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Antiparasitic activities of novel ruthenium/lapachol complexes

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ABSTRACT

The present study describes the synthesis, characterization, antileishmanial and antiplasmodial activities of novel diimine/(2,2′-bipyridine (bipy), 1,10-phenanthroline (phen), 4,4′-methylbipyridine (Me-bipy) and 4,4′-methoxybipyridine (MeO-bipy)/phosphine/ruthenium(II) complexes containing lapachol (Lap, 2-hydroxy-3-(3-33 methyl-2-buthenyl)-1,4-naphthoquinone) as bidentate ligand. The $[Ru(Lap)(PPh_3)_2(bipy)]PF_6$ (**1**), $[Ru(Lap)(PPh_3)_2(Me\text{-}bipy)]PF_6$ (**2**), $[Ru(Lap)(PPh_3)_2(MeO\text{-}bipy)]PF_6$ **(3)** and$[Ru(Lap)(PPh_3)_2(phen)]PF_6$ (**4**) complexes, PPh_3 = triphenylphospine, were synthesized from the reactions of *cis*-$[RuCl_2(PPh_3)_2(X\text{-}bipy)]$ or *cis*-$[RuCl_2(PPh_3)_2(phen)]$, with lapachol. The $[RuCl_2(Lap)(dppb)]$ **(5)** [dppb = 1,4-*bis*(diphenylphosphine)butane] was synthesized from the *mer*-$[RuCl_3(dppb)(H_2O)]$ complex. The complexes were characterized by elemental analysis, molar conductivity, infrared and UV–vis spectroscopy, $^{31}P\{^1H\}$ and ^1H NMR, and cyclic voltammetry. The Ru(III) complex, $[RuCl_2(Lap)(dppb)]$, was also characterized by the EPR technique. The structure of the complexes $[Ru(Lap)(PPh_3)_2(bipy)]PF_6$ and $[RuCl_2(Lap)(dppb)]$ was elucidated by X-ray diffraction. The evaluation of the antiparasitic activities of the complexes against *Leishmania amazonensis* and *Plasmodium falciparum* demonstrated that lapachol–ruthenium complexes are more potent than the free lapachol. The $[RuCl_2(Lap)(dppb)]$ complex is the most potent and selective antiparasitic compound among the five new ruthenium complexes studied in this work, exhibiting an activity comparable to the reference drugs.

1. Introduction

Leishmaniasis and malaria are diseases caused by protozoan parasites and are characterized by high morbidity. It is estimated that leishmania disease causes about seventy thousand deaths annually and malaria kills around 1 million children only in Africa [1]. The first line treatment for leishmaniasis still relies on the use of pentavalent antimonials, although other drugs are also used for the treatment of *Leishmania* infection, such as pentamidine isethionate, amphotericin B and miltefosine [2,3]. Malaria treatment relies on the use of quinoline-based drugs, such as chloroquine, primaquine and mefloquine, as well as antifolates and artemisinin derivatives, depending on the parasite's susceptibility [4]. Common problems with these antiparasitic drugs are severe side effects and development of drug resistance. Based on this scenery, the research of new active compounds against these parasites is pivotal.

The *Tabebuia* genus, belonging to the bignoniaceae plant family, is widely used in the traditional medicine in South America [5,6]. Among the active secondary metabolites present in this genus, 2-hydroxy-3-(3-methyl-2-buthenyl)-1,4-naphthoquinone (lapachol, Fig. 1) is one of the most studied. Lapachol is endowed with anticancer and antimicrobial properties [7,8]. Because of its antiproliferative activity, lapachol has been employed as a prototype for the design and synthesis of new anticancer and antimicrobial agents. This has led to the identification of fewer lapachol derivatives with an enhanced activity [9–12].

Like other naphthoquinones [13,14], lapachol is a feasible ligand for the preparation of coordinating or organometallic compounds. In fact, there are some findings showing that lapachol–metal complexes are biologically more active than the free molecule [15–18]. Ruthenium complexes are considered to be one of the most promising types of metal compounds for cancer treating, due its interesting chemical properties, such as: versatility in ligand exchange, octahedral geometry and variability of oxidation states [19,20]. Recently it was observed that the lapachol–Ru(II) complex is a more potent anticancer agent than lapachol–Os(II) and Rh(III) complexes [18], suggesting that the use of ruthenium is promising to improve the biological activity of lapachol.

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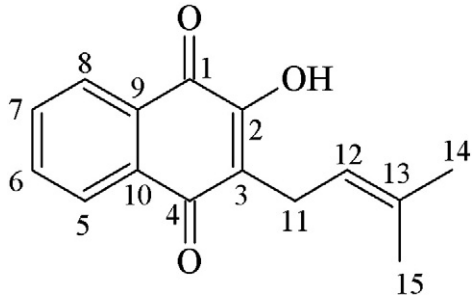

Fig. 1. Lapachol structure.

Therefore, the present study describes the synthesis, characterization, antileishmanial and antiplasmodial activities of novel diimines (2,2′-bipyridine (bipy), 1,10-phenantroline (phen), 4,4′-methylbipyridine (Me-bipy) and 4,4′-methoxybipyridine (MeO-bipy) and monophosphine ruthenium(II) and (III) complexes containing lapachol as a bidentate ligand.

2. Experimental section

2.1. Materials for synthesis

Solvents were purified by standard methods. All chemicals used were of reagent grade or comparable purity. The $RuCl_3 \cdot 3H_2O$ was purchased from Degussa or Aldrich. The ligands 1,4-*bis*(diphenylphosphino)butane (dppb), triphenylphosphine (TPP), bipy, Me-bipy, MeO-bipy and phen were used as received from Aldrich.

2.2. Instrumentation

Elemental analyses were performed in a Fisons EA 1108 model (Thermo Scientific). The IR spectra of the powder complexes were recorded using CsI pellets in the 4000–200 cm^{-1} region in a Bomen–Michelson FT MB-102 instrument. The UV–Visible (UV–vis) spectra of the complex were recorded in CH_2Cl_2 solution, in a Hewlett Packard diode array—8452A. The electron paramagnetic resonance (EPR) spectrum was measured in solid state at −160 °C using a Varian E-109 instrument, recorded at the X band frequency, within a rectangular cavity (E-248) fitted with a temperature controller. Cyclic voltammetry (CV) experiments of the complexes in solution were promoted in an electrochemical analyzer BAS model 100B. These experiments were carried out at room temperature, in CH_2Cl_2 containing 0.10 M $Bu_4N^+ClO_4^-$ (TBAP) (FlukaPurum) as support electrolyte, and using an one-compartment cell, with both working and auxiliary electrodes, which were stationary Pt foils, while the reference electrode was Ag/AgCl, 0.10 M TBAP in CH_2Cl_2. Under these conditions, the ferrocene is oxidized at 0.43 V (Fc^+/Fc).

All NMR experiments were run on a BRUKER, DRX400 MHz equipment, in a BBO 5 mm probe, at 298 K, and TMS (tetramethylsilane) for internal reference. For ^1H and ^{13}C NMR, DMSO-d_6 was used as solvent, while CH_2Cl_2 was used as solvent for ($^{31}P\{^1H\}$) NMR. The splitting of proton, carbon and phosphorus resonances was reported as s = singlet and m = multiplet.

2.3. X-ray crystallography

Blue single crystals of complexes **(1)** and **(5)** were grown by slow evaporation of a dichloromethane/*n*-hexane solution. X-ray diffraction experiments were carried out using a suitable crystal mounted on glass fiber, and positioned on the goniometer head. Intensity data were measured with the crystal at room temperature on an Enraf–Nonius Kappa-CCD diffractometer with graphite monochromated MoKα radiation (λ = 0.71073 Å). The cell refinements were performed using the software Collect [21] and Scalepack [22], and the final cell parameters were obtained on all reflections. Data reduction was carried out using the software Denzo-SMN and Scalepack [22]. The structures were solved by the Direct method using SHELXS-97 [15] and refined using the software SHELXL-97 [23]. A Gaussian method implemented was used for the absorption corrections [24]. Non-hydrogen atoms of the complexes were unambiguously located, and a full-matrix, least-square refinement of these atoms with anisotropic thermal parameters was carried out. The aromatic C–H hydrogen atoms were positioned stereochemically and were refined with fixed individual displacement parameters [$U_{iso}(H) = 1.2\ U_{eq}(Csp^2)$] using a riding model with an aromatic, C–H bond length fixed at 0.93 Å. Methylene groups of the dppb ligand in the complex **(5)**, and methine group of the lapachol were set as isotropic with a thermal parameter 20% greater than the equivalent isotropic displacement parameter of the atom to which each one was bonded, whereas methyl groups were set with $U_{iso}(H)$ values of $1.5U_{eq}(C_{methyl})$. Tables were generated by WinGX [25] and the structure representations by ORTEP-3 [18] and MERCURY [21]. The main crystal data collections and structure refinement parameters for **(1)** and **(5)** are summarized in Table 1.

2.4. Synthesis

All the solvents used in this work were of reagent quality and used without further purification. Lapachol was obtained according to the procedure described in [24]. The precursors *cis*-[$RuCl_2(PPh_3)_2$(X-bipy)] (X = H, methyl (Me) and methoxy (MeO)) and *cis*-[$RuCl_2(PPh_3)_2$ (phen)] were prepared according to literature [26,27]. Typically [100.0 mg; 0.1 mmol] of the [$RuCl_2(PPh_3)_3$] was dissolved in degassed 20 mL of dichloromethane (Merck) and N-heterocyclic (X-bipy or

Table 1
Crystal data and structure refinement for complex [Ru(Lap)$(PPh_3)_2$(bipy)]PF_6 **(1)** and [$RuCl_2$(Lap)(dppb)] **(5)**.

	[Ru(Lap)$(PPh_3)_2$(bipy)]PF_6	[$RuCl_2$(Lap)(dppb)]
Empirical formula	$[RuC_{61}H_{51}N_2O_3P_2]PF_6$	$[RuC_{43}H_{41}Cl_2O_3P_2]$
Formula weight	1168.02	839.67
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell dimensions		
a (Å)	15.950(5)	9.1790(1)
b (Å)	16.744(5)	29.6950(5)
c (Å)	20.316(5)	14.7120(3)
β (°)	93.151(5)	104.564(1)
Volume ($Å^3$)	5418(3)	3881.20(11)
Z	4	4
Density calculated (Mg/m^3)	1.432	1.437
μ (mm^{-1})	0.447	0.663
F(000)	2392	1724
Crystal size (mm^3)	0.26 × 0.28 × 0.53	0.11 × 0.19 × 0.29
θ range (°)	2.96 to 26.76°	2.94 to 26.75°
Index ranges	−20 ≤ h ≤ 20 −19 ≤ k ≤ 21 −25 ≤ l ≤ 23	−11 ≤ h ≤ 8 −37 ≤ k ≤ 37 −18 ≤ l ≤ 18
Reflections collected	36,197	27,401
Independent reflections	11,479 [R(int) = 0.0423]	8251 [R(int) = 0.0617]
Completeness to θ	99.4%	99.7%
Max. and min. transmission	0.942 and 0.795	0.947 and 0.867
Data/restraints/parameters	11,479/0/687	8251/0/462
Goodness-of-fit on F^2	1.209	1.129
Final R indices [I > 2sigma(I)]	R1 = 0.0566, wR2 = 0.1321	R1 = 0.0376, wR2 = 0.0724
R indices (all data)	R1 = 0.0669, wR2 = 0.1386	R1 = 0.0692, wR2 = 0.0776
$\Delta\rho_{max.}$ and $\Delta\rho_{min.}$ ($e.Å^{-3}$)	0.553 and −0.641	0.557 and −0.541