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In Silico Evaluation of Daechualkaloid A Maltoxazine as a Potential Acetylcholinesterase Inhibitor for Alzheimer's Disease: A Docking, DFT, and SwissADME-Based Study

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ABSTRACT: Daechualkaloid A Maltoxazine is a naturally occurring alkaloid derived from medicinal plants traditionally used in Asian medicine for treating various diseases. Due to its potential neuroprotective properties, it was investigated in this study for its inhibitory activity against acetylcholinesterase (AChE), a key therapeutic target in Alzheimer's disease (AD). In this study, we employed in silico approaches, including density functional theory (DFT) and molecular docking, to evaluate the structural stability and binding affinity of Daechualkaloid A Maltoxazine. DFT calculations were carried out using Gaussian 09W software at the B3LYP/6-311G** level of theory. Molecular docking was performed using PyRx software against the AChE protein (PDB ID: 1EVE) to determine the binding interactions. Additionally, SwissADME analysis was conducted to predict the pharmacokinetic and drug-likeness properties of the compound. DFT results revealed HOMO and LUMO energy values of -5.622 eV and -1.415 eV, respectively, indicating a stable molecular configuration with an energy gap of 4.207 eV. Molecular docking showed a strong binding affinity with a docking score of -8.6 kcal/mol. Key interactions included hydrogen bonding with residues GLY119 and SER200 of AChE. The SwissADME analysis demonstrated favorable pharmacokinetic properties, including optimal lipophilicity, water solubility, gastrointestinal absorption, and the ability to cross the blood-brain barrier. The compound also met several drug-likeness criteria, including Lipinski's rule of five. The in-silico findings suggested that Daechualkaloid A Maltoxazine has promising potential as an acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. However, as this study is limited to computational predictions and a single target, further experimental validation/ investigations are necessary to confirm its therapeutic potential.

Keywords: Daechualkaloid A Maltoxazine; SwissADME analysis; Physiochemical properties; DFT calculations; Molecular docking

1. Introduction

Alzheimer's disease (AD) is marked by a progressive decline in functional and cognitive abilities and is the most prevalent form of neurodegenerative disorder (Liu et al., 2021). It is now among the top global death-causing diseases, responsible for over 1.6 million mortalities each year (Collaborators et al., 2021).

In 2019, 50 million individuals were affected by AD, with a rapidly aging global population, this number is estimated to surpass 150 million by 2050 (Zingel et al., 2021). The cost of managing AD is expected to soar to 2 trillion dollars by 2030, posing a challenge of a heavy financial burden on healthcare systems (Wimo et al., 2017). The most widely accepted pathological explanation of AD is the cholinergic hypothesis, which proposes that a deficit in acetylcholine levels contributes to the cognitive decline observed in AD patients. Consequently, the development of acetylcholinesterase inhibitors (AChE-Is) has become a central strategy for symptom management in AD (Mohamed and PN Rao, 2011). Current FDA-approved AChE inhibitors, such as donepezil, rivastigmine, and galantamine, have demonstrated clinical efficacy in mild to moderate AD (Pettenati et al., 2003). However, these drugs exhibit several limitations, including short-lived benefits due to upregulation of AChE expression, poor brain vs. peripheral selectivity, and undesirable side effects such as insomnia, vivid dreams, gastrointestinal distress, and urinary incontinence. Moreover, treatment discontinuation often leads to rapid worsening of symptoms (Mesulam, 1999). Therefore, the discovery of novel AChE-Is with better brain selectivity, improved tolerability, and enhanced pharmacokinetic profiles remains a critical area of research.

Daechualkaloid A Maltoxazine (DAM) is a naturally occurring alkaloid (table 1) featuring oxygen and nitrogen heterocyclic moieties. It has been isolated from medicinal plants such as *Zizyphus jujuba var. inermis* and *Nicotiana tabacum*. *Zizyphus jujuba var. inermis*, a member of the Rhamnaceae family, is widely used in Asian traditional medicine for its reported anticancer, antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, sedative, and anxiolytic effects (Rodriguez Villanueva and Rodriguez Villanueva, 2017). Structurally, DAM contains a ketone functional group and an ether bridge—features commonly associated with pharmacological activity. Similar heterocyclic alkaloids have demonstrated diverse biological effects, including neuroprotective, anti-inflammatory, and antimicrobial activities (Saxena, 2007). A wide range of natural alkaloids, including isoquinoline derivatives, indole-based scaffolds, and terpenoid/steroid alkaloids, have been explored for their potential in AD therapy (Mukherjee et al., 2007). Among these, rivastigmine (Birks and Evans, 2015) and galantamine (Lilienfeld, 2002) are available for managing symptoms of mild to moderate AD patients. Despite the promising pharmacological profile of DAM and its structural resemblance to other bioactive alkaloids, it has not yet been explored as a potential acetylcholinesterase inhibitor through computational approaches. Given the limitations of current drugs and the need for new therapeutic candidates, this study aims to evaluate DAM as a potential anti-Alzheimer's agent using modern computer-aided drug design (CADD) techniques.

CADD integrates bioinformatics and cheminformatics tools to predict a compound's biological activity, pharmacokinetics, and safety profile. It significantly reduces the time, cost, and failure rate of traditional experimental drug discovery (Al-Otaibi et al., 2021). Among these tools, density functional theory (DFT) allows for the evaluation of molecular stability and reactivity by analyzing quantum chemical descriptors such as HOMO-LUMO energies (Bello Roba and Umar Bello; Golea et al., 2023). Additionally, ADME analysis is essential for predicting pharmacokinetic behavior, which drastically reduces the failures in the clinical phase (Hay et al., 2014). Similarly, Docking, an applied computational technique, is used to evaluate how strongly a ligand or a small molecule can bind to the target, predictions of binding affinities (Hantoush et al., 2022).

In this study, we employed an integrated in silico approach—comprising DFT calculations, molecular docking, and SwissADME profiling—to evaluate the electronic properties, drug-likeness, and inhibitory potential of Daechualkaloid A Maltoxazine (DAM) against acetylcholinesterase. The findings provide useful computational insights into DAM's potential as an anti-Alzheimer's agent. The study is limited to theoretical predictions and a single biological target, necessitating further experimental validation and broader biological exploration.

2. Methodology

2.1. Density Functional Theory (DFT) Calculations

DFT calculations were performed using the Gaussian 09W software package (https://gaussian.com/glossary/g09) (Bayrakdar et al., 2015). Geometry optimization and energy minimization of the compound were carried out at the level B3LYP using 6-311G** basis set

(Krishnan et al., 1980). The calculations were performed in the gas phase at default settings. GaussView 6.0 was used for drawing structure and for visualization of optimized geometries, frontier molecular orbitals, and electrostatic potential maps. After frequency and geometry optimization, Global reactivity descriptors such as ionization potential, electron affinity, chemical hardness, softness, electronegativity, chemical potential, and electrophilicity indices (including electroaccepting and electrodonating powers) were calculated based on the frontier molecular orbital energies (HOMO and LUMO) derived from DFT calculations. These parameters were estimated using well-established theoretical relationships grounded in Koopmans' theorem and conceptual density functional theory (DFT), as described in the literature (Afrdi et al., 2024; Koopman, 1931).

2.2. ADME Analysis

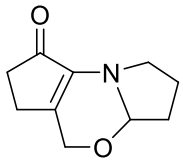
The physiochemical and pharmacokinetic properties of the targeted phytochemical were evaluated using the SwissADME web tool (http://www.swissadme.ch/) (Daina et al., 2017). The structure was uploaded in SMILE format for its properties evaluation, like physiochemical, lipophilicty, solubility, drug-likeness, medicinal chemistry, and pharmacokinetic properties.

2.3. Molecular Docking

Molecular docking studies were performed using PyRx virtual screening software (https://pyrx.sourceforge.io). The protein structure was retrieved from the Protein Data Bank (PDB ID: 1EVE) and prepared using Discovery Studio. Water molecules, hetero atoms, and other ligands were removed for accurate docking. The ligand was taken from the KNApSAck database (https://www.knapsackfamily.com/knapsack_core/top.php).

The protein and ligand were saved in appropriate formats, and their binding energies were measured for their ligand-protein interactions.

Table 1: Name, SMILE, KNApSAck_ID, and Structures of DAM

Compound	SMILE	C_ID	Structure
Daechualkaloid A Maltoxazine	O=C1CCC2=C1N3C(CCC3)OC2	C00055390	

1. Results

3.1 Density Functional Theory (DFT) Calculations

3.1.1 Chemical Reactivity Descriptors

DFT calculations were performed using Gaussian 09W. **Figure 1** shows the optimized geometry of Daechualkaloid A Maltoxazine **(DAM)** taken from Gaussian calculations. The Chemical reactivity descriptors help identify active sites, determining stability and reactivity, and predict interactions between drug candidates and biological targets. Different chemical reactivity descriptors were calculated, including the HOMO-LUMO energy gap, ionization potential, electron affinity, chemical potential, hardness and softness, and others. The chemical reactivity descriptors such as ionization potential, corresponds to HOMO, and Electron affinity, corresponds to LUMO, values are -5.622 eV, -1.415 eV respectively. These values contribute to the HOMO-LUMO energy gap of 4.207, which reflects the difference in the energy of highest occupied molecular orbit (HOMO) and lowest un-occupied molecular orbit (LUMO). Chemical Potential (μ), hardness (η), and softness (S) values are -3.519 eV, 2.104 eV, and 0.475 eV^{-1} respectively, suggesting a moderate reactivity profile. Electrophilicity Index (ω) for C1 is 2.944 eV, which qualifies the stabilization energy gained upon acquiring additional electronic charge. Additionally, the electron-donating power (ω+) and electron-accepting power (ω-) are 1.446 and 4.966 au, respectively, emphasizing the dual ability of a molecule to accept and donate electrons. The electronegativity (χ) is observed as 3.519 eV, while the back-donation Energy is -0.526 eV, indicating modest stabilization due to electron back-donation effects. The maximum amount of electronic charge that a system can accept (ΔNmax) is calculated as 0.836 eV.

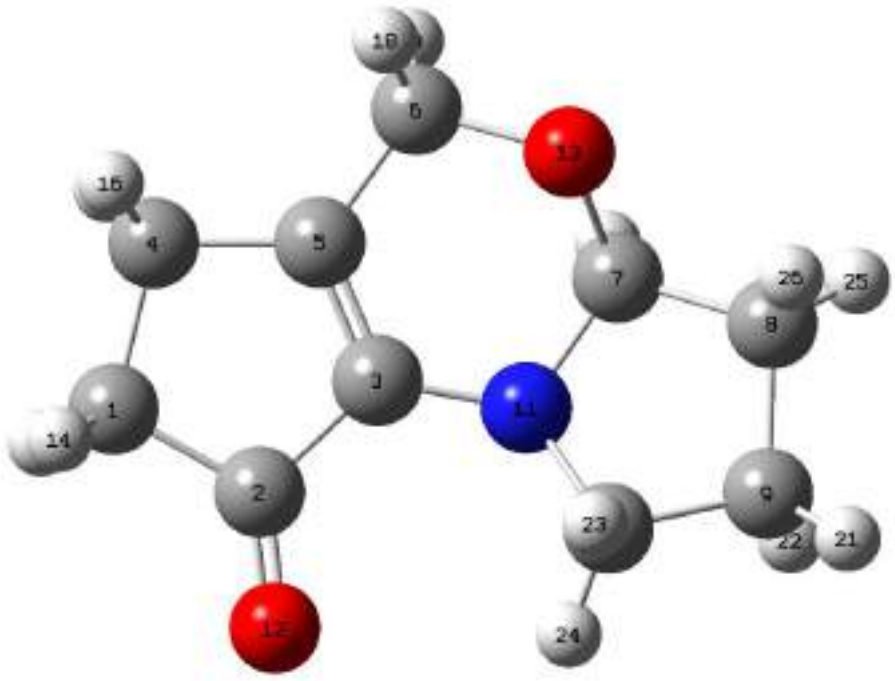


Figure 1: Optimized Geometry of DAM

Table 2. The Chemical Reactivity Descriptors of DAM

Parameters (eV)	**Values**
Ionization Potential (I), H (–I) (eV)	-5.622
Electron Affinity (A), L (–A) (eV)	-1.415
Energy Gap (ΔE) (eV)	4.207
Chemical Potential (μ) (eV)	-3.519
Hardness (η) (eV)	2.104
Softness (S) (eV^{-1})	0.475
Electrophilicity Index (ω) (eV)	2.944
Electrophilicity Index (ω+) (au)	1.446
Electrophilicity Index (ω-) (au)	4.966
Electronegativity (χ) (eV)	3.519
Back-donation Energy (eV)	-0.526
Maximum Electron Transfer (eV) (ΔNmax)	0.836

H (–I): highest occupied molecular orbital energy (HOMO), **L (–A)**: lowest unoccupied molecular orbital energy (LUMO), **ΔE (L–H)**: energy gap (HOMO-LUMO gap), **μ**: chemical potential, **η**: chemical hardness, **S**: chemical softness, **ω:** electrophilicity index, ω^+: electron donating power, ω^-: electron accepting power, **ΔNmax**: maximum number of electrons transferred, $\Delta\varepsilon_{back\text{-}donat}$: back-donation energy.

3.2.2 Thermodynamic Properties of DAM

Table 3 represents the thermochemical quantity values of the compounds. The main thermodynamic quantities ΔE (au), ΔH (au), and ΔG (au) for the C1 are 593.8171, 593.8054, and 593.8538, respectively. Similarly, the $\Delta E_{thermal}$ value of the compound is 144.806 kcal/mol, reflecting the internal energy of the system at the given temperature. In the same way, the entropy is calculated as 101.802 cal/mol K, indicating the degree of disorder in the system. The CV value of the compound is determined as 42. 259 cal/ mol K. Dipole moment (DM) determined as 2.5854 debye, suggesting moderate polarity, while Polarizability (α) is reported as 117.0460 au.

Table 3: The Thermochemical Quantities, Polarizability, and Dipole Moment values of DAM

Parameters	Values
ΔE(au)	-593.8171
ΔH(au)	593.8054
ΔG (au)	593.8538
$\Delta E_{thermal}$ (kcal/mol)	144.806
Cv (cal/molK)	42.259
S (cal/molK)	101.802
DM (debye)	2.5854
α (au)	117.0460

ΔE: electronic energy, **ΔH**: enthalpy, **ΔG**: gibbs free energy, **$\Delta E_{thermal}$**: thermal energy, **Cv**: heat capacity at constant volume, **S:** entropy, **DM**: dipole moment, **α**: polarizability.

3.2.3 Frontier Molecular Orbitals (FMO) and Molecular Electrostatic Map (MEP)

Figure 2(A) shows the molecular orbitals of **DAM**. The HOMO was predominantly spread over the six membered ring and adjacent electron-withdrawing carbonyl group. In contrast, the LUMO was mainly distributed over the carbonyl carbon and nearby atoms, highlighting these areas as electrophilic centers susceptible to nucleophilic attack. **Figure 2(B)** illustrates the MEP surface of the MAD, which visualizes the electrophilic and nucleophilic regions. The red region indicates high electron density and negative electrostatic potential, while the blue region shows low electron density and positive electrostatic potential. The red region over the oxygen atom indicates a nucleophilic center suitable for interaction with an electrophilic site. Contrary to this, the light blue surface around hydrogen and carbon atoms represents an electrophilic region exposed to nucleophiles.

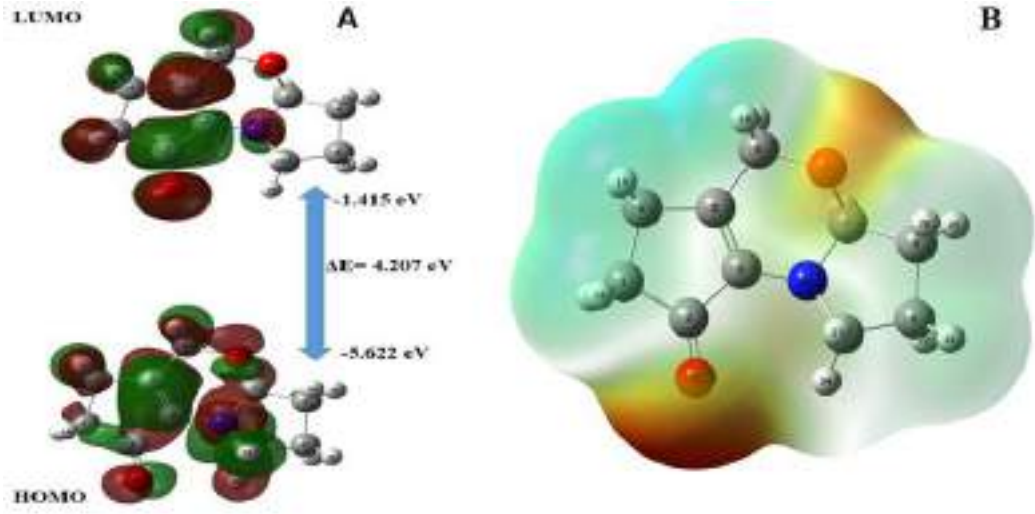


Figure 2: Frontier Molecular Orbitals **(A)** and Molecular Electrostatic Potential Surface **(B)** of DAM

3.3. ADME Analysis

SwissADME results of **DAM** are depicted in **Figure 3,** which shows the physiochemical properties, lipophilicity, solubility, pharmacokinetic properties, druglikeness properties, and medicinal chemistry.

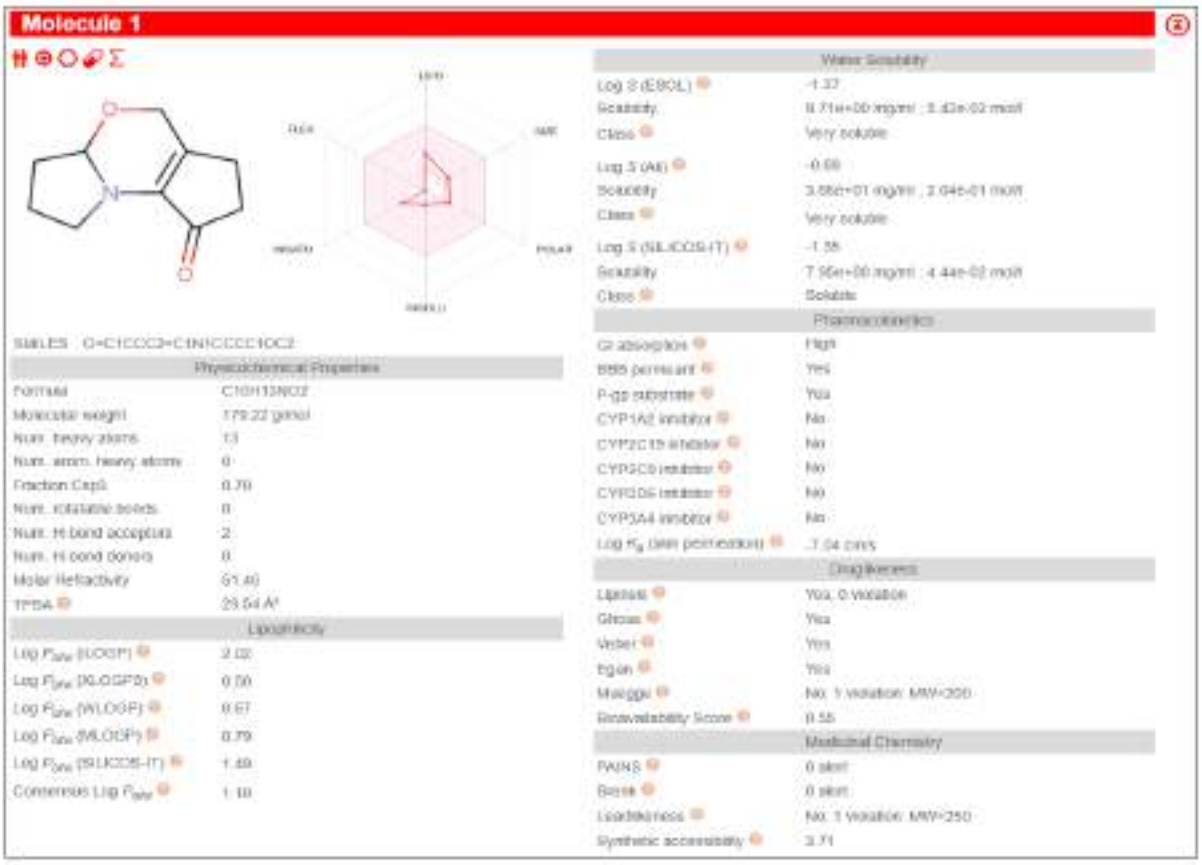


Figure 3: SwissADME results of DAM

3.3.1 Physicochemical properties

The physicochemical properties of the compound were evaluated using SwissADME, depicted in the **Figure 3** The compound has a molecular weight of 179.22 g/mol, which falls within the acceptable range. According to Lipinski's rule of five, a suitable range for molecular weight is typically between 100-600 g/mol, supporting the potential of the compound to pass through biological membranes. The number of heavy atoms was found to be 13, which contributes to the compound's synthesis feasibility and permeability. The compound is missing aromatic heavy atoms. The fraction of Sp3-hybridized carbon for the compound is 0.7. Additionally, the number of rotatable bonds of the compound is 0, which is within the optimal range: 0-11, which represents good molecular flexibility. The number of hydrogen bond acceptors is 2, while the number of hydrogen bond donors is 0. These values comply with Lipinski's rule of five (HBA ≤ 10, HBA<5). The molar refractivity value is 51.46, indicating a good binding affinity of the compound with the target. TPSA value is 29.54 A2, suggesting a good membrane permeability.

3.3.2. Lipophilicity

Lipophilicity indicates the membrane permeability potential of a drug, influencing the drug distribution in the body. SwissADME software determines the lipophilicity of the compound as a consensus logP value, which is the average of different algorithms, given in **Figure 3**. The compound showed a 1.1 consensus logP value.

3.3.3 Water solubility

The first step for drug absorption in the body is its solubility in aqueous body fluids, i.e, saliva, stomach acid, or blood. The water solubility of the compound was determined by three methods, which show "very soluble" for ESOL and Ali method, and 'soluble' for SILIOC-IT method **Figure 3.**

3.3.4 Pharmacokinetics

Absorption refers to how a drug enters the bloodstream from the site of administration, such as the gut or muscle. This process determines a drug's bioavailability, which is the fraction of the drug that reaches systemic circulation. The white region of the Boiled Egg Model (**Figure 4**) indicates high gastrointestinal (GI) absorption, suggesting good oral bioavailability. Similarly, distribution describes how a drug spreads

throughout the body, including its ability to reach the tissues and fluids. It is influenced by factors such as blood blood-brain-barrier (BBB), which may affect its central nervous system availability. The yellow region of the Boiled Egg Model shows the BBB crossing ability of a compound. The compound is a P-gp substrate, which can be knocked out of the body. Drug metabolism involves biotransformation, often mediated by cytochrome P450 (CYP450). Absence of CYP450 enzyme inhibition in **DAM** is favorable because it indicates a low risk of drug-drug interaction and has fewer metabolic side effects. The skin permeability expressed as log Kp is -0.74 cm/s, indicating moderate transdermal diffusion potential **Figure 3**.

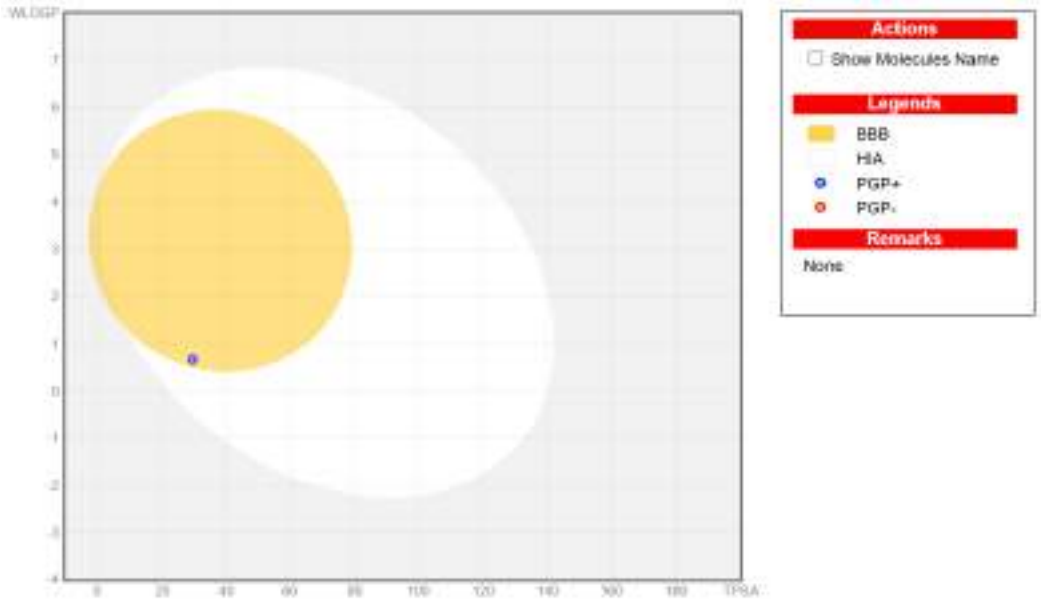


Figure 4: Boiled Egg Model of DAM

3.3.5. Drug-likeness and Medicinal Chemistry

Regarding drug-likeness properties, **DAM** showed only one violation, which pertains to its molecular weight being below 250. Despite this, the compound complies with major drug-likeness rules, including Lipinski, Ghose, Veber, and Egan filters. Muegge rule is not fully met due to a minor molecular weight issue (the optimum range is 200-600, and 179.92 is under 200). Bioavailability score is 0.55, indicating a reasonable likelihood of oral bioavailability. Furthermore, **DAM** showed zero alerts for both PAINS (Pan Assay Interference Compounds) and Brenk filters, indicating the absence of any problematic substructures typically associated with false positives or problematic pharmacophores. Synthetic accessibility is 3.71, suggesting moderate ease of synthesis **Figure 3**.

3.3.6. Molecular Docking

Molecular docking was performed between DAM and acetylcholinesterase (AChE), which is a potential target in Alzheimer's disease, using the PyRx virtual screening tool. -8.6 kcal/m docking score was recorded as a result of interaction between the ligand and the protein structure **(Table 4)**. The docking study also showed significant interaction between the ligand and the amino acid residues. TRP84, GLY119, SER200, and HIS440 residues of the targeted proteins showed interactions. Residues GLY119 and SER200 form conventional hydrogen bonding with the ligand at distances of 3.20 Å and 2.95 Å, respectively. Similarly, the TRP84 and HIS440 amino acid residues of the protein structure each form one pi-alkyl bond with the ligand at distances of 4.09 and 4.80 Å. These interactions give stability to the ligand-protein complex, making it more stable **Figure 5.**

Table 4: Docking results of MAD with AChE (1EVE).

Compound	Binding affinity	Distance b/w Lig-Residue	Residues Involved	Type of Interactions
DAM	-8.6	2.95 3.20 4.80 4.09	Ser200 GLY119 HIS440 TRP84	Conventional H-bond Conventional H-bond Pi-Alkyl Pi-Alkyl

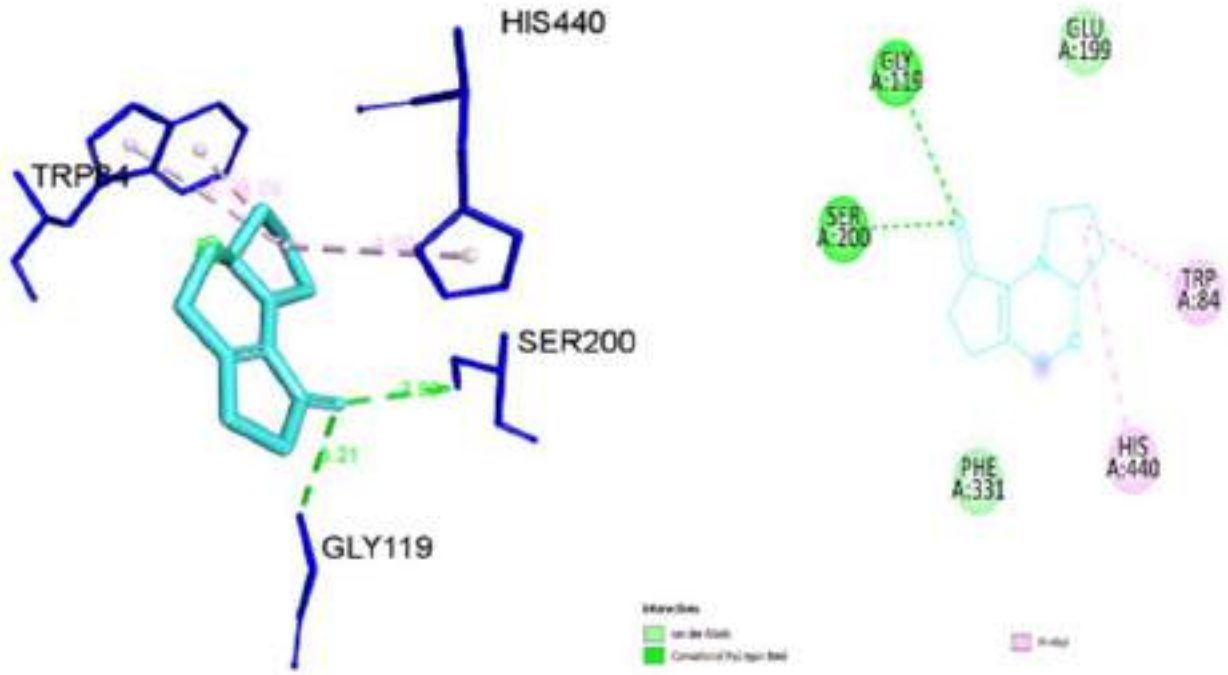


Figure 5: 2D and 3D visualization of docking interactions of DAM with AChE.

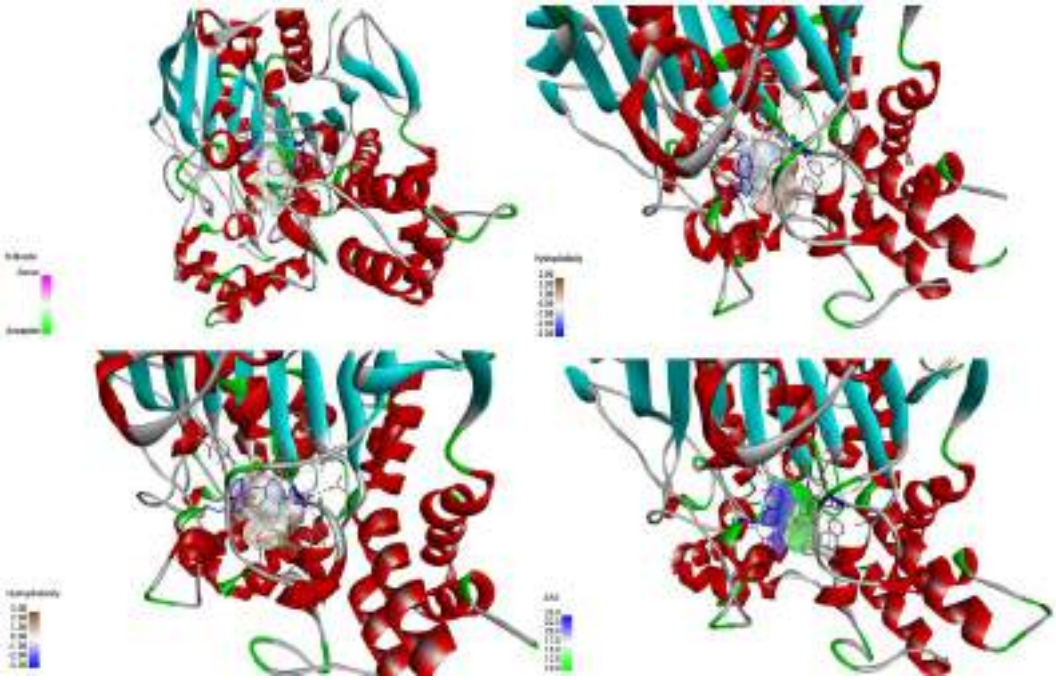


Figure 6*:* Molecular docking visualization of DAM within the active site of the target protein.

4. Discussion

Determining the stability and reactivity of the compound is crucial for its potential use in drug design. In this respect, the chemical reactivity descriptors provide insights into the molecule's behavior under biological conditions and its suitability for interacting with biomolecular targets. Electronic properties such as a lower ionization potential (I) -5.622 eV value are associated with antioxidant potential, reflecting the compound's ability to donate electrons easily. This suggests that the compound serves as a free radical scavenger, enhancing its therapeutic relevance. Along with other descriptors, electron affinity is given in **Table 2**, which is -1.415 eV. The negative value shows the compound's ability to accept electrons, supporting the electrochemical stability of the compound and the potency of the compound to form stable complexes with biological targets. The energy gap between the highest occupied molecular orbit and the lowest unoccupied molecular orbit determines stability and reactivity **(Afrdi et al., 2024)**. HOMO-LUMO energy gap is 4.207, which indicates a moderate level of chemical stability, and the compound is desirable for biological interactions.

The Chemical Potential (μ) shows the ability of the ligand to gain more electrons hence; it is given as negative electronegativity. Its more negative value indicates more stability -3.519 eV. Hardness (η) is the resistance to change in its e-cloud; its value is determined as 2.104 eV. Softness (S), the ease of deformation, is measured as 0.475 eV^{-1}, which indicates a moderate degree of chemical reactivity. Electrophilicity Index (ω) calculated as 2.944 eV, the value shows that the compound is good for interactions with biological nucleophiles. Similarly, Electrophilicity Index (ω+) is the electron-donating power of a compound (Parr et al., 1999; Roy et al., 2005), calculated as 1.446 au. Electrophilicity Index (ω-), on the other hand, is electron-accepting power and measured as 4.966 au. Electron donating power (ω+) is less than electronegativity (χ) calculated as 3.519 eV, which denotes that THE compound is ambivalent; this dual character suggests that the compound has the potential to bind with diverse biological targets.

Table 2 represents the thermochemical quantities, dipole moment, and polarizability values of the compounds. The main thermodynamic quantities ΔE (au), ΔH (au), and ΔG (au) for **DAM are** calculated as 59.8171, 593.8054, and 593.8538, respectively, predicting the favorability of biological interactions. Also, the ΔEthermal value of the compound is 144.806 kcal/mol, indicating sufficient energy for metabolic transformations. In the same way, the entropy is calculated as 101.802 cal/mol K, suggesting good thermodynamic feasibility in molecular binding. The CV value of the compound is determined as 42. 259 cal/ mol K indicates a compound's ability to store thermal energy, which is relevant to time-dependent biological reactions. DM (dipole moment) is 2.5854 debye, suggesting a balanced distribution of charge, enhancing interactions with polar molecules, i.e., nucleic acid or protein. Polarizability ($\boldsymbol{\alpha}$) is reported as 117.0460 au, indicating that the molecules' electronic cloud can be easily distorted, contributing to drug-target interactions such as strong van der Waals and dispersion interactions.

The HOMO-LUMO orbitals of the investigated compounds are presented in **Figure 2 (A)**. These figures demonstrate the spatial distribution of HOMO and LUMO of the compound under investigation. The HOMO extends over aromatic rings and heteroatoms or localized over central conjugated system. This signifies that these electron-rich regions show the electron-donating ability of a molecule. The potential site for electrophilic attack is near oxygen and nitrogen due to the concentration of electron density near those atoms. LUMO, in contrast, shows regions of electron deficiency, particularly carbonyl, which is the favorable site for nucleophilic attack. The analysis supports the narrative that the molecule shows ambient reactivity with distant electrophilic and nucleophilic regions.

MEP of a compound shows various colors, and the electrostatic potential of a compound could be studied through this color-coded map on MEP. The charge distribution of the compound is represented as;

a. Blue shows the presence of an electron-deficient or positively charged region.
b. Light blue signifies a mildly electron-deficient area.
c. Red represents a negatively-charged or electron-rich area. Yellow highlights the slightly electron-rich region
d. Green indicates a neutral region (Feizi-Dehnayebi et al., 2022)

It is evident from the image that the most negatively charged region-red area, is located around the oxygen atoms, whereas the positively

charged surfaces are positioned over the hydrogen atoms.

The primary objective of drug discovery is to identify compound that exhibits good absorption, distribution, metabolism, and excretion (ADME) properties with a favorable pharmacokinetics and pharmacodynamics profile. An ideal drug should possess features such as oral bioavailability, high target specificity, absence of off-target effects, and minimal toxicity (Egan et al., 2000). A safe and effective drug molecule adheres to Lipinski's rule of five, which argues that for effective drug absorption the molecular weight should be equal or less than 500 Da, Partition co-efficient logP or lipophilicity should be ≤ 5, HBA (hydrogen bond acceptor) parameters and number of nitrogen and oxygen (N + O atoms) should be < 10, and HBD (hydrogen bond donor) parameter should be < 5 (Lipinski et al., 1997). If a compound violates more than one of above-mentioned conditions, then it will not be considered safe for oral administration. The compound C1 does not violate any of these conditions. It has a molecular weight of 179.22 g/mol, indicating its potential suitability for oral drug development. To reach the systemic circulation drug must pass through cell membranes, and small molecules can move via passive diffusion across cell membranes, following a concentration gradient (LZ, 1991).

The number of heavy atoms is 13, supporting the compound's synthesis feasibility and good permeability. Similarly, the compound has no aromatic heavy atoms, which may influence its metabolic stability and interaction with biological targets. Furthermore, to get insights into molecular three-dimensional complexities, the calculation of the Fsp3 value is pivotal. It reflects the degree of carbon saturation and is calculated as the number of sp3 hybridized carbons divided by the total number of carbon atoms (Dehelean et al., 2020). DAM showed a fraction Csp3 value of 0.70, suggesting a high degree of saturation and potentially favorable pharmacokinetic properties. The number of rotatable bonds for the compound is 0, which represents good molecular flexibility. For a drug to be effective in the CNS, the count of rotatable bonds should be fewer than 8 (Pajouhesh and Lenz, 2005).

Molar refractivity of **DAM** is 51.46, which represents the binding affinity of the compound with the target, giving insight into size and polarizability. MR shows how easily an electric field of a compound can be distorted when it interacts with the electric field of other molecules. A higher MR value suggests greater polarizability, which can enhance molecular interactions, particularly at the receptor binding site (Kumar et al., 2021). Optimal MR value reflects a good fit for the receptor binding site. It correlates with lipophilicity, which facilitates the ability of a compound to cross cellular membranes. This, in turn, affects the compound's absorption, distribution, and bioavailability. MR is also important for QSAR (quantitative structure activity relationship) studies to predict the biological activity of the compound (Saxena, 1995). The topographical polar surface area (TPSA) value is 29.54 $Å^2$, indicating good passive membrane permeability, which represents apparent polarity (Daina et al., 2017). The compound complies fully with RO5, suggesting good bioavailability.

LogP (octanol/water partition coefficient) values obtained from various predictive models fall within the optimum range determined by the RO5 rule, indicating suitable lipophilicity. High value of $LogP_{0/w}$ indicates hydrophobicity, which means more affinity of the drug for lipid membranes. Low value of $LogP_{0/w}$ indicates hydrophilicity, which means more affinity of the compound for the aqueous phase (Kumer et al., 2022). Drug distribution in the body is studied via lipophilicity. It influences the compound's ability to interact with the biological membranes. Generally, the higher the logP value indicates the higher the lipophilicity, which can enhance membrane permeability and also affect drug solubility. Lipophilicity depends on molecular size, polarity, and hydrogen bonds in a compound (Zerroug et al., 2019). Consensus logP value is 1.10, while iLOGP, XLOGP3, and other Log values lie within the optimal range (0-3), suggesting the compound's potential for effective absorption and distribution (Pastewska et al., 2022). Water solubility was also predicted through different methods through SwissADME software, which shows "very soluble" for ESOL and Ali method, and 'soluble' for SILIOC-IT method. (Aqueous solubility is within range (-4-0.5 log mol/L) (Taylor and Triggle, 2007).

The pharmacokinetic properties showed that **DAM** exhibits a high gastrointestinal (GI) absorption value, which indicates good oral bioavailability. Distribution of the drug in the body largely depends on blood blood-brain barrier (BBB), p-gp substrate, and skin permeability. Blood blood-brain barrier separates the central nervous system (CNS) from the peripheral nervous system. It serves as the agent to clean the toxins and metabolic waste from the

brain into to blood. BBB plays a critical role in CNS homeostasis by regulating the movement of nutrients between the brain and the blood (Pardridge, 1990). The results show that **DAM** can cross the blood-brain-barrier and has the potential to be used as CNS CNS-targeted drug. Unfortunately, DAM is a P-gp substrate, which means it can be recognized by glycoprotein P-gp, which is a membrane-bound protein responsible for transporting substances in and out of cells. Being a P-gp substrate indicates that a compound could be actively pumped in and out of a cell by glycoprotein, which can influence drug absorption, distribution, and resistance. While P-gp substrate may reduce intracellular drug accumulation, it is leveraged in designing the targeted drug delivery strategies, especially in the treatment of certain diseases, i.e., diseases associated with the CNS (Endicott and Ling, 1989).

When two drugs compete for the same enzyme binding site, the inhibition of these enzymes becomes a major factor in determining drug-drug interaction (Domínguez-Villa et al., 2021). This inhibition reduces the elimination or metabolism of various used drugs and therapeutic agents. Thus, CYP inhibition results in toxicity or elevated plasma concentrations. CYP2D6 is crucial because it has high genetic variability, and individuals with reduced enzyme activity experience decreased drug effectiveness or adverse reactions (Manikandan and Nagini, 2018). CYP2C19 is responsible for detoxifying carcinogens and plays a role in metabolizing various drugs or activating environmental procarcinogens. CYP2C9 exhibits potential for metabolizing drugs that have a narrow therapeutic window (Daly et al., 2017). Absence of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 inhibitors indicates that **DAM** shows no risk of drug-drug interaction and no side effects. Skin permeability (log Kp) value is -0.74 cms-1, which is relevant considering thermal delivery options.

The structural features of a compound are referred to as drug-likeness, which play an important role in drug development. Several parameters, including Lipinski's rule, blood–brain barrier (BBB) penetration, CYP1A2 interaction, hydrogen bond donors (HBD), topological polar surface area (TPSA), gastrointestinal (GI) absorption, and bioavailability, are evaluated in this context. Only one violation is observed at molecular weight lesser than 250, Lipinski, Ghose (good size and atom count), Veber (TPSA complies and low number of rotatable bonds), and Egan (good predictions of permeability) are all complying except Muegge due to minor molecular weight issue (optimum range is 200-600, and 179.92 is under 200). **DAM** has all the characteristics essential for any therapeutic molecule. It has minimum toxicity and maximum efficacy and can reach the target site with a bioavailability score of 0.55.

Positive values in drug likeness score indicate favorable drug-like properties. A compound might have toxicity risk due to primary or binary mutagenic, irritant, tumorigenic, and reproductive effects, which is evaluated by risk score. PAINS zero alert indicated the safety of a drug. Similarly, Brenk is also zero alert, which indicates the absence of any problematic substructures. Synthetic accessibility is 3.71, indicating the synthesis of DAM is not tough.

The docking results indicate significant binding affinity of the MAD with acetylcholinesterase enzyme, involved in Alzheimer's disease. Visualization of results revealed a number of interactions with the amino acid residues of the target protein. The carbonyl oxygen of the five-ring fragment makes two hydrogen bonds with the GLY119 and SER200 amino acids, anchoring it near the enzyme's catalytic site. The hydrogen bond distances between the residue and oxygen atom are 2.93 and 3.21, which fall within the optimal hydrogen bond range, suggesting good stability and specificity.

Pi-alkyl contacts were noticed between the ligand's aromatic portion and Trp84 and His440 amino acid residues of the protein, at distances of 4.09 and 4.80. Interaction with Trp84 at the peripheral anionic site (PAS) indicates a probable two different inhibitory mechanisms. First, resisting the catalytic activity of acetylcholine hydrolysis and second, blocking β-amyloid aggregation. These mechanisms are pivotal in Alzheimer's disease pathology. To summarize, the binding of DAM with the key active site residues of the protein suggests DAM as a good inhibitor of AChE.

5. Conclusions

In this study, computational evaluation of Daechualkaloid A Maltoxazine against Alzheimer's disease was performed. Density functional theory (DFT) calculations indicate low reactivity of the compound because of its high HOMO-LUMO energy gap, while the electrophilicity index (ω) indicates Strong interaction with nucleophilic sites. Molecular docking studies revealed binding affinities

towards acetylcholinesterase (AChE), which is a potential Alzheimer's target. Similarly, ADME profiling highlighted satisfactory physicochemical properties and favorable pharmacokinetic properties, including lipophilicity, gastrointestinal absorption, water solubility, and blood-brain barrier permeability. Despite the promising computational results, suggesting significant therapeutic potentials of Daechualkaloid A Maltoxazine against Alzheimer's disease, the absence of experimental validation and consideration of only one target represent important limitations of the current study. Therefore, further computational and experimental studies will add more value to its therapeutic capabilities. Despite the promising computational results, the absence of experimental validation and consideration of only one target represent important limitations of the current study.

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