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In silico Docking Studies of Novel Pyrazole Derivatives for Glaucoma

K. Banupriya[1], R. Girija[2] and S. Aruna[3]
Research Scholar, PG & Research Department of Chemistry, Queen Mary's College, Chennai-04

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Corresponding Author Email: mgbanugovind@gmail.com

Abstract
Purpose: To explore newer computational approaches in the design of novel CAS inhibitors for the treatment of glaucoma. **Methods:** An *in-silico* molecular docking was utilized to design a novel CAs inhibitors for the treatment of glaucoma. The designed novel molecules were theoretically evaluated to predict their pharmacokinetic properties and toxic effects. Lead molecules were screened out in virtual screening technique on the basis of low binding energies obtained in AutoDock based molecular docking simulation. **Results:** Out of ten top lead compounds screened, ZINC01729523 and ZINC04692015 were promising, having shown potent inhibition of myocilin, good pharmacokinetic properties and absence of any toxic effects. **Conclusion:** Docking studies of molecular libraries containing a large number of ligands is very useful for short-listing of potential lead molecules for further structure-based discovery of antiglaucoma-drugs.

Keywords
Pyrazole derivatives, AutoDock, glaucoma and CAs.

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INTRODUCTION
Heterocyclic compounds occur widely in nature. Nitrogen containing heterocyclic molecules constitute the largest portion of these chemical entities, which are part of many natural products [1-3]. Further, pyrazole derivatives are also used as chelating agents and inhibitors. pyrazoles have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis [4]. Glaucoma is a group of eye diseases that can lead to blindness by damaging the optic nerve. The eye continuously produces a fluid, called the aqueous, that must drain from the eye to maintain healthy eye pressure. Worldwide, the number of people with glaucoma was estimated at over 64.3 million in 2013 and is expected to increase to 76.0 million in 2020.There are four main types of glaucoma: Chronic glaucoma, Acute glaucoma, Secondary and developmental glaucoma. Glaucoma is a worldwide leading cause of irreversible vision loss. Because it may be asymptomatic until a relatively late stage, diagnosis is frequently delayed. The glaucoma is a group of progressive optic neuropathies characterized by degeneration of retinal ganglion cells and resulting changes in the optic nerve head [5] The biological basis of glaucoma is poorly understood and the factors contributing to its progression have not been fully characterized [6]. Glaucoma affects more than 70 million people worldwide with approximately 10% being bilaterally

blind, making it the leading cause of irreversible blindness in the world [3]. Traditionally, glaucoma is diagnosed by increased intra-ocular pressure (IOP), which is a very crucial factor in its pathogenesis. The normal range of IOP in healthy individuals is 10 to 21mmHg [7]. Systemic inhibitors are useful in reducing elevated intraocular pressure (IOP) characteristic to this disease, as they represent the most efficient physiological treatment of glaucoma [8] . Carbonic anhydrases (CAs; also known as carbonate dehydratases) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes. Many of the CA isozymes involved in these processes are important therapeutic targets with the potential to be inhibited to treat a range of disorders including oedema, glaucoma, obesity, cancer, epilepsy and osteoporosis [9]. Carbonic anhydrases (CAs) are ubiquitous zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide and water to a bicarbonate ion and proton [10-15]. Treatment strategies to prevent glaucoma and the consequent irreversible vision loss are based on the reduction of IOP by using topically acting or systemic hypotensive drugs [16-19]. Sulphonamide CAIs, among which acetazolamide, dichlorophenamide (systemically-acting agents) as well as dorzolamide and brinzolamide (topically acting drugs), are commonly used anti-glaucoma agents [20].

MATERIALS AND METHODS

Uniprot

The Universal Protein Resource (UniProt) provides a stable, comprehensive, freely accessible, central resource on protein sequences and functional annotation. It contains a large amount of information about the biological function of proteins derived from the research literature. ((https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2238893)).

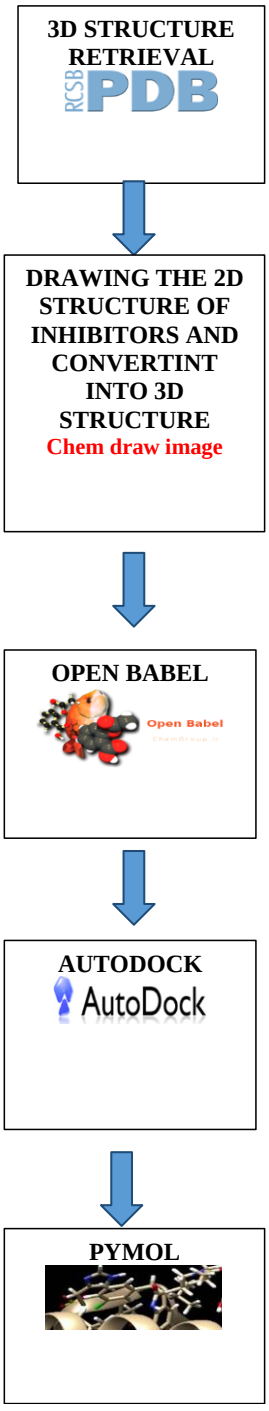
Protein structure preparation

The Protein Data Bank archive (PDB) has served as the single repository of information about the 3D structures of proteins, nucleic acids, and complex assemblies. The 3D crystal structure of the targeted glaucoma protein 4WR7 was retrived from the protein Data Bank (PDB) (www.rcsb.org/pdb) structural and active site studies of the protein were done by using Pymol molecular visualization software (Senthil Raja,et al.,(2011)).

Chemdraw

ChemDraw is the drawing tool of choice for chemists to create publication-ready, scientifically intelligent drawings for use in ELNs, databases and publications and for querying chemical databases. Chemists who use ChemDraw to predict properties are able to save time and reduce costs by identifying compounds that are likely to have the desired properties before actually synthesizing them. Chemists can also save time and increase data accuracy using ChemDraw to generate spectra, construct correct IUPAC names, and calculate reaction stoichiometry (https://www.lib.ncsu.edu/faq/what-is-chemdraw-and-how-do-i-access-it).

METHODOLOGY:



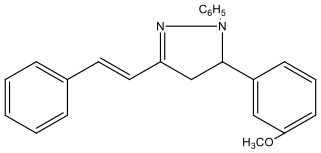
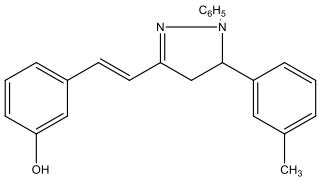
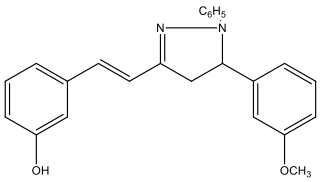
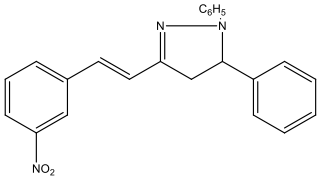
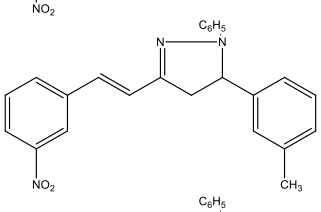
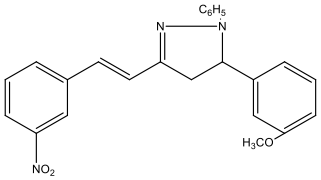
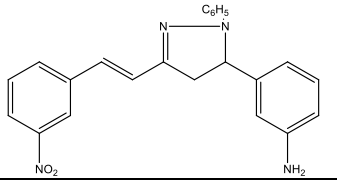
RESULT AND DISCUSSION:

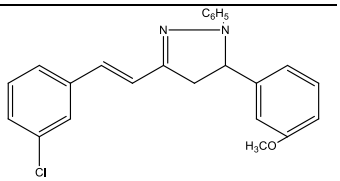
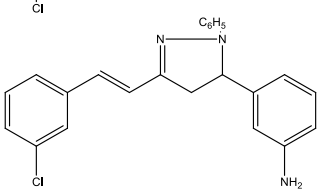
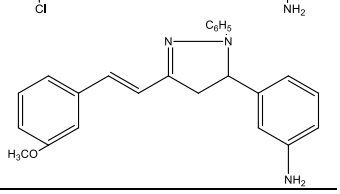
1. STRUCTURE RETRIEVAL:

The 3D crystal structure of the targeted eye disorder protein of CAs (ID.4WR7) was retrieved from the Protein Data Bank (PDB).

2. Preparation of Ligand:

For further docking analysis of the Substituted Pyrazole derivatives compounds are taken. The two-dimensional structures of the ligand were generated using the Chemdraw tool. This software contains tools for 2D cleaning, 3D optimization and viewing. These data are saved as a molecular format file (MDL MOL format). The molecular format converter tool (Open Babel) is used to convert this file into the PDB format and is used during docking analysis. The structure and molecular formula of Substituted Pyrazole derivatives compounds was shown in Table.1

COMPOUND	COMPOUND NAME	MOLECULAR WEIGHT	2D STRUCTURE
1a	(E)-5-(4-methoxyphenyl)-1-phenyl-3-styryl-4,5-dihydro-1H-pyrazole	**354.44**	
1b	(E)-3-(2-(1-phenyl-5-(m-tolyl)-4,5-dihydro-1H-pyrazol-3-yl) vinyl) phenol	354.44	
1c	(E)-3-(2-(5-(3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)vinyl)phenol	370.44	
1d	(E)-3-(3-nitrostyryl)-1,5-diphenyl-4,5-dihydro-1H-pyrazol	369.42	
1e	(E)-3-(3-nitrostyryl)-1-phenyl-5-(m-tolyl)-4,5-dihydro-1H-pyrazol	383.44	
1f	(E)-5-(3-methoxyphenyl)-3-(3-nitrostyryl)-1-phenyl-4,5-dihydro-1H-pyrazol	399.44	
1g	(E)-3(3-(3-nitrostyryl)-1-phenyl-4,5-dihudro-1H-pyrazol-5-yl) aniline	384.43	

1h	(E)-3-(3-chlorostyryl)-5-(3-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole	388.48	
1i	(E)-3-(3-(3-chlorostyryl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) aniline	373.88	
1j	(E)-3-(3-(3-methoxystyryl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl) aniline	369.46	

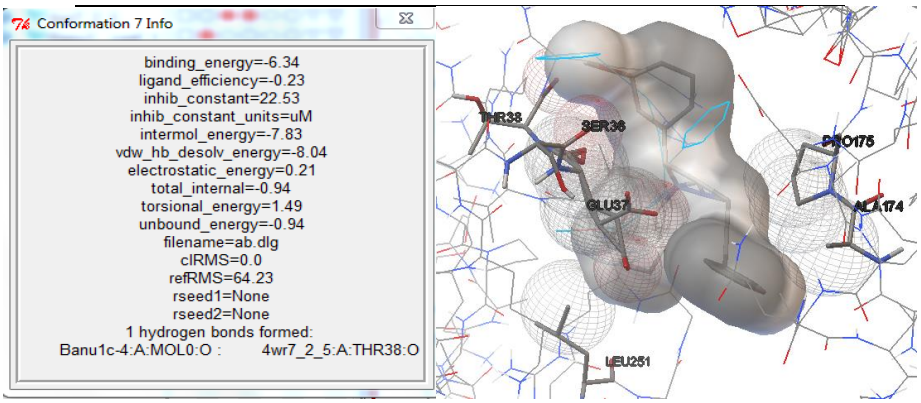
DOCKING STUDY:

Banupriya et.al Prepared substituted chalcones which are treated with phenyl hydrazine hydrochloride in ethanol were refluxed 8hrs in the presence of 10% NaOH produced substituted pyrazole derivatives. The compounds are purified and recrystallized from ethanol. The recrystallized compounds are further purified with column chromatography. The characterization of the compounds are done using IR, H^1 NMR, C^{13} NMR,Mass. That pyrazole derivatives are subjected to docking studies [2].

The inhibitors docked with CAs using Autodock software (Version 4.2). The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. Autodock results were analyzed to study the interactions and the binding energy of the docked structure. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were visualized using the PyMol visualizer tool. A bond is formed between two atoms by overlapping the atomic orbitals. This overlap of atomic orbitals to form molecular orbitals occurs only at certain distances between the atom. When the amino acid residues of the active site is closer, then the interactions is much higher than the other sites.

Table 1. Docking interaction between CAs and 1a

CAs		1a	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
GLU 37	OE1	N	3.4	-6.34
GLU 37	OE1	N	3.1	



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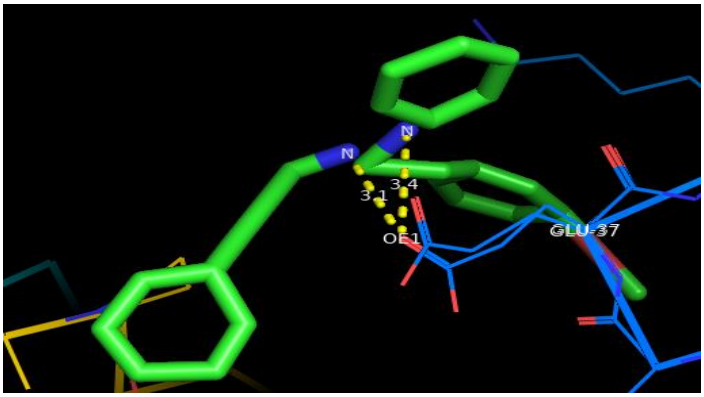


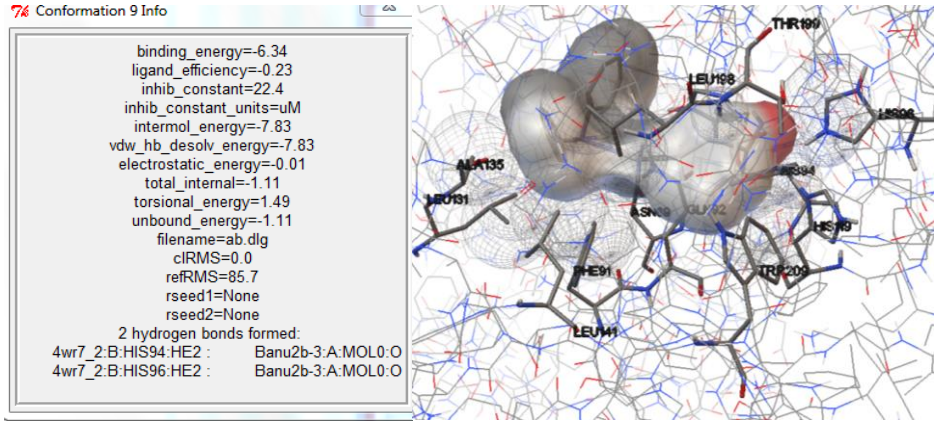
(c)

Molecular docking study of the 1a against CAs

Figure 2. Docking of CAs and 1a (a) Binding energy (b)Interaction between CAS and 1a as visualized using Auto Dock (c) Hydrogen bond forms between CAS and 1a is visualized using PYMOL

Table. Docking interaction between CAs and 1b

GLAUCOMA		1b	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
THR 199	OG1	O	3.1	-6.34
HIS 96	NE2	O	2.7	
HIS 94	NE2	O	2.7	
HIS 119	ND1	O	3.3	



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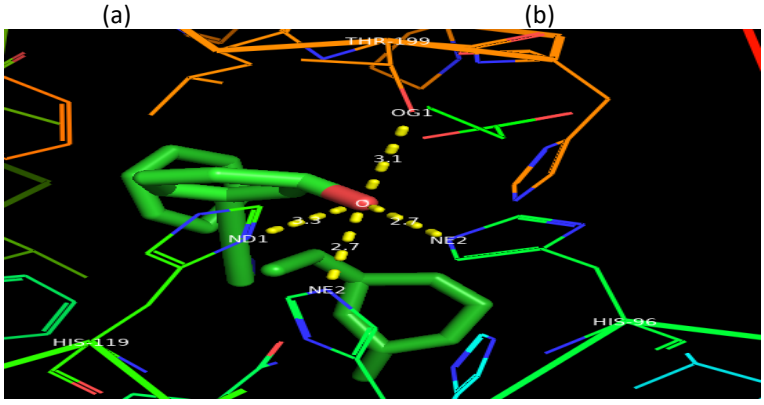


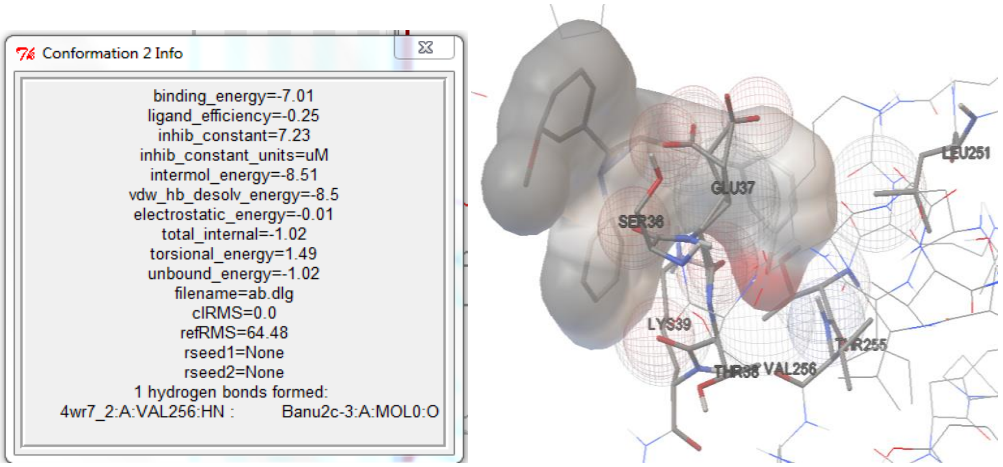
(c)

Molecular docking study of the 1b against CAs

Figure 5. Docking of CAs and 1b (a) Binding energy (b)Interaction between CAs and 1b as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1b is visualized using PYMOL

Table. Docking interaction between CAs and 1C

CAS		1C	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
VAL 256	N	O	2.9	
GLU 37	O	O	2.8	
GLU 37	O	O	2.6	-7.01



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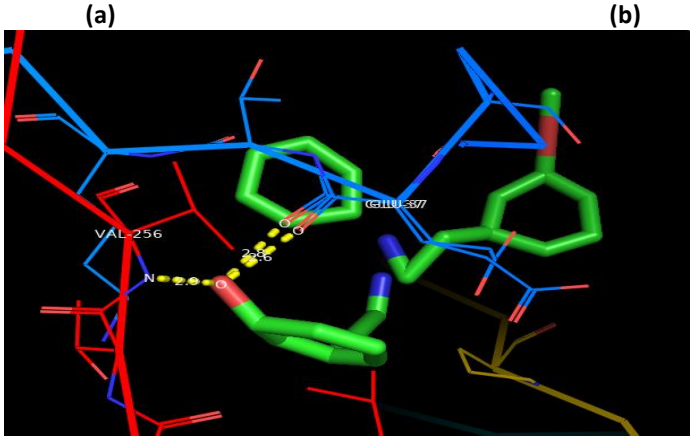


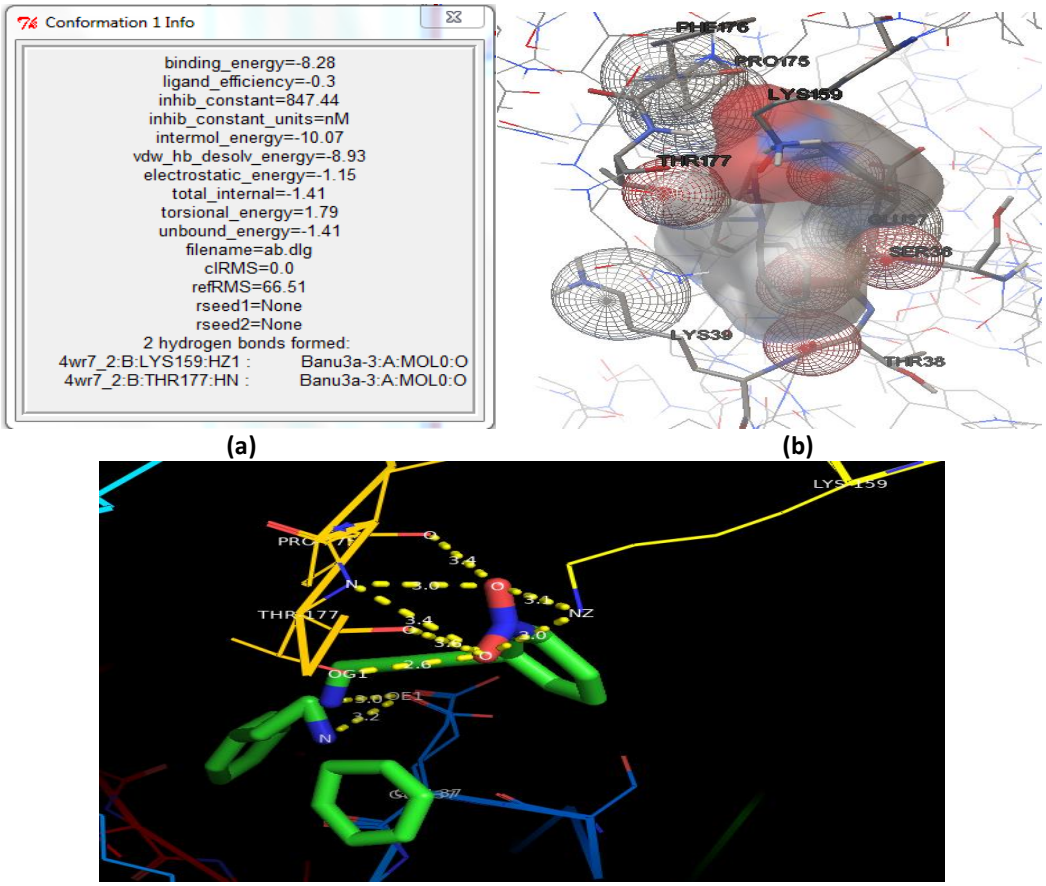
(c)

Molecular docking study of the 1C against CAs

Figure 6. Docking of CAs and 1C (a) Binding energy (b)Interaction between CAs and 1C as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1C is visualized using PYMOL

Table. Docking interaction between CAs and 1d

CAs		1d	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
THR 177	N	O	3.4	-8.28
THR 177	N	O	3.0	
THR 177	OG1	O	2.6	
PRO 175	O	O	3.4	
GLU 37	OE1	N	3.0	
GLU 37	OE1	N	3.2	
LYS 159	NZ	O	3.1	
LYS 159	NZ	O	3.0	

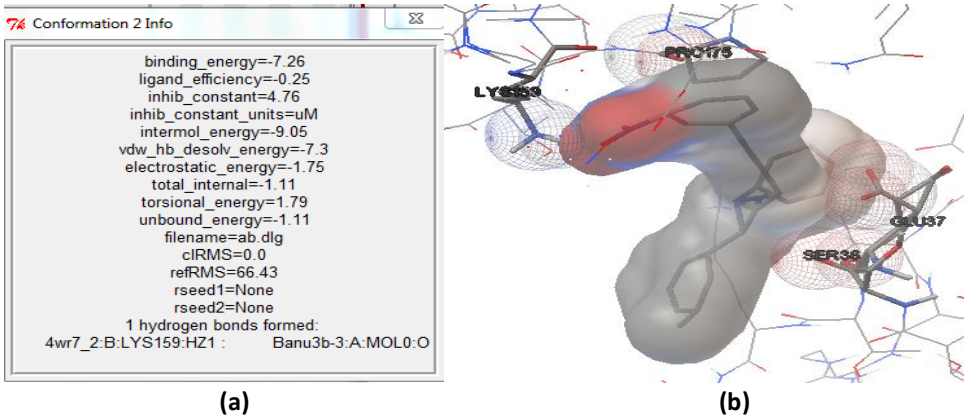


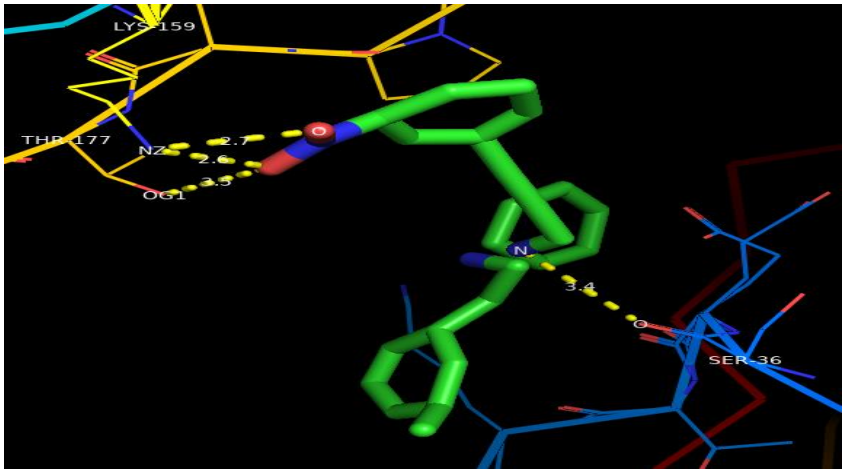
Molecular docking study of the 1d against CAs

Figure 8. Docking of CAs and 1d (a) Binding energy (b)Interaction between CAs and 1d as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1d is visualized using PYMOL

Table. Docking interaction between CAs and 1e

CAs		1e	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
SER 36	N	O	3.4	-7.26
THR 177	OG1	O	3.5	
LYS 159	NZ	O	2.7	
LYS 159	NZ	O	2.6	





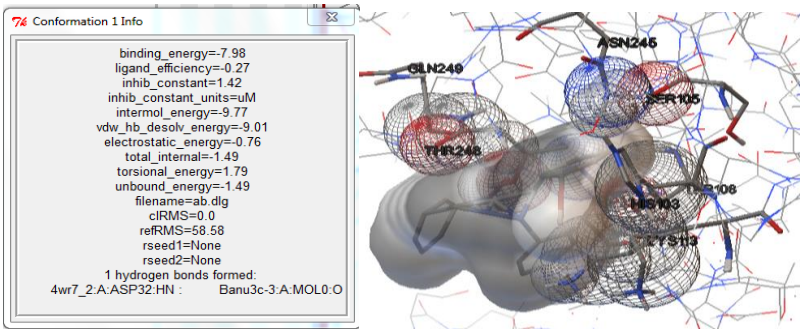
(c)

Molecular docking study of the 1e against CAs

Figure 9. Docking of CAs and 1e (a) Binding energy (b)Interaction between CAs and 1e as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1e is visualized using PYMOL

Table. Docking interaction between CAs and 1f

CAs		1f	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
ASP 32	N	O	2.8	-7.98
THR 248	OG1	N	3.5	
GLN 249	O	O	2.9	
GLN 249	O	N	3.2	



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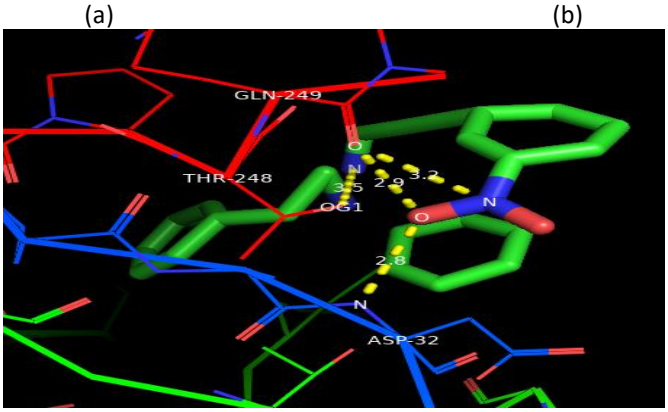


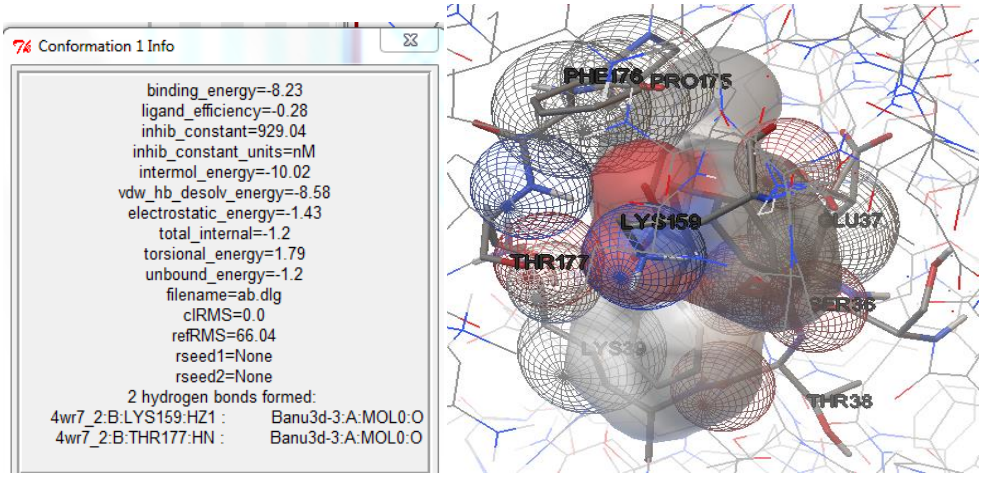
(c)

Molecular docking study of the 1f against CAs

Figure 10. Docking of CAs and 1f (a) Binding energy (b)Interaction between CAs and 1f as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1f is visualized using PYMOL

Table. Docking interaction between CAs and 1g

CAs		1g	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
THR 177	O	O	3.4	-8.23
THR 177	OG1	O	2.8	
THR 177	N	O	3.3	
THR 177	N	O	2.8	
GLU 37	OE1	N	3.5	
GLU 37	OE1	N	3.6	
LYS 159	NZ	O	3.0	
LYS 159	NZ	O	2.8	
PRO 175	O	O	3.5	



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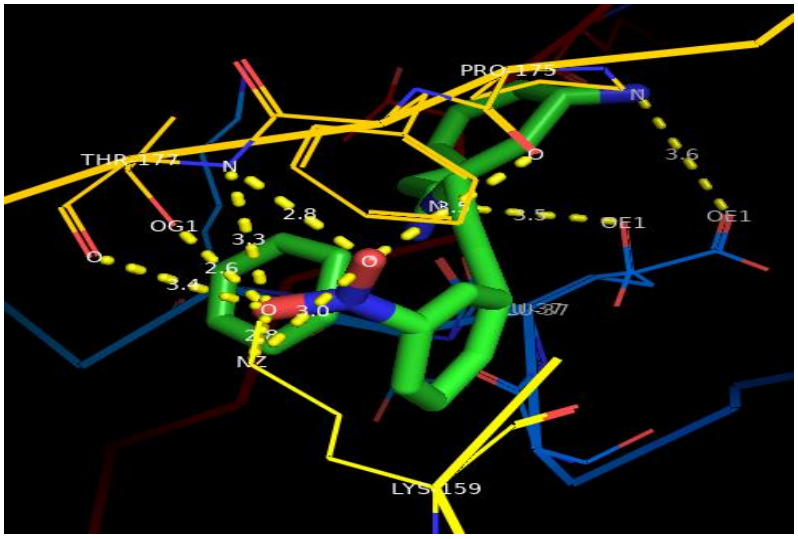


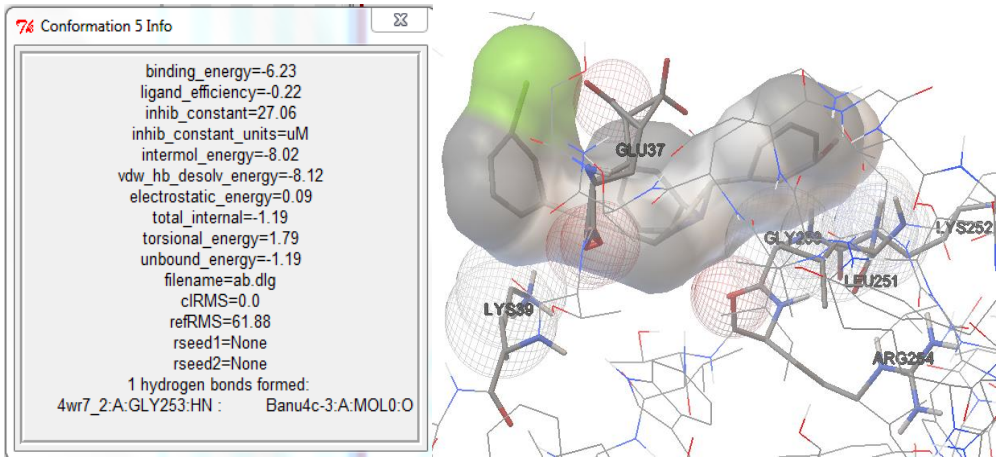
(c)

Molecular docking study of the 1g against CAs

Figure 11. Docking of CAs and 3D (a) Binding energy (b)Interaction between CAs and 1g as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1g is visualized using PYMOL

Table. Docking interaction between CAs and 1h

CAs		1h	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
ARG 254	O	N	3.0	-6.23
ARG 254	O	N	3.1	
GLY 253	N	O	3.2	
LYS 159	N	O	3.2	



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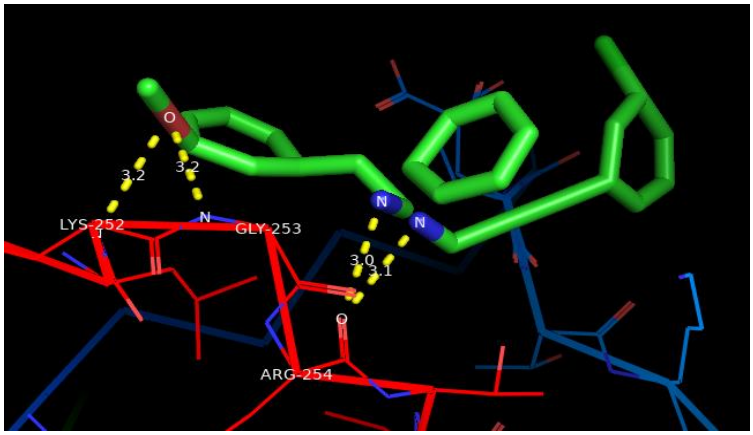


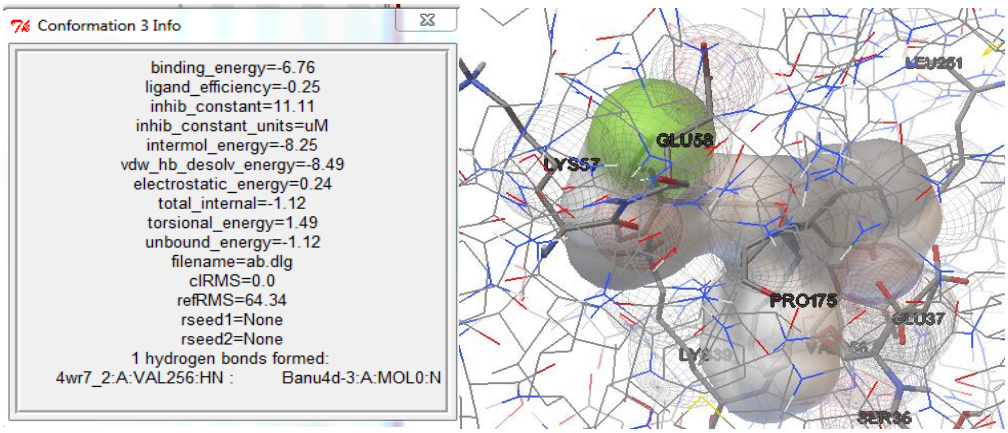
(c)

Molecular docking study of the 1h against CAs

Figure 13. Docking of CAs and 4C (a) Binding energy (b)Interaction between CAs and 1h as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1h is visualized using PYMOL

Table. Docking interaction between CAs and 1i

CAs		1i	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
GLU 37	O	N	2.9	-6.76
GLU 37	OE1	N	3.3	



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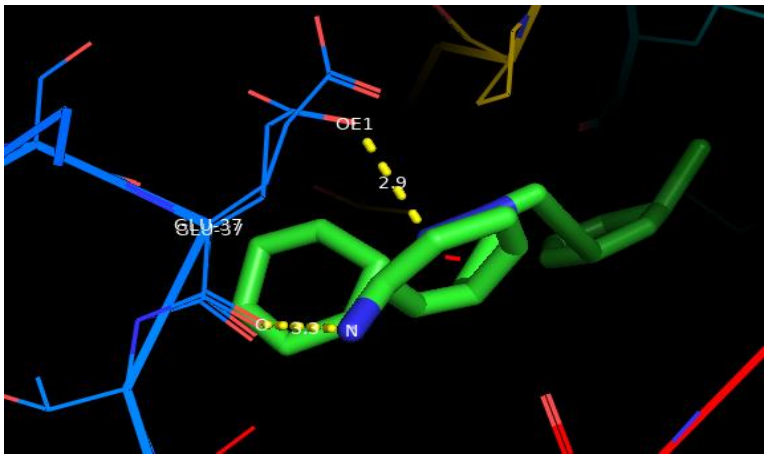


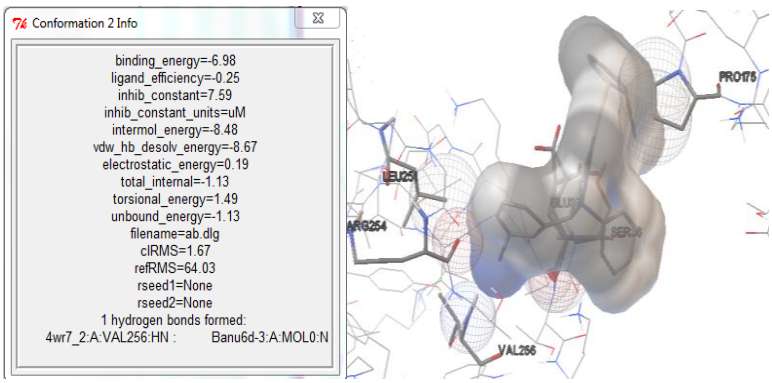
(c)

Molecular docking study of the 1i against CAs

Figure 14. Docking of CAs and 1i (a) Binding energy (b)Interaction between CAs and 1i as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1i is visualized using PYMOL

Table. Docking interaction between CAs and 1j

CAs		1j	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
GLU 37	O	N	3.2	-6.98
GLU 37	O	N	3.9	
GLU 37	OE1	N	2.7	
GLU 37	OE1	N	2.9	



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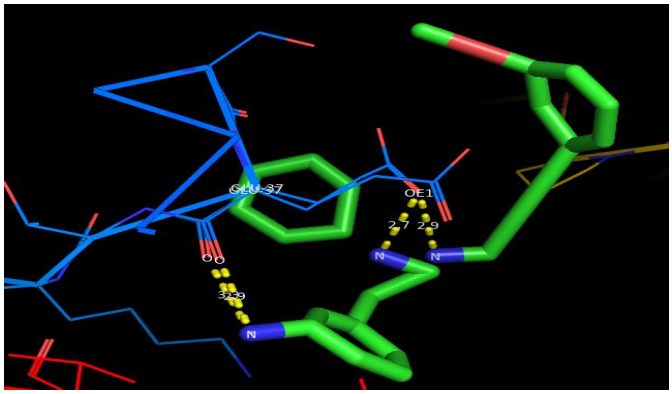


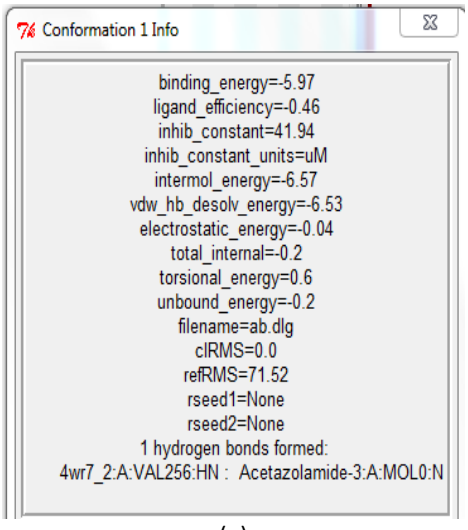
(c)

Molecular docking study of the 1j against CAs

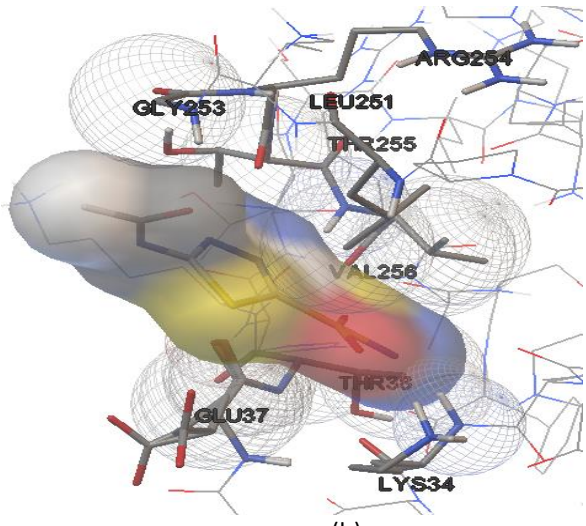
Figure 18. Docking of CAs and 1j (a) Binding energy (b)Interaction between CAs and 1j as visualized using Auto Dock (c) Hydrogen bond forms between CAs and 1j is visualized using PYMOL

Table. Docking interaction between CAs and Actazolamide

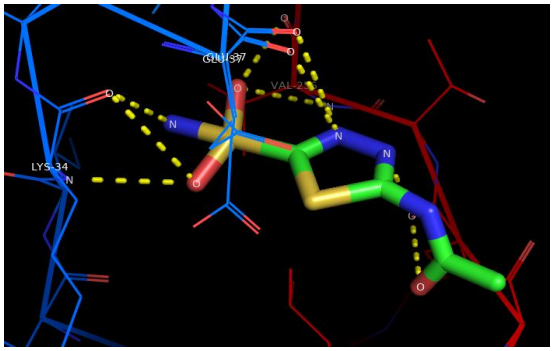
CAS		ACTAZOLAMIDE	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
LYS 34	N	O	3.2	-5.97
LYS 34	O	O	3.4	
LYS 34	O	N	2.7	
GLU 37	O	N	3.5	
GLU 37	O	N	3.1	
VAL 256	O	O	3.0	
VAL 256	N	O	3.4	
VAL 256	O	N	2.8	
VAL 256	O	O	3.1	



(a)



(b)



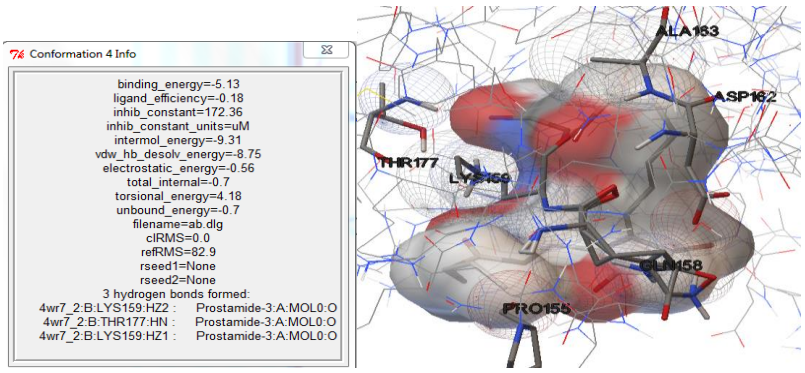
(c)

Molecular docking study of the Actazolamide against CAs

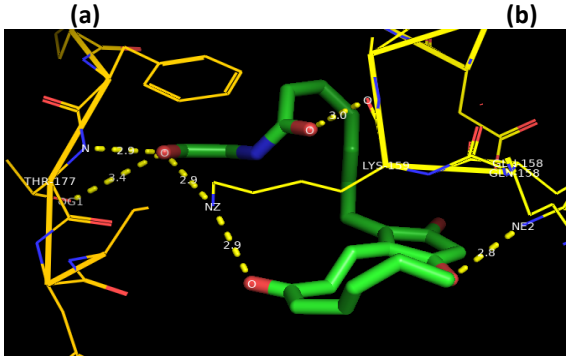
Figure 19. Docking of CAs and Actazolamide (a) Binding energy (b)Interaction between CAs and Actazolamide as visualized using Auto Dock (c) Hydrogen bond forms between CAs and Actazolamide is visualized using PYMOL

Table. Docking interaction between CAs and Prostamide

CAs		PROSTAMIDE	DISTANCE Å	BINDING SCORE (Kcal/mol)
RESIDUE	ATOM	ATOM		
GLN 158	NE2	O	2.8	-5.13
LYS 159	O	O	3.0	
THR 177	N	O	2.9	
THR 177	OG1	O	3.4	
THR 177	NZ	O	2.9	
THR 177	NZ	O	2.9	



(a) **(b)**



(c)

Molecular docking study of the Prostamide against CAs

Figure 20. Docking of CAs and Prostamide (a) Binding energy (b)Interaction between CAs and Prostamide as visualized using Auto Dock (c) Hydrogen bond forms between CAs and Prostamide is visualized using PYMOL

Table. OVERALL DOCKING RESULT OF GLAUCOMA

S.NO	COMPOUND NAME	KEY RESIDUE	DOCKING ENERGY (Kcal/mol)	NO.OF. HYDROGEN BONDS
1.	1a	GLU 37, GLU 37	-6.34	1
2.	1b	THR 199, HIS 96, HIS 94, HIS 119	-6.34	2
3.	1c	VAL 256, GLU 37, GLU 37	-7.01	1
4.	1d	THR 177, THR 177, THR 177, PRO 175, GLU 37, GLU 37, LYS 159, LYS 159	-8.28	2
5.	1e	SER 36, THR 177, LYS 159, LYS 159	-7.26	1
6.	1f	ASP 32, THR 248, GLN 249, GLN 249	-7.98	1
7.	1g	THR 177, THR 177, THR 177, THR 177, GLU 37, GLU 37, LYS 159, LYS 159, PRO 175	-8.23	2
8.	1h	ARG 254, ARG 254, GLY 253, LYS 159	-6.23	1
9.	1i	GLU 37, GLU 37	-6.76	1
10.	1j	GLU 37, GLU 37, GLU 37, GLU 37	-6.98	1
11.	Acetazolamide	LYS 34, LYS 34, LYS 34, GLU 37, GLU 37, VAL 256, VAL 256, VAL 256, VAL 256	-5.97	1
12.	Prostamide	GLN 158, LYS 159, THR 177, THR 177, THR 177, THR 177	-5.13	3

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CONCLUSION

Molecular Docking Insilico Studies using Autodock 4.2 is very useful in shortlisting lead compounds for treating glaucoma. Nearly ten compounds are subjected to AutoDock studies. All the compounds show promising result with a inhibition of Glaucoma and all the compounds are compared with the standard drug Acetazolide and Prostamide. Out of ten compounds two compounds can serve as promising lead compounds: 1b ((E)-3-(2-(1-phenyl-5-(m-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)vinyl)phenol) and 1g ((E)-3(3-(3-nitrostyryl)-1-phenyl-4,5-dihudro-1H-pyrazol-5-yl)aniline). Lead compounds posses two hydrogen bonds and they can be further used for structure-based discovery of Novel drugs for the treatment of glaucoma.

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