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Enantiopure Indolizinoindolones with in vitro Activity against Blood- and Liver-Stage Malaria Parasites

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Dedicated to Prof. Ana M. Lobo on the occasion of her 70th birthday.

Malaria continues to be a major cause of morbidity and mortality to this day, and resistance to drugs like chloroquine has led to an urgent need to discover novel chemical entities aimed at new targets. Here, we report the discovery of a novel class of potential antimalarial compounds containing an indolizinoindolone scaffold. These novel enantiopure indolizinoindolones were synthesized, in good-to-excellent yields and excellent diastereoselectivities, by cyclocondensation reaction of (*S*)- or (*R*)-tryptophanol and 2-acyl benzoic acids, followed by intramolecular α-amidoalkylation. Interestingly, we were able to synthesize for the first time 7,13b-*cis* indolizinoindolones in a two-step route. The novel compounds showed promising activity against erythrocytic stages of the human malaria parasite, *Plasmodium falciparum*, and liver stages of the rodent parasite *Plasmodium berghei*. In particular, an (*S*)-tryptophanol-derived isoindolinone was identified as a promising starting scaffold to search for novel antimalarials, combining excellent activity against both stages of the parasite's life cycle with low cytotoxicity and excellent metabolic and chemical stability in vitro.

Introduction

Malaria is a major cause of morbidity and mortality in many regions of the world. The World Health Organization (WHO) estimated a total of 198 million cases of malaria, which resulted in 584000 deaths in 2013. African populations, particularly children younger than five years old, are the most affected by this disease.[1]

Human malaria is caused by five species of *Plasmodium* parasites, with *P. falciparum* and *P. vivax* leading to most of the observed disease and *P. falciparum* to most deaths.[2] Unfortunately, due to their ability to rapidly develop drug resistance, *Plasmodium* parasites continue to be a major challenge in drug development.[3] Currently, the WHO recommends the use of artemisinin-based combination therapies for treating uncomplicated *P. falciparum* malaria (World Malaria Report 2014, WHO, Geneva).[4] However, resistance to artemisinins is emerging in Southeast Asia.[5] The resistance of malaria parasites to available drugs, along with the complexity of the *Plasmodium* life cycle, are among the main reasons why malaria remains such a burden to humanity. As a consequence, new drugs for the treatment and prevention of malaria are urgently needed.

Plasmodium parasites enter their mammalian hosts as sporozoites, injected through the bite of female *Anopheles* mosquitoes. After infection, malaria parasites invade and develop within liver cells. The liver phase precedes the infection of red blood cells which is responsible for the symptomatic phase of the disease.[6] Most drug discovery and development is focused on the asexual erythrocytic stages of *Plasmodium* infection, which, in the case of *P. falciparum*, can be maintained and propagated in culture. Conversely, the liver stage occurs only once in each infective cycle, and its study requires the isolation of parasites from the salivary glands of infected mosquitoes.[7] While treating malaria requires the elimination of blood-stage parasites, the parasite liver stage presents a promising target for malaria chemoprophylaxis.[8] *P. vivax* and *P. ovale*, but not *P. falciparum*, also form liver hypnozoites, dormant parasite forms that are insensitive to most available drugs, and may lead to relapses of clinical illness after treatment. Drugs acting against hypnozoites are required to achieve radical cure of *P. vivax* and *P. ovale* infections. Considering potential benefits for chemoprophylaxis and treatment, it is important to identify

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new chemotypes acting against both liver and erythrocytic stages of malaria parasites.

Indole-based compounds are known to possess a wide range of biological activities,[9] including antimalarial activity.[10] In particular, the spiroindolone KAE 609 (currently in phase I clinical trials as an antimalarial) is active against *P. falciparum*,[11] and several indole alkaloids (e.g. cryptolepine, and dihydrousambarensine) have shown potent antimalarial activity.[12]

Moreover, synthetic indoloisoquinolines **1** (Figure 1), synthesized from enantiopure tryptophanol, were recently reported to have low micromolar activity against a chloroquine-resistant *P. falciparum* strain.[13] Also, (*R*)-phenylalaninol-derived bicyclic lactams **2** (Figure 1) were recently described to inhibit different strains of *P. falciparum* with no cytotoxicity against mammalian cells.[14] However, there are no reports of similar compounds acting against both liver and erythrocytic stages of malaria parasites.

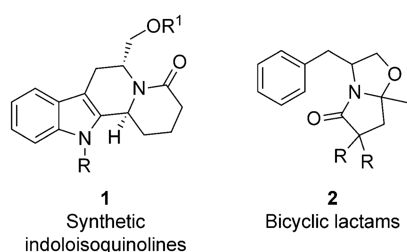

Figure 1. Structures of indoloisoquinolines and bicyclic lactams with antimalarial activity.

Here, we describe the design, synthesis, and structure–activity relationship study of enantiopure benzoindolizinoindolones (Figure 2) as inhibitors of *P. falciparum* erythrocytic-stage and *P. berghei* liver-stage parasites. The purpose of this study was to assess the effect of the isoindolinone and indole moieties merged in the same molecule on the antiplasmodial activity. We now report that benzoindolizinoindolones combine good activity against *Plasmodium* erythrocytic and liver stages, with low cytotoxicity and high metabolic stability.

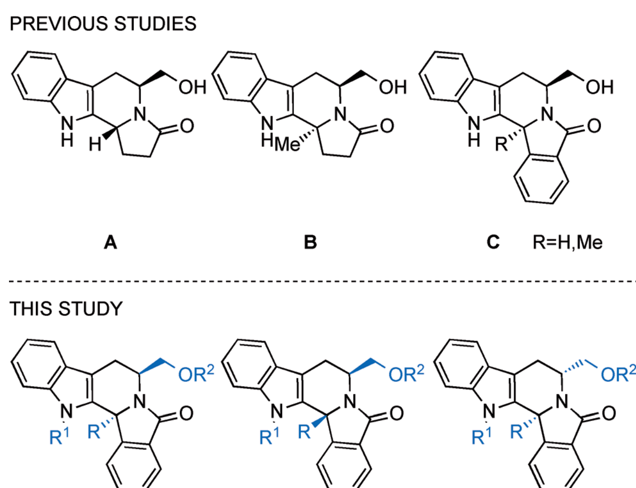

Figure 2. Structures of the enantiopure indolizinoindolones reported previously and discussed in this study.

Results and Discussion

Chemistry

The indolizinoindolones were prepared by an enantioselective two-step route that involved: 1) the stereoselective cyclocondensation of a racemic keto-acid with enantiopure tryptophanol and 2) a subsequent stereocontrolled cyclization on the aromatic ring taking advantage of the masked *N*-acyl iminium ion present in the resulting tricyclic compounds. The aminoalcohol used as the chiral inductor in the cyclocondensation reaction not only constitutes the source of chirality but is also used to assemble the final target polycyclic products.[15]

In particular, we synthesized a series of tryptophanol-derived oxazoloisoindolinones, which after Pictet–Spengler cyclization led to the enantiopure indolizinoindolones. This approach has already proved valuable for the synthesis of complex indole alkaloids in which the Pictet–Spengler cyclization is a key step. Specifically, enantiopure indolizinoindolones **A** and **B** have been prepared by Allin and co-workers in 43–55% yields (Figure 2).[16a,b] However, there is only a report of the application of this methodology to the asymmetric synthesis of benzoindolizinoindolones. This approach uses (*S*)-tryptophanol and keto-carboxylic acids in microwave-irradiation conditions, leading directly to *trans*-diastereoisomer benzoindolizinoindolones **C** (Figure 2).[16c]

In this work, the enantiopure tryptophanol-derived oxazoloisoindolinones were synthesized, in good chemical yields and excellent stereoselectivity, using the same reaction conditions reported before using phenylalaninol as the chiral inductor.[15b] In particular, cyclocondensation reaction of (*S*)- or (*R*)-tryptophanol with different oxo-acids, led to one of the two possible enantiopure stereoisomeric oxazoloisoindolinone products. As in the phenylalaninol-derived lactams, the relative stereochemistry of the oxazolidine moiety in the tryptophanol-derived oxazoloisoindolinones was found to be *cis*. A subsequent intramolecular aminoalkylation on the indole 2-position, taking advantage of the masked *N*-acyliminium moiety, led to the target indolizinoindolones in good yields.

We started by synthesizing compounds **3a**–**b** (Scheme 1), which were prepared in yields of 80–84% by refluxing a toluene solution of (*S*)-tryptophanol and the appropriate 2-acyl benzoic acid derivative, under Dean–Stark conditions. Bearing in mind the importance of the absolute stereochemical configuration of biologically active compounds, compounds **4a**–**b** (Scheme 1) were synthesized starting from (*R*)-tryptophanol. Using the same reaction conditions, compound **4a** (enantiomer of compound **3a**) was prepared in 76% yield and compound **4b** (enantiomer of compound **3b**) was obtained in 75% yield. The absolute configuration of the stereogenic center of the products generated in this step was unambiguously established by X-ray crystallographic analysis of lactam **3a** (Figure 3) and by comparing the carbon NMR chemical shifts with the corresponding phenylalaninol derivatives.[15b]

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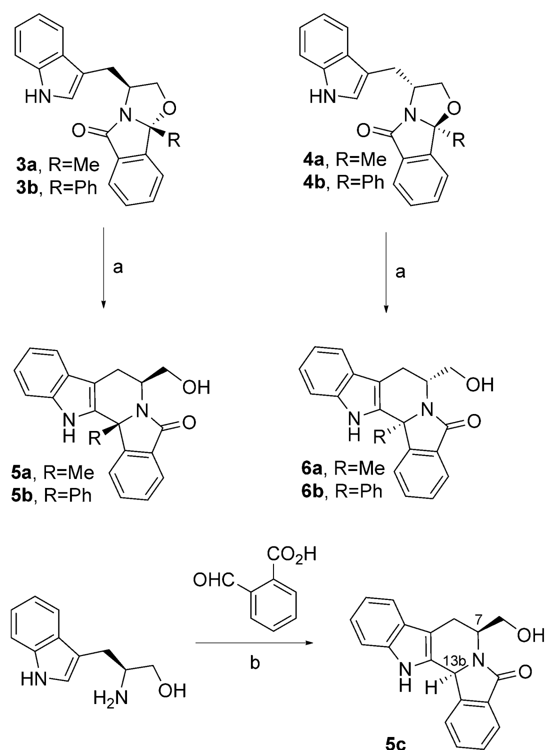


Scheme 1. Synthesis of enantiopure benzoindolizinoindolones **5a**–**c** and **6a**–**b**. *Reagents and conditions*: a) CH_2Cl_2, $BF_3{\cdot}OEt_2$, RT, 3 h, **5a**: 64%, **5b**: 63%, **5c**: 42%, **6a**: 65%, **6b**: 75%; b) toluene, reflux, 16 h, 42%.

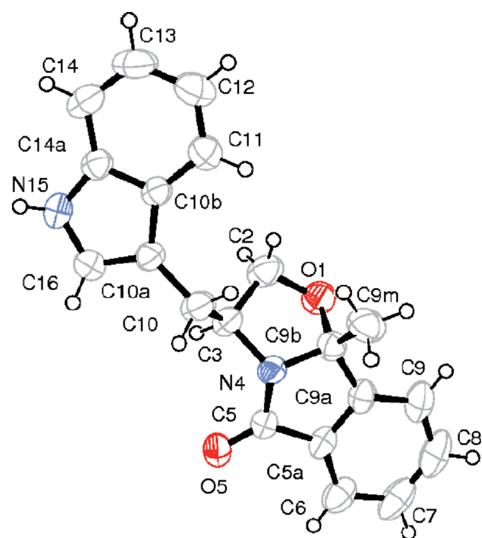


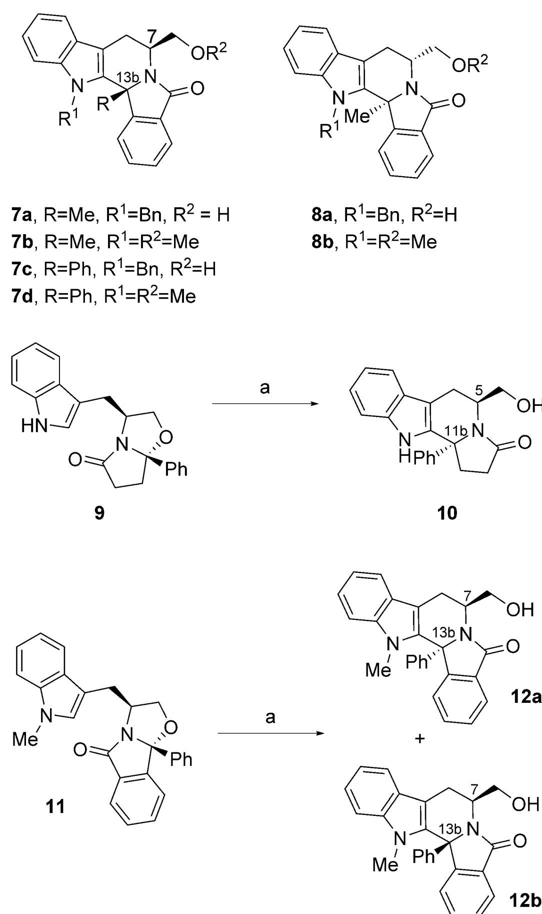
Figure 3. X-ray crystallographic structure of lactam **3a**. Crystallographic information file (CIF) data can be found in the Supporting Information.

Cyclization of compounds **3a**–**b** and **4a**–**b** by intramolecular α-amidoalkylation on the indole 2-position took place stereoselectively by treatment with $BF_3{\cdot}OEt_2$ to give the 7,13b-*cis* indole derivatives **5a**–**b** and **6a**–**b**, as single stereoisomers detectable by NMR spectroscopy.

Cyclocondensation of (*S*)-tryptophanol with 2-formylbenzoic acid led directly to 7,13b-*trans* indole derivative **5c** (Scheme 1). This result is consistent with that reported by Jida et al. using microwave conditions for the cyclocondensation of (*S*)-tryptophanol with 2-formylbenzoic acid.[16c]

Based on the structure–activity relationship (SAR) study performed by Allin for indoloisoquinolines,[13] we decided to study the effect on activity of hydroxymethyl O-protection and indole N-protection with a methyl or benzyl group. Compounds **7a**, **7c**, and **8a** were obtained after reaction of **5a**, **5b**, and **6a**, respectively, with benzyl bromide in the presence of sodium hydride. N,O-Dimethylation of compounds **5a**, **5b**, and **6a** led to compounds **7b**, **7d**, and **8b** (Scheme 2).

A stereoselective cyclocondensation also occurred starting from 3-benzoylpropionic acid. Compound **9** was obtained, in 90% yield, after cyclocondensation reaction of (*S*)-tryptophanol and 3-benzoylpropionic acid. Treatment of compound **9** with $BF_3{\cdot}OEt_2$ led to *trans*-diastereoisomer indolizinoindolone **10** in 77% yield (Scheme 2) as a single diastereoisomer (detectable by NMR spectroscopy). Interestingly, the stereochemistry at C-5 was opposite to that observed for compound **5b** under the same reaction conditions. This result seems to suggest that the presence of the aromatic ring in the isoindolinone moiety (compound **5b**) can eventually act as an element of stereocontrol during the intramolecular cyclization reaction.



Scheme 2. Indolizinoindolones **7a**–**d** and **8a**–**b**, and synthesis of indole derivatives **10**, **12a**, and **12b**. *Reagents and conditions*: a) CH_2Cl_2, $BF_3{\cdot}OEt_2$, RT, 2–3 h, **10**: 77%, **12a**: 58% **12b**: 24%.

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In order to obtain *N*-methyl indole derivatives with the hydroxyl group unprotected, we synthesized compound **11** starting from compound **3b**. Interestingly, treatment of compound **11** with $BF_3 \cdot OEt_2$, led to the formation of the two C-13b epimeric indole derivatives (**12a** and **12b**) in a ratio of 2.5:1 (Scheme 2).

The configuration of the new stereogenic centers formed during the Pictet–Spengler cyclization reaction was determined using ^{13}C NMR spectroscopy and X-ray crystallography. The carbon chemical shift of C-7 appears around 50-52 ppm for compounds **5a**, **5b**, and **12b**, diastereoisomers with *cis* substituents at positions C-7 (hydroxymethyl group) and C-13b (R-group). When the R and the hydroxymethyl groups are *trans*, carbons C-7 (for compounds **5c**, and **12a**) and C-5 (for compound **10**) are less shielded and appear at higher ppm values (above 54.5 ppm) than those of the *cis* isomers. The absolute configuration of the stereogenic center generated in this step was unambiguously established by X-ray crystallographic analysis of compound **12b** (Figure 4).

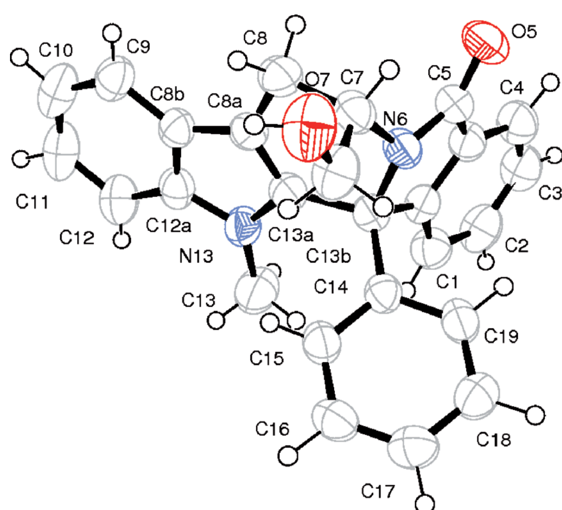

Figure 4. X-ray crystallographic structure of compound **12b**. Crystallographic information file (CIF) data can be found in the Supporting Information.

Table 1. Antiplasmodial activities and cytotoxicity against Huh-7 human hepatoma cells of compounds **5–8**, **10**, and **12**.

Compound	IC_{50} [μM] Blood stage[a,c]	IC_{50} [μM] Liver stage[b,c,d]	CC_{50} [μM] Huh-7[c,d]
5a	1.6±0.02	6.5±1.1	>200
5b	1.1±0.2	ND	ND
5c	>10	ND	ND
6a	>10	ND	ND
6b	4.2±0.04	3.9±0.4	>200
7a	6.0±0.8	5.8±0.08	88.1±18.2
7b	1.5±0.3	4.7±0.5	82.6±12.0
7c	2.7±0.1	6.9±1.9	>200
7d	2.3±0.09	8.3±1.6	>200
8a	8.4±2.3	ND	ND
8b	2.5±0.6	ND	ND
10	>10	ND	ND
12a	2.4±0.1	2.2±0.4	>200
12b	1.2±0.1	0.6±0.07	76.2±18.4
Primaquine	ND	9.5±2.3	ND
Chloroquine	0.14	ND	ND

[a] *Plasmodium falciparum* W2. [b] *Plasmodium berghei*. [c] Data represent the mean±SD of *n*=3 independent experiments performed in triplicate. [d] ND=not done.

Blood and liver schizontocidal activities and cytotoxicity

To evaluate the potential of the synthesized compounds as blood-stage antimalarials, they were screened against the chloroquine-resistant W2 strain of *P. falciparum* (Table 1). Compounds **5a–b**, **6b**, **7a–d**, **8a–b**, and **12a–b** exhibited IC_{50} values ranging from 1 to 8 μM. In particular, compounds **5a–b** with R=methyl and R=phenyl, respectively, had good antiplasmodial activity, with IC_{50} values ranging from 1 to 2 μM. Also, substitution of the methyl or phenyl group at position C-13b by a hydrogen, with opposite stereochemistry, led to loss of activity (e.g. compounds **5a** and **5b**, versus the counterpart **5c**). Protection of the indole nitrogen atom with a benzyl group had a small impact on activity (e.g. compounds **5a** and **5b**, versus the counterparts **7a** and **7c**, respectively). Moreover, the N,O-dimethylation of compounds **5a** and **5b** did not improve their activity (e.g. compounds **7b** and **7d**). In general, compounds **5** and **7**, derived from (*S*)-tryptophanol, were more active than the corresponding enantiomers **6** and **8** (derived from (*R*)-tryptophanol). The isoindolinone moiety seems to have an important role for activity. In fact, the substitution of the isoindolinone moiety for a pyrrolidone group resulted in loss of activity (compound **10**, versus the pyrrolidone counterpart **5b**). Finally, the results show that the methylation of the hydroxy group and the stereochemistry of position C-13b do not affect activity (compounds **7d**, versus the counterparts **12a** and **12b**).

The compounds were also evaluated for their activity against liver stages of the rodent parasite *P. berghei*. Most compounds significantly decreased the parasite load in Huh-7 human hepatoma cells when compared to untreated controls (Figure 5). Only compounds **5c** and **6a** were less active than primaquine. Except for compound **6b**, the compounds derived from (*S*)-tryptophanol were more active than their corresponding enantiomers, derived from (*R*)-tryptophanol. The IC_{50} values were determined for the most active compounds (**5a**, **6b**, **7a–d**, **12a–b**). All compounds presented an IC_{50} lower than 7 μM (Table 1). When R was a methyl group, protection of the nitrogen atom with a methyl or benzyl group had a small impact on activity (e.g. compounds **7a–d** versus compound **5a**). However, the stereochemistry of position C-13b had some effect on activity. In particular, compound **12b** was nearly 4 times more active than its diastereoisomer **12a**.

The in vitro cytotoxicity of the active compounds was then evaluated using Huh-7 cells. Remarkably, most of the compounds were not cytotoxic at concentrations up to 200 μM (Table 1). Only, compounds **7a–b** and **12b** led to cytotoxicity at concentrations between 76 and 88 μM, a result that indicates very low toxicity.

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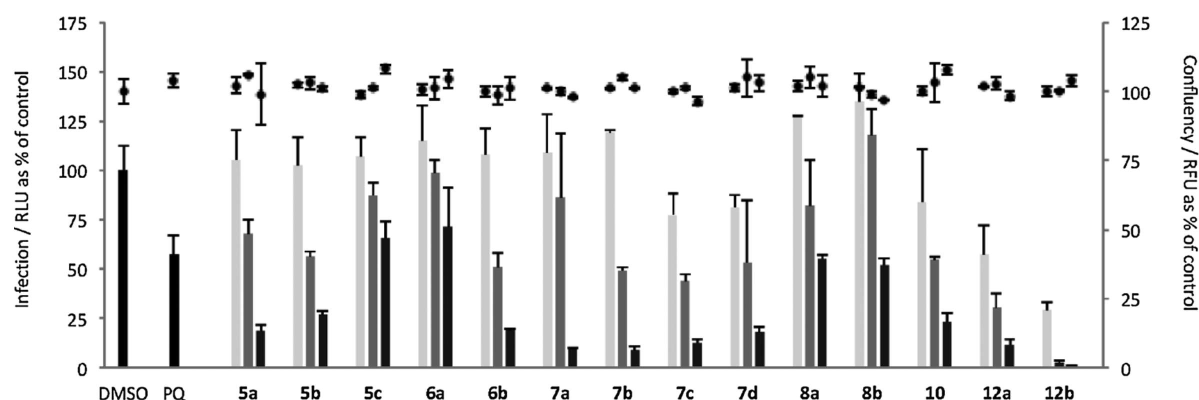

Figure 5. In vitro activity of compounds **5 a–c**, **6 a–b**, **7 a–d**, **8 a–b**, **10**, and **12 a–b** against *P. berghei* liver stages. Infection (bars, in relative luminescence units, RLU) and cell confluency (circles, in relative fluorescence units, RFU) of human hepatocellular carcinoma cells (Huh7) are shown at 1 μM (light grey), 5 μM (dark grey), and 10 μM (black) of each compound. Primaquine (PQ) at 10 μM was included in the assay as control. Results are expressed as mean ± SD (triplicate wells).

Metabolic stability

The stability of compounds **12 a** and **12 b** was briefly evaluated. First, chemical stability in pH 7.4 phosphate buffer and metabolic stability in human plasma and rat liver microsomes at 37 °C were evaluated. Both compounds were stable in phosphate buffer and plasma for the duration of the assays (three days). In addition, compounds **12 a** and **12 b** underwent slow degradation when incubated in rat microsomes with an NADPH-regenerating system, with half-lives of 1.5 h and 1.7 h, respectively. Overall, these data indicate that both **12 a** and **12 b** showed excellent metabolic and chemical stability.

Conclusions

Herein we report a set of novel dual-stage antimalarial compounds based on the indolizinoindolone scaffold. These compounds were designed to combine the indole and isoindolinone scaffolds. Cyclization of oxazoloisoindolinones derived from enantiopure tryptophanol allowed the enantioselective synthesis of indolizinoindolone derivatives. It should be highlighted that we described here for the first time the synthesis of 7,13b-*cis* indolizinoindolones in a two-step route. Moreover, indolizinoindolones displayed in vitro activity against the erythrocytic and liver stages of malaria parasites. Importantly, there seems to be a preference for (*S*)-tryptophanol-derived isoindolinones for activity. In addition, the activity against liver stages of *P. berghei* was nearly four times greater for the 7,13b-*cis* diastereoisomer with a phenyl group at C-13b compared with the activity obtained for the 7,13b-*trans* diastereoisomer. This demonstrates that the stereochemistry of the target compounds has a critical role in activity against the liver stage of malaria parasites. From this study, compound **12 b** emerged as the most promising indolizinoindolone, combining excellent activity against both stages of the parasite's life cycle with low cytotoxicity and excellent metabolic and chemical stability in vitro. To our knowledge, this is the first report of this scaffold demonstrating activity against both blood and liver stages of *Plasmodium* parasites, and overall, indolizinoindolones are promising lead compounds for the development of new dual-stage antimalarials.

Experimental Section

Chemistry

General: All reagents and solvents obtained from commercial suppliers and were used without further purification. Melting points were determined using a Kofler camera Bock monoscope M. The infrared spectra were collected on a Shimadzu IRAffinity-1 FTIR infrared spectrophotometer (Kyoto, Japan). Microanalyses were performed in a Thermo Scientific FLASH 2000 Series CHNS/O analyzer (Waltham, MA, USA) and are within ±0.5 % of theoretical values. Analysis Merck Silica Gel 60 F254 plates were used for analytical thin-layer chromatography; flash chromatography was performed on Merck Silica Gel (200–400 mesh). The specific rotation values were measured in a PerkinElmer 241 MC polarimeter (Waltham, MA, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz Ultra-Shield (Billerica, MA, USA). ^{1}H NMR spectra were recorded at 400 MHz. ^{13}C NMR spectra were recorded at 100 MHz. ^{1}H and ^{13}C NMR chemical shifts are reported in δ (ppm) referenced to the solvent used and the proton coupling constants *J* in are reported in Hz. Spectra were assigned using appropriate correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple quantum coherence (HMQC) sequences.

CCDC 1051839 and 1051840 contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

General preparation of compounds 3 a–b, 4 a–b, 5 c, and 9

To a stirred solution of the appropriate aminoalcohol ((*S*)- or (*R*)-tryptophanol) (1 equiv) in toluene, was added the appropriate β-oxo-acid (1.1 equiv). The mixture was heated at reflux under inert atmosphere using Dean–Stark conditions. At the end of the reaction, the solvent was removed under vacuum. The product was obtained after flash chromatography.

(3*S*,9b*R*)-3-((1*H*-Indol-3-yl)methyl)-9b-methyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9b*H*)-one (3 a): Following the general proce-

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dure, to a solution of (*S*)-tryptophanol (0.2 g, 1.05 mmol) in toluene (10 mL) was added 2-acetylbenzoic acid (0.19 g, 1.16 mmol). The reaction time was 16 h, and the eluent for flash chromatography was EtOAc/*n*-hexane (2:1). The product was obtained as white crystals (0.26 g, 81 %); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.92 (s, 1 H, NH), 7.74–7.65 (m, 3 H), 7.59 (m, 2 H, H-ar), 7.38–7.34 (m, 2 H, H-ar), 7.08 (t, *J* = 7.1 Hz, 1 H, H-ar), 7.01 (t, *J* = 7.4 Hz, 1 H, H-ar), 4.41–4.29 (m, 2 H, CH and OCH$_2$), 4.14 (dd, *J* = 8.0, 6.1 Hz, 1 H, OCH$_2$), 3.25 (dd, *J* = 14.6, 5.1 Hz, 1 H, CH$_2$-indole), 3.12 (dd, *J* = 14.6, 8.1 Hz, 1 H, CH$_2$-indole), 1.67 ppm (s, 3 H, CH$_3$).

(3*S*,9b*R*)-3-((1*H*-Indol-3-yl)methyl)-9b-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9b*H*)-one (3 b): Following the general procedure, to a solution of (*S*)-tryptophanol (0.16 g, 0.84 mmol) in toluene (10 mL) was added 2-benzoylbenzoic acid (0.21 g, 0.93 mmol). The reaction time was 16 h, and the eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was obtained as a white solid (0.27 g, 84 %); 1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.87 (s, 1 H, NH), 7.75–7.73 (m, 1 H, H-ar), 7.63–7.58 (m, 4 H, H-ar), 7.48–7.42 (m, 3 H, H-ar), 7.37–7.29 (m, 3 H, H-ar), 7.24 (s, 1 H, H-2-indole), 7.08–7.05 (m, 1 H, H-ar), 6.99–6.95 (m, 1 H, H-ar), 4.53 (m, 2 H, H-3 and OCH$_2$), 3.89–3.83 (m, 1 H, OCH$_2$), 2.95 (dd, *J* = 14.6, 5.8 Hz, 1 H, CH$_2$-indole), 2.61 ppm (dd, *J* = 14.6, 7.7 Hz, 1 H, CH$_2$-indole).

(3*S*,7a*S*)-3-((1*H*-Indol-3-yl)methyl)-7a-phenyltetrahydropyrrolo[2,1-b]oxazol-5(6*H*)-one (9): Following the general procedure, to a solution of (*S*)-tryptophanol (0.1 g, 0.53 mmol) in toluene (10 mL) was added 3-benzoylpropionic acid (0.10 g, 0.58 mmol). The reaction time was 3 h, and the eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was obtained as a white solid (0.18 g, 90 %); ^{1}H NMR (CDCl$_3$): δ = 8.07 (s, 1 H, NH), 7.51 (m, 2 H, H-ar), 7.47–7.29 (m, 5 H, H-ar), 7.17 (t, *J* = 7.5 Hz, 1 H, H-ar), 7.11–7.01 (m, 2 H, H-ar), 4.68–4.45 (m, 1 H, H-3), 4.16 (m, 1 H, H-2), 3.68–3.54 (m, 1 H, H-2), 3.08 (dd, *J* = 14.7, 6.1 Hz, 1 H, CH$_2$-indole), 2.85 (dd, *J* = 17.0, 8.4 Hz, 1 H, CH$_2$), 2.71–2.38 (m, 3 H, CH$_2$ and CH$_2$-indole), 2.32–2.17 ppm (m, 1 H, CH$_2$).

(3*R*,9b*S*)-3-((1*H*-Indol-3-yl)methyl)-9b-methyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9b*H*)-one (4 a): Following the general procedure, to a solution of (*R*)-tryptophanol (0.1 g, 0.53 mmol) in toluene (15 mL) was added 2-acetylbenzoic acid (0.10 g, 0.58 mmol). The reaction time was 16 h, and the eluent for flash chromatography was EtOAc/*n*-hexane (3:7). The product was obtained as white crystals (0.13 g, 76 %); ^{1}H NMR spectrum was found to be identical to the one obtained for compound **3 a**.

(3*R*,9b*S*)-3-((1*H*-Indol-3-yl)methyl)-9b-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9b*H*)-one (4 b): Following the general procedure, to a solution of (*R*)-tryptophanol (0.2 g, 1.1 mmol) in toluene (25 mL) was added 2-benzoylbenzoic acid (0.26 g, 1.16 mmol). The reaction time was 16 h, and the eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was obtained as a white solid (0.28 g, 70 %); ^{1}H NMR spectrum was found to be identical to the one obtained for compound **3 b**.

(7*S*,13b*S*)-7-(Hydroxymethyl)-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (5 c): Following the general procedure, to a solution of (*S*)-tryptophanol (0.1 g, 0.53 mmol) in toluene (20 mL) was added 2-formylbenzoic acid (0.09 g, 0.59 mmol). The reaction time was 16 h, and the eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was obtained as colorless needles (0.07 g, 42 %); ^{1}H NMR spectrum was found to be identical to the one reported in the literature.[16c]

General preparation of compounds 5a–b, 6a–b, and 10

To a solution of the proper tryptophanol-derived starting material in anhydrous CH$_2$Cl$_2$ was added dropwise BF$_3$·OEt$_2$ (4 equiv). The mixture was kept at room temperature under inert atmosphere for 3 h. H$_2$O was added and the solution was extracted two times with CH$_2$Cl$_2$ (same volume as used in the reaction). The organic layer was dried and concentrated, and the crude residue was purified by flash chromatography.

(7*S*,13b*R*)-7-(Hydroxymethyl)-13 b-methyl-7,8,13,13 b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (5 a): Following the general procedure, to a solution of compound **3 a** (0.06 g, 0.18 mmol) in CH$_2$Cl$_2$ (5 mL) was added BF$_3$·OEt$_2$ (92 μL, 0.73 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (7:3). The product was recrystallized in EtOAc/MeOH to afford a white solid (0.04 g, 64 %); mp: 292–294 °C; $[\alpha]_D^{22} = +190.0$ (c = 2.6, MeOH); ^{1}H NMR (400 MHz, MeOD): δ = 8.09 (d, *J* = 8.0 Hz, 1 H, H-ar), 7.78 (d, *J* = 7.6 Hz, 1 H, H-ar), 7.68 (t, *J* = 7.6 Hz, 1 H, H-ar), 7.49 (t, *J* = 7.4 Hz, 1 H, H-ar), 7.40 (d, *J* = 7.4 Hz, 1 H, H-ar), 7.37 (d, *J* = 8.4 Hz, 1 H, H-ar), 7.10 (t, *J* = 7.4 Hz, 1 H, H-ar), 6.99 (t, *J* = 7.4 Hz, 1 H, H-ar), 5.14 (m, 1 H, CH), 3.82 (m, 2 H, OCH$_2$), 2.99 (d, *J* = 16.0 Hz, 1 H, CH$_2$-indole), 2.88 (dd, *J* = 16.0, 2.6 Hz, 1 H, CH$_2$-indole), 1.88 ppm (s, 3 H, CH$_3$); ^{13}C NMR (101 MHz, MeOD): δ = 172.0 (C=O), 151.7 (Cq), 138.5 (Cq), 134.0 (Cq), 133.9 (CH-ar), 130.1 (CH-ar), 129.7 (Cq), 128.1 (CH-ar), 124.7 (Cq), 123.3 (CH-ar), 123.0 (CH-ar), 120.1 (CH-ar), 119.3 (CH-ar), 112.1 (CH-ar), 105.9 (Cq), 64.3 (C-13b), 63.1 (OCH$_2$), 52.4 (C-7), 29.0 (CH$_3$), 23.3 ppm (CH$_2$-indole); IR (KBr): $\tilde{\nu}$ = 3337 (N–H/O–H), 1667 cm^{-1} (C=O); Anal. calcd for C$_{20}$H$_{18}$N$_2$O$_2$·0.15 H$_2$O: C 74.81, H 5.76, N 8.73, found: C 74.47, H 5.68, N 8.73.

(7*S*,13b*R*)-7-(Hydroxymethyl)-13 b-phenyl-7,8,13,13 b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (5 b): Following the general procedure, to a solution of compound **3 b** (0.15 g, 0.38 mmol) in CH$_2$Cl$_2$ (10 mL) was added BF$_3$·OEt$_2$ (190 μL, 1.52 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was recrystallized in EtOAc/*n*-hexane to afford white crystals (0.09 g, 63 %); mp: 305 °C (decomposition); $[\alpha]_D^{22} = -83.6$ (c = 1.4, MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.60 (s, 1 H, NH), 7.89 (d, *J* = 7.6 Hz, 1 H, H-ar), 7.81 (d, *J* = 7.6 Hz, 1 H, H-ar), 7.65 (td, *J* = 7.6, 0.8 Hz, 1 H, H-ar), 7.54 (t, *J* = 7.6 Hz, 1 H, H-ar), 7.50 (d, *J* = 8.0 Hz, 1 H, H-ar), 7.47 (d, *J* = 8.0 Hz, 1 H, H-ar), 7.29–7.27 (m, 3 H, H-ar), 7.17 (t, *J* = 7.6 Hz, 1 H, H-ar), 7.04–7.02 (m, 1 H, H-ar), 6.99–6.97 (m, 2 H, H-ar), 4.93–4.85 (m, 1 H, CH), 4.80 (dd, *J* = 6.5, 4.4 Hz, 1 H, OH), 3.07 (d, *J* = 16.2 Hz, 1 H, CH$_2$-indole), 3.04–2.97 (m, 1 H, OCH$_2$), 2.87 (dd, *J* = 16.2, 7.2 Hz, 1 H, CH$_2$-indole), 2.83–2.76 ppm (m, 1 H, OCH$_2$); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 168.3 (C=O), 150.0 (Cq), 141.8 (Cq), 136.7 (Cq), 132.9 (CH-ar), 130.4 (Cq), 129.8 (Cq), 128.9 (CH-ar), 128.5 (2×CH-ar), 128.2 (CH-ar), 127.0 (2×CH-ar), 126.3 (CH-ar), 123.8 (CH-ar), 123.4 (CH-ar), 122.2 (CH-ar), 119.1 (CH-ar), 118.6 (CH-ar), 111.5 (CH-ar), 107.0 (Cq), 66.8 (OCH$_2$), 61.4 (C-13b), 49.9 (C-7), 21.7 ppm (CH$_2$-indole); IR (KBr): $\tilde{\nu}$ = 3347 (N–H/O–H), 1672 cm^{-1} (C=O). Anal. calcd. for C$_{25}$H$_{20}$N$_2$O$_2$·0.5H$_2$O: C 77.10, H 5.45, N 7.19, found: C 78.68, H 5.32, N 7.31.

(7*R*,13b*S*)-7-(Hydroxymethyl)-13 b-methyl-7,8,13,13 b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (6 a): Following the general procedure, to a solution of compound **4 a** (0.85 g, 2.67 mmol) in CH$_2$Cl$_2$ (50 mL) was added BF$_3$·OEt$_2$ (1.3 mL, 10.68 mmol). The eluent for flash chromatography was EtOAc/MeOH (19:1). The product was recrystallized in EtOAc/MeOH to afford white crystals (0.56 g, 65 %); mp: 297–299 °C; $[\alpha]_D^{22} = -215.5$ (c = 0.7, MeOH); ^{1}H NMR spectrum was found to be identical to the one obtained for compound **5 a**.

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(7*R*,13b*S*)-7-(Hydroxymethyl)-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (6b): Following the general procedure, to a solution of compound **4b** (0.10 g, 0.26 mmol) in CH_2Cl_2 (10 mL) was added $BF_3{\cdot}OEt_2$ (130 μL, 1.05 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (3:7). The product was recrystallized in EtOAc to afford white solids (0.075 g, 75%); $[\alpha]_D^{22} = +83.1$ ($c = 2.0$, MeOH); ^{1}H NMR spectrum was found to be identical to the one obtained for compound **5b**; Anal. calcd for $C_{25}H_{20}N_2O_2$: C 78.92, H 5.31, N 7.37, found: 78.87, H 5.24, N 7.37.

(5*S*,11b*R*)-5-(Hydroxymethyl)-11b-phenyl-5,6,11,11b-tetrahydro-1*H*-indolizino[8,7-b]indol-3(2*H*)-one (10): Following the general procedure, to a solution of compound **9** (0.05 g, 0.14 mmol) in CH_2Cl_2 (5 mL) was added $BF_3{\cdot}OEt_2$ (70 μL, 0.58 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (2:1). The product was recrystallized in EtOAc/*n*-hexane to afford white solids (0.037 g, 77%); mp: 315–317 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta =$ 11.48 (s, 1H, NH), 7.44–7.28 (m, 7H, H-ar), 7.13 (t, $J = 7.6$ Hz, 1H, H-ar), 7.02 (t, $J = 7.6$ Hz, 1H, H-ar), 4.06–3.98 (m, 2H, OCH_2), 3.29–3.25 (m, 1H, CH), 2.88 (dd, $J = 15.6$, 11.2 Hz, 1H, CH_2-indole), 2.77–2.68 (m, 2H, CH_2-indole and CH_2), 2.61–2.53 (m, 1H, CH_2), 2.47–2.40 (m, 1H, CH_2), 2.26–2.18 ppm (m, 1H, CH_2); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta = 176.8$ (C=O), 144.1 (Cq), 136.3 (Cq), 135.7 (Cq), 128.6 (2×CH-ar), 127.6 (CH-ar), 126.3 (Cq), 126.1 (2×CH-ar), 121.5 (CH-ar), 118.9 (CH-ar), 118.1 (CH-ar), 111.42 (CH-ar), 108.6 (Cq), 68.4 (Cq), 61.7 (OCH2), 55.1 (C-5), 33.9 (CH_2), 30.7 (CH_2), 23.1 ppm (CH_2-indole); IR (KBr): $\tilde{\nu} = 3186$ (N–H/O–H), 1663 cm^{-1} (C=O). Anal. calcd for $C_{21}H_{20}N_2O_2$: C 78.23, H 6.27, N 8.69, found: C 78.03, H 5.79, N 8.63.

General procedure for N-benzylation

To a stirred solution of the adequate indolizinoindolone derivative in dimethylformamide (DMF, 5 mL) under inert atmosphere and ice bath, was added NaH (1.5 equiv of 60% dispersion in mineral oil), and the mixture was stirred for 30 min. After this period benzyl bromide (1.5 equiv) was added, and the reaction was allowed to stand at room temperature for 24 h. The reaction was then quenched with ice-cold water (15 mL) and extracted several times with ethyl acetate (2×15 mL). The organic phase was washed with brine (2×15 mL), dried over Na_2SO_4, and the solvent evaporated. The crude residue was purified by flash chromatography.

(7*S*,13b*R*)-13-Benzyl-7-(hydroxymethyl)-13b-methyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (7a): The general procedure was followed, starting from compound **5a** (0.086 g, 0.267 mmol), benzyl bromide (0.05 mL, 0.40 mmol), and NaH 60% (0.016 g, 0.40 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was recrystallized in EtOAc/*n*-hexane and afforded colorless needles (0.035 g, 32%); mp: 122–125 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.85$ (d, $J = 7.6$ Hz, 1H, H-ar), 7.54 (m, 2H, H-ar), 7.41 (t, $J = 7.4$ Hz, 1H, H-ar), 7.34 (td, $J = 7.4$, 0.8 Hz, 1H, H-ar), 7.25–7.20 (m, 3H, H-ar), 7.11 (m, 2H, H-ar), 7.08–7.01 (m, 1H, H-ar), 6.83–6.78 (m, 2H, H-ar), 5.83 (d, $J = 18.0$ Hz, 1H, CH_2-Ph), 5.76 (d, $J = 18.0$ Hz, 1H, CH_2-Ph), 5.22 (qd, $J = 7.4$, 2.2 Hz, 1H, CH), 3.95 (m, 2H, OCH_2), 3.24 (dd, $J = 15.9$, 7.4 Hz, 1H, CH_2-indole), 2.99 (dd, $J = 15.9$, 2.2 Hz, 1H, CH_2-indole), 1.94 ppm (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 170.0$ (C=O), 148.3 (Cq), 137.9 (Cq), 137.5 (Cq), 134.9 (Cq), 132.1 (CH-ar), 131.1 (Cq), 129.0 (2×CH-ar), 128.9 (CH-ar), 127.5 (CH-ar), 126.8 (Cq), 125.8 (2×CH-ar), 124.4 (CH-ar), 123.2 (CH-ar), 123.0 (CH-ar), 120.2 (CH-ar), 118.9 (CH-ar), 110.5 (CH-ar), 108.4 (Cq), 64.7 (C-13b), 63.7 (OCH_2), 51.0 (C-7), 49.3 (CH_2-Ph), 28.8 (CH_3), 23.1 ppm (CH_2-indole); IR (KBr): $\tilde{\nu} = 3350$ (O–H); 1676 cm^{-1} (C=O); Anal. calcd for $C_{27}H_{24}N_2O_2{\cdot}0.25\ H_2O$: C 78.52, H 5.99, N 6.78, found: C 78.85, H 6.38, N 6.14.

(7*S*,13b*R*)-13-Benzyl-7-(hydroxymethyl)-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (7c): The general procedure was followed, starting from compound **5b** (0.04 g, 0.10 mmol), benzyl bromide (0.019 mL, 0.158 mmol), and NaH 60% (0.006 g, 0.158 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (3:7). The product was recrystallized in EtOAc/*n*-hexane and afforded white solids (0.044 g, 89%); mp: 291–293 °C; ^{1}H NMR ($CDCl_3$): $\delta = 7.89$ (d, $J = 7.6$ Hz, 1H, H-ar), 7.60–7.58 (m, 1H, H-ar), 7.35–7.27 (m, 4H, H-ar), 7.19–6.93 (m, 10H, H-ar), 6.68 (d, $J = 7.2$ Hz, 2H, H-ar), 5.67 (d, $J = 17.9$ Hz, 1H, CH_2-Ph), 5.50 (d, $J = 17.9$ Hz, 1H, CH_2-Ph), 5.24 (m, 1H, CH), 3.47–3.31 (m, 3H, CH_2OH and CH_2-indole), 2.87 ppm (d, $J = 16.2$ Hz, 1H, CH_2-indole); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 170.4$ (C=O), 147.9 (Cq), 141.2 (Cq), 137.8 (Cq), 136.8 (Cq), 132.4 (CH-ar), 132.0 (Cq), 131.7 (CH-ar), 129.3 (CH-ar), 129.1 (2×CH-ar), 129.0 (CH-ar), 128.6 (2×CH-ar), 128.0 (2×CH-ar), 127.3 (Cq), 127.0 (Cq), 125.9 (2×CH-ar), 125.3 (CH-ar), 123.9 (CH-ar), 123.3 (CH-ar), 120.3 (CH-ar), 119.1 (CH-ar), 110.70 (CH-ar), 110.4 (Cq), 69.6 (Cq), 62.7 (OCH_2), 50.9 (C-7), 50.3 (CH_2-Ph), 22.7 ppm (CH_2-indole); Anal. calcd for $C_{32}H_{26}N_2O_2{\cdot}0.75H_2O$: C 79.39, H 5.74, N 5.79, found: C 79.40, H 5.50, N 5.82.

(7*R*,13b*S*)-13-Benzyl-7-(hydroxymethyl)-13b-methyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (8a): The general procedure was followed, starting from compound **6a** (0.08 g, 0.25 mmol), benzyl bromide (0.05 mL, 0.377 mmol), and NaH 60% (0.016 g, 0.377 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was recrystallized in EtOAc/*n*-hexane and afforded white solids (0.041 g, 40%); ^{1}H NMR spectrum was found to be identical to the one obtained for compound **7a**.

General procedure for N,O-dimethylation

To a stirred solution of the adequate indolizinoindolone derivative in dry DMF (5 mL) under inert atmosphere and an ice bath, was added NaH (3 equiv of 60% dispersion in mineral oil). The mixture was allowed to stir for 30 min and methyl iodide (3 equiv) was added. The reaction was further stirred at room temperature for 16 h. After this period, ice cold H_2O (20 mL) was added, and the mixture was extracted with EtOAc (3×20 mL). The organic phase was washed with brine (3×20 mL), dried over Na_2SO_4, and the solvent evaporated. The crude residue was adsorbed onto SiO_2 and purified by flash chromatography.

(7*S*,13b*R*)-7-(Methoxymethyl)-13,13b-dimethyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (7b): The general procedure was followed, starting from compound **5a** (0.1 g, 0.31 mmol), methyl iodide (0.06 mL, 0.942 mmol), and NaH 60% (0.038 g, 0.942 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (1:1). The product was recrystallized in EtOAc/*n*-hexane and afforded pale-yellow crystals (0.096 g, 88%); mp: 150–152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.96$ (d, $J = 8.0$ Hz, 1H, H-ar), 7.91 (d, $J = 7.2$ Hz, 1H, H-ar), 7.62 (dt, $J = 8.0$, 0.8 Hz, 1H, H-ar), 7.48 (m, 2H, H-ar), 7.33 (d, $J = 8.3$ Hz, 1H, H-ar), 7.26 (dt, $J = 7.2$, 1.2 Hz, 1H, H-ar), 7.13 (dt, $J = 7.4$, 0.8 Hz, 1H, H-ar), 5.45 (m, 1H, CH), 4.16 (s, 3H, NCH_3), 3.70 (dd, $J = 9.5$, 7.6 Hz, 1H, OCH_2), 3.63 (dd, $J = 9.5$, 8.1 Hz, 1H, OCH_2), 3.40 (s, 3H, OCH_3), 3.10 (dd, $J = 15.8$, 7.0 Hz, 1H, CH_2-indole), 2.92 (d, $J = 15.8$ Hz, 1H, CH_2-indole), 2.03 ppm (s, 3H,

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CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 169.1$ (C=O), 149.1 (Cq), 138.3 (Cq), 134.5 (Cq), 132.2 (CH-ar), 131.3 (Cq), 128.7 (CH-ar), 126.6 (Cq), 124.5 (CH-ar), 122.7 (CH-ar), 122.6 (CH-ar), 119.7 (CH-ar), 118.9 (CH-ar), 109.2 (CH-ar), 107.6 (Cq), 72.2 (OCH_2), 64.1 (C-13b), 58.7 (OCH_3), 47.5 (C-7), 33.4 (NCH_3), 28.3 (CH_3), 23.2 ppm (CH_2-indole); IR (KBr): $\tilde{\nu} = 1697\ cm^{-1}$ (C=O); Anal. calcd for $C_{22}H_{22}N_2O_2$: C 76.27, H 6.41, N 8.10, found: 75.98, H 6.49, N 8.12.

(7*S*,13b*R*)-7-(Methoxymethyl)-13-methyl-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (7d): The general procedure was followed, starting from compound **5b** (0.045 g, 0.12 mmol), methyl iodide (0.02 mL, 0.355 mmol), and NaH 60% (0.014 g, 0.355 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (1:9). The product was recrystallized in EtOAc/*n*-hexane and afforded white crystals (0.028 g, 58%); mp: 235–237 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.88$ (d, $J = 7.2$ Hz, 1H, H-ar), 7.64 (d, $J = 8.0$ Hz, 1H, H-ar), 7.59–7.50 (m, 3H, H-ar), 7.37–7.23 (m, 5H, H-ar), 7.14 (t, $J = 7.2$ Hz, 1H, H-ar), 6.97 (d, $J = 7.2$ Hz, 2H, H-ar), 4.45 (m,1H, OCH_2), 4.25 (dd, $J = 9.6$, 4.4 Hz, 1H, OCH_2), 3.76 (s, 3H, NCH_3), 3.63–3.56 (m, 1H, CH), 3.34 (s, 3H, OCH_3), 3.18–3.15 ppm (m, 2H, CH_2-indole); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 169.6$ (C=O), 148.6 (Cq), 139.2 (Cq), 138.0 (Cq), 134.7 (Cq), 133.4 (Cq), 131.9 (CH-ar), 129.1 (2×CH-ar), 128.8 (2×CH-ar), 128.6 (2×CH-ar), 126.2 (Cq), 124.8 (CH-ar), 124.2 (CH-ar), 122.7 (CH-ar), 119.8 (CH-ar), 119.1 (CH-ar), 112.2 (Cq), 109.2 (CH-ar), 73.7 (OCH_2), 72.1 (Cq), 58.9 (OCH3), 52.3 (C-7), 33.6 (NCH_3), 26.1 ppm (CH_2-indole); Anal. calcd for $C_{27}H_{24}N_2O_2$: C 79.38, H 5.93, H 6.86, found: C 79.25, H 5.89, N 6.87.

(7*R*,13b*S*)-7-(Methoxymethyl)-13,13b-dimethyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (8b): The general procedure was followed, starting from compound **6a** (0.1 g, 0.31 mmol), methyl iodide (0.06 mL, 0.942 mmol), and NaH 60% (0.04 g, 0.942 mmol). The eluent for flash chromatography was EtOAc/*n*-hexane (2:1). The product was recrystallized in EtOAc/*n*-hexane and afforded pale-yellow crystals (0.096 g, 88%); ^1H NMR spectrum was found to be identical to the one obtained for compound **7b**.

(3*S*,9b*R*)-3-((1-Methyl-1*H*-indol-3-yl)methyl)-9b-phenyl-2,3-dihydrooxazolo[2,3-a]isoindol-5(9b*H*)-one (11): To a solution of 0.125 g of compound **3b** (0.33 mmol) in DMF (7.5 mL) at 0 °C and under N_2 atmosphere, 0.03 g of NaH 60% (0.72 mmol, 2.2 equiv) was added. After stirring for 20 min, 0,03 mL of methyl iodide (0.49 mmol, 1.5 equiv), was added then slowly allowed to warm to room temperature over 3 h. The reaction was then portioned with 20 mL of cold water, the phases were separated, and the aqueous phase was extracted with EtOAc (2×20 mL). All the organic extracts were combined, washed with brine, dried, and concentrated to get the crude product. The mixture was recrystallized from EtOAc to afford compound **11** as a crystalline white solid (0.104 g, 80%); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.81$ (dd, $J = 5.2$, 3.1 Hz, 1H, H-ar), 7.64 (m, 2H), 7.50 (m, 3H, H-ar), 7.39 (m, 3H, H-ar), 7.25 (m, 3H, H-ar), 7.09 (t, $J = 7.4$ Hz, 1H, H-ar), 6.97 (s, 1H, H-ar), 4.70 (m, 1H, H-3), 4.46 (t, $J = 8.1$ Hz, 1H, H-2), 3.99 (dd, $J = 8.3$, 7.1 Hz, 1H, H-2), 3.72 (s, 3H, NCH_3), 3.21 (dd, $J = 14.7$, 5.9 Hz, 1H, CH_2-indole), 2.66 ppm (dd, $J = 14.7$, 9.3 Hz, 1H, CH_2-indole).

(7*S*,13b*R*)-7-(Hydroxymethyl)-13-methyl-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one and (7*S*,13b*S*)-7-(hydroxymethyl)-13-methyl-13b-phenyl-7,8,13,13b-tetrahydro-5*H*-benzo[1,2]indolizino[8,7-b]indol-5-one (12a and 12b): To a solution of compound **11** (0.207 mg, 0.53 mmol) in dry dichloromethane (5 mL) under an atmosphere of N_2, was added 4 equiv of $BF_3{\cdot}OEt_2$ (0.14 mL, 1,1 mmol) at room temperature. The mixture was kept at room temperature under inert atmosphere with stirring. After 2 h the solvent was evaporated to dryness and the residue was purified by flash chromatography using EtOAc/*n*-hexane (1:2) as eluent.

12a: Obtained as a white solid (0.12 g, 58%); mp: 263–265 °C (EtOAc/*n*-hexane); $[\alpha]_D^{22} = +23.7$ ($c = 4.3$, MeOH); ^1H NMR (400 MHz, $[D_6]DMSO$): $\delta = 7.88$ (d, $J = 7.6$ Hz, 1H, H-ar), 7.79 (d, $J = 7.4$ Hz, 1H, H-ar), 7.71 (t, $J = 7.5$ Hz, 1H, H-ar), 7.62 (t, $J = 7.4$ Hz, 1H, H-ar), 7.54 (d, $J = 7.6$ Hz, 1H, H-ar), 7.44 (d, $J = 8.2$, 1H, H-ar), 7.42–7.35 (m, 3H, H-ar), 7.21 (t, $J = 7.6$ Hz, 1H, H-ar), 7.09 (t, $J = 7.6$ Hz, 1H, H-ar), 6.89 (d, $J = 6.4$ Hz, 2H, H-ar), 4.99 (t, $J = 5.8$ Hz, 1H, OH), 4.34–4.17 (m, 1H, CH_2O), 4.01 (dd, $J = 11.4$, 5.8 Hz, 1H, CH_2O), 3.73 (s, 3H, NCH_3), 3.33 (s, 1H, CH), 3.05 (dd, $J = 15.4$, 3.8 Hz, 1H, CH_2-indole), 2.96 ppm (dd, $J = 15.4$, 11.4 Hz 1H, CH_2-indole); ^{13}C NMR (101 MHz, $[D_6]DMSO$): $\delta = 168.5$ (C=O), 148.1 (Cq), 138.8 (Cq), 137.7 (Cq), 134.4 (Cq), 132.6 (CH-ar), 132.4 (Cq), 129.4 (CH-ar), 129.0 (CH-ar), 129.0 (CH-ar), 128.1 (CH-ar), 125.5 (Cq), 125.5 (CH-ar), 123.4 (CH-ar), 122.4 (CH-ar), 119.5 (CH-ar), 118.7 (CH-ar), 111.3 (Cq), 109.9 (CH-ar), 71.8 (C-13b), 61.8 (OCH_2), 54.5 (C-7), 33.6 (NCH_3), 24.7 ppm (CH_2-indole); IR (KBr): $\tilde{\nu} = 3283$ (N–H), 1697 cm^{-1} (C=O); Anal. calcd for $C_{26}H_{22}N_2O_2$: C 79.22, H 5.64, N 7.11, found: C 79.00, H 5.37, N 6.93.

12b: Obtained as a white solid (0.05 g, 24%); mp: >280 °C (EtOAc/*n*-hexane); $[\alpha]_D^{22} = +23.7$ ($c = 4.3$, MeOH); ^1H NMR (400 MHz, $[D_6]DMSO$): $\delta = 7.84$ (t, $J = 7.4$ Hz, 2H, H-ar), 7.68 (t, $J = 7.4$ Hz, 1H, H-ar), 7.61 (t, $J = 7.4$ Hz, 1H, H-ar), 7.54 (d, $J = 7.6$ Hz, 1H, H-ar), 7.51 (d, $J = 8.2$ Hz, 1H, H-ar), 7.38–7.27 (m, 3H, H-ar), 7.24 (t, $J = 7.6$ Hz, 1H, H-ar), 7.10 (t, $J = 7.6$ Hz, 1H, H-ar), 6.84 (d, $J = 4.8$ Hz, 2H, H-ar), 4.99–4.82 (m, 1H, CH), 4.81–4.70 (m, 1H, OH), 3.90 (s, 3H, NCH_3), 3.12–3.05 (m, 2H, CH_2-indole and OCH_2), 2.92 (dd, $J = 16.0$, 6.9 Hz, 1H, CH_2-indole), 2.65–2.57 ppm (m, 1H, OCH_2); ^{13}C NMR (101 MHz, $[D_6]DMSO$): $\delta = 168.1$ (C=O), 149.0 (Cq), 140. 7 (Cq), 137.8 (Cq), 132.9 (CH-ar), 131.8 (Cq), 131.1 (Cq), 129.3 (CH-ar), 128.6 (CH-ar), 127.9 (CH-ar), 126.1 (Cq), 125.3 (CH-ar), 123.5 (CH-ar), 122.6 (CH-ar), 119.4 (CH-ar), 118.7 (CH-ar), 110.0 (CH-ar), 108.4 (Cq), 68.9 (C-13b), 61.0 (OCH_2), 50.2 (C-7), 34.4 (NCH_3), 22.2 ppm (CH_2-indole); IR (KBr): $\tilde{\nu} = 3283$ (N–H), 1697 cm^{-1} (C=O); Anal. calcd for $C_{26}H_{22}N_2O_2{\cdot}0.75\ H_2O$: C 76.59, H 5.82, N 6.87, found: C 76.26, H 5.38, N 6.78.

^1H and ^{13}C NMR spectra for compounds **5a**, **5b**, **10**, **12a**, and **12b**, as well as additional crystallographic information can be found in the Supporting Information.

Biology

In vitro activity assay against the blood stage of *P. falciparum* infection: Human red blood cells infected with ~1% parasitemia of ring-stage *P. falciparum* synchronized with 5% sorbitol were incubated with tested compounds in 96-well plates at 37 °C for 48 h in RPMI-1640 medium, supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4, 10% heat-inactivated human serum (or 0.5% Albumax, 2% human serum), and 100 μM hypoxanthine under an atmosphere of 3% O_2, 5% CO_2, 91% N_2. After 48 h, the cells were fixed in 2% HCHO in phosphate-buffered saline (PBS), transferred into PBS with 100 mM NH_4Cl, 0.1% Triton X-100, and 1 nM YOYO-1, and then analyzed in a flow cytometer (FACSort, Becton Dickinson, Franklin Lakes, NJ, USA; $\lambda_{ex} = 488$ nm, $\lambda_{em} = 520$ nm) to count infected erythrocytes. IC_{50} values were calculated using GraphPad PRISM 5 software (La Jolla, CA, USA).

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In vitro activity against the liver stage of *P. berghei* infection: Inhibition of liver-stage infection by test compounds was determined by measuring the luminescence intensity of Huh-7 cells infected with a firefly-luciferase-expressing *P. berghei* line, as previously described.[17] Briefly, Huh-7 cells, a human hepatoma cell line, were cultured in 1640 RPMI medium supplemented with 10% *v/v* fetal bovine serum, 1% *v/v* nonessential amino acids, 1% *v/v* penicillin/streptomycin, 1% *v/v* glutamine, and 10 mM HEPES, pH 7, and maintained at 37 °C with 5% CO_2. For infection assays, Huh-7 cells (1.0×10^4 per well) were seeded in 96-well plates the day before drug treatment and infection. The medium was replaced by medium containing the appropriate concentration of each compound approximately 1 h prior to infection with sporozoites freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes. An amount of the dimethyl sulfoxide (DMSO) solvent equivalent to that present in the highest compound concentration was used as control. Sporozoite addition was followed by centrifugation at 1700 g for 5 min. Parasite infection load was measured 48 h after infection by a bioluminescence assay (Biotium).

Assay of cytotoxicity: Compound cytotoxicity was evaluated following a 48 h incubation of Huh7 cells with increasing concentrations of each compound. Cell confluence was then assessed by the AlamarBlue assay (Invitrogen, UK), following the manufacturer's instructions.

Stability assays: High-performance liquid chromatography (HPLC) measurements were carried out using a VWR HITACHI assembly (Tokyo, Japan) equipped with an L-2400 UV detector, an L-2300 column oven, and an L-2130 pump. An injection valve equipped with a 20 μL sample loop was used. The separation was performed on a LichroCART RP-18 (5 μm, 250–4 mm) analytical column (Merck). MeOH/phosphate buffer 10 mM, pH 3.7 (70:30, *v*:*v*) was used as eluent. Elution was performed at a solvent flow rate of 1 mL min^{-1}. Chromatograms were monitored by UV/Vis detection at 285 nm. All analyses were performed at 37 °C.

Stability in pH 7.4 phosphate buffer: 10.0 μL of a 10^{-2} M stock solution of compounds **12a** or **12b** in DMSO was added to 2.5 mL of K_3PO_4 buffer solution (pH 7.4, 0.5 M) at 37 °C. At appropriate intervals, samples were removed and analyzed by HPLC using the methodology previously described. The stability was assessed for a period of 72 h.

Stability in human plasma: Human plasma was obtained from the pooled, heparinized blood of healthy donors and was frozen and stored at −20 °C prior to use. A 10^{-2} M stock solution of compound **12a** or **12b** in DMSO (10.0 μL) was incubated at 37 °C in human plasma diluted to 80% (*v/v*, 2.5 mL) with K_3PO_4 buffer (pH 7.4, 0.5 M). At appropriate intervals, aliquots (200 μL) were removed and added to acetonitrile (200 μL) to quench the reaction and precipitate plasma proteins. These samples were vortexed and centrifuged, and the supernatant was analyzed by HPLC using the methodology previously described. The stability was assessed for a period of 48 h.[18]

Stability in rat microsomes: Male rat pooled liver microsomes (Sprague-Dawley) BD Gentest were used. A mixture of purified water (570 μL), K_3PO_4 (160 μL , pH 7.4, 0.5 M), NADPH Regenerating System Solution A (40 μL, BD Biosciences Cat. No. 451220), NADPH Regenerating System Solution B (8 μL, BD Biosciences Cat. No. 451200), and microsomes (20 μL) was incubated for 10 min at 37 °C in a water bath before addition of a 10^{-2} M stock solution of the tested compounds (**12a** and **12b**) in DMSO (2 μL). At appropriate intervals, aliquots (80 μL) were removed and added to ice-cold CH_3CN (80 μL). These samples were mixed and centrifuged, and the supernatant was analyzed by HPLC using the methodology previously described. The percentage of unmetabolized compounds was quantified by HPLC.[19] The viability of the rat microsomes was verified by evaluating their CYP2E1-catalyzed *p*-nitrophenol hydroxylation capacity, applying a methodology described elsewhere.[20]

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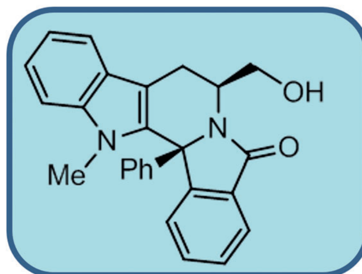
All about antimalarials: A novel class of potential antimalarial compounds containing an indolizinoindolone scaffold showed promising activity against erythrocytic stages of the human malaria parasite, *Plasmodium falciparum*, and liver stages of the rodent parasite *Plasmodium berghei*. The most promising compound exhibited IC_{50} values of 1.2 and 0.6 μM for the erythrocytic and liver stages, respectively, with excellent metabolic and chemical stability.

Novel antimalarials



IC_{50} (blood stage) = 1.2 μM
IC_{50} (liver stage) = 0.6 μM

N. A. L. Pereira, Â. Monteiro, M. Machado, J. Gut, E. Molins, M. J. Perry, J. Dourado, R. Moreira, P. J. Rosenthal, M. Prudêncio, M. M. M. Santos**

Enantiopure Indolizinoindolones with in vitro Activity against Blood- and Liver-Stage Malaria Parasites

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