Bioorganometallic Chemistry

DOI: 10.1002/anie.201202939

On the Biological Properties of Alkynyl Phosphine Gold(I) Complexes**

*Andreas Meyer, Christoph P. Bagowski, Malte Kokoschka, Maria Stefanopoulou, Hamed Alborzinia, Suzan Can, Danielle H. Vlecken, William S. Sheldrick, Stefan Wölfl, and Ingo Ott**

Gold complexes have a long tradition in the treatment of the symptoms of rheumatoid arthritis.[1,2] Therapeutically used drugs include mainly gold(I) thiolates (e.g. aurothiomalate and auranofin), which belong to the group of disease-modifying antirheumatic drugs (DMARDs) that are used to slow down or stop the progression of this severe and disabling rheumatic disorder. Interestingly, in vitro studies on cultured tumor cells have also indicated the considerable potential of this class of metallodrugs for tumor chemotherapy, and thioredoxin reductase is one of the enzymes identified as a critical target.[3–9] Intensified research on the development of gold antitumor drugs has led to many active species such as gold(I) complexes with phosphine, thiolate, chloride, and carbene ligands as well as gold(III) derivatives.[3,4,10–12]

However, a major issue in the development of new bioactive gold complexes is the preparation of complexes that show suitable stability under physiological conditions.[13–16] Gold complexes with alkynyl ligands, which are widely used because of their catalytic and luminescent properties,[17] might display reasonably stable coordinative bonds. In fact, recent initial reports on the bioactivity of alkynyl gold complexes indicate that this type of organometallic complex offers opportunities for the development of new chemotherapeutics against cancer and infectious diseases.[18] Despite these prospectives, only three studies on the biological potential of alkynyl gold complexes have been reported so far.[19–21]

Here, we present the outcome of a pilot study aimed at establishing the biological profile of alkynyl phosphine gold(I) complexes. Our study shows that the critical target enzyme thioredoxin reductase can be efficiently and selectively inhibited and that cysteine and selenocysteine residues are presumably the sites of molecular interaction with the enzyme. Moreover, we quantified the cellular uptake of the complexes, established their effects on tumor cell metabolism and mitochondrial respiration, and investigated their anti-angiogenic properties in zebrafish embryos.

A series of six alkynyl gold(I) complexes (**1**–**6**, see Figure 1) was prepared by reacting the respective alkynes with chloro(triphenylphosphine)gold(I). The structures were confirmed by ^{1}H, ^{13}C, ^{31}P NMR, and IR spectroscopy and

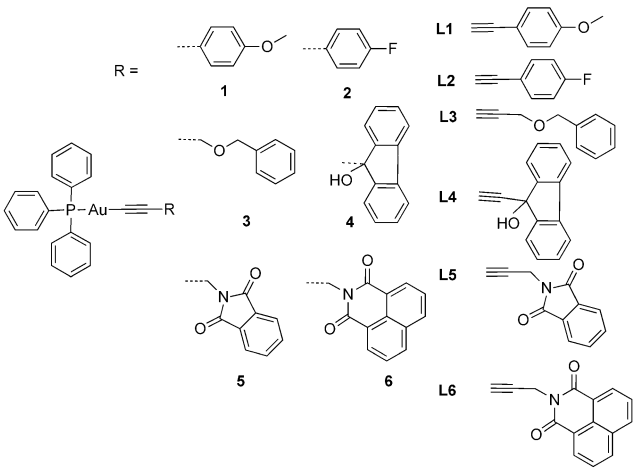

Figure 1. Overview of the investigated gold complexes **1**–**6** and the alkynes **L1**–**L6**.

their high purity was determined by elemental analysis (see the Supporting Information). The disappearance of the alkyne proton signal of the free ligands in the ^{1}H NMR spectra is an indication of the complex formation. The resulting complexes were readily soluble in organic solvents such as chloroform, dichloromethane, and dimethylformamide (DMF). For application in the biological assays, stock

[*] A. Meyer, Prof. Dr. I. Ott
Institute of Medicinal and Pharmaceutical Chemistry
Technische Universität Braunschweig
Beethovenstrasse 55, 38106 Braunschweig (Germany)
E-mail: ingo.ott@tu-bs.de
Homepage: http://www.pharmchem.tu-bs.de/forschung/ott/
Prof. Dr. C. P. Bagowski
Klinik für Innere Medizin, Ernst-Moritz Arndt University Greifswald
Friedrich Löffler Strasse 23a, 17475 Greifswald (Germany)
Dr. M. Kokoschka
Centre for Electrochemical Sciences-CES
Ruhr-Universität Bochum, 44780 Bochum (Germany)
Dr. M. Stefanopoulou, Prof. Dr. W. S. Sheldrick
Lehrstuhl für Analytische Chemie
Ruhr-Universität Bochum, 44780 Bochum (Germany)
Dr. H. Alborzinia, S. Can, Prof. Dr. S. Wölfl
Institut für Pharmazie und Molekulare Biotechnologie
Ruprecht-Karls-Universität Heidelberg
Im Neuenheimer Feld 364, 69120 Heidelberg (Germany)
M. Sc. D. H. Vlecken
Institute of Biology, Department of Molecular and Cellular Biology
University of Leiden, AL Leiden (The Netherlands)

[**] Financial support by the Deutsche Forschungsgemeinschaft (DFG, project FOR630), the EU, and the state of North Rhine Westphalia in the framework of the HighTech.NRW is gratefully acknowledged.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201202939.

solutions of the compounds were prepared in DMF and diluted with the respective assay buffers and media.

Proliferation assays with **1**–**6** in MCF-7 breast adenocarcinoma and HT-29 colon carcinoma cells confirmed the strong antiproliferative activity of the alkynyl phosphine gold(I) complexes (see Table 1). The observed IC_{50} values between 0.8 and 12.0 μM were in the range of the activities of established cytostatics such as cisplatin and 5-fluorouracil and recently studied gold(I) phosphine derivatives.[22,23] The metal-free alkyne ligands **L1**–**L6** were investigated as controls, and as expected they showed no activity against tumor cell proliferation.

Table 1: Antiproliferative effects and enzyme inhibitory activity of alkynyl gold(I) complexes and alkynes expressed as IC_{50} values [μM] obtained in two or three independent experiments.

	HT-29	MCF-7	TrxR	GR	GR/TrxR[a]
L1	>100	>100	>100	>250	n.a.[b]
L2	>100	>100	>100	>250	n.a.[b]
L3	>100	>100	>100	>250	n.a.[b]
L4	>100	>100	>100	>250	n.a.[b]
L5	>100	>100	>100	>250	n.a.[b]
L6	>100	>100	>100	>250	n.a.[b]
1	5.0±0.3	1.0±0.2	0.045±0.034	10.3±4.4	228
2	1.6±0.7	2.2±0.4	0.359±0.054	19.3±7.9	54
3	5.2±1.5	0.8±0.5	0.047±0.007	19.1±6.0	406
4	2.3±1.2	3.8±0.1	1.400±0.251	65.7±6.7	47
5	4.5±0.3	0.8±0.3	0.423±0.113	14.9±11.9	35
6	12.0±3.5	2.2±0.6	0.337±0.113	31.1±7.0	92

[a] Ratio of GR vs. TrxR inhibition. [b] n.a.: not applicable.

In order to evaluate the extent of cellular uptake, we measured the gold content of HT-29 cells exposed to 5.0 μM of the complexes over a period of 6 h by a method based on high-resolution continuum source atomic absorption spectroscopy (HRCS-AAS).[24] Differences in the observed cytotoxicity values of Table 1 could not be explained by these experiments, although the experiments indicated a faster uptake of the complexes with smaller alkynyl ligands (see the Supporting Information for details).

As mentioned above, thioredoxin reductase (TrxR) represents a critical pharmacological target for bioactive gold(I) species and is significantly involved in pathophysiological processes including cancer and rheumatic diseases.[5,25] Besides the prospective target enzyme TrxR, the structurally and functionally similar glutathione reductase (GR, from yeast) was used as a reference to check the specificity of the enzymatic inhibition.

The strongest inhibition of TrxR was observed with **1** and **3**, which exhibited IC_{50} values in the low nanomolar range (see Table 1). GR was inhibited with more than 200-fold higher IC_{50} values in the micromolar range. This clearly demonstrates that alkynyl gold(I) complexes can achieve tremendous selectivity for the inhibition of TrxR closely resembling key features of the gold(I) lead compound auranofin (9 nM against TrxR and 15 μM against GR in the same assay).[26] Complexes **2**, **5**, and **6** were approximately eight times less active against TrxR, and for **4** the highest value against TrxR was observed (1.4 μM). However, as outlined above, these differences in activity against TrxR (**1**, **3** ⩾ **2**, **5**, **6** > **4**) were not translated into a substantially higher cytotoxic activity of **1** and **3** relative to that of **2** and **4**–**6**. The metal-free alkynes **L1**–**L6** showed no activity.

The interaction of gold complexes with the active center of TrxR is supposedly dominated by ligand-exchange processes between the coordinated ligands at the gold center and cysteine/selenocysteine residues of the enzyme.[7,27,28] In the assay for TrxR inhibition the enzyme was initially exposed to the complexes for 75 min to allow these ligand-exchange reactions to achieve equilibrium conditions. In order to check for the time dependency of the enzymatic reaction we measured the inhibition of TrxR by **1** close to its IC_{50} concentration (0.050 μM) immediately after adding the complex to the enzyme. In fact this experiment indicated a time-dependent process since the activity was decreased to only 79(±12)% of that of the untreated control. After 10 min of exposure the enzymatic activity already dropped to approximately 50% and remained close to this value, which indicates that the kinetic processes involved in the interaction with TrxR are completed within a few minutes.

Based on their exceptionally strong TrxR inhibitory properties complexes, **1** and **3** were selected for further comparative studies. To study the relative strengths of the Au–C and Au–P bonds, the bond dissociation energies of the structurally closely related complexes **1**–**3** were evaluated theoretically by DFT calculations.[29–31] The calculated Au–C bond dissociation energies were in the range of 270 to 278 kJ mol^{-1}, while the Au–P bond dissociation energies ranged between 173 and 175 kJ mol^{-1} (see the Supporting Information). Accordingly both types of bonds are stable from a theoretical viewpoint with the phosphines representing the more weakly coordinated ligands. However, because of the relatively small differences between the respective gold complexes themselves, further conclusions concerning the kinetics of the interaction with TrxR could not be drawn.

Mass spectrometry can be used to identify possible binding partners on the molecular level. Here we used the selenocysteine-containing model peptide Ala-Gly-Sec-Val-Gly-Ala-Gly-Leu-Ile-Lys (AGUVGAGLIK) to check for binding to selenocysteine, which is present in the active site of TrxR. Incubation of the selenopeptide with the most active TrxR inhibitors **1** and **3** for 75 min, 24 h, and 48 h followed by MS analysis led in all cases to molecular ions at m/z 1133, which corresponds to a naked gold atom attached to the peptide. Additional peaks were observed in both cases for peptide coordination by the gold(alkynyl) and the gold(triphenylphosphine) fragment. MS/MS spectra of the molecular ion at m/z 1133 contained appropriate modified and unmodified series of b^+ and y^+ fragment ions and confirmed the selenocysteine residue as the major gold binding site (see Figure 2).

Influences on tumor cell metabolism can be measured by using an advanced sensor chip system, which makes it possible to continuously monitor drug effects on cell metabolism as well as cell adhesion properties. The effects of **1** and **3** on cell impedance, cell respiration, and extracellular acidification were studied in MCF-7 cells in concentrations of 5 and 10 μM

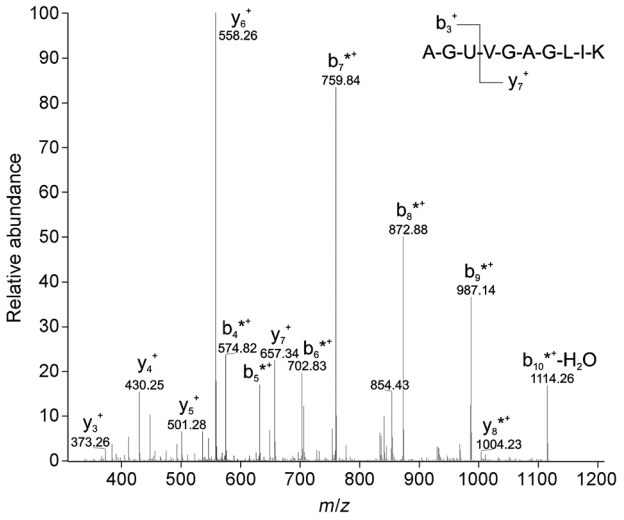

Figure 2. MS/MS spectrum of the molecular ion [peptide + Au]$^+$ at *m/z* 1133 formed by ionization of a 1:1 mixture of complex **3** with the selenopeptide H-AGUVGAGLIK-OH after 75 min of incubation at 37 °C. Signal groups marked with an asterisk (*) stem from species that contain one gold atom. Similar results were obtained after 24 h and 48 h of exposure and with **1** (see the Supporting Information).

(see Figure 3). A decrease of cell impedance is the consequence of changes in the cell membrane properties (e.g. cell–cell or cell–matrix contacts or cell adhesion) and reflects major cellular morphological changes. Cell impedance was substantially decreased in a dose- and time-dependent manner with both **1** and **3**. In both concentrations used, **3** was clearly more effective. The sharp decrease of oxygen consumption reflects the immediate inhibitory effects of **1** and **3** on cellular respiration. Compound **3** was also more effective in decreasing the extracellular acidification of the cells, which is related to a lowered glycolysis rate. Complex **1** triggered only minor activity in this case. Concomitant reduction of impedance and glycolysis with **3** at 10 μM clearly indicates efficient killing of cells at this concentration.[32] Complexes **1** and **3** show some similarities to gold(I) complexes with N-heterocyclic carbene (NHC) ligands and cisplatin, but also differences in their effects on MCF-7 cell metabolism.[24,26,32] Both cisplatin and gold(I) NHC complexes triggered a compensatory enhancement of glycolysis when respiration was inhibited, which was not observed with **1** and **3**. Similar to **1** and **3**, gold(I) NHC complexes caused an immediate decrease of cell respiration but cisplatin affected respiration only after extended exposure periods. Cell impedance declined after approximately 8–10 h with all compounds.

The strong and immediate decrease in respiration clearly indicates mitochondria as a major target organell, which also had been described previously for gold metallodrugs.[10,24,33,34] Accordingly, we studied the effects of **1** and **3** on the respiration of functionally active mitochondria (see Figure 4). In this assay the oxygen consumption of freshly isolated mouse liver mitochondria was measured using an oxygen sensor system. Complex **3** initially stimulated mitochondrial respiration in concentrations of 5 and 10 μM (as reflected by an increased oxygen consumption in this assay) and eventually inhibited mitochondrial functions with longer exposure (> 240 min). This indicates that complex **3** acts as a weak oxidative decoupling agent and only after longer treatment inhibits mitochondrial respiration. With this property it significantly differs from recently studied gold(I) complexes, which had demonstrated strong inhibition of mitochondrial respiration.[26] Complex **1** showed generally the same properties as **3**, but in this case the effects were only minor. The gold-free ligand **L3** was used as a negative control and as expected its effects could not be distinguished from those of the untreated control. chloro(triphenylphosphine)-gold(I) was used as a positive control and inhibited mitochondrial respiration strongly at a concentration of 10 μM (see the Supporting Information for the results with both controls).

Another feature highly relevant for the design of anticancer metallodrugs is the triggering of anti-angiogenic effects, which have been reported for various examples including ruthenium, cobalt, and iridium coordination compounds.[23,35–39] Anti-angiogenic effects are generally related to the reduction of blood vessel formation in growing tumors.

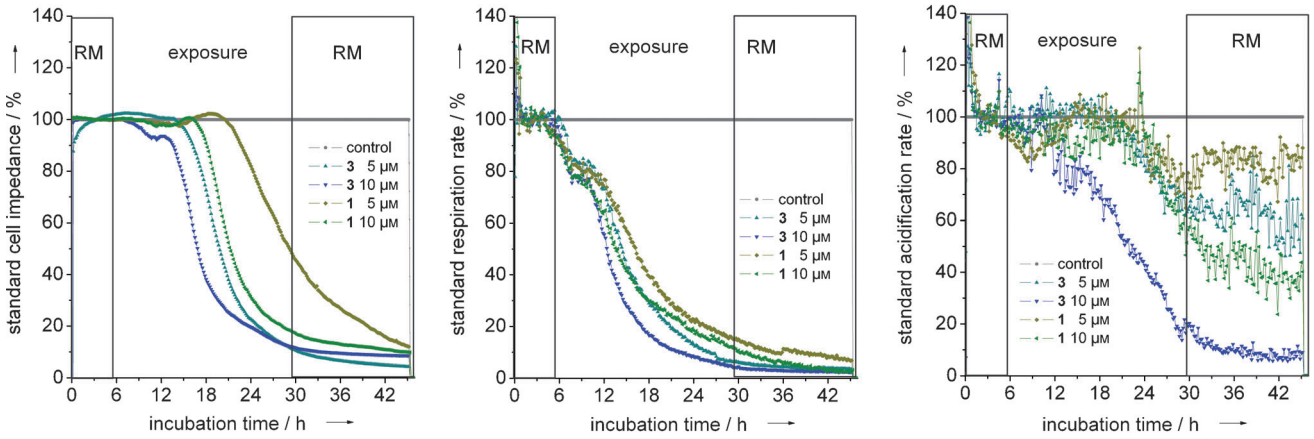

Figure 3. Real-time analysis of MCF-7 cell metabolism in response to exposure to 5 and 10 μM solutions of complexes **1** and **3**. Cell impedance (left), standard respiration rate (middle), and standard extracellular acidification rate (right). Treatment started after an equilibration time of about 5 h and continued for 24 h. RM: running medium (no compound).

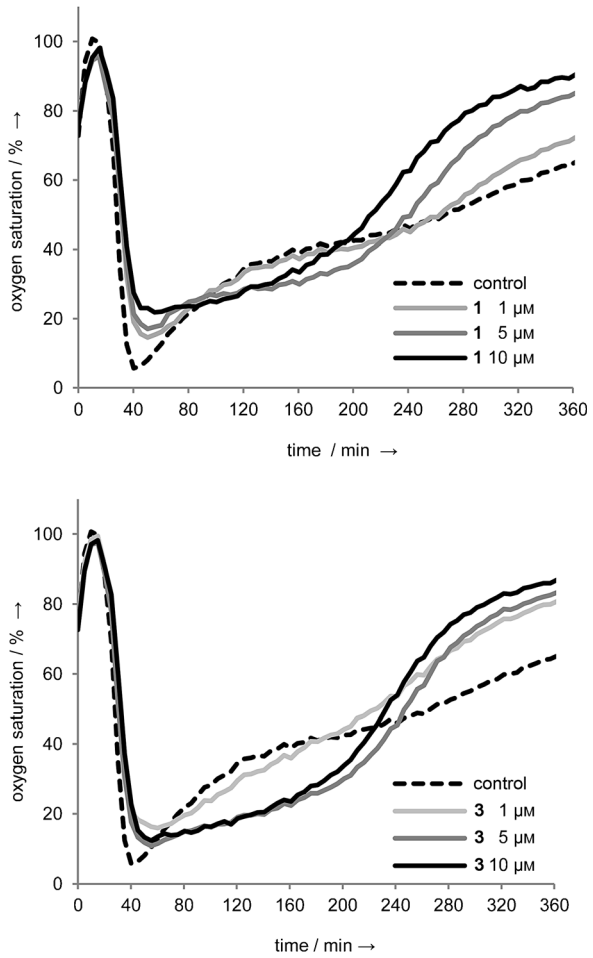

Figure 4. Time course of the oxygen saturation of medium containing freshly isolated mouse liver mitochondria. Compounds were added in concentrations of 1, 5, and 10 μM. Top: **1**, bottom: **3.** Normal mitochondrial activity leads to a decrease in oxygen saturation (control). In comparison inhibition of mitochondrial respiration leads to higher oxygen saturation and an enhancement of respiration results in a lower oxygen saturation. Rotenone, an inhibitor of respiratory chain complex I, leads to continuously high levels of oxygen saturation and carbonyl cyanide 3-chlorophenylhydrazone (CCCP), which acts as a decoupling agent, causes an increased oxygen consumption (not shown).

As a result the malignant tissue ultimately "starves" owing to the reduction of necessary nutrients. This approach is considered a practicable strategy for anticancer drug development.[40] Recently experimental in vivo assays using developing zebrafish embryos have demonstrated a huge potential as an animal model in the development of anti-angiogenic agents because of the fast and reliable results and the practical applicability for drug screening procedures.[41]

Interestingly, the alkynyl gold complexesused in this study displayed significant effects on the blood vessel formation in developing zebrafish embryos, whereas the metal-free ligands **L1** and **L3** remained completely inactive (see Figure 5 and the Supporting Information). Thus, after 48 h and 72 h of exposure to **1** and **3** in nontoxic concentrations (0.1 and 1.0 μM), relevant defects in vessel formation could be noted in

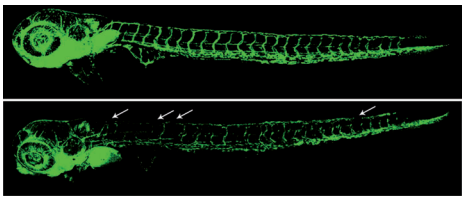

Figure 5. Blood vessel formation in developing zebrafish embryos (transgenic zebrafish line, Tg:fli1/e GFP) was monitored three days after fertilization; top: **L1** (0.1 μM), bottom: **3** (0.1 μM). Examples of effects on vessel formation are marked with arrows.

more than 90% of the treated embryos. The known anti-angiogenic drug thalidomide[42] was used as a positive reference in the same concentrations and exhibited significant but lower activity (approximately half of the embryos were affected, see the Supporting Information). These results strongly indicate that the activity of the gold complexes is related to that of the intact metal species. This result is of particular importance since we could show in previous reports that chloro gold(I) phosphines were not active in this assay and that the activity of related naphthalimide-containing gold(I) derivatives was linked to the presence of the organic naphthalimide ligand.[23,35] The results reported here thus imply that the alkynyl gold(I) moiety can be used to obtain active angiogenesis inhibitors.

In summary our study shows that alkynyl gold complexes of the type alkynyl(triphenylphosphine)gold(I) exhibit a promising potential as future chemotherapeutics. They trigger antiproliferative effects and are generally strong inhibitors of TrxR with a high selectivity over the related enzyme glutathione reductase. Moreover, effects against tumor cell metabolism and mitochondrial respiration were observed as well as significant anti-angiogenic properties in zebrafish embryos. TrxR is an enzyme with various physiological and pathophysiological functions.[43,44] Among other pathways it is involved in cell proliferation, apoptosis, and angiogenesis and it is relevant for several diseases including cancer. It has to be clarified in future studies whether the observed biological effects of alkynyl gold(I) complexes are directly connected to the inhibition of TrxR or the result of the interaction with different pathways. A number of molecular targets have been reported for structurally diverse gold species and hence they might also be relevant for the bioactivity of alkynyl(phosphine)gold(I) complexes.[6,7] For example, this might include the interaction with PARP-1,[45] phosphatases,[46,47] and cathepsins.[48,49]

The results obtained in this study clearly warrant further studies on the biochemistry of alkynyl gold complexes, the identification of additional targets, as well as the development of structure–activity relationships.

Received: April 17, 2012
Revised: May 17, 2012
Published online: July 29, 2012

Keywords: angiogenesis · bioorganometallic chemistry · cellular uptake · gold complexes · thioredoxin reductase

[1] R. Eisler, *Inflammation Res.* **2003**, *52*, 487.
[2] B. M. Sutton, E. McGusty, D. T. Walz, M. J. DiMartino, *J. Med. Chem.* **1972**, *15*, 1095.
[3] I. Ott, *Coord. Chem. Rev.* **2009**, *253*, 1670.
[4] S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori, *Med. Res. Rev.* **2010**, *30*, 550.
[5] A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, *Coord. Chem. Rev.* **2009**, *253*, 1692.
[6] C. Gabbiani, L. Messori, *Anticancer Agents Med. Chem.* **2011**, *11*, 929.
[7] K. P. Bhabak, B. J. Bhuyan, G. Mugesh, *Dalton Trans.* **2011**, *40*, 2099.
[8] E. Meggers, *Chem. Commun.* **2009**, 1001.
[9] C.-M. Che, F.-M. Siu, *Curr. Opin. Chem. Biol.* **2010**, *14*, 255.
[10] P. J. Barnard, S. J. Berners-Price, *Coord. Chem. Rev.* **2007**, *251*, 1889.
[11] S. J. Berners-Price, A. Filipovska, *Metallomics* **2011**, *3*, 863.
[12] C.-M. Che, R. W.-Y. Sun, *Chem. Commun.* **2011**, *47*, 9554.
[13] C. F. Shaw, *Chem. Rev.* **1999**, *99*, 2589.
[14] M. T. Coffer, C. F. Shaw, A. L. Hormann, C. K. Mirabelli, S. T. Crooke, *J. Inorg. Biochem.* **1987**, *30*, 177.
[15] S. M. Cottrill, H. L. Sharma, D. B. Dyson, R. V. Parish, C. A. M. Auliffe, *J. Chem. Soc. Perkin Trans. 2* **1989**, 53.
[16] N. A. Malik, G. Otiko, P. J. Sadler, *J. Inorg. Biochem.* **1980**, *12*, 317.
[17] J. C. Lima, L. Rodriguez, *Chem. Soc. Rev.* **2011**, *40*, 5442.
[18] J. C. Lima, L. Rodriguez, *Anticancer Agents Med. Chem.* **2011**, *11*, 921.
[19] E. Schuh, S. M. Valiahdi, M. A. Jakupec, B. K. Keppler, P. Chiba, F. Mohr, *Dalton Trans.* **2009**, 10841.
[20] C.-H. Chui, R. S.-M. Wong, R. Gambari, G. Y.-M. Cheng, M. C.-W. Yuen, K.-W. Chan, S.-W. Tong, F.-Y. Lau, P. B.-S. Lai, K.-H. Lam, C.-L. Ho, C.-W. Kan, K. S.-Y. Leung, W.-Y. Wong, *Bioorg. Med. Chem.* **2009**, *17*, 7872.
[21] E. Vergara, E. Cerrada, A. Casini, O. Zava, M. Laguna, P. J. Dyson, *Organometallics* **2010**, *29*, 2596.
[22] H. Scheffler, Y. You, I. Ott, *Polyhedron* **2010**, *29*, 66.
[23] C. P. Bagowski, Y. You, H. Scheffler, D. H. Vlecken, D. J. Schmitz, I. Ott, *Dalton Trans.* **2009**, 10799.
[24] R. Rubbiani, S. Can, I. Kitanovic, H. Alborzinia, M. Stefanopoulou, M. Kokoschka, S. Mönchgesang, W. S. Sheldrick, S. Wölfl, I. Ott, *J. Med. Chem.* **2011**, *54*, 8646 – 8657.
[25] S. Gromer, S. Urig, K. Becker, *Med. Res. Rev.* **2004**, *24*, 40.
[26] R. Rubbiani, I. Kitanovic, H. Alborzinia, S. Can, A. Kitanovic, L. A. Onambele, M. Stefanopoulou, Y. Geldmacher, W. S. Sheldrick, G. Wolber, A. Prokop, S. Wölfl, I. Ott, *J. Med. Chem.* **2010**, *53*, 8608.
[27] a) S. Urig, K. Fritz-Wolf, R. Reau, C. Herold-Mende, K. Toth, E. Davioud-Charvet, K. Becker, *Angew. Chem.* **2006**, *118*, 1915; *Angew. Chem. Int. Ed.* **2006**, *45*, 1881.
[28] C. Gabbiani, G. Mastrobuoni, F. Sorrentino, B. Dani, M. P. Rigobello, A. Bindoli, M. A. Cinellu, G. Pieraccini, L. Messoria, A. Casini, *Med. Chem. Commun.* **2011**, *2*, 50.
[29] Gaussian 09 (Revision A.2), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, **2009**.
[30] K. L. Schuchardt, B. T. Didier, T. Elsethagen, L. S. Sun, V. Gurumoorthi, J. Chase, J. Li, T. L. Windus, *J. Chem. Inform. Modeling* **2007**, *47*, 1045.
[31] D. Feller, *J. Comput. Chem.* **1996**, *17*, 1571.
[32] H. Alborzinia, S. Can, P. Holenya, C. Scholl, E. Lederer, I. Kitanovic, S. Wölfl, *Plos One* **2011**, *6*, e19714.
[33] P. J. Barnard, M. V. Baker, S. J. Berners-Price, D. A. Day, *J. Inorg. Biochem.* **2004**, *98*, 1642.
[34] J. L. Hickey, R. A. Ruhayel, P. J. Barnard, M. V. Baker, S. J. Berners-Price, A. Filipovska, *J. Am. Chem. Soc.* **2008**, *130*, 12570.
[35] I. Ott, X. Qian, Y. Xu, D. H. W. Vlecken, I. J. Marques, D. Kubutat, J. Will, W. S. Sheldrick, P. Jesse, A. Prokop, C. P. Bagowski, *J. Med. Chem.* **2009**, *52*, 763.
[36] a) I. Ott, B. Kircher, C. P. Bagowski, D. H. W. Vlecken, E. B. Ott, J. Will, K. Bensdorf, W. S. Sheldrick, R. Gust, *Angew. Chem.* **2009**, *121*, 1180; *Angew. Chem. Int. Ed.* **2009**, *48*, 1160.
[37] P. Nowak-Sliwinska, J. R. v. Beijnum, A. Casini, A. A. Nazarov, G. Wagnieres, H. v. d. Bergh, P. J. Dyson, A. W. Griffioen, *J. Med. Chem.* **2011**, *54*, 3895.
[38] a) A. Wilbuer, D. H. Vlecken, D. J. Schmitz, K. Kräling, K. Harms, C. P. Bagowski, E. Meggers, *Angew. Chem.* **2010**, *122*, 3928; *Angew. Chem. Int. Ed.* **2010**, *49*, 3939.
[39] L. Morbidelli, S. Donnini, S. Filippi, L. Messori, F. Piccioli, P. Orioli, G. Sava, M. Ziche, *Br. J. Cancer* **2003**, *88*, 1484.
[40] E. M. Bridges, A. L. Harris, *Biochem. Pharmacol.* **2011**, *81*, 1183.
[41] K. Stoletov, R. Klemke, *Oncogenesis* **2008**, *27*, 4509.
[42] R. J. D'Amato, M. S. Loughnan, E. Flynn, J. Foklman, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4082.
[43] A. Holmgren, J. Lu, *Biochem. Biophys. Res. Commun.* **2010**, *396*, 120.
[44] E. S. J. Arnér, *Biochim. Biophys. Acta. Gen. Subj.* **2009**, *1790*, 495.
[45] F. Mendes, M. Groessl, A. A. Nazarov, Y. O. Tsybin, G. Sava, I. Santos, P. J. Dyson, A. Casini, *J. Med. Chem.* **2011**, *54*, 2196.
[46] M. R. Karver, D. Krishnamurthy, R. A. Kulkarni, N. Bottini, A. M. Barrios, *J. Med. Chem.* **2009**, *52*, 6912.
[47] Q. Wang, N. Janzen, C. Ramachandran, F. Jirik, *Biochem. Pharmacol.* **1997**, *54*, 703.
[48] E. Weidauer, Y. Yasuda, B. K. Biswal, M. Cherny, M. N. G. James, D. Brömme, *Biol. Chem.* **2007**, *388*, 331.
[49] A. Chircorian, A. M. Barrios, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5113.

