

Ruthenium(II) and osmium(II) 1,2,3-triazolylidene organometallics: a preliminary investigation into the biological activity of 'click' carbene complexes†

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Taking advantage of the facile and versatile synthetic properties of 'click' 1,2,3-triazolylidene N-heterocyclic carbenes (tzNHC's), a range of new organometallic Ru(II) and Os(II) arene complexes containing functionalised tzNHC ligands, $[M(\eta^6\text{-}p\text{-cymene})(\text{tzNHC})\text{Cl}_2]$ [$M = \text{Ru(II)}, \text{Os(II)}$], have been synthesised and fully characterised, including the X-ray crystal structure of one of the Os(II) complexes. The tzNHC ligands remain coordinated to the metal centres under relevant physiological conditions, and following binding to the model protein, ubiquitin. The *in vitro* cytotoxicity of the compounds towards human ovarian cancer cells is dependent on the substituent on the tzNHC ligand but is generally $<50 \mu\text{M}$ and in some cases $<1 \mu\text{M}$, whilst still retaining a high degree of selectivity towards cancer cells over healthy cells ($1.85 \mu\text{M}$ in A2780 ovarian cancer cells *versus* $435 \mu\text{M}$ in human embryonic kidney cells in one case).

Introduction

The introduction and concurrent success of cisplatin and other platinum-based drugs in the clinic has undoubtedly transformed cancer chemotherapy.¹ However, the problems associated with platinum agents, in particular their lack of selectivity towards cancer cells, has resulted in the search for alternative metal-based agents which overcome this limitation.² In this regard, ruthenium(III) coordination compounds, notably NAMI-A and KP1019 (Fig. 1), are under clinical development.^{3,4} As the proposed mechanism of action of such compounds is thought to involve reduction *in vivo*, organometallic Ru(II) compounds have also started to attract attention.^{5,6} Within this field, significant efforts have centered around the development of the RAPTA-based (Ru Arene PTA, pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane) compounds⁷ and ethylene diamine [Ru-arene-en]⁺ compounds⁸ (Fig. 1). Although complexes containing ligands other than phosphines and amines have been investigated for their biological applications,^{9,10} one class of ligands which seems to have been largely neglected are N-heterocyclic carbenes (NHC's), which is

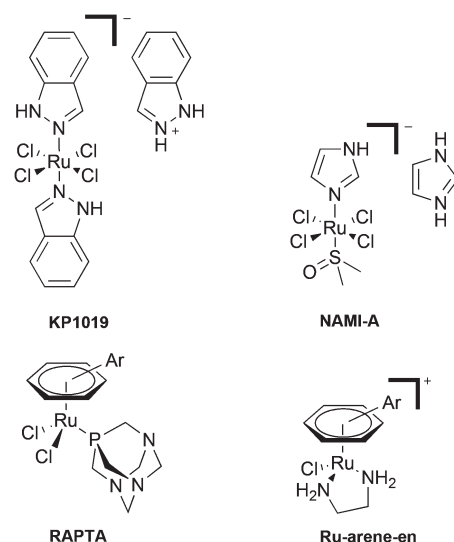


Fig. 1 Structures of selected Ru(III) and Ru(II) complexes developed as anti-tumour agents.

somewhat surprising given the large research efforts and success of Ru-NHC complexes in catalytic applications.¹¹

The potential of metal-based NHC complexes as anti-cancer agents has been reviewed only recently, with the bulk of the research centered on either Group 10 or 11 metal complexes.^{12–14} However, Ru(II) NHC complexes have been touched upon, and show promising results in terms of enzyme inhibition and *in vitro* anti-proliferative effects.^{15,16}

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Furthermore, studies on zebrafish embryos have demonstrated that a selection of the complexes are essentially non-toxic,¹⁷ a somewhat significant observation considering the high toxicity of the clinically used platinum drugs.

Although relatively new,¹⁸ one class of topical NHC ligands, which to the best of our knowledge remain unexplored in biological applications, are the 1,2,3-triazolyldiene N-heterocyclic carbenes.^{19,20} We are particularly interested in this class of NHC as the facile, modular synthetic route *via* the CuAAC (copper catalysed azide alkyne cycloaddition) 'click' reaction offers the ability to readily introduce a number of functional groups onto the parent triazole^{21,22} and ultimately the metal complex²³ *e.g.* for use in cellular targeting or enzyme inhibition.²⁴ Furthermore, the resulting strong metal–ligand bond of the organometallic complex²⁵ may prove advantageous in a highly complex biological environment.

Herein, we report the synthesis, characterisation and, to the best of our knowledge, the first investigation into the biological activity of ruthenium(II) and osmium(II) arene compounds containing 1,2,3-triazolyldiene NHC ligands.

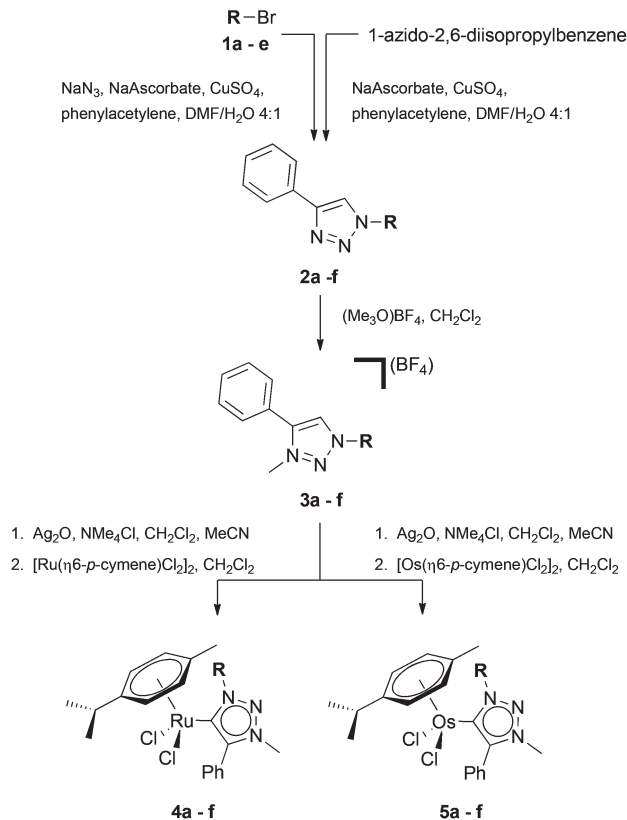
Results and discussion

Synthesis and characterisation

Ruthenium(II) and osmium(II) piano stool complexes containing functionalised tzNHC ligands were synthesised (Scheme 1) using an adaption of the procedure reported by Albrecht *et al.*¹⁸

In brief, the facile one-pot CuAAC methodology described by Crowley and co-workers was used to synthesise the 1,4-disubstituted-1,2,3-triazoles **2a–e**, eliminating the need to isolate the potentially explosive azide intermediates.^{26,27} Conversely, **2f** was synthesised directly from 1-azido-2,6-diisopropylbenzene. Regioselective methylation of the triazoles **2a–f** at the N3 position was carried out using $[\text{Me}_3\text{O}][\text{BF}_4]$ to afford **3a–f**.²⁸ Subsequent reaction of the triazolium salts **3a–f** with Ag_2O , followed by addition of either $[\text{Ru}(\eta^6\text{-}p\text{-cymene})\text{Cl}_2]_2$ or $[\text{Os}(\eta^6\text{-}p\text{-cymene})\text{Cl}_2]_2$, afforded the mononuclear piano stool complexes in good yields.¹⁸ Using this method we were able to introduce the simple alkyl (ethyl **4a** and **5a**, hexyl **4b** and **5b**, dodecyl **4c** and **5c**), benzyl (**4d** and **5d**), and diisopropylphenyl (**4f** and **5f**) substituents onto the tzNHC metal complex. Furthermore, as a proof of principle that the methodology can be extended to incorporate biologically relevant functional groups such as glucosyl-based moieties, we also synthesised compounds containing a 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl group as a substituent on the tzNHC (**4e** and **5e**). All the metal compounds were isolated as yellow to orange solids which were soluble in a range of solvents (MeOH, CH_2Cl_2 , MeCN, acetone, DMSO), and in addition, the presence of the carbohydrate substituent on **4e** and **5e** imparted good water solubility to the complexes.

Characterisation data corroborate the expected structures of the metal carbene complexes. Namely, the pre-carbene proton of the triazolium salts **3a–f** (H-1, *ca.* 8.6–8.8 ppm, CDCl_3 , ESI⁺)



Scheme 1 Route used to prepare Ru(II) and Os(II) 1,2,3-triazolyldiene NHC complexes. **1a/2a/3a/4a/5a**: R = ethyl; **1b/2b/3b/4b/5b**: R = *n*-hexyl; **1c/2c/3c/4c/5c**: R = *n*-dodecyl; **1d/2d/3d/4d/5d**: R = benzyl; **1e/2e/3e/4e/5e**: R = 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl; **2f/3f/4f/5f**: R = 2,6-diisopropylphenyl.

is no longer present in the ^1H NMR spectra of the metal complexes **4a–e** and **5a–e**, and the α protons on the N1 triazolyldiene substituent show a slight downfield shift (*ca.* 0.2 ppm) in the ^1H NMR spectra relative to the triazolium salt precursors **3a–e**. The $^{13}\text{C}\{^1\text{H}\}$ chemical shift of the carbene centre (C-1) differs slightly for the ruthenium (*ca.* 160 ppm) and osmium (*ca.* 148 ppm) complexes.

The main ion observed in the ESI mass spectra of the complexes arises from fragmentation *via* the loss of a chloride ligand to give $[\text{M} - \text{Cl}]^+$ type species.

The molecular structure of **5d** was unequivocally verified by single crystal X-ray crystallography on crystals grown at 4 °C by the slow diffusion of diethylether into a dichloromethane solution of **5d**. The molecular structure of **5d** is shown in Fig. 2 and selected bond parameters are given in the caption.

As expected, the complex adopts a piano-stool motif, comprising the *p*-cymene 'seat', with the two chloride ligands and the tzNHC ligand representing the three 'legs'. The Os–tzNHC bond length $[\text{Os}(1) - \text{C}(1)]$ is 2.068(10) Å, with the benzyl N(1) substituent orientated in such a manner to minimize interactions with the *p*-cymene ring. The $\text{Os} \cdots p\text{-cymene}$ centroid distance is [1.675(5) Å], with no appreciable tilt or offsetting of the *p*-cymene ring observed.



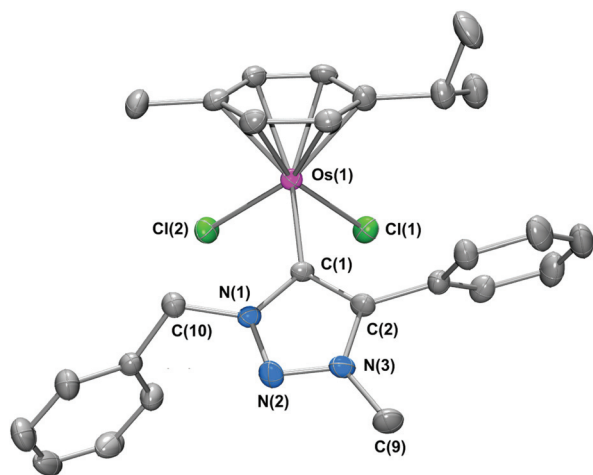


Fig. 2 Molecular structure of **5d**-CH₂Cl₂ with selected atom labelling. Hydrogen atoms and the CH₂Cl₂ solvate have been omitted for clarity. Thermal ellipsoids are drawn at 30% probability. Selected bond lengths (Å) and angles (°): Os(1)–C(1) 2.068(10), Os(1)–Cl(1) 2.420(3), Os(1)–Cl(2) 2.445(3); C(1)–Os(1)–Cl(1) 85.7(3), C(1)–Os(1)–Cl(2) 90.9(3), Cl(1)–Os(1)–Cl(2) 82.53(11).

Behaviour in solution

The chloride ligand(s) of ruthenium arene compounds such as those of the RAPTA and [Ru-arene-en]⁺ series (Fig. 1) undergo hydrolysis in aqueous solution, giving aquated species, which are proposed to be the active form of the compounds inside cells.^{29,30} The rate of this hydrolysis process has been suggested to influence the biological activity of ruthenium drugs therefore, it is useful to evaluate the behaviour of new compounds under pseudo-physiological conditions.^{31,32} As such, the solution characteristics of the new ruthenium(II) and osmium(II) tzNHC complexes were probed by a combination of UV-Vis spectroscopy and ¹H NMR spectroscopy, and compared to the RAPTA series of compounds, in particular RAPTA-C, ([Ru(η⁶-*p*-cymene)Cl₂(PTA)]), due to the structural similarity. In aqueous phosphate buffered solution (10 mM, pH 7.4), the UV-Vis spectra of **4e** was stable and unchanged from the initial solution over a period of 7 days. However, the UV-Vis trace differed when the spectrum was acquired in saline phosphate buffered solution (10 mM, pH 7.4, [Cl[−]] 150 mM), indicating that under the former conditions hydrolysis of one, or both, of the chloride ligand(s) had already taken place. Unlike RAPTA-C, which reaches an equilibrium after *ca.* 20 min,²⁹ hydrolysis of **4e** must be very rapid. Moreover, as with RAPTA-C, the process is suppressed under relatively high physiological chloride concentrations. Furthermore, although the ¹H NMR spectrum of **4e** (D₂O) is rather complicated due to the presence of a number of different species (tentatively assigned as hydrolysis products with the loss of either one or both chloride ligands), there was no evidence to suggest the loss of either the *p*-cymene or tzNHC ligands. The electrospray mass spectra of an aqueous solution of **4e** also provides evidence which points towards the loss of one or both of the chloride ligands, with the ions corresponding to both [4e-Cl]⁺ and [4e-2Cl]²⁺ species present (see ESI†). As with **4e**,

the osmium(II) complex **5e** also appears to undergo immediate hydrolysis in aqueous phosphate buffered solution.

Biological activity

The biological activity of the compounds was initially explored by determining their cytotoxicity against two human tumour (A2780 and A2780R) and non-tumour (HEK) cell lines (Table 1).

The new complexes show promising activity profiles, notably, a number of compounds display low micromolar activity against the tumour cell lines (<1 μM), which contrasts with other bifunctional ruthenium arene compounds such as RAPTA-C.³³ This difference may be related to the relatively fast hydrolysis process, at least in part, as relationships between the rate of hydrolysis (activation) and cytotoxicity have been demonstrated.³⁴ Importantly, in all cases the tzNHC complexes show selectivity towards the tumour cell lines (A2780 and A2780R) over the non-tumourigenic HEK cell line – this is exemplified by **4a** which has a *ca.* 200-fold selectivity for the A2780 cell line over the HEK cell line. In addition, such a marked *in vitro* selectivity profile in this assay shows improvements over the selectivity characteristics of cisplatin. It is also evident that increasing the lipophilicity of the complex (brought about by modifying the tzNHC alkyl sidechain) influences the *in vitro* activity of the complexes,³⁵ with the activity trends being **4a** < **4b** < **4c** and **5a** < **5b** < **5c**, presumably due to increased uptake into cells.³⁶ The relationship between the metal ion employed, *i.e.* Ru(II) *vs.* Os(II), and the cytotoxicity for the reported tzNHC complexes follows no obvious trends, and prediction of the level of biological activity upon switching the metal centre from Ru(II) to Os(II) is not straightforward. For example, whilst the *in vitro* cytotoxicity of RAPTA-C and OsAPTA-C ([Os(η⁶-*p*-cymene)Cl₂(PTA)]) is of a similar order of magnitude,³⁷ when the PTA ligand is replaced by a carbohydrate-based phosphine in a similar series of compounds, the Ru(II) complexes are more active than the Os(II) analogues.³⁸

As RAPTA complexes are believed to interact with proteins in preference to DNA,³⁹ we also probed the interaction of **4b** with the model protein ubiquitin (Ub) using HR-ESI mass

Table 1 Cytotoxicity (IC₅₀, μM) of selected compounds at 72 h

	A2780 ^a	A2780R ^b	HEK ^c
4a	1.85 ± 0.29	25.6 ± 5.5	435 ± 25
5a	27.8 ± 12.3	112 ± 13	168 ± 11
4b	0.31 ± 0.08	1.77 ± 0.04	29.0 ± 1.8
5b	4.97 ± 0.03	14.2 ± 3.3	16.5 ± 1.4
4c	0.12 ± 0.02	0.23 ± 0.02	1.80 ± 0.20
5c	0.12 ± 0.02	0.33 ± 0.06	2.59 ± 0.34
4d	4.82 ± 0.12	37.8 ± 7.8	64.0 ± 6.1
5d	1.41 ± 0.06	2.36 ± 0.46	31.7 ± 5.0
4e	41.0 ± 8.2	>200	>200
5e	19.9 ± 2.3	105 ± 5	>200
4f	0.21 ± 0.05	1.10 ± 0.12	1.85 ± 0.12
5f	0.13 ± 0.03	0.21 ± 0.01	2.89 ± 0.03
Cisplatin	4.3 ± 0.5	18.2 ± 1.0	15.3 ± 0.5

^a Human ovarian carcinoma cells. ^b Human ovarian carcinoma cells – acquired resistance to cisplatin. ^c Human embryonic kidney cells.



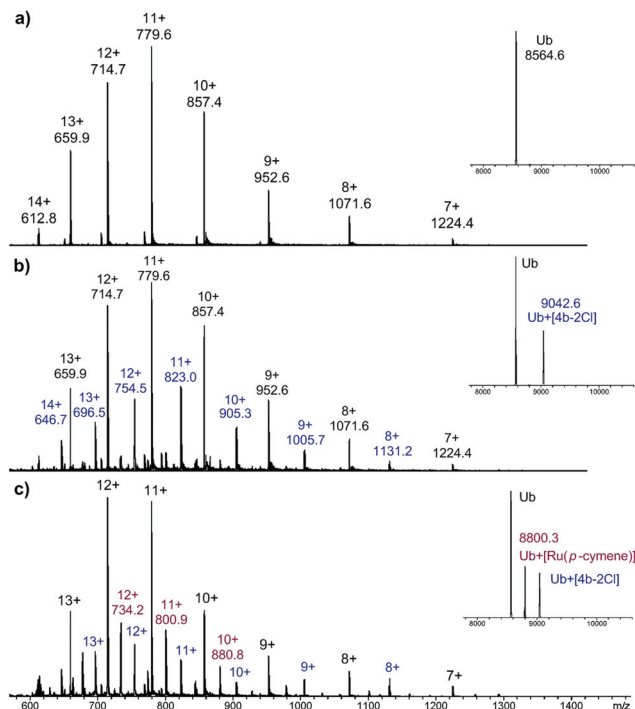


Fig. 3 ESI mass spectra of (a) Ub; (b) Ub + 4b, (1:5 ratio) 1 day incubation and (c) Ub + 4b, (1:5 ratio) 5 day incubation, with deconvoluted mass spectra shown as insets.

spectrometry. Complex 4b was incubated with Ub (5:1) at 37 °C and the mass spectra were recorded after 1 and 5 days (Fig. 3).

After 1 day, peaks corresponding to a 1:1 Ub-complex adduct is observed, assigned as Ub + [4b-2Cl], with the tzNHC fragment still bound to the metal centre. After 5 days, partial loss of the tzNHC ligand was observed, and although 4b was present in excess, the mass spectrum still indicated the presence of unmodified Ub. This reactivity is quite distinct to that of RAPTA-C (see ESI†), which reacts far more readily with Ub, with loss of the pta ligand observed after 1 day, and prolonged incubation leading to the complete disappearance of free Ub. The osmium(II) tzNHC complex responded differently again, and was not found to coordinate to Ub under the employed conditions (see ESI†). The low reactivity of the tzNHC compounds towards Ub, and presumably other relevant proteins, may prove advantageous, as with some drugs (in particular cisplatin), interactions with proteins are believed to deactivate the drug and/or lead to adverse side effects.⁴⁰

Conclusions

For the first time, we have demonstrated that the relatively new tzNHC ligands offer considerable potential in medicinal inorganic/organometallic chemistry. The ruthenium(II) and osmium(II) η^6 -arene complexes with these ligands show an excellent activity profile against a range of cell lines, and in some instances, such as compound 4a, show excellent selectivity towards tumour cell lines over non tumour lines. In

keeping with previously studied Ru(II) arene complexes, the tzNHC examples also undergo hydrolysis in aqueous solution, but in contrast, do not react with model proteins such as Ub as readily. Different groups can be easily introduced onto the tzNHC ligand *via* the initial CuAAC click reaction. Thus, a number of different functionalities, *e.g.* targeting groups, enzyme inhibitors, optically active fluorophores/lumophores, may be introduced, allowing diversity to be included in the complexes, an important aspect with respect to drug discovery.

Experimental

General

[Ru(η^6 -p-cymene)Cl₂]₂,⁴¹ and [Os(η^6 -p-cymene)Cl₂]₂,⁴² were prepared using literature procedures, all other reagents and solvents were obtained from commercial sources and used without further purification. The general synthetic protocol for the synthesis of the metal tzNHC complexes 4a-f and 5a-f is outlined below, full synthetic details and characterisation data for all compounds in this study (including 1,4-disubstituted-1-2-3-triazoles 2a-f and the corresponding triazolium salts 3a-f) are included in the ESI.†

General synthetic procedure for Ru(II) and Os(II) tzNHC complexes 4a-f and 5a-f

The triazolium salt (1 equiv.), Ag₂O (0.5 equiv.) and NMe₄Cl (1 equiv.) were stirred in CH₂Cl₂-MeCN (1:1, 10 mL) in a foil-covered flask for 18 h to give a cloudy white solution. The solvent was removed and the residue redissolved in CH₂Cl₂. [M(η^6 -p-cymene)Cl₂]₂ (M = Ru or Os) (0.5 equiv.) was added and the solution stirred for a further 90 min. The solution was then filtered through a short column of celite to remove the salt by-products, concentrated under reduced pressure and precipitated with either Et₂O or hexane at -4 °C. The solid was isolated by filtration, washed with either Et₂O or hexane and dried *in vacuo*.

X-Ray crystallography

Single crystal X-ray diffraction data for 5d·CH₂Cl₂ were collected using graphite monochromated Cu K α radiation (λ = 1.54180 Å) on an Agilent Technologies SuperNova dual system in combination with an Atlas CCD detector. Data reduction was carried out using CrysAlis PRO.⁴³ The structure was solved by direct methods (SHELXS-97) and refined against all F^2 data (SHELXL-97 and Shelxle).^{44,45} Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were positioned geometrically and refined using a riding model with $d(\text{CH}_{\text{aro}})$ = 0.93 Å, U_{iso} = 1.2 $U_{\text{eq}}(\text{C})$ for aromatic, $d(\text{CH})$ = 0.98 Å, U_{iso} = 1.2 $U_{\text{eq}}(\text{C})$ for CH, 0.97 Å, U_{iso} = 1.2 $U_{\text{eq}}(\text{C})$ for CH₂ and 0.96 Å, U_{iso} = 1.2 $U_{\text{eq}}(\text{C})$ for CH₃ (Table 2).

Cell culture conditions and cytotoxicity assay

Human A2780 and A2780cisR ovarian carcinoma and HEK (human embryonic kidney) cells were obtained from the



Table 2 Crystal data and structure refinement parameters for **5d**·CH₂Cl₂

CCDC code	960089
Empirical formula	C ₂₇ H ₃₁ Cl ₄ N ₃ Os
Formula weight	729.55
Temperature	298(2) K
Wavelength	1.54180 Å
Crystal system	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>n</i>
Unit cell dimensions	<i>a</i> = 6.38330(10) Å, <i>b</i> = 28.5636(6) Å, <i>c</i> = 15.9512(3) Å; β = 98.391(2)°
Volume	2877.25(9) Å ³
<i>Z</i>	4
Density (calculated)	1.684 Mg m ⁻³
Absorption coefficient	11.946 mm ⁻¹
<i>F</i> (000)	1432
Crystal size	0.33 × 0.17 × 0.10 mm ³
Theta range for data collection	3.09 to 67.48°
Index ranges	−7 ≤ <i>h</i> ≤ 5, −20 ≤ <i>k</i> ≤ 34, −16 ≤ <i>l</i> ≤ 19
Reflections collected	10 726
Independent reflections	5178 [<i>R</i> (int) = 0.0360]
Completeness to theta = 67.48°	99.8%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.3919 and 0.1083
Refinement method	Full-matrix least-squares on <i>F</i> ²
Data/restraints/parameters	5178/0/317
Goodness-of-fit on <i>F</i> ²	1.192
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> ₁ = 0.0641, <i>wR</i> ₂ = 0.1693
<i>R</i> indices (all data)	<i>R</i> ₁ = 0.0713, <i>wR</i> ₂ = 0.1732
Largest diff. peak and hole	2.700 and −2.486 e Å ⁻³

European Collection of Cell Cultures (Salisbury, UK). A2780 and A2780R cells were grown routinely in RPMI-1640 medium, while HEK cells were grown with DMEM medium, with 10% foetal calf serum (FCS) and antibiotics at 37 °C and 5% CO₂. Cytotoxicity was determined using the MTT assay (MTT = 3(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 µL of cell solution (approximately 20 000 cells) per well and pre-incubated for 24 h in medium supplemented with 10% FCS. Compounds were prepared as DMSO solutions then immediately dissolved in the culture medium and serially diluted to the appropriate concentration, to give a final DMSO concentration of 0.1%. 100 µL of drug solution was added to each well and the plates were incubated for another 72 h. Subsequently, MTT (5 mg mL⁻¹ solution) was added to the cells and the plates were incubated for a further 2 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 590 nm using a multiwell plate reader and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from at least two independent experiments, each comprising three micro-cultures per concentration level.

Ubiquitin binding studies

The compound was incubated with Ubiquitin (from bovine red blood cells, min 90%, Sigma Aldrich) in MilliQ water at a molar

ratio of 5 : 1 at 37 °C. Immediately prior to analysis, an aliquot of the incubation solution was diluted with water-acetonitrile-trifluoroacetic acid (50 : 50 : 0.1) to yield a final protein concentration of 0.5 µM, and introduced by direct infusion into the ESI source of the mass spectrometer at a flow rate of 5 µL min⁻¹ (source voltage, +4.5 kV; capillary temperature, 180 °C; sheath gas flow, 4 L min⁻¹). Data were processed with DataAnalysis 4.0 SP5 software (Bruker Daltonics).

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Notes and references

- 1 N. J. Wheate, S. Walker, G. E. Craig and R. Oun, *Dalton Trans.*, 2010, **39**, 8113–8127.
- 2 G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.*, 2010, **54**, 3–25.
- 3 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.
- 4 C. G. Hartinger, M. A. Jakupec, S. Zorbas-Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P. J. Dyson and B. K. Keppler, *Chem. Biodiversity*, 2008, **5**, 2140–2155.
- 5 A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen and G. Sava, *J. Inorg. Biochem.*, 2012, **106**, 90–99.
- 6 W. H. Ang and P. J. Dyson, *Eur. J. Inorg. Chem.*, 2006, 3993–3993.
- 7 W. H. Ang, A. Casini, G. Sava and P. J. Dyson, *J. Organomet. Chem.*, 2011, **696**, 989–998.
- 8 P. C. A. Bruijninx and P. J. Sadler, *Adv. Inorg. Chem.*, 2009, **61**, 1–62.
- 9 B. Therrien, *Coord. Chem. Rev.*, 2009, **253**, 493–519.
- 10 G. S. Smith and B. Therrien, *Dalton Trans.*, 2011, **40**, 10793–10800.
- 11 G. C. Vougioukalakis and R. H. Grubbs, *Chem. Rev.*, 2010, **110**, 1746–1787.
- 12 W. Liu and R. Gust, *Chem. Soc. Rev.*, 2013, **42**, 755–773.
- 13 A. Gautier and F. Cisnetti, *Metallomics*, 2012, **4**, 23–32.
- 14 L. Mercs and M. Albrecht, *Chem. Soc. Rev.*, 2010, **39**, 1903–1912.
- 15 L. Oehninger, H. Alborzina, S. Ludewig, K. Baumann, S. Wölfl and I. Ott, *ChemMedChem*, 2011, **6**, 2142–2145.
- 16 L. Oehninger, M. Stefanopoulou, H. Alborzina, J. Schur, S. Ludewig, K. Namikawa, A. Munoz-Castro, R. W. Koster, K. Baumann, S. Wölfl, W. S. Sheldrick and I. Ott, *Dalton Trans.*, 2013, 1657–1666.



- 17 J. M. Alfaro, A. Prades, M. del Carmen Ramos, E. Peris, J. Ripoll-Gómez, M. Poyatos and J. S. Burgos, *Zebrafish*, 2010, **7**, 13–21.
- 18 P. Mathew, A. Neels and M. Albrecht, *J. Am. Chem. Soc.*, 2008, **130**, 13534–13535.
- 19 J. D. Crowley, A.-L. Lee and K. J. Kilpin, *Aust. J. Chem.*, 2011, **64**, 1118–1132.
- 20 K. F. Donnelly, A. Petronilho and M. Albrecht, *Chem. Commun.*, 2013, **49**, 1145–1159.
- 21 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
- 22 M. Meldal and C. W. Tornøe, *Chem. Rev.*, 2008, **108**, 2952–3015.
- 23 K. J. Kilpin, E. L. Gavey, C. J. McAdam, C. B. Anderson, S. J. Lind, C. C. Keep, K. C. Gordon and J. D. Crowley, *Inorg. Chem.*, 2011, **50**, 6334–6346.
- 24 K. J. Kilpin and P. J. Dyson, *Chem. Sci.*, 2013, **4**, 1410–1419.
- 25 D. Yuan and H. V. Huynh, *Organometallics*, 2012, **31**, 405–412.
- 26 J. D. Crowley, P. H. Bandeen and L. R. Hanton, *Polyhedron*, 2010, **29**, 70–83.
- 27 J. D. Crowley and P. H. Bandeen, *Dalton Trans.*, 2010, **39**, 612–623.
- 28 K. J. Kilpin, U. S. D. Paul, A.-L. Lee and J. D. Crowley, *Chem. Commun.*, 2011, **47**, 328–330.
- 29 C. Scolaro, C. G. Hartinger, C. S. Allardyce, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1743–1748.
- 30 K. J. Kilpin, S. M. Cammack, C. M. Clavel and P. J. Dyson, *Dalton Trans.*, 2013, **42**, 2008–2014.
- 31 W. H. Ang, E. Daldini, C. Scolaro, R. Scopelliti, L. Juillerat-Jeannerat and P. J. Dyson, *Inorg. Chem.*, 2006, **45**, 9006–9013.
- 32 F. Wang, A. Habtemariam, E. P. L. van der Geer, R. Fernández, 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.
- 33 W. H. Ang, *Ecole Polytechnique Federale de Lausanne (EPFL)*, PhD Thesis, 2007.
- 34 A. F. A. Peacock, S. Parsons and P. J. Sadler, *J. Am. Chem. Soc.*, 2007, **129**, 3348–3357.
- 35 A. K. Renfrew, L. Juillerat-Jeanneret and P. J. Dyson, *J. Organomet. Chem.*, 2011, **696**, 772–779.
- 36 A. Kurzwernhart, W. Kandioller, C. Bartel, S. Bächler, R. Trondl, G. Mühlgassner, M. A. Jakupec, V. B. Arion, D. Marko, B. K. Keppler and C. G. Hartinger, *Chem. Commun.*, 2012, **48**, 4839–4841.
- 37 A. Dorcier, W. H. Ang, S. Bolaño, L. Gonsalvi, L. Juillerat-Jeannerat, G. Laurenczy, M. Peruzzini, A. D. Phillips, F. Zanobini and P. J. Dyson, *Organometallics*, 2006, **25**, 4090–4096.
- 38 M. Hanif, A. A. Nazarov, C. G. Hartinger, W. Kandioller, M. A. Jakupec, V. B. Arion, P. J. Dyson and B. K. Keppler, *Dalton Trans.*, 2010, **39**, 7345–7352.
- 39 B. Wu, M. S. Ong, M. Groessl, Z. Adhireksan, C. G. Hartinger, P. J. Dyson and C. A. Davey, *Chem.-Eur. J.*, 2011, **17**, 3562–3566.
- 40 A. R. Timerbaev, C. G. Hartinger, S. S. Aleksenko and B. K. Keppler, *Chem. Rev.*, 2006, **106**, 2224–2248.
- 41 M. A. Bennett and A. K. Smith, *J. Chem. Soc., Dalton Trans.*, 1974, 233–241.
- 42 H. Werner and K. Zenkert, *J. Organomet. Chem.*, 1988, **345**, 151–166.
- 43 *Crysalis PRO, release 1.171.35.21*, Agilent Technologies, 2012.
- 44 G. M. Sheldrick, *Acta Crystallogr., Sect. A: Fundam. Crystallogr.*, 2008, **64**, 112–122.
- 45 C. B. Hübschle, G. M. Sheldrick and B. Dittrich, *J. Appl. Crystallogr.*, 2011, **44**, 1281–1284.

