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Synthesis, Characterization and Biological Studies of Mixed Ligands Nicotinamide-Trimethoprim Complexes

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Abstract

A series of transition metal ions have been employed in the synthesis of mixed-ligand complexes from nicotinamide and trimethoprim. The prepared complexes were characterized using physicochemical and spectroscopic methods of analysis such as melting point determination, conductivity measurements, CHN elemental analysis and infrared spectroscopy. The results showed the coordination of the ligands to the central metal ions in a bidentate manner. The results of the antimicrobial activity of the complexes carried out against strains of *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Klebsiella* spp., *Escherichia coli and Candida* spp. showed a comparative better activity in the synthesized complexes as compared with the ligands.

Keywords: Nicotinamide, trimethoprim, mixed ligand-metal complexes, antimicrobial activity

1. Introduction

The development and clinical use of antibacterials during the 20th century substantially reduced mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitro-glycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered and developed [1, 2]. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. Paralleled to this, there has been an alarming increase in resistance of bacteria, fungi, viruses and parasites to multiple existing agents [3-5]. Antimicrobial resistance, which is the ability of a microbe to resist the effects of medication previously used to treat them, is now a major threat to public health [6, 7]. Resistant microbes are increasingly difficult to treat, requiring alternative medications or higher doses – hence calls for new antibiotic therapies have been issued, but new drug-development is becoming rarer [8]. This search has led to efforts in the field of bioinorganic chemistry. Heterocyclic compounds play a significant role in many biological systems, especially N-donor ligand systems being a component of several vitamins and drugs such as nicotinamide. Nicotinamide, chemically 3-pyridine carboxamide is the compound with the chemical formula $C_6H_6N_2O$ (Figure 1a). Basically, being an amide derivative of nicotinic acid, nicotinamide is also called niacinamide, niacin, nicotinic acid amide and vitamin B3. Because nicotinamide has pyridine ring, it gives characteristic pyridine reactions [9, 10]. Nicotinamide is known as a component of the vitamin B complex as well as a component of the coenzyme, nicotinamide adenine dinucleotide (NAD). These are more important for transfer of hydrogen in the cell breath. The presence of pyridine ring in numerous naturally abundant compounds, adducts of nicotinamide are also scientific interest. Therefore, the structure of nicotinamide has been the subject of

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many studies [11].

Trimethoprim chemically known as 5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine, the structure of which is shown in Figure 1b, belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors [12]. It is a synthetic derivative of trimethoxybenzyl-pyrimidine with antibacterial and antiprotozoal properties. As a pyrimidine inhibitor of bacterial dihydrofolate reductase, trimethoprim binds tightly to the bacterial enzyme, blocking the production of tetrahydrofolic acid from dihydrofolic acid. The antibacterial activity of this agent is potentiated by sulfonamides. It is used in prophylaxis treatment and urinary tract infections [13]. Most gram-negative and gram-positive microorganisms are sensitive to trimethoprim, but resistance can develop when the drug is used as a single therapy [14].

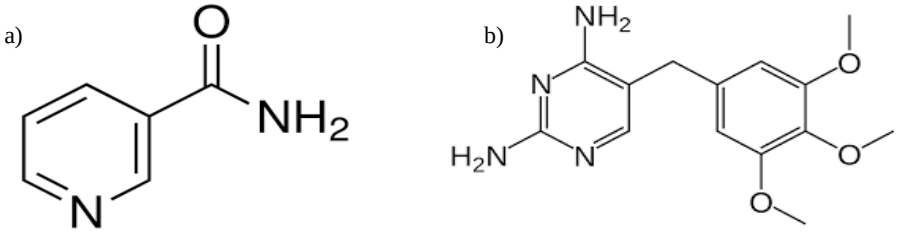

Figure 1. Chemical Structure of Nicotinamide (a) and Trimethoprim (b)

Mixed ligands complexes have been studied because of their importance in biological systems [7-8, 15]. Few reports are available on the mixed metal complexes of trimethoprim [16]. Thus, this work is aimed at the synthesis, characterization and biological studies of mixed ligand complexes of nicotinamide and trimethoprim.

2. Materials and Methods

2.1 Collection of Materials

All chemicals where obtained from Sigma-Aldrich and used without purification. Elemental analysis (C, H, N) were carried out by standard methods. Physico-chemical analysis such as solubility, melting point and conductivity measurement were carried out at Chemistry Department, University of Ilorin using a HANNA instrument conductivity meter at cell constant 1.34. IR spectra were recorded in the 4000–400 cm^{-1} region with a Perkin Elmer Spectrum One FTIR spectrophotometer using KBr pellets. Electronic spectra were recorded by a Shimadzu 3600/UV–Vis spectrophotometer. Antimicrobial activity of the synthesized complexes was by screening the complexes against the tested organisms. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Pseudosomanas aureginosa* and *Candida* spp. were obtained from the Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

2.2 Synthesis of the Complexes

All metal complexes were obtained according to a general procedure: a solution of a metal salt (1 mmol) dissolved in 5 mL of methanol was added to a solution of trimethoprim ligand (1 mmol) in 5 mL of distilled water and finally 5 mL of methanol was added to the mixture and the mixture was heated under reflux. Lastly, the solution of nicotinamide (1 mmol) in distilled water (10 mL) was added dropwise to a stirring solution. It was allowed to reflux for 5 hr. The resulting solution was left for 20–22 days for crystallization at room temperature. The precipitate formed were filtered and washed with cold water and dried in vacuum. The mixed ligand metal complexes were prepared according to Equation 1.

$$MCl_2.2H_2O + TMP + NIC \rightarrow [M(TMP)(NIC)(H_2O)_2]Cl_2 \quad (1)$$

where M is the metal ions, NIC is nicotinamide and TMP is trimethoprim.

2.3 Antimicrobial Screening and Procedure

The method adopted by Matangi *et al.* [15] was adopted in studying the antimicrobial activity. 7 gram of nutrient agar was weighed into a 250 mL conical flask, 250 mL distilled water (sterilized for 24 h) was mixed with the agar and it was covered properly with cotton wool and foil paper so as to avoid contamination. The solution was heated for 15 min so as to dissolve the nutrient agar, it was sterilized for 24 h in an autoclave. The nutrient agar was introduced into the Petri dish and was allowed to set properly to solidify. A 1 cm hole was bored at the centre of the plate with the aid of a hole borer and was allowed to remove the cracked hole so as to view the bottom of the Petri dish. The antibacterial activity of the ligands and complexes were determined at various concentrations: 200 mg/L and 500 mg/L. These various concentrations of complexes and ligands solutions were poured gently into the hole bored in the Petri dish and left covered. This was left in the incubator for 24 h to allow the outgrowth of the microorganism. The zone inhibitory of the complexes and ligands were determined.

3. Results and Discussions

The complexes were prepared by the reaction of nicotinamide and trimethoprim with metal salts in the ratio 1:1:1. The melting point obtained for each of the complexes is higher than the melting point of the ligands ranging from 198-272^{o}C (Table 1). This shows that there is coordination between the ligands and their metal salts, thereby resulting to complexation [17]. The results of the elemental analysis of some of the complexes are presented in Table 2 and the results of the electronic spectra of the ligands and their complexes are presented in Table 3.

Table 1. Some Physical Properties of Nicotinamide, Trimethoprim and their Complexes

Ligands/ complexes	State	Colour	Melting point (^{0}C)	Conductivity (μScm^{2})
Nicotinamide	Crystalline	White	128-131	2.9
Trimethoprim	Crystalline	White	199-203	2.7
[Mn(NIC)(TMP)(H_2O)$_2$].Cl_2	Crystalline	Pink	198–200	107
[Cu(NIC)(TMP)(H_2O)$_2$].Cl_2	Crystalline	Green	245 –247	14
[Co(NIC)(TMP)(H_2O)$_2$].Cl_2	Crystalline	Pink	270- 272	60.5
[Fe(NIC)(TMP)(H_2O)$_2$].Cl_2	Powdery	Orange	250 –252	13.2
[Zn(NIC)(TMP)(H_2O)$_2$].Cl_2	Powdery	White	220 – 222	1.6

Table 2. Elemental Analysis of some Metal Complexes

Complexes	% Calculated (% Found)		
	% C	% H	% N
[Ni(NIC)(TMP)(H_2O)$_2$].C_{l2}	41.77 (41.59)	4.38 (4.79)	14.61 (14.51)
[Co(NIC)(TMP)(H_2O)$_2$].Cl_2	41.76 (41.61)	4.38 (4.36)	14.61 (14.44)
[Fe(NIC)(TMP)(H_2O)$_2$].Cl2	47.63 (47.65)	3.37 (3.38)	5.85 (5.87)

.**Table 3.** The Electronic Spectra for the Free Ligands and its Complexes.

Ligands/Complexes	Wavelength (nm)	Energies (cm^{-1})	Tentative Assignment
Trimethoprim	312	32051	n → π*
Nicotinamide	293	34130	n → π*
[Mn(TMP)(NIC)(H_2O)$_2$].Cl_2	318	31447	n → π*
[Cu(TMP)(NIC)(H_2O)$_2$].Cl_2	680	14706	MLCT
[Co(TMP)(NIC)(H_2O)$_2$].Cl_2	317	31546	n → π*
[Fe(TMP)(NIC)(H_2O)$_2$].Cl_2	299	33445	n → π*
[Zn(TMP)(NIC)(H_2O)$_2$].Cl_2	303	33003	n → π*

Table 4. Characteristic IR Bands (cm^{-1}) of the Ligands and its Complexes.

Ligands/Complexes	*ν*(N-H)	*ν*(C=C)	*ν*(N-O)	*ν*(C-N)	*ν*(M-N)	*ν*(M-O)
Trimethoprim	3313	------	1382	1245		
Nicotinamide	3365	1620	-----	1394		
[Mn(TMP)(NIC)(H_2O)$_2$].Cl_2	3467	1631	1333	1056	502	418
[Cu(TMP)(NIC)(H_2O)$_2$].Cl_2	3421	1658	1422	1127	606	528
[Co(TMP)(NIC)(H_2O)$_2$].Cl_2	3403	1587	1338	1126	529	406
[Fe(TMP)(NIC)(H_2O)$_2$].Cl_2	3406	1589	1342	1039	524	432
[Zn(TMP)(NIC)(H_2O)$_2$].Cl_2	3415	1637	1381	1047	525	434

The infrared spectra of the ligands and the complexes are shown in Table 4. In nicotinamide, the band at 3365 cm^{-1} was assigned to *ν*(N-H), the bands at 1394 cm^{-1} was assigned to *ν*(C-N), both of the bands have been shifted to a different frequency in the synthesized complexes. This indicates that the coordination of the nicotinamide occurs through the nitrogen of the pyrimidine group and the nitrogen of the amine group [18]. Nicotinamide acts as a bidentate ligand. In the infrared spectra of trimethoprim, the band at 1125 cm^{-1} was assigned to *ν*(C-O). The reaction of nicotinamide and trimethoprim with the metal salts was evidenced by the shifting of the band 3365 cm^{-1} which is assigned to *ν*(N-H) to a different frequency in the complexes. From the results obtained in conductivity measurements, it was observed that the complexes are non-electrolyte [19]. In order to clarify the mode of bonding and the effect of the metal ion on the ligand, the FT-IR spectra of the Ligands and metal complexes have been compared and assigned on the basis of careful comparison. The FT-IR spec-

tra of the ligands and its metal complexes are listed in Table 4. Trimethoprim possesses seven potential donor sites; two pyrimidinyl N atoms, two NH_2 groups on the pyrimidine rings and three methoxy groups. So, the *v*as(N-H) and *v*s(N-H) modes of the pyrimidine-NH_2 groups in the free TMP are assigned to the strong and sharp bands at 3469 and 3313 cm^{-1}, respectively, which are affected by the presence of hydrogen bonds [20]. In all the complexes, the bands *v*(N-H) due to asymmetric and symmetric vibrations, are present in the region 3467–3403 cm^{-1}; these bands shifted significantly with respect to those of the ligand. This confirms that the metal ion is coordinated to the trimethoprim through the two N atoms that belong to NH_2 groups. *v*(C-N) of conjugated cyclic system of the ligand is lowered in complex with respect to ligand. This is an indication of bonding of metal ion through two–NH_2 nitrogen of conjugated cyclic system [21].

Table 5. Comparison of the Antimicrobial Activity of the Free Ligands and some of their Complexes

	E. coli		***P. a***		***S. a***		***K.* spp.**		***C.* spp.**	
	Concentration of ligands and mixed complexes (mg/L)									
Ligands/complexes	200	500	200	500	200	500	200	500	200	500
Nicotinamide	0	0	20	0	0	30	0	0	0	0
Trimethoprim	53	40	0	0	30	0	0	0	0	0
$[Mn(NIC)(TMP)(H_2O)].Cl_2$	51	20	0	25	60	50	0	0	0	17
$[Cu(NIC)(TMP)(H_2O)].Cl_2$	50	51	0	0	0	0	0	0	0	16
$[Ni(NIC)(TMP)(H_2O)].Cl_2$	54	50	0	17	0	40	0	0	0	15

The antibacteria activities of the ligands and complexes were tested against five bacteria species; *Escherichia coli*, *Pseudosomanas aureginosa*, *Staphylococcus aureus*, *Klebsiella* spp., and *Candida* spp. The antibacterial activities of the compounds were estimated based on the average size of inhibition zone formed around the well on the seeded agar plate. The measurements are taken in millimetres (mm). The result shows that the inhibitory activities of the metal complexes were more pronounced as compared to the parent ligands. This indicates that the mixed metal complexes have more antibacterial inhibitory property especially against the *Escherichia coli*. Lower or Lack of inhibitory effect of some of the complexes may probably be due to the substitution effect during ligation. Also, there was no inhibitory activity on the solvent used in dissolving the compounds, so the solvent is not adding any inhibitory values to the result.

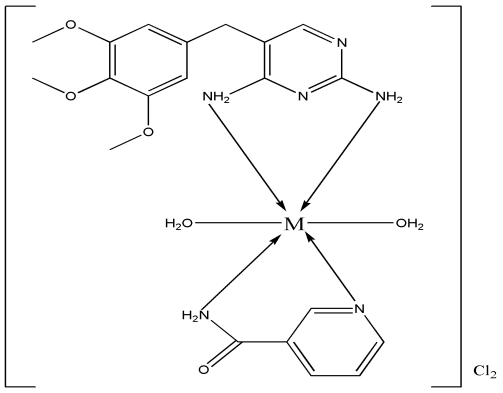

Figure 2. Proposed Structure of $[M(NIC)(TMP)(H_2O)_2].Cl_2$
[M = Mn(II), Cu(II), Fe(II), Zn(II), Co(II)]

The proposed structure shows that the ligands act as bidentate towards the central metal ion and two water molecules coordinated to the metal within the coordination sphere, to give an octahedral geometry for the synthesized complexes.

4. Conclusions

In this study, metal(II) complexes of mixed ligands of trimethoprim and nicotinamide have been prepared and characterized by analytical and spectroscopic methods. The isolated complexes are stable in air, insoluble in water and common organic solvents, but completely soluble in dimethylsulphoxide. The elemental analysis, colour, melting point and the formula weight of the complexes were given in Tables 1 and 2 and agree very well with the molecular formula proposed. The combined results of the physical and spectroscopic studies confirmed the formation of complexes. The analytical data show the composition of the metal complexes to be $[Cu(NIC)(TMP)(H_2O)_2]Cl_2$, $[Zn(NIC)(TMP)(H_2O)_2]Cl_2$, $[Mn(NIC)(TMP)(H_2O)_2]Cl_2$, $[Co(NIC)(TMP)(H_2O)_2]Cl_2$ and $[Fe(NIC)(TMP)(H_2O)_2]Cl_2$. The ligands forming chelating complexes. The molar conductivity measurements of all the compounds were carried out in DMSO ($\sim 1.10^{-3}$ M solutions). The conductivity data of the complexes are very low and they can be regarded as non-electrolytes. Chloride ions in all

complexes have been determined by titration with $AgNO_3$. Moreover, the complexes were subjected to antimicrobial activity tests against *Staphylococcus aures*, *Pseudomonas aureginosa*, *Klebsiella* spp., *Candida* spp. and *Esherichia coli*. The result shows that the complexes have higher inhibitory activities against *Esherichia coli* and other species used compared with their original ligands.

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