Antimalarial Pharmacology and Therapeutics of Atovaquone

Gemma L Nixon[1*], Darren M Moss[1*], Alison E Shone[1], David Lalloo[1], Nicholas Fisher[1], Paul M O'Neill[2], Stephen A Ward[1] and Giancarlo A Biagini[1]**

[1]*Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*

[2]*Department of Chemistry, Liverpool University, Liverpool L69 7ZD, UK*

* These authors contributed equally to the study

** Author for correspondence. Tel:+441517053151, Fax:+441517053371, Biagini@liverpool.ac.uk

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Summary

Atovaquone is used as a fixed dose combination with proguanil (Malarone™), either for treating children and adults with uncomplicated malaria or as a chemoprophylaxis for preventing malaria in travellers. Indeed in the US, between 2009-2011, Malarone™ prescriptions accounted for 70% of all antimalarial pre-travel prescriptions. In 2013 the patent for Malarone™ will expire, potentially resulting in a wave of low-cost generics. Furthermore, the malaria scientific community has a number of antimalarial quinolones, with a related pharmacophore to atovaquone, at various stages of pre-clinical development. With this in mind, it is timely here to review the current knowledge of atovaquone, with the purpose of aiding decision making of clinicians and drug developers involved in the future use of atovaquone generics or atovaquone derivatives.

Introduction

Atovaquone is the end product of half a century of research by many groups who researched the antiparasitic properties of numerous structurally related compounds.[1-6] Currently Atovaquone is used as a fixed dose combination with proguanil (Malarone™), for the treatment of children and adults with uncomplicated malaria or as a chemoprophylactic agent for preventing malaria in travellers.[7, 8] Between 2009 and 2011 in the US Malarone™ accounted for 70% of all antimalarial pre-travel prescriptions.[9]

The development of atovaquone as an antimalarial drug began over 50 years ago when the outbreak of World War 2 caused substantial shortages in the supply of quinine.[10] Intense

efforts in America led to thousands of structurally diverse compounds being investigated, several of which were hydroxynaphthoquinones. Modest antimalarial activity when administered to ducks infected with *Plasmodium lophurae* resulted in a robust lead optimisation programme generating more than 300 quinones, some of which demonstrated greater activity than quinine in the duck assay. However, when administered to malaria patients these compounds were devoid of any activity due to poor absorption and rapid metabolism.[11, 12] Attempts to solve these problems and produce an orally active quinine were unsuccessful both then and when the problem was re-visited in the 1960s.[13] Research in the 1960s did however lead the development of Lapinone (1), which was given intravenously and had activity against *Plasmodium vivax* (Figure 1).[14]

The use of quinones as antimalarial agents was then reinvestigated in the 1980s by a group at the Wellcome Research Laboratories. More meaningful studies could be carried out at this time due to the development of test systems using the human parasite *Plasmodium falciparum in vitro* or in *Aotus* monkeys. The aim of this study was to design a quinone with good antimalarial activity against *P. falciparum* combined with good metabolic stability in humans. Several 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone analogues (2 and 3) were synthesised with the metabolically labile 4' position of the cyclohexyl ring substituted with a range of groups.[15, 16] Several of these quinones demonstrated a potency of ~1 nM towards *P. falciparum in vitro* but only atovaquone (4) was inert to human liver microsomes.[17, 18] The trans isomer of atovaquone is substantially more potent than corresponding cis isomer. The chemical synthesis of atovaquone was originally disclosed in 1991 in US patent No.4981874. This route gave a poor yield of 4% atovaquone calculated from only the last two steps (Figure 2A).[19]

Williams and Clark then published a variant of this methodology (Figure 2B) in which oxalate (11) was used to produce racemic compound (9) in 43% yield and the ester by-product (12) in 38% yield.[20] Conversion to atovaquone was then achieved as described in Figure 2A. The disadvantages of this process are the column chromatography required to separate (9) from (12) and the same poor yield problem will still prevail in the final two steps.

Both processes described so far also involve the use of silver nitrate, a heavy metal which can be difficult to remove and whose use is tightly regulated. The recently patented (WO 2010/001379) synthesis seen in Figure 2C offers an improved synthesis of atovaquone as it is higher yielding and doesn't involve the use of heavy metals.[21]

A common problem with all the routes so far is that large amounts of the potentially useful, yet significantly less potent cis isomer of atovaquone are disregarded as only the trans isomer is required. There are two literature procedures that address this problem. Reacting the cis isomer of atovaquone, atovaquone intermediates or isomeric mixtures thereof with a strong acid results in a clean epimerization to the corresponding trans isomer and thus to high yields of trans atovaquone.[22] Heating the cis isomer at reflux in organic solvent also carries out this transformation.[23]

With the patent relating to Malarone due to expire in 2013 the synthesis of atovaquone will be exploited to its full potential as generic versions of the drug are likely to become common place. This will in turn have a marked effect on the cost of goods as currently the high cost of atovaquone is frequently prohibitive in its use by the endemic population within countries affected by malaria. Increased availability and use of the drug will also have an effect on the clinical efficacy of atovaquone and factors such as access, sustainability, and resistance need to be considered.[24] Furthermore, the malaria scientific community has a number of antimalarial quinolones, with a related pharmacophore to atovaquone, at various stages of pre-clinical development.[25-30]

Pharmacodynamics

Mode of Action. Atovaquone is a competitive inhibitor of ubiquinol, specifically inhibiting the mitochondrial electron transport chain at the bc_1 complex.[31] Inhibition of bc_1 activity results in a loss of mitochondrial function.[32, 33] During the intra-erythrocytic stage of infection, a key role of the parasite mitochondrion is to provide orotate for pyrimidine biosynthesis through the activity of dihydroorotate dehydrogenase (DHODH). Consistent with this, inhibition of the bc_1 complex by atovaquone affects the concentrations of metabolites in the pyrimidine biosynthetic pathway.[34, 35] Indeed, transgenic *P. falciparum* parasites expressing ubiquinone-independent yeast DHODH have been shown to display an atovaquone-resistant phenotype.[36] In addition, a recent study suggests that a further cellular consequence of mitochondrial inhibition by atovaquone is the inhibition of purine biosynthesis.[37] Blood stage parasite death as a result of atovaquone is relatively slow compared to other antimalarials such as artemisinin and chloroquine.[25, 38] This feature appears to be consistent with other mitochondrial-acting antimalarials and is possibly due to the drug acting only on late trophozoites and not on the earlier “ring” stages.[25] Atovaquone is however active against liver stages, resulting in its utility as a prophylaxis drug, however it is not believed to be active against “dormant” hypnozoites.[8, 39]

Mechanism of Parasite Resistance to Atovaquone/Malarone™. Although the crystal structure of the *P. falciparum* cytochrome bc_1 complex is not available, details of atovaquone binding to cytochrome *b* have been elucidated based on studies performed on model organisms and molecular modelling. These studies, that include Electron Paramagnetic Resonance spectroscopy of the Rieske [2Fe-2S] cluster, site-directed mutagenesis of model organism cytochrome *b*, and gene sequencing of atovaquone-resistant *Plasmodium* species, demonstrate that atovaquone is most likely a competitive inhibitor of the parasite's cytochrome *b* quinol oxidation (Q_o) site (Figure 3).[28, 40]

Malarone™ drug failure has been associated with a mis-sense point mutation at position 268 in cytochrome *b*, exchanging tyrosine for serine (Y268S) or, less frequently, asparagine (Y268N).[41-45] Position 268 in cytochrome *b* is highly conserved across all phyla and is located within the "ef" helix component of the Q_o site which is putatively involved in ubiquinol binding. The resultant atovaquone-resistant growth IC_{50} phenotype of these mutants is some 1000-fold higher than susceptible strains, however this is accompanied by a ~40 % reduction in the V_{max} of the bc_1 complex, suggestive of a significant fitness cost to the parasite.[46]

It is well documented that atovaquone monotherapy gives rise to *de novo* resistance very rapidly.[47, 48] However, the underlying reason for this phenomenon has not been determined and, as discussed in the next section, may be partially explained by pharmacokinetic considerations (related to the physicochemical properties of atovaquone) as well as hitherto untested considerations related to the molecular target such as for example the effect of an increased mutation rate of mitochondrially-encoded genes such as cytochrome *b* compared to nuclear encoded genes.[49] Furthermore, it has been reported that an *in vitro* atovaquone resistant parasite line has been generated in the laboratory possessing wild-type cyt *b*.[50] The mechanism underpinning the parasite's atovaquone resistant phenotype in this strain remains to be elucidated.

Pharmacokinetics

The pharmacokinetic parameters of atovaquone in the currently utilised formulation (Malarone™, 250 mg atovaquone + 100 mg proguanil) have been determined (Figure 4).[51] Median atovaquone plasma AUC (h.µM), $t_{1/2}$ (h), C_{max} (µM) and t_{max} (h) were 295, 87.2, 3.74, 3.25, respectively, following single-dose and 254, 55.9, 13.8 and 4.00, respectively, upon reaching steady-state. The similar AUC values observed between single-dose and steady-state dosing suggests no unexpected accumulation of atovaquone following repeated

administration, although this may be due to saturation of plasma atovaquone concentrations and an increase in atovaquone concentrations in tissues cannot be ruled out.

Atovaquone IC_{50} against susceptible malaria *in vitro* is very low, ranging from 1 to ~3.5 nM.[31, 52, 53] This has resulted in the belief that atovaquone plasma concentrations (around 1-10 µM, see Figure 4) are sufficient to produce total suppression of malaria. However, atovaquone shows extremely high levels of plasma protein binding (>99.5%) and therefore the concentration of un-bound atovaquone is likely to be significantly lower.[54] Extrapolations of Pharmacokinetic-Pharmacodynamic dynamics using *in vitro* data should therefore be treated with caution.

At present, there are no established minimum effective plasma concentrations of atovaquone for malaria prophylaxis. However, a clear correlation between atovaquone steady-state plasma concentration and treatment success has been established in *Pneumocystis* pneumonia in patients with AIDS.[55] Atovaquone plasma concentrations of 10 to <15 µg / mL and 15 to <20 µg / mL resulted in 79% and 95% treatment success, respectively. Furthermore, there have been case reports of atovaquone treatment failure in antimalarial therapy that were not explained by drug resistance mutations, and patients with body weight >100 kg have a marked increased chance of treatment failure compared to <100 kg patients, both of which suggest drug concentration may be a factor in determining treatment failure.[42, 56, 57] The prediction of atovaquone therapy failure and resistance selection using drug concentration parameters has the potential to improve current patient therapy and an investigation determining a PK-PD relationship is warranted.

Absorption. Absorption of atovaquone shows dose-limitation, with maximum absorption observed using 750 mg tablets.[58] Poor drug solubility was suggested as the cause of this limit to absorption, and this led to the development of an atovaquone liquid suspension formulation, which showed improved *Pneumocystis* pneumonia treatment success compared to the tablet formation.[59]

The bioavailability of 750 mg atovaquone when taken with food was 23% in HIV-infected patients.[60] Combining data from six clinical trials, the inter-patient variability of atovaquone bioavailability is substantial and has been determined at 107%, which is likely due to the drug's low solubility and the effects of food.[60-62]

The oral absorption of atovaquone increased when taken with a high fat meal (2 slices of toast with 56 g butter, with 3.9-fold exposure compared to fasted), whereas a minimal-fat meal (2 slices of toast) had minimal impact on absorption.[62] Consequently, it is recommended that atovaquone be taken with a high-fat meal. However, a recent *in vitro* study showed that atovaquone IC_{50} increased 20-fold when serum used in the assay was taken from a subject recently given a high-fat meal, compared to serum from a fasted subject (0.5 ng / mL to 12 ng / mL, $p < 0.01$).[63] A correlation between high serum triglyceride concentrations and high atovaquone IC_{50} was observed, suggesting reduced free (unbound) atovaquone concentrations due to increased drug-fat binding. The clinical relevance of this finding is unknown, but the impact to atovaquone PK is likely to be transient and is unlikely to outweigh the benefit of increased atovaquone absorption.

Dissolution of atovaquone tablets increases in the presence of milk, and therefore the presence of milk in meals may increase atovaquone bioavailability in patients.[61] This may provide an alternative strategy to high-fat meals when aiming to maximise the bioavailability of atovaquone, although this has not been shown clinically.

Distribution. Atovaquone is highly bound to plasma protein (>99.5%) and shows high affinity for human serum albumin, although the low drug clearance rate suggests that atovaquone may also accumulate in tissues, where it is protected from biliary clearance.[54] In a study of atovaquone population pharmacokinetics, the volume of distribution of atovaquone was 7.98 L / kg, although individual values were markedly linked to body weight; volume of distribution shows a linear increase with increased patient body weight.[60]

Metabolism. Under normal conditions, there is no evidence that atovaquone is significantly metabolised in humans, or that metabolism is required for drug elimination. It may be possible that certain enzymes could be induced and therefore lead to increased atovaquone biotransformation, but this has not been demonstrated.

Elimination. Atovaquone pharmacokinetics is characterised by an extremely long elimination half life of around 50 to 84 hours.[58, 62, 64] Elimination is primarily via the liver, with almost undetectable amounts (<0.6%) of drug being eliminated via the kidney.[65] Over 90% of drug excreted in bile was in the parent form. Elimination of atovaquone is complicated by the possibility of enterohepatic recirculation of drug, which may help explain atovaquone pharmacokinetic profiles where reduction and then increases in drug concentration are seen with time.

In a study of atovaquone population pharmacokinetics, the oral clearance of atovaquone was increased in patients with higher body weight, with 60% increased clearance seen in an 80 kg patient compared to a 40 kg patient.[60] In the same study, the average oral clearance of atovaquone was higher in Oriental (8.49 L / h) and Malay (9.13 L / h) subjects compared to white (1-7.6 L / h) subjects.[60]

Drug interactions

Atovaquone is highly bound to plasma protein (>99.5%) and shows high affinity for human serum albumin.[54] Furthermore, the half life of atovaquone is long, ranging around 50 to 84 hours and the major limiting factor to atovaquone clearance is likely its plasma protein binding.[58, 62, 64] This suggests that any drug which reduces atovaquone plasma protein binding may potentially alter atovaquone tissue distribution and/or clearance. However, the authors can find no published articles investigating the drug-mediated displacement of atovaquone from plasma protein and the clinical impact of these interactions, and this area requires further research. The interaction observed between atovaquone and antiretrovirals, where efavirenz, lopinavir and ritonavir (all highly protein-bound drugs) reduced atovaquone plasma concentrations in HIV-infected patients, may involve atovaquone plasma-protein displacement, although this was not demonstrated.[66] This emphasises the importance of establishing the interactions between antimalarials, including atovaquone, and antiretrovirals.

The potential for atovaquone to displace other protein-bound drugs has been investigated. A case study has recently been published which describes a potential interaction between the anticoagulant drug warfarin and atovaquone, where the author suggests that atovaquone caused an increase in free warfarin concentrations to super-therapeutic levels.[67] A separate investigation found that atovaquone did not alter the pharmacokinetics of the antiepileptic drug phenytoin, another highly protein-bound drug which is susceptible to displacement interactions.[68] The evidence that atovaquone can compete with other drugs for plasma protein binding is lacking, although further investigations are required to fully understand this potential factor in atovaquone pharmacokinetics.

Atovaquone exposure is markedly decreased when taken concomitantly with the antibiotic drug rifampicin and therefore co-administration of atovaquone and rifampicin is not recommended.[69] The mechanism behind this interaction is not fully understood, although the ability of rifampicin to induce activity of metabolism enzymes and drug transporters is assumed to be responsible. However, no metabolite of atovaquone has been identified in

humans, and the impact of individual enzymes and transporters on atovaquone disposition is unclear.

There is evidence that atovaquone can inhibit cytochrome P450 enzymes, although data has been generated *in vitro* and the relevance to clinical drug interactions is unknown. Atovaquone inhibited the metabolism of 50 µM 7-benzyloxy-4-(trifluoromethyl)-coumarin (BFC) by recombinant CYP3A4, with an IC_{50} of 4.7 µM.[51] Similarly, sulfamethoxazole (SMX) metabolism by recombinant CYP2C9 was inhibited by atovaquone, with a K_i of 15 µM.[70] However, when atovaquone was pre-incubated with human serum and centrifuge-filtered to remove protein before use, no CYP2C9 inhibitory activity was observed. A recent case study described a HIV-infected female with a marked increase in plasma concentrations of antiretroviral drugs etravirine (+55%) and unboosted saquinavir (+274%) following atovaquone / proguanil prophylaxis.[71] In the same study, raltegravir plasma concentrations were unchanged following atovaquone/proguanil prophylaxis. The evidence that atovaquone/proguanil prophylaxis increases exposure of etravirine and saquinavir (both cytochrome P450 substrates) but not raltegravir (no affinity for cytochrome P450 enzymes) suggests atovaquone, proguanil, or indeed both drugs, may be inhibiting cytochrome P450 activity.[72-74]

Co-administration of atovaquone and the nucleoside reverse transcriptase inhibitor zidovudine increased the exposure (33% increase in $AUC_{0\text{-}8h}$, $p < 0.05$) and decreased the oral clearance (25% reduction, $p < 0.05$) of zidovudine in HIV-infected patients.[75] Furthermore, patients taking atovaquone showed a trend towards lower zidovudine-glucuronide plasma concentrations (6% reduction in $AUC_{0\text{-}8h}$, $p < 0.1$) and a significant decrease in the ratio between zidovudine-glucuronide and plasma concentrations (30% reduction, $p < 0.05$). Atovaquone exposure was unchanged when co-administered with zidovudine.

The atovaquone-mediated 33% increase in zidovudine exposure is in itself unlikely to cause increased hematologic toxicity, although caution is advised in patients taking additional drugs with similar toxicity profiles to zidovudine.[75] Also, increased zidovudine plasma concentrations and reduced zidovudine glucuronidation may potentially lead to increased formation of the cytochrome P450-mediated zidovudine metabolite, 3'-amino-3'-deoxythymidine, which shows seven-fold higher toxicity in bone marrow cells compared to the parent drug.[76]

The increased exposure and decreased clearance of zidovudine suggests that atovaquone is inhibiting the glucuronidation of zidovudine. The primary enzyme involved in zidovudine glucuronidation is uridine 5'-diphospho-glucuronosyltransferase (UGT) 2B7.[77] Therefore, clearance of UGT2B7 substrates, such as the anti-HIV drug efavirenz, may also be influenced by atovaquone and further investigations are warranted in this area.[77]

Atovaquone did not alter the exposure of the anti-HIV protease inhibitor drug indinavir in healthy volunteers.[78] Indinavir is a substrate of the drug efflux transporter, ABCB1, and the absence of any effect of atovaquone on indinavir pharmacokinetics suggests that atovaquone is not altering the activity of ABCB1, although this has not been confirmed.[79]

Safety and Toxicology

Atovaquone has been found to be generally well tolerated and causes few side effects. Adverse events are generally mild and include rash, fever, vomiting, diarrhoea, abdominal pain and headache. Indeed, overdoses as large as 31,500 mg have been reported causing little or no symptomatology.[80]

Conclusion

Despite the extensive use of Atovaquone-Proguanil, there remains a considerable knowledge gap concerning its pharmacology. The rollout of generics following the expiry of the patent will undoubtedly see an increase in Atovaquone-Proguanil usage that will be closely followed by an increase in the treatment failures. Clearly, if the community is to manage this issue and develop improved derivatives, more effort needs to be placed into understanding the PK-PD mechanisms underpinning Atovaquone-Proguanil treatment failure.

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Transparency Declaration

None to declare.

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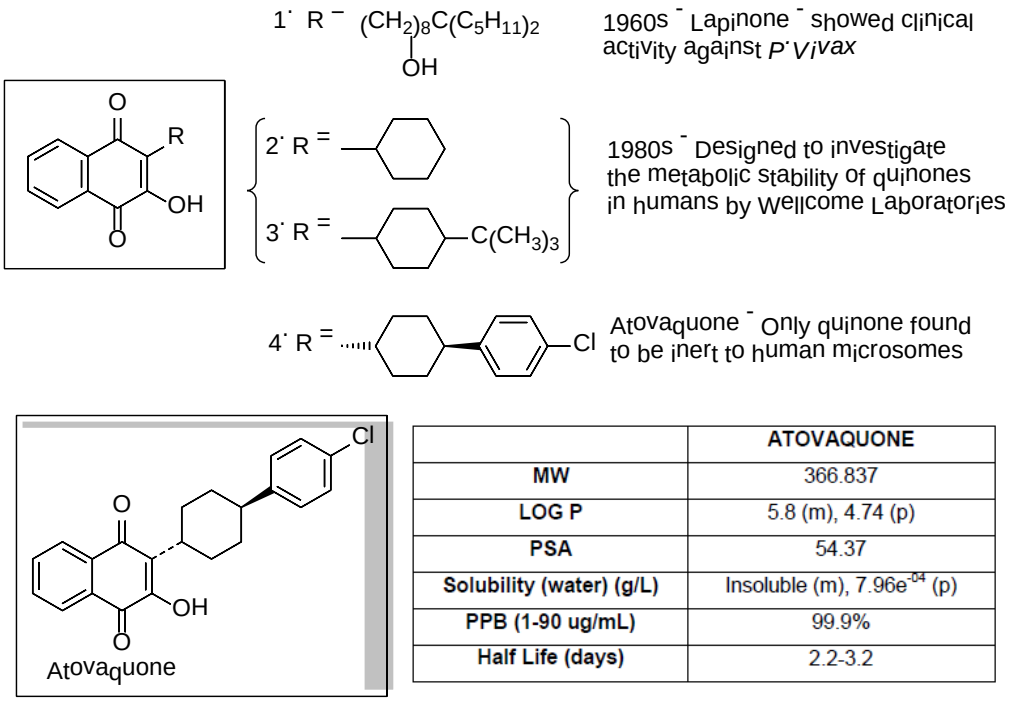
Figures



	ATOVAQUONE
MW	366.837
LOG P	5.8 (m), 4.74 (p)
PSA	54.37
Solubility (water) (g/L)	Insoluble (m), $7.96e^{-04}$ (p)
PPB (1-90 ug/mL)	99.9%
Half Life (days)	2.2-3.2

Figure 1. Historical development of atovaquone and its PK properties (m-measured, p-predicted).

Figure 2A: The original synthesis of Atovaquone.

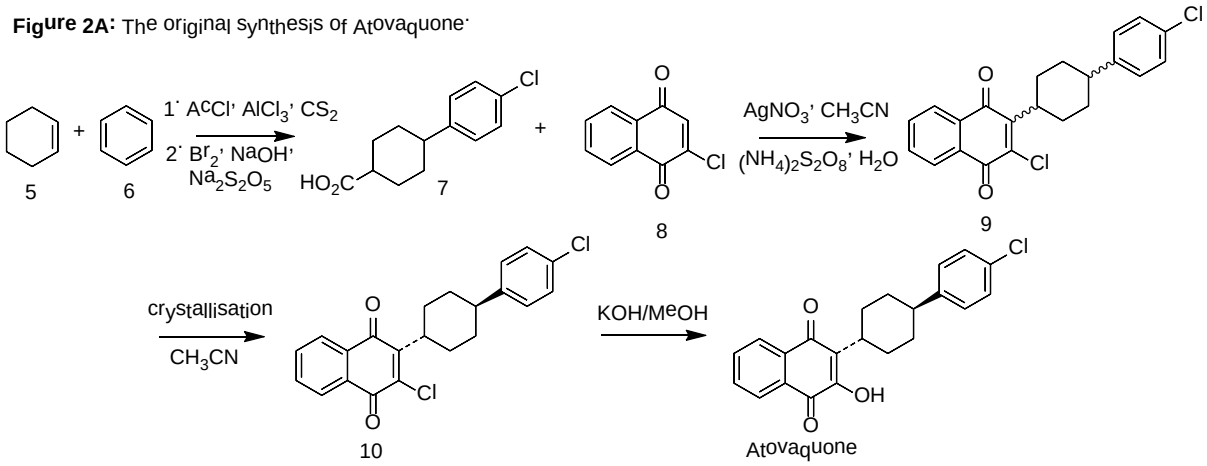


Figure 2B: Williams and Clarke Atovaquone Synthesis.

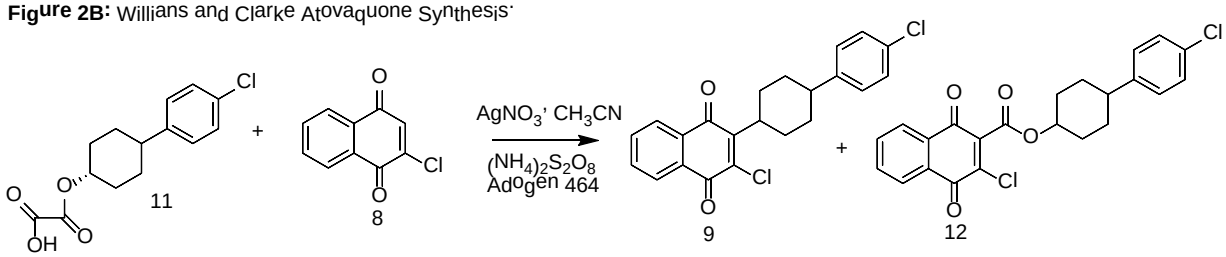


Figure 2C: Improved Atovaquone Synthesis.

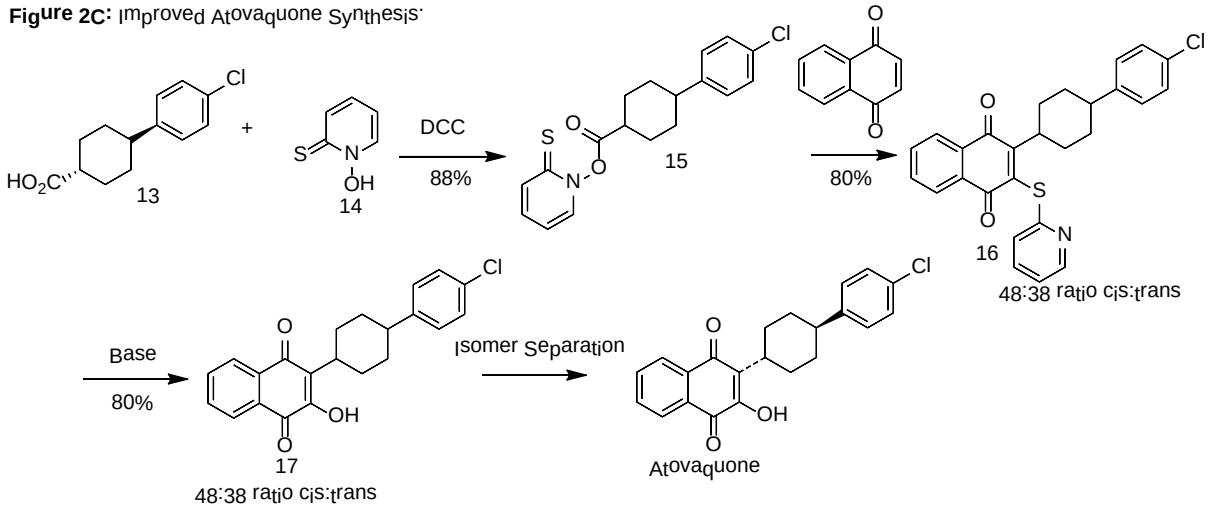


Figure 2: Synthetic routes used to synthesise atovaquone

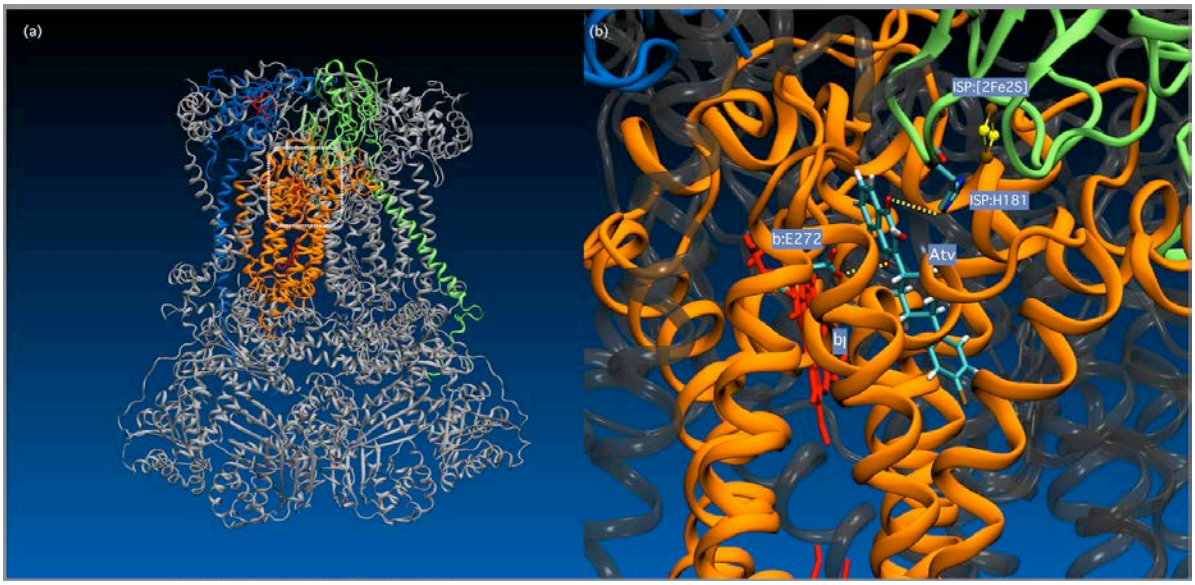
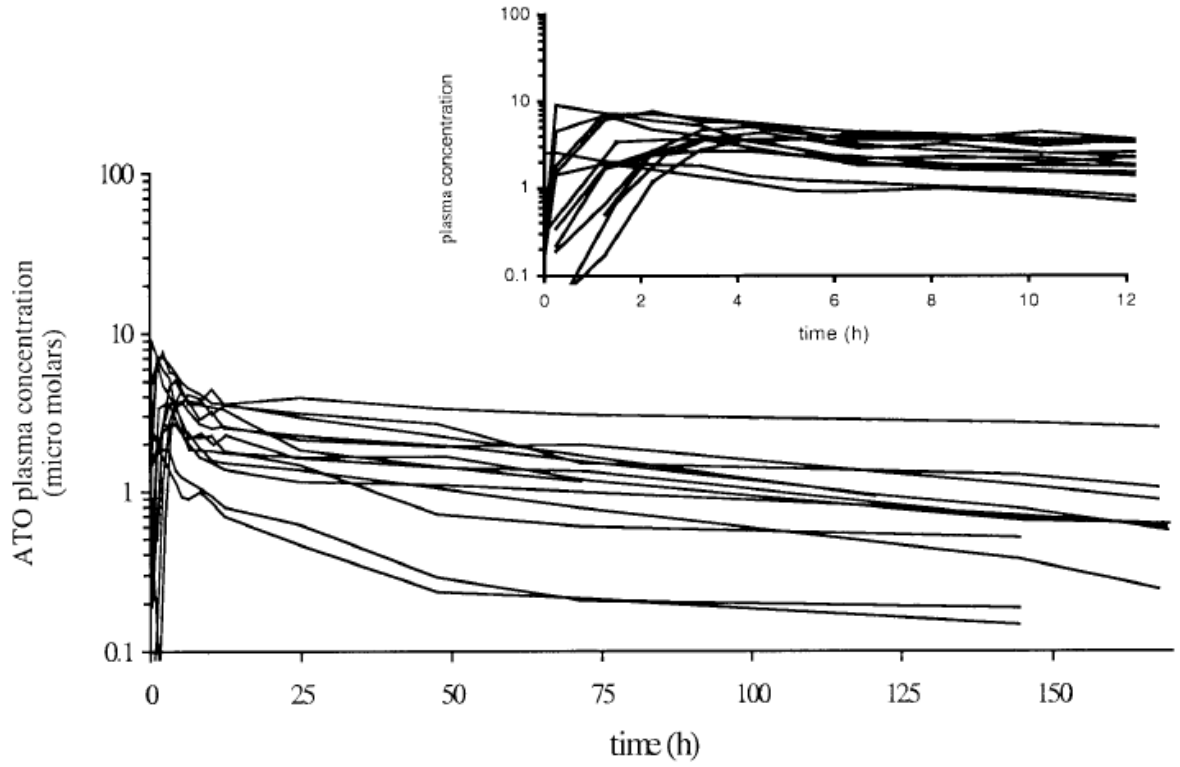


Figure 3: Panel (a). Cartoon representation of the yeast cytochrome bc_1 complex (3CX5.PDB, with atovaquone modelled at the Q_o site (boxed area).[81] The bc_1 complex is a structural and functional homodimer with a molecular mass of approximately 480 kDa, consisting of 10 discrete subunits per monomer in yeast and *P. falciparum*. The electron-transferring catalytic unit of one monomer is highlighted; cytochrome *b* is represented in orange, cytochrome c_1 in blue and the Rieske iron-sulpur protein (ISP) in green. Haem groups (cyt *b* and cyt c_1) are shown in red. The remaining subunits of the complex are rendered in grey. **Panel (b)** Molecular model of atovaquone (ATO) bound to the Q_o site of the bc_1 complex. Subunits are coloured as in panel (a). Atovaquone was modelled into the Q_o site of cytochrome *b* as described by *Fisher N et al*.[46] Hydrogen-bonding interactions between the naphthoquinone headgroup of atovaquone and sidechains of Glu-272 (cyt *b*) and His-181 (ISP) are indicated by yellow lines. The positions of haem b_l (cyt *b*) and the ISP [2Fe2S] cluster are also shown.



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560 **Figure 4.** Atovaquone plasma concentration-time profile after single dose of Malarone in 13

561 healthy individuals. Used with permission from the study by Thaper *et al*.[51]

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