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Synthesis, characterization, X-ray structure and *in vitro* antimycobacterial and antitumoral activities of Ru(II) phosphine/diimine complexes containing the "$SpymMe_2$" ligand, $SpymMe_2$ = 4,6-dimethyl-2-mercaptopyrimidine

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ABSTRACT

The reaction of *cis*-[$RuCl_2$(dppb)(N–N)], dppb = 1,4-bis(diphenylphosphino)butane, complexes with the ligand $HSpymMe_2$, 4,6-dimethyl-2-mercaptopyrimidine, yielded the cationic complexes [Ru($SpymMe_2$)(dppb)(N–N)]PF_6, N–N = bipy (**1**) and Me-bipy (**2**), bipy = 2,2′-bipyridine and Me-bipy = 4,4′-dimethyl-2,2′-bipyridine, which were characterized by spectroscopic and electrochemical techniques and X-ray crystallography and elemental analysis. Additionally, preliminary *in vitro* tests for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv ATCC 27264 and antitumor activity against the MDA-MB-231 human breast tumor cell line were carried out on the new complexes and also on the precursors *cis*-[$RuCl_2$(dppb)(N–N)], N–N = bipy (**3**) and Me-bipy (**4**) and the free ligands dppb, bipy, Me-bipy and $SpymMe_2$. The minimal inhibitory concentration (MIC) of compounds needed to kill 90% of mycobacterial cells and the IC_{50} values for the antitumor activity were determined. Compounds **1**–**4** exhibited good *in vitro* activity against *M. tuberculosis*, with MIC values ranging between 0.78 and 6.25 μg/mL, compared to the free ligands (MIC of 25 to >50 μg/mL) and the drugs used to treat tuberculosis. Complexes **1** and **2** also showed promising antitumor activity, with IC_{50} values of 0.46 ± 0.02 and 0.43 ± 0.08 μM, respectively, against MDA-MB-231 breast tumor cells.

1. Introduction

Tuberculosis (TB) has reemerged as one of the leading causes of death in the world, reaching a million deaths annually [1]. The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* and co-infections with AIDS have aggravated this serious situation and, since resistance decreases the effectiveness of most available antitubercular agents [2], there is an urgent need to develop new drugs to help reduce the global burden of tuberculosis, as has been well documented [3]. There is much evidence that complexes of metals such as silver, copper and iron, are effective antitubercular agents [4–6]. Ruthenium complexes, including organometallics [7], containing ligands such as diimines [8], phosphines [9], Schiff bases. [10] and thiosemicarbazones [11], have also been tested as antibacterial agents against a series of bacteria [7,8,11,12]. In some cases it has been shown that ruthenium complexes containing organic drugs as ligands can overcome resistance developed by bacteria to the organic compounds alone [13,14]. Another possible pharmaceutical application for metal complexes exploits their antitumoral activity. The success of cisplatin as an anticancer agent has stimulated the search for cytotoxic compounds with a more acceptable toxicity profile and enhanced activity [15,16]. Molecules containing other heavy metals of groups 8, 9 and 10 of the periodic table with a variety of ligands have constantly been evaluated as potential anticancer agents in a number of human tumor cells [17,18]. Specifically, ruthenium is an interesting alternative to platinum [19]. It has a range of oxidation states (II, III and IV) accessible under physiological conditions and its compounds are generally less toxic than platinum ones [20,21]. Two ruthenium-based anticancer drugs, NAMI-A – [ImH][*trans*-$RuCl_4$(DMSO)(Im)] and KP1019 – [H_2Ind][*trans*-

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$RuCl_4(HInd)_2$] (HInd = 1*H*-indazole), have successfully completed phase 1 clinical trials and are scheduled to enter phase 2 trials in the near future [22–24]. Phosphine complexes of various transition metals (Au, Ag, Cu, Ru, Rh, Pt, Pd) have been evaluated as potential antitumor agents in various human tumor cell lines [25–30]. In the Au complexes, for instance, the observed activity has been attributed to the presence of the phosphine ligands, since similar complexes without them had a very low activity [31]. In this sense, the metal center acts as a carrier of the real drug, protecting it from oxidation or other reactions in the cellular medium [32]. This paper describes the synthesis, spectroscopic and electrochemical characterization of two new complexes with the general formula [Ru(SpymMe$_2$)(dppb)(N–N)]PF_6, N–N = bipy (**1**) and Me-bipy (**2**). Additionally, the X-ray structure of **1** is presented and discussed. Finally, preliminary *in vitro* tests of antimycobacterial and antitumor activities of **1** and **2** and also of the biological activities of the precursors *cis*-[$RuCl_2$(dppb)(N–N)] [33,34], N–N = bipy (**3**) and Me-bipy (**4**), and the free ligands dppb, bipy, Me-bipy and SpymMe$_2$, are presented and discussed.

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), 2,2′-bipyridine (bipy), 4,4′-dimethyl-2,2′-bipyridine (Me-bipy) and 4,6-dimethyl-2-mercaptopyrimidine (HSpymMe$_2$) were used as received from Aldrich. The *cis*-[$RuCl_2$(dppb)(N–N)], N–N = bipy (**3**) or Me-bipy (**4**), complexes were prepared according to published procedures [33,34].

2.2. Instrumentation

The infrared spectra were recorded using CsI pellets in an FTIR Bomem–Michelson 102 spectrometer in the 4000–200 cm^{-1} region. UV–visible (UV–vis) spectra were recorded in a HP8452A (diode array) spectrophotometer. All NMR experiments were performed at 293 K on a Bruker spectrometer, 9.4 T, observing ^1H at 400.13, ^{13}C at 100.61 and $^{31}P\{^1H\}$ at 161.98 MHz. The NMR spectra were recorded in $CDCl_3$, with TMS (^1H and ^{13}C) and 85% H_3PO_4 ($^{31}P\{^1H\}$) as internal and external references, respectively. The splitting of proton, carbon and phosphorus resonances, respectively, in the reported ^1H, ^{13}C and $^{31}P\{^1H\}$ NMR spectra are defined as s = singlet, d = doublet, t = triplet, and m = multiplet. Cyclic voltammetry experiments were carried out at room temperature in CH_2Cl_2 containing 0.10 M Bu_4NClO_4 (TBAP) (Fluka Purum), with a BAS-100B/W Bioanalytical Systems Inc. electrochemical analyzer; the working and auxiliary electrodes were stationary Pt foils; a Luggin capillary probe was used and the reference electrode was Ag/AgCl. Under these conditions the ferrocene is oxidized at 0.43 V (Fc^+/Fc). The microanalyses were performed in the Microanalytical Laboratory of Universidade Federal de São Carlos, São Carlos (SP), with an EA 1108 CHNS microanalyser (Fisions Instruments).

2.3. X-ray crystallography

Orange crystals of **1** $\cdot$ $0.2H_2O$ were grown by slow evaporation of a dichloromethane/*n*-hexane solution. The crystal was mounted on an Enraf–Nonius Kappa-CCD diffractometer with graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation. The final unit-cell parameters were based on all reflections. Data collections were made using the COLLECT program [35]; integration and scaling of the reflections were performed with the HKL DENZO-SCALEPACK system of programs [36]. Absorption correction was carried out by the Gaussian method [37]. The structure was solved by direct methods with SHELXS-97 [38]. The model was refined by full-matrix least squares on F^2 by means of SHELXL-97 [39]. All hydrogen atoms were stereochemically positioned and refined with a riding model. The ORTEP view shown in Fig. 1 was prepared using ORTEP-3 for Windows [40]. Hydrogen atoms on the aromatic rings were refined isotropically, each with a thermal parameter 20% greater than the equivalent isotropic displacement parameter of the atom to which it is bonded. The data collections and experimental details are summarized in Table 1, and selected bond distances and angles are given in Table 2.

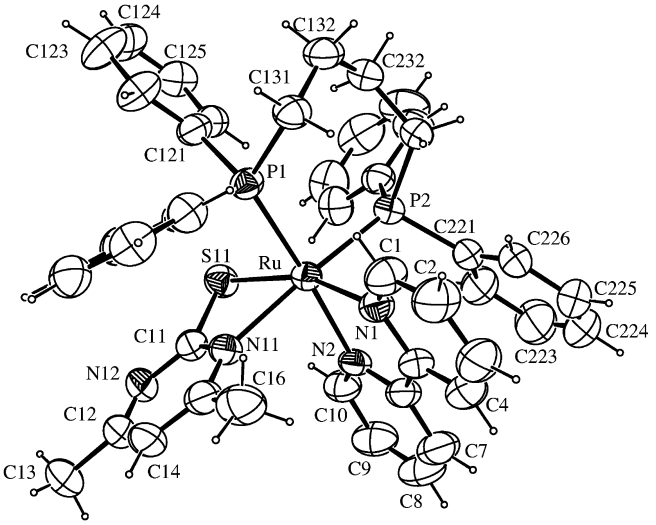


Fig. 1. ORTEP view of the cation complex [Ru(SpymMe$_2$)(dppb)(bipy)]$PF_6 \cdot 0.2H_2O$ with labeled atoms and 50% probability ellipsoids.

Table 1
Crystallographic data and refinement details for [Ru(SpymMe$_2$)(dppb)(bipy)]$PF_6 \cdot 0.2H_2O$

Empirical formula	$C_{44}H_{43}N_4P_2SRuPF_6 \cdot 0.2H_2O$
Formula weight	971.47
Temperature (K)	293(2)
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	
a (Å)	13.2520(3)
b (Å)	14.3737(4)
c (Å)	23.2284(6)
β (°)	98.265(1)
Volume (Å^3)	4378.6(2)
Z	4
Density (calculated) (g cm^{-3})	1.474
Absorption coefficient (mm^{-1})	0.578
$F(000)$	1984
Crystal size (mm^3)	0.20 × 0.16 × 0.04
θ range for data collection (°)	3.11–26.50
Limiting indices	$-16 \leqslant h \leqslant 16$ $-17 \leqslant k \leqslant 18$ $-29 \leqslant l \leqslant 28$
Reflections collected	29,800
Independent reflections (R_{int})	9011 (0.1246)
Data/restraints/parameters	9011/0/543
Goodness-of-fit on F^2	1.026
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0465, wR_2 = 0.1052
R indices (all data)	R_1 = 0.0800, wR_2 = 0.1201
Peak and hole/e Å^{-3}	0.477 and −0.887

Table 2
Selected bond lengths (Å) and angles (°) for complex [Ru(SpymMe$_2$)(dppb)(bipy)]$PF_6 \cdot 0.2H_2O$, with estimated standard deviations in parentheses

Ru–N(1)	2.117(3)	N(1)–Ru–N(2)	77.0(1)	N(2)–Ru–P(1)	176.02(7)
Ru–N(2)	2.139(2)	N(1)–Ru–N(11)	96.5(1)	N(11)–RuP(1)	92.92(7)
Ru–N(11)	2.177(2)	N(2)–Ru–N(11)	83.41(9)	P(2)–Ru–P(1)	94.02(3)
Ru–P(2)	2.3092(8)	N(1)–Ru–P(2)	88.96(7)	N(1)–Ru–S(11)	161.28(7)
Ru–P(1)	2.3326(8)	N(2)–Ru–P(2)	89.81(6)	N(2)–Ru–S(11)	91.95(8)
Ru–S(11)	2.4059(9)	N(11)–Ru–P(2)	170.09(8)	N(11)–Ru–S(11)	66.87(8)
S(11)–C(11)	1.720(4)	N(1)–Ru–P(1)	101.94(7)	P(2)–Ru–S(11)	106.31(3)

2.4. Synthesis

The thiolate derivatives [Ru(SpymMe$_2$)(dppb)(N–N)]PF_6, N–N = bipy (**1**) and Me-bipy (**2**) were prepared by reacting the *cis*-[$RuCl_2$(dppb)(N–N)], N–N = bipy (**3**) and Me-bipy (**4**) precursors (0.064 mmol; ≅50.0 mg) with excess of the 4,6-dimethyl-2-mercaptopyrimidine ligand (0.099 mmol; ≅14.0 mg) and 0.20 mmol (32.6 mg) of NH_4PF_6 in methanol (20 mL) under an Ar atmosphere for 12 h. The final orange solutions were concentrated to ca. 3 mL and excess of water was added in order to obtain orange precipitates. The solids were filtered off, well rinsed with water (3 × 5 mL) and diethyl ether (3 × 5 mL) and dried *in vacuo*.

2.4.1. [Ru(SpymMe$_2$)(dppb)(bipy)]PF_6 (**1**)

Yield: 58 mg (91%). Anal. Calcd for $C_{44}H_{43}F_6N_4P_3RuS$: exptl (calc) C, 54.61 (54.60); H, 4.72 (4.48); N, 5.93 (5.79); S, 3.72 (3.31). $^{31}P\{^1H\}$ NMR: δ(ppm) 44.5 (d); 39.3 (d), $^2J_{pp}$ = 36.8 Hz. ^1H NMR (400.21 MHz, $CDCl_3$, 298 K): δ(ppm) 9.42 (d, 1H, ^3J = 5.66 Hz); 8.86 (d, 1H, ^3J = 4.0 Hz); 8.44 (d, 1H, ^3J = 7.43 Hz); 8.27 (d, 1H, ^3J = 8.06 Hz); 8.12 (t, 1H, ^3J = 8.08 Hz); 7.78 (t, 1H, ^3J = 7.76 Hz); 7.58 (t, 1H, ^3J = 8.51 Hz); 7.44 (t, 1H, ^3J = 7.47 Hz) (aromatic hydrogens for bipy); 7.34–6.56 (overlapped signals, 20H aromatic hydrogens for dppb); 4.0–1.0 (8H, CH_2 of dppb); 5.67 (s, 1H of SpymMe$_2$); 2.15 (s, 3H, CH_3 of SpymMe$_2$); 0.48 (s, 3H, CH_3 of SpymMe$_2$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ(ppm) 19.0 (s, CH_3 of SpymMe$_2$), 20.3 (s, CH_2), 23.1 (s, CH_2), 24.4 (s, CH_3-SpymMe$_2$), 29.9 (d, $^1J_{CP}$ 29, CH_2), 30.2 (d, $^1J_{CP}$ 29, CH_2), 114.8 (C5-SpymMe$_2$), 122.8 (C3-bipy), 124.6 (C5-bipy), 126.4–138.5 (m, Ph), 138.3 (C4-bipy), 138.7 (C4-bipy), 150.8 (C6-bipy), 155.4 (C4-SpymMe$_2$), 157.2 (C6-bipy), 158.7 (C6-SpymMe$_2$), 165.5 (C2-bipy), 166.3 (C2-bipy), 185.3 (C2–SpymMe$_2$). UV–vis (CH_2Cl_2, 10^{-5} M): λ/nm (ε/M^{-1} cm^{-1}) 296 (2.62×10^4), 302 (2.61×10^4), 426 (4.49×10^3).

2.4.2. [Ru(SpymMe$_2$t)(dppb)(Me-bipy)]PF_6 (**2**)

Yield: 55 mg (86%). Anal. Calcd for $C_{46}H_{47}F_6N_4P_3RuS$: exptl (calc) C, 55.32 (55.47); H, 4.98 (4.76); N, 5.75 (5.63); S, 3.42 (3.22). $^{31}P\{^1H\}$ NMR: δ(ppm) 45.0 (d); 39.8 (d), $^2J_{pp}$ = 36.8 Hz.^1H NMR (400.21 MHz, $CDCl_3$, 298 K): δ(ppm) 9.15 (d, 1H, ^3J = 5.29 Hz); 8.65 (d, 1H, ^3J = 5.75 Hz); 8.32 (s, 1H); 8.16 (s, 1H); 7.58 (t, 2H, ^3J = 8.37 Hz) (aromatic hydrogens for Me-bipy); 2.63 (s, 3H, CH_3); 2.41 (s, 3H, CH_3') (aliphatic hydrogens for Me-bipy; 7.45–6.60 (overlapped signals, 20H aromatic hydrogens for dppb); 4.0–1.0 (8H, CH_2 of dppb); 5.67 (s, 1H of SpymMe$_2$); 2.15 (s, 3H, CH_3 of SpymMe$_2$); 0.50 (s, 3H, CH_3 of SpymMe$_2$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ(ppm) 19.5 (s, CH_3 of SpymMe$_2$), 20.3 (s, CH_2), 20.9 (s, 2CH_3-Me-bipy), 21.3 (s, CH_2), 24.3 (s, CH_3-SpymMe$_2$), 30.3 (d, $^1J_{CP}$ 31, CH_2), 30.4 (d, $^1J_{CP}$ 28, CH_2), 114.6 (C5-SpymMe$_2$), 123.9 (C3-Me-bipy), 125.6 (C5-Me-bipy), 127.5-138.5 (m, Ph), 150.0 (C6-Me-bipy), 150.5, 151.5, 155.0 (C4-SpymMe$_2$), 156.3 (C6-Me-bipy), 158.3 (C6-SpymMe$_2$), 162.5 (C4-Me-bipy), 162.7 (C4-Me-bipy), 169.2 (C2-Me-bipy), 170.6 (C2-Me-bipy), 183.9 (C2-SpymMe$_2$). UV–vis (CH_2Cl_2, 10^{-5} M): λ/nm (ε/M^{-1} cm^{-1}) 290 (2.66×10^4), 300 (2.66×10^4), 408 (5.25×10^3).

2.5. Antimycobacterial activity assay

Antimycobacterial activities of each tested compound and of the standard drug isoniazid (Difco laboratories, Detroit, MI, USA) were determined in triplicate in sterile 96-well flat bottomed microplates (Falcon 3072; Becton Dickinson, Lincoln Park, NJ, USA) and Middlebrook 7H9 Broth (Difco) supplemented with oleic acid–albumin–dextrose–catalase (OADC) enrichment (BBL/Becton Dickinson, Sparks, MD, USA). The tested compound concentrations ranged from 0.15 to 250 μg/mL and the isoniazid from 0.015 to 1.0 μg/mL. The microplate Alamar Blue assay [41] (MABA) was used to measure the minimal inhibitory concentration (MIC) for the tested compounds (minimum concentration necessary to inhibit 90% growth of *M. tuberculosis* H_{37}Rv ATCC 27294). The development of a pink color in the wells was taken as indicating bacterial growth and the maintenance of a blue color as the contrary. Thus, MIC was assumed to be the lowest concentration able to inhibit the change of color from blue to pink. The fluorescence of the dye was measured in a SPECTRAfluor Plus microplate reader (Tecan®) in bottom reading mode with excitation at 530 nm and emission at 590 nm.

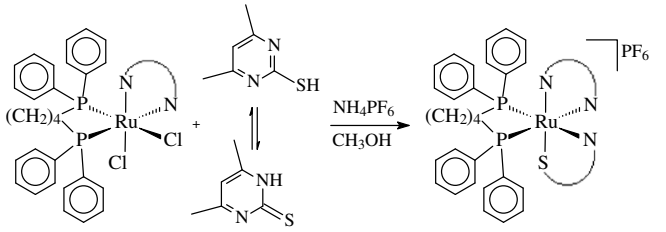
2.6. Cell culture assay (MDA-MB-231)

In vitro cytotoxicity assays on cultured human tumor cell lines still represent the standard method for the initial screening of antitumoral agents. Thus, as a first step in assessing their pharmacological properties, the new ruthenium complexes were assayed against a human breast tumor cell line MDA-MB-231 (ATCC No. HTB-26). The cells were routinely maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), at 37 °C in a humidified 5% CO_2 atmosphere. After reaching confluence, the cells were detached by trypsinization and counted. For the cytotoxicity assay, 5×10^4 cells $well^{-1}$ were seeded in 200 μL of complete medium in 96-well assay microplates (Corning Costar). The plates were incubated at 37 °C in 5% CO_2 for 24 h to allow cell adhesion, prior to drug testing. All tested compounds were dissolved in sterile DMSO (stock solution with maximum concentration of 20 mM) and diluted to 5, 2, 1, 0.5, 0.2, 0.02 and 0.002 mM. From each of these dilute samples 2 μL aliquots were added to 200 μL medium (without FBS) giving a final concentration of DMSO of approximately 1% and a final concentration of the complex diluted about 100 times. Cells were exposed to the compounds for a 24 h-period. Cell respiration, as an indicator of cell viability, was determined by the mitochondrial-dependent reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan [42]. MTT solution (0.5 mg/mL) was added to cell cultures and incubated for 3 h, after which 100 μL of isopropanol was added to dissolve the precipitated formazan crystals. The conversion of MTT to formazan by metabolically viable cells was monitored in an automated microplate reader at 570 nm. The percent cell viability was calculated by dividing the average absorbance of the cells treated with the test compounds by that of the control; % cell viability was plotted against drug concentration (logarithmic scale) to determine the IC_{50} (drug concentration at which 50% of the cells are viable relative to the control), with the error estimated from the average of 3 trials.

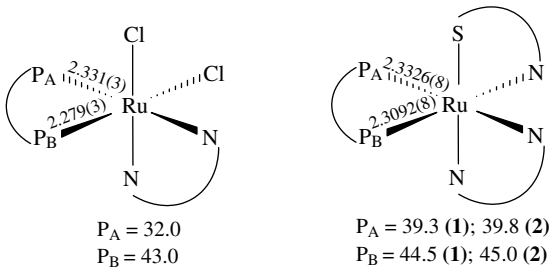
3. Results and discussion

3.1. Synthesis of the complexes

The chemical reactivity of the SpymMe$_2$ ligand with complexes such as *cis*-[$RuCl_2$(dppb)(N–N)] [33] allowed us to synthesize com-



Scheme 1. Synthesis of the ruthenium (II) compounds.



Scheme 2. $^{31}P\{^1H\}$ chemical shifts and Ru–P distances for complexes **1**–**2** and precursors (av.).

plexes with the general formula $[Ru(SpymMe_2)(dppb)(N–N)]PF_6$ (N–N = bipy and Me-bipy), containing three chelated ligands, in mild conditions by simple chlorides exchange (see Scheme 1).

3.2. Structural studies

The X-ray structure of $[Ru(SpymMe_2)(dppb)(bipy)]PF_6$ (**1**) · $0.2H_2O$ was determined and an ORTEP view showing the atom numbering scheme is projected in Fig. 1. Selected bond lengths and angles are presented in Table 2. The crystal structure of the complex consists of separated discrete molecular units and solvated water molecules. Compound **1** · $0.2H_2O$ crystallizes in the monoclinic system, space group $P2_1/n$, with the Ru center adopting a distorted octahedral coordination geometry formed by three bidentate ligands. The phosphorus atoms are disposed *trans* to nitrogen atoms, one from the bipy and other from the $SpymMe_2$ ligand. The sulfur atom is positioned *trans* to the remaining bipy nitrogen atom.

The Ru–P bonds of 2.3092(8) and 2.3326(8) Å are within the normal range found for Ru(II) tertiary phosphine complexes [43–48]. The P–Ru–P angle in the seven-membered ring of dppb is 94.02(3)°, comparable with the values previously observed for **3** [33]. The average Ru–N (bipy) distance of 2.128 Å in complex **1** is longer than that observed in the precursor **3** (2.09 Å), probably due to the less efficient back-donation from Ru^{II} in **1**. The same effect probably causes the Ru–N ($SpymMe_2$) distance 2.177(2) Å to be considerable longer than that observed in the complex $[Ru(SpymMe_2,\text{-}N,\text{-}S)(SpymMe_2,\text{-}S)(NO)(dppe)]PF_6$ [49] 2.105(9) Å. The Ru–S distance of 2.4059(9) Å is essentially identical to those observed in other complexes containing the 'SpymMe$_2$' or similar thiolate ligands [49–51]. The $SpymMe_2$ ligand dimensions in complex **1** (C(11)–S(11) = 1.720(4) Å, C(11)–N(11) = 1.347(5) Å and N(11)–C(11)–S(11) = 110.7(2)°) are consistent with deprotonation and coordination of the heterocycle to the Ru center [52]. The C–S bond distance of 1.720(4) Å is significantly longer than the expected C=S double-bond distance of 1.62 Å, but shorter than a C–S single-bond distance of 1.81 Å [53], suggesting a partial double bond character [54]. The four-membered ring formed by the S,-N chelating heterocyclic thiolate is inherently strained [52]. This is evidenced in **1** by the small S(11)–Ru–N(11) angle of 66.87(8)°.

3.3. Characterization of the compounds

The $^{31}P\{^1H\}$ NMR spectra of **1** and **2** in $CDCl_3$ present a typical AX spin systems with chemical shifts at 44.5 (d); 39.3 (d) and 45.0 (d); 39.8 (d) ppm, respectively, with $^2J_{P-P}$ = 36.8 Hz, indicating the magnetic nonequivalence of the two phosphorus atoms. Precursor complexes **3** and **4** presents a pair of doublets at 43.0 and 32.0 ppm with $^2J_{P-P}$ = 32.0 Hz in their $^{31}P\{^1H\}$ spectra [33,55]. In the precursors, the high-field doublet corresponds to the P *trans* N, as previously described [33]. These assignments are based on an empirical linear correlation established between the crystallographically determined Ru–P distances in a series of Ru-dppb complexes and the corresponding ^{31}P chemical shifts observed in solution, with the chemical shifts becoming more high-field with increasing Ru–P bond lengths [33,46]. Based on this previous information, we suggest that in the new complexes the high-field doublet belongs to the P *trans* (N–N) nitrogen, because the Ru–P distance of 2.3326(8) Å (*trans* N–N) is longer than that observed for the Ru–P *trans* N of the $SpymMe_2$ ligand (2.3092(8) Å) (see Scheme 2).

The ^1H NMR spectra of **1** and **2** showed signals for the phosphine phenyl groups as a series of multiplets in the δ 7.50–6.60 ppm region, corresponding to 20 hydrogen atoms. The diimine (N–N) proton resonances are observed in the typical region. For **1**, the expected eight aromatic proton environments (relative intensity 1H each) appear in the 9.42–7.44 ppm region. For **2**, the six aromatic protons of Me-bipy are observed in the 9.15–7.58 ppm region, while two additional singlets at 2.63 and 2.41 ppm are observed for the CH_3 groups. The coordination of the $SpymMe_2$ thiolate ligand is verified by three characteristic singlet signals at 5.67, 2.15 and 0.48 ppm for **1** and at 5.67, 2.15 and 0.50 ppm for **2**, corresponding to the only hydrogen atom of the pyrimidine ring and to the two methyl groups attached to the pyrimidine ring, respectively. The IR spectra of the new complexes also confirm the presence of the $SpymMe_2$ ligand coordinated to the metal. The band attributable to the ν(N–H), which appears at 3200–3100 cm^{-1} in free thiones, is absent in **1** and **2**, indicating that the $SpymMe_2$ is coordinated in the deprotonated form [56]. The electronic spectra of **1** and **2** show three bands in the UV region, assigned as intra-ligand transitions by means of comparison with the free ligands (dppb and $SpymMe_2$). One band observed in the visible region results from a metal-to-ligand charge transfer transition, probably involving both diimine and $SpymMe_2$ ligands. Mass spectra (ESMS) of **1** and **2** show the parent molecular ion at m/z 823 and 851 $[M-PF_6]^+$, respectively, in agreement with the assigned above formulations. No fragmentation was observed in the experimental conditions utilized. The cyclic voltammetric experiments for **1** and **2** were carried out in CH_2Cl_2 solution. The electrochemical behavior of these complexes was similar to that observed for the precursors **3** and **4**. A quasi-reversible process was observed for each complex, corresponding to an one-electron Ru^{II}/Ru^{III} redox process. As expected, the $E_{1/2}$ values found for the new complexes were considerably more anodic than those observed for the respective precursors, by approximately 0.30 V (Table 3), indicating a more stabilized Ru center in the new derivatives than in their precursors. This stabilization is supposed to be due to the substitution of two monoanionic donor chlorides by a single monocharged chelating $SpymMe_2$ unit, resulting in the positive-charged product complexes [57]. Cyclic voltammograms are shown in Supplementary Material Figs. S3 and S4).

3.4. Antimycobacterial activity

The compounds were investigated for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strains by the MABA method [41]. The minimum inhibitory concentrations

Table 3
Electrochemical data for complexes **1**–**4** in CH_2Cl_2 vs. Ag/AgCl

Complex	$E_{1/2}/V$[a]	$\Delta E_p/V$[b]
1[c]	0.92	0.18
2[c]	0.88	0.16
3[d]	0.65	0.15
4[d]	0.60	0.17

[a] *Scan rate*: 100 mV s^{-1}.
[b] $\Delta E_p = E_{pa} - E_{pc}$.
[c] This work.
[d] Ref. [55].

Table 4
MIC value (μg/mL and μM) of antimycobacterial activity of Ru complexes, free ligands and reference drug

Identification	Compound	MIC (μg/mL)	MIC (μM)
1	$[Ru(SpymMe_2)(dppb)(bipy)]PF_6$	0.78	0.80
2	$[Ru(SpymMe_2)(dppb)(Me\text{-}bipy)]PF_6$	0.78	0.78
3	*cis*-$[RuCl_2(dppb)(bipy)]$	3.9	5.17
4	*cis*-$[RuCl_2(dppb)(Me\text{-}bipy)]$	6.25	7.99
Ligand	dppb	>50	>117.2
Ligand	bipy	25	160.1
Ligand	Me-bipy	25	135.7
Ligand	$HSpymMe_2$	25	178.3
Reference drug	Isoniazid	0.03	0.36

(MICs) found for the ruthenium complexes, free ligands and isoniazid are shown in Table 4.

According to these tests, compounds **1** and **2** exhibited promising activity, with MIC values of 0.80 and 0.78 μM, respectively. The results indicate that these complexes have stronger *in vitro* activity than that of ethambutol (MIC 5.62 μM) or gatifloxacin (MIC 0.99 μM), and quite similar to that of the main drug isoniazid (MIC 0.36 μM), which is the clinically used as a first-line drug in several schemes of conventional tuberculosis treatment [3]. Complexes **3** and **4** (MIC of 5.17 and 7.99 μM, respectively) were also active, but to a lesser extent. Interestingly, the MIC values for free ligands were several times higher than those observed for the complexes **1**–**4**.

3.5. Antitumoral activity

The cells were exposed to each compound for a period of 24 h, in order to allow them reach DNA or any other biological target. The new complexes **1** and **2**, the precursors **3** and **4** and the uncoordinated dppb, bipy, Me-bipy and $HSpymMe_2$ ligands were tested against the MDA-MB-231 cells. For comparison, the cytotoxicity of cisplatin was evaluated under the same experimental conditions. The IC_{50} values, calculated from the dose-survival curves generated by the MTT assay obtained after 24 h drug treatment, are shown in Table 5. Fig. 2 shows the cell viability vs. concentration plots for complexes **1** and **2**.

The new complexes **1** and **2** were very active against the human tumor cell line with IC_{50} values of 0.46 ± 0.02 and 0.43 ± 0.08 μM, respectively. Interestingly, the precursor complexes **3** and **4** were also cytotoxic to the MDA-MB-231 cells, but with IC_{50} values more than 30-fold higher than those observed for **1** and **2**. At this point is relevant to mention that the complexes *cis*-$[RuCl_2(dppb)(N\text{–}N)]$ (**3** and **4**) are considered pro-drugs because in DMSO solution occurs a rapid exchange of one chloride forming the cationic species $[RuCl(DMSO)(dppb)(N\text{–}N)]Cl$. By contrast, complexes **1** and **2** are very stable in DMSO solutions at least for a 24 h-period. The free ligands were also evaluated in the same experimental conditions and showed a complete lack of activity without appreciable cytotoxicity at concentrations as high as 200 μM. In the literature, most ruthenium-containing complexes studied for their antitumor activity have a pair of *cis*-orientated chloro ligands with some reports indicating the possibility of a mechanism involving DNA binding [17,58–63]. More recently some papers have described that the main factor responsible for the antitumor activity of ruthenium complexes is the binding with serum proteins such as albumin and transferrin, despite that the exact role of such binding plays in the mechanism of the action of metallodrugs remains to be elucidated [64]. In our complexes, much higher activity was achieved when the *cis*-chlorides of the *cis*-$[RuCl_2(dppb)(N\text{–}N)]$ pre-

Table 5
IC_{50} values for the MDA-MB-231 cell line of Ru complexes, free ligands and cisplatin measured in DMSO solution.

Identification	Compound	IC_{50} (μM)
1	$[Ru(SpymMe_2)(dppb)(bipy)]PF_6$	0.46 ± 0.02
2	$[Ru(SpymMe_2)(dppb)(Me\text{-}bipy)]PF_6$	0.43 ± 0.08
3	*cis*-$[RuCl_2(dppb)(bipy)]$	22.6 ± 10
4	*cis*-$[RuCl_2(dppb)(Me\text{-}bipy)]$	13.8 ± 1.3
Ligand	dppb	>200
Ligand	bipy	>200
Ligand	Me-bipy	>200
Ligand	$HSpymMe_2$	>200
Reference drug	Cisplatin	63 ± 5

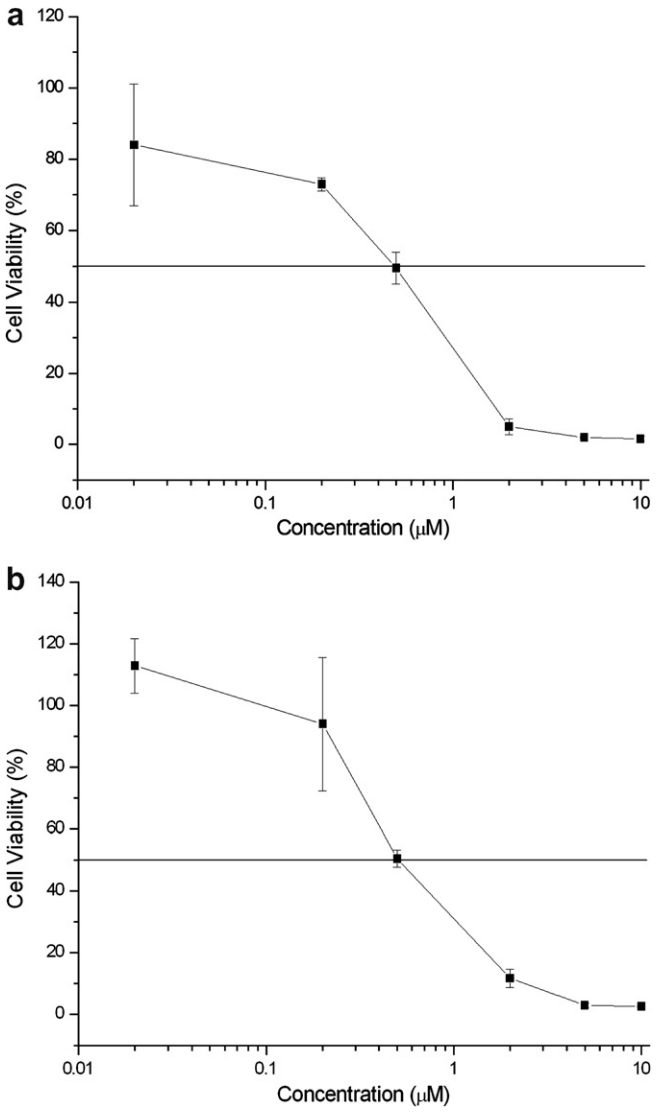


Fig. 2. Cell viability vs. concentration plot for complexes **1** (a) and **2** (b), with error bars representing the 95% confidence interval.

cursors (**3** and **4**) were replaced by the $SpymMe_2$ ligand, forming the new derivatives *cis*-[Ru($SpymMe_2$)(dppb)(N–N)]PF_6 (**1** and **2**). These data indicate that different cytotoxic mechanism may occur with these two classes of complexes. A major structural difference between complexes **1** and **2** on the one hand and with **3** and **4** on the other consists in the presence of three chelated ligands in the former while **3** and **4** show only two chelated and two monodentate ligands. Considering this fact, complexes **3** and **4** may be too reactive with components of the cell culture medium and/or the cells such a way their activity were decreased when compared with **1** and **2**. From the above results, it appears that higher cytotoxicity of these complexes is associated with the presence of the three stable bidentate chelating ligands in their structures. Thus, one possible explanation for the activity of **1** and **2** is that they are coordinatively saturated and rigid complexes, and are able to bind non-covalently the DNA as recently reported for others Ru(II) complexes containing diimines ligands [65]. Even relative to cisplatin (reference metallodrug), our complexes, including **3** and **4**, are much more active on the MDA-MB-231 cell line, indicating their potential usefulness as antitumoral agents.

4. Conclusions

In this investigation two new ruthenium phosphine/diimine complexes [Ru($SpymMe_2$)(dppb)(N–N)]PF_6, N–N = bipy (**1**) and Me-bipy (**2**), were synthesized and characterized. The complexes were characterized by spectroscopy, cyclic voltammetry and X-ray crystallography. Antimicrobial activity assays of the new complexes provided evidence that they are potential agents against mycobacterial infections, specifically against *M. tuberculosis* H37Rv. Further studies should also include the evaluation and improvement of intracellular uptake of the complexes, as well as testing them for intramacrophagic clearance of mycobacterial infections. Such studies could shed light on their mechanisms of action in mycobacterial species. The *in vitro* antitumor activity test utilizing the MDA-MB-231 human tumor cell line indicated a high degree of cytotoxicity in both **1** and **2.** Our results show that the presence of the $SpymMe_2$ ligand, replacing the chlorides ligands of the precursors, forming cationic species, represents a highly advantageous modification of the phosphine/diimine ruthenium complexes, leading to species even more active than cisplatin. Currently, we are working on new derivatives in order to understand what factor is mainly responsible for the observed activities.

5. Abbreviations

dppe	1,2-bis(diphenylphosphino)ethane
dppb	1,4-bis(diphenylphosphino)butane
$HSpymMe_2$	4,6-dimethyl-2-mercaptopyrimidine
$SpymMe_2$	deprotonated 4,6-dimethyl-2-mercaptopyrimidine
bipy	2,2′-bipyridine
Me-bipy	4,4′-dimethyl-2,2′-bipyridine
ESMS	electrospray mass spectrometry
IC_{50}	drug concentration at which 50% of the cells are viable relative to the control
MABA	microplate alamar blue assay
MIC	minimal inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TMS	tetramethylsilane

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Appendix A. Supplementary material

UV–vis spectra and cyclic voltammograms for complexes **1** and **2** (Figs. S1, S2, S3 and S4) are included as supplementary materials. CCDC 663381 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2008.05.009.

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