Synthesis, Characterization, DFT calculations and molecular Docking study of Novel Copper mixed ligands complex on Drosophila melanogaster for insecticidal properties

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Abstract

In this study, the novel mixed ligand complex of copper is prepared from (Z)-N'-((E)-2-hydroxy-3,5-diiodobenzylidene)-N,N-dimethylcarbamohydrazono-thioic acid schiff base(H_2L)and 2,2'-bypyridine. The characterization has been done by NMR, elemental analysis, IR and HRMS. Computation calculations based on DFT showed that the HOMO-LUMO energy gap is -5.81 eV for ligand and -5.53 eV for the Cu(II) complex and the [Cu(L)(bipy)] is having square pyramidal geometry.

The binding energy values obtained from the molecular docking with acetylcholinesterase enzymes of Drosophila melanogaster for ligand (H_2L) and [Cu(L)(bipy)] are -6.8 and -7.4 (kcal/mol) respectively. These values showed that the ligand (H_2L) and [Cu(L)(bipy)] are having affinity for the target enzyme.

Keywords: Mixed ligand complex, acetylcholinesterase enzymes, Drosophila melanogaster.

Introduction

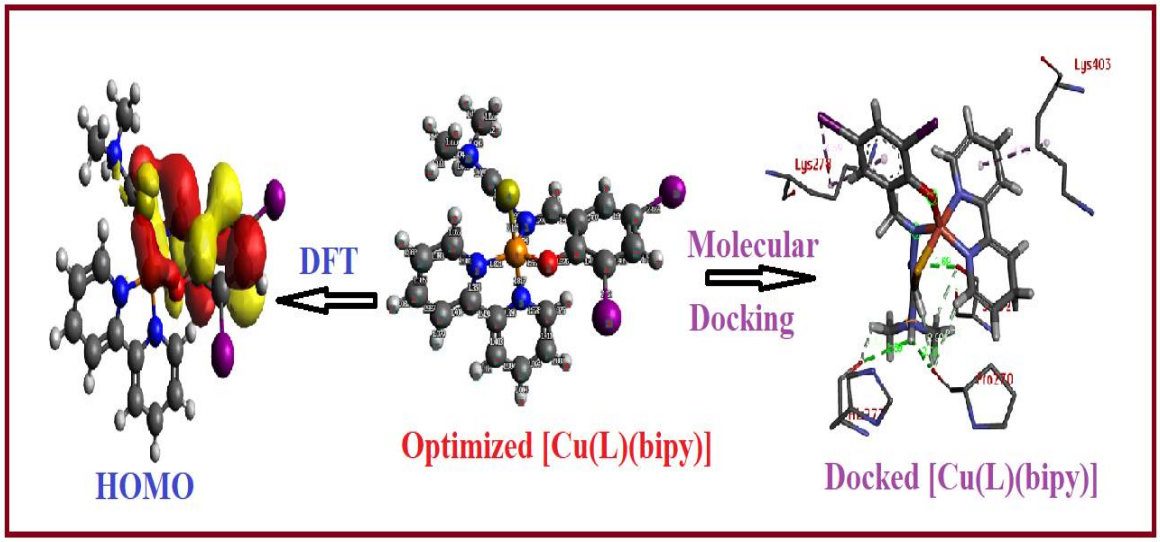
The metal complexes derived from Schiff bases are always in the research due to the ease of preparation and wide applications of theses complexes. The Schiff bases contain the azomethine group and show many biological properties like antibacterial, antimicrobial, antioxidant, antifungal, anticancer and antimalarial activities.[1,2] Over the past few decades, the copper chelates with Schiff bases were studied for varies applications in food industry, dye industry, analytical chemistry, catalysis etc.[3,4]

Schiff bases of metal complexes show many biological activities like anti-inflammatory, antidepressant, antiglycation, antibacterial. The metal complexes show insecticidal as well as plant growth regulator properties.[5] Molecular docking study is *in silico* method which provides the information about all the possible binding interactions of drug molecule with binding site of target.[6] So by using *in silico* methods, we can design the molecules with maximum biological activity and minimum side effect.

The acetylcholinesterase enzyme is important enzyme of insect where the most of insecticides like organophosphorus (OP) and neonecotinoides bind and kill the insect.[7] Our present investigation deals with the molecular docking study of newly synthesized copper complexes with the acetylcholinesterase of Drosophila melanogaster (common fruit flies).

Material and Methods

Materials used for synthesis: The chemicals used were obtained from Sigma-Aldrich and were used without further purification.



Graphical Abstract

Characterization techniques: The characterization was done by using elemental analysis and spectroscopic techniques. The FT-IR spectra of the compounds were recorded on a Shimadzu IR Affinity-1S Fourier transform infrared spectrophotometer in the range 4000-400 cm^{-1} using KBr pellets. The electronic spectra of the compounds were taken on a Thermoscientific UV-Vis recording spectrophotometer evolution-3000 in quartz cells. Melting point was measured on a Boetius micro melting point apparatus. The NMR of ligand is obtained from Bruker Avance 400/Avlll HD-300(FT NMR) instrument. The HRMS is obtained from Waters Alliance e2695/ HPLC-TQD Mass spectrometer.

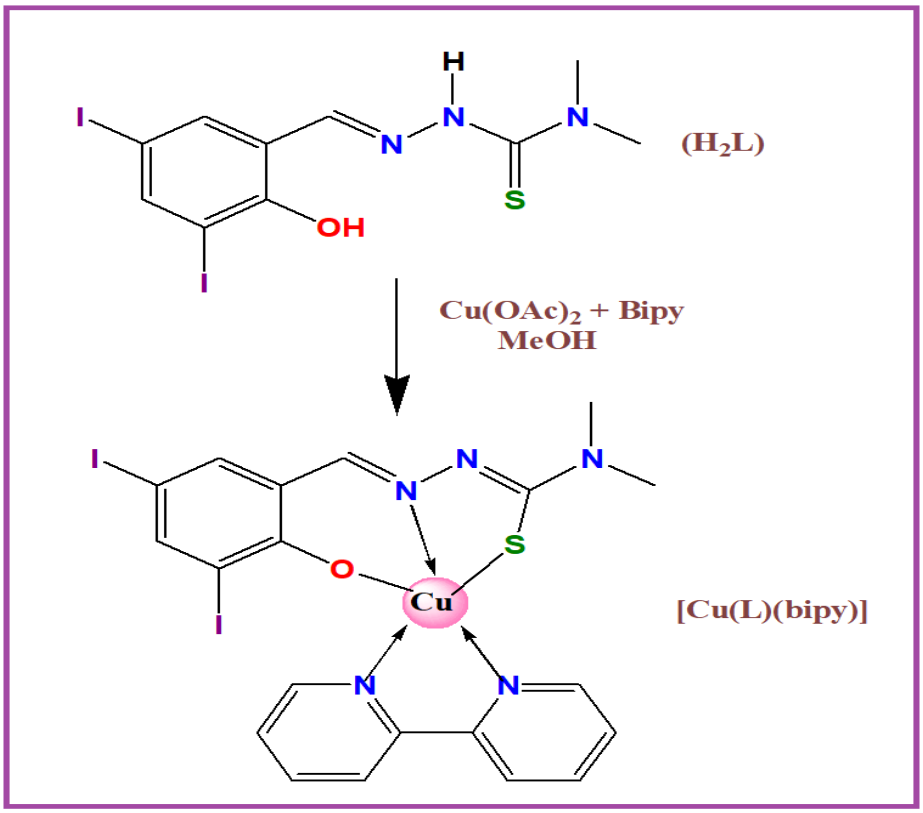
Computational methodology: We used the ORCA software package with Avogadro to do the computational analysis of ligand and complexes.[8,9] In ORCA software, the BP86 parameter at def2-SVP level was used for calculations. The spectral calculations were performed by using TD-DFT and the parameters BP86 parameter at def2-SVP level for ligand and B3LYP parameter at def2-TZVP (-f) for complex was used for electronic spectra calculations. An IR spectrum was obtained at the basic set at def2-SVP level of ORCA software.[10]

Molecular Docking: The molecular docking studies were performed using the Autodock Vina docking program.[11] Open source software Discovery Studio Visualizer is used to see the docking results. The acetylcholinesterase (PDB-1DX4) enzyme of Drosophila melanogaster was used for binding site for interaction. The A segment of acetylcholinesterase enzyme is used for docking.[12]

Results and Discussion

Synthesis of Thiosemicarbazone ligand: Thiosemicarbazone (H_2L) ligand was synthesized as white crystalline product by refluxing 3,5-diiodosalicylaldehyde (5 mmol) with 4,4-dimethyl-3-thiosemicarbazide (5 mmol) in 1:1 ratio in EtOH for 4hr.The white crystalline product was obtained, which was filtered, washed and recrystallized from MeOH and stored in a desiccator over $CaCl_2$. Yield: 88%. m.p.:195-200 °C. UV-Vis (MeOH) **λ** (nm): 253,360.

Synthesis of copper (II) complexes (1): $Cu(OAc)_2.H_2O$ (2.0 mmol) dissolved in 1ml of H_2O and MeOH solution (10 mL) of thiosemicarbazone (H_2L) ligand (2.0 mmol) was added with constant stirring. The reaction mixture was stirred continuously for 2h. To the above reaction mixture, the second ligand (2, 2'-bipyridine) MeOH solution (5 mL) was added with stirring and stirring continued for 1 hour. The blue colored reaction mixture was filtered to remove solid impurities and left for slow evaporation. After 2 days, blue colored powered products were collected, washed and recrystallized with MeOH and stored in the desiccators over $CaCl_2$. Yield: 82 %, m.p.251-253°C. UV-Vis (MeOH) **λ** (nm): 280,595. Elemental analysis of Ligand and Cu (II) complex is listed in table 1. The synthesis is shown in scheme 1.



Scheme 1: Synthesis of [Cu(L)(bipy)]

Table 1
Analytical and physical data of ligand and its copper (II) complexes.

Molecular formula	Colour/% yield	Elemental analysis: % found/ Calcd.		
		C	H	N
$C_{10}H_{11}I_2N_3OS(H_2L)$	White (88)	25.48 (25.59)	2.31 (2.34)	8.84 (8.85)
$C_{20}H_{17}CuI_2N_5OS$	Blue (82)	34.66 (34.68)	2.47 (2.48)	10.10 (10.12)

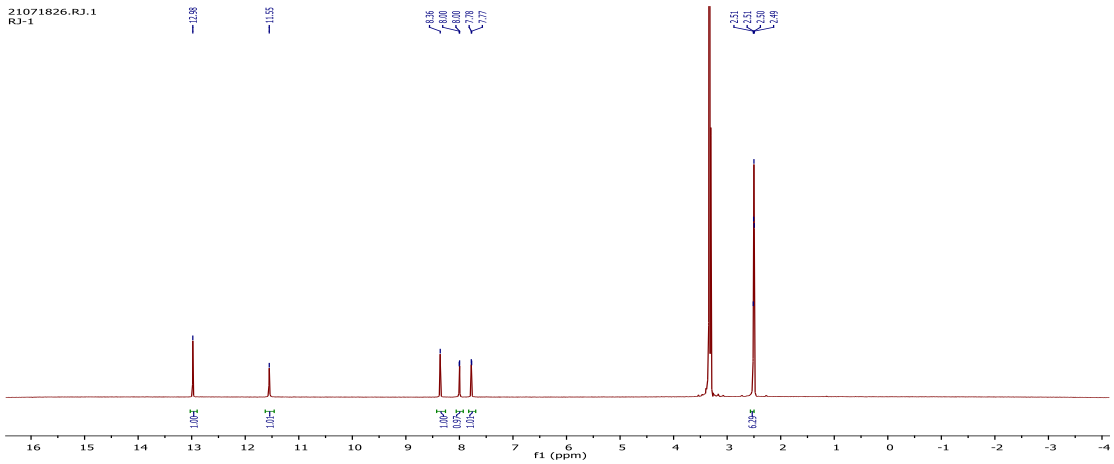


Figure 1: NMR of Ligand H_2L

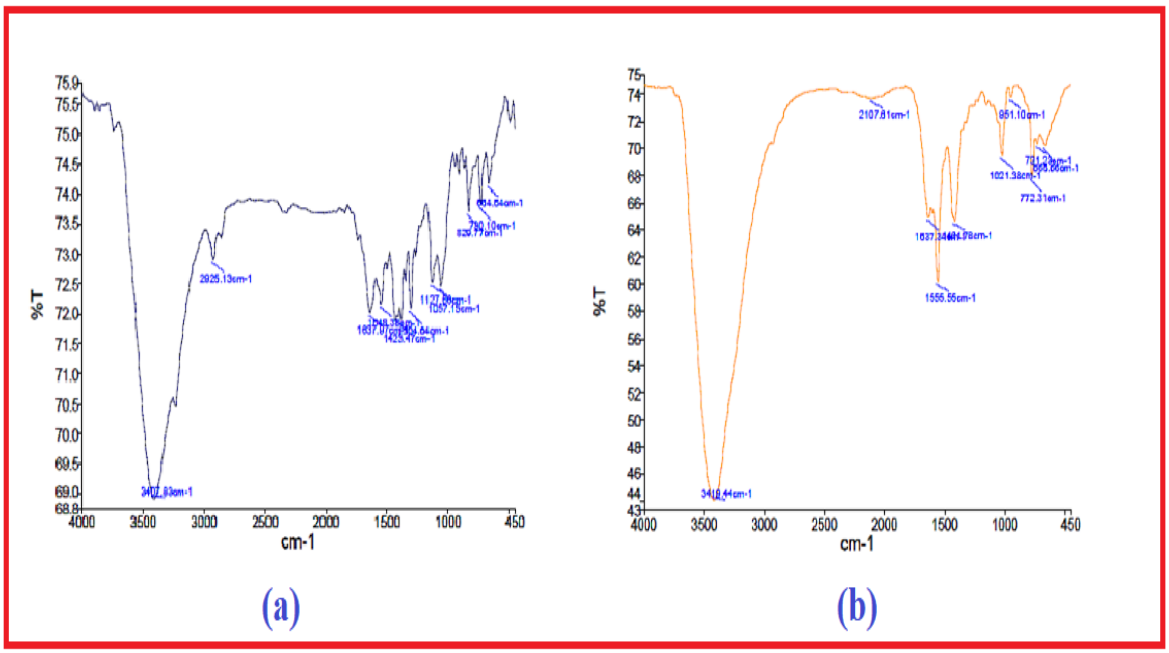


Figure 2: IR Spectra (a) Ligand (H_2L) and (b) Cu (II) Complex [Cu(L)(bipy)]

Characterization using Spectral Techniques

NMR Spectral analysis: The H-NMR of the ligand was taken in DMSO. The spectra shown in figure 1 has the peaks for 11 protons which confirmed the structure of ligand. Furthermore, there is upfield peak signal for six protons (methyl protons) at δ 2.51 of terminal methyl groups attached to nitrogen, two peaks containing one proton each are at δ 8.00 and δ 8.36 showing the aromatic protons of the ligand. The peak for OH group proton is at δ 12.98 and NH group proton is at δ 11.55 which are in downfield region due to the electronegativity of nitrogen and oxygen.[13]

IR spectral analysis: The experimental IR spectra are obtained for the ligand as well as for the complex of copper. The ligand co-ordinates with the metal through N, O, S atoms and the IR graph (Figure 2) showed that there is

decrease in the stretching vibration frequency for C-S bond [1057cm^{-1}in H_2L, 1021.38 cm^{-1} in Cu (II) complex]. This decrease in C-S double bond vibrational frequency is due to the ligand tautomerization during complexation. The N=C bond stretching vibration frequency showed slight decrease [1637.97 cm^{-1} in H_2L, 1637.34 cm^{-1} in Cu (II) complex]. The OH stretching frequency is missing in the complex due to the deprotonation during the complex formation (2925.13 cm^{-1} in H_2L).[14]

Mass Spectra analysis: HRMS data is obtained for the ligand and complex. In the HRMS of the ligand (Figure 3), the molecular ion peak absence showed that the molecule is unstable and fragmented rapidly in high energy electronic beam. In the ligand fragments, the m/z ratio are at odd peaks due to odd numbers of nitrogen atoms in the thiosemicarbazide ligand.[15] The complexes HRMS are shown in figure 4. The formation of complex is confirmed by the mass data.

Computational analysis

Optimized Geometry: The computational calculations based on DFT provide the structure of compound with minimum energy and maximum stability.[16] The molecular optimized geometries of ligand (H_2L) and metal chelates are presented in figure 5. The selected bond length (Å) and bond angles (°) for the optimized structures of ligand and Cu (II) complex are given in table 2. Computational calculations show that the bond length of the Cu-N is longer than the single covalent bond (Cu-S or Cu-O) which showed that these bonds are co-ordinate bonds. Here H_2L is a tridentate ligand, binding through N, O and S atoms. The co-ligands are bidentate and combine through N(2) and N(5) to have five coordination at the copper(II) center metal ion. Computational molecular optimized investigation indicated square pyramidal geometry around the Cu (II) ions.

Frontier molecular orbitals and Electronic spectra: In ligand, the charge is distributed on the aromatic ring pi-system and on the outer delocalized pi -system at HOMO and LUMO as shown in figure 6. In complex (1), the figure 7 showed that the charge density is more spread on the ligand (H_2L) in HOMO and LUMO. The energy values of FMO are useful to find out the hardness, softness and electrophilicity properties of molecule.[17,18] These properties of the formed Ligand (H_2L) and novel Cu complex are listed in table 3. Computational UV-visible spectra data and theoretical electronic transitions of the ligand and copper complexes are tabulated in table 4 and spectra in figure 6 and figure 7.

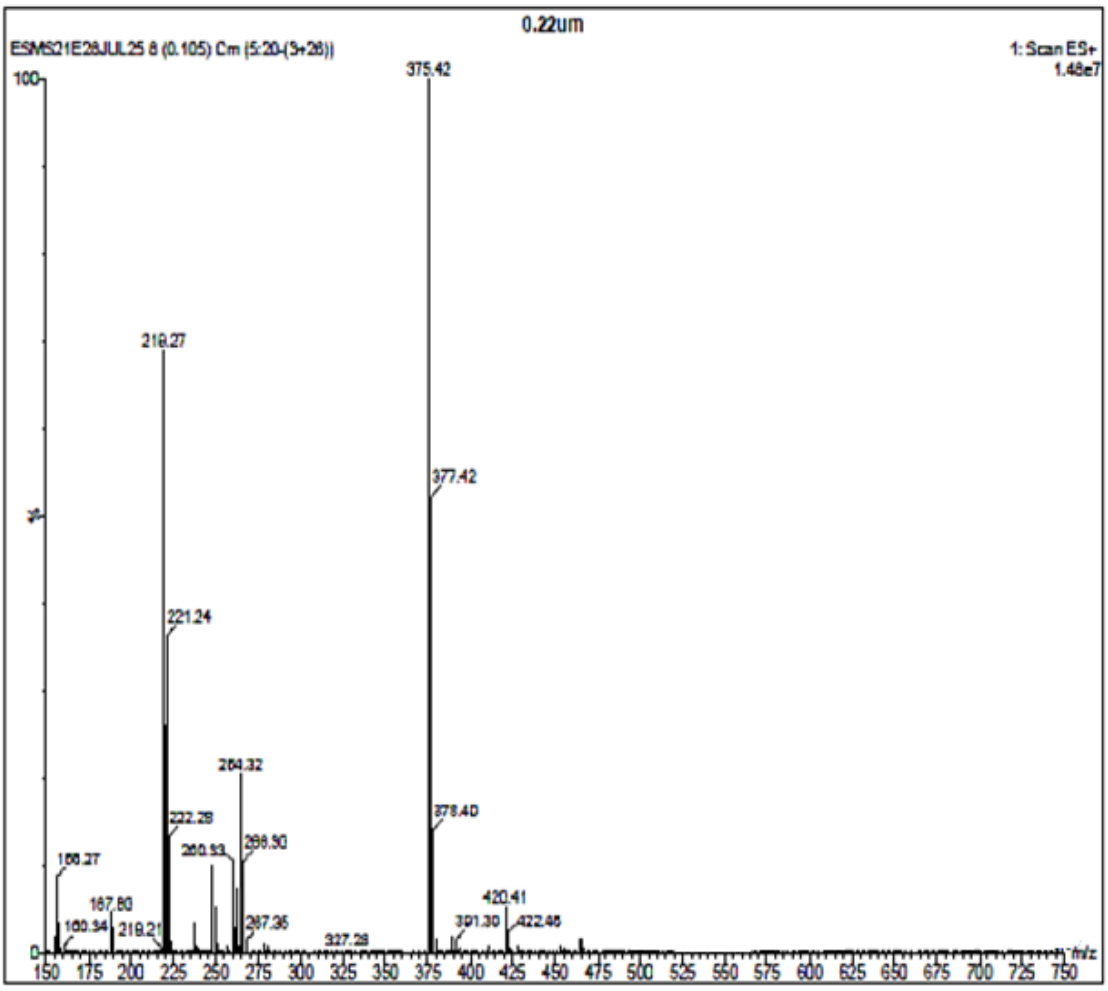


Figure 3: HRMS of Ligand (H_2L)

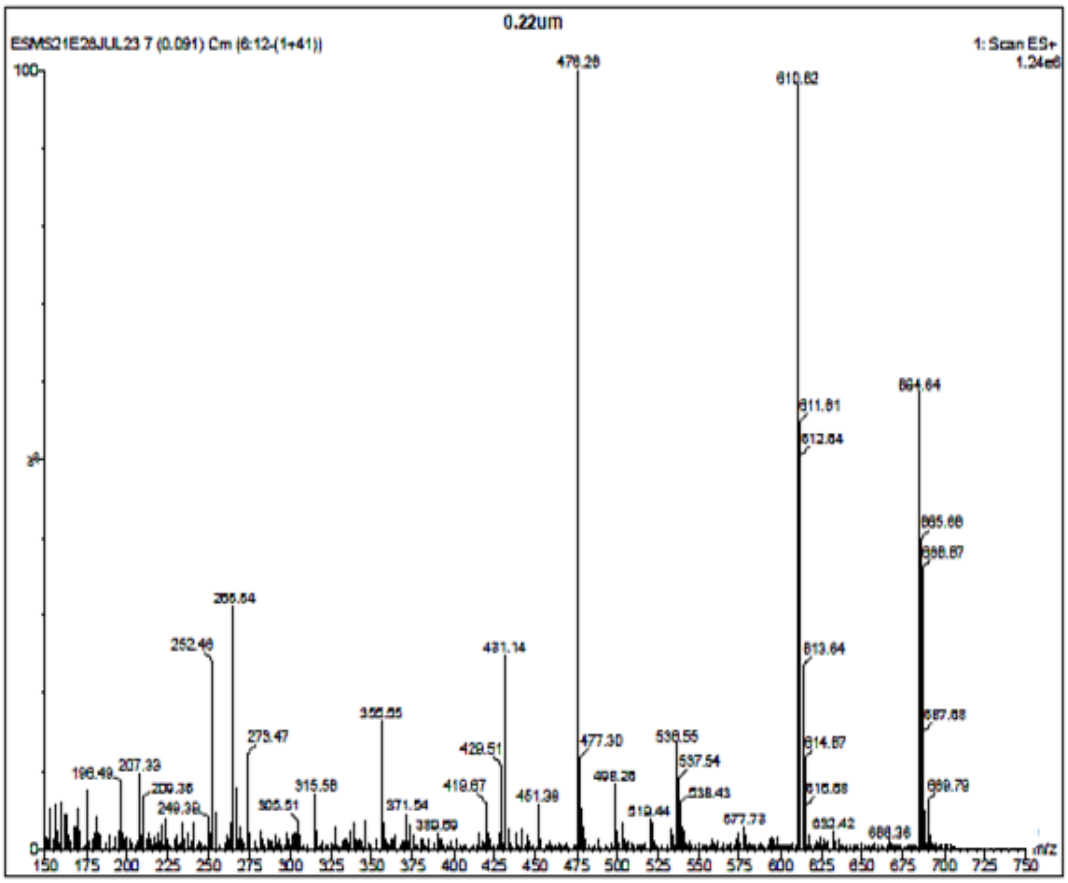


Figure 4: HRMS of Cu(II) complex [Cu(L)(bipy)]

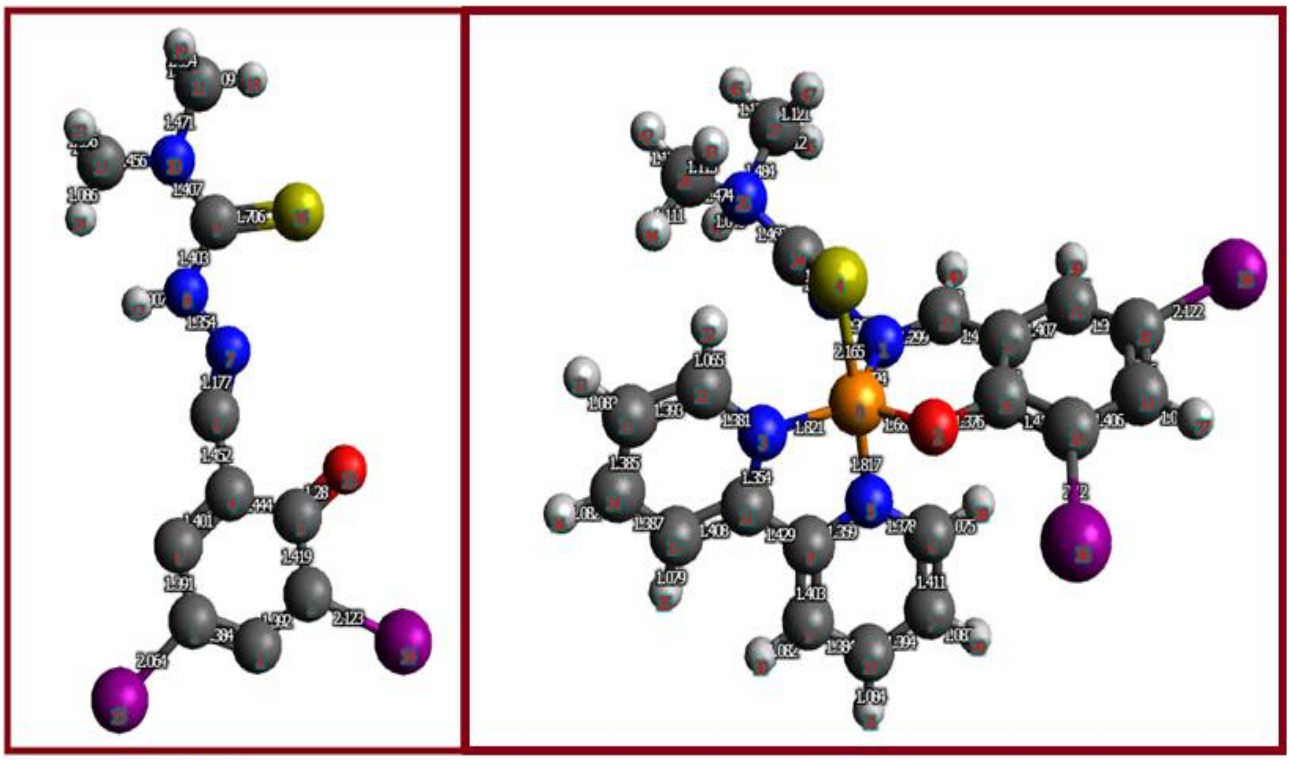


Figure 5: Optimized geometry (a) Ligand H_2L and (b) Cu (II) Complex [Cu(L)(bipy)]

Table 2
Bond lengths (Å) and bond angles (°) for optimized structures of ligand and complexes.

Molecule	Bond distance (Å)	Bond angle (°)
Ligand (H_2L)	N(9)-N(10) = 1.4263	N(9)-N(10)-C(11) = 120.9
	N(9)-C(7) = 1.3411	N(9)-N(10)-C(7) =113.6
	N(10)-C(11)=1.3955	N(10)-C(11)-S(15) = 124.0
	C(11)-S(15) =1.6944	N(10)-C(11)-N(12) =112.9
	C(3)-I(4) = 2.0832	C(16)- C(7)-N(9) =117.5
[Cu(L)(bipy)]	Cu(0)-N(2) = 1.9725	N(2)-Cu(0)-N(5) = 96.5
	Cu(0)-N(5) = 1.8634	N(2)-Cu(0)-N(3) = 90.7
	Cu(0)-N(3) =1.9602	N(2)-Cu(0)-S(4) = 85.0
	Cu(0)-S(4) = 2.231	N(5)-Cu(0)-N(3) =102.6
	Cu(0)-O(1) =1.8568	N(3)-Cu(0)-S(4) =74.0
	S(4)-N(24) =1.7721	O(1)-Cu(0)-S(4) =72.5
	N(3)-N(23) = 1.452	O(1)-Cu(0)-N(5) =106.6
	O(1)-C(16) = 1.3438	O(1)-Cu(0)-N(3) = 92.7

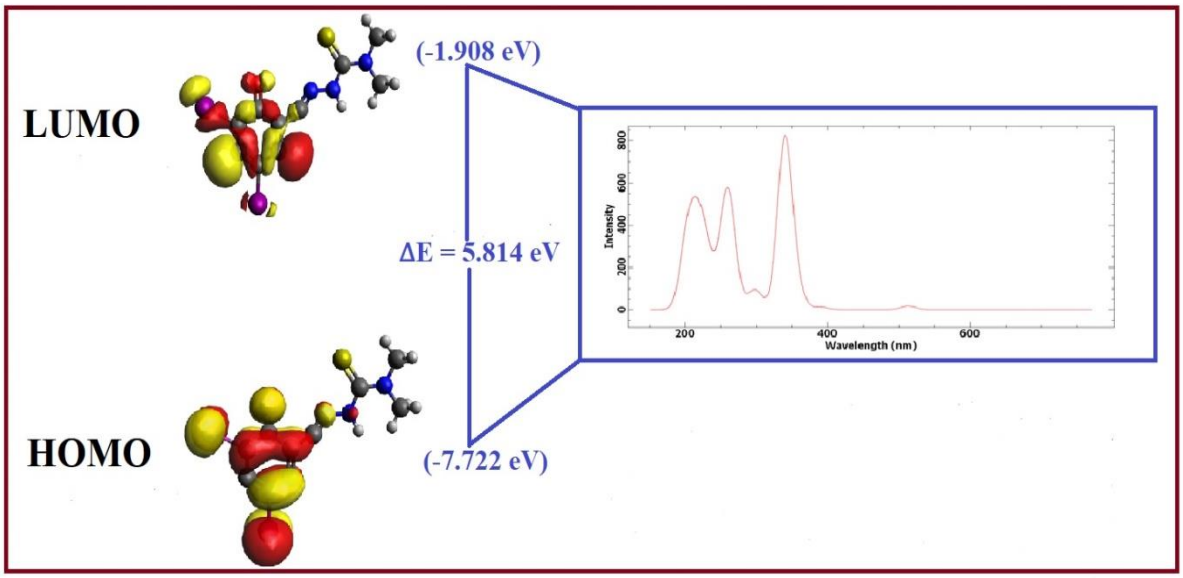


Figure 6: FMO of Ligand (H_2L) with Computational Electronic Spectra

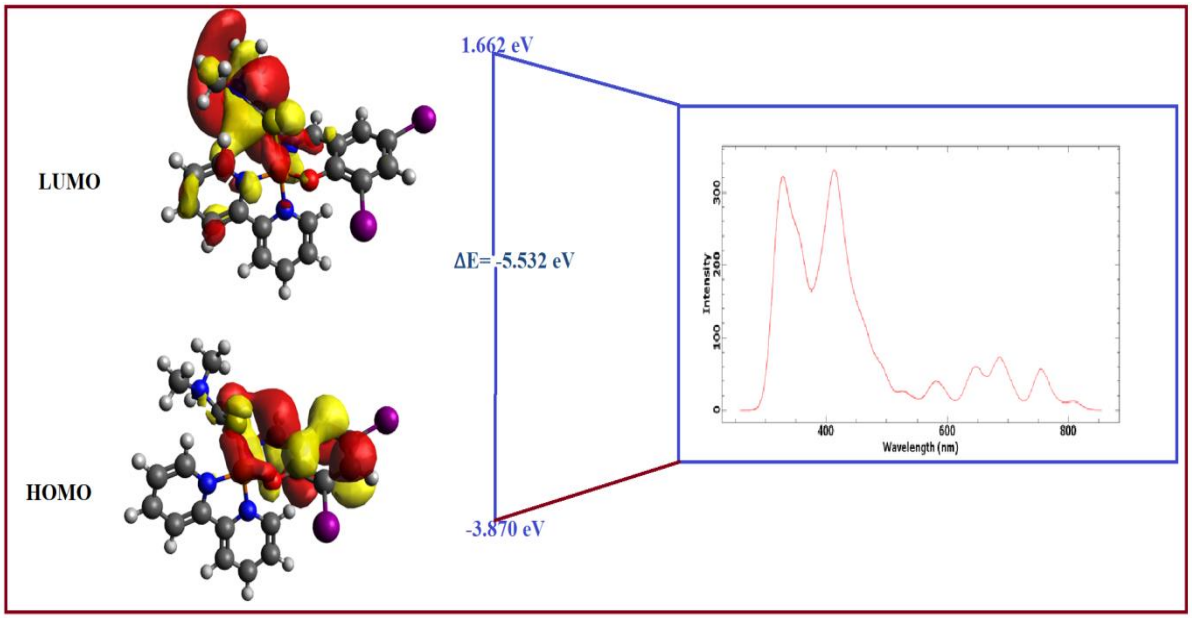


Figure 7: FMO of Cu (II)Complex [Cu(L)(bipy)] with Computational Electronic Spectra

During the theoretical calculations of electronic spectra of complexes, open shell are deducted which showed that the copper (II) complex is paramagnetic in nature. As the intensities of the peaks between 250 nm to 350 nm are very sharp, these peaks are due to the symmetrically allowed π-π* transitions in the ligand (H_2L). In the ligand (H_2L), there are more than one nitrogen atoms, so there are different centers for the n-π* transitions. The energies required for these transitions are less compared to π-π*, so n-π* transitions occurred at longer wavelength and these n-π* transitions are not symmetrically allowed, so there intensities are also less than π-π* transitions[42].

In Cu (II) complex due to increase in extent of delocalization, the π-π* and n-π* transition shifted slightly towards the longer wavelength. The complex [Cu(L)(bipy)] showed absorption at 594.9 nm probably due to MLCT and as the energy required for d-d transitions is less, so the transitions at 669.6 nm, 833.8 nm and 845.1 nm are probably d-d transitions.[19]

Molecular Docking Interactions: The molecular docking was done on the acetylcholinesterase enzyme of Drosophila melanogaster. For the best docked confirmation, the binding energies for ligand (H_2L) and [Cu(L)(bipy)] are -6.8 and -7.4 (kcal/mol) respectively.[20] The interaction of ligand with the target is shown in figure 8 and for complex it is shown in figure 10. The binding energies are negative both for ligand and Cu(II) complex values, thus both the ligand and the Cu(II) complex are having ability to bind with target. The Cu(II) complex is having high binding ability compared to ligand. The intermolecular interactions types and distance are shown in the table 5. The SER: 238(2.25 Å) and TYR:370 (4.10 Å) of the enzyme are involved in the hydrogen bonding with the ligand while in the Cu (II) complex, the SER: 329 (2.69 Å), PRO:270 (2.23 Å) and HIS: 273 (2.89 Å) amino acids of enzyme participated in hydrogen bonding.

The distance of hydrogen bonding satisfies the criteria of real docking (≤ 3.5 Å) except TYP: 370 where the bond length is slight longer.[21] Non-covalent interactions like electrostatic interactions and hydrophobic interactions also played important role for the binding of molecule at the target site of enzyme and protein.[22] The surficial properties of acetylcholinesterase enzyme for non-covalent interactions are shown in figure 9 for ligand and in figure 10 for complex. The distances of these interactions are in the range of real docking and all these interactions hold the molecule in the pocket of binding site.

Table 3
Energy of FMO of ligand (H_2L) and copper (II) complexes.

FMO	Energy(eV)	
	Ligand(H_2L)	[Cu(L)(bipy)]
LUMO	-1.908	1.662
HOMO	-7.722	-3.870
ΔE *	-5.814	-5.532
Electron Affinity (A)	1.908	-1.662
Chemical Potential (μ)	-1.454	0.331
Chemical Hardness (η)	2.907	2.766
Chemical Softness (S)	0.344	0.361
Electrophilicity Index (ω)	0.363	0.019
Ionization Energy (I)	7.772	3.870
Electronegativity (χ)	1.454	-0.331

Table 4
Theoretical electronic transition for ligand and Cu(II) complex.

Molecule	Wavelength(nm)	Energy(cm^{-1})	Absorption	Transition
H_2L	259.7	38085.2	0.3568	π-π*
	298.2	33534.0	0.0815	π-π*
	338.8	29512.4	0.5044	π-π*
	342.2	29225.3	0.1931	π-π*
	356.7	28032.8	0.0786	n-π*
	390.5	2506.2	0.0116	n-π*
[Cu(L)(bipy)]	575.6	19172.8	0.0620	MLCT
	652.9	16808.6	0.1681	d-d transition
	685.6	14935.0	0.1972	d-d transition
	761.8	11992.8	0.1561	d-d transition

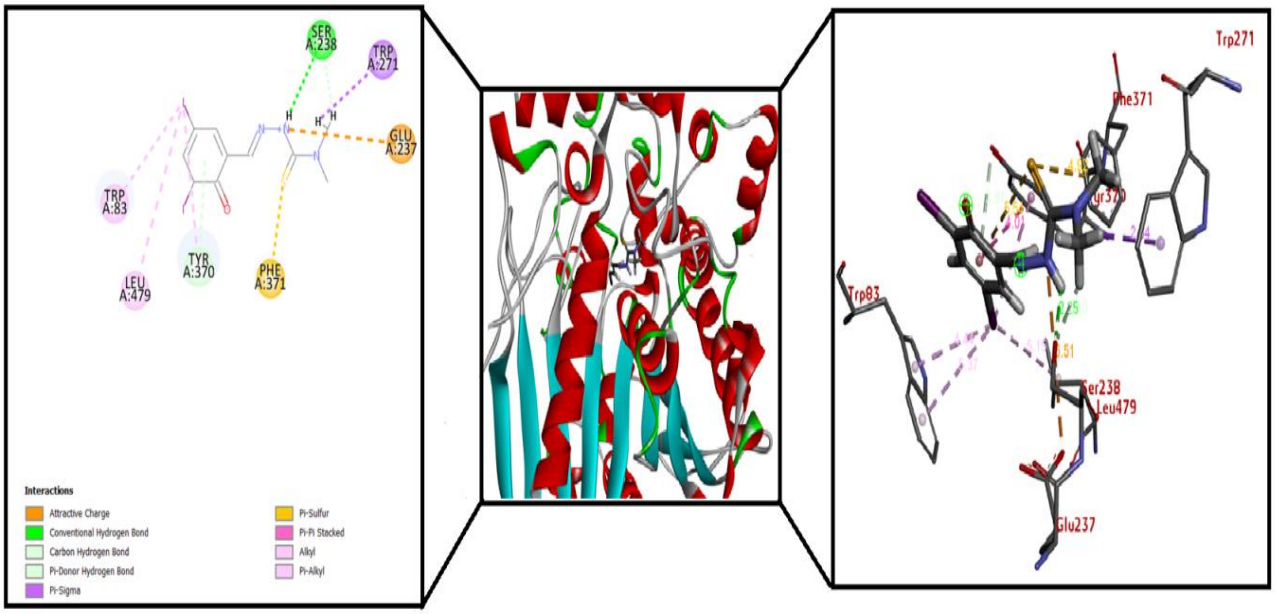


Figure 8: Representation of 2D and 3D interactions at binding site of acetylcholinesterase enzyme of Drosophila melanogaster with the ligand.

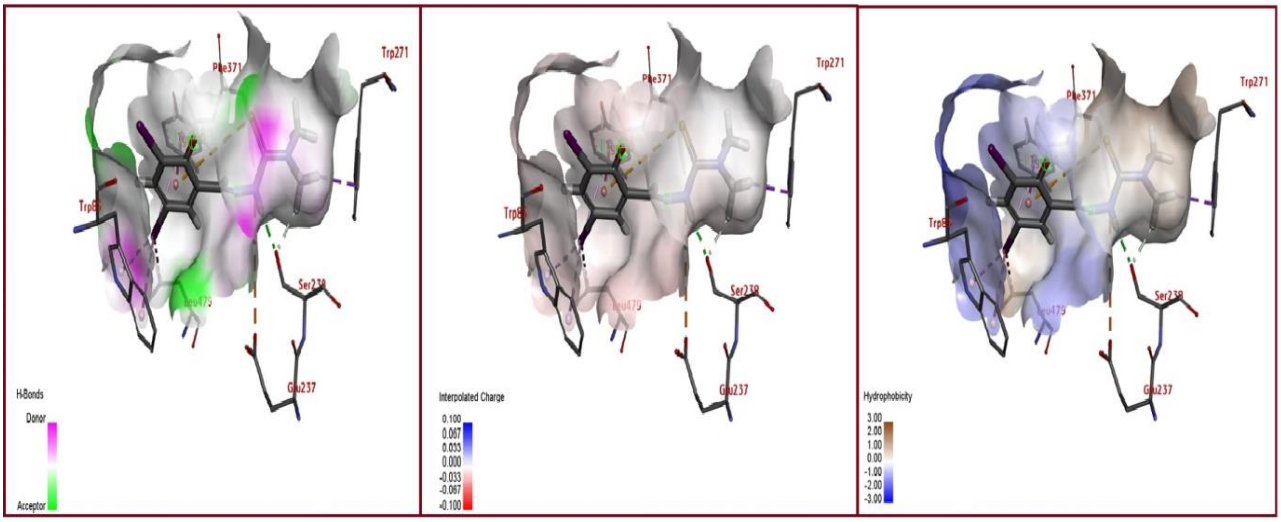


Figure 9: Binding site surface interactions of acetylcholinesterase enzyme of Drosophila melanogaster with the ligand (H_2L)

Table 5
Binding energies and intermolecular interaction at binding site of acetylcholinesterase enzyme of Drosophila melanogaster

Molecule	Binding energy (kcal/mol)	Hydrogen bond	Electrostatic interaction	Hydrophobic interactions
H_2L	-6.8	SER:238(2.25 Å) TYR:370(4.10 Å)	PHE:371(5.54 Å) GLU:237(5.51 Å)	TRP:83 (4.44 Å) TRP:271(2.64 Å) LEU:479 (5.37 Å)
[Cu(L)(bipy)]	-7.4	SER: 329 (2.69 Å) PRO:270 (2.23 Å) HIS: 273 (2.89 Å)	PRO:270 (2.90 Å) HIS: 273 (2.53 Å)	LYS: 278 (4.93 Å) LYS: 403 (4.75 Å)

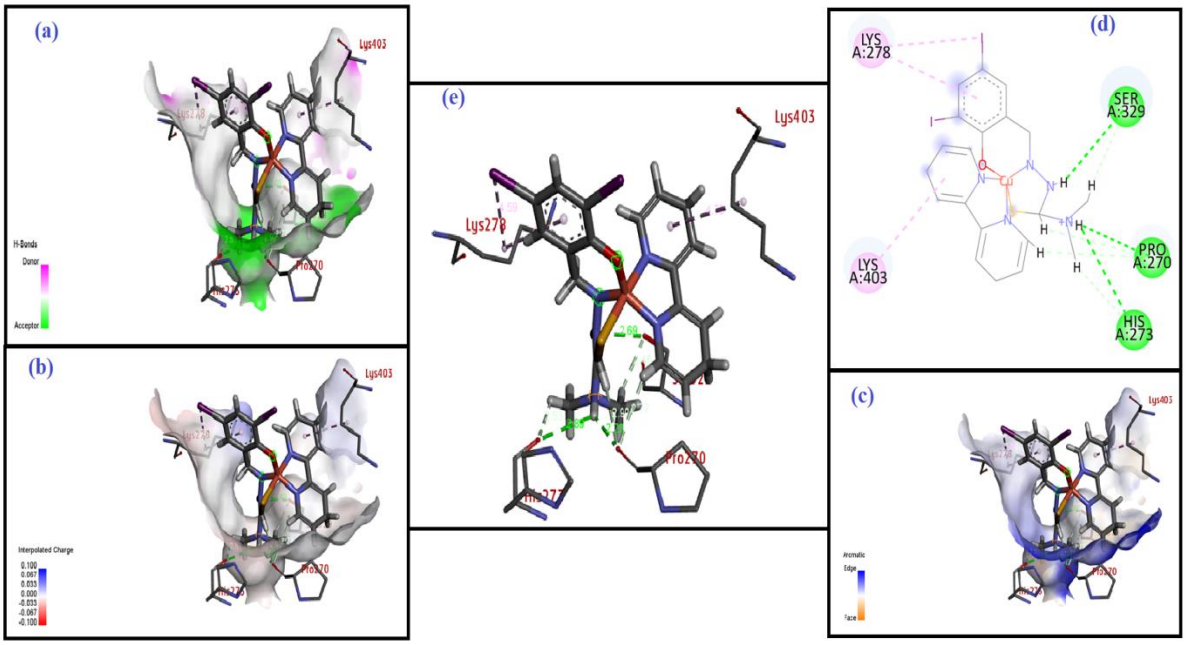


Figure 10: (a), (b) and (c) represent the Binding site surface interactions of acetylcholinesterase enzyme of Drosophila melanogaster with Cu (II) complex [Cu(L)(bipy)] and (d) and (e) represent the 3D interaction at binding site of target

Conclusion

The theoretical Computational DFT calculations showed that both ligand and Cu (II) complex are stable compounds. The *in silico* molecular docking studies showed that the binding at the target side of acetylcholinesterase enzyme is thermodynamically favored due to the negative binding energies for ligand and Cu (II) complex. Thus, these compounds are the promising candidates for the future research in the field for the insecticidal activity.

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