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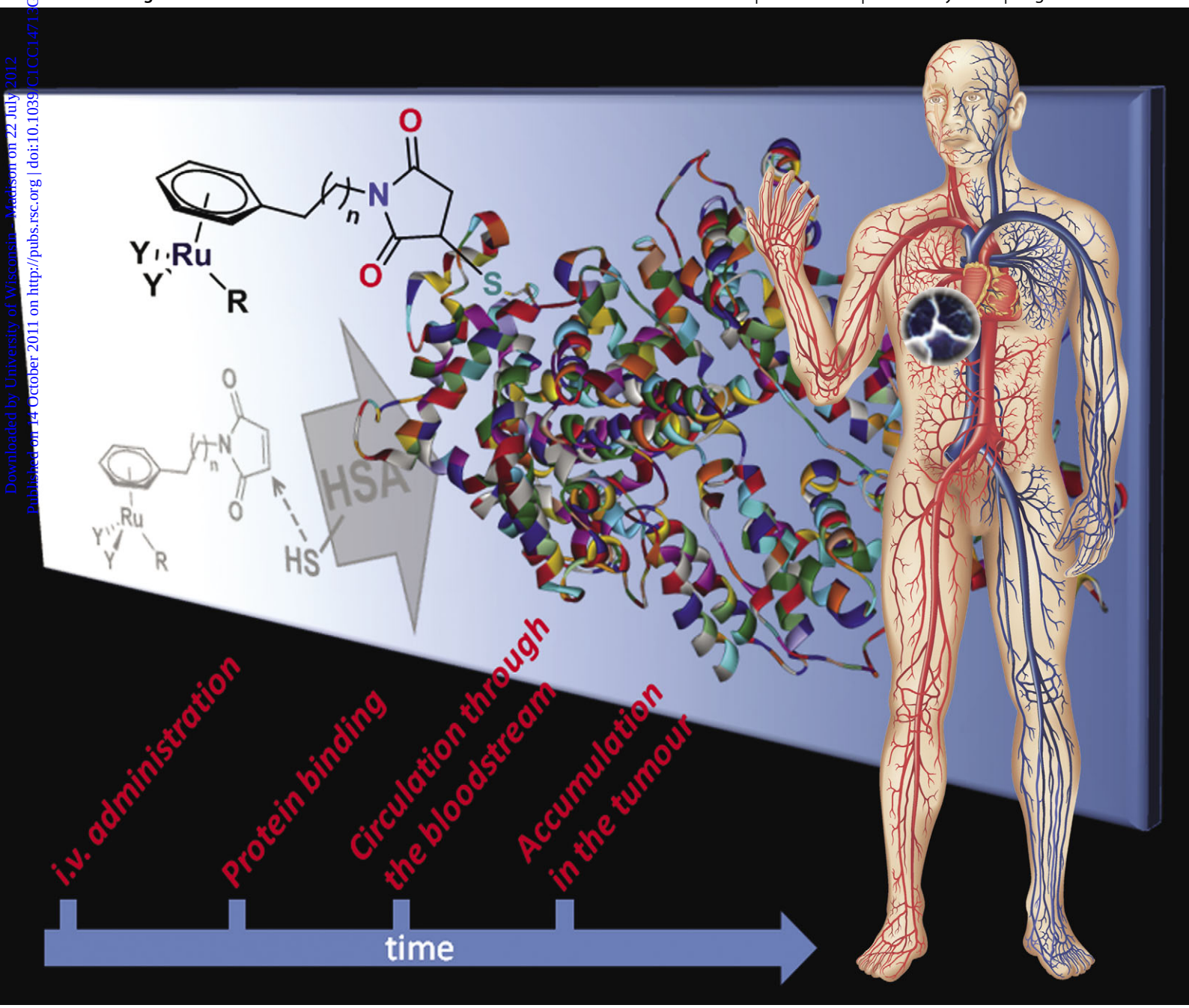

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COMMUNICATION

Maleimide-functionalised organoruthenium anticancer agents and their binding to thiol-containing biomolecules†‡

Muhammad Hanif,[ab] Alexey A. Nazarov,[c] Anton Legin,[a] Michael Groessl,[c] Vladimir B. Arion,[a] Michael A. Jakupec,[a] Yury O. Tsybin,[c] Paul J. Dyson,[c] Bernhard K. Keppler[a] and Christian G. Hartinger*[ad]

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Ru^{II}(arene) anticancer compounds with maleimide functionality were prepared to allow selective interaction with thiol-containing biomolecules and thereby enforcing the selective delivery of the compounds to the tumour.

Metal-based drugs are applied for the treatment of different diseases and in particular platinum complexes are among the most widely used anticancer chemotherapeutics.[1] One of the major drawbacks of Pt-based drugs is their low degree of selectivity for tumour tissue, which results in a number of serious side effects during the medication.[1] Several different strategies have been proposed to overcome the limitations of Pt complexes, and targeting *via* tethering Pt to macromolecular drug carriers appears promising.[2] Notably, Kratz and co-workers reported on Pt complexes linked to maleimides,[3] known to react selectively with thiols of biomolecules[4] for transport of bioactive moieties by human serum albumin (HSA) in the blood serum. Such macromolecules can extravasate into the tumour tissue *via* leaky vessels exploiting the enhanced permeability and retention (EPR) effect. The EPR effect is characterised by increased permeability from the blood vessels into tumours because of rapid and defective angiogenesis. Furthermore, drug accumulation is improved by a dysfunctional lymphatic drainage in tumours.[5]

More recently, bioorganometallics and in particular anticancer Ru(arene) complexes were found to exhibit promising biological activity.[6–8] We have developed a series of Ru(arene) compounds in which the arene is functionalised with a maleimide moiety (Fig. 1), following a recently reported strategy.[9] In addition, the Ru coordination sphere is completed with chlorido leaving groups and P-based co-ligands, *i.e.*, carbohydrate-derived phosphites and 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]-decane (pta) and for comparison the bulky and lipophilic triphenylphosphine, resembling drug candidates with known anticancer activity.[6,10,11] Ru(arene)(pta) complexes have been shown to inhibit tumours *in vivo*,[12] in particular with respect to metastases which are often difficult to treat.[13] On the other hand Ru(arene) moieties linked to sugar derivatives were developed to target a cytotoxic moiety selectively into the tumour by exploiting the high energy demand of tumours which can only be satisfied by glycolysis due to insufficient supply *via* the blood stream.[11,14,15]

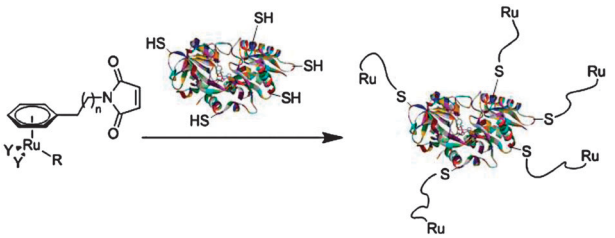

Fig. 1 Strategy for tagging bioactive Ru complexes to thiol-containing biomolecules.

Complexes **1–4** were prepared by reacting dimeric $[Ru(\eta^6\text{-}N\text{-benzylmaleimide})Cl_2]_2$ with the respective ligand in CH_2Cl_2 or dimethylformamide (DMF; Scheme 1). The compounds were characterised by NMR spectroscopy, electrospray ionisation mass spectrometry (ESI MS), and elemental analysis (see Supporting information for details‡). The $^{31}P\{^1H\}$ NMR spectra feature several signals which coalesce at elevated temperature, indicating the presence of a single species (see Fig. S1‡). Such behaviour has neither been observed for the parent compound [dichlorido(η^6-*p*-cymene)-(1,3,5-triaza-7-phosphaadamantane)ruthenium(II)] (RAPTA-C) nor for related sugar-phosphite complexes with their *p*-cymene ligands.[5,11] The bulky maleimide substituent causes hindered rotation along the Ru-$arene_{centroid}$ axis compared to methyl/isopropyl residues.

[a] *University of Vienna, Institute of Inorganic Chemistry, Waehringer Str. 42, A-1090 Vienna, Austria. E-mail: christian.hartinger@univie.ac.at; Fax: +43-1-4277-9526; Tel: +43-1-4277-52609*
[b] *Department of Chemistry, COMSATS Institute of Information Technology Abbottabad Campus, University Road, Abbottabad-22060, Pakistan*
[c] *Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland*
[d] *The University of Auckland, School of Chemical Sciences, Private Bag 92019, Auckland 1142, New Zealand*

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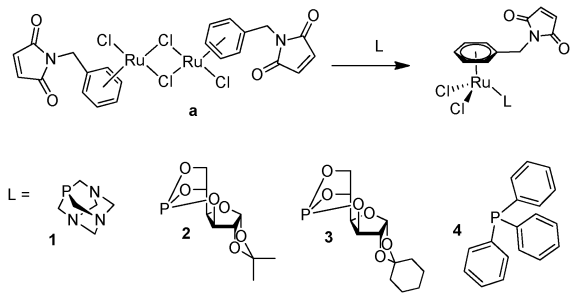
‡ Electronic supplementary information (ESI) available: Materials and Methods, Synthetic procedures, NMR, SEC and ESI-MS studies on the binding to thiols, X-ray diffraction data. CCDC 837010. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1cc14713g



Scheme 1 Synthesis of $Ru^{II}(\eta^6$-*N*-benzylmaleimide) anticancer compounds.

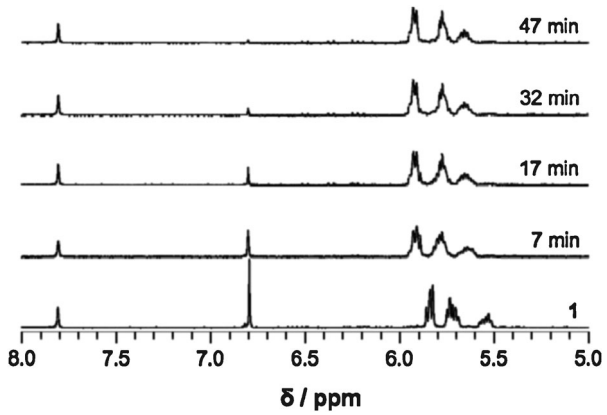


Fig. 2 ^{1}H NMR spectra of the reaction of **1** with Cys (1 : 1.2) in D_2O/100 mM NaCl. d_7-DMF was used as internal standard (7.8 ppm).

Single crystals of **1** suitable for X-ray diffraction were grown by slow diffusion of diethyl ether into a DMF/methanol solution (the molecular structure of **1** is shown in Fig. S2‡). The ruthenium–centroid$_{arene}$ distance and the Ru-donor atom bond lengths are similar to those for RAPTA-C for which two crystallographically independent molecules were found in the unit cell (see Table S2‡).[5] However, the P–Ru–Cl and the Cl–Ru–Cl angles are very different which is due to the different substituents at the arene moiety.

In order to determine the selectivity of thiols for the maleimide functionality of **1** but not for its Ru centre, the compound was reacted with the biomolecules Cys, glutathione (GSH), *N*-acetylcysteine (NAC) and *N*-acetylcysteine methyl ester (*N*-AcCysMe) in a molar ratio of 1 : 1.2 (complex : Cys) in D_2O/100 mM NaCl (pH 5.5) at room temperature. NaCl was added to suppress aquation of the Ru centre.[11,16] The ^{1}H NMR spectra show that the maleimide CH protons at *ca.* 6.8 ppm disappear rapidly relative to the d_7-DMF internal standard assuming pseudo-first order kinetics (Fig. 2 and 3), accompanied by labilisation of the arene–Ru bond and resulting in additional signals at approximately 7.2 ppm (within 1 day, see Fig. S3‡).

In the case of Cys, GSH and NAC the reactions proceeded fairly quickly and within approximately 60 min, **1** was quantitatively converted into the respective adduct (Fig. 3). The reaction with *N*-AcCysMe in D_2O/100 mM NaCl was too rapid to be followed by NMR spectroscopy, which is related to the pH in the incubation solution.

Furthermore, the influence of aquation of **1** was studied in order to address the role of the metal centre on the reaction

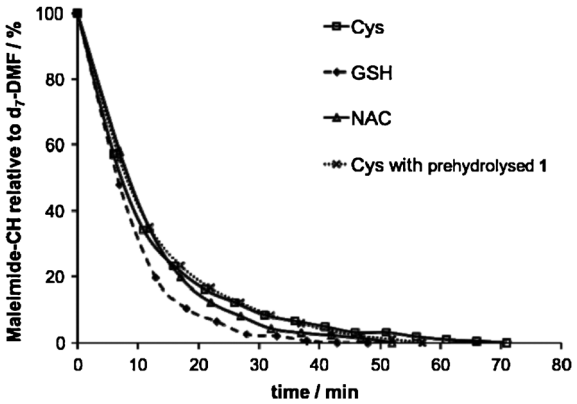


Fig. 3 Time course of the decrease of maleimide CH signals due to reaction with Cys (k = 0.07 min^{-1} [0.988]), GSH (k = 0.13 min^{-1} [0.985]) and NAC (k = 0.09 min^{-1} [0.998]) in D_2O as compared to the reaction of prehydrolysed **1** with Cys (k = 0.08 min^{-1} [0.995]). The data was normalised to the signal of d_7-DMF as internal standard.

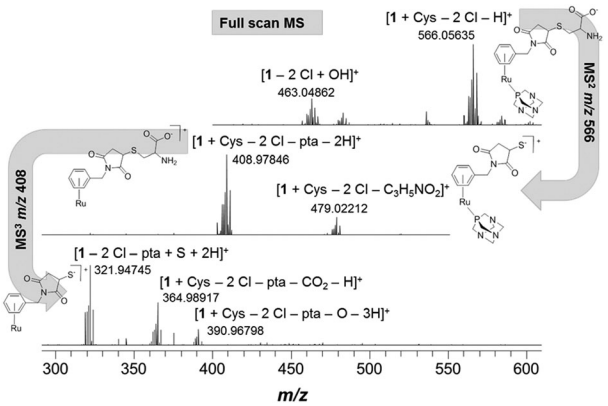


Fig. 4 MS^n study to characterise the formed adduct in the reaction of **1** with Cys in aqueous solution; top: full scan mass spectrum, middle: MS^2 of parent ion at m/z 566, bottom: MS^3 of parent ion at m/z 408.

with thiols. Compound **1** was allowed to hydrolyse for 24 h in D_2O (indicated by a shift in the $^{31}P\{^1H\}$ NMR spectra from approximately −32 to −28 ppm) and afterwards Cys was added. Similar kinetics were observed indicating high selectivity for the nucleophilic addition of the thiol to the maleimide moiety, especially under physiological conditions (phosphate buffer pH 7.4, 100 mM NaCl; data not shown).

In addition to the NMR studies, ESI-Fourier transform ion cyclotron resonance (FT-ICR)-mass spectra were recorded in order to unambiguously characterise the formed species in the reaction of **1** with the biological thiols. The reaction between **1** and Cys in a molar ratio of 1 : 1 yielded a species at m/z 566.05635 (Fig. 4 and S4‡), which can be assigned to the $[\mathbf{1} + Cys - 2Cl - H]^+$ ion, accompanied by a decrease of the signal at m/z 463.04862 $[\mathbf{1} - 2Cl + OH]^+$.

Fragmentation of the adduct ion at m/z 566 by collision-induced dissociation resulted in the formation of two main main product ions at m/z 408.97846 and 479.02212 (Fig. 4), which were identified as fragments with the pta ligand and with a part of the Cys residue cleaved, respectively. An MS^3 experiment with m/z 408 as precursor ion revealed the sequential cleavage of the Cys backbone by decarboxylation

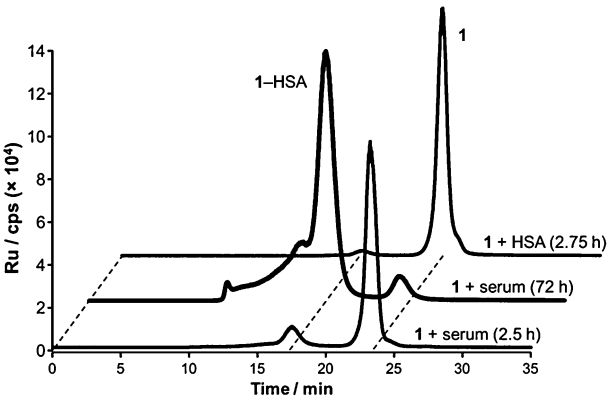


Fig. 5 SEC–ICP-MS chromatograms for the reaction with human serum albumin and human serum (1 : 1).

with the S remaining bound to the maleimide residue (Fig. 4). These data indicate that the maleimide group preferentially reacts with Cys and that the metal centre is not directly involved in the adduct formation. Similar observations were made for the reactions with GSH and NAC. In the reaction of **1** with NAC the most abundant peak was assigned to $[\mathbf{1} - 2\,Cl + NAC - H]^+$ (m/z 608.06700) and with GSH to $[\mathbf{1} - 2\,Cl + GSH - H]^+$ (m/z 752.12089).

In an attempt to characterise the reaction of **1** with HSA, ^{1}H NMR spectroscopy and size exclusion chromatography hyphenated to inductively coupled plasma mass spectrometry (SEC–ICP-MS) studies were performed on reaction mixtures containing **1** and HSA in molar ratios from 1 : 1 to 2 : 1 (protein : **1**; see Fig. 5 and S5‡). The maleimide CH protons are overlapped by proton signals of aromatic amino acids of HSA, however, the peaks assignable to d_7-DMF and the maleimide moiety were clearly distinguishable. The NMR spectra indicate a significantly lower reactivity to HSA compared to the small molecules. A similar picture was seen in the SEC–ICP-MS in which only a small amount of Ru was detected bound to HSA after about 3 h. However, after 72 h in serum the majority of Ru was found in the 60–80 kDa fraction containing HSA (Fig. 5 and Fig. S6‡ for SEC–UV/vis chromatograms).

The *in vitro* activity of **1–4** was established in human ovarian (CH1), colon (SW480) and non-small cell lung cancer (A549) cells and compared to RAPTA-C (Table 1). In the chemosensitive cell line CH1, all maleimide-functionalised compounds are more active than the parent compound RAPTA-C. The role of the phosphorus-containing ligand on the anticancer activity becomes evident when comparing the data for SW480 and A549 cells, in which the compounds bearing more liphophilic carbohydrate-derived or triphenylphospine ligands are significantly more active than the pta analogue and RAPTA-C.

In conclusion, we have functionalised anticancer Ru^{II}(arene) compounds with maleimide moieties that undergo selective reaction with thiol-containing biological nucleophiles *via* the maleimide residue. This behaviour was observed in presence of amino acids as well as of HSA, having an accessible

Table 1 *In vitro* anticancer activity (mean IC_{50} values ± standard deviation) of **1–4** in human ovarian (CH1), colon (SW480) and non-small cell lung cancer (A549) cells (exposure time 96 h)

	IC_{50} values/μM		
Compound	CH1	SW480	A549
1	26 ± 1	191 ± 49	>640
2	14 ± 1	13 ± 1	63 ± 4
3	15 ± 2	13 ± 1	92 ± 31
4	15 ± 3	22 ± 4	116 ± 5
RAPTA-C	65 ± 15	170 ± 60	>640

Cys residue (Cys34). These data indicate that Cys containing biomolecules could act as carriers for such a compound class as evidenced by their cytotoxicity toward cancer cells. Indeed, we can speculate that HSA can deliver and ultimately release the Ru(arene) fragments although further work must be carried out to confirm this hypothesis.

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Notes and references

1 M. A. Jakupec, M. Galanski, V. B. Arion, C. G. Hartinger and B. K. Keppler, *Dalton Trans.*, 2008, 183–194.
2 S. van Zutphen and J. Reedijk, *Coord. Chem. Rev.*, 2005, **249**, 2845–2853.
3 A. Warnecke, I. Fichtner, D. Garmann, U. Jaehde and F. Kratz, *Bioconjugate Chem.*, 2004, **15**, 1349–1359.
4 P. Haquette, M. Salmain, K. Svedlung, A. Martel, B. Rudolf, J. Zakrzewski, S. Cordier, T. Roisnel, C. Fosse and G. Jaouen, *ChemBioChem*, 2007, **8**, 224–231.
5 C. S. Allardyce, P. J. Dyson, D. J. Ellis and S. L. Heath, *Chem. Commun.*, 2001, 1396–1397.
6 C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**, 391–401.
7 A. Bergamo and G. Sava, *Dalton Trans.*, 2011, **40**, 7817–7823.
8 G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.*, 2011, **54**, 3–25.
9 P. Haquette, B. Talbi, S. Canaguier, S. Dagorne, C. Fosse, A. Martel, G. Jaouen and M. Salmain, *Tetrahedron Lett.*, 2008, **49**, 4670–4673.
10 P. J. Dyson, *Chimia*, 2007, **61**, 698–703.
11 I. Berger, M. Hanif, A. A. Nazarov, C. G. Hartinger, R. O. John, M. L. Kuznetsov, M. Groessl, F. Schmitt, O. Zava, F. Biba, V. B. Arion, M. Galanski, M. A. Jakupec, L. Juillerat-Jeanneret, P. J. Dyson and B. K. Keppler, *Chem.–Eur. J.*, 2008, **14**, 9046–9057.
12 S. Chatterjee, S. Kundu, A. Bhattacharyya, C. G. Hartinger and P. J. Dyson, *JBIC, J. Biol. Inorg. Chem.*, 2008, **13**, 1149–1155.
13 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.
14 I. Berger, A. A. Nazarov, C. G. Hartinger, M. Groessl, S.-M. Valiahdi, M. A. Jakupec and B. K. Keppler, *ChemMedChem*, 2007, **2**, 505–514.
15 C. G. Hartinger, A. A. Nazarov, S. M. Ashraf, P. J. Dyson and B. K. Keppler, *Curr. Med. Chem.*, 2008, **15**, 2574–2591.
16 C. Scolaro, C. G. Hartinger, C. S. Allardyce, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1743–1748.