Check for updates

View Article Online
View Journal

Dalton Transactions

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: D. Azar, H. Audi, S. Farhat, M. El Sibai, R. Abi-Habib and R. S. Khnayzer, *Dalton Trans.*, 2017, DOI: 10.1039/C7DT02255G.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the author guidelines.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the ethical guidelines, outlined in our author and reviewer resource centre, still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/dalton

Please do not adjust margins

ROYAL SOCIETY OF CHEMISTRY
View Article Online
DOI: 10.1039/C7DT02255G

Dalton Transactions

COMMUNICATION

Phototoxicity of Strained Ru(II) Complexes: Is it the Metal Complex or the Dissociating Ligand?

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Daniel Azar,[a] Hassib Audi,[a] Stephanie Farhat,[a] Mirvat El-Sibai,[a] Ralph Abi-Habib[a] and Rony S. Khnayzer[a,*]

Open Access Article. Published on 13 July 2017. Downloaded on 14/07/2017 11:43:32.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

A photochemically dissociating ligand in $Ru(bpy)_2(dmphen)Cl_2$ [bpy = 2,2'-bipyridine; dmphen = 2,9-dimethyl-1,10-phenanthroline] was found to be more cytotoxic on ML-2 cancer cell line than $Ru(bpy)_2(H_2O)_2^{2+}$ and the prototypical cisplatin. Our findings illustrate the potential potency of diimine ligands in photoactivatable Ru(II) complexes.

Cancer is a disease associated with high mortality rates in the entire world.[1] Limited numbers of metal-based chemotherapeutic agents are clinically approved, among which is the prototypical drug cisplatin.[2] This Pt(II) complex was associated with side-effects and drug-resistance problems among patients.[3, 4] On one hand, photoactivated chemotherapeutic drugs (PACT)[5] rely on the selective, localized and stoichiometric release of cytotoxic substances following controlled light irradiation.[6, 7] Using this technique, tumor can be targeted with less side effects than conventional chemotherapy since prodrugs are ideally inert in the dark at the concentration used.[5, 8] When transition metal complexes are deployed, the photochemical reactivity can be modulated by virtue of structural modifications.[9] The photophysical tuning of the excited state can be achieved via fine design of the organic ligand framework.[10] On the other hand, photodynamic therapy (PDT) drugs act through the catalytic production of $^1O_2$ and subsequently reactive oxygen species (ROS) that will induce cell death.[11] More recently, research on the photochemical release of cytotoxic agents through ligand dissociation of PACT is incrementing.[12-15] A significant tuning of photochemical reactivity was afforded by ligand functionalization around metal centers.[9] In sterically congested Ru(II) polypyridyl complexes bearing alkyl or phenyl substituents on the 2,9 positions of 1,10-phenanthroline or 6,6' positions of 2,2'-bipyridine, photochemical ligand ejection follows the population of a dissociative triplet metal-centered excited state (^{3*}MC).[16-18] The latter can be thermally populated from the triplet metal-to-ligand charge transfer state (^{3*}MLCT).[19] As the distortions from the regular octahedral geometry increase, the formation of the ^{3*}MC state was increasingly favored.[16-18] In recent studies, attention has been paid to the role of $Ru(bpy)_2(H_2O)_2^{2+}$ product formed as a result of photochemical dissociation of sterically strained diimine ligands.[13] This photoproduct is believed to mimic cisplatin's mode of action by cross-linking DNA.[20] However, along with the release of these species, side-products consisting of free ligands are also formed. Recently, the 2,9-dimethyl-1,10-phenanthroline (dmphen) ligand was used in an examples of chiral Ru(II) complexes containing a 2,3-dihydro-1,4-dioxino[2,3-f]-1,10-phenanthroline (dop)[15] ligand or hydroxyquinoline ligand[21]. In addition, structurally related compounds to dmphen were used in conjunction with bipyridine such as dmdop (2,3-dihydro-1,4-dioxino[2,3-f]-2,9-dimethyl-1,10-phenanthroline)[15] or dpq (dipyrido[3,2-f:2',3'-h]-quinoxaline)[13]. All these examples furnished Ru(II) complexes that demonstrated a potential application as PACT drugs in cancer therapy. In general, the biological role of the dissociated ligands is ill-characterized and mostly assumed to be inert. While this assumption is correct for a variety of examples tested on a number of cell lines, we found that the free dmphen is significantly more toxic than $Ru(bpy)_2(H_2O)_2^{2+}$ co-product on ML-2 cell lines (non-adherent Acute Myeloid Leukemia (AML) cells). Despite the fact that the utilization of PACT which produce multiple potentially potent species might complicate the mechanistic studies in biological media, we believe it has a major impact on future photosensitizer design and application. In that perspective, the metal center can be merely used as a carrier for highly cytotoxic ligands. The latter would be selectively released inside or in the vicinity of cancer cells upon light activation. Notably, the photochemistry of caged Ru compounds has been utilized for the release of biologically active molecules such as serotonin,[22] CO,[23] NO,[24] etc.

$Ru(bpy)_2(dmphen)(PF_6)_2$ and $Ru(bpy)_2(dmphen)Cl_2$ were synthesized and characterized using a variety of techniques, see ESI.[25] The quantum yield of ligands photoejection from $Ru(bpy)_2(dmphen)(PF_6)_2$ was measured following 442 nm He/Cd laser excitation (Fig. S4). A value of 0.32±0.03 % was obtained in acetonitrile which is comparable to previously reported sterically strained $Ru(m\text{-}bpy)_3(BF_4)_2$ [m-bpy = 6-methyl-2,2'-bipyridine] and one order of magnitude larger than $Ru(bpy)_3(PF_6)_2$.[26] Note that these quantum yield values are acquired for complexes bearing non-coordinating counter-ions in acetonitrile as a coordinating solvent. NMR spectroscopy in CD_3CN revealed that either bpy or dmphen

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

Electronic Supplementary Information (ESI) available: Experimental section, NMR spectra, HPLC chromatogram, ESI-MS, UV-vis spectra and cytotoxicity data. See DOI: 10.1039/x0xx00000x

Dalton Transactions Accepted Manuscript

Please do not adjust margins

View Article Online
DOI: 10.1039/C7DT02033J

ligands can dissociate from $Ru(bpy)_2(dmphen)(PF_6)_2$ forming a mixture of solvent-bound Ru(II) products[27] and free ligands in ~ 1:1 (bpy:dmphen) ratio (Fig. S5).

The photochemical ligand dissociation of the water-soluble $Ru(bpy)_2(dmphen)Cl_2$ was studied in acetonitrile and water under a versatile broadband irradiation (white LED light, see ESI for details). Under those experimental conditions, the half-life in acetonitrile was found to be 5 times less in water (~ 5 min) than in acetonitrile (~ 25 min) (Fig. 1). The rate of ligands release was slower in water than in acetonitrile which is attributed to a larger solubility of the ejected ligand in the latter as well as the preference of Ru(II) center to acetonitrile over aquo ligands. It is worth noting that $Ru(bpy)_2(dmphen)Cl_2$ was stable in the dark as evidenced by the lack of change in UV-vis spectra after a week of storage in aqueous solution.

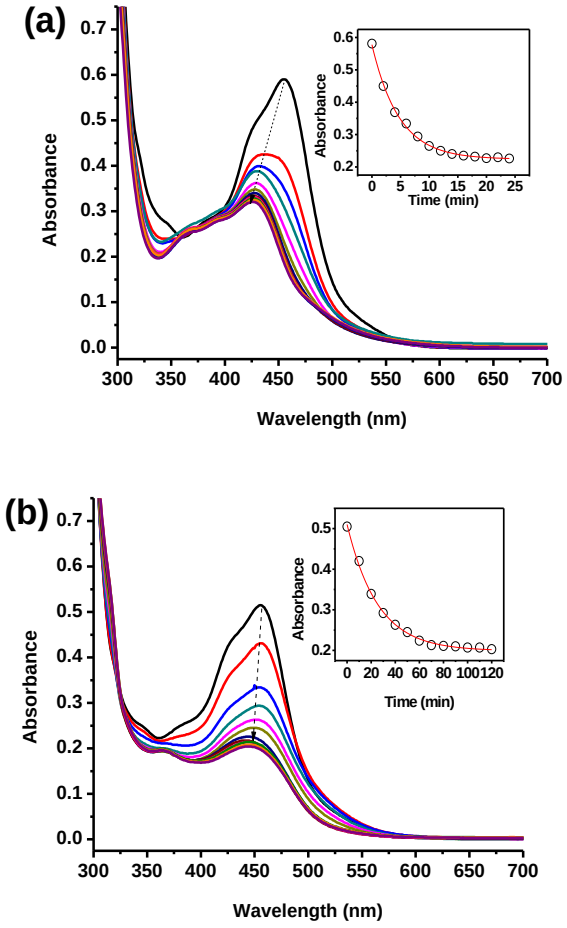


Fig. 1 Absorption spectra of a stirred solution of $Ru(bpy)_2(dmphen)Cl_2$ (30 µM) irradiated with white LED light (see ESI for more details) placed 2 cm far from a 1 cm pathlength quartz cuvette in (**a**) acetonitrile recorded at 2 min interval and (**b**) water at 10 min intervals. Dashed arrows indicate the progress of the charge transfer absorption peak. The inset represents the change of absorbance at 450 nm as a function of irradiation time.

Previously reported PACT agents bearing substituted bipyridil ligands such as $Ru(bpy)_2(dmbpy)Cl_2$ [dmbpy = 6,6'-dimethyl-2,2'-bipyridine] displayed a quantitative photoejection of the dmbpy ligand.[13] However, it is not uncommon to have the non-substituted ligand, such as bipyridine, dissociate from a sterically encumbered ruthenium complex. Sauvage and coworkers have previously reported the photochemical dissociation of bipyridine from $Ru(bpy)_2(dpph)(PF_6)_2$ [dppH = 2,9-diphenyl-1,10-phenanthroline].[27] Since ligand release likely occurs through a stepwise one nitrogen dissociation of the diimine moiety,[28] the re-coordination of the rigid phenanthroline based moiety is more efficient than the flexible bipyridine ligands leading to competitive photo-induced ligand dissociation of dmphen and bpy ligands from $Ru(bpy)_2(dmphen)Cl_2$. In addition, steric interactions around the metal can be relieved via an assymetrical distortion of the octahedral geometry and Ru-N bond elongation labilizing both dmphen and bpy ligands.[29] The cytotoxicities of $Ru(bpy)_2(dmphen)Cl_2$, $Ru(bpy)_2Cl_2$, dmphen, bpy, an equimolar mixture of $Ru(bpy)_2Cl_2$ and dmphen, and cisplatin were measured on ML-2 cancer cell line (Fig. 2 and Table 1).

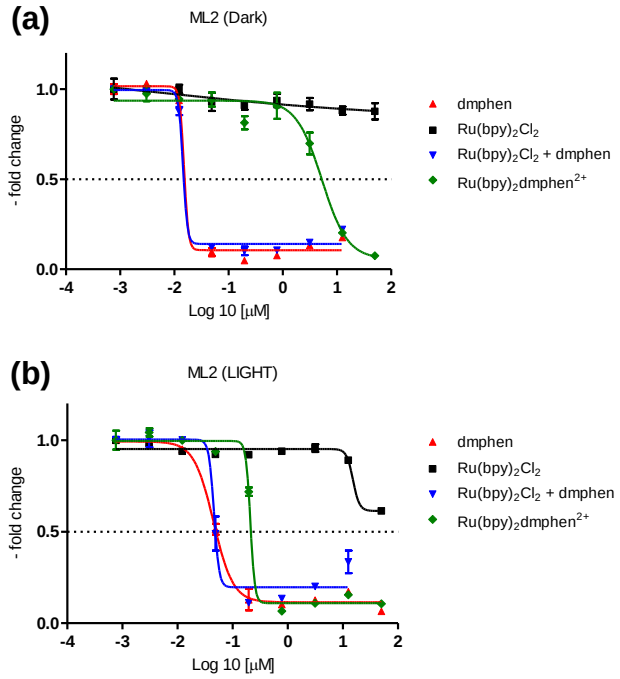


Fig. 2 Cytotoxicity data acquired on ML2 cell line of $Ru(bpy)_2dmphenCl_2$, $Rubpy_2Cl_2$, 2,9-dimethyl-1,10-phenanthroline (dmphen), and a mixture of $Rubpy_2Cl_2$ and dmphen in the dark (**a**) and upon blue-light (see ESI for more details) excitation (**b**).

Table 1 IC_{50} on ML2 cell lines expressed in µmol/L of $Ru(bpy)_2dmphenCl_2$, $Rubpy_2Cl_2$, 2,9-dimethyl-1,10-phenanthroline (dmphen), 2,2'-bipyridine (bpy), and a mixture of $Rubpy_2Cl_2$ and dmphen, in the dark and upon light activation. Cisplatin and bpy controls (Fig. S6) were acquired in the dark.

Compound	Dark	Light
$Ru(bpy)_2dmphenCl_2$	5.5 µM	0.2 µM
$Rubpy_2Cl_2$	> 100 µM	>100 µM
dmphen	0.02 µM	0.04 µM
bpy	> 100 µM	
$Rubpy_2Cl_2$+dmphen	0.02 µM	0.04 µM
cisplatin	4.0 µM	

Open Access Article. Published on 13 July 2017. Downloaded on 14/07/2017 11:43:32.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Dalton Transactions Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

View Article Online
DOI: 10.1039/C7DT01922G

Open Access Article. Published on 13 July 2017. Downloaded on 14/07/2017 11:43:32.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

The photoresponsive $Ru(bpy)_2(dmphen)Cl_2$ was tested in the dark and upon photoactivation revealing a phototoxicity index (PI = [IC_{50} dark]/[IC_{50} light]) of 27.5, with IC_{50} in the dark of 5.5 µM and in the light of 0.2 µM (Fig. 2 and Table 1). A blue LED light source (see ESI for details) was used to provide metal-to-ligand charge transfer (MLCT) photoexcitation. The IC_{50} of the dmphen ligand was found ~ 0.02-0.04 µM (difference between light and dark is not significant due to plate to plate variability) with and without the presence of $Ru(bpy)_2Cl_2$ indicating that the former is significantly more potent than the latter. Notably, this ligand was also more potent than the prototypical cisplatin complex which possessed an IC_{50} of 4.0 µM when measured under dark conditions, Fig. S6. It is worth noting that the bpy ligand was found to have no potency at concentrations lower than 50 µM on ML-2 cells with IC_{50} > 100 µM, Fig. S6. In previous studies, bpy exhibited a moderate cytotoxicity (IC_{50} ~ 30 µM) on chronic myelogenous leukemia cell line (K562) whereas no potency was detected on MDA-MB-231 and MCF-7 cells.[30, 31] To further substantiate our findings, $Ru(bpy)_2Cl_2$, a thermal and photochemical precursor of $Ru(bpy)_2(H_2O)_2^{2+}$ (Fig. S8),[20, 32] exhibited marginal cytotoxicity with and without irradiation (IC_{50} > 100 µM in the dark and upon light activation, Fig. 2 and Table 1). These results are consistent with a previous cytotoxicity assessment on L1210 and HeLa cells whereby $Ru(bpy)_2(H_2O)_2^{2+}$ was shown to lack DNA interstrand cross-linking efficiency.[33] Furthermore, $Ru(bpy)_2(H_2O)_2^{2+}$ was not found to be a potent cysteine protease enzyme inhibitor in a study on isolated enzymes and human cell lysates.[34] The photoproducts of $Ru(bpy)_2(dmphen)Cl_2$ were assessed in water using ESI-MS experiments under identical irradiation conditions used in the biological studies. In aqueous medium, it was found that both bpy and dmphen dissociate in a ratio of ~3:2 (bpy:dmphen) to form the corresponding polypyridyl Ru(II) aquo species, Fig. S7. In addition, ESI-MS (Fig. S7-S8) results were supportive of the photochemical formation of $Ru(bpy)_2(H_2O)_2^{2+}$ from $Ru(bpy)_2Cl_2$ and $Ru(bpy)_2(dmphen)Cl_2$ in water. Based on these data combined, the significant phototoxicity of $Ru(bpy)_2(dmphen)Cl_2$ on ML-2 cancer cell line can be largely attributed to the released dmphen ligand rather than the formation of $Ru(bpy)_2(H_2O)_2^{2+}$ which was found to be minimally potent under our experimental conditions. Peculiarly, dmphen was also previously found to be potent on L1210 cell line with IC_{50} ~ 0.25 µM.[35] The mechanistic function of metal binding chelators, such as dmphen, was proposed to be through two plausible pathways: binding to free essential metals or to trace-metal contaminants in cell culture media.[35] Both mechanisms lead to the formation of biologically active metal-chelate complexes.[35] Despite the fact that our data clearly shows the potential role of dmphen ligand in dictating the photobiological activity of the $Ru(bpy)_2dmphenCl_2$ complex, we can't rule out the contribution of other possible photoproducts such as $Ru(bpy)(dmphen)(H_2O)^{2+}$ which was detected by ESI-MS, Fig. S7. In addition, besides water, there are multiple potential ligands in biological media leading to the formation of multiple species that could bind DNA or target specific organelles within the cell.[20]

Conclusions

$Ru(bpy)_2(dmphen)Cl_2$, a sterically congested and photochemically labile Ru(II) complexes, was investigated against ML-2 Acute Myeloid Leukemia (AML) cancer cell line. Upon visible light irradiation in water, either bpy or dmphen ligands dissociate to form polypyridyl Ru(II) aquo species. The ligand ejection was more rapid in acetonitrile than in water likely due to the better solvation of photoproducts in the former solvent. $Ru(bpy)_2Cl_2$, a thermal and photochemical precursor to $Ru(bpy)_2(H_2O)_2^{2+}$,[32] was found to be minimally potent relative to the highly cytotoxic dmphen when tested independently on ML-2 cancer cell line in the dark and upon photoactivation. These experiments clearly indicate that the ruthenium center can act as a carrier to a cytotoxic diimine ligand. In addition, these findings unveil the potential role of dissociating ligands in the biological mechanism of action in strained polypyridyl Ru(II) produgs. Finally, this work may aid the development and understanding of new caged PACT drugs containing cytotoxic phenanthroline or bipyridine derivatives.

Acknowledgements

RSK acknowledges 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. L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-Tieulent and A. Jemal, *CA Cancer J. Clin.*, 2015, **65**, 87-108.
2. A. S. Abu-Surrah and M. Kettunen, *Curr. Med. Chem.*, 2006, **13**, 1337-1357.
3. V. Cepeda, M. A. Fuertes, J. Castilla, C. Alonso, C. Quevedo and J. M. Perez, *Anti-cancer Agent Me.*, 2007, 3-18.
4. M. A. Fuertes, C. Alonso and J. M. Perez, *Chem. Rev.*, 2003, **103**, 645-662.
5. N. J. Farrer, L. Salassa and P. J. Sadler, *Dalton Trans.*, 2009, 10690-10701.
6. R. Ackroyd, C. Kelty, N. Brown and M. Reed, *Photochem. Photobiol.*, 2001, **74**, 656-669.
7. M. R. Detty, S. L. Gibson and S. J. Wagner, *J. Med. Chem.*, 2004, **47**, 3897-3915.
8. D. E. J. G. J. Dolmans, D. Fukumura and R. K. Jain, *Nat. Rev. Cancer*, 2003, **3**, 380.
9. C. Mari, V. Pierroz, S. Ferrari and G. Gasser, *Chem. Sci.*, 2015, **6**, 2660-2686.
10. N. A. Smith and P. J. Sadler, *Phil. Trans. R. Soc. A*, 2013, **371**.
11. M. C. DeRosa and R. J. Crutchley, *Coord. Chem. Rev.*, 2002, **233–234**, 351-371.
12. R. N. Garner, J. C. Gallucci, K. R. Dunbar and C. Turro, *Inorg. Chem.*, 2011, **50**, 9213-9215.
13. B. S. Howerton, D. K. Heidary and E. C. Glazer, *J. Am. Chem. Soc.*, 2012, **134**, 8324-8327.
14. J. D. Knoll, B. A. Albani and C. Turro, *Acc. Chem. Res.*, 2015, **48**, 2280-2287.
15. A. N. Hidayatullah, E. Wachter, D. K. Heidary, S. Parkin and E. C. Glazer, *Inorg. Chem.*, 2014, **53**, 10030-10032.
16. J.-P. Collin, D. Jouvenot, M. Koizumi and J.-P. Sauvage, *Inorg. Chem.*, 2005, **44**, 4693-4698.
17. P. Mobian, J.-M. Kern and J.-P. Sauvage, *Angew. Chem. Int. Ed.*, 2004, **43**, 2392-2395.
18. V. W. Yam, E. Baranoff, F. Barigelletti, S. Bonnet, J.-P. Collin, L. Flamigni, P. Mobian and J.-P. Sauvage, in *Photofunctional Transition Metal Complexes*, Springer Berlin Heidelberg, 2007, vol. 123, pp. 41-78.
19. J. V. Caspar and T. J. Meyer, *J. Am. Chem. Soc.*, 1983, **105**, 5583-5590.
20. T. N. Singh and C. Turro, *Inorg. Chem.*, 2004, **43**, 7260-7262.
21. D. K. Heidary, B. S. Howerton and E. C. Glazer, *J. Med. Chem.*, 2014, **57**, 8936-8946.

Dalton Transactions Accepted Manuscript

View Article Online
DOI: 10.1039/C7DT02255G

22. L. Zayat, M. Salierno and R. Etchenique, *Inorg. Chem.*, 2006, **45**, 1728-1731.
23. M. A. Wright and J. A. Wright, *Dalton Trans.*, 2016, **45**, 6801-6811.
24. P. C. Ford, *Acc. Chem. Res.*, 2008, **41**, 190-200.
25. E. V. Dose and L. J. Wilson, *Inorg. Chem.*, 1978, **17**, 2660-2666.
26. Q. Sun, S. Mosquera-Vazquez, L. M. Lawson Daku, L. Guénée, H. A. Goodwin, E. Vauthey and A. Hauser, *J. Am. Chem. Soc.*, 2013, **135**, 13660-13663.
27. A.-C. Laemmel, J.-P. Collin and J.-P. Sauvage, *Eur. J. Inorg. Chem.*, 1999, **1999**, 383-386.
28. S. Tachiyashiki, H. Ikezawa and K. Mizumachi, *Inorg. Chem.*, 1994, **33**, 623-625.
29. H. Ichida, S. Tachiyashiki and Y. Sasaki, *Chem. Lett.*, 1989, **18**, 1579-1580.
30. D. A. Paixão, I. M. Marzano, E. H. L. Jaimes, M. Pivatto, D. L. Campos, F. R. Pavan, V. M. Deflon, P. I. d. S. Maia, A. M. Da Costa Ferreira, I. A. Uehara, M. J. B. Silva, F. V. Botelho, E. C. Pereira-Maia, S. Guilardi and W. Guerra, *J. Inorg. Biochem.*, 2017, **172**, 138-146.
31. P. P. Silva, W. Guerra, G. C. dos Santos, N. G. Fernandes, J. N. Silveira, A. M. da Costa Ferreira, T. Bortolotto, H. Terenzi, A. J. Bortoluzzi, A. Neves and E. C. Pereira-Maia, *J. Inorg. Biochem.*, 2014, **132**, 67-76.
32. A. Vaidyalingam and P. K. Dutta, *Anal. Chem.*, 2000, **72**, 5219-5224.
33. O. Novakova, J. Kasparkova, O. Vrana, P. M. van Vliet, J. Reedijk and V. Brabec, *Biochemistry*, 1995, **34**, 12369-12378.
34. T. Respondek, R. N. Garner, M. K. Herroon, I. Podgorski, C. Turro and J. J. Kodanko, *J. Am. Chem. Soc.*, 2011, **133**, 17164-17167.
35. A. Mohindru, J. M. Fisher and M. Rabinovitz, *Nature*, 1983, **303**, 64-65.

Open Access Article. Published on 13 July 2017. Downloaded on 14/07/2017 11:43:32.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

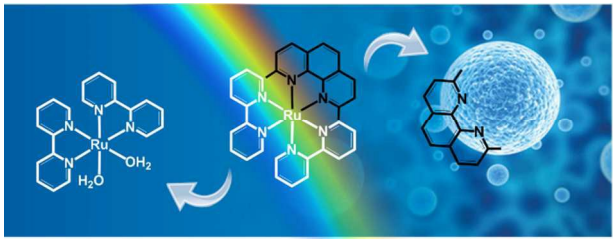
Dalton Transactions Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

View Article Online
DOI: 10.1039/C7DT02255G

A table of contents entry:

This work exemplifies the potential potency of photochemically ejected ligands from strained Ru(II) polypyridyl complexes used in photoactivated chemotherapy.



Open Access Article. Published on 13 July 2017. Downloaded on 14/07/2017 11:43:32.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Dalton Transactions Accepted Manuscript