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# A PULSE RADIOLYSIS STUDY OF FREE RADICALS FORMED BY ONE ELECTRON OXIDATION OF THE ANTIMALARIAL DRUG PYRONARIDINE.

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## ABSTRACT

Free radicals from one-electron oxidation of the antimalarial drug pyronaridine have been studied by pulse radiolysis. The results show that pyronaridine is readily oxidised to an intermediate semiiminoquinone radical by inorganic and organic free radicals, including those derived from tryptophan and acetaminophen. The pyronaridine radical is rapidly reduced by both ascorbate and caffeic acid. The results indicate that the one-electron reduction potential of the pyronaridine radical at neutral pH lies between those of acetaminophen (707 mV) and caffeic acid (534 mV). The pyronaridine radical decays by a second order process which DFT calculations (UB3LYP/6-31+G\* ) suggest is a disproportionation reaction. Important calculated dimensions of pyronaridine, its phenoxy and aminyl radical as well as the iminoquinone are presented.

**KEYWORDS:-** Pyronaridine, free radical, pulse radiolysis, oxidation, DFT antimalarial

## INTRODUCTION

The massive problem of endemic and drug-resistant malaria in tropical countries, especially that due to potentially fatal infections with *Plasmodium falciparum*, has led to the development of a wide range of antimalarial drugs [1]. Pyronaridine (Figure 1) was introduced as an antimalarial agent in the 1970's as a development of the existing antimalarial drug amodiaquine [2,3]. Although an effective antimalarial agent, amodiaquine has the potential to induce potentially fatal hepatotoxicity [4] and has now been withdrawn from use, except in the treatment of acute and resistant infections. Toxicity of amodiaquine results from oxidation of the aminophenol function, probably through the intermediate formation of the semiiminoquinone radical [5], and formation of a reactive iminoquinone [6-9]. In comparison, pyronaridine shows less clinical toxicity but retains some of the biochemical properties associated with amodiaquine toxicity such as oxidation by peroxidases, iminoquinone formation, glutathione depletion and cytotoxicity [6]. These reactions of the aminophenol function in antimalarial drugs reflect the well known toxicity of the same group within acetaminophen (N-acetylaminophenol, APAP) [10]. The pyronaridine molecule is normally formulated for clinical use as the tetrphosphate and Figure 1 indicates the pK<sub>a</sub> values for proton loss at the various sites in the molecule [11]. Pyronaridine is of particular interest since it has been reported to be active against multidrug-resistant strains of *Plasmodium* [12], inhibits *Plasmodium falciparum* topoisomerase II [13] and is being evaluated for world wide prophylactic use against all strains (drug resistant and sensitive) of malaria [1].

{FIGURE 1 }

The propensity for oxidation of the aminophenol function in both amodiaquine and pyronaridine is involved not only in toxic side effects but may also be involved in their modes of antimalarial action. In the intra-erythrocytic stage the malaria parasite degrades haemoglobin and utilises the released amino acids for its own catabolism [14]. The heme that is simultaneously released is potentially toxic to the parasite through reactions that induce oxidative stress and contribute to the pathophysiology of fatal cerebral malaria [15]. Biocrystallization of the free heme, which may be a spontaneous or enzymically promoted process [16], produces redox inactive  $\beta$ -hematin, also known as hemozoin or malaria pigment [17]. This eliminates oxidative stress due to free heme and allows the parasite to survive. Compounds that inhibit heme biocrystallization also possess antimalarial activity [18]. Recent results indicate that pyronaridine forms a complex with hematin that inhibits further biocrystallization [19]. This is now considered to be the mode of action rather than inhibition of parasite topoisomerase [13]. Such interactions appear to depend on a slipped offset interaction [3, 18] rather than the previously assumed  $\pi$ - $\pi$  interactions between drug and hematin, with the drug acting as a partial electron donor.

Pulse radiolysis studies have previously been used to study the redox behaviour of phenols and aminophenols and the properties of the phenoxyl and semi(imino)quinone radicals formed by one-electron oxidation [20,21]. Pulse radiolysis studies of both APAP [22] and amodiaquine [23] have been reported. The present pulse radiolysis study has been undertaken to assess the reactivity and reduction potential of the intermediate free radical formed by one-electron oxidation of pyronaridine.

## MATERIALS AND METHODS

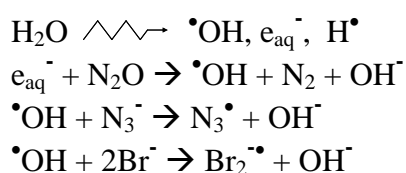
Pyronaridine tetrphosphate was a gift from Professor D Warhurst (London School of Hygiene and Tropical Medicine). The model compound *N*-(4-hydroxy-3,5-bis(pyrrrolidin-1-ylmethyl)phenyl)acetamide, SA48, was prepared by a published procedure [24]. Other chemicals used were of Analar grade and solutions were prepared in water obtained from a Millipore Milli Q unit or equivalent.

Pulse radiolysis was undertaken using the Daresbury linear accelerator with pulses of 12 MeV electrons [25]. The radiation dose was approximately 6 Gy per pulse with a pulse length of 200 ns. The solution was irradiated in a quartz capillary cell with an optical pathlength of 2.5 cm and dosimetry was performed with an air saturated solution of KSCN (10 mmol dm<sup>-3</sup>).

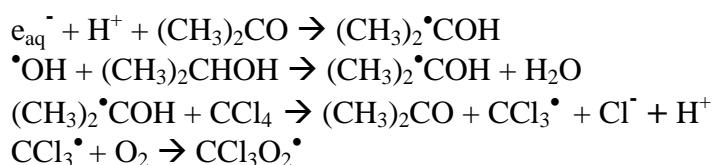
## RESULTS

### 1. Oxidation of pyronaridine by inorganic radicals

The oxidizing inorganic radicals N<sub>3</sub><sup>•</sup> (E<sub>o</sub>' 1.33 V [26]) and Br<sub>2</sub><sup>•-</sup> (E<sub>o</sub>' 1.66 V [26]) were produced by pulse radiolysis of N<sub>2</sub>O-saturated solutions containing the corresponding salt:-



In addition, the oxidizing trichloromethylperoxyl radical, CCl<sub>3</sub>O<sub>2</sub><sup>•</sup> (E<sub>o</sub>' 1.3 V [26]), was produced in solutions saturated with N<sub>2</sub>O/O<sub>2</sub> (4:1 v/v) containing acetone, propan-2-ol and CCl<sub>4</sub> :-



At pH < 7, both azidyl radical and dibromide radical anion reacted with pyronaridine to produce a product radical with absorption maxima in the measured difference spectrum at 540 and 630 nm (Figures 2A and 2B). The difference spectra also displayed bleaching in the region of the long wavelength absorption maximum of pyronaridine at 430 nm (Figure 2B). As the pH was increased the transient absorption spectrum resulting from oxidation by azidyl radicals (Figure 2A) was transformed to one with absorption maxima at 490 and 600 nm, with isobestic points at ca 555 and 630 nm. The transient spectra obtained by oxidation of pyronaridine by the trichloromethylperoxyl radical at pH 7.7 (Figure 2B) was very similar to that formed by reaction of azidyl radical at the same pH value. The similarity in transient absorption spectra at a particular pH value produced by the different oxidizing free

radicals indicates that reaction occurs by simple one-electron oxidation and that the shift in the spectrum with pH results from deprotonation of the radical.

{FIGURE 2}

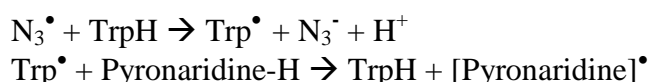
For comparison, the transient absorption spectra obtained by one-electron oxidation of the model compound 4-amino-2,6-bis(1-pyrrolidinylmethyl)-phenol (SA48, Figure 1) are shown in Figure 3. The transient spectra show maxima at 450 nm at pH 6.8 and 500 nm at pH 12.8, very similar to those observed previously for APAP [22] with the change resulting from deprotonation of the phenoxyl radical at the nitrogen atom with a  $pK_a$  of 11.1. Second order rate constants for reaction of oxidizing free radicals with pyronaridine and related compounds are shown in Table 1. Azidyl radicals were found to react with pyronaridine, amodiaquine, APAP and SA48 at neutral pH with rate constants in excess of  $10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ , close to the diffusion controlled limit and consistent with the high reduction potential for  $N_3^\bullet$ . In alkaline solution, the rate constants all increase due to deprotonation of the phenolic group ( $pK_a$  ca 10). However, measurements with pyronaridine were limited by it being virtually insoluble at  $\text{pH} > 10$ . Deprotonation of the pyrrolidine groups in pyronaridine and SA28 appear to have little effect on the rate of oxidation by the azidyl radical. At neutral pH, the second order rate constant for reaction of dibromide radical anion decreases by two orders of magnitude in the order pyronaridine > amodiaquine > APAP and is taken to reflect the both influence of the positively charged pyrrolidine groups and the lower reduction potentials for amodiaquine and pyronaridine compared with APAP (see below). The electrophilic trichloromethylperoxyl radical ( $\text{CCl}_3\text{O}_2^\bullet$ ) was also found oxidize pyronaridine very rapidly with a second order rate constant of  $1.8 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . These results suggest that the aminophenol moiety of pyronaridine is the principle site for reaction with oxidizing free radicals.

{FIGURE 3}

{TABLE 1}

## 2. Free radical interactions between pyronaridine and organic compounds

In aqueous solution at neutral pH, tryptophan was oxidised to the neutral indolyl radical ( $\lambda_{\text{max}}$  520 nm) by azidyl radicals. The indolyl radical from tryptophan is relatively oxidising ( $E_o'$  1.015 V [27]) and in the presence of pyronaridine was found to react, as shown by the formation of the characteristic 640 nm absorption of the pyronaridine radical at neutral pH as illustrated in Figure 4.



The second order rate constant for oxidation of pyronaridine by tryptophanyl radicals was found to be  $(8.0 \pm 0.4) \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  from the second order plot in the inset to Figure 4. The lower rate constant by over an order of magnitude compared with that determined with the inorganic radicals described above is due to the comparatively lower reduction potential of the tryptophanyl radical. The semiquinone free radical from APAP ( $E_o'$  707 mV [22]) was also found to

oxidise pyronaridine to the free radical with an apparent second order rate constant of  $\sim 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  as illustrated in Figure 5.

{FIGURE 4}

{FIGURE 5}

Ascorbate is highly reducing with  $E_o'$  ( $\text{Asc}^{\bullet-}, \text{H}^+/\text{AscH}$ ) 300 mV [20]. Accordingly in solutions containing pyronaridine and lower concentrations of ascorbate, the absorption at 640 nm of the pyronaridine radical formed by oxidation with azidyl radical at neutral pH was found to decay exponentially with first order rates increasing with ascorbate concentration as illustrated in Figure 6. The inset to Figure 6 shows the second order plot giving a second order rate constant of  $(1.4 \pm 0.1) \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . Caffeic acid ( $E_o'$  534 mV [28]) was similarly found to reduce the pyronaridine radical with a second order rate constant of  $(5.6 \pm 0.4) \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ .

{FIGURE 6}

These free radical interactions between species with the known reduction potentials demonstrate that the one electron [reduction](#) potential of the pyronaridine radical at neutral pH lies between that of APAP (707 mV) and caffeic acid (534 mV). It was not possible to undertake the usual experiments to determine transient equilibria with redox standards at high pH (>12) [20] due to the insolubility of pyronaridine under these conditions.

### 3. Decay of the pyronaridine radical

The radical described above formed from the one-electron oxidation of pyronaridine was unstable and decayed on a millisecond timescale. The decay of the difference spectrum at pH 6.7 is illustrated in Figure 7. The radical peaks at 540 and 640 nm decay and are replaced by a much less intense residual absorbance peaking in the region of 550 – 600 nm. At all wavelengths the decay could be fitted to a second order process plus a residual product absorbance. The decay at 640 nm is shown in the inset to Figure 7 and gave a second order rate constant for decay ( $2k_2$ ) of  $(45.2 \pm 0.1) \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . [This value is based on an extinction coefficient of  \$4,700 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}\$  at 640 nm, assuming quantitative oxidation of pyronaridine by azidyl radical.](#) The observed second order decay could be explained by either a radical termination (i.e. dimerization) or a disproportionation reaction. The second possibility appears to be more consistent with steric hindrance imposed by two methylene pyrrolidiny groups occupying both *ortho*-phenolic positions and with the residual absorption found during pulse radiolysis. In this case the product spectrum at 550 – 600 ns belongs to the iminoquinone that has been previously discussed in relation to the toxic side effects of this drug [6]. [Preliminary mass spectral investigations of the products from radiolysis of a nitrous oxide saturated solution of pyronaridine containing sodium azide have revealed the formation of the quinone \*\*3a\*\* \(Figure 8\).](#)

{FIGURE 7}

The structural details of pyronaridine and the one- and two-electron oxidised products were studied using density functional theory (DFT) methodology with the Gaussian03 program [29] to ascertain which route was thermodynamically favoured since most investigators have assumed that disproportionation is the favoured decay mode [Figure 8]. Similar combined pulse radiolytic – DFT approaches have proved successful in explaining the decay of *ortho*-substituted transient semi-iminoquinones involved in pheomelanogenesis [30].

{FIGURE 8}

For the DFT study, the input model for pyronaridine (**1**) was built in two parts. First the moiety based on the 7-chloro-2-methoxybenzo[*b*][1,5]naphthyridine heterocyclic system was attached via an NH substituent to a phenyl ring. There are only two variables, namely the C1-C2-N1-C3 and C2-N1-C3-C4 torsion angles between the aromatic rings (see Figure 9 for atom identification), and optimum values were obtained from previous calculations [31]. The second variable involves the orientations of the 4-amino-2,6-*bis*(pyrrolidin-1-ylmethyl)phenol fragment and we used experimental data from the CCDC [32]) in particular DUTTUH, DUTVAP, SOPBEE and VIMYEV which had very similar conformations. The resulting complete structural model was then fully optimised; subsequently, starting models for **2a**, **2b** and **3a** were built by removing the appropriate hydrogen atom(s) from the optimised 1 and then fully optimised using the UB3LYP/6-31+G\* methodology.

The enthalpies of the reactions  $\mathbf{1} - \text{H}^\bullet \Rightarrow \mathbf{2a}$  and  $\mathbf{1} - \text{H}^\bullet \Rightarrow \mathbf{2b}$  were then studied. All entities were geometry optimised and the enthalpies of reaction were calculated as 87.1 and 87.6 kcal mol<sup>-1</sup> respectively. Therefore, there is little significant difference between the energies of the phenoxyl and aminyl radicals. These values compare favourably with the free energy (76.7 kcal mol<sup>-1</sup>) for the found in the related molecule 4,6-di-*tert*-butyl-2-*tert*-butylimino-semiquinone in which the phenoxyl group is also sterically hindered [33].

{FIGURE 9}

In contrast, the enthalpy of the reaction  $\mathbf{1} - 2\text{H}^\bullet \Rightarrow \mathbf{3a}$  was calculated as 154.97 kcal mol<sup>-1</sup>. This can be compared favourably with the enthalpies for the formation of 2a + 2b, or indeed 2\*2a or 2\*2b which would have a combined enthalpy of ca 175 kcal mol<sup>-1</sup>. Thus the disproportionation reaction of radicals 2a and 2b to form 3a is favoured by ca 20 kcal mol<sup>-1</sup>. The structures of **1**, **2a**, **2b** and **3a** are shown in Figure 9 with important dimensions compared in Table 2.

{TABLE 2}

It will be noted that there is, as expected, a significant change in geometry when the hydrogen on N1 is removed in **3a**. The main change is a decrease in the N1-C3 bond length by 0.136 Å which is accompanied by a change in conformation as the C2-N1-C3-C4 torsion angle changes from 139.4° to -173.6° so that the arrangement around the C3-N1 double bond is approximately planar. This increase in conjugation will cause a corresponding shift in the product absorption maximum as observed

experimentally for the iminoquinone product in Figure 7. By contrast the C1-C2-N1-C3 torsion angle changes from 145.3° in **1** to 62.5° in **3a** twisting further away from planarity and concomitant with a slight increase in the C2-N1 bond length which has less double bond character increasing slightly from 1.375 to 1.385 Å. The structures of the radicals **2a** and **2b** show some variations. In **2a** the C-O7 bond length is 1.257 Å, close to that for a double bond; the C-C bonds in the six-membered ring starting adjacent to the carbonyl are 1.469, 1.376, 1.418, 1.421, 1.373, 1.466 Å showing that the ring loses some of its aromatic character but not all. Thus the comparable distances in the iminoquinone **3a** are 1.492, 1.349, 1.464, 1.466, 1.349, 1.493 Å. The N1-C3 in **2a** bond has slightly more double bond character than in **1** and the C2-N1-C3-C4 torsion angle increases to 172.2°. By contrast the torsion angles in **2b** are almost exactly the same as in **3a**. The N1-C3 bond length at 1.343 Å retains some double bond character but is still significantly longer than the 1.296 Å found in **3a**.

There is an additional change in that in **1**, there is an **intermolecular** hydrogen bond between O7-H and N5 with an O7...N5 distance of 2.718 Å. This is maintained in **2b** with a distance of 2.686 Å but with the removal of the hydrogen atom on O7, as in **2a** or **3a**, this distance increases to 3.361 and 3.338 Å respectively. These results are consistent with conclusions drawn in Section 1 that the aminophenol moiety is the reaction site with oxidizing free radicals. Radicals such as **2a** and/or **2b** could arise through interaction of oxidizable groups with free heme(II) released during the parasite mediated catabolism of haemoglobin [34], and could contribute, in part, to the antimalarial action of compounds containing the *para*-amino phenol moiety [3].

## CONCLUSIONS

Pyronaridine is readily oxidized to the radical species with a one-electron reduction potential at pH 7 for the radical species between ca 530 and 700 mV as defined by observed reactions with caffeic acid and the acetaminophen semiiminoquinone radical respectively. The result shows that pyronaridine is more readily oxidised than acetaminophen and accounts for the ease with which the drug is metabolised to toxic intermediates. The radical decays by a second order process which is suggested on the basis of spectral evidence and calculation to be a disproportionation resulting in formation of the iminoquinone that is responsible for reaction with thiols and protein conjugation *in vivo*.

## SUPPLEMENTARY MATERIAL

Coordinates of the optimised structures of **1**, **2a**, **2b**, **3a** [may be found in the Supplementary Material](#)

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**Table 1** – Second order rate constants (units,  $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$ ) for reaction at neutral pH (unless otherwise indicated) of some inorganic radicals with pyronaridine and related compounds. <sup>1</sup>From reference [23]. <sup>2</sup>From reference [22].

| Radical species                  | Pyronaridine   | Amodiaquine <sup>1</sup> | APAP <sup>2</sup>   | SA48  |
|----------------------------------|--|--------------------------|---|---|
| $\text{N}_3^\bullet$             | $3.5 \times 10^9$ (pH 6.8)<br>$5.2 \times 10^9$ (pH 9.2) | $1.2 \times 10^9$        | $3.8 \times 10^9$ (pH 7.1)<br>$5.8 \times 10^9$ (pH 11.1) | $2.4 \times 10^9$ (pH 6.8)<br>$3.2 \times 10^9$ (pH 12.8) |
| $\text{Br}_2^{\bullet-}$         | $3.0 \times 10^9$ (pH 6.8)                               | $2.1 \times 10^8$        | $2.5 \times 10^7$   | ----  |
| $\text{CCl}_3\text{O}_2^\bullet$ | $1.8 \times 10^9$ (pH 7.7)                               | ----                     | ----  | ----  |

**Table 2** - Dimensions in **1**, **2a**, **2b** and **3a**, distances, Å; torsion angles, °.

|             | <b>1</b> | <b>2a</b> | <b>2b</b> | <b>3a</b> |
|-------------|----------|-----------|-----------|-----------|
| C2-N1       | 1.375    | 1.388     | 1.401     | 1.385     |
| N1-C3       | 1.432    | 1.393     | 1.343     | 1.296     |
| C1-C2-N1-C3 | 145.3    | 135.5     | 63.4      | 62.5      |
| C2-N1-C3-C4 | 139.4    | 172.2     | -170.9    | -173.6    |
| O7...N5     | 2.718    | 3.361     | 2.686     | 3.338     |

## FIGURE LEGENDS

**FIGURE 1** Structures of the antimalarial drugs pyronaridine (malaridine; Drug 7351 or 4-[(7-chloro-2-methoxybenzo[b]-1,5-naphthyridin-10-yl)amino]-2,6-bis(1-pyrrolidinylmethyl)-phenol) and amodiaquine (4-(7-chloroquinolin-4-ylamino)-2-((diethylamino)methyl)phenol), together with that of the model compound SA48 (*N*-(4-hydroxy-3,5-bis(pyrrolidin-1-ylmethyl)phenyl)acetamide). The table indicates the  $pK_a$  values and sites of ionization in the pyronaridine molecule (from reference [11]).

**FIGURE 2** A: Transient spectra formed by oxidation of pyronaridine by azidyl radical in  $N_2O$ -saturated solutions containing pyronaridine ( $50 \mu\text{mol dm}^{-3}$ ) and sodium azide ( $0.1 \text{ mol dm}^{-3}$ ) at pH 5.4 [40  \$\mu\text{s}\$  after the pulse](#) (●), pH 6.7 [40  \$\mu\text{s}\$  after the pulse](#) (○), pH 7.6 [20  \$\mu\text{s}\$  after the pulse](#) (■) and pH 8.8 [20  \$\mu\text{s}\$  after the pulse](#) (▲). B:- Transient spectra from oxidation of pyronaridine ( $50 \mu\text{mol dm}^{-3}$ ) by  $\text{Br}_2^{\bullet}$  in  $N_2O$ -saturated solution containing KBr ( $0.1 \text{ mol dm}^{-3}$ ) at pH 6.8 [40  \$\mu\text{s}\$  after the pulse](#) (□), and by trichloromethylperoxyl radical at pH 7.7 [50  \$\mu\text{s}\$  after the pulse](#) (◆) in a solution saturated with  $N_2O/O_2$  (4:1 v/v) and containing propan-2-ol ( $3.3 \text{ mol dm}^{-3}$ ), acetone ( $1.4 \text{ mol dm}^{-3}$ ) and carbon tetrachloride ( $12 \text{ mmol dm}^{-3}$ ). [Dose = 9 Gy per pulse.](#) The [absorption spectrum](#) of [unirradiated](#) pyronaridine ( $50 \mu\text{mol dm}^{-3}$ ) at pH 8.8 is shown for comparison (solid line).

**FIGURE 3** Transient absorption spectra from one-electron oxidation of SA48 by azidyl radicals at pH 6.8 (◆) and at pH 12.8 (□).

**FIGURE 4** Oxidation pyronaridine by tryptophan radicals demonstrated by formation of the pyronaridine radical transient absorption at 640 nm in  $N_2O$ -saturated solutions at pH 7 containing  $\text{NaN}_3$  ( $0.1 \text{ mol dm}^{-3}$ ) and tryptophan ( $2.5 \text{ mmol dm}^{-3}$ ) (a); and together with pyronaridine at concentrations of 50 (b); 100 (c); 150 (d) and 200 (e)  $\mu\text{mol dm}^{-3}$ . [INSET: effect of tryptophan concentration on the first order rate for formation of the transient absorbance at 640 nm fir the above solutions.](#)

**FIGURE 5** The transient absorption change in an  $N_2O$ -saturated solution at pH 7 containing APAP ( $4 \text{ mmol dm}^{-3}$ ) and pyronaridine ( $1 \text{ mmol dm}^{-3}$ ) recorded at 640 nm.

**FIGURE 6** Reduction of the pyronaridine radical recorded at 640 nm by pulse radiolysis of  $N_2O$ -saturated solutions of pyronaridine ( $1 \text{ mmol dm}^{-3}$ ) and  $\text{NaN}_3$  ( $0.1 \text{ mol dm}^{-3}$ ) at pH 6.8 alone and with increasing concentrations of ascorbate (90, 180, 300 and 500  $\mu\text{mol dm}^{-3}$ ). **INSET:-** Second order plots for the reduction of pyronaridine radical absorption at 640 nm and pH 6.8 by ascorbate (■) and caffeic acid (□).

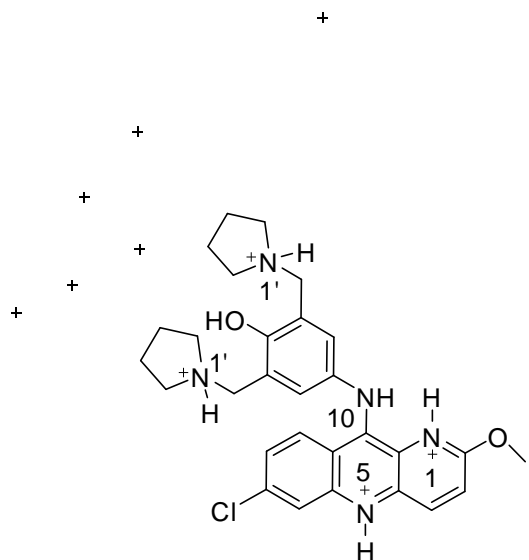
**FIGURE 7** Decay of the transient difference spectra on a millisecond timescale following pulse radiolysis of an  $N_2O$ -saturated solution of pyronaridine

( $50 \mu\text{mol dm}^{-3}$ ) containing sodium azide ( $0.1 \text{ mol dm}^{-3}$ ) and phosphate buffer ( $20 \text{ mmol dm}^{-3}$ ) at pH 6.7. Spectra are shown at delays after the pulse of  $50 \mu\text{s}$  (■),  $200 \mu\text{s}$  (□),  $500 \mu\text{s}$  (◆),  $1.5 \text{ ms}$  (○) and  $8 \text{ ms}$  (\*). Inset: decay of the transient absorption at  $640 \text{ nm}$ .

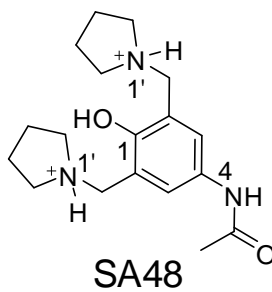
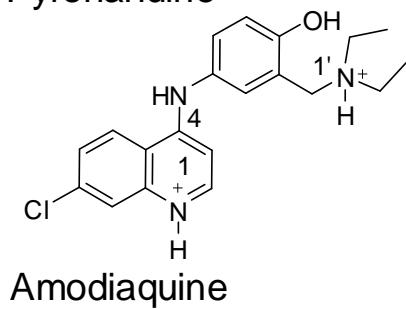
FIGURE 8 Disproportionation of Pyronaridine radicals. **1**: pyronaridine); **2a**: phenoxyl radical; **2b**: aminyl radical; **3a** Pyronaridine quinone: 4-(7-chloro-2-methoxybenzo[*b*][1,5]naphthyridin-10-ylimino)-2,6-bis(pyrrolidin-1-ylmethyl)cyclohexa-2,5-dienone.

FIGURE 9 Structure of pyronaridine **1**, the two radicals **2a** and **2b**, and the iminoquinone **3a**.



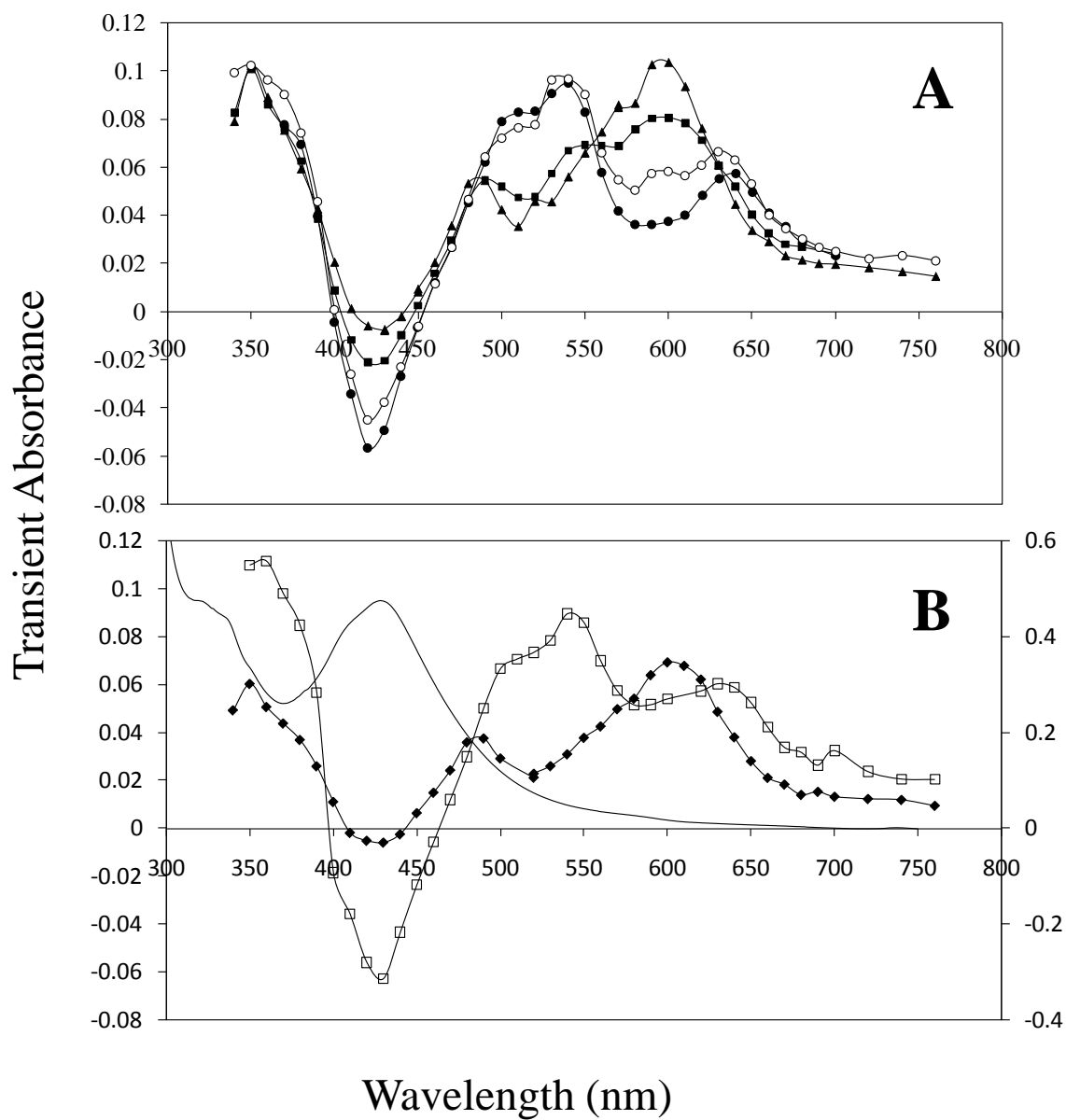


Pyronaridine

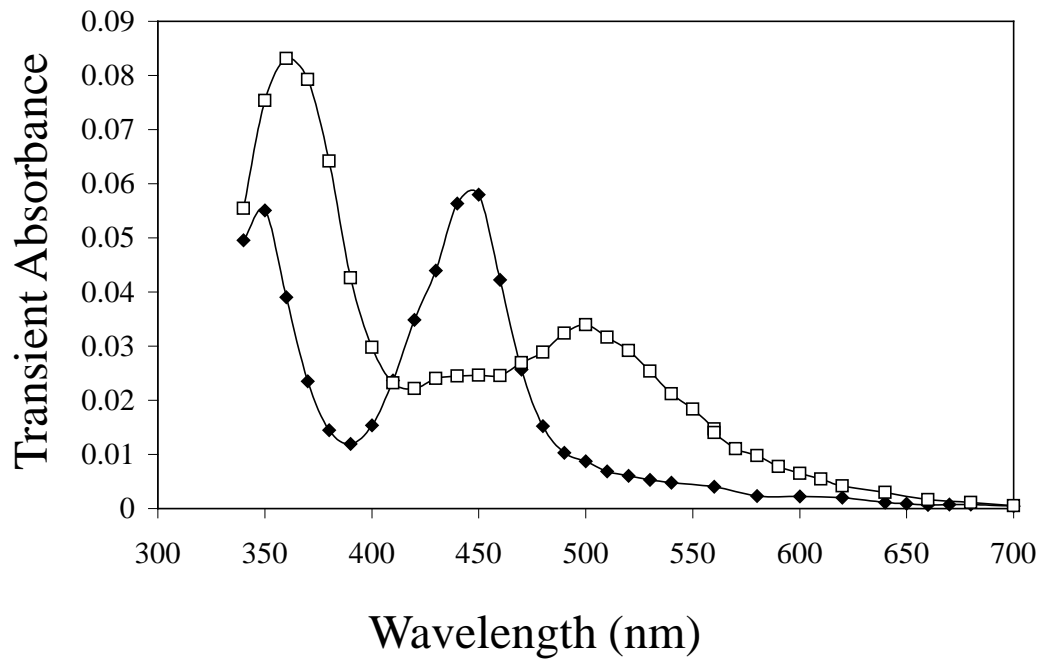


**FIGURE 1**

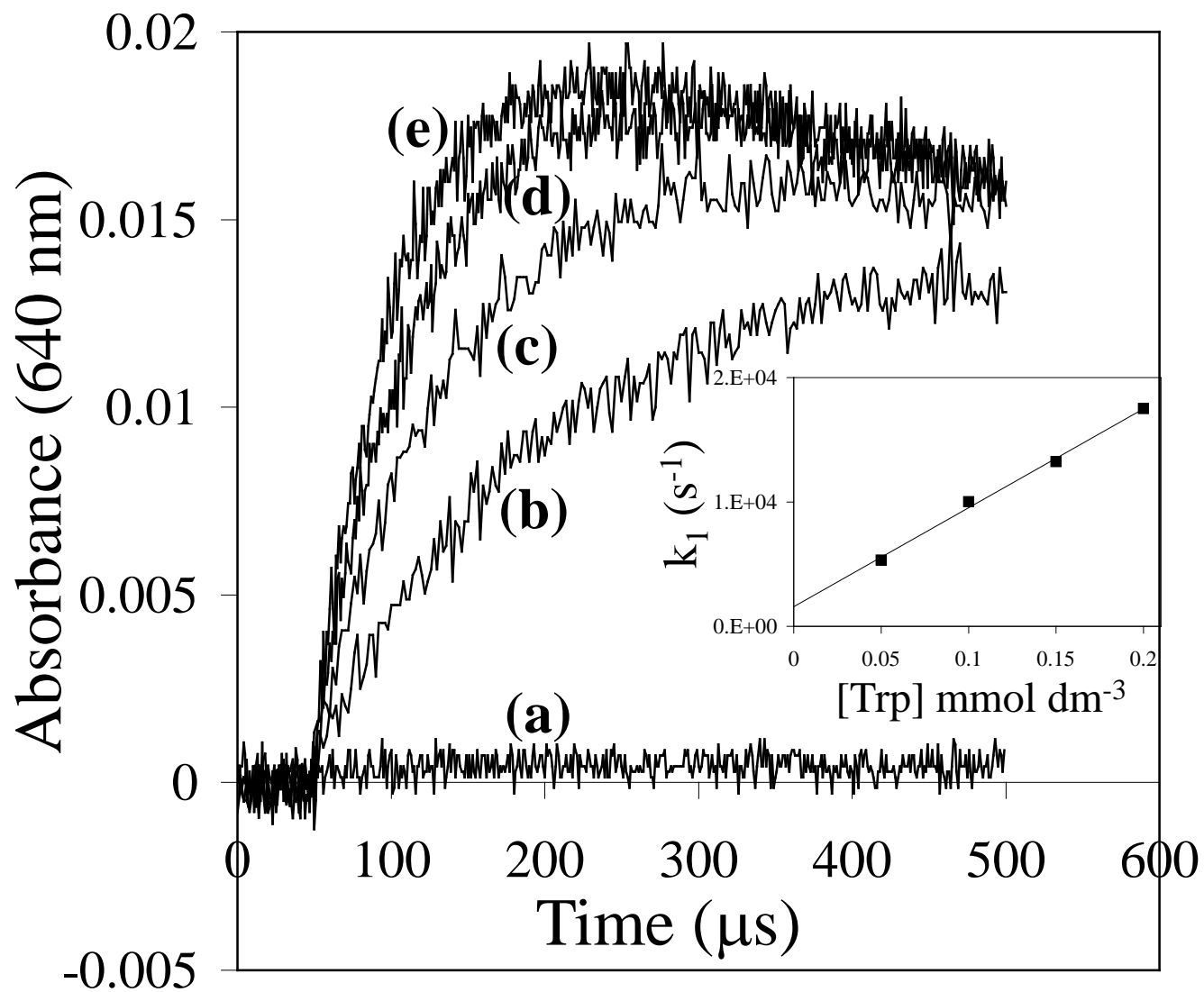
**FIGURE 2**



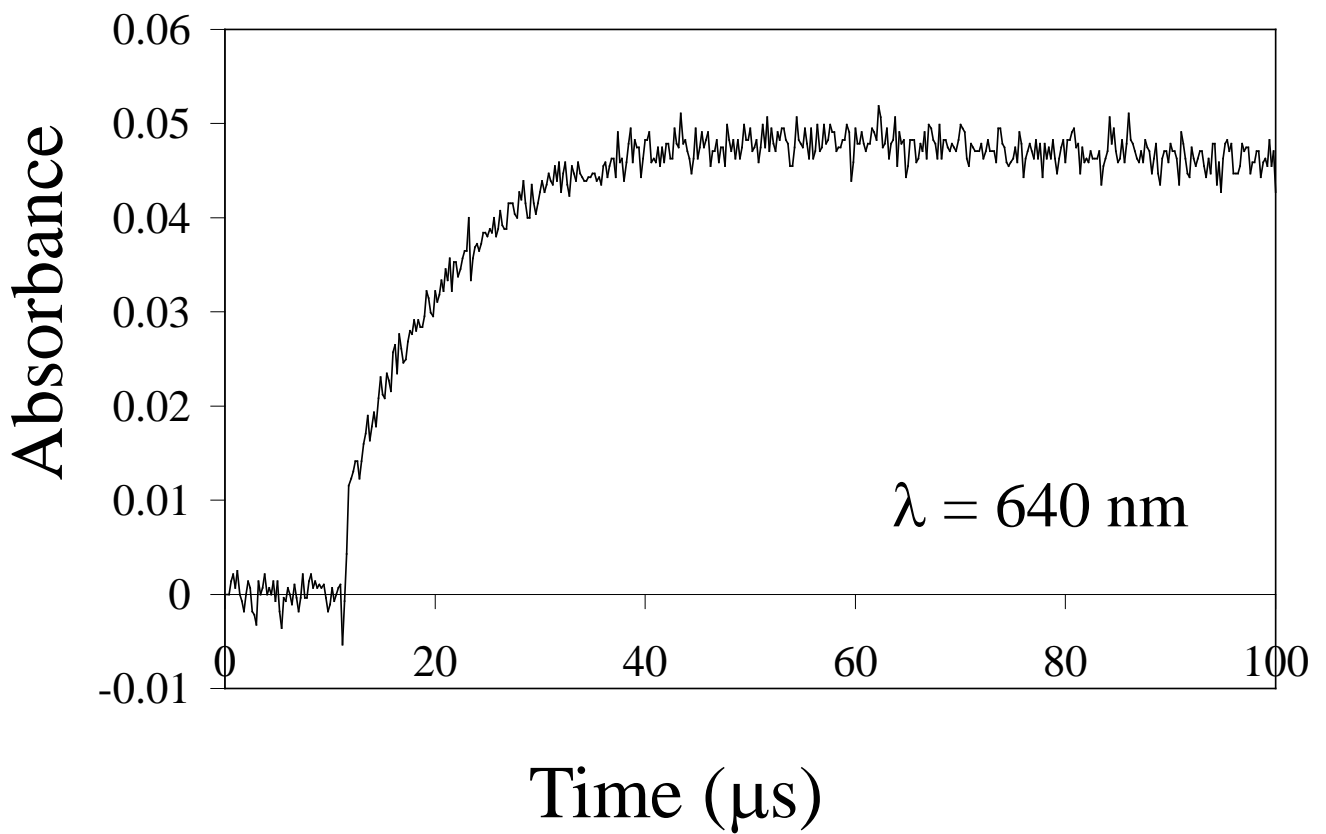
**FIGURE 3**



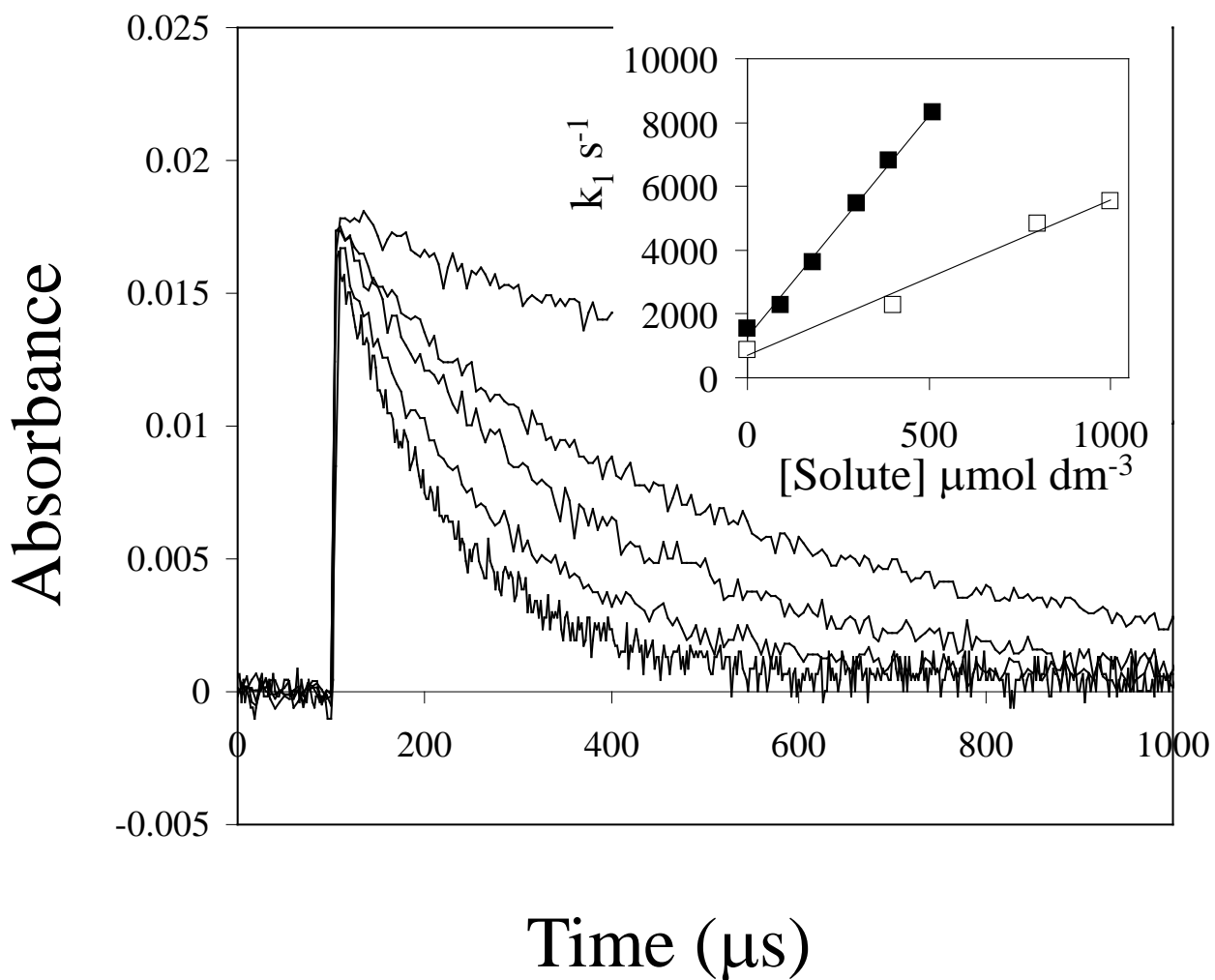
**FIGURE 4**



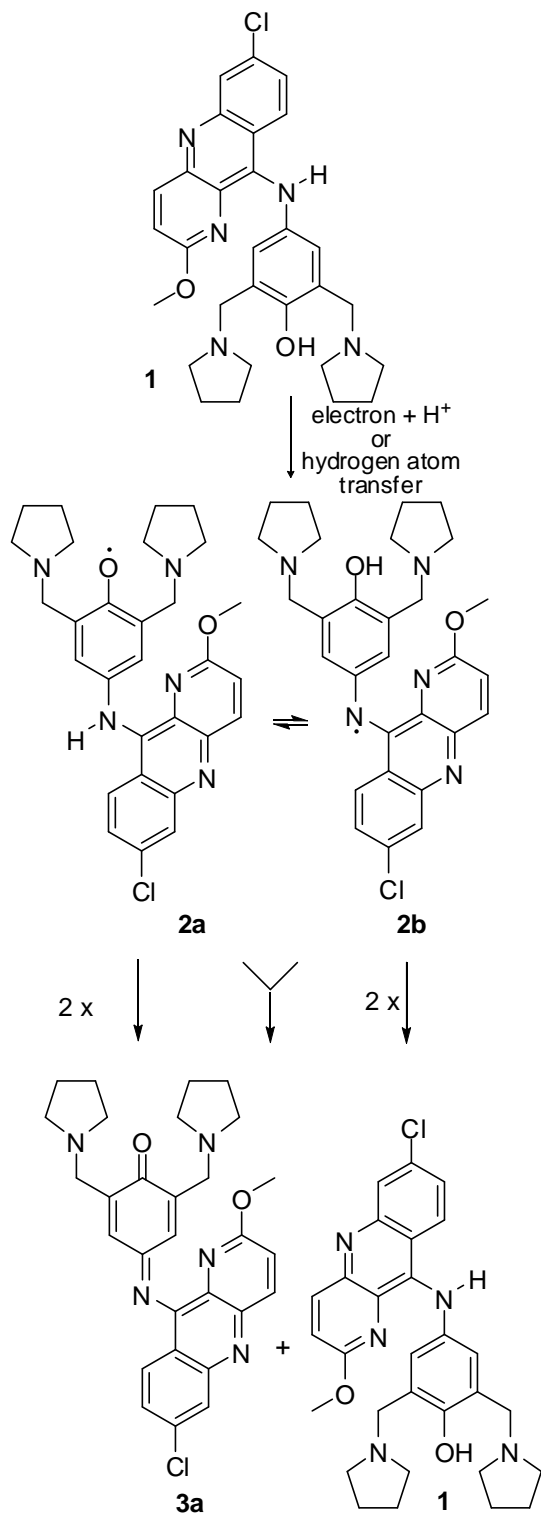
**FIGURE 5**



**FIGURE 6**

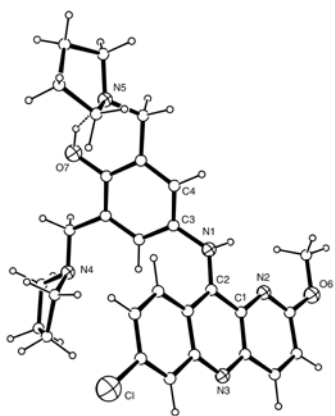




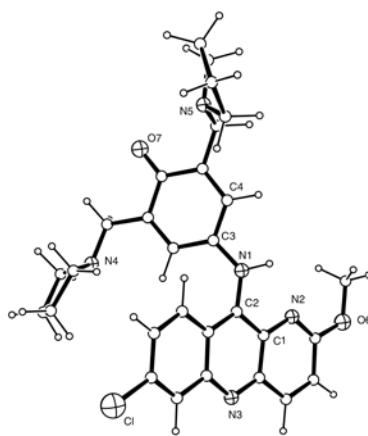


**FIGURE 8**

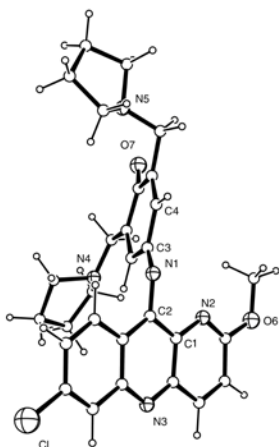




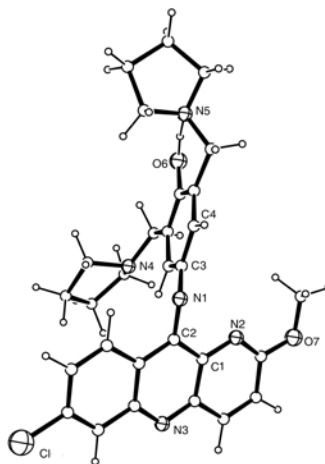
**1**



**2a**



**3a**



**2b**

**FIGURE 9**

# A PULSE RADIOLYSIS STUDY OF FREE RADICALS FORMED BY ONE ELECTRON OXIDATION OF THE ANTIMALARIAL DRUG PYRONARIDINE.

F.M.D.Ismail, M.G.B.Drew, S.Navaratnam and R.H.Bisby

## SUPPLEMENTARY MATERIAL

### A – RESULTS FROM DFT CALCULATIONS

### B – DATA FROM ELECTROSPRAY MASS SPECTROMETRY

### A -- RESULTS FROM DFT CALCULATIONS

**1**: pyronaridine [malaridine, Drug 7351 or Pyronaridine (4-[(7-chloro-2-methoxybenzo[b]-1,5-naphthyridin-10-yl)amino]-2,6-bis(1-pyrrolidinylmethyl)-phenol); **2a**: phenoxy radical; **2b**: aminyl radical; **3a** Pyronaridine quinone: 4-(6-chloro-2-methoxyacridin-9-ylimino)-2,6-bis(pyrrolidin-1-ylmethyl)cyclohexa-2,5-dienone.

=====

### STRUCTURE OF 1

SCF Done: E(RB+HF-LYP) = -2008.82200761 A.U. after 19 cycles

Standard orientation:

-----

| Center | Atomic | Atomic | Coordinates (Angstroms) |           |           |
|--------|--------|--------|-------------------------|-----------|-----------|
| Number | Number | Type   | X                       | Y         | Z         |
| 1      | 6      | 0      | -1.509253               | -1.872551 | -0.558419 |
| 2      | 6      | 0      | -0.387286               | -1.283390 | 0.029153  |
| 3      | 7      | 0      | 0.914835                | -1.648679 | -0.444302 |

-----

|    |    |   |           |           |           |
|----|----|---|-----------|-----------|-----------|
| 4  | 6  | 0 | 1.988642  | -0.814107 | -0.645392 |
| 5  | 6  | 0 | 3.280059  | -1.347494 | -0.377022 |
| 6  | 6  | 0 | 4.427375  | -0.525192 | -0.585063 |
| 7  | 7  | 0 | 4.378412  | 0.740434  | -1.026829 |
| 8  | 6  | 0 | 3.162077  | 1.237601  | -1.324834 |
| 9  | 6  | 0 | 1.924364  | 0.506009  | -1.178837 |
| 10 | 6  | 0 | 0.719081  | 1.124493  | -1.628784 |
| 11 | 6  | 0 | 0.711273  | 2.395977  | -2.149540 |
| 12 | 6  | 0 | 1.929630  | 3.120193  | -2.236021 |
| 13 | 17 | 0 | 1.884503  | 4.755687  | -2.885470 |
| 14 | 6  | 0 | 3.119777  | 2.570448  | -1.843117 |
| 15 | 1  | 0 | 4.057160  | 3.109081  | -1.927109 |
| 16 | 1  | 0 | -0.210974 | 2.849030  | -2.498270 |
| 17 | 1  | 0 | -0.213989 | 0.577550  | -1.574192 |
| 18 | 6  | 0 | 5.701490  | -1.113762 | -0.284407 |
| 19 | 6  | 0 | 5.773835  | -2.398709 | 0.166932  |
| 20 | 6  | 0 | 4.551169  | -3.128729 | 0.335374  |
| 21 | 8  | 0 | 4.697204  | -4.395912 | 0.787900  |
| 22 | 6  | 0 | 3.510022  | -5.171168 | 0.981271  |
| 23 | 1  | 0 | 2.967218  | -5.290155 | 0.037868  |
| 24 | 1  | 0 | 3.856499  | -6.139216 | 1.346074  |
| 25 | 1  | 0 | 2.853090  | -4.697314 | 1.717727  |
| 26 | 7  | 0 | 3.366223  | -2.639167 | 0.085831  |
| 27 | 1  | 0 | 6.714506  | -2.884784 | 0.404085  |
| 28 | 1  | 0 | 6.588938  | -0.505479 | -0.433022 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 29 | 1 | 0 | 1.229308  | -2.571169 | -0.151057 |
| 30 | 6 | 0 | -0.557493 | -0.399200 | 1.101605  |
| 31 | 6 | 0 | -1.829722 | -0.071387 | 1.572002  |
| 32 | 1 | 0 | -4.850872 | -0.681295 | 0.789554  |
| 33 | 6 | 0 | -2.036363 | 0.867935  | 2.747469  |
| 34 | 7 | 0 | -0.879055 | 1.697720  | 3.051599  |
| 35 | 6 | 0 | -0.677640 | 2.835314  | 2.146325  |
| 36 | 6 | 0 | 0.382474  | 3.694993  | 2.861697  |
| 37 | 6 | 0 | 0.233787  | 3.319205  | 4.365832  |
| 38 | 6 | 0 | -0.917350 | 2.297475  | 4.388387  |
| 39 | 1 | 0 | -0.802536 | 1.525980  | 5.158366  |
| 40 | 1 | 0 | -1.884573 | 2.811524  | 4.567973  |
| 41 | 1 | 0 | 1.155937  | 2.863912  | 4.741640  |
| 42 | 1 | 0 | 0.014402  | 4.186351  | 4.997518  |
| 43 | 1 | 0 | 1.386168  | 3.451529  | 2.499101  |
| 44 | 1 | 0 | 0.219525  | 4.761947  | 2.677598  |
| 45 | 1 | 0 | -0.364990 | 2.499405  | 1.153143  |
| 46 | 1 | 0 | -1.621817 | 3.406223  | 2.024751  |
| 47 | 1 | 0 | -2.261844 | 0.264536  | 3.638035  |
| 48 | 1 | 0 | -2.939148 | 1.481108  | 2.566324  |
| 49 | 1 | 0 | 0.304628  | 0.050717  | 1.584420  |
| 50 | 1 | 0 | -1.373365 | -2.555535 | -1.394331 |
| 51 | 8 | 0 | -4.186924 | -0.370001 | 1.463737  |
| 52 | 6 | 0 | -2.950788 | -0.680004 | 0.973908  |
| 53 | 6 | 0 | -2.796006 | -1.598760 | -0.084358 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 54 | 6 | 0 | -3.998528 | -2.331456 | -0.647400 |
| 55 | 7 | 0 | -5.171013 | -1.463386 | -0.822781 |
| 56 | 6 | 0 | -5.058673 | -0.480585 | -1.914438 |
| 57 | 6 | 0 | -6.491607 | 0.060770  | -2.066830 |
| 58 | 6 | 0 | -7.402070 | -1.090764 | -1.544789 |
| 59 | 6 | 0 | -6.418435 | -2.191709 | -1.104712 |
| 60 | 1 | 0 | -6.745240 | -2.744898 | -0.217730 |
| 61 | 1 | 0 | -6.260661 | -2.922802 | -1.920245 |
| 62 | 1 | 0 | -8.005743 | -0.751735 | -0.696820 |
| 63 | 1 | 0 | -8.092900 | -1.458577 | -2.309900 |
| 64 | 1 | 0 | -6.625897 | 0.962828  | -1.461439 |
| 65 | 1 | 0 | -6.707634 | 0.329520  | -3.105528 |
| 66 | 1 | 0 | -4.322192 | 0.289287  | -1.666530 |
| 67 | 1 | 0 | -4.725753 | -0.981112 | -2.843387 |
| 68 | 1 | 0 | -3.727393 | -2.827286 | -1.597338 |
| 69 | 1 | 0 | -4.297482 | -3.125679 | 0.052042  |

-----

**STRUCTURE OF 3a**

SCF Done: E(RB+HF-LYP) = -2007.57446479 A.U. after 18 cycles

Standard orientation:

-----

| Center | Atomic | Atomic | Coordinates (Angstroms) |          |           |
|--------|--------|--------|-------------------------|----------|-----------|
| Number | Number | Type   | X                       | Y        | Z         |
| 1      | 6      | 0      | -2.004275               | 0.756707 | -1.351592 |

-----

|    |    |   |           |           |           |
|----|----|---|-----------|-----------|-----------|
| 2  | 6  | 0 | -0.704737 | 0.305475  | -0.843573 |
| 3  | 7  | 0 | 0.344849  | 0.900972  | -1.317432 |
| 4  | 6  | 0 | 1.650443  | 0.523787  | -1.051105 |
| 5  | 6  | 0 | 2.165471  | -0.741911 | -1.428215 |
| 6  | 6  | 0 | 3.568822  | -0.982523 | -1.221603 |
| 7  | 7  | 0 | 4.420109  | -0.099137 | -0.687605 |
| 8  | 6  | 0 | 3.932531  | 1.110980  | -0.347591 |
| 9  | 6  | 0 | 2.555505  | 1.485855  | -0.527869 |
| 10 | 6  | 0 | 2.145214  | 2.799104  | -0.157125 |
| 11 | 6  | 0 | 3.038953  | 3.698044  | 0.372315  |
| 12 | 6  | 0 | 4.393277  | 3.308438  | 0.561270  |
| 13 | 17 | 0 | 5.506629  | 4.480690  | 1.252109  |
| 14 | 6  | 0 | 4.839813  | 2.059848  | 0.215752  |
| 15 | 1  | 0 | 5.873075  | 1.760499  | 0.352375  |
| 16 | 1  | 0 | 2.727312  | 4.700959  | 0.644889  |
| 17 | 1  | 0 | 1.109784  | 3.085366  | -0.311151 |
| 18 | 6  | 0 | 4.068691  | -2.271818 | -1.612480 |
| 19 | 6  | 0 | 3.229069  | -3.189956 | -2.165014 |
| 20 | 6  | 0 | 1.849210  | -2.827189 | -2.339967 |
| 21 | 8  | 0 | 1.081569  | -3.778939 | -2.912444 |
| 22 | 6  | 0 | -0.301247 | -3.469465 | -3.134945 |
| 23 | 1  | 0 | -0.399966 | -2.599602 | -3.791172 |
| 24 | 1  | 0 | -0.719075 | -4.358960 | -3.608243 |
| 25 | 1  | 0 | -0.807001 | -3.263018 | -2.187295 |
| 26 | 7  | 0 | 1.336787  | -1.678983 | -1.992203 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 27 | 1 | 0 | 3.560326  | -4.173103 | -2.482870 |
| 28 | 1 | 0 | 5.124510  | -2.473216 | -1.457466 |
| 29 | 6 | 0 | -0.693117 | -0.726684 | 0.195588  |
| 30 | 6 | 0 | -1.834567 | -1.288714 | 0.646918  |
| 31 | 6 | 0 | -1.867575 | -2.364317 | 1.710536  |
| 32 | 7 | 0 | -0.616082 | -2.508966 | 2.436463  |
| 33 | 6 | 0 | -0.398934 | -1.518566 | 3.499361  |
| 34 | 6 | 0 | 0.800158  | -2.077699 | 4.289868  |
| 35 | 6 | 0 | 0.776135  | -3.607644 | 3.999976  |
| 36 | 6 | 0 | -0.460403 | -3.805361 | 3.105713  |
| 37 | 1 | 0 | -0.341319 | -4.605568 | 2.366663  |
| 38 | 1 | 0 | -1.349180 | -4.045585 | 3.724082  |
| 39 | 1 | 0 | 1.683351  | -3.911995 | 3.468135  |
| 40 | 1 | 0 | 0.712675  | -4.208379 | 4.913039  |
| 41 | 1 | 0 | 1.737353  | -1.632171 | 3.941137  |
| 42 | 1 | 0 | 0.710180  | -1.852283 | 5.357450  |
| 43 | 1 | 0 | -0.216132 | -0.523733 | 3.080518  |
| 44 | 1 | 0 | -1.293469 | -1.445572 | 4.150837  |
| 45 | 1 | 0 | -2.098244 | -3.317626 | 1.215569  |
| 46 | 1 | 0 | -2.722153 | -2.174520 | 2.386647  |
| 47 | 1 | 0 | 0.258758  | -1.037241 | 0.613435  |
| 48 | 1 | 0 | -1.976212 | 1.552111  | -2.092979 |
| 49 | 8 | 0 | -4.168205 | -1.529925 | 0.331948  |
| 50 | 6 | 0 | -3.145826 | -0.909578 | 0.042702  |
| 51 | 6 | 0 | -3.169478 | 0.211313  | -0.943979 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 52 | 6 | 0 | -4.518447 | 0.672822  | -1.441030 |
| 53 | 7 | 0 | -5.171478 | 1.548899  | -0.474772 |
| 54 | 6 | 0 | -4.666606 | 2.921932  | -0.427164 |
| 55 | 6 | 0 | -5.704559 | 3.665358  | 0.436709  |
| 56 | 6 | 0 | -7.008690 | 2.826577  | 0.285736  |
| 57 | 6 | 0 | -6.617940 | 1.682136  | -0.666958 |
| 58 | 1 | 0 | -7.119468 | 0.734819  | -0.442506 |
| 59 | 1 | 0 | -6.857661 | 1.954661  | -1.716095 |
| 60 | 1 | 0 | -7.321431 | 2.425265  | 1.254901  |
| 61 | 1 | 0 | -7.842590 | 3.413893  | -0.112384 |
| 62 | 1 | 0 | -5.383895 | 3.694511  | 1.483011  |
| 63 | 1 | 0 | -5.830277 | 4.700889  | 0.103886  |
| 64 | 1 | 0 | -3.654526 | 2.954868  | -0.010833 |
| 65 | 1 | 0 | -4.624607 | 3.362350  | -1.446270 |
| 66 | 1 | 0 | -4.404528 | 1.166802  | -2.426734 |
| 67 | 1 | 0 | -5.155813 | -0.206426 | -1.577022 |

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**STRUCTURE OF 2a**

SCF Done: E(UB+HF-LYP) = -2008.18201945 A.U. after 33 cycles

Standard orientation:

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| Center | Atomic | Atomic | Coordinates (Angstroms) |   |   |
|--------|--------|--------|-------------------------|---|---|
| Number | Number | Type   | X                       | Y | Z |

-----



|    |    |   |           |           |           |
|----|----|---|-----------|-----------|-----------|
| 1  | 6  | 0 | -2.039188 | 1.479846  | -0.038479 |
| 2  | 6  | 0 | -0.765675 | 0.872080  | -0.209479 |
| 3  | 7  | 0 | 0.333363  | 1.559932  | 0.299203  |
| 4  | 6  | 0 | 1.659961  | 1.167283  | 0.416881  |
| 5  | 6  | 0 | 2.642688  | 2.111483  | 0.032456  |
| 6  | 6  | 0 | 4.020757  | 1.740717  | 0.128985  |
| 7  | 7  | 0 | 4.446841  | 0.553607  | 0.580251  |
| 8  | 6  | 0 | 3.515470  | -0.315939 | 1.018607  |
| 9  | 6  | 0 | 2.094813  | -0.056772 | 0.989121  |
| 10 | 6  | 0 | 1.221891  | -1.001424 | 1.605336  |
| 11 | 6  | 0 | 1.702966  | -2.158036 | 2.167405  |
| 12 | 6  | 0 | 3.096946  | -2.433530 | 2.123320  |
| 13 | 17 | 0 | 3.671327  | -3.938291 | 2.823521  |
| 14 | 6  | 0 | 3.983966  | -1.547851 | 1.572599  |
| 15 | 1  | 0 | 5.052038  | -1.734530 | 1.561351  |
| 16 | 1  | 0 | 1.032235  | -2.861092 | 2.650292  |
| 17 | 1  | 0 | 0.158391  | -0.795683 | 1.649794  |
| 18 | 6  | 0 | 4.981582  | 2.716007  | -0.302496 |
| 19 | 6  | 0 | 4.562099  | 3.931096  | -0.755378 |
| 20 | 6  | 0 | 3.151226  | 4.196367  | -0.790592 |
| 21 | 8  | 0 | 2.814103  | 5.421942  | -1.240428 |
| 22 | 6  | 0 | 1.420531  | 5.748925  | -1.313434 |
| 23 | 1  | 0 | 0.963987  | 5.712951  | -0.319258 |
| 24 | 1  | 0 | 1.386426  | 6.763759  | -1.711327 |
| 25 | 1  | 0 | 0.896335  | 5.056891  | -1.979400 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 26 | 7 | 0 | 2.232515  | 3.339719  | -0.425972 |
| 27 | 1 | 0 | 5.249004  | 4.702312  | -1.087699 |
| 28 | 1 | 0 | 6.033786  | 2.453324  | -0.247218 |
| 29 | 1 | 0 | 0.254093  | 2.572164  | 0.236418  |
| 30 | 6 | 0 | -0.658849 | -0.343470 | -0.931757 |
| 31 | 6 | 0 | -1.771485 | -0.930056 | -1.489833 |
| 32 | 6 | 0 | -1.691732 | -2.161284 | -2.365889 |
| 33 | 7 | 0 | -0.452725 | -2.915744 | -2.234482 |
| 34 | 6 | 0 | -0.400668 | -3.806150 | -1.068290 |
| 35 | 6 | 0 | 0.828169  | -4.696883 | -1.333202 |
| 36 | 6 | 0 | 0.995178  | -4.671513 | -2.881458 |
| 37 | 6 | 0 | -0.169203 | -3.794073 | -3.374823 |
| 38 | 1 | 0 | 0.076585  | -3.200830 | -4.262793 |
| 39 | 1 | 0 | -1.048638 | -4.423173 | -3.622065 |
| 40 | 1 | 0 | 1.955293  | -4.223027 | -3.156986 |
| 41 | 1 | 0 | 0.960497  | -5.671973 | -3.325005 |
| 42 | 1 | 0 | 1.716398  | -4.288163 | -0.841077 |
| 43 | 1 | 0 | 0.677049  | -5.709218 | -0.944156 |
| 44 | 1 | 0 | -0.326819 | -3.231926 | -0.139733 |
| 45 | 1 | 0 | -1.321162 | -4.422484 | -1.008665 |
| 46 | 1 | 0 | -1.785909 | -1.829875 | -3.409699 |
| 47 | 1 | 0 | -2.581596 | -2.790728 | -2.181676 |
| 48 | 1 | 0 | 0.310464  | -0.800412 | -1.102849 |
| 49 | 1 | 0 | -2.103713 | 2.398445  | 0.544251  |
| 50 | 8 | 0 | -4.093568 | -0.772588 | -1.956631 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 51 | 6 | 0 | -3.091739 | -0.299322 | -1.363779 |
| 52 | 6 | 0 | -3.181289 | 0.922367  | -0.558017 |
| 53 | 6 | 0 | -4.545730 | 1.533738  | -0.350549 |
| 54 | 7 | 0 | -5.295008 | 0.876826  | 0.714026  |
| 55 | 6 | 0 | -4.827865 | 1.141906  | 2.074884  |
| 56 | 6 | 0 | -5.950722 | 0.572505  | 2.963382  |
| 57 | 6 | 0 | -7.221090 | 0.628174  | 2.063932  |
| 58 | 6 | 0 | -6.718406 | 1.214738  | 0.731002  |
| 59 | 1 | 0 | -7.219101 | 0.794430  | -0.147892 |
| 60 | 1 | 0 | -6.868629 | 2.315351  | 0.712426  |
| 61 | 1 | 0 | -7.629293 | -0.375800 | 1.910857  |
| 62 | 1 | 0 | -8.015312 | 1.244362  | 2.498078  |
| 63 | 1 | 0 | -5.725547 | -0.460187 | 3.248503  |
| 64 | 1 | 0 | -6.063074 | 1.151499  | 3.885998  |
| 65 | 1 | 0 | -3.854832 | 0.674345  | 2.253967  |
| 66 | 1 | 0 | -4.713289 | 2.233846  | 2.250322  |
| 67 | 1 | 0 | -4.445712 | 2.623767  | -0.156597 |
| 68 | 1 | 0 | -5.119321 | 1.411813  | -1.275464 |

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**STRUCTURE OF 2b**

SCF Done: E(UB+HF-LYP) = -2008.18284432 A.U. after 27 cycles

Standard orientation:

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| Center | Atomic | Atomic | Coordinates (Angstroms) |   |   |
|--------|--------|--------|-------------------------|---|---|
| Number | Number | Type   | X                       | Y | Z |

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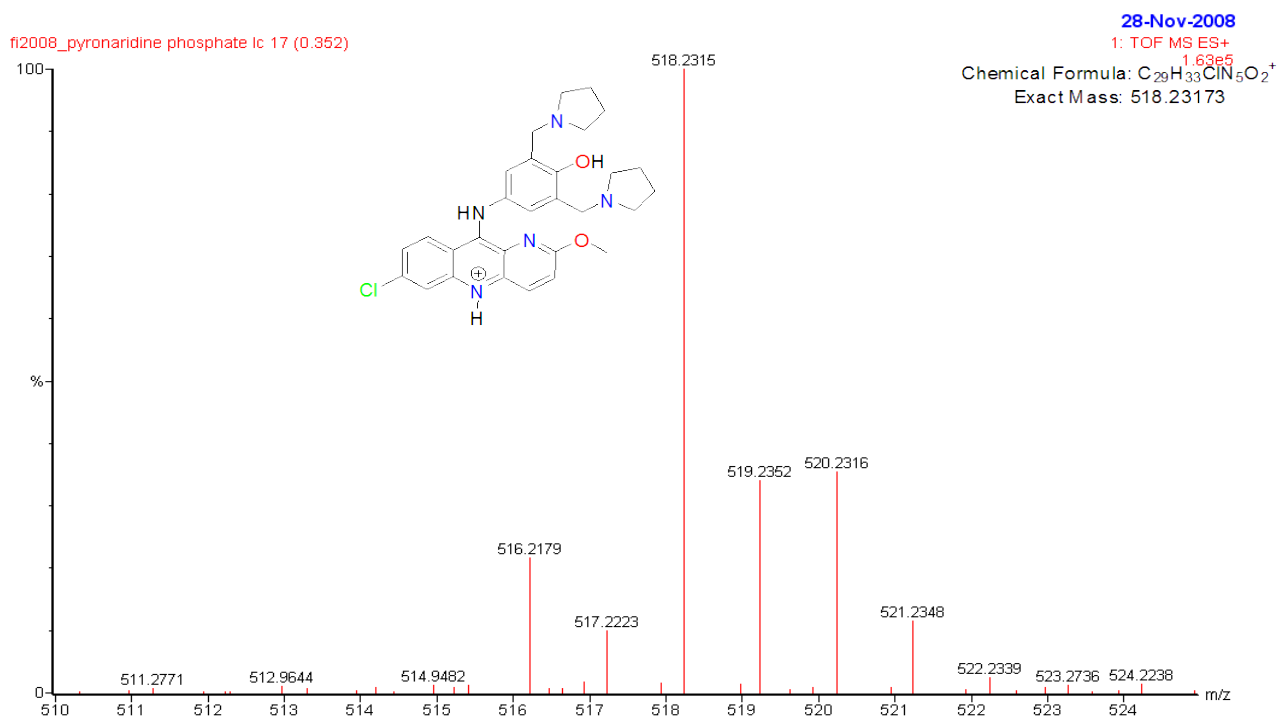
|    |    |   |           |           |           |
|----|----|---|-----------|-----------|-----------|
| 1  | 6  | 0 | 1.820453  | -0.267508 | -1.696406 |
| 2  | 6  | 0 | 0.561344  | -0.017170 | -1.049686 |
| 3  | 7  | 0 | -0.552109 | -0.337772 | -1.728757 |
| 4  | 6  | 0 | -1.819944 | 0.038382  | -1.265541 |
| 5  | 6  | 0 | -2.232396 | 1.380753  | -1.130600 |
| 6  | 6  | 0 | -3.602600 | 1.630727  | -0.770318 |
| 7  | 7  | 0 | -4.515329 | 0.677750  | -0.538516 |
| 8  | 6  | 0 | -4.116453 | -0.603612 | -0.663619 |
| 9  | 6  | 0 | -2.778089 | -0.977036 | -1.034834 |
| 10 | 6  | 0 | -2.441687 | -2.358211 | -1.131892 |
| 11 | 6  | 0 | -3.376587 | -3.332398 | -0.880428 |
| 12 | 6  | 0 | -4.697851 | -2.951232 | -0.515185 |
| 13 | 17 | 0 | -5.869977 | -4.225856 | -0.198002 |
| 14 | 6  | 0 | -5.070031 | -1.636262 | -0.404487 |
| 15 | 1  | 0 | -6.075733 | -1.343831 | -0.124332 |
| 16 | 1  | 0 | -3.125031 | -4.385133 | -0.958681 |
| 17 | 1  | 0 | -1.429465 | -2.627258 | -1.422458 |
| 18 | 6  | 0 | -3.998623 | 3.006885  | -0.660792 |
| 19 | 6  | 0 | -3.096829 | 3.999245  | -0.899311 |
| 20 | 6  | 0 | -1.757106 | 3.625769  | -1.261955 |
| 21 | 8  | 0 | -0.925153 | 4.668088  | -1.495880 |
| 22 | 6  | 0 | 0.418600  | 4.360602  | -1.888845 |
| 23 | 1  | 0 | 0.425528  | 3.775637  | -2.814095 |
| 24 | 1  | 0 | 0.898550  | 5.328426  | -2.039842 |

|    |   |   |           |           |           |
|----|---|---|-----------|-----------|-----------|
| 25 | 1 | 0 | 0.929488  | 3.793258  | -1.104833 |
| 26 | 7 | 0 | -1.336600 | 2.397819  | -1.374374 |
| 27 | 1 | 0 | -3.349809 | 5.052700  | -0.838753 |
| 28 | 1 | 0 | -5.030236 | 3.214451  | -0.392639 |
| 29 | 6 | 0 | 0.616294  | 0.455891  | 0.305824  |
| 30 | 6 | 0 | 1.809012  | 0.642767  | 0.974658  |
| 31 | 1 | 0 | 4.894431  | 0.077710  | 0.409137  |
| 32 | 6 | 0 | 1.841581  | 1.109879  | 2.420051  |
| 33 | 7 | 0 | 0.610985  | 0.809781  | 3.138442  |
| 34 | 6 | 0 | 0.454515  | -0.598046 | 3.517793  |
| 35 | 6 | 0 | -0.729622 | -0.586740 | 4.505471  |
| 36 | 6 | 0 | -0.753952 | 0.866523  | 5.065369  |
| 37 | 6 | 0 | 0.449179  | 1.550421  | 4.392209  |
| 38 | 1 | 0 | 0.287730  | 2.615675  | 4.190601  |
| 39 | 1 | 0 | 1.352028  | 1.458735  | 5.031327  |
| 40 | 1 | 0 | -1.684142 | 1.370898  | 4.784781  |
| 41 | 1 | 0 | -0.677595 | 0.899495  | 6.157169  |
| 42 | 1 | 0 | -1.666437 | -0.821116 | 3.989715  |
| 43 | 1 | 0 | -0.594950 | -1.335133 | 5.293329  |
| 44 | 1 | 0 | 0.276647  | -1.224680 | 2.637208  |
| 45 | 1 | 0 | 1.374263  | -0.973588 | 4.013309  |
| 46 | 1 | 0 | 1.987308  | 2.199269  | 2.434242  |
| 47 | 1 | 0 | 2.724609  | 0.679241  | 2.926904  |
| 48 | 1 | 0 | -0.308990 | 0.631276  | 0.845381  |
| 49 | 1 | 0 | 1.787167  | -0.621862 | -2.724518 |

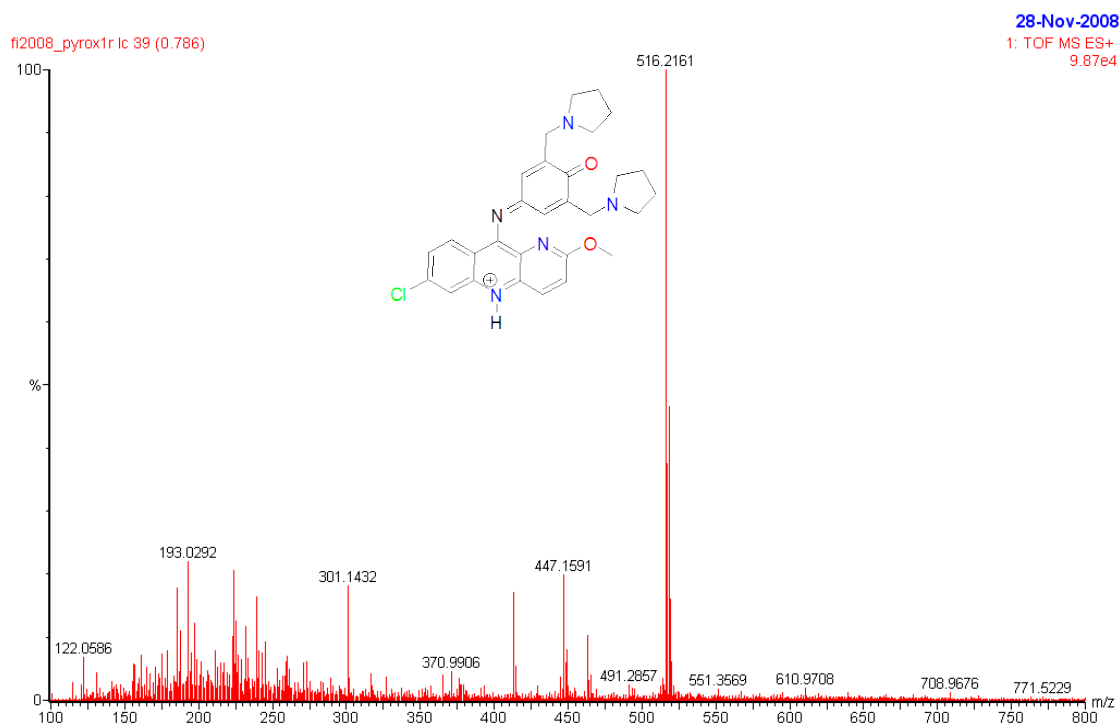
|    |   |   |          |           |           |
|----|---|---|----------|-----------|-----------|
| 50 | 8 | 0 | 4.193879 | 0.557517  | 0.949974  |
| 51 | 6 | 0 | 3.026061 | 0.392233  | 0.287650  |
| 52 | 6 | 0 | 3.025659 | -0.048771 | -1.064840 |
| 53 | 6 | 0 | 4.341852 | -0.199293 | -1.804824 |
| 54 | 7 | 0 | 5.363771 | -0.878427 | -0.995774 |
| 55 | 6 | 0 | 5.125686 | -2.324578 | -0.822313 |
| 56 | 6 | 0 | 6.453884 | -2.852471 | -0.250286 |
| 57 | 6 | 0 | 7.524712 | -1.837102 | -0.749394 |
| 58 | 6 | 0 | 6.724543 | -0.793032 | -1.551797 |
| 59 | 1 | 0 | 7.111364 | 0.226811  | -1.455246 |
| 60 | 1 | 0 | 6.715061 | -1.050369 | -2.627049 |
| 61 | 1 | 0 | 8.038550 | -1.367427 | 0.095301  |
| 62 | 1 | 0 | 8.289365 | -2.310371 | -1.373409 |
| 63 | 1 | 0 | 6.420676 | -2.872568 | 0.843788  |
| 64 | 1 | 0 | 6.655114 | -3.873600 | -0.587739 |
| 65 | 1 | 0 | 4.262302 | -2.503786 | -0.175198 |
| 66 | 1 | 0 | 4.912056 | -2.786241 | -1.803867 |
| 67 | 1 | 0 | 4.178132 | -0.731011 | -2.758801 |
| 68 | 1 | 0 | 4.737177 | 0.795350  | -2.054651 |

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## B – DATA FROM ELECTROSPRAY MASS SPECTROMETRY



**Supp Figure 1:** Positive ion high resolution electrospray mass spectrum of authentic protonated Pyronaridine (7-chloro-10-(4-hydroxy-3,5-bis(pyrrolidin-1-ylmethyl)phenylamino)-2-methoxybenzo[b][1,5]naphthyridin-5-ium). Note presence of peak with reduced intensity ascribed to spontaneous formation of iminoquinone during electrospray conditions.

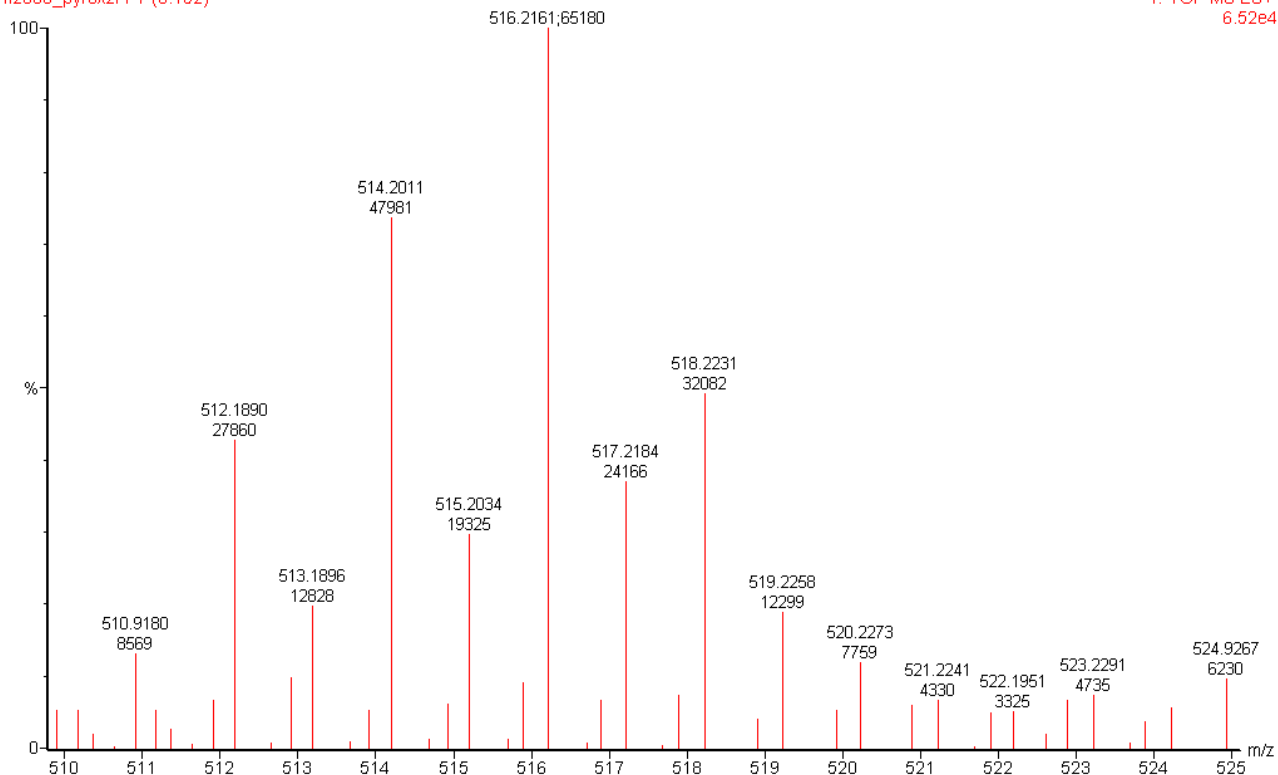


**Supp Figure 2:** Positive ion high resolution electrospray mass spectrum of authentic protonated iminoquinone (7-chloro-2-methoxy-10-(4-oxo-3,5-bis(pyrrolidin-1-ylmethyl)cyclohexa-2,5dienylideneamino) benzo[b][1,5]naphthyridin-5-ium)

28-Nov-2008

1: TOF MS ES+  
6.52e4

fi2008\_pyrox2r1 7 (0.152)



**Supp Figure 3:** Positive ion high resolution electrospray mass spectrum revealing presence of imnoquinone in pulse radiolysed sample.