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RESEARCH ARTICLE

In Vitro Evaluation of Antifungal Activity of Synthesized Novel Zn(II) Complex with Schiff base Ligand

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Abstract

Objectives: The current study is concerned with creating a Zn(II) complex with a novel schiff base ligand and to evaluate the Zn(II) metal complex's in-vitro antifungal efficacy against *Candida albicans, Candida glabrata, Candida krusei*, and *Candida tropicali*. **Methods:** N, N'-bis(2-hydroxy-3-methoxybenzaldehyde)-2-phenyl-1,3-propanediamine was synthesized by using 2-hydroxy-3-methoxybenzaldehyde and 2-phenyl-1,3-propanediamine. Metal precursor $Zn(OAc)_2.2H_2O$ react with the ligand to form Zn(II) metal complex. The structure of the synthesis complex was confirmed by elemental analysis i.e. 1H-NMR, IR and MS spectroscopy. **Findings :** Zn(II) schiff base complexes have unique characteristics and unusual reactivity, which operate as a foundation for the development of complex molecules with potential use in a wide range of applications. One of the most typical fungal infections is candidiasis. The findings suggest that the Zn(II) metal complex could be a promising antifungal agent. **Novelty:** Characterization and unique properties of the newly synthesized zinc complex and its potential as a novel class of antifungal agent.

Keywords: Schiff Base; N; N'bis(2hydroxy3methoxybenzaldehyde)2phenyl1; 3propanediamine; Antifungal; Candidiasis; Candida

1 Introduction

Due to the physicochemical, catalytic, photochromic, biological, and medicinal properties, transition metals became more intriguing with the advent of coordination chemistry by being given lone pairs from electron-rich nitrogen and/or oxygen atoms [(1)]. The azomethine-containing species produce a superior class of ligands that are unrivalled in their variety, similarity to naturally occurring biological molecules, and relevance to metabolic processes occurring in biological systems [(2)]. So many researchers [(3–6)] collectively emphasize the paramount importance and versatile applications of N2O2 Schiff base ligands and their corresponding metal complexes in the fields of coordination chemistry and theoretical studies. These ligands serve as essential building blocks for constructing diverse transition metal complexes, enabling precise control over coordination geometries and electronic properties, which in turn,

holds significant implications for catalysis, materials science, and the development of functional materials. Additionally, the integration of spectroscopic and computational investigations enhances our understanding of these complexes' structural and electronic features, underscoring their enduring relevance in advancing our knowledge of coordination chemistry and theoretical chemistry, with far-reaching implications for various scientific and technological domains.

Ni(II), Cu(II), and Zn(II) Schiff bases complexes have unique characteristics and unusual reactivity that serve as a foundation for the development of complex molecules with potential applications in numerous industries [7,8]. These novel and peculiar features open a fresh range for research into their potential biological, analytical, and medicinal applications [9]. Numerous Ni(II), Cu(II), and Zn(II) Schiff base complexes are utilised as anti-inflammatory, antipyretic, chemosensor, anti-diabetic [10], anti-bacterial [11], anti-cancer, and anti-HIV medications [12](human immunodeficiency virus).

Many researchers have been synthesized metal complexes with Schiff base ligands and tested for antibacterial activity [13–16]. The significant antibacterial activity displayed by these metal complexes. This finding underscores the potential of Schiff base ligands and their respective metal complexes i.e. Zr(IV), Cu(II) Ni(II) and Zn(II) as promising candidates for combating bacterial infections. The antibacterial properties observed in these compounds hold considerable importance in addressing the global challenge of antibiotic resistance and emphasize their potential utility as novel antimicrobial agents, bridging the fields of coordination chemistry and biomedical research for the development of effective antibacterial therapies.

A type of yeast called Candida can be found in minute amounts on the skin, in the stomach, and in the mouth of people [17,18]. It can result in a dangerous infection if it expands rapidly. Candidiasis, a disease mostly brought on by *Candida albicans, Candida krusei, Candida glabrata*, and *Candida tropicalis*, is a condition brought on by these yeasts. There are several issues with managing Candida infections, including the scarcity of powerful anticandidal medications, their toxicity, and their high price. Additionally, the extended use of these medications has resulted in an increase in the incidence of Candida species strains that are resistant to medicines. The creation of novel and potent antifungal agents is urgently required due to the challenges involved in the treatment of candidal infections. A number of studies have been conducted on activity of Zn(II) metal complexes on various microorganisms. However, there are limited number of studies on effect of Zn(II) metal complexes with different ligands over Candida species and whatever results were obtained were less effective. Joseyphus and Nair in 2008 conducted research in which analyzed the effect of synthesized Schiff base ligand and Zn(II) Schiff base complexes on fungi i.e. *C. albicans*. The results of the ligands show moderate activity on *C. albicans* species while Zn(II) complexes shows less activity against *C. albicans*. In another study done in 2006 by Chohan et al. examined how *C. albicans* and *C. glabrata*, two fungi, were affected by synthesised Schiff base ligands and Zn(II) Schiff base complexes. Ligand and related Zn(II) metal complexes showed little efficacy against *C. albicans* and *C. glabrata* when the findings were compared to those obtained with the common medications miconazole and amphotericin B. In a study done in 2005, Osowole et al. examined the impact on fungi like *C. albicans* of synthesised Schiff base ligand and Zn(II) Schiff base complexes. The ligand and associated Zn(II) metal complex exhibited no effect on *C. albicans*. Hence, the current study reports the preparation and antifungal activity of novel Zn(II) metal complex using agar well diffusion method, possessing interesting biological activity against Candida species. A novel antifungal medication is developed in the current research work, which concentrated on the synthesis and structural elucidation of schiff base complexes with Zn(II).

2 Methodology

2.1 General experimental procedures

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The 1H-NMR spectrum were recorded in $CDCl_3$andDMSO-d6 at 400 MHz on a Brucker NMR instrument, using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon /Xenon as FAB gas.

2.2 Synthesis of the schiff base ligand

Ethanolic (25 mL) solution of 2-hydroxy-3-methoxybenzaldehyde (2 mmol) was added an ethanolic (25 mL) solution of the 2-phenyl-1,3-propanediamine (1 mmol). The reaction mixture was refluxed for 3.0 hrs [19]. The resulting solution was cooled to room temperature and the precipitates obtained were collected by suction filtration and washed with cold ethanol to afford the desired Schiff base [N,N'-bis(2-hydroxy-3-methoxybenzaldehyde)-2-phenyl-1,3-propanediamine].

Analysis: IR (KBr, cm^{-1}): 3455(-OH), 3064 (Ar., =CH), 2943 (-CH), 1631(-HC=N), 1166 (C-O). 1H NMR ($CHCl_3$, δppm): 14.52 (s, 2H, Two –OH group), 8.27 (s, 2H, Two –CH=N group), 3.92 (s, 6H, Two –OCH_3 group), 6.14 (s, 1H, -CH-N), 6.63-7.22 (m. 11H).MS(m/z): 390 (M+), 359, 328, 311, 294 etc. Calculated formula $C_{23}H_{22}N_2O_4$. Yield: 81 %.

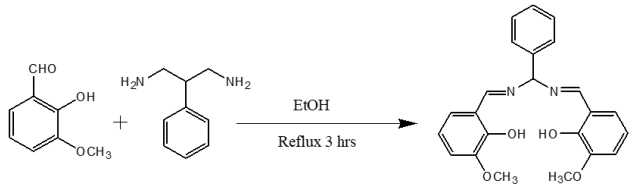

Fig 1. Synthesis of Ligand

2.3 General procedure for the synthesis of the complex

In order to synthesize the zinc(II) complexes [Zn(L)], the metal precursor $Zn(OAc)_2.2H_2O$ (0.220 g, 1 mmol) was added to a hot methanolic solution (30 mL) of the corresponding ligand [20] (1.0 mmol). The resultant mixture was refluxed for 3 h and then it was filtered, washed thoroughly with methanol and dried.

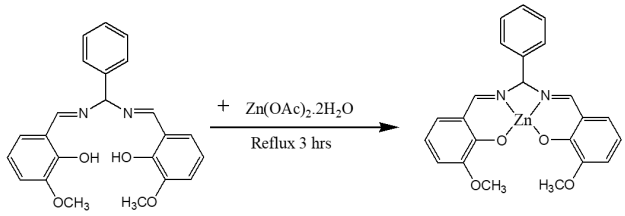

Fig 2. Synthesis of Metal Complex

Analysis: IR (KBr, cm^{-1}): 3062 (Ar., =CH), 2924 (-CH), 1620 (-HC=N), 1298 (C-O), 542 (M-N), 410 (M-O). 1H NMR (DMSO-d6, δppm): 8.32 (s, 2H, Two –CH=N group), 3.97 (s, 6H, Two $–OCH_3$ group), 6.18 (s, 1H, -CH-N), 6.67-7.38 (m. 11H). Calculated formula $C_{23}H_{20}N_2O_4Zn$. Yield: 68 %, Yellow in colour, The complex is more likely to dissolve and remain stable in aqueous solutions.

2.4 Determination of Antifungal Assay

In this experiment agar well diffusion method is used because it is relatively simple and straightforward to perform. It can be adapted to test a wide range of compounds, including metal complexes, and different microorganisms. It allows for the simultaneous screening of multiple compounds on a single plate. The agar well diffusion method provides qualitative or quantitative results by measuring the diameter of the inhibition zones around the wells. Larger inhibition zones generally indicate higher antimicrobial activity.

By using the agar well diffusion method, the experimental plant's antifungal activity was examined against *C. albicans, C. glabrata, C. Krusei*, and *C. tropicalis* [21]. These yeasts were subcultured onto Sabouraud's dextrose agar (SDA) from Merck (Germany), and they were then incubated at different temperatures for 24 hours and 2–5 days, respectively. The concentration of the fungal spore suspensions in sterile PBS was set at 106 cells/ml. rolling on the agar medium's surface after dipping a sterile swab into the fungus suspension. The plates were dried for 15 minutes at room temperature. Using a sterile glass tube, 10 mm-diameter wells spaced roughly 7mm apart were punched in the culture media. Each well-received 0.1 ml of various diluted fresh extracts until the point of saturation. At 37°C, plates were incubated. Bioactivities were measured by measuring the diameter of the inhibitory zone after 24-hour incubation (in mm). Means were computed for each experiment, which was carried out in triplicate.

3 Results and Discussion

3.1 Characterization of ligand

The mass spectrum of this ligand showed a molecular ion peak at m/z 390 [M+] with other important peak at 359, 328, 311, 294 etc. The molecular formula was determined as $C_{23}H_{22}N_2O_4$ with the help of 1H-NMR & elemental investigation. IR spectrum

Table 1. Antifungal activity of Zn(II)complex

	Zone of inhibition (mm)			
Tested Drug (Synthesized Metal Complex)	***C. albicans***	***C. glabrata***	***C. krusei***	***C. tropicalis***
TD1 (20μL/well)	19	21	18	17
TD2 (40μL/well)	23	24	21	19
TD3 (60μL/well)	26	27	23	21
TD4 (80μL/well)	32	28	25	24
Itraconazole (20μL/well)	30	28	26	25

TD1: 20μL/well Synthesized Metal Complex was used; TD2: 40μL/well Synthesized Metal Complex was used; TD3: 60μL/well Synthesized Metal Complex was used; TD4: 80μL/well Synthesized Metal Complex was used.

(cm^{-1}, KBr) of ligand confirmed the presence of –OH group by showing absorption at 3455 cm^{-1}. The presence of aromatic =CH group was established by the absorption at 3064 cm^{-1}. The absorption at 2943 cm^{-1} and 1631 cm^{-1} indicated the presence of –CH and -CH=N group respectively.The 1HNMR spectrum ($CDCl_3$, δppm) showed a sharp singlet at 14.52 (s, 2H, Two –OH group) which indicated the presence of –OH groups. One another singlet was observed at 3.92 (s, 6H, Two -OCH_3) for six protons, were ascertained to two methoxy groups at position C3and C3'. Two another singlets were observed at 8.27 (s, 2H, Two –CH=N group) and 6.14 (s, 1H, -CH-N) which indicates the presence of –CH=N- and -CH-N= respectively. A multiplet peak was observed between δ 6.63-7.22 (m, 11H, Ar-H) for remaining eleven aromatic hydrogen. On the basis of above observation and discussion this ligand was identified as N,N'-bis(2-hydroxy-3-methoxybenzaldehyde)-2-phenyl-1,3-propanediamine.

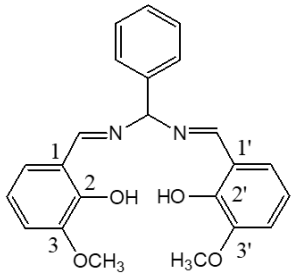

Fig 3. N, N'-bis(2-hydroxy-3-methoxybenzaldehyde)-2-phenyl-1,3-propanediamine

3.2 Characterization of Zn(II complex)

The comparison between the selected bands in the FT-IR spectra of Schiff base ligand and its metal complexwas analyzed. In the FT-IR spectra of complexes, the ν (HC=N) and ν (C–O) bands shifted to lower and higher wavenumbers, respectively, in comparison with their corresponding free ligand thereby indicating a coordinative interaction between the iminic nitrogen and phenolic oxygen atoms with central metals. The iminic nitrogen and phenolic oxygen coordination could also be confirmed by the appearance of weak bands located at the low wavenumbers which are assigned to (M–N) and (M–O) at 542 cm^{-1} and 410 cm^{-1} respectively. Hydroxyl group peak also absent in Zn(II) complex.1H NMR spectra of the ligand was recorded in deuterated chloroform ($CDCl_3$), while the spectra of the complexes were recorded in DMSO-d6. In the 1H NMR spectra of the ligand signal at δ = 14.52 ppm, which corresponds to the phenolic (-OH)group is disappeared in the spectra of the zinc complex. This clearly indicates that the Schiff base ligand is coordinated as an anionic ligand to the Zn(II) ions. In the 1H NMR spectra of the complex, the signal at 8.32is attributed to the H–C=N- proton of imine, for zinc complex. There is a slight shift of the imine proton to down field in the obtained 1H NMR spectra of the zinc complex compared to the ligand one. This shift confirms the coordination of the phenolic oxygen and imine nitrogen to metal ions. The aromatic eleven protons of the Zn(II) complex appear in the range of 6.72–7.38 (m, 11H, Ar-H) as multiplet peak. Based on these observed chemical shift difference, Zn(II) complex with the ligand [N,N'-bis(2-hydroxy-3-methoxybenzaldehyde)-2-phenyl-1,3-propanediamine] is confirmed and assumed that coordination number of Zn in this complex is six [22].

3.3 Antifungal Activity

Results from the synthetic Zn(II) metal complex were significant. According to the study's findings, the compounds prevents the growth of *Candida albicans, Candida glabrata, Candida krusei*, and *Candida tropicalis*. Itraconazole used as the reference point for measuring antifungal activity. Itraconazole inhibited *C. albicans, C. glabrata, C. krusei*, and *C. tropicalis* with zones of inhibition measuring 30mm, 28mm, 26mm, and 25mm, respectively. Compared to the control standard (30mm zone of inhibition), sample TD4 demonstrated superior antifungal efficacy against *C. albicans*. While samples TD1, TD2, and TD3 had zones of inhibition for the same fungi that were 19mm, 23mm, and 26mm, respectively, less effective against fungi than the control standard. In comparison to the control standard (28mm zone of inhibition), sample TD4 demonstrated comparable antifungal efficacy against *C. glabrata*. While the antifungal activity of samples TD1, TD2, and TD3 was smaller than that of the control standard, with a zone of inhibition of the same fungi of 21mm, 24mm, and 27mm, respectively. In comparison to the control standard, all other samples TD1 to TD4 displayed decreased antifungal activity against *C. krusei* and *C. tropicalis*.

4 Conclusion

The synthesized complex was characterized using various physico and spectrochemical techniques. The results showed that the stoichiometry in complexes is 1:1 (ligand:metal). The coordination sites are azomethine nitrogens and phenolic oxygens as evident from 1H-NMR and FT-IR. The zinc complex the apical sites were involved in coordination with water molecules to increase the coordination number from four to six to make distorted octahedral geometry instead of square planar around the metal atom. Comparatively, these outcomes are in line with previous research [(23–25)] in the field of Schiff base complexes, as seen in the referenced papers. Sample TD4 showed greater antifungal activity against *C. albicans* as compared to the control standard (30mm zone of inhibition). Zones of inhibition for the same fungus were 19 mm, 23 mm, and 26 mm, smaller for samples TD1, TD2, and TD3, respectively, than for the control standard. New Metal complexes reported here represent a new class of antifungal which might induce strong in-vitro antifungal effect on Candida species and hence they are comparatively more active than control.

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