Research article

**Biological studies of Schiff base, 2-(1-hydroxycyclohexyl) phenyl methylene hydrazine carbothioamide and its Cu(II) and Zn(II) metal complexes**

Prasuna M Kamatam¹, Vanitha Sanambatla¹, Seshiaiah Kalluru²*

¹ Department of Chemistry, INIUA University, Ananthapuram, Andhra Pradesh, India
²Department of Chemistry, SV University, Tirupati, Andhra Pradesh, India

**ABSTRACT**

Condensation of 1-hydroxycyclohexyl phenyl ketone with hydrazine carbothioamide produced a new Schiff base, 2-(1-hydroxycyclohexyl) phenyl methylene hydrazine carbothioamide. The Cu(II) and Zn(II) metal complexes of the Schiff base were prepared. The different spectroscopic methods such as FT-IR, UV-Visible, ¹H NMR, and ¹³C NMR spectroscopy were used to elucidate the structural characteristics of the synthesized compounds. Further, the biological applications of the prepared Schiff bases and its metal complexes were studied for the antibacterial activity. The antimicrobial investigation was carried out against different Gram positive and Gram negative bacteria like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*. According to the findings, the prepared Cu(II) and Zn(II) metal complexes are more biologically active than Schiff base ligand, and among all tested bacterial species, *Staphylococcus aureus* growth was most inhibited by prepared compounds.

**Keywords:** 1-hydroxycyclohexyl phenyl ketone, Hydrazine carbothioamide, Schiff base, Cu (II), Zn (II)

Received - 21-12-2021, Accepted- 24-03-2022

**Correspondence:** Seshiaiah Kalluru* seshaiahsvu@gmail.com

Department of Chemistry, SV University, Tirupati, Andhra Pradesh, India

**INTRODUCTION**

The complexes of Cu(II) and Zn(II) ion containing Schiff bases possess remarkable properties as catalysts in various biological systems, antimicrobial activities, antiviral activities, antifungal activities, anti-inflammatory activities, antiradical activities, insecticidal activities, antitumor and cytotoxic activities. They are also useful in the disciplines of analytical chemistry, agrochemistry, pharmaceuticals, polymers, and dyes. The importance of this metal complexes as essential bioactive chemicals in vitro and in vivo has sparked a surge of interest in these compounds as possible treatment agents for a variety of ailments.

In coordination chemistry, Schiff bases are most frequently utilised as chelating ligands. Schiff bases with donor atoms such as N, O, S, etc. exhibit structural similarities to neutral biological systems and are used in biological systems owing to the existence of an imine group. Thiosemicarbazide and its derivatives as ligands with possible sulphur and nitrogen atoms are interesting and have gotten a lot of attention, not only for their structural chemistry but also for their usefulness in the medical and pharmaceutical fields. Antibacterial, antitumor, antifungal, antiproliferative, antidiabetic, herbicidal, anticancer, anti-inflammatory actions are among the biological activities shown by these compounds. Because they are used as starting materials in the synthesis of commercial goods, Schiff bases are an important family of chemicals. Schiff bases are also considered as favoured ligands because of their capacity to form complexes with various transition metals and serve as catalysts in a variety of processes. The chelating ability of the thiosemicarbazide moiety is good, and this feature can be improved in thiosemicarbazone by inserting a suitable aldehyde or ketone with an additional donor atom to make the ligand polydentate. Anticancer and antibacterial properties have been documented for many Schiff base transition metal complexes. Some medications have been observed to have more action when supplied as metal complexes rather than as free molecules. As a result, Schiff base transition metal complexes may provide an undiscovered source of medicines. Complexes of transition and non-transition metals with Schiff base ligands have recently emerged as promising materials for electronic applications, owing to their excellent photo and electroluminescent properties, as well as their ease of synthesis, which allows for structural modification for material optimization. These are intensively investigated because of their flexibility, selectivity and sensitivity towards the central metal atom, structural and biological similarities, and the inclusion of an imine group (-C≡N-) that confers biological activity.

Owing to its antimicrobial properties of the Schiff bases
and metal complexes, this paper describes the synthesis of Schiff base, 2-(1-hydroxycyclohexyl) phenyl methylene hydrazine carbothioamide and its Cu (II) and Zn (II) complexes. The prepared compounds are characterized by spectral techniques such as FT-IR, $^1$H & $^{13}$C NMR, UV-Visible spectroscopy. The antibacterial activity of all the prepared compounds is screened against the selected bacteria.

MATERIALS AND METHODS

Materials

The starting chemicals such as 1-hydroxycyclohexyl phenyl ketone and hydrazine carbothioamide was acquired from Sigma, India. Solvents like methanol, ethanol and also Cu(II) and Zn(II) metal salts were purchased from Merck, India.

Synthesis of Schiff base, 2-(1-hydroxycyclohexyl) phenyl methylene hydrazine carbothioamide

0.01 moles of 1-hydroxycyclohexyl phenyl ketone was dissolved in 20 ml of ethanol and added 0.01 moles of hydrazine carbothioamide with constant stirring. The reaction mixture was refluxed for 6 hours at 70°C. The resulting mixture was cooled to room temperature, filtered, and then recrystallized. The recrystallized Schiff base was dried in hot air oven.

Synthesis of Cu (II) and Zn (II) metal complexes

The metal salts and ligand were taken in 1:2 molar ratios in ethanol. A solution of the appropriate metal salt (0.001 mmol) [MX$_2$, Where M = Cu (II) (or) Zn (II)] dissolved in 15 ml ethanol was added to a hot ethanolic solution of the ligand (0.002 mmol). The mixture was refluxed for 3 hours at 60°C. The complexes were precipitated by cooling the mixture to room temperature. The mixture was then filtered and washed with ethanol. Finally, the solid was dried in vacuum desiccators.

RESULTS AND DISCUSSION

FT-IR Studies

The functional group that are present in the prepared compounds were identified using the FT-IR spectroscopy (Make: Bruker Alpha). The Figure 2 visualizes the FT-IR spectra of the prepared Schiff base and its metal complexes. The dual band in the range of 3150 cm$^{-1}$ and 3300 cm$^{-1}$ can be assigned to the -N-H stretching present in the compound. The aromatic and aliphatic C-H stretching bands are so weak at 3056 cm$^{-1}$ and 2914 cm$^{-1}$. The important imine group stretching was seen at 1620 cm$^{-1}$. The band at 1485 cm$^{-1}$ can assigned to C=S stretching in the compound. The aromatic C=C stretching was seen at 1445 cm$^{-1}$. The FT-IR data confirms the successful preparation of Schiff base. In the case of metal complexes, all the notable functional groups stretching frequencies are lowered which indicates the coordination with metal atom and also the intensities are also decreased.
**1H NMR Studies**

The NMR spectroscopy (Model: Bruker Biospin Gmbh) is a powerful tool to investigate the structure of the organic compound. The $^1$H NMR spectra of prepared Schiff base was recorded and given in the Figure 4. The hydrogens of the non-aromatic cyclohexyl ring were seen in the range of 1.0 ppm to 2.6 ppm. The hydrogen atom of the hydroxy group was seen at 3.7 ppm. The aromatic hydrogens were seen at 6.8 ppm and 7.0 ppm. Finally, the hydrogens of the hydrazine carbothioamide were seen at 7.8 ppm and 8.2 ppm. The proton NMR data was aligned with the Schiff base structure and confirms the successful synthesis of Schiff base from corresponding ketone and amine.

**13C NMR Studies**

The carbon NMR spectroscopy (Model: JEOL 400 MHz Spectrometer) is an important tool to know about the carbon atoms structure in the compound. The $^{13}$C NMR spectra was recorded for the Schiff base and given in the Figure 3. The peaks in the 20 ppm, 32 ppm, 68 ppm region were assigned to the cyclohexyl fragment. The aromatic carbons are seen in the range from 128 ppm to 138 ppm. The most important imine group carbon was seen at 157 ppm. The peak at 178 ppm can assign to carbon atom of the thioamide group. By this data, the carbon NMR spectra insights the successful synthesis of Schiff base.
UV-Visible Studies

The UV-Visible or electronic spectra (Model: Carey UV-Vis Spectrometer) was used to elucidate different electronic transitions in the prepared compounds. The absorption bands in the range 265 nm and 289 nm can be assigned to the more energy band, \( \pi - \pi^* \) and low energy band, \( n - \pi^* \) of the Schiff base.

**Figure 5:** UV-Visible spectral image of Schiff base, Cu(II) and Zn(II) complexes

The aromatic and imine transitions are overlapped and visualized as a broad shoulder band in the spectrum. In the case of metal complexes, the above said transition bands are subjected to bathochromic shift which indicates the successful chelation with metal ions. And additional band present in the spectrum of Cu (II) complexes may be due to d-d transitions. In the case of Zn (II) complexes the absence of band in that region due to \( d^{10} \) configuration of zinc and d-d transition is not possible.

Antibacterial Studies

Certain Gram positive (Staphylococcus aureus and Bacillus subtilis) and gram negative (Escherichia coli and Pseudomonas aeruginosa) were screened against the prepared Schiff base and its Cu(II) and Zn(II) complexes for their antibacterial activity by Agar paper disc method. Zone of inhibition values are given in mm against all the four tested bacteria. Streptomycin was taken as a standard drug for calculating the zone of inhibition values. All the prepared compounds and standard drug were dissolved in DMSO. From the inhibition experimental values, it was noted that the metal complexes showed higher activity compared to Schiff base on the bacteria. The Cu(II) and Zn(II) are more active than the bare Schiff base and having good antibacterial activity. So, the Cu(II) and Zn(II) metal complexes are more active in the antibacterial activity and reached good values of inhibition of growth of bacteria. Among the all prepared compounds, the Cu(II) complex exhibited highest antibacterial activity.

**Table 1:** Antibacterial activity of Schiff base and Cu (II) and Zn (II) metal complexes

| Compounds       | Gram’s positive bacteria | Gram’s negative bacteria |
|-----------------|--------------------------|--------------------------|
|                 | Bacillus subtilis | Staphylococcus aureus | Escherichia coli | Pseudomonas aeruginosa |
| Schiff base     | 0.7                    | 0.7                      | 0.8             | 0.6                     |
| Cu(II) complex  | 0.8                    | 2.2                      | 2.6             | 1.7                     |
| Zn(II) complex  | 1.2                    | 2.0                      | 2.1             | 1.8                     |
| Streptomycin (Standard Drug) | 1.7         | 2.8                      | 3.4             | 2.3                     |

**Figure 6:** Antibacterial activities of Schiff base, Cu (II) and Zn (II) complexes
CONCLUSIONS
A new Schiff base, 2-(1-hydroxycyclohexyl) phenyl methylene hydrazine carbothioamide and its Cu(II) and Zn(II) metal complexes were synthesized and biological applications of the synthesized compounds like antibacterial activity was investigated in the current study. The characterization was carried out using different instrumental methods such as FT-IR, UV-Visible, $^1$H NMR, and $^{13}$C NMR spectroscopy. Some common Gram positive and negative bacteria (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) was selected for screening antibacterial activity of the prepared Schiff base and its metal complexes. From the antibacterial experiments, the Cu(II) and Zn(II) complexes having the good zone of inhibition values whereas bare Schiff base was less active against all the bacteria. Among the prepared compounds, Schiff base Cu(II) complex was more active towards the all tested bacterial species.

REFERENCES
1. Gou Y, Li J, Fan B, 2017. Structure and biological properties of mixed-ligand Cu(II) Schiff base complexes as potential anticancer agents. Eur. J. Med. Chem. 134, Pages- 207-217.
2. Galini M, Salehi M, Kubicki M, 2017. Structural characterization and electrochemical studies of Co(II), Zn(II), Ni(II) and Cu(II) Schiff base complexes derived from 2-(E)-(2-methoxyphenylimino)methyl)-4-bromophenol; Evaluation of antioxidant and antibacterial properties. Inorganica Chim. Acta. 461, Pages- 167-173.
3. Saif M, El-Shafiy HF, Mashaly MM, 2016. Synthesis, characterization, and antioxidant/cytotoxic activity of new chromone Schiff base nano-complexes of Zn(II), Cu(II), Ni(II) and Co(II). J. Mol. Struct. 1118, Pages- 75-82.
4. Joce MV, Metilda P, 2021. Synthesis, characterization and biological applications of curcumin-lysine based Schiff base and its metal complexes. J. Coord. Chem. 74, Pages- 2395-2406.
5. Palaniammal A, Vedanayaki S, 2021. Synthesis, spectroscopic, anti-microbial, anti-cancer and DNA cleavage of mono nuclear copper and cobalt (II) complexes with thiosemicarbazone ligand. J. Mol. Struct. 1157, Pages- 381-394.
6. Al-Amiery AA, Al-Majedy YK, Ibrahim HH, 2012. Catalytic activity of Schiff base complexes in oxidation of cyclohexane. J. Mol. Liq. 240, Pages- 486-496.
7. Sakthivel RV, Sankudevan P, Vennila P, 2021. Experimental and theoretical analysis of molecular structure, vibrational spectra and biological properties of the new Co(II), Ni(II) and Cu(II) Schiff base metal complexes. J. Mol. Struct. 1233, 130097.
8. Assey GE, Mgohamwende R, 2020. A review of Titanium, Vanadium and Chromium transition metal Schiff base complexes with biological and catalytic activities. Pharm. Pharmacol. Int. J. 8, Pages- 136-146.
9. Johannes K, Ryjul WS, Seth MC, 2021. Metal complexes for therapeutic applications, Trends Chem. 3, Pages- 523-534.
10. Kostova I, Saso L, 2013. Advances in research of Schiff-base metal complexes as potent antioxidants. Curr. Med. Chem. 20, Pages- 4609-4632.
11. Ghanghas P, Choudhary A, Kumar D, 2021. Coordination metal complexes with Schiff bases: Useful pharmacophores with comprehensive biological applications. Inorg. Chem. Commun. 130, 108710.
12. More MS, Joshi PG, Mishra YK, 2019. Metal complexes driven from Schiff bases and semicarbazones for biomedical and allied applications: a review. Mater. Today Chem. 14, 100195.
13. Ritter E, Przybylisky P, Brzezinski B, 2009. Schiff Bases in Biological Systems, Curr. Org. Chem. 13, Pages- 241-249.
14. Xu Y, Shi Y, Lei F, 2020. A novel and green cellulose-based Schiff base-Cu (II) complex and its excellent antibacterial activity. Carbohydr. Polym. 230, 115671.
photoluminescent and magnetic properties of complexes of zinc(II) and copper(II) with Schiff-base ligands derived from 2,6-diacetylpyridine. Polyhedron 138, Pages- 225-231.

28. Omidi S, Kakanejadifard A, 2020. A review on biological activities of Schiff base, hydrazone, and oxime derivatives of curcumin. RSC Adv, 10, Pages- 30186-30202.

29. Arunadevi A, Raman N, 2020. Biological response of Schiff base metal complexes incorporating amino acids – a short review. J. Coord, Chem. 73, Pages- 2095- 2116.

30. Liu X, Hamon J, 2019. Recent developments in penta-, hexa- and heptadentate Schiff base ligands and their metal complexes. Coord, Chem. Rev. 389, Pages- 94-118.