

Spectral, Magnetic and Biological Studies of Zn^{II} Complexes of Schiff Bases Derived From 4-(*O*-Methoxyphenyl)-2-Aminothiazole

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ABSTRACT

In the present Zn^{II} complexes of Schiff bases (SB-1 to SB-7) derived from 4-(*o*-methoxyphenyl)-2-aminothiazole and R substituted salicylaldehyde (R = H, 3-Me, 4-Me, 5-Me, 3-OMe and 5-Br) and 2-hydroxy-1-naphthaldehyde *o*-hydroxyaldehydes were synthesized, characterized and tested for the bioactivity against Gram positive and Gram negative group of organisms. The as-synthesized complexes were characterized by elemental, spectral and spectroscopic methods for measuring magnetic susceptibility, conductivity and assayed for biological activities. The complexes showed 1:2 metal : ligand stoichiometry (ML₂) and an octahedral geometry. The complexes showed an antibacterial activity against *Bacillus subtilis* 2063 and *Escherichia coli* 2931, and antifungal activity by preventing the dimorphic switching in *Candida albicans*.

INTRODUCTION

The methods to treat the emerging diseases and the increasing number of multidrug resistant microbial pathogens have in the medical community (Desai *et al.*, 2013; Muroi *et al.*, 2004; Pfeltz and Wilkinson, 2004; Roberts, 2004; Tenover and McDonald, 2005) have called for the search for new bioactive compounds that will effectively inhibit these pathogens. In recent years, literature review shows that Schiff bases have shown promising applications in medicinal and pharmaceutical (Anand *et al.*, 2012). This could happened because the Schiff bases act as good ligand and provide many potential binding sites for complexation of diverse metal ions like Cu (II), Co(II), Ni(II), or Zn(II). Therefore, Schiff bases have been used as chelating ligands in coordination chemistry (Kashyap *et al.*, 2012; More *et al.*, 2014; More *et al.*, 2013). The metal ion in such complexes can be coordinated by the imine nitrogen atom and also by the other active centers present in the molecule. This can lead to many interesting catalytic and potential properties.

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The metal complexes in Schiff can modify both magnitude and direction of the pharmacological activity of the initial organic compounds (ligands) as a result of changes in their size, shape, charge density distribution, and redox potential (Patel, 2010; Wu *et al.*, 2011).

Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases are generally excellent chelating agents, especially when a functional group like -OH or -SH is present close to the azomethine group so as to form a five or six membered ring with the metal ion. Although numerous reports are available on the biological applications of the Schiff bases and its derivatives, particular antibacterial (Anand *et al.*, 2012), reports on the use of Schiff bases as the inhibitors of dimorphic switching in *Candida albicans* is yet not reported. *C. albicans* is an important opportunistic pathogens, which causes. *C. albicans* is an opportunistic fungal pathogen mainly causing infections among immunocompromised individuals (Buchheidt *et al.*, 2000), leading to superficial mycoses to life threatening systemic candidiasis.

Generally, the infections associated with *C. albicans* are ascribed to the capability of this fungus to switch between unicellular budding yeast to multicellular, filamentous mycelial or hyphal form (Felk *et al.*, 2002).

Several studies in the past have reported that the hyphal form of *C. albicans* is more invasive compared with budding yeast (Felk *et al.*, 2002; Hausauer *et al.*, 2005; Lossinsky *et al.*, 2006). Therefore, encouraged by this facts together with our previous work (More *et al.*, 2014; More *et al.*, 2013), in the present study we decided to synthesize Schiff bases derived from 4-(*o*-methoxyphenyl)-2-aminothiazole, R- substituted salicylaldehyde (R = H, 3-Me, 4-Me, 5-Me, 3-OMe and 5-Br) and 2-hydroxy-1-naphthaldehyde and explore its application not only as a bacterial but also as an inhibitor of dimorphic switching in *C. albicans*.

MATERIALS AND METHODS

Synthesis Zinc^{II} Complexes

An ethanolic solution of 0.01 mole of zinc acetate (Thomas Baker and Co., London) was added to the ethanolic solution containing 0.02 mole of *o*-hydroxyaldehyde and 0.02 mole of substituted 2-aminothiazole or 0.02 mole of Schiff bases. The reaction mixture was refluxed on a water bath for 30 minutes. On standing overnight the dark orange red coloured crystal separated, which were filtered, washed with ethanol and dried under vacuum.

Physical measurements

Carbon, hydrogen, nitrogen and sulphur analyses of thiazole Schiff bases and the complexes were performed by conventional microanalytical techniques. Molecular weights were determined by cryoscopic method using benzene as the solvent. The estimation of zinc was carried out gravimetric methods. The Ultraviolet and visible spectra were recorded in ethanol on Perkin Elmer Uv-visible 25 lambda spectrometer using quartz cells at room temperature. The Infrared spectra were recorded in KBr pellets on Shimadzu FT-IR 8400 spectrometer in the range 400-4000 cm⁻¹.

Antibacterial activity

It was performed by disc diffusion method. In short, a paper disc of 6 mm was prepared and sterilized by autoclave. Around 10 uL of different Schiff bases were loaded on the disc and kept on the nutrient agar plate previously spread with approximately 10⁶ cells/mL of each *Bacillus subtilis* and *Escherichia coli*, separately. The plates were incubated at 37 °C for 16 hrs and observed for the zone of inhibition around disc.

Antifungal activity

It was performed by germ tube inhibition assay. In short, 10⁴ cells/ well of *Candida albicans* (yeast form) were inoculated in yeast extract-peptone-dextrose broth in presence of various concentrations (0-500ug/L) of zinc complexes of Schiff bases, and incubated for 3 hrs at 30 °C. After incubations, cells were observed for the formation of germ tube under microscope (40X). The percent formation of germ tube is calculated from the control, which is treated as 100 percent.

RESULTS AND DISCUSSIONS

Characterizations

All the complexes are orange red coloured crystalline solids and melt with decomposition above 240 °C without showing the sharp melting points. The complexes are soluble in chloroform, DMF, DMSO, benzene and nitrobenzene. Elemental analysis data suggest 1:2 (metal: ligand) stoichiometry (ML₂) for the complexes (Table 1). Molecular weight determination data and very low molar conductance values (<10³Ω⁻¹cm²mol⁻¹) in nitrobenzene solution indicate that the complexes are monomeric and non-electrolytic in nature. The Zn^{II} complexes are diamagnetic. The IR spectral data is given in Table 2. The absence of ν OH mode (band at ~2900 cm⁻¹ in the ligands) in the complexes suggest the deprotonation of phenolic OH of the ligand and its coordination through O atom. The ν C-O mode for the complexes was observed at ~1330 cm⁻¹ (as against ~1280 cm⁻¹ for the ligand) and ν C=N mode at mode at ~1580 cm⁻¹ (as against ~1630 cm⁻¹ for the ligands). The shifting of ν C-O towards higher frequency and lowering of ν C=N in the complexes, as compared to the ligands, suggests that the coordination to the central metal atom takes place through oxygen of the phenolic OH group and nitrogen of the azomethine group. (The shifting of ν C-O towards higher frequency in the complexes is due to conversion of hydrogen bonded structure to a covalent metal bonded structure (Kovacic, 1967; Tayssei P and Charette, 1963). The coordination of nitrogen to the central metal atom reduces the electron density and thus causes reduction in >C=N frequency (Kovacic, 1967; Tayssei and Charette, 1963). In the light of above discussion, we propose that coordination to the central metal atom takes place through oxygen of the phenolic OH group, nitrogen of the azomethine group and nitrogen of the thiazole moiety. The ligands (SB-1 to SB-7) thus behave as NNO donor ligands.

Biological activities

Literature survey reveals that aminothiazole, Schiff bases and their complexes exhibits anti-quorum sensing (Buchheidt *et al.*, 2000), antibiofilm (Wu *et al.*, 2011), antibacterial, antifungal, anticancer and antitubercular activities (Felk *et al.*, 2002; Hausauer *et al.*, 2005). In present studies we have tested the Zn^{II} complexes of thiazole Schiff bases for the evaluation of antibacterial activity against *Bacillus subtilis* 2063 and *Escherichia coli*. Out of all Schiff bases tested for the antibacterial activity, Schiff bases 6 and 7 showed an antibacterial activity. Whereas the compounds could act on Gram positive *B. subtilis*, they could not act on Gram negative organisms, *E. coli*. This could be due the difference in the cell wall composition between *B. subtilis* and *E. coli*. The cells of *E. coli* possess an extra outer membrane around the cell wall, called lipopolysaccharide, which may hinder the penetration of the Schiff bases 6. The antibacterial activity of the metal complexes can be explained on the basis of chelation theory. Chelation reduces the polarity of the metal ion, because positive charges of the metal are partially shared with the donor atoms present in the ligands and there may

Table 1: Elemental Analysis data of Zn^{II} complexes.

Complex	R	C% Found (Calc)	H% Found (Calc)	N% Found (Calc)	S % Found (Calc)	Zn% Found (Calc)	μ_{eff} BM (Expt)
ZnL ₂ Ligand=SB-1	H	59.55 (59.69)	3.70 (3.83)	8.30 (8.18)	9.50 (9.37)	9.35 (9.55)	Diamagnetic
ZnL ₂ Ligand=SB-2	3-CH ₃	60.58 (60.67)	4.08 (4.24)	7.70 (7.86)	8.83 (9.00)	9.03 (9.18)	Diamagnetic
ZnL ₂ Ligand=SB-3	4-CH ₃	60.40 (60.67)	4.12 (4.24)	7.65 (7.86)	9.15 (9.00)	8.98 (9.18)	Diamagnetic
ZnL ₂ Ligand=SB-4	5-CH ₃	60.43 (60.67)	4.15 (4.24)	7.70 (7.86)	8.81 (9.00)	9.01 (9.18)	Diamagnetic
ZnL ₂ Ligand=SB-5	3-OCH ₃	58.23 (58.10)	3.90 (4.06)	7.38 (7.52)	6.50 (8.61)	8.97 (8.78)	Diamagnetic
ZnL ₂ Ligand=SB-6	5-Br	48.31 (48.50)	2.79 (2.87)	6.50 (6.65)	7.50 (7.61)	7.60 (7.76)	Diamagnetic
ZnL ₂ Ligand=SB-7		64.20 (64.32)	3.98 (3.85)	6.98 (7.14)	8.03 (8.17)	8.45 (8.33)	Diamagnetic

Table 2: Important IR frequencies of Zn^{II} complexes (cm⁻¹).

Sr. No.	Complex	ν OH	ν C=N	ν C-O	Phenyl and Thiazole Ring vibrations
1	ZnL ₂ Ligand=SB-1	Absent	1570	1325	3138,2932, 1518,1497.
2	ZnL ₂ Ligand=SB-2	Absent	1589	1325	3150, 2912, 1528, 1499.
3	ZnL ₂ Ligand=SB-3	Absent	1593	1310	3052, 2911, 1574, 1464.
4	ZnL ₂ Ligand=SB-4	Absent	1589	1325	3150, 2912, 1528, 1499.
5	ZnL ₂ Ligand=SB-5	Absent	1593	1336	3150, 2935, 1501, 1427.
6	ZnL ₂ Ligand=SB-6	Absent	1589	1313	3140, 2933, 1516, 1448.
7	CoL ₂ Ligand=SB-7	Absent	1580	1320	3075,2935 1537, 1493.

be π -electron delocalization over the whole chelation. This phenomenon increases the lipophilic character of the metal chelate and favors its permeation more efficiently through the lipid layer of the microorganism, thus destroying them more forcefully. The other factors like solubility, conductivity and bond length between the metal and ligand also increase the biological activity. The MIC results are given in table no 3. As seen we could only found the zone of inhibition around the cells of *B. subtilis*.

Table 3: Diameter of Zone of Inhibition by Zn^{II} complexes of SB-6 and SB-7.

Conc (µg/mL)	Diameter of Zone of Inhibition (mm)			
	<i>Bacillus subtilis</i>		<i>Escherichia coli</i>	
	Zn ^{II} comp SB-6	Zn ^{II} comp SB-7	Zn ^{II} comp SB-6	Zn ^{II} comp SB-7
0	No Zone	No Zone	No Zone	No Zone
62.5	No Zone	No Zone	No Zone	No Zone
125	No Zone	No Zone	No Zone	No Zone
250	10	8	No Zone	No Zone
500	14	11	No Zone	No Zone

As mentioned earlier, *C. albicans* is a dimorphic opportunistic pathogen causing both superficial and systemic infections, and the most important aspect involved in the pathogenesis of this fungus is its ability to exist as both yeast and mycelium with filamentous hyphae (dimorphism) depending on host environment (Torosantucci *et al.*, 2004). The hyphal form of this fungus is more invasive and is also involved in the secretion of various proteases and lipases which facilitate tissue invasion of the

fungus (Hausauer *et al.*, 2005). This dimorphic switching is significant for fungi as it act as a regulatory element in the pathogenesis of this fungus (Liu, 2002). In the past, efforts have been directed to curb this dimorphic using both synthetic and natural products, however, Schiff bases have not been tried.

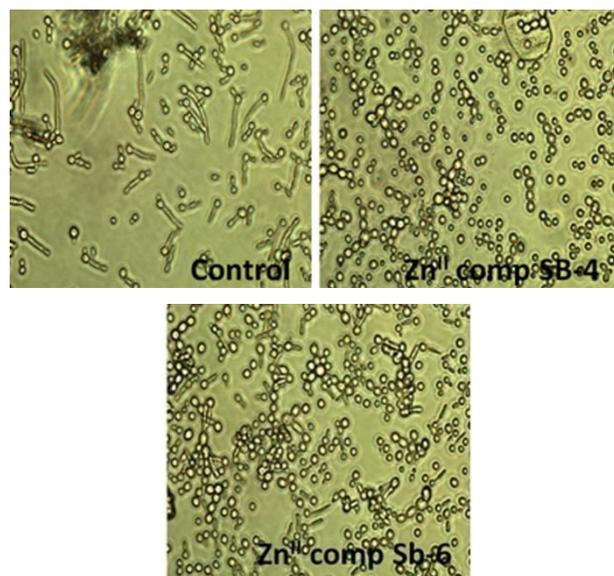


Fig. 1: The inhibition of germ tube formation in *C. albicans*. The controls yeasts forms shows the formation of germ tube, where such germ tube formation is inhibited in presence of Schiff bases.

When we incubated the yeast forms of *C. albicans* with various concentrations of Schiff bases of zinc complexes for 6 hrs, we could observe the inhibition of germ tube in presence of zinc complexes of SB-4 and SB-6 (figure 1) at 250 and 125 ug/mL, respectively. The other zinc complexes of Schiff bases did not show any inhibition. The control shows the formation of germ tube. The percent inhibition of germ tube formation, respectively, in presence of zinc complexes of SB-4 and SB-6 was 100 and 95%.

The formation of germ tube is the first step in the switching of yeast form of *C. albicans* to hyphae form. Since, the germ tube is inhibited by these compounds, the switching to hyphae form and its invasion in the tissue is prevented. The currently available molecular technologies will aid in further elucidating the molecular mechanism of action of these complexes.

CONCLUSION

The Zn^{II} complexes of thiazole Schiff bases under study are orange coloured shining crystalline solids and undergo decomposition above 240 °C without showing sharp melting points. They are soluble in chloroform, DMSO, DMF, and nitrobenzene etc. solvents. Elemental analysis data suggested 1:2 metal-ligand stoichiometry (ZnL₂) for the complexes. Molecular weight determination data and very low conductance value in nitrobenzene suggested that the complexes are monomeric and non-electrolytic in nature. Electronic spectral data suggested an octahedral geometry for the complexes. The magnetic susceptibility measurements suggested that the complexes are diamagnetic. Whereas a zone of inhibition was observed around the disc of Zn^{II} complexes of Schiff bases SB-6 and SB-7 against *B. subtilis* characterized Zn^{II} complexes of Schiff bases as an antibacterial, the inhibition of germ tube formation in *C. albicans* in presence of Zn^{II} complexes of Schiff bases SB-4 and SB-6 characterized Zn^{II} complexes of Schiff bases as an inhibitor of dimorphic switching.

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