Molecular Docking of Fluvastatin and Teneligliptin Targeting AT1R in Hypertension

By

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

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Declaration

It is hereby declared that

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

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Approval

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Ethics Statement

The study does not involve any kind of animal trial and human trial.

Abstract

Hypertension, or high blood pressure is a major risk factor causing cardiovascular diseases such as cerebrovascular stroke and ischemic heart disease. It is now considered as one of the most common disease and over 1.13 billion people are suffering from hypertension all over the world. The protein AT1R (octapeptide receptor that belongs to class A GPCR) plays an important role in vascular smooth muscle contraction and aldosterone secretion which results in hypertension. AT1R protein is responsible for developing hypertension via some biosynthetic pathways such as Ca/IP3 pathway. AT1 receptor antagonists have become the drug of choice for the patients who are suffering from hypertension and other cardiovascular disease by controlling overexpression of AT1 receptor. In this study, drug repurposing and other *in silico* computational methods have been used in order to find potential AT1R antagonists which will be used to inhibit and control the production of AT1R. PyRx, Discovery studio, Ramachandran plot and ProSa web server were used in this study. Initially over 160 small molecules were selected and docked with the AT1R protein based on binding affinity and stability ten potential drugs which were finally selected. The aim of this study was to find drugs which targeted and blocked AT1R to prevent its activation in order to control hypertension. The study shows that fluvastatin and teneligliptin have high binding affinities towards AT1R and could be used as anti-hypertensive drugs for hypertension by inhibiting the activation of AT1R.

Keywords: Angiotensin receptor; Hypertension; Drug repurposing; Molecular docking; Drug-protein interaction.

Dedication

Dedicated to my parents and supervisors, professor Dr. Eva Rahman Kabir and Mohammad Kawsar Sharif Siam

Acknowledgement

I would like to begin by thanking the Almighty who gives me all the strength and patience to complete this project work. Without the endless help of numerous individuals who are acknowledged here, this project would not have been possible to complete.

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List of Acronyms

ACE	Angiotensin Converting Enzyme
RAS	Renin-Angiotensin System
RAAS	Renin–Angiotensin–Aldosterone System
AT1R	Angiotensin II receptor type 1
FDA	Food and Drug Administration
WHO	World Health Organization
GHO	Global Health Observatory
ADME	Absorption, Distribution, Metabolism and Excretion
VS	Virtual Screening
GPCR	G-protein-coupled receptors
CVD	Cardiovascular Diseases
Ang II	Angiotensin II
AT1 or AGTR1	Angiotensin II Receptor Type 1
AT2 or AGTR2	Angiotensin II Receptor Type 2
AT4	Angiotensin II Receptor Type 4
ECL	Extracellular Loop
ICL	Intracellular Loop
MAPK	Mitogen-Activated Protein Kinase
IP3	Inositol-1,4,5-Triphosphate
PKC	Protein Kinase C
COX	Cyclo-oxygenase

OLM	Olmesartan
ARBs	Angiotensin Receptor Blockers
NIH	National Institutes of Health
PDE5	Phosphodiesterase Type 5
HTS	High-Throughput Screening
CADD	Computer-Aided Drug Design
BP	Blood Pressure
HMG-CoA	Hydroxymethylglutaryl Co-enzyme
LDL	Low Density Lipoprotein
NCBI	National Center for Biotechnology Information
ILE	Isoleucine
SER	Serine
VAL	Valine
TYR	Tyrosine
TRP	Tryptophan
NSAID	Non-Steroidal Anti-inflammatory Drugs

Chapter 1

Introduction

Hypertension or high blood pressure is a major risk factor causing cardiovascular diseases such as cerebrovascular stroke and ischemic heart disease. According to the World Health Organization's (WHO) Global Health Observatory (GHO) data, 1.13 billion people are suffering from hypertension all over the world (Chockalingam, Facc, C, & C, 2016). Hypertension is a condition characterized by high blood pressure which is normally equal to or greater than 140/90 mmHg for a long period of time. Hypertension is also known as "silent killer". It can be managed but there is no way to cure high blood pressure. For that reason, it is dangerous for patients who are suffering from high blood pressure (Rodriguez-iturbe, Pons, & Johnson, 2017). Interrelationships between environmental and genetic factors are likely to contribute in the pathophysiology of hypertension. In addition, lifestyle-related factors, hypovitaminosis D, angiotensin converting enzyme (ACE) gene polymorphism, metabolic syndrome and low birth weight are considered important contributors to hypertension, especially in Bangladesh (Islam & Majumder, 2012). Angiotensin II (Ang II) is an octapeptide which is produced by the renin-angiotensin system (RAS). It plays a vital role in the pathophysiology of hypertension. Cardiovascular homeostasis is regulated by vasoactive hormone which acts on both the blood volume and the vascular resistance (Cappelli, Mohr, Gallelli, Rizzo, Anzini, Vomero, Mennuni, Ferrari, Makovec, Menziani, Benedetti, et al., 2004). Blood pressure (BP) is often difficult to control. Either the blood pressure is not frequently and properly measured, or the physician fails to react properly in the period of elevated blood pressure values, or proper, adequate and optimal

treatment is not provided in time, or the patient behaves carelessly in case of taking the necessary medication regularly (Jordan, Kurschat, Reuter, & Jordan, 2018).

JNC 7 (the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure) claims a very high, strong, consistent, independent, predictive and etiologically significant and risky relationship between high blood pressure and cerebral-stroke (Polonikov, et al., 2011). According to the National Health and Nutrition Examination Survey between 2005 and 2010, it has been measured that in the US, the percentage of treatment-eligible adult population who are suffering from hypertension would decline from 20.3% to 19.2%. Moreover, according to the 7th Joint National Committee guideline, the percentage of treatment-eligible adult population with hypertension who are aged 60 years and older would decrease from 68.9% to 61.2% (Handler, 2015). The reason behind this decreasing number of incidences of stroke in recent periods is the reduction of blood pressure (Jordan et al., 2018).

In this recent era, hypertension has become an important medical and public health issue because of its rate of increasing the risks of cardiovascular and kidney disease (Islam & Majumder, 2012). The most convenient methods to find a cure and solution for this devastating disease is drug repurposing. As discovering and establishing a new drug in a market is both time consuming and expensive, drug repurposing seems like the most effective and efficient method to find out a viable cure for human race (Roder & Thomson, 2015). In the drug repurposing process, already established and marketed drugs are being utilized because this method is convenient, cheap and less time consuming in comparison to novel drug discovery. Thus, it is considered a convenient and useful choice to find a medication which is preferable for

hypertension. In this study, the molecular target was AT1R (Angiotensin II receptor type 1) and the anti-hypertensive effect of anti-diabetic, statins, anti-inflammatory drugs were investigated.

1.1 Different Types of Hypertension

Hypertension is a chronic and progressive disease that is responsible for heart failure, stroke and ultimately death if it is not treated properly. The World Health Organization (WHO) reports that 54% of strokes and 47% of cases of ischemic heart disease are directly associated with high blood pressure, which is thus known as one of the main risk factors for cardiovascular morbidity and mortality (Jordan et al., 2018). Worldwide people are suffering from different types of hypertension. Among all, primary or essential and secondary hypertension are common and 90% of hypertensive patients are dealing with these two types of hypertension (Fogoros, 2018).

1.1.1 Essential Hypertension

As the exact reason which is responsible for high blood pressure is still unknown in large number of patients, these patients fall in a group which is known as primary or essential hypertension (Rodriguez-iturbe et al., 2017). Though there are currently no expectable clinical methods for detecting a specific cause of elevating blood pressure for patients with essential hypertension, the patients can experience some events like frequent headaches, dizziness, or nose bleeds and tiredness. Essential hypertension has a potential role in cardiovascular disease in adults, congestive heart failure, and is commonly associated with diabetes mellitus, cerebrovascular accidents, and renal failure, but early and proper treatment of hypertension can decrease the significant rate of subsequent cardiovascular morbidity and death (Bao, Threefoot, Srinivasan, & Berenson, 1995).

1.1.2 Secondary Hypertension

Sometimes the causes responsible for high blood pressure is known and this type of high blood pressure is called secondary hypertension (Rodriguez-iturbe et al., 2017). Reason behind secondary hypertension include renal artery stenosis, hyperaldosteronism, or pheochromocytoma in the differential diagnosis, especially in younger patients and those who find it difficult to control blood pressure (Jordan et al., 2018).

1.1.3 Other or Resistant Hypertension

Resistant hypertension is a usual clinical problem that both the primary care clinicians and specialists are facing in everyday practice. The patients with resistant hypertension are prescribed different classes of three antihypertensive agents at optimal dose (Calhoun et al., 2008). Population and clinical surveys in North America, Europe and Australia reported that 50% to 75% of people with hypertension are being treated with antihypertensive agents, but the blood pressure at target levels are not achieved (Adler, 2001).

1.2 Drug Repurposing

Drug repurposing is a process by which identification and development of new therapeutic and medical indications for existing or already established drugs and also withdrawn or rejected drugs or compounds can be found with the help of experimental and computational techniques (Ashburn & Thor, 2004, Ishida, Konishi, Ebner, & Springer, 2016). The process is also known as drug repositioning, drug rescue or drug re-profiling (Ishida et al., 2016). According to the current system of drug discovery, the process of identifying, developing and registering of new drugs normally require 10–17 years to complete and costs up to USD$1.5 billion which is expensive and time-consuming and almost 70–90 % of clinical trials of drugs are failing (Roder &

Thomson, 2015). In comparison with the novel drug discovery method, drug repurposing is a more convenient process in the medical history as it ensures lower costs and shorter time, smaller corporate infrastructure, an increase in the chance of possibility of approval by the FDA (Krouse, Gray, Macdonald, & Mccray, 2014), reduction in resources required for developing any given therapy (Naveja, Dueñas-gonzález, & Medina-franco, 2011) and minimization of the early clinical trials which includes information regarding side effects, pharmacokinetics and drug-drug interaction (Ishida et al., 2016). It is a promising approach that accelerates drug discovery process. Although many examples of drug repurposing are available for drugs, new therapeutic uses were discovered by serendipity rather than systematic approach (Naveja et al., 2011). As existing drugs are already established and toxicological and pharmacological data are known, repurposing these can be much cheaper and faster than traditional methods of finding and developing a new drug (Morgan, Campbell, Yu, Sponseller, & Muster, 2012). For the treatment of rare or orphan diseases which do not have any proven or established treatment yet, drug repurposing has become very useful (Ishida et al., 2016).

US National Institutes of Health (NIH) started to allocate funding for projects that focuses on drug repurposing with several goals which ranges from searching and identifying drugs to obtain smoking cessation, to drugs able to slow down Alzheimer's disease progression (Naveja et al., 2011). The idea of repurposing a drug produces a number of accomplished events where drugs are being used for a totally different purpose than they were previously intended to (Charbel et al., 2013). Cancer treatment is one of the common examples of drug repurposing. Numerous examples of such drugs used in drug repurposing are available. One most common example is Thalidomide, which was basically developed as a sedative in 1957 by a company Chemie Grunenthal in Germany. Initially, pregnant women were found to use it for managing their

morning sickness (Matthews & Mccoy, 2003). But later from the market, this drug was withdrawn as it caused polyneuritis and inhibited proper formation of human embryo. It also caused miscarriages and babies with deformed limbs. Approximately ten thousand children in fourty-six countries had been reported with birth defects because of the intake of thalidomide (Lenz, Knapp, & Clinic, 1962), but later anticancer indications were found which worked on prostate cancer and refractory multiple myeloma (Amato, Loughnan, Flynn, & Folkman, 1994). US-FDA approved thalidomide in 2006 for testing purpose to check the ability of treating multiple myeloma in combination with dexamethasone (Lenz et al., 1962).

Another example of repurposing is Sildenafil. Initially Sildenafil was identified for the treatment of angina pectoris, but failed in efficacy during clinical trial. In 1998, FDA gave sildenafil the approval for the treatment of erectile dysfunction, but later it was found that sildenafil targets phosphodiesterase type 5 (PDE5) which was highly expressed in both the penis and in lungs (Naveja et al., 2011). Again sildenafil was repurposed and got FDA approval for the management of pulmonary arterial hypertension due to its minimal toxicities and well-tolerance (Lee, Chiao, & Tsang, 2005). Thus, sildenafil was re-profiled twice. Ropinirole was identified as an antihypertensive agent but later marketed in 1997 for treating Parkinson's disease. Moreover, Galantamine was launched in 1960s for treating paralysis and as anesthesia but now approved in many countries for managing mild to moderate Alzheimer's disease (Ashburn & Thor, 2004). Pregabalin which has a chemical similarity with gabapentin is another example of drug repositioning. Originally it was intended to treat epileptic disorders, but later it was found that it can be useful as seizure medications, in anxiety problems and neuropathic disorders (Abagyan & Totrov, 2001). Since conventional approach of drug discovery and development is highly expensive and requires time, scientists and researchers are considering drug repurposing process

(Connor & Roth, 2005). Through drug repositioning process, the problems can be solved which are commonly faced during drug discovery (Schuster, Laggner, & Langer, 2005). Because of high cost and risks related with new drug molecule development, drug repurposing process is preferred by scientists (Cuatrecasas & Cuatrecasas, 2006). The ideas behind drug repositioning could be initiated from serendipitous observations, from novel, or from technology platforms used to determine repositioning opportunities. Repurposing process includes several steps such as idea validation and exploring which is just the beginning. Though repurposing is an economical process in comparison with de novo drug discovery process, there are some challenges associated with repurposing process. (Ashburn & Thor, 2004). Furthermore, drug repurposing is preferable and convenient due to some advantages which includes minimization of the uses of resources, lower cost and time saving (Siavelis, Bourdakou, Athanasiadis, Spyrou, & Nikita, 2015) compared to the conventional drug development process. This is because that target drug has been acknowledged in the society for several years and the pharmacological and toxicological data have already been established (Munos, 2009). Recently the aim of drug discovery is shifting from a single-target to a multitarget approach with the help of drug repurposing (Méndez-lucio, Naveja, & Vite-caritino, 2018).

1.3 *In Silico* Drug Designing

Recently an evolutional change in drug discovery process has been encountered due to the implementation of computational methods which enables to identify, design and develop new drug more rapidly and at convenient cost (Zoete, Grosdidier, & Michielin, 2009). Pharmaceutical industries currently find an interest in the improvement of computational models in order to predict target drug pharmacokinetics (Colmenarejo, n.d.). One of the most promising benefits of computational drug designing process is that it gives the opportunity to evaluate any published

data in a systematic way (Kotz & Editor, 2013). Implementation of computational approach in drug discovery and development process is achieving popularity and appreciation. There are different terms practiced in drug repurposing, one of which is *in silico* (Kapetanovic, 2008). *In silico* can be defined as performing functions with the help of computer or through computer stimulations. *In silico* has become more popular and useful process due to using software to identify, analyze biological and medical data and develop drugs with new indications (Ekins, Mestres, & Testa, 2007). Moreover, in order to develop and test pharmacology hypothesis, *in silico* methods have become more popular recently. *In silico* methods have modern high-performance computing system which include information regarding quantitative structure-activity relationships, pharmacophores, homology models, machine learning, data analyzing and data analysis tools. It also has advantages of discovering and optimizing old molecules in order to increase binding affinity to a specific target, to perform target-based screening and profiling, to improve ADME (absorption, distribution, metabolism and excretion), toxicological properties and physicochemical properties to anticipate biological activity (Ekins, Mestres, & Testa, 2007). For drug repurposing, *in silico* virtual screening (VS) approaches are widely used all over the world which are based on two major approaches: a) structure-based and b) ligand-based. Automated molecular docking is an important and unavoidable part of the computational biology or *in silico* process. By applying computer-based methods in drug discovery, virtual screening (VS) eliminates the drawbacks of traditional high-throughput screening (HTS) (Bielska et al., 2011). Virtual screening can be described by ranking and scoring molecules in order to their affinity for specific target in the large chemical libraries (Oprea & Matter, 2004). In virtual screening, docking process helps to analyze the orientation of molecules in order to predict the ligand-target binding affinity between the two molecules by using a scoring function (Naveja et

al., 2011). Since scoring functions are usually used to assume each type of interactions and tightness of interaction for estimating binding free energy, it is still considered as an optimal process for docking in *in silico* process. However, scoring function skips some critical criteria in binding affinity (Kapetanovic, 2008, Eldridge, Murray, Auton, Paolini, & Mee, 1997). *In silico* modeling use the help of high-performance computers in order to design and repurpose drugs since it requires less time and minimum resources. *In silico* methods are preferable than conventional drug discovery because of its promising advantages in chemical synthesis and biological testing. In the process of molecular docking, multiple ligand-protein binding is evaluated based on all the conformations and orientations in order to ensure the most stable complex (Kapetanovic, 2008). The data collected from the suitable orientation, ligand-protein binding affinity, tightness of interaction and binding free energy can be anticipated by *in silico* process (Eldridge et al., 1997).

1.3.1 Molecular Docking

A vast influence of computational technologies in identification and development of computer-aided drug design (CADD) has been observed in the field of drug repurposing method (Chen, 2011). Molecular docking is a technique which is defined as interaction of molecules with a receptor (Supriya, Shankar, Lalitha, Dastgiri, & Babu, 2017). The main objective of molecular docking is to evaluate the position, orientation and conformation of specific binding site of a small-molecule, ligand with target macromolecule. Along with that, docking provides the opportunity to understand the basic interactions between the ligand and its receptor. Normally docking process is a combination of both a search algorithm that used to evaluate several ligands (sometimes protein) conformations, and a scoring function for determining the true binding mode and affinities. Usually more than 30 different docking programs are used in computational

process (Zoete et al., 2009). There are two types of docking is available, rigid docking, where the molecules are rigid and ligand is binding in 3D space of protein by using scoring function and flexible docking, where molecules are flexible resulting a complex structure formation (Supriya et al., 2017). Different criteria are required for ligands such as flexibility, size, lipophilicity and hydrophilicity in order to evaluate ligand-protein interaction in several docking approaches. (Bursulaya, Totrov, Abagyan, & Iii, 2004). Docking algorithms are classified into two major classes according to the flexible ligand conformation approaches: a) algorithms that try to completely adjust the ligand into the protein's binding pocket by matching (geometrically, chemically, energetically etc.), and b) algorithms that create an optimal ligand conformation by fixing an energy optimization problem (Bursulaya et al., 2004). Docking approach helps in combining and screening databases of molecules and also gives information about 3D structures of target proteins which contributes a vital role in computational drug discovery (Xue & Bajorath, 2000). Docking methods have received full-size interest in latest years because of targeting those molecules for which experimentally established structures are not available. For example, by the help of docking process scientists and researchers are targeting molecules belonging to the superfamily of G-protein-coupled receptors (GPCRs) in order to find new therapeutic indications (Bissantz, Bernard, Hibert, & Rognan, 2003).

1.4 Angiotensin II (type 1) Receptor

The renin–angiotensin–aldosterone system (RAAS) is an enzymatic cascade which consists of enzymes, peptides and the receptors and is produced by the kidneys. RAAS has a vital role in homeostasis. The disturbance of RAAS results in the development of cardiovascular diseases (CVD) including hypertension, atherosclerosis and heart failure (Drapala, Sikora, & Ufnal, 2014). Renin–angiotensin–aldosterone system (RAAS) is responsible for producing angiotensin

II (Ang II) *in vivo* from angiotensin I with the help of angiotensin converting enzyme (ACE). Angiotensin II is chemically an octapeptide that has an important function in the pathophysiology of hypertension. Angiotensin II, also known as vasoactive hormone regulates the cardiovascular homeostasis by maintaining a balance between vascular resistance and blood volume (Cappelli, Mohr, Gallelli, Rizzo, Anzini, Vomero, Mennuni, Ferrari, Makovec, Menziani, Benedetti, et al., 2004). Angiotensin II activates some receptors including the G-protein-coupled angiotensin receptors which consist of seven transmembrane domains. Furthermore, G-protein-coupled angiotensin receptors are grouped into three subtype receptors: 1) angiotensin II receptor type 1 (AT1 or AGTR1) , angiotensin II receptor type 2 (AT2 or AGTR2) , and angiotensin II receptor type 4 (AT4) (Watts, Kanagy, & Lombard, n.d.). Usually two methods were followed to determine the angiotensin receptor subtypes. One of these method is binding affinities or antagonist specification method and another one is molecular cloning method (Wong, Ii, Ii, & Ang, 2016). Angiotensin II has a tendency to bind with both type I (AT1) and type II (AT2) receptor subtypes but AT1 receptor contains maximum tendency to regulate most of the cardiovascular functions along with controlling and maintaining oxidative stress, aldosterone secretion, vasoconstriction, renal sodium resorption, vasopressin release, cardiac and vascular cell hypertrophy, sympathetic stimulation, and cell proliferation (Nickenig, 2004a). Angiotensin II receptor type 1 was selected as our protein of choice because excessive activation of AT1R with the help of angiotensin II (Ang II) can cause cardiovascular disease and it is also responsible for the development of insulin based diabetes (Sanni, Lyngsø, Gammeltoft, & Hansen, n.d.). Research has showed that AT1 receptor antagonists has become the drug of choice for the patients who are suffering from hypertension, other cardiovascular disease and type 2 diabetes by controlling overexpression of AT1 receptor (Karamyan, 2011).

1.4.1 Structure of Angiotensin II Receptor Type 1 (AT1R Protein)

Angiotensin II receptor type 1 (AT1R) is an octapeptide receptor that belongs to class A GPCR, having a structural similarity with chemokine receptors and opioid receptors (Zhang, Unal, Gati, et al., 2015). Among all angiotensin II receptors, AT1R has sub divided into two classes AT1A and AT1B which are similar in structure and give similar binding affinities. As AT1 receptor is a G-protein coupled receptor, it consists of seven transmembrane α-helix and 359 amino acids (Watts et al., n.d.). AT1 receptor protein also includes an extracellular N-terminus, three extracellular loops (ECL), an intracellular C-terminus, three intracellular loops (ICL) and amphipathic helix in its structure. Two disulfide bonds are present in AT1R in order to shape the extracellular side of AT1R by linking the N-terminus with ECL3 and helix III and ECL2. There are three intracellular loops (ICL) in AT1R namely ICL1, ICL2 and ICL3 which help to connect helices and C-terminal (Zhang, Unal, Gati, et al., 2015). By using serial femtosecond crystallography and lipidic cubic phase crystallization methods, the crystal structure of AT1R was established. By studying the established crystal structure of AT1R, it is found that sodium binding pocket and the amino acids such as Asn111 and Asn295 in transmembrane domain III and VII of AT1R protein contribute to its receptor activation (Young, Nguyen, Chedrawe, Rainey, & Dupré, 2017).

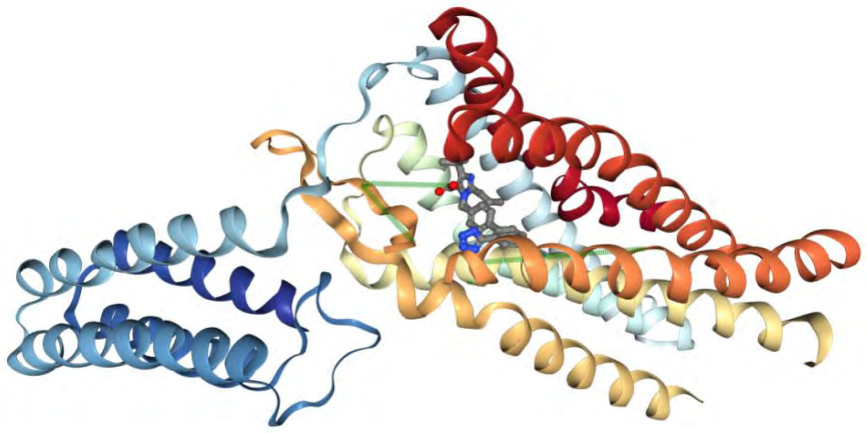


Figure 1: Crystal structure of angiotensin II receptor type 1 (AT1R) (Zhang, Unal, Desnoyer, et al., 2015).

1.4.2 Mechanism of Action of AT1R in Hypertension

In human, AT1 receptors are present in different parts of the body such as many glands, the endometrial blood vessels and pulmonary artery smooth muscle cells (De Gasparo, Catt, Inagami, Wright, & Unger, n.d., 2018). Angiotensin II activates AT1 receptor which is responsible for many cellular responses such as smooth muscle contraction, aldosterone secretion, neuronal activation, neurosecretion, DNA and protein synthesis, ion transport, and cell growth and proliferation. AT1 receptor is a G protein-coupled receptor (GPCR) which consists of seven hydrophobic transmembrane helices in the cell membrane (Cappelli, Mohr, Gallelli, Rizzo, Anzini, Vomero, Mennuni, Ferrari, Makovec, Menziani, De Benedetti, et al., 2004). AT1R receptor has an important role in vasoconstriction which results in hypertension or increased blood pressure along with other cardiovascular complications (Siragy, 2000). The mechanism of AT1R producing hypertension is shown in figure 2.

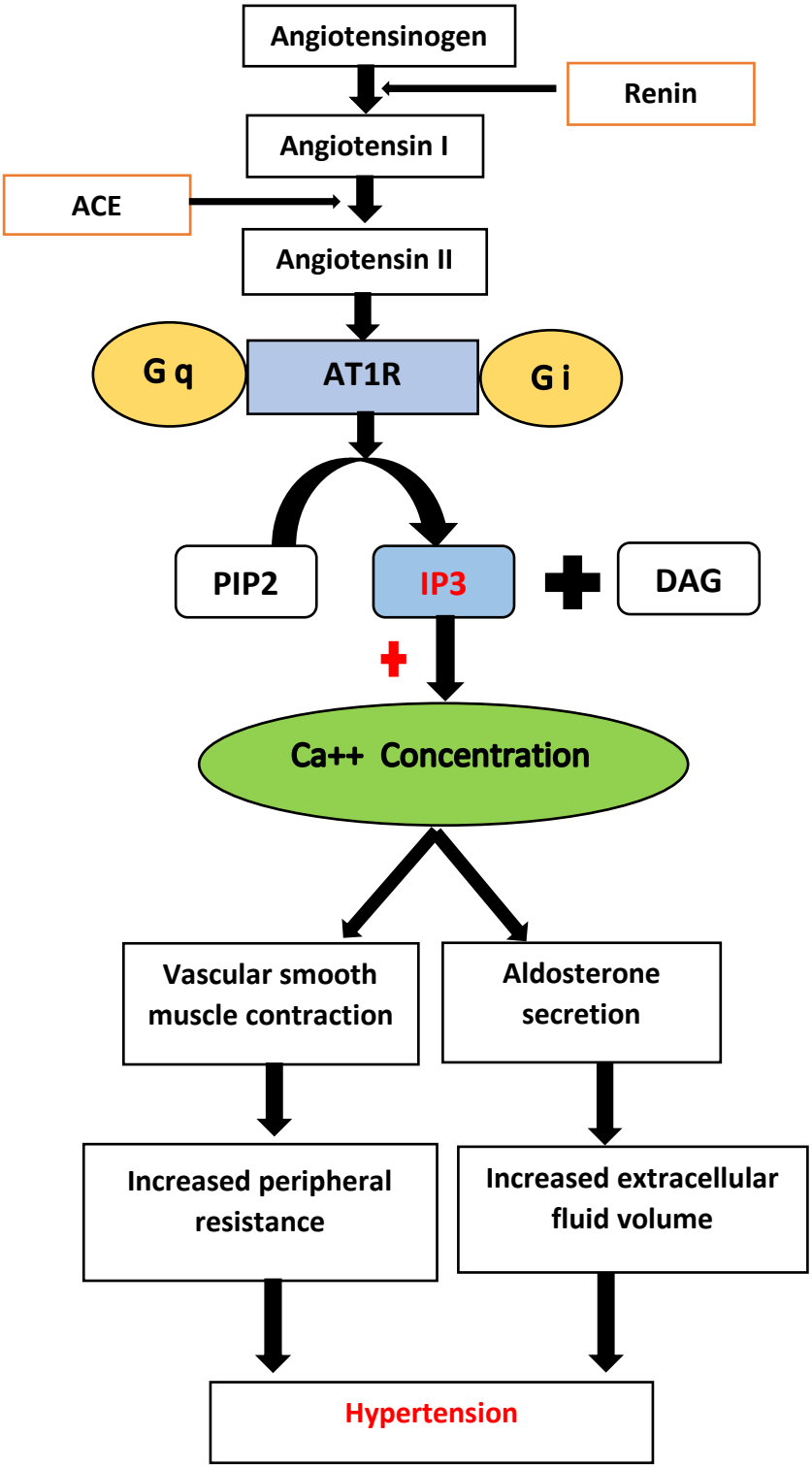


Figure 2: Mechanism of action of AT1R in human body (Barbosa-filho et al., 2006).

The mechanism of AT1R in producing hypertension (Figure 2) starts from the conversion of angiotensinogen into angiotensin I with the help of renin. When angiotensin I converts into

angiotensin II by angiotensin converting enzyme (ACE), it binds with AT1R receptor and results in AT1R activation. AT1R mainly coupled to G i and G q. The binding of angiotensin II with AT1R resulting in conformational changes in protein molecule. Activation of AT1R mediates signal transduction through several plasma membrane effector systems which include enzyme for instance phospholipase A2, phospholipase C, phospholipase D, adenylyl cyclase and ion channels (De Gasparo et al., n.d.). Moreover, AT1 receptor also uses Ca/IP3 pathway to mediate many cellular response such as vascular smooth muscle contraction, MAPK activities, and EGF receptor activation (Wong, 2015). When AT1R activates via Ang II, it produces primary signal transduction activities by activating adenylyl cyclase which controls intracellular cAMP levels and induces vasoconstriction. In other words, AT1 receptor has an important role in the activation of second messengers at the cellular level that includes inositol-1,4,5-triphosphate (IP 3) and diacylglycerol (DAG) which result in increasing Ca++ ion concentration inside the cell. The increase level of Ca++ ion causes vascular smooth muscle contraction followed by increasing peripheral resistance which results in hypertension. Moreover, aldosterone secretion also increases due to the increased level of Ca++ concentration followed by increasing extracellular fluid volume and results in hypertension (Barbosa-filho et al., 2006). In addition, cardiovascular diseases, hypertension and insulin resistance diabetes develop due to increased AT1 receptor activation by Angiotensin II (Sanni et al., n.d. 2017).

1.4.3 Functions of Angiotensin II Receptor Type 1 (AT1R)

Angiotensin II is a biologically active molecule of the renin-angiotensin system which is produced from Angiotensin I via angiotensin-converting enzyme (ACE) and plays a vital role in regulating blood pressure and electrolyte balance by binding with cell-surface receptors such as

AT1R and AT2R (Bergsma et al., 1992). Angiotensin II receptor type 1 (AT1R) is a G protein-coupled receptor (GPCR), which is located in heart, brain, liver, and kidneys is responsible for maintaining normal blood pressure and fluid and electrolyte homeostasis. Excess level of Angiotensin can cause overactivation of AT1R which can induce many diseases, for instance hypertension, cardiovascular hypertrophy, angina, myocardial infarction, stroke and insulin based diabetes (Zhang, Unal, Desnoyer, et al., 2015). As angiotensin II type 1 receptor is present in blood vessels, heart, kidney, adrenal glands, brain and pituitary, it mediates many physiological effects related to these organs. In blood vessels Ang II binds with AT1 receptors, which produce smooth muscle contraction, intimal hyperplasia and angiogenesis. Ang II is responsible for causing cellular proliferation after binding with AT1R which results in increasing cardiac contractility, myocardial hypertrophy, collagen synthesis and myocardial fibrosis. Moreover, Ang II stimulates AT1 receptors to increase the release of aldosterone from adrenal cortex which results in increasing rate of sodium and fluid regulation and increasing blood pressure. When angiotensin binds with AT1R in brain and pituitary, it commands posterior pituitary to release arginine vasopressin (antidiuretic hormone) which triggers the brain region that produce thirst. This results in hypertension and increased drinking behavior (Siragy, 2000). Furthermore, angiotensin II is a potent vasoconstrictor, which binds with AT1R in kidney. By binding AT1R, the efferent arteriolar constriction and glomerular filtration increase which results in increasing total peripheral resistance and blood pressure (Schmieder, 2005). Overexpression of AT1R can also cause excessive brain inflammation, autoimmune disorder and cognitive loss (Saavedra, 2012). Thus, AT1R is a good target for the treatment and drug development of hypertension and cardiovascular diseases. The alpha helices, beta sheets and the coils of AT1R are shown in Figure 3, where red portions represent alpha helices, yellow portions represent beta

sheets and green portions are coils. This is the results of protein folding and makes the structure of the protein stable.

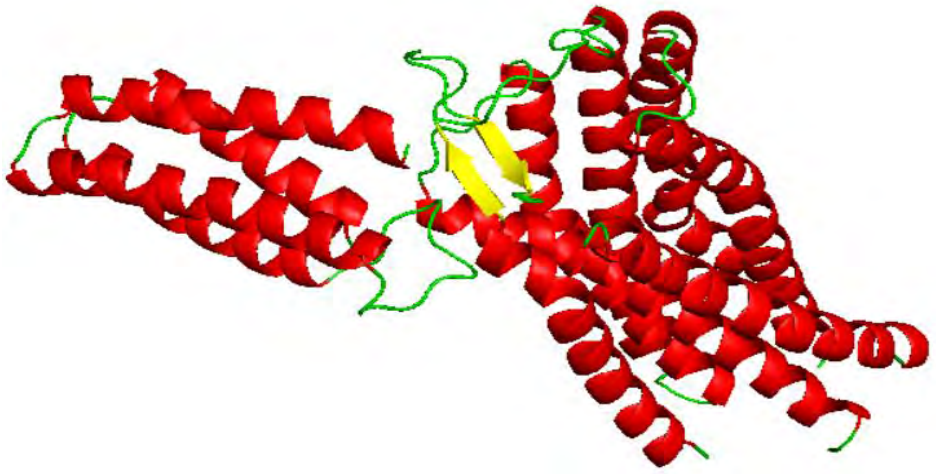
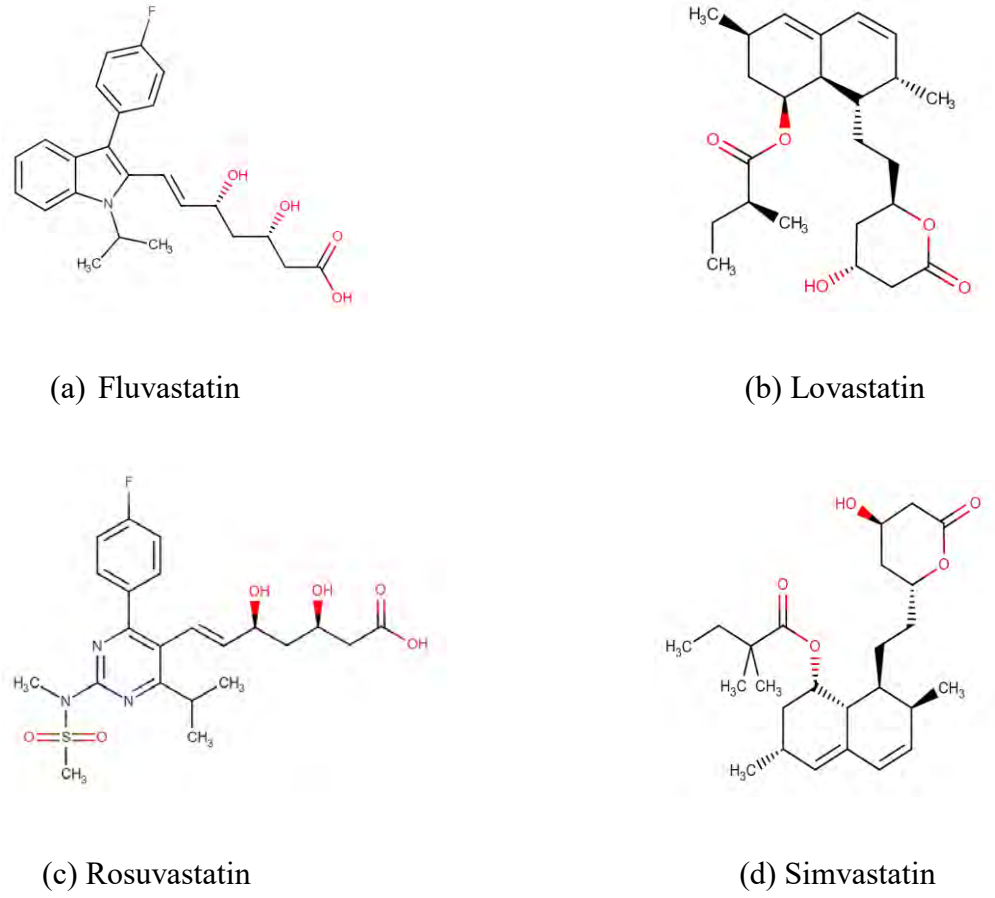


Figure 3: Structure of AT1R (obtained from PyMOL (version 1.8.4.0) (Seeliger & De Groot, 2010).

1.5 Statin Drugs and its Relation to Hypertension

Statin drugs are a class of drugs that originate from fungus. It was first isolated from the fungus *Penicillium citrinum* (Endo, 1988). Statin can be obtained either from fungus or synthetically. Example of fungal derived statins are lovastatin, simvastatin and pravastatin. atorvastatin, Cerivastatin, fluvastatin, pitavastatin and rosuvastatin are examples of synthetically derived statins (Shuhaili, Samsudin, Stanslas, Hasan, & Thambiah, 2017). Usually statins are used to inhibit the hydroxymethylglutaryl-CoA (HMG-CoA) which is a reductase enzyme and structurally similar to hydroxymethylgtutaryl coenzyme A. Statin drugs are used to treat hypercholesterolemia by inhibiting a major step of biosynthesis pathway of cholesterol (Istvan & Deisenhofer, 2001). Due to the good tolerance of statin drugs, these are known as powerful and efficient medication for preventing cardiovascular disease, atherosclerosis and reduction of plasma cholesterol levels (Stancu & Sima, 2001). Statins are used to decrease cellular cholesterol

level by hindering the HMG-CoA reductase enzyme to perform its function which result in reduced concentration of cholesterol in the liver. Moreover, this HMG-CoA inhibition in the liver increases the expression of LDL-receptors, which cause enhancement of the clearance rate of LDL-cholesterol from the blood circulation (Sirtori, 2014). Figure 4 gives the structures of a few statin drugs that are used to treat cardiovascular diseases and hypercholesteremia.



(a) Fluvastatin (b) Lovastatin

(c) Rosuvastatin (d) Simvastatin

Figure 4: Structures of some Statin Drugs (obtained from NCBI). (a) Structure of Fluvastatin, (b) Structure of Lovastatin, (c) Structure of Rosuvastatin and (d) Structure of simvastatin (Istvan & Deisenhofer, 2012).

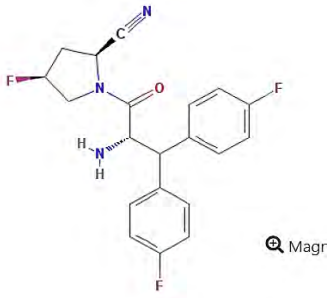
Statins have been widely reported to cause 30% reduction in major coronary events by the help of hypolipidemic therapy in hypercholesterolemic patients. Thus, without a doubt it is established that hypolipidemic therapy is safe enough to decrease morbidity and mortality. These

studies also claim that statin treatment could be considered as a secondary therapy for the patients with normal cholesterol levels who are suffering from myocardial infarction, hypertension and other cardiovascular disease (Nickenig, 2004). Scientists have proven that hypercholesterolemia is associated with high level of AT1 receptors expression by angiotensin II which increase the risk of angiotensin II-induced blood pressure in humans (Nickenig et al., 1999). The study aims to inhibit the excessive expression of AT1R protein in order to control high blood pressure.

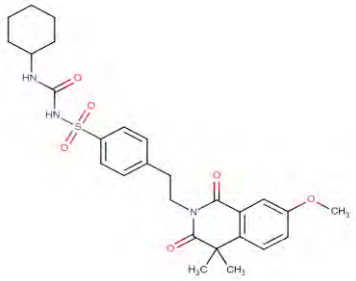
1.6 Anti-diabetic Drugs and its Relation to Hypertension

Diabetes has become an area of concern in the medical and research sectors as it is a chronic, progressive disease in which blood glucose levels remain higher than normal (Pullenayegum, Associate, Epidemiology, & Sherifali, 2010). There are two types of diabetes which include type 1 diabetes (autoimmune disease) and type 2 diabetes. Both type of diabetes requires antidiabetic medications to control blood glucose level in order to lead a healthy life. Recently a significant number of drugs are being used to treat hyperglycemia. There are five different types of oral antidiabetic drugs available in the market to treat type 2 diabetes (Dave P. Macfarlane, Paterson, & Fisher, 2007). Examples of some antidiabetic drugs are metformin, denagliptin, glipizide, teneligliptin, glisoxepide and sitagliptin (Jansen et al., 2014). Type 2 diabetes causes many cardiovascular disease, retinopathy and nephropathy. As chronic hyperglycemia is responsible for cardiovascular disease, it increases rate of morbidity and mortality (D P Macfarlane, Paterson, & Fisher, 2007). Patients with type II diabetes has a high tendency to develop arterial hypertension. Scientists claim that oral antidiabetic drugs can be used to treat hypertension. Sulfonylureas (e.g. glyburide), which is an antidiabetic drug has an effect in arterial pressure and metformin can be used to reduce arterial hypertension in humans (Peuler, Soltis, Grove, State, &

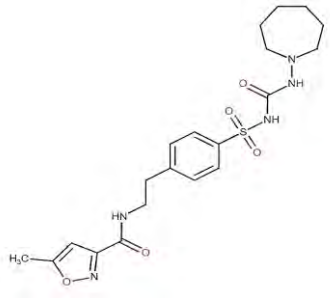
Grove, 1997). Figure 5 gives the structures of a few antidiabetic drugs that are used to treat diabetes.



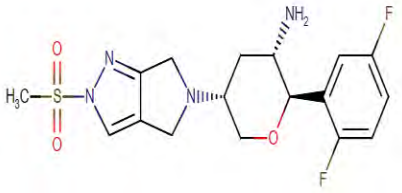
(a) Denagliptin



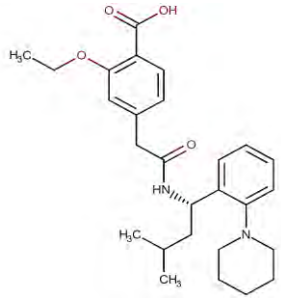
(b) Gliquidone



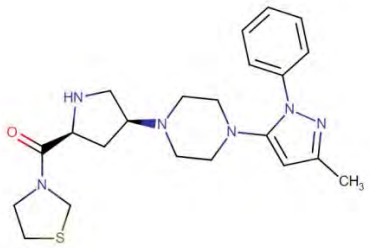
(c) Glisoxepide



(d) Omarigliptin



(e) Repaglinide



(f) Teneligliptin

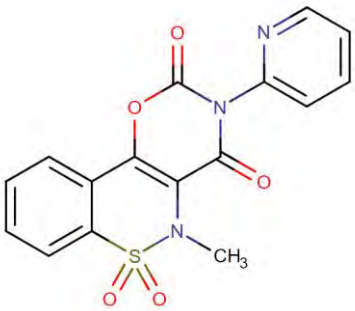
Figure 5: Structures of some Antidiabetic Drugs (obtained from NCBI). (a) Structure of Denagliptin, (b) Structure of Gliquidone, (c) Structure of Glisoxepide, (d) Structure of Omarigliptin, (e) Structure of Repaglinide and (f) Structure of Teneligliptin (Kushwah & Katti, 2015)

Scientists claim that insulin resistance and diabetes are associated with high level of AT1 receptors expression which is activated by angiotensin II and increase the risk of angiotensin II-induced blood pressure in humans (Sanni et al., n.d. 2018). In this study, a high rate of expression of AT1R protein was inhibited. Since antidiabetic agents have already shown some anti-hypertensive effect in some studies, they were chosen for this study.

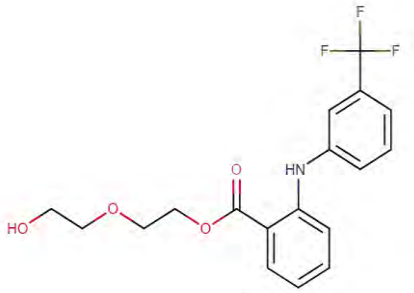
1.7 Anti-inflammatory Drugs and its Relation to Hypertension

Anti-inflammatory drugs are a class of drugs that are originated from certain plants which is usually used to treat inflammatory disease by providing relief from pain, fever and inflammation. Usually there are two classes of drugs used to treat inflammation which include steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAID). Example of some steroidal anti-inflammatory drugs are flumethasone and medrysone and some non-steroidal anti-inflammatory drugs are droxicam, etofenamate and balsalazide (Rainsford, 2007). There are two cyclo-oxygenase (COX) enzyme systems present in the human body which are COX-1 and COX-2. COX-1 involves the production of prostaglandin and thromboxane that are responsible for controlling gastrointestinal, renal, vascular functions whereas COX-2 involves the production of prostaglandin and is responsible for producing inflammation, pain and fever. NSAIDs are used to block the COX-2 pathway in order to treat inflammation and other inflammatory diseases (Vane & Botting, 1996). Some inflammatory diseases have shown a tendency to increase the chance of cardiovascular disease. Angiotensin II (Ang II) has an impact on the formation of inflammatory disease and is also known as a powerful proinflammatory mediator. Some research claim that inflammatory diseases are related with high level of AT1 receptors expression by angiotensin II and increase the risk of angiotensin II-induced blood pressure in humans (Chang

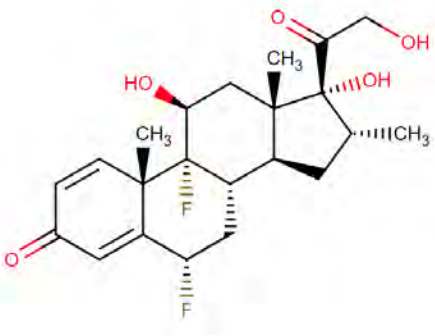
& Wei, 2015). Figure 6 gives the structures of a few anti-inflammatory drugs that are used to treat inflammatory diseases.



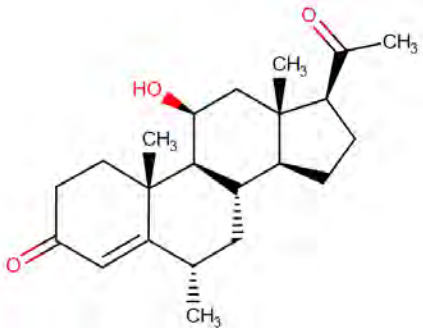
(a) Droxicam



(b) Etofenamate



(c) Flumethasone



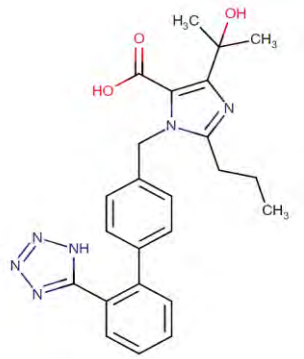
(d) Medrysone

Figure 6: Structures of some Anti-inflammatory Drugs (obtained from NCBI). (a) Structure of Droxicam, (b) Structure of Etofenamate, (c) Structure of Flumethasone and (d) Structure of Medrysone (Rome & Lands, 1975).

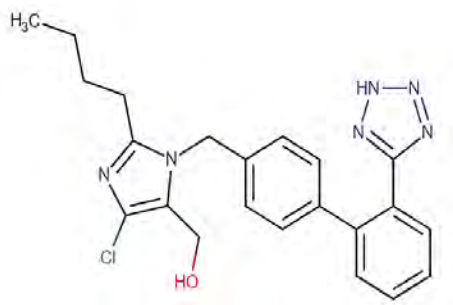
In this study, we are about to inhibit the high rate of expression of AT1R protein. As inflammation occurs due to the overexpression of AT1R which is also responsible for hypertension, we used some anti-inflammatory drugs in this study for the inhibition of AT1R.

1.8 Angiotensin Receptor Blockers and its relation to Hypertension

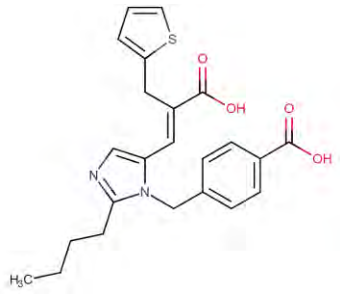
Angiotensin receptor blockers (ARBs) are a new class of drugs that are known as potential antihypertensive agent. ARBs are used to treat anxiety, depression, protect cerebral blood flow during stroke and other cardiovascular diseases such as heart attack, angina and hypertension. These drugs act by blocking the angiotensin ii type 1 receptor (AT1R). By blocking AT1R, these drugs mainly balance the level of angiotensin ii in the body which result in controlling many physiological effects such as maintaining blood pressure, salt and water balance and cardiovascular function and structure (Saavedra, 2012). This class of drugs are the selective blockers of AT1R. They are selectively blocking the effects of the renin angiotensin system which result in controlling the formation of angiotensin ii in the body and the cardiovascular and cardiorenal systems. Losartan, first drug belongs to ARBs class which is approved by FDA. Other examples of this class of drugs are valsartan, olmesartan, eprosartan and telmisartan (Miura, Karnik, & Saku, 2011). In this study, over expression of AT1R protein was inhibited. Since angiotensin receptor blockers (ARBs) have already used as anti-hypertensive drug, they were chosen as reference drugs for this study. Figure 7 gives the structures of few ARBs that are used to treat hypertension and cardiovascular diseases.



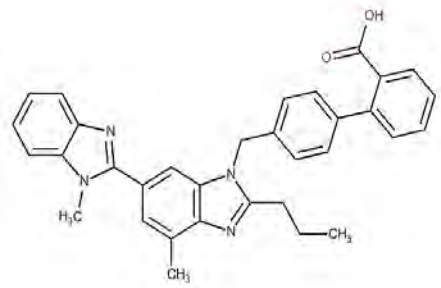
(a) Olmesartan



(b) Losartan



(c) Eprosartan



(d) Telmisartan

Figure 7: Structures of some Angiotensin Receptor Blockers (obtained from NCBI). (a) Structure of Olmesartan, (b) Structure of Losartan, (c) Structure of Eprosartan and (d) Structure of Telmisartan (Miura et al., 2011)

1.9 Rationale of the Study

Angiotensin II receptor type 1 is the protein that is considered a target for hypertension therapies because it is activated by angiotensin II enzyme and its overexpression can cause many other cardiovascular diseases (Zhang, Unal, Desnoyer, et al., 2015). A potential and promising therapeutic strategy can be used to control hypertension by inhibition of the enzymatic function of AT1R with different classes of drugs and small molecules (Egami, Murohara, et al., 2003). Hypertension has become a crucial and pliable risk factor for heart diseases such as heart attack, angina, stroke. Moreover, hypertension is considered a leading reason behind the mortality and morbidity caused by cardiovascular disease in the world (Tadevosyan, Maclaughlin, & Karamyan, 2011). Drug repurposing can be considered as an alternative to find effective medication for hypertension and cardiovascular disease. It has become a convenient method as it saves money, time and resources than conventional drug discovery process (Ashburn & Thor, 2004). In this study we used different classes of drugs including anti-diabetic, anti-inflammatory and statin drugs for drug repurposing purpose in order to find out a potential candidate (AT1R inhibitor) for the management of hypertension as well as cardiovascular diseases.

Chapter 2

Methodology

2.1 Aim of the Study

By using molecular docking and computational techniques, the antihypertensive activity of anti-diabetic drugs, statin drugs and anti-inflammatory drugs were determined in this *in silico* study.

The first part of the methodology involved past literature review on the specific topic and was done thoroughly. Then drugs were listed which included different classes of drugs, followed by establishing the binding affinities between receptors and ligands which were determined by conducting molecular docking. To complete computational docking, 3D structures of the macromolecule or protein and ligands or small molecules or drugs were important. Therefore, the aim was to find antihypertensive indications of anti-diabetic drugs, statin drugs and anti-inflammatory drugs by the inhibition of AT1R.

2.2 Software and Online Tools used for Molecular Docking

In this *in silico* study, different types of software and online tools such as PyMol, PyRx, Discovery Studio, OpenBabel and DrugBank were used. The list is given below in Table 1

Table 1: Software used for in silico study

Sl No.	Software and Online Tools Used	Versions	Reference
1.	PyRx	0.8	(Dallakyan, 2016)

2.	PyMol	1.8.4.0	(Seeliger & De Groot, 2010)
3.	DrugBank	5.1.1	(Wishart et al., 2018)
4.	Open Babel	2.4.0	(Boyle et al., 2011)
5.	Discovery Studio	17.2.0.16349	(Eid, Zalewski, Smieško, Ernst, & Vedani, 2013)

PyRx was used for the purpose of docking in order to determine the binding affinities of the drugs to the protein molecules. To visualize and validate the drugs and the protein, PyMol was used. The purpose of using DrugBank was to obtain the structure of drugs. OpenBabel was the software used for converting sdf files which were obtained from PubChem into pdb files. By using Discovery Studio, amino acids of the proteins with which the drugs bound were determined. Along with that software, some databases such as RCSB-PDB, PubChem, ProSa-web server and Rampage were also utilized to complete this *in silico* study.

2.3 Steps in Molecular Docking

In the process of molecular docking, several steps are involved which are illustrated with the help of a flowchart (Figure 8). This flowchart indicates the schematic steps such as obtaining protein and drug molecules, molecular docking, visualization and validation which were involved in molecular docking process.

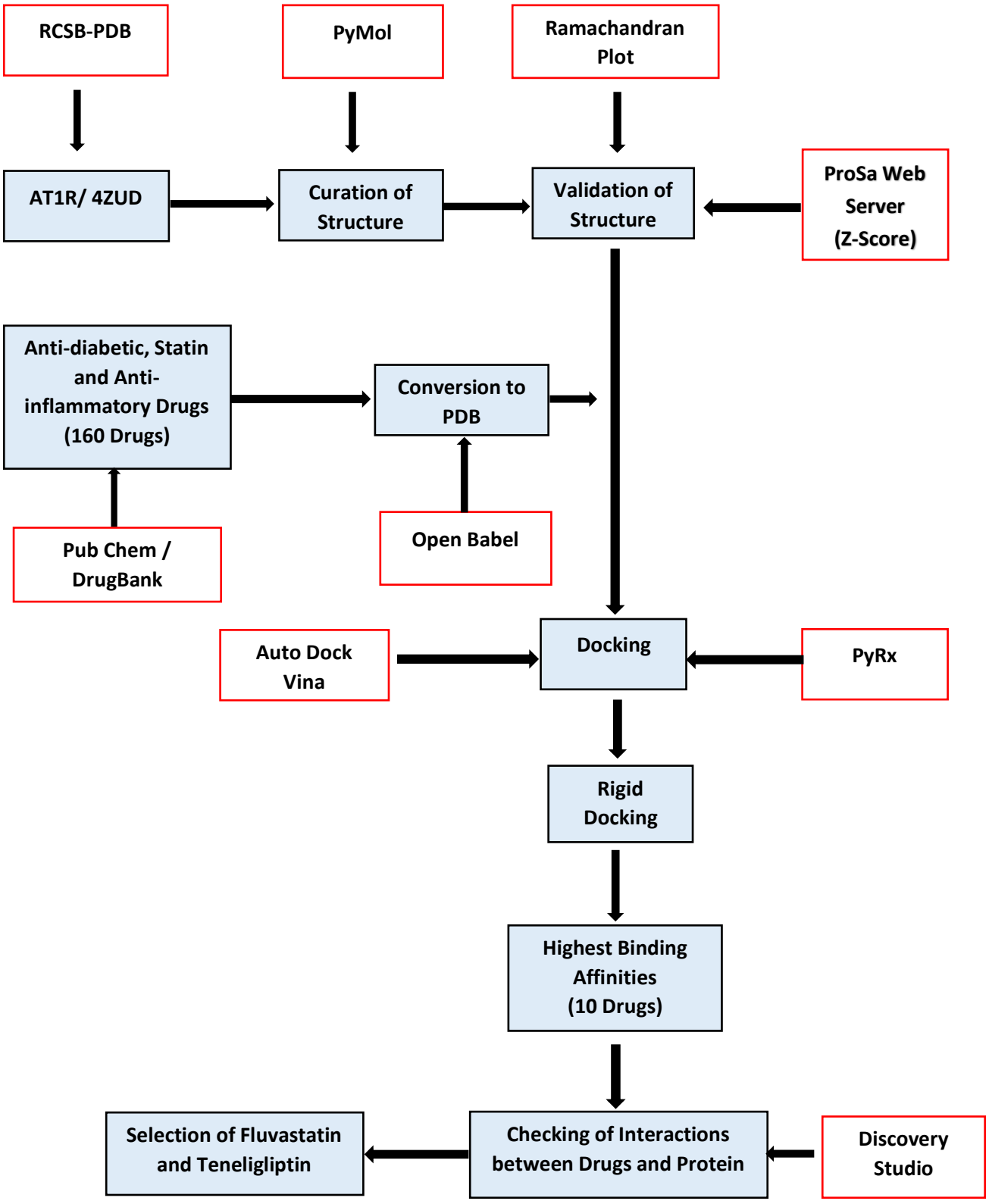


Figure 8: Flow Chart with the steps used in molecular docking.

2.3.1 Collecting Protein and Drugs Molecules

According to Figure 8. first the protein of our interest AT1R (4ZUD) was downloaded from RCSB PDB (Protein Data Bank) database. All the files present in the RCSB PDB database are pdb files of the proteins (Garg et al., 2016). In this databank, 3D structures of different macromolecules for instance proteins and nucleic acids are available. These macromolecules might be found in all living organisms ranging from humans to bacteria. The understanding of these macromolecules are important for drug development, human health and disease prevention (Bielska et al., 2011). Then, the protein structure was curated using the software, PyMoL for eliminating other hetero atoms (water molecules) to simplify the work. The protein molecule contained Olmesartan as ligands which were also deleted. PubChem and DrugBank were used to obtain files of anti-diabetic drugs, statin drugs and anti-inflammatory drugs. The drug files obtained from DrugBank were in pdb format and 3D structure which did not require any changes. But the drug files obtained from PubChem were in sdf format and 3D structure and they were required to convert. These sdf format files then converted to pdb format by using conversion software, OpenBabel.

2.3.2 Steps used in Molecular Docking

After collecting drugs and protein, molecular docking of all the drugs were completed using the software, PyRx. Two types of molecular docking include rigid docking and flexible docking. Only rigid docking was done for this *in silico* study. From the ligand preparation option of preferences, all the torsions were inactivated before conducting molecular docking. Then, the AT1R protein molecule and drug molecules were loaded in PyRx. The protein molecule was marked as macromolecule and the drug molecules were marked as ligands from the auto-dock

option. As the protein of interest consists of only one chain, no changes were required for the docking. Before conducting the auto-dock vina, the entire protein was covered by maximizing vina search space. Then the auto-dock vina was performed. Several results were obtained indicating binding affinities with a negative sign. This negative sign appeared because the reaction was an exothermic reaction. The more negative the value was, the greater was the binding affinity. Then the pdbqt files obtained from docking were saved.

2.3.3 Steps Involved in Validation and Visualization Process

There are several methods available for the validation process. In this process, validation was done by using PyMOL and discovery studio software. First, the saved pdbqt files of drug molecule which was obtained as a result of protein docking was opened in PyMOL. The curated protein file without ligand was also opened. Drug molecule and protein formed a complex and this complex was then saved. The drug-protein complex was opened in discovery studio. Then in a different window, protein structure without ligand was also opened. In the protein structure, hydrogens and the ligands were added followed by defining these ligands. Discovery studio provides some important information including the actual distance, amino acids, categories of the bond and types of bonding between the ligand and the protein. All the data obtained from discovery studio were documented and recorded. For some drugs screenshots were taken as well. Moreover, for the validation process, ProSa web server and Ramachandran Plot were used (Sheik, Sundararajan, Hussain, & Sekar, 2002). Ramachandran plot was used twice. One was before docking the drug and then again after docking the drug to analyze any changes in the psi and phi angles caused by the drug-protein binding.

Chapter 3

Results and Validation

3.1 *In Silico* Binding of AT1R with Anti-diabetic, Statin and Anti-inflammatory Drugs

PubChem and DrugBank.ca websites were used to download the small molecules or drugs which belong to anti-diabetic class of drugs, statin class of drugs and anti-inflammatory class of drugs. The drug molecules obtained from PubChem were in sdf format and 3D structure and they were converted to pdb format with the help of OpenBabel. Then PyRx was used for molecular docking. During molecular docking, AT1R protein was marked as the macromolecule and the different drugs were marked as ligands. Only rigid docking was performed and then based on binding affinities drugs were selected. The chances of drug-protein binding are increased with the exothermic binding affinity. Simvastatin and lovastatin showed great binding affinities with AT1R protein, but these two drugs were not selected as in 2016, these were withdrawn from the market ("Why FDA pulled cholesterol drugs off market | Formulary Watch," n.d.). The results of rigid docking are incorporated in Table 2. In rigid docking, both the protein and the drugs are of rigid in nature.

Table 2: Rigid docking results of different classes of Drug Molecules with AT1R using PyRx, Version 0.8 (Dallakyan & Olson, 2015).

Sl No.	Drugs	Rigid Docking Binding Affinities (kcal/mol)
1.	Gliquidone	-10.6
2.	Glisoxepide	-9.8
3.	Omarigliptin	-10.2
4.	Repaglinide	-10.4
5.	Teneligliptin	-10.9
6.	Fluvastatin	-11.0
7.	Lovastatin	-10.1
8.	Simvastatin	-10.3
9.	Droxicam	-9.8
10.	Etofenamate	-9.5
11.	Flumethasone	-10.5
12.	Medrysone	-10.7

Table 3: Rigid docking results of Angiotensin Receptor Blockers (ARBs) with AT1R using PyRx, Version 0.8 (Dallakyan & Olson, 2015).

Sl No.	Drugs	Rigid Docking Binding Affinities (kcal/mol)
1.	Eprosartan	-9.5
2.	Losartan	-11.5
3.	Olmesartan	-12.4
4.	Telmisartan	-11.6

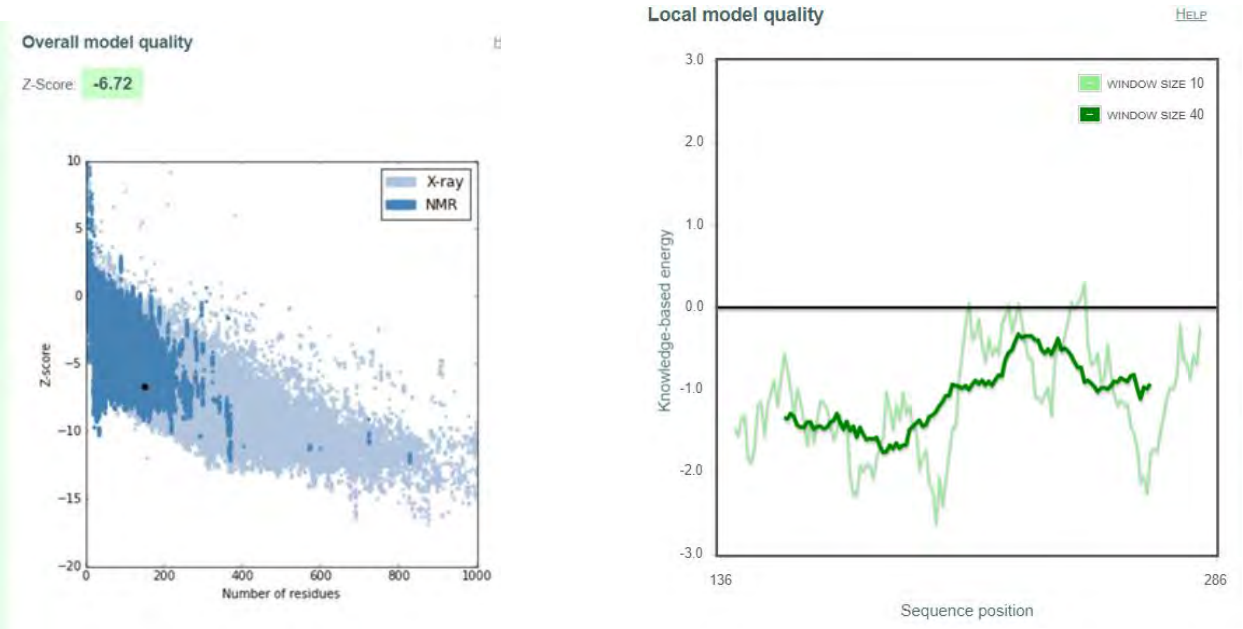
Angiotensin receptor blockers (ARBs) such as eprosartan, losartan, olmesartan and telmisartan were docked by using PyRx and the binding affinities were observed (Table 3). These were the potential drugs that are used for the treatment of hypertension and other cardiovascular diseases. Docking results of these angiotensin receptor blockers were used as a reference in this study in order to evaluate the result.

3.2 Validation

The results obtained in this *in silico* study required validation. The validation of obtained results were done by using ProSa web server, by analyzing the Ramachandran plot of the protein (without ligand) and the protein-drug complex, and by visualization using PyMol and Discovery Studio.

3.2.1 Validation of the Structure of AT1R

After downloaded the protein from RCSB-PDB, it was curated with the help of PyMol. Then the structure of AT1R was validated by using ProSa web server. Figure 9 showed the z-score of AT1R and also the residue score of AT1R.



(a) The z-score of AT1R (b) The plot of residue score of AT1R

Figure 9: (a) Shows the z-score of AT1R obtained from ProSA Web Server. (b) Shows the plot of residue score of AT1R. The z-score of AT1R is -6.72 (Wiederstein & Sippl, 2007).

Figure 9 showed the z-score of the protein which was -6.72. The higher the value is far from zero towards the negative, is considered as a better value. The residues were seen on the NMR region.

3.2.2 Visualization

Visualization is very important for this *in silico* study and it is done by using PyMol and Discovery Studio.

3.2.2.1 Visualization using PyMol

In this study, PyMol was used which involved visualization of the protein-drug complex. Olmesartan (OLM), ligand which was already attached with the protein, AT1R was used as a reference for those drugs or small molecules that were bound to the protein, AT1R. Figure 10 and figure 11 represented the superimposition of drugs with the olmesartan molecule. It also showed that these drugs and olmesartan were binding in the same binding pocket or not. Figure 10. and figure 11 also gave an idea about the orientation of drugs and Olmesartan. Here blue color drug represented the ligand that was already present in the protein and the red color of drug represented our selected drugs in this study.

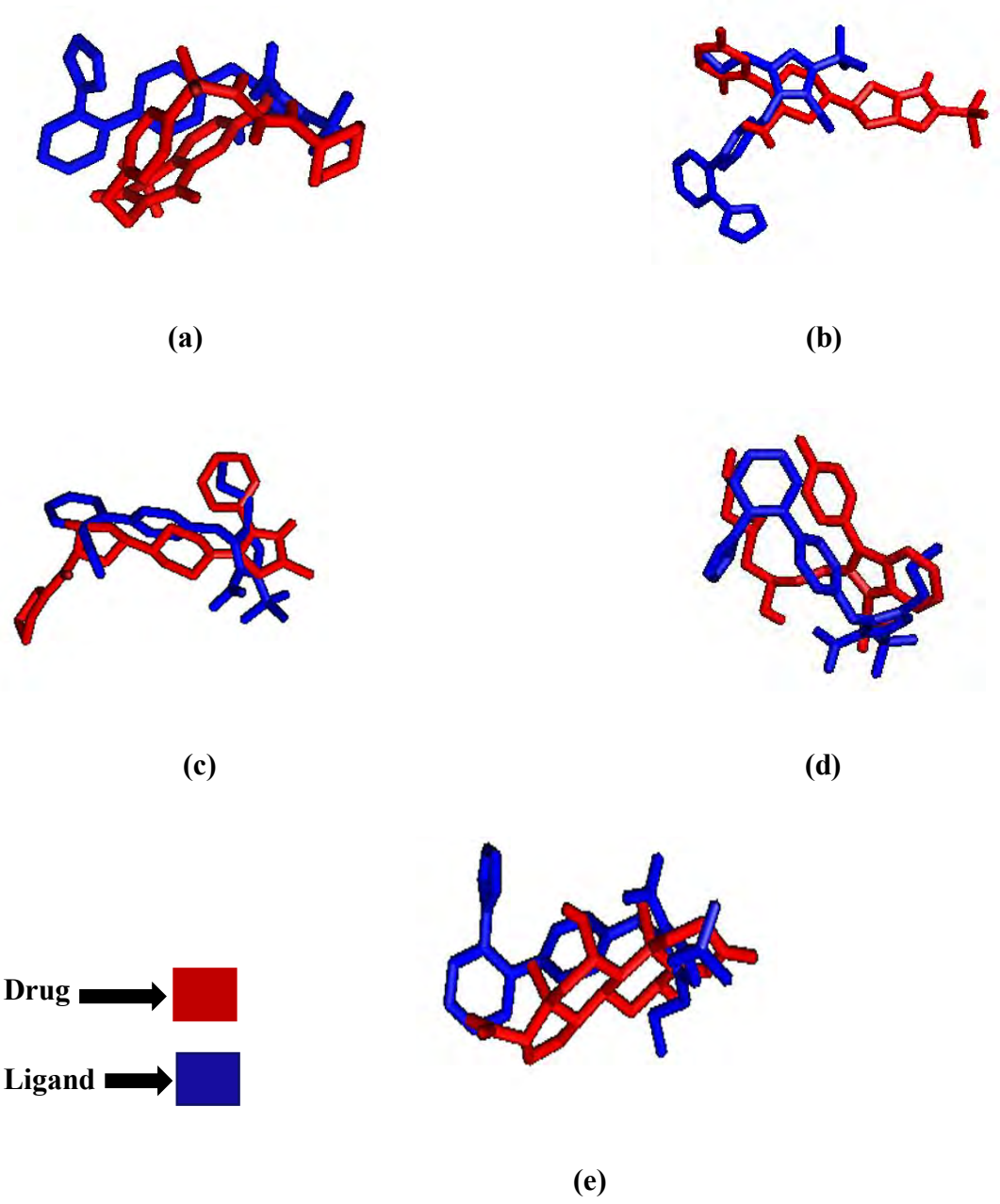


Figure 10: (a) Superimposition of Olmesartan with Gliquidone. (b) Superimposition of Olmesartan with Omarigliptin. (c) Superimposition of Olmesartan with Teneligliptin. (d) Superimposition of Olmesartan with Fluvastatin. (e) Superimposition of Olmesartan with Medrysone. Drugs bind with ligand, Olmesartan in the same binding pocket of protein, AT1R. Superimposition of Drugs and Olmesartan are in the same orientation. Red color represent the drugs and blue color represents ligand, olmesartan (visualization using PyMOL version 1.8.4.0) (Seeliger & De Groot, 2010).

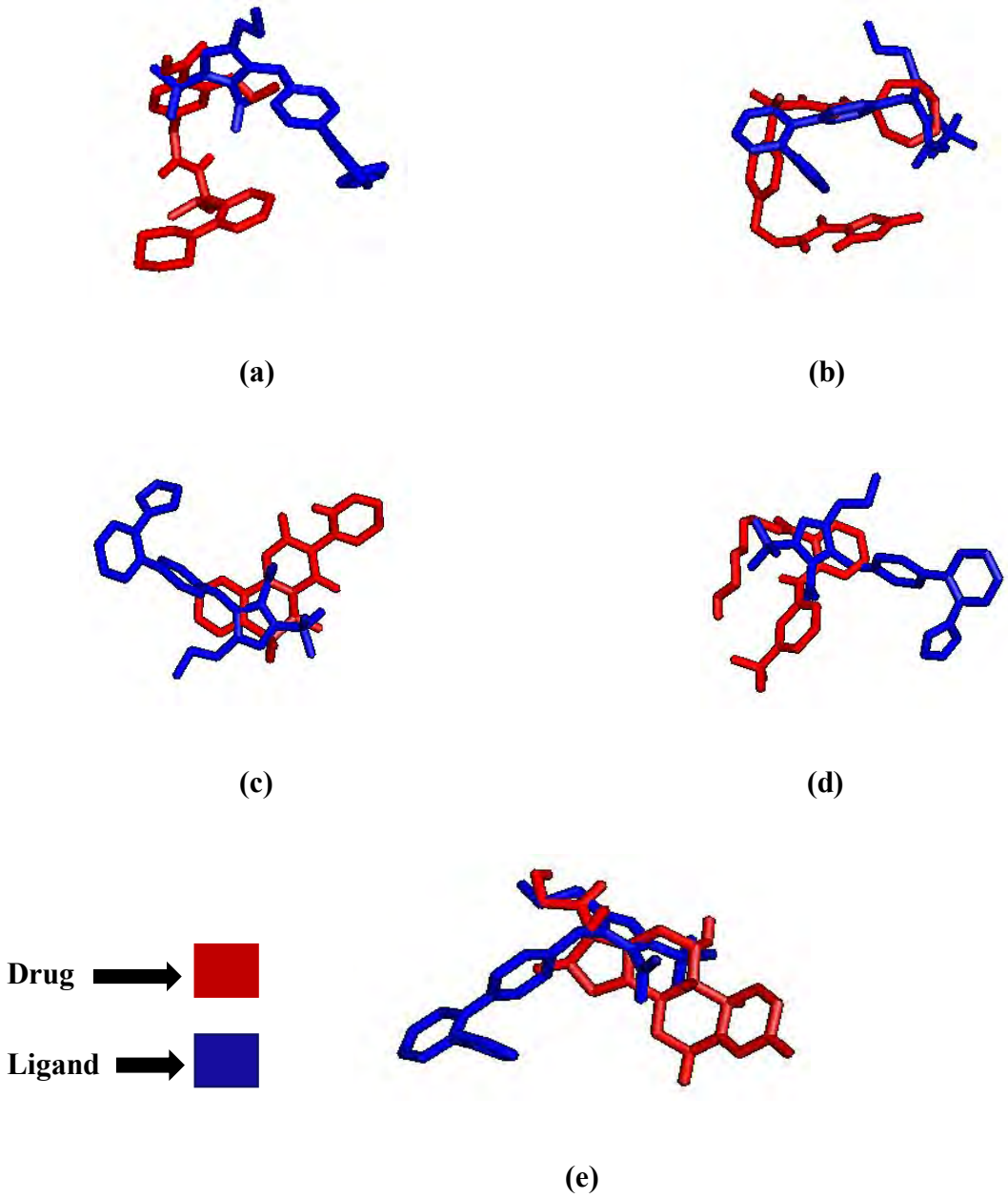


Figure 11: (a) Superimposition of Olmesartan with Repaglinide. (b) Superimposition of Olmesartan with Glisoxepide. (c) Superimposition of Olmesartan with Droxicam. (d) Superimposition of Olmesartan with Etofenamate. (e) Superimposition of Olmesartan with Flumethasone. Drugs bind with ligand, Olmesartan in the same binding pocket of protein, AT1R. Superimposition of Drugs and Olmesartan are in the opposite orientation. Red color represent the drugs and blue color represents ligand, olmesartan (visualization using PyMOL version 1.8.4.0) (Seeliger & De Groot, 2010)

3.2.2.2 Visualization using Discovery Studio

Discovery Studio is a software used for visualization in this study. It helped to collect information about the interactions between protein and the ligands. Discovery studio also provided ideas about the types of bond, categories and distance between drug molecules and protein. It also gave ideas about the amino acids with which the bonds were formed. The pdb files of drug-protein complex were opened in discovery studio and then all the interactions of drugs with the amino acids of protein were observed. The interactions of most drugs were observed with the amino acids lining the binding pocket of the protein.

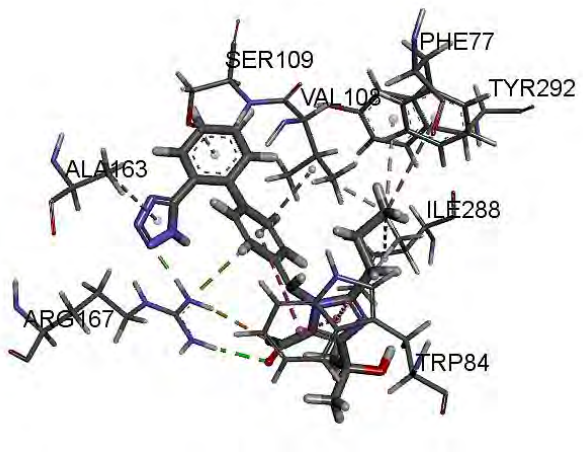
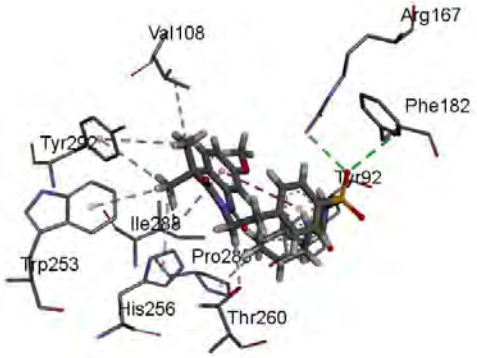


Figure 12: Non-bond Interactions of Olmesartan Ligand with AT1R (obtained from Discovery Studio) (Eid et al., 2013)

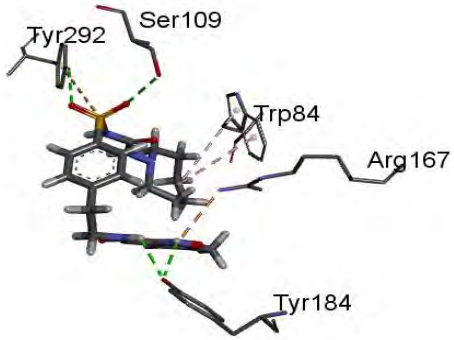
Figure 12 showed the amino acids of protein, AT1R with which the ligand, Olmesartan was bound. These amino acids were ARG167 (aa Arginine), ALA163 (aa Alanine), SER109 (aa Serine), VAL108 (aa Valine), PHE77 (aa Phenylalanine), TYR292 (aa Tyrosine), ILE288 (aa Isoleucine), TRP84 (aa Tryptophan) which was used in validation.

3.2.2.2.1 *In Silico* Binding of AT1R with Drugs

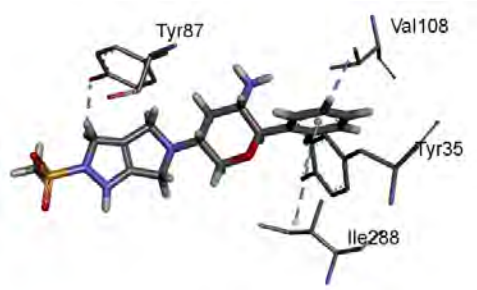
Discovery Studio was used to see the interaction between drugs and AT1R. In figure 13 the interactions between amino acids of protein, AT1R and ten drugs from three different classes such as gliquidone, glisoxepide, omarigliptin, repaglinide, teneligliptin, fluvastatin, droxicam, etofenamate, flumethasone and medrysone were observed.



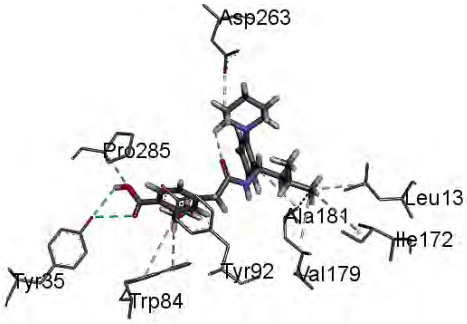
(a)



(b)



(c)



(d)

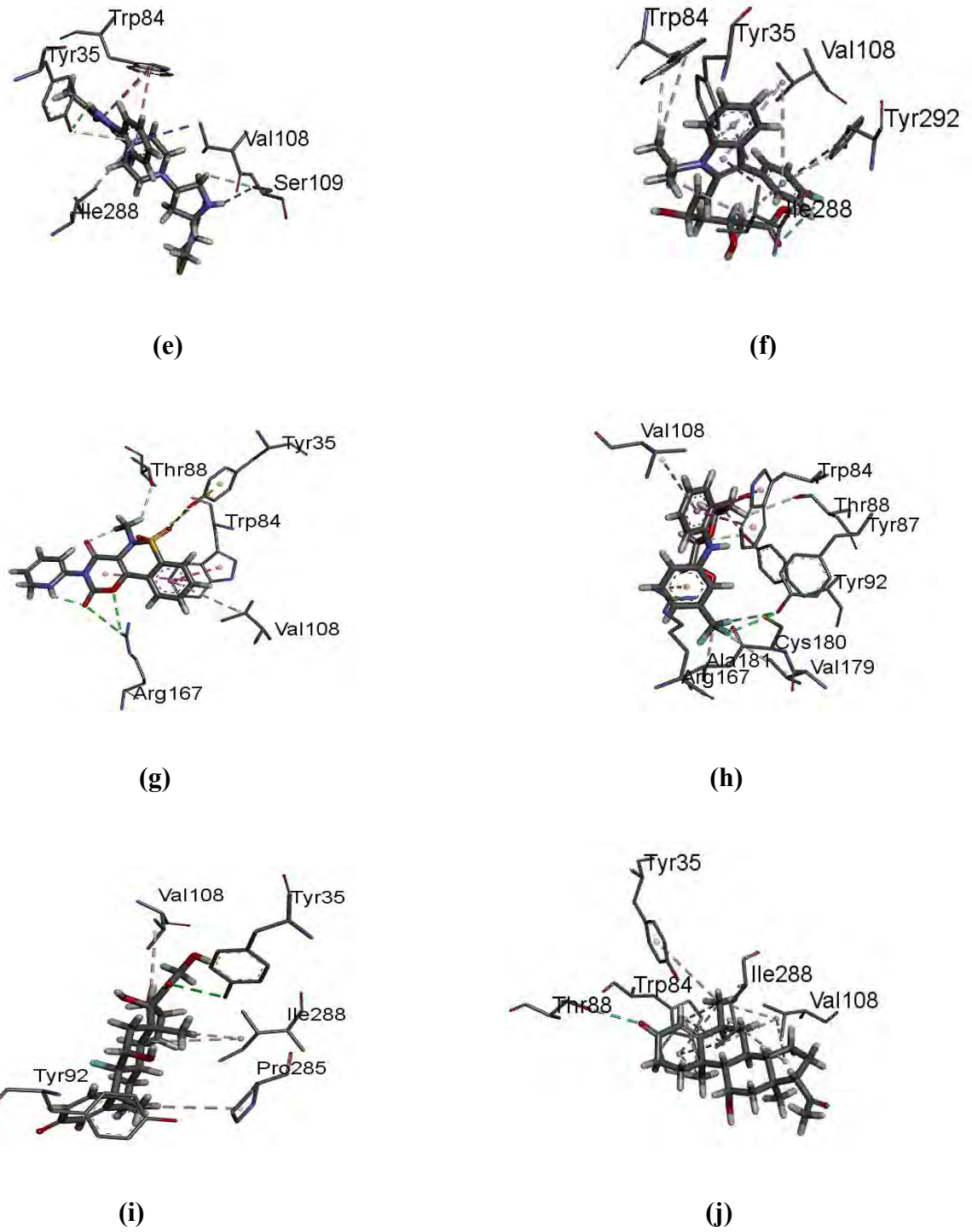


Figure 13: Non-bond Interactions of AT1R with (a) Gliquidone, (b) Glisoxepide, (c) Omarigliptin, (d) Repaglinide, (e) Teneligliptin, (f) Fluvastatin, (g) Droxicam, (h) Etofenamate, (i) Flumethasone and (j) Medrysone (obtained from Discovery Studio) (Eid et al., 2013)

3.2.3 Validation of Protein using Ramachandran Plot (without Ligand)

The Ramachandran plot used to assume the conformation of the backbone of a given polypeptide chain more quantitatively starting from knowledge of its amino acid sequences (Kertz, 2011). The Ramachandran plot helped to obtain some information which includes number of residues in favored region, in allowed region and in outlier region.

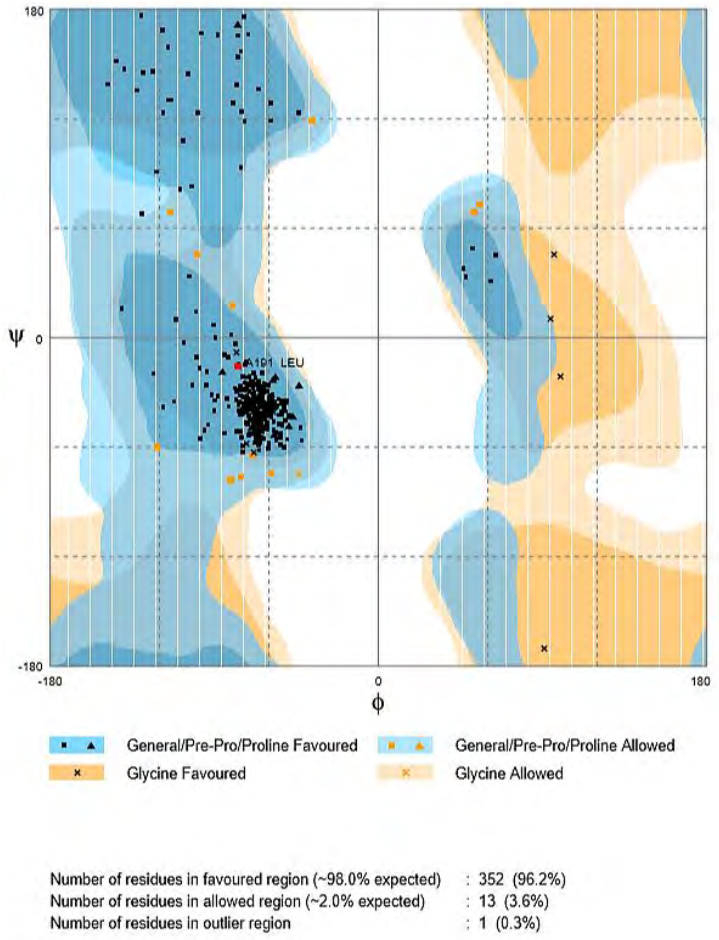
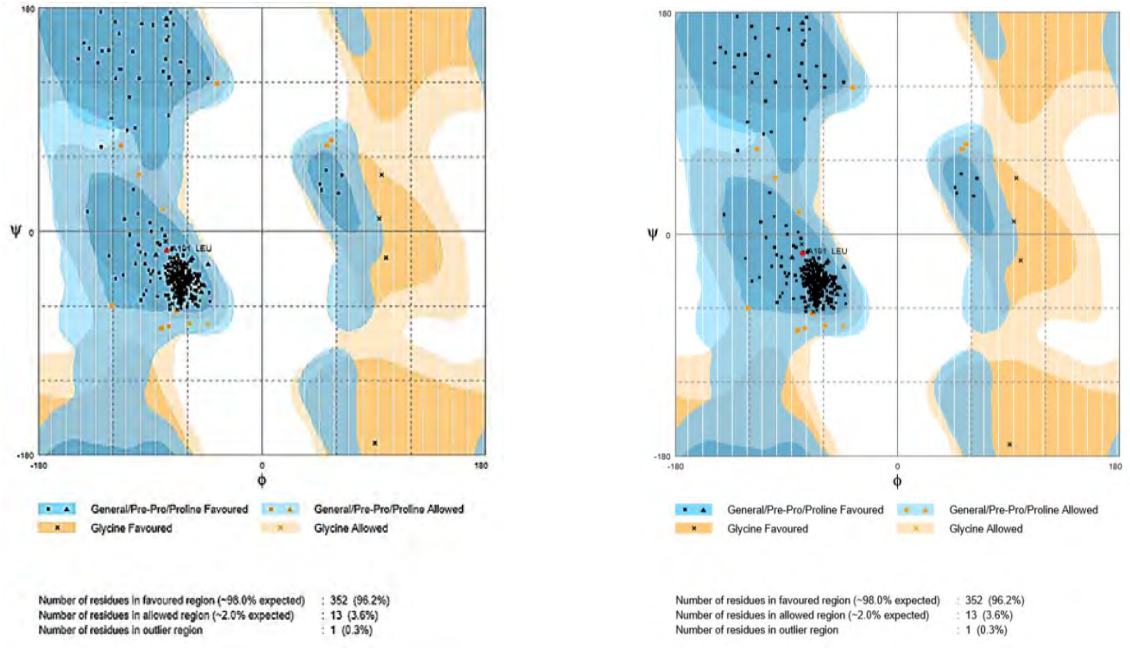


Figure 14: Ramachandran Plot of Human AT1R without Ligand where number of residues in favored region (~98.0% expected): 352(96.2%). Number of residues in allowed region (~2.0% expected): 13(3.6%). Number of residues in outlier region 1(0.3%) (Kertz, 2011).

3.2.3 Validation of Protein using Ramachandran Plot (with Ligand)

Ramachandran Plot was again done for potential drugs such as Fluvastatin and Teneligliptin with AT1R (figure 15).



(a) Fluvastatin

(b) Teneligliptin

Figure 15: Ramachandran Plot of Human Protein, AT1R bound to (a) Fluvastatin and (b) Teneligliptin (obtained from Rampage) where number of residues in favored region (~98.0% expected): 352(96.2%).Number of residues in allowed region (~2.0% expected): 13(3.6%). Number of residues in outlier region 1(0.3%) (Kertz, 2011).

Chapter 4

Discussion

Hypertension has become an important medical and public health issue which is responsible for many cardiovascular diseases such as heart attack, stroke, myocardial infarction and kidney disease. Hypertension also causes medical complications in about 5-10% of pregnancies and is ranked as the second leading cause of maternal death during pregnancy specially in the developed world (Kattah & Garovic, 2013). Renin angiotensin II enzymes plays an important role in activating the G-protein-coupled angiotensin receptors such as AT1R which consist of seven transmembrane domains. AT1 receptor shows high tendency in regulating most of the cardiovascular functions along with aldosterone secretion, vasoconstriction and renal sodium resorption etc. (Miura et al., 2011). After activation of AT1R with the help of angiotensin ii, it produces hypertension by following some biosynthetic pathways such as Ca/IP3 pathway and calcium channel opening (De Gasparo et al., n.d.). Moreover, activation of AT1R shows a significant effect in the pathogenesis of human type 2 diabetes, tumor-related angiogenesis and growth (Egami, Matsuishi, et al., 2003). For establishing cancer therapeutics, a better understanding of the function of AT1R in producing hypertension is important.

For this study, AT1R, a protein which is responsible for producing hypertension was targeted. AT1R protein was downloaded from RCSB PDB (Protein Data Bank) database. Then docking was performed after removing the heteroatoms such as water and Olmesartan. The protein was visualized by PyMol and Discovery Studio and validated by using ProSa web server and Ramachandran Plot. Generally rigid docking produced better results compared to flexible docking because in rigid docking both the protein and drugs are remain rigid and the drug can

easily bind in the protein pocket. Only rigid docking was carried out in this study. In the present study, high binding affinities of drugs with AT1R helped in the choice of the anti-diabetic, statin and anti-inflammatory drugs. A high binding affinity gave an idea about a link between these three classes of drugs and AT1R, but the extent was still unknown. Based on binding affinity ten drugs were selected for further study. Moreover, Angiotensin receptor blockers (ARBs) such as eprosartan, losartan, olmesartan and telmisartan were also docked by using PyRx and high binding affinities were obtained. The binding affinities of these angiotensin receptor blockers were used as a reference and helped to compare the binding affinities of selected three classes of drugs in this study.

Fluvastatin had a highest binding affinity of -11.0 kcal/mol and teneligliptin had second highest binding affinity of -10.9 kcal/mol among these ten drugs. The affinities were close to established antihypertensive drugs such as losartan (-11.5 kcal/mol) and higher than eprosartan (-9.5 kcal/mol).

To analyze the link between drugs and protein more accurately, non-bonded interactions, bond distances and type of bonds were evaluated in Discovery studio. Discovery studio was used as a validation tool which helps to visualize the drug-protein complex more precisely. It also gave more distinct ideas about the interaction of the protein with the ligand. With AT1R, drugs such as gliquidone, glisoxepide, omarigliptin, repaglinide, teneligliptin, fluvastatin, lovastatin, simvastatin, droxicam, etofenamate, flumethasone and medrysone were observed to interact with different amino acids of AT1R. Among these twelve drugs from three different classes, ten were selected for further study as lovastatin and simvastatin were withdrawn from market ("Why FDA pulled cholesterol drugs off market | Formulary Watch," n.d.). The bonds that were formed between drugs and protein include hydrogen bond and hydrophobic bond, halogen bond and

electrostatic bond. Moreover, hydrogen bond included conventional hydrogen bond, pi-donor hydrogen bond and carbon hydrogen bond. And hydrophobic bonds included pi-alkyl bonds, pi-pi T-shaped bond, alkyl bond, pi-sigma bond, pi-pi stacked bond, pi-cation bond and pi-sulfur bond. All the hydrogen bonds ranged from 2-4 Å but those hydrogen bonds ranged from 2-3 Å were considered to be decent (Langkilde et al., 2008). Hydrophobic bonds help in enhancing the binding affinity (Onofrio et al., 2014).

With AT1R, fluvastatin was observed to interact with amino acids such as TYR35 (aa Tyrosine), TYR292 (aa Tyrosine), ILE288 (aa Isoleucine), TRP84 (aa Tryptophan) and VAL108 (aa Valine). Most of the bonds observed were hydrogen bonds, hydrophobic bonds and halogen bonds. Hydrogen bonds included pi-donor hydrogen bond, and hydrophobic bonds included halogen (fluorine) bond, pi-sigma, pi-pi T-shaped, alkyl and pi-alkyl bonds. Hydrophilic bonds help to increase the binding affinity of fluvastatin.

Several bonds were formed between teneligliptin and AT1R. Between teneligliptin and AT1R, five hydrogen bonds and five hydrophobic bonds were formed. The amino acids involved in the hydrogen bonds between teneligliptin and AT1R were TYR35 (aa Tyrosine) and SER109 (aa Serine). Amino acids involved in the hydrophobic bonds between teneligliptin and AT1R were VAL108 (aa Valine), TRP84 (aa Tryptophan) and ILE288 (aa Isoleucine). The types of hydrogen bonds include conventional hydrogen bond and carbon hydrogen bond. The types of hydrophobic bonds include pi-donor hydrogen bond, pi-sigma, pi-pi shaped, pi-pi stacked and pi-alkyl bonds. Some of the hydrogen bonds formed between teneligliptin and AT1R were ranged from 2-3 Å which was considered to be decent.

The protein was validated using Ramachandran plot and ProSa web server. The obtained z-score was -6.72. The greater the value is far from zero towards the negative, is considered as a better

value. The residues were seen on the NMR region but it did not fall within the X ray region. The Ramachandran plot was used to determine the conformation of the backbone of a given polypeptide chain more quantitatively starting from knowledge of its amino acid sequences (Kertz, 2011). With the help of Ramachandran plot, it was possible to obtain some important information which included number of residues in favored region, in allowed region and in outlier region. 98% of residues in favored region the expected but a value over 90% was considered to be acceptable. For residues in allowed region, 2% was the expected value and a deviation of ±5% is acceptable. The expected value of residues in outlier region should be as less as possible (Kertz, 2011). Figure 15 represented the number of amino acid residues are seen in the favored region is 96.2% and the number of residues is seen in the allowed region is 3.6% and the number of residues in the outlier region is 0.3%. Thus, no significant changes were observed, which mean no serious changes have been occurred in the conformation of psi and phi bonds after binding of drugs with AT1R and the amino acids of the protein were at the place where they were supposed to be. Moreover, fluvastatin could be beneficial in the management of hypertension (Horiuchi, Cui, Li, & Li, 2003) and teneligliptin also shown effective in the treatment of cardiovascular diseases (Homma et al., 2017). Thus, in this study we are predicting that these two drugs, fluvastatin and teneligliptin could be potential AT1R inhibitors.

Chapter 5

Conclusion

The *in silico* study showed that anti-diabetic drugs, statin drugs and anti-inflammatory drugs could be effective inhibitors of AT1R and suggests that these drugs may play a significant role in inhibition of the protein, AT1R. All the data such as binding affinities, non-bond interactions help to predict that these classes of drugs could be effective on hypertension and also in cardiovascular diseases.

Chapter 6

Future Work

No *in vitro* and *in vivo* studies have been performed yet. Further studies and *in vitro* tests could be performed to confirm the potential interactions of ligands with AT1R. If the results of *in vitro* tests are satisfactory, the drug could be considered effective as a potent antagonist of AT1R. Then *in vivo* tests could be performed to ensure that these drugs are safe and clinically useful as anti-hypertensive drugs for the management of hypertension and cardiovascular diseases. Thus, it could provide a direction to repurpose similar types of drugs in order to find antihypertensive activities in future.

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