http://dx.doi.org/10.5935/0103-5053.20150161

*J. Braz. Chem. Soc.*, Vol. 26, No. 9, 1838-1847, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

# Ruthenium(II) Complexes Containing Anti-Inflammatory Drugs as Ligands: Synthesis, Characterization and *in vitro* Cytotoxicity Activities on Cancer Cell Lines

***Junai C. S. Lopes,[a,b] Jaqueline L. Damasceno,[c] Pollyanna F. Oliveira,[c] Adriana P. M. Guedes,[d] Denise C. Tavares,[c] Victor M. Deflon,[e] Norberto P. Lopes,[f] Marcos Pivatto,[b] Alzir A. Batista,[d] Pedro I. S. Maia[g] and Gustavo Von Poelhsitz*[,b]***

[a]*Instituto Federal Norte de Minas Gerais, Campus Pirapora, CP 54, 39270-000 Pirapora-MG, Brazil*

[b]*Instituto de Química, Universidade Federal de Uberlândia, CP 593, 38400-902 Uberlândia-MG, Brazil*

[c]*Universidade de Franca, 14404-600 Franca-SP, Brazil*

[d]*Departamento de Química, Universidade Federal de São Carlos, CP 676, 13561-901 São Carlos-SP, Brazil*

[e]*Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, 13566-590 São Carlos-SP, Brazil*

[f]*Núcleo de Pesquisa em Produtos Naturais e Sintéticos (NPPNS), Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto-SP, Brazil*

[g]*Instituto de Ciências Naturais, Exatas e Educação, Universidade Federal do Triângulo Mineiro, 38064-200 Uberaba-MG, Brazil*

The synthesis, characterization and cytotoxic activity of *cis*-[Ru(dicl)(dppm)$_2$]PF$_6$ and *cis*-[Ru(ibu)(dppm)$_2$]PF$_6$, (dppm = 1,1-bis(diphenylphosphine)methane; dicl = diclofenac anion and ibu = ibuprofen anion), are described in this work. Complexes were characterized by elemental analysis, Fourier transform infrared spectroscopy (FTIR), UV-Vis, $^{31}$P{$^1$H} nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRESIMS). X-ray structure of *cis*-[Ru(ibu)(dppm)$_2$]PF$_6$ is also described. Preliminary calf thymus DNA (ct-DNA) binding studies were carried out by UV-Vis and viscosity experiments, with results suggesting the existence of electrostatic interactions between ruthenium complexes and ct-DNA. Cytotoxicity assays were carried out on a panel of human cancer cell lines and a human normal cell line. Complexes displayed a high to moderate cytotoxicity with $IC_{50}$ ranging from 5 to 47 μmol L$^{-1}$. *cis*-[Ru(ibu)(dppm)$_2$]PF$_6$ was found to be the most active, with $IC_{50}$ values lower than cisplatin. The degree of cytotoxicity was maintained for the normal cell line, although *cis*-[Ru(ibu)(dppm)$_2$]PF$_6$ exhibited a similar selectivity to that of cisplatin but with a higher activity for at least two tumor cell lines which evidences a promising anticancer candidate and selects this complex for further experiments.

**Keywords:** ruthenium(II) complexes, cytotoxic activity, sodium diclofenac, sodium ibuprofen, dppm

## Introduction

The disseminated use of cisplatin and other platinum based metallodrugs as chemotherapeutic agents against ovarian, bladder and testicular cancers, among others, is still a key aspect for the development of the medicinal inorganic chemistry.[1-6] In the search for coordination compounds which are active against tumors and less toxic than cisplatin, ruthenium compounds emerge as the most promising candidates.[5-7] Their interesting biological features include the mechanism of action, toxicity and biodistribution which differ from those of classical platinum compounds and might therefore be active against cisplatin resistant human cancers.[5,7-11] In the last years, three ruthenium complexes (Figure 1) have entered in clinical trials: [InH][*trans*-RuCl$_4$(In)$_2$] (KP1019) and Na[*trans*-RuCl$_4$(In)$_2$] (In = indazole) (NKP1339) that displayed high activity in primary tumor models and [ImH][*trans*-RuCl$_4$(DMSO)(Im)] (NAMI-A) which showed effect against solid tumor metastases.[9,12-15]

*e-mail: gustavo@iqufu.ufu.br

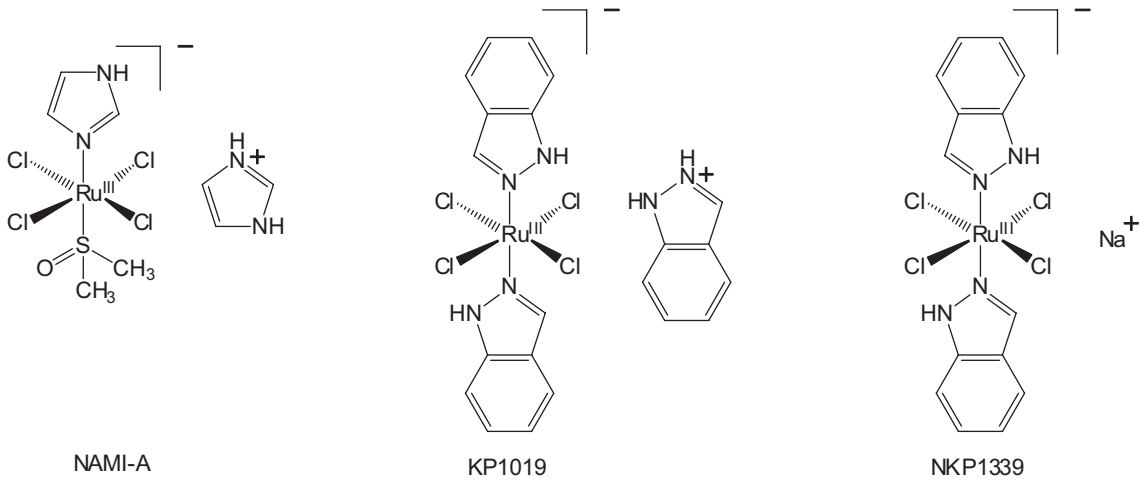



**Figure 1.** Anticancer ruthenium(III) based complexes: NAMI A, KP1019 and NKP1339.

A previous work from our group presented biological results from the diphosphinic ruthenium(II) precursor *cis*-[$RuCl_2(dppm)_2$] and its derivative with 2-pyridinecarboxylic acid anion ($pic^-$), the complex [$Ru(pic)(dppm)_2$]$PF_6$, where the $pic^-$ ligand coordinates on *N,O*-bidentate mode. The antimycobacterial activity against MTB $H_{37}Rv$ of this complex was evaluated and the minimum inhibitory concentration (MIC) value was in the low micromolar range.[16] Some additional studies performed with the analogous [$Ru(pic)(dppe)_2$]$PF_6$, dppe = 1,2-bis(diphenylphosphine)ethane, revealed a high antibacterial activity against *S. aureus*, *C. albicans* and *M. smegmatis.*[17] This last complex also presented a relatively low acute oral toxicity in mice.[17]

Due to this background of promising biological results for complexes containing the *cis*-[$Ru(P–P)_2$] unit, P–P = diphosphine, our current strategy consists in evaluating derivatives with different chelating moiety by replacing the chlorido ligands in order to select new cytotoxic agents against tumor cells. In the present work anti-inflammatory molecules were chosen as co-ligands to explore the possible synergic effect between the *cis*-[$Ru(dppm)_2$] unit and these biologically active ligands. Diclofenac and ibuprofen are non-steroidal anti-inflammatory drugs (NSAIDs) currently used in clinical medicine due to their antipyretic, antiarthritic, analgesic and anti-inflammatory properties, acting by inhibition of cyclooxygenases (COX-1 and COX-2).[18-20]

In this work the synthesis and characterization of two new ruthenium(II) derivatives with formula [$Ru(dicl)(dppm)_2$]$PF_6$ (**1**) and [$Ru(ibu)(dppm)_2$]$PF_6$ (**2**) are reported. Furthermore, preliminary binding properties to calf thymus DNA (ct-DNA) and *in vitro* tests of cytotoxic activities against a panel of human cell lines are presented and discussed.

## Experimental

### General

Solvents were purified by standard methods. All chemicals used were of reagent grade or comparable purity. The $RuCl_3 \cdot 3H_2O$ and the ligands 1,1-bis(diphenylphosphino) methane (dppm), sodium diclofenac and racemic sodium ibuprofen were used as received from Aldrich. The ct-DNA was purchased from Sigma Chemical Co. Ltd. The *cis*-[$RuCl_2(dppm)_2$] precursor complex was prepared according to the literature method.[21]

### Instrumentation

Infrared spectra (IR) were obtained on a PerkinElmer Spectrum Two spectrophotometer equipped with an attenuated total reflectance (ATR) sample holder and ZnSe crystal. The spectra were recorded in the range of 4000-600 $cm^{-1}$ with a 4 $cm^{-1}$ resolution. UV-Vis spectroscopy was performed on a Shimadzu UV2501 PC spectrophotometer using cuvettes with a 1 cm path length and methanol as solvent. $^{31}P\{^1H\}$ nuclear magnetic resonance (NMR) was performed on a Bruker DRX 400 MHz spectrometer with a BBO 5 mm probe at 298 K. The NMR spectra were recorded in $CH_2Cl_2$ using a capillary of $D_2O$ to get the lock and with $H_3PO_4$ (85%) as external reference. Conductance data, obtained at 298 K on $1 \times 10^{-3}$ mol $L^{-1}$ methanol solutions of the complexes, were measured with a Tecnopon MCA 150 conductometer. Elemental analyses were performed on a Perkin Elmer 2400 Series II CHNS/O microanalyser. High-resolution mass spectra (HRESIMS) with electrospray ionization were measured on an ultrOTOF (Bruker Daltonics) spectrometer, operating in the positive mode. Methanol was

used as solvent system and the samples were infused into the ESI source at a flow rate of 5 μL $min^{-1}$. The calculated values for the charged complex ions were made by using ChemBioDraw Ultra 14.0.

### Synthesis

The precursor *cis*-[$RuCl_2(dppm)_2$] (0.103 mmol; 100 mg) was solubilized in 50 mL of methanol, followed by the direct addition of sodium diclofenac (0.120 mmol; 37.2 mg) or sodium ibuprofen (0.120 mmol; 26.6 mg), respectively, for synthesis of the complexes **1** and **2**. The resulting solution was stirred at room temperature for a 6 h period. The final solution was concentrated to ca. 5 mL and an aqueous solution of $NH_4PF_6$ (0.150 mmol; 24.4 mg) was added for the precipitation of a yellow solid. The solid was filtered off and washed with water (3 × 5 mL) and diethyl ether (3 × 5 mL) and dried under reduced pressure.

#### [Ru(dicl)(dppm)$_2$]$PF_6$ (**1**)

Yield: 60.0 mg (85.5%); anal. calcd. for $C_{64}H_{54}Cl_2F_6NO_2P_5Ru$: exptl. (calcd.) C 58.68 (58.57), H 4.16 (4.26), N 1.07 (1.18); λ / nm (ε / L $mol^{-1}$ $cm^{-1}$) 229 ($4.60 \times 10^4$), 257 ($2.90 \times 10^4$), 288 ($7.60 \times 10^3$), 338 ($2.10 \times 10^3$); IR (ATR) ν / $cm^{-1}$ 3344, 3058, 3026, 2925, 2854, 1565, 1522, 1484, 1468, 1452, 1436, 1392, 1366, 1314, 1100, 1000, 949, 876, 837, 778, 731, 715, 694; $^{31}P$ {$^1H$} NMR (162.0 MHz, $CH_2Cl_2/D_2O$) $\delta$ 8.2 (t, 2P, *J* 39.0 Hz), –12.8 (t, 2P, *J* 39.0 Hz); –144.7 (sep, 1P, *J* 711 MHz, $PF_6^-$); HRESIMS *m/z* calcd. for $C_{64}H_{54}Cl_2NO_2P_4Ru$ $[M - PF_6]^+$: 1164.1520; found: 1164.1520.

#### [Ru(ibu)(dppm)$_2$]$PF_6$ (**2**)

Yield: 57.0 mg (65.4%); anal. calcd. for $C_{63}H_{61}F_6O_2P_5Ru$: exptl. (calcd.) C 61.84 (62.02), H 5.47 (5.04); λ / nm (ε / L $mol^{-1}$ $cm^{-1}$) 227 ($5.60 \times 10^4$), 257 ($3.50 \times 10^4$), 340 ($2.90 \times 10^3$); IR (ATR) ν / $cm^{-1}$ 3057, 2951, 2927, 2864, 1516, 1485, 1461, 1436, 1420, 1373, 1282, 1191, 1161, 1145, 1121, 1092, 1026, 1000, 905, 873, 834, 784, 763, 735, 727, 714, 694; $^{31}P\{^1H\}$ NMR (162.0 MHz, $CH_2Cl_2/D_2O$) $\delta$ 8.3 (t, 2P, *J* 38.0 Hz), –12.3 (t, 2P, *J* 38 Hz); –144.7 (sep, 1P, *J* 711 Hz, $PF_6^-$); HRESIMS *m/z* calcd. for $C_{63}H_{61}O_2P_4Ru$ $[M - PF_6]^+$: 1075.2660; found: 1075.2660.

### X-ray crystallography

Yellow crystals of the complex **2** were grown by slow evaporation of a dichloromethane solution at room temperature. The data collection was performed using Mo-Kα radiation (λ = 0.71073 Å) on a BRUKER APEX II Duo diffractometer. Standard procedures were applied for data reduction and absorption correction. The structure was solved with SHELXS97 using direct methods[22] and all non-hydrogen atoms were refined with anisotropic displacement parameters with SHELXL97.[23] The hydrogen atoms were calculated at idealized positions using the riding model option of SHELXL97.[23] Table 1 presents detailed information about the structural determination.

**Table 1.** Crystallographic data and structural refinement details for complex **2**

| | |
|---|---|
| Empirical formula | $C_{63}H_{61}F_6O_2P_5Ru$ |
| Molar mass / (g $mol^{-1}$) | 1220.04 |
| Temperature / K | 296 |
| Crystal system | orthorhombic |
| Space group | Pna2(1) |
| Unit cell dimension | |
| a / Å | 23.7204 (7) |
| b / Å | 14.4607(5) |
| c / Å | 17.3534(6) |
| V / $Å^3$ | 5952.5(3) |
| Z | 4 |
| Density (calcd.) / (g $cm^{-3}$) | 1.361 |
| Absorption coefficient / $mm^{-1}$ | 0.459 |
| F (000) | 2512 |
| Crystal size / $mm^3$ | 0.43 × 0.42 × 0.30 |
| θ range data collection / degree | 1.65 to 25.04 |
| Index range | –24 ≤ h ≤ 28; |
| | – 17 ≤ k ≤ 17; |
| | – 15 ≤ l ≤ 20 |
| Reflection collected | 21774 |
| Independent reflection | 9009 [R(int) = 0.0190] |
| Completeness (to θ = 25.04°) / % | 99.5 |
| Absorption correction | semi-empirical from equivalents |
| Data / restraint / parameter | 9009 / 16 / 766 |
| Goodness-of-fit on $F^2$ | 1.041 |
| Final R indices [I > 2sigma(I)] | $R_1$ = 0.0243, $wR_2$ = 0.0579 |
| R indices (all data) | $R_1$ = 0.0275, $wR_2$ = 0.0600 |
| Absolute structure parameter | – 0.011(16) |
| Largest diff. peak and hole | 0.308 and – 0.189 e.$Å^{-3}$ |

### DNA titration and viscosity experiments

A standard solution of ct-DNA was prepared in tris-HCl buffer (5 mol $L^{-1}$ tris-HCl, pH 7.2). The concentration of this ct-DNA solution was measured from its absorption intensity at 260 nm using the molar absorption coefficient value of 6600 $mol^{-1}$ L $cm^{-1}$. Solutions of ruthenium complexes **1** and **2** used in the experiments were prepared in Tris-HCl buffer containing 2% of dimethyl sulfoxide (DMSO). In the titration experiments, different concentrations of the ct-DNA were used while the ruthenium complex was at 20 μmol $L^{-1}$.

Viscosity experiments were carried out using an Ostwald viscometer maintained at a constant temperature of 25 °C in

a thermostatic bath. The viscosity of the ct-DNA solution was measured in the presence of increasing amounts of the complexes **1** and **2**. The flow times were measured with an automated timer. Each sample was measured three times, and an average flow time was calculated. The obtained data are presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio ([Ru]/[DNA]), where $\eta$ is the viscosity of ct-DNA in the presence of the complexes and $\eta_0$ is the viscosity of ct-DNA alone in buffer solution.[24-26]

### Human cell lines and culture conditions

For the experiments, four different human cell lines from the 4th through 12th passages were used: HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma), MO59J (glioblastoma) and GM07492A (normal lung fibroblasts). The different cell lines were maintained as monolayers in plastic culture flasks (25 $cm^2$) containing HAM-F10 plus Dulbecco's Modified Eagle Medium (DMEM), 1:1 (Sigma-Aldrich) or only DMEM, depending on the cell line, supplemented with 10% foetal bovine serum (Nutricell) and 2.38 mg $mL^{-1}$ Hepes (Sigma-Aldrich) at 37 °C in a humidified 5% $CO_2$ atmosphere. Antibiotics (0.01 mg $mL^{-1}$ streptomycin and 0.005 mg $mL^{-1}$ penicillin; Sigma-Aldrich) were added to the medium to prevent bacterial growth.

### Cell viability assay related to human cell lines

Cytotoxic activity on the cell lines was assessed using the Colorimetric Assay *in vitro* Toxicology-XTT Kit (Roche Diagnostics) according to the manufacturer's instructions. For the experiments, $1 \times 10^4$ cells were seeded into microplates with 100 µL of culture medium (1:1 HAM F10 + DMEM or DMEM alone) supplemented with 10% fetal bovine serum containing concentrations of the ruthenium complexes ranging from 1.5625 to 1600 µg $mL^{-1}$. Negative (no treatment), solvent (0.02% DMSO) and positive (25% DMSO) controls were included. Positive controls comprising cisplatin (Sigma-Aldrich, ≥ 98% purity) were included. After incubation at 36.5 °C for 24 h, the culture medium was removed and cells were washed with 100 µL of phosphate-buffered saline (PBS) to remove the treatments, after which they were exposed to 100 µL of HAM-F10 culture medium without phenol red. Then, 25 µL of XTT were added and the cells were incubated at 36.5 °C for 17 h. The absorbance of the samples was determined using a multi-plate reader (ELISA-Tecan-SW Magellan vs 5.03 STD 2P) at a wavelength of 450 nm and a reference length of 620 nm.

### Statistical analysis related to human cell line assays

Cytotoxicity was assessed using the $IC_{50}$ response parameter (50% cell growth inhibition) calculated with the GraphPad Prism program, plotting cell survival against the respective concentrations of the treatments. One-way ANOVA was used for the comparison of means ($p < 0.05$). The selectivity index was calculated by dividing the $IC_{50}$ value of the isolated compounds on GM07492-A cells by the $IC_{50}$ value determined for human cancer cells.

## Results and Discussion

### Synthesis

The reaction of sodium salts of diclofenac and ibuprofen with the ruthenium(II) diphosphine precursor complex *cis*-$[RuCl_2(dppm)_2]$ resulted in the products **1** and **2** by chlorido exchange under mild conditions as showed in Scheme 1.

The yellow ruthenium(II) complexes **1** and **2** were isolated as pure solids from methanol, in reasonable to good yields. The elemental analyses are described in experimental section and they agreed well with the proposed formulations. The molar conductance values measured in methanol at room temperature range from 98 to 104 S $cm^2$ $mol^{-1}$, revealing the 1:1 electrolytic nature of these complexes.[27] Complexes are air stable both in the solid state and in DMSO solutions as evaluated by $^{31}P\{^1H\}$ NMR and UV-Vis experiments for a period of 48 h.

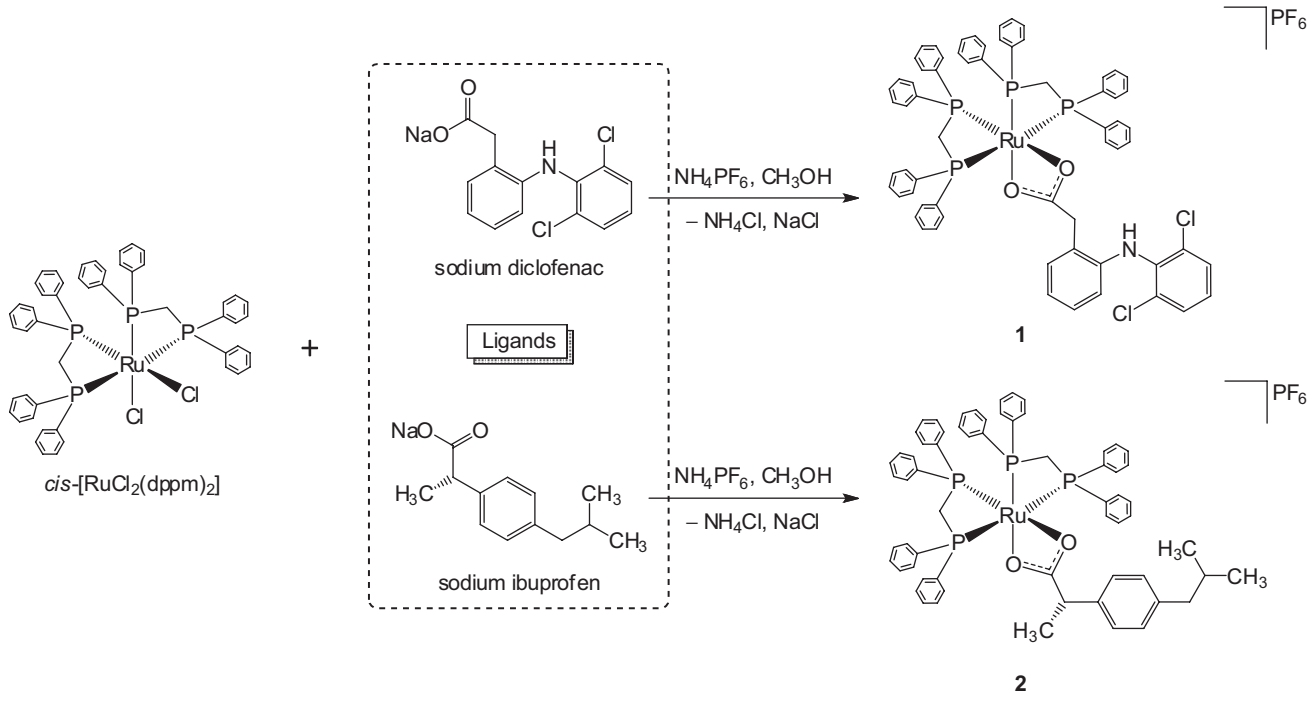
### Infrared spectroscopy

The infrared spectra (IR) of complexes **1** and **2** shows the typical asymmetric $\nu_{as}(COO^-)$ and symmetric $\nu_s(COO^-)$ carboxylate stretching frequencies at 1522; 1452 $cm^{-1}$ (**1**) and 1516; 1461 $cm^{-1}$ (**2**), respectively, as showed in Figure 2.

The $\Delta\nu$ values of 70 $cm^{-1}$ for complex **1** and 55 $cm^{-1}$ for **2** are indicative of a $\eta^2$ binding mode of the carboxylate group.[21] In addition, for complex **1**, characteristic vibrational modes of the diclofenac ligand at 3344 and 1100 $cm^{-1}$ were observed, corresponding to νNH and νPh–Cl, respectively. For both compounds the characteristic P–F stretch of the $PF_6^-$ counterion was seen at 837 $cm^{-1}$.[28] Most of the vibrational modes observed were characteristic of the dppm ligands occurring practically at same frequencies observed for the precursor *cis*-$[RuCl_2(dppm)_2]$.

### X-ray structure analyses

X-ray structure analyses of the complex **2** confirm the IR spectroscopy data. An ORTEP drawing of **2** showing the




**Scheme 1.** Route for the synthesis of complexes **1** and **2**.

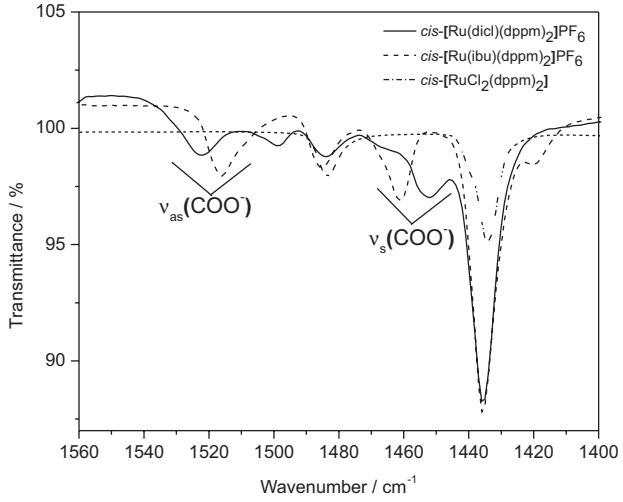



**Figure 2.** IR spectra of *cis*-[Ru(dicl)(dppm)$_2$]PF$_6$ (**1**), *cis*-[Ru(ibu)(dppm)$_2$]PF$_6$ (**2**) and *cis*-[RuCl$_2$(dppm)$_2$] in the range of 1560-1400 cm$^{-1}$.

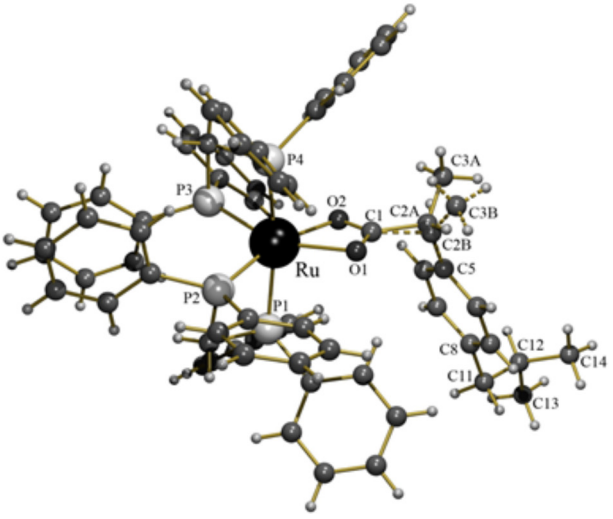



**Figure 3.** ORTEP view of the cation complex *cis*-[Ru(ibu)(dppm)$_2$]$^+$ (**2**) showing the two disordered forms of ibuprofen ligand. The PF$_6^-$ counterion was omitted for clarity.

atom numbering scheme is depicted in Figure 3. Selected bond lengths and angles are presented in Table 2.

Two disordered positions were refined for the fragment which includes the chiral center C(2) as well as the methyl and hydrogen groups attached to it. The two sites are shown together in Figure 3. The solid lines (labeled A) indicate the bonds between the atoms with higher occupation factor (68.4%), whereas the dashed lines (labeled B) represent the species with the lower occupation (31.6%). Plots showing the major and minor components of the disordered structure observed in **2** separately can be found as supplementary information (Figures S1 and S2). The reason for the disorder can clearly be derived from the orientation of the ligand due to the presence of the chiral center C(2), and consequently, two complex species with the ligand in *R* (major component) and *S* (minor component) configurations could be detected in the solid structure of the compound. Since the ligand used was a racemic mixture of ibuprofen, it is reasonable that both *R* and *S* isomers react with ruthenium(II) precursor. In fact, the $^{31}P\{^1H\}$ NMR of a powder of complex **2** also shows two set of signals with relative integration 60:40, revealing that these species are also preserved in solution

**Table 2.** Selected bond distances and angles for complex **2**

| Bond | Bond distance / Å | Bond angle | Angle / degree |
|---|---|---|---|
| Ru(1)–O(2) | 2.1676(18) | P(3)–Ru(1)–P(2) | 92.01(2) |
| Ru(1)–O(1) | 2.217(2) | O(2)–Ru(1)–P(4) | 92.17(5) |
| Ru(1)–P(3) | 2.2811(7) | P(2)–Ru(1)–P(1) | 70.93(2) |
| Ru(1)–P(2) | 2.2958(7) | P(3)–Ru(1)–P(4) | 72.36(2) |
| Ru(1)–P(4) | 2.3204(5) | O(1)–Ru(1)–P(1) | 95.52(5) |
| Ru(1)–P(1) | 2.4092(6) | P(4)–Ru(1)–P(1) | 170.69(2) |
| O(1)–C(1) | 1.268(4) | O(1)–Ru(1)–P(3) | 154.73(6) |
| O(2)–C(1) | 1.251(4) | O(2)–Ru(1)–P(2) | 163.77(6) |
| | | O(2)–Ru(1)–O(1) | 59.34(9) |

(see solution studies). Furthermore, the observation of the two types of isomers in the same crystal is not very common. This can be explained as a case of static disorder where **2** presents the *R* and *S* configurations distributed among different unit cells.[29]

This compound crystallizes in the orthorhombic system, space group Pna2(1), with the Ru center adopting a distorted octahedral coordination geometry formed by two *cis*-chelating diphosphine ligands and the bidentate ($\eta^2$) carboxylate group of the ibuprofen ligand. The distortions are caused by chelation angles of 70.93(2) and 72.36(2)° imposed by the methylene bridge of dppm ligands and especially by the carboxylate group with an O(1)–Ru–O(2) angle of only 59.34(9)°. This small angle found for the carboxylate group is very similar to that observed for ruthenium(II) complexes containing coordinated acetate and other carboxylates in the bidentate fashion.[30-32] The Ru–P bond lengths vary from 2.3204(5) to 2.4092(6) Å for mutually *trans* disposed phosphorus atoms and from 2.2811(7) to 2.2958(7) Å for phosphorus atoms *trans* positioned to oxygen atoms from de carboxylate group. These marked differences clearly illustrate the greater *trans*-influence of phosphorus when compared with oxygen.[31-33] The carboxylate ligand is coordinated with a certain degree of asymmetry as illustrated by the Ru–O distances of 2.1676(18) and 2.217(2) Å. These values are in the range reported for similar compounds.[30-35] This asymmetry probably is due some weak interactions of the phenyl group of ibuprofen with adjacent phenyl rings of dppm. This kind of asymmetry was previously observed for ruthenium(II) ferrocenylcarboxylates.[34]

### $^{31}P\{^{1}H\}$ Nuclear magnetic resonance (NMR) spectroscopy

The $^{31}P\{^{1}H\}$ NMR spectra of complexes **1** and **2** show typical patterns of species containing two *cis* positioned diphosphines and equal ligands completing the octahedral coordination sphere. For complex **1** a pair of triplets that integrate in 1:1 ratio with chemical shifts at 8.2 and –12.8 ppm was observed. The splitting pattern was consistent with an $A_2X_2$ ($\Delta\nu / J = 87$) assignment similar to those described for analogous complexes.[34] For complex **2** a slightly different behavior was observed. In the more deshielded region two triplets with very close chemical shifts (8.4 and 8.2 ppm) appeared, besides one triplet in the shielded region (–12.3 ppm), as showed in Figure 4.

The integration of the triplets at 8.4 and 8.2 ppm are in the 1:1 ratio with the triplet at –12.3 ppm. This behavior clearly indicates the presence of a mixture of two very similar species and based on the integration of each line of the signals close to 8 ppm it is found a 60:40 ratio between the species. This ratio is in agreement with the two configurations of ibuprofen ligand observed in crystal structure of the complex **2** as previously discussed. The splitting pattern is also consistent with an $A_2X_2$ assignment with $\Delta\nu / J = 88$ and 87 for each one of the configurations. In addition, since the $PF_6^-$ counterion was utilized, it was observed the characteristic septet due to the phosphorus-fluorine coupling with chemical shift centered at –144.6 ppm for both complexes.

### High-resolution mass spectrometry (HRESI)

Mass spectra of complexes containing ruthenium are typical for their isotopic pattern demonstrated by the presence of $^{96}$Ru (5.5%), $^{98}$Ru (1.9%), $^{99}$Ru (12.7%), $^{100}$Ru (12.6%), $^{101}$Ru (17.1%), $^{102}$Ru (31.6%) and $^{104}$Ru (18.6%) isotopes, with the nuclide abundance in parentheses. Furthermore, complex **1** had a ligand with chlorine ($^{35}$Cl (75.8%) and $^{37}$Cl (24.2%)) which contributes with an additional isotopic pattern (Figure 5). The high-resolution mass spectra of the compounds **1** and **2** were recorded and the obtained data confirm the established pattern (Figures 5a and 5b). In this study, the *m/z* values listed below in the text refer to the peak of the most abundant element corresponding to the $^{102}$Ru isotope. The HRMS spectra were acquired in the positive mode and the charged complex ions were observed at *m/z* 1164.1520 $[M]^+$ (**1**) and 1075.2660 $[M]^+$ (**2**), in agreement with calculated values for $C_{64}H_{54}Cl_2NO_2P_4Ru$, 1164.1520 and $C_{63}H_{61}O_2P_4Ru$, 1075.2660, respectively. Collision-induced dissociation (CID) experiments (MS/MS) with an increasing collisional energy using $N_2$ as collision gas under the selected ions at *m/z* 1164.1520 $[M]^+$ (**1**) and 1075.2660 $[M]^+$ (**2**), showed a fragmentation pathway just for complex **1** even in higher collisional energies. The loss of 295 u was proposed for a neutral elimination of the ligand (diclofenac, acid form) (Figure 5c).

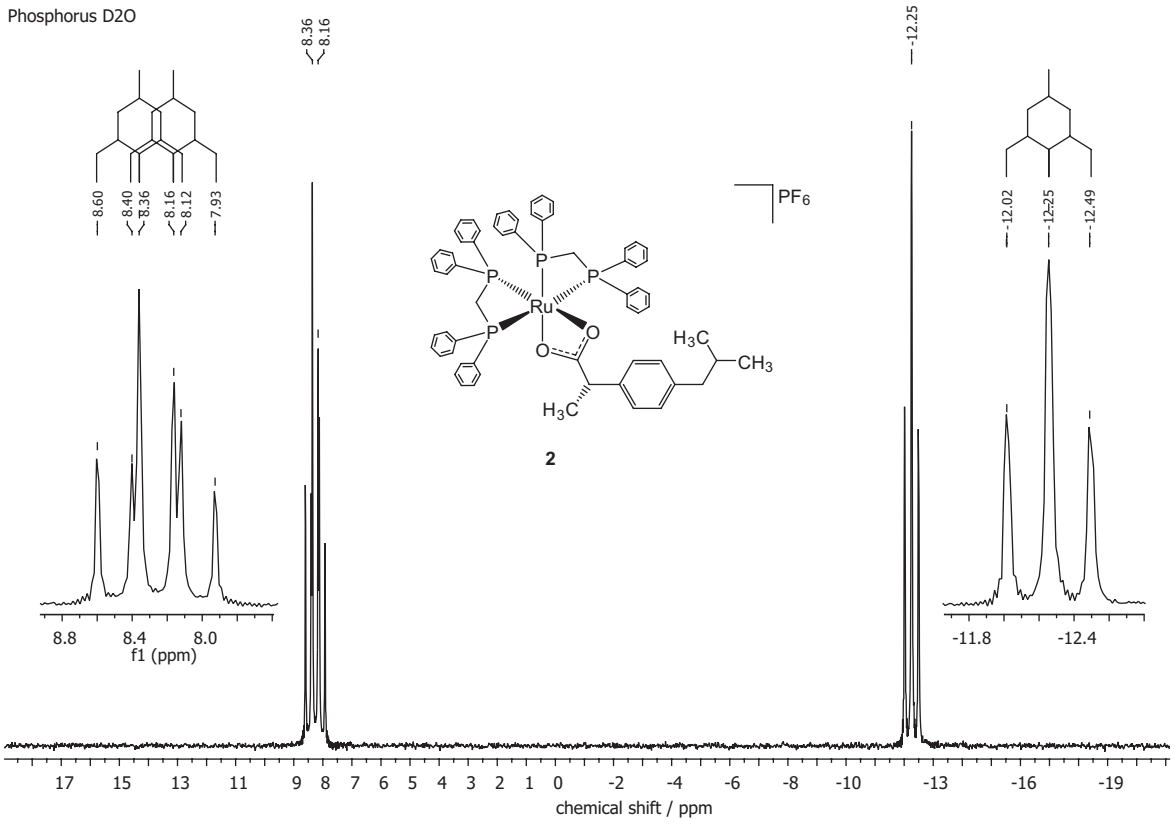



**Figure 4.** $^{31}P$ [$^{1}H$] NMR spectrum (162.0 MHz, $CH_2Cl_2/D_2O$) of complex 2.

## Ct-DNA binding studies: UV-Vis spectrophotometrical and viscosity studies

In an attempt to study the nature of the ruthenium complexes interactions with ct-DNA, UV-Vis absorption spectra were obtained by titration of the complexes with increasing concentrations of ct-DNA. The electronic spectra of complexes **1** and **2** showed an intense absorption peak around 264 nm, which could be attributed to an intraligand $\pi$-$\pi^*$ transition of the coordinated groups in the complex, that has been selected to study the spectral changes with ct-DNA addition. Both complexes displayed the same behavior in which absorption decreases with ct-DNA titration, however, this characteristic is attributed only to dilution effects. This was demonstrated by titration of the complexes with buffer solution (not containing ct-DNA) in which the same absorption decrease was observed. All these spectra are showed in supplementary information (Figure S4). These data showed that these complexes do not exhibit covalent or intercalative interactions with ct-DNA.[36,37] Due to the very weak interaction (hypochromism < 3%) was not possible determine the intrinsic binding constant ($K_b$) between the ruthenium complexes and ct-DNA.

The possible mode of interaction between complexes and ct-DNA was also evaluated by viscosity experiments. It is well known that classical intercalators, such as ethidium bromide, lead to an increase in the viscosity of ct-DNA because separation of the base pairs occurs to accommodate the intercalator. A covalent DNA-binding mode may cause its fragmentation, thus decreasing the ct-DNA viscosity.[38-40] However, complexes **1** and **2,** exhibited essentially no effect on the viscosity of ct-DNA as demonstrated in a plot $(\eta/\eta_o)^{1/3}$ *versus* [complex]/[DNA] showed in supplementary information (Figure S5). This result is consistent with existence of electrostatic interactions between ruthenium complexes and ct-DNA.[25,26] Considering the molecular structure and positive charge of the complexes, electrostatic interactions involving the negatively charged phosphate groups of ct-DNA are expected.

## *In vitro* cytotoxic activity

The human cell lines were exposed to the ruthenium(II) complexes and cisplatin for a period of 24 h, in order to allow them reach DNA or any other biological target. The $IC_{50}$ values, calculated from the dose-survival curves generated by the XTT assays obtained after drug treatment are shown in Table 3.

Complexes **1** and **2** have showed, in general, high cytotoxicity against all the human tumor cell lines assayed

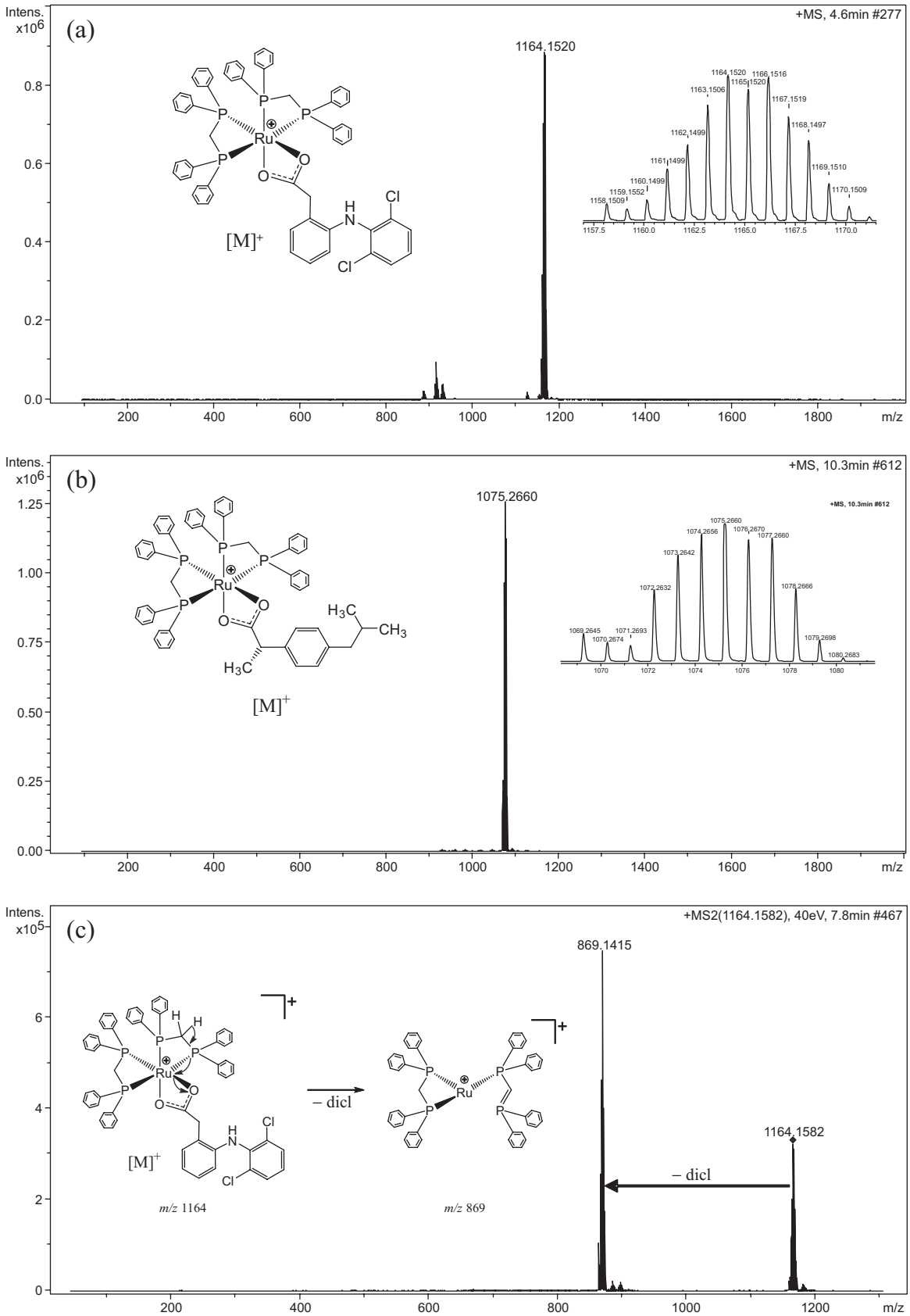



**Figure 5.** ESI mass spectra of *cis*-[Ru(dicl)(dppm)$_2$]$^+$ (**1**) and *cis*-[Ru(ibu)(dppm)$_2$]$^+$ (**2**). (a) HRESI-MS spectrum of *cis*-[Ru(dicl)(dppm)$_2$]$^+$ *m/z* 1164.1520 [M]$^+$ (calcd. for $C_{64}H_{54}Cl_2NO_2P_4Ru$, 1164.1520); (b) HRESI-MS spectrum of *cis*-[Ru(ibu)(dppm)$_2$]$^+$ *m/z* 1075.2660 [M]$^+$ (calcd. for $C_{63}H_{61}O_2P_4Ru$, 1075.2660) and (c) ESI-MS/MS spectrum of *m/z* 1164.1520.

**Table 3.** Inhibitory activity of ruthenium(II) complexes and cisplatin against normal and tumor cell lines, expressed as $IC_{50}$

| Compound | Cell line | | | |
|---|---|---|---|---|
| | HepG2[a] | MCF-7[b] | MO59J[c] | GM07492A[d] |
| | $IC_{50}$ / (μmol $L^{-1}$) | | | |
| *cis*-[$RuCl_2(dppm)_2$] | 108 ± 28 | 191 ± 13 | 134 ± 10 | 66 ± 4 |
| **1** | 7.6 ± 0.9 | 47 ± 6 | 8 ± 2 | 2 ± 1 |
| **2** | 5 ± 3 | 9 ± 3 | 6.5 ± 0.2 | 6 ± 2 |
| Cisplatin | 6.3 ± 0.7 | 34 ± 4 | 22 ± 4 | 26 ± 3 |

[a]HepG2: hepatocellular carcinoma; [b]MCF-7: breast adenocarcinoma; [c]MO59J: glioblastoma; [d]GM07492A: normal lung fibroblasts.

with $IC_{50}$ values ranging from 5 to 9 μmol $L^{-1}$, except for complex **1** in MCF-7 cells that showed a moderate cytotoxicity as presented in Table 3. Complex **2** displayed higher activity than **1** in all the tumor cell lines assayed with similar $IC_{50}$ values independent of the cell line. This non-selective activity of complex **2** was not observed for complex **1** that was much less cytotoxic in MCF-7 cells. Compared with the reference metallodrug cisplatin, complex **1** displayed approximately the same cytotoxic activity for HepG2 and MCF-7 cells and a three times increased activity for MO59J cells. Complex **2** had similar activity to cisplatin for HepG2 cells and a four times increased activity against MCF-7 and MO59J cells. The selectivity index (SI) (SI = $IC_{50}$ GM07492A / $IC_{50}$ human tumor cell line) was smaller than 1 for all the cell lines assayed for complex **1,** while for complex **2** the SI values are very close to 1, both indicating a low selectivity.

Under the same experimental conditions cisplatin also displayed SI values close to 1 for MCF-7 and MO59J tumor cell lines. The precursor complex *cis*-[$RuCl_2(dppm)_2$] was less active than the complexes **1** and **2** by factors ranging from 4.1 to 21.6. A similar increase in toxicity was observed against the normal cell line GM07492A. These data clearly indicates that the exchange of two chlorido ligands by a bidentate anti-inflammatory molecule makes the *cis*-$[Ru(dppm)_2]^{2+}$ unity complex more cytotoxic, probably due to the different lipophilicity and consequent entrance in the cells. Further experiments concerning the amount of ruthenium complex that gets inside the cells as well as its intracellular targets, beyond DNA, are required to understand details of the observed activity and will be a point of study in a near future.

## Conclusions

In this investigation two new ruthenium(II) complexes containing dppm and the anions of anti-inflammatory drugs diclofenac and ibuprofen with formula [Ru(dicl)$(dppm)_2$]$PF_6$ (**1**) and [Ru(ibu)$(dppm)_2$]$PF_6$ (**2**) were synthesized and characterized by elemental analysis, X-ray crystallography, spectroscopic and spectrometric methods. The spectroscopic analyses are in agreement with a chelated coordination through the carboxylate group, for the diclofenac and ibuprofen ligands. The crystallographic studies for the ibuprofen derivative revealed two configurations for the ligand in the crystalline structure. Viscosity experiments suggest an electrostatic interaction between ct-DNA and complexes **1** and **2**. The *in vitro* cytotoxicity activity assays of the complexes indicate a high activity against three human tumor cell lines. Indeed one of the complexes was more active than cisplatin against two tumor cells. Interestingly, exchanging chlorido ligands of the *cis*-[$RuCl_2(dppm)_2$] by diclofenac and ibuprofen resulted in higher cytotoxic activity probably due to the differences in lipophilicity upon complexation influencing the amount of compound that gets inside the cells. Further studies are necessary to verify the biological targets of this class of ruthenium(II) complexes. Although these complexes displayed low selectivity they present potential for the treatment of breast adenocarcinoma and gliobastoma since they present similar SI value to that of cisplatin but a higher activity, so they could be used in lower concentrations.

## Supplementary Information

Supplementary information is available free of charge at http://jbcs.org.br as PDF file.

Coordinates and other crystallographic data have been deposited with the CCDC, deposition code CCDC 1040297. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

## Acknowledgements

We thank CNPq, CAPES, FAPESP (Grant 2009/54011-8), the Minas Chemical Network and FAPEMIG (Grant APQ-04010-10). The authors are also thankful to the Grupo de Materiais Inorgânicos do Triângulo-GMIT research group supported by FAPEMIG (APQ-00330-14).

## References

1. Jamieson, E. R.; Lippard, S. J.; *Chem. Rev.* **1999**, *99*, 2467.
2. Wang, D.; Lippard, S. J.; *Nat. Rev. Drug Discov.* **2005**, *4*, 307.

3. Dasari, S.; Tchounwou, P. B.; *Eur. J. Pharmacol.* **2014**, *740*, 364.
4. Wheate, N. J.; Walker, S.; Craig, G. E.; Oun, R.; *Dalton Trans.* **2010**, *39*, 8113.
5. Mjos, K. D.; Orvig, C.; *Chem. Rev.* **2014**, *114*, 4540.
6. Barry, N. P. E.; Sadler, P. J.; *Chem. Commun.* **2013**, *49*, 5106.
7. Hartinger, C. G.; Zorbas-Seifried, S.; Jakupec, M. A.; Kynast, B.; Zorbas, H.; Keppler, B. K.; *J. Inorg. Biochem.* **2006**, *100*, 891.
8. Ang, W. H.; Casini, A.; Sava, G.; Dyson, P. J.; *J. Organomet. Chem.* **2011**, *696*, 989.
9. Bergamo, A.; Gaiddon, C.; Schellens, J. H. M.; Beijnen, J. H.; Sava, G.; *J. Inorg. Biochem.* **2012**, *106*, 90.
10. Kostova, I.; *Curr. Med. Chem.* **2006**, *13*, 1085.
11. Bratsos, L.; Jedner, S.; Gianferrara, T.; Alessio, E.; *Chimia* **2007**, *61*, 692.
12. Pillozzi, S.; Gasparoli, L.; Stefanini, M.; Ristori, M.; D'Amico, M.; Alessio, E.; Scaletti, F.; Becchetti, A.; Arcangeli, A.; Messori, L.; *Dalton Trans.* **2014**, *43*, 12150.
13. Trondl, R.; Heffeter, P.; Kowol, C. R.; Jakupec, M. A.; Berger, W.; Keppler, B. K.; *Chem. Sci.* **2014**, *5*, 2925.
14. Hartinger, C. G.; Jakupec, M. A.; Zorbas-Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K.; *Chem. Biodivers.* **2008**, *5*, 2140.
15. Oszajca, M.; Kulis, E.; Stochel, G.; Brindell, M.; *New J. Chem.* **2014**, *38*, 3386.
16. Pavan, F. R.; Von Poelhsitz, G.; Nascimento, F. B.; Leite, S. R. A.; Batista, A. A.; Deflon, V. M.; Sato, D. N.; Franzblau, S. G.; Leite, C. Q. F.; *Eur. J. Med. Chem.* **2010**, *45*, 598.
17. Pavan, F. R.; Von Poelhsitz, G.; Cunha, L. V. P.; Barbosa, M. I. F.; Leite, S. R. A.; Batista, A. A.; Cho, S. H.; Franzblau, S. G.; Camargo, M. S.; Resende, F. A.; Varanda, E. A.; Leite, C. Q. F.; *PloS One* **2013**, *8*, e64242.
18. Wang, J.; Hughes, T. P.; Kok, C. H.; Saunders, V. A.; Frede, A.; Groot-Obbink, K.; Osborn, M.; Somogyi, A. A.; D'Andrea, R. J.; White, D. L.; *Br. J. Cancer* **2012**, *106*, 1772.
19. Moser, P.; Sallmann, A.; Wiesenberg, I.; *J. Med. Chem.* **1990**, *33*, 2358.
20. Dahl, J. B.; Kehlet, H.; *Br. J. Anaesth.* **1991**, *66*, 703.
21. Sullivan, B. P.; Meyer, T. J.; *Inorg. Chem.* **1982**, *21*, 1037.
22. Sheldrick, G. M.; *SHELXS-97*; *Program for Crystal Structure Resolution*; University of Göttingen, Germany, 1997.
23. Sheldrick, G. M.; *SHELXL-97; Program for Crystal Structures Analysis*; University of Göttingen, Germany, 1997.
24. Cohen, G.; Eisenberg, H.; *Biopolymers* **1969**, *8*, 45.
25. Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B.; *Biochemistry* **1993**, *32*, 2573.
26. Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B.; *Biochemistry* **1992**, *31*, 9319.
27. Geary, W. J.; *Coord. Chem. Rev.* **1971**, *7*, 81.
28. Nakamoto, K.; *Infrared and Raman Spectra of Inorganic and Coordination Compounds,* 5th ed.; Wiley-Interscience: New York, 1997.
29. Muller, P.; Herbst-Irner, R.; Spek, A. L.; Schneider, T. R.; Sawaya, M. R.; *Crystal Structure Refinement: a Crystallographer´s Guide to SHELXL*, Oxford University Press: New York, 2006.
30. Jia, G. C.; Rheingold, A. L.; Haggerty, B. S.; Meek, D. W.; *Inorg. Chem.* **1992**, *31*, 900.
31. Murray, A. H.; Yue, Z.; Wallbank, A. I.; Cameron, T. S.; Vadavi, R.; MacLean, B. J.; Aquino, M. A. S.; *Polyhedron* **2008**, *27*, 1270.
32. Lucas, N. T.; Powell, C. E.; Humphrey, M. G.; *Acta Crystallogr. C* **2000**, *56*, 392.
33. Coe, B. J.; Glenwright, S. J.; *Coord. Chem. Rev.* **2000**, *203*, 5.
34. Wyman, I. W.; Burchell, T. J.; Robertson, K. N.; Cameron, T. S.; Aquino, M. A. S. *Organometallics* **2004**, *23*, 5353.
35. Sanchez-Delgado, R. A.; Thewalt, U.; Valencia, N.; Andriollo, A.; Marquezsilva, R. L.; Puga, J.; Schollhorn, H.; Klein, H. P.; *Inorg. Chem.* **1986**, *25,* 1097.
36. Zhang, Q. L.; Liu, J. G.; Chao, H.; Xue, G. Q.; Ji, L. N.; *J. Inorg. Biochem.* **2001**, *83*, 49.
37. Kalaivani, P.; Prabhakaran, R.; Dallemer, F.; Vaishnavi, E.; Poornima, P.; Vijaya Padma, V.; Renganathan, R.; Natarajan, K.; *J. Organomet. Chem.* **2014**, *762*, 67.
38. Sellamuthu, A.; Ravishankaran, R.; Karande, A. A.; Kandaswamy, M.; *Dalton Trans.* **2012**, *41*, 12970.
39. Zivec, P.; Perdih, F.; Turel, I.; Giester, G.; Psomas, G.; *J. Inorg. Biochem.* **2012**, *117*, 35.
40. Navarro, M.; Castro, W.; Higuera-Padilla, A. R.; Sierraalta, A.; Abad, M. J.; Taylor, P.; Sanchez-Delgado, R. A.; *J. Inorg. Biochem.* **2011**, *105*, 1684.


*Submitted: April 23, 2015*
*Published online: June 30, 2015*

**FAPESP has sponsored the publication of this article.**