RSC Advances

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Mitigating UVA Light Induced Reactivity of 6-Thioguanine through Formation of a Ru(II) Half-Sandwich Complex

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Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

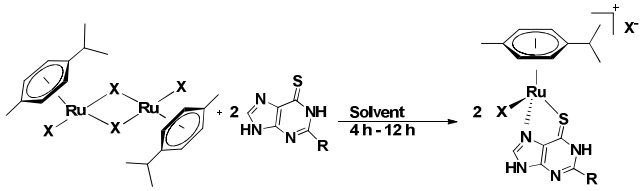
The organometallic complex of (η^6-cymene)Ru(II)Br with 6-thioguanine (6-TG) shows better photostability than the biologically active 6-thioguanine which is used as an immunosuppressant and as an anticancer agent.

Leukemia is a dreaded cancer of the blood and bone marrow. Among the various drugs available for treating leukemia, 6-thioguanine (**6-TG**) is the drug of choice since 1966, especially for treating acute lymphatic leukemia (ALL).[1] Unfortunately **6-TG**, which is also given as an immunosuppressant to patients who have undergone organ transplants, causes severe side effects. Karran and coworkers have skillfully pinned down the source of the problem to a reaction undergone by **6-TG** on irradiation with UVA light.[2] The major products formed are guanine-6-sulfonate (G^{SO3}) and reactive oxygen species (ROS) resulting in skin cancer.[3, 4] Hence, ways and means of modulating the reactivity of **6-TG** are of great interest.

Organometallic half-sandwich Ru(II) complexes are known to have significant anticancer activity. [5-13] The complexes bind to the enzyme, transferrin, which is taken up selectively by cancer cells overexpressing transferrin receptors, thereby increasing internalization.[14, 15] So using **6-TG** as an ancillary ligand for the half-sandwich Ru(II) moiety should result in photoreactivity modulation of **6-TG** and deliver the complex to cancer cells through the transferrin enzyme. To this end, we synthesized and studied half-sandwich complexes of **6-TG** and the related 6-mercaptopurine (**6-MP**).

Half-sandwich η^6-arene ruthenium complexes of thiopurine as an ancillary ligand are rare.[16] We synthesize the target complexes through a convenient reaction of the ruthenium(II) dimer [(η^6-cymene)RuX_2]$_2$ (X = Cl or, Br) with thiopurines in a suitable solvent. The chloro complexes of Ru(II) with thiopurine precipitate from the reaction mixture in dichloromethane during the course of the reaction in reasonable yields. In the case of the bromido complexes, the reaction was carried out in methanol and pure complexes were precipitated by addition of diethyl ether (**Scheme 1**). The complexes are characterized by standard spectroscopic techniques and analytical data. Three of the complexes are also amenable to single crystal X-ray structure analysis. Single crystals are obtained by slow diffusion of diethyl ether into a solution of the complexes in DMF. Based on the structural and ^{1}H NMR data, it is clear that the complexes have the expected half-sandwich structure, with the ancillary ligand coordinating through S and N in κ^2 fashion resulting in dissociation of X^- which is present in the lattice as a hydrogen-bond stabilized counter ion.

All bond distances, Ru-S, Ru-N and those between the cymene and Ru are as expected.[11, 17] The important bond lengths and bond angles for the complexes are listed in **Table S1** and **S2**. Crystallographic data and refinement parameters for all complexes are listed in **Table S3**.



Scheme 1: Synthesis of half-sandwich Ru(II) complexes with thiopurines

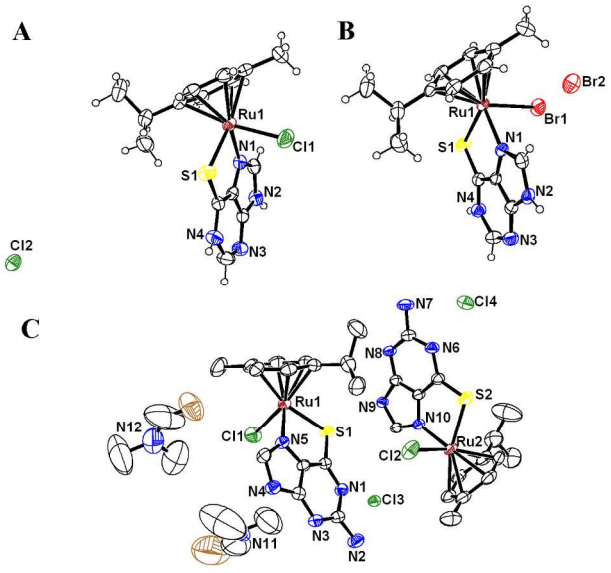


Figure 1: ORTEP view of **RuMPCl** (**A**), **RuMPBr** (**B**) and **RuTGCl.DMF** (**C**) at 40% thermal ellipsoid. Hydrogen atoms are omitted in structure **C** to improve clarity.

The ORTEP representation of the molecular structures of the complexes is shown in **Figure 1**. The lattice abounds in H bonding between the counter anion and ancillary ligands and in the case of **RuTGCl**, intermolecular H-bonding further stabilizes the lattice (**Figure S1**).

^{1}H NMR spectra of these complexes in dry d_6-DMSO are

completely consistent with the molecular structure (**Figure S2**). The thioguanine complex dissolved in D_2O/H_2O in 1:4 ratio showed very little change in water suggesting significant stability of the complexes in water (**Figure S3a**). However, ^{1}H NMR spectra of **RuMPCl** or **RuMPBr** complexes reveal the formation of a mixture of species. However, it is difficult to decipher the nuclearity of the RuMPX complexes from the ^{1}H NMR spectra (**Figure S3b**).

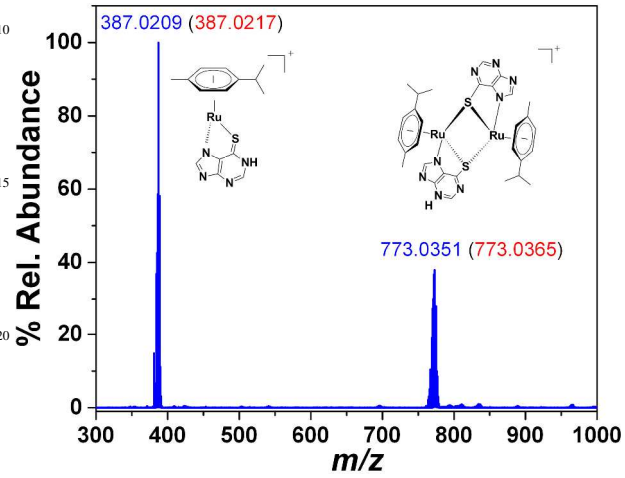
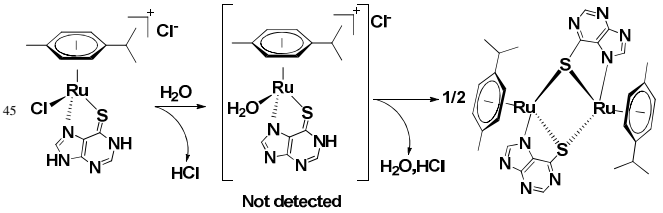


Figure 2: ESI-MS spectrum of **RuMPCl** in water.

Further characterization of the complexes formed on reaction with water as a function of time was attempted using ESI-MS spectrometry (**Table S5**). We expected the formation of an aqua complex as observed earlier for organometallic ruthenium complexes with ethylenediamine and 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane, (PTA) ligands.[17-19] However, ESI-MS analysis showed formation of a dimeric species and not the aqua complex (**Figure 2**). The singly charged protonated dinuclear ruthenium species and a singly charged mononuclear species were the most abundant ions in the ESI-MS spectra (**Figure S4**). It is possible that the major species is dinuclear and it is fragmented during ESI-MS acquisition resulting in higher amount of mononuclear species. Based on these studies we suggest the following steps in the hydrolysis and dimerization similar to the reactions observed in the case of 2-mercaptobenzothiazole half-sandwich complexes with Ru(II)[11] (**Scheme 2**).



Scheme 2: Plausible steps in the formation of the dimeric species.

Using a home built photoreactor, for which the luminosity was standardized with an actinometer,[20] the photochemical behavior of the complexes and of **6-TG** was studied (**Figure S5** and **S6**). Aqueous solutions of the ruthenium complexes (0.1 mM) were irradiated for 10 min and the UV-Vis spectral changes were followed. Under these conditions, solutions of **RuTGBr** were quite stable whereas solutions of **RuTGCl** showed some decrease in its concentration.

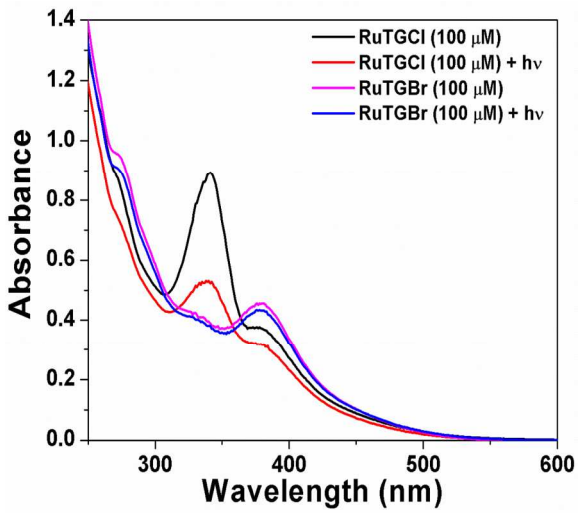


Figure 3: UV-Vis spectra of ruthenium complexes before and after 10 min irradiation with UVA light

ESI-MS spectra of **RuTGX** complexes before and after photoirradiation showed the peak at *m/z* 402.03 corresponding to $[(\eta^6$-cymene)Ru(**6-TG**-H)$]^+$ to be the most abundant species, irrespective of the halide in the complex and a small peak at m/z 803.05 corresponding to a dinuclear species. After irradiation, the spectral features were mostly unchanged (**Figure S7**). To confirm the photostability of these complexes, ^{1}H NMR studies were also carried out. Three sets of samples each containing 8 mg of **RuTGCl** or **RuTGBr** dissolved in 5 mL of water were irradiated separately 30 min, 10 min or kept in the dark. The samples were then lyophilized and ^{1}H NMR spectrum of the residue recorded (**Figure S8**). ^{1}H NMR spectra suggested that both complexes were quite stable *i.e.*, no leaching of the ancillary ligand and the arene cap was observed after irradiation. Stability of **RuTGBr** in the presence of UVA light was further confirmed by using 4-hydroxy benzoic acid as an internal calibrant for ^{1}H NMR spectra (**Figure S9**). No significant changes were observed after photoirradiation in the ratios of internal calibrant with cymene peaks of **RuTGBr**. Based on these experiments one can say that under conditions which cause **6-TG** to undergo photooxidation, **RuTGBr** is quite stable. Leaching of the arene group was not observed contrary to earlier reports on some half sandwich complexes.[21]

Table 1: *In vitro* growth inhibition of human cancer cell lines and LogP values of $[(\eta^6$-cymene)$Ru^{II}X(SN)]$ complexes

Compounds	GI_{50} (μM, 48 h)			LogP
	K562	Jurkat	Molt-4	
RuMPCl	<0.1	34.7	<0.1	-1.45 ± 0.06
RuTGCl	<0.1	<0.1	<0.1	-0.63 ± 0.09
RuMPBr	<0.1	42.2	32.1	-0.90 ± 0.04
RuTGBr	<0.1	<0.1	<0.1	-1.42 ± 0.25
6-TG	<0.1	<0.1	9.4	-
6-MP	<0.1	<0.1	<0.1	-

Having established the greater photostability and solution

behavior of these complexes relative to **6-TG**, it remained to be seen if they have better or equivalent anticancer activity. Growth inhibition (GI_{50}) of all complexes and **6-TG** were checked against K562 (chronic myelogenous leukemia), Jurkat (leukemic T cell lymphoblast) and Molt-4 (leukemic T cell lymphoblast) cell lines by the sulphorhodamine B (SRB) assay.[22] The results are listed in **Table 1**. GI_{50} values suggest that all complexes are highly active against the K562 cell line. Cytotoxicity values of the complexes against the K562 cell line are comparable to the active drug, 6-thioguanine (**6-TG**) and 6-mercaptopurine (**6-MP**). In the Jurkat cell line, **6-MP** complexes are inactive. However, **6-TG** complexes are quite active. It was also observed that against the Molt-4 cell line, **RuMPCl**, **RuTGCl** and **RuTGBr** are more active than **6-TG**. It was observed that all complexes have a negative LogP value, and are quite soluble in water. So **RuTGX** complexes are suitable, like **6-TG,** for oral administration to the patient.

In conclusion, the aqueous solutions of **RuTGBr**, are stable in the presence of UVA light in contrast to **6-TG**. **RuTGCl** and **RuTGBr** show significant anticancer activity against three leukemia cell lines. Clearly, the ruthenium complex of **6-TG** (**RuTGBr**) is a potential alternative to the photosensitive 6-thioguanine as an immunosuppressant and in the treatment of various malignant leukemia.

Authors would like to thank DST, New Delhi, for generous funding. RM thanks CSIR, New Delhi for a Senior Research Fellowship. Authors would like to thank Dr. A. Juvekar of ACTREC, Mumbai for growth inhibition data.

Notes and references

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† Electronic Supplementary Information (ESI) available: Detailed experimental procedures, ^{1}H and ^{13}C NMR data, hydrolysis studies of all complexes by ^{1}H NMR and ESI-MS, photoreaction of **6-TG**, photostability of **RuTGCl** and **RuTGBr** by ^{1}H NMR. CCDC number for **RuMPCl**, **RuMPBr** and **RuTGCl.DMF** are 879121, 967545 and 967547, respectively. See DOI: 10.1039/b000000x/

1. P. Karran and N. Attard, *Nat Rev Cancer*, 2008, **8**, 24-36.
2. P. O'Donovan, C. M. Perrett, X. Zhang, B. Montaner, Y.-Z. Xu, C. A. Harwood, J. M. McGregor, S. L. Walker, F. Hanaoka and P. Karran, *Science*, 2005, **309**, 1871-1874.
3. X. Ren, F. Li, G. Jeffs, X. Zhang, Y.-Z. Xu and P. Karran, *Nucleic Acids Res.*, 2010, **38**, 1832-1840.
4. X. Zou, H. Zhao, Y. Yu and H. Su, *J. Am. Chem. Soc.*, 2013, **135**, 4509-4515.
5. Y. K. Yan, M. Melchart, A. Habtemariam and P. J. Sadler, *Chem. Commun.*, 2005, 4764-4776.
6. N. P. E. Barry and P. J. Sadler, *Chem. Commun.*, 2013, **49**, 5106-5131.
7. C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**, 391-401.
8. G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.*, 2010, **54**, 3-25.
9. A. Kurzwernhart, W. Kandioller, C. Bartel, S. Bachler, R. Trondl, G. Muhlgassner, M. A. Jakupec, V. B. Arion, D. Marko, B. K. Keppler and C. G. Hartinger, *Chemical Communications*, 2012, **48**, 4839-4841.
10. S. Das, S. Sinha, R. Britto, K. Somasundaram and A. G. Samuelson, *J. Inorg. Biochem.*, 2010, **104**, 93-104.
11. R. Mitra, S. Das, S. V. Shinde, S. Sinha, K. Somasundaram and A. G. Samuelson, *Chem. Eur. J.*, 2012, **18**, 12278-12291.
12. I. Bratsos, E. Mitri, F. Ravalico, E. Zangrando, T. Gianferrara, A. Bergamo and E. Alessio, *Dalton Trans.*, 2012, **41**, 7358-7371.
13. L. Leyva, C. Sirlin, L. Rubio, C. Franco, R. Le Lagadec, J. Spencer, P. Bischoff, C. Gaiddon, J.-P. Loeffler and M. Pfeffer, *Eur. J. Inorg. Chem.*, 2007, **2007**, 3055-3066.
14. Z. M. Qian, H. Li, H. Sun and K. Ho, *Pharma. Rev.*, 2002, **54**, 561-587.
15. W. Guo, W. Zheng, Q. Luo, X. Li, Y. Zhao, S. Xiong and F. Wang, *Inorg. Chem.*, 2013, **52**, 5328-5338.
16. K. Yamanari, R. Ito, S. Yamamoto, T. Konno, A. Fuyuhiro, K. Fujioka and R. Arakawa, *Inorg. Chem.*, 2002, **41**, 6824-6830.
17. F. Wang, H. Chen, S. Parsons, I. D. H. Oswald, J. E. Davidson and P. J. Sadler, *Chem. Eur. J.*, 2003, **9**, 5810-5820.
18. F. Wang, A. Habtemariam, E. P. van der Geer, R. Fernandez, M. Melchart, R. J. Deeth, R. Aird, S. Guichard, F. P. Fabbiani, P. Lozano-Casal, I. D. Oswald, D. I. Jodrell, S. Parsons and P. J. Sadler, *Proceedings of the National Academy of Sciences of the United States of America*, 2005, **102**, 18269-18274.
19. K. J. Kilpin, S. M. Cammack, C. M. Clavel and P. J. Dyson, *Dalton Trans.*, 2013, **42**, 2008-2014.
20. T. Lehóczki, É. Józsa and K. Ősz, *J. Photochem. Photobiol., A*, 2013, **251**, 63-68.
21. W. Weber and P. C. Ford, *Inorg. Chem.*, 1986, **25**, 1088-1092.
22. N. J. M. Sanghamitra, M. K. Adwankar, A. S. Juvekar, V. Khurajjam, C. Wycliff and A. G. Samuelson, *Ind. J. Chem.*, 2011, **50A**, 465-473.