IN SILICO MOLECULAR PROPERTIES AND DOCKING STUDIES OF NEW SCHIFF BASES OF ISATIN DERIVATIVES AS PROSPECTIVE AChE INHIBITORS FOR ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a longer-term neurodegenerative condition characterized by a gradual decline in cognitive abilities, mostly as a result of neuronal loss in the hippocampus and cerebral cortex, with the frontal as well as temporal lobes showing the first alterations. The cholinergic system is pivotal in learning, memory consolidation, retrieval, and controlling aspects of cognitive function. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are essential enzymes that facilitate the breakdown of acetylcholine, and the inhibition of these enzymes is a recommended treatment option for mild to moderate AD. Donepezil, rivastigmine, galantamine, and tacrine are approved by the FDA as AChE inhibitors that provide symptomatic benefit by enhancing cholinergic neurotransmission, although they do not modify disease progression and are often associated with adverse effects.

Isatin, an endogenous indole derivative naturally present in mammalian tissues and abundant in the hippocampus, has emerged as a promising scaffold for designing neuroprotective agents. It has the capacity to cross via the blood-brain barrier, and its documented antioxidant, anti-aggregation, enzyme-inhibitory, and neuroprotective activities make it an attractive template for developing multifunctional anti-Alzheimer compounds.

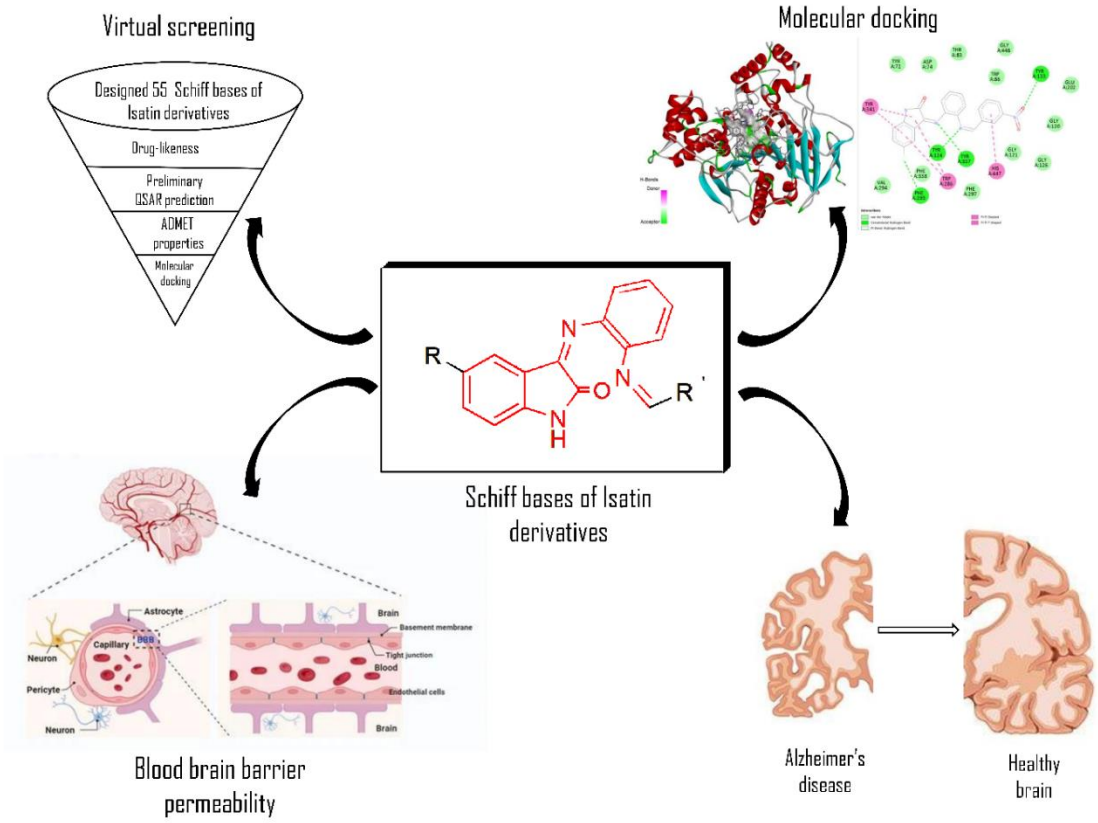
This study examines a set of newly Schiff base derivatives of isatin were rationally designed based on known pharmacophoric features associated with cholinesterase inhibition. Physiochemical pharmacokinetic and toxicity properties were evaluated using Molinspiration, Swiss ADME and Osiris property explorer. Molecular docking studies were carried out using a validated docking protocol PyRx to predict the binding affinity and interaction patterns of the designed compounds with the active site of AChE. Donepezil was used as the reference standard. The ligand target interactions were analysed using Biovia Discovery studio visualizer.

Docking studies demonstrated strong binding affinities toward AChE, with all proposed derivatives showing better interactions compared with the standard drug, donepezil. The results are promising, but molecular docking only gives us guesses. It needs to be tested in both *in vitro* and *in vivo* to confirm its ability to inhibit enzymes, protect neurons, and have good pharmacokinetic-properties.

KEYWORDS

Alzheimer's disease, AChE inhibitor, Isatin, *In-silico* studies, Molinspiration, Swiss ADME, Osiris Property Explorer, Molecular docking, Donepezil.

GRAPHICAL ABSTRACT



INTRODUCTION

Alzheimer's disease is a progressively debilitating neurological disorder characterized by irreversible decline in memory, cognition, and behavioral function. Neuropathological changes predominantly involve neuronal loss and synaptic dysfunction in the hippocampus and cerebral cortex, with early involvement of the frontal and temporal regions followed by widespread cortical degeneration, although the speed of progression differs from person to person [1]. The loss of cognitive abilities, including thinking, remembering, and reasoning, to the point where it interferes with day-to-day activities is known as dementia. Alzheimer's disease (AD) is the most prevalent kind of dementia. Cholinergic neuron destruction, oxidative stress, neuritic plaques, and neurofibrillary tangles due to amyloid-beta peptide (Aβ) deposition in the most affected region of the brain are the features of AD. The clinical manifestations of AD typically include impaired memory retention, language disturbances, and visuospatial deficits. Motor or sensory problems, walking difficulties, and seizures usually appear only in the later stages of the illness [2-6]. At first, AD was thought to be rare, but later it was accepted as a typical aspect of aging [7]. The WHO predicts that in the next century, AD will be more prevalent than AIDS, cancer, and cardiovascular diseases. Over 46 million people are predicted to have AD by 2050, a threefold rise. [8,9]

Numerous risk factors have been connected to Alzheimer's disease. The primary risk factor for Alzheimer's is becoming older. Traumatic brain injury, depression, cardiovascular and cerebrovascular illness, parental age, smoking, a family history of dementia, and elevated homocysteine levels have all been linked to an increased risk of Alzheimer's disease [10,11].

Among the various neurochemical alterations observed in AD, degeneration of the cholinergic system is one of the most consistently reported findings. A class of enzymes known as cholinesterase converts the neurotransmitter acetylcholine (ACh) into choline and acetic acid. This breakdown helps the cholinergic neuron return to its normal resting state after being active [12]. Excessive enzymatic activity leads to reduced synaptic availability of Ach, contributing significantly to cognitive impairment in AD patients. ACh plays an important role in memory. It helps with learning new information, storing it, stabilizing it, updating it, forgetting unnecessary details, and recalling it when needed [13]. Cholinesterase enzymes are of two types, Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE).

AchE is a crucial component of neuromuscular connections and cholinergic brain synapses. AChE inhibitors are an effective way to treat AD. Currently, the FDA has approved four AChE

inhibitors to treat Alzheimer's disease: Tacrine, Donepezil, Rivastigmine, and Galantamine. Additionally, the only drug that can block the N-methyl-aspartate (NMDA) receptor is memantine. [14,15] Additionally, several naturally derived compounds, including curcumin, varenicline, huperzine, resveratrol and cycloastragenol have been approved by the FDA for the management of AD [16,17].

The active site of AChE is structurally complex and consists of several functional regions, including the esteratic site, the oxyanion hole, the anionic subsite, the acyl-binding pocket, the peripheral anionic site, and residues belonging to the omega loop, each contributing to substrate binding and enzyme activity. Consequently, molecules capable of interacting with multiple subsites within AChE are considered advantageous for effective enzyme inhibition [18].

1. Esteratic subsite (catalytic triad) – Ser 203, His 447, Glu 334
2. Oxyanion hole - Gly121, Gly122, Ala204
3. Anionic subsite – Trp 86, Tyr 133, Glu 202, Gly 448, Ile 451
4. Acyl-binding pocket – Trp 236, Phe 295, Phe 297, Phe 338
5. Peripheral anionic subsite (PAS) – Asp 74, Tyr 124, Ser 125, Trp 286, Tyr 337, Tyr 341
6. Omega loop residues – Thr 83, Asn 87, Pro 88

Donepezil is a piperidine-based drug that reversibly inhibits acetylcholinesterase. It is highly selective for AChE and shows much weaker activity towards BuChE [19]. Although donepezil is widely prescribed for AD, its administration is often limited by adverse reactions. Mild effects commonly involve sleep irregularities, digestive upset, and muscle-related complaints, whereas excessive dosing can lead to serious systemic effects such as lowered blood pressure, respiratory distress, and reduction in the heart rate [20].

Isatin (1*H*-indole-2,3-dione), an endogenous indole derivative found in mammalian tissues and enriched in the hippocampus, has attracted increasing interest as a multifunctional scaffold for neuroprotective drug design. Its ability to cross the blood–brain barrier, along with reported antioxidant, anti-aggregation, enzyme-inhibitory, and neuroprotective activities, makes isatin a promising candidate for the development of novel anti-Alzheimer agents [21,22].

Isatin and its scaffolds are found to have diverse pharmacological profile, including anti-depressant [23], anti-diabetic [24,25], anti-fungal [26], anti-inflammatory [27], anti-viral [28], anti-bacterial [29], anti-tubercular [30], analgesic [31] etc. Based on earlier findings, exogenously administered isatin can access the CNS through blood-brain barrier passage efficiently, and its

brain-penetrating derivatives have shown neuroprotective and anti-Alzheimer's effects in preclinical studies [32]. Isatin derivatives have shown inhibitory effects on several enzymes associated with neurodegeneration, including cholinesterase, carbonic anhydrase, and MAO-B. They also interfere with protein aggregation and exhibit notable neuroprotective and antioxidant activities [33]. According to theoretical research of the compounds' pharmacokinetic and toxicological characteristics, isatin derivatives have good oral bioavailability and can penetrate the blood–brain barrier, making them potential candidates for novel AD drugs.

Currently approved AChE inhibitors, including donepezil, rivastigmine, galantamine, and tacrine, offer symptomatic relief in mild to moderate AD [34,35]. However, these drugs do not halt disease progression and are often associated with adverse effects such as gastrointestinal disturbances and hepatotoxicity, highlighting the need for safer and more effective therapeutic alternatives. Based on these considerations, the present work focuses on the rational design of novel Schiff bases of isatin derivatives and to evaluate their *in silico* molecular property prediction and docking studies against human AChE. This study aims to identify promising candidates with favorable binding characteristics and drug-like properties suitable for further optimization and acts as a lead molecule for AD.

MATERIALS AND METHODS

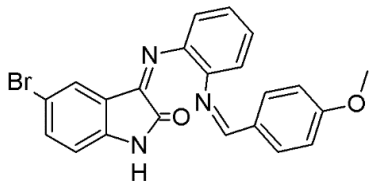
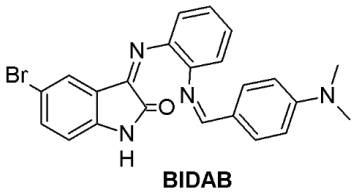
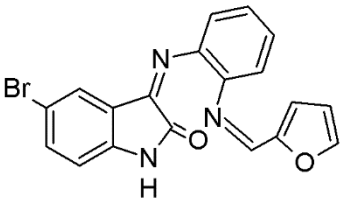
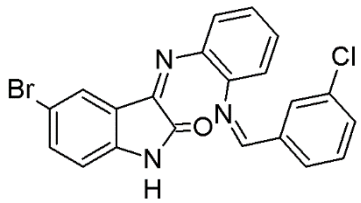
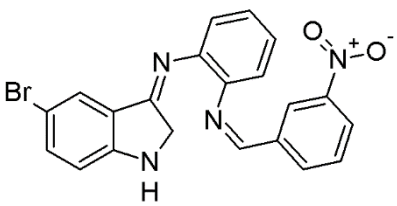
In this study, we evaluated the suggested isatin derivatives using a variety of software programs. The 2D and 3D structures of ligands were drawn using Marvin Sketch. Marvin Sketch was used to translate the developed ligands into Protein Data Bank (PDB) format. The Lipinski rule of five qualities was predicted using Molinspiration software. You may calculate physicochemical descriptors and forecast pharmacokinetic characteristics and druglike nature using Swiss ADME software. The toxicity profile, including mutagenicity, tumorigenicity, irritancy, and reproductive impact, was predicted using OSIRIS molecular property explorer program.

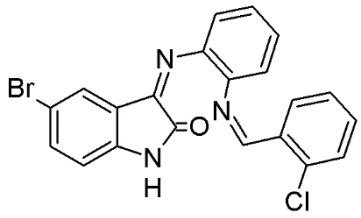
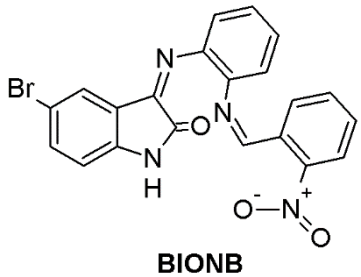
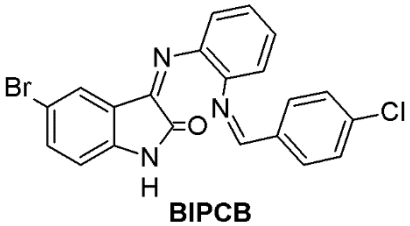
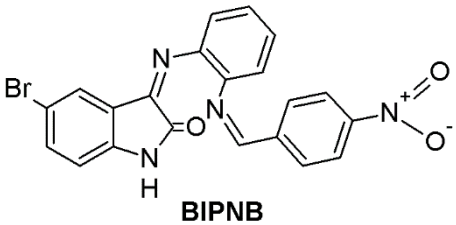
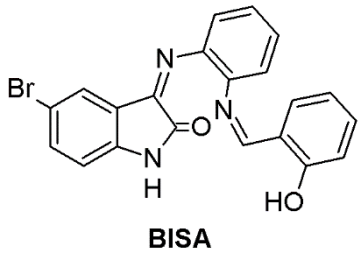
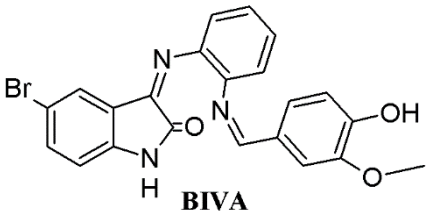
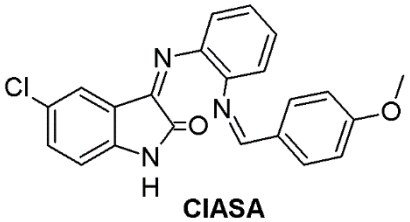
For Molecular Docking, the 3D structure of protein was taken from RCSB PDB database. Molecular docking study were performed for the designed compounds using PyRx – Virtual Screening Tool [36]. The protein ligand interactions were visualized using Biovia Discovery studio visualizer.

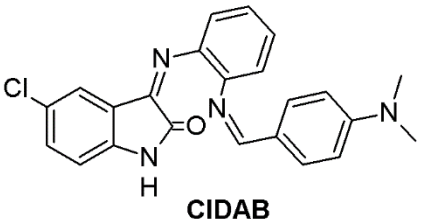
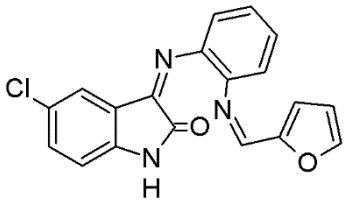
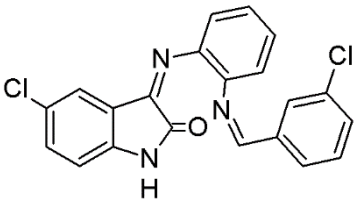
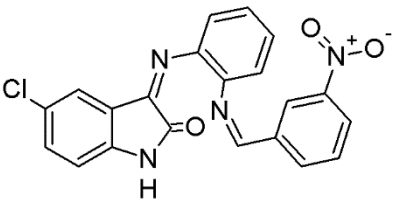
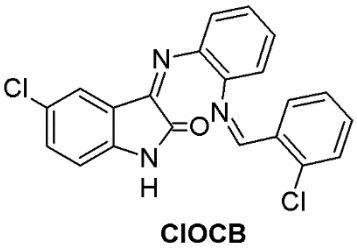
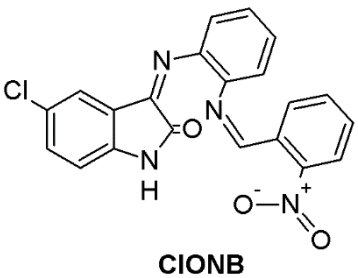
PROPOSED DERIVATIVES

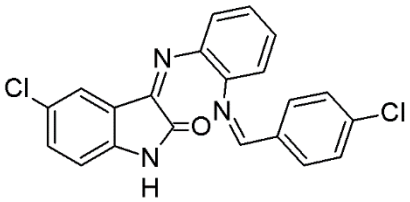
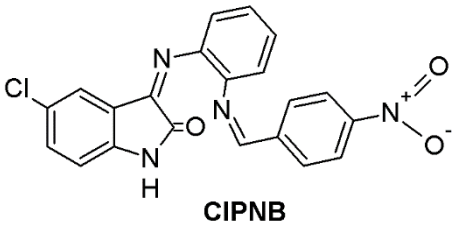
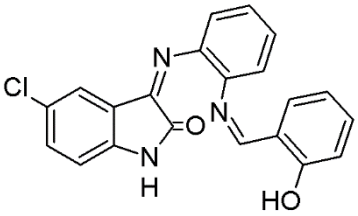
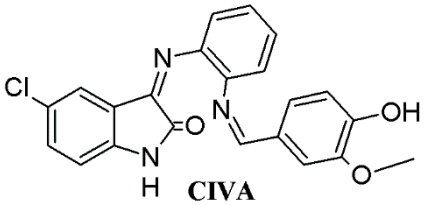
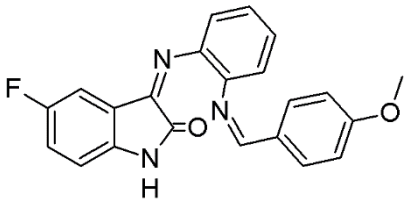
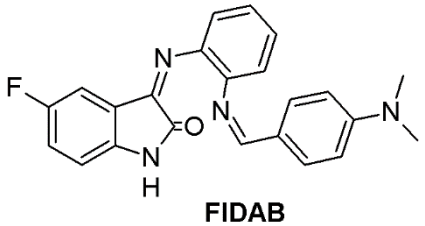
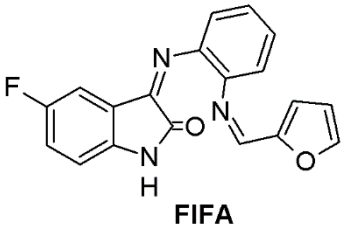
The structure of proposed derivatives were drawn by Marvin sketch software was shown in table 1.

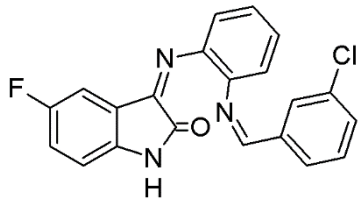
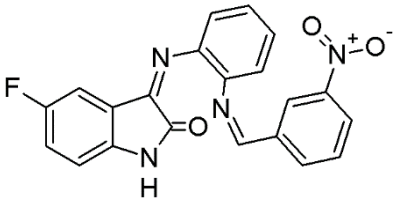
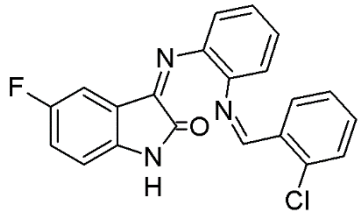
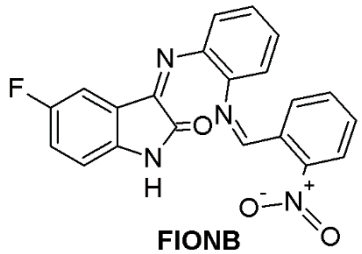
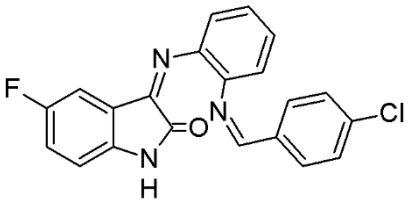
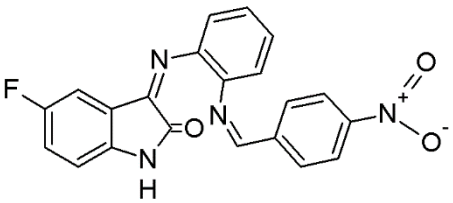
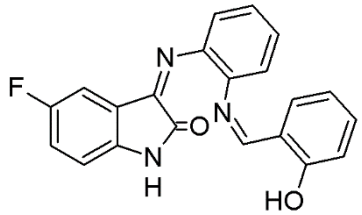
Table 1: Structure of proposed Isatin derivatives

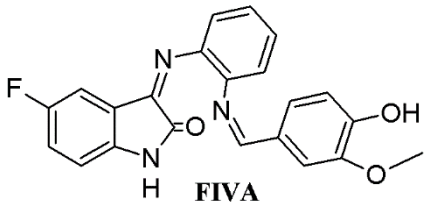
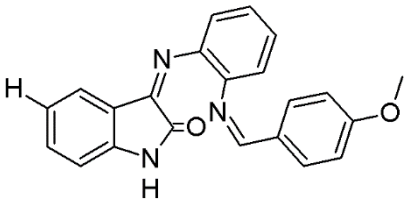
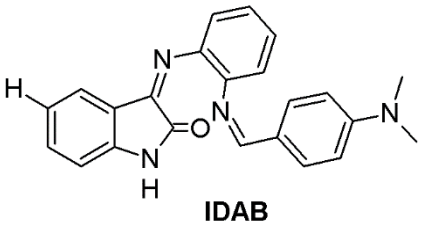
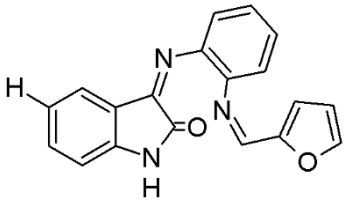
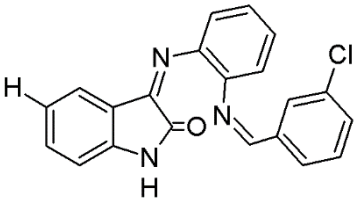
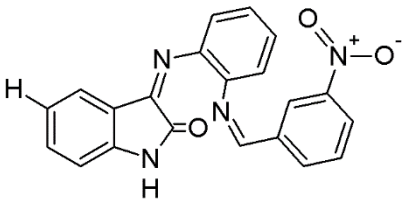
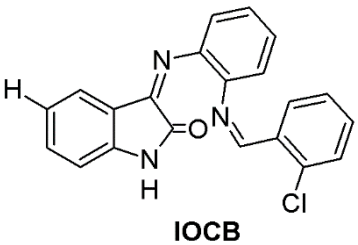
S. NO.	COMPOUND CODE	STRUCTURE OF DESIGNED ISATIN DERIVATIVES	IUPAC NAMES
1.	BIASA	 BIASA	(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(4-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one
2.	BIDAB	 BIDAB	(3*Z*)-5-bromo-3-{[2-({(*Z*)-[4-(dimethylamino)phenyl]methylidene} amino)phenyl]imino}-1,3-dihydro-2*H*-indol-2-one
3.	BIFA	 BIFA	(3*Z*)-5-bromo-3-[(2-{[(*Z*)-furan-2-ylmethylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one
4.	BIMCB	 BIMCB	(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(3-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one
5.	BIMNB	 BIMNB	(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(3-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one

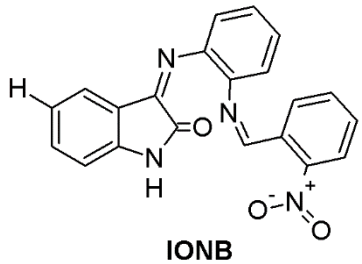
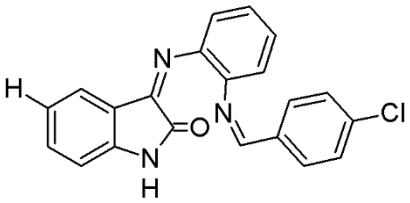
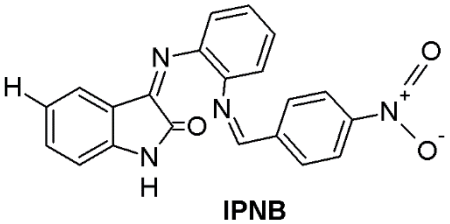
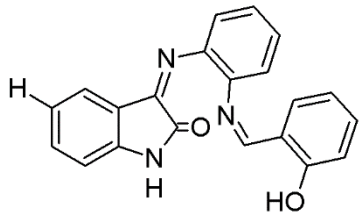
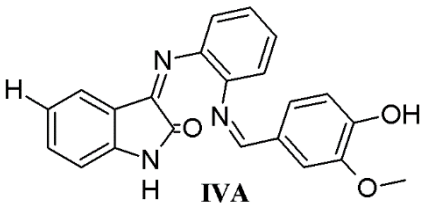
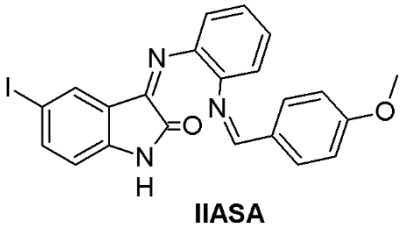
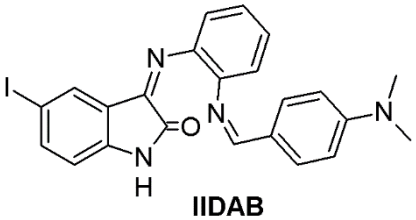
6.	**BIOCB**	 **BIOCB**	**(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(2-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
7.	**BIONB**	 **BIONB**	**(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(2-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
8.	**BIPCB**	 **BIPCB**	**(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(4-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
9.	**BIPNB**	 **BIPNB**	**(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(4-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
10.	**BISA**	 **BISA**	**(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(2-hydroxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
11.	**BIVA**	 **BIVA**	**(3*Z*)-5-bromo-3-[(2-{[(*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
12.	**CIASA**	 **CIASA**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(4-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**

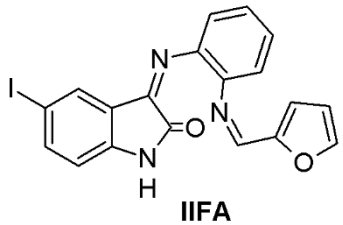
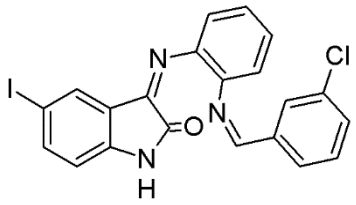
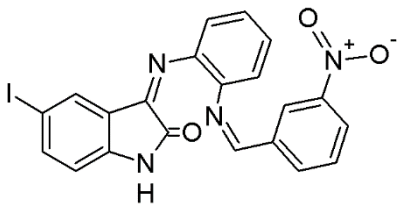
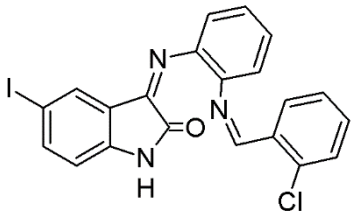
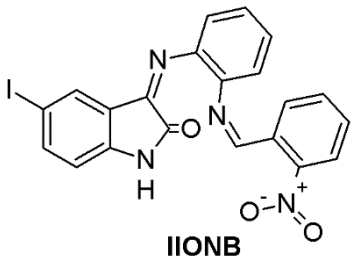
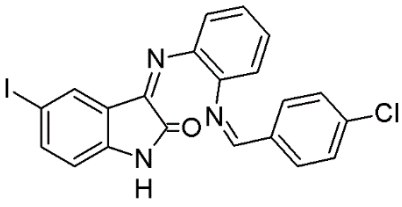
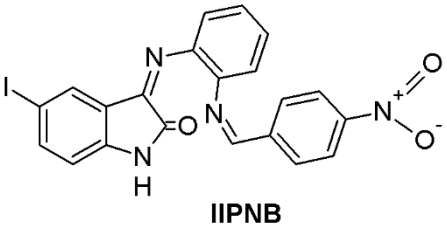
13.	**CIDAB**	 **CIDAB**	**(3*Z*)-5-chloro-3-{[2-({(*Z*)-[4-(dimethylamino)phenyl]methylidene}amino)phenyl]imino}-1,3-dihydro-2*H*-indol-2-one**
14.	**CIFA**	 **CIFA**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-furan-2-ylmethylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
15.	**CIMCB**	 **CIMCB**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(3-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
16.	**CIMNB**	 **CIMNB**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(3-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
17.	**CIOCB**	 **CIOCB**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(2-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
18.	**CIONB**	 **CIONB**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(2-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**

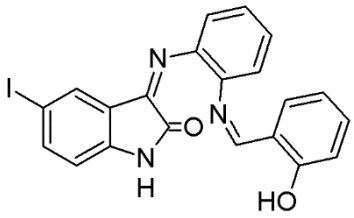
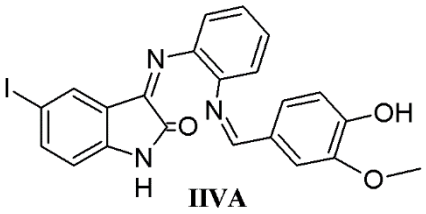
19.	**CIPCB**	 **CIPCB**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(2-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
20.	**CIPNB**	 **CIPNB**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(4-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
21.	**CISA**	 **CISA**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(2-hydroxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
22.	**CIVA**	 **CIVA**	**(3*Z*)-5-chloro-3-[(2-{[(*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
23.	**FIASA**	 **FIASA**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-(4-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
24.	**FIDAB**	 **FIDAB**	**(3*Z*)-3-{[2-({(*Z*)-[4-(dimethylamino)phenyl]methylidene}amino)phenyl]imino}-5-fluoro-1,3-dihydro-2*H*-indol-2-one**
25.	**FIFA**	 **FIFA**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-furan-2-ylmethylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**

26.	**FIMCB**	 **FIMCB**	**(3*Z*)-3-[(2-{[(*Z*)-(3-chlorophenyl)methylidene]amino} phenyl)imino]-5-fluoro-1,3-dihydro-2*H*-indol-2-one**
27.	**FIMNB**	 **FIMNB**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-(3-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
28.	**FIOCB**	 **FIOCB**	**(3*Z*)-3-[(2-{[(*Z*)-(2-chlorophenyl)methylidene]amino} phenyl)imino]-5-fluoro-1,3-dihydro-2*H*-indol-2-one**
29.	**FIONB**	 **FIONB**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-(2-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
30.	**FIPCB**	 **FIPCB**	**(3*Z*)-3-[(2-{[(*Z*)-(4-chlorophenyl)methylidene]amino} phenyl)imino]-5-fluoro-1,3-dihydro-2*H*-indol-2-one**
31.	**FIPNB**	 **FIPNB**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-(4-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
32.	**FISA**	 **FISA**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-(2-hydroxyphenyl)methylidene]amino}phenyl)i mino]-1,3-dihydro-2*H*-indol-2-one**

33.	**FIVA**	 **FIVA**	**(3*Z*)-5-fluoro-3-[(2-{[(*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
34.	**IASA**	 **IASA**	**(3*Z*)-3-[(2-{[(*Z*)-(4-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
35.	**IDAB**	 **IDAB**	**(3*Z*)-3-{[2-({(*Z*)-[4-(dimethylamino)phenyl]methylidene}amino)phenyl]imino}-1,3-dihydro-2*H*-indol-2-one**
36.	**IFA**	 **IFA**	**(3*Z*)-3-[(2-{[(*Z*)-furan-2-ylmethylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
37.	**IMCB**	 **IMCB**	**(3*Z*)-3-[(2-{[(*Z*)-(3-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
38.	**IMNB**	 **IMNB**	**(3*Z*)-3-[(2-{[(*Z*)-(3-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
39.	**IOCB**	 **IOCB**	**(3*Z*)-3-[(2-{[(*Z*)-(2-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**

40.	**IONB**	 **IONB**	**(3*Z*)-3-[(2-{[(*Z*)-(2-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
41.	**IPCB**	 **IPCB**	**(3*Z*)-3-[(2-{[(*Z*)-(4-chlorophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
42.	**IPNB**	 **IPNB**	**(3*Z*)-3-[(2-{[(*Z*)-(4-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
43.	**ISA**	 **ISA**	**(3*Z*)-3-[(2-{[(*Z*)-(2-hydroxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
44.	**IVA**	 **IVA**	**(3*Z*)-3-[(2-{[(*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
45.	**IIASA**	 **IIASA**	**(3*Z*)-5-iodo-3-[(2-{[(*Z*)-(4-methoxyphenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
46.	**IIDAB**	 **IIDAB**	**(3*Z*)-3-{[2-({(*Z*)-[4-(dimethylamino)phenyl]methylidene}amino)phenyl]imino}-5-iodo-1,3-dihydro-2*H*-indol-2-one**

47.	**IIFA**	 **IIFA**	**(3*Z*)-3-[(2-{[(*Z*)-furan-2-ylmethylidene] amino}phenyl)imino]-5-iodo-1,3-dihydro-2*H*-indol-2-one**
48.	**IIMCB**	 **IIMCB**	**(3*Z*)-3-[(2-{[(*Z*)-(3-chlorophenyl)methylidene] amino}phenyl)imino]-5-iodo-1,3-dihydro-2*H*-indol-2-one**
49.	**IIMNB**	 **IIMNB**	**(3*Z*)-5-iodo-3-[(2-{[(*Z*)-(3-nitrophenyl)methylidene] amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
50.	**IIOCB**	 **IIOCB**	**(3*Z*)-3-[(2-{[(*Z*)-(2-chlorophenyl)methylidene] amino}phenyl)imino]-5-iodo-1,3-dihydro-2*H*-indol-2-one**
51.	**IIONB**	 **IIONB**	**(3*Z*)-5-iodo-3-[(2-{[(*Z*)-(2-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**
52.	**IIPCB**	 **IIPCB**	**(3*Z*)-3-[(2-{[(*Z*)-(4-chlorophenyl)methylidene] amino}phenyl)imino]-5-iodo-1,3-dihydro-2*H*-indol-2-one**
53.	**IIPNB**	 **IIPNB**	**(3*Z*)-5-iodo-3-[(2-{[(*Z*)-(4-nitrophenyl)methylidene]amino}phenyl)imino]-1,3-dihydro-2*H*-indol-2-one**

54.	**IISA**	 **IISA**	**(3*Z*)-3-[(2-{[(*Z*)-(2-hydroxyphenyl)methylidene] amino}phenyl)imino]-5-iodo-1,3-dihydro-2*H*-indol-2-one**
55.	**IIVA**	 **IIVA**	**(3*Z*)-3-[(2-{[(*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]amino} phenyl)imino]-5-iodo-1,3-dihydro-2*H*-indol-2-one**

IN SILICO **MOLECULAR PROPERTIES OF PROPOSED DERIVATIVES**

1. Physicochemical properties using Molinspiration software

Lipinski's RO5 is important for evaluating an oral drug's bioavailability. According to the rule, compounds with molecular weight <500, calculated logP <5, hydrogen bond donors <5 (OH and NH groups), and hydrogen bond acceptors <10 (N and O atoms) have a strong potential for oral bioavailability. According to Lipinski's criterion, a drug taken orally often has no more than one violation [37].

2. Drug-likeness and ADME properties using Swiss ADME software

A molecule can only act as an effective drug if it reaches its biological target in adequate amounts and remains in an active form long enough to trigger the intended biological response. Therefore, modern drug development evaluates absorption, distribution, metabolism, and excretion (ADME) much earlier in the discovery pipeline, when many candidate compounds are being considered but only limited physical samples are available.

For lipophilicity, XLOGP3 should be in the range from − 0.7 to + 6.0 and it is considered as lipophilicity descriptors. GI absorption indicates how well a molecule can pass through the gastrointestinal tract after oral administration, which is essential for achieving good bioavailability. BBB permeation describes the ability of a compound to cross the blood–brain barrier and reach the central nervous system.

The Bioavailability Score estimates the probability that a compound will show at least 10% oral bioavailability in rats or have detectable permeability in Caco-2 cells. This semi-quantitative rule-based method uses factors such as total charge, TPSA, and Lipinski rule

violations to group molecules into four categories, with corresponding probabilities of 11%, 17%, 56%, or 85%.

Lead-likeness is related to drug-likeness, but lead compounds are expected to undergo further chemical modifications that usually increase their size and lipophilicity. Therefore, lead molecules need to be smaller and less hydrophobic than typical drug-like compounds. Because of this, it is important for chemists to evaluate whether a molecule is suitable to begin lead-optimization work. Synthetic accessibility is an important criterion during compound selection. The SA score indicates how easy a molecule is to make, with values from 1 meaning very simple to synthesize and 10 meaning highly challenging [38].

3. Toxicity assessment using ORISIS molecular property explorer software

The cLogP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water, reflects its hydrophilicity. Compounds with high cLogP values are less hydrophilic and often show poor absorption or permeation. For good oral absorption, the cLogP should generally be below 5. The aqueous solubility of a molecule also plays a major role in its absorption and distribution. Low solubility usually leads to poor absorption, so avoiding poorly soluble compounds is an important goal in drug design. Drug-likeness describes how closely a molecule matches the typical features of approved drugs. It is assessed using properties such as cLogP, molecular weight, structural fingerprints and topological descriptors. The drug score combines drug-likeness, cLogP, solubility, molecular weight and toxicity risk factors into a single value that helps estimate the compound’s overall potential as a drug candidate. The toxicity prediction identifies possible risks such as mutagenicity, teratogenicity, irritation or reproductive effects. These alerts indicate that the compound’s structure may be harmful under the corresponding toxicity category [39].

4. Molecular Docking Study

X-ray crystal structure

The protein data bank [available on https://www.rcsb.org/] provided the X-ray crystal structure of human acetylcholinesterase (AChE) (PDB ID: 4PQE) [40]. The resolution of the AChE domain's X-ray crystal structure was 2.90 Å. (Figure 1)

Figure 1: Crystal structure of Human Acetylcholinesterase

Preparation of Protein:

By filling in the missing residues, the receptor's crude Protein Data Bank (PDB) structure was improved. Hydrogen atoms were added while water molecules were eliminated. Docking simulation was performed using the optimized receptor, which was stored as a.pdb file.

Preparation of ligand

The newly designed Schiff bases of isatin derivatives were drawn using Marvin Sketch, and the 'Clean 3D' option was applied to arrange all the ligands in proper three-dimensional form and converted into Protein Data Bank (PDB) format.

Molecular docking

Molecular docking studies were carried out to understand how the designed derivatives interact with the target protein for their potential anti-Alzheimer activity. The docking were performed using PyRx software. After docking, the output file contain best computational binding pose were open into BIOVIA Discovery Studio Visualizer and created 3D and 2D ligand-receptor interactions.

RESULTS AND DISCUSSION

Designing:

Based on research on the active binding site of AChE published in the literature, a number of Schiff bases of isatin were created. the several pharmacophores that have been used in this context, as well as the structural characteristics for successful contact with the receptor. Isatin derivatives with a halogen and a substituted aromatic ring that meet the hydrophobic, hydrophilic, and aromatic conditions for binding at the target's active site were created using the bioisosterism principle.

Druglikeness studies:

Evaluating the drug-likeness of the proposed compounds according to Lipinski's rule of five, table 2 showed that all the compounds are expected to be orally accessible since they do not violate Lipinski's rule of five. For lipophilicity, the acceptable XLOGP3 range is −0.7 to +6.0, and all the compounds fall within this limit.

Table 2: Physicochemical properties by using Molinspiration software

Compound code	M.W.	mi Log P	TPSA	n atoms	n OH	n OHNH	No. of rot bonds	No. of violations
BIASA	434.29	5.57	66.82	28	5	1	4	1
BIDAB	447.34	5.61	60.83	29	5	1	4	1
BIFA	394.23	4.77	70.73	25	5	1	3	**0**
BIMCB	438.71	6.16	57.59	27	4	1	3	1
BIMNB	449.26	5.44	103.41	29	7	1	4	1
BIOCB	438.71	6.14	57.59	27	4	1	3	1
BIONB	449.26	5.42	103.41	29	7	1	4	1
BIPCB	438.71	6.19	57.59	27	4	1	3	1
BIPNB	449.26	5.47	103.41	29	7	1	4	1
BISA	420.27	5.45	77.82	27	5	2	3	**0**
BIVA	450.29	4.85	87.05	29	6	2	4	1
CIASA	389.84	5.43	66.82	28	5	1	4	1
CIDAB	402.88	5.48	60.83	29	5	1	4	1
CIFA	349.78	4.63	70.73	25	5	1	3	**0**

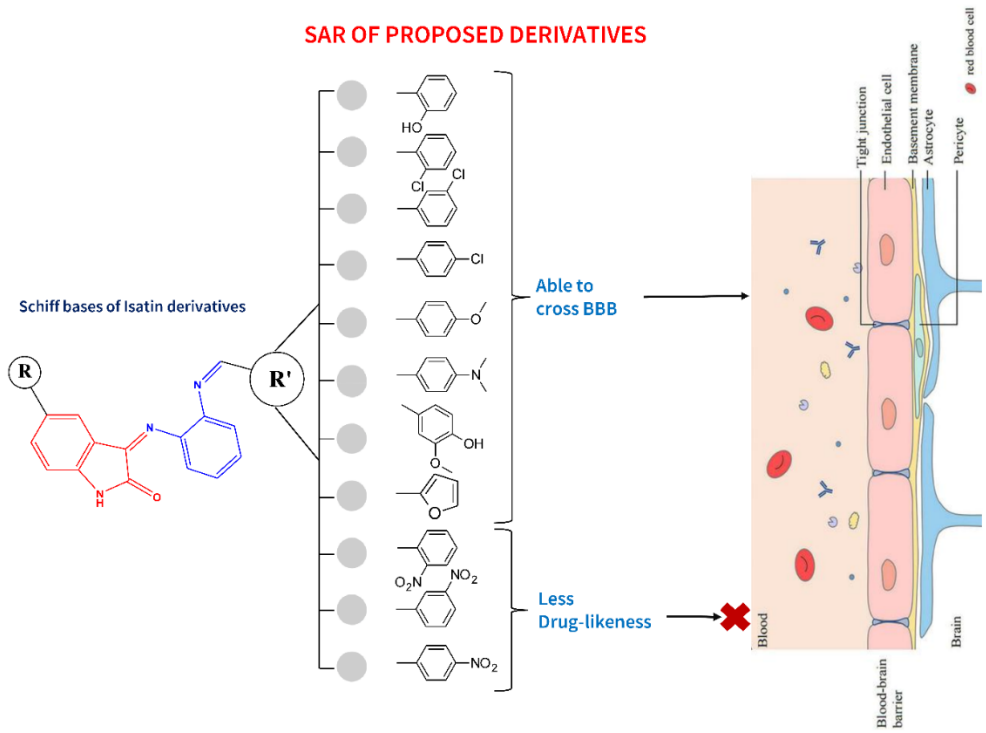
CIMCB	394.26	6.03	57.59	27	4	1	3	1
CIMNB	404.81	5.31	103.71	29	7	1	4	1
CIOCB	394.26	6.01	57.59	27	4	1	3	1
CIONB	404.81	5.29	103.41	29	7	1	4	1
CIPCB	394.26	6.05	57.59	27	4	1	3	1
CIPNB	404.81	5.34	103.41	29	7	1	4	1
CISA	375.81	5.32	77.82	27	5	2	3	1
CIVA	405.84	4.72	87.05	29	6	2	4	**0**
FIASA	373.39	3.95	66.82	28	5	1	4	**0**
FIDAB	386.43	3.99	60.83	29	5	1	4	**0**
FIFA	333.32	3.15	70.73	25	5	1	3	**0**
FIMCB	377.81	4.54	57.59	27	4	1	3	**0**
FIMNB	388.36	3.83	103.41	29	7	1	4	**0**
FIOCB	377.81	4.52	57.59	27	4	1	3	**0**
FIONB	388.36	3.8	103.41	29	7	1	4	**0**
FIPCB	377.81	4.57	57.59	27	4	1	3	**0**
FIPNB	388.36	3.85	103.41	29	7	1	4	**0**
FISA	359.36	3.83	77.82	27	5	2	3	**0**
FIVA	389.39	3.23	87.05	29	6	2	4	**0**
IASA	355.4	4.78	66.82	27	5	1	4	**0**
IDAB	368.44	4.83	60.83	28	5	1	4	**0**
IFA	315.33	3.98	70.73	24	5	1	3	**0**
IMCB	359.82	5.38	57.59	26	4	1	3	1
IMNB	370.37	4.66	103.41	28	7	1	4	**0**
IOCB	359.82	5.35	57.59	26	4	1	3	1
IONB	370.37	4.63	103.41	28	7	1	4	**0**
IPCB	359.82	5.4	57.59	26	4	1	3	1
IPNB	370.37	4.68	103.41	28	7	1	4	**0**
ISA	341.37	4.66	77.82	26	5	2	3	**0**
IVA	371.4	4.06	87.05	28	6	2	4	**0**
IIASA	481.29	5.84	66.82	28	5	1	4	1
IIDAB	494.34	5.88	60.83	29	5	1	4	1
IIFA	441.23	5.04	70.73	25	5	1	3	1
IIMCB	485.71	6.44	57.59	27	4	1	3	1

IIMNB	496.26	5.72	103.41	29	7	1	4	1
IIOCB	485.71	6.41	57.59	27	4	1	3	1
IIONB	496.26	5.69	103.41	29	7	1	4	1
IIPCB	485.71	6.46	57.59	27	4	1	3	1
IIPNB	496.26	5.74	103.41	29	7	1	4	1
IISA	467.27	5.72	77.82	27	5	2	3	1
IIVA	497.29	5.12	87.05	29	6	2	4	1

Pharmacokinetic properties using Swiss ADME:

All the compounds were found to have high GI absorption and compounds has favorable physicochemical properties and are likely to be effectively absorbed when given orally. The blood brain barrier permeation expresses the relative affinity of the drug for the brain tissue. It is observed from the table 3 that compounds BIASA, BIDAB, BIFA, BIMCB, BIOCB, BIPCB, BISA, CIASA, CIDAB, CIFA, CIMCB, CIOCB, CIPCB, CISA, FIASA, FIDAB, FIFA, FIMCB, FIOCB, FIPCB, FISA, IASA, IDAB, IFA, IMCB, IOCB, IPCB, ISA, IIASA, IIDAB, IIFA, IIMCB, IIOCB, IIPCB and IISA were predicted to able to penetrate the BBB whereas the remaining compounds were predicted to have no BBB penetration.

Figure 2: SAR of proposed derivatives



All the compounds were found to have a bioavailability score of 0.55 and displaying a likelihood of being an oral drug candidate. Lead-likeness is similar to drug-likeness, but lead compounds are usually smaller and less lipophilic because they will be further modified during drug development. Among the proposed compounds, only four derivatives such as FIFA, IFA, ISA and IIASA met the lead-likeness criteria.

The SA score reflects the synthetic feasibility of a molecule, where 1 indicates very easy synthesis and 10 indicates a very difficult synthesis. All the compounds showed SA scores between 2.99 and 3.46, suggesting that they are relatively easy to synthesize.

Table 3: Drug-likeness and ADME properties by using Swiss ADME software

Compound code	Lipinski rule	Log P (XLOP3)	GI absorption	BBB	Bioavailability score	Leadlikeness	Synthetic accessibility
BIASA	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
BIDAB	No violation	4.67	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.38
BIFA	No violation	3.94	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.21
BIMCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
BIMNB	No violation	4.37	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.24
BIOCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
BIONB	No violation	4.37	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.31
BIPCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
BIPNB	No violation	4.37	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.21
BISA	No violation	4.19	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.14
BIVA	No violation	4.16	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.24
CIASA	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
CIDAB	No violation	4.61	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.27
CIFA	No violation	3.88	High	**Yes**	0.55	No; 1 violation: XLOP3>3.6	3.11
CIMCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
CIMNB	No violation	4.31	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.14
CIOCB	No violation	5.11	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.04
CIONB	No violation	4.31	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.8	3.21
CIPCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
CIPNB	No violation	4.31	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.11

CISA	No violation	4.13	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.04
CIVA	No violation	4.1	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
FIASA	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.12
FIDAB	No violation	4.08	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.33
FIFA	No violation	3.35	High	**Yes**	0.55	**Yes**	3.17
FIMCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
FIMNB	No violation	3.78	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.2
FIOCB	No violation	4.58	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.08
FIONB	No violation	3.78	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.26
FIPCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.8	3.12
FIPNB	No violation	3.78	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.17
FISA	No violation	3.6	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.8	3.09
FIVA	No violation	3.57	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.19
IASA	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.12
IDAB	No violation	3.98	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.24
IFA	No violation	3.25	High	**Yes**	0.55	**Yes**	3.11
IMCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
IMNB	No violation	3.68	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.12
IOCB	No violation	4.48	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.7	2.99
IONB	No violation	3.68	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.8	3.2
IPCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.9	3.12
IPNB	No violation	3.68	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.8	3.09
ISA	No violation	3.5	High	**Yes**	0.55	**Yes**	3.01
IVA	No violation	3.47	High	No	0.55	No; 1 violation: MW>350	3.1
IIASA	No violation	3.25	High	**Yes**	0.55	**Yes**	3.11
IIDAB	No violation	4.63	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.46
IIFA	No violation	3.9	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.27
IIMCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12
IIMNB	No violation	4.33	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.3
IIOCB	No violation	5.13	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.2
IIONB	No violation	4.33	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.8	3.37
IIPCB	No violation	5.17	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.12

IIPNB	No violation	4.33	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.6	3.28
IISA	No violation	4.15	High	**Yes**	0.55	No; 2 violations: MW>350, XLOP3>3.7	3.23
IIVA	No violation	4.12	High	No	0.55	No; 2 violations: MW>350, XLOP3>3.5	3.31

Toxicity studies using OSIRIS property explorer:

From the data in Table 4, it is observed that about 25 compounds showed no mutagenic, tumorigenic, irritant, or reproductive toxicity. All compounds were free from Mutagenic effect and reproductive effect. Compound BIDAB, BISA, CIDAB, CIVA, FIDAB, FIVA, IDAB, IVA, IIDAB and IIVA were found to be Tumorigenic effect. Compound BIFA, CIFA, FIFA, IFA and IIFA were found to be Irritant effect.

Drug-likeness describes how similar a molecule is to approved drugs. About 15 of the proposed compounds had drug-likeness scores between –0.7 and –7.95, indicating that they are not structurally suitable for drug development in their current form and may need further chemical modification to improve their potential as lead molecules.

Table 4: Toxicity assessment by using ORISIS molecular property explorer software

Compound code	cLog P	Solubility	Drug likeness	Drug score	Mutagenic effect	Tumorigenic effect	Irritant effect	Reproductive effect
BIASA	3.69	-5.8	2.44	0.72				
BIDAB	3.65	-5.82	1.31	0.42				
BIFA	2.95	-5.46	2.22	0.64				
BIMCB	4.36	-6.52	2.23	0.66				
BIMNB	2.84	-6.24	**-2.92**	0.41				
BIOCB	4.36	-6.52	2.73	0.67				
BIONB	2.84	-6.24	**-4.9**	0.39				
BIPCB	4.36	-6.52	3.13	0.68				
BIPNB	2.84	-6.24	**-7.95**	0.39				
BISA	3.41	-5.49	2.24	0.75				
BIVA	3.34	-5.5	2.68	0.59				
CIASA	3.57	-5.7	4.65	0.8				
CIDAB	3.53	-5.72	3.51	0.47				

CIFA	2.83	-5.37	4.43	0.7				
CIMCB	4.24	-6.42	4.4	0.74				
CIMNB	2.72	-6.14	**-0.7**	0.56				
CIOCB	4.24	-6.42	4.89	0.75				
CIONB	2.72	-6.14	**-2.68**	0.45				
CIPCB	4.24	-6.42	5.28	0.75				
CIPNB	2.72	-6.14	**-5.73**	0.42				
CISA	3.29	-5.39	4.45	0.83				
CIVA	3.22	-5.54	4.88	0.65				
FIASA	3.06	-5.28	2.89	0.83				
FIDAB	3.03	-5.3	1.76	0.47				
FIFA	2.32	-4.94	2.67	0.7				
FIMCB	3.74	-6	2.68	0.78				
FIMNB	2.21	-5.72	**-2.47**	0.47				
FIOCB	3.74	-6	3.18	0.79				
FIONB	2.21	-5.72	**-4.45**	0.44				
FIPCB	3.74	-6	3.58	0.8				
FIPNB	2.21	-5.72	**-7.5**	0.44				
FISA	2.79	-4.97	2.69	0.85				
FIVA	2.72	-4.98	3.13	0.67				
IASA	2.96	-4.97	4.27	0.87				
IDAB	2.93	-4.98	3.14	0.51				
IFA	2.22	-4.63	4.05	0.73				
IMCB	3.64	-5.68	4.06	0.82				
IMNB	2.11	-5.41	**-1.09**	0.56				
IOCB	3.64	-5.68	4.63	0.82				
IONB	2.11	-5.41	**-3.01**	0.47				
IPCB	3.64	-5.68	4.96	0.82				
IPNB	2.11	-5.41	**-6.12**	0.45				
ISA	2.69	-4.65	4.14	0.89				
IVA	2.62	-4.67	4.56	0.7				
IIASA	3.4	-5.98	4.68	0.71				

IIDAB	3.37	-6	3.54	0.41				
IIFA	2.66	-5.65	4.46	0.63				
IIMCB	4.08	-6.7	4.47	0.66				
IIMNB	2.55	-6.42	**-0.67**	0.49				
IIOCB	4.08	-6.7	4.95	0.66				
IIONB	2.55	-6.42	**-2.65**	0.39				
IIPCB	4.08	-6.7	5.35	0.66				
IIPNB	2.55	-6.42	**-5.7**	0.36				
IISA	3.12	-5.67	4.48	0.74				
IIVA	3.05	-5.69	4.91	0.56				

Molecular Docking:

The elucidation of interactions between the designed derivatives and target is crucial to check whether the compounds are able to mimic the binding mode of the substrate. Docking studies showed that the designed compound were fit well in the active site pocket made up of the flowing key residues, Ser 203, His 447, Glu 334, Gly121, Gly122, Ala204, Trp 86, Tyr 133, Glu 202, Gly 448, Ile 451, Trp 236, Phe 295, Phe 297, Phe 338, Asp 74, Tyr 124, Ser 125, Trp 286, Tyr 337, Tyr 341, Thr 83, Asn 87, Pro 88. The interactions were compared to the reference drug Donepezil.

The deigned derivatives were exhibited significant binding affinities in the range of -7.8 to -10.9 kcal/mol when compared to standard drug [Donepezil -7.7 kcal/mol]. As represented in table 5, all the proposed derivatives were produced potent binding affinity when compared to the standard drug Donepezil towards the target AChE (PDB ID: 4PQE)

Table 5: Docking scores of proposed derivatives and standard drug on target AChE

S. No.	Compound Code	Binding affinity (kcal/mol)	Interacting residues (Conventional hydrogen bond interaction)
1.	BIASA	-8.3	Tyr A:124, Ser A:293
2.	BIDAB	-8.4	Gln A:413, Asn A:533
3.	BIFA	-8.5	Tyr A:72, Ser A:293
4.	BIMCB	-8.3	Glu A:313
5.	BIMNB	-9	Asn A:233, Gln A:413

6.	BIOCB	-8.2	Ser A:293
7.	BIONB	-8.5	Ser A:293
8.	BIPCB	-8.3	Glu A:313
9.	BIPNB	-8.9	Ser A:293, Tyr A:337
10.	BISA	-8.3	Asn A:233, Glu A:313, Gln A:413
11.	BIVA	-8.9	Arg A:296, Glu A:313
12.	CIASA	-8.4	Tyr A:124, Ser A:293
13.	CIDAB	-8.3	Gln A:413, Asn A:533
14.	CIFA	-7.8	-
15.	CIMCB	-8.3	Glu A:313
16.	CIMNB	-8.9	Asn A:233, Gln A:413
17.	CIOCB	-8.2	-
18.	CIONB	-8.3	-
19.	CIPCB	-8.3	Glu A:313
20.	CIPNB	-9.2	Tyr A:72, Phe A:295
21.	CISA	-8.3	Asn A:233, Glu A:313, Gln A:413
22.	CIVA	-8.9	Arg A:296, Glu A:313, Pro A:368
23.	FIASA	-8.4	Tyr A:124, Ser A:293
24.	FIDAB	-8.4	Ser A:293
25.	FIFA	-8.9	Ser A:293, Phe A:295, Arg A:296
26.	FIMCB	-8.3	Glu A:313
27.	FIMNB	-10.9	Tyr A:124, Tyr A:133, Tyr A:337, Phe A:295
28.	FIOCB	-9	Tyr A:124, Ser A:293
29.	FIONB	-8.3	Asn A:233, Gln A:413
30.	FIPCB	-8.9	Tyr A:124, Tyr A: 341, Ser A:293
31.	FIPNB	-8.9	Tyr A:124, Tyr A: 337, Ser A:293
32.	FISA	-8.7	Tyr A:124, Ser A:293
33.	FIVA	-10	Tyr A:124, Tyr A: 337, Phe A:295
34.	IIASA	-8.3	Tyr A:124, Ser A:293
35.	IIDAB	-8.4	Ser A:293
36.	IIFA	-8.8	Ser A:293, Phe A:295, Arg A:296
37.	IIMCB	-8.3	Glu A:313

38.	IIMNB	-9	Asn A:233, Gln A:413
39.	IIOCB	-8.3	-
40.	IIONB	-8.3	Asn A:233, Gln A:413
41.	IIPCB	-8.4	Glu A:313
42.	IIPNB	-8.9	Tyr A:124, Tyr A: 337, Ser A:293
43.	IISA	-8.3	Asn A:233, Glu A:313, Gln A:413
44.	IIVA	-8.9	Arg A:296, Glu A:313, Pro A:368
45.	IASA	-8.2	Tyr A:124, Ser A:293
46.	IDAB	-8.4	Gln A:413, Asn A:533
47.	IFA	-9.5	Asp A:74, Tyr A:124, Tyr A: 337
48.	IMCB	-8.4	Tyr A:124
49.	IMNB	-9.8	Tyr A:124, Ser A:125
50.	IOCB	-8.8	Ser A:293, Tyr A:341
51.	IONB	-8.3	Ser A:293
52.	IPCB	-8.6	Tyr A:124
53.	IPNB	-8.7	Tyr A:124, Tyr A: 337, Ser A:293
54.	ISA	-8.2	Asn A:233, Glu A:313
55.	IVA	-8.7	Arg A:296, Glu A:313
56.	Donepezil	-7.7	-

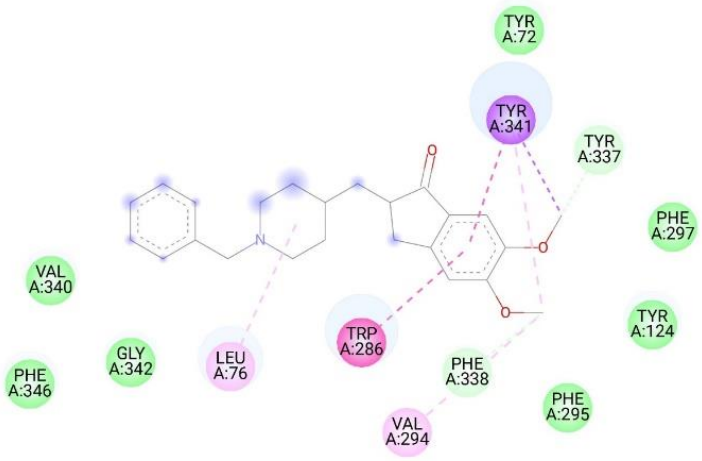
Anionic sub-site: Tyr A:133

Acyl binding pocket: Phe A:295

Peripheral anionic sub-site: Asp A:74, Tyr A:124, Tyr A: 337

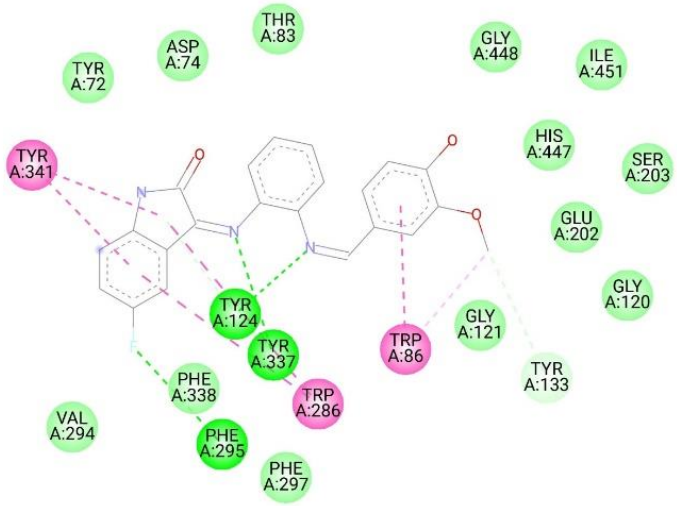
The binding pose of Donepezil (Figure 3) showed Pi-sigma interaction with Tyr A:341, Pi-Pi Stacked interaction with the residues Trp A:286 and Tyr A:341, Pi- alkyl interaction with the residues Leu A:76, Val A:294 and Tyr A:341, Carbon hydrogen bond with the residues Tyr A:337 and Phe A:338 and Van der waals interactions with residues Tyr A:72, Tyr A:124, Phe A:295, Phe A:297, Val A: 340, Gly A:342 and Phe A:346.

Figure 3: 2D interaction of the reference compound Donepezil with the active site residues of 4PQE



Compound FIVA showed the significant binding energy for AChE at -10 kcal/mol. Compound FIVA showed conventional hydrogen bonds with the residues Tyr A:124, Tyr A: 337 and Phe A:295, Carbon hydrogen bond with residues Tyr A:124 and Tyr A:133, pi-pi interactions with the residues Trp A:86, Trp A:286 and Tyr A:341, pi-alkyl interaction with residue Trp A:86, Van der waals interaction with the residues Tyr A:72, Asp A:74, Thr A:83, Gly A:120, Gly A:121, Glu A:202, Ser A:203, Val A:294, Phe A:297, Phe A:338, His A:447, Gly A:448 and Ile A:451. (Figure 4)

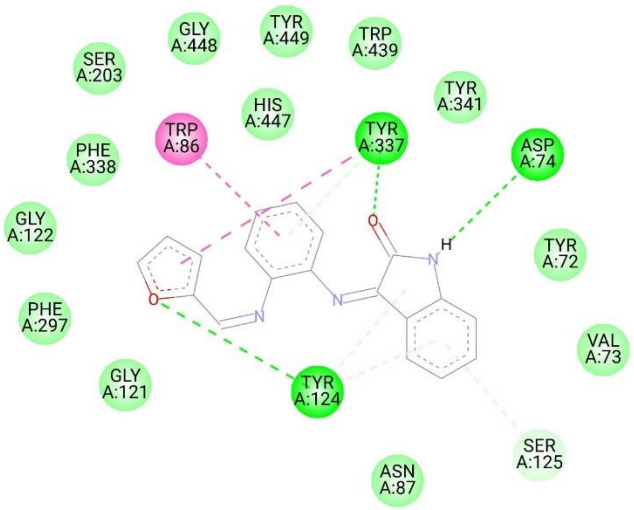
Figure 4: 2D interaction of the FIVA with the active site residues of 4PQE



Compound IFA showed the lowest binding energy for AChE at -9.5 kcal/mol. Compound IFA showed Conventional hydrogen bonds with the residues Asp A:74, Tyr A:124 and Tyr A: 337, pi-donor hydrogen bond with the residues Tyr A:124, Ser A:125 and Tyr A:337,

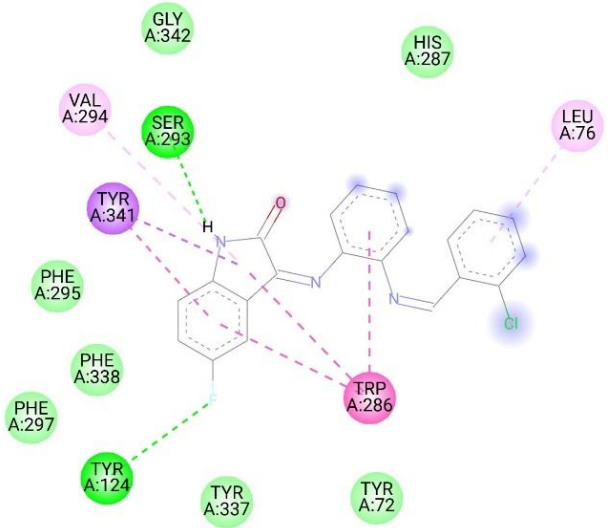
pi-pi interactions with the residues Trp A:86 and Tyr A:337, Van der waals interactions with the residues Tyr A:72, Val A:73, Asn A:87, Gly A:121, Gly A:122, Ser A:203, Phe A:297, Tyr A:341, Phe A:338, Trp A:439, His A:447, Gly A:448 and Tyr A:449. (Figure 5)

Figure 5: 2D interaction of the IFA with the active site residues of 4PQE



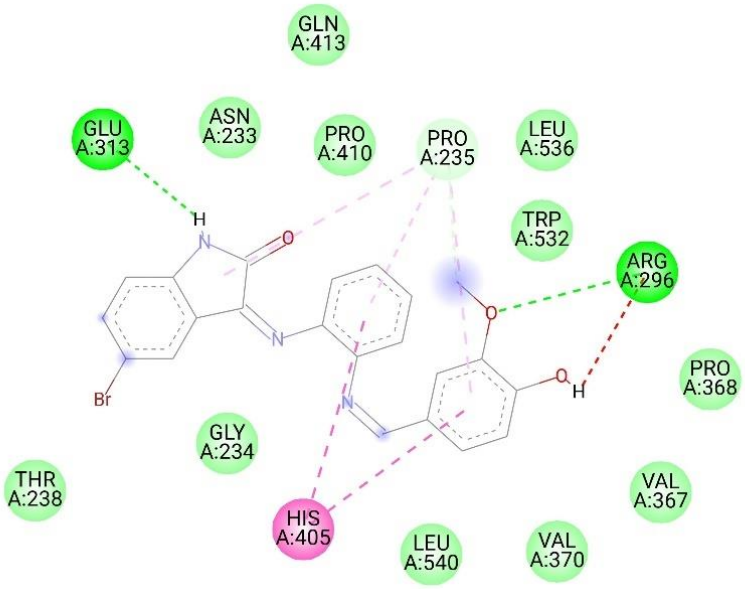
Compound FIOCB showed the lowest binding energy for AChE at -9 kcal/mol. Compound FIOCB showed conventional hydrogen bond with the residues Tyr A:124 and Ser A:293, pi-pi stacked interaction with the residue Trp A:286, pi-alkyl interaction with the residues Leu A:76 and Val A:294, Pi-sigma interaction with the residue Tyr A:341, Van der waals interaction with the residues Tyr A:72, His A:287, Phe A:295, Phe A:297, Phe A:338 and Gly A:342.

Figure 6: 2D interaction of the FIOCB with the active site residues of 4PQE



Compound BIVA showed the lowest binding energy for AChE at -8.96 kcal/mol. Compound BIVA showed Conventional hydrogen bond with the residues Arg A:296 and Glu A:313, Carbon hydrogen bond with the residue Pro A:235, Pi-pi T-shaped interaction with the residue His A:405, Pi-alkyl interaction with the residue Pro A:235, Van der waals interactions with the residues Asn A:233, Gly A:234, Thr A:238, Val A:367, Pro A:368, Val A:370, Pro A:410, Gln A:413, Trp A:532, Leu A:536 and Leu A:540.

Figure 7: 2D interaction of the BIVA with the active site residues of 4PQE



CONCLUSION

Based on molecular docking, all the proposed Schiff bases of isatin derivatives showed excellent binding affinity towards the target protein better than the standard drug (Donepezil). All the proposed derivatives have been studied for their physiochemical and pharmacokinetic properties and the results were according to the Lipinski's rule of 5. In the present study, the proposed Schiff base of isatin derivatives containing (methoxyphenyl)methylidene, [(dimethylamino)phenyl] methylidene, furan-2-ylmethylidene, (3-chlorophenyl)methylidene, (2-chlorophenyl) methylidene, (4-chlorophenyl)methylidene, (2-hydroxyphenyl)methylidene, and (4-hydroxy-3-methoxyphenyl)methylidene substituents demonstrated favorable BBB permeability. This enhanced brain penetration can be attributed to the presence of electron-donating groups, heteroaromatic rings and optimal lipophilicity, which collectively support passive diffusion across the BBB. Further, these derivatives were found to be potent inhibitor of AChE as evidenced from the molecular docking study which was compared with the standard drug Donepezil.

In contrast, the derivatives substituted with (2-nitrophenyl)methylidene, (3-nitrophenyl)methylidene, and (4-nitrophenyl)methylidene failed to cross the BBB. The poor permeability of these compounds may be associated with the strong electron-withdrawing nature of the nitro group, increased polarity and reduced lipophilicity, which are unfavorable for BBB penetration. Overall, the findings highlight that the nature and position of substituents on the Schiff bases of isatin derivatives play a vital role in modulating BBB permeability, providing valuable insights for the rational design of isatin-based scaffolds targeting central nervous system disorders. This study concludes that these designed derivatives can offer a novel category of lead compounds for developing AChE inhibitors used to treat Alzheimer's diseases.

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CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest that could have influenced the work reported in this manuscript.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

AI DECLARATION

Generative or Assistive Artificial Intelligence tools were not used for the creation of chemical structure, figures, or graphical abstract at any stage during the development of this manuscript.

REFERENCES

1. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. Nature reviews disease primers. **2015** Oct 15;1(1):1-8. https://doi.org/10.1038/nrdp.2015.56

2. Greene JD, Baddeley AD, Hodges JR. Analysis of the episodic memory deficit in early Alzheimer's disease: evidence from the doors and people test. Neuropsychologia **1996**; 34:537-551. https://doi.org/10.1016/0028-3932(95)00151-4
3. Pillon B, Deweer B, Agid Y, Dubois B. Explicit memory in Alzheimer's, Huntington's, and Parkinson's diseases. Arch Neurol **1993**; 50:374-379. https://doi.org/10.1001/archneur.1993.00540040036010
4. Price BH, Gurvit H, Weintraub S, Geula C, Leimkuhler E, Mesulam M. Neuropsychological patterns and language deficits in 20 consecutive cases of autopsy-confirmed Alzheimer's disease. Arch Neurol **1993**;50: 931-7. https://doi.org/10.1001/archneur.1993.00540090038008
5. Esteban-Santillan C, Praditsuwan R, Ueda H, Geldmacher DS. Clock drawing test in very mild Alzheimer's disease. J Am Geriatr Soc **1998**; 46:1266-1269. https://doi.org/10.1111/j.1532-5415.1998.tb04543.x
6. Kirk A, Kertesz A. On drawing impairment in Alzheimer's disease. Arch Neurol **1991**; 48:73-77. https://doi.org/10.1001/archneur.1991.00530130083024
7. Schachter AS, Davis KL. Alzheimer's disease. Dialogues in clinical neuroscience. **2000** Jun 30;2(2):91-100. https://doi.org/10.31887/DCNS.2000.2.2/asschachter
8. Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M. World Alzheimer report **2015**. The global impact of dementia: an analysis of prevalence, incidence, cost and trends (Doctoral dissertation, Alzheimer's Disease International).
9. Maresova P, Mohelska H, Dolejs J, Kuca K. Socio-economic aspects of Alzheimer's disease. Current Alzheimer Research. **2015** Nov 1;12(9):903-911.
10. Nicolas G, Acuña-Hidalgo R, Keogh MJ, Quenez O, Steehouwer M, Lelieveld S, Rousseau S, Richard AC, Oud MS, Marguet F, Laquerrière A. Somatic variants in autosomal dominant genes are a rare cause of sporadic Alzheimer's disease. Alzheimer's & Dementia. **2018** Dec;14(12):1632-1639. https://doi.org/10.1016/j.jalz.2018.06.3056
11. Liljegren M, Waldö ML, Rydbeck R, Englund E. Police interactions among neuropathologically confirmed dementia patients: prevalence and cause. Alzheimer Disease & Associated Disorders. **2018** Oct 1;32(4):346-50. https://doi.org/10.1097/WAD.0000000000000267

12. Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. Current neuropharmacology. **2013** May 1;11(3):315-335.
13. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: Targeting the Cholinergic System. Curr Neuropharmacol. **2016**;14(1):101-115.
14. Giacobini E. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacological research. **2004** Oct 1;50(4):433-440. https://doi.org/10.1016/j.phrs.2003.11.017
15. Saxena M, Dubey R. Target enzyme in Alzheimer's disease: Acetylcholinesterase inhibitors. Current topics in medicinal chemistry. **2019** Feb 1;19(4):264-275. https://doi.org/10.2174/1568026619666190128125912
16. Cummings JL. Alzheimer's disease. New England Journal of Medicine. **2004** Jul 1;351(1):56-67. https://doi.org/10.1056/NEJMra040223
17. Gajendra K, Pratap GK, Poornima DV, Shantaram M, Ranjita G. Natural acetylcholinesterase inhibitors: a multi-targeted therapeutic potential in Alzheimer's disease. European Journal of Medicinal Chemistry Reports. **2024** Aug 1;11:100154. https://doi.org/10.1016/j.ejmcr.2024.100154
18. Wiesner J, Kříž Z, Kuča K, Jun D, Koča J. Acetylcholinesterases–the structural similarities and differences. Journal of enzyme inhibition and medicinal chemistry. **2007** Jan 1;22(4):417-424. https://doi.org/10.1080/14756360701421294
19. Racchi M, Mazzucchelli M, Porrello E, Lanni C, Govoni S. Acetylcholinesterase inhibitors: novel activities of old molecules. Pharmacological research. **2004** Oct 1;50(4):441-451. https://doi.org/10.1016/j.phrs.2003.12.027
20. Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics. Molecular medicine reports. **2019** Aug;20(2):1479-1487. https://doi.org/10.3892/mmr.2019.10374
21. Priyanka V. Gandhi, Shubham R. Burande, Manoj S. Charde, Rita D. Chakole. A review on Isatin and its derivatives: synthesis, reactions and applications. J Adv Sci Res **2021**; 12 (4): 01-11. https://doi.org/10.55218/JASR.202112401
22. Chinnasamy RP, Sundararajan R, Govindaraj S. Synthesis, characterization, and analgesic activity of novel schiff base of isatin derivatives. J Adv Pharm Technol Res. **2010** Jul;1(3):342-347. https://doi.org/10.4103/0110-5558.72428
23. Rahim F, Malik F, Ullah H, Wadood A, Khan F, Javid MT, Taha M, Rehman W, Rehman AU, Khan KM. Isatin based Schiff bases as inhibitors of α-glucosidase:

Synthesis, characterization, *in vitro* evaluation and molecular docking studies. Bioorganic chemistry. **2015** Jun 1;60:42-48. https://doi.org/10.1016/j.bioorg.2015.03.005

24. Noori M, Davoodi A, Iraji A, Dastyafteh N, Khalili M, Asadi M, Mohammadi Khanaposhtani M, Mojtabavi S, Dianatpour M, Faramarzi MA, Larijani B. Design, synthesis, and *in silico* studies of quinoline-based-benzo [d] imidazole bearing different acetamide derivatives as potent α-glucosidase inhibitors. Scientific Reports. **2022** Aug 18;12(1):14019. https://doi.org/10.1038/s41598-022-18455-7
25. Alomari M, Taha M, Rahim F, Selvaraj M, Iqbal N, Chigurupati S, Hussain S, Uddin N, Almandil NB, Nawaz M, Farooq RK. Synthesis of indole-based-thiadiazole derivatives as a potent inhibitor of α-glucosidase enzyme along with in silico study. Bioorganic chemistry. **2021** Mar 1;108:104638. https://doi.org/10.1016/j.bioorg.2021.104638
26. Khan S, Ullah H, Hussain R, Khan Y, Khan MU, Khan M, Sattar A, Khan MS. Synthesis, in vitro bio-evaluation, and molecular docking study of thiosemicarbazone-based isatin/bis-Schiff base hybrid analogues as effective cholinesterase inhibitors. Journal of Molecular Structure. **2023** Jul 15;1284:135351. https://doi.org/10.1016/j.molstruc.2023.135351
27. Alagarsamy V, Meena S, Ramseshu KV, Raja Solomon V, Thirumurugan K. Synthesis and pharmacological evaluation of 3-(4-chloro phenyl)-2-substituted-3H-quinazolin-4-ones as analgesic and anti-inflammatory agents. Drug Development Research. **2008** Jun;69(4):226-233. https://doi.org/10.1002/ddr.20249
28. Pandeya SN, Sriram D, Nath G, DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4′-chlorophenyl) thiazol-2-yl] thiosemicarbazide. European Journal of Pharmaceutical Sciences. **1999** Oct 1;9(1):25-31. https://doi.org/10.1016/S0928-0987(99)00038-X
29. Alagarsamy V, Giridhar R, Yadav MR, Revathi R, Ruckmani K, De Clercq E. AntiHIV, Antibacterial and Antifungal Activities of Some Novel 1, 4-disubstituted-1, 2, 4-triazolo [4, 3-a] quinazolin-5 (4H)-ones. Indian Journal of Pharmaceutical Sciences. **2006** Jul 1;68(4). https://doi.org/10.4103/0250-474X.27840
30. Tran VH, Nguyen QD, Le NV. Study on the Anti-Tuberculosis Effect of Some Thiosemicarbazones and IsonicotinylHydrazone Derivatives of Isatin and 5-Halo-Isatin. Tap. Chi. Dou Hoc. **2002**;8:15-17.

31. Sridhar SK, Sreenivasulu M, Ramachandran S, Kishore Gnana Sam S, Saravanan M. Synthesis, Analgesic and Anti-inflammatory activity of n-Mannich bases of (4'-substituted)-2-phenyl indoles. Indian drugs. **2001**;38(10):531-534.
32. Medvedev A, Kopylov A, Buneeva O, Kurbatov L, Tikhonova O, Ivanov A, Zgoda V. A neuroprotective dose of isatin causes multilevel changes involving the brain proteome: prospects for further research. International Journal of Molecular Sciences. **2020** Jun 11; 21(11):4187. https://doi.org/10.3390/ijms21114187
33. Mavroidi B, Kaminari A, Matiadis D, Hadjipavlou-Litina D, Pelecanou M, Tzinia A, Sagnou M. The prophylactic and multimodal activity of two isatin thiosemicarbazones against Alzheimer's disease *in vitro*. Brain sciences. **2022** Jun 19;12(6):806. https://doi.org/10.3390/brainsci12060806
34. Carney G, Bassett K, Wright JM, Maclure M, McGuire N, Dormuth CR. Comparison of cholinesterase inhibitor safety in real-world practice. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2019 Jan 1;5:732-9. https://doi.org/10.1016/j.trci.2019.09.011
35. Pardo-Moreno T, González-Acedo A, Rivas-Domínguez A, García-Morales V, García-Cozar FJ, Ramos-Rodríguez JJ, Melguizo-Rodríguez L. Therapeutic approach to Alzheimer's disease: current treatments and new perspectives. Pharmaceutics. 2022 May 24;14(6):1117. https://doi.org/10.3390/pharmaceutics14061117
36. Kondapuram SK, Sarvagalla S, Coumar MS. Docking-based virtual screening using PyRx Tool: autophagy target Vps34 as a case study. Molecular docking for computer-aided drug design 2021 Jan 1 (pp. 463-477). Academic Press. https://doi.org/10.1016/B978-0-12-822312-3.00019-9
37. Dr. Dhrubo Jyoti Sen, Dr. Pruthviraj K. Chaudhary, Upasana D. Sharma, Manthankumar V. Oza, Dhruvee H. Raval, Dushyant P. Chaudhary. Molinspiration is inspiration to molecule by software in drug discovery. World J Pharm Med Res **2025**; 11(5), 165-173.
38. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports. **2017** Mar 3;7(1):42717. https://doi.org/10.1038/srep42717
39. Nalini CN, Deepthi SR, Ramalakshmi N, Uma G. Toxicity risk assesment of isatins. Rasayan Journal of chemistry. **2011**;4(4):829-33.

40. Seliem IA. Design, eco-friendly synthesis, and molecular docking studies of isatin hybrids as promising therapeutic agents (anticancer, anticholinesterase inhibitor, α-glucosidase inhibitor, and anti-MRSA). RSC advances. 2025;15(39):32309-32327. https://doi.org/10.1039/D5RA04722F