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A Single-Pot Synthesis of Atovaquone: An Antiparasitic Drug of Choice

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Supporting Information

ABSTRACT: The present article relates to a practical, economically viable, and validated at industrial scale, single-pot synthetic route for preparation of atovaquone, one of the most versatile antiparasitic drugs of choice used for the prophylaxis and treatment of diseases such as pneumocystis, toxoplasmosis, babesiosis, coccidiosis, and malaria. However, owing to the extremely poor yields of synthesis and very high doses of treatment (due to poor bioavailability) the cost of treatment with this drug is not affordable by the patients in need, particularly in the third world countries where these diseases are most prevalent. Unlike most of the reported processes which use 2-chloronaphthoquinone and pure *trans*-4-chlorophenyl cyclohexane carboxylic acid, our process is based on the decarboxylative alkylation of isomeric mixture of 4-chlorophenyl cyclohexane carboxylic acid with 1,4-naphthoquinone to give 42% overall yield of atovaquone, 10 times higher than from the reported process (4%) from the innovators of this drug.

INTRODUCTION

About 3.3 billion people, almost half of the world's population, is at risk of malaria, and therefore, undoubtedly the last few decades have seen significant progress towards treatment of malaria and efforts to develop new antimalarial agents. Artemisinins, having proven themselves to be life-saving drugs,[1−3] however, are not useful for malaria prophylaxis[4,5] because of their incapability of acting upon the initial liver stages of the malaria parasite. Malarone, a fixed dose combination of atovaquone with proguanil hydrochloride marketed by GlaxoSmithKline, on the other hand, stands alone for its versatility because it is useful for the treatment as well as casual prophylaxis of malaria in chloroquine-sensitive and -resistant strains of *Plasmodium falciparum*.[6] Atovaquone acts by inhibiting the mitochondrial electron transport system and thereby nucleotide biosynthesis,[7−10] and proguanil by synergistically blocking the alternate routes of electropotential generation via adenine nucleotide carriers (ANC) and decreasing the effective concentration of atovaquone required for killing the parasite[11−13] Atovaquone alone is also used against *Pneumocystis carinii*[14] and tachyzoite and cyst forms of *Toxoplasma gondii*[15,16] and is marketed as Mepron by GlaxoSmithKline for the treatment of pneumonia in patients intolerant to the first-line therapy using trimethoprim-sulphamethoxazole (TMP-SMX).[17] Treatment of babesiosis is yet another important indication of atovaquone when given in combination with azithromycin.[18] However, the only factor which has prevented Malarone from becoming first-line treatment for malaria is its very high cost as compared to other antimalarial drugs, including artemisinins. Indeed, there is an unmet need to develop an economically viable synthesis of atovaquone[19] to make this drug affordable to patients in the third world.

The first synthesis of atovaquone (**1**) reported by Hudson et al.[20,21] relies on the generation of a free radical of *trans*-4-chlorophenylcyclohexane from its carboxylic acid (**3**) using $AgNO_3$ and ammonium persulfate following a method reported by Jacobsen et al.[22] and subsequent condensation with 2-chloronaphthoquinone (**2**) to give chloroatovaquone (**4**). Hydrolysis of **4** with methanolic KOH gives the required product (**1**) in 4−4.5% overall yield in two steps (Scheme 1). The first report on the free radical-mediated alkylation of 2-hydroxy-1,4-naphthoquinone with cycloalkyl carboxylic acids, in general, was published in 1948 by Fieser[23] using diacyl peroxide to give respective products in a range from 5 to 6% (Scheme 2); however, it was not extended for the synthesis of **1**.

Two more processes along similar lines using 2-hydroxy-1,4-naphthoquinone (**13**) (Scheme 3)[24] and 2,3-dichloro-1,4-naphthoquinone (**15**) (Scheme 4)[25] instead of **2**, have recently been reported to give atovaquone in 23% and 38% yields, respectively. The main disadvantage of both processes is the cost and nonavailability of starting materials [2-hydroxy-1,4-naphthoquinone (**13**) and 2,3-dichloro-1,4-naphthoquinone (**15**)] and lower overall yields of the final product.

An additional route which starts from 4-(4-chlorophenyl)-cyclohexane oxalate (**8**)[26] and **2**, is reported to yield chloroatovaquone (**4**) in 43% yield, and would ultimately yield 25% or less of atovaquone if extrapolated using Hudson's[21] hydrolytic transformation of **4** to atovaquone (**1**), (Scheme 5). The most recent synthesis from GlaxoSmithKline[27] (Scheme 6) and an additional route using Barton's method[28] are novel from the chemistry point of view, but may not prove to be economical as both use exotic reagents and catalysts.

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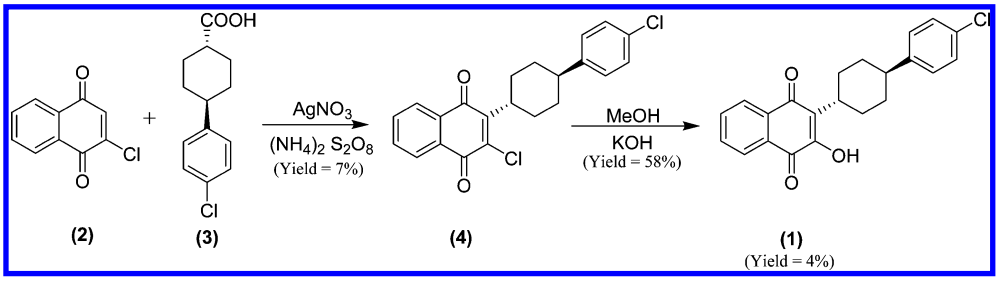
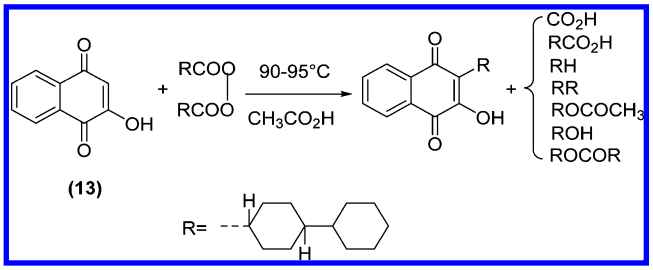
Scheme 1. Hudson's synthesis of atovaquone (1)



Scheme 2. Fieser's free radical-mediated alkylation of 2-hydroxy-1,4-naphthoquinone (13)



RESULTS AND DISCUSSION

The method reported by Hudson et al.[20,21] appeared to be too impractical for a large-scale preparation of atovaquone because of its poor yields. However, the reported process was repeated to examine and understand the factors responsible for lower yields. In addition, pure samples of the various intermediates were also obtained for quantitative monitoring of this condensation step.

Reactions of **2** with **3** [Scheme 7; Example (x) below] were performed in triplicate in the presence of powdered $AgNO_3$ and ammonium persulfate in acetonitrile/water as per the published method to get a semisolid mass after reaction work-up. Assay using HPLC and pure samples of **4**, **4a**, **6**, and **6a** (quantitative) revealed the crude product to be a mixture of mainly *trans*-isomer (**4**) (8−8.5%), *cis*-isomer (**4a**) (9.5−10%), the dechlorinated product as a mixture of **6** and **6a** (13−14%), and a substantial amount of unidentified polymeric material. Crystallization of the crude product thus obtained under reported conditions gave 97−98% pure **4** in 6−6.5% isolated yields. Hydrolysis of the latter following Hudson's[21] conditions gave pure atovaquone in 57−60% yield (maximum overall yield on **3** was ∼4%).

The learnings gleaned from these experiments are that the conditions and yields published by Hudson et al.[21] are reproducible, free radical-mediated alkylation of chloronaphthoquinone is responsible for the majority of the yield loss of the target product (i.e., atovaquone), the poor efficiency in the isolation of **4** from the crude product also contributes to the lower yields of atovaquone in 6−6.5% yields, the formation of dechlorinated product (a mixture of *trans*-**6** and *cis*-**6a** isomers) in the free radical-assisted reaction is a major source of yield loss, and the formation of the *cis*-isomer (**4a**) occurs even when pure *trans*-**3** is used as an alkylation agent in the first step.

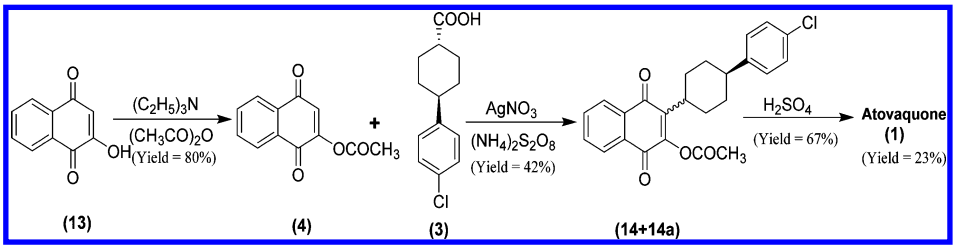
Free radical-mediated reactions in general are not very clean, but the formation of **6** and **6a** in high percentage was an important observation and suggested that, if the formation of these side products were avoided, then the overall yield would be more than double. It was found to be a challenge to reduce this to practice; however, rather than minimizing the formation of **6**, what appeared more interesting was to plan the synthesis of atovaquone (**1**) through this intermediate **6** itself. This was a novel approach that prompted the examination of the condensation of 1,4-naphthoquinone (**5**) with **3** to prepare **6** and exploration of its transformation into atovaquone (**1**).

Gratifyingly,[29] the reaction of **5** with **3** following Hudson's[21] conditions [Scheme 8; Example (i)] occurred smoothly. The crude product obtained from the reaction (run in triplicate) was analyzed to assess ratios and yields of **6** and **6a**, which was found to be in a range of 55−61% (Table 1). It is pertinent to mention that the reaction of **2** and **3** gave a yield of <19% under identical conditions (see Table 2). As detailed in Scheme 5, the crude product obtained from the reaction of **5** and **3** was crystallized from acetonitrile affording 97% pure *trans*-(**6**) in ∼20% yield.

After establishing the feasibility of the synthesis of **6** from **5**, the next step was the transformation to atovaquone which was envisioned by chlorinating **6** and subjecting the expected dichloro product **7** to dehydrohalogenation under standard conditions [(Scheme 9, Example (iii)].

The chlorination of **6** in acetic acid proceeded well to give the dichloro product[30] (**7**) with quantitative conversion of **6**. Though the isolation of the dichloro intermediate was not necessary, it was isolated and analyzed by HPLC. The analysis confirmed the presence of **7** as a mixture of two isomers in a ratio of 30:60 along with ∼7% of monochlorinated product **4**. The presence of ∼7% dehydrohalogenated product (**4**) in the reaction mass was encouraging and demonstrated the ease with

Scheme 3. Synthesis of atovaquone using 2-hydroxy-1,4-naphthoquinone (13)



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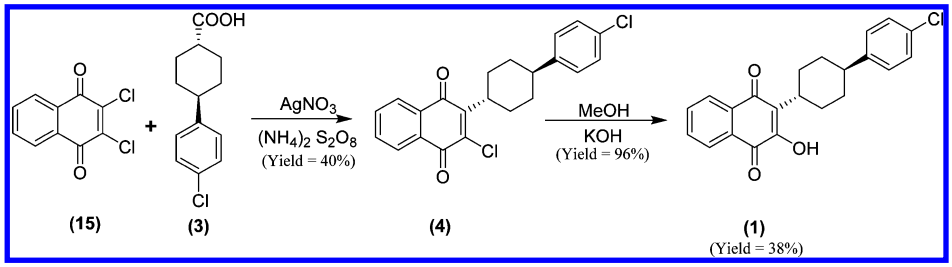
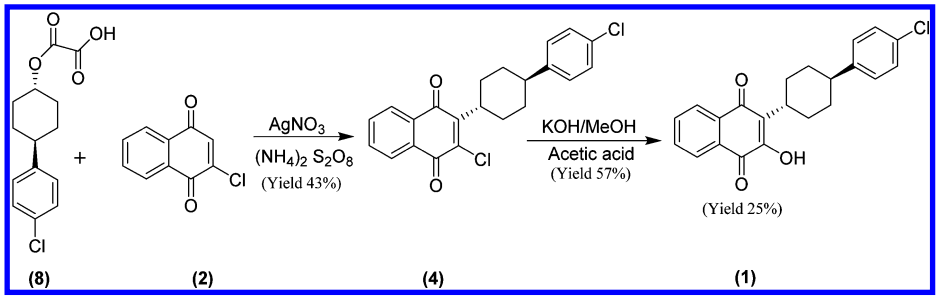
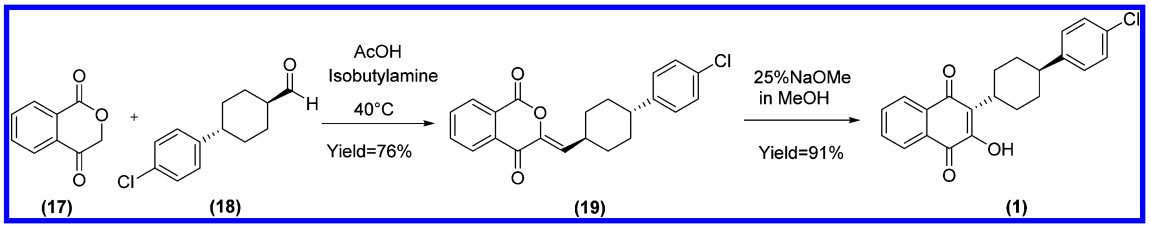
Scheme 4. Synthesis of atovaquone using 2,3-dichloro-1,4-naphthoquinone (15)



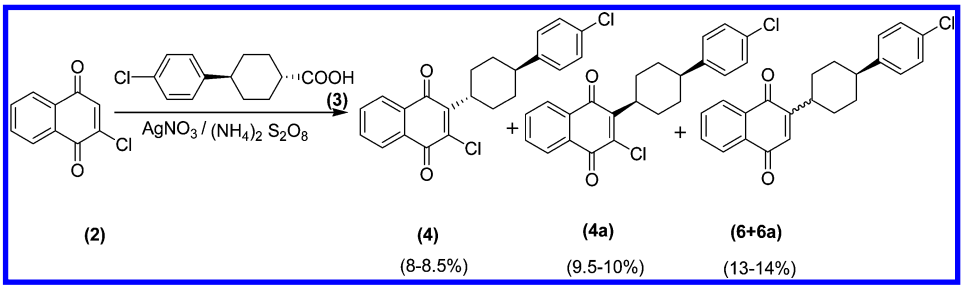
Scheme 5. Williams' synthesis of atovaquone (1)



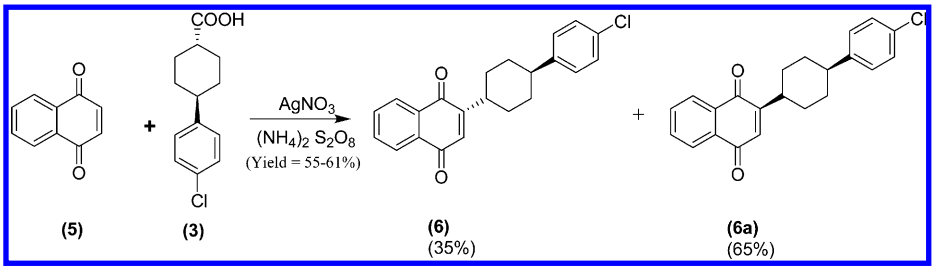
Scheme 6. GlaxoSmithKline's synthesis of atovaquone (1)



Scheme 7. Validation of Hudson's condensation of 2 with 3



Scheme 8. Condensation of 1,4-naphthoquinone (5) with 4-(4-chlorophenyl) cyclohexane carboxylic acid (3)



which elimination of hydrochloric acid was taking place from **7**. The mixture of **7** and **4** on treatment with anhydrous sodium acetate in acetic acid gave **4** as a single product. Hydrolysis of **4** with methanolic KOH, as previously presented, gave quantitative yield of atovaquone (**1**) of high purity (overall yield =13.5% on **3**, see Scheme 9).

It was encouraging to obtain 3−4-fold increase in yield in comparison to the original synthesis reported by Hudson;[21] however, it was also very clear that if atovaquone was to replace artemisinin as a first-line treatment for malaria, the synthesis could not afford to lose a significant amount of product as its unwanted isomer (**6a**), (almost 60% of the reaction products)

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Table 1. Reaction of 4-(4-chlorophenyl) cyclohexane carboxylic acid (3) and 1,4-naphthoquinone (5) following the method given in Example (i)

sr. no.	example no.	ratio of *cis*-isomer (**6a**) %	ratio of *trans*-isomer (**6**) %	HPLC assay of both isomers (**6** + **6a**) %
1	(i)-A	61.18	38.8	55.5
2	(i)-B	63.20	36.79	59.8
3	(i)-C	65.10	34.89	61.4

Table 2. Reaction of 4-(4-chlorophenyl) cyclohexane carboxylic acid (3) and 2-chloro-1,4-naphthoquinone (2) following the method given in Example (ix)

sr. no.	example no.	ratio of *cis*-isomer (**4a**) %	ratio of *trans*-isomer (**4**) %	HPLC assay of both isomers (**4** + **4a**) %
1	(x)-A	51.85	48.15	18.9
2	(x)-B	50.67	49.32	16.8
3	(x)-C	45.87	54.13	18.2

invariably formed during reaction of **3** with **5**. Since epimerization in a free radical-mediated reaction is typical, it was realized that it may not be possible to avoid the formation of **6a** in the condensation of **5** and **3**. The only practical way to improve overall yield of atovaquone would be utilizing the formation of **6a** and isomerization to the desired stereochemistry (**6**). A literature search did not reveal any method to convert **6a** directly to **6** via isomerization. However, the literature search led to reports claiming isomerization of *cis*-atovaquone (**1a**) as well as acetyl atovaquone (2-[4-(4-chlorophenyl)cyclohexyl]-3-acetoxy-1,4-naphthoquinone) to **1**.[24] Hence, the goal could in theory be achieved by isomerization at the final step, provided a practical method to convert **6a** into **1a** was available. Chlorination of **6a** and dehydrochlorination of the resulting dichloro intermediate (**7**) led to **4a** in 99% yield (Scheme 10). The basic hydrolysis of **4a** did indeed afford **1a**. The overall yield of **1a** starting from **6a** was 65%.

After establishing the successful transformation of **6a** to **4a** and **1a**, attention was focused on the isomerisation of **1a** to **1**. Thermal conditions as reported by Verma et al.[31]were initially investigated. Unfortunately, the reported results could not be repeated. The conditions, (99% H_2SO_4), as disclosed by Antonio Nardi et al.,[24] also did not yield the desired results. It was disappointing; however, the report on the formation of unwanted products (Scheme 11) in a similar study[32] on *cis*-2-hydroxy-3-(4-butylcyclohexyl)-1,4-naphthoquinone (**9**) at 55 °C further substantiated our findings that these conditions are somewhat not suitable for the desired transformation.

Being convinced that the acid-catalyzed isomerisation of **1a** to **1** should proceed; efforts continued and ultimately the desired transformation proceeded in nearly quantitative yield at ambient temperature (25−30 °C) when 90% H_2SO_4 was used. After our discloser[29] on the isomerisation of **1a** to **1**, another report claiming a similar transformation appeared;[33] however, inferior results in terms of yield and purity of atovaquone were obtained.

The current process takes care of most of the issues highlighted above wherein the chlorination is carried out on the mixture of **6** and **6a** to get dichloro product (**7**) as a mixture of isomers, which upon dehydrohalogenation, followed by hydrolysis, yields atovaquone as a mixture of *cis*- *trans*-isomers (**1a** and **1**). Upon treatment with 90% aqueous sulfuric acid as disclosed above, the mixture of **1a** + **1** leads to the desired product—atovaquone (**1**) through a single-pot synthesis, in 42% overall yield on the mixture of *trans*- and *cis*-4-chlorophenyl cyclohexanecarboxylic acid (**3** and **3a**), almost 10 times higher than reported by Hudson et al.[21] [Scheme 12; Example (xi)]

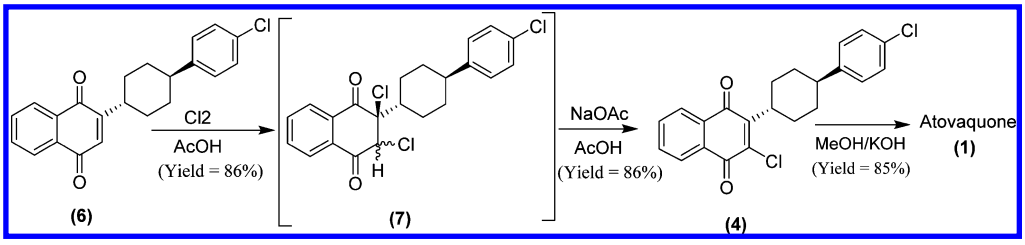
CONCLUSION

The work reported in this paper discusses a novel and cost-effective single-pot practical synthesis of atovaquone which takes care of most of the issues related to the synthesis and yield of this versatile antiparasitic drug, atovaquone. Unlike most of the reported processes which use 2-chloronaphthoquinone and pure *trans*-4-chlorophenyl cyclohexane carboxylic acid, our process is based on the decarboxylative alkylation of an isomeric mixture of 4-chlorophenyl cyclohexane carboxylic acid with 1,4-naphthoquinone to give 42% overall yield of atovaquone, 10 times higher than that from the reported process (4%) by the innovators of this drug.

EXPERIMENTAL SECTION

Example (i): *trans*-2-[4-(4-Cyclohexyl)chlorophenyl]-1,4-naphthoquinone (6). To a stirred solution of silver nitrate (2.13 kg, 12.54 mol) dissolved in 30 L water, *trans*-4-(4-chlorophenyl)cyclohexane carboxylic acid (**3**), (15.0 kg, 62.89 mol) was added. To this was added acetonitrile (75 L) under stirring. The reaction mixture was heated to reflux, and 1,4-naphthoquinone (**5**), (12.0 kg, 75.94 mol) was added in three lots. Ammonium persulfate (35.85 kg, 157.2 mol) dissolved in 90 L water was added over a period of 1 h to the stirred solution and maintained under reflux for half an hour. The reaction mass was cooled to 30−32 °C and extracted with 2 × 30 L methylene chloride. The organic layer was first washed with 30 L water and then with 2 × 30 L 10% sodium carbonate aqueous solution, followed by water until the pH became neutral. The reaction mass was distilled to remove methylene chloride and was stirred in 9.0 L acetonitrile and filtered. The

Scheme 9. Transformation of 6 to 4 by classical chlorination/dechlorination method



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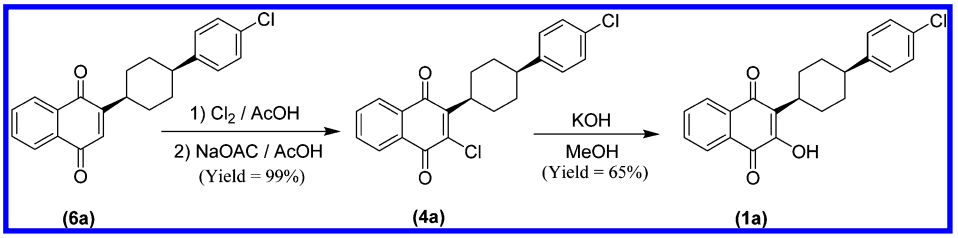
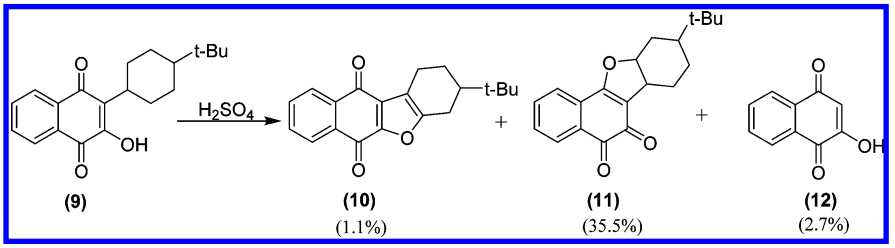
Scheme 10. Transformation of unwanted isomer (6a) into *cis*-isomer of atovaquone (1a)

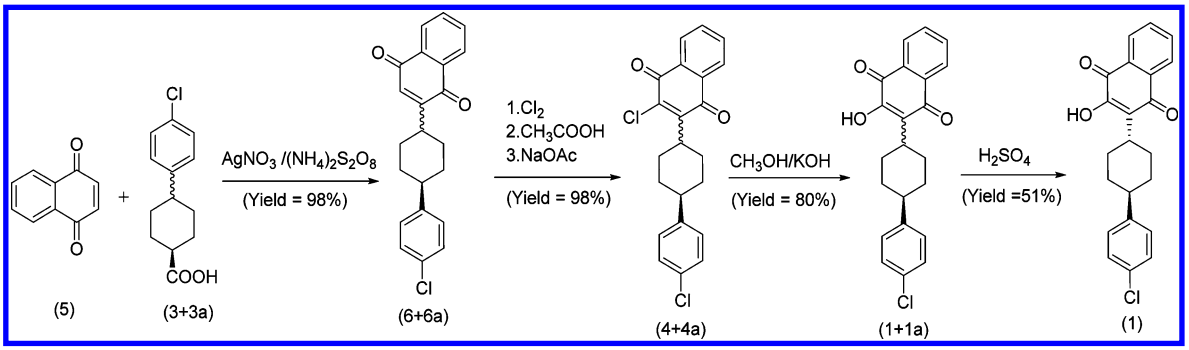


Scheme 11. Hudson's study on H_2SO_4-catalyzed degradation of *cis*-2-hydroxy-3-(4-butylcyclohexyl)-1,4-naphthoquinone (9)[a]



[a] *cis-trans*-2-Hydroxy-3-(4-butylcyclohexyl)-1,4-naphthoquinone (**9**), 3-*tert*-butyl-1,2,3,4-tetrahydrobenzo(b)naphtho[2,3-*d*]furan-6−11-quinone (**10**), 9-*tert*-butyl-6b,7,8,9,10,10a,-hexahydrobenzo-[*b*]naphtho[2,1-*d*]furan-5,6,quinine (**11**), 2-hydroxynaphthoquinone (**12**).

Scheme 12. Single-pot synthesis of atovaquone (1) from naphthoquinone (5) and isomeric mixture of 4-chlorophenyl cyclohexane carboxylic acid (3 and 3a)



solid thus obtained was heated to reflux in 2 × 14 L acetonitrile, cooled to 30−32 °C to crystallize, and filtered to get **6**. Yield: 4.6 kg (20.8%); mp 147−149 °C (uncorrected). IR (KBr) ν 780, 823, 1091, 1259, 1305, 1490, 1591, 1661, 2856, 2939, 3497 cm^{-1}; ^1H NMR ($CDCl_3$ with 0.03% TMS, 400 MHz): δ_H 8.08−8.15 (2H, m, H13 and H16), 7.74−7.77 (2H, m, H14 and H15), 7.28−7.30 (2H, d, J = 8.5 Hz, H19 and H21), 7.18−7.20 (2H, d, J = 8.5 Hz, H18 and H22.), 6.81 (1H, d, J = 1.2 Hz, H12), 2.99−3.05 (1H, tt, H4), 2.55−2.62 (1H, tt, H1), 2.01−2.05 (4H, m, H3 and H5), 1.42−1.72 (4H, m, H2 and H6). ^{13}C NMR ($CDCl_3$ with 0.03% TMS, 100 MHz) δ 185.8, 184.8, 155.7, 145.3, 133.7, 133.1, 132.5, 131.9, 131.7, 128.5, 128.2, 126.8, 126.0, 43.4, 36.2, 33.9, 32.2. HRMS calcd for $C_{22}H_{19}O_2Cl$ $[M + H]^+$ 351.11518, found 351.11544. The HPLC analysis using hypersil BDS, acetonitrile/water/methanol/ortho-phosphoric acid (525:300:175:5), 3 mL/min, 25 °C, shows purity more than 97% with *cis*-isomer being less than 0.5%.

Example (ii): Recycling of Silver Nitrate. To the stirred aqueous solution (180 L) obtained from the above experiment were gradually added silver nitrate (1.07 kg, 6.27 mol) and *trans*-4-(4-chlorophenyl)cyclohexane carboxylic acid (**3**, 15.0 kg, 62.89 mol). The reaction mixture was heated to reflux, and 1,4-naphthoquinone (**2**, 12.0 kg, 75.94 mol) was added in three lots. Ammonium persulfate (35.85 kg, 157.2 mol) dissolved in 90 L aqueous solution obtained from the above experiment was added to it over a period of 1 h. and maintained under reflux for half an hour. The reaction mixture was then cooled to 30−32 °C and extracted with 3 × 30 L methylene chloride. The organic layer was first washed with 50 L water followed by washing with 3 × 30 L 10% sodium carbonate aqueous solution, followed by water until the pH became neutral. The organic layer was distilled to eliminate methylene chloride and was stirred in 12.0 L acetonitrile and filtered. The solid thus obtained was further leached in 14 L acetonitrile to get **6** of 97.5% purity. Yield: 4.46 kg (19.98%). Mp 147−149 °C (uncorrected).

Example (iii): 2-[4-(4-Chlorophenyl)cyclohexyl]-2,3-dichloro-2,3-dihydro-1,4-naphthoquinone(7). To a stirred suspension of 2-[4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (**6**), (4.5 kg, 12.83 mol) in glacial acetic acid (22.5 L) was passed chlorine gas at about 20 °C. After completion, the reaction mass was filtered, washed with 0.5 L glacial acetic acid and then with water until the pH of the washing became neutral. The product was dried at 30−32 °C; Yield 4.65 kg (85.9%). The HPLC analysis using hypersil BDS, acetonitrile/water/methanol/*ortho*-phosphoric acid (525:300:175:5), 3 mL/min, 25 °C, showed it to be a mixture of two isomers in ratio of 30% and 60%, respectively, along with contamination of ∼7% monochlorinated product (**4**). The

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crude product as such was used in the next step without further purification.

Example (iv): 2-[4-(4-Chlorophenyl)cyclohexyl]-3-chloro-1,4-naphthoquinone (4). The crude product obtained from the above experiments (4.5 kg, 10.70 mol) was suspended in glacial acetic acid (36 L) and anhydrous sodium acetate (1.32 kg) was added to the reaction mixture. The latter was heated to reflux for 1 h, and 124 L water was added to the cooled reaction mixture. The precipitated product was filtered off, dried at 65 °C, and recrystallized from 360 L acetonitrile to get **4**. Yield: 3.66 kg (89.0%); mp185−187 °C; IR (KBr) ν 830, 848, 1083, 1282, 1458, 1566, 1593, 1668, 3071 cm^{-1}; ^1H NMR ($CDCl_3$ with 0.03% TMS, 400 MHz): δ 8.10−8.16 (m, 2H), 7.73−7.80 (m, 2H), 7.28−7.30 (d, 2H), 7.19−7.21 (d, 2H), 3.32−3.40 (tt, 1H), 2.66−2.74 (tt, 1H), 2.30−2.41 (m, 2H), 2.01−2.05 (m, 2H), 1.80−1.85 (m, 2H), 1.53−1.64 (m, 2H). The HPLC analysis using hypersil BDS, acetonitrile/water/methanol/*ortho*-phosphoric acid (525:300:175:5), 3 mL/min, 25 °C, confirmed the purity of *trans*-isomer to be more than 99.5%.

Example (v): Preparation of Atovaquone (1). 2-[4-(4-Chlorophenyl)cyclohexyl]-3-chloro-1,4-naphthoquinone (**4**), 3.5 kg, 9. 09 mol) was suspended in 105 L methanol, and potassium hydroxide (5.1 kg, 91.07 mol) dissolved in 35 L water was added under heating to 65−68 °C over a period of 20 min. Further, the reaction mixture was refluxed for 45 min and cooled to 30−32 °C and filtered. The filtrate was acidified with 50% aqueous hydrochloric acid to precipitate the product, which was filtered, dried, and recrystallized from 300 L acetonitrile to obtain **1**. Yield: 2.83 kg (85%); mp 219−221 °C; IR (KBr) ν 728, 822, 1089, 1278, 1347, 1369, 1490, 1595, 1659, 2855, 2926, 3377 cm^{-1}; ^1H NMR ($CDCl_3$ with 0.03% TMS, 400 MHz): δ 8.08−8.16 (m, 2H), 7.68−7.80 (m, 2H), 7.55 (s, 1H), 7.27−7.29 (d, 2H), 7.18−7.20 (d, 2H), 3.15−3.23 (tt, 1H), 2.62−2.69 (tt, 1H), 2.16−2.26 (m, 2H), 1.97−2.01 (m, 2H), 1.77−1.80 (m,2H), 1.54−1.65 (m, 2H). The HPLC analysis using hypersil BDS, acetonitrile/water/methanol/*ortho*-phosphoric acid (525:300:175:5), 3 mL/min, 25 °C, confirmed the purity of atovaquone to be more than 99.8%, without a detectable amount of *cis*-isomer and no other single impurity more than 0.05%.

Example (vi): Preparation of Atovaquone (1). To a solution of silver nitrate (14.17 g, 0.08 mol) dissolved in 200 mL water were added *trans*-4-(4-chlorophenyl)cyclohexane carboxylic acid (**3**) 100 g (0.42 mol) and acetonitrile (500 mL). The solution was heated to reflux followed by addition of 1,4-naphthoquinone (**5**), (80 g, 0.51 mol). A solution of ammonium persulfate (239 g; 1.05 mol) in water (600 mL) was added dropwise to the above solution and reflux continued for half an hour. The reaction mass was then cooled to 30−32 °C and extracted with methylene chloride. The organic layer was first washed with water, followed with 10% sodium carbonate aqueous solution, and then with water until the pH was neutral. The organic layer was concentrated to afford a solid mass which was dissolved in glacial acetic acid (745 mL), and chlorine gas was passed at about 20 °C under continuous stirring, and the reaction was monitored by TLC. After the complete disappearance of the starting material, nitrogen gas was purged to remove excess chlorine from the reaction mass. Anhydrous sodium acetate (52.3 g) was then added, and the reaction mass was heated to reflux for 90 min. The reaction mass was cooled and quenched into a mixture of methylene chloride and water. The methylene chloride was washed with water until the pH was neutral. The solvent was then concentrated to get 155 g of 2-[4-(4-chlorophenyl)cyclohexyl]-3-chloro-1,4-naphthoquinone (**4** + **4a**) which was dissolved in 4.5 L methanol. A solution of potassium hydroxide (225 g, 4.02 mol) in water (1550 mL) was added dropwise under heating over a period of 1 h. The reaction mass was then refluxed for 2 h and cooled to 30−32 °C and filtered. The filtrate was acidified with 50% aqueous hydrochloric acid to precipitate the solid mass as a mixture of *trans*- and *cis*-atovaquone (**1** + **1a**), yield 125 g (81.3%).

Example (vii): Preparation of *cis*-Atovaquone (1a) from *cis*-2-[4-(4-chlorophenyl) cyclohexyl]-1,4-naphthoquinone (6a). To a stirred suspension of *cis*-2-[4-(4-chlorophenyl) cyclohexyl]-1,4-naphthoquinone (**6a**), (5.0 g, 0.014 mol) in glacial acetic acid (37.5 mL) was passed chlorine gas at about 20 °C under continuous stirring, and the reaction was monitored by TLC. After the complete disappearance of the starting material, nitrogen gas was purged to remove excess chlorine from the reaction mass. Anhydrous sodium acetate (1.47 g) was then added, and the reaction mass was heated to reflux for 90 min. The reaction mass was cooled and quenched into a mixture of methylene chloride and water. The methylene chloride was washed with water until neutral pH. The solvent was then concentrated to get 5.5 g *cis* 2-[4-(4-chlorophenyl) cyclohexyl]-3-chloro-1,4-naphthoquinone (**4a**) which was dissolved in 165 mL methanol. A solution of potassium hydroxide (8 g, 0.14 mol) in water (55 mL) was added dropwise under heating over a period of 1 h. The reaction mass was then refluxed for 2 h and cooled to 30−32 °C and filtered. The filtrate was acidified with 50% aqueous hydrochloric acid to precipitate a solid mass which on recrystallization from acetonitrile gave 3.4 g (65%) *cis*-atovaquone (**1a**).

Example (viii): Preparation of Atovaquone (1). To a solution of silver nitrate (14.17 g, 0.08 mol) dissolved in 200 mL water were added *trans*-4-(4-chlorophenyl)cyclohexane carboxylic acid (**3**) [100 g (0.42 mol)] and acetonitrile (500 mL). The solution was heated to reflux followed by addition of 1,4-naphthoquinone (**5**), (80 g, 0.51 mol). A solution of ammonium persulfate (239 g; 1.05 mol) in water (600 mL) was added dropwise to the above solution, and reflux was continued for half an hour. The reaction mass was then cooled to 30−32 °C and stirred with ethyl acetate (400 mL) and the lower aqueous layer was allowed to settle and was removed; the upper ethyl acetate layer was washed with 10% sodium carbonate aqueous solution, and then with water until neutral pH. The organic layer was concentrated to afford a solid mass which was dissolved in glacial acetic acid (375 mL) in the same pot, and chlorine gas was passed at about 20 °C under continuous stirring; the reaction was monitored by TLC. After the complete disappearance of the starting material, nitrogen gas was purged to remove excess chlorine from the reaction mass. Anhydrous sodium acetate (52.3 g) was then added, and the reaction mass was heated to reflux for 90 min. The acetic acid was distilled out completely to get 2-[4-(4-chlorophenyl)-cyclohexyl]-3-chloro-1,4-naphthoquinone (**4** + **4a**) which was dissolved in 2.25 L methanol. A solution of potassium hydroxide (225g, 4.02 mol) in water (1550 mL) was added dropwise under reflux over a period of 1 h. The reaction mass was then refluxed for 2 h, cooled to 30−32 °C, and acidified with 50% aqueous hydrochloric acid to precipitate a solid mass as a mixture of *trans*- and *cis*-atovaquone (**1** + **1a**), yield 130 g (84.6%).

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Example (ix): Epimerization of Mixture of Atovaquone (1 + 1a). The *cis*-/*trans*-isomeric mixture (130 g) obtained from the above Example (viii) was added to 1140 mL of 90% sulfuric acid and stirred at 28−30 °C. The reaction was monitored by HPLC, and when the *cis*-isomer had reached a level of less than 0.5%, the reaction mixture was quenched into ice−water, filtered, and washed with water until neutral pH. The crude product was purified by column chromatography on silica gel (1% ethyl acetate/hexane) affording 64.5 g atovaquone (yield 42% from **3**). The HPLC analysis using hypersil BDS, acetonitrile/water/methanol/*ortho*-phosphoric acid (525:300:175:5), 3 mL/min, 25 °C, confirmed the purity of atovaquone to be more than 99.8%, without a detectable amount of *cis*-isomers and no other single impurity more than 0.05%.

Example (x): 2-Chloro-[4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (4). A mixture of 2-chloro-1,4-naphthoquinone (**2**), (8.07 g, 0.04 mol), *trans* 4-(4-chlorophenyl)-1-cyclohexane carboxylic acid (**3**), (10 g, 0.04 mol), and silver nitrate (2.14 g,0.01 mol) was heated to vigorous reflux in 80 mL acetonitrile. A solution of ammonium persulfate (24.0 g, 10.11 mol) in 100 mL water was added over a period of 1.0 h. The mixture was refluxed for 3 h, then cooled in ice for 30 min, extracted with 2 × 50 mL boiling MDC, washed with water, and distilled off when a yellow−brown mass was obtained. The HPLC and mass spectra of this material showed the presence of *cis*- and *trans*-isomers of 2-chloro-[4-(4-chlorophenyl) cyclohexyl]-1,4-naphthoquinone (**4a** and **4**) as well as the mixture of *cis*- and *trans*-isomers of 2-[4-(4-chlorophenyl)cyclohexyl]-1,4-naphthoquinone (**6** and **6a**).

Example (xi): Preparation of Atovaquone (1) from a mixture of 3 and 3a. To a solution of silver nitrate (19.33 g, 0.11 mol) dissolved in 272 mL water were added *cis*- and *trans*-4-(4-chlorophenyl)cyclohexane carboxylic acid [200 g (0.8385 mol)] (**3** + **3a**) and acetonitrile (680 mL). The solution was heated to reflux followed by addition of 1,4-naphthoquinone (**5**) (108.11 g, 0.68 mol). A solution of ammonium persulfate (325.3 g; 1.43 mol) in water (816 mL) was added dropwise to the above solution, and reflux was continued for 30 min. The reaction mass was then cooled to 30−32 °C and extracted with methylene chloride. The organic layer was first washed with 50% hydrochloric acid followed by 10% sodium carbonate aqueous solution, then 50% hydrochloric acid, and then with water until neutral pH. The organic layer was concentrated to dryness, glacial acetic acid (1.02L) was added, and chlorine gas was passed at about 20 °C. After the complete disappearance of the starting material (as monitored by TLC), nitrogen gas was purged to remove excess chlorine from the reaction mass. Anhydrous sodium acetate (63.09 g, 0.77 mol) was then added, and the reaction mass was heated to reflux for 120 min. The reaction mass was cooled and quenched into a mixture of methylene chloride and water. The methylene chloride was then washed with water until neutral pH. The solvent was concentrated to obtain the residue containing a mixture of compound **4** and **4a**, and the residue was then stirred with 6.39 L methanol. A solution of potassium hydroxide (306.4 g, 5.47 mol) in water (2.038 L) was added dropwise under heating over a period of 2 h. The reaction mass was then refluxed for 2.5 h and cooled to 30−32 °C and filtered. The filtrate was neutralized with 50% aqueous hydrochloric acid to precipitate a mixture of **1** and **1a**. It was then filtered and washed with water until neutral pH and dried in an oven at 65 °C. Dry atovaquone (**1**+**1a**) (207.6 g) was obtained which was added to 2.076 L of 90% sulfuric acid and stirred at 28−30 °C for 4 h. The reaction was monitored by HPLC, and when the *cis*-isomer (**1a**) was less than 0.5%, the reaction mixture was quenched into ice−water, filtered, and washed with water until neutral pH. The crude product was purified by column chromatography on silica gel (1% ethyl acetate/hexane) affording atovaquone (**1**) (126 g). Yield: 41%. HPLC purity: 99.8%.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR for structures no. 1, 4 and 6; details of HRMS measurements and HRMS spectrum structure no. 6, ^{13}C NMR spectrum and 2D NMR spectrum for structure no. 6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ REFERENCES

(1) Sutherland, C. J.; Ord, R.; Dunyo, S.; Jawara, M.; Drakeley, C. J.; Alexander, N.; Coleman, R.; Pinder, M.; Walraven, G.; Targett, G. *PLoS Med.* **2005**, *2*, e92.

(2) Mclntosh, H. M.; Olliaro, P. *Med. Trop (Marseille)* **1998**, *58* (3 Suppl.), 61−62.

(3) Gabor, A. B. *Pharmacol. Ther.* **2001**, *90* (2−3), 261−265.

(4) Olumese, P. *Guidelines for the Treatment of Malaria*; World Health Organization: Geneva, 2006.

(5) Lucy, C. O.; Chris, J. D.; Teun, B.; Christopher, J. W.; Azra, C. G. *PLoS Med.* **2008**, *5* (11), e226.

(6) Marra, F.; Salzman, J. R.; Ensom, M. H. *Ann. Pharmacother.* **2003**, *37* (9), 1266−1275.

(7) Fry, M.; Pudney, M. *Biochem. Pharmacol.* **1992**, *43*, 1545−1553.

(8) Srivastava, I. K.; Rottenberg, H.; Vaidya, A. B. *J. Biol. Chem.* **1997**, *272*, 3961−3966.

(9) Biagini, G. A.; Vinayavejakul, P.; O'Neill, P. M.; Bray, P. G.; Ward, S. A. *Antimicrob. Agents Chemother.* **2006**, *50*, 1841−1851.

(10) Seymour, K. K.; Yeo, A. E.; Rieckmann, K. H. *Ann. Trop. Med. Parasitol.* **1997**, *91*, 603−609.

(11) Srivastava, I. K.; Vaidya, A. B. *Antimicrob. Agents Chemother.* **1999**, *43* (6), 1334−1339.

(12) Painter, H. J.; Morrisey, J. M.; Mather, M. W.; Vaidya, A. B. *Nature.* **2007**, *446*, 88−91.

(13) Painter, H. J.; Morrisey, J. M.; Vaidya, A. B. *Antimicrob. Agents Chemother.* **2010**, *54* (12), 5281−5287.

(14) Baggish, A. L.; Hill, D. R. *Antimicrob. Agents Chemother.* **2002**, *46*, 1163−1173.

(15) Romand, S.; Pudney, M.; Derouin, F. *Antimicrob. Agents Chemother.* **1993**, *37*, 2371−2378.

(16) Guttendge, W. E.; Latter, V. S. Hudson, A. T. Naphthoquinones for the treatment and prophylaxis of Pneumocystis carinii infections. EP0362996 B1. 27 Apr 1994; *Chem. Abstr.* **1990**, *113*, 152064.

(17) Hughes, W. T.; LaFon, S. W.; Scott, J. D.; Masur, H. J. *Infect. Dis.* **1995**, *171*, 1295−1301.

(18) Wittner, M.; Lederman, J.; Tanowitz, H. B.; Rosenbaum, G. S.; Weiss, L. M. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 219−222.

(19) Nixon, G. A.; Moss, D. M.; Shone, A. E.; Lalloo, D. G.; Fisher, N.; O'Neill, P. M.; Ward, S. A.; Biagini, G. A. *J. Antimicrob. Chemother.* **2013**, 1−9.
(20) Hudson, A. T. Randall, A. W. Naphthoquinone derivatives. EP123238. 1 July, 1992; *Chem. Abstr.* **1985**, *102*, 113082.
(21) Hudson, A. T. Latter, V. Medicaments. EP0580185A1. 26 Jan, 1994; *Chem. Abstr.* **1990**, *113*, 152064.
(22) Jacobsen, N.; Torrsell, K. J. *Liebigs Ann. Chem.* **1972**, *736*, 135−147.
(23) Fieser, L. *J. Am. Chem. Soc.* **1948**, *70* (10), 3174−3215.
(24) Antonio, N.; Mara, S.; Annibale, S. Stefano, M. Process for the preparation of trans-2,3-disubstituted naphthoquinones. U.S. Patent 7,842,840 B2. 30 Nov, 2010; *Chem. Abstr.* **2008**, *149*, 576277.
(25) Saralya, S. S.; Shasikumar, S. H.; Shashipraba; Kanakamajalu, S.; Koottungalamadhom, R. R.; Ananathalakshmi V.; Govindarajalu, J.; Rao, K. S. Nagarajan, K. Preparation of naphthoquinone compounds using 2,3-dihalonaphthoquinone. U.S. Patent Appl. 0004024 A1. 6 Jan 2011; *Chem. Abstr.* **2009**, *151*, 425364.
(26) Williams, D. R.; Clark, M. P. *Tetrahedron Lett.* **1998**, *39*, 7629−7632.
(27) Dwyer, A. N.; Gordon, A. Urquhart, M. Novel process. WO/2012/080243 A2. 21 June, 2012; *Chem. Abstr.* **2012**, *157*, 133525.
(28) Zhu, F.; Qiao, H. Bekhazi, M. A process for preparing atovaquone and associate intermediates. WO/2010/001379 A1. 7 Jan, 2010; *Chem. Abstr.* **2010**, *152*, 119262.
(29) Kumar, A.; Dike, S. Y.; Mathur, P. K.; Thankachen, B. N.; Sharma, B.; Kore, S. S. Buchude, V. S. Process for preparation of atovaquone and novel intermediates thereof. U.S. Patent 7,847,127 B2. 7 Dec, 2010; *Chem. Abstr.* **2010**, *152*, 429385.
(30) Romily, C. A.; Peter, B. D.; Mare, D. L.; Larsen, D. S. *J. Chem. Soc., Perkin Trans.* **1983**, *2*, 271−279.
(31) Verma, S. S.; Patel, D. J. Dwivedi, S. D. Process for preparation of atovaquone and the conversion of cis-isomer to trans-isomer. WO/2008/122988 A1. 16 Oct, 2008; *Chem. Abstr.* **2008**, *149*, 471210.
(32) Hudson, A. T.; Randall, A. W. *Eur. J. Med. Chem.* **1986**, 271−275.
(33) Zhu, F. Bekhazi, M. Process for the epimerization of atovaquone isomer, atovaquone intermediates and mixture thereof. U.S. Patent 0144347 A1. 16 June, 2011; *Chem. Abstr.* **2010**, *152*, 153712.