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Laboratory note

Synthesis and antimalarial activity of new atovaquone derivatives

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ABSTRACT

In this paper we describe the design and synthesis of 18 derivatives of the antimicrobial atovaquone which were substituted at the 3-hydroxy group by ester and ether functions. The compounds were evaluated in vitro for their activity against the growth of *Plasmodium falciparum*, the malaria causing parasite. All the compounds showed potent activity, with IC_{50} values in the range of 1.25–50 nM, comparable to those of atovaquone and much higher than chloroquine or quinine.

1. Introduction

Malaria is a devastating disease, which is endemic in about 100 developing countries putting 2.2 billion people at risk [1,2]. *Plasmodium falciparum* is the parasite responsible for most malaria cases (80%), which often prove fatal. The burden of malaria has not declined, partly because the parasites have become resistant to the available drugs [3–5]. The most recent estimates indicate that there are more than 500 million clinical cases of malaria annually on the planet, a number that has nearly doubled since 1998 [6].

The antimalarial activity of naphthoquinones has been widely reported [7]. Atovaquone, a hydroxy naphthoquinone, with potent activity against *Pneumocystis carinii* [8] and leishmaniasis [9], has been shown to be a potent antimalarial drug [10]. Atovaquone is a lipophilic analogue of ubiquinone (coenzyme Q), an important component of the mitochondrial electron transfer system in cells. The antimalarial activity of atovaquone has been attributed to its interference with mitochondrial electron transport in the parasite, specifically at the cytochrome *c* reductase complex [11], that results in a collapse of the mitochondrial membrane potential. The activity of the parasitic enzyme dihydroorotate dehydrogenase is also inhibited because of its dependence on a functional mitochondrial electron transport chain. As dihydroorotate dehydrogenase is a key enzyme in pyrimidine biosynthesis, its inhibition by atovaquone disrupts plasmodial DNA synthesis and replication [12–14].

Atovaquone is characterized by poor bioavailability [15], but this is increased by a factor of 2–3 if it is administered with food with a high lipid content. Moreover this compound is known to bind 99.9% to plasma proteins. In a previous study, we showed that methylation of the hydroxy moiety of atovaquone led to a compound exhibiting similar activity as atovaquone and more active than chloroquine and quinine against *P. falciparum* [16]. In an effort to optimize these results and notably to improve the absorption without a lipid-rich food intake, and to decrease the plasma protein binding by increasing the lipophilicity, we have synthesized two series of drugs by adding various esters (compounds **1** and **2**) and ethers (compounds **3**) to the 3-hydroxy group of atovaquone. The ester derivatives can be considered as prodrugs with the expectation of improved bioavailability. The lipophilicity and physico-chemical properties of these compounds were modulated by the introduction of: (i) saturated or unsaturated carbon chain of different lengths ($R = CH_3–(CH_2)_n$, $n = 0$, 4, 6, 7, 8, 16) and (ii) a phenyl group, eventually substituted with halogen atoms (Cl, F) in the para position.

These atovaquone derivatives were prepared as shown in Scheme 1. All these compounds were evaluated in vitro for their antimalarial activity.

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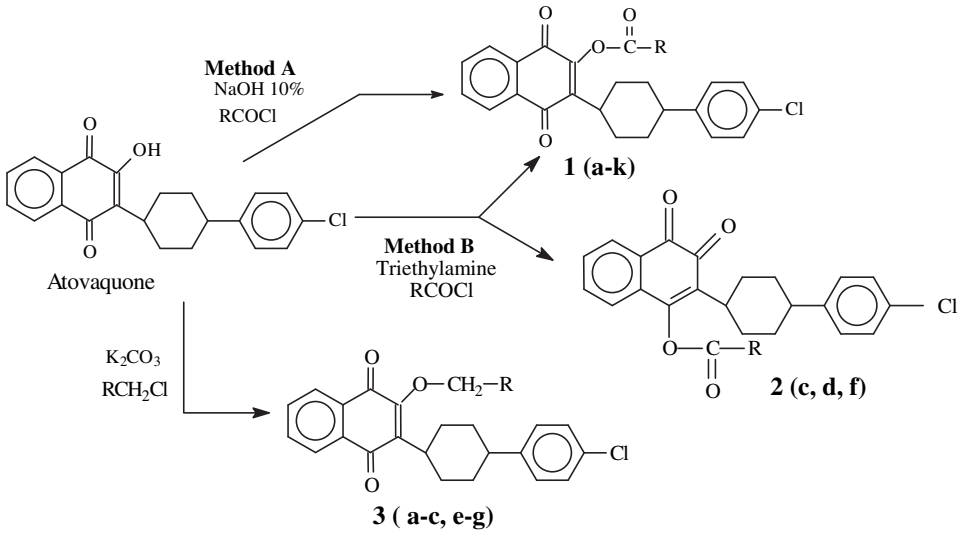


Scheme 1. Synthesis of atovaquone derivatives **1**(**a**–**k**), **2**(**c**, **d**, **f**), **3**(**a**–**c**, **e**–**g**).

2. Chemistry

All the syntheses were carried out starting from atovaquone. Ester derivatives were synthesized by two different methods. Compounds **1**(**a**, **b**, **e**, **g**–**k**) were obtained by the condensation of atovaquone with suitable acid chlorides in the presence of NaOH (10%) (Method A).

If NaOH was replaced by triethylamine (Method B), we obtained a mixture of compounds **1** and **2**. The products **1**(**c**, **d**, **f**) and **2**(**c**, **d**, **f**) were separated by column chromatography.

Condensation of atovaquone with the corresponding halogenated compound, in the presence of K_2CO_3, gave the ether derivatives **3**(**a**–**c**, **e**–**g**).

3. Pharmacology

In vitro activity against *P. falciparum* was determined using chloroquine, quinine and atovaquone as reference compounds. The results of the biological evaluation are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth and are listed in Table 1.

4. Results and discussion

We determined the in vitro antimalarial activity of the atovaquone derivatives after one blood cycle (48 h) contact with *P. falciparum* (Table 1). The atovaquone derivatives of series **1** and **3** exhibited potent antimalarial activity showing half-maximal inhibition concentration (IC_{50}) in the nanomolar range. All the ester derivatives **1**(**a**–**f**, **h**–**k**) but one (**1g**) had very similar IC_{50} in the very low nanomolar range between 1 and 3 nM, comparable to that of atovaquone and much higher than chloroquine or quinine. The presence of an alkyl chain that contains less than 8 carbon atoms does not affect the antimalarial activity. Similarly, replacement of the alkyl chain by an unsaturated chain (**1k**) or an aromatic ring **1**(**b**, **h**–**j**) substituted or not by a halogen atom, did not significantly modify the results. Only a major increase in the length of the alkyl chain (compound **1g**) led to a significant loss of antimalarial activity.

The ester derivatives appear more potent than the ether analogues whose IC_{50} are 5 nM or higher. With an ether substituent at the position 2 of the atovaquone, an aromatic ring (**3b**) had no effect while an alkyl chain **3**(**c**, **e**, **f**) slightly decreased the antimalarial activity, with a significantly more marked effect with the long hexadecyl chain (**3g**).

Only one compound of the series **2** was tested. The compound **2f** with a nonanoyl chain showed no activity against the growth of the malarial parasites until 100 nM, while its isomer **1f**, with functionally similar substitutes at position 2, gave an IC_{50} of 1.9 nM.

5. Conclusion

In this paper we describe the synthesis of 18 new atovaquone derivatives in which the 3-hydroxy group was derivatized by an ester or ether function. Most of the compounds showed high activity against the growth of *P. falciparum* in vitro, with IC_{50} values below 10 nM for 15 of them. Ester derivatives at the hydroxyl group of atovaquone gave compounds with the highest activity, in the

Table 1
Structure and in vitro antimalarial activity for atovaquone analogues.

Compounds	R	IC_{50} (nM)
1a	$-CH_3$	1.5
1b	$-C_6H_5$	1.25
1c	$-(CH_2)_4-CH_3$	1.25
1d	$-(CH_2)_6-CH_3$	1.5
1e	$-(CH_2)_7-CH_3$	3.1[a]
1f	$-(CH_2)_8-CH_3$	1.9
1g	$-(CH_2)_{16}-CH_3$	52[a]
1h	$-C_6H_4-4F$	1.6
1i	$-CH_2-C_6H_4-4F$	1.75
1j	$-CH_2-C_6H_4-4Cl$	1.65
1k	$-CH{=}CH-C_6H_5$	1.45
2c	$-(CH_2)_4-CH_3$	–
2d	$-(CH_2)_6-CH_3$	–
2f	$-(CH_2)_8-CH_3$	>100[a]
3a	$-CH_3$	5.55
3b	$-C_6H_5$	7.65
3c	$-(CH_2)_4-CH_3$	13.5
3e	$(CH_2)_7-CH_3$	14
3f	$-(CH_2)_8-CH_3$	11
3g	$-(CH_2)_{16}-CH_3$	32[a]
Atovaquone		0.75
Chloroquine		125
Quinine		180

[a] Tested compound after dissolution in DMF.

same range as atovaquone and much higher than chloroquine or quinine. In these series, the optimum activity was observed in compounds with a carbon chain of between 1 and 8 methylene groups or a phenyl moiety.

Most of these compounds had potent activity and the ADME evaluations (see Table S1, Supplementary material) using the *ADME Boxes v 4.0* software showed that for several of the series, while the increase in lipophilicity did not lead to a major improvement in the oral bioavailability; they were still in the same range as atovaquone. Further research is necessary therefore to determine whether these compounds have potential as antimalarial drugs.

6. Experimental protocols

6.1. Chemistry

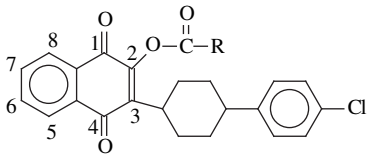
2-[*E*-4-(4-chlorophenyl)-cyclohexyl]-3-hydroxy-1,4-naphthoquinone (atovaquone) was a gift from Glaxo-Smith-Kline Laboratories (Evreux, France). All other reagents were purchased from Aldrich.

Melting points were determined with a DSC-50 Shimadzu apparatus. Infrared spectra were recorded on a Perkin–Elmer 983G spectrophotometer. ^{13}C and ^{1}H NMR spectra were recorded using a Brucker AM 250 MHz spectrometer. For the ^{1}H NMR data, chemical shifts are reported in parts per millions (δ, ppm) downfield from $CHCl_3$ as an internal standard, multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quadruplet) or m (multiplet). The microanalyses were performed in the Microanalytical Laboratory of ENSIACET in Toulouse, France, and the results obtained were within ±0.4% of the theoretical values. Reactions were monitored by thin-layer chromatography (TLC) and product mixtures were purified by column chromatography using silica gel 60 F-254, 70–200 mesh. All yields are calculated for analytically pure materials.

6.1.1. Method A: the general procedure for the synthesis of compounds **1**(***a***, ***b***, ***e***, ***g***–***k***)

To a solution of 2.7 mmoles (1 g) of atovaquone in CH_2Cl_2 (50 mL), 40 mL of aqueous NaOH solution (10%) were added and the solution was stirred for 4 h at room temperature. Then 7 mmoles of the appropriate acid chloride were added and stirring was maintained for 48 h. The reaction mixture was washed with water (3×50 mL) and the organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was crystallized from ethanol.

All these compounds **1**(**a**–**k**) gave the same IR absorption bands: I.R., KBr, ν cm^{-1}: 2924, 2907, 2851 (CH, CH_2, CH_3); 1767 (CO, ester); 1674, 1652 (CO, quinone); 1619, 1590 (C=C).



*6.1.1.1. Acetic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1a**).* Yield: 83%; m.p.: 163.1 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.14–8.05 (m, 2H, H_5, H_8), 7.78–7.68 (m, 2H, H_6, H_7), 7.26 (m, 2H, ArH), 7.17 (m, 2H, ArH), 3.07 and 2.59 (2m, 2H, 2CH cyclohexyl), 2.16 (s, 3H, CH_3), 2.08–1.47 (m, 8H, $4CH_2$ cyclohexyl). ^{13}C NMR, $CDCl_3$, δ ppm: 184.50, 178.35, 168.11 (3C=O), 154.4 (C_2); 145.10 (C Ar), 142.10 (C_3), 135.20, 135.07 (C_6 and C_7), 131.80, 131.59, 130.79 (3C Ar), 128.45, 128.35, 127.95 (4CH Ar), 126.80 (C_5 and C_8), 43.28, 34.24 (2CH cyclohexyl), 33.99, 29.91 ($4CH_2$, cyclohexyl), 21.8 (CH_3). Anal. Calcd for $C_{24}H_{21}ClO_4$ (408.5).

*6.1.1.2. Benzoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1b**).* Yield: 46%; m.p.: 150 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.23 (m, 2H, ArH), 8.17–8.07 (m, 2H, H_5, H_8), 7.80–7.66 (m, 3H, H_6, H_7, ArH), 7.60–7.52 (m, 2H, ArH), 7.21 (m, 2H, ArH), 7.11 (m, 2H, ArH), 3.18 and 2.48 (2m, 2H, 2CH cyclohexyl), 1.96–1.43 (m, 8H, $4CH_2$ cyclohexyl). ^{13}C NMR, $CDCl_3$, δ ppm: 184.52, 178.26, 164.12 (3C=O), 151.79 (C_2), 145.51, 142.25 (2C Ar), 141.90 (C_3), 134.35, 134.22 (C_6 and C_7), 133.81 (CH Ar), 132.39, 131.59, 130.73 (3C Ar), 130.66, 128.92, 128.44, 128.15 (6CH Ar), 126.95 (2CH Ar), 126.57 (C_5 and C_8), 43.18, 35.89 (2CH cyclohexyl), 34.19, 30.04 ($4CH_2$ cyclohexyl). Anal. Calcd for $C_{29}H_{23}ClO_4$ (470.5).

*6.1.1.3. Nonanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1e**).* Yield: 63%; m.p.: 79 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.12–8.05 (m, 2H, H_5, H_8), 7.76–7.73 (m, 2H, H_6, H_7), 7.26 (m, 2H, ArH), 7.15 (m, 2H, ArH), 3.07 (m, 1H, CH cyclohexyl), 2.71 (t, 2H, $COCH_2$), 2.58 (m, 1H, CH cyclohexyl), 2.17–1.29 (m, 20H, CH_2 cyclohexyl, CH_2 aliphatics), 0.87 (t, 3H, CH_3). Anal. Calcd for $C_{31}H_{35}ClO_4$ (506.5).

*6.1.1.4. Octadecanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1g**).* Yield: 26%; m.p.: 69 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.13–8.05 (m, 2H, H_5, H_8), 7.76–7.68 (m, 2H, H_6, H_7), 7.25 (m, 2H, ArH), 7.16 (m, 2H, ArH), 3.06 (m, 1H, CH cyclohexyl), 2.70 (t, 2H, $COCH_2$), 2.58 (m, 1H, CH cyclohexyl), 2.02–1.24 (m, 38H, CH_2 cyclohexyl, CH_2 aliphatics), 0.86 (t, 3H, CH_3). Anal. Calcd for $C_{40}H_{53}ClO_4$ (632.5).

*6.1.1.5. 4-Fluoro-benzoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1h**).* Yield: 25%; m.p.:191 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.30–8.25 (m, 2H, H_5, H_8), 8.20–8.10 (m, 2H, ArH), 7.82–7.70 (m, 2H, H_6, H_7), 7.30–7.25 (m, 4H, ArH), 7.15–7.10 (m, 2H, ArH), 3.19 and 2.53 (2m, 2H, 2CH cyclohexyl), 2.07–1.27 (m, 8H, CH_2 cyclohexyl). Anal. Calcd for $C_{29}H_{22}ClFO_4$ (488.5).

*6.1.1.6. 4-Fluoro-phenyl-acetic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1i**).* Yield: 20%; m.p.: 155 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.16–8.08 (m, 2H, H_5, H_8), 7.80–7.67 (m, 2H, H_6, H_7), 7.49–7.44 (m, 2H, ArH), 7.29 and 7.14 (2m, 6H, ArH), 4.01 (s, 2H, CO–CH_2–), 2.90 and 2.31 (2m, 2H, 2CH cyclohexyl), 1.87–1.28 (m, 8H, CH_2 cyclohexyl). ^{13}C NMR, $CDCl_3$, δ ppm: 184.34, 178.13, 168.45 (3C=O), 160.70 (C Ar), 153.03 (C_2), 145.45 (C Ar), 142.09 (C_3), 134.99, 134.24 (C_6 and C_7), 132.84, 132.31, 131.63 (3C Ar), 131.39, 131.28 (2CH Ar), 130.58 (C Ar), 128.21, 128.09, 126.97, 126.93 (4CH Ar), 126.49, 126.01 (C_5 and C_8), 115.96, 115.67 (2CH Ar), 43.23 (CH cyclohexyl), 40.37 (CH_2), 35.74 (CH cyclohexyl), 30.93, 29.38 ($4CH_2$ cyclohexyl). Anal. Calcd for $C_{30}H_{24}ClFO_4$ (502.5).

*6.1.1.7. 4-Chlorophenyl-acetic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1j**).* Yield: 20%; m.p.: 167 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.15–8.08 (m, 2H, H_5, H_8), 7.84–7.69 (m, 2H, H_6, H_7), 7.43 (m, 4H, ArH), 7.29 (dd, 2H, ArH), 7.14 (dd, 2H, ArH), 4.00 (s, 2H, CO–CH_2–), 2.89 and 2.35 (2m, 2H, 2CH cyclohexyl), 1.88–1.28 (m, 8H, CH_2 cyclohexyl). Anal. Calcd for $C_{30}H_{24}Cl_2O_4$ (519).

*6.1.1.8. Cinnamoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1k**).* Yield: 27%; m.p.: 181.8 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.18–8.11 (m, 2H, H_5, H_8), 7.97 (d, 1H, –CH=C<u>H</u>–CO), 7.81–7.66 (m, 4H, ArH), 7.49 (m, 3H, H_6, H_7, ArH), 7.26 (m, 2H, ArH), 7.17 (m, 2H, ArH), 6.75 (d, 1H, –CH=C<u>H</u>–C_6H_5), 3.15 and 2.56 (2m, 2H, CH cyclohexyl), 2.08–1.27 (m, 6H, 3CH_2 cyclohexyl), 1.43 (m, 2H, CH_2 cyclohexyl). ^{13}C NMR, $CDCl_3$, δ ppm: 184.60, 178.39, 164.20 (3C=O), 151.52 (C_2), 148.60 (CH=), 145.58 (C Ar), 142.09 (C_3),

133.88 (C_6 and C_7), 133.76, 132.41, 131.59, 131.24 (4C Ar), 129.11, 128.62, 128.45, 128.18, 126.95 (9CH Ar), 126.57 (C_5 and C_8), 115.58 (CH=), 43.23, 35.98 (2CH cyclohexyl), 34.24, 29.64 ($4CH_2$ cyclohexyl). Anal. Calcd for $C_{31}H_{25}ClO_4$ (496.5).

*6.1.2. Method B: the general procedure for the synthesis of compounds **1**(**c**, **d**, **f**) and **2**(**c**, **d**, **f**)*

To a solution of atovaquone (2.7 mmoles, 1 g) in 1,2-dichloroethane (50 mL) triethylamine (7.1 mmoles, 1 mL) was added dropwise. After stirring at room temperature for 10 min, 2.7 mmoles of the appropriate acid chloride were added and stirring was maintained for six days. The reaction mixture was washed with water and the organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product obtained was chromatographed with cyclohexane/1,2-dichloroethane (85/15) as eluent. Two compounds were isolated and crystallized from ethanol.

*6.1.2.1. Hexanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1c**).* Yield 51%; m.p.: 108.9 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.12–8.05 (m, 2H, H_5, H_8), 7.77–7.67 (m, 2H, H_6, H_7), 7.29 (m, 2H, ArH), 7.18 (m, 2H, ArH), 3.10 (m, 1H, CH cyclohexyl), 2.75 (t, 2H, $COCH_2$), 2.60 (m, 1H, CH cyclohexyl), 2.02–1.44 (m, 14H, CH_2 cyclohexyl, CH_2 aliphatics), 0.99 (t, 3H, CH_3). ^{13}C NMR, $CDCl_3$, δ ppm: 184.56, 178.35, 171.11 (3C=O), 151.51 (C_2), 145.53 (C Ar), 141.80 (C_3), 134.14, 133.72 (C_6 and C_7), 132.37 (C Ar), 131.65, 131.39 (2C Ar), 130.69, 128.50, 128.13 (4CH Ar), 126.86, 126.47 (C_5 and C_8), 43.28 (CH cyclohexyl), 35.90 (CH_2 aliphatic), 34.24 (CH cyclohexyl), 33.99 ($2CH_2$ cyclohexyl), 31.27 (CH_2 aliphatic), 29.91 ($2CH_2$ cyclohexyl), 24.53, 22.37 ($2CH_2$ aliphatics), 13.97 (CH_3). Anal. Calcd for $C_{28}H_{29}ClO_4$ (464.5).

*6.1.2.2. Octanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1d**).* Yield: 30%; m.p.: 85.1 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.16–8.05 (m, 2H, H_5, H_8), 7.78–7.68 (m, 2H, H_6, H_7), 7.26 (m, 2H, ArH), 7.16 (m, 2H, ArH), 3.08 (m, 1H, CH cyclohexyl), 2.71 (t, 2H, $COCH_2$), 2.58 (m, 1H, CH cyclohexyl), 2.14–1.20 (m, 18H, CH_2 cyclohexyl, CH_2 aliphatics), 0.89 (t, 3H, CH_3). Anal. Calcd for $C_{30}H_{33}ClO_4$ (492.5).

*6.1.2.3. Decanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinon-2-yl ester (**1f**).* Yield: 25%; m.p.: 69 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.13–8.05 (m, 2H, H_5, H_8), 7.78–7.67 (m, 2H, H_6, H_7), 7.25 (m, 2H, ArH), 7.16 (m, 2H, ArH), 3.07 (m, 1H, CH cyclohexyl), 2.71 (t, 2H, $COCH_2$), 2.58 (m, 1H, CH cyclohexyl), 2.07–1.26 (m, 22H, CH_2 cyclohexyl, CH_2 aliphatics), 0.86 (t, 3H, CH_3). Anal. Calcd for $C_{32}H_{37}ClO_4$ (520.5).

All these compounds **2**(**c**, **d**, **f**) had the same IR absorption bands: I.R., KBr, ν cm^{-1}: 2929, 2872 (CH, CH_2, CH_3); 1762 (CO, ester); 1674 (CO, quinone); 1592 (C=C).

*6.1.2.4. Hexanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,2-naphthoquinon-2-yl ester (**2c**).* Yield: 6%; m.p.: 198.8 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.30 (dd, 1H, H_8), 7.91 (dd, 1H, H_5), 7.73–7.62 (m, 2H, H_6, H_7), 7.28 (m, 2H, ArH), 7.19 (m, 2H, ArH), 3.22 (m, 1H, CH cyclohexyl), 2.90 (t, 2H, $COCH_2$), 2.69 (m, 1H, CH cyclohexyl), 2.09–1.58 (m, 14H, CH_2 cyclohexyl, CH_2 aliphatics), 1.03 (t, 3H, CH_3). Anal. Calcd for $C_{28}H_{29}ClO_4$ (464.5).

*6.1.2.5. Octanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,2-naphthoquinon-2-yl ester (**2d**).* Yield: 5%; m.p.: 178 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.27 (dd, 1H, H_8), 8.05 (dd, 1H, H_5), 7.70–7.59 (m, 2H, H_6, H_7), 7.26 (m, 2H, ArH), 7.16 (m, 2H, ArH), 3.19 (m, 1H, CH cyclohexyl), 3.03 (t, 2H, $COCH_2$), 2.66 (m, 1H, CH cyclohexyl), 2.16–1.24 (m, 18H, CH_2 cyclohexyl, CH_2 aliphatics), 0.89 (t, 3H, CH_3). Anal. Calcd for $C_{30}H_{33}ClO_4$ (492.5).

*6.1.2.6. Decanoic acid 3-(*E*-4-parachlorophenylcyclohexyl)-1,2-naphthoquinon-2-yl ester (**2f**).* Yield: 25%; m.p.: 169 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.27 (dd, 1H, H_8), 7.90 (dd, 1H, H_5), 7.69–7.62 (m, 2H, H_6, H_7), 7.24 (m, 2H, ArH), 7.15 (m, 2H, ArH), 3.18 (m, 1H, CH cyclohexyl), 2.86 (m, 2H, $COCH_2$), 2.65 (m, 1H, CH cyclohexyl), 2.16–1.23 (m, 22H, CH_2 cyclohexyl, CH_2 aliphatics), 0.87 (t, 3H, CH_3). ^{13}C NMR, $CDCl_3$, δ ppm: 183.77, 180.03, 169.10 (3C=O), 158.05 (C_4), 145.93, 132.77 (2C Ar), 131.81(C_6 or C_7), 131.49, 131.11 (2C Ar), 128.44 (C_6 or C_7), 128.20, 126.25 (4CH Ar), 126.01, 123.20 (C_5 and C_8), 122.00 (C_3), 43.28 (CH cyclohexyl), 34.70, 31.81, 30.46 ($5CH_2$ aliphatics), 29.95 (CH_2 cyclohexyl), 29.83 (CH cyclohexyl), 29.73 (CH_2 cyclohexyl), 27.75 (CH_2 aliphatic), 25.65 ($2CH_2$ cyclohexyl), 25.40, 22.64 ($2CH_2$ aliphatics), 14.09 (CH_3). Anal. Calcd for $C_{32}H_{37}ClO_4$ (520.5).

*6.1.3. The general procedure for the synthesis of compounds **3**(**a**–**c**, **e**–**g**)*

1.36 mmoles (0.2 g) of K_2CO_3 were added to a solution of 1.36 mmoles (0.5 g) of atovaquone in anhydrous butanone (50 mL) and the mixture heated under reflux for 30 min. Then 1.36 mmoles of the appropriate halogenated derivative were added and the mixture was stirred and refluxed for 14 h. After cooling, the mixture was filtered and the organic phase evaporated under reduced pressure. The residue was purified by recrystallization from ethanol.

All these compounds gave the same IR absorption bands: I.R., KBr, ν cm^{-1}: 2954, 2910, 2050 (CH, CH_2, CH_3); 1663, 1652 (CO, quinone); 1593 (C=C).

*6.1.3.1. 2-Ethoxy-3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone (**3a**).* Yield: 68%; m.p.: 114 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.08–8.00 (m, 2H, H_5, H_8), 7.72–7.63 (m, 2H, H_6, H_7), 7.27 (m, 2H, ArH), 7.16 (m, 2H, ArH), 4.37 (q, 2H, $\underline{CH_2}$–CH_3), 3.20 (m, 1H, CH cyclohexyl), 2.61 (m, 1H, CH cyclohexyl), 2.23–1.51 (m, 8H, CH_2 cyclohexyl), 1.46 (t, 3H, CH_2–$\underline{CH_3}$). ^{13}C NMR, $CDCl_3$, δ ppm: 185.52, 181.89 (2C=O), 158.07 (C_2), 146.00 (C Ar), 137.77, 133.08 (C_6 and C_7), 132.37 (C Ar), 132.23 (C_3), 131.49, 131.46 (2C Ar), 128.40, 128.18 (2CH Ar), 126.80 (C_5 and C_8), 126.36, 125.90 (2CH Ar), 70.09 (CH_2 aliphatic), 43.27, 35.18 (2CH cyclohexyl), 34.42, 29.89 ($4CH_2$ cyclohexyl), 14.07 (CH_3). Anal. Calcd for $C_{24}H_{23}ClO_3$ (394.5).

*6.1.3.2. 2-Benzyloxy-3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone (**3b**).* Yield: 17%; m.p.: 128 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.08–8.03 (m, 2H, H_5, H_8), 7.74–7.66 (m, 2H, H_6, H_7), 7.47–7.09 (m, 9H, ArH), 5.37 (s, 2H, CH_2–C_6H_5), 3.08 (m, 1H, CH cyclohexyl), 2.46 (m, 1H, CH cyclohexyl), 2.07–1.23 (m, 8H, CH_2 cyclohexyl). ^{13}C NMR, $CDCl_3$, δ ppm: 185.43, 181.84 (2C=O), 157.10 (C_2), 146.00 (C Ar), 139.99 (C_3), 136.54 (C Ar), 135.01 (C_6 and C_7), 133.89, 132.85, 131.45 (3C Ar), 128.72, 128.65 (2CH Ar), 128.40, 128.21, 128.16, 127,00 (7CH Ar), 126.39, 126.03 (C_5 and C_8), 75.56 (CH_2), 43.13, 35.63 (2CH cyclohexyl), 34.40, 29.72 ($4CH_2$ cyclohexyl). Anal. Calcd for $C_{29}H_{25}ClO_3$ (456.5).

*6.1.3.3. 2-Hexanoxy-3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone (**3c**).* Yield: 30%; m.p.: 94 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.07–7.99 (m, 2H, H_5, H_8), 7.72–7.63 (m, 2H, H_6, H_7), 7.28–7.15 (m, 4H, ArH), 4.31 (t, 2H, OCH_2), 3.20 (m, 1H, CH cyclohexyl), 2.60 (m, 1H, CH cyclohexyl), 2.24–1.34 (m, 16H, CH_2 cyclohexyl, CH_2 aliphatics), 0.91 (t, 3H, CH_3). ^{13}C NMR, $CDCl_3$, δ ppm: 185.52, 181.89 (2C=O), 158.07 (C_2), 146.00 (C Ar), 133.77, 133.08 (C_6 and C_7), 132.37, 131.49, 131.46 (3C Ar), 128.49 (C_3), 128.43 (2C Ar), 128.19 (2C Ar), 126.32, 125.93 (C_5 and C_8), 74.09 (CH_2 aliphatic), 43.36 (CH cyclohexyl), 35.36, 31.58 ($2CH_2$ aliphatics), 30.39 (CH cyclohexyl), 29.94, 25.64 ($4CH_2$ cyclohexyl), 25.54, 22.69 ($2CH_2$ aliphatics), 14.07 (CH_3). Anal. Calcd for $C_{28}H_{31}ClO_3$ (450.5).

*6.1.3.4. 2-Nonanoxy-3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone (**3e**).* Yield: 41%; m.p.: 83.4 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.07–7.99 (m, 2H, H_5, H_8), 7.70–7.05 (m, 2H, H_6, H_7), 7.27–7.15 (m, 4H, ArH), 4.31 (t, 2H, OCH_2), 3.20 (m, 1H, CH cyclohexyl), 2.60 (m, 1H, CH cyclohexyl), 2.19–1.27 (m, 22H, CH_2 cyclohexyl, CH_2 aliphatics), 0.86 (t, 3H, CH_3). Anal. Calcd for $C_{31}H_{37}ClO_3$ (492.5).

*6.1.3.5. 2-Decanoxy-3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone (**3f**).* Yield: 22%; m.p.: 74 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.07–7.99 (m, 2H, H_5, H_8), 7.73–7.62 (m, 2H, H_6, H_7), 7.25 (dd, 2H, ArH), 7.17 (dd, 2H, ArH), 4.31 (t, 2H, OCH_2), 3.20 (m, 1H, CH cyclohexyl), 2.60 (m, 1H, CH cyclohexyl), 2.24–1.20 (m, 24H, CH_2 cyclohexyl, CH_2 aliphatics), 0.85 (t, 3H, CH_3). Anal. Calcd for $C_{32}H_{39}ClO_3$ (506.5).

*6.1.3.6. 2-Octadecanoxy-3-(*E*-4-parachlorophenylcyclohexyl)-1,4-naphthoquinone (**3g**).* Yield: 26%; m.p.: 66 °C; ^{1}H NMR, $CDCl_3$, δ ppm: 8.07–7.99 (m, 2H, H_5, H_8), 7.72–7.63 (m, 2H, H_6, H_7), 7.28–7.14 (m, 4H, ArH), 4.31 (t, 2H, OCH_2), 3.20 (m, 1H, CH cyclohexyl), 2.60 (m, 1H, CH cyclohexyl), 2.24–1.23 (m, 40H, CH_2 cyclohexyl, CH_2 aliphatics), 0.86 (t, 3H, CH_3). Anal. Calcd for $C_{40}H_{55}ClO_3$ (618.5).

6.2. Pharmacology

The drug effects on the growth of *P. falciparum* (Nigerian strain) in vitro were measured in microtiter plates according to the method described by Desjardins [17]. The final volume in each well was 200 μL, consisting of 50 μL of complete medium (RPMI 1640 + 10% AB^+ human serum) with or without drug and 150 μL of *P. falciparum* infected erythrocyte suspension (1.5% final hematocrit and 0.6% parasitemia). The drugs were dissolved in DMSO and then further diluted in culture medium so that the final DMSO concentration never exceeded 0.25%.

After 48 h incubation at 37 °C, 30 μL of complete medium containing 0.6 μCi of [^{3}H] hypoxanthine were added to each well. After a further 18 h at 37 °C, the cells were lyzed using an automatic cell harvester and the parasite macromolecules, including radioactive nucleic acids, were retained on glass fiber filters. After adding 1 mL of scintillation cocktail, the filters were counted for radioactivity in a liquid scintillation counter.

Background radioactivity was obtained from incubating non-infected erythrocytes under the same conditions. Parasitic viability was expressed as IC_{50} which is the drug concentration leading to 50% inhibition of parasite growth. The results are the means of two independent experiments performed in triplicate.

6.3. Pharmacokinetic parameters

The bioavailability (%F) of the molecules was calculated *in silico* using the program ADME Boxes v. 4.0 [18].

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Appendix. Supplementary data

Table S1: Structure of atovaquone derivatives together with their MW, HBA[a], (HBD[b] is 0), log *P*, %F[c] calculated values and in vitro antimalarial activity for atovaquone analogues.

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.07.021.

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