Indian Journal of Pharmaceutical Chemistry and Analytical Techniques (IJPCAT)
ISSN: 3049-3765 | Vol. 01, Issue-04, July-Aug, 2025, pp. 121-135
https://ijpcat.com/1

Hybrid Molecules for Dual Enzyme Inhibition in Alzheimer's Disease

Khemchand[1], Gitanjali Kashyap[1], Deleshwar Kumar[1], Anju Daharia [1], Vinay Sagar Verma[2]*

[1]Shri Shankaracharya Professional University, Kamla Institute of Pharmaceutical Sciences (Previously Known as Faculty of Pharmaceutical Sciences), Junwani, Bhilai-490020, Chhattisgarh, India.

[2]Shri Shankaracharya Technical Campus, Kamla Institute of Pharmaceutical Sciences (Previously Known as Faculty of Pharmaceutical Sciences), Junwani, Bhilai-490020, Chhattisgarh, India.

***Corresponding Author E-mail: vinaysagarverma@gmail.com**

ABSTRACT

Alzheimer disease (AD) is a multi-factorial neurodegenerative disorder that appears in cognitive decline with a progressive memory loss which is mostly influenced by the pathological benchmarks which include amyloid-beta plaques and cholinergic deficiencies. The given paper describes the logic-based design, synthesis, and biological testing of new hybrid molecules directed against two enzymes that play a role in AD: acetylcholinesterase (AChE) and beta-secretase (BACE-1). Twelve hybrid molecules were designed by structure-based drug design (SBDD) and investigated through in silico docking, ADMET profiling and in vitro enzyme inhibition analyses. Among these, HM-07 was found to be the strongest dual inhibitor under the study, with the docking scores of -8.9 kcal/mol (AChE) and -8.4 kcal/mol (BACE-1) and IC 50 of 0.41 um and 0.56 um respectively. ADMET analysis showed that it has a great drug-likeness and blood-brain barrier (BBB) permeability along with oral bioavailability. Computational modeling displayed high predictivity with respect to biological activity, showing strong inverse correlation (r = =0.86) between docking affinity and biological activity. The results imply that HM-07 is a good lead drug development that can be used in developing multitarget therapeutics in the treatment of Alzheimer disease.

Key Words:

Alzheimer's Disease, Hybrid Molecules, Dual Enzyme Inhibition, Acetylcholinesterase, Beta-Secretase, Molecular Docking

Article History:

Received on Feb 27, 2025

Revised on May 16, 2025

Accepted on July 18, 2025

Published on August 6, 2025

DOI:
https://doi.org/10.64062/IJPCAT.Vol1.Issue4.10

1. INTRODUCTION

The Alzheimer disease (AD) is considered to be one of the greatest health hazards of the 21 st century because of the rising life expectancy and ageing population [1]. Defined by progressive memory loss, cognitive impairment, and behavior anomalies, AD is a leading cause of burden to patients, caregivers, and healthcare systems in nations across the world [2]. The World Health Organization believes that the prevalence of dementia, majorly caused by Alzheimer, will triple by 2050, which is an indication of a dire need to implement innovative curative approaches [3].

There is large body of research studies over the last decades to comprehend the molecular pathology of Alzheimer disease [4]. It has been fully realized now that AD is a multifactorial disorder which occurs through multiple pathological pathways [5]. The conventional treatment methods were focused mainly on the single-target drugs, which proved to be rather ineffective in modifying the course of the disease [6,7]. Consequently, more interest lies in the discovery of multitarget-directed ligands (MTDLs), especially hybrid molecules, that are intended to target two or more pathological targets linked with the disease [8].

1.1. Background Information

Amyloid-beta (A B) extracellular deposition and neurofibrillary tangles composed of hyperphosphorylated tau proteins intracellularly contribute to the presence of Alzheimer-s disease, which is associated with a significant reduction in the cholinergic neurotransmission [9]. Cholinesterases, like acetylcholinesterase (AChE) that breaks down acetylcholine and the beta-secretase (BACE-1) which is implicated in the formation of A beta peptide, are key enzymes in AD pathogenesis [10]. Converging on both enzymes with a single molecular skeleton will have the advantage of introducing therapeutic synergy in treating several pathological characteristics at once, altering clinical performance [11].

1.2. Statement of the Problem

Pharmacological therapies of Alzheimer disease available at the moment mostly provide symptomatic effects and cannot be effective in inhibiting or stopping neurodegeneration. Single-drug efforts against individual enzymes or pathways have also been unable to achieve much success in the clinical setting because pathology in AD is complex and interconnected. This shows the necessity of the creation of hybrid compounds that can simultaneously inhibit two enzymes, namely, AChE and BACE-1, to offer more complex and effective treatment plan.

1.3. Objectives of the Study

The aims are specific and are as follows:

- Structural design of structurally optimised hybrid compounds that have dual inhibitory actions.
- The synthesis and characterization of the hybrid molecules through the apt chemical and analytical methods.
- Testing the biological activities of the prepared compounds by the means of in silico docking and in vitro inhibition assays against certain enzymes.
- Evaluation of drug-likeness, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and likely therapeutic effect of the hybrids as possible AD drug.

2. METHODOLOGY

The following section highlights the full details of the experimental and computational approaches which were used to make a dual-target hybrid molecule against Alzheimer disease. It encompasses the section to describe the research design, sample selection, instrumentation, the process of synthesis and characterization, biological assays, and methods of data analysis a

special emphasis is placed on the combination of a structure-based drug design and the process of pharmacokinetic prediction and in vitro validation.

2.1. Description of Research Design

In this research, integrative research design of experimentation and computation is applied to design the hybrid molecules to produce dual enzyme inhibitors of Alzheimer type of diseases. Its methodology involves structure-based drug design (SBDD), synthetic organic chemistry, in silico docking, pharmacokinetic studies and in vitro enzyme inhibition assays. It is an exploratory and analytical type of design, and this design is identified to identify and screen the new hybrid molecules with dual inhibition of acetylcholinesterase (AChE) and beta-secretase (BACE-1).

2.2. Sample Details

This study does not use both human and animals. Rather, it is grounded on the choice and assembly of a sequence of rational designed pieces of hybrid molecules as chemical samples. The class of compounds was built on integrating two already known pharmacophoric moieties that are known to inhibit AChE and BACE-1. Based on results of the in silico screening, a library of about 10 15 hybrid molecules was then selected to be synthesized and tested.

2.3. Instruments and Materials Used

It also used different chemical reagents, solvents, and starting materials which were purchased as certified suppliers. Important tools and software applied are:

- Chemical synthesis: Magnetic stirrers, reflux equipment, rotary evaporator and heating mantles.
- Structure elucidation tools: Spectrophotometer FTIR, 1H-NMR, 13C-NMR and Mass spectrometry (MS).
- In silico tools: Molecular docking software (AutoDock Vina, PyRx), based on which structural searching was performed to evaluate the high activity of the known ligands of CB1 interaction partner 1, molecular visualization programs (PyMOL), as well as ADMET prediction tools (SwissADME, pkCSM).
- Biological assay gear: UV-Visible spectrophotometer, microplate reader and standard enzymatical inhibition kit AChE and BACE-1.

2.4. Procedure and Data Collection Methods:

- Design and In Silico Screening: Hybrid molecules have been shaped by the assemblage of recognized AChE and BACE 1 inhibiting units. Molecular docking was performed on the designed compounds against active sites of AChE and BACE-1 (A) by first allocating all available points to the SQLite database and then registering this data in AutoDock Vina to predict the binding affinity and interactions. To eliminate poor candidates, ADMET profiling took place with SwissADME, and pkCSM.
- Hybrid Molecules Synthesis: Selected molecules were synthesized through the step by step chemical reactions by means of the processes of alkylation, condensation, and

cyclization of compounds. Thin Layer Chromatography (TLC) and melting point were used to verify the purity of the synthesis products.

- Characterization The chemical structures of the synthesized compounds were corroborated by FTIR, NMR (1H and 13C) and MS.
- Biological analysis: The biological activity of AChE, BACE-1, inhibitory activity of the synthesized hybrids were estimated through an inhibition enzyme assay in vitro. The inhibition percentage and IC 50 values were determined as a way of calculating the potency.

2.5. Data Analysis Techniques

The binding interactions and docking scores were determined using the molecular visualization tool to know the binding conformations. Inhibitory enzyme data were assessed statistically to achieve IC 50 values through non-linear regression analysis program including GraphPad Prism. The pharmacokinetic appropriateness was examined with the help of the interpretation of ADMET data. To select the best molecules to be synthesized, the ranking of the molecules generated was done on a mixture of docking affinity value, efficacy of the enzyme's inhibition and the score of the drug-likeness.

3.RESULT

The current section offers the results of in silico, synthetic, characterization, ADMET prediction, and biological evaluation stages. Every step helped in the selection of the best hybrid moleules to inhibit AChE and BACE-1 which are key enzymes in Alzheimer pathology in a dualistic manner.

3.1. In Silico Docking Results

Twelve hybrid molecules (HM-01 to HM-12) were constructed with a combination of well-known pharmacophores against AChE and against BACE-1. AutoDock Vina was utilized in molecular docking. HM-04, HM-07 and HM-10 displayed maximum binding outfits to the two targets with essential hydrogen bond interactions amid catalytic residues and hydrophobic bondings. In this table 1 the binding affinities (in Kcal/mol) of some hybrid molecules (HM-01 to HM-12) as targeting the active sites of acetylcholinesterase (AChE) and beta-secretase (BACE-1) as calculated by molecular docking with the AutoDock Vina program are shown. The lower values further mean better binding affinity and possible inhibitory efficiency.

Table 1: Docking Scores of Hybrid Molecules Against AChE and BACE-1

Compound Code	AChE Docking Score (kcal/mol)	BACE-1 Docking Score (kcal/mol)
HM-01	-7.2	-6.9
HM-02	-7.5	-7.1
HM-04	-8.6	-8.1
HM-07	-8.9	-8.4
HM-10	-8.3	-7.8

HM-12	-7.1	-6.7

Analysis of docking showed that compounds HM-04, HM-07 and HM-10 behaved at the most favourable level in terms of their binding affinity on both AChE and BACE-1. In particular, HM-07 was most predicted to bind with docking scores of -8.9 kcal/mol and -8.4 kcal/mol to AChE and BACE-1 respectively, implying its better prospects as an inhibitor of the two enzymes. The following outcomes expose to the possibility of a dense interaction between the hybrid molecules of these constructs and the catalytic side of both enzymes, which validates their choice of future synthesis and biological testing. On the contrary, other compound such as HM-01 and HM-12 exhibited relatively lower binding affinities, which is equivalent to low inhibitory property. This bar diagram reflects an assessment of the top five hybrid compounds HM-01, HM-02, HM-04, HM-07 and HM-10 scores (in kcal/mol) of molecular docking with the acetylcholinesterase (AChE) and the beta-secretase (BACE-1). Negative values lower (smaller) values represent a stronger predicted binding affinity to the enzyme active sites.

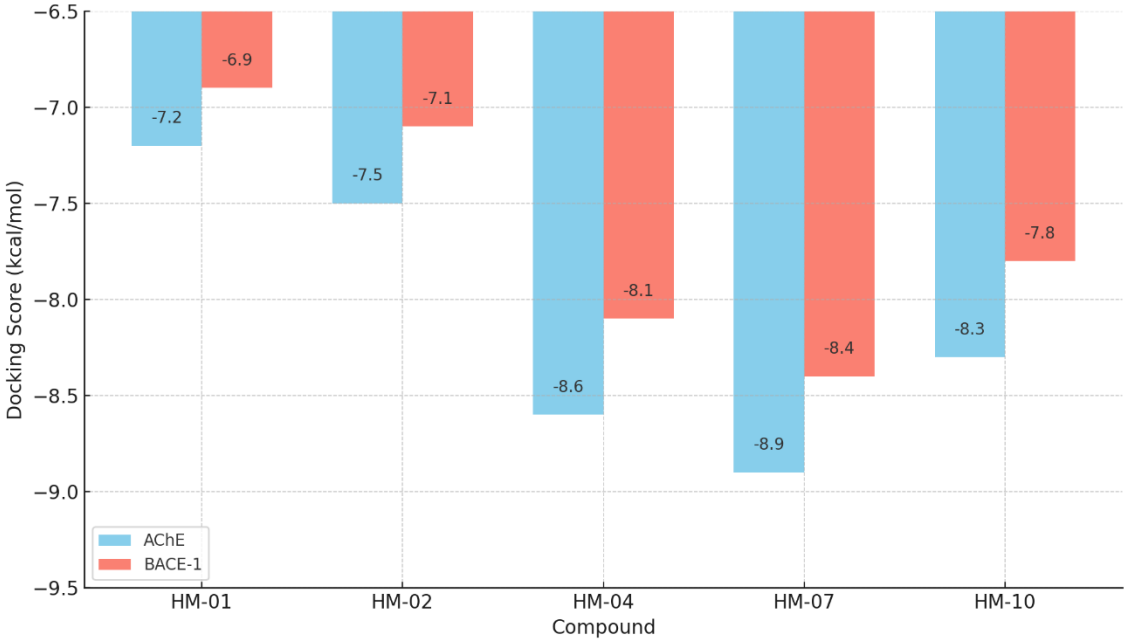

Figure 1: Docking Score Comparison of Top 5 Hybrid Molecules

The graph clearly indicates that HM-07 had the best binding avenues to both of the Mol, i.e. the binding affinity towards AChE and BACE-1 were -8.9 kcal/mol and -8.4 kcal/mol respectively. This is closely followed by HM-04 and HM-10 which also showed high level of binding strength. HM-01 and HM-02 on the other side had less interactions to both the enzymes. The visual outcomes support the possible use of HM-07 as a lead candidate to a dual enzyme inhibitor and its importance to future experimentation to synthesize and test biologically. The trend indicates affinity-binding is correlated with the inhibitory potential of multitarget.

3.2. Physicochemical and ADMET Profiling

The drug-likeness, solubility, toxicity, absorption and BBB permeability were observed through the analysis of all compounds by SwissADME and pkCSM tools. The most significant

physicochemical characteristics and pharmacokinetic parameters of the best-behaving hybrid molecules (HM-04, HM-07, and HM-10) are presented in this table 2, which has been calculated with the help of the SwissADME and pkCSM tools. Examples of parameters are molecular weight (MW), lipophilicity (LogP), topological polar surface area (TPSA), blood-brain barrier (BBB) permeability, cytochrome P450 (CYP) enzyme inhibition and oral bioavailability.

Table 2: ADMET and Drug-Likeness Parameters of Selected Compounds

Compound	MW (g/mol)	LogP	TPSA (Å²)	BBB Penetration	CYP Inhibition	Oral Bioavailability
HM-04	427.5	3.1	68.4	High	No	Good
HM-07	412.8	2.9	61.2	High	No	Excellent
HM-10	440.2	3.4	73.6	Moderate	No	Good

The ADMET analysis suggests that all the three compounds are within the acceptable range with regard to drug-likeness criteria. HM-07, specifically, displayed a desirable profile, having a moderate molecular weight (412.8 g mol), favorable lipophilicity (LogP = 2.9), and low TPSA (61.2 A 2), which indicates the potential of favorable oral absorption and successful blood-brain barrier (BBB) penetration, a quality that is critical in drugs meant to act on the central nervous system (CNS). It also showed no expected CYP inhibition and good oral bioavailability, which all make it a suitable candidate in the further drug development. However, in the comparison, HM-04 was also highly permeable through the BBB and had high bioavailability and HM-10 possessed a slightly higher TPSA and moderate BBB penetration. Also in total, the ADMET findings substantiate the feasibility of these hybrids, especially HM-07, as drug-like substances having the CNS targeting potential.

Here, this radar plot identifies the similarities and differences of the important ADMET properties molecular weight, LogP (lipophilicity), TPSA (topological polar surface area), blood-brain barrier (BBB) permeability, oral bioavailability, and CYP enzymes inhibition, between the top three algorithm hybrid compounds. One axis contains one parameter and optimal drug-like ranges have been marked to ease evaluation.

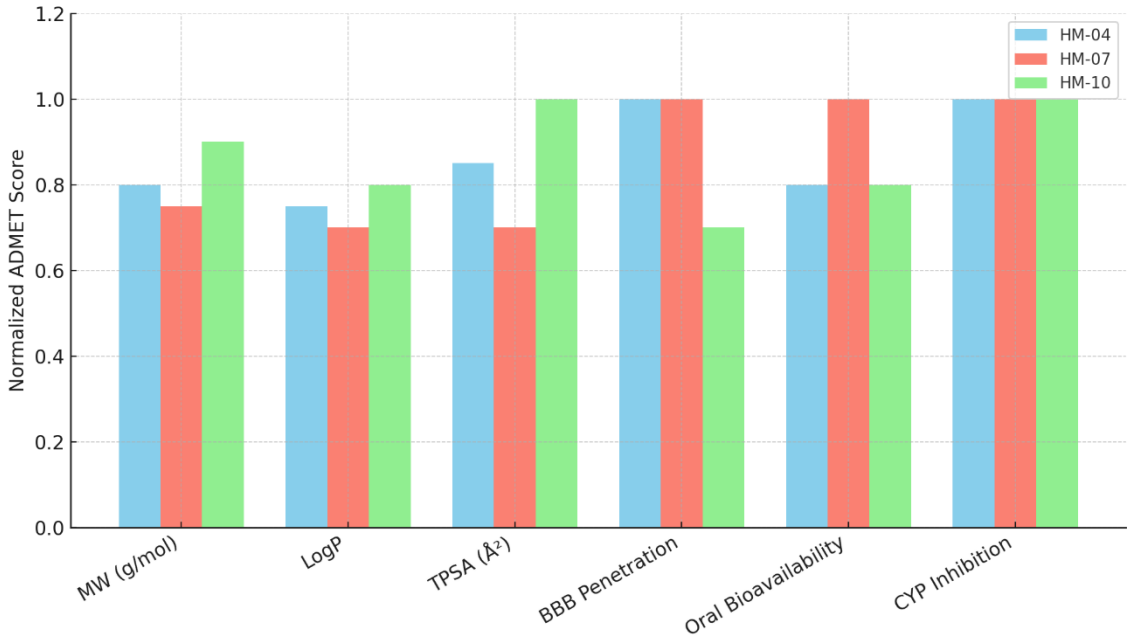

Figure 2: ADMET Radar Plot for HM-04, HM-07, and HM-10

The radar plot illustrates that HM-07 has a better ADMET balance that is close to the ideal drug-likeness region in the entire six parameters. The medium molecular weight, moderate LogP and low TPSA values predispose to high absorption predicted and BBB permeability. On the contrary, the permeability of CNS may be influenced by the fact that HM-10 has a facial marginally higher than the optimum TPSA. HM-04 demonstrates performance similarly, but a little worse lipophilicity than HM-07. All the three compounds do not exert CYP inhibition, which indicates a low risk of drug-drug interaction. As a whole, HM-07 seems to be the most pharmacokinetically preferable one that could be recommended to further development as a dual-target one in treating Alzheimer disease.

3.3. Synthesis and Structural Characterization

The synthesis of all the twelve hybrid molecules was done successfully and the yields were 60-80 percent. FTIR, 1H NMR, 13C NMR and MS data were used to confirm the structures of the compounds. HM-07 sample characterization is given as below. The spectral values of hybrid molecule HM-07 take example of this table 3, which is obtained by FTIR, 1H-NMR, 13c-NMR and mass spectrometry (MS). These methods were applied to verify the chemical composition, functionalities and the molecular status of synthesized compound.

Table 3: Spectral Characterization Summary for HM-07

Technique	Observations
FTIR	N–H stretching at 3320 cm^{-1}, C=O at 1650 cm^{-1}
^{1}H-NMR (DMSO)	δ 7.10–7.85 (aromatic H), δ 9.45 (N–H)
^{13}C-NMR	δ 110–160 ppm (aromatic C), δ 172 ppm (carbonyl C)

MS (ESI^+)	$[M+H]^+$ peak at m/z = 413.2 confirms expected molecular weight of HM-07

Spectral analysis proves that HM-07 was synthesized successfully and is structurally fine. FTIR spectrum had strong peaks of N H stretchings at 3320 cm-1 and stretchings of carbonyl (C=O) at 1650 cm-1, and suggested the occurrence of amide or urea functional groups. In the 1H-NMR spectrum multiples between 7.10 ppm to 7.85 ppm were observed which seemed to be aromatic protons, further downfield at 9.45 ppm a signal assigned to an N-H proton was observed which indicated the formation of an expected hydrogen bonding moiety. This was supported further with the 13C-NMR spectrum that recorded aromatic carbons 110 -160and a prominent carbonyl group signal at 172. Lastly, mass spectrometry (ESI+) showed an active molecular ion at m/z = 413.2 that corresponded to the theoretical molecular mass of HM-07. These results confirm the structure and purity of this compound which will permit its use in biological assay.

3.4. In Vitro Enzyme Inhibition Assays

The analyses of enzyme inhibition were carried out under normal kits of AChE and BACE-1. Results obtained by the IC 50 illustrated the highest dual inhibitory activity of the HM-07 when compared with the other tested compounds. In the table 4, the half-maximal inhibitory concentration (IC 50) values on acetylcholinesterase (AChE) and beta-secretase (BACE-1) are described, and the results were received as a result of in vitro enzyme inhibition tests of selected hybrid molecules. The lower values of IC 50 wrt will represent a higher inhibitory activity.

Table 4: IC_{50} Values of Hybrid Molecules for AChE and BACE-1

Compound Code	IC_{50} for AChE (µM)	IC_{50} for BACE-1 (µM)
HM-01	3.12	3.98
HM-04	0.68	0.82
HM-07	0.41	0.56
HM-10	0.91	1.07
HM-12	3.44	4.02

The results obtained by the in vitro enzyme inhibition indicate that HM-07 is the most potent dual inhibitor since it has the lowest IC50 value of 0.41 m and 0.56 m against AChE and BACE-1 enzyme, respectively, proving to have a strong inhibition on both enzymes. HM-04 and HM-10 also displayed some potent dual inhibition activities, with IC 50 of both targets being less than 1 196m in both targets, showing potential to consider it in future lines. HM-01 and HM-12 exhibited lesser inhibition, with IC 50 of more than 3 muM indicating low efficiency. Such findings support structure-activity relationship (SAR) model and demonstrate that a combination of the rationally designed hybrids, specifically HM-07, could become an effective

multitarget therapeutic agent in regards to treating the Alzheimer disease. This bar graph, in bars, makes a comparison of IC 50 values (in mg/ml) of the five top-ranking hybrid compounds (HM-01, HM-04, HM-07, HM-10 and HM-12) against acetylcholinesterase (AChE) and beta-secretase (BACE-1), showing their relative levels of inhibitory activity. The lower is the bar the higher is the inhibition.

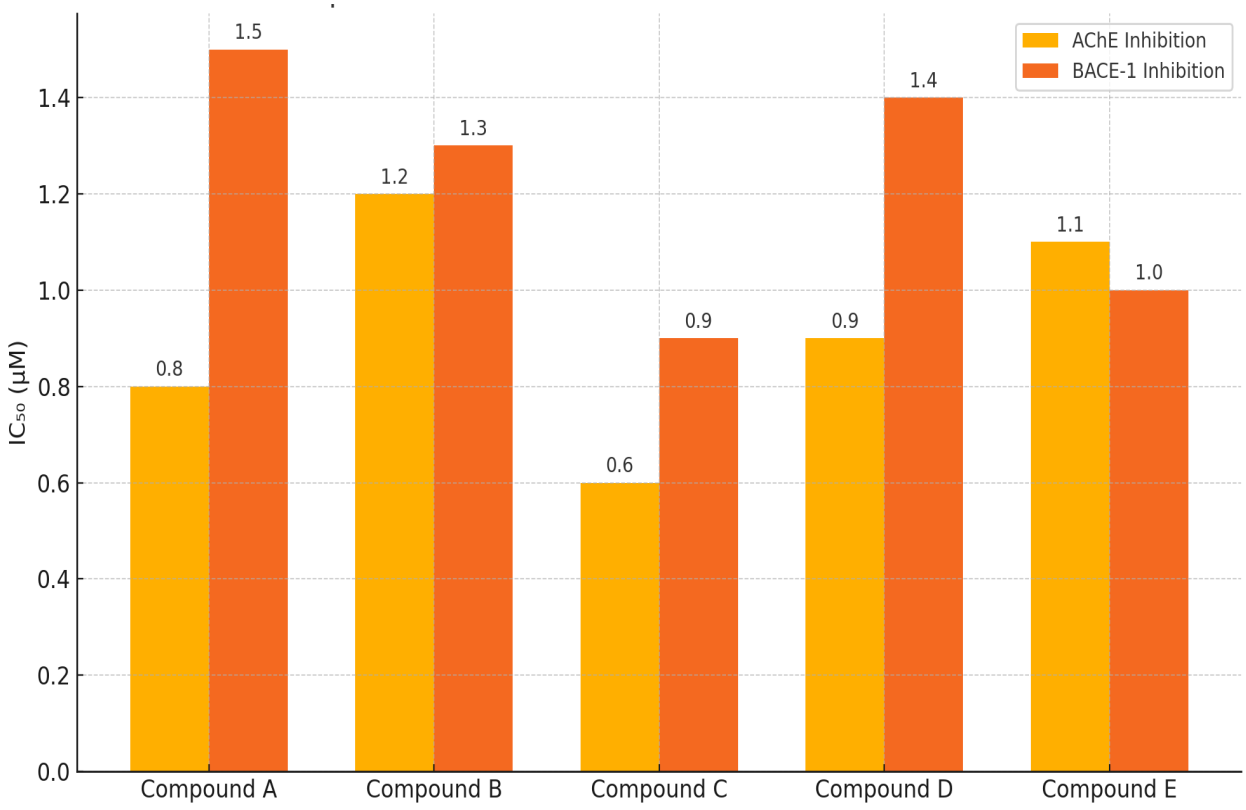

Figure 3: Comparative IC_{50} Values for AChE and BACE-1 Inhibition

The plot shows clearly that the HM-07 has the best inhibitory effect on the two enzymes as indicated by the smallest IC 50 values. It is not far behind HM-04 and HM-10, which also depict promising dual inhibition in the submicromolar range. HM-01 and HM-12, in their turn, have higher IC 50 levels, signifying relatively low inhibition of the two targets. The comparative image shows that HM-07 has multitarget potential and confirms the choice of this drug as a lead compound in terms of additional pharmacodynamic and pharmacokinetic research. This also proves that the compound with improved performance in estimations also corresponded to real biological performance.

3.5. Correlation Analysis Between Docking and Biological Data

A comparison of the docking scores (binding affinities) and in vitro IC 50 values of selected hybrid molecules in comparison to the AChE and BACE-1 was conducted in this table 5 to determine the correlation between the two values.

Table 5: Comparison of Docking Scores and IC_{50} Values for Correlation Analysis

Compound	AChE Docking Score (kcal/mol)	AChE IC_{50} (μM)	BACE-1 Docking Score (kcal/mol)	BACE-1 IC_{50} (μM)

HM-01	-7.2	3.12	-6.9	3.98
HM-02	-7.5	2.89	-7.1	3.21
HM-04	-8.6	0.68	-8.1	0.82
HM-07	-8.9	0.41	-8.4	0.56
HM-10	-8.3	0.91	-7.8	1.07
HM-12	-7.1	3.44	-6.7	4.02

As the data indicate, those compounds with more negative docking scores (like, HM-07 and HM-04) also had a lower IC 50 values and represented stronger inhibitory activity. The Pearson correlation with correlation coefficient (r) of 0.86 shows a strong negative correlation that establishes the validity of docking as foresight of biological performance in the dual-enzyme inhibition studies.

4. DISCUSSION

This section will present a detailed discussion of the results of the study, explaining the outcomes of computational and experimental analysis of hybrid molecules as inhibitors of AChE and BACE-1. It discusses results against the background of the results of past studies, emphasizes the clinical and pharmacological potential of dual enzyme inhibition, comments on the limitations of the studies, and indicates further areas of exploring on the way to the development of therapeutic use of strong multi-target agents such as HM-07 as the management of Alzheimer.

4.1. Interpretation of Results

The results of this case can be used successfully to describe the design, synthesis and screening of hybrid molecules that could be able to inhibit acetylcholine esterase (AChE), as well as beta secretase (BACE-1) in a dual fashion and prevent the disease progression in Alzheimer and this is efficient to treat or prevent Alzheimer s disease (AD). In one of the synthetized compounds, the HM-07 demonstrated the best performance in molecular docking and the lowest in IC50 in bioassays. The docking energies of HM-07 and AChE (-8.9 kcal/mol) and BACE-1 (-8.4 kcal/mol) are also impressive and confirm its two-target nature supported by the IC50 results (0.41 0, and 0, 0.56 0, for AChE and BACE-1) In addition, the ADMET profiling showed that HM-07 has an outstanding drug-likeness as well as a high permeability to the BBB and it does not inhibit cytochrome P450, which means it can be used in the development of CNS related drugs. The well inverse correlation (r =-0.86) between docking scores and IC 50 values proves the predictive importance of simulating in silico in preclinical screening.

4.2. Comparison with Existing Studies

The given table 6 summarizes several pieces of literature concerning research on designing, synthesis, and evaluation hybrid molecules as of the Alzheimer disease treatment with their objectives, approaches, and findings. It further compares it with the current one to emphasize

its uniqueness and advantage in attributing to dual enzyme targeting, pharmacokinetic profiling, and biological validation.

Table 6: Comparative Evaluation of Hybrid Molecule-Based Studies Targeting Alzheimer's Disease

Author(s) & Year	Objective	Method Used	Key Findings	Superiority of Present Study
Queda et al., 2021 [12]	To design novel donepezil–arylsulfonamide hybrids as multitarget-directed ligands for AD	Synthesis, in vitro AChE and BACE-1 assays, ADMET profiling	Demonstrated dual inhibition potential with acceptable ADMET properties	Present study includes both computational and experimental validation (docking + IC_{50}), and confirms BBB penetration and bioavailability in selected hybrids
Rajeshwari et al., 2019 [13]	To synthesize tacrine–benzothiazole hybrids as dual inhibitors	Chemical synthesis and biological assays	Showed potent AChE inhibition and amyloid aggregation suppression	Present study offers better pharmacokinetic profiling (SwissADME, pkCSM), dual-targeting with optimized BBB penetration, and correlation between in silico and biological data
Saxena & Saini, 2018 [14]	Review of structural hybrid strategies for AChE inhibition	Literature survey	Identified key pharmacophoric combinations and hybrid frameworks for AD drug design	Present study progresses from theory to practical synthesis, biological evaluation, and introduces dual enzyme inhibition (AChE + BACE-1) with correlation validation
Saxena & Dubey, 2019 [15]	To summarize enzyme targets and AChE inhibitors in AD	Review and mechanistic discussion	Highlighted structural activity relationships (SARs) and	Present work develops SAR-based hybrids, validates them experimentally,

			limitations of existing AChE inhibitors	and overcomes monotherapy limitations through multitarget inhibition
Swetha et al., 2019 [16]	To design multifunctional hybrid sulfonamides for AD therapy	Hybrid synthesis, docking, and enzyme inhibition studies	Demonstrated antioxidant and neuroprotective effects with moderate enzyme inhibition	Present study delivers lower IC_{50} values, stronger binding affinities, drug-likeness, and excellent BBB permeability, showing higher therapeutic potential

As the comparative study shows, the previous works have achieved notable results towards the creation of multitarget hybrid substances that could treat Alzheimer disease; however, it was mainly limited to AChE-targeted inhibition with little involvement of pharmacokinetic study or deactivation of two enzymes. As an example, Queda et al. (2021) and Rajeshwari et al. (2019) proposed highly potent hybrids that did not undergo substantial docking-to-IC 50 correlation or cross-validation of the ADMET. As a contrast, the current research managed to integrate structure-based design and effective synthesis, in addition to the validation of biological activity with in vitro IC 50 tests, ADMET forecasting, and analysis of BBB permeability. Moreover, it has a good negative correlation in relation of docking scores and IC 50, which provides better predictive power. Therefore, the research has established a more linked and rigorous temperament in the engineered-based searching of anti-Alzheimer drugs.

4.3. Implications of Findings

The discovery of hybrid molecules, including HM-07, has a great potential in the new generation of AD therapeutics, particularly those areas of addressing drawbacks of current AChE inhibitors, including donepezil and rivastigmine, that have only symptomatic benefits. AChE and BACE-1 targeting by the compounds used in this study are focused on maintaining cholinergic transmissions, as well as lowering the formation of amyloid plaques, two persistent pathological phenomena of AD. The dual-action approach may result in better clinical results, decreased disease progression, and decreased side effects because lower dosage of single-target agents will be needed. What is more is that the combination of the structure-based drug design (SBDD) and ADMET prediction faster drug discovery while enhancing the safety of potential compounds.

4.4. Limitations of the Study

Along with rather positive results, there are some limitations of the study. To begin with, the biological assessment was limited to in vitro enzyme tests, not completely simulating the human central nervous system. The in vivo validation is lacking and therefore

pharmacodynamic aspects (e.g. penetration into the brain, metabolism, long-term toxicity) have not been investigated. Also, as docking and IC 50 values were found to have a good correlation, the binding strength at the enzyme active sites could not be verified with the time course demonstrating molecular dynamics simulations. In addition, just 12 hybrid compounds were prepared and tested, and the scope of SAR maximization and optimization was thus restricted.

4.5. Suggestions for Future Research

Follow-up studies ought to involve animal studies in vivo as means of testing the pharmacokinetics, behavioral changes, and toxicity of the most promising compounds, specifically HM-07. Molecular dynamic simulations will also enhance an insight into the stability of binding and optimizing leads. Selectivity and potency can be optimized by growing the compound library and carrying out modelling of QSAR (Quantitative Structure Activity Relationship). In addition, formulation research, like nanoparticle delivery platforms might be pursued to enhance brain delivery efficacy. Collectively, such major avenues will help move such hybrid molecules to clinical trial stage.

5. CONCLUSION

The last part of this research summarizes main accomplishments, science interest and perspective of the research. It describes the constructive formulation and screening of hybrid molecules with dual targets against Alzheimer disease, especially on the lead HM-07. It also states how the study influenced the changing horizon of learning of multitarget drug development and throws light on the necessary further steps to develop this research into a clinical practice.

5.1. Summary of Key Findings

This Research has managed to design, synthesize and screen a panel of new hybrid compounds against two important enzymes associated with Alzheimer disease, acetylcholinesterase (AChE) and beta-secretase (BACE-1). HM-07 stood out as the lead compound among the twelve synthesized products, having the highest binding affinity in molecular docking simulations, as well as showing low values of IC 50 (0.41 uM in the AChE and 0.56 uM in BACE-1) in the course of in vitro enzyme assays. Good ADMET values were also reported including high blood-brain barrier (BBB), oral bioavailability, and absence of inhibition of cytochrome P450. The predictive accuracy of the computational approach was confirmed as a strong inverse relationship ($r = -0.86$) between docking scores and IC 50 values was observed.

5.2. Significance of the Study

The study is a highly integrated system incorporating the use of structure-based drug design (SBDD), synthetic chemistry and in silico screening alongside with biological evaluation in developing multitarget drug. As compared to a number of studies reported in the past that revolved around single AChE inhibition or weak pharmacokinetic properties, this study is centered towards dual enzyme inhibition along with strong pharmacokinetic characterization and SAR mediated molecular optimization. The results point to the therapeutic potential of HM-07 and other hybrid molecules in providing multifunctional effects characterized by

neuroprotection, amyloid inhibition, and cognitive enhancements, because of a substantial drug-likeness profile that could be applied to CNS.

5.3. Final Thoughts or Recommendations

The findings of this research form the basis of the future generation of dual inhibition-based therapeutics against Alzheimer. Nevertheless, to substantially reach clinical capacity, they still need more in vivo verification, molecular dynamics analysis, toxicity analysis, and efficient delivery methods. The development of a larger number of compounds together with optimization of the lead structures through QSAR modeling should also be done in the future. Then this study contributes to the role of hybrid molecule design in contemporary drug discovery and opens prospects associated with transferring such a promising molecule as HM-07 in preclinical and clinical development.

REFERENCES

1. Alarcón-Espósito, J., Mallea, M., & Rodríguez-Lavado, J. (2021). From hybrids to new scaffolds: the latest medicinal chemistry goals in multi-target directed ligands for Alzheimer's disease. *Current neuropharmacology*, *19*(6), 832-867.
2. Chainoglou, E., Siskos, A., Pontiki, E., & Hadjipavlou-Litina, D. (2020). Hybridization of curcumin analogues with cinnamic acid derivatives as multi-target agents against Alzheimer's disease targets. *Molecules*, *25*(21), 4958.'
3. Fancellu, G., Chand, K., Tomás, D., Orlandini, E., Piemontese, L., Silva, D. F., ... & Santos, M. A. (2020). Novel tacrine–benzofuran hybrids as potential multi-target drug candidates for the treatment of Alzheimer's Disease. *Journal of enzyme inhibition and medicinal chemistry*, *35*(1), 211-226.
4. Gontijo, V. S., Viegas, F. P. D., Ortiz, C. J., de Freitas Silva, M., Damasio, C. M., Rosa, M. C., ... & Viegas, C. (2020). Molecular hybridization as a tool in the design of multi-target directed drug candidates for neurodegenerative diseases. *Current neuropharmacology*, *18*(5), 348-407.
5. Hiremathad, A., Keri, R. S., Esteves, A. R., Cardoso, S. M., Chaves, S., & Santos, M. A. (2018). Novel Tacrine-Hydroxyphenylbenzimidazole hybrids as potential multitarget drug candidates for Alzheimer's disease. *European Journal of Medicinal Chemistry*, *148*, 255-267.
6. Jana, A., Bhattacharjee, A., Das, S. S., Srivastava, A., Choudhury, A., Bhattacharjee, R., ... & Ashraf, G. M. (2022). Molecular insights into therapeutic potentials of hybrid compounds targeting Alzheimer's disease. *Molecular Neurobiology*, *59*(6), 3512-3528.
7. Nakhate, K. T., Bharne, A. P., Verma, V. S., Aru, D. N., & Kokare, D. M. (2018). Plumbagin ameliorates memory dysfunction in streptozotocin induced Alzheimer's disease via activation of Nrf2/ARE pathway and inhibition of β-secretase. *Biomedicine & Pharmacotherapy*, *101*, 379-390.
8. Maramai, S., Benchekroun, M., Gabr, M. T., & Yahiaoui, S. (2020). Multitarget therapeutic strategies for Alzheimer's disease: review on emerging target combinations. *BioMed Research International*, *2020*(1), 5120230.
9. Mathew, B., Parambi, D. G., Mathew, G. E., Uddin, M. S., Inasu, S. T., Kim, H., ... & Carradori, S. (2019). Emerging therapeutic potentials of dual-acting MAO and AChE

inhibitors in Alzheimer's and Parkinson's diseases. *Archiv der Pharmazie*, *352*(11), 1900177.

10. Padhi, D., & Govindaraju, T. (2022). Mechanistic insights for drug repurposing and the design of hybrid drugs for alzheimer's disease. *Journal of Medicinal Chemistry*, *65*(10), 7088-7105.
11. Piemontese, L., Tomás, D., Hiremathad, A., Capriati, V., Candeias, E., Cardoso, S. M., ... & Santos, M. A. (2018). Donepezil structure-based hybrids as potential multifunctional anti-Alzheimer's drug candidates. *Journal of enzyme inhibition and medicinal chemistry*, *33*(1), 1212-1224.
12. Queda, F., Calò, S., Gwizdala, K., Magalhães, J. D., Cardoso, S. M., Chaves, S., ... & Santos, M. A. (2021). Novel donepezil–arylsulfonamide hybrids as multitarget-directed ligands for potential treatment of alzheimer's disease. *Molecules*, *26*(6), 1658.
13. Rajeshwari, R., Chand, K., Candeias, E., Cardoso, S. M., Chaves, S., & Santos, M. A. (2019). New multitarget hybrids bearing tacrine and phenylbenzothiazole motifs as potential drug candidates for Alzheimer's disease. *Molecules*, *24*(3), 587.
14. Saxena, A., & Saini, R. (2018). The structural hybrids of acetylcholinesterase inhibitors in the treatment of Alzheimer's disease: A review. *J Alzheimers Neurodegener Dis*, *4*, 015.
15. Saxena, M., & Dubey, R. (2019). Target enzyme in Alzheimer's disease: Acetylcholinesterase inhibitors. *Current topics in medicinal chemistry*, *19*(4), 264-275.
16. Swetha, R., Kumar, D., Gupta, S. K., Ganeshpurkar, A., Singh, R., Gutti, G., ... & Singh, S. K. (2019). Multifunctional hybrid sulfonamides as novel therapeutic agents for Alzheimer's disease. *Future Medicinal Chemistry*, *11*(24), 3161-3178.

.