Antiproliferative Agents

International Edition: DOI: 10.1002/anie.201603931
German Edition: DOI: 10.1002/ange.201603931

# Ferrocenyl Quinone Methide–Thiol Adducts as New Antiproliferative Agents: Synthesis, Metabolic Formation from Ferrociphenols, and Oxidative Transformation

*Yong Wang, Marie-Aude Richard, Siden Top,* Patrick M. Dansette, Pascal Pigeon, Anne Vessières, Daniel Mansuy,* and Gérard Jaouen**

***Abstract:*** *Ferrociphenols (**FCs**) and their oxidized, electrophilic quinone methide metabolites (**FC-QMs**) are organometallic compounds related to tamoxifen that exhibit strong antiproliferative properties. To evaluate the reactivity of **FC-QMs** toward cellular nucleophiles, we studied their reaction with selected thiols. A series of new compounds resulting from the addition of these nucleophiles, the **FC-SR** adducts, were thus synthesized and completely characterized. Such conjugates are formed upon metabolism of **FCs** by liver microsomes in the presence of NADPH and thiols. Some of the **FC-SR** adducts exhibit antiproliferative properties comparable to those of their **FC** precursors. Under oxidizing conditions they either revert to their **FC-QM** precursors or transform into new quinone methides (QMs) containing the SR moiety, **FC-SR-QM**. These results provide interesting data about the reactivity and mechanism of antiproliferative effects of **FCs**, and also open the way to a new series of organometallic antitumor compounds.*

We are currently witnessing strong contributions to biological science by new structures from the domain of inorganic chemistry.[1] Long overshadowed by an all-encompassing interest in organometallic complexes in catalysis, the bioorganometallic chemistry of transition metals has slowly revealed its unique potential, particularly in terms of medicinal applications,[2] including optimized space-filling for enzyme inhibition purposes,[3] redox activity on specific targets,[4] and antiproliferative effects by ruthenium catalysts on cancer cells.[5] We can add to these functional attributes the property of intracellular multi-targeting of some anticancer candidates, which could be of interest in delaying or inhibiting problems of drug resistance.[6] In light of this, bioorganometallic chemistry clearly demands further exploration, and indeed, the current growth in interest in this area underlines this.[7]

In this context, we designed and studied organometallic structures with strong antiproliferative potential, namely ferrociphenols, which have the unusual property of possessing a ferrocenyl–ene–phenol motif that is active on cancer cells in a redox environment,[8] allowing the generation of a primary active metabolite of the QM-type.[9] Products **FC1**, **FC2**, and **FC3** are typical of the active ferrociphenols related to tamoxifen; they are oxidized by chemical oxidants or by liver microsomes into QMs **FC1-QM**, **FC2-QM**, and **FC3-QM**, respectively (Figure 1).[9b,10] Biologically, these

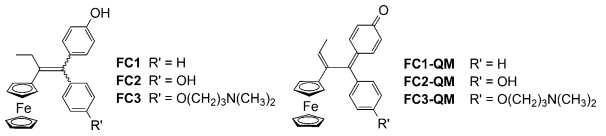



***Figure 1.*** Ferrociphenols (**FCs**) and the corresponding QMs (**FC-QMs**).

species operate via senescence and apoptosis mechanisms, depending on the concentration of compounds and the nature of cancer cells.[11] This type of behavior may provide access to the treatment of cancers that are currently incurable because they fail to respond to proapoptotic stimuli.

QMs are active species that can react with various nucleophiles inside the cell, such as peptides or proteins bearing thiols or selenols. Such reactions between QMs and nucleophiles in vivo could lead to cell death by interference with oxidative stress or inactivation of enzymes.[12] We recently reported that targeting thioredoxin reductases by ferrocenyl QMs could affect the cellular redox balance in Jurkat cancer cells and may be partly responsible for the antiproliferative activity of ferrociphenols.[13] Therefore, it becomes particularly important to undertake a study on the reaction of ferrocenyl QMs with selected nucleophiles. Herein, we present our results on the reaction of thiol nucleophiles such as glutathione (GSH), *N*-acetyl-L-cysteine methyl ester (NACM), and mercaptoethanol (ME) with **FC-QMs**. A series of new compounds resulting from this reaction, the **FC-SR** adducts, were thus synthesized chemically and also identified upon metabolism of **FCs** by liver microsomes in the presence of NADPH and thiols. These organometallic thiol adducts not only exhibit potent anti-

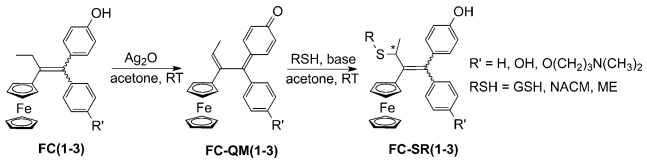
[*] Dr. Y. Wang, Dr. M.-A. Richard, Dr. S. Top, Dr. P. Pigeon, Dr. A. Vessières, Prof. G. Jaouen
Sorbonne Universités, UPMC Univ Paris 06
CNRS UMR 8232, IPCM, 75005 Paris (France)
and
PSL, Chimie ParisTech
11 rue Pierre et Marie Curie, 75005 Paris (France)
E-mail: siden.top@chimie-paristech.fr
gerard.jaouen@chimie-paristech.fr
Dr. P. M. Dansette, Dr. D. Mansuy
Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601 CNRS, Université Paris Descartes
PRES Paris Cité Sorbonne
45 rue des Saints Pères, 75270 Paris Cedex 06 (France)
E-mail: Daniel.Mansuy@parisdescartes.fr

Supporting information for this article can be found under: http://dx.doi.org/10.1002/anie.201603931.

proliferative properties but also show unique behavior under oxidative conditions.

An efficient synthesis of the desired **FC-SR** adducts involved a nucleophilic attack of thiols on **FC-QMs** in the presence of a base (Scheme 1). These adducts were formed as a mixture of stereoisomers because of the existence of *Z*- and




***Scheme 1.*** General method for the synthesis of the **FC-SR** adducts.

*E*-isomers at the level of the double bond and the presence of a chiral carbon (for the stereoisomers of the **FC1-SR** adducts see the Supporting Information, Chart SI1). Their structures were established by various techniques, including by X-ray crystallographic study of **FC1-ME**, which confirmed the 1,8-addition of ME on the QM scaffold (Figure 2).[14] Under physiological conditions (50 mM phosphate buffer, 37 °C), we observed the formation of around 40% **FC3-SR** from the incubation of **FC3-QM** in the presence of excess NACM or ME at pH 5. This underlined the high reactivity of ferrocenyl QMs toward thiols, which made the 1,8-Michael-type addition possible, even at an acidic pH that was much lower than the $pK_a$ value of thiol deprotonation (ca. 8.3).

Incubation of ferrociphenols with rat liver microsomes in the presence of NADPH (liver microsomes and NADPH: LM) and various thiols, led to **FC-SR** adducts, as well as the cyclized indene **FC-CP** and allylic alcohols, **FC-AA** (Scheme 2). These metabolites were already observed under identical incubations performed in the absence of thiols.[10]

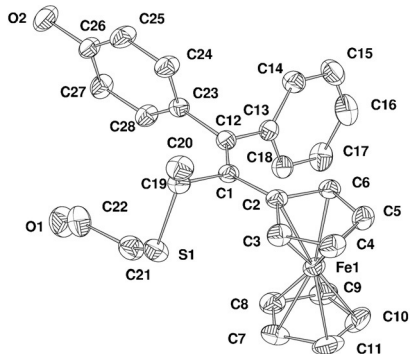
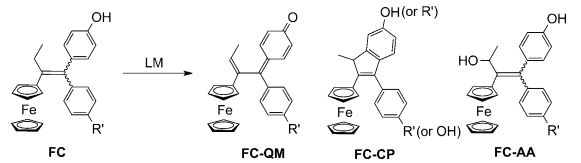



***Figure 2.*** Molecular structure of **FC1-ME** as its (*S*)-*E* form, thermal ellipsoids are shown at 50%.




***Scheme 2.*** Products obtained from incubation of **FC(1–3)** with liver microsomes and NADPH (LM); R' = H, OH, $O(CH_2)_3NMe_2$.

The **FC-SR** metabolites exhibited HPLC retention times and MS characteristics identical to those found for authentic samples. Upon microsomal incubation of **FC1–3** in the presence of GSH, NACM, or ME, the proportion of conjugates formed relative to all metabolites varied between 5–40%. Interestingly, the adducts formed in the presence of GSH represented about 40% of all metabolites for **FC2** and **FC3**, while this proportion decreased to only about 10% for **FC1**. By comparison, microsomal incubation of 4-hydroxytamoxifen, **4-OHTAM**, in the presence of GSH was reported to give a very small amount of QM-GSH adduct.[15]

The antiproliferative activity of synthesized **FC-SR** compounds against hormone-refractory breast cancer MDA-MB-231 cells is shown in Table 1. We have already shown that QMs, **FC1-QM** and **FC3-QM**, were less active than their parent compounds, **FC1** and **FC3**.[9b] This weaker activity could be attributed to the chemical reactivity and instability of QMs in the incubation medium. Interestingly, the antiproliferative effects of adducts **FC1-ME** and **FC1-NACM** were close to that of **FC1**, and even better than that of **FC1-QM**. The same phenomenon was also found for compounds **FC2-ME** and **FC2-NACM**, which had similar $IC_{50}$ values around 0.9 μM that were comparable to those of their ferrociphenol precursor. By contrast, the GSH adducts **FC1-SG** and **FC3-SG**, exhibited $IC_{50}$ values higher than 10 μM. The weak activity of hydrophilic **FC-SG** compounds may be due to heightened difficulties in cell membrane penetration. In the case of the **FC3** series, **FC3-ME** and **FC3-NACM** were three- and 10-times less active than **FC3**, respectively. As in the case of the GSH adducts, it is likely that this decreased cytotoxicity could be due to the more difficult membrane penetration of these adducts, which contain two hydrophilic side chains. It is assumed that these **FC-SR** adducts will exhibit much higher antiproliferative effects, when formed inside the cells as metabolites of ferrociphenols, at the level of the endoplasmic reticulum and close to important cell targets. The **FC-SR** adducts constitute a new class of potent antiproliferative compounds. Moreover, our results suggest that the capture of **FC-QMs** by nucleophilic thiols in vivo may preserve the antiproliferative effects of the ferrociphenols and their QM metabolites by avoiding the formation of inactive indene **FC-CP** metabolites (Scheme 2).

The **FC-SR** adducts share the same ferrocenyl–ene–phenol motif as their **FC** parent compounds. Thus, the

***Table 1:*** Antiproliferative activity of ferrocenyl compounds against MDA-MB-231 cells.

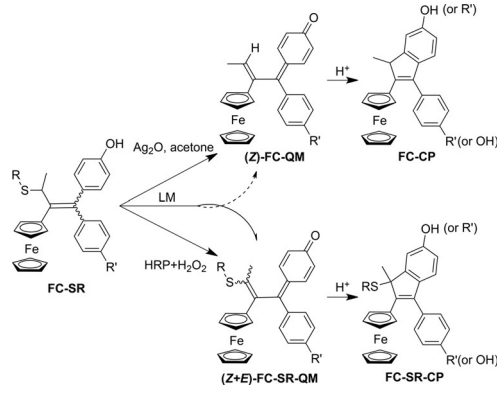
| Compound | $IC_{50}$ [μM][a] | Compound | $IC_{50}$ [μM][a] |
|---|---|---|---|
| **FC1** | $1.5 \pm 0.1$[c] | **FC2-NACM**[b] | $1.0 \pm 0.2$ |
| **FC1-QM** | $7.2 \pm 0.5$[c] | **FC3** | 0.5[c] |
| **FC1-ME**[b] | $2.2 \pm 0.1$ | **FC3-QM** | $1.8 \pm 0.2$[c] |
| **FC1-NACM**[b] | $1.5 \pm 0.1$ | **FC3-ME**[b] | $1.6 \pm 0.3$ |
| **FC1-SG**[b] | $>10$ | **FC3-NACM**[b] | $4.8 \pm 0.2$ |
| **FC2** | $0.6 \pm 0.1$[c] | **FC3-SG**[b] | $>10$ |
| **FC2-ME**[b] | $0.9 \pm 0.1$ | **4-OHTAM** | $30 \pm 0.6$[d] |

[a] Measured after 5 days of culture (mean of two independent experiments in quadruplicate (± SD)). [b] Mixture of all stereoisomers. [c] Values from Ref. [10]. [d] $LC_{50}$ values obtained from Ref. [18].

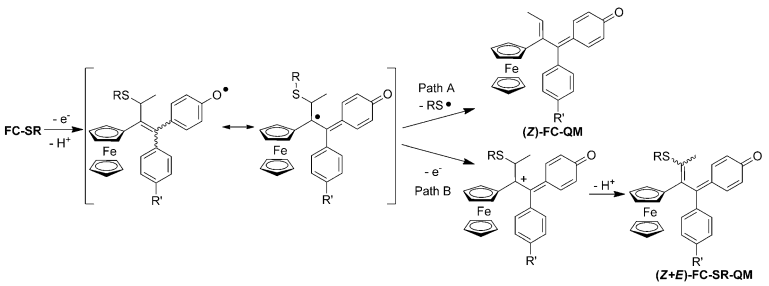
  

antiproliferative activity of **FC-SR** could come from their ability to be oxidized into QMs. To explore this possibility, we first studied the chemical oxidation of **FC-SR** by $Ag_2O$ in acetone (Scheme 3). After 30 min at 20 °C, the $^1H$ NMR spectrum of the mixture clearly showed the quantitative formation of **FC-QM**. This observation indicated that the **FC-SR** adducts arising from the addition of thiols on **FC-QMs** may revert back to their **FC-QM** precursors in the presence of an oxidant. Interestingly, the **FC-QMs** obtained from this reaction were mainly in their *Z*-form, whereas the **FC-QMs** formed from direct oxidation of **FCs** were predominantly in their *E*-form.[9b] Thus, both precursor compounds, **FCs** and their **FC-SR** metabolites, generated **FC-QMs** under chemical oxidation conditions. The reversibility of the addition of thiols to QMs or α,β-unsaturated carbonyl compounds has been well-studied and is referred to as a thiyl radical pathway.[16] The reversible reaction of **4-OHTAM-QM** with GSH was also reported to occur under physiological conditions.[15] In the case of the reaction of **FC-SR** with $Ag_2O$, it is likely that a quinone radical derived from a one-electron oxidation of **FC-SR** is formed initially. Elimination of a SR radical would then lead to **FC-QM** (Scheme 4, Path A). The formation of (*Z*)-**FC-QM** as the major isomer may be explained by a steric effect. SR is a bulky group compared to H and the methyl group, and SR should position itself at the opposite side of the ferrocenyl group to minimize steric hindrance (Scheme 5). Subsequently, elimination of the SR group from this position should lead to the formation of (*Z*)-**FC-QM**.

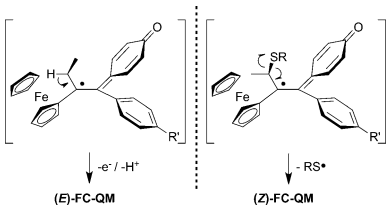
To further evaluate the possible fates of the **FC-SR** adducts under a wider range of physiological oxidative conditions, we have studied their oxidation by the horseradish peroxidase (HRP)/$H_2O_2$ system or by rat liver microsomes in the presence of NADPH. Incubation of **FC3-SG** or **FC3-NACM** with $H_2O_2$/HRP for just a few seconds, led to the formation of a major new product that could be detected by UV/Vis spectroscopy and LC-MS. The new product showed a strong absorbance around 415 nm that is characteristic of QMs, and its mass spectrum exhibited a molecular ion corresponding to **FC3-SG-QM** (or **FC3-NACM-QM**), which is derived from a two-electron oxidation of the starting **FC3-SR** adduct (Scheme 4, Path B). This product appeared only transiently, as its acid-catalyzed cyclization led to the corresponding indene compound **FC3-SR-CP** (Scheme 3) and prevented the isolation of the novel **FC3-SR-QM** compound.




***Scheme 3.*** Fate of the **FC-SR** adducts under oxidizing conditions.




***Scheme 4.*** Proposed oxidative evolution of **FC-SR** adducts.

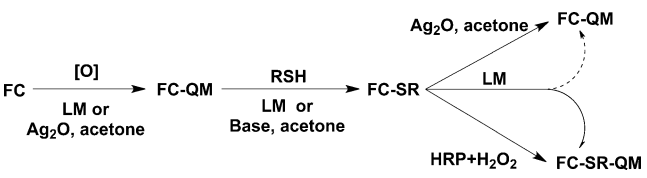



***Scheme 5.*** Proposed mechanism for the formation of (*E*)- and (*Z*)-**FC-QM**.

Incubation of the **FC3-SR** adducts (SR = SG, NACM) with liver microsomes in the presence of NADPH led to indene derivatives, **FC3-SR-CP** and **FC3-CP**, that should result from the acid-catalyzed cyclization of **FC3-SR-QM** and **FC3-QM. FC3-CP** was characterized by comparison of its HPLC retention time and MS spectrum with those of a previously described authentic sample.[10] **FC3-SR-CP** was characterized by its UV/Vis spectroscopy and mass spectrometry. The **FC3-SR-CP** products were the major metabolites, as the **FC3-SR-CP/FC3-CP** ratio was around 85:15 and 98:2 in the case of SR = SG and SR = NACM, respectively.

The above results indicate that oxidation of **FC-SR** adducts may either produce **FC-QM** precursors, or lead to new QMs retaining the SR moiety (**FC-SR-QMs**), as a function of the oxidizing medium (Schemes 3 and 6). Oxidation of **FC-SR** by $Ag_2O$ led to **FC-QMs**, whereas oxidation by $H_2O_2$/HRP or by liver microsomes in the presence of NADPH led mostly to **FC-SR-QMs**. Under these oxidizing conditions, which are closer to those found in vivo, the presence of a ferrocenyl group in proximity to the quinone radical (formed upon one-electron oxidation of **FC-SR**) would favor the formation of a ferrocenyl α-carbenium ion (Scheme 4, Path B). Subsequently, this ion would lose a proton to give **FC-SR-QM**.

In comparison, an HPLC study of the slow evolution of **FC3-SG** in phosphate buffer over one month showed the formation of only 5–10 % of the indene product **FC3-SG-CP**,




***Scheme 6.*** Steps involved in the oxidation of ferrociphenols.

and the absence of **FC3-CP**. By contrast, under the same conditions the **4-OHTAM-SG** adduct led to **4-OHTAM-QM**.[15] This result indicates that the ferrocenyl group plays an important role; it facilitates the formation of a quinone carbenium ion from the quinone radical intermediate, which is derived from the one-electron oxidation of **FC-SR**. The well-known ability of metallocenes to stabilize adjacent α-carbenium ions should explain the formation of the quinone carbenium ion shown in Scheme 4, and the generation of organometallic QM-retaining thiols **FC-SR-QM** after loss of a proton. The role of ferrocene as a carbenium ion "inducer" is a supplement to the ferrocene toolbox employed in organometallic chemistry. Previously we reported ferrocene in the role of an intramolecular oxidation "antenna" and a stabilized carbenium ion "modulator".[9a,17] The various roles played by the ferrocenyl group on the ferrociphenol scaffold could explain the improved antiproliferative activity of ferrociphenols when compared to tamoxifen.

In summary, the aforementioned results indicate that the metabolism of ferrociphenols **FC(1–3)** by liver microsomes in the presence of NADPH and thiols lead to new metabolites, which result from the 1,8-Michael addition of thiols on QM intermediates **FC-QM(1–3)**. Some of the **FC-SR** adducts exhibited antiproliferative effects toward hormone-resistant cancer cells, and these effects were comparable to those exhibited by the corresponding ferrociphenols. Moreover, these adducts can be further oxidized to give novel QM metabolites, which can participate in events leading to cell death. In particular, oxidation of these metabolites by liver microsomes in the presence of NADPH led to new QMs containing the sulfur moiety **FC-SR-QMs**, together with minor amounts of **FC-QMs**. This data reveals a unique mode of metabolic oxidation of ferrociphenols **FCs**, where two classes of reactive, electrophilic metabolites are formed successively. The **FC-QMs** and **FC-SR-QMs**, as well as possible intermediate carbenium ions stabilized by the ferrocene moiety (Scheme 4), could lead to irreversible damage of cell macromolecules, and may be at the origin of the antiproliferative effects of ferrociphenols. This unique ferrociphenol metabolic oxidation profile may offer new strategies for determining the mechanistic action of ferrociphenols, and allow the rational design of new organometallics for the treatment of resistant cancers.

## Acknowledgements

Agence Nationale de la Recherche (ANR-10-BLAN-706, Mecaferrol) and Feroscan are gratefully acknowledged for financial support. We thank P. Herson (Labex Michem, Université Pierre et Marie Curie, Paris, IPCM) for the X-ray structure determinations.

**Keywords:** antitumor agents · glutathione · liver microsomes · Michael addition · organometallic compounds

**How to cite:** *Angew. Chem. Int. Ed.* **2016**, *55*, 10431–10434
*Angew. Chem.* **2016**, *128*, 10587–10590

Received: May 18, 2016
Published online: June 8, 2016

[1] N. P. E. Barry, P. J. Sadler, *Chem. Commun.* **2013**, *49*, 5106–5131.
[2] E. A. Hillard, A. Vessieres, G. Jaouen in *Medicinal Organometallic Chemistry*, *Vol. 32* (Eds.: G. Jaouen, N. Metzler-Nolte), Springer, Heidelberg, **2010**, pp. 81–117.
[3] M. Dörr, E. Meggers, *Curr. Opin. Chem. Biol.* **2014**, *19*, 76–81.
[4] P. Messina, E. Labbé, O. Buriez, E. A. Hillard, A. Vessières, D. Hamels, S. Top, G. Jaouen, Y. M. Frapart, D. Mansuy, C. Amatore, *Chem. Eur. J.* **2012**, *18*, 6581–6587.
[5] J. J. Soldevila-Barreda, I. Romero-Canelón, A. Habtemariam, P. J. Sadler, *Nat. Commun.* **2015**, *6*, 6582.
[6] J. M. Hearn, I. Romero-Canelon, A. F. Munro, Y. Fu, A. M. Pizarro, M. J. Garnett, U. McDermott, N. O. Carragher, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E3800–E3805.
[7] a) G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3–25; b) C. G. Hartinger, N. Metzler-Nolte, P. J. Dyson, *Organometallics* **2012**, *31*, 5677–5685; c) F. Cisnetti, A. Gautier, *Angew. Chem. Int. Ed.* **2013**, *52*, 11976–11978; *Angew. Chem.* **2013**, *125*, 12194–12196; d) M. A. Cinellu, I. Ott, A. Casini, in *Bioorganometallic Chemistry*, Wiley, **2014**, pp. 117–140; e) A. A. Nazarov, C. G. Hartinger, P. J. Dyson, *J. Organomet. Chem.* **2014**, *751*, 251–260.
[8] a) S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché, G. Jaouen, *Chem. Eur. J.* **2003**, *9*, 5223–5236; b) M. Görmen, P. Pigeon, S. Top, E. A. Hillard, M. Huché, C. G. Hartinger, F. de Montigny, M.-A. Plamont, A. Vessières, G. Jaouen, *ChemMedChem* **2010**, *5*, 2039–2050.
[9] a) E. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem. Int. Ed.* **2006**, *45*, 285–290; *Angew. Chem.* **2005**, *117*, 291–296; b) D. Hamels, P. M. Dansette, E. A. Hillard, S. Top, A. Vessieres, P. Herson, G. Jaouen, D. Mansuy, *Angew. Chem. Int. Ed.* **2009**, *48*, 9124–9126; *Angew. Chem.* **2009**, *121*, 9288–9290; c) G. Jaouen, S. Top in *Advances in Organometallic Chemistry and Catalysis* (Ed.: J. L. P. Armando), Wiley, New Jersey, **2014**, pp. 563–580.
[10] M.-A. Richard, D. Hamels, P. Pigeon, S. Top, P. M. Dansette, H. Z. S. Lee, A. Vessières, D. Mansuy, G. Jaouen, *ChemMedChem* **2015**, *10*, 981–990.
[11] a) A. Vessières, C. Corbet, J. M. Heldt, N. Lories, N. Jouy, I. Laios, G. Leclercq, G. Jaouen, R.-A. Toillon, *J. Inorg. Biochem.* **2010**, *104*, 503–511; b) C. Bruyère, V. Mathieu, A. Vessières, P. Pigeon, S. Top, G. Jaouen, R. Kiss, *J. Inorg. Biochem.* **2014**, *141*, 144–151; c) G. Jaouen, A. Vessieres, S. Top, *Chem. Soc. Rev.* **2015**, *44*, 8802–8817.
[12] a) S. J. Dougan, A. Habtemariam, S. E. McHale, S. Parsons, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11628–11633; b) F. Giannini, G. Suss-Fink, J. Furrer, *Inorg. Chem.* **2011**, *50*, 10552–10554.
[13] A. Citta, A. Folda, A. Bindoli, P. Pigeon, S. Top, A. Vessières, M. Salmain, G. Jaouen, M. P. Rigobello, *J. Med. Chem.* **2014**, *57*, 8849–8859.
[14] The supplementary crystallographic data for this paper is contained in 1454701 (**FC1-ME**). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[15] P. W. Fan, F. Zhang, J. L. Bolton, *Chem. Res. Toxicol.* **2000**, *13*, 45–52.
[16] F. Dénès, M. Pichowicz, G. Povie, P. Renaud, *Chem. Rev.* **2014**, *114*, 2587–2693.
[17] Y. Wang, P. Pigeon, S. Top, M. J. McGlinchey, G. Jaouen, *Angew. Chem. Int. Ed.* **2015**, *54*, 10230–10233; *Angew. Chem.* **2015**, *127*, 10368–10371.
[18] F. Zhang, P. W. Fan, X. Liu, L. Shen, R. B. van Breemen, J. L. Bolton, *Chem. Res. Toxicol.* **2000**, *13*, 53–62.