Ruthenium(II)-phosphine complexes cause mitochondrial dysfunction in lung cancer cells

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RESUMO

The use of metal-based compounds as anticancer agents has been rising with the development of cisplatin and other platinum-based drugs. However, the use of these compounds is limited due to their side effects and resistance. Aiming to overcome this issue, other metals have been intensively studied as potential anticancer agents, such as Ruthenium. In this work, we studied six Ruthenium(II)-diphosphine compounds containing different mercapto ligands (N-S), with general formula [Ru(N-S)(dppm)$_2$]Cl (dppm=1,1-bis(diphenylphosphino)methane). These compounds were characterized by different techniques such as NMR, HRMS, IR, UV-Vis and XRD, and their purity confirmed by elemental analysis. Positive log P values in n-octanol/PBS indicated their preference for the organic phase. Cytotoxicity experiments revealed promising IC_{50} values on A549 lung cancer cells, 0.48 μM and 0.80 μM for [Ru(mtz)(dppm)$_2$]Cl (**1**) and [Ru(mmi)(dppm)$_2$]Cl (**2**), respectively (mtz and mmi are 2-mercapto-2-thiazoline and mercapto-1-methylimidazole in their deprotonated forms). It should mention that both complexes were more cytotoxic than cisplatin control. Based on these promising results, **1** and **2** were studied biologically in depth. Migration and clonogenic assays were performed in A549 lung cancer cells. Also, both complexes are capable of affecting the mitochondrial functions, disrupting the mitochondrial potential and respiration in these cells. Taken together, our findings provide valuable insights into the cytotoxic potential of Ruthenium-phosphine-based complexes.

Keywords: medicinal inorganic chemistry, ruthenium, mitochondrial damage, cancer, cytotoxicity.

Introduction

The use of metal-based compounds as anticancer agents has been rising with the development of cisplatin and other platinum-based drugs (1). Aiming to overcome the side effects and resistance, other metals have been intensively studied as potential anticancer agents. Ruthenium, exhibiting chemical and structural properties different from those presented by the platinum complexes, arises as a promising alternative for the development of novel metal-based compound for medicinal applications (2). In this context, we report the synthesis and characterization of six Ru(II)-diphosphine complexes containing different mercapto ligands (Figure 1, 1–6).

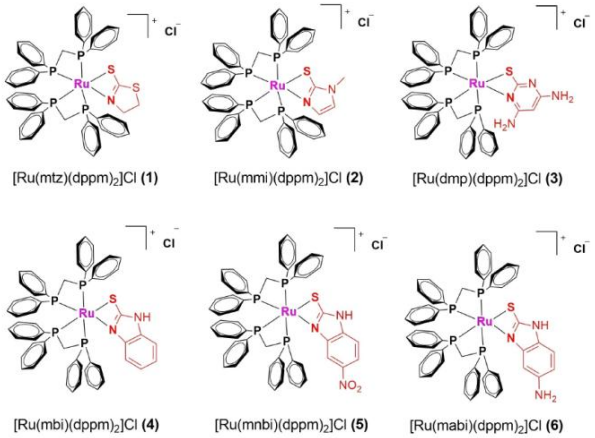

Figure 1. Chemical structures of Ruthenium complexes (1-6).

After confirming the stability of these complexes, their cytotoxicity was investigated in different cancer cells. Based on their promising results, complexes **1** and **2** were further studied. Migration and clonogenic assays were performed in A549 lung cancer cells. Also, both complexes were capable of affecting the mitochondrial functions, disrupting the mitochondrial potential and respiration. Overall, we demonstrate that ruthenium-phosphine-mercapto compounds are efficient cytotoxic anticancer agents

Experimental

Synthesis of complexes 1–6

The complexes were obtained from the *cis*-[$RuCl_2$(dppm)$_2$] precursor. To a solution of a mercapto ligand (0.12 mmol) and $NaHCO_3$ (0.21 mmol) in methanol previously degassed, *cis*-[$RuCl_2$(dppm)$_2$] (0.16 mmol) was dded. The system was kept under stirring and reflux for approximately 12 h. The volume of the solution was reduced and the powder was filtered off, washed with water and ethyl ether, and dried under reduced pressure.

Results and Discussion

Synthesis and characterization

The Ru(II) complexes **1–6** were obtained by refluxing the precursor [$RuCl_2(dppm)_2$] and the respective mercapto ligands in MeOH in presence of $NaHCO_3$ (Yield: 45–83%) (Figure 1). All complexes were characterized by NMR, IR, UV-Vis and conductivity. As example, the $^{31}P\{^1H\}$ NMR spectrum for **1** shows different signals at 3.86 (ddd, 1P), -1.07 (ddd, 1P) and -17.28 ppm (ddd, 2P), indicating the formation of the product (Figure 2). ^1H NMR spectrum revealed the aliphatic protons from dppm ligand at 2.0–6.0 ppm, while the aromatic protons from dppm/mercapto ligands can be found in the region between 6.5–8.0 ppm. Their purity was confirmed by elemental analysis. Complexes **1**, **2**, **4** and **5** were crystallized in DCM or DCM:MeOH, and their structures confirmed by single crystal XRD.

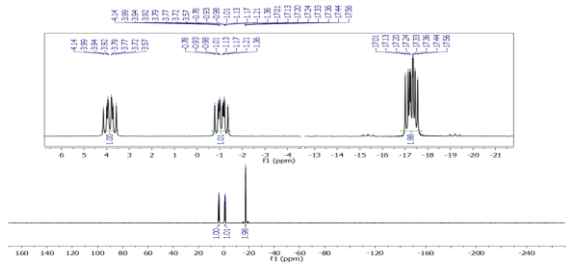

Figure 2. $^{31}P\{^1H\}$ NMR spectrum of 1 in DMSO-d6, 298 K.

Stability and Physicochemical Properties

First, the stability of the complexes was investigated in DMSO and DMSO/PBS by UV-Visible. No significant changes were observed in their spectra during 48 h, indicating their integrity. Additionally, solutions of these complexes in DMSO and DMSO/DMEM were also studied by $^{31}P\{^1H\}$ NMR. Again, no significant changes were observed after 48 h, indicating their stability. Positive log P values indicated that all complexes are mainly found in the organic phase, highlighting **5** as the most lipophilic compound (Figure 3).

Biological investigation

The cytotoxicity of complexes **1–6** was investigated in different cell lines via alamar blue (resazurin) fluorometric assay. In general, the compounds had a better performance on A549 lung cancer cells with IC_{50} ranging from 0.48 to 13.55 μM. The best results were obtained for 1 and 2 (IC_{50} = 0.48 and 0.80 μM, respectively), which were 27 and 16 times more active than cisplatin and 26 and 15 times more active than precursor *cis*-[$RuCl_2(dppm)_2$] (Figure 3).

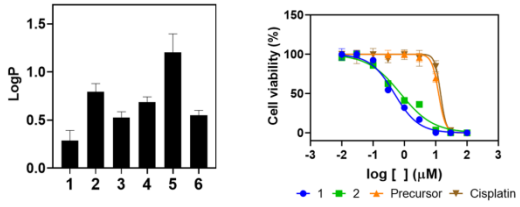

Figure 3. (A) Partition coefficient of 1–6 between PBS/octanol. (B) Cell viability (A549) after treatment with the compounds.

Due their promising results, complexes **1** and **2** were selected for further studies. As phosphine-based complexes cause mitochondrial dysfunction (3), we investigate the ability of **1** and **2** to damage mitochondria via JC-1 assay. As presented in Figure 4, both complexes were able to affect the MMP, as indicated in the green fluorescence of JC-1 monomeric form.

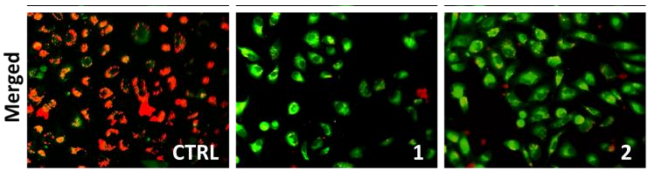

Figure 4. Fluorescence images of the JC-1 dye detected in A549 cancer cells treated for 24 h with 1 and 2.

To obtain better insights about this mechanism, we investigated the mitochondrial respiratory chain. The results revealed different oxygen consumption rates from cells in the absence and presence of **1** and **2** (Figure 5). In general, both complexes affected the OXPHOS as compared to controls which present a normal respiration profile. Also, the levels of ATP are drastically affected on A549 cells upon incubation with these complexes, suggesting as mitochondrial dysfunction.

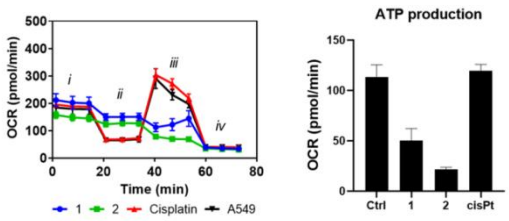

Figure 5. Mito stress test profile after 4 h of treatment with 1 and 2 showing the oxygen consumption rate after treatment with specific electron-transport chain inhibitors. ATP production levels after treatment with 1 and 2 for 4 h.

Conclusions

We report the synthesis and the biological investigation of six Ru(II)-diphosphine complexes as potential anticancer agents. Complexes **1** and **2** are cytotoxic on different cell lines, highlighting their effect against A549 cells. Both compounds affected the mitochondrial membrane potential and the oxygen consumption rate, confirming mitochondrial dysfunction. Our findings provide valuable insights into the cytotoxic potential of Ruthenium based compounds containing phosphine moieties.

Acknowledgements

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1. L. Kelland, *Nat. Rev. Cancer* **2007**, 7, 573–584.
2. E. Boros, P. J. Dyson, G. Gasser, *Chem.* **2020**, 6, 41–60.
3. R. J. Mitchell *et al.*, *Inorg. Chem*. **2023**, 62, 10940–10954.