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New ruthenium(II)/phosphines/diimines complexes: Promising antitumor (human breast cancer) and *Mycobacterium tuberculosis* fighting agents

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ABSTRACT

The synthesis and characterization of ruthenium compounds of the type $[RuCl_2(P)_2(N–N)]$ $[(P)_2 = (PPh_3)_2$, dppb = 1,4-bis(diphenylphosphino)butano; dppp = 1,3-bis(diphenylphosphino)propane; N–N = 5,5′-dimethyl-2,2′dipyridyl (5,5′-mebipy) or 4,4′-dimethyl-2,2′dipyridyl (4,4′-mebipy)] are described. The complexes were characterized using elemental analysis, UV–Vis and infrared spectroscopies, cyclic voltammetry, and X-ray crystallography. *In vitro* evaluation of the complexes, using the MTT methodology, revealed their cytotoxic activities in a range of 5.4–15.7 μM against the MDA-MB-231 breast tumor cells and showed that, in this case, they are more active than the reference metallodrug cisplatin. The *in vitro* antimycobacterial activities of the complexes had their Minimum Inhibitory Concentration (MIC) for MTB cell growth measured, by the REMA method. The MICs for these complexes were found to be between 12.5 and 25.0 μg/mL. The results are comparable with the "second line" drug cycloserine (MIC = 12.5–50.0 μg/mL), commonly used in the treatment of TB.

1. Introduction

Since the discovery of the cisplatin as an antitumor agent in the sixties, extensive research with transition metal ions has been done and ruthenium has attracted a considerable interest as the basis for new potential metallodrugs to treat cancer diseases [1–3]. Metallopharmaceuticals have enormous potential to help overcome the limitations of current therapies in cancer due to their variety of geometries and chemical reactivities [4]. The activities of these compounds depend on the metal ion, their ligands and the structure of the compounds. Thus, NAMI-A, imidazolium *trans*-[tetrachloro(dimethylsulfoxide)(1H-imidazole)ruthenate(III)], and KP1019, indazolium *trans*-[tetrachlorobis(1H-indazole) ruthenate(III)], are potential antitumor drugs that are in clinical trials having successfully completed phase I. The success of these two compounds reinforces the scope to continue searching for new drugs that may have the cure, or for diminishing unwanted effects of cancer treatment. Also, the bifunctional complexes of the general structural composition $[Ru(\eta^6\text{-arene})X_2(PTA)]$ (PTA = 1,3,5-triaza-7-phosphaadamantane) (RAPTA) showed antimetastatic properties and generally low toxicity comparable to those observed for NAMI-A [3].

The interest in synthesizing new metal compounds is in diminishing the diverse side effects, as nephrotoxicity, toxicity, nausea and vomiting, in cancer patients treated with the cisplatin. In the last five years we have been synthesizing some ruthenium/phosphine/diimine complexes, which are showing promising activity against cancer and also against *Mycobacterium tuberculosis* [MT] [5–9]. The bacterium *M. tuberculosis* is the tuberculosis agent, which is responsible for the death of about 2 million people annually [10]. The treatment of the illness is expensive, long, causes some side effects and encounters resistance. Therefore many researchers are looking for new alternatives in the treatment of this illness such as some ruthenium and gold complexes that have been found to be very promising, showing minimum inhibitory concentrations (MICs) comparable to or even better than some reference drugs used in the treatment of tuberculosis [7–9,10].

In this work, four new ruthenium complexes were synthesized, characterized, their X-rays structures were determined, and their

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citotoxicity tested against MDA-MB-231 (human breast cancer) cancer cells and against MT.

2. Experimental

2.1. Materials and measurements

All manipulations were carried out under purified argon using standard Schlenk techniques. Reagent grade solvents were appropriately distilled and dried before use. All chemicals used were of reagent grade or comparable purity. All chemicals were purchased from Aldrich or Fluka and were used as received. The precursors $[RuCl_2(PPh_3)_3]$ and $[RuCl_2(dppb)(PPh_3)]$ were prepared using published procedures [11–13].

The IR spectra of the complexes were recorded on a FTIR Bomem-Michelson 102 spectrometer in the 4000–200 cm^{-1} region using solid samples pressed in CsI pellets. The NMR spectra of $^{31}P\{^1H\}$ were recorded using a BRUKER, DRX400 tesla equipment, in a BBO 5 mm probe at room temperature, and dichloromethane was used as solvent, using a capillary containing D2O. The molar conductance measurements (Λ) were carried out in dichloromethane at 25 °C, using concentrations of 1.0×10^{-3} M for the complexes. Cyclic voltammetry (CV) experiments were carried out at room temperature in CH_2Cl_2 containing 0.100 M Bu_4NClO_4 (TBAP) (Fluka Purum) using a BAS-100B/W Bioanalytical Systems Instrument. The working and auxiliary electrodes were stationary Pt foils; the reference electrode was of Ag/AgCl, in a Luggin capillary probe. The electronic spectra were obtained through a scanning done on a Hewlett–Packard diode array, model 8452A, spectrophotometer. The microanalyses were performed at the Microanalytical Laboratory at the Federal University of São Carlos, São Carlos, São Paulo, using a FISIONS CHNS, mod. EA 1108 micro analyzer.

2.2. Synthesis

2.2.1. Synthesis of $[RuCl_2(PPh_3)_2(5,5'\text{-mebipy})]$ (**1**)

The complex was obtained by reacting 0.500 g (0.520 mmol) of $[RuCl_2(PPh_3)_3]$ dissolved in 20.0 mL of dichloromethane, previously deaerated, and after that 0.106 g (0.570 mmol) of 5,5′-mebipy was added. For the syntheses of the complex (**1**) and (**2**) the solutions were refluxed for 2 h, after that the volumes were diminished until 2 mL and diethyl ether was added for the precipitation of the desired product. The solids were filtered off, well rinsed with diethyl ether (3 × 5 mL) and dried in vacuum. Yield 0.413 g (90%) Experimental *Anal.* Calc. for $C_{48}H_{42}P_2N_2Cl_2Ru$: C, 65.84; H, 4.66; N, 3.22. Found: C, 65.46; H, 4.81; N, 3.18%. Molar conductance (μS/cm, CH_2Cl_2) 3.2. $^{31}P\{^1H\}$ NMR (162 MHz, CH_2Cl_2/D_2O) δ(ppm) 27.6 (s) Selected IR(cm^{-1}; w = weak; s = strong): 1604 [w, ν(C=N)], 1481[s, ν(C=N)], 1435 [s, ν(C=N)], 517 [s, ν(Ru–P)], 461 [w, ν(Ru–N)], 303 e 287 [w, ν(Ru–Cl)]. UV–Vis, CH_2Cl_2 λ_{max}/nm ($\varepsilon/M^{-1}\,cm^{-1}$): 272 (22 391), 306 (21 361), 460 (1458).

2.2.2. Synthesis of cis-$[RuCl_2(dppb)(5,5'\text{-mebipy})]$ (**2**)

The complex was obtained by reacting 0.300 g (0.350 mmol) of $[RuCl_2(dppb)(PPh_3)]$ dissolved in 20.0 mL of dichloromethane, previously deaerated, and after that 0.071 g (0.380 mmol) of 5,5′-mebipy was added. Yield 0.246 g (90%). Experimental *Anal.* Calc. for $C_{40}H_{40}Cl_2N_2P_2Ru$: C, 61.24; H, 5.22; N, 3.68. Found: C, 61.38; H, 5.15; N, 3.58%. Molar conductance (μS/cm, CH_2Cl_2) 4.5. $^{31}P\{^1H\}$ NMR (162 MHz, CH_2Cl_2/D_2O) δ(ppm) 45.4 (d) and 31.8 (d) $^2J_{p-p}$(Hz) 32.7. Selected IR(cm^{-1}): 1637 [w, ν(C=N)], 1475 [s, ν(C=N)], 1433 [s, ν(C=N)], 519 [s, ν(Ru–P)], 436 [w, ν(Ru–N)], 301 e 267 [w, ν(Ru–Cl)]. UV–Vis CH_2Cl_2 λ_{max}/nm ($\varepsilon/M^{-1}\,cm^{-1}$): 312 (16594), 424 (2330).

2.2.3. Synthesis of cis-$[RuCl_2(dppp)(N–N)]$; N–N = 5,5′-mebipy (**3**) or (4,4′-mebipy) (**4**)

These complexes were synthesized by reacting 0.300 g (0.340 mmol) of $[RuCl_2(PPh_3)_2(N–N)]$, [N–N = 5,5′-mebipy (**3**) or 4,4′-mebipy (**4**)] in 30.0 mL of a mixture of dichlorometane:benzene (1:1) with 0.210 g (0.510 mmol) of dppp [1,3-bis(diphenilphosphine) propane]. For the syntheses of the complex (**3**) and (**4**) the solutions were refluxed for 4 days. (**3**): Yield: 0.223 g (85%). Experimental *Anal.* Calc. for $C_{39}H_{38}Cl_2N_2P_2Ru$: C, 60.90; H, 5.27; N, 3.27. Found: C, 60.94; H, 4.98; N, 3.64%. Molar conductance (μS/cm, CH_2Cl_2) 1.7. $^{31}P\{^1H\}$ NMR (162 MHz, CH_2Cl_2/D_2O) δ(ppm) 38, (d) and 30.2 (d) $^2J_{p-p}$(Hz) 42,2. Selected IR (cm^{-1}): 1618 [w, ν(C=N)], 1483 [s, ν(C=N)], 1433 [s, ν(C=N)], 517 [s, ν (Ru–P)], 444 [w, ν(Ru–N)], 301 and 274 [w, ν(Ru–Cl)]. UV–Vis CH_2Cl_2, λ_{max}/nm ($\varepsilon/M^{-1}\,cm^{-1}$): 292 (13885), 424 (1954).

(**4**): Yield: 0.236 g (90%). Experimental *Anal.* Calc. for $C_{39}H_{38}Cl_2N_2P_2Ru$: C, 61.05; H, 5.01; N, 3.40. Found: C, 60.94; H, 4.98; N, 3.64%. Molar conductance (μS/cm, CH_2Cl_2) 2.0. $^{31}P\{^1H\}$ NMR (162 MHz, CH_2Cl_2/D_2O) δ(ppm) 39.3 (d) and 28.8 (d) $^2J_{p-p}$(Hz) 42.0.Selected IR(cm^{-1}): 1585 [w, ν (C=N)], 1481[s, ν(C=N)], 1433 [s, ν(C=N)], 515 [s, ν(Ru–P)], 442 [w, ν(Ru–N)], 303 e 276 [w, ν(Ru–Cl)]. UV–Vis CH_2Cl_2 $\lambda_{max.}$/nm ($\varepsilon/M^{-1}\,cm^{-1}$): 306 (14252), 424 (1947).

2.3. Cell culture assay in 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-diphenyl tetrazolium bromide] to formazan [14]. 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 three trials.

2.4. Antimycobacterial activity assay

Antimycobacterial activities of each tested compound and of the standard drug isoniazide (Difco laboratories, Detroit, MI, USA) were determined in triplicate in 96 sterilized flat bottomed micro-plates (Falcon 3072; Becton Dickinson, Lincoln Park, NJ, USA) and Middlebrook 7H9 Broth (Difco) supplemented with oleic