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Synthesis and antimalarial evaluation of novel isocryptolepine derivatives

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ABSTRACT

A series of mono- and di-substituted analogues of isocryptolepine have been synthesized and evaluated for in vitro antimalarial activity against chloroquine sensitive (3D7) and resistant (W2mef) *Plasmodium falciparum* and for cytotoxicity (3T3 cells). Di-halogenated compounds were the most potent derivatives and 8-bromo-2-chloroisocryptolepine displayed the highest selectivity index (106; the ratio of cytotoxicity (IC_{50} = 9005 nM) to antimalarial activity (IC_{50} = 85 nM)). Our evaluation of novel isocryptolepine compounds has demonstrated that di-halogenated derivatives are promising antimalarial lead compounds.

1. Introduction

The development of new antimalarial therapies represents an important contribution to global health and natural products are a valuable source of potential lead compounds.[1,2] Invariably semi-synthetic derivatives of natural compounds are developed to improve biopharmaceutical and/or pharmacological properties, as demonstrated with artemisinin.[3]

The West African climbing shrub *Cryptolepis sanguinolenta* is a component of several traditional herbal remedies used to treat a variety of maladies, including malaria.[4] Cryptolepine (5-methyl-5*H*-indolo[3,2-*b*]quinoline) (**1**) was the major bioactive alkaloid isolated from this shrub and has been found to possess antibacterial,[5,6] antihyperglycemic,[7] antimuscarinic,[8] antifungal[9] and antimalarial activity (Fig. 1).[10,11]

Cryptolepine (**1**) has also been found to intercalate non-specifically into DNA and inhibit topoisomerase II, resulting in undesirable cytotoxicity that could negate its therapeutic potential.[12–14] Investigations of cryptolepine analogues have found that the cytotoxicity and antimalarial activity of the parent alkaloid can be improved by the addition of ring substituents. A range of alkylated, halogenated and nitrated cryptolepine derivatives have been synthesized and biologically evaluated in recent years.[15–18] Typically, the halogenated derivatives have demonstrated improved bioactivity, with 2,7-dibromocryptolepine the most promising antimalarial candidate (IC_{50} = 49 nM; K1 strain *P. falciparum*).[18]

Of the other alkaloids isolated from *Cryptolepis sanguinolenta*, neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline) (**2**) and isocryptolepine (5-methyl-11*H*-indolo[3,2-*c*]quinoline) (**3a**) were the only ones found to possess significant antimalarial activity, albeit less potent than **1**.[19] Several synthetic and biological investigations of substituted neocryptolepine derivatives have been undertaken but no compound has proved sufficiently bioactive for therapeutic use.[20–22] Many of its derivatives, however, possess increased antimalarial activity and lower cytotoxicity in comparison to the parent alkaloid.

A small number of isocryptolepine derivatives have previously been synthesized,[23,24] and the synthesis of two compounds (2-chloroisocryptolepine and 8-chloroisocryptolepine), coincidental to our work, was recently reported.[25] However, the antimalarial activity of these compounds has not been described thus far.

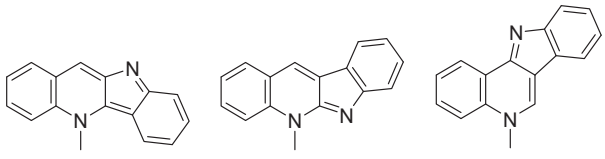

Figure 1. Structures of cryptolepine (**1**), neocryptolepine (**2**) and isocryptolepine (**3a**).

Abbreviations: CQ, chloroquine; CQR, chloroquine resistant; CQS, chloroquine sensitive; NBS, *N*-bromosuccinimide; NCS, *N*-chlorosuccinimide; PPA, polyphosphoric acid; NOE, nuclear overhauser effect.

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Although both **3a** and **2** are less active than **1**, they have similar selectivity indices[19] and it is unclear why **3a** has been neglected. We report the synthesis and antimalarial activity of eight isocryptolepine derivatives that were assessed for potential as lead compounds for new antimalarial agents. Derivatives with substituents in similar positions to derivatives of **1** and **2**[17,18,21] were synthesized, to enable direct comparison. Compounds were evaluated for their in vitro antiplasmodial activity and cytotoxicity.

2. Results and discussion

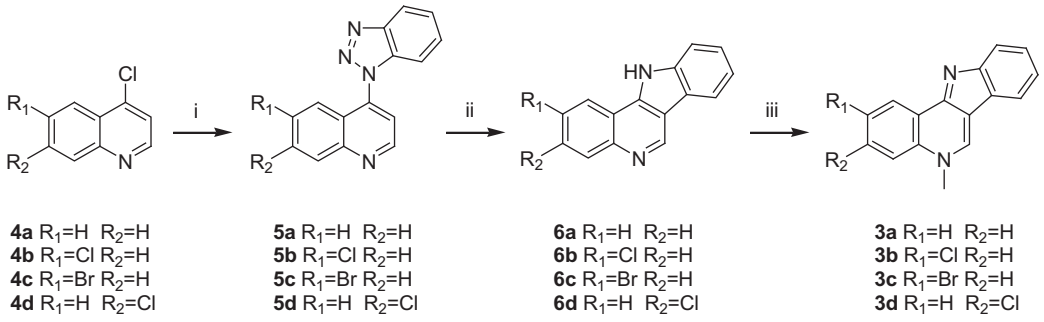
2.1. Chemistry

The parent alkaloid, isocryptolepine (**3a**) was prepared as described previously by Molina et al.[26] (Scheme 1). 4-Chloroquinoline (**4a**) was initially thermally coupled with benzotriazole to give 4-(1-benzotraizolyl)quinoline (**5a**). Intermediate **5a** was subsequently cyclized, in the presence of polyphosphoric acid (PPA), to yield 11*H*-indolo[3,2-*c*]quinoline (**6a**). Compound **6a** was *N*-methylated with iodomethane to yield the hydroiodide salt of **3a**, which was converted to its free base and characterization data were consistent with previous reports.[11,26–30]

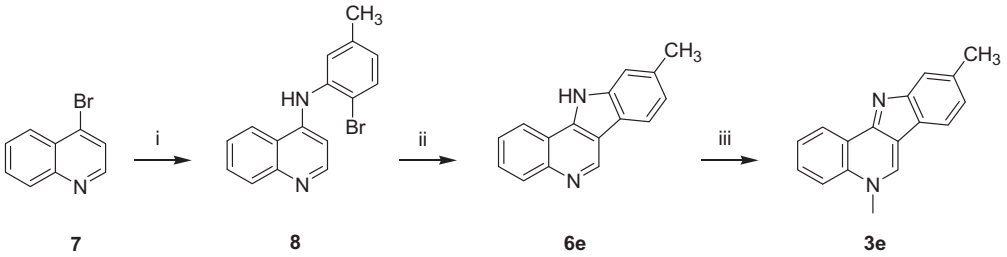
Our synthetic strategy for analogues of **3a** was inspired by a recent report of the synthesis of neocryptolepine derivatives from substituted 2-chloroquinolines, via a modification of the Molina method.[20] Substituted 4-chloroquinolines **4b–d** were coupled to benzotriazole, producing 4-(1-benzotraizolyl)quinolines **5b–d** in good yields (70–78%). Subsequent cyclisation gave the chloro substituted 11*H*-indolo[3,2-*c*]quinolines **6b** and **6d** in moderate yield (77–78%) but low yield of the bromo substituted 11*H*-indolo[3,2-*c*]quinoline **6c** (54%). Intermediates **6b–d** were treated with iodomethane to give the isocryptolepine derivatives **3a–d** and the reaction proceeded smoothly when acetonitrile was used as the reaction solvent. Higher yields were obtained with a substituent at R_1 (88–90%) while *N*-methylation was less efficient with a substituent at R_2 (61%).

In contrast to the above halogenated compounds, the 9-methyl analogue **3e** was synthesized by a modification of the method reported by Jonckers et al.[31] for **3a** (Scheme 2). Initially 5-methyl-2-bromoaniline was coupled to 4-bromoquinoline (**7**) via a palladium catalyzed Buchwald-Hartwig reaction to generate the intermediate 4-(2-bromo-5-methylphenylamino)quinoline (**9**) in moderate yield (76%). Intermediate **8** was subjected to cyclization via a palladium catalyzed intramolecular Heck reaction to yield 9-methyl-11*H*-indolo[3,2-*c*]quinoline (**6e**) in moderate yield (69%). Finally treatment of the cyclic intermediate **6e** with iodomethane in acetonitrile afforded 9-methylisocryptolepine (**3e**) in good yield (84%).

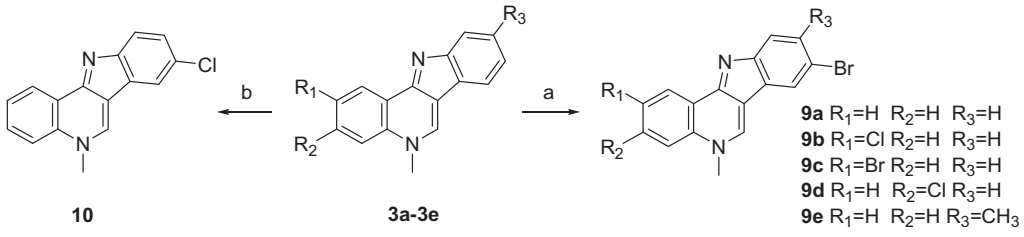
A further range of halogenated derivatives **9a–e** and **10** were prepared via aromatic electrophilic substitution (Scheme 3).



Scheme 1. Synthesis of compounds **3a–d**. [a]Reagents and Conditions: (i) Benzotriazole, 110–120 °C, 30 min; (ii) PPA, 130–140 °C, 3–4 h; (iii) CH_3I, CH_3CN, reflux, 20 h.



Scheme 2. Synthesis of compound **3e**. [a]Reagents and Conditions: (i) 2-bromo-5-methylaniline, $Pd_2(dba)_3$ (1 mol %), XANTPHOS (2 mol %), Cs_2CO_3, dioxane, reflux, 24 h; (ii) $Pd(OAc)_2$ (2 mol %), BINAP (2 mol %), K_2CO_3, DMF, 150 °C, 24 h; (iii) CH_3I, CH_3CN, reflux, 20 h.



Scheme 3. Synthesis of compounds **10** and **9a–e**. [a]Reagents and Conditions: (a) NBS, 150 °C; (b) NCS, 150 °C.

Bromination of **3a** and mono-substituted derivatives **3b–e** was achieved by reaction with *N*-bromosuccinimide (NBS) via adaption of literature methods.[32] At room temperature, or elevated temperatures, the use of one molar equivalent of NBS exclusively produced 8-substituted derivatives **9a–e** in good yield (71–80%). Attempts to chlorinate **3a** with *N*-chlorosuccinimide (NCS) gave 8-chloroisocryptolepine (**10**) in low yield (41%), possibly due to the lower reactivity of this halogenating reagent. The ring position of electrophilic substitution, in compounds **9a–e** and **10**, was confirmed by selective 1D NOE NMR spectroscopic analysis.

2.2. In vitro antiplasmodial activity

The hydrochloride salts of isocryptolepine derivatives, and the parent **3a**, were evaluated for their in vitro antiplasmodial activity against the chloroquine sensitive strain (CQS) 3D7 and the chloroquine resistant strain (CQR) W2mef of *P. falciparum*. The results are summarized in Table 1. The parent alkaloid **3a** was found to possess in vitro antimalarial activity (IC_{50} = 1177 nM) against CQR W2mef comparable to previous data (IC_{50} = 780 nM).[19] Similarly CQ displayed in vitro antimalarial activity (IC_{50} = 144 nM) against the CQR strain at comparable levels to published data (IC_{50} = 171–246 nM).[17–19,33] The mono-substituted isocryptolepine compounds **3d**, **3e**, **9a** and **10a** showed improved activity compared to **3a**, against both strains. Di-substituted derivatives **9b–e** were also more active than **3a** against both strains, and generally more active than the mono-substituted counterparts. The di-halogenated derivative **9b** showed the greatest activity (IC_{50} = 85 nM, CQR W2mef) and our order of activity is the same as that reported for a series of cryptolepine derivatives.[17] Onyeibor et al.[17] found that 7-bromo-2-chlorocryptolepine, 7-bromo-3-chlorocryptolepine and 2,7-dibromocryptolepine were the most active derivatives and the analogous derivatives in the present study were **9b**, **9d** and **9c**. The di-substituted derivatives with substituents on opposing sides of the molecule (i.e., **9b**) were more active than those with substituents on the same aromatic ring (i.e., **9e**). The di-bromo compound **9c** was the only derivative to have greater activity against CQR W2mef compared to CQS 3D7.

2.3. In vitro cytotoxicity

The hydrochloride salts of isocryptolepine derivatives, and the parent **3a**, were evaluated for their in vitro cytotoxicity against 3T3 cells (mouse embryonic fibroblasts). The results are summarized in Table 1 and selectivity indices were calculated using the CQR (W2mef) antiplasmodial activity data. The majority of derivatives (**3d**, **3e**, **9a**, **9c–e** and **10**) had a similar cytotoxicity compared to the parent **3a**, with IC_{50} values ranging from 1970 to 2640 nM. The compound 8-bromo-2-chloroisocryptolepine (**9b**) was an exception, being 4-fold less cytotoxic than the parent alkaloid. The most potent antimalarial derivatives, **9b**, **9c** and **9d** had selectivity indices of 106, 23 and 26 respectively. Therefore compound **9b** (8-bromo-2-chloroisocryptolepine) represents the most selective of this series of compounds.

3. Conclusion

Eight derivatives of isocryptolepine have been evaluated for in vitro antiplasmodial activity against CQS and CQR strains of *P. falciparum*. All were found to be more active than the parent compound, with 8-bromo-2-chloroisocryptolepine (**9b**) being the most potent derivative. In vitro cytotoxicity against 3T3 cells has identified **9b** as the only derivative to have reduced cytotoxicity in comparison to the parent compound and a selectivity index >100. As compound **9b** displays antimalarial activity in the low nano-molar range, comparable to lumefantrine[33] (IC_{50} = 56 nM; CQR W2mef), **9b** represents a novel promising lead in antimalarial drug development.

4. Experimental section

4.1. Chemistry

Starting materials **4b**, **4c** and **7** were prepared as previously reported.[34–36]DMF was dried over $CaSO_4$ and distilled under reduced pressure. Dioxane was dried over sodium before distillation under nitrogen from sodium/benzophenone. Other solvents, reagents and reactants were available commercially and used as received from the supplier. Analytical TLC was performed on silicagel 60 F_{254} (Macherey–Nagel) and flash chromatography was performed using Silica gel 60 (0.040–0.063 mm, Fluka). Characterization of novel compounds was carried out using NMR spectroscopy and mass spectrometry. NMR spectra were recorded with Varian-Gemini (200 MHz, ^1H; 50 MHz, ^{13}C), Bruker AV400 (400 MHz, ^1H; 100 MHz, ^{13}C) or Bruker AV600 (600 MHz, ^1H) spectrometers. Coupling constants (*J*) are expressed in Hertz (Hz) and assignment of ^1H and ^{13}C spectra were routinely made with the aid of 2D experiments. Mass spectra (MS) were obtained using a HP5896 or VG Autospec high-resolution mass spectrometer. High resolution mass spectrometry (HRMS) was used to determine the accurate mass of the molecular ion in-lieu of elemental analysis. Melting points (Mp) were recorded with a Barnstead Electrothermal digital melting-point apparatus. The purity of derivatives for biological testing were assessed by analytical HPLC (purity >96%) on an Apollo C18 column (4.6 × 150 mm, 5 μm) eluting with 20–80% acetonitrile in water (0.5% formic acid) over ten min at a flow rate of 1.5 mL/min.

Table 1
In vitro antiplasmodial activity against *P. falciparum*, in vitro cytotoxicity against 3T3 cells and selectivity indices of substituted isocryptolepines

Compound[a]	Antiplasmodial activity[b] (nM)		Cytotoxicity[b] (nM)	Selectivity index [c]
	3D7	W2mef		
3a, Isocryptolepine	665 ± 221	1177 ± 390	2188 ± 351	1.9
3d, 3-Chloroisocryptolepine	130 ± 12	316 ± 205	2263 ± 460	7.3
3e, 9-Methylisocryptolepine	448 ± 83	760 ± 268	2067 ± 331	2.7
9a, 8-Bromoisocryptolepine	85 ± 33	184 ± 47	1970 ± 360	11
9b, 8-Bromo-2-chloroisocryptolepine	57 ± 14	85 ± 5.6	9005 ± 3754	106
9c, 2,8-Dibromoisocryptolepine	127 ± 98	112 ± 12	2585 ± 533	23
9d, 8-Bromo-3-chloroisocryptolepine	50 ± 4	100 ± 16	2640 ± 663	26
9e, 8-Bromo-9-methylisocryptolepine	62 ± 39	131 ± 55	2500 ± 352	19
10, 8-Chloroisocryptolepine	117 ± 16	218 ± 35	2104 ± 304	9.6
CQ	20 ± 27	144 ± 11	71,954 ± 19,532	500

[a] Tested as hydrochloride salts.
[b] Mean IC_{50} ± standard deviation; at least three separate determinations.
[c] Cytotoxic/antiplasmodial ratio.

4.1.1. General procedure for the synthesis of 4-(1-benzotriazolyl)quinolines (5a–d)

Benzotriazole and the appropriate quinoline compound (**4a–d**) were heated at 110–120 °C for 30 min. The resulting solid was cooled to room temperate and collected by filtration (washing with H_2O). The solid obtained was subsequently recrystallized from ethanol to give **5a–d**.

4.1.2. 4-(1-Benzotriazolyl)quinoline (5a)

Prepared from **4a** (1.62 g, 9.89 mmol) and benzotriazole (1.30 g, 10.88 mmol). White crystalline solid; yield 77% (1.87 g). Mp 132–133 °C (Lit.[37] mp 132–133 °C).

4.1.3. 4-(1-Benzotriazolyl)-6-chloroquinoline (5b)

Prepared from **4b** (425 mg, 2.15 mmol) and benzotriazole (274 mg, 2.30 mmol). White crystalline solid; yield 77% (464 mg). Mp 186–187 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.45–7.55 (3H, m, H-4′, H-5′ and H-6′), 7.61 (1H,d, J = 4.8 Hz, H-3), 7.75 (1H, dd, J = 8.8, 2.2 Hz, H-7), 7.86 (1H, d, J = 2.2 Hz, H-5), 8.20 (1H, d, J = 8.8 Hz, H-8), 8.22 (1H, dd, J = 8.8, 1.2 Hz, H-7′), 9.10 (1H, d, J = 4.8 Hz, H-2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 109.1, 116.7, 119.9, 121.6, 122.9, 124.2, 128.3, 130.9, 132.8, 133.7, 138.9, 145.3, 147.7, 149.7. MS (EI): 99 (44), 126 (15), 127 (18), 134 (31), 162 (49), 164 (19), 190 (29), 216 (17), 217 (81), 252 (100), 253 (19), 254 (35), 280 (37). HRMS (EI): 280.0515 ($C_{15}H_9N_4Cl$ [M]$^+$ requires 280.0516).

4.1.4. 4-(1-Benzotriazolyl)-6-bromoquinoline (5c)

Prepared from **4c** (401 mg, 1.65 mmol) and benzotriazole (212 mg, 1.78 mmol). White crystalline solid; yield 70% (376 mg). Mp 181–182 °C. ^{1}H NMR(200 MHz, $CDCl_3$): δ 7.46–7.57 (3H, m, H-4′. H-5′ and H-6′), 7.61 (1H,d, J = 4.4 Hz, H-3), 7.91 (1H, dd, J = 9.2, 2.2 Hz, H-7), 8.05 (1H, d, J = 1.8 Hz, H-5), 8.14 (1H, d, J = 8.8 Hz, H-8), 8.24 (1H, dd, J = 7.6, 1 Hz, H-7′), 9.13 (1H, d, J = 4.6 Hz, H-2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 109.1, 116.6, 119.9, 121.8, 123.4, 124.2, 124.9, 128.3, 130.9, 132.9, 133.5, 138.8, 145.4, 147.9, 149.8. MS (EI): 100 (19), 127 (20), 190 (29), 206 (18), 208 (17), 216 (23), 217 (100), 218 (20), 296 (55), 298 (58), 324 (17), 326 (17). HRMS (EI): 324.0005 ($C_{15}H_9N_4Br$ [M]$^+$ requires 324.0011).

4.1.5. 4-(1-Benzotriazolyl)-7-chloroquinoline (5d)

Prepared from **4d** (392 mg, 1.98 mmol) and benzotriazole (265 mg, 2.22 mmol). White crystalline solid; yield 78% (432 g). Mp 190–192 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.48 (1H, d, J = 8.4 Hz, H-4′), 7.52 (1H, t, J = 7.8 Hz, H-6′), 7.56 (1H, dd, J = 9.0, 2.4 Hz, H-6), 7.59 (1H, t, J = 7.8 Hz, H-5′), 7.62 (1H, d, J = 4.8 Hz, H-3), 7.83 (1H, d, J = 9.0 Hz, H-5), 8.24 (1H, d, J = 8.4 Hz, H-7′), 8.29 (1H, d, J = 1.8 Hz, H-8), 9.14 (1H, d, J = 4.2 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 110.3, 117.0, 120.9, 125.2, 129.1, 129.3, 129.4, 133.8, 137.2, 141.0, 146.4, 150.4, 151.5. MS (EI): 99 (20), 135 (17), 162 (38), 190 (21), 217 (62), 252 (100), 253 (21), 254 (34), 280 (25). HRMS (EI): 280.0520 ($C_{15}H_9N_4Cl$ [M]$^+$ requires 280.0516).

4.1.6. General procedure for the synthesis of 11*H*-indolo[3,2-*c*]quinolines (6a–d)

To the appropriate 4-(1′-benzotriazolo)quinoline (**5a-5d**), polyphosphoric acid (PPA) was added and the mixture heated at 130–140 °C until the formation of nitrogen ceased (1–4 h). The reaction mixture was cooled, quenched with water and the precipitate collected by filtration. The residue was re-suspended in water, made alkaline with 10% NaOH (*aq*) and the precipitate collected by filtration. The product was purified by washed the solid with DCM (unless otherwise stated) to give **6a–d**.

4.1.7. 11*H*-indolo[3,2-*c*]quinoline (6a)

Prepared from **5a** (204 mg, 0.83 mmol) and PPA (7.17 g). Cream solid; yield 84% (152 mg). Mp >300 °C (Lit.[26] mp 336–337 °C).

4.1.8. 2-Chloro-11*H*-indolo[3,2-*c*]quinoline (6b)

Prepared from **5b** (608 mg, 2.17 mmol) and PPA (19.48 g). Cream solid; yield 77% (422 mg). Mp >350 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.36 (1H, td, J = 7.5, 0.6 Hz, H-8), 7.52 (1H, td, J = 7.8, 0.6 Hz, H-9), 7.73–7.75 (2H, m, H-3 and H-10), 8.14 (1H, d, J = 9.0 Hz, H-4), 8.33 (1H, dd, J = 7.8, 0.6 Hz, H-7), 8.64 (1H, d, J = 2.4 Hz, H-1), 9.61 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 111.9, 114.7, 117.8, 120.1, 120.7, 121.1, 121.5, 125.8, 128.1, 129.8, 131.4, 138.7, 143.6, 145.1. MS (EI): 252 (100), 253 (18), 254 (34). MS (EI): 217 (11), 252 (100), 253 (18), 254 (34). HRMS (EI): 252.0453 ($C_{15}H_9N_2Cl$ [M]$^+$ requires 252.0454).

4.1.9. 2-Bromo-11*H*-indolo[3,2-*c*]quinoline (6c)

Prepared from **5c** (675 mg, 2.08 mmol) and PPA (16.71 g). Wash with MeOH; cream solid; yield 54% (332 mg). Mp >350 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ .34 (1H, td, J = 7.5, 0.8 Hz, H-8), 7.50 (1H, ddd, J = = 8.2, 7.0, 0.8 Hz, H-9), 7.75 (1H, dt, J = 7.2, 0.8 Hz, H-10), 7.83 (1H, dd, J = 8.8, 2.0 Hz, H-3), 8.06 (1H, d, J = 9.2 Hz, H-4), 8.32 (1H, dt, J = 7.6, 0.8 Hz, H-7), 8.85 (1H, d, J = 2.0 Hz, H-1), 9.62 (1H, s, H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.3, 114.9, 118.3, 118.7, 120.3, 120.7, 121.7, 124.7, 125.9, 130.8, 131.7, 139.0, 139.2, 144.0, 145.4. MS (EI): 190 (19), 216 (22), 217 (40), 296 (100), 297 (22), 298 (98), 299 (17). HRMS (EI): 295.9944 ($C_{15}H_9N_2Br$ [M]$^+$ requires 295.9949).

4.1.10. 3-Chloro-11*H*-indolo[3,2-*c*]quinoline (6d)

Prepared from **5d** (993 mg, 3.54 mmol) and PPA (31.32 g). Cream solid; yield 78% (695 mg). Mp >310 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.35 (1H, td, J = 7.5, 0.6 Hz, H-8), 7.51 (1H, ddd, J = 8.4, 7.2, 0.6 Hz, H-9), 7.73 (1H, dd, J = 8.4, 0.6 Hz, H-10), 7.74 (1H, dd, J = 8.7, 2.1 Hz, H-2), 8.16, (1H, d, J = 2.4 Hz, H-4), 8.33 (1H, dd, J = 7.5, 0.6 Hz, H-7), 8.56 (1H, d, J = 8.4 Hz, H-1), 9.62 (1H, s, H-6), 12.88 (1H, br s, N-H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 111.8, 114.5, 115.5, 120.0, 120.6, 121.5, 124.0, 125.7, 125.9, 128.1, 132.2, 138.7, 139.3, 145.7, 145.9. MS (EI): 217 (16), 252 (100), 253 (20), 254 (33). HRMS (EI): 252.0454 ($C_{15}H_9N_2Cl$ [M]$^+$requires 252.0454).

4.1.11. 9-Methyl-11*H*-indolo[3,2-*c*]quinoline (6e)

To a degassed solution of $Pd(OAc)_2$ (4.2 mg, 2 mol %) and BINAP (12 mg, 2 mol %) in dry DMF (8 mL), K_2CO_3 (2.51 g, 18.16 mmol) and **8** (282 mg, 0.90 mmol) was added. The flask was flushed with nitrogen and the mixture heated at 150 °C for 24 h under nitrogen. After cooling, the mixture was filtered through celite and washed with DCM (40 mL). The solvent was removed in vacuo and the residue purified by flash column chromatography eluting with DCM and EtOAc (1:1 increasing to 1:0). Cream solid; yield 69% (144 mg). Mp >340 °C (d). ^{1}H NMR (600 MHz, DMSO-d_6): 2.52 (3H, s, CH_3), 7.16 (1H, dd, J = = 7.8, 1.2 Hz, H-8), 7.51 (1H, d, J = 0.6 Hz, H-10), 7.66 (1H, ddd, J = 8.1, 6.9, 1.2 Hz, H-2), 7.72 (1H, ddd, J = 8.1, 6.9, 1.2 Hz, H-3), 8.11 (1H, dd, J = 8.4, 0.6 Hz, H-4), 8.17 (1H, d, J = 7.8 Hz, H-7), 8.53 (1H, dd, J = 8.4, 1.2 Hz, H-1), 9.53 (1H, s, H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.7, 111.7, 114.4, 117.2, 119.6, 119.8, 122.0, 122.2, 125.6, 127.8, 129.5, 135.2, 139.3, 139.6, 144.6, 145.3. MS (EI): 231 (63), 232 (100), 233 (19). HRMS (EI): 232.1002 ($C_{16}H_{12}N_2$ [M]$^+$ requires 232.1000).

4.1.12. General procedure for the synthesis of 3a–e

To a solution of the appropriate 11*H*-indolo[3,2-*c*]quinoline (**6a–e**) in CH_3CN, iodomethane was added and the mixture refluxed for 20 h under nitrogen. The reaction mixture was then cooled, the solvent removed in vacuo and the residue dissolved

in a 1:1 solution of 30% NH_4OH (*aq*) and DCM. The organic layer was extracted with DCM, dried ($MgSO_4$) and the residue purified by flash column chromatography. A gradient solvent mixture of DCM:EtOH:NH_3 was used, starting with 100:0:1 and increasing to 100:4:1 to give the indicated isocryptolepine **3a–e**.

4.1.13. Isocryptolepine (3a)

Prepared from **6a** (128 mg, 0.58 mmol) and iodomethane (3.5 mL, 56.22 mmol). Yellow crystalline solid; yield 94% (128 mg). Mp 138–139 °C (Lit.[38] mp 134–135 °C). HPLC purity, 99.9%.

4.1.14. 2-Chloroisocryptolepine (3b)

Prepared from **6b** (51 mg, 0.20 mmol) and iodomethane (1.3 mL, 20.9 mmol). Yellow crystalline solid; yield 88% (47 mg). Mp 248–249 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.19 (3H, s, NCH_3), 7.26 (1H, t, J = 7.5 Hz, H-8), 7.45 (1H, t, J = 7.5 Hz, H-9), 7.79–7.81 (2H, m, H-3 and H-10), 8.02 (1H, d, J = 9.0 Hz, H-4), 8.10 (1H, d, J = 7.2 Hz, H-7), 8.66 (1H, d, J = 2.4 Hz, H-1), 9.25 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.0 (NCH_3), 116.4 (C-6a), 118.5 (C-4), 119.5 (C-10), 119.7 (C-7), 120.0 (C-8), 121.9 (C-11b), 122.4 (C-1), 125.4 (C-6b), 125.5 (C-9), 128.8 (C-3), 129.5 (C-2), 133.8 (C-4a), 138.1 (C-6), 151.3 (C-11a), 154.4 (C-10a). MS (EI): 205 (28), 266 (100), 267 (20), 268 (34). HRMS (EI): 266.0603 ($C_{16}H_{11}N_2Cl$ [M]$^+$ requires 266.0611). The spectra data acquired was in agreement with that previously published.[25]

4.1.15. 2-Bromoisocryptolepine (3c)

Prepared from **6c** (206 mg, 0.69 mmol) and iodomethane (4.3 mL, 69.07 mmol). Yellow crystalline solid; yield 90% (194 mg). Mp 262–263 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.19 (3H, s, NCH_3), 7.26 (1H, t, J = 7.5 Hz, H-8), 7.45 (1H, ddd, J = 7.8, 7.2, 0.6 Hz, H-9), 7.80 (1H, d, J = 7.8 Hz, H-10), 7.92 (1H, dd, J = 9.6, 2.1 Hz, H-3), 7.96 (1H, d, J = 9.0 Hz, H-4), 8.10 (1H, d, J = 7.2 Hz, H-7), 8.82 (1H, d, J = 2.4 Hz, H-1), 9.27 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.0 (NCH_3), 116.6 (C-6a), 117.7 (C-2), 118.6 (C-10), 119.5 (C-4), 119.9 (C-7), 120.0 (C-8), 122.3 (C-11b), 125.5 (C-6b), 125.6 (C-9 and C-1), 131.4 (C-3), 134.1 (C-4a), 138.1 (C-6), 151.2 (C-11a), 154.5 (C-10a). MS (EI): 189 (16), 231 (44), 310 (100), 311 (19), 312 (88), 313 (18). HRMS (EI): 310.0113 ($C_{16}H_{11}N_2Br$ [M]$^+$ requires 310.0106).

4.1.16. 3-Chloroisocryptolepine (3d)

Prepared from **6d** (438 mg, 1.73 mmol) and iodomethane (11 mL, 176.7 mmol). Yellow crystalline solid; yield 61% (281 mg). Mp 268–270 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.21 (3H, s, NCH_3), 7.25 (1H, t, J = 7.5 Hz, H-8), 7.44 (1H, ddd, J = 7.8, 7.2, 0.6 Hz, H-9), 7.71 (1H, dd, J = 9.0, 1.8 Hz, H-2), 7.79 (1H, br d, J = 8.4 Hz, H-10), 8.09–8.10 (2H, m, H-4 and H-7), 8.73 (1H, d, J = 9.0 Hz, H-1), 9.27 (1H, s, H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.2 (NCH_3), 116.8 (C-6a), 117.2 (C-4), 118.6 (C-10), 119.6 (C-11b), 119.7 (C-7), 120.1 (C-8), 125.4 (C-2), 125.6 (C-6b), 125.7 (C-1), 125.8 (C-9), 133.9 (C-3), 136.2 (C-4a), 138.4 (C-6), 152.1 (C-11a), 154.7 (C-10a). MS (FAB): 147 (18), 267 (100), 268 (23), 269 (35). HRMS (FAB): 267.0685 ($C_{16}H_{12}N_2Cl$ [M+H]$^+$ requires 267.0689). HPLC purity, 98.9%.

4.1.17. 9-Methylisocryptolepine (3e)

Prepared from **6e** (222 mg, 0.95 mmol) and iodomethane (5.9 mL, 94.77 mmol). Yellow crystalline solid; yield 84% (197 mg). Mp 259–260 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.58 (3H, s, CH_3), 3.97 (3H, s, NCH_3), 7.09 (1H, ddd, J = 7.8, 1.2, 0.6 Hz, H-8), 7.52–7.55 (2H, m, H-2 and H-4), 7.61 (1H, ddd, J = 9.0, 7.8, 1.2 Hz, H-3), 7.73 (1H, d, J = 7.8 Hz, H-7), 7.77 (1H, br s, H-10), 8.12 (1H, s, H-6), 8.86 (1H, dd, J = 7.2, 2.1 Hz, H-1). ^{13}C NMR (50 MHz, DMSO-d_6): δ 21.7 (CH_3), 42.1 (NCH_3), 116.0 (C-6a), 117.4 (C-4), 117.9 (C-10), 119.1 (C-7), 120.4 (C-11b), 121.5 (C-8), 122.7 (C-6b), 123.8 (C-1), 125.1 (C-2), 129.2 (C-3), 134.9 (C-9), 135.3 (C-4a), 137.8 (C-6), 151.8 (C-11a), 153.7 (C-10a). MS (EI): 231.1 (19), 245.1 (35), 246.1 (100), 247.1 (19). HRMS (EI): 246.1151 ($C_{17}H_{14}N_2$ [M]$^+$ requires 246.1157). HPLC purity, 98.7%.

4.1.18. 4-(2-Bromo-5-methylphenylamino)quinoline (8)

To a degassed solution of $Pd_2(dba)_3$ (21.7 mg, 1 mol %) and XANTPHOS (29.8 mg, 2.1 mol %) in dry dioxane (10 mL), Cs_2CO_3 (1.08 g, 3.31 mmol), 4-bromoquinoline (500 mg, 2.40 mmol) and 2-bromo-5-methylaniline (490 mg, 2.63 mmol) were added. The flask was flushed with nitrogen and the mixture refluxed for 24 h under nitrogen. After cooling, the reaction mixture was filtered through Celite, washing with DCM (120 mL). The solvent was removed in vacuo and the residue purified by flash column chromatography eluting with DCM and EtOAc (50:50 increasing to 20:80). Off-white solid; yield 76% (572 mg). Mp 155–156 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.33 (3H, s, CH3), 6.84 (1H, dd, J = 8.4, 2.4 Hz, H-4′), 7.02 (1H, d, J = 5.4 Hz, H-3), 7.34 (1H, d, J = 2.4 Hz, H-6′), 7.52 (1H, d, J = 7.8 Hz, H-3′), 7.54 (1H, ddd, J = 8.4, 7.2, 1.2 Hz, H-6), 7.71 (1H, ddd, J = 8.4, 7.2, 1.2 Hz, H-7), 8.01 (1H, d, J = 8.4 Hz, H-8), 8.09 (1H, d, J = 8.4 Hz, H-5), 8.63 (1H, d, J = 4.8 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.3, 102.8, 114.5, 119.8, 120.8, 124.2, 126.3, 127.1, 128.3, 130.5, 133.3, 137.4, 139.0, 147.1, 148.4, 148.9. MS (EI): 218 (31), 231 (15), 232 (23), 233 (100), 234 (18), 312 (27), 314 (28). HRMS (EI): 312.0263 ($C_{16}H_{13}N_2Br$ [M]$^+$ requires 312.0262).

4.1.19. General procedure for the synthesis of 9a–d and 10

To a solution of the appropriate isocryptolepine (**3a-3e**) in dry DMF, *N*-bromosuccinimide (NBS) or *N*-chlorosuccinimide (NCS) was added and the solution heated at 150 °C overnight (unless otherwise stated). The reaction was then cooled, quenched with water and basified with 10% NaOH (aq). The precipitated obtained was collected and purified by flash column chromatography (eluting with DCM:EtOH:NH_3; 100:0:1 increasing to 100:4:1) or recrystallisation (from the solvent indicated) to give the respective isocryptolepine derivative **9a–d** or **10**.

4.1.20. 8-Bromoisocryptolepine (9a)

Prepared from **3a** (395 mg, 1.70 mmol) and NBS (344 mg, 1.93 mmol), 20 h. Recrystallisation solvent: EtOH; yellow crystalline solid; yield 74% (392 mg). Mp 257–258 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.25 (3H, s, NCH_3), 7.54 (1H, dd, J = 8.4, 1.8 Hz, H-9), 7.71–7.74 (2H, m, H-2 and H-10), 7.85 (1H, ddd, J = 8.7, 7.2, 1.5 Hz, H-3), 8.05 (1H, d, J = 9.0 Hz, H-4), 8.30 (1H, d, J = 2.4 Hz, H-7), 8.76 (1H, dd, J = 8.1, 1.5 Hz, H-1), 9.39 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.3 (NCH_3), 111.7 (C-8), 115.2 (C-6a), 117.5 (C-4), 120.0 (C-10), 121.0 (C-11b), 122.0 (C-7), 123.8 (C-1), 125.4 (C-2), 127.5 (C-6b), 127.6 (C-9), 129.4 (C-3), 135.4 (C-4a), 139.2 (C-6), 153.0 (C-10a and C-11a). MS (EI): 189 (17), 215 (16), 216 (20), 231 (21), 310 (100), 311 (22), 312 (100), 313 (19). HRMS (EI): 310.0110 ($C_{16}H_{11}N_2Br$ [M]$^+$ requires 310.0106). HPLC purity, 99.5%.

4.1.21. 8-Bromo-2-chloroisocryptolepine (9b)

Prepared from **3b** (116 mg, 0.44 mmol) and NBS (86.6 mg, 0.49 mmol), 2 h. Yellow crystalline solid; yield 77% (118 mg). Mp 265–266 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.22 (3H, s, NCH_3), 7.54 (1H, dd, J = 8.4, 2.1 Hz, H-9), 7.73 (1H, d, J = 8.4 Hz, H-10), 7.85 (1H, dd, J = 9.6, 2.7 Hz, H-3), 8.08 (1H, d, J = 9.6 Hz, H-4), 8.30 (H, d, J = 2.4 Hz, H-7), 8.66 (1H, d, J = 2.4 Hz, H-1), 9.36 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.4 (NCH_3), 112.1 (C-8), 115.5 (C-6a), 120.0 (C-4), 120.2 (C-10), 122.0 (C-11b) 122.1 (C-7), 122.4 (C-1), 127.4 (C-6b), 127.9 (C-9), 129.1 (C-3), 129.9 (C-2), 133.9 (C-4a), 139.3 (C-6), 151.8 (C-11a), 153.0 (C-10a). MS (EI):

188 (17), 215 (21), 310 (22), 312 (22), 344 (72), 346 (100), 347 (19), 348 (25). HRMS (EI): 343.9727 ($C_{16}H_{10}N_2ClBr$ $[M]^+$ requires 343.9716). HPLC purity, 98.3%.

4.1.22. 2,8-Dibromoisocryptolepine (9c)

Prepared from **3c** (175 mg, 0.56 mmol) and NBS (106 mg, 0.59 mmol), 20 h. Yellow crystalline solid; yield 71% (155 mg). Mp 324–326 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.29 (3H, s, CH_3), 7.55 (1H, dd, J = 8.8, 2.2 Hz, H-9), 7.74 (1H, d, J = 8.8 Hz, H-10), 8.00 (1H, dd, J = 8.8, 2.2 Hz, H-3), 8.05 (1H, d, J = 9.2 Hz, H-4), 8.33 (H, d, J = 2.0 Hz, H-7), 8.83 (1H, d, J = 2.0 Hz, H-1), 9.44 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.4 (NCH_3), 112.2 (C-8), 115.6 (C-6a), 118.2 (C-2), 120.3 (C-4 and C-10), 122.2 (C-7), 122.4 (C-11b), 125.6 (C-1), 127.4 (C-6b), 128.0 (C-9), 131.9 (C-3), 134.3 (C-4a), 139.6 (C-6), 151.8 (C-11a), 152.9 (C-10a). MS (EI): 215 (18), 309 (21), 311 (20), 388 (52), 390 (100), 391 (19), 392 (50). HRMS (EI): 387.9189 ($C_{16}H_{10}N_2Br_2$ $[M]^+$ requires 387.9211). HPLC purity, 96.5%.

4.1.23. 8-Bromo-3-chloroisocryptolepine (9d)

Prepared from **3d** (202 mg, 0.76 mmol) and NBS (149 mg, 0.84 mmol), 24 h. Recrystallisation solvent: MeOH and H_2O; yellow crystalline solid; yield 71% (187 mg). Mp 247–250 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.23 (3H, s, CH_3), 7.54 (1H, dd, J = 8.7, 2.1 Hz, H-9), 7.73 (1H, d, J = 8.4 Hz, H-10), 7.76 (1H, dd, J = 8.4, 1.8 Hz, H-2), 8.16 (1H, d, J = 1.8 Hz, H-4), 8.31 (H, d, J = 1.8 Hz, H-7), 8.74 (1H, d, J = 8.4 Hz, H-1), 9.40 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.4 (NCH_3), 112.0 (C-8), 115.7 (C-6a), 117.4 (C-4), 119.6 (C-11b), 120.2 (C-10), 122.2 (C-7), 125.6 (C-2), 125.7 (C-1), 127.4 (C-6b), 127.9 (C-9), 134.0 (C-3), 136.1 (C-4a), 139.6 (C-6), 152.4 (C-11a), 153.2 (C-10a). MS (FAB): 344 (18), 345 (80), 346 (39), 347 (100), 348 (25), 349 (26). HRMS (FAB): 344.9798 ($C_{16}H_{11}N_2ClBr$ $[M+H]^+$ requires 344.9794). HPLC purity, 97.6%.

4.1.24. 8-Bromo-9-methylisocryptolepine (9e)

Prepared from **3e** (160 mg, 0.65 mmol) and NBS (137 mg, 0.77 mmol), 20 h. Yellow crystalline solid; yield 80% (168 mg). Mp 266–267 °C. ^{13}C NMR (400 MHz, DMSO-d_6): δ 2.53 (3H, s, CH_3), 4.23 (3H, s, NCH_3), 7.70 (1H, t, J = 7.2 Hz, H-2), 7.75 (1H, s, H-10), 7.84 (1H, ddd, J = 8.4, 7.2, 1.4 Hz, H-3), 8.03 (1H, d, J = 8.8 Hz, H-4), 8.32 (1H, s, H-7), 8.74 (1H, dd, J = 8.0, 1.2 Hz, H-1), 9.32 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.2 (CH_3), 42.1 (NCH_3), 114.9 (C-8), 115.3 (C-6a), 117.4 (C-4), 120.1 (C-10), 121.0 (C-11b), 122.5 (C-7), 123.8 (C-1), 125.1 (C-2), 125.4 (C-6b), 129.2 (C-3), 133.1 (C-9), 135.4 (C-4a), 138.3 (C-6), 153.3 (C-11a), 154.1 (C-10a). MS (EI): 98 (54), 229 (21), 230 (21), 245 (54), 246 (16), 324 (100), 326 (94), 327 (17). HRMS (EI): 324.0255 ($C_{17}H_{13}N_2Br$ $[M]^+$ requires 324.0262). HPLC purity, 97.5%.

4.1.25. 8-Chloroisocryptolepine (10)

Prepared from **3a** (222 mg, 0.96 mmol) and NCS (146 mg, 1.09 mmol), 24 h. Recrystallisation solvent: EtOH; yellow crystalline solid; yield 41% (99 mg). Mp 257–259 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.25 (3H, s, CH_3), 7.40 (1H, dd, J = 9.0, 2.1 Hz, H-9), 7.72 (1H, t, J = 7.5 Hz, H-2), 7.77 (1H, d, J = 9.0 Hz, H-10), 7.85 (1H, ddd, J = 8.5, 7.0, 1.2 Hz, H-3), 8.05 (1H, d, J = 8.4 Hz, H-4), 8.16 (1H, d, J = 2.4 Hz, H-7), 8.75 (1H, dd, J = 7.8, 1.2 Hz, H-1), 9.38 (1H, s, H-6). ^{13}C NMR (50 MHz, DMSO-d_6): δ 42.6 (NCH_3), 114.8 (C-6a), 117.8 (C-4), 118.7 (C-10), 119.2 (C-7), 120.2 (C-11b), 123.8 (C-1), 124.3 (C-8), 125.4 (C-2), 125.9 (C-9), 126.2 (C-6b), 129.9 (C-3), 135.4 (C-4a), 140.0 (C-6), 150.5 (C-11a), 151.6 (C-10a). MS (EI): 266 (100), 267 (20), 268 (33). HRMS (EI): 266.0602 ($C_{16}H_{11}N_2Cl$ $[M]^+$ requires 266.0611). HPLC purity, 97.6%. The spectra data acquired was in agreement with that previously published.[25]

4.1.26. Conversion of 3a, 3d, 3e, 9a–9e and 10 to hydrochloride salts

Compounds were dissolved in a minimal amount of methanol, and concentrated HCl added dropwise. The volume of solvent was reduced *in vacuo*, the resultant precipitate collected by filtration and dried under high vacuum, before purity analysis by HPLC and biological evaluation.

4.2. Biological evaluation

4.2.1. In vitro activity against *P. falciparum*

The laboratory-adapted *Plasmodium falciparum* strains 3D7 and W2mef were cultured in RPMI 1640 HEPES (Sigma–Aldrich) supplemented with 92.6 mg/L L-glutamine (Sigma–Aldrich), 500 μg/L gentamicin, 50 mg/L L-hypoxanthine (Sigma–Aldrich) and 10% v/v pooled human plasma.[33,39,40] Cultures were incubated at 37 °C in a low oxygen atmosphere (3–9%). Stock solutions were prepared in 50% v/v DMSO and distilled water (**3a**, **3e**, **9a**, **9e** and **10**), 80% v/v DMSO and distilled water (**3d**, **9b**, **9c** and **9d**), or distilled water (CQ) and stored in the absence of light at 0 °C. Aliquots were freshly diluted to a working standard with RPMI and added in triplicate to 96-well plates (final DMSO concentration <0.05% per well and had no effect on parasite growth). Infected erythrocytes were added (final parasitemia 0.5% and 1.5% hematocrit) and parasite growth was measured using the [^{3}H]-hypoxanthine growth inhibition assay.[33,41] To the drug-parasite suspensions, [^{3}H]-hypoxanthine (Perkin Elmer) was added (final concentration of 0.5 μCi/well). The plates were incubated for 48 h and then subjected to a freeze-thaw process before harvesting onto 96-well glass-fibre filtermats using a Havaster 96 (Tomtec Incorporated). Filtermats were counted on a 1450 Microbeta Plus liquid scintillation counter (Wallac). Chloroquine diphosphate was used as a positive control for antimalarial activity and drug-free controls (uninfected and infected) were included in each test. The assay was performed a minimum of three separate times for each compound and IC_{50} values were determined by non-linear regression analysis of log-dose–response curves (Graphpad Prism 4.0).

4.2.2. In vitro cytotoxicity

3T3 cells (mouse embryonic fibroblasts) were cultured and maintained in RPMI 1640 (Invitrogen) supplemented with 2 mM L-alanyl-L-glutamine (GlutaMAX; Invitrogen), 100 U/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen) and 10% fetal calf serum. 24 h prior to testing, cells were added to 96-well plates pre-coated with 1% gelatine at 7500 cells per well (final well volume 100 μL) and incubated at 37 °C in a 5% CO_2 humidified atmosphere. Stock solutions were prepared as for the antimalarial evaluation. Aliquots were freshly diluted with RPMI to a working standard, serial dilutions conducted and 100 μL of each solution added in quadruplicate to 96-well plates. The potential DMSO effect was countered by including a vehicle control arm to the experiments. The plates were incubated for 48 h and chemosensitivity was assessed using the MTT assay.[42,43] The media was removed from each well and 100 μL of 1 mg/mL MTT (Sigma) in RPMI was added. Plates were incubated for 60 min at 37 °C, the supernatant was removed and 100 μL DMSO added to each well. The absorbance of each plate was measured using an automated plate reader (Bio-Rad Laboratories) at a wavelength of 595 nm. Isocryptolepine hydrochloride was used as a positive control for cytotoxicity and a drug-free control was included in each test. The assay was performed a minimum of three separate times for each compound and IC_{50} values were determined by non-linear regression analysis of log-dose–response curves, after correction for the DMSO effects (Graphpad Prism 4.0).

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References and notes

1. Lee, K. H. J. *Nat. Prod.* **2010**, *73*, 500.
2. Newman, D. J.; Cragg, G. M. J. *Nat. Prod.* **2007**, *70*, 461.
3. White, N. J. *Science* **2008**, *320*, 330.
4. Sofowora, A. *Medicinal Plants and Traditional Medicine in Africa*, 1st ed.; John Wiley and Sons: Chichester, UK, 1982. pp 221–223.
5. Boakye-Yiadom, K.; Heman-Ackah, S. M. J. *Pharm. Sci.* **1979**, *68*, 1510.
6. Cimanga, K.; Pieters, L.; Claeys, M.; Vanden Berghe, D.; Vlietinck, A. J. *Planta Med.* **1991**, *57*, A98.
7. Bierer, D. E.; Fort, D. M.; Mendez, C. D.; Luo, J.; Imbach, P. A.; Dubenko, L. G.; Jolad, S. D.; Gerber, R. E.; Litvak, J.; Lu, Q.; Zhang, P.; Reed, M. J.; Waldeck, N.; Bruening, R. C.; Noamesi, B. K.; Hector, R. F.; Carlson, T. J.; King, S. R. J. *Med. Chem.* **1998**, *41*, 894.
8. Rauwald, H. W.; Kober, M.; Mutschler, E.; Lambrecht, G. *Planta Med.* **1992**, *58*, 486.
9. Ablordeppey, S. Y.; Fan, P.; Li, S.; Clark, A. M.; Hufford, C. D. *Bioorg. Med. Chem.* **2002**, *10*, 1337.
10. Kirby, G. C.; Paine, A.; Warhurst, D. C.; Noamese, B. K.; Phillipson, J. D. *Phytother. Res.* **1995**, *9*, 359.
11. Grellier, P.; Ramiaramanana, L.; Millerioux, V.; Deharo, E.; Shrével, J.; Frappier, F.; Trigalo, F.; Bodo, B.; Pousset, J. L. *Phytother. Res.* **1996**, *10*, 317.
12. Bonjean, K.; De Pauw-Gillet, M. C.; Defresne, M. P.; Colson, P.; Houssier, C.; Dassonneville, L.; Bailly, C.; Greimers, R.; Wright, C.; Quetin-Leclercq, J.; Tits, M.; Angenot, L. *Biochemistry* **1998**, *37*, 5136.
13. Dassonneville, L.; Bonjean, K.; De Pauw-Gillet, M. C.; Colson, P.; Houssier, C.; Quetin-Leclercq, J.; Angenot, L.; Bailly, C. *Biochemistry* **1999**, *38*, 7719.
14. Dassonneville, L.; Lansiaux, A.; Wattelet, A.; Wattez, N.; Mahieu, C.; Van Miert, S.; Pieters, L.; Bailly, C. *Eur. J. Pharmacol.* **2000**, *409*, 9.
15. Arzel, E.; Rocca, P.; Grellier, P.; Labaeïd, M.; Frappier, F.; Guéritte, F.; Gaspard, C.; Marsais, F.; Godard, A.; Quéguiner, G. J. *Med. Chem.* **2001**, *44*, 949.
16. Lavrado, J.; Paulo, A.; Gut, J.; Rosenthal, P. J.; Moreira, R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1378.
17. Onyeibor, O.; Croft, S. L.; Dodson, H. I.; Feiz-Haddad, M.; Kendrick, H.; Millington, N. J.; Parapini, S.; Phillips, R. M.; Seville, S.; Shnyder, S. D.; Taramelli, D.; Wright, C. W. J. *Med. Chem.* **2005**, *48*, 2701.
18. Wright, C. W.; Addae-Kyereme, J.; Breen, A. G.; Brown, J. E.; Cox, M. F.; Croft, S. L.; Gökçek, Y.; Kendrick, H.; Phillips, R. M.; Pollet, P. L. J. *Med. Chem.* **2001**, *44*, 3187.
19. Van Miert, S.; Hostyn, S.; Maes, B. U. W.; Cimanga, K.; Brun, R.; Kaiser, M.; Mátyus, P.; Dommisse, R. A.; Lemière, G.; Vlietinck, A.; Pieters, L. J. *Nat. Prod.* **2005**, *68*, 674.
20. El Sayed, I.; Van der Veken, P.; Steert, K.; Dhooghe, L.; Hostyn, S.; Van Baelen, G.; Lemière, G.; Maes, B. U. W.; Cos, P.; Maes, L.; Joossens, J.; Haemers, A.; Pieters, L.; Augustyns, K. J. *Med. Chem.* **2009**, *52*, 2979.
21. Jonckers, T. H. M.; Van Miert, S.; Cimanga, K.; Bailly, C.; Colson, P.; De Pauw-Gillet, M. C.; Van den Heuvel, H.; Claeys, M.; Lemière, F.; Esmans, E. L.; Rozenski, J.; Quirijnen, L.; Maes, L.; Dommisse, R.; Lemière, G. L. F.; Vlietinck, A.; Pieters, L. J. *Med. Chem.* **2002**, *45*, 3497.
22. Van Miert, S.; Jonckers, T.; Cimanga, K.; Maes, L.; Maes, B.; Lemière, G. L.; Dommisse, R. A.; Vlietinck, A.; Pieters, L. *Exp. Parasitol.* **2004**, *108*, 163.
23. Dhanabal, T.; Sangeetha, R.; Mohan, P. S. *Tetrahedron* **2006**, *62*, 6258.
24. Meyers, C.; Rombouts, G.; Loones, K. T. J.; Coelho, A.; Maes, B. U. W. *Adv. Synth. Catal.* **2008**, *350*, 465.
25. Kumar, D.; Kumar, N. M.; Rao, V. S. *Chem. Lett.* **2009**, *38*, 156.
26. Molina, A.; Vaquero, J. J.; Garcia-Navio, J. L.; Alvarez-Builla, J.; De Pascual-Teresa, B.; Gago, F.; Rodrigo, M. M.; Ballesteros, M. J. *Org. Chem.* **1996**, *61*, 5587.
27. Kumar, R. N.; Suresh, T.; Mohan, P. S. *Tetrahedron Lett.* **2002**, *43*, 3327.
28. Murray, P. E.; Mills, K.; Joule, J. A. J. *Chem. Res-M* **1998**, 1435.
29. Pousset, J. L.; Martin, M. T.; Jossang, A.; Bodo, B. *Phytochemistry* **1995**, *39*, 735.
30. Sharaf, M. H. M.; Schiff, P. L. *Jr.; Tackie, A. N.; Phoebe, C. H., Jr.; Martin, G. E. J. Heterocycl. Chem.* **1996**, *33*, 239.
31. Jonckers, T. H. M.; Maes, B. U. W.; Lemière, G. L. F.; Rombouts, G.; Pieters, L.; Haemers, A.; Dommisse, R. A. *Synlett* **2003**, 615.
32. Barraja, P.; Diana, P.; Lauria, A.; Passannanti, A.; Almerico, A. M.; Minnei, C.; Longu, S.; Congiu, D.; Musiu, C.; La Collo, P. *Bioorg. Med. Chem.* **1999**, *7*, 1591.
33. Wong, R. P. M.; Salman, S.; Ilett, K. F.; Siba, P. M.; Mueller, I.; Davis, T. M. E. *Antimicrob. Agents Chemother.* **2011**, *55*, 1194.
34. Lin, A. J.; Loo, T. L. J. *Med. Chem.* **1978**, 21.
35. Riegel, B.; Lappin, G. R.; Adelson, B. H.; Jackson, R. I.; Albisetti, C. J., Jr.; Dodson, R. M.; Baker, R. H. J. *Am. Chem. Soc.* **1946**, 68.
36. Margolis, B. J.; Long, K. A.; Laird, D. L. T.; Ruble, J. C.; Pulley, S. R. J. *Org. Chem.* **2007**, 72.
37. Kermack, W. O.; Storey, N. E. J. *Chem. Soc.* **1950**, 607.
38. Miki, Y.; Kuromatsu, M.; Miyatake, H.; Hamamoto, H. *Tetrahedron Lett.* **2007**, *48*, 9093.
39. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
40. Scheibel, L. W.; Ashton, S. H.; Trager, W. *Exp. Parasitol.* **1979**, *47*, 410.
41. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
42. Mosmann, T. J. *Immunol. Methods* **1983**, *65*, 55.
43. Fox, S. A. *Kusmaity; Loh, S. S. W.; Dharmarajan, A. M.; Garlepp, M. J. Biochem. Biophys. Res. Commun.* **2005**, *337*, 983.