Antitumor Agents

Deutsche Ausgabe: DOI: 10.1002/ange.201503048
Internationale Ausgabe: DOI: 10.1002/anie.201503048

Organometallic Antitumor Compounds: Ferrocifens as Precursors to Quinone Methides**

Yong Wang, Pascal Pigeon, Siden Top, Michael J. McGlinchey, and Gérard Jaouen**

Abstract: *The synthesis and chemical oxidation profile of a new generation of ferrocifen derivatives with strong antiproliferative behavior in vitro is reported. In particular, the hydroxypropyl derivative* $HO(CH_2)_3C(Fc)=C(C_6H_4OH)_2$ ***(3b)*** *exhibited exceptional antiproliferative activity against the cancer cell lines HepG2 and MDA-MB-231 TNBC, with* IC_{50} *values of 0.07 and 0.11 μM, respectively. Chemical oxidation of* ***3b*** *yielded an unprecedented tetrahydrofuran-substituted quinone methide (QM) via internal cyclization of the hydroxyalkyl chain, whereas the corresponding alkyl analogue* CH_3CH_2-$C(Fc)=C(C_6H_4OH)_2$ *merely formed a vinyl QM. The ferrocenyl group in* ***3b*** *plays a key role, not only as an intramolecular reversible redox "antenna", but also as a stabilized carbenium ion "modulator". The presence of the oxygen heterocycle in* ***3b-QM*** *enhances its stability and leads to a unique chemical oxidation profile, thus revealing crucial clues for deciphering its mechanism of action in vivo.*

In the search for organometallic antitumor compounds with behavior differentiated from the primary targeting of DNA, an approach based on common metals such as Fe has already proven to be of interest.[1] Many ferrocenyl species showing contrasting antiproliferative effects,[2] in particular the family of acyclic or ansa ferrocifens (Figure 1) are seen as innovative drug candidates.[3] At low concentrations, they operate principally via a mechanism of senescence,[4] although in some cases apoptosis is possible,[4a] through at least partially targeting enzymes of a redox system, for example, thioredoxin reductase (TrxR),[5] that are overexpressed in cancer cells, while leaving healthy cells untouched.[3c,d] They show IC_{50} values of around 0.6 μM for acyclic systems to 0.09 μM for ansa molecules with triple-negative MDA-MB-231 cancer cells, and have been formulated for in vivo studies in breast cancer and glioma .[3d,6] These products are of great potential interest since senescence could be an alternative route for cancers resistant to proapoptotic stimuli, which play a major role in the incidence of mortality due to this type of pathology.[4b]

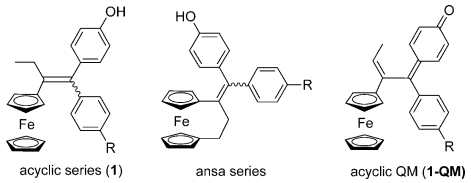


Figure 1. Acyclic and ansa organometallic compounds derived from the ferrocifen family (R = H or OH), and the key acyclic quinone methide (QM).

Ideally, to enable the establishment of a new mechanism of action, a new generation of products is required. These products should involve an unequivocally characterized primary metabolite and they must also exhibit lower IC_{50} values than those of the acyclic series. Previously, the molecules in Figure 1 were modified by changing the metals and functional groups, and attaching various chains.[7] With replacement of the alkyl chain by a hydroxypropyl group, we present the first observations on what may become a new generation of ferrocifen derivatives (Figure 2 A) with strong antiproliferative potential. Moreover, they yielded a novel quinone methide (QM) that is key to the new behavior.

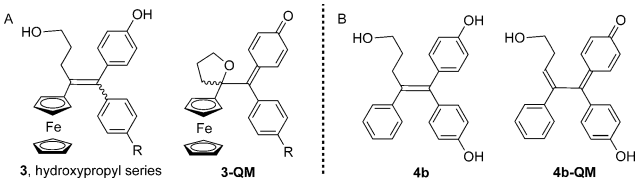


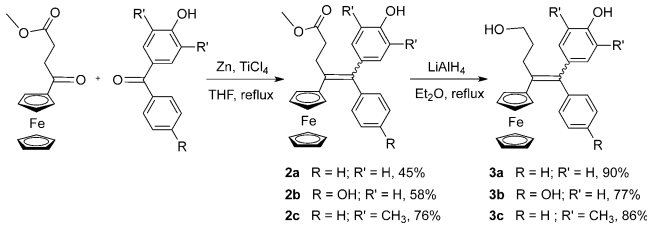
Figure 2. A) The new hydroxypropyl series **3**, derived from the acyclic ferrocifen family, and the novel tetrahydrofuran-substituted **3-QM**. B) The purely organic equivalent **4b** and the acyclic vinyl **4b-QM**.

Efficient syntheses of the desired hydroxypropyl-alkenes **3** proceeded *via* McMurry cross-coupling[8] to give compounds **2a–c** and $LiAlH_4$ reduction of the esters to the corresponding alcohols **3** (Scheme 1). Monophenol analogues **2a**, **2c**, **3a**, and **3c** were obtained as mixtures of *Z* and *E* isomers. The corresponding tamoxifen organic derivatives (series **4**) were prepared analogously. X-ray crystal structures of **3a** and **3b** are shown in Figure S1 in the Supporting Information.

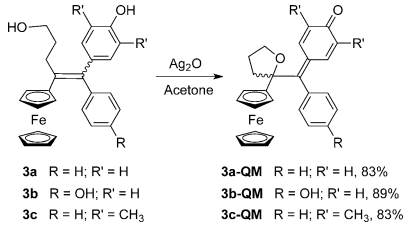
[*] Dr. Y. Wang, Dr. P. Pigeon, Dr. S. Top, Prof. G. Jaouen
Sorbonne Universités, UPMC Univ Paris 06
UMR 8232, IPCM, 75005 Paris (France)
and
CNRS, UMR 8232, IPCM, 75005 Paris (France)
and
PSL, Chimie ParisTech
11 rue Pierre et Marie Curie, 75005 Paris (France)
E-mail: siden.top@chimie-paristech.fr
gerard.jaouen@chimie-paristech.fr
Prof. M. J. McGlinchey
UCD School of Chemistry and Chemical Biology
University College Dublin, Belfield, Dublin 4 (Ireland)

[**] Y. W. thanks the PGG foundation, the PSL University and Feroscan for financial support. We thank P. Herson (Université Pierre et Marie Curie, Paris, IPCM and LabEx Michem) for the X-ray structure determinations.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201503048.



Scheme 1. Syntheses of the ferrocenyl compounds in series **2** and **3**.



Scheme 2. Synthesis of the quinone methides.

Lipophilicity values and antiproliferative effects against the estrogen-receptor-negative (ER−) MDA-MB-231 breast cancer cells are reported in Table 1, together with values obtained for the acyclic diphenol compound Fc(Et)C=C-$(C_6H_4OH)_2$. Compared to the organic compound **4b**, the ferrocenyl moiety increases the lipophilicity of the new molecules, while the presence of polar groups decreases their lipophilicity. Compound **3b** shows exceptional antiproliferative effects on the hormone-independent cell line MDA-MB-231, with an IC_{50} value around 0.11 μM, which is markedly superior to that of its ester precursor **2b** and that of the acyclic diphenol compound. The tamoxifen derivative **4b**, the organic analogue of **3b**, shows limited cytotoxicity against MDA-MB-231 ($IC_{50} \approx 50$ μM), a result that underlines the key role played by the ferrocenyl substituent in the generation of antiproliferative effects (tamoxifen itself has an IC_{50} value of 40 μM).[9]

Table 1: IC_{50} values with MDA-MB-231 cells and log Po/w results for selected ferrocenyl compounds.

Compound	IC_{50} [μM][a]	logPo/w
1 (R = OH)[b]	0.64 ± 0.06	5.0
4b	50.78 ± 1.33	3.6
2a	4.09 ± 1.67	5.0/5.3
2b	0.44 ± 0.09	4.4
2c	1.50 ± 0.17	5.4/6.2
3a	1.16 ± 0.02	4.5/4.9
3b	0.11 ± 0.02	4.2
3c	1.23 ± 0.05	5.0/5.6

[a] Measured after 5 days of culture (mean of two independent experiments ± SD). [b] Values from Ref. [1a].

Compound **3b** was selected for further tests on human tumor cell lines, which revealed that it could prevent the growth of the pancreatic carcinoma cell line Mia-PaCa with an IC_{50} value of 1.23 μM and shows excellent antiproliferative activity against liver hepatocellular carcinoma, with an IC_{50} value of 0.07 μM on HepG2 cells. This exceptional antiproliferative behavior led to further exploration of its framework to try to decipher the active motif.

We find that compounds **1-QM** (Figure 1) are the key metabolites upon metabolism of the acyclic alkyl chain series **1** by rat liver microsomes and they can also be prepared by chemical oxidation.[3e,f] To help unravel the reasons for the excellent antiproliferative effect of **3b**, chemical oxidation was used to prepare selected putative metabolites (Scheme 2). NMR and X-ray crystallography data confirmed their structures as novel tetrahydrofuran-substituted **3-QM** systems involving cyclization of the hydroxyalkyl chain rather than the previously reported behavior of acyclic **1-QM**, which contains a conjugated *trans* double bond.[3e]

The QM structure of **3c-QM** was established by X-ray crystallography (Figure 3) and the cyclic ether confirms the intramolecular reaction of the hydroxy function on the carbon center adjacent to the ferrocenyl group.

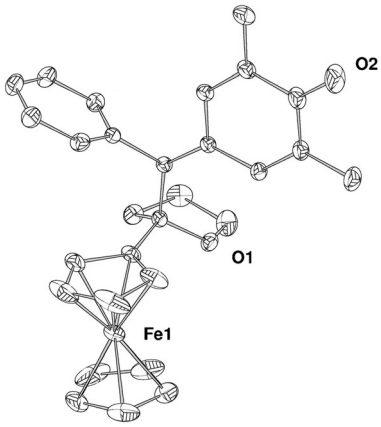
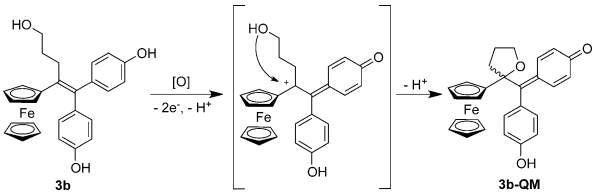


Figure 3. Molecular structure of **3c-QM** with thermal ellipsoids shown at 50% probability.

For the diphenol **1** (R = OH), an archetypical example of the first generation of ferrocifen acyclic species (Figure 1), the corresponding QM proved to be quite unstable and exhibited evidence of decomposition before oxidation was complete. In contrast, **3b-QM** derived from the hydroxypropyldiphenol precursor showed reasonable stability over least 4 weeks when kept at −20 °C in the solid state. Moreover, its stability in solution was also significantly improved (Figures S2, S3), except in the weakly acidic solvent chloroform, in which it started to decompose. The half-life in acetone was approximately 30 h, but was even longer in DMSO, where the compound remained unchanged for at least two days with a half-life of around six weeks.

The formation of the quinone ring was entirely consistent with the mechanism already proposed for acyclic species, whereby the ferrocenyl unit acts as a kind of intramolecular oxidation "antenna", such that oxidation of the phenol group leads to a carbenium ion.[10] However, from this point the process can vary owing to the presence of the terminal hydroxy function, such that the carbenium ion undergoes nucleophilic attack to form a heterocycle rather than a double

bond (Scheme 3). Oxidation of compounds **4**, the purely organic analogues of the ferrocenyl alcohol derivatives **3**, yielded only the acyclic vinyl QM systems (Figure 2B). The absence of a significant NMR peak between 3.5 and 4.5 ppm,

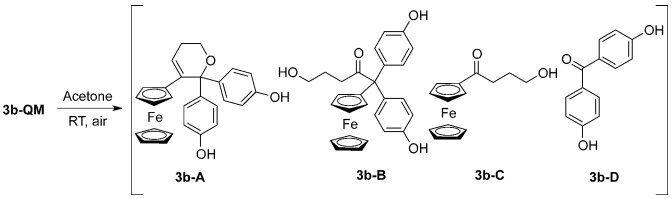


Scheme 3. Proposed mechanism for the formation of the novel heterocyclic ferrocenyl QM species.

which is characteristic of OCH_2 in a tetrahydrofuran ring, indicates that the oxidation of **4** did not yield a QM heterocycle. NMR peaks characteristic of the acyclic QM appeared transiently, but decomposition prior to complete oxidation prevented isolation of the QM, even for compound **4c**, in which the ferrocenyl group of **3c** was replaced by a phenyl group. Thus, for the organic analogue **4**, oxidation predominantly gives the vinyl QM, whereas in the ferrocenyl series **3**, oxidation furnishes a novel QM that bears a tetrahydrofuran ring. These quite distinct results, together with the well-known fact that organometallic complexes adjacent to a double bond favor the stabilization of α-carbenium ions, let us deduce that the ferrocenyl group not only plays the role of intramolecular oxidation "antenna" but also acts as a "modulator" and facilitates trapping of the hydroxy function by the carbenium ion, thereby leading to tetrahydrofuran ring formation. To the best of our knowledge, the **3-QM** species is the first tetrahydrofuran-substituted QM reported to date, which may indicate the potential for structural diversity in quinone chemistry.

Freshly synthesized **3a-QM**, **3b-QM**, and **3c-QM** were also cytotoxic against MDA-MB-231 cells (IC_{50} values of 1.89, 4.39, and 6.00 μM, respectively). The IC_{50} values of stable **3a-QM** and **3c-QM** were the same as those of their parent molecules **3a** and **3c**. The higher value for **3b-QM**, relative to **3b**, could be due to its relatively better chemical reactivity, since strong nucleophiles may be present in the incubation medium. Nevertheless, this behavior suggests that **3b** should have remarkable intrinsic antiproliferative properties and motivated us to explore the chemical oxidation profiles of **3b** and **3b-QM**.

The oxidative evolutio of **3b-QM** is more complex than that of **1-QM** (Scheme 4).[3e,5] Its half-life in acetone was around 30 h, and all of its derivatives were stable enough to be isolable upon complete decomposition. The four products, **3b-A**, **3b-B**, **3b-C**, and **3b-D**, which had not previously been observed in the metabolic processes of the **1-QM** series,[5] were identified by NMR or X-ray crystallography (**3b-A**; Figure S1). For the acyclic ferrocifen derivatives, the major byproduct in each case, during or after oxidation, was an indene product resulting from acid-mediated cyclization,[3e,5] but the presence of the tetrahydrofuran ring leads to different



Scheme 4. Metabolic Stability Profile of **3b-QM**.

species resulting from involvement of the oxygen atom of the alkyl chain.

As shown in Scheme S1, under the slightly acidic conditions in acetone, protonation of the quinone **3b-QM** can form a carbenium ion that evolves through several pathways. The first is ring expansion by migration of the adjacent oxygen, which places the carbocation adjacent to the ferrocenyl group, and subsequent proton loss to yield **3b-A**. Secondly, a pinacol rearrangement can give compound **3b-B**.[11] Finally, the carbenium ion can react with traces of water to give a pinacol-type intermediate that gives **3b-C** and **3b-D** on further oxidation. This radical oxidation parallels a previous report on [3]-ferrocenophane derivatives.[11]

The cytotoxicity of **3b-QM** against MDA-MB-231 cells was consistent with its chemical derivatives **3b-A** and **3b-B** (IC_{50} values of 2.03 and 4.14 μM, respectively). Compounds **3b-QM**, **3b-A**, **3b-B**, **3b-C**, and **3b-D** were the major products during the chemical oxidation and subsequent decomposition of **3b**. The cytotoxicity values obtained from the precursor **3b** strongly suggest that, when incubated with live cancer cells, **3b** should generate a remarkable intrinsically electrophilic metabolite that is probably native **3b-QM**. The evolution of **3b** proposed above occurred through chemical methods without the involvement of other nucleophiles. It provides some clues that prodrug **3b** could generate several possible carbenium ions in vivo that could be captured by nucleophiles, such as thiols or selenols, inside the cells.[5] Thus possible cross-coupling with reactive nucleophiles or a related protein could lead to cell death. Another pathwaythat could be relevant to the cytotoxicity is oxidative cleavage of pinacol-type analogues.[11]

In summary, modification of the alkyl chain in the original acyclic derivatives yielded **3b**, which bears a terminal hydroxyalkyl group and exhibits exceptional antiproliferative activity against liver hepatocellular carcinoma cells (HepG2) and ER− breast cancer cells (MDA-MB-231), with IC_{50} values of 0.07 and 0.11 μM, respectively. Chemical oxidation of **3b** yielded an unprecedented tetrahydrofuran-substituted QM via internal cyclization of the alkyl chain, which was identified as a possible key primary metabolite. The ferrocenyl group not only plays the role of intramolecular reversible redox "antenna" but also acts as a stabilized carbenium ion "modulator". Resulting from these structural changes, **3b-QM** exhibits moderate stability and a unique chemical oxidation profile, which reveals crucial clues that may help us decipher its mechanism of action in vivo. Future work will focus on gaining insight into the mechanism of action of these novel species, especially in the presence of healthy cells and

selected nucleophiles, thus opening the way to a new generation of unique potential drug candidates.

Keywords: antitumor agents · drug discovery · ferrocene · metabolism · quinones

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 10230–10233
Angew. Chem. **2015**, *127*, 10368–10371

[1] a) E. Hillard, A. Vessieres, G. Jaouen, in *Medicinal Organometallic Chemistry*, *Vol. 32* (Eds.: G. Jaouen, N. Metzler-Nolte), Topics in Organometallic Chemistry, Springer, Berlin, **2010**, pp. 81–117; b) S. S. Braga, A. M. S. Silva, *Organometallics* **2013**, *32*, 5626–5639.

[2] a) S. B. Deepthi, R. Trivedi, L. Giribabu, B. Sridhar, P. Sujitha, C. G. Kumar, *J. Organomet. Chem.* **2014**, *774*, 26–34; b) M. Librizzi, A. Longo, R. Chiarelli, J. Amin, J. Spencer, C. Luparello, *Chem. Res. Toxicol.* **2012**, *25*, 2608–2616; c) A. G. Harry, W. E. Butler, J. C. Manton, M. T. Pryce, N. O'Donovan, J. Crown, D. K. Rai, P. T. M. Kenny, *J. Organomet. Chem.* **2014**, *766*, 1–12; d) B. Balaji, B. Balakrishnan, S. Perumalla, A. A. Karande, A. R. Chakravarty, *Eur. J. Med. Chem.* **2014**, *85*, 458–467; e) J. d. J. Cázares-Marinero, S. Top, A. Vessières, G. Jaouen, *Dalton Trans.* **2014**, *43*, 817–830; f) P. Marzenell, H. Hagen, L. Sellner, T. Zenz, R. Grinyte, V. Pavlov, S. Daum, A. Mokhir, *J. Med. Chem.* **2013**, *56*, 6935–6944.

[3] a) D. Plażuk, A. Vessières, E. A. Hillard, O. Buriez, E. Labbe, P. Pigeon, M. A. Plamont, C. Amatore, J. Zakrzewski, G. Jaouen, *J. Med. Chem.* **2009**, *52*, 4964–4967; b) M. Görmen, P. Pigeon, S. Top, E. A. Hillard, M. Huche, C. G. Hartinger, F. de Montigny, M. A. Plamont, A. Vessieres, G. Jaouen, *ChemMedChem* **2010**, *5*, 2039–2050; c) Q. Michard, G. Jaouen, A. Vessieres, B. A. Bernard, *J. Inorg. Biochem.* **2008**, *102*, 1980–1985; d) E. Allard, C. Passirani, E. Garcion, P. Pigeon, A. Vessieres, G. Jaouen, J. P. Benoit, *J. Controlled Release* **2008**, *130*, 146–153; e) 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; f) 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.

[4] a) A. Vessières, C. Corbet, J. M. Heldt, N. Lories, N. Jouy, I. Laïos, 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.

[5] A. Citta, A. Folda, A. Bindoli, P. Pigeon, S. Top, A. Vessieres, M. Salmain, G. Jaouen, M. P. Rigobello, *J. Med. Chem.* **2014**, *57*, 8849–8859.

[6] a) A. L. Lainé, E. Adriaenssens, A. Vessieres, G. Jaouen, C. Corbet, E. Desruelles, P. Pigeon, R. A. Toillon, C. Passirani, *Biomaterials* **2013**, *34*, 6949–6956; b) A. L. Lainé, A. Clavreul, A. Rousseau, C. Tétaud, A. Vessieres, E. Garcion, G. Jaouen, R. A. Toillon, C. Passirani, *Nanomedicine* **2014**, *10*, 1667–1677.

[7] G. Jaouen, S. Top, in *Advances in Organometallic Chemistry and Catalysis* (Ed.: A. J. L. Pombeiro), Wiley, Hoboken, **2014**, pp. 563–580.

[8] P. Pigeon, M. Gormen, K. Kowalski, H. Mueller-Bunz, M. J. McGlinchey, S. Top, G. Jaouen, *Molecules* **2014**, *19*, 10350–10369.

[9] S. Groleau, J. Nault, M. Lepage, M. Couture, N. Dallaire, G. Bérubé, R. C. Gaudreault, *Bioorg. Chem.* **1999**, *27*, 383–394.

[10] P. Messina, E. Labbe, O. Buriez, E. A. Hillard, A. Vessieres, D. Hamels, S. Top, G. Jaouen, Y. M. Frapart, D. Mansuy, C. Amatore, *Chem. Eur. J.* **2012**, *18*, 6581–6587.

[11] M. Görmen, P. Pigeon, E. A. Hillard, A. Vessières, M. Huche, M. A. Richard, M. J. McGlinchey, S. Top, G. Jaouen, *Organometallics* **2012**, *31*, 5856–5866.

Received: April 16, 2015
Published online: July 14, 2015

