Targeting SPARC Protein: Search for Potential Inhibitors for the Treatment of Colorectal Cancer

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
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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 thesis titled “Targeting SPARC Protein: Search for Potential Inhibitors for the Treatment of Colorectal Cancer” submitted by Mahin Ferdous Chowdhury (20346005), of Summer, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Colorectal cancer is the second most deadly cancer worldwide. The search for successful treatment by targeted therapy for colorectal cancer is ongoing, and recent advances look promising. *In silico* study using molecular docking is one way of analyzing approved drugs to repurpose them. Multiple studies have shown the involvement of SPARC protein in the growth and metastasis of colorectal cancer, contributing to its selection as a promising drug target for this study. Molecular docking was carried out and binding affinities of drugs of the five different therapeutic classes with the protein were assessed. Moreover, superimposition and non-bond interactions of the drugs with the protein were visualized and evaluated. Lastly, pharmacokinetic properties of these drugs were analyzed. It was concluded that Griseofulvin and Lemborexant were the most promising candidates for the treatment of colorectal cancer.

Keywords: Colorectal cancer (CRC); SPARC; drug repurposing; molecular docking.

Dedication

Dedicated to my parents

Acknowledgement

First and foremost, all praises and glory to Allah SWT, the All-Knowing and All-Wise, Who has bestowed me with all the knowledge, wisdom and patience and helped me in all my trials and tribulations.

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List of Acronyms

CRC	Colorectal Cancer
EMT	Epithelial-mesenchymal Transition
vHTS	Virtual High-throughput Screening
SPARC	Secreted Protein Acidic and Rich in Cysteine
ECM	Extracellular Matrix
FS	Follistatin-like
EC	Extracellular
PPIs	Proton Pump Inhibitors
GERD	Gastroesophageal Reflux Disease
AE	Adverse Events
5-FU	5-Fluorouracil
ITP	Immune Thrombocytopenic Purpura
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
LG-UTUC	Low-grade Upper Tract Urothelial Cancer
%HOA	Percent Human Oral Absorption

Chapter 1

Introduction

1.1 Cancer

According to the National Cancer Institute, cancer is defined as a collection of diseases characterized by the abnormal proliferation of cells that have mutated leading to the invasion of other cells and tissues. These cells can invade almost all organs of the body and can metastasize as well (spread to distant sites of the body via the blood and lymphatic systems) (Gaidai et al., 2023). The word cancer is also used as neoplasm or malignant tumor (World Health Organization, 2019). Table 1 shows the prevalence and percentage of the different types of cancer, and Table 2 shows their mortality rates.

Table 1: Prevalence and Percentage of Different Types of Cancer (Global Cancer Burden Growing, amidst Mounting Need for Services, 2024)

Type of Cancer	**Prevalence (Number of new patients)**	**Percentage of Total Incidence**
Lung cancer	2.5 million	12.4%
Female breast cancer	2.3 million	11.6%
Colorectal cancer	1.9 million	9.6%
Prostate cancer	1.5 million	7.3%
Stomach cancer	9,70,000	4.9%

Table 2: Mortality Rate of Different Types of Cancer (Global Cancer Burden Growing, amidst Mounting Need for Services, 2024)

Type of Cancer	**Number of Cancer Deaths**	**Percentage**
Lung cancer	1.8 million	18.7%
Colorectal cancer	9,00,000	9.3%
Liver cancer	7,60,000	7.8%
Breast cancer	6,70,000	6.9%
Stomach cancer	660,000	6.8%

It is predicted that the world will see a 77% increase in cancer incidence by 2050, accounting for over 35 million new cancer cases, an estimated 20 million increase from 2022.

Tumor development involves a multistep process known as carcinogenesis, where several etiologies are involved (M. Kulesz-Martin et al., 2018). The activation and progression of cancer involves multiple genes, such as oncogenes. An oncogene is a cellular gene (proto-oncogene) that has undergone mutation and fusion with another gene or is overexpressed, making it dysfunctional. Oncogenes usually facilitate carcinogenesis by deregulating cell division or eliminating apoptosis in cancer cells (Brown, 2021). It comprises of four stages namely tumor initiation, tumor promotion, malignant conversion, and tumor progression. When cells get exposed to harmful chemicals, the covalent bonds in DNA get damaged resulting in certain mutational events like the triggering of proto-oncogenes and deactivation of tumor suppressor genes. The mutations, irrespective of their order, accumulate over time and result in multistage carcinogenesis (Weston & Harris, 2003).

Accordingly, there are different cancer treatments depending on cancer type and how advanced it is. The common cancer treatments include chemotherapy, hormone therapy, immunotherapy, hyperthermia, radiation therapy, targeted therapy, surgery etc (*Types of Cancer Treatment - NCI*, n.d.).

1.2 Colorectal Cancer

Colorectal cancer (CRC) makes up approximately 10% of all cancer incidences, and the third most frequently encountered cancer worldwide. Globally, it is ranked second in causing most cancer-related deaths (*Colorectal Cancer*, n.d.). The term CRC has originated from three distinct aspects (Alzahrani et al., 2021). The first aspect postulates that both the cancers (colon cancer and rectal cancer) originate in the large intestine making them a single type of cancer. The second aspect depicts that the colonic and rectal walls share identical anatomical structure

of the mucosa, muscular layer and, partially, the serosa, in addition to their comparable histology. The homogeneous functions of the colorectal tract, comprising of fluid resorption, concentration and transportation of stool and its excretion makes up the third aspect of CRC. The condition in which normal epithelium of the colon and rectum gets mutated into a precancerous lesion (adenomatous intermediate) and, ultimately, to an invasive carcinoma (adenocarcinoma) is defined as colorectal cancer (CRC).

People with colon cancer usually do not exhibit symptoms in the beginning. However, when the symptoms appear, they depend on the size of the growth and location in the large intestine. It is generally identified by the following symptoms (*Colon Cancer - Symptoms and Causes - Mayo Clinic*, n.d.):

- Abnormal bowel activity, such as constipation or frequently occurring diarrhea.
- Bleeding of the rectum or stool containing blood.
- Continuous abdominal discomfort including painful cramps and gas.
- A feeling of insufficient bowel movements.
- Constantly feeling weak or tired.
- Weight loss for unknown reasons.

The development of CRC involves multiple risk factors. People having one or more of the following factors are more vulnerable to CRC (*Colon Cancer - Symptoms and Causes - Mayo Clinic*, n.d.):

- Age (usually older than 50)
- Ethnicity
- A personal history of CRC or polyps (According to (Fearnhead et al., 2002))

- Inflammatory bowel disorders
- Inherited conditions such as familial adenomatous polyposis and Lynch syndrome
- High fat and low fiber diet
- Lack of exercise
- Diabetes, obesity, smoking
- Alcohol

1.3 Current Treatment Strategies for Colorectal Cancer and Their Drawbacks

Patients with cancer are usually treated with chemotherapy, surgery and radiation or a combination of these if it is in an advanced stage (Alzahrani et al., 2021). A combination of cytotoxic and targeted antineoplastic agents is typically used to treat patients suffering from metastatic CRC (Mármol et al., 2017). A variety of drug combinations consisting of Folfiri (folinic acid, fluorouracil and irinotecan) and Folfox (folinic acid, fluorouracil and oxaliplatin) has emerged as a first-line treatment over the past few years. Although chemotherapy is used by healthcare practitioners as the primary mode of cancer treatment, their nonspecific toxicity against rapidly dividing cells and the development of secondary resistances still makes them a less preferred choice for the optimum treatment of CRC (García-Aranda & Redondo, 2019).

1.4 Drug Repurposing

A process by which applications of approved or investigational drugs are identified that are an alternative option to the existing medical indication is called drug repurposing, also known as drug repositioning or reprofiling (Pushpakom et al., 2018). Many incurable and orphan diseases have been treated by repurposed drugs over the past few decades. For instance, metformin, a

popular antidiabetic drug was concluded to have anticancer properties as it reduces the occurrence of multiple cancers, as well as inhibits the proliferation and migration of cancer cells, activates apoptosis, and reduces EMT (epithelial-mesenchymal transition) and metastasis (Kulkarni et al., 2023). Another example of drug repurposing is the use of antibiotics to treat cancer. Some of the popular antibiotics namely clarithromycin, pioglitazone and treosulfan have been repurposed for being used to treat patients with non-small cell lung cancer, whereas, doxorubicin can treat breast, lung, stomach, ovarian, bladder and thyroid cancers (Kulkarni et al., 2023).

Drugs selected for repurposing may also be tested directly for Phase II clinical trials. The rationale behind this is that these drugs already have all safety, preclinical and efficacy data available which greatly assists the researchers to make accurate conclusions at every stage of the development process. The researchers also have a reduced risk of failure with these drugs (Kulkarni et al., 2023).

1.5 *In silico* Drug Designing

In silico drug designing is a section of computer-aided drug design used in the discovery, optimization and validation of drugs that were screened from a vast library of drug molecules. It primarily focuses on compounds which are more likely to bind to the protein targets present in the body which, in turn, increases the number of drugs screened from the large library (virtual high-throughput screening [vHTS]) (Zoete et al., 2009). *In silico* drug designing has gained momentum in recent years because of its significant role in the facilitation of rapid and lost-cost drug designing which greatly helped researchers screen large drug libraries in a short amount of time and develop or repurpose drugs that would meet unmet clinical needs.

1.5.1 Molecular Docking

Drug repurposing involves the molecular docking technique, a computational strategy based on the structure of the drug for the prediction of complementarity of binding site between the ligand (usually, a drug) and its target (usually, a receptor) (Pushpakom et al., 2018). It is a computational technique that employs over 30 computational tools and algorithms (Zoete et al., 2009), such as AutoDock Vina, Discovery Studio, AutoDock GOLD etc (Agu et al., 2023).

The entire docking process can be roughly divided into two interconnected steps. It begins by examining multiple ligand conformations in the target protein's active binding pocket which is followed by the use of a scoring function to rank these conformations (Meng et al., 2012). It is expected that sampling algorithms simulate the binding mode occurring in experiments whereas the scoring function will mark it highest among all generated conformations.

Pushpakom et al. (2018) explained the process of molecular docking as:

- Conventional docking: One target but multiple ligands. Researchers prefer this type of docking when they need to examine a set of drug molecules against a single target involved in a particular disease.
- Inverse docking: Multiple targets but one ligand. In this type of docking, prior knowledge is not available regarding a single drug target involved in a disease. For this reason, a group of targets are taken into consideration and investigated against the library of drugs to predict the interactions between them and select drugs for repurposing.

Molecular docking greatly helps in the drug development and optimization process by providing fundamental information regarding the ligand-target interactions. It helps researchers in estimating the affinity of the drugs for the selected target much before its synthesis.

1.6 Targeted Protein: SPARC (Secreted Protein Acidic and Rich in Cysteine)

Secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin or BM-40, is a glycoprotein of the extracellular matrix (ECM), with a molecular weight of 32kDa. It is primarily involved in a variety of functions namely tissue remodeling, wound repair, and cell migration and differentiation (Naito et al., 2021). This protein was initially discovered by Termine JD who isolated it from non-collagenous fetal bovine bone extract and found it to be its primary component (Jiang et al., 2023). The presence and location of SPARC may vary depending on the age of the individual. From early development till mid-gestation, SPARC is present in the interface between the epithelium and mesenchyme and in the mesenchyme. However, the protein is notably absent from the epithelium in adults and is only found in the muscularis mucosa of the colon (Drev et al., 2019).

The structure of SPARC resembles the other proteins of the same family. Its domain comprises of three sections: a "cysteine-rich follistatin-like (FS) domain, an acidic N-terminal domain, and an *a*-helical extracellular (EC) calcium-binding domain with an EF-hand motif" (Jiang et al., 2023). It can bind to several extracellular components owing to its unique structural features.

Poor prognosis was observed in cancer patients with high SPARC expression. Thus, SPARC has the potential to be used as a significant indicator of prognosis in CRC undergoing chemoradiotherapy (Jiang et al., 2023).

1.7 Selection of Drugs

A list of drug classes was prepared randomly to determine their potential in the treatment of CRC, namely statins, antihypertensive drugs, antifungal agents, sedatives and hypnotics, and

proton pump inhibitors (PPIs). All the selected drugs were FDA-approved and majority of them are currently available in the market for the treatment of a variety of indications.

1.7.1 Statins

A class of drugs that works to inhibit the functions of HMG-CoA reductase enzyme competitively is known as statins or HMG-CoA reductase inhibitors. This enzyme is essential for mevalonate synthesis which is a naturally occurring substance involved in the production of cholesterol in the human body. There are 7 types of statins currently available in the market namely atorvastatin (Figure 1), fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin.

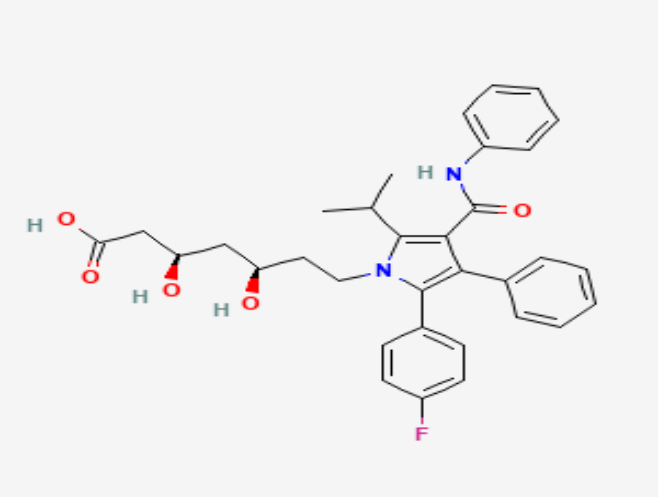


Figure 1: 2D Structure of Atorvastatin (Retrieved from PubChem)

Statins made a revolutionary change in the treatment of hypercholesterolemia because of their significant effect in lowering blood cholesterol level (Stancu & Sima, 2001). In addition, they exert antiatherosclerotic and pleiotropic effects independent of their hypolipidemic action. Statins have limited adverse effects and are well tolerated by the body. In recent years, statins have been repurposed extensively and can be used in the treatment of prostate cancer, breast cancer, non-Hodgkin lymphoma, hepatocellular carcinoma, glioblastoma (Liu et al., 2023), head and neck cancer (Bourguillon et al., 2021), lung cancer (Amin et al., 2022) etc.

1.7.2 Antihypertensive Drugs

Antihypertensives make up a popular class of drugs used to lower blood pressure in patients with hypertension. Only a small portion of patients are prescribed with a single antihypertensive drug as the conventional prescription usually involves two or more such agents (Schiffrin, 2010). For this study, four notable classes of antihypertensive drugs namely β-blockers, calcium channel blockers, diuretics and renin-angiotensin system inhibitors have been selected (Feldman et al., 2015).

1.7.3 Antifungal Agents

Drugs which selectively inhibit fungal pathogens inside the host either by inhibiting their growth or by destroying them without inflicting any harm to the host are called antifungal agents (Dennis M. Dixon & Thomas J. Watsh, 1996). Only a handful of antifungal drugs are effective nowadays for their use in eliminating fungal pathogens causing severe infections (Seyedmousavi et al., 2017). Some popular antifungal drugs include azoles like fluconazole, itraconazole, and ketoconazole and allylamines like terbinafine etc.

1.7.4 Anxiolytics, Sedatives and Hypnotics

At present times, anxiolytics and sedatives are mainstay in two types of anesthetic practice, namely to relieve anxiety in patients before certain surgical procedures and as adjuvants during anesthesia (Barends & Absalom, 2016). There is a wide array of these drugs available in the market. Examples include benzodiazepines (alprazolam, clonazepam, lorazepam), barbiturates (secobarbital, phenobarbital, mephobarbital), doxepine, zolpidem etc.

1.7.5 Proton Pump Inhibitors (PPIs)

Starting from their introduction more than 25 years ago, proton pump inhibitors (PPIs) have become the most commonly used, safe and effective drug in the management of disorders caused by gastric acid such as gastroesophageal reflux disease (GERD) and peptic ulcer disease (Strand et al., 2017). The drugs must activate within the parietal cells in order to get ionized which will facilitate the formation of covalent disulfide bonds with cysteine residues of the H+/K+ ATPase (Ward & Kearns, 2013). The proton pump gets inactivated i.e., no longer secretes gastric acid when PPI binds to it. Examples of PPIs include lansoprazole, omeprazole (Figure 2), pantoprazole, rabeprazole, and esomeprazole.

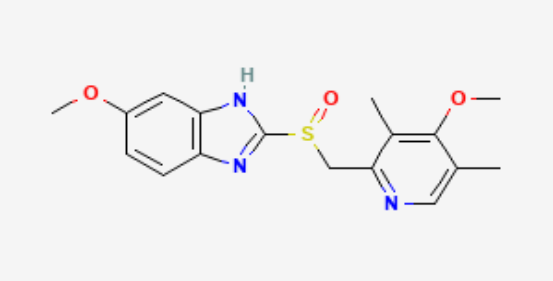


Figure 2: 2D Structure of Omeprazole (Retrieved from PubChem)

1.8 Aim of the Study

The study aims to find potent inhibitors of SPARC protein from the approved list of synthetic drugs that may be used in the treatment of CRC.

1.9 Rationale

Cancer takes the life of millions of people each year making it one of the deadly diseases worldwide. The treatment of cancer can be difficult because of frequent development of drug resistance and omnipresent adverse effects. The conventional chemotherapy used in cancer treatment can be expensive, time-consuming and poses significant risk to the patients.

Patients who have undergone chemotherapy face enormous adverse events (AE) with varied severity. 5-Fluorouracil (5-FU), the most widely used antineoplastic agent used to treat CRC has a wide range of toxicities, such as diarrhea, vomiting, immune thrombocytopenic purpura (ITP), mucositis, neutropenia, hand and foot syndrome etc. (Fotheringham et al., 2019). Approximately, one-fifth of the total patients treated with 5-FU suffer from severe toxicities reaching grade 3/4 and 0.5%-1% of patients experience fatal toxicity. Oxaliplatin, another agent frequently used in combination with 5-FU, causes sensory peripheral neuropathy, a disabling condition which affects the patient's quality of life. Thus, it is necessary to develop safer and effective alternative treatment options for CRC.

Researchers have made tremendous advancements over time in molecular oncology facilitating the generation of exceptionally specific therapies which can disrupt genes or proteins essential for the cancer cell's growth and survival and thereby, prompt apoptosis in these cells. In addition, these advanced treatments are preferred to be mainstay in the treatment of cancer due to their significantly reduced adverse effects compared to conventional treatment options (García-Aranda & Redondo, 2019). Drug repurposing can substantially contribute to this aspect by proposing potential candidates already approved by the FDA for treatment of other indications to be used in treating cancer. Nowadays, pharmaceutical companies and research institutions are more inclined to repurposing already approved drugs because of their shortened development cycle, reduced expenditure and shorter approach in bringing alternative effective treatments to patients compared to the complicated and lengthy conventional drug discovery and development processes (Parvathaneni et al., 2019).

Chapter 2

Methodology

Several databases such as PubMed, Elsevier, Nature, Springer etc. were studied and pertinent information regarding CRC, its pathogenesis, symptoms, risk factors and current treatment strategies was collected. Significant details regarding *in silico* drug designing, molecular docking and SPARC protein were assembled as well for the purpose of the study. This was followed by the performance of molecular docking to find out the binding affinities between the ligands and the protein (macromolecule). After that screening of the selected synthetic drugs was carried out with the protein to find out the best possible drugs for the treatment of CRC.

2.1 Online Software, Tools and Databases Used for Molecular Docking and Visualization

As this is an *in silico* study, a number of different software and tools such as PyMOL, Discovery Studio, Autodock Vina etc. were used for molecular docking, visualization and screening purposes. In addition, databases like RCSB-PDB and PubChem were used to obtain the three-dimensional (3D) structure of the protein and the selected drugs respectively. These software and tools were essential for the accurate and expeditious conduct of the study.

Table 3: Online Software and Tools Used in the Study

Serial No.	Software and Tools	Version
01	PyMOL	1.7.4
02	OpenBabel GUI	2.4.1
03	Autodock Vina	1.5.6
04	Autodock Tools	1.5.6
05	Discovery Studio	16.1.0.15350
06	QikProp	-

2.2 Molecular Docking and Visualization

Journals were analyzed to get an idea about the proteins involved in the development, progression and metastasis of CRC. Screening of multiple journals showed that progression and metastasis of CRC was significantly dependent on SPARC, thereby making it a favorable target for the discovery of novel CRC treatments (Naito et al., 2021) (Drev et al., 2019). Molecular docking, visualization and screening of the selected synthetic drugs was then carried out.

2.2.1 Macromolecule (Protein) Preparation

The PDB structure of the SPARC protein (PDB ID: 2V53) was downloaded from RCSB-PDB. In order to curate the protein, all the ligands and water molecules were removed from it using PyMOL. Following this, the protein was converted into PDBQT file using AutoDock Vina.

2.2.2 Ligand Preparation

The 3D structure of all the selected synthetic drugs were downloaded from PubChem database in SDF format. Using OpenBabel GUI, the SDF files of these drugs were converted into PDB format. As rigid-docking is performed in this study, the drugs were then converted into PDBQT files by making all the active bonds non-rotatable.

2.2.3 Steps in Molecular Docking

After the preparation of the protein and selected drugs, site-specific molecular docking was carried out using AutoDock Vina. The drugs demonstrating strong binding affinities were taken to the next stage. A stronger binding affinity of the synthetic drugs toward the protein was indicated by a lower (or more negative) value (Kabir et al., 2021). Following this, PyMOL was used to check the superimposition of these drugs with the standard drug. The superimposed drugs were further screened using Discovery Studio Visualizer to investigate the protein-ligand interactions. This step gave information regarding the amino acids involved, their distance, category and types of bonds. Similarities were drawn between the amino acids involved with the drugs and that of the standard drug. The last step was to assess the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of the drugs using QIKPROP software.

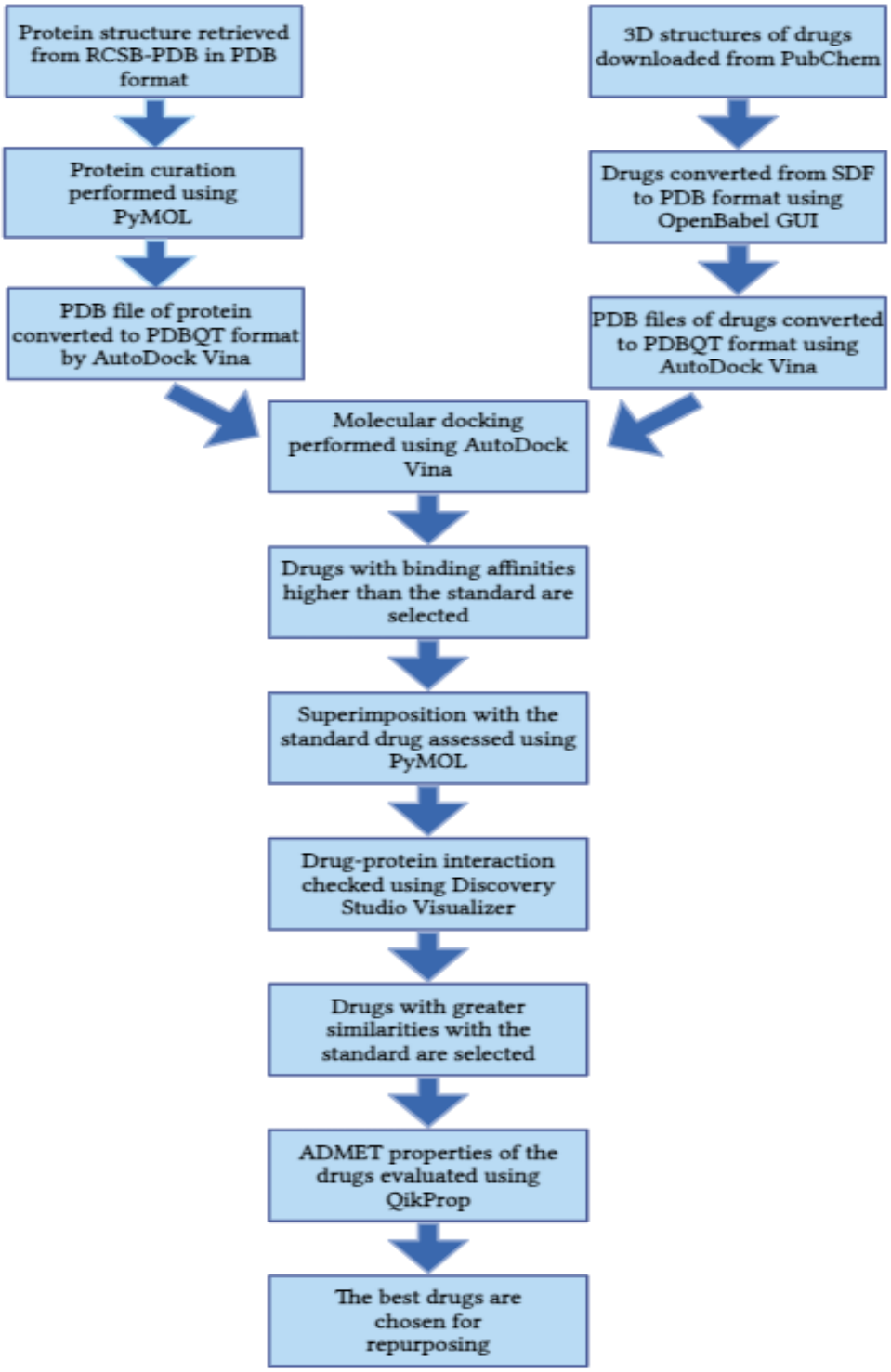


Figure 3: Schematic Representation of Molecular Docking and Screening Process

Chapter 3

Results

3.1 Protein Structure

RCSB-PDB database was used to retrieve the PDB structure of the desired SPARC protein (PDB ID: 2V53). Its expression system was *Homo sapiens* and the resolution was 3.20Å (Table 4 and Figure 4).

Table 4: Overview of SPARC Protein Retrieved from RCSB-PDB

PDB ID of Protein	Name of Protein	Expression System	Resolution	Mutation	Chains	Publication Year
2V53	Crystal structure of a SPARC-collagen complex	*Homo sapiens*	3.2Å	No	A, B, C, D, E	2008

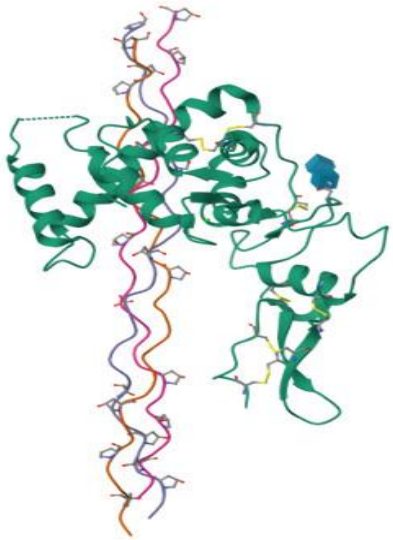


Figure 4: 3D Structure of SPARC Protein (Retrieved from RCSB-PDB)

3.2 *In silico* Screening of Selected Synthetic Drugs

3.2.1 Selection of Standard Drug

Mitomycin (PubChem CID: 5746), an established inhibitor of SPARC protein, was chosen as the standard drug (Kuan-Chou Lin et al., 2023). It is a cytotoxic antibiotic which is used as anticancer therapy in patients suffering from late-stage stomach, metastatic colorectal cancer and pancreatic cancer. In the 1950s, it was first discovered from cultures of *Streptomyces caespitosus* by Japanese microbiologists.

Mitomycin (Figure 5) is an alkylating agent responsible for the cross-linking in the complementary strands of DNA double helix and results in the inhibition of DNA synthesis at lower concentration and RNA and protein synthesis at higher concentrations. It is also commonly indicated in the treatment of female breast cancer, mouth (oral) cancer, pharyngeal cancer, gastrointestinal cancer, peritoneal carcinomatosis, urothelial carcinoma etc. Additionally, it is used as an adjunct therapy in patients undergoing ab externo glaucoma surgery.

As the binding affinity of Mitomycin appeared to be -7.2 kcal/mol, therefore drugs having binding affinities greater than this value were taken for further screening.

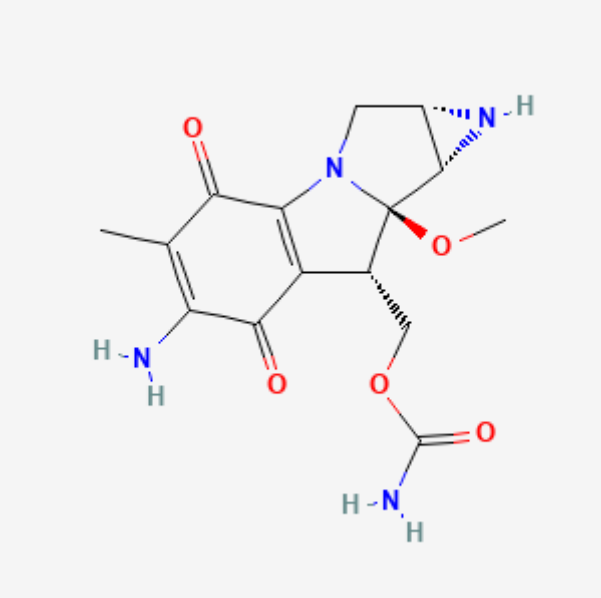


Figure 5: 2D Structure of Mitomycin (Retrieved from PubChem)

3.2.2 Rigid Docking of the Ligands

The five classes of drugs were taken into account for rigid docking. As mentioned before, the standard drug is Mitomycin whose binding affinity with the protein of interest (PDB ID: 2V53) was found to be -7.2 kcal/mol. This value was taken as the threshold for selected ligands and those having binding affinities greater than this value (lower or more negative value) were considered for further screening (Tables 5-9).

Table 5: Rigid Docking Results of Statins having Higher Binding Affinities than Mitomycin

Serial No.	Statins	Binding Affinity (kcal/mol)
01	Atorvastatin	-8.6
02	Cerivastatin	-7.3
03	Ezetimibe	-9.6
04	Fluvastatin	-8.6
05	Lovastatin	-8.8
06	Mevastatin	-9.2
07	Pitavastatin	-8.8
08	Pravastatin	-8.6
09	Rosuvastatin	-8.8
10	Simvastatin	-9.3

Table 6: Rigid Docking Results of Antihypertensive Drugs having Higher Binding Affinities than Mitomycin

Serial No.	Antihypertensive Drugs	Binding Affinity (kcal/mol)
01	Benazepril	-9.1
02	Cilazapril	-8.6
03	Enalapril	-8
04	Enalaprilat	-8.2
05	Lisinopril	-8
06	Moexipril	-7.3
07	Naftopidil	-8.3
08	Perindopril	-7.7
09	Quinapril	-9.3
10	Ramipril	-9.7
11	Trandolapril	-9.9
12	Urapidil	-7.7
13	Carteolol	-7.4
14	Carvedilol	-7.7
15	Esmolol	-7.3
16	Labetalol	-7.8
17	Nebivolol	-9.3
18	Penbutolol	-7.9

19	Talinolol	-8.6
20	Amlodipine	-7.8
21	Clevidipine	-7.6
22	Diltiazem	-7.5
23	Isradipine	-7.7
24	Levamlodipine	-7.7
25	Nicardipine	-8.9
26	Nimodipine	-7.5
27	Nisoldipine	-7.9
28	Verapamil	-7.4
29	Azilsartan	-10.1
30	Candesartan	-9.1
31	Eprosartan	-8.7
32	Fimasartan	-9.2
33	Forasartan	-8.5
34	Irbesartan	-9.1
35	Losartan	-8.7
36	Olmesartan	-9.3
37	Pratosartan	-9.1
38	Tasosartan	-8.9
39	Telmisartan	-8.2
40	Valsartan	-9.2
41	Bendroflumethiazide	-8.2
42	Chlorthalidone	-7.8
43	Eplerenone	-8.6
44	Furosemide	-7.3
45	Indapamide	-8.2
46	Metolazone	-7.7
47	Spironolactone	-8.1
48	Torsemide	-7.6
49	Xipamide	-7.9

Table 7: Rigid Docking Results of Antifungal Agents having Higher Binding Affinities than Mitomycin

Serial No.	Antifungal Agents	Binding Affinity (kcal/mol)
01	Griseofulvin	-7.4
02	Ketoconazole	-7.8
03	Terbinafine	-7.7
04	Terbinafine Hydrochloride	-7.7
05	Terconazole	-7.5
06	Voriconazole	-7.6

Table 8: Rigid Docking Results of Anxiolytics, Sedatives and Hypnotics having Higher Binding Affinities than Mitomycin

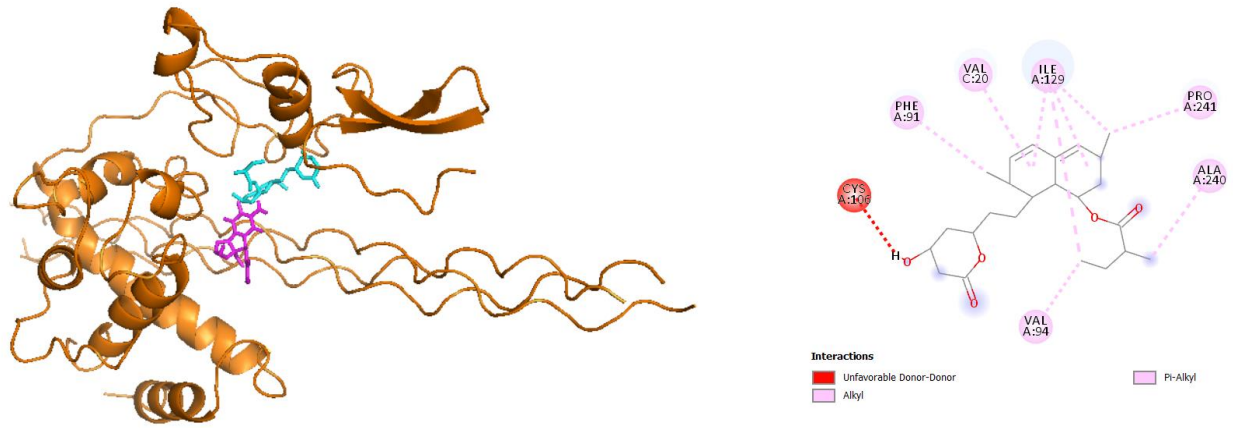
Serial No.	Anxiolytics, Sedatives and Hypnotics	Binding Affinity (kcal/mol)
01	Alprazolam	-7.8
02	Clonazepam	-7.4
03	Lorazepam	-7.5
04	Chlordiazepoxide	-7.8
05	Temazepam	-7.6
06	Midazolam	-7.4
07	Triazolam	-7.9
08	Flurazepam	-7.7
09	Clorazepate	-8.1
10	Oxazepam	-8.2
11	Quazepam	-7.5
12	Estazolam	-7.3
13	Remimazolam	-7.6
14	Buspirone	-8.6
15	Zolpidem	-8.3
16	Suvorexant	-8.4
17	Eszopiclone	-7.6
18	Lemborexant	-8.5
19	Daridorexant	-8.2
20	Doxepin	-7.6
21	Zaleplon	-7.3

Table 9: Rigid Docking Results of Proton Pump Inhibitors (PPIs) having Higher Binding Affinities than Mitomycin

Serial No.	Proton Pump Inhibitors (PPIs)	Binding Affinity (kcal/mol)
01	Dexlansoprazole	-8.3
02	Esomeprazole	-8.2
03	Lansoprazole	-8.2
04	Omeprazole	-8.2
05	Pantoprazole	-7.9
06	Rabeprazole	-7.7

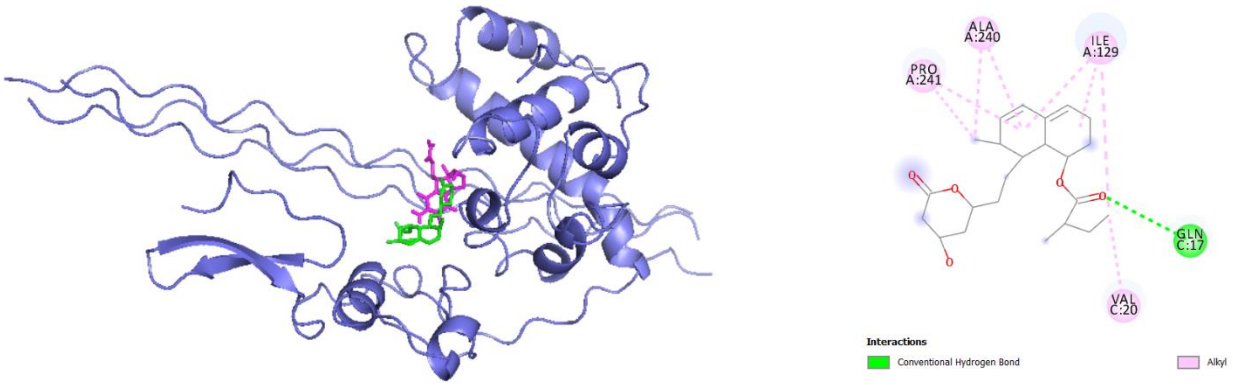
3.2.3 Superimposition of Ligands with Standard Drug

Ligands having binding affinities higher than the standard drug were further evaluated using PyMOL. The software was used to observe whether the ligands superimpose on the standard drug or not. A total of 70 drugs from the selected drug classes were found superimposed at identical binding pockets as the standard drug. The superimposition of some well-performed drugs is shown along with their interactions with the SPARC protein (Figure 6-12).



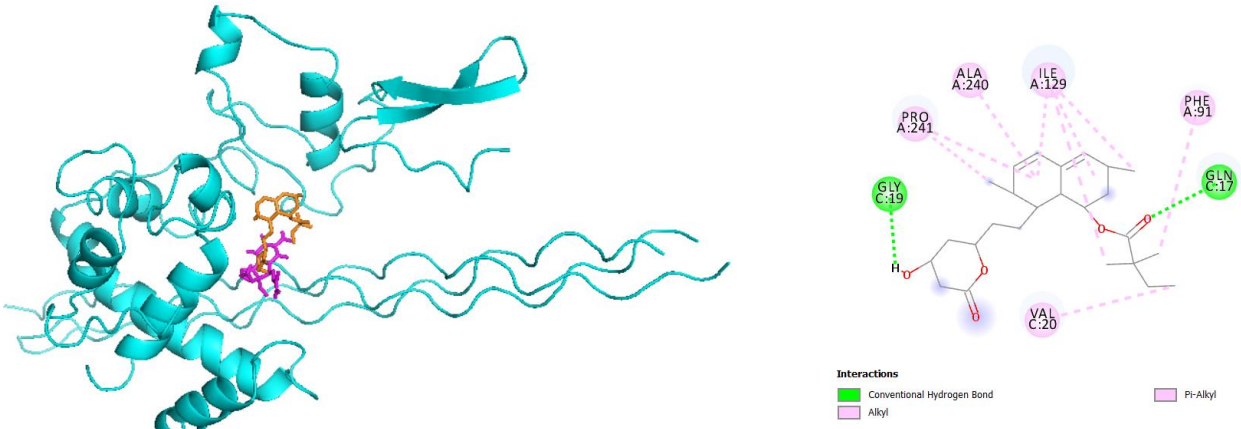
(a) (b)

Figure 6: (a) Superimposition of Mitomycin (magenta) and Lovastatin (cyan) with SPARC Protein, (b) 2D Diagram of SPARC-Lovastatin Interaction



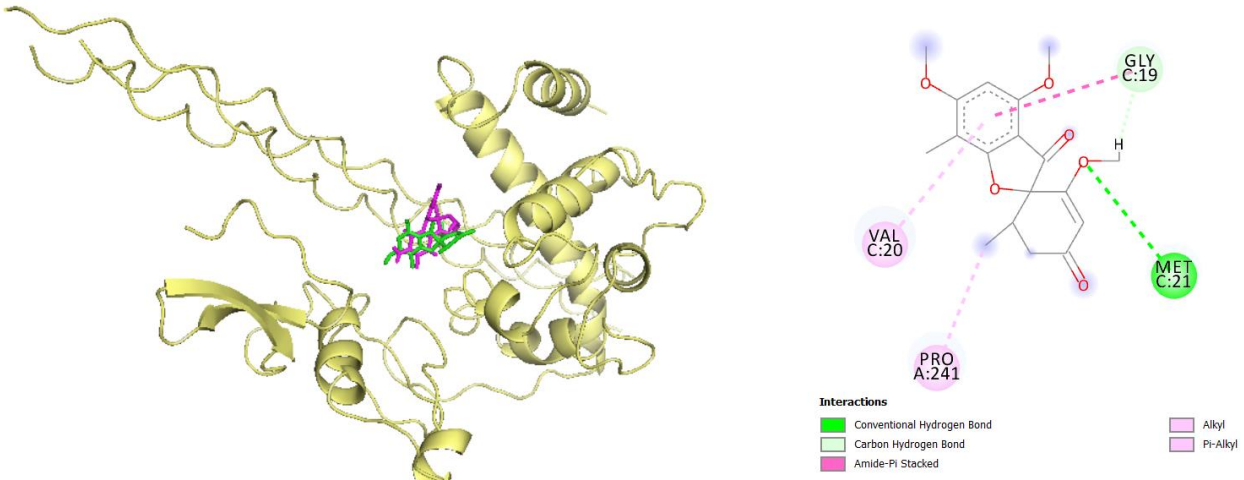
(a) (b)

Figure 7: (a) Superimposition of Mitomycin (magenta) and Mevastatin (green) with SPARC Protein, (b) 2D Diagram of SPARC-Mevastatin Interaction



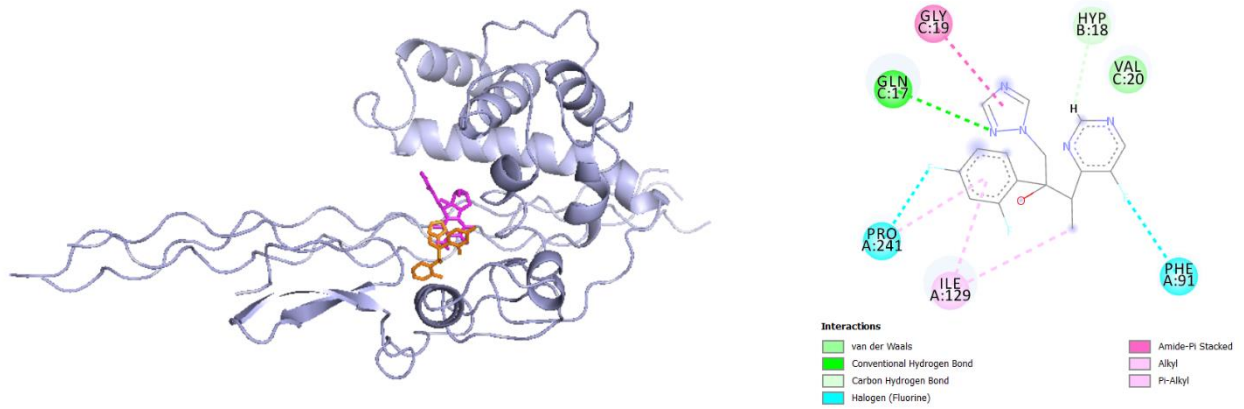
(a) (b)

Figure 8: (a) Superimposition of Mitomycin (magenta) and Simvastatin (orange) with SPARC Protein, (b) 2D Diagram of SPARC-Simvastatin Interaction



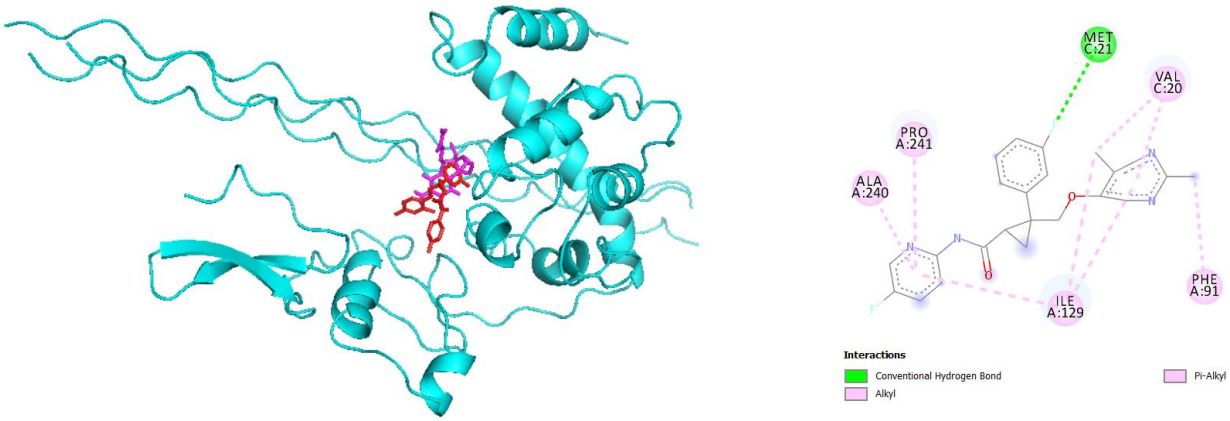
(a) (b)

Figure 9: (a) Superimposition of Mitomycin (magenta) and Griseofulvin (green) with SPARC protein, (b) 2D Diagram of SPARC-Griseofulvin Interaction



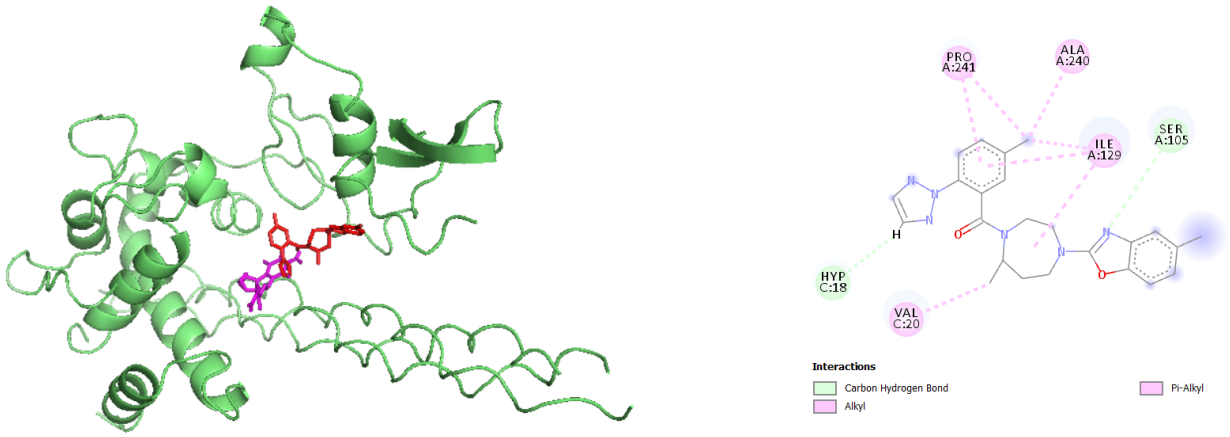
(a) (b)

Figure 10: (a) Superimposition of Mitomycin (magenta) and Voriconazole (orange) with SPARC Protein, (b) 2D Diagram of SPARC-Voriconazole Interaction



(a) (b)

Figure 11: (a) Superimposition of Mitomycin (magenta) and Lemborexant (red) with SPARC Protein, (b) 2D Diagram of SPARC-Lemborexant Interaction



(a) (b)

Figure 12: (a) Superimposition of Mitomycin (magenta) and Suvorexant (red) with SPARC protein, (b) 2D Diagram of SPARC-Suvorexant Interaction

3.2.4 Non-bond Interaction Visualization through Discovery Studio

Ligands that effectively superimposed on the standard drug, Mitomycin were evaluated using Discovery Studio to visualize their interactions with the target protein. Discovery Studio Visualizer is employed to investigate the similarities in the amino acids involved in the bonds

between the protein and ligands. The distance, category and types of bonds give valuable information regarding the drug-protein interactions as well.

In order to evaluate the degree of drug-protein interactions, presence of some useful parameters like hydrogen bonds, hydrophobic bonds and halogen bonds were taken into consideration. Hydrophobic interactions and hydrogen bonding are crucial for stabilization of ligands at the interface of the protein structure as well as facilitate the improvement of binding affinity and drug efficacy (Varma et al., 2010). Moreover, non-covalent interactions like halogen bonding enhance inhibitor recognition (Lu et al., 2009), binding affinity and also improves binding selectivity (Lu et al., 2012). Therefore, the presence of these bonds will be an indicator of strong interactions between the protein and ligands.

3.2.4.1 Non-bond Interactions of SPARC (2V53) with Standard Drug, Mitomycin

In case of Mitomycin, amino acids MET21, VAL20, ILE129 and PRO241 of the SPARC protein are observed to interact with the ligand (Figure 13). The distance ranges from 3 to 5 Å (Table 10). The types of bonds are conventional hydrogen bond, alkyl bond and pi-alkyl bond. There are two hydrogen bonds and four hydrophobic bonds present which indicates a strong interaction between the protein and Mitomycin.

Table 10: Ligand-protein Interactions Between SPARC Protein (2V53) and Mitomycin

Amino acids involved in the interaction	Distance	Category	Types of bonds
C:MET21:N - :UNL1:O	3.24362	Hydrogen Bond	Conventional Hydrogen Bond
D:VAL20:N - :UNL1:O	3.25169	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:C - A:ILE129	4.38204	Hydrophobic	Alkyl
:UNL1:C - A:PRO241	4.84025	Hydrophobic	Alkyl
:UNL1:C - C:VAL20	3.68497	Hydrophobic	Alkyl
:UNL1 - C:VAL20	4.57505	Hydrophobic	Pi-Alkyl

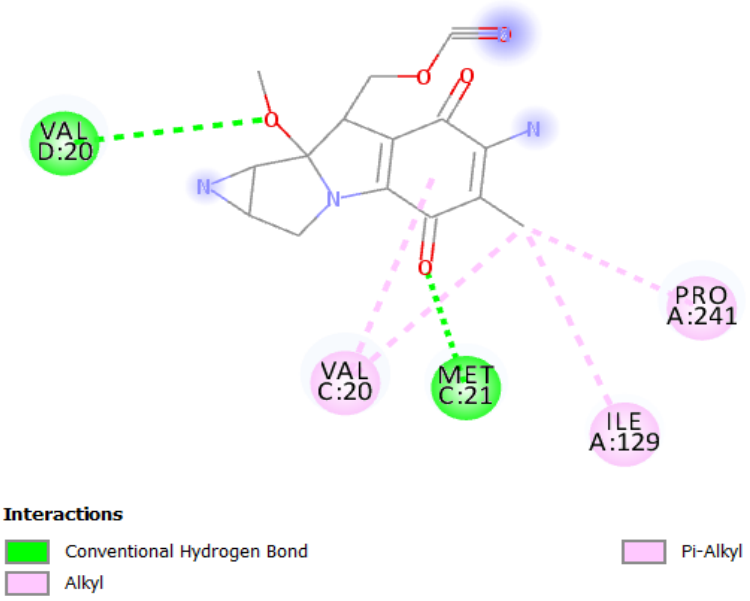


Figure 13: 2D Diagram of SPARC-Mitomycin Interaction

3.2.4.2 Non-bond Interactions of SPARC (2V53) with the Ligands

All the drugs mentioned below (Table 11-17) have three or more amino acids identical to the standard drug, Mitomycin. A notable presence of hydrogen bonds and hydrophobic interactions are observed suggesting strong ligand-protein interactions and making them suitable candidates for further screening.

Table 11: Ligand-protein Interactions Between SPARC Protein (2V53) and Lovastatin

Amino acids involved in the interaction	Distance	Category	Types of bonds
A:ILE129 - :UNL1	4.22019	Hydrophobic	Alkyl
A:ILE129 - :UNL1	4.40732	Hydrophobic	Alkyl
A:ALA240 - :UNL1:C	4.35654	Hydrophobic	Alkyl
C:VAL20 - :UNL1	5.28374	Hydrophobic	Alkyl
:UNL1:C - A:ILE129	3.95063	Hydrophobic	Alkyl
:UNL1:C - A:PRO241	3.84642	Hydrophobic	Alkyl
:UNL1:C - A:VAL94	4.63946	Hydrophobic	Alkyl
:UNL1:C - A:ILE129	5.19993	Hydrophobic	Alkyl
A:PHE91 - :UNL1:C	4.8152	Hydrophobic	Pi-Alkyl

Table 12: Ligand-protein Interactions Between SPARC Protein (2V53) and Mevastatin

Amino acids involved in the interaction	Distance	Category	Types of bonds
C:GLN17:NE2 - :UNL1:O	2.99153	Hydrogen Bond	Conventional Hydrogen Bond
A:ILE129 - :UNL1	4.05616	Hydrophobic	Alkyl
A:ILE129 - :UNL1	5.36902	Hydrophobic	Alkyl
A:ALA240 - :UNL1	4.87771	Hydrophobic	Alkyl
A:ALA240 - :UNL1:C	4.38995	Hydrophobic	Alkyl
A:PRO241 - :UNL1	5.34235	Hydrophobic	Alkyl
:UNL1:C - A:PRO241	3.84619	Hydrophobic	Alkyl
:UNL1:C - A:ILE129	4.00233	Hydrophobic	Alkyl
:UNL1:C - C:VAL20	4.46999	Hydrophobic	Alkyl

Table 13: Ligand-protein Interactions Between SPARC Protein (2V53) and Simvastatin

Amino acids involved in the interaction	Distance	Category	Types of bonds
C:GLN17:NE2 - :UNL1:O	2.87367	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - C:GLY19:O	1.85267	Hydrogen Bond	Conventional Hydrogen Bond
A:ILE129 - :UNL1	4.0838	Hydrophobic	Alkyl
A:ILE129 - :UNL1	5.38266	Hydrophobic	Alkyl

A:ALA240 - :UNL1	5.04129	Hydrophobic	Alkyl
A:PRO241 - :UNL1	5.14687	Hydrophobic	Alkyl
:UNL1:C - A:PRO241	3.7659	Hydrophobic	Alkyl
:UNL1:C - A:ILE129	5.43617	Hydrophobic	Alkyl
:UNL1:C - A:ILE129	4.6821	Hydrophobic	Alkyl
:UNL1:C - C:VAL20	4.62887	Hydrophobic	Alkyl
A:PHE91 - :UNL1:C	5.40874	Hydrophobic	Pi-Alkyl

Table 14: Ligand-protein Interactions Between SPARC Protein (2V53) and Griseofulvin

Amino acids involved in the interaction	**Distance**	**Category**	**Types of bonds**
C:MET21:N - :UNL1:O	3.16376	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H12 – C:GLY19:O	2.91885	Hydrogen Bond	Carbon Hydrogen Bond
C:GLY19:C,O; VAL20:N - :UNL1	4.03216	Hydrophobic	Amide-Pi Stacked
:UNL1:C – A:PRO241	4.36855	Hydrophobic	Alkyl
:UNL1 – C:VAL20	4.99274	Hydrophobic	Pi-Alkyl

Table 15: Ligand-protein Interactions Between SPARC Protein (2V53) and Voriconazole

Amino acids involved in the interaction	**Distance**	**Category**	**Types of bonds**
C:GLN17:NE2 - :UNL1:N	3.13118	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H13 – B:HYP18:OD1	2.72319	Hydrogen Bond	Carbon Hydrogen Bond
A:PHE91:O - :UNL1:F	3.58941	Halogen	Halogen (Fluorine)
A:PRO241:N - :UNL1:F	3.47138	Halogen	Halogen (Fluorine)
C:GLY19:C,O; VAL20:N - :UNL1	4.31038	Hydrophobic	Amide-Pi Stacked
:UNL1:C – A:ILE129	5.02777	Hydrophobic	Alkyl
:UNL1 – A:ILE129	4.59687	Hydrophobic	Pi-Alkyl
:UNL1 – A:PRO241	5.14495	Hydrophobic	Pi-Alkyl

Table 16: Ligand-protein Interactions Between SPARC Protein (2V53) and Lemborexant

Amino acids involved in the interaction	Distance	Category	Types of bonds
C:MET21:N - :UNL1:F	3.38745	Hydrogen Bond; Halogen	Conventional Hydrogen Bond; Halogen (Fluorine)
:UNL1:C - A:ILE129	3.74292	Hydrophobic	Alkyl
:UNL1:C - C:VAL20	3.6833	Hydrophobic	Alkyl
A:PHE91 - :UNL1:C	5.30857	Hydrophobic	Pi-Alkyl
:UNL1 - A:ILE129	4.67165	Hydrophobic	Pi-Alkyl
:UNL1 - A:ALA240	4.04529	Hydrophobic	Pi-Alkyl
:UNL1 - A:PRO241	5.298	Hydrophobic	Pi-Alkyl
:UNL1 - A:ILE129	5.12616	Hydrophobic	Pi-Alkyl
:UNL1 - C:VAL20	5.31844	Hydrophobic	Pi-Alkyl

Table 17: Ligand-protein Interactions Between SPARC Protein (2V53) and Suvorexant

Amino acids involved in the interaction	Distance	Category	Types of bonds
A:SER105:CB - :UNL1:N	3.4457	Hydrogen Bond	Carbon Hydrogen Bond
:UNL1:H28 - C:HYP18:O	2.78463	Hydrogen Bond	Carbon Hydrogen Bond
A:ILE129 - :UNL1	5.25002	Hydrophobic	Alkyl
A:ALA240 - :UNL1:C	3.40461	Hydrophobic	Alkyl
:UNL1:C - C:VAL20	5.19089	Hydrophobic	Alkyl
:UNL1:C - A:ILE129	4.20329	Hydrophobic	Alkyl
:UNL1:C - A:PRO241	4.29858	Hydrophobic	Alkyl
:UNL1 - A:ILE129	4.79198	Hydrophobic	Pi-Alkyl
:UNL1 - A:PRO241	4.5589	Hydrophobic	Pi-Alkyl

3.2.5 Evaluation of Pharmacokinetic Properties in admetSAR

It is customary to assess the pharmacokinetic properties of the selected drugs before their evaluation in in-vitro tests. Multiple properties and descriptors were analyzed using QIKPROP to get a clear understanding of the pharmacokinetic properties of these drugs. These include %HOA (Percent Human Oral Absorption), QPPCaco, QPPMDCK, QPlogKhsa, CNS,

QPlogBB and PSA. %HOA is an indicator of predicted human oral absorption. Caco-2 cell permeability (a gut-blood barrier model) is represented by QPPCaco whereas QPPMDCK indicates MDCK cell permeability (a blood-brain barrier model). QPlogKhsa predicts the binding between a drug and human serum albumin and CNS, QPlogBB and PSA represents the drug's permeability to the central nervous system (Hage-Melim et al., 2020). The pharmacokinetic properties of the selected ligands along with the standard drug, Mitomycin is mentioned in Table 18.

Table 18: Pharmacokinetic Properties of the Standard Drug and Selected Ligands

Molecules	Absorption	Distribution			CNS Permeability		
	%HOA	QPPCaco	QPPMDCK	QPlogKhsa	CNS	QPlogBB	PSA
Mitomycin (standard drug)	38.878	6.937	2.54	-0.492	-2	-1.631	168.074
Lovastatin	100	718.894	346.275	0.514	-1	-0.926	81.3
Mevastatin	100	625.056	297.689	0.455	-2	-1.031	84.414
Simvastatin	100	784.019	380.303	0.617	-1	-0.86	80.016
Griseofulvin	100	2115.373	2269.8	-0.503	0	-0.075	81.775
Voriconazole	100	1218.465	2148.426	-0.134	0	-0.38	67.469
Lemborexant	100	1672.473	2744.585	0.573	0	-0.328	74.772
Suvorexant	100	2044.964	2635.181	0.402	0	-0.057	68.639

All the selected drugs have 100% oral absorption (>80% high). Griseofulvin showed high permeability through Caco-2 cells which indicates good intestinal permeability (QPPCaco; >500 nm/s great). All the other drugs except Lovastatin, Mevastatin, and Simvastatin demonstrated acceptable MDCK cell permeability (QPPMDCK; >500 nm/s great) as well. It is notable that drugs should minimally bind to plasma proteins like human serum albumin as it greatly impacts the availability of the drug in the systemic circulation. The predicted binding to human serum albumin is represented by QPlogKhsa (−1.5 to 1.5) and all the selected ligands

are compliant to this parameter, indicating that they are sufficiently available within the systemic circulation and thus, can reach the target organs.

In order for the drugs to be used in the treatment of CRC and to avoid any psychotropic side effects, they must not cross the blood-brain barrier. The blood/brain partition coefficient, QPlogBB has a recommended range of -3 to 1.2 which is an indicator of the drug's entry to the central nervous system. Lastly, the drugs will not cross the blood-brain barrier if the PSA is >60 (Hage-Melim et al., 2020). As the CNS, QPlogBB and PSA values of the selected drugs are within the acceptable range, it can be predicted that these drugs may not cross the barrier.

Based on QIKPROP analysis, the absorption, distribution and CNS permeability properties of Griseofulvin (antifungal agent) and Lemborexant (sedative and hypnotic) are optimum and therefore, chosen as potential candidates for the treatment of CRC.

Chapter 4

Discussion

CRC is considered one of the most fatal cancers worldwide, taking the lives of millions of people every year. Its treatment can be strenuous because of frequent development of drug resistance and multiple adverse effects. Current treatment options available are expensive, time-consuming and pose significant risk to the patients including diarrhea, vomiting, immune thrombocytopenic purpura (ITP), mucositis, neutropenia, hand and foot syndrome, cardiac symptoms etc. Thus, drug repurposing was performed with the intention of finding safer and effective potential drug candidates for CRC. SPARC protein was targeted in this study as it was found to be overexpressed in CRC cells and silencing of SPARC leads to the suppression of multiple pathways like axon guidance pathway, MAPK, TGF-β and p53 signaling pathways etc. (Naito et al., 2021).

For this study, the PDB structure of the SPARC protein (PDB ID: 2V53) was downloaded from RCSB-PDB and curated in PyMOL by removing all the ligands and water molecules. Following this, the protein was converted into PDBQT file using AutoDock Vina. Mitomycin, a known inhibitor of SPARC protein, was taken as the standard drug. The PDBQT files of all the selected drugs along with the standard drug were prepared in AutoDock Vina following the standard procedure. After the preparation of the protein and selected ligands, site-specific rigid docking was carried out using AutoDock Vina and those demonstrating higher binding affinities than the standard drug were taken to the next stage. Superimposition of these ligands were checked using PyMOL which was followed by 3D-visualization of the ligands in Discovery Studio in order to draw similarities between the amino acids involved. The binding of the standard drug, Mitomycin with SPARC protein included four amino acids namely MET21, VAL20, ILE129, PRO241. Thus, ligands having three or more identical amino acids

with the standard drug were selected and lastly, their pharmacokinetic properties were evaluated using QIKPROP.

Among the selected ligands, Griseofulvin (antifungal agent) and Lemborexant (sedative and hypnotic) were selected as the most potential candidate for the treatment of CRC. The binding affinities of these drugs were -7.4 and -8.5 and kcal/mol respectively which is higher than that of the standard drug, Mitomycin (-7.2 kcal/mol). Moreover, these drugs superimposed quite well with the standard and had three or more common amino acids indicating strong protein-ligand interactions. Lastly, pharmacokinetic properties of these potential candidates were analyzed. The two candidates had significantly higher oral absorption than the standard drug, Mitomycin. Their QPPCaco and QPPMDCK values demonstrated remarkably higher intestinal permeability and renal permeability as well. Moreover, they showed no CNS permeability as the values for CNS, QPlogBB and PSA were within the recommended range.

Therefore, screening of drugs belonging to the five drug classes suggested Griseofulvin and Lemborexant to be potential candidates for the treatment of CRC. Future research may include molecular dynamic simulation, *in vitro* and *in vivo* studies to further establish the findings of this study.

Chapter 5

Conclusion

The study used *in silico* molecular docking approaches to repurpose existing approved drugs for finding alternative treatment options for CRC. As SPARC protein is overexpressed and plays an essential role in the development and progression of CRC, it was targeted and five classes of drugs were randomly chosen and screened. This study concludes that Griseofulvin and Lemborexant showed the most favorable results during screening and hence, may be suggested as promising candidates for further studies.

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