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Exploring [1, 1'-bianthracene]-9, 9', 10, 10'-tetrone from *Senna hirsuta* as a potential acetylcholinesterase inhibitor for alzheimer's disease treatment: An *In-Silico* investigation

Kalpana Rawat[1], Disha Tewari[2], Amisha Bisht[3], Subhash Chandra[1*]
[1] Department of Botany, Computational Biology & Biotechnology Laboratory, Soban Singh Jeena University, Almora, Uttarakhand, India
[2] Department of Biotechnology, Kumaun University, Bhimtal, Uttarakhand, India
[3] Department of Botany, Pt. Badridutt Pandey Campus Bageshwar, Soban Singh Jeena University, Almora, Uttarakhand, India

Abstract
Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with the dysfunction of acetylcholinesterase (AChE), a key enzyme in neurotransmitter regulation. This study focused on constructing a phytochemical library of 71 compounds isolated from *Senna hirsuta*. Using a systematic in silico approach, including molecular docking and ADMET profiling, [1,1'-Bianthracene]-9,9',10,10'-tetrone (CID: 6737485) emerged as a promising candidate. The compound demonstrated a strong binding affinity with AChE, achieving a binding energy of -12.4 kJ/mol, which outperformed the reference molecule (-10.6 kJ/mol), indicating significant therapeutic potential. Drug-likeness and ADMET assessments further support its potential as a novel compound for AD treatment. Experimental validation is essential to confirm its efficacy and advance its development as a treatment for AD.

Keywords: Alzheimer's disease, acetylcholinesterase inhibitors, molecular docking, ADMET

Introduction
Alzheimer's disease (AD) is a progressive neurological disorder characterized by gradual memory loss, declining cognitive abilities, diminished physical function, and ultimately death due to brain cell degeneration [1]. Projections indicate that by 2050, AD will affect one in every 85 people worldwide [2], creating a significant social and economic burden [3]. The disease results from dementia caused by impaired nerve impulse transmission due to neurodegeneration. Therefore, effective treatments aim to enhance nerve transmission. One approach to improving nerve function is to inhibit acetylcholinesterase (AChE), an enzyme that reduces acetylcholine (ACh) levels in the synaptic cleft. By suppressing AChE activity, higher ACh concentrations are maintained, improving electrical impulse conduction. Anticholinesterase inhibitors can slow AD progression, enhance cognitive function, and improve the quality of life for patients [4]. Numerous natural and synthetic AChE inhibitors are being explored in clinical trials for AD treatment, with donepezil being a widely used drug for treating mild-to-moderate symptoms [5]. Crystal structures of donepezil bound to both Torpedo californica AChE (TcAChE) and human AChE (hAChE) have revealed insights into the enzyme-inhibitor interaction [6, 7]. Donepezil binds to the active site of AChE, forming stable interactions that inhibit the enzyme. AChE also accelerates amyloid fibril formation through interaction with amyloid beta (Aβ) peptide at its peripheral anionic sites [8, 9], and donepezil has been shown to reduce Aβ aggregation by interacting with these sites [10]. Although donepezil provides cognitive and functional improvements in the early stages of treatment, its effectiveness wanes in later stages [11, 12] highlighting the need for novel therapeutic options. In this study, we focused on plant-based compounds, as traditional medicine has long recognized the therapeutic potential of plant-derived substances, often with fewer side effects compared to synthetic drugs. We constructed a detailed phytochemical library of 71 compounds isolated from *Senna hirsuta*, a flowering plant in the Fabaceae family, native to Central and South America but also naturalized in various regions. By employing a systematic in silico approach, we utilized molecular docking to predict the binding interactions of these compounds with key targets, ADMET profiling to evaluate their pharmacokinetic properties, molecular dynamics (MD) simulations to assess the stability of these interactions, and MM/PBSA binding free energy calculations to estimate the binding affinity. Through these methods, we aimed to identify promising therapeutics for Alzheimer's disease (AD), advancing the search for more effective treatments for this complex neurodegenerative disorder.

Methodology
Construction of Phytochemical library
To construct the phytochemical library, *Senna hirsuta*, a plant from the Fabaceae family, was selected due to its long history in traditional medicine and its potential therapeutic properties. Native to Central and South America, *Senna hirsuta* is known to contain bioactive compounds with various medicinal benefits. A thorough literature review was conducted to identify the phytochemicals isolated from this plant. From the collected data, 71 compounds, including flavonoids, alkaloids, and other secondary metabolites, were chosen for further analysis. This library of 71 phytochemicals was then used for systematic in silico screening to identify potential acetylcholinesterase (AChE) inhibitors for the treatment of Alzheimer's disease.

Receptor and ligand preparation
The resolved crystal structure of Acetylcholinesterase (AChE) enzyme (PDB ID: 7D9P) was retrieved from the Protein Data Bank (https://www.rcsb.org). For protein preparation, all water molecules, ions, and nonspecific molecules were removed from the protein molecule using

PyMOL software. Further, the addition of hydrogen atoms to the receptor molecule was carried out by using the MG Tools of AutoDock Vina software. The structure of the protein was saved in PDB format for further analysis. The SDF format of 3D structure of all 71 compounds and the inhibitor already bound with crystal structure of protein i.e. (2S)-2-[[4-fluoranyl-1-[(2-fluorophenyl) methyl] piperidin-4-yl] methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one or H0R were downloaded from PubChem server. Then OpenBabel (version 2.3.1) [13] was used to convert SDF format files to PDB format. For the preparation of the ligands, hydrogens were added to all the compounds and energy minimization was done with UFF force field using conjugate-gradient algorithm by PyRx software. Later all the compounds were converted into pdbqt format.

Molecular docking

To find potential candidates against Acetylcholinesterase (AChE), molecular docking was carried out to screen ligands with high affinity at the binding site of enzyme by pyVSvina, a python-based tool for Virtual Screening of a library of ligands against a protein receptor using Autodock Vina. For docking, a three-dimensional grid box was set into X = 13.9, Y = 43.2, and Z = 27.2 grid points, and the grid spacing was 23.1x 16.3x 21.0 Å for X, Y and Z coordinates respectively. The number of exhaustiveness was set to eight for predicting the accurate result. Throughout the molecular docking process, the ligand molecules were flexible and the receptor was kept as rigid. Finally, the result in the form of binding energy was extracted from the software. The best confirmations with the low binding energy or docking score as compared to HOR were chosen for further analysis.

Visualization

The 2D interactions of protein-ligand complexes including hydrogen bonds and the bond lengths were analyzed by using PLIP (Protein Ligand Interaction Profiler) online web-server [14] while 3D visualization analysis studies were performed by using PyMol molecular visualization tool version 2.1.0 [15].

Drug-likeness and ADMET analysis

The compounds that were finalized by Autodock Vina after virtual screening were further proceeded to predict their drug-likeness and extensive ADMET analysis. Drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis are crucial steps to evaluate the potential of compounds for further development. For the compounds selected from the *Senna hirsuta* phytochemical library, drug-likeness was assessed using various parameters such as molecular weight, lipophilicity (LogP), hydrogen bond donors and acceptors, and the presence of any structural alerts for toxicity. These properties help predict whether the compounds are likely to be orally bioavailable and to have favorable pharmacokinetic properties. The drug-likeness and ADMET properties were evaluated through the online web-server SwissADME [16].

Result & discussion

Molecular docking

Before performing the molecular docking, validation of the protocol was done by Re-docking the reference molecule (HOR) into the active site of acetylcholinesterase (AChE) enzyme. The result indicated that the docked HOR was completely superimposed with co-crystallized HOR in PDB. The docked HOR showed interaction with the same amino acid residues by hydrogen and hydrophobic bonds as found in the crystal structure in PDB (Figure 1).

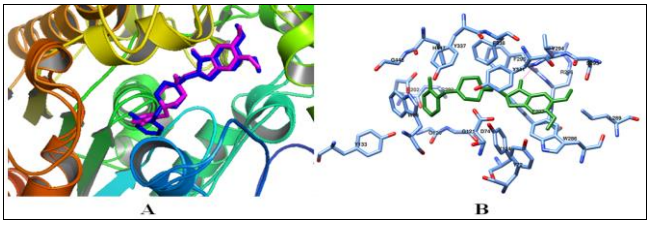

Fig 1: (A) Three-dimensional visualization of the active binding site of the AChE protein and (B) detailed illustration of the residues participating in active site interactions.

After that, to screen novel compounds against AChE, the molecular docking of 71 compounds was performed with target protein AChE using pyVSvina. The docking results are ranked based on binding energies. Out of the 71 compounds, [1,1'-Bianthracene]-9,9',10,10'-tetrone (CID: 6737485) was identified as the most promising candidate based on its low binding energy and high binding affinity, as presented in the table 1. The compound exhibited a binding energy of -12.4 kJ/mol, indicating stronger binding affinity and stability with AChE compared to the reference compound HOR (-10.6 kJ/mol). Consequently, [1,1'-Bianthracene]-9,9',10,10'-tetrone advanced to drug-likeness and toxicity prediction analysis.

Table 1: Binding energy of the reference molecule and the top hit compound identified through molecular docking.

	Ligands	Binding Energy (kcal/mol)
Reference molecule or already bound inhibitor of receptor	(2S)-2-[[4-fluoranyl-1-[(2-fluorophenyl) methyl] piperidin-4-yl] methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one	-10.6
Novel compounds screened	[1,1'-Bianthracene]-9,9',10,10'-tetrone	-12.4

Visualization

PLIP (Protein Ligand Interaction Profiler) online web-server was used to visualize the protein–ligand interactions [14]. This tool helped in identifying and mapping the specific binding interactions, providing insights into the molecular interactions that contribute to the stability and affinity of the protein-ligand complex.

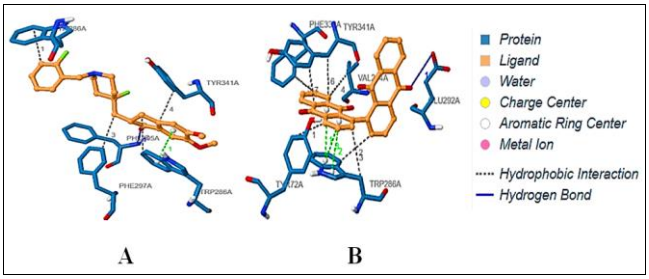

Fig 2: Protein-ligand interactions illustrating hydrogen bonds and hydrophobic interactions between (A) HOR-AChE and (B) the hit compounds with Ache

The docked pose of the best hit compound and reference molecule i.e., HOR with AChE is shown in Figure 2.

Protein-ligand interactions, including hydrogen bonds and hydrophobic interactions between HOR-AChE and the hit compounds, are summarized in Table2.

Table 2: Protein-ligand interactions illustrating hydrogen bonds and hydrophobic interactions between HOR-AChE and the hit compounds with Ache

Hydrophobic Interactions						Hydrogen Bonds				
Protein –Ligand Complex	**Residue**	**AA**	**Distance**	**Ligand Atom**	**Protein Atom**	**Residue**	**AA**	**Distance H-A**	**Distance D-A**	**Donor Angle**
AChE-HOR	86A	TRP	3.79	5027	782	295A	PHE	2.05	3.03	159.02
	286A	TRP	3.84	5010	2581					
	297A	PHE	3.55	5008	2695					
	341A	TYR	3.43	5011	3084					
AChE-[1,1'-Bianthracene]-9,9',10,10'-tetrone	72A	TYR	3.73	5022	648	292A	GLU	3.46	4	119.25
	286A	TRP	3.67	5009	2576					
	286A	TRP	3.99	5031	2571					
	294A	VAL	3.91	5027	2654					
	338A	PHE	3.85	5028	3055					
	341A	TYR	3.74	5027	3081					
	341A	TYR	3.31	5024	3084					

Drug-likeness and ADMET properties

The results of drug-likeness and ADMET properties are presented in Table 3. The drug-likeness and ADMET analysis indicate that [1,1'-Bianthracene]-9,9',10,10'-tetrone has favorable properties that make it a promising candidate for future Alzheimer's disease research. These analyses suggest that the compound possesses the necessary characteristics, such as optimal absorption, distribution, metabolism, excretion, and toxicity profiles, making it suitable for further investigation as a potential therapeutic agent for Alzheimer's disease.

Table 3: Drug-likeness and ADMET properties of [1, 1'-Bianthracene]-9, 9', 10, 10'-tetrone.

[1,1'-Bianthracene]-9,9',10,10'-tetrone		
Physicochemical Properties	Formula	$C_{28}H_{14}O_4$
	Molecular weight	414.41 g/mol
	Num. rotatable bonds	1
	Num. H-bond acceptors	4
	Num. H-bond donors	0
Pharmacokinetics	Human Intestinal Absorption	HIA+
	Caco-2 Permeability	Caco2+
	CYP450 2C9 Substrate	Non-substrate
	CYP450 2D6 Substrate	Non-substrate
	CYP450 3A4 Substrate	Non-substrate
	CYP450 2D6 Inhibitor	Non-inhibitor
	CYP450 2C19 Inhibitor	Non-inhibitor
	CYP450 3A4 Inhibitor	Non-inhibitor
	CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
	AMES Toxicity	Non-AMES toxic
	Carcinogens	Non-carcinogens
Drug likeness	Lipinski	Yes; 0 violation
	Ghose	Yes
	Veber	Yes

Conclusion

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder, highlighting the urgent need to discover effective drug candidates for its treatment. Bioinformatics tools offer a promising approach to accelerate drug discovery efforts against this challenging disease. In this study, we aimed to identify novel acetylcholinesterase (AChE) inhibitors from compounds derived from *Senna hirsuta* using a comprehensive in silico workflow. A library of 71 compounds was constructed and subjected to molecular docking, drug-likeness evaluation, and ADMET analysis. The findings suggest that [1,1'-Bianthracene]-9,9',10,10'-tetrone is a promising candidate for AChE inhibition. However, further *in vitro* and *in vivo* studies are essential to validate its pharmacological effects and therapeutic potential against AD.

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