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Molecular Docking Study of Isatin Derivatives with Acetylcholinesterase Enzyme to Develop a Novel Anti-Alzheimer Drug

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ABSTRACT

Alzheimer disease (AD), a deadly neurological condition that worsens over time, affects older adults and is identified by a decline in memory and cognitive ability. Alzheimer's is a complex and diverse condition and the pathophysiology is yet unknown. Acetylcholinesterase (AChE) inhibition provides a dual effect as a cognitive enhancer and prevents the deposition of amyloid beta peptide, which renders it a significant target for anti-Alzheimer therapy. AChE was thus chosen as the target. Based on the pharmacophoric characteristics of the target, the current study discusses the construction of a virtual library of 100 new Isatin derivatives that target the *Acetylcholinesterase* enzyme as inhibitors. By employing molecular docking using Autodock Tools 4.2 (1.5.6), the effectiveness of the ligand towards the target protein (4EY7) was assessed. The ligands were optimized by predicting drug-like qualities like ADMET properties and toxicity profiles using *Molinspiration* and *Osiris*. A lead molecule with an optimum docking score, non-toxicity and good oral bioavailability was identified.

INTRODUCTION

Indole and its derivatives have a special function in a number of biological processes in heterocyclic compounds. Isatin is a major indole derivative that is being researched as a potential treatment for illnesses of the central nervous system. Since it is an endogenous chemical, the hippocampus region of the brain has a particularly high concentration despite being spread throughout the tissues and fluids of mammals. Isatin has a wide range of pharmacological effects, including those against cancer, tuberculosis, fungal infections, bacteria, antioxidants, inflammatory pain, convulsants, Alzheimer's disease, and HIV.

Dr. Alois Alzheimer, a German physician, originally described Alzheimer's disease (AD) in 1908. It is a neurodegenerative condition that progresses fatally and is characterized by declines in memory and cognitive ability. Elderly people and those with 60–80% dementia are the groups most affected. Since AD is a complicated disorder, its pathogenesis is still understood, however, the majority of theories claim that it is correlated with cholinergic depletion, amyloid beta fibril production, neurofibrillary tangle deposition, and tau protein. Neuro-inflammation, oxidative stress, and gene mutations for the Amyloid Precursor Protein (APP), Presenilins 1 and 2 (PSEN1, PSEN2), and Apolipoprotein E (ApoE) are further neurological abnormalities.

Acetylcholinesterase (AChE), N-Methyl-D-Aspartate Receptor, Amyloid beta protein, tau protein, beta-secretase, glycogen synthase kinase (GSK3), and phosphodiesterase are some of the targets that are currently being studied for the treatment of AD. AChE has a significant catalytic role in the hydrolysis of acetylcholine and a non-catalytic role in promoting the synthesis of amyloid beta peptides. The inhibition of AChE enhances acetylcholine levels and prevents the accumulation of Amyloid beta peptide. Thus, in the present study, AChE was chosen as the target, since it offers a more precise framework for future medication development.

Materials and Methods:

1. Constructing of virtual library:

Based on the knowledge of pharmacophoric features like Hydrogen bond acceptor (HBA), Hydrogen bond donor (HBD), and aromatic ring features that are essential for biological

activity, a virtual library consisting of 100 new Isatin derivatives were created as potent Acetylcholinesterase inhibitors.

2. Molecular Docking Studies:

a. Protein Preparation:

The crystallized three-dimensional structure of the protein *Acetylcholinesterase* was obtained from the RCSB Protein Data Bank as PDB ID: 4EY7 Homosapien, Resolution 2.35Å. The obtained protein was prepared by removing the co-crystallized ligands, cofactors and water molecules using Molegro Molecular Viewer and saved as pdb format.

b. Ligand Preparation:

The two-dimensional chemical structure of 100 novel ligands was identified using the Zinc 15 database and sketched using Chem Draw Ultra 12.0, saved in MDL mol format. The sketched ligands were energy-minimized using Chem 3D Pro 12.0.

c. Docking studies:

In computational drug discovery, one of the virtual screening techniques is docking. The molecules that passed the ADMET properties were further screened for binding energy using Autodock Tools 4.2 (1.5.6). Autodock is an automated docking tool designed to predict the binding properties and conformation of protein-ligand complexes and is used to screen a huge library of compounds. Autodock uses the Lamarckian genetic algorithm and force-field-based scoring functions to determine the binding energy of protein-ligand complexes. From the Autodock results, a lead molecule was identified with the best binding properties to the enzyme acetylcholinesterase, and the hydrogen bond interactions were viewed using the Molegro Molecular Viewer.

3. ADMET Properties

Additionally, all the selected molecules were screened by ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties through Molinspiration, Swiss ADME and Osiris Property Explorer.

Molinspiration is an online software tool to evaluate the in silico pharmacokinetic properties of ligands. It is based on the Chris Lipinski rule of five, which is used to identify small molecules that can be absorbed and permeated easily in vivo systems.

The criteria are:

- Molecular weight less than 500 daltons
- Log P Partition coefficient less than 5
- Less than 5 Hydrogen bond donors
- Less than 10 Hydrogen bond acceptors

It is employed to predict Molecular weight, log P, Total Polar Surface Area (TPSA), number of Hydrogen bond acceptors and donors, number of atoms, number of rotatable bonds, and also predict the bioactivity score of ligands.

Swiss ADME property is an online software tool to evaluate the physicochemical properties, lipophilicity, solubility, BBB permeant, and Bioavailability score.

Osiris Property Explorer is an online chem-informatics tool employed to determine the toxic potential of designed molecular compounds. It predicts toxicities such as tumorigenicity, mutagenicity, irritant effect and reproductive effect.

A lead molecule with non-toxic and good oral bioavailability was identified from the results of ADMET properties.

Results and Discussion:

Virtual Library Scaffolds:

100 molecule of Isatin derivatives was designed based on the pharmacophoric features of the *Acetylcholinesterase* enzyme and sketched using Chem Draw software.

Molecular docking studies:

The bonding interactions of 100 compounds with the target were used to identify the active site of acetylcholinesterase inhibitors. The table below shows the anticipated activity for each designed ligand docking score.

LIGAND	DOCKING SCORE (kcal/mol)	LIGAND	DOCKING SCORE (kcal/mol)	LIGAND	DOCKING SCORE (kcal/mol)
Lig 1	-12.3	Lig 35	-11.4	Lig 69	-9.29
Lig2	-12.22	**Lig 36**	**-8.04**	Lig 70	-8.93
Lig3	-8.83	Lig 37	-8.4	Lig 71	-10.57
Lig 4	-8.0	Lig 38	-9.16	Lig 72	-8.19
Lig 5	-11.05	Lig 39	-9.91	Lig 73	-9.66
Lig 6	-4.82	Lig 40	-11.09	Lig 74	-11.28
Lig 7	-5.19	Lig 41	-9.21	Lig 75	-9.64
Lig 8	-7.33	Lig 42	-8.08	Lig 76	-10.82
Lig 9	-9.09	Lig 43	-8.9	Lig 77	-7.7
Lig 10	-10.0	Lig 44	-9.48	Lig 78	-7.6
Lig 11	-9.71	Lig45	-8.63	Lig 79	-7.48
Lig 12	-9.68	Lig 46	-9.05	Lig 80	-9.5
Lig 13	-11.43	Lig 47	-7.73	Lig 81	8.67
Lig14	-8.72	Lig 48	-10.37	Lig 82	-7.42
Lig 15	-9.2	Lig 49	-10.08	Lig 83	-9.65
Lig 16	-8.8	Lig 50	-8.4	Lig 84	-10.02
Lig 17	-6.9	**Lig 51**	**-8.01**	Lig 85	-9.56
Lig 18	-9.61	Lig 52	-6.53	Lig 86	-10.83
Lig 19	-8.71	**Lig 53**	**-7.09**	Lig 87	-9.87
Lig 20	-10.97	Lig 54	-9.65	Lig 88	-9.64
Lig21	-11.24	Lig 55	-8.62	Lig 89	-7.82
Lig 22	-10.69	Lig 56	-10.07	Lig 90	-10.42
Lig 23	-8.71	Lig 57	-7.88	Lig 91	-8.35
Lig 24	-11.25	Lig 58	-8.31	Lig 92	-9.38
Lig 25	-7.77	Lig 59	-8.17	Lig 93	-8.86
Lig 26	-7.64	Lig 60	-6.47	Lig 94	-9.95
Lig 27	-6.13	Lig 61	-7.96	Lig 95	-9.47
Lig 28	-6.98	Lig 62	-9.67	Lig 96	-10.3
Lig 29	-7.39	Lig 63	-9.85	Lig 97	-10.04
Lig 30	**-7.11**	**Lig 64**	**-10.77**	Lig 98	-9.48
Lig 31	-8.26	Lig 65	-9.57	Lig 99	-7.24
Lig 32	-8.33	Lig 66	-8.76	Lig 100	-7.67
Lig 33	-8.07	Lig 67	-8.34		
Lig 34	-8.49	Lig 68	-9.62		

ADMET PROPERTIES:

These 100 molecules were screened for ADMET properties (Absorption, Distribution, Metabolism, Excretion) using Molinspiration, Swiss ADME. Osiris property explorer. Out of these 5 molecules were selected for a further phase of lead molecule declaration. The following table depicts the important ADMET properties that determine the biological outcome using software.

Molinspiration

Cpd ID	**MW**	**Log P**	**TPSA**	**Lipinski rule Violation**	**H-bond donor**	**H-bond acceptor**	**Rotatable bonds**
1	363.46	3.99	46.84	0	0	5	5
2	376.49	4.79	50.50	0	0	5	3
3	390.27	2.98	62.53	0	1	6	3
4	389.29	4.59	50.50	0	0	5	3
5	326.44	2.83	49.63	0	1	5	3

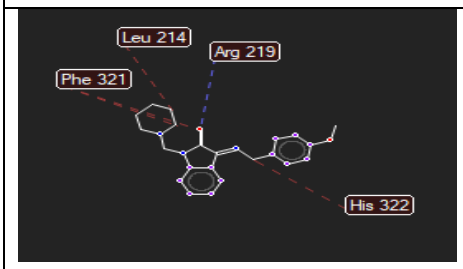
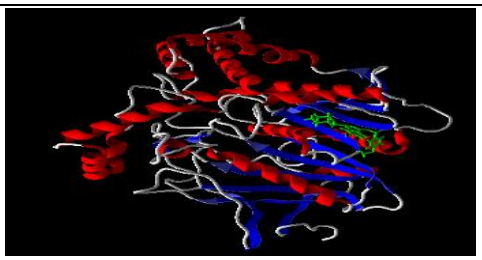
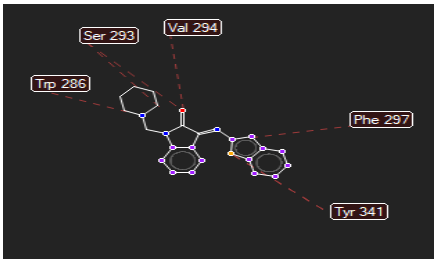
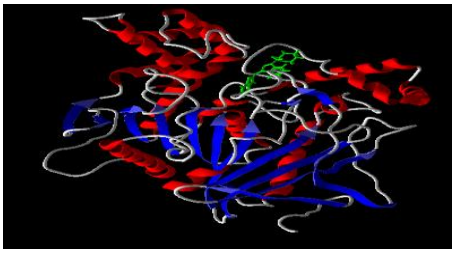
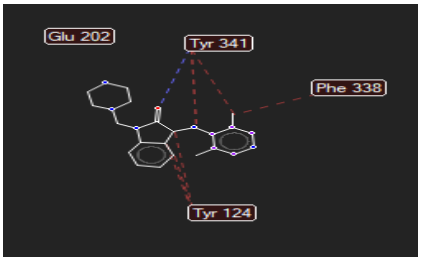
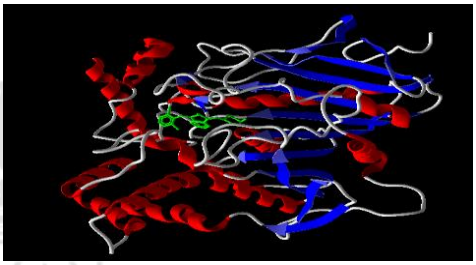
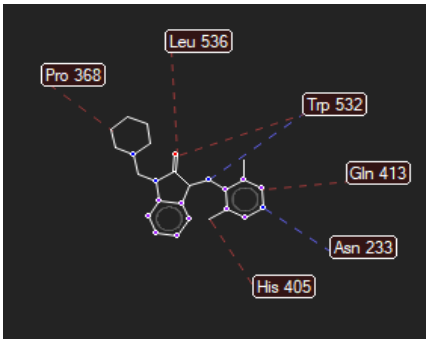
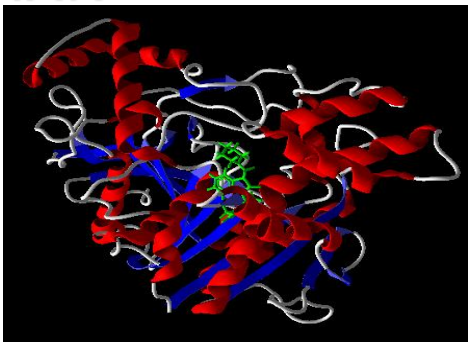
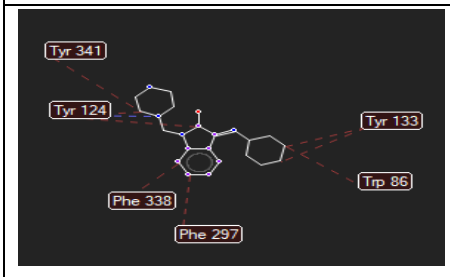
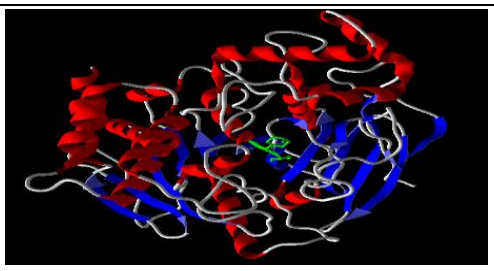
Swiss ADME

GI Absorption	BBB permeability	Bioavailability score	Skin permeability	CYP enzyme inhibitor
High	Yes	0.55	-5.91cm/s	Yes

Osiris Property explorer

Mutagenicity	Tumorigenicity	Irritant	Reproductive effect
No	No	No	No

Docking Interactions

2D Amino acid interactions	3D interactions
	
	
	
	
	

From the 2D view, the hydrogen bond interactions with residues Arg 219, Tyr 341, Trp 532, Asn 233, Tyr 124. In this most interactions are seen with Tyr 341 and Tyr 124 residues. The

steric interactions with residue include Leu 214, Phe 321, His 322, Trp 286, Ser 293, Val 294, Phe 297, Tyr 341, Tyr 124, Phe 338, Leu 536, Pro 368, Gln 413, His 405, Tyr 133, Trp 86. Most interactions are seen with Phe 297, Tyr 341, Tyr 124and Phe 338 residues.

CONCLUSION:

Clinical data suggested that a decline in acetylcholine level is mostly seen in Alzheimer's disease. In this study, Acetylcholinesterase was chosen as the anti-alzheimer target. Ligands were designed as AChE inhibitors and subjected to *in silico* drug-likeness prediction using *Molinspiration* software and toxicity screening with *Osiris Property Explorer*. Designed ligands were further subjected to docking studies using *Autodock Tools 4.2(1.5.6)* software and the binding energy was calculated. From the docking results, the key binding interactions of highly active ligands with the amino acids were presented. Among the docked compounds the Isatin Mannich base derivatives 30, 36, 51, 53 and 64 with binding energies -7.11, -8.04, -8.01, -7.09 and -10.77 Kcal/mol respectively. They were found to be non-toxic, possess good drug-likeness and have good binding affinity with the AChE enzyme. These compounds can act as potential leads and further computational studies, synthesis and invitro evaluation will be carried out in future works.

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