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Cryptolepine analogues containing basic aminoalkyl side-chains at C-11: Synthesis, antiplasmodial activity, and cytotoxicity

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Abstract—A series of cryptolepine derivatives has been synthesized through the incorporation of short basic side-chains in the C-11 position of the 10*H*-indolo[3,2-*b*]quinoline scaffold. Their antiplasmodial activity was evaluated in vitro against the chloroquine resistant *Plasmodium falciparum* W2 strain, showing IC_{50} values between 22 and 184 nM, while their cytotoxicity was assessed using HUVEC cells, revealing three compounds with a selectivity ratio higher than 10. The most selective of these derivatives, **4d**, with a selectivity ratio of 46, was also the least cytotoxic of the series.

Malaria is a major infectious disease in tropical and subtropical regions, caused by parasites of *Plasmodium* species, the most lethal of which is *P. falciparum*.[1] Chloroquine (CQ) **1** (Fig. 1) has been the mainstay of malaria chemotherapy for 50 years, but its use is now limited by the spread of CQ-resistant *P. falciparum* strains.[2] Drug resistance results from the loss of activity on erythrocyte-stage parasites, which are responsible for the clinical symptoms of the disease. Thus, it is urgent to find new blood schizontocides to combat the spread of multi-drug resistant *P. falciparum* strains.[3]

Cryptolepine (5-methyl-10*H*-indolo[3,2-*b*]quinoline) **2** (Fig. 1) is the major alkaloid from the roots of the west African climbing shrub *Cryptolepis sanguinolenta*, an herbal drug used in traditional medicine for the treatment of malaria.[4,5] Cryptolepine and its hydrochloride, **3** (Fig. 1), display potent in vitro antiplasmodial activity[6] but also present cytotoxic properties that preclude their clinical use.[7] The cytotoxicity can be ascribed to the ability of cryptolepine to intercalate into DNA and inhibit topoisomerase II as well as DNA synthesis.[8,9]

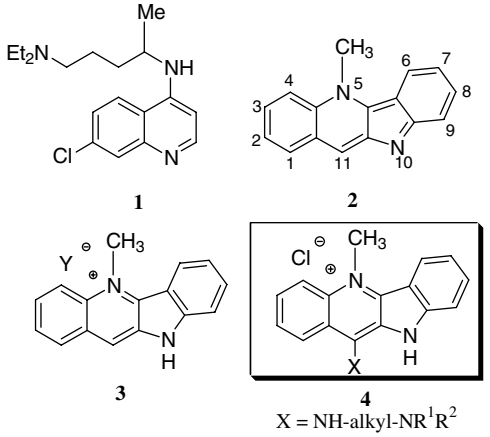

Figure 1. Structures of chloroquine, **1**, cryptolepine, **2**, cryptolepine hydrochloride (**3**, Y = Cl), cryptolepine triflate (**3**, Y = OTf), and cryptolepine derivatives with basic side-chains at C-11, **4**.

However, studies on the possible mode of antimalarial action suggest that cryptolepine is able to inhibit hemozoin formation (i.e., the heme detoxification process),[10,11] which is also the mechanism reported for 4-aminoquinolines (e.g., **1** and amodiaquine).[12,13] A basic amino side-chain is a major chemical feature required for 4-aminoquinoline accumulation in the acid (pH 5.5) food vacuole of the parasite, the site of the host hemoglobin digestion.[14] Based on this structural requirement, we designed a novel group of cryptolepine

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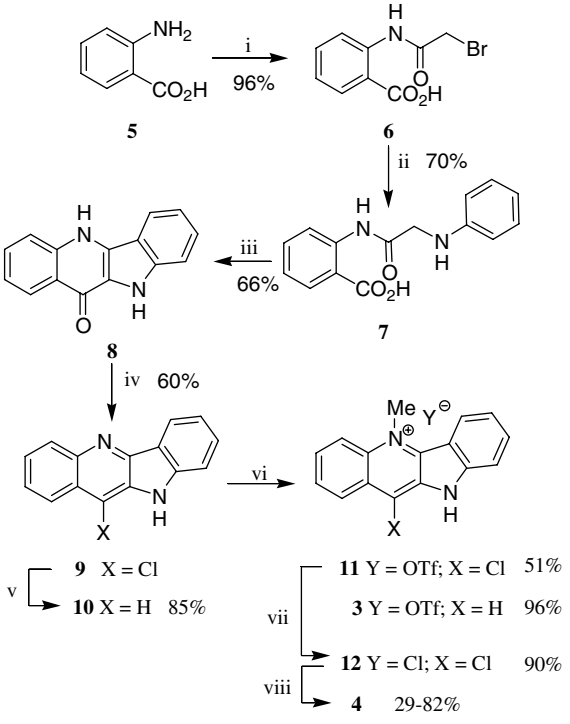
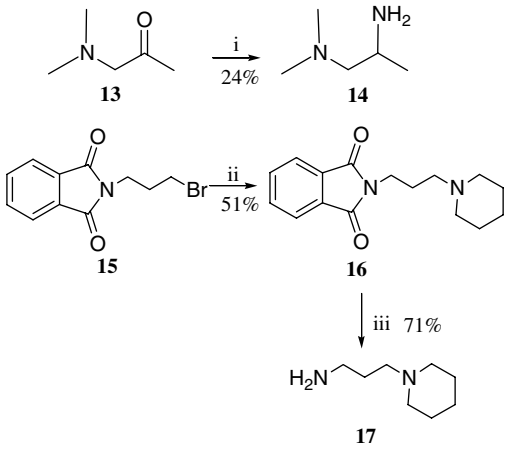
analogues, **4** (Fig. 1), that incorporate an alkyldiamino side-chain at C-11, aiming to increase accumulation in the parasite food vacuole. We now report the synthesis and evaluation of the antiplasmodial activity and cytotoxicity of compounds **4**.

Cryptolepine hydrochloride, **3** (Y = Cl), and its C-11 alkyldiamine derivatives, **4**, were synthesized via 11-chloroquindoline intermediate, **9**,[15] according to the route depicted in Scheme 1. Anthranilic acid, **5**, was treated with bromoacetyl bromide to afford the bromoacetyl derivative **6**, which was treated with aniline to give compound **7**. Acid-catalyzed cyclization of **7** with polyphosphoric acid (PPA) gave the quindolone **8**, which, by reaction with $POCl_3$, gave the corresponding 11-chloroquindoline **9**.[15] Hydrogenation of **9** with 10% Pd-C at 60 psi provided the quindoline **10**.[16] Reaction of **9** and **10** with methyl triflate formed the corresponding triflate **11** and cryptolepine triflate (**3**, Y = OTf).[17] Initial attempts to prepare compounds **4** involved the reaction of triflate **11** with alkyldiamines, but the final product contained always triflate and chloride anions. Alternatively, compound **11** was first treated with sodium carbonate and then with HCl to give the key intermediate 11-chlorocryptolepine hydrochloride, **12**.[17] Finally, cryptolepine derivatives containing an alkyldiamine side-chain at C-11, **4**, were obtained by reacting **12** with the appropriate amine, in 29–82% yield.[18] Most of the alkyldiamines used in this final step were purchased and used without further purification. However, for **4c** and **4i**, the corresponding amines required for the substitution reaction with **12** were synthesized according



Scheme 1. Synthesis of cryptolepine derivatives **4a**–**j**. Reagents and conditions: (i) Bromoacetyl bromide, DMF/Dioxane, rt; (ii) aniline, DMF, 120 °C; (iii) PPA, 130 °C; (iv) $POCl_3$, 120 °C; (v) H_2 Pd-C 10%, NaOAc, AcOH, 60 psi; (vi) **9** or **10**, methyl triflate, anhydrous toluene, rt; (vii) (a) 5% Na_2CO_3; (b) HCl–Et_2O; (viii) RNH_2, AcOEt, reflux.



Scheme 2. Synthesis of alkyldiamines required for compounds **4c** and **4i**. Reagents and conditions: (i) NH_4OAc, $NaBH_3CN$, anhydrous $MgSO_4$, dry methanol, reflux; (ii) piperidine, TEA, CH_2Cl_2, reflux; (iii) hydrazine, EtOH, reflux.

to the route depicted in Scheme 2. Thus, N^1,N^1-dimethylpropane-1,2-diamine, **14**, used for synthesis of **4c**, was prepared by reductive amination of *N*,*N*-dimethylaminopropanone, **13**, with ammonium acetate and $NaBH_3CN$. 3-Piperidin-1-ylpropan-1-amine, **17**, used for synthesis of **4i**, was prepared using a Gabriel synthesis starting with the phthalimide **15** (Scheme 2).

Compounds **4a**–**j** were tested in vitro against CQ-resistant W2 *P. falciparum* strain.[19] The corresponding IC_{50} values, together with their cytotoxicity determined using human umbilical vein endothelial cells (HUVEC), are presented in Table 1. As it appears from the data, IC_{50} values for **4a**–**j** range from 22 to 184 nM, which represent a significant improvement in antiplasmodial activity over the cryptolepine, **3**, (755 nM) and chloroquine (249 nM).[20] Inspection of the data in Table 1 allows the following conclusions to be drawn.

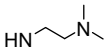
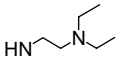
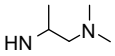
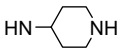
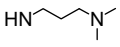
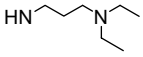
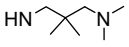
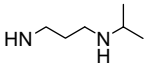
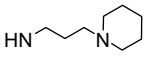
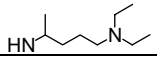
First, compounds containing side-chains with three carbon atoms (e.g., **4e**, **f**, **h**, **i**) generally present better antiplasmodial activity than those with two carbon atoms (e.g., **4a**–**c**) or four carbon atoms (**4j**).

Second, branched side-chains reduce significantly the activity independently of side-chain length (two carbon atoms: **4c** vs **4a**, **b**; three carbons atoms: **4g** vs **4e**, **f**, **h**, **i**); the exception to this trend is observed for the analogue with the less flexible piperidine side-chain, that is, **4d**.

Third, terminal secondary and tertiary alkylamine groups do not affect antiplasmodial activity as shown by the IC_{50} values for **4h** when compared with those of its *N*,*N*-dimethyl and *N*,*N*-diethyl counterparts, **4e** and **4f**, respectively.

Fourth, the cytotoxicity/antiplasmodial ratio for most of the compounds **4** is >1, indicating some selectivity against the parasite. Compound **4d** emerged as the most selective (selectivity ratio of ca. 46) as well as the less cytotoxic (ca. 2 times less cytotoxic than cryptolepine) of the present series. In contrast, compounds with

Table 1. Antiplasmodial activity against *P. falciparum* W2 strain and cytotoxicity in HUVEC cells of cryptolepine analogues **4**

Compound	X	Antiplasmodial IC_{50} [a,b] W2[b]	Cytotoxicity IC_{50}[a,c] HUVEC	Cyto./antipl. ratio
CQ,**1**		249[20]	—	
3	H	755 (±1)	1180 (±1)	1.6
4a		50 (±8)	161 (±1)	3.2
4b		82 (±23)	674 (±1)	8.2
4c		132 (±7)	617 (±1)	4.7
4d		44 (±1)	2042 (±1)	46.4
4e		30 (±6)	155 (±2)	5.2
4f		32 (±5)	114 (±1)	3.6
4g		184 (±21)	82 (±1)	0.5
4h		22 (±2)	794 (±1)	36.1
4i		36 (±2)	1161 (±1)	32.3
4j		127 (±2)	35 (±1)	0.3

IC_{50} values are in nM.
[a] IC_{50} values are means of at least three determinations.
[b] Ref. 20.
[c] Ref. 23.

branched side-chains seem to be particularly cytotoxic in the HUVEC cell line (i.e., **4g** and **4j** are 14 and 34 times more cytotoxic than cryptolepine), with selectivity ratios of ca. 0.4. Worthy of note, the most cytotoxic compound, **4j**, contains the chloroquine alkylamino substituent. Cytoxicity displayed by compounds **4** may reflect electrostatic or hydrogen-bonding interactions between the amine side-chain and the phosphate backbone of DNA.[21,22]

In conclusion, a number of cryptolepine analogues, **4**, containing an alkyldiamine side-chain at C-11 have been synthesized using a single key intermediate, 11-chlorocryptolepine, **12**. Six of these cryptolepine analogues display potent antiplasmodial activity against the *P. falciparum* W2 CQ-resistant strain, with IC_{50} values ranging from 22 to 82 nM. The highest cytotoxicity/antiplasmodial ratio was observed for the piperidine derivative **4d**. These results indicate that introducing an alkyldiamino side-chain at position C-11 of cryptolepine is a promising approach for further optimization against drug-resistant malaria parasites.

Acknowledgments

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18. 5-Methyl-11-(piperidin-4-ylamino)quindolinium hydrochloride (**4d**). To a suspension of **12** (50.0 mg, 0.165 mmol, 1 equiv) in AcOEt (5 mL) was added commercial piperidin-4-amine (29.7 mg, 0.296 mmol, 31.1 μL, 1.8 equiv). Reaction mixture was refluxed for 24 h and after this period, the formed precipitate was collected, washed with diethyl ether, and dried, to give **4d**, 66% (40.3 mg) yield, as an orange solid, mp 325–327 °C. ^{1}H NMR (400 MHz, DMSO) δ_H (ppm) 8.66 (d, J = 8.3 Hz, 1H), 8.56 (d, J = 8.6 Hz, 1H), 8.29 (d, J = 6.2 Hz, 1H), 8.09 (dd, J = 8.6, 5.6 Hz, 1H), 7.97 (d, J = 6.3 Hz, 1H), 7.84 (dd, J = 6.2, 5.6 Hz, 1H), 7.81 (dd, J = 6.3, 5.6 Hz, 1H) 7.44 (dd, J = 8.3, 5.6 Hz, 1H), 4.80 (s, 3H), 4.14 (d, J = 12.8 Hz, 2H), 3.85 (t, J = 11.6 Hz, 2H), 3.55–3.45 (m, 1H), 2.28 (t, J = 11.6 Hz, 2H), 2.10–1.97 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ_C (ppm) 151.71; 149.09; 142.53; 137.11; 136.92; 130.89; 130.32; 129.94; 126.08; 124.86; 123.15; 119.41; 119.00; 55.70; 52.11; 44.08; 35.22. Anal. (C, H, N): Calcd for $C_{21}H_{23}ClN_4{\cdot}2HCl{\cdot}0.4H_2O$: C, 56.42; H, 5.82; N, 12.53; Found: C, 56.52 H, 6.01 N, 13.80. ESI-TOF/MS (*m*/*z*): Calcd for $C_{21}H_{23}N_4^+$: 331.1923; Found: 331.1917 Yields for remaining compounds: **4a** 62%; **4b** 35%; **4c** 29%; **4e** 34%; **4f** 82%; **4g** 78%; **4h** 56%; **4i** 38% and **4j** 30%.
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23. The cytotoxicity evaluation of the series of compounds studied was made using human umbilical vein endothelial cells (HUVEC). The degree of cell injury was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, in which cell viability is assessed by mitochondrial-dependent reduction of MTT to formazan. IC_{50} values were determined from graphics of degree of cell injury over inhibitor concentrations with GraphPad Prism software. Experimental details are provided as Supporting Information.