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Synthesis, characterization and antibacterial susceptibility testing of manganese complexes of doxycyline with bipyridine and phenanthroline

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Abstract. Three manganese complexes of the antibiotic doxycyline viz.: manganese doxycyline, $[MnDox_2]Cl_2{\cdot}2H_2O$ (**1**), and manganese doxycyline with bipyridine, $[MnDox_2(bpy)]Cl_2{\cdot}8H_2O$ (**2**), and phenanthroline, $[MnDox_2(phen)]Cl_2{\cdot}8H_2O$ (**3**), as the ancillary ligand were synthesized and characterized by FT-IR, elemental analysis and electrospray mass spectroscopy. The three complexes show good solubility in DMF and DMSO. Data obtained from spectroscopic techniques used show that doxycycline coordinates to the central manganese atom through the oxygen of the amide group and the carbonyl oxygen atom of ring A while bipyridine/phenanthroline coordinates through the two diimine nitrogen atoms. The stoichiometry of manganese-doxycycline is 1:2 and octahedral geometry is the preferred coordination in all the complexes.

Keywords: manganese, doxycycline, coordination, antibacterial, synthesis.

1. Introduction

Manganese is an example of metal ions which form part of the structures of enzymes of most plants and animals which they require for growth. A human body contains a total of about 16 mg of manganese [1]. Manganese as Mn^{2+} is analogous to Mg^{2+} except that it is redox active with +2, +3 and +4 oxidation states having relevance in biology [2]. At least four enzymes – the photosystem II, manganese catalase, manganese superoxide dismutase and ribonucleotide reductase use manganese complex at their catalytic centers which are required for the redox activities of these enzymes [3].

Two possible indirect interactions of manganese ions on DNA structure rather than direct coordination of the ions to DNA have been identified. The first interaction involves the neutralization of the negative charge of the phosphate backbone and stabilization of the double helical structure. The second interaction mode is the prevents DNA renaturation by interaction with sites of the bases that are not involved in base pairing [4].

NMR studies have shown that manganese has affinity for GC residue with a preference for the N7 atom of guanine [5, 6] and some manganese complexes interact with the intra-molecular quadruplex by a one-side external π-stacking [7, 8]. Metallochaperones are metal receptor proteins reported to act in the intracellular trafficking of metal ions [9].

Some manganese complexes have been demonstrated to possess antimicrobial properties. For instance, manganese complexes incorporating 1,10-phenathroline (phen) and malonic acid (mal) (e.g. $[Mn(phen)_2(H_2O)_n]^{2+}$ and $[Mn(phen)_2(mal)]{\cdot}2H_2O$), efficiently inhibit the growth of *C. albicans in vitro* at 37 ^oC [10-12]. Similarly, Mn(II) complexes containing 1,10-phenanthroline and dicarboxylate ligands which possess low toxicity to *mellonella* larvae, VERO and A549 mammalian cells have been reported to strongly inhibit the viability of *M. tuberculosis* strains, H37Rv and CDC1551 [12]. Manganese(II) complexes of thiosemicarbazones which possess low cytotoxicity were reported as potential anti - *M. tuberculosis* agents. Their selectivity index (SI) values were comparable or higher than first line drugs tuberculosis drugs [13]. The activities of these manganese complexes were attributed to high oxidation potentials, suggesting that biological activity might be a function of redox processes [14]. Under conditions of oxidative stress, manganese can also act as a cofactor substitute for iron in iron-containing enzymes [15].

The attention of scientists have also been drawn to the possible *in vivo* application of manganese(II) macrocycles in pain management [16] while some manganese porphyrin complexes have shown biological activity such as SOD-mimics with low aqueous toxicity [17]. Some other manganese-porphyrin complexes cleave DNA by oxidative damage in the presence of oxygen donors [18, 19] while some are capable of catalyzing dismutation of the superoxide anion and are a current focus for developing SOD mimics as drugs because of their low *in vitro* toxicity [20].

In our continuous effort to investigate the effect of complexation of metal ions to biologically relevant ligands [21-25], we have synthesized manganese-doxycycline complex and mixed doxycycline manganese complexes with bipyridine and phenanthroline and characterized them by FT-IR, elemental analysis and electrospray mass spectroscopy. The rationale was based on the aforementioned vital roles manganese and its compounds play in biological systems, and the pleiotropic properties of tetracyclines.

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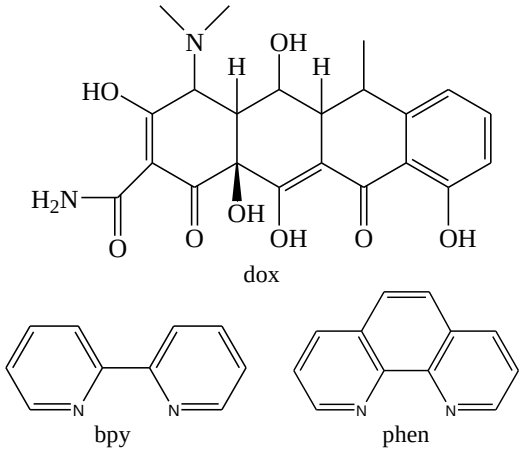
2. Experimental

2.1. Materials and measurements

Doxycycline hyclate was a gift from Neimeth International Pharmaceuticals Plc, Nigeria and fresh solutions were used to ensure stability while 2,2'-bipyridine and 1,10-phenanthroline monohydrate were from SDFCL, India. All other chemicals and reagents are of analytical grade and used without further purifications. Infrared spectra were recorded on Shimadzu FT-IR-8400 on samples pressed in KBr pellets.



Scheme 1. Doxycycline (dox), 2, 2'-bipyridine (bpy) and 1, 10-phenanthroline (phen).

2.2. Syntheses of the complexes

[MnDox$_2$]Cl$_2$·2H$_2$O (1). 2 mmol of Doxycycline hyclate and 1 mmol of $MnCl_2·4H_2O$ were stirred in methanol for 5 hours at room temperature. The resulting yellow solution was filtered, and the filtrate was allowed to evaporate slowly at room temperature. The precipitated off-white crystals were then filtered and washed with methanol before vacuum drying.

Calculated for [MnDox$_2$]Cl$_2$·2H$_2$O (%): C, 50.39; H, 4.81; N, 5.34. Found (%): C, 51.23; H, 5.50; N, 5.29. FT-IR (KBr, ν/cm^{-1}): 1672.34, 1614.47, 1587.47, 1550.82, 1531.53, 1518.03, 1454.38, 1373.36, 1350.22, 1315.50, 1240.27, 1201.69, 1172.76, 1139.97, 1089.82, 1062.81, 1035.81, 993.37,939.36, 885.36, 850.64, 821.70, 800.49, 783.13, 707.90, 667.39, 621.10, 563.23, 542.02, 532.37, 497.65, 434.00, 406.99. ESI-MS: [Mn+3(Dox)-1H$_2$O]$^{2+}$ = 686, [Mn+2(Dox)+H$_2$O] = 964.9. Decomposition temperature: 203-205 ^{o}C.

[MnDox$_2$(bpy)]Cl$_2$·8H$_2$O (2). A mixture of 1 mmol of Doxycycline hyclate and 1 mmol of $MnCl_2·4H_2O$ was stirred in methanol for 1 hour followed by addition of 1 mmol of 2,2'-bipyridine and further stirring for another 3 hours at room temperature. The resulting yellow solution was filtered, and the filtrate was allowed to evaporate slowly at room temperature. The precipitated off-white crystals were then filtered and washed with methanol before vacuum drying. Calculated for [MnDox$_2$(bpy)]Cl$_2$·8H$_2$O (%): C, 49.40; H, 5.37; N, 6.40. Found (%): C, 48.58; H, 4.82; N, 5.87. UV-Vis (H$_2$O, nm): 232, 280, 344. FT-IR (KBr, ν/cm^{-1}): 1672.34, 1664.62, 1612.54, 1599.04, 1550.82, 1531.53, 1510.31, 1500.67, 1446.66, 1375.29, 1352.14, 1315.50, 1242.20, 1201.69, 1172.76, 1139.97, 1089.82, 1058.96, 1035.81, 1010.73, 995.30, 939.36, 904.64, 885.36, 850.64, 819.77, 800.49, 773.48, 734.90, 707.90, 667.39, 623.03 565.16, 545.87, 532.37, 499.58, 459.07, 435.93. Decomposition temperature: 205-206 ^{o}C.

[MnDox$_2$(phen)]Cl$_2$·8H$_2$O (3). A mixture of 1 mmol of Doxycycline hyclate and 1 mmol of $MnCl_2·4H_2O$ in methanol was stirred for 1 hour followed by addition of 1 mmol of 1,10-phenanthroline and further stirring for another 3 hours at room temperature. The resulting yellow solution was filtered, and the filtrate was allowed to evaporate slowly at room temperature. The precipitated off-white crystals were then filtered and washed with methanol before vacuum drying. Calculated for [MnDox$_2$(phen)]Cl$_2$·8H$_2$O (%): C, 50.31; H, 5.28; N, 6.29. Found (%): C, 50.13; H, 5.22; N, 5.83. FT-IR (KBr, ν/cm^{-1}): 1664.62, 1612.54, 1587.47, 1570.11, 1550.82, 1533.46, 1518.03, 1496.81, 1452.45, 1425.44, 1329.00, 1242.20, 1217.12, 1170.83, 1132.25, 1062.82, 1039.67, 1003.02, 935.51m 883.43, 846.78, 825.56, 804.34, 725.26, 713.69, 665.46, 615.31, 584.45, 545.87, 499.58, 459.07, 430.14. ESI-MS: Mn+Dox+2(Phen) = 862.1 and [MnDox2Phen + Na]$^{2+}$ = 575.1 Decomposition temperature: 207-209 ^{o}C.

3. Results and discussion

3.1. Characterization

The complex **1** was prepared from the reaction of $MnCl_2·4H_2O$ with doxycycline while complexes **2** and **3** were prepared from the reactions of $MnCl_2·4H_2O$ with doxycycline and bipyridine or phenanthroline for complex **2** and **3** respectively. All the complexes were obtained in good yield and characterized by elemental analysis, FT-IR and thermal profile.

The results of the elemental analyses are in accordance with the proposed formula: [MnDox$_2$]Cl$_2$·2H$_2$O (**1**), [MnDox$_2$ (bpy)]Cl$_2$·8H$_2$O (**2**) and [MnDox$_2$(phen)]Cl$_2$·8H$_2$O (**3**) where Dox, bpy and phen are doxycyline, bipyridine and phenanthroline respectively.

Complexes **1** and **3** were also characterized by ESI-MS in positive ionization mode. Complex **1** has peaks corresponding to [Mn+3(Dox)-1H$_2$O]$^{2+}$ = 686, [Mn+3(Dox)-1H$_2$O] = 1370 and [Mn+2(Dox)+H$_2$O] = 964.9 while complex **3** has peaks corresponding to [Mn+Dox+2(Phen)] = 862.1 and [MnDox2Phen + Na]$^{2+}$ = 575.1. The contribution of sodium is from the mass spectrometer while the detection of three molecules of doxycycline in the structure of **1** was due to the lability of the complex.

The infrared spectra of the complexes were compared to that of the ligand and were assigned based on previous published report [21]. The stretching frequency of doxycycline hydroxyl shifted from 3452 to 3508 cm^{-1} in complexes **1** and **2** and 3512 cm^{-1} in complex **3**, a shift of 46 cm^{-1} for complexes **1** and **2** and 60 cm^{-1} for complex **3**. However, the amide NH$_2$ band at 3331 cm^{-1} is nearly unchanged in the complexes except in complex **3**. This suggests that coordination took place at ring A of the ligand and that oxygen of the hydroxyl group is involved in coordination.

The amide I band υ(C=O) of doxycycline changed shape and shifted from 1678 to 1672 cm^{-1} in complexes **1** and **2** and 1664 cm^{-1} in complex **3** suggesting the involvement of oxygen of the amide group in coordination. The carbonyl stretching υ(C=O) on rings A and C at 1616 cm^{-1} and 1587 cm^{-1} respectively are essentially unchanged eliminating the possibility of the participation of both carbonyl groups in coordination. Other absorptions associated to the amide group of ring A of doxycyline appear at the same frequencies for the complexes and doxycycline but with completely different shapes in the complexes indicating the participation of oxygen of the amide group in coordination. The coordination of the oxygen of the amide group of ring A also led to the shift in stretching frequency of $\delta(NH_2)$ and $\upsilon(C\text{-}NH_2)$ of doxycycline from 1244 and 1219 to 1240 and 1201, 1242 and 1201 and 1242 and 1217 for complexes **1**, **2** and **3** respectively. This confirms that oxygen of the amide group is involved in coordination to manganese.

The bands at 1500 and 1496 cm^{-1} of complexes **2** and **3** can be attributed to the stretching frequencies υ(C=N) of 2,2'-bipyridine and 1,10-phenanthroline respectively while the bands at 734 and 725 cm^{-1} can be attributed to the υ(C-N-C) stretching of the diamine moiety of complexes **2** and **3** respectively. The new absorptions at 434, 435 and 436 cm^{-1}; 497, 499 and 499 cm^{-1}; and at 563, 565 and 584 cm^{-1} in complexes **1**, **2** and **3** respectively are attributed to Mn-O bond while the ones at 459 in complexes **2** and **3** are attributed to Mn-N bond. Similar coordination mode has been proposed for 1:1 copper complex of doxycycline [21].

Table 1. Summary of FT-IR spectra assignment of complexes **1** - **3** (wavenumber in cm^{-1})

Doxycycline	1	2	3	Assignment
3452, 3331, 3290, 3217	3508, 3333, 3288, 3213	3508, 3333, 3290, 3209	3512, 3319, 3296, 3242, 3200	N-H and O-H stretch
3010	3034	3024, 3039	3082, 3057	Ar-H stretch
1678	1672, 1664	1672, 1664	1664	Amide I C=O
1616	1614	1612	1612	C=O on ring *A*
	1587	1599	1587, 1570	C=O on ring *C*
1244, 1219	1240, 1201	1242, 1201	1242, 1217	Ring A amide group
	497, 459, 434	499, 459, 435	499, 459, 436	Mn-O and Mn-N bond

3.2. Antimicrobial studies

Table 2. Antiplasmodial activity of doxycycline, lincomycin and the complexes

S/N	Complexes	Concentration (µg/ml)	Relative activity to Dox	Reference
1.	$CuDox_2$	14	0.71	21
2.	CubpyDox	9	1.11	21
3.	CuphenDox	1.8	5.56	21
4.	Cudppzdox	1.1	9.09	21
6.	**1**	>100	ND	This work
7.	**2**	5	2	This work
8.	**3**	97	0.10	This work
	Reference Drug			
A	Dox hyclate	10		This work
B	Lincomycin hydrochloride	>100		This work
C	Chloroquine diphosphate	0.02		This work

Dox = doxycycline, bpy = 2,2'-bipyridine, phen =1.10-phenanthroline, dppz = dipyrido[3,2-a:2',3'-c]phenazin

Table 3. Antibiotic resistance pattern of doxycycline and complexes 1-3 against test bacterial isolates (a) *Staphylococcus aureus* and (b) *Klebsiella pneumonia*

a.

Agents	Zone of Inhibition (mm) against concentration in mg/ml			
	0.5	1.0	1.5	2.0
Dox	15	15	16	17
1	17	18	19	20
2	13	14	16	18
3	13	15	16	17

b.

	Zone of Inhibition (mm) against concentration in mg/ml			
	0.5	1.0	1.5	2.0
Dox	11	12	13	15
1	16	18	21	22
2	10	12	13	14
3	13	15	15	16

Among the manganese complexes, only complex **2** showed good activity which is twice as effective as the parent ligand doxycycline. The phenanthroline complex (**3**) and the binary complex **1** has less significant activity. While only **1** has higher inhibitory activity than doxycycline against *Staphylococcus aureus*, both **1** and **3** have higher inhibitory activity against *Klebsiella pneumonia* than doxycycline. This trend showed that the biological activity of the complexes was not a function of the planarity of the diimine ligands.

4. Conclusions

Three manganese complexes of the antibiotic doxycycline have been synthesized and well characterized by elemental analysis, FT-IR and electrospray mass spectroscopies. The stoichiometric ratio of manganese-doxycycline in all the complexes is 1:2. The three complexes possess antimicrobial activities in the same range with doxycycline against *Staphylococcus aureus* and *Klebsiella pneumonia***.** Complex **2** exhibited the highest antiplasmodial activity among the three complexes. This complex (**2**) also has higher antiplasmodial activity than the corresponding copper complex.

Conflict of interest

The authors declare that there is no conflict of interests.

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