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Thermoresponsive organometallic arene ruthenium complexes for tumour targeting†

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Application of mild hyperthermia can increase the cytotoxicity of anticancer drugs in tumour cells. In this report, we describe low molecular weight thermoactive ruthenium-based drugs with fluorous chains that are selectively triggered by mild hyperthermia. The organometallic complexes were prepared, characterized, and evaluated for their *in vitro* cytotoxicity against a panel of human cancer cell lines and non-cancerous immortalized cells. The compounds show considerable chemo-thermal selectivity towards cancer cells (*ca.* 5 μM *versus* >500 μM for healthy cells) for the compound with the longest fluorous chain.

Introduction

Platinum-based anticancer drugs including cisplatin, carboplatin and oxaliplatin lack selectivity towards cancerous cells and therefore their therapeutic application causes severe side-effects such as nephrotoxicity,[1–3] neurotoxicity,[4,5] nausea and vomiting.[6,7] In contrast, ruthenium-based chemotherapeutics present fewer side-effects compared to platinum-based drugs. Although ruthenium-based compounds are not currently employed in the clinic, two ruthenium(III) compounds, namely KP1019[8] and NAMI-A,[9] completed phase I clinical trials and are currently in phase II trials. The different toxicity profiles of platinum- and ruthenium-based compounds remain unclear, although several reasons have been proposed.[10] Irrespective of the full mechanistic differences it is not unreasonable that DNA targeting by platinum compounds leads to the severe side-effects due to the ubiquitous nature of this target. Interestingly, organoruthenium (piano-stool) complexes with the structural composition $[Ru^{II}(\eta^6\text{-arene})X_2(PTA)]$ (PTA = 1,3,5-triaza-7-phosphaadamantane), known as RAPTA compounds, exhibit anti-metastatic[11] and anti-angiogenic[12] properties coupled with a relatively low toxicity comparable to that observed for NAMI-A.[13]

In an effort to improve drug selectivity it is possible to enhance the activity of a compound at the tumour site by applying external techniques or inducers.[14] One such strategy combines chemotherapy with tumour localised mild hyperthermia.[15–17] A slight increase of the local temperature differentiates tissues, healthy ones adapting easily while cancerous cells, with a disorganized and compact vascular structure, have difficulties in dissipating the heat. Some chemotherapeutics exhibit increased activity under mild hyperthermia (40.5–42 °C),[18] even though they are also cytotoxic under normal conditions, and were not intentionally designed for this application. The thermosensitivity of small molecule drugs can be enhanced by attaching them to thermoresponsive macromolecules, *e.g.* liposomal drug carriers[19–24] or micelles that are insoluble at 37 °C and become soluble under hyperthermia, enabling them to cross the cell membrane where they release their drug content.[25,26] Replacing macromolecules with low molecular weight thermosensitive drugs remains an attractive alternative approach. As proof of concept, rationally designed thermoactive derivatives of the organic drug chlorambucil (CLB)[27,28] have been recently designed and were found to be essentially inactive at 37 °C and activated by mild hyperthermia (41 °C) *in vitro*.[29] Recently, the synthesis and biological evaluation (under normal conditions) of some short to medium length fluorous chain bipyridine cisplatin derivatives have been reported.[30,31] Similar types of compounds (amphiphilic fluoroalkylated bipyridine platinum and palladium complexes) have also been tested in liposomal formulations.[32–34] Liposomal formulations of platinum-based drugs, with the rational that liposomal delivery can increase drug bioavailability and also accumulation at the tumour site as a consequence of the enhanced permeability and retention (EPR) effect, are now in clinical trials.[35–38] Herein, ruthenium(II)–arene derivatives (Fig. 1) modified with fluorous chains in order to endow them with thermoresponsive properties[39–41] are described.

The general structure of these new ruthenium(II)–arene complexes is similar to that of RAPTA-C (Fig. 1) – the PTA ligand being replaced with the desired fluorous or alkyl derivatized pyridine ligands. The two labile chloride ligands allow activation *via* hydrolysis following cellular internalization.[11,42] Pyridine was selected as the coordinating moiety based on the widespread use of such ligands in the domain.[43–51] The fluorous and alkyl chains are connected to the pyridine ligand *via* an

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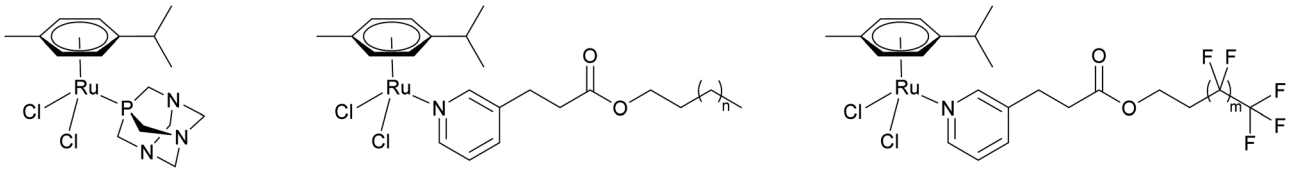

Fig. 1 Structure of RAPTA-C and the new ruthenium(II)–arene complexes derivatized with alkyl or fluoroalkyl 'ponytails'.

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ester linker that may, in principle, be hydrolysed by intracellular enzymes such as esterases.[41,52,53]

Results and discussion

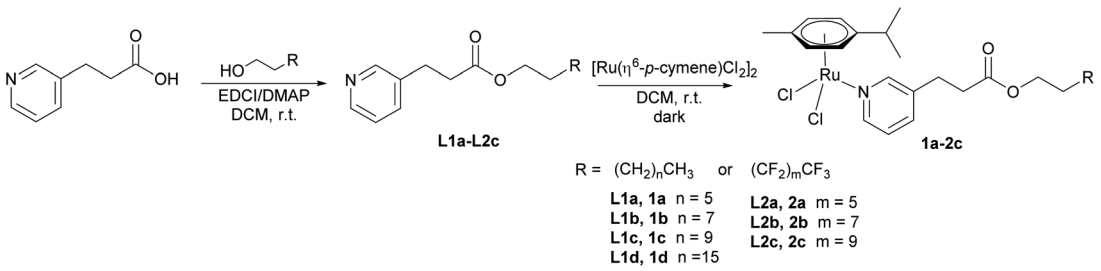
The proposed approach implies a straightforward synthetic pathway and, consequently, the new derivatives, containing either an alkyl or fluorous chain, were synthesized in two steps using modified pyridine ligands as shown in Scheme 1. The pyridine ligands were obtained in good yield (70–87%) using a standard procedure starting from commercially available 3-pyridine-propionic acid and the corresponding alkyl or fluoroalkyl alcohols. In the second step the pyridine ligands were reacted with the dimer, $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$, in anhydrous, degased dichloromethane in the dark under an inert atmosphere. The complexes were isolated by precipitation in good yield (71–87%).

All the compounds have been fully characterized (^1H, ^{13}C and where appropriate ^{19}F NMR spectroscopy, ESI mass spectrometry, IR spectroscopy and elemental analysis: see ESI for details†). The formation of the ester ligands (both alkyl and perfluoroalkyl derivatives) is accompanied by a deshielding of around 0.4 ppm of the protons in the alpha position relative to the oxygen atom, and subsequent complexation to the ruthenium center *via* the pyridine N-atom is accompanied by a deshielding of *ca.* 0.4 ppm for the two pyridine protons in the alpha position to the nitrogen atom and of a deshielding of *ca.* 5 ppm for the respective carbon atoms. There is only little change in position of the proton signals of the *p*-cymene ring in comparison to those observed in the parent dimer $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2]_2$. The structures of the compounds were further corroborated by ESI-MS. The most abundant peaks observed in the spectra of the ligands are those assigned to $[M + H]^+$ ions, whereas the spectra of the pyridine Ru(II)-*p*-cymene complexes are dominated by species assigned to $[M - Cl]^+$ ions. Apart from the ^{19}F NMR spectra and the very specific ^{13}C NMR profile, the presence of the fluorous chain is also clearly evidenced from the IR spectra with the presence of a strong large peak between 1110 and 1250 cm^{-1}. A peak at *ca.* 1730 cm^{-1} confirms the presence of the ester C=O group.

In vitro anticancer activity

The cytotoxicity of the modified pyridine ligands and their corresponding complexes has been assessed in various cancer cell lines (cisplatin-sensitive A2780 and resistant A2780cisR ovarian carcinoma, MCF-7 and MDA-MBA-231 breast carcinomas and A549 human lung carcinoma) and human embryonic kidney (HEK 293) cells (used as a model for normal cells). Cytotoxicity studies were carried out at 37 °C for 72 hours and at 41 °C for 2 hours followed by 70 hours at 37 °C to simulate hyperthermia in the tested cell lines (Table 1).

Distinct thermosensitive behaviour of the compounds is present, but needs to be evident against the majority of the tested cancerous cell lines in order to be considered as effective. In this respect, complex **2c** exhibits considerable differences of up to at least two orders of magnitude (maximum concentrations tested were 500 μM) and hence exhibits ideal thermoresponsive behaviour. In all cases, complex **2c** remains inactive at normal body temperature (IC_{50} values >500 μM) and becomes toxic towards tumour cells after a 2 hour hyperthermia signal (IC_{50} values ranging from 5.0 to 42 μM in the various cancer cell lines). Strikingly, the ligand in **2c**, *i.e.* **L2c**, shows no thermoactivity or cytotoxicity against the screened cell lines except on MCF-7 breast cancer with a negligible (non-thermoresponsive) toxicity of 237 μM at 37 °C and 284 μM under hyperthermia. Moreover, **2c** shows selectivity towards cancerous cells with a weak cytotoxicity under mild hyperthermia against HEK 293 cells.



Scheme 1 Synthesis of ligands **L1a–L2c** and the ruthenium-*p*-cymene complexes **1a–2c**.

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Table 1 IC_{50} values determined for the ligands **L1a–L2c** and complexes **1a–1d** and **2a–2c** in A2780, A2780cisR, A549, MCF-7, MDA-MB-231 and HEK 293 cell lines at 37 °C and under hyperthermia (2 h at 41 °C followed by 70 h at 37 °C – labeled 41 °C in the table)

	A2780 (μM)		A2780cisR (μM)		MCF-7 (μM)		MDA-MB-231 (μM)		A549 (μM)		HEK 293 (μM)	
Compound	37 °C	41 °C	37 °C	41 °C	37 °C	41 °C	37 °C	41 °C	37 °C	41 °C	37 °C	41 °C
L1a	>500	263 ± 12	>500	389 ± 27	>500	458 ± 16	>500	459 ± 27	303 ± 17	358 ± 20	>500	338 ± 3
L1b	>500	98 ± 7	224 ± 23	>500	>500	133 ± 13	>500	>500	>500	323 ± 18	>500	153 ± 12
L1c	>500	69 ± 4	69 ± 4	97 ± 15	315 ± 48	110 ± 7	487 ± 9	>500	96 ± 3	100 ± 9	189 ± 10	206 ± 1
L1d	>500	40 ± 4	88 ± 5	>500	301 ± 75	>500	>500	>500	>500	>500	>500	>500
L2a	>500	181 ± 12	141 ± 9	>500	209 ± 5	136 ± 1.8	362 ± 24	>500	364 ± 8	>500	>500	>500
L2b	476 ± 164	243 ± 35	192 ± 7	>500	>500	>500	>500	>500	>500	>500	>500	>500
L2c	>500	>500	>500	>500	237 ± 25	284 ± 31	>500	>500	>500	>500	>500	>500
1a	>500	23 ± 1	114 ± 1	482 ± 18	339 ± 73	218 ± 4	328 ± 22	100 ± 2	>500	>500	155 ± 17	324 ± 10
1b	>500	49 ± 1	>500	362 ± 14	319 ± 87	108 ± 2	>500	473 ± 20	>500	>500	>500	45 ± 3
1c	113 ± 2	49 ± 1	84 ± 1	48 ± 1	>500	63 ± 4	>500	96 ± 8	391 ± 14	123 ± 8	86 ± 10	42 ± 1
1d	>500	15 ± 1	>500	27 ± 1	>500	17 ± 1	70 ± 8	>500	>500	42 ± 12	>500	149 ± 9
2a	>500	52 ± 2	111 ± 1	>500	>500	70 ± 4	275 ± 19	67 ± 9	355 ± 43	>500	270 ± 18	160 ± 6
2b	44 ± 1	15 ± 1	25 ± 2	21 ± 1	38 ± 2	25 ± 2	36 ± 2	31 ± 2	43 ± 1	40 ± 2	>500	>500
2c	>500	10 ± 1	>500	42 ± 2	>500	5.0 ± 0.3	>500	36 ± 5	>500	33 ± 7	>500	132 ± 5

The incorporation of the fluorous chain appears to give rise to the thermoactive effect. Indeed, complex **1c**, the hydrocarbon analogue of **2c**, exhibits a totally different profile to **2c**. It is active in some cell lines at 37 °C and also moderately toxic against non-tumourigenic HEK 293 cells with an IC_{50} value of 86 μM. Complexes with shorter alkyl chains, *i.e.* **1a** and **1b**, show generally poor activity against cancerous and non-cancerous cells. Complex **1d**, with the longest alkyl chain, exhibits good thermoactivity except in the MDA-MB-231 cell line with an IC_{50} of 70 μM at 37 °C and >500 μM under hyperthermia. Similarly, its ligand alone, **L1d**, is only thermoactive in A2780 cells. Indeed, against A2780cisR and MCF-7 cells, **L1d** is more active than the corresponding complex at normal body temperature, but loses activity under hyperthermia, a behaviour shared with ligands **L1b**, **L2a**, **L2b** and even complex **2a**.

Excluding the cisplatin-resistant cell line, only **L2a** is less cytotoxic under hyperthermia against MDA-MB-231 and A459 cells. Ligands **L1d** and **L2b** are inactive at both temperatures in the other cell lines. Ligands with the longest, bulky chains, *i.e.* **L2b**, **L2c** and **L1d**, are the least active ligands across the panel of cell lines. In A2780 cells the alkylated ligands show increasing cytotoxicity under hyperthermia as the chain length increases, possibly due to increased lipophilicity.

Compounds containing the shorter fluorinated chains do not exhibit a thermoactivity comparable to **2c**. Consequently, the length of the fluorous chain has a significant impact on the potential thermoactive behaviour, which is consistent with the results from the study of chlorambucil modified with fluorinated chains.[29] Nevertheless, **2b** is remarkably cytotoxic and selective towards cancerous cells compared to normal cells, whereas the activity of **2a** is not affected by mild hyperthermia in a systematic manner, presumably due to the short fluorous chain.

Cellular uptake

Cellular uptake studies were conducted on the lead complex, *i.e.* **2c**, to determine the dependency of uptake on temperature in cancerous and non-cancerous cells. A 2 hour heating at 41 °C was used to simulate the hyperthermia signal during a 24 hour incubation prior to measurement. At 37 °C **2c** is internalized three fold more in the A2780 ovarian cancer cell line compared to the normal HEK 293 cells (Fig. 2). Under mild hyperthermia, internalization of **2c** in A2780 cells increases whereas heat has little impact on uptake into HEK 293 cells. These data are consistent with the tumour cell selectivity observed for **2c**. It should be noted, however, that while uptake of **2c** into cancer cells exceeds that in the HEK 293 cells, uptake alone does not explain the vast differences in cytotoxicity following heat treatment. In this context the difficulties cancer cells have dissipating heat[54,55] must also make them more susceptible to cell death induced by the internalized compound.

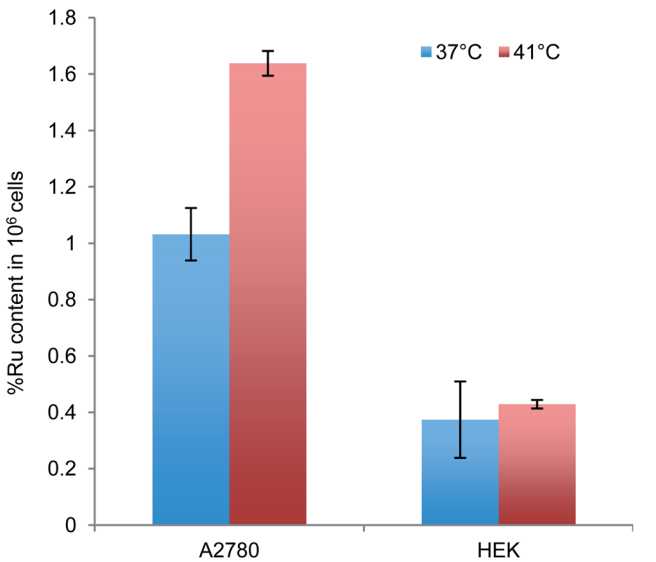

Fig. 2 Cellular uptake of **2c** in A2780 and HEK 293 cell lines with and without a 2 hour hyperthermia signal at 41 °C. Error bars represent Standard Deviation.

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Conclusions

Organometallic ruthenium complexes with a long fluorous appendage exert selective cytotoxicity toward tumour cells under mild hyperthermia. Long fluorous chains are required to obtain relevant thermoresponsive behaviour. For the lead compound, *i.e.* **2c**, it is noteworthy that the fluorous ligand alone is not cytotoxic under any of the applied conditions whereas the ruthenium complex demonstrates considerable differences under normal and thermal conditions (*ca.* 5 μM *versus* >500 μM) and selectivity towards cancer cells over healthy HEK 293 cells. Discrimination between cancerous and normal cells may be attributed to more extensive internalization by cancer cells compared to normal cells combined with the fact that the tumoural cells are sensitized to the cytotoxic agents under mild hypothermia. This discovery opens the way towards the rational design of other thermoactive anticancer drugs.

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Notes and references

1 X. Yao, K. Panichpisal, N. Kurtzman and K. Nugent, *Am. J. Med. Sci.*, 2007, **334**, 115–124.
2 N. Pabla and Z. Dong, *Kidney Int.*, 2008, **73**, 994–1007.
3 P. D. Sanchez-Gonzalez, F. J. Lopez-Hernandez, J. M. Lopez-Novoa and A. I. Morales, *Crit. Rev. Toxicol.*, 2011, **41**, 803–821.
4 B. Bhhatarai and P. Gramatica, *Chem. Res. Toxicol.*, 2010, **23**, 528–539.
5 R. G. van der Hoop, C. J. Vecht, M. E. L. van der Burg, A. Elderson, W. Boogerd, J. J. Heimans, E. P. Vries, J. C. van Houwelingen, F. G. I. Jennekens, W. H. Gispen and J. P. Neijt, *N. Engl. J. Med.*, 1990, **322**, 89–94.
6 L. X. Cubeddu, I. S. Hoffmann, N. T. Fuenmayor and A. L. Finn, *N. Engl. J. Med.*, 1990, **322**, 810–816.
7 B. Bhhatarai and P. Gramatica, *Chem. Res. Toxicol.*, 2010, **23**, 277.
8 C. G. Hartinger, M. A. Jakupec, S. Zorbas-Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P. J. Dyson and B. K. Keppler, *Chemistry & Biodiversity*, 2008, **5**, 2140–2155.
9 J. M. Rademaker-Lakhai, D. van den Bongard, D. Pluim, J. H. Beijnen and J. H. M. Schellens, *Clin. Cancer Res.*, 2004, **10**, 3717–3727.
10 C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**, 391–401.
11 C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2005, **48**, 4161–4171.
12 P. Nowak-Sliwinska, J. R. van Beijnum, A. Casini, A. A. Nazarov, G. Wagnieres, H. van den Bergh, P. J. Dyson and A. W. Griffioen, *J. Med. Chem.*, 2011, **54**, 3895–3902.
13 A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen and G. Sava, *J. Inorg. Biochem.*, 2012, **106**, 90–99.
14 N. P. Barry, O. Zava, J. Furrer, P. J. Dyson and B. Therrien, *Dalton Trans.*, 2010, **39**, 5272–5277.
15 C. M. Wendtner, S. Abdel-Rahman, M. Krych, J. Baumert, L. H. Lindner, A. Baur, W. Hiddemann and R. D. Issels, *J. Clin. Oncol.*, 2002, **20**, 3156–3164.
16 H. I. Robins, J. D. Cohen, C. L. Schmitt, K. D. Tutsch, C. Feierabend, R. Z. Arzoomanian, D. Alberti, F. d'Oleire, W. Longo, C. Heiss, *et al.*, *J. Clin. Oncol.*, 1993, **11**, 1787–1794.
17 J. A. Dickson and S. K. Calderwood, *Nature*, 1976, **263**, 772–774.
18 R. D. Issels, *Eur. J. Cancer*, 2008, **44**, 2546–2554.
19 D. Needham, G. Anyarambhatla, G. Kong and M. W. Dewhirst, *Cancer Res.*, 2000, **60**, 1197–1201.
20 L. H. Lindner, M. E. Eichhorn, H. Eibl, N. Teichert, M. Schmitt-Sody, R. D. Issels and M. Dellian, *Clin. Cancer Res.*, 2004, **10**, 2168–2178.
21 L. Li, T. L. M. ten Hagen, D. Schipper, T. M. Wijnberg, G. C. van Rhoon, A. M. M. Eggermont, L. H. Lindner and G. A. Koning, *J. Controlled Release*, 2010, **143**, 274–279.
22 G. Kong, G. Anyarambhatla, W. P. Petros, R. D. Braun, O. M. Colvin, D. Needham and M. W. Dewhirst, *Cancer Res.*, 2000, **60**, 6950–6957.
23 A. M. Ponce, Z. Vujaskovic, F. Yuan, D. Needham and M. W. Dewhirst, *Int. J. Hyperthermia*, 2006, **22**, 205–213.
24 S. Unezaki, K. Maruyama, N. Takahashi, M. Koyama, T. Yuda, A. Suginaka and M. Iwatsuru, *Pharm. Res.*, 1994, **11**, 1180–1185.
25 J. Andrew Mackay and A. Chilkoti, *Int. J. Hyperthermia*, 2008, **24**, 483–495.
26 D. E. Meyer, B. C. Shin, G. A. Kong, M. W. Dewhirst and A. Chilkoti, *J. Controlled Release*, 2001, **74**, 213–224.
27 J. E. Ultmann, G. A. Hyman and A. Gellhorn, *JAMA, J. Am. Med. Assoc.*, 1956, **162**, 178–183.
28 S. Sachanas, G. A. Pangalis, T. P. Vassilakopoulos, P. Korkolopoulou, F. N. Kontopidou, M. Athanasoulia, X. Yiakoumis, C. Kalpadakis, G. Georgiou, S. Masouridis, M. Moschogiannis, P. Tsirkinidis, V. Pappis, S. I. Kokoris, M. P. Siakantaris, P. Panayiotidis and M. K. Angelopoulou, *Leuk. Lymphoma*, 2011, **52**, 387–393.
29 C. M. Clavel, O. Zava, F. Schmitt, B. H. Kenzaoui, A. A. Nazarov, L. Juillerat-Jeanneret and P. J. Dyson, *Angew. Chem., Int. Ed.*, 2011, **50**, 7124–7127.
30 T. T. Chang, S. V. More, N. Lu, J. W. Jhuo, Y. C. Chen, S. C. Jao and W. S. Li, *Bioorg. Med. Chem.*, 2011, **19**, 4887–4894.
31 K. E. Elwell, C. Hall, S. Tharkar, Y. Giraud, B. Bennett, C. Bae and S. W. Carper, *Bioorg. Med. Chem.*, 2006, **14**, 8692–8700.
32 N. Garelli, P. Vierling, J. L. Fischel and G. Milano, *Eur. J. Med. Chem.*, 1993, **28**, 235–242.
33 N. Garelli and P. Vierling, *Biochim. Biophys. Acta*, 1992, **1127**, 41–48.
34 N. Garelli and P. Vierling, *Inorg. Chim. Acta*, 1992, **194**, 247–253.
35 F. Kratz, I. A. Muller, C. Ryppa and A. Warnecke, *ChemMedChem*, 2008, **3**, 20–53.
36 D. Peer, J. M. Karp, S. Hong, O. C. FaroKHzad, R. Margalit and R. Langer, *Nat. Nanotechnol.*, 2007, **2**, 751–760.
37 M. J. Hannon, *Pure Appl. Chem.*, 2007, **79**, 2243–2261.

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38 S. Taurin, H. Nehoff and K. Greish, *J. Controlled Release*, 2012, **164**, 265–275.
39 K. Niikura, K. Nambara, T. Okajima, Y. Matsuo and K. Ijiro, *Langmuir*, 2010, **26**, 9170–9175.
40 F. R. Carrel and P. H. Seeberger, *J. Org. Chem.*, 2008, **73**, 2058–2065.
41 B. Hungerhoff, H. Sonnenschein and F. Theil, *J. Org. Chem.*, 2002, **67**, 1781–1785.
42 W. H. Ang, E. Daldini, C. Scolaro, R. Scopelliti, L. Juillerat-Jeannerat and P. J. Dyson, *Inorg. Chem.*, 2006, **45**, 9006–9013.
43 J. G. Malecki, R. Kruszynski, M. Jaworska, P. Lodowski and Z. Mazurak, *J. Organomet. Chem.*, 2008, **693**, 1096–1108.
44 G. Suss-Fink, F. A. Khan, L. Juillerat-Jeanneret, P. J. Dyson and A. K. Renfrew, *J. Cluster Sci.*, 2010, **21**, 313–324.
45 J. Grau, V. Noe, C. Ciudad, M. J. Prieto, M. Font-Bardia, T. Calvet and V. Moreno, *J. Inorg. Biochem.*, 2012, **109**, 72–81.
46 C. A. Vock, C. Scolaro, A. D. Phillips, R. Scopelliti, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2006, **49**, 5552–5561.
47 A. Bacchi, G. Cantoni, M. R. Chierotti, A. Girlando, R. Gobetto, G. Lapadula, P. Pelagatti, A. Sironi and M. Zecchini, *CrystEngComm*, 2011, **13**, 4365–4375.
48 F. Y. Wang, A. Habtemariam, E. P. L. van der Geer, R. Fernandez, M. Melchart, R. J. Deeth, R. Aird, S. Guichard, F. P. A. Fabbiani, P. Lozano-Casal, I. D. H. Oswald, D. I. Jodrell, S. Parsons and P. J. Sadler, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 18269–18274.
49 Y. Fu, A. Habtemariam, A. M. Pizarro, S. H. van Rijt, D. J. Healey, P. A. Cooper, S. D. Shnyder, G. J. Clarkson and P. J. Sadler, *J. Med. Chem.*, 2010, **53**, 8192–8196.
50 N. Gligorijevic, S. Arandelovic, L. Filipovic, K. Jakovljevic, R. Jankovic, S. Grguric-Sipka, I. Ivanovic, S. Radulovic and Z. L. Tesic, *J. Inorg. Biochem.*, 2012, **108**, 53–61.
51 K. D. Camm, A. El-Sokkary, A. L. Gott, P. G. Stockley, T. Belyaeva and P. C. McGowan, *Dalton Trans.*, 2009, 10914–10925.
52 B. Hungerhoff, H. Sonnenschein and F. Theil, *Angew. Chem., Int. Ed.*, 2001, **40**, 2492–2494.
53 P. Beier and D. O'Hagan, *Chem. Commun.*, 2002, 1680–1681.
54 C. W. Song, *Cancer Res.*, 1984, **44**, 4721–4730.
55 F. K. Storm, W. H. Harrison, R. S. Elliott and D. L. Morton, *Cancer Res.*, 1979, **39**, 2245–2251.