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RESEARCH ARTICLE

Novel Phenolic Hydrazide–Hydrazones as Multi-Target Anti-Alzheimer Agents: Docking and ADMET Studies

Ankit Agnihotri*, Reema Sinha, Abhishek Kashyap

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative ailment characterised by increasing cognitive impairment, memory loss and decreased cholinergic neurotransmission. Acetylcholinesterase (AChE) inhibitors are still one of the most efficient therapeutic strategies for symptomatic treatment of AD; nevertheless, the existing medications have significant drawbacks such as unpleasant effects and limited efficiency. Herein, creation and evaluation of a series of new phenolic hydrazide-hydrazone derivatives as possible AChE inhibitors on the basis of molecular docking and ADMET investigations are reported. Ten ligands (A01–A10) were rationally developed from previously described hydrazide–hydrazone pharmacophores with antioxidant and neuroprotective effects. Molecular docking investigations were conducted against crystal structure of AChE protein (PDB ID: 7E3H) using PyRx and AutoDock Vina software . The proposed compounds exhibited good binding affinity towards the active site of AChE with docking scores ranging from −10.4 to −11.8 kcal/mol. Among the synthesised analogues, A04, A06 and A10 showed superior docking scores and favourable interactions with important amino acid residues such as TYR337, TYR341 and GLY122 equivalent to the standard medicine donepezil (-11.9 kcal/mol). ADMET analysis using SwissADME showed adequate drug similarity, favourable pharmacokinetic features and conformity to Lipinski's criterion for majority of the substances. The phenolic moiety may also have antioxidant and neuroprotective benefits. In conclusion, the present investigation reveals that phenolic hydrazide-hydrazone derivatives have potential anti-Alzheimer properties and they might be used as lead compounds for further synthesis, biological assessment and creation of safer and more efficient AChE inhibitors.

Keywords: In-silico studies, Alzheimer's disease, AChE, Hydrazone Derivatives, Drug-Likeness, Drug Design.

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INTRODUCTION

AD is a progressive brain disease for which no treatment exists. It is one of the biggest public health problems of the 21st century. [1] In 2023, there are about 55 million people worldwide with it, and if no new medications are developed, this figure is expected to double by 2050. [2] It accounts for 60 to 80 percent of all cases of dementia. The disease gradually robs memory, language, and reasoning, and the capacity to manage behaviour. [3] Those with it are unable to do basic activities like dressing or eating independently and need a lot of support from caretakers. Alzheimer's disease has a profound impact on families, health systems, and economies around the globe. Dementia is expected to cost the world more than $1.3 trillion in 2019. [4,5] This is predicted to go up a lot in the approaching decades. The position is worsening in developing countries such as India due to the growing aging population. Millions of people are deprived of sufficient neurological care, specialist services, or novel pharmaceutical treatment. Decades of extensive research and several clinical trials later, there's no cure for the disorder and no medicine to change its course. It only goes to show how desperate people are for other therapeutic options. [6] The neuropathological hallmarks of Alzheimer's disease (AD) include the extracellular accumulation of amyloid-beta (Aβ) plaques derived from abnormal processing of the amyloid precursor protein (APP), intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein, neuroinflammation, synaptic dysfunction, and progressive neuronal loss, especially in the hippocampus, entorhinal cortex, and neocortex. [7] A prominent and well-documented pathogenic element is a marked drop in cholinergic neurotransmission in the brains of patients with the disease (Fig. 1), as a consequence of degeneration of cholinergic neurons and a concomitant reduction of acetylcholine (ACh) levels in the synaptic cleft. [8,9]

The cholinergic hypothesis of Alzheimer's disease was developed in the 1970s and 1980s and suggested that the cognitive deficits associated with AD are directly

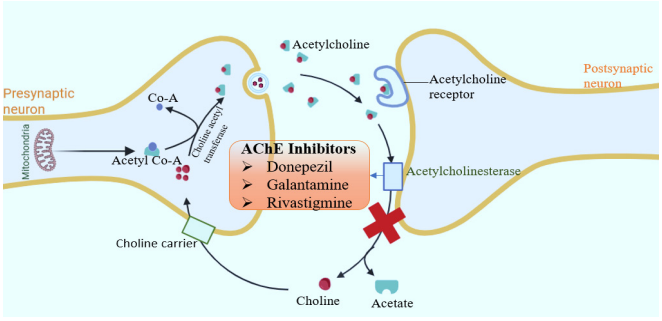


Figure 1: Schematic representation of acetylcholine neurotransmission and the role of acetylcholinesterase (AChE) and its inhibitors.

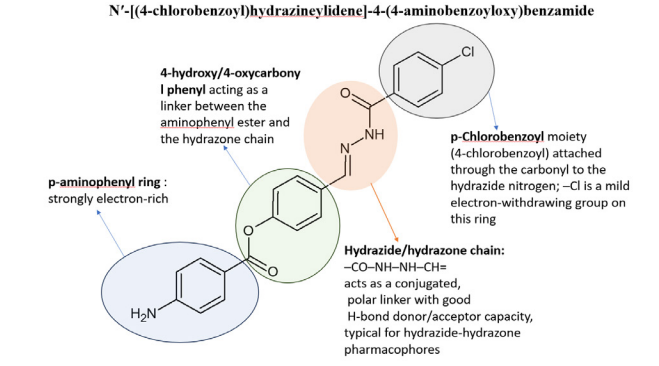
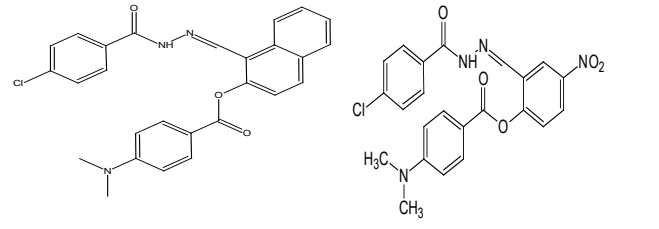


Figure 2: Structure Of Novel Hydrazide-Hydrazone Compounds



1 2

Fig. 3: Images of scaffolds designed based on studies of prior developed Acetylcholine inhibitors

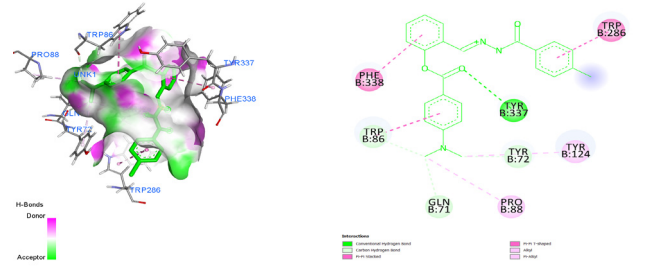


Figure 4 : Interactions of ligand A01

related to the loss of central cholinergic function. This is the main reason why acetylcholinesterase (AChE) inhibitors are the best therapy available. [10,11] These drugs inhibit the action of acetylcholinesterase, which normally breaks down acetylcholine in the synapse very rapidly. This results in a large and persistent level of synaptic ACh. It relieves the cholinergic deficiency and enhances cognitive function. Up to present, only donepezil, rivastigmine, galantamine, and tacrine are approved AChE inhibitors for AD. But tacrine was pulled off the market because it was shown to wreak havoc on the liver. [12] The AChE inhibitors used in the clinic are helpful but have all substantial dangers. They may make you sick, with vomiting, diarrhea, and a slower heart rate. They are also not very excellent at working or healing ailments. They do not protect the brain, modify the course of a disease, and in rare situations may be highly toxic to the liver. These problems have led to the endeavor of making and testing new generations of AChE inhibitors with improved pharmacological profiles, more selectivity, the ability to cross the blood-brain barrier more readily, and the ability to act on numerous pathogenic processes of Alzheimer's disease at the same time. [13] Hydrazides react with carbonyl compounds to provide the pharmacophore -C(=O)-NH-N=CH-. They are a particularly significant family of chemical compounds that have a huge range of pharmacological properties. [14] The hydrazone functional group is a very important building block in medicinal chemistry. It may have a lot of different effects on living organisms. It may be an anticonvulsant, antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, and most importantly, it is a cholinesterase blocker. The phenolic hydrazide-hydrazones are more biochemically significant than other molecules of this structural family, since they include a phenol group with at least one hydroxyl group connected to the aromatic ring. The phenolic hydroxyl groups have been found to possess antioxidant, metal chelating, and neuroprotective properties.[15,16]

Hydrazide-Hydrazone Compounds as Pharmacophores

Chemistry and Synthesis of Hydrazide-Hydrazones

Hydrazide-hydrazones are a class of nitrogen-containing organic compounds, The general structure of the designed hydrazide-hydrazone scaffold is shown in Figure 2. which have an amide nitrogen (hydrazide; R-CO-NH-NH2) and imine nitrogen (hydrazone; R2C=N-NH-) in the same molecular structure. [14] They are often prepared by the reaction of an acid hydrazide with an aldehyde or ketone in acid medium or with catalysts. During the condensation, the nucleophilic addition of the terminal amino group of the hydrazide to the carbonyl molecule occurs. This results in a hemiorthoamide intermediate, which subsequently eliminates water to provide the

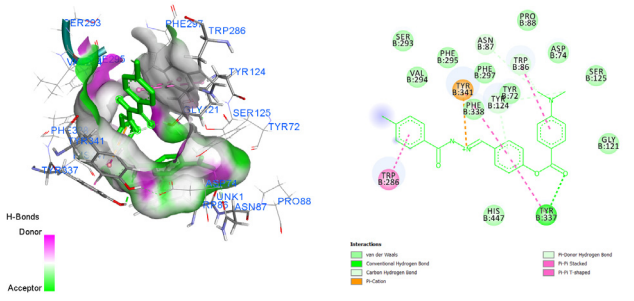


Figure 5: Interactions of ligand A02

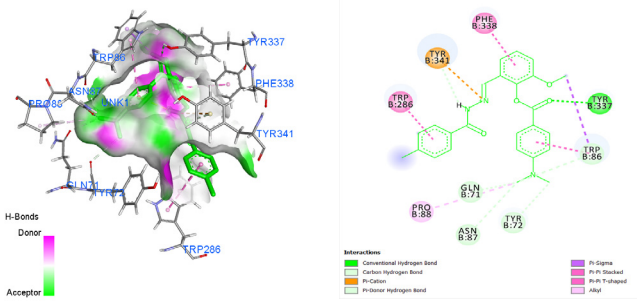


Figure 6: Interactions of ligand A03

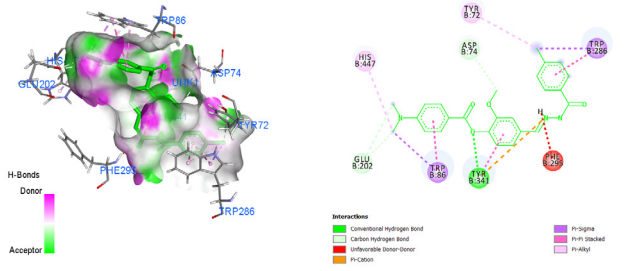


Figure 7: Interactions of ligand A04

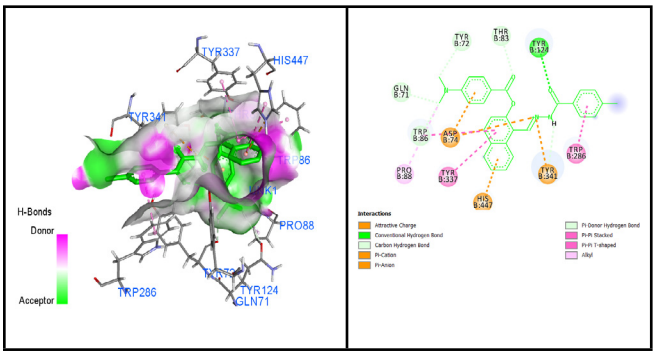


Figure 8: Interactions of ligand A05

stable hydrazone. Hydrazide-hydrazones are particularly valuable intermediates in combinatorial and medicinal chemistry since the reaction is generally straightforward, provides several products, and works with a wide variety of substrates.[15]

The hydrazide-hydrazone scaffold may be utilized to synthesize a myriad of different structures. The hydrazide component (aromatic, heteroaromatic, or aliphatic) and the carbonyl component (aromatic aldehydes, heteroaromatic aldehydes, aliphatic ketones, alpha-keto acids) can be varied to produce libraries of structurally diverse compounds with systematically changing physico-chemical and pharmacological properties. [17] Hydrazide-hydrazones are widely used in medicinal chemistry due to their modifiability and the drug-like property of the scaffold, such as molecular weight, lipophilicity, hydrogen bond donor and acceptor capacity, and rotatable bond count.[14,17]

Biological Activities of Hydrazide-Hydrazones

The literature is replete with information on the pharmacological profile of hydrazide-hydrazone compounds. One of the earliest big uses of pharmaceuticals was in the battle against TB. Isoniazid (hydrazide of isonicotinic acid), a key component of TB treatment, reacts with intracellular NAD and NADP cofactors in Mycobacterium tuberculosis to create hydrazone adducts. [The hydrazide-hydrazones have been researched extensively as anticancer agents. The majority of these chemicals have been shown to kill cancer cells by blocking topoisomerase, inserting DNA, and inducing apoptosis. [18,19]

The significant acetylcholinesterase inhibitory action of hydrazide-hydrazone compounds has been shown by many published studies and is of utmost importance to our work. Several SAR investigations have shown that aromatic rings, particularly those capable of pi-pi stacking, are important for the aromatic gorge residues of AChE. Strong AChE inhibition is obtained when the electron-withdrawing or electron-donating substituents at certain places are combined with the hydrazone nitrogen atoms that may form hydrogen bonds with the CAS and PAS residues. [20,21] Dual binding inhibitors are reported with IC50s in the nanomolar to tens of micromolar range that are as effective or better than those already on the market. The pharmacophore of these inhibitors could interact with the catalytic active site and the peripheral anionic site. [22,20]

Significance of the Phenolic Moiety

The phenolic hydroxyl group in the hydrazide-hydrazone scaffold imparts many pharmacologically beneficial features to this molecule. Phenols are among the most studied antioxidant functional groups in nature and pharmacological development. They can donate hydrogen atoms to quench reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and peroxynitrite, which are much higher in the brains of people with Alzheimer's disease due to

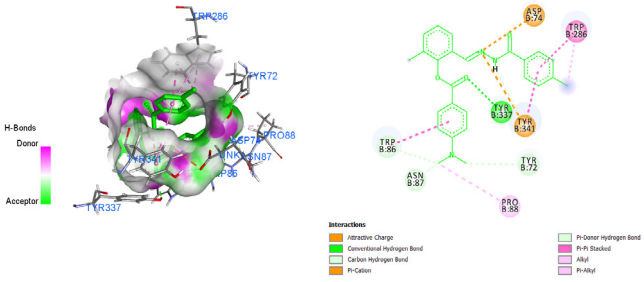


Figure 9: Interactions of ligand A06

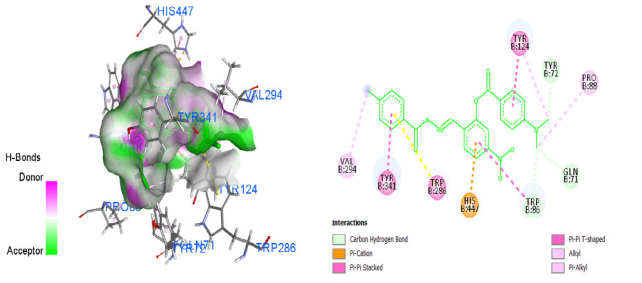


Figure 10: Interactions of ligand A07

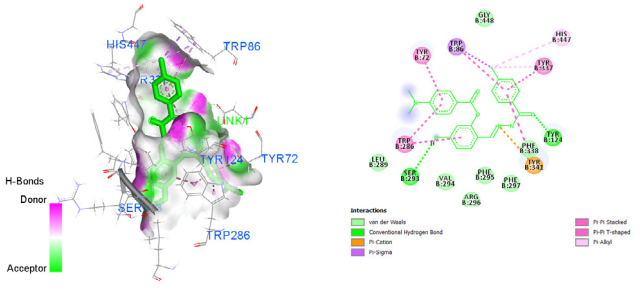


Figure 11: Interactions of ligand A09

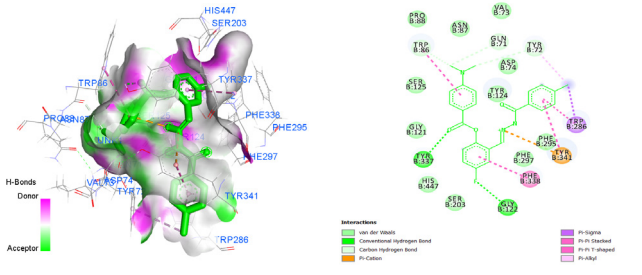


Figure 12: Interactions of ligand A10

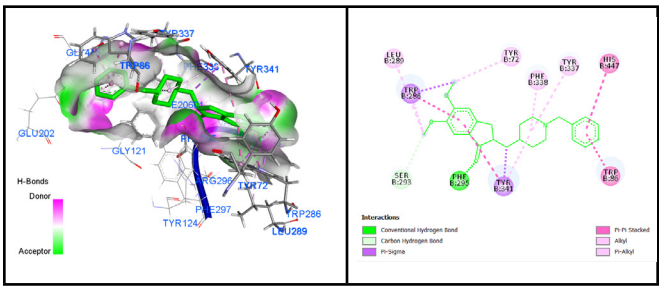


Figure 13: Interactions of the standard ligand Donepezil

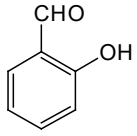
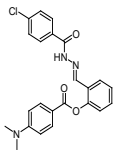
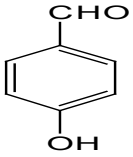
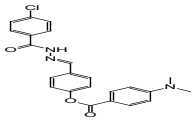
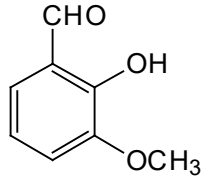
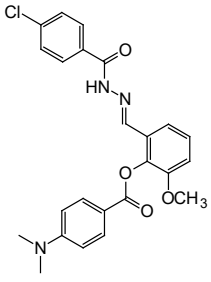
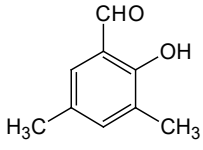
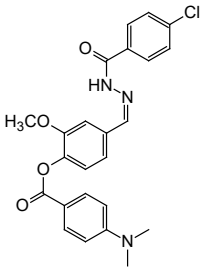
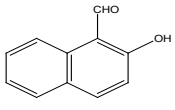
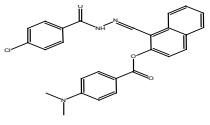
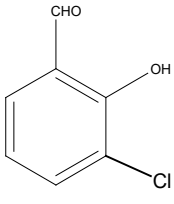
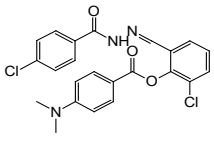
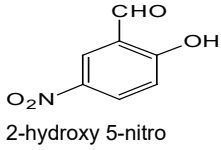
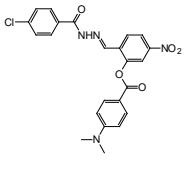
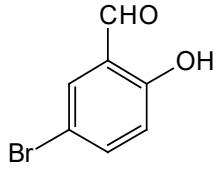
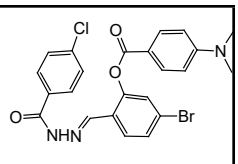
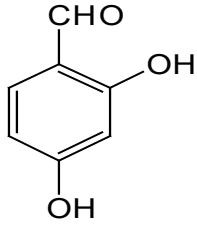
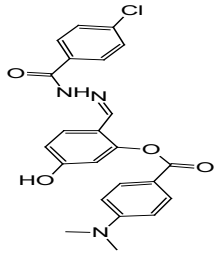
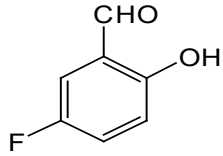
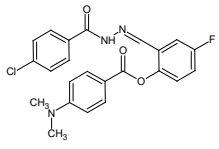
problems with mitochondria and oxidative reactions occurring when metals are present. Phenolic hydrazide-hydrazone compounds are a multitarget drug strategy that simultaneously targets the cholinergic deficiency and the oxidative stress component of AD development. This is achieved by incorporating phenolic antioxidant activity into the scaffold of an AChE inhibitor. [23,24] Phenolic hydroxyl groups are good at binding transition metal ions, especially copper (Cu2+) and zinc (Zn2+). If these ions are not in equilibrium, they may aggregate amyloid-beta and make it hazardous to neurons. Copper and zinc ions bind to the amyloid-beta peptide at the residues His6, His13, His14, and Asp1. This leads them to aggregate into neurotoxic oligomers. Their redox characteristics are also changed, enabling them to create hydrogen peroxide and hydroxyl radicals via Fenton-type processes. Metal-chelating phenolic hydrazide-hydrazones might be three-fold pharmacologically beneficial, i.e., they could inhibit AChE and hence potentiate cholinergic action, protect neurones by antioxidant activity, and reduce metal-catalyzed Abeta aggregation and oxidative stress via metal binding. [25]

Phenolic hydrazide-hydrazones are promising candidates for rational drug design against AChE for Alzheimer's disease based on their structural features in Figure 3. Hydrazones have nitrogen atoms that may bind with hydrogen to important sections of the AChE active site. The aromatic rings may stack on each other and interact with the aromatic gorge of the enzyme in a water-repelling manner. The phenolic hydroxyl groups may also form hydrogen bonds and act as antioxidants that could protect the brain in additional ways than just preventing the enzyme from functioning. This scaffold

Table 1: Designed 10 ligands from scaffold 1, and their obtained docking score by docking studies.

S. No.	*Ligand Code*	*Docking Score*
	A01	-10.4
	A02	-10.9
	A03	-10.8
	A04	-11.8
	A05	-10.6
	A06	-11.6
	A07	-11.3
	A08	-10.6
	A09	-10.5
	A10	-11.6
	Standard (Donepezil)	-11.9

Table 2: Substituted Benzaldehydes with Synthesized final compound

Compound code	*Substituted benzaldehyde (R)*	*Structures of the synthesized compound*
A01	 2-Hydroxy benzaldehyde	
A02	 4-Hydroxy benzaldehyde	
A03	 2-hydroxy-3methoxy benzaldehyde	
A04	 2-hydroxy 3,5-dimethyl benzaldehyde	
A05	 2-hydroxy naphthaldehyde	
A06	 2-hydroxy 3-chloro benzaldehyde	
A07	 2-hydroxy 5-nitro benzaldehyde	
A08	 2-Hydroxy 5-bromo benzaldehyde	
A09	 2,4 dihydroxy benzaldehyde	
A10	 2-Hydroxy 5fluoro benzaldehyde	

is also highly useful for the optimization of medicinal chemistry campaigns since it is simple to create, flexible in structure, and has drug-like physical and chemical features.[26,27] The structures of the synthesized compounds derived from substituted benzaldehydes are presented in Table 2.

MATERIALS AND METHODS

Docking Work Performed

Hardware and software utilised in the study

For the current docking investigations, a 64-bit Microsoft Surface 2 laptop with Windows 11 Pro installed in a single language, an Intel(R) Core (TM) i7-8650U CPU @ 1.90GHz (2.11 GHz), installed 16 GB random access memory (RAM), and a 1 TB SD drive was employed.

Procedure

The Apo form of the protein was retrieved from the PDB file by using PDB Swiss Viewer. Then the ligand was extracted from the protein ligand complex, and a new PDB file was formed. A library SDF file was utilized, and twenty distinct versions were used. Open Babel software was then used to generate photos of the scaffolds from 1. These pictures were derived from the corresponding existing 4-BNIB for each scaffold from 1. Phenolic hydrazone Hydrazide analogues were used as a scaffold to design twenty ligands.

Table 3: Binding energy and Conventional hydrogen bond

S.no	Compound code	Conventional Hydrogen Bond	Distance	Binding energy ((kcal/mol)
1	A01	TYR337	2.63064	-10.4
2	A02	TYR337	2.75895	-10.9
3	A03	TYR337	2.39707	-10.8
4	A04	TYR341	2.82788	**-11.8**
5	A05	TYR124	2.52587	-10.6
6	A06	TYR337	2.65741	-11.6
7	A07	---------	-------	-11.3
8	A08	--------	--------	-10.6
9	A09	TYR124	2.81852	-10.5
10	A10	GLY122 TYR337	2.81501 2.578	-11.6
11	Donepezil	PHE295	2.06267	-11.9

Table 4: Data of ADME studies of design molecules (SWISS ADME)

	Compound Code	*Molecular Weight*	*Num. H-bond acceptors*	*Num. H-bond donors*	*Log P o/w (Log P) (XLOGP3)*	*Drug Likeness (Lipinski*
1.	A01	421.88 g/mol	4	1	4.99	Yes, 1 Violation
2.	A02	421.88 g/mol	4	1	4.99	Yes, 1 Violation
3.	A03	451.90 g/mol	5	1	4.96	Yes, 0 Violation
4.	A04	451.90 g/mol	5	1	4.96	Yes, 0 Violation
5.	A05	471.93 g/mol	4	1	6.61	Yes, 1 Violation
6.	A06	456.32 g/mol	4	1	5.62	Yes, 1 Violation
7.	A07	467.88 g/mol	6	2	4.36	Yes, 0 Violation
8.	A08	500.77 g/mol	4	1	5.68	Yes, 2 Violation
9.	A09	437.88 g/mol	5	2	4.64	Yes, 0 Violation
10.	A10	439.87 g/mol	5	1	5.09	Yes, 1 Violation
11.	Donepezil* (Standard drug)	415.95 g/mol	4	0	3.92	Yes, 0 violation

The reference ligand and protein were prepared for docking using the PyRx virtual screening tool and the Open Babel and Vina wizards. The library of compounds from the SDF was transferred to the PDBQT format and docked to the Apo version of the protein using the ligand libraries.[28] Ligands were prepared and converted to 2D, SD, and other relevant formats using the ACD/ChemSketch I ACS style in the Open Babel GUI. The images of the protein ligand complex were made using BIOVIA Discovery Studio Visualizer (Studio 2020). The crystallographic PDB file was downloaded online from the Protein Data Bank (PDB). [29,30]

The binding site interactions of the ligand with the protein

The binding site interaction of acetylcholinesterase (AChE) enzyme and ligand complex was shown by using BIOVIA Discovery Studio Visualizer 2020 with catalytic triad and oxyanion hole residues. Protein PDB file 7E3H was used to search for the catalytic triad residues and the oxyanion hole residues in the protein-ligand interaction site. This allowed us to identify and validate the binding region in the downloaded PDB file of the protein-ligand complex of 7E3H. The binding location of the acetylcholinesterase (AChE) inhibitors was shown to include the catalytic triad residues Ser122, His269, and Asp239. We studied the residues in the ligand binding site of the protein PDB file 7E3H, which we downloaded from https://www.rcsb.org (Protein Data Bank). The catalytic triad Ser122, His269, and Asp239 were detected at the initial ligand-protein interaction site. The oxyanion hole residues Gly50, Ala51, Met123, and Gly124 were also located at the ligand-protein binding site.[31]

Protein Preparation

The X-ray crystallographic structure of monoacylglycerol lipase complexed with the ligand (Donepezil) is used in this investigation, and the PDB ID is 7E3H. We downloaded the PDB resources in PDB file format for the protein and ligand 3D X-ray crystallographic structure from https://www.rcsb.org/ (Protein Science - 2017 - Williams – Mol Probity More and Better Reference Data for Improved All-atom Structure Validation. Pdf, n.d.). The APO version of the protein was generated using the MOLPROBITY service. [34,35] The protein was obtained from the MOLPROBITY service by submitting the PDB file of the acetylcholinesterase (AChE) protein molecule (PDB ID: 7E3H) in the protein production method. Then we added hydrogens to it, and we used the "Make Flipkin kinemages illustrating any Asn, Gln, or His flips" option to flip the residues and look at all the atom connections and form of the molecule. When the application finishes, click "download" to start downloading the final protein file. Swiss PDB reader was used to generate the Apo form of the protein and prepared for protein production and docking with the help of reference ligands (Donepezil) and ligands of different styles. [32]

Ligand Preparation

Using a Swiss PDB reader, the original ligand (Donepezil) was removed from the protein ligand complex and saved as a separate file. The saved file was then used as the reference ligand for docking. The single, separate scaffold prepared in this study was aimed to produce new compounds that may inhibit acetylcholinesterase. The new scaffolds were built with several features comparable to the molecule that was made earlier. Using these scaffolds, we generated 10 distinct variations. The 10 compounds from the scaffold are listed in Table 1 and were employed as ligands. All the derivatives' molecular structures were generated using the ACD/ChemSketch drawing application with ACS style and OpenBabel GUI software. [33]

Also, the wizard in the PyRx virtual screening tool uses Open Babel to prepare the ligands for docking simulations by placing them in PDBQT format.

All ligands are produced as small as possible throughout the preparation procedure. Then ligands are prepared in PDBQT format, and the SDF file of the ligand library is imported. The Vina wizard of the virtual screening application PyRx was used to connect the stabilized structures of proteins and ligands. [31]

Validation Process

The authenticity of the acetylcholinesterase (AChE) protein crystal structure was evaluated by using an inhibitor ligand (Donepezil) (PDB ID: 7E3H) 9-NITRO-5,12-DIHYDRO-7H-BENZO[2,3]AZEPINO[4,5-B]INDOL-6-ONE]. This was done by redocking the separated ligand file (ATU) that was obtained from the original PDB file with the co-crystallized structure of the ligand and the Apo form that was built from the acetylcholinesterase protein file from 7E3H to the exact binding site coordinates. The grid coordinate properties were noted for future use. [34]

Docking of Prepared Protein and Ligand

Ligand docking was accomplished using the Vina wizard of the PyRx virtual screening software. The Apo protein file was uploaded to the MOLPROBITY Server, and the produced protein file was downloaded. The obtained file was then used for the redocking analysis and the docking of the freshly synthesized carbamate derivative ligands (ligands 4–10). The apo form of the protein and a separate

ligand file obtained from the protein-ligand complex were also generated using the Swiss PDB Viewer. [35]

The SMILES strings of the generated ligands were utilized to construct a ligand library in SDF format using the Open Babel tool. The ligands were then preprocessed using the Open Babel wizard included in the PyRx virtual screening tool and converted into the AutoDock PDBQT format.

We then performed docking activities using the AutoDock Vina wizard.

The grid box for the grid manufacturing process was 25 × 25 x 25 in the x, y, and z axes. The attribute value coordinates were derived from the original PDB file using the binding site interactions between the protein and ligand ATU. There was a discrepancy of 0.375 between grid points. The grid picked has X, Y, and Z values of -13.07, 20.8, and -9.6, respectively. The AutoDock Vina wizard included in the PyRx virtual screening tool was used to dock each ligand to the produced protein structure.[38] The binding energies and hydrogen-bond interactions of the docked ligands are summarized in Table 3. All docking findings based on various structural designs were examined using BIOVIA Discovery Studio Visualizer 2020.[36]

The designed ligands and their docking scores are summarized in Table 1. The binding interactions of ligand A01 with AChE are presented in Figure 4, The docking interaction profile of ligand A02 is shown in Figure 5, Figure 6 illustrates the binding interactions of ligand A03 within the active site of AChE, The molecular interactions of ligand A04 with key amino acid residues are depicted in Figure 7, The docking pose and interaction map of ligand A05 are shown in Figure 8, Figure 9 presents the interaction pattern of ligand A06 with the target protein, The binding interactions observed for ligand A07 are illustrated in Figure 10, The interaction profile of ligand A09 with AChE is shown in Figure 11, The docking interactions of ligand A10 are presented in Figure 12, The interaction pattern of the reference drug donepezil with AChE is depicted in Figure 13.

ADME Studies Performed:

Further research needs to be performed on the adsorption, distribution, metabolism, and excretion (ADME) studies on the molecules to explore the likelihood of a created molecule being used as an important therapeutic agent in the treatment of sickness. The ADME research was carried out using the SWISSADME web-based program (http://www.swissadme.ch/), where several characteristics such as lipophilicity (iLOGP, XLOGP3, WLOGP, MLOGP and SILICOS-IT), water solubility [Log S (ESOL), Log S (Ali) and Log S (SILICOS-IT)] were predicted. It predicts drug-kinetic characteristics (GI absorption, BBB permeation, P-gp substrate CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, inhibitor, Log Kp), medicinal chemistry (PAINS, Brenk, Leadlikeness, Synthetic accessibility), and druglikeness (Bioavailability Score Lipinski, Muegge, Ghose, Veber) using the SWISSADME web-based application.[37,38]

The SWISSADME web-server is developed and managed by the Molecular Modelling Group at the Swiss Institute of Bioinformatics. SMILES of different structures were uploaded, and the RUN was clicked to receive results. [39]

Toxicity Studies Performed

The toxicology studies were done using the online ADMET prediction tool of the pkCSM web server database, which offers access to the research of chemicals either by drawing or uploading in SMILES format. [42] Toxicity data include: AMES (carcinogenic potential of a substance), maximum tolerated dose (human), hERG I inhibitor, hERG II inhibitor, oral rat chronic toxicity (LOAEL), oral rat acute toxicity (LD50), skin sensitization, hepatotoxicity, T. pyriformis toxicity, and minnow toxicity studies. [40]

RESULTS

Performed Docking Studies

Target site identification

- *2D & 3D Interactions of derivative analogue with 7E3H*

ADMET S Analysis (SwissADME)

SwissADME is a free online tool and is intended for evaluating the pharmacokinetic and physicochemical characteristics of small molecules. It is especially useful in the processes of Drug discovery and Drug development. Based on well-established models and algorithms, users can input chemical structures to receive predictions on characteristics including lipophilicity, water solubility, pharmacokinetic profiles, and drug-likeness. SwissADME/T provides insightful information on their potential for ADME/T.

CONCLUSION

This paper reports the rational design and in-silico evaluation of new phenolic hydrazide-hydrazone derivatives as possible acetylcholinesterase (AChE) inhibitors for the treatment of Alzheimer's disease. Alzheimer's disease is one of the most difficult neurodegenerative diseases in the world owing to the lack of total treatment and the low efficacy of existing medications. Therefore, the identification of safer, more efficient and multifunctional therapeutic agents is of great relevance.

In the present work, ten new ligands (A01–A10) were developed based on the hydrazide–hydrazone pharmacophore and tested by molecular docking tests against the enzyme AChE (PDB ID: 7E3H). The docking data indicated that majority of the compounds showed good binding affinities with docking scores from −10.4 to −11.8 kcal/mol, which was similar to the standard medication donepezil (−11.9 kcal/mol). Among all the compounds A04, A06 and A10 showed the most promising interactions with key amino acid residues located in the catalytic and peripheral active regions of the enzyme showing their substantial inhibitory potential. Additional contact investigations revealed the existence of hydrogen bonding, π-π stacking and hydrophobic interactions with critical residues such as TYR337, TYR341, GLY122 and PHE295 which are important for enzyme inhibition and ligand stabilisation. These interactions imply that the proposed compounds could successfully suppress the activity of acetylcholinesterase and increase cholinergic neurotransmission in Alzheimer's disease patients.

Besides docking analysis, ADMET prediction studies showed the majority of the compounds had acceptable pharmacokinetic and drug-likeness features with a minimum number of Lipinski rule violations. In the proposed scaffold, the phenolic hydroxyl group may also exhibit antioxidant and metal-chelating capabilities which can contribute to reducing oxidative stress and amyloid-beta aggregation related to the advancement of Alzheimer's disease. This is why these chemicals might be seen as multifunctional therapeutic agents addressing many pathogenic pathways of AD at once. In conclusion, we have shown that phenolic hydrazide-hydrazone derivatives are attractive lead compounds for the creation of novel anti-Alzheimer medicines. The work provides a good platform for further medicinal chemistry optimisation and biological studies. However, more in-vitro enzyme inhibition experiments, in-vivo pharmacological investigations, toxicity profiles and clinical assessments are needed to evaluate the therapeutic potential, safety and effectiveness of these drugs before their probable use in clinical practice.

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