

Design of New N-Salicylidene Alanine Transition Metal Schiff-Base Complexes with Zn (II), Mn (II), Fe (II), and Fe (III): Synthesis, Characterization, Toxicity, and Computational Studies of the Potential Metal-Ligand Binding Sites

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ABSTRACT

We have proposed the structures and potential metal-ligand binding sites of four N-Salicylidene alanine transition metal Schiff-base complexes with Zn (II), Mn (II), Fe(II) and Fe(III) using both experimental and computational methods. Different models of the structures were fully optimized and analyzed using density functional theory (DFT) B3LYP method and the binding modes revealed that models 4a, 5c, 6c, and 7f correspond to complexes I, II, III, and IV respectively. The geometry adopted are tetrahedral, trigonal bipyramidal, octahedral, and octahedral respectively for complexes I, II, III, and IV. Characterization of the complexes were done using

melting point/decomposition temperatures, solubility test, FT-IR and UV-visible spectroscopy. All the complexes were seen to have a different melting point from the amino acid; alanine, which was used in the synthesis. The complexes were soluble in water and most polar solvents, which is important for intended use in biological systems as drugs. In addition, the experimental and calculated IR spectra of the complexes revealed prominent stretching frequencies including the -C=N-imine group that are similar within 5-10 % margin to the most stable structures 4a, 5c, 6c, and 7f. Furthermore, the UV-visible studies of complex I showed three prominent electronic transitions, two prominent transitions for complex II, three prominent transitions for complex III and three prominent transitions for complex IV in both the experimental and computational model structures. These electronic transitions were assigned to the $\pi \rightarrow \pi^*$ and HOMO-1 $\rightarrow$ LUMO+2, HOMO-4 $\rightarrow$ LUMO, HOMO $\rightarrow$ LUMO for complex I, $\pi \rightarrow \pi^*$ and HOMO-5 $\rightarrow$ LUMO-5, HOMO $\rightarrow$ LUMO+1 for complex II, $^2T_{2g} \rightarrow ^2E_g$, $\pi \rightarrow \pi^*$ and HOMO-2 $\rightarrow$ LUMO+1, and HOMO $\rightarrow$ LUMO+1 for complex III, $\pi \rightarrow \pi^*$ and HOMO-3 $\rightarrow$ LUMO+1, HOMO-3 $\rightarrow$ LUMO for complex IV. 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

The recent study and development of new therapeutic schiff-base complexes in the field of bio-inorganic chemistry, biochemistry and medicinal chemistry has been in an exponential growth over the years. This is because most of these schiff-base complexes play an important role in biological systems owing to the presence of the imine or azomethine group and the recognition that many of these complexes can be used as models that could mimic and/or interact with biologically important molecules for the mitigation, control and/or cure of many diseases. Previous research has shown that schiff-bases derived from amino acids contain bidentate or multidentate binding sites to transition metals and biomolecules with application in drug design and development. These metal complexes can be synthesized through the reaction between the Schiff-base ligands derived from amino acids and a metal precursor in a suitable solvent and under different experimental conditions. Many Schiff-base complexes show excellent catalytic and biological activity in various reactions such as polymerization reactions, reduction of thionyl chloride, oxidation of organic compounds, reduction of ketones, aldol reactions, Henry reaction, epoxidation etc, which gives us the need and motivation to expand the synthesis of schiff-base ligands derived from amino acids and their subsequent binding to transition metals. In addition, schiff-base complexes have been used industrially and exhibit a wide range of biological activities and/or properties such as anti-fungal, anti-malarial, anti-bacterial, anti-diabetic, anti-cancer, anti-proliferative, anti-tumor, antimicrobial, antioxidant, etc agents including their use as dyes and insecticides. Schiff-bases and their complexes also have the ability to interact with DNA to mitigate or control different diseases [1-13]. The discovery of cisplatin, auranofin, and other chemotherapeutic drugs in the early 1990s, the exploration of the binding between schiff-bases and metals as potential drug candidates has continued to motivate scientists in the process of drug development, design and discovery [14-17]. In this paper, we will present routes towards the development and design of new

compounds synthesized between Schiff-bases derived from salicylaldehyde, alanine and their binding to transition metals. The transition metals used in this research are Zn (II), Mn(II), Fe(II), and Fe(III). In addition, we will determine the toxicity of these compounds and the potential binding modes between the Schiff-bases and the metals using computational B3LYP density functional theory (DFT) method. The characterization of the complexes will include melting/decomposition temperatures, solubility in different solvents, Fourier Transform Infra-red spectroscopy (FT-IR), ultra violet-visible spectroscopy (UV-Vis), etc. The computational DFT studies will compare the results with the experimental results and predict the possible binding sites in the complexes.

EXPERIMENTATION

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

Synthesis of N-Salicylidene Alanine Zn (II) Complex [N-S-ala-Zn (II)]-I

Sodium hydroxide (NaOH; 0.0112 mols) and alanine ($C_3H_7NO_2$; 0.0112 mols) were completely dissolved in a 250 mL beaker containing 30.0 mL of water with the help of a magnetic stirrer at room temperature. An equimolar amount of salicylaldehyde (0.0112 mols) was added drop wise to the resulting mixture while stirring continuously for 45 minutes. A 30.0 mL aqueous solution of zinc (II) chloride heptahydrate ($ZnCl_2 \cdot 7H_2O$; 0.0056 mols) was slowly added onto the resulting solution and allowed to react for an additional 45 minutes in the fume hood. The resulting mixture was concentrated to about 60 % of the original volume to initiate crystallization. The mixture was allowed in the fume hood for three days and the resulting yellow powder was filtered through suction, air dried and stored in a vial for subsequent characterization. The percentage yield was 80 %.

Synthesis of N-Salicylidene Alanine Mn (II) Complex [N-S-ala-Mn (II)]-II

Similarly, (NaOH; 0.0112 mols) and alanine ($C_3H_7NO_2$; 0.0112 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 (0.0112 mols) was added drop wise to the mixture allowed to for 40 minutes. A 20.0 mL aqueous solution of anhydrous manganese (II) chloride ($MnCl_2$; 0.0056 mols) was slowly added onto the resulting mixture and allowed to react for an additional 1 hour in the fume hood. The mixture was allowed in the fume hood for four days and the resulting yellow 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 85 %.

Synthesis of N-Salicylidene Alanine Fe (II) Complex [N-S-ala-Fe (II)]-III

Sodium hydroxide (NaOH; 0.0112 mols) and alanine ($C_3H_7NO_2$; 0.0112 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 (0.0112 mols) was then added drop wise to the resulting mixture allowed to react for 50 minutes. A 30.0 mL aqueous solution of Iron (II) chloride monohydrate ($FeCl_2 \cdot H_2O$; 0.0056 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 55 % of its original volume and allowed in the fume hood for five days. The resulting light

red 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 87 %.

Synthesis of N-Salicylidene Alanine Fe (III) Complex [N-S-ala-Fe (III)]-I

Sodium hydroxide (0.0112 mols) and alanine (0.0112 mols) were dissolved in a 250 mL beaker containing 40.0 mL of water while stirring continuously with the help of a magnetic stirrer at room temperature. An equimolar amount of salicylaldehyde (0.0112 mols) was added drop wise to the resulting mixture while stirring. The reaction was allowed to go on for 45 mins and a 30.0 mL aqueous solution of Iron (III) chloride pentahydrate ($\text{FeCl}_3 \cdot 5\text{H}_2\text{O}$; 0.0056 mols) was slowly added onto the resulting solution and allowed to react for an additional 45 minutes in the fume hood. The resulting mixture was concentrated to 60 % of the original volume to initiate crystallization. After four days, a dark purple powder was filtered through suction, recrystallized in ethanol-water mixture (50/50), air dried and stored in a vial for subsequent characterization. The percentage yield was 90 %.

Toxicity Test of the 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 [18-19].

In this paper, an aqueous solution of the complexes (N-Salicylidene alanine Zn (II)-I, N-Salicylidene alanine Mn (II)-II, N-Salicylidene alanine Fe (II)-III, and N-Salicylidene alanine Fe (III)-IV were prepared with a slightly higher antibiotic concentration (1.0 μM) than those used in the Kirby-Bauer assay (30.0 μg). 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 [20].

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 [21-25]. The structural geometries were fully optimized and all elements were analysed 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 [26-30]. 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 [31-, 35]. The calculated spectra of the different structural geometries would be compared to the experiment results to determine the potential binding mode of the alanine Schiff-base ligands to the transition metal centers. The geometric parameters (bond lengths and bond angles) of the most stable optimized structures are shown in Table S1 of the supporting materials.

RESULTS AND DISCUSSION

Melting Point/Decomposition Temperature and Reaction Time

The first characterization technique of a synthesized compound is to determine the melting/decomposition and/or boiling point. 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 acid (alanine) from which they were synthesized. The melting points/decomposition temperatures of all our complexes were different from the melting point of the amino acid use, indicating that new compounds were synthesized. The melting point of alanine was observed at a precise and sharp temperature of 300 °C compared to N-Salicylidene alanine Zn(II) complex-I that melts at a temperature below 250 °C while changing colour from white to yellow, N-Salicylidene alanine Mn(II) complex-II melts below 350 °C and changes colour from yellow to orange, N-Salicylidene alanine Fe(II)-III complex melts below 250 °C with colour changing from light red to dark red, N-Salicylidene alanine Fe (III)-IV complex decompose at temperatures below 250 °C while changing colour from dark purple to black. The results of melting point/decomposition temperatures of the complexes are presented in Table 1

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

Complex	M.Pt/Decomp.T/°C	Reaction Time /mins	Yield (%)	Colour
N-S-ala-Zn (II)-I	250	110	80	yellow
N-S-ala-Mn (II)-II	350	120	85	yellow
N-S-ala-Fe (II)-III	250	130	87	light red
N-S-ala-Fe (III)-IV	250	110	90	dark purple
Alanine	300	-	-	white

Solubility Test

Solubility of compounds is one of the fundamental requirements to ascertain the effectiveness of a compound intended to be used as a drug. The solubility of our complexes was determined in different solvents and the results will enable us to perform other techniques. This was done manually by dissolving approximately 0.100 g sample of each complex in approximately 10.00 mL of solvent. The solubility results are presented in table 2. It is important to note that our complexes were all soluble in water and other polar solvents and insoluble in non-polar solvents with increased solubility of the complexes observed in a warmer solvent.

Table 2: Solubility of the Complexes

Complexes	Water	Ethanol	methanol	chloroform	hexane	D ₂ O
N-S-ala-Zn (II)-I	s	S	Ss	l	i	s
N-S-ala-Mn (II)-II	s	S	Ss	l	i	s
N-S-ala-Fe (II)-III	s	S	Ss	l	i	s
N-S-ala-Fe (III)-IV	s	S	Ss	l	i	s

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

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

Fourier transform infrared (FT-IR) spectroscopy was used to determine the vibrations of the atoms in the complexes. This will enable us to identify the different stretching frequencies of all the functional groups and other atoms present in the complexes. This was done using a FLUKA table top FT-IR instrument. The IR results of the prominent peaks measured in wavenumbers (cm^{-1}) are shown in table 3. The FT-IR spectra of the transition metal complexes reveal that the different Schiff-base ligands bind to the metal centers in a multidentate form, in which the coordinate bond takes through the nitrogen atom of the imine ($\text{C}=\text{N}$) group, deprotonated the aromatic- O^- (hydroxyl group), deprotonated Ac-O^- (carboxylate group), water, and/or counter anionic ligands to the transition metal ions. In all the complexes, a slight shift in the strong azomethine ($\text{C}=\text{N}$) stretching frequencies is due to the distorted structures or geometries adopted when the ligands bind to the metal centers. Generally, the weaker stretching frequencies were observed for the deprotonated aromatic- O^- (hydroxyl group), Ac-O^- (carboxylate group), water, and/or the counter anion.

The imine $\nu(\text{C}=\text{N})$ stretching frequency was observed between $1646\text{--}1655\text{ cm}^{-1}$ in complex I, $1596\text{--}1631\text{ cm}^{-1}$ in complex II, $1560\text{--}1655\text{ cm}^{-1}$ in complex III, and $1541\text{--}1521\text{ cm}^{-1}$ in complex IV. The aromatic and aliphatic $\nu(\text{C-H})$ stretching vibrational bands were seen at 3062 cm^{-1} and 2900 cm^{-1} respectively in complex I, 3060 cm^{-1} and 2900 cm^{-1} respectively in complex II, 3054 cm^{-1} (aromatic) in complex III, 2977 cm^{-1} (aromatic) for complex IV. The symmetric or asymmetric $\nu(\text{O-H})$ vibrates at 3468 cm^{-1} in complex I, $3180\text{--}3367\text{ cm}^{-1}$ in complex II, 3240 cm^{-1} in complex III, 3225 cm^{-1} in complex IV. The $\nu(\text{C-O})$ group vibrates at a stretching frequency of 1465 cm^{-1} in complex I, 1442 cm^{-1} in complex II, 1442 cm^{-1} in complex III, 1466 cm^{-1} in complex IV. The carbonyl $\nu(\text{C=O})$ stretching frequency was seen at 2750 cm^{-1} for complex I, between $2250\text{--}2642\text{ cm}^{-1}$ in complex II, between $2345\text{--}2374\text{ cm}^{-1}$ in complex III, between $2281\text{--}2374\text{ cm}^{-1}$ in complex IV. We observed a slight shift in the vibrational frequencies of all the functional groups in all the complexes and this is due to the distorted structures after coordination to the central metal ion when compared to literature values [1,5,11-13].

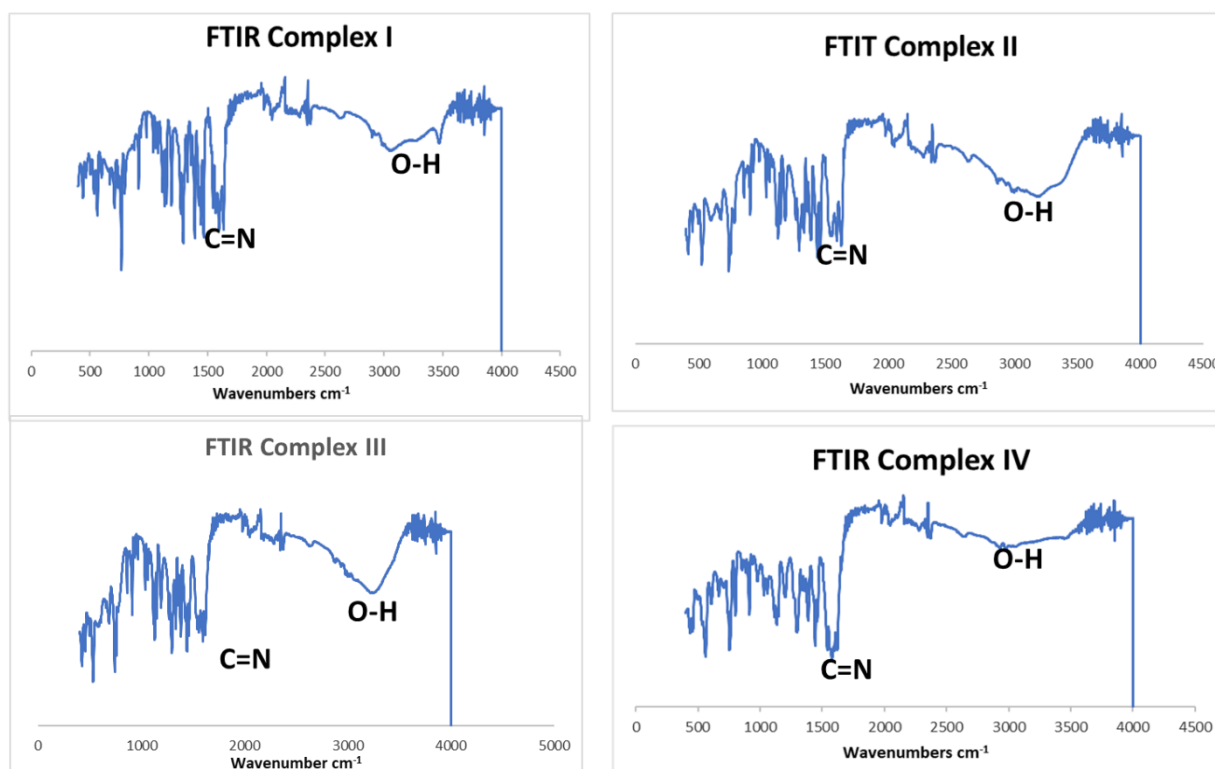


Figure 2: Experimental FTIR spectra for Complexes I, II, IV and VI showing prominent peaks. Other FTIR are available in the supporting material

The characteristic experimental FTIR values (in cm^{-1}) for the complexes are presented in table 3.

Table 3. Experimental FTIR 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	1645-1655	3062-2900	3468	1465	2750
II	1596-1631	3060-2900	3180-3367	1442	2250
III	1560-1655	3054	3240	1442	2345-2374
IV	1541-1621	2977	3225	1466	2281-2374

UV-Visible Spectra and Energies of the Complexes

All the complexes are coloured, 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.

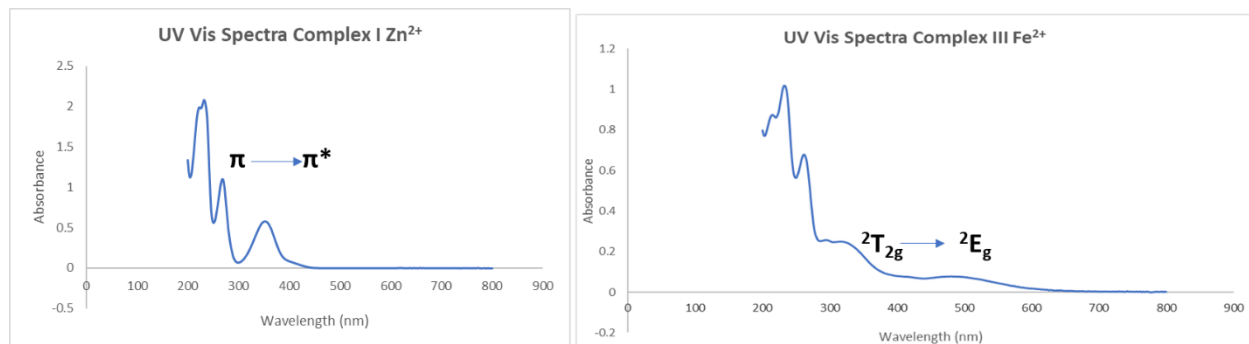


Figure 3: Experimental UV-visible spectra for Complexes I, and III showing prominent absorption peaks and transitions. Other UV-Vis spectra are available in the supporting material

The yellow N-salicylidene alanine Zn (II); complex **I** showed three peaks on the UV-visible plot. These peaks are due to charge-charge transfer as a result of the π -electron system in the aromatic benzene ring. The three peaks observed at 234 nm, 270 nm and 354 nm was assigned to the $\pi \rightarrow \pi^*$ transition in the aromatic benzene ring. Similarly, complex **II**; N-salicylidene alanine Mn (II) (yellow) showed two peaks at 276 nm and 358 nm on the UV-vis plots and these peaks are due to the $\pi \rightarrow \pi^*$ transition in the aromatic benzene ring. In addition, the light red complex **III**; N-salicylidene alanine Fe (II) showed one prominent peak at 320 nm, which was assigned to the ${}^2T_{2g} \rightarrow {}^2E_g$ transition. The two additional peaks observed at 236 nm and 265 nm were assigned to the $\pi \rightarrow \pi^*$ transition in the aromatic benzene ring. In complex **IV** [(Fe (III))], the peaks observed at 230 nm, 264 nm and 334 nm were assigned to the $\pi \rightarrow \pi^*$ of the aromatic benzene ring.

The maximum wavelengths and energies of the transitions are represented in table 4.

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

Complex; colour	Max. wavelength ($\lambda_{\max}$, nm)	Energy	Electronic Transition
I; yellow	234	8.50×10^{-19}	$\pi \rightarrow \pi^*$
	270	7.37×10^{-19}	$\pi \rightarrow \pi^*$
	354	5.62×10^{-19}	$\pi \rightarrow \pi^*$
II; yellow	276	7.21×10^{-19}	$\pi \rightarrow \pi^*$
	358	5.56×10^{-19}	$\pi \rightarrow \pi^*$
III; light red	320	6.22×10^{-19}	${}^2T_{2g} \rightarrow {}^2E_g$
	236, 265	8.42×10^{-19}	$\pi \rightarrow \pi^*$
IV; dark purple	230, 264, 334, 508	8.65×10^{-19}	$\pi \rightarrow \pi^*$

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 on the complexes

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

S = isolates were susceptible to growth, R = isolates were resistant to growth

COMPUTATIONAL STUDIES ON THE COMPLEXES

Computational density functional theory (DFT) was used to investigate the binding sites of the Schiff base ligands to the different metal centers: Zn (II), Mn (II), Fe (II), and Fe (III). Different metal-alanine Schiff-base structures were proposed and optimized and 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 Alanine Zn (II) Complex, I

Based on our previous study [9], we used the deprotonated hydroxide functional group alanine Schiff-base ligand to investigate the binding mode of the Schiff-base ligand to zinc (II) metal. As shown in Figure 4, five zinc-alanine Schiff base structure models were successfully optimized. Models 4a and 4b show four coordinated pseudo-tetrahedral geometry with deprotonated alanine Schiff-base structure providing three binding sites and water or chlorine respectively as the fourth binding site. Models 4a and 4b have three oxygen donor sites and one nitrogen donor site and two oxygen donor sites, one nitrogen and one chlorine donor sites respectively to zinc. Models 4c and 4d show a coordination number of five with two water or one water-one chlorine donor sites respectively. Similarly, models 4c and 4d have four oxygen donor and one nitrogen donor sites and three oxygen donor sites, one nitrogen and one chlorine donor sites respectively to zinc. The six-coordinated complex 4e is only feasible with a 2:1 alanine Schiff-base ligands to one zinc metal. Model 4e has four oxygen donor sites and two nitrogen donor sites to zinc. The five models of N-S-ala-Zn (II) are shown in figure 4.

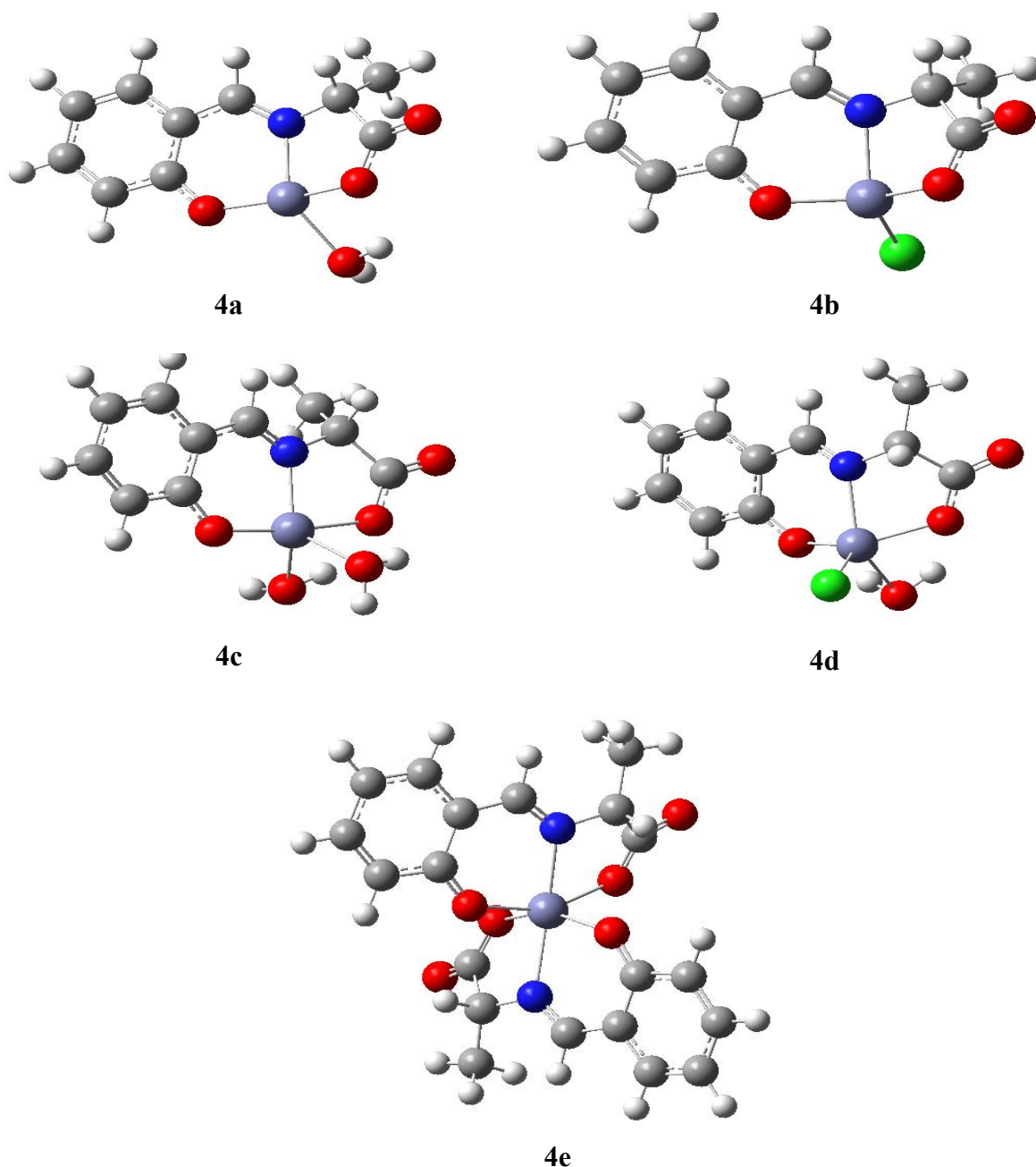


Figure 4: Proposed structural geometries of Zn-Alanine Schiff-base models

From Figure 4 and computational details, we can see that all the five optimized models of alanine Schiff-base Zn (II) complexes (**4a-4e**) were stable in terms of bond lengths. The most significant changes were elongations in the Zn-O bond lengths for the H₂O and the Zn-Cl ligands. For example, Zn-O bond lengths of structure **4a** are 1.916 Å and 1.982 Å for the deprotonated hydroxyl and carboxylate groups respectively, while Zn-O distance for the H₂O ligand is 2.139 Å. Similarly, the chloride ligand also affects the bond lengths in the complexes. For example, Zn-N bond length increases from 2.041 Å in **4a** to 2.111 Å in **4b**. A similar elongation in Zn-N bond length was observed from 2.062 Å in **4c** to 2.156 Å in **4d**. It is important to note that all the bond lengths were elongated in the more crowded, five-coordination sphere of zinc. For

example, Zn-O bond lengths in **4c** and **4d** has an elongation of 0.03 to 0.11 Å when compared to **4a** and **4b**. Furthermore, the greatest structural changes in bond lengths were observed in structure **4e**. For example, the Zn-O and Zn-N bond lengths in **4e** show an elongation of 0.14 to 0.24 Å when compared to **4a**. This implies that bond lengths increase with coordination or complexity of the complexes.

Generally, it was observed that the C-O-Zn bond angles were much larger than the O-Zn-N bond angles. The presence of H₂O and Cl⁻ ligands bonded to Zn (II) causes a decrease in the C-O-Zn and O-Zn-N bond angles. For example, in structure **4a**, the bond angle of C1-O1-Zn is 125.46° while the bond angle of O1-Zn-N1 is 95.42°. Similarly, in structure **4c**, the C1-O1-Zn bond angle is 126.63° while the bond angle of O1-Zn-N1 is 93.37°. In the octahedral coordinated structure **4e**, the C1-O1-Zn bond angle reaches a maximum value of 132.23° while the O1-Zn-N1 bond angle reaches a minimum value of 81.83°.

The IR spectra of structures **4a** – **4e** were computed and the results are shown in Figure 5. Comparing the experimental (Figure 2) and calculated IR spectra of N-S-ala-Zn (II) complex (figure 4), we can see the profile of calculated IR spectra of **4a** closely resembles the experimental IR spectra of complex I. Similarly, the IR stretching frequency observed at 1797 cm⁻¹ was assigned to the carbonyl $\nu(-C=O)$ group, the stretching frequency observed at 1692 cm⁻¹ was assigned to the imine $\nu(-C=N-)$ functional group, and the peaks observed at 500 cm⁻¹ and 717 cm⁻¹ were assigned to the rocking vibration modes of water. Various peaks around 3500 - 3800 cm⁻¹ were assigned to symmetric vibration of water. While the absolute values of the calculated IR peaks differ slightly from the experimental values, the peak at 1797 cm⁻¹ for the imine $\nu(-C=N-)$ functional group and the O-H peaks at 500, 1700 and 3500 cm⁻¹ regions agree well with the experiment results [10-11].

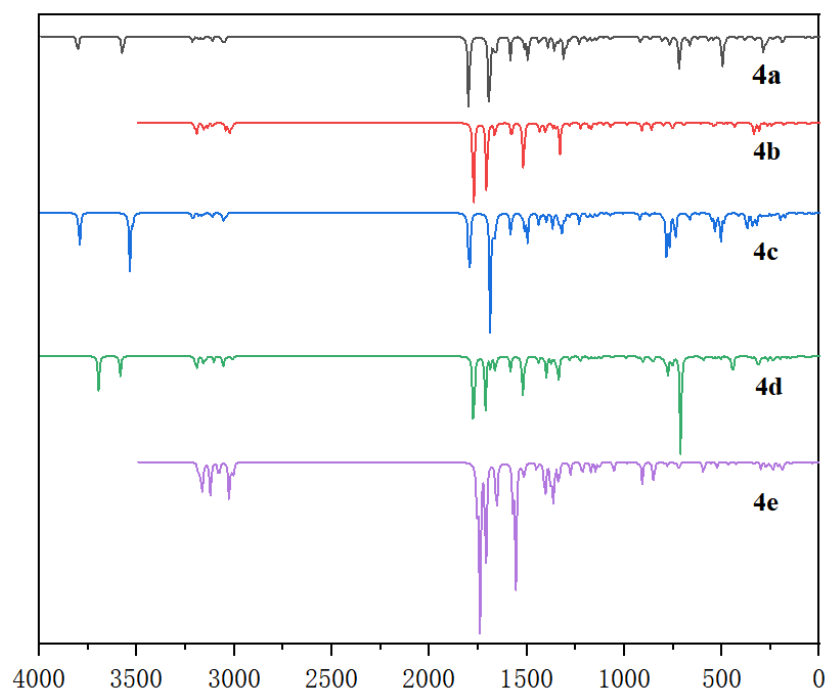


Figure 5: The calculated IR spectra of model structures 4a – 4e.

In addition, the calculated UV-vis spectra of structures **4a** – **4e** were computed and the results are presented in Figure 5. From the UV-Vis spectra in figure 5, the calculated spectra profile of structure **4a** matches well with the experiment UV-Vis spectra of complex **I**. Three prominent peaks predicted were observed at 212 nm, 247 nm, and 347 nm. These transitions were assigned to HOMO-1 → LUMO+2, HOMO-4 → LUMO, HOMO → LUMO, in agreement with the experimental UV-vis transitions observed at 234 nm, 270 nm and 354 nm. Based on the modeling results, the most probable or proposed structure for complex **I** is **4a**.

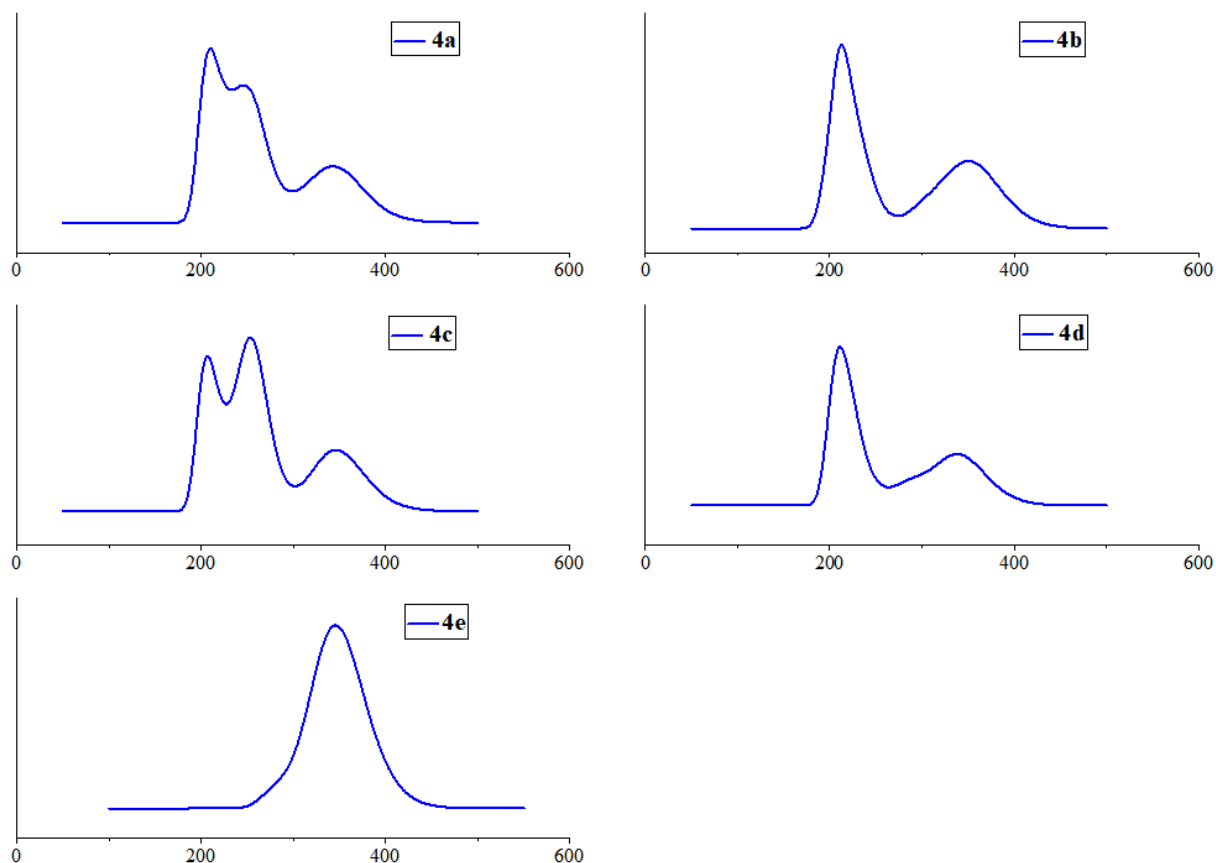


Figure 6: The calculated UV-vis spectra of model structures 4a-4e

Computational Studies on N-Salicylidene Alanine Mn (II) Complex, II

Similarly, six deprotonated N-S-ala-Mn (II) complex II models were completely optimized. The models in structures **5a** and **5b** show a tetrahedral geometry with three oxygen donors, one nitrogen donor sites to Mn (II) in **5a** and two oxygen donors, one nitrogen donor and one chloride donor sites to Mn (II) in **5b**. Models **5c** and **5d** have a coordination number of five around the Mn (II) metal with two H₂O ligands in **5c** and one H₂O and one chloride ligand in **5d**. In models **5e** and **5f**, Mn (II) has a coordination number of six (octahedral geometry) with three H₂O ligands in **5e** and four oxygen donor atoms and two nitrogen donors in a 1:2 metal:ligand ratio in **5f**.

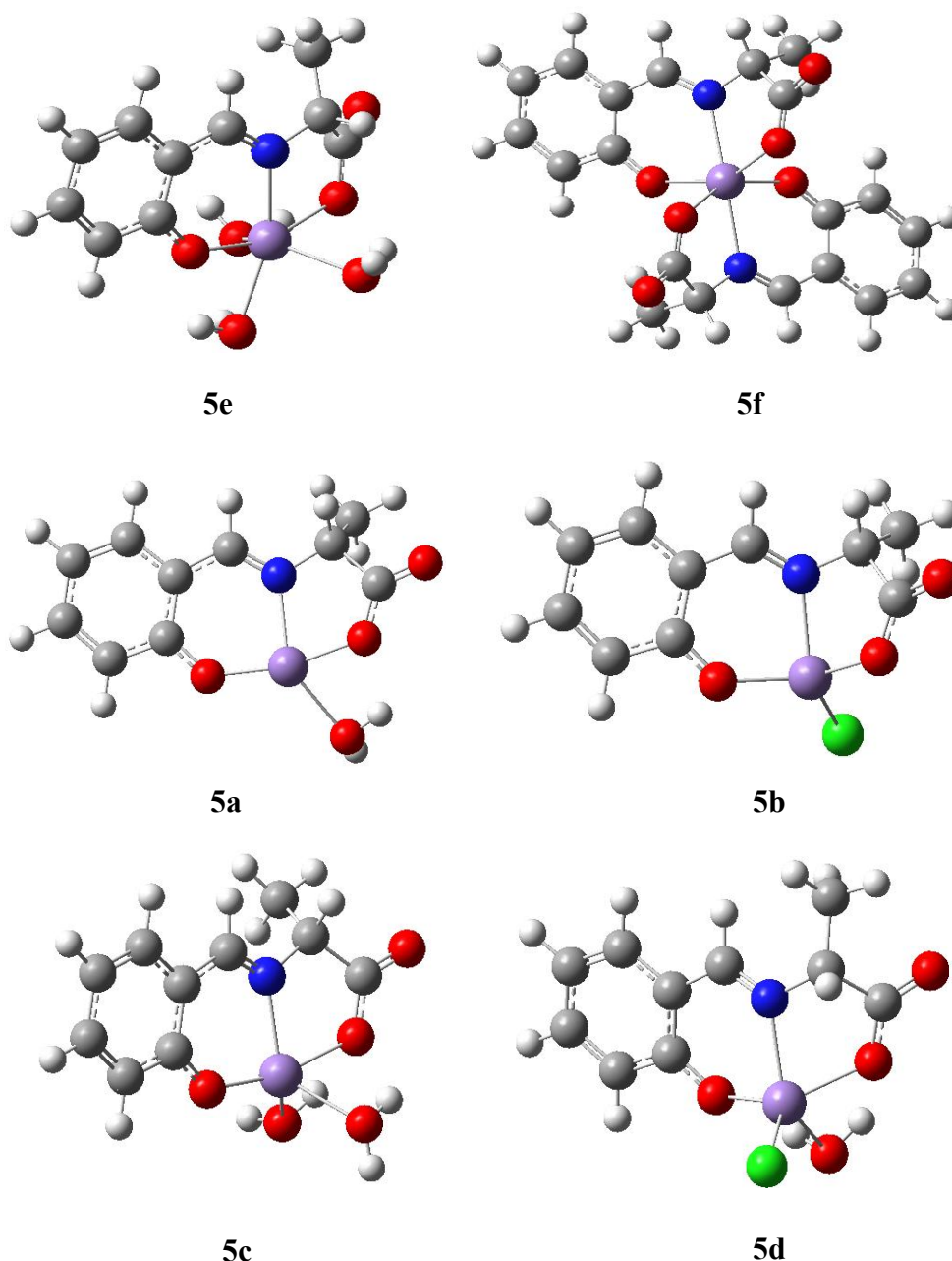


Figure 7: Proposed structural geometries of N-S-ala-Mn (II) complex II

From the computational analysis (table S1) and figure 7, we can observe that the bond length of Mn and the donor atoms increases with increase in the coordination number around the metal center. For example, the Mn-O1 bond lengths are 1.923 Å, 1.952 Å, and 2.020 Å respectively in models **5a**, **5c**, and **5e**. A similar elongation pattern was observed for the Mn-N bond lengths in models **5a**, **5c**, and **5e**. The longest Mn-O and Mn-N bond lengths were observed in model **5f** where two N-Salicylidene alanine ligands are bonded to Mn (II). For example, Mn-O2 bond length increased from 2.005 Å in **5a** to 2.161 Å in **5f**, while the Mn-N bond length increased from 2.138 Å in **5a** to 2.307 Å in **5f**.

Interestingly, the bond angles around the center of Mn (II) shows an opposite decreasing trend with increase in coordination number. For example, the O1-Mn-N1 bond angles for **5a**, **5c**, and **5e** are respectively, 89.92°, 88.92°, and 86.43°. This is due to increase in coordination around the metal center. The smallest bond angles were observed for model **5f** with O1-Ni-N1 and O2-Ni-N1 bond angles of 78.57° and 73.73° respectively.

The calculated IR spectra of models **5a** – **5f** are shown in Figure 8. Comparing these results with the experimental IR spectra of N-Salicylidene alanine Mn (II) complex, **II** (figure 2), the profile of calculated IR spectra of **5c** closely resemble the experimental IR spectra. The prominent peak observed at 1794 cm⁻¹ was assigned to the stretching of carbonyl $\nu(\text{C}=\text{O})$ group, the peak 1682 cm⁻¹ was assigned to the imine $\nu(\text{-C}=\text{N-})$ functional group, and the peaks 515 and 770 cm⁻¹ were assigned to the rocking vibration modes of water. The peaks observed between 3000 - 3800 cm⁻¹ were assigned to symmetric $\nu(\text{O-H})$ vibrations of water molecule. While the absolute values of the calculated IR peaks differ slightly, the imine $\nu(\text{-C}=\text{N-})$ vibrational peak at 1682 cm⁻¹ and the $\nu(\text{O-H})$ peaks at 500, 1700 and 3000-3800 cm⁻¹ agree well with the experiment results for complex **II**.

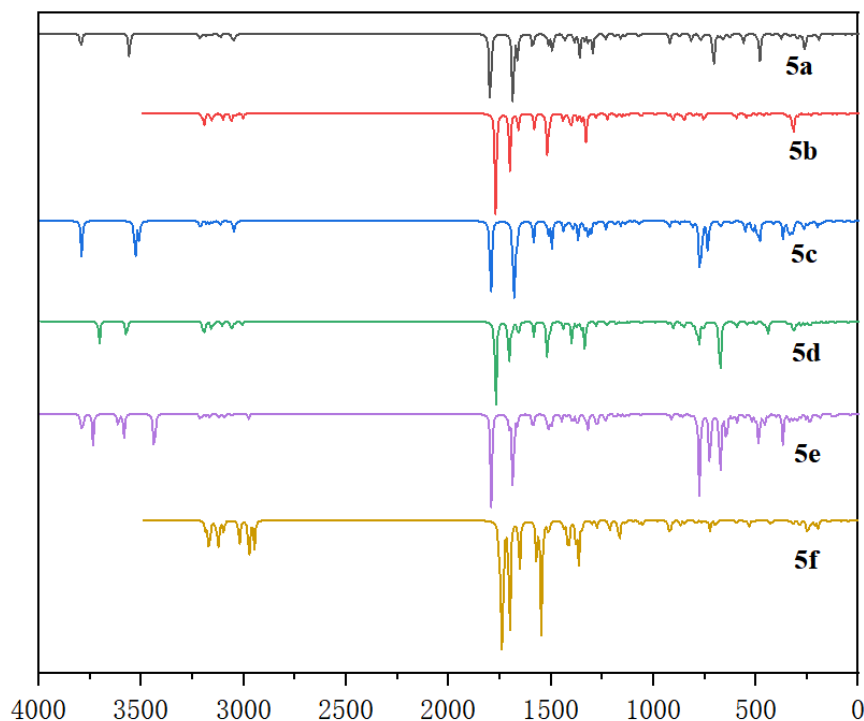


Figure 8: The calculated IR spectra of model structures 5a – 5f.

Similarly, the computed UV-Vis spectra of the model structures 5a-5f are shown in Figure 9. The calculated IR spectra of model **5c** agrees with the experiment results. The prominent peaks observed at 258 nm and 366 nm agrees with the experimental IR peaks (276 nm and 358 nm) observed for complex **II**. The two transitions were assigned to the HOMO-5→LUMO-5, and HOMO → LUMO+1. Therefore, based on comparison between the experimental and computational results, the most probable structure for complex **II** is **5c**.

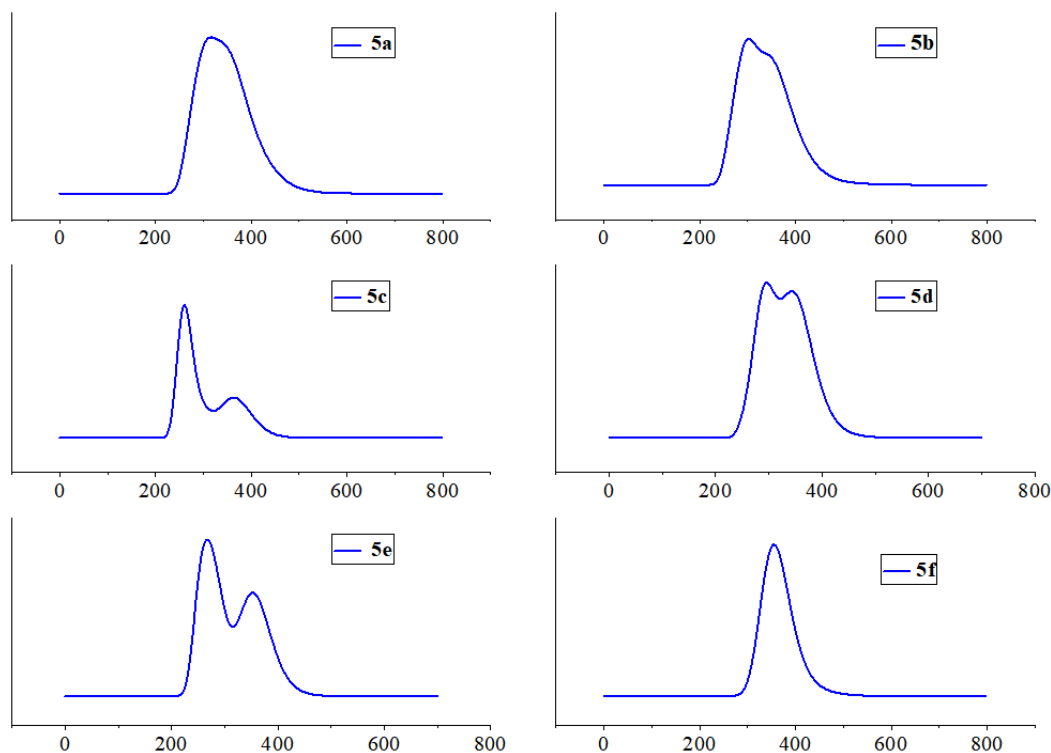


Figure 9: The calculated UV-vis spectra of model structures 5a -5f.

Computational Studies on N-Salicylidene Alanine Fe (II) Complex III

Computational studies were carried on five models of N-S-ala-Fe(II) complex III. The optimized structures of the five models adopt either the tetrahedral or octahedral geometry around the Fe(II) metal center (see figure 10). The tetrahedral model **6a** contains one H₂O ligand while the tetrahedral model **6b** contains one chloride ligand. On the other hand, the octahedral model **6c** contains three H₂O ligands, the octahedral model **6d** contains two H₂O ligands and one chloride ligand and the octahedral model **6e** contains four oxygen donor atoms and two nitrogen donor atoms with a 1:2 metal:ligand ratio.

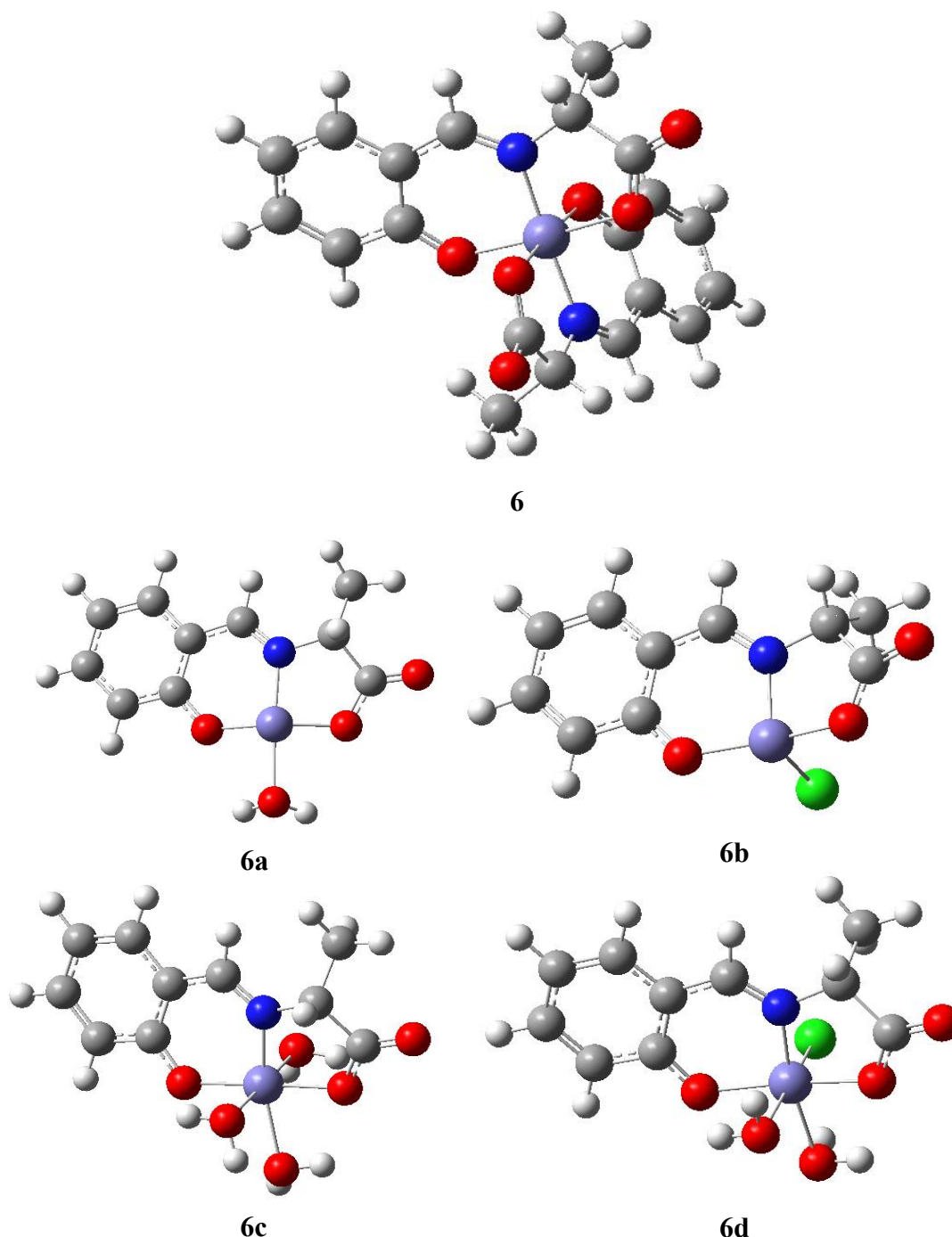


Figure 10: Proposed structural models of N-S-ala-Fe (II) complex III

From figure 10 and the computational details (table S1), the most significant differences between the five optimized models of complex **III** is from the coordination number around the Fe (II) center and the *trans* effect. For example, in **6a** the Fe-O bond lengths between the carboxylate oxygen donor and the hydroxyl oxygen donor atoms and the Fe (II) are respectively 1.820 Å and 1.835 Å, while H₂O-Fe (II) distance is 2.029 Å. The *trans* position of the H₂O ligand N donor atom elongates the Fe-H₂O bond length and shortens the Fe-N bond length. A similar

trend of observed with **6b** with the chloride ligand replacing the H₂O ligand. Further examination reveal that the Fe-N bond length decreases from 1.887 Å in **6a** to 1.863 Å in **6b**. This trend is reversed in models **6c** and **6d** with an elongation in the Fe-N bond length from 1.897 Å respectively. In addition, an increase in the coordination number around the Fe(II) center causes an elongation in all the corresponding metal-ligand bonds. For example, Fe-O bond lengths in **6c** and **6d** are 0.03- 0.11 Å longer than those in **6a** and **6b**. The greatest structural changes are observed in the octahedral model **6e**, where two Schiff-base ligands are boded to the Fe (II) center. This increase in coordination number around the Fe(II) center destabilizes the structure, thereby elongating the bond lengths. For example, the Fe-O and Fe-N bond lengths in **6e** are 0.14 - 0.24 Å longer than the corresponding bond lengths in **6a**.

The calculated IR spectra of structures **6a** – **6e** are shown in Figure 11. By comparing the calculated IR spectra with the experimental IR spectra of N-S-ala-Fe (II) complex **III** (figure 2), we see that the profile of calculated IR spectra of **6c** resembles the experimental IR spectra of complex **III**. The calculated IR vibrational peak at 1790 cm⁻¹ was assigned to the stretching of carbonyl $\nu(\text{C}=\text{O})$ functional group, the peak 1690 cm⁻¹ was assigned to the imine $\nu(\text{C}=\text{N})$ functional group, and the peaks 495 and 732 cm⁻¹ were assigned to the rocking vibration modes of water. The different peaks observed between 3500 - 3800 cm⁻¹ were assigned to symmetric vibration of the $\nu(\text{O}-\text{H})$ stretching in water. While the absolute values of the calculated IR peaks differ slightly from the experimental IR peaks, the peaks observed at 500, 1700 and 3500 cm⁻¹ agrees well with the experiment IR results.

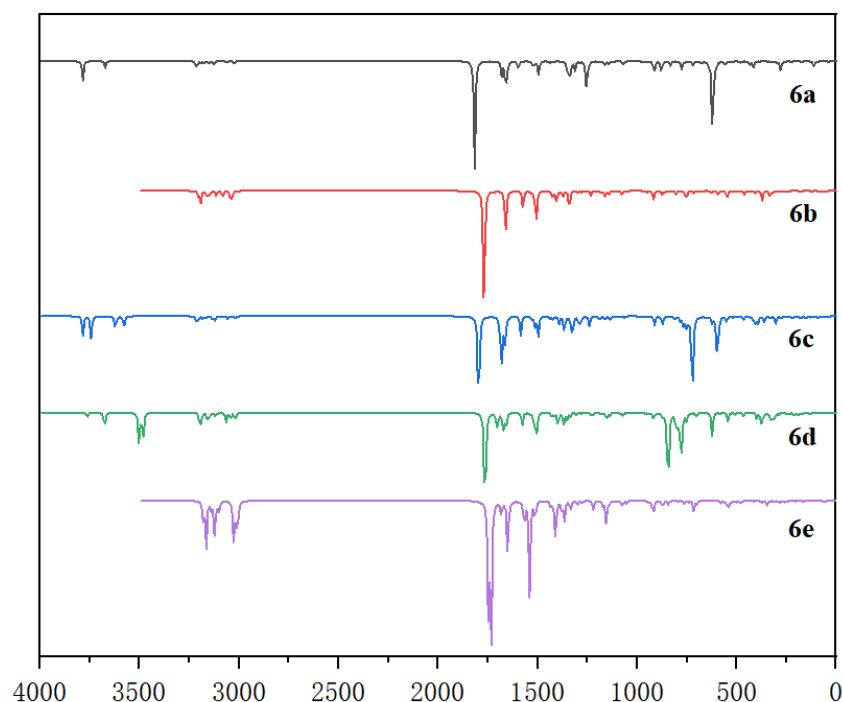


Figure 11: The calculated IR spectra of model structures 6a – 6e.

The calculated UV-vis spectra of the model structures **6a** – **6e** are shown in figure 12. From the calculated UV-vis, model **6c** agrees well with the experiment UV-vis spectra of complex **III**. The

two prominent peaks observed at 315 nm and 375 nm are assigned to the HOMO-2→LUMO+1, and HOMO→LUMO+1 electronic transition. Based on the computational details of the calculated IR and UV-vis spectra, the most probable structure of complex **III** is mode **6c**.

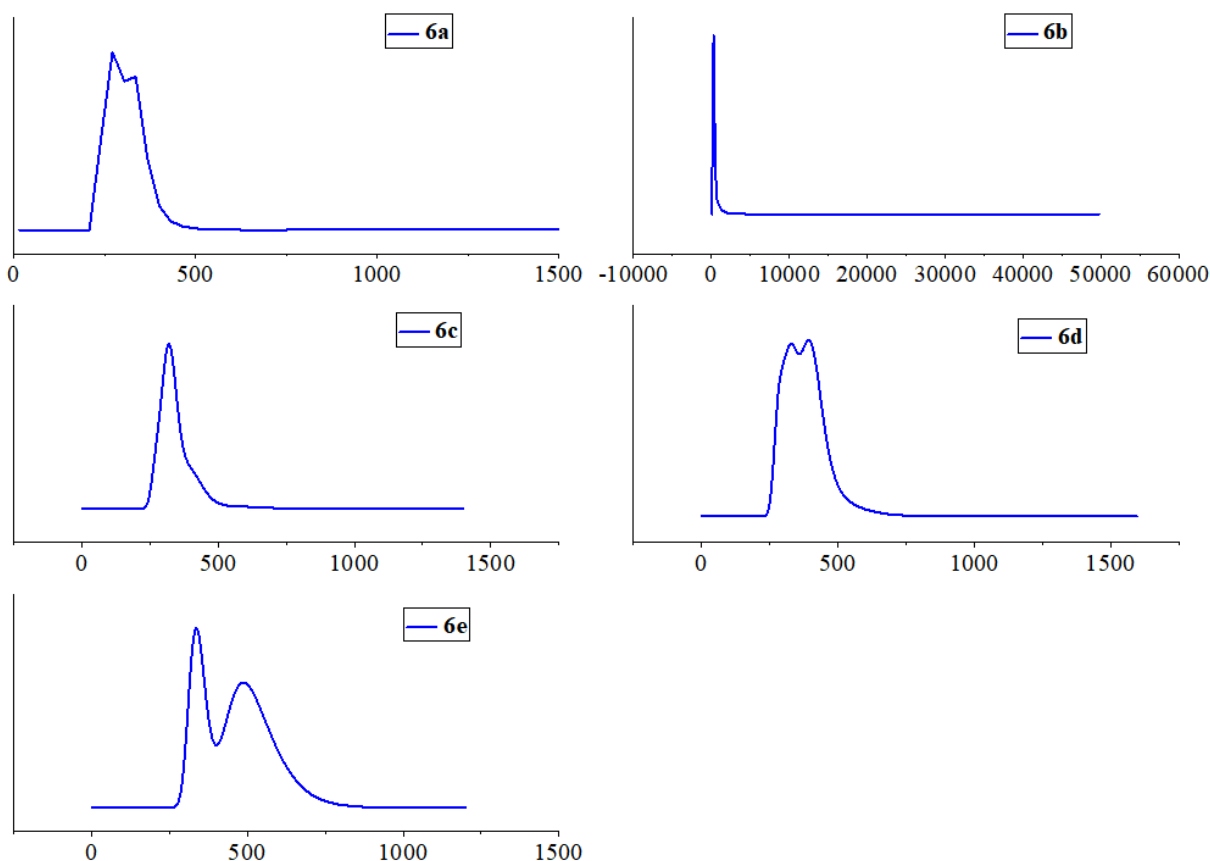


Figure 12: The calculated UV-vis spectra of model structures 6a -6e.

COMPUTATIONAL STUDIES ON N-SALICYLIDENE ALANINE FE (III) COMPLEX IV

Computational analysis on the binding modes between N-Salicylidene alanine Schiff-base ligand and Fe (III) included seven model structures. The seven models were completely optimized and the structures **7a-7g** are shown in figure 13. Models **7a** and **7b** have tetrahedral geometries around the Fe (III) with the fourth ligand being H₂O in **7a** and chlorine in **7b**. Models **7c** and **7d** have a coordination number of five around the Fe (III) center with two H₂O ligands in **7c** and one H₂O and one chloride ligands in **7d**. Models **7e** and **7f** have octahedral geometries around Fe (III) center with two H₂O and one chloride ligands in **7e** and three H₂O ligands in **7f**. Model **7g** also has an octahedral geometry around Fe(III) center with four oxygen donor sites and two nitrogen donor sites from two N-Salicylidene alanine Schiff-base ligands.

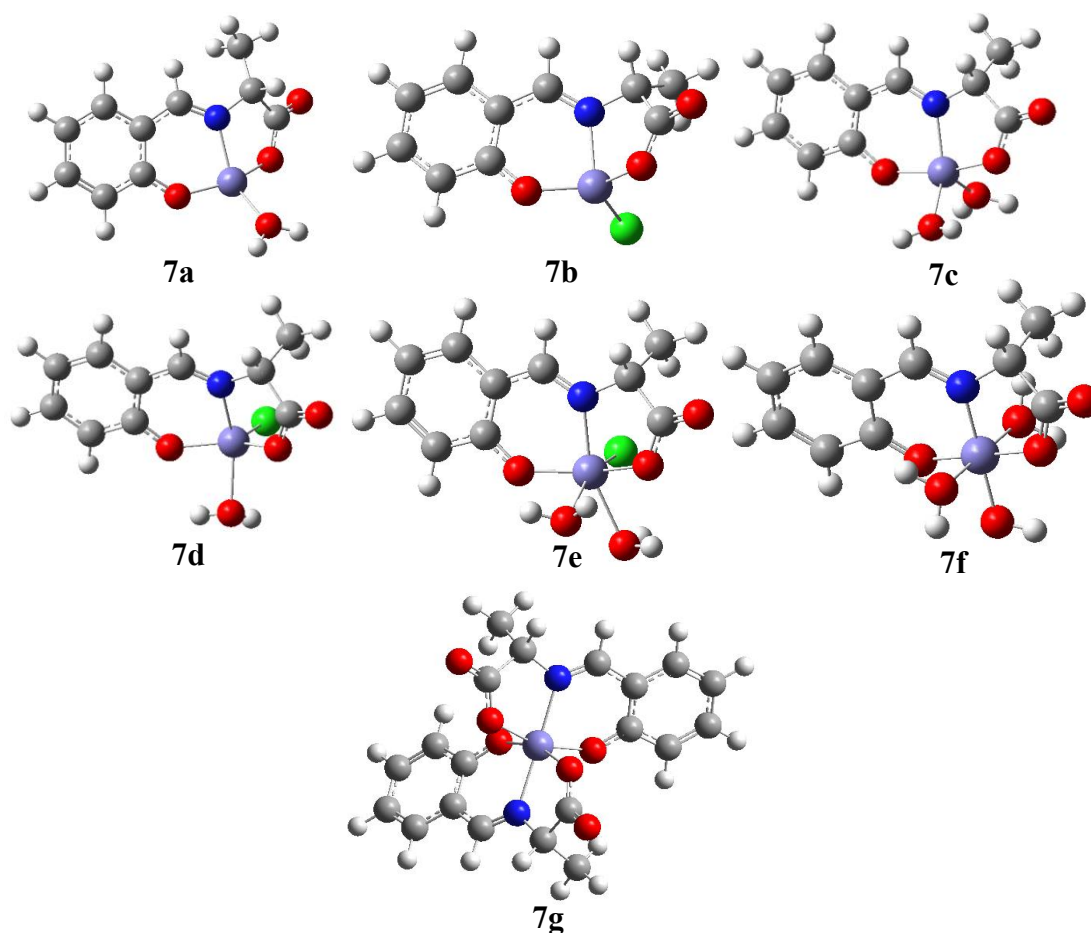


Figure 13: Proposed structural geometries of N-Salicylidene alanine Fe(III) models IV

From computational details (Table S1) and the model structures of N-S-ala-Fe(III) complex IV (figure 13), all the Fe-N bond lengths are longer than the corresponding Fe-O bond lengths. This is probably due to the complexity of the different models of complex IV. For example, in model 7a, the Fe-O1(hydroxyl oxygen) and Fe-O2 (carboxylate oxygen) bond lengths are respectively 1.864 Å and 1.844 Å while the Fe-N bond length is 2.079 Å. The shorter Fe-O bond lengths suggest the stronger binding of oxygen to the Fe(III) metal center. Comparing the models in complex III and complex IV, it is observed that the Fe (II)-N bond lengths in complex III are shorter than the Fe (III)- N bond lengths in complex IV. In addition, the Fe (III)-O bond lengths are longer and weaker than that of Fe (II)-O except when combined with a single water. For example, for model 6a-6e, the Fe(II)-N bond lengths are respectively 1.887 Å, 1.863 Å, 1.897 Å, 1.904 Å, 1.927 Å, while the corresponding Fe(III)-N bond lengths in model 7a-7g are respectively 2.079 Å, 2.056 Å, 2.113 Å, 2.116 Å, and 2.159 Å. Similarly, The Fe(II)-O bond lengths in model 6a are 1.820 Å and 1.835 Å respectively, while the corresponding Fe(III)-O bond lengths in model 7a are 1.864 Å and 1.844 Å. This may be due to the interaction between Fe (II) and a single water and the fact that complex III is neutral.

By comparing the bond angles in complex III and IV, it was observed that the C-O-Fe (II) bond angle in complex III in the same binding mode is smaller than the C-O-Fe (III) bond angle in

complex **IV**. For example, for model **6a-6e** the C1-O1-Fe bond angles are respectively 127.41 °, 126.19 °, 123.58 °, 125.11 °, 125.78 ° while corresponding bond angles in model **7a-7g** are 134.36 °, 131.80 °, 136.23 °, 133.88 °, 132.75 ° respectively. This may be due to the stronger binding between oxygen donor atoms and Fe (III), shorter bond lengths, and increased repulsion between coordinating atoms. On the contrary, the bond angles between O-Fe (II)-N in complex **III** are greater than that of O-Fe (III)-N bond angles in complex **IV**. For example, the bond angles between O1-Fe-N in model **6a-6e** are respectively 96.34 °, 95.80 °, 96.30 °, 94.15 °, 92.49 ° while the O1-Fe-N bond angles in model **7a-7g** are 89.63 °, 90.08 °, 87.23 °, 86.45 °, and 83.84 ° respectively. This may be due to the stronger binding between N-donor atom and Fe (II) compared to the bonding between N-donor atom and Fe (III), resulting in shorter bond lengths and larger bond angles.

Using computation DFT technique, we calculated IR spectra of models **7a - 7g** and the results are shown in figure 14. By comparing the calculated IR spectra of the seven models with the experimental IR spectra of complex **IV** (figure 2), we observe that the profile of calculated IR spectra of **7f** resembles the experimental IR spectra of complex **IV**. The stretching frequency of the peak observed at 1802 cm⁻¹ was assigned to the carbonyl $\nu(\text{C=O})$ stretching of carbonyl functional group, the peak at 1679 cm⁻¹ was assigned to the imine $\nu(\text{-C=N-})$ functional group, the peaks between 480-700 cm⁻¹ were assigned to the rocking vibration modes of water, and the peaks observed around 3755 cm⁻¹ were assigned to symmetric vibration of water. While the absolute values of the calculated IR peaks differ slightly from the experimental IR spectra of complex **IV**, the peaks observed at 500, 1700 and 3800 cm⁻¹ agrees well with the experiment results of complex **IV**.

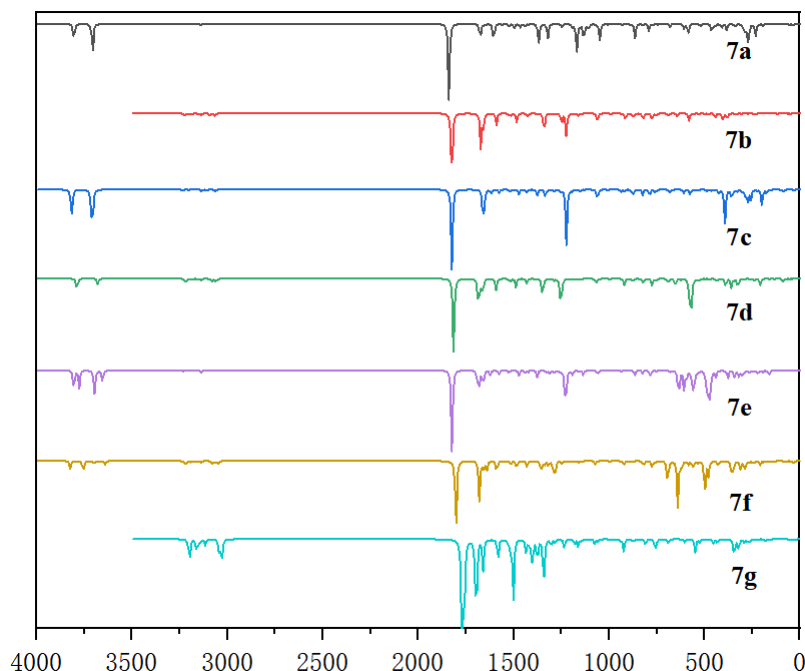


Figure 14: The calculated IR spectra of model structures 7a - 7g.

On the other hand, the calculated UV-vis spectra of models **7a** – **7g** are shown in figure 15. Similarly, the calculated spectra profile of **7f** matches well with the experiment UV-vis spectra of complex **IV**. The prominent peaks observed at 452 nm and 692 nm were assigned to the HOMO-3→LUMO+1, and HOMO-3→LUMO electronic transitions. Based on these modeling IR and UV-Vis results, the most probable structure of complex **IV** is **7f**.

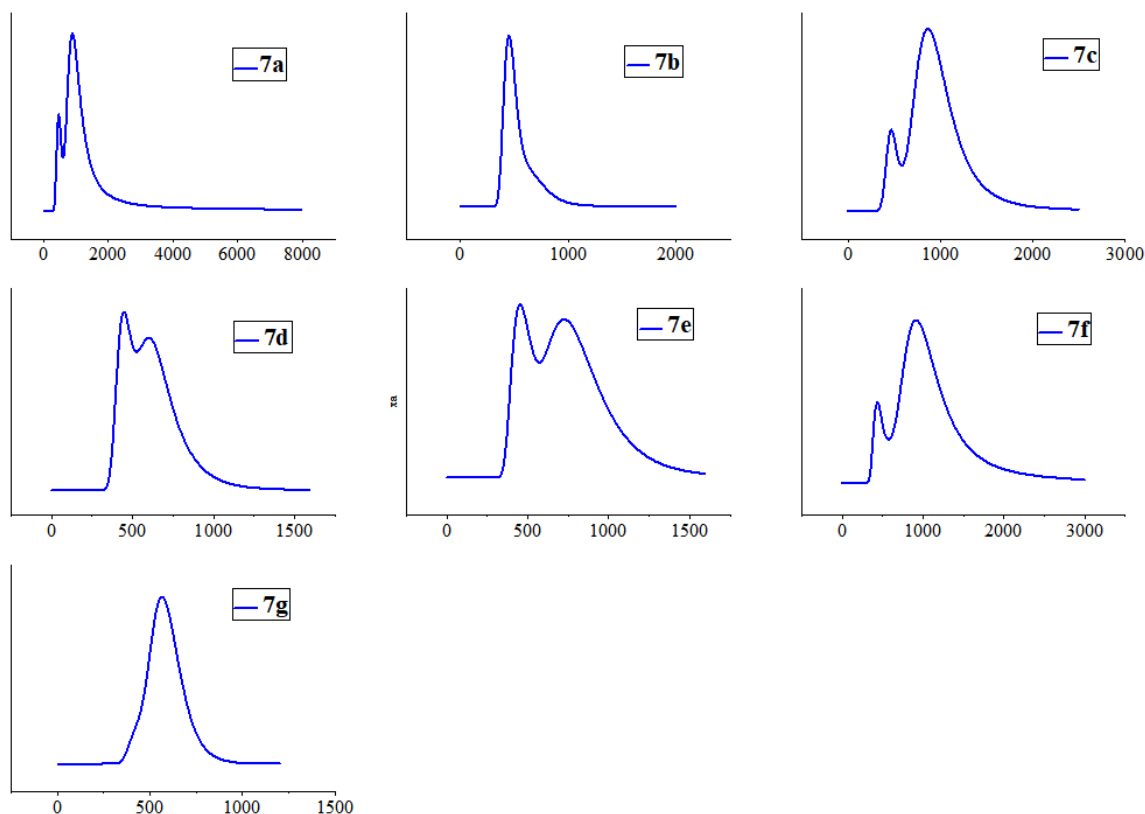


Figure 15: The calculated UV-vis spectra of model structures 7a-7g

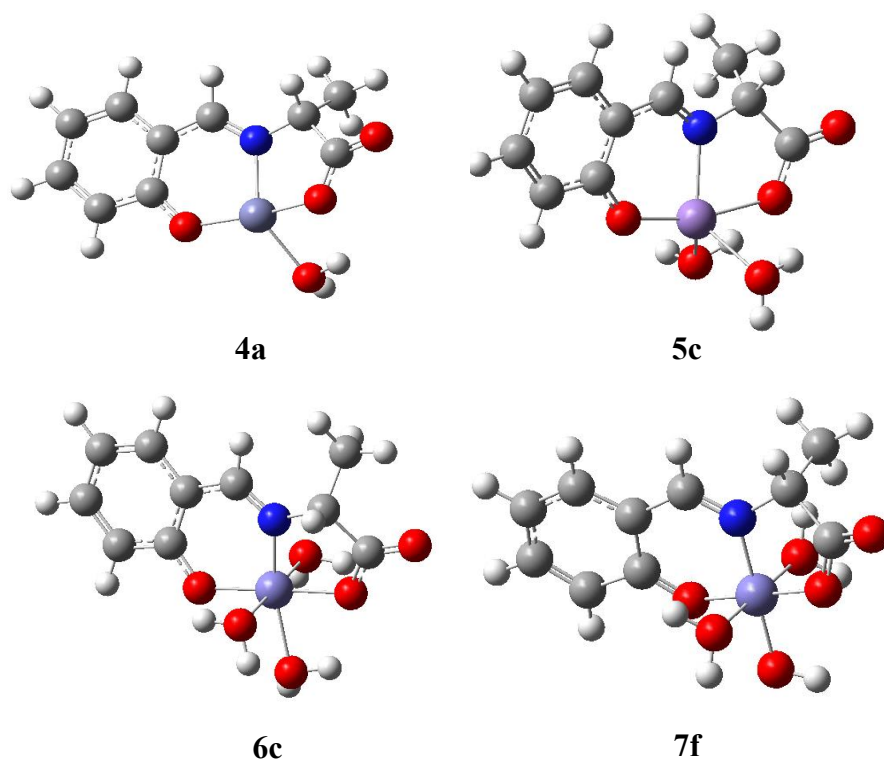


Figure 16: Proposed structure of the complexes

CONCLUSION

Four Schiff-base complexes derived from amino acid alanine and transition metals: N-Salicylidene alanine Zn (II)-complex **I**, N-Salicylidene alanine Mn(II)-complex **II**, N-Salicylidene alanine Fe(II)-complex **III**, and N-Salicylidene alanine Fe(III)-complex **IV** have been synthesized and characterized using basic analytical techniques such as melting point/decomposition temperatures, solubility, FT-IR and UV-Visible spectroscopies. In Addition, the binding sites of the Schiff-base ligands to the transition metal centers were determined using density functional theory (DFT) B3LPY technique and the calculated results compared to the experimental results. All the complexes have different melting points/decomposition temperatures from the amino acid alanine, indicating the formation of new compounds, were soluble in water and other polar solvents with solubility increasing in the warm solvents. This is very crucial in the design and development of new therapeutic drugs. Furthermore, the calculated FT-IR and UV-Visible spectra of the complexes agree with the experimental results. These results were used to determine the binding sites between the Schiff-base ligands and the transition metal centers. Complex **I** adopts a tetrahedral geometry with three oxygen donor sites from deprotonated hydroxyl and carboxylate groups of the Schiff-base ligand, H₂O ligand and imine nitrogen donor atom of the Schiff-base ligand. Similarly, complexes **II**, **III**, and **IV** adopt trigonal bipyramidal, octahedral, and octahedral geometry with two, three, and three H₂O molecules respectively bonded to the central metal atoms. Based on the results, the modeled structures **4a**, **5c**, **6c**, and **7f** respectively correspond to complexes **I**, **II**, **III**, and **IV**. Finally, 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. This is a very important consideration for the design and development of a potential drug candidate.

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