Antimicrobial and *in silico* molecular docking studies of trivalent metal complexes of tridentate Schiff base ligand

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Introduction

In biological processes, inorganic compounds play a crucial role, and it has been established that many organic compounds used in medicine do not have a purely organic mode of action [1-3]. Infectious diseases, which are directly related to bacteria exhibiting multiple resistance to antibiotics, have increased the world's mortality rate, resulting in about 17 million deaths globally per year in children and the elderly.

Therefore, the need to develop novel antibiotics with excellent mechanisms of action has become an urgent biomedical necessity. Schiff base ligands bearing azomethine (>C=N–) functional group, possessing N, O, and S-donor atoms, have been established as versatile pharmacophores for design and development of various biologically active compounds [3-5].

Metal-organic compounds play a critical role in many biological systems, and it has been well-known that various organic compounds utilized in medicine are triggered by metal ion metabolism [2-4]. Oxygen and nitrogen donor Schiff bases transition metal complexes are of interest, because of their ability to adopt an uncommon configuration. Schiff bases are a significant set of organic compounds specially studied owing to their various applications [1]. Tridentate donor ligands formed by heterocyclic Schiff bases have displayed some metal complexes stabilization character, having the coordination to occur via the nitrogen and oxygen-donor atoms [2].

In effort geared towards the development of chemotherapeutic agents with antimicrobial activities, we report the synthesis, characterization of metal(III) complexes of the category: [M(L)(H2O)nCly] obtained from the interaction of metal^{3+} ion salts with organic N, N, O-Schiff base (HL) (n, y = 1 or 2 and M = Cr(III) [**C1**], Ru(III) [**C2**], Fe(III) [**C3**], Al(III) [**C4**], and Ti(III) [**C5**]). Compounds were characterized by CHNS, molar conductance, XRD, UV-Vis, TGA/DTA, FTIR, and NMR. The FT-IR spectral data showed that the ligand HL coordinates to the metal ions via the azomethine group nitrogen atom and hydroxyl group oxygen atom of the o-vanillin moiety, while the M(III) complexes exhibited an octahedral geometry. The XRD analysis showed that the complexes possess crystallite sizes [1,3,5]. The DFT calculations at B3LYP and the LANL2DZ method for the HL and complexes (C1-C5) support the experimental results of the geometric parameters.

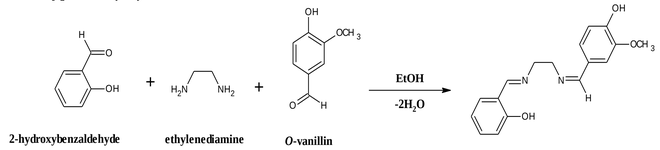
R= H o aryl, alkyl

R^1, R^2 = H, aryl, alkyl

Schiff base structure

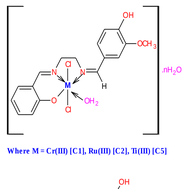
Synthesis of trivalent metal complexes of tridentate Schiff base ligand

Synthesis of the ligand: 4-{(*Z*)-[(2-{(*E*)-[(2-hydroxyphenyl)methylidene]amino}ethyl)imino]methyl}-2-methoxyphenol (HL)

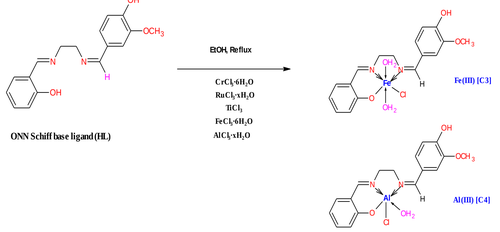


2-hydroxybenzaldehyde ethylenediamine *O*-vanillin

Synthesis of the Schiff base ligand (HL)



Where M = Cr(III) [C1], Ru(III) [C2], Ti(III) [C5]



Synthetic pathway and proposed structures of the Metal(III) complexes of ONN Schiff base ligand (HL)

DFT calculations

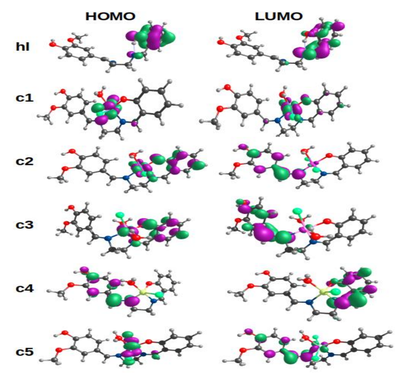
The theoretical calculations including the representations of the frontier orbitals HOMO and LUMO were performed using Gaussian 09 W and Gauss View 6.0 software for the synthesized ligand (**HL**) and its corresponding Cr(III) [**C1**], Ru(III) [**C2**], Fe(III) [**C3**], Al(III) [**C4**], and Ti(III) [**C5**] complexes (**C1-C5**). The molecular morphologies and chemical structures of all the synthesized complexes were optimized using the B3LYP/LANL2DZ scheme in the gas phase[5]

Antimicrobial activity

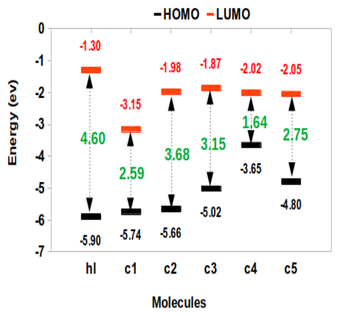
The synthesized ligand (**HL**) and its complexes (**C1-C5**) were screened *vis-a-vis* the disk diffusion plate method. The selected bacterial strains: *Escherichia coli, α-hemolytic streptococcus*, and *Staphylococcus aureus and* fungi, namely: *Aspergillus candideus, Penicillium cephalosporin*, and *Aspergillus niger* [4].

Molecular docking

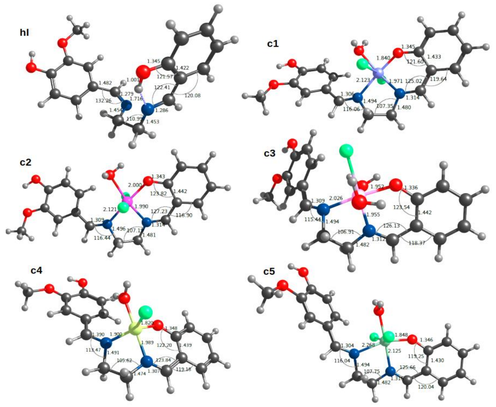
All the molecular docking designs were accomplished using AutoDock 4.2 software and AutoDock Tools ADT. Based on the in vitro results and in an attempt to emphasize the interactions binding the studied molecules to the tested strains, we have docked **HL**, **C2** and **C5** against *Staphylococcus aureus* topoisomerase II DNA gyrase A, *Streptococcus pneumoniae* topoisomerase II DNA gyrase B, and *Escherichia coli* topoisomerase II DNA gyrase B, with the respective PDB IDs: **2XCT**, **5BOD**, and **5L3J** [5,6].



Electronic surface of the HOMO and LUMO for the ligand (HL) and metal complexes (C1-C5)



The HOMO (black), LUMO (red), and the band gap (green) for the ligand HL and the metal complexes (C1-C5)



Optimized structures of the ligand (HL) and metal complexes (C1-C5), showing the selected bond distances and angles

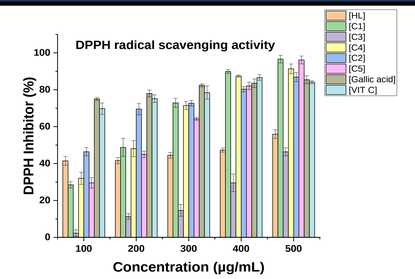
Biological Studies

Antioxidant capacity

Two kinds of free radicals are used for in vitro antioxidant activity studies of the Schiff base ligand (HL) and its trivalent metal complexes (C1-C5) viz DPPH-2,2-diphenyl-1-picrylhydrazyl and ABTS-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Ascorbic acid (vitamin C), Rutin, and Gallic acid were engaged as standard agents [7-9].

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The scavenging potentials of **C1-C5**, and the standards amplified in a dose-dependent fashion (Figure 4). The scavenging potential order could be ranked as: [Gallic acid] > [Vitamin C] > [**C2**] > [**C4**] > [G] > [**C5**] > [**HL**] > [**C3**] with the IC_{50} ± SEM values being respectively 0.84 ± 1.73 > 1.17 ± 1.14 > 1.69 ± 2.68 > 1.98 ± 1.36 > 2.02 ± 1.47 > 2.21 ± 1.75 > 4.11 ± 1.58 > 5.74 ± 1.30 μM [7,8].

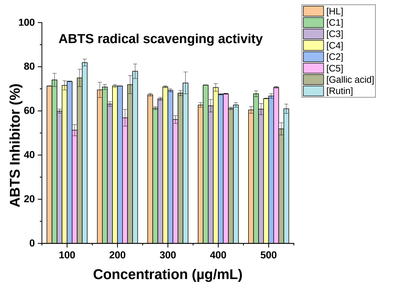


DPPH free radical scavenging assays of HL and its M(III) complexes (n = 3)

ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging assay

The bond interactions between the metal ions and the ligand (**HL**) brought about an increase in spectrum activity as compared to that of the free ligand (15.32 ± 5.3 μM) and a decrease in spectrum activity as compared to that of the standards used [Gallic acid (1.22 ± 1.08 μM), rutin (2.86 ± 0.92 μM)]. The uptake of the ABTS$^+$ radical by **HL** and **C1-C5** was established to possess modest to high activities [7,8].

However, **C5** presented significantly better ABTS scavenging activity with an IC_{50} value of 8.70 ± 2.78 μM and a correlation coefficient of 0.976 R^2, while the remaining complexes **C2**, **C1**, **C3**, and **C4** gave IC_{50} ± SEM values of 8.81 ± 3.43, 10.49 ± 3.24, 12.02 ± 4.60 and 15.01 ± 4.98 μM, respectively. The ABTS rummaging activity arrangement can be hierarchical in the order of: [Gallic acid] > [Rutin] > [**C5**] > [**C2**] > [**C1**] > [**C3**] > [**C4**] > [**HL**] [7,8].



ABTS activities of HL, trivalent metal complexes, and standard drugs (n = 3)

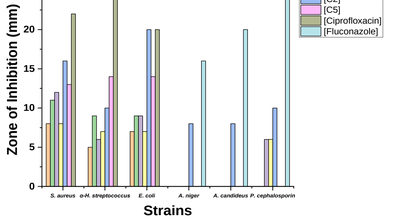
Antimicrobial analysis

The antimicrobial potentials were considered in terms of inhibition zone values (mm) are depicted by a bar graph in Figures. From the data, it can be perceived that the complexes showed a higher zone of inhibition (mm) than the free Schiff base (**HL**). However, the actions of the complexes (**C1-C5**) were found to be lower than that of the standard drug ciprofloxacin.

It is worth noting that the pathogenic isolates were more sensitive (larger zone of inhibition) to [**C2**] than other complexes. The inhibition zones observed for **C2** were *E. coli* (20 mm), *α-H. streptococcus* (10 mm), *S. aureus* (16 mm), *A. candideus* (8 mm), *P. cephalosporin* (10 mm), and *A. niger* (8 mm). The highest antibacterial activity of the studied agents was observed against *S. aureus* and *E. coli*.

Furthermore, (**C2**) displayed the highest antifungal activity against *P. cephalosporin*, with an inhibition zone of 10 mm), whereas complexes (**C3**) and (**C4**) showed moderate antifungal actions (6 mm). The other complexes were found to be inactive against the fungal pathogens. In addition, (**C2**) was observed as the only compound to exhibit moderate antifungal activities against fungal pathogens: *A. niger* and *A. candideus* with an inhibition zone of 8 mm.

From the current study, it is seen that the antibacterial prospective of the complexes can be in the order of: [**C2**] > [**C5**] > [**C1**] > [**C3**] > [**C4**] > **HL** [7-9].



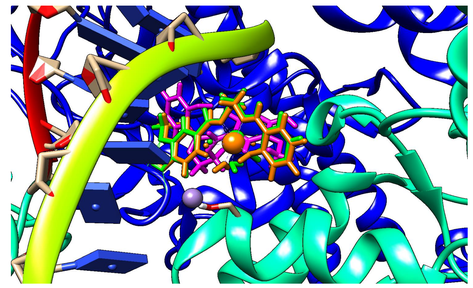
Antimicrobial activity of the Schiff base (HL) and its M(III) complexes

In silico molecular docking studies

The most favorable binding conformation of the ligands (**HL**, **C2**, and **C5**) to the tested targets (**2XCT**, **5BOD**, and **5L3J**) was considered in each case [6]. To emphasize the interactions' categories that might bind the two most effective complexes **C2** and **C5** as well as their free ligand **HL** to the screened bacterial strains, a molecular docking simulation was carried out against selected pathogenic proteins from each species investigated, namely *S. aureus topoisomerase* II DNA gyrase A (PDB ID: **2XCT**), *S. pneumoniae topoisomerase* II DNA gyrase B (PDB ID: **5BOD**) and *E. coli topoisomerase* II DNA gyrase B (PDB ID: **5L3J**).

The docking results showed good binding energies and promising maximum interactions (Table 1). All the free binding energies were found to be negative, in the range of −4.96 to −6.10 kcal/mol for **2XCT**, with **C2** showing the highest value.

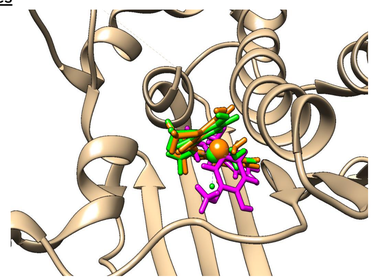
Moreover, the binding energies of the studied compounds in the active sites of the receptors **5BOD** and **5L3J** were found to vary from −5.68 to −7.24 kcal/mol for **5BOD** and from −5.73 to −6.26 kcal/mol for **5L3J**, with **HL** displaying slightly better docking scores than the other studied molecules for both cases. The molecular docking results obtained from this study showed some similarity to other studies reporting Schiff bases and their metal complexes [7-10].



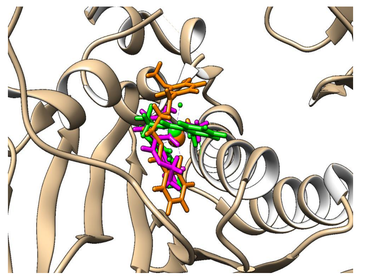
Overlapping of the three studied molecules in the receptor's binding pockets (HL is highlighted in magenta, C2 in green, and C5 in orange)

Table 1. Binding energies of HL ligand and its trivalent metal complexes

Ligand/Receptor	ΔG (kcal/mol)
***Staphylococcus aureus* (PDB ID: 2XCT)**	
HL	– 4.96
C2	– 6.10
C5	– 6.00
***Streptococcus pneumoniae* (PDB ID: 5BOD)**	
HL	– 7.24
C2	– 5.96
C5	– 5.68
***Escherichia coli* (PDB ID: 5L3J)**	
HL	– 6.26
C2	– 6.18
C5	– 5.73



Docking poses' overlap of the complexes' binding pockets built up of the studied molecules (HL, C2, C5) and the target 5BOD (HL is highlighted in magenta, C2 in green, and C5 in orange)



Docking poses' overlap of the complexes' binding pockets built up of the studied molecules (HL, C2, C5) and the target 5BOD (HL is highlighted in magenta, C2 in green, and C5 in orange)

Conclusions

The complexes displayed good to excellent antioxidant and antimicrobial behaviours. Specifically, the antibacterial activities of C2 and C5 are a door opener to future investigation of the potential antibacterial and chemotherapeutic activities of these complexes.

References

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