Molecular docking, drug-likeness, *in-silico* ADMET and adverse effect prediction of some novel Spirooxindole derivatives for Alzheimer's disease

[1]Tanjima Tarique Laskar, [2]Monica Arora

[1]Student,[2]Teacher
Department of Pharmaceutical Chemisty
Al-Ameen College of Pharmacy, Bengaluru, India

***Abstract*- Spiro oxindole is a versatile scaffold which has been extensively studied in pharmaceutical and synthetic chemistry. In the medicinal field, a number of compounds featuring spirooxindole motif have displayed excellent pharmacological activities. Spirooxindole derivatives have a highly precious synthetic framework due to their diversified biological properties and it is found to play a key role in the treatment of Alzheimer's disease by preventing amyloid fibril formation at early stages of this disease. Recent progress and development in the bioactivity profiles of spirooxindole derivatives have demonstrated their notable position in present-day drug discovery studies. In this study, five spirooxindole derivatives were designed and *in-silico* studies such as drug likeliness, molecular docking and ADMET studies were performed. All the designed derivatives were found to exhibit docking scores higher than the standard drug Donepezil and toxicity studies has also revealed that the designed compounds didn't exhibit any toxicity and are considered to be safe. Among the designed derivatives, compound A1 showed highest binding affinity in docking study and it has also showed Blood Brain Barrier (BBB) permeability in ADME studies which is essential for treatment of early stages of Alzheimer disease. Furthermore, it can be considered that the multidirectional development of novel drugs containing this spirooxindole scaffold will continue to be the research hotspot in the field of medicinal chemistry in future.**

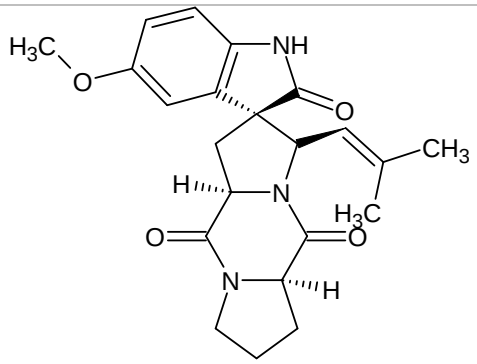
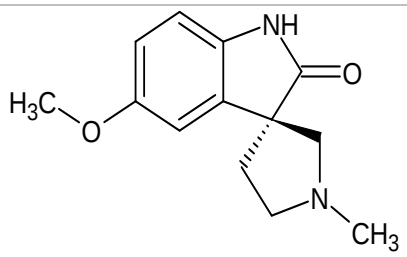
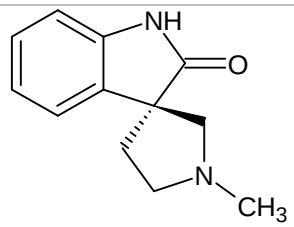
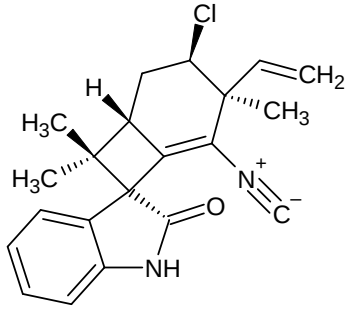
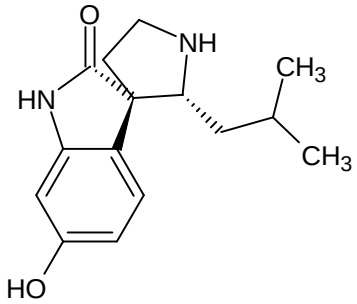
***Keywords*: Alzheimer's disease, Molecular Docking, drug-likeliness, ADME, Toxicity studies**

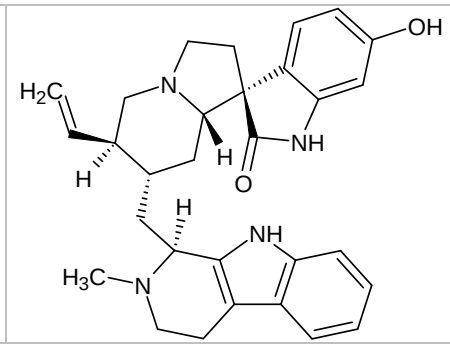
I. INTRODUCTION

Alzheimer disease's (AD) is the most common cause of dementia in the elderly patient which is a progressive neurodegenerative disorder that accounts for 60% - 70% of cases of progressive cognitive impairment in elderly patients[1]. According to relevant global statistics AD affects about 3% of elderly patients aged between 65-74[2]. Although the origin of this cognitive impairment has not yet been fully explored, many risk factors are thought to have contribution for the progression of this disease including inflammation, oxidative stress, amyloid-β (Aβ) deposits, low acetylcholine levels and aggregation of τ protein[3]. AD must be differentiated from other causes of dementia such as reversible dementia, Parkinson's disease with dementia, Vascular dementia, dementia with Lewy bodies and frontotemporal dementia[4]. The most distinctive lesions present within the brain of AD patients are the neuritic senile plaques and neurofibrillary tangles. The prominent pathological features of AD are granulovacuolar degeneration, Hirano bodies, neuronal and dendritic loss, neuropil threads, dystrophic neurites, cerebrovascular amyloid, as well as generalized atrophy of the brain. The most striking intraneuronal change seen within the brains of AD patients are paired helical filaments of neurofibrillary tangles[5]. To date, several researchers have focused on design and development of new novel drug molecules to target the AD, while some drugs are already approved by FDA for treatment of AD which involves inhibition of cholinesterase enzyme (Donepezil and Galantamine). However due to the short half-lives, low therapeutic efficacy, low bioavailability and high toxicity of these reported drugs there is still and urge to develop new, more potent and less toxic compounds for treatment of AD[6].

Spirooxindole based heterocycles have been extensively studied due to their structural framework which is suitable for various pharmacological targets as well as for various biological activities including anticancer, anti-inflammatory, antimicrobial activities while several studies have reported their effectiveness as cholinesterase inhibitors[7-15]. The unique structural feature is the spiro ring fused at the C3 position of the oxindole core with varied heterocyclic motifs which seem to be a promising candidate for drug discovery as it incorporates both oxindoles and other heterocyclic moieties simultaneously[16]. Spirooxindoles are used for development of new lead compounds with improved solubility for drug discovery due to their excellent binding efficiency in the binding pockets of target protein[17-20].

Table 1: Various naturally obtained spirooxindole derivatives are as follows-

Natural alkaloids	Structures	Description
Spirotryprostatin A		It is an indolic alkaloid. Chemically it is 2,5-Diketopiperazine class of natural products obtained from the fungus *Aspergillus fumigatus*. Spirotryprostatin A have been found to possess anti-mitotic activities and as such they have become the great interest for cancer studies[21]
Horsfiline		It is an oxindole alkaloid belonging to the class of spiroindolone. It is found in the plant *Horsfieldia superba* which is used in traditional herbal medicine system due to their analgesic effect[21]
Coerulescine		It is an oxindole alkaloid found in the plant Horsfieldia superba. It is found to exhibit analgesic effects[22]
Welwitindolinone A		It is alkaloid extracted from certain blue-green algae that may be active against drug resistance. It has a structure similar to that of hapalindole with a cyclobutane ring. Welwitindolinone A isonitrile is a natural product found in *Hapalosiphon welwitschii* and *Fischerella muscicola* [22]
Elacomine		(+)-Elacomine is a natural anticancer agent which is a hemiterpene spiro oxindole alkaloid obtained from Elaeagnus commutate. It is toxic to melanoma cells with no effect on normal human cell lines which allows elacomine for a uniquely targeted treatment for melanoma[21,22]

Strychnofoline		It is a Strychnos alkaloid obtained from the leaves of *Strychnos usambarensis* and it is widely employed as an anticancer agent[21,22]

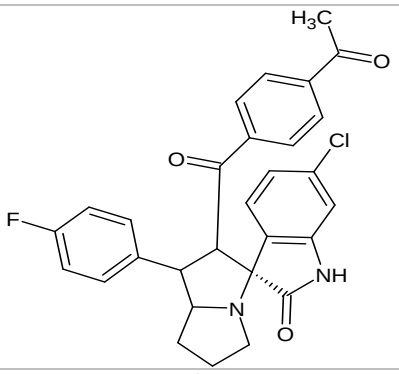
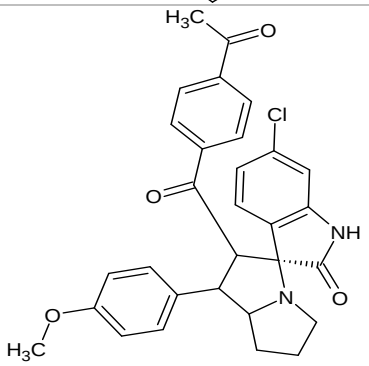
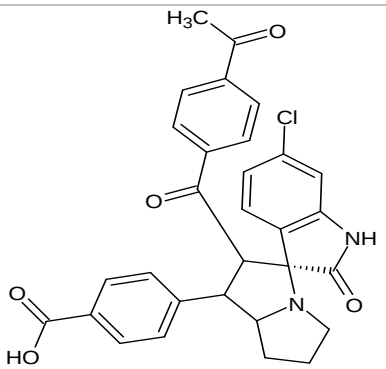
II. MATERIALS AND METHODS

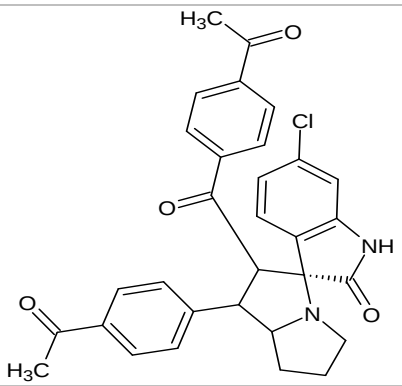
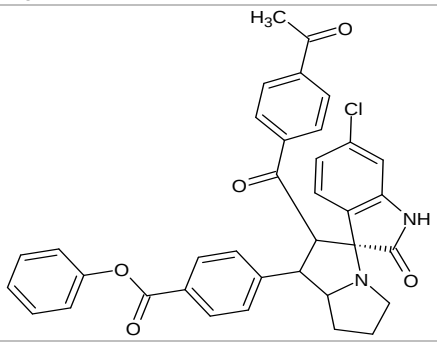
a. Ligand preparation

The test compounds are five congeneric spirooxindole derivatives (A1, A2, A3, A4, A5), shown in Table 2 were generated in ChemSketch software and saved in MDL Molfiles. Thereafter the compounds were imported into Chem 3D software and the compounds were optimized by minimizing energy to achieve the optimal (lowest energy) stable structures and the optimized ligands were saved in PDB file format. Following the energy minimization step, the ligands were imported into Biovia Discovery studio and polar hydrogens were added to the optimized structures and saved in Protein Data Bank files. The physical properties of the designed compounds are obtained from ChemSketch software tool which determines the molecular formula, molecular weight, polarizability, molar refractivity, molar volume, Parachor, Index of refraction, Surface tension and density of the compounds.

Donepezil is an approved marketed drug for treatment of Alzheimer's disease and it is used a reference drug for docking studies. The structure of Donepezil was obtained from PubChem.

Table 2: Structures of sketched ligands with their properties

Ligands code	Structures	Characteristics
A1		**Molecular Formula**: $C_{36}H_{29}ClN_2O_5$ **Formula Weight**: 605.07886 **Molar Refractivity**:164.81 ± 0.4 cm^3 **Molar Volume**: 425.1 ± 5.0 cm^3 **Parachor**: 1235.0 ± 6.0 cm^3 **Index of Refraction**: 1.702 ± 0.03 **Surface Tension**: 71.2 ± 5.0 dyne/cm **Density**:1.42 ± 0.1 g/cm^3 **Polarizability**: 65.33 ± 0.5 $10^{-24}cm^3$
A2		**Molecular Formula**: $C_{30}H_{27}ClN_2O_4$ **Formula Weight**: 514.99938 **Molar Refractivity**: 139.98 ± 0.4 cm^3 **Molar Volume**: 370.2 ± 5.0 cm^3 **Parachor**: 1056.0 ± 6.0 cm^3 **Index of Refraction**: 1.680 ± 0.03 **Surface Tension**: 66.1 ± 5.0 dyne/cm **Density:** 1.39 ± 0.1 g/cm^3 **Polarizability**: 55.49 ± 0.5 $10^{-24}cm^3$
A3		**Molecular Formula**: $C_{30}H_{25}ClN_2O_5$ **Formula Weight**: 528.9829 **Molar Refractivity**: 139.89 ± 0.4 cm^3 **Molar Volume**: 358.3 ± 5.0 cm^3 **Parachor**: 1057.8 ± 6.0 cm^3 **Index of Refraction**: 1.709 ± 0.03 **Surface Tension**: 75.9 ± 5.0 dyne/cm **Density**: 1.47 ± 0.1 g/cm^3 **Polarizability**: 55.46 ± 0.5 $10^{-24}cm^3$

A4		**Molecular Formula**: $C_{31}H_{27}ClN_2O_4$ **Formula Weight**: 527.01008 **Molar Refractivity**: 142.99 ± 0.4 cm^3 **Molar Volume**: 377.1 ± 5.0 cm^3 **Parachor**: 1080.9 ± 6.0 cm^3 **Index of Refraction**: 1.682 ± 0.03 **Surface Tension**: 67.4 ± 5.0 dyne/cm **Density**: 1.39 ± 0.1 g/cm^3 **Polarizability**: 56.68 ± 0.5 $10^{-24}cm^3$
A5		**Molecular Formula**: $C_{36}H_{29}ClN_2O_5$ **Formula Weight**: 605.07886 **Molar Refractivity**: 164.81 ± 0.4 cm^3 **Molar Volume**: 425.1 ± 5.0 cm^3 **Parachor**: 1235.0 ± 6.0 cm^3 **Index of Refraction**: 1.702 ± 0.03 **Surface Tension**: 71.2 ± 5.0 dyne/cm **Density**: 1.42 ± 0.1 g/cm^3 **Polarizability**: 65.33 ± 0.5 $10^{-24}cm^3$

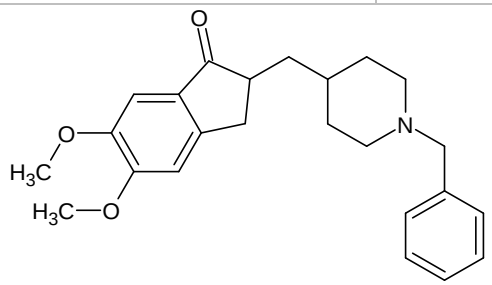


Figure 1: Molecular structure of Donepezil {2-((1-Benzylpiperidin-4-yl) methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one}

b. Protein preparation

The 3D crystal structure of the target protein used for docking evaluation was obtained from the RCSB protein data bank files (PDB ID:4ACX) with a resolution of 2.00 Å which was identified from the literature studies and saved in PDB file format[24]. The protein was imported into Biovia Discovery Studio and the protein was prepared by removing the water molecules, complexed ions, non-standard amino acids, associated ligands and all non-protein elements. Then polar hydrogen atoms were added to the protein structure and it was saved in Protein Data Bank Files.

c. Molecular docking

Molecular Docking has become a very useful tool for drug discovery due to its ability of predicting ligand conformation and binding mode to the receptor binding site[25].The docking study between the target and the ligands was carried out by using the software PyRx. The software was utilized in predicting the ligand-protein interactions and it is characterized by its flexibility and speed in executing docking operations. The process begins by importing the library of ligands (tests and reference compounds) into the software followed by loading of crystal structure of protein. The grid box was adjusted for ligands and protein active site interactions. The docking process is run to obtain the docking score for calculating the binding affinity with RMSD value zero.

Post docking analysis and visualization of 2D interactions of compounds with active site amino acid residues was carried out using Biovia Discovery Studio.

d. Drug-likeliness prediction

Computational technology has improved the success rate by reducing experimental drug trials hence, it serves as a vital tool for identification of drug candidate. The drug likeliness of the designed compounds was predicted using Molinspiration software. Molinspiration is a web-based software which is used to obtain parameter such as MiLogP, TPSA, drug likeness scores etc. MiLogP is calculated as sum of fragment-based contributions and correction factors and it is used to check good permeability across the cell membrane. Partition coefficient or Log P is an important parameter used in rational drug design to measure the hydrophobicity of molecules. Hydrophilic/lipophilic nature of a drug molecule affects drug-receptor interactions, absorption, bioavailability, metabolism as well as the toxicity of molecules. Molecular Polar Surface Area is calculated based on sum of fragment contributions of O and N-centered polar fragments. Total polar surface area (TPSA) defines the hydrogen bonding potential of a molecule and is a very good predictor of drug transport properties such as blood brain barrier penetration, intestinal absorption, bioavailability etc. Calculation of volume is based on group contributors. Number of rotatable bonds measures the flexibility of molecules and it is a very good descriptor of absorption and bioavailability of drugs. Bioactivity of the drug can be checked by calculating the activity score of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor, enzyme inhibitor. For

organic molecules if the probability of bioactivity score is greater than zero (>0) then it is active, if it is between-5.0-0.0 then it is moderately active, if less than -5.0 (< -5.0) then it is inactive. The drug likeness scores were calculated by considering MiLogP (partition coefficient), molecular weight, number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor, number of violations, number of rotatable bonds and volume[26].

e. ADMET prediction

Many potential therapeutic agents fail to make its way to the stages of clinical trials because of their unfavourable ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) parameters. For a molecule to be considered as a drug candidate it must obey the Lipinski, Veber and Igan rules which are based on evaluation of ADME properties and bioavailability of molecules by oral administration[27]. Compounds whose physicochemical properties do not meet at least two of the Lipinski rule, Veber rule and Igan rules are not suitable to be developed as drug candidate. The evaluation of two factors such as number of rotatable bonds(n-ROTB) and Topological polar surface area (TPSA) allows us to know if the mode of ligand-receptor interactions is flexible or inflexible[28].The ADME properties were evaluated with the aid of Swiss ADME which is an online ADME prediction tool (http://www.swissadme.ch.)[29]

Toxicity risk assessment provides and insight about probable side effects of compounds that may be useful for further processing in drug development and discovery. The various toxicological profile can be measured by means of precomputed set of structural fragments. The toxicity parameters of the ligands were determined by using the Protox II software[30].

f. Adverse effect prediction

To obtain the predicted biological activity profile for the compounds, only structural formula is necessary; thus, prediction is possible even for virtual structure designed in computer but not synthesized yet. PASS Online predicts over 4000 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence o gene expression, etc.

III. RESULTS AND DISCUSSION

a. Evaluation of drug-likeness properties

The first aim of this study is to predict the drug-likeliness or drug-like properties of the five molecules (A1, A2, A3, A4, A5) in order to describe the biological activity of the compounds. The second aim is to identify the bioactivity of the molecules with drug-like properties in order to study their interaction with the binding site of target protein. In Table 3 and 4, we present the evaluation results of the drug-like properties and bioactivities of molecules obtained from Molinspiration software.

For TPSA, when it is less than 140 $Å^2$ and the number of rotatable bonds is less than 10, the compounds become more flexible and it is able to interact more with the target receptor. We note from Table 3 that the TPSA values for all five compounds are less than 140 $Å^2$ and also the compounds have n-ROTB value less than 10, so it can be assumed that all five compounds can interact flexibly with the target receptor. The value of miLogP is just above 5 which suggests that the compounds may have good permeability across cell membrane. The no. of hydrogen bond donors is < 5, no. of hydrogen bond acceptors is <10, Molecular weight of most of the compounds are just above 500Da and no. of violations for Lipinski rule is 2 which suggests that the compounds can be a potential candidate for synthesis.

For bioactivity score, as seen in Table 4, the compounds almost falls in the standard region which clearly indicates that the compound possesses the required properties to act as potential drugs with very little modifications in the chemical structure.

Table 3: ADME properties of the five designed compounds

Compounds code	natoms	TPSA	n-rotb	MW	miLogP	nOHNH	nON	nviolations	volume
A1	36	66.48	4	502.97	5.54	1	5	2	428.89
A2	37	75.71	5	515.01	5.43	1	6	2	449.51
A3	38	103.78	5	528.99	5.29	2	7	2	450.96
A4	38	83.55	5	527.02	5.28	1	6	2	459.51
A5	44	92.78	7	605.09	7.24	1	7	2	523.34

Table 4: Bioactivity score of the compounds

Compound code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
A1	-0.26	-0.44	-0.52	-0.51	-0.33	-0.47

A2	-0.29	-0.50	-0.55	-0.52	-0.34	-0.48
A3	-0.24	-0.49	-0.54	-0.41	-0.28	-0.41
A4	-0.25	-0.48	-0.52	-0.50	-0.29	-0.45
A5	-0.43	-0.96	-0.82	-0.75	-0.36	-0.73

b. Molecular docking

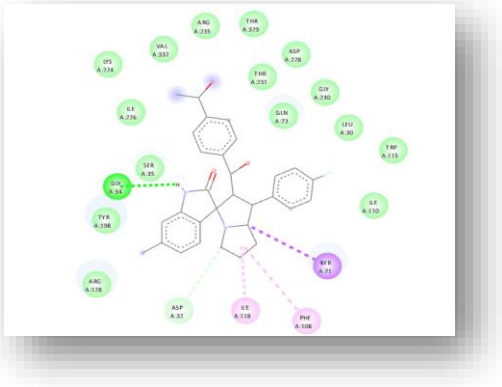
A docking study was carried by taking a target protein for Alzheimer's disease from RCSB protein data bank files (PDB ID:4ACX) with a resolution of 2.00 Å which was identified from the literature studies. The designed compounds were docked by using PyRx virtual screening tool. The lowest docking pose energy with lower root mean square deviation (RMSD=0) was selected as the docking score with best protein affinity (Table 5). Docking study of Spirooxindole derivatives with target protein was carried out by taking Donepezil as a standard drug of the respective target. All the derivatives (A1, A2, A3, A4, A5) strongly inhibited the target protein with binding affinity of −10.1, −9.7, −9.6, and −9.6, -11.1 kcal/mol, respectively while that of standard Donepezil was found to be -8.4 kcal/mol. Docking analysis of all the spirooxindole derivatives showed that all the designed compounds exhibited good binding affinity compared to the reference drug Donepezil.

Spirooxindole derivatives were further analysed for their interaction with 4ACX target protein using Biovia Discovery Studio which is presented in Table 6. Interaction of A1 with 4ACX involved one conventional hydrogen bond, the bond formed involved GlyA:34, one Carbon- Hydrogen interactions with AspA:32, one Pi-Sigma interactions with TyrA:71, two Alkyl interactions with PheA:108 and IleA:118. Interaction of A2 involves three Conventional Hydrogen bond interactions with GlyA:34, ArgA:235, GlnA:73, two Carbon- Hydrogen interactions with AspA:32and GlyA:230, one Pi-Sigma interactions with TyrA:71, , two Alkyl interactions with PheA:108 and IleA:118, one Pi-Alkyl interaction with LeuA:30. Interaction of A3 involves four Conventional Hydrogen bond interactions with GlyA:34, GlnA:73, TyrA:198 and ThrA:232, one Carbon- Hydrogen interactions with GlyA:230, one Pi-Sigma interactions with TyrA:71, two Alkyl interactions with ValA:332 and IleA:118. Interaction of A4 involves three Conventional Hydrogen bond interactions with GlyA:34 GlnA:73 and LysA:224, one Carbon- Hydrogen interactions with GlyA:230, one Pi-Pi T-Shaped with TyrA:71, one Unfavourable Donor-Donor interaction with TyrA:158. Interaction of A5 involves one Conventional Hydrogen bond interactions with GlnA:73, three alkyl interactions with IleA:118, AlaA:39 and IleA:126, two Pi-Alkyl interactions with ValA:69, IleA:126, one Pi-Pi T-Shaped with TyrA:71, one Pi-Donor Hydrogen Bond with TrpA:76 and the interaction of standard drug Donepezil involves one Conventional Hydrogen bond interactions with ThrA:232 and one Pi-Pi Stacked with TyrA:71.

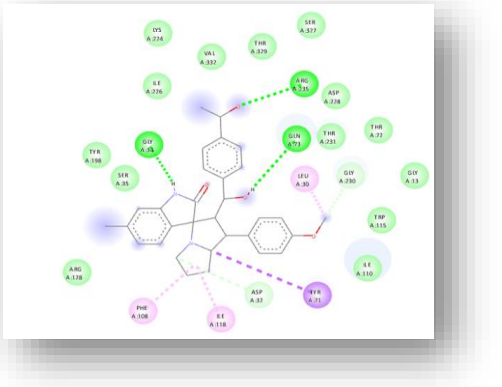
Table 5: Interaction of amino acids with target protein along with binding affinity score

Compound code	**Binding Affinity (kcal/mol)**	**Conventional Hydrogen bond interactions**	**Carbon-Hydrogen interactions**	**Pi-Sigma interactions**	**Alkyl interactions**	**Pi-Alkyl**	**Pi-Pi T-Shaped**	**Unfavourable Donor-Donor**	**Pi-Donor Hydrogen Bond**	**Pi-Pi Stacked**
A1	-10.0	GlyA:34	AspA:32	TyrA:71	PheA:108, IleA:118					
A2	-9.7	GlyA:34, ArgA:235, GlnA:73	AspA:32, GlyA:230	TyrA:71	PheA:108, IleA:118	LeuA:30				
A3	-9.6	GlyA:34, GlnA:73, TyrA:198, ThrA:232	GlyA:230	TyrA:71	IleA:118, ValA:332					
A4	-9.6	GlyA:34, GlnA:73, LysA:224	GlyA:230				TyrA:71	TyrA:158		
A5	-11.1	GlnA:73			IleA:118, AlaA:39, IleA:126	ValA:69, IleA:126,	TyrA:71		TrpA:76	
Donepezil	-8.4	ThrA:232								TyrA:71

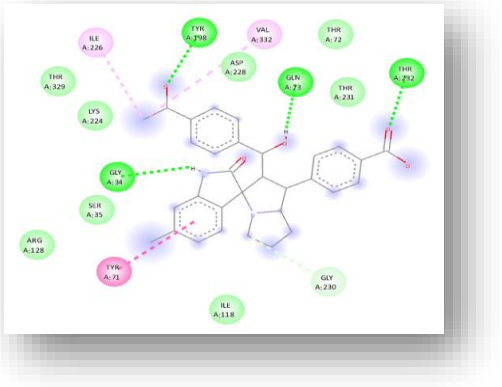
Table 6: 2D Interaction of the compounds with various amino acid residues of target protein 4ACX



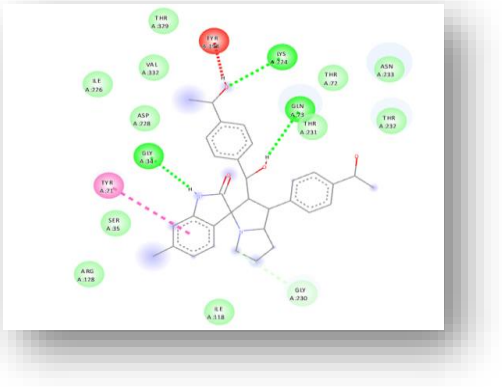
A1



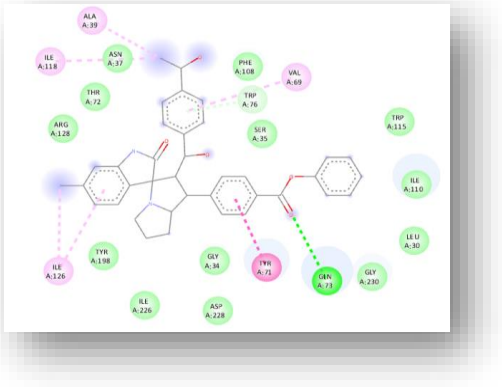
A2



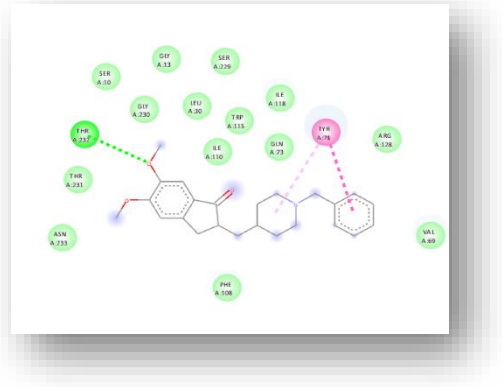
A3



A4



A5



Donepezil

c. ADME prediction

The results on predictive data for pharmacokinetics, bioavailability and drug-likeness of established five spirooxindole derivatives are shown in Table 7-8.

In Table 7, for pharmacokinetics prediction, the gastrointestinal (GI) absorption rate was obtained higher for A1, there presence is blood-brain permeability, it is CYP450 2C9 and CYP450 3A4 inhibitor with skin permeation value of -6.10 cm/s. For A2, gastrointestinal (GI) absorption rate was obtained higher, it is P-glycoprotein substrate, inhibitor of CYP450 2C19, CYP450 3A4 and CYP450 2C9 with skin permeation value of -6.27 cm/s. For A3, gastrointestinal (GI) absorption rate was obtained higher, it is P-glycoprotein substrate, inhibitor of CYP450 2C19, CYP450 3A4 and CYP450 2C9 with skin permeation value of -8.28 cm/s. For A4, gastrointestinal (GI) absorption rate was obtained higher, it is P-glycoprotein substrate, inhibitor of CYP450 2C19 and CYP450 3A4 with skin permeation value of -6.55 cm/s. For A5, gastrointestinal (GI) absorption rate was obtained higher, inhibitor of CYP450 2C9 and CYP450 2D6 with skin permeation of -5.79 cm/s. Among all the designed compounds, A1 is showing permeability for Blood-Brain-Barrier so it can be useful for the treatment of Alzheimer disease by preventing amyloid fibril formation at early stages of Alzheimer's disease.

Table 7: Pharmacokinetics prediction of Spirooxindole derivatives

Compounds code	Gastro-intestinal absorption	Blood-Brain permeant	P-glycoprotein substrate	CYP450 1A2 inhibitor	CYP450 2C19 inhibitor	CYP450 2C9 inhibitor	CYP450 2D6 inhibitor	CYP450 3A4 inhibitor	Skin permeation as log Kp (cm/s)
A1	High	Yes	No	No	No	Yes	No	Yes	-6.10
A2	High	No	Yes	No	Yes	Yes	No	Yes	-6.27
A3	High	No	Yes	No	No	Yes	No	Yes	-8.28
A4	High	No	Yes	No	Yes	Yes	No	Yes	-6.55
A5	High	No	No	No	No	Yes	Yes	No	-5.79

The prediction of bioavailability score, lipophilicity and water solubility values for all studied compounds are shown in table 8. All the compounds except A5 have shown lipophilicity: XLOGP3 value between normal range i.e within -0.7 to +5.0 and solubility logS not higher than 6 which suggests that the compounds A1, A2, A3, A4 have drug-likeliness properties

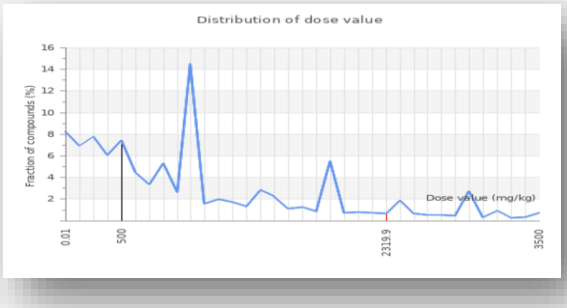
Table 8: Bioavailability, lipophilicity and water solubility prediction of the compounds

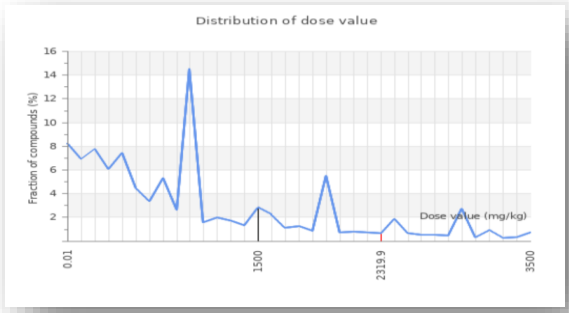
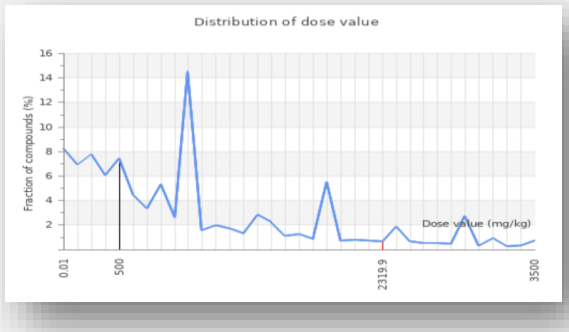
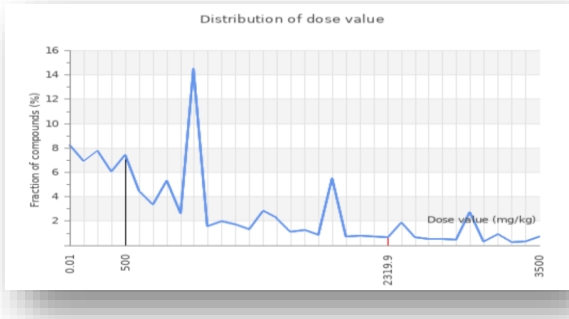
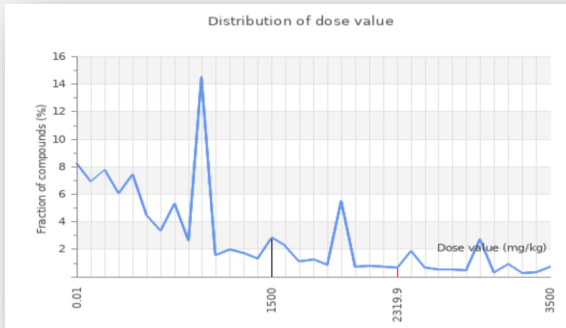
Compounds code	Bioavailability score	Water solubility as logS	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT
A1	0.55	-5.96	3.16	4.60	4.95	3.83	5.62
A2	0.55	-5.88	3.42	4.47	4.40	3.11	5.27
A3	0.55	-4.24	3.00	1.75	4.09	3.05	4.66
A4	0.55	-5.76	3.22	4.18	4.59	2.96	5.68
A5	0.17	-7.26	3.88	5.91	5.61	4.21	6.24

d. Toxicity prediction

The risk assessment and safety profile of the compounds ere predicted using Protox II which is used for prediction of various toxicity end points such as acute toxicity, hepatotoxicity, immunotoxicity, hepatotoxicity, mutagenicity, cytotoxicity, carcinogenicity, toxicity targets and adverse outcome pathways. The oral toxicity prediction of the compounds is shown in the following figures in table 9 which represents the graphical presentation of the predicted dose value of the drug compounds and it also highlights that the designed compounds are devoid of toxic effects.

Table 9: Prediction of dose distribution value

Compounds code	Dose distribution value
A1	

A2	
A3	
A4	
A5	

The Protox-II server showed that the compounds A1, A3 and A4 has LD_{50}value of 500mg/kg and compounds A2, A5 have LD_{50}value of 1500mg/kg and the graphical accuracy of prediction is 67.38%. Class 4 is the predicted toxicity of the drug compound as shown in the table 10.

Table 10: Prediction of oral acute toxicity, class, average similarity and prediction accuracy

Compound Code	Predicted LD_{50} (mg/kg)	Predicted Toxicity Class	Average similarity (%)	Prediction accuracy (%)
A1	500	4	55.78	67.38
A2	1500	4	56.33	67.38
A3	500	4	58.33	67.38

A4	500	4	57.82	67.38
A5	1500	4	54.53	67.38

The prediction of organ toxicity of all the compounds was examined and liver toxicity was found to be inactive with a probability score of 0.65,0.61, 0.60, 0.62 and 0.60 respectively. Toxicity endpoints of carcinogenicity was found to be inactive with a probability score of 0.59, 0.57, 0.60,0.59 and 0.53 respectively. For immunotoxicity the toxicity endpoints were found to be inactive with a probability score of 0.77, 0.67, 0.99, 0.94 and 0.77 respectively. Toxicity endpoints of mutagenicity was found to be inactive with a probability score of 0.64, 0.65,0.67, 0.65 and 0.66 respectively. Toxicity endpoints of cytotoxicity was found to be inactive with a probability score of 0.72, 0.67, 0.66, 0.70, and 0.66 respectively. Therefore, the probability scores of all the predicted compounds indicates that the compounds maintain the safety profile with no probability of toxicological outcomes.

Table 11: Prediction of organ toxicity, Toxicity end points, Tox21-Nuclear receptor signalling pathways, Tox21-Stress response pathways for compound A1

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatoxicity	Dili	Inactive	0.65
Toxicity end points	Carcinogenicity	Carcino	Inactive	0.59
Toxicity end points	Immunotoxicity	Immuno	Inactive	0.77
Toxicity end points	Mutagenicity	Mutagen	Inactive	0.64
Toxicity end points	Cytotoxicity	Cyto	Inactive	0.72
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.75
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligan Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.90
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.87
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligan Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.93
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.95
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.95
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.60
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.86
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5(ATAD5)	sr_atad5	Inactive	0.87

Table 12: Prediction of organ toxicity, Toxicity end points, Tox21-Nuclear receptor signalling pathways, Tox21-Stress response pathways for compound A2

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatoxicity	Dili	Inactive	0.61
Toxicity end points	Carcinogenicity	Carcino	Inactive	0.57
Toxicity end points	Immunotoxicity	Immuno	Inactive	0.67
Toxicity end points	Mutagenicity	Mutagen	Inactive	0.65
Toxicity end points	Cytotoxicity	Cyto	Inactive	0.67
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.78
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligan Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.89
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.85
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligan Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.94
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.91
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.91
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.64
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.84
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5(ATAD5)	sr_atad5	Inactive	0.90

Table 13: Prediction of organ toxicity, Toxicity end points, Tox21-Nuclear receptor signalling pathways, Tox21-Stress response pathways for compound A3

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatoxicity	Dili	Inactive	0.60
Toxicity end points	Carcinogenicity	Carcino	Inactive	0.60
Toxicity end points	Immunotoxicity	Immuno	Inactive	0.99
Toxicity end points	Mutagenicity	Mutagen	Inactive	0.67
Toxicity end points	Cytotoxicity	Cyto	Inactive	0.66
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.80
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligan Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.90
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.87
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligan Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.86
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.92
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.92
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.65
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.86
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5(ATAD5)	sr_atad5	Inactive	0.89

Table 14: Prediction of organ toxicity, Toxicity end points, Tox21-Nuclear receptor signalling pathways, Tox21-Stress response pathways for compound A4

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatoxicity	Dili	Inactive	0.62
Toxicity end points	Carcinogenicity	Carcino	Inactive	0.59
Toxicity end points	Immunotoxicity	Immuno	Inactive	0.94
Toxicity end points	Mutagenicity	Mutagen	Inactive	0.65
Toxicity end points	Cytotoxicity	Cyto	Inactive	0.70
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.75
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligan Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.90
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.87
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligan Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.91
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.94
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.94
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.61
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.85
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5(ATAD5)	sr_atad5	Inactive	0.90

Table 15: Prediction of organ toxicity, Toxicity end points, Tox21-Nuclear receptor signalling pathways, Tox21-Stress response pathways for compound A5

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatoxicity	Dili	Inactive	0.60
Toxicity end points	Carcinogenicity	Carcino	Inactive	0.53
Toxicity end points	Immunotoxicity	Immuno	Inactive	0.77
Toxicity end points	Mutagenicity	Mutagen	Inactive	0.65
Toxicity end points	Cytotoxicity	Cyto	Inactive	0.66
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.79
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligan Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.89
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.86
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligan Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.90
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.90
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.90
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.65
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.85
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5(ATAD5)	sr_atad5	Inactive	0.90

e. Adverse effect prediction

The possible adverse effects of all the compounds are predicted using Pass Online software and the probable adverse effects of the designed drugs are given below in table 16.

Table 16: Adverse effects of the designed compounds

Compound code	Adverse effects
A1	Chorea, Myoclonus, Delirium, Dystonia, Dysarthria, QT interval prolongation, Torsades de pointes
A2	Myoclonus, Chorea, Hypotonia, Dysarthria, Keratopathy, Choreoathetosis, QT interval prolongation
A3	Myoclonus, Xerostomia, Urinary retention, Excitibility, Chorea, Hypotonia, Dysarthria, Keratopathy, Choreoathetosis, QT interval prolongation

A4	Chorea, Myoclonus, Delirium, Dystonia, Dysarthria, Keratopathy, Parkinsonism, Retroperitoneal fibrosis, Akathisia, Silorrhea, Laryngospasm
A5	Retroperitoneal fibrosis, Reproductive dysfunction, Glaucoma, Chorea, Keratopathy, Myoclonus, Choreoathetosis, Delirium, Sedative

IV. CONCLUSION

In the present research, the theoretical evaluation of binding affinities (kcal/mol) of some Spirooxindole derivatives with target protein (PDB ID:4ACX) was carried out in order to validate their potency. The molecular docking results showed a good docking score ranged from – 9.6 kcal/mol to – 11.1 kcal/mol signifying that the molecules can bind more tightly with the active site of the target when compared to the standard drug Donepezil. In addition, the results of the drug-likeness and ADME properties have revealed that the newly designed drug molecules have good pharmacokinetic properties which are predicted to be orally bioavailable, less toxic, and good absorption. Furthermore, the increased number of H-bonds formed by the designed drug compounds have interaction distances less than 3.0 Å which gives the structural insight to support the claim why it is able to bind tightly with the active pocket of the targeted enzyme. Among all the designed derivatives, A1 has showed Blood Brain Barrier (BBB) permeability in ADME studies which is essential for treatment of early stages of Alzheimer disease and it follows all the essential criteria of an ideal drug candidate. The outcome of the research strengthens the relevance of these compounds as promising drug candidates for the treatment of Alzheimer disease which could help the medicinal chemists and pharmaceutical professionals in further designing and synthesis of more potent compounds for treatment of Alzheimer disease.

V. ACKNOWLEDGEMENT

Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, University e-Library and various other sources for their cooperation and guidance in writing this *in-silico* research.

REFERENCES:

1. Cummings JL, Cole G. Alzheimer disease. Jama. 2002 May 8;287(18):2335-8.doi:10.1001/jama.287.18.2335
2. Barakat A, Alshahrani S, Al-Majid AM, Ali M, Altowyan MS, Islam MS, Alamary AS, Ashraf S, Ul-Haq Z. Synthesis of a new class of spirooxindole–benzo [b] thiophene-based molecules as acetylcholinesterase inhibitors. Molecules. 2020 Oct 13;25(20):4671.doi:https://doi.org/10.3390/molecules25204671
3. Wang Z, Wang Y, Li W, Mao F, Sun Y, Huang L, Li X. Design, synthesis, and evaluation of multitarget-directed selenium-containing clioquinol derivatives for the treatment of Alzheimer's disease. ACS Chemical Neuroscience. 2014 Oct 15;5(10):952-62. oi:https://doi.org/10.1021/cn500119g
4. Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. Disease-a-month: DM. 2010 Sep;56(9):484. doi: 10.1016/j.disamonth.2010.06.001
5. Smith MA. Alzheimer disease. International review of neurobiology. 1998 Jan 1;42:1-54.doihttps://doi.org/10.1016/S0074-7742(08)60607-8
6. Giacobini E. Selective inhibitors of butyrylcholinesterase: a valid alternative for therapy of Alzheimer's disease?. Drugs & aging. 2001 Dec;18:891-8.
7. Panda SS, Jones RA, Bachawala P, Mohapatra PP. Spirooxindoles as Potential Pharmacophores. Mini reviews in medicinal chemistry. 2017 Jan 1;17(16):1515-36.doi: 10.2174/1389557516666160624125108
8. Kia Y, Osman H, Kumar RS, Murugaiyah V, Basiri A, Perumal S, Razak IA. A facile chemo- regio and stereoselective synthesis and cholinesterase inhibitory activity of spirooxindole–pyrrolizine–piperidine hybrids. Bioorganic & medicinal chemistry letters. 2013 May 15;23(10):2979-83. doihttps://doi.org/10.1016/j.bmcl.2013.03.027
9. Kia Y, Osman H, Kumar RS, Basiri A, Murugaiyah V. Synthesis and discovery of highly functionalized mono-and bis-spiro-pyrrolidines as potent cholinesterase enzyme inhibitors. Bioorganic & medicinal chemistry letters. 2014 Apr 1;24(7):1815-9. doihttps://doi.org/10.1016/j.bmcl.2014.02.019
10. Kia Y, Osman H, Kumar RS, Murugaiyah V, Basiri A, Perumal S, Wahab HA, Bing CS. Synthesis and discovery of novel piperidone-grafted mono-and bis-spirooxindole-hexahydropyrrolizines as potent cholinesterase inhibitors. Bioorganic & medicinal chemistry. 2013 Apr 1;21(7):1696-707. doi:https://doi.org/10.1016/j.bmc.2013.01.066
11. Osman H, Kumar RS, Basiri A, Murugaiyah V. Ionic liquid mediated synthesis of mono-and bis-spirooxindole-hexahydropyrrolidines as cholinesterase inhibitors and their molecular docking studies. Bioorganic & medicinal chemistry. 2014 Feb 15;22(4):1318-28. doihttps://doi.org/10.1016/j.bmc.2014.01.002
12. Arumugam N, Almansour AI, Kumar RS, Kotresha D, Saiswaroop R, Venketesh S. Dispiropyrrolidinyl-piperidone embedded indeno [1, 2-b] quinoxaline heterocyclic hybrids: Synthesis, cholinesterase inhibitory activity and their molecular docking simulation. Bioorganic & Medicinal Chemistry. 2019 Jun 15;27(12):2621-8.doihttps://doi.org/10.1016/j.bmc.2019.03.058
13. Barakat A, Soliman SM, Alshahrani S, Islam MS, Ali M, Al-Majid AM, Yousuf S. Synthesis, X-ray single crystal, conformational analysis and cholinesterase inhibitory activity of a new spiropyrrolidine scaffold tethered benzo [b] thiophene analogue. Crystals. 2020 Feb 15;10(2):120.doi:https://doi.org/10.3390/cryst10020120
14. Goyal D, Kaur A, Goyal B. Benzofuran and indole: promising scaffolds for drug development in Alzheimer's disease. ChemMedChem. 2018 Jul 6;13(13):1275-99.doi:https://doi.org/10.1002/cmdc.201800156

15. Kumar RS, Ali MA, Osman H, Ismail R, Choon TS, Yoon YK, Wei AC, Pandian S, Manogaran E. Synthesis and discovery of novel hexacyclic cage compounds as inhibitors of acetylcholinesterase. Bioorganic & medicinal chemistry letters. 2011 Jul 1;21(13):3997-4000.doi:https://doi.org/10.1016/j.bmcl.2011.05.003
16. Yu B, Yu DQ, Liu HM. Spirooxindoles: Promising scaffolds for anticancer agents. European journal of medicinal chemistry. 2015 Jun 5;97:673-98.doi:https://doi.org/10.1016/j.ejmech.2014.06.056
17. Lotfy G, El Sayed H, Said MM, Aziz YM, Al-Dhfyan A, Al-Majid AM, Barakat A. Regio-and stereoselective synthesis of new spirooxindoles via 1, 3-dipolar cycloaddition reaction: Anticancer and molecular docking studies. Journal of Photochemistry and Photobiology B: Biology. 2018 Mar 1;180:98-108.doi:https://doi.org/10.1016/j.jphotobiol.2018.01.026
18. Lotfy G, Said MM, El Sayed H, El Sayed H, Al-Dhfyan A, Aziz YM, Barakat A. Synthesis of new spirooxindole-pyrrolothiazole derivatives: Anti-cancer activity and molecular docking. Bioorganic & medicinal chemistry. 2017 Feb 15;25(4):1514-23doi:https://doi.org/10.1016/j.bmc.2017.01.014
19. Lotfy G, Aziz YM, Said MM, El Ashry ES, El Tamany ES, Barakat A, Ghabbour HA, Yousuf S, Ul-Haq Z, Choudhary MI. Synthesis of oxindole analogues, biological activity, and in silico studies. ChemistrySelect. 2019 Sep 20;4(35):10510-6.doi:https://doi.org/10.1002/slct.201901228
20. Xia M, Ma RZ. Recent progress on routes to spirooxindole systems derived from isatin. Journal of Heterocyclic Chemistry. 2014 May;51(3):539-54.doi:https://doi.org/10.1002/jhet.1114
21. Zhang M, Yang W, Qian M, Zhao T, Yang L, Zhu C. Iodine-promoted three-component reaction for the synthesis of spirooxindoles. Tetrahedron. 2018 Mar 1;74(9):955-61.doi: 10.1016/j.tet.2018.01.001
22. Divar M, Zomorodian K, Sabet R, Moeini M, Khabnadideh S. An efficient method for synthesis of some novel spirooxindole-4H-pyran derivatives. Polycyclic Aromatic Compounds. 2021 Aug 19;41(7):1549-62doi:https://doi.org/10.1080/10406638.2019.1686405
23. Yu Q, Guo P, Jian J, Chen Y, Xu J. Nine-step total synthesis of (−)-strychnofoline. Chemical Communications. 2018;54(9):1125-8.doi: https://doi.org/10.1039/C7CC08938D
24. Swahn BM, Holenz J, Kihlström J, Kolmodin K, Lindström J, Plobeck N, Rotticci D, Sehgelmeble F, Sundström M, von Berg S, Fälting J. Aminoimidazoles as BACE-1 inhibitors: The challenge to achieve in vivo brain efficacy. Bioorganic & medicinal chemistry letters. 2012 Mar 1;22(5):1854-9.doi:https://doi.org/10.1016/j.bmcl.2012.01.079
25. Chtita S, Belhassan A, Aouidate A, Belaidi S, Bouachrine M, Lakhlifi T. Discovery of potent SARS-CoV-2 inhibitors from approved antiviral drugs via docking and virtual screening. Combinatorial chemistry & high throughput screening. 2021 Mar 1;24(3):441-54.doi: https://doi.org/10.2174/1386207323999200730205447
26. Tareq Hassan Khan M. Predictions of the ADMET properties of candidate drug molecules utilizing different QSAR/QSPR modelling approaches. Current drug metabolism. 2010 May 1;11(4):285-95.doi: 10.2174/138920010791514306
27. Hansch C, Leo A, Mekapati SB, Kurup A. Qsar and adme. Bioorganic & medicinal chemistry. 2004 Jun 15;12(12):3391-400.doi: https://doi.org/10.2174/138920010791514306
28. Jin Z, Wang Y, Yu XF, Tan QQ, Liang SS, Li T, Zhang H, Shaw PC, Wang J, Hu C. Structure-based virtual screening of influenza virus RNA polymerase inhibitors from natural compounds: Molecular dynamics simulation and MM-GBSA calculation. Computational biology and chemistry. 2020 Apr 1;85:107241doi: https://doi.org/10.1016/j.compbiolchem.2020.107241
29. Chtita S, Aouidate A, Belhassan A, Ousaa A, Taourati AI, Elidrissi B, Ghamali M, Bouachrine M, Lakhlifi T. QSAR study of N-substituted oseltamivir derivatives as potent avian influenza virus H5N1 inhibitors using quantum chemical descriptors and statistical methods. New Journal of Chemistry. 2020;44(5):1747-60.doi: https://doi.org/10.1039/C9NJ04909F
30. 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.