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In vivo antimalarial activity of novel 2-hydroxy-3-anilino-1, 4-naphthoquinones obtained by epoxide ring-opening reaction

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ABSTRACT

1,4-Naphthoquinone derivatives are known to have relevant activities against several parasites. Among the treatment options for malaria, atovaquone, a 1,4-naphthoquinone derivative, is widely applied in the treatment and prophylaxis of such disease. Based on the structure simplification of atovaquone, we designed and synthesized four novel naphthoquinoidal derivatives. The compounds were obtained by the underexplored epoxide-opening reaction of 1,4-naphthoquinone using aniline derivatives as nucleophiles. The antiplasmodial activity of the synthesized compounds was performed in vivo using Peter's 4 days suppression test. Significant parasitemia reduction and increased survival were observed for some of the compounds.

1,4-Naphthoquinones are a class of compounds broadly studied in organic synthesis, medicinal chemistry and natural products chemistry.[1] Within these studies, the antimalarial activity has been widely reported for several 1,4-naphthoquinones.[2] The antiparasitic activity of the quinones is related to several mechanisms such as the competitive inhibition of the cytochrome bc1 complex, generation of reactive oxygen species, enzymatic inhibition (e.g., glutathione reductase, dihydroorotate dehydrogenase and glycerol glyceraldehyde-3-phosphate dehydrogenase) alkylation of biomolecules, depletion of glutathione, among others.[3]

Lawsone derivatives containing the 2-hydroxy-1,4-naphthoquinone scaffold are highly explored for the development of novel antiparasitics. Atovaquone, parvaquone and buparvaquone are 3-substituted-2-hydroxy-1,4-naphthoquinones clinically applied as antimalarial (atovaquone) and antipneumocystic (parvaquone and buparvaquone) agents, which exemplifies the potentiality of such scaffold in the development of novel drugs. Additionally, some studies indicate the potential application of natural or synthetic 2-amino-1,4-naphthoquinones and 2-amino-1,2-naphthoquinones as antiplasmodial agents.[4]

Despite the pharmacological relevance of aminated and hydroxylated naphthoquinoidal derivatives and the wide structural diversity of 1,4-naphthoquinones, 2-amino-3-hydroxy-1,4-naphthoquinone is an uncommon structural fragment only observed in few examples of natural products and bioactive compounds.[5] Recently 2-(4-phenylpiperazinyl)-3-hydroxy-1,4-naphthoquinone **1**,[6] which can be seen as a ring bioisostere of atovaquone, was shown to have inhibitory effects against *Plasmodium falciparum*.[6]

Based on the structures of atovaquone and **1**, we propose the simplification of the common scaffold by modifying the spacer group (SG) between the naphthoquinoidal moiety and the benzene ring (Atovaquone, SG = cyclohexane; and **1**, SG = piperazine). In our proposal, the aliphatic rings of these two compounds are substituted by a nitrogen atom to produce 3-anilino-substituted hydroxynaphthoquinones (Fig. 1).

The most common synthetic methodology for obtaining 2-amino-3-hydroxy-1,4-naphthoquinones and derivatives involves the nucleophilic substitution of halonaphthoquinones, followed by hydrolysis depending on the starting material.[7] Moreover, azide,[8] nitro or hydrazine reduction,[9] amination of aryliodonium ylides of 2-hydroxy-1,4-naphthoquinones,[10] and oxidation of 2-amino-1,4-naphthoquinones are somewhat useful strategies.[11] In this work, we describe a two-step method to synthesize novel 2-hydroxy-3-amino-1,4-naphthoquinone derivatives from

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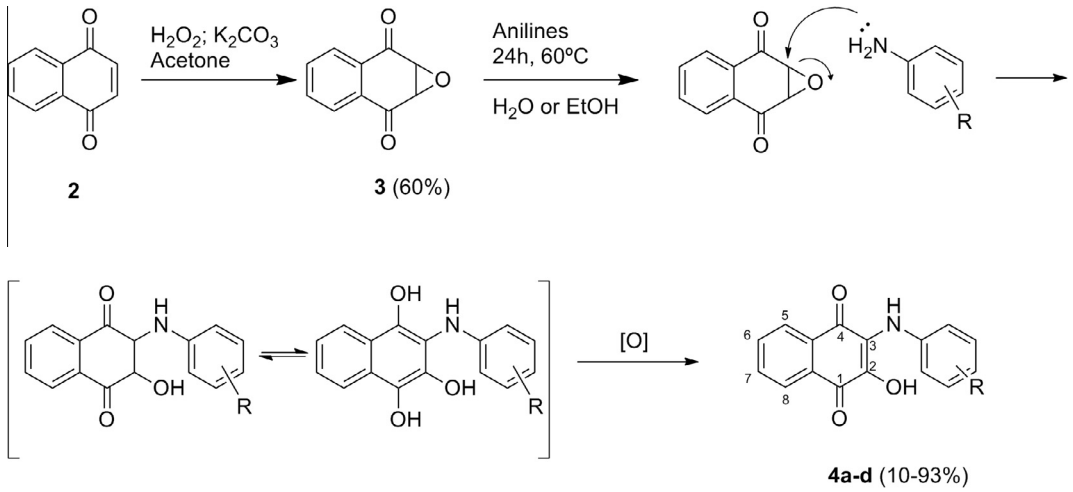

Figure 1. Synthesis and reaction mechanism of the 2-anilino-3-hydroxy-1,4-naphthoquinone obtained herein.

1,4-naphthoquinone **1**, based on aminolysis of epoxide **2** in aqueous or non-aqueous media. Although epoxide ring-opening reactions are widely described in the literature, there are only few examples describing the nucleophilic addition to 2,3-epoxy-1,4-naphthoquinone **3**.[12]

Herein, we report the reactivity of **3** with four anilines to generate the respective 2-hydroxy-3-anilino-1,4-naphthoquinone derivatives **4a**–**d** as purple solids (Table 1). The epoxide **3** was prepared by oxidation of 1,4-naphthoquinone **2**, in the presence of hydrogen peroxide, and used after recrystallization (Fig. 1).[13] The results indicate that, following the ring-opening reaction, an oxidation step regenerates the quinone ring (Fig. 1). In all cases, the typical procedure involved the addition of the aniline to a stirring solution of **3**, in water or ethanol, at 60 °C, until complete consumption of **3** (observed in TLC). All reactions were reproducible in gram scale. All compounds were purified by flash chromatography.[14]

In order to increase reaction yields, 1 equiv of six Lewis acids ($ZnCl_2$, CuI, $Cu(OAc)_2$, $CuCl_2$, $InCl_3$, InI), previously described as catalysts for epoxide ring-opening reaction,[15] were tested in the aqueous reaction of **3** with 2-chloro-aniline. Additionally, 5 mol % of $Sc(OTf)_3$ in dichloromethane was also tested. In comparison with noncatalyzed aqueous reaction, the time needed to entirely consume of precursor **3** observed in thin layer chromatography, was reduced in the presence of catalysts (especially for $ZnCl_2$). Impelled by these results, $ZnCl_2$ was also tested in aqueous reactions with the three other anilines. In all cases time for precursor consumption was improved to approximately 2 h, with reduction in the yield of **4b**, expressive increment in the yield of **4c**, and no significant effect in the yields of **4a** and **4d** (Table 1).

The structure of **4a**,[16] **4b**,[17] **4c**[18] and **4d**[19] were confirmed based on ^{1}H NMR, ^{13}C NMR and HRMS-ESI data. A common pattern of signals could be identified in ^{1}H NMR spectra, where the four hydrogen atoms of the naphthoquinoidal ring (H-5 to H-8) originates two signals (near 8.0 ppm and 7.6 ppm). Additionally, the aromatic signals observed for the aniline moiety were similar to the respective starting anilines, however with slight shielding. In the ^{13}C NMR, carbonyl carbons were detected as two signal near 181 and 179 ppm, while the others carbons appear as eight signals between 120 and 140 ppm, as expected.

The antiplasmodial activity of compounds **4a**–**d** was assayed in vivo using a Peter's[20] four days suppression test protocol with some modifications.[21] Groups of six adult Swiss albino mice infected with *Plasmodium berghei* NK65 were treated for four consecutive days with compounds **4a**–**d** (100 mg/kg/day), chloroquine (10 mg/kg/day positive control group) and vehicle (negative control group). Parasitemia was determined by blood smear exam on

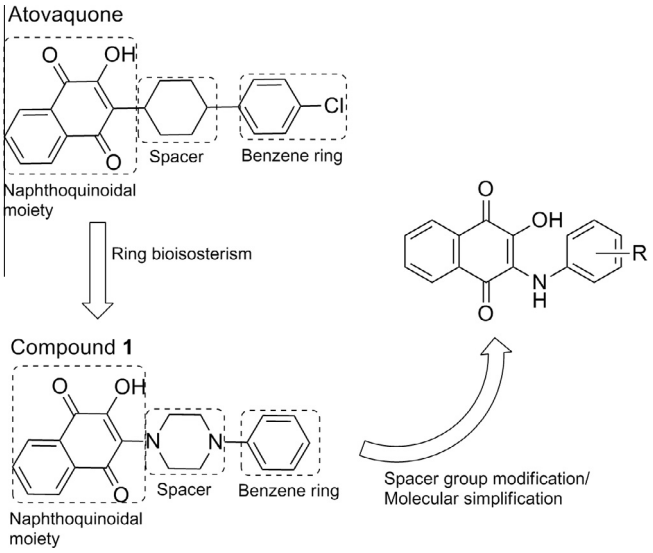

Figure 2. Evolution of strategies to design novel antimalarial derivatives.

Table 1
Reaction of 2,3-epoxy-1,4-naphthoquinone **3** with nucleophiles on ethanol and water yielding the respective 2-anilino-3-hydroxy-1,4-naphthoquinones

Nucleophile	Prod.	Yield[a] (%)			Data
		A	B	C	
Aniline	**4a**	88.0	94.7	65.2	Ref.14
2-chloro-aniline	**4b**	50.0	23.7	9.5	Ref.15
4-methoxy-aniline	**4c**	61.6	92.2	93.0	Ref.16
2,6-dimethyl-aniline	**4d**	48.5	55.0	30.5	Ref.17

[a] A—water; B—water + Zinc chloride (1 equiv), C—ethanol.

Table 2
Effects of compounds **4a**, **4b** and **4d** over parasitemia

Compd	Parasitemia (%)		Parasitemia inhibition (%)	
	Day 5	Day 7	Day 5	Day 7
4a	0.7	2.2	53.3	18.5
4c	0.5	2.0	66.6	25.9
4d	1.5	2.7	0	0
Cont.	1.5	2.7	0	0

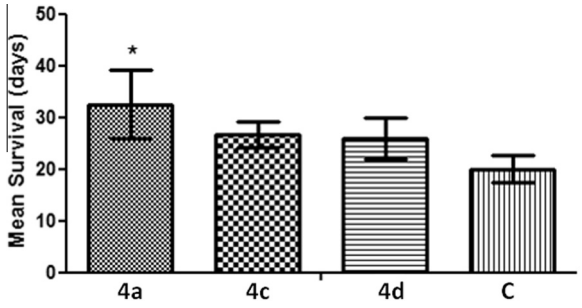

Figure 3. Mean survival of animals treated with **4a**, **4b** and **4d** and the negative control.

the 5th and 7th days after parasite inoculation from which parasite growth inhibition of each drug was calculated.[22] The compounds were considered partially active if parasitemia decreased by 30% or more.[20] Mortality was monitored in all the groups for four weeks after inoculation.

As shown in Table 2, significant parasitemia reduction was observed for compounds **4a** and **4c**, mainly at day five after parasite inoculation when parasite growth inhibition was 53% and 66% respectively. No relevant activity over parasitemia was observed in the group of animals treated with compound **4d**. Since the activities were tested in vivo, this result may be related to poor pharmacokinetic profile and bioavailability of this compound. The compound **4b** could not be tested due to solubility problems. The positive control group, treated with chloroquine, showed a parasite growth inhibition of 98%, showing the necessity of chemical modifications to obtain antiplasmodial activity consistent for clinical tests. It is worth to note that the drugs were administered via gavage, consequently, first pass metabolism may have significant influence over their activities. Future tests with subcutaneous administration and higher attack doses will enable us to outline in detail the potentiality of these compounds.

The mean survival of the animals under different therapeutic regimens is shown in Figure 2. While the animals treated with chloroquine survived for more than 60 days after parasite inoculation, the group treated with vehicle showed a mean survival of 20 ± 2.7 days. Our results (Fig. 3) indicate a slight higher survival for the animals treated with **4c** and **4d** (26.0 ± 4.0 and 26.7 ± 2.5 days, respectively), however with no statistical significance (*T*-test $p > 0.05$ when compared to control group). Compound **4a**, however, showed a statistically significant enhancement of mean survival (32.5 ± 6.6, *T*-test $p < 0.01$ when compared to control group).

The antiplasmodial activity observed for **4a** and **4c** gives supports to the potential use of 2-amino-3-hydroxy-1,4-naphthoquinone derivatives as antiplasmodial agents, which justifies further studies for the development of a library of 2-hydroxy-3-amino-1,4-naphthoquinone derivatives applying a diversity-oriented synthesis approach. In this study, we were able to obtain compound **3** in moderate yields, in multi-gram, scale and compounds **4a**–**d** were obtained in moderate to good yields with a simple work-up. Three of the four novel compounds were active in vivo against *P. berghei* NK65.

Due to their structural similarity with atovaquone these novel compounds may probably act through similar mechanism. Atovaquone is believed to inhibit electron flow in aerobic respiration, by binding to cytochrome b in place of ubiquinone in the parasite mitochondria. Such inhibition hampers the activity of dihydroorotate dehydrogenase, an enzyme linked to the mitochondrial electron transport system that is required in the de novo synthesis of pyrimidines.[23] Additionally, the redox potential of naphthoquinones is suggested to cause an oxidative stress which may also be responsible for some antiparasitic activity of this class of compounds. Further studies will furnish molecular evidences of the mechanism of action of the antiplasmodial compounds showed herein.

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14. (a) General method for reaction of **3** with anilines in water: The aniline (3 equiv) was added to a suspension of **3** in water, at 60 °C. After the consumption of **3**, the precipitate was filtered through reduced pressure, dried in a dessicator purified by flash chromatography. (b) General method for reaction of **3** with anilines in ethanol: The aniline (3 equiv) was added to a solution of **3** in ethanol, at 60 °C. After the consumption of **3**, the solvent was distilled and the product was purified by flash chromatography.
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16. 2-Hydroxy-3-(phenylamino)-1,4-naphthoquinone, **4a**, dark purple solid purified by flash chromatography (hexane/ethyl acetate 4:1). Yield: 95.1% (water) 65.2% (ethanol) ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 8.0 (m, 2H), 7.6 (m, 2H), 7.3 (t, 2H), 7.08 (t, 1H), 7.0 (d, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 182.3, 179,5. 138.9, 136.3, 134.0, 133.6, 133.0, 130.8, 130.3, 128.2, 126.6, 126.0, 124.7, 123.4,

121.4, 122.7; HRMS-ESI: $[M+H]^+$ calcd for $C_{16}H_{12}NO_3$, 266.0817; found, 266.0812; IV (ATR) ν (cm^{-1}): 3357, 3290, 1647, 1612, 1570, 1512, 1500, 1445, 1380, 1332, 1264, 711, 694. Melting point:>215 °C

17. 2-[(2-Chlorophenyl)amino]-3-hydroxy-1,4-napthoquinone, **4b**, dark purple solid purified by flash chromatography (hexane/ethyl acetate 9:1). Yield: 60.5% (water) 40.3% (ethanol) ^1H NMR (500 MHz, $CDCl_3$) δ_H: 8.04–7.99 (m, 2H), 7.65–7.60 (m, 2H), 7.31–7.29 (dd, J = 7.5 Hz, 2.0 Hz, 1H), 7.15–7.12 (dt, J = 7.5 Hz, 7.5 Hz, 2.0 Hz, 1H), 6.93–6.90 (dt, J = 7.5 Hz, 7.5 Hz, 2.0 Hz, 1H), 6.83–6.81 (dd, J = 7.5 Hz, 2.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 181.9, 179,7. 137.1, 135.9, 134.0, 133.8, 130.9, 130.1, 129.0, 126.7, 126.4, 126.1, 125.2, 123.8, 123.4, 122.2; HRMS-ESI: $[M+Na]^+$ calcd for $C_{16}H_{10}ClNNaO_3$, 322.0247; found, 322.0241 ; IV (ATR) ν (cm^{-1}): 3739, 3313, 2922, 2852, 2347, 1660, 1625, 1592, 1573, 1516, 1467, 1376, 1327, 1295, 1264, 1226, 1179, 1136, 1075, 1013, 979, 793, 750, 720, 601, 554; Melting point: 155 °C
18. 2-Hydroxy-3-[(4-methoxyphenyl)amino]-1,4-napthoquinone, **4c**, dark purple solid purified by flash chromatography (hexane/ethyl acetate 4:1). Yield: 61,6% (water) 17.3% (ethanol) ^1H NMR(500 MHz, $CDCl_3$) δ_H: 8.03–8.06 (t, J = 8.5 Hz, 2H), 7.60–7.57 (m, 2H), 7.02–6.98 (d, J = 8.3 Hz, 2H), 6.88–6.84 (d, J = 8.3 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 182.5, 179.3. 156.4, 135.5, 134.1, 133.4, 132.0, 130.8, 130.6, 126.7, 126.0, 126.8, 123.8, 113.6, 55.6, HRMS-ESI: $[M+Na]^+$ calcd for $C_{17}H_{13}NNaO_4$, 318,0742; found, 318,0736; IV (ATR) ν (cm^{-1}): 3743, 3422, 3315, 2924, 2854, 1642, 1619, 1573, 1509, 1462, 1380, 1328, 1265, 1118, 1079, 1016, 981, 822, 795, 716, 589. Melting point: 141 °C.
19. 2-[(2,6-Dimethylphenyl)amino]-3-hydroxy-1,4-napthoquinone, **4d**, dark purple solid purified by flash chromatography (hexane/ethyl acetate 9:1). Yield: 62.2% (water) 30.5% (ethanol) ^1H NMR (500 MHz, $CDCl_3$) δ_H: 7.99–7.97 (d, 7.2 Hz, 1H), 7.92–7.91 (d, 7.2 Hz, 1H), 7.61–7.52 (m, 2H), 7.06–7.0 (m, 3H), 2.17 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 181.0, 178,0. 135.8, 134.9, 133.6, 132.9, 132.0, 129.6, 127.0, 126.5, 125.7, 125.3, 124.7, 17.1; HRMS-ESI: $[M+H]^+$ calcd for $C_{18}H_{16}NO_3$, 294,1125 found: 294,1124; IV (ATR) ν (cm^{-1}): 3739, 3422, 3376, 3289, 3065, 2956, 2922, 2847, 2644, 1974, 1779, 1710, 1649, 1619, 1591, 1572, 1478, 1382, 1317, 1296, 1266, 1223, 1075, 1024, 975, 916, 879, 789, 775, 719, 569. Melting point: 151 °C.
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21. Adult Swiss albino mice weighing 20 ± 2 g were infected intraperitoneally with infected blood containing 1×10^5 *P. berghei* NK65. The mice were randomly allocated to groups of three to five animals per cage. The compounds were diluted in a Tween 20 solution with distilled water (final concentration of 2% Tween 20), 200 μL of the concentration of test solution was administrated orally, using the gavage technique, to each animal in dose of 100 mg/kg/day. The chloroquine was dissolved in distilled water and was administered orally as an antimalarial control reference at a dose of 10 mg/kg/day. A negative control group consisted of a 2% Tween 20 solution in distilled water that was orally administered to each animal.
22. Blood smears from all the mice were prepared, fixed with methanol, stained with Giemsa and examined microscopically (1000× magnification). The parasitemia was determined by counting the number of parasitized erythrocytes out of 1000–3000 in random fields of the microscope. Parasite growth inhibition in the drug-treated groups was defined as the parasitemia in the nontreated control group minus the parasitemia in the treated control group, divided by the parasitemia in the nontreated control group, expressed in percentages.
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