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In Silico Identification of Novel Indole Analogues as Potent β - Secretase Inhibitors

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ABSTRACT

Alzheimer's disease is a serious health issue with serious social and economic consequences. The availability of drugs based on symptoms is not adequate, and treatment of AD remains difficult. It is vital to take care of all necessary measures in the context of existence, which is becoming more difficult for the human brain to handle. The condition is brought on by the body's multidirectional pathology, which necessitates the employment of the multi-target-directed ligand (MTDL) strategy. Based on this, the current work with the indole fused pyrimidine inspired molecule as a lead provides hope for a novel lead for AD. To evaluate the β-Secretase inhibitory activity of designed indole fused pyrimidine analogues using in silico docking studies. In silico docking studies were carried out using Auto Dock Vina. The docking scores of indole fused pyrimidine analogues varied between -7.0 to -7.8kcal/mol. When compared to the standard docking score, test compound IP-2 was shown to be more significant. The further development of potent inhibitors for the treatment of Alzheimer's disease from these molecular docking analyses. Further investigations on the above compounds are necessary to develop potential chemical entities for the prevention and treatment of Alzheimer's disease.

INTRODUCTION

Alzheimer's disease is a brain disease that gradually impairs thinking and memory abilities as well as the capacity to do even the most basic tasks. Alzheimer's disease concerns A total of 121,499 deaths from AD were reported in official death certificates in 2019, making Alzheimer's disease the sixth-leading cause of death in the country. Alzheimer's disease was the seventh-leading cause of death in 2020 and 2021, when COVID-19 made its way into the top ten causes of death list. Among Americans aged 65 and older, Alzheimer's disease continues to be the fifth-leading cause of mortality. Deaths from heart disease, HIV, and stroke all declined between 2000 and 2019, but recorded deaths from AD developed by more than 145%. The COVID-19 pandemic in 2020 most likely made this trajectory of AD mortality worse. More than 11 million family members and other unpaid caregivers provided an estimated 18 billion hours of care to people with Alzheimer's or other dementias in 2022.

More than 25% of COVID-19 patients experience neurological symptoms such headaches, anosmia, hyposmia, and memory loss. Viral entry into the central nervous system (CNS) and/or the ensuing neuroinflammation, both of which are associated with an increased risk for Alzheimer's disease (AD), may have neurological consequences. Therapeutic BACE1 inhibition has the potential to revolutionise Alzheimer's disease treatment.

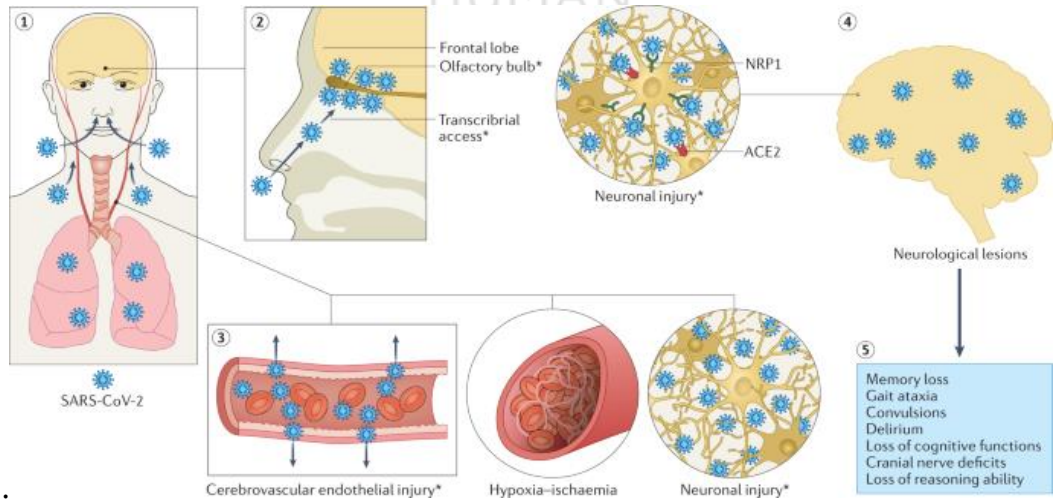


Figure - 1 Routes of the spread of SARS-CoV-2 across the CNS and the possible mechanisms involved in neuronal injury, AD

Indole molecules produced by the gut microbiota possess essential biological activities, including neuroprotection and antioxidant properties. Since then, research has cemented additional characteristics of these substances, including anti-inflammatory, immunoregulatory, and amyloid anti-aggregation features. By targeting multiple pathologic mechanisms simultaneously, certain indoles may be excellent candidates to ameliorate neurodegeneration. We propose that the management of the microbiota to induce a higher production of neuroprotective indoles (e.g., indole propionic acid) will promote brain health during aging. This area of research represents a new therapeutic paradigm that could add functional years of life to individuals who would otherwise develop dementia.

The importance of pyrimidine nucleus in the AD management has been proven and reported by several scientists. The substituted pyrimidine derivatives were generally found with the multi target approaches like anti-cholinesterase (AChE and BuChE), anti-Aβ-aggregation (AChE- and self-induced) and anti-β-secretase (BACE-1) inhibitory activity, in an effort to identify lead, multifunctional candidates as part of our multi-targeted approach to treat AD.

Based on the above facts, the present work focused on designing two different heterocyclic nucleuses Indole fused pyrimidone to inhibit β-Secretase and to assess its activity through insilico molecular docking studies.

METHODOLOGY

Table: 1: Software required

SI. No	SOFTWARE	USAGE
01	ChemdrawUltra8.0	3-D drawing, optimizing and calculating various Physicochemical descriptors of the proposed molecules
02	ACD/Chem Sketch	To Create and modify images of chemical structures
03	Mol inspiration tool	Predict Physicochemical properties and Bioactivity
04	Pro Tox-II	Prediction of Toxicity
05	PyRX	Virtualscreeningsoftwareforcomputationaldrugdiscoverythatcanbeusedtoscreenlibrariesof compounds against potential drug target
06	Auto dock vina	Molecular Docking
07	Accelrys-Discovery Studio	For the analysis of docking results

Chem Draw Ultra and Chem Sketch

The computationally designed lead compounds were drawn by using Chem Draw Ultra 8.0. developed by Cambridge Pvt.Ltd. Chemical and physical information about lead molecules can be easily computed using molecular descriptors or chemical descriptors. It is essential in the areas of quantitative structure property relationships and quantitative structure activity relationships

PREPARATION OF LIGAND

In order to provide a protein-ligand complex with improved binding energy, the lead molecules that are docked must be a good representation of the actual ligand. For this, the lead molecules need to fulfill the requirements.

- ❖ Must be prepared in PDB format and must have all hydrogens.
- ❖ It must contain a single molecule that has no covalent bonds to the receptor, with no fused fragments such as counter ions and solvents. Must contain realistic bond lengths and bond angles.

Design of Ligand

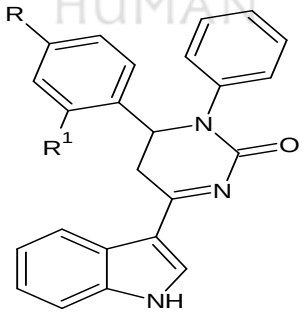


Table: 2 Derivatives

COMPOUND CODE	R	R^1
IP_1	H	H
IP_2	Cl	H
IP_3	OH	H
IP_4	OH	OH
IP_5	CH_3	H

Lipinskis Rule of Five:

Lipinskis rule of five is a rule of thumb to evaluate drug likeness or to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug pharmacokinetics in the human body, namely its absorption, distribution, metabolism and excretion.

Pro-Tox II

ProTox-II, a virtual lab for the prediction of toxicities of small molecules.

The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments. It is based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

PROTEIN SELECTION AND PREPARATION OF PROTEIN

The selected protein/receptor target which has the specific biological activity was downloaded in the RCSB PDB format using PDB ID 2IRZ from Protein Data Bank (www.rcsb.org). Prior to docking, the protein crystal structures are prepared by removing the ligands and water molecules from the protein and adding hydrogen atoms to optimize hydrogen bonds.

β Secretase

The β secretase, widely known as β-site amyloid precursor protein cleaving enzyme 1 (BACE1), initiates the production of the toxic amyloid β (Aβ) that plays a crucial early part in Alzheimer's disease pathogenesis. β secretase is a key therapeutic target for reducing cerebral Aβ concentrations in Alzheimer's disease, and clinical development of BACE1 inhibitors is being actively pursued. Although BACE1 inhibitor drug development has proven challenging, several promising BACE1 inhibitors have recently begun human clinical trials. The safety and effectiveness of these drugs are being tested at present in healthy individuals and patients with Alzheimer's disease, and will shortly be tested in individuals with pre-

symptomatic Alzheimer's disease. Although hopes are high that BACE1 inhibitors might be effective for the prevention or treatment of Alzheimer's disease, concerns have been raised about potential mechanism-based side effects of these drugs.

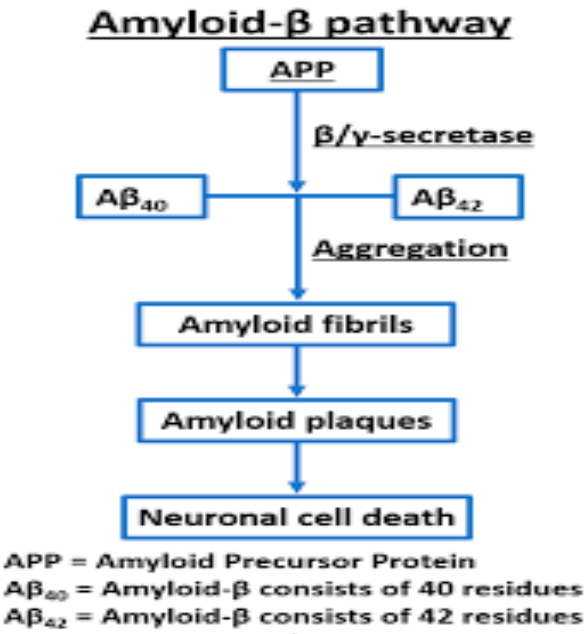


Figure – 2 Role of β- secretase in neuronal cell death

***In silico* Molecular Docking Studies**

To perform the docking studies of the compounds by using a nuclear protein, **β Secretase**

(Protein ID: 2IRZ), was obtained from the RCSB Protein Data Bank. (www.rcsb.org). All the water molecules in the crystal structure were deleted, bond orders were assigned, hydrogens were added and the protein was then further refined for docking studies by using Auto Dock Vina.

RESULT AND DISCUSSION

COMPUTER AIDED DRUG DESIGN

Ligand Validation (www.molinspiration.com)

Analysis of Lipinski's rule of five revealed that all the selected 5 lead compounds were showed zero violation and therefore suitable for biological evaluation.

EVALUATION OF LIPINSKI'S RULE OF FIVE OF DESIGNED MOLECULE USING MOLINSPIRATION SOFTWARE

The rule of five analyses helped to raise awareness about properties and structural features that make more or less drug-like. The rules do not predict whether a compound is pharmacologically active or not. The rule is important for drug development where a pharmacologically lead structure is optimized step-wise for increased activity and selectivity as well as drug-likeness property. All the designed compounds were subjected to Lipinski's rule of five .The evaluation was done by using web browser software called molinspiration software of the synthesized compounds none of them violate the rules and logP value less than 5.

Table: 3

code	Log P	TPSA	No of atoms	MW	noN	Noh NH	No of violations	nrotb	Volume
IP1	5.30	48.46	28	365.44	4	1	1	3	333.65
IP2	5.98	48.46	29	399.88	4	1	1	3	347.19
IP3	4.82	68.69	29	381.44	5	2	0	3	341.67
IP4	4.74	88.92	30	397.43	6	3	0	3	349.69
IP5	5.75	48.46	29	379.46	4	1	1	3	350.21

EVALUATION OF DRUG-LIKENESS SCORE USING pkCSM PHARMACOKINETIC PROPERTY

Table:4 Absorption

Code	Water solubility	$CaCO_2$ Permeability	Intestinal absorption	Skin permeability	p-glycoprotein in substrate	p-glycoprotein in 1 and 2 inhibitor
IP_1	-6.139	1.0	90.72	-2.762	YES	YES
IP_2	-6.084	1.25	88.96	-2.725	NO	NO
IP_3	-5.607	0.34	87.742	-2.735	YES	YES
IP_4	-4.588	0.397	85.903	-2.743	YES	YES
IP_5	-5.997	1.252	90.424	-2.727	YES	YES

Table: 5 Distribution

Code	VDss(Human)	Fraction unbound	BBB permeability	CNS permeability
IP_1	0.097	0.069	0.348	-0.753
IP_2	0.147	0.014	0.392	-0.752
IP_3	-0.048	0.02	-0.182	-1.441
IP_4	-0.419	0	-0.774	-1.588
IP_5	0.149	0.019	0.405	-0.752

Table: 6 Metabolism

Code	CYP2D6 Substrate	CYP3A4 Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
IP_1	Yes	Yes	Yes	Yes	Yes	Yes	Yes
IP_2	No	Yes	Yes	Yes	Yes	No	Yes
IP_3	No	Yes	Yes	Yes	Yes	No	Yes
IP_4	No	Yes	Yes	Yes	Yes	Yes	No
IP_5	No	Yes	Yes	Yes	Yes	No	Yes

Table:7 Excretion

Code	Tolerance clearance	Renal OCT2 substrate
IP_1	0.554	No
IP_2	0.151	No
IP_3	0.489	No
IP_4	0.491	No
IP_5	0.591	No

Optimization of the ADME (absorption, distribution, metabolism and excretion) properties of the drug molecule is often the most difficult and challenging part of the whole drug discovery process . The ADME profile will also have a major impact on the likelihood of success of a drug.

The drug likeness properties of the designed compounds were done by using online software named pkCSM. The software predicts the water solubility, calcium carbonate permeability, skin permeability, intestinal absorption, p – glycoprotein 1 and 2 inhibitor. The software predicts distribution (VDss human, Fraction unbound, BBB permeability, Cs permeability,) metabolism (CYP2D6 substrate, CYP3A4 substrate, CYP1A2 substrate, CYPC19 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor) excretion (total clearance and renal OCT2 substrate).The ADME properties of the 5 lead molecules are all with in the acceptable range for human beings , indicating that found in the present study can be utilized for the purpose of developing new drugs.

MOLECULAR DOCKING

Docking Result for Alzheimer's disease

❖ The selected novel indole pyrimidone derivatives were screened and against the enzyme 2IRZ.

❖ The molecular docking study was carried out into the active site of β Secretase (protein ID: 2IRZ) using Auto dock vina. Molecular modelling of the designed molecules showed significant fitting to a β Secretase enzymes (-7.0 to -7.8kcal/mol) as compared to standard Donepezil (-7.3 kcal/mol).

❖ Docking scores for anti-Alzheimer's activity are presented in the Table - 8 and the 2D structure of the selected lead compounds is shown in **Figure 3- 8.**

PyRx results of anti-Alzheimer's activity

Table: 8 Docking results of designed compound towards 2IRZ by using PyRx software.

Sl.No	Code	Binding energy (Kcal/mol)
1	IP_1	-7.4
2	IP_2	-7.8
3	IP_3	-7.4
4	IP_4	-7.7
5	IP_5	-7.5
6	Donepezil	-7.3

DOCKING POSES OF DESIGNED COMPOUNDS WITH 2D REPRESENTATION AGAINST 2IRZ PROTEIN

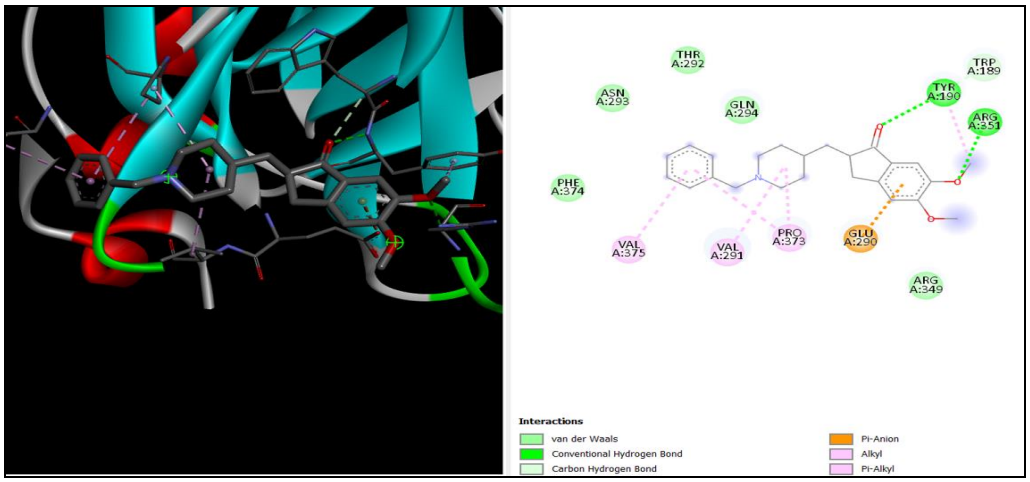


Figure - 3 Standard against 2IRZ

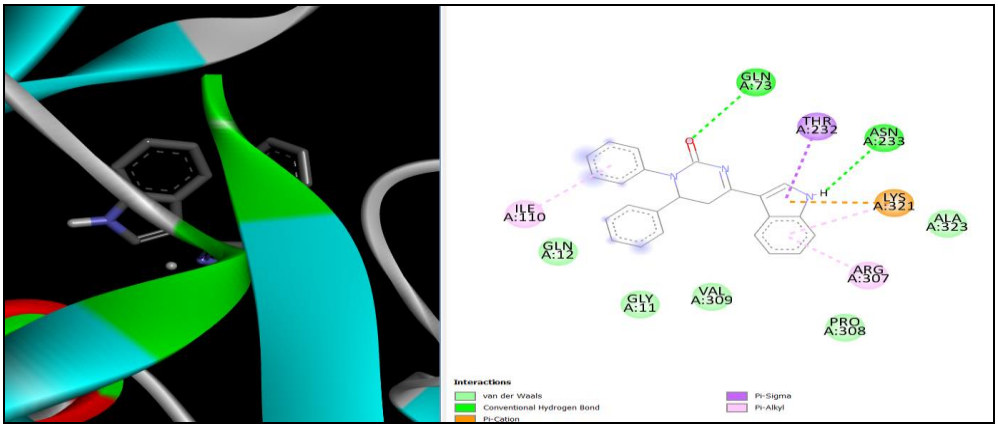


Figure – 4 Ligand IP-1 against 2IRZ

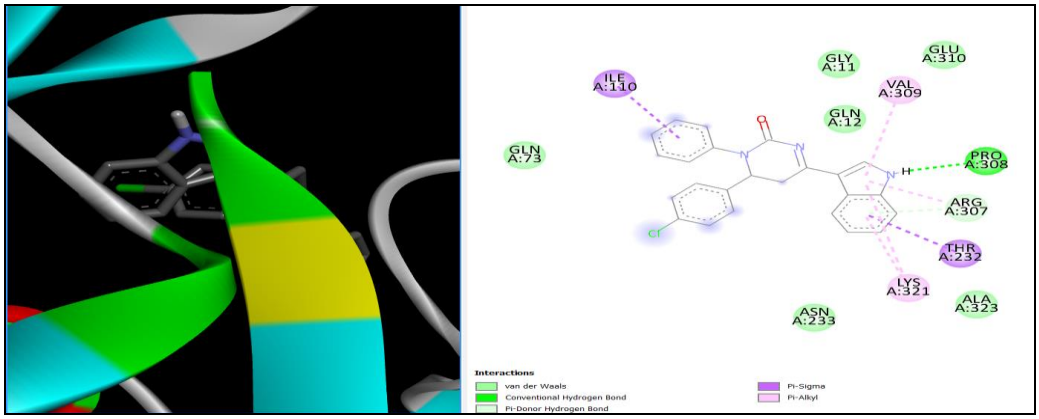


Figure - 5 Ligand IP-2 against 2IRZ

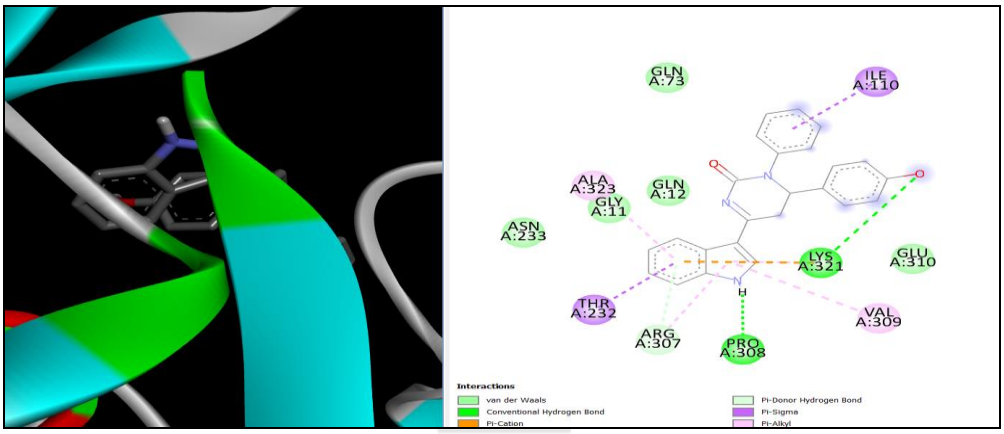


Figure - 6 Ligand IP-3 against 2IRZ

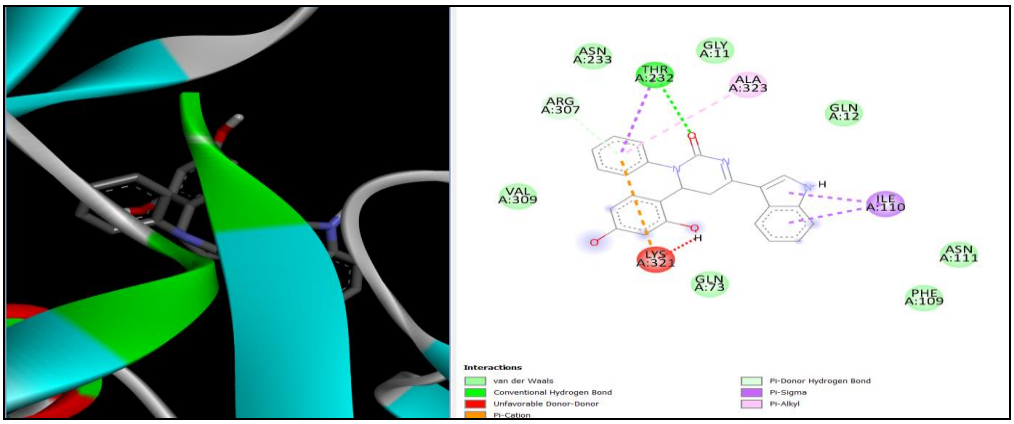


Figure - 7 Ligand IP-4 against 2IRZ

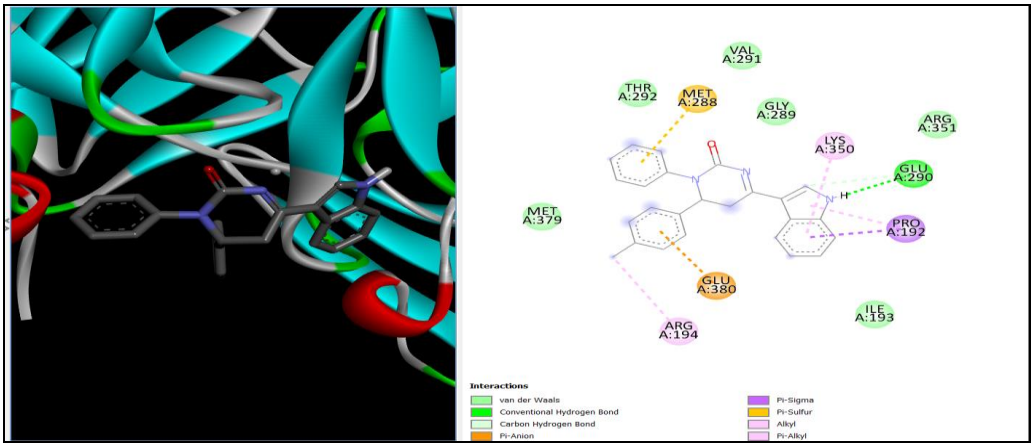
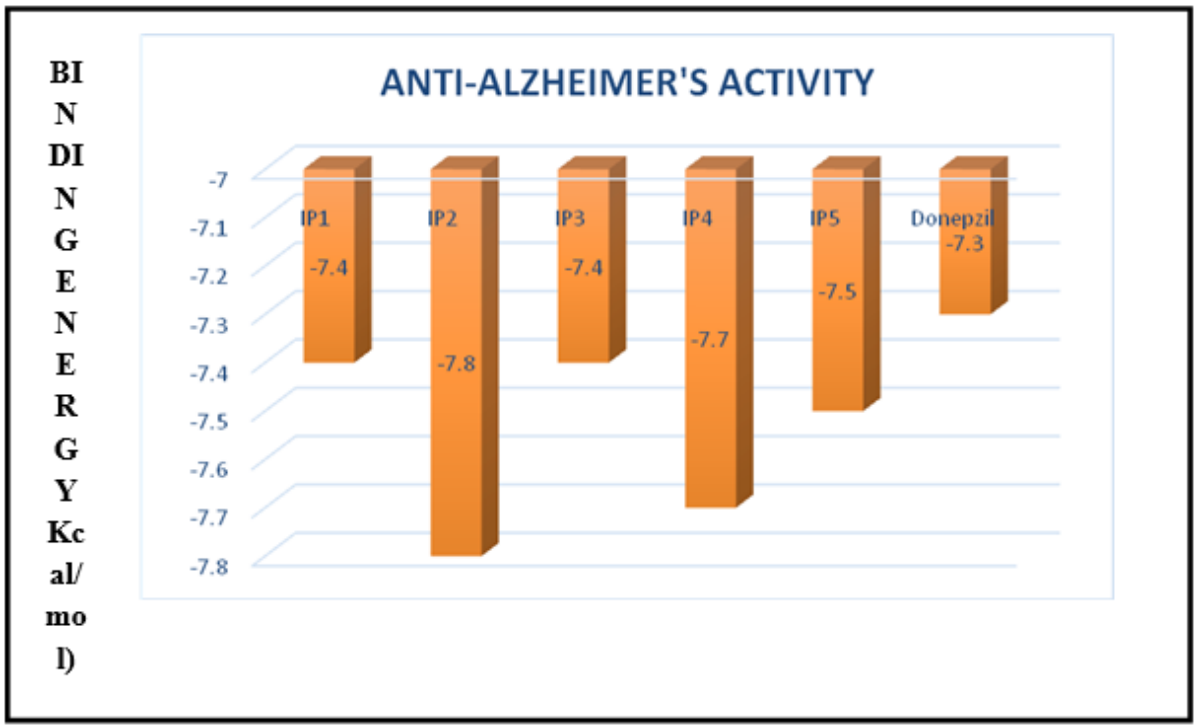


Figure - 8 Ligand IP-5 against 2IRZ



COMPOUND CODE

The PyRx results between the interactions of 2IRZ inhibitor with the designed compounds have given an account result of -7.8 kcal/mol binding energy for the IP_2.

CONCLUSION

The present study conclude that identified a finest molecule of indole-fused-pyrimidine analogue IP-2 (Figure 4) as an innovative drug candidate who was docked against β-Secretase in a deliberate attempt to discover an MTDL, able to interfere with diverse key

target points of AD Neurodegeneration. The molecular properties were calculated from suitable computational tools like molinspiration and toxicity by protox-II. These lead molecules were investigated for drug-like properties by calculating Lipinski's rule of five using molinspiration. All of the selected lead molecules showed zero violations of the rule of 5, which indicates good bioavailability.

This compound IP-2 is well thought-out as a lead molecule, and need to synthesis and evaluates its potency against Alzheimer's disease through molecular level and in vivo studies.

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