Anti-Cancer Complexes

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A Macrocyclic Ruthenium(III) Complex Inhibits Angiogenesis with Down-Regulation of Vascular Endothelial Growth Factor Receptor-2 and Suppresses Tumor Growth In Vivo

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Abstract: *A macrocyclic ruthenium(III) complex [Ru^{III}-(N_2O_2)Cl_2]Cl (**Ru-1**) is reported as an inhibitor of angiogenesis and an anti-tumor compound. The complex is relatively non-cytotoxic towards endothelial and cancer cell lines in vitro, but specifically inhibited the processes of angiogenic endothelial cell tube formation and cancer cell invasion. Moreover, compared with known anti-cancer ruthenium complexes, **Ru-1** is distinct in that it suppressed the expression of vascular endothelial growth factor receptor-2 (VEGFR2), and the associated downstream signaling that is crucial to tumor angiogenesis. In addition, in vivo studies showed that **Ru-1** inhibited angiogenesis in a zebrafish model and suppressed tumor growth in nude mice bearing cancer xenografts.*

Ruthenium complexes have attracted a surge of interest as alternatives to platinum-based anti-cancer agents.[1] Ruthenium complexes commonly exist in oxidation states of +2 and +3 under physiological conditions. The electron-rich Ru^{II} metal center is often stabilized by π-acceptor ligands in solution and in the solid state. In the absence of π-accepting ligands, ruthenium complexes are more prone to ligand exchange reactions with biomolecules, whereas the Ru^{III} counterparts are generally less substitution-active. This phenomenon is interpreted as "activation by reduction".[2] The reducing microenvironment of tumors may favor the reduction of Ru^{III} complexes to Ru^{II} species that could coordinate more rapidly to molecular targets.[3]

A number of ruthenium complexes have been shown to possess both in vitro and in vivo anti-cancer activities.[1–11] One example is (ImH)[*trans*-Ru^{III}(Im)(DMSO)Cl_4] (NAMI-A). NAMI-A is generally non-cytotoxic towards solid tumors but effective against tumor metastasis and angiogenesis.[4] Several possible targets of NAMI-A, including cell adhesion molecules, the extracellular matrix, and cytoskeleton proteins, have been described.[5] Another promising anti-cancer active ruthenium complex is InH[Ru^{III}(In)$_2Cl_4$] (KP-1019) where In = indazole. Although the chemical structures of KP-1019 and NAMI-A are similar, changing axial ligand has been observed to significantly alter the cellular uptake and hydrolytic stability.[6] The biological activity of KP-1019 is also different from NAMI-A. KP-1019 is cytotoxic against cisplatin-resistant colorectal carcinoma, and it did not exhibit specific anti-metastasis activity.[7] In recent years, organoruthenium complexes have also been reported to display anti-cancer activities.[8] Sadler and Dyson developed "piano-stool" anti-cancer organoruthenium(II) arene complexes, namely RM- and RAPTA-complexes containing ethylenediamino and phosphoadamatane ligands, respectively. The RM-type complexes generally exhibit promising in vitro cytotoxicity,[9] and the RAPTA-type is active against tumor angiogenesis.[10] The complex [Ru^{II}(Phen)(CN)(CH_3CN)$_2$]PF_6 (RDC11) was found to display significant in vitro and in vivo anti-cancer activities against several human cancer cell lines and human cancer xenografts in mice.[11] In this study, a macrocyclic ruthenium(III) complex [Ru^{III}(N_2O_2)Cl_2]Cl (**Ru-1**), exhibiting promising anti-angiogenesis activity and reduction of invasiveness of cancer cells, is described. Complex **Ru-1** was found to down-regulate the vascular endothelial growth receptor-2 (VEGFR2), which is an essential signaling protein for endothelial cell angiogenesis.[12] Furthermore, **Ru-1** was also shown to exhibit in vivo anti-angiogenic activity in a zebrafish model and anti-tumor activity in nude mice bearing human breast carcinoma xenografts.

We previously reported the synthesis and reactivities of the ruthenium complexes supported by the N_2O_2 ligand.[13] In this study, the anti-cancer activity of ruthenium(III) complexes containing N_2O_2 ligands with different axial ligands were examined and compared with the reported anti-cancer ruthenium complexes, including NAMI-A and [Ru(η^6-C_6H_6)Cl(en)]PF_6.[4,14] The structures of the ruthenium(III) complexes in this work are depicted in Figure 1. Reaction of K_2[$RuCl_5(H_2O)$] with the N_2O_2 ligand in refluxing ethanol yielded **Ru-1**. Complex **Ru-2** was prepared by heating **Ru-1** with excess silver(I) perchlorate in water followed by recrystallization. The solution stability of **Ru-1** against biological reducing agents was examined by UV/Vis absorption spectrophotometry. For **Ru-1** in DMSO solution, the 377 nm absorption peak, assigned to Cl^- to Ru^{III} ligand-to-metal charge transfer (LMCT), remained stable for hours (Figure S1A in the Supporting Information). In phosphate-buffered saline (PBS), the 371 nm absorption peak decreased gradually by 43% over a period of 10 min (Figures 2A and

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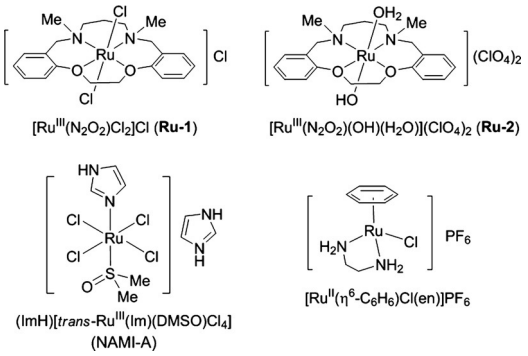

Figure 1. Chemical structures of the ruthenium complexes in this work.

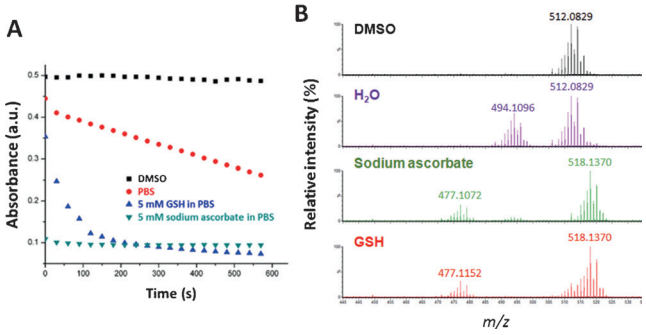

Figure 2. Solution stability of **Ru-1**. A) The absorbance at 377 nm (for **Ru-1** in DMSO) and that at 371 nm (for **Ru-1** in PBS) were monitored over 10 min. B) High-resolution ESI-MS analysis of DMSO and aqueous solution of **Ru-1** and aqueous solutions of **Ru-1** incubated with excess reducing agent (2 equiv sodium ascorbate or 5 equiv GSH) for 10 min.

S1B). In the presence of sodium ascorbate or glutathione (GSH), the decrease of absorbance at 371 nm was markedly accelerated (Figures 2A and S1). The solution stability of **Ru-1** was further examined by high-resolution electrospray ionization–mass spectrometry (HR-ESI-MS). The HR-ESI-MS spectrum of a DMSO solution of **Ru-1** was dominated by $[Ru^{III}(N_2O_2)Cl_2]^+$ species ($m/z = 512.0829$), and no significant change was observed up to 48 h (Figures 2B and S2). In aqueous solution, an intense signal corresponding to the species of $[Ru^{III}(N_2O_2)(OH)Cl]^+$ ($m/z = 494.1096$), attributed to the hydrolysis of **Ru-1**, was observed after incubation for 10 min (Figure S2A). Upon reacting **Ru-1** with excess sodium ascorbate or GSH, the formation of $[Ru^{II}(N_2O_2)Cl]^+$ ($m/z = 477.1072$) was observed (Figures 2B and S2A). Incubation with excess reducing agent for 24 and 48 h revealed formation of a hydrolysis product $[Ru^{III}(N_2O_2)(OH)_2]^+$ ($m/z = 476.1446$) and multiple Ru^{II} species (Figure S2). The reduction of **Ru-2** by sodium ascorbate could also be observed, as shown by the presence of the Ru^{II} species $[Ru^{II}(N_2O_2)(OH)(CH_3CN)]^+$ (Figure S3B). Furthermore, the cyclic voltammogram of **Ru-1** in CH_3CN showed a reversible Ru^{III}/Ru^{II} couple at -0.26 V vs. $Ag/AgNO_3$ (0.29 V vs. NHE; Figure S4). Thus, spontaneous reduction of **Ru-1** is likely to occur in the reducing cellular environment, which contains ascorbate and GSH at millimolar concentrations.[15] It is noteworthy that NAMI-A has been reported with a $Ru^{III/II}$ potential of 0.25 V vs. NHE[6a] and rapid reduction to Ru^{II} species by GSH or ascorbate.[3] In addition, NAMI-A was shown to undergo immediate hydrolysis in phosphate buffer, with dissociation of chloride or DMSO ligand within minutes.[16] Despite the greatly different structures, **Ru-1** shares several similar chemical properties with NAMI-A, including the comparable reduction potential and instability against hydrolysis/reduction.

The in vitro cytotoxicities of the ruthenium complexes were examined by MTT assay, and the cytotoxicities of NAMI-A and $[Ru(\eta^6\text{-}C_6H_6)Cl(en)]PF_6$ were also examined for comparison. Both **Ru-1** and **Ru-2** were relatively non-cytotoxic against HeLa (human cervical epithelial carcinoma), MDA-MB231 (human breast carcinoma), and MS-1 (mouse pancreatic islet endothelial) cells with IC_{50} values >100 μM (Table S1). Extended incubation up to 96 h also did not result in a significant increase in cytotoxicity (Figure S5). Moderate cytotoxicity of **Ru-1** against other human cancer cells lines was observed with IC_{50} ranges of 27 to 74 μM (Table S1).

The cellular uptake of the ruthenium complexes was determined by inductively coupled plasma mass spectrometry (ICP-MS). In MS-1 or MDA-MB231 cells treated with the ruthenium complexes for 18 h, the cellular uptake of **Ru-1** was markedly higher than that of **Ru-2** (Figure 3). Noticeably, the

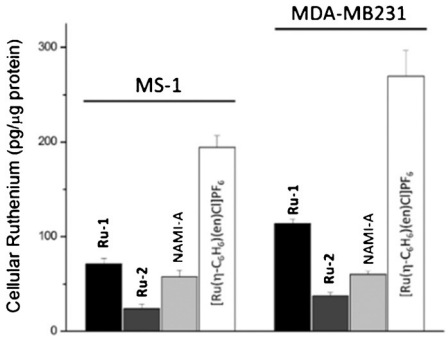

Figure 3. The cellular uptake of ruthenium complexes by MS-1 endothelial cells or MDA-MB231 breast cancer cells as determined by ICP-MS. Error bars = standard deviation.

uptake of **Ru-1** and NAMI-A were comparable in MS-1 cells, while **Ru-1** exhibited about 1.9-fold higher uptake than NAMI-A in MDA-MB231 cells. The ruthenium(II) arene complex $[Ru(\eta^6\text{-}C_6H_6)Cl(en)]PF_6$ displayed significantly higher uptake among the studied complexes in both cell types. Compared to **Ru-1**, **Ru-2** ($[Ru^{III}(N_2O_2)(H_2O)(OH)]^{2+}$) has a higher charge and a tendency to bind to extracellular components through substitution of water ligand, rendering it difficult to enter the cells.

The anti-angiogenesis activities of the ruthenium complexes in terms of their abilities to inhibit the tube formation of MS-1 endothelial cells were investigated, using NAMI-A as a reference. **Ru-1** exhibited marked inhibition of tube formation (Figure 4A). No significant effect was observed in cells treated with **Ru-2**. The anti-angiogenic activities of **Ru-1** were also confirmed by concentration-dependent inhib-

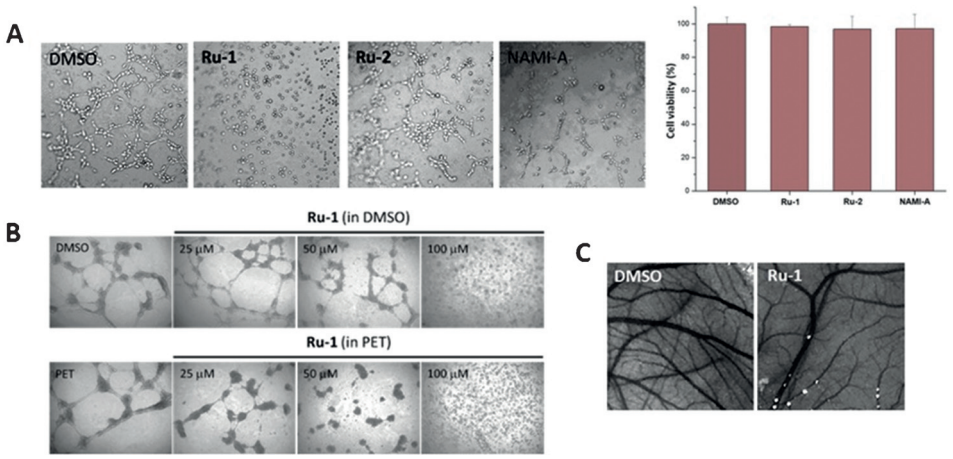

Figure 4. Anti-angiogenic properties of **Ru-1**. A) Inhibition of MS-1 endothelial cell tube formation by **Ru-1**. The tube formation activity was inhibited by **Ru-1** (100 μM) but not by **Ru-2** (100 μM). NAMI-A was used as a reference compound. No significant reduction of cell viability was observed after treatment of the ruthenium complexes as examined by MTT assay. Error bars = standard deviation. B) The tube formation activity of HUVEC was completely inhibited by **Ru-1** (100 μM). Similar results were observed when DMSO or PET was used as the solvent. C) Inhibition of angiogenesis in chicken chorioallantoic membrane (CAM) by **Ru-1**. The figures show the angiographic images of ex ovo CAM on embryonic development day 10 (EDD 10).

ition of tube formation in human umbilical vein endothelial cells (HUVEC; Figure 4B). The effect of organic solvents as the vehicle of **Ru-1** was also examined by using DMSO to replace PET (polyethylene glycol 400/ethanol/Tween-80, 6:3:1, v/v/v); the latter is a commonly used solvent in drug formulation. Comparable inhibition profiles were observed with **Ru-1** in PET as in DMSO (Figure 4B). We employed a chicken embryo chorioallantoic membrane (CAM) assay to study the ex ovo anti-angiogenic activity of **Ru-1**. The complex was administered topically to the CAM, starting from embryo development day 7 (EDD7), daily for three days. In the embryos treated with **Ru-1**, the vascular formation was inhibited, as indicated by a larger area of avascular zones compared to those of DMSO control (Figure 4C).

The effects of **Ru-1** and **Ru-2** on cancer cell invasiveness were examined by Transwell invasion assay.[17] The invasive MDA-MB231 breast cancer cells were seeded on the Matrigel-coated membrane and treated with the ruthenium complexes or vehicle for 24 h. As shown in Figure 5, complex **Ru-1** inhibited the invasion of cells through the Matrigel-coated membrane by 25.4% at 50 μM and by 73.6% at 100 μM. In contrast, **Ru-2** exhibited no significant effect on the cell invasion.

Cancer cells overexpress and secrete vascular endothelial growth factor (VEGF), which promotes angiogenesis and metastasis by binding to the VEGFR expressed on endothelial cell surface.[18] Thus, the VEGFR pathway is an important drug target of anti-cancer medicine.[19] VEGFR2 is activated via tyrosine phosphorylation, leading to activation of downstream kinases, such as protein kinase B (Akt) and extracellular regulated protein kinase 1/2 (ERK1/2), and endothelial cell proliferation.[12] We examined the effect of **Ru-1** on VEGFR2 expression and the downstream signaling pathways. Treatment of MS-1 endothelial cells with **Ru-1** induced a time- and concentration-dependent inhibition of VEGFR2 protein levels (Figure 6A,B). **Ru-1** treatment also inhibited the phosphorylation of ERK1/2 and Akt. In MDA-MB231 cancer cells, **Ru-1** treatment down-regulated the phosphorylation of VEGFR2 and Akt (Figure 6A). We also found that VEGFR2 protein expression was specifically inhibited by treatment of **Ru-1**, but not by NAMI-A or $[Ru(\eta^6\text{-}C_6H_6)Cl(en)]PF_6$ (Figure 6C). Noticeably, quantitative reserve transcription polymerase chain reaction (qRT-PCR) showed that **Ru-1** suppressed VEGFR2 mRNA expression in MS-1 cells (Figure 6D). The qRT-PCR analyses indicated that **Ru-1** downregulates VEGFR2

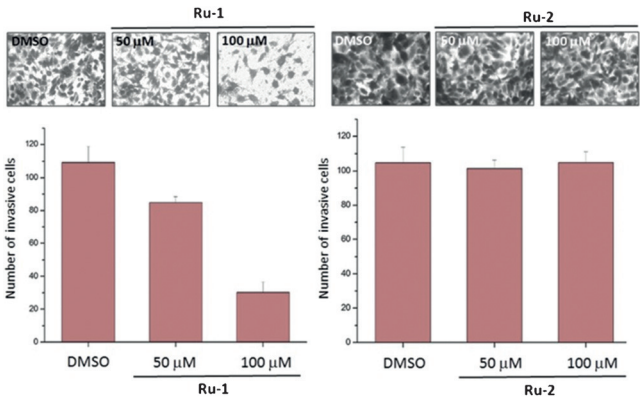

Figure 5. Complex **Ru-1** inhibited invasion of MDA-MB231 breast cancer cells at 24 h posttreatment as determined by Matrigel-Transwell migration assay. Error bars represent standard deviation.

expression at the transcriptional level. Reports on the effect of ruthenium complexes on VEGFR-mediated angiogenesis are sparse. A ruthenium(II) complex with a 2,6-bis(benzimidazolyl)-pyridine ligand was shown to block the expression and phosphorylation of VEGFR2.[20] A ruthenium(II) tris-bipyridyl complex conjugated with a peptoid targeting VEGFR2 was demonstrated to inhibit the phosphorylation of VEGFR2.[21] Our study indicated that the ruthenium(III) complex with a neutral macrocyclic N_2O_2 ligand, **Ru-1**, reduces the expression of VEGFR2 in endothelial cells and inhibits the downstream survival signaling pathways of ERK and Akt. As VEGFR2 functions as the major determinant of angiogenesis and exerts its pro-angiogenic functions through PI3K/Akt/mTOR and Ras/Raf-1/ERK1/2 pathways,[22] inhibition of VEGFR2 expression and the associated downstream pathways could account for the anti-angiogenic activity of **Ru-1**.

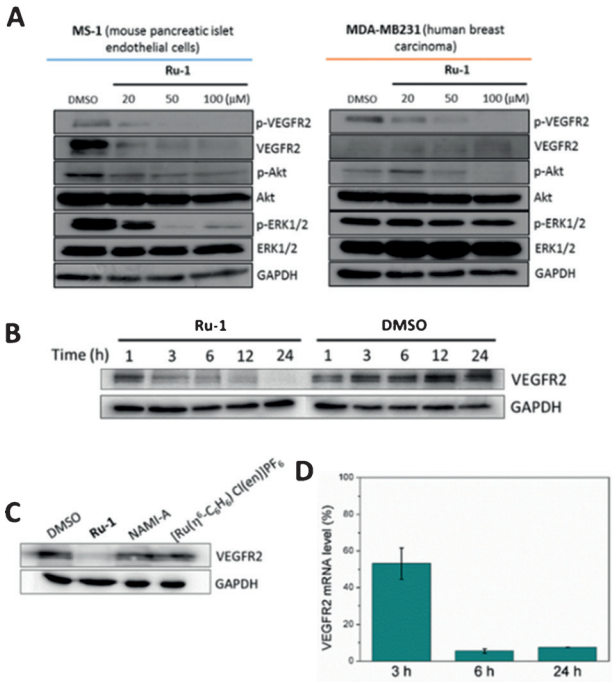

Figure 6. Complex **Ru-1** inhibited VEGFR2 expression and VEGFR signaling in MS-1 cells. A) Effects of **Ru-1** on the VEGFR signaling (phosphorylation of VEGFR2, Akt, and ERK) at 24 h posttreatment (the corresponding effects in MDA-MB231 cells are shown for comparison). B) VEGFR2 expression of cells treated with **Ru-1** (100 μM) or DMSO as revealed by Western blotting. C) VEGFR2 protein expression in MS-1 cells treated with DMSO, **Ru-1** (100 μM), NAMI-A (150 μM), or $[Ru(\eta^6\text{-}C_6H_6)Cl(en)]PF_6$ (50 μM) for 24 h. D) VEGFR2 mRNA expression at 3, 6, and 24 h posttreatment of **Ru-1** (100 μM) compared with the vehicle control as determined by qRT-PCR. Error bars = standard deviation.

The in vivo anti-angiogenic activity of **Ru-1** was assessed using transgenic Tg(*flk1*:mCherry) zebrafish embryos expressing red fluorescence reporter protein under the control of the *flk1/vegfr2* gene promoter, and serves as a marker protein of blood vessels.[23] Figure 7 and Table 1 show the anti-angiogenic effect of **Ru-1**, as revealed by reduced formation of intersegmental vessels (ISV, green arrows) in 68% of embryos examined at 48-hours postfertilization (hpf). Moreover, **Ru-1** did not appear to induce toxicity, and no embryo was found dead after the exposure to the complex. We also examined whether **Ru-1** could inhibit

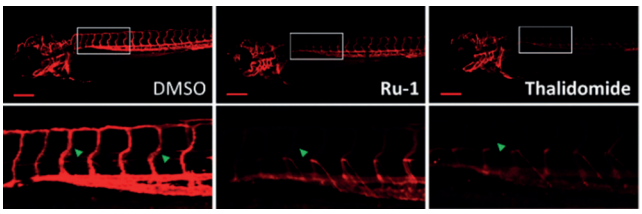

Figure 7. Anti-angiogenic effects of **Ru-1** in Tg(*flk1*:mCherry) zebrafish model. Zebrafish embryos were incubated with **Ru-1** (100 μM) and thalidomide (20 μM) was used as the positive control. The formation of blood vessels was revealed by ISV (green arrows) growth at 48-hpf and observed under confocal microscopy (scale bar = 500 μm).

Table 1: Proportion of embryo population with suppressed ISV growth.

	Suppressed ISV growth (%sample)	Survival (%sample)	Sample size
Vehicle	0	100	17
Ru-1 (100 μM)	68	100	19
Thalidomide (20 μM)	80	79	19

tumor growth in vivo. Nude mice xenografted with MDA-MB231 breast cancer cells were treated with **Ru-1** (20 mg kg^{-1}) through intravenous (i.v.) injection twice per week. Treatment of the mice with **Ru-1** for 17 days resulted in significant inhibition of tumor growth by 75% compared to the solvent control group (Figure 8A), with no animal death or weight loss (Figure 8B).

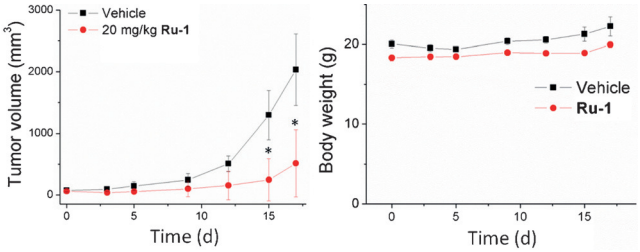

Figure 8. Complex **Ru-1** inhibits in vivo tumor growth in nude mice bearing MDA-MB231 xenografts. A) Tumor volumes of mice after treatment of **Ru-1** (20 mg kg^{-1}) or PET (vehicle control). B) Body weight of mice after treatment of **Ru-1** or PET (vehicle control). $*p \leq 0.05$ (*t*-test).

In conclusion, we have discovered a macrocyclic ruthenium(III) complex **Ru-1**, which is relatively non-cytotoxic, exhibits anti-angiogenic, anti-metastatic, and promising in vivo anti-tumor activities in association with down-regulation of the VEGFR2 expression and the downstream signaling pathways. Importantly, the special mechanism of action of **Ru-1** is not shared by the anti-cancer ruthenium complexes NAMI-A and the ruthenium(II)-arene complex.

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[1] a) A. Bergamo, G. Sava, *Chem. Soc. Rev.* **2015**, *44*, 8818–8835; b) A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen, G.

Sava, *J. Inorg. Biochem.* **2012**, *106*, 90 – 99; c) S. L.-F. Chan, R. W.-Y. Sun, M.-Y. Choi, Y. Zeng, L. Shek, S. S.-Y. Chui, C.-M. Che, *Chem. Sci.* **2011**, *2*, 1788 – 1792; d) A. Levina, A. Mitra, P. A. Lay, *Metallomics* **2009**, *1*, 458 – 470; e) R. W.-Y. Sun, M. F.-Y. Ng, E. L.-M. Wong, J. Zhang, S. S.-Y. Chui, L. Shek, T.-C. Lau, C.-M. Che, *Dalton Trans.* **2009**, 10712 – 10716; f) A. Bergamo, G. Sava, *Dalton Trans.* **2007**, 1267 – 1272; g) W. H. Ang, P. J. Dyson, *Eur. J. Inorg. Chem.* **2006**, 4003 – 4018; h) M. J. Clarke, *Coord. Chem. Rev.* **2002**, *232*, 69 – 93.

[2] M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev.* **1999**, *99*, 2511 – 2534.

[3] M. Ravera, S. Baracco, C. Cassino, P. Zanello, D. Osella, *Dalton Trans.* **2004**, 2347 – 2351.

[4] a) A. Vacca, M. Bruno, A. Boccarelli, M. Coluccia, D. Ribatti, A. Bergamo, S. Garbisa, L. Sartor, G. Sava, *Br. J. Cancer* **2002**, *86*, 993 – 998; b) G. Sava, K. Clerici, I. Capozzi, M. Cocchietto, R. Gagliardi, E. Alessio, G. Mestroni, A. Perbellini, *Anti-Cancer Drugs* **1999**, *10*, 129 – 138.

[5] a) L. Brescacin, A. Masi, G. Sava, A. Bergamo, *J. Biol. Inorg. Chem.* **2015**, *20*, 1163 – 1173; b) C. Casarsa, M. T. Mischis, G. Sava, *J. Inorg. Biochem.* **2004**, *98*, 1648 – 1654; c) G. Sava, S. Zorzet, C. Turrin, F. Vita, M. Soranzo, G. Zabucchi, M. Cocchietto, A. Bergamo, S. DiGiovine, G. Pezzoni, L. Sartor, S. Garbisa, *Clin. Cancer Res.* **2003**, *9*, 1898 – 1905.

[6] a) M. Groessl, E. Reisner, C. G. Hartinger, R. Eichinger, O. Semenova, A. R. Timerbaev, M. A. Jakupec, V. B. Arion, B. K. Keppler, *J. Med. Chem.* **2007**, *50*, 2185 – 2193; b) M. A. Jakupec, E. Reisner, A. Eichinger, M. Pongratz, V. B. Arion, M. Galanski, C. G. Hartinger, B. K. Keppler, *J. Med. Chem.* **2005**, *48*, 2831 – 2837.

[7] a) A. Bergamo, A. Masi, M. A. Jakupec, B. K. Keppler, G. Sava, *Met.-Based Drugs* **2009**, *16*, 681270; b) S. Kapitza, M. A. Jakupec, M. Uhl, B. K. Keppler, B. Marian, *Cancer Lett.* **2005**, *226*, 115 – 121.

[8] a) C. G. Hartinger, N. Metzler-Nolte, P. J. Dyson, *Organometallics* **2012**, *31*, 5677 – 5685; b) G. Gasser, N. Metzler-Nolte, *Curr. Opin. Chem. Biol.* **2012**, *16*, 84 – 91; c) A. F. A. Peacock, P. J. Sadler, *Chem. Asian J.* **2008**, *3*, 1890 – 1899.

[9] a) 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, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18269 – 18274; b) A. Bergamo, A. Masi, A. F. A. Peacock, A. Habtemariam, P. J. Sadler, G. Sava, *J. Inorg. Biochem.* **2010**, *104*, 79 – 86.

[10] a) C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava, P. J. Dyson, *J. Med. Chem.* **2005**, *48*, 4161 – 4171; b) P. Nowak-Sliwinska, J. R. van Beijnum, A. Casini, A. A. Nazarov, G. Wagnières, H. van den Bergh, P. J. Dyson, A. W. Griffioen, *J. Med. Chem.* **2011**, *54*, 3895 – 3902.

[11] X. Meng, M. L. Leyva, M. Jenny, I. Gross, S. Benosman, B. Fricker, S. Harlepp, P. Hébraud, A. Boos, P. Wlosik, P. Bischoff, C. Sirlin, M. Pfeffer, J.-P. Loeffler, C. Gaiddon, *Cancer Res.* **2009**, *69*, 5458 – 5466.

[12] S. P. Ivy, J. Y. Wick, B. M. Kaufman, *Nat. Rev. Clin. Oncol.* **2009**, *6*, 569 – 579.

[13] a) W. W. Y. Lam, S.-M. Yiu, D. T. Y. Yiu, T.-C. Lau, W.-P. Yip, C.-M. Che, *Inorg. Chem.* **2003**, *42*, 8011 – 8018; b) C.-M. Che, W.-T. Tang, W.-T. Wong, T.-F. Lai, *J. Am. Chem. Soc.* **1989**, *111*, 9048 – 9056.

[14] a) R. E. Aird, J. Cummings, A. A. Ritchie, M. Muir, R. E. Morris, H. Chen, P. J. Sadler, D. I. Jodrell, *Br. J. Cancer* **2002**, *86*, 1652 – 1657; b) R. E. Morris, R. E. Aird, P. del Socorro Murdoch, H. Chen, J. Cummings, N. D. Hughes, S. Parsons, A. Parkin, G. Boyd, D. I. Jodrell, P. J. Sadler, *J. Med. Chem.* **2001**, *44*, 3616 – 3621.

[15] F. Q. Schafer, G. R. Buettner, *Free Radical Biol. Med.* **2001**, *30*, 1191 – 1212.

[16] M. Bacac, A. C. G. Hotze, K. van der Schilden, J. G. Haasnoot, S. Pacor, E. Alessio, G. Sava, J. Reedijk, *J. Inorg. Biochem.* **2004**, *98*, 402 – 412.

[17] A. Albini, *Pathol. Oncol. Res.* **1998**, *4*, 230 – 241.

[18] L. M. Ellis, D. J. Hicklin, *Nat. Rev. Cancer* **2008**, *8*, 579 – 591.

[19] M. Kowanetz, N. Ferrara, *Clin. Cancer Res.* **2006**, *12*, 5018 – 5022.

[20] H. Lai, Z. Zhao, L. Li, W. Zheng, T. Chen, *Metallomics* **2015**, *7*, 439 – 447.

[21] J. Lee, D. G. Udugamasooriya, H.-S. Lim, T. Kodadek, *Nat. Chem. Biol.* **2010**, *6*, 258 – 260.

[22] A.-K. Olsson, A. Dimberq, J. Kreuger, L. Claesson-Welsh, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 359 – 371.

[23] S.-W. Jin, D. Beis, T. Mitchell, J.-N. Chen, D. Y. R. Stainier, *Development* **2005**, *132*, 5199 – 5209.

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