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Exploring the Inhibitory Potential of Chinese Bittersweet Alkaloid I Against GSK-3β: DFT, Molecular Docking, NCI, and ADME Study

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ABSTRACT: Alkaloids have drawn attention due to their potential role as a therapeutic inhibitor of glycogen synthase kinase-3β (GSK-3β), as it is an essential enzyme associated with neurodegenerative and metabolic disorders. The current study combines Density Functional Theory (DFT), molecular docking, Non-Covalent Interaction (NCI) analysis, and ADME, comprehensively evaluating the inhibitory potential of Chinese Bittersweet Alkaloid I against GSK-3β. Molecular docking revealed a binding energy of –6.1 kcal/mol, showing two conventional H-bonds with ANS62 and THR22, reflecting strong binding within the active region of the protein. DFT calculations at the B3LYP/6-311G level provided interpretations into the molecule's electronic reactivity, with a HOMO–LUMO energy gap of 4.075 eV, balanced electrophilicity (0.142 a.u), hardness (2.037 eV), softness (0.490 eV^{-1}), and well-defined reactive sites supported by molecular electrostatic potential mapping. The NCI analysis proved the presence of significant van der Waals and weak attractive interactions taking part into ligand stability. ADME estimation demonstrated favorable physicochemical properties, good drug-likeness, acceptable pharmacokinetic behavior, and advantageous gastrointestinal absorption, but was unable to cross blood blood-brain barrier. These integrated computational results suggest that Chinese Bittersweet Alkaloid I is a potential scaffold for the development of GSK-3β inhibitors, demanding further experimental validation as computational studies inherit limitations.

Keywords: GSK-3β; Binding Energy; Pharmacokinetics; Molecular Docking; NCI Analysis; DFT calculations

1. INTRODUCTION

Glycogen synthase kinase-3b (GSK-3b) is a multifunctional serine/threonine kinase that is currently brought to the center of the stage in cellular physiology as a regulatory molecule (Saraswati et al., 2018). Contrary to most other kinases, GSK-3b is constitutively active at rest, making it able to regulate a great variety of biological processes, as it keeps levels of activation of the signalling proteins in glucose metabolism, mitochondrial homeostasis, synaptic plasticity, neurodevelopment, and inflammatory responses constant (Maqbool et al., 2016). It phosphorylates over 100 proteins transcription factors, metabolic enzymes,

signalling proteins, and structural components, therefore affecting cell growth, differentiation, survival, or death and gene expression (Hulcová et al., 2018a; Wassan et al., 2025).

An accumulating body of evidence holds GSK-3b hyperactivation guilty in the cause and pathogenesis of diverse chronic illnesses(Woodgett, 1990). Overactive GSK-3β in the central nervous system is involved in tau hyperphosphorylation, the development of neurofibrillary tangles, and amyloid-β synthesis, as well as dysfunction, neuroinflammation, and neuronal death - organizational processes in the pathology of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD)(Liu et al., 2025a). On the same note, GSK-3b dysregulation has been associated with impaired insulin signaling, which is relevant to Type 2 Diabetes Mellitus, as well as with oncogenic processes such as cell proliferation, migration, survival, and drug resistance through pathways including the Wnt/b-catenin and PI3K/Akt pathways (Manoukian and Woodgett, 2002). This extensive pathological applicability altogether makes GSK-3b one of the most promising molecular targets of therapeutic interventions in various domains of disease (Liu et al., 2025b).

Medicinally important plants often contain alkaloids, flavonoids, terpenoids, phenolics, etc., which may have multi-mechanistic action on and multi-target effect and potentially lower toxicity than synthetic small molecules (Mansoor et al., 2025). Specifically, the alkaloids, or aromatic substances containing nitrogen, are famous for their anti-cancer, neuroprotective, anti-inflammatory, anti-oxidant, and other pharmacological effects (Afridi et al., 2025; Wang et al., 2025). Many studies have shown that certain alkaloids, particularly from the Amaryllidaceae family, inhibit GSK-3b and positively affect neurodegenerative and cancer models (Hulcová et al., 2018b). This supports the potential of GSK-3b inhibitors as drug targets. However, many bioactive alkaloids from less-studied medicinal plants still need further research. One such example is *Celastrus angulatus*, or Chinese bittersweet, which has shown significant anti-inflammatory, neuroprotective, and anticancer properties due to its alkaloids (Wakabayashi et al., 1988; Yin et al., 1999). Alkaloid I from *C. angulatus* has not been studied for its potential to inhibit GSK-3β, although some other constituents may be explored as kinase inhibitors, representing a significant research opportunity (Xu et al., 2022).

Modern drug-discovery pipelines are unequivocally embracing computational chemistry and in-silico technologies. These approaches enable efficient and cost-effective screening of extensive compound libraries, significantly reducing the need for time-consuming, complex synthesis and wet-lab tests (Lin et al., 2020). As an example, the optimized molecular geometry, electronic structure, charge distribution, global reactivity descriptors, frontier molecular orbitals (HOMO-LUMO), and chemical stability can be computed with the aid of Density Functional Theory (DFT) (Schleder et al., 2019). The Binding pose and affinity of a ligand within the active or allosteric site of a target protein, GSK-3b, can be determined using the molecular docking techniques and applied to the crystallographic structure of a target protein (Abdullahi and Adeniji, 2020; Mujtaba et al., 2025f). Further, Non-Covalent Interaction analysis (NCI analysis) can be used to elucidate the web of weak interactions (hydrogen bonds, p-p stacking, van der Waals contacts, and electrostatics) that hold protein-ligand complexes together. Toxicity measurements and ADME (Absorption, Distribution, Metabolism, and Excretion) measurements give information on the pharmacokinetics and drug-likeness of candidate molecules (Bang et al., 2025). This fact is crucial in prioritizing compounds in terms of their therapeutic potential.

The current study assesses anti-GSK-3b (Glycogen Synthase Kinase 3 Beta) inhibitory properties of Chinese Bittersweet Alkaloid I using a holistic computational workflow. It focuses on elucidating the molecular aspects of interaction, determining the estimate of the binding affinity and stability, explaining important binding interactions, and evaluating drug-like properties. The suggested multi-level in silico testing makes a strong theoretical basis for further experimental validation and can be very helpful in the discovery of new natural product-based GSK-3b inhibitors**.**

2. MATERIALS AND METHODS

2.1 Methodology of Molecular Docking

Molecular docking of Chinese Bittersweet Alkaloid I, Figure 1, with GSK-3β (PDB ID: 4E3F) was performed using PyRx. The structure of the protein was obtained from the Protein Data Bank and brought into the Discovery Studio. It was cleaned by eliminating water molecules as well as heteroatoms. Afterwards, it was converted into PDBQT format. The grid box was set to cover the entire protein structure through a non-targeted docking approach, enabling the ligand

to inspect all the possible binding sites. Default Vina parameters were used to run the docking, and the energy minimum pose was selected. The docking conformations and non-covalent interactions were inspected and displayed by using Discovery Studio Visualizer, which includes hydrogen bonding, hydrophobic interactions, and key amino acid contacts.

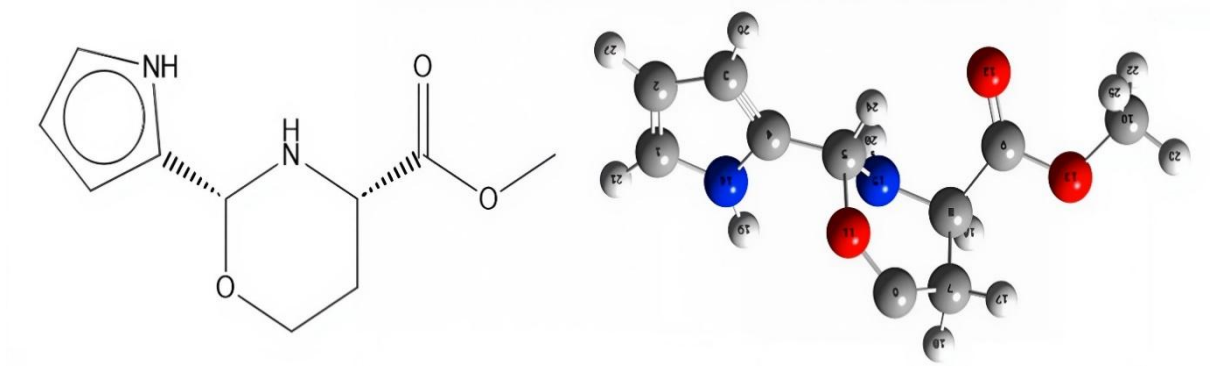

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Figure 1: 2D and 3D Optimized Geometry of CBAI

2.2 Analysis by DFT

Density Functional Theory (DFT) measurements were run to evaluate the electronic properties of Chinese Bittersweet Alkaloid I. All calculations were performed using the B3LYP functional with basis set 6-311G. The energy minimum molecular configuration was used for all later calculations. The highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) energies are used to study the global chemical reactivity descriptors of the studied molecule such as Electron affinity (A), Ionization potential (I), chemical softness (S), chemical potential (μ), electrophilicity index (ω), electronegativity (χ) and chemical hardness (η). The descriptors of chemical reactivity and stability of the molecules based on DFT were calculated according to Koopman's theorem (Koopmans, 1934):

$$I = -EHOMO$$

$$A = -ELUMO$$

$$\eta = \frac{ELUMO - EHOMO}{2}$$

$$\omega = \frac{\mu 2}{2\eta}$$

$$\mu = \frac{ELUMO + EHOMO}{2}$$

$$\chi = -\mu$$

2.3 NCI Analysis

The molecular structures of the compound were designed and built initially using GaussView06. Structure optimizations and measurements of electronic structures were performed via Gaussian 09 with the B3LYP/6-311G functional and basis set. For Non-Covalent interaction (NCI) analysis, wave function data produced from the Gaussian 09 were examined using Multiwfn, and final NCI surfaces were visualized with VMD, allowing the color-coded iso-density surfaces to recognize attractive, repulsive, and van der Waals ligand-receptor connections. Plot-based visualizations of computed information, energy plots, and NCI indices were designed through Gnuplot to enable analysis of trends and interactions among electronic properties. All the figures were further refined and enhanced for improved clarity and quality with the help of IrfanView.

2.4 SwissADME Analysis

An online tool, SwissADME (http://www.swissadme.ch), was used to evaluate the pharmacokinetic profile of Chinese Bittersweet Alkaloid I (CBAI). The SMILE format of the compounds was generated, which is essential for the SwissADME server. All predicted parameters were saved and utilized to determine the drug-like profile as well as the pharmacokinetic compatibility of the compound.

3 Results

3.1 Molecular Docking

Molecular docking was performed between Chinese Bittersweet Alkaloid I (CBAI) with GSK-3β (PDB ID: 4E3F), resulting in a -6.1 kcal/m docking score, which indicates the strong interaction between the ligand and the protein structure **(Table 1)**. The docking study also showed a significant interaction between the ligand and the amino acid residues. ALA65, ASN62, THR200, PRO202, and HIS94 residues of the targeted proteins showed interactions. PRO202 forms a carbon-carbon-hydrogen bond with the ligand. The ligand forms conventional hydrogen bonding with ASN62 and THR200 residues. These bonds are hydrogen bond acceptors. Similarly, the ligand makes an alkyl and pi-alkyl bond with the HIS94 and ALA65 residues of the protein structure. These bonding types are hydrogen bond donors. This ligand-protein complex exhibits a strong affinity due to the dual nature of its interactions, making it more stable **(Figure 2).**

Table 1: Docking results of CBAI with GSK-3β.

Compound	Binding affinity	Residues Involved	Type of Interactions
Chinese Bittersweet Alkaloid I (CBAI)	-6.1	ASN62	Conventional H-bond
		THR220	Conventional H-bond
		PRO202	Carbon Hydrogen Bond
		HIS94	Pi-Alkyl and Alkyl
		ALA65	Pi-Alkyl and Alkyl

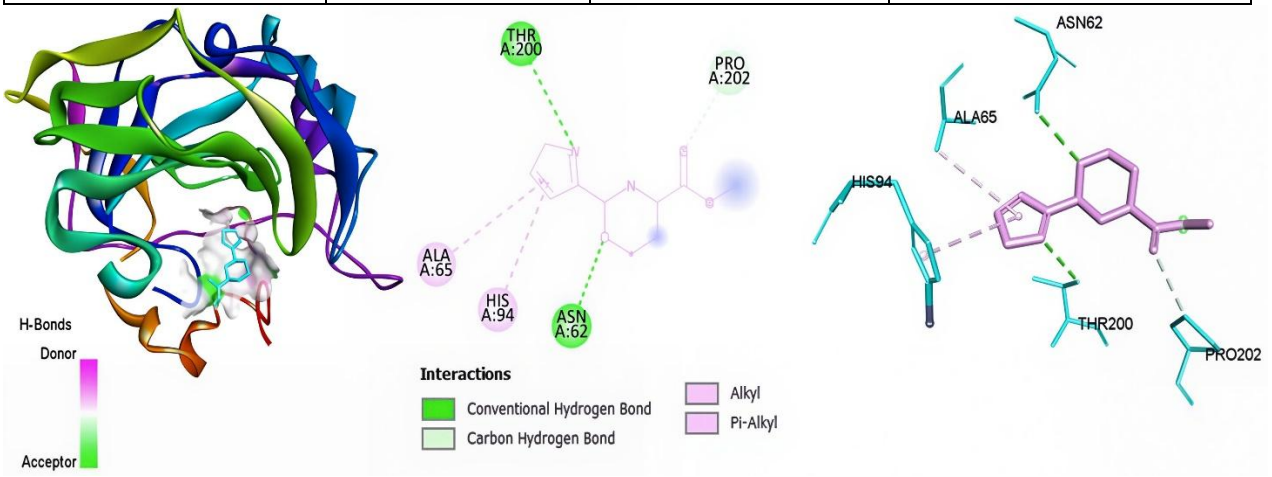

Figure 2: 3D and 2D visualization of Docking Interactions

3.2 DFT calculations

The DFT tool is very important in modern quantum chemistry because it determines structural, thermodynamic, and electronic properties of molecules.

3.2.1 Frontier molecular orbitals and Molecular electrostatic potential surface

The descriptors of chemical reactivity and stability of the molecules based on DFT were calculated Equations (1) – (6) according to Koopman's theorem. The values of FMOs including HOMO and LUMO are 4.999 eV and 0.924 eV respectively and their energy gap (Moreover, the HOMO and LUMO orbitals energy are used to study the global chemical reactivity descriptors of the studied molecules such as Electron affinity (A), Ionization potential (I), chemical softness (S), chemical potential (μ), electrophilicity index (ω), electronegativity (χ) and chemical hardness (η).

The moderate energy gap (4.075 eV) tells us about the chemical stability of the molecule, but it can still involve charge-transfer processes. The molecular orbitals of CBAI are shown in Figure 3. The HOMO was distributed over the electron-withdrawing

carbonyl group. Contrary to this, the LUMO was mainly spread over the carbonyl carbon and nearby atoms. These electrophilic centers are open to nucleophilic attack. MESP surface of the CBAI was shown in Figure 3, which pictures the electrophilic and nucleophilic centers. High and low electron density were indicated by the red region and the blue region, respectively. The nucleophilic center was spread over the oxygen atom, suggesting suitable interactions for the electrophilic center. In contrast, the electrophilic region was around nitrogen-hydrogen (NH), susceptible to nucleophiles.

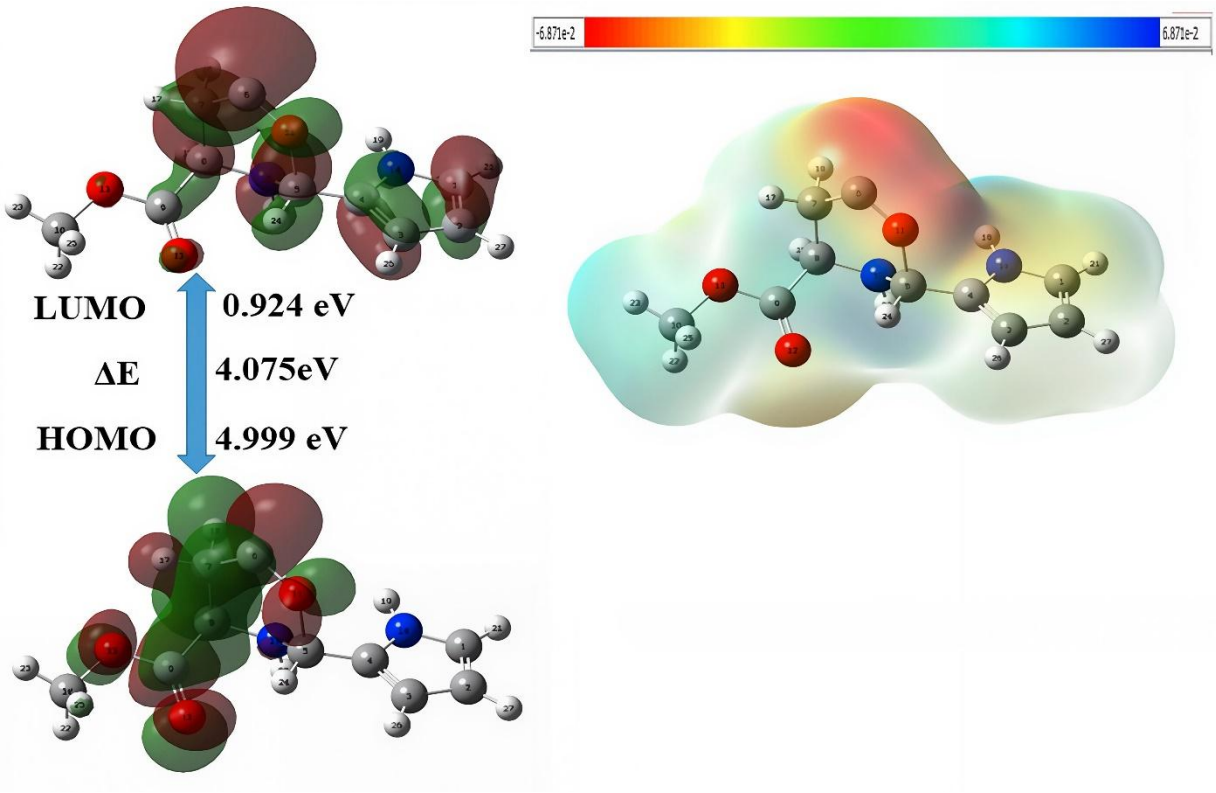

Figure 3: Frontier Molecular Orbitals and Molecular Electrostatic Potential Mapping

3.2.2 Global reactivity descriptors

Global reactivity descriptors are used to predict the stability and reactivity of the molecule. **Table 2** shows the calculated value of ionization potentials (4.999 eV), electron affinities (0.924 eV), chemical hardness (2.037 eV), chemical softness (0.490 eV^{-1}), chemical potential (-2.961 eV), as well as electrophilicity index (2.152 eV) and electronegativity (2.961 eV) for the studied molecule. The value of polarizability (α) is 134.245 au. Thermodynamic parameters, including heat capacity and entropy, are also studied. The values of heat capacity and entropy are 52.373 cal/mol-kelvin and 120.874 cal/mol-kelvin, respectively. These parameters suggest the thermal stability of the molecule.

Table 2: Chemical Descriptor Values of Studied Compound

Parameters	**Values**	**Parameters**	**Values**
Ionization Potentials (-H)	4.999 eV	Electrophilicity Index (ω−)	0.142 a.u.
electron affinities (-L)	0.924 eV	Electronegativity (χ)	2.961 eV
Energy Gap (ΔE)	4.075 eV	Polarizability (α)	134.245 a.u
Chemical Potential (μ)	-2.961 eV	Heat capacity (ΔH)	52.373 cal/mol-k
Hardness (η)	2.037 eV	Entropy (S)	120.874 cal/mol-k
Softness (S)	0.490 eV^{-1}	Electrophilicity Index (ω)	2.152 eV

3.2.3 Non-covalent Interaction Analysis

NCI analysis helps to visualize different types of weak interactions. Strong interactions, van der Waals, and steric effects are represented by blue, green, and red regions, respectively. Reduced density gradient (RDG) plot further supports NCI analysis, as depicted in **Figure 4.** (λ_2)p values indicate these weak interactions. Strong interactions (hydrogen bonding) are represented by the blue region that has negative zero values. The green region shows weak van der Waals interactions, and its values are close to zero. Steric repulsions are represented by the red region that has positive zero values. NCI analysis and RDG plot contribute to the overall stability of the ligand with protein structure.

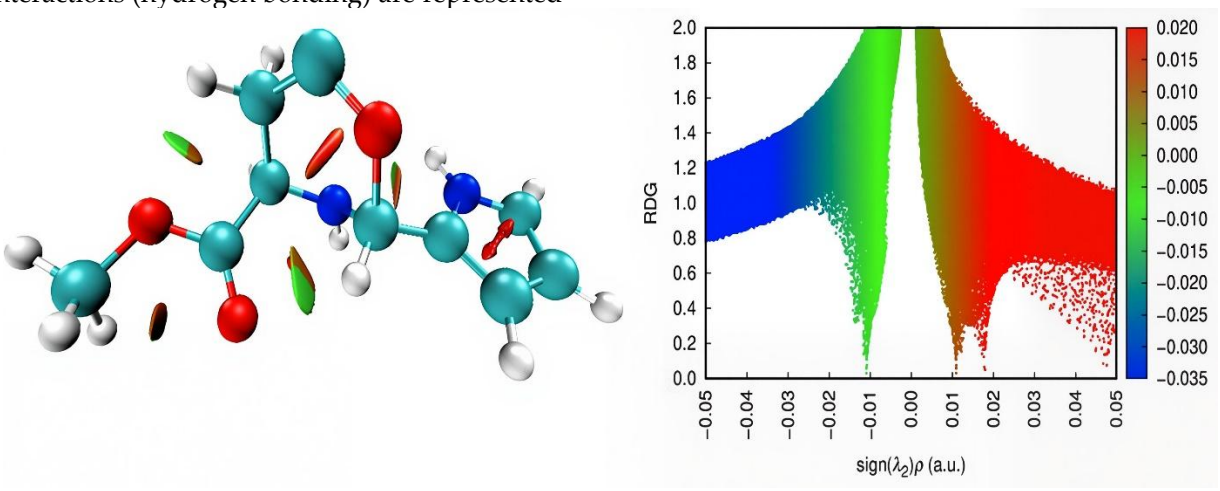

Figure 4: Non-Covalent Interactions and RDG Plot

4 ADME Analysis

ADME analysis done by the SwissADME tool, which reveals the physicochemical properties, lipophilicity, solubility, pharmacokinetic properties, druglikeness properties, and medicinal chemistry (**Figure 5**).

4.1 Physicochemical properties

SwissADME was used to evaluate the physicochemical properties of the compound, as illustrated in **Figure 5.** The molecular formula of the compound is $C_{10}H_{14}N_2O_3$. The molecular weight of the compound is 210.23 g/mol, which is within a reasonable range. Lipinski's rule of five states that the acceptable range for molecular weight is generally between 100-600 g/mol. The compound's synthesis, feasibility, and permeability are related to the number of heavy atoms, which was found to be 15. The compound has aromatic heavy atoms and has a 0.50 value of the fraction of Sp3-hybridized carbon. Furthermore, it has 3 rotatable bonds, which are in the optimal range: 0-11, which correlates to good molecular flexibility. It has 4 hydrogen bond acceptors (HBA) and 2 hydrogen bond donors (HBD). These values are related to Lipinski's rule of five (HBD<5, HBA ≤ 10). The value of molar refractivity is 56.77, showing that the compound has good binding affinity with the target. The compound has a 63.35 Å^2 TPSA value, revealing that the compound has favorable membrane permeability.

4.2 Lipophilicity

Drug lipophilicity determines its membrane permeability potential, which influences the drug distribution in the body. The lipophilicity of the compound was determined by SwissADME software as a consensus logP value. It is the average of different algorithms, given in **Figure 5**. The consensus logP value of the compound is 0.64.

4.3 Water solubility

The water solubility of a drug is the first step for its absorption in the body in aqueous body fluids, i.e, saliva, stomach acid, or blood. The determination of water solubility of the compound was done by three methods, showing that the drug is "very soluble" for the ESOL and Ali method, and 'soluble' for the SILIOC-IT method, depicted in **Figure 5**.

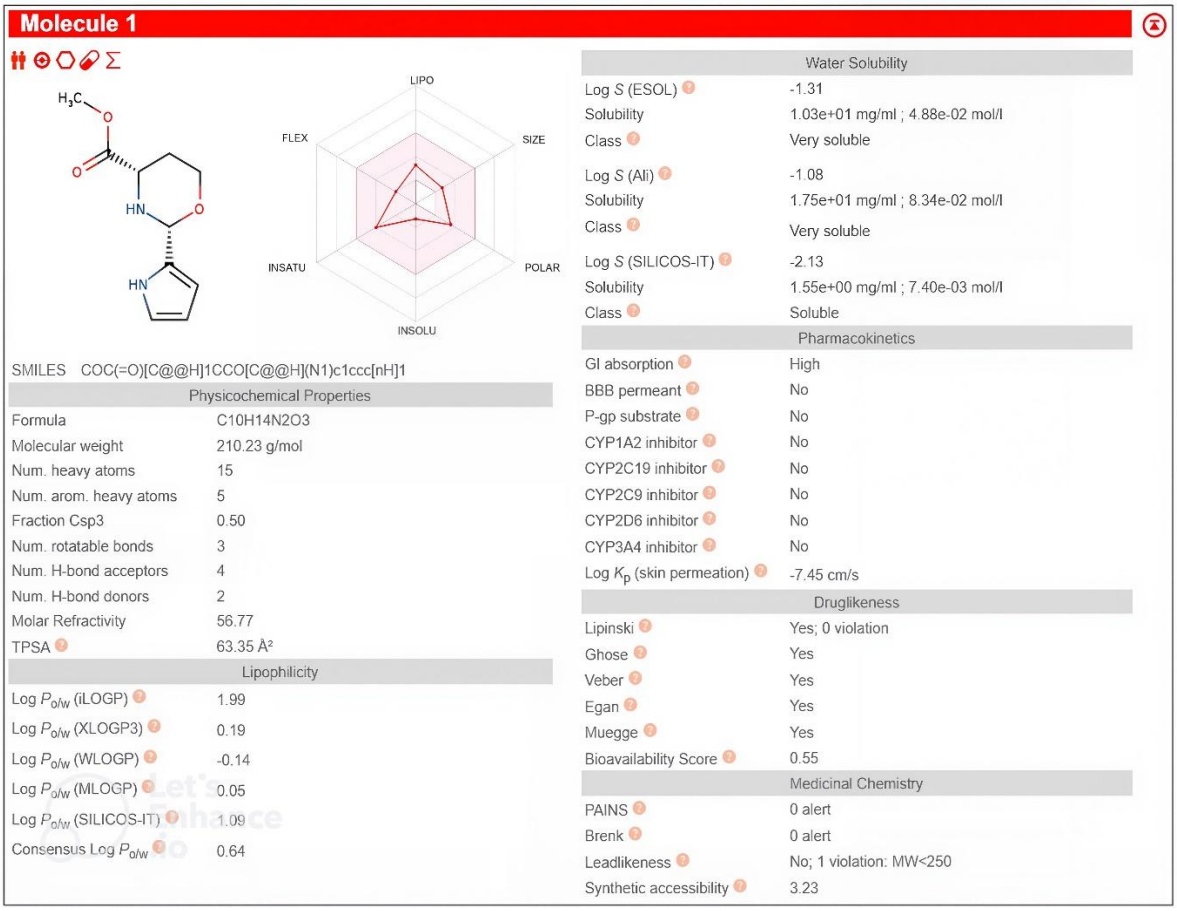

Figure 5: Physicochemical and Pharmacokinetic Properties

4.4 Pharmacokinetics

Drug absorption is a process of entering the bloodstream from the administration site, such as the oral, gut, or muscle. The drug's bioavailability is the fraction of the drug reaching the body's circulation. It is determined by absorption. The compound has high gastrointestinal (GI) absorption reflecting high oral bioavailability, which is depicted by the white region of the Boiled Egg Model (**Figure 6**). Similarly, blood blood-brain-barrier (BBB) is the factor that may affect its central nervous system availability. The yellow region of the Boiled Egg Model shows the BBB crossing ability, indicating the compound cannot pass through the BBB. The compound cannot be knocked out of the body because it is not a P-gp substrate. Biotransformation occurs during drug metabolism, often regulated by cytochrome P450 (CYP450). Chinese Bittersweet Alkaloid I shows the absence of CYP450 enzyme inhibition, which is favorable because drug-drug interactions and metabolic side effects are low. log Kp value is -7.45 cm/s, which represents skin permeability. This value indicates no transdermal permeation potential, **Figure 5.**

4.5. Drug-likeness and Medicinal Chemistry

Chinese Bittersweet Alkaloid I follows major drug-likeness rules, including Lipinski, Ghose, Veber, and Egan filters. Muegge rule is not fully followed due to a molecular weight issue (the optimum range is 200-600, and 210.23 is below 250). The compound has only one violation, which is its molecular weight less than 250, regarding drug-likeness properties. The bioavailability score of the compound is 0.55, suggesting an acceptable likelihood of oral bioavailability. Additionally, zero alerts were recorded for both PAINS (Pan Assay Interference Compounds) and Brenk filters. This indicates the absence of any problematic pharmacophores. The value of synthetic accessibility is 3.23, suggesting that it has moderate ease of synthesis **Figure 5**.

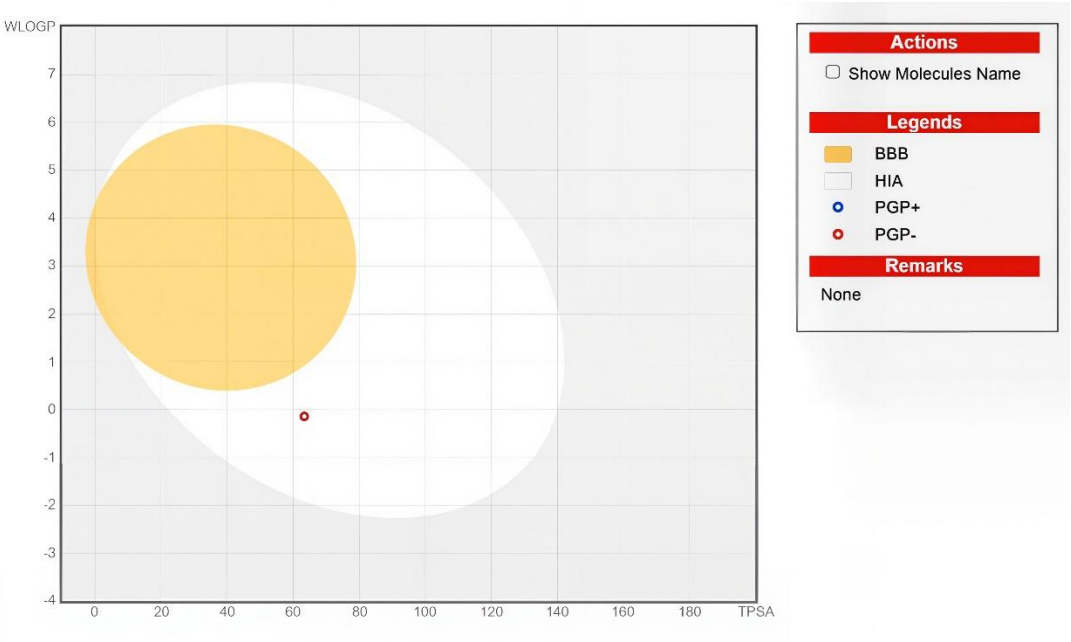

Figure 5: Boiled-Egg Model

4. Discussion

Molecular docking analyzes the mechanism of interaction between the receptor sites and inhibitors (Czechtizky and Hamley, 2015). It has great importance to predict the interactions between ligands and their protein targets in the drug discovery field. The mechanisms of selectivity can be easily found by docking ligands with protein targets (Singh et al., 2017). The docking score indicates a moderately strong binding affinity between Chinese Bittersweet Alkaloid I (CBAI) with GSK-3β**.** The results visualized a number of ligand interactions with the amino acid residues of the protein target. The oxygen and nitrogen of the six and five-membered rings form two hydrogen bonds with the ASN62 and THR200 amino acids, respectively, **Figure 2**. These stable interactions anchor it near the active site of the enzyme**.** Pi-alkyl contacts were observed between the HIS94 and ALA65 residues of the protein and the ligand's aromatic portion. Interactions with PRO202 are carbon-hydrogen bonds. CBAI has occupied the active site of the enzyme and blocked it, revealing the docking score and binding of CBAI with the key active site residues of the protein, indicating CBAI as a good GSK-3β inhibitor.

Density function theory (DFT) studies are essential for understanding intermolecular interactions and designing structures having desired drug properties (Mujtaba et al., 2025e). The analysis of stability and reactivity of the compound is important in drug design. In this respect, the global reactivity descriptors give information regarding molecular behavior under a biotic environment and its interaction selectivity with receptor targets (Mujtaba et al., 2025b). The charge donating or accepting capacity of a chemical species is gained by the ionization potential (I) and the electron affinity (A). The ionization potential of a compound is the minimum amount of energy required to give up an electron. The tendency of a neutral atom or molecule to accept an extra electron is electron affinity. A low value of ionization potentials reveals a good character to lose electrons, while a high value of electronic affinity shows a good character to receive electrons (Koopmans, 1934). The HOMO indicates the ability to donate an electron of a chemical species, while the LUMO represents the electron-accepting potential (Mujtaba et al., 2025a). The HOMO and LUMO tell us about the kinetic stability and chemical reactivity of the molecule due to their participation in chemical stability. Lower LUMO energy values guide the molecule to accept electrons, while high HOMO values direct the molecule to lose electrons. The chemical stability of a compound is indicated by the energy gap parameter. A lower energy gap value indicates the molecule is less stable and more reactive, while a higher value shows the compound is more stable and less reactive (Layaida et al.,

2023). Hence 4.075 eV energy gap shows the chemical stability and less reactivity of the compound.

HOMO energy (EHOMO) and LUMO energy (ELUMO) have been used to estimate the ionization potential (I) and the electron affinity (A), respectively, in simple molecular orbital theory approaches. Electronic properties such as a lower value (-4.999 eV) of ionization potential (I) are related to antioxidant properties due to their ability to lose electrons easily. In this respect, compound acts as a free radical scavenger, enhancing its therapeutic use. Electron affinity value (-0.924 eV) shows the ability to form a stable complex with biological targets.

The interpretation of molecular stability and reactivity has been done by chemical hardness (η) and softness (S). The resistance to change in electron distribution or charge transfer is chemical hardness. It measures the deformation of the electron density and of the chemical reactivity related to the HOMO-LUMO gap (Flores-Holguín et al., 2021). Half of the HOMO-LUMO energy gap is equal to the global hardness (η), which is an important stability criterion. Its large value indicates high stability and low chemical reactivity. Favorable reactions occur involving soft molecules with a low energy gap, while hard molecules are highly stable and less reactive. According to the results in **Table 1**, it has a higher hardness value 2.037 eV and a low softness value 0.490 eV^{-1}. High hardness and low softness values show the molecule's resistance to deformation and moderate chemical reactivity degree.

The parameter for studying the reactivity of organic molecules that participate in polar reactions is the electrophilicity index (ω) (Mujtaba et al., 2025d). The electrophilicity index (ω) measures the electron-accepting tendency of a species. The electrophilicity index represents the stabilization of the system when it receives extra electrons from the environment (Parr and Pearson, 1983). The component is a good electrophile when it has a high value of (ω), and its (ω) lower value leads the component to be a good nucleophile. The value of the electrophilicity index (ω) is 2.152 eV, which shows that it can accept electrons due to its electrophilic carbonyl center. The Electrophilicity index (ω+) has a lower value of 0.034 a.u. Than the electrophilicity index (ω−) value 0.142 a.u. This results in the electron accepting capacity being higher than the electron donating capacity. The compound shows good nucleophilic character as compared to the electrophilic one. The overall ω+ indicates that Chinese Bittersweet Alkaloid I has both nucleophilic and electrophilic character, suggesting its ability to form complexes with diverse biological targets.

The chemical potential (μ) is the tendency for electrons to leak from a system in equilibrium. The compound is less stable or more reactive when it has a higher electron potential. The chemical potential of the compound (–2.961 eV) is higher. This result is closely aligned with the hardness (η). The negative value of the chemical potential of a molecule is electronegativity (χ), and Mulliken defined it as the average of the HOMO and LUMO energies (Mulliken, 1934). The tendency of an atom to attract electrons to it is electronegativity (χ). Table 2 shows that the compound has a higher electronegativity value (2.961 eV), so it is the best electron attractor. This result is aligned with the (A) calculation. The value of polarizability (134.245 au) shows that the electronic cloud of the molecule can be easily distorted. It contributes to drug-target interactions, such as strong van der Waals interactions. Thermochemical quantities such as heat capacity (52.373 cal/mol-kelvin) indicate sufficient energy for metabolic transformations. Meanwhile, the entropy (101.802 cal/mol K) shows good thermodynamic feasibility in molecular binding. These parameters indicate the thermal stability of the molecule.

MESP is used to analyze the net electrostatic effect formed at that point by the total charge dispersion (Gadre et al., 2021). It is a visual method for the relative polarity of molecules as well as the positive and negative electrostatic potentials' location (Layaida et al., 2023; Mujtaba et al., 2025c). Different colors are used to express electrostatic potential values on the component map. Negative, positive, and neutral electrostatic potentials are interpreted by red, blue, and green colors, respectively (Garza et al., 2013). The analysis of the physicochemical properties of a compound is also done by the MESP surface. The surfaces of HOMO and LUMO of the molecule were based on the optimized structure. The MOs were expressed by red, blue, and green areas in Figure 3 with opposite phases. The red color indicates the negative phase (Oxygen atom) that is available for electrophilic attack, while the positive phase (NH bond) is indicated by blue color, susceptible to nucleophilic attack. Green color represents the neutral part of the molecule that is less reactive. HOMO and LUMO distribution is over the Chinese Bittersweet Alkaloid I. The carbonyl group cannot donate

electrons, but it will accept electrons. It shows the electrophilic nature of the carbonyl group. The susceptibility to nucleophilic and electrophilic attack shows the dipolar nature of the compound, and it can also be involved in intermolecular interactions.

Drug discovery is mainly to find a compound that has good pharmacokinetic and pharmacodynamic features, including absorption, distribution, metabolism, and excretion. The new drug should be selective, target specific, have good oral absorptivity, be distributed, metabolized with no or low toxic effects, and eliminated as allowing one dosage per day (Egan et al., 2000). The new drug should be according to the Lipinski rule of five. This rule states that a new compound should be aligned with molecular mass<500, HBD<5, HBA ≤ 10, and log P<5 for its better absorption and permeability (Sardar, 2023). These parameters are related to optimum solubility in water, absorption in the intestine, and eventually bioavailability. The oral activity problems are most probable due to missing these parameters. CBAI does not violate these conditions except for molecular weight. Its molecular mass is 210.23 g/mol, suggesting that it is suitable for oral drug development. A drug has to reach the systemic circulation, passing cell membranes. Small molecules can pass through cell membranes by passive diffusion, following a concentration gradient (LZ, 1991).

Heteroatoms play a necessary role in some drug metabolism, like nitrogen in 4-hydroxy isoleucine (St. Jean Jr and Fotsch, 2012). Likewise, drug metabolization involves redox reactions (heteroatom dealkylation, hydroxylation, heteroatom oxygenation, reduction, and dehydration) that produce active metabolites (Obach, 2013). The heavy atoms are 15, supporting the feasible synthesis and good permeability of the compound.

The fraction of sp3-hybridized carbon in a molecule is represented by Fsp3. It is calculated by taking the ratio of sp3 carbon atoms to the total number of atoms. This factor predicts the carbon saturation and spatial structure of a compound (Dehelean et al., 2020). Fsp3 has an optimal range >0.42, and marketed drugs (almost 84 %) fall under this criterion (Kombo et al., 2013). Its higher value than the reasonable range shows complex synthesis and does not promise good performance. The drugs have to follow this restriction. The studied compound falls under the required threshold value. It suggests a high degree of saturation and favorable pharmacokinetic capabilities.

The optimal values for the rotatable bond and topological polar surface area (TPSA) parameters are ≤ 10 and ≤ 140 Å2, respectively suggested by experiments. The absorption and bioavailability will be poor if these factors have higher values (Clark, 1999). The studied compound follows the optimal range of rotatable bonds and TPSA, indicating better absorption, membrane permeability, bioavailability, and computational adaptability of the compound (Daina et al., 2017). The safe range of molar refractivity values is 40–130, and our compound has its favorable (56.77) value. It represents the compound's binding affinity with the target, clarifying size and polarizability (Mujtaba et al., 2025b).

Lipophilicity evaluates the distribution of a drug in the body. It influences the membrane permeability of the drug. The factors that affect lipophilicity are molecular size, polarity, and hydrogen bonds in a compound (Zerroug et al., 2019). Lipophilicity is determined as the log of the partition coefficient between n-octanol and water (log P). The relation between log P and permeability is nonlinear, and permeability decrease suggests low and high values of log P. The deviated values show lower permeability through the lipid membrane (Hansch and Clayton, 1973). In the SwissADME tool, the Log P values are analyzed as the average of iLOGP, XLOGP3, WLOGP3, MLOGP3, Silico Log P, and consensus LogP. The studied compound falls in the safe range of log P Value (optimal value< 5), indicating good lipophilicity, effective distribution of the compound (Pastewska et al., 2022).

Water solubility is important for oral bioavailability and absorption of a drug (Bhalani et al., 2022). Three methods are used to predict the water solubility of the compound. ESOL (first method) determines solubility based on molecular structure, weight, heavy atoms in the aromatic system, and the number of rotatable bonds. The second method, Log S (Ali), predicts solubility by taking TPSA into the calculations, while the third method, Log (SILICOS-IT), calculates the negative logarithm of water solubility of a compound through the fragmental method. The values of LogS are − 10 to 0 (-10 in solubility, -6 poor solubility, -4 solubility, -2 very solubility, and 0 high solubility) (Shweta et al., 2019; Taylor and Triggle, 2007). Our compound showed high solubility. The Log S (Ali) method also shows high solubility. Since solubility determines oral absorption and bioavailability, so, solubility tests are done in the laboratory to validate the drug-likeness analysis of

compounds. The Boiled-Egg Model evaluates the gastrointestinal absorption, the blood-brain barrier, and the P-gp substrate level of the drug. Its white and yellow yolk indicate the GI absorption and BBB passing ability of the drug (Ahmed et al.). The studied compound showed high GI absorption and cannot cross the BBB, indicating not used for CNS-related diseases.

The bioavailability score (ABS) predicts the probability of a compound showing measurable bioavailability (10%) in rats or in Caco-2 cells. Compounds following the rule of five show a 0.55 ABS value, and 0.17 if they disobey. ABS also explains the absorption status of compounds (Martin, 2005). CBAI showed favorable bioavailability (0.55). P-gp ejects the drug for the protection of the CNS from foreign compounds in the body. Its excessive production results in cancer due to the resistance of many drugs in some cells. P-gp affects the drug's bioavailability due to its interaction with P-gp, hindering its beneficial uses (Akhtar et al., 2011)**.** CBAI is not a P-gp substrate, indicating it is not ejected out via P-gp. The compound can easily perform its healing role.

Drug elimination is possible via drug and cytochromes P450 (CYP450) interactions, as these isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) are important for metabolism. Drugs (50–90%) are substrates of these CYP450. These isoenzymes' inhibition results in drug-drug interaction, causing toxicity and side-effects due to decreased removal and accumulation of drugs (Manikandan and Nagini, 2018). CYP2C19 detoxifies carcinogens and metabolizes different drugs or triggers procarcinogens. CYP2C9 metabolizes drugs, having a narrow therapeutic range. CBAI has not shown inhibition to CYP450, suggesting no drug-drug interaction and no adverse effects. The compound's skin permeability (log Kp) value is -7.45 cm-1, indicating no skin permeation potential. Rule-based five filters are used to predict drug-like properties in the SwissADME tool, which various pharmaceutical companies also use. Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) are these filters. CBAI follows all the parameters of these filters, suggesting the drug is therapeutically safe (Muegge et al., 2001). CBAI has good oral bioavailability, solubility, permeability, absorptivity, distribution, and metabolization with no side effects and can be easily synthesized. Further evaluation of CBAI was done by NCI and RDG plot that illustrates the hydrogen bonding, van der Waals' interactions, and steric repulsions represented by blue, green, and red regions, respectively. Despite a moderate docking score, these interactions stabilize the binding of ligand and enzyme, blocking its catalytic site. To summarize, the results of molecular docking, DFT calculations, ADME, and NCI analysis suggest that CBAI is a good inhibitor of GSK-3β.

5. Conclusion

To conclude, this study using integrated computational approaches validates Chinese Bittersweet Alkaloid I (CBAI) as a potential GSK-3β inhibitor. **CBAI** exhibits favorable interactions with GSK-3β, displaying a binding affinity of –6.1 kcal/mol, supported by several key interactions, particularly conventional Hydrogen Bonding, which suggests that the compound may inhibit the activity of the GSK-3β protein. It was also concluded from the high HOMO-LUMO energy gap that the compound is relatively stable in a biological environment, making it a potential drug candidate. NCI analysis highlights the contribution of weak noncovalent interactions, primarily van der Waals forces, to the stabilization of the ligand–protein complex. These findings are further supported by ADME predictions, showing that the compound does not inhibit major CYP isoforms and is not a P-glycoprotein (P-gp) substrate, suggesting a favorable pharmacokinetic profile. Moreover, the compound has better physicochemical properties, high solubility, favorable medicinal chemistry, and zero violations of common drug-likeness filters, further enhancing its drug potential, making it less suitable for central nervous system–related disorders. This characteristic may also reduce the risk of CNS-associated side effects. Collectively, these results suggest that CBAI represents a promising drug scaffold, warranting further experimental validation and structural optimization.

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