Antitumor Agents

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Ferrocenyl Quinone Methides as Strong Antiproliferative Agents: Formation by Metabolic and Chemical Oxidation of Ferrocenyl Phenols**

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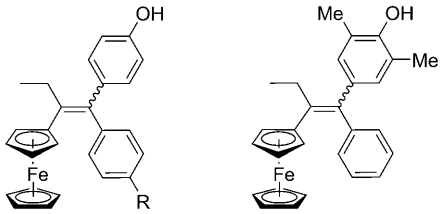
The modification of the biological effects of some biologically active molecules by ferrocene is an active field of study.[1,2] The addition of a ferrocenyl moiety to selected polyaromatic phenols,[3] amines, and amides[4] can potentiate their antiproliferative effects against breast and prostate cancer cells. For example, hydroxytamoxifen, the active metabolite of the breast cancer drug tamoxifen, shows limited toxicity against hormone-refractory breast cancer cells (IC_{50} for MDA-MB-231: 29 μM).[5] However, when a phenyl group is replaced by a ferrocene moiety, the resulting "hydroxyferrocifen" displays a dramatic improvement in toxicity ($IC_{50} = 0.5$ μM).[6] In 2006, we proposed in this journal a novel mechanism of activation of ferrocene phenols: their ferrocene-mediated oxidation to quinone methide (QM) metabolites.[7] This work was subsequently featured as part of a Highlight article.[8] Quinone metabolites are potentially cytotoxic species,[9–11] and, when ferrocene is present, QM formation takes place at comparatively mild (i.e. biologically relevant) oxidation potentials. Furthermore, the observation that some cancer cells are under heightened oxidative stress[12] suggests that this class of redox-activated compounds may show an interesting selectivity profile.

The formation of ferrocenyl QMs has been supported only by electrochemical experiments until now.[7] We herein show that QMs do form upon metabolism of **1**–**4** by rat liver microsomes and can also be prepared by chemical oxidation of **1**–**4** with Ag_2O. These new organometallic QMs have been completely characterized by mass spectrometry and ^1H and ^{13}C NMR spectroscopy, and one crystal structure has been obtained. These compounds were also found to exhibit marked antiproliferative behavior against hormone-independent breast cancer cells. This study thus prefigures the preclinical development of ferrocenyl phenols currently underway.



1 R = H (*E* + *Z*)
2 R = $O(CH_2)_3N(CH_3)_2$ (*E* + *Z*)
3 R = OH

4 (*E* + *Z*)

To establish whether QMs could be formed during metabolism of ferrocene phenols **1**–**4** in mammals, we studied the transformation of these complexes by liver microsomes containing the main enzymes responsible for xenobiotics metabolism. Incubation of 100 μM **3** with an aerobic suspension of rat liver microsomes (1 μM cytochrome P450) in phosphate buffer (pH 7.4) containing 1 mM nicotinamide adenine dinucleotide phosphate (NADPH) for 30 min at 37 °C led to the formation of a new major product that was detected by HPLC–MS and purified by preparative HPLC. Its mass spectrum (ESI^+) exhibited a molecular ion at *m*/*z* 422 [M^+] corresponding to the QM derived from two-electron oxidation of **3**, whose mass spectrum was characterized by a molecular ion at *m*/*z* 424 [M^+]. Fragmentation of the ion at *m*/*z* 422 by MS–MS led to an ion at *m*/*z* 356 that results from the loss of 66 u, which could correspond to a cyclopentadiene moiety.

Identical microsomal incubations with monophenolic compounds **1** and **4** led to the formation of the corresponding QM metabolites as major products. These metabolites were

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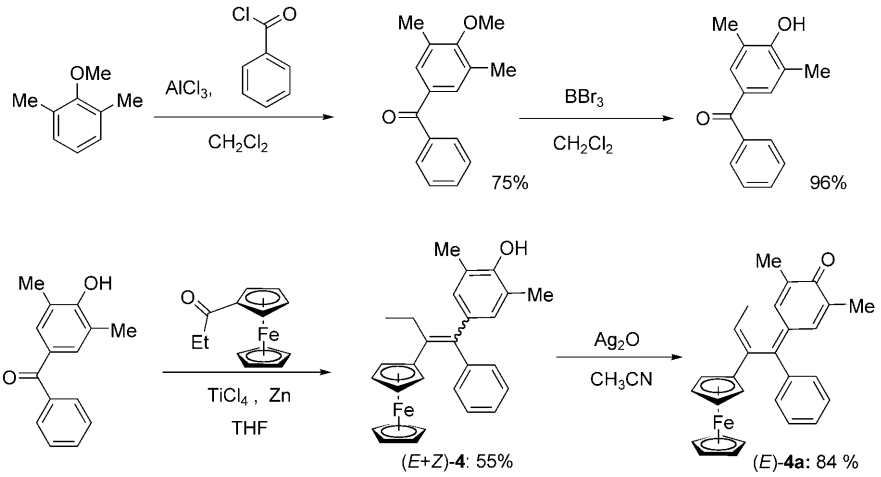
Supporting information for this article (experimental conditions, synthesis, ^1H and ^{13}C NMR spectroscopy and mass spectrometry for **1a**, **2a**, **4**, and **4a** and intermediates; melting points and elemental analysis data for **4** and **4a**; NOESY, HMQC, and COSY studies for **1a** and **2a**, and ORTEP diagrams for **4** and **4a**) is available on the WWW under http://dx.doi.org/10.1002/anie.200903768.

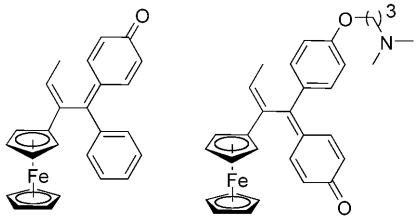
also identified by HPLC–MS: a molecular ion [M^+] at m/z 406 and 434 for the metabolite of **1** and **4**, respectively, and MS–MS fragments corresponding to the loss of 15 and 66 u (loss of CH_3 and C_5H_6, respectively) were observed for the two metabolites. Finally, incubation of the ferrocifen compound related to tamoxifen (**2**) also led to the formation of a QM metabolite that exhibited a MS molecular ion at m/z 508 [M+H$^+$], whereas the mass spectrum of **2** showed a molecular ion at m/z 510.

These data strongly suggest that QM metabolites are generally formed upon oxidation of the studied ferrocenyl phenols by liver microsomes. As these QMs appeared to be stable in the conditions used for their identification, we decided to prepare them by chemical oxidation. It is noteworthy that the chemically prepared QMs exhibited HPLC retention times and MS spectra identical to those of the corresponding QM metabolites formed in microsomal incubations.

Chemical oxidation was performed by dissolving **1**,[13] **2**,[14] **3**,[1] and newly synthesized **4** (1 mmol; Scheme 1) in a small volume of distilled acetonitrile and adding freshly prepared Ag_2O (5 equiv). After filtration and evaporation of the solvent under reduced pressure, the corresponding QMs **1a** (88% yield), **2a** (79%), and **4a** (84%) were obtained in greater than 95% purity after 45, 90, and 10 min, respectively. For all compounds, the color rapidly changed from orange to dark red, and NMR spectroscopy confirmed a structural change to a QM; the appearance of a doublet and a quartet in the ^{1}H NMR spectra at around $\delta = 1.68$ and 6.44 ppm, respectively, indicated the formation of a double bond on the ethyl group. Furthermore, ^{13}C NMR spectroscopy indicated the formation of a C=O bond as the peak corresponding to the C–OH bond in **1**, **2**, and **4** shifted from around $\delta =$ 153 ppm to $\delta = 187$ ppm. As observed above, the mass spectra of **1a, 2a**, and **4a** showed the loss of two protons from their parent molecules. For compound **3**, however, although we were able to observe the appearance of characteristic QM peaks in the NMR spectra, QM **3a** was unstable and started decomposing before oxidation was complete.



Scheme 1. Synthesis of **4** and **4a**.



(*E*)-**1a** (*E*)-**2a**

Compounds **1**, **2**, and **4** were obtained as 50/50 mixtures of *Z* and *E* isomers. With respect to the double bond of the terminal alkene function, the QM could exist as a *Z* or *E* isomer. Surprisingly, only one isomer was visible in the NMR spectra of **1a**, **2a**, and **4a**. NOESY NMR experiments of **1a** and **2a** showed that the methyl allylic protons point away from the ferrocenyl group towards either the quinone (**1a**) or the aromatic ring (**2a**), and that the vinylic proton points towards the ferrocene moiety, giving the compounds *E* configuration.

Compound **4a** proved to be stable enough to afford X-ray quality red plates from a CH_2Cl_2/pentane mixture (Figure 1).[15] The QM structure of **4a** was confirmed by comparison of its bond lengths with those of **4** and literature values for those of the QM 2,5-dimethylfuchsone.[16] The C18–O1 bond is significantly shorter for **4a** than for **4**, suggesting C=O bond character. Similarly, the C1–C15 and C2–C3 bonds are shorter in **4a** than in **4**, indicating C=C bond character. In contrast, the C1–C2 bond is longer in **4a** than in **4**, which is consistent with a C–C bond. Another feature is the alternating long and short bonds in the quinone group in **4a**, which is characteristic of a 1,4-unsaturated ring.

The methide double bond in **4a** is slightly distorted (dihedral angle of 7.93°); this distortion may be explained by the bulkiness of the different groups involved, which try to minimize steric interactions with one another. The molecule is quite strained; the ferrocene moiety is in an eclipsed configuration, and an angle of 5.86° can be observed between the two cyclopentadienyl (Cp) rings. The obtained *E* configuration is explained by the observation that the C2–C3 double bond and the upper cyclopentadiene ring are nearly in the same plane (torsion angle of 18.97°), which means that if the C=C bond had *Z* configuration, the methyl protons would strongly interact with the Cp ring. As free rotation is possible around the C1–C2 bond, steric interactions with both rings can thus be minimized.

Parent molecules **1** and **2** show strong antiproliferative (cytotoxic) effect on hormone-independent breast cancer cells (MDA-MB-231) and yield IC_{50} values of 1.1 and 0.5 μM, respectively.[14,17] Freshly synthesized **1a** and **2a** were also toxic against MDA-MB-231 cells (IC_{50} values of 21.7 ± 4.3 and 4.2 ± 1 μM, respectively). These higher values for **1a** and **2a**,

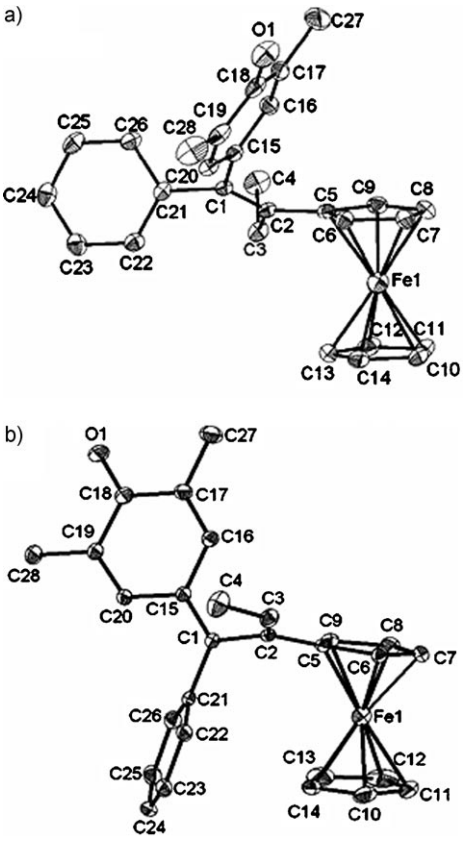


Figure 1. ORTEP views of a) (*Z*)-**4** and b) (*E*)-**4a** with thermal ellipsoids presented at 50% probability. Selected bond lengths [Å] for **4**: C18–O1 1.402(4), C15–C16 1.401(4), C16–C17 1.399(4), C17–C18 1.407(5), C15–C1 1.510(4), C1–C2 1.361(4), C2–C3 1.536(4); for **4a:** C18–O1 1.246(3), C15–C16 1.459(4), C16–C17 1.361(4), C17–C18 1.481(4), C15–C1 1.381(4), C1–C2 1.512(4), C2–C3 1.343(4).

relative to those **1** and **2**, could be due to their chemical instability, as strong electrophiles, in the incubation medium. These IC_{50} values nonetheless suggest that **1a** and **2a** should have remarkable intrinsic antiproliferative properties. Moreover, since **1a** and **2a** are formed as major metabolites of **1** and **2** inside the cells, at the level of the endoplasmic reticulum, they should play a key role in the anticancer properties of ferrocenyl phenols. The previously proposed mechanism of oxidative activation of such ferrocenyl phenols is therefore established.

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[15] Intensity data were collected at 200 K with a Enraf–Nonius Kappa CCD diffractometer equipped with a CCD two-dimensional detector [$\lambda\ Mo_{K\alpha} = 0.71073$ Å]. Data were collected in 236 frames (**4**) and 345 frames (**4a**·CH_2Cl_2) with ϕ and ω scans (width of 1.5° and exposure time of 75 s per frame for **4** and 2° and 40 s for **4a**·CH_2Cl_2). Data reduction was performed with EVALCCD software (Duisenberg & Scheurs 1990–2000). Data were corrected for Lorentzian and polarization effects, and a semi-empirical absorption correction based on symmetry equivalent reflections was applied by using the SADABS program [G. M. Sheldrick, SADABS, Program for Scaling and Correction of Area Detector Data, University of Göttingen, Göttingen, Germany, **1997**, R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33]. Lattice parameters were obtained from least-squares analysis of 91 (**4**) and 143 (**4a**·CH_2Cl_2) reflections. The structure was solved by direct methods and refined by full matrix least-squares analysis, based on F^2, using the Crystals software package [Betteridge et al., **2003**]. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were located with geometrical restraints in the riding mode.
4: $C_{28}H_{28}FeO$; $M = 436.38$; monoclinic; $P21/n$; $a = 9.319(1)$, $b = 12.066(1)$, $c = 20.305(1)$ Å, $\beta = 94.717(9)°$, $V = 2275.4(4)$ Å^3; $Z = 4$; $0.21 \times 0.18 \times 0.15$ mm^3; $\rho_{calcd} = 1.27$; $\mu = 0.679$; $2\Theta_{max} = 60°$; total reflections: 22754; independent reflections: 6597; $R(I > 2\sigma(I)) = 0.0546$, $wR2(all) = 0.1387$, $S = 0.9016$; highest residual electron density 1.11 e Å^{-3}.
4a·CH_2Cl_2: $C_{29}H_{28}Cl_2FeO$; $M = 519.29$; triclinic, $P\bar{1}$; $a = 8.904(1)$, $b = 9.6247(8)$, $c = 15.404(2)$ Å, $\alpha = 92.208(7)$, $\beta = 101.598(8)$, $\gamma = 90.581(7)°$. $V = 1291.99(18)$ Å^3; $Z = 2$; $0.26 \times 0.19 \times 0.12$ mm^3; $\rho_{calcd} = 1.33$; $\mu = 0.810$; $2\Theta = 60°$; total reflections: 26816; independent reflections: 7517; $R\ (I > 2\sigma(I)) = 0.0562$, $wR2$ (all) = 0.1392, S = 1.0357; highest residual electron density 1.10 e Å^{-3}. CCDC 736717 (**4**) and 736718 (**4a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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