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A Multiaction and Multitarget Ru(II)-Pt(IV) Conjugate Combining Cancer Activated Chemotherapy and Photodynamic Therapy to Overcome Drug Resistant Cancers

Johannes Karges,^[a] Thirumal Yempala,^[b] Mickaël Tharaud,^[c] Dan Gibson,^{*[b]} and Gilles Gasser^{*[a]}

Abstract: Cancer has emerged as one of the deadliest diseases worldwide. Pt(II) complexes are commonly used to treat this condition. To reduce their side effects and improve their pharmacological properties, Pt(IV) complexes are being developed as prodrug candidates that are activated by reduction in cancer cells. Concomitantly, photodynamic therapy (PDT) has received increasing attention over the last years. Among other compounds studied as photosensitizers (PSs), Ru(II) polypyridine complexes have gained much attention over the recent years due to their attractive characteristics. In this article, the first example of a novel Pt(IV)-Ru(II) conjugate, which combines cancer activated chemotherapy with photodynamic therapy, is presented. Upon entering the cancer cell, the Pt(IV) centre is reduced to Pt(II) and the axial ligands including the Ru(II) complex and phenylbutyrate are released. As each component has its individual target, the conjugate exerts a multitarget and multiaction effect with (photo-)cytotoxicity values upon irradiation up to clinically relevant 595 nm in the low nanomolar range in various (drug resistant) 2D monolayer cancer cells and 3D multicellular tumour spheroids.

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Introduction

To date, the cure and treatment of cancer remains a major challenge in modern medicine. Cisplatin (*cis*-diamminedichloridoplatinum(II)) and its platinum based derivatives oxaliplatin and carboplatin are the clinically most frequently used anticancer drugs worldwide. Despite their enormous clinical success, the application of these compounds is limited due to severe side effects (e.g., kidney damage, nausea, vomiting and bone marrow suppression), low cancer cell selectivity and, more worryingly, an increasing number of platinum resistant tumours.^[1] A known concept to improve the therapeutic outcome of chemotherapy is to administer cisplatin and its derivatives in combination with other biologically active molecules such as histone deacetylase inhibitors (Vorinostat)^[2], retinoid receptor regulators (vitamin D derivatives)^[3] or microtubule network disturbing compounds (e.g., paclitaxel, vinblastine)^[4] to generate a drug mixture with multiple targets and actions. Despite improvements in the treatment outcomes, the application of these drug mixtures is limited as the different components may reach the target after different circulation times leading to non-ideal drug doses.^[5] To overcome these drawbacks, research efforts are devoted towards the development of novel Pt(IV) based compounds, which can be activated on site. As the compound enters the cell, the metal centre can be reduced from Pt(IV) to Pt(II), releasing its axial ligands. To improve the efficiency of the treatment, the axial ligands themselves can be biologically active molecules such as targeting moieties, carbon nanotubes, protein-binding moieties or receptor-binding peptides.^[6] Recently, the use of phenylbutyrate (PhB), which is a histone deacetylase inhibitor that de-condenses chromatin to improve the interaction of cisplatin with DNA, was demonstrated.^[7] As a consequence, Pt(IV) prodrugs with PhB as an axial ligand were shown to have a highly improved therapeutic profile, causing DNA damage and epigenetic effects.^[8] Complementary to chemotherapy, cancer treatment using photodynamic therapy (PDT) has received increasing attention over the recent years. In PDT, a photosensitizer (PS) is either locally or systemically injected. Upon excitation at a specific wavelength, the PS acts as a photocatalyst to generate reactive oxygen species (ROS) and ultimately trigger cell death.^[9] Worthy of note, Sadler and co-workers have recently reported the use of photoactivatable Pt(IV) complexes which can act by non-classical mechanism.^[10] Among the investigated classes of compounds, metal complexes^[11] and especially

Ru(II) polypyridine complexes have received increasing attention due to their attractive chemical and photophysical properties including high water solubility, high ROS production, chemical stability and photostability.^[12] Despite recent research efforts, the majority of studied Ru(II) polypyridine complexes are excited using blue or UV-A light.^[13] Due to light scattering effects and absorption by biological compartments, the light penetration depth in the tissue is poor at these wavelengths, limiting the treatment of deep seated or large tumors.^[14] To tackle this issue, some of us have recently reported the DFT-guided design of Ru(II) polypyridine complexes with a strong absorption red shift towards the biological spectral window (600-900 nm). In this pursuit, the complex [Ru(4,7-diphenyl-1,10-phenanthroline)₂(4,4'-dimethyl-2,2'-bipyridine)]²⁺, which showed a phototoxic effect from 480 nm up to clinically relevant 595 nm, was unveiled.^[15] Herein, a novel Ru(II)-Pt(IV) conjugate (**Ru-Pt**), which combines cancer activated chemotherapy and PDT by acting on multiple targets inside the cancer cell with several biological mechanism to overcome drug resistances, is described. Upon entering cancer cells, the Pt(IV) metal centre is reduced to Pt(II) releasing PhB and the Ru(II) polypyridine complex. The Ru(II) complex was found to selectively accumulate in the Golgi apparatus where it is able to generate singlet oxygen (¹O₂) upon irradiation at various wavelength ranging from 480 up to clinically relevant 595 nm.

Results and Discussion

To ensure that the photophysical and biological properties of each component are not influenced, the moieties were conjugated with a long aliphatic linker. The linking unit between the Ru(II) and the Pt(IV) complex was synthesised starting from 4,4'-dimethyl-2,2'-bipyridine, which was asymmetrically oxidised to the carboxylic acid using SeO₂.^[16] The generated 4'-methyl-2,2'-bipyridinyl-4-carboxylic acid was treated with SOCl₂ to yield the acid chloride intermediate, which was used to attach *tert*-butyl-*N*-(6-aminohexyl)carbamate by peptide bond formation.^[17] The generated 2,2'-bipyridine derivative was then coordinated to dichlorobis(4,7-diphenyl-1,10-phenanthroline)ruthenium(II), which was previously synthesised from 4,7-diphenyl-1,10-phenanthroline and RuCl₃. Finally, the *tert*-butoxycarbonyl protection group was removed using trifluoroacetic acid to give the Ru(II) polypyridine complex **Ru**. To facilitate the conjugation of **Ru** to the **Pt-PhB-OH** the axial OH group of the Pt(IV) was activated to form **Pt-PhB-MS** (MS=monosuccinimidylcarbonate), whose synthesis was prepared in a similar manner to the structurally related derivatives.^[8b] Both carboxylates (link to PhB) and carbamate (link to Ru) detach from the Pt^{IV} following reduction.^[8c] The **Ru** complex was then coupled with **Pt-PhB-MS** in presence of diisopropylethylamine to yield the final **Ru-Pt** complex. The synthesis and characterisation of the complexes are described in the SI (Scheme S1, Figure S1-S8).

The photophysical properties of **Ru** were compared with **Ru-Pt** to evaluate its potential as a PDT PS and to investigate whether the conjugation to the Pt(IV) complex influenced them (Table S1). As expected, the absorption and emission spectra of **Ru** and **Ru-Pt** (Figure S9-S10) showed no significant differences. Importantly, the spectra showed an absorption tail towards the biological spectral window potentially enabling the treatment of deep-seated and large tumours as the light penetration depth directly correlates with the used wavelengths. Worthy of note, previous studies have shown that PSs can show a phototoxic effect although their extinction coefficients are below 100 M⁻¹cm⁻¹.^[12a,15] The conjugate was found to have a large Stokes shift, implying minimal interference between excitation and emission. The luminescence quantum yields of **Ru** and **Ru-Pt** were found to be similar ($\Phi_{em, Ru} = 2.0 \%$, $\Phi_{em, Ru-Pt} = 2.1 \%$), which is in the same range as other published Ru(II) polypyridine complexes.^[18] Both compounds were found to have excited state lifetimes in the

Table 1. IC₅₀ values (μM) in the dark and upon irradiation at 480 (10 min, 3.1 J/cm²) and 595 nm (120 min, 22.5 J/cm²) for **Pt-PhB-OAc**, **Ru** and **Ru-Pt** in comparison to Protoporphyrin IX (PpIX) in cancerous in human cervical carcinoma (HeLa) and non-cancerous retinal pigment epithelium (RPE-1) cells. Average of three independent measurements.

	HeLa						RPE-1					
	dark	480 nm	PI	dark (120 min)	595 nm	PI	dark	480 nm	PI	dark (120 min)	595 nm	PI
Pt- PhB-OAc	18.94 ± 0.83	-	-	-	-	-	81.49 ± 6.54	-	-	-	-	-
Ru	13.57 ± 0.28	1.65 ± 0.11	8.2	6.13 ± 0.38	0.58 ± 0.16	10.9	4.22 ± 0.42	0.82 ± 0.17	5.1	2.5 ± 0.36	0.62 ± 0.30	4.0
RuPt	3.26 ± 0.07	0.31 ± 0.06	10.5	1.41 ± 0.13	0.13 ± 0.07	10.8	4.38 ± 0.57	0.61 ± 0.29	7.2	2.47 ± 0.49	0.45 ± 0.18	5.5
PpIX	>100	2.50 ± 0.13	> 40.0	81.26 ± 8.34	1.52 ± 0.92	53.4	> 100	3.84 ± 0.19	> 26.3	83.5 ± 6.71	1.93 ± 0.36	43.3

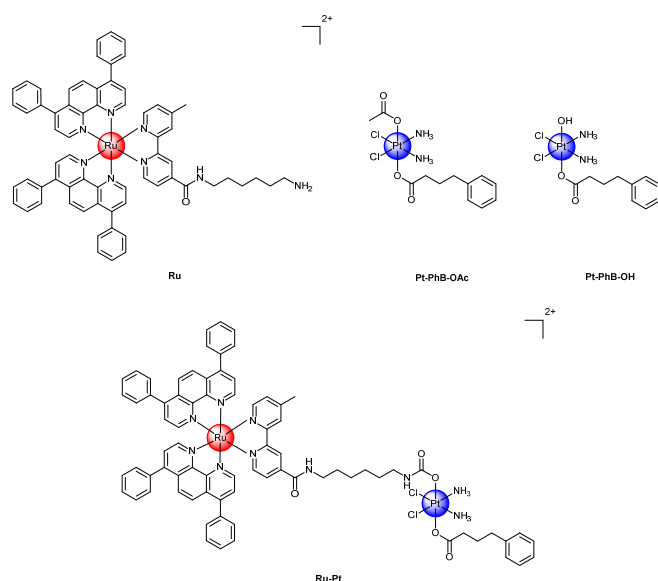


Figure 1. Chemical structures investigated in this work. **Ru** was isolated as a PF₆⁻ salt whereas **Ru-Pt** was isolated as a TFA⁻ salt.

nanosecond range (Figure S11-S12) in an air saturated environment (147-183 ns) and in degassed environment (1003-1054 ns). Importantly, the presence of air had a drastic effect on the lifetime, indicating that the excited state of the complex can interact with molecular oxygen (³O₂) to generate singlet oxygen (¹O₂). For quantification of the amount of ¹O₂ produced upon light irradiation, the singlet oxygen quantum yields were determined by two methods: 1) directly by measuring the phosphorescence signal of ¹O₂, 2) indirectly by capturing ¹O₂ with a reporter molecule and monitoring its change by absorption spectroscopy.^[19] The characteristic emission signal at 1270 nm in the infrared region using the direct method confirmed the production of ¹O₂ upon light exposure. Importantly, **Ru** and **Ru-Pt** are able to produce ¹O₂ upon irradiation at 450 and 540 nm. The measurements revealed high ¹O₂ quantum yields of 45-64 % in CH₃CN and of 3-6 % in an aqueous solution (Table S2). Overall, these studies showed that **Ru-Pt** has attractive photophysical properties for applications as a PDT agent and the conjugation to the Pt(IV) centre did not significantly influence the photophysical properties of the Ru(II) complex.

The stability of the conjugate was then investigated as previous studies have shown that this could be problematic for metal complexes.^[20] The conjugate was incubated in the dark in H₂O at room temperature and physiological pH and the absorption recorded in time intervals up to 48 h (Figure S13). Additionally, the complex was analysed by HPLC after a 48 h incubation in H₂O (Figure S14). As no significant differences were observed, the stability in an aqueous solution is indicated.

After a chemical and photophysical characterisation of the conjugate, its potential activity as a chemotherapeutic agent and PS for PDT was investigated. The cytotoxicity of **cisplatin**, **Pt-PhB-OH**, **Pt-PhB-OAc**, **Ru** and **Ru-Pt** towards non-cancerous retinal pigment epithelium (RPE-1) and cancerous human cervical carcinoma (HeLa) cells in the dark as well as upon irradiation at various wavelengths between 480 – 595 nm was determined (Table 1, Table S3). In both investigated cell lines the conjugate **Ru-Pt** (IC_{50, HeLa} = 3.26 ± 0.07 μM, IC_{50, RPE-1} = 4.38 ± 0.57 μM) showed a higher cytotoxicity in the dark than **Ru** (IC_{50, HeLa} = 13.57 ± 0.28 μM, IC_{50, RPE-1} = 4.22 ± 0.42 μM) or the platinum based complexes **cisplatin**, **Pt-PhB-OH** or **Pt-PhB OAc** (IC_{50, HeLa} = 5.9 – 18.9 μM, IC_{50, RPE-1} = 27.7 – 81.4 μM) alone. All Pt(IV) prodrugs and importantly **Ru-Pt** showed a 1.3 - 4.7 times higher cytotoxicity in the cancerous cell line in comparison to the non-cancerous cell line. This effect was previously described in the literature and is caused by the preferential reduction of the Pt(IV) centre to Pt(II) in cancer cells, which are hypoxic. Strikingly, upon irradiation at various wavelengths between 480 – 595 nm, the **Ru-Pt** conjugate is able to cause a phototoxic effect. Thanks to the additional PDT effect, the cytotoxicity improved drastically, with IC₅₀ values in the nanomolar range (IC_{50, HeLa} = 0.13 – 0.39 μM, IC_{50, RPE-1} = 0.45 – 0.61 μM). Overall, these results indicate the beneficial effect of combining the properties of the Pt(II) complex as a chemotherapeutic agent and the Ru(II) complex as a PS as a conjugate. Interestingly, **Ru-Pt** showed a slightly higher cytotoxicity in cancerous cells in comparison to non-cancerous cells.

Capitalising on these preliminary results, the potential of **Ru-Pt** to overcome resistance was further investigated in depth in human ovarian carcinoma (A2780) cells and its cisplatin resistant line (A2780 cis) as well as its doxorubicin resistant line (A2780 ADR). The uptake mechanism of the **Ru-Pt** conjugate was studied in A2780 cells by blocking different cellular pathways and determining the amount of Ru and Pt inside the cells (Figure 2a) by inductively coupled plasma mass spectrometry (ICP-MS).^[21] The incubation with the cationic transporter inhibitor (tetraethylammonium chloride) did not significantly influence the cellular uptake, suggesting that this pathway does not majorly contribute to the internalisation of the compound. The incubation at lower temperature (4 °C) and with metabolic inhibitors (2- deoxy-D-glucose and oligomycin) decreased the uptake, indicating that the internalisation is energy dependent. In contrast, the incubation with endocytotic inhibitors (ammonium chloride or chloroquine) drastically decreased the uptake. Overall, these results indicate

that **Ru-Pt** is internalised primarily by an energy dependent endocytotic mechanism. Worthy of note, the recently reported Ru-Pt metallacage was internalised by the same pathway.^[22] Importantly, similar levels of Ru and Pt were detected inside the cells indicating that the Ru-Pt is stable in the cell culture medium and was internalised as a single moiety.

Following this, the localisation of the released Ru(II) complex and Pt(II) complex (cisplatin) were investigated by extraction of the major cellular organelles (nucleus, mitochondria, lysosomes, Golgi apparatus, endoplasmic reticulum) and determination of the amount of Ru and Pt inside of each by ICP-MS (Figure 2b). As expected, Ru and Pt were found in different cellular compartments, indicating that the Pt(IV) centre is reduced and the axial ligands (**Ru** and PhB) released and that the conjugate is acting

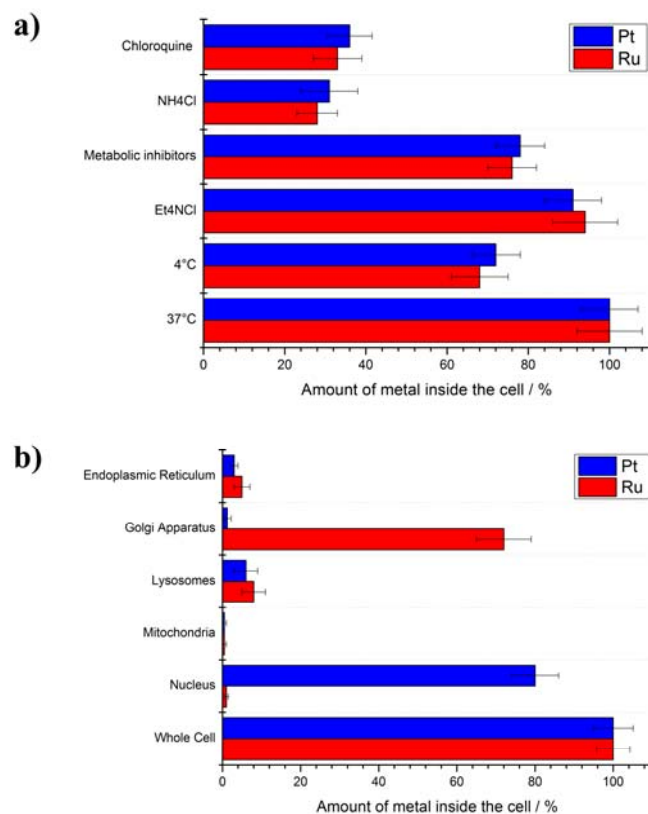


Figure 2. a) Cellular uptake mechanism study of **Ru-Pt** (10 μ M) in A2780 cells in the presence of different inhibitors/conditions by determination of the amount of Ru and Pt inside the cells by ICP-MS. Endocytic inhibition: NH₄Cl (50 mM) or chloroquine (100 μ M), metabolic inhibition: 2-Deoxy-D-glucose (50 mM) and oligomycin (5 μ M), cation transporter inhibition: Et₄NCl, low temperature: incubation at 4°C, control: incubation at 37°C. **b)** Cellular localisation of Ru and Pt of the conjugate **Ru-Pt** (10 μ M) in A2780 cells inside the major cellular organelles (endoplasmic reticulum, golgi apparatus, lysosomes, mitochondria, nucleus, whole cell) after 4 h incubation in the dark, extraction of their cellular organelles and determination of the amount of Ru and Pt inside each organelle by ICP-MS.

simultaneously in different cellular organelles. As anticipated, the vast majority of Pt was found in the nucleus. This finding is in agreement with the in-depth investigation of the anticancer drug cisplatin, which is targeting the nucleus and binding to DNA. On the contrary, the majority of Ru was found in the Golgi apparatus. Additionally, small amounts of Pt and Ru were also found in the lysosomes and the endoplasmic reticulum. Taken together, these results indicate that **Ru-Pt** is internalised through an engulfing mechanism, in which the cell membrane draws the complex from the outside to the inside by formation of intracellular transport vesicles. These can then interact with the endomembrane system consisting of the lysosomes, Golgi apparatus and endoplasmic reticulum, where they release their content. As small amounts of Ru and Pt were both found in the lysosomes and the endoplasmic reticulum, it tends to demonstrate that the **Ru-Pt** conjugate is majorly released in these organelles. In a further process, the Pt(IV) centre is reduced and the Ru and Pt moieties localise in their respective target organelles (Golgi apparatus and nucleus).

Table 2. IC₅₀ values (μM) in the dark and upon irradiation at 480 (10 min, 3.1 J/cm²) and 595 nm (60 min, 11.3 J/cm²) for **cisplatin**, **Pt-PhB-OH**, **Pt-PhB-OAc**, **Ru** and **Ru-Pt** in comparison to Protoporphyrin IX (PpIX) in the human ovarian carcinoma (A2780), human cisplatin resistant ovarian carcinoma (A2780 cis) and human doxorubicin resistant ovarian carcinoma (A2780 ADR) cell lines. Average of three independent measurements.

	A2780					A2780 cis					A2780 ADR				
	dark	480 nm	PI	595 nm	PI	dark	480 nm	PI	595 nm	PI	dark	480 nm	PI	595 nm	PI
cisplatin	4.54 ± 0.65	-	-	-	-	19.53 ± 1.11	-	-	-	-	8.97 ± 0.71	-	-	-	-
Pt-PhB-OAc	1.39 ± 0.22	-	-	-	-	6.17 ± 0.36	-	-	-	-	3.69 ± 0.32	-	-	-	-
Pt-PhB-OH	1.07 ± 0.24	-	-	-	-	5.95 ± 0.37	-	-	-	-	2.56 ± 0.34	-	-	-	-
Ru	15.35 ± 0.89	2.86 ± 0.52	5.4	4.30 ± 0.66	3.6	17.26 ± 0.90	4.67 ± 0.43	3.7	7.10 ± 0.92	2.4	18.60 ± 0.71	3.41 ± 0.42	5.5	4.47 ± 0.35	4.2
Ru-Pt	0.98 ± 0.22	0.08 ± 0.04	12.3	0.16 ± 0.05	6.1	1.38 ± 0.29	0.10 ± 0.03	13.8	0.19 ± 0.06	7.1	1.13 ± 0.28	0.09 ± 0.03	12.6	0.17 ± 0.04	6.6
PpIX	>100	4.53 ± 0.61	22.1	8.19 ± 1.13	12.2	>100	6.95 ± 1.46	14.4	8.94 ± 1.53	11.2	>100	7.12 ± 1.96	14.0	10.38 ± 1.62	9.6

To study the efficiency of the conjugate against drug resistant cell lines, the cytotoxicity of **cisplatin**, **Pt-PhB-OH**, **Pt-PhB-OAc**, **Ru** and **Ru-Pt** in the dark and upon irradiation at 480 (10 min, 3.1 J/cm²) and 595 nm (60 min, 11.3 J/cm²) against A2780, A2780 cis and A2780 ADR was investigated. All complexes (Table 2) were found to have lower cytotoxic values in all ovarian cancer cell lines in comparison to the HeLa and RPE-1 cell lines (Table 1). These results show the enormous potential of the conjugate **Ru-Pt** with cytotoxicity values in the dark in the low micromolar range (IC_{50, dark} = 0.98 – 1.38 μM). It is worth mentioning that a PS should be ideally non-toxic in the dark. The irradiation drastically improved the cytotoxic profile (IC_{50, 480nm} = 0.08 – 0.10 μM, IC_{50, 595nm} = 0.16 – 0.19 μM) with PI values between 6.1 – 13.8. While the cytotoxicity of **cisplatin**, **Pt-PhB-OH** and **Pt-PhB-OAc** is lower in the resistant cell lines A2780 cis and A2780 ADR, the **Ru-Pt** conjugate overcomes the resistance even in the dark and strikingly, by irradiation the cytotoxicity in all cell lines is further improved by a factor of ten. Comparing the cytotoxicity of **Ru** and **Ru-Pt** under irradiation we see that **Ru-Pt** is significantly more potent than **Ru** in all A2780 cell lines. Taken together, these results confirm the beneficial effect of the conjugation of the Ru(II) and Pt(IV) complex to the conjugate **Ru-Pt**.

Encouraged by these promising results, the mechanism of action of **Ru-Pt** was further examined by determining its cell death pathway. For this study, the cytotoxic profile of **cisplatin**, **Ru** in the dark, **Ru-Pt** in the dark and upon irradiation at 480 nm (10 min, 3.1 J/cm²) at its IC₅₀ concentration in A2780 cells was measured in the presence of autophagy (3-methyladenine), apoptosis (Z-VAD-FMK), paraptosis (cycloheximide) or necrosis (necrostatin-1) inhibitors (Figure 3a). The incubation with autophagy, paraptosis and necrosis inhibitors did not significantly influence the cell viability of **cisplatin**, **Ru** and **Ru-Pt** in the dark, indicating that these pathways are not primarily accessed for triggering of their cell death. In contrast, preincubation with an apoptosis inhibitor increased the survival of the cells treated with **cisplatin**, indicating that this mechanism is majorly contributing to the cell death. This result is in agreement with a study of cell death pathways of **cisplatin** in human ovarian carcinoma cells.^[23] In addition, elevated survival levels for the preincubation with an apoptotic inhibitor and treatment with **Ru** in the dark were detected, suggesting an apoptosis induced cell death mechanism. Therefore, it was not surprising to observe an apoptotic cell death pathway for **Ru-Pt** in the dark. Importantly, upon irradiation of **Ru-Pt**, the cell survival is highly increased with apoptotic and paraptotic inhibitors, indicating a cell death mechanism for the phototoxic effect involving both pathways. Worthy of note, several PDT agents were previously reported in the literature to cause cell death by apoptosis and paraptosis pathways.^[24]

For further examination of the cell death mechanism, its dependency on caspase 3/7 pathways was investigated using a Caspase-Glo 3/7 assay. These caspases are known executors of extrinsic and intrinsic apoptosis mechanism.^[25] The caspase 3/7 activity was measured in A2780 cells for **cisplatin**, **Ru** and **Ru-Pt** in the dark and upon irradiation at 480 nm (10 min, 3.1 J/cm²) using half of their IC₅₀ concentration (Figure 3b). As a positive control for caspase activity, the kinase inhibitor staurosporin was used. **Cisplatin**, **Ru** and **Ru-Pt** in the dark did not significantly increase caspase 3/7 activity, ruling out this pathway for cell death. The result for **cisplatin** is in agreement with a recent study in the same cell line that showed that apoptosis is induced by ERK/p53/PUMA activation.^[26] In contrast to this, upon light irradiation and treatment with **Ru** and **Ru-Pt**, the caspase 3/7 activity is highly elevated, suggesting that the phototoxic effect is caused by these pathways. Worthy of note, other Ru(II) polypyridine complexes were also found to generate a cytotoxic effect by this pathway.^[27]

After evaluation of the (photo-)cytotoxicity on 2D monolayer cells, the effect of **Ru-Pt** on 3D multicellular tumour spheroids (MCTS) was investigated. This is of special interest as many anticancer agents have failed translation from 2D monolayer cells to *in vivo* models due to compromised drug delivery. MCTS can mimic the pathological conditions found in solid tumors such as proliferation gradients and its hypoxic centre and can therefore be used for the assessment of drug delivery.^[28] A2780 MCTS with a diameter of about 400 μm were incubated with increasing concentrations of **Ru-Pt** for 12 h. After this time, the cytotoxic effect in the dark and upon irradiation at 480 nm (10 min, 3.1 J/cm²) and 595 nm (120 min, 22.5 J/cm²) was determined upon measurement of the ATP concentration of living cells inside the MCTS. **Ru-Pt** showed a strong cytotoxic effect (IC_{50, dark} = 7.32 ± 0.41 μM), indicating that the conjugate is able to completely penetrate the MCTS. Importantly, as the cytotoxicity is shifting to the nanomolar range (IC_{50, 480nm} = 0.49 ± 0.16 μM, IC_{50, 595nm} = 0.68 ± 0.27 μM) upon irradiation at 480 nm and 595 nm, the synergetic effect of the Pt(IV) complex as a chemotherapeutic agent and the Ru(II) complex as a PDT agent is demonstrated.

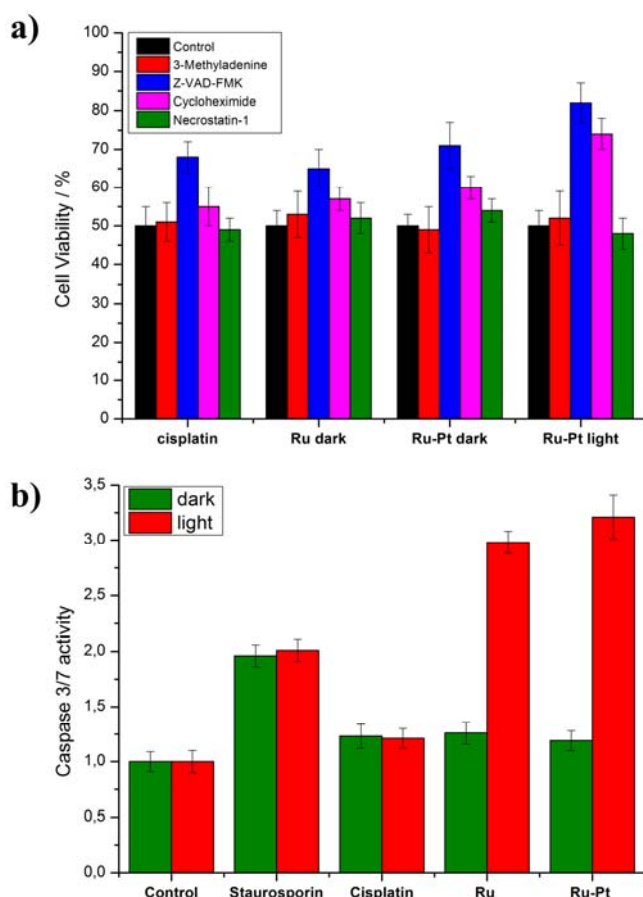


Figure 3. Cell death mechanism study in A2780 cells in the dark or upon irradiation at 480 nm (10 min, 3.1 J/cm²) **a)** upon treatment with the IC₅₀ value of the corresponding compound and in the presence of different inhibitors by determination of the cell viability. Autophagy inhibitor: 3-Methyladenine (100 μ M), apoptosis inhibitor: Z-VAD-FMK (20 μ M), paraptosis inhibitor: Cycloheximide (0.1 μ M), necrosis inhibitor: Necrostatin-1 (60 μ M). **b)** upon treatment with a concentration of half of the IC₅₀ value of the corresponding compound by determination of the caspase 3/7 activity.

Conclusion

In summary, in this article, a novel conjugate (**Ru-Pt**) combining a Pt(IV) complex as a chemotherapy drug and a Ru(II) complex as a photosensitizer for long wavelength photodynamic therapy, which targets several cellular organelles and acts by various cytotoxic mechanism, is reported. The conjugate is able to enter cancerous cells by an energy depended endocytosis mechanism. While being stable in an aqueous solution, the Pt(IV) centre is reduced inside cancerous cells to Pt(II) and releases its axial ligands. Phenylbutyrate acts as a histone deacetylase inhibitor and can de-condense the chromatin, improving the binding of the Pt(II) complex (cisplatin) to DNA. The Ru(II) complex was found to accumulate at the Golgi apparatus. Next to the cytotoxic effect exerted by the anticancer properties of cisplatin, the Ru(II) complex can act as a photosensitizer to catalytically generate singlet oxygen from 480 up to clinically relevant 595 nm. Thanks to the multitargeting and multi-action mechanisms based on a combination of apoptosis and paraptosis pathways, (photo-)cytotoxicity values in the low nanomolar range were observed in various 2D monolayer cells as well as 3D MCTS. Importantly, the conjugate showed a synergetic effect and was able to overcome drug resistance, which is found in many clinically treated tumors. We strongly believe that this novel conjugate and the approach of combining a Pt(IV) complex as a chemotherapeutic drug and a Ru(II) complex as a photosensitizer for photodynamic therapy has a great potential for further development.

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- [1] a) A.-M. Florea, D. Büsselberg, *Cancers* **2011**, 3, 1351-1371; b) C. Cullinane, G. B. Deacon, P. R. Drago, A. P. Erven, P. C. Junk, J. Luu, G. Meyer, S. Schmitz, I. Ott, J. Schur, L. K. Webster, A. Klein, *Dalton Trans.* **2018**, 47, 1918-1932; c) Z. Wang, Z. Deng, G. Zhu, *Dalton Trans.* **2019**, 48, 2536-2544; d) M. Hanif, C. G. Hartinger, *Future Med. Chem.* **2018**, 10, 615-617; e) C. G. Hartinger, in *Advances in Bioorganometallic Chemistry* (Eds.: T. Hirao, T. Moriuchi), Elsevier, **2019**, 157-172; f) D. Cirri, M. G. Fabbrini, A. Pratesi, L. Ciofi, L. Massai, T. Marzo, L. Messori, *BioMetals* **2019**, 32, 813-817.
- [2] C.-H. Pan, Y.-F. Chang, M.-S. Lee, B. C. Wen, J.-C. Ko, S.-K. Liang, M.-C. Liang, *BMC Cancer* **2016**, 16, 857.
- [3] M. Milczarek, S. ROSIŃSKA, M. Psurski, M. Maciejewska, A. Kutner, J. Wietrzyk, *Anticancer Res.* **2013**, 33, 433-444.
- [4] Y. H. Kim, S. W. Shin, B. S. Kim, J. H. Kim, J. G. Kim, Y. J. Mok, C. S. Kim, H. S. Rhyu, J. H. Hyun, J. S. Kim, *Cancer* **1999**, 85, 295-301.
- [5] a) S. Dasari, P. Bernard Tchounwou, *Eur. J. Pharmacol.* **2014**, 740, 364-378; b) E. Boros, P. J. Dyson, G. Gasser, 2020 *Chem* **6**, 41-60; c) A. Sharma, J. F. Arambula, S. Koo, R. Kumar, H. Singh, J. L. Sessler, J. S. Kim, *Chem. Soc. Rev.* **2019**, 48, 771-813.
- [6] a) T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* **2016**, 116, 3436-3486; b) M. D. Hall, T. W. Hambley, *Coord. Chem. Rev.* **2002**, 232, 49-67; c) W. H. Ang, I. Khalaila, C. S. Allardyce, L. Juillerat-Jeanneret, P. J. Dyson, *J. Am. Chem. Soc.* **2005**, 127, 1382-1383; d) K. Suntharalingam, Y. Song, S. J. Lippard, *Chem. Commun.* **2014**, 50, 2465-2468; e) E. Petruzzella, R. Sirota, I. Solazzo, V. Gandin, D. Gibson, *Chem. Sci.* **2018**, 9, 4299-4307; f) R. Ma, Y. Wang, L. Yan, L. Ma, Z. Wang, H. C. Chan, S.-K. Chiu, X. Chen, G. Zhu, *Chem. Commun.* **2015**, 51, 7859-7862; g) M. V. Babak, Y. Zhi, B. Czarny, T. B. Toh, L. Hooi, E. K.-H. Chow, W. H. Ang, D. Gibson, G. Pastorin, *Angew. Chem. Int. Ed.* **2019**, 58, 8109-8114; h) J. X. Ong, C. S. Q. Lim, H. V. Le, W. H. Ang, *Angew. Chem. Int. Ed.* **2019**, 58, 164-167; i) J. Z. Zhang, E. Wexselblatt, T. W. Hambley, D. Gibson, *Chem. Commun.* **2012**, 48, 847-849; j) L. Cubo, T. W. Hambley, P. J. Sanz Miguel, A. Carnero, C. Navarro-Ranninger, A. G. Quiroga, *Dalton Trans.* **2011**, 40, 344-347; k) L. Ma, N. Wang, R. Ma, C. Li, Z. Xu, M.-K. Tse, G. Zhu, *Angew. Chem. Int. Ed.* **2018**, 57, 9098-9102; l) G. Thiabaud, L. Harden-Bull, Y.-J. Ghang, S. Sen, X. Chi, J. L. Bachman, V. M. Lynch, Z. H. Siddik, J. L. Sessler, *Inorg. Chem.* **2019**, 58, 7886-7894; m) N. Muhammad, N. Sadia, C. Zhu, C. Luo, Z. Guo, X. Wang, *Chem. Commun.* **2017**, 53, 9971-9974; n) X. Wang, X. Wang, S. Jin, N. Muhammad, Z. Guo, *Chem. Rev.* **2019**, 119, 1138-1192.
- [7] a) S. D. Gore, M. A. Carducci, *Expert Opin. Inv. Drug.* **2000**, 9, 2923-2934; b) R. R. Rosato, S. Grant, *Cancer Biol. Ther.* **2003**, 2, 31-38.
- [8] a) H. Kostrhunova, E. Petruzzella, D. Gibson, J. Kasparkova, V. Brabec, *Chem. Eur. J.* **2019**, 25, 5235-5245; b) R. Raveendran, J. P. Braude, E. Wexselblatt, V. Novohradsky, O. Stuchlikova, V. Brabec, V. Gandin, D. Gibson, *Chem. Sci.* **2016**, 7, 2381-2391; c) J. J. Wilson, S. J. Lippard, *Inorg. Chem.* **2011**, 50, 3103.
- [9] a) D. E. Dolmans, D. Fukumura, R. K. Jain, *Nat. Rev. Cancer* **2003**, 3, 380-387; b) S. Bonnet, *Dalton Trans.* **2018**, 47, 10330-10343; c) F. Heinemann, J. Karges, G. Gasser, *Acc. Chem. Res.* **2017**, 50, 2727-2736.
- [10] a) C. Vallotto, E. Shaili, H. Shi, J. S. Butler, C. J. Wedge, M. E. Newton, P. J. Sadler, *Chem. Commun.* **2018**, 54, 13845-13848; b) C. A. Wootton, C. Sanchez-Cano, A. F. Lopez-Clavijo, E. Shaili, M. P. Barrow, P. J. Sadler, P. B. O'Connor, *Chem. Sci.* **2018**, 9, 2733-2739; c) N. A. Kratochwil, J. A. Parkinson, P. J. Bednarski, P. J. Sadler, *Angew. Chem. Int. Ed.* **1999**, 38, 1460-1463.
- [11] a) L. K. McKenzie, H. E. Bryant, J. A. Weinstein, *Coord. Chem. Rev.* **2019**, 379, 2-29; b) L. Zeng, P. Gupta, Y. Chen, E. Wang, L. Ji, H. Chao, Z.-S. Chen, *Chem. Soc. Rev.* **2017**, 46, 5771-5804; c) C. Imberti, P. Zhang, H. Huang, P. J. Sadler, *Angew. Chem. Int. Ed.*, **2020**, 59, 61-73; d) V. Novohradsky, A. Rovira, C. Hally, A. Galindo, G. Viguera, A. Gandioso, M. Svitelova, R. Bresoli-Obach, H. Kostrhunova, L. Markova, J. Kasparkova, S. Nonell, J. Ruiz, V. Brabec, V. Marchán, *Angew. Chem. Int. Ed.* **2019**, 58, 6311-6315; e) J. Pracharova, G. Viguera, V. Novohradsky, N. Cutillas, C. Janiak, H. Kostrhunova, J. Kasparkova, J. Ruiz, V. Brabec, *Chem. Eur. J.* **2018**, 24, 4607-4619; f) A. Zamora, G. Viguera, V. Rodríguez, M. D. Santana, J. Ruiz, *Coord. Chem. Rev.* **2018**, 360, 34-76.
- [12] a) S. Monro, K. L. Colón, H. Yin, J. Roque III, P. Konda, S. Gujar, R. P. Thummel, L. Lilge, C. G. Cameron, S. A. McFarland, *Chem. Rev.* **2019**, 119, 797-828; b) A. Li, C. Turro, J. J. Kodanko, *Acc. Chem. Res.* **2018**, 51, 1415-1421; c) J. Liu, C. Zhang, T. W. Rees, L. Ke, L. Ji, H. Chao, *Coord. Chem. Rev.* **2018**, 363, 17-28; d) F. E. Poynton, S. A. Bright, S. Blasco, D. C. Williams, J. M. Kelly, T. Gunnlaugsson, *Chem. Soc. Rev.* **2017**, 46, 7706-7756; e) M. Jakubaszek, J. Rossier, J. Karges, J. Delasoie, B. Goud, G. Gasser, F. Zobi, *Helv. Chim. Acta* **2019**, 102, e1900104; f) J. Shum, P. K.-K. Leung, K. K.-W. Lo, *Inorg. Chem.* **2019**, 58, 2231-2247; g) K. Qiu, Y. Chen, T. W. Rees, L. Ji, H. Chao, *Coord. Chem. Rev.* **2019**, 378, 66-86; h) J. Karges, M. Jakubaszek, C. Mari, K. Zarschler, B. Goud, H. Stephan, G. Gasser, *ChemBioChem*, **2019**, accepted, DOI: 10.1002/cbic.201900419; i) Y. Ellahioui, M. Patra, C. Mari, R. Kaabi, J. Karges, G. Gasser, S. Gómez-Ruiz, *Dalton Trans.* **2019**, 48, 5940-5951; j) B. S. Howerton, D. K. Heidary, E. C. Glazer, *J. Am. Chem. Soc.* **2012**, 134, 8324-8327; k) J. D. Knoll, C. Turro, *Coord. Chem. Rev.* **2015**, 282-283, 110-126; l) A. M. Palmer, B. Peña, R. B. Sears, O. Chen, M. E. Ojaimi, R. P. Thummel, K. R. Dunbar, C. Turro, *Philos. Trans. R. Soc. A* **2013**, 371, 20120135; m) J. Karges, F. Heinemann, F. Maschietto, M. Patra, O. Blacque, I. Ciofini, B. Spingler, G. Gasser, *Biorg. Med. Chem.* **2019**, 27, 2666-2675; n) S. A. McFarland, A. Mandel, R. Dumoulin-White, G. Gasser, *Curr. Opin. Chem. Biol.* **2020**, 56, 23-27; o) W. Streciwilk, A. Terenzi, X. Cheng, L. Hager, Y. Dabiri, P. Prochnow, J. E. Bandow, S. Wölfl, B. K. Keppler, I. Ott, *Eur. J. Med. Chem.* **2018**, 156, 148-161.

- [13] a) H. Yin, M. Stephenson, J. Gibson, E. Sampson, G. Shi, T. Sainuddin, S. Monro, S. A. McFarland, *Inorg. Chem.* **2014**, 53, 4548-4559; b) J. Karges, O. Blacque, P. Goldner, H. Chao, G. Gasser, *Eur. J. Inorg. Chem.*, **2019**, 3704-3712, DOI: 10.1002/ejic.201900569; c) J. Karges, O. Blacque, M. Jakubaszek, B. Goud, P. Goldner, G. Gasser, *J. Inorg. Biochem.* **2019**, 198, 110752.
- [14] a) K. Ogawa, Y. Kobuke, *Anti-Cancer Agents Me.* **2008**, 8, 269-279; b) B. C. Wilson, W. P. Jeeves, D. M. Lowe, *Photochem. Photobiol.* **1985**, 42, 153-162.
- [15] J. Karges, F. Heinemann, M. Jakubaszek, F. Maschietto, C. Subecz, M. Dotou, O. Blacque, M. Tharaud, B. Goud, E. V. Zahinos, B. Spingler, I. Ciofini, G. Gasser, *ChemRxiv*, doi: 10.26434/chemrxiv.11336669.
- [16] T. Banerjee, S. Rawalekar, A. Das, H. N. Ghosh, *Eur. J. Inorg. Chem.* **2011**, 2011, 4187-4197.
- [17] E. J. McLaurin, A. B. Greytak, M. G. Bawendi, D. G. Nocera, *J. Am. Chem. Soc.* **2009**, 131, 12994-13001.[18] a) M. J. Cook, A. P. Lewis, G. S. McAuliffe, V. Skarda, A. J. Thomson, J. L. Glasper, D. J. Robbins, *J. Chem. Soc. Perkin Trans. 2* **1984**, 1293-1301; b) A. Juris, V. Balzani, F. Barigelli, S. Campagna, P. I. Belser, A. Von Zelewsky, *Coord. Chem. Rev.* **1988**, 84, 85-277.
- [19] a) J. Karges, P. Goldner, G. Gasser, *Inorganics* **2019**, 7, 4; b) J. Karges, G. Gasser, *Inorg. Chim. Acta* **2019**, 119196.
- [20] a) U. Basu, J. Karges, F. Chotard, C. Balan, P. Le Gendre, G. Gasser, E. Bodio, R. Malacea Kabbara, *Polyhedron* **2019**, 172, 22-27; b) A. K. Renfrew, J. Karges, R. Scopelliti, F. D. Bobbink, P. Nowak-Sliwinska, G. Gasser, P. Dyson, *ChemBioChem* **2019**, 20, 2876.
- [21] a) J. Karges, U. Basu, O. Blacque, H. Chao, G. Gasser, *Angew. Chem. Int. Ed.*, **2019**, 58, 14334-14340; b) J. Karges, O. Blacque, H. Chao, G. Gasser, *Inorg. Chem.* **2019**, 58, 12422-12432.
- [22] Z. Zhou, J. Liu, T. W. Rees, H. Wang, X. Li, H. Chao, P. J. Stang, *Proc. Natl. Acad. Sci.* **2018**, 115, 5664-5669.
- [23] M. G. Ormerod, C. F. O'Neill, D. Robertson, K. R. Harrap, *Exp. Cell Res.* **1994**, 211, 231-237.
- [24] a) D. Kessel, *Photochem. Photobiol.* **2019**, 95, 119-125; b) V. Rapozzi, F. D'Este, L. E. Xodo, *J. Porphyr. Phthalocyanines* **2019**, 23, 410-418.
- [25] J. M. Adams, *Genes Dev.* **2003**, 17, 2481-2495.
- [26] H. Song, M. Wei, W. Liu, S. Shen, J. Li, L. Wang, *Histol. Histopathol.* **2018**, 33, 73-79.
- [27] a) C. Qian, J.-Q. Wang, C.-L. Song, L.-L. Wang, L.-N. Ji, H. Chao, *Metallomics* **2013**, 5, 844-854; b) H. Huang, P. Zhang, B. Yu, Y. Chen, J. Wang, L. Ji, H. Chao, *J. Med. Chem.* **2014**, 57, 8971-8983.
- [28] a) P. A. Netti, D. A. Berk, M. A. Swartz, A. J. Grodzinsky, R. K. Jain, *Cancer Res.* **2000**, 60, 2497-2503; b) J. Friedrich, C. Seidel, R. Ebner, L. A. Kunz-Schughart, *Nat. Protoc.* **2009**, 4, 309.