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Synthesis and evaluation of a novel series of indoloisoquinolines as small molecule anti-malarial leads

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ABSTRACT

A group of novel synthetic indoloisoquinolines was prepared and its potential as a novel series of small-molecule anti-malarial leads was assessed. The structure–activity relationship on variation of three distinct regions of chemical space was investigated. A lead compound was generated with an activity close to that observed for a known anti-malarial natural product, dihydrousambarensine, that shares the indoloisoquinoline template structure.

Malaria continues to pose a significant global health and socio-economic burden on those regions where it is endemic. Whilst substantial investment in the delivery of front-line artemisinin-based combination therapies and use of insecticide impregnated bednets have seen a fall in the mortality attributed to malaria over recent years, data from 2009 show that this disease still imposes a significant impact both in terms of morbidity (~255 million cases) and mortality (~781,000 deaths) annually.[1] A major challenge is the narrow drug discovery pipeline, a problem exacerbated by recent reports of artemisinin treatment failure in South East Asia.[2,3] In recent years, however, high throughput screening of small-molecule libraries as well as natural products derived from plants and marine organisms have sought to seed this pipeline with diverse and novel chemical entities.[4–6] Indole alkaloids isolated from various *Strychnos* species of plants have been demonstrated to show in vitro selectivity and activity against both chloroquine resistant (CQR) and sensitive (CQS) isolates of *Plasmodium falciparum*, the aetiological agent of the most severe form of human malaria.[7,8] Of these compounds characterized, dihydrousambarensine, **1**, emerged as an interesting candidate showing altered levels of activity between CQR and CQS isolates, with a clear preferred activity against CQR isolates (0.85 μM vs 0.03 μM for CQS and CQR, respectively).[8] Over recent years our research group has become expert in the stereoselective synthesis of indole alkaloid targets and we have recently reported the asymmetric synthesis of alkaloids including harmicine **2**, deplancheine **3** and 12*b*-epidevinylantirhine **4** (Fig. 1).[9] Given the structural similarity of dihydrousambarensine, **1**, to these compounds we have initiated a research program to investigate the structure–activity relationship (SAR) on the indoloisoquinoline template **5**. Examination of a collection of heterocyclic compounds originating from our historical research efforts identified 10 compounds that shared some structural similarity to dihydrousambarensine. Of these, the indoloisoquinoline derivative **6a** (Fig. 2) was identified as a potential lead compound for this investigation (IC_{50} of 20 μM, Table 1). Based on this structure we decided to undertake an initial investigation of three areas of chemical space (Fig. 2) with the aim of gaining an increased appreciation of the resulting activity that could be realised by simple chemical modification of the readily available indoloisoquinoline core **5a**, namely: (i) the indole N-substituent (R); (ii) the hydroxymethyl O-substituent (R^1); (iii) alkenic substitution on or around the lactam ring (R^2).

Compound **5a** was prepared by our previously reported route from L-tryptophan,[10] and utilized as a starting point for further structural diversity in our investigation. Symmetrical alkylation of the *N*- and *O*-groups was readily achieved by the general procedure outlined in Scheme 1 to yield compounds **7a–c**.

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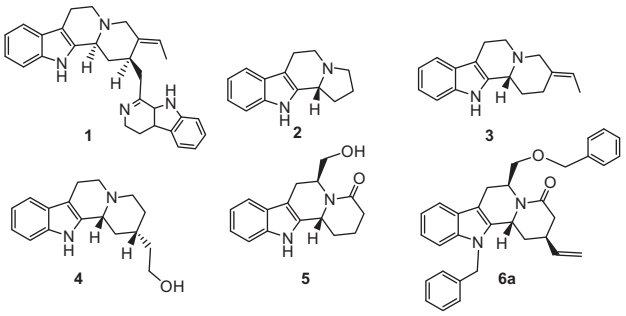
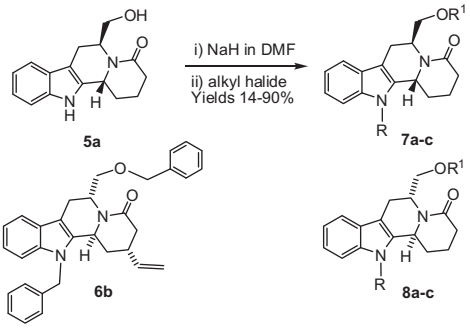


Figure 1. The indoloisoquinoline template and anti-malarial leads.



Scheme 1. Synthesis of indoloisoquinoline targets.

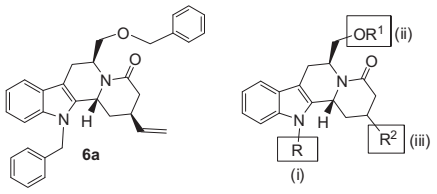


Figure 2. Initial lead compound and proposed sites of chemical modification on the indoloisoquinoline template.

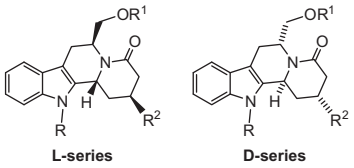


Figure 3. Alternative template structures of the lead indoloisoquinoline compounds.

We were mindful of the fact that the absolute stereochemistry present within the indoloisoquinoline core of the anti-malarial natural product dihydrousambarensine, **1**, did not match that of our initial series of compounds and, due to the known potential differences in activity of enantiomeric series of biologically active compounds, we also prepared the analogous series of diastereoisomeric compounds, **8a–c**, by following the same series of alkylation reactions from the diastereoisomeric template **5b**, itself produced from the D-enantiomer of the original tryptophan precursor (Fig. 3).[10] Unsymmetrically substituted compounds **9–17** (Table 1) were prepared by a modified procedure in which the appropriate precursor (**5a** or **5b**) underwent stepwise derivatization. Initial alkylation using NaH in DMF with 1.5 equiv of the alkyl halide gave the *N*-alkyl product followed (if required) by a second alkylation on the hydroxymethyl oxygen atom (using NaH in DMF with 2 equiv of the appropriate alkyl halide). We have also prepared and investigated the enantiomer **6b** of our lead compound from the initial screen, **6a**. The synthesis of **6b** followed the previously reported synthesis of **6a** albeit starting from the D-enantiomer of the original tryptophan precursor.[11] It is worth noting at this point that compounds **5**, **7–17** lacked the presence of the vinyl substituent (R^2) on the lactam ring.

Anti-malarial activity was determined using an adaptation of the lactate dehydrogenase (LDH) assay.[12,13] Assays to determine the 50% inhibitory concentration (IC_{50}) were carried out using intraerythrocytic cultures of *P. falciparum* lines Dd2 (chloroquine resistant) and 3D7 (chloroquine sensitive). It is immediately apparent from Table 1 that the compounds tested in this screen show a significant increase in antiplasmodial activity ($p = 0.015$) on moving from the L-tryptophan to the D-tryptophan series, highlighting the importance of absolute stereochemistry in this indoloisoquinoline template. Indeed the more active D-series of compounds has the same absolute stereochemistry at the ring junction within the heterocyclic core as the natural product that formed the basis of this study (dihydrousambarensine, **1**) and also shares the relationship between stereochemistry and activity with other natural products from the Frédérich study.[8]As a result we therefore decided to continue our own study with compounds derived from the D-tryptophan enantiomer.

Themes that emerge from this study include the apparent preference for a benzyl substituent on the indole nitrogen atom (R), with a methyl (as in **11b**) or allyl (as in **12b**) substituent at R^1 leading to the highest levels of activity in this screen (both have an IC_{50} value of 1.3 μM, Table 1). An increase in steric bulk at R^1 on moving

Table 1
Structure–activity relationships of indoloisoquinoline derivatives[14]

Compound L-series	R	R^1	R^2	IC_{50} (μM) Dd2	Compound D-series	R	R^1	R^2	IC_{50} (μM)Dd2
5a	H	H	H	71	**5b**	H	H	H	56
6a	Benzyl	Benzyl	vinyl	20	**6b**	Benzyl	Benzyl	Vinyl	13
7a	Methyl	Methyl	H	30	**8a**	Methyl	Methyl	H	29
7b	Allyl	Allyl	H	16	**8b**	Allyl	Allyl	H	13
7c	Benzyl	Benzyl	H	12	**8c**	Benzyl	Benzyl	H	3.5
					9	Benzyl	H	H	5
					10	Allyl	H	H	41
11a	Benzyl	Methyl	H	32	**11b**	Benzyl	Methyl	H	1.3
12a	Benzyl	Allyl	H	35	**12b**	Benzyl	Allyl	H	1.3
					13	Benzyl	Propyl	H	2.8
					14	Benzyl	Cyclohexylmethyl	H	19
					15	Cyclohexylmethyl	Allyl	H	9
					16	Cyclobutylmethyl	Allyl	H	7
					17	4-Pyridyl methyl	Allyl	H	2.1

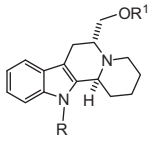


Figure 4. Tertiary amine derivatives **18** and **19**.

from an allyl (in **12b**) to either a propyl group, as in **13**, or to a cyclohexyl ring, **14**, gave a fall in activity. The propyl moiety may be viewed as being less planar, and consequently more degrees of freedom, than allyl as a result of the introduction of the double bond in the latter. The fully saturated cyclohexyl moiety would be expected to show conformational preference for a chair rather than the planarity present in the original aromatic ring of **12b**. The difference in activity between **8c** (R^1 = benzyl) and **14** (R^1 = cyclohexylmethyl) is significant and again points to the preference for some degree of planarity in the ring component, and also possibly an increase in electron density, within the R^1 substituent.

Variation of the ring moiety within the R grouping in **12b** leads to significant falls in activity with compounds **15** and **16**. Each of these analogues contains an all-carbon ring structure at R, but neither alternative group (cyclohexylmethyl nor cyclobutylmethyl) shares the electronic or steric properties of the aromatic ring contained within the original benzyl substituent at R in **12b**.

Compound **17** retains almost the same level of activity as **12b** on introduction of an alternative nitrogen-bearing pyridylmethyl substituent at R. Indeed, one would expect that the steric and electronic properties of the pyridylmethyl group would closely mimic that of the benzyl substituent at R in **12b**, albeit with an increase in the overall basicity of pyridyl analogue, **17**. In this current series of compounds an increase in the basic nature of the compounds does not seem to lead to a corresponding increase in activity, a fact supported by the work on compounds **18** and **19** (Fig. 4), discussed below.

With the results of the pyridyl analogue **17** in hand we decided to explore an alternative mode to introduce a higher level of basicity to the series. Tertiary amine analogues **18** and **19** (Table 2) were prepared by amide group reduction of compounds **11b** and **12b** respectively (using lithium aluminium hydride in dry THF).

Table 2
Activity of tertiary amine analogues

Compound Yield (%)	R	R^1	IC_{50} (μM) Dd2
18 (45%)	Benzyl	Me	3.3
19 (12)	Benzyl	Allyl	1.5

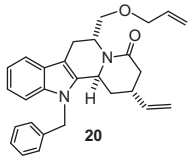


Figure 5. Most active synthetic indoloisoquinoline.

Comparing compound **18** with its amide analogue **11b** (Table 1) we observe a slight decrease in activity on removal of the lactam functionality. Compound **19**, bearing an allyl substituent at R^1, shows comparable activity (IC_{50} 1.5 μM) to its amide parent **12b** (IC_{50} 1.3 μM). Removal of the lactam carbonyl group to generate tertiary amines does not therefore lead to an apparent increase in antiplasmodial activity in this series.

Based on the observed increase in activity of **12b** over **8c** we decided to prepare compound **20** (Fig. 5), incorporating the *O*-allyl, *N*-benzyl substitution pattern of **12b** but now introducing the vinyl group present at R^2 in the original lead compound **6a**. Compound **20** was prepared by a route that is analogous to the preparation of compound **6b**, albeit starting from the *N*-benzyl, *O*-allyl compound, **12b**. Compound **20** was found to have an IC_{50} of 1.1 μM, and is our most active compound to date. It is interesting to compare this activity with that of compounds **6a** and **6b** (Table 1) which share the presence of the vinyl substituent at R^2. Simply replacing the *O*-benzyl substituent of **6b** by *O*-allyl (in **20**) leads to a considerable increase in activity. Although close to the margin for error, compound **20**, containing the vinyl substituent at R^2, *is* more active than its precursor **12b** and the original vinyl-containing leads **6a** and **6b**.

Finally, compounds **21** and **22**(Fig. 6) were prepared by typical selenoxide-induced unsaturation chemistry in order to investigate an alternative modification of the lactam ring, effecting a change in ring conformation on introduction of a conjugated double bond into this ring.

On comparing these results to those of the 'parent' compounds **12b** and **8c** there is no significant change in activity of their unsaturated analogues, **21** and **22** respectively (Table 3).

In this study we set out to investigate the structure–activity relationships of a range of synthetic indoloisoquinolines sharing some structural similarities to the known antiplasmodial natural product dihydrousambarensine, **1**. Through a series of rational structural modifications we have developed our compound series from initial activities of around 70 μM to our current lead compound, **20**, with an IC_{50} of 1.1 μM. This level of inhibition is comparable, if not better, than those previously reported for monoindole alkaloid moieties isolated from *Strychnos* spp.[8] Of note is the fact that, like most indole alkaloids, the inhibitory effects reported here for the CQR isolate Dd2 were not significantly different from those determined from the CQS isolate 3D7 (see Supplementary data).

Table 3
Activity of unsaturated lactam derivatives

Compound Yield (%)	R	R^1	IC_{50} (μM) Dd2
21 (25%)	Benzyl	Allyl	1.7
22 (40)	Benzyl	Benzyl	3.6

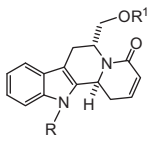


Figure 6. Unsaturated indoloisoquinolines **21** and **22**.

We have identified the following general structural properties as having an effect on antiplasmodial activity in the synthetic indoloisoquinolines:

(i) absolute chirality of the template: compounds derived from D-tryptophan are preferred;
(ii) the steric bulk and a degree of planarity within R is important, with the presence of an aromatic ring being preferred;
(iii) introduction of allyl at R^1 can lead to an increase in activity;
(iv) an increase in basicity at R, or within the lactam ring, does not lead to an increase in activity;
(v) introducing unsaturation to the lactam ring does not lead to an increase in activity;
(vi) introduction of substitution, currently a vinyl group, at R^2 can lead to an increase in activity.

That chirality of the template, and the size and nature of substitutions at the R and R^1 position, correlates with the antiplasmodial activity in this compound series suggesting an interaction with a specific parasite target. Compounds **8c** and **20** when added to *P. falciparum* culture at 5 μM appear to induce major morphological alterations in the trophozoite stage of parasite development (data not shown), although this provides only very provisional insights to a potential target for the indoloisoquinoline target. Introduction of a vinyl substituent and the resulting levels of biological activity in compound **20** tantalizingly suggest that additional exploration of the R^2 position is desirable, with the aim of extending the indoloisoquinoline core to produce analogues that more closely mimic the structural properties found in this region of chemical space within the bisindole alkaloid, dihydrousambarensine, **1**. Such modifications, and more detailed analysis of the temporal and spatial effect of these compounds on parasite growth and development, will form the basis of future studies in our group and our results will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data (representative experimental conditions and characterization data for the synthesis of compounds **8a**, **9**, **11b**, **12b**, **19**,**20**, and **21**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.071.

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13. Intraerythrocytic cultures of *P. falciparum* were maintained in standard continuous culture.[14] Cultures were maintained at 37 °C in a 1% O_2: 3% CO_2: 96% N_2 environment. When required, cultures were synchronised to ring stages using the sorbitol lysis technique.[15]All compounds were solubilised in 100% dimethyl sulphoxide (DMSO) to a 100 mM stock (stored at −20 °C), with dilution to appropriate concentration made in complete *P. falciparum* cell culture medium immediately prior to use. Assays were carried out in a 96-well microtitre plate using an initial 200 μl of 2% haematocrit 1% ring stage culture and five-fold dilutions of drug (800 μM–51.2 nM). 100% growth was established from cultures where no drug was added, and 0% from cultures subjected to a supralethal dose (100 nM) of artemether. Following 48 h of incubation, 25 μl of resuspended culture were transferred to a fresh 96-well microtitre plate and 100 μl of Malstat reagent (0.1 M Tris pH9.1, 0.2 M sodium lactate, 10 μM acetylpyridine adenine dinucleotide (APAD), 0.2% Triton X-100) and 25 μl of NBT/PES (16 μg ml^{-1}nitrobluetetrazolium, 0.239 M phenazineethosulphate) added and mixed by pipetting. After 1hr incubation in the dark at room temperature, the absorbance at 650 nm was measured using a Scientific Biotek EL800 plate reader. The mean % growth of at least $n = 6$ assays was plotted using a log dose–response curve and the IC_{50} extrapolated using GraphIT (v3 Erithacus Software).
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