2020

Design of New Ruthenium Complexes for Photoactivated Chemotherapy

Lindsey Paul
University of Richmond

Design of New Ruthenium Complexes for Photoactivated Chemotherapy

by

Lindsey Paul

Honors Thesis

in

Program in Biochemistry and Molecular Biology

University of Richmond

Richmond, VA

April 24, 2020

Advisor: Dr. Mike Norris

This thesis has been accepted as part of the honors requirements

in the Program in Biochemistry and Molecular Biology.

_Mike Norris_____ ____4/20/20_____

(advisor signature) *(date)*

_Julie Pollock_____ _____4/20/20_____

(reader signature) *(date)*

ABSTRACT

Photoactivated chemotherapy (PACT) offers a targeted approach to cancer treatment through selective drug activation. Substitutionally labile ruthenium-based prodrugs undergo ligand-loss when irradiated, producing an unbound ligand and a Ru-aqua complex. We report the synthesis and cytotoxicity of several new ruthenium-centered complexes and their irradiation products for use in PACT. A series of complexes were synthesized in order to study the effects of structural differences on cell viability. Cell viability was tested on T47D human breast cancer cells in the presence of compound to determine cytotoxicity and dose-response. While neither the Ru-complexes nor their ligands demonstrated cytotoxicity, their Ru-aqua dissociation complexes all demonstrated cytotoxic effects at increasing concentrations. The results indicate the potential for the synthesized Ru-complexes to be used in PACT.

INTRODUCTION

Cancer is the second leading cause of death globally, accounting for one in six deaths. The World Health Organization's International Agency for Research on Cancer reported over 9.6 million cancer-related deaths and 18 million new cases of cancer in 2018, and those numbers are expected to increase in coming years.[i] Traditionally, cancer patients have been treated with surgery, chemotherapy, and radiation. Modern surgery can be minimally invasive and performed without damage to surrounding tissues, but its use depends on tumor location and the disease progression, as well as the relative health of the patient. Chemotherapy and radiation have also been found to be successful for different types of cancer, but both are nonspecific and can consequently cause severe and sometimes fatal side effects through interactions with healthy

cells.[ii] Contemporary treatments such as hormone-based therapy, gene therapy, stem cell therapy, and immunotherapy are promising for certain disease indications and progression states, but are relatively new fields of exploration and have not yet been proven successful across a wide range of cancer types.[iii] There is a clear need for additional cancer treatments that are selective for cancer cells.

Photoactivated chemotherapy (PACT), a term first utilized by Sadler *et al*. in 2009, describes a selective approach to treating cancer through the activation of a prodrug using photoirradiation.[iv,v] Most PACT molecules involve a transition metal complex that is activated by the photo-induced dissociation of a coordinated ligand, acting as a protecting group.[vi] Upon the cleavage of a ligand from a PACT molecule through irradiation, one or both photoproducts may exhibit cytotoxicity, generally through interactions with DNA or proteins, to induce cell apoptosis, autophagy, or necrosis.[vii,viii] Due to the targeted nature of light irradiation, PACT offers a more selective approach to cancer treatment and a promising avenue for exploration within bioinorganic anticancer therapeutics.

PACT is often compared to photodynamic therapy (PDT), a similar anticancer approach utilizing photoirradiation to create radical oxygen species through excited-state electron transfer, leading to cell death due to oxidative stress.[ix] But while PDT has progressed further along the development pipeline and has even been approved for clinical use for certain disease indications, it relies upon the presence of oxygen, which makes it ineffective in treating hypoxic tumors. It has also been known to cause a strong immune response.[x] PACT research can build upon the knowledge gained from the developmental achievements of PDT, including the application of fiber-optic technology for delivering light to tumors, while offering a wider range of possible uses, especially within oncology.[xi]

Much of the literature concerning PACT involves the study of ruthenium metal complexes due to their unique optical properties, tunable properties such excitation wavelength and kinetics of ligand dissociation, and minimal toxicity compared to other metals.[xii,xiii,xiv,xv] Recently, ruthenium polypyridyl complexes have entered clinical development for cancer treatment, though none have progressed to the point of regulatory approval.[xvi] Additionally, while many ruthenium complexes have been investigated for use as PACT agents, none have advanced to clinical trials. For many of the ruthenium PACT agents reported in the literature, it is unclear whether the anticancer agent is the ruthenium photodissociation product or the dissociated ligand.[xvii] Detailed elucidation of the mechanisms of cellular uptake and cytotoxicity for ruthenium PACT molecules is needed to better understand the potential for these molecules to demonstrate safety and efficacy in a clinical setting.[xviii] Optimization of the wavelength of activation and photodynamic window of ruthenium PACT molecules also remain a challenge; the proper wavelength for penetration can be tissue-specific, adding to the challenge of therapeutic design.[xix] The systematic study of new ruthenium PACT molecules could provide greater clarification about the chemical properties necessary for anticancer activity and related mechanisms. Here, we present the synthesis, characterization, and cytotoxic activity of three novel ruthenium PACT complexes (Figure 1) and their irradiation products (Figure 2). The cytotoxic activity of the irradiation products was tested separately to evaluate whether the cytotoxic effects might originate from the ruthenium photodissociation product or the dissociated ligand. The complexes were designed using bipyridine-derived ligands with different functional groups to provide a better understanding of effects of these chemical differences on cytotoxicity (Figure 3).

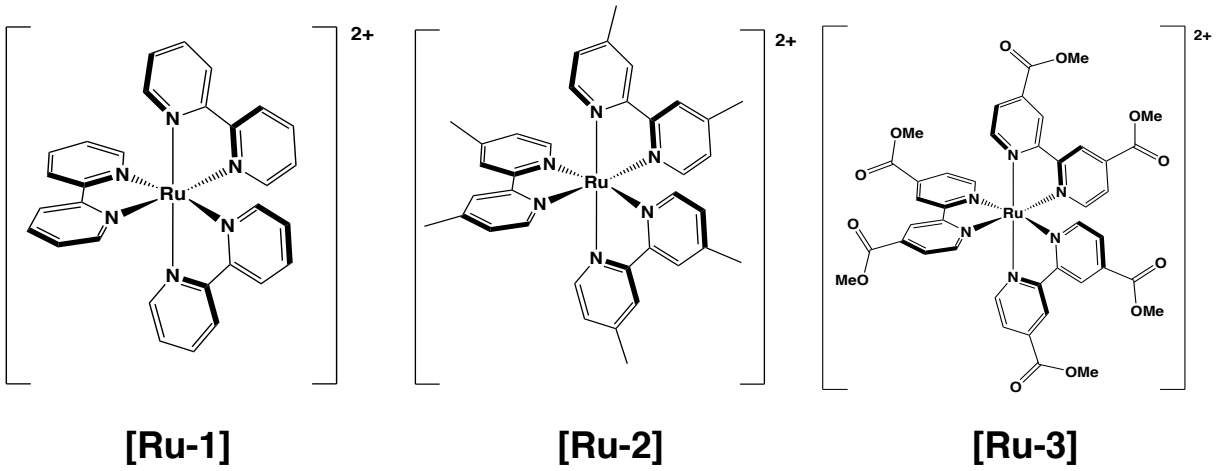


Figure 1. Chemical structures of ruthenium complexes **[Ru-1]**, **[Ru-2]**, and **[Ru-3]**.

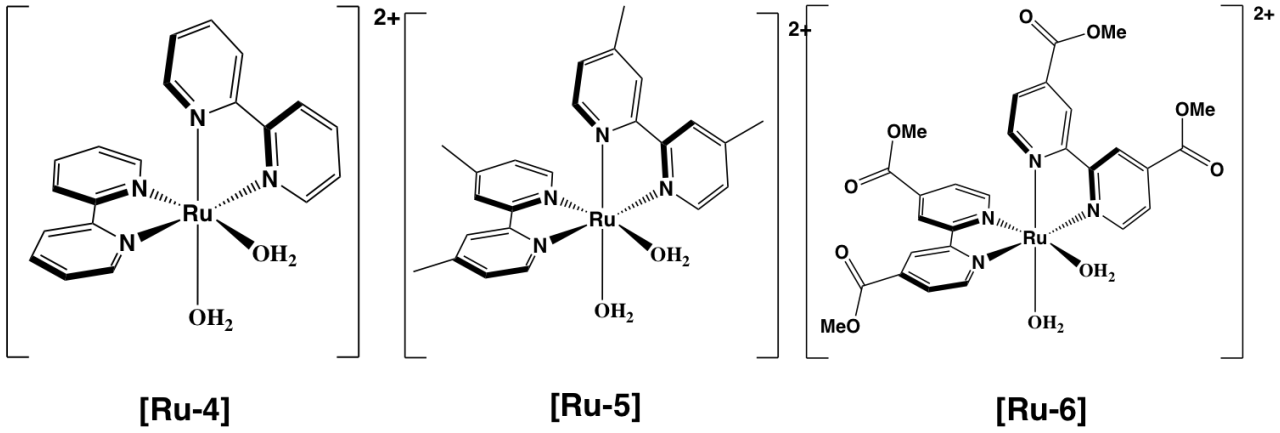


Figure 2. Chemical structures of ruthenium-aqua complexes **[Ru-4]**, **[Ru-5]**, and **[Ru-6]**.

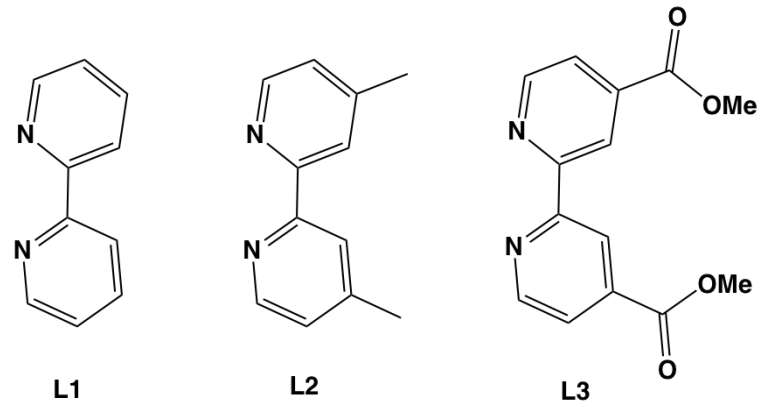
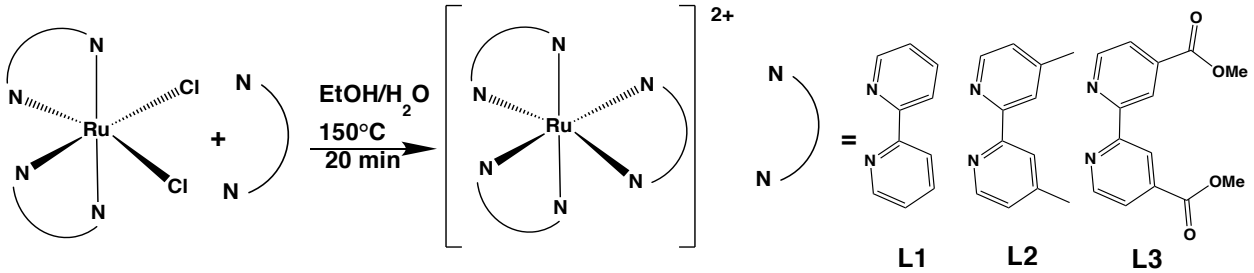


Figure 3. Chemical structures of ligands **L1** (bpy), **L2** (bpy-Me_2), and **L3** (bpy-$(COOMe)_2$).

EXPERIMENTAL SECTION

Materials and Methods. 2,2'-Bipyridine (bpy), 2,2'-Bipyridine-4,4'-dicarboxylic acid, and 1-Methylbenzimidazole were purchased from Combi-Blocks. 2,2'-bi-4-picoline (bpy-Me_2) and benzimidazole were purchased from Oakwood. Silver nitrate was purchased from Alfa-Aesar. Magnesium sulfate was purchased from Sigma-Aldrich. All were used as received. RuL_2Cl_2 complexes were prepared as described in the literature.[xx] All other reagents were ACS grade and used without additional purification. Microwave reactions were carried out in a CEM Discover SP microwave synthesizer. ^1H spectra were recorded on a Bruker 500 MHz spectrometer or a Bruker 300 MHz spectrometer.

General Synthesis of $[RuL_3]^{2+}$ Complexes. In a typical procedure, RuL_2Cl_2 (300 mg, 0.619 mmol) and **L** (0.619 mmol) were added to a microwave vessel along with 1:1 EtOH/H_2O (20 mL). The reaction was heated at 150 °C for 20 min. The resulting solution was then filtered and the solvent was removed from the filtrate on a rotary evaporator. The solid residue was collected and washed with Et_2O. The PF_6^- salts of the complexes were obtained *via* salt metathesis where an aqueous solution of NH_4PF_6 was added to a solution of the chloride salt in H_2O. Orange precipitates formed in all cases, which were filtered and washed with Et_2O.



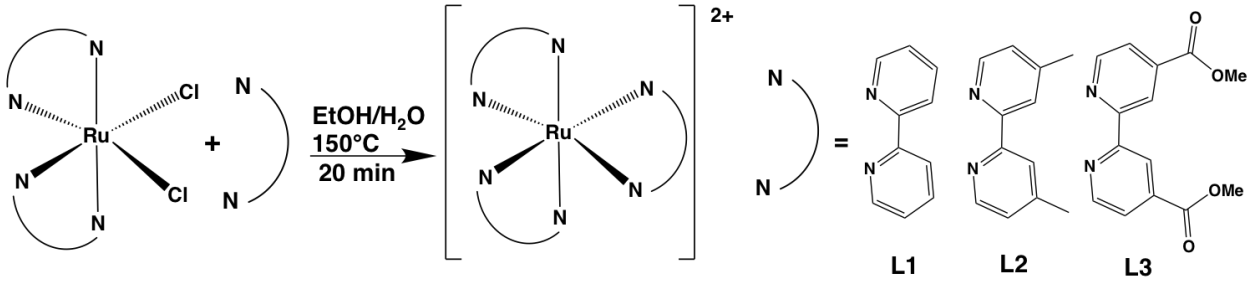
Scheme 1. General synthesis of $[RuL_3]^{2+}$ complexes.

[Ru-1]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.66 (s, 1H), 8.86 (t, J = 7.5 Hz, 3H), 8.78 (d, J = 7.5 Hz, 1H), 8.270 – 8.062 (m, 5H), 7.95 (d, J = 5.7 Hz, 1H), 7.855 – 7.703 (m, 4H), 7.616 – 7.471 (m, 4H), 7.38 (t, J = 7.2 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 5.68 (d, J = 8.4 Hz, 1H).

[Ru-2]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71(m, 6H), 7.53 (m, 6H), 7.33 (m, 6H).

[Ru-3]. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.41 (s, 6H), 7.99 (s, 6H), 7.86 (s, 6H), 3.97 (s, 18H), 2.50 (s, 18H).

General Synthesis of $[RuL_2(OH_2)_2]^{2+}$ Complexes. In a typical procedure, RuL_2Cl_2 (0.619 mmol) and 210 mg $AgNO_3$ (1.24 mmol) were added to a flask along with 1:1 MeOH/H_2O (20 mL). The reaction was heated at 40 °C for 60 min. The resulting solution was then filtered through celite and the solvent was removed from the filtrate on a rotary evaporator. The solid residue was collected and washed with Et_2O.



Scheme 2. General synthesis of $[RuL_2(OH_2)_2]^{2+}$ complexes.

[Ru-5]. ^{1}H NMR (500 MHz, Methanol-d_4) δ 9.21 (d, J = 5.8 Hz, 2H), 8.63 (s, 2H), 8.42 (s, 2H), 7.81 (m, 2H), 7.47 (d, *J* = 5.9 Hz, 2H), 7.08 (d, *J* = 6.4 Hz, 2H), 2.78 (d, *J* = 18.4 Hz, 6H), 2.49 (d, *J* = 13.9 Hz, 6H).

[Ru-6]. ^{1}H NMR (500 MHz, Methanol-d_4) δ 9.54 (d, J = 20.0 Hz, 2H), 9.24 (d, J = 56.0 Hz, 2H), 9.01 (d, J = 69.0 Hz, 2H), 8.43 (d, J = 85.3 Hz, 2H), 8.02 (d, J = 30.5 Hz, 2H), 7.67 (d, J = 32.6 Hz, 2H), 4.15 (m, 6H), 4.02 (m, 6H).

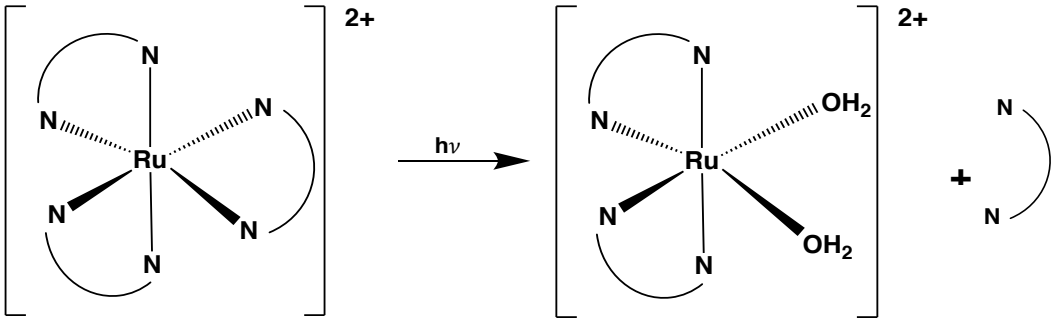
Synthesis of L_3. 2,2'-Bipyridine-4,4'-dicarboxylic acid (1.00 g, 4.0 mmol) was added to a flask along with 18 mL MeOH and 2 mL H_2SO_4. The reaction was heated at 75 °C overnight. The product was extracted in 50 mL dichloromethane with three rinses of H_2O, dried with magnesium sulfate, and isolated by removal of the solvent on a rotary evaporator. Yield: 76.5%.

Cell Viability. The cytotoxic effects of the compounds on T47D human breast cancer cells was measured using the CellTiter-Blue® Cell Viability Assay.[xxi] T47D cells were cultured in RPMI-1640 media with L-glutamine (GenClone) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) and 100 U/mL Penicillin-100 μg/mL Streptomycin (GenClone) at 37°C and 5% CO_2. For each experiment, the cells were seeded into a 96-well plate with 5,000 cells/well (100 μL/well) and grown for 2-4 days. Then the cells were washed with PBS. Indicated concentrations of compound were added to the wells in triplicate. After 24 hours of incubation at 37°C, the cells were incubated for 2 hours in the presence of 0.15 mg/mL resazurin in PBS (pH 7.4). Fluorescence was measured using an excitation wavelength of 555 nm and an emission wavelength of 585 nm using a SpectroMax i3 plate reader (Molecular Devices).

RESULTS AND DISCUSSION

For a compound to be suitable for PACT, it should not be cytotoxic until activated by light irradiation. This allows for clinical treatment to be selective for cancer cells, as light irradiation can be targeted in patients to activate a prodrug in only specific tumors and tissues. In this study,

each series of synthesized complexes could be considered suitable for PACT if the $[RuL_3]^{2+}$ complex was biologically inactive and at least one of the irradiation products was found to be cytotoxic. It is hypothesized that upon irradiation, a ligand dissociates from the $[RuL_3]^{2+}$ complex, and water molecules from the surrounding environment coordinate to the ruthenium metal center in its place (Scheme 3). Ruthenium complexes **[Ru-4]**, **[Ru-5]**, and **[Ru-6]** were synthesized to mimic the likely irradiation products of **[Ru-1]**, **[Ru-2]**, and **[Ru-3]**, respectively. All synthesized compounds were characterized using ^{1}H NMR spectroscopy. Their cytotoxic effects on T47D cells were then evaluated to determine whether **[Ru-1]**, **[Ru-2]**, and **[Ru-3]** have potential for use in photoactivated chemotherapy. The cytotoxicity of the ruthenium irradiation products and individual ligands were tested separately, since it is important to know where the toxicity originates when considering a molecule for PACT.



Scheme 3. General scheme for light-activated ligand dissociation from $[RuL_3]^{2+}$ complexes.

None of the $[RuL_3]^{2+}$ complexes demonstrated cytotoxicity, as cell survival, measured in fluorescence, did not decrease in the presence of increasing concentrations of these compounds (Figure 4). Since these complexes were designed to be used as prodrugs, these preliminary results confirm the possibility that any of these three compounds could be suitable for PACT, given that one of their irradiation products proves to be cytotoxic.

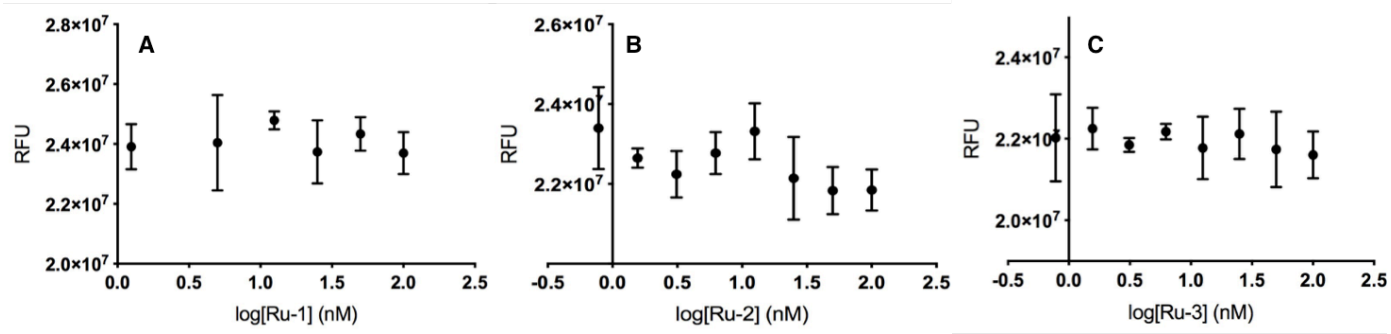


Figure 4. Cytotoxicity dose responses of $[RuL_3]^{2+}$ complexes in T47D cells. (A) **[Ru-1]**; (B) **[Ru-2]**; (C) **[Ru-3]**. n = 3.

Excitingly, all three of the $[Ru(L_2)(OH_2)_2]^{2+}$ complexes displayed strong cytotoxic activity, with significant cell death occurring in the presence of higher concentrations of these complexes (Figure 5). The EC_{50} values calculated for each of these complexes are shown in Table 1. Since the $[RuL_3]^{2+}$ complexes did not demonstrate cytotoxicity but the $[Ru(L_2)(OH_2)_2]^{2+}$ did, these results exhibit promising potential for **[Ru-1]**, **[Ru-2]**, and **[Ru-3]** to be used as PACT prodrugs. While each of the $[Ru(L_2)(OH_2)_2]^{2+}$ complexes clearly show a trend toward cytotoxicity, more studies of compounds **[Ru-4]** and **[Ru-6]** are needed to demonstrate their ability to cause complete cell death at high concentrations, as seen with **[Ru-5]** (Figure 5B).

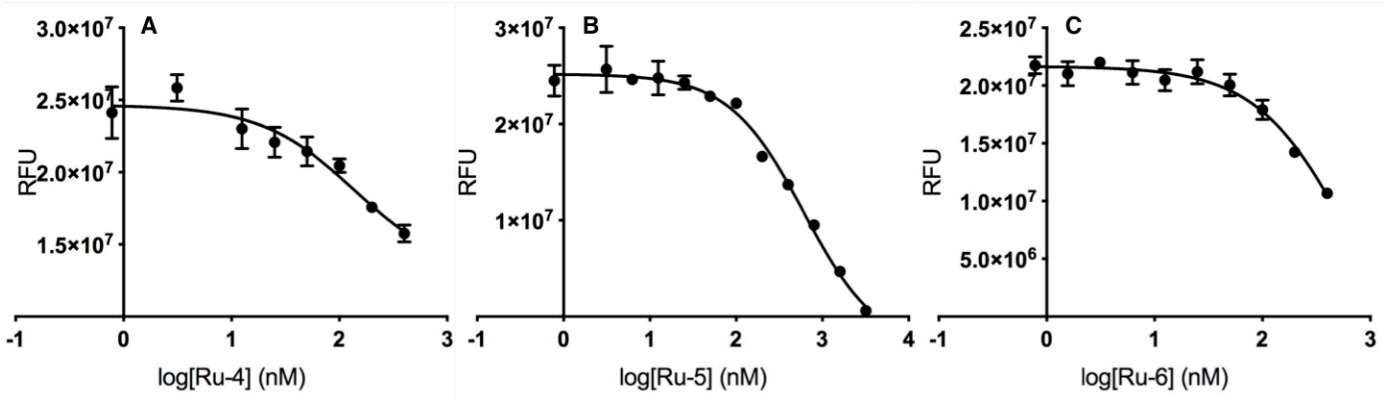


Figure 5. Cytotoxicity dose responses of $[RuL_2(OH_2)_2]^{2+}$ complexes in T47D cells. (A) **[Ru-4]**; (B) **[Ru-5]**; (C) **[Ru-6]**. n = 3.

Compound	log(EC_{50}) (nM)	Standard Error	95% Confidence Interval	EC_{50} (nM)	95% Confidence Interval (CI)	R^2	Degrees of freedom
[Ru-4]	**2.14**	**0.1761**	**(1.765 - 2.611)**	**138**	**(56.98 - 408.2)**	**0.8836**	**21**
[Ru-5]	**2.785**	**0.04657**	**(2.689- 2.884)**	**609.6**	**(488.6 - 765.4)**	**0.9847**	**33**
[Ru-6]	**2.812**	**0.183**	**(2.518 – 3.338)**	**659.1**	**(329.8 - 2179)**	**0.9524**	**27**

Table 1. The cytotoxicity EC_{50} values of **[Ru-4]**, **[Ru-5]**, and **[Ru-6]** in T47D cells. Data were collected using a CellTiter-Blue® Cell Viability Assay and analyzed using least-squares regression. EC_{50} values are averages from assays run in triplicate.

Similar to the $[RuL_3]^{2+}$ complexes, none of the ligands themselves demonstrated cytotoxicity at the concentrations tested (Figure 6). Based on these results, it is likely that if the $[RuL_3]^{2+}$ complexes were to be photoactivated, the resulting cytotoxicity would come from the ruthenium photoproduct and not from the dissociated ligand, since the ligands showed no cytotoxicity on their own. The cytotoxicity of the ligands was generally more variable than that of the ruthenium complexes, possibly due to issues with solubility. Though each of the complexes and ligands tested were dissolved in ethanol prior to adding them to the cell media for cell treatment, **L1** was dissolved in DMSO because of increased solubility compared to ethanol.

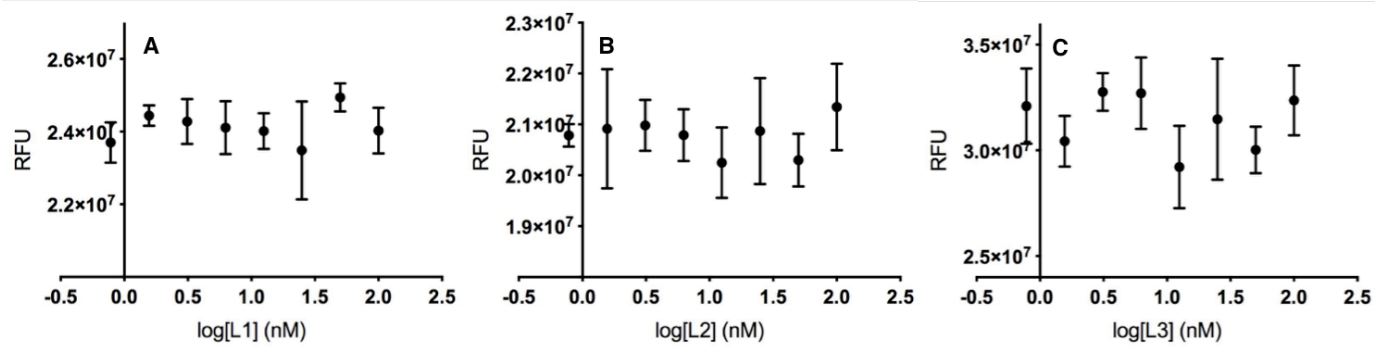


Figure 6. Cytotoxicity dose responses of ligands in T47D cells. (A) **L1**; (B) **L2**; (C) **L3**. n = 3.

T47D cells were chosen as an appropriate cellular model for testing the cytotoxicity of these compounds because of the potential for PACT to be used to treat triple-negative and multidrug-resistant breast cancer and because methods of light delivery to breast tissue have already been established.[xxii,xxiii] Further studies in other cell lines, such as A549 human lung carcinoma cells, should be conducted to confirm the results found in the T47D cells and evaluate the possibility of using these compounds to treat other cancer types.

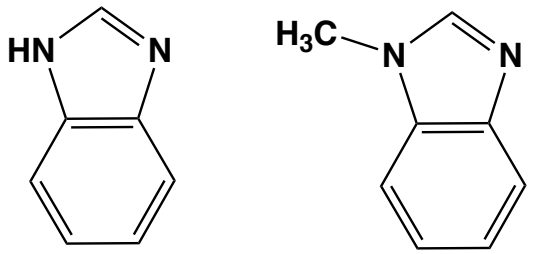
In drug design, EC_{50} values are often used to determine the potency of a given compound. They represent the concentration of compound needed to cause half of the maximal biological response. For a lead small molecule compound in a drug discovery pipeline, it is generally agreed that compounds with EC_{50} values below 1 μM are good candidates for further optimization.[xxiv] A comparison of the EC_{50} values calculated for **[Ru-4]**, **[Ru-5]**, and **[Ru-6]** reveals that **[Ru-4]** is significantly more potent than **[Ru-5]**, with EC_{50} values of 138 nM (56.98-408.2) and 609.6 nM (448.6-765.4), respectively. The EC_{50} value of **[Ru-6]** is the highest at 659.1 nM (329.8-2179), but the error is too large to determine whether this is significantly higher than either of the other two complexes. The fact that these compounds have EC_{50} values in the nM range indicates they are effective drug candidates. From these data, it seems that the complexes become less potent with increasing ligand size, although additional studies must be

done to confirm this trend and it may instead be due to other factors, such as electronic properties that vary significantly across the series. It is possible that the electron-donating character of the methyl groups on **L2** and the electron-withdrawing character of the carboxymethyl groups on **L3** affected the potency of **[Ru-5]** and **[Ru-6]**, but further investigation is necessary to explore these trends.

Along with potency, ease of ligand dissociation is another important factor in determining whether a compound might make an effective PACT prodrug. While **[Ru-4]** might have been the most potent of the three ruthenium photoproducts synthesized, this does not necessarily mean **[Ru-1]** is the best candidate for further investigation, as it could prove to be more difficult to photoactivate than **[Ru-2]** or **[Ru-3]**. The wavelength at which ligand dissociation occurs is also an essential factor in determining whether a compound can be used to treat tumors in different tissue areas, as different tissues absorb different wavelengths of light. The chemical differences between the ligands on the complexes studied might cause them to dissociate at different wavelengths and be optimal for treatment of different cancer types. The photoactivation of the $[RuL_3]^{2+}$ compounds studied here is yet to be explored.

One idea for increasing the ease of ligand photodissociation from ruthenium PACT complexes is to design complexes with monodentate ligands rather than bidentate ligands. Designing complexes with cytotoxic ligands is also an important strategy in optimizing the potency of the photoactivated compounds. Combining these two properties, there are examples in the literature of cytotoxic ruthenium complexes containing monodentate benzimidazole-derived ligands.[xxv,xxvi,xxvii] For this reason, we studied the cytotoxicity of two monodentate ligands, benzimidazole and 1-methylbenzimidazole (Figure 7). Though neither of these compounds

demonstrated cytotoxicity (Figure 8), this avenue of inquiry may be worth pursuing. We have not yet proceeded with coordinating these ligands to ruthenium complexes.



Benzimidazole **1-Methylbenzimidazole**

Figure 7. Chemical structures of benzimidazole and 1-methylbenzimidazole.

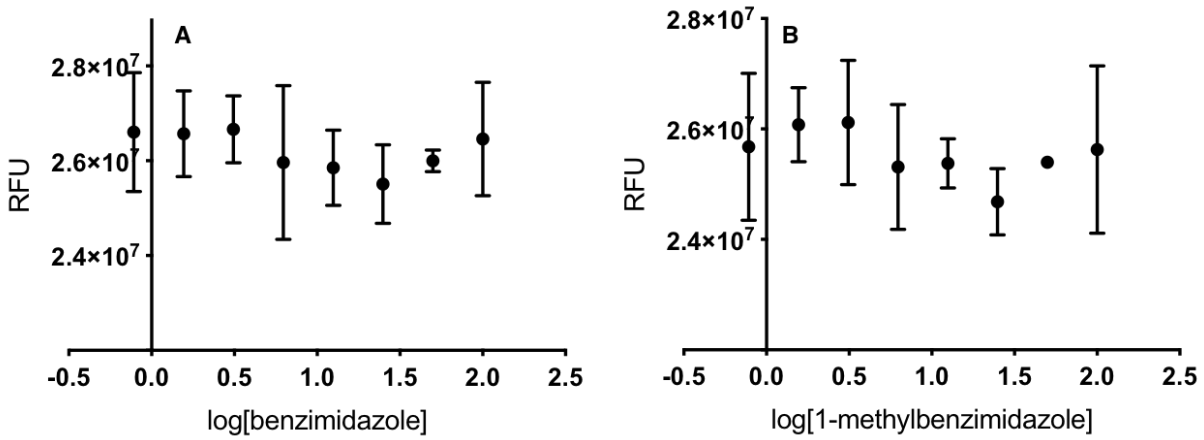


Figure 8. Cytotoxicity dose responses of ligands in T47D cells. (A) Benzimidazole; (B) 1-methylbenzimidazole. n = 3.

CONCLUSIONS

In conclusion, we have demonstrated the cytotoxic properties of three novel ruthenium complexes and their ligand dissociation products. While the ruthenium complexes and their ligands exhibited no effect on cell viability, their Ru-aqua dissociation complexes were demonstrated to be cytotoxic at increasing concentrations. The potency of these complexes were

investigated and compared. The findings of this study demonstrate the potential use of **[Ru-1]**, **[Ru-2]**, and **[Ru-3]** as PACT compounds.

ACKNOWLEDGMENT

I would like to thank Dr. Mike Norris for welcoming me into his lab, helping me design this project, and mentoring me throughout its completion and during my entire college career. I would also like to thank Dr. Julie Pollock for training me in cell culturing techniques and allowing me to use her lab for this project. Additionally, I would like to thank Melissa Gu for synthesizing some of the materials used to create the ruthenium complexes described in this paper, as well as the rest of the members of the Norris lab. This work was supported by the University of Richmond.

---

[i] "All Cancers: Globocan 2018." *World Health Organization International Agency for Research on Cancer*, March, 2019. http://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf

[ii] Bonnet, Sylvestre. "Why Develop Photoactivated Chemotherapy?" *Dalton Transactions* 47, no. 31 (2018): 10330–43. https://doi.org/10.1039/C8DT01585F.

[iii] Tsimberidou, A. M.; Fountzilas, E.; Nikanjam, M.; Kurzrock, R. Review of Precision Cancer Medicine: Evolution of the Treatment Paradigm. *Cancer Treat. Rev.* **2020**, *86*, 102019. https://doi.org/10.1016/j.ctrv.2020.102019.

[iv] Rijt, Sabine H. van, and Peter J. Sadler. "Current Applications and Future Potential for Bioinorganic Chemistry in the Development of Anticancer Drugs." *Drug Discovery Today* 14, no. 23–24 (December 2009): 1089. https://doi.org/10.1016/j.drudis.2009.09.003.

[v] Bonnet, 2018.

[vi] Bonnet, 2018.

[vii] Cuello-Garibo, J.-A.; S. Meijer, M.; Bonnet, S. To Cage or to Be Caged? The Cytotoxic Species in Ruthenium-Based Photoactivated Chemotherapy Is Not Always the Metal. *Chemical Communications* **2017**, *53* (50), 6768–6771. https://doi.org/10.1039/C7CC03469E.

[viii] Zeng, L.; Gupta, P.; Chen, Y.; Wang, E.; Ji, L.; Chao, H.; Chen, Z.-S. The Development of Anticancer Ruthenium(II) Complexes: From Single Molecule Compounds to Nanomaterials. *Chem Soc Rev* **2017**, *46* (19), 5771–5804. https://doi.org/10.1039/c7cs00195a.

[ix] Bonnet, 2018.

[x] Bonnet, 2018.

[xi] Wilson, B. C.; Patterson, M. S. The Physics, Biophysics and Technology of Photodynamic Therapy. *Phys Med Biol* **2008**, *53* (9), R61-109. https://doi.org/10.1088/0031-9155/53/9/R01.

[xii] Farrer, Nicola J., Luca Salassa, and Peter J. Sadler. "Photoactivated Chemotherapy (PACT): The Potential of Excited-State d-Block Metals in Medicine." *Dalton Transactions (Cambridge, England : 2003)*, no. 48 (December 28, 2009): 10690–701. https://doi.org/10.1039/b917753a.

[xiii] Ndagi, Umar, Ndumiso Mhlongo, and Mahmoud E Soliman. "Metal Complexes in Cancer Therapy – an Update from Drug Design Perspective." *Drug Design, Development and Therapy* 11 (March 3, 2017): 599–616. https://doi.org/10.2147/DDDT.S119488.

[xiv] Rijt *et al*., 2009.

[xv] Hartinger, Christian G., Stefanie Zorbas-Seifried, Michael A. Jakupec, Bernd Kynast, Haralabos Zorbas, and Bernhard K. Keppler. "From Bench to Bedside – Preclinical and Early Clinical Development of the Anticancer Agent Indazolium Trans-[Tetrachlorobis(1H-Indazole)Ruthenate(III)] (KP1019 or FFC14A)." *Journal of Inorganic Biochemistry*, Special Issue Containing Contributions from the 12th International Conference on Biological Inorganic Chemistry, 100, no. 5 (May 1, 2006): 891–904. https://doi.org/10.1016/j.jinorgbio.2006.02.013.

[xvi] Alessio, E.; Messori, L. The Deceptively Similar Ruthenium(III) Drug Candidates KP1019 and NAMI-A Have Different Actions. What Did We Learn in the Past 30 Years? *Met Ions Life Sci* **2018**, *18*. https://doi.org/10.1515/9783110470734-011.

[xvii] Cuello-Garibo *et al.*, 2017.

[xviii] Zeng *et al*., 2017.

[xix] Bonnet, S. Shifting the Light Activation of Metallodrugs to the Red and Near-Infrared Region in Anticancer Phototherapy. *Comments on Inorganic Chemistry* **2015**, *35* (4), 179–213. https://doi.org/10.1080/02603594.2014.979286.

[xx] Norris, M. R.; Concepcion, J. J.; Glasson, C. R. K.; Fang, Z.; Lapides, A. M.; Ashford, D. L.; Templeton, J. L.; Meyer, T. J. Synthesis of Phosphonic Acid Derivatized Bipyridine Ligands and Their Ruthenium Complexes. *Inorg. Chem.* **2013**, *52* (21), 12492–12501. https://doi.org/10.1021/ic4014976.

[xxi] CellTiter-Blue® Cell Viability Assay: Technical Bulletin. *Promega Corporation* #TB317, **2016**. https://www.promega.com/-/media/files/resources/protocols/technical-bulletins/101/celltiter-blue-cell-viability-assay-protocol.pdf?la=en

[xxii] Lowdell, C. P.; Ash, D. V.; Driver, I.; Brown, S. B. Interstitial Photodynamic Therapy. Clinical Experience with Diffusing Fibres in the Treatment of Cutaneous and Subcutaneous Tumours. *Br. J. Cancer* **1993**, *67* (6), 1398–1403. https://doi.org/10.1038/bjc.1993.259.

[xxiii] Kim, A.; Zhou, J.; Samaddar, S.; Song, S. H.; Elzey, B. D.; Thompson, D. H.; Ziaie, B. An Implantable Ultrasonically-Powered Micro-Light-Source (MLight) for Photodynamic Therapy. *Sci Rep* **2019**, *9* (1), 1395. https://doi.org/10.1038/s41598-019-38554-2.

[xxiv] Stocks, Michael. "Chapter 3 - The Small Molecule Drug Discovery Process – from Target Selection to Candidate Selection." In *Introduction to Biological and Small Molecule Drug Research and Development*, edited by Robin Ganellin, Stanley Roberts, and Roy Jefferis, 81–126. Oxford: Elsevier, 2013. https://doi.org/10.1016/B978-0-12-397176-0.00003-0.

[xxv] Bhattacharyya, S.; Purkait, K.; Mukherjee, A. Ruthenium(II) p-Cymene Complexes of a Benzimidazole-Based Ligand Capable of VEGFR2 Inhibition: Hydrolysis, Reactivity and Cytotoxicity Studies. *Dalton Trans.* **2017**, *46* (26), 8539–8554. https://doi.org/10.1039/C7DT00938K.

[xxvi] Lari, M.; Martínez-Alonso, M.; Busto, N.; Manzano, B. R.; Rodríguez, A. M.; Acuña, M. I.; Domínguez, F.; Albasanz, J. L.; Leal, J. M.; Espino, G.; García, B. Strong Influence of Ancillary Ligands Containing Benzothiazole or Benzimidazole Rings on Cytotoxicity and Photoactivation

of Ru(II) Arene Complexes. *Inorg Chem* **2018**, *57* (22), 14322–14336. https://doi.org/10.1021/acs.inorgchem.8b02299.
[xxvii] Yellol, J.; Pérez, S. A.; Buceta, A.; Yellol, G.; Donaire, A.; Szumlas, P.; Bednarski, P. J.; Makhloufi, G.; Janiak, C.; Espinosa, A.; Ruiz, J. Novel C,N-Cyclometalated Benzimidazole Ruthenium(II) and Iridium(III) Complexes as Antitumor and Antiangiogenic Agents: A Structure–Activity Relationship Study. *J. Med. Chem.* **2015**, *58* (18), 7310–7327. https://doi.org/10.1021/acs.jmedchem.5b01194.