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Stereoselective preparation of pyridoxal 1,2,3,4-tetrahydro-β-carboline derivatives and the influence of their absolute and relative configuration on the proliferation of the malaria parasite *Plasmodium falciparum*

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ABSTRACT

We have selectively synthesized by *Pictet–Spengler* condensation of tryptophan and pyridoxal the four stereoisomers of a pyridoxal β-carboline derivative that was designed to inhibit the proliferation of *Plasmodium falciparum*. Biological investigation of the four compounds revealed that they all inhibit the growth of *P. falciparum*. With an IC_{50} value of 8 ± 1 μM, the highest inhibitory effect on the proliferation of the parasite was found for the 1,3-*trans*-substituted tetrahydro-β-carboline that was obtained from D-tryptophan. Lower activity was found for its enantiomer, while the two diastereomeric *cis*-products were markedly less effective. Apparently a distinct spacial orientation of the carboxyl group of the substituted tetrahydropyridine unit of the compounds is needed for high activity, while the absolute configuration of the molecules is of lesser importance.

1. Introduction

Malaria is an infectious disease, widely-spread in tropical and sub-tropical areas in South America, Asia, and, in particular, sub-saharan Africa. It is caused by a parasite of the genus *Plasmodium*, the most virulent being *Plasmodium falciparum*. Malaria causes more than 2 million fatalities per worldwide year, and more than 300 million new infections occur annually. Since there are no vaccines against malaria available as yet and because the few existing antimalarials are progressively losing their efficacy due to the development of drug resistance, there is a vital need for new and potent antiplasmodials. As a new strategy to interfere with the parasite's metabolism, poisoning of pyridoxal 5-phosphate-dependent ornithine decarboxylase (*Pf*ODC) was suggested by Müller et al.[1] With their initial investigation they showed that a pyridoxyl-amino acid derivative, 1,2,3,4-tetrahydro-β-carboline derivative **PT3** (Fig. 1), is a promising candidate for further examination. **PT3** showed a distinct antiplasmodial activity, inhibiting the proliferation of cultured *P. falciparum* with an IC_{50} value of 14 μm, while leaving mammalian cells largely unaffected.[1]

PT3, however, was available in just a very small amount: it arose as a minor side product upon the reductive amination of pyridoxal with tryptophan methyl ester. Due to the small amount of sample, **PT3** was only characterized with a mass spectrum. More detailed data that would have revealed the isomeric purity as well as the stereochemical identity of the material were not obtained. Hence, the preliminary biological study could not assign the observed inhibitory effect of **PT3** to a specific isomeric form of the compound. More material and access to all four stereoisomers of **PT3**, compounds *cis*-**1** and *trans*-**1** in both enantiomeric forms (Fig. 1; the symbols D and L referring to the configuration of the tryptophan moiety), was thus needed to be able to complete the biological evaluation of **PT3**.

In this report we describe the preparation of all four isomeric 1,2,3,4-tetrahydro-β-carboline derivatives of the type **1** and the results of their evaluation as antiplasmodial agents.

2. Results and discussion

2.1. Synthesis

The most direct method to prepare 1,3 disubstituted 1,2,3,4-tetrahydro-β-carboline derivatives is the *Pictet–Spengler* condensation

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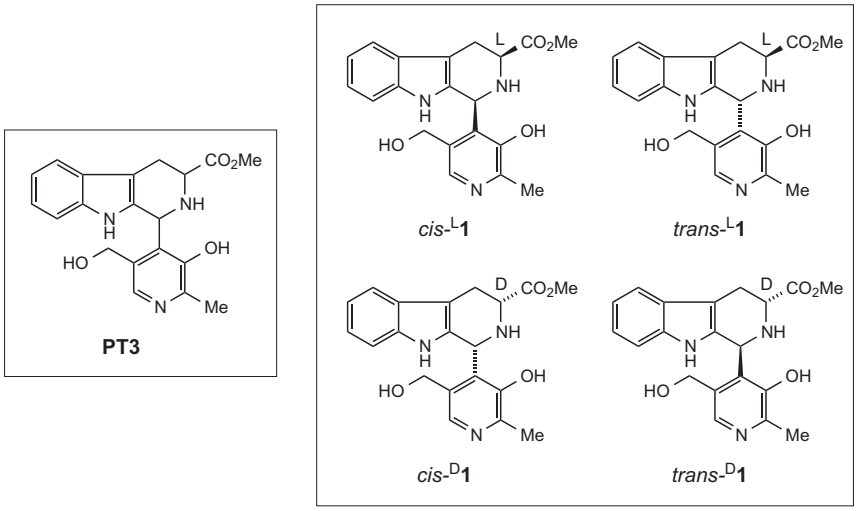


Figure 1. Target structures to be investigated with regards to their antiplasmodial activity.

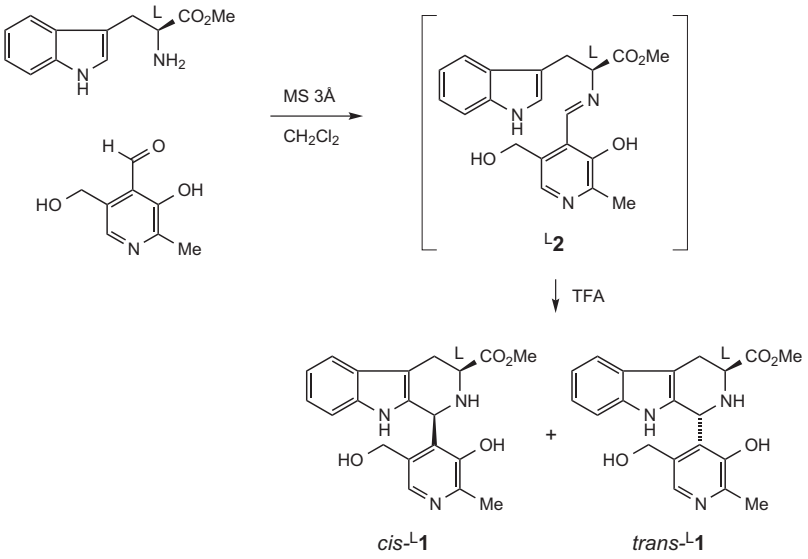
of tryptamine derivatives with aldehydes,[2,3] and most possibly, **PT3** arose from just this reaction, which easily could have proceeded in competition with the intended reductive amination of tryptophan methyl ester with pyridoxal.

The *Pictet–Spengler* condensation of tryptophan esters with aldehydes has been described previously. It was studied in more detail by Cook[4–8] and Bailey[9–11] and their co-workers, particularly also with regards to the stereochemical outcome of the reaction. It was shown that thermodynamic control of the process leads preferentially to *trans*-configured products, in particular when *N*-benzyl tryptophan methyl ester was used as the starting material. Kinetic control, on the other hand, leads predominantly to the *cis*-configured products, however with only moderate selectivities in most cases. Depending on the reaction conditions, also partial racemization was observed.

For the synthesis of the target compounds of type **1**, *Pictet–Spengler* condensation of D- and L-tryptophan methyl esters with pyridoxal was performed (Scheme 1). Following the procedure of Bailey et al. described for the kinetically controlled transformation,[9] tryptophan methyl ester, either dissolved as the free base or liberated in situ from its hydrochloric salt by the addition of 1.1 equiv of NEt_3, was treated with pyridoxal hydrochloride in the presence of molecular sieves in CH_2Cl_2 at reflux for 15–60 min to allow the formation of imine **2**. Cyclization to the tetrahydro-β-carboline products *cis*-**1** and *trans*-**1** was then effected by the addition of an excess of trifluoroacetic acid (TFA), while heating was continued for an additional 3–5 h.

The reaction products were found to depend on the exact reaction conditions. If 30 min were provided for the imine formation, and the subsequent acid treatment was performed for 3.5 h, the reaction delivered a 75% yield of the *cis*- and *trans*-β-carboline derivatives in a ratio of 93:7. The two compounds, however, only arose as racemates. If, on the other hand, only a short time was allowed for imine formation and TFA was added after just 15 min of mixing the starting materials, only a little racemization occurred (er >95:5). The desired products, however, arose in the unsatisfactory low chemical yield of 15% (*cis*/*trans* ratio of 95:5).

From these initial results we concluded that racemization occurs during imine formation, prior to the cyclization, and that imine formation has to be effected as completely as possible prior to the addition of TFA because it is inhibited by the excess of the acid. That racemization is a problem that can occur in the course



Scheme 1. *Pictet–Spengler* condensation of tryptophan methyl ester with pyridoxal, shown with L-tryptophan derivative as the starting material.

of the *Pictet–Spengler* condensation was discovered earlier. It was studied in more detail with the reaction of tryptophan methyl ester with benzaldehyde.[9] Loss of enantiomeric purity proved particularly prominent when the condensations were performed at elevated temperatures and in the presence of catalytic amounts of acid. Mechanistically, the racemization was rationalized by an imine (or iminium ion) tautomerism by which imine ^{L}**3** is in equilibrium with ^{D}**3** via compound **4** (or their protonated forms, Scheme 2). Considering the function of pyridoxal in biological processes it is readily conceivable that the corresponding imine–imine isomerisation, the reversible interconversion of ^{L}**2** and ^{D}**2**, is favored for the pyridoxyl as compared to the phenyl derivative. Due to the double activation of H-C(α) of the tryptophan moiety by the carboxyl and the pyridoximine groups, slightly basic conditions may lead to high isomerization rates. However, to circumvent the problem by working under purely acidic conditions is not feasible since imine formation is largely blocked when an excess of acid is present. In fact, when the hydrochlorides of both components of the transformation were reacted with each other at −15 °C and, to effect cyclization, at 23 °C for a prolonged period of time, the desired products were obtained in only 32% yield (as a *cis*/*trans* mixture of 56:44). In addition, the enantiomeric ratio of these products turned out to be only 88:12, indicating that the effect of avoiding basic conditions was offset by the effect of a longer reaction time under slightly acidic conditions.

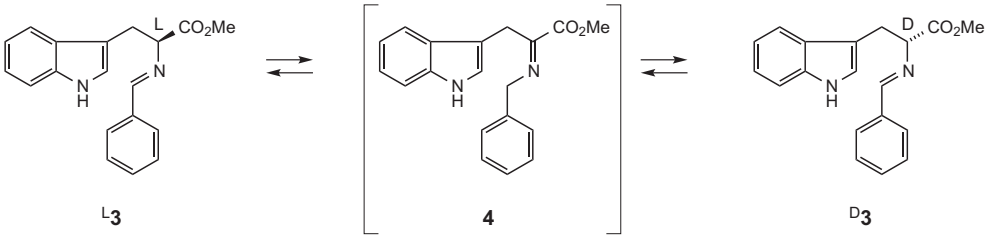
The best conditions to realize satisfactory chemical yields and high enantiomeric purities was to perform the imine formation under slightly basic conditions at low temperature over a prolonged period of time, followed by cyclization under strongly acidic conditions. Thus, tryptophan methyl ester was allowed to react with pyridoxal hydrochloride at −15°C for 18 h, and subsequently with TFA for an additional 18 h until cyclization was complete. This procedure delivered 79% of a mixture of *cis*-**1** and *trans*-**1** in a ratio of 75:25 and enantiomeric ratios of >95:5. The low *cis*/*trans* selectivity might appear as a drawback of this procedure. However, it was in fact beneficial for our purpose since we were interested in obtaining suitable amounts of both isomers for biological testing.

The diastereoisomeric products *cis*-**1** and *trans*-**1** were separated by column chromatography—the minor *trans* isomer eluting as the first component. Unfortunately, chromatography was associated with a considerable loss of material. The ^{1}H and ^{13}C NMR spectra of the two compounds show the typical signals reported for 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines as well as the expected signals for the pyridoxyl portions, proving the connectivity of the molecules. In the NMR spectra, particularly well observed in the ^{13}C NMR spectra, duplication of some of the signals was observed, indicating that the compounds are present as two conformers. For the stereochemical assignment of the compounds, several methods were applied. The relative configurations of the stereogenic centers in the structures were deduced by the ^{13}C NMR method reported by Ungemach et al.,[12] by ROESY spectroscopy, and by comparison of calculated and measured IR spectra of the two compounds. According to the investigation of Ungemach, the stereoisomer for which the carbon nuclei ^{13}C(1) and ^{13}C(3) are more deshielded can be assigned to the *cis*-configured compound. Accordingly, the second-eluting compound, with the respective ^{13}C signals found at δ 53.9 and 56.8 ppm, was deduced to be *cis*-**1** and the first-eluting component, with the corresponding signals registered at δ 50.3 and 54.4 ppm, to *trans*-**1**. This assignment was supported by the ROESY spectra of the two compounds. A distinct cross peak for the signals of H-C(1) and H-C(3) can be recognized in the spectrum of *cis*-**1** but not so in the spectrum of *trans*-**1**. Thus, the *cis* relationship of the two H atoms in *cis*-**1** is confirmed. Finally, the calculated IR spectra of *cis*-**1** and *trans*-**1** show distinctive differences in the regions of $1/\lambda$ 1200 cm^{-1} and 1250 cm^{-1}, which find their match in the experimental spectra of the two compounds.

The assignment of the absolute configurations in the several compounds of the type **1** is based on chemical considerations only. Since no mechanistic path for the *Pictet–Spengler* reaction is conceivable that would induce controlled inversion of the configuration at the stereogenic center of the starting amino acid, the absolute configuration (of the major enantiomer) at C(3) is assumed to be retained. Thus, the dextrorotary products obtained from l-tryptophan methyl ester, (+)-*cis*-^{L}**1** and (+)-*trans*-^{L}**1**, were assigned to be of (*S*)-configuration at C(3) of the tetrahydro-β-carboline frameworks, the levorotary products, (−)-*cis*-^{D}**1** and (−)-*trans*-^{D}**1**, resulting from the D-amino acid of (*R*)-configuration at this respective center. The enantiomeric purities of the samples were determined with the *cis*-isomers, which were base-line separated upon HPLC on a chiral stationary phase (*Nucleodex beta-PM*, *Macherey*–Nagel). We were not able to directly determine the enantiomeric ratio of the *trans*-isomers because these isomers did not separate sufficiently upon chiral HPLC. Since, however, racemization was shown to occur prior to the *Pictet–Spengler* cyclization, the enantiomeric ratio should be the same for both isomers deriving from the same batch.

2.2. Biological evaluation

The antiplasmodial activities of the four isomeric tetrahydro-β-carbolines (+)-*cis*-^{L}**1** (er >95:5), (+)-*trans*-^{L}**1**(er >95:5), (−)-*cis*-^{D}**1** (er = 88:12), and (−)-*trans*-^{D}**1** (er = 88:12) were investigated as described previously for **PT3**.[1] Thus, cultured *P. falciparum* was treated with the four compounds, and the growth inhibitory effects on the parasites was determined with the [^{3}H]-hypoxanthine incorporation assay.[13] The results are shown in Figure 2. As can be recognized from the graph, all four isomers of **1** show antiplasmodial activity. Strongest inhibition with an IC_{50} of 8 ± 1 μM was observed with (−)-*trans*-^{D}**1**, the *trans* product deriving from D-tryptophan, followed by (+)-*trans*-^{L}**1** (IC_{50} = 22 ± 3 μM). The inhibitory effects of the two *cis*-configured products (+)-*cis*-^{D}**1** and (−)-*cis*-^{L}**1** were almost the same (IC_{50} = 108 ± 11 μM and 91 ± 2 μM), but markedly lower than those of the *trans*-products. Since **PT3** showed an IC_{50} value of 14 μM in the original study,[1] it has to be assumed that the respective sample was mainly composed of the *trans*-configured isomer(s).



^{L}3 4 ^{D}3

Scheme 2. Proposed racemization of tryptophan imines by imine–imine tautomerism.

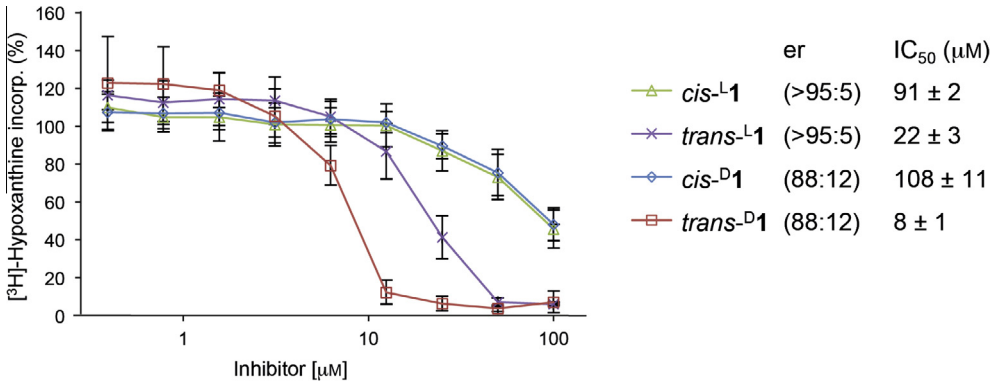


Figure 2. Effect of the enantiomerically enriched compounds of type **1** on cultured *P. falciparum*. Proliferation was determined after 48 h incubation by the [^{3}H]-hypoxanthine incorporation assay described in the Section 3. The experiment was conducted in at least three independent analyses, each in triplicate. Error bars and indicated errors in the IC_{50} values represent standard deviations.

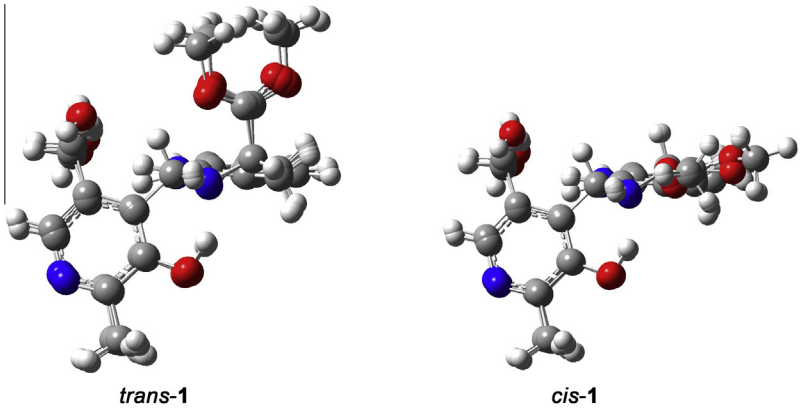


Figure 3. Overlay of the six lowest energy conformers of *trans*-**1** and *cis*-**1**, calculated with *Gaussian* on the DFT level with B3LYP functional and 6-31(d,p) basis set.

The structural dependency of the inhibitory effects is rather interesting. Evidently, the overall shape rather than a particular absolute configuration of a stereogenic center within the compounds is important for the biological activity. While in the *trans*-configured compounds the carboxyl group is directed almost perpendicular to the plane of the tetrahydro-β-carboline framework, the same group is located 'in-plane' in the case of the *cis*-configured products (Fig. 3). Possibly this characteristic is preferred for the molecules to fit into the enzymatic pocket. Surprisingly (−)-*trans*-^{D}**1**, the derivative of D-tryptophan, is the most active compound. Since L-ornithine is the substrate for the targeted *Pf*ODC it would be expected that a derivative of L-tryptophan would best fit into the active site of the enzyme. But this is not the case. This suggests that compounds of type **1** may not be targeting the originally envisioned *Pf*ODC but a different enzyme of the rather large family of PLP-dependent enzymes.[14]

2.3. Conclusion

We have shown that the four isomeric products of the *Pictet–Spengler* condensation of tryptophan and pyridoxal can be prepared in enantiomerically enriched form. However, partial racemization could not be prevented, even when rather mild conditions were used. For the preparation of enantiomerically pure compounds and for the specific synthesis of either the *cis* or the *trans* isomeric forms, more optimization of the reaction conditions would be necessary. The inhibitory effects on the proliferation of *P. falciparum* are, as expected, different for the four stereoisomeric compounds, and, with an IC_{50} value of 8 μM for *trans*-^{D}**1** rather promising. Apparently, since the *trans*-isomers show higher inhibitions than the *cis*-compounds, the active sites of the affected PLP dependent enzymes of *P. falciparum* presumably accommodate best molecules that have their carboxylic group located axially at the heterocycle, standing perpendicularly to the plane of the tetrahydro-β-carboline framework. This can be regarded as a key structural feature that should be considered when new analogous structures are developed with the goal to obtain new antimalarials that show activieties similar to established drugs with IC_{50} values in the nM range.

3. Experimental

3.1. General for synthesis and analysis

D-Tryptophan methyl ester hydrochloride was prepared according to the literature,[15] and the remaining starting materials and reagents were purchased from *Sigma–Aldrich*. The solvents CH_2Cl_2 and MeOH used for the reactions were of *puriss.* grade, absolute, stored over molecular sieve and also purchased from Sigma-Aldrich. Solvents used for column chromatography and thin layer chromatography (TLC) were of *techn.* grade. The reactions were carried out under N_2 in oven-dried (100 °C) glass equipment and monitored by TLC for completion. Thin layer chromatography (TLC): *Merck* tlc plates silica gel *60* on aluminum with fluorescence indicator F_{254}, with the indicated solvent system; the spots were visualized by UV light (254 and 366 nm). Column chromatography: *Fluka* silica gel *60* (40–63 μm), containing 0.1% Ca, with the indicated solvent system. Mp: Büchi *510*; heating rate 2 °C min^{-1};

range 2/3 to fully molten. Specific optical rotation $[\alpha]_D$: *JASCO* P-2000 polarimeter; concentration in g per 100 ml. IR and VCD spectra: ChiralIR-2X (*Bio Tools*), $1/\lambda$ in cm^{-1}, *s* = strong, *m* = medium, *w* = weak. ^1H NMR spectra: *Bruker* AC-600 (600 MHz), δ in ppm relative to the solvent peak (acetone, $\delta = 2.05$), coupling constant *J* in Hz. ^{13}C NMR spectra: *Bruker* AC-600 (150 MHz), δ in ppm rel. to the solvent (acetone, $\delta = 206.7$), multiplicities from DEPT-135 and DEPT-90 experiments. High resolution electrospray ionization mass spectrometry (HR-ESI-MS): Samples were analyzed with a *Waters Acquity* UPLC (*Waters*, Milford, USA) connected to an *Acquity* eλ detector and a *Bruker maXis* QTof high-resolution mass spectrometer (*Bruker Daltonics*, Bremen, Germany). An *Acquity BEH* C18 HPLC column (1.7 μm, 1 × 50 mm, *Waters*) was used with a mixture of H_2O + 0.1% HCOOH (A) and CH_3CN + 0.1% HCOOH (B) solvent (0.1 ml flow rate, linear gradient from 5% to 98% B within 4 min followed by flushing with 98% B for 1 min). UV(DAD) spectra were recorded between 200 and 600 nm at 1.2 nm resolution and 20 points s^{-1}. The mass spectrometer was operated in the positive electrospray ionization mode at 4000 V capillary voltage, −500 V endplate offset, with a N_2 nebulizer pressure of 1.6 bar and dry gas flow of 8 l min^{-1} at 200 °C. MS acquisitions were performed in the mass range of *m*/*z* 50–2000 at 20,000 resolution (full width at half maximum) and 1.5 Hz spectra rate. Prior to analysis, masses were calibrated between *m*/*z* 158 and 1450 below 2 ppm accuracy with a 2 mm soln. of HCO_2Na.

3.2. Methyl (1*S*,3*S*)- and (1*R*,3*S*)-1-(3-hydroxy-5-hydroxymethyl-2-methylpyridin-4-yl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (*cis*-^{L}**1** and *trans*-^{L}**1**)

Procedure with one component used as hydrochloride salt and the other as free base. L-Tryptophan methyl ester (1.91 g, 8.74 mmol, 1.0 equiv) and pyridoxal hydrochloride (1.96 g, 9.61 mmol, 1.1 equiv) were added to CH_2Cl_2 (50 ml) over activated molecular sieves (MS 3 Å, 8.5 g) at −15 °C. The mixture was stirred for 18 h at this temperature, then TFA (2.00 ml, 26.2 mmol, 3.0 equiv) was added. Stirring at −15 °C was continued for 1 h, then the mixture was slowly warmed to 23 °C (over 5 h), and stirring was continued for another 18 h. The suspension was filtered through a plug of cotton to remove the molecular sieves, the solids were washed with MeOH, and the solvent was evaporated in vacuo. The crude product was passed through a short column of SiO_2 (200 g, CH_2Cl_2/MeOH 10:1) to afford a material consisting primarily of the two desired products *cis*-^{L}**1** and *trans*-^{L}**1** in a ratio of 75:25 (yield: 2.54 g, 6.92 mmol, 79%). Column chromatography (500 g SiO_2, CH_2Cl_2/MeOH/NH_3 100:5:1) afforded *trans*-^{L}**1** (114 mg, first eluting isomer), *cis*-^{L}**1** (357 mg), and a mixture of the two isomers (779 mg). This mixture was re-chromatographed (250 g SiO_2) to afford a second batch of *trans*-^{L}**1** (80 mg) and *cis*-^{L}**1** (322 mg) and again some of the mixture of the two isomers (237 mg). Mass balance for the chromatographies: approximately 50% of the material was lost upon chromatography. Overall yields of the purified products, obtained as off-white amorphous solids: *trans*-^{L}**1** (194 mg, 0.53 mmol, 6%), *cis*-^{L}**1** (679 mg, 1.85 mmol, 21%). The enantiomeric ratio of *cis*-^{L}**1** (*er* >95:5) was determined by HPLC on a *Nucleodex beta-PM* column (particle size 5 μm, 200 × 4 mm, *Macherey–Nagel*, Düren, Germany) with 40% MeCN, 60% H_2O (containing 2% of Et_3NHOAc) as the eluent and using a flow rate of 0.7 ml min^{-1}. The areas of the signals detected at 224 and 300 nm (UV-DAD) at R_t = 15.5 min and 18.4 min for *cis*-^{L}**1** and *cis*-^{D}**1**, respectively, were used to calculate the ratio of the two enantiomers. The enantiomeric *trans*-^{L}**1** and *trans*-^{D}**1** were not separated (not even partially) under the given conditions.

Data of *cis*-^{L}**1** (major isomer; er >95:5, no signal detected in HPLC for *cis*-^{D}**1**): mp: 193.7–194.3 °C (decomp., precipitate obtained upon evaporation of the solvents after chromatography). $[\alpha]_D^{25}$ +59.6 (*c* 0.27, DMSO). On-line UV(DAD) (MeCN/H_2O/HCO_2H; in nm): λ_{min} 243, λ_{max} 281, λ_{min} 286, λ_{max} 289, λ_{infl} 319, λ_{sh} 331. IR: 3273*m*, 3057*w*, 2736*w*, 2334*w*, 1748*s*, 1388*m*, 1234*s*, 1139*m*, 1021*s*, 733*s*, 721*s*. ^1H NMR (CD_3COCD_3, the sample appears to be a mixture of atropisomers in a ratio of approx. 53:47; some signals are broadened or duplicated and overlapping; signal interpretation supported by a COSY and ROESY spectrum; numbering of the carbons according to the β-carboline nomenclature): 11.88/11.86 (2 *br. s*, Ar—OH); 9.64 (*br. s*, CH_2OH); 7.95/7.94 (2*s*, pyridyl H); 7.50 (*d*, *J* = 7.2, arom. C(5)H); 7.22 (*d*, *J* = 7.8, arom. C(8)H); 7.05–6.99 (symm. *m* with 12 lines, AB portion of ABMX system, C(6)H and C(7)H); 6.03–6.00 (*m* with broadened lines, C(1)H, couples with N(2)H and homoallylic with C(4)H_2); 4.96 (*br. s*, N(9)H); 4.94–4.93 (*m* with broadened signals, 1 H of CH_2OH); 4.76–4.73 (*m* with broadened signals, 1 H of CH_2OH); 4.18/4.15 (2 *br. s*, N(2)H); 4.07–4.05 (*m* with broadened signals, $CHCO_2$); 3.84 (*s*, CO_2CH_3); 3.29 (*ddd*, *J* = 15.1, 3.5, 1.4, C(4)H_{cis}); 3.03 (*ddd*, *J* = 15.1, 11.7, 2.6, C(4)H_{trans}); 2.23 (*s*, pyridyl CH_3). ROESY: crosspeak at 6.03–6.00 (*m* of C(1)H)/4.07–4.05 (*m* of C*H*CO_2) indicative for the *cis* relationship of the respective H-atoms. ^{13}C NMR (CD_3COCD_3; some signals are duplicated; numbering of the signals according to the β-carboline (n) and the pyridine (n′) nomenclature): 172.9 (*s*, *C*$OOCH_3$); 153.0/152.8 (2 *s*, pyridyl C(5′)); 148.93/148.86 (2 *s*, pyridyl C(4′)); 141.1/141.0 (2 *d*, pyridyl C(6′)H); 137.6/137.5 (2 *s*, C(8a)); 132.82/132.79/132.7/132.6 (4 *s*, 2 arom. C); 130.89/130.86 (2 *s*, 1 arom. C); 127.8/127.7 (2 *s*, 1 arom. C); 122.4 (*d*, C(6)H); 119.9 (*d*, C(7)H); 118.8 (*d*, C(5)H); 112.1/112.0 (2 *d*, C(8)H); 108.32/108.27 (*s*, C(4)); 61.0/60.9 (2 *t*, CH_2OH); 56.9/56.8 (2 *d*, C(3)H); 53.9/53.8 (2 *d*, C(1)H), 52.7 (*q*, CO_2CH_3); 26.0/25.9 (2 *t*, C(4)H_2); 19.2 (*q*, pyridyl CH_3). HR-ESI-MS: calcd. for $C_{20}H_{22}N_3O_4^+$ ($[M+H]^+$): *m*/*z* 368.16048; found: 368.16078.

Data of *trans*-^{L}**1** (minor isomer; er not determined but possibly the same as for *cis*-^{L}**1** (>95:5)): mp: 169.0–169.9 °C (precipitate obtained upon evaporation of the solvents after chromatography). $[\alpha]_D^{25}$ −10.6 (*c* 0.26, DMSO). On-line UV(DAD) (MeCN/H_2O/HCO_2H; in nm): λ_{min} 246, λ_{max} 281, λ_{min} 286, λ_{max} 290, λ_{infl} 319, λ_{sh} 329. IR: 3305*m*, 2952*m*, 2906*m*, 2358*w*, 1731*m*, 1709*m*, 1348*m*, 1219*s*, 1129*m*, 1015*s*, 745*s*. ^1H NMR (CD_3COCD_3, the sample appears to be a mixture of atropisomers in a ratio of approx. 50:50; some signals are broadened or duplicated and overlapping; signal interpretation supported by a COSY and ROESY spectrum; numbering of the carbons according to the β-carboline nomenclature)): 12.02/12.00 (2 *br. s*, Ar—OH); 9.73 (*br. s*, CH_2OH), 7.95 (*s*, pyridyl H); 7.48 (*d*, *J* = 7.5, C(5)H); 7.19, (*d*, *J* = 7.9, C(8)H); 7.03–6.97 (well-structured *m*, AB portion of ABMX system, C(6)H and C(7)H); 6.26–6.27 (*m* with broadened lines, C(1)H); 5.12 (*br. s*, N(9)H); 4.95–4.92 (*m*, 5 broadened lines, 1H of CH_2OH); 4.77–4.74 (*m*, 6 lines, 1H of CH_2OH); 4.48–4.47 (*m* with broadened signals, $CHCO_2$); 4.36/4.32 (2 *br. s*, N(2)H); 3.66 (*s*, CO_2CH_3); 3.46 (*dt*, *J* = 15.6, 1.5, C(4)H_{cis}); 3.35 (*ddd*, *J* = 15.7, 6.2, 2.3, C(4)H_{trans}); 2.23 (*s*, pyridyl CH_3). ROESY: missing crosspeak at 6.26–6.27 (*m* of C(1)H)/4.48–4.47 (*m* of $CHCO_2$) indicative for the *trans* relationship of the respective H-atoms. ^{13}C NMR (CD_3COCD_3; some signals are duplicated; numbering of the signals according to the β-carboline (n) and the pyridine (n′) nomenclature): 174.0 (*s*, *C*$OOCH_3$); 153.2/153.0 (2 *s*, pyridyl C(5′)); 149.0/148.9 (2 *s*, pyridyl C(4′)); 141.3/141.2 (2 *d*, pyridyl C(6')H); 137.3/137.2 (2 *s*, C(8a)); 132.6, 131.8/131.7, 131.27/131.25 (5 *s*, 3 arom. C); 127.8/127.8 (2 *s*, 1 arom. C); 122.4 (*d*, C(6)H); 119.8 (*d*, C(7)H); 118.8 (*d*, C(5)H); 112.0/111.9 (2 *d*, C(8)H); 106.9/106.8 (2 *s*, C(4)); 60.9/60.8 (2 *t*, CH_2OH); 54.5/54.4 (2 *d*, C(3)H), 52.4 (*q*, CO_2CH_3); 50.3/50.2 (2 *d*, C(1)H); 24.00/23.97 (2 *t*, C(4)H_2); 19.2 (*q*, pyridyl CH_3). HR-ESI-MS: calcd for $C_{20}H_{22}N_3O_4^+$ ($[M+H]^+$): *m*/*z* 368.16048; found: 368.16088.

3.3. Methyl (1*R*,3*R*)- and (1*S*,3*R*)-1-(3-hydroxy-5-hydroxymethyl-2-methylpyridin-4-yl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-b]indole-3-carboxylate (*cis*-^{D}**1** and *trans*-^{D}**1**)

Procedure with both starting materials used as hydrochloride salts and without addition of TFA. D-Tryptophan methyl ester hydrochloride (1.15 g, 4.52 mmol, 1.0 equiv) and pyridoxal hydrochloride (1.18 g, 5.80 mmol, 1.3 equiv) were added to CH_2Cl_2 (30 ml) over activated molecular sieves (3 Å, 7.0 g) at −15 °C. The mixture was stirred for 24 h at this temperature and, then it was slowly warmed to 23 °C (over 5 h), and stirring was continued for another 18 h. The suspension was filtered through a plug of cotton to remove the molecular sieves, the solids were washed with MeOH, and the solvent was evaporated in vacuo. The crude product was passed through a short column of SiO_2 (200 g, CH_2Cl_2/MeOH 10:1) to afford a material consisting primarily of the two desired products *cis*-^{D}**1** and *trans*-^{D}**1** in a ratio of 56:44 (yield: 537 mg, 1.46 mmol, 32%). Column chromatography (500 g SiO_2, CH_2Cl_2/MeOH/NH_3 100:5:1) afforded *trans*-^{D}**1** (129 mg), *cis*-^{D}**1** (198 mg), and a mixture of the two isomers (98 mg). Re-chromatography of this mixture and again of the mixture obtained therefrom resulted in additional *trans*-^{D}**1** (6 + 7 mg), *cis*-^{D}**1** (10 + 19 mg), and a remaining mixture of the two isomers (35 mg). Overall yields of the purified products, obtained as off-white amorphous solids: *trans*-^{D}**1** (142 mg, 0.39 mmol, 9%), *cis*-^{D}**1** (227 mg, 0.62 mmol, 14%). The enantiomeric ratio of *cis*-^{D}**1** (er = 88:12) was determined by HPLC as described above for *cis*-^{L}**1**.

Data of *cis*-^{D}**1** (major isomer, er = 88:12): mp: 192.0–192.4 °C (decomp., precipitate obtained upon evaporation of the solvents after chromatography). $[\alpha]_D^{25}$ −53.6 (*c* 0.27, DMSO). UV, IR, and NMR data identical to those of *cis*-^{L}**1**. HR-ESI-MS: calcd. for $C_{20}H_{22}N_3O_4^+$ ($[M+H]^+$): *m*/*z* 368.16048; found: 368.16120.

Data of *trans*-^{D}**1** (minor isomer; er not determined but possibly the same as for *cis*-^{D}**1** (8:12)): mp: 168.60–169.9 °C (precipitate obtained upon evaporation of the solvents after chromatography). $[\alpha]_D^{25}$ +6.0 (*c* 0.25, DMSO). UV, IR, and NMR data identical to those of *trans*-^{L}**1**. HR-ESI-MS: calcd for $C_{20}H_{22}N_3O_4^+$ ($[M+H]^+$): *m*/*z* 368.16048; found: 368.16092.

3.4. Inhibition assays on cultured *P. falciparum*

P. falciparum 3D7-strain was maintained in continuous culture according to Trager and Jensen[16] with modification.[13] The parasites were grown in human erythrocytes (O+), RPMI 1640 medium supplemented with 25 mm HEPES, 20 mm aq $NaHCO_3$ soln, and 0.5% AlbuMAX II (*Invitrogen*, Germany) at 4% hematocrit. The cells were cultivated in 90 mm *Petri* dishes (*Nunc*, Denmark) and incubated at 37 °C in the presence of 90% N_2, 5% O_2, and 5% CO_2. The impact of the four pyridoxyl-adducts of type **1** on the erythrocytic stages of *P. falciparum* was determined by using the [^{3}H]-hypoxanthine incorporation assay as described previously,[13] and the IC_{50} values were calculated from at least three independent sigmoidal inhibition curves as shown in Figure 2 using *GraphPad Prism 4.0*. Chloroquine was used as positive control to confer optimal assay conditions. The individual IC_{50} values including standard deviations (SD) for the four samples are: 91 ± 2 μM (*cis*-^{L}**1**, er >95:5), 22 ± 3 μM (*trans*-^{L}**1**, estimated er >95:5), 108 ± 11 μM (*cis*-^{D}**1**, er = 88:12), and 8 ± 1 μM (*trans*-^{D}**1**, estimated er = 88:12).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.057. These data include MOL files and InChiKeys of the most important compounds described in this article.

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