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Synthesis and Antimalarial Activities of New Hybrid Atokel Molecules

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The currently spreading resistance of the malaria parasite *Plasmodium falciparum* to artemisinin-based combination therapies makes an urgent need for new efficient drugs. Aiming to kill artemisinin-resistant *Plasmodium*, a series of novel hybrid drugs named Atokels were synthesized and characterized. Atokels are based on an 8-amino- or 8-hydroxyquinoline entity covalently bound to a 1,4-naphthoquinone through a polyamine linker. These drugs have been designed to target the parasite mitochondrion by their naphthoquinone moiety reminiscent of the antimalarial drug atovaquone, and to trigger a damaging oxidative stress due to their ability to chelate metal ions in order to generate redox active complexes in situ. The most effective Atokel drug shown a promising antimalarial activity (IC_{50} = 622 nM on an artemisinin-resistant *P. falciparum* strain) and no cytotoxicity at 50 μM indicating a specific antiplasmodial mode of action.

Introduction

Malaria is a major public health issue for more than three billion of people living in endemic areas, and also a significant threat for millions of travelers. Five different *Plasmodium* species are responsible for infection of 230 million people and death of more than 400,000 people each year, making malaria one of the three main infectious causes of death with tuberculosis and AIDS.[1]

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Currently, treatment of malaria relies on chemotherapy by using medicines acting on the blood stage of this parasitic disease. Artemisinin-based combination therapies (ACTs) that associates a derivative of the naturally occurring endoperoxide artemisinin (ART) to an antimalarial drug belonging to another therapeutic class, are the current recommended drug regimens. Then, most commonly used ACTs consist of an artemisinin derivative (dihydroartemisinin, or artemether, or artesunate) associated to mefloquine, lumefantrine, amodiaquine, sulfadoxine-pyrimethamine, piperaquine, or pyronaridine.[2] Unfortunately, the emergence of *Plasmodium falciparum* resistance to artemisinin derivatives and ACTs in southeast Asia threatens world's malaria control and may reduce in the near future the efficacy of the first-in-class antimalarial drugs.[3,4] Therefore, it is urgent to develop new drugs efficient against currently resistant malaria parasites.[5]

The antimalarial activity of ART is based on intraparasitic reductive activation of the peroxidic bond of the drug by iron(II)-heme generated by the parasite induced hemoglobin catabolism, and subsequent alkylation of heme by the drug derived C-centered radical.[6] Understanding of the main feature of the mechanism of action of artemisinin prompted us and later several other research groups to design synthetic peroxide hybrid molecules such as trioxaquines[5b,7–9] as new antimalarial drugs. Unfortunately, *P. falciparum* parasites resistant to both artemisinin derivatives and a partner drug such as mefloquine or piperaquine recently emerged.[10–13] Moreover, it was demonstrated that trioxaquines exhibited cross-resistance with artemisinin, in both the experimental artemisinin-resistant parasite line (F32-ART) and in Cambodian field isolates. Trioxaquine drug pressure in vitro selected trioxaquine-resistant parasites with the same genetic trait (K13 mutation) than artemisinin-resistant parasites, thus raising concerns about the potential place of endoperoxide-based antiplasmodials in the future therapeutic arsenal.[14]

Then, with the aim to kill multiresistant strains, we designed new hybrid antimalarial drugs with a dual mode of action, due

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to the presence within a single molecule of two pharmacophores that are not necessarily expected to act on the same biological target.[9] As pharmacophores, we used the naphthoquinone ring reminiscent of atovaquone and different 2-substituted-8-aminoquinolines related to the TDMQ series (Figure 1). Atovaquone has a potent antimalarial activity,[15] and is until to now the only antimalarial drug active on artemisinin-resistant *Plasmodium* strains in vitro.[13,16] This drug behaves as a competitive inhibitor of ubiquinone and is able to block the parasite respiratory chain, a mechanism that remains active in resistant and quiescent *Plasmodium* parasites.[16] On the other hand, TDMQ drug-candidates (Figure 1) have been designed as copper chelators, in order to target the copper dyshomeostasis in the brain of patients with Alzheimer's disease (AD).[17–19]

The ability of several TDMQ chelators to limit the oxidative stress created by storage of copper in amyloids is due to their specific affinity for copper(II). In fact, TDMQ20 or TDMQ22 providing a perfect square-planar N_4-coordination sphere around copper(II) have very strong affinities for copper(II) ($\log K_{app} = 15–17$), and are consequently able to fully inhibit the oxidative stress induced by Cu–Aβ_{1-16}. In contrast, TDMQ5 or TDMQ19 providing a distorted N_3X-coordination to copper, exhibit moderate affinities for copper(II) ($\log K_{app} = 10$). Consequently, TDMQ5 and TDMQ19 are able to provide a Cu^{II}/Cu^{I} redox cycle and, consequently, trigger ROS (reactive oxygen species) production.

In order to disrupt the mitochondrial activity of artemisinin-resistant parasites (and, consequently, kill them), our idea was to associate the naphthoquinone moiety of atovaquone to a chelator based on a TDMQ skeleton able to recruit in situ redox-active metal ions and, consequently, able to create an oxidative stress within mitochondria that are rich in oxygen and electrons.

Here, we report synthesis and physico-chemical properties of such naphthoquinone-chelator hybrid molecules, named Atokel, and also their biological activities on *Plasmodium* parasites.

Results and Discussion

Synthesis and Characterization

Structural modulations of the Atokel series were carried out by preparing compounds with hydrogen and/or chlorine substituents at C5 and C7 of the 8-aminoquinoline nucleus (R_5 and R_7, Table 1a). In fact, other factors being equal, these substituents allowed to modulate affinity of TDMQ ligands for copper(II) by 2 orders of magnitude.[18] The role of i) substituent at position 3′ of the naphthoquinone moiety (R_3', Table 1a), ii) substituents at the R_3'-R_8' positions of the naphthoquinone (Table 1b), and iii) a 8-hydroxyquinoline moiety to be compared to the drugs based on an 8-aminoquinoline (compounds **3**, **4**, and **14**, Table 1c, to compare to **1**, **2**, and **13**, respectively) were also investigated. This series includes 5′,8′-dihydroxy-1′,4′-naphthoquinone derivatives (**Atokel-19** to **-24**), a scaffold known to have η^2 metal chelating properties.[20] We also tried to synthesize Atokel derivatives with a hydroxyl group at C3′, like in atovaquone itself, by deprotection of the C3′-tosylate derivatives. Unfortunately, all attempts to get 3′-OH-Atokel derivatives failed until to now.

The syntheses of Atokel derivatives are summarized in Schemes 1, 3 and 4. We presented separately synthetic pathways of atokel derivatives containing the 8-aminoquinoline moiety (Scheme 1), the 8-hydroxyquinoline moiety (scheme 3) and the 8-aminoquinoline moiety and a 3′-tosylate on the naphthalene-1,4-dione moiety (Scheme 4). Each intermediate compound was numbered as "X-Atokel-n", where X stands for the specific letter of the given intermediate, followed by the target Atokel, with n = 1–24. **Atokel-1-8** and **Atokel-13-24** have been synthesized from the commercially available 3,5-dichloroaniline or 3-chloro-2-nitroaniline (**A**, Scheme 1A), or 2-methyl-8-nitroquinoline (**C**, Scheme 1A) or 2-methyl-8-hydroxyquinoline (= 2-methylquinolin-8-ol, L, Scheme 3).

Oxidation of the 2-methyl-8-nitroquinoline ring to a 2-vinyl-8-nitroquinoline by using $FeCl_3$ and $K_2S_2O_8$, was a pivotal step

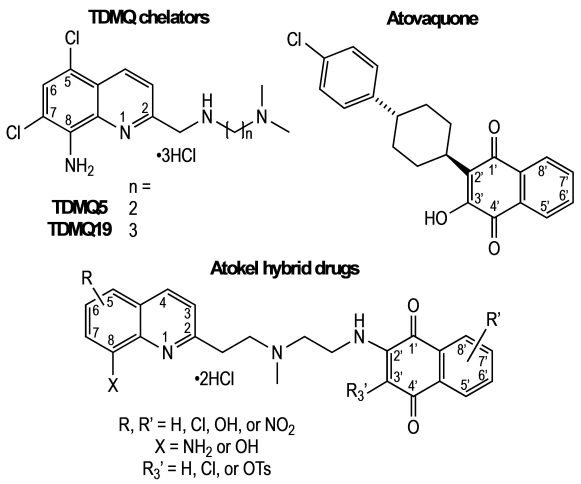
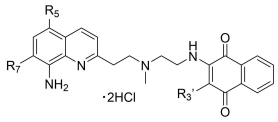


Figure 1. Structures of TDMQ chelators, atovaquone, and the Atokel hybrid drugs.

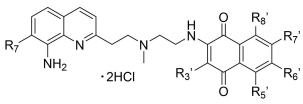
Table 1. Modulation of the structure of atovaquone-chelator (Atokel) drugs.

a) Variation of R_5 and R_7 of the 8-aminoquinoline, and of R_3' of the naphthoquinone



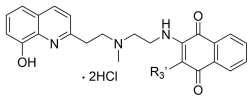
R_5	R_7	R_3'	Atokel-n with n=
H	H	H	**1**
Cl	Cl	H	**5**
H	Cl	H	**7**
H	H	Cl	**2**
Cl	Cl	Cl	**6**
H	Cl	Cl	**8**
H	H	OTs	**13**
Cl	Cl	OTs	**15**
H	Cl	OTs	**16**

b) Variation of R_3'-R_8' of the naphthoquinone



R_7	R_3'	R_5'	R_6'	R_7'	R_8'	Atokel-n with n=
H	Cl	H	H	H	NO_2	**17**
H	Cl	NO_2	H	H	H	**18**
H	H	OH	Cl	Cl	OH	**19**
H	Cl	OH	H	H	OH	**24**
Cl	Cl	H	H	H	NO_2	**20**
Cl	Cl	NO_2	H	H	H	**21**
Cl	H	OH	Cl	Cl	OH	**22**
Cl	Cl	OH	H	H	OH	**23**

c) 8-Hydroxyquinolines for comparison with 8-aminoquinolines



R_3'	Atokel-n with n=
H	**3**
Cl	**4**
OTs	**14**

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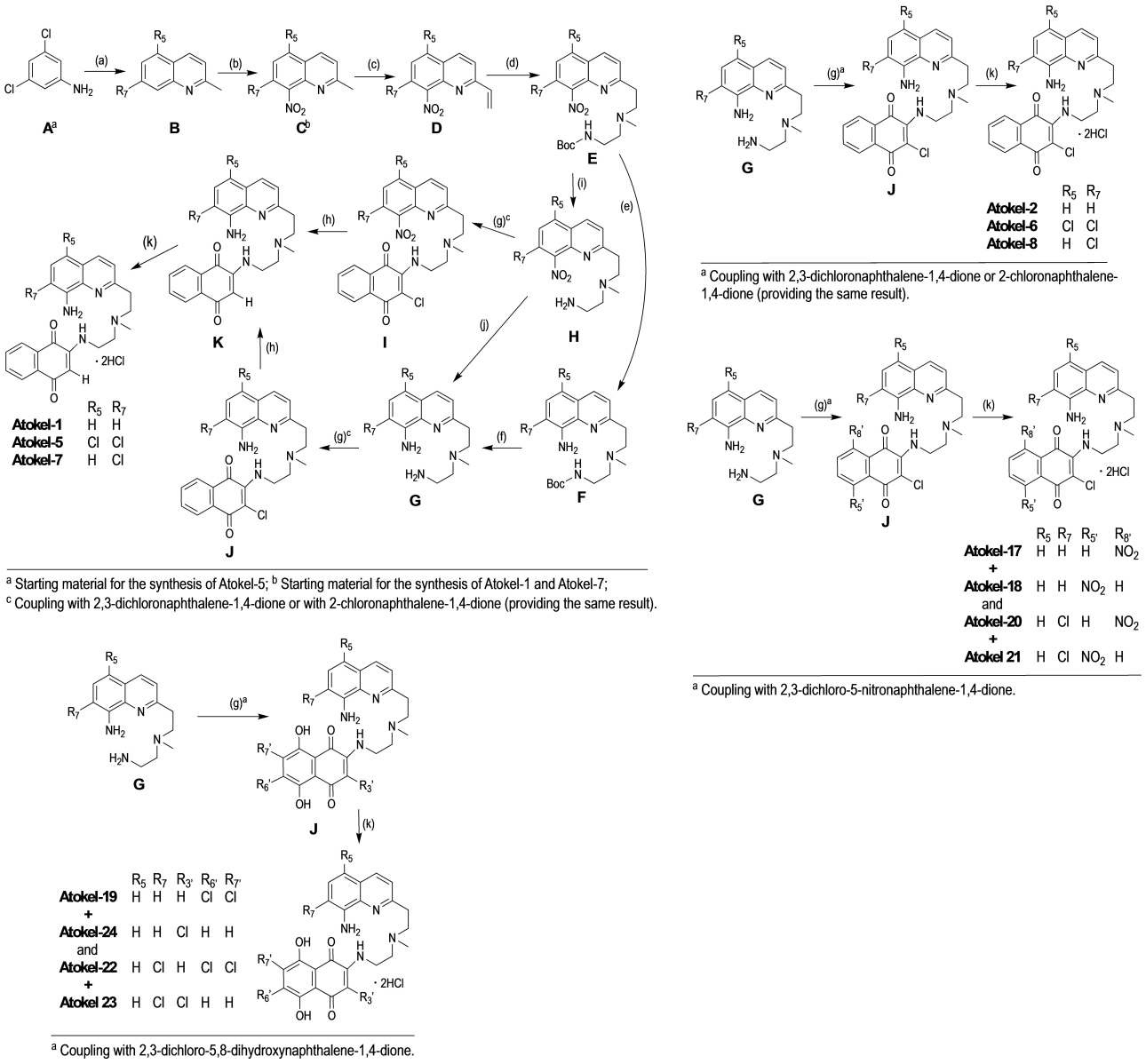

[a] Starting material for the synthesis of Atokel-5; [b] Starting material for the synthesis of Atokel-1 and Atokel-7; [c] Coupling with 2,3-dichloronaphthalene-1,4-dione or with 2-chloronaphthalene-1,4-dione (providing the same result).

[a] Coupling with 2,3-dichloronaphthalene-1,4-dione or 2-chloronaphthalene-1,4-dione (providing the same result).

[a] Coupling with 2,3-dichloro-5-nitronaphthalene-1,4-dione.

[a] Coupling with 2,3-dichloro-5,8-dihydroxynaphthalene-1,4-dione.

Scheme 1. General scheme of the syntheses of Atokel ligands without substituent on the 1,4-naphthoquinone residue (panel A, **Atokel-1**, **-5**, and **-7**), with a 3′-chloro-1,4-naphthoquinone (panel C, **Atokel-2**, **-6** and **-8**), with a 5′,8-dihydroxy-1,4-naphthoquinone moiety (panel B, **Atokel-19**, **-22**, **-23**, and **-24**), and with 5′- or 8′-nitro-1,4-naphthoquinone (panel D, **Atokel-17**, **-18**, **-20**, and **-21**). Reaction conditions of each step are the following: (a) CH_3CHO, 12 M HCl, 4 h; (b) HNO_3/H_2SO_4, RT, 1 h; (c) $FeCl_3$, $K_2S_2O_8$, *N*,*N*-dimethylacetamide (DMA), 110 °C, 20 min to 3.5 h; (d) Cs_2CO_3, *tert*-butyl(2-(methylamino)ethyl)carbamate, 1,4-dioxane, overnight; (e) Pd/C 5 wt%, $N_2H_4{\cdot}H_2O$, *i*-PrOH, 80 °C, 5 min to 1 h; (f) CF_3COOH, CH_2Cl_2, RT, overnight; (g) 2-chloro-1,4-naphthoquinone or 2,3-dichloro-1,4-naphthoquinone (panels A and C), or 2,3-dichloro-5-nitro-1,4-naphthoquinone (panel D), or 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (panel B), K_2CO_3 or not/or CH_3COOH, CH_3CN, RT, overnight; (h) Pd/C 5 wt%, $N_2H_4{\cdot}H_2O$, i-PrOH, 80 °C, 5 min to1 h; (i) CF_3COOH, CH_2Cl_2, RT, overnight; (j) Pd/C 5 wt%, $N_2H_4{\cdot}H_2O$, i-PrOH, 80 °C, 5 min to 40 min; (k) HCl, EtOH, CH_2Cl_2, 1 h.

[Scheme 1A, step (c)] to introduce the polyamine side chain (Compound **E**). The nitro group was then reduced by Pd/C (5 wt%) and hydrazine, to afford intermediate **F**. Deprotection of the distal amine by removal of the butyloxycarbonyl group was achieved using trifluoroacetic acid, to yield **G**. The terminal primary amine then reacted with the commercially available 5-nitro-2,3-dichloro-1,4-naphthoquinone (**Atokel-17** and **-18**), or 5,8-dihydroxy-2,3-dichloro-1,4-naphthoquinone (=naphthazarin, **Atokel-19**, **-20**, **-23**, and **-24**), or 2,3-dichloro-1,4-naphthoquinone (other Atokel derivatives) in acetonitrile to obtain the naphthoquinone-chelator coupling products **J** (Scheme 1), **Q** (Scheme 3), or **X** (Scheme 4). Protonation by HCl then afforded Atokel-2, -6, -8, -17 and 24 (Scheme 1B–D), or Atokel-3 and -4 (Scheme 3), or Atokel-13-16 (Scheme 4). Atokel-1, 5, and 7 were obtained by reductive dechlorination of the corresponding C2′–Cl derivatives (intermediate **J** of Atokel-2, 6, and 8, respectively, Scheme 1A) in the presence of palladium on carbon (5 wt%) and $N_2H_4{\cdot}H_2O$. Alternatively, **F-Atokel-7** was also obtained by dechlorination of **E-Atokel-5** at position 5 of the quinoline (concomitant with reduction of 8-NO_2) upon reduc-

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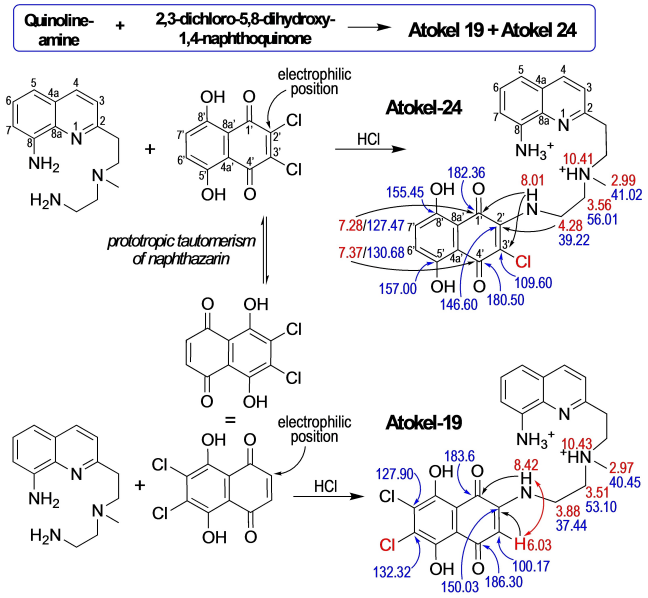
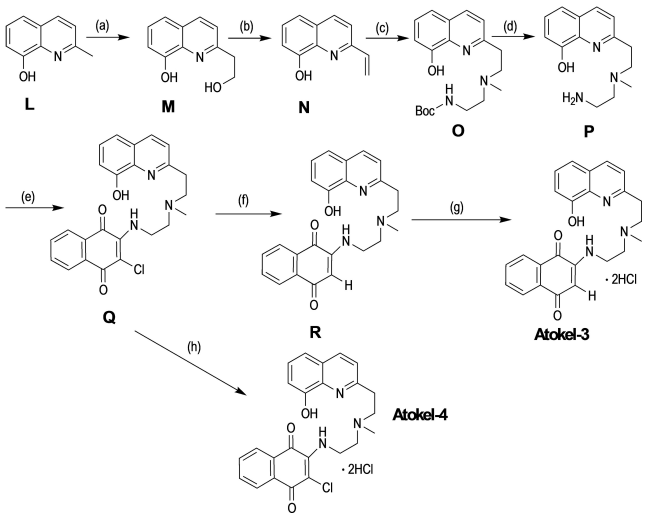
tion by palladium on carbon (30 wt%) and $N_2H_4{\cdot}H_2O$. The next steps of the synthetic routes of **Atokel-7-8** were similar to that described for preparation of **Atokel-5-6**. It should be noted that reaction of the intermediate **G** with 5-nitro-2,3-dichloro-1,4-naphthoquinone provided both **Atokel-17** and **Atokel-18** (R_7 = H), or **Atokel-20** and **Atokel-21** (R_7 = Cl) (Scheme 1D), due to the equivalent nucleophilic attack of the primary amine on the indiscernible C2′ and C3′ positions of the dichloronaphthoquinone.



Scheme 2. Reaction of a quinoline-amine derivative with 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone, giving rise to the **Atokel-19/24** couple. The red and blue labels stand for the NMR chemical shifts of protons (600 MHz) and carbon atoms (150 MHz), respectively. The black arrows stand for HMBC correlations, the red double arrow stands for ROESY correlation.



Scheme 3. General scheme of the syntheses of **Atokel-3-4**: (a) *n*-BuLi, paraformaldehyde, THF, 3 h; (b) 1) MsCl, Et3 N, CH_2Cl_2, RT, 3 h, 2) NaOH (1 M), EtOH, reflux, 5 h; (c) *tert*-butyl (2-(methylamino)ethyl)carbamate, CH_3COOH, CH_3OH, 70 °C, overnight; (d) CF_3COOH, CH_2Cl_2, RT, overnight; (e) 2,3-dichloronaphthalene-1,4-dione or 2-chloronaphthalene-1,4-dione, CH_3CN, RT, overnight; (f) Pd/C 5 wt%, $N_2H_4{\cdot}H_2O$, *i*-PrOH, 80 °C, 5 min-1 h; (g) HCl, EtOH, CH_2Cl_2; (h) HCl, EtOH, CH_2Cl_2.

Interestingly, coupling of the intermediate G with 5,8-dihydroxy-2,3-dichloro-1,4-naphthoquinone provided a couple of Atokel derivatives with different substitution patterns of the naphthoquinone ring: a 3′-H-6′,7′-Cl_2-5,8-$(OH)_2$-, or a 3′-Cl-6′,7′-H_2-5,8-$(OH)_2$-naphthoquinone (couples **Atokel-19/24** and **Atokel-22/23**, where R_7 = H or Cl, respectively, Scheme 1B). This feature is due to the already reported prototropic tautomerism of naphthazarin derivatives,[21] and the resulting Atokel derivatives have been separated and unambiguously characterized by 2D NMR correlations. This tautomerism and the subsequent NMR characterization of **Atokel-19** and **-24** are shown in Scheme 2. The protons at C6′ and C7′, and Cl at C3′ were unambiguously characterized in **Atokel-24**. Conversely, the H–C3′ was identified in **Atokel-19**, with ROESY correlation with the C2′–NH.

Atokel-3 and **-4**, which are the 8-hydroxyquinoline analogues of the 8-aminoquinoline derivatives **Atokel-1** and **-2**, respectively, were obtained starting from the 8-hydroxy-2-methyl quinoline (Scheme 3, **L**). The functionalization of the C2-methyl group was achieved by using *n*-butyllithium at −78 °C, followed by addition of paraformaldehyde, to yield the 2-hydroxyethyl derivative **M**. Then, reaction with methanesulfonyl chloride and triethylamine, followed by sodium ethylate afforded the C2-vinyl derivative (**N**, Scheme 3). Then, the α-NH_2-ω-Boc-diamine chain reated with the C2-vinyl group. Subsequent acidic deprotection of the distal primary amine gave rise to intermediate **P** (Scheme 3). This primary amine derivative reacted with 2,3-dichloro-1,4-naphthoquinone derivative in the presence of K_2CO_3 in acetonitrile, to afford the derivative **Q**. Protonation of **Q** yielded **Atokel-4.** Dechlorination of **Q** in the presence of Pd/C 5 wt%, followed by protonation, afforded **Atokel-3** (Scheme 3).

The 3′-tosylated derivatives **Atokel-13-16** were synthesized as described in Scheme 4. The commercially available 2,3-dichloro-1,4-naphthoquinone **S** was reacted with sodium nitrite under reflux in methanol/acetone, to give 2-hydroxy-3-nitronaphthalene-1,4-dione **T**. This compound was reduced by sodium dithionite to afford 2-hydroxy-3-aminonaphthalene-1,4-dione **U**. Then, 2,3-dihydroxynaphthalene-1,4-dione **V** was obtained by hydrolysis of the amino group with 30% sulfuric acid under reflux. The α,β-diol reacted with tosyl chloride in presence of triethylamine, to afford the 2,3-ditosylated derivative **W**. The 2,3-ditosylnaphthalene-1,4-dione then reacted with the distal primary amine of the side chain of the quinoline derivatives **G** (Scheme 1A) or **P** (Scheme 3) to provide, after protonation with HCl, **Atokel-13-16**. Several attempts were carried out in order to remove the 3′-OTs substituent and to generate the 3′-hydroxyl function present in atovaquone itself. Unfortunately, all these attempts failed, even in presence of titanium isopropoxide associated to trimethylsilyl chloride and magnesium powder.[22]

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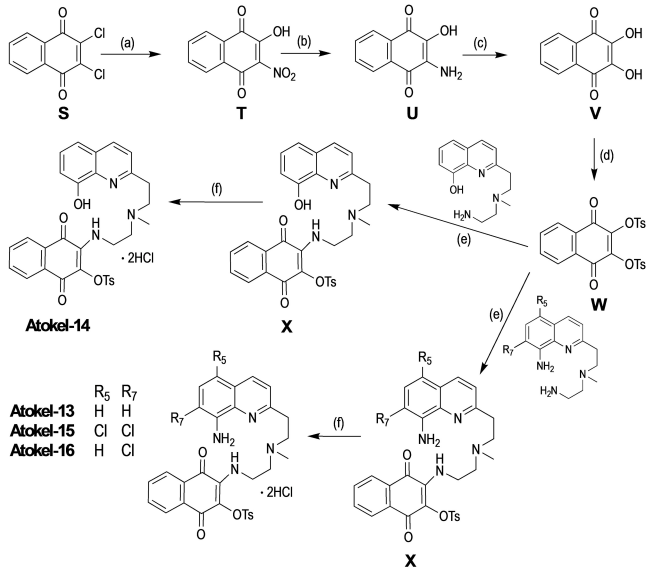

Scheme 4. General scheme of the syntheses of **Atokel-13-16**: (a) $NaNO_2$, acetone/methanol (1/1, v/v), reflux, 3 h; (b) $Na_2S_2O_4$, H_2O, RT, 2 h; (c) H_2SO_4 (30 %), reflux; (d) TsCl, Et_3N, THF, RT, 24 h; (e) in presence of compound **G** (Scheme 1) or **P** (Scheme 3), K_2CO_3, CH_3CN, RT, overnight; (f) HCl, EtOH, CH_2Cl_2.

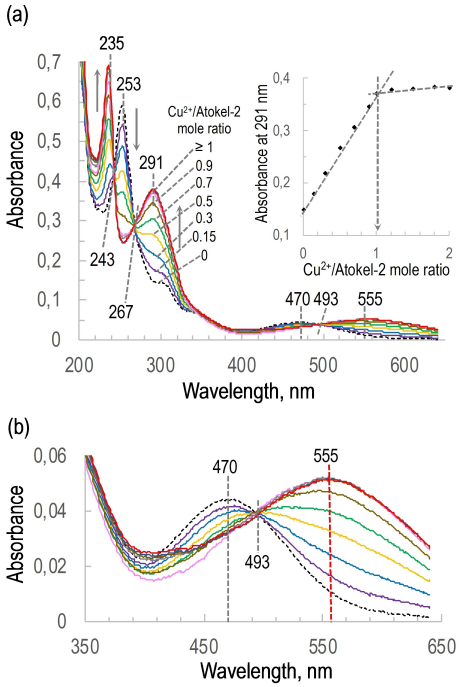

Figure 2. (a) Titration of **Atokel-2** AR: bold face font (15 μM) by $CuCl_2$, from 0 (dashed trace) to 5 mol equiv. of Cu, in Tris-buffered saline pH 7.4. Arrows indicate the decrease of free **Atokel-2** (253 nm) and increase of Cu^{II}-**Atokel-2** (235, 291 and 555 nm). Insert: Absorbance at 291 nm against the Cu^{2+}/**Atokel-2** molar ratio. (b) Extension of the 350–650 nm wavelength range.

Metal Chelation by Atokel Ligands

Titration of Atokels by Cu^{II}

Stoichiometry of the Cu^{II}-Atokel Complexes. Titration of all Atokel ligands was carried out by addition of aliquots of a $CuCl_2$ aqueous solution in an Atokel ligand solution (15 μM in Tris buffer 200 μM, pH 7.4), except **Atokel-15** and **-16** that could not be extensively studied due to their poor aqueous solubility. Chelation of copper was monitored by UV-Vis spectroscopy. The results allow to investigate (i) the behavior of ligands based on an 8-aminoquinoline moiety compared to ligands based on a 8-hydroxyquinoline (**Atokel-1** or **-2** compared to **Atokel-3** or **-4**, respectively), (ii) the role of non-coordinating Cl and NO_2 substituents either on the quinoline or on the naphthoquinone moieties, (iii) the evidence of the role of potentially coordinating hydroxyl or tosyl (OTs) substituents on the naphthoquinone moiety (**Atokel-24** compared to **Atokel-2**, or **Atokel-14** compared to **Atokel-3**).

First of all, the titrations by Cu^{2+} of all Atokel ligands without 3′-OTs or 5′,8′-OH naphthoquinone, whether they contained an 8-NH_2 or an 8-OH quinoline, resulted in immediate and complete copper coordination in a complex having 1/1 Cu^{II}/L stoichiometry. As examples, the titrations of **Atokel-2** and **-4** are depicted in Figures 2 and 3, respectively. Upon metalation by copper(II), the main absorption band of **Atokel-2** (253 nm) decreased, and was splitted into two absorption bands (235 and 291 nm) assigned to the Cu^{II}-**Atokel-2** complex (Figure 2). In addition, the small band of the ligand at 470 nm underwent a bathochromic shift to 555 nm. Well defined isosbestic points detected at 243 nm, 267 nm and 493 nm, and the plot of absorbance at 291 nm with respect to the Cu/Atokel-2 molar ratio in the mixture (Figure 2a, Insert) unambiguously confirmed the metalation of Atokel-2 as a 1/1 complex.

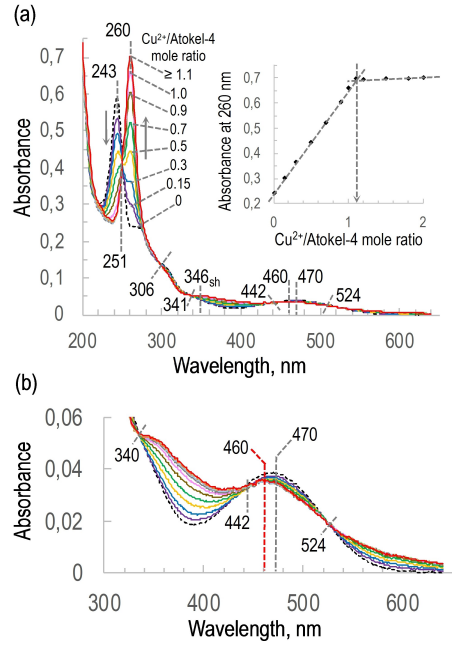

Figure 3. (a) Titration of **Atokel-14** (15 μM) by $CuCl_2$, from 0 (dashed trace) to 5 mol equiv. of Cu, in Tris-buffered saline pH 7.4. Arrows indicate the decrease of free **Atokel-4** (243 nm) and increase of Cu^{II}-**Atokel-4** (260 nm). Insert: Absorbance at 260 nm against the Cu^{2+}/**Atokel-14** molar ratio. (b) Extension of the 300–650 nm wavelength range.

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Upon metalation of the 8-OH group of **Atokel-4**, the absorbances of the free ligand at 260 nm and 460 nm, were shifted to 243 and 470 nm, assigned to a 1/1 Cu-**Atokel-4** complex, with clear isosbestic points (251, 306, 340, 442 and 524 nm) (Figure 3). Very similar results were observed for **Atokel-1-8**, **-7-18**, and **-20-21**: the Cl and NO_2 substituents, despite slight changes of the absorbance wavelengths, did not induce significantly different coordination behaviors.

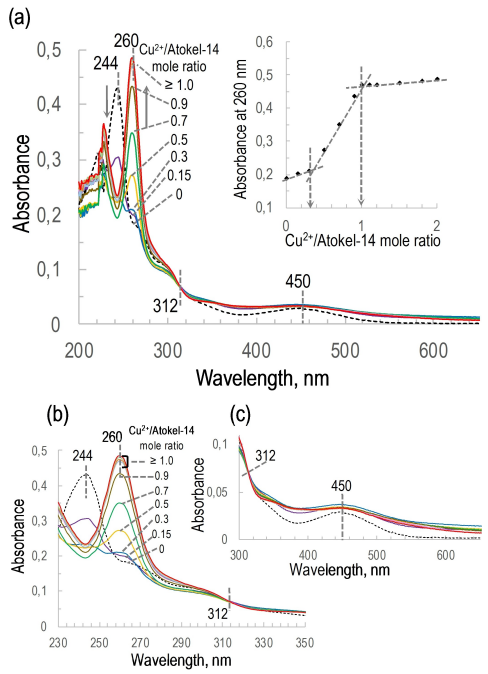

Figure 4. (a) Titration of **Atokel-14** (15 μM) by $CuCl_2$, from 0 (dashed trace) to 5 mol equiv. of Cu, in Tris-buffered saline pH 7.4. Arrows indicate the decrease of free **Atokel-14** (244 nm) and increase of Cu^{II}-**Atokel-14** (260 nm). Insert: Absorbance at 260 nm against the Cu^{2+}/**Atokel-14** molar ratio. (b) Extension of the 230–350 nm wavelength range. (c) Extension of the 300–650 nm wavelength range.

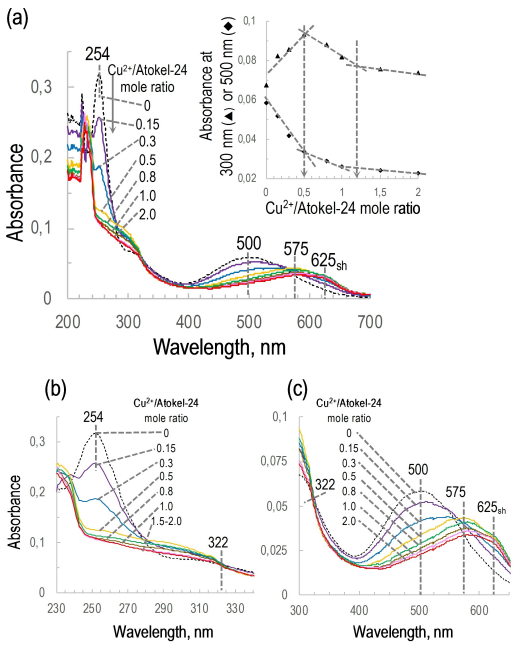

Figure 5. (a) Titration of **Atokel-24** AR: bold face font (15 μM) by $CuCl_2$, from 0 (dashed trace) to 5 mol equiv. of Cu, in Tris-buffered saline pH 7.4. Arrows indicate the decrease of free **Atokel-24** (254 and 500 nm) and increase of Cu^{II}-**Atokel-24** (575 nm). Insert: Absorbance at 300 nm and 500 nm against the Cu^{2+}/**Atokel-24** molar ratio. (b) Extension of the 230–350 nm wavelength range. (c) Extension of the 300–650 nm wavelength range.

For Atokels bearing a 3′-OTs substituent (**Atokel-13** and **-14**), or a 5′,8′-naphthoquine moiety (=naphthazarin, **Atokel-19**, **-20**, **-21**, and **-24**), 1/1 Cu^{II}-Atokel complexes were also evidenced, along with complexes with structures CuL_3 (L=**Atokel-14**, Figure 4) or CuL_2 (L=**Atokel-24**, Figure 5). Along with the plot of absorbance with respect to Cu/L ratio (Figures 4 and 5, Inserts), the presence of complexes having several ligands per Cu ion was assessed by the detection of specific isosbestic points for Cu/L molar ratios below or equal to 0.3 in the case of **Atokel-14** (257 and 271 nm), or to 0.5 in the case of **Atokel-24** (278 and 575 nm). These results are consistent with the coordination of copper(II) by two η^2-naphthazarin ligands[20] (for **Atokel-24**) or three monodentate tosylates (for **Atokel-14**) at low Cu/L molar ratios, followed by rearrangement to more stable 1/1 complexes while the available Cu is no more a limiting factor.

Affinity of Atokels for Cu^{II}. The affinity of copper(II) for Atokel ligands in complexes with Cu/L 1/1 stoichiometry was evaluated as previously reported,[18,23] using nitriloacetate (NTA) or ethylene diamine (EDA) as competitive ligands {log K_{app} [Cu-NTA or Cu-EDA]=10.3 or 7.8, respectively[24]}. The main results are reported in Table 2. For Atokels bearing a 8-aminoquinoline and no 5′,8′-hydroxyl substituents, the log K_{app} [Cu–L] values at pH 7.4 were found to be moderate, in the range 9.1-6.5. From **Atokel-1** {log K_{app} [Cu–L]=8.9}, substitution of the ligand by Cl resulted in gradual decrease of copper(II) affinity, with log K_{app} [Cu–L]=7.6, 7.2, and 6.5 for **Atokel-7** (7-Cl), **Atokel-5** (5,7-Cl_2) and **Atokel-6** (5,7,3′-Cl_3), respectively. This moderate but consistent withdrawal electronic effect of chlorine was already evidenced in the TDMQ series.[18]

Conversely, the presence of the donating 5′- or 8′-NO_2 resulted in slight increase of affinity (≈+0.5 log unit) of the ligand for copper(II), with log K_{app} [Cu–L]=9.1–9.2 for L=**Atokel-17** or **-18** (having a NO_2 at C8′ or C5′, respectively), compared to 8.5 for **Atokel-2** (having no NO_2 substituent). Similarly, log K_{app} [Cu–L] was 8.0 or 7.7 for L=**Atokel-20** or **-21**, respectively, compared to 7.3 for **Atokel-8**. The 3′-OTs substituent did not induce any significant change in copper affinity {log K_{app} [Cu–L]=8.9 or 9.1, for **Atokel-1** or **-13**, respectively}. All Atokels bearing a coordinating naphthazarin (**Atokel-19**, **-20**, **-21**, and **-24**) exhibited Cu affinities in the range log K_{app}=10.2–10.5, roughly 2 log units higher than the corresponding analogs without hydroxyls at positions 5′ and 8′ {log K_{app}=10.2 for **Atokel-23** or **-24**, compared to 7.3 and 8.5 for **Atokel-8** and **-2**, respectively}. Atokel ligands based on a 8-hydroxyquinoline motif exhibited log K_{app} values in the range of 10.2 to 10.5 (**Atokel-3**, **-4**, and **-14**).

One should note that, in the TDMQ series, tetradentate chelators based on an 8-aminoquinoline substituted by a chelating polyamine side chain, such moderate copper affinity (log K_{app}≤10.5) was found for ligands that offered a N_3Cl

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Table 2. Affinity of Atokel ligands for copper(II) in Tris buffer, pH 7.4. Selected data.

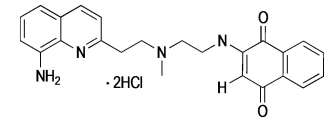
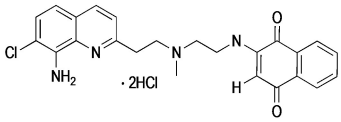
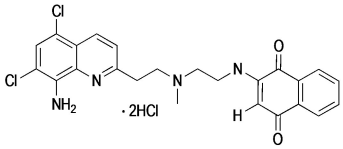
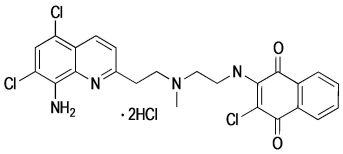
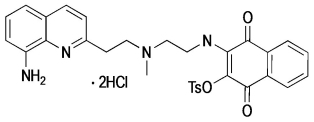
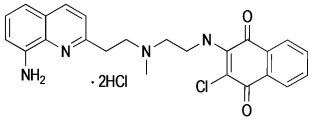
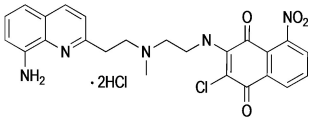
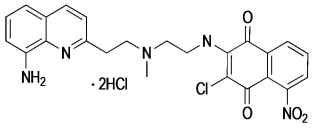
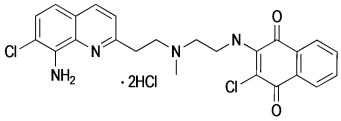
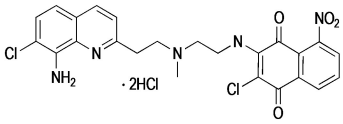
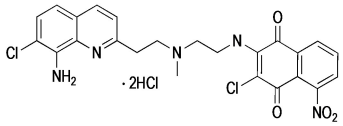
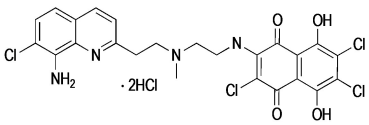
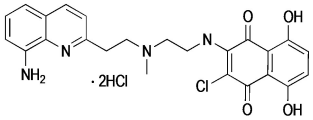
with n =	L=Atokel-n	log K_{app} [Cu–L]
1		8.9
7		7.6
5		7.2
6		6.4
13		9.1
2		8.5
17		9.1
18		9.2
8		7.3
20		8.0
21		7.7
23		10.2

Table 2. continued

with n =	L=Atokel-n	log K_{app} [Cu–L]
24		10.2

coordination sphere to copper(II). The corresponding Cu-TDMQ complexes were able to oxidize ascorbate in vitro, suggesting that the geometry and steric constraint of such ligands are able to accomodate both copper(II) and copper(I).

Titration of Atokels by $ZnCl_2$ and $FeCl_3$

Titration by Zinc. Titration of all Atokel ligands was carried out using $ZnCl_2$ as metal source, and monitored by UV-Vis spectroscopy. All the derivatives based on an 8-aminoquinoline moiety and bearing no hydroxy substituent on the naphthoquinone ring, exhibited unchanged spectra up to 20 mole equivalents of metal with respect to Atokel, indicating that they were not significantly able to chelate zinc(II).

For Atokels bearing a C5′,C8′-naphthoquine moiety (= naphthazarin, **Atokel-19**, **-20**, **-21**, and **-24**), half-metallation was obtained after addition of 30 to 150 mol equivalents of metal salt and 300 to 1000 mol equivalent of zinc were required to achieve full metallation. These data indicated that these ligands have a very low affinity for zinc(II), with calculated log K_{app} (pH 7.4) values below 3.5–3.0. As a matter of example, titration of Atokel-4 by $ZnCl_2$ is provided as Supporting Information (Figure S1). This low affinity chelation of zinc(II) by the naphthazarin moiety is consistent with metal complexes already reported for this motif.[20] So, the selectivity of Atokel ligands for the redox active copper(II) with respect to zinc(II) was at least 7 orders of magnitude.

On the other hand, all Atokels based on a 8-hydroxyquinoline moiety (**Atokel-2**, **-4**, **-14**) were found to exhibit some significant affinity for zinc, with log K_{app} (pH 7.4) values below 4 (half metallation at 7–9 mol equivalents of metal salt). This result is consistent with the lack of metal specificity of 8-hydroxyquinolines, compared to 8-aminoquinolines as previously observed.[25,26]

Titration by Iron. Titration of **Atokel-2**, **-3**, **-4**, **-14**, and **-24** was carried out with $FeCl_3$, and monitored by UV-Vis spectroscopy. No significant change occurred in the spectra upon addition of 50 mol equivalents of iron(III), indicating that Atokel have no significant affinity for iron(III) in these conditions, regardless of their substitution pattern was (8-amino- or 8-hydroxyquinoline, hydrogen, chlorine, or tosylate at C3′, or dihydroxy substitution at C5′ and C8′ of the naphthoquinone moiety).

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Ability of Cu-Atokel Complexes to Induce the Reduction of Dioxygen

The aerobic oxidation of ascorbate induced by the 1/1 Cu-Atokels complexes was measured by kinetic UV-visible, as an indirect evaluation of their ability to reduce dioxygen and, consequently, to produce the damaging hydroxyl radicals.[18,27] Each Atokel ligand was first incubated with $CuCl_2$ and the chelation of copper(II) was confirmed by UV-Vis spectroscopy {[Atokel] = 11 μM in Hepes buffer pH 7.4, Cu/L molar ratio = 1/1.1}. An aliquot of aqueous ascorbate was then added in the UV-Vis cuvette, and the absorbance of ascorbate was immediately monitored at 265 nm. A selection of results is reported in Figure 6 and Table 3. First of all, all Atokel ligands, except for **Atokel-3**, in presence of one mol equivalent of copper(II), induced rapid oxidation of ascorbate, with conversion values higher than 60% after 10 minutes and full ascorbate conversion before 30 minutes of reaction time (Figure 6). The ascorbate oxidation was not correlated to the presence of a 8-amino- or a 8-hydroxyquinoline in the Atokel ligand (85% and 61% for 8-amino **Atokel-2** and **-13**, respectively, compared to 78% and 91% for the 8-hydroxy derivatives **Atokel-4** and **-14**, respectively, Table 3). Similarly, it was not correlated with the 3′-substituent (91%, 85% and 61% for **Atokel-1**, **-2**, and **-13**, respectively), or with the 5′,8′-dihydroxy pattern of the naphthoquinone moiety (85% and 93% for **Atokel-2**, and **-24**, respectively). Only **Atokel-3** in the presence of copper(II) exhibited a low redox activity, with 5% of ascorbate oxidation at 10 min (16% at 30 min), while its 3′-chlorinated and 3′-tosylated analogues **Atokel-4** and **-14** induced 78% and 91% of ascorbate oxidation, respectively. At this point, there is no clear correlation between the substitution pattern of Atokel ligands and the redox activity of their copper complexes.

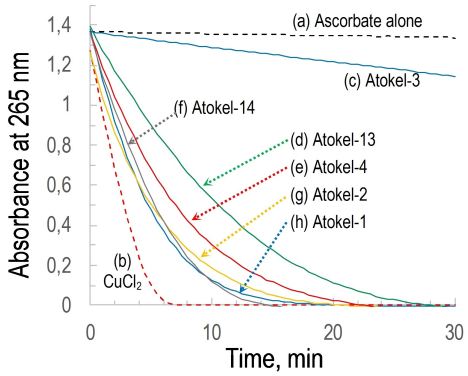

Figure 6. Kinetic UV-Vis spectrum (265 nm) of aerobic ascorbate oxidation, in the presence of (a) no additive, (b) $CuCl_2$, or L=Atokel-n + $CuCl_2$, with Cu/L mol ratio = 1/1, with L = **Atokel-3** (c), **-13** (d), **-4** (e), **-2** (g), or **-1** (h). [Atokel] = 11 μM, [Cu^{2+}] = 10 μM, [ascorbate] = 100 μM, in Hepes 50 mM, pH 7.4.

Table 3. Aerobic ascorbate oxidation by Cu-Atokel complexes, with Cu/L stoichiometries = 1/1 or 1/2.[a] Measured by decrease of ascorbate absorbance at 265 nm. [b] Ascorbate autoxidation (no Atokel, no Cu). [c] - Ascorbate oxidation in the presence of $CuCl_2$. AR: This part of the title of Table 3 is redundant with footnotes of the Table.

Atokel-n n =	Cu/L molar ratio	Ascorbate oxidation at 10 min (%)[a]
1	1/1	91
2	1/1	85
3	1/1	5
4	1/1	78
8	1/1	100
17	1/1	97
13	1/1	61
	1/2	41
14	1/1	91
	1/2	5
19	1/1	75
	1/2	39
22	1/1	91
	1/2	29
24	1/1	93
	1/2	66
–[b]	–	1
–[c]	–	100

[a] Measured by decrease of ascorbate absorbance at 265 nm; [b] ascorbate autoxidation (no Atokel, no Cu); [c] ascorbate oxidation in the presence of $CuCl_2$.

Since Atokel ligands bearing a 3′-OTs- or 5′,8′-$(OH)_2$-naphthoquinone were found able to form copper complexes with Cu/L stoichiometries 1/3 or 1/2, respectively, their ability to oxidize ascorbate in presence of 0.5 equiv. of copper(II) was investigated.

The comparison of ascorbate oxidation by several Atokel ligands in presence of 1 or 0.5 mol equiv. of copper(II) is reported in Figure 7 and Table 3. At 10 min of reaction time, **Atokel-22** and **-24** induced 29% and 66% of ascorbate oxidation in presence of 0.5 equiv. of copper, respectively, while ascorbate conversion in presence of 1 mol equiv. of copper was determined to 91% and 93%, respectively. **Atokel-14** induced only 5% of ascorbate oxidation at 10 min in presence of 0.5 mol equiv. of copper, while oxidation was higher than 90% in presence of 1 mol equiv. of copper. These results confirm that these ligands generate different copper complexes having different redox behaviors, according to the available quantity of copper.

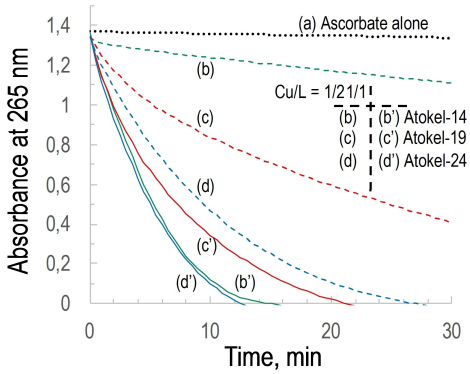

Figure 7. Kinetic UV-Vis spectrum (265 nm) of aerobic ascorbate oxidation, in presence of (a) no additive, or L=Atokel-n + $CuCl_2$, with Cu/L mole ratio = 1/1 (full traces) or 1/2 (dashed traces). L = **Atokel-14** (b, b′), **-19** (c, c′), or **-24** (d, d′). [Atokel] = 11 μM; [Cu^{2+}] = 5 or 10 μM; [ascorbate] = 100 μM, in Hepes 50 mM, pH 7.4.

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Biological Activity of Atokel Derivatives

Antimalarial Activity

The antiplasmodial activities of these hybrid molecules have been evaluated in vitro on the strain of *P. falciparum* F32-ART.[28] On this strain, the IC_{50} value of atovaquone, the reference antimalarial drug routinely tested in our laboratory, was 0.8 ± 0.2 nM. Consequently, the Atokel derivatives having a IC_{50} value higher than 1 μM, 1000-fold higher than that of atovaquone were considered inefficient. The percentage of F32-ART *Plasmodium* growth inhibition by Atokel drugs at 1 μM was found in the range 0–40% (Table 4), except for the more active Atokel-13 (72%). The IC_{50} value of Atokel-13 on the F32-ART strain was 622 ± 9 nM. Atovaquone acts by inhibiting the mitochondrial cytochrome bc_1 complex (cyt bc_1) of *Plasmodium*.[29] The X-ray structure of mitochondrial cyt bc_1 from *Saccharomyces cerevisiae* with atovaquone bound indicates that a polarized H-bond to His181 of the Rieske protein in cyt bc_1 interacts at 2.8 Å with the deprotonated hydroxyl group at C3′ of the drug ($pK_a = 6.9$).[29] So, the lack of this C3′–OH group in the Atokel series might be, at least in part, responsible for the low antimalarial activity of these compounds.

However, beside this OH group, interaction of atovaquone with the Qo site of the protein involves the protons and carbonyl functions of the naphthalene-1,4-dione moiety (with the highly conserved residues Val-136 and Pro-271 on one hand, and Met-139, Gly-143 and Ile-269, on the other hand).[29] Consequently, any derivative containing the naphthalene-1,4-dione residue (even without hydroxyl at C2 or the specific side-chain of atovaquone) may retain some affinity for the target protein.

Cytotoxicity

The IC_{50} value of **Atokel-13** against Vero cells was largely up to 50 μM (corresponding to the maximum concentration tested, with no cytotoxicity at this concentration). Then, the selectivity index of **Atokel-13** was > 80 (> 50/0.6). In the same conditions, the IC_{50} of atovaquone against Vero cells was 8 μM, indicating a selectivity index of this drug around 1×10^4.

Conclusion

A series of ligands based on an 8-amino- or 8-hydroxyquinoline covalently bound to a 1,4-naphthoquinone through a polyamine linker, were synthesized and fully characterized. These ligands exhibited an affinity for copper(II) with log K_{app} values in the range 7–11, and a high selectivity for copper(II) with respect to zinc(II) and iron(III). Despite a rational design that aimed to target the mitochondrion and to trigger a damaging oxidative stress, the synthesized Atokel ligands exhibited a deceiving antimalarial activity, that might due in part to the lack of hydroxyl substituent at C3′. The obtained results do not allow to deduce a possible correlation between the drug structures, their ability to induce ROS production in presence of a redox active metal ion like copper, and their biological activity. The determination of the structures of metalated Atokel complexes might provide useful information for pharmacomodulation in order to improve the antiplasmodial activity of these hybrid molecules.

Experimental Section

Reagents and Methods

All solvents and commercially available reagents were purchased from Sigma-Aldrich, Fluka, Acros, TCI, or BLD Pharmatech, and were used without further purification. copper(II)- zinc(II) and iron(III) cloride were used as source of metal ions. ^{1}H- and ^{13}C NMR spectra were recorded on a Brucker Avance Neo 600 spectrometer et 298 K except otherwise stated, or on Bruker Avance 400 or Avance 500 spectrometers. Chemical shift values are given in ppm, using tetramethylsilane (TMS) as the external standard. All spectra have been recorded in the ranges −1 to 13 ppm for ^{1}H, and −10 to 210 ppm for ^{13}C. Full characterization of compounds by high resolution NMR (600 MHz in ^{1}H, 150 MHz in ^{13}C) is routinely based on COSY, ROESY, HSQC and HMBC analyses, at variable temperature when necessary. For all final Atokel ligands, the NMR signals have been listed along with assignment in the logic order of Atokel ligands building: first, quinoline positions from 1 to 8, then the substituent at C2, and finally the naphthoquinone ring. HPLC analyses were carried out using a GL Sciences Inc. equipment with InertSustain C18 column (5 μm, 4.6 mm x 150 mm), flow rate was 1.0 mL min^{-1}, UV detection at $\lambda = 254$ nm. ESI$^+$-Mass spectra were obtained on ThermoFisher or Xevo G2 QTOF (Waters). UV-visible spectra were recorded from 200 to 700 nm, at 25 °C, on a Evolution 300 (ThermoFisher) or Cary 3500 spectrophotometers equipped with magnetic stirring.

Synthesis and Characterization of Atokels

The Atokel ligands and their synthetic intermediates have been prepared according to general protocols depicted in Schemes 1, 3, and 4. Each compound was numbered as "X-Atokel-n", where X stands for the letter of the given intermediate in Schemes 1, 3, and 4, followed by the target Atokel, with n = 1–24.

Atokel-1

8-Nitro-2-vinylquinoline (D-Atokel-1). To solution of 2-methyl-8-nitroquinoline (**C**, Scheme 1A, 0.5 g, 2.65 mmol) in DMA (5 mL), $FeCl_3$ (13.0 mg, 0.08 mmol) and $K_2S_2O_8$ (1.43 g, 5.3 mmol) were added. The mixture was stirred at 110 °C for 15 min, and quenched with aqueous ammonium hydroxide in an ice water bath. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/hexane, 3/1, v/v). After evaporation of the solvent, compound **D-Atokel-1** was obtained as a white solid (106 mg, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.17$ (d, $J = 8.6$ Hz, 1H), 7.96–7.93 (m, 2H), 7.64 (d, $J = 8.6$ Hz, 1H), 7.52 (t, $J = 8.0$ Hz, 1H), 6.99 (dd, $J = 17.6$, 10.8 Hz, 1H), 6.42 (d, $J = 17.6$ Hz, 1H), 5.71 ppm (d, $J = 10.8$ Hz, 1H).

tert-Butyl(2-(methyl(2-(8-nitroquinolin-2-yl)ethyl)amino)ethyl)carbamate (E-Atokel-1). To a mixture of **D-Atokel-1** (1.2 g, 6.0 mmol) and Cs_2CO_3 (3.9 g, 12.0 mmol) in 1,4-dioxane (10 mL) was added *tert*-butyl (2-(meth-

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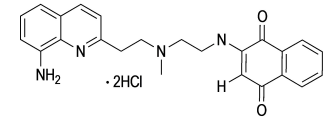
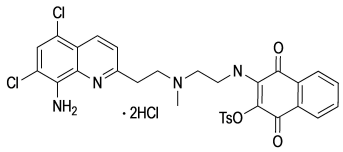
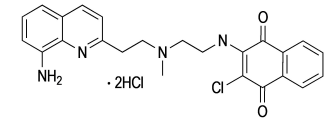
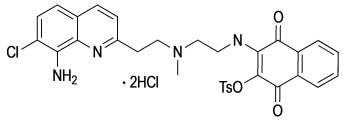
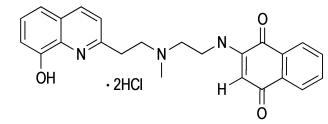
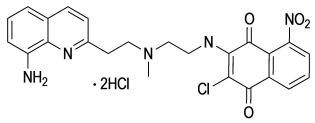
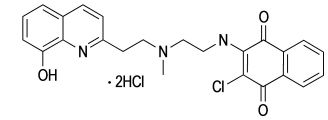
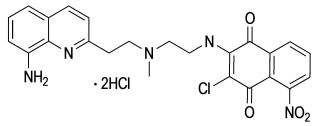
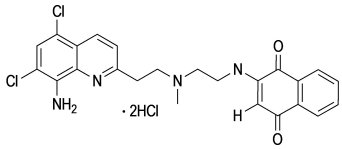
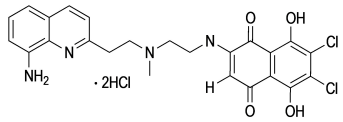
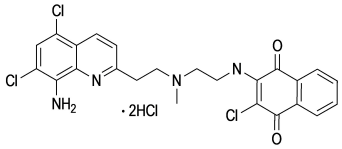
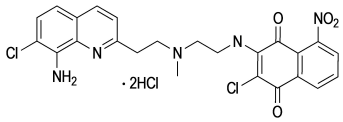
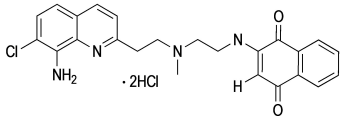
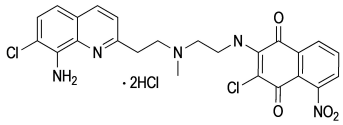
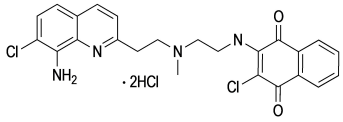
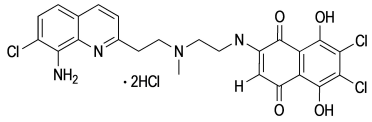
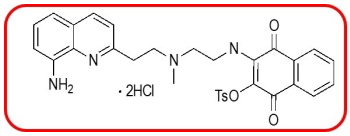
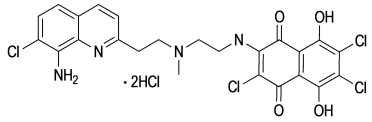
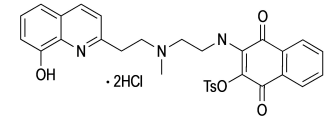
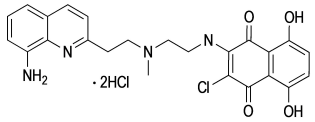
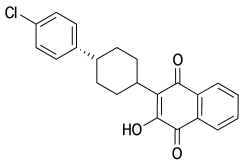
Table 4. Antimalarial activity of Atokel derivatives against the F32-ART strain of *P. falciparum*. The inhibition of parasite growth at 1 μM of drug concentration is provided, as well as the IC_{50} values (in parentheses). The IC_{50} value of atovaquone on F32-ART *P. falciparum* is given for comparison. For details, see Experimental Section.

with n =	L=Atokel-n	% of inhibition of *P. falciparum* at 1 μM[a] (IC50, μM)	with n =	L=Atokel-n	% of inhibition of *P. falciparum* at 1 μM[a] (IC50, μM)
1		10 (> 1)	15		21 (> 1)
2		11 (> 1)	16		40 (> 1)
3		5 (> 1)	17		8 (> 1)
4		19 (> 1)	18		3 (> 1)
5		0 (> 1)	19		6 (> 1)
6		4 (> 1)	20		22 (> 1)
7		5 (> 1)	21		7 (> 1)
8		35 (> 1)	22		0 (> 1)
13		72 (0.62 ± 0.01)[b]	23		29 (> 1)
14		27 (> 1)	24		22 (> 1)
For comparison: Atovaquone		IC50[c] 0.8 ± 0.2 nM			

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ylamino)ethyl)carbamate (2.09 g, 12.0 mmol). The reaction mixture was stirred at room temperature for 16 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 60/1, v/v). After evaporation of the eluent, target compound was obtained as brown oil (1.95 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.15$ (d, $J = 8.4$ Hz, 1H), 7.98–7.95 (m, 2H), 7.54 (t, $J = 8.0$ Hz, 1H), 7.44 (d, $J = 8.4$ Hz, 1H), 4.91 (brs, 1H), 3.18–3.16 (m, 4H), 2.95 (t, $J = 6.8$ Hz, 2H), 2.55 (brs, 2H), 2.30 (s, 3H), 1.40 ppm (s, 9H).

2-Chloro-3-((2-(methyl(2-(8-nitroquinolin-2-yl)ethyl)amino)ethyl)amino)naphthalene-1,4-dione (I-Atokel-1). To a solution of **E-Atokel-1** (400 mg, 1.07 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (7 mL) dropwise over a 15 min period, in an ice bath. The resulting mixture was stirred for 2 h at room temperature, then was then poured onto ice, neutralized with aqueous ammonium hydroxide. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product **H-Atokel-1** was dissolved in acetonitrile (6 mL) was added 2,3-dichloronaphthalene-1,4-dione (233 mg, 1.07 mmol). The reaction mixture was stirred at room temperature for 24 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v). After evaporation of the eluent, target compound was obtained as red solid (370 mg, 74%). The same product was obtained in 66% yield, when using 2-chloronaphthalene-1,4-dione as starting material. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 8.38$ (d, $J = 7.6$ Hz, 1H), 8.12–8.04 (m, 2H), 7.90–7.54 (m, 6H), 6.81 (brs, 1H), 3.76 (br, 2H), 3.06 (br, 2H), 2.87 (br, 2H), 2.61 (br, 2H), 2.27 ppm (s, 3H).

2-((2-((2-(8-Aminoquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)naphthalene-1,4-dione (K-Atokel-1). Palladium on carbon (11 mg, 5 wt%) and hydrazine hydrate 80% (0.5 mL) were added to a solution of **J-Atokel-2** (210 mg, 0.48 mmol) in isopropanol (5 mL). The mixture was stirred at 80 °C for 1 h. The reaction mixture was poured into water and extracted with dichloromethane (3×20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v.). Evaporation of the solvent afforded **Atokel-1** as a red solid (156 mg, 81%). Alternatively, **K-Atokel-1** was obtained from **I-Atokel-1** under the same reaction conditions. ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.87$–7.81 (m, 3H), 7.66 (td, $J = 7.6$ Hz, $J = 1.2$ Hz, 1H), 7.55 (td, $J = 7.6$ Hz, $J = 1.2$ Hz, 1H), 7.22 (d, $J = 8.4$ Hz, 1H), 6.88 (t, $J = 8.0$ Hz, 1H), 6.79 (d, $J = 8.0$ Hz, 1 H), 6.67 (d, $J = 7.2$ Hz, 1 H), 5.47 (s, 1 H), 3.12 (t, $J = 6.4$ Hz, 2 H), 3.02 (t, $J = 6.0$ Hz, 2H), 2.94 (t, $J = 6.0$ Hz, 2H), 2.65 (t, $J = 6.4$ Hz, 2H), 2.36 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 183.2$, 180.3, 157.6, 148.5, 143.7, 137.7, 136.1, 134.2, 133.5, 131.7, 130.4, 127.2, 126.2, 125.9, 125.3, 121.8, 115.4, 110.3, 98.9, 56.0, 54.2, 41.4, 39.1, 35.7 ppm. ESI$^+$-MS: calcd for $C_{24}H_{25}N_4O_2$: 401.08 ([M + H]$^+$); found: 401.19.

Atokel-1. To a solution of **K-Atokel-1** in dichloromethane was added excess hydrochloric acid solution (2.0 N in ethanol). The precipitate was filtered, washed with ethanol and dried under reduced pressure to give Atokel-1 as a yellow solid (100%). mp: 107–110 °C. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 10.47$ (brs, 1H), 8.35 (d, $J = 8.4$ Hz, 1H), 8.02–7.90 (m, 2 H), 7.84 (t, $J = 7.5$ Hz, 1H), 7.77–7.72 (m, 3H), 7.60–7.53 (m, 3H), 5.93 (s, 1H), 3.90 (br, 2H), 3.77 (br, $J = 5.6$ Hz, 2H), 3.53 (br, 4H), 2.96 ppm (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 182.3$, 181.6, 157.9, 148.7, 139.5, 137.7, 135.3, 133.4, 132.8, 132.4, 130.8, 127.7, 126.9, 126.4, 125.8, 123.4, 121.1, 101.0, 53.2, 52.9, 40.6, 37.0, 31.6 ppm. ESI$^+$-MS: calcd for $C_{24}H_{25}N_4O_2$: 401.08 ([M + H]$^+$); found: 401.19. Elemental analysis calcd (%) for $C_{24}H_{24}N_4O_2 \cdot 2.3HCl \cdot 0.9H_2O \cdot 0.4\ C_2H_5OH$ (apparent MW = 520.78): C 57.20, H 5.94, N 10.76, found: C 57.20, H 5.96, N 10.78. HPLC (CH_3OH/H_2O, 35/65, v/v, 0.2 vol% TFA): $R_t = 19.8$ min, purity 95%.

Atokel-2

tert-Butyl(2-((2-(8-aminoquinolin-2-yl)ethyl)(methyl)amino)ethyl)carbamate (F-Atokel-1). Palladium on carbon (40 mg, 5 wt%) and hydrazine hydrate 80% (2.4 mL) were added to a solution of E-Atokel-1 (0.8 g, 2.14 mmol) in isopropanol (12 mL). The mixture was stirred at 80 °C for 13 min. The reaction mixture was poured into water and extracted with dichloromethane (3× 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (CH_2Cl_2/CH_3OH, 40/1, v/v.). Evaporation of the solvent afforded **F-Atokel-1** as a yellow oil (0.66 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.96$ (d, $J = 8.4$ Hz, 1H), 7.28–7.24 (m, 2H), 7.10 (d, $J = 8.4$ Hz, 1H), 6.90 (d, $J = 7.6$ Hz, 1H), 4.91 (br, 3H), 3.19 (q, $J = 5.3$ Hz, 2H), 3.09 (t, $J = 6.8$ Hz, 2H), 2.90 (t, $J = 6.8$ Hz, 2H), 2.53 (t, $J = 5.6$ Hz, 2H), 2.30 (s, 3H), 1.41 ppm (s, 9H).

N^1-(2-(8-Aminoquinolin-2-yl)ethyl)-N^1-methylethane-1,2-diamine (G-Atokel-1). To a stirred solution of **F-Atokel-1** (1 g, 2.9 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (12 mL) dropwise over a 15 min period, in an ice bath. The resulting mixture was stirred for 2 h at room temperature, and was then poured onto ice, neutralized with aqueous ammonium hydroxide. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (ethyl acetate/isopropanol/25% ammonium hydroxide, 5/2/0.6, v/v/v). After evaporation of the solvent, **G-Atokel-1** was isolated as a yellow oil (760 mg, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.94$ (d, $J = 8.4$ Hz, 1H), 7.26–7.22 (m, 2H), 7.09 (d, $J = 8.0$ Hz, 1H,), 6.87 (d, $J = 7.2$ Hz, 1H), 4.42 (br, 4H), 3.07 (t, $J = 6.4$ Hz, 2H), 2.89 (t, $J = 6.4$ Hz, 2H), 2.79 (t, $J = 6.0$ Hz, 2H), 2.53 (t, $J = 6.0$ Hz, 2H), 2.25 ppm (s, 3H).

2-((2-((2-(8-Aminonaphthalen-2-yl)ethyl)(methyl)amino)ethyl)amino)-3-chloronaphthalene-1,4-dione (J-Atokel-2). To a solution of **G-Atokel-1** (370 mg, 1.5 mmol) in acetonitrile (4 mL) was added 2,3-dichloronaphthalene-1,4-dione (344 mg, 1.5 mmol) or 2-chloronaphthalene-1,4-dione (288 mg, 1.5 mmol). The reaction mixture was stirred at room temperature for 24 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v). After evaporation of the eluent, the target compound was obtained as a red solid (328 mg or 368 mg, 50% or 56%, when starting from 2,3-dichloronaphthalene-1,4-dione or 2-chloronaphthalene-1,4-dione, respectively). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.82$ (d, $J = 7.6$ Hz, 1H),7.79 (d, $J = 8.4$ Hz, 1H), 7.71 (d, $J = 7.6$ Hz,1H), 7.58 (td, $J = 7.6$ Hz, $J = 1.2$ Hz, 1H), 7.49 (td, $J = 7.6$ Hz, $J = 1.2$ Hz, 1H), 7.15 (d, $J = 8.4$ Hz, 1H), 6.84 (t, $J = 8.0$ Hz, 1H), 6.74 (d, $J = 8.0$ Hz, 1H), 6.56 (d, $J = 7.2$ Hz, 1H), 3.88 (m, 2H), 3.12 (t, $J = 6.8$ Hz, 2H), 3.03 (t, $J = 6.8$ Hz, 2H), 2.69 (t, $J = 6.0$ Hz, 2H), 2.42 ppm (s, 3H,); ^{13}C NMR (100 MHz, CD_3OD): $\delta =$ 179.1, 176.5, 157.5, 143.6, 137.7, 136.1, 134.2, 132.4, 132.0, 127.2, 126.2, 126.1, 125.6, 121.8, 115.4, 110.3, 56.1, 55.6, 41.4, 41.3, 35.6 ppm. HPLC: $R_t = 16.5$ min; ESI$^+$-MS calcd for $C_{24}H_{24}ClN_4O_2$: 435.15 ([M + H]$^+$); found: 435.11.

Atokel-2. Protonation of **J-Atokel-2** afforded **Atokel-2**, in the same experimental conditions as described above for the preparation of **Atokel-1** from **K-Atokel-1**. **Atokel-2** was isolated in form of a yellow

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solid (100%). mp: 145–148 °C. ^{1}H NMR (600 MHz, DMSO-d_6), δ, ppm: 7.57 (H3), 8.37 (H4), 7.55 (H5), 7.74 (H6), 7.60 (H7), 3.55 [2H, C2–CH_2], 3.91 [2H, C2–CH_2–CH_2], 2.99 [3H, C2-$(CH_2)_2$–HN^+–CH_3], 3.55 [2H, CH_2–CH_2–NH–C2′], 4.20 [2H, CH_2–NH–C2′], 7.69 [1H, *H*N–C2′], 7.99 (H5′), 7.85 (H6′), 7.77 (H7′), 7.97 (H8′). ^{13}C NMR, δ, ppm: 157.48 (C2), 123.33 (C3), 137.73 (C4), 127.72 (C5), 31.65 (C2–CH_2), 53.45 (C2–CH_2–CH_2), 40.90 [C2-$(CH_2)_2$–HN^+–CH_3], 55.92 (CH_2–CH_2–NH–C2′), 39.09 (CH_2–CH_2–NH–C2′), 180.54 (C1′), 145.96 (C2′), 135.30 (C3′), 176.00 (C4′), 132.10 (C4a′), 126.22 (C5′), 135.30 (C6′), 133.35 (C7′), 127.06 (C8′), 130.90 (C8a′). At 298 K, C4a, C6, C7, and C8 were not detected. Complete assignment of the quinoline motif was possible at 353 K: ^{1}H NMR, δ, ppm: 7.52 (H3), 8.28 (H4), 7.44 (H5), 7.46 (H6), 7.35 (H7). ^{13}C NMR, δ, ppm: 156.47 (C2), 122.89 (C3), 137.73 (C4), 127.90 (C4a), 127.19 (C5), 120.13 (C6), 115.88 (C7), 137.48 (C8), 138.40 (C4a). C2-$(CH_2)_2$–HN^+–CH_3 was not detected. HMBC correlations were detected between *H*N–C2′ (7.69 ppm) and C1′ (180.54 ppm) on one hand, and C3′ (135.30 ppm) on the other hand. ESI$^+$-MS: calcd for $C_{24}H_{24}ClN_4O_2$: 435.15 ([M+H]$^+$); found: 435.11. Elemental analysis calcd (%) for $C_{24}H_{23}ClN_4O_2 \cdot 2.0HCl \cdot 0.2H_2O \cdot 0.3CH_2Cl_2 \cdot 0.3\ C_2H_5OH$ (apparent MW = 549.10): C 54.30, H 5.09, N 10.17, found: C 54.47, H 5.09, N 10.07. HPLC (CH_3OH/H_2O, 35/65, v/v, 0.2 vol% TFA): R_t = 19.8 min, purity 98%.

Atokel-4

2-(2-Hydroxyethyl)quinolin-8-ol (M-Atokel-3). *n*-Butyllithium (4.3 mL, 6.9 mmol) was added dropwise to a stirring solution of 8-hydroxyquinaldine (0.5 g, 3.1 mmol) in tetrahydrofuran (10 mL) at −78 °C for 2 h, under Ar. Paraformaldehyde (377 mg, 12.4 mmol) was added at −78 °C, and the temperature was allowed to raise room temperature over a period of 2 h. The mixture was quenched by addition of saturated ammonium chloride solution (10 mL) at −78 °C and extracted with dichloromethane. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure, and the residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 25/1, v/v) to give **M-Atokel-3** as a white solid (446 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.31–7.26 (m, 2H), 7.15 (d, *J* = 7.6 Hz, 1H), 5.18 (br, 2H), 4.13 (t, *J* = 6.0 Hz, 2H), 3.19 ppm (t, *J* = 6.0 Hz, 2H).

2-Vinylquinolin-8-ol (N-Atokel-3). Triethylamine (184 μL, 13.75 mmol) and methanesulfonyl chloride (91 mg, 8.25 mmol) were added to a solution of **M-Atokel-3** (50 mg, 2.75 mmol) in dichloromethane (2.5 mL) at 0 °C, and the mixture was stirred at room temperature for 3 h. The solvent was evaporated, the resulting solid was solubilized in the minimum of EtOH (0.9 mL), and an aqueous solution of sodium hydroxide (1 M, 3 mL) was added. The mixture was stirred for 5 h at reflux, and then neutralized with HCl 1 M to pH = 7. The solution was extracted with dichloromethane, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with CH_2Cl_2 as eluent, to give **N-Atokel-3** as a white solid (36 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 6.97 (dd, *J* = 17.6, 10.8 Hz, 1H), 6.29 (d, *J* = 17.6 Hz, 1H), 5.63 ppm (d, *J* = 10.8 Hz, 1H).

tert-Butyl(2-((2-(8-hydroxyquinolin-2-yl)ethyl)(methyl)amino)ethyl)carbamate (O-Atokel-3). To a mixture of **N-Atokel-3** (1.3 g, 7.6 mmol) was added to a stirring solution of *tert*-butyl (2-(methylamino)ethyl)carbamate (3.06 g, 15.2 mmol) in methanol (20 mL), and then 2 mL of acetic acid were added and the mixture was stirred at 70 °C for 24 h. It was then concentrated under reduced pressure. Dichloromethane was added, and the mixture was washed with saturated aqueous $NaHCO_3$ and water. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure, and the residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 20/1, v/v) to give **O-Atokel-3** as a yellow oil (2.3 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 4.91 (brs, 1H), 3.19 (q, *J* = 4.2 Hz, 2H), 3.11 (t, *J* = 7.2 Hz, 2H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.54 (t, *J* = 6.0 Hz, 2H), 2.30 (s, 3H), 1.42 ppm (s, 9H).

2-(2-((2-Aminoethyl)(methyl)amino)ethyl)quinolin-8-ol (P-Atokel-3). To a stirred solution of **O-Atokel-3** (450 mg, 1.3 mmol) dissolved in CH_2Cl_2 (5 mL) at 0 °C, trifluoroacetic acid (7 mL) was added. After 3 h at room temperature, the solution was neutralized with aqueous ammonium hydroxide in an ice bath. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduce pressure. The crude mixture was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 20/1, v/v) to give **P-Atokel-3** as a yellow oil (320 mg, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 7.2 Hz, 1H), 3.80 (brs, 2H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.79 (t, *J* = 6.0 Hz, 2H), 2.51 (t, *J* = 6.0 Hz, 2H), 2.31 ppm (s, 3H).

2-Chloro-3-((2-((2-(8-hydroxyquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino) naphthalene-1,4-dione (Q-Atokel-4). To mixture of **P-Atokel-3** (400 mg, 0.92 mmol) and K_2CO_3 (270 mg, 1.1 mmol) in acetonitrile (10 mL) was added 2-chloronaphthalene-1,4-dione (626 mg, 1.8 mmol). The reaction mixture was stirred at room temperature for 24 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH, 20/1, v/v). After evaporation of the eluent, the target compound was obtained as a orange solid (370 mg, 52%). The same compound was obtained in a 48% yield when using 2,3-dichloronaphthalene-1,4-dione as material. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.92 (d, *J* = 8.4 Hz, 1H),7.87 (d, *J* = 7.6 Hz, 1H), 7.75 (d, *J* = 7.6 Hz,1H), 7.64 (td, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H), 7.54 (td, *J* = 7.6 Hz, *J* = 1.2 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 6.95–6.92 (m, 2H), 6.72–6.69 (m, 1H), 3.77 (t, *J* = 6.0 Hz, 2H), 3.03 (t, *J* = 6.0 Hz, 2H), 2.94 (t, *J* = 6.0 Hz, 2H), 2.60 (t, *J* = 6.0 Hz, 2H), 2.35 ppm (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ = 179.1, 176.4, 158.7, 152.0, 137.7, 136.4, 134.3, 132.3, 132.1, 129.9, 129.5, 127.4, 127.0, 126.2, 126.3, 125.7, 122.3, 117.5, 110.4, 56.0, 55.6, 41.2, 34.9, 31.7 ppm. ESI$^+$-MS: calcd for $C_{24}H_{23}ClN_3O_3$: 436.14 ([M+H]$^+$); found: 436.14.

Atokel-4. From **Q-Atokel-4**, **Atokel-4** was prepared using the procedure above described for the preparation of **Atokel-1** from **J-Atokel-1** [Scheme 1, steps (h) and (n)]. **Atokel-4** was a yellow solid (yield = 96%). mp: 167–170 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.42 (brs, 1H), 8.70 (s, 1H), 7.97 (m, 2H), 7.90–7.80 (m, 2H), 7.79–7.73 (m, 1H), 7.68 (s, 1H), 7.57 (m, 2H), 7.38 (d, *J* = 5.3 Hz, 1H), 4.20 (br, *J* = 5.4 Hz, 2H), 3.76 (br, 2H), 3.71 (br, 2H), 3.50 (br, 2H), 2.96 ppm (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 180.5, 175.9, 155.9, 150.3, 145.9, 143.5, 135.2, 133.3, 132.1, 130.7, 129.5, 128.7, 126.9, 126.2, 123.7, 118.5, 115.3, 56.0, 53.9, 40.5, 39.3, 29.6 ppm. ESI$^+$-MS: calcd for $C_{24}H_{23}ClN_3O_3$: 436.14 ([M+H]$^+$); found: 436.14. Elemental analysis calcd (%) for $C_{24}H_{22}ClN_3O_3 \cdot 2.0HCl \cdot 0.2CH_2Cl_2$ (apparent MW = 525.81): C 55.28, H 4.68, N 7.99, found: C 55.55, H 4.41, N 7.96; HPLC (CH_3OH/H_2O, 35/65, v/v, 0.2 vol% TFA): R_t = 33.7 min, purity 97%.

Atokel-3

2-((2-((2-(8-Hydroxyquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)naphthalene-1,4-dione (R-Atokel-3). Palladium on carbon (12 mg, 5 wt%) and hydrazine hydrate 80% (1 mL) were added to a solution of Q-Atokel-4 (230 mg, 0.57 mmol) in

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isopropanol (4 mL). The mixture was stirred at 80 °C for 20 min. The reaction mixture was poured into water and extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (CH_2Cl_2/MeOH, 20/1, v/v.). Evaporation of the solvent afforded **R-Atokel-3** as a orange solid (185 mg, 86 %). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.94 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.78 (d, J = 7.6 Hz,1H), 7.65 (t, J = 7.6 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 6.98–6.95 (m, 2H), 6.77–6.75 (m, 1H), 6.54 (s, 1H), 3.11 (t, J = 5.6 Hz, 2H), 3.05 (t, J = 6.4 Hz, 2H), 2.94 (t, J = 6.4 Hz, 2H), 2.65 (t, J = 5.6 Hz, 2H), 2.36 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 183.2, 180.3, 158.9, 152.1, 148.5, 137.9, 136.3, 134.3, 133.5, 131.8, 130.4, 127.4, 126.2, 125.9, 125.3, 122.3, 117.5, 110.3, 98.9, 55.8, 54.1, 41.4, 39.0, 35.3 ppm. ESI$^+$-MS: calcd for $C_{24}H_{24}N_3O_3$: 402.17 ([M + H]$^+$); found: 402.21.

Atokel-3. The same protonation as described above for the preparation of **Atokel-1** afforded **Atokel-3** as an orange red solid (100 %). mp: 167–170 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ, ppm: 7.83 (H3), 8.73 (H4), 7.57 (H5), 7.57 (H6), 7.36 (H7), 3.66 [2H, C2–CH_2], 3.73 [2H, C2–CH$_2$–CH_2], 2.95 [3H, C2-(CH$_2$)$_2$–HN$^+$–CH_3], 3.44 [2H, CH_2–CH$_2$–NH–C2′], 3.73 [2H, CH_2–NH–C2′], 7.71 [1H, HN–C2′], 5.92 (H3′), 7.94 (H5′), 7.85 (H6′), 7.75 (H7′), 7.97 (H8′). ^{13}C NMR (150 MHz, DMSO-d_6), δ, ppm: 155.92 (C2), 123.59 (C3), 142.37 (C4), 118.30 (C5), 129.18 (C6), 114.74 (C7), 150.79 (C8), 29.89 (C2–CH$_2$), 53.74 (C2–CH$_2$–CH$_2$), 40.12 [C2-(CH$_2$)$_2$–HN$^+$–CH$_3$], 52.70 (CH$_2$–CH$_2$–NH–C2′), 37.15 (CH$_2$–CH$_2$–NH–C2′), 181.67 (C1′), 148.75 (C2′), 101.05 (C3′), 182.37 (C4′), 133.35 (C4a′), 125.88 (C5′), 135.38 (C6′), 132.89 (C7′), 124.43 (C8′), 132.88 (C8a′). C8a, HO–C8, and C2-(CH$_2$)$_2$–HN$^+$–CH$_3$ were not detected. HMBC correlations were detected between HN–C2′ (7.71 ppm) and C1′ (181.67 ppm), and between H5′ (7. 94 ppm) and C4′ (182.37 ppm). ESI$^+$-MS: calcd for $C_{24}H_{24}N_3O_3$: 402.17 ([M + H]$^+$); found: 402.21. Elemental analysis calcd (%) for $C_{24}H_{23}N_3O_3 \cdot 2.0HCl \cdot 1.3H_2O \cdot 0.3CH_2Cl_2$ (apparent MW = 523.28): C 55.78, H 5.43, N 8.03, found: C 55.67, H 5.42, N 7.89. HPLC (CH_3OH/H_2O, 35/65, v/v, 0.2 vol % TFA): R_t = 28.9 min, purity 93 %.

Atokel-5

5,7-Dichloro-2-methylquinoline (B-Atokel-5). To solution of 3,5-dichloroaniline (2.0 g, 12.3 mM) in HCl (12 м, 8 mL) at 0 °C, acetaldehyde was added dropwise under stirring. The reaction mixture was kept at 0 °C for 15 min, and the temperature was gradually raised to 80 °C and stirred at 80 °C for 4 h. The resulting mixture was poured into ice-cold water and neutralized with aqueous ammonium hydroxide. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (ethyl acetate/petroleum ether, 1/20, v/v). After, evaporation of the eluent, **B-Atokel-5** was obtained as a yellow solid (1.80 g, 69 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.39 (d, J = 8.4 Hz, 1H), 7.94 (s, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 2.75 ppm (s, 3H).

5,7-Dichloro-2-methyl-8-nitroquinoline (C-Atokel-5). To a stirred solution of **B-Atokel-5** (2.00 g, 9.5 mM) in neat sulfuric acid (10 mL), was added dropwise fuming nitric acid (2 mL) over a 1 h period, at room temperature. The resulting mixture was stirred for an additional hour, and was then poured into ice. The mixture was allowed to warm to ambient temperature, and neutralized with aqueous ammonium hydroxide. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (ethyl acetate/petroleum ether, 1/10, v/v). By evaporation of the solvent, **C-Atokel-5** was isolated as a yellow solid (1.82 g, 91 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, J = 8.8 Hz, 1H), 7.63 (s, 1H), 7.50 (d, J = 8.8 Hz, 1H), 2.76 ppm (s, 3H).

5,7-Dichloro-8-nitro-2-vinylquinoline (D-Atokel-5). To a solution of **C-Atokel-5** (1.0 g, 3.9 mM) in DMA (5 mL), $FeCl_3$ (19.0 mg, 0.117 mmol) and $K_2S_2O_8$ (1.05 g, 7.8 mmol) were added. The mixture was stirred at 110 °C for 1.5 h, and quenched with water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (EtOAc/petroleum ether, 1/10, v/v). After evaporation of the eluent, compound **D-Atokel-5** was obtained as a brown solid (430.3 mg, 41 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.50 (d, J = 7.2 Hz, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.64 (s, 1H), 6.96 (dd, J = 17.6, 10.8 Hz, 1H), 6.45 (d, J = 17.6 Hz, 1 H), 5.78 ppm (d, J = 10.4 Hz, 1H).

tert-Butyl (2-((2-(5,7-dichloro-8-nitroquinolin-2-yl)ethyl)(methyl)amino)ethyl) carbamate (E-Atokel-5). The procedure was similar to that described for the preparation of **E-Atokel-1**. **E-Atokel-5** was obtained as a yellow oil (83 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.48 (d, J = 8.8 Hz, 1H), 7.66 (s, 1H), 7.60 (d, J = 8.8 Hz, 1H), 5.37 (brs, 1H), 3.36 (m, 4H), 3.23 (br, 2H), 2.84 (br, 2H), 2.53 (s, 3H), 1.41 ppm (s, 9H).

N^1-(2-(5,7-Dichloro-8-nitroquinolin-2-yl)ethyl)-N^1-methylethane-1,2-diamine (H-Atokel-5). The procedure was similar to that described for the preparation of **H-Atokel-1**. **H-Atokel-5** was obtained as a yellow oil (98 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.43 (d, J = 8.8 Hz, 1H), 7.61 (s, 1H), 7.54 (d, J = 8.8 Hz, 1H), 5.75 (brs, 2H), 3.14 (t, J = 6.8 Hz, 2 H), 2.94-2.91 (m, 4 H), 2.64 (t, J = 6.0 Hz, 2H), 2.53 (s, 3H), 1.41 ppm (s, 9H).

2-Chloro-3-((2-((2-(5,7-dichloro-8-nitroquinolin-2-yl)ethyl)(methyl)amino)ethyl) amino)naphthalene-1,4-dione (I-Atokel-5). To a mixture of **H-Atokel-5** (370 mg, 1.08 mmol) and K_2CO_3 (250 mg, 1.3 mmol) in acetonitrile (6 mL) was added 2-chloronaphthalene-1,4-dione (208 mg, 1.08 mmol). The reaction mixture was stirred at room temperature for 24 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v). After evaporation of the eluent, the target compound was obtained as red solid (520 mg, 90 %). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.47 (d, J = 8.8 Hz, 1H), 7.90–7.88 (m, 2H), 7.80–7.76 (m, 2H), 7.70–7.69 (m, 2H), 6.48 (brs, 1H), 3.73 (q, J = 5.2 Hz, 2H), 3.10 (t, J = 6.0 Hz, 2H), 2.87 (t, J = 6.0 Hz, 2H), 2.57 (t, J = 5.2 Hz, 2H), 2.28 ppm (s, 3H).

2-((2-((2-(8-Amino-5,7-dichloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino) naphthalene-1,4-dione (K-Atokel-5). Palladium on carbon (32 mg, 5 wt %) and hydrazine hydrate 80 % (2 mL) were added to a solution of **I-Atokel-5** (510 mg, 0.96 mmol) in isopropanol (10 mL). The mixture was stirred at 80 °C for 17 min. The reaction mixture was poured into water and extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v.). Evaporation of the solvent afforded **K-Atokel-5** as a orange-red solid (340 mg, 76 %). In addition, **K-Atokel-5** can also be obtained with a similar procedure that described for the preparation of **K-Atokel-1**, by using **J-Atokel-6** as starting material (yield = 90 %). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.21 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.63 (t, J = 8.4 Hz, 1H), 7.44 (t, J = 8.4 Hz, 1H), 6.85 (s, 1H), 5.46 (s, 1H), 3.13-3.10 (m, 4H), 3.07 (t, J = 5.2 Hz, 2H), 2.70 (t, J = 5.2 Hz, 2H), 2.46 ppm (t, 3H); ESI$^+$-MS: calcd for $C_{24}H_{23}Cl_2N_4O_2$: 469.12 ([M + H]$^+$); found: 469.12.

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Atokel-5. The same procedure as described above for the preparation of **Atokel-1** afforded **Atokel-5** as a orange red solid (100%). mp: 179–182 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.62 (H3), 8.35 (H4), 7.59 (H6), 3.50 [2H, C2–CH_2], 3.79–3.90 [2H, C2–CH$_2$–CH_2], 10.72 [1H, C2-(CH$_2$)$_2$–HN$^+$–CH$_3$], 2.93 [3H, C2–(CH$_2$)$_2$–HN$^+$–CH_3], 3.35–3.50 [2H, CH_2–CH$_2$–NH–C2′], 3.71 [2H, CH_2–NH–C2′], 7.67 [1H, HN–C2′], 5.91 (H3′), 7.95 (H5′), 7.84 (H6′), 7.74 (H7′)), 7.99 (H8′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 156.70 (C2), 123.84 (C3), 133.63 (C4), 123.84 (C4a), 115.30 (C5), 127.55 (C6), 112.00 (C7), 141.27 (C8), 137.40 (C8a), 31.74 (C2–CH$_2$), 53.62 (C2–CH$_2$–CH$_2$), 40.30 [C2-(CH$_2$)$_2$–HN$^+$–CH$_3$], 52.74 (CH$_2$–CH$_2$–NH–C2′), 37.16 (–CH$_2$–CH$_2$–NH–C2′), 181.70 (C1′), 101.07 (C3′), 182.25 (C4′), 125.85 (C5′), 135.35 (C6′), 132.87 (C7′), 126.39 (C8′). The C2′, C4a′, C8a′ were not detected. The C8-H_3N$^+$ was not detected. HMBC correlations were detected between HN–C2′ (7.67 ppm) and C1′ (177.85 ppm) and C3′ (101.07 ppm), and between H4 (8.35 ppm) and C5 (115.30 ppm). ESI$^+$-MS: calcd for $C_{24}H_{23}Cl_2N_4O_2$: 469.12 ([M + H]$^+$); found: 469.12. Elemental analysis calcd (%) for $C_{24}H_{22}Cl_2N_4O_2 \cdot 1.7HCl \cdot 0.2CH_2Cl_2$ (apparent MW = 548.33): C 53.01, H 4.43, N 10.22, found: C 53.09, H 3.85, N 10.01. HPLC (CH_3OH/H_2O, 55/45, v/v, 0.2 vol% TFA): R_t = 18.5 min, purity 93%.

Atokel-6

tert-Butyl(2-((2-(8-amino-5,7-dichloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)carbamate (F-Atokel-6). The product was prepared starting from E-Atokel-5, with a similar procedure as described for the preparation of **F-Atokel-1** from **E-Atokel-1** (yield = 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31 (d, J = 8.6 Hz, 1H), 7.41 (s, 1H), 7.36 (d, J = 8.6 Hz, 1H), 5.32 (brs, 2 H), 4.88 (brs, 1 H), 3.20 (q, J = 5.6 Hz, 2H), 3.10 (t, J = 7.3 Hz, 2 H), 2.92 (d, J = 7.3 Hz, 2H), 2.54 (t, J = 5.6 Hz, 2H), 2.30 (s, 3H), 1.41 ppm (s, 9H).

N^1-(2-(8-Amino-5,7-dichloroquinolin-2-yl)ethyl)-N^1-methylethane-1,2-diamine (G-Atokel-6). Palladium on carbon (11 mg, 5 wt%) and hydrazine hydrate 80% (170 μL) were added to a solution of **H-Atokel-5** (50 mg, 0.15 mmol) in isopropanol (2 mL). The mixture was stirred at 80 °C for 7 min. The reaction mixture was poured into water and extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (ethyl acetate/isopropanol/25% ammonium hydroxide, 5/2/0.6, v/v/v). Evaporation of the solvent afforded **G-Atokel-5** as a yellow oil (36 mg, 80%). In addition, the preparation of **G-Atokel-5** can be also obtained by removing the -Boc group using an excess of trifluoroacetic acid (yield = 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31 (d, J = 8.4 Hz, 1H), 7.41 (s, 1H), 7.37 (d, J = 8.4 Hz, 1H), 5.33 (brs, 2H), 3.13 (t, J = 7.6 Hz, 2H), 2.90 (t, J = 7.6 Hz, 2H), 2.75 (t, J = 6.0 Hz, 2H), 2.50 (t, J = 6.0 Hz, 2H), 2.31 ppm (s, 3H).

2-((2-((2-(8-Amino-5,7-dichloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-3-chloronaphthalene-1,4-dione (J-Atokel-6). To mixture of **G-Atokel-5** (40 mg, 0.13 mmol) and K_2CO_3 (22 mg, 0.16 mmol) in acetonitrile (2 mL) was added 2,3-dichloronaphthalene-1,4-dione (30 mg, 0.13 mmol). The reaction mixture was stirred at room temperature for 24 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v). After evaporation of the eluent, the target compound was obtained as a red solid (54 mg, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.20 (d, J = 8.6 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.79 (m, 2H), 7.70 (t, J = 7.4 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.23 (s, 1H), 6.66 (brs, 1H), 6.00 (s, 2H), 3.78 (q, J = 6.0 Hz, 2H), 3.06 (t, J = 6.4 Hz, 2H), 2.94 (t, J = 6.4 Hz, 2H), 2.60 (t, J = 6.0 Hz, 2H), 2.31 ppm (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 179.6, 174.3, 160.1, 140.9, 140.9, 140.8, 137.5, 135.2, 132.9, 132.8, 130.3, 126.7, 126.6, 126.1, 123.9, 123.5, 115.45, 111.8, 56.5, 55.60, 42.3, 35.8, 29.5 ppm. ESI$^+$-MS: calcd for $C_{24}H_{22}Cl_3N_4O_2$: 503.08 ([M + H]$^+$); found: 503.08.

Atokel-6. The same protonation procedure as described above for the preparation of **Atokel-1** afforded **Atokel-6** as a orange red solid (100%). mp: 193–195 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.34 (brs, 1H), 8.36 (d, J = 8.5 Hz, 1H), 7.98 (t, J = 8.1 Hz, 2H), 7.84 (t, J = 7.0 Hz, 1H), 7.77 (t, J = 7.5 Hz, 1H), 7.67–7.53 (m, 3H), 3.85–3.78 (m, 2H), 3.49 (m, 4H), 3.46–3.36 (m, 2H), 2.96 ppm (d, J = 4.6 Hz, 3H). The low solubility of this derivative, even in DMSO-d_6 and in pyridine-d_5) prevented its ^{13}C NMR analysis. ESI$^+$-MS: calcd for $C_{24}H_{22}Cl_3N_4O_2$: 503.08 ([M + H]$^+$); found: 503.08. Elemental analysis calcd (%) for $C_{24}H_{21}Cl_3N_4O_2 \cdot 1.8HCl \cdot 0.2CH_2Cl_2$ (apparent MW = 586.42): C 49.57, H 3.99, N 9.55, found: C 49.86, H 3.25, N 9.44. HPLC (CH_3OH/H_2O, 55/45, v/v, 0.2 vol% TFA): $R_{t=}$ 15.5 min, purity 90%.

Atokel-7

7-Chloro-2-methyl-8-nitroquinoline (C-Atokel-7). The procedure was similar to that described for the preparation of **B-Atokel-5**. **C-Atokel-7** was obtained as a yellow solid (77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 2.73 ppm (s, 3H).

7-Chloro-8-nitro-2-vinylquinoline (D-Atokel-7). The procedure was similar to that described for the preparation of **D-Atokel-5**. **D-Atokel-7** was obtained as a yellow solid (33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (d, J = 8.6 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 8.6 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 6.94 (dd, J = 17.6, 10.8 Hz, 1H), 6.39 (d, J = 17.5 Hz, 1H), 5.72 ppm (d, J = 10.8 Hz, 1H).

tert-Butyl(2-((2-(7-chloro-8-nitroquinolin-2-yl)ethyl)(methyl)amino)ethyl)carbamate (E-Atokel-7). The procedure was similar to that described for the preparation of **E-Atokel-1**. **E-Atokel-7** was obtained as a yellow oil (94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (d, J = 8.5 Hz, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 4.80 (brs, 1H), 3.22–3.07 (m, 4H), 2.89 (t, J = 6.8 Hz, 2H), 2.51 (t, J = 5.6 Hz, 2H), 2.27 (s, 3H), 1.40 ppm (s, 9H).

N^1-(2-(7-Chloro-8-nitroquinolin-2-yl)ethyl)-N^1-methylethane-1,2-diamine (H- Atokel-7). The procedure was similar to that described for the preparation of **H-Atokel-1**. **H-Atokel**-7 was obtained as a yellow oil (96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (d, J = 8.5 Hz, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1H), 7.45 (d, J = 8.5 Hz, 1H), 3.14 (t, J = 6.8 Hz, 2H), 3.07 (br, 2H), 2.93 (t, J = 6.8 Hz, 2H), 2.86 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 6.0 Hz, 2H), 2.28 ppm (s, 3H).

2-Chloro-3-((2-((2-(7-chloro-8-nitroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-4a,8a-dihydronaphthalene-1,4-dione (I-Atokel-7). The procedure was similar to that described for the preparation of **I-Atokel-5**. **I-Atokel-7** was obtained as a red solid (79%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.43 (d, J = 8.5 Hz, 1H), 8.12 (d, J = 8.9 Hz, 1H), 7.91 (d, J = 7.5 Hz, 1H), 7.84–7.59 (m, 5H), 6.67 (brs, 1H), 3.75 (q, J = 5.5 Hz, 2H), 3.08 (t, J = 6.0 Hz, 2H), 2.86 (br, 2H), 2.60 (br, 2H), 2.28 ppm (s, 3H).

2-((2-((2-(8-Amino-7-chloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)naphthalene-1,4-dione (K-Atokel-7). The procedure was similar to that described for the preparation of **K-Atokel-5**. **Atokel-7** was obtained as a red solid (86%). In addition, **Atokel-7** can be obtained with a similar procedure as that described for the preparation of **Atokel-5**, by using **Atokel-8** as starting material (yield = 94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.07 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 7.5 Hz, 1H), 7.82 (t, J = 7.3 Hz, 1H), 7.71 (t, J = 7.3 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 6.99 (d, J = 8.7 Hz, 1H), 6.84 (br, 1H), 5.86

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(brs, 2H), 5.65 (s, 1H), 3.21 (q, $J=5.6$ Hz, 2H), 3.07 (t, $J=6.8$ Hz, 2H), 2.93 (t, $J=6.8$ Hz, 2H), 2.66 (t, $J=5.6$ Hz, 2H), 2.32 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta=181.7$, 181.5, 159.2, 148.4, 140.9, 137.5, 136.6, 135.2, 133.6, 132.5, 130.6, 127.2, 126.3, 125.8, 125.8, 122.7, 114.9, 113.0, 100.0, 56.4, 54.5, 42.4, 39.7, 36.1 ppm. ESI$^+$-MS calcd for $C_{24}H_{24}ClN_4O_2$: 435.16 ([M+H]$^+$); found: 435.16.

Atokel-7. The same procedure as described above for the preparation of Atokel-1 afforded Atokel-7 as a red solid (94%). mp: 177–181 °C. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=10.95$ (brs, 1H), 8.23 (d, $J=8.4$ Hz, 1H), 7.97 (m, 2H), 7.84 (t, $J=7.3$ Hz, 1H), 7.79–7.63 (m, 2H), 7.48 (d, $J=8.4$ Hz, 1H), 7.39 (d, $J=8.7$ Hz, 1H), 7.12 (d, $J=8.7$ Hz, 1H), 6.59 (brs, 2H), 5.92 (s, 1H), 3.83–3.72 (m, 4H), 3.50–3.38 (m, 4H), 2.93 ppm (d, $J=4.3$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta=182.2$, 181.7, 155.9, 148.7, 140.3, 138.2, 136.4, 135.3, 133.4, 132.8, 130.8, 128.2, 126.4, 126.3, 125.8, 122.7, 115.3, 114.2, 101.0, 53.7, 52.7, 40.1, 37.2, 31.5 ppm. ESI$^+$-MS calcd for $C_{24}H_{24}ClN_4O_2$: 435.16 ([M+H]$^+$); found: 435.16. Elemental analysis calcd (%) for $C_{24}H_{23}ClN_4O_2\cdot1.9HCl\cdot0.1CH_2Cl_2$ (apparent MW=512.69): C 56.46, H 4.93, N 10.93, found: C 56.35, H 4.62, N 10.65. HPLC (CH_3OH/H_2O, 50/50, v/v, 0.2 vol% TFA): $R_t=13.3$ min, purity 88%.

Atokel-8

tert-Butyl(2-((2-(8-amino-7-chloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)carbamate (F-Atokel-8). The procedure was similar to that described for the preparation of **F-Atokel-5**. **F-Atokel-8** was obtained as a yellow oil (96%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.95$ (d, $J=8.4$ Hz, 1H), 7.32 (d, $J=8.8$ Hz, 1H), 7.26 (d, $J=8.4$ Hz, 1H), 7.03 (d, $J=8.8$ Hz, 1H), 5.31 (brs, 2H), 4.88 (brs, 1H), 3.20 (q, $J=5.6$ Hz, 2H), 3.10 (t, $J=6.8$ Hz, 2H), 2.90 (s, $J=6.8$ Hz, 3H), 2.54 (t, $J=6.0$ Hz, 2H), 2.3 (s, 3H), 1.41 ppm (s, 9H).

N^1-(2-(8-Amino-7-chloroquinolin-2-yl)ethyl)-N^1-methylethane-1,2-diamine (G-Atokel-8). The procedure was similar to that described for the preparation of **G-Atokel-5**. **G-Atokel-8** was obtained as a yellow oil (84%). In addition, **G-Atokel-8** can be also obtained by removing the Boc group with an excess of trifluoroacetic acid (yield=100%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.89$ (d, $J=8.4$ Hz, 1H), 7.27 (d, $J=8.8$ Hz, 1H), 7.20 (d, $J=8.4$ Hz, 1H), 6.98 (d, $J=8.8$ Hz, 1H), 5.67 (brs, 2H), 5.31 (brs, 1H), 3.03 (t, $J=7.2$ Hz, 2H), 2.89-2.84 (m, 4H), 2.57 (t, $J=6.4$ Hz, 2H), 2.23 ppm (s, 3H).

2-((2-((2-(8-Amino-7-chloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-3-chloronaphthalene-1,4-dione (J-Atokel-8). The procedure was similar to that described for the preparation of **J-Atokel-6**. **J-Atokel-8** was obtained as a yellow oil (72%). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=8.06$ (d, $J=8.4$ Hz, 1H), 7.94 (d, $J=7.6$ Hz, 1H), 7.84 (d, $J=7.6$ Hz, 1H), 7.80 (td, $J=7.5$, 1.2 Hz, 1H), 7.71 (td, $J=7.5$, 1.2 Hz, 1H), 7.38 (d, $J=8.4$ Hz, 1H), 7.17 (d, $J=8.7$ Hz, 1H), 6.97 (d, $J=8.7$ Hz, 1H), 6.89 (brs, 1H), 5.83 (s, 2 H), 3.79 (q, $J=6.0$ Hz, 2 H), 3.02 (t, $J=6.0$ Hz, 2H), 2.89 (t, $J=6.8$ Hz, 2H), 2.62 (t, $J=6.0$ Hz, 2H), 2.29 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta=179.7$, 175.5, 159.0, 140.9, 137.5, 136.7, 135.1, 132.9, 132.4, 130.4, 127.2, 126.7, 126.1, 125.8, 122.7, 114.9, 112.9, 56.6, 56.2, 42.0, 41.9, 36.0 ppm. ESI$^+$-MS calcd for $C_{24}H_{23}Cl_2N_4O_2$: 469.12 ([M+H]$^+$); found: 469.12.

Atokel-8. The same protonation as described above for the preparation of **Atokel-1** afforded **Atokel-8** as a red solid (96%). mp: 170–174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=10.70$ (brs, 1H), 8.22 (d, $J=8.4$ Hz, 1H), 8.06–7.93 (m, 2H), 7.85 (t, $J=7.2$ Hz, 1H), 7.77 (t, $J=7.4$ Hz, 1H), 7.64 (t, $J=6.4$ Hz, 1H), 7.48 (d, $J=8.4$ Hz, 1H), 7.38 (d, $J=8.7$ Hz, 1H), 7.11 (d, $J=8.8$ Hz, 1H), 4.88 (br, 2 H), 4.17 (q, $J=6.4$ Hz, 2H), 3.83 (s, 2H), ≈3.5 (br, 2H), 3.48 (t, $J=7.8$ Hz, 2H), 2.95 ppm (d, $J=4.6$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta=180.5$, 175.9, 155.9, 153.4, 145.9, 140.6, 137.9, 136.6, 135.2, 133.3, 132.1, 130.7, 128.7, 128.1, 126.9, 126.3, 126.2, 122.7, 119.6, 115.2, 113.9, 55.9, 53.8, 49.1, 33.0, 31.7 ppm. ESI$^+$-MS calcd for $C_{24}H_{23}Cl_2N_4O_2$: 469.12 ([M+H]$^+$); found: 469.12. Elemental analysis calcd (%) for $C_{24}H_{22}Cl_2N_4O_2\cdot1.9HCl\cdot0.1CH_2Cl_2$ (apparent MW=547.13): C 52.91, H 4.44, N 10.24, found: C 53.08, H 3.75, N 10.05. HPLC (CH_3OH/H_20, 50/50, v/v, 0.2 vol% TFA): $R_t=12.1$ min, purity 96%.

Atokel-13

2-Hydroxy-3-nitronaphthalene-1,4-dione (T-Atokel-13). To the solution of 2,3-dichloronaphthalene-1,4-dione (S-Atokel-13) (1 g, 4.4 mmol) in acetone/methanol (1/1, v/v, 40 mL), $NaNO_2$ (1.52 g, 22.0 mmol) was added and the mixture was refluxed for 45 min. Then the mixture was cooled to room temperature, the solvent was evaporated under vacuum, and the residue was acidified with concentrated HCl (≈2.0 mL) in order to convert the insoluble salt of nitroquinone into the soluble acid form. The mixture was filtered, the NaCl precipitate was washed repeatedly with acetone. The crude product was obtained after evaporation of the solvent, and then purified by silica gel flash chromatography (ethyl acetate/methanol, 20/1, v/v). Evaporation of the solvent afforded **T-Atokel-13** as a red solid (700 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=8.04$ (d, $J=7.6$ Hz, 1H), 7.91 (d, $J=7.6$ Hz, 1H), 7.83 (t, $J=7.4$ Hz, 1H), 7.69 (t, $J=7.4$ Hz, 1H), 3.44 ppm (brs, 1H).

2-Amino-3-hydroxynaphthalene-1,4-dione (U-Atokel-13). To the solution of **T-Atokel-13** (7.2 g, 32.85 mmol) in H_2O (20 mL), a solution of $Na_2S_2O_4$ (25.7 g, 147.61 mmol) in H_2O (80 mL) was added. The mixture was stirred at room temperature for 2 h until the reaction was complete (TLC monitoring). The purple black precipitate of aminoquinone was filtered, washed with H_2O (2× 50 mL), purified by silica gel flash chromatography (ethyl acetate/methanol, 20/1, v/v). Evaporation of the solvent afforded **U-Atokel-13** as a purple black (6.0 g, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=9.48$ (brs, 1H), 7.86 (d, $J=7.2$ Hz, 2H), 7.84 (d, $J=7.2$ Hz, 2H), 7.71 (t, $J=7.2$ Hz, 1H), 7.65 (t, $J=7.2$ Hz, 1H), 5.95 ppm (s, 2H).

2,3-Dihydroxynaphthalene-1,4-dione (V-Atokel-13). To a stirred solid of **U-Atokel-13**, H_2SO_4 (30%, 3 mL) was added and refluxed for 8 h to hydrolyze the amine. The solution was cooled and extracted with EtOAc (3×20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (ethyl acetate/methanol, 20/1, v/v) to give **V-Atokel-3** as a red solid (36 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=7.93$ (dd, $J=5.6$ Hz, $J=3.2$ Hz, 2H), 7.77 (dd, $J=5.6$ Hz, $J=3.2$ Hz, 2H), 7.33 ppm (brs, 2H).

1,4-Dioxo-1,4-dihydronaphthalene-2,3-diyl-bis(4-methylbenzenesulfonate) (W-Atokel-13). To a solution of **V-Atokel-13** (30 mg, 0.16 mmol) in THF (0.5 mL) was added Et_3N (128 mg, 1.28 mmol). A solution of tosyl chloride (TsCl, 300 mg, 1.60 mmol) in THF (1.5 mL) was slowly added over 15 min at 0 °C, in an ice-water bath. The ice water bath was then removed and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was poured into water and extracted with dichloromethane (3×20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (CH_2Cl_2/hexane, 3/1, v/v). Evaporation of the solvent afforded **W-Atokel-13** as a yellow solid (37 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.12$ (br, 2H), 7.79–7.78 (m, 6H), 7.33–7.26 (m, 4H), 2.48 ppm (s, 6H).

3-((2-((2-(8-Aminoquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-1,4-dioxo-1,4,4a,8a-tetrahydronaphthalen-2-yl-4-methylbenzenesulfonate (X-Atokel-13). To mixture of **G-Atokel-1** (10 mg, 0.04 mmol) and K_2CO_3 (7 mg, 0.05 mmol) in THF (2 mL) was added **T-Atokel-13** (20 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 12 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over

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Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 20/1, v/v). After evaporation of the eluent, the target compound was obtained as a red solid (11.6 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.00 (d, J = 8.4 Hz, 1H), 7.94–7.86 (m, 4H), 7.84 (t, J = 7.0 Hz, 1H), 7.74 (t, J = 6.8 Hz, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 6.99 (s, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 5.75 (s, 3H), 3.05-2.98 (m, 2H), 2.87 (t, J = 6.9 Hz, 2H), 2.59 (t, J = 5.8 Hz, 2H), 2.41 (s, 3H), 2.27 ppm (s, 3H). ESI$^+$-MS calcd for $C_{31}H_{31}N_4O_5S$: 571.20 ([M + H]$^+$); found: 571.20.

Atokel-13. To a solution of **X-Atokel-13** in dichloromethane was added excess hydrochloric acid solution (2.0 N in ethanol). The organic phase was evaporated and dried under reduced pressure to give **Atokel-13** as a yellow solid (100%). mp: 167–170 °C; ^{1}H NMR (400 MHz, DMSO-d_6) : δ = 10.41 (brs, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 7.6 Hz, 1H), 7.86 (t, J = 7.4 Hz, 1H), 7.78 (t, J = 6.4 Hz, 2H), 7.71 (d, J = 6.4 Hz, 1H), 7.56 (m, 3H), 7.48 (d, J = 8.0 Hz, 2H), 4.03 (br, J = 5.7 Hz, 2H), 3.87 (br, 2H), 3.51 (t, J = 7.2 Hz, 2H), 3.44 (t, J = 7.2 Hz, 2H), 2.93 (s, 3H), 2.45 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.5, 176.1, 158.0, 146.0, 141.1, 139.6, 137.8, 137.7, 135.7, 133.5, 133.5, 131.8, 131.6, 130.3, 130.2, 128.9, 127.7, 126.9, 126.9, 126.2, 126.0, 125.7, 123.4, 54.6, 53.1, 40.5, 37.9, 31.5, 21.7 ppm. ESI$^+$-MS calcd for $C_{31}H_{31}N_4O_5S$: 571.20 ([M + H]$^+$); found: 571.20. Elemental analysis calcd (%) for $C_{31}H_{30}N_4O_5S \cdot 2.0HCl \cdot 0.3H_2O \cdot 0.2\ C_2H_5OH \cdot 0.4CH_2Cl_2$ (apparent MW = 692.17): C 55.18, H 5.04, N 8.09, found: C 55.23, H 4.89, N 8.06. HPLC (eluent CH_3OH/H_2O, 55/45, 0.2 vol% TFA): R_t = 11.9 min, purity 95%.

Atokel-14

3-((2-((2-(8-Hydroxyquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-1,4-dioxo-1,4,4a,8a-tetrahydronaphthalen-2-yl 4-methylbenzenesulfonate (X-Atokel-14). The procedure was similar to that described for the preparation of **X-Atokel-13**, but using P-Atokel-3 (Scheme 3) as 8-hydroxyquinoline derivative. **X-Atokel-14** was obtained as a red solid (16%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.29 (brs, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.01–7.79 (m, 5H), 7.74 (t, J = 7.2 Hz, 1H), 7.45 (m, 3H), 7.23 (s, 2H), 6.96–6.94 (m, 1H), 6.92 (s, 1H), 3.50 (br, 2H), 3.07 (br, 2H), 2.92 (br, 2H), 2.59 (br, 2H), 2.41 (s, 3H), 2.28 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.4, 175.6, 159.2, 152.9, 145.7, 138.2, 136.5, 135.8, 133.9, 133.2, 131.9, 130.2, 129.8, 128.7, 126.8, 126.1, 125.8, 122.9, 117.9, 111.2, 56.2, 55.7, 42.1, 40.9, 36.1, 21.6 ppm. ESI$^+$-MS calcd for $C_{31}H_{30}N_3O_6S$: 571.18 ([M + H]$^+$); found: 571.18.

Atokel-14. The same procedure as described above for the preparation of **Atokel-1** afforded **Atokel-14** as a red solid (100%). mp: 90–95 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.88 (brs, 1H), 8.50 (s, 1H), 7.91–7.22 (m, 13H), 3.99–3.42 (m, 11H), 2.92 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.5, 176.2, 160.4, 156.0, 146.0, 141.3, 135.8, 135.8, 133.6, 133.5, 130.3, 129.1, 128.9, 126.9, 126.2, 123.3, 118.4, 55.7, 54.5, 53.7, 38.2, 30.2, 21.7 ppm. ESI$^+$-MS calcd for $C_{31}H_{30}N_3O_6S$: 572.18 ([M + H]$^+$); found: 572.18. Elemental analysis calcd (%) for $C_{31}H_{29}N_3O_6S \cdot 1.7HCl \cdot 1.5H_2O \cdot 0.2\ C_2H_5OH \cdot 0.1CH_2Cl_2$ (apparent MW = 678.36): C 55.77, H 5.22, N 6.16, found: C 55.68, H 5.26, N 6.00. HPLC (CH_3OH/H_2O, 55/45, v/v, 0.2 vol% TFA): R_t = 15.1 min, purity 87%.

Atokel-15

3-((2-((2-(8-Amino-5,7-dichloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-1,4-dioxo-1,4,4a,8a-tetrahydronaphthalen-2-yl 4-methylbenzenesulfonate (X-Atokel-15). The procedure was similar to that described for the preparation of **X-Atokel-13**, but using **G-Atokel-5** (Scheme 1) as 8-aminoquinoline synthon. **X-Atokel-15** was obtained as a red solid (42%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.22 (d, J = 8.6 Hz, 1H), 7.91–7.79 (m, 4H), 7.78–7.69 (m, 2H), 7.57 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 8.0 Hz, 2H), 7.25 (s, 1H), 6.64 (s, 1H), 5.99 (s, 2H), 3.48 (br, 2H), 3.09 (t, J = 6.0 Hz, 2H), 2.93 (d, J = 6.0 Hz, 2H), 2.56 (br, 2H), 2.43 (s, 3H), 2.28 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.0, 175.6, 159.0, 145.8, 140.9, 137.5, 135.8, 133.1, 133.0, 130.2, 129.6, 128.7, 126.8, 126.7, 126.7, 126.1, 123.8, 123.5, 115.4, 111.8, 55.4, 55.3, 45.8, 40.9, 36.1, 21.6 ppm. ESI$^+$-MS calcd for $C_{31}H_{29}Cl_2N_4O_5S$: 639.12 ([M + H]$^+$); found: 639.12.

Atokel-15. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-15** as a red solid (100%). mp: 130–134 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.59 (H3), 8.34 (H4), 7.62 (H6), 3.48 [2H, C2–CH_2], 3.76–3.88 [2H, C2–CH_2–CH_2], 10.78 [1H, C2-$(CH_2)_2$–HN^+–CH_3], 2.91 [3H, C2–$(CH_2)_2$–HN^+–CH_3], 3.45–3.33 [2H, CH_2–CH_2–NH–C2′], 3.99 [2H, CH_2–NH–C2′], 7.79 [1H, HN–C2′], 7.90 (H5′), 7.85 (H6′), 7.78 (H7′), 8.00 (H8′), 7.93 [2H, H2″–Tos], 7.47 [2H, H3″–tosyl], 2.44 [3H, H_3C–C4″–tosyl]. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 156.68 (C2), 123.81 (C3), 133.54 (C4), 123.85 (C4a), 115.15 (C5), 127.52 (C6), 112.07 (C7), 141.24 (C8a), 31.69 (C2–CH_2), 53.37 (C2–CH_2–CH_2), 40.20 [C2-$(CH_2)_2$–HN^+–CH_3], 54.46 (CH_2–CH_2–NH–C2′), 38.08 (–CH_2–CH_2–NH–C2′), 181.57 (C1′), 137.25 (C2′), 126.00 (C3′), 176.14 (C4′), 131.60 (C4a′), 126.21 (C5′), 135.81 (C6′), 133.46 (C7′), 126.91 (C8′), 130.19 (C8a′), 133.52 [tosyl–C1″], 128.87 [tosyl–C2″], 130.26 [tosyl–C3″], 145.93 [tosyl–C4″], 21.70 [H_3C–C4″–tosyl]. At 353 K, the HN–C2′ was high-field shifted (7.54 ppm) compared to 298 K (7.79 ppm), and exhibited HMBC correlations with C1′ (181.57 ppm) and C3′ (126.00 ppm). In addition, an HMBC correlation was detected between C1′ (181.57 ppm) and H8′ (8.00 ppm); a COSY correlation was evidenced between HN–C2′ and H_2C–HN–C2′ (3.99 ppm), and ROE correlations were evidenced between H2″–tosyl (7.93 ppm) and H_2C–HN–C2′ (3.99 ppm), H_2C–CH_2–HN–C2′ (3.45–3.33 ppm), and HN^+–CH_3 (2.91 ppm). ESI$^+$-MS calcd for $C_{31}H_{29}Cl_2N_4O_5S$: 639.12 ([M + H]$^+$); found: 639.12. Elemental analysis calcd (%) for $C_{31}H_{28}Cl_2N_4O_5S \cdot 2.0HCl \cdot 0.1CH_2Cl_2$ (apparent MW = 712.46): C 51.81, H 4.22, N 7.77, found: C 51.92, H 3.85, N 7.63. HPLC (CH_3OH/H_2O, 70/30, v/v, 0.2 vol% TFA): R_t = 11.2 min, purity 98%.

Atokel-16

3-((2-((2-(8-Amino-7-chloroquinolin-2-yl)ethyl)(methyl)amino)ethyl)amino)-1,4-dioxo-1,4,4a,8a-tetrahydronaphthalen-2-yl 4-methylbenzenesulfonate (X-Atokel-16). The procedure was similar to that described for the preparation of **X-Atokel-13**, but starting from **G-Atokel-7**. **X-Atokel-16** was obtained as a red solid (80%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.07 (d, J = 8.4 Hz, 1H), 7.92–7.88 (m, 3H), 7.85–7.81 (m, 2H), 7.73 (t, J = 7.4 Hz, 1H), 7.46–7.41 (m, 3H), 7.19 (d, J = 8.8 Hz, 1H), 7.00 (d, J = 8.8 Hz, 1H), 6.86 (t, J = 5.2 Hz, 1H), 5.82 (s, 2H), 3.49 (q, J = 5.6 Hz, 2H), 3.05 (t, J = 6.8 Hz, 2H), 2.89 (t, J = 6.8 Hz, 2H), 2.58 (t, J = 6.0 Hz, 2H), 2.42 (s, 3H), 2.27 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.2, 175.6, 159.1, 145.7, 140.9, 137.5, 136.7, 135.8, 133.9, 133.1, 131.9, 130.2, 129.8, 128.7, 127.2, 127.2, 126.8, 126.1, 125.8, 125.7, 122.7, 114.9, 113.0, 56.1, 55.6, 42.1, 40.6, 36.1, 21.6 ppm. ESI$^+$-MS calcd for $C_{31}H_{30}ClN_4O_5S$: 605.16 ([M + H]$^+$); found: 605.16.

Atokel-16. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-16** as a red solid (100%). mp: 115–120 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.88 (brs, 1H), 8.21 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.94–7.90 (m, 3H), 7.85 (t, J = 7.6 Hz, 1H), 7.80–7.75 (m, 2H), 7.54–7.43 (m, 3H), 7.39 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 5.73 (brs, 2H), 4.00 (br, 2H), 3.79 (br, 2H), 3.47–3.35 (m, 4H), 2.90 (s, 3H), 2.44 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 181.5, 176.1, 155.8, 145.9, 141.3,

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140.8, 137.7, 136.8, 135.7, 133.5, 133.5, 131.6, 130.3, 130.2, 128.8, 128.1, 126.9, 126.3, 126.2, 126.1, 122.6, 120.5, 119.6, 115.0, 113.7, 54.5, 53.6, 38.1, 32.6, 31.7, 21.7 ppm. ESI$^+$-MS calcd for $C_{31}H_{30}ClN_4O_5S$: 605.16 ([M + H]$^+$); found: 605.16. Elemental analysis calcd (%) for $C_{31}H_{29}ClN_4O_5S \cdot 2.0HCl \cdot 0.15CH_2Cl_2$ (apparent MW = 690.76): C 54.16, H 4.57, N 8.11, found: C 54.14, H 4.40, N 7.89. HPLC (CH_3OH/H_2O, 60/40, v/v, 0.2 vol% TFA): R_t = 20.9 min, purity 95%.

Atokel-17 and Atokel-18

J-Atokel-17 *and* ***J-Atokel-18***. The procedure was similar to that described for the preparation of **J-Atokel-5**. **J-Atokel-17** and **J-Atokel-18** were obtained as red solids with yields of 35% and 28%, respectively. ^{1}H NMR for **J-Atokel-17** (500 MHz, DMSO-d_6): δ = 8.25 (d, J = 7.5 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.05 (t, J = 8.0 Hz, 2H), 7.38 (d, J = 8.5 Hz, 1H), 7.13 (t, J = 7.5 Hz, 2H), 6.95 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 7.5 Hz, 1H), 5.82 (s, 2H), 3.81 (br, 2H), 3.06 (t, J = 6.0 Hz, 2H), 2.97 (t, J = 6.0 Hz, 2H), 2.68 (br, 2H), 2.37 ppm (s, 3H). ^{1}H NMR for J-Atokel-18 (500 MHz, DMSO-d_6): δ = 8.08–8.04 (m, 2H), 8.00 (d, J = 7.5 Hz, 1H), 7.90 (t, J = 8.0 Hz, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.17 (br, 1H), 7.09 (s, 1H), 6.90 (s, 1H), 6.68 (d, J = 7.0 Hz, 1H), 5.76 (s, 2H), 3.80 (br, 2H), 2.96 (br, 2H), 2.88 (br, 2H), 2.62 (br, 2H), 2.28 ppm (s, 3H).

Atokel-17. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-17** as an oxblood red solid (100%). mp: 166–169 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.59 (H3), 8.40 (H4), 7.75 (H5), 7.56 (H6), 7.62 (H7), 3.56 [2H, C2–CH_2], 3.90 [2H, C2–CH$_2$–CH_2], 2.97 [3H, C2-(CH$_2$)$_2$–HN$^+$–CH_3], 3.51 [2H, CH_2–CH$_2$–NH–C2′], 4.15 [2H, CH_2–NH–C2′], 7.83 [1H, *H*N–C2′], 8.21 (H5′), 8.03 (H6′), 8.08 (H7′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 157.53 (C2), 123.40 (C3), 137.71 (C4), 127.73 (C4a), 123.82 (C5), 127.00 (C6), 120.00 (C7), 139.20 (C8a), 31.60 (C2–*C*H$_2$), 53.41 (C2–CH$_2$–*C*H$_2$), 40.96 [C2-(CH$_2$)$_2$–HN$^+$–*C*H$_3$], 55.70 (*C*H$_2$–CH$_2$–NH–C2′), 39.31 (–*C*H$_2$–*C*H$_2$–NH–C2′), 177.85 (C1′), 146.50 (C2′), 110.30 (C3′), 174.23 (C4′), 133.20 (C4a′), 128.73 (C5′), 136.43 (C6′), 126.98 (C7′), 148.30 (C8′), 121.71 (C8a′). The C8 and the acidic proton C2-(CH$_2$)$_2$–*H*N$^+$–CH$_3$ were not detected. HMBC correlations were detected between *H*N–C2′ (7.83 ppm) and C1′ (177.85 ppm), and between H5′ (8.21 ppm) and C4′ (174.23 ppm). ESI$^+$-MS calcd for $C_{24}H_{23}ClN_5O_4$: 480.14 ([M + H]$^+$); found: 480.12. Elemental analysis calcd (%) for $C_{24}H_{22}ClN_5O_4 \cdot 2.0HCl \cdot 0.1H_2O \cdot 0.1CH_2Cl_2 \cdot 0.5\ C_2H_5OH$ (apparent MW = 586.17): C 51.43, H 4.71, N 11.95, found: C 51.37, H 4.69, N 11.98. HPLC (CH_3OH/H_2O, 35/65, v/v, 0.2 vol% TFA): R_t = 16.5 min, purity 94%.

Atokel-18. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-18** as an oxblood red solid (100%). mp: 155–159 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.58 (H3), 8.38 (H4), 7.71 (H5), 7.54 (H6), 7.53 (br, H7), 3.56 [2H, C2–CH_2], 3.92 [2H, C2–CH$_2$–CH_2], 10.52 [1H, C2-(CH$_2$)$_2$–*H*N$^+$–CH$_3$], 2.99 [3H, C2-(CH$_2$)$_2$–HN$^+$–CH_3], 3.56 [2H, CH_2–CH$_2$–NH–C2′], 4.22 [2H, CH_2–NH–C2′], 7.92 [1H, *H*N–C2′], 8.09 (H6′), 7.95 (H7′), 8.17 (H8′). ^{13}C NMR (150 MHz, DMSO-d_6): 157.50 (C2), 123.35 (C3), 137.73 (C4), 127.83 (C4a), 127.04 (C6), 139.21 (C8a), 31.68 (C2–*C*H$_2$), 53.46 (C2–CH$_2$–*C*H$_2$), 40.90 [C2-(CH$_2$)$_2$–HN$^+$–*C*H$_3$], 55.82 (CH$_2$–CH$_2$–NH–C2′), 39.33 (–CH$_2$–*C*H$_2$–NH–C2′), 178.83 (C1′), 146.60 (C2′), 110 (C3′), 172.80 (C4′), 122.50 (C4a′), 148.20 (C5′), 128.42 (C6′), 134.60 (C7′), 129.34 (C8′), 132.20 (C8a′). The C5, C7, and C8 were not detected (general broadening of the signals of the aminoquinoline ring), C3′ was very broad at 298 K, but detected at 333 K. HMBC correlations were detected between *H*N–C2′ (7.92 ppm) and C1′ (178.83 ppm), and between H8′ (8.17 ppm) and C1′. ESI$^+$-MS calcd for $C_{24}H_{23}ClN_5O_4$: 480.14 ([M + H]$^+$); found: 480.14. Elemental analysis calcd (%) for $C_{24}H_{22}ClN_5O_4 \cdot 2.0HCl \cdot 0.1H_2O \cdot 0.3CH_2Cl_2 \cdot 0.5\ C_2H_5OH$ (apparent MW = 603.15): C 50.38, H 4.65, N 11.61, found: C 50.31, H 4.65, N 11.63. HPLC (CH_3OH/H_2O, 35/65, v/v, 0.2 vol% TFA): R_t = 13.0 min, purity 97%.

Atokel-19 and Atokel-24

J-Atokel-19 *and* ***J-Atokel-24***. To mixture of G-Atokel-1 (100 mg, 0.41 mmol) and a few drops of CH_3COOH in acetonitrile (5 mL), 2,3-dichloro-5,8-dihydroxynaphthalene-1,4-dione (106 mg, 0.41 mmol) was added. The reaction mixture was stirred at room temperature for 24 h, and quenched with water. After extraction with dichloromethane, the organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 100/1, v/v). After evaporation of the eluent, **J-Atokel-19** and **J-Atokel-24** were obtained as purple black solid (42 mg, 21%) and red solid (11 mg, 6%), respectively. ^{1}H NMR for J-Atokel-19 (500 MHz, DMSO-d_6): δ = 15.07 (brs, 1H), 11.91 (brs, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.61 (s, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.73 (d, J = 6.5 Hz, 1H), 5.73 (s, 1H), 3.41 (s, 2H), 3.20–3.14 (m, 4H), 2.91 (s, 2H), 2.53 ppm (s, 3H). ^{1}H NMR for J-Atokel-24 (500 MHz, DMSO-d_6): δ = 13.09 (s, 1H), 11.90 (brs, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.36–7.28 (m, 3H), 7.21 (d, J = 9.5 Hz, 1H), 7.06 (t, J = 8.0 Hz, 1H), 6.87 (d, J = 7.5 Hz, 1H), 6.71 (d, J = 7.5 Hz, 1H), 3.90 (d, J = 5.0 Hz, 2H), 3.06–3.04 (m, 4H), 2.74 (s, 2H), 2.39 ppm (s, 3H).

Atokel-19. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-19** as a purple black solid (100%). mp: 175–179 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.58 (H3), 8.37 (H4), 7.69 (H5), 7.57 (H6), 7.78 (H7), 3.57 [2H, C2–CH_2], 3.94 [2H, C2–CH$_2$–CH_2], 10.43 [1H, C2-(CH$_2$)$_2$–*H*N$^+$–CH$_3$], 2.97 [3H, C2-(CH$_2$)$_2$–HN$^+$–CH_3], 3.51 [2H, CH_2–CH$_2$–NH–C2′], 3.88 [2H, CH_2–NH–C2′], 8.42 [1H, *H*N–C2′], 6.03 (H3′), 14.50 (*H*O–C5′), 8.5 (br, *H*O–C8′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 157.60 (C2), 123.38 (C3), 137.68 (C4), 127.70 (C4a), 120.56 (C5), 126.96 (C6), 124.66 (C7), 139.40 (C8a), 31.71 (C2–*C*H$_2$), 53.14 (C2–CH$_2$–*C*H$_2$), 40.45 [C2-(CH$_2$)$_2$–HN$^+$–*C*H$_3$], 53.10 (*C*H$_2$–CH$_2$–NH–C2′), 37.44 (–*C*H$_2$–*C*H$_2$–NH–C2′), 183.60 (C1′), 150.03 (C2′), 100.17 (C3′), 186.30 (C4′), 110.55 (C4a′), 152 (C5′), 132.32 (C6′), 127.90 (C7′), 152 (C8′), 111.46 (C8a′). C8 was not detected. HMBC correlations were detected between *H*N–C2′ (8.42 ppm) and C1′ (183.60 ppm), between CH_2–NH–C2′ (3.88 ppm) and C2′ (150.03 ppm), between H3′ (6.03 ppm) and C2′, and between *H*O–C5′ (14.50 ppm) and C4a′ (110.55 ppm). A ROESY correlation was detected between *H*N–C2′ (8.42 ppm) and H3′ (6.03 ppm). ESI$^+$-MS calcd for $C_{24}H_{23}Cl_2N_4O_4$: 501.11 ([M + H]$^+$), found: 501.13. Elemental analysis calcd (%) for $C_{24}H_{22}Cl_2N_4O_4 \cdot 1.9HCl \cdot 0.3H_2O \cdot 0.2CH_2Cl_2 \cdot 0.9\ C_2H_5OH$ (apparent MW = 634.49): C 49.22, H 4.81, N 8.83, found: C 49.30, H 4.79, N 8.73. HPLC (CH_3OH/H_2O, 55/45, v/v, 0.2 vol% TFA): R_t = 12.1 min, purity 95%).

Atokel-24. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-24** as a red solid (100%). mp: 83–87 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.58 (H3), 8.38 (H4), 7.73 (H5), 7.55 (H6), 7.61 (H7), 3.56 [2H, C2–CH_2], 3.92 [2H, C2–CH$_2$–CH_2], 10.41 [1H, C2-(CH$_2$)$_2$–*H*N$^+$–CH$_3$], 2.99 [3H, C2-(CH$_2$)$_2$–HN$^+$–CH_3], 3.56 [2H, CH_2–CH$_2$–NH–C2′], 4.28 [2H, CH_2–NH–C2′], 8.01 [1H, *H*N–C2′], 7.37 (H6′), 7.27 (H7′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 157.57 (C2), 123.34 (C3), 137.67 (C4), 127.75 (C4a), 124.00 (C5), 127.00 (C6), 120.00 (C7), 31.60 (C2–*C*H$_2$), 53.42 (C2–CH$_2$–*C*H$_2$), 41.02 [C2-(CH$_2$)$_2$–HN$^+$–*C*H$_3$], 56.01 (*C*H$_2$–CH$_2$–NH–C2′), 39.22 (–*C*H$_2$–*C*H$_2$–NH–C2′), 182.36 (C1′), 146.60 (C2′), 109.60 (C3′), 180.50 (C4′), 110.64 (C4a′), 157,00 (C5′), 130.68 (C6′), 127.47 (C7′), 155.45 (C8′), 111.59 (C8a′). C5-C7 signals were broaden, detectable only by XL-SOFAST HSQC sequence. C8 and C8–NH_3^+ were not detected. C3′ was detected only by HMBC correlation with *H*N–C2′, at 338 K. A ROESY correlation was

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detected between H4 and H5, and HMBC correlations were detected between CH_2–NH–C2′ (4.28 ppm) and C2′ (146.6 ppm), *H*N–C2′ (8.01 ppm) and Cl–C3′ (109.6), *H*N–C2′ (8.01 ppm) and C1′ (182.36 ppm), H7′ (7.28 ppm) and C1′, H6′ (7.37 ppm) and C4′ (180.5 ppm), respectively. ESI$^+$-MS calcd for $C_{24}H_{24}ClN_4O_4$: 467.14 ([M+H]$^+$), found: 467.14. Elemental analysis calcd (%) for $C_{24}H_{23}ClN_4O_4 \cdot 2.0HCl \cdot 1.0H_2O \cdot 0.5CH_2Cl_2 \cdot 0.4\ C_2H_5OH$ (apparent MW = 618.74): C 49.11, H 4.95, N 9.06, found: C 48.99, H 4.83, N 8.97. HPLC (CH_3OH/H_2O, 45/55, v/v, 0.2 vol% TFA): R_t = 10.6 min, purity 96%.

Atokel-20 and Atokel-21

J-Atokel-20 *and* ***J-Atokel-21***. The procedure was similar to that described for the preparation of **J-Atokel-5**. J-Atokel-20 and J-**Atokel-21** were obtained as red solids with 39% and 45%, yields, respectively. ^{1}H NMR for **J-Atokel-20** (500 MHz, $CDCl_3$): δ = 8.28 (d, *J* = 6.5 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 6.86 (s, 2H), 6.15 (brs, 1H), 5.21 (s, 2H), 3.81 (q, *J* = 5.5 Hz, 2H), 3.06 (t, *J* = 4.5 Hz, 2H), 3.04 (t, *J* = 4.5 Hz, 2H), 2.61 (t, *J* = 5.5 Hz, 2H), 2.41 ppm (s, 3H). ^{1}H NMR for J-Atokel-21 (500 MHz, $CDCl_3$): δ = 7.92 (d, *J* = 8.5 Hz, 2H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.24 (d, *J* = 4.0 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 5.23 (s, 2H), 3.82 (s, 2H), 2.64 (t, *J* = 5.5 Hz, 2H), 2.42 ppm (s, 3H).

Atokel-20. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-20** as a red solid (100%). mp: 171–172 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.50 (H3), 8.26 (H4), 7.14 (H5), 7.40 (H6), 3.52 [2H, C2–CH_2], 3.83 [2H, C2–CH_2–CH_2], 11.17 [1H, C2-$(CH_2)_2$–*H*N$^+$–CH_3], 2.94 [3H, C2-$(CH_2)_2$–HN$^+$–CH_3], 3.47 [2H, CH_2–CH_2–NH–C2′], 4.13 [2H, CH_2–NH–C2′], 7.85 [1H, *H*N–C2′], 8.21 (H5′), 8.03 (H6′), 8.08 (H7′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 155.70 (C2), 122.73 (C3), 137.85 (C4), 126.32 (C4a), 115.13 (C5), 128.18 (C6), 113.86 (C7), 140.80 (C8), 31.73 (C2–CH_2), 53.84 (C2–CH_2–CH_2), 40.60 [C2-$(CH_2)_2$–HN$^+$–CH_3], 55.92 (CH_2–CH_2–NH–C2′), 39.48 (–CH_2–CH_2–NH–C2′), 177.80 (C1′), 146.60 (C2′), 100.50 (C3′), 174.40 (C4′), 133.25 (C4a′), 128.68 (C5′), 136.37 (C6′), 127.04 (C7′), 148.25 (C8′), 121.68 (C8a′). HMBC correlations were detected between *H*N–C2′ (7.85 ppm) and C1′ (177.80 ppm), and between H5′ (8.21 ppm) and C4′ (174.40 ppm). ESI$^+$-MS calcd for $C_{24}H_{22}Cl_2N_5O_4$: 514.10 ([M+H]$^+$), found: 514.10. Elemental analysis calcd (%) for $C_{24}H_{21}Cl_2N_5O_4 \cdot 1.8HCl \cdot 0.1CH_2Cl_2 \cdot 0.3\ C_2H_5OH$ (apparent MW = 602.30): C 49.26, H 4.15, N 11.63, found: C 49.16, H 4.00, N 11.50. HPLC (CH_3OH/H_2O, 45/55, v/v, 0.2 vol% TFA): R_t = 16.7 min, purity 95% (+ 2% Atokel-21, R_t = 16.7 min).

Atokel-21. The same procedure as described above for the preparation of Atokel-13 afforded Atokel-21 as a red solid (100%). mp: 175–179 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.51 (H3), 8.27 (H4), 7.14 (H5), 7.39 (H6), 3.54 [2H, C2–CH_2], 3.83 [2H, C2–CH_2–CH_2], 10.48 [1H, C2-$(CH_2)_2$–*H*N$^+$–CH_3], 2.95 [3H, C2-$(CH_2)_2$–HN$^+$–CH_3], 3.49 [2H, CH_2–CH_2–NH–C2′], 4.20 [2H, CH_2–NH–C2′], 7.93 [1H, *H*N–C2′], 8.08 (H6′), 7.93 (H7′), 8.16 (H8′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 155.92 (C2), 122.74 (C3), 137.96 (C4), 126.33 (C4a), 115.18 (C5), 128.15 (C6), 114.03 (C7), 140.62 (C8), 136.67 (C8a), 31.67 (C2–CH_2), 53.85 (C2–CH_2–CH_2), 40.76 [C2-$(CH_2)_2$–HN$^+$–CH_3], 55.80 (CH_2–CH_2–NH–C2′), 39.46 (–CH_2–CH_2–NH–C2′), 178.70 (C1′), 146.48 (C2′), 172.60 (C4′), 122.54 (C4a′), 148.12 (C5′), 128.39 (C6′), 134.50 (C7′), 129.23 (C8′), 121.68 (C8a′). C3′ and C8a′ were not detected. HMBC correlations were detected between *H*N–C2′ (7.93 ppm) and C1′ (178.70 ppm), and between H8′ (8.16 ppm) and C1′. ESI$^+$-MS calcd for $C_{24}H_{22}Cl_2N_5O_4$: 514.10 ([M+H]$^+$), found: 514.10. Elemental analysis calcd (%) for $C_{24}H_{21}Cl_2N_5O_4 \cdot 1.9HCl \cdot 0.1CH_2Cl_2 \cdot 0.2\ C_2H_5OH$ (apparent MW = 601.34): C 48.94, H 4.07, N 11.65, found: C 49.06, H 3.97, N 11.66. HPLC (CH_3OH/H_2O, 45/55, v/v, 0.2 vol% TFA): R_t = 13.6 min, purity 96% (+ 2% of Atokel-20, R_t = 16.7 min).

Atokel-22 and Atokel-23

J-Atokel-22 *and* ***J-Atokel-23***. The same procedure as described above for the preparation of **J-Atokel-5** afforded a mixture of **J-Atokel-22** and **J-Atokel-23**. These two regioisomers were separated by silica gel flash column chromatography (CH_2Cl_2/CH_3OH, 100/1, v/v), that afforded **J-Atokel-22** and **J-Atokel-23** as purple black solid (118 mg, 35%) and red solid (13 mg, 4%), respectively. ^{1}H NMR for J-Atokel-22 (500 MHz, $CDCl_3$): δ = 14.27 (s, 1H), 11.94 (brs, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.17 (brs, 1H), 5.51 (s, 1H), 5.13 (br, 2H), 3.13 (q, *J* = 5.5 Hz, 2H), 3.09 (t, *J* = 5.5 Hz, 2H), 3.05 (t, *J* = 5.0 Hz, 2H), 2.73 (t, *J* = 5.0 Hz, 2H), 2.43 ppm (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ = 184.9, 181.7, 158.3, 152.3, 150.1, 148.0, 140.9, 137.3, 136.8, 132.3, 127.2, 125.9, 124.8, 122.8, 114.7, 113.0, 110.8, 110.3, 98.6, 55,3, 54,2, 42.0, 39.3, 34.9 ppm. ^{1}H NMR for J-Atokel-23 (500 MHz, $CDCl_3$): δ = 13.06 (s, 1H), 11.32 (brs, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 9.5 Hz, 1H), 7.05 (d, *J* = 9.5 Hz, 1H), 6.95 (d, *J* = 9.0 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.49 (br, 1H), 5.19 (s, 2H), 3.92 (q, *J* = 5.5 Hz, 2H), 3.12 (t, *J* = 5.5 Hz, 2H), 3.05 (t, *J* = 5.5 Hz, 2H), 2.66 (t, *J* = 5.5 Hz, 2H), 2.44 ppm (s, 3H).

Atokel-22. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-22** as a purple black solid (100%). mp: 165–170 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.46 (H3), 8.21 (H4), 7.09 (H5), 7.35 (H6), 3.47 [2H, C2–CH_2], 3.77 and 3.88 [2x1H, C2–CH_2–CH_2], 10.9 [1H, C2-$(CH_2)_2$–*H*N$^+$–CH_3], 2.95 [3H, C2-$(CH_2)_2$–HN$^+$–CH_3], 3.20 and 3.51 [2x1H, CH_2–CH_2–NH–C2′], 3.79 [2H, CH_2–NH–C2′], 8.33 [1H, *H*N–C2′], 6.00 (H3′), 14.8 (O*H*–C5′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 155.70 (C2), 122.70 (C3), 137.80 (C4), 126.30 (C4a), 114.90 (C5), 127.90 (C6), 119.40 (C7), 141.00 (C8), 31.80 (C2–CH_2), 53.60 (C2–CH_2–CH_2), 40.30 [C2-$(CH_2)_2$–HN$^+$–CH_3], 52.50 (CH_2–CH_2–NH–C2′), 37.60 (–CH_2–CH_2–NH–C2′), 183.77 (C1′), 150.00 (C2′), 100.20 (C3′), 186.30 (C4′), 110.57 (C4a′), 152.17 (C5′), 132.32 (C6′), 127.9 (C7′), 152.41 (C8′). The C8–NH_3^+ and C8a′ were not detected. A ROESY correlation was detected between *H*N–C2′ (8.33 ppm) and H3′ (6.00 ppm). HMBC correlations were detected between *H*N–C2′ (8.33 ppm) and C1′ (183.77 ppm), and between O*H*–C5′ (14.8 ppm) and C4′ (186.30, ^4J), C5′ (152.17 ppm) and C6′ (132.32 ppm). ESI$^+$-MS calcd for $C_{24}H_{22}Cl_3N_4O_4$: 535.07 ([M+H]$^+$), found: 535.07. Elemental analysis calcd (%) for $C_{24}H_{21}Cl_3N_4O_4 \cdot 1.7HCl \cdot 0.1CH_2Cl_2 \cdot 0.3\ C_2H_5OH$ (apparent MW = 620.10): C 47.84, H 4.02, N 9.04, found: C 47.74, H 3.94, N 8.95. HPLC (CH_3OH/H_2O, 65/35, v/v, 0.2 vol% TFA): R_t = 12.1 min, purity 86%).

Atokel-23. The same procedure as described above for the preparation of **Atokel-13** afforded **Atokel-23** as a red solid (100%). mp: 175–177 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.45 (H3), 8.18 (H4), 7.08 (H5), 7.33 (H6), 3.45 [2H, C2–CH_2], 3.80 and 3.87 [2x1H, C2–CH_2–CH_2], 2.97 [3H, C2-$(CH_2)_2$–HN$^+$–CH_3], 3.45 and 3.56 [2x1H, CH_2–CH_2–NH–C2′], 4.18 [2H, CH_2–NH–C2′], 7.84 [1H, *H*N–C2′], 7.34 (H6′), 7.24 (H7′). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 155.47 or 155.65 (C2), 122.65 (C3), 137.50 (C4), 126.18 (C4a), 114.97 (C5), 130.73 (C6), 113.49 (C7), 137.12 (C8), 31.89 (C2–CH_2), 54.04 (C2–CH_2–CH_2), 40.90 [C2-$(CH_2)_2$–HN$^+$–CH_3], 55.96 (CH_2–CH_2–NH–C2′), 39.45 (–CH_2–CH_2–NH–C2′), 182.50 (C1′), 146.52 (C2′), 109.20 (C3′), 180.70 (C4′), 110.47 (C4a′), 157,09 (C5′), 127.98 (C6′), 127.51 (C7′), 155.65 or 155.47 (C8′), 111.38 (C8a′). The C8–NH_3^+ and C2-$(CH_2)_2$–*H*N$^+$–CH_3 were not detected. HMBC correlations were detected between *H*N–C2′ (7.84 ppm) and Cl–C3′ (109.2 ppm), *H*N–C2′ (7.84 ppm) and C1′ (182.50 ppm), H7′ (7.24 ppm) and C1′ (182.50 ppm), H6′ (7.34 ppm) and C4′ (180.7 ppm), respectively. Some of them were detectable only at 353 K. ESI$^+$-MS calcd for $C_{24}H_{23}Cl_2N_4O_4$: 501.11 ([M+H]$^+$), found: 501.11. Elemental analysis calcd (%) for $C_{24}H_{22}Cl_2N_4O_4 \cdot 1.9HCl \cdot 0.1CH_2Cl_2 \cdot 0.3\ C_2H_5OH$ (apparent MW = 592.05): C 50.03, H 4.40, N 9.45, found: C 50.00, H 4.30, N 9.40. HPLC (CH_3OH/H_2O, 50/50, v/v, 0.2 vol% TFA): R_t = 17.2 min, purity 96%.

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Ligand/Metal Stoichiometry in Presence of Cu^{2+} or Zn^{2+}

Titration of Atokel ligands by $CuSO_2 \bullet 5H_2O$ or $ZnSO_2 \bullet 7H_2O$ was carried out as reported in Ref. [18], except the following: Tris buffer was 200 μм, the Atokel stock solutions were in DMSO, and the final DMSO concentration in the cuvette was below 1 vol%. Titration by $FeCl_3$ was carried out as reported in Ref. [29], up to 50 mol equiv. of metal salt in methanol/Tris buffer 200 μM, pH 7.4, 50/50, v/v. Total variation of volume in the cuvette after addition of 50 mole equiv. of Fe^{3+} was below 4 vol%.

Evaluation of Cu^{II} or Zn^{II} to Ligand Affinity Constants

The affinity constants of Atokel ligands for copper(II) and zinc(II) were evaluated as previously reported, either directly from UV-visible titrations (for Zn) or using a competitive chelator, namely ethylenediamine (EDA) or sodium nitriloacetate (NTA).[18,23,30]

Oxidation of Ascorbic Acid by Cu-Atokel Complexes

The oxidation of ascorbate in presence of Atokel ligands and copper was carried out as reported in Ref. [18]. The final concentrations in the cuvette were as follows: Atokel, 11 μм; $CuCl_2$, 10 or 5 μм, and sodium ascorbate, 100 μм in Hepes buffer, 50 мм, pH 7.4, containing less than 2 vol% of DMSO. For each kinetic experiment, the absorbance at 265 nm was corrected by subtracting the own absorbance or each copper complex at 265 nm at t_0 from the raw data.

Evaluation of Antiplasmodial Activity of Atokels and Cytotoxicity

The *Plasmodium falciparum* strain F32-ART, an artemisinin-resistant line obtained by artemisinin drug pressures,[28] was cultivated in vitro as previously described[31] with some modifications.[7] The antiplasmodial activity of the molecules was performed in 2 independant experiments by SYBR Green I method[32] on synchronized ring-stage parasites at 1% parasitemia treated with different concentrations of each drug for 48 h, in 96-well culture plates.[16] The drug concentrations tested were in the ranges 0.1 nм–1 μм for **Atokel-1-7**, and 0.1 μм - 50 μм for **Atokel-8-24**. All the compounds were dissolved in DMSO and serial dilutions were carried out in RPMI 1640 to yield a final DMSO concentration of 0.5% in the well, a concentration that was verified as not affecting parasite growth. Each concentration tested was done in triplicate. The molecules were then washed off three times in PBS. The parasitized red blood cells were lysed by a cycle of freezing/thawing and subsequently probed with SYBR Green for fluorescence reading on Microplate Fluorescence Reader (excitation: 485 nm, emission: 528 nm). The control parasite culture (i.e., RPMI with 0.5% DMSO) was referred to as 100% growth. The IC_{50} values (50% Inhibitory Concentration of parasite growth) were calculated using GraphPad Prism software 7 (GraphPad Software, San Diego, CA, USA) by drawing the curve: % inhibition versus log of drug concentration. Because for all Atokels tested, except **Atokel-13**, their activity against *P. falciparum* in vitro was at least 1000-fold lower than that of atovaquone, we decided to present, for each molecule, the percentage of inhibition of *P. falciparum* obtained at 1 μм (Table 4). For **Atokel-13** and atovaquone (tested in parallel as drug control), 3 and 6 independent experiments were respectively conducted and their IC_{50} values were reported as a mean of the values ± standard error of the mean (SEM).

The cytotoxicity of **Atokel-13** and of atovaquone as antiplasmodial drug control (with phenol 0.5% as control of cytotoxicity for each experiment), was carried out 3 times, on Vero cells. The drug concentrations tested ranged from 5 nм to 50 μм. The security index was calculated as the ratio of cytotoxicity/antiplasmodial activity.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: malaria · metal chelator · oxidative stress · quinoline · quinone

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