**New Luminescent Polynuclear Metal Complexes with Anticancer Properties: Toward Structure–Activity Relationships**

Margot Wenzel, Andrea de Almeida, Emilia Bigaeva, Paul Kavanagh, Michel Picquet, Pierre Le Gendre, Ewen Bodio, and Angela Casini

**ABSTRACT:** A series of new heterodinuclear luminescent complexes with two different organic ligands have been synthesized and characterized. A luminescent Ru(II)(polypyridine) moiety and a metal-based anticancer fragment (AuCl, (p-cymene)RuCl2, (p-cymene)OsCl3, (Cp*)RhCl2, or Au-thioglucose) are the two general features of these complexes. All of the bimetallic compounds have been evaluated for their antiproliferative properties in vitro in human cancer cell lines. Only the complexes containing an Au(I) fragment exhibit antiproliferative activity in the range of cisplatin or higher. The photophysical and electrochemical properties of the bimetallic species have been investigated, and fluorescence microscopy experiments have been performed successfully. The most promising bimetallic cytotoxic complexes (i.e., with the Au-thioglucose scaffold) have shown to be easily taken up by cancer cells at 37 °C in the cytoplasm or in specific organelles. Interestingly, experiments repeated at 4 °C showed no uptake of the bimetallic species inside cells, which confirms involvement of active transport processes. To evaluate the role of glucose transporters in the cell uptake of the gold complexes, inhibition of the GluT-1 (glucose transporter isoform with high level of expression in cancer cells) was achieved, showing only scarce influence on the compounds’ uptake. Finally, the observed absence of interactions with nucleic acid model structures suggests that the gold compounds may have different intracellular targets with respect to cisplatin.

**INTRODUCTION**

Cancer is the second most frequent cause of death in the industrialized world after cardiovascular diseases. In this area, several research efforts have demonstrated that the unique properties of metal ions can be exploited in the design of new anticancer drugs that have different mechanisms of pharmacological activity, with respect to classical organic drugs, and can be used for cancer cell-targeted approaches. Within this field, polynuclear compounds are a relatively new and successful approach in metal-based cancer chemotherapy, as shown for example by the trinuclear Pt(II) compound BBR3464 evaluated in clinical trials (Figure 1). This concept of multinuclearity has been performed successfully. The most promising bimetallic cytotoxic complexes (i.e., with the Au-thioglucose scaffold) have shown to be easily taken up by cancer cells at 37 °C in the cytoplasm or in specific organelles. Interestingly, experiments repeated at 4 °C showed no uptake of the bimetallic species inside cells, which confirms involvement of active transport processes. To evaluate the role of glucose transporters in the cell uptake of the gold complexes, inhibition of the GluT-1 (glucose transporter isoform with high level of expression in cancer cells) was achieved, showing only scarce influence on the compounds’ uptake. Finally, the observed absence of interactions with nucleic acid model structures suggests that the gold compounds may have different intracellular targets with respect to cisplatin.

A greater challenge in this concept of multinuclearity consists in the combination of two (or more) different metal containing moieties, requiring a design of suitable ligands to coordinate selectively one metal and the other. The idea is that the combination of two different metal-based compounds into a unique structure might improve their anticancer properties thanks to the multiplication of the potential biological targets and to the new physicochemical properties of the generated bimetallic species. In recent years, a few successful examples have been described in the literature by us and others (Figure 1). In some cases, a significant improvement of the cytotoxic activity of the heterobimetallic entity has been observed in comparison with the parent monometallic complexes or with an equimolar mixture of both of them.

In this context, the synthesis of a series of heterobimetallic complexes based on Pt(II) and Au(I) units was described by us. Cell viability studies have shown potent antiproliferative activity toward cancer cells for both the mono- and dinuclear complexes. Interestingly, the biological properties were significantly improved by replacement of the chlorido ligand of Au(I) by 1-thio-β-D-glucose tetraacetate, as previously observed for other Au(I) cytotoxic complexes. Exploration of the cellular uptake, subcellular distribution, as well as the fate of these metallo-drugs (mono or poly nuclear)
inside cells is of major importance to get insights into their mechanism of action. Thus, there is an increasing need for imaging methods that allow the direct mapping of the subcellular distribution of metal-based therapeutics, while preserving important morphological information on the cell.

Among the various strategies to achieve metal compounds imaging in biological environments, fluorescence microscopy is certainly one of the most explored, and an increasing number of publications reporting on bifunctional metal compounds bearing fluorescent moieties for both therapeutic and imaging applications (so-called *theranostic* agents) have appeared.23−26 Thus, several examples of such fluorescent metal complexes are present in the literature, including Pt(II) complexes tagged with fluorescent rhodamine-type moieties,27 gold,28 zinc29 and copper30 complexes bearing both fluorescent and cytotoxic ligands, cobalt complexes with coumarin ligands,31 as well as organometallic ruthenium compounds.32 Within this frame, we recently developed series of cytotoxic metal complexes featuring a porphyrin33,34 or the dipyrromethene fluorophore (BODIPY, boron-dipyrromethene)35,36 as highly fluorescent coordinating ligands (Figure 2). Moreover, some of us reported on cytotoxic silver(I) and gold(I) N-heterocyclic carbene (NHC) complexes bearing a fluorescent anthracenyl ligand,37 and on gold(I) NHC38 or phosphine39 compounds bound to a coumarin moiety (Figure 2).

Following these promising results, we describe here a new series of dinuclear heterobimetallic compounds featuring a luminescent Ru(bipy)2(N∧N) moiety and a second metal-based unit with potential as anticancer agents (Figure 3). To link these two metal-based fragments together, we designed two bifunctional ligands bearing both a phosphine scaffold as well as a bidentate N∧N ligand. Specifically, the first ligand (1) is based on a bipyridine (bipy) moiety while the second one (2) is based on a dipyridylamine (dipy) skeleton and has been recently described by us.17 Both ligands are able to coordinate Ru(II) ions via their N-donor groups to achieve the corresponding luminescent ruthenium polypyridyl complexes Ru(bipy)3 and Ru(bipy)2(dipy), respectively. Afterward, coordination of the phosphine unit in the mononuclear ruthenium complexes to Ru(II)-, Os(II)-, Au(I)-, and Rh(III)-based scaffolds has been envisaged and resulted in the synthesis of two series of heteronuclear complexes: series I based on the Ru(bipy)3 moiety, and series II based on the Ru(bipy)2(dipy) scaffold, respectively (Figure 3).

In addition, the replacement of the chlorido ligand bound to Au(I) ions by 1-thio-β-D-glucose 2,3,4,6-tetraacetate allowed the generation of aurano-fin-like derivatives within both series. Aurano-fin is an antiarthritic drug currently being investigated for potential therapeutic application including cancer.40 Interestingly, it has been hypothesized that the thio-sugar unit in aurano-fin, and in other metal compounds, is acting as a true substrate for the glucose active-transport system, and can be used to increase the uptake of the resulting metallo-drugs.41 Thus, the two new series of bimetallic compounds have been tested for their cytotoxic effects on various cancer cell lines (A2780S, A2780R, and A549) in comparison to cisplatin. The possibility of an interaction of representative compounds with DNA (deoxyribonucleic acid) has also been assessed by gel electrophoresis. Moreover, fluorescence microscopy studies of some of the bimetallic complexes were performed to evaluate the metal uptake via GLUT-1 transporters and distribution in cancer cells.

![Figure 1. Examples of homo- and heteropolymetallic complexes described as anticancer agents.](image1)

![Figure 2. Luminescent metal-based complexes with biological activity.](image2)
RESULTS AND DISCUSSION

Synthesis and Characterization. Initially, the synthesis of the ruthenium monometallic complexes 1-PF$_6$ and 1-Cl was achieved, which requires the generation of the non-commercially available (4′-methyl-[2,2′-bipyridin]-4-yl)methanamine 3 in three steps as described in the literature. Then, the reaction proceeds in three additional steps, starting with the coupling reaction of 3 with 4-iodobenzoyl chloride and followed by the complexation of the ruthenium precursor (Ru(bipy)$_2$Cl$_2$ with the functionalized bipyridine derivative 4 via the exchange of the two chlorido ligands (Scheme 1). At this stage, counteranion exchange reaction can be performed by addition of aqueous saturated KPF$_6$ solution, giving rise to the complex 1-PF$_6$. The pallado-catalyzed coupling reaction between the iodo derivatives 5 (5-PF$_6$ or 5-Cl) and diphenylphosphine gave the desired phosphines 1-Cl and 1-PF$_6$ in 83% yield (Scheme 1).

The same strategy was applied to the synthesis of the dipyridylamine-phosphine based ruthenium complex 2 starting from compound 6, whose synthesis has been recently described by us (Scheme 2). The desired product 2 was obtained in more than 81% (global yield).

Afterward, the ruthenium mononuclear complexes 1-PF$_6$, 1-Cl, and 2 were metalated at the terminal phosphine with gold, arene-ruthenium, pentamethylcyclopentadienyl-rhodium or arene-osmium.
derivatives under mild conditions. The complexation reactions were monitored by $^{31}$P NMR (nuclear magnetic resonance), and a significant chemical shift was observed going from the free phosphines in 1-PF$_6$, 1-Cl, and 2 (singlet at around −5 ppm) to the desired bimetallic complexes (singlet at around 33 ppm for Au derivatives, 26 ppm for the Ru ones, 31 ppm for the Rh one, and −12 ppm for the Os one). All complexation reactions afforded the bimetallic complexes in good to excellent yields (Table 1).

In previous studies, we and others noticed that replacing the chlorido ligand of a phosphine gold chloride complex by a thioglucose tetracetate could lead to a moderate to dramatic increase of its cytotoxicity. Thus, we decided to perform the same experiment on the gold compounds 1A-Cl and 2A by reaction with one equivalent of thioglucose tetracetate and sodium hydroxide to obtain respectively the complexes 1D and 2D (Scheme 3). The reactions were monitored by $^{31}$P NMR, and...
a chemical shift of +5 ppm was observed between the starting phosphine-gold-chloride complexes and the phosphine-gold-thioglucose products. Noteworthy, the shape of the signal goes from a sharp singlet to a broad one, characteristic feature of this exchange of ligands on phosphine gold complexes.44

**Photophysical and Electrochemical Properties.** The mononuclear ruthenium complexes 1-PF6 and 2 exhibit in DMSO (dimethylsulfoxide) a typical MLCT (metal-to-ligand charge transfer) absorption band at 457 and 433 nm and a luminescence emission band at 535 and 539 nm, respectively (Table 2). These

<table>
<thead>
<tr>
<th>complexes</th>
<th>λabs (nm)</th>
<th>λem (nm)</th>
<th>Φ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ru(bipy)3]Cl2</td>
<td>454</td>
<td>630</td>
<td>8</td>
</tr>
<tr>
<td>1-PF6</td>
<td>457</td>
<td>535</td>
<td>19</td>
</tr>
<tr>
<td>1A-PF6</td>
<td>457</td>
<td>535</td>
<td>14</td>
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<td>IB</td>
<td>456</td>
<td>533</td>
<td>14</td>
</tr>
<tr>
<td>IC</td>
<td>457</td>
<td>536</td>
<td>15</td>
</tr>
<tr>
<td>1D</td>
<td>456</td>
<td>593</td>
<td>13</td>
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<tr>
<td>2</td>
<td>433</td>
<td>539</td>
<td>12</td>
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<td>531</td>
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<td>429</td>
<td>533</td>
<td>&lt; 1</td>
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<tr>
<td>2D</td>
<td>436</td>
<td>545</td>
<td>6</td>
</tr>
</tbody>
</table>

Values taken from ref 45.

Table 2. Absorption and Emission Wavelengths and Quantum Yields of Luminescence of the Mono- (1-PF6 and 2) and Bimetallic (1A-PF6,1D and 2A-2D) Complexes Measured in Degassed DMSO at 298 K, Using [Ru(bipy)3]Cl2 as Internal Reference

Table 3. Voltammetric Parameters Evaluated for 1D Recorded in DMSO Containing 0.1 M TBAP

<table>
<thead>
<tr>
<th>redox couple</th>
<th>assignment</th>
<th>E′ c (V)</th>
<th>E c (V)</th>
<th>ΔE p (V)</th>
<th>E′ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ru3+/2+</td>
<td>0.85</td>
<td>0.77</td>
<td>0.077</td>
<td>0.81</td>
</tr>
<tr>
<td>II</td>
<td>Ru3+/2+</td>
<td>−1.65</td>
<td>−1.73</td>
<td>0.086</td>
<td>−1.69</td>
</tr>
<tr>
<td>III</td>
<td>Ru3+/2+</td>
<td>−1.82</td>
<td>−1.91</td>
<td>0.088</td>
<td>−1.87</td>
</tr>
<tr>
<td>IV</td>
<td>Ru0/1+</td>
<td>−2.06</td>
<td>−2.15</td>
<td>0.091</td>
<td>−2.11</td>
</tr>
</tbody>
</table>

Scans were recorded at a glassy carbon working electrode (3 mm diameter) at a scan rate of 0.1 V/s. All potentials (V) were measured vs. Fc+/Fc redox couple.

The value of +0.8 V vs Fc+/Fc recorded for [Ru(bipy)3](3+/2+) in DMF (0.1 M Bu4NPF6).47 The slight discrepancy in redox potential (+0.01 V) observed is likely due to sensitivity of the Fc+/Fc redox couple (used as an internal reference)46 to the different solvent and electrolyte composition employed.49 In addition, the presence of additional functional groups on the coordinating bipyridine ligand linking to the Au moiety complicates direct electrochemical comparison to [Ru(bipy)3](3+/2+). For example, the electron-donating 4'-methyl group located on the bipyridine ligand is expected to shift E′(−) by approximately −0.03 V, compared to [Ru(bipy)3](3+/2+), as predicted by the Lefler ligand electrochemical series.48 All redox couples (I, II, III, and IV) are quasi-reversible (ΔE p values range from ~0.07 to 0.09 V), with similar ΔE p values to those of [Ru(bipy)3](3+/2+) for corresponding redox couples.47 This suggests that the relatively rapid heterogeneous electron transfer rates for these redox couples are not altered by attachment to the Au moiety. Further evidence that the metal centers remain electronically independent (uncoupled) is indicated by the lack of significant change in voltammetric response between compound 1D and [Ru(bipy)3](3+/2+). Strong electronic coupling of metal centers would be expected to alter the voltammetric response for one or both metal centers, compared to their mononuclear forms, as observed for electronically coupled binuclear complexes.51 The scarcely defined voltammetric peak at ~1.06 V vs Fc+/Fc that can be tentatively attributed to Au(I) reduction52 suggests that the Au metal center is relatively inaccessible to heterogeneous electron transfer in DMSO solvent within this potential range (~2.3 to 1.2 V vs Fc+/Fc), thereby restricting Ru–Au cooperative effects.

Preliminary stability studies of the complexes have been performed by 31P NMR and UV–visible spectrophotometry in PBS (phosphate buffer saline) buffer over 48 h (see Supporting Information for details). Most of the complexes showed no significant change in the resulting spectra. In some cases, a slight decrease of the MLCT band appeared, due to a partial fluorescence imaging. Notably, replacing the chlorido ligand on gold by the thioglucose tetracetate moiety allows the partial recovery of the luminescence (Φ = 6%) and the possible use of 2D for fluorescence imaging.

It is worth mentioning that cyclic voltammetric (CV) measurements on the Ru–Au bimetallic compound 1D also indicate the absence of an effect of the coupling of two redox active centers (Ru(II) and Au(I)) within the same molecule. Figure S-4 shows the CV of compound 1D recorded in DMSO containing 0.1 M TBAP (tetrabutylammonium perchlorate) as supporting electrolyte. Clearly, the redox couples I, II, III, and IV can be attributed to Ru3+/2+ (metal-based), Ru2+/1+, Ru1+/0, and Ru0/1− (ligand-based) redox transitions, respectively.47 The redox potential (E′(−)) of +0.81 V vs Fc+/Fc (ferrocene) evaluated for the metal centered Ru3+/2+ couple, shown in Table 3, is close to
precipitation of the compounds in solution. Fortunately, even after 48 h, the absorbance decreased less than 30%. Noteworthy, neither new band appearance nor band disappearance has been noticed. Therefore, we can conclude that there is few or no degradation of the luminescent ruthenium-based moiety of the reported fluorescent metal compounds within the time frame of the experiment (few hours). Regarding the phosphine metal part, the stability of analogues has been investigated in previous works by UV–visible spectroscopy, as well as $^{31}$P and $^1$H NMR, which showed no decomposition after 48 h.$^{17,36}$

**Antiproliferative Effects and DNA Interaction Studies.** The bimetallic complexes 1A-PF$_6$-1D and 2A-2D were initially tested for their antiproliferative properties using the classical MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay against various cancer cell lines, including the human ovarian carcinoma sensitive (A2780S) and cisplatin-resistant (A2780cisR) cells and the human lung carcinoma (A549) cell lines. The obtained results are reported in Table 4. Overall, the cytotoxic effect of these complexes appears to be dependent on different factors.

<table>
<thead>
<tr>
<th>compound</th>
<th>A2780S</th>
<th>A2780R</th>
<th>A549</th>
</tr>
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<tbody>
<tr>
<td>1A-PF$_6$</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1B</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1C</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>1D</td>
<td>2.7 ± 0.9</td>
<td>6.4 ± 1.0</td>
<td>26.5 ± 2.6</td>
</tr>
<tr>
<td>2A</td>
<td>12.0 ± 3.7</td>
<td>12.7 ± 1.4</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>2B</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>2C</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>2D</td>
<td>1.4 ± 0.3</td>
<td>3.1 ± 0.9</td>
<td>5.7 ± 0.5</td>
</tr>
<tr>
<td>cisplatin</td>
<td>2.5 ± 0.9</td>
<td>35.0 ± 3.5</td>
<td>8.0 ± 0.5</td>
</tr>
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</table>

The insertion of Rh, Ru, and Os groups to be coupled to the Ru(poly(pyridyl)) compounds resulted in complexes with poor or no activity in the tested cell lines, independently on the type of luminescent tag moiety and linker type. This observation is consistent with the previous findings that similar ruthenium and rhodium complexes are, in general, less cytotoxic than platinum-based anticancer agents.$^{53,54}$ On the other hand, the luminescent tag linker seems to play an important role in the case of the heteronuclear Ru–Au complexes: in fact, 1A-PF$_6$ with the “bipy” linker, has no toxic effects on the tested cell lines, while 2A, bearing the “dipy” linker, is moderately toxic in most cell lines. Interestingly, the only complexes displaying significant antiproliferative effects were the heteronuclear Ru–Au complexes 1D, 2A, and 2D and these compounds were more effective on the cisplatin-resistant cell line A2780cisR than cisplatin itself, indicating lack of cross-resistance and a different mechanism of action from the platinum drug. This hypothesis has been confirmed by DNA electrophoresis experiment (Figure 4), which demonstrated the absence of interaction between complex 2D and plasmid pUC19 DNA.

Moreover, compound 2D has a similar potency in all tested cell lines, while compound 1D has an IC$_{50}$ (half maximal inhibitory concentration) against A549 cells of 1 order of magnitude higher. This fact may indicate that the mechanism of activity of the two metal compounds may be different in this cell line. Furthermore, among these three Ru–Au derivatives, the most lipophilic compounds 1D and 2D, containing a conjugated bioactive molecule (thioglucose moiety) were even more toxic against all cell lines than the parent compounds, 1A-PF$_6$ and 2A respectively (with chloride as ancillary ligand). This observation suggests that the increase of cytotoxic potency of complexes 1D and 2D, with respect to the other complexes, might derive from an improved uptake in cancer cells.

**Fluorescence Microscopy.** To assess the imaging properties of our heteronuclear complexes in vitro, fluorescence microscopy was used, allowing us to gain insights into the uptake and localization of these luminescent compounds. Thus, the gold(I)–ruthenium (II) compounds 1D, 2A, and 2D were selected, due to their promising cytotoxic effects and sufficient fluorescence quantum yields. The human ovarian A2780 and lung A549 cells were grown onto a sterile chamber slides and treated with the luminescent compounds as described in the Experimental Section.

Evaluation of active transport mechanisms which may be involved in the uptake of the complexes was performed incubating cells with the metal complexes at either 37 or 4 °C, respectively. At low temperatures, active and facilitated passive transport mechanisms are commonly inhibited. Figure 5 shows the low fluorescence of complex 2A on A2780 at 4 °C in A2780 cells after 3 h incubation. At this temperature the luminescence of the compound is very low and this is representative for all the tested complexes in both cell lines (data not shown). Therefore, the uptake of the complexes should occur by active or facilitated transport. Figure 6 shows that complex 1D has enhanced intracellular fluorescence intensity in both cell lines. Complex 2D is the most cytotoxic compound of the series despite being one of the least luminescent. Thus, the low fluorescence intensity of this complex may lead to scarce detection by fluorescence microscopy, rather than the low uptake, although the need of longer incubation times for uptake cannot be excluded.

Interestingly, compounds 1D and 2D present quite different intracellular localization: while 2D appears to have a diffuse localization in the cytoplasm, complex 1D seems to accumulate in organelles in the nuclei, in both A2780 and A549 cells. This is different for example from what has been observed in the case of previously reported lipophilic mononuclear Ru(bipy)$_2$ complexes which showed membrane localization.$^{55}$ Interestingly, Gottschald et al. presented ruthenium poly(pyridyl) complexes...
with peripherally attached sugar substituents. In this case, fluorescence microscopy revealed that D-glucose conjugated complexes possess a clear intracellular distribution with a granular pattern as observed for complex 1D. Adding a targeting moiety to a complex, such as thioglucose, may increase its uptake by aiming at the glucose transporters. GluT-1 is a glucose transporter isoform known by having a high level of expression on A549 cells. To investigate the transport of our Ru–Au complexes via this isoform, we used a known inhibitor (WZB117, 3-fluoro-1,2-phenylene bis(3-hydroxybenzoate)) for this transporter and expected to see a decrease in uptake of the complexes bearing the targeting moiety, complexes 1D and 2D.
Unfortunately, due to its fluorescent properties, complex 2D was not detected in these cells. As shown in Figure 6, a 30 min preincubation of cells with 50 μM of the inhibitor did not affect the uptake of complex 1D. These results argue against the role of the Glut-1 isoform as transporter in the uptake of the Ru−Au complexes bearing the thioglucose targeting moiety but do not exclude the possibility that other isoforms are involved.

Since the uptake of compound 2D could not be followed by fluorescence microscopy, we investigated the effect of WZB117 in the toxicity of complex 2D in A2780 cells. Thus, the toxicity of both the Glut-1 inhibitor and compound 2D were evaluated at 24 and 72 h, by measuring the cell viability using an MTT assay. The cells were incubated with either WZB117 and compound 2D alone or coincubation of compound 2D with several concentrations of WZB117. The inhibitor was not toxic up to a concentration of 0.5 μM at 24 h and very low toxicity up to 20 μM at 72 h. The concentrations of complex 2D used were 1 and 5 μM for both incubation times. Our results show that coincubation of the Ru(II)−Au(I) complex with the Glut-1 inhibitor did not affect the toxicity of the complex (data not shown), suggesting that Glut-1 transporter is not involved in the uptake of this complex, as shown for complex 1D by fluorescence microscopy.

**CONCLUSIONS**

Two series of novel heterobimetallic complexes possessing a luminescent Ru(poly(pyridine)) scaffold have been synthesized and characterized. All the compounds have been studied for their photophysical properties, showing typical MLCT absorption bands between 420 and 460 nm, and emission bands centered around 535 nm (595 nm when possessing the chloride counteranion). While the quantum yield of the complexes in deuterated solvent (13C) as an internal standard. Alternatively, 85% H2PO4 (10% and 15% BF3·OEt2 (10%) in CDC13 were used as external standards. The coupling constants are reported in Hertz. All aromatic positions, ortho, meta, para, are defined using phosphorus as main group. All aromatic positions in pyridines are numbered as follows:

Infrared spectra were recorded on a Bruker Vector 22 FT-IR (Fourier transformed infrared) spectrophotometer (transmission mode) equipped with the ATR “golden gate” or on a Bruker Vertex 70v FT-IR spectrophotometer. Elemental analyses were performed on a Thermo Electron Flash EA 1112 Series analyzer. UV−visible absorption spectra were recorded on a JASCO V630 BIO spectrometer. The steady-state fluorescence emission spectra were obtained by using a JASCO FP8560 spectrophotoluminometer instrument.

2. Synthesis, 1-PF6 (or 1-Cl). In a round-bottom flask under argon and equipped with a cooling system, we introduced 461 mg (or 372 mg) of 4,000 mmol, 0.002 equiv) of Pd(OAc)2 dissolved in 20 mL of distilled acetonitrile. 113 μL (0.814 mmol, 2 equiv) of distilled triethylamine and 71 μL (0.407 mmol, 1 equiv) of diphenylphosphine were then added, and the flask under argon was closed by a rubber septum. After 4 h, the reaction mixture was stirred at 85 °C overnight. After it was cooled at room temperature, the solvent was removed by rotary evaporation, and the crude product purified by column chromatography on silica (elucent = 100% acetonitrile/10% distilled water/1 KNO3 aq. saturated); yield 403 mg (or 328 mg) (0.338 mmol, 83%).

1H NMR (500.13 MHz, CD3Cl2): δ = 8.51 (m, 1 H, CH (3’)), 8.44 (m, 4 H, 4 × CH-bipyridine), 8.34 (m, 1 H, CH (5’)), 8.07 (m, 4 H, 4 × CH-bipyridine), 7.96 (m, 1 H, CH (5’)), 7.87 (dd, 2 H, JCH = 8.9 Hz and JHH = 1.4 Hz, 2 × CH-Ar), 7.79–7.68 (m, 4 H, 4 × CH-bipyridine), 7.62–7.32 (m, 18 H, 2 × CH-Ar + 10 × CH-PPh3 + 6 × CH-bipyridine), 7.25 (m, 1 H, CH (3’)) 4.81 (d, 2 H, JHH = 5.6 Hz, CH2), 2.57 (s, 3 H, CH3). 31P{1H} NMR (151.74 MHz, CD3Cl2):

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δ = −5.40 (s, PPh3) (−144.5 (hept. J_{H-P} = 7.12 Hz, PPh3) 

1^1C{1H}NMR (500.13 MHz, CDCl3): δ = 8.50 (m, 1 H, CH(3)), 8.45 (m, 4 H, 4 × CH-bipyridine), 8.35 (broad s, 1 H, CH(5)), 8.10 − 8.02 (m, 5 H, 4 × CH-bipyridine + CH (5')), 7.78 − 7.71 (m, 4 H, 4 × CH-bipyridine), 7.65 − 7.44 (m, 20 H, 4 × CH-PhAr + 6 × CH-bipyridine)), 7.26 (m, 1 H, CH(3)), 4.84 (d, 2 H, J_{H-Ph} = 5.8 Hz, CH2), 2.60 (s, 3 H, CH3). ^31P{1H}NMR (202.46 MHz, CDCl3): δ = −32.3 (s, PPh3), −144.5 (hept. J_{H-P} = 7.12 Hz, PPh3), ^31C{1H}NMR (125.76 MHz, CDCl3): δ = 161.6 (s, CO), 157.0 (s, C^{iv}-bipyridine), 156.8 (s, C^{iv}-bipyridine), 156.7 (s, 2 × C^{iv}-bipyridine), 156.6 (s, C^{iv}-bipyridine), 156.2 (s, 1A-PF6), 152.8 (J_{H-P} = 29.6 Hz, C^{iv}-Ar), 151.5 (s, CH-bipyridine), 151.3 (s, 2 × CH-bipyridine), 151.2 (J_{H-P} = 35.0 Hz, 2 × C^{iv}-PhP), 151.2 (s, CH-bipyridine), 150.5 (s, 2 × CH-bipyridine), 150.1 (s, 2 × CH-bipyridine) + 151.7 − 139.8 (m, 4 × CH-bipyridine + 2 × CH-Ar), 134.2 (J_{H-P} = 14.3 Hz, 4 × CH-bipyridine), 134.3 (J_{H-P} = 15.7 Hz, 2 × CH-Ar), 133.1 (s, C^{iv}-bipyridine), 132.7 (s, C^{iv}-bipyridine), 132.4 (s, C^{iv}-CONH), 132.3 (d, J_{H-P} = 2.7 Hz, 2 × CH-PhP, para), 129.4 (J_{H-P} = 10.6 Hz, 4 × CH-bipyridine, meta), 128.9 (broad s, CH(3)), 128.2 − 127.9 (m, 3 × (CH(5) and 6')), 125.3 (s, CH(5)), 124.2 (s, CH-bipyridine), 124.1 (s, CH-bipyridine), 124.0 (s, CH-bipyridine), 123.9 (s, CH-bipyridine), 123.4 (s, CH(3)), 121.9 (s, CPh2), 121.1 (s, CH3), 121.1 (s, CH3), 121.1 (s, CH3), 121.1 (s, CH3), 121.1 (s, CH3), 121.1 (s, CH3). ESI-MS (CH3Cl/MeOH, positive mode) exact mass for [C72H63N5O9P4Ru2]^{+} ([M + Na]^+): theoretical m/z 548.6092: found m/z 548.60857 (err. 0.138 ppm). UV–Vis (DMSO): λ_{max} (nm) (ε, mol−1 cm−1) 457 (7200) (golden gate diamant: δ (cm−1) 3409 (ε_{NH amide}), 3565 (ε_{1HC}Am), 1638 (ε_{c-CO amide}), 1619 (ε_{C=C amide}).

In a Schlenk tube under argon were introduced 146 mg (0.123 mmol, 1 equiv) of 1-PF6 and 38 mg (0.061 mmol, 0.5 equiv) of [p-(pyr-cymene)RuCl]2. 

Degassed benzene (3 mL) was added, and the reaction was stirred at room temperature in the dark during 3 h. The desired coordination product directly precipitated from the mixture.

The red precipitate was isolated by filtration and dried to get 1B as a red powder; yield 170 mg (0.114 mmol, 93%).

^31P{1H}NMR (500.13 MHz, CDCl3): δ = 8.47 (broad s, 1 H, CH(3)), 8.45 − 8.41 (m, 4 H, 4 × CH-bipyridine), 8.34 (broad s, 1 H, CH(5)), 8.08 − 8.03 (m, 4 H, 4 × CH-bipyridine), 7.87 − 7.70 (m, 11 H, 11 × CH-bipyridine + 2 × CH-Ar + 2 × CH-PhP, meta), 7.52 − 7.42 (m, 12 H, 10 × CH-bipyridine, 2 × CH (6 and 6'))), 7.26 (broad d, 1 H, J_{H-P} = 5.5 Hz, CH(3')), 5.27 (d, 2 H, J_{H-P} = 6.6 Hz, 2 × CH-Ar-pyrimine), 5.03 (d, 2 H, J_{H-P} = 6.3 Hz, 2 × CH-arpyrine), 4.76 (broad d, 2 H, J_{H-P} = 6.3 Hz, CH2), 2.78 (hept. 1H, J_{H-P} = 7.0 Hz, CH-PPh), 2.58 (s, 3 H, CH3), 1.90 (s, 3 H, CH3), 1.17 (d, 6 H, J_{H-P} = 7.0 Hz, 2 × CH2-PhP). ^31P{1H}NMR (202.46 MHz, CDCl3): δ = 25.6 (s, PPh3), −144.4 (hept. J_{H-P} = 71.9 Hz, PPh3), ^31C{1H}NMR (125.76 MHz, CDCl3): δ = 159.7 (s, CO), 157.4 (s, C^{iv}-bipyridine), 157.2 (s, C^{iv}-bipyridine), 156.6 (s, C^{iv}-bipyridine), 156.2 (s, C^{iv}-bipyridine), 151.9 (s, CH-bipyridine), 151.7 (s, CH-bipyridine), 151.6 (m, 4 × CH-bipyridine), 151.5 (d, J_{H-P} = 41.4 Hz, CH-Ar), 151.4 (s, C^{iv}-bipyridine), 150.9 (s, CH-bipyridine), 150.5 (s, CH-bipyridine), 148.8 (s, C^{iv}-CONH), 138.3 (s, 2 × CH-bipyridine), 138.2 (s, 2 × CH-bipyridine), 134.9 (s, CH(5)), 134.7 (d, J_{H-P} = 46.9 Hz, 2 × CH-PPh), 134.6 (m, 2 × CH-Ar), 131.0 (m, 2 × CH-Ar), 129.3 (s, CH(3)), 128.7 − 128.2 (m, 10 × CH-PhP + 2 × CH (6 and 6')), 128.5 (s, CH(5)), 126.4 − 124.3 (m, 12 × CH-bipyridine), 123.7 (s, CH(3)), 89.2 (m, 2 × CH-Ar-pyrimine), 88.0 (m, 2 × CH-Ar-pyrimine), 79.1 (s, C^{iv}-pyrene), 79.0 (s, C^{iv}-pyrene), 79.0 (s, C^{iv}-pyrene), 43.0 (CH2), 30.9 (CH2-PPh), 22.1 (s, CH3), 21.5 (s, CH3), 18.0 (s, CH3), ESI-MS (CH3Cl/MeOH, positive mode) exact mass for [C72H63N5O9P4Ru2]^{+} ([M + Na]^+) theoretical m/z 603.08793: found m/z 603.08895 (err. 2.929 ppm). UV–Vis (DMSO): λ_{max} (nm) (ε, mol−1 cm−1) 456 (5000) (golden gate diamant and FIR): δ (cm−1) 3395 (ε_{NH amide}), 3076 (ε_{1HC}Am), 1639 (ε_{C-CO amide}), 1621 (ε_{C=N amide}, 321 (ε_{ν(CN)}).

1A-PF6 (or 1A-CI). In a Schlenk tube under argon were introduced 106 mg (or 87 mg) (0.089 mmol, 1 equiv) of 1-PF6 (or 1-CI) and 29 mg (0.089 mmol, 1 equiv) of [AuCl(H2)]Cl. Degassed benzene (3 mL) was added, and the reaction was stirred at room temperature in the dark during 3 h. The desired coordination product directly precipitated from the mixture.

The red precipitate was isolated by filtration and dried to get 1A-PF6 (or 1A-CI) as a red powder; yield 117 mg (or 99 mg) (0.082 mmol, 92%).
In a Schlenk tube under argon were introduced 210 mg (0.174 mmol, 1 equiv) of 1A-Cl, dissolved in 3 mL of degassed acetonitrile at 0 °C. In another Schlenk tube under argon were mixed 64 mg (0.174 mmol, 1 equiv) of thiglucoside tetraacetate in 3 mL of acetone and 174 μL of 1 M NaOH (0.174 mmol, 1 equiv). This mixture was stirred 10 min at room temperature in the dark, and was then added at 0 °C on the bimetallic solution. The reaction was stirred at room temperature in the dark during 3 h. The solvent was evaporated, and distilled dichloromethane (5 mL). The salts were removed by filtration under argon; the filtrate was evaporated and dried to obtain the desired product as a yellow red powder; yield 153 mg (0.100 mmol, 57%).

1H NMR (500.13 MHz, CD2Cl2): δ = 9.66 ( broad 1 H, NH), 8.73 ( d, 1H, J

H = 3.0 Hz, CH (3)), 8.85–8.53 ( m, 4 H, 4 × CH-chloride), 8.49 ( broad s, 1 H, CH (3)), 8.23–8.20 ( dd, 2H, J

H = 8.2 Hz and J

H = 2.0 Hz, 2 × CH-chloride), 8.15–8.06 ( m, 4 H, 4 × CH-chloride), 7.84–7.83 ( broad d, 1H, J

H = 5.6 Hz, CH (5), 7.80–7.73 ( m, 3H, 3 × CH-chloride), 7.68–7.45 ( m, 19 H, 4 × CH-Ar + 10 × CH-PPh3 + 5 × CH-pyrene), 7.27 ( m, 1 H, CH (3)), 5.20–5.07 ( m, 4 H, 4 × CH-glucose), 4.85 ( d, 2 H, J

H = 5.9 Hz, CH (3)), 3.80 ( m, 3 H, CH2NH and CHCH2OC, 2.61 ( s, 3 H, CH3), 2.03–1.99 ( m, 12 H, 4 × CH-glucose), 1.31–1.29 ( d, 6 H, J

H = 7.0 Hz, 2 × CH2-Pr), 1.24 ( s, 6 H, CH3).

13C NMR (125.76 MHz, CDCl3): δ = 198.7 (s, C=CH-CONH), 170.4 ( s, C=CH-CONH), 169.9 ( s, 2 × C=COCH3), 169.4 ( s, 2 × COCH3), 157.0 (d, J

C-P = 43.2 Hz, C=CH-Ar), 156.9–156.8 (m, 6 × C=CH-bipyridine), 156.7 ( m, 2 × C=CH-bipyridine and C=CH-Ar), 151.7 (s, CH (5)), 151.3 ( m, 5 × CH-chloride), 150.3–150.2 ( m, 2 × CH-bipyridine), 137.9 ( s, 2 × CH-bipyridine), 137.7 ( s, 2 × CH-bipyridine), 136.7 (d, J

H = 40.3 Hz, 2 × C=CH-PPh3), 134.4 ( d, J

H = 2.8 Hz, 2 × CH2-Pr), 134.3 ( d, J

H = 2.9 Hz, 4 × CH-Ph3-meta), 134.3 ( s, 2 × CH2-Pr, para), 134.1 ( s, CH-bipyridine), 131.9 ( broad s, CH3), 129.3 ( d, J

H = 11.6 Hz, 4 × CH-Ph3ortho), 128.7 ( d, J

H = 8.9 Hz, 2 × CH-Ar), 127.1 ( s, 2 × CH-bipyridine), 125.6 ( s, CH (5)), 124.4 ( s, 2 × CH-bipyridine), 124.3 ( s, 2 × CH-bipyridine), 123.9 ( s, CH (3)), 75.8 ( s, CHCH2OC), 73.4 ( s, 2 × CH-glucose), 70.3 ( s, CH-glucose), 56.4 ( s, CH4), 53.0 ( s, CH-glucose), 42.7 ( s, CH2), 21.2 ( s, CH4), 20.5 ( s, 3 × CH3), ESI-MS (CH3OH, positive mode) exact mass for C59H52Cl2N7O1P1 (M+H)+: found m/z 859.57208; calculated m/z 859.57208.

2C. In a Schlenk tube under argon were introduced 49 mg (0.042 mmol, 1 equiv) of Ru(bipy)3(dipy)PPh3 (2) and 13 mg (0.021 mmol, 0.5 equiv) of [(Cp*RhCl)2]. Degassed benzene (3 mL) was added, and the reaction mixture was stirred 4 h in the dark at room temperature. The desired coordination product precipitated; it was filtered and dried under vacuum to obtain 2C as a red powder; yield 63 mg (0.042 mmol, 99%).

1H NMR (500.13 MHz, CD2Cl2): δ = 8.75–8.18 ( m, 10 H, 10 × CH-bipy), 8.11–8.04 ( m, 4 H, 2 × CH (3 and 3′) + 2 × CH-bipy), 7.85–7.68 ( m, 7 H, CH (4′) + 6 × CH-bipy), 7.67–7.62 ( m, 2 H, 2 × CH (2 and 2′)), 7.59–7.47 ( m, 14 H, 10 × CH-Ph3 + 4 × CH-Ar), 7.26–7.09 ( m, 2 H, 2 × CH (1 and 1′)), 6.20 ( broad d, 1 H, J

H = 7.2 Hz, CH (4)), 1.72 ( s, 15 H, 5 × CH3), 1.31 ( d, J

H = 144.6 Hz, PPh3), 114.6 ( hept, J

H = 7.1 Hz, PPh3).

13C NMR (125.76 MHz, CDCl3): δ = 38.8 ( d, J

H = 40.3 Hz, 2 × C=CH-PPh3), 134.5 ( m, 153.3 × 4 × C(IV)), 131.5 ( m, 151.3 × 4 × C(IV)), 130.6 ( s, 2 × CH (1 and 1′)), 139.5 ( m, C(IV) + CH (4′)), 138.7 ( s, broad, 3 × CH (2′, 3 and 3′)), 135.3 ( s, 2 × CH-bipy), 135.0 ( s, 2 × CH-PPh3 ortho), 134.5 ( m, 4 × CH-XPhP2 ortho + para), 133.9 ( s, C6H5-CH2), 132.5 ( m, C6H5-PPh3).
11.5 (s, 2 × CH-bipy), 13.13 (s, 2 × CH-bipy), 128.7 (m, 4 × CH-PPh₂ meta + 2 × CH-Ar), 128.4 (broad s, 4 × CH-bipy), 127.3–127.1 (m, 3 × CH (2) + CH (4) + 2 × C¹IV-PPh₂), 100.0 (s, 2 × CH-Ar), 46.4 (s, 8 × C¹IV-Cp³), 10.1 (s, CH₃), 9.1 (s, 2 × CH₂), 8.8 (s, 2 × CH).

ESI-MS (CH₂Cl₂/MeOH, positive mode) exact mass for C₉₇H₁₁₀Cl₂N₇O₁P₁Rh₂Ru₂ ([M⁺], theoretical m/z 590.57619): found m/z 590.5744 (err. 0.006 ppm). UV (DMSO): δ = 3307 (ν (E)= cm⁻¹) 3077 (ν O amide), 2931 (ν CH₃), 2876 (ν (CH₂)₃), 1620 (ν (P=O amide), 1559 (ν (C=O)), 1538 (ν (C=O))).

3. In a round-bottom flask under argon equipped with a cooling system, were introduced 150 mg (0.350 mmol, 1 equiv) of 4-iodo-N-(4'-methyl-[2,2'-bipyridin]-4-yl)methylbenzamide (4) and 169 mg (0.350 mmol, 1 equiv) of cis-dichlorobis(2,2'-bipyridine)ruthenium(II) dihydrate. A degassed 1:1 mixture of ethanol and chloroform (10 mL) was added, and the reaction was stirred under argon at room temperature for 3 h. The residue was finally purified by silica gel chromatography (eluent 100 acetone/10 distilled water/1% KNO₃, aq. saturated); yield 368 mg (0.897 mmol, 93%).

H NMR (300.13 MHz, CDCl₃): δ = 3.85 (broad s, 2 × CH₂-bipy). The desired crude product obtained by precipitation during 48 h in the dark. The mixture was let to return to room temperature. The solvent was evaporated, and distilled dichloromethane (5 mL) was added. The salts were removed by filtration under argon; the filtrate was evaporated and dried to obtain the product as a bright red powder; yield 103 mg (0.072 mmol, 71%).

4. In a Schlenk tube under argon were introduced 81 mg (0.101 mmol, 1 equiv) of 1-thio-bipyridine, and stirred 5 min. In another Schlenk tube under argon were introduced 37 mg (0.101 mmol, 1 equiv) of 1-methoxyvinyl-1H-benzotriazole (3,4) and 81 mg (0.101 mmol, 1 equiv) of 1-methoxyvinyl-1H-benzotriazole (3,4). The salts were removed by filtration under argon; the filtrate was evaporated and dried to obtain the desired product as a white powder; yield 1.460 g (3.641 mmol, 92%).

5. In a round-bottom flask under argon equipped with a cooling system, were introduced 129 mg (0.101 mmol, 1 equiv) of Ru(bpy)₃(dipyAulCl)₂ (2A), dissolved in distilled acetone (5 mL). The reaction mixture was cooled down to 0 °C and stirred 5 min. In another Schlenk tube under argon were introduced 37 mg (0.101 mmol, 1 equiv) of 1-thio-bipyridine, and the reaction mixture was stirred 10 min at room temperature in the dark. This solution was slowly added to the previous one at 0 °C, and the resulting reaction mixture was stirred 3 h at room temperature. The solvent was evaporated, and distilled dichloromethane (5 mL) was added. The salts were removed by filtration under argon; the filtrate was evaporated and dried to obtain the desired product as a bright red powder; yield 103 mg (0.072 mmol, 71%).

6. In a round-bottom flask under argon, protected from light and equipped with a cooling system, were introduced 1053 g (3.960 mmol, 1 equiv) of 4-iodo-N-(4'-methyl-[2,2'-bipyridin]-4-yl)methylbenzamide (4) and 169 mg (0.350 mmol, 1 equiv) of cis-dichlorobis(2,2'-bipyridine)ruthenium(II) dihydrate. A degassed 1:1 mixture of ethanol and chloroform (10 mL) was added, and the reaction was stirred under argon at room temperature for 3 h. The residue was finally purified by silica gel chromatography (eluent 100 acetone/10 distilled water/1% KNO₃, aq. saturated); yield 368 mg (0.897 mmol, 93%).

H NMR (300.13 MHz, CDCl₃): δ = 3.85 (broad s, 2 × CH₂-bipy). The desired crude product obtained by precipitation during 48 h in the dark. The mixture was let to return to room temperature. The solvent was evaporated, and distilled dichloromethane (5 mL) was added. The salts were removed by filtration under argon; the filtrate was evaporated and dried to obtain the desired product as a white powder; yield 103 mg (0.072 mmol, 71%).
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\(^{1}J_{HF-H} = 8.1 \text{ Hz and } ^{1}J_{HF-H} = 0.87 \text{ Hz, } 2 \times CH (3 \text{ and } 7' )\), 7.23 (dt, 2 H, \(^{1}J_{HF-H} = 8.6 \text{ Hz and } ^{1}J_{HF-H} = 2.1 \text{ Hz}, 2 \times CH-Ar), 7.18 (\text{dd}, 2 \text{H, } ^{1}J_{HF-H} = 7.4 \text{ Hz, } ^{1}J_{HF-H} = 4.8 \text{ Hz and } ^{1}J_{HF-H} = 0.96 \text{ Hz, } 2 \times CH (4 \text{ and } 4' )\).

\(^{13}C(\text{H}) \text{ NMR (125.76 MHz, CDCl₃): } \delta = 170.3 (s, \text{C}-\text{C}), 155.1 (s, 2 \times \text{C-ppyridine}), 148.9 (s, 2 \times \text{CH (1 and } 1')\), 138.2 (s, 2 \times \text{CH (2 and } 2')\), 137.5 (s, 2 \times \text{CH-Ar}), 135.3 (s, \text{C}-\text{C,C(H,CO)}), 130.6 (s, 2 \times \text{CH−Ar}), 122.1 (s, 2 \times \text{CH (3 and } 3')\), 121.7 (s, 2 \times \text{CH (4 and } 4')\), 97.9 (s, C). ESI-MS (CH₂Cl₂/MeOH, positive mode) exact mass for \[\text{C}_3\text{H}_2\text{N}_7\text{O}_1\text{I}_1\text{Ru}_1, \left[\text{M}^+\right]_2^+, \text{theoretical mass/s} \text{2} = 402.00978\text{;} \text{found mass/s} \text{2} = 402.01004(\text{err. 0.064 ppm).}

Elemental analysis for \(\text{C}_2\text{H}_4\text{N}_2\text{O}_1\text{I}_1\text{Ru}_1 \text(flux overnight in the dark).}

In a round-bottom flask under argon and equipped with a cooling system, were introduced 1.079 g (2.691 mmol, 1 equiv) of 4-iodo- \(\text{C}_3\text{H}_2\text{N}_7\text{O}_1\text{I}_1\text{Ru}_1\) (407.52182 Da): found C 50.89, H 3.01, N 10.47; \text{C 50.78, H 3.01, N 10.63.}

IR (golden gate diamant): \(\nu_{\text{cm}^{-1}} = 3418 (\nu_{\text{C=O}} \text{amide}), 3001 (\nu_{\text{C=H}} \text{amide}), 2935 (\nu_{\text{C−H amide}}), 144.6 (\text{hept, } 1\text{H, CON}), 129.1 (s, 2 \times \text{C−CON}), 127.3 (s, 2 \times \text{C−IV-bipyridine}), 118.9 (s, 2 \times \text{C−IV-bipyridine}), 116.3 (s, 2 \times \text{CH−Ar}), 99.1 (s, \text{C−IV-bipyridine})ruthenium(II) dihydrate. A degassed 1:1 water/aqueous solution and the residue was complete medium. Solutions of the compounds were prepared by diluting a freshly prepared stock solution (10−2 M in DMSO) of the corresponding compound in aqueous media (RPMM or DMEM depending on the cell lines). The percentage of DMSO in the culture medium never exceeded 0.2%; at this concentration DMSO has no effect on the cell viability. Cisplatin (Sigma-Aldrich) stock solutions were prepared in Milli-Q water. Afterward, the intermediate dilutions of the compounds were added to the wells (200 μL) to obtain a final concentration ranging from 0.01 to 200 μM, and the cells were incubated for 72 h. Following 72 h drug exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyldiazotetrazolium bromide (MTT) was added to the cells at a final concentration of 500 μg/mL incubated for 3–4 h, then the culture medium was removed and the violet formazan dissolved in DMSO. The optical density of each well (96-well plates) was quantified in quadruplicate at 540 nm using a multwell plate reader (iEMS Reader MF, Labsystems, Bioconcept) and the percentage of surviving cells was calculated from the ratio of absorbance between treated and untreated cells. The IC₅₀ value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (±SE) of at least three independent experiments.

5. Fluorescence Microscopy. Sterile 8-well chambers mounted on a slide with cover (Thermo Scientific) were coated with 10 mg/mL solution of poly-1-lysine hydrobromide (Sigma-Aldrich) in sterile water (400 μL per well). After 24 h, the wells were washed with PBS and coated plates were used to culture the cells, A2780 and A549, at a density of 5 × 10⁵ cells per well, with the respective complete medium (RPMM for A2780, DMEM for A549). After 24 h, the culture medium was removed and replaced with fresh medium, without FCS, containing 0.01, 0.5, 5, or 10% of complete medium. The percentage of DMSO in the culture medium never exceeded 0.2%: at this concentration DMSO has no effect on the cell viability. The human embryonic kidney (HEK293), human lung cancer (A549), for 3 h at 37 °C with Glut-1 inhibitor WZB117 (EMD Chemicals) at a concentration of 50 μM. Following the preincubation, the solution of inhibitor was removed and the cells were incubated with the various solutions of compounds, freshly prepared in medium without FCS (RPMM for A2780, DMEM for A549), for 3 h at 37 °C. At the end of incubation time, the cells were rapidly washed with cold PBS and fixed with 2% paraformaldehyde (Klinopas) for 30 min at 4 °C. Nuclei were stained using DAPI solution of 10 μg/mL (4′,6-diamidino-2-phenylindole (DAPI), dihydrochloride), Invitrogen) in PBS and incubated for 15 min at room temperature, in the dark. Following incubation, cells were permeabilized with 0.2% Triton X-100 for 20 min at 4 °C and treated with 1 μg/mL of propidium iodide for 10 min at room temperature. Cells were washed twice with PBS and then prepared for visualization by removal of the wells were and drying of the glass slide. Slides were then mounted with a glass slipcover with 20% PBS-glycerol (Sigma) and analyzed under a fluorescence microscope (DM 4000 B, Leica). PI was excited at 547 nm (emission wavelength 572 nm) and the compounds at 358 nm (emission wavelength 461 nm, DAPI filter). The acquired images were obtained using individual filters and a combined image, overlaying the fluorescence acquired with the two filters, was obtained using the Leica microscope software.

6. Electrophoresis with Plasmid DNA. Aliquots of pUC19 plasmid DNA (10 μL, 20 μL/mL) in buffer (5 mM Tris/Cl, 50 mM NaClO₄, pH 7.4) were incubated with different concentrations of the compounds (in the range 0.1 and 1 metal complex/DNAbp) at 37 °C overnight. After the incubation period, the samples were loaded in 1% agarose gel. Samples were separated by electrophoresis for ~1 h at 80 V in Tris-acetate/EDTA (ethylenediaminetetraacetic acid) buffer (TAE). Afterward, the gel was stained for 30 min in EBTr (ethidium bromide).

7. Glut-1 Inhibition Assay. A2780 and A549 cells were grown on a coated glass slide for 24 h with the respective complete medium, at a density of 5 × 10⁵ cells per well. After 24 h, the medium was removed and the cells were preincubated for 30 min at 37 °C with Glut-1 inhibitor WZB117 (EMD Chemicals) at a concentration of 50 μM. Following the preincubation, the solution of inhibitor was removed and the cells were incubated with the various solutions of compounds, freshly prepared in medium without FCS (RPMM for A2780, DMEM for A549), for 3 h at 37 °C. At the end of incubation time, cells were rapidly washed with cold PBS and fixed with 2% paraformaldehyde (Klinopas) for 30 min. Nuclei were stained using DAPI solution of 10 μg/mL (4′,6-diamidino-2-phenylindole, dihydrochloride), Invitrogen) in PBS and incubated for 15 min at room temperature, in the dark. Following incubation, cells were permeabilized with 0.2% Triton X-100 for 20 min at 4 °C and treated with 1 μg/mL of propidium iodide for 10 min at room temperature. Cells were washed twice with PBS and then prepared for visualization by removal of the wells were and drying of the glass slide. Slides were then mounted with a glass slipcover with 20% PBS-glycerol (Sigma) and analyzed under a fluorescence microscope (DM 4000 B, Leica). PI was excited at 547 nm (emission wavelength 572 nm) and the compounds at 358 nm (emission wavelength 461 nm, DAPI filter). The acquired images were obtained using individual filters and a combined image, overlaying the fluorescence acquired with the two filters, was obtained using the Leica microscope software.

Electrochemistry. Cyclic voltamograms were recorded using a PalmSens EmStat3+ potentiostat. Samples (~2 mmol) were dissolved in DMSO containing 0.1 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte and 1 mM ferrocene (Fc) as an internal reference. Voltamograms were recorded in a single compartment, glassy carbon electrode (0.1 mL volume) containing 0.1 mmol/L potassium ferricyanide (K₃[Fe(CN)₆]), theoretical mass/s = 407.52182 Da): found mass/s = 407.52212 Da (err. 0.0473 ppm). IR (golden gate diamant): \(\nu_{\text{cm}^{-1}} = 3355 (\nu_{\text{C=O}} \text{amide}), 2935 (\nu_{\text{C=H amide}}), 1722 (\nu_{\text{C=O}} \text{amide}), 1328 (\nu_{\text{C=O}})\).
compounds at 358 nm (emission wavelength 461 nm, DAPI filter). The acquired images were obtained using individual filters and a combined image, overlaying the fluorescence acquired with the two filters, was obtained using the Leica microscope software.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.5b02910.

Stability studies of the complexes and complete tables of photophysical and CV data (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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