EurJIC
European Journal of Inorganic Chemistry

A Journal of ChemPubSoc Europe

Accepted Article

Title: Photoactivatable Ru(II) complex bearing 2,9-diphenyl-1,10-phenanthroline: unusual photochemistry and significant potency on cisplatin-resistant cell lines

Authors: Najwa Mansour, Stephanie Mehanna, Mohamad A. Mroueh, Hassib Audi, Kikki Bodman-Smith, Costantine F. Daher, Robin I. Taleb, Mirvat El-Sibai, and Rony S. Khnayzer

To be cited as: *Eur. J. Inorg. Chem.* 10.1002/ejic.201800194

Link to VoR: http://dx.doi.org/10.1002/ejic.201800194

WILEY-VCH

Photoactivatable Ru(II) complex bearing 2,9-diphenyl-1,10-phenanthroline: unusual photochemistry and significant potency on cisplatin-resistant cell lines

Najwa Mansour,[a,b] Stephanie Mehanna,[a,b] Mohamad A. Mroueh,[c] Hassib Audi,[a] Kikki Bodman-Smith,[b] Costantine F. Daher,[a] Robin I. Taleb,[a] Mirvat El-Sibai,[a] and Rony S. Khnayzer[a,*]

[a] Department of Natural Sciences, Lebanese American University, Beirut 1102-2801, Lebanon.

[b] Faculty of Health and Medical Sciences, Department of Microbial and Cellular Sciences, University of Surrey.

[c] School of Pharmacy, Department of Pharmaceutical Sciences, Lebanese American University.

KEYWORDS cancer, photoactivatable chemotherapy, ruthenium polypyridyl complexes.

*Corresponding author: e-mail: rony.khnayzer@lau.edu.lb, URL: http://sas.lau.edu.lb/natural-sciences/people/rony-khnayzer.php

Abstract

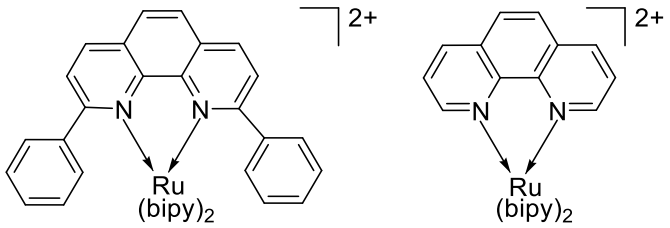
The current study investigates $[Ru(bipy)_2(dpphen)]Cl_2$ [where bipy= 2,2'-bipyridine and dpphen = 2,9-diphenyl-1,10-phenanthroline] (complex **1**) for photoactivatable chemotherapy (PACT) application on five cancer cell lines. $[Ru(bipy)_2(phen)]Cl_2$ [where phen = 1,10-phenanthroline] (complex **2**) was included as an unstrained control. Upon excitation with visible light, complex **2** proved to be photostable while complex **1** underwent a quantitative dissociation of the bipy ligand and formation of a Ru(II) polypyridyl aqua complex in water. Complex **1** demonstrated only marginal activity in the dark; its cytotoxicity increased significantly upon photoactivation with a high phototoxicity index (PI = [IC_{50} dark] / [IC_{50} light]) ranging from 39.2-fold in A549 to over 100-fold in MDA-MB-231. Complex **2**, on the other hand, did not show much difference in anticancer activity between dark and light conditions. Importantly, the IC_{50} of the photoproduct of complex **1** was several folds lower than that of cisplatin in all tested cell lines. Furthermore, the dissociating ligand (bipy) was biologically inert in almost all cell lines investigated confirming that phototoxicity was mediated primarily by the Ru aqua complex that is released upon irradiation. In conclusion, the Ru-centered complex **1** could represent a potential photoactivatable chemotherapeutic drug that increases selectivity to tumors and offers alternative treatment in the light of increasing cisplatin resistance.

Introduction

Platinum-based complexes have been extensively used as chemotherapeutic agents since the discovery of cisplatin, which was proved to treat a variety of cancers.[1] However, due to acquired resistance and dose-limiting side effects, other metals have been investigated.[2] Among these metals, Ru has gained interest due to the tunable physical and chemical properties of the associated complexes. Furthermore, variation in ligand framework allows Ru compounds to target specific organelles within cancer cells.[3] Moreover, the Ru(II) state was found to be the most active form in the hypoxic environment of tumors thus conferring selectivity to cancer cells.[4] The cellular uptake of some Ru complexes by transferrin receptors which are overexpressed in cancer cells further increases tumor selectivity and accordingly limits systemic toxicity.[5] Once inside the cell, Ru prodrugs can undergo light-mediated activation classified as photodynamic therapy (PDT) or photoactivated chemotherapy (PACT). The latter uses light to change the chemical and physical properties of the Ru complex by altering its electronic state thus generating a form of the molecule that mediates cytotoxicity through DNA binding, ligand ejection or caging mechanisms. Unlike PDT, PACT is not dependent on the presence of oxygen and thus offers a greater advantage in targeting cancer cells which are hypoxic.[6,7] Strained Ru complexes are particularly attractive as light-activated agents because they absorb in the visible range and are kinetically inert and photochemically labile. Although most Ru(II) polypyridyl complexes with pseudo-octahedral geometry are rather stable upon irradiation, those having a distorted octahedral geometry undergo a rapid deactivation of the excited state due to the decreased ligand-field splitting. This induces ligand dissociation resulting from the population of the ^{3}d-d state which is thermally accessed from the ^{3}MLCT excited state. There are many approaches for the use of Ru complexes in PACT depending on the photoactivation strategy adopted. Ru(II) polypyridyl complexes with distorted octahedral geometry can undergo ligand dissociation upon irradiation and possibly bind DNA in a similar fashion to cisplatin.[6,8] Using this approach, Glazer and coworkers prepared a series of Ru(II) complexes bearing methyl substituents near the coordination sphere.[8] $[Ru(bipy)_2(dmbipy)]^{2+}$ [where dmbipy = 6,6'-dimethyl-2,2'-bypiridyl] was tested on leukemia and lung cancer cell lines. As expected, photoejection of the dimethyl substituted ligands upon irradiation resulted in the formation $[Ru(bipy)_2(H_2O)_2]^{2+}$ and the free dmbipy photoproducts in aqueous solution. When added to pUC19 plasmid, the light-activated complex induced DNA photobinding as evidenced by delayed migration of the supercoiled DNA on the gel and decreased

EtBr intercalation. DNA damage occurred only upon photoactivation which was correlated with a decreased cell viability in leukemia and lung cancer cells, as compared to dark conditions.[8] Another methylated derivative, $[Ru(bipy)_2(dmdpq)]^{2+}$ [where dmdpq = 2,9'-dimethyl-dipirido[3,2-*f*:2',3'-*h*]-quinoxaline] formed the same aquated complex as above following the photoejection of the straining ligand. However, the photoactivated complex exhibited a dual effect on DNA, inducing both photobinding and photocleavage.[8] Later, the same group used a similar strategy to explore $[Ru(bipy)_2(dmdop)]^{2+}$ [where dmdop = 2,3-dihydro-1,4-dioxino [2,3-*f*]-2,9-dimethyl-1,10-phenanthroline] and $[Ru(dmphen)_2(dop)]^{2+}$ [where dmphen = 2,9-dimethyl-1,10-phenanthroline].[9] The latter exhibited photoinduced DNA damage via covalent binding whereas the former induced both single-stranded breaks (SSBs) and covalent binding to DNA. However, phototoxicity against leukemia cells did not linearly correlate with the degree of DNA damage.[9] Turro and coworkers investigated the photoinduced binding of *cis*-$[Ru(bipy)_2(NH_3)_2]^{2+}$ to DNA to mimic the mode of action of cisplatin. The resulting complex, *cis*-$[Ru(bipy)_2(H_2O)(OH)]^{+}$, was shown to bind covalently to 9-methyl and 9-ethyl guanine as well as to single-stranded and double-stranded DNA.[10] In another study, Turro and coworkers evaluated four $[Ru(bipy)_2(L)]^{2+}$ complexes where L corresponded to bisthioether (3,6-dithiaoctane or 1,2-bis(phenylthio)ethane) or nitrogen bidentate (ethylenediamine or 1,2-dianilinoethane) ligands. The bis-aqua complexes resulting from ligand exchange of bisthioether were shown to efficiently bind guanosine 5-monophosphate as well as linear double-stranded DNA.[11] Gasser and coworkers investigated $[Ru(II)(bipy)_2dppz]^{2+}$ derivatives [where dppz = dipyrido[3,2-a:2′,3′-c] phenazine]. When irradiated, all complexes produced O_2 as efficiently as photosensitizers available in the market and were shown to intercalate DNA. Complexes with the highest nuclear accumulation and intercalative potential induced efficient DNA photocleavage which reflected in higher phototoxicity in HeLa cells. [12] Other ligand frameworks were also tested and showed potential use for PACT with DNA being the main biological target. MacFarland and coworkers explored three strained Ru(II) metal-organic dyads bearing the crowded 6,6'-dmb ligand (6,6′-dimethyl-2,2′-bipyridine) and imidazo[4,5-*f*] [1,10]phenanthroline (IP) appended with n-thiophene units (nT; n = 1 to 3). The three complexes were shown to have a dual mode of action; as the number of thiophene units decreased, the dissociation rate of the 6,6'-dmb ligand increased which correlated with the formation of cisplatin-like DNA adducts, whereas the complex with the highest n number had the highest singlet oxygen yield which translated in more DNA single-stranded breaks. All

three complexes exhibited a significantly higher phototoxicity than cisplatin in tested cancer cell lines.[13] Bonnet and coworkers were the first to develop Ru(II) prodrugs with a *trans* configuration that allows photoactivation with green light. [Ru(biqbipy)(dmso)Cl]Cl [where biqbipy = 6,6′-bis[N-(isoquinolyl)1-amino]-2,2′-bipyridine)] and [Ru(biqbipy)(Amet)(HAmet)]PF_6 [where HAmet = N-acetyl-L-methionine, Amet = deprotonated N-acetyl-L-methionine] were shown to eject the *trans* ligands and become bis-aquated. The latter interacted more strongly with DNA than the prodrug, however, their confirmed apoptotic activity could not be conclusively attributed to this interaction.[14] Glazer's group investigated Ru(II) polypyridyl complexes with one or two bisquinoline ligands. Upon photoactivation with high wavelengths (700 or 800 nm), the resulting bis-aqua complexes were found to bind DNA and proved to be cytotoxic against leukemia cells.[15] While most studies focused on the role of Ru(II) aqua complexes in inducing cytotoxicity, we have investigated the possible contribution of the dissociating ligand to the biological activity of $[Ru(bipy)_2(dmphen)]Cl_2$ [where dmphen = 2,9-dimethyl-1,10-phenanthroline] on ML-2 Acute Myeloid Leukemia cell line. Results of cell viability assays showed that the toxicity of dmphen was significantly higher than the aqua complex formed and thus proved that the Ru(II) center can act as a cage for the cytotoxic ligand. These findings uncovered the important role of the coordinating ligands in the biological activity of strained Ru(II) polypyridyl complexes.[16] Similarly, another report by Bonnet and coworkers illustrated that the photoreleased 6,6'-dimethyl-2,2'-bipyridine (dmbipy) ligand is responsible for the phototoxicity of $[Ru(bipy)_2(dmbipy)]^{2+}$, whereas the Ru bis-aqua photoproduct was found to be biologically inert.[17] Turro and coworkers previously reported similar results on the pseudo-octahedral complex $[Ru(tpy)(5CNU)_3]^{2+}$ (3) [where tpy = 2,2′:6′,2″-terpyridine and 5CNU = 5-cyanouracil].[18] Upon excitation with visible light, the ligand 5CNU was ejected from the caging Ru(II) complex and the photoproduct exerted a dual cytotoxic activity; 5CNU induced cell death through the inhibition of pyrimidine catabolism whereas the Ru(II) center was capable of binding DNA[18]. In the present study, two Ru(II) polypyridyl complexes were investigated: $[Ru(bipy)_2(dpphen)]Cl_2$ (complex **1**) which is sterically strained by the phenyl groups on the 2,9-positions of dpphen, along with the unstrained control $[Ru(bipy)_2(phen)]Cl_2$ (complex **2**), Figure **1**.



1 **2**

Figure 1. Structures of complexes **1** and **2** where bipy = 2,2'-bipyridine.

The photochemistry of $[Ru(bipy)_2(dpphen)](PF_6)_2$ was previously studied by Sauvage and coworkers who reported the quantitative ejection of bipy in acetonitrile instead of the hindering dpphen ligand thus generating the photoproduct $[Ru(bipy)(dpphen)(CH_3CN)_2]^{2+}$.[19] Unlike phenanthroline derevatives, bipy was shown to be inactive in cancer cells since it only forms electrostatic interactions with DNA and is unable to intercalate.[20] In the present work, the photochemistry of $[Ru(bipy)_2(dpphen)](PF_6)_2$ was also re-evaluated in acetonitrile for comparison with reported results and the chloride salt of complex **1** was explored as a potential photochemotherapeutic candidate. The photochemistry of the chloride complex was examined in water to determine if bipy photorelease would occur and the phototoxicity of the new product was evaluated on a panel of cancer cell lines to probe the PACT of complex **1**.

Results

In all photochemical and photobiological experiments, the light output from a home-built blue LED setup [21] (460 nm, ~100 mW/cm^2) was used to provide the excitation source. This light source provides a direct metal-to-ligand charge transfer (MLCT) excitation. The extinction coefficient of the MLCT transition for complex **1** was 14,000 ± 1,000 $M^{-1}cm^{-1}$ at 448 nm in both acetonitrile ($[Ru(bipy)_2(dpphen)](PF_6)_2$) and water ($[Ru(bipy)_2(dpphen)]Cl_2$), Figure **2**. The peak at 285 nm can be assigned to intra-ligand $\pi \rightarrow \pi^*$ transition.

10.1002/ejic.201800194

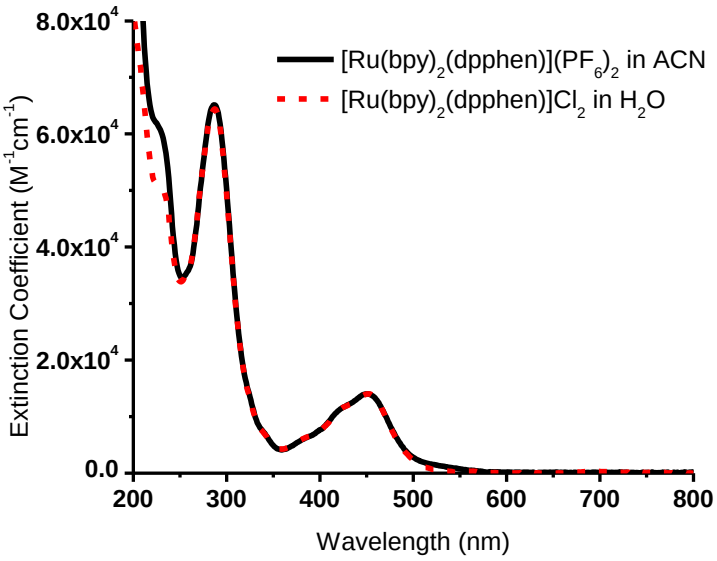


Figure 2. UV-vis spectra of complex **1** as hexafluorophosphate and chloride salts in acetonitrile and water, respectively.

To determine the chemical composition of the photochemical products, ^{1}H NMR was performed on $[Ru(bipy)_2(dpphen)](PF_6)_2$ **(**before and after photoactivation) along with the free ligands dpphen and bipy for comparison. As shown in Figure 3, the ^{1}H NMR spectrum in CD_3CN of the sample after irradiation showed a quantitative conversion of the $[Ru(bipy)_2(dpphen)](PF_6)_2$ into photoproducts. The resulting solution contained peaks assigned to the free bipy (labeled with circles) while dpphen could not be detected among the photolysis products. A broadening of the proton signals assigned to the hydrogen atoms at the ortho and meta positions of the phenyl groups of $[Ru(bipy)_2(dpphen)](PF_6)_2$ was observed before photoactivation and disappeared in the photoproducts.

10.1002/ejic.201800194

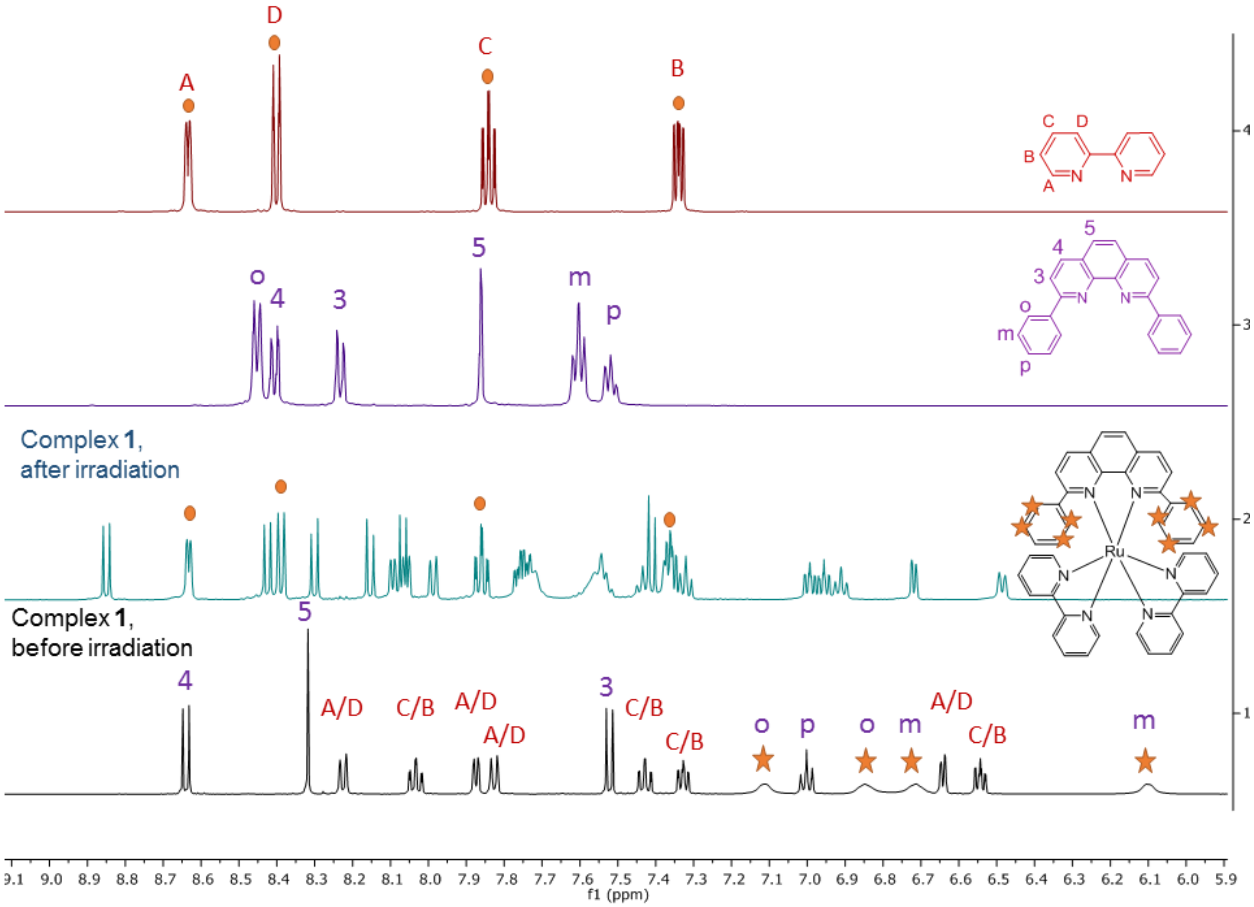


Figure 3. ^{1}H NMR spectra (500 MHz) in CD_3CN of complex **1** $[Ru(bipy)_2(dpphen)](PF_6)_2$ before photolysis (black), after 6 h of photolysis (green) as well as the free ligands 2,9-diphenyl-1,10-phenanthroline (dpphen) (purple) and 2,2'-bipyridine (bipy) (light blue). The full spectra are found in Figure S9. Peaks marked with a circle are for the bipy free ligand, the ones marked with stars are assigned to the ortho and meta hydrogens of the phenyl groups in complex **1**. The remaining assigned peaks were done based on the expected chemical shift of the bipy (red) and dpphen (violet) ligands. A or D peaks and C or B peaks could not be distinguished in complex **1**.

The photochemistry of $[Ru(bipy)_2(dpphen)]Cl_2$ in water was confirmed by LC/MS. While only the peak assigned to $[Ru(bipy)_2(dpphen)]^{2+}$ appeared at m/z = 373.20 $[M\text{-}2Cl]^{2+}$ (Figure **4** left) before irradiation, bipy was identified at m/z = 157.13 $[M+H]^{+}$ after irradiation along with the aquated form of the photoproduct, $[Ru(bipy)(dpphen)(H_2O)]^{2+}$ assigned at m/z = 303.60 $[M\text{-}2Cl]^{2+}$, Figure **4**. Dpphen, which produced a signal at m/z = 333.33 $[M+H]^{+}$ (Figure S10) was absent in the photoproduct in water. LC/MS spectra of complex **2** before and after irradiation presented a peak

at m/z = 297.07 $[M-2Cl]^{2+}$ (Figure S11), and therefore demonstrate that complex **2** did not undergo any detectable ligand dissociation upon blue light irradiation.

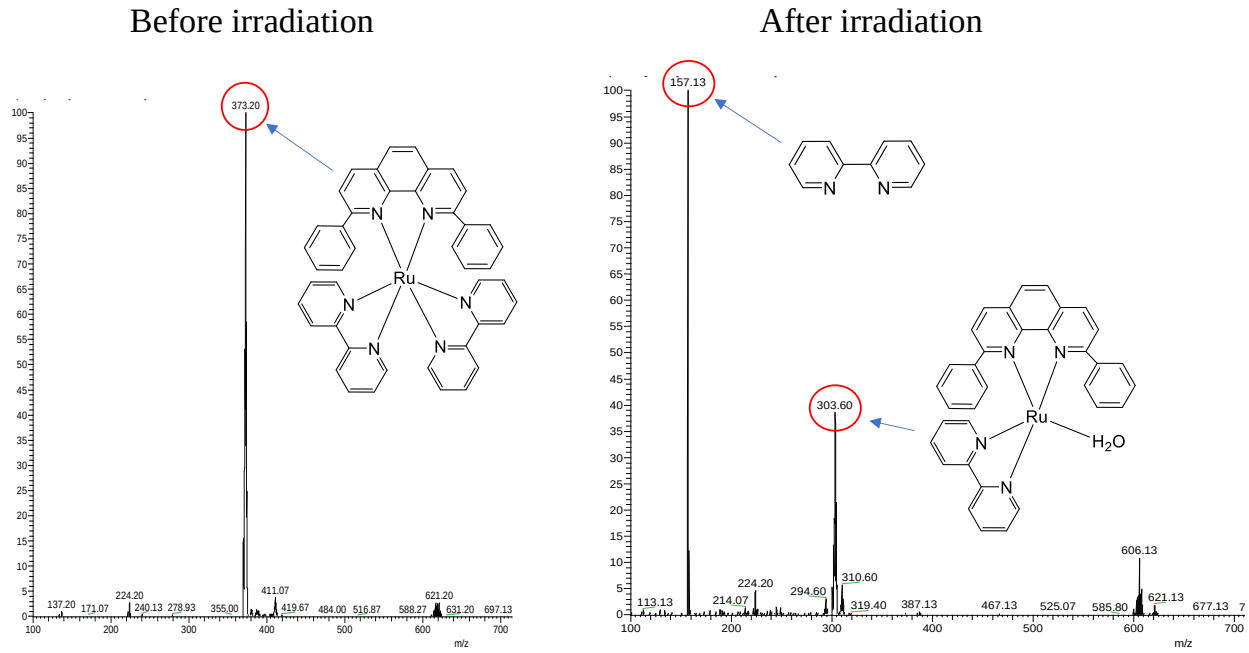


Figure 4. ESI-MS spectra of $[Ru(bipy)_2(dpphen)]Cl_2$ (15 µM) in water before (left) and after (right) photolysis with blue light for 30 min at an output of 100 mW/cm^2 . The peak at m/z = 606.1 depicts the singly charged $[Ru(bipy)(dpphen)(OH)]^+$ and at 303.6 for $[Ru(bipy)(dpphen)(H_2O)]^{2+}$.

The UV-vis spectra of $[Ru(bipy)_2(dpphen)]Cl_2$ (45 µM) were measured as a function of irradiation time (460 nm, 100 mW/cm^2), Figure S12. The half-life ($t_{1/2}$) in water was found to be 4.3 min whereas it was 2.2 min in acetonitrile. As expected, $t_{1/2}$ in water was larger than in acetonitrile since the latter provides enhanced solvation of the photoproducts.[16] However, it is important to note that the rate of photoejection is dependent on the concentration and volume of the solution as well as the incident power density. In the NMR experiment, the concentration was >100-folds larger than the ESI-MS, hence the time required to complete photolysis was 6 h in Figure **3** and 30 min in Figure **4** even though photolysis under identical concentration is faster in acetonitrile than water.

The quantification of cellular uptake of complex **1** was performed using ICP-MS. The optimum uptake time was used to set the incubation period prior to irradiation in order to maximize cellular concentration of prodrug. While at t = 0 h no Ru could be detected (C < 2 ng/10^6 cells), the uptake started within the first hour (2.73 ng/10^6 cells) then increased until it reached a maximum between

6 and 24 h of approximately 11 ng/10^6 cells, Figure **5**. WST-1 kit was used to assess the anticancer activity of complexes **1** and **2** and controls on five cell lines: murine melanoma B16-F10, human alveolar adenocarcinoma A549, triple negative human breast adenocarcinoma MDA-MB-231, human colorectal adenocarcinoma HT29 and human colon adenocarcinoma Caco-2. The cell survival assay of complex **1** was performed to assess its biological activity, before and after photoactivation. Cisplatin was used as a positive control and complex **2** as an unstrained control. While complex **2** showed no significant activity in both dark and light conditions (IC_{50} > 100 µM) (Figure **6** and Table **1**), complex **1** exhibited different cytotoxicity profile. In the dark, its IC_{50} values ranged from 55.1 µM in B16-F10 to higher than 100 µM in the other cells lines. But, upon irradiation, the cytoxicity of complex **1** increased dramatically with IC_{50} values ranging from 0.82 µM in B16-F10 cells to a maximum of 2.55 µM in A549 cells. Calculation of the phototoxicity index (PI = [IC_{50} dark] / [IC_{50} light]) showed PI values ranging from 39.2-fold in A549 to greater than 100-fold in MDA-MB-231 (Table **1**). According to Table **1,** the IC_{50} values of cisplatin ranged between 7.70 µM in B16-F10 cells and greater than 10 µM in HT-29 cells. Also, the dissociating ligand bipy was found to be biologically inert on all cell lines (IC_{50} over 100 µM) except on MDA-MB-231 where an IC_{50} of 37.5 µM was recorded (Figure **6** and Table **1**).

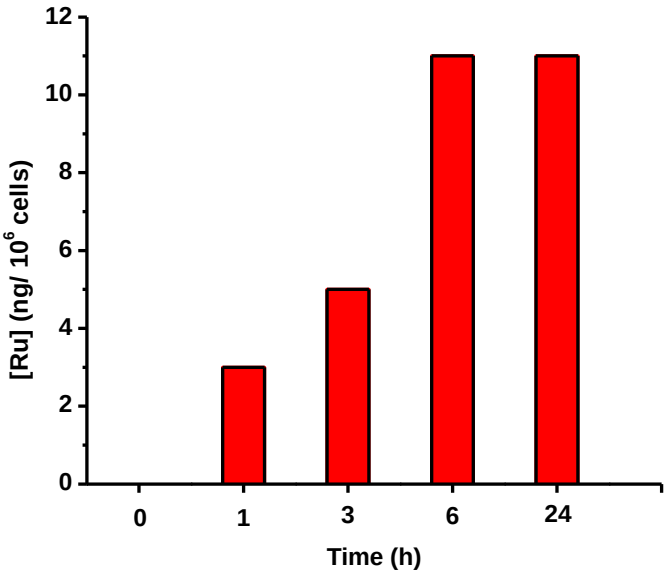


Figure 5. ICP-MS analysis using the EPA 200-7/8 M method for compound **1** uptake by A549 cells at different time points. Results are expressed in ng/10^6 cells.

10.1002/ejic.201800194

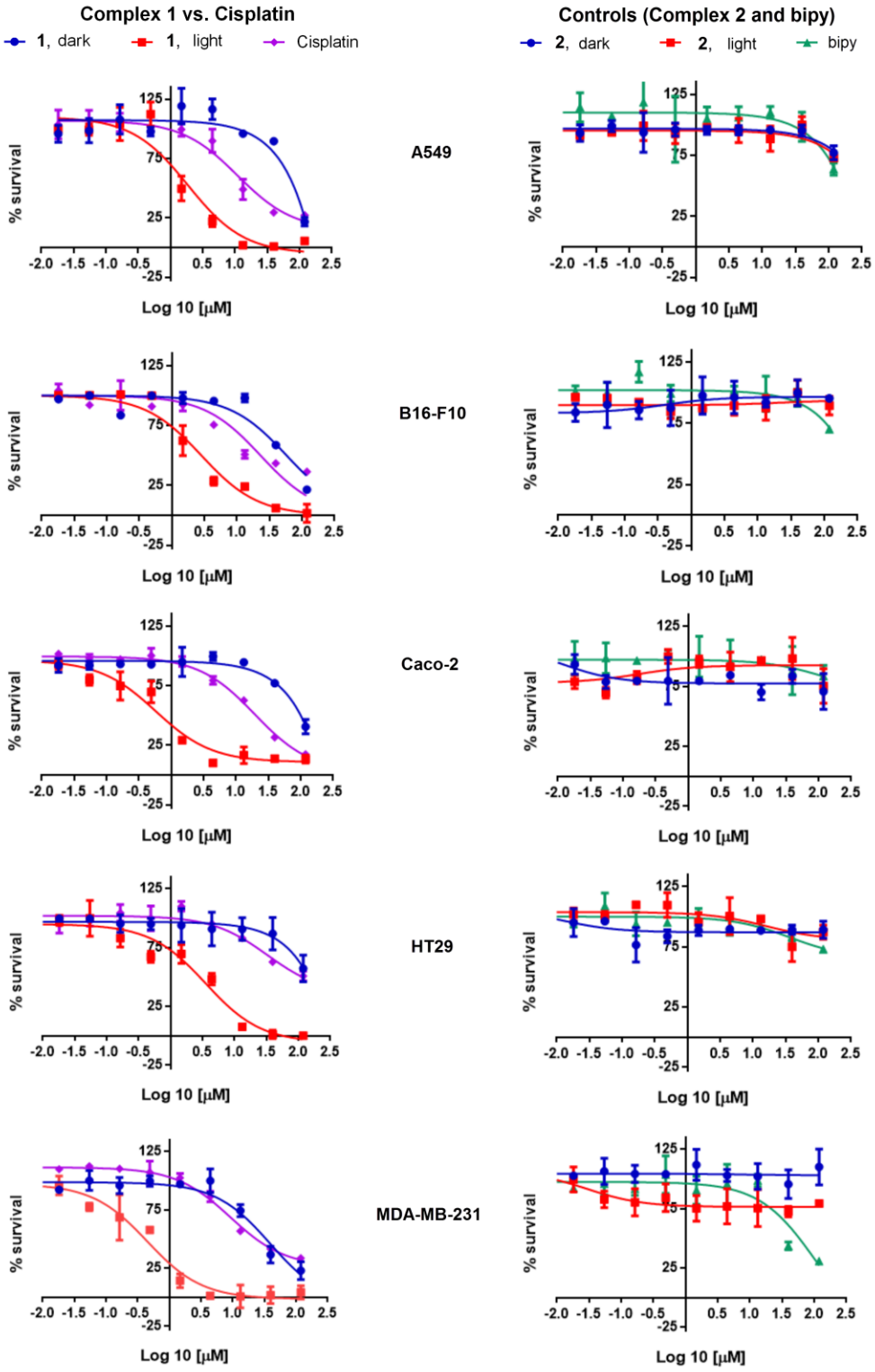


Figure 6. Cytotoxic effect of complexes **1**, **2**, cisplatin and bipy on A549, B16-F10, Caco-2, HT29 and MDA-MB-231. Cells were treated with 3-fold dilutions of all agents starting at 120 µM. Complexes **1** and **2** were irradiated with blue LED at a power of 100 mW/cm^2. The effect of bipy and cisplatin was only evaluated in the dark. IC_{50} values are reported in Table **1**.

Table 1. IC_{50} values in dark/light and phototoxicity indices (PI) of complexes **1** and **2** on various cell lines. Data on the free bipy ligand and cisplatin are reported only in the dark. Numbers are averages of three separate experiments and each experiment included samples in triplicates. Numbers are expressed as mean ± SEM.

	IC_{50}			
Cell Line	**Compound 1**	**Compound 2**	**bipy free ligand**	**Cisplatin**
A549	Dark: >100[a] Light: 2.55[b] ± 1.74 PI: >39.2	Dark: >100[a] Light: >100[a] PI: ---	>100[a]	7.74[b] ± 1.00
B16	Dark: 55.1[a] ± 5.84 Light: 0.82[b] ± 0.83 PI: >68.8	Dark: >100[c] Light: >100[c] PI: ---	>100[c]	7.70[d] ± 0.19
Caco-2	Dark: >100[a] Light: 1.48[b] ± 0.22 PI: >67.6	Dark: >100[a] Light: >100[a] PI: ---	>100[a]	21.8[c] ± 0.53
HT-29	Dark: >100[a] Light: 1.98[b] ± 2.35 PI: >50.5	Dark: >100[a] Light: >100[a] PI: ---	>100[a]	>100[a]
MDA-MB-231	Dark: >100[a] Light: 1.00[b] ± 0.78 PI: >100	Dark: >100[a] Light: >100[a] PI: ---	37.5[c]± 0.14	30.9[c] ± 0.82

[a, b, c, or d] In each row, values that are not sharing the same letter are significantly different ($p < 0.05$).

Discussion

The use of prodrugs in anticancer therapy has recently become a very attractive approach mainly because of its potential selectivity to tumors which could reduce systemic toxicity and acquired resistance induced by conventional chemotherapy.[6] Ru(II) polypyridyl complexes having a distorted octahedral geometry have gained interest as prodrugs due to their chemical stability in the dark and their ability to undergo ligand dissociation upon visible light excitation.[8] In the present study, we have exploited these features to synthesize the complex $[Ru(bipy)_2(dpphen)]Cl_2$ (complex **1**) and explore its cytotoxic potential *in vitro* upon photoactivation. The complex is sterically strained by virtue of introducing a phenyl group on the 2,9-positions of the phenanthroline group. It is intuitive to think that the sterically hindering ligand (dpphen) is expected to dissociate from the complex upon photoactivation, however results showed that the ligand bipy was ejected when the complex was irradiated in acetonitrile thus generating the photoproduct $[Ru(bipy)(dpphen)(CH_3CN)_2]^{2+}$. Such behavior could be related to the distorted

geometry of the complex, an effect that might be attributed to the twisted bipy moieties or prolonged Ru-N(bipy) distances. Therefore, the ejection of bipy would be easier since the rotation of the pyridine ring about the C^2-$C^{2'}$ bond becomes possible after the first Ru-N bond is broken.[19] This is not the case with the dpphen whose plane is resistant to distortion, thus rendering the ligand less labile. This unexpected ligand substitution is perhaps due to the asymmetrical distortion of the complex.[19] To confirm these results, we have conducted ^{1}H NMR and ESI-MS studies on the photochemistry of the complex in deuterated acetonitrile and water respectively. The MLCT absorption of complex **1** was measured by UV/vis spectrophotometry and recorded at around 448 nm (blue range). This wavelength was the basis for choosing blue LED (460 nm) to induce photolysis for characterization and biological studies. When performed on the irradiated complex, ^{1}H NMR suggested that only bipy was photolabilized in CD_3CN which is consistent with Sauvage's results.[19] At room temperature, broadening of the proton signals was observed for hydrogen atoms assigned to the ortho and meta positions of the phenyl groups of $[Ru(bipy)_2(dpphen)](PF_6)_2$ before photoactivation. This ^{1}H NMR broadening[22] could be attributed to the intramolecular π-π stacking[23] of the phenyl groups at 2,9 positions of the 1,10-phenanthroline and the pyridine ring of the ancilliary ligands. In this perspective, the lack of dissociation of the dpphen ligand could be due to two main factor: (1) the rigidity of the 1,10-phenanthroline ligand as compared to 2,2'-bipyridine which possesses a free rotation around C^2-$C^{2'}$ (2) possible intramolecular π-π stacking of the phenyl groups which likely increases the activation energy for dissociation of this ligand. LC-MS analysis confirmed that $[Ru(bipy)_2(dpphen)]Cl_2$ has a similar photochemical behavior in water with a quantitative release of bipy upon irradiation. Similarly to cisplatin, complex **1** is expected to undergo aquation and this was also verified by LC-MS which identified another photolysis product $[Ru(bipy)(dpphen)(H_2O)]^{2+}$. However, the electrospray ionization used by the LC-MS may affect the aquation process as exemplified by the detection of ligand deficient $[Ru(bipy)_2]^{2+}$, $[Ru(bipy)_2(H_2O)_2]^{2+}$, and $[Ru(bipy)_2(H_2O)(OH)]^{+}$ for similar photoproducts in water.[8,10,17] Therefore, the results obtained above do not necessarily translate the actual number of aqua ligands bound to the Ru center in solution. Moreover, the complex was highly stable in the solid state as well as in water solution under dark conditions, as verified by unchanged ^{1}H NMR and LC-MS spectra respectively after months of storage. This constitutes a practical convenience since when protected from light, the complex can be stored for a long period with no signs of degradation.

Unlike complex **1**, complex **2** did not undergo photolysis and was found to be photostable as confirmed by ^{1}H NMR and LC-MS data (Figure S11) which were identical before and after irradiation of the complex. Complex **2,** with distorted octahedral geometry, therefore exhibits completely different photochemical properties as compared to complex **1**.

The main purpose of photochemotherapy is to mediate phototoxicity in cancer cells while minimizing damage to healthy tissues that are not irradiated.[24] To be considered for photochemotherapeutic applications, complex **1** should be primarily active upon photolysis and thus exhibit a large difference in cytotoxicity between dark and light conditions, as measured by the phototoxicity index (PI). Cellular uptake of complex **1** was maximized to about 11 ng/10^6 cells after 6-24 h of incubation, which compares well with other Ru anticancer drug uptake.[25] PI values measured by cell survival assays were consistently high among all cell lines tested (they ranged from >39-fold in A549 to >100-fold in MDA-MB-231). This was the result of high difference in toxicity between dark and light conditions; while complex **1** showed little effect in the dark (with a minimum IC_{50} value of 55 μM in murine melanoma cells, B16-F10), cytotoxicity increased considerably upon irradiation (IC_{50} between 0.83 μM in B16-F10 cells to 2.6 μM in A549 cells). As compared to cisplatin, the photoactivated form of complex **1** induced higher toxicity to all cell lines tested and the difference was mostly noticeable in human colorectal adenocarcinoma cells (HT-29) which were found to be resistant to cisplatin (IC_{50} > 100 μM). This finding is consistent with previous studies that confirmed the refractory response of colon cancer cells to cisplatin due to their ability to induce DNA repair. [26] The free bipy ligand was included in cell survival assays to test whether the dissociating ligands contribute to phototoxicity of complex **1**. Results showed that the bipy ligand was not potent (IC_{50} >100 μM) in all cell lines tested except in MDA-MB-231 where IC_{50} was still high (37.5 μM). These results were comparable to the data obtained by our group on ML-2 Acute Myeloid Leukemia cell line[16] and in line with previous results that showed moderate to no potency of bipy on K562, MDA-MB-231 and MCF-7 cells.[27,28] This favors the hypothesis that phototoxicity of complex **1** is predominantly mediated by the Ru-aqua photoproduct rather than the liberated ligand. According to Lincoln and Nordén, dark toxicity can be reduced by altering the structure of the Ru complex in a way to avoid DNA binding or intercalation. This can be achieved by replacing phenanthroline ligands by bipyridine.[29] Our results suggest that complex **1** and complex **2** had reduced DNA interaction *in vitro* which significantly reduced their dark toxicity. However, upon irradiation, bipy was photoejected from complex **1**

leading to product $[Ru(bipy)(dpphen)(H_2O)]^{2+}$ as a cytotoxic agent. Complex **2**, on the other side proved to be inactive in both dark and light conditions on all cell lines tested (IC_{50} > 100 μM) which is in accordance with the results found by Glazer and coworkers.[8] pUC8 plasmid gel electrophoresis experiments[30] using Ru(II) polypyridine complexes have previously demonstrated potential DNA single and double-stranded breaks[11]. However, these findings do not necessary correlate with the mechanism of compound **1** in living cells. A thorough study is currently underway to elucidate the *in vitro* mechanistic details of this compound.

Conclusions

Ru(II) polypyridyl complexes with distorted octahedral geometry are potential candidates for photochemotherapy due to their tunable photochemical properties. $[Ru(bipy)_2(dpphen)]Cl_2$ (complex **1**) was able to mediate cytotoxicity selectively upon photoejection of the inert bipy ligand and release of a highly toxic Ru(II) aqua complex. $[Ru(bipy)_2(phen)]Cl_2$ (complex **2**) control was shown to be photostable and biologically inert in the dark and upon photoexcitation. Complex **1** exhibited superior cytotoxicity and selectivity upon photoexcitation when compared with cisplatin, and may be considered for potential application in PACT. Further *in vitro* and *in vivo* investigations are underway to elucidate the biological mechanism of action.

Experimental Section

Instrumentation

Elemental analysis was provided by Atlantic Microlab and HR-ESI MS data were acquired at Michigan State University Mass Spectrometry Core Facility. MALDI-TOF MS spectra were collected on a 4800 MALDI TOF/TOF MS/MS Analyzer instrument (Applied Biosystems) operated by a 4000 Series Explorer software. Briefly, aqueous solutions of Ru(II) complexes (120 μM) were premixed with CHCA matrix (saturated α-cyano-4-hydroxy-cinnamic acid in a mixture containing 1:1 of 50% acetonitrile and 0.1% TFA. The resulting samples were then spotted on a stainless-steel plate (Opti-TOF TM 384 Well Insert), dried by vacuum and inserted for data acquisition. 13 calibration points were included on each plate (4700 Proteomics Analyzer Calibration mixture). UV/vis absorption was measured by a Cary 60 spectrophotometer from Agilent Technologies). NMR spectra were acquired on an AC500 Bruker spectrometer (^{1}H NMR at 500 MHz). Chemical shifts were recorded in delta (δ) units and expressed as ppm values

relatively to the internal standard TMS. LC-MS/MS data were recorded by Ultimate 3000 RSLC/TSQ Endura system (ThermoScientific). Briefly, aqueous solutions of complexes **1** and **2** (20 µM) were prepared and infused by direct injection into the heated ESI probe [(2 µL injection at a flow rate of 100 µL/min of LC-MS grade water containing 0.05% formic acid, sheath gas (2.71 L/min), auxilliary gas (5.78 L/min), sweep gas (1.5 L/min), ion transfer tube temperature (325 °C), vaporizer temperature (200 °C), positive ion spray voltage (3700 V)]. ICP-MS was conducted at the American University of Beirut (environment core lab) on Agilent 7500 ICP-MS at 0.3 seconds (integration time/mass) with 3 repetitions and an uptake speed of 1 rotation per second. Blue LED was obtained from LED Engin (LZ4-40B208-000) and operated at 50% of its full power (130 lumen). The lamp was regulated by an LED dimmer (home-built) using a Philips Xitanium LED driver LEDINTA0700C210FO and light was condensed using a focusing lens with narrow beam output (LLNS-1T06-H). Star LED heat sink was used to cool the system.

Materials

Cis-bis(2,2′-bipyridine)dichlororuthenium(II) hydrate ($Ru(bipy)_2Cl_2 \cdot 2H_2O$), 1,10-phenanthroline, silica gel, sephadex LH 20, Dowex 22 chloride, and all other chemicals and solvents were purchased from Aldrich and used without any further purification. LC-MS grade water and formic acid were from Fisher Chemical. DMEM medium, FBS and penicillin G sodium salt were purchased from Aldrich and WST-1 reagent (tetrazolium salt) was obtained from Roche©. All cell lines used were from the American Type Culture Collection (ATCC).

Synthesis of $[Ru(bipy)_2(dpphen)](PF_6)_2$ (Complex 1)

2,9-diphenyl-1,10-phenanthroline was synthesized according to a published procedure.[31] Synthesis of the complex was performed according to a modified procedure.[19] $Ru(bipy)_2Cl_2 \cdot 2H_2O$ (100 mg, 0.2 mmol) and 2,9-dpphen (69 mg, 0.19 mmol) were mixed with ethylene glycol (8 mL) in a pressure vessel. The solution was degassed for 1 h under argon pressure then refluxed for six h. After cooling at room temperature, filtration was carried out using micropores (0.45 µm). The product was precipitated by adding a saturated solution of KPF_6.[2] The precipitate was filtered then purified by column chromatography (silica gel, 92% acetonitrile, 7% H_2O, 1% KNO_3). Yield: 153.5 mg, 72%. ^1H NMR (CD_3CN, 500 MHz): δ = 8.66 (d, *J* = 8 Hz, 2H), 8.34 (s, 1H), 8.24 (d, *J* = 8 Hz, 2H), 8.06 (td, *J* = 7.73, 1.47 Hz, 2H), 7.89 (d, *J* = 6.22 Hz, 2H), 7.84 (d, *J* = 7.98 Hz, 2H),

7.54 (d, *J* = 8.29 Hz, 2H), 7.45 (td, *J* = 7.71 Hz, 1.46 Hz, 2H), 7.35 (dq, *J* = 6.02 Hz, 1.3 Hz, 2H), 7.14 (br. s, 2H), 7.02 (t, *J* = 7.56 Hz, 2 H), 6.87 (br. s, 2H), 6.74 (br. s, 2H), 6.66 (d, *J* = 4.93 Hz, 2 H), 6.56 (td, *J* = 6.09, 1.3 Hz, 2H), 6.13 (br. s, 2 H). ^{13}C NMR (CD_3CN, 500 MHz): δ = 168.91, 158.99, 157.05, 154.02, 151.96, 149.55, 139.96, 138.65, 138.16, 137.06, 131.48, 129.96, 129.63, 129.02, 127.60, 127.57, 125.33, 124.14. Elemental Anal. Calcd for $C_{44}H_{32}F_{12}N_6P_2Ru.H_2O$: C, 50.15; H, 3.25; N, 7.98. Found: C, 50.32; H, 3.17; N, 7.98. HRMS (ESI/QTOF) m/z $[M]^{2+}$ Calcd for $C_{44}H_{30}N_6Ru$ 373.0872; found 373.0903. MS (MALDI/TOF) m/z, $[M]^+$ 746.15. UV/Vis (ACN): λ_{max}(ε $M^{-1}cm^{-1}$) 448 nm (14,000). For biological testing the hexafluorophosphate salt was converted to chloride salt using Dowex chloride ion exchange resins to promote the solubility of the complex in water.

Synthesis of $[Ru(bipy)_2(phen)]Cl_2$ (Complex 2)

Synthesis was performed according to a modified procedure.[32] $Ru(bipy)_2Cl_2 \cdot 2H_2O$ (100 mg, 0.20 mmol) and 1,10-phenanthroline (39.64 mg, 0.22 mmol) were added to a solution of 1:1 EthOH:H_2O (8 mL) in a round bottom flask. The mixture was stirred under argon for 1 h then refluxed for three h. After cooling at room temperature, solvents were evaporated, and the residue was dissolved in methanol (2 mL) and added dropwise to rapidly stirred diethyl ether (300 mL) producing an orange precipitate that was collected by vacuum filtration and washed thoroughly with ether. The compound was then purified on a sephadex LH-20 column with methanol as eluent and finally dried under reduced pressure. Yield: 121.4 mg; 91 %. ^{1}H NMR (CD_3CN, 500 MHz): δ = 8.63 (dd, *J* = 8.27 Hz, 1.26 Hz, 2H), 8.60 (d, *J* = 7.99 Hz, 2H), 8.55 (d, *J* = 8.11 Hz, 2H), 8.26 (s, 2H), 8.10 (m, 4H), 7.99 (td, *J* = 7.63 Hz, 1.49 Hz, 2H), 7.85 (d, *J* = 6.4 Hz, 2H), 7.74 (dd, *J* = 8.26 Hz, 5.25 Hz, 2H), 7.53 (d, *J* = 5.64 Hz, 2H), 7.45 (dq, *J* = 6.31 Hz, 1.33 Hz, 2H), 7.22 (dq, *J* = 6.31 Hz, 1.33 Hz, 2H). ^{13}C NMR (CD_3CN, 500 MHz): δ = 158.26, 157.99, 153.45, 152.96, 152.81, 148.49, 138.79, 138.66, 137.80, 132.00, 129.05, 128.47, 128.35, 127.03, 125.30, 125.23. Elemental Anal. Calcd for $C_{32}H_{24}Cl_2N_6Ru.3H_2O$: C, 53.49; H, 4.21; N, 11.7. Found: C, 53.25; H, 4.53; N, 11.42. HRMS (ESI/QTOF) m/z $[M]^{2+}$ Calcd for $C_{32}H_{22}N_6Ru$ 297.0557; found 297.0568. MS (MALDI/TOF) m/z, $[M]^+$ 594.07. UV/Vis (H_2O): λ_{max}(ε $M^{-1}cm^{-1}$) 448 nm (16,000).[20]

^{1}H NMR and LC-MS/MS of complexes 1 and 2 to evaluate their photochemistry

The photochemistry of complex **1** (PF_6 salt) was determined by ^{1}H NMR in deuterated acetonitrile (CD_3CN), after which the complex was converted to a chloride salt and subjected an LCMS analysis in order to determine whether the complex exhibits the same photochemical transformation in water. Complex **2** was evaluated by LC-MS and all measurements were performed before and after irradiation. For ^{1}H NMR studies, samples were irradiated for 6 h as opposed to 35 min for LC-MS (at an output of 100 mW/cm^2) and the ligands dpphen and bipy were included as controls to allow their detection in photolysis products.

Determination of the rate of ligand dissociation of complex 1 by UV-vis spectroscopy

The chloride form of complex **1,** $[Ru(bipy)_2(dpphen)]Cl_2$ was irradiated with blue LED light (460 nm, 100 mW/cm^2) in water and acetonitrile for different durations. UV-vis absorption spectra were then acquired to determine the half-life of the complex under identical conditions.

Quantification of cellular uptake by ICP-MS

Quantification of cellular uptake was carried out by ICP-MS following a modified procedure.[33] Briefly, 1.0 x 10^6 cells (A549) were plated in 60 mm petri dishes and allowed to adhere for 12 h at 37° with 5% CO_2. Complexes **1** (30 µM) were then added and the plates were incubated for 1, 3, 6, 12 and 24 h. Cells were then harvested by scraping, precipitated by centrifugation at 800 RCF for 20 minutes at 4° and washed 3 times with PBS. The pellets were resuspended in milli-Q water, transferred to a glass vial containing 65% HNO_3 and completely digested at 120 °. The product obtained was then dissolved in 1 mL milli-Q water containing 2% HNO_3. Quantification of Ru was performed using Agilent 7500 inductively coupled plasma mass spectrometry (ICP-MS): EPA 200-7/8 M method and data were analyzed using ICP-MS Mass Hunter B.01.01 software.

Cell survival assay

Murine melanoma cell line B16-F10, human alveolar adenocarcinoma cell line A549, triple negative human breast adenocarcinoma cell line MDA-MB-231, human colorectal adenocarcinoma cell line HT29 and human colon adenocarcinoma Caco-2 cell line were maintained in DMEM containing 10% FBS and 1.5% pen-strep at 37° with 5% CO_2. Cells were seeded in a 96 well-plate at a concentration of 10^4 cells/well and allowed to adhere for 6 h at 37° with 5% CO_2. Treatment with serial dilutions of complexes **1** and **2**, cisplatin and the ligands

dpphen and bipy was performed, followed by a 12 h incubation period. Cells were then irradiated with blue LED purchased from LED Engin possessing 460 nm peak wavelength and operated at 50% of its 130 lumen full power which was controlled by a home-built LED dimmer (Light output ~ 100 mW/cm^2). The light was focused on the plate using a focusing lens with narrow beam output. Irradiation was typically done for 35 minutes for "light conditions" or else the plates were left in the dark for all other experiments. Cell proliferation was assessed after 72 h using WST-1 reagent (Roche©) according to the manufacturer's instructions and data were analyzed using GraphPad Prism 6 software (GraphPad Software). Cell viability was expressed as percent survival relatively to the negative control (non-treated cells). IC_{50} was calculated from 3 separate experiments using a non-linear regression (curve fit) on GraphPad Prism 6 software (GraphPad Software) and expressed as mean ± SEM, calculated using Microsoft Excel (version 1705, 2016). Two-way analysis of variance (ANOVA) was used to determine statistical significance (GraphPad) and differences between all groups were considered significant if $p < 0.05$ (Computed by Tukey's multiple comparisons test).

Acknowledgements

We would like to acknowledge financial support from the School Research and Development Council at the Lebanese American University and the Lebanese National Council for Scientific Research (Ref: 05-06-14).

References

(1) Arora, R.; Thakur, J. S.; Azad, R. K.; Mohindroo, N. K.; Sharma, D. R.; Seam, R. K. Cisplatin-Based Chemotherapy: Add High-Frequency Audiometry in the Regimen. *Indian J. Cancer* **2009**, *46* (4), 311–317.

(2) Cepeda, V.; Fuertes, M. A.; Castilla, J.; Alonso, C.; Quevedo, C.; Pérez, J. M. Biochemical Mechanisms of Cisplatin Cytotoxicity. *Anticancer Agents Med. Chem.* **2007**, *7* (1), 3–18.

(3) Bergamo, A.; Gaiddon, C.; Schellens, J. H. M.; Beijnen, J. H.; Sava, G. Approaching Tumour Therapy Beyond Platinum Drugs: Status of the Art and Perspectives of Ruthenium Drug Candidates. *J. Inorg. Biochem.* **2012**, *106* (1), 90–99.

(4) Schluga, P.; Hartinger, C. G.; Egger, A.; Reisner, E.; Galanski, M.; Jakupec, M. A.; Keppler, B. K. Redox Behavior of Tumor-Inhibiting ruthenium(III) Complexes and Effects of Physiological Reductants on Their Binding to GMP. *Dalton Trans. Camb. Engl. 2003* **2006**, No. 14, 1796–1802.

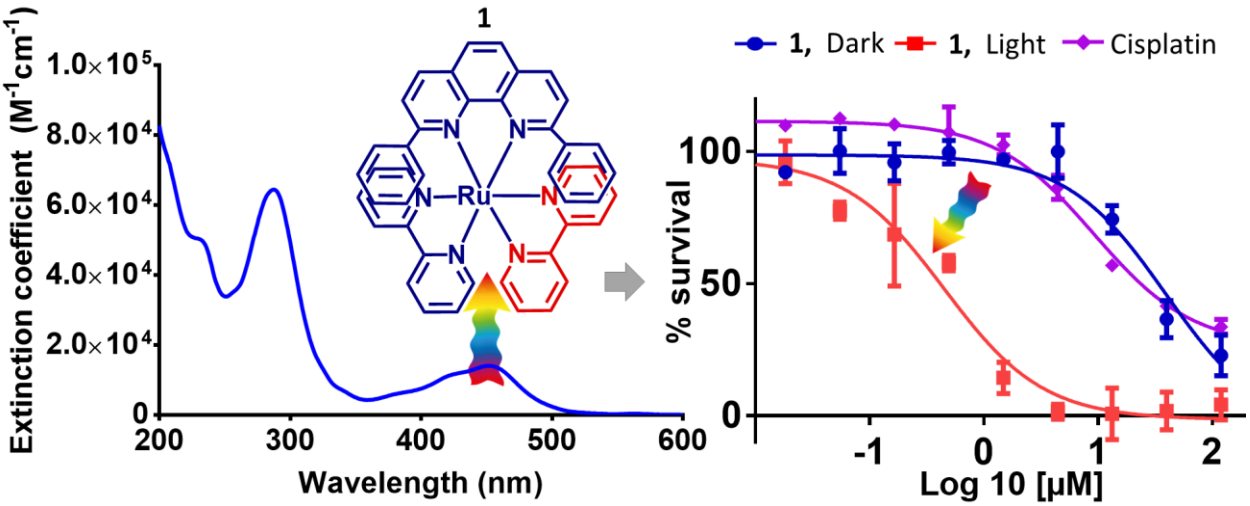
(5) Guo, W.; Zheng, W.; Luo, Q.; Li, X.; Zhao, Y.; Xiong, S.; Wang, F. Transferrin Serves As a Mediator to Deliver Organometallic Ruthenium(II) Anticancer Complexes into Cells. *Inorg. Chem.* **2013**, *52* (9), 5328–5338.

(6) Mari, C.; Pierroz, V.; Ferrari, S.; Gasser, G. Combination of Ru(II) Complexes and Light: New Frontiers in Cancer Therapy. *Chem. Sci.* **2015**, *6* (5), 2660–2686.

(7) Schatzschneider, U. Photoactivated Biological Activity of Transition-Metal Complexes. *Eur. J. Inorg. Chem.* **2010**, *2010* (10), 1451–1467.
(8) Howerton, B. S.; Heidary, D. K.; Glazer, E. C. Strained Ruthenium Complexes Are Potent Light-Activated Anticancer Agents. *J. Am. Chem. Soc.* **2012**, *134* (20), 8324–8327.
(9) Hidayatullah, A. N.; Wachter, E.; Heidary, D. K.; Parkin, S.; Glazer, E. C. Photoactive Ru(II) Complexes With Dioxinophenanthroline Ligands Are Potent Cytotoxic Agents. *Inorg. Chem.* **2014**, *53* (19), 10030–10032.
(10) Singh, T. N.; Turro, C. Photoinitiated DNA Binding by Cis-[Ru(bpy)2(NH3)2]2+. *Inorg. Chem.* **2004**, *43* (23), 7260–7262.
(11) Garner, R. N.; Joyce, L. E.; Turro, C. Effect of Electronic Structure on the Photoinduced Ligand Exchange of Ru(II) Polypyridine Complexes. *Inorg. Chem.* **2011**, *50* (10), 4384–4391.
(12) Mari Cristina; Pierroz Vanessa; Rubbiani Riccardo; Patra Malay; Hess Jeannine; Spingler Bernhard; Oehninger Luciano; Schur Julia; Ott Ingo; Salassa Luca; et al. DNA Intercalating RuII Polypyridyl Complexes as Effective Photosensitizers in Photodynamic Therapy. *Chem. – Eur. J.* **2014**, *20* (44), 14421–14436.
(13) Sainuddin, T.; Pinto, M.; Yin, H.; Hetu, M.; Colpitts, J.; McFarland, S. A. Strained Ruthenium Metal–organic Dyads as Photocisplatin Agents with Dual Action. *J. Inorg. Biochem.* **2016**, *158*, 45–54.
(14) Rixel, V. H. S. van; Siewert, B.; L. Hopkins, S.; C. Askes, S. H.; Busemann, A.; A. Siegler, M.; Bonnet, S. Green Light-Induced Apoptosis in Cancer Cells by a Tetrapyridyl Ruthenium Prodrug Offering Two Trans Coordination Sites. *Chem. Sci.* **2016**, *7* (8), 4922–4929.
(15) Wachter, E.; Heidary, D. K.; Howerton, B. S.; Parkin, S.; Glazer, E. C. Light-Activated Ruthenium Complexes Photobind DNA and Are Cytotoxic in the Photodynamic Therapy Window. *Chem. Commun.* **2012**, *48* (77), 9649–9651.
(16) Azar, D. F.; Audi, H.; Farhat, S.; El-Sibai, M.; Abi-Habib, R. J.; Khnayzer, R. S. Phototoxicity of Strained Ru(II) Complexes: Is It the Metal Complex or the Dissociating Ligand? *Dalton Trans.* **2017**, *46* (35), 11529–11532.
(17) Cuello-Garibo, J.-A.; Meijer, M. S.; Bonnet, S. To Cage or to Be Caged? The Cytotoxic Species in Ruthenium-Based Photoactivated Chemotherapy Is Not Always the Metal. *Chem. Commun. Camb. Engl.* **2017**, *53* (50), 6768–6771.
(18) Sgambellone, M. A.; David, A.; Garner, R. N.; Dunbar, K. R.; Turro, C. Cellular Toxicity Induced by the Photorelease of a Caged Bioactive Molecule: Design of a Potential Dual-Action Ru(II) Complex. *J. Am. Chem. Soc.* **2013**, *135* (30), 11274–11282.
(19) Laemmel, A.-C.; Collin, J.-P.; Sauvage, J.-P. Efficient and Selective Photochemical Labilization of a Given Bidentate Ligand in Mixed Ruthenium(II) Complexes of the Ru(phen)2L2+ and Ru(bipy)2L2+ Family (L = Sterically Hindering Chelate). *Eur. J. Inorg. Chem.* **1999**, *1999* (3), 383–386.
(20) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. Mixed-Ligand Complexes of ruthenium(II): Factors Governing Binding to DNA. *J. Am. Chem. Soc.* **1989**, *111* (8), 3051–3058.
(21) Audi, H.; Azar, D. F.; Mahjoub, F.; Farhat, S.; El Masri, Z.; El-Sibai, M.; Abi-Habib, R. J.; Khnayzer, R. S. Cytotoxicity Modulation of ruthenium(II) Tris-Bathophenanthroline Complexes with Systematically Varied Charge. *J. Photochem. Photobiol. Chem.* **2018**, *351* (Supplement C), 59–68.
(22) Sun, H.; Ye, K.; Wang, C.; Qi, H.; Li, F.; Wang, Y. The Π−π Stacked Geometries and Association Thermodynamics of Quinacridone Derivatives Studied by 1H NMR. *J. Phys. Chem. A* **2006**, *110* (37), 10750–10756.
(23) He, L.; Ma, D.; Duan, L.; Wei, Y.; Qiao, J.; Zhang, D.; Dong, G.; Wang, L.; Qiu, Y. Control of Intramolecular Π–π Stacking Interaction in Cationic Iridium Complexes via Fluorination of Pendant Phenyl Rings. *Inorg. Chem.* **2012**, *51* (8), 4502–4510.

(24) Dickerson, M.; Sun, Y.; Howerton, B.; Glazer, E. C. Modifying Charge and Hydrophilicity of Simple Ru(II) Polypyridyl Complexes Radically Alters Biological Activities: Old Complexes, Surprising New Tricks. *Inorg. Chem.* **2014**, *53* (19), 10370–10377.
(25) Romero-Canelón, I.; Pizarro, A. M.; Habtemariam, A.; Sadler, P. J. Contrasting Cellular Uptake Pathways for Chlorido and Iodido Iminopyridine Ruthenium Arene Anticancer Complexes. *Met. Integr. Biometal Sci.* **2012**, *4* (12), 1271–1279.
(26) Gharehbaghi, K.; Szekeres, T.; Yalowitz, J. A.; Fritzer-Szekeres, M.; Pommier, Y. G.; Jayaram, H. N. Sensitizing Human Colon Carcinoma HT-29 Cells to Cisplatin by Cyclopentenylcytosine, in Vitro and in Vivo. *Life Sci.* **2000**, *68* (1), 1–11.
(27) Paixão, D. A.; Marzano, I. M.; Jaimes, E. H. L.; Pivatto, M.; Campos, D. L.; Pavan, F. R.; Deflon, V. M.; Maia, P. I. da S.; Da Costa Ferreira, A. M.; Uehara, I. A.; et al. Novel copper(II) Complexes with Hydrazides and Heterocyclic Bases: Synthesis, Structure and Biological Studies. *J. Inorg. Biochem.* **2017**, *172*, 138–146.
(28) Silva, P. P.; Guerra, W.; Dos Santos, G. C.; Fernandes, N. G.; Silveira, J. N.; da Costa Ferreira, A. M.; Bortolotto, T.; Terenzi, H.; Bortoluzzi, A. J.; Neves, A.; et al. Correlation between DNA Interactions and Cytotoxic Activity of Four New Ternary Compounds of copper(II) with N-Donor Heterocyclic Ligands. *J. Inorg. Biochem.* **2014**, *132*, 67–76.
(29) Lincoln, P.; Nordén, B. DNA Binding Geometries of Ruthenium(II) Complexes with 1,10-Phenanthroline and 2,2‘-Bipyridine Ligands Studied with Linear Dichroism Spectroscopy. Borderline Cases of Intercalation. *J. Phys. Chem. B* **1998**, *102* (47), 9583–9594.
(30) Rahman, A.-; Choudhary, M. I. *Frontiers in Anti-Cancer Drug Discovery, Volume (1)*; Bentham Science Publishers, 2011.
(31) Dietrick-Buchecker, C. O.; Marnot, P. A.; Sauvage, J. P. Direct Synthesis of Disubstituted Aromatic Polyimine Chelates. *Tetrahedron Lett.* **1982**, *23* (50), 5291–5294.
(32) Caspar, J. V.; Meyer, T. J. Photochemistry of tris(2,2’-bipyridine)ruthenium(2+) Ion (Ru(bpy)32+). Solvent Effects. *J. Am. Chem. Soc.* **1983**, *105* (17), 5583–5590.
(33) Chen, L.; Peng, F.; Li, G.; Jie, X.; Cai, K.; Cai, C.; Zhong, Y.; Zeng, H.; Li, W.; Zhang, Z.; et al. The Studies on the Cytotoxicity in Vitro, Cellular Uptake, Cell Cycle Arrest and Apoptosis-Inducing Properties of Ruthenium Methylimidazole Complex [Ru(MeIm)4(p-cpip)]2 +. *J. Inorg. Biochem.* **2016**, *156*, 64–74.

10.1002/ejic.201800194



In the present study, $[Ru(bipy)_2(dpphen)]Cl_2$ [where bipy= 2,2'-bipyridine and dpphen = 2,9-diphenyl-1,10-phenanthroline] (complex **1**) was shown to eject the bipy ligand upon light activation resulting in the formation of an aqua complex in water. The complex exhibited a significantly higher toxicity upon irradiation (up to over 100-fold increase) on five cancer cell lines including cisplatin-resistant ones. The free bipy ligand displayed minimal potency and therefore phototoxicity was attributed to the formation of the aqua complex.