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Synthesis and Biological Activity of Copper(Ii) Schiff Base Complexes as Potential Agents for Tuberculosis Therapy

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Abstract
*Four new copper (II) Schiff base complexes (**CuL1-CuL4**) were synthesized by reaction of $CuCl_2.2H_2O$ with Schiff bases **L1-L4** derived from condensation of 2-aminopyridine with substituted saliclyaldehydes. The compounds were characterized by elemental analysis, infrared (IR), nuclear magnetic resonance (NMR), electronic absorption and molar conductivity. The Schiff bases reacted as bidentate ligands towards copper (II) to yield complexes with a 1:1 (M:L) molar ratio. Evaluation of the in-vitro anti-tuberculosis activity against mycobacterium tuberculosis $H_{37}RV$ using the proportion method showed the complexes exhibited enhanced in-vitro anti-tuberculosis activity against the bacteria compared to the free ligands and reference compound (INH).*
Keywords: 2-aminopyridine, Schiff bases, Copper (II) complexes, *In vitro* anti-tuberculosis activity, *Mycobacterium tuberculosis*

Introduction
The high rate of mortality and co-morbidity of tuberculosis (TB) with HIV makes TB one of the greatest health problems in the world[1, 2]. Over the years, *Mycobacterium tuberculosis* (*M.TB*) the causative bacteria for TB has acquired resistance to nearly all the first-line anti-tuberculosis drugs such as isoniazid (INH), rifampicin, pyraziamide, ethambutol etc., making it difficult to treat the infection clinically. This led to renewed interest in the search for new classes of active compounds against *M.TB*. Since the discovery of cisplatin, a metal-based anti-cancer drug [3], the development of metal-based therapeutic agents have been actively pursued. The development of new anti-tuberculosis agents is currently focused on molecular modification of the existing compounds with anti-TB effect[4, 5] and the screening of new classes of compounds active against *M.tuberculosis* [6-8].
We are currently interested in the use of Schiff bases, particularly those from salicylaldehyde because of their use as models for biological systems. These compounds are reported to show antibacterial [9, 10], anticonvulsant [11], antitumor [12] and anti-tuberculosis [13] activities. In addition, the compounds function as excellent chelating ligands with transition metals ion [14-15]. Schiff base metal complexes have been investigated as models for antibacterial [16] antifungal [17] and anti-tuberculosis[18] .
Reports on the pyridine moiety as pharmacophore for the anti-tuberculosis activity of most drugs [19] prompted our investigation of compounds containing 2-aminopyridine as potential leads in the search for new anti-tubercular drugs.

Materials and Method
All chemicals and solvents used were obtained commercially from Aldrich Chemicals Ltd and used without further purification.
Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. The Infrared (IR) spectra were recorded on an FTS 7000 series Digilab Win-IR Pro spectrophotometer equipped with a selenium ATR (attenuated total reflectance) accessory. ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) were recorded on using deuterated chloroform ($CDCl_3$) as solvent with TMS as internal standard on a Varian Mercury 300 MHz spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 CHNS/O analyzer. Electronic spectra were recorded at room temperature using freshly prepared solutions of the Schiff bases on a Cecil Super Aquarius 9000 series UV-Vis spectrophotometer with a 1 cm quartz cell. The quantitative determination of the metal ions was performed using Perkin-Elmer Atomic Absorption spectrometer. Antituberculosis screening was performed at the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

Typical synthesis of Schiff bases
A solution of aldehyde (0.20 mmol) in ethanol (10 mL) was slowly added to a stirred solution of 2-aminopyridine (0.20 mmol) in ethanol (10 mL) with the addition of two drops of formic acid. The `mixture was heated to reflux at 60 ^{o}C for 6 h and then allowed to cool to room temperature. The precipitate formed was

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collected by filtration, recrystallized from ethanol-hexane (1:1) and dried over silica gel in a desiccator to afford the desired Schiff base [20].

Typical synthesis of Schiff base copper(II) complexes

A solution of copper (II) chloride ($CuCl_2.2H_2O$) (1.1 mmol.) in hot ethanol: water (5 ml, 1:1(v/v)) was added to a solution of the Schiff base (1.0 mmol.) in hot absolute ethanol (15 ml). The resulting mixture was stirred under refluxed at 60 ^oC for 6h and allowed to cool to room temperature. The solid obtained was collected by filtration, washed severally with ethanol:water (1:1 v/v)and dried over silica gel in a dessicator

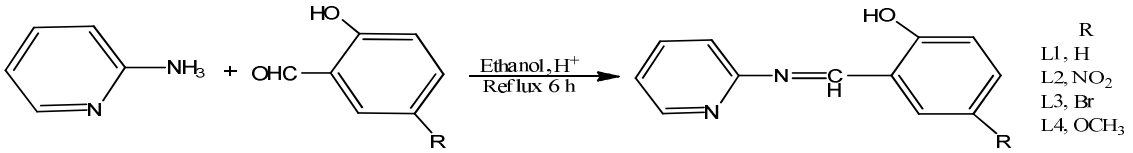
Anti-tuberculosis study

The anti-tuberculosis test was performed using the proportion method [21, 22]. Lowenstein-Jensen (LJ) medium was prepared by blending freshly laid chicken eggs with mineral salt and 2% malachite green. A stock solution of the test compound in DMF (0.04 mg/ml) was prepared, filtered through 0.22 µg pore membrane and diluted with LJ medium to give solutions with concentrations 0.4, 0.2 and 0.1µg/ml. Each solution (7-10 ml) was poured into a sterile universal container and inspissated at 85^oC for 45 min (LJ slope). Control experiments were set up using a growth media without the test compound and with the solvent. The LJ slopes were inoculated with 10^{-2} and 10^{-4} CFU/ml of *M.TB* H_{37RV} and incubated at 37^oC for 28 days. The active compounds were further incubated for 14 days. INH was used as a reference compound.

Results

Physical, analytical and spectroscopic results of the Schiff bases and the corresponding metal complexes.

The Schiff bases **L1-L4** were isolated in good yields from the condensation reaction of 2-aminopyridine with salicylaldehyde (**L1**), 5-nitrosalicylaldehyde (**L2**), 5-bromosalicylaldehyde (**L3**) and 5-methoxysalicylaldehyde (L4) (Scheme I). Treatment of ligands **L1-L4** with Cu(II) chloride gave complexes **CuL1-CuL4** with a metal:ligand ratio of 1:1. Physical, analytical and spectroscopic data for the compounds are summarized in Tables 1, and 2.



Scheme I: Reaction scheme for the synthesis of Schiff bases L**1-L4**

Table 1: Physical and analytical data of compounds L1-CuL4

Compound	Empirical formula (M.wt)	% yield)	m.p (^oC)	Microanalysis calcd (found) C	H	N	Cu
L1	$C_{12}H_{10}N_2O$ (198)	66	62-64	72.71 (72.33)	5.08 (5.03)	14.10 (14.00)	-
CuL1	$C_{12}H_{15}ClCuN_2O_4$ (349)	54	164-166	41.15 (41.66)	4.32 (3.94)	8.00 (8.12)	18.14 (20.55
L2	$C_{12}H_9N_3O_3$ (243)	46	182-184	59.26 (59.14)	3.73 (3.56)	17.28 (16.96)	-
Cul2	$C_{14}H_{16}ClCuN_3O_5$ (405)	57	249-256	41.49 (42.18)	3.98 (3.32)	10.37 (11.06)	15.68 (15.54
L3	$C_{12}H_9N_2OBr$ (277)	81	138-140	52.01 (51.96)	3.27 (3.21)	10.10 (9.88)	-
CuL3	$C_{12}H_{10}ClBrCuN_2O_2$ (390)	62	289-295	36.66 (38.41)	2.56 (2.50)	7.13 (7.25)	16.16 (16.67
L4	$C_{13}H_{10}N_2O_2$ (228)	75	82-84	68.42 (68.32)	5.26 (5.28)	12.28 (12.14)	-
CuL4	$C_{15}H_{19}ClCuN_2O_4$(389)	53	208-209	46.16 (47.01)	4.91 (4.55)	7.18 (7.98)	16.28 (16.91

M.wt = molecular weight (g/mol)

Antituberculosis activity.

The *in-vitro* anti-tuberculosis activity of the investigated compounds was evaluated against *M.TB* H37Rv at a concentration of 0.4-0.1 µg/ml on 10^{-2} and 10^{-4} CFU/ml of the standard strain using the modified proportion method [22]. The results are presented in Table 3. The unsubstituted ligand (L1) and its metal complex exhibited no activity at all concentrations used and on both 10^{-2} and 10^{-4} CFU/ml of *M.TB* H37Rv. However, the nitro containing compound (L2) and its complex exhibited activity at all the concentration used when compared to the reference compound (INH) which showed highest activity at 0.2 µg/ml. The bromo substituted (L3) ligand show the same level of activity as the complex. Compound L4, the methoxy containing ligand exhibited increased

anti-tuberculosis activity in the presence of metal ion at 10^{-2} CFU/ml of 0.4 µg/ml and 10^{-4} CFU/ml on 0.2 µg/ml.

Table 2. Characteristic IR and NMR bands for Schiff bases and metal complexes.

Compound	IR bands (cm^{-1})						δ_{TMS} (ppm) HC=N	
	v(OH)	*v*(C=N)	*v*(C-O)	*v*(C=N)py	*v*(M-O)	*v*(M-N)	δ_H	δ_C
L1	3058	1603	1276	-	-	-	9.41	161.84
CuL1	-	1595	1286	863	516	451	-	-
L2	3331	1595	1285	-	-	-	9.53	162.89
CuL2	3311	1605	1247	850	482	444	-	-
L3	-	1608	1276	-	-	-	9.34	160.87
CuL3	-	1587	1281	888	511	477	-	-
L4	3021	1598	1271	-	-	-	9.37	157.50
CuL4	-	1614	1278	886	521	467	-	-

Table 3: *In-vitro* anti-tuberculosis activity of Schiff bases and their metal complexes on *M.TB* H37Rv.

Compound	0.4 y		0.2 y		0.1 y	
	10^{-2} b	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}
L1	20	2	35	3	62	7
CuL1	16	2	22	2	30	5
L2	0	0	0	0	0	0
CuL2	0	0	0	0	0	0
L3	10	0	12	0	30	6
CuL3	6	0	10	0	24	2
L4	24	0	33	6	41	10
CuL4	0	0	12	0	26	4
INH	0	0	0	0	12	8

Sensitivity values >= inactive, <1 = active; y is concentration in µg/ml ; b = CFU/ml

Discussion

Spectroscopic results of the Schiff bases and the corresponding metal complexes.

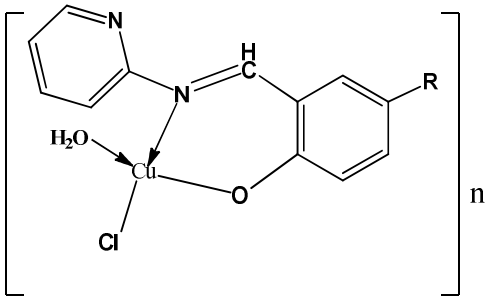
The IR spectra of the ligands (Table 2) show important band in the region 1595-1608 cm^{-1} attributed to ν(C=N) (azomethine) mode confirming the formation of the Schiff bases. This was further supported by a singlet observed at 9.34-9.53 ppm and 163.40 and 167.63 ppm in the ^{1}H and ^{13}C NMR spectra respectively. The metal complexes are soluble in common organic solvents such as DMF and DMSO. The low molar conductance values of the metal complexes 3.20-8. $4ohm^{-1}cm^{2}mol^{-1}$ reveal their non-electrolytic nature [23].

IR spectra of the metal complexes

A study and comparison of the IR spectra of the ligands and the metal complexes suggest that the Schiff bases (**L1-L4**) act as bidentate ligands in nature with the azomethine nitrogen and phenolic oxygen as the two coordination sites. Diagnostic IR spectra bands of the ligands and their metal complexes are presented in Table 2.

The characteristic absorption bands of the ligands occurred at 3331-3021 cm^{-1} , 1595-1608 cm^{-1} and 1271-1286 cm^{-1} due to *v*(OH), *v*(C=N) and *v*(C-O) vibrations respectively. The band due to phenolic OH group disappeared in the spectra of the complexes indicating the involvement of oxygen of the phenolic group in coordination. In addition, a considerable shift was observed in the *v*(C-O) frequency as a consequence of coordination of the phenolic oxygen of the ligands to the metal ion [24]. The band due to azomethine nitrogen atom of the Schiff bases shifted to either a lower or higher frequency at 1587-1614 cm^{-1} upon complexation indicating the involvement of the nitrogen of the azomethine group in coordination [25] . In the spectra of all the metal complexes, new bands appeared at 482-521 cm^{-1} and 444-477 cm^{-1} assigned to *v*(M-O) and *v*(M-N) vibrations respectively confirming complexation of the metal and ligand [26].

Based on the spectroscopic and analytical data square planar geometry was proposed for all the metal complexes as shown in figure 1.



n = H_2O, L1A
C_2H_5OH, L2A and L4A

Figure 1: Proposed structure for metal complexes.

Antituberculosis activity

A comparative study of the Schiff bases and their metal complexes show that some of the complexes exhibited anti-tuberculosis activity over the free ligand. The observed increased anti-tuberculosis of some of the metal over the ligand and the reference compound (INH) is in agreement with most of the published work on biological assay of Schiff bases and their transition metal complexes [6,7] and is because of increase in cell permeability. The lipid membrane which surrounds the *M.TB* cell favours passage of lipid soluble materials and metal complexes have high lipid character over the ligands [27].

In general, the most active compounds against *M.TB* H37Rv are the nitro containing compound (**L2** and **L2A**) which exhibited activity at lowest concentration 0.1 µg/ml when compared with the reference compound which showed highest activity at 0.2 µg/ml. This result is in agreement with previous reports [28,12] that the presence of a nitro group increased antimycobacterial activity of the Schiff base compounds.

Conclusion

The design and synthesis of new Schiff base Copper (II) complexes for antitubercular study have been successfully demonstrated. The metal complexes showed enhanced inhibitory activity towards *Mycobacterium tuberculosis* strain compared to the free ligands. Compounds containing the nitro group (**L2**) and its metal complex (**L2A**) inhibited the growth of the organism at minimal concentrations compared to the reference compound (INH) and can be considered as a good starting point to develop new lead compounds for the management of tuberculosis.

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