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In Silico pharmacological target based novel molecules design and validation for Tumor, Glaucoma and Hypertension using molecular docking studies

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ABSTRACT

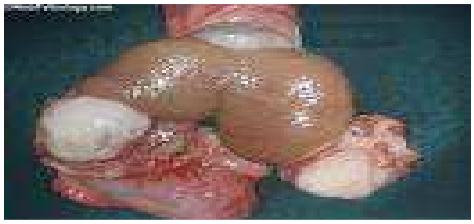
Carbonic anhydrase is the target which is responsible for glaucoma,beta adrenergic receptor is the target for hypertension and in dna ,NAD+ADP-ribosyltransferase is responsible for tumor.The drugs which are satisfying Lipinsky rules for antitumor are cisplatin,cyclophosphamide,dactinomycin,ifosfamide and mitomycin.The drugs for glaucoma are:acetazolamide,brinzolamide,dichorphenamide,ethoxzolamide and metazolamide.The drugs for hypertensionare:acebutolol,alprenolol,atenolol,betaxolol,bis prolol, metoprolol, nebivolol, oxprenolol.Toxicity levels for those drugs were predicted using ADME pharma algorithms. These drugs were drawn and singlepoint,geometry optimization,molecular dynamicswere performed inorder to get good orientation.Docking was performed to find the best drug .R group of those best drugs was modified by replacing different functional groups in its position,those were optimized using different algorithms.Docking was performed for those analogs with the targets.The molecule with the greatest fitness score will have the maximum binding affinity. This leads to a better and efficient drug which is devoid of side effects to the prior one.Properties of better drugs were calculated accurately by using ADME/ToxWEB (pharma algorithms)

Keywords: Glaucoma, Hypertension,Tumor,ADME,Docking.

INTRODUCTION

TUMOR

Tumor is an abnormal mass of tissue. Tumors are a classic sign of inflammation, and can be benign or malignant (cancerous). There are dozens of different types of tumors. Their names usually reflect the kind of tissue they arise in



Tumor

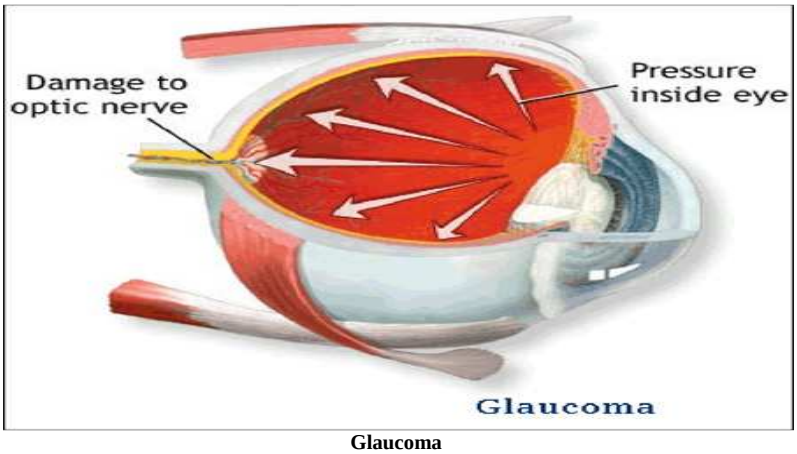
In general, tumors appear to occur when there is a problem with the dividing of cells in the body. Typically, the division of cells in the body is strictly controlled. New cells are created to replace older ones or to perform new functions. Cells that are damaged or no longer needed die to make room for healthy replacements.If the balance of cell division and death is disturbed, a tumor may form.Problems with the body's immune system can lead to tumors. Tobacco causes more deaths from cancer than any other environmental substance.Treatment also varies based on the type of tumor, whether it is benign or malignant, and its location. If the tumor is benign (meaning it has no potential to spread) and is located in a "safe" area where it will not cause symptoms or affect the function of the organ, sometimes no treatment is needed.Sometimes benign tumors may be removed for cosmetic reasons, however. Benign tumors of the brain may be removed because of their location or harmful effect on the surrounding normal brain tissue.If a tumor is malignant, treatments include:

- Surgery
- Radiation
- Chemotherapy
- A combination of these methods

If the cancer is in one location, the goal of treatment is usually to remove the tumor with surgery. If the tumor has spread to local lymph nodes only, sometimes these can also be removed. If all of the cancer cannot be removed with surgery, the options for treatment include radiation and chemotherapy, or both. Some patients require a combination of surgery, radiation and chemotherapy.However, lymphoma is rarely treated with surgery. Chemotherapy and radiation therapy are most often used for treating lymphoma.

GLAUCOMA

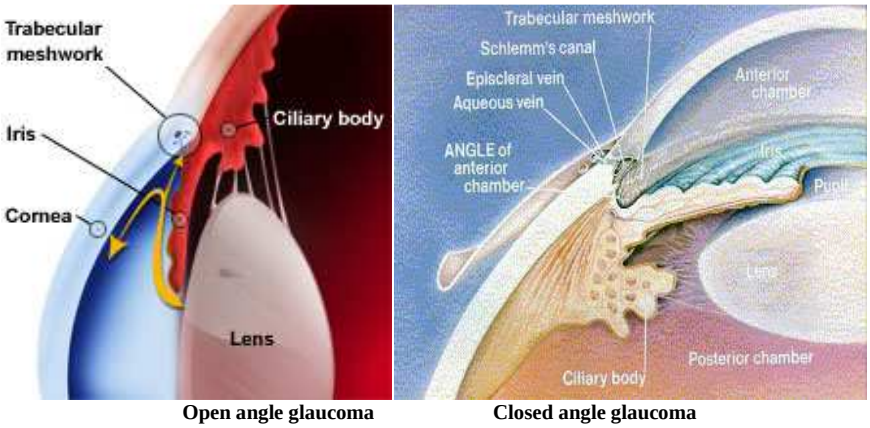
Glaucoma is a disease of the major nerve of vision, called the optic nerve .The optic nerve receives light from the retina and transmits impulses to the brain that we perceive as vision. Glaucoma is characterized by a particular pattern of progressive damage to the optic nerve that generally begins with a subtle loss of side vision (peripheral vision) [1]. If glaucoma is not diagnosed and treated, it can progress to loss of central vision and blindness.Glaucoma is usually, but not always, associated with elevated pressure in the eye (intraocular pressure). Generally, it is this elevated eye pressure that leads to damage of the eye (optic) nerve. In some cases, glaucoma may occur in the presence of normal eye pressure. This form of glaucoma is believed to be caused by poor regulation of blood flow to the optic nerve [2].



Glaucoma

There are 6 types of glaucoma:

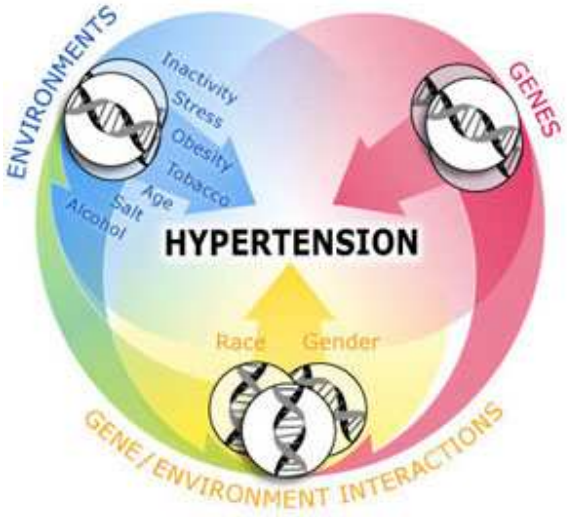
Primary angle-closure glaucoma, Low-tension or normal-tension glaucoma, Primary open-angle glaucoma, Neovascular glaucoma, Toxic glaucoma, Absolute glaucoma [3].



Open angle glaucoma **Closed angle glaucoma**

HYPERTENSION

Hypertension is a chronic medical condition in which the blood pressure is elevated. It is also referred to as high blood pressure or shortened to HT, HTN or HPN. The word "hypertension", by itself, normally refers to systemic, arterial hypertension. Hypertension can be classified as either essential (primary) or secondary. Essential or primary hypertension means that no medical cause can be found to explain the raised blood pressure. It is common. About 90-95% of hypertension is essential hypertension Secondary hypertension indicates that the high blood pressure is a result of (*i.e.*, secondary to) another condition, such as kidney disease or tumours(adrenal adenoma or pheochromocytoma).Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure Even moderate elevation of arterial blood pressure leads to shortened life expectancy [4]. At severely high pressures, defined as mean arterial pressures 50% or more above average, a person can expect to live no more than a few years unless appropriately treated. Beginning at a systolic pressure (which is peak pressure in the arteries, which occurs near the end of the cardiac cycle when the ventricles are contracting) of 115 mmHg and diastolic pressure (which is minimum pressure in the arteries, which occurs near the beginning of the cardiac cycle when the ventricles are filled with blood) of 75 mmHg (commonly written as 115/75 mmHg), cardiovascular disease (CVD) risk doubles for each increment of 20/10 mmHg.



Hypertension

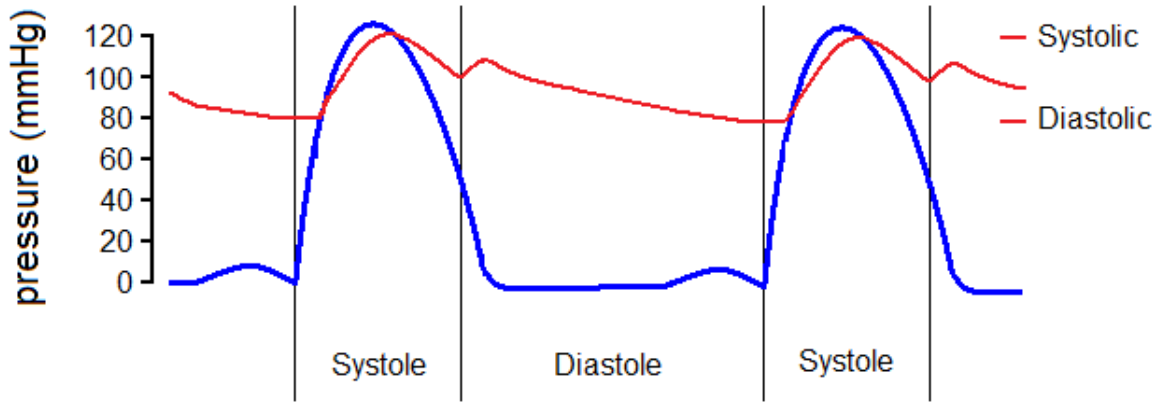
CLASSIFICATION

A recent classification recommends blood pressure criteria for defining normal blood pressure, prehypertension, hypertension (stages I and II), and isolated systolic hypertension, which is a common occurrence among the elderly [5]. These readings are based on the average of seated blood pressure readings that were properly measured during 2

or more office visits. In individuals older than 50 years, hypertension is considered to be present when a person's blood pressure is consistently at least 140 mmHg systolic or 90 mmHg diastolic. Patients with blood pressures over 130/80 mmHg along with Type 1 or Type 2 diabetes, or kidney disease require further treatment [6]. Resistant hypertension is defined as the failure to reduce blood pressure to the appropriate level after taking a three-drug regimen (include thiazide diuretic) Guidelines for treating resistant hypertension have been published in the UK, and US. Excessive elevation in blood pressure during exercise is called exercise hypertension. The upper normal systolic values during exercise reach levels between 200 and 230 mm Hg.Exercise hypertension may be regarded as a precursor to established hypertension at rest [7].

Table1- classification

Classification	Systolic pressure		Diastolic pressure	
	mmHg	kPa (kN/m^2)	mmHg	kPa (kN/m^2)
Normal	90–119	12–15.9	60–79	8.0–10.5
Prehypertension	120–139	16.0–18.5	80–89	10.7–11.9
Stage 1	140–159	18.7–21.2	90–99	12.0–13.2
Stage 2	≥160	≥21.3	≥100	≥13.3
Isolated systolic hypertension	≥140	≥18.7	<90	<12.0



graph

Aim and Scope of the Present Investigation:

To design a better drug candidate with more efficacy and less side effects based on the docking scores. Carbonic anhydrase is responsible for causing glaucoma by forming aqueous humor. Carbonic anhydrase inhibitors brinzolamide (Azopt), acetazolamide (Diamox) ,Dichlorphenamide decrease bicarbonate formation from ciliary processes in the eye, thus decreasing formation of Aqueous humor. Carbonic anhydrase inhibitors reduce intraocular pressure by partially suppressing the secretion of aqueous humor (inflow), although the mechanism by which they do this is not fully understood. Evidence suggests that HCO^{3-} ions are produced in the ciliary body by hydration of carbon dioxide under the influence of carbonic anhydrase and diffuse into the posterior chamber which contains more Na^+ and HCO^{3-} ions than does plasma and consequently is hypertonic. Water is then attracted to the posterior chamber by osmosis, resulting in a drop in pressure. β-blockers are among the most widely used drugs in the prevention and treatment of cardiovascular disease, although they are associated with increased peripheral resistance. Third-generation β-blockers avoid this adverse effect by inducing vasodilation through different mechanisms. In particular, nebivolol, a highly selective blocker of β_1- adrenergic receptors, is the only β-blocker known to induce vascular production of nitric oxide , the main endothelial vasodilator. The specific mechanism of nebivolol is particularly relevant in hypertension, where nitric oxide dysfunction occurs. Indeed, nebivolol is able to reverse endothelial dysfunction. Nebivolol induces nitric oxide production via activation of β_3- adrenergic receptors, which can explain the good metabolic profile observed after treatment with this drug. Moreover, nebivolol can also stimulate the β_3-adrenergic receptor-mediated production of nitric oxide in the heart, and this stimulation can result in a greater protection against heart failure. In conclusion, nebivolol has a unique profile among

antihypertensive drugs, adding to a very high selectivity against β_1 adrenergic receptors, and an agonist action on β_3 receptors and nitric oxide (NO), which has led to clinically significant improvements in hypertensive patients. The selective action of antbiotic mytomycin on DNA (NAD + ADP ribosyl transferase)Inhibits the action thereby leading to relatively much slower process of DNA breakdown [8,9,10,11].

MATERIALS AND METHODS

BLAST:
Basic Local Alignment Search Tool. It is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences.BLAST searches for high scoring sequence alignments between the query sequence and sequences of the model organisms and it also gives functional and evolutionary relationship between the sequences as well as it helps to identify members of gene families.It uses smith –waterman algorithm.BLAST is available on NCBI homepage [12].

SDSC BIOLOGY WORKBENCH: Biology workbench is a web based tool which allows searching of protein and nucleic acid sequence databases and for analyzing sequence data.

a)CLUSTAL W :CLUSTAL W performs multiple sequence alignment on proteins or nucleotide sequences by inserting gaps.Evolutionary relationships can be obtained through the dendrogram.

b)TEX SHADE: This tool gives coloured pattern of identical,similar and conserved residues between the query and model organism's sequences.The patterns of residues are aligned using local alignment.

c)BOX SHADE: This tool gives coloured pattern of identical,similar and conserved residues between the query and model organism's sequences.The patterns of residues are aligned using global alignment.

d)CLUSTAL DISTANCE MATRIX: The matrix contains data that shows relationships between a given set of elements (DNA and Protein sequences). Values in the matrix file show distance, similarity or identity between different sequences.The evolutionary distance between the organisms is measured in light year.

EXPASY (Expert Protein Analysis System):
Proteomics server of the Swiss Institute of Bioinformatics (SIB) is dedicated to the analysis of protein sequences and structures as well as 2D PAGE [13].

TOOLS USED FOR STRUCTURAL ANALYSIS FROM EXPASY
PRIMARY STRUCTURE PREDICTION:
Protparam (Protein parameters)
Protparam is a tool which allows the computation of various physical and chemical parameters for a given protein. The parameters include the molecular weight, theoretical PI, amino acid composition, atomic composition, Extinction Coefficient, Estimated half life, instability index, aliphatic index and Grand Average of Hydropathy (GRAVY). It is used for **Primary Structure Analysis** of a Protein sequence.

SECONDARY STRUCTURE PREDICTION
HNN (Hierarchical Neural Network)
Secondary structure prediction tool and works on machine learning process with high accuracy.It gives the information about the percentage of alpha helices,beta sheets ,coils,turns.

TERTIARY STRUCTURE PREDICTION
CPH MODELS (Comparative Homology Modelling)
It is an automated neural network based protein modelling server. It helps to predict tertiary structure of proteins [14].
HYPERCHEM
HyperChem is a versatile molecular modeler and editor and a powerful computational package. It offers many types of molecular and quantum mechanics calculations. The following actions can be performed by HyperChem:
- Building and Displaying Molecules
- Optimizing the Structures of Molecules

- Investigating the Reactivity of Molecules and Functional Groups
- Generating and Viewing Orbital and Electronic Plots
- Evaluating Chemical Pathways and Mechanisms
- Studying the Dynamic Behavior of Molecules

GOLD (Genetic Optimization for Ligand Docking)

Gold uses genetic algorithm to provide docking of flexible ligand and a protein with flexible hydroxyl groups. Otherwise the protein is considered to be rigid. This makes it a good choice when the binding pocket contains amino acids that form hydrogen bonds with the ligand.

GOLD offers a choice of scoring functions: GoldScore, ChemScore and User Defined Score. The solutions are known to have 70-80% accuracy when tested on complexes extracted from PDB. GOLD will only produce reliable results if it is used properly and correct atom typing for both protein and ligand is particularly important [15,16].

LIGAND SCOUT:

It will perform pharmacophore analysis of a complex molecule by showing the amino acid interactions with a specific ligand complex

ADME/Tox WEB

It gives the accurate information for the parameters involved in the lipinsky rules for ligands.It is the most accurate algorithm for the information about the ligands [17,18,19].

METHODOLOLGY:*Step1:sequence analysis*

a)*Retreival of sequence from NCBI(National center for biotechnololgy information)*

Target proteins i.e carbonic anhydrase,beta1 adrenergic receptor and NAD+ADP-ribosyltransferase sequence in FASTA format was retrieved from the NCBI.The function of the query sequence was obtained from "CDD(conserved domains database)".

Step2:Phylogenetic analysis

Step3:structural analysis of protein

The selected protein was subjected to structural analysis for prediction of its primary,secondary and tertiary structures.this structural analysis gives us a pdb id for our target protein by which the structure of the protein was downloaded from pdb.

Step 4:HYPERCHEM:

Force – Field Selection

Selecting a force field is done before you invoke the Model Builder because the Model Builder assigns atom types to each atom according to the force field that we specify. The force-field chosen is AMBER2 from

Setup → Molecular Mechanics → Amber
Setup → Select Parameter Set → AMBER2

Inhibitors for the above targets was selected, which are then sketched and their energy was optimized by performing single point,energy minimization,molecular dynamics to obtain stable structures.

Single – Point Energy Calculations

Single point calculation determines molecular properties, such as energy or spin density, of a defined molecular structure. It gives the measure of the energy of a molecule without altering it [20].

Geometry -Optimization

Energy Minimization or Optimization alters the geometry of the molecule and lowers its energy to yield a molecule of more stable conformation.

Molecular Mechanics Optimization: Parameters
Algorithm: Polak-Ribiere (Conjugant gradient)

Molecular Dynamics Calculations

Molecular dynamics is carried out to anneal the system to obtain a lower energy minimum. It simulates the evolution of a system over time, producing a trajectory of atomic positions and velocities. The dynamics are run in 3 optional steps of heat, run and cool.

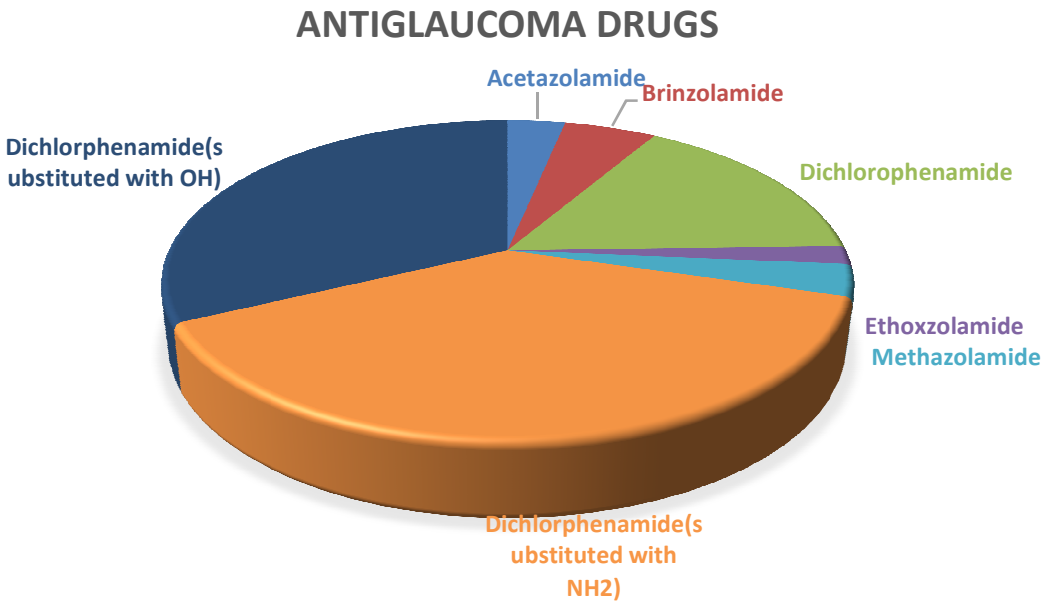
Step 5:Docking

The protein structure was retrieved from PDB using the CPH result. The eighteen optimized ligand molecules and the respective proteins was docked under scrutiny using GOLD software.The molecules with that protein are docked using GOLD software, which works, on Genetic Algorithm. Based on the parameters such as fitness score,vanderwaals interactions obtained from the gold score and score,DG ,obtained from the chemscore , three better drugs were obtained from the eighteen molecules.Two analogs of each drug were created by replacing the hydrophilic region on the target molecule with other functional groups (considered at random). The drug molecules and the six analogs are then studied by performing various energy, simulation as well as QSAR calculations .Those analogs were docked with the proteins again and by comparing the docking scores with the previous scores a better efficient drug was obtained for each group.

CALCULATIONS AND RESULTS

Anti Glaucoma Drugs

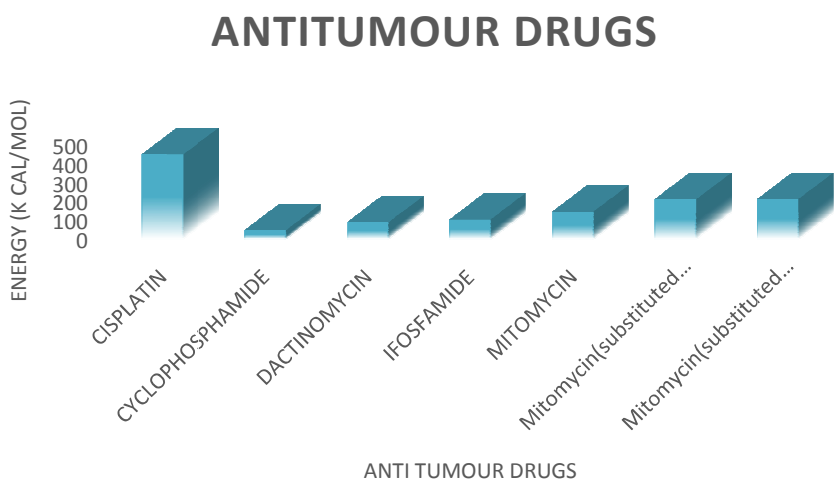
MOLECULE	ENERGY(Kcal/mol)
Acetazolamide	132.51
Brinzolamide	211.76
Dichlorphenamide	657.27
Ethoxzolamide	74.96
Methazolamide	130.29
Dichlorphenamide(Substituted with NH_2)	1562.64
Dichlorphenamide(substituted with OH)	1310.49



Anti Tumor Drugs

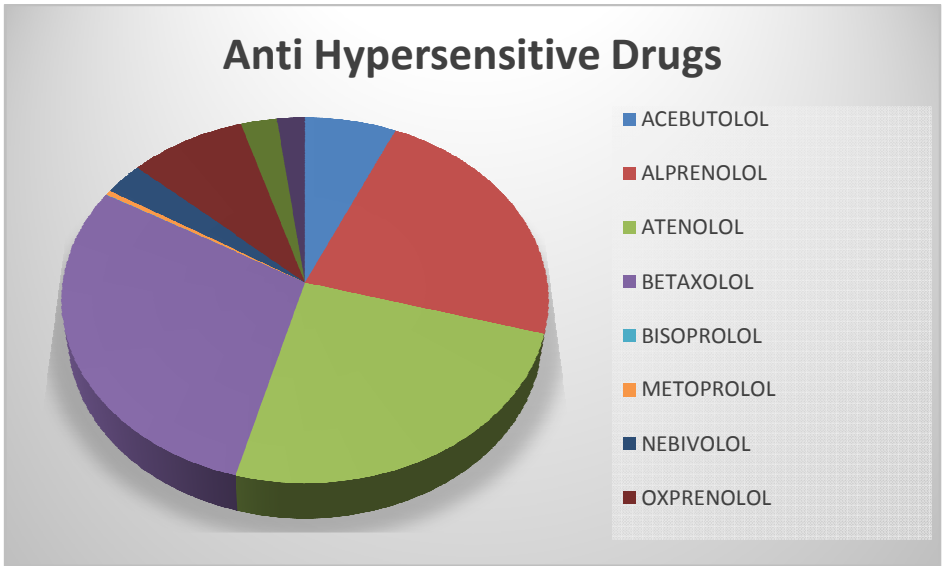
MOLECULE	ENERGY (Kcal/mol)
Cisplatin	451.72
Cyclophosphamide	45.38
Dactinomycin	88.10
Ifosfamide	103.98
Mitomycin	145.12

Mitomycin(Substituted With Cl)	212.26
Mitomycin(Substituted With F)	213.28



Anti Hypertensive Drugs

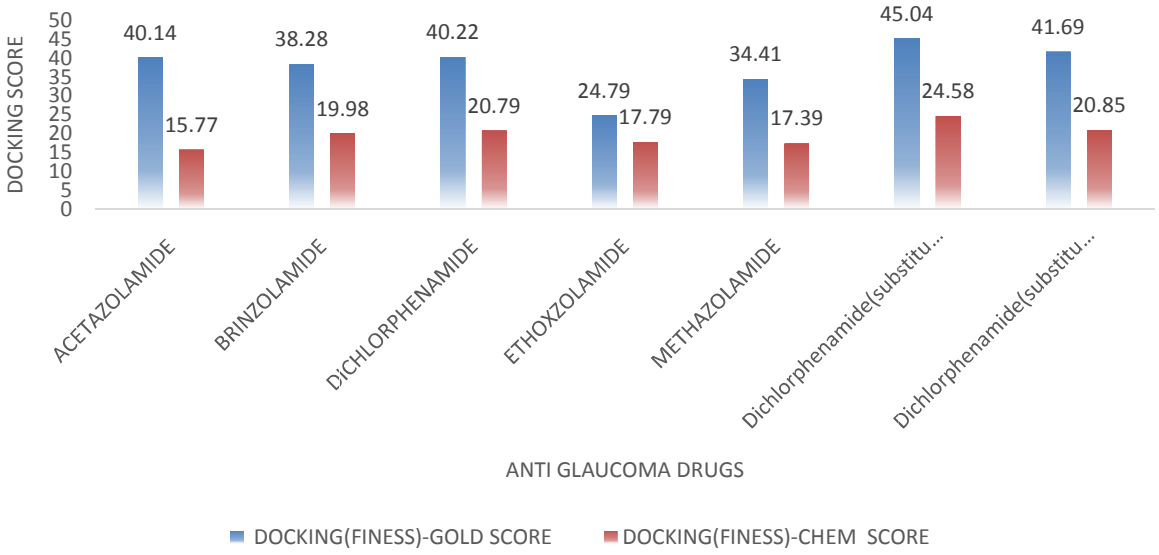
MOLECULE	**ENERGY** (Kcal/mol)
Acebutolol	76.23
Alprenolol	261.46
Atenolol	283.79
Betaxolol	336.08
Bisoprolol	0.337
Metoprolol	4.64
Nebivolol	33.01
Oxprenolol	97.81
Nebivolol(Substituted With NH_2)	30.23
Nebivolol (Substituted With F)	23.37



Docking Results

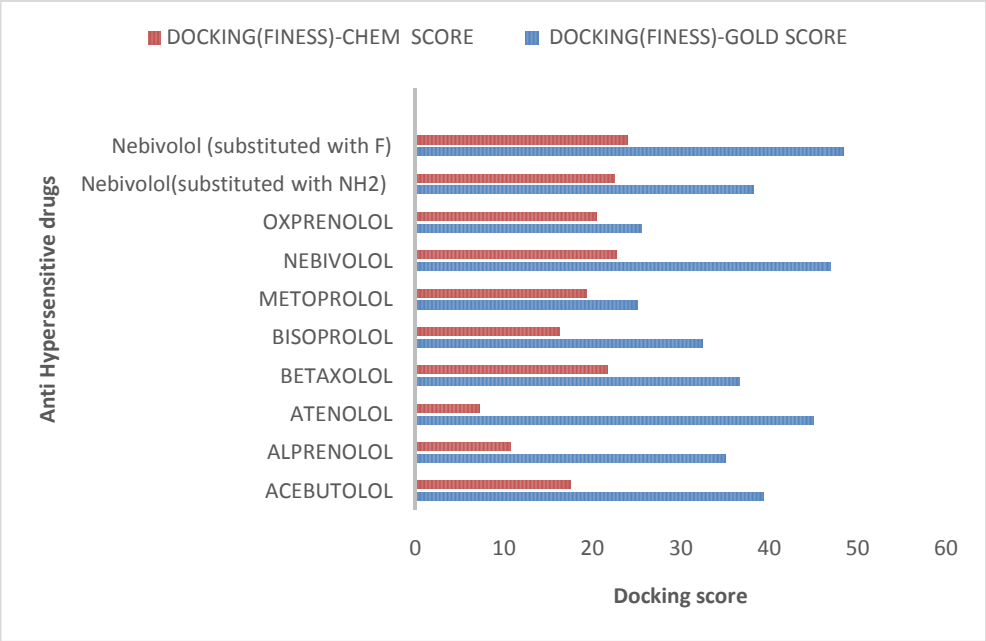
Anti Glaucoma Drugs

MOLECULE	DOCKING(FINESS)-GOLD SCORE	DOCKING(FINESS)-CHEM SCORE
Acetazolamide	40.14	15.77
Brinzolamide	38.28	19.98
Dichlorphenamide	40.22	20.79
Ethoxzolamide	24.79	17.79
Methazolamide	34.41	17.39
Dichlorphenamide(Substituted With NH_2)	45.04	24.58
Dichlorphenamide(Substituted With OH)	41.69	20.85



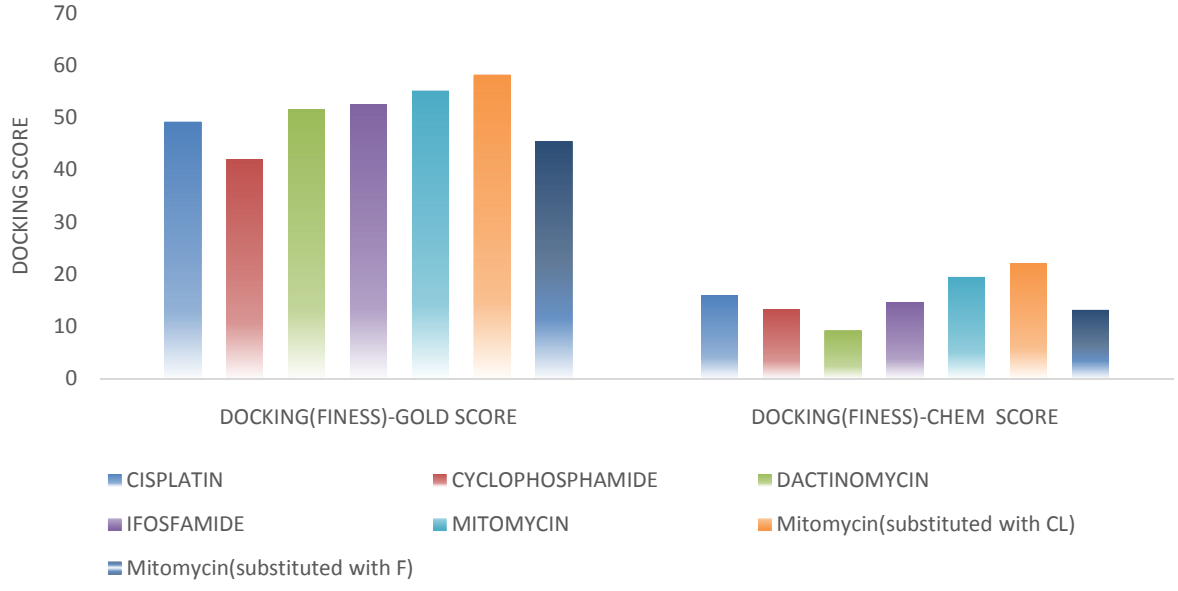
Anti Hypertensive Drugs Results

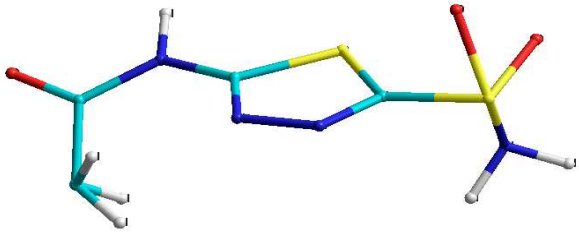
MOLECULE	DOCKING(FINESS)-GOLD SCORE	DOCKING(FINESS)-CHEM SCORE
Acebutolol	39.31	17.52
Alprenolol	35.04	10.71
Atenolol	45.04	7.24
Betaxolol	36.61	21.75
Bisoprolol	32.41	16.23
Metoprolol	25.07	19.33
Nebivolol	46.97	22.77
Oxprenolol	25.55	20.45
Nebivolol(Substituted With NH_2)	38.20	22.47
Nebivolol (Substituted With F)	48.44	24.02



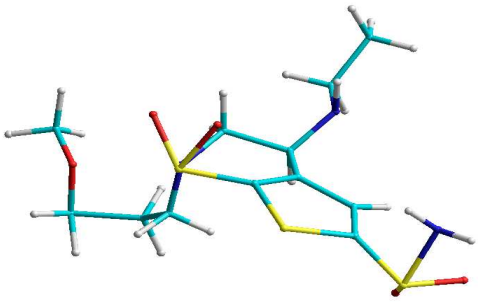
Anti Tumor Drugs

MOLECULE	DOCKING(FINESS)-GOLD SCORE	DOCKING(FINESS)-CHEM SCORE
Cisplatin	49.14	16.00
Cyclophosphamide	42.05	13.26
Dactinomycin	51.60	9.19
Ifosfamide	52.60	14.53
Mitomycin	55.10	19.46
Mitomycin(Substituted With CL)	58.16	22.10
Mitomycin(Substituted With F)	45.50	13.13

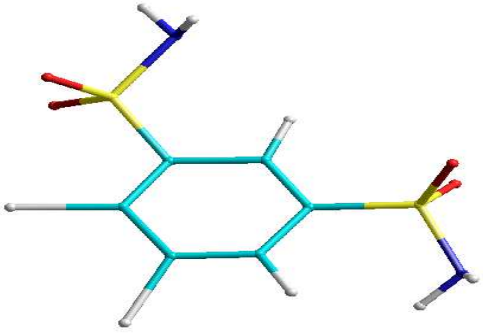




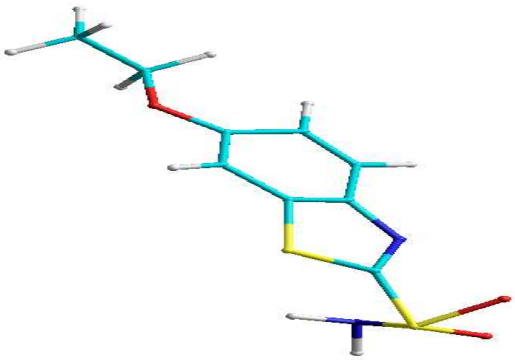
ACETAZOLAMIDE



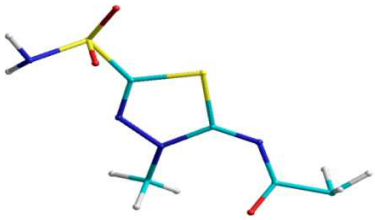
BRINZOLAMIDE



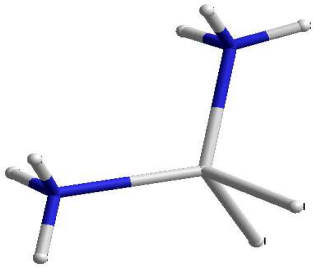
DICHLORPHENAMIDE



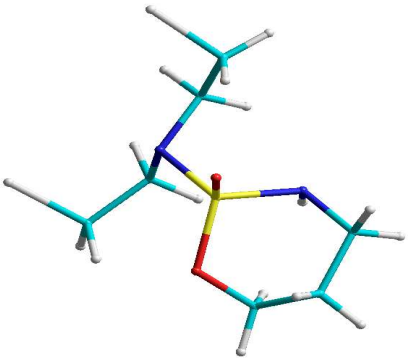
ETHOXZOLAMIDE



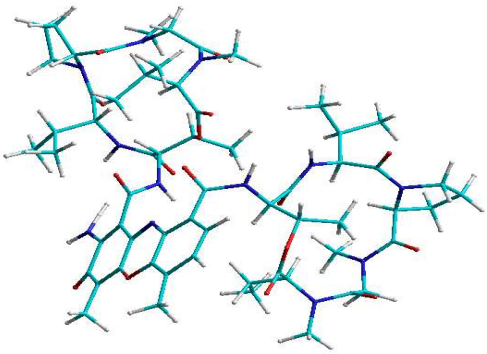
METHAZOLAMIDE



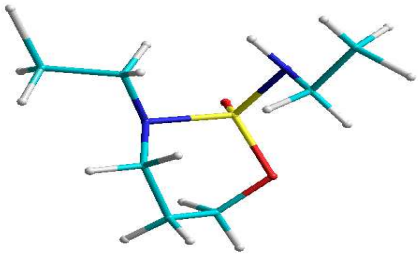
CISPLATIN



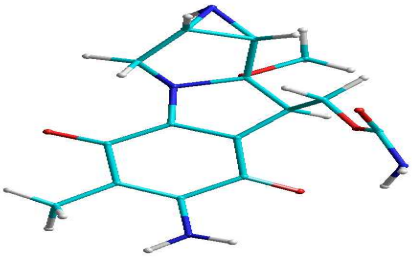
CYCLOPHOSPHAMIDE



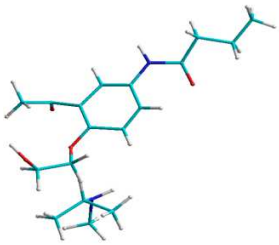
DACTINOMYCIN



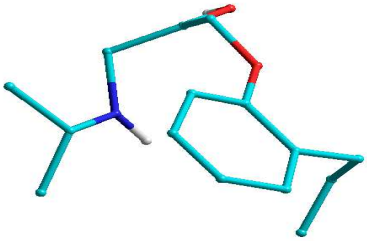
IFOSFAMIDE

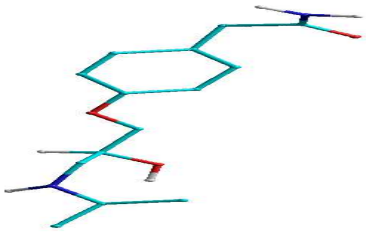


MITOMYCIN



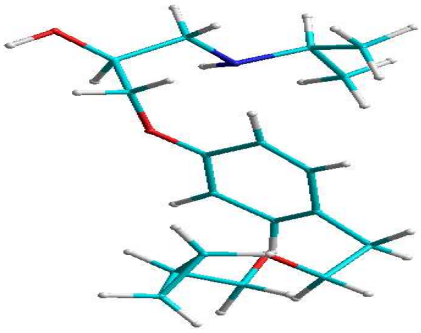
ACEBUTOLOL



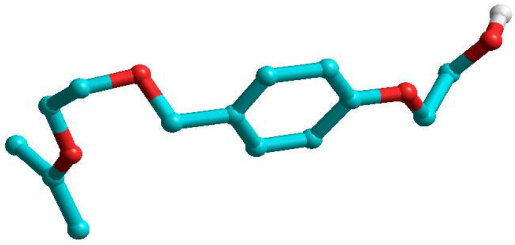


ATENOLOL

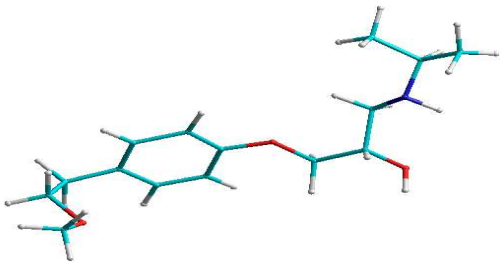
ALPRENOLOL



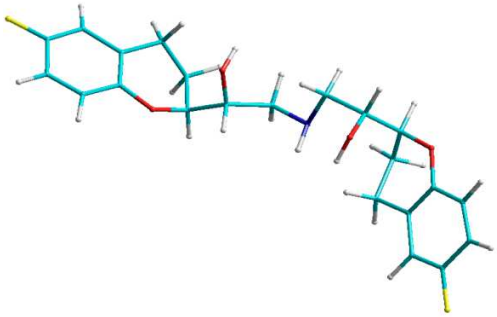
BETAXOLOL



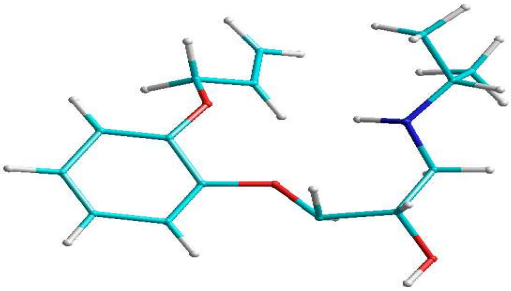
BISOPROLOL



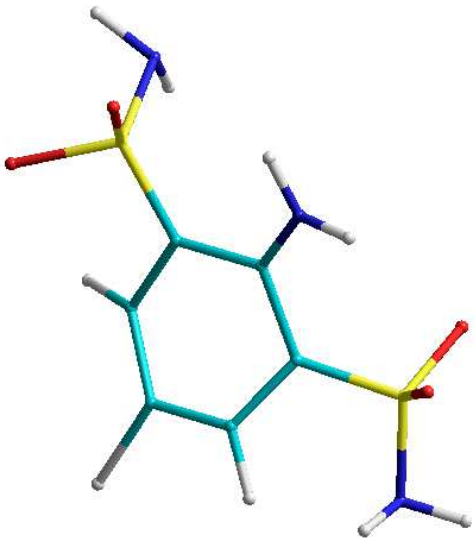
METOPROLOL



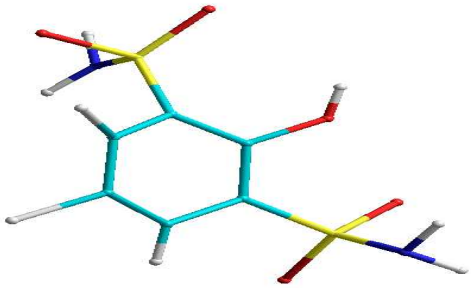
NEBIVOLOL



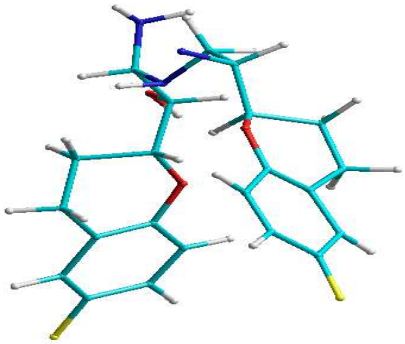
OXPRENOLOL



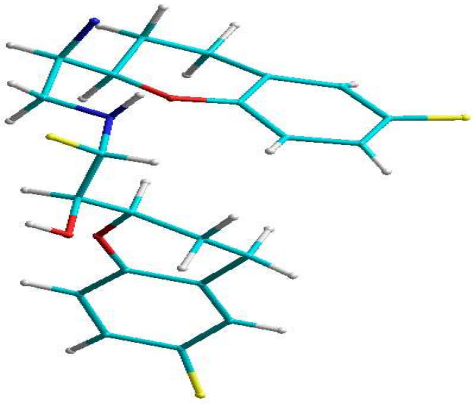
Dichlorphenamide(substituted with NH2)



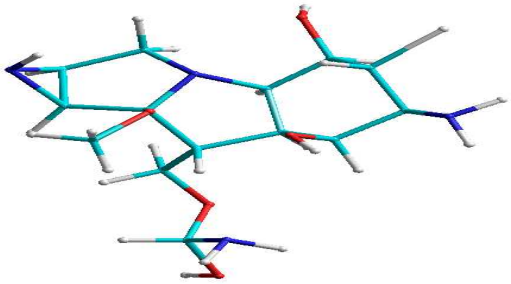
Dichlorphenamide(substituted with OH)



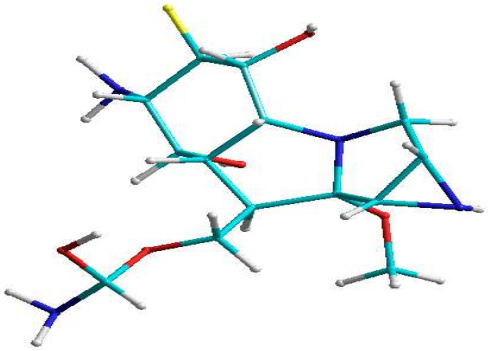
Nebivolol(substituted with NH2)



Nebivolol (substituted with F)



Mitomycin(substituted with CL)



Mitomycin(substituted with F)

CONCLUSION

We have identified the following targets(carbonic anhydrase,beta1 adrenergic receptor,ribosyl transferase)and found their evolutionary principle by using the sequence similarity search tool called as Blast.conserved domains are found by using tex shade and box shade tools. eighteen drugs which are satisfying Lipinsky rules were taken for glaucoma,hypertension and tumor.we have performed molecular docking studies using gold software including goldscore and chemscore scoring functions.Based on the parameters like gold score(fitness score,vanderwaals interactions),chem score(score,DG)we found out mitomycin,dichlorphenamide and nebivolol as the better drugs. R group of each better drugs was modified by replacing two different functional groups in its position for better potency.Finally we consider mitomycin substituted with cl,dichlorphenamide substituted with NH2,nebivolol substituted with F as the best drugs based on the above parameters and insilico tools.

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