RESEARCH ARTICLE

Design, Synthesis, *In-silico* Study and Biological Evaluation of Some Heterocyclic Derivatives as Cholinesterase Inhibitors

Rupali Jinturkar*, Pallavi Patil

PES Modern College of Pharmacy, Nigdi, Pune- Savitribai Phule Pune University, Pune, Maharashtra, India

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ABSTRACT

The study involves design, *in-silico* evaluation and synthesis of new Tacrine-based heterocyclic derivatives as neuroprotective agents. The need for efficient treatments that can target several pathogenic pathways is vital given the rising incidence of numerous disorders involving neuronal degeneration and subsequent neuronal death, which need neuroprotective agents. By combining Tacrine with a heterocyclic scaffold, the study seeks to take advantage of its dual binding ability of the enzyme acetylcholinesterase (AChE). A comprehensive literature survey was conducted, to identify suitable heterocyclic moieties with reported cholinesterase (ChE) inhibitory and neuroprotective activity. In the present research new Tacrine-thiazolidine derivatives were designed and has been synthesized and later checked for AChE inhibitory activity. The structural characterization was done using various spectroscopic techniques, including IR, ^{1}H-NMR and Mass Spectroscopy. Acetylcholinesterase inhibitory activities of the synthesized compounds were diagnosed. Among the synthesized compounds, compound S2M1, which contains unsubstituted phenyl ring on thiazolidinone linked to Tacrine-Nitrogen through Ethylene Bridge, exhibited the most significant inhibitory effects against AChE, with 56.41% as a percent inhibition of AChE compared to that of scopolamine. In-silico insights shown very good binding interactions of compound S2M1 with AChE enzyme. Additionally, in silico assessments indicated that all synthesized compounds possess favourable drug-like characteristics and are predicted to be non-toxic, but amongst all S2M1 proves to be the most competent derivative as AChE inhibitor.

Keywords: Neuroprotectives, Heterocyclic, Design, in-silico, Synthesis.

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Conflict of interest: None

INTRODUCTION

Neurodegeneration involves incremental and gradual diminishment of structure or function of neurons, ultimately may lead to neuronal death—these conditions lead to impairments in cognition and movement. Its complex pathophysiology encompassing cholinergic depletion, amyloid-β deposition, oxidative stress, and excitotoxicity necessitates the development of multifunctional therapeutic agents capable of addressing multiple disease pathways simultaneously.

Tacrine, the first centrally acting acetylcholinesterase (AChE) inhibitor approved for AD treatment, offered transient symptomatic relief but was withdrawn due to hepatotoxicity. Despite this, it remains a valuable scaffold for designing multitarget-directed ligands (MTDLs) by virtue of its well-characterized binding to both the binding site of protein[1,2].

Hybridizing Tacrine with additional pharmacophores like azetidine through appropriate linkers can enhance inhibitory potency, reduce aggregation, and impart neuroprotective properties. For instance, modified Tacrine hybrids such as tacrine–chromene conjugates exhibited potent effects as inhibitors of acetyl and Butyryl choliesterase, moreover effects on BACE-1, MAO-B inhibition, Aβ aggregation, antioxidant activity, and metal-chelating capabilities—highlighting their multifunctional potential[3].

Similarly, other tacrine conjugates, including those with thiadiazole moieties, were found to inhibit cholinesterases, block NMDA receptors, and exhibit radical scavenging activity[4]. As per literature, Thiazolidine moiety has shown promising neuroprotective activity[5-10]. Also, the hepatotoxicity of the Tacrine moiety has been observed due to unsubstituted amino nitrogen. Any kind of substitution on it has shown reduced hepatotoxicity. Confirming this, the synthesized compounds in present research work, when evaluated prove themselves as non-toxic. And, hence the current research includes substitution of thiazolidinone ring on Tacrine nitrogen through appropriate linker.

MATERIALS AND METHODS

Design

ADMET Properties

The PKCSM online server was used to forecast the compounds' ADMET (Absorption, Metabolism, Distribution, Toxicity and Excretion) characteristics in order to evaluate drug-likeness. The dataset demonstrated overall good ADME profiles and no toxicity. Ultimately, compounds with acceptable BBB permeability indicators were selected for subsequent molecular docking analysis.

**Author for Correspondence: rupalijinturkar@gmail.com*

Molecular Docking Study

The target protein (PDB ID: AChE-7XN1) was fetched from Protein Data Bank. The selection of appropriate target protein was done based on the standard parameters as mentioned in Table 1 and 3D image was downloaded from RCSB Protein Data Bank as shown in Figure 1. Refinement of the protein was carried out using tool Chimera v1.16 to produce a superior structure for docking: After energy minimization, all extraneous components were removed to avoid interference during docking. The ligand structure was fetched from PubChem and first refined in Marvin Sketch (ChemAxon, v22.13): addition of hydrogens was done, cleaning up of 2D and 3D structures was done, multiple conformers generated, and the one with lowest energy saved as a mol2 file. Further optimization of this ligand was done in Chimera using AM1-BCC charges. Both protein and ligand files were then converted to PDBQT format via AutoDock Tools so that they could be used with AutoDock Vina[11-15].

Molecular Dynamics Simulation (MDs)

Molecular dynamics (MD) simulations play a paramount role in assessing the flexibility and dynamic behaviour of proteins. This computational technique is extensively applied to study interactions between protein-ligand complexes. The MD simulations study was conducted on the dock complexes of ligand with top docking score (S2M1) using the Desmond 2020.1 from Schrödinger, LLC. The root mean square deviation (RMSD) was computed.

Synthesis

Every reagent was obtained from Sigma-Aldrich. Precoated Merck thin-layer chromatography (TLC) plates were used to track the reaction's progress, and a 9:1 solvent solution consisting of DCM and methanol was used. EI digital melting point equipment was used to determine the samples' melting points. The synthesised compounds' infrared spectra were obtained with a JASCO M-4100 Fourier transform infrared (FTIR) spectrophotometer. A Bruker Avance DRX300 NMR spectrometer was used to record proton nuclear magnetic resonance (^1H-NMR) spectra in DMSO. Signal multiplicities are defined as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m), and chemical shifts are expressed in δ units (ppm) with respect to tetramethylsilane (TMS) as the internal standard. An Agilent 6320 ion-trap mass spectrometer (Chromein, Pune) was used to acquire mass spectra. Synthetic scheme was followed as shown in Figure 2. And derivatives were synthesized as per Table 2.

Procedure for Step I: Synthesis of N1-(1,2,3,4-tetrahydroacridine-9-yl)ethane-1,2-diamine

A mixture of 9-CHLORO-1, 2, 3, 4-TETRAHYDROACRIDINE (2 mmol), 1,2-diaminoethane (12 mmol), KI as a catalyst (0.05 g) and 1-pentanol (5 mL) were mixed and heated to oil bath reflux (140-160 °C) for 12 hours, and the most of the solvent and the unreacted diamine were removed under reduced pressure. The reaction mixture was allowed to cool to room temperature, and then mixed with 50 ml of Dichloromethane and then washing was done with 10% sodium hydroxide solution (1 × 50 mL) and water (2 × 40 mL). The organic layer was dried over Na2SO4, filtered, vacuum evaporator was used to remove solvent and get solid residue, and the residues' purification was done using column chromatographic technique by elution method. Mobile phase used was DCM: methanol = 9:1. Colour of product was brown[16,17].

Procedure for Step II: Synthesis of Target Compounds of Series 1 (S2M1-S2M8)

N1-(1,2,3,4-tetrahydroacridine-9-yl)ethane-1,2-diamine (1mmol) and respective aldehydes (1mmol) were stirred independently in toluene at Room temp, followed by addition of mercapto acetic acid (Thioglycolic acid) (1mmol) and refluxed for 5-6 hrs at temp 120 degree in oil bath. Thin layer chromatographic techniques was used to track the completion of the reaction using DCM:methanol in the ratio of 9:1 as a solvent system. Toluene was removed by vacuum evaporator and then fragment separation was done using column chromatography. Product obtained were analysed for spectral characterization.

Biological Evaluation

After completion of the synthesis, the designed molecules were subjected to Acute Oral Toxicity studies on male Wister rats.

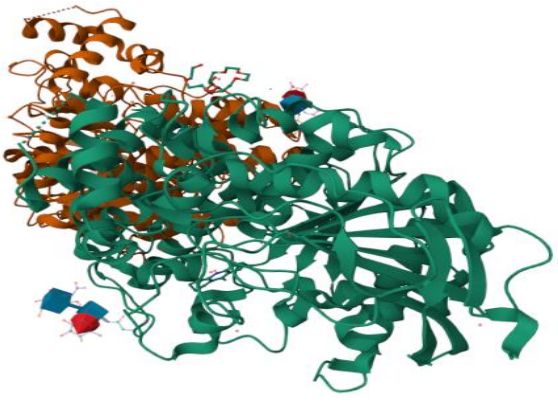


Figure 1: 3D structure of AChE Protein with PDB id 7XN1 from RCSB Protein Data Bank

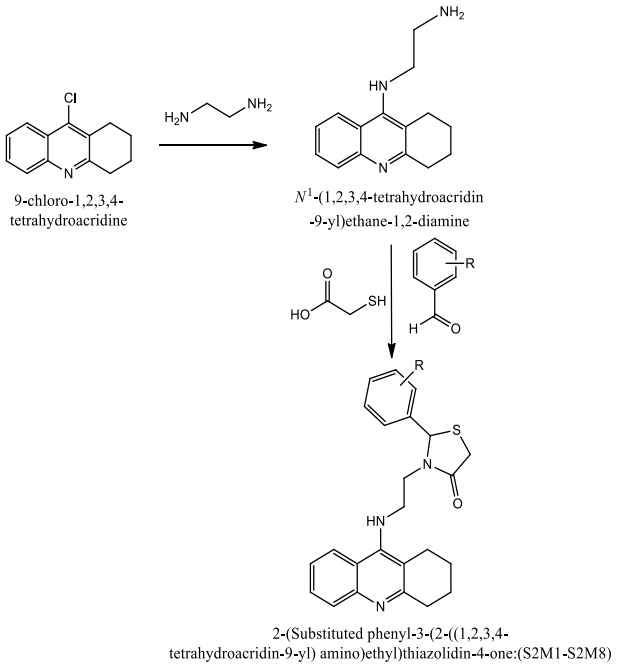


Figure 2: The scheme for synthesis of target molecules (S2M1-S2M8)

Table 1: Comparison between standard values and values of retrieved protein for validation of protein selected for docking study

Parameters	Details	Standards
Protein Id	7XN1	-
Method of experiment	X-RAY Diffraction	-
Classification	Hydrolase	-
Mutation	No	No
Resolution	2.85 Å	Near about 3.00 Å

Table 2: The substituents for the target compounds

CODE	-R
S2M1	-H
S2M2	4- F
S2M3	4-Cl
S2M4	4-OCH3
S2M5	3-Cl
S2M6	4-NO2
S2M7	4-OH
S2M8	3-OH

Referring to the molecular docking evaluation results, only top 4 molecules were subjected to biological evaluation as cholinesterase inhibitors. The biological evaluation involved estimation of biochemical Parameter i.e. Acetylcholinesterase inhibitory activity.

After Animal Ethical committee approval (letter no. MCP/IAEC/011/2025), for biological evaluation Wister male rats were procured and kept for observation for 7 days in which they were fed normally. Then they were grouped in to seven groups namely Normal control, Scopolamine induced control and Tacrine standard control and four test compound groups containing 6 animals in each group. After 14 days of treatment with test compounds and standard, the animals were sacrificed and their brain homogenate were used for estimating the acetylcholinesterase concentration using Ellman's method[18-21].

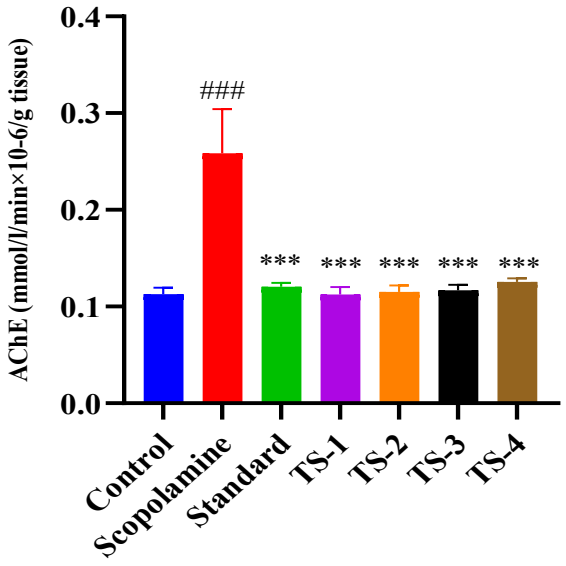
RESULTS AND DISCUSSION

Design

The designing part of research involved linking the two scaffolds namely Tacrine and Thiazolidine having proven biological activity against neurodegeneration. The designed molecules were subjected to *in-silico* evaluation for ADMET, Molecular docking and dynamics.

ADMET Study

The web server pkCSM was preferred to predict the various physicochemical properties and therapeutic potential by testing drug likeliness of all proposed compounds. Based on their calculated physicochemical and lipophilicity values, the collection of target compounds (S2M1–S2M8) cooperated well with well-known drug-likeness filters. As water solubility plays a critical role in bioavailability, Log S values were also evaluated, indicating that these compounds are soluble as can be seen in Table 3.



Graph 1: Effects of oral administration of treatments on brain AChE concentration (mmol/l/min×10^{-6}/g tissue)

Molecular Docking Study

Molecular docking with AutoDock Vina v1.2.6 was performed for the target compound against AChE (PDB ID: 7XN1). Docking outcomes were evaluated based on binding affinity (docking score) and the nature of interactions at the enzyme's active site (see Table 6). The 2D and 3D interaction maps for compound S2M1 docked with 7XN1 are shown in Figures 3 and Figure 4. The top docking score for S2M1 against AChE (7XN1) was -9.1 kcal/mol, as determined by Vina, indicating a strong predicted interaction with the active site residues.

MD Simulation

After 100 ns, the simulation analysis demonstrates that the compound S2M1 interacts with the protein molecule in a nearly constant phase until the simulation is finished. By computing the Root Mean Square Deviation (RMSD), the docked complexes' stability and structural changes were evaluated. Favourable stability of the protein-ligand complex is usually indicated by low fluctuations and a stable RMSD of the backbone atoms. AChE, the co-crystal, and the chemical S2M1 all achieved equilibrium and stability over the course of the molecular dynamics simulation. As seen in Figures 5 and 6, this suggests that the docked complexes have stabilized and are interacting well with the protein residues.

The results of molecular docking and molecular dynamics simulation revealed that the compound S2M1 interacts with the binding pocket forming a Hydrophobic Interactions with TYR72A (3.17), TRP86A (3.64), TRP286A (3.45), TRP286A (3.95), PHE297A (3.34), PHE338A (3.87), TYR341A (3.28), TYR341A (3.22), TRP439A (3.33). Also, shows Hydrogen Bonding with: TYR337A (2.84) and π-Stacking with TYR337A (3.96).Thus indicating good affinity and interaction between target molecule S2M1 and Protein 7NX1.

Synthesis

Melting point, FTIR, 1 H-NMR, mass spectrometry and elemental analysis characterized all the synthesized molecules. The synthesized molecules showed single peak during chromatographic assessments.

Step I product: N1-(1,2,3,4-tetrahydroacridine-9-yl)ethane-1,2-diamine

Yield 69.2%, Brown solid; FTIR (KBr) v/cm-1: 3372(NH str.), 2943(C-H str, aro.), 2870(C-H str, ethylene CH2),

1640(C=N), 1310(Aro. C-C str), 1219(C-N str), 760(NH wag); 1H NMR (400MHz, CDCl3), d [ppm]: 1.63-1.82(m,4H, CH2CH2), 1.83-2.08(t,2H,CH2), 2.55-3.16(t,2H,CH2), 7.38-7.42(tt,1H,CH), 7.74-7.84(tt,1H,CH), 8.00-8.05(dd,1H,CH), 8.24-8.27(dd,1H,CH), 3.89-3.96(t,2H,CH2), 2.46-2.50(t,2H,CH2), 3.16(t,NH), 3.97-4.17(t,2H,NH2); C15H19N3 ; calculated: C, 74.65; H, 7.94; N, 17.41; (M+1): 242.16.

Target Molecule S2M1: 2-phenyl-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl)thiazolidin-4-one

Yield 65.2%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3351 (NH str), 2927 (C-H str-aro.), 2664(C-H str (CH_2-CH_2), 1667(C=O ring str.), 1426(Aro.C-C str), 1181(C-N str.), 779 (NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]: 1.88-1.90 (m,4H, CH_2CH_2), 2.39-2.55(t,2 H, CH_2), 2.96-3.23(t,2H, CH_2), 3.08-3.34(t,2H, CH_2), 3.49-3.59(t,2H, CH_2), 3.85(s,2H, CH_2), 3.86-3.98(t,NH), 5.85(s,1H), 7.30-7.33(t,3H, CH), 7.36-7.44(d,1H, CH), 7.46-7.57(d,1H), 7.59-7.77(tt,1H), 7.86-7.88(tt,1H), 7.90-7.92(dd,1H), 8.23-8.25(dd,1H)N; $C_{24}H_{25}N_3OS$; calculated: C, 71.43; H, 6.24; N, 10.41; O, 3.96; S, 7.95; (M+1): 404.18.

Target Molecule S2M2: 2-(4-fluorophenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino) ethyl) thiazolidin-4-one

Yield 55.8%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3260(NH str), 2940(C-H str (aro), 2870(C-H str(ethelene), 1666(C=O ring str), 1509(Aro C-C str), 1223(C-F str), 760(NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]:1.78-1.95(m,4H, CH_2CH_2), 2.49-2.50(t,2H, CH_2), 2.96-3.03(t,2H, CH_2), 3.08-3.14(t,2H, CH_2), 3.49-3.59(t,2H, CH_2), 3.65(s,2H, CH_2), 3.86-3.97(t,NH), 5.89(s,1H, CH), 7.14-7.22(d,2H, CH_2), 7.39-7.43(d,2H, CH_2), 7.48-7.52(tt,1H,CH), 7.79-7.85(tt,1H,CH), 7.86-7.88(dd,1H,CH), 8.27-8.29(dd,1H,CH); $C_{24}H_{24}FN_3OS$; calculated: C, 68.38; H, 5.74; F, 4.51; N, 9.97; O, 3.80; S, 7.61; (M+1): 422.17.

Target Molecule S2M3: 2-(4-chlorophenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl) thiazolidin-4-one

Yield 55.8%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3332(NH stretch), 2927(C-H stretch (aromatic), 2870(C-H stretch (ethylene), 1666(C=O ring stretch), 1521(Aromatic C-C stretch), 1089(Aryl C-Cl stretch), 756(NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]:1.56-1.80(m,4H, CH_2CH_2), 2.49-2.55(t,2H,CH_2), 2.98-3.03(t,2H, CH_2), 3.08-3.12(t,2H, CH_2), 3.57-3.64(t,2H, CH_2), 3.71(s,2H, CH_2), 3.86-3.89(t,NH), 5.89(s,1H), 7.25-7.35(d,2H,CH_2), 7.37-7.40(d,2H, CH_2), 7.76-7.80(tt,1H,CH), 7.79-7.85(tt,1H,CH), 7.88-7.90(dd,1H,CH), 8.25-8.27(dd,1H,CH); $C_{24}H_{24}ClN_3OS$; calculated: C, 65.81; H, 5.52; Cl, 8.09; N, 9.59; O, 3.65; S, 7.32; (M+2): 439.13.

Target Molecule S2M4: 2-(4-methoxyphenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl) thiazolidin-4-one

Yield 52.4%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3331(NH str), 2934(C-H str(aro), 2850(C-H str (ethylene), 1667(C=O ring str), 1514(Aro C-C str), 1256(Aryl C-O-C), 1112, 1176(Aryl C-O str), 841(NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]: 1.79-2.49(m,4H,CH_2CH_2), 2.49-2.50 (t,2H, CH_2), 2.99-3.04(t,2H, CH_2), 3.06-3.12(t,2H, CH_2), 3.23-3.26(t,2H, CH_2), 3.63(s,2H, CH_2), 3.73(s,3H, OCH_3), 3.74-3.82(t,NH), 5.81(s,1H,CH), 6.85-6.87(d,2H), 7.24-7.26(d,2H), 7.47-7.50(tt,1H), 7.77-7.81(tt,1H), 7.89-7.91(dd,1H), 8.26-8.28(dd,1H); $C_{25}H_{27}N_3O_2S$; calculated: C, 69.26; H, 6.28; N, 9.69; O, 7.38; S, 7.40; (M+1): 434.19.

Target Molecule S2M5: 2-(3-chlorophenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl) thiazolidin-4-one

Yield 42.4%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3274(NH str), 3061 (C-H str (aro), 2925(C-H str(ethylene), 1671(C=O ring str), 1579(Aro C=C), 1403(Aro C-C stretch), 1196(Aryl C-Cl str), 757(NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]: 1.79-1.86(m,4H, CH_2CH_2), 2.49-2.56(t,2H,CH_2), 2.94-2.95(t,2H, CH_2), 3.01-3.08(t,2H, CH_2), 3.56-3.60(t,2H, CH_2), 3.70(s,2H, CH_2), 3.86-3.94(t,NH), 5.86(s,1H,CH), 7.28-7.30(d,1H,CH), 7.37-7.40(t,1H,CH), 7.66-7.68 (d,1H,CH), 7.70(s,1H,CH), 7.81-7.84 (tt,1H, CH), 7.88-7.92(tt, 1H,CH), 8.20-8.22(dd,1H,CH), 8.39-8.41(dd,1H,CH); $C_{24}H_{24}ClN_3OS$; calculated: C, 65.83; H, 5.51; Cl, 8.05; N, 9.56; O, 3.67; S, 7.33; (M+2): 439.13.

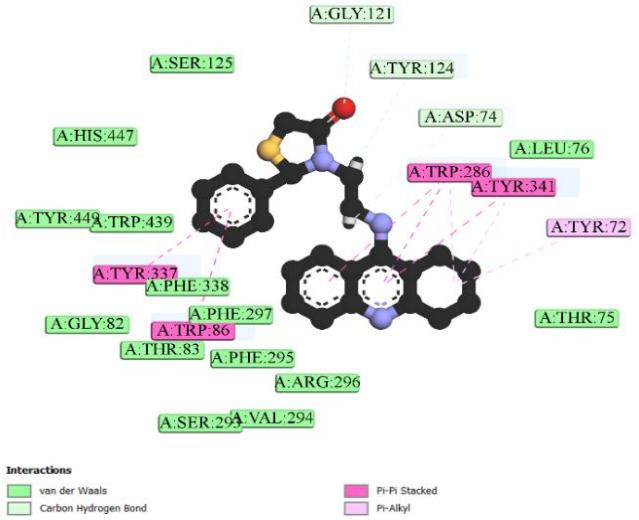


Figure 3: 2D Image of interactions between target molecule S2M1 and protein 7XN1

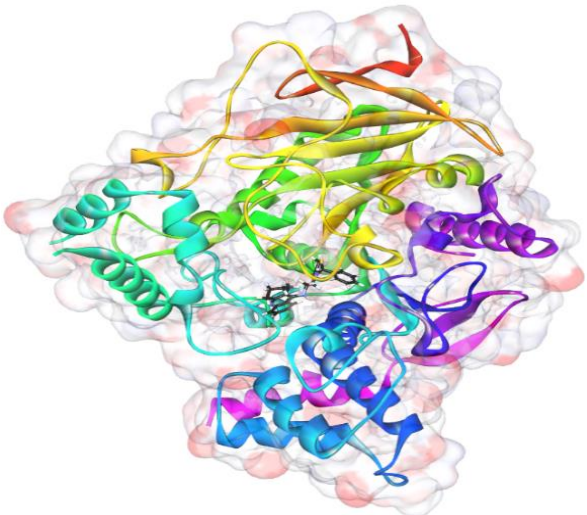


Figure 4: 3D Image of interactions between target molecule S2M1 and protein 7XN1

Table 3: The physicochemical, pharmacokinetics and drug likeness of target compounds (S2M1-S2M8)

Comp.	Physicochemical Properties				Lipophilicity	Solubility	P'kinetics		Drug likeness				
	MW	RB	HBA	HBD	cLog P	Log S	GI	BBB	Lipinski	Ghose	Veber	Egan	Muegge
S2M1	403.17	5	2	1	3.23	-3.56	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M2	421.17	5	3	1	3.30	-3.72	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M3	437.13	5	2	1	3.42	-3.15	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M4	434.16	6	3	1	3.64	-3.63	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M5	437.13	5	2	1	3.47	-3.15	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M6	449.40	6	4	2	3.39	-3.22	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M7	419.17	5	3	2	2.79	-3.42	High	Yes	Yes	Yes	Yes	Yes	Yes
S2M8	419.17	5	3	2	3.05	-3.42	High	Yes	Yes	Yes	Yes	Yes	Yes

MW: Molecular weight, HBA: number of hydrogen bond acceptors, HBD: number of hydrogen bond donors, RB: number of rotatable bonds, cLog P: Partition coefficient, Log S: Water solubility, GI: Gastrointestinal absorption, BBB: Blood-brain barrier permeant. Solubility class: -4 < soluble < -2

Target Molecule S2M6: 2-(4-nitrophenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl) thiazolidin-4-one

Yield 42.4%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3354(NH str), 2924(C-H str(aro), 2850(C-H str(ethylene), 1664(C=O ring str), 1522 with 1346(Aro. Nitro str), 1447(Aro C-C str), 832(NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]: 1.60-1.80(m,4H,CH_2CH_2), 2.49-2.50(t,2H, CH_2), 2.98-3.15(t,2H, CH_2), 3.18-3.26(t,2H,CH_2), 3.63-3.67(t,2H, CH_2), 3.81(s,2H, CH_2), 3.90-3.94 (t,NH), 6.06(s,1H,CH), 7.45-7.49(tt,1H,CH), 7.60-7.62(d,2H,CH), 7.76-7.80(tt,1H,CH), 7.86-7.88 (dd,1H,CH), 8.13-8.15(d,2H,CH), 8.25-8.27 (dd,1H,CH); $C_{24}H_{24}N_4O_3S$; calculated: C, 64.27; H, 5.39; N, 12.49; O, 10.70; S, 7.15; (M+1): 449. 16.

Target Molecule S2M7: 2-(4-hydroxyphenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl) thiazolidin-4-one

Yield 50.4%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3428(NH str), 3321(OH str), 2917(C-H str(aro), 2851(C-H str (ethylene), 1611(C=O ring str), 1541, 1428(Aro C-C str), 895 (NH wag); ^{1}H NMR (400MHz, $CDCl_3$), d [ppm]: 1.78-1.95(m,4H, CH_2CH_2), 2.49-2.50(t,2H, CH_2), 2.96-3.03(t,2H, CH_2), 3.08-3.14(t,2H, CH_2), 3.49-3.59(t,2H, CH_2), 3.65(s,2H, CH_2), 3.86-3.97 (t,NH), 5.35(s,1OH), 5.89(s,1H, CH_2), 6.64-6.66 (d,2H,CH,CH), 7.48-7.52(tt,1H,CH), 7.79-7.85 (tt,1H, CH), 7.86-7.88 (d, 2H, CH, CH), 7.86-7.88 (dd,1H,CH), 8.27-8.29(dd,1H,CH) ; $C_{24}H_{25}N_3O_2S$; calculated: C, 68.71; H, 6.01; N, 10.02; O, 7.63; S, 7.64; (M+1): 420.17.

Target Molecule S2M8: 2-(3-hydroxyphenyl)-3-(2-((1,2,3,4-tetrahydroacridin-9-yl)amino)ethyl) thiazolidin-4-one

Yield 52.4%, yellow-Brown solid; FTIR (KBr) v/cm-1: 3403(NH), 3378(OH str), 2977(C-H str(aro), 2824(C-H str(ethylene), 1623(C=O ring str, 1541, 1403(Aro C-C str), 897(NH wag); 1H NMR (400MHz, CDCl3), d [ppm]: 1.79-1.86(m,4H, CH2 CH2), 2.49-2.56(t,2H, CH2), 2.94-2.95(t,2H, CH2), 3.01-3.08(t,2H, CH2), 3.56-3.60(t,2H, CH2), 3.70(s,2H, CH2), 3.86-3.94 (t,1NH), 5.36(s,1OH), 5.86 (s,1H, CH), 6.98- 7.00(d,1H,CH), 7.00-7.27(t,1H,CH), 7.30-7.68(d,1H,CH), 7.70 (s,1H,CH), 7.81-7.84(tt,1H,CH), 7.88-7.92(tt,1H,CH), 8.20-8.22(dd,1H,CH), 8.39-8.41(dd,1H,CH); C24H25 N3O2S; calculated: C, 68.71; H, 6.01; N, 10.02; O, 7.63; S, 7.64; (M+1): 420.17.

Biological Evaluation

The acetylcholinesterase inhibitory activity data of the some target compounds is as per Table 5 and Graph 1.

The concentration of AChE enzyme in normal control group was found to be 0.1127/mmol/l /min×10^{-6}/g tissue. There was significant (###p<0.001) increase in brain AChE concentration in the scopolamine-treated group as compared to normal control group and the increase was found to be 53.28%. Treatment with standard and all the derivatives (4mg/kg, p.o.) significantly (***p<0.001) reduced the brain AChE concentration (mmol/l/min×10^{-6}/g tissue) as compared to scopolamine-treated animals and the reduction was found to be 53.28 %, 56.41%, 55.37%, 54.79%, and 44.71 % respectively. Indicating that target

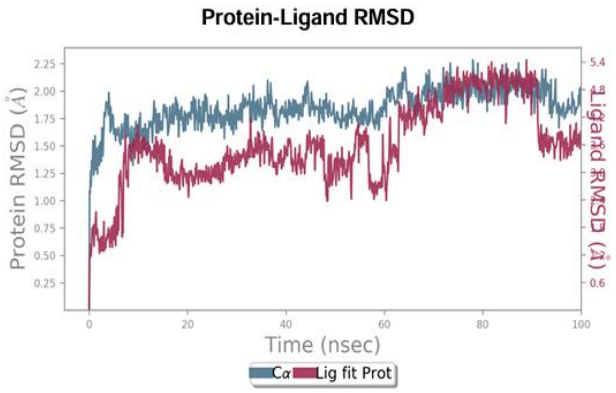


Figure 5: MD simulation analysis of 100 ns trajectories of RMSD of Cα backbone of 7XN1 and target molecule S2M1

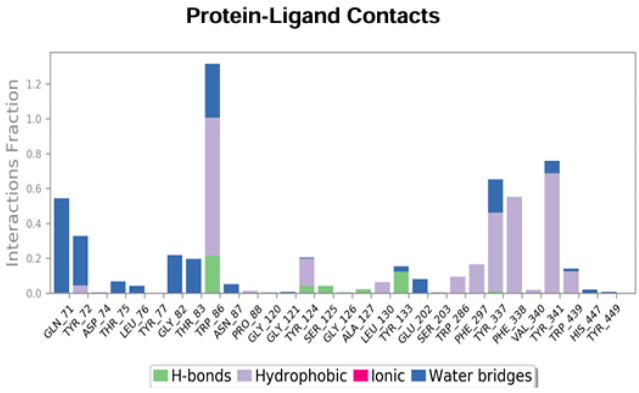


Figure 6: Bar graph of Protein-ligand contacts of (A) 7XN1_S2M1 showing interaction fraction of amino acid residues over the period of simulation

Table 4: Molecular docking interaction between Synthesized Molecules with 7XN1

Code	R	Docking score (kcal/mol)
S2M1	-H	-11.32
S2M2	4- F	-10.09
S2M3	4-Cl	-9.909
S2M4	4-OCH3	-8.963
S2M5	3-Cl	-9.381
S2M6	4-NO2	-10.211
S2M7	4-OH	-9.255
S2M8	3-OH	-9.046
THA	--	-9.166

Table 5: Assay results for Anticholinesterase inhibition activities of synthesized compounds

Comp. code	-R	Animal study code	% reduction in AChE concentration
S2M1	-H	TS-1	56.41%
S2M2	4- F	TS-3	55.37%
S2M3	4-Cl	TS-4	54.79%
S2M6	4-NO2	TS-2	44.71 %
Standard	Tacrine	Standard	53.28 %

compound S2M1 has shown marked inhibition of AChE as compared to other molecules. Inhibition seems to be reduced in an order of compound with -NO2 substitution, -F substitution and -Cl substitution.

CONCLUSION

Finding chemicals that inhibit the cholinesterase enzyme to prevent neurodegeneration is the goal of this research. Tacrine-thiazolidine merging compounds were therefore created and described for this reason. All the designed compounds were synthesized successfully and evaluated for Acetylcholinesterase inhibition. In-silico study predicted the good binding affinity of all the designed compounds towards the target protein AChE (PDB id: 7XN1). But amongst all compound S2M1 showed best affinity and interaction with the target, followed by electron withdrawing group substituted derivatives. When actual Biological evaluation using animals was conducted, it showed good inhibition by S2M1. Apart from it, results showed presence of electron withdrawing groups (NO_2, F, Cl) on the 4-substituted benzene ring attached to thiazolidine, significantly increases cholinesterase inhibitory activity. The most promising compound as inhibitor of cholinesterase S2M1 (4-H) compound, followed by electron withdrawing substitution. It was concluded that the compound S2M1, being the most potent among those tested, holds promise as a drug candidate, provided it can be validated in in vivo studies.

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