

Full Length Research Paper

Antibacterial activity of metal complexes of antifolate drug pyrimethamine

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Ag(I), Co(II) and Cu(II) complexes of pyrimethamine [5-(4-chlorophenyl)-6-ethyl pyrimidinediamine] have been synthesized and characterized by elemental analysis, conductivity measurement, FTIR and UV-Vis spectroscopy. Pyrimethamine act as a monodentate ligand through the pyrimidinic N(1) atom and coordinate the metal ions to give rise to a 4-coordinate tetrahedral and 6-coordinate octahedral for the Co(II) and Cu(II) complexes and linear coordination geometry for the Ag(I) complexes. The *in vitro* antimicrobial activities of the complexes against some bacteria were determined using the macro dilution method. The Ag(I) complexes showed enhanced activity and their minimum inhibitory concentration and minimum bacteria concentration were determined.

Key words: Pyrimethamine, metal complexes, antimicrobial activity, antibiotic, drug resistance.

INTRODUCTION

Compounds containing pyrimidine rings play a significant role in biological systems (Saha and Kar, 1977). Pyrimethamine is an antifolate drug widely used in malarial chemotherapy. It inhibits the bacterial enzymes dihydrofolate reductase (Macreadie et al., 2000; Simo et al., 2000). Pyrimethamine is generally administered in conjunction with a sulphonamide resulting in a synergistic effect due to the sequential inhibition of enzymatic steps in the folate synthesis provided by the combination (Sirawaraporn and Yuthavong, 1986; Chulay et al., 1984). It is an effective drug against protozoan parasites, such as *Toxoplasma gondii* and *Plasmodium falciparum*, is poorly water soluble, weakly basic (pka 7.34) and exhibit marked variation in oral bioavailability (Onyeji et al., 2009). At present, disease-causing microbes that have become resistant to antibiotic drug therapy are on the increase and resistance against antimicrobial agents is becoming a public health problem worldwide (Levy, 1998; Cohen, 1992; Neu, 1992). In the search for novel therapy against resistant organism, the modification of existing drug by combination to a metal centre has gain-

ed attention in recent years (Navarro et al., 1997; Delhaes et al., 2001).

The efficacies of some therapeutic agents are known to increase upon co-ordination, thus metal-based drug is seen as promising alternatives for possible replacement for some of the current drugs. A number of antibiotics such as bleomycin, streptonigrin and bacracin have been reported to function properly upon coordination with metal ions (Li-June, 2003). Metal complexes as pharmaceuticals have received considerable attention in the development of anticancer agents using platinum, ruthenium and other metals, with greater efficacy and reduced toxic side effects (Timerbaev et al., 2006). Vanadium compounds, either alone or in combination with other agents, have the potential to serve as anti-diabetic agents (Srivastava 1995). Studies on Cu(II) with pyrimethamine have been reported and the complex showed some activity against resistant strain of *Plasmodium falciparum* and *Trypanosoma brucei rhodosiense* (Ajibade and Kolawole, 2008). The recognition of the potential employment of metal complexes and chelates in therapeutic application provides useful outlet for basic research in transition metal chemistry (Obaleye et al., 1997) and in line with this application we present the synthesis, characterization and antimicrobial studies of Ag(I), Co(II) and Cu(II) complexes of pyrimethamine drug.

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MATERIALS AND METHODS

Materials

All metal salts, reagents and chemicals are of analytical grade and they were used without further purification. Melting point determination was carried out using Gallenkamp melting point apparatus, conductivity measurement was carried out using CON 6/TDS 6 Hand-held conductivity/TDS Meter. FTIR spectra were obtained on a Perkin-Elmer Lambda 25 Model System 2000 FT-IR spectrophotometer in the range 4000 - 370 cm^{-1} . UV-Vis spectra was obtained on an Elmer Lambda 25 UV-VIS spectrophotometer. *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 19582), *Enterobacter cloacae* (ATCC 13047), and *Proteus vulgaris* (CSIR 0030) typed cultures as obtained from the American Typed Culture Collection (ATCC) were used.

Synthesis of the metal complexes

Pyrimethamine (4 mmol, 0.995 g) was dissolved in 50 mL of methanol and heated slightly for the drug to dissolve completely. This was followed by drop-wise addition of corresponding metal salts in methanol in 2:1 mole ratios for Cu(II) and Co(II) complexes and 1:1 for silver complexes. In some cases, 1 mole of diphenylphosphinoethane (DPPE) was added. The resultant solution in each case was refluxed for 6 h, evaporated under reduced pressure and the precipitate filtered off and washed with methanol and diethylether and dried in vacuum.

Antimicrobial study

The metal complexes were dissolved in minimum amount of dimethylformamide and diluted with water. Mueller-Hinton agar medium suitable for testing the sensitivity of clinically important pathogens towards antibiotics, and nutrient broth were prepared with the standard preparatory techniques. Typed culture organisms comprising gram positive and gram negative bacteria isolates were sub-cultured (Broth inoculation of organisms) and placed in an incubator with temperature of 37 - 39°C for 18 - 24 h. Sensitivity testing to ascertain the activities of the metal complexes was determined using agar well diffusion method as described (Irobi et al., 1994; Russell and Furr, 1977) with little modifications.

Minimum inhibitory concentration (MIC)

Duplicates of two fold serial dilution of complexes were prepared from a solution of metal complexes in sterilized distilled water. 2 ml of different concentration of the prepared solutions was added to 18 ml of pre-sterilized molten nutrient agar at a temperature of 50°C to give final concentrations of 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 and 0.0078125 mg/mL. The medium was poured to sterile Petri-dishes to allow it to set. The dry surface of the nutrient agar medium was then streaked with sub-cultured bacterial isolates. Plates were incubated after streaking, in an incubator at 37°C for 24 h, and examined for the presence or absence of growth (Akinpelu and Kolawole, 2004). MIC was taken as the lowest concentration that will prevent the bacteria growth.

Minimum bacteria concentrations (MBC)

MBC of metal complexes were determined using (Olorundare et al.,

1992). Samples of organisms were taken from plates which were used for the MIC test that were with no visible growth and sub cultured on to freshly prepared nutrient agar medium by streaking. These plates were incubated at 37°C for 24 h. The MBC was taken as the lowest concentration of the complexes that did not allow any bacterial growth on the surface of the agar plates.

RESULTS

The analytical data for the complexes are presented in Table 1, percentage yield, melting point / decomposition temperature and conductivity measurement of the complexes as well as their colour are presented in Table 2. The physical properties of the complexes showed that their colour varied from grey-white for the silver complexes to pink and green for copper and cobalt complexes. The complexes are stable solid with melting point ranging from 160 - 238°C. Conductivity measurements of the complexes as presented in Table 2 showed that the complexes are non-electrolytes in solution. Selected infrared bands for the metal complexes and the pyrimethamine drug are presented in Table 3. A comparison of the IR spectrum of the ligand with the metal complexes showed that the N-H band in the pyrimethamine experience little or no change in the complexes while the C=N bond shifted in the complexes as compared to the ligand. The electronic spectra of the complexes also confirm the coordination of the metal ions to pyrimethamine and are in accord with the geometries proposed for the complexes. The *in vitro* antimicrobial screening of the complexes showed that the Co(II) and Cu(II) complexes does not possess any antimicrobial activity while the Ag(I) complexes have some antimicrobial properties. The zones of inhibition of the Ag(I) complexes are presented in Table 4. The minimum inhibitory concentration (MIC) and the minimum bacteria concentration (MBC) of these complexes were determined. The results are presented in Tables 5 and 6.

DISCUSSION

Metal complexes of pyrimethamine drug were prepared by the reaction of the appropriate metal salts with pyrimethamine drug in methanol. The complexes were isolated as air stable solids. Elemental analysis revealed the composition of the metal complexes. All the metal complexes melted/ decomposed below 250°C and their solubility studies showed that they are generally insoluble in both polar and non-polar solvent but soluble in polar coordinating solvents such as dimethylsulfoxide and dimethylformamide. The conductivity measurements in DMF at 10^{-3} mol dm^{-3} showed that the complexes are non-electrolyte in solution. The analytical data, colour, melting point/ decomposition temperature, molecular formula and conductivity data of the complexes are presented in Table 1. Ag(I) complexes formed a two coordinate specie

Table 1. Analytical data of complexes

Compound	Molecular formula	Formula weight	Analytical data found (Calculated)		
			C	H	N
[Ag(pyrm) ₂ Cl]·3H ₂ O	C ₂₄ H ₃₂ N ₈ Cl ₃ O ₃ Ag	694.79	41.64 (41.49)	3.99 (4.64)	16.74 (16.13)
[Ag(pyrm)CH ₃ COO]	C ₁₄ H ₁₆ N ₄ ClO ₂ Ag	415.63	40.66 (40.46)	3.25 (3.88)	13.36 (13.48)
[Cu(pyrm) ₂ Cl ₂]·H ₂ O	C ₂₄ H ₂₈ N ₈ Cl ₄ OCu	649.89	43.80 (44.35)	4.45 (4.34)	17.60 (17.24)
[Cu(pyrm) ₂ (CH ₃ COO) ₂]	C ₂₈ H ₃₂ N ₈ Cl ₂ O ₄ Cu	679.06	50.22 (49.52)	4.87 (4.75)	18.27 (16.50)
[Cu(pyrm) ₂ (CH ₃ COO) ₂ DPPE]·H ₂ O	C ₅₄ H ₅₈ N ₈ Cl ₂ O ₅ P ₂ Cu	1095.49	58.81 (59.20)	5.18 (5.34)	10.42 (10.23)
[Co(pyrm) ₂ (CH ₃ COO) ₂ DPPE]·3H ₂ O	C ₅₄ H ₆₂ N ₈ Cl ₂ O ₇ P ₂ Co	1126.91	57.16 (57.55)	5.24 (5.55)	10.23 (9.94)
[Co(pyrm) ₂ Cl ₂ DPPE]·2H ₂ O	C ₅₀ H ₅₄ N ₈ Cl ₄ O ₂ P ₂ Co	1061.71	56.91 (56.56)	4.97 (5.13)	10.74 (10.55)

Table 2. Some physical properties of the complexes.

Compound	Colour	M.P/D.T (°C)	Cond. (μS)	(%) Yield
[Ag(pyrm) ₂ Cl]·CH ₃ OH	Gray	238	3.70	97
[Ag(pyrm)CH ₃ COO]	White	199	60.80	74
[Cu(pyrm) ₂ Cl ₂]·H ₂ O	Purple	218	46.70	96
[Cu(pyrm) ₂ (CH ₃ COO) ₂]	Green	208	2.77	95
[Cu(pyrm) ₂ (CH ₃ COO) ₂ DPPE]·H ₂ O	Green	160	13.99	89
[Co(pyrm) ₂ (CH ₃ COO) ₂ DPPE]·3H ₂ O	Pink	236	4.19	98
[Co(pyrm) ₂ Cl ₂ DPPE]·2H ₂ O	Dark Green	206	43.60	97

Table 3. Relevant infrared frequencies (cm⁻¹) for the ligand and the complexes.

Compound	ν(C=N)	ν(N-H)s	ν(N-H)as	ν(CO ₂)
Pyrimethamine	1576	3468	3311	
[Ag(pyrm) ₂ Cl]·CH ₃ OH	1559	3468	3312	
[Ag(pyrm)CH ₃ COO]	1578	3468	3339	1597, 1433
[Cu(pyrm) ₂ Cl ₂]·H ₂ O	1560	3441	3315	
[Cu(pyrm) ₂ (CH ₃ COO) ₂]	1560	3468	3316	1478
[Cu(pyrm) ₂ (CH ₃ COO) ₂ DPPE]·H ₂ O	1560	3468	3313	1479
[Co(pyrm) ₂ (CH ₃ COO) ₂ DPPE]·3H ₂ O	1562	3440	3394	1479
[Co(pyrm) ₂ Cl ₂ DPPE]·2H ₂ O	1577	3440	3312	

by coordinating to one molecule of pyrimethamine, while the Co(II) and Cu(II) complexes formed a tetrahedral and octahedral coordination depending on the reactants involved in the synthesis process, with the metal coordinating with two molecules of pyrimethamine each (Figure 2).

The IR spectra of the ligands and complexes were

compared and assigned on the basis of careful comparison Table. The N-H stretching frequencies of the pyrimethamine molecule undergoes a slight shift in all the complexes. These could be attributed to the overlap of $\nu(\text{N-H})$ and $\nu(\text{O-H})$ of methanol or inter and intra molecular hydrogen bonding (Ajibade and Kolawole, 2008). The pyrimidinic N(1) atom which appears as

Table 4. Sensitive patters of zones of inhibition exhibited by complexes on some pathogens.

Microorganism	[Ag ₂ (pyrm) ₂ Cl]·3H ₂ O mg/ml	[Ag(pyrm)CH ₃ COO] mg/ml
<i>Staphylococcus aureus</i>	20.5	22.0
<i>Streptococcus faecalis</i>	18.5	21.5
<i>Bacillus cereus</i>	10.5	15.5
<i>Bacillus pumilus</i>	9.5	16.5
<i>Escherichia coli</i>	9.0	14.0
<i>Pseudomonas aeruginosa</i>	8.0	18.5
<i>Enterobacter cloacae</i>	15.5	19.5
<i>Proteus vulgaris</i>	7.5	12.5

Table 5. The minimum inhibitory concentrations (MIC) exhibited by the complexes against the bacteria isolates.

Microorganism	[Ag ₂ (pyrm) ₂ Cl]·3H ₂ O mg/ml	[Ag(pyrm)CH ₃ COO] mg/ml
<i>Staphylococcus aureus</i>	0.03125	0.0625
<i>Streptococcus faecalis</i>	0.03125	0.0625
<i>Bacillus cereus</i>	0.03125	0.0625
<i>Bacillus pumilus</i>	0.03125	0.0625
<i>Escherichia coli</i>	0.03125	0.0625
<i>Pseudomonas aeruginosa</i>	0.03125	0.0625
<i>Enterobacter cloacae</i>	0.03125	0.0625
<i>Proteus vulgaris</i>	0.0625	0.0625

a prominent single band at 1576 cm⁻¹ in the parent drug shift to different position in the complexes. This may be attributed to the fact that coordination of the parent drug to the metal ions preferentially takes place through the N(1) atom (Ajibade et al, 2006). The presence of the acetate ion in some complexes is confirmed by bands at about 1479 cm⁻¹ (Simo et al; 2000; Ajibade et al 2007).

The silver complexes occur as a two coordinate linear species. Their electronic spectra do not have a band in the visible region as a result of the absence of a d - d transition which is indicative of a d¹⁰ Ag(I) configuration. Ag(I) complexes possess a transition band at about 250 nm which is a characteristics of a metal charge 4d - 4s transition at relatively high energies (Kunkely and Vogler, 2006). The electronic spectra of [Cu(pyrm)₂(CH₃COO)DPPE]·H₂O showed a broad band in the region 700 nm expected for a d - d transition of an octahedral Cu(II) complex. The broadness of the band could be attributed to the overlapping of several bands as a result of strong Jahn-Teller distortion expected in a d⁹ ion (Ajibade et al., 2006). In the electronic spectra of the Co(II) complexes, [Co(pyrm)₂(Cl)₂DPPE]·2H₂O show two bands of weak to medium intensity at 600 nm and 690 nm respectively which may tentatively be assigned to ⁴T_{1g}(F) → ⁴T_{1g} and ⁴T_{1g}(F) → ⁴T₁(P) transitions of pseudo-octahedral cobalt(II). (Ajibade et al., 2006). [Co(pyrm)₂(CH₃COO)DPPE]·3H₂O shows a single weak transition at about 450 nm which can be attributed to the d - d transition of a d⁷

Co(II).

Antimicrobial test

Selectivity test of metal complexes on bacteria isolates revealed that the silver complexes possess antimicrobial activities compared to the pyrimethamine drug which did not show any activity. The Ag(I) complexes exhibited activities against all eight bacterial isolates comprising of both gram-positive and gram-negative organisms. These showed that the Ag(I) complexes possess a broad spectrum activity. *P. vulgaris* shows the least zone of inhibition with 7.5 mm and 12.5 mm in [Ag(pyrm)₂Cl]·3H₂O and [Ag(pyrm)CH₃COO] respectively. While *Staphylococcus aureus* has the highest zone of inhibition with 20.5 mm and 22 mm for [Ag(pyrm)₂Cl]·3H₂O and [Ag(pyrm)CH₃COO] respectively as shown in Table 4. The MIC of the silver complexes as presented in Table 5 shows that [Ag₂(pyrm)₂Cl]·3H₂O has an MIC value of 0.03125 mg/mL against all the organisms except for *P. vulgaris* with a value of 0.0625 mg/ml. And for the complex [Ag(pyrm)CH₃COO], the MIC value against all the organism is 0.0625 mg/ml. This results indicated that [Ag(pyrm)₂Cl]·3H₂O has stronger activity. The MBC (minimum bacteria concentration) value which is the lowest concentration of the metal complex that will prevent the bacteria growth was performed. [Ag(pyrm)₂Cl]·3H₂O

Table 6. The minimum bacteria concentrations (MBC) exhibited by the complexes against the bacteria isolates

Microorganism	[Ag(pyrm) ₂ Cl]·3H ₂ O mg/ml	[Ag(pyrm)CH ₃ COO] mg/ml
<i>Staphylococcus aureus</i>	0.03125	0.0625
<i>Streptococcus faecalis</i>	0.03125	0.0625
<i>Bacillus cereus</i>	0.03125	0.0625
<i>Bacillus pumilus</i>	0.03125	0.0625
<i>Escherichia coli</i>	0.03125	0.0625
<i>Pseudomonas aeruginosa</i>	0.03125	0.0625
<i>Enterobacter cloacae</i>	0.0625	0.0625
<i>Proteus vulgaris</i>	0.0625	0.0625

has MBC value of 0.03125 mg/mL against all bacteria isolate except *Enterobacter cloacae* and *P. vulgaris* with 0.0625 mg/mL MBC value. [Ag(pyrm)CH₃COO] has MBC value of 0.0625 mg/mL against all the bacteria isolates as shown in Table 5. This result along with MIC results further reveals that [Ag(pyrm)₂Cl]·3H₂O has a stronger antimicrobial activity.

Conclusion

Air stable Ag(I), Co(II) and Cu(II) complexes of pyrimethamine have been synthesized and characterized. Based on their electronic spectral results, a linear geometry has been suggested for the Ag(I) complexes, tetrahedral geometry and an octahedral geometry for Co(II) and Cu(II) complexes. The antimicrobial screening of the complexes showed that the Ag(I) complexes have enhanced activity, with {[Ag₂(pyrm)₂].0.7CH₃OH} showing a stronger antimicrobial potential at an MIC value of 0.03125 mg/ml.

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