

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

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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 humans¹ but certainly essential for some organisms.²⁻⁸ 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.⁹

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.¹⁰ Vanadium has also been shown to have some ability to lower

cholesterol levels and blood pressure at low doses in humans.⁹

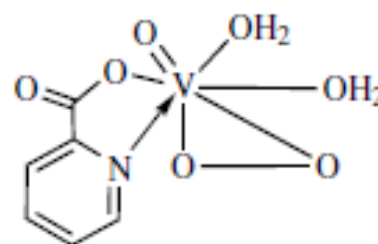


Fig.1: Oxoperoxopicolinatovanadium(V) [mpV(pic)] with pentagonal bipyramidal geometry

Vanadium ions have many structural roles shown by its structural and electronic analogy to phosphorus.^{8,10} It is an enzyme co-factor¹ and is found in certain tunicates^{9,10,11} and possibly mammals. VOSO₄ has been reported to be a potent inhibitor for *Escherichia coli* alkaline phosphatase^{12,13} and the aqueous V^{IV} chemistry has been described in detail to explain this phenomenon.¹⁴ The most well-known V^{IV} species is the vanadyl cation (VO²⁺-cation, [VO(H₂O)₅]²⁺ (Figure 2)).¹⁵⁻¹⁷

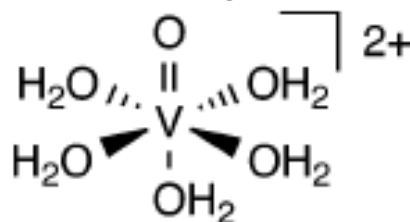


Fig.2: Vanadyl cation, the most common in aqueous solution

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 ($5 \pm 1 \times 10^5 \text{ M}^{-1}$) than its S, enantiomer ($8 \pm 1 \times 10^4 \text{ M}^{-1}$). 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.¹⁸

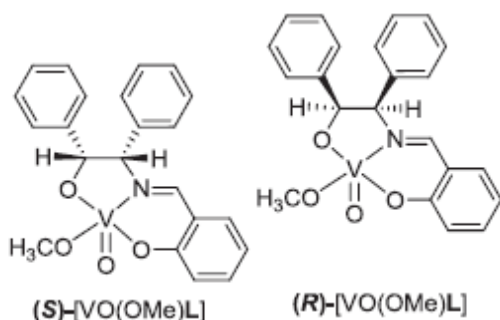


Fig.3: Vanadium(V) schiff base complexes

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 10^5 M^{-1} range.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 [VOCl(dppz)₂]⁺ (Figure 4) when activated with near-IR light at 750 nm.²⁰

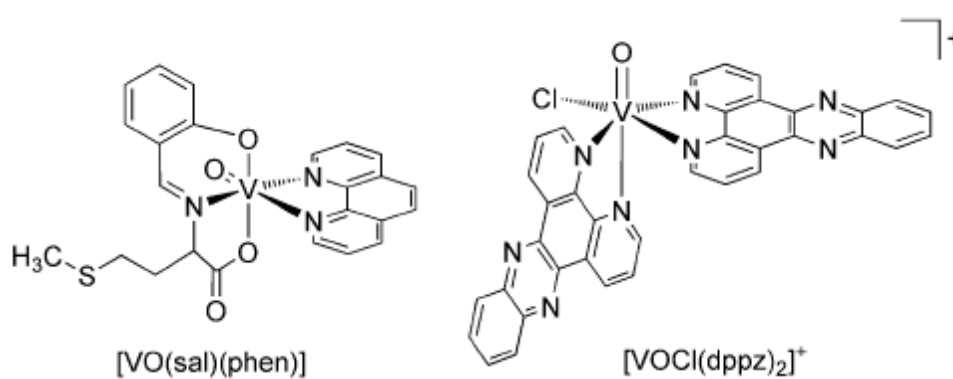


Fig.4: Oxovanadium(IV) complexes with diamine ligands

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.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 ElementarAnalyseSystemeVario ® MICRO VI 6.2 GmbH. Melting points were taken on Jenway digital melting point apparatus and were uncorrected.

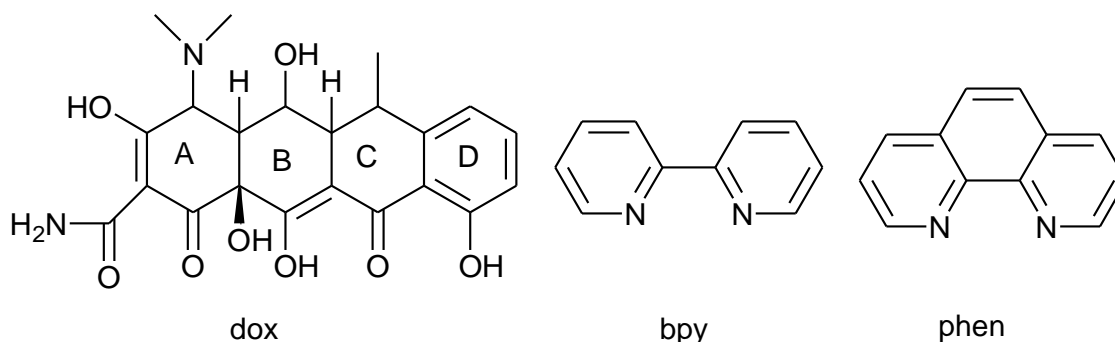


Fig.5: Structures of ligands used.

Synthesis of the complexes**Synthesis of [(VO)₃Dox(H₂O)₄(OH)₂] (1)**

0.126 g (0.5 mmol) of VOSO₄ 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 redissolved 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 [(VO)₃Dox(H₂O)₄bpy] (2)

0.126 g (0.5 mmol) of VOSO₄ 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 redissolved 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, 6.42. 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 [(VO)₃Dox(H₂O)₄phen] (3)

0.126 g (0.5 mmol) of VOSO₄ 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 0.1100 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 redissolved 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.²² Quantitative assessment of *in vitro* antiplasmodial activity was determined with the parasite lactate dehydrogenase assay using a modified method of Makler and Hinrichs.²³ 20 mg mL⁻¹ stock solution in 100% DMSO of the test samples were prepared to and stored at -20 °C. Further dilutions were prepared on the

day of the experiment. Chloroquine diphosphate (CQDP) was used as the reference drug. A full dose-response experiment was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC₅₀-value). Samples were tested at a starting concentration of 100 µg mL⁻¹, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg mL⁻¹. Reference drug (CQDP) was tested at a starting concentration of 1000 ng mL⁻¹. The highest concentration of the solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC₅₀-values were obtained using a non-linear dose response curve fitting analysis via Graph Pad Prism v.4.0 software.

III. RESULTS AND DISCUSSION**Synthesis and characterization of complexes 1-3**

Trinuclear oxovanadium complexes of doxycycline have been synthesized and characterized. The complexes were obtained in good yield and are stable in solid state and in solution at ambient conditions. Data obtained from FT-IR and elemental analysis are in agreement with the proposed molecular formulae for the complexes.

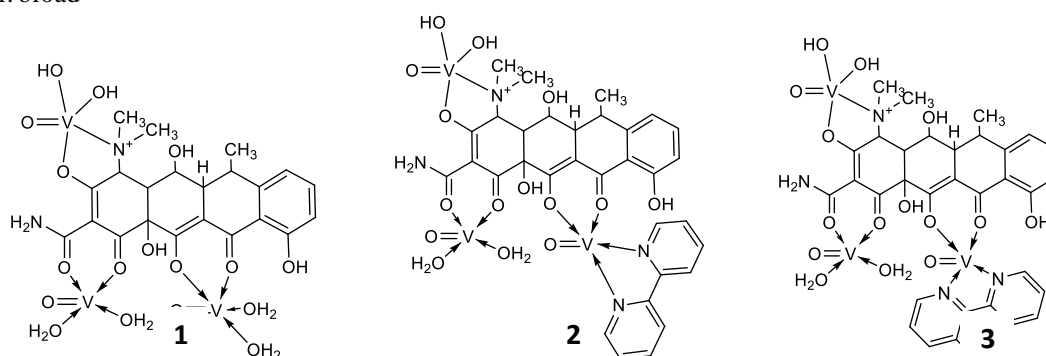
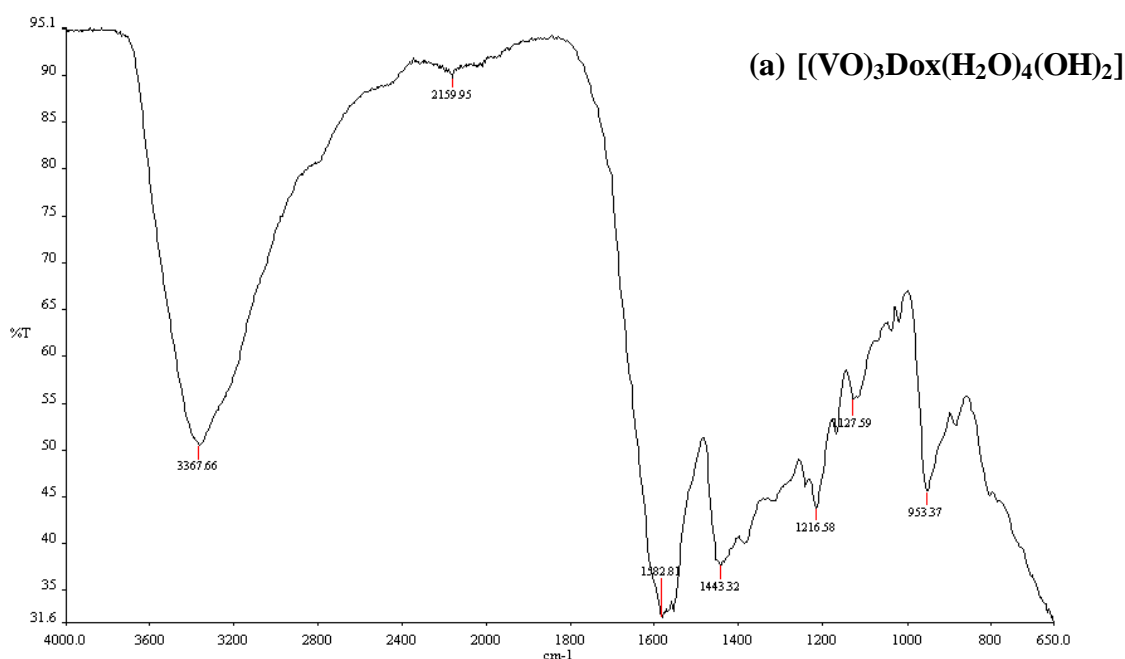
Amide II absorption at 1520 is absent in all the complexes except in VODoxphen (3) which appears at 1519 cm⁻¹ (weak). Strong band at 1678 cm⁻¹ due to carbonyl stretching of ring A, ν(C=O), is absent in all the complexes indicating its coordination to VO²⁺. Carbonyl stretching ν(C=O) on ring C at 1616 cm⁻¹ in doxycycline shifted to 1582, 1584 and 1582 cm⁻¹ in VODox (1), VODoxbpy (2) and VODoxphen (3) respectively. ν(COH) + δ(CH₃) 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.²⁴

Two bands at 1244 and 1219 cm⁻¹ in FTIR spectrum of doxycycline assigned to δ(NH₂) and ν(C-NH₂) are essentially unchanged in the new complexes indicating that NH₂ is not involved in coordination in the complexes. The stretching frequency of the V=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(C-N-C) of diimine appeared at 765, 732 for VODoxbpy(2) and 723 VODoxphen(3) while C=N of diimine appeared at 885 and 848 for VODoxbpy(2) and VODoxphen (3) respectively.

Table.1: Diagnostic bands in the FT-IR Spectra of complexes 1-3 (wavenumber in cm^{-1})

VODox (1)	VObpyDox (2)	VODoxphen (3)	Assignment
3367 str, br	3366 str, br	3342 br	ν -O-H
			Amide I C=O absent in the complex
1582	1584	1582, 1519	Amide II absorption $\delta\text{NH}_2 + \nu\text{C-NH}_2$
1443	1495, 1442	1445, 1426	$\delta(\text{COH}) + \delta(\text{CH}_3)$ of BCD chromophore
1216	1242	1217	δNH_2 and $\nu\text{C-NH}_2$ (no change)
953	952	963	$\nu(\text{V=O})$
	885	848	C=N of diamine
	765, 732	723	$\nu(\text{C-N-C})$ of diamine
		572, 549, 530, 523	O=V-Ligand bond

str: strong; br: broad

Fig.7: proposed structures of the complexes $[(\text{VO})_3\text{Dox}(\text{H}_2\text{O})_4(\text{OH})_2]$ (1), $[(\text{VO})_3\text{Dox}(\text{H}_2\text{O})_4\text{bpy}]$ (2) and $[(\text{VO})_3\text{Dox}(\text{H}_2\text{O})_4\text{phen}]$ (3)(a) $[(\text{VO})_3\text{Dox}(\text{H}_2\text{O})_4(\text{OH})_2]$

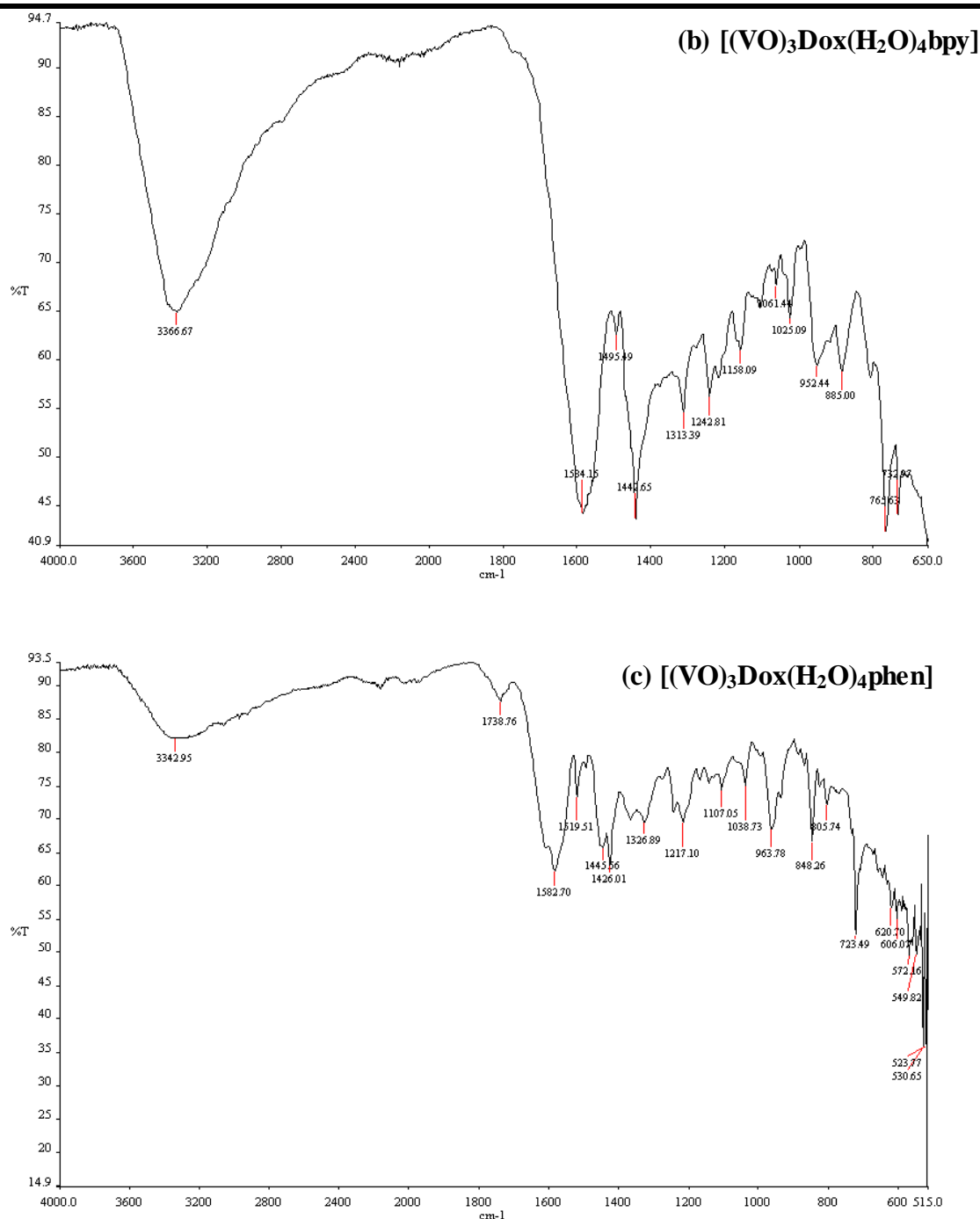


Fig.6: FTIR spectra of (a) 1, (b) 2 and (c) 3

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 ($\mu g/ml$)	RELATIVE ACTIVITY TO DOX
12.	VODox 1	20	0.5
13.	VODoxbpy 2	13	0.77

14.	VODoxphen 3	30	0.33
A	Dox Hyclate	10	
B	Lin Hydrochloride	>100	
C	CQ diphosphate	0.02	

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** possess comparable antiparasmodial 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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