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Synthesis, characterization and antibacterial studies on some metal(ii) complexes of 4-amino-6-hydroxy-2mercaptopyrimidine.

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ABSTRACT:Metal(II) complexes of 4-amino-6-hydroxy-2-mercaptopyrimidine were synthesized and characterised by electronic and infrared spectroscopies, conductance, melting point and room temperature magnetic susceptibility measurements {M = Mn, Fe, Co, Ni, Cu and Zn}. The molar conductance measurements in DMSO revealed that the Co(II) complex was a 1:1 electrolyte, and the Cu(II) and Zn(II) complexes were 1:2 electrolytes respectively. Infrared spectra indicated that the ligand coordinated through N_2 chromophores. Electronic spectra and magnetic moments revealed that all the complexes were octahedral and paramagnetic with the exceptions of the Cu(II) and Zn(II) complexes which were square-planar/ tetrahedral and diamagnetic. In addition, the Mn(II) and Cu(II) complexes exhibited antiferromagnetism. The antibacterial studies revealed that the metal complexes were mostly active against *B. cereus* and *S. aureus*, exhibited selective activity towards *P. mirabilis* and were mostly inactive against *E. coli*, *P. aeruginosa*, *K. oxytoca* and *S. aureus*.

Keywords: antibacteria, antiferromagnetism, electrolyte, geometry, spin-crossover.

Introduction

Pyrimidines are renowned for their various biological activities such as, anticancer, antimalaria, antimicrobial and HIV inhibitors (Hafez and El-Gazza, 2009, Reddy and Reddy, 2000, Roy et al., 2007, Sakthilatha and Rajavel, 2013). They are also the basic nucleus of DNA and RNA (Hadjikakou et al., 2000, Ma et al., 2005). Many ligands have been synthesized that contained appended pyrimidine moieties and their metal complexes are found to have very good biological properties for example many pyrimidinyl Schiff base, pyrimidine dithiocarbamate and (pyrimidinylsulfinyl) benzamidazoles complexes have good anticancer and anti-bacteria activities (Khan and Asnani, 2011, Osowole et al., 2010, Osowole et al., 2011, Osowole et al., 2012, Sakthilatha and Rajavel 2013). Literature shows that some work has been done on metal(II) complexes of 4,6-diamino-2-thiopyrimidine, 4,6-diamino-2-methylthiopyrimidine, diaminopyrimidine, 4,6-diamino-5hydroxy-2-mercaptopyrimidine, pyrimethamine and mercapto pyrimidines (Ajibade and Idemudia, 2013, Hadjikakou et al., 2000, Ma et al., 2005, Gutierrez et al., 1987, Sahar et al., 2008, Mostafa and Badria, 2008). However, no work has been reported on the metal(II) complexes of 4-amino-6-hydroxy-2-mercaptopyrimidine. This work is new, and is an extension of our earlier studies on metal(II) complexes of various chloropyrimidines (Osowole and Oni, 2013, Osowole and Oni, 2013), as a continuation of our research activities on various metal(II) mixed ligand complexes of various aminopyrimidines and 2,2'-bipyridine/1,10phenanthroline with interesting structures and biological properties. Thus, our aims are to synthesise these mixed ligand metal complexes and investigate their magnetic properties for possible cooperative phenomena like ferro- and antiferromagnetisms, as well as their suitability as broad spectrum antibacterial agents invitro.

Material And Methods

Chemicals

4-amino-6-hydroxy-2-mercaptopyrimidine monohydrate, CuCl₂.2H₂O, Mn(COOCH₃)₂. 4H₂O, Zn(NO₃)₂.6H₂O, Co(NO₃)₂.6H₂O, Ni(COOCH₃)₂.4H₂O and FeSO₄.7H₂O were purchased from Aldrich and British Drug Houses Ltd (BDH) and were used as received. The solvents were purified by distillation.

Physical measurement

The solid reflectance and infrared spectra for the complexes were recorded on a Perkin-Elmer $\lambda 25$ and Perkin Elmer FT-IR BX spectrophotometers respectively. Electrolytic conductivities in DMSO and melting points were determined using electrochemical analyser and Mel-Temp electrothermal machine respectively, while the room temperature magnetic measurements at 303K were done by using Sherwood Susceptibility balance MSB Mark 1.

Preparation of the metal(II) complexes

The metal(II) complexes were prepared by adding $0.21 \text{ g} - 0.37 \text{ g} (1.24 \times 10^{-3} \text{ moles})$ of the hydrated metal(II) acetates, chlorides, sulphates and nitrates in bits to a stirring solution of $0.40 \text{ g} (2.48 \times 10^{-3} \text{ moles})$ of 4-amino-6-hydroxy-2-mercapto pyrimidine in 30 mL of 70 % ethanol. The pH of the resulting homogeneous coloured solutions were adjusted to 9 with triethylamine, and then refluxed for 3 h. The precipitates formed were filtered, washed with 70% ethanol and dried over anhydrous CaCl₂.

Antimicrobial Studies

Antibacterial tests on the ligand and metal(II) complexes were done using agar diffusion technique. The surface of Muller Hinton's agar in a petri dish was inoculated uniformly with 0.3 mL of 18 h old test bacteria cultures.10 mg/mL solution of each complex in DMSO was added to a 9 mm well bore unto the agar. The plates were allowed to stand on the bench for 30 minutes followed by incubation at 37°C for 24 h then inhibitory zones were measured as a measure of antibacterial activities of the complexes. The experiments were conducted in duplicates and streptomycin was used as a reference drug.

Results And Discussion

The equations for the formation of the metal(II) complexes are given in equations 1-5 and the metal complexes formed in moderate yields of 40-60%.

$2\mathrm{Mn}(\mathrm{OAc})_2.4\mathrm{H}_2\mathrm{O} + 4\mathrm{L} \rightarrow [{\mathrm{Mn}(\mathrm{L})_2}_2(\mathrm{OAc})_2]. 4\mathrm{H}_2\mathrm{O} + 2\mathrm{HOAc}$	(1)
$FeSO_4.7H_2O + 2L \rightarrow [Fe(L)_2(SO_4)] + 7H_2O$	(2)
$CuCl_2$. $2H_2O + 2L \rightarrow [Cu(L)_2]Cl_2 + 2H_2O$	(3)
$Co(NO_3)_2.6H_2O + 2L \rightarrow [Co(L)_2(NO_3)(H_2O)]NO_3 + 5H_2O$	(4)
$Zn(NO_3)_2.6H_2O + 2L \rightarrow [Zn(L)_2](NO_3)_2.2H_2O + 4H_2O$	(5)

The ligand had a decomposition temperature of 312°C, whereas its metal complexes decomposed in the range 132-234°C, confirming coordination. The complexes were all slightly soluble in water, methanol, ethanol, nitromethane and methylene chloride but were soluble in DMSO. The analytical data, colours, % metal, %yields, melting points, molar conductivity and room temperature magnetic moments for the complexes are presented in Table 1.

Molar conductance and infrared spectra measurements

The molar conductivities of all the complexes in DMSO showed that the Mn(II), Co(II) and Fe(II) complexes had very low molar conductance values in the range 4.35⁻⁵.16 Ω^{-1} cm²mol⁻¹ and thus were covalent. The Co(II) complex had a value of 59.2 Ω^{-1} cm²mol⁻¹, the Cu(II) and Zn(II) complexes had molar conductance values of 98.7 Ω^{-1} cm²mol⁻¹ and 81.0 Ω^{-1} cm²mol respectively which indicated 1^{:1} and 1^{:2} electrolytes . Literature reported values in the range 51.5 - 59.2 Ω^{-1} cm²mol⁻¹ and 84.2 - 101.9 Ω^{-1} cm²mol⁻¹ as 1^{:1} and 1^{:2} electrolytes respectively (Geary, 1971).

Electronic spectra and magnetic moments

The Mn(II) complex spectra showed two absorption bands at 12.82 kK and 23.70 kK assigned to ${}^{6}A_{1g} \rightarrow {}^{4}A_{1g}(v_1)$ and ${}^{6}A_{1g} \rightarrow {}^{4}E_{g}(v_2)$ transitions of a 6-coordinate octahedral geometry. The effective magnetic moment of Mn(II) complexes are expected to be close to the spin-only value of 5.90 B.M. since the ground term is ${}^{6}A_{1g}$. A room temperature moment of 3.15 B. M was observed for this complex which is indicative of antiferromagnetism operating through a dimeric structure (Figure 1, Agwara et al., 2010, Osanai et al., 2006). The Fe(II) complex had two absorption bands at 15.23 kK and 22.22 kK typical of high spin and low spin octahedral geometries, and were assigned to ${}^{5}T_{2g} \rightarrow {}^{5}E_{g}$ and ${}^{1}A_{1g} \rightarrow {}^{1}T_{1g}$ transitions. An observed moment of 3.07 B.M was complementary of equilibrium between high spin \leftrightarrow low spin (spin crossover) octahedral forms (Salmon et al., 2009).

Similarly, the Co(II) complex had two absorption bands at 19.23 kK and 22.73 kK, assigned to ${}^{4}A_{2g} \rightarrow {}^{4}T_{1g}(F)$ (v₂) and ${}^{4}A_{2g} \rightarrow {}^{4}T_{1g}(P)(v_3)$ transitions of a 6-coordinate octahedral geometry. This geometry was corroborated by a room temperature magnetic moment of 4.60 B.M since moments in the range 4.6 – 5.2 B.M is usually observed for octahedral Co(II) (Roy et al., 2007).

The Ni(II) complex showed two absorption bands at 14.71 kK and 23.81 kK typical of an octahedral geometry, and were assigned to ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g}(v_{2})$ and ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g}(P)(v_{3})$ transitions respectively. This geometry was supported by an observed room temperature magnetic moment of 3.77 B. M since moments in the range 3.7–4.00 B. M were reported for distorted octahedral Ni(II) complexes (Kalagouda et al., 2006).

The Cu(II) complex displayed two bands at 12.20 kK and 22.22 kK, and were assigned to ${}^{2}B_{1g} \rightarrow {}^{2}A_{1g}$ and ${}^{2}B_{1g} \rightarrow {}^{1}E_{1g}$ transitions of a four coordinate square planar geometry, because tetrahedral and octahedral Cu(II) complexes usually have a single band below and above 10.0 kK respectively. Interestingly, this Cu(II) complex was diamagnetic due to very strong antiferromagnetic interactions, operating through Cu-Cu bond (Figure1, Osanai et al., 2006).

The Zn(II) complex had only charge transfer transitions from metal to ligand at 12.70 kK and 16.67 kK since no d - d transition is expected. This complex was expectedly diamagnetic and tetrahedral (Agwara, et al., 2012).



Figure 1. The proposed structures for the Mn(II) and Cu(II) complexes

Infrared spectra

The $u(NH_2)$ vibrations in the metal-free ligand, 4-amino-2-mercaptopyrimidine, was observed at 3395 cm⁻¹ (Ajibade and Idemudia, 2013, Hadjikakou et al., 2000). This band shifted to 3428 - 3484 cm⁻¹ in the metal(II) complexes due to coordination of the N donor atom of the amino group (Ma et al., 2005, Gutierrez et al., 1987). The u(C=N) band in the metal-free ligand, 4-amino-2-mercaptopyrimidine, at 1654 cm⁻¹ shifted to 1651 - 1657 cm⁻¹ in the complexes indicating the involvement of N donor atom of u(C=N) in coordination

(Sahar et al., 2008). The v(C=C) stretching frequencies in the ligand at 1423 cm⁻¹ also shifted to 1423 – 1424 cm⁻¹ in the complexes due to coordination (Khan and Asnani, 2011). The bands due to v(M-N) and v(M-O) / v(M-Cl) were absent in the spectrum of metal-free ligand, 4-amino-2-mercaptopyrimidine but were observed in the range 527 – 581 cm⁻¹ and 335 – 379 cm⁻¹ in the metal complexes, as further evidence of coordination (Mostafa and Badria, 2008).

Antibacterial activities

The results are presented in Figure 2 and Table 3. The metal(II) complexes were generally not active against Gram negative bacteria, *that is, E. coli, P. mirabilis, P. aeruginosa* and *K. oxytoca* but showed very good activities against Gram positive *B. cereus and S. aureus* with inhibitory zones range of 11.0-33.0 mm and 11.0-20.0 mm respectively. Generally, the metal complexes that were active, had higher activity than the ligand, 4-amino-2-mercaptopyrimidine, due to chelation which reduces the polarity of the metal ion as a result of the partial sharing of its positive charge with the donor group and possible π – electron delocalization within the aromatic ring.



Figure 2. The histogram of the antibacterial activities of the ligand and metal complexes.

This increases the lipophilic character of the complexes and hence favouring its permeation through the lipid layers of the cell membrane (Nishat and Rahis-ud-din, 2004). The inactivity of some the metal(II) complexes were attributed to their probable lipophobic nature and as such could not penetrate through the lipid membrane of the bacteria (Nair et al., 2006). The Zn(II) complex had the best activity, and was active against four organisms, that is, *B. cereus, E. coli, P. mirabilis* and *S. aureus* with inhibitory zones range of 11.0-20.0 mm. The Co(II) and Mn(II) complexes were both active against three organisms, that is, *B. cereus, P. mirabilis* and *S. aureus, P. mirabilis*, and *K. oxytoca* with inhibitory zones range of 11.0-19.0 mm and 11.0-16.0 mm respectively. Similarly, the Ni(II) and Cu(II) complexes were both active against same two organisms, that is, *B. cereus* and *S. aureus* with inhibitory zones of 17.0 mm and 16.0 mm, and 33.0 mm and 13.0 mm respectively. The Fe(II) complex had least activity, and was active against one organism, *that is, B. cereus* with an inhibitory zone of 13.0 mm. Thus, the decreasing order of antibacterial activities of the complexes was Zn(II) complex > Co(II) complex ~ Mn(II) complex > Cu(II) complex ~ Ni(II) complex > Fe(II) complex. Expectedly, Streptomycin was more active than all the metal complexes and the Zn(II) complex had a broad spectrum anti-bacteria activity against *B. cereus, E. coli, P. mirabilis*, and *S. aureus* with inhibitory zones range of 11.0-20.0 mm.

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Complex	Molar mass	Colour	% Yield	% Metal		D.t (°C)	$\begin{array}{c} \text{D.t} & \uparrow_{\text{m}} \\ \text{(oC)} & (\Omega^{\cdot 1} \text{cm}^2 \text{mol}^{\cdot 1}) \end{array}$	
				Expr	Theo			
L	161.18	Cream		-	-	*312	-	-
$[{Mn(L)_2}_2(OAc)_2].4H_2O$	1016.90	Brown	40	9.45	10.82	132	5.16	3.15
$[Fe(L)_2(SO_4)]$	474.23	Brown	60	12.29	11.78	204	4.65	3.07
$[C_0(L)_2((NO_3)H_2O)]NO_3.H_2O$	541.31	Green	40	10.60	10.89	190	59.2	4.60
$[Ni(L)_2(OAc)_2]$	499.12	Green	50	11.44	11.76	166	4.35	3.77
$[{Cu(L)_2}Cl_2]_2$	913.60	Green	50	15.78	14.01	232	98.7	D
$[Zn(L)_2](NO_3)_2.2H_2O$	547.74	White	40	11.97	11.94	234	81.0	D

Table 1. Analytical data for the metal(II) complexes

Key: L = 4-amino-6-hydroxy-2-mercaptopyrimidine, * = melting point point, ^m = molar conductance, D. t = Decomposition temperature.

Table 2. Infrared spectra	of metal(II)	complexes
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Complex	$v(NH_2)$	u(C=N)	v(C=C)	v(M-N	v(M-O)/	Electronic Spectra(kK)
					v(M-Cl)	
L	3395b	1654s	1423m	-	-	32.26, 38.46
$[{Mn(L)_2}_2(OAc)_2].4H_2O$	3435b	1655s	1424s	573b	357m	12.82, 23.70, 32.47
$[Fe(L)_2(SO_4)]$	3428b	1656s	1423s	581b	368m	15.23, 22.22, 32.47, 38.46
$[C_0(NO_3)(L)_2(H_2O)]NO_3.H_2O$	3484b	1657s	1423s	547b	368m	19.23, 22.73, 36.76
[Ni(L) ₂ (OAc) ₂]	3458b	1653s	1423s	527b	379m	14.71 ,23.81, 32.25
$[{Cu(L)_2}Cl_2]_2$	3432b	1656s	1424s	565b	369s	12.20, 22.22, 30.30, 37.04
$[Zn(L)_2](NO_3)_2.2H_2O$	3460b	1651s	1423m	538b	335s	12.70,16.67, 32.25

Key L =4-amino-2-mercaptopyrimidine, s = strong, m = medium, b = Broad

Table 3. Antibacterial of metal(II) Complexes

Complexes	B. cereus	E. coli	P. mirabilis	P. aeruginosa	K. oxytoca	S. aureus
L	R	11.0±2.8	R	R	R	13.0 ± 5.7
$[{Mn(L)_2}_2(OAc)_2].4H_2O$	R	R	13.0 ± 5.7	R	$16.0{\pm}1.4$	11.0 ± 2.8
$[Fe(L)_2(SO_4)]$	13.0 ± 5.7	R	R	R	R	R
[Co(NO ₃)(L) ₂ (H ₂ O)]NO ₃ .H ₂ O	11.0 ± 2.8	R	19.0 ± 2.8	R	R	12.0 ± 4.2
$[Ni(L)_2(OAc)_2]$	17.0±0	R	R	R	R	16.0 ± 1.4
$[{Cu(L)_2}Cl_2]_2$	33.0 ± 2.8	R	R	R	R	13.0 ± 5.7
$[Zn(L)_2(H_2O)_2](NO_3)_2$	12.0 ± 4.2	11.0±0.0	13.0 ± 5.7	R	R	20.0 ± 1.4
Streptomycin	40.0±1.4	34.0 ± 4.2	$56.0{\pm}1.4$	34.0 ± 1.4	26.0 ± 1.4	38.0 ± 7.1

Key: L = 4-amino-2-mercaptopyrimidine, R = Resistance

Conclusion

From the infrared spectra data, the ligand behaved as bidentate and coordinated to the metal ions through the nitrogen atoms of amino and the imine of the 4-amino-2-mercaptopyrimidine. Electronic spectra and magnetic moments revealed that all the complexes were octahedral and paramagnetic with the exceptions of the Cu(II) and Zn(II) complexes which were square-planar/ tetrahedral and diamagnetic. The antibacterial studies revealed that the Zn(II) complex had a broad spectrum anti-bacteria activity against *B. cereus, E. coli, P. mirabilis,* and *S. aureus* with inhibitory zones range of 11.0-20.0 mm.

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