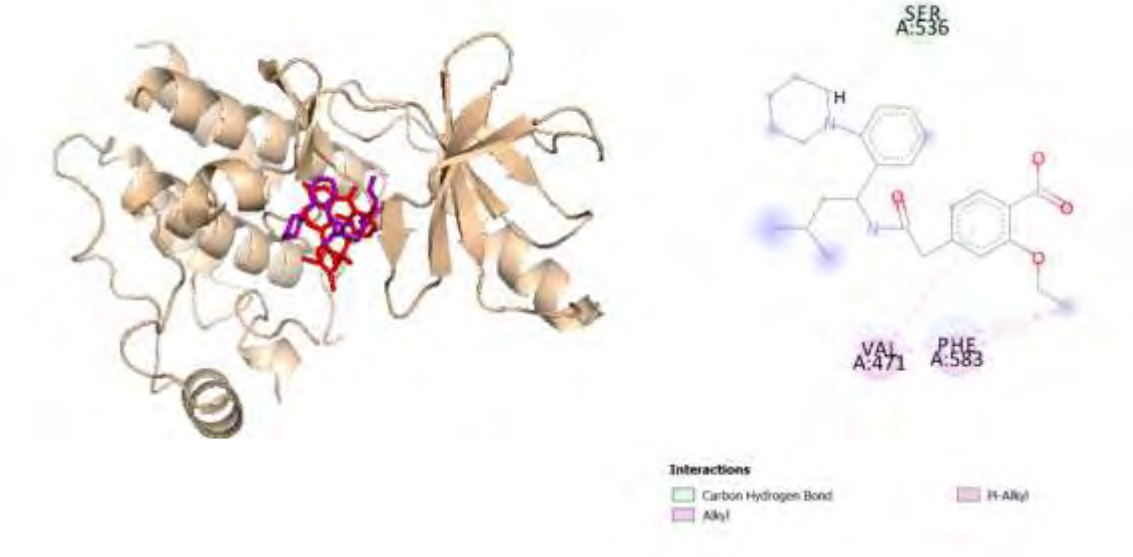
(a) **(b)**

Figure 3:(a) Superimposed binding mode of Vemurafenib (red) and Irbesartan (cyan) with BRAF^{V600E} & (b) 2D diagram of BRAF^{V600E}-Irbesartan interaction.



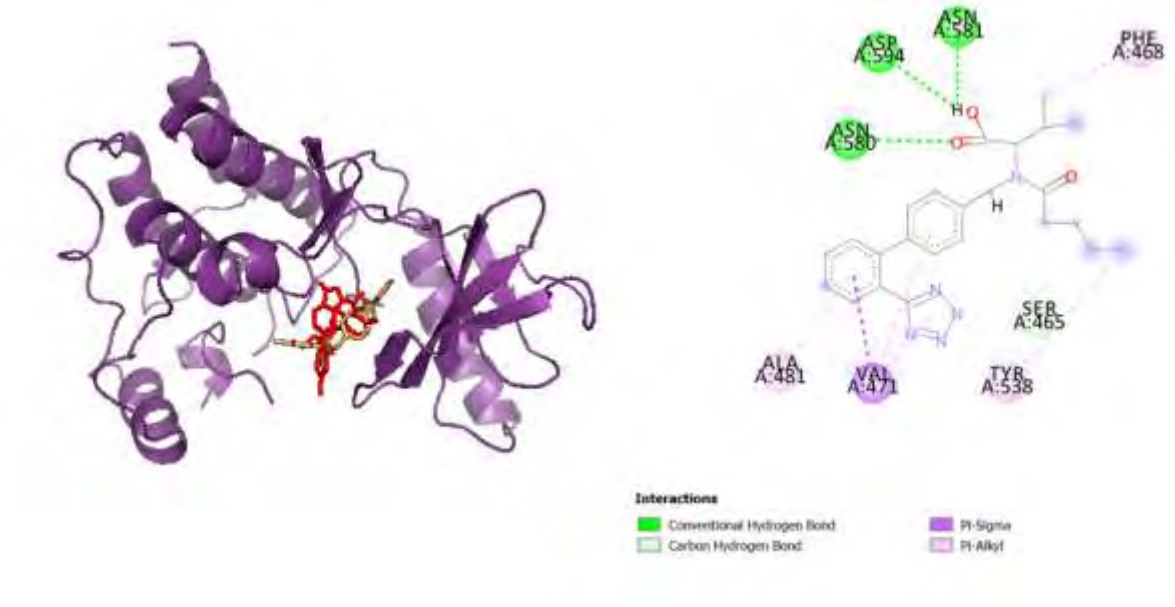
(a) **(b)**

Figure 4:(a) Superimposed binding mode of Vemurafenib (red) and Pitavastatin (green) withBRAF^{V600E} & (b) 2D diagram of BRAF^{V600E}-Pitavastatin interaction.



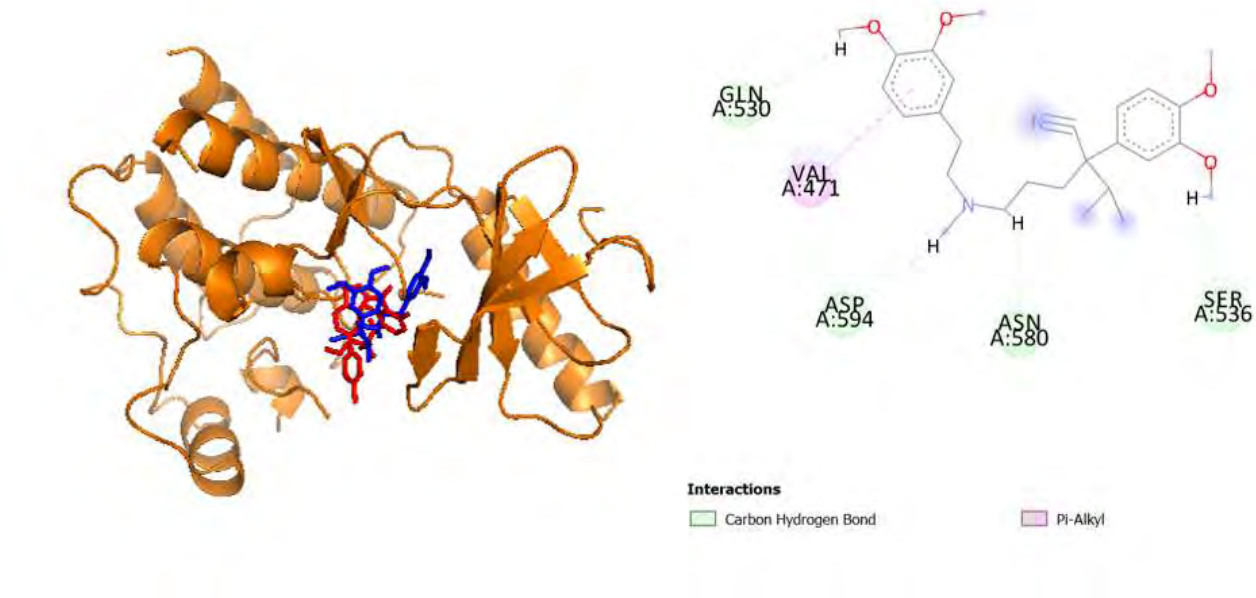
(a) **(b)**

Figure 5:(a) Superimposed binding mode of Vemurafenib (red) and Repaglinide (purple) with BRAF^{V600E} & (b) 2D diagram of BRAF^{V600E}-Repaglinide interaction.



(a) **(b)**

Figure 6:(a) Superimposed binding mode of vemurafenib (red) and Valsartan (pale-yellow) with BRAF^{V600E} & (b) 2D diagram of BRAF^{V600E}-Valsartan interaction.



(a) **(b)**

Figure 7:(a) Superimposed binding mode of Vemurafenib (red) and Verapamil (blue) with BRAF^{V600E} & (b) 2D diagram of BRAF^{V600E}-Verapamil interaction.

3.3 Interaction Between Protein and Ligand Analysis

In this research, the amino acid sequence and types of the bonds were observed through Discovery Studio. Ligands that superimposed with the reference drug were chosen to identify their interaction with the protein and to determine the common amino acid sequence (Table 2), distance and types of the bonds.

Table 2: Bonding Interaction between protein and ligand to identify amino acid sequence.

Serial No.	Name of Drug	Amino acid Sequence
01	Vemurafenib	ASN581, SER536, ASN580, PHE468, VAL471
02	Fimasartan (Anti-hypertensive)	VAL471
03	Valsartan (Anti-hypertensive)	ASN580, ASN581, VAL471, PHE468
04	Olmesartan (Anti-hypertensive)	SER536, VAL471, ASN581
05	Irbesartan (Anti-hypertensive)	ASN581, VAL471
06	Eprosartan (Anti-hypertensive)	ASN580, VAL471
07	Candesartan (Anti-hypertensive)	VAL471, SER536
08	Azilsartan (Anti-hypertensive)	VAL471, SER536
09	Ramipril (Anti-hypertensive)	SER536, VAL471
10	Lisinopril (Anti-hypertensive)	ASN580, VAL471
11	Enalapril (Anti-hypertensive)	VAL471
12	Telmisartan (Anti-hypertensive)	PHE468, VAL471
13	Quinapril (Anti-hypertensive)	VAL471
14	Moexipril (Anti-hypertensive)	SER536, PHE468, VAL471
15	Trandolapril (Anti-hypertensive)	PHE468, VAL471
16	Benazepril (Anti-hypertensive)	SER536
17	Terazosin (Anti-hypertensive)	VAL471
18	Prazosin (Anti-hypertensive)	ASN581, VAL471
19	Phenoxybenzamine (Anti-hypertensive)	VAL471
20	Phentolamine (Anti-hypertensive)	ASN580, VAL471
21	Reserpine (Anti-hypertensive)	ASN580, SER536, VAL471

22	Labetalol (Anti-hypertensive)	VAL471
23	Barnidipine (Anti-hypertensive)	SER536, VAL471
24	Triamterene (Anti-hypertensive)	VAL471
25	Metolazone (Anti-hypertensive)	VAL471
26	Chlortalidone (Anti-hypertensive)	VAL471
27	Indapamide (Anti-hypertensive)	VAL471, PHE468
28	Bendroflumethiazide (Anti-hypertensive)	VAL471
29	Furosemide (Anti-hypertensive)	VAL471
30	Bumetanide (Anti-hypertensive)	VAL471
31	Spironolactone (Anti-hypertensive)	SER536, VAL471
32	Eplerenone (Anti-hypertensive)	VAL471
33	Doxazosin (Anti-hypertensive)	ASN580, VAL471
34	Verapamil (Anti-hypertensive)	ASN580, SER536, VAL471
35	Lercanidipine (Anti-hypertensive)	SER536, VAL471
36	Linagliptin (Anti-diabetic)	SER536, VAL471
37	Sitagliptin (Anti-diabetic)	ASN581, PHE468, VAL471
38	Empagliflozin (Anti-diabetic)	VAL471
39	Canagliflozin (Anti-diabetic)	ASN580
40	Dapagliflozin (Anti-diabetic)	VAL471
41	Acarbose (Anti-diabetic)	SER536, ASN580
42	Repaglinide (Anti-diabetic)	SER536, VAL471
43	Nateglinide (Anti-diabetic)	VAL471
44	Gliquidone (Anti-diabetic)	VAL471
45	Glimepiride (Anti-diabetic)	SER536
46	Simvastatin (Anti-cholesterol)	VAL471, PHE468
47	Pravastatin (Anti-cholesterol)	SER536, VAL471, PHE468
48	Pitavastatin (Anti-cholesterol)	SER536, VAL471
49	Lovastatin (Anti-cholesterol)	VAL471
50	Fluvastatin (Anti-cholesterol)	SER536, VAL471
51	Atorvastatin (Anti-cholesterol)	SER536

3.4 Pharmacokinetic Parameter Analysis

The pharmacokinetic characteristics of the reference drug and selected drugs which have at least three amino acid sequences in common with the reference drug Vemurafenib are shown in Table 3 (Shaikh & Siu, 2016; User Manual, 2015).

Table 3: Pharmacokinetic properties of reference drug and selected drugs.

(http://gohom.win/ManualHom/Schrodinger/Schrodinger_20152_docs/qikprop/qikprop_user_manual.pdf)

Molecule	**Absorption**	**Distribution**			**CNS Permeability**		
	%HOA	**QPPCaco**	**QPPMDCK**	**QPlogKhsa**	**CNS**	**QPlogBB**	**PSA**
Vemurafenib	**93.191**	**226.779**	**490.885**	**0.504**	**-2**	**-1.086**	**101.878**
Indapamide	80.453	302.054	266.041	-0.275	-2	-1.067	100.094
Irbesartan	96.955	416.615	192.012	0.507	-2	-1.382	100.889
Pitavastatin	90.403	95.108	83.54	0.439	-2	-1.224	99.365
Repaglinide	88.776	293.58	180.16	0.488	-1	-0.916	89.013
Valsartan	81.627	87.744	58.935	-0.243	-2	-1.432	121.895
Verapamil	100	918.908	499.495	0.591	1	-0.324	62.28

**Percentage of human oral absorption (%HOA), intestinal permeability (QPPCaco2) in nm/s, Coefficient of binding to human serum albumin (QPlogKhSa), Central Nervous System activity (CNS), Van der Waals surface area of polar nitrogen and oxygen atoms (PSA), IC50 on QPlogHERG, renal permeability (QPPMDCK) in nm/s, brain/blood partition coefficient (QPlogBB).*

Among the other candidates, (Table 3) Verapamil showed the highest human oral absorption (100%). This indicates it has higher bioavailability, higher solubility and higher absorption from site of application to blood stream. Intestinal permeability as indicated by QPPCaco (recommended >500) and QPMDCK (recommended >500) values were also within the recommended range and were better compared to the other chosen candidates. The QPlogKhsa (recommended -1.5 to 1.5) value for Verapamil (0.591) indicates suitable binding to human serum albumin. All these values show good distribution properties.

The CNS permeability properties as shown by CNS value (recommended -2 to 2), QPlogBB value (recommended -3 to 1.2) and PSA value (recommended 7-200) were also within the recommended range for Verapamil. All these values demonstrate Verapamil as a suitable candidate compared to the others (Table 3).

Chapter 4

Discussion

According to previous reports, in metastatic colorectal cancer, the BRAF mutation leads to an inadequate chemotherapy response and a lower patient survival rate. $BRAF^{V600E}$ mutation and its overexpression is considered to be responsible for 80% of carcinomas and the remaining 20% is for V600K (Dain Md Opo et al., 2022; Leonetti et al., 2018; Yan et al., 2022). The goal of this study was to identify potential compounds through computer-based drug design that could target BRAF overexpression by drug repurposing. Drug repurposing or repositioning uses drug molecules with an approved indication for another novel pharmacological action (Pamplona et al., 2023). The study began with the idea to find out the relation between the three classes of drugs (anti-cholesterol, anti-diabetic and anti-hypertensive drugs) and CRC. Molecular docking used virtual screening by using different FDA-approved medications (Umar et al., 2020). The first step was to analyse the binding affinity of around 120 drugs with the target protein (PDB ID:3OG7) through AutoDock Vina and AutoDock tools. The binding affinities were compared to the reference drug, Vemurafenib. The drugs which had better binding affinity than the reference drug (<-8.5kcal/mol) were then chosen for superimposition using PyMOL. Drugs that overlapped with the reference drug in the same binding pocket were chosen. Then the interaction between protein and the chosen ligands, specific amino acid sequence, types of bonds, category and distance through Discovery Studio were observed. The binding of the reference drug (Vemurafenib) with BRAF included 5 amino acids: ASN581, SER536, ASN580, PHE468 and VAL471. Drugs which had at least three or more similar amino acids with the reference were selected. The pharmacokinetic properties of these drugs were analysed using QikProp (Schrodinger Software).

Six candidates Indapamide (anti-hypertensive), Irbesartan (anti-hypertensive), Pitavastatin (anti-cholesterol), Repaglinide (anti-diabetic), Valsartan (anti-hypertensive) and Verapamil (anti-hypertensive) were considered the potential BRAF inhibitors. Based on the overall results Verapamil (anti-hypertensive drug) was considered the most potential inhibitor of $BRAF^{V600E}$ to treat colorectal cancer as it showed three amino acid SER536, ASN580 and VAL471 common with the reference drug. Verapamil showed both hydrogen and hydrophobic bonds with the targeted $BRAF^{V600E}$ like the reference drug. The pharmacokinetic parameter analysis revealed Verapamil had 100% of %HOA (recommended >80%), which indicates that the drug has higher bioavailability, higher solubility and higher absorption from site of application to blood stream. QPPCaco indicates the intestinal permeability which was higher for Verapamil (anti-hypertensive) than the reference drug (Vemurafenib) and other candidates (Table 3). A value greater than 500 is considered as standard and has a great permeability (Muthiah et al., 2021).The value for Verapamil obtained was 918.908 indicating a satisfactory permeability. The blood-brain barrier, however, poses a significant obstacle to drug delivery to the central nervous system. A molecule with a high degree of lipophilicity and a low molecular weight can readily pass the barrier but too polar substances are unable to move across the membrane. A value of QPlogBB (-3 to 1.2) is considered acceptable. On the other hand, QPMDCK value of >500 indicates standard permeability. The QPlogBB value for Verapamil was -0.324 which was within the recommended range (-3 to1.2). Also, the QPMDCK value was 499.495 which is close to the recommended value (500) and it can be considered to have satisfactory permeability (Muthiah et al., 2021). Moreover, on a scale of -2 (inactive) to +2, CNS activity indicates the expected central nervous system activity. As the Verapamil CNS value falls under this range so it can be said that it has good activity on CNS. A positive value indicates it will easily cross the BBB and will not give toxic effect as it is within the range (Lohou et al., 2019).

The recommended range for the QPlogKhsa value, which shows the binding to human serum albumin, is -1.5 to 1.5. The extent to which a drug binds to the proteins in blood plasma may have an impact on the drug's effectiveness. The unbound drug shows higher efficacy and can easily cross the cell membrane than the protein bound drug (Amengor et al., 2022). The value of QPlogKhsa for Verapamil was 0.59 which demonstrated high efficacy and good distribution properties.

After screening and analyzing the pharmacokinetic parameters for 56 drugs, the current study proposes Verapamil (anti-hypertensive drug) as a suitable candidate compared to other candidates for further experiments. MDS (molecular dynamic simulation) can be carried out to further validate the findings. Future work may also include biological assays, *in vitro* studies and *in vivo* studies (Kabir et al., 2021).

Chapter 5

Conclusion

The study proposes Verapamil, an anti-hypertensive drug as a potential inhibitor of $BRAF^{V600E}$. 11% of people who have hypertension, have a greater risk of developing CRC as hypertension blocks cell apoptosis and increase abnormal cell growth. Exploring the potential of Verapamil as a $BRAF^{V600E}$ inhibitor introduces an exciting paradigm shift in cancer therapeutics. By repurposing a well-established anti-hypertensive drug, this study aims to offer a novel approach to target an oncogenic mutation that plays a central role in CRC. Future studies may include evaluation of the synergistic effects of combining Verapamil with existing BRAF inhibitors for enhanced therapeutic efficacy. Further *in vitro* and *in vivo* studies could be done to elucidate how Verapamil exerts its inhibitory effects on $BRAF^{V600E}$ and its downstream targets.

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