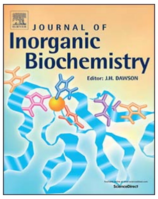
Journal of Inorganic Biochemistry 176 (2017) 66–76

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio



Selective Ru(II)/lawsone complexes inhibiting tumor cell growth by apoptosis

Katia M. Oliveira[a], Luna-Dulcey Liany[b], Rodrigo S. Corrêa[c], Victor M. Deflon[d], Marcia R. Cominetti[b], Alzir A. Batista[a,*]

[a] Departamento de Química, Universidade Federal de São Carlos, CP 676, CEP 13565-905, São Carlos, SP, Brazil
[b] Departamento de Gerontologia, Universidade Federal de São Carlos, CP 676, CEP 13565-905, São Carlos, SP, Brazil
[c] Departamento de Química, Universidade Federal de Ouro Preto, CEP 35400-000 Ouro Preto, MG, Brazil
[d] Instituto de Química de São Carlos, Universidade de São Paulo, CEP 13560-970 São Carlos, SP, Brazil

ARTICLE INFO

Keywords:
Ruthenium complexes
Lawsone
Cytotoxicity
Lung cancer

ABSTRACT

New Ru(II) complexes with lawsone (law) characterized as *trans*-[Ru(law)$(PPh_3)_2$(N-N)]PF_6, where PPh_3 means triphenylphosphine and N-N is 2,2′-bipyridine (**1**), 4,4′-dimethyl-2,2′-bipyridine (**2**), 4,4′-dimethoxy-2,2′-bipyridine (**3**), 1,10-phenanthroline (**4**) or 4,7-diphenyl-1,10-phenanthroline (**5**), induce apoptosis in tumor cells. Cytotoxicity of the complexes against the tumor cell lines DU-145 (prostate cancer cells), MCF-7 (breast cancer cells), A549 (lung cancer cells) and lung non-tumor cell line MRC-5 demonstrated promising IC_{50} values, lower than those found for the cisplatin, a drug used as a reference. Due to the high cytotoxic activity and selectivity against A549 cells line, complex (**5**) was selected for detailed assays. The complex (**5**) inhibits cells migration in concentrations in a nanomolar range, inducing tumor cell death by apoptosis, as confirmed by flow cytometry experiments. Furthermore, the antiproliferative activity of complex (**5**) on A549 tumor cells is attributed to a cell cycle arrest at the Sub G1 phase, followed by a decrease in the number of cells at the S phase. In addition, the interaction of the complexes (**1–5**) with CT-DNA was evaluated by circular dichroism, in which no changes in the secondary structure of DNA were observed, suggesting a weak interaction of the complexes with the biomolecule. On the other hand, complexes (**1–5**) showed a higher interaction with human serum albumin (HSA) by non-covalent van der Waals forces and hydrogen bonding, resulting in static quenching.

1. Introduction

After accidentally discovering cisplatin, the search for metallic compounds endowed with antitumor activities has become a topical area of research in inorganic and coordination chemistry [1,2]. Several four-coordinated platinum(II) analogs have been developed, such as carboplatin and oxaliplatin, however, many of these platinum compounds have showed resistance and high toxicity, increasing side effects, such as nephrotoxicity, neurotoxicity, nausea and vomiting [3–5]. Therefore, the development of new anticancer metallodrugs has focused on complexes with other metals, such as ruthenium, gold, osmium, gallium and others, seeking to obtain compounds with different modes of action [6,7]. The variation of the metal ion provides versatility in terms of designing new drugs, taking into account the variety of oxidation states of the metal center, the coordination number and geometries of the complexes, allowing for adjustments in their chemical reactivity [8,9].

In this context, ruthenium complexes are very promising bioactive agents [10]. So far, the ruthenium complexes, NAMI-A (*trans*-[$RuCl_4$(1*H*-imidazole)(DMSO-S)]), KP1019 (*trans*-[$RuCl_4$(1*H*-indazole)$_2$]), and NKP-1339 (sodium [*trans*-$RuCl_4$(1*H*-indazole)$_2$]) have been evaluated in clinical trials and are effective in treating metastatic tumors [11,12]. Recently, half-sandwich η^6-arene-Ru(II) complexes have been introduced into clinical trials. The complex RAPTA-C ([$RuCl_2$(η^6-p-cymene)(PTA)], PTA = 1,3,5-triaza-7-phosphaadaman tane) stands out due to its effective antimetastatic activity in vivo, and low cytotoxicity in vitro [13–15]. This makes these compounds even more attractive, given that the objective of cancer chemotherapy is to eliminate tumor metastasis which is mainly responsible for their ineffectiveness of chemotherapy [9,16].

In metal complexes, the ligands also perform an essential role in the rational design of new drugs because they can increase the biological activity of the compounds, by recognizing specific targets and even changing the chemical reactivity of the complex in the body [8]. Introducing ligands with a previous biological activity can lead to synergistic effects with the metal center or with the complex and is a good

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* Corresponding author.
E-mail addresses: kmoliveiraq@gmail.com (K.M. Oliveira), daab@ufscar.br (A.A. Batista).

http://dx.doi.org/10.1016/j.jinorgbio.2017.08.019
Received 3 May 2017; Received in revised form 8 August 2017; Accepted 24 August 2017

strategy for metallodrug development. Based on this aspect, a class of natural compounds known as naphthoquinone is associated with many biological activities, such as antibacterial, antifungal, antimalarial and anticancer [17–21]. An example of a naturally occurring compound is known as Lawsone (2-hydroxy-1,4-naphthoquinone), commonly extracted from *Lawsonia alba* [22]. The lawsone can coordinate metals in a bidentate way, O,O$^-$, forming 5-membered ring [23–25]. In fact, some metal complexes containing lawsone and derivatives as ligands have demonstrated higher biological activity than free lawsone [4,21,26]. Recently, Hartinger and co-workers have reported half-sandwich Ru(II) arene complexes containing lawsone and its derivatives as ligands, which exhibited cytotoxic activity against SW480, CH1/PA-1 and A549 tumor cell lines [4].

As part of our ongoing effort to design new metallodrug candidates with antitumor activity, in recent years our research group has reported some ruthenium complexes containing phosphines and diimines as ligands with promising results against this disease [27–30]. Therefore, in this paper we have synthesized and characterized five new ruthenium complexes containing phosphines, diimines and lawsone as ligands. The cytotoxicity assays, in vitro, of the ruthenium complexes against human tumor cell lines, including breast, prostate and lung and against one lung non-tumor human cell line, were carried out using the MTT method. In addition, we analyzed the effect of ruthenium complexes on cell morphology and migration, as well as the mechanism of cell death induced and changes in cell cycle arrest by flow cytometry. Furthermore, their ability to interact with CT-DNA and HSA were investigated.

2. Experimental

2.1. Material and methods

All chemicals used to prepare the complexes and buffer solution are of analytical grade or chemically pure grade. All the synthesis of the complexes was carried under argon atmosphere. The $RuCl_3{\cdot}3H_2O$, triphenylphosphine (PPh_3), N-N = 2,2′-bipyridine (bipy), 4,4′-dimethyl-2,2′-bipyridine (mebipy), 4,4′-dimethoxy-2,2′-bipyridine (meobipy), 1,10-phenanthroline (phen), 4,7-diphenyl-1,10-phenanthroline (phphen) and lawsone (law) were used as received from Aldrich. KPF_6, salts used for buffer preparation, CT-DNA (Calf Thymus), HSA (Human Serum Albumin) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Aldrich.

2.2. Physical measurements

The NMR experiments ($^{31}P\{^1H\}$,$^{13}C\{^1H\}$, ^1H) were recorded on a 9.4 T Bruker Avance III spectrometer in DMSO-d_6. The $^{31}P\{^1H\}$ shifts are reported in relation to H_3PO_4 85%. The experiments of ^1H-^1H gCOSY, ^1H-^{13}C gHSQC, and ^1H-^{13}C gHMBC were carried out for complete characterization of ruthenium complexes. Elemental analyses (C, H and N) were performed on a Fisons EA 1108 model (Thermo Scientific) equipment. UV–Visible (UV–vis) was recorded on a Hewlett Packard diode array – 8452A spectrophotometer. IR spectra between 4000 and 200 cm^{-1} were registered using as CsI pellets on a Bomem-Michelson FT-MB-102 instrument. Fluorescence spectra were performed by using a fluorimeter Synergy/H1-Biotek. The circular dichroism spectra were obtained from a spectropolarimeter Jasco J-720, using the quartz cuvette circular of 1 cm path length. The electrochemical experiments were carried using a BAS, model 100B at room temperature in CH_2Cl_2 containing 0.10 M tetrabutylammonium perchlorate (TBAP) (Fluka Purum) as a support electrolyte. The working and auxiliary electrodes were stationary Pt foils, and the reference electrode was Ag/AgCl, 0.10 M TBAP in CH_2Cl_2, in a Luggin capillary probe. Conductivity values were obtained using a MeterLab CDM2300 at room temperature.

2.3. Synthesis and characterization

2.3.1. Synthesis of cis,trans-$[RuCl_2(PPh_3)_2(N\text{-}N)]$, general procedure

The synthesis of the complex precursors was carried out following procedures described by Batista and coworkers [31]. For this, the N-N ligand (0.25 mmol) was added to the solution of $[RuCl_2(PPh_3)_3]$ (0.20 mmol, 0.2 g) in 20 mL of CH_2Cl_2. The reaction was kept in agitation under argon atmosphere for 1 h. After that, the solution volume was reduced to approximately 2 mL and ethyl ether was added to precipitate a brown solid. The solid was filtered, washed with ethyl ether and dried under vacuum.

2.3.2. Synthesis of trans-$[Ru(law)(PPh_3)_2(N\text{-}N)]PF_6$, general procedure

To obtain the new complexes (**1–5**), the lawsone (0.18 mmol; 0.031 g) was dissolved in 50 mL of a mixture of CH_2Cl_2:CH_3OH (1:1 v/v) in a Schlenk flask, containing 80 μL of triethylamine. Then, (0.12 mmol) of *cis,trans*-$[RuCl_2(PPh_3)_2(N\text{-}N)]$ and (0.24 mmol; 0.044 g) KPF_6 was added. The solution was kept under reflux, inert atmosphere and was stirred for 12 h. The solution with a dark color was concentrated to ca. 5 mL, and water was added to precipitate a powder. The solids were filtered off, washed with water and diethyl ether (3 × 15 mL each) and dried under vacuum.

trans-$[Ru(law)(PPh_3)_2(bipy)]PF_6$ (**1**): Yield: 49.66 mg (77%). Anal. Calc. for $[C_{56}H_{43}F_6N_2O_3P_3Ru]{\cdot}1/2\ CH_2Cl_2$: exp. (calc) C, 60.92 (61.15); H, 4.02 (3.94); N, 2.52 (2.55) %. Molar conductivity in CH_2Cl_2, 33.96 S $cm^2\ mol^{-1}$. IR (Selected bands, cm^{-1}): $\nu(C_1{=}O)$ 1602, $\nu(C_4{=}O)$ 1618, $\nu(C_2{-}O)$ 1091. $^{31}P\{^1H\}$ NMR δ(ppm): 30.68 (s). ^1H NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 5.53 (s, 1H, law), 9.86 (m, 1H, bipy), 9.37 (m, 1H, bipy), 8.14–6.97 (overlapped signals, 30H aromatic hydrogen for PPh_3, 6H for bipy and 4H for law). ^{13}C NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm) 198.55 ($C_1{=}O$ of law), 181.52 ($C_4{=}O$ of law), 170.46 ($C_2{-}O$ of law).

trans-$[Ru(law)(PPh_3)_2(mebipy)]PF_6$ (**2**): Yield: 48.02 mg (75%). Anal. Calc. for $[C_{58}H_{47}F_6N_2O_3P_3Ru]$ exp. (calc) C, 61.95 (61.76); H, 4.78 (4.20); N, 2.33 (2.48) %. Molar conductivity in CH_2Cl_2, 40.20 S $cm^2\ mol^{-1}$. IR (Selected bands, cm^{-1}): $\nu(C_1{=}O)$ 1600, $\nu(C_4{=}O)$ 1610, $\nu(C_2{-}O)$ 1093. $^{31}P\{^1H\}$ NMR δ(ppm): 30.06 (s). ^1H NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 2.30 (s, 3H, CH_3 of mebipy), 2.41 (s, 3H, CH_3 of mebipy), 5.59 (s, 1H, law), 9.65 (m, 1H, bipy), 9.23 (m, 1H, bipy), 8.16–6.98 (overlapped signals, 30H aromatic hydrogen for PPh_3, 4H for mebipy and 4H for law). ^{13}C NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 198.53 ($C_1{=}O$ of law), 181.46 ($C_4{=}O$ of law), 170.63 ($C_2{-}O$ of law).

trans-$[Ru(law)(PPh_3)_2(meobipy)]PF_6$ (**3**): Yield: 50.83 mg (80%). Anal. Calc. for $[C_{58}H_{47}F_6N_2O_5P_3Ru]$ exp. (calc) C, 60.31 (60.05); H, 4.18 (4.08); N, 2.12 (2.41) %. Molar conductivity in CH_2Cl_2, 31.60 S $cm^2\ mol^{-1}$. IR (Selected bands, cm^{-1}): $\nu(C_1{=}O)$ 1599, $\nu(C_4{=}O)$ 1614, $\nu(C_2{-}O)$ 1092. $^{31}P\{^1H\}$ NMR δ(ppm): 31.46 (s). ^1H NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 3.85 (s, 3H, CH_3 of meobipy), 3.91 (s, 3H, CH_3 of meobipy), 5.47 (s, 1H, law), 9.51 (m, 1H, meobipy), 9.05 (m, 1H, meobipy), 8.12–7.00 (overlapped signals, 30H aromatic hydrogen for PPh_3, 4H for meobipy and 4H for law). ^{13}C NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 198.59 ($C_1{=}O$ of law), 181. 30 ($C_4{=}O$ of law), 170.75 ($C_2{-}O$ of law).

trans-$[Ru(law)(PPh_3)_2(phen)]PF_6$ (**4**): Yield: 46.14 mg (73%). Anal. Calc. for $[C_{58}H_{43}F_6N_2O_3P_3Ru]{\cdot}1/2\ CH_2Cl_2$ exp. (calc) C, 62.03 (61.98); H, 3.95 (3.86); N, 2.33 (2.49) %. Molar conductivity in CH_2Cl_2, 39.30 S $cm^2\ mol^{-1}$. IR (Selected bands, cm^{-1}): $\nu(C_1{=}O)$ 1599, $\nu(C_4{=}O)$ 1616, $\nu(C_2{-}O)$ 1096. $^{31}P\{^1H\}$ NMR δ(ppm): 30.08 (s). ^1H NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 5.59 (s, 1H, law), 10.24 (m, 1H, phen), 9.70 (m, 1H, phen), 8.47–6.82 (overlapped signals, 30H aromatic hydrogen for PPh_3, 6H for phen and 4H for law). ^{13}C NMR (400 MHz, DMSO-d_6, 298 K): δ(ppm): 198.83 ($C_1{=}O$ of law), 181.54 ($C_4{=}O$ of law), 170.68 ($C_2{-}O$ of law).

trans-$[Ru(PPh_3)_2(law)(phphen)]PF_6$ (**5**): Yield: 37.80 mg (70%). Anal. Calc. For $[C_{70}H_{51}F_6N_2O_3P_3Ru]$ exp. (calc) C, 65.36 (65.88); H,