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Synthesis and Evaluation of New Antimalarial Analogues of Quinoline Alkaloids Derived from *Cinchona ledgeriana* Moens ex Trimen

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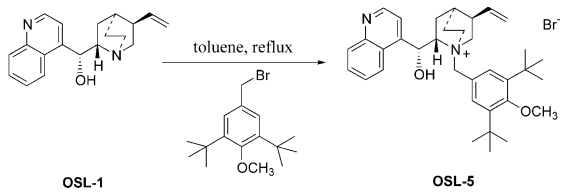
Abstract—In the course of attempts to develop antimalarial drugs, we have designed and synthesized a series of quinoline alkaloide derivatives. Three of them, *N*-(4-methoxy-3,5-di-*tert*-butylbenzyl)cinchonidinium bromide (OSL-5), *O*-benzyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)cinchonidinium bromide (OSL-7), and *N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)quininium bromide (OSL-14) show potent activity against *Plasmodium falciparum*.

Introduction

Malaria, one of the most important infectious diseases in the world, is caused by several species of the genus *Plasmodium*, a protozoan parasite that is transmitted to humans by *Anopheles* mosquitoes. *Plasmodium falciparum* is the most virulent human malaria parasite and is responsible for more deaths in Africa than any other parasitic disease.[1] Despite extensive efforts to eradicate the insect vector using insecticides and the development of several types of synthetic antimalarial agents, the incidence of malaria is still increasing, in large part due to the development of resistance in parasites and mosquitoes to available drugs and insecticides.[2–4] Predictions for trends in global warming have brought forecasts that human malaria will spread into regions presently too cool for supporting mosquito vectors.[5] Thus, there is a great need for new antimalarial agents and insecticides, ideally with different modes of action and chemical structures than currently used compounds.

Historically, plant secondary metabolites have played an important role as antimalarial agents. Quinine, a quinoline alkaloid derived from the bark of *Cinchona ledgeriana* Moens ex Trimen, is the oldest known natural antimalarial drug. Quinine remains an essential drug in treating severe manifestations of *falcifarum* malaria and has served as a lead compound for development of the 8-aminoquinoline and 4-aminoquinoline classes of antimalarial agents.[6,7]

In our laboratory, we have been extensively investigating development of antimalarial chemotherapy based on new target sites.[8–10] In addition, we have been involved in an extensive search for naturally occurring alternatives to currently used larvicides.[11,12] Recently, we synthesized 3 quinoline alkaloids derived from cinchonidine (Schemes 1 and 2).[13,14] Furthermore, 9 quinoline alkaloids derived from cinchonine, quinine, and quinidine were synthesized. These alkaloids were evaluated



Scheme 1.

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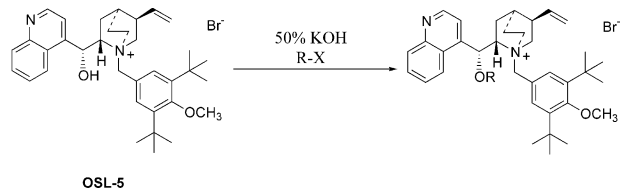

Scheme 2.

for their activities against *P. falciparum*. We report here methods for synthesis and structural determination of these quinoline analogues and their in vitro antimalarial activities.

Results and Discussion

Four naturally occurring quinoline alkaloids (OSL-1–OSL-4) and 12 synthetic analogues (OSL-5–OSL-16) (Fig. 1) were evaluated for in vitro antimalarial activity against *P. falciparum* according to methods previously described.[9,10] The results are summarized in Table 1. The four natural quinolines showed strong antimalarial activities as determined by an assay based on inhibition of ^{3}H-hypoxanthine uptake. However, the synthetic quinoline analogues showed relatively less antimalarial activity, compared with their natural parent compounds or chloroquine ($IC_{50} = 190$ nM), a widely used antimalarial drug. Only OSL-14 ($IC_{50} = 380$ nM) possessed similar antimalarial activity to its parent quinoline alkaloid, quinine (OSL-4) ($IC_{50} = 370$ nM). These findings suggest addition of 3,5-di-*tert*-butyl-4-methoxybenzyl bromide onto the nitrogen atom in the quinuclidine moiety of the parent compound created a conformational change leading to decreased antimalarial activity.

Increased antimalarial activity of several synthetic quinolines was seen with an assay of development, that measures the formation of new ring-stage parasites after 48 h of incubation with inhibitors. Two cinchonidine analogues (OSL-5, $IC_{50} = 160$ nM; OSL-7, $IC_{50} = 63$ nM) and one quinine analogue (OSL-14, $IC_{50} = 99$ nM) showed potent antimalarial activity, compared with their parent compounds, cinchonidine (OSL-1; $IC_{50} = 290$ nM) and quinine (OSL-4; $IC_{50} = 120$ nM). These findings showed that the synthetic quinoline analogues were active, but not as rapidly potent as the parent compounds against *falciparum* malaria. Addition of 3,5-di-*tert*-butyl-4-methoxybenzyl bromide onto the nitrogen atom in the quinuclidine moiety (OSL-5 or OSL-14) appeared to augment antimalarial activity relative to the parent compounds, cinchonidine or quinine. An additional

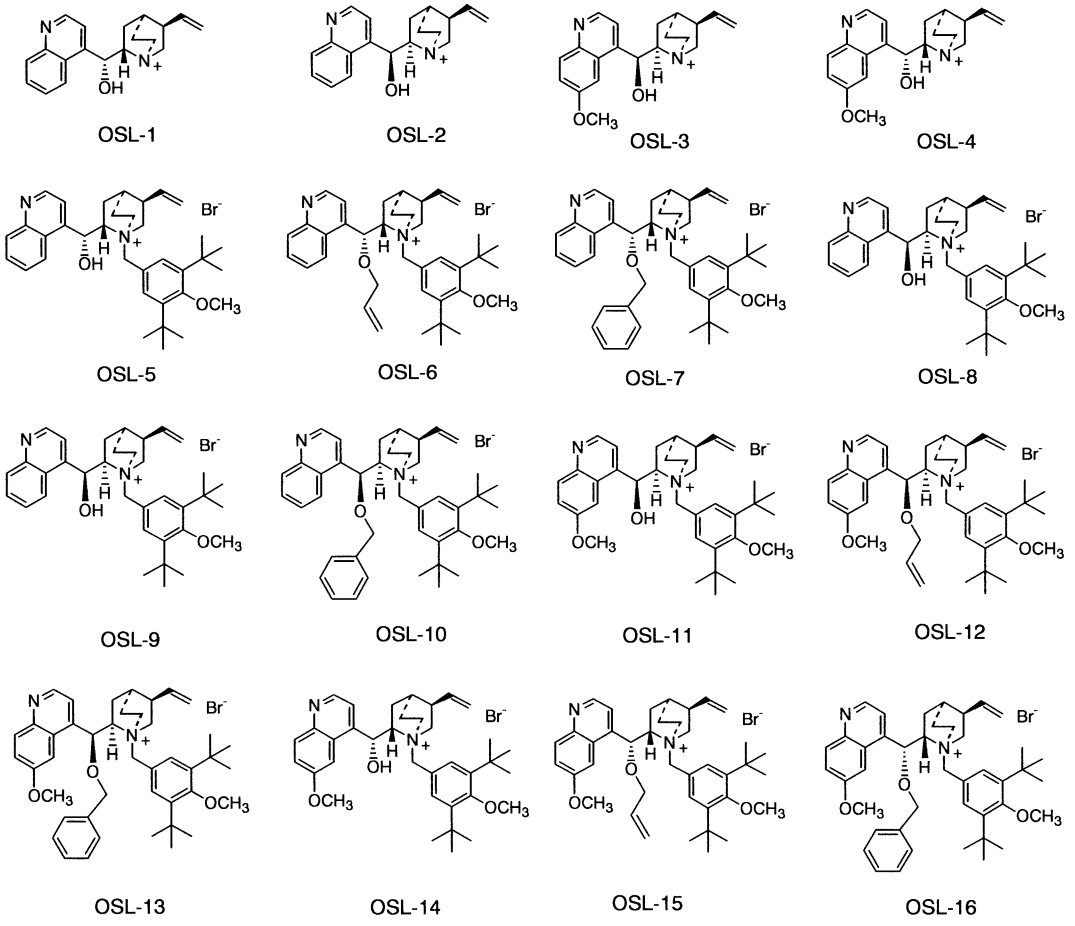

Figure 1. Structures of four naturally occurring quinoline alkaloids (OSL-1–4) and 12 synthesized analogues (OSL-5–16).

Table 1. Antimalarial activities (IC_{50}) of natural (OSL-1–4) and synthetic quinoline alkaloids (OSL-5–16) and chloroquine based on two in vitro antiparasitic assays

Compd	^{3}H-Hypoxanthine uptake (nM)	Parasite development (nM)
OSL-1	380	290
OSL-2	220	51
OSL-3	160	4
OSL-4	370	120
OSL-5	510	160
OSL-6	1100	580
OSL-7	520	63
OSL-8	360	160
OSL-9	800	530
OSL-10	1100	120
OSL-11	1000	510
OSL-12	1200	230
OSL-13	410	100
OSL-14	380	99
OSL-15	1000	410
OSL-16	620	150
Chloroquine	190	36

structural change by benzyl group on hydroxyl group (OSL-7, $IC_{50} = 63$ nM) also increased the antimalarial activity of cinchonidine 4.6-fold ($IC_{50} = 290$ nM).

Our results offer strong evidence these synthetic quinoline alkaloids (OSL-7 and OSL-14) have similar antimalarial potency to their natural parent compounds, cinchonidine and quinine. These particular new compounds show promise as alternatives for currently used drugs in malarial chemotherapy.

Experimental

General

^{1}H NMR spectra were recorded on a Bruker AC 300 and a AC 200F spectrometers. ^{13}C NMR spectra were recorded at 50 MHz on a Bruker AC 200F spectrometer. Optical rotations were measured with JASCO-DIP-1000 digital polarimeter. Melting points were determined using an electrothermal apparatus and are uncorrected. Mass spectra were recorded on Shimadzu QP 5050A. Cinchonidine (OSL-1), cinchonine (OSL-2), quinidine (OSL-3) and quinine (OSL-4) were purchased from Aldrich.

Chemistry

***N*-(4-Methoxy-3,5-di-*tert*-butylbenzyl) cinchonidinium bromide (OSL-5).** 3,5-Di-*tert*-butyl-4-methoxybenzyl bromide (4.38 g, 14 mmol) was added to a suspension of cinchonidine (2.94 g, 10 mmol) in toluene (70 mL) and the mixture stirred at reflux for 4 h (Scheme 1). The reaction mixture was cooled to room temperature, evaporated, and the residue was crystallized from diethyl ether/CH_2Cl_2 to give a dark brown solid. Further purification of the residue was performed by flash chromatography (93:7, dichloromethane/methanol) which afforded the desired product (91%, 5.57 g) as brown solid.

$[\alpha]_D^{25}$ −77.8 ($c = 2$, $CHCl_3$); mp 210–212 °C; IR (film, cm^{-1}) 3504, 3040, 3000, 2950, 1700, 1585, 1500, 1460, 1450, 1410, 1389, 1352, 1262, 1225, 1210, 1115, 1010; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (s, 19H), 1.69 (m, 1H), 2.05 (s, 1H), 2.14–2.20 (m, 2H), 2.66 (m, 1H), 3.30 (m, 1H), 3.45 (m, 1H), 3.64 (d, $J = 11.4$ Hz, 1H), 3.68 (s, 3H), 3.79 (t, $J = 8.5$ Hz, 1H), 4.86 (t, $J = 11.0$ Hz, 1H), 5.01 (d, $J = 10.3$ Hz, 1H), 5.08 (s, 1H), 5.13 (d, $J = 5.9$ Hz, 1H), 5.50–5.62 (m, 1H), 5.74 (d, $J = 11.0$ Hz, 1H), 6.56 (d, $J = 6.5$ Hz, 1H), 6.74 (d, $J = 6.2$ Hz, 1H), 7.58–7.61 (m, 2H), 7.69 (s, 2H), 7.74 (d, $J = 4.4$ Hz, 1H), 8.01–8.07 (m, 2H), 8.85 (d, $J = 4.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.54, 24.85, 26.68, 32.01, 35.98, 37.88, 51.33, 60.37, 61.12, 63.59, 64.14, 64.33, 68.53, 117.87, 120.14, 121.06, 122.78, 127.61, 129.15, 130.42, 132.37, 136.47, 144.84, 144.95, 148.02, 150.08, 161.19; MS (EI) *m*/*z* 527, 472, 456, 210, 165.

***O*-Allyl-*N*-(4-methoxy-3,5-di-*tert*-butylbenzyl)cinchonidinium bromide (OSL-6).** Allyl bromide (0.64 mL, 7.5 mmol) and 2.8 mL of 50% of aq KOH (25.0 mmol) was added to a suspension of *N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)cinchonidinium bromide (3.03 g, 5.0 mmol) in 40 mL of CH_2Cl_2. The resulting mixture was stirred for 5 h (Scheme 2). The mixture was diluted with 40 mL of water and extracted with CH_2Cl_2 (3×40 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel (93:7, dichloromethane/methanol) to give product (92%, 2.98 g) as yellow solid.

$[\alpha]_D^{25}$ −142.5 ($c = 2$, $CHCl_3$); mp 220–221 °C; IR (film, cm^{-1}) 3407, 3000, 2949, 1704, 1625, 1596, 1567, 1510, 1502, 1491, 1470, 1454, 1400, 1350, 1210, 1115, 1070, 1010; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47–1.50 (s, 19H), 2.09–2.17 (m, 3H), 2.63 (s, 1H), 3.34–3.47 (m, 3H), 3.75 (s, 3H), 4.01 (m, 1H), 4.28 (m, 2H), 4.64 (d, $J = 11.5$ Hz, 2H), 5.01 (d, $J = 8.4$ Hz, 1H), 5.05 (d, $J = 10.5$ Hz, 1H), 5.32–5.43 (m, 3H), 5.77 (m, 1H), 6.18 (m, 1H), 6.23 (s, 1H), 6.31 (d, $J = 11.5$ Hz, 1H), 7.70 (s, 3H), 7.80 (t, $J = 7.4$ Hz, 1H), 7.93 (m, 1H), 8.14 (d, $J = 8.39$ Hz, 1H), 8.71 (d, $J = 8.48$ Hz, 1H), 8.97 (d, $J = 4.39$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.99, 22.54, 25.28, 27.02, 31.99, 35.92, 37.77, 42.30, 51.04, 59.39, 60.31, 62.72, 64.34, 65.73, 70.30, 115.55, 118.40, 119.17, 120.04, 121.14, 124.40, 125.10, 129.10, 129.85, 130.28, 132.37, 132.47, 136.29, 139.88, 144.82, 148.40, 149.40, 161.18; MS (EI) *m*/*z* 567, 470, 394, 268, 167.

***O*-Benzyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)cinchonidinium bromide (OSL-7).** $[\alpha]_D^{25}$ −78.7 ($c = 2$, $CHCl_3$); mp 190 °C; IR (film, cm^{-1}) 3406, 3060, 2952, 1625, 1615, 1597, 1541, 1537, 1450, 1410, 1388, 1351, 1260, 1225, 1210, 1114, 1060, 1010; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 18H), 1.64–1.74 (m, 2H), 2.02–2.09 (m, 1H), 2.30 (m, 1H), 2.55 (s, 1H), 3.09 (t, $J = 11.1$ Hz, 2H), 3.70 (s, 3H), 4.10 (d, $J = 7.0$ Hz, 1H), 4.21 (m, 1H), 4.58–4.74 (m, 3H), 4.98 (d, $J = 10.2$ Hz, 2H), 5.27 (d, $J = 17.1$ Hz, 1H), 5.74 (m, 1H), 5.86 (m, 1H), 6.52 (d, $J = 7.6$ Hz, 1H), 6.65 (s, 1H), 7.33–7.48 (m, 8H), 8.08 (t, $J = 8.1$ Hz, 1H), 8.25 (m, 1H), 8.39 (d, $J = 8.9$ Hz, 1H), 8.64 (s, 1H), 9.29 (d, $J = 8.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ

22.40, 25.01, 26.98, 32.11, 36.00, 37.80, 50.82, 59.23, 61.42, 64.32, 65.23, 70.01, 72.62, 111.74, 118.74, 127.66, 129.16, 129.72, 130.60, 132.46, 134.06, 135.53, 136.03, 136.54, 137.68, 139.34, 147.71, 152.27, 153.07; MS (EI, 70 eV) *m*/*z* 617. 552. 355. 268. 207. 136.

***N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)cinchoninium bromide (OSL-8).** $[\alpha]_D^{25}$ 79.0 ($c=2$, $CHCl_3$); mp 190 °C; IR (film, cm^{-1}) 3504, 3040, 3000, 2950, 1700, 1585, 1500, 1460, 1450, 1410, 1389, 1352, 1262, 1225, 1210, 1115, 1010; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.38 (s, 18H), 1.59–1.63 (m, 3H), 2.02 (m, 1H), 2.42 (s, 1H), 2.92 (m, 1H), 3.38 (m, 1H), 3.68 (s, 3H), 3.96 (m, 1H), 4.15 (m, 2H), 5.09–5.29 (m, 4H), 5.91 (m, 1H), 6.38 (s, 1H), 6.76 (s, 1H), 7.46 (t, $J=8.4$ Hz, 2H), 7.62 (s, 2H), 7.75 (d, $J=4.1$ Hz, 1H), 7.91 (d, $J=8.1$ Hz, 1H), 8.06 (d, $J=7.8$ Hz, 1H), 8.69 (d, $J=4.1$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.40, 23.98, 27.27, 32.15, 36.00, 38.16, 56.13, 62.98, 64.32, 64.90, 67.13, 118.02, 121.34, 122.42, 123.38, 127.65, 129.73, 133.15, 136.00, 145.09, 149.82, 161.04.

***O*-Allyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)cinchoninium bromide (OSL-9).** $[\alpha]_D^{25}$ 105.9 ($c=2$, $CHCl_3$); mp 210–220 °C; IR (film, cm^{-1}) 3407, 3000, 2949, 1704, 1625, 1596, 1567, 1510, 1502, 1491, 1470, 1454, 1400, 1350, 1210, 1115, 1070, 1010; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (s, 18H), 1.50 (m, 1H), 1.82 (m, 1H), 1.97 (m, 1H), 2.32 (m, 1H), 2.54 (m, 1H), 2.86 (m, 1H), 3.52 (t, $J=12.0$ Hz, 1H), 3.73 (s, 3H), 3.90 (m, 1H), 4.09–4.16 (m, 3H), 4.29 (dd, $J=4.8$ Hz, 1H), 4.39 (d, $J=11.9$ Hz, 1H), 5.23 (d, $J=17.2$ Hz, 1H), 5.32 (d, $J=10.4$ Hz, 1H), 5.38 (d, $J=4.1$ Hz, 1H), 5.43 (s, 2H), 5.86–5.94 (m, 1H), 6.08–6.16 (m, 2H), 6.29 (d, $J=11.6$ Hz, 1H), 7.66 (s, 3H), 7.80 (t, $J=7.6$ Hz, 1H), 7.97 (s, 1H), 8.13 (d, $J=8.4$ Hz, 1H), 8.88 (d, $J=8.4$ Hz, 1H), 8.96 (d, $J=4.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.06, 23.51, 27.26, 32.02, 35.94, 37.71, 54.22, 55.34, 61.86, 64.33, 70.29, 117.96, 119.92, 121.07, 125.01, 129.78, 130.34, 132.39, 135.59, 139.52, 144.89, 149.35, 161.23.

***O*-Benzyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)cinchoninium bromide (OSL-10).** $[\alpha]_D^{25}$ 77.5 ($c=2$, $CHCl_3$); mp 210 °C; IR (film, cm^{-1}) 3406, 3060, 2952, 1625, 1615, 1597, 1541, 1537, 1450, 1410, 1388, 1351, 1260, 1225, 1210, 1114, 1060, 1010; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.46 (s, 18H), 1.78–1.96 (m, 3H), 2.31–2.47 (m, 2H), 2.63–2.81 (m, 1H), 3.37 (m, 1H), 3.73 (s, 3H), 3.99–4.23 (m, 3H), 4.32 (d, $J=11.1$ Hz, 1H), 4.80 (m, 1H), 4.84 (d, $J=11.1$ Hz, 1H), 5.08 (d, $J=17.6$ Hz, 1H), 5.26 (d, $J=10.7$ Hz, 1H), 5.41 (m, 1H), 5.89 (m, 1H), 6.12 (m, 1H), 6.31 (s, 1H), 7.40–7.84 (m, 7H), 7.89 (s, 1H), 7.94 (m, 1H), 8.04 (s, 1H), 8.18 (d, $J=8.3$ Hz, 1H), 8.88 (d, $J=8.3$ Hz, 1H), 9.02 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.92, 23.45, 27.21, 32.02, 35.89, 37.48, 53.69, 55.24, 61.48, 64.24, 65.25, 71.69, 73.48, 117.64, 118.96, 120.87, 124.99, 128.64, 128.90, 129.24, 132.34, 135.62, 139.47, 144.65, 149.21, 161.07.

***N*-(3,5-di-*tert*-Butyl-4-methoxybenzyl)quinidinium bromide (OSL-11).** $[\alpha]_D^{25}$ 105.1 ($c=2$, $CHCl_3$); mp 223–225 °C; ^1H NMR ($CDCl_3$, 200 MHz); δ 1.38 (s, 18H), 1.77–1.79 (m, 1H), 1.85 (m, 1H), 2.45–2.51 (m, 3H), 2.95–2.98 (m, 1H), 3.38 (s, 2H), 3.69 (s, 3H), 3.84–3.89 (m, 1H), 3.94 (s, 3H), 4.11–4.14 (m, 1H), 4.50–4.55 (m, 1H), 4.75 (d, $J=12.0$ Hz, 1H), 5.17 (s, 1H), 5.20 (d, $J=6.8$ Hz, 1H), 5.74 (d, $J=12.4$ Hz, 1H), 5.93–6.02 (m, 1H), 6.50 (d, $J=4.0$, 1H), 6.69–6.71 (m, 1H), 7.24–7.27 (m, 1H), 7.30 (d, $J=2.4$ Hz, 1H), 7.59 (s, 2H), 7.67 (d, $J=4.4$ Hz, 1H), 7.92 (d, $J=9.2$ Hz, 1H), 8.60 (d, $J=4.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.78, 24.55, 27.68, 32.41, 36.30, 38.54, 50.60, 54.17, 56.37, 56.87, 64.16, 64.66, 65.45, 68.45, 102.24, 118.19, 120.67, 121.25, 121.31, 126.19, 131.69, 132.56, 136.09, 143.18, 144.14, 144.91, 147.31, 158.02, 161.30.

***O*-Allyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)quinidinium bromide (OSL-12).** $[\alpha]_D^{25}$ 133.8 ($c=2$, $CHCl_3$); mp 224–225 °C; ^1H NMR ($CDCl_3$, 200 MHz); δ 1.47 (s, 18H), 2.03 (m,1H), 2.57 (m, 3H), 2.91 (m, 1H), 3.52 (m, 3H), 3.74 (s, 3H), 4.19 (m, 4H), 4.31 (m, 2H), 4.68 (m, 2H), 5.21 (m, 2H), 5.30–5.44 (m, 3H), 5.94 (m, 2H), 6.08 (m, 2H), 7.37–7.42 (m, 2H), 7.59 (m, 3H), 8.04 (d, $J=8.4$ Hz, 1H), 8.83 (d, $J=4.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 22.22, 23.41, 27.01, 31.88, 37.40, 38.72, 53.99, 55.34, 61.98, 64.28, 70.33, 104.65, 116.19, 117.60, 119.44, 129.02, 129.66, 131.56, 132.15, 132.49, 135.65, 137.50, 144.84, 165.21.

***O*-Benzyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)quinidinium bromide (OSL-13).** $[\alpha]_D^{25}$ 98.8 ($c=2$, $CHCl_3$); mp 245–247 °C; ^1H NMR ($CDCl_3$, 200 MHz); δ 1.46 (s, 18H), 1.91–2.05 (m, 3H), 2.49–2.55 (m, 2H), 2.74–2.89 (m, 1H), 3.28 (m, 1H), 3.73 (m, 3H), 4.00–4.13 (m, 1H), 4.33–4.39 (m, 5H), 4.71–4.83 (m, 2H), 5.13–5.24 (m, 3H), 5.30 (m, 1H), 5.76–5.93 (m, 1H), 6.46 (m, 1H), 6.79 (m, 1H), 7.40–7.43 (m, 5H), 7.67 (d, $J=9.2$ Hz, 2H), 8.05–8.09 (m, 2H), 8.34 (m, 2H), 8.89 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.97, 23.58, 27.10–30.92, 31.98, 35.89, 37.57, 53.95, 55.81, 57.95, 61.33, 63.40, 64.33, 65.31, 72.02, 105.15, 118.00, 120.57, 121.81, 127.81, 129.12, 129.49, 129.61, 132.12, 133.87, 135.18, 135.40, 144.96, 145.63, 150.85, 160.92, 161.39.

***N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)quininium bromide (OSL-14).** $[\alpha]_D^{25}$ −130.8 ($c=2$, $CHCl_3$); mp 225–227 °C; ^1H NMR ($CDCl_3$, 200 MHz); δ 1.38 (s, 18H), 1.69 (m, 2H), 2.10 (m, 2H), 2.35–2.47 (m, 2H), 2.67–2.71 (m, 1H), 2.89–2.93 (m, 1H), 3.18–3.22 (m, 1H), 3.56–3.64 (m, 2H), 3.70 (s, 3H), 4.01 (s, 3H), 4.43 (d, $J=7.3$ Hz, 1H), 5.04–5.15 (m, 2H), 5.57–5.69 (m, 1H), 6.21 (d, $J=12.2$ Hz, 1H), 6.62 (d, $J=7.3$ Hz, 1H), 6.80 (d, $J=7.3$ Hz, 1H), 7.15–7.44 (m, 3H), 7.56 (s, 1H), 7.67 (d, $J=4.5$ Hz, 1H), 8.08 (d, $J=7.0$ Hz, 1H), 8.77 (d, $J=4.4$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.46, 24.74, 26.76, 31.92, 35.95, 38.09, 51.03, 56.49, 61.49, 63.03, 64.37, 64.76, 70.15, 101.87, 118.15, 120.21, 120.49, 120.98, 128.18, 132.13, 132.31, 136.33, 143.13, 145.21, 147.62, 158.23.

***O*-Allyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)quininium bromide (OSL-15).** $[\alpha]_D^{25}$ −256.2 ($c=2$, $CHCl_3$); mp 202–205 °C; ^1H NMR ($CDCl_3$, 200 MHz); δ 1.45 (s, 18H), 2.12 (m, 1H), 2.30–2.41 (m, 3H), 2.69 (m, 1H), 3.31–3.51 (m, 3H), 3.74 (s, 3H), 4.14–4.17 (m, 4H), 4.33

(m, 2H), 4.70 (m, 2H), 5.07 (d, $J = 10.4$ Hz, 2H), 5.26–5.43 (m, 3H), 5.74–5.91 (m, 1H), 5.99–6.13 (m, 1H), 6.31 (m, 2H), 7.38 (m, 2H), 7.63 (m, 3H), 8.05 (d, $J = 11.0$ Hz, 1H), 8.82 (d, $J = 4.7$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.98, 23.59, 27.11, 30.92, 31.98, 35.90, 37.58, 53.95, 55.82, 57.96, 61.34, 63.40, 64.34, 65.36, 72.19, 105.16, 118.00, 120.57, 121.82, 127.82, 129.13, 129.50, 129.61, 132.13, 133.88, 135.19, 135.40, 144.97, 145.64, 150.86, 160.92, 161.39.

***O*-Benzyl-*N*-(3,5-di-*tert*-butyl-4-methoxybenzyl)quininium bromide (OSL-16).** $[\alpha]_D^{25}$ −174.6 ($c = 2$, $CHCl_3$); mp 246–248 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.44 (s, 18H), 1.53 (s, 2H), 2.05 (m, 1H), 2.49 (m, 1H), 3.09 (m, 1H), 3.19–3.31 (m, 2H), 3.72 (s, 3H), 4.10–4.24 (m, 5H), 4.70–4.86 (m, 2H), 5.03–5.30 (m, 3H), 5.50–5.56 (m, 2H), 5.76–5.88 (m, 1H), 6.30–6.32 (m, 2H), 7.33–7.59 (m, 7H), 8.04–8.07 (m, 2H), 8.28–8.33 (m, 2H), 8.86 (d, $J = 4.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.73, 24.79, 25.26, 27.31, 32.06, 35.95, 37.84, 38.32, 45.89, 50.50, 57.92, 58.62, 61.33, 64.27, 67.64, 71.56, 99.26, 105.02, 118.53, 120.79, 121.08, 127.89, 128.79, 129.16, 129.64, 130.18, 131.83, 132.47, 133.88, 136.06, 136.35, 141.36, 144.77, 153.22, 157.69, 160.79, 161.17.

Biology

Antimalarial assay. All experiments were with W2-strain (chloroquine-resistant) parasites, cultured in human erythrocytes using standard methods. Two antimalarial assays were carried out for each compound according to procedures described in the literature.[9] In brief, for [^{3}H]hypoxanthine uptake assays, ring-stage parasites were incubated with multiple concentrations of compounds (from 100X stocks in DMSO) for 24 h, [^{3}H]hypoxanthine was added for an additional 18 h, and cells were harvested and counts representing incorporated hypoxanthine were quantified. For development assays, parasites were incubated with different concentrations of compounds for 48 h, beginning at the ring stage. At the completion of the incubation, Giemsa-stained smears were evaluated microscopically, and ring-stage parasites per 1000 erythrocytes were counted. Both assays were performed twice for each inhibitor, each [^{3}H]hypoxanthine assay was performed in triplicate each concentration. All values were normalized to percent control (1% DMSO) activity and 50% inhibitory concentrations (IC_{50}) were calculated using the Prism 3.0 program (GraphPad Software).[15]

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