



Synthesis, Characterization, and Toxicity Studies on Three Amino Acid Schiff-Base Ligands and Their Potential Binding Sites to Ni (II) ion: Comparison with Computational Density Functional Theory (DFT) Studies

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ABSTRACT

Three Schiff-base ligands derived from amino serine, leucine and phenylalanine and salicylaldehyde, benzaldehyde and N-4-dimethylaminobenzaldehyde respectively were successfully synthesized and characterized. In addition, three Ni (II) complexes were synthesized *in situ* and characterized with the above Schiff-bases. The structures of these complexes were investigated experimentally and

computationally using density functional theory (DFT) technique to determine the potential binding environment with the Ni (II) center. Simulation of different models of the complexes were fully optimized using computational DFT B3LYP technique and the results revealed that models 4a, 5a, and 6a correspond to complexes-I, II, & III respectively. All the three complexes adopt a stable square planar geometry around the Ni (II) center. The complexes were characterized using melting point/decomposition temperatures, solubility test, FT-IR and UV-visible spectroscopy and the results compared with calculated results. The melting points/decomposition temperatures of all the complexes were different from the melting points of the starting amino acids used in their syntheses and the complexes were found to be soluble in water and other polar solvents. Experimental FT-IR and UV-Visible results agree with calculated results within accepted levels (5-10 %). The experimental imine $\nu(\text{C}=\text{N})$ stretching frequencies of all the three complexes agrees well with the calculated results while the rest of the stretching frequencies show some discrepancies. Furthermore, the UV-Visible spectra the three electronic transitions HOMO-6 $\rightarrow$ LUMO, HOMO-2 $\rightarrow$ LUMO+1, and HOMO $\rightarrow$ LUMO+1 for complex-I, two transitions HOMO-2 $\rightarrow$ LUMO+1 and HOMO-1 $\rightarrow$ LUMO for complex-II, and two transitions HOMO-7 $\rightarrow$ LUMO and HOMO $\rightarrow$ LUMO+1 for complex-III. The complexes were non-toxic towards prokaryotic gram positive (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus mutants*) and gram negative (*Aquaspirillum serpens* *Escherichia coli*) bacterial and eukaryotic (*Saccharomyces cerevisiae*) bacterial.

Keywords: Design, Synthesis, N-Salicylidene Alanine, Density functional Theory, Toxicity

INTRODUCTION

In recent years, the use of Schiff-base ligands and their transition metal complexes is gaining substantial applications as bioactive compounds in biochemistry and medicinal chemistry. This is due to their vital role in bonding with representation group elements and transition metal coordination chemistry inherent from their simple synthetic methods and variety in simple and complex structures. In addition, Schiff-base ligands and their complexes play crucial roles pharmacological processes owing to their binding affinities to biological molecules and in the design and development of new chemotherapeutic drugs. Schiff-bases are formed by reacting any primary amine and a carbonyl (aldehyde or ketone) using specific reagents and reaction conditions and identified by the characteristic imine or azomethine ($-\text{C}=\text{N}-$) functional group. The multidentate Schiff-base ligands act as chelating compounds in coordination chemistry and can bind to the transition metals and biomolecules with a lot of application in biological systems. Over the years, Schiff-bases and their transition metal complexes have been shown or tested as antibacterial, anticancer, antifungal, antitumor, antiviral, antioxidant, anti-inflammatory agents including catalysis and insecticides [1-12]. In this article, we will present comparative studies on the binding modes of three Schiff-base ligands derived from three amino acids; serine, leucine and phenylalanine and their complexes with Ni (II). The toxicity of the complexes and their binding modes to the Ni (II) center using computational B3LYP density functional theory (DFT) technique. Characterization of the Schiff-bases and the complexes would be performed using melting points/decomposition temperatures, solubility, FT-IR and UV-Visible spectroscopies and the results compared with calculated results to propose the adopted structures.

MATERIALS AND METHODS

All the chemicals and solvents mentioned in this research were used as purchased without any modification and all the complexes were synthesized *in-situ*.

Synthesis of N-Salicylidene Serine Schiff-Base and the Ni (II) Complex-I

The same parameters were used to synthesize the Schiff-base and its complex, but the Schiff-base was isolated with acetic acid while the complex was synthesized *in-situ*.

Sodium hydroxide (NaOH; 0.0095 mols) and serine ($C_3H_7NO_3$; 0.0095 mols) were completely dissolved in 40.0 mL of water in a 250 mL beaker with the help of a magnetic stirrer at room temperature. An equimolar amount of salicylaldehyde (0.0095 mols) was added drop wise to the mixture allowed to for 40 minutes. A 40.0 mL aqueous solution of anhydrous nickel (II) nitrate ($Ni(NO_3)_2$; 0.00475 mols) was slowly added onto the resulting mixture and allowed to react for an additional 50 minutes in the fume hood. The mixture concentrated to 50 % of its original volume and allowed in the fume hood for four days. The light blue powder was filtered via suction, recrystallized in ethanol-water mixture (50/50), air dried and stored in a vial for subsequent characterization. The percentage yield was 87 %.

Synthesis of N-Benzylidene Leucine Schiff-Base and the Ni (II) Complex-II

Similarly, the same parameters were used to synthesize the Schiff-base and its complex, but the Schiff-base was isolated with acetic acid while the complex was synthesized *in-situ*

Sodium hydroxide (NaOH; 0.0114 mols) and leucine ($C_6H_{13}NO_2$; 0.0114 mols) were completely dissolved in 40.0 mL of water in a 250 mL beaker with the help of a magnetic stirrer at room temperature. An equimolar amount of benzaldehyde (0.0114 mols) was then added drop wise to the resulting mixture allowed to react for 50 minutes. A 30.0 mL aqueous solution of anhydrous Nickel (II) nitrate ($Ni(NO_3)_2$; 0.0057 mols) was slowly added onto the above mixture and allowed to react for an additional 1 hour in the fume hood. The mixture was concentrated to about 60 % of its original volume and allowed in the fume hood for five days. The resulting light blue powder was filtered via suction, recrystallized in ethanol-water mixture (60/40), air dried and stored in a vial for subsequent characterization. The percentage yield was 90 %.

Synthesis of N-4-dimethylaminobenzylidene Phenylalanine Schiff-Base and the Ni (II) Complex-III

Similarly, the same parameters were used to synthesize the Schiff-base and its complex, but the Schiff-base was isolated with acetic acid while the complex was synthesized *in-situ*. Sodium hydroxide (NaOH; 0.0121 mols) and leucine ($C_9H_{11}NO_2$; 0.0121 mols) were completely dissolved in 40.0 mL of water in a 250 mL beaker with the help of a magnetic stirrer at room temperature. An equimolar amount of benzaldehyde (0.0121 mols) was then added drop wise to the resulting mixture allowed to react for 40 minutes. A 30.0 mL aqueous solution of anhydrous Nickel (II) nitrate ($Ni(NO_3)_2$; 0.00605 mols) was slowly added onto the above mixture and allowed to react for an additional 1 hour in the fume hood. The mixture was concentrated to about 60 % of its original volume and allowed in the fume hood for five days. The resulting light blue powder was filtered via suction, recrystallized in ethanol-water mixture

(60/40), air dried and stored in a vial for subsequent characterization. The percentage yield was 88 %.

Toxicity Test of the Schiff-Bases and Complexes

The Kirby-Bauer assay was used to test the toxicity of the complexes for bacterial growth against the following gram-positive and gram-negative bacterial species: *Aquaspirillum serpens*, *Escherichia coli* strain K12, *Saccharomyces cerevisiae* strain Alpha 1, *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Streptococcus mutants*. The Kirby-Bauer assay is one of the most sensitive tests used for bacterial growth against prokaryotes and eukaryotes in microbiology as reported in our previous publications [4,13-15].

In this research, an aqueous solution of the Schiff-bases (N-Salicylidene serine, N-Benzylidene leucine and N-4-dimethylaminobenzylidene phenylalanine) and their Ni (II) complexes (I, II, III) were prepared with a slightly higher antibiotic concentration (1.0 μ M) than those used in the Kirby-Bauer assay (30.0 μ g). The antimicrobial and antimicrobial-free discs used in this research were purchased from Becton, Dickinson and Company located in New Jersey. The antimicrobial-free discs were placed in a sterile 2.0 mL microcentrifuge tube containing the aqueous solution of each compound to be tested and centrifuged for 5 minutes at $12,000 \times g$ to remove all air bubbles from the discs. The discs were stored over-night at room temperature before being transferred onto the culture plates. Each compound in the disk was placed at intervals of more than 24 mm apart, center to center, on the same 100 mm inoculated Mueller-Hinton culture plate. There were three culture plates of the same organism tested per sample, these served as biological replicates in case there was inhibition of organism growth. The incubated plates were observed in log phase of growth to prevent bacteria with slower growth from skewing results [16].

Computational Method

The computational method used in this paper was the density functional theory (DFT) technique B3LYP embedded in G09 with 6-31G(d,p) all electron basis set [17-21]. The structural geometries of the Schiff-bases and complexes were fully optimized and all elements were analyses using this basis set with the exception of metals, which used the LANL2DZ basis set for the valence electrons with the corresponding effective core potentials (ECPs). The lowest energy structure conformations were confirmed by separate frequency calculations using the same method and basis set [22-26]. The UV-Visible absorption spectra simulated using TD-DFT method and infrared (IR) spectra of the complexes were all modeled from the most stable conformation of the optimized structures [27-30]. The calculated spectra of the different structural geometries would be compared to the experiment results to determine the most stable and potential binding mode of the Schiff-base ligands to the Ni (II) centers. The geometric parameters (bond lengths and bond angles) of the most stable optimized structures are shown in Table S1 of the supporting materials and figure 1 shows the optimized structures of the Schiff-Bases.

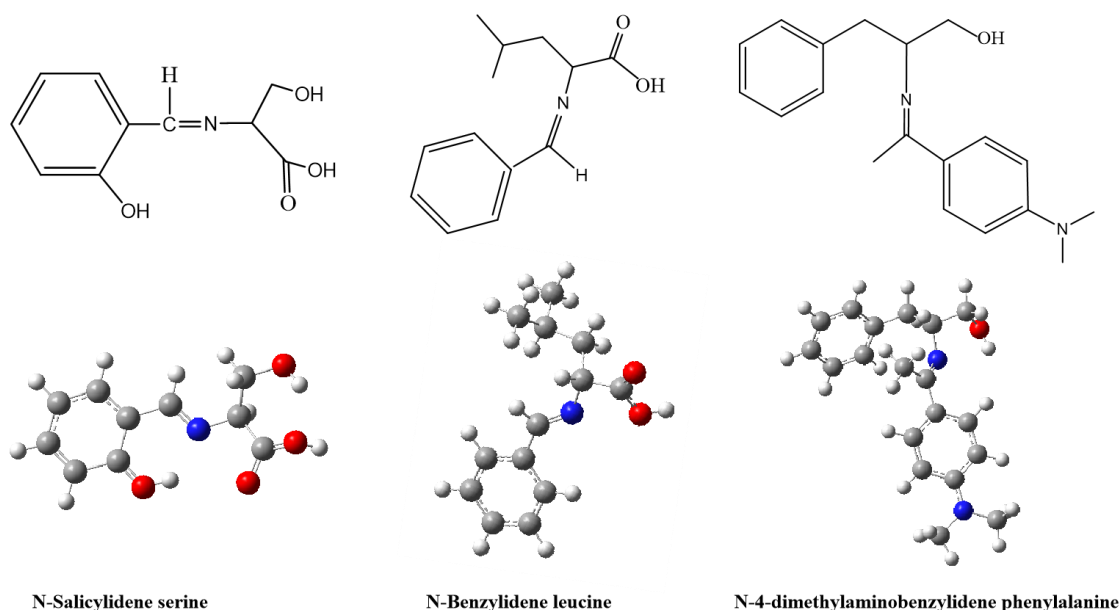


Figure 1. Optimized structures of the Schiff-Bases.

RESULTS AND DISCUSSION

Melting Point/Decomposition Temperature and Reaction Time

The most common and first method to characterize or determine a newly synthesized compound is the melting/decomposition and/or boiling points. The melting points/decomposition temperatures of our compounds were determined using the digital melting point apparatus and this were compared to the melting point/decomposition temperatures of the amino acids; serine, leucine and phenylalanine, from which they were synthesized. The melting points/decomposition temperatures of all our compounds were observed to be different from the melting points of the amino acid use, indicating that new compounds were synthesized.

The melting point of serine, leucine and phenylalanine were observed at a precise and sharp temperature of 220 °C, 286 °C, and 270 °C respectively. N-Salicylidene serine Schiff-base decomposed at temperatures greater than 300 °C, N-Benzylidene leucine melts around 205 °C, while N-4-dimethylaminobenzylidene phenylalanine melts around 200 °C respectively. Similarly, N-Salicylidene serine Ni (II) complex-I decompose at temperatures above 320 °C and changes colour from light blue to black, N-Benzylidene leucine Ni (II) complex-II melts at 210 °C and changes colour from light blue to green, N-4-dimethylaminobenzylidene phenylalanine Ni (II) complex-III decomposes at temperatures above 210 °C and changes colour from light blue to minty green. The melting point of alanine, serine, leucine and phenylalanine agrees well with the literature values [2]. The melting point/decomposition temperatures of the compounds are presented in table 1.

Table 1: Melting point/Decomposition Temperature and Reaction Times of the complexes

Compound	M.Pt/Decomp.T/°C	Reaction Time /mins	Yield (%)
N-Salicylidene serine	>300	110	80
N-Benzylidene leucine	205	120	85
N-4-dimethylaminobenzylidene phenylalanine	200	130	87
Complex-I	320	135	87
Complex-II	210	145	90
Complex-III	210	125	88
Serine	220	-	-
Leucine	286	-	-
Phenylalanine	270	-	-

Solubility Test

The effectiveness of any compound intended to be used as a drug candidate is its solubility. The solubility of our compounds was determined in different solvents and the results will enable us to perform other techniques that need soluble compounds. The solubility of the compounds was done manually by dissolving approximately 0.100 g sample of each compound in about 10.00 mL of solvent. The solubility results are presented in table 2. From table 2, we can observe that all the compounds were soluble in water and other polar solvents and insoluble in non-polar solvents with solubility increasing in the warm solvent.

Table 2: Solubility of the Complexes

Compound	Water	Ethanol	methanol	chloroform	hexane	D ₂ O
N-Salicylidene serine	s	s	ss	i	i	s
N-Benzylidene leucine	s	s	ss	i	i	s
N-4-dimethylaminobenzylidene phenylalanine	s	s	ss	i	i	s
Complex-I	s	s	ss	i	i	s
Complex-II	s	s	ss	i	i	s
Complex-III	s	s	ss	i	i	s

s = soluble; i = insoluble; ss = sparingly soluble

Fourier Transform Infra-red (FT-IR) Spectra of the Complexes

The FT-IR spectroscopy was used to identify the vibrational stretching frequencies between atoms in functional groups. This was done using a FLUKA table top FT-IR instrument. The imine $\nu(\text{C}=\text{N})$ stretching frequency for complex-I, was observed between $1576\text{--}1651\text{ cm}^{-1}$, between $1595\text{--}1618\text{ cm}^{-1}$ in complex-II, and between $1591\text{--}1626\text{ cm}^{-1}$ in complex-III. The aromatic and aliphatic $\nu(\text{C}-\text{H})$ stretching vibrational bands were seen at 2940 cm^{-1} (aromatic) in complex-I, between $2885\text{--}2952\text{ cm}^{-1}$ (aromatic) in complex-II, and between $2985\text{--}3029\text{ cm}^{-1}$ (aromatic) in complex-III. The symmetric or asymmetric $\nu(\text{O}-\text{H})$ peaks vibrate between $3385\text{--}3568\text{ cm}^{-1}$ in complex-I, $3298\text{--}3356\text{ cm}^{-1}$ in complex-II, and at 3554 cm^{-1} in complex-III. The $\nu(\text{C}-\text{O})$ group vibrates at a stretching frequency between $1381\text{--}1467\text{ cm}^{-1}$ in complex-I, between $1383\text{--}1409\text{ cm}^{-1}$ in complex-II, and at 1403 cm^{-1} in complex-III. The carbonyl $\nu(\text{C}=\text{O})$ stretching frequency was observed between $2282\text{--}2372\text{ cm}^{-1}$ in complex-I, and between $2050\text{--}2373\text{ cm}^{-1}$ in complex-II. A slight shift in the vibrational frequencies was observed for all the groups in all the

complexes and this is due to the distorted structures adopted after coordination of the Schiff-base ligands to Ni (II) center when compared to literature values [1,5,11-13]. The FT-IR results are shown in figure 2 and table 3.

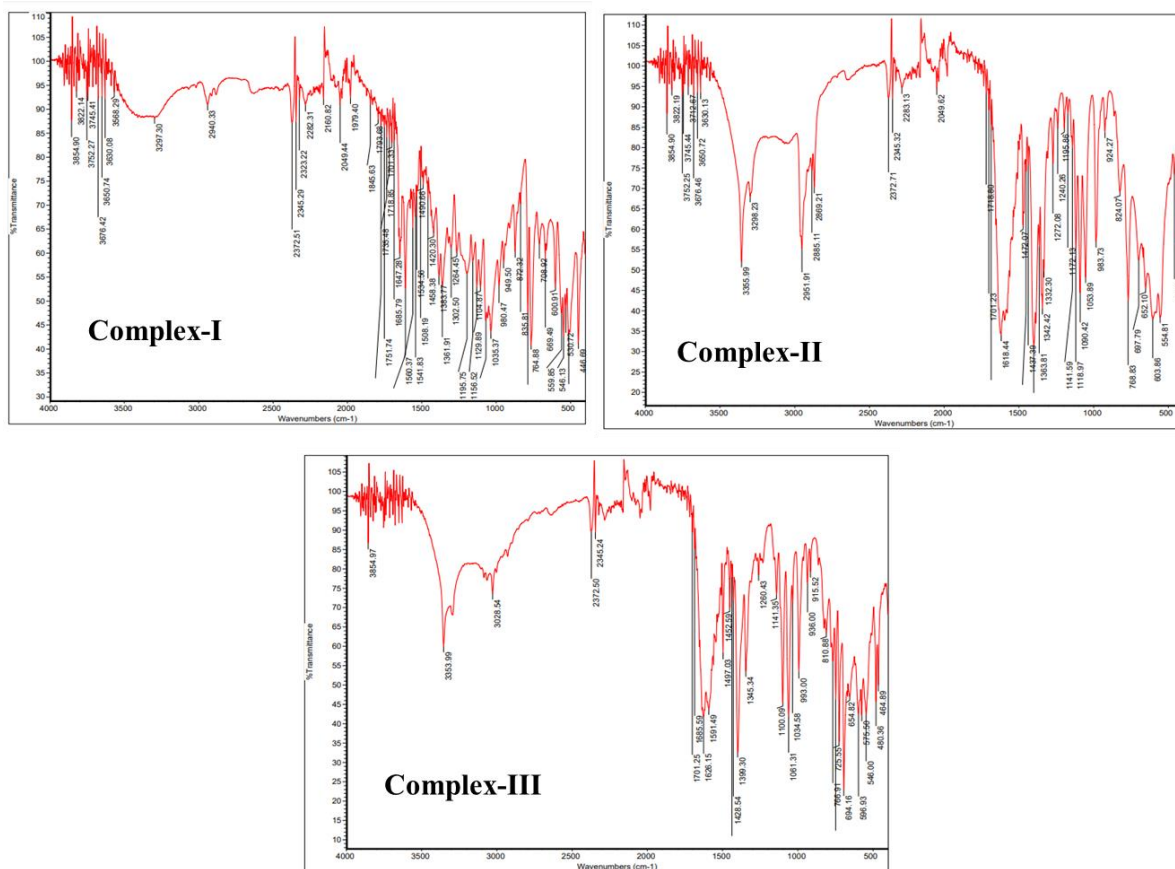


Figure 2. Experimental FT-IR spectra for Complexes I, II, and III showing prominent peaks.

Table 3: Experimental FT-IR values (in cm^{-1}) of prominent peaks for the complexes

Complex	$\nu(\text{C}=\text{N})/\text{cm}^{-1}$	$\nu(\text{C}-\text{H})_{\text{aromatic}} - \nu(\text{C}-\text{H})_{\text{aliphatic}}/\text{cm}^{-1}$	$\nu(\text{O}-\text{H})/\text{cm}^{-1}$	$\nu(\text{C}-\text{O})/\text{cm}^{-1}$	$\nu(\text{C}=\text{O})/\text{cm}^{-1}$
I	1576-1651	2940	3385-3568	1381-1647	2282-2372
II	1595-1618	1885-2952	3298-3356	1383-1409	2050-2373
III	1591-1626	2985-3029	3554	1403	-

UV-Visible Spectra and Energies of the Complexes

All the complexes had a blue colour, thereby implying that they can undergo electronic transitions in both tetrahedral (square planar) and octahedral environments. The electronic UV-visible spectra showing some prominent peaks are shown in figure 3, and table 4 represents the maximum wavelengths, energy and electronic transitions of the complexes.

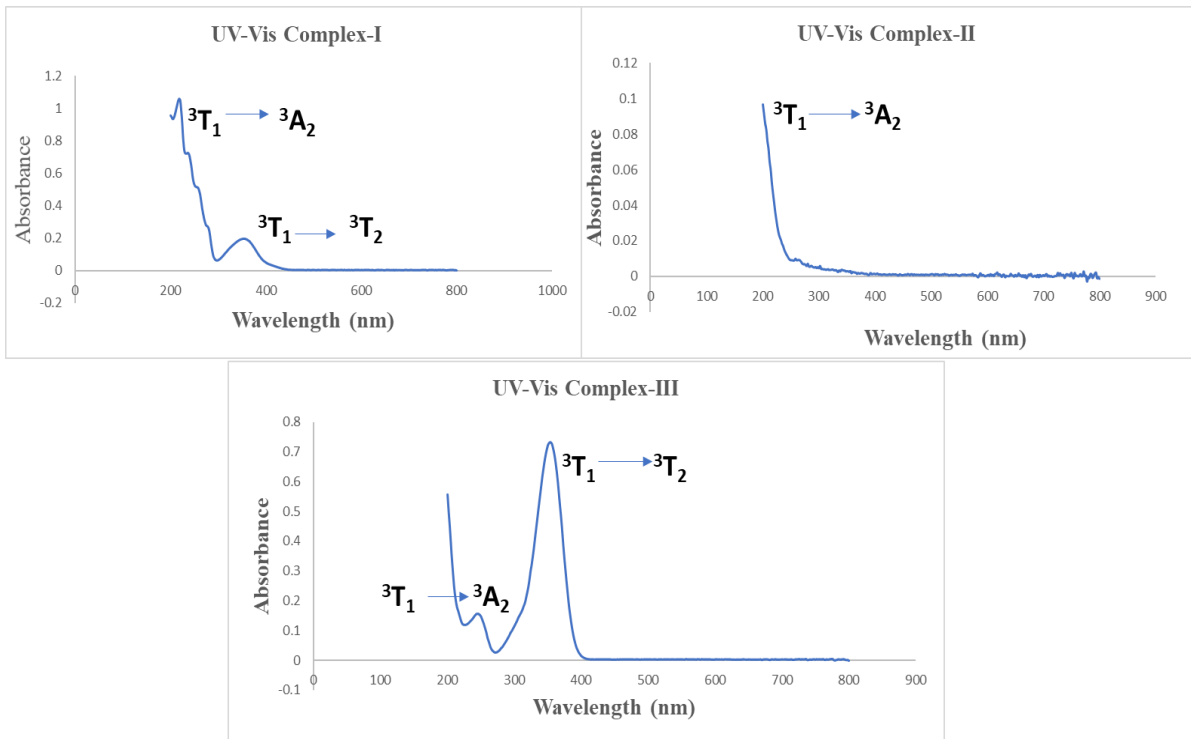


Figure 3: Experimental UV-visible spectra for Complexes I, II, and III showing prominent absorption peaks and transitions.

From figure 3, the two peaks seen at 220 nm, and 358 nm in complex-I, N-salicylidene serine Ni (II) were assigned to the ${}^3T_1 \rightarrow {}^3A_2$ and ${}^3T_1 \rightarrow {}^3T_2$ respectively. The third weak peak observed around 278 nm was assigned to the $\pi \rightarrow \pi^*$ transition. Complex-II; N-salicylidene leucine Ni (II) showed two peaks at 202 nm (strong) and 274 nm (weak) which were also assigned to the ${}^3T_1 \rightarrow {}^3A_2$ and ${}^3T_1 \rightarrow {}^3T_2$ respectively. Similarly, complex-III; N-4-dimethylaminobenzylidene phenylalanine Ni (II) showed a weak peak at 250 nm and a broad peak at 356 nm can were assigned to the ${}^3T_1 \rightarrow {}^3A_2$ and ${}^3T_1 \rightarrow {}^3T_2$ respectively. It is important to note that all the complexes absorbed at approximately the same wavelength, which is expected because they are all bonded to the same Ni (II) center.

Table 4: Electronic transitions showing the maximum wavelength and energy in the Complexes

Complex; colour	Max. wavelength (λ_{max} , nm)	Energy	Electronic Transition
I; light blue	220		${}^3T_1 \rightarrow {}^3A_2$
	278		$\pi \rightarrow \pi^*$
	358		${}^3T_1 \rightarrow {}^3T_2$
II; light blue	202		${}^3T_1 \rightarrow {}^3A_2$
	274		${}^3T_1 \rightarrow {}^3T_2$
III; blue	250		${}^3T_1 \rightarrow {}^3A_2$
	356		${}^3T_1 \rightarrow {}^3T_2$

TOXICITY AND BACTERIAL GROWTH INHIBITION PROPERTIES OF THE COMPLEXES

In order to investigate toxicity and/or cytotoxicity of the complexes, the Kirby-Bauer assay test was used to determine the growth inhibition properties of the complexes against bacterial cells; prokaryotes and a eukaryote. The bacterial cells used include: gram negative *Aquaspirillum serpens* and *Escherichia coli*, and gram-positive *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Streptococcus mutants*. The results revealed that the complexes were growth resistance or non-toxic against gram-negative, gram-positive and eukaryotic bacterial cells. The results including all replicates are shown in table 5.

Table 5: Kirby-Bauer Susceptibility Test

Prokaryotes	Complexes (I, II, & III)	Positive control	Negative control
Gram negative			
<i>Aquaspirillum serpens</i>	R	S	R
<i>Escherichia coli</i>	R	S	R
Gram positive			
<i>Staphylococcus aureus</i>	R	S	R
<i>Staphylococcus epidermis</i>	R	S	R
<i>Streptococcus mutants</i>	R	S	R
Eukaryote			
<i>Saccharomyces cerevisiae</i>	R	S	R

COMPUTATIONAL STUDIES ON THE COMPLEXES

Density functional theory (DFT) computational method was used to simulate and investigate the binding environment between of the Schiff base ligands and the Ni (II) center. In order to accomplish this, different models and binding modes around Ni (II) were proposed and fully optimized. The most stable geometries were used to propose the binding environment of the Schiff-bases to the metal centers. The results are discussed in this section.

Computational Studies on N-Salicylidene Serine Ni (II) Complex-I

After complete optimization, two Ni (II)-N-Salicylidene serine Schiff-base structural models were proposed with a tetrahedral or square planar environment around Ni (II) center (figure 4). Based on the biding modes, a square planar geometry is most probable around the Ni (II) center with three oxygen binding (carboxylate O, hydroxyl O, and H₂O ligand) and one nitrogen donor site of the imine group in **4a**. In model **4b**, the H₂O ligand is replaced by the Cl⁻ ligand.

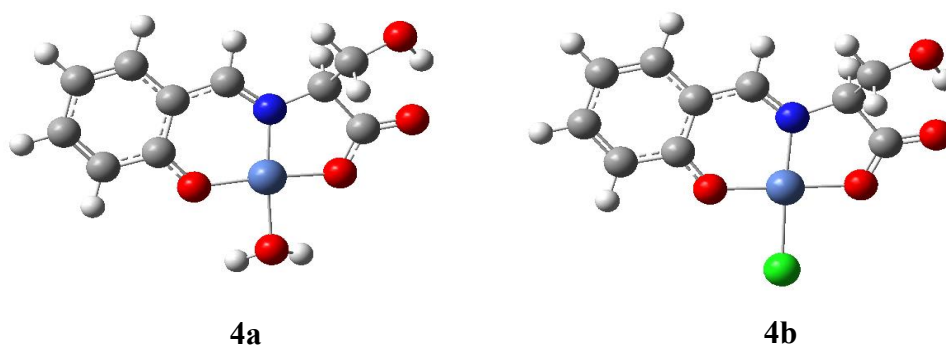


Figure 4. Optimized structural geometries of complex-1.

From the computational details (table S1) and figure 4, we observed that the Ni-N bond length and the Ni-O were slightly elongated in **4b**, where Ni (II) bonds to a Cl⁻ ligand) than in **4a**, where Ni (II) bonds to a H₂O ligand. For example, the Ni-N bond length elongates by 0.06 Å from 1.841 Å in **4a** to 1.898 Å in **4b**. Similarly, an elongation of between 0.03-0.04 Å was observed for the Ni-O2 bond length in **4b** than the Ni-O1 in **4a**. Contrary to the elongation of the Ni-N and Ni-O bond lengths, we observed a slight decrease in the C-O bond length from **4a** to **4b**. For example, the C1-O1 bond length decreases from 1.308 Å in **4a** to 1.289 Å in **4b**. The same trend was seen for the C-O bond length from the aromatic ring (hydroxyl O) with a decrease of 0.02 Å in C10-O3 bond length. No significant variation in bond lengths were observed for the rest of bonds in the two models. This further confirms the square planar geometry around the Ni (II) center.

Furthermore, a slight distortion in the square plane structure was observed around the Ni (II) center. For example, the N-Ni-O1 and N-Ni-O2 bond angles are respectively 97.60° and 93.83° in **4a** and 87.85° and 85.50° in **4b**. The most significant change in bond angles were observed for the C-O-Ni compared to the O-Ni-N. For example, the C-O-Ni bond angles are respectively 126.32° and 128.18° for **4a** and **4b**. The increase in bond angle in **4b** may be due to the greater electronegativity of oxygen atom compared to the nitrogen atom. This means that structure **4a** is slightly more stable than **4b**. The calculated IR spectra of **4a** and **4b** are shown in figure 5. By observation, the calculated IR profile of **4a** resembles the experimental FT-IR spectra of complex-I (figure 2). The stretching mode at 1767 cm⁻¹ was assigned to the ν(C=O) stretching frequency, the peak at 1679 cm⁻¹ was assigned to the imine ν(-C=N-) group, the peak at 1122 cm⁻¹ was assigned to the ν(C-O) stretching frequency, and the peaks at 587 cm⁻¹ and 1501 cm⁻¹ were assigned to the ν(O-H) rocking vibrations in water. Similarly, the peaks between 3500-3800 cm⁻¹ were assigned to symmetric or asymmetric ν(O-H vibrations in water. We observe a slight variation in the stretching frequencies, but the imine stretching frequency and the vibrations around 600,1100, 1700 and 3700 cm⁻¹ agrees with the experimental results.

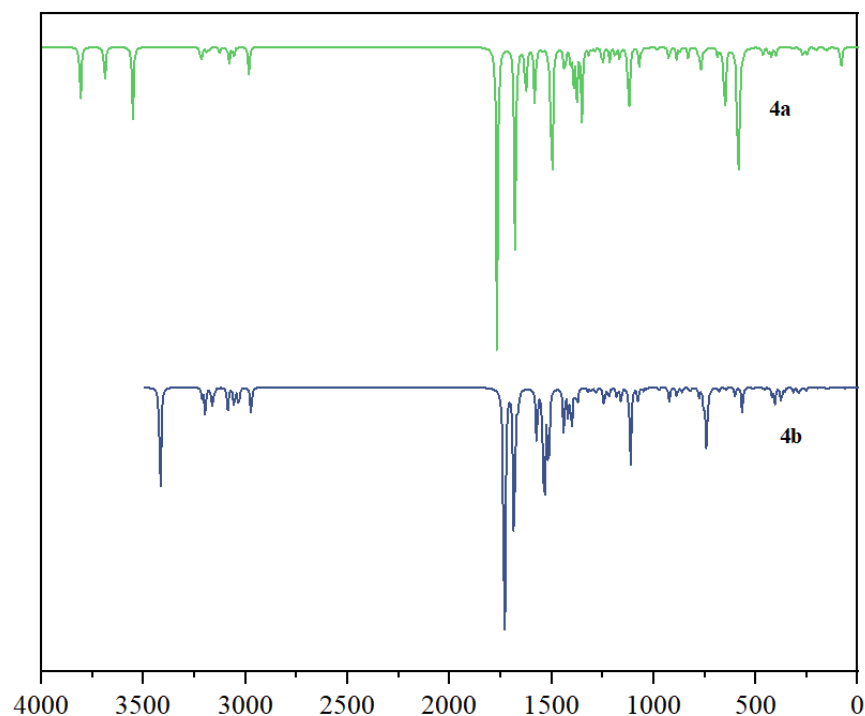


Figure 5. The calculated IR spectra of model 4a and 4b.

The calculated UV-vis spectra of **4a** and **4b** were compared with the experimental results (figure 6). Similarly, the calculated UV-Vis spectra **4a** matches with the experiment results. Three prominent peaks were observed at 236 nm, 283nm, and 373 nm and this corresponds to the HOMO-6→LUMO, HOMO-2→LUMO+1, and HOMO→LUMO+1 electronic transition. Based on these modeling results, the most probable structure of complex-I is **4a**.

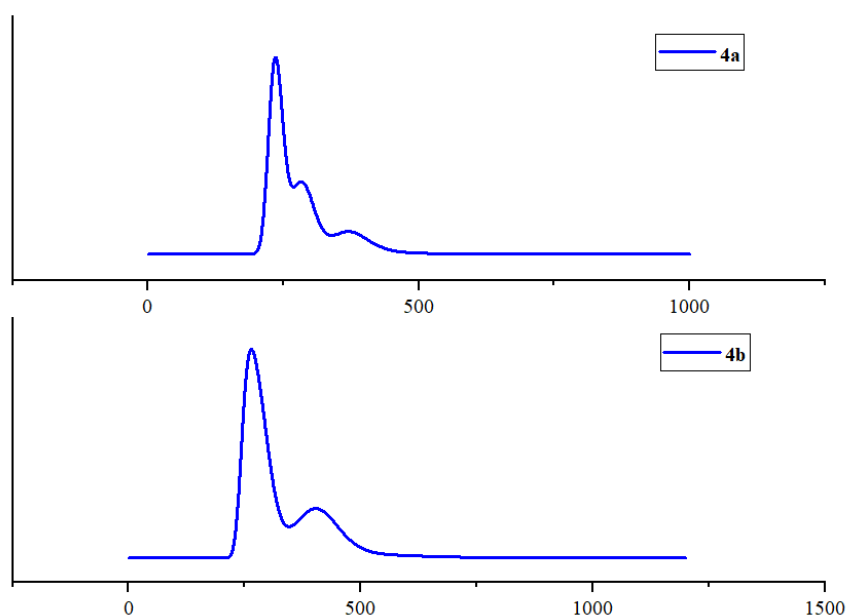


Figure 6. The calculated UV-vis spectra of 4a and 4b.

Computational Studies on N-Benzalidene Leucine Ni (II) Complex-II

The possible binding environment of complex-II to the Ni (II) center was studied using computational DFT simulation. After optimization, three models of complex-II were proposed as shown in figure 7. Similarly, the three models adopt a square geometry around the Ni (II) center with two donor sites from the Schiff-base ligand (imine N atom, and carboxylate O), and the third and fourth donor sites coming either from two H₂O ligands (**5a**), or one H₂O and one Cl⁻ ligands (**5b**). The third model **5c** is made of two Schiff-base ligands with donor sites each.

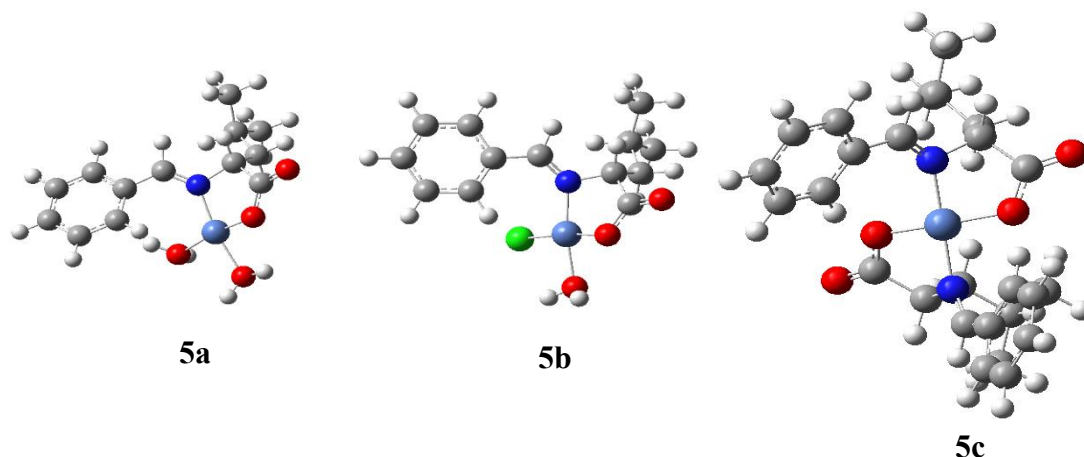


Figure 7. Optimized structural geometries of Complex-II.

From figure 7 and the computational details in table S1, we observed that the Ni-O bond length from H₂O donor ligand is greater than that of Ni-O bond length from carboxylate oxygen donor ligand. For example, the Ni-O bond length for the two H₂O donor ligands in **5a** are respectively 1.970 Å (Ni-O1) and 1.946 Å (Ni-O3) while the bond length of Ni-O2 from the carboxylate oxygen donor ligand is 1.798 Å. This trend was also observed for **5b** from 1.844 Å to 1.939 Å respectively for the Ni-O bond length from H₂O ligand and Ni-O bond length from the carboxylate donor ligand. Interestingly, the Ni-O bond lengths from the carboxylate oxygen donor, the H₂O donor ligand, and the Ni-N bond length in **5b** were slightly shorter than those in **5a**. For example, Ni-N bond length decreases from 1.900 Å in **5a** to 1.895 Å in **5b**. The observed reduction in bond length may be due to the presence of the Cl⁻ ligand, which affects the Ni-N bond length. In the three models (**5a**, **5b** and **5c**), the square planar geometry is slightly distorted with a smaller N-Ni-O bond angle from the carboxylate ligand compared to the N-Ni-O bond angle from the H₂O ligand or N-Ni-Cl bond angle from the Cl⁻ ligand. For example, the O1-Ni-N1 and O1-Ni-Cl bond angles are respectively 101.61° and 100.75° for **5a** and **5b** while the O2-Ni-N1 bond angles in **5a**, **5b**, and **5c** are respectively 85.88°, 84.74°, and 83.65°. The rest of the bond angles formed with Ni (II) are similar. For example, O2-Ni-N1* and N1-Ni-O2* bond angles are 96.17° and 96.85° respectively while the O2-Ni-N1 and O2*-Ni-N1* bond angles are 83.65° and 83.83° respectively. Similarly, the calculated IR spectra of **5a**, **5b**, and **5c** (figure 8) were compared with the experimental FT-IR spectra of complex-II (figure 2). From the results, the calculated IR spectra of **5a** closely resembles the experimental spectra of complex-II. The IR peak at 1852 cm⁻¹ was assigned to the stretching of carbonyl ν(C=O) group, the peak at 1691 cm⁻¹ was assigned to the imine (-C=N-) functional group, and the peaks observed at 552 and 1661 cm⁻¹ were assigned to the ν(O-H) rocking vibration in H₂O. The peaks between 3500-3800

cm^{-1} were assigned to symmetric or asymmetric $\nu(\text{O-H})$ vibration in water or hydroxyl groups. There is agreement between the calculated and experiment results for the imine peak and other peaks observed at 600, 1700 and 3700 cm^{-1} .

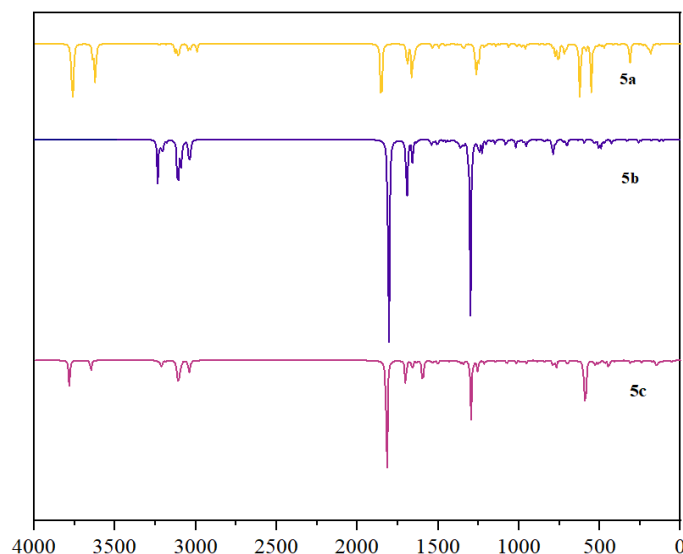


Figure 8. The calculated IR spectra for **5a**, **5b**, and **5c**.

The calculated UV-Vis spectra of **5a**, **5b** and **5c** are shown in figure 9. By comparison, we can observe that the calculated UV-Vis spectra of **5a** resembles the experiment UV-Vis spectra of complex-**II**. The prominent peaks observed at 270 nm and 330 nm corresponding to the HOMO-2→LUMO+1 and HOMO-1→LUMO electronic transition. Based on these modeling results, the most probable structure of complex-**II** is **5a**.

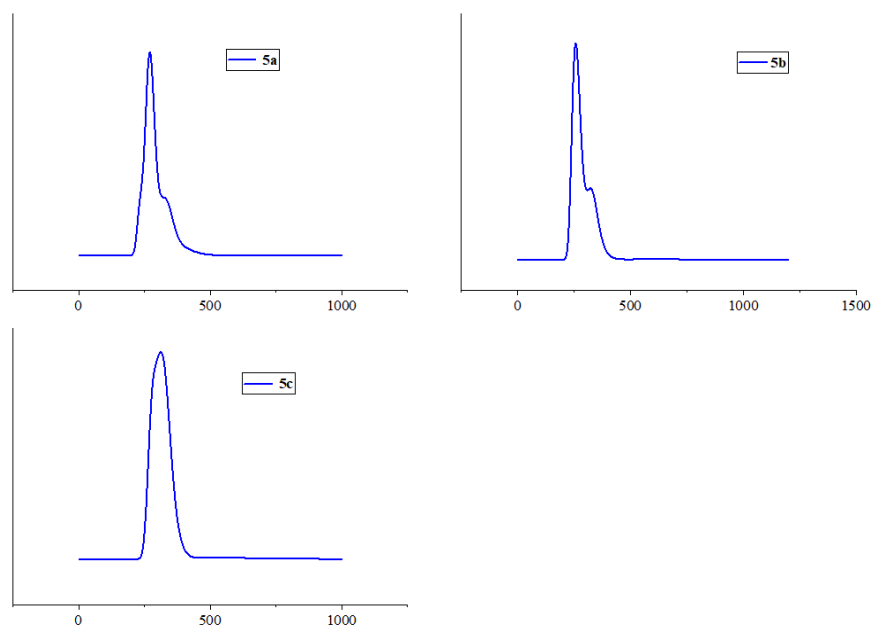


Figure 9. The calculated UV-Vis spectra of **5a**, **5b**, and **5c**.

Computational Studies on N-4-dimethylaminobenzylidine Phenylalanine Complex-III

Three stable models of N-4-dimethylaminobenzylidine phenylalanine complex-III were analyzed after complete optimization to investigate the binding mode of the Schiff-base ligand to the Ni(II) as shown in figure 10. The three models display a stable square planar geometric around the Ni(II) bonding environment, where two chelating sites from ligands (imine nitrogen and oxygen from deprotonated hydroxy group). The third and fourth bonding sites come from the oxygen of two H₂O ligands (**6a**) or one H₂O and one Cl⁻ ligands (**6b**). Model **6c** is a four coordinated complex containing two Schiff-base ligands, each with two chelating sites.

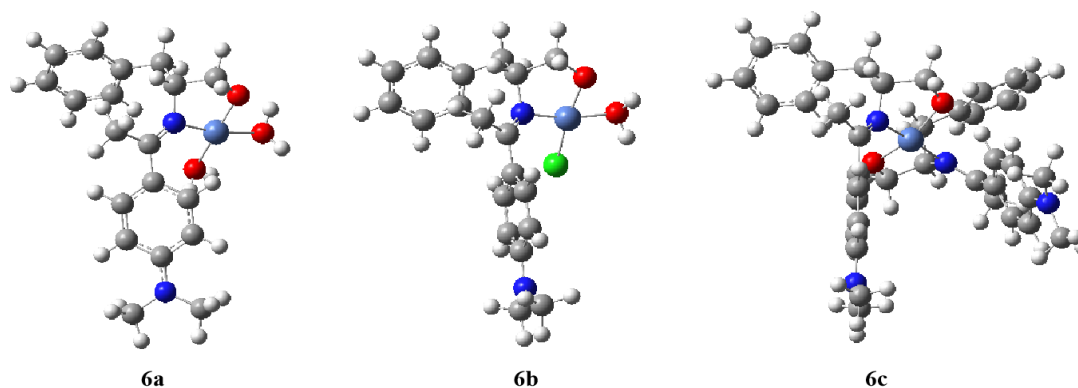


Figure 10. Optimized structural geometries of Complex-III.

From figure 10, Ni(II) is bonded to two H₂O ligands in **6a**, and one H₂O and one Cl⁻ ligands in **6b** and from computational details in table S1, we observed that the Ni-O bond lengths from the H₂O ligand is longer than the Ni-O bond length from the deprotonated hydroxide oxygen. For example, Ni-O bond lengths from deprotonated hydroxide oxygen bond in **6a** and **6b** are respectively 1.789 Å and 1.827 Å, while the Ni-O bond lengths from the H₂O ligands in **6a** and **6b** are respectively 1.987 Å and 1.953 Å with an elongation of 0.13-0.20 Å. We also observed that the Ni-O bond lengths from the H₂O ligands in **6b** are shorter than those in **6a**. For example, Ni-O1 bond lengths in **6a** and **6b** are 1.987 Å and 1.953 Å respectively. In addition, there is an elongation in Ni-N bond length from **6a** (1.906 Å) to **6b** (1.950 Å). This may be due to steric hindrance between chlorine and the aromatic ring in **6b** that causes weakening of the bonds. The N-Ni-O bond angle in **6b** is significantly greater than the O-Ni-Cl bond angle. For example, N-Ni-O2 and N-Ni-Cl bond angles are respectively 99.29°, 106.76°, The O1-Ni-O3 bond angles in **6a** and **6b** are 82.53°, 82.17° respectively. The geometrical shape in **6a** is closer to a square compared to the other; the O1-Ni-O2 bond angle is 90.14°. Model **6c** is slightly distorted with the O3-Ni-O3* and N-Ni-N* bond angles of 155.66° and 173.14° respectively. It may be due to steric hindrance caused by the two Schiff-base ligands.

The calculated IR spectra of **6a**, **6b**, and **6c** are shown in figure 11. We observed that the calculated IR spectra of **6a** resembles the experimental FT-IR spectra of complex-III (figure 2). By examining the stretching mode of the peaks, the peak at 1637 cm⁻¹ was assigned to the imine ν(-C=N-) functional group, the peak at 1418 cm⁻¹ was assigned to the ν(-C-N-) functional group and the peaks between 500-800 cm⁻¹ were assigned to the ν(O-H) rocking vibration in water. The peaks observed between 3500-3800 cm⁻¹ were assigned to symmetric or asymmetric ν(O-H) vibration in water or hydroxyl groups. While the absolute values of the calculated IR peaks

differ slightly, the imine peak and the peaks observed at 600, 1400 and 3700 cm^{-1} regions agree well with the experiment results.

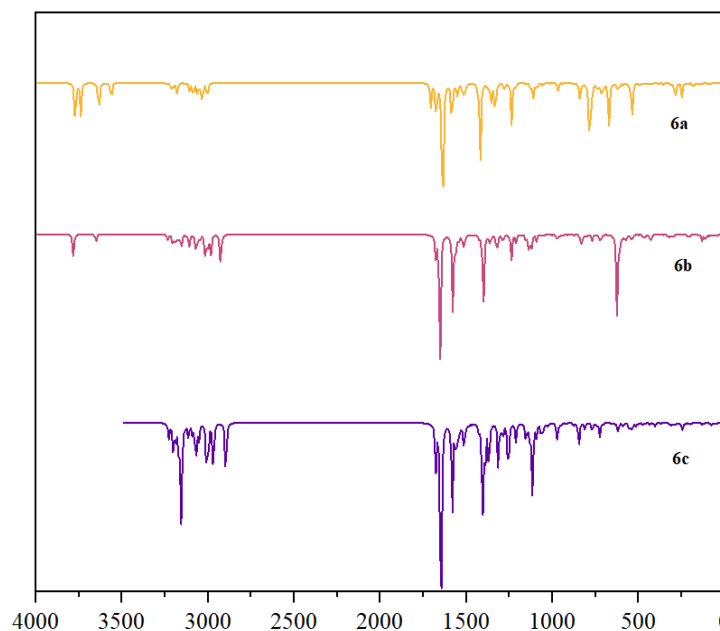


Figure 11. The calculated IR spectra of model structures 6a, 6b, and 6c.

Figure 12 shows the calculated UV-Vis spectra of **6a**, **6b**, and **6c**. From the results in figure 12, we see that the calculated spectra of **6a** resembles the experiment UV-Vis spectra of complex-**III**. The prominent peaks observed at 264 nm and 341 nm correspond to the HOMO-7→LUMO and HOMO→LUMO+1 electronic transition. Based on these modeling results, the most probable structure of complex-**III** is **6a**.

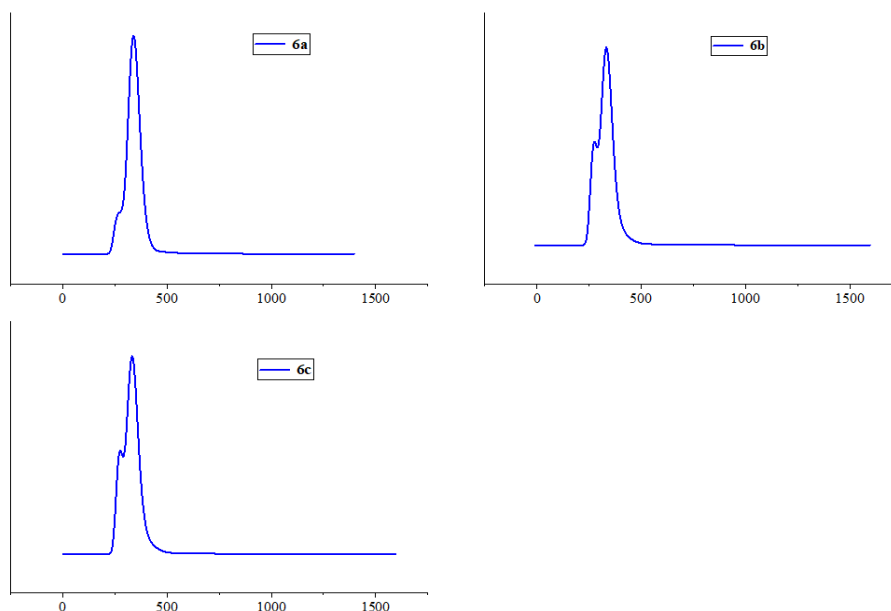


Figure 12. The calculated UV-vis spectra of 6a, 6b, and 6c.

CONCLUSION

Three Schiff-base ligands derived from serine, leucine, phenylalanine and salicylaldehyde, benzaldehyde and N-4-dimethylaminobenzaldehyde respectively and their Ni (II) complexes were synthesized and characterized. In addition, computational DFT B3LYP technique was used to simulate different models of the complexes to investigate the binding modes between the Schiff-base ligands and the Ni (II) center. The experimental and computational results confirmed that all three complexes adopt the square planar geometry that correspond to models **4a**, **5a**, and **6a** respectively for complexes-**I**, **II**, and **III**. Furthermore, the FT-IR and UV-Visible spectra the three complexes revealed three electronic transitions HOMO-6→LUMO, HOMO-2→LUMO+1, and HOMO→LUMO+1 for complex-**I**, two electronic transitions HOMO-2→LUMO+1 and HOMO-1→LUMO for complex-**II**, and two electronic transitions HOMO-7→LUMO and HOMO→LUMO+1 for complex-**III**. The three complexes were non-toxic towards prokaryotic gram positive (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus mutants*) and gram negative (*Aquaspirillum serpens* *Escherichia coli*) bacterial and eukaryotic (*Saccharomyces cerevisiae*) bacterial.

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