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Anti-tubercular Activity of Ruthenium (II) Complexes with Polypyridines

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Abstract

A series of nine polypyridyl-ruthenium (II) complexes (N-ligands = 2,2′-bipyridines; 2,2′-6′,2′-terpyridines, di-alkyloxy-2,2′-6,2-bipyridine-3,3′-di-carboxylates), were tested against *Mycobacterium tuberculosis* (MBT). The complex (**11**) showed remarkable activity against MBT as compared to other complexes, (1–10). The aquo ligand of complex (**11**), as opposed to other chloro and acetonitrile derivatives, appears to play a key role in the antitubercular potency of this new class of metal-based compounds.

Keywords: *Ruthenium (II) complexes, polypyridines, anti-tubercular activity*

Introduction

Despite the ready availability of effective treatments, tuberculosis still remains a major threat worldwide. The emergence of drug resistant strains of *Mycobacterium tuberculosis*, particularly multiple drug resistant strains (MDR) [1–4] has complicated the treatment protocol and raised the concern that tuberculosis may once again become an incurable disease in future. For this reason it is critical to discover new drug therapies with more aggressive mechanism to work against resistant strains of *Mycobacterium tuberculosis*. Numerous reviews recently reported in the literature are a proof of the renewed interest towards this pathology [5–12].

A number of such selected metal based compounds contain metal-oxygen (M-O) hemilabile bonds e.g., (M-O=M-OH_2)[13,14,22] or (M-$SOMe_2$)[16] which have shown interesting and potential anti-tumor or antibacterial activity (Figure 1).

Isoniazid derived copper(II) and nickel(II) complexes with antimycobacterial *in vitro* activity have been reported [13]. The metal complexes of 2-(1′/2′-hydroxynaphthyl)-benzoxazoles also showed significant activity (MIC <3.12 μg/mL)[14]. In an other report a nickel(II) binuclear complex has displayed a significant activity with MIC 10-fold lower than that of Rifampicin and reaches almost equal to Isoniazid, so far the sole established anti-tuberculosis drug which possesses the same MIC value against *M. tuberculosis*.

Instead, charged mononuclear complexes of 2,6-diacetylpyridine and bis-benzoylhydrazone in different mixtures showed MICs >12.5 mg/ mL [15]. Na[*trans*-$RuCl_4(Me_2SO)(Im)$] (Im = imidazole), a ruthenium(III) complex shows encouraging anti-tumour and anti-metastatic properties [16].

Since 1969, Dwyer et al [17] reported the bacteriostatic action of metal complexes of 2,2′-bipyridyl

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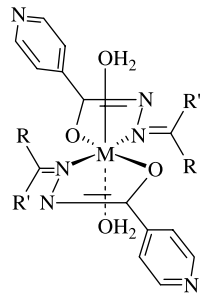

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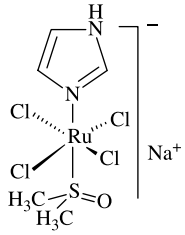
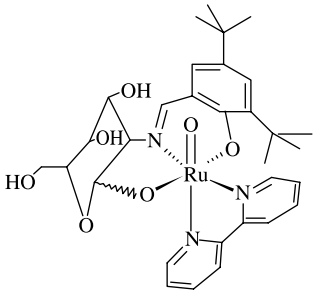
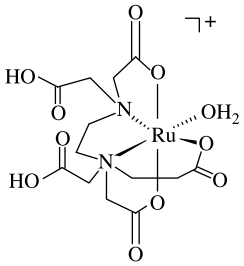


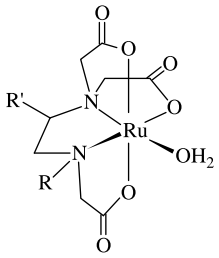
[Ru(Im)(Cl)$_4$(DMSO)]$^-$Na$^+$ Ref.[16]



[(SugarA)(Bpy)RuIV(=O)]$^+$Cl$^-$; Ref.[23]



[Ru(dtpa)(OH$_2$)]+ ; Ref.[22]



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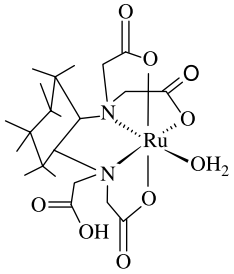
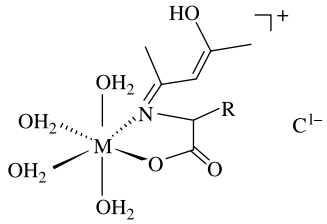
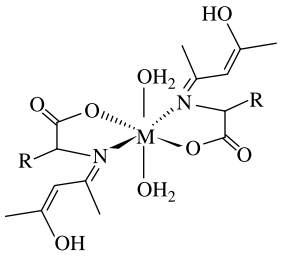


[Ru(cdta)(OH$_2$)] ; Ref.[22]



M = Co(II), Cu(II), Ni(II) or Zn(II); Ref.[23]



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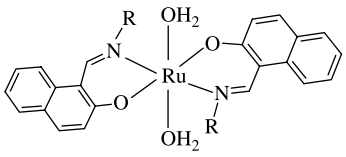

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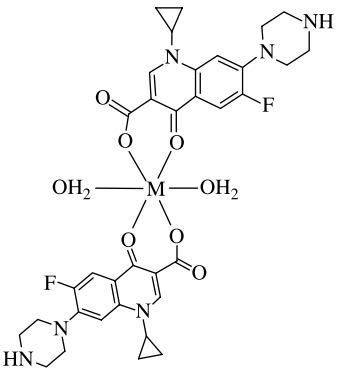

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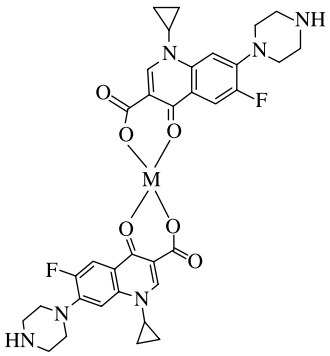

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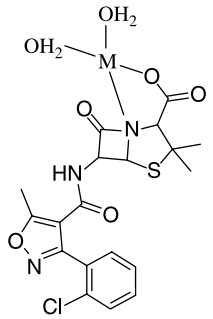

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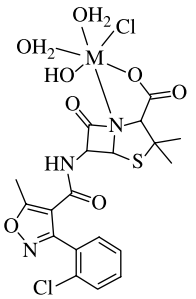
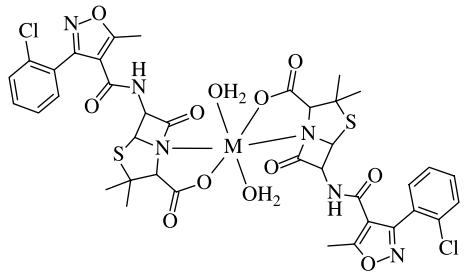


M = Co(II), Ni(II) or Zn(II); Ref.[26]



M = Co(II), Ni(II) or Zn(II); Ref.[26]

Figure 1. Examples of some bioactive transition metal complexes containing precursors of Metal-OH$_2$ or Metal = O moieties.

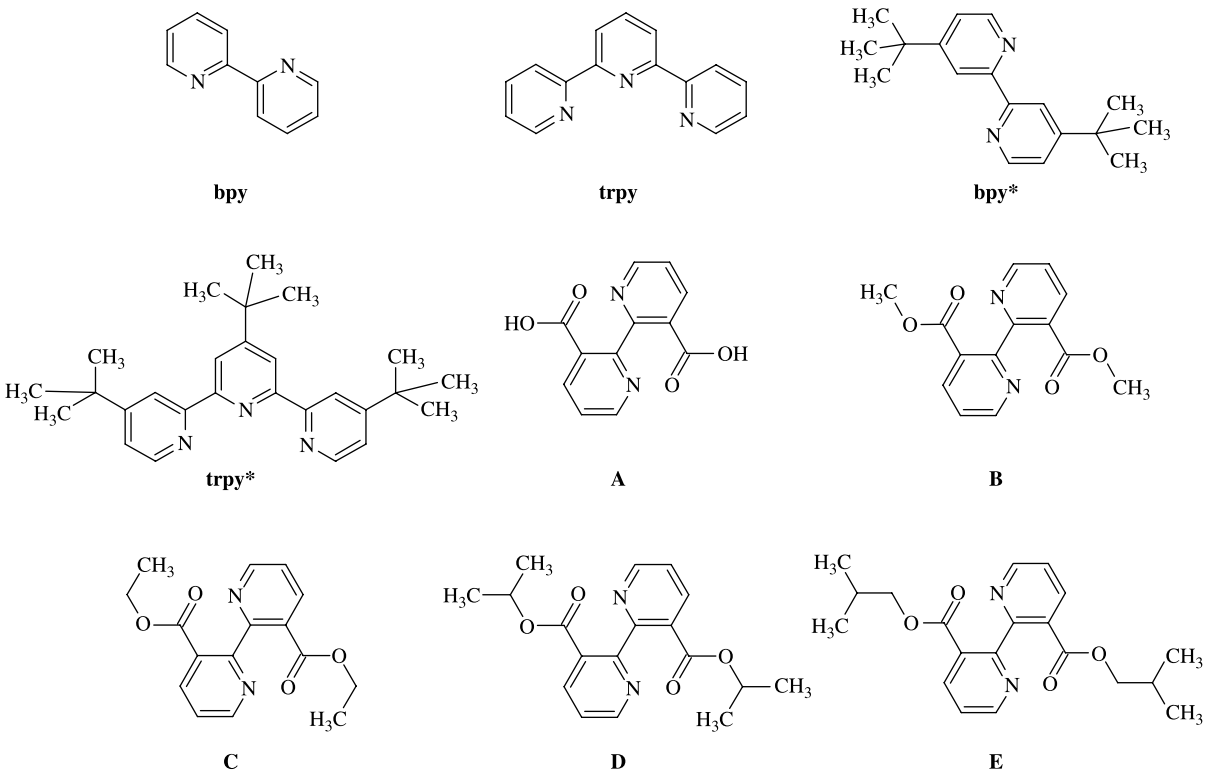


Figure 2. Structures of tested polypyiridines compounds.

against selected Gram-positive, Gram-negative and acid-fast bacteria. With the same idea, we have prepared and wish to report some Ruthenium complexes bearing substituted polypyridyl ligands (2,2′-bipyridines or 2,2′-6′,2″-terpyridines) bearing Ru(II)-X moieties where X = Cl, CO, CH_3CN or H_2O. We have also made a genuine effort to investigate and report in this paper antitubercular screening results of these complexes (Figure 2). These ruthenium complexes, derived from the coordination of bipyridyl and/or terpyridyl ligands, can be obtained through a simpler and economical synthetic method. The starting material, 2,2′-bipyridine-3,3′-dicarboxylic acid, was obtained in good yield from commercially available 1,10-phenanthroline by its oxidation using the $KMnO_4$ as described previously [18a].

Materials and methods

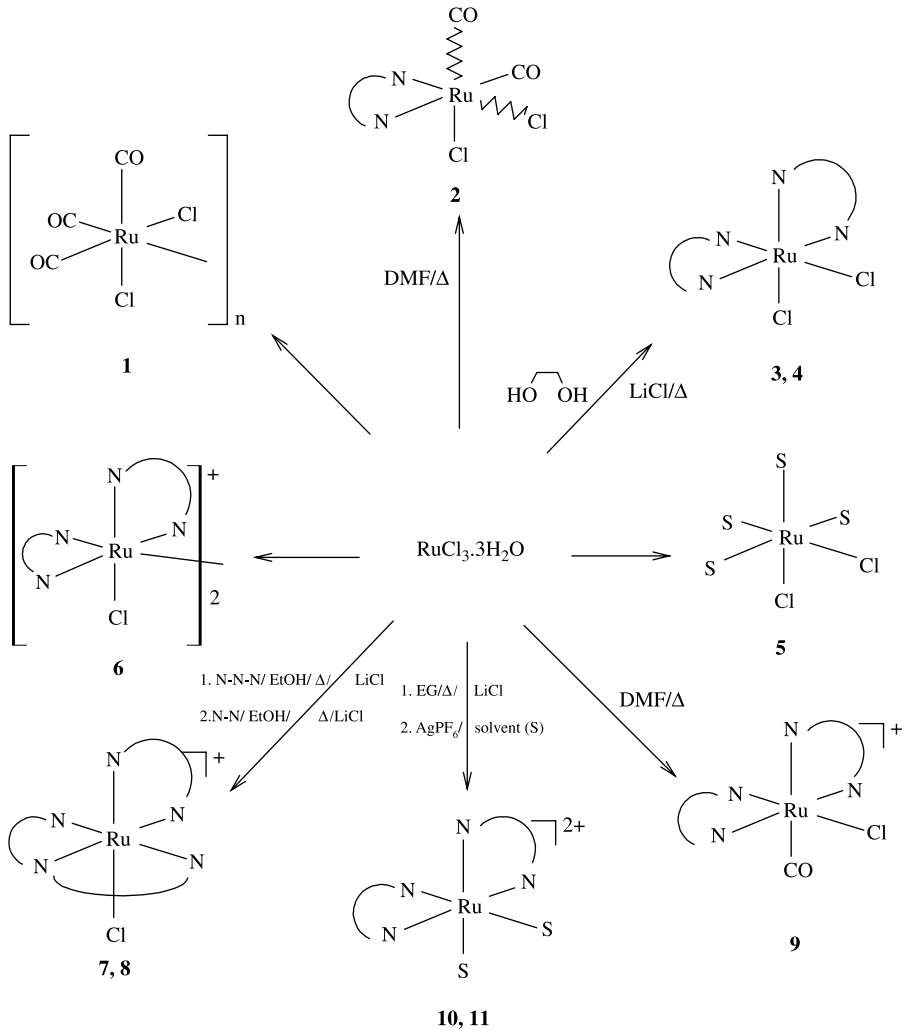
All materials and solvents were of reagent grade as received from commercial sources. Diethyl-2,2′-bipyridine-3,3′-dicarboxylate (**C**) was similarly synthesized and coordinated to ruthenium(II) as described in our previous work and depicted in literature.[22] ^{1}H NMR spectra were recorded on AC 250 MHz NMR Bruker Spectrometer at ambient temperature and chemical shifts were reference to the internal tetramethylsilane. Infrared spectra were recorded in KBr pellets using a Perkin-Elmer 1310 spectrophotometer. Mass spectra were determined by platform II Micromass (ESI +, CH_3CN/H_2O: 50/50). Elemental analyses were performed by CNRS Service Central d'Analyse Vernaison (France).

Antitubercular Activity

Primary screening was conducted at 6.25 μg mL^{-1} against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [19]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against *M. tuberculosis* H37Rv to determine the MIC using MABA. MIC is defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to controls[19].

General procedure for the preparation of dialkyl-2, 2′-bipyridine-3,3′-dicarboxylates B–E

*Diethyl-2,2′-bipyridine-3,3′-dicarboxylate (**C**).* The ligand **C** was prepared by a similar procedure as described in our previous work [18]. The 2,2′-bipyridine-3,3′-dicarboxylic acid was prepared from 1,10-phenanthroline by a literature procedure. Compound acid (600 mg, 2.5 mmol) and thionyl chloride (12 mL) were refluxed for 5 h. The excess amount of thionyl chloride was distilled off and the



1: n =2
2: N-N = C
3: N-N = bpy
4: N-N = D
5: S = DMSO
6: N-N = bpy*
7: N-N = bpy*; N-N-N = trpy
8: N-N = bpy; N-N-N = trpy*
9: N-N = phenanthroline
10: N-N = D; S = CH_3CN
11: N-N = D; S = H_2O

Scheme 1. Synthesis of ruthenium (II) complexes (**1**–**11**).

residue dried in vacuum for 5 h. Toluene (20 mL) and ethanol (2 mL) were added to it and refluxed for 3 h. Chloroform (40 mL) was added and the mixture was treated with a cold solution of sodium bicarbonate (2.5%). The organic layer was dried on sodium sulphate and the solvent removed in vacuum, giving 733 mg of crude product. The mixture was purified by chromatography on silica gel column using ether as eluent to afford **C** as a white solid (670 mg, 83%).

Selected data for ligand (**C**): Mp = 81–82°C. IR (KBr, cm^{-1}): 1695 (C=O, s), 1535 (C=C, w), 1415 (C=N, m), 1260 (C-O, w). ^{1}H-NMR (250.14 MHz, $CDCl_3$): 8.73 (dd; 2H, H6/6′, J = 4.8 and 1.7 Hz), 8.37 (dd, 2H, H4/4′, J = 7.9 and 1.7 Hz), 7.42 (dd, 2H, H5/5′, J = 7.9 and 4.8 Hz), 4.95 (m, 2H, CH, J = 6.3 Hz), 0.97 (d, 12H, $4CH_3$, J = 6.3 Hz). Anal. Cal. for $C_{18}H_{20}N_2O_4$ (328.37): C 65.85, H 6.10, N 8.53; found: C 65.78, H 6.22, N 8.38%. MS: $[M]^+$ [m/e = 329.10] (Cal. for $C_{18}H_{20}N_2O_4$: 328.371).

cis-*Dichloro-[{N,N′-bis-(di-iso-propyl-2,2′-bipyridine-3,3′-dicarboxylate)}]Ru(II).mono-hydrate: [Ru(D)$_2$Cl$_2$].H$_2$O.* Following the same literature procedure, the complex *cis*-(Cl/Cl)-[$RuCl_2$(**D**)$_2$].H_2O was synthesized: 0.262 g (1 mmol) of $RuCl_3.3H_2O$ and 0.600 g (2 mmol) of ligand (**D**) were combined in 15 mL of ethyleneglycol. The mixture was heated gently for 25 min and allowed to cool to room temperature. The violet solid was then precipitated with water, filtered off and washed three

Table I. Biological activity of free N-ligands and their ruthenium (II) complexes against *Mycobacterium tuberculosis in vitro.* [19].

Compound (TAACF code)	Structure type	Assay	MIC (μg/mL)	% Inhibition	Activity
A (153306)	N-N	Alamar	>6.25	0	negative
B (143051)	N-N	Alamar	>6.25	27	negative
C (143052)	N-N	Alamar	>6.25	26	negative
D (143053)	N-N	Alamar	>6.25	39	negative
E (143054)	N-N	Alamar	>6.25	28	negative
1 (155609)	$[Ru(CO)_3Cl_2]_2$	Alamar	>6.25	0	negative
2 (155593)	$Ru(C)(CO)_2Cl_2$	Alamar	>6.25	0	negative
3 (155613)	$Ru(bpy)_2Cl_2$	Alamar	>6.25	0	negative
4 (155614)	$Ru(D)_2Cl_2$	Alamar	>6.25	27	negative
5 (155605)	$Ru(Me_2SO)_4Cl_2$	Alamar	>6.25	0	negative
6 (155607)	$[Ru(bpy\star)_2Cl]_2^{2+}$	Alamar	>6.25	34	negative
7 (155608)	$Ru(bpy\star)(trpy)Cl]^+$	Alamar	>6.25	37	negative
8 (155604)	$[Ru(bpy)(trpy\star)C]^+$	Alamar	>6.25	30	negative
9 (155606)	$[Ru(Phen)_2(CO)Cl]^+$	Alamar	>6.25	0	negative
10 (155615)	$[Ru(D)_2(CH_3CN)_2]^{2+}$	Alamar	>6.25	0	negative
11 (155616)	$[Ru(D)_2(OH_2)_2]^{2+}$	Alamar	< 6.25	**100**	positive

times respectively with water and diethyl ether and dried under vacuum. An X-ray quality crystal of *cis*-(Cl/Cl)-$[RuCl_2(D)_2].H_2O$ was obtained from the recrystallization in acetone-ethanol.

Selected data for *cis*-(Cl/Cl)-$[RuCl_2(D)_2].H_2O$ (**2**): Yield 82%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.36 (d, 2H, J = 5.4 Hz, H^6), 8.25 (d, 2H, J = 7 Hz, H^4), 7.78 (d, 2H, J = 6.8 Hz, $H^{4'}$), 7.7 (m, 2H, $H^{6'}$), 7.63 (dd, 2H, J = 7.4 and 5.4 Hz, H^5), 6.9 (dd, 2H, J = 6.8 and 7.2 Hz, $H^{5'}$), 4.24 (m, 8H, CH_2-O), 1.23 (m, 12H, CH_3). IR (KBr) ν 1731 (vs), 1576 (s) cm^{-1}. Anal. Calc. (found) for $C_{32}H_{32}N_4Cl_2O_8Ru.H_2O$: C 48.61 (48.27), H 4.33 (4.17), N 7.09 (6.93%); Cyclic voltammetry (CH_3CN, 0.1 M TBAH, Pt/ECS): $E_{1/2}$ = 0.54 V (ΔEp = 80 mV).

The electrochemical and preliminary study of cyclic voltammetry was carried out and showed reversible and electrochemical stability properties of complex *cis*-(Cl)-$[RuCl_2(D)_2].H_2O$.

Results and discussion

Chemistry

Scheme 1 depicts the general reaction used to prepare ruthenium (II) complexes **1**–**11** which have been published previously[8]. and Ref In our first published work, complexes containing (N-N)Ru, $(N\text{-}N)_2$Ru, (N-N)(N-N-N)Ru moieties were prepared from the reaction of ($RuCl_3.3H_2O$) and (N-N and/or N-N-N) ligands in the presence of a mixture of ethanol as solvent and Et_3N as a reducing agent, or a solvent alone which could play the reducing and solvolysis roles as that of dimethylformamid (DMF) or ethylenglycol (EG). [18a–c] The coordination of substituted bipyridines (bpy⋆, **A**–**E**) and substituted terpyridine (trpy⋆) instead of unsubstituted polypyridines (bpy and trpy), under the same conditions, lead to higher, but still modest, water soluble complexes.

Antimycobacterial activity

The antimycobacterial activity of the compounds was determined to identify the compounds having inhibitory activity against *M. tuberculosis*. Interesting results were obtained from these assays and data is reported in Table I. The *in vitro* antimycobacterial activities of these polypyridine ligands and their ruthenium complexes **1**–**11** were inferior to that of isoniazid against *M. tuberculosis* H37Rv. Further, the free N,N-ligands **A**–**E** had either little or no activity (0–39%inhibition). However, none of the compounds showed activity against *M. tuberculosis* H37Rv suggesting that the compounds possess no specific anti-tubercular activity. This could probably be due to their low absorption (MIC >6.25 μg/mL) against *M. tuberculosis*. However, data in Table I shows that only the ruthenium-aquo complex **11** of ligand **D** showed a high antitubercular activity compared to the rest of the free ligands (**A**–**E**), neutral complexes **1**–**5**, monocationic or dicationic complexes **6**–**10** (Table I).

In this case, the degree of lipophilicity of the carboxylate substituent does not correlate positively with the antitubercular activity. A direct influence of the redox properties from the ruthenium may appears to be more important for such an activity (Table I).

In addition to structure-activity relationships, an essential investigation is required to establish the relationship between redox potential and structure-activity which would help to understand the mechanism of metal-aquo complexes like complex **11** to inhibit various diseases such as tuberculosis, cancer and HIV. It would also be possible to address the significant area by testing their effect(s) on four cell lines, one of which has normal topoisomerase I, protease and three others have mutant (ethambutol, camptothecin and cisplatin-resistant) enzymes. If there were a difference in GI50 value this would indicate that an enzyme is a critical target for the metal based drug. A further consideration relates to the poor solubility

of the Ru-OH_2 derivatives in water; for the cell line assays to function it is important to prepare derivatives that are more soluble. Substituting the ester groups at 3,3′ position, with some other groups such as sugars or amides is possible and might generate the desired effects.

Interestingly, solvatation of ruthenium-Cl moiety of complex **4** with water molecules in the presence of AgTf has been demonstrated to be a powerful and optimal method for Ru-OH_2 complexes in the preparation of a new efficient metal-based anti-tubercular class of compounds. This work provides for the first time a simple method for the preparation of a wide range of such compounds which are bioactive and could be clinically used as anti-tubercular agent.

The important anti-tubercular activity of this class of compounds suggests a promising novel approach to the design of prospective and significantly potential compounds for treating other bacterial infections.

As a guide for future work, the data reported herein indicates that the **Ru-OH_2** compounds have a definite potential efficacy that merits development through modification of both the lipophicity of bipyridyl ligands and the nature of the metal ion.

Conclusion

In this paper we report an efficient metal based anti-tubercular agent having Ru-OH_2 coordination which highlights the significant feature of the presence of the metal-aquo moiety in the coordination of bioactive molecule to the metal. Antitumor and anti-HIV screening studies on complex **11** are in progress at the National Cancer Institute (NCI) which will help us in elucidating the redox potential /activity relationships.

Acknowledgements

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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