Trinuclear Oxovanadium Complexes of Doxycycline: Synthesis, Characterization and Antiplasmodial Studies

Joshua A. Obaleyé1 and Olufunso O. Abosede2,*

1Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria
2Present address: Department of Chemistry, Federal University Otuoke, P.M.B. 126, Yenagoa, Bayelsa State.
* Corresponding author

Abstract—Variable oxidation states of vanadium and strong binding ability of doxycycline have been exploited to synthesize three new oxovanadium complexes of doxycycline. The structures of the new complexes were validated by elemental analysis (C, H, N) and FTIR spectroscopy. The ratio of oxovanadium to doxycycline is 1:3 in all the three complexes. Doxycycline coordinates to the first and second vanadium using phenolic oxygen and nitrogen atoms at ring A as well as the amide and keto oxygen atoms of ring A. The third vanadium binds to phenolic and keto oxygen atoms on rings B and C to form complex 1. 2,2'-bipyridine and 1,10-phenanthroline coordinate to the third oxovanadium replacing the two aqua ligands attached to it in complexes 2 and 3 respectively. Oxovanadium forms 5-coordinate complexes in all the three complexes. Antiplasmodial studies showed that complex 2 have comparable activity with the parent drug, doxycycline, while all three complexes have higher activities than lincomycin.

Keywords—doxycycline; iron(III); diimine; antibacterial; DNA Binding; antiplasmodial.

I. INTRODUCTION

Vanadium is a trace element which is beneficial and possibly essential in humans but certainly essential for some organisms. Vanadium exists in many oxidation states: −3, −1, 0, + 1, + 2, + 3, + 4, and +5 although thermodynamically and kinetically possible oxidation states under physiological conditions are + 5, + 4, and +3. The most notable action of vanadium ion and vanadium compounds is their insulin-mimetic activity and ability to reduce blood sugar levels from high to normal though the oxidation state most relevant to insulin action has not been established.

A monoperoxovanadate(V) complex, oxoperoxopicolinatovanadium(V) dehydrate [mpV(pic)] (Figure 1), has been shown to achieve a 20% decrease in plasma glucose in STZ-diabetic rats when administered by intraperitoneal or subcutaneous injection. Vanadium has also been shown to have some ability to lower cholesterol levels and blood pressure at low doses in humans.

Vanadium ions have many structural roles shown by its structural and electronic analogy to phosphorus. It is an enzyme co-factor and is found in certain tunicates and possibly mammals. VOSO₄ has been reported to be a potent inhibitor for Escherichia coli alkaline phosphatase and the aqueous V⁴⁺ chemistry has been described in detail to explain this phenomenon.

The most well-known V⁴⁺ species is the vanadyl cation (VO²⁺-cation, [VO(H₂O)₅]²⁺ (Figure 2).

The CT DNA, protein binding (bovine serum albumin), DNA cleavage and cytotoxic activities of chiral V(V) Schiff base complexes, (S)-[VO(OMe)L] and (R)-[VO(OMe)L] (Figure 3), have been reported. Both enantiomers of the same complex showed efficient groove or surface binding with DNA, the (R)-[VO(OMe)L] enantiomer exhibiting stronger DNA binding affinity (5 ± 1 x 10⁻⁵ M⁻¹) than its S, enantiomer (8 ± 1 x 10⁻⁴ M⁻¹). The
R enantiomer efficiently cleaved the DNA in the presence of white fluorescence light via mechanistic pathway that involves the formation of singlet oxygen. The R enantiomer also displayed stronger BSA binding and cytotoxic activity.\textsuperscript{18}

Chakravarty and co-workers have shown that heteroleptic oxovanadium(IV) compounds with salicylidene and N,N-heterocyclic ligands like phen, dpq, or dppz coordinated to the metal e.g. [VO-(sal)(phen)] (Figure 4) bind to double-stranded DNA with a \( K_b \) value in the \( 10^9 \text{ M}^{-1} \) range.\textsuperscript{19} Although the chemical nuclease activity in the dark was poor, light-induced double-strand cleavage was observed upon excitation at both 365 and 750 nm through a singlet oxygen formation pathway, whereas neither the ligands or vanadyl sulfate alone showed any activity.

Both singlet oxygen and hydroxyl radical formation were identified in mechanistic studies with various quenchers and radical scavengers of the bis-dppz complex \([\text{VOCl}(\text{dppz})_2]^+\) (Figure 4) when activated with near-IR light at 750 nm.\textsuperscript{20}

A recent review on the applications of vanadium-based compounds in industrial processes such as in catalysis, batteries, metal-organic frameworks as well as possible pharmacological applications has been published.\textsuperscript{21}

## II. EXPERIMENTAL

### Materials and methods

All reagents and solvents were of analytical grade and used without further purifications. Doxycycline hyclate was a gift from Neimeth International Pharmaceuticals Plc, Lagos, Nigeria. Fresh solutions were prepared to ensure stability; 1,10-phenanthroline monohydrate and vanadyl sulphate were obtained from S. D. Fine Chemicals Ltd., India and used as received. Chloroquine diphosphate was obtained from Sigma. UV/Vis spectra were recorded on a Jasco UV-vis spectrophotometer. Infrared spectra were recorded on samples pressed in KBr pellets. Elemental analyses were taken on Elementar AnalysenSysteme Vario \textregistered\  MICRO VI 6.2 GmbH. Melting points were taken on Jenway digital melting point apparatus and were uncorrected.

![Fig.3: Vanadium(V) Schiff base complexes](image1)

![Fig.4: Oxovanadium(IV) complexes with diamine ligands](image2)

![Fig.5: Structures of ligands used.](image3)
Synthesis of the complexes

Synthesis of $\text{[VO}_3\text{Dox(H}_2\text{O})_4\text{(OH)}_2\text{]}$ (1)

0.126 g (0.5 mmol) of VOSO$_4$ was added to 0.256 g (0.5 mmol) doxycycline hyclate in water-methanol and the solution stirred for 2 hours. The resulting solution was set aside at room temperature to obtain green solid which was redisolved in methanol and purified by column chromatography using alumina as stationary phase and acetone and methanol as eluent. Calculated: C, 35.22; H, 4.43; N, 3.73. Found: C, 34.82; H, 3.75; N, 3.57. FT-IR (KBr, v/cm$^{-1}$): 3367, 1582, 1443, 1216, 1127, 953.

Synthesis of $\text{[VO}_3\text{Dox(H}_2\text{O})_4\text{bpy]}$ (2)

0.126 g (0.5 mmol) of VOSO$_4$ was dissolved in 3 ml water and 0.256 g (0.5 mmol) doxycycline hyclate and 5 ml methanol added. The solution was stirred at ambient conditions for 2 hours and 0.078 g (0.5 mmol) of 2,2-bipyridine was added as solid and stirring continued for another 1 hour. The resulting solution was allowed to stand at room temperature and the green solid obtained was redisolved in methanol and purified by column chromatography using alumina as stationary phase and acetone and methanol as eluent. Calculated: C, 44.05; H, 4.51; N, 6.25. Found: C, 44.99; H, 4.10; N, 6.65. FT-IR (KBr, v/cm$^{-1}$): 3366, 1584, 1495, 1442, 1313, 1242, 1158, 1061, 1025, 952, 885, 765, 732.

Synthesis of $\text{[VO}_3\text{Dox(H}_2\text{O})_4\text{phen]}$ (3)

0.126 g (0.5 mmol) of VOSO$_4$ was dissolved in 2 ml water and 0.260 g (0.5 mmol) doxycycline hyclate and 10 ml methanol added. The solution was stirred at ambient conditions for 2 hours and 0.110 g (0.5 mmol) of 1,10-phenanthroline was added as solid and stirring continued for another 1 hour. The resulting solution was allowed to dry by setting aside at room temperature. The dark green glassy solid obtained the next day was redisolved in methanol and purified by column chromatography using alumina as stationary phase and acetone and methanol as eluent. Calculated: C, 45.55; H, 4.38; N, 6.25. Found:C, 45.48; H, 3.70; N, 6.32. FT-IR (KBr, v/cm$^{-1}$): 3342, 1738, 1582, 1519, 1445, 1426, 1326, 1217, 1107, 1038, 963, 848, 805, 723, 620, 606, 572, 549, 530, 523.

Antiplasmodial study

Samples of all compounds were tested in triplicate against chloroquine-sensitive (NF54) strains of *Plasmodium falciparum*. Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified procedure of Trager and Jensen. Quantitative assessment of in vitro antimalarial activity was determined with the parasite lactate dehydrogenase assay using a modified method of Makler and Hinrichs. Two bands at 1244 and 1219 cm$^{-1}$ in FTIR spectrum of doxycycline assigned to δ (NH$_3$) and ν(C=NH$_2$) are essentially unchanged in the new complexes indicating that NH$_3$ is not involved in coordination in the complexes. The stretching frequency of the ν = O group expected in the range 960 ± 50 cm$^{-1}$ are the new strong absorptions at 953, 952 and 963 cm$^{-1}$ in VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. ν (COH) + δ (CH$_3$) of BCD chromophore at 1558 cm$^{-1}$ also shifted to 1443, 1442 and 1445 cm$^{-1}$ for VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. This suggests there is considerable change in the structure and probably conformation in the doxycycline ring of the formed complexes due to the trinuclear nature of the complexes formed. Similar coordination modes proposed for the new complexes have been previously reported for dinuclear Vanadium-tetracycline complexes. Two bands at 1244 and 1219 cm$^{-1}$ in FTIR spectrum of doxycycline assigned to δ (NH$_3$) and ν(C=NH$_2$) are essentially unchanged in the new complexes indicating that NH$_3$ is not involved in coordination in the complexes. The stretching frequency of the ν = O group expected in the range 960 ± 50 cm$^{-1}$ are the new strong absorptions at 953, 952 and 963 cm$^{-1}$ in VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. ν (COH) + δ (CH$_3$) of BCD chromophore at 1558 cm$^{-1}$ also shifted to 1443, 1442 and 1445 cm$^{-1}$ for VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. This suggests there is considerable change in the structure and probably conformation in the doxycycline ring of the formed complexes due to the trinuclear nature of the complexes formed. Similar coordination modes proposed for the new complexes have been previously reported for dinuclear Vanadium-tetracycline complexes. Two bands at 1244 and 1219 cm$^{-1}$ in FTIR spectrum of doxycycline assigned to δ (NH$_3$) and ν(C=NH$_2$) are essentially unchanged in the new complexes indicating that NH$_3$ is not involved in coordination in the complexes. The stretching frequency of the ν = O group expected in the range 960 ± 50 cm$^{-1}$ are the new strong absorptions at 953, 952 and 963 cm$^{-1}$ in VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. ν (COH) + δ (CH$_3$) of BCD chromophore at 1558 cm$^{-1}$ also shifted to 1443, 1442 and 1445 cm$^{-1}$ for VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. This suggests there is considerable change in the structure and probably conformation in the doxycycline ring of the formed complexes due to the trinuclear nature of the complexes formed. Similar coordination modes proposed for the new complexes have been previously reported for dinuclear Vanadium-tetracycline complexes. Two bands at 1244 and 1219 cm$^{-1}$ in FTIR spectrum of doxycycline assigned to δ (NH$_3$) and ν(C=NH$_2$) are essentially unchanged in the new complexes indicating that NH$_3$ is not involved in coordination in the complexes. The stretching frequency of the ν = O group expected in the range 960 ± 50 cm$^{-1}$ are the new strong absorptions at 953, 952 and 963 cm$^{-1}$ in VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. ν (COH) + δ (CH$_3$) of BCD chromophore at 1558 cm$^{-1}$ also shifted to 1443, 1442 and 1445 cm$^{-1}$ for VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. This suggests there is considerable change in the structure and probably conformation in the doxycycline ring of the formed complexes due to the trinuclear nature of the complexes formed. Similar coordination modes proposed for the new complexes have been previously reported for dinuclear Vanadium-tetracycline complexes.
Table 1: Diagnostic bands in the FT-IR Spectra of complexes 1-3 (wavenumber in cm⁻¹)

|                | VO_3Dox (1)  | VO_3BpyDox (2) | VO_3Doxphen (3) | Assignment |
|----------------|--------------|----------------|-----------------|------------|
| 3367 str, br   | 3366 str, br | 3342 br        |                 | υ-O-H      |
| 1582           | 1584         | 1582, 1519     |                 | Amide I C=O absent in the complex |
| 1443           | 1495, 1442   | 1445, 1426     | δ(CH₃) of BCD chromophore |
| 1216           | 1242         | 1217           | δNH₂ + δ(C-NH₂) (no change) |
| 953            | 952          | 963            |                  | ν(V=O)     |
| 885            | 848          |                | C=N of diamine  |
| 765, 732       |              | 723            | ν(C-N-C) of diamine |
|                |              | 572, 549, 530, 523 | O=V-Ligand bond |

str: strong; br: broad

Fig. 7: proposed structures of the complexes [(VO)₃Dox(H₂O)₂(OH)₂] (1), [(VO)₃Dox(H₂O)₂bpy] (2) and [(VO)₃Dox(H₂O)₂phen] (3)

(a) [(VO)₃Dox(H₂O)₄(OH)₂]
Antiplasmodial activity study

The minimum inhibitory concentration and relative activity—IC$_{50}$ (parental compound)/IC$_{50}$ (metal complex) of doxycycline and the vanadyl complexes 1-3 are presented in Table 2.

**Table 2: Antiplasmodial activity of doxycycline and vanadium complexes**

| S/N | COMPLEXES     | CONCENTRATION (μg/ml) | RELATIVE ACTIVITY TO DOX |
|-----|---------------|-----------------------|--------------------------|
| 12. | VODox 1       | 20                    | 0.5                      |
| 13. | VODoxbpy 2    | 13                    | 0.77                     |

Fig.6: FTIR spectra of (a) 1, (b) 2 and (c) 3
For the vanadium complexes, the bpy complex (2) was seen to have similar activity to the parent ligand doxycycline while the binary complex (1) and phenanthroline complex (3) were found to be two and two-and-half fold less effective than the parent ligand respectively. This shows that the planarity of the polypyridyl ligands is not a criterion for the activity of these complexes. Though the activities of all these complexes against chloroquine sensitive (CQS) strain of Plasmodium falciparum (NF54) are lower than the parent drug, doxycycline and chloroquine, they all have higher activities than lincomycin.

IV. CONCLUSION

Three new oxovanadium complexes of doxycycline have been synthesized and structurally characterized. FTIR and elemental analyses data confirmed the formation of both single ligand doxycycline complex (1) and mixed ligand doxycycline complexes with 2,2'-bipyridine (2) and 1,10-phenanthroline (3). Complex 2 possesses comparable antiplasmodial activity against chloroquine sensitive NF54 and higher activity than lincomycin. These complexes are quite soluble in water and very stable at ambient conditions. This work has shown that formation of single ligand and mixed ligand complexes hold promise to finding stable drugs with equal therapeutic efficacies.

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