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Biomimetic oxidation of praziquantel catalysed by metalloporphyrins

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Abstract

This paper reports an HPLC–MS/MS investigation of praziquantel (anthelmintic agent) oxidation by iodosylbenzene in an organic medium, using iron(III) tetraarylporphyrins (where aryl = phenyl, pentafluorophenyl, 2-nitrophenyl, 2-trifluoromethylphenyl and mesityl) and manganese(III) tetraphenylporphyrin as catalysts. The majority of the oxidation products have been identified by sequential MS and NMR analyses. The selectivities of the oxidations by these cytochrome P-450 models are studied and compared. Time dependent reaction profiles suggest that with all the catalysts, the initial oxidation occurs predominantly at the 7-position. With Fe(T2TFMPP)Cl the yield and selectivity (64%) for 7-hydroxypraziquantel remain high even after 24 h reaction, whereas with the other catalysts this initial product is further oxidized to di- and trihydroxypraziquantel. The Fe(TFPP)Cl system results in the higher yield (11%) of *cis*- and *trans*-4′-hydroxypraziquantel, the major metabolites from in vivo and in vitro metabolism of praziquantel by cytochrome P-450 monooxygenases.

Keywords: Drug metabolism; Metalloporphyrin; Oxidation; Praziquantel; LC–MS

1. Introduction

The oxidative metabolism of drugs by cytochrome P-450 monooxygenases has been extensively studied in the last two decades [1]. The driving force has been to obtain a thorough understanding of the metabolic pathways and of the biological properties of the active metabolites. However, there are several problems associated with the use of biological systems in studying drug metabolism and identifying unknown metabolites, such as the difficulty of their isolation from the biological matrix, due to their hydrophilic character, their low yields and the ethical problems of using animals for research [2].

An alternative approach is to use biomimetic catalysts, such as metalloporphyrins (MeP), to study the behavior of biologically active compounds under oxidative conditions [2–5]. It has been shown that oxidations catalysed by metalloporphyrins can mimic the in vivo metabolism of some drugs, yielding synthetic metabolites [2]. In these systems, metabolite candidates can be obtained in relatively large amounts to provide samples for pharmacological testing and to aid identification of in vivo metabolites. Secondly, unstable metabolites can be identified and sometimes isolated under controlled reaction conditions. Thirdly and importantly, the number of animal experiments used for drug testing can be reduced [6].

MeP have been thoroughly studied as catalysts for the oxidation of simple compounds such as alkanes and alkenes [7–13], but there are relatively few reported studies of

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drug oxidation: some examples include lidocaine, odapipam, ABT-200; ABT-418, aminopyrine [2], acetaminophen [4,14] and others [5,15].

In this paper, we report a study on praziquantel (PZQ) oxidation using metalloporphyrins as catalysts. PZQ, 2-(cyclohexylcarbonyl)-1,2,3,6,7,11β-hexahydro-4*H*-pyrazino-[2,1a]-isoquinolin-4-one, is an effective anthelmintic agent that exhibits broad activity against trematodes and cestodes, particularly for *Schistosoma* and *Taenia* infections [16–18]. After oral administration to humans, drug absorption is very rapid and is followed by a fast and extensive metabolism by the cytochrome P-450 monooxygenases, producing mainly *trans*-4′-monohydroxypraziquantel (*trans*-4′-OH) [19–21]. In contrast, in vitro incubation with rat, mouse, rabbit or human liver microsomes yields *cis*-4′-monohydroxypraziquantel (*cis*-4′-OH) as the main product [20,22–24]. Other mono-, di- and trihydroxylated metabolites as well as glucoronide conjugates were reported by Lerch and Blaschke [24] and more recently by Meier and Blaschke [25].

In the present study, PZQ oxidation was carried out in organic solution, using iodosylbenzene (PhIO) as oxygen donor. The catalysts used were iron(III) and manganese(III) 5,10,15,20-tetraphenylporphyrin and the iron(III) derivatives of four other tetraarylporphyrins: 5,10,15,20-tetrakis(mesityl)porphyrin [H_2(TMP)], 5,10,15,20-tetrakis (pentafluorophenyl)porphyrin [H_2(TFPP)], 5,10,15,20-tetrakis(2-nitrophenyl)-β-octachloroporphyrin [H_2(T2NPCl$_8$P)] and 5,10,15,20-tetrakis(2-trifluoromethylphenyl)porphyrin [H_2(T2TFMPP)] (Fig. 1). The latter four ligands were selected because they are more resistant to degradation and, the three electron-deficient ligands [H_2(TFPP), H_2(T2NPCl$_8$P) and H_2(T2TFMPP)] result in more reactive metalloporphyrin species than the parent ligand, 5,10,15,20-tetraphenylporphyrin [H_2(TPP)] [10–12]. The aim was to identify the most reactive sites of PZQ and to determine conditions that lead to selective oxidation of some sites. The products obtained in the oxidation reactions were characterized by liquid chromatography–mass spectrometry (LC–MS/MS) and the major product, 7-hydroxypraziquantel, was also analysed by ^{1}H and ^{13}C NMR spectroscopy (2D HMBC and HMQC).

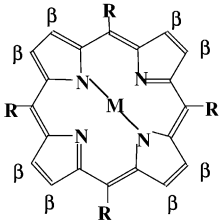
2. Materials and methods

PZQ was obtained from Merck (Germany). *Trans*-4′-OH (containing 15% of *cis*-4′-OH) was kindly provided by G. Blaschke from the Institute of Pharmaceutical Chemistry, University of Münster, Germany. PhIO was synthesized according to a previously described method [26]. The porphyrins H_2(TPP), H_2(TFPP) and Fe(TMP)Cl were purchased from Midcentury (USA). H_2(T2TFMPP) was synthesized according to a previously described method [27]. Manganese or iron insertion into the free base porphyrins was carried out by adapting the methodology described by Adler et al. [28]. The perchlorinated porphyrin, Fe(T2NPCl$_8$P)Cl, was obtained according to Wijesekera et al. [29].

Methanol (MeOH) and acetonitrile (MeCN) were of HPLC grade (EM Science, USA). Deuterated chloroform ($CDCl_3$) was obtained from Aldrich. All other chemicals were analytical-reagent grade and were used without further purification. The water used for the mobile phase preparation was purified with a Milli-Q Plus System (Millipore, USA).

2.1. Oxidation procedure

The reaction mixtures (2 mL) in 1,2-dichloroethane (DCE) or MeCN contained 6.0 mM PZQ, 0.3 mM metalloporphyrin and 9.0 mM PhIO. For the experiments with



Metalloporphyrin	R		M
TPP	Phenyl	H	Fe, Mn
TMP	2,4,6-triCH_3-Phenyl	H	Fe
TFPP	F_5Phenyl	H	Fe
T2TFMPP	2-CF_3-Phenyl	H	Fe
T2NPCl$_8$P	2-NO_2-Phenyl	C l	Fe

Fig. 1. Structures of metalloporphyrin catalysts used in this study.

Mn(TPP)Cl, 3 mM imidazole was also added [30]. All experiments were carried out at room temperature, in air, in a glass vessel equipped with a magnetic stirring bar. Reaction times were measured after the addition of PhIO. At regular intervals, the magnetic stirring was stopped and an aliquot of reaction mixture (10 μL) was withdrawn and after porphyrin extraction, it was analysed by HPLC. Porphyrin extraction was carried out adding hexane (90 μL) and mobile phase (100 μL) to the aliquot removed from the mixture reaction. The mixture was vortex mixed and centrifuged; the lower phase (mobile phase) was then injected into the chromatographic system. This work-up procedure did not remove unreacted praziquantel or the oxidation products.

2.2. HPLC analysis

The analytical HPLC analyses were performed on a Shimadzu liquid chromatograph equipped with an LC-10AS solvent pump, a 7125 Rheodyne injector with 20 μL loop, an SPD-10A spectrophotometric detector (λ = 220 nm) and a CR6-A integrator. The separation of drug and oxidation products was carried out on a Lichrospher CN column, 5 μm particle size (125 mm × 4 mm) supplied by Merck (Germany). The analytical column was protected by a Lichrospher CN guard column (4 mm × 4 mm, Merck). Elution was carried out at a flow-rate of 1.0 mL/min using methanol:water (15:85 v/v) as the mobile phase. The preparative HPLC analyses for monohydroxypraziquantel isolation were performed using a 1000 μL loop and a Shim-Pack CN column, 5 μm particle size (250 mm × 20 mm), protected by a CN guard column (Shimadzu, Japan). Elutions were carried out at a flow-rate of 9.0 mL/min using methanol:water (30:70 v/v) as the mobile phase.

2.3. LC–MS/MS analysis

The LC–MS/MS system used for the analysis of PZQ consisted of a Varian 5000 pump, a Rheodyne 7125 injector with a 20 μL loop and a Finnigan MAT LCQ mass spectrometer (USA) equipped with an ion-trap mass spectrometer and an atmospheric pressure chemical ionisation interface. The vaporizer temperature was set at 450 °C and nitrogen was applied as the sheath gas at a flow-rate of 60 (arbitrary units). The heated capillary was maintained at 150 °C. Mass analysis was performed in the positive ion mode with the source current set at 5 μA, and the potentials of tube lens and capillary set at 45 and 31 V, respectively. The chromatographic analyses were carried out as described above.

One of the PZQ oxidation products (peak eluting at 12 min) was characterized using an LC triple-stage quadrupole (Micromass, Manchester, UK), fitted with a Z-electrospray interface operated in the positive ion mode. The source block and desolvation temperatures were 100 and 250 °C, respectively. Nitrogen was used as both drying and nebulizing gas at 51 and 548 L/h, respectively. Argon was used as collision gas at a pressure of approximately 3.5×10^{-3} mbar. The HPLC eluent was split by a Valco valve and a flow-rate of approximately 0.1 mL/min was introduced into the stainless steel capillary probe held at 3.5 kV. The cone and collision cell voltage were 45 V and 30 eV, respectively.

2.4. Isolation and NMR analyses of monohydroxypraziquantel

Seven reaction mixtures (2 mL each) in DCE containing 10 mM PZQ, 0.3 mM Fe(TFPP)Cl and 12 mM PhIO were prepared to obtain sufficient monohydroxypraziquantel for NMR characterization. The reactions were carried out as described above and after 45 min, the reaction mixtures were worked up by adding hexane (18 mL) and methanol:water 7:3 (20 mL). The mixtures were vortex mixed and centrifuged and the lower phases were removed, evaporated, dissolved in the mobile phase and then injected into the chromatographic system. The eluent fraction, containing the oxidation product, was evaporated and the residue was dissolved in $CDCl_3$ and analysed by NMR (^{1}H, 2D HMBC and 2D HMQC) spectroscopy. The spectra were recorded on a Bruker AMX-500 and DRX-400 and chemical shifts (δ) were referenced to the solvent signal.

3. Results and discussion

3.1. Characterization and identification of the oxidation products of PZQ

A representative HPLC chromatogram from an oxidation mixture of PZQ catalysed by a MeP, [Fe(TFPP)Cl], after appropriate work-up, is shown in Fig. 2. In addition to *cis*- and *trans*-4′-OH, which were identified by comparing their retention times with those of authentic standards, other mono- and di-, and tri-hydroxylated products were also detected. LC–MS/MS analyses were used to confirm the identities of *cis*- and *trans*-4′-OH and to obtain additional structural information on the other products. Table 1 shows the fragments obtained from the MS/MS studies as well as the proposed structures for the products.

3.2. Mass spectral analysis of PZQ

The chromatogram in Fig. 2 shows a peak at 26 min, corresponding to PZQ, and its mass spectrum and the MS/MS spectra of the fragment ions are shown in Fig. 3A–D, respectively. A proposed MS fragmentation scheme to account for the ions observed is shown in Fig. 4. The parent ion (PI) of PZQ, at *m*/*z* 313, is formed by protonation of one of the amide groups [31]. The PZQ MS^2 spectrum, Fig. 3B, indicates the parent ion fragments to give species **A** (*m*/*z* 203), corresponding to the loss of cyclohexylketene. The MS^3 fragmentation of **A**

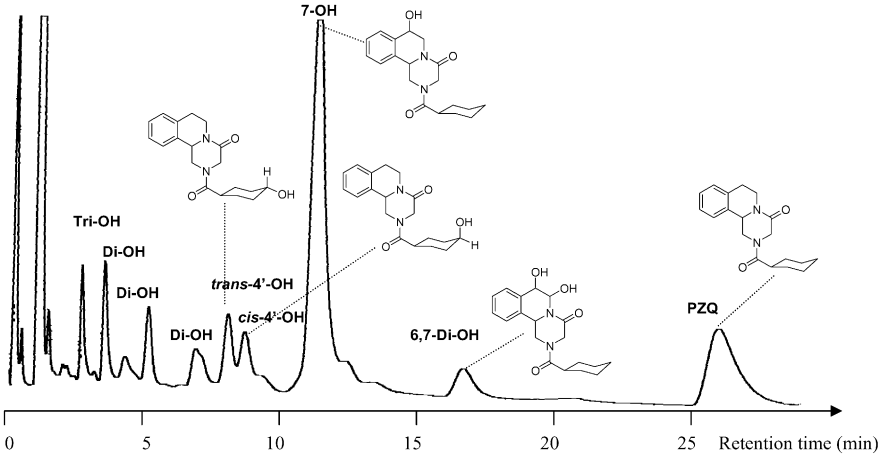


Fig. 2. HPLC chromatogram of PZQ and oxidation products obtained from the reaction of PZQ with PhIO, catalysed by Fe(TFPP)Cl in MeCN; reaction time = 45 min. Conditions: Lichrospher CN column, 5 μm particle size (125 mm × 4 mm), mobile phase: methanol:water (15:85 v/v), flow-rate of 1.0 mL/min, detection at 220 nm.

(Fig. 3C) shows an elimination of 29, assigned to $H_2C{=}NH$, arising from retrocyclisation to give **B** (*m*/*z* 174). A minor pathway gives the benzylic cation **D**, *m*/*z* 132, which is probably formed by loss of HNCO from fragment **B**. Finally, the MS^4 fragmentation of *m*/*z* 174 (Fig. 3D) shows the π-assisted loss of 28, assigned to CO, to give the benzylic cyclic cation **C** (*m*/*z* 146).

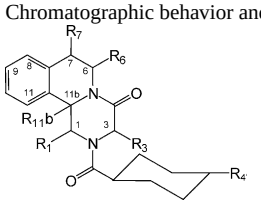
3.3. Products arising from oxidation on the cyclohexyl ring

The presence of fragment **A**, with *m*/*z* 203, in the mass spectra of the oxidation products was key to establish whether oxidation had occurred on the cyclohexyl ring or on the remainder of the molecule. Thus, the fragmentation patterns of three products, *cis*-4′-OH (*m*/*z* 329), *trans*-4′-OH (*m*/*z* 329) and a compound assigned to the 4′-ketone (*m*/*z* 327), each have in common fragment **A**, indicating that oxidation has occurred in the cyclohexyl ring (Table 1). The ketone which is only a minor product in the oxidation catalysed by $Fe(T2NPCl_8P)Cl$ probably arises by further oxidation of both *cis*- and *trans*-4′-OH. For none of the other products was fragment **A** observed in the mass spectra and it can be reasonably assumed that they involve oxidation of the hexahydropyrazinoisoquinoline part of PZQ.

3.4. Products arising from oxidation of PZQ on the hexahydropyrazinoisoquinoline skeleton

MS analysis of the major product (retention time, t_R = 12.0 min) in all the oxidation experiments shows it is monohydroxylated and isomeric with *cis*- and *trans*-4′-OH, *m*/*z* 329 (Table 1). However, its MS fragmentation does not give cation **A** but rather species with *m*/*z* 311, 201 and 173.

Table 1
Chromatographic behavior and mass spectrum fragmentation for the products obtained in the PZQ oxidation reactions



Retention time (min)	Oxidation product	Parent ion	Diagnostic fragments
3.1	Tri-OH	361	343 (**PI** − 18), 325 (343 − 18), 199 (dihydro **E**)
4.6	Di-OH	345	345 → 327 (**PI** − 18), 309 (327 − 18), 327 → 309 (327 − 18), 309 → 199 (dihydro **E**)
5.4	Di-OH	345	345 → 327 (**PI** − 18), 327 → 309 (327 − 18), 217 (**E** + 16), 217 → 189 (**F** + 16), 171 (dihydro **F**)
7.0	Di-OH	345	345 → 327 (**PI** − 18), 309 (327 − 18), 199, 199 → 171 (dihydro **F**)
7.5	*Trans*-4′-OH	329	329 → 311 (**PI** − 18), 203 (**A**), 203 → 174 (**B**), 132 (**D**), 174 → 146 (**C**)
8.6	*Cis*-4′-OH	329	329 → 311 (**PI** − 18), 203 (**A**), 311 → 203 (**A**), 203 → 174 (**B**), 132 (**D**)
9.8	4′-Ketone	327	327 → 203 (**A**), 203 → 174 (**B**), 174 → 146 (**C**)
12.0[a]	7-OH	329	329 → 311 (**PI** − 18), 201 (**E**), 173 (**F**)
16.0	6,7-Di-OH	345	345 → 327 (**PI** − 18), 327 → 217 (**E** + 16), 217 → 146 (dihydro **D** + 16)
26.0	PZQ	313	313 → 203 (**A**), 203 → 174 (**B**), 132 (**D**), 174 → 146 (**C**)

[a] Product analysed by LC–MS/MS using a triple quadrupole spectrometer; PZQ, $R_1 = R_3 = R_6 = R_7 = R_{11b} = R_{4'} = H$.

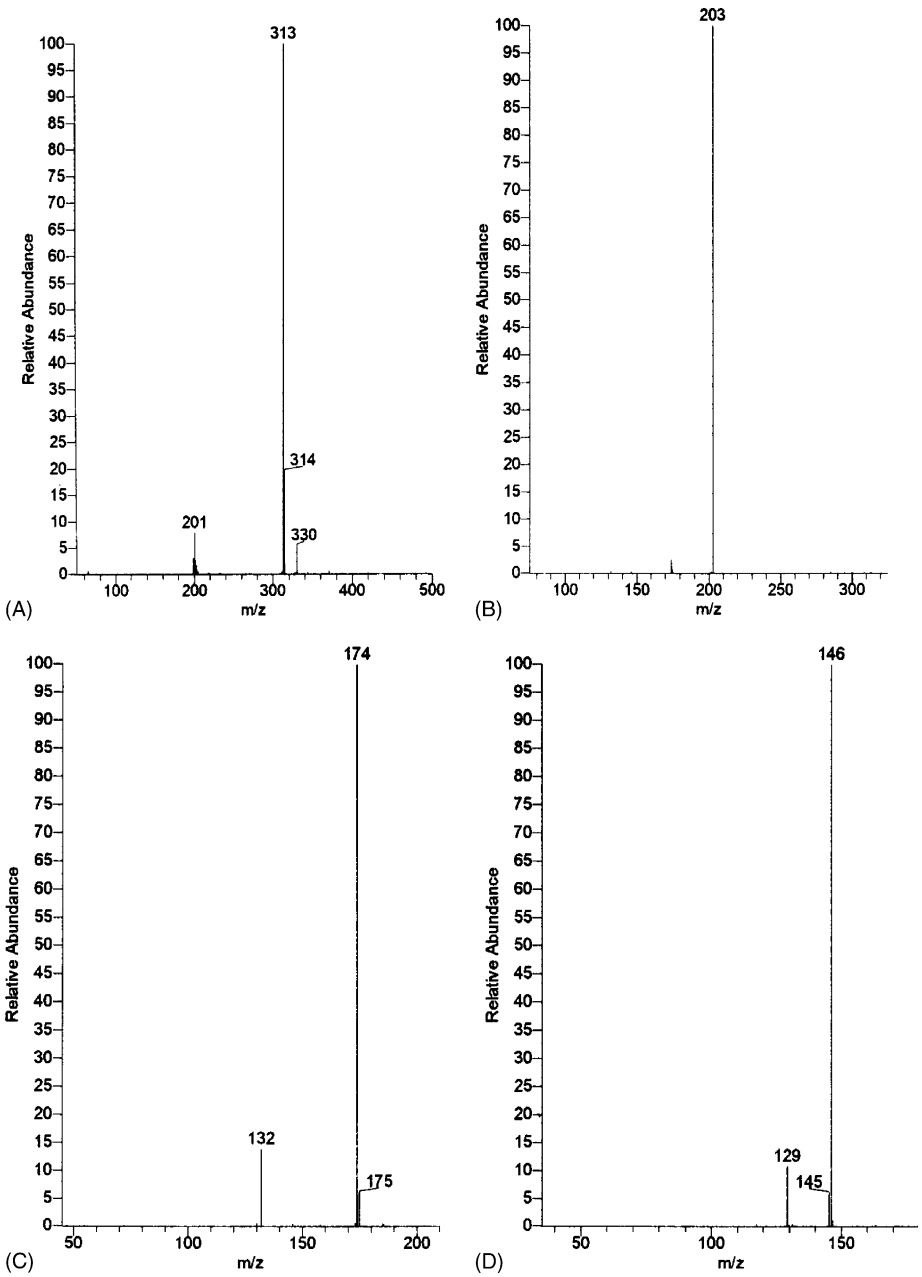


Fig. 3. Mass spectrum for PZQ and MS/MS fragmentations.

The aromatic ring can be eliminated as the site of hydroxylation since, based on the known selectivity of oxidations catalysed by metalloporphyrins, this is unlikely; furthermore, a phenolic product would not give fragment ions with the *m*/*z* values observed (e.g. loss of water, see below). Thus the hydroxylation must occur at either positions 1, 3, 6, 7 or 11b with fragments with *m*/*z* 311 and 201 arising from loss of water followed by cyclohexylketene, respectively. A proposed fragmentation pathway, assuming the product is 7-hydroxypraziquantel (7-OH) (see below), is shown in Fig. 5. Comparison of the MS/MS fragmentations of the equivalent cations **A** and **E**, in Figs. 4 and 5, respectively, shows that the double bond in the latter favours elimination of CO rather than the CH_2=NH loss observed with **A**. Whether this change in fragmentation pathway is due to electronic or structural differences between the two species is unclear.

At first sight, position 11b might be expected to be the preferred site for hydroxylation since the C–H bond is both tertiary and benzylic. However, the active oxidants in reactions catalysed by metalloporphyrins are considered to be electrophilic species, such as oxoiron(IV) porphyrin π radical cations ($O{=}FeP^{\bullet+}$), and oxidation at position 11b would be disfavoured by the electron-withdrawing effect of the α-amido-nitrogen. Likewise positions 1, 3 and 6 which are also α to an amido-nitrogen would be relatively unreactive. The remaining C–H bonds, at position 7, which are benzylic and

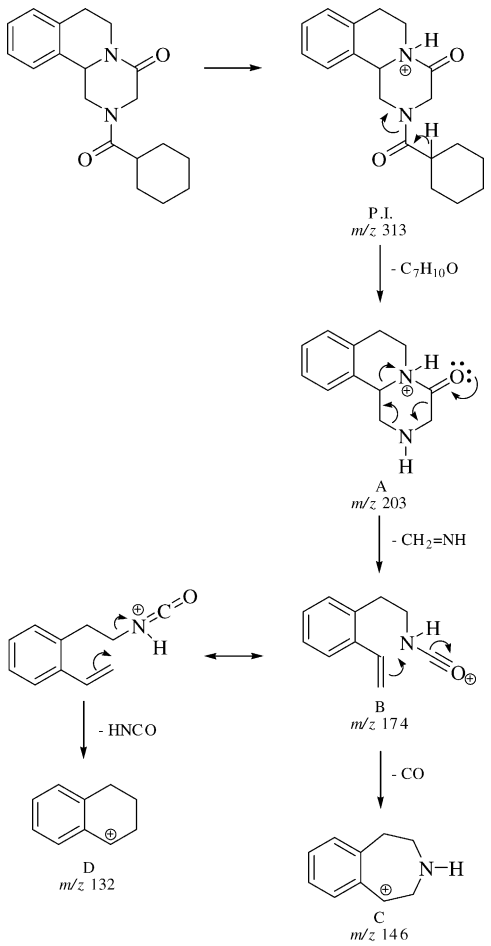


Fig. 4. Proposed MS/MS fragmentation routes for PZQ.

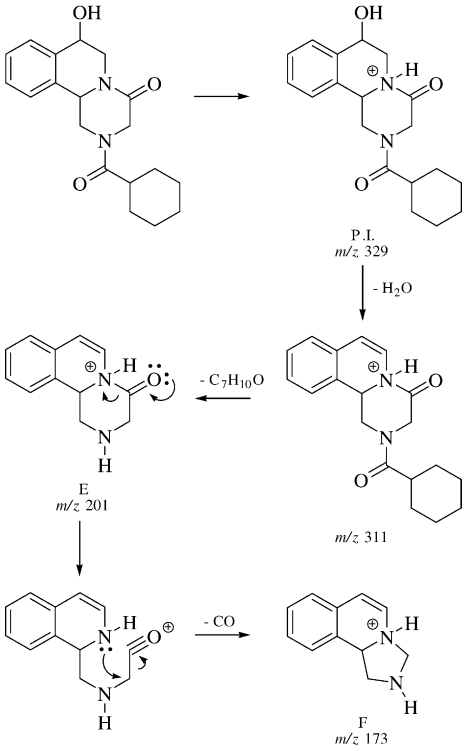


Fig. 5. Proposed alternative MS/MS fragmentation pathway for 6- or 7-OH derivatives of PZQ.

β to an amido-nitrogen are most likely to be the preferred point of attack. Furthermore, compared with 11b, position 7 is less sterically hindered and more accessible to the relatively hindered active oxidants in these systems. These arguments suggest that the major monohydroxylated product in all the oxidations is 7-OH with attack occurring on the less sterically hindered pseudo-axial C–H bond. To confirm the preferred oxidation at C-7, the reaction was scaled up and the product isolated by HPLC. The ^{1}H NMR data from the oxidation product were compared to those from of the PZQ and are presented in Table 2. 2D HMBC and HMQC analyses (Fig. 6) confirmed the proposed ^{1}H chemical shifts, indicative of oxidation at C-7. The HMBC spectrum showed ^{3}J correlations between δ 7.56 (H-8) and δ 81.2 (C-7) and between δ 4.75 (H-6a) and δ 166.8 (C-4) as well as ^{2}J correlations between δ 4.75 (H-6a) and δ 81.1 (C-7) and between δ 4.54 (H-3a) and δ 166.8 (C-4). The HMQC spectrum showed a ^{1}J correlation between δ 7.56 (H-8) and δ 129.1 (C-8). The presence of the OH group in C-7 could also be supported when δ H-8 values from the oxidation product (δ 7.56) and PZQ (δ 7.23) were compared. Based on the NMR results, it can be concluded that oxidation occurs preferably at C-7. Therefore, it also follows that the four dihydroxy-products are most likely to be 1,7-, 3,7-, 6,7- and 7,11b-dihydroxypraziquantel (1,7-Di-OH, 3,7-Di-OH, 6,7-Di-OH and 7,11b-Di-OH, respectively) arising from the further oxidation of 7-OH. This is supported by the time dependence of the reaction profiles and is discussed below.

Assignment of the structures of the four dihydroxypraziquantels on the basis of mass spectra is not unambiguous. However, it seems likely that the compound that elutes at

Table 2
^{1}H NMR shifts[a], multiplicity[b] and *J*-values[c] from PZQ[d] and 7-OH PZQ[d]

H	PZQ	7-OH PZQ
1a	5.17 (dd); 2.7; 13.4	5.22 (brd); 13.3
1b	2.85 (m)	2.79 (m)
3a	4.48 (d); 17.6	4.54 (d); 17.4
3b	4.08 (d); 17.6	4.07 (d); 17.4
6a	4.81 (m)	4.75 (m)
6b	2.85 (m)	3.14 (m)
7a,b	2.85 (m)	–
7	–	3.82 (m)
8	7.23 (m)	7.56 (brs)
9, 10, 11	7.23 (m)	7.24 (m)
11b	4.81 (m)	4.75 (m)
1′	2.29 (m)	2.37 (m)
2′–6′	1.60 (m)	1.71 (m)

[a] Values in ppm (δ).
[b] Values in Hz.
[c] Multiplicities: brs, broad singlet; d, doublet; dd, double doublet; brd, broad doublet; m, multiplet.
[d] 400 MHz; solvent: $CDCl_3$.

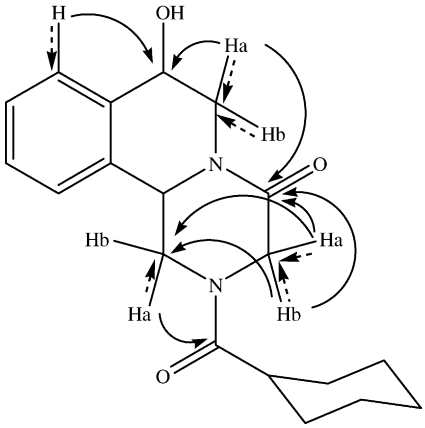


Fig. 6. Important correlations observed in HMBC (straight arrows) and HMQC (dashed arrows) spectra from 7-OH PZQ (500 MHz, $CDCl_3$).

16.0 min in Fig. 2 is 6,7-Di-OH, since in the MS/MS analyses it is the only dihydroxycompound that eliminates a single water molecule; the other three isomers show the loss of two waters. The elimination of water from 6,7-Di-OH product would give an enol which would tautomerise in preference to the loss of water.

MS/MS spectra of the dihydroxy-compounds with t_R of 4.6, 5.4 and 7.0 min show some similarities and differences. Thus they all give ions with *m*/*z* values 345, 327 and 309 from the parent ion and fragments from loss of one and two water molecules, respectively. Two of the compounds (t_R = 4.6 and 7.0 min) also show a fragment with *m*/*z* 199, corresponding **E** in Fig. 5, arising from the loss of cyclohexylketene from the cation with *m*/*z* 309. With the third dihydroxy-isomer, however, this was not detected and instead an ion with *m*/*z* 217 corresponding to the loss of one water molecule and a cylcohexylketene was observed. Unfortunately the data are insufficient to assign the HPLC peaks to specific dihydroxypraziquantel products.

Depending on the catalytic system and conditions, trihydroxylated products were also observed at 3.1 min (*m*/*z* at 361). MS characterization of these products was not carried out because this peak arose from a mixture of compounds.

4. The dependence of the oxidation of PZQ on the metalloporphyrin catalyst

The product yields were obtained by HPLC analyses of the reaction mixtures and were standardised against PZQ consumed, assuming that the parent compound and oxidation products have approximately the same molar absorptivity at 220 nm. Fig. 7A–D shows the product time profiles for the reactions of PZQ using Fe(T2TFMPP)Cl, Fe(TFPP)Cl, Fe(T2NPCl_8P)Cl and Fe(TMP)Cl, respectively in DCE. The main oxidation products of PZQ after 24 h, with all the metalloporphyrin catalysts are reported in Table 3. No products were detected from control experiments in the absence of a catalyst. This study shows that the MeP-catalysed oxidation of PZQ by PhIO leads to the formation of several products and their distribution depends on the meso-aryl substituents on the porphyrins. The initial rate of reaction was also calculated from the total yield of oxidation products obtained in the first 5 min of the reaction (Table 3).

The time dependence studies suggest that in all the systems PZQ is first hydroxylated in the 7-position and that this is further hydroxylated to Di-OH (mainly the product with t_R = 5.4 min) and Tri-OH products. The best yield of 7-OH was obtained using Fe(T2TFMPP)Cl as the catalyst, whereas Fe(TMP)Cl, Fe(T2NPCl_8P)Cl and Fe(TFPP)Cl led to higher yields of Di-OH and Tri-OH (Table 3). This is in marked contrast to the in vivo and in vitro metabolism of PZQ which favours hydroxylation of the cyclohexyl ring rather than the rest of the molecule, giving *cis*- and *trans*-4′-OH as the main metabolites [20–24]. Clearly, although the cytochrome P-450 models bring about the same oxidation processes as the

Table 3
Products obtained in the oxidation of PZQ with PhIO catalysed by Fe and Mn porphyrins in organic medium

Catalyst system		Initial reaction rate[a] (μM/min)	Conversion of PZQ (%)	Yield (%)[b]				
				7-OH	*Trans*-4′-OH	*Cis*-4′-OH	Di-OH[d]	Tri-OH
Fe(TPP)Cl	DCE	432	57	41	–	–	5	9
	MeCN	255	26	4	<1	<1	14	5
Fe(TFPP)Cl	DCE	805	56	7	2	9	1	34
	MeCN	534	60	20	1	1	21	10
Fe(T2NPCl_8P)Cl[c]	DCE	678	70	10	5	2	21	7
Fe(T2TFMPP)Cl	DCE	960	77	64	1	–	2	7
Fe(TMP)Cl	DCE	184	44	17	<1	1	17	8
Mn(TPP)Cl	DCE	218	51	38	<1	<1	1	1
	MeCN	81	54	45	–	–	1	5

Reactions carried out at room temperature, in air, for 24 h. PZQ, 6.0 mM; metalloporphyrin, 0.3 mM; iodosylbenzene, 9.0 mM; solvent, 2 mL DCE (dichloroethane); MeCN, acetonitrile.

[a] Initial reaction rates obtained from the extent of oxidation in the first 5 min of reaction.

[b] Yields after 24 h based on the amount of substrate used.

[c] The 6% of 4′-ketone.

[d] Di-OH is dihydroxy species at 5.4 min.

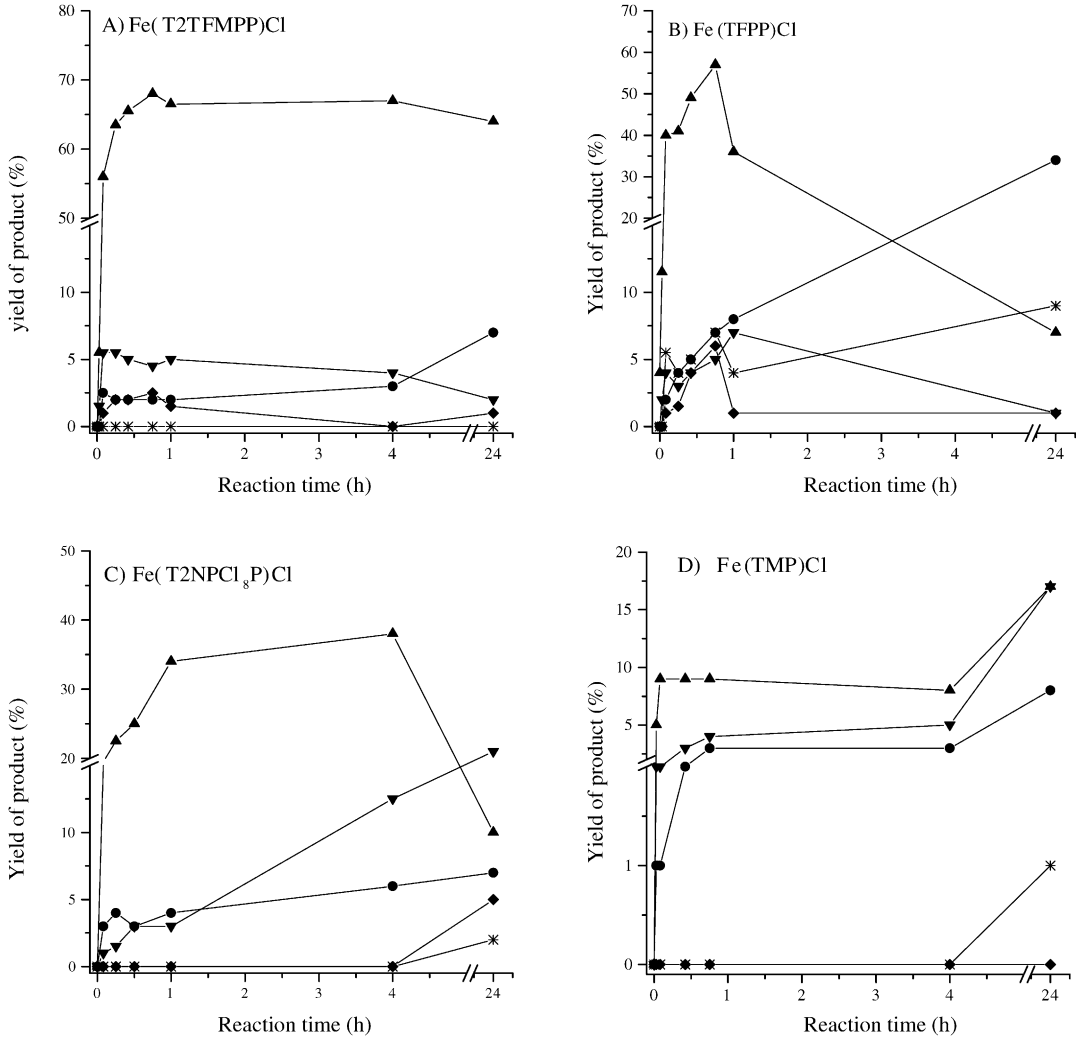


Fig. 7. Time dependence profiles of PZQ oxidations. Reaction conditions: PZQ, 6.0 mM; MeP, 0.3 mM; iodosylbenzene, 9.0 mM; DCE, 2 mL, at room temperature, in air. Reaction followed for 24 h. 7-OH (▲); *trans*-4′-OH (♦); *cis*-4′-OH (✳); Di-OH (t_R = 5.4 min) (▼); Tri-OH (●).

enzymes, the selectivities of both systems are quite different suggesting that with the latter the approach of PZQ towards active oxidant is more constrained by the protein matrix. Only with Fe(TFPP)Cl and Fe(T2NPCl$_8$P)Cl are significant amounts of *cis*- and *trans*-4′-OH formed and their further oxidation product, 4′-ketone, was only observed in appreciable quantities (6% yield) using Fe(T2NPCl$_8$P)Cl.

The oxidation of PZQ in DCE, using Fe(TPP)Cl resulted in an initial reaction rate of 432 μM product min^{-1} and 57% conversion of praziquantel after 24 h. The more electron-deficient iron porphyrin, Fe(T2TFMPP)Cl, led to an increase in the rate and consumption of praziquantel (960 μM product min^{-1}, 77% conversion), (Fig. 7A). Interestingly, the more reactive catalyst showed the greater selectivity, giving ∼65% of 7-OH. The Fe(TPP)Cl/PhIO system was the first successful model developed for cytochrome P-450 [32], although its catalytic activity is usually low due to the ready destruction of the porphyrin ring [9]. It was expected that using Fe(T2TFMPP)Cl would lead to a higher consumption of praziquantel, however, the increased selectivity was unexpected.

Using another electron-deficient catalyst, Fe(TFPP)Cl, also led to a high initial reaction rate (805 μM product min^{-1}, Fig. 7B) and a high initial selectivity for 7-OH (57% after 45 min). However, with this system a longer reaction time led, as expected, to the further reaction of 7-OH to give Tri-OH products (34% Tri-OH after 24 h). Changing the solvent with this catalyst to MeCN resulted in a decrease in the initial rate of reaction and less extensive further oxidation to polyhydroxylated products.

The very electron-deficient catalyst, Fe(T2NPCl$_8$P)Cl, resulted in a high conversion of PZQ and the initial selectivity for 7-hydroxylation was lost with further reaction (Fig. 7C). After 24 h, 70% of PZQ had reacted to give 10% 7-OH, 2% *cis*-4′-OH, 5% *trans*-4′-OH, 21% Di-OH, 6% 4′-ketone and 7% Tri-OH. These results indicate that with this robust iron porphyrin PZQ is extensively oxidized in a 24 h reaction. It is noteworthy that with this polyhalogenated metalloporphyrin,

oxidations with dioxygen as the oxidant are also a possible source of some of the oxidation products [33].

The sterically hindered, electron-rich catalyst, Fe(TMP)Cl, was, as expected, the least reactive of the iron(III) porphyrins (initial rate of 184 μM product min^{-1}). However, even with this catalyst, after 24 h, 44% of the praziquantel was consumed.

The initial reaction rates for PZQ oxidation catalysed by the Mn(TPP)Cl in DCE (218 μM min^{-1}) and MeCN (81 μM min^{-1}) (Table 3) were significantly lower than those of the corresponding FeP-catalysed reactions (432 and 255 μM min^{-1}, respectively). However, the overall product selectivity for both systems was similar.

5. Conclusions

The initial hydroxylation of PZQ by PhIO with all the catalytic systems [Fe(TPP)Cl, Fe(T2TFMPP)Cl, Fe(TFPP)Cl, Fe(T2NPCl_8P)Cl, Fe(TMP)Cl and Mn(TPP)Cl] occurs preferentially at the 7-position, giving 7-OH (Fig. 7). The reactions with catalysts Fe(TPP)Cl, Fe(T2TFMPP)Cl and Mn(TPP)Cl are selective toward 7-OH, even after 24 h, whereas with Fe(TFPP)Cl and Fe(T2NPCl_8P)Cl, a significant amount of this product is converted to the di- and trihydroxylated compounds (Fig. 7B and C) in the later stages of the reaction. This suggests that electronic factors are affecting the reactivity of the active oxidant leading to extensive further oxidation to di and tri-hydroxylated products. Interestingly the bulky *ortho*-substituents on the meso-aryl groups, such as trifluoromethyl- in Fe(T2TFMPP)Cl, nitro- in Fe(T2NPCl_8P)Cl and methyl in Fe(TMP)Cl, do not appear to have a significant effect on the product distribution.

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