Oxidation of antiparasitic 2-substituted quinolines using metalloporphyrin catalysts: scale-up of a biomimetic reaction for metabolite production of drug candidates

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A study comparing the chemical oxidation of drug candidates with cell-based metabolism (liver microsomes, hepatocytes) shows that biomimetic oxidation can replace the biological approach, thus allowing access to large quantities of metabolites, which can also be used for the preparation of new products.

In the course of drug discovery, features such as absorption, distribution, metabolism and excretion (ADME) are determinant parameters that need to be studied at the earliest stages.[1] In mammals, cytochromes P450 (CYP450) are primarily responsible for the oxidative metabolism of endogenous and exogenous products.[2]

Cell-based metabolism studies of active compounds using liver microsomes, hepatocytes, or microfungi present several advantages over *in vivo* studies in animals,[3] but the difficulty of isolation from the biological medium and small quantities are severe drawbacks.[4] Thus metalloporphyrin (MEP) catalyzed oxidations have been proposed as a biomimetic alternative approach to the cell-based metabolism study of bioactive compounds.[4–10]

Certain MEPs in the presence of active oxygen donors form metallo-oxo species that mimic the active sites of cytochrome enzyme systems.[11] This strategy presents the advantage of producing several metabolite candidates, helping in the identification of the cell-based and/or *in vivo* produced metabolites. As part of ongoing studies to determine the metabolites of 2-substituted quinolines prepared in our laboratories as agents against the parasitic disease leishmaniasis,[12,13] previous studies characterized several metabolites of 2-propylquinoline produced *in vitro* by liver microsomes, hepatocytes and recombinantly expressed enzymes using LC/MS analyses.[14]

In this communication we report the biomimetic chemical oxidation of 2-substituted quinolines catalyzed by either chloromanganese(tetra-2,6-dichlorophenylporphyrin) (Mn(TDCPP)Cl), or chloromanganese(tetraphenylchlorofluoroporphyrin) (Mn(TPCFP)Cl). The results show that these synthetically prepared products are similar to those obtained by cell-based experiments. To our knowledge this is the first time that such metabolites have been produced in gram quantities.[15] Thus our approach allows us to transform the metabolites further into functionalized products that are being biologically tested and used for the preparation of more highly elaborated compounds.

The MEPs used in this study were prepared as already described by Lindsey *et al.*[16] from the corresponding porphyrinogen followed by metallation. The oxidation reactions were performed as follows: 1 g of substrate **2a** or **2b** in a three-neck round bottomed flask with 0.133 g (2.5 mol%) of either Mn(TDCPP)Cl, or Mn(TPCFP)Cl, and 10 mol% of imidazole in 1:1 dichloromethane/acetonitrile (18 mL) open to the atmosphere were stirred at room temperature. Imidazole (50 mol%) and H_2O_2 (35%, 2.6 mL) in acetonitrile (25 mL) were slowly added *via* an addition funnel. The reaction was stopped after TLC analysis showed no further evolution of the reaction mixture (around 2 h). After evaporation of the solvent the crude reaction mixture was purified by repeated flash chromatography on silica gel (with 7/3 cyclohexane:EtOAc as eluent), affording several fractions (with purity checked by HPLC and in the range of 88.1 to 99.7%) which were analyzed by ^{1}H and ^{13}C NMR spectroscopy as well as mass spectrometry.

Interestingly, when 2-propylquinoline **2a** was oxidized as described above, racemic compound **3a** was isolated in 17% yield[17] and identified as one of the metabolites previously proposed on the basis of HPLC/mass analysis from *in vitro* rat microsome experiments (Fig. 1).[14] It is worth noting that **3a**, obtained in such quantities, can be fully characterized (see spectroscopic data[17]).

Compound **4a**, which was not observed in the cell-based experiments, was isolated as a single diastereomer (whose stereochemistry has not been elucidated yet) as the major product (36% yield).[17] With an authentic sample in hand, it could be interesting to check if **4a** is ever produced *in vitro* by hepatocytes (**4a** may have reacted with nucleophiles such as glutathione through an enzymatic (glutathione transferase) or non-enzymatic pathway).[14,18] Starting material **2a** was also recovered in 15% yield. Several other compounds present in low quantities could not be characterized. However when compound **2a** was oxidized by a different MEP under the same reaction conditions as described above, a new compound **5a** was obtained (14% yield),[17] together with metabolite candidates **3a** and **4a** (in 13 and 39% yield, respectively) and starting material **2a** recovered in 9% yield. The ketone **5a** corresponds to an over-oxidized metabolite (2-(2-hydroxypropyl)quinoline) produced by human liver microsomes *in vitro* and by rat CYP450 isoenzyme (primarily CYP2B1 and 2A6) oxidation[14] (Fig. 1). When the reaction was performed under the same reaction conditions as described above but without MEP catalyst, starting material **2a** was recovered unchanged and no oxidized products were observed.

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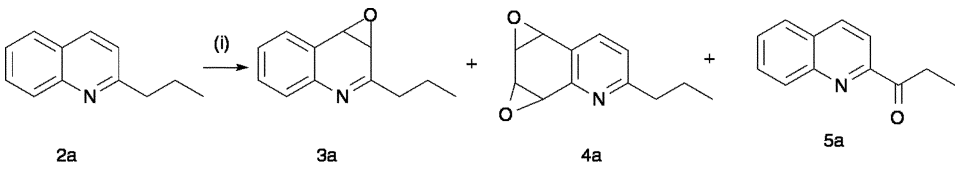


(i) MEP, H_2O_2, imidazole, CH_2Cl_2/CH_3CN (1:1)

MEP = Mn(TDCPP)Cl; **3a** (17%), **4a** (36%), **5a** (0%)
MEP = Mn(TPCFP)Cl; **3a** (13%), **4a** (39%), **5a** (14%)

Fig. 1 MEP catalyzed oxidation of **2a**.

We have also studied the MEP catalyzed oxidation of another antiparasitic 2-substituted quinoline, compound **2b**, which has shown promising *in vivo* antileishmanial activity in an animal model of visceral leishmaniasis.[19] When quinoline **2b** was treated as above in the presence of a catalytic amount of Mn(TPCFP)Cl, compound **3b** was obtained in a trace amount,[20] together with the other monoepoxy compounds **3c** and **3d**, whereas the diepoxide **4b** was isolated as a single diastereomer (again its stereochemistry has not been elucidated yet) in 35% yield.[20] It is interesting to note that a minor product **4c**, which could be produced from **4b** by the addition of two molecules of water, was characterized and isolated in 3% yield (Fig. 2). When the reaction was performed with Mn(TDCPP)Cl all five compounds were obtained in slightly different yields (see Fig. 2). Even with longer reaction time, starting material **2b** was not completely consumed under these reaction conditions; optimization of the process is still under investigation. Under the reaction conditions used in this study, no side-chain oxidation of **2b** was observed. This illustrates the high chemoselectivity of the MEP oxidation system. In fact when the oxidation of **2b** was attempted in the absence of MEP, no reaction occurred and the starting material was recovered unchanged.

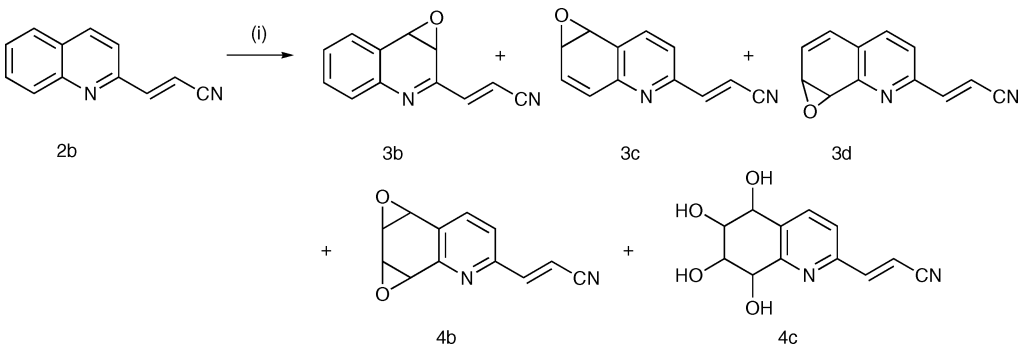
These results show that 2-substituted quinolines, when treated by MEP and H_2O_2 in the presence of imidazole in dichloromethane/acetonitrile, give mainly oxidized products that possess one or two epoxide rings on the aromatic moiety. Side-chain oxidation is a minor event for the propyl group (electron donor substituent) and not observed in the presence of the acrylonitrile (electron withdrawing group). Because MEP oxidation produces these metabolites on the gram scale, they can now be used as intermediates for the preparation of more elaborated structures, as shown in Fig. 3.

For instance, product **4a** was treated in wet acetone in the presence of a catalytic amount of sulfuric acid for 4 h to give the expected tetraol **6a** in 80% yield.[17] Manganese dioxide oxidation of **6a** led then to the bis-oxidized product **7a**,[17] which is stable, although tautomeric with **8a** (Fig. 3).

In conclusion, this study shows that the MEP catalyzed oxidation of 2-substituted quinolines mimics the CYP450 oxidation responsible for drug metabolism in mammals. Thus, it can be used as a chemical model of drug metabolism, since identical compounds have been obtained by both methods. Furthermore, new metabolite candidates, not yet characterized elsewhere, have also been produced, which could help in the characterization of metabolites produced *in vitro*/*in vivo*.

The preparative scale of this approach should be emphasized, since the oxidized compounds can now be independently studied for their bioactivity (results to be published in due course) and also be used as key building blocks for the access of more highly elaborated products such as the 5,6,7,8-tetrahydroxyquinoline derivative **8a**. Generalization of this method for metabolite production of active principles used in the pharmaceutical industry is in progress.

To a greater extent, biomimetic chemical oxidation of any chemical substance ought to be studied in order to gain



(i) CH_2Cl_2/CH_3CN (1:1), MEP, H_2O_2, imid.

MEP = Mn(TDCFP)Cl; **3b** (trace), **3c** (3%), **3d** (2%), **4b** (35%), **4c** (3%)
MEP = Mn(TDCPP)Cl; **3b** (trace), **3c** (5%), **3d** (trace), **4b** (18%), **4c** (8%)

Fig. 2 MEP catalyzed oxidation of **2b**.

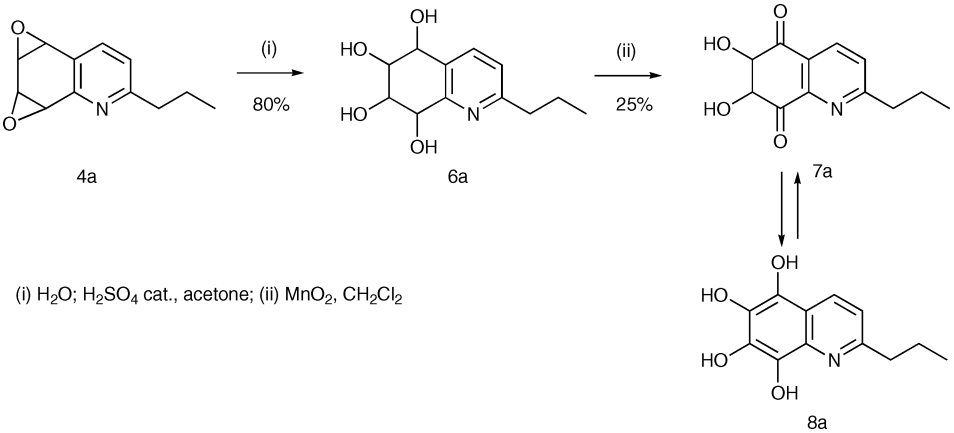


Fig. 3 Acid opening and oxidation of **4a**.

knowledge regarding metabolite candidates for toxicological studies. Improvements in metalloporphyrin-based oxidation reactions can be envisaged to give higher yields and better substrate selectivity, which might ultimately lead to more regio-, chemo- and enantioselective oxidations.[21]

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17 **Caution**, epoxides of aromatic compounds are suspected to be involved in the carcinogenesis of such compounds! Selected spectroscopic data (^{1}H, ^{13}C NMR, mass) of new compounds obtained after Mn(TPCFP)Cl catalyzed oxidation of **2a** (1 g): compound **3a** (140 mg): ^{1}H (300 MHzCDCl$_3$) δ 8.12 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 7.5 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.48 (t, J = 8.1 Hz, 1H), 4.71 (d, J = 5.1 Hz, 1H), 4.14 (d, J = 5.1 Hz, 1H), 2.30 (m, 2H), 1.36 (m, 2H), 0.61 (t, J = 7.2 Hz, 3H); ^{13}C (75 MHz, CDCl$_3$) δ 202.9, 146.9, 133.9, 130.1, 129.6, 129.3, 124.4, 60.4, 57.3, 42.5, 16.1, 13.2; EIMS (70 eV) *m/z* (%) 187 (M, 34), 172 (36), 159 (100), 143 (10), 103 (10), 77 (9); IR cm^{-1} 2965, 1725, 1525, 1345, 1260, 1015; compound **4a** (431 mg): ^{1}H (300 MHz, CDCl$_3$) δ 7.59 (d, J = 7.8 Hz, 1H), 7.08 (d, J = 7.8 Hz, 1H), 4.05 (m, 1H), 4.03 (m, 1H), 3.85 (d, J = 4.0 Hz, 1H), 3.67 (d, J = 4.0 Hz, 1H), 2.74 (t, J = 7.4 Hz, 2H), 1.73 (hex, J = 7.4 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C (75 MHz, CDCl$_3$) δ 162.8, 151.3, 138.7, 124.5, 122.6, 55.3, 54.4, 53.1, 50.7, 39.9, 22.8, 13.6; EIMS (70 eV) *m/z* (%) 203 (M, 37), 202 (39), 188 (18), 175 (100), 159 (12), 146 (45), 117 (11), 77 (12); IR cm^{-1} 2960, 2925, 2855, 1725, 1580, 1275; compound **5a** (152 mg): ^{1}H (300 MHz, CDCl$_3$) δ 8.25 (d, J = 8.4 Hz, 1H), 8.18 (dt, J = 8.4, 0.4 Hz, 1H), 8.12 (d, J = 8.6 Hz, 1H), 7.86 (dd, J = 8.0, 1.0 Hz, 1H), 7.77 (td, J = 7.0, 1.6 Hz, 1H), 7.63 (td, J = 6.8, 1.2 Hz, 1H), 3.42 (q, J = 7.4 Hz, 2H), 1.26 (t, J = 4.0 Hz, 3H); ^{13}C (75 MHz, CDCl$_3$) δ 203.0, 153.0, 147.1, 136.7, 130.5, 129.8, 129.5, 128.3, 127.6, 118.1, 30.8, 8.0; EIMS (70 eV) *m/z* (%) 185 (M, 17), 157 (54), 129 (100), 101 (25), 77 (15); IR cm^{-1} 2975, 1690, 1560, 1360, 1115, 935; compound **6a** (260 mg): ^{1}H (300 MHz, CDCl$_3$) δ 7.51 (d, J = 7.5 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 4.45 (brs, 2H), 4.13 (brs, 1H), 3.84 (brs, 1H), 2.66 (t, J = 7.5 Hz, 2H), 1.65 (m, 2H), 0.90 (t, J = 6.9 Hz, 3H); ^{13}C (75 MHz, CDCl$_3$) δ 162.1, 151.2, 139.3, 128.4, 123.5, 71.8, 66.2, 57.5, 53.7, 39.6, 22.9, 13.7; EIMS (70 eV) *m/z* (%) 239 (M, 18), 147 (9), 129 (45), 112 (90), 84 (40), 70 (84), 57 (100) ; IR cm^{-1} 3330, 2960, 1600, 1035; compound **7a** (10 mg): ^{1}H (300 MHz, CDCl$_3$) δ 8.18 (d, J = 8.1 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 4.56 (d, J = 3.6 Hz, 1H), 4.11 (d, J = 3.6 Hz, 1H), 2.87 (t, J = 7.8 Hz, 2H), 1.80 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H); ^{13}C (75 MHz, CDCl$_3$) δ 190.8, 163.9, 151.4, 137.1, 132.0, 124.2, 71.1, 57.6, 54.0, 40.6, 22.6, 13.8; EIMS (70 eV) *m/z* (%) 235 (M, 15), 203 (41), 188 (40), 175 (100), 159 (22), 146 (14), 77 (15); IR cm^{-1} 2920, 1710, 1590, 1260, 1115.
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20 Mn(TDCPP)Cl catalyzed oxidation of **2b** (1 g): compound **3b** (trace): EIMS (70 eV) *m/z* (%) 196 (M, 17), 168 (20), 140 (15), 129 (15), 128 (100); compound **3c** (18.8 mg): ^{1}H (300 MHz, CDCl$_3$) δ 7.95 (d, J = 7.8 Hz, 1H), 7.41 (d, J = 15.9 Hz, 1H), 7.25 (d, J = 7.5 Hz, 1H), 6.94 (brd, J = 9.6 Hz, 1H), 6.80 (dd, J = 9.9, 3.9 Hz, 1H), 6.64 (d, J = 16.2 Hz, 1H), 4.49 (d, J = 3.9 Hz, 1H), 4.17 (td, J = 3.9, 1.5 Hz, 1H); ^{13}C (75 MHz, CDCl$_3$) δ 151.6, 150.9, 148.1, 138.2, 133.1, 131.3, 129.8, 122.5, 117.8, 101.5, 56.1, 53.0; EIMS (70 eV) *m/z* (%) 196 (M, 100), 179 (12), 168 (17), 145 (30), 143 (20); IR cm^{-1}2920, 2215, 1565, 1455, 970;

compound **3d** (10.1 mg): ^{1}H (300 MHz, $CDCl_3$) δ 7.62 (d, $J = 7.8$ Hz, 1H), 7.41 (d, $J = 16.2$ Hz, 1H), 7.32 (d, $J = 7.8$ Hz, 1H), 6.75 (brd, $J = 9.6$ Hz, 1H), 6.72 (d, $J = 15.3$ Hz, 1H), 6.60 (dd, $J = 9.6$, 3.6 Hz, 1H), 4.64 (d, $J = 3.6$ Hz, 1H), 4.19 (td, $J = 3.6$, 1.5 Hz, 1H);^{13}C (75 MHz, $CDCl_3$) δ 151.5, 149.7, 147.8, 146.0, 136.2, 129.6, 128.8, 128.2, 124.3, 117.9, 101.1, 58.1, 53.7; EIMS (70 eV) *m/z* (%) 196 (M, 100), 168 (34), 145 (12), 140 (12), 70 (9), 63 (9); compound **4b** (205.3 mg): ^{1}H (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 7.6$ Hz, 1H), 7.35 (d, $J = 16.0$ Hz, 1H), 7.26 (d, $J = 7.6$ Hz, 1H), 6.67 (d, $J = 16.0$ Hz, 1H), 4.08 (m, 1H), 4.06 (m, 1H), 3.88 (d, $J = 4.0$ Hz, 1H), 3.73 (d, $J = 4.0$ Hz, 1H); ^{13}C (75 MHz, $CDCl_3$) δ 153.0, 151.0, 147.2, 139.6, 129.4, 124.1, 117.7, 102.0, 55.5, 54.8, 52.9, 50.5; EIMS (70 eV) *m/z* (%) 212 (M, 32), 183 (100), 155 (86), 128 (18), 102 (14), 77 (11); IR cm^{-1} 3070, 3020, 2920, 2850, 2220, 1730, 1570, 1455, 960. compound **4c** (15.2 mg): ^{1}H (300 MHz, $CDCl_3$) δ 7.96 (d, $J = 7.8$ Hz, 1H), 7.66 (d, $J = 16.2$ Hz, 1H), 7.65 (d, $J = 7.8$ Hz, 1H), 6.81 (d, $J = 16.2$ Hz, 1H), 5.14 (m, 1H), 4.74 (m, 1H), 4.10 (dd, $J = 3.6$, 0.3 Hz, 1H), 3.94 (m, 1H); ^{13}C (75 MHz, $CDCl_3$) δ 155.2, 153.1, 149.2, 142.2, 132.8, 126.6, 119.5, 103.2, 69.6, 58.5, 57.2, 54.0; EIMS (70 eV) *m/z* (%) 248 (M, 13), 213 (100), 196 (28), 185 (52), 167 (52), 155 (35), 129 (20), 102 (20), 77 (27); IR cm^{-1} 3300, 2925, 2220, 1745, 1570, 1445, 960.

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