EXPLORING MOLECULAR TARGETS FOR REPOSITIONING OF HYPERTENSIVE DRUGS

A PROJECT REPORT

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF

THE DEGREE

OF

MASTER OF TECHNOLOGY IN
BIOINFORMATICS ENGINEERING

Submitted by:

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JUNE-2020

CANDIDATE'S DECLARATION

I Bhawna Singh, 2K18/BIO/02 of M.Tech (Bioinformatics), hereby declare that the project Dissertation titled "EXPLORING MOLECULAR TARGETS FOR REPOSITIONING OF HYPERTENSIVE DRUGS" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirements for the award of the degree of Master of Technology, is original and not copied from any source with proper citation. This work has not previously formed the basis for the award of the Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation titled **"EXPLORING MOLECULAR TARGETS FOR REPOSITIONING OF HYPERTENSIVE DRUGS"** which is submitted by Bhawna Singh (2K18/BIO/02),Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ABSTRACT

Drug repositioning or drug repurposing or drug profiling is the discovery of new applications for approved or failed drug.. Drug repositioning is the development of new approved drug applications.The cost of bringing a medicine to the market is around one million which include clinical and preclinical trials.Repositioning of drugs help in cutting down costs as well as time involve in intial validation and authorization . The procedure involved in Drug repositioning is generally performed during the drug development phase to modify or extend an active molecule's distribution line. On a fundamental level, repositioning opportunities exist because drugs perturb multiple biological entities and engage themselves in multiple biological processes. Therefore, a drug can play multiple roles or perform a various mode of actions that are responsible for its pharmacology.Hypertension, is a condition that causes increase in the risk of cardiovascular diseases. In this study an attempt has been made to reposition hypertensive drugs for different diseases by exploring molecular targets of hypertensive drugs.Consider that they often need to be administered for long periods of time, often over whole life timeSide effects although present, have been found safe enough to be used for such long durations, hence repurposing these drugs for other diseases may be beneficial with limited side effects.

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LIST OF ACRONYM

ACRONYM NAMES	DESCRIPTION
PDB	PROTEIN DATA BANK
GPCR	G PROTEIN COUPLED RECEPTOR

Chapter 1 INTRODUCTION

The standard method of drug production requires vast quantities of time and energy before a compound is labored into the the market . Despite huge investments a lead molecule often has has minimal chances of entering the open market. The research molecule's itinerary remains unpredictable in its entire lifecycle. This situation causes pharmaceutical companies to discover new drugs on dreams.Drug g repositioning is one of the feasible choices for beginners in the area of new drug science .Drug discovery is the process of identification of biologically active small molecules against different disease conditions.

Classical drug discovery starts from the identification of disease target, lead compound identification and optimization, ADMET studies and finally to market. Developing a Single molecule may take 10−17 years and the success rate can be as low as 0.01%.. The global annual budgets of R&D became $ 130 billion with fewer new drugs. The numbers of new drugs or New Molecular Entities (NME) released in the market are decreased and there is acute pressure on the R&D circle to increase the number of candidate drugs in the late stage pipeline.

These NMEs have to go through a number of pharmacokinetic and toxicity studies for their release into market. Molecules with potential drug like activities are evaluated simultaneously for their toxicity effects in cell and animal models.

After a strenuous and systematic evaluation of drug activity and other properties, several drug like molecules may have to be dropped because of undesired bio-distribution and toxicity.A new concept called “drug repositioning” is being emerged in the pharmaceutical R&D circle. Drug repositioning is the process of finding new uses outside the scope of the original medical indication for which the drug is prescribed A repositioned drug can go directly to preclinical testing and clinical trials and save the initial 6−9 years essentially needed for the development of a new drug, with reduced risk and costs..

Examples of drug repositioning

Drugs	MECHANISM OF ACTION	ORIGINAL INDICATION	NEW INDICATION
Duloxetine	Non-selective Serotonin reuptake inhibitor	Depression	urinary incontinence
Sibutramine	Non - selective Serotonin reuptake inhibitor	Depression	Obesity
Paclitaxel	Attaches polymerization of tubulin	Cancer	Restenosis

Chapter2 REVIEW OF LITERATURE

2.1HYPERTENSION

High blood pressure, also called vital signs (HBP), can be a long-term medical condition when the vital sign within the arteries rises continuously. High blood pressure generally does not cause any symptoms. Prolonged high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, atria fibrillation, peripheral arterial disease, vision loss,chronic kidney disease, and dementia. Significantly higher signaling is assessed as primary (significant) hypertension or hypertension. About 90-95% of primary cases, described as a high appreciation of the importance of an unhealthy lifestyle and genetic factors. Increasing risk factors include excessive salt intake, excess weight, smoking, and alcohol abuse.The remaining5-10%ofcasesareclassifiedas secondary,which is defined as one of the most important secondary symptoms, such as chronic mental disorders, decreased kidney function, mental disorders, or the use of birth control pills. Blood pressure is expressed by twomeasurements, systolic and diastolic pre ssures, which are very high and low pressures, respectively. In most adults, the most common symptom of rest is within the range of 100-130 millimeters ofmercury(mmHg)systolicand 60-80 mmHg diastolic.Inmany adults, high blood pressure is present if the effective blood pressure persists at or above140 / 90 mmHg.

2.2TARGETS FOR HYPERTENSION

2.2.1CALCIUM CHANNEL

Calciumblockers reduce bloodpressure bypreventing calciumfrom enteringthe heart cells and blood vessels. Calcium causes the guts and nerves to determine more tightly. By blocking calcium, calcium blockers allow the blood vessels to relax and open. Some calcium blockers on the site have an additional chance of reducing your heart rate, which can lower your blood pressure, decrease chest pain (angina), and control irregular heartbeat.CCB decreases blood pressure by reducing the calcium limit or the rate at which calcium resides in the heart muscle and cell walls that are not like that. Calcium stimulates the heart to enter into a stronger contract. When calcium mobility is limited, your heart's contractions are weak with each stroke, and your blood vessels can relax. This leads to a reduction in blood pressure. Calcium blockers are drugs used to lowerblood pressure.They work byslowing the flow of calcium into the guts' cells and the vessel's walls, making it easier for the guts to pump and increase blood vessels. Because of this, guts do not require hard work and vital signals.

2.2.2BETA ADRENERGIC RECEPTOR

Beta-adrenergic receptors are found in cells of guts tissue, smooth muscle, nerves, kidneys, and other tissues that are part of the sympathetic system and causestress responses, especially whe n stimulated by epinephrine (adrenaline). Beta-blockers disrupt the binding of epinephrine receptors and otherstress hormonesand weaken the effects of stress hormones. Beta-blockers work by blocking the effects of the hormone epinephrine. Beta-blockers make your heart beat faster and with less energy, lowering your blood pressure. Beta-blockers also help open up your arteries and blood vessels to improve blood flow.

2.2.3ALDOSTERONE RECEPTOR

Aldosterone is a hormone protected by the adrenal gland. The molecule is involved in signaling that regulates blood pressure. The basic site of aldosterone activation is mineralocorticoid receptors in the epithelial kidney cells within the proximal cells of the distal tubule and the collecting ducts.
The main action of aldosterone is the storage of sodium and water, while aldosterone can also stimulate myocardial fibrosis and promote cardiac hypertrophy and remodeling. Also, aldosterone can directly alter endothelial function by reducing gas availability and stimulating the vascular inflammatory response.

2.3.4ANGIOTENSIN CONVERTING ENZYMES

ACE inhibitors produce vasodilation by inhibiting the formation of angiotensin II. This vasoconstrictor is ac tivated by the proteolytic action of renin (released by the kidneys) by circulating angiotensinogen to form a angiotensin I. Angiotensin I then convert to angiotensin II by angiotensinconverting enzyme.Angiotensin II b uildsup arteries and arteries by binding to AT receptors located in thesympathetic muscle, which is coupled to the Gq protein and thusan IP3 signal transductionpathway. Angiotensin II also facilitates the release of norepinephrine from sympathetic adrenergic pain and prevents the regeneration of norepinephrine through these nerves. This effect of angiotensin II augments is a sensitive function in the guts and blood vessels.ACE inhibitors Dilate artery and veins by blocking angiotensinII formationand prevents bradykinin metabolism. This vasodilation reduces blood pressure, preloading, and loading back into the guts.

2.3TYPES OF RECEPTORS

2.3.1Ion Channel-Linked Receptors

The channel-coupled receptors bind tothe ligand and openthe channel through amembrane that allows certain ions to interact. To construct the channel,this type of cell-less receptor has a deep membrane spanning region.In order tointeract with the phospholipid carboxylic acid tails that form in the center of the cell wall, many amino acids within the membrane-spanning regionare hydrophobicin nature. On the other hand,the amino acids that comprisetheinnerchannellineare hydrophilic to allow the passage of water or ions. When the ligand binds tothe outer region of the channel, within the protein structure that allows the ions such as sodium, calcium, magnesium, and hydrogen to pass through.On theotherhand, the

amino acids that line the insideofthe channelarehydrophilic to allowforthesection of water or particles.At the point whena ligand ties to the extracellular area of the channel, there's a conformational change inside the protein's structure that licenses particles like sodium, calcium,magnesium, and hydrogen to go through.

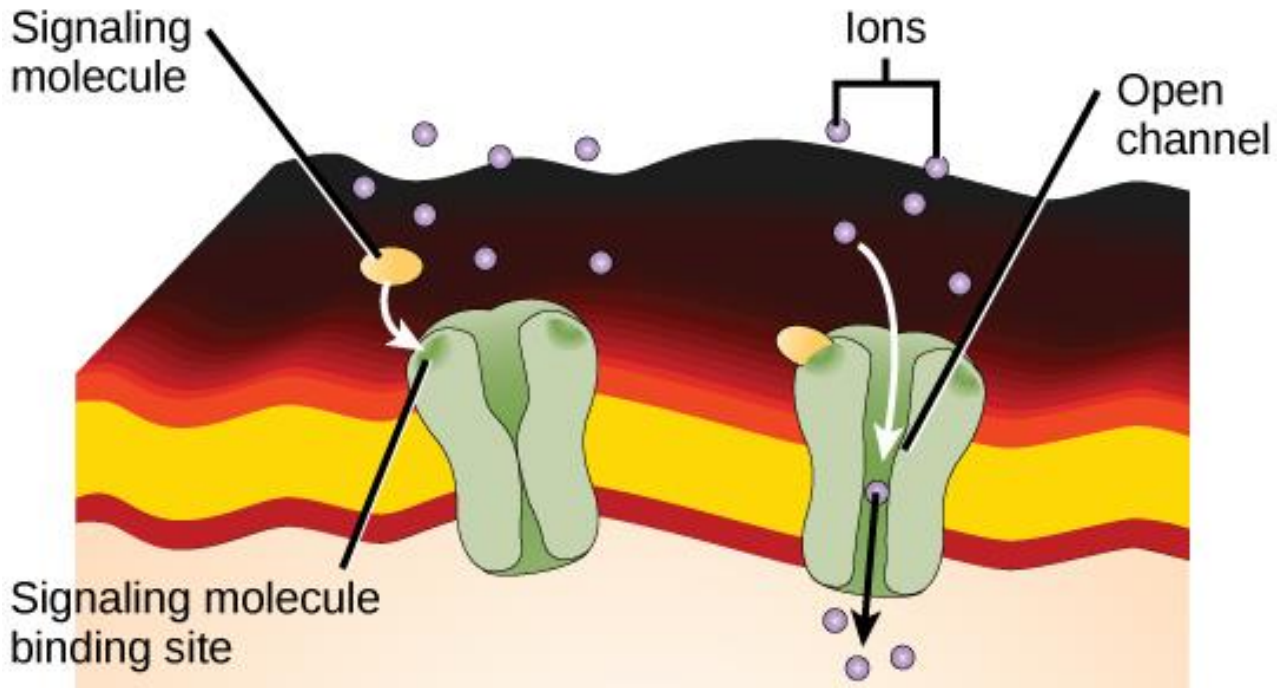


FIGURE1:IONCHANNEL LINKED RECEPTOR

2.3.2GPCR

G-protein-related receptors bind tothe ligand and activate a membraneproteincalled G-protein. The G-protein used then contacts the ion channel or enzymeinside the membrane. All G-proteincoupled receptors have seven transmembranedomains, but each receptor has its own unique extracellular domain and G-protein-binding site. -

CellsignaingusingGproteincoupled receptorsoccursas a series of cyclicevents. Before the ligand binds, the ina ctive Gprotein canbind the newly exposed site to a specific receptor through its binding. When G protein binds to areceptor, the active state change activates Gprotein, which releases GDP anddow nloads GTP. G-protein self-assembly then diffuses into the α subunit and thus the β subunit. One or both of those G-protein fragments can also be ready to use other proteins as a result. Later, GTP in the active G-protein signal is activated in GDP and thus the β subunit is inactive. Humidity re-assembles to make protein-G inactive, and as a result the cycle starts over.

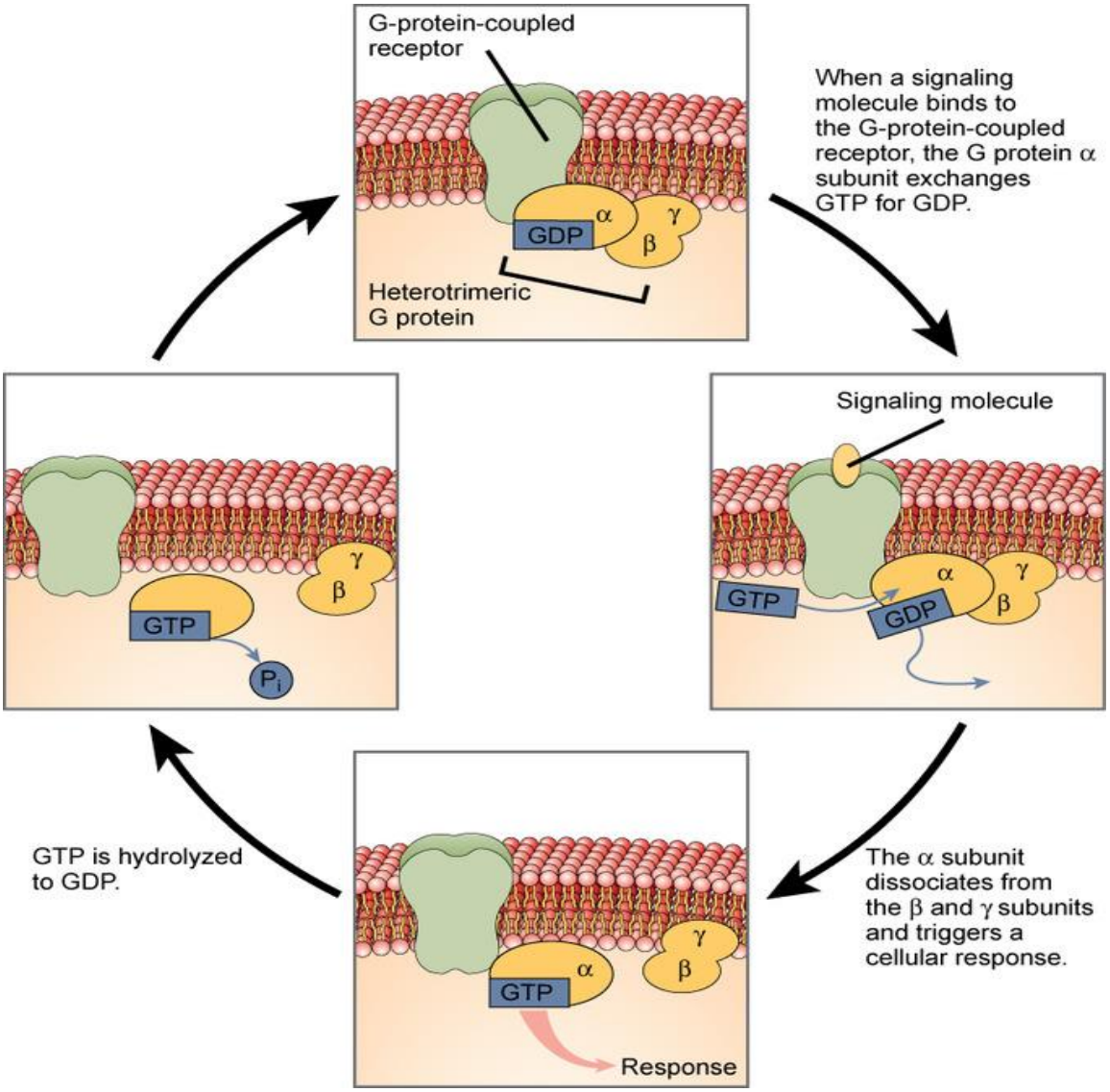
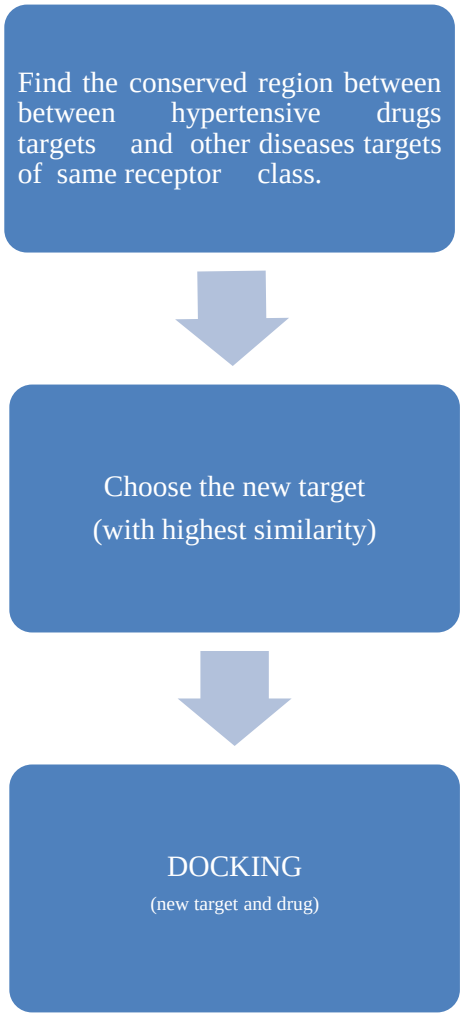


FIGURE2:GPCR

2.3.3ENZYME LINKED RECEPTOR

The enzyme-linked receptors are cell-surface receptors that have intrinsic enzyme-related domains. In some cases, the intracellular domain of the receptor itself isan enzyme or enzyme-linked receptor has an intracellular domain that interactsdirectly with the enzyme. Enzyme-linked receptors usually have large external and internal domains, but the membrane-bounding region contains a single alpha-helical strand region of the peptide strand. When the ligand binds to the outer domain, the signal is passed through the membrane and activatesthe enzyme, which triggers the sequence of events within the cell that eventuallyleads to the reaction.

Chapter 3 METHODOLOGY


Find the conserved region between between hypertensive drugs targets and other diseases targets of same receptor class.
Choose the new target
(with highest similarity)
DOCKING
(new target and drug)

3.1 BLAST

Find the conserved region between hypertensive drugs targets and other diseases targets of same receptor class. and select the new targets having highest similarity with hypertensive drug targets.

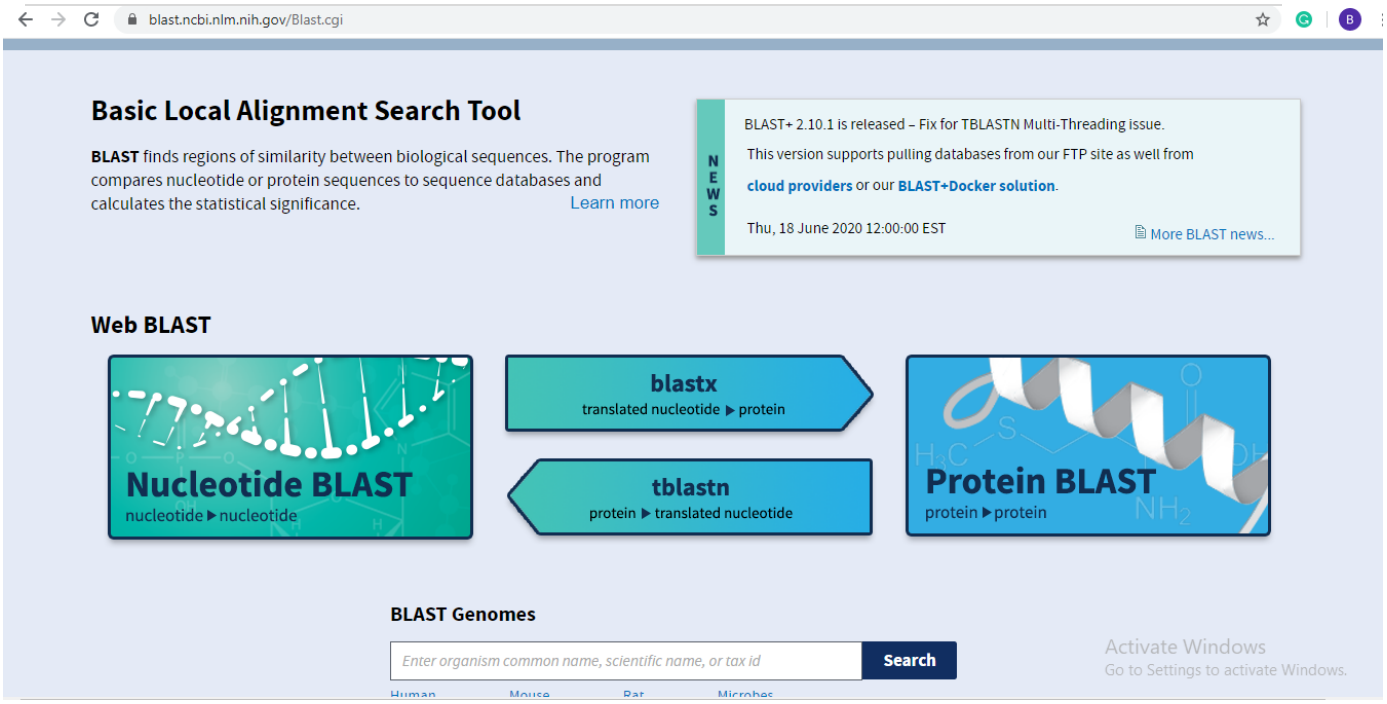


FIGURE3:BLAST

3.2Target from PDB

X ray crystallographic structure of all the new targets protein was downloaded from protein databank(http://www.rcsb.org).

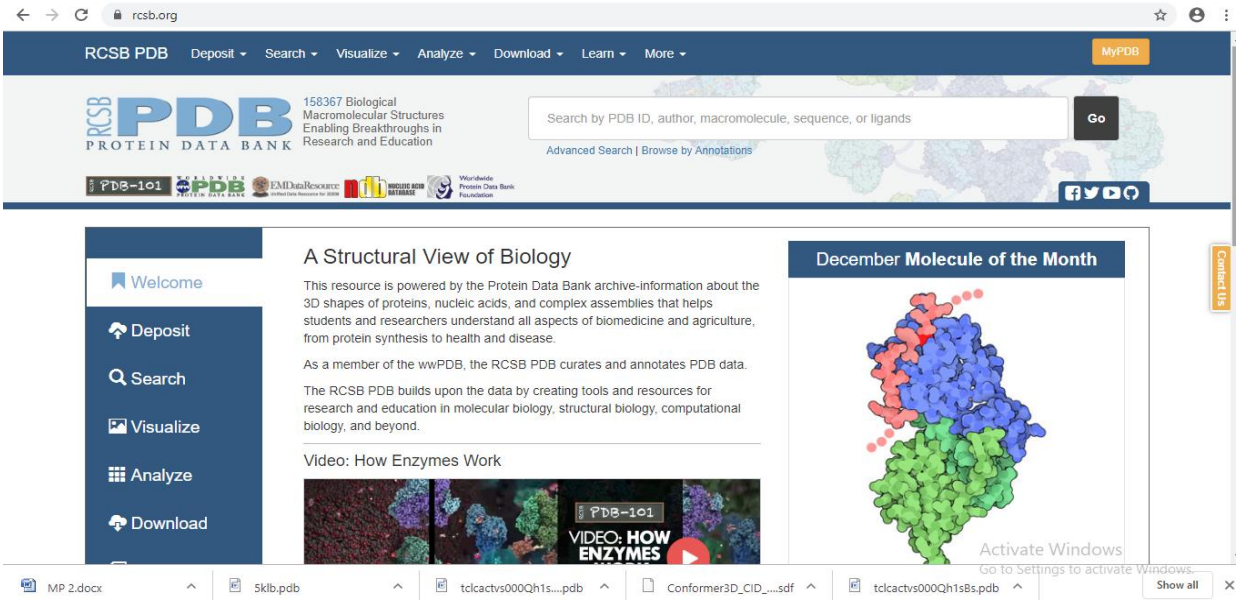


FIGURE4:PDB

3.3 Ligand from PUBCHEM

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Structure of hypertensive drugs were retrieved from PubChem database(http://pubchem.ncbi.nlm.nih.gov).

These drugs for hypertension were downloaded in SDF format and converted into PDB.

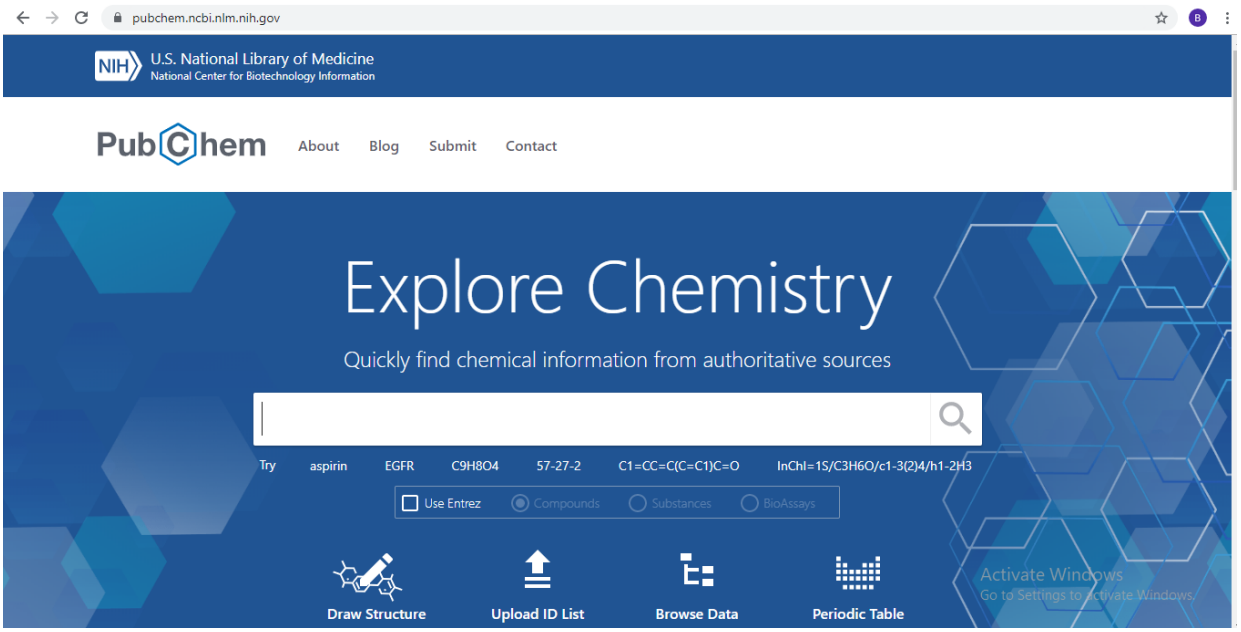

FIGURE5:PUBCHEM

3.4docking

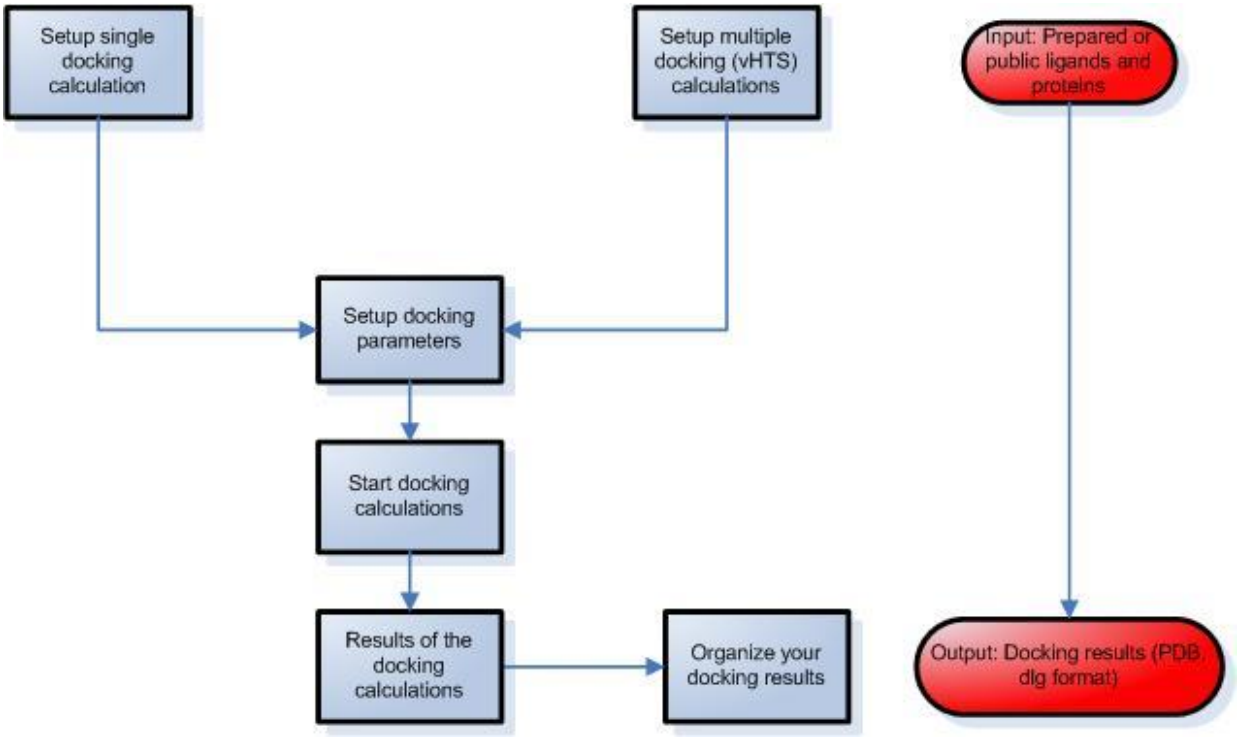

FIGURE6: DOCK PREP

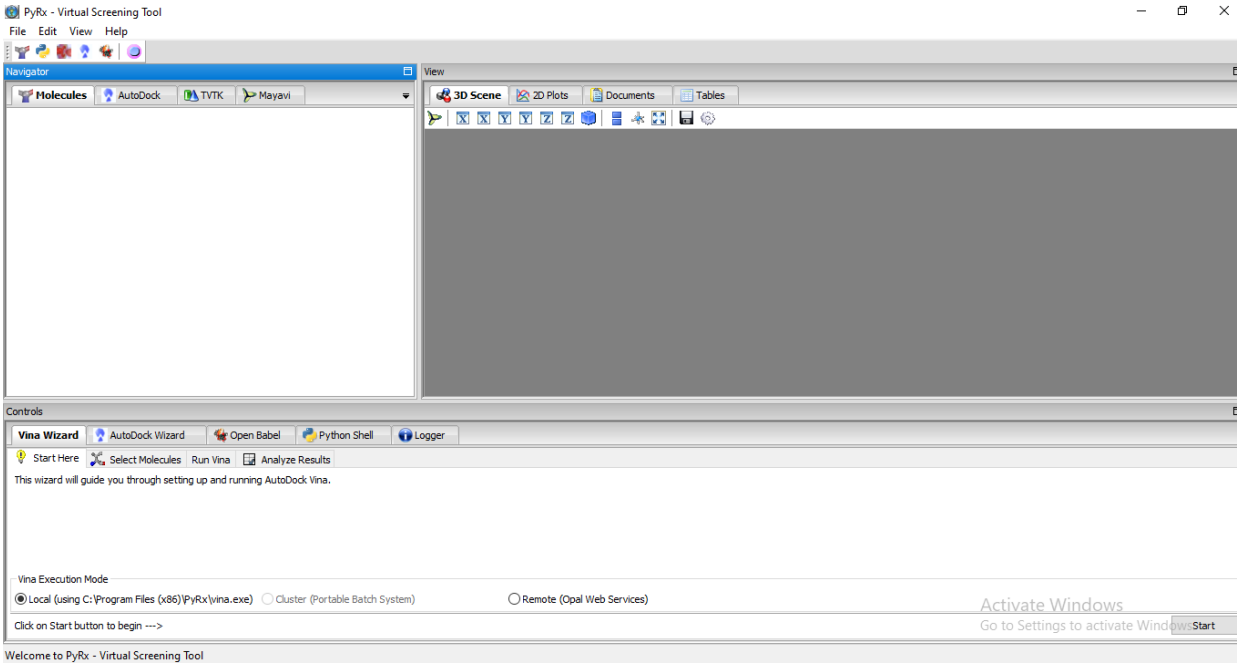

FIGURE7 :PYRX

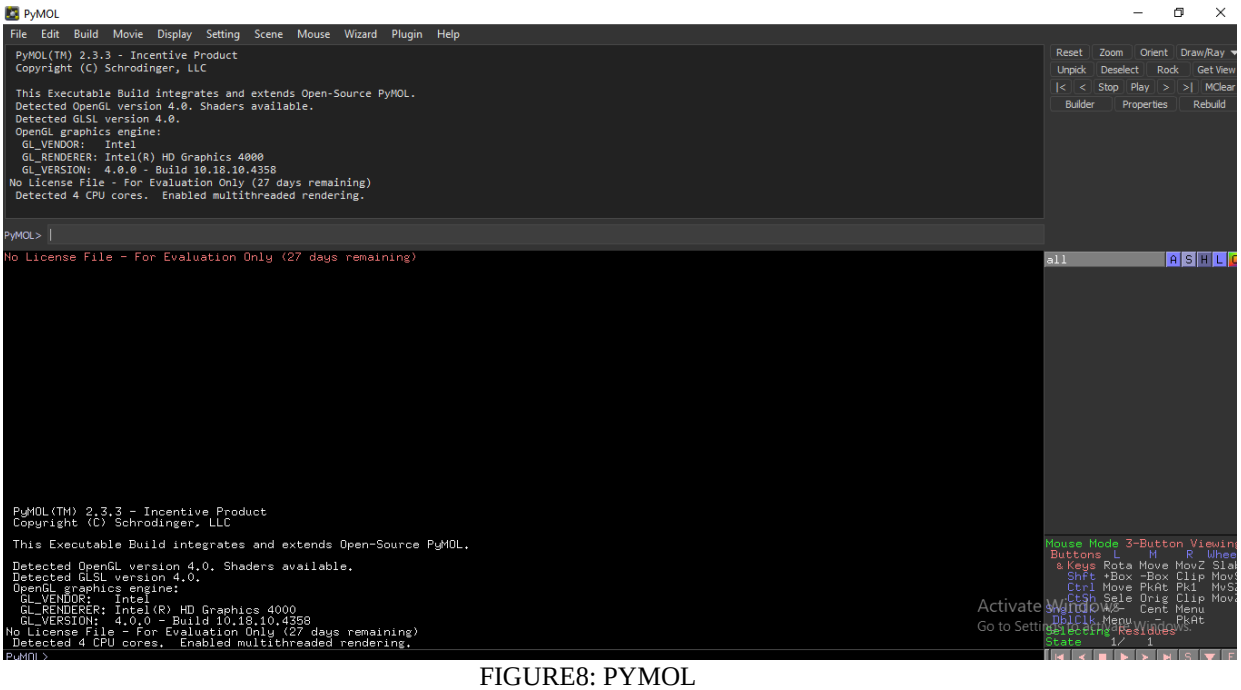

FIGURE8: PYMOL

Chapter 4 RESULTS AND DISCUSSION

Drug discovery is an expensive and time consuming process.This process preceeds through three stages ,first discovery where new compounds are screened and identified then preclinical stages where compounds are tested invitro and animal models and clinical development drugs tested in human beings. Using already existing drugs for different diseases reduced development cost and time due to availability of previously collected pharmokinetics,toxicology and safety data.

Nowdays ,due to hectic and busy life hypertension or high blood pressure become very common problem mainly in youngster.Hypertension is associated with increased cardiovascular risks which include heart attack and stroke.Hypertension is a major cause of premature mortality. consider that they often need to be administered for long periods of time, often over whole life timeSide effects although present, have been found safe enough to be used for such long durations, hence repurposing these drugs for other diseases may be beneficial with limited side effects.

No new antihypertensive medication has been introduced to clinical practice sine 2007therefore drug repurposing of already existing drugs for hypertension become very important.

5.1 DRUGS FOR HYPERTENSION

Table 2 shows repositioned drugs for hypertension(From minor projects). These are different drugs that worked for different diseases here, we repositioned these drugs as antihypertensive agents.

ACCESSION NO	DRUG	DISEASES	TARGETS
DB00555	Lamotrigine	Bipolar disorder	Calcium channel
DB12093	Tetrahydropalmatine	Schizophrenia	Calcium channel
DB00492	Fosinopril	Diabetes	Angiotensin Converting enzyme
DB00726	Trimipramine	Depresion	Beta adrenergic receptor
DB00715	Paroxetine	Depresion	Beta adrenergic receptor

TABLE1:REPOSITIONED DRUGS

5.2 TARGETS AND THEIR RECEPTOR CLASS

Now, we repositioned hypertensive drugs by identifying new targets for these repositioned drugs. We can identify new targets for hypertensive drugs by comparing the similarity percentage of repositioned drug targets with other targets of the same receptor class.

TARGETS	RECEPTOR CLASS
Calcium channel	ION CHANNEL RECEPTOR
Calcium channel	ION CHANNEL RECEPTOR
Angiotensin Converting enzyme	ENZYME LINKED RECEPTOR
Beta adrenergic receptor	GPCR
Beta adrenergic receptor	GPCR

TABLE2:SHOWING TARGETS AND THEIR RECEPTOR CLASS

5.2.1 Calcium channel percentage indicates that the calcium channel receptor shows the highest similarity with the serotonin receptor. receptors belong to the ion channel receptor class. The below table shows the similarity percentage of calcium channel receptors with other targets.

TARGET	SIMILARITY PERCENTAGE
Nicotini acetylcholine	48%
Zinc activated ion channel	43%
GABA	28.85%
Glutamate receptor	31.25%
Serotonin	54.55%

TABLE3:SHOWING SIMILARITY PERCENTAGE OF CALCIUM CHANNEL WITH OTHER TARGETS

5.2.2 Angiotensin converting enzyme belong to the enzyme linked rreceptor class. The below table shows the similarity percentage of angiotensin converting enzyme with other targets. The similarity percentage indicates that the angiotensin converting enzyme shows the highest similarity with sumo converting enzymes.

TARGET	SIMILARITY PERCENTAGE
Epidermal growth factor receptor	23.64%
Glial cell derived neutrophic factor	20 %
Trk neutrophin receptor	19%
Toll like	30%
Sumo converting enzyme	58.33%
Androgen reeptor	27.59%
Endothelian converting enzyme	31.25%

TABLE4:SHOWING SIMILARITY PERCENTAGE OF ANGIOTENSIN CONVERTING ENZYME WITH OTHER TARGETS

5.2.3Beta adrenergic receptor belong to the GPCR class. The below table shows the similarity percentage of beta adrenergic receptor with other targets. The similarity percentage indicates that the beta adrenergic receptor shows the highest similarity with opoid receptor.

TARGET	SIMILARITY PERCENTAGE
Chemokine receptor	24.02%
Angionestin receptor	24%
Bradykinin	23.68%
Opoid receptor	53.85%
Somatostatin receptor	25.46%
Galamin receptor	20.33%
Relaxin receptor	18%
Melatonin receptor	34%
Eicosanoid receptor	36.76%
Alpha adrenergic receptor	34.31%
Dopamine receptor	36.76%
Histamine receptor	29.74%

TABLE5:SHOWING SIMILARITY PERCENTAGE OF BETA ADRENERGIC RECEPTOR WITH OTHER TARGETS

5.3 DRUGS WITH NEW TARGETS

Based on the similarity percentage of repositioned drugs targets with other targets ,table 6 shows repositioned drugs with new targets that worked on different diseases.

5.5

DRUGS	NEW TARGET	DISEASES
Lamotrigine	Serotonin receptor	Depresion
Tetrahydropalmatine	Serotonin receptor	Depression
Fosinopril	Sumo converting enzyme	Alzheimer diseases
Trimipramine	Opoid receptor	Cancer
Paroxetine	Opoid receptor	Cancer

Table 6:SHOWING DRUGS WITH NEW TARGETS

5.4 DOCKING OF DRUGS WITH NEW TARGET

Now,to identify new therapeutics uses of repositioned hypertensive drugs we perform docking of repositioned drugs with new targets.

DRUGS	NEW TARGET	DOCKING SCORE
Lamotrigine	Serotonin receptor	-7.07
Tetrahydropalmatine	Serotonin receptor	-6.95
Fosinopril	Sumo converting enzyme	-6.25
Trimipramine	Opoid receptor	-7.24
Paroxetine	Opoid receptor	-8.58

TABLE7:SHOWING DOCKING SCORE

Based on docking score ,Paroxentine shows minimum binding energy .So , we can repositioned paroxetine for the treatment of cancer.

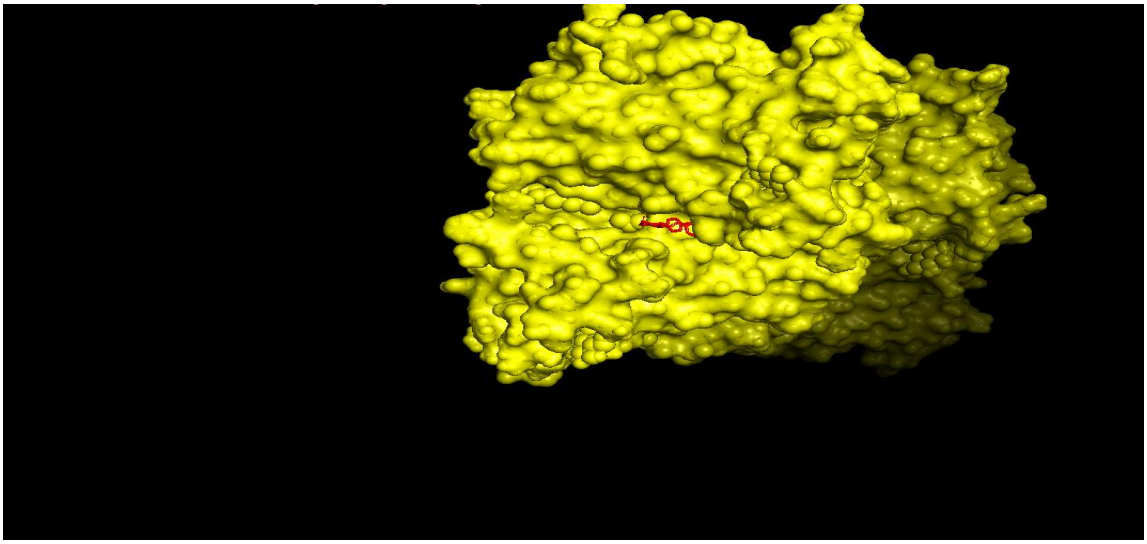

FIGURE 10:Lamotrigine docked to serotonin receptor

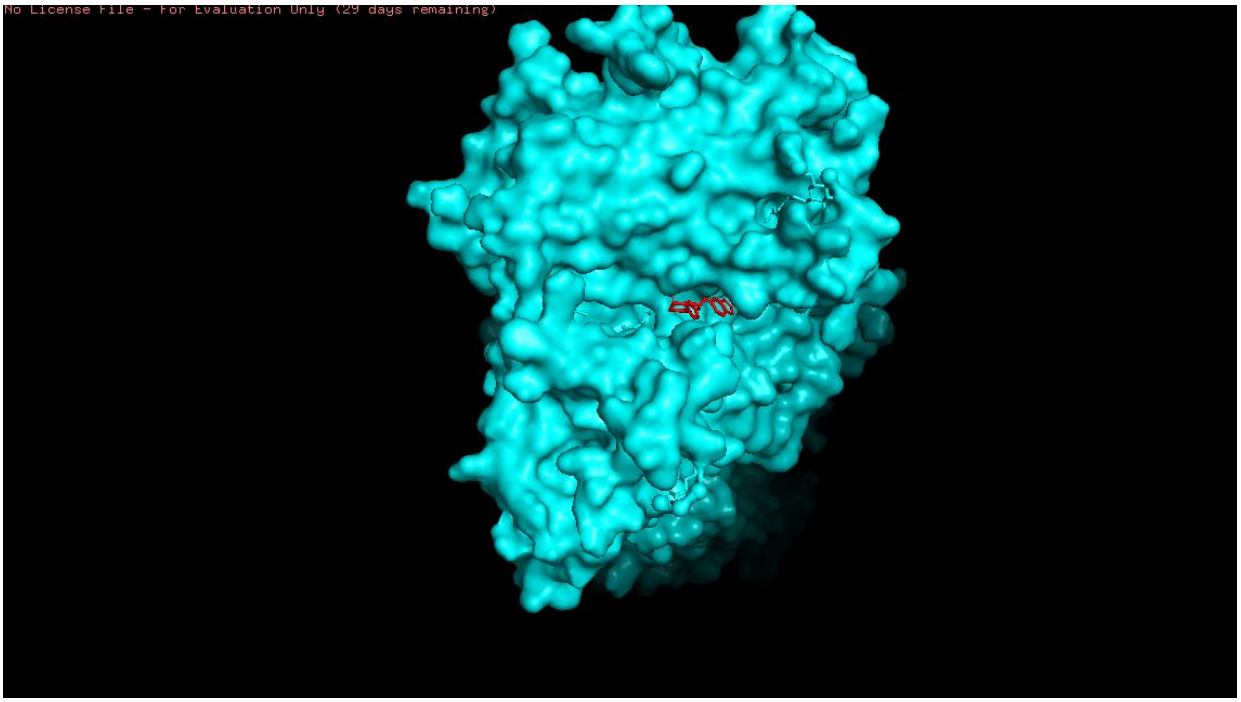

FIGURE11:Tetrahydropalmatine docked to serotonin receptor

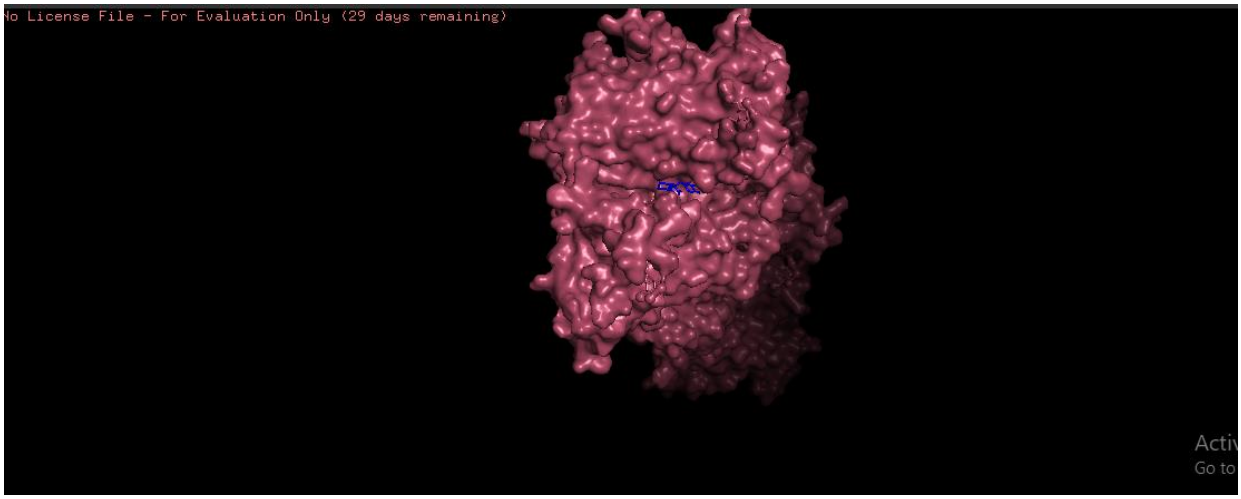

FIGURE 12: Fosinopril docked toSUMO converting enzyme

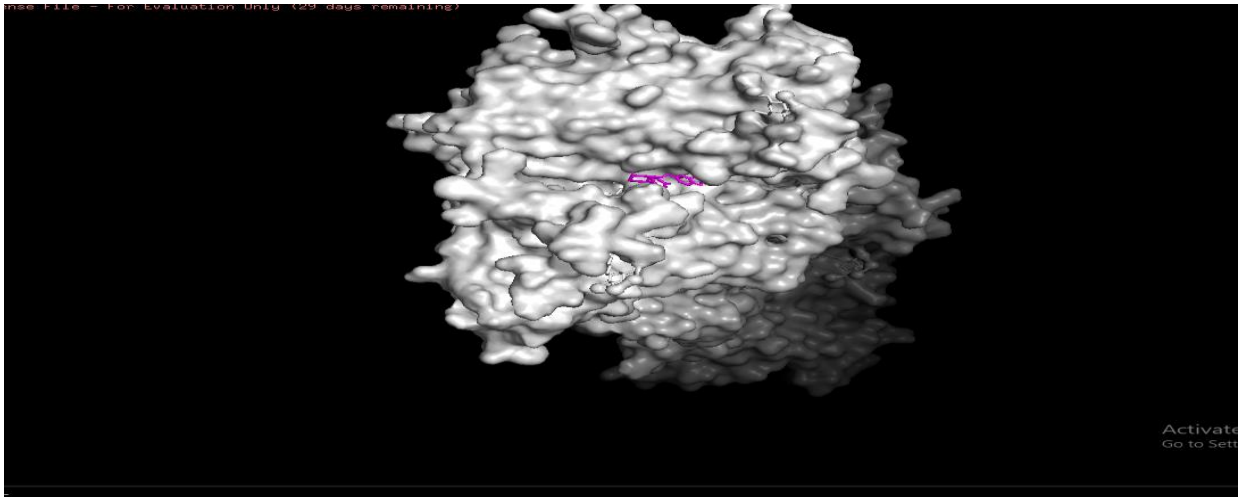

FIGURE13:Trimipramine docked to opoid receptor

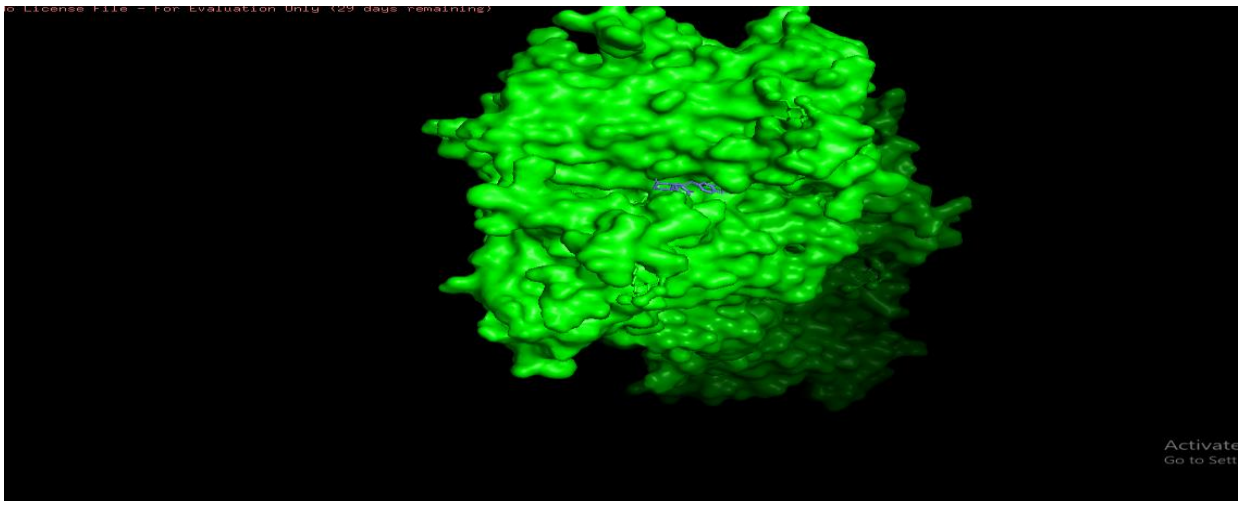

FIGURE14:Paroxetine docked to beta opoid receptor

Chapter 5 CONCLUSION

Drug discovery and development is time consuming,costly and extremely risky business. The cost of bringing a medicine to the market is around one million which include clinical and preclinical trials.Repositioning of drugs help in cutting down costs as well as time involve in intial validation and authorization We discovered new drugs for hypertension i.e lamotrigine,tetrahydropalmatine,fosinopril,trimipramine,paroxetine from already existing drugs by using docking. . consider that these hypertensive drugs often need to be administered for long periods of time, often over whole life time.Side effects although present, have been found safe enough to be used for such long durations, hence repurposing these drugs for other diseases may be beneficial with limited side effects.

In this study an attempt has been made to repositioned these hypertensive drugs for different diseases by exploring their molecular targets (Beta adrenergic receptor,calcium channel,aldosterone receptor,angiotensin converting enzyme)..Firstly, facilitate the information about therapeutics targets and their receptor class, then find the conserved region between hypertensive drugs targets and other diseases targets of same receptor class. and select the new targets having highest similarity with hypertensive drug targets.New targets are opoid receptor,serotoin receptor,sumo converting enzyme.By using docking calculate the binding affinity of hypertensive drugs with new targets for different diseases and choose the drugs with highest affinity for new targets for drug repositioning. Based on docking score ,Paroxentine shows minimum binding energy .So , we can repositioned paroxetine for the treatment of cancer.

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