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SYNTHESIS, CHARACTERIZATION AND MOLECULAR DOCKING STUDIES OF ARYLOXYPROPANOLAMINES

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ABSTRACT

Molecular docking was done on protein which is Turkey Beta-1 Adrenergic receptor with Stabilising mutations and bound against Isoprenaline (2Y03) is selected for docking with a ligand,1-(4-(3-(4-amino-5-(4-chlorophenyl) pyrimidin-2-ylamino)-2-hydroxypropoxy)phenyl)ethanone (AOP 1) as ligand which forms protein-ligand complex having high binding affinity & is selected for synthesis by taking p-hydroxy acetophenone & epichlorhydrin as substrates & reactants respectively. Methanol and amines are also used as reactants.

KEYWORDS: 1-(4-(3-(4-amino-5-(4-chlorophenyl)pyrimidin-2 -ylamino)-2-hydroxypropoxy)phenyl)ethenone (AOP 1), Turkey Beta-1 Adrenergic receptor with Stabilising mutations and bound against Isoprenaline (2Y03).

INTRODUCTION

Molecular docking is a Computer Aided Drug Design (CADD) which involves formation of ligand-macromolecule complex. With the availability of 3D structure of macromolecule, we can determine the preferred binding mode of ligand to active site of macromolecule to form a stable complex. By using different softwares, the binding affinity of ligand to macromolecule is analysed. CADD is not only used to find out possible binders or inhibitors, it also evaluates strength of binding affinity between the ligand and macromolecule. It is useful to know the binding strength of a group of compounds or derivatives to determine which derivative is the best binder or inhibitor.

The theory of molecular docking involves substrate recognition process. It is explained by lock and key concept. In this concept, the binding of the ligand (small molecule or substrate protein) will be fitted to receptor active site. The general representation of molecular docking is shown in Fig. 1 and the Lock and Key concept was represented in Fig. 2.

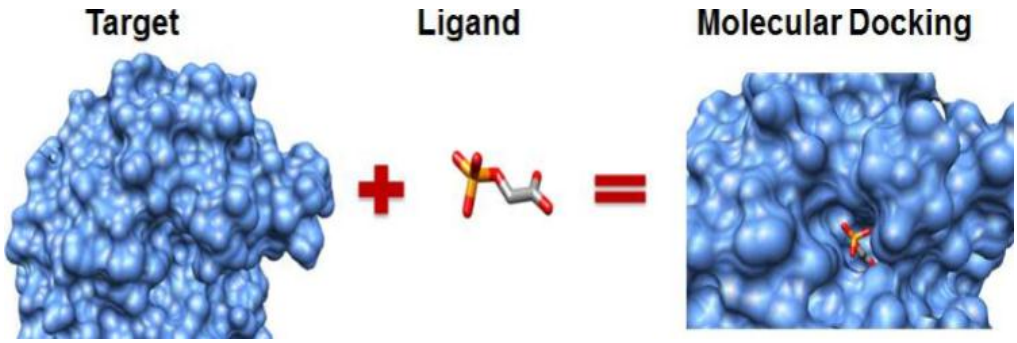


Fig. 1: Representation of molecular docking.

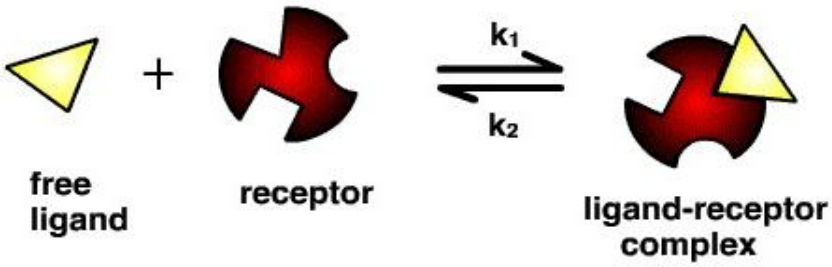


Fig 6: Lock & key concept.

APPLICATIONS OF DOCKING

- **Virtual screening (hit identification):** Docking with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.
- **Drug Discovery (lead optimization):** Docking can be used to predict in where and in which relative orientation a ligand binds to a protein (binding mode or pose). This information may in turn be used to design more potentand selective analogue.
- **Bioremediation:** Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes.
- **Drug-DNA Interaction:** Molecular docking plays a prominent role in initial prediction of drugs binding properties of nucleic acid. This information establishes the correlation between drug's molecular structure and its cytotoxicity.

- The potency and/or the selectivity of the drug candidate towards the protein, can therefore be improved.

STEP BY STEP PROCEDURE FOR DOCKING

A. Selecting of Macromolecule: Three-dimensional structure of protein molecule, Turkey beta-1 adrenergic receptor with stabilising mutations and bound against Isoprenaline (2Y03) is selected for docking with 1-(4-(3-(4-amino-5-(4-chlorophenyl)pyrimidin-2-ylamino)-2-hydroxypropoxy)phenyl)ethenone (AOP 1) as ligand. The receptor 2Y03 is downloaded from the Protein Data Bank (PDB) which is in PDB format. The structure of protein is shown in Fig. 3.

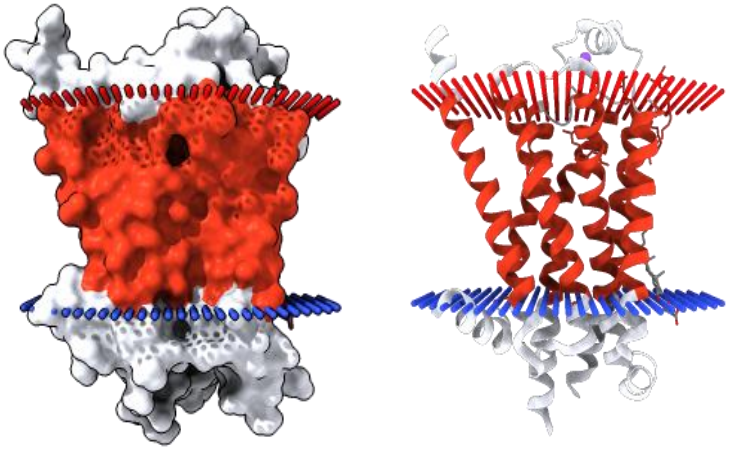


Fig 3: **Turkey beta-1 adrenergic receptor with Stabilizing mutations and bound against Isoprenaline.**

B. Autodocking of Macromolecule: After the protein is retrieved from PDB, protein should be Auto dock. It is done by using the PyRx software. PyRx software contains Navigator, View & Control bar. Under Navigator bar there are sub sections named Molecule, Auto dock, TVTK, andMayavi. Under View bar there are 3D scene, 2D plots, Documents, and Tables. Control bar having Vina wizard, Auto dock wizard, Open babel, Python shell, and Logger. In the software, FILE is used to select option ―LOAD MOLECULE (as shown in Fig 4).

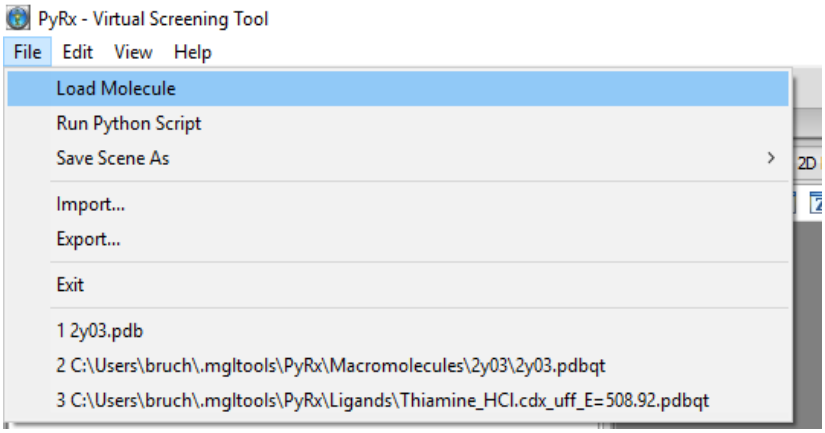


Fig 4: Step 1 – selection of load molecule option in software.

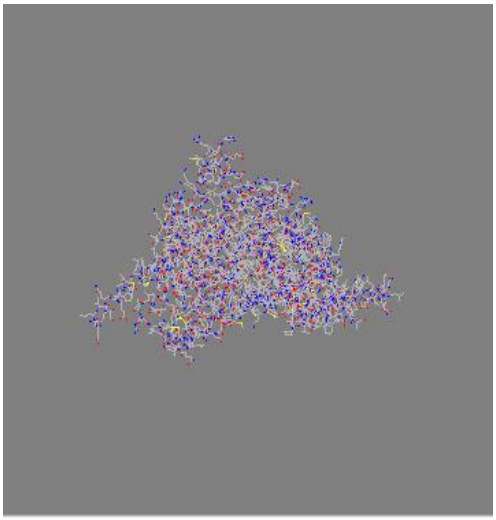


Fig 5: Uploaded 3D structure of Protein.

Under the sub-section ―Molecule 2Y03 is present, right click on 2Y03 protein, shows the option AUTO DOCK. Selection of the Auto dock further shows, make ligand &make macromolecule. Select the option MAKE MACROMOLECULE to get the Auto docked protein molecule (as shown in Fig 10).

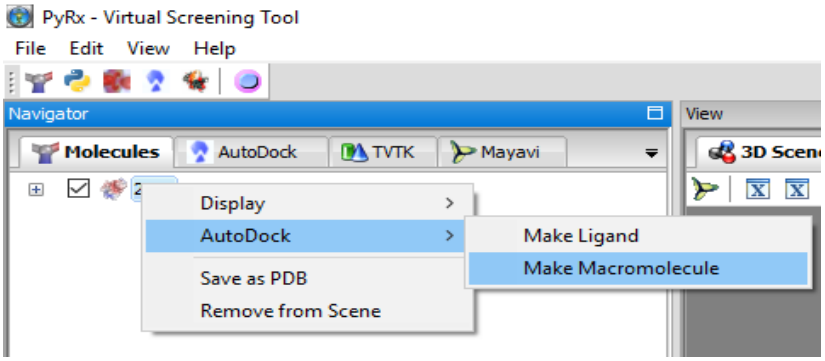


Fig 6: Step 2- Making of protein molecule into macromolecule.

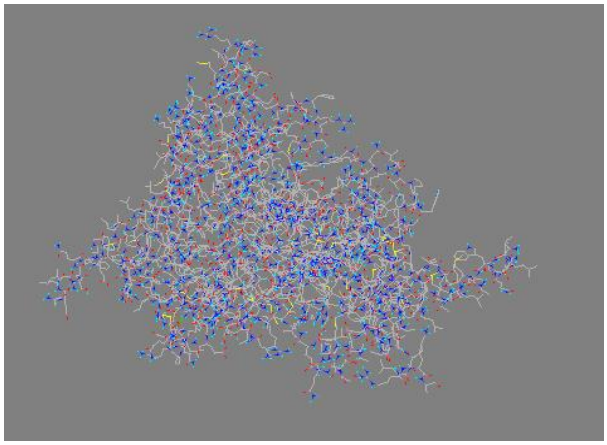


Fig 7: Macromolecule of Protein.

C. Selecting The Ligand: The selected ligand which is1-(4-(3-(4-amino-5-(4-chlorophenyl)pyrimidin-2-ylamino)-2-hydroxypropoxy)phenyl)ethenone(which is formed by taking the Epoxide derivative as reactant with substrate Pyrimethamine), is drawn using ChemDraw and is downloaded in SDF format. The SDF format of ligand molecule is loaded by using the FILE in which the option—IMPORT is selected (asshown in Fig 8).

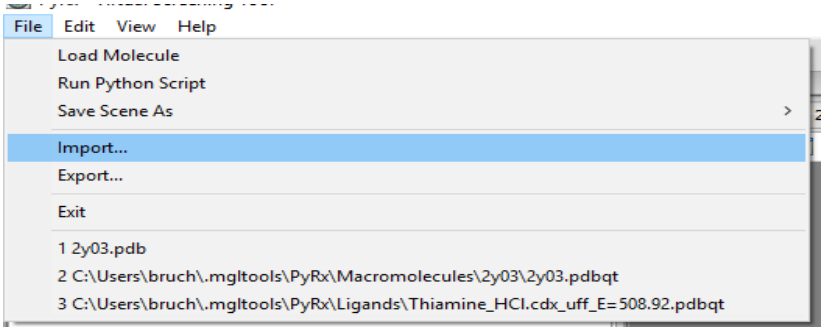


Fig 8: Step 3- Selection of import option in software.

Click on the chemical table file to load ligand in SDF form.

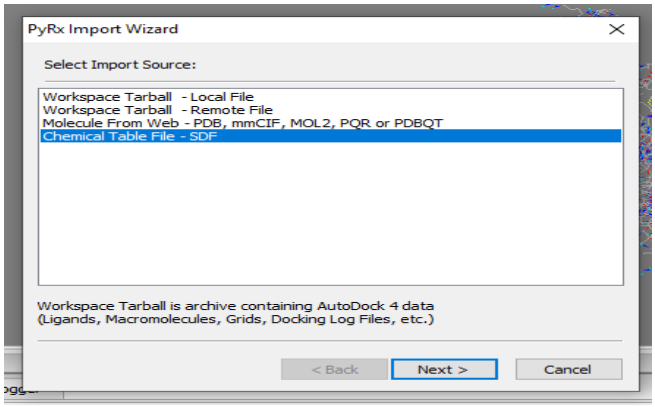


Fig 9: Step 4 – Selecting the ligand in SDF form.

In the —View bar, Open babel is seen only when structure appears in —View bar. Open babel of control bar is seen with Title, Molecular formula, Molecular weight, Number of atoms of the loaded ligand. Right click on selected ligand in the open babel select the - MINIMISE SELECTED. We get Gibb's free energy i.e., E value of the product E= 213.97 (as shown in Fig 10).

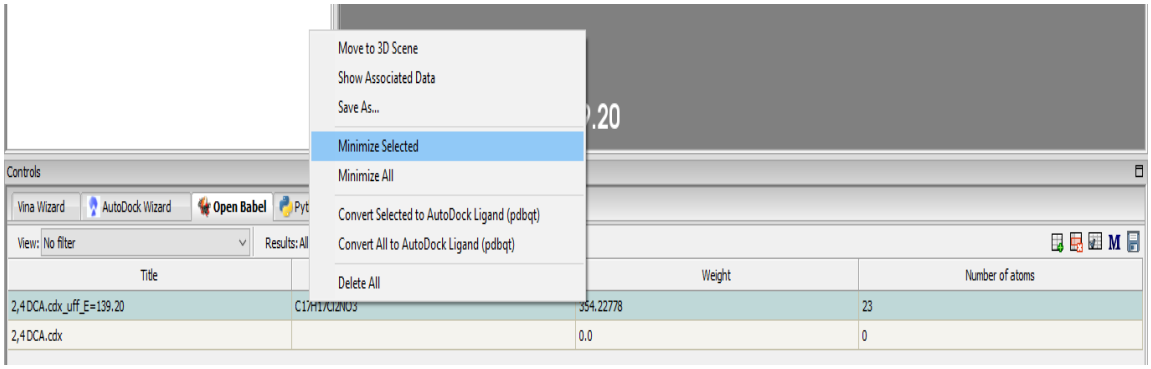


Fig 10: Step 5 – Minimising the ligand.

Again, right click on the selected ligand in the open babel, select the option CONVERT SELECTED TO AUTODOCK LIGAND (pdbqt), as shown in Fig 11.

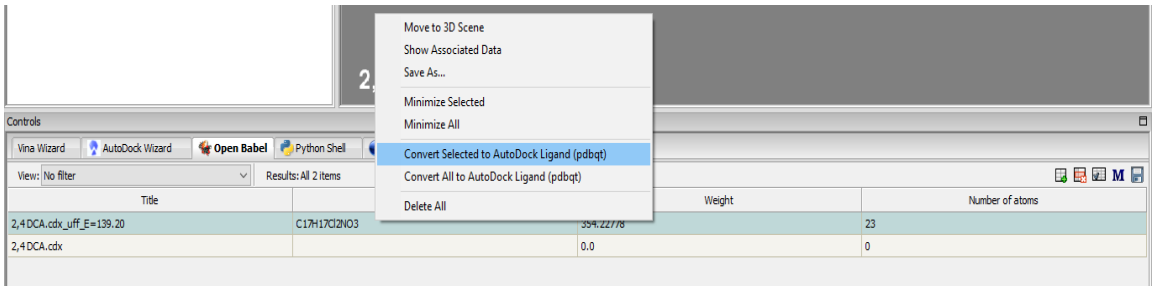


Fig 11: Step 6 – Converting the ligand to AutoDock ligand (pdbqt).

Go to Vina wizard from controls bar and click START and FORWARD for formation of protein-ligand complex, as shown in Fig 12 and Fig 13.

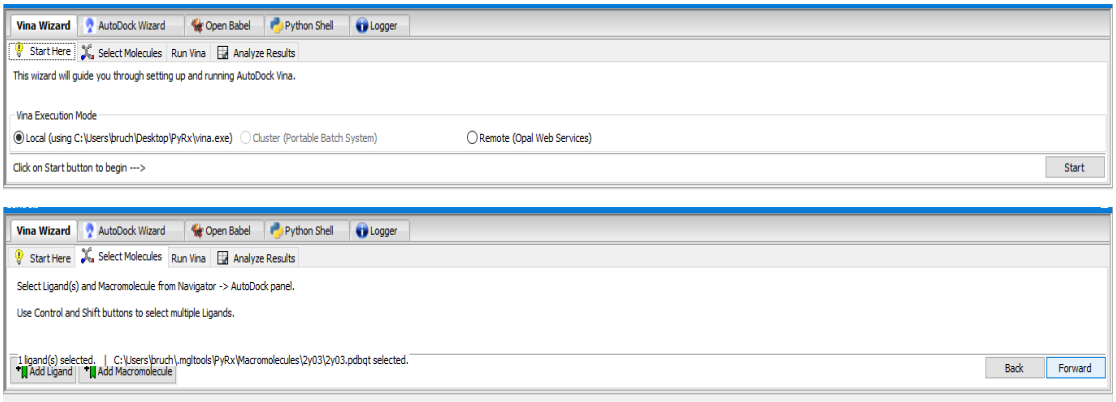


Fig 12: Step 7 – Click start and forward options.

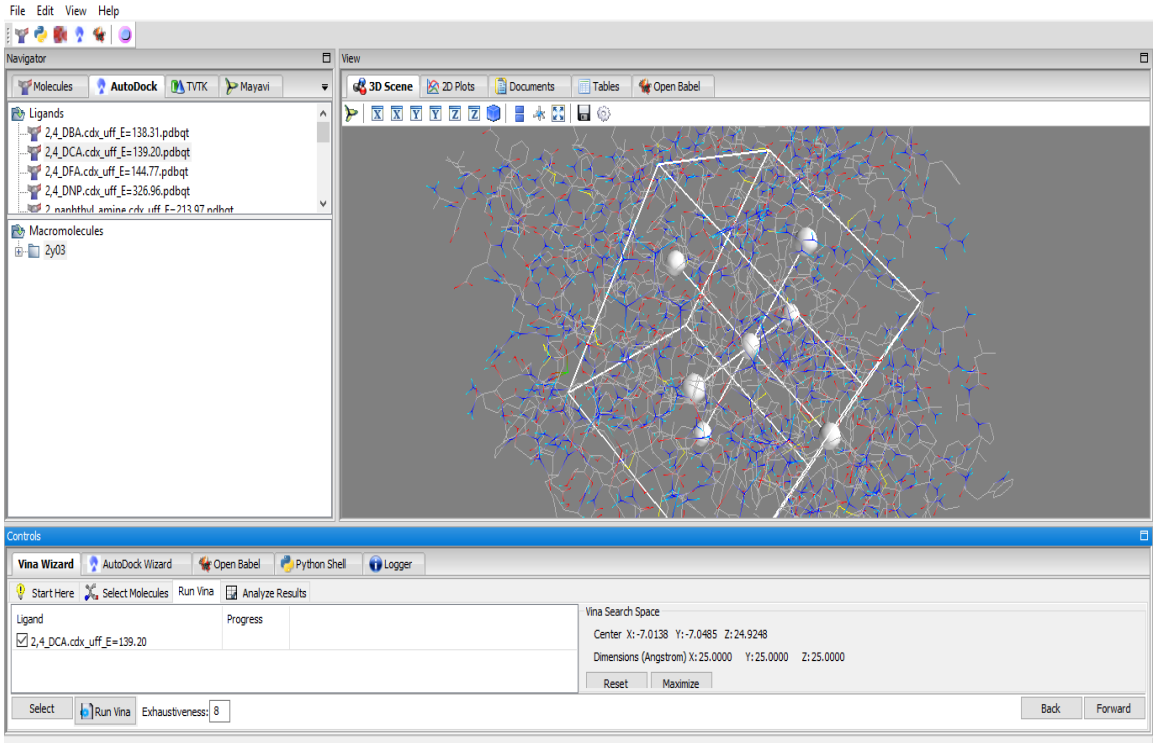


Fig 13: Step 8 – Click forward option again.

Click the ―RUN VINA, docking score function runs and results appear. Download the SDF format of the protein-ligand complex for the evaluation, as shown in Fig 14 and Fig 15.

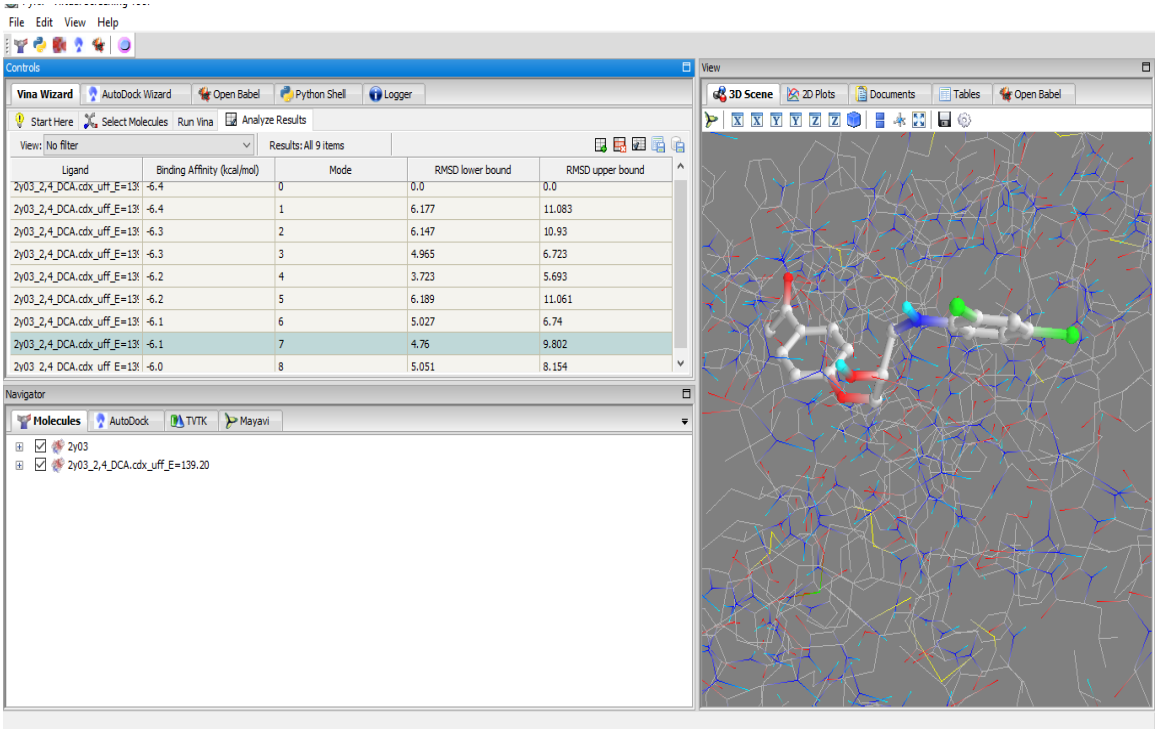


Fig 15: Docking results appear.

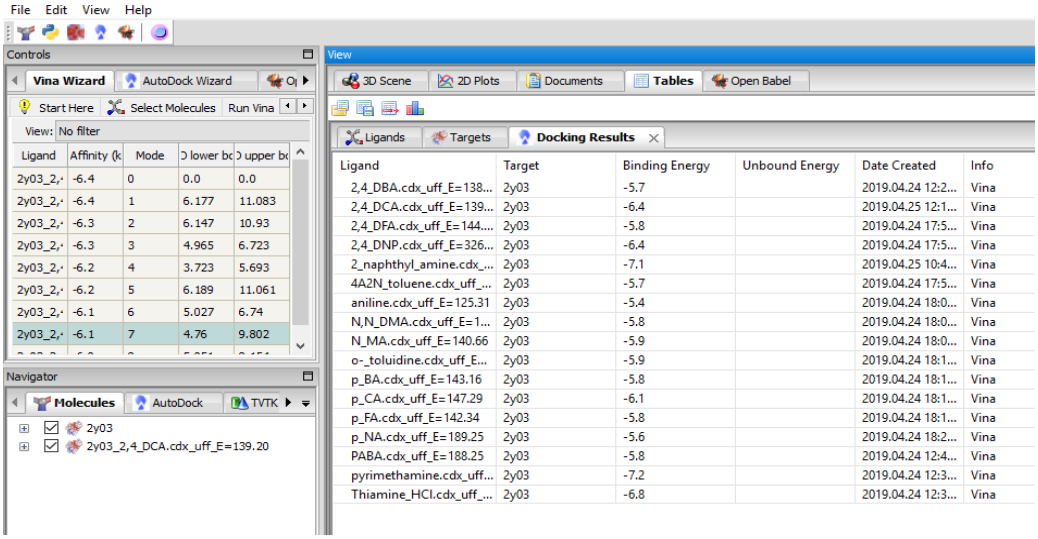
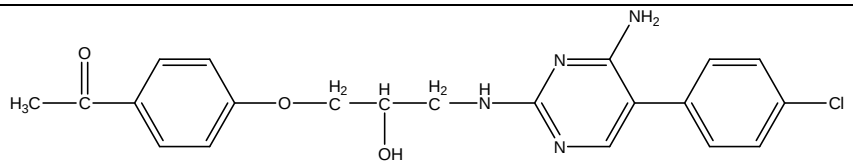
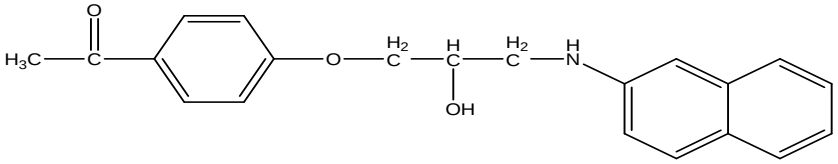
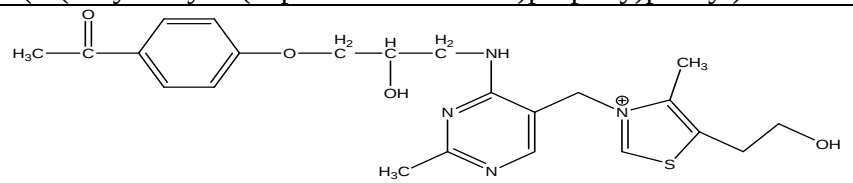
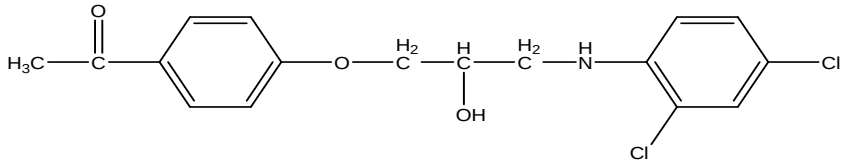


Fig 16: SDF format of the results.

RESULTS OF DOCKING

Molecular docking was performed on 17 derivatives of Aryoxypropanolamines with the protein mentioned above. The docking scores of best 4 molecules, out of 17, were mentioned in the table below.

Code	Structure of ligand	Docking score
AOP 1	 1-(4-(3-(4-amino-5-(4-chlorophenyl)pyrimidin-2-ylamino)-2-hydroxypropoxy)phenyl)ethenone	**-7.2**
AOP 2	 1-(4-(2-hydroxy-3-(naphthalen-3-lamino)propoxy)phenyl)ethanone	**-7.1**
AOP 3	 1-(4-(3-(5-((5-(2-hydroxyethyl)-4-methylthiazol-3(2H)-yl)methyl)-2-methylpyrimidin-4-ylamino)-2-hydroxypropoxy)phenyl)ethenone	**-6.8**
AOP 4	 1-(4-(3-(2,4-dichlorophenylamino)-2-hydroxypropoxy)phenyl)ethenone	**-6.4**

GENERAL METHOD OF SYNTHESIS OF ARYLOXYPROPANOLAMINES

- **Chemicals**

4- hydroxy acetophenone, 0.6M methanolic sodium hydroxide solution, Solution of epichlorhydrin in methanol, Epoxide, Dry methanol, anhydrous isopropylamine (99%), 2N hydrochloric acid, Ether, 11N sodium hydroxide, Water.

- **Apparatus**

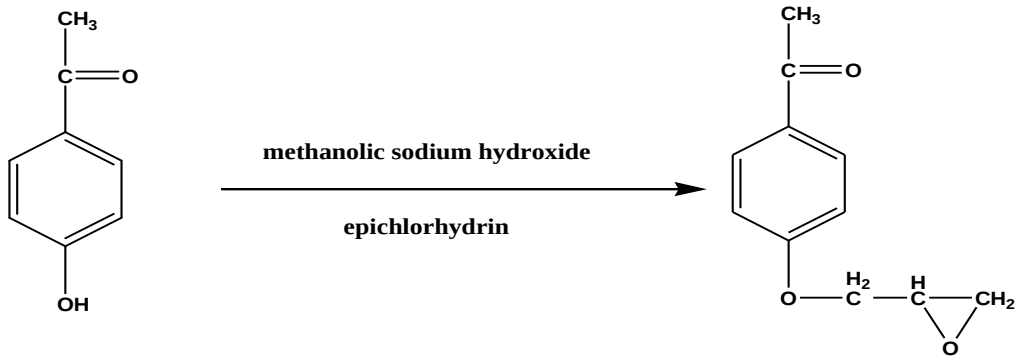
Round bottomed flask, Reflux condenser, Water bath, Conical flasks, Beakers, Test tubes, Glass rods, Melting point apparatus, Thermometer.

- **Procedure**

Step I- Formation of Epoxide

4-hydroxy acetophenone (0.005moles) was dissolved in 10ml of 0.6M methanolic sodium hydroxide solution, which was added over an hour into a solution of epichlorhydrin (0.1mole) in methanol. The mixture was left overnight to yield the crude product. It was bittered, washed with water and methanol, and dried. In case, a solid precipitate was not formed on completion of the reaction, the excess of epichlorhydrin and methanol were removed by evaporation under vacuum. The oily residue was taken in ethyl acetate and allowed to stay overnight. The epoxide was separated in the form of oil in high yield. The residue so obtained was used as such for the next step.

The chemical reaction was shown in Scheme 1.



4-hydroxy acetophenone **Epoxide derivative**

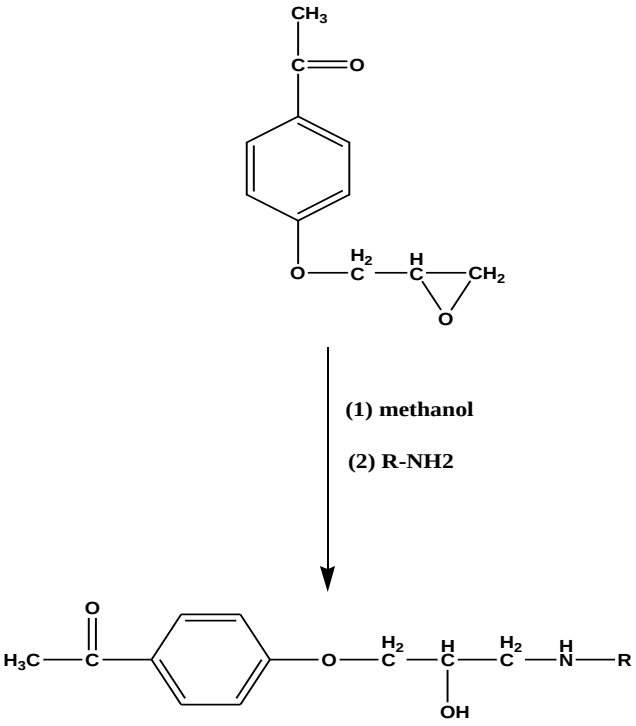
(Scheme 1).

Step II- Preparation of Aryloxypropanolamines

0.02moles of epoxide was dissolved in dry methanol and a large excess of anhydrous isopropylamine (99%) was added. It was refluxed on water bath until the reactions was

completed. At the end of the reaction, the excess isopropylamine was evaporated to dryness in vacuum. The residue obtained was extracted between 2N hydrochloric acid and ether. The hydrochloric acid layer was basified with 11N sodium hydroxide. The product was filtered, dried and re-crystallised from ethyl acetate. In case, the product obtained was found to be hygroscopic, it is converted into the oxalate salts. A solvated solution of oxalic acid in ethanol was added equimolar quantities to an ethanolic solution of the compound. This solution was left over-night and precipitate was filtered and dried.

The chemical reaction was shown in Scheme 2.



(Scheme 2).

The synthesis of the best three derivatives of Aryloxypropanolamines were synthesized in the same procedure as mentioned above. "**R**" is replaced with the ligands taken for docking.

CHARACTERIZATION

Table 1: Physical parameters of three Aryloxypropanolamines with high docking score.

S.no	Molecule Name	Molecular Formula	Molecular Weight (gm)	Melting Point (°c)	% Yield
1.	AOP 1	$C_{21}H_{21}ClN_4O_3$	412.87	224	78.2
2.	AOP 2	$C_{21}H_{21}O_3N$	335.4	207	64.5
3.	AOP 3	$C_{23}H_{29}O_4N_4S$	457.57	198	60.8

Biological Evaluation

In view of various biological and pharmacological importances of different Aryoxypropanolamines, it is felt worthwhile to evaluate them for possible activities. The compounds obtained in the present study were screened for anti-hypertensive activity. Moreover, the results obtained on the Aryloxypropanolamines in our laboratory possessed significant anti-hypertensive activity.

Anti-hypertensive Studies

The reported Aryloxypropanolamines possess anti-hypertensive activity. The compounds prepared during the present work were tested for anti-hypertensive activity.

EXPERIMENTAL PROCEDURE

Ex-vivo inotropic and chronotropic effect on isolated frog heart

The frog was pithed and destroyed by passing a stiletto through the occipito-atlantic junction. The anterior chest wall was opened and a pericardiectomy was performed to expose the heart. The one end of the aorta, inferior vena cava was identified. A small cut inferior vena cava and a Syme's cannula was inserted towards the heart. A steady flow of the perfusion Frog-Ringer solution containing oxygenated, fluid of the following composition: NaCl 6.5, KCl 0.14, CaCl2 0.12, and NaHCO3 0.2, NaH2PO4 0.01, Glucose 2.0 in g /L. A steady flow of the perfusion Frog-Ringer solution was perfused through this cannula and there was an opening in the cannula through which drugs could be injected by pushing a capillary tube attached to a syringe through an injection needle. A very thin hook was attached to the apex of beating heart and connects it to force transducer on power lab to find the cardiac parameter such as force of contraction, heart rate and cardiac output. The normal contraction of frog heart was recorded on smoked drum as described previously. Sub-sequentlyinjected0.1 mg of adrenaline, in a sequential order and noted the change in the rate and amplitude of contraction. Maintained 15min gap between the administration of each dose of drug and test samples. (Gattusoetal., Kulkarn, 1999).

RESULTS

The cardiogram obtained, when AOP 1, AOP 2 AND AOP 3 were administered are shown in Fig. 17, Fig, 18 and Fig.19. The heart rate and cardiac output of Frog's Heart, when AOP 1, AOP 2 and AOP 3 were administered.

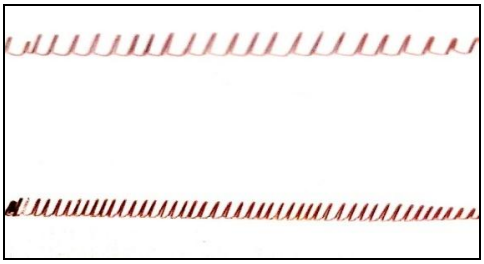


Figure 17: Action of AOP 1 on Frog's Heart.

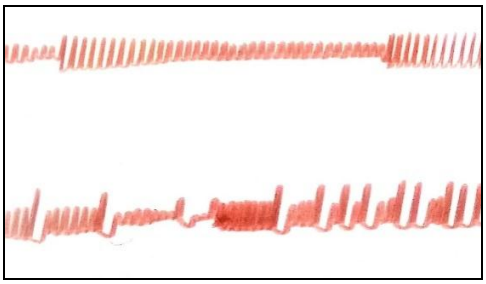


Figure 18: Action of AOP 2 on Frog's Heart.

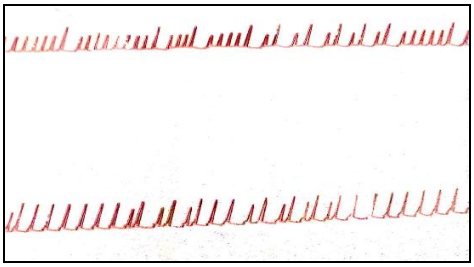


Figure 18: Action of AOP 3 on Frog's Heart.

Table 2: Anti-hypertensive studies of AOP 1.

S.no.	Type of solution	Heart rate	Cardiac output
1.	Normal	98	14
2.	Drug solution	76	9

Table 3: Anti-hypertensive studies of AOP 2.

S.no.	Type of solution	Heart rate	Cardiac output
1.	Normal	96	15
2.	Drug solution	81	13

Table 4: Anti-hypertensive studies of AOP 3.

S.no.	Type of Solution	Heart Rate	Cardiac Output
1.	Normal	96	12
2.	Drug solution	85	10

SUMMARY AND CONCLUSION

Molecular docking is a Computer Aided Drug Design (CADD) which involves formation of ligand-macromolecule complex. With the availability of 3D structure of macromolecule, we can determine the preferred binding mode of ligand to active site of macromolecule to form a stable complex.

Aryloxypropanolamines are important class of β-adrenergic blocking agents (β-blockers) and extensively used in medicinal chemistry for the treatment of hypertension, angina pectoris, glaucoma, anxiety, and obesity.

The receptor or protein, Turkey Beta-1 Adrenergic receptor with Stabilising mutations and bound against Isoprenaline (2Y03) was docked with various Aryloxypropanolaminederivatives. Among the 17 derivatives of Aryloxypropanolamines, 1-(4-(3-(4-amino-5-(4-chlorophenyl) pyrimidin-2-ylamino)-2-hydroxypropoxy) phenyl) ethenone; (AOP1) was best docked with a Docking score of -7.2 and Gibb's free energy of 476.65.

Aryloxypropanolamines AOP 1, AOP 2 and AOP 3 were screened for anti-hypertensive activity. For this purpose, a frog's heart was used to observe the activity of different Aryloxypropanoalmines. Based on the results obtained, it is confirmed that, AOP 1 has better action on the heart rate and cardiac output compared to AOP 2 and AOP 3, and therefore, AOP 1 shows better anti-hypertensive activity.

It is concluded that AOP 1 have high docking score and is selected as the best derivative of Aryloxypropanolamies with better anti-hypertensive activity compared to others.

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