Role of Statins as Antagonists of SPARC in Gastric Cancer

A project submitted

by

Rad Shahmat

ID: 14146007

Session: Spring 2014

to

The Department of Pharmacy

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh

September, 2018

Dedicated to my parents for their constant love and support

Certification Statement

This is to certify that the project titled “Role of Statins as Antagonists of SPARC in Gastric Cancer” submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Professor Dr Eva Rahman Kabir, Chairperson, Department of Pharmacy, BRAC University. Throughout the project, I have given appropriate credit where I have used the language, ideas or writings of another.

Signed,

_____

Countersigned by the Supervisor,

_____

Acknowledgement

I would like to begin by thanking the Almighty from whom I got the strength to complete this project work. This project would not have been possible without numerous individuals who are recognized here.

Firstly, I would like to express my deepest gratitude and respect to my most esteemed supervisor Professor Dr. Eva Rahman Kabir (Chairperson, Department of Pharmacy, BRAC University), without whom I could not have completed this project. Her constant guidance and support towards my research based project helped me grow as a research student as well as a future researcher.

I would thank Mohammad Kawsar Sharif Siam (Senior Lecturer, Department of Pharmacy, BRAC University) who I am deeply grateful for his valuable scientific input.

I would also like to thank Dr. Raeed Jamiruddin (Assistant Professor, Department of Pharmacy, BRAC University) for his oversight and recommendations during my project.

My sincere thanks to Md. Sohanur Rahman, Shannon Sherwin Moreino (Teaching Assistants, Department of Pharmacy, BRAC University) and Nahid Akter Tonima (Lab Officer, Department of Pharmacy, BRAC University) for their constant support.

Lastly, I would like to thank my family for their constant love and support throughout this journey. Who inspired me to work harder to complete this thesis work.

Abstract

Cancer refers to the abnormal and uncontrollable growth of cells in the body. It is the second leading cause of death in the world and in 2015 alone claimed the lives of 8.8 million people. The protein SPARC (Secreted Protein Acidic and Rich in Cystine) plays an important role in wound healing, bone formation, tumor progression, fibrosis and angiogenesis. Maintaining the normal level of SPARC in the human body is very important as deviation from the normal level of SPARC in the body can hamper bone mineralization, cell proliferation and extracellular matrix synthesis process. SPARC acts as both cancer promoter and cancer suppressor, which makes it a possible target for anti-cancer drug development. In gastric cancer, SPARC acts as a tumor promoter and downregulating the production of it showed better prognosis of the disease. In this study, drug repurposing and other *in silico* computational techniques have been used to find a potential statin antagonist of SPARC which will inhibit and lower the production of SPARC in gastric cancer cells. PyRx was used to carry out the molecular docking of statin drugs with SPARC and Discovery studio was used to check the drug-protein interaction. Ramachandran plot and z-score were used to validate the protein structure of SPARC. The z-score of SPARC was found to be -7.91. Initially over 200 small molecules were chosen and docked with the SPARC protein, which included two classes of drugs, anti-inflammatory drugs and statins. Among them statin drugs were finally chosen on the basis of binding affinity and stability. The potential statin drugs which were finally selected were atorvastatin, simvastatin, pitavastatin and pravastatin. Among them, atorvastatin showed one of the highest binding affinity of -9.6Kcal/Mol. The aim of this study was to find a statin drug which targeted and bound to SPARC and prevented its expression and atorvastatin fulfilled the criteria. The study shows that atorvastatin, for its high binding affinity towards SPARC could be used as an anti-cancer drug for gastric cancer by reducing the expression of SPARC.

Table of Contents

List of Tables

List of Figures

Abbreviations

SPARC	Secreted Protein Acidic and Rich in Cystine
FDA	Food and Drug Administration
HMG-CoA	Hydroxymethylglutaryl-CoA
LDL	Low Density Lipoprotein
CHD	Coronary Heart Disease
ACS	Acute Coronary Syndrome
MI	Myocardial Infraction
NAG	N-Acetyl-D-Glucosamine
NMR	Nuclear Magnetic Resonance
TRP	Tryptophan
GLN	Glutamine
PRO	Proline
LEU	Leucine
ASN	Asparagine
ASP	Aspartate
ILE	Isoleucine
TYR	Tyrosine
HIS	Histidine
LYS	Lysine
GLU	Glutamate

1. Introduction

Cancer is a term given to a large group of diseases that may affect any part of the body, generally referring to the uncontrolled and abnormal growth of cells in the body. These abnormal cells are termed as malignant or cancer cells. There are over 200 types of cancer which are defined by the name of the tissue from which the cancer cells originated from like breast cancer, lung cancer or gastric cancer (C. P. Davis, n.d.). All cancer arises when normal cells transform into tumor cells from a pre-cancerous lesion which eventually transform into malignant cancer cells. These changes could occur due to a person's genetic factor or external agents. The external agents consist of biological carcinogens, physical carcinogens and chemical carcinogens (Plummer et al., 2016).

Cancer develops as a consequence of damage to the gene in normal cells. It starts to develop when a number of mutations take place in the gene that governs the cells ability to grow and divide. These cells also contain tumor suppressing genes, which delay the progression of cancer. A significant amount of mutation of the gene is required to transform a normal cell into cancer cell. When cancer cells grow they also create new mesh of blood vessels to deliver nutrients to the cancer cells, which is called angiogenesis (Seyfried, n.d.).

Cancer, being the second leading reason of death in the world, has forced scientists to seek new forms of medication for its treatment. Drug repurposing is one of the methods to find a cure for this devastating disease. Since discovering a new drug is both time consuming and expensive, drug repurposing seems like the most effective method to find a viable cure (Roder & Thomson, 2015). Usually, drugs that have been previously established are utilized for the drug repurposing process. As the method is cheap and does not take as much time in comparison to novel drug discovery, it is the best choice to find a medication for cancer. In this study, SPARC (Secreted Protein Acidic and Rich in Cystine) was the molecular target and the anti-cancer effect of statins were investigated.

1.1. Gastric Cancer

Gastric cancer is the second leading cause of death correlating to cancer. It is also the fourth most common cancer in the world. (Sitarz et al., 2018). The distribution of gastric cancer differs around the globe, but people from Asian countries account for more cases of gastric cancer than European and American countries combined (Carcas, 2014).

Histologically there are two subtypes of this disease, diffuse type and intestinal type gastric cancer (Lauren, 1965). The most frequently diagnosed subtype of gastric cancer in high-risk populations is the intestinal type gastric cancer. It is more frequently diagnosed in older males than females and the tumors have a gross ulcerated appearance. The tumorigenesis of this subtype is correlated with *H. pylori* bacteria infection. Severe *H. pylori* infection causes several histological changes to the gastric cell line which eventually forms a malignant lesion (Henson, Dittus, Younes, Nguyen, & Albores-Saavedra, 2004). The diffuse subtype of gastric cancer is more aggressive and occurs more frequently in younger people. It occurs frequently in both males and females. It could be linked to *H. pylori* infection but the most frequent reason for this cancer is the loss of expression of the tumor suppressor gene E-cadherin. Diffuse type gastric tumors usually invade the adjacent structures such as duodenum and esophagus without gland formation (Barber et al., 2008).

1.2. Drug Repurposing

Drug repurposing is the process of identifying and developing new uses for existing approved drugs, withdrawn or rejected drugs using experimental and computational methods (Ashburn & Thor, 2004). This method promises to reduce the cost and risks of drug development programs and can significantly speed up the identification of new drugs (Jesús Naveja, Dueñas-González, & Medina-Franco, 2016). As the toxicological and pharmacological data of existing drugs are already known, repurposing them can prove to be much cheaper and faster than conventional methods of finding a new drug (Morgan, Campbell, Yu, Sponseller, & Muster, 2012). This concept of repurposing a drug has given rise to a number of successful events where drugs are used for a different purpose than they were initially intended for which includes cancer treatment (Charbel Issa et al., 2013)

There are several examples of such drugs. One well-known example is Thalidomide, which was originally found as a sedative by the German pharmaceutical corporation Grünenthal in 1957. It was initially used to treat morning sickness of pregnant woman. It was later withdrawn from the market as it was found to cause polyneuritis and prevented the human embryo to develop properly and caused babies to be born with deformed limbs. More than ten thousand children in fourty-six countries had been born with birth

defects due to the use of thalidomide (Lenz & Knapp, 1962), but later it was found to have anticancer properties on prostate cancer and refractory multiple myeloma (D'Amato, Loughnan, Flynn, & Folkman, 1994). For testing the ability to treat multiple myeloma in combination with dexamethasone, thalidomide received US-FDA approval in 2006 (Lengauer & Rarey, 1996). Another example is itraconazole, which was initially an anti-fungal drug and later found to have anti-cancer properties, particularly for prostate and non-small cell lung cancer (Huijgens et al., 1999). Another example of drug repurposing is pregabalin, which is a chemical cousin of gabapentin. Originally it was used to treat epileptic disorders, but drug repurposing showed that it can also be used in treating anxietyproblems, neuropathic disorders and as seizure medications (Abagyan & Totrov, 2001).

Scientists are now looking forward to drug repurposing as the conventional method of drug discovery and development is highly expensive and takes years to complete (O'Connor & Roth, 2005). Due to the great cost and risks associated with new molecule development, people are now becoming more involved in drug repurposing (Cuatrecasas, 2006). Moreover, drug repurposing is found to be cheaper than the novel drug development since the candidate drug has been accepted in the medical community for many years (Munos, 2009). As a result, people get the drug far more rapidly than conventional drug discovery programs. In the last few years, scientists have established various approaches for drug repurposing and these approaches are- a) using chemically identical drugs for the treatment of the same disease, b) using drugs which show similar side effects, c) targeting drugs which have resemblance in molecular activity and, d) targeting drugs that have analogous molecular pathology (Opera et. al., 2011).

Drug repositioning is thought to offer significant benefits over de novo drug discovery (Opera 2008). The problems encountered during drug discovery could be reduced through drug repositioning (Schuster & Laggner, 2005). New drugs could be launched in the market at a faster rate with the exploitation of existing drug knowledge (Lindsay, 2003). Repositioning of drugs which are already accepted has the advantage of minimizing the cost and saving time due to the already existing pharmacological and toxicological data (Stuart, 2004). This method can be used to identify a new therapeutic indication for a drug that has a therapeutic activity for a particular disease. This method

also helps to find the gaps among the drug–target interaction matrix. It also possesses a safety and efficacy data at some stages in medical studies (Schuster & Laggner, 2005).

1.3. *In silico* Drug Designing

The main focus of drug repurposing is chemical screening to invent novel drug–target interactions, where unexpected therapeutic action, technological and financial limitations show up as a large-scale process (Ashburn & Thor, 2004). There are different techniques of drug repurposing, one of which is *in silico* method. *In silico* is an expression which means the function was performed in computer or via computer simulation. *In silico* methods have advantages of modern high-performance computing system for safe virtual screening and thus represent a new approach (Zimmerman, 2004). The need of virtual screening is vital for large-scale of drugs and working with data sets. The limiting factor is the validation and accuracy of the approaches used for the prediction of drug–protein interactions. For drug repurposing, docking is used which is a part of the computational biology or *in silico* process. It is a process, which calculates the ideal orientation of one molecule to a second when they bind to each other to form a stable complex (Ning, et al., 2010). The knowledge of the desired orientation may be used to predict the binding affinity between the two molecules used, for example, scoring functions (Eldridge et al., 1997).

The designing and repurposing of drugs *in silico* employs high performance computers to screen drugs virtually. Virtual screening is very important for large scale characterization of drugs and working with data sets. The validation and accuracy of the drug protein interaction is the limiting factor in this process. Docking is used to identify drug-protein interactions and predicts the preferred orientation of one molecule to other in situations where two molecules bind with each other to form a stable complex. According to the data obtained on preferred orientation, binding affinity and the strength of association between the drug and the protein can be predicted (Eldridge, Murray, Auton, Paolini, & Mee, 1997).

1.3.1. Molecular Docking:

The objective of molecular docking is to evaluate the possible binding geometries of a ligand with a target with a known 3D structure (Luo & Sharp, 2002). Usually, docking

procedures contain both a search algorithm for the investigation of different ligand (and sometimes protein) conformations, and a scoring function for the calculation of ligand binding affinities (Boresch, Tettinger, Leitgeb & Karplus, 2003). Preferably, the scoring function should be able to ascertain a solution with the precise ligand binding mode from alternative solutions, and eventually be able to rank a set of ligands according to experimental binding affinity (Woo & Roux, 2005). In principle, the first principle of thermodynamics should be used to calculate the binding affinity (Rodinger et al., 2008).

1.4. Selection of a Gastric Cancer Protein

SPARC, also called BM-40 or osteonectin, is a secreted glycoprotein that works in tissue remodeling (Hohenester, Maurer, & Timpl, 1997). SPARC was chosen as our protein of choice because in gastric cancer, the cancer cell lines showed variable levels of SPARC. SPARC transcript and protein level were up-regulated in the cases of diffuse type and intestinal type gastric cancer and it correlated with poor prognosis and invasiveness of the cancer (Wang et al., 2004). Research has showed that down-regulating SPARC in high expressing gastric cell lines inhibited their growth and expression (Yin et al., 2010).

1.4.1. Structure of SPARC

The gene corresponding to SPARC, the SPARC gene was initially thought of as a bone matrix and an endothelial basement membrane protein (Neveen Said, 2016). SPARC comprises of an acidic N-terminal segment, a follistatin-like (FS) domain and an EF-hand calcium-binding (EC) domain (Figure 1.1). The two domains interact by a small interface that requires the EF-hand pair of the EC domain. One face of SPARC has residues, which relate to cell binding and cell spreading inhibition. The opposite face of SPARC has the binding site for collagens and N-linked carbohydrates. The FS domain which is elongated is related to serine protease inhibitors of the Kazal family in structure (Hohenester et al., 1997).

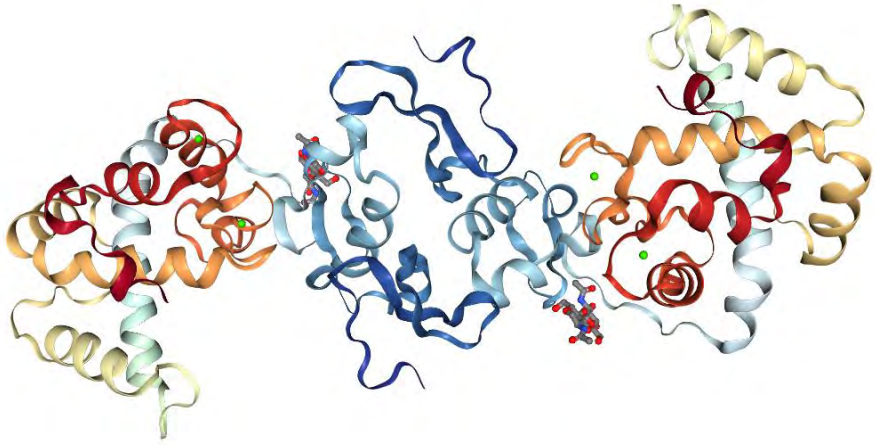


Figure 1.1. Crystal structure of SPARC (Hohenester et al., 1997)

1.4.2. Function of SPARC

SPARC is a protein which is specific to bone and selectively binds to collagen and hydroxyapatite. When SPARC binds to insolubilized type 1 collagen, it forms a complex which binds to free calcium ions and synthetic apatite crystals. The SPARC-Collagen complexes nucleate mineral phase deposition from metastable balanced salt solutions. The protein is found in higher levels in the matrix and is localized to mineralized bone trabeculae. It is a protein which is tissue specific, that links the collagen phases and bone mineral and also initiates active mineralization in normal skeletal tissue (Termine et al., 1981).

SPARC increases the effect of apoptosis by supplementing the signaling cascade in a caspase-8 dependent manner. This has no relation to death receptor stimulation and it leads to downstream involvement of apoptosis (Tang & Tai, 2007). Figure 1.2 shows the pathways by which SPARC functions in apoptosis. SPARC functions in the cytoskeletal rearrangement, regulation of cell adhesion, proliferation, tissue remodeling and matrix assembly (Bradshaw, 2016). SPARC also helps in wound healing, bone formation, tumor progression, fibrosis and angiogenesis. Angiogenesis refers to endothelial cell proliferation, migration, extracellular matrix synthesis and degradation. In some cases SPARC promotes angiogenesis and in others it shows anti-angiogenetic activity (Rivera, Bradshaw, & Brekken, 2011).

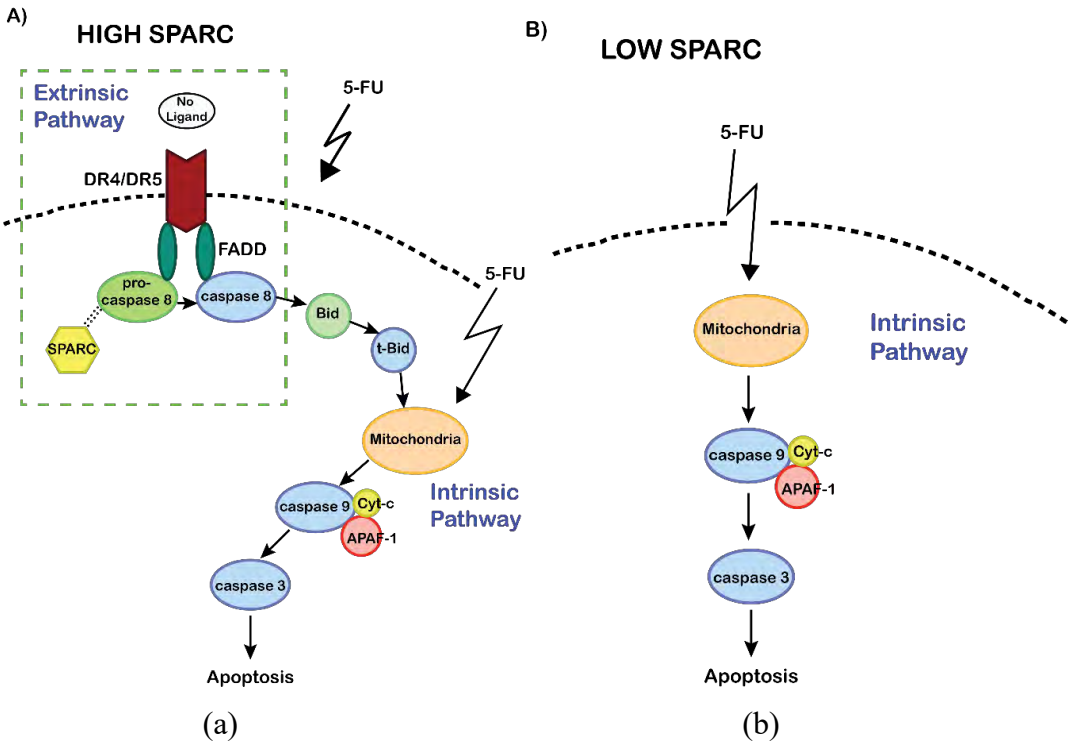


Figure 1.2. SPARC-induced apoptosis (Rahman, Chan, & Tai, 2011)

(a) SPARC interacts with pro-caspase 8 and cleaves it to caspase 8, which upon exposure to chemotherapy activates the mitochondrial pathway of apoptosis.

(b) at lower SPARC levels, apoptosis only occurs independent of caspase 8.

1.4.3. SPARC and its relation to Cancer Progression

There is a growing evidence that SPARC has an important role in a selection of cancers, but there is no concrete model explaining the facts of how it functions and how it contributes to the progression and development of cancer. SPARC plays a multi-faceted contextual role depending on the type of cancer, the surrounding milieu and the type of cell. SPARC acts both as tumor suppressor and tumor promoter depending on these factors (Neveen Said, 2016). SPARC is expressed in tumors along with its surrounding stroma in several cancers in contrast to the normal tissue, but depending on the cancer its expression pattern is different. For example, high levels of SPARC expression have been reported in melanoma, breast cancer, glioblastomas and gastric cancer. This expression pattern indicates a possible role of SPARC in tumor progression. However, other types of cancer shows lower level of SPARC such as colorectal cancer, ovarian cancer,

pancreatic cancer and acute myelogenous leukemia. This pattern of expression indicates an inhibitory role of SPARC in tumor formation. (Neveen Said, Frierson, Sanchez-Carbayo, Brekken, & Theodorescu, 2013).

SPARC serves as tumor suppressor for several types of cancer such as bladder cancer, ovarian cancer, cervical cancer and colorectal cancer. Studies have revealed that the transcription and expression of SPARC were significantly downregulated in bladder cancer cell line. High SPARC expression is related to increased disease free survival (Neveen Said et al., 2013). SPARC is also abundantly expressed in advanced phases of ovarian cancer. However, new evidence shows that SPARC normalizes the tumor microenvironment and counters tumor growth (N. Said et al., 2007). A screening study to identify hyper methylated genes in invasive cervical cancer showed that SPARC had the highest frequency of aberrant methylation (Parwani, 2007). Overexpression of SPARC and high serum level of it is correlated with the development of cervical cancer (SHI et al., 2016). According to clinical studies and experimental cell models, SPARC is also considered to be a tumor suppressor in colorectal cancer. In animal xenografts, exogenous SPARC along with chemotherapy proved to be highly effective in tumor regression (Tai, Dai, Owen, & Chen, 2005).

SPARC also serves as tumor promoter in some cancer types. In gastric cancer, where the cell lines of human gastric cancer showed variable levels of SPARC, it acts as a tumor suppressor. In the issues of diffuse type and intestinal type gastric cancer, SPARC transcript and protein level were up-regulated and it was correlated with poor prognosis and invasiveness of the cancer (Wang et al., 2004). Down-regulating SPARC in high expressing gastric cell lines showed that it inhibited their growth and expression (Yin et al., 2010). Thus, SPARC is a good target for cancer therapy and drug development.

The alpha helices, beta sheets and the coils of SPARC are shown in Figure 1.3. Red portions indicate alpha helices; yellow portions indicate beta sheets and green portions are coils. They are the result of protein folding and gives stability to the structure of the protein.

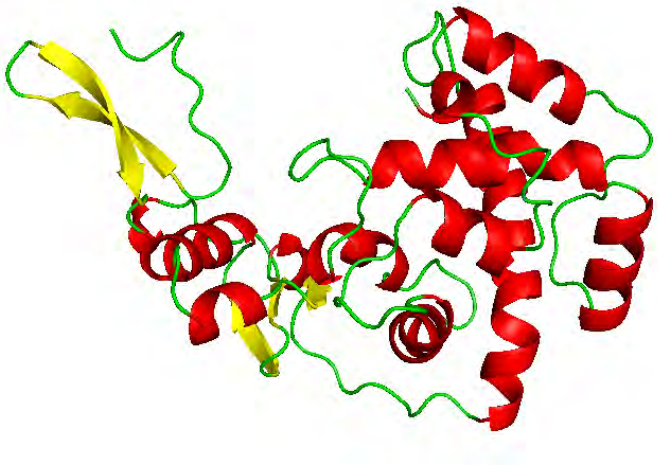


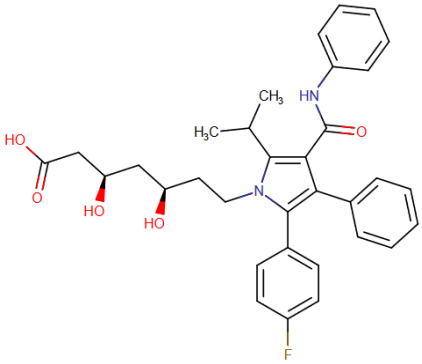
Figure 1.3. Structure of SPARC (obtained from PyMOL version 1.8.4.0) (Seeliger & de Groot, 2010)

1.5. Statin Drugs and its relation to cancer

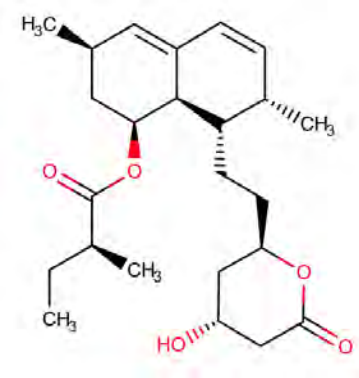
Statins are a class of drugs that originate from fungus. It was first isolated from the fungus *Penicillium citrinum.* They are lipid lowering drugs and they are used in the treatment of cardiovascular diseases (Endo, 1988). Figure 1.4 gives the structure of a few statin drugs.

Statins inhibit the hydroxymethylglutaryl-CoA (HMG-CoA) reductase enzyme. Structurally they are related to hydroxymethylgtutaryl coenzyme A. Statins inhibit a key step of the biosynthetic pathway of sterols. This makes them a powerful medication to lower cholesterol levels, and for this reason it is also widely used for prevention of cardiovascular diseases (Sirtori, 2014).

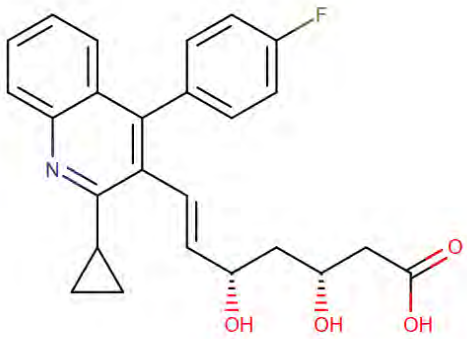
Statins decrease cellular cholesterol content by preventing the HMG-CoA reductase enzyme to function. This restricts the cholesterol biosynthesis and decreases the concentration of cholesterol in the liver. This increases the expression of LDL-receptors in the liver cell membranes, which enhances the clearance of LDL-cholesterol from the blood circulation (Sirtori, 2014).



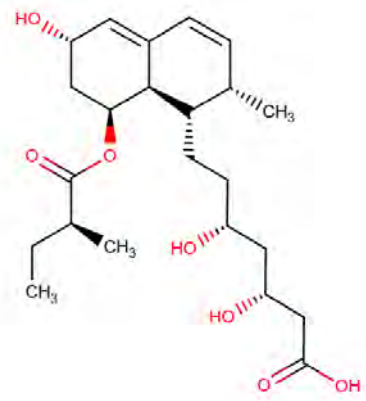
(a) Atorvastatin



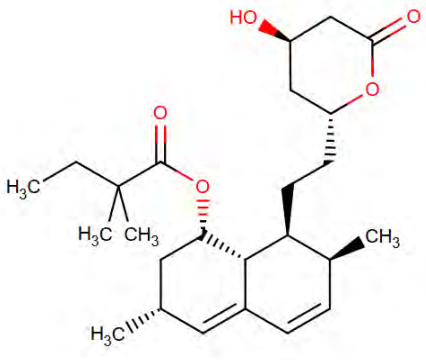
(b) Lovastatin



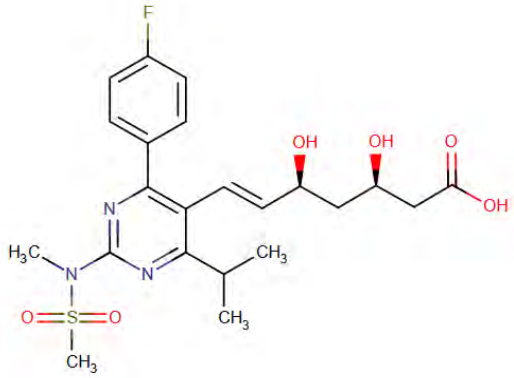
(c) Pitavastatin



(d) Pravastatin



(e) Simvastatin



(f) Rosuvastatin

Figure 1.4. Structures of some Statin Drugs (obtained from NCBI) (Wishart et al., 2018) (a) Structure of Atorvastatin, (b) Structure of Lovastatin, (c) Structure of Pitavastatin, (d) Structure of Pravastatin, (e) Structure of Simvastatin, (f) Structure of Rosuvastatin

Statins have been widely reported to have positive activity in ovarian cancers. But the concentration of statins which is required for cell death is a lot higher than the concentration that was achieved in patients who were treated for hypercholesterolemia. But statins can cause myopathy in higher doses. To prevent this problem, statins could be used along with other drugs to reduce the adverse side effects. Combination of BH3 mimetic ABT-737 or pictilisib with pitavastatin gave more effective results than pitavastatin alone. Pictilisib or ABT-737 increased cell death caused by pitavastatin in ovarian cancer treatment (De Wolf, De Wolf, & Richardson, 2018). In our studies, we wanted to suppress the expression of SPARC protein. As statins have already been known for their anti-cancer effects, they were chosen for the study and the drug atorvastatin showed a promising binding affinity of -9.6 Kcal/Mol.

1.5.1. Atorvastatin

Belonging to the statin class of drugs, atorvastatin is a lipid lowering drug which is primarily used as the primary and secondary prevention of Coronary Heart Disease (CHD). In people suffering from acute coronary syndrome (ACS), atorvastatin reduces the risk of myocardial infraction (MI), angina and other cardiovascular events. As it functions as a competitive inhibitor of HMG-CoA reductase by reducing the levels of mevalonate, it is used for the treatment of hypercholesterolemia and hypertriglyceridemia. It primarily functions in the liver. Atorvastatin decreases the level of cholesterol in the liver and thus upregulates the hepatic LDL receptors; this in turn, increases the uptake of cholesterol from the liver, causing a decrease in the plasma LDL levels (Chauvin, Drouot, Barrail-Tran, & Taburet, 2013). Research has shown that atorvastatin could be an anti-cancer drug of choice for breast cancer and thyroid cancer (Wilke et al., 2014). The drug was chosen for the study since its ability to bind to SPARC and suppressing it have not been extensively studied yet. Figure 1.5 shows the structure of atorvastatin.

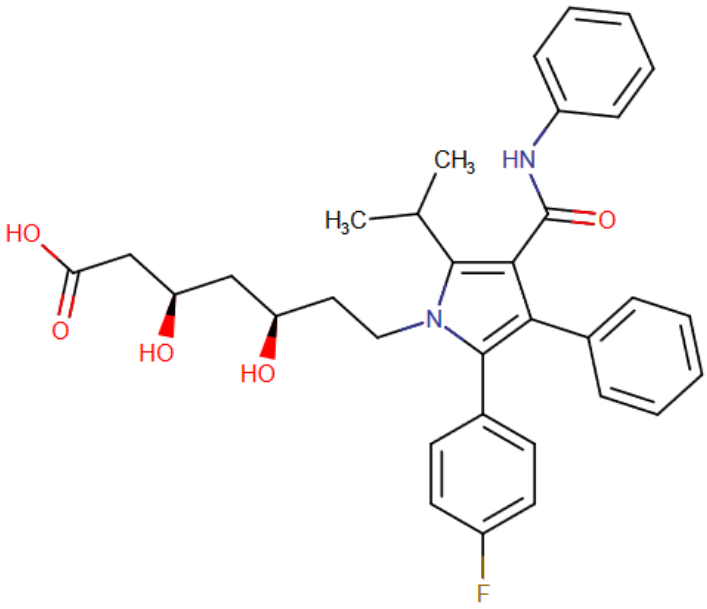


Figure 1.5. Structure of Atorvastatin (obtained from NCBI) (Wishart et al., 2018)

1.6. Rationale of the Study

Huge amount of resources are invested every year in search of a valid and viable cure or treatment for cancer. Yet, cancer is the second largest reason of death in the world after cardiac diseases and gastric cancer is the second most common type of cancer. Drug repurposing can be used to find an alternate effective medication for gastric cancer. Alternative approaches to discovering anticancer drugs has become a necessity because of the high cost, increasing failure rate, limited efficacy, poor safety, lengthy testing and designing process and poor bioavailability of the cancer drug development process (Gupta, Sung, Prasad, Webb, & Aggarwal, 2013) and would save costs by evading clinical activities.

2. Methodology

The first part of the methodology consisted of a thorough review of past literature on the specific topic, followed by establishing the binding affinities that persisted between receptors and ligands which were determined by utilizing molecular docking. 3D structures of the macromolecule and ligands or small molecules were necessary to carry out computational docking. Statin drugs may prove beneficial in the inhibition of SPARC protein. Which will help treat gastric cancer.

2.1. Software used for molecular Docking, Visualization and Validation

A variety of software and online tools were used for this *in silico* study, shown in Table 2.1.

Table 2.1. Software used for *in silico* study

Sl No.	Software and Online Tools Used	Versions	Reference
1.	PyMol	1.8.4.0	(Seeliger & de Groot, 2010)
2.	PyRx	0.8	(Dallakyan & Olson, 2015)
3.	Discovery Studio	17.2.0.16349	(Eid, Zalewski, Smieško, Ernst, & Vedani, 2013)
4.	ConSurf	2010	(Ashkenazy et al., 2016)
6.	DrugBank	5.1.1	(Wishart et al., 2018)
7.	Open Babel	2.4.0	(O'Boyle et al., 2011)

PyMol was used to visualize the protein and the drug molecules. PyRx was used for the docking of the drugs to the protein molecules. Discovery Studio was used to determine the amino acids with which the drug molecules were bound with the protein. ConSurf online tool was used to determine the amino acids which fell in the conserved and variable regions. The structures of the drug molecules were obtained from the DrugBank.

OpenBabel was the software used to convert sdf files obtained from PubChem to pdb files.

2.2. Steps Involved in Molecular Docking

The steps involved in the process of molecular docing are illustrated with the help of a flowchart (Figure 2.1). As depicted in Figure 2.0 the protein of interest SPARC (1BMO) was downloaded from RCSB PDB (Protein Data Bank) database. RCSB PDB provides pdb files of the protein (Garg et al., 2016). This databank has the 3D structures of different macromolecules such as proteins and nucleic acids. These macromolecules could be found in all living beings ranging from humans to bacteria. The protein structure was curated in PyMoL. As the chain A and chain B are the same, one of them was deleted to simplify the work. The protein molecule contained Calcium Ions and N-Acetyl-D-Glucosamine as ligands. They were also deleted.

The files for the statin drugs were obtained from PubChem and DrugBank. The files collected from DrugBank were in pdb format and they did not require any changes. The files collected from PubChem were in sdf format and they were converted to pdb format using OpenBabel, which is a conversion software.

Using the software named PyRx, molecular docking was conducted. Docking can be done by two approaches. One of them is Flexible Docking and the other is Rigid Docking. For this computational study, rigid docking was used. In the preference option all the torsions were inactivated. The protein molecule of SPARC and drug molecules were loaded in PyRx. The protein molecule was set as macromolecule and the drug molecules were set as ligands. As the protein of interest has two identical chains, only one chain was used for the docking. Before running the auto-dock vina, the vina search space was maximized to ensure that the entire protein was covered. The auto-dock vina was then carried out. Various results were generated showing binding affinities that showed a negative sign. It showed a negative sign because the reaction was an exothermic reaction. The more negative the number was, the greater the binding affinity was. The pdbqt files of the docking were saved.

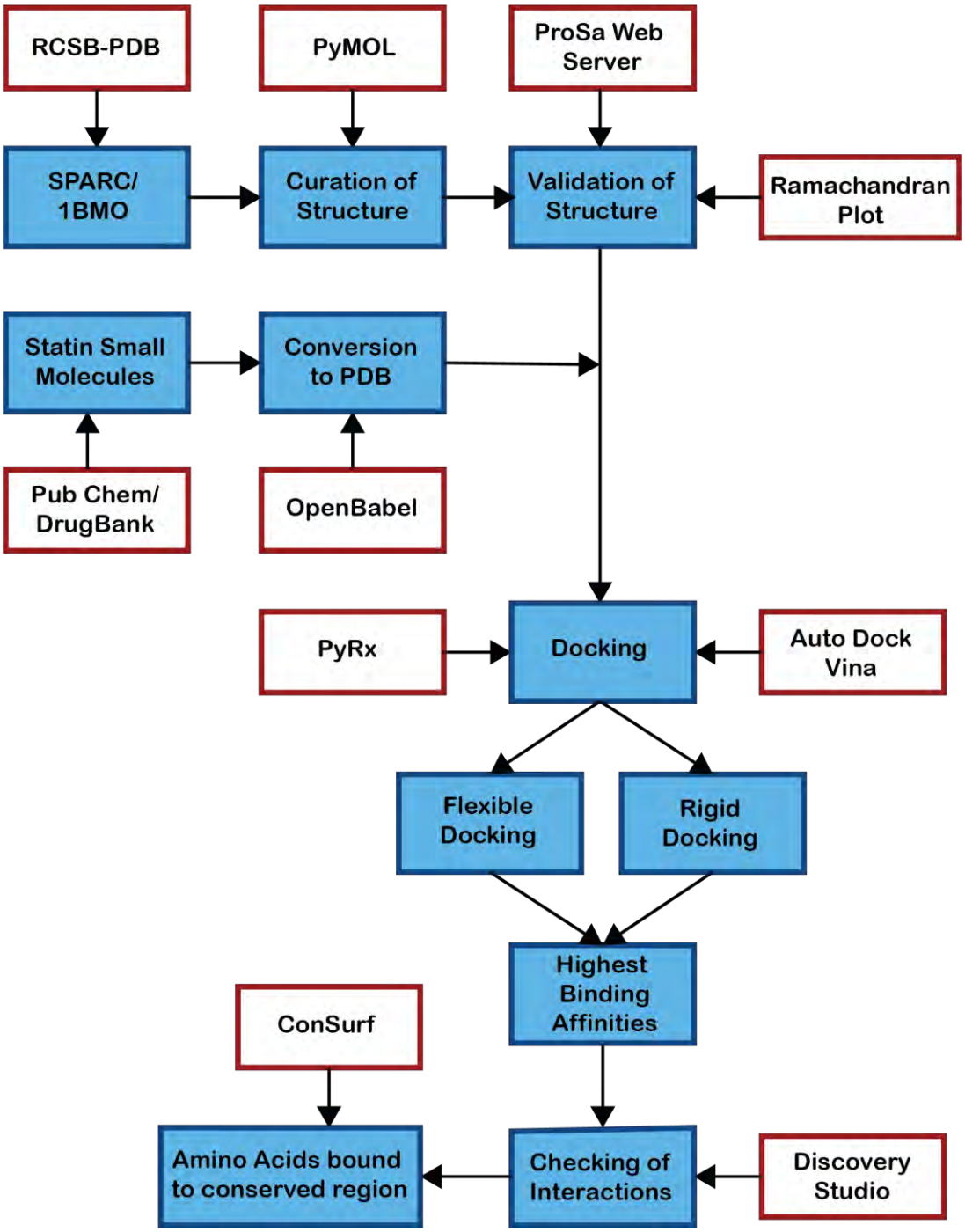


Figure 2.1. Flow Chart indicating the steps involved in molecular docking

Several methods were used for the validation process. PyMOL and discovery studio were used for visualization. The pdbqt file which was found as a result of docking protein with drug molecule was saved. This pdbqt file was opened in PyMOL and the file of the

protein without ligand was also opened. This drug protein complex was then saved. It was then opened in discovery studio. The protein structure without ligand was also opened in a different window. Hydrogens were added to the structure and the ligands were added to the protein. Then the ligands were defined. Discovery studio provides a strong representation of the actual distance, bonding types, amino acids and categories of the bond between the ligand and the protein molecule. The data of the distance, bonding types, amino acids and categories of the bond were recorded and screenshots were also taken.

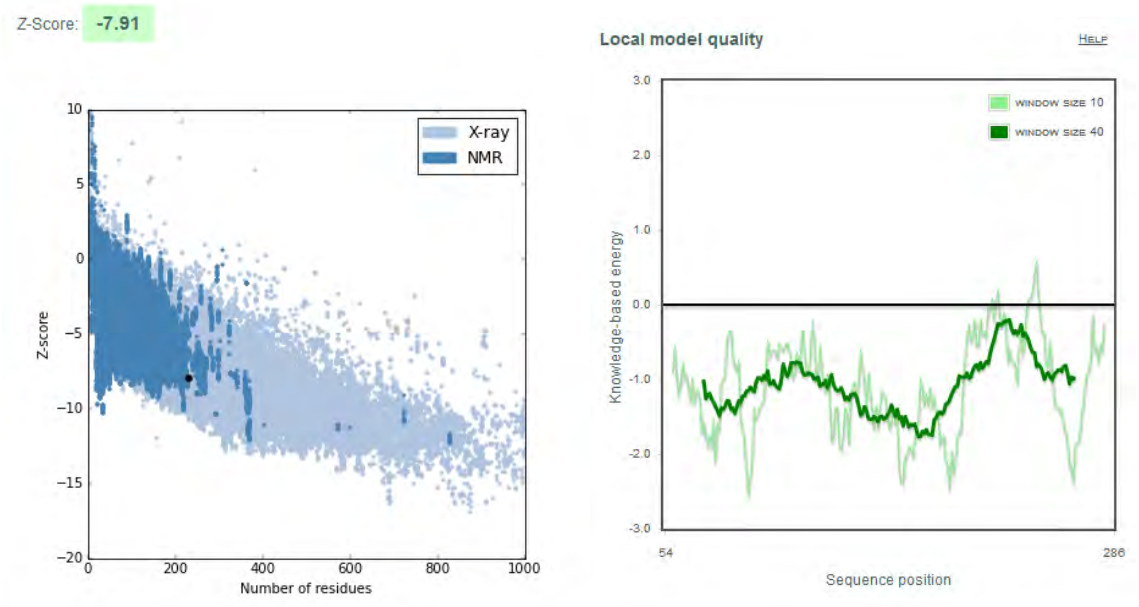
Ramachandran Plot, ProSa WebServer and Consurf were also used for the validation process. Ramachandran plot was once generated before docking the drug and then again after docking the drug to determine if the drug caused any changes in the psi and phi angles. The SPARC protein was then opened with an online tool named ConSurf, which indicates the conserved regions of the protein. ConSurf was used to determine if the drugs were bound with amino acid which were in the conserved regions or with amino acids which are prone to mutation.

3. Results and Validation

The results obtained in the study have been discussed in this section.

3.1. Validation of the Structure of SPARC

The pdb file of SPARC was downloaded from RCSB-PDV. It was curated with PyMOL. The hetero atoms or water, N-Acetyl-D-Glucosamine, the ligand and calcium ions were removed. The z-score of the protein after curation was -7.91 (Figure 3.1). The greater the value is away from zero towards the negative, the better. The residues were seen on the X ray region. However, it did not fall within the NMR region.



(a) **(b)**

Figure 3.1. (a) The z-score of SPARC (obtained from ProSA Web Server), (b) The plot of residue score of SPARC (obtained from ProSA Web Server) (Sippl, 1993)

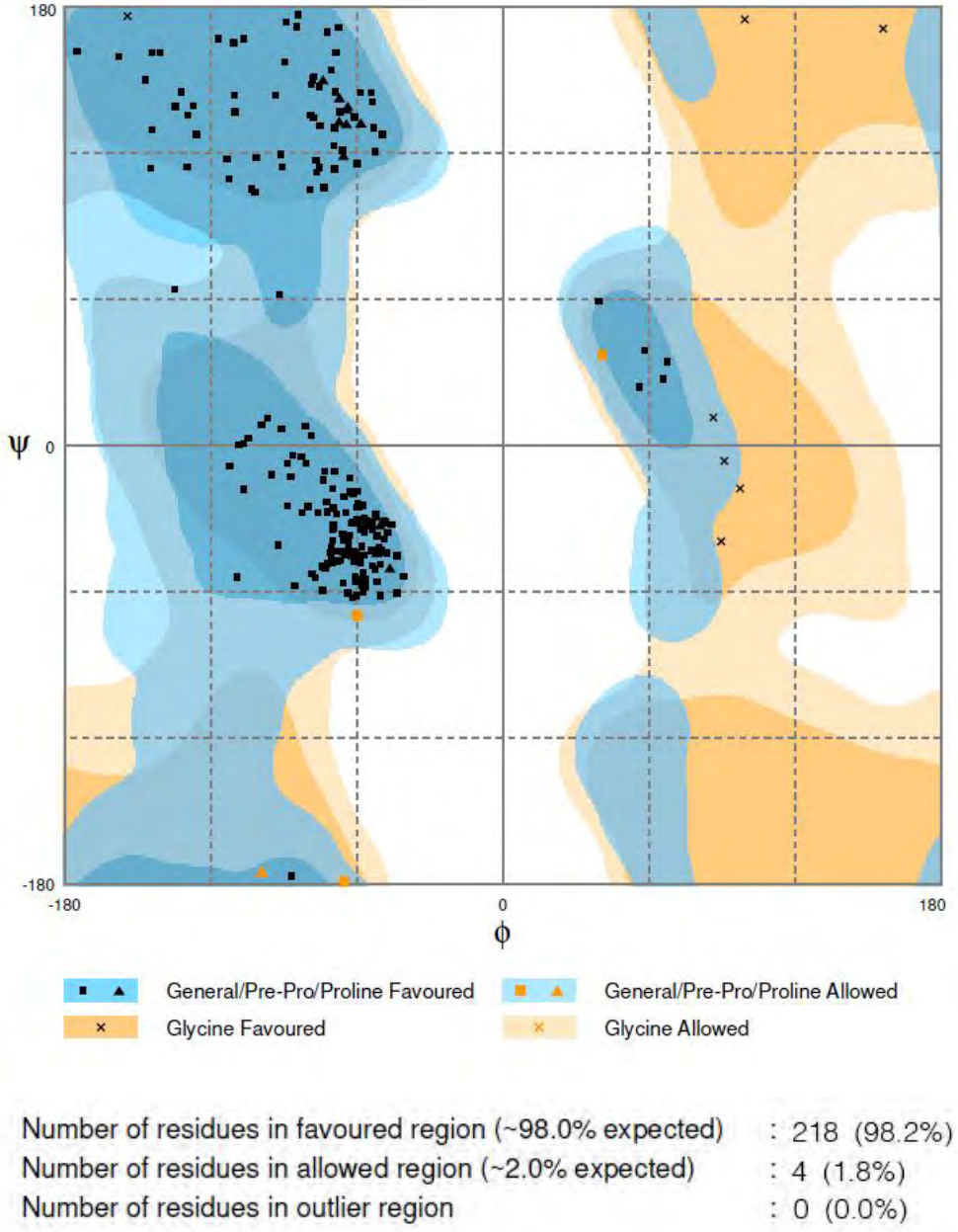


Figure 3.2. Ramachandran Plot showing the energy minimization of the whole protein, SPARC (obtained from Rampage) (Lovell et al., 2003)

The Ramachandran plot helps to predict the conformation of the backbone of a given polypeptide chain more quantitatively starting from knowledge of its amino acid sequences. Figure 3.2. shows that the number of amino acid residues in the favored region is 98.2% and the number of residues in the allowed region is 1.8%. No residues are seen in the outlier region.

3.2. *In silico* binding of SPARC with Statins

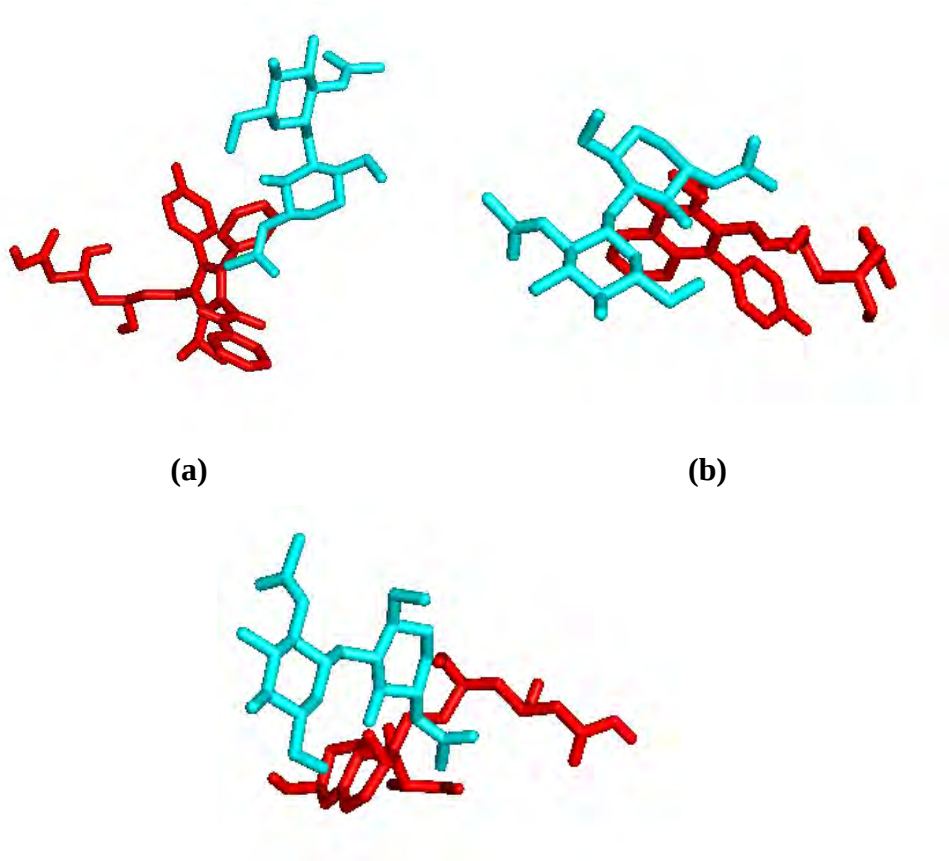
Small molecules which belong to statin class of drugs were downloaded from PubChem and DrugBank.ca website respectively. The molecules which were downloaded from PubChem were in sdf format. They were converted to pdb format using OpenBabel. Molecular docking was carried out using PyRx. SPARC was set as the macromolecule and the statin drugs were set as ligands. Both rigid and flexible docking were carried out and then drugs were selected based on their binding affinities. The more exothermic the binding affinity is, the more the probability of the drug binding to the protein is. Pitavastatin, atorvastatin and pravastatin had the highest binding affinities. Although having high binding affinity, simvastatin and lovastatin were not chosen as they were withdrawn from the market in 2016 ("Why FDA pulled cholesterol drugs off market | Formulary Watch," n.d.). The results of docking are summarized in Table 3.1.

Table 3.1. Flexible and rigid docking results of statins with SPARC using PyRx, Version 0.8 (Dallakyan & Olson, 2015). In the case of flexible docking of drugs with SPARC, the drugs are of flexible nature and the protein is rigid and in rigid docking, both the protein and the drugs are of rigid nature.

Statin Drugs	Flexible Docking Affinity (kcal/mol)	Rigid Docking Affinity (kcal/mol)
Atorvastatin	-9.5	-9.6
Lovastatin	-8.6	-9.2
Pitavastin	-8.1	-10
Pravastatin	-8.2	-9.3
Simvastatin	-8.70	-8.7

3.2.1. Visualization using PyMol

Validation using PyMol involves visualization of the protein molecule with which the drug small molecules or ligands are bound. The protein molecule with the ligand as first loaded in PyMOL and then the output pdbqt files obtained from docking using PyRx were also loaded in PyMOL. The main ligand of SPARC, N-Acetyl-D-Glucosamine (NAG) was used as a reference for the drug small molecule that was bound to the protein. Figure 3.3. shows the superimposition of drugs with the NAG molecule and Figure 3.4. shows drugs attached to the binding pockets.



(a) **(b)**

(c)

Figure 3.3. (a) Superimposition of NAG with Atorvastatin. (b) Superimposition of NAG with Pitavastatin. (c) Superimposition of NAG with Pravastatin. Statins bind to the same substrate binding pocket as the reference ligand NAG. (visualization using PyMOL version 1.8.4.0) (Seeliger & de Groot, 2010)

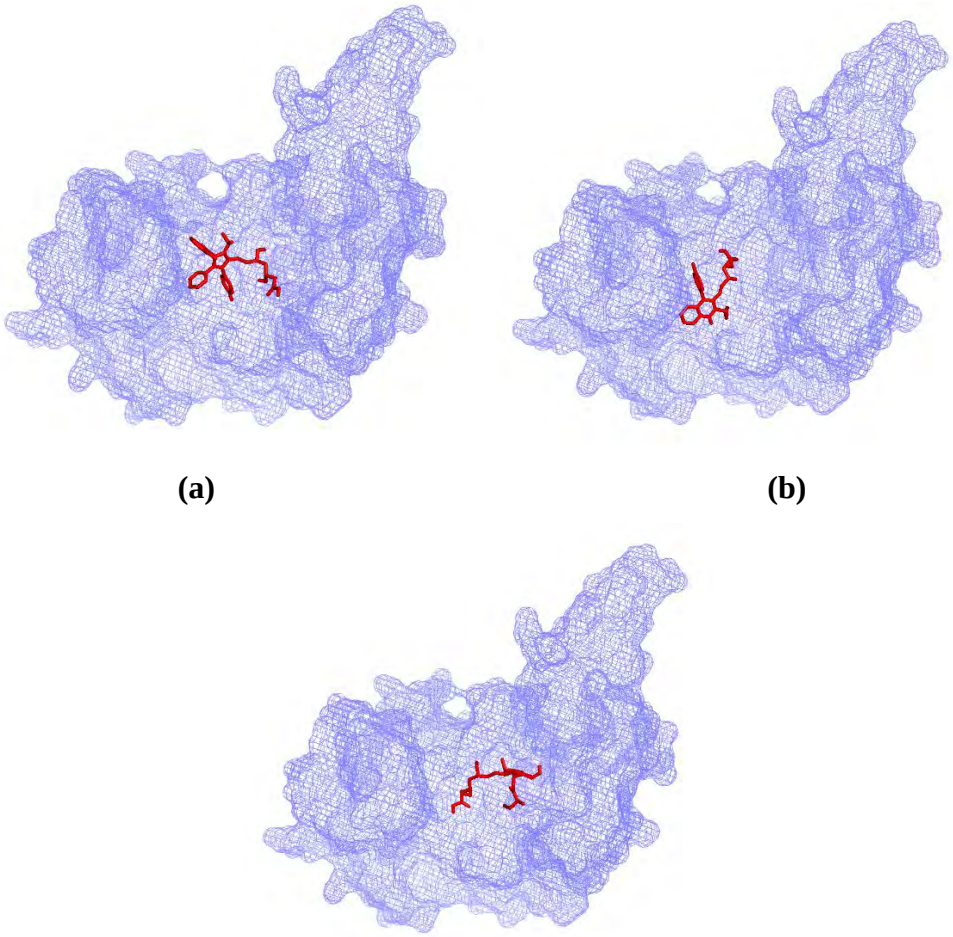


Figure 3.4. (a) Atorvastatin in the binding pocket of SPARC, (b) Pitavastatin in the binding pocket of SPARC, (c) Pravastatin in the binding pocket of SPARC (visualization using PyMOL version 1.8.4.0) (Seeliger & de Groot, 2010)

3.2.2. Visualization using Discovery Studio

To get an idea about the interactions between protein and the ligands, discovery studio was used. The types of the bond along with the amino acids with which the bonds are formed could also be observed using discovery studio. It also shows the distance between the protein and the small molecules. The amino acid interactions, bond length and bond types of the ligand with the protein can be compared with the reference ligand using it. The pdb files of the protein drug complex were opened in discovery studio and the interactions with the ligand were observed. The drugs interacted with the amino acids lining the binding pocket of the protein.

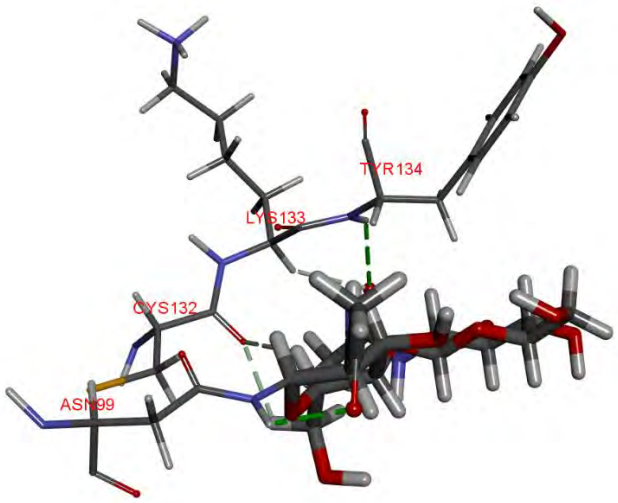


Figure 3.5. Non-bond Interactions of NAG Ligand with SPARC (obtained from Discovery Studio) (Eid et al., 2013)

Table 3.2. Non-bond interactions involved in the binding of NAG to SPARC (Visualized using Discovery Studio Version 17.2.0.16349)

Type of Bond	Amino Acid. Ligand and atom interaction	Distance in angstroms (Amino Acid-Ligand)	Category
Conventional Hydrogen Bond	ASN99	2.3101	Hydrogen Bond
Conventional Hydrogen Bond	TYR134	2.7556	Hydrogen Bond
Carbon Hydrogen Bond	LYS133	2.55655	Hydrogen Bond
Carbon Hydrogen Bond	CYS132	2.85156	Hydrogen Bond
Carbon Hydrogen Bond	CYS132	2.57029	Hydrogen Bond

Several hydrogen bonds were formed between NAG and SPARC. Five hydrogen bonds were formed. The distances of the hydrogen bond ranged from 2.3 – 2.8 angstroms (Table 3.2). The important amino acids in the bonding of NAG to SPARC included

ASN99 (aa Asparagine), TYR134 (aa Tyrosine), LYS (aa Lysine) and CYS132 (aa Cysteine) (Figure 3.5).

3.2.2.1. *In Silico* Binding of SPARC with Atorvastatin

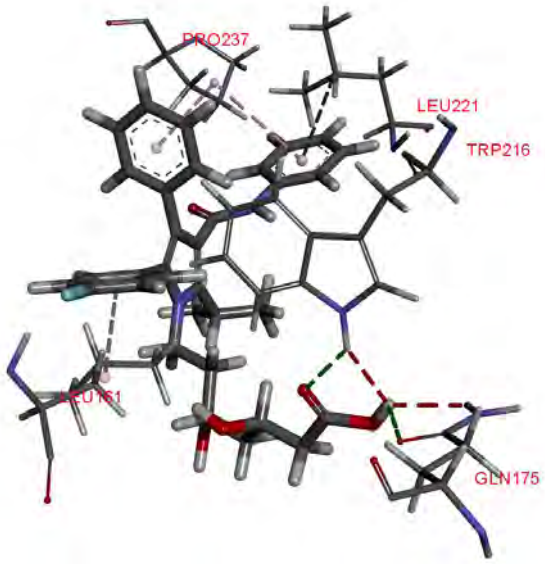


Figure 3.6. Non-bond Interactions of Atorvastatin with SPARC (obtained from Discovery Studio) (Eid et al., 2013)

Table 3.3. Non-bond interactions involved in the binding of Atorvastatin to SPARC (Visualized using Discovery Studio Version 17.2.0.16349)

Ligand	Binding Affinity (kCal/ mol)	Type of Bond	Amino Acid. Ligand and atom interaction	Distance in angstroms (Amino Acid-Ligand)	Category
Atorvastatin	-9.6	Conventional Hydrogen Bond	B:TRP216:HE1 - :UNL1:O	2.45032	Hydrogen Bond
		Conventional Hydrogen Bond	:UNL1:H - B:GLN175:OE 1	2.95999	Hydrogen Bond

		Pi-Alkyl	:UNL1 - B:PRO237	4.57758	Hydrophobic
		Pi-Alkyl	:UNL1 - B:LEU221	5.377	Hydrophobic
		Pi-Alkyl	:UNL1 - B:PRO237	4.03443	Hydrophobic
		Pi-Alkyl	:UNL1 - B:LEU161	5.26418	Hydrophobic

Several bonds were formed between atorvastatin and SPARC. The types of bond include hydrogen bonds and hydrophobic bonds where two of them are hydrogen bonds and four of them are hydrophobic (Table 3.3). The first hydrogen bond formed between TRP216 (aa Tryptophan) and oxygen atom of atorvastatin having a bond length of 2.45 angstroms. The second hydrogen bond formed between GLN175 (aa Glutamine) and atorvastatin having a bond length of 2.96 angstroms. The first hydrophobic bond was formed between PRO237 (aa Proline) and pi-alkyl groups of atorvastatin with a bond length of 4.58 angstroms. The other hydrophobic bonds formed includes LEU221 (aa Leucine), PRO237 (aa Proline) and LEU161 (aa Leucine) with the pi-alkyl groups of atorvastatin with bond lengths of 5.38, 4.03 and 5.26 angstroms respectively. Hydrophobic interactions have a very significant role in nonpolar molecules. Davis and Teague found in their study that hydrophobic interactions greatly influence the affinity with which a ligand binds to its receptor molecule. They also revealed that hydrophobic bonds can increase the binding per methyl group by around 3.2 times (A. M. Davis & Teague, 1999) Figure 3.6. shows these amino acids bound to the NAG molecule. Atorvastatin does not bind with any amino acids with which the reference ligand NAG or drug pravastatin binds. Pitavastatin and atorvastatin both bind with proline, but the number is different. Atorvastatin binds with PRO237, while pitavastatin binds with PRO137 and while atorvastatin forms Pi-Alkyl bind with proline, pitavastatin forms Alkyl bond.

3.2.2.2. *In Silico* Binding of SPARC with Pitavastatin

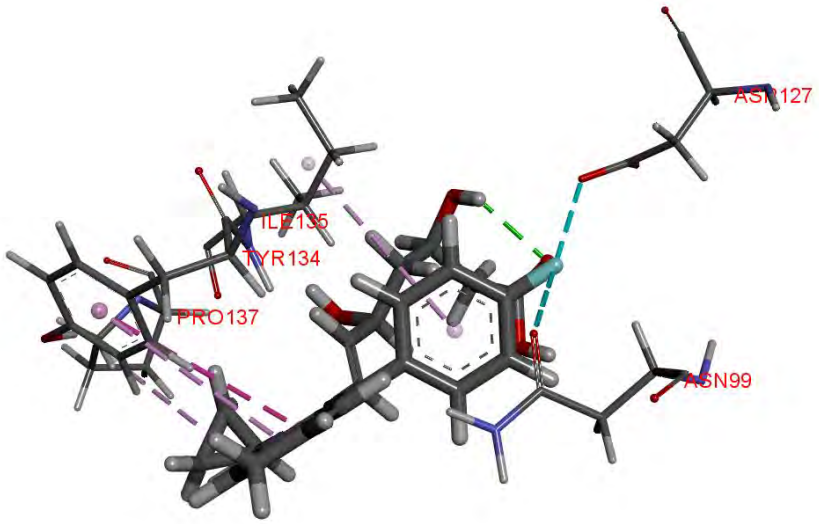


Figure 3.7. Non-bond Interactions of Pitavastatin with SPARC (obtained from Discovery Studio) (Eid et al., 2013)

Table 3.4. Non-bond interactions involved in the binding of Pitavastatin to SPARC (Visualized using Discovery Studio Version 17.2.0.16349)

Ligand	Binding Affinity (kCal/ mol)	Type of Bond	Amino Acid. Ligand and atom interaction	Distance in angstroms (Amino Acid-Ligand)	Category
Pitavastatin	-10	Conventional Hydrogen Bond	:UNL1:H - :UNL1:O	2.34449	Hydrogen Bond
		Halogen (Fluorine)	B:ASN99:OD1 - :UNL1:F	3.56015	Halogen
		Halogen (Fluorine)	B:ASP127:OD2 - :UNL1:F	2.89077	Halogen
		Pi-Pi T-shaped	B:TYR134 - :UNL1	5.45902	Hydrophobic

		Alkyl	:UNL1 - B:PRO137	3.96232	Hydrophobic
		Pi-Alkyl	B:TYR134 - :UNL1	5.34816	Hydrophobic
		Pi-Alkyl	:UNL1 - B:ILE135	5.33274	Hydrophobic

Several bonds were formed between pitavastatin and SPARC. The types of bonds include conventional hydrogen bond, halogen bond, pi-pi T-shaped, alkyl and pi alkyl bonds (Table 3.4). One hydrogen bond, two halogen bonds and four hydrophobic bonds were formed between pitavastatin and SPARC. The hydrogen bond was formed between an unknown amino acid of SPARC and pitavastatin with a distance of 2.34 angstroms. The first halogen bond formed was between ASN99 (aa Asparagine) and the F of pitavastatin with a bond length of 3.56 angstroms; the other halogen bond was between ASP127 (aa Aspartate) and F of pitavastatin with the bond length of 2.89 angstroms. Four hydrophobic bonds included bonds between pitavastatin and amino acids, PRO137 (aa Proline), ILE135 (Isoleucine) and two TYR134 (aa Tyrosine), the distances being 3.96, 5.33, 5.4 and 5.34 angstroms. Hydrophobic bonds can strongly increase the binding affinities as a drug binds to its receptor. Thus, these allowed a greater binding affinity between SPARC and pitavastatin. Figure 3.7. shows these amino acids bound to the pitavastatin molecule. From the table 3.4 we can also observe that pitavastatin binds to the same amino acids ASN99 (aa Asparagine) and TYR134 (aa Tyrosine) as the reference ligand NAG. Like atorvastatin, it also binds with amino acid proline. But while atorvastatin forms Pi-Alkyl bind with proline, pitavastatin forms Alky bond.

3.2.2.3. *In Silico* Binding of SPARC with Pravastatin

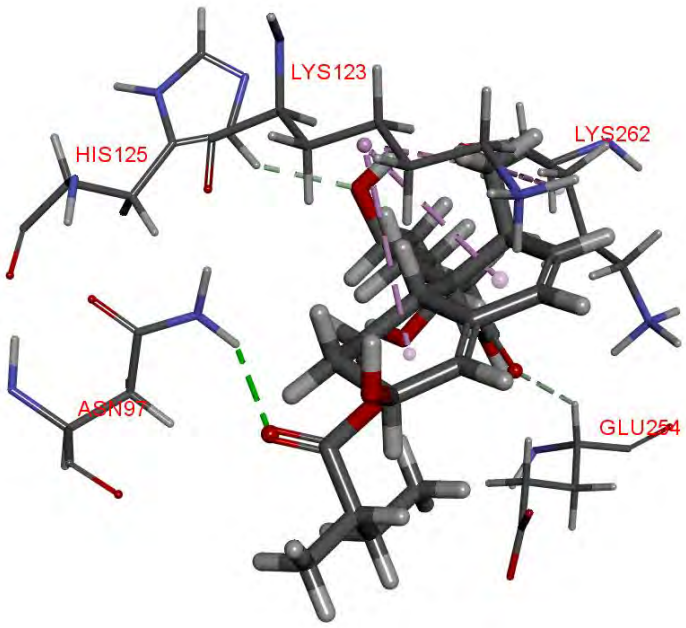


Figure 3.8. Non-bond Interactions of Pravastatin with SPARC (obtained from Discovery Studio) (Eid et al., 2013)

Table 3.5. Non-bond interactions involved in the binding of Pravastatin to SPARC (Visualized using Discovery Studio Version 17.2.0.16349)

Ligand	Binding Affinity (kCal/mol)	Type of Bond	Amino Acid. Ligand and atom interaction	Distance in angstroms (Amino Acid-Ligand)	Category
Pravastatin	-9.3	Conventional Hydrogen Bond	B:ASN97:HD21 - :UNL1:O	2.39775	Hydrogen Bond
		Carbon Hydrogen Bond	B:HIS125:HD2 - :UNL1:O	2.46988	Hydrogen Bond
		Carbon Hydrogen	B:GLU254:HA - :UNL1:O	2.54792	Hydrogen Bond

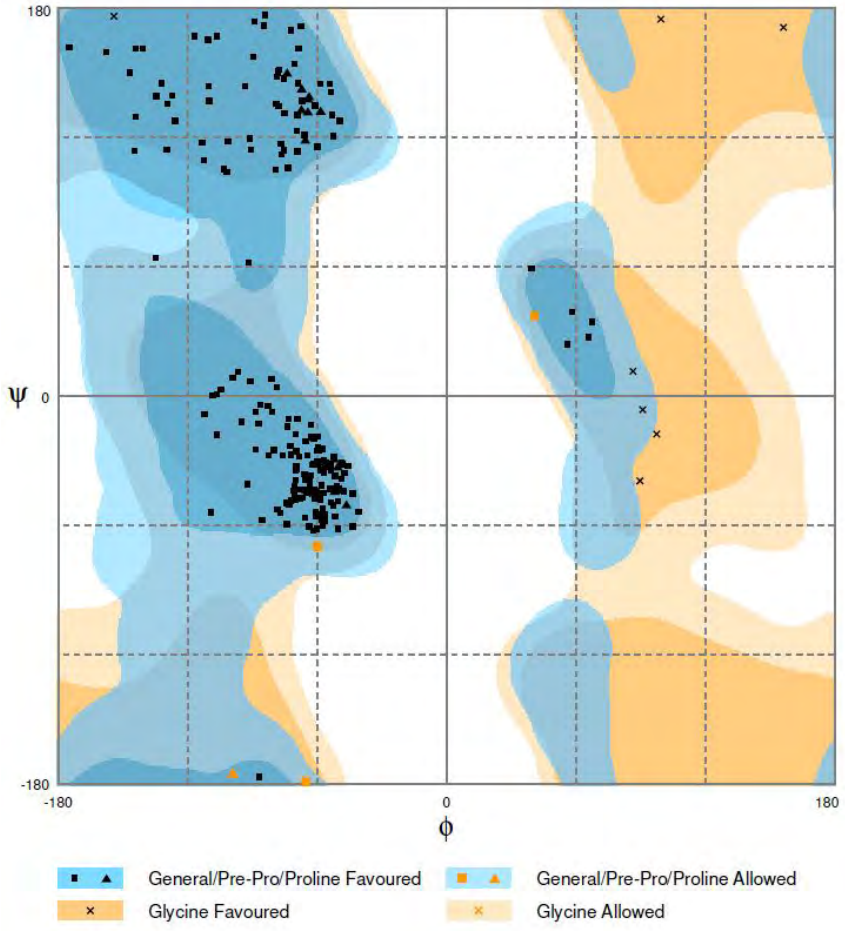
		Bond			
		Alkyl	B:LYS123 - :UNL1	4.1365	Hydrophobic
		Alkyl	:UNL1 - B:LYS123	4.26367	Hydrophobic
		Alkyl	:UNL1:C - B:LYS123	3.89959	Hydrophobic
		Alkyl	:UNL1:C - B:LYS262	4.29303	Hydrophobic

Several bonds were formed between atorvastatin and SPARC. The types of bond include hydrogen bonds and hydrophobic bonds, where three of them are hydrogen bonds and four of them are hydrophobic (Table 3.5). The first hydrogen bond formed between ASN97 (aa Asparagine) and oxygen atom of pravastatin having a bond length of 2.4 angstroms. The second hydrogen bond formed between HIS125 (aa Histidine) and oxygen atom of pravastatin having a bond length of 2.47 angstroms. The third hydrogen bond formed between GLU254 (aa Glutamate) and pravastatin with bond length of 2.55 angstroms. The first hydrophobic bond was formed between LYS123 (aa Lysine) and alkyl groups of pravastatin with a bond length of 4.14 angstroms. The other hydrophobic bonds formed includes LYS262 (aa Lysine) and two LYS123 (aa Lysine) with the alkyl groups of pravastatin with bond lengths of 4.3, 4.26 and 3.9 angstroms respectively. Figure 3.8. shows these amino acids bound to the pravastatin molecule. Pravastatin does not bind with any amino acid with which atorvastatin, pitavastatin or the reference ligand NAG binds.

3.3. Validation of Drug-Protein Complex

Ramachandran plots of protein bound to drugs were generated for further validation. ConSurf was also used to identify the conserved regions.

3.3.1. Validation using Ramachandran plot



Number of residues in favoured region (~98.0% expected)	: 218 (98.2%)
Number of residues in allowed region (~2.0% expected)	: 4 (1.8%)
Number of residues in outlier region	: 0 (0.0%)

Figure 3.9. Ramachandran Plot showing the energy minimization of the protein, SPARC bound to statins (obtained from Rampage) (Lovell et al., 2003)

The Ramachandran plot helps to predict the conformation of the backbone of a given polypeptide chain more quantitatively starting from knowledge of its amino acid sequences (Lovell et al., 2003). From figure 3.9. we can see that the number of amino acid residues in the favored region is 98.2% and the number of residues in the allowed region is 1.8%. No residues are seen in the outlier region. No significant changes were observed, which indicates no notable changes have occurred in the conformation of psi and phi bonds after binding of drugs with SPARC.

3.3.2 Validation using ConSurf

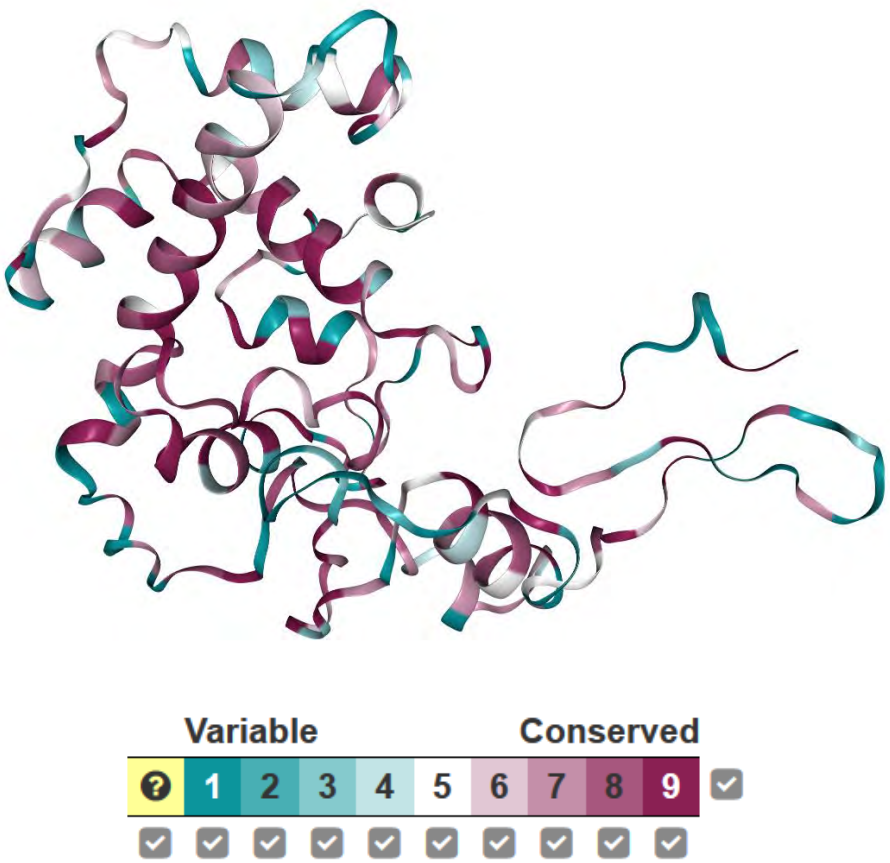


Figure 3.10. Conserved regions and variable regions of SPARC (obtained from ConSurf) (Ashkenazy et al., 2016)

SPARC was then uploaded in ConSurf, which is an online tool showing the conserved regions of the protein. A conserved region indicates that the part had remained unchanged for a very long time and is considered functionally important; they are not subjected to frequent mutations (Krishnan, Li, & Issac, 1998). Figure 3.10. shows the conserved and variable regions of SPARC. Considering the non-bond interactions observed in Discovery Studio, the amino acids that were involved in drug binding, TRP216 (aa Tryptophan), GLN175 (aa Glutamine), PRO237 (aa Proline), LEU221 (aa Leucine), LEU161 (aa Leucine), ASN99 (aa Asparagine), ASP127 (aa Aspartate), ILE135 (Isoleucine), TYR134 (aa Tyrosine), ASN97 (aa Asparagine), HIS125 (aa Histidine), and LYS262 (aa Lysine) lie in conserved regions. On the other hand, PRO137 (aa Proline), GLU254 (aa Glutamate) and LYS123 (aa Lysine) lie in the variable regions.

3.4. Selection of Statin Drug

The statin drugs that were chosen made no significant changes to the bond angles of psi and phi bonds. From the various results obtained, the drug pitavastain showed the best binding affinity and interaction with SPARC. However though, it showed high binding affinity, validation using ConSurf showed that PRO137 (aa Proline) which binds to pravastatin, falls in the variable region. Pravastatin had the lowest binding affinity among the three. Further validation using ConSurf showed that GLU254 (aa Glutamate) and LYS123 (aa Lysine) which binds to pravastatin, falls in the variable region. Previous studies have also shown that pravastatin's role in cancer is controversial (Bujanda et al., 2016). Thus, in this study we are suggesting that atorvastatin, which has the second highest binding affinity of -9.6 kCal/mol could inhibit abnormal cell mitosis via inhibition of the SPARC biosynthetic pathway and could be a better choice of drug in the treatment of gastric cancer.

4. Discussion

SPARC is a secreted glycoprotein that works in tissue remodeling and also serves as a tumor promoter in gastric cancer cell lines were it shows variable levels of SPARC (Hohenester et al., 1997). The transcription of SPARC and its level was upregulated in gastric cancer cell lines and has been correlated with invasiveness and poor prognosis of the disease (Wang et al., 2004). Down regulating the expression of SPARC in these cell lines show that it inhibits the growth and expression of cancer (Yin et al., 2010). Thus, inhibitors of SPARC could be used as anticancer drugs. Again SPARC increases the effect of apoptosis by supplementing the signaling cascade in a caspase-8 dependent manner. This has no relation to death receptor activation and it leads to downstream involvement of apoptosis (Tang & Tai, 2007). A better understanding of the function of SPARC in tumor progression is thus vital for establishing cancer therapeutics.

For this purpose, SPARC, a protein which is explicitly expressed in gastric cancer cells was targeted. Docking was carried out after removing the B chain along with heteroatoms such as water and N-Acetyl-D-Glucosamine. The protein was validated using z-score value, Verify3D, Ramachandran Plot and Errat. The z-score of SPARC was found to be -7.9, which is considered as a satisfactory outcome. The greater the value is away from zero towards the negative, the better. The residues were seen on the X ray region. However, it did not fall within the NMR region.

Docking is a computational technique that helps determine the binding affinities of drugs with the protein and both rigid and flexible docking were carried out. In the present study, rigid docking produced better results compared to flexible docking. The high binding affinities helped in the choice of the statin drugs. A high binding affinity suggested a link between statin drugs and SPARC, but the extent was unknown. To find out the link more precisely, non-bonded interactions, bond distances and types were looked up in Discovery studio. Discovery studio was used as a validation tool which provided a more distinct idea about the interaction of the protein with the ligand.

With SPARC, atorvastatin was observed to interact with GLN175 (aa Glutamine), PRO237 (aa Proline), LEU221 (aa Leucine) and LEU161 (aa Leucine). All the bonds were Hydrogen Bond and Hydrophobic Bond. Hydrogen bond included conventional Hydrogen Bond. And hydrophobic bonds included pi-alkyl bonds. All the hydrogen

bonds ranged from 2-3 Å which was considered to be decent. Hydrophobic bonds help in enhancing the binding affinity.

Ramachandran plot was plotted to show the energy minimization of the entire protein. Ramachandran plot was another method that was used for validation of SPARC as well as the SPARC-drug complexes. The energy minimization plot showed same results with both the SPARC and the SPARC-drug complexes. The number of amino acid residues in the favored region was 98.2%, the number of amino acids in the allowed region was 1.8%. No residues were observed in the outlier region. The results were close to the expected results. Expected results for amino acid residues in the favored and allowed region are 98% and 2% respectively. In case of residues in outlier region, the percentage is expected to be as less as possible. Values over 90% are considered acceptable for residues on favored region and a deviation of ±5% is allowed in the allowed region. Same results were obtained with the SPARC-drug complexes; the results indicated that the amino acids were located in desirable locations after the protein complex was formed and no undesirable or harmful effects would be caused in the body. Previous studies have shown that in and pitavastatin exhibited anticancer properties. However when the drug was administered at high doses, pitavastatin showed side effects like rhabdomyolysis. (Al-Qatati & Aliwaini, 2017; De Wolf, De Wolf, & Richardson, 2017).

Consurf (Ashkenazy et al., 2016) results showed that the amino acids TRP216 (aa Tryptophan), GLN175 (aa Glutamine), PRO237 (aa Proline), LEU221 (aa Leucine), LEU161 (aa Leucine), ASN99 (aa Asparagine), ASP127 (aa Aspartate), ILE135 (Isoleucine), TYR134 (aa Tyrosine),ASN97 (aa Asparagine), HIS125 (aa Histidine), and LYS262 (aa Lysine) lie in highly conserved regions; this indicates that atorvastatin could possibly act as a potential SPARC inhibitor to prevent gastric cancer progression.

5. Conclusion

The current *in silico* study shows that the statin drug, atorvastatin could be an effective inhibitor of SPARC and suggests that atorvastatin may play a significant role in suppressing gastric cancer progression and tumor development.

Previous studies were found to state its use as an anticancer drug. However, no *in vivo* studies have been carried out yet. Further studies and *in vivo* tests could be performed to confirm the ligands potential interaction with SPARC. If the *in vivo* tests prove the drug to be effective as a potent antagonist of SPARC, a safe and clinically useful anti-cancer drug for gastric cancer could be attained.

References:

Ashburn, T. T., & Thor, K. B. (2004). Drug repositioning: Identifying and developing new uses for existing drugs. *Nature Reviews Drug Discovery*, *3*(8), 673–683. https://doi.org/10.1038/nrd1468

Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., & Ben-Tal, N. (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Research*, *44*(W1), W344-50. https://doi.org/10.1093/nar/gkw408

Barber, M., Murrell, A., Ito, Y., Maia, A.-T., Hyland, S., Oliveira, C., … Fitzgerald, R. (2008). Mechanisms and sequelae of E-cadherin silencing in hereditary diffuse gastric cancer. *The Journal of Pathology*, *216*(3), 295–306. https://doi.org/10.1002/path.2426

Bradshaw, A. D. (2016). The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? *Journal of Molecular and Cellular Cardiology*, *93*(2015), 156–161. https://doi.org/10.1016/j.yjmcc.2015.11.014

Bujanda, L., Rodríguez-González, A., Sarasqueta, C., Eizaguirre, E., Hijona, E., Marín, J. J. G., … Cosme, A. (2016). Effect of pravastatin on the survival of patients with advanced gastric cancer. *Oncotarget*, *7*(4), 4379–4384. https://doi.org/10.18632/oncotarget.6777

Carcas, L. P. (2014). Gastric cancer review. *Journal of Carcinogenesis*, *13*, 14. https://doi.org/10.4103/1477-3163.146506

Charbel Issa, P., Gillies, M. C., Chew, E. Y., Bird, A. C., Heeren, T. F. C., Peto, T., … Scholl, H. P. N. (2013). Macular telangiectasia type 2. *Progress in Retinal and Eye Research*, *34*, 49–77. https://doi.org/10.1016/j.preteyeres.2012.11.002

Chauvin, B., Drouot, S., Barrail-Tran, A., & Taburet, A.-M. (2013). Drug–Drug Interactions Between HMG-CoA Reductase Inhibitors (Statins) and Antiviral Protease Inhibitors. *Clinical Pharmacokinetics*, *52*(10), 815–831. https://doi.org/10.1007/s40262-013-0075-4

D'Amato, R. J., Loughnan, M. S., Flynn, E., & Folkman, J. (1994). Thalidomide is an inhibitor of angiogenesis. *Proceedings of the National Academy of Sciences*, *91*(9), 4082–4085. https://doi.org/10.1073/pnas.91.9.4082

Dallakyan, S., & Olson, A. J. (2015). Small-Molecule Library Screening by Docking with PyRx (pp. 243–250). https://doi.org/10.1007/978-1-4939-2269-7_19

Davis, A. M., & Teague, S. J. (1999). Hydrogen Bonding, Hydrophobic Interactions, and Failure

of the Rigid Receptor Hypothesis. *Angewandte Chemie International Edition*, *38*(6), 736–749. https://doi.org/10.1002/(SICI)1521-3773(19990315)38:6<736::AID-ANIE736>3.0.CO;2-R

Davis, C. P. (n.d.). Cancer Causes, Types, Treatment, Symptoms & Signs. Retrieved August 27, 2018, from https://www.medicinenet.com/cancer/article.htm#cancer_facts

De Wolf, E., De Wolf, C., & Richardson, A. (2018). ABT-737 and pictilisib synergistically enhance pitavastatin-induced apoptosis in ovarian cancer cells. *Oncology Letters*, *15*(2), 1979–1984. https://doi.org/10.3892/ol.2017.7516

Eid, S., Zalewski, A., Smieško, M., Ernst, B., & Vedani, A. (2013). A molecular-modeling toolbox aimed at bridging the gap between medicinal chemistry and computational sciences. *International Journal of Molecular Sciences*, *14*(1), 684–700. https://doi.org/10.3390/ijms14010684

Eldridge, M. D., Murray, C. W., Auton, T. R., Paolini, G. V., & Mee, R. P. (1997). Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *Journal of Computer-Aided Molecular Design*, *11*(5), 425–445. https://doi.org/10.1023/A:1007996124545

Endo, A. (1988). Chemistry, biochemistry, and pharmacology of HMG-CoA reductase inhibitors. *Klinische Wochenschrift*, *66*(10), 421–427. https://doi.org/10.1007/BF01745510

Garg, V. K., Avashthi, H., Tiwari, A., Jain, P. A., Ramkete, P. W., Kayastha, A. M., & Singh, V. K. (2016). MFPPI - Multi FASTA ProtParam Interface. *Bioinformation*, *12*(2), 74–77. https://doi.org/10.6026/97320630012074

Gupta, S. C., Sung, B., Prasad, S., Webb, L. J., & Aggarwal, B. B. (2013). Cancer drug discovery by repurposing: teaching new tricks to old dogs. *Trends in Pharmacological Sciences*, *34*(9), 508–517. https://doi.org/10.1016/j.tips.2013.06.005

Henson, D. E., Dittus, C., Younes, M., Nguyen, H., & Albores-Saavedra, J. (2004). Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: increase in the signet ring cell type. *Archives of Pathology & Laboratory Medicine*, *128*(7), 765–770. https://doi.org/10.1043/1543-2165(2004)128<765:DTITIA>2.0.CO;2

Hohenester, E., Maurer, P., & Timpl, R. (1997). Crystal structure of a pair of follistatin-like and EF-hand calcium-binding domains in BM-40. *EMBO Journal*, *16*(13), 3778–3786.

https://doi.org/10.1093/emboj/16.13.3778

Huijgens, P. C., Simoons-Smit, A. M., Van Loenen, A. C., Prooy, E., Van Tinteren, H., Ossenkoppele, G. J., & Jonkhoff, A. R. (1999). Fluconazole versus itraconazole for the prevention of fungal infections in haemato-oncology. *Journal of Clinical Pathology*, *52*(5), 376–380. https://doi.org/10.1136/jcp.52.5.376

Jesús Naveja, J., Dueñas-González, A., & Medina-Franco, J. L. (2016). *Drug Repurposing for Epigenetic Targets Guided by Computational Methods*. *Epi-Informatics: Discovery and Development of Small Molecule Epigenetic Drugs and Probes*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802808-7.00012-5

Krishnan, A., Li, K.-B., & Issac, P. (1998). *In silico biology. In Silico Biology* (Vol. 4). IOS Press. Retrieved from https://content.iospress.com/articles/in-silico-biology/isb00123

LAUREN, P. (1965). THE TWO HISTOLOGICAL MAIN TYPES OF GASTRIC CARCINOMA: DIFFUSE AND SO-CALLED INTESTINAL-TYPE CARCINOMA. AN ATTEMPT AT A HISTO-CLINICAL CLASSIFICATION. *Acta Pathologica et Microbiologica Scandinavica*, *64*, 31–49. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14320675

Lenz, W., & Knapp, K. (1962). Thalidomide embryopathy. *Archives of Environmental Health*, *5*(2), 14–19. https://doi.org/10.1080/00039896.1962.10663250

Lovell, S. C., Davis, I. W., Arendall, W. B., de Bakker, P. I. W., Word, J. M., Prisant, M. G., … Richardson, D. C. (2003). Structure validation by Cα geometry: ϕ,ψ and Cβ deviation. *Proteins: Structure, Function, and Bioinformatics*, *50*(3), 437–450. https://doi.org/10.1002/prot.10286

Morgan, R. E., Campbell, S. E., Yu, C. Y., Sponseller, C. A., & Muster, H. A. (2012). Comparison of the Safety, Tolerability, and Pharmacokinetic Profile of a Single Oral Dose of Pitavastatin 4 mg in Adult Subjects With Severe Renal Impairment Not on Hemodialysis Versus Healthy Adult Subjects. *Journal of Cardiovascular Pharmacology*, *60*(1), 42–48. https://doi.org/10.1097/FJC.0b013e318256cdf0

Neveen Said. (2016). Annals of Carcinogenesis Role of SPARC in Cancer ; Friend or Foe, *1*(1), 1–11.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*(1), 33. https://doi.org/10.1186/1758-2946-3-33

Parwani, A. V. (2007). Discovery of Novel Methylation Biomarkers in Cervical Carcinoma by Global Demethylation and Microarray Analysis. *Yearbook of Pathology and Laboratory Medicine*, *2007*, 167–169. https://doi.org/10.1016/S1077-9108(08)70356-6

Plummer, M., de Martel, C., Vignat, J., Ferlay, J., Bray, F., & Franceschi, S. (2016). Global burden of cancers attributable to infections in 2012: a synthetic analysis. *The Lancet Global Health*, *4*(9), e609–e616. https://doi.org/10.1016/S2214-109X(16)30143-7

Rahman, M., Chan, A. P. K., & Tai, I. T. (2011). A peptide of sparc interferes with the interaction between Caspase8 and Bcl2 to resensitize Chemoresistant tumors and enhance their regression in vivo. *PLoS ONE*, *6*(11). https://doi.org/10.1371/journal.pone.0026390

Rivera, L. B., Bradshaw, A. D., & Brekken, R. A. (2011). The regulatory function of SPARC in vascular biology. *Cellular and Molecular Life Sciences*, *68*(19), 3165–3173. https://doi.org/10.1007/s00018-011-0781-8

Roder, C., & Thomson, M. J. (2015). Auranofin: Repurposing an Old Drug for a Golden New Age. *Drugs in R and D*, *15*(1), 13–20. https://doi.org/10.1007/s40268-015-0083-y

Said, N., Frierson, H. F., Sanchez-Carbayo, M., Brekken, R. A., & Theodorescu, D. (2013). Loss of SPARC in bladder cancer enhances carcinogenesis and progression. *The Journal of Clinical Investigation*, *123*(2), 751–766. https://doi.org/10.1172/JCI64782

Said, N., Socha, M. J., Olearczyk, J. J., Elmarakby, A. A., Imig, J. D., & Motamed, K. (2007). Normalization of the Ovarian Cancer Microenvironment by SPARC. *Molecular Cancer Research*, *5*(10), 1015–1030. https://doi.org/10.1158/1541-7786.MCR-07-0001

Seeliger, D., & de Groot, B. L. (2010). Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *Journal of Computer-Aided Molecular Design*, *24*(5), 417–422. https://doi.org/10.1007/s10822-010-9352-6

Seyfried, T. N. (n.d.). How does cancer develop? - Petrafoundation. Retrieved August 27, 2018, from http://www.petrafoundation.com/how-does-cancer-develop/

SHI, D., JIANG, K., FU, Y., FANG, R., LIU, X., & CHEN, J. (2016). Overexpression of SPARC correlates with poor prognosis in patients with cervical carcinoma and regulates cancer cell epithelial-mesenchymal transition. *Oncology Letters*, *11*(5), 3251–3258. https://doi.org/10.3892/ol.2016.4399

Sippl, M. J. (1993). Recognition of errors in three-dimensional structures of proteins. *Proteins: Structure, Function, and Genetics*, *17*(4), 355–362.

https://doi.org/10.1002/prot.340170404

Sirtori, C. R. (2014a). The pharmacology of statins. *Pharmacological Research*, *88*, 3–11. https://doi.org/10.1016/j.phrs.2014.03.002

Sirtori, C. R. (2014b). The pharmacology of statins. *Pharmacological Research*, *88*, 3–11. https://doi.org/10.1016/j.phrs.2014.03.002

Sitarz, R., Skierucha, M., Mielko, J., Offerhaus, G. J. A., Maciejewski, R., & Polkowski, W. P. (2018). Gastric cancer: epidemiology, prevention, classification, and treatment. *Cancer Management and Research*, *10*, 239–248. https://doi.org/10.2147/CMAR.S149619

Tai, I. T., Dai, M., Owen, D. A., & Chen, L. B. (2005). Genome-wide expression analysis of therapy-resistant tumors reveals SPARC as a novel target for cancer therapy. *Journal of Clinical Investigation*, *115*(6), 1492–1502. https://doi.org/10.1172/JCI23002

Tang, M. J., & Tai, I. T. (2007). A novel interaction between procaspase 8 and SPARC enhances apoptosis and potentiates chemotherapy sensitivity in colorectal cancers. *Journal of Biological Chemistry*, *282*(47), 34457–34467. https://doi.org/10.1074/jbc.M704459200

Termine, J. D., Kleinman, H. K., Whitson, S. W., Conn, K. M., McGarvey, M. L., & Martin, G. R. (1981). Osteonectin, a bone-specific protein linking mineral to collagen. *Cell*, *26*(1 PART 1), 99–105. https://doi.org/10.1016/0092-8674(81)90037-4

Wang, C. S., Lin, K. H., Chen, S. L., Chan, Y. F., & Hsueh, S. (2004). Overexpression of SPARC gene in human gastric carcinoma and its clinic-pathologic significance. *British Journal of Cancer*, *91*(11), 1924–1930. https://doi.org/10.1038/sj.bjc.6602213

Why FDA pulled cholesterol drugs off market | Formulary Watch. (n.d.). Retrieved July 19, 2018, from http://www.formularywatch.com/feature-articles/why-fda-pulled-cholesterol-drugs-market

Wilke, M., Göbel, A., Rauner, M., Benad-Mehner, P., Schiitze, N., Fussel, S., … Rachner, T. D. (2014). Zoledronic acid and atorvastatin inhibit αvβ33-mediated adhesion of breast cancer cells. *Journal of Bone Oncology*, *3*(1), 10–17. https://doi.org/10.1016/j.jbo.2014.02.001

Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., … Wilson, M. (2018). DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Research*, *46*(D1), D1074–D1082. https://doi.org/10.1093/nar/gkx1037

Yin, J., Chen, G., Liu, Y., Liu, S., Wang, P., Wan, Y., … Gao, H. (2010). Downregulation of SPARC expression decreases gastric cancer cellular invasion and survival. *Journal of*

Experimental and Clinical Cancer Research, *29*(1), 1–9. https://doi.org/10.1186/1756-9966-29-59

---------- END ----------