EXPLORING ISCHEMIC STROKE: "TARGETING PROTEINS MODULATORS INVOLVED IN STROKE BY STREPTOMYCES COMPOUNDS USING MOLECULAR DOCKING STUDIES"

(PROJECT-REPORT)

Submitted in partial fulfilment of the requirements for the award of Master of Science in Medical Biotechnology and clinical research

By

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(DEEMED TO BE UNIVERSITY)

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Sandhiya (41101010)** who carried out the final year project entitled ***"Exploring Ischemic Stroke: Targeting proteins modulators involved in Stroke by streptomyces compounds using molecular docking studies"*** under our supervision from December 2022 to May 2023.

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DECLARATION

I, **SANDHIYA R (41101010),** hereby declare that the Project Report entitled "***Exploring Ischemic Stroke: Targeting proteins modulators involved in Stroke by streptomyces compounds using molecular docking studies***" done by me under the guidance of **Dr. R RAJESH KANNA** at **Sathyabama Institute of Science and Technology, Chennai** is submitted in partial fulfilment of the requirements for the award of Master of Science in Medical Biotechnology and clinical research.

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ABSTRACT

Ischemic stroke is a type of stroke that occurs when there is a blockage or obstruction in a blood vessel that supplies blood to the brain, resulting in a lack of blood flow and oxygen to brain cells. This can lead to brain damage and can have serious, long-lasting effects on a person's cognitive and physical abilities. The blockage can be caused by a blood clot or a buildup of fatty deposits and cholesterol in the blood vessels. Ischemic strokes are the most common type of stroke, accounting for approximately 87% of all strokes. It is a major cause of disability and mortality, and current treatments have limited effectiveness. Four Ischemic stroke targets (COX1, ACE-2, PDE3 & α2AP) were selected and docked with 3173 compounds of streptomeDB (which follows Lipinski rule of five), and the top 4 compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone) commonly interacting with the four targets shows a high binding affinity with the target on the active site. The COX1 shows interaction with (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone), ACE-2 shows interaction with (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone), PDE3 shows interaction with (D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone) & α 2AP shows the interaction with (D-Olivosyl-jadomycin-Gly & Gutolactone). This may be considered a piece of evidence indicating the interaction of the lead compounds with that of the target. The identified lead molecules can serve as starting points for the development of novel therapies for ischemic stroke.

Keywords: Natural compounds, Stroke, Cardiovascular diseases, Targets.

TABLE OF CONTENTS

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	i
	LIST OF TABLE AND FIGURE	iv
1	**INTRODUCTION**	1
	1.1 Acute Management of Stroke Initial Treatment	2
	1.2 Thrombolytic Therapy	4
	1.2.1 Role of Anticoagulants	5
	1.2.2 Anticoagulation for Acute Ischemic Stroke	5
	1.3 Direct thrombin inhibitors and Factor Xa inhibitors	7
	1.3.1 Direct thrombin inhibitors	8
	1.3.2 Factor Xa inhibitors	9
	1.4 Haemorrhages	10
	1.5 Ischemic Stroke and Neurologic Deficits	10
	1.6 Potential drug targets	11
	1.6.1 COX-1 (Cyclooxygenase-1)	12
	1.6.2 ACE2 (Angiotensin I converting enzyme 2)	12
	1.6.3 PDE3 (Phosphodiesterase3)	12
	1.6.4 Alpha 2 Antiplasmin	13
	1.6.5 PDE5A (cGMP-specific phosphodiesterase 5A)	13

	1.6.6 PPARγ (Peroxisome proliferator-activated receptors)	13
	1.6.7 P53 (Tumor protein P53)	14
	1.6.8 SOD1 (Superoxide dismutase [Cu-Zn])	14
	1.6.9 PAI-1 (Plasminogen activator inhibitor-1)	14
	1.6.10 Coagulation factor II (Prothrombin)	14
	1.6.11 NOS3 (Nitric oxide synthase 3)	14
	1.6.12 AChE (Acetylcholinesterase)	15
	1.6.13 P2Y12 (P2Y purinoceptor 12)	15
2	**LITERATURE SURVEY**	16
3	**AIM AND SCOPE**	19
	3.1 Aim	19
	3.2 Scope	19
4	**METHODS AND MATERIALS**	20
	4.1 Target Identification	21
	4.2 Ligand Screening	22
	4.3 Pharmacokinetic Analysis	22
	4.4 Molecular Docking	22
5	**RESULTS**	24
	5.1 Target Identification	24
	5.2 Identification of Ligands	25
	5.3 Molecular Docking	31
	5.4 Discussion	36
6	**SUMMARY AND CONCLUSION**	38
	BIBLIOGRAPHY	39

LIST OF TABLES AND FIGURES

TABLE	DESCRIPTION	PAGE NO
1.1	General Management of Patients with Acute Stroke	3
5.1	List of identified targets for Ischemic Stroke	24
5.2	The list of 250 compounds with low binding energy	26
5.3	Identified lead compounds from sterptomeDB database with their physiochemical properties	30
FIGURE	**DESCRIPTION**	**PAGE NO**
4.1	The general workflow of performing molecular docking	20
5.1	The Venn diagram and docking score	30
5.2	The 2-D diagram of the interactions between the target (COX1) and lead compounds	31
5.3	The 2-D diagram of the interactions between the target (ACE-2) and lead compounds	32
5.4	The 2-D diagram of the interactions between the target (PDE3) and lead compounds	33
5.5	The 2-D diagram of the interactions between the target (α2AP) and lead compounds	34

CHAPTER 1

INTRODUCTION

Stroke is the second largest cause of death and the primary cause of disability worldwide. The incidence of stroke is rising along with the global population of those 65 and older, which is expanding faster than all other age groups. Additionally, the overall burden of stroke is shifting towards younger age groups, especially in low- and middle-income nations. When the blood supply to the brain is interrupted or diminished, it can cause a stroke, which results in the death of brain cells. Ischemic stroke happens when a blood clot or fatty deposit known as plaque blocks an artery that delivers blood to the brain. This obstruction may develop in the skull or in the neck. Typically, clots begin in the heart and move through the circulatory system. A clot may separate on its own or stick to an artery. The brain doesn't receive enough blood or oxygen when a brain artery is blocked, and cells begin to deteriorate. When plaque separates from an artery and goes to the brain, it causes an ischemic stroke. Additionally, plaque can accumulate in the arteries supplying blood to the brain, narrowing them to the point where an ischemic stroke results. It accounts for around 87% of all stroke cases, making it the most prevalent form. According to the World Health Organisation (WHO). Worldwide, there were 5.5 million fatal strokes in 2019, out of an anticipated 13.7 million new cases. The burden of stroke is predicted to rise in the coming years due to aging populations and changes in lifestyle risk factors such as poor diets, inactivity, and cigarette use. The incidence of stroke is highest in low- and middle-income nations. Ischemic stroke has a large financial cost in addition to high death and morbidity rates. Since many stroke survivors endure long-term problems that necessitate continuing medical care and support, the morbidity rates for ischemic stroke can be quite high. About two-thirds of stroke survivors will be disabled to some extent, and one-third will need rehabilitation in a specialized institution, according to the American Heart Association. Additionally, depression, anxiety, and other mental health disorders may be more common in stroke survivors. The location and degree of the stroke, as well as the person's age, health status, and other circumstances, can all affect how severe the morbidity rates are. The total morbidity rates for ischemic stroke, however, emphasize the significance of successfully avoiding and managing this condition to lessen the burden on patients, families, and healthcare systems. The annual anticipated direct and indirect costs of stroke, which

include expenses for medical care, rehabilitation, and lost productivity, are in the billions of dollars. Rehabilitation, including physical therapy, speech therapy, and occupational therapy, can help individuals recover from the consequences of stroke and improve their quality of life. Physical effects: A stroke may result in a variety of physical disabilities, including paralysis, weakness, and sensory abnormalities. The location and severity of the brain damage determine how severe these disabilities are. For instance, whereas a stroke that affects the sensory cortex can cause a loss of sensation on one side of the body, a stroke that affects the motor cortex can cause weakness or paralysis on that side of the body. These physical limitations can make it challenging for people to carry out daily tasks, work, and socialize. Cognitive effects: Stroke can have an impact on a person's cognitive function, including their memory, attention, and language abilities. These limitations may make it more difficult for a person to carry out regular tasks, work, and interact with others. Communication issues may arise in stroke survivors, which may result in social isolation and sadness. Emotional effects: Strokes can also have emotional effects like despair, anxiety, and personality changes. These emotional changes may have an impact on a person's general quality of life as well as their ability to recover. Stroke-related speech issues, such as slurred speech or trouble finding the correct words, are another complication of the disease. Due to this, people may find it challenging to communicate with others, which may cause dissatisfaction and isolation. Fatigue: Stroke survivors may feel worn out or exhausted, which might limit their capacity to carry out everyday tasks and could hinder their rehabilitation. Stroke patients may experience difficulty swallowing, which raises their risk of choking and aspiration pneumonia. A stroke can result in vision issues such as double vision or vision loss in one or both eyes. It also increases the likelihood of seizures, which can cause further brain damage.

1.1 Acute Management of Stroke Initial Treatment

The goal for the acute management of patients with stroke is to stabilize the patient and to complete initial evaluation and assessment, including imaging and laboratory studies, within 60 minutes of patient arrival. Critical decisions focus on the need for intubation, blood pressure control, and determination of risk/benefit for thrombolytic intervention. Hypoglycaemia and hyperglycaemia need to be identified and treated early in the evaluation. Not only can both produce symptoms that mimic ischemic stroke, but they can also aggravate ongoing neuronal ischemia. Administration

of glucose in hypoglycaemia produces profound and prompt improvement, while insulin should be started for patients with stroke and hyperglycaemia. Ongoing studies will help to determine the optimal level of glycemic control. Hyperthermia is infrequently associated with stroke but can increase morbidity. Administration of acetaminophen, by mouth or per rectum, is indicated in the presence of fever (temperature >100.4° F [38° C]). Supplemental oxygen is recommended when the patient has a documented oxygen requirement. To date, there is conflicting evidence on whether supernormal oxygenation improves outcomes. Optimal blood pressure targets remain to be determined. Many patients are hypertensive on arrival. American Stroke Association guidelines have reinforced the need for caution in lowering blood pressure acutely. In the small proportion of patients with stroke who are relatively hypotensive, pharmacologically increasing blood pressure may improve flow through critical stenoses. Serial monitoring and interventions, when necessary, early in the clinical course and eventual stroke rehabilitation and physical and occupational therapy are the ideals of management. In patients with transient ischemic attacks (TIAs), failure to recognize the potential for near-term stroke, failure to perform a timely assessment for stroke risk factors, and failure to initiate primary and secondary stroke prevention exposes the patient to undue risk of stroke and exposes clinicians to potential litigation. TIAs confer a 10% risk of stroke within 30 days, and one-half of the strokes occurring after a TIA occurred within 48 hours.

Table 1.1 General Management of Patients with Acute Stroke

Blood glucose	Treat hypoglycaemia with D50 Treat hyperglycaemia with insulin if serum glucose >200 mg/dL
Blood pressure	See recommendations for thrombolysis candidates and noncandidates (Table 3)
Cardiac monitor	Continuous monitoring for ischemic changes or atrial fibrillation
Intravenous fluids	Avoid D5W and excessive fluid administration IV isotonic sodium chloride solution at 50 mL/h unless otherwise indicated
Oral intake	NPO initially; aspiration risk is great, avoid oral intake until swallowing assessed

Oxygen	Supplement if indicated ($Sa0_2$< 94%)
Temperature	Avoid hyperthermia; use oral or rectal acetaminophen and cooling blankets as needed

1.2 Thrombolytic Therapy

Current treatments for acute ischemic stroke include IV thrombolytic therapy with tissue-type plasminogen activator (t-PA) and endovascular therapies using stent retriever devices. A 2015 update of the American Heart Association/American Stroke Association guidelines for the early management of patients with acute ischemic stroke recommends that patients eligible for intravenous t-PA should receive intravenous t-PA even if endovascular treatments are being considered and that patients should receive endovascular therapy with a stent retriever if they meet criteria. Newer stroke trials have explored the benefit of using neuroimaging to select patients who are most likely to benefit from thrombolytic therapy and the potential benefits of extending the window for thrombolytic therapy beyond the guideline of 3 hours with t-PA and newer agents. CT angiography may demonstrate the location of vascular occlusion. CT perfusion studies are capable of producing perfusion images and together with CT angiography are becoming more available and utilized in the acute evaluation of stroke patients. The Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE) trial suggested that there might be benefit of administering IV t-PA within 3-6 hours of stroke onset in patients with small ischemic cores on diffusion-weighted magnetic resonance imaging (MRI) and larger perfusion abnormalities (large ischemic penumbras). The Desmoteplase In Acute Ischemic Stroke (DIAS) trial sought to show the benefit of administering desmoteplase in patients within 3-9 hours of onset of acute stroke with a significant mismatch (>20%) between perfusion abnormalities and ischemic core on diffusion-weighted MRI. Larger randomized trials of desmoplas were negative (>20%) between perfusion abnormalities and ischemic core on diffusion-weighted MRI. Larger randomized trials of desmoplasia were negative.

1.2.1 Role of Anticoagulants

Anticoagulation is the controlled therapeutic inhibition of blood clotting by means of appropriate drugs (i.e., anticoagulants). The role of anticoagulants in the treatment of cerebral ischemia has changed. For many years, anticoagulation was used routinely in acute ischemic stroke. However, in the past 2 decades, randomized, controlled studies have helped to better define the role of anticoagulants in the acute treatment and prevention of stroke. In addition, several new oral and parenteral anticoagulants are in different stages of clinical trials for use in the prophylaxis of ischemic thromboembolic stroke.

1.2.2 Anticoagulation for Acute Ischemic Stroke

Current data do not support the routine use of anticoagulation for acute ischemic stroke. Several randomized, controlled trials that used IV heparinoids, subcutaneous low-molecular-weight heparin (LMWH), or subcutaneous unfractionated heparin (UFH) early after ischemic stroke failed to show a significant overall benefit of treatment over controls. The International Stroke Study (IST) compared aspirin with subcutaneous UFH at 2 different doses (5000 units or 12,500 units bid); no difference in morbidity and mortality from stroke was shown between the group treated with aspirin and the group treated with UFH. In addition, although UFH seemed to decrease the risk of pulmonary embolism and deep venous thrombosis (DVT), it increased the risk of hemorrhagic complications. A systematic review by the Cochrane collaboration demonstrated that anticoagulation (with UFH, LMWH, heparinoids, oral anticoagulants, or thrombin inhibitors) did not decrease the odds of death or development of dependency from stroke. Although anticoagulants prevented pulmonary embolism, they also increased the risk of hemorrhage, leading to the conclusion that anticoagulation cannot be recommended for the treatment of acute ischemic stroke. The last trial evaluating early intravenous anticoagulation with UFH was published in 1986. It showed no benefit in the treatment arm compared with the control arm. An exception to the lack of benefit from anticoagulation might be in patients with acute ischemic stroke ipsilateral to a severe stenosis or occlusion of the internal carotid artery. In the TOAST (Trial of Org 10172 in Acute Stroke Treatment) trial, this group appeared to benefit from early IV administration of the LMWH danaparoid. However, this was a post hoc analysis with a small number of individuals,

so the effect of chance cannot be excluded. Therefore, further research is needed to confirm the findings.

If early anticoagulation after ischemic stroke is indicated but UFH is contraindicated because of large brain infarctions, hemorrhagic infarctions, or pronounced microangiopathic changes in the brain, LMWH (in a body-weight–adapted dose) could be used because of lower bleeding risk, although this recommendation is not based on solid evidence. In patients with acute ischemic stroke and atrial fibrillation, a controlled, randomized study (Heparin in Acute Embolic Stroke Trial [HAEST]) failed to show the superiority of LMWH (dalteparin 100 IU/kg subcutaneously bid) to aspirin (160 mg/d). On the basis of this evidence, patients with acute ischemic stroke and atrial fibrillation should be treated with aspirin in the acute phase (and then placed on anticoagulation).

When long-term anticoagulation is indicated, the use of UFH or LMWH has been advocated to serve as a bridge while a therapeutic international normalized ratio (INR) is achieved with warfarin. A small pilot study found that LMWH (enoxaparin 1 mg/kg subcutaneously bid) was safer than IV UFH for this purpose in patients with subacute cerebral ischemia. However, further studies are needed to confirm this finding before this approach can be recommended generally.

Despite evidence from the randomized clinical trials discussed above, anticoagulation continues to be recommended for some specific clinical situations. These recommendations are based on uncontrolled studies and expert opinion. Even among experts there is disagreement about the best level of anticoagulation, route of administration, timing and duration of treatment, use of a bolus dose, and safety of the therapy, given the severity of neurologic deficits, size of infarction on baseline computed tomography (CT), vascular distribution, or presumed cause of stroke.

Some of the indications currently proposed by many experts for early full-dose IV heparin after stroke or transient ischemic attack (TIA) include the following: Conditions with potential high risk of early cardiogenic reembolization, such as atrial fibrillation with proven intracardial thrombus on echocardiography, artificial valves, left atrial or ventricular thrombi, or myocardial infarction during the last 4 weeks. Symptomatic dissection of the arteries supplying the brain (after exclusion of subarachnoid hemorrhage on CT scan). Symptomatic extracranial or intracranial arteriosclerotic stenosis with crescendo TIAs or early progressive stroke. Basilar artery occlusion before or after intra-arterial pharmacological or mechanical thrombolysis. Known

hypercoagulable states (eg, protein C and S deficiencies, activated protein C [APC] resistance, antithrombin deficiency, relevant titer of antiphospholipid antibodies). Cerebral venous sinus thrombosis. The use of anticoagulation in cerebral venous sinus thrombosis is based on open case series with no controls. Anticoagulation has been used even in the presence of hemorrhagic infarctions typical of this condition. Authors have reported good outcomes compared with historical controls. Conclusive data are lacking about the management of anticoagulation in patients with hemorrhagic conversion of ischemic brain infarction or primary cerebral hemorrhage who have an absolute indication for anticoagulation for the prevention of embolism (ie, atrial fibrillation or mechanical heart valves). Small retrospective case series of patients with urgent need for anticoagulation (eg, with artificial heart valves) showed a better outcome for those treated with full-dose IV heparin (only after normalization of INR values by administration of prothrombin complex and/or other warfarin antagonists) than for those treated with low-dose subcutaneous heparin; however, these studies lack concomitant control subjects, thus making any conclusions about true efficacy and safety difficult. At the present time, patients with acute ischemic stroke treated with intravenous recombinant tissue plasminogen activator (rtPA; see alteplase) clearly should not be treated with anticoagulation for at least 24 hours post thrombolysis.

1.3 Direct thrombin inhibitors and Factor Xa inhibitors

Novel oral anticoagulants (NOACs) include apixaban, dabigatran, rivaroxaban, and edoxaban. NOACs are alternatives to warfarin for high-risk patients (including those with a history of stroke) who have atrial fibrillation. Apixaban, edoxaban, and rivaroxaban inhibit Factor Xa, whereas dabigatran is a direct thrombin inhibitor. Apixaban and dabigatran were shown to be superior to warfarin for the prevention of stroke and systemic embolism, while rivaroxaban and edoxaban were shown to be equivalent. The rates of intracranial hemorrhage are lower for NOACs compared with warfarin. Dabigatran carries a higher risk of gastrointestinal bleeding compared with warfarin, and it appears to increase the risk of myocardial infarction. These medications have not been compared against each other.

1.3.1 Direct thrombin inhibitors

The RE-LY study evaluated the efficacy and safety of 2 different doses of dabigatran relative to warfarin in more than 18,000 patients with atrial fibrillation. Patients were randomized to 1 of 3 arms: (1) adjusted dose warfarin, (2) dabigatran 110 mg bid, or (3) dabigatran 150 mg bid. Dabigatran 110 mg was noninferior to warfarin for the primary efficacy endpoint of stroke or systemic embolization, while dabigatran 150 mg was significantly more effective than warfarin or dabigatran 110 mg. Major bleeding occurred significantly less often with dabigatran 110 mg than with warfarin; dabigatran 150 mg had similar bleeding to warfarin. Dabigatran, a competitive, direct thrombin inhibitor, was approved by the US Food and Drug Administration in 2010 for the prevention of stroke and thromboembolism associated with nonvalvular atrial fibrillation. The dose is 150 mg PO bid (decrease to 75 mg PO bid with renal impairment). When converting from warfarin, discontinue warfarin and initiate dabigatran when INR < 2.0.

The FDA approved a monoclonal antibody reversal agent (idarucizumab [Praxbind]) for patients treated with dabigatran when reversal of dabigatran's anticoagulant effects is needed for emergency surgery or urgent procedures, or in the event of life-threatening or uncontrolled bleeding. Idarucizumab is specific for reversing dabigatran, although other NOAC reversal agents are currently in clinical trials or awaiting FDA approval (eg, andexanet alfa, PER977). Accelerated approval for idarucizumab was based on interim analysis of the Re-VERSE AD trial. Investigators found that, among 39 patients who had been receiving dabigatran and required an urgent procedure were then given idarucizumab, 36 underwent their urgent procedure—with 33 (92%) having normal hemostasis during the event. Two of the remaining patients had mildly abnormal bleeding (with slight oozing), while just one had moderately abnormal yet controlled bleeding. Among 35 of 51 patients who had serious bleeding were able to be assessed, hemostasis, as determined by local investigators, was restored at a median of 11.4 hours. Guidelines from the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA)/Heart Rhythm Society (HRS) on atrial fibrillation have been updated to include the use of oral direct thrombin inhibitors (ie, dabigatran). The guidelines include a class Ib recommendation (ie, treatment is useful/effective based on a single randomized trial) for dabigatran. The guidelines recommend dabigatran may be used as an alternative to warfarin for the prevention of stroke and systemic thromboembolism in patients with

paroxysmal-to-permanent atrial fibrillation and risk factors for stroke or systemic embolization. Patients with atrial fibrillation who are not candidates include those with prosthetic heart valves or hemodynamically significant valve disease, severe renal failure (creatinine clearance ≤15 mL/min), or advanced liver disease.

1.3.2 Factor Xa inhibitors

Apixaban (Eliquis) was approved by the FDA in December 2012. Approval was based on 2 clinical trials. The ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial compared apixaban with warfarin for the prevention of stroke or systemic embolism in patients with atrial fibrillation and at least one additional risk factor for stroke. Results showed that apixaban was superior to warfarin in preventing stroke or systemic embolism, causing less bleeding, and resulting in lower mortality.

Rivaroxaban (Xarelto) was approved in 2011 to reduce the risk of stroke and systemic embolism in patients with nonvalvular AF. Approval was based on the ROCKET-AF double-blind trial (n >14,000), in which the risk of major bleeding was similar for rivaroxaban and warfarin, but a significantly lower risk of intracranial hemorrhage and fatal bleeding was seen with rivaroxaban when compared with warfarin.

Edoxaban (Savaysa) was approved by the FDA in January 2015 to reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. In the ENGAGE AF-TIMI 48 trial (n=21,105), edoxaban was noninferior to warfarin with respect to the prevention of stroke or systemic embolism. Edoxaban was associated with significantly lower rates of major bleeding ($P < 0.001$) and death from cardiovascular causes (P=0.01%) compared with warfarin. In May 2018, coagulation factor Xa recombinant (AndexXa) was approved for patients treated with rivaroxaban or apixaban, when reversal of anticoagulation is needed because of life-threatening or uncontrolled bleeding. Approval was supported by data from two Phase 3 ANNEXA studies (ANNEXA-R and ANNEXA-A), which evaluated the safety and efficacy of Andexxa in reversing the anticoagulant activity of the Factor Xa inhibitors rivaroxaban and apixaban in healthy older volunteers. Results demonstrated a rapid and significant reversal of anti-Factor Xa (FXa) activity. Anti-FXa activity was reduced among apixaban-treated participants by 94% compared with 21% for placebo ($p< 0.001$). A 92% reduction of anti-FXa activity was observed in the rivaroxaban-treated participants compared with 18% for placebo ($p< 0.001$). In the ANNEXA-4 trial, 67

patients who had acute major bleeding within 18 hours after administration of an FXa inhibitor received coagulation factor Xa recombinant. After the IV bolus plus 2-hour IV infusion, the median anti-FXa activity decreased by 89% from baseline among patients receiving rivaroxaban and by 93% among patients receiving apixaban. Assessment at 12 hours after the infusion adjudicated clinical hemostasis as excellent or good in 37 of 47 patients in the efficacy analysis (79%; 95% CI, 64 to 89). Thrombotic events occurred in 12 of 67 patients (18%) during the 30-day follow-up.

1.4 Haemorrhages

A bleeding risk stratification scheme called HEMORR$_2$ HAGES has been validated in at least a dataset of anticoagulated patients. The score is calculated as the sum of the following risk factors for bleeding: Hepatic or renal disease, Ethanol abuse, Malignancy, Old age (>75 y), Rebleeding, Reduced platelet counts or platelet dysfunction, Hypertension that is uncontrolled, Anemia, Genetic factors, Elevated fall risk, Stroke.

1.5 Ischemic Stroke and Neurologic Deficits

Thrombolytics restore cerebral blood flow in some patients with acute ischemic stroke and may lead to improvement or resolution of neurologic deficits. Thrombolytic therapy is of proven and substantial benefit for select patients with acute cerebral ischemia. The evidence base for thrombolysis in stroke includes 21 completed randomized controlled clinical trials enrolling 7152 patients, using various agents, doses, time windows, and intravenous or intra-arterial modes of administration. Data from these trials are congruent in supporting the following conclusions: Intravenous fibrinolytic therapy at the cerebral circulation dose within the first 3 hours of ischemic stroke onset offers substantial net benefits for virtually all patients with potentially disabling deficits. Intravenous fibrinolytic therapy at the cerebral circulation dose within 3-4.5 hours offers moderate net benefits when applied to all patients with potentially disabling deficits. MRI of the extent of the infarct core (already irreversibly injured tissue) and the penumbra (tissue at risk but still salvageable) can likely increase the therapeutic yield of lytic therapy, especially in the 3- to 9-hour window. Intra-arterial fibrinolytic therapy in the 3- to 6-hour window offers moderate net benefits when applied to all patients with potentially disabling deficits and large artery cerebral thrombotic occlusions.

1.6 Potential drug targets

Drug target identification is a vital phase in the drug discovery process. A drug target is a molecule or molecular pathway involved in a disease process that can be manipulated by a medication to achieve a therapeutic effect. Drug target identification is a complicated and multi-disciplinary process involving a variety of methodologies and approaches.

Studying the biology of the disease and the molecular processes involved in its development and progression is one method for identifying therapeutic targets. This can include researching the molecular interactions between proteins and other biomolecules involved in the disease process, analysing gene expression patterns, finding critical signalling pathways, and studying the molecular interactions between proteins and other biomolecules involved in the disease process. Another method for identifying therapeutic targets is to employ high-throughput screening techniques to identify tiny compounds that bind to specific proteins or other molecular targets. This method may entail screening vast libraries of chemicals to identify prospective drug candidates with the desired biological activity.

Once prospective pharmacological targets have been identified, researchers can employ a variety of methodologies to further confirm their therapeutic potential, such as cell-based assays, animal models, and clinical trials. These studies can aid in determining the safety and efficacy of possible medication candidates, as well as providing crucial information on their mechanism of action and potential adverse effects.

Compounds and anti-stroke target were obtained from streptomedb database and integrity, correspondingly. According to molecular docking energy, top compounds for 14 targets were chosen,

- COX-1 (Cyclooxygenase-1)
- ACE2 (Angiotensin I converting enzyme 2)
- PDE3 (Phosphodiesterase3)
- Alpha 2 Antiplasmin
- PDE5A (cGMP-specific phosphodiesterase 5A)
- PPARγ (Peroxisome proliferator-activated receptors)
- P53 (Tumor protein P53)

- SOD1 (Superoxide dismutase [Cu-Zn])
- PAI-1 (Plasminogen activator inhibitor-1)
- Coagulation factor II (Prothrombin)
- NOS3 (Nitric oxide synthase 3)
- AChE (Acetylcholinesterase)
- P2Y12 (P2Y purinoceptor 12)

1.6.1 COX-1 (Cyclooxygenase-1)

COX-1 plays a crucial role in maintaining platelet aggregation, COX-1 is responsible for the synthesis of prostaglandins that protect the stomach lining, regulate blood flow to the kidneys, and promote platelet aggregation, which is necessary for blood clotting. COX-1 is essential for the synthesis of Thromboxane A2 (TXA2), which stimulates platelet aggregation and vasoconstriction, and thus exerts hemostatic/thrombogenic effect. Pharmacological inhibition of TxA_2 synthesis leads to the inhibition of platelet aggregation. Therefore, the inhibition of COX-1 may have both beneficial and harmful effects in stroke, depending on the timing, dosage, and individual patient factors.

1.6.2 ACE2 (Angiotensin I converting enzyme 2)

ACE2 metabolizes angiotensin II (Ang II), a potent vasoconstrictor and pro-inflammatory molecule, to Ang-(1-7), which has vasodilatory and anti-inflammatory effects. By reducing Ang II levels and increasing Ang-(1-7) levels, ACE2 may protect against the deleterious effects of Ang II in stroke. ACE2 may have anti-inflammatory effects in the brain, which could help to reduce neuronal damage and improve recovery after stroke.

1.6.3 PDE3 (Phosphodiesterase3)

PDE3 inhibitors, which prevent the breakdown of cAMP and cGMP, have been examined as potential treatments for stroke. One mechanism by which PDE3 inhibitors may help in stroke is by stimulating vasodilation, or the relaxation of blood vessels, which can increase blood flow to the brain. PDE3 inhibitors have been shown to increase cerebral blood flow and improve neurological outcomes in animal models of stroke. PDE3 inhibitors may be beneficial in stroke is by protecting neurons from

damage. PDE3 inhibitors have been shown to reduce the production of reactive oxygen species (ROS) and to inhibit apoptosis, or programmed cell death, in neurons.

1.6.4 Alpha 2 Antiplasmin

High level of Alpha 2 Antiplasmin, an ultrafast, covalent inhibitor of plasmin, have been linked in humans to increase the risk of ischemic stroke and failure of tissue plasminogen activator therapy. A2AP works by binding to plasmin, which prevents it from breaking down fibrin, the protein that forms the structural framework of blood clots. This prevents the blood clot from dissolving, allowing it to remain in place and prevent further bleeding. Hence A2AP plays an important role in regulating the formation and breakdown of blood clots in stroke and other cardiovascular diseases

1.6.5 PDE5A (cGMP-specific phosphodiesterase 5A)

DE5A inhibition has been recommended as a potential treatment method in stroke due to its capacity to boost cGMP levels and improve vasodilation and blood flow in the brain. Furthermore, PDE5A inhibition has been demonstrated to have anti-inflammatory and anti-apoptotic effects in the brain, which may be useful in stroke. PDE5A inhibition can increase neuroprotection in stroke by activating protein kinase G (PKG), which is downstream of cGMP. PKG activation can activate a variety of signalling pathways that protect neurons from injury and promote neuroplasticity and neurogenesis.

1.6.6 PPARγ (Peroxisome proliferator-activated receptors)

PPAR has been proven to have neuroprotective properties in stroke patients. When engaged, PPAR can decrease inflammation and oxidative damage while also promoting neurogenesis and angiogenesis. Several research have been conducted to study the mechanism by which PPAR exerts its neuroprotective effect in stroke. One putative mechanism is the control of the blood-brain barrier (BBB). The BBB is a specialised structure that regulates the passage of chemicals between the blood and the brain. The BBB is disturbed during a stroke, resulting in an influx of immune cells and inflammatory mediators into the brain, which can worsen tissue damage. PPAR has been found to protect the integrity of the BBB by regulating the expression of tight junction proteins and decreasing the production of pro-inflammatory cytokines.

1.6.7 P53 (Tumor protein P53)

The mechanism involves the control of the inflammatory response following a stroke. p53 has been demonstrated to regulate the expression of many pro-inflammatory cytokines, including TNF-alpha and IL-6, which are likely to contribute to stroke-induced brain injury.

1.6.8 SOD1 (Superoxide dismutase [Cu-Zn])

SOD1 reduces oxidative stress in the brain by transforming damaging superoxide radicals into less hazardous molecules. SOD1 possesses anti-inflammatory characteristics in addition to antioxidant action. Stroke-induced inflammation can activate a variety of inflammatory pathways, exacerbating brain injury and cell death.

1.6.9 PAI-1 (Plasminogen activator inhibitor-1)

It suppresses the function of tissue plasminogen activator (tPA), which is in charge of dissolving blood clots. PAI-1 can contribute to the formation and stability of blood clots in the context of stroke, which can lead to ischemic damage in the brain. This sets off a chain of events that causes the coagulation system to activate and blood clots to develop. tPA normally aids in the breakdown of these clots and the restoration of blood flow to the affected location. PAI-1, on the other hand, limits the function of tPA, which might result in the formation of stable blood clots, further restricting blood flow to the brain.

1.6.10 Coagulation factor II (Prothrombin)

It is a protein that is essential for the coagulation (clotting) process. When a blood artery is injured, prothrombin is transformed to thrombin by the action of other clotting factors such as factor X and factor V.

1.6.11 NOS3 (Nitric oxide synthase 3)

NOS3 plays a complex and diversified involvement in stroke. While NOS3-derived NO can improve blood flow and reduce ischemic injury, excessive NO generation or reduced NOS3 function might cause brain damage and raise the risk of stroke.

1.6.12 AChE (Acetylcholinesterase)

AChE may contribute to stroke by breaking down AChE in the brain. AChE is engaged in several physiological activities, including blood flow modulation and cerebral blood

flow maintenance. When AChE levels are reduced as a result of AChE action, blood flow to the brain is restricted, which can exacerbate the consequences of stroke.

1.6. 13 P2Y12 (P2Y purinoceptor 12)

The stimulation of platelets and the production of blood clots are the mechanisms by which P2Y12 contributes to stroke. Platelets become activated and attach to the site of injury when a blood vessel is damaged. P2Y12 activation causes the release of more platelet activators, which results in the formation of a blood clot. This clot may subsequently migrate to the brain and obstruct blood flow, resulting in ischemic damage.

CHAPTER 2

REVIEW OF LITERATURE

Over 80% of strokes occur in the elderly (people over the age of 65), and patient outcomes following a stroke are heavily influenced by age. The increased sensitivity of the elderly to ischemic stroke is linked to many alterations in the old brain. The risk factor profiles and mechanisms of ischemia damage differ between young and senior stroke patients. Elderly folks frequently receive ineffective therapy and have poorer outcomes after a stroke than younger people. The majority of preclinical investigations of neuroprotective medicines have used young animals, which may explain why these treatments have failed to translate in people (Chen et al., 2010)

Investigate the efficacy of natural thrombolytic medicines in the treatment of stroke. Natural sources of thrombolytic activity have been reported, and active compounds have been identified and characterised. A total of ten natural molecules were chosen for docking investigations (Alshehri et al., 2022)

The data supporting pre-hospital and emergency stroke care, including the use of emergency medical services protocols for stroke identification, intravenous thrombolysis in acute ischemic stroke, including updates to recommended patient eligibility criteria and treatment time windows, and advanced imaging techniques with automated interpretation to identify patients with large areas of brain at risk but no large completed infarcts who are likely to benefit (Phipps & Cronin, 2020)

The latest evidence and therapeutic recommendations for evaluation and treatment of adults with acute ischemic stroke. The intended audiences are prehospital care providers, physicians, allied health professionals, and hospital administrators who are responsible for the management of acute (Jauch et al., 2013)

The ischemic cascade, a complex series of neurochemical processes triggered by transient or permanent focal cerebral ischemia that includes cellular bioenergetic failure, excitotoxicity, oxidative stress, blood-brain barrier dysfunction, microvascular injury, hemostatic activation, post-ischemic inflammation, and finally cell death of neurons, glia, and endothelial cells (Brouns & De Deyn, 2009)

Lipids are crucial components of many biological processes and play critical roles in the pathophysiology of many prevalent neurological illnesses. Furthermore, fatty acid-binding proteins (FABPs), a family of lipid chaperone proteins, have been implicated in the onset or progression of several neurodegenerative diseases, including Alzheimer's and Parkinson's disease, in recent years. However, there has been little focus on the roles of FABPs in ischemic stroke. demonstrated that neural tissue-associated FABPs play a role in the pathophysiology of ischemic brain injury in mice. the literature published in the last decade that has reported on the links between FABPs and ischemia, and summarise the important regulatory mechanisms of FABPs implicated in ischemic injury. Potential FABPs that could be used as therapeutics (Guo et al., 2022)

Largest unmet medical need indications in medicine, and reactive oxygen species generating NADPH oxidase type 4 (Nox4) as a main causative treatment target. There are both traditional protein-protein interactions and metabolite-dependent interactions. Based on this protein-metabolite network, a gene ontology-based semantic similarity ranking was developed to identify acceptable synergistic targets for network pharmacology. The nitric oxide synthase (Nos1–3) gene family is the closest target to Nox4. Indeed, when we combine a NOS and a NOX inhibitor at subthreshold concentrations, pharmacological synergy as evidenced by reduced cell death, reduced infarct size, stabilised blood-brain barrier, reduced reoxygenation-induced leakage, and preserved neuromotor function, all in a supraadditive manner. Thus, protein-metabolite network analysis, for example, guilt by association, can predict and pair synergistic mechanistic disease targets for systems medicine driven network. Approaches that lower the chance of failure in single-target and symptom-based medication discovery and therapy may be developed in the future. (Casas et al., 2019)

For the treatment of ischemic stroke, novel, effective, and safe medications are required. Although circulating protein biomarkers with causal genetic evidence are promising drug targets, no systematic proteome screen has been performed. (Chong et al., 2019)

Epidemiological research has enhanced our understanding of stroke risk factors, and clinical trials have shown that reducing risk variables can reduce stroke risk. Stroke risk factors are categorised as traditional or innovative, and they can also be

adjustable or non-modifiable. Choose traditional risk factors for ischemic stroke, primary and secondary prevention measures, and areas of research development. Stroke treatment should be comprehensive, including patient, community, and medical personnel education, evaluation of individual risk factors, and assessment of overall stroke risk (Jose Rafael Romero, 2007)

Stroke has a substantial worldwide health impact, and patients currently have few therapeutic treatment alternatives. Pre-clinical research mainly relies on rodent stroke models, however the restrictions associated with using these systems alone have meant that translation of therapeutic molecules to the clinic has not been very successful to yet. Zebrafish illness modelling provides a potentially complementary platform for pre-clinical chemical screening to enhance the drug discovery process in translational stroke research (Crilly et al., 2022)

There is still more to be discovered about anti-stroke medications and molecular mechanisms. More than 60000 compounds from the traditional Chinese medicine (TCM) database were computationally analysed and docked to the 15 known anti-stroke targets in order to screen prospective known to inhibit-stroke compounds. To validate docking results, 192 anti-stroke plants for clinical therapy and 51 existing anti-stroke medicines were employed. A total of 2355 anti-stroke potential compounds were obtained. Between these chemical compounds, 19 are identical in structure to 16 current medications, a portion of which has been used for anti-stroke treatment. In addition, anti-stroke plants were significantly enriched in these candidate compounds. The compound-target-plant network was built using the given results. The structure of the network exposes the likely molecular mechanism of these drugs' anti-stroke activity. The majority of potential chemicals and anti-stroke plants are known to interact with the target NOS3, PSD-95, and PDE5A. subsequently found 35 anti-stroke chemicals with favourable features using the ADMET filter. The 35 potential anti-stroke compounds provide a chance to produce new anti-stroke medications while also improving studies on the molecular causes of anti-stroke (Liu et al., 2017)

CHAPTER 3

AIM AND SCOPE

3.1 Aim

The aim of this study is to identify potential drug targets and lead molecules for the treatment of ischemic stroke using zebrafish models and behavioural analysis. Ischemic stroke is a major public health concern and there is a need for new and more effective therapies.

3.2 Scope

The screening of a library of compounds to identify potential lead molecules and also involve the characterization of the identified lead molecules in terms of their mechanisms of action and potential for clinical translation. The study aims to contribute to the development of more effective therapies for ischemic stroke, which could have significant benefits for patients and the wider healthcare system. The use of zebrafish models and behavioural analysis offers a promising approach for drug discovery and lead molecule identification, which could be applied to other neurological disorders as well.

CHAPTER 4

MATERIALS AND METHODS

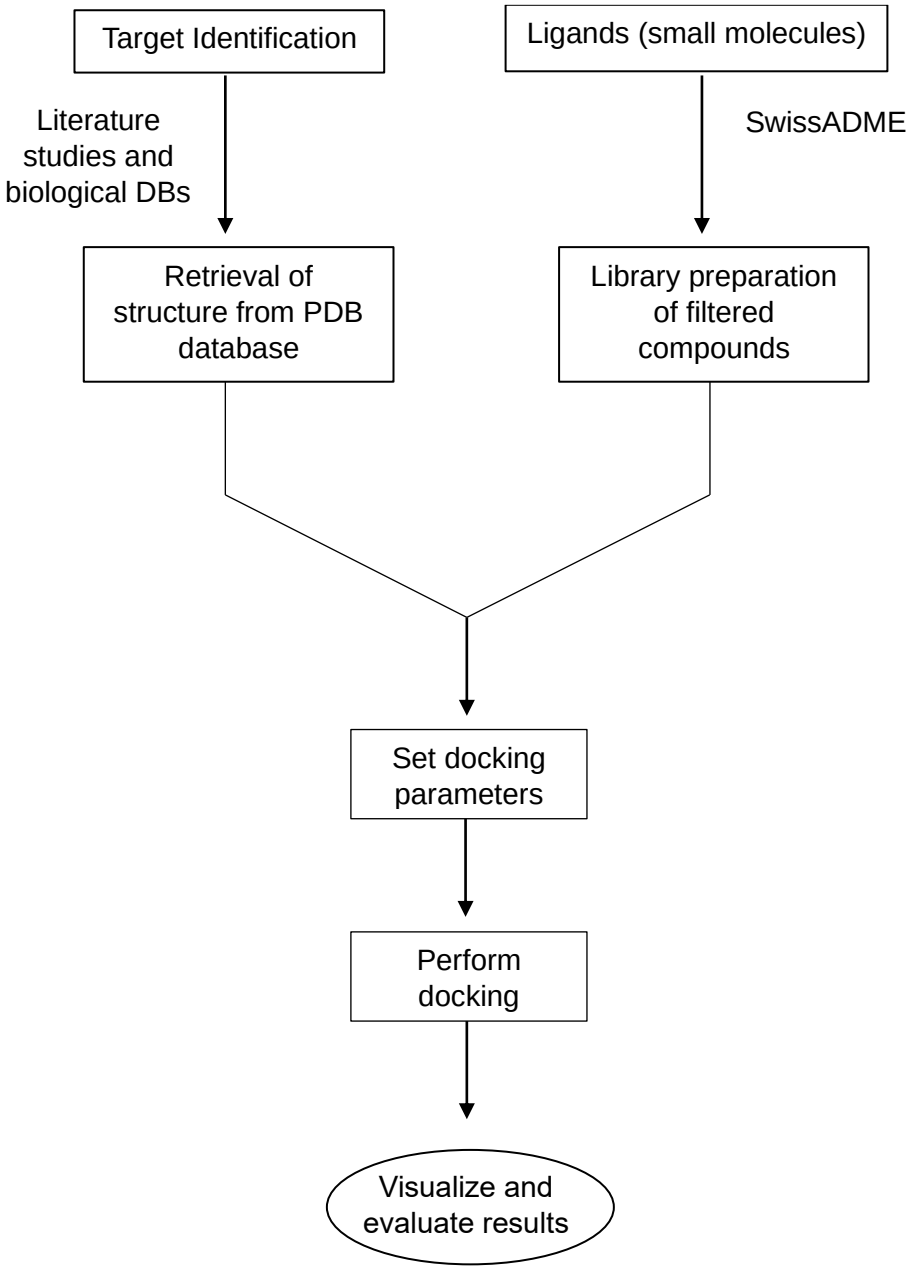


Figure4.1 The general workflow of performing molecular docking

4.1 Target Identification

The target will be obtained from Uniport and PDB, and heteroatoms will be eliminated. The Protein Data Bank (PDB) library of 3D structural data for major biological molecules (proteins, DNA, and RNA) is a vital resource for fundamental biology, health, energy, and biotechnology researchers and learners. Through an online information portal and a downloadable data archive, the PDB provides access to 3D structural information for the components of life found in all living creatures on the planet. The 3D structure of a biological macromolecule is critical for understanding how it affects human and animal health and disease.

The primary purpose of the PDB is to provide researchers with access to experimentally determined structures, which are essential for understanding how the structure and function of biomolecules interact. The PDB contains information on the atomic coordinates of a protein or nucleic acid, as well as accompanying experimental data, ligands, ions, and intermolecular interactions.

UniProt provides information on the sequence and function of proteins. It serves as the major source for annotated protein sequence data, which includes information about the protein's localization, interactions, and post-translational modifications, as well as functional and structural information. UniProt (PIR) is a collaborative effort of the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource.

UniProt provides access to several other protein sequence databases, including UniProtKB/Swiss-Prot, UniProtKB/TrEMBL, and UniRef. UniProtKB/Swiss-Prot is a database of protein sequences that have undergone experimental characterisation. UniProtKB/TrEMBL, on the other hand, is a database of protein sequences that have yet to be experimentally characterised. It was produced computationally. Protein sequences that are closely related are grouped together in the UniRef database to avoid redundancy. In addition, UniProt provides tools and resources for protein sequence analysis, such as BLAST (Basic Local Alignment Search Tool), which is an algorithm and programme for comparing primary biological sequence information, such as amino-acid sequences of proteins or nucleotides of DNA and/or RNA sequences.

4.2 Ligand Screening

StreptomeDB is a database of natural products produced by Streptomyces bacteria, which have been identified as potential lead compounds for drug development. Lead molecules are compounds that have been identified as potential starting points for the development of new drugs. Lead molecules can also be useful in identifying new drug targets. The structure and function of a lead molecule and its target protein, researchers can gain insights into the biological pathways and mechanisms involved in disease. This information can be used to develop new drugs that target these pathways or mechanisms.

4.3 Pharmacokinetic Analysis

SWISSADME is an open web tool created by the Swiss Institute of Bioinformatics (SIB) that predicts the pharmacokinetic properties of small molecules. It uses computational models to predict ADME (absorption, distribution, metabolism, and excretion) properties of compounds. The molecules will be screened based on Lipinski's rule of five, which is a set of rules utilized in medication discovery and development. (Lipinski, 2004)

Lipinski's ROF

•Molecular weight [between 200- 500 Daltons]

•LogP (partition coefficient) [≤ 5]

•Number of hydrogen bond donors [≤ 5]

•Number of hydrogen bond acceptors [≤ 10]

•Rotatable bonds [≤ 9]

4.4 Molecular Docking

Molecular docking is a computational procedure that attempts to predict noncovalent binding of macromolecules or, more commonly, a macromolecule (receptor) and a small molecule (ligand) efficiently, beginning with their unbound structures, structures obtained from MD simulations, homology modelling, and so on. The objective is to anticipate the bound conformations as well as the binding affinity. Auto Dock Vina is an open-source molecular docking programme. Dr. Oleg Trott created and

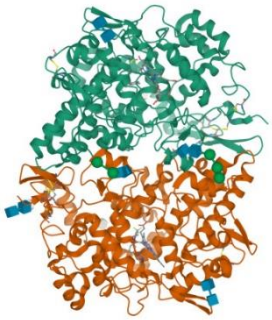
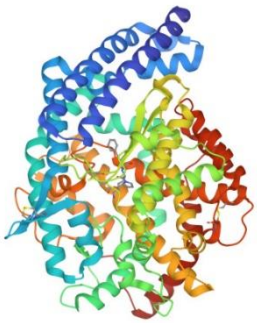
implemented it in the Molecular Graphics Lab (now CCSB) at The Scripps Research Institute. AutoDock is a collection of docking automation tools. It is intended to anticipate how tiny compounds, such as substrates or drug candidates, bind to a known 3D structural receptor. It has been changed and refined throughout time to offer additional functionalities, and several engines have been built.

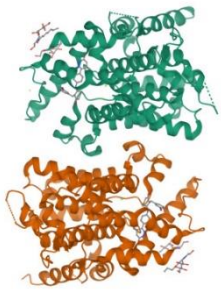
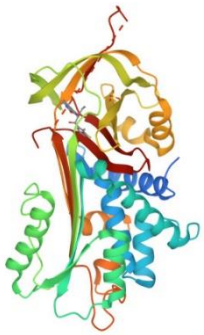
CHAPTER 5

RESULTS

5.1 Target Identification

Table 5.1. List of identified targets for Ischemic Stroke

Name	PDB ID	Protein structure	Method	Active sites
COX-1	3KK6		X-RAY DIFFRACTION	ALA527_ARG120_HIS90_ILE523_LEU352_LEU359_LEU384_PHE518_TRP387_TYR355_TYR385_VAL116_VAL349
ACE2	1O86		X-RAY DIFFRACTION	ALA354_GLU376_HIS353_HIS513_LYS511_PHE457_THR282_TRP279_VAL380

PDE3	1SO2		X-RAY DIFFRACTION	GLN988_HIS948_ILE938_ILE955_LEU895_PHE991_PRO941_SER990_THR952
α2AP	7AEL		X-RAY DIFFRACTION	ILE335_ILE379_LEU333_LEU376_LEU425_LEU427_LEU459_MET303_MET458_PHE455_PHE457_PRO377_THR428_TRP277

The 3-D structure of the targets (3KK6,1O86,1SO2,7AEL) was extracted from the PDB in the Sdf format. The above mentioned four targets play a crucial role in the disease mechanism and shown to have high inhibiting activity. Active sites are the region where the lead molecules bind and show the inhibition activity.

5.2 Identification of ligands

StreptomeDB provides a user-friendly interface that allows users to search for natural products based on various criteria, such as chemical structure, biosynthetic pathway, or biological activity. Users can also explore the biosynthetic pathways of natural products and identify the genes responsible for their production. In addition, StreptomeDB includes tools for analyzing and visualizing the data, such as interactive metabolic maps and structure-activity relationship (SAR) plots. These tools can help researchers to identify new drug leads or to optimize existing natural products for improved efficacy.

Table5.2. The list of 250 compounds with low binding energy

	ACE2		antiplasmin		cox1		PDE3
AbyssomicinP	-9.9	4Demethylamino4oxostaurosporine	-10.2	SpirotoamideB	-12	Uncialamycin	-13.9
GuanitrypmycinA12	-9.9	BombyxamycinB	-9.9	22E5E7E9R10R102S3S23Dimethyloxiran2yl10hydroxy379trimethyldeca257trien1yl56dimethoxy3methylpyridin4ol	-9.8	SpirotoamideB	-12.7
Jadomycin	-9.9	CHEMBL94678	-9.9	2468NonatetraenamideN2hydroxy5oxo1cyclopenten1yl92methylphenyl2E4E6E8E	-9.6	3Hydroxy3NacetylholyrineA	-12.4
NiizalactamC	-9.9	3Hydroxy3NacetylholyrineA	-9.8	263Methylbut2enyl1Hindol3ylacetonitrile	-9.5	3NAcetylholyrineA	-12.1
StaurosporineM1	-9.9	3NDemethyl4hydroxystaurosporine	-9.7	5HydroxyherboxidieneA1	-9.5	7OxoK252b	-12
9Hydroxyl3Nacetyl4Hydroxylstaurosporine	-9.8	7OxoK252d	-9.7	SDNPA	-9.5	RLDigitoxosylarcyriaflavinA	-12
K252aMe	-9.8	GuanitrypmycinA12	-9.7	3Z5E7Z9Z12R13E15E17Z19Z21Z23R• 12• Hydroxy• 23• methyl• 1• azacyclotetracosa• 35791315171921• nonaen• 2• one	-9.4	3NDemethyl4hydroxystaurosporine	-11.9
Prexiamycin	-9.8	GuanitrypmycinB11	-9.6	910Anthracenedione2ethyl	-9.4	4Demethylamino4oxostaurosporine	-11.8
10epideOHHSAF	-9.7	K252d	-9.6	Anhydrosek4b	-9.4	3ODemethyl4Ndemethyl4Nacetyl4epistaurosporine	-11.7
GuanitrypmycinA11	-9.7	NiizalactamC	-9.6	NanaomycinA	-9.3	9Hydroxy3NacetylholyrineA	-11.6
IzumiphenazineB	-9.7	7OxoMLR52	-9.5	JuglomycinB	-9.2	9Hydroxyl3Nacetyl4Hydroxylstaurosporine	-11.6
MycotrienolI	-9.7	GuanitrypmycinA11	-9.5	NanaomycinalphaA	-9.2	BaraphenazineD	-11.6
NocardioazineA	-9.7	IsofuranonaphthoquinoneG	-9.5	Phebestin	-9.2	3NFormylholyrineA	-11.5
CifednamideA	-9.6	K252a	-9.5	PyridinopyroneA	-9.2	7OxoK252d	-11.3
ProdigiosinR1	-9.6	7Oxostaurosporine	-9.4	11Deoxylandomycinone	-9.1	BE12406B	-11.3
Progeldanamycin	-9.6	AminoansamycinD	-9.4	4Demethylamino4oxostaurosporine	-9.1	K252a	-11.3
SteptocarbazoleA	-9.6	AminoansamycinF	-9.4	Chrysophanol	-9.1	StaurosporineM1	-11.3
TiancimycinA	-9.6	PhenazinolinD	-9.4	EN7	-9.1	FradcarbazoleC	-11.2
XanthobaccinC	-9.6	AntibioticK252a	-9.3	Rk270a	-9.1	GuanitrypmycinA11	-11.2
Aureoverticillactam	-9.5	C07349	-9.3	Tetrangomycin	-9.1	NAcetylNdemethylmayamycin	-11.2
BombyxamycinB	-9.5	NocardioazineA	-9.3	36disubstitutedIndoleB	-9	Sespenine	-11.2
GuanitrypmycinC11	-9.5	PhenazinolinE	-9.3	6Hydroxy1phenazinecarboxylicacid	-9	AlbacarcinM	-11.1
PhenazinolinC	-9.5	3-Chloro-68-dihydroxy-8-alpha-lapachone	-9.2	Austramide	-9	AminoansamycinF	-11.1
UCN02	-9.5	3NAcetylholyrineA	-9.2	CuevaeneA	-9	GuanitrypmycinB12	-11.1
7OxoMLR52	-9.4	3ODemethyl4Ndemethyl4Nacetyl4epistaurosporine	-9.2	DOlivosyljadomycinGly	-9	SteptocarbazoleA	-11.1

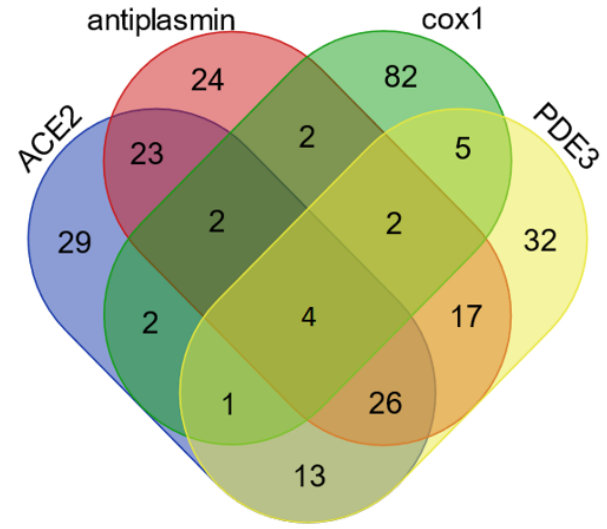
AntibioticK252a	-9.4	7OxoholyrinA	-9.2	JadomycinG	-9	StreptoanthraquinoneA	-11.1
BaraphenazineD	-9.4	9Hydroxy3NacetylholyrineA	-9.2	Kinanthraquinone	-9	UCN02	-11.1
BorrelidinCR1	-9.4	ArcyriaflavinA	-9.2	L29141	-9	4NFormyl7oxoholyrinA	-11
Butylcycloheptylprodigiosin	-9.4	BE24566B	-9.2	NPhenylnaphthalen2amine	-9	7OxoMLR52	-11
C07349	-9.4	FormicapyridineA	-9.2	Z23Dehydroanhydrocycloheximide	-9	Ansaetherone	-11
Eriamycin	-9.4	Jadomycin	-9.2	6Dimethylallylindole3carbaldehyde	-8.9	BE12406A	-11
FI3	-9.4	Lugdunomycin	-9.2	ActinofuranoneB	-8.9	StreptocarbazoleD	-11
JadomycinA	-9.4	MLR52	-9.2	Cryptolepinone	-8.9	19Methoxyxiamycin	-10.9
NiizalactamB	-9.4	NeoabyssomicinE	-9.2	CyclolTrplTyr	-8.9	9HydroxyK252c	-10.9
PhenazinolinA	-9.4	3NFormylholyrineA	-9.1	Heptanedioicacidbisbenzoyloxyamide	-8.9	Jadomycin	-10.9
RLDigitoxosylarcyriaflavinA	-9.4	5HydroxyherboxidieneA1	-9.1	NanaomycinbetaA	-8.9	Tan1030A	-10.9
Tan1030A	-9.4	9Hydroxyl3Nacetyl4Hydroxylstaurosporine	-9.1	Streptoseolactone	-8.9	12Dehydrotetracycline	-10.8
Uncialamycin	-9.4	BaraphenazineE	-9.1	2E114Aminophenyl59dihydroxy468trimethyl11oxoundec2enoicAcid	-8.8	3DemethylRK1409	-10.8
XiamycinE	-9.4	HolyrineA	-9.1	3Z6S3Benzylidene62Sbut2ylpiperazine25dione	-8.8	3HydroxyholyrineA	-10.8
3Hydroxy3NacetylholyrineA	-9.3	HolyrineB	-9.1	4Benzoxazolecarboxylicacid22hydroxy6methylphenyl	-8.8	BaraphenazineA	-10.8
3NFormylholyrineA	-9.3	Ikarugamycin	-9.1	ActinofuranoneG	-8.8	C07349	-10.8
9DAmicetosylrabelomycin	-9.3	K252aMe	-9.1	Anthraquinone	-8.8	Chelocardin	-10.8
AnsatrienolJ	-9.3	PhenazinolinA	-9.1	Cryptolepine	-8.8	EN7	-10.8
BMCHP	-9.3	PhenazinolinB	-9.1	GuanitrypmycinC11	-8.8	FluostatinH	-10.8
BorrelidinM	-9.3	4NFormyl7oxoholyrinA	-9	Kinobscurinone	-8.8	FormicapyridineA	-10.8
CapsimycinB	-9.3	7Hydroxystaurosporine	-9	Phenazine16dicarboxylicacid	-8.8	HatomarubiginA	-10.8
GuanitrypmycinB11	-9.3	AminoansamycinG	-9	RK286C	-8.8	Idarubicin	-10.8
Gutolactone	-9.3	AnanstrepA	-9	StreptorubinB	-8.8	L29141	-10.8
K252a	-9.3	BaraphenazineD	-9	6Hydroxyphenazine1carboxamide	-8.7	7Hydroxystaurosporine	-10.7
Piceamycin	-9.3	Gutolactone	-9	AminoansamycinF	-8.7	EchosideB	-10.7
RK1409B	-9.3	MycotrienolII	-9	BombyxamycinB	-8.7	NiizalactamB	-10.7
3NAcetylholyrineA	-9.2	17Demethoxyreblastatin	-8.9	C12451	-8.7	StreptocarbazoleE	-10.7
3ODemethyl4Ndemethyl4Nacetyl4epistaurosporine	-9.2	Griseoviridin	-8.9	Calarene	-8.7	AntibioticK252a	-10.6
7Oxostaurosporine	-9.2	GuanitrypmycinB12	-8.9	DiolmycinB1	-8.7	BE24566B	-10.6

BC194	-9.2	PhenazinolinC	-8.9	EntDDHK	-8.7	Gilvocarcina	-10.6
BaraphenazineE	-9.2	10epideOHHSAF	-8.8	EpiDDHK	-8.7	IzumiphenazineB	-10.6
BorrelidinA	-9.2	7OxoK252b	-8.8	LactomycinC	-8.7	Lugdunomycin	-10.6
DOlivosyljadomycinGly	-9.2	Aureoverticillactam	-8.8	Oxychloraphin	-8.7	LysoquinoneTH1	-10.6
EN7	-9.2	BaraphenazineC	-8.8	Terbinafine	-8.7	MDN0170	-10.6
Ethylisoallocholate	-9.2	BaraphenazineF	-8.8	Tuberine	-8.7	Resistoflavin	-10.6
Griseoviridin	-9.2	C12177	-8.8	TubermycinBsodiumsalt	-8.7	Staurosporineaglycone	-10.6
PhenazinolinD	-9.2	CytochalasinH	-8.8	3Acetonylidene7prenylindolin2one	-8.6	17Demethoxyreblastatin	-10.5
PontemazineA	-9.2	Eriamycin	-8.8	4Benzoxazolecarboxylicacid22hydroxy4methylphenyl	-8.6	7OxoholyrinA	-10.5
StreptocarbazoleD	-9.2	GilvocarcinM	-8.8	9Phenanthrenol	-8.6	CapsimycinB	-10.5
StreptocarbazoleE	-9.2	GuanitrypmycinC11	-8.8	ActinofuranoneH	-8.6	CytochalasinJ	-10.5
19Carbonylxiamycin	-9.1	IDMF	-8.8	AloesaponarinII	-8.6	EchosideD	-10.5
3NDemethyl4hydroxystaurosporine	-9.1	IzumiphenazineB	-8.8	Ambotz5500136	-8.6	Ekatetrone	-10.5
4DeacetylgriseusinA	-9.1	JadomycinA	-8.8	CHEBI70050	-8.6	GuanitrypmycinC11	-10.5
5FluorothaxtominA	-9.1	L29141	-8.8	CHEMBL1268	-8.6	Gutolactone	-10.5
6epiAlteramideB	-9.1	Oxiamycin	-8.8	CHEMBL2180322	-8.6	HolyrineA	-10.5
9Hydroxy3NacetylholyrineA	-9.1	1222Methyl5propan2ylphenoxyethoxyethylimidazole	-8.7	CHEMBL381959	-8.6	MLR52	-10.5
AminoansamycinF	-9.1	28NMethylikarugamycin	-8.7	FerroverdinC	-8.6	MayamycinB	-10.5
AnthracimycinBII2619	-9.1	A204sodium	-8.7	GuanitrypmycinB11	-8.6	Naphthopyranomycin	-10.5
AranciamycinG	-9.1	AnthracimycinBII2619	-8.7	Panobinostat	-8.6	Oviedomycin	-10.5
BE24566B	-9.1	AntibioticX14885A	-8.7	Z64Hydroxy3methylbut2en1ylindolin2one	-8.6	Phebestin	-10.5
BorrelidinH	-9.1	Aotaphenazine	-8.7	12Deoxo12hydroxy8Omethyltetrangomycin	-8.5	Progeldanamycin	-10.5
CHEMBL400132	-9.1	AranciamycinG	-8.7	1Phenazinecarboxylicacid	-8.5	RK286C	-10.5
Diastaphenazine	-9.1	BaraphenazineG	-8.7	Albidopyrone	-8.5	3• Methyltetraphene• 167812• pentol	-10.4
GuanitrypmycinB12	-9.1	KapurimycinA3	-8.7	CHEMBL94678	-8.5	CHEBI70050	-10.4
IDMF	-9.1	Lavendamycin	-8.7	CarpatamideH	-8.5	DOlivosyljadomycinGly	-10.4
K252d	-9.1	NocardioazineB	-8.7	Desoxyerythrolaccin	-8.5	Griseoviridin	-10.4
KapurimycinA3	-9.1	PenicisteroidC	-8.7	Flavanone	-8.5	HatomarubiginB	-10.4
Kinanthraquinone	-9.1	12Dehydrotetracycline	-8.6	Gutolactone	-8.5	IsofuranonaphthoquinoneG	-10.4

LansaiB	-9.1	19Carbonylxiamycin	-8.6	NSC247562	-8.5	JadomycinA	-10.4
MDN0170	-9.1	AnandinB	-8.6	RK270C	-8.5	Lactimidomycin	-10.4
MayamycinB	-9.1	BorregomycinC	-8.6	StreptoglutarimideJ	-8.5	NaphthacemycinA1	-10.4
Metacycloprodigiosin	-9.1	BorrelidinCR1	-8.6	1Phenazinecarboxylicacidmethylester	-8.4	PhenazinolinB	-10.4
NocardioazineB	-9.1	Borrelidin	-8.6	1Phenazinylaceticacid	-8.4	Phenazoviridin	-10.4
PhenazinolinB	-9.1	Butylcycloheptylprodigiosin	-8.6	4Benzoxazolecarboxylicacid22hydroxy5methylphenyl	-8.4	Sapurimycin	-10.4
PhenazinolinE	-9.1	CytochalasinJ	-8.6	4ZAnnimycin	-8.4	TiancimycinA	-10.4
SCHEMBL13009061	-9.1	DOlivosyljadomycinGly	-8.6	ActinoquinolineA	-8.4	8OMethyltetrangulol	-10.3
StreptorubinB	-9.1	Diastaphenazine	-8.6	Caboxamycin	-8.4	AnsatrienolK	-10.3
7Hydroxystaurosporine	-9	Ekatetrone	-8.6	Chalcone	-8.4	BaraphenazineG	-10.3
7OxoholyrinA	-9	FluostatinH	-8.6	Flavone	-8.4	BorregomycinB	-10.3
AbyssomicinQ	-9	IIQB	-8.6	FridamycinF	-8.4	ElmenolG	-10.3
AnthracimycinB	-9	Lavendamycinmethylester	-8.6	HeraclemycinD	-8.4	FL120B	-10.3
BaraphenazineG	-9	MayamycinB	-8.6	HydroxymariloneC	-8.4	GilvocarcinM	-10.3
BorregomycinD	-9	NAcetylNdemethylmayamycin	-8.6	Lactimidomycin	-8.4	GuanitrypmycinA12	-10.3
C12177	-9	Piceamycin	-8.6	Physcion	-8.4	HolyrineB	-10.3

On the sterptomeDB database, there were a total of 6000 compounds present. The compounds were filtered based on Lipinski's rule of five and 3173 were retrieved. The docking was performed with the 3173 compounds with the targets, out of which the top 250 compounds that are mentioned in the above Table were considered based on their low binding energies. Finally, the 4 compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1, and Gutolactone) that were in common interactions with the targets (COX1, ACE-2, PDE3 & α2AP) were identified.

(q)



(r)

	ACE2	antiplasmin	cox1	PDE3
Aminoansamycin F	-9.1	-9.4	-8.7	-11.1
Dolivosyljadomycin Gly	-9.2	-8.6	-9	-10.4
GuanitrypmycinC1-1	-9.5	-8.8	-8.8	-10.5
Gutolactone	-9.3	-9	-8.5	-10.5

Figure 5.1 Image (q) represents the Venn diagram of the acquired four targets (COX1, ACE-2, PDE3 & α2AP) and the image (r) shows the docking score generated by the Auto dock Vina.

There was a total of top 4 compounds selected from the list of compounds obtained after the filtering of the Lipinski rule of five. These 4 compounds have no violation and matched every rule of Lipinski.

Table 5.3. Identified lead compounds from sterptomeDB database with their physiochemical properties

S. No	Name	Molecular weight	LogP	HBD	HBA
1	Aminoansamycin F	422.43058	2.1585	4.0	8.0
2	D-Olivosyl-jadomycin-Gly	493.46212	1.5353	3.0	10
3	Guanitrypmycin C1-1	482.49398	1.594	5.0	9.0
4	Gutolactone	476.51618	0.449	3.0	9.0

5.3 Molecular docking

The identified four lead compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone) were docked with each of the four targets (COX-1, ACE2, PDE3 & α2AP) individually using Auto dock Vina. The purple arrow in the Fig 5.1 (I) indicates the Hydrogen bond formation between the lead molecule and the target in the required active site.

(I)

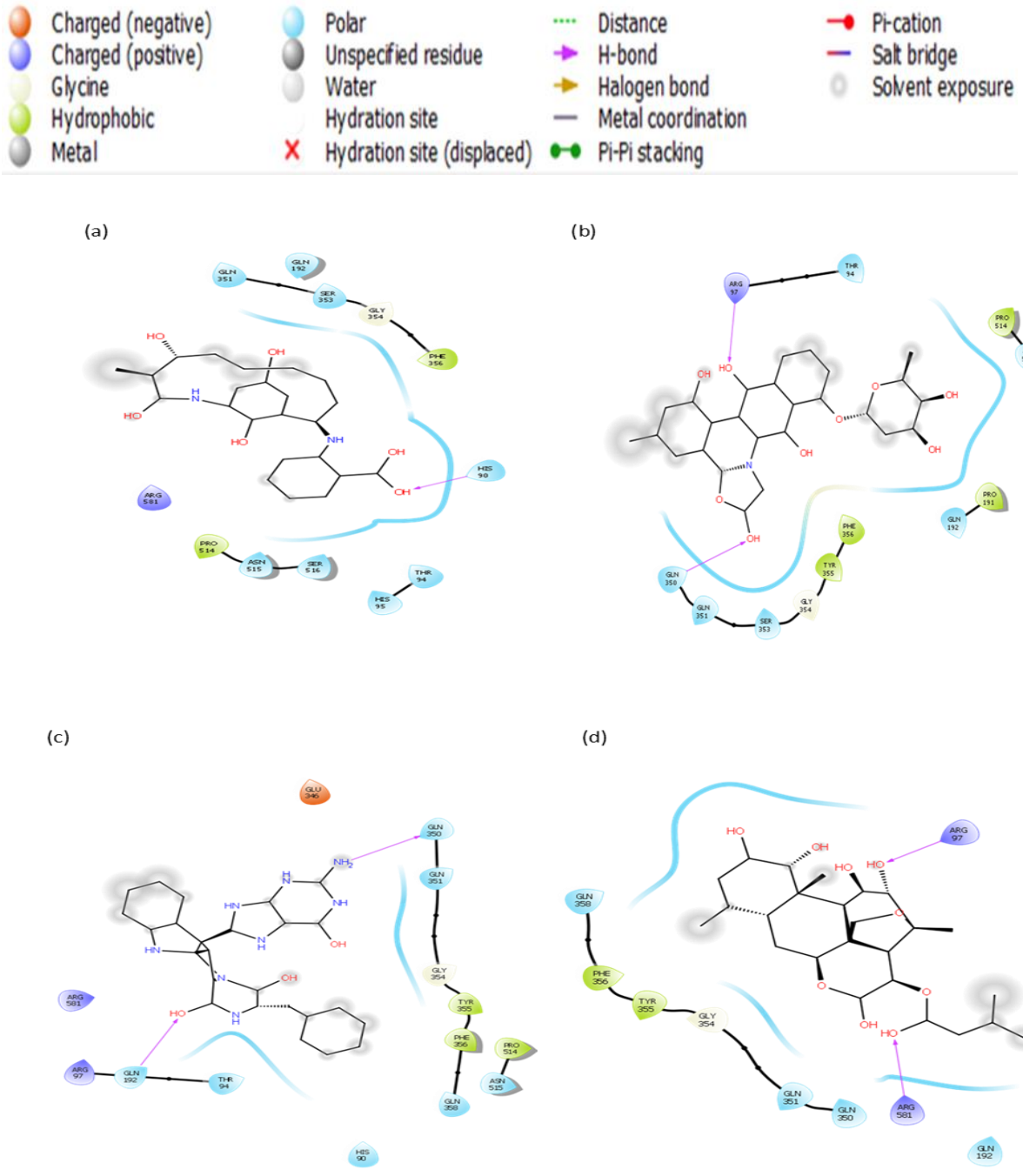


Figure 5.2 The image (ɪ) shows the information of Amino acid, bonds and ions. The images from (a) to (d) represents the 2-D diagram of the interactions between the target (COX1) and lead compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone)

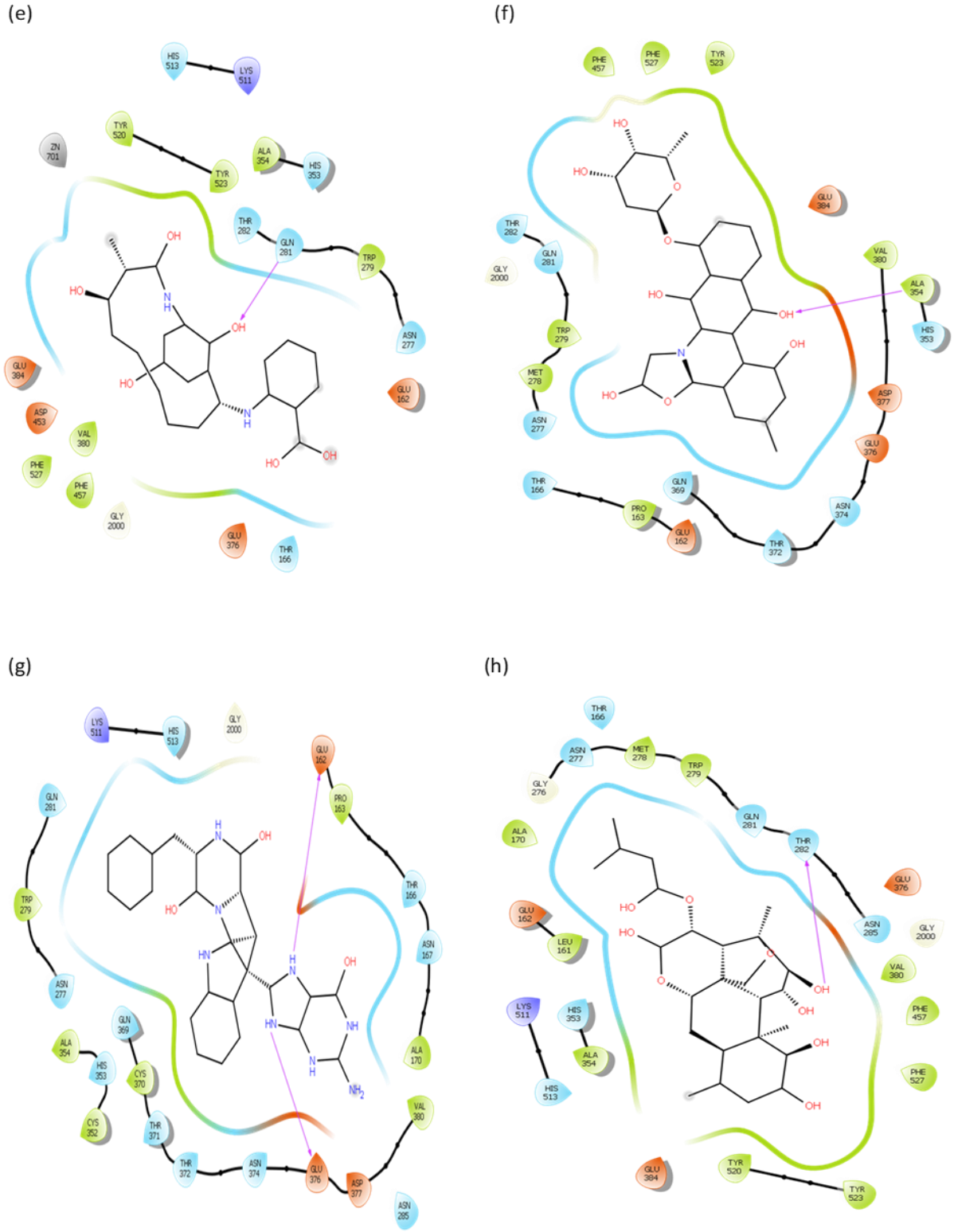
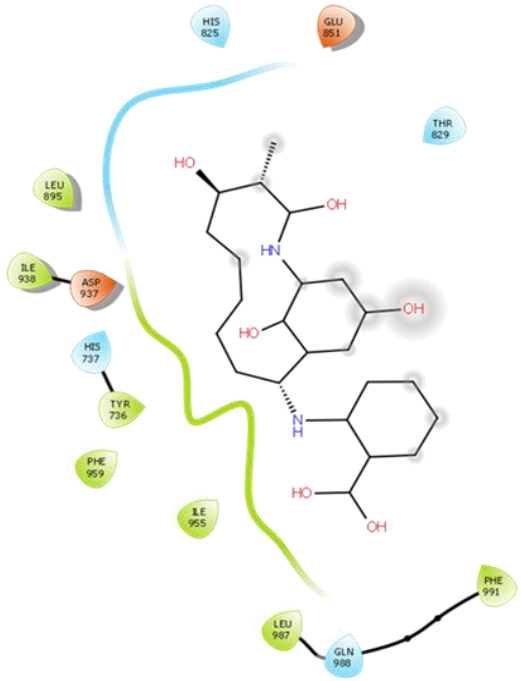
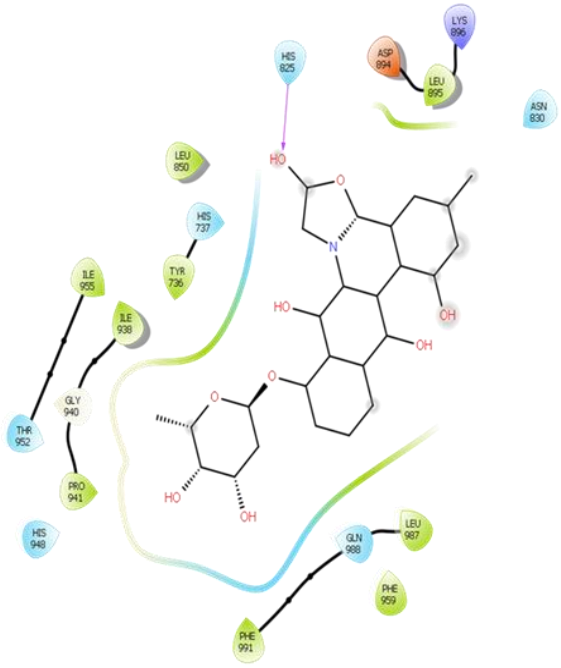


Figure 5.3 The images from (e) to (h) represents the 2-D diagram of the interactions between the target (ACE-2) and lead compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone)

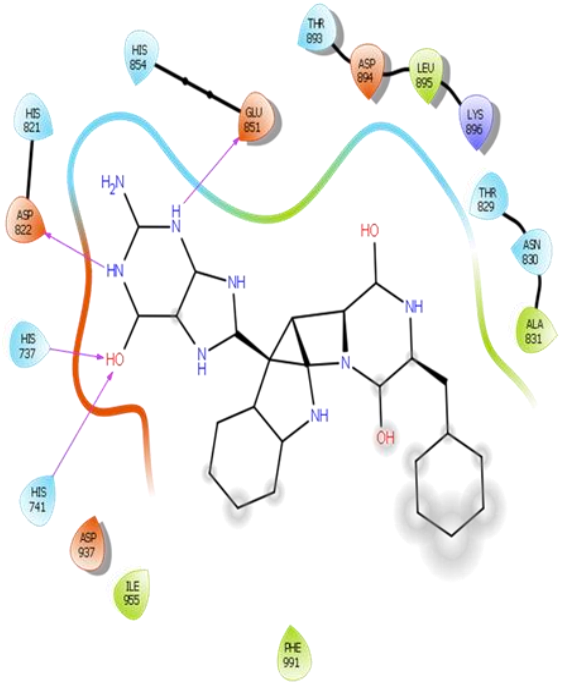
(i)



(j)



(k)



(l)

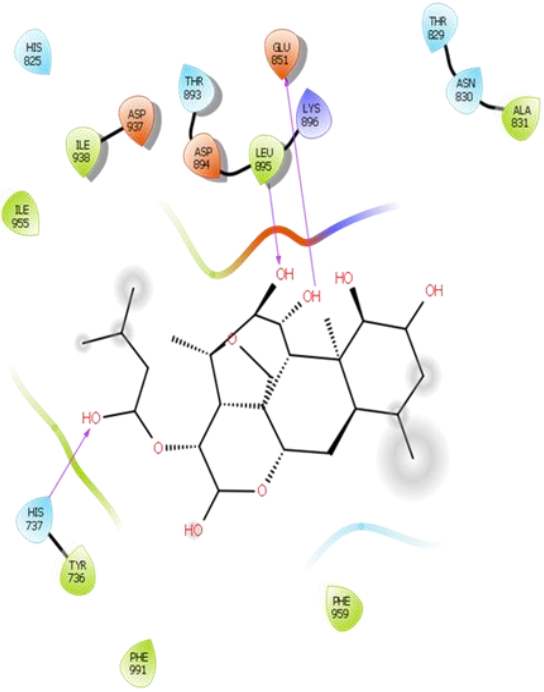


Figure 5.4 The images from (i) to (l) represents the 2-D diagram of the interactions between the target (PDE3) and lead compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone)

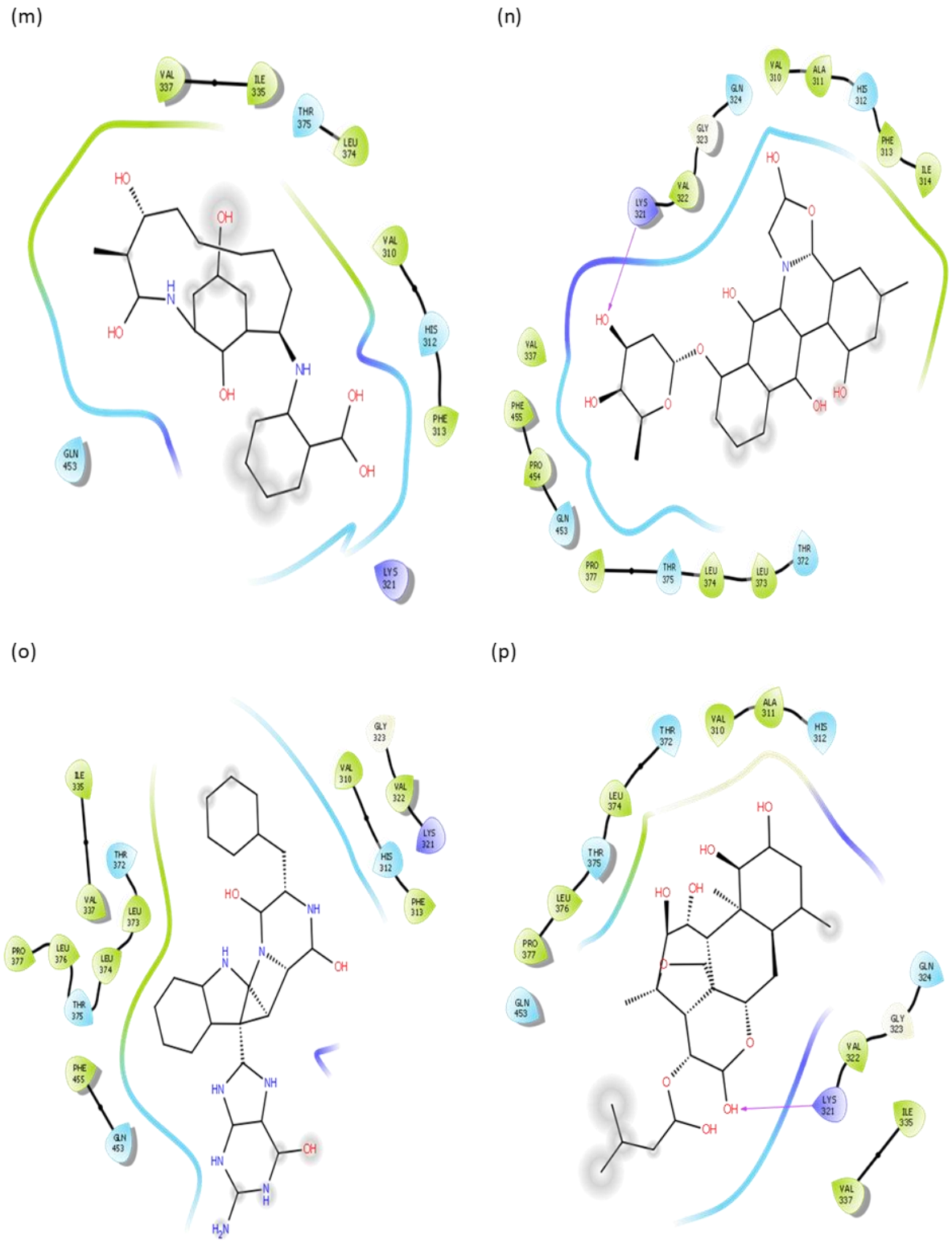


Figure 5.5 Figure 5.3 The images from (m) to (p) represents the 2-D diagram of the interactions between the target (α2AP) and lead compounds (Aminoansamycin F, D-Olivosyl-jadomycin-Gly, Guanitrypmycin C1-1 & Gutolactone)

The active sites for the target (COX1) are ALA527, ARG120, HIS90, ILE523, LEU352, LEU359, LEU384, PHE518, TRP387, TYR355, TYR385, VAL116, & VAL349. The hydrogen bond formation for the target (COX1) with the lead molecules (a) Aminoansamycin F were HIS90, (b) D-Olivosyl-jadomycin-Gly were ARG97 & GLN350, (c) Guanitrypmycin C1-1 were GLN192 & GLN350 and (d) Gutolactone were ARG97 & ARG581.

The active sites for the target (ACE-2) are ALA35, GLU376, HIS353, HIS513, LYS511, PHE457, THR282, TRP279, & VAL380. The hydrogen bond formation for the target (COX1) with the lead molecules (e) Aminoansamycin F were GLN281, (f) D-Olivosyl-jadomycin-Gly were ALA354, (g) Guanitrypmycin C1-1 were GLU162 & GLU376 and (h) Gutolactone were THR282.

The active sites for the target (PDE3) are GLN988, HIS948, ILE938, ILE955, LEU895, PHE991, PRO941, SER990, & THR952. The hydrogen bond formation for the target (COX1) with the lead molecules (i) Aminoansamycin F; no hydrogen bond interaction was found, (j) D-Olivosyl-jadomycin-Gly were HIS825, (k) Guanitrypmycin C1-1 were HIS737, HIS741, ASP822 & GLU851 and (l) Gutolactone were HIS737, GLU851, & LEU895.

The active sites for the target (α2AP) are ILE335, ILE379, LEU333, LEU376, LEU425, LEU427, LEU459, MET303, MET458, PHE455, PHE457, PRO377, THR428, & TRP277. The hydrogen bond formation for the target (COX1) with the lead molecules (m) Aminoansamycin F; no hydrogen bond interaction was found, (n) D-Olivosyl-jadomycin-Gly were LYS321, (o) Guanitrypmycin C1-1; no hydrogen bond interaction was found and (p) Gutolactone were LYS321.

5.4 Discussion

In silico molecular docking is a computational approach which clearly predicts the bonding between two different particles. This method included, algorithms like molecular stimulation, molecular dynamics and fragment-based methods. In recent years, many research works have been focused on the development of antithrombolytic (antiplatelet and anticoagulant) activity however *in silico* molecular docking of these compounds were limited to develop drugs against cardiovascular diseases such as stroke. Molecular docking studies revealed good docking score, glide energy and glide model. Aminoansamycin F showed maximum docking score. The maximum negative glide energy is highly favourable and the docking score of Aminoansamycin showed great compliances. Prediction of interaction energies between receptor and ligand are the important task in molecular docking (Babaheydari et al., 2013). Virtual screening uses docking and docking scores of each and every analyzed compound. In silico molecular docking is a computational method that accurately forecasts the interaction of two distinct particles. Algorithms such as molecular stimulation, molecular dynamics, and fragment-based approaches were used in this method. Many studies have been carried out over the past few decades to develop antithrombolytic (antiplatelet and anticoagulant) activity, but in silico molecular docking of these compounds has been limited in order to develop drugs against cardiovascular diseases such as stroke. Molecular docking investigations found that the docking score, glide energy, and glide model were all satisfactory. The highest docking score was achieved by aminoansamycin F. The highest negative glide energy is extremely advantageous, and Aminoansamycin's docking score demonstrated strong compliances. The prediction of interaction energies between receptor and ligand is a critical step in molecular docking (Babaheydari et al., 2013). Docking is employed in virtual screening and docking scores for each analysed chemical. The method used is based on predicting the binding affinities and modes of a given molecule to a target via docking to an X-ray crystallographic structure. The technique relies on docking to an X-ray crystallographic structure to forecast the affinity for binding and pathways that connect a particular molecule to a target. Lines between protein residues and ligand atoms were used to explain the ligand-protein interaction. Plant phytochemicals comprise a variety of substances such as flavonoids, steroids, and phenols, which form complexes with various soluble proteins. Glycosides

in the plant extract were found to lower blood pressure and to be cardioprotective (Gong et al., 2011, Cheng et al., 2003, Okwu, 2001). Molecular docking is a highly effective method for determining the affinity of the therapeutic agent selected and a macromolecular target site. The hydrophobic interaction, Van der Waal's forces, and electrostatic attraction forces were used to calculate the glide score (Elokely and Doerksen, 2013). Lipinski's rule of five is applied to assess the chemical properties of the lead molecule, which influence membrane permeability and define lead molecule bioavailability within cells (Ursu et al., 2011 All of the lead compounds used in this experiment followed Lipinski's rule of five.

CHAPTER 6

SUMMARY & CONCLUSION

The lead molecules obtained from the natural products of Streptomyces bacteria shows high binding affinity for inhibiting the four targets. This is the first step in identifying novel compounds for the disease target, however further advanced animal studies and pre-clinical studies are required to check the efficacy of the compound in the biological system, in the future these lead compounds may be evolved as potential drugs for the treatment of Ischemic stroke.

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