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Synthesis and biological evaluation of chlorthalidone schiff base and their metal complexes as inhibitors of dihydrofolate reductase of *Pneumocystis carinii*

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ABSTRACT

Present work deals with synthesis and biological evaluation of Cu(II), Ni(II), Zn(II) and Co(II) metal complexes derived from chlorthalidone condensed with trihydroxybenzaldehyde as potent inhibitors of dihydrofolate reductase (DHFR) from *Pneumocystis carinii* (pc). Structural characterizations were performed using ¹H and ¹³C NMR, MASS, IR, UV-Vis spectrometer and elemental analysis; designed compounds were then evaluated by enzyme assay against dihydrofolate reductase of *P.carinii*. The Cu(II) was found to be remarkably selective inhibitor of *Pneumocystis carinii* DHFR. Other ligand and metal complexes exhibited moderate activity. This study shows that benzenesulfonamide moiety increases the potency of the compounds which further increases on coordination with metal ions.

Keywords: Chlorthalidone, schiff base metal complex, DHFR inhibitor, docking, *P.carinii* inhibitors.

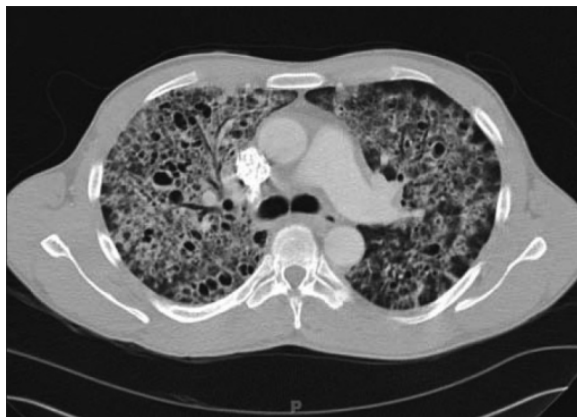
INTRODUCTION

Fungus affecting human beings are spreading worldwide and causing life threatening diseases [1]. HIV infected person is susceptible to be attacked by *Pneumocystis carinii*, causing severe infections, due to their loss of immunity (Figure 1). An enzyme dihydrofolate reductase (DHFR) present in *P.carinii*, catalyzes folic acid to dihydro and tetrahydro folic acid, is essential for cell growth and division. Inhibition of DHFR can promote their negative growth. In the past 50 years this active target have attracted interest of many workers to evolve drug as anticancer (methotrexate), antibacterial (trimethoprim) and antiprotozoal (pyrimethamine). Many drugs have developed for treatment causing by *P.carinii*, which include co-administration of the selective but weak DHFR inhibitor. Trimethoprim or pyrimethamine are given in combination with sulfonamides to enhance potency [2]. Some antifolates, Trimetrexate and Piritrexim co-administered with Leucovorin are also used for the treatment of these infections [3-5]. The gene that encodes in bacterial, human and parasite enzymes (DHFR) have now been recognized and conserved. With the help of this knowledge many selective inhibitors have been exploited for

particular pathogens. For example, pyrimethamine and cycloguanil bind strongly to *P. falciparum* DHFR active site, but not to that of humans.

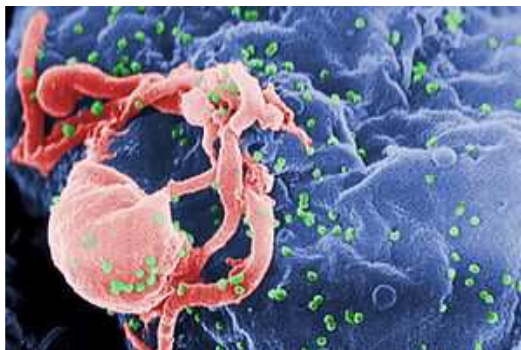
Figure 1: *Pneumocystis carinii*, High resolution computed tomography showing the hallmark of PCP in a clinical setting of immune compromise

Note the ground-glass attenuation with a geographic or mosaic distribution



In eukaryotic cell, resistance to DHFR inhibitors develops in three major ways: changes in drug transport or accumulation, overexpression of the wild type enzyme or point mutations in the DHFR gene that reduce the binding affinity of the inhibitor. With resistance to common antimicrobial agents and their toxicity, the demands for new potent drug candidate have been increased for the patient suffering from pulmonary infection [6]. As *Pneumocystis* infection can be progressive and the success of therapy is related to the intensiveness of disease, at the time of the initiation of therapy, early therapy is essential. Transition metal complexes have attracted attention in exploring their role in such antimicrobial activities [7]. Various sulfonamide and thiazide schiff bases have also been extensively investigated because of their bioactivity, using enzyme inhibition assay e.g. dihydrofolate reductase inhibition assay have been performed to restrict the growth of microbes [8,10].

Figure 2: Scanning electron micrograph of HIV-1, in green, budding from cultured lymphocytes



Chlorthalidone is diuretic thiazide drugs have been well studied for their bioactivity as antibiotics and tumor metastasis inhibitors (Figure 2). It has also been studied for their protease inhibition activity of HIV-1 using molecular docking [11]. This drug is used to treat hypertension, originally marketed as Hygroton in the USA. Compared with other medications of the thiazide class, chlorthalidone has the longest duration of action, but a similar diuretic effect at maximal therapeutic doses [12]. It is often used in the management of hypertension and edema. No research works have yet performed on chlorthalidone for the treatment of infections caused by *P.carinii* [13]. In the present work we now report the potent activities of chlorthalodone schiff base and their transition metal complexes as inhibitors of DHFR from *P.carinii*.

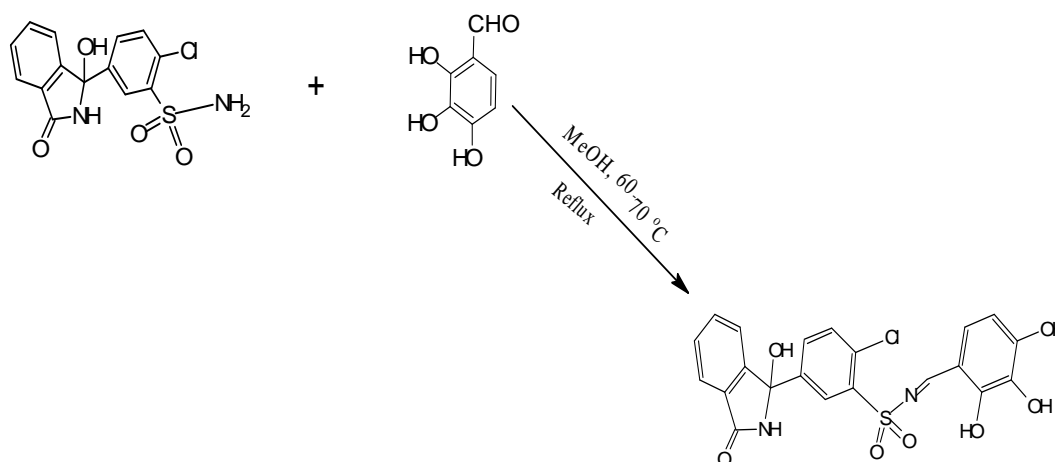
MATERIALS AND METHODS

All chemicals used were of analytical grade. Diuretic drug Chlorthalidone was purchased from IPCA laboratories, Ratlam (M.P.), which was recrystallized and analyzed for percentage purity using HPLC. Metal salts used were purchased from Merck chemicals and recrystallized using methanol. Methanol was purchased from Merck chemicals and redistilled using magnesium turnings; anhydrous methanol was collected in a dark colored glass bottle.

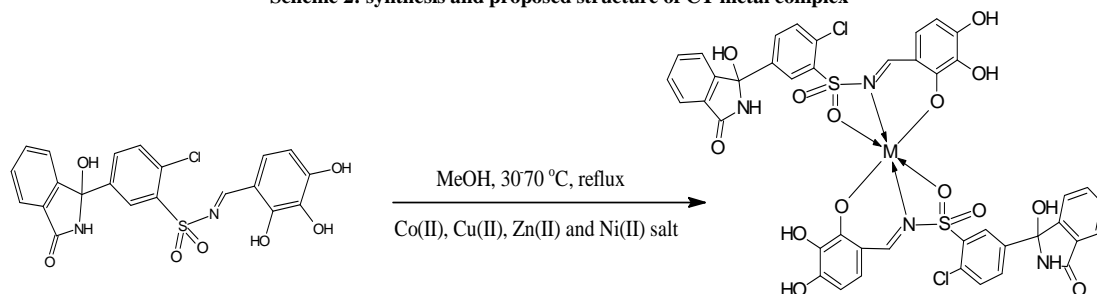
2.1. General procedure for the synthesis of ligand (CT), (2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl)methylidene]benzenesulfonamide)(L) and their transition metal complexes

Schiff base ligand was prepared according to **Scheme 1**. To an ethanol solution (20 ml) of chlorthalidone (0.02 mole) a trihydroxybenzaldehyde (0.02 mole) solution in ethanol 20 ml was added with constant stirring. Later on the solution was refluxed for 3 hr. As the reaction completed solution was cooled at room temperature, solvent was removed under reduced

Scheme 1: synthesis of Schiff base ligand from chlorthalidone 2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl)methylidene]benzenesulfonamide,(CT)



Scheme 2: synthesis and proposed structure of CT metal complex



where, M=Co(II),Cu(II), Zn(II) and Ni(II)

pressure, yellow crystals were separated. Obtained crystals were washed thoroughly with ethanol, afforded TLC pure products in good yield [14,15]. The transition metal complexes have been synthesized by refluxing the ethanolic solution of ligand with metal salts for four hour, purification was followed by the same procedure reported for the ligand (Scheme 1 and scheme 2).

2.1.1. {2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl) methylidene]benzenesulfonamide} CT ligand

Pale yellow powder, Yield: 76% (1.22 g); mp: 179–184 °C; λ_{max} : 410 nm (1.56); IR (KBr, cm^{-1}): 3120 (NH, pyrrolidine), 1631 (HC=N imine), 1435 (C-N, pyrrolidine), 1570, 1300, 3229 (C-OH), 1694 (C=O pyrrolidine), 840.49 (S-N), 1014 (S=O); ^1H NMR (DMSO- d_6 , 400 MHz, ppm): 13.5 (C_{25} -OH), 8.83 (NH pyrrolidine), 8.54 (CH=N imine), 4.73 (C_2 -OH indol), 7.27-8.54 (C-H benzene ring); ^{13}C NMR (DMSO- d_6 , d, ppm): 147.6 (C_{25}), 166.1 (C_{23} imine), 102.1 (C_2 -OH pyrrolidine), 130.9 (C_{14} -Cl), 168.7 (C_5 =O), 145.7 (C_{13} -S); Anal. Calcd. For: $\text{C}_{21}\text{H}_{15}\text{ClN}_2\text{O}_7\text{S}$ (474.871): C (53.11%), H (3.18%), Cl (7.47%), N (5.9%), O (23.58%), S (6.75%).

2.1.2. {2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl) methylidene]benzenesulfonamide} CTZ complex

Yellow powder; Yield: 91% (2.07 g); mp: 210 °C; UV-Vis: λ_{max} 401 nm (1.89); IR (KBr, cm^{-1}): 2831 (NH, pyrrolidine), 1590 (HC=N imine), 1435 (C-N, pyrrolidine), 1570, 1300, 1694 (C=O pyrrolidine), 915.49 (S-N), 1380 (M-O), 520 (M-N); ^1H NMR (DMSO- d_6 , 400 MHz, ppm): 8.83 (NH pyrrolidine), 8.10 (CH=N imine), 6.6 (C_2 -OH pyrrolidine), 7.01-8.10 (C-H benzene ring); ^{13}C NMR (DMSO- d_6 , d, ppm): 150.7 (C=N imine), 102.1 (C-OH pyrrolidine), 130.1 (C-Cl), 130.5 (C_5 =O), 144.5 (C_{13} -S); Anal. Calcd. : $\text{C}_{42}\text{H}_{28}\text{Cl}_2\text{N}_4\text{ZnO}_{14}\text{S}_2$ (1013.13): C (49.79%), H (2.79%), Cl (7%), N (5.53%), O (22.11%), S (6.33%), Zn (6.46%).

2.1.3. {2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl) methylidene]benzenesulfonamide} CTN complex

Yellowish green powder; Yield: 91% (2.07 g); mp: 210 °C; λ_{max} : 395 nm (2.01); IR (KBr, cm^{-1}): 2850 (NH, pyrrolidine), 1610 (HC=N imine), 1435 (C-N, pyrrolidine), 1300, 1694 (C=O pyrrolidine), 920.71 (S-N), 1387 (M-O), 523 (M-N); ^1H NMR (DMSO- d_6 , 400 MHz, ppm): 8.83 (NH pyrrolidine), 8.10 (CH=N imine), 6.6 (C_2 -OH pyrrolidine), 7.01-8.10 (C-H benzene ring); ^{13}C NMR (DMSO- d_6 , d, ppm): 150.7 (C=N imine), 102.1 (C-OH pyrrolidine), 130.1 (C-Cl), 130.5 (C_5 =O), 144.5 (C_{13} -S); Anal. Calcd. : $\text{C}_{42}\text{H}_{28}\text{Cl}_2\text{N}_4\text{NiO}_{14}\text{S}_2$ (1006.420): C (50.12%), H (2.8%), Cl (7.05%), N (5.57%), Ni (5.83%), O (22.26%), S (6.37%).

2.1.4. {2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl) methylidene]benzenesulfonamide} CTCu complex

Light green powder; Yield: 91% (2.07 g); mp: 196 °C; λ_{max} : 442 nm (1.96); IR (KBr, cm^{-1}): 3045 (NH, pyrrolidine), 1625 (HC=N imine), 1435 (C-N, pyrrolidine), 1300, 1694 (C=O pyrrolidine), 917.54 (S-N), 1390 (M-O), 528 (M-N); ^1H NMR (DMSO- d_6 , 400 MHz, ppm): 8.83 (NH pyrrolidine), 8.10 (CH=N imine), 6.6 (C_2 -OH pyrrolidine), 7.01-8.10 (C-H benzene ring); ^{13}C NMR (DMSO- d_6 , d, ppm): 150.7 (C=N imine), 102.1 (C-OH pyrrolidine), 130.1 (C-Cl), 130.5 (C_5 =O), 144.5 (C_{13} -S); Anal. Calcd. : $\text{C}_{42}\text{H}_{28}\text{Cl}_2\text{CuN}_4\text{O}_{14}\text{S}_2$ (1011.272): C (49.88%), H (2.79%), Cl (7.01%), Cu (6.28%), N (5.54%), O (22.15%), S (6.34%).

2.1.5. {2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl) methylidene]benzenesulfonamide} CTCo complex

Light green powder; Yield: 91% (2.07 g); mp: 196 °C; λ_{max} : 390 nm (2.94); IR (KBr, cm^{-1}): 3029 (NH, pyrrolidine), 1601 (HC=N imine), 1435 (C-N, pyrrolidine), 1300, 1694 (C=O pyrrolidine), 928.67 (S-N), 1386 (M-O), 532 (M-N); ^1H NMR (DMSO- d_6 , 400 MHz, ppm): 8.83 (NH pyrrolidine), 8.10 (CH=N imine), 6.6 (C_2 -OH pyrrolidine), 7.01-8.10 (C-H benzene ring); ^{13}C NMR (DMSO- d_6 , d, ppm): 150.7 (C=N imine), 102.1 (C-OH pyrrolidine), 130.1 (C-Cl), 130.5 (C_5 =O), 144.5 (C_{13} -S); Anal. Calcd. : $\text{C}_{42}\text{H}_{28}\text{Cl}_2\text{CoN}_4\text{O}_{14}\text{S}_2$ (1006.659): C (50.11%), H (2.8%), Cl (7.04%), Co (5.85%), N (5.57%), O (22.25%), S (6.37%).

2.2. In vitro biological activity**2.2.1. Antibacterial activity**

In this research work the antibacterial activity of schiff base ligand (L), and their metal(II) complexes were studied against four *E.coli*, *S.aureus*, *S.pyruogenes*, *B.cereus* using agar-well diffusion method, according to the literature protocol [16]. Obtained results were compared with those of standard drug trimethoprim. Bacterial culture was incubated for 24 hr into nutrient broth. By using a sterilized cork borer (7 mm diameter), wells were then dug in the culture plates. Test compounds dissolved in DMSO were added (0.2 μl) to these wells and left for 2 hr at 4 °C. Culture plates were incubated at 30 °C for 18–24 hr. Inhibition zones formed on the medium were measured as millimeters (mm) diameter [17].

2.2.2. Antifungal activity

Schiff base and their metal(II) complexes were studied for their activity against *T. longifusus*, *C. albican*, *A. flavus* and *P.carinii* fungal strains according to procedure reported elsewhere [18,19]. Obtained results were compared with those of standard drug miconazole and recorded in Table 3 and 4. All the compounds were dissolved in DMSO, fungi were cultivated in sabouraud dextrose agar (Merck). Tested samples were applied to the culture plates and incubated at 36 °C for 48 hr. At the end of the incubation period, minimum inhibition concentration (MIC) was recorded as the lowest concentrations of the substances that gave no visible turbidity.

2.2.3. Inhibition of dihydrofolate reductase

The chlorthalidone schiff base and their Zn(II), Cu(II), Co(II) and Ni(II) complexes evaluated for their ability to inhibit dihydrofolate reductase from *P.carinii*, using continuous spectrophotometric assay measuring oxidation at 340 nm at 37 °C. The expressed genes transcribed by *E.coli*, cells were lysed and enzyme was extracted. The methodologies of the assay are described elsewhere [20]. All results were observed as % inhibition and IC₅₀ values.

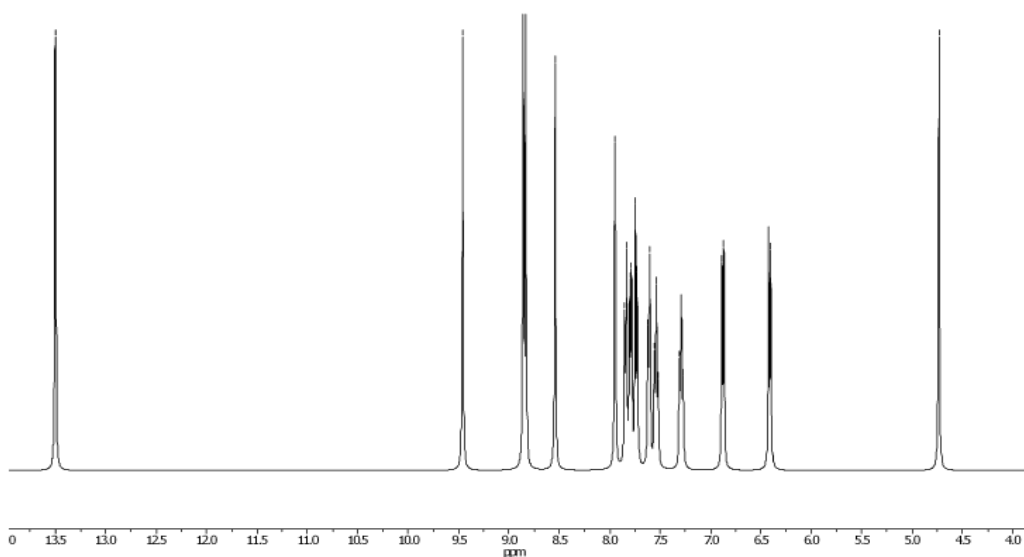
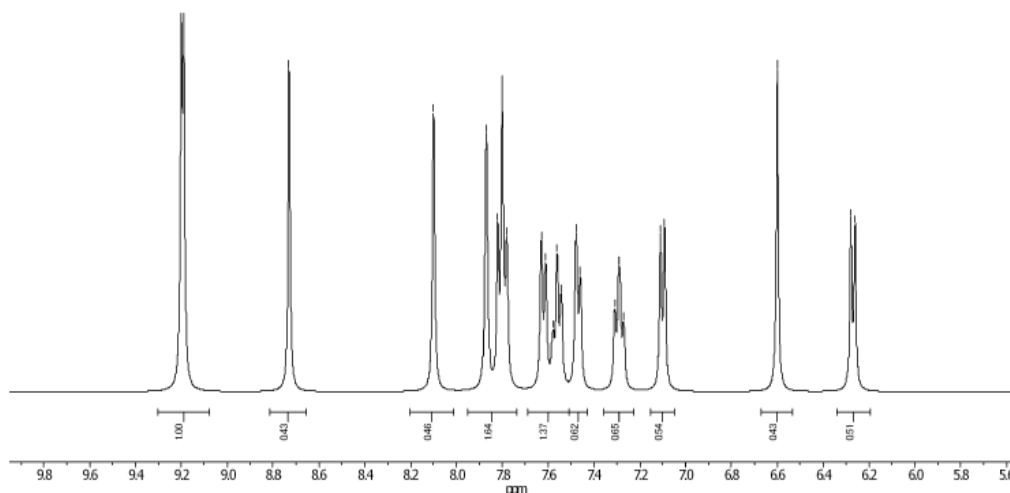
RESULTS AND DISCUSSION

The Schiff base ligand and its Cu(II), Ni(II),Co(II) and Zn(II), complexes were synthesized and characterized by spectroscopic and elemental analysis techniques. The complexes were found to be air stable. The ligand and metal complexes were soluble only in CH₃OH and DMSO at room temperature. The composition of ligands was consistent with their mass spectral, nuclear resonance and IR data.

3.1. Spectroscopic characterization of ligand and their metal complexes

3.1.1. ¹H NMR spectra

The ¹H NMR spectrum of Schiff base ligand and their metal(II) complexes in deuterated DMSO exhibited signals consistent with the proposed structure [21]. Aromatic proton H-28 and H-29 appears between 6.40 and 6.88 ppm as doublets. The pyrrolidine (-OH) appeared at 4.73 ppm as singlet. Aromatic (C-OH) appeared at 9.4, 13.5 and 8.83 ppm as a singlet due to deshielding of higher electronegative (-O) atom (Figure 3). The pyrrolidine proton (-C-NH-C) was observed at 8.83 ppm and imine proton (CH=N) at 8.54 ppm. The phenyl proton H-7 and H-8 appeared as triplet of doublet at 7.27 and 7.52 ppm respectively. Doublet of doublet was observed for H-6 and H-9 at 7.55 and 7.72 ppm respectively. The H-12 and H-16 was appeared as multiplate between 7.74- 7.94 ppm. Deprotonation of (C-OH) proton at position O-30 of Cu(II), Co(II), Ni(II) and Zn(II) complexes, confirms the complex formation and their geometry. On complex formation, imine proton (-CH=N) shifted to less downfield at 8.10 ppm and aromatic proton (-CH) was observed from 7.09-7.86 ppm more downfield than ligand due the increase in steric hindrance caused by increase in electron density (Figure 3 & 4). The hydroxyl group (Ar-OH) shifted to less downfield at 9.18-9.20 ppm due to shielding effect upon complex formation [22].

Figure 3: ^1H NMR chemical shift of CT ligandFigure 4: ^1H NMR chemical shift of CT Zn(II) complex

3.1.2. ^{13}C NMR spectra

Schiff base ligand and their complexes were analyzed for ^{13}C NMR in Bruker's 400 MHz NMR using DMSO-d_6 as solvent. The ^{13}C NMR spectral information is reported along with their possible assignments in the experimental section and all the carbons were found in the expected region. The ^{13}C NMR spectra of Schiff base ligand displayed the imine ($\text{C}=\text{NH}$) carbon at 166.15, pyrrolidine carbons in the region at position 102.16 ppm, 130.51 ppm, 145.01 ppm and 168.47 ppm. All carbons of phenyl group in ligand appeared at 111.31 ppm, 116.12 ppm, 147.60, 149.61 ppm, 129.80 and 132.69 ppm. The C_{25} and C_{27} shows more downfield due to presence of electronegative ($-\text{OH}$) group.

The conclusion obtained from these studies provided further support to the mode of bonding explained in the IR and ^1H NMR spectral data. The spectra of Cu(II) , Zn(II) , Ni(II) and Co(II) complexes of all exhibited downfield shifting of azomethine and pyrrolidine carbon from 166.04-150.77 ppm and 153.4-156.6 ppm in the spectra of ligands to 161.2-162.59 ppm and 154.72-158.30 ppm in the spectra of their metal(II) complexes respectively, indicating the coordination of azomethine and pyrrolidine nitrogen to the metal ion. Similarly, the phenyl carbon of ligand existing near the coordination sites showed downfield shift from 163.1 ppm in the spectra of free ligand to 164.25 ppm in the

spectra of the metal(II) complexes which were attached with hetero sulphur and nitrogen of sulphonamide moiety and pyrrolidine ring respectively. All other carbons of the ligands in the spectra of the metal (II) complexes underwent downfield shifting by 0.24–0.8 ppm due to the increased conjugation and coordination with the metal.

3.1.3. IR spectra

The characteristic IR spectra of ligand and their metal (II) complexes possessed potential donor sites azomethine linkage, pyrrolidine hydroxyl (-OH), sulphonamide (C-S) and pyrrolidine (N-H) groups which have tendency to coordinate with the metal ions. The IR spectra of the ligand exhibited peaks at 3120–3127 cm^{-1} correspond to vibration of N-H (pyrrolidine). In chlorthalidone these peaks were observed at 3325 and 1715 cm^{-1} , due to sulphonamide amino (-NH₂) vibrations. In the spectra of the ligands a new sharp band appeared at 1608 cm^{-1} assigned to the azomethine linkage (C=N) [23]. A strong peak was observed at 1700 cm^{-1} corresponds to C-Cl bond. The hydroxyl (O-H) bond of benzene moiety appeared at 3329 cm^{-1} , another frequency observed at 1693 cm^{-1} correspond to C=O from pyrrolidine ring (Table 1). In all the metal complexes, a new band appeared at 520–532 cm^{-1} due to M–N vibration indicating the coordination of nitrogen atom with the metal ions [24,25]. The appearance of a weak band at 545 cm^{-1} assigned to weak M–N vibrations, confirm the coordination and geometry of metal complexes. The disappearance of O-H proton and in turn appearance of new band at 2358–2521 cm^{-1} correspond to two O-H group attached to phenyl ring.

Table 1. IR vibrational spectra of ligand and complexes

Compounds	IR frequencies ν cm^{-1}							
	HC=N	NH ₂	M-N	M-O	Ar-OH	Ar-H	C-NH-C	C=O
Ligand	1608	3120	-	-	3329	1273	3120	1693
CTZ	1590	-	520	1380	2358	1140	2831	1623
CTN	1610	-	523	1387	2400	938	2850	1640
CTCu	1625	-	528	1390	2521	996	3045	1661
CTCo	1601	-	532	1386	2386	1250	3029	1610

The formation of M-O bond confirmed deprotonation and coordination of hydroxyl group (O-H) to the metal atom. It also indicates coordination of sulphonamide moiety to the central metal ion. All other bands remain unchanged in the spectra of all ligands and their corresponding metal complexes.

3.1.4. Mass spectra

The mass spectral data and fragmentation pattern of schiff base ligand and their metal complexes justifies the formation of the proposed structures and their bonding pattern. The spectra of ligand showed molecular ion peak m/z 474 (Calcd.474.827) of [C₂₁H₁₅CIN₂O₇S], which loses a hydrogen (H) as a radical to give most stable fragment at m/z 473 of [C₂₁H₁₄CIN₂O₇S]⁺. Its base peak [C₁₄H₁₁CIN₂O₄S]⁺, was observed at m/z 338. The molecular ion peak of Cu(II), Co(II), Zn(II) and Ni(II) complex was observed at m/z ; 1011, 1006, 1013 and 1006 respectively. The first fragmentation pattern followed the cleavage of S-C, C=N bonds confirming the proposed structure of ligand and metal complexes.

3.2. Biological activity

3.2.1. In vitro antibacterial study

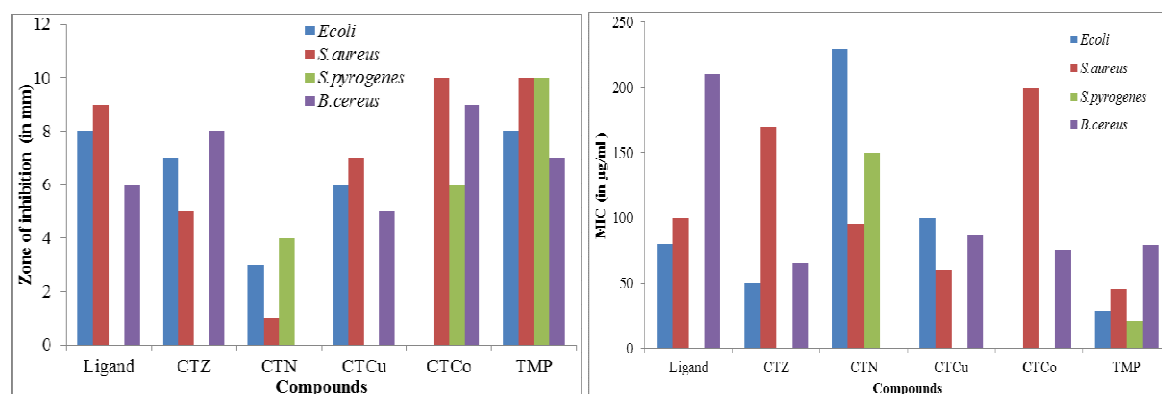
The schiff base ligand and complexes were studied for their antibacterial activity against four bacterial strain *E.coli*, *S.aureus*, *S.pyrogenes* and *B.cereus* using disk diffusion method. All the compounds exhibited moderate to significant inhibitory effects on the growth of selected bacterial strains (Figure 5) [26,27]. Data showed that ligand and their Co(II) complex have higher activity against *S.aureus*. However Co(II) and Zn(II) complex were found to be most effective against *S.pyrogenes*.

Table 2. Zone of inhibition of CT ligand and their complexes against some bacterial strains

Compounds	Zone of inhibition (in 10 mm)			
	<i>E.coli</i>	<i>S.aureus</i>	<i>S.pyrogenes</i>	<i>B.cereus</i>
Ligand	8	9	-	6
CTZ	7	5	-	8
CTN	3	1	4	-
CTCu	6	7	-	5
CTCo	-	10	6	9
^a TMP	8	10	10	7

^aTMP: Trimethoprim, inhibition zone was measured in 10 mm.

Figure 5: Zone of inhibition and MIC of CT ligand and their metal complexes against selected bacteria



Zn(II) and Co(II) complex exhibited higher potency to inhibit the growth of *B. cereus*. Obtained result also showed that Ni(II) complex have poor activity against all the strain. Ligand and Zn(II) complex were found to be most active against *E. coli*. Zn (II) complex exhibited lowest MIC 50 µg/ml and 65 µg/ml was against *E. coli* and *B. cereus* respectively. MIC of 60 µg/ml against *S. aureus* was found correspond to Cu(II) complex. The results are shown in Table 2 and Table 3.

Table 3. Minimum inhibitory concentration (MIC) of Schiff base and their metal complexes against selected bacterial culture

Compounds	Minimum inhibitory concentration (µg/ml)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. pyrogenes</i>	<i>B. cereus</i>
Ligand	80	100	-	210
CTZ	50	170	-	65
CTN	230	95	150	-
CTCu	100	60	-	87
CTCo	-	200	-	75
^a TMP	29	45	21	79

^aTMP: Trimethoprim

3.2.2. In vitro antifungal study

In this research work Co(II), Ni(II), Cu(II) and Zn(II) metal complexes of 2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl) methylidene]benzenesulfonamide) have been prepared and their antifungal activities were evaluated. The minimum inhibitory concentration (MIC) of tested compounds were recorded against *T. longifusus*, *C. albican*, *A. flavus* and *P. carinii* are shown in Tables 4 and 5.

Table 4. Zone of inhibition against selected fungal culture

Compounds	Zone of inhibition (in 30 mm)			
	<i>T. longifusus</i>	<i>C. albican</i>	<i>A. flavus</i>	<i>P. carinii</i>
Ligand	24	9	28	22
CTZ	7	20	13	26
CTN	19	16	25	11
CTCu	-	-	17	23
CTCo	-	-	10	11
Miconazole	29	14	21	20

It was found that ligand possessed excellent activity against *T. longifusus*, however Co(II) and Cu(II) have no activity against *T. longifusus* and *C. albican*. Ligand and Ni(II) complex were found to be most effective against *A. flavus*. It was found that ligand, Cu(II) and Zn(II) complex possessed maximum activity against *P. carinii*. Cu(II) complex have lower MIC value 50 µg/ml (Figure 6) against *P. carinii*. Lowest MIC of Co(II) complex was found to be 60 µg/ml for *A. flavus* [26]. However Ni(II) complex exhibited lowest MIC 50 µg/ml for *C. albican*. MIC of other compounds is shown in Table 5.

Figure 6: comparison of antifungal activity and MIC of ligand and their metal complexes against selected fungal culture

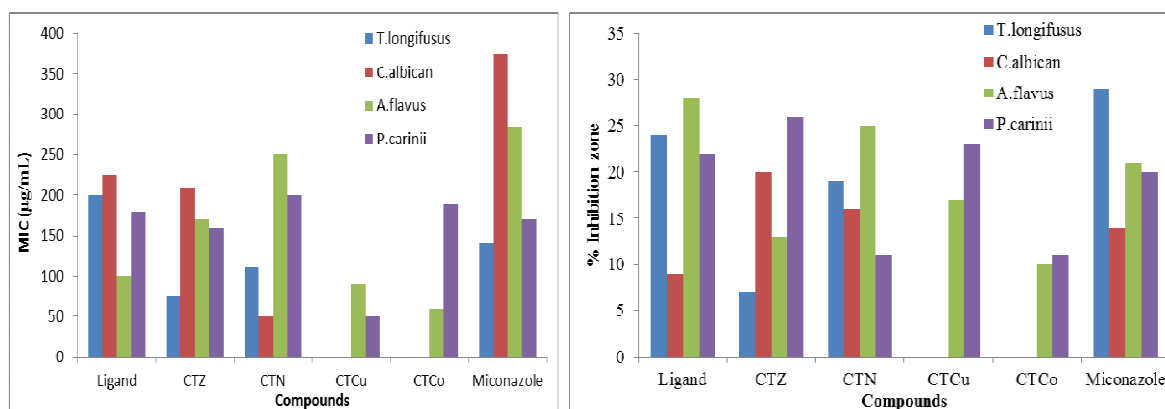


Table 5. Minimum inhibitory concentration against fungal culture

Compounds	Minimum inhibitory concentration (µg/ml)			
	<i>T.longifusus</i>	<i>C.albican</i>	<i>A.flavus</i>	<i>P.carinii</i>
Ligand	200	225	100	180
CTZ	75	210	170	160
CTN	110	50	250	200
CTCu	-	-	90	50
CTCo	-	-	60	190
Miconazole	140	375	285	170

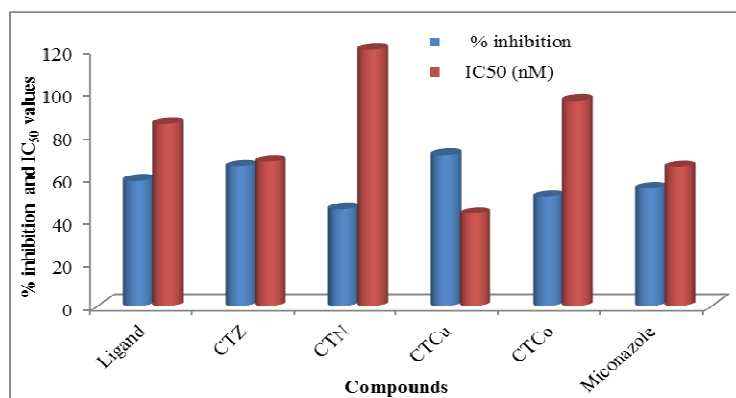
3.2.3. *PcDHFR* inhibition assay

The CT schiff base ligand and their metal ion complexes, were evaluated for their activities to inhibit the growth of dihydrofolate reductase from *P.carinii* (*PcDHFR*). The methodology was used according to the protocol, previously reported [28]. The obtained results were compared with the standard drug Miconazole. The IC_{50} -values observed are shown in Table 6. The electronegative bridges between benzenesulfonamide ring system and distal substitution showed moderate activity. It was observed that Cu(II) complex shows the activity of 70.5% inhibition IC_{50} - 43.1 nM/mL) with higher efficacy than other compounds. Ligand have the potency to inhibit 58.6% of *PcDHFR* (IC_{50} - 85.0 nM/ml) and Zn(II) complex have the activity of 65.3% (IC_{50} - 67.6 nM/ml).

Table 6. Inhibition concentrations (IC_{50} , µM) of Schiff base ligand and their metal complexes against DHFR from *P. carinii*

Compounds	% inhibition	IC_{50} (nM)
Ligand	58.6	85.0
CTZ	65.3	67.6
CTN	45.4	119.7
CTCu	70.5	43.1
CTCo	50.9	95.8
Miconazole	55.0	65.0

Figure 7: Comparison of inhibition activity of ligand and metal complexes against DHFR of *P.carinii*



Ni(II) complex has comparatively lowest activity of IC₅₀ 119.7 nM/ml with 45.4% inhibitory activity. The Co(II) complex shows the 50.9% activity of IC₅₀ - 95.8 nM/ml. Percentage inhibition of the ligand and complexes are shown in Table 6. Comparative percentage inhibition and IC₅₀ values are also shown in Figure 7.

CONCLUSION

The ionicity of M-N bonds in the tested compounds seems to correlate with their growth inhibitory action which may be due to high positive charge on the M²⁺ ion, and the high negative charge on the N atoms. This improves its antibacterial and antifungal activity. Objective of the present program is to achieve a favorable toxicity profile by applying the soft drug concept, it is still highly desirable to suppress host toxicity at the site of administration. Experimental results showed that ligand have less inhibition activity as compared to the Cu(II) and Zn(II) complexes. Ni(II) complex have the lowest activity to inhibit the *PcDHFR* from *P.carinii*. Schiff base ligand and their metal complexes exhibited their moderate to good activities against selected fungal and bacterial strain. This results observed by this experiments can serve as valuable research tools and guide for further selection of compounds as targets for synthesis of a new drug moiety for the inhibition of diseases caused by *P.carinii*.

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Abbreviations

CT-2-chloro-5-[(2R,4E)-4-ethylidene-2-hydroxy-3-methylidene-5-oxopyrrolidin-2-yl]-N-[(E)-(2,3,4-trihydroxyphenyl)methylidene]benzenesulfonamide; **DHFR**- Dihydrofolate reductase; **CTZ**, **CTNi**, **CTCu**, **CTCo**- CT Schiff base Zn(II), Ni(II), Cu(II) and Co(II) complex; **TMP**- Trimethoprime.

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