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Research Article

Green Solvent-Free Microwave Synthesis, Spectroscopic and Computational (DFT) Characterization, and Molecular Docking of the *Bis*-Schiff Base Derived from Hexamethylenediamine and Benzaldehyde

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ABSTRACT

Schiff bases are organic compounds characterized by the imine functional group (C=N) formed by condensing primary amines and carbonyl compounds. Their versatile chemical properties and broad range of applications have led to ongoing interest in their synthesis and functionality. This paper presents a green synthesis of ***EE-bis***-Schiff base from hexamethylenediamine and benzaldehyde using solvent-free conditions and microwave irradiation, enhancing reaction efficiency while minimizing environmental impact. Characterization of the synthesized compound was conducted via FT-IR spectroscopy and CHN analysis. Furthermore, comprehensive Density Functional Theory (DFT) studies were performed at the B3LYP/6-311g(d,p) level of theory, providing insights into the molecular structure, stability, and electronic properties of **EE-bis**-Schiff base. Computationally molecular structure, thermodynamic stability, infrared spectrum analysis, ultraviolet-visible absorption spectrum, nuclear magnetic resonance (NMR) spectra analysis, HOMO–LUMO energies, quantum molecular descriptors, map of electrostatic potential, and Mulliken atomic charges analysis of this Schiff base were studied. Also, molecular docking of **EE-bis**-Schiff base with **1AJ6**, **6LU7**, and **1HSG** receptors was done.

Keywords: Schiff base, hexamethylenediamine, Benzaldehyde, molecular docking, Density Functional Theory (DFT), green synthesis

1. Introduction

Hugo Schiff first synthesized Schiff bases in the 19th century, and they have since been of great interest due to their versatile chemical properties and potential applications. Schiff bases are organic compounds characterized by an imine functional group (C=N) formed by the condensation reaction of a primary amine and a carbonyl compound (aldehyde or ketone)[1-2]. Modifications in reactants (using substituted amines or carbonyls) can lead to a diverse range of Schiff bases with unique properties. Many Schiff bases are colored compounds and exhibit useful UV-Vis absorption properties.Their solubility and stability can vary widely depending on substituents.Schiff bases are important in coordination chemistry,they can act as ligands, forming complex compounds with transition metals, studied for their catalytic activities, as dyes, and used in sensors[3-5]. Some Schiff bases show biological activities such as antimicrobial, antifungal, and anticancer properties, making them valuable in pharmaceuticals[6-9]. Ongoing studies focus on synthesizing Schiff bases with improved biological activity and lower toxicity for medicinal applications. These organic compoundsremain a significant area of chemical research due to their versatility and wide range of applications.

Recent studies aim to optimize their synthesis and expand their utility in various scientific fields. There is growing interest in environmentally friendly synthetic routes, including microwave irradiation and solvent-free conditions.Microwave-assisted synthesis of Schiff bases is a modern approach that offers several advantages over traditional methods, such as reduced reaction time, improved yields, and better final product purity. Microwave irradiation accelerates the reaction by providing uniform heating throughout the reaction medium. This can enhance the reacting species' energy, facilitating Schiff bases' formation more efficiently than conventional heating.The advantages of microwave-assisted synthesis are speed (reactions can often be completed in minutes instead of hours, which is typical for traditional

methods), higher yields (enhanced reaction kinetics frequently lead to higher yields of the desired product), selectivity (the uniform heating can improve selectivity, reducing the formation of by-products), and (environmentally friendly, many procedures can be performed with little or no solvent, making it a greener approach to synthesis) [10-13].

Density Functional Theory (DFT) is a powerful computational quantum mechanical method widely used to investigate molecules' electronic structure and properties, including Schiff bases. DFT is based on the principle that the ground state properties of a many-electron system can be determined from the electron density rather than the wave function. DFT utilizes approximate exchange-correlation functionals to calculate properties like energy, geometry, and electronic distribution. DFT can model various synthesis pathways for Schiff bases, predicting optimized molecular structures, revealing bond lengths, angles, and dihedral angles that correlate with experimental data, and identifying transition states. DFT helps understand the condensation reaction mechanism between amines and carbonyl compounds, allowing researchers to predict the stability of intermediates.It provides insights into the reactivity of Schiff bases in various chemical processes, including nucleophilic and electrophilic reactions. DFT calculates electronic structure and molecular orbitals, demonstrating how the electron density is distributed in Schiff bases. This can reveal information about the nature of bonding,likethe double bond character in C=N. It assists in predicting absorption spectra by simulating electronic transitions, which can help in understanding theirchromophore properties. Itis employed to explore Schiff bases in the context of organic electronics, photovoltaic materials, and sensors, investigating properties like charge transport and stability.These computational approaches complement experimental methods by allowing detailed theoretical predictions that guide the design and application of Schiff bases in various fields, including materials science and medicinal chemistry[14-16].

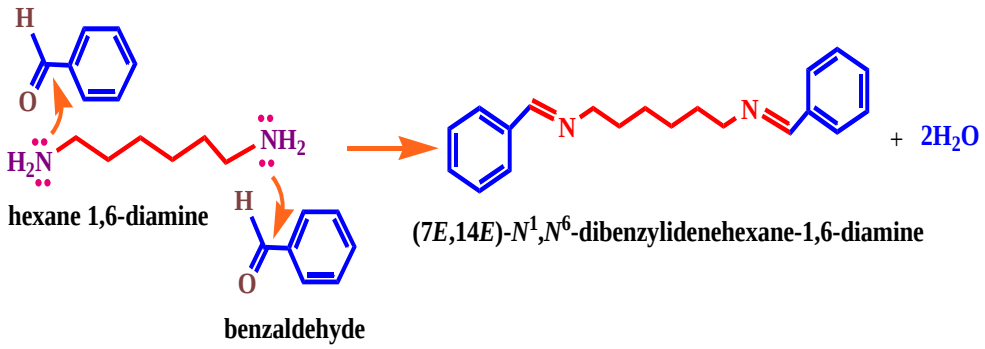
Molecular docking is a computational technique widely used in drug discovery to predict the preferred orientation of one molecule (the ligand, which can be a Schiff base) when it binds to a target protein or receptor. This orientation can help understand the ligand's interaction and affinitytoward the target, aiding in the design of new drugs based on Schiff bases or related compounds[17-20].

The development of sustainable synthetic methodologies has gained significant attention in organic chemistry. This paper reports a green synthesis of an***EE-bis***-Schiff base formed by the reaction of hexamethylenediamine with benzaldehyde (**Scheme 1**). In 2017, K. N. Jean-Baptiste and colleagues synthesized this bis-Schiff base classically [21]. They have survivedthe antibacterial properties of ***EE-bis***-Schiff base, but it was inactive on all the strains tested. Employing environmentally friendly techniques, the synthesis was conducted under solvent-free conditions, utilizing microwave irradiation to enhance reaction efficiency and reduce energy consumption. FT-IR spectroscopy and CHN analysis characterized the resulting bis-Schiff base. A comprehensive DFT study at B3LYP/6-311g(d, p) level of theory was also performed to explore the synthesized compound's molecular structure, stability, and electronic properties. The theoretical calculations provided insight into the charge distribution and geometry of ***EE-bis***-Schiff base, confirming its potential for applications in catalysis and material science.

Molecular Docking studies were conducted on the bis-Schiff base to assessits ability to bind to specific targets. Even though it did not exhibit antibacterial properties, running molecular docking studies can still yield valuable insights that may inform future research or development efforts.

2. Experimental

The synthesis commonly involves the reaction of a primary diamine with benzaldehyde, resulting in the removal of water (dehydration). This reaction is typically simple and can be carried out under mild conditions.Research has explored the use of various catalysts (acidic or basic) and solvent systems (including eco-friendly solvents) to enhance yield and reaction efficiency. A microwave synthesizer or a suitable domestic microwave can be used; however, the former often provides better control over reaction parameters. Microwave-assisted synthesis has become a widely adopted technique in academic and industrial settings, significantly reducing reaction times and increasing yields.The microwave radiation heats the mixture of diamine and benzaldehyde, promoting the condensation reaction that leads to the di-imine formation (***EE-bis***-Schiff base, **Scheme 1**).



Scheme.1. Synthesis reaction of (7E, 14E)-N^1,N^6-dibenzylidene)hexane-1,6-diamine(***EE-bis***-Schiff base or **EE-Sb**)

Instruments and materials

A dedicated microwave synthesis lab instrument was employed to facilitate the rapid synthesis of the Schiff base. The system was calibrated for controlled power output and temperature monitoring during the reaction. The melting point was measured using the Stuarts SMP10 melting point apparatus. Carbon, hydrogen, and nitrogen percentages were determined by microanalyses (Heraeus Elemental analyzer). The IR spectrum (KBr discs) on an 8101 Shimadzu FTIR spectrophotometer was recorded. Benzaldehyde, ethanol,

andhexamethylenediamine were obtained from Sigma-Aldrich commercial supplier (purity ≥ 99%). Hexamethylenediamine was stored under an inert atmosphere to prevent moisture absorption. Benzaldehyde was purified former using distillation. Ethanol was used directly without further purification. An acid catalyst,acetic acid, was used in small amounts to facilitate the condensation reaction between hexamethylenediamine and benzaldehyde.All reagents used were of analytical grade or higher, and standard solutions were prepared for calibration purposes in spectroscopic analyses.

Synthesis and characterization of EE-bis-Schiff base

Benzaldehyde (0.2mmol) and hexane-1,6-diamine (0.1mmol) were combined in a microwave-safe vessel. The microwave was set to the appropriate power (300 watts) for 15 minutes. The reaction was monitored by sampling at intervals and using the TLC technique to check for the formation of the Schiff base. Once the reaction was complete, allow the mixture to cool to obtain the crude product. The obtainedprecipitate was filtered and recrystallized in ethanol. $C_{20}H_{24}N_2$ R_f: 0.57 in hexane/acetone (50;50), yield: 83%, m.p.,198.6°*C*; IR (KBr discs, cm^{-1})2925; 2856; and 1638.

Calculation methods and computational programs

All electronic structure calculations were performed using the Gaussian09 suite of programs [22]. Optimized geometry of **EE-Sb** was fully optimized using the hybrid DFT of B3LYP which employs the three-parameter Becke exchange functional, B3[23,24] with the Lee-Yang-Parr nonlocal correctional functional LYP [25] along with the polarized 6-311g (p, d) [26] basis sets in the gas phase. Analysis of the vibrational frequencies is used to ensure the absence of imaginary values in the vibrational mode calculations of the minima. The GaussView6[27] program was used for preprocessing, structure modification, and analyses of structures, Mulliken charges, frequencies, and chemical shifts. The potential electrostatic map

was typically generated using DFTcalculations and visualized by GaussView 6. The UV-Vis absorption spectrum of titled compoundwas calculated by the TD/DFT method.

MolecularDocking Method

The resulting 3D conformations of **EE-Sb**were minimized using theMMFFforce field to remove any steric clashes and ensure docking studies.The target proteins (receptors: **1STE**, **1Z11**, and **1AJ6**) were selected from the Protein Data Bank (PDB)(http://www.rcsb.org/pdb/info.html). Before docking, the protein was prepared by removing water molecules, adding missing atoms, and optimizing the structure. The protonation states of the amino acids were adjusted according to the pH relevant to the biological context of the study.Molecular docking was performed using AutoDock Vina4 software [28]. The grid box was defined to encompass the active site of the receptor, providing sufficient space for ligand binding.

The docking results were analyzed based on binding affinity scores and positional data. Key interactions between **EE-Sb**and the active site of the protein were examined through visual inspection using the PyMOL molecular visualization tool[29, 30]. The top-ranked binding poses were further assessed for hydrogen bonds, hydrophobic interactions, and other relevant docking metrics.The docking protocol was validated by redocking a co-crystallized ligand from the PDB structure to determine the accuracy and reliability of the computational method.

A root means square deviation (RMSD) of less than 2 Å was considered acceptable for successful redocking[31].The results were analyzed using Discovery Studio 4.0 software, AutoDock Tools, and LigPlotsoftware[32-34].

3. Results and Discussion

Molecular Structure of the Bis-SchiffBase

EE-Sb (synthesized Schiff base) is a stable diimine because it does not undergo imine-enamine tautomerism. Geometrical isomerism in Schiff bases influences their characteristics and arises from the presence of a C=N bond, resulting in various spatial configurations of the substituents. Depending on the groups linked to the carbon and nitrogen, it may exhibit various configurations resulting in Z and E isomers. Large groups adjacent to the C=N bond may stress specific conformations, affecting isomer stability. Grasping geometrical isomerism in Schiff bases is crucial for their reactivity, characteristics, and uses in multiple domains, such as medicinal chemistry and materials science. DFT calculations can assist in assessing the stability of various isomers and their corresponding energies.

Every imine bond (N=CH) can exist in two geometric forms, leading to four geometric isomers (i.e., ZZ, EZ, ZE, and EE) for a bis-Schiff base featuring two imine bonds. In this case, since the identical groups link to both imine bonds, only three geometric isomers can be present for the produced Schiff base. All three geometrical isomers (three diastereomers) were optimized using the DFT/B3LYP/6-311g (p, d) method, and their internal energies and dipole moments were calculated (refer to **Table 1**). **Table 1** illustrates that the EE-geometrical isomer is the most stable form. Aryl and alkyl substituents exhibit steric hindrance, resulting in the ZZ-isomer and EZ-isomer energies being 7.98 and 15.23 kcal/mol higher than that of the EE-isomer, respectively. As a result, this study highlights the attributes of the EE-isomer. Three-dimensional models (generated using GussView06 software) of these three configurational isomers, along with several structural parameters and atomic numbers, are shown in **Figure 1**.

The dipole moment quantifies a molecule's polarity, indicating the charge distribution in Schiff bases. It may influence their solubility, reactivity, and intermolecular interactions.

Table 1 shows that the dipole moment (μ, Debye) of **EE-Sb** is zero, categorizing it as a non-polar molecule because of its symmetry. Conversely, the EZ and ZZ diastereomers are regarded as polar molecules due to their dipole moment values.

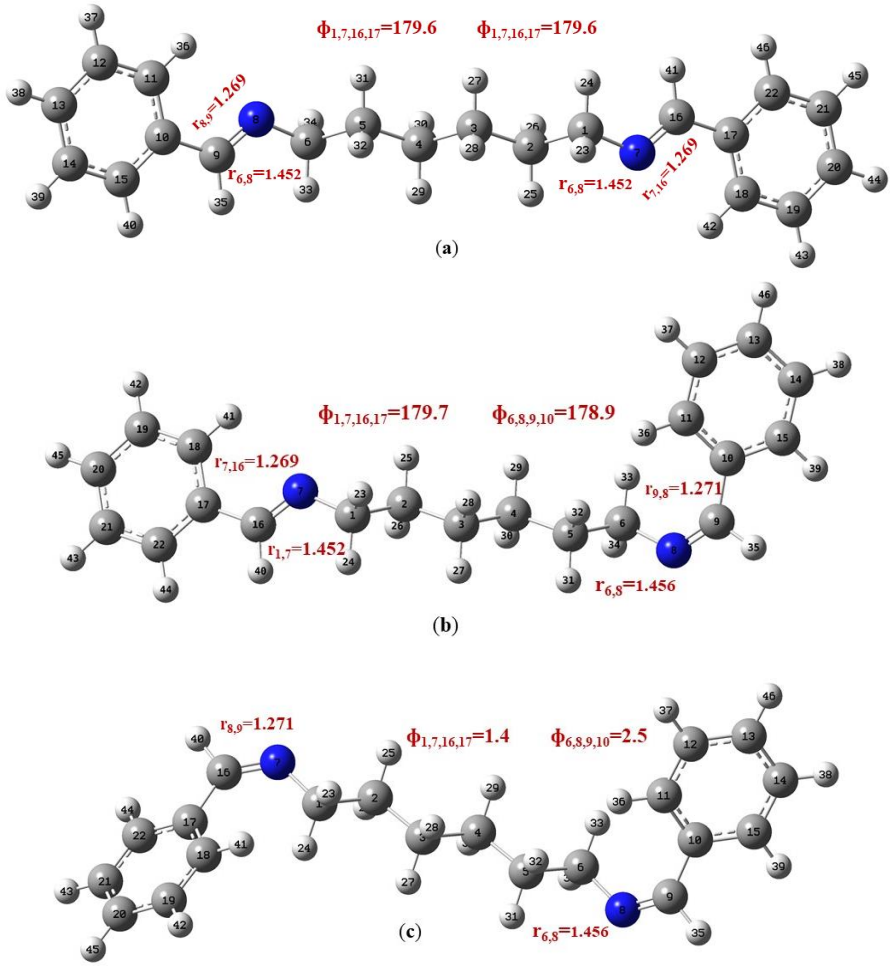


Figure .1. The 3D structures of three geometrical isomers (**a**) *EE*,(**b**) *EZ*, and (**c**) *ZZ*

Table .1. Calculated internal energies and dipole moments of three isomers of the *bis*-Schiff base at B3LYP/6-311G (d, p) level of theory

bis-Schiff base	Internal Energy (*au*)	ΔE(*au*)	ΔE(*kcal/mol*)	μ (D)
EE	-886.2959	0.00	0.00	0.00
EZ	-886.2832	0.01	7.98	1.05
ZZ	-886.2716	0.02	15.23	1.31

Figure 1 shows the lengths of the $r_{C=N}$ and r_{C-N} bonds in various diastereomers are nearly identical. These bonds are 1.272 *Å*and 1.45 *Å*, respectively. The Φ_{CCNC} dihedral angle exhibits a configuration characteristic of an imine bond. The 180ºvalue is associated with the *E*-geometric isomer, while Φ_{CCNC} equaling zero degree indicates the *Z*-isomer.

Thermodynamic Stability

The thermodynamic stability of Schiff bases can be analysed using a combination of experimental techniques and computational methods like DFT. DFT calculations can provide the energies of reactants, products, and intermediates, allowing for the estimation of formation energies and stability.Understanding the thermodynamic stability of Schiff bases is critical for their application in catalysis, pharmaceuticals,dye development, and drug design, where the stability of intermediates or final products is paramount. The factors influencing the thermodynamic stability of Schiff bases are electronic effects and steric hindrance, solvent interactions, and tautomerism. Electron-withdrawing groups stabilize the imine bond by stabilizing the positive charge at the nitrogen. In contrast, electron-donating groups may destabilize the imine linkage by increasing electron density around nitrogen [35, 36].In **EE-Sb** resonance between the aromatic ring (mesomerism effect) and the imine group increases the stability of this base.

The thermodynamic parameters of **EE-Sb** were calculated at B3LYP/6-311G (d, p) level of theory, these data are listed in **Table 2**. A positive Gibbs free energy change (ΔG^{o}),enthalpy change(ΔH^{o}), and entropy (ΔS^{o}) during the formation of **EE-Sb** indicatethat the formation is thermodynamically unfavoured at room temperature.

Table . 2. Calculated thermodynamic parametersof **EE-Sb** in the gas phase.

ΔH°(*kcal/mol*)	**ΔG°**(*kcal/mol*)	**ΔE°**(*kcal/mol*)	**ΔS°**(*kcal/K.mol*)
3.35	5.91	3.35	3.33

Infrared spectrum analysis

Infrared (IR) spectroscopy is a valuable technique for characterizing molecular structures, including Schiff bases. The IR spectrum provides information about the functional groups and the molecular environment within a compound based on the absorption of infrared light at specific wavelengths [37-39].

The calculated and experimentalIR spectra of **EE-Sb** are shown in **Figure 1S**. Wave number and intensityof vibrational modes are listed in**Table 1S.**According to the 3N-6 formula, **EE-Sb** has 132 wave numbers, but 48 vibrational modes are IR inactive or non-active modes (their epsilon values are 0.00) because of molecular symmetry.If the epsilon (ε) value is zero for a vibrational mode, this typically means that the vibrational mode does not result in a change in dipole moment. This means that the mode cannot be detected using infrared spectroscopy because it does not interact with IR radiation.

All of the calculated wave numbers are scaled by multiplyingby a factor of 0.967 (concerning the **C**omputational **C**hemistry **C**omparison and **B**enchmark **D**ata **B**ase (CCCBDB)). The stretching vibration of the C=N bond is a key feature in the Schiff bases' IR spectrum, typically appearing around 1600–1640 cm^{-1}. This band is often strong and can be used to confirm the presence of the imine functional group.According to **Table 1S,** imine bond vibrations are in 1662.39cm^{-1} (stretching, symmetrical) and 1662.86cm^{-1} (stretching, asymmetrical) (experimental value is1638 cm^{-1}).

Aliphatic C-H stretching vibrations can be found in the 2850–3000 cm^{-1} region. Aromatic C-H stretching typically appears slightly higher, around 3000–3100 cm^{-1}. For **EE-Sb**, symmetrical stretching of aliphatic CH bonds isat 2864.81, 2902.77, 2914.97, 2924.01, 2925.65, 2934.83, and 2966.79 cm^{-1} region. The aromatic C-H stretching modes are at3051.39, 3051.40 cm^{-1} (unsymmetrical stretching of one ring) and 3093.53, 3082.23 cm^{-1}

(symmetrical stretching) regions. For these vibrational modes, experimental values are 2936 and 2856 cm^{-1}.

Bending modes of C-H bonds, particularly from alkyl groups, may appear around 1350–1470 cm^{-1}. Bending vibrations of this base are as follows: 1364.21 cm^{-1}($\delta_{CH\text{-}NCH}$,twisting), 1438.34, 1447.63, and 1466.87 cm^{-1}(δ_{CH2},twisting), and 1434.18, 1475.24 cm^{-1} (δ_{CH2},wagging).

The region below 1500 cm^{-1} is referred to as the fingerprint region and contains many complex vibrations. The specific patterns in this region can provide additional information regarding the molecular structure and functional groups present.

Ultraviolet-Visible Absorption Spectrum

Ultraviolet-Visible (UV-Vis) spectroscopy is a powerful analytical technique used to study the electronic transitions in a molecule. For Schiff bases, the UV-Vis absorption spectrum provides valuable insights into their electronic structure, conjugation, and functional groups. The wavelength of maximum absorbance (λ_{max}) for Schiff bases typically occurs in the range of 250 to 400 *nm*. The exact position of λ_{max}can vary depending on the specific structure of the Schiff base and the substituents. The degree of conjugation in the Schiff base has a significant impact on its UV-Vis spectrum, and the absorption peak may shift to a longer wavelength via resonance [40-43].

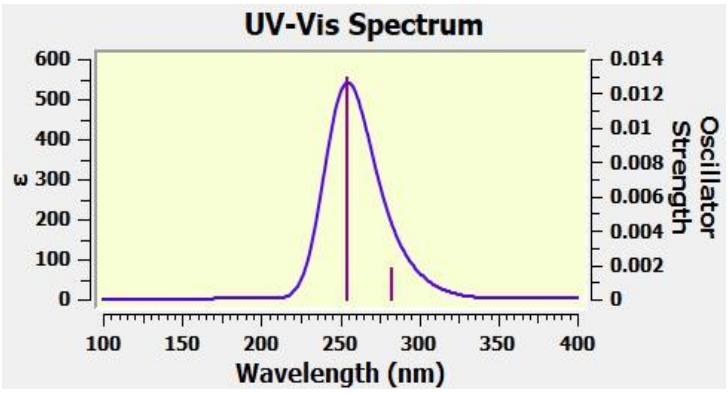


Figure. 2. Calculated UV-Vis spectrum of **EE-Sb** Schiff base.

UV-Vis absorption spectrum of **EE-Sb** was calculated by the TD/DFT method (**Figure 2**).Due to the presence of π electrons in the C=N bond and aromatic ring, it exhibitsa strong absorption band at254 nm (oscillator strength=0.0129). Oscillator strength measures the probability of an electronic transition. A value of 0.0129 indicates a weak transition, meaning the molecule has a low probability of absorbing photons at that specific wavelength. This band is a result of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions.The intensity of the absorption peaks can provide information about the molar absorptivity (ε) and the nature of the transitions. Stronger absorptions usually indicate more allowed transitions, while less intense peaks may suggest forbidden transitions or less efficient overlap of molecular orbitals.

Nuclear Magnetic Resonance (NMR) spectra analysis

NMR spectroscopy is a powerful tool for the structural elucidation and characterization of Schiff bases. It is essential for characterizing them, revealing valuable structural information through the examination of chemical shifts, multiplicity, multiple patterns, integration, and coupling constants. By carefully interpreting NMR spectra, we can deduce the presence and arrangement of functional groups, verify the formation of Schiff bases, and explore their conformations and interactions in chemical reactions[44, 45].

The ^{1}H and ^{13}CNMR spectra of **EE-Sb** were calculated in the gas phase andare shown in **Figures 3**and **4**. The data in **Table 3** represent the aromatic protons that appear downfield (higher ppm), generally around 6-8 ppm. The proton attached to the nitrogen of the imine group (C=N) typically shows a distinct signal, often around 8-10 ppm, due to its de-shielding effect from the electronegative nitrogen. Protons on the carbon adjacent to the imine group may also resonate downfield, usually around 3-6 ppm, depending on the electronic environment. If the imine proton shows coupling with neighbouring protons, it can indicate whether they are in a cis or trans configuration relative to the imine bond.Data in **Table 3** shows that two hydrogens in CH=N groups (H41, H35) are at 8.45 ppm, while two aromatic

hydrogens (near the N imine, H42 and H36) are deshielded, exhibiting a chemical shift at 8.41 ppm. Eight aromatic hydrogens appear at 7.52 ppm. The aliphatic hydrogens of CH_2 are not chemically equivalent. The simulated spectrum shows a distinct peak for each of the aliphatic hydrogens, but experimentally, they appear as multiplepeaks. In the ^{13}CNMR spectrum, four types of aromatic carbons are expected; however, due to the steric orientation of the nitrogen atoms, all aromatic carbons are not equivalent to each other. The carbons of the imine groups are at 160.86 ppm, and three types of aliphatic carbons are found at 61.87, 30.83, and 27.15 ppm, respectively. In both the experimental and simulated spectra, the chemical shift values are in good agreement.

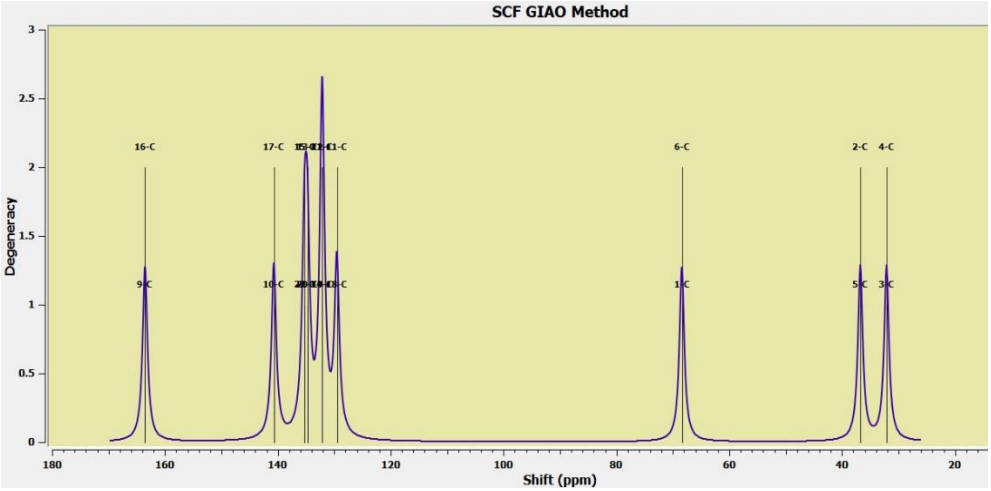


Figure. 3. Calculated ^{13}CNMR spectrum of **EE-Sb** Schiff base.

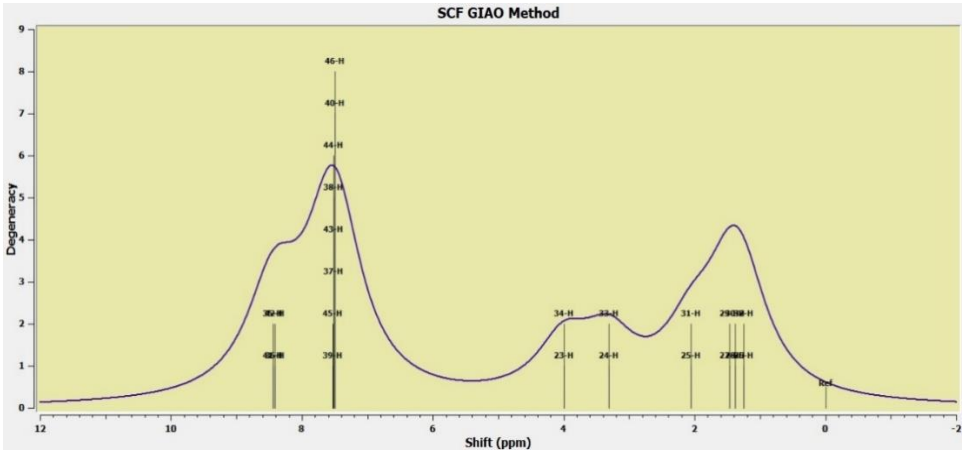


Figure .4. Calculated ^{1}HNMR spectrum of **EE-Sb** Schiff base.

Table . 3. The calculated and experimental chemical shifts (δ, *ppm*)of **EE-Sb** Schiff base.

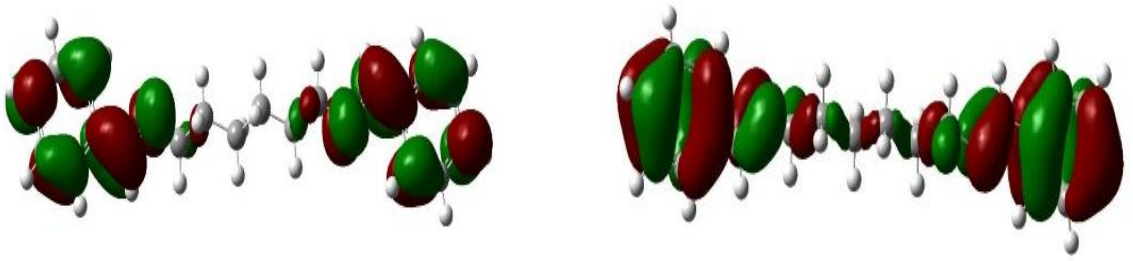
Types of atoms	^{13}CNMR		Types ofatoms	^{1}HNMR	
	Experimental[41]	**calculated**		**Experimental** [41]	**Calculated**
9, 16	160.86	163.53	**41,35**	8.19 (s, 2H),	8.45
10, 17	-	140.70	**36,42**		8.41
22, 15	136.31	135.28	**37,38,39,40, 43,44,45,46**	7.32-7.65(m, 10H)	7.52
20, 13	134.46	134.77	**23,34**	1.35-3.51(m, 12H)	4.00
14, 21	130.47	132.17	**24,33**		3.31
19, 12	128.55	132.11	**25,31**		2.06
18, 11	128.03	129.54	**27,29**		1.46
1, 6	61.87	68.40	**28,30**		1.38
5, 2	30.83	36.77	**26,32**		1.25
3, 4	27.15	32.13			

HOMO–LUMO Analysis and Quantum Molecular Descriptors

HOMO-LUMO (frontier molecular orbitals) analysis is a vital aspect of understanding the electronic properties of molecular systems, including Schiff bases. These compounds often display intriguing electronic characteristics due to the presence of the imine functional group (C=N). The HOMO is associated with the molecule's capacity to donate electrons, while the LUMO represents its ability to accept electrons. For Schiff bases, the HOMO is typically localized around the nitrogen and adjacent carbon atoms, whereas the LUMO may be more delocalized. Insights gained from HOMO-LUMO analysis can guide the design of Schiff bases for various applications, including sensors, organic electronics, and pharmaceuticals, where their electronic properties are crucial. By examining the energy levels and spatial distributions of these orbitals, we can predict the reactivity, stability, and potential applications of these compounds. Computational chemistry methods are commonly employed to conduct these analyses effectively [46-49].

Plots of FMOs for **EE-Sb** are presented in **Figure 5**. **EE-Sb** exhibits π-conjugation due to the presence of C=N bonds alongside aromatic rings, which can enhance electron

delocalization. The energy difference between the HOMO and LUMO, or energy gap, is crucial for understanding the stability and reactivity of the molecule. This energy gap is also related to the optical properties of Schiff bases. The wavelength of light absorbed corresponds to the energy difference between these orbitals, making this analysis useful for predicting UV-Vis absorption spectra. Research articles and spectroscopic studies indicate that a larger gap (>4.5 eV) generally signifies a more stable, less reactive molecule that absorbs in the UV region. Smaller gaps (~3–4 eV) suggest increased reactivity and absorption closer to visible light. The energy gap of 4.56 eV between the HOMO and LUMO in ***EE-bis-*** Schiff base indicates the amount of energy required to excite an electron from the HOMO to the LUMO. This reflects that the molecule is less reactive and more stable. Additionally, it suggests that **EE-Sb** is less likely to engage in electron transfer reactions or absorb visible light, which can influence its optical properties.



HOMO **LUMO**

Figure. 5. Calculated plots of frontier molecular orbital surfaces for**EE-Sb** Schiff base

Quantum molecular descriptors are mathematical functions derived from quantum mechanical calculations that characterize the chemical and physical properties, behavior, interaction capabilities, stability, and activities of molecules, including Schiff bases [50, 51]. Quantum molecular descriptors include ionization potential (IP, the tendency of a system to change in response to variations in particle number or concentration), electron affinity (A), global hardness (η), global softness (σ, which indicates how easily the molecule can donate

electrons), and electrophilicity index (ψ). These descriptors can be derived from HOMO and LUMO energies using the corresponding equations (see **Table 4**).

Table .4. The calculated quantum molecular descriptors of **EE-Sb** Schiff base

Parameter	value
E_{HOMO} / *eV*	-7.26
E_{LUMO} / *eV*	-2.70
E_{HOMO}–E_{LUMO} (ΔE) / *eV*	4.56
Ionization potential [IP = $-E_{HOMO}$], *eV*	7.26
Electron affinity [A = $-E_{LUMO}$], *eV*	2.70
Global hardness [η =(IP − A)/2], *eV*	2.28
Global softness [σ = 1/η], *eV*	0.44
Electrophilicity index [ψ = $\chi^2/2\eta$], *eV*	5.44
α_{ave}(in *a.u.*)	254.7187
α_{ave}(in*esu*)	37.749×10^{-24}

Global softness (σ) is a measure of a molecule's chemical reactivity, defined as the inverse of its hardness (η). It indicates how easily the electron cloud can be distorted or how reactive the molecule is. Typically, global softness ranges from near zero (very hard, less reactive molecules) to large values (very soft, highly reactive molecules). These ranges are as follows: hard molecules: σ ≈ 0.01–0.1 eV^{-1}, moderately soft: σ ≈ 0.1–1 eV^{-1}, and very soft/reactive: σ > 1 eV^{-1}. Softness increases as the HOMO-LUMO gap decreases, as a small gap means electrons can be more easily excited or transferred. Since softness is the reciprocal of hardness (η), and hardness relates to the HOMO-LUMO gap, a typical softness value for a stable organic molecule might be in the vicinity of 0.1–0.5 eV^{-1}. For the **EE-Sb** molecule, the softness value is σ=0.44, indicating that this Schiff base is moderately soft.

Ionization potential (IP), as a quantum molecular descriptor, typically ranges from about 6 to 12 eV for most organic and inorganic molecules. Ionization potential measures the energy required to remove an electron from a neutral molecule or atom. Lower IP values (~6–8 eV) indicate molecules that are easier to ionize, often associated with higher reactivity or

electron-donating ability. Higher IP values (~9–12 eV) correspond to more stable, less reactive molecules with strong bonds. The specific IP value depends on the molecular structure, atomic composition, and the computational method used to estimate it. For the **EE-Sb** molecule, the ionization potential value is IP=7.26, indicating that this Schiff base is reactive.

The electrophilicity index (ψ) is a quantum molecular descriptor that quantifies a molecule's ability to accept electrons; it typically ranges from approximately 0.0 to 2.0 eV, with most organic molecules falling below 1.0 eV. Low values (~0.0–0.5 eV) indicate molecules with weak electrophilic character, making them less likely to accept electrons. Moderate values (~0.5–1.0 eV) suggest a moderate level of electrophilicity. High values (~1.0–2.0 eV) indicate strong electrophiles that readily accept electrons in reactions. The exact value depends on the molecule's electronic structure, and higher electrophilicity suggests a greater capacity to participate in electron-acceptor reactions, such as nucleophilic attacks.

Polarizability measures how easily the electron cloud of a molecule can be distorted by an external electric field. The value given, 37.749×10^{-24}*esu*, indicates the extent to which the electron distribution in **EE-Sb** can be polarized. **EE-Sb** has a relatively high capacity for dipole formation in response to an electric field. This can influence its interactions with other molecules, such as in solvation, bonding, and reaction mechanisms. It may also affect optical properties, including the absorption and scattering of light, which are important in many applications, such as dyes and sensors. The polarizability can be influenced by the electronic structure and steric factors.

Mulliken atomic charges analysis

Mulliken atomic charge analysis is extensively utilized in computational chemistry to estimate charge distribution within a molecule based on its electronic structure. In a Schiff

base, this analysis can offer insights into the electronic environment of the involved atoms, particularly the nitrogen and carbon in the imine group. Mulliken atomic charge analysis of a Schiff base provides valuable information regarding the electron distribution within the molecule, aiding in the prediction of its behavior in chemical reactions, its reactivity, and its interactions with other molecules. The Mulliken charges are derived from the overlap populations between atomic orbitals, where the charge on each atom is defined based on the electron density distribution surrounding that atom [52, 53]. The overall distribution of charges can indicate the electron-withdrawing or donating nature of various substituents attached to the Schiff base, influencing its reactivity and physical properties. However, Mulliken charges can sometimes present a misleading picture, as they tend to overestimate the charge on more electronegative atoms while underestimating the charges on less electronegative ones.

The calculated electronic charge–density distributions (Mulliken atomic charges) of **EE-Sb** are summarized in **Table 5**. Results indicate that all hydrogen atoms possess a net positive charge. The H35 and H41 atoms (CH=N, 0.061e) exhibit a lower positive atomic charge compared to the other hydrogen atoms. This is attributed to the presence of an electronegative imine group and the resonance effect of the aromatic ring. Nitrogen atoms carry a significant negative charge (-0.291e) due to their lone pair of electrons and involvement in the double bond with carbon. The charge distribution can reflect the nitrogen atom's basicity and potential interactions in chemical reactions. The carbon atoms in the imine groupshavea partial positive charge (C-9 and C-16, 0.132e) due to resonance effects. This charge distribution highlights its susceptibility to nucleophilic attack. All of the other carbon atoms have negative charges, which is due to adjacent electronegative nitrogen atoms. These data clearly show that **EE-Sb** is reactive toward substitution reactions.

Table .5. Calculated Mulliken atomic charges of **EE-Sb**

C-atom	charge	H-atom	charge	H-atom	charge
C-1	-0.072	C-19	-0.095	H-35	0.061
C-2	-0.205	C-20	-0.081	H-36	0.100
C-3	-0.213	C-21	-0.095	H-37	0.098
C-4	-0.213	C-22	-0.067	H-38	0.097
C-5	-0.205	H-23	0.114	H-39	0.095
C-6	-0.072	H-24	0.075	H-40	0.088
C-9	0.132	H-25	0.116	H-41	0.061
C-10	-0.114	H-26	0.103	H-42	0.100
C-11	-0.023	H-27	0.104	H-43	0.098
C-12	-0.095	H-28	0.105	H-44	0.097
C-13	-0.081	H-29	0.104	H-45	0.095
C-14	-0.095	H-30	0.105	H-46	0.088
C-15	-0.067	H-31	0.116	**N-atom**	**charge**
C-16	0.132	H-32	0.103	N-7	-0.291
C-17	-0.114	H-33	0.075	N-8	-0.291
C-18	-0.023	H-34	0.114		

Map of Electrostatic Potential (MESP)

The electrostatic potential map illustrates how the electric potential varies in the molecular structure. MESP provides a visual method to understand the relative polarity of a molecule. The map shows regions of high and low electron density[54-56]. Electrostatic potential maps are often color-coded. Typically, red indicates regions of high negative charge density, blue indicates high positive charge density, and green represents neutral areas. Areas of the molecule that have dipole moments will show distinct potential variations. Thus, the MESP map has been primarily used to identify electrophilic and nucleophilic sites, as well as in studies of biological recognition and hydrogen bonding interactions.

Such a mapped electrostatic potential surface has been plotted for the title compound using GaussView 06 employing B3LYP/6-311G(d,p) level of theory. The nitrogen atom in **EE-Sb**,

due to its electronegativity, attracts electron density, leading to regions of negative potential. Conversely, areas near the carbon show relatively positive potential due to their partial positive charge. The MESP map indicates that the negative regions (red) correspond to the nitrogen atoms; therefore, electrophilic attack can occur at these sites. The positive regions (blue) are over the hydrogen atoms of **EE-Sb**, hence, nucleophilic attack can take place in these regions. **Figure 6**provides a visual representation of the chemically active sites and comparative reactivity of the atoms.

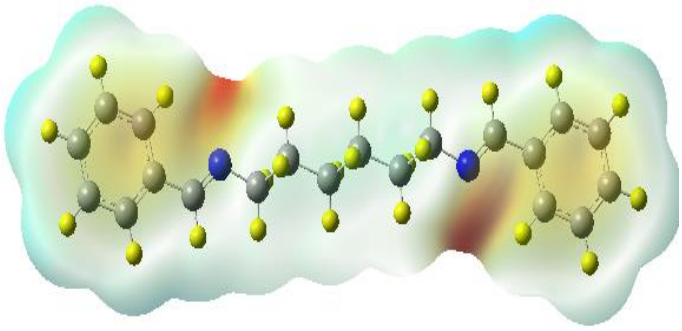


Figure 6. Electrostatic Potential Map of **EE-Sb** Schiff base (obtained by Gussview06 visualization software)

Analysis of Docking study

The output from molecular docking provides insights into the pose (orientation and conformation) of the ligand within the binding site, binding energies, and interactions formed during docking (such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions). Docking studies can help predict the potential biological activity of Schiff bases by evaluating their ability to bind to specific targets. This is particularly valuable in drug discovery for identifying promising candidates for further development [57-60]. Molecular docking can be employed to investigate how different substituents on the Schiff base influence its binding affinity and interactions with the target. This aids researchers in understanding the contributions of specific structural features to biological activity. Once a Schiff base demonstrates promising activity in docking studies, further modifications can be implemented to enhance its binding affinity, specificity, or pharmacokinetic properties.

Molecular docking can inform these modifications by predicting how changes will impact binding. Docking can be utilized in virtual screening strategies to assess extensive libraries of Schiff bases or related compounds, aiding in the identification of potential hits for further experimental validation. The docking results may indicate that the Schiff base binds effectively to the active site, forming hydrogen bonds and hydrophobic contacts, suggesting a mechanism through which it exerts its inhibitory effect. Further optimization may involve modifying the substituent groups on the Schiff base to enhance binding affinity based on the docking predictions. Through this computational approach, researchers can predict binding modes, affinities, and the potential biological activities of Schiff bases, ultimately aiding in the rational design of new therapeutics. By refining the docking simulations and analyzing the interactions, scientists can make informed decisions in the drug discovery process [61-65].

Molecular docking Results

It is essential to determine the dimensions of the Cartesian grid box at the beginning of docking studies. Properly defining this box ensures the docking process covers the relevant binding site comprehensively, allowing accurate prediction of ligand-receptor interactions. An incorrectly sized grid might miss the active site or include irrelevant regions, reducing the reliability of the results. So, setting the right grid box dimensions is a crucial preparatory step in molecular docking. The dimensions of the Cartesian grid box for the protein receptors typically depend on the specific docking study and the size of the binding site. They are usually set to encompass the active site with some margin for flexibility. **Table 6** presents the centers and dimensions of the Cartesian grid box of the protein receptors studied for conducting molecular docking with **EE-Sb (**ligand). The results of molecular docking studies using AutoDock are shown in **Table 7** and **Figure7**and include binding energies, **EE-Sb (**ligand) interactions, hydrogen bonds, and hydrophobicity. The optimal combination with the

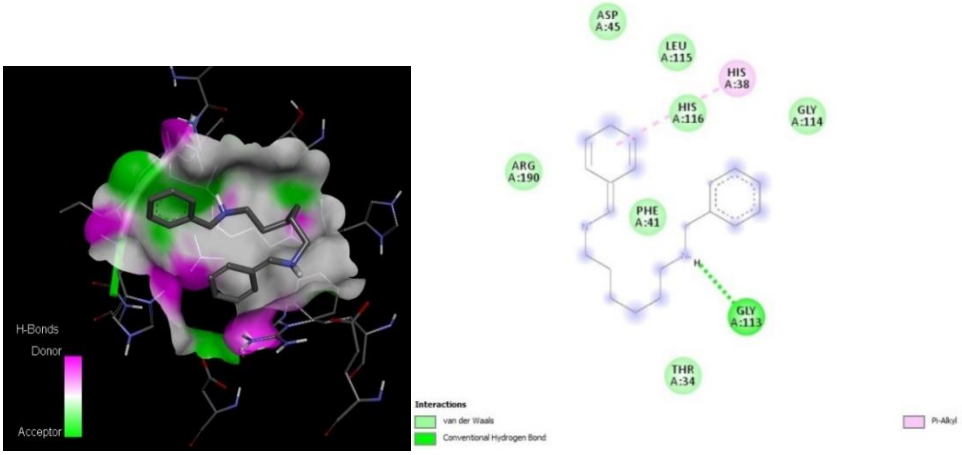
lowest affinity energy was chosen as the outcome of docking. The affinity of the ligand for the receptor binding site increases with decreasing energy. The results were compared to doxorubicin, the medication of choice, and were analyzed using MGLTools. Qualitative studies show in **Figure 7**, that of the total amino acids of the proposed active site in receptors of **1AJ6**, **6LU7**, and **1HSG**only amino acids (ASP30 and TYR32 at the distances of 2.37 and 2.29 Å), (THR216 at the distance of 2.03 Å) and (ASN46 and LYS103 at the distances of 2.93 and 2.23 Å), respectively, with the nitrogen atoms of the imine group in the formation hydrogen bonds are involved. As depicted in this figure, the ligand-receptor interaction of **1AJ6**, **6LU7**, and **1HSG**also indicates the location of this ligand with a hydrophobic interaction position, with the number of 8, 9, and 10 amino acids, respectively. The results of molecular docking showed that the studied ligand performed well and had an acceptable affinity energy. Considering that the studied ligand has two imine segments, it is expected to be a good inhibitor. The binding results of the synthesized ligand showed that this compound can bind to the active site of the protein receptors investigated in the present study and partially inhibits these receptors. Based on the binding results, the inhibitory potential of the studied ligand is different from the active site of protein receptors. The lowest affinity energy corresponds to the **1Z11** receptor with an inhibition constant (Ki) of 15.20μM, and the highest affinity energy corresponds to the **1STE** receptor with a Ki of 798.04μM. Doxorubicin has been used as the reference drug, with ligand(**Figure 7**)was examined. In **Figure 7**, carbon atoms are gray, oxygen atoms are red, and nitrogen atoms are blue.

Table .6. Centers and Cartesian size of the grid box for the studied protein receptors

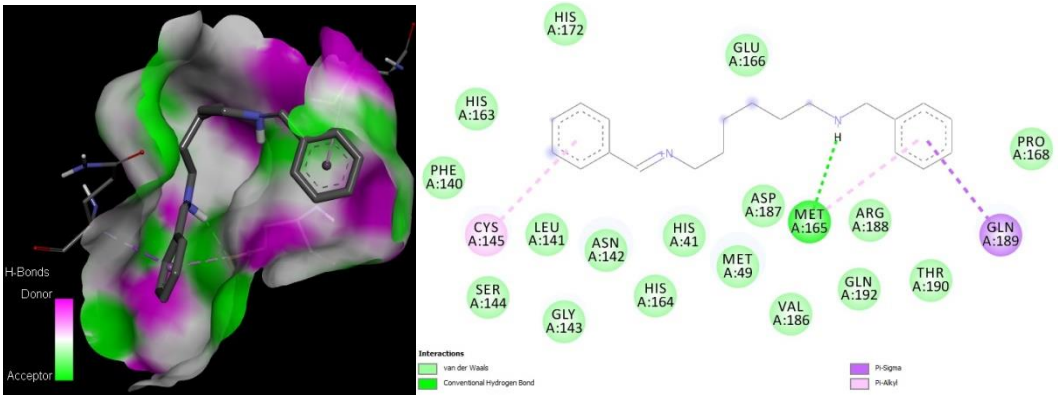
Receptor	Center			Size		
	X	Y	Z	X	Y	Z
1AJ6	59.2	5.6	45.0	79.3	29.3	66.7
6LU7	-26.9	12.5	55.6	-3.3	36.1	79.3
1HSG	59.3	6.4	39.3	79.5	30.0	63.0

Table. 7. The affinity energy and interactions between**EE-Sb** and the amino acids of the active site of the receptors.

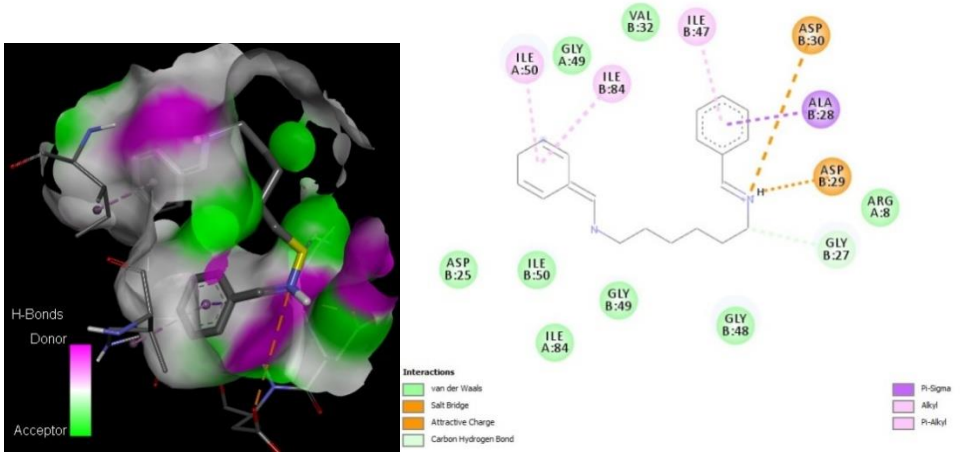
Receptor	Affinity energy ($kcalmol^{-1}$)	Ki (µM)	Hydrogen interactions (distance, Å)	Hydrophobic interactions
1AJ6	-5.85	51.32	GLY113(2.00)	ASP45-ARG190-LEU115-HIS116-PHE41-THR34-GLY114
6LU7	-5.80	56.52	MET165(2.17)	PHE140-HIS163-HIS172-GLU166-LEU141-SER144-GLY143-ASN142-HIS164-HIS41-MET164-HIS41-MET49-ASP187-VAL186-GLN192-ARG188-THR190-PRO168
1HSG	-5.61	77.13	---	ASP25-ILE50-ILE84-GLY49-GLY48-ARG8-VAL32-GLY49
Doxorubicin	-6.98	7.71	Ala 835 (4.25); Leu 837 (3.38); gTyr 740 (5.59); Glu 842 (5.41); Glu 842 (5.19)	Tyr845, Glu844, His846, Leu846, Gly839, Val741, Glu738,Asn676



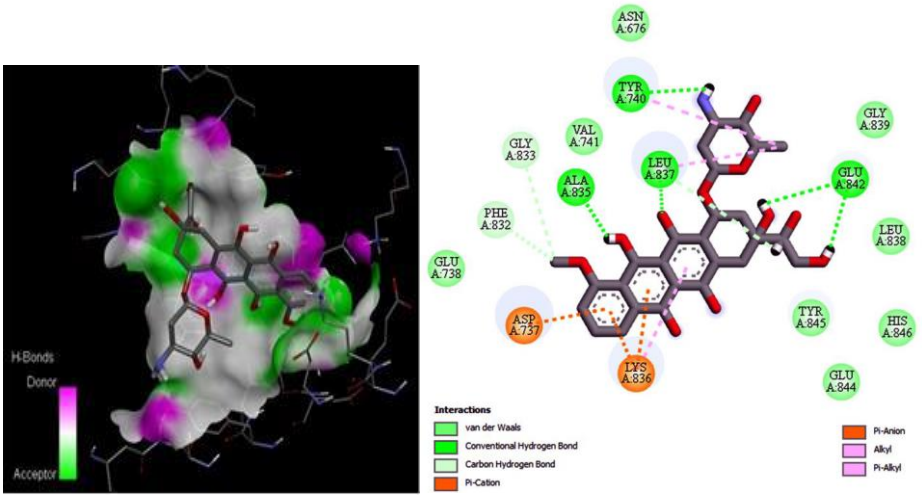
(a)1AJ6



(b)6LU7



(c)1HSG



(d) Doxorubicin

Figure .7. EE-Sb (ligand) interaction with the binding sites of receptors.

In the molecular docking studies of most of the ligand interactions with the introduced receptors, the part of **EE-Sb** that contains the phenyl rings is completely placed in a hydrophobic pocket of the active site of the receptors and the best orientation to create a hydrogen bond forit provides molecules (**Figure7**). A part of the molecule, which includes the phenyl ring and the nitrogen atom of the imine group, forms Van der Waals, pi-alkyl, pi-anion, etc. Electrostatic interactions with the side chains of amino acids in the active site of the receptors.

Quantitative studies of some docking results, including intermolecular energy, van der Waals energy, electrostatic energy, total internal energy, docking energy, affinity energy, and inhibition constant Ki are given in **Table 8**. The results obtained from the output specify the docking simulation for the studied ligand in the interaction with the active site of the receptors. The obtained values for affinity energy, docking energy, indicating the probable effectiveness of the inhibition of this ligand in the activity of 1Z11 receptor.In this molecular simulation, the calculated binding energy and docking energy represent a combination of intramolecular energy with free torsional energy and the ligand's internal energy, respectively [66, 67].

Table. 8. Molecular docking parameters (kcalmol^{-1}) for **EE-Sb**-receptor interaction

Receptor	**Parameters**						
	Unbound system energy	Dihedral free energy	total internal energy	Electrostatic energy	V_{dW}+H_{bond}+desolv energy	intermolecular energy	Docked energy
1AJ6	+0.86	+4.18	+0.86	-2.65	-8.56	-11.20	-10.64
6LU7	+1.17	+4.18	+1.17	-2.72	-9.51	-12.23	-11.06
1HSG	-0.02	+4.18	-0.02	-3.59	-8.55	-12.14	-12.16

Conclusion

In this study, a bis-Schiff basewas successfully synthesized through microwave-assisted condensation of benzaldehyde with hexane-1,6-diamine, achieving an 83% yield, whereasthe classic synthesis yield is 61%, and confirmed via spectroscopic and thermal analysis. Computational investigations using DFT/ B3LYP/6-311g (p, d) elucidated the stable geometrical configuration of the EE-isomer, highlighting its non-polar nature and structural features critical for potential biological interactions. Overall, the integrated experimental and theoretical approach provides valuable insights into the structural, thermodynamic, and interactive aspects of this bis-Schiff base, laying a foundation for future development in

medicinal and material science applications. The IR spectra confirmed the presence of key functional groups, notably the C=N imine bond, with vibrational modes closely matching computational predictions. UV-Vis analysis revealed a distinct absorption at 254 nm, consistent with $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, reflecting the conjugated electronic system within the molecule. The NMR spectra demonstrated good agreement between simulated and experimental chemical shifts, validating the computational approach. HOMO–LUMO analysis indicated a relatively wide energy gap (4.56 eV), implying the molecule's stability and moderate reactivity, supported by quantum descriptors. Overall, the synergy between experimental data and computational modeling enhances our understanding of **EE-Sb**'s properties, paving the way for future functional and application-based investigations. Mulliken charge analysis revealed regions of high nucleophilicity and electrophilicity, particularly highlighting the reactivity of nitrogen and carbon atoms involved in the imine groups. The electrostatic potential surface further illustrates active sites susceptible to nucleophilic or electrophilic attack, informing the compound's possible reactivity pathways. Docking results demonstrated that **EE-Sb** can effectively bind to multiple protein receptors, with binding energies comparable to known drugs like doxorubicin, and form stable interactions through hydrogen bonds and hydrophobic contacts. Especially notable is the strong affinity observed with the **1Z11** receptor, suggesting promising inhibitory potential. Overall, these findings suggest that **EE-Sb** possesses favorable electronic and binding characteristics, supporting its potential as a candidate for further development in therapeutic applications. Molecular docking studies demonstrated favorable binding affinities of the compound toward target proteins, suggesting promising bioactive properties.

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