Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 5155–5164 **5155**
DOI: 10.1021/jm100410f

Spirotetrahydro β-Carbolines (Spiroindolones): A New Class of Potent and Orally Efficacious Compounds for the Treatment of Malaria

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Received April 1, 2010

The antiplasmodial activity of a series of spirotetrahydro β-carbolines is described. Racemic spiroazepineindole (**1**) was identified from a phenotypic screen on wild type *Plasmodium falciparum* with an in vitro IC_{50} of 90 nM. Structure−activity relationships for the optimization of **1** to compound **20a** (IC_{50} = 0.2 nM) including the identification of the active 1*R*,3*S* enantiomer and elimination of metabolic liabilities is presented. Improvement of the pharmacokinetic profile of the series translated to exceptional oral efficacy in the *P. berghei* infected malaria mouse model where full cure was achieved in four of five mice with three daily doses of 30 mg/kg.

Introduction

Malaria remains a persistent public health problem for about 40% of the global population, mainly in sub-Saharan Africa. *Plasmodium falciparum*, the most relevant malaria parasite, is estimated to infect 300−500 million people per year and result in over 863000 deaths in 2008 of which approximately 90% were children.[1] Malaria control programs relying on disease prevention and artemisinin combination therapies (ACT[a]) have been extremely effective in reducing the disease burden. However, recent reports of increased tolerance to artemisinin derivatives in *Plasmodium falciparum* suggest that we may soon lose the last and only widely effective antimalarial drugs.[2]

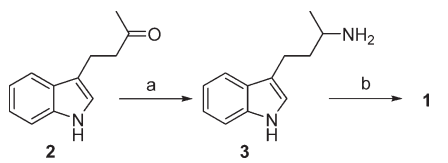
In an effort to discover new antimalarial leads, a high throughput, whole cell screen based on the proliferation of *P. falciparum* was performed and led to the identification of racemic spiroazepineindole **1**.[3] The compound displayed moderate potency against both wild type (NF54) and chloroquine resistant (K1) strains of the parasite, and when tested in the *Plasmodium berghei* infected mouse model,[4] a single 100 mg/kg dose of **1** resulted in a 96% reduction in parasitemia (activity). Encouraged by these preliminary results, an optimization effort to increase potency and in vivo activity of **1** was initiated. The structure−activity relationships (SAR) and in vivo efficacy of this new class of antimalarial is described.[5]

Chemistry

To reconfirm the in vitro potency of the hit, racemic **1** was prepared by reductive amination of ketone **2** followed by a Pictet−Spengler cyclization[6] with 5-bromoisatin, to provide spiroazepineindole **1** in 66% yield (Scheme 1).[7] The reaction conditions were expected to provide an equal mixture of four stereoisomers consisting of two pairs of the 1*R*,3*S*, 1*S*,3*R*, and 1*S*,3*S*, 1*R*,3*R* enantiomers. Instead, the reaction produced a ~9:1 diastereomeric excess favoring one pair of enantiomers.[8] To determine which pair of enantiomers were formed in excess, chiral separation followed by X-ray crystallography revealed that the more favored pair consisted of the 1*R*,3*S* and 1*S*,3*R* stereoisomers (data not shown). Interestingly, when tested in vitro, the individual enantiomers displayed markedly different potencies on the parasite where **1a** was greater than 250-fold more potent than **1b** (Figure 1) on strain NF54.

All spiroazepineindole derivatives (**1**, **4**, and **6**) were prepared according to Scheme 1 starting from the appropriate indoleamine, while the spirotetrahydro β-carboline derivatives (**17**−**20**) were synthesized from the corresponding substituted indoles (Scheme 2).[9] Briefly, Vilsmeyer−Haack formylation of the indole followed by condensation with

Scheme 1. Synthesis of Racemic **1**



Reagents and conditions: (a) $NaBH_3CN$, NH_4OAc, rt; (b) 5-bromoisatin, *p*-TsOH·H_2O (cat), 110 °C.

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[a]Abbreviations: ACT, artemisinin combination therapies; AS, artesunate; CQ, chloroquine; CYP450, cytochrome P450; PAMPA, parallel artificial permeability assay; hERG, human ether-a-go-go related gene.

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Published on Web 06/22/2010

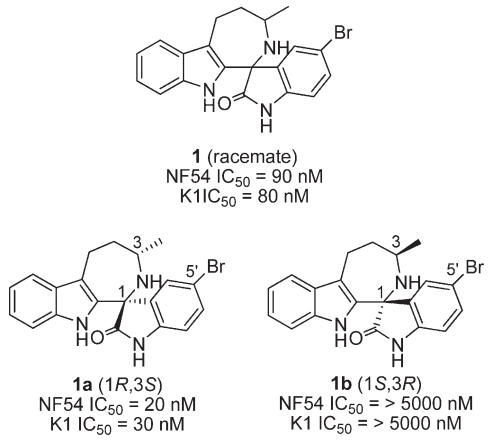


Figure 1. In vitro activity of spiroindolone **1** and the readily accessible stereoisomers.

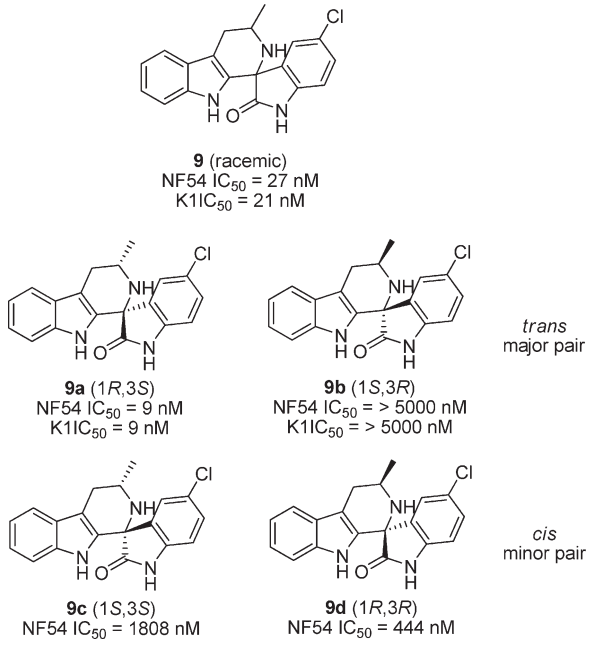


Figure 2. In vitro activity of spiroindolone **9** and its four stereoisomers.

nitroethane provided the corresponding nitroalkene. Next, reduction of the nitroalkene with lithium aluminum hydride, followed by condensation with 5-chloroisatin provided racemic **17**−**20** with a high degree of diastereoselectivity. The individual 1*R*,3*S* and 1*S*,3*R* enantiomers were then resolved by chiral chromatography and could be readily identified and differentiated by optical rotation.

In general, the minor pair of diastereomers was not isolated from the reaction mixture except in a few cases. For example, in the case of **9**, also prepared according to Scheme 2, all four stereoisomers were isolated and tested individually for potency. The minor 1*S*,3*S* and 1*R*,3*R* pair of stereoisomers were found to be less potent than the corresponding active 1*R*,3*S* stereoisomer (Figure 2). This trend was found to be consistent across the class and in all cases where all four stereoisomers were isolated. From these results, we concluded that the 1*R*,3*S* configuration was the required stereochemistry for activity on *P. falciparum*.

Diasteroselectivity in the Pictet−Spengler reaction is well documented and has been studied by several groups.[10] We sought to take advantage of the high degree of diastereoselectivity observed in our system by using enantiopure *S*-α-methyl indoleamines instead of the racemic mixtures. This would directly provide the corresponding 1*R*,3*S* enantiomer and obviate the need for chiral resolution, as only a single pair of diastereomers would be formed.[11]

Enantiopure **9a** and **9b** were obtained from the corresponding *S*- and *R*-α-methyl indoleamines respectively. (*S*)-2-(1*H*-Indol-3-yl)-1-methyl-ethylamine (**22**) was prepared from D-tryptophanol after complete reduction of the hydroxymethyl

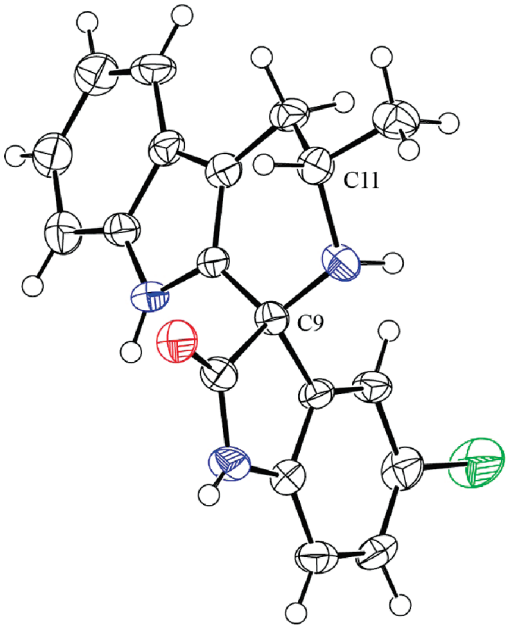
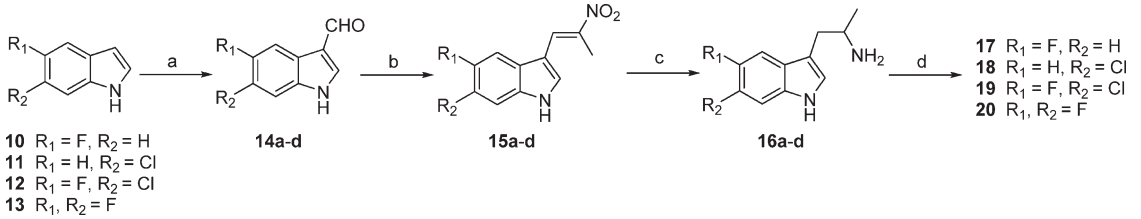


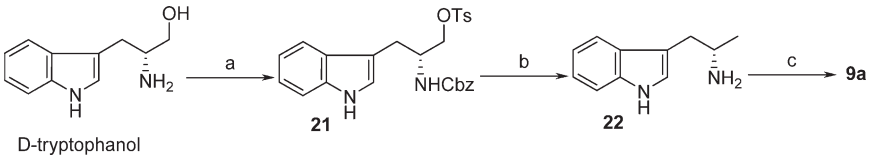
Figure 3. Crystal structure of the active 1*R*,3*S* enantiomer of **9a** (only one of the two independent molecules is shown). Atomic displacement ellipsoids are drawn at the 50% probability level, hydrogen atoms drawn as spheres of arbitrary radius. For clarity only the stereogenic centers are labeled (C9*R*, C11*S*).

Scheme 2. Racemic Route to the Spirotetrahydro β-Carbolines Series



Reagents and conditions: (a) $POCl_3$, DMF; (b) nitroethane, ammonium acetate, reflux; (c) $LiAlH_4$, THF, reflux; (d) 5-chloroisatin, *p*-TsOH·H_2O (cat), 110 °C.

Scheme 3. Synthesis of the Active Enantiomer of **9a** from D-Tryptophanol



Reagents and conditions: (a) (1) CbzCl, Na_2CO_3, rt; (2) *p*-TsCl, TEA, rt; (b) H_2, $Pd(OH)_2$, rt; (c) 5-chloroisatin, *p*-TsOH·H_2O (cat), 110 °C.

Table 1. In Vitro Antimalarial Activity and Structure−Activity Relationships of the Spiroindolones

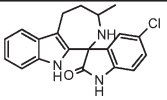
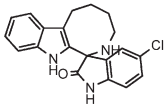
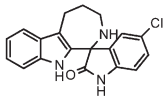
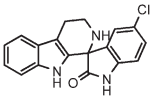
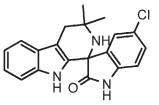
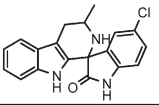
compound	structure	activity on *P. falciparum* strains		comment
		NF54 IC_{50} (nM)	K1 IC_{50} (nM)	
4		84	n.d.	racemate-1*R*,3*S* and 1*S*,3*R*
5		> 5000	> 5000	racemate
6		86	83	racemate
7		126	104	racemate
8		857	819	racemate
9		27	21	racemate-1*R*,3*S* and 1*S*,3*R*

Table 2. Key Physicochemical Properties of the Spiroindolones[a]

compd	solubility (pH 6.8) (μg/mL)	logP	$clogD_{7.4}$	pK_a^1/pK_a^2
1a	79	3.9	4.0	5.2/nd
9a	194	3.4	3.5	5.1/10.4
17a	140	3.6	3.8	4.8/nd
18a	8	4.8	4.4	5.2/10.6
20a	170	4.0	3.9	4.9/nd

[a] Data were experimentally determined except $clogD_{7.4}$; nd, not determined.

to the corresponding methyl group (Scheme 3).[12] Subsequent cyclization with 5-chloroisatin in the presence of catalytic acid provided enantiopure **9a** after purification. The reduction of the tosyl group could also be achieved with lithium aluminum hydride with no observable racemization of the stereogenic center. The opposite, 1*S*,3*R* enantiomer (**9b**) was obtained from L-tryptophanol using the same procedure.

An X-ray crystal structure of **9a** unambiguously assigned the 1*R*,3*S* configuration to the active enantiomer (Figure 3) and showed that both potency and the corresponding stereochemistry of the spirotetrahydro β-carboline series was consistent with that of the spiroazepineindole series (**1a**).

The only drawback of this potentially ideal route is the limited availability of chiral, substituted α-methyl indoleamines or D-tryptophan derivatives. Because of this, we chose the racemic route to compounds **17**−**20** utilizing a chiral resolution step as the preferred synthesis to explore SAR. Upon identifying biologically active racemates in vitro, the potency of a particular compound could always be improved by isolation or synthesis of the corresponding 1*R*,3*S* enantiomer.

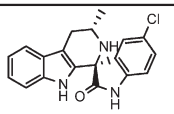
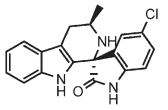
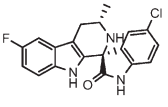
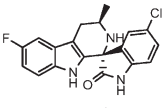
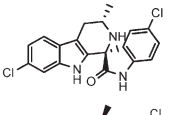
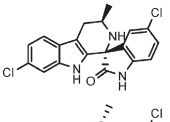
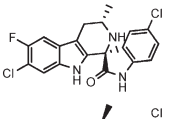
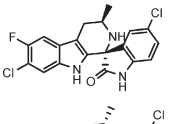
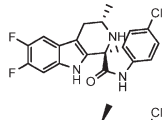
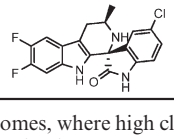
Results and Discussion

Structure−Activity Relationships. On the basis of the difference in potencies between the enantiomers, we surmised that the spirocenter was an essential feature of the scaffold, and indeed its removal lead to an almost complete loss of activity for the series (see Supporting Information). Hence to readily access the spirocenter, we incorporated the oxindole moiety via isatins in all of the derivatives described.

Replacement of the 5′-bromide of **1** with other halogens had negative effects except for the 5′-chloro derivative (**4**). Although a number of additional mono- and disubstituted oxindole derivatives were prepared, only the monosubstituted, 5′-chloro derivatives had the optimum balance between potency, favorable pharmacokinetics, and synthetic accessibility.

Simplifying the structure by removing the C3 stereogenic center and increasing the size of the central ring azepine ring

Table 3. Comparison of the Pharmacokinetic Profile of the Spirotetrahydro β-Carbolines[c]

compound	structure	hepatic CL_{int}[a]		CYP 2C9	in vivo CL[b]	NF54
		mouse	human	inhibition (μM)	mL/min/kg	IC_{50} (nM)
9a		high	high	1.51	49.66	9
9b		low	low	> 10.00	n.d.	> 5000
17a		high	med	1.72	24.01	3
17b		med	low	> 10.00	n.d.	182
18a		low	low	4.11	60.08	4
18b		low	low	> 10.00	n.d.	116
19a		low	low	5.42	9.75	0.9
19b		low	low	> 10.00	2.58	77
20a		low	low	7.35	8.53	0.2
20b		low	low	> 10.00	4.14	83

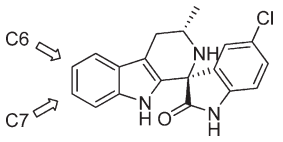
[a] intrinsic clearance in liver microsomes, where high clearance corresponds to poor stability in the presence of liver enzymes. [b] Measured in mice at a single 5 mg/kg iv dose. [c] nd, not determined.

by one carbon lead to an inactive compound (**5**: IC_{50} > 5000 nM); however, azepineindole (**6**) and tetrahydro-β-carboline (**7**) derivatives retained moderate potency (Table 1). Interestingly for the azepineindoles, the similar potency between **4** and **6** suggests that the C3 methyl is not essential in this series. In contrast, the C3 methyl enhances potency in the tetrahydro β-carboline series, where an almost five-fold increase in activity is observed with the methyl group present (**7** vs **9**). Substitutions at C3 were found to be extremely limited. Only methyl or trifluoromethyl was tolerated because increasing the steric bulk to *gem*-dimethyl (**8**), hydroxymethyl, *n*-propyl, or methyl ester significantly decreased activity even with the required stereochemistry (see Supporting Information).

Optimizing the Pharmacokinetic Profile. The spiroindolones as a class proved to be an exceptionally high quality scaffold from a medicinal chemistry perspective (Table 2). Most derivatives prepared displayed favorable in vitro solubility and permeability (PAMPA and Caco-2). Derivatives generally did not show cytotoxicity across several human cell lines nor was there significant binding in a panel of human receptors, kinases, and ion channels (IC_{50} > 10 μM). Moreover the spiroindolones display low cardiotoxicity potential (hERG), and genotoxicity potential (miniaturized Ames test and high content micronucleus), hence no effort to improve these properties was required.[13]

In addition to in vitro potency, significant differences between pairs of enantiomers were observed with respect to metabolic stability and CYP450 inhibition. Compounds **9a** and **9b** proved to be representative of the types of trends we observed for most analogues in Table 3. More specifically, the less active, or 1*S*,3*R*, enantiomers displayed superior metabolic stability in the presence of liver microsomes, had low to medium in vivo clearance, and showed little or no propensity for drug−drug interactions (CYP450 liabilities). In contrast, the biologically active 1*R*,3*S* enantiomers often displayed high clearance with poor microsomal stability and were found to inhibit CYP 2C9 preferentially although were generally inactive against CYP 3A4 and CYP 2D6 isoforms (Table 3).

To address the metabolic stability of **9a** and to reduce its CYP 2C9 liabilities, we identified susceptible sites on the molecule which could be modified or blocked by chemical means and translate into a longer half-life.



Focusing on the western part of the molecule, positions C6 and C7 stood out as susceptible metabolic sites prone to oxidation and thus high clearance. The racemic 6-fluoro derivative (**17**) showed a moderate improvement in metabolic stability, however, upon separation of the two enantiomers (**17a** and **17b**), only the less active 1*S*,3*R* enantiomer displayed the desired stability profile in vitro (Table 2). Moreover the 6-fluoro substitution had no effect on decreasing CYP 2C9 inhibition although potency of the active enantiomer (**17a**) had improved slightly. Interestingly, despite the high clearance and low stability predicted from in vitro assays, **17a** showed a nearly two-fold improvement in clearance over **9a** in vivo.

In contrast to **17**, when the individual enantiomers of **18** were profiled, the active 1*R*,3*S* enantiomer (**18a**) showed improved microsomal stability that was similar to **18b**. In addition a three-fold improvement in potency on the parasite and lower CYP 2C9 inhibition was achieved over **9a** (Table 2). Combining both C6 and C7 substitutions had an additive affect on potency and further improved the pharmacokinetic properties. Compounds **19a** and **20a** now displayed subnanomolar potency on the parasite with the desired in vitro stability and decreased CYP 2C9 liabilities. Thus C7 substitutions were critical in increasing metabolic stability, minimizing drug−drug interaction, and increasing in vitro potency.

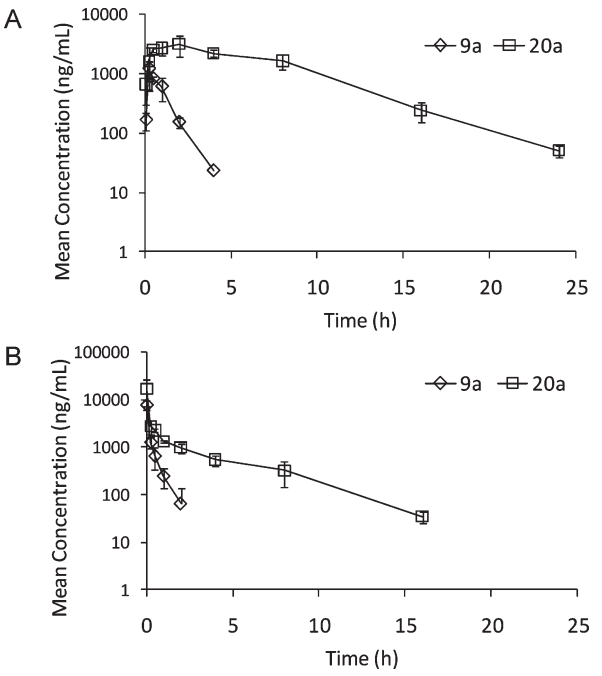


Figure 4. In vivo PK profile of **9a** and **20a** showing a plot of plasma concentration vs time for **9a** (◇) and **20a** (□) administered orally (A) and intravenously (B) to mice. Plasma concentrations are indicated in ng/mL ± standard deviation (n = 3).

In Vivo Pharmacokinetic Profile of 9a and 20a. The marked difference in the in vitro PK profile of unsubstituted (**9a**) and substituted (**20a**) spiroindolones translated well in vivo (Figure 4 and Table 4). Following intravenous administration, **9a** displayed a moderate total systemic clearance (55% hepatic blood flow), moderate volume of distribution (0.91 L/kg or 1.2 times the total volume of body water), and short half-life of 0.42 h. The relatively low oral exposure of **9a** (3.88 μM·h) resulted in modest oral bioavailability (13%). In contrast, after intravenous administration, **20a** displayed a higher volume of distribution, 2.7 times the total body water (1.60 L/kg). Similar to the low in vitro clearance observed in liver microsomes, the total systemic clearance was lower (~10% hepatic blood flow) and the compound had a markedly longer half-life than **9a**. Although the oral absorption (T_{max}) of **20a** was significantly slower than **9a**, the compound reached both higher peak plasma concentrations and 18-fold greater exposure (AUC_∞) than **9a**. The increased exposure translated to an apparent bioavailability of 53%. Moreover the C_{max} of **20a** in mice is about 40000-fold above the *Plasmodium falciparum* IC_{50} suggesting lower doses for efficacy and decreased potential for adverse toxicity.

Table 5. Single Dose Efficacies of **9a** and **20a** in the *P. berghei* Mouse Model[a]

	1 × 30 mg/kg		1 × 100 mg/kg	
compd	activity (%)	survival (d)	activity (%)	survival (d)
CQ	99.5	9.0	99.6	12.5
AS	95.6	5.8	98.0	7.0
9a	99.9	10.7	99.9	14.3
20a	99.6	12.0	99.4	13.0

[a] Control survival, 6−7 days; CQ, chloroquine; AS, artesunate; cure, no parasites at day 30; compounds were formulated in 10% ethanol, 30% PEG400, 6% vitamin E TPGS.

Table 6. Multiple Dose Efficacies of **9a** and **20a** in the *P. berghei* Mouse Model[a]

	3 × 10 mg/kg		3 × 30 mg/kg	
compd	activity (%)	survival (d)	activity (%)	survival (d)
CQ	99.8	13.6	99.8	14.3
AS	96.0	8.1	99.0	12.2
9a	99.9	14.6	99.9	23.8
20a	99.8	17.2	99.8	29.4

[a] Control survival, 6−7 days; CQ, chloroquine; AS, artesunate; cure, no parasites at day 30; compounds were formulated in 10% ethanol, 30% PEG400, 6% vitamin E TPGS.

Table 4. Pharmacokinetic Parameters for **9a** and **20a** Following Oral Dosing at 25 mg/kg and iv Dosing at 5 mg/kg in Mice[a]

	oral PK parameters					intravenous PK parameters		
compd	C_{max} (μM)	T_{max} (h)	AUC_∞ (μM·h)	$T_{1/2}$ (h)	F (%)	V_{ss} (L/kg)	CL (mL/min/kg)	$T_{1/2}$ (h)
9a	3.58	0.25	3.88	0.66	13	0.91	49.66	0.42
20a	8.32	2.00	71.44	3.18	53	1.60	8.53	2.94

[a] C_{max}, maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; AUC_∞, area under the curve extrapolated to infinity; V_{ss}, volume of distribution at steady state; CL, clearance; $T_{1/2}$, half-life; F, oral bioavailability.

Collectively, the data suggests that the excellent in vitro potency, good bioavailability, long oral exposure, and half-life (3 h) of **20a** is amenable to a single or low dose oral treatment for malaria. To evaluate the oral efficacy of **9a** and **20a**, the compounds were tested in the *P. berghei* infected malaria mouse model.

In Vivo Efficacy in the *P. berghei* Malaria Mouse Model. Average mouse survival results using two different oral dosing regimens of **9a** and **20a** are presented along with chloroquine (CQ) and artesunate (AS) as comparisons (Table 5). The spiroindolones show excellent in vivo activity. A single oral dose of **9a** (30 mg/kg) reduced parasitemia on day three by more than 99% and prolonged average mouse survival to 10.7 days. Increasing the dose to 100 mg/kg had only a marginal effect on mouse survival (14.3 days) and may be attributed to the high clearance of the compound. At single doses, the increased absorption (high C_{max}) and greater exposure (AUC) of **20a** did not lead to a significant improvement in efficacy. A single oral dose of 30 mg/kg reduced parasitemia by more than 99% and prolonged mouse survival for 12 days while increasing the dose to 100 mg/kg lead to essentially no change in mouse survival. However, under these conditions, both spiroindolones were comparable to CQ (99.6%, 12.5 days) and outperformed AS (98.0% and 7.3 days) in terms of average mouse survival.

In a multiple oral dosing regimen, differences between **9a** and **20a** became more apparent where, **20a** proved to be superior to **9a**, CQ, and AS (Table 6). Three oral daily doses of 10 mg/kg resulted in 17.2 days average mouse survival while 29.4 days survival was achieved with 3 × 30 mg/kg oral doses (full cure in four out of five mice). In addition, neither toxicity nor behavioral changes after drug administration was observed in any of the animals treated with **9a** and **20a** at any dosing regimen.

The in vivo efficacy data suggests that despite the high clearance and short half-life, **9a** is a highly efficacious antimalarial when administered orally and comparable to standard antimalarials in the *P. berghei* mouse model. Optimizing the metabolic stability and improving potency resulted in increased oral absorption, exposure, and efficacy in mice. The in vivo profile and efficacy of **20a** is consistent with pharmacokinetic properties required for a single dose or low dose cure for malaria.

The spiroindolones represents a promising new antimalarial chemotype, displaying exceptional in vitro activity and parasitemia reduction at low doses in the *P. berghei* mouse model. Although the mechanism of action (MoA) and molecular target is currently unknown, preliminary evidence suggests that the spiroindolones function by a different mechanism than the heme detoxification pathway of 4-aminoquinolines and instead may act by inhibiting protein synthesis within the parasite.[14] Moreover, the observation that only the 1*R*,3*S* enantiomer is biologically active implies the presence of a discrete molecular target. Further preclinical evaluation of this series of compounds is currently ongoing.

Experimental Section

General Methods. Reagents and solvents were purchased from Aldrich, Acros, or other commercial sources and used without further purification. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates from Merck. Compounds were visualized under UV light, ninhydrin, or phosphomolybdic acid (PMA) stain. NMR spectra were obtained on a Varian 300 MHz Mercury NMR or Bruker 500 MHz Ultrashield spectrometer using $CDCl_3$, DMSO-d_6, or MeOD-d_4 as solvents. Preparative chiral separation was performed on a Chiracel OD-H column (2 cm × 25 cm) with 90:10 hexanes:ethanol as the mobile phase. Analytical chiral chromatography was carried out on a Chiracel AD-H column and all single enantiomers reported were greater than 98% e.e. Compound purity was determined by LC/MS and HPLC and carried out on an Agilent LC110 HPLC equipped with a Waters Symmetry Shield RP18, 3.5 μm, 4.6 mm × 150 mm column using a gradient (13 min) of 95:5 H_2O (0.1% formic acid):CH_3CN to 5:95 H_2O (0.1% formic acid):CH_3CN. The purity of all compounds reported were >95% measured at 254 nm. The specific rotation reported for all compounds was measured on a Jasco P-1020 polarimeter at 25 °C with sample concentration and solvent noted where appropriate.

X-ray Structure Determination. Intensity data collection for **9a** was performed on the PX II beamline at the Swiss Light Source in Villigen at a wavelength of 0.71073 Å, which corresponds to Mo Kα radiation. The structure was solved by dual-space recycling methods and refined by full-matrix least-squares on F^2 using the SHELXTL program suite. The asymmetric unit contains two independent molecules. Non-hydrogen atoms were refined with anisotropic displacement parameters; hydrogen atoms were calculated in idealized positions and refined using a riding model. The crystals are twinned (twin law −1 0 0 0 −1 0 0 0 1). CCDC 758814 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Crystal data for **9a**: colorless rod from chloroform/hexadecane, size 0.04 × 0.02 × 0.01 mm^3, $C_{19}H_{16}ClN_3O$, M_r = 337.80, trigonal space group $P3_2$ (No. 145) with a = 14.834(2), c = 13.354(6) Å, V = 2544.8(7) $Å^3$, Z = 6, D_c = 1.323 $g \cdot cm^{-3}$, 19289 reflections measured, 5109 independent (R_{int} = 0.0429), 2.20° < θ < 24.10°, T = 100 K, 444 parameters, 1 restraint, R_1 = 0.0279, wR_2 = 0.0637 for 4987 reflections with $I > 2\sigma(I)$, R_1 = 0.0294, wR_2 = 0.0645 for all 5109 data, GoF = 1.048, res el dens = +0.13/−0.17 $e \cdot Å^{-3}$. Absolute structure parameter x = 0.08(6).

3-(1*H*-Indol-3-yl)-1-methyl-propylamine (3). Ammonium acetate (4.45 g, 57.7 mmol) and sodium cyanoborohydride (0.37 g, 5.9 mmol) were added to a solution of 4-(1*H*-indol-3yl)-butan-2-one (**2**) (1.0 g, 5.3 mmol) in methanol (20 mL) and stirred at room temperature. After 4 h, the reaction mixture was quenched by slow addition of 1 N hydrochloric acid and adjusted to pH ∼ 2. The mixture was concentrated in vacuo and diluted with dichloromethane. The aqueous phase was adjusted to pH ∼ 12 with 4 N NaOH and washed with dichloromethane (3 × 50 mL). The combined organic phases were dried over sodium sulfate and concentrated in vacuo. The residue was purified by flash chromatography to afford **3** (622 mg, 62%) as an oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.71 (s, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.08 (d, J = 2.1 Hz, 1H), 7.04 (td, J = 8.1, 1.5 Hz, 1H), 6.95 (td, J = 7.2, 1.2 Hz, 1H), 2.81 (m, 1H), 2.70 (m, 2H), 1.61 (m, 2H), 1.02 (d, J = 6.3 Hz, 3H). MS (ESI) m/z 189.0 $(M + H)^+$.

5′-Bromo-3-methyl-3,4,5,10-tetrahydro-2*H*-spiro[azepino[3,4-*b*]-indole-1,3′-indol]-2′(1′*H*)-one (1). *p*-Toluenesulfonic acid (0.29 g, 1.51 mmol) was added to a flask charged with **3** (1.00 g, 5.32 mmol) and 5-bromoisatin (1.20 g, 1.32 mmol) in *n*-butanol (11 mL) and heated to 120 °C. After 16 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with 0.5 M NaOH and saturated aqueous NaCl. The organic layer was separated and dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to provide racemic **1** as dark-brown solid (1.4 g, 66%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (s, 1H), 9.96 (s, 1H), 8.14 (s, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.26 (d, J = 2.4 Hz, 1H), 7.16 (dd, J = 6.3, 1.2 Hz, 1H), 6.99 (td, J = 6.9, 1.5 Hz, 1H), 6.94 (td, J = 6.9, 1.5 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 3.89 (m, 1H), 3.11 (ddd,

J = 12.0, 8.1, 4.5 Hz, 1H), 2.86 (ddd, J = 12.9, 8.7, 4.2 Hz, 1H), 2.79 (d, J = 5.7 Hz, 1H), 2.07 (m, 1H), 1.65 (dtd, J = 12.2, 8.4, 3.9 Hz, 1H), 1.05 (d, J = 6.6 Hz, 3H). MS (ESI) m/z 397.0 $(M + H)^+$. The racemate (**1**) was separated into its enantiomers by chiral chromatography under the conditions described in General Methods. The absolute configuration was determined by X-ray crystal analysis and characterized below.

(1*R*,3*S*)-5′-Bromo-3-methyl-3,4,5,10-tetrahydro-2*H*-spiro[azepino-[3,4-*b*]indole-1,3′-indol]-2′(1′*H*)-one (1a). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (s, 1H), 9.96 (s, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.26 (d, J = 1.8 Hz, 1H), 7.16 (dd, J = 7.0, 1.2 Hz, 1H), 6.99 (td, J = 6.9, 1.5, 1H), 6.94 (td, J = 6.9, 1.2 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 3.88 (m, 1H), 3.11 (ddd, J = 12.0, 7.8, 4.2 Hz, 1H), 2.87 (ddd, J = 12.9, 8.7, 3.9 Hz, 1H), 2.79 (d, J = 6.0 Hz, 1H), 2.07 (m, 1H), 1.64 (dtd, J = 12.9, 8.7, 4.2 Hz, 1H), 1.04 (d, J = 6.6 Hz, 3H). MS (ESI) m/z 397.0 $(M + H)^+$; $[\alpha]_D^{25}$ = +226.9° (c = 1.0 g/L, methanol).

(1*S*,3*R*)-5′-Bromo-3-methyl-3,4,5,10-tetrahydro-2*H*-spiro[azepino-[3,4-*b*]indole-1,3′-indol]-2′(1′*H*)-one (1b). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (s, 1H), 9.96 (s, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.26 (d, J = 1.5 Hz, 1H), 7.16 (bd, J = 7.8 Hz, 1H), 6.98 (td, J = 6.9, 1.5 Hz, 1H), 6.94 (td, J = 7.2, 1.2 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 3.89 (m, 1H), 3.11 (ddd, J = 11.7, 7.8, 4.2 Hz, 1H), 2.84 (ddd, J = 12.9, 8.7, 4.2 Hz, 1H), 2.79 (d, J = 6.0 Hz, 1H), 2.07 (m, 1H), 1.64 (dtd, J = 12.9, 8.7, 4.2 Hz, 1H), 1.04 (d, J = 6.4 Hz, 3H). MS (ESI) m/z 397.0 $(M + H)^+$; $[\alpha]_D^{25}$ = −254.9° (c = 1.0 g/L, methanol)

5′-Chloro-3-methyl-3,4,5,10-tetrahydro-2*H*-spiro[azepino[3,4-*b*]-indole-1,3′-indol]-2′(1′*H*)-one (4). Compound **4** was prepared according to Scheme 1 with 5-chloroisatin. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (s, 1H), 9.95 (s, 1H), 8.25 (s, 1H), 7.46 (bd, J = 8.4 Hz, 1H), 7.32 (dd, J = 8.1, 2.4 Hz, 1H), 7.16 (m, 2H), 6.96 (m, 2H), 6.91 (d, J = 8.1 Hz, 1H), 3.89 (m, 1H), 3.11 (m, 1H), 2.86 (m, 1H), 2.07 (m, 1H), 1.64 (m, 1H), 1.04 (d, J = 6.6 Hz, 3H). MS (ESI) m/z 352.0 $(M + H)^+$.

5′-Chloro-2,3,4,5,6,11-hexahydrospiro[azocino[3,4-*b*]indole-1,3′-indol]-2′(1′*H*)-one (5). *p*-Toluenesulfonic acid (0.020 g, 1.06 mmol) was added to a flask charged with 4-(1*H*-indol-3-yl)-butylamine[15] (0.10 g, 0.53 mmol) and 5-chloroisatin (0.11 g, 0.58 mmol) in absolute ethanol (5 mL) and heated to 100 °C. After 26 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with 0.5 M NaOH and saturated aqueous NaCl. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by reverse phase chromatography to provide **5** as dark-brown solid (14 mg, 8%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.54 (s, 1H), 8.45 (bs, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.33 (m, 2H), 7.05 (m, 2H), 6.96 (m, 1H), 6.29 (bs, 1H), 2.54 (m, 1H), 2.40 (m, 3H), 1.39 (m, 4H). MS (ESI) m/z 352.0 $(M + H)^+$.

5′-Chloro-3,4,5,10-tetrahydro-2*H*-spiro[azepino[3,4-*b*]indole-1,3′-indol]-2′(1′*H*)-one (6). *p*-Toluenesulfonic acid (0.11 g, 0.57 mmol) was added to a flask charged with 3-(1*H*-indol-3-yl)-propylamine[15] (0.10 g, 0.57 mmol) and 5-chloroisatin (0.12 g, 0.63 mmol) in absolute ethanol (5 mL) and heated to 100 °C. After 15 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with 0.5 M NaOH and saturated aqueous NaCl. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to provide **6** as an orange solid (17 mg, 9%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.56 (s, 1H), 10.10 (s, 1H), 7.46 (dd, J = 7.0, 1.5 Hz, 1H), 7.32 (dd, J = 8.4, 2.2 Hz, 1H), 7.15 (m, 2H), 7.00 (dd, J = 6.9, 1.5 Hz, 1H), 6.97 (dd, J = 6.9, 1.8 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 3.47 (m, 2H), 3.02 (m, 3H), 1.98 (m, 2H). MS (ESI) m/z 338.0 $(M + H)^+$.

5′-Chloro-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (7). *p*-Toluenesulfonic acid (0.12 g, 0.62 mmol) was added to a flask charged with tryptamine (0.10 g, 0.62 mmol) and 5-chloroisatin (0.13 g, 0.69 mmol) in absolute ethanol (6 mL) and then heated to 100 °C. After 23 h, the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with 0.5 M NaOH and saturated aqueous NaCl. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to provide **7** as an orange solid (6 mg, 3%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.57 (s, 1H), 10.48 (s, 1H), 7.47 (d, J = 7.2 Hz, 1H), 7.32 (dd, J = 8.1, 2.1 Hz, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 7.02 (m, 2H), 6.95 (d, J = 8.1 Hz, 1H), 3.59 (m, 1H), 3.12 (m, 2H), 2.80 (m, 2H). MS (ESI) m/z 324.0 $(M + H)^+$.

5′-Chloro-3,3-dimethyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (8). Sodium hydroxide (2.88 g, 72.0 mmol) was added to a solution of gramine (12.0 g, 69.0 mmol) in 2-nitropropane (44 mL, 490 mmol) and heated to reflux. After 18 h, the reaction was cooled to room temperature and a 10% aqueous acetic acid (60 mL) added. After stirring for an additional hour, the mixture was diluted with water and extracted with diethyl ether. The organic phase was separated and washed with water then dried over $MgSO_4$ to provide 3-(2-methyl-2-nitropropyl)-1*H*-indole as a dark-brown oil (16.5 g, quantitative), which was used in the next step without further purification.

A solution of hydrazine hydrate (5.68 g, 114 mmol) in 95% ethanol (6.5 mL) was added to a well stirred suspension of 3-(2-methyl-2-nitropropyl)-1*H*-indole (8.5 g, 39.0 mmol) and Raney Ni in 95% ethanol (75 mL) and heated to reflux. After 12 h, the reaction was filtered through celite and concentrated in vacuo. The residue was recrystallized from isopropanol to provide 1-(1*H*-indol-3-yl)-2-methylpropan-2-amine (2.8 g, 38%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.85 (s, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.03 (td, J = 6.9, 1.2 Hz, 1H), 6.95 (td, J = 7.2, 1.2 Hz, 1H), 2.68 (s, 2H), 1.02 (s, 6H). MS (ESI) m/z 189.0 $(M + H)^+$.

p-Toluenesulfonic acid (0.022 g, 0.117 mmol) was added to solution of 1-(1*H*-indol-3-yl)-2-methylpropan-2-amine (0.110 g, 0.59 mmol) and 5-chloroisatin (0.106 g, 0.59 mmol) in absolute ethanol (6 mL) and heated to 130 °C in a sealed tube. After 16 h, the reaction was cooled to room temperature and concentrated in vacuo. The residue was purified by flash chromatography to provide **8** (98 mg, 43%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.55 (s, 1H), 10.51 (s, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.28 (dd, J = 8.4, 2.1 Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H), 7.00 (m, 3H), 6.90 (d, J = 8.4 Hz, 1H), 2.76 (d, J = 14.7 Hz, 1H), 2.67 (d, J = 14.7 Hz, 1H), 1.84 (s, 1H), 1.30 (s, 3H), 1.29 (s, 3H). MS (ESI) m/z 352.0 $(M + H)^+$.

5′-Chloro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol-2′(1′*H*)-one (9). *p*-Toluene-sulfonic acid (0.113 g, 0.57 mmol) was added to mixture of 1-(1*H*-indol-3-yl)propan-2-amine (0.100 g, 0.57 mmol) and 5-chloroisatin (0.115 g, 0.63 mmol) in absolute ethanol (5.5 mL) and heated to 100 °C. After 18 h, the reaction was cooled to room temperature and diluted with ethyl acetate and then subsequently washed with 1 M HCl, water, and 1 M NaOH. The organic phase was separated and dried over Na_2SO_4 and concentrated in vacuo. The residue was redissolved in a minimun amount of ethyl actate and triturated with methylene choride to provide **9** (131 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H), 10.42 (s, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.31 (dd, J = 8.4, 2.4 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 2.4 Hz, 1H), 6.99 (m, 1H), 6.92 (d, J = 8.4 Hz, 2H), 3.93 (m, 1H), 3.05 (d, J = 6.3 Hz, 1H), 2.79 (dd, J = 15.0, 3.6 Hz, 1H), 2.41 (dd, J = 15.0, 10.5 Hz, 1H), 1.18 (d, J = 6.3 Hz, 3H). MS (ESI) m/z 338.0 $(M + H)^+$.

Preparation of 9a from D-Tryptophanol. Benzyl chloroformate (0.374 mL, 2.29 mmol) was added dropwise to a suspension of sodium carbonate (482 mg, 3.92 mmol) and D-tryptophanol (500 mg, 2.27 mmol) in a 1:1 solution of water and acetone (22.6 mL) at 0 °C. After addition, the cooling bath was removed and the reaction stirred at room temperature. After two hours, the reaction was carefully acidified to pH 2 with concentrated HCl and diluted with water. The aqueous layer was washed with ethyl acetate and the combined

organic phases separated and dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography column to give the corresponding *N*-Cbz-D-tryptophanol (428 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.76 (s, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.39−7.25 (m, 5H), 7.12−7.05 (m, 3H), 6.96 (t, J = 7.8 Hz, 1H), 4.99 (s, 2H), 4.68 (t, J = 5.4 Hz, 1H), 3.73 (m, 1H), 3.36 (m, 2H), 2.91 (dd, J = 14.4, 6.0 Hz, 1H), 2.73 (dd, J = 14.1, 6.0 Hz, 1H). MS (ESI) m/z 325.0 $(M + H)^+$.

p-Toluenesulfonyl chloride (199 mg, 1.05 mmol) was added to a solution of *N*-Cbz-D-tryptophanol (320 mg, 0.988 mmol) and triethylamine (267 μL, 1.93 mmol) in dry dichloromethane (2.8 mL) at 0 °C and allowed to warm to room temperature. After 18 h, the reaction was concentrated in vacuo and the residue was purified by flash chromatography to give the corresponding tosylate (580 mg, 100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.81 (s, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.50−7.25 (m, 10H), 7.08 (m, 2H), 6.95 (t, J = 7.2 Hz, 1H), 4.95 (s, 2H), 4.00 (m, 2H), 3.89 (m, 1H), 2.80 (d, J = 7.2 Hz, 2H), 2.39 (s, 3H). MS (ESI) m/z 479.0 $(M + H)^+$.

Palladium(II) hydroxide (72.7 mg) was added to a solution of the tosylate intermediate (580 mg, 1.21 mmol) in absolute ethanol (36 mL) and stirred at 1 atm H_2 atmosphere at room temperature. After two hours, the reaction was filtered through celite and concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to provide (*S*)-2-(1*H*-indol-3-yl)-1-methyl-ethylamine (169 mg, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.78 (s, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.05 (td, J = 7.2, 1.2 Hz, 1H), 6.95 (td, J = 7.2, 1.2 Hz, 1H), 3.08 (m, 1H), 2.62 (d, J = 7.8 Hz, 2H), 0.98 (d, J = 6.3 Hz, 3H). MS (ESI) m/z 175.0 $(M + H)^+$.

(1*R*,3*S*)-5′-Chloro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol-2′(1′*H*)-one (9a). *p*-Toluenesulfonic acid (16.8 mg, 0.088 mmol) was added to solution of (*S*)-2-(1*H*-indol-3-yl)-1-methyl-ethylamine (0.153 g, 0.881 mmol) and 5-chloroisatin (0.176 g, 0.969 mmol) in dry ethanol (3.1 mL) and heated to 110 °C in a sealed tube. After 16 h, the reaction was cooled to room temperature and concentrated in vacuo. The residue was purified by flash chromatography to provide **9a** (135 mg, 45%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H), 10.42 (s, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.31 (dd, J = 8.4, 2.1 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 7.03 (m, 2H), 6.98 (m, 1H), 6.92 (d, J = 8.1 Hz, 1H), 3.92 (m, 1H), 3.05 (d, J = 6.0 Hz, 1H), 2.79 (dd, J = 14.9, 3.3 Hz, 1H), 2.41 (dd, J = 4.5, 2.5 Hz, 1H), 1.18 (d, J = 6.0 Hz, 3H). MS (ESI) m/z 338.0 $(M + H)^+$; $[\alpha]_D^{25}$ = +253.6° (c = 0.104 g/L, methanol).

(1*S*,3*R*)-5′-Chloro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (9b). Compound **9b** was prepared using the same procedure starting from L-tryptophanol. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.46 (s, 1H), 10.42 (s, 1H), 7.43 (d, J = 7.5 Hz, 1H), 7.31 (dd, J = 8.4, 2.1 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 7.03 (m, 2H), 6.98 (m, 1H), 6.92 (d, J = 8.1 Hz, 1H), 3.92 (m, 1H), 3.06 (d, J = 6.0 Hz, 1H), 2.78 (dd, J = 14.9, 3.6 Hz, 1H), 2.41 (dd, J = 4.5, 2.5 Hz, 1H), 1.18 (d, J = 6.3 Hz, 3H). MS (ESI) m/z 338.0 $(M + H)^+$; $[\alpha]_D^{25}$ = −269.8° (c = 0.104 g/L, methanol).

The following procedure for racemic **20** was applied generally for the preparation of racemic **17**, **18**, and **19** starting from the corresponding halogenated indoles.

5,6-Difluoro-1*H*-indole-3-carbaldehyde (14d). Phosphorus oxychloride (180 mL, 1.96 mol) was added dropwise to dry dimethylformamide (1.4 L) at −20 °C over 30 min and stirred at −5 °C. After one hour, a solution of 5, 6-difluoroindole (200 g, 1.31 mol) in dry dimethylformamide (600 mL) was added dropwise to the above mixture at −20 °C. The cooling bath was removed and the mixture allowed to warm to room temperature. After one hour, the reaction was poured onto ice, basified with solid KOH, and stirred for an additional 15 min. The resulting precipitate was washed with 10% ethyl acetate in petroleum ether to give **14d** (180 g, 76%) as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.26 (bs, 1 H), 9.92 (s, 1H), 8.36 (s, 1H), 7.94 (dd, J = 8.2, 2.8 Hz, 1H), 7.57 (dd, J = 6.4, 4.4 Hz, 1H). MS (ESI) m/z 180.0 $(M - H)^+$.

5,6-Difluoro-3-(2-nitro-propenyl)-1*H*-indole (15d). A solution of 5,6-difluoro-1*H*-indole-3-carbaldehyde (200 g, 1.11 mol) in nitroethane (2 L) was refluxed with ammonium acetate (72.4 g, 0.94 mol). After 2 h, the reaction mixture was concentrated in vacuo to remove nitroethane, diluted with ethyl acetate, and washed with water and brine then dried over anhydrous Na_2SO_4. The organic layer was concentrated in vacuo and precipitated from 10% ethyl acetate in petroleum ether to provide **15d** (172 g, 65%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.29 (bs, 1H), 8.39 (s, 1H), 8.05 (s,1H), 7.99 (dd, J = 8.2, 3.6 Hz, 1H), 7.52 (dd, J = 6.8, 4.0 Hz, 1H), 2.47 (s, 3H). MS (ESI) m/z 237.0 $(M - H)^+$.

2-(5,6-Difluoro-1*H*-indol-3-yl)-1-methyl-ethylamine (16d). A solution of **15d** (170 g, 0.714 mol) in dry THF (2 L) was added dropwise to a suspension of lithium aluminum hydride (95 g, 2.5 mol) in THF (1 L) at 0 °C then heated to reflux. After two hours, the reaction mixture was cooled to 0 °C and quenched by slow addition of brine then diluted with ethyl acetate and filtered through celite. The filtrate was dried over anhydrous Na_2SO_4 and concentrated to give **16d** (150 g crude) as a viscous brown syrup. The residue was used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.18 (bs, 1H), 7.3 (dd, J = 10.0, 4.0 Hz, 1H), 7.12 (dd, J = 8.6, 5.2 Hz, 1H), 7.05 (s, 1H), 3.25 (m, 1H), 2.8 (dd, J = 7.2, 11.6 Hz, 1H), 2.61 (dd, J = 10.6, 8.4 Hz, 1H), 1.16 (d, J = 8.0 Hz, 3H). MS (ESI) m/z 211.0 $(M + H)^+$.

5′-Chloro-6,7-difluoro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (20). *p*-Toluenesulfonic acid (13.6 g, 0.071 mol) was added to a mixture of **16d** (150 g, 0.714 mol) and 5-chloroisatin (90.8 g, 0.5 mol) in absolute ethanol (1.5 L) and heated to reflux. After 20 h, the reaction was cooled to room temperature and concentrated in vacuo. The residue was diluted with ethyl acetate and washed with 2N HCl. The organic phase was separated and washed sequentially with saturated aqueous NaOH, water, and brine. The organic layer was separated and dried over anhydrous Na_2SO_4 and concentrated to provide a brown residue, which was triturated with dichloromethane to provide racemic **20** (110 g, 41%) as a light-yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.65 (s, 1H), 10.48 (s, 1H), 7.43 (dd, J = 10.8, 4.0 Hz, 1H), 7.32 (dd, J = 8.8, 2.6 Hz, 1H), 7.1 (dd, J = 9.2, 6.0 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.92 (d, J = 10.8 Hz, 1H), 3.90 (m, 1H), 3.08 (d, J = 8.0 Hz, 1H), 2.76 (m, 1H), 2.33 (m, 1H), 1.16 (d, J = 8.8 Hz, 3H). MS (ESI) m/z 374.0 $(M + H)^+$. The racemate (**20**) was separated into its enantiomers by chiral chromatography under the conditions described in General Methods.

(1*R*,3*S*)-5′-Chloro-6,7-difluoro-3-methyl-2,3,4,9-tetrahydrospiro-[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (20a). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (bs, 1H), 10.49 (bs, 1H), 7.44 (m, 1H), 7.33 (dd, J = 8.5, 2.0 Hz, 1H), 7.11 (m, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 3.91 (m, 1H), 3.10 (bd, J = 6.0 Hz, 1H), 2.76 (dd, J = 15.0, 3.5 Hz, 1H), 2.38 (dd, J = 15.5, 10.5 Hz, 1H), 1.17 (d, J = 6.5 Hz, 3H). MS (ESI) m/z 374.0 $(M + H)^+$; $[\alpha]_D^{25}$ = +220.8° (c = 0.100 g/L, methanol).

(1*S*,3*R*)-5′-Chloro-6,7-difluoro-3-methyl-2,3,4,9-tetrahydrospiro-[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (20b). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.66 (bs, 1H), 10.49 (bs, 1H), 7.44 (m, 1H), 7.33 (dd, J = 8.0, 2.0 Hz, 1H), 7.11 (m, 1H), 7.04 (d, J = 1.5 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 3.91 (m, 1H), 3.10 (bd, J = 5.5 Hz, 1H), 2.76 (dd, J = 15.0, 3.5 Hz, 1H), 2.38 (dd, J = 15.0, 10.5 Hz, 1H), 1.19 (d, J = 7.0 Hz, 3H). MS (ESI) m/z 374.0 $(M + H)^+$; $[\alpha]_D^{25}$ = −230.9° (c = 0.100 g/L, methanol).

5′-Chloro-6-fluoro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (17). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.53 (s, 1H), 10.47 (s, 1H), 7.32 (dd, J = 8.4, 1.8 Hz, 1H), 7.19 (dd, J = 9.9, 2.4 Hz, 1H), 7.13 (dd, J = 8.9, 4.5 Hz, 1H), 7.04 (d, J = 1.8 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 6.85 (td, J = 8.9, 2.7 Hz, 1H), 3.91 (m, 1H), 3.35 (bs, 1H), 2.76 (dd, J = 14.9, 3.9 Hz, 1H), 2.38

(dd, J = 15.2, 10.2 Hz, 1H), 1.17 (d, J = 5.1 Hz, 3H). MS (ESI) m/z 356.0 $(M + H)^+$.

(1*R*,3*S*)-5′-Chloro-6-fluoro-3-methyl-2,3,4,9-tetrahydrospiro-[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (17a). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.53 (s, 1H), 10.48 (s, 1H), 7.32 (dd, J = 8.3, 2.2 Hz, 1H), 7.20 (dd, J = 9.8, 2.3 Hz, 1H), 7.16 (dd, J = 8.8, 4.6 Hz, 1H), 7.06 (d, J = 1.5 Hz, 1H), 6.93 (d, J = 6.0 Hz, 1H), 6.87 (dt, J = 6.9, 1.8 Hz, 1H), 3.95 (m, 1H), 3.35 (bs, 1H), 2.77 (dd, J = 15.0, 3.7 Hz, 1H), 2.40 (dd, J = 15.0, 10.6 Hz, 1H), 1.18 (d, J = 6.4 Hz, 3H). MS (ESI) m/z 356.1 $(M + H)^+$; $[\alpha]_D^{25}$ = +225.5° (c = 0.122 g/L, methanol).

(1*S*,3*R*)-5′-Chloro-6-fluoro-3-methyl-2,3,4,9-tetrahydrospiro-[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (17b). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.55 (s, 1H), 10.51 (s, 1H), 7.32 (dd, J = 8.4, 2.4 Hz, 1H), 7.20 (dd, J = 10.1, 2.4 Hz, 1H), 7.14 (dd, J = 8.8, 4.5 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 6.86 (td, J = 9.3, 2.4 Hz, 1H), 3.93 (m, 1H), 3.36 (bs, 1H), 2.76 (dd, J = 15.0, 3.6 Hz, 1H), 2.39 (dd, J = 15.2, 10.5 Hz, 1H), 1.17 (d, J = 6.3 Hz, 3H). MS (ESI) m/z 357.0 $(M + H)^+$; $[\alpha]_D^{25}$ = −204.4° (c = 0.113 g/L, methanol).

5′,7-Dichloro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (18). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.79 (s, 1H), 10.50 (s, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.32 (dd, J = 8.1, 2.4 Hz, 1H), 7.17 (d, J = 1.5 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 6.98 (dd, J = 8.4, 1.8 Hz, 1H), 6.93 (d, J = 8.4 Hz, 1H), 3.91 (m, 1H), 3.11 (bd, J = 5.5 Hz, 1H), 2.78 (dd, J = 15.2, 3.9 Hz, 1H), 2.39 (dd, J = 15.0, 10.8 Hz, 1H), 1.17 (d, J = 6.3 Hz, 3H). MS (ESI) m/z 373.0 $(M + H)^+$.

(1*R*,3*S*)-5′,7-Dichloro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (18a). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64 (bs, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.34 (bs, 1H), 7.29 (dd, J = 8.1, 2.4 Hz, 1H), 7.18 (d, J = 1.6 Hz, 1H), 7.15 (d, J = 2.0 Hz, 1H), 7.09 (dd, J = 8.4, 1.7 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 4.20 (m, 1H), 2.95 (dd, J = 15.4, 3.8 Hz, 1H), 2.53 (dd, J = 15.4, 10.4 Hz, 1H), 1.31 (d, J = 6.4 Hz, 3H), 1.21 (d, J = 6.2 Hz, 1H). MS (ESI) m/z 372.1 $(M)^+$; $[\alpha]_D^{25}$ = +285.4° (c = 0.101 g/L, methanol).

(1*S*,3*R*)-5′,7-Dichloro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (18b). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (bs, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.35 (bs, 1H), 7.27 (dd, J = 8.4, 2.1 Hz, 1H), 7.17 (d, J = 1.6 Hz, 1H), 7.14 (d, J = 2.0 Hz, 1H), 7.09 (dd, J = 8.4, 1.7 Hz, 1H), 6.86 (d, J = 8.3 Hz, 1H), 4.21 (m, 1H), 2.95 (dd, J = 15.4, 3.8 Hz, 1H), 2.39 (dd, J = 15.4, 10.4 Hz, 1H), 1.31 (d, J = 6.4 Hz, 3H), 1.21 (d, J = 6.2 Hz, 1H). MS (ESI) m/z 372.1 $(M)^+$; $[\alpha]_D^{25}$ = −258.9° (c = 0.104 g/L, methanol).

5′,7-Dichloro-6-fluoro-3-methyl-2,3,4,9-tetrahydrospiro[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (19). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.69 (s, 1H), 10.51 (s,1H), 7.43 (d, J = 10.0 Hz, 1H), 7.32 (bd, J = 8.3 Hz, 1H), 7.26 (d, J = 6.5 Hz, 1H), 7.04 (bs, 1H), 6.93 (d, J = 8.3 Hz, 1H), 3.91 (m, 1H), 3.12 (bd, J = 5.5 Hz, 1H), 2.77 (bd, J = 14.2 Hz, 1H), 2.38 (dd, J = 14.2, 10.9 Hz, 1H), 1.16 (d, J = 6.5 Hz, 1H). MS (ESI) m/z 390.0 $(M)^+$.

(1*R*,3*S*)-5′,7-Dichloro-6-fluoro-3-methyl-2,3,4,9-tetrahydrospiro-[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (19a). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.69 (s, 1H), 10.51 (s, 1H), 7.43 (d, J = 10.0 Hz, 1H), 7.33 (dd, J = 8.0, 2.2 Hz, 1H), 7.27 (d, J = 6.5 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 3.91 (m, 1H), 3.13 (bd, J = 6.2 Hz, 1H), 2.74 (dd, J = 15.0, 3.0 Hz, 1H), 2.35 (dd, J = 15.0, 10.3 Hz, 1H), 1.15 (d, J = 6.0 Hz, 3H). MS (ESI) m/z 392.0 $(M + 2H)^+$; $[\alpha]_D^{25}$ = +255.4° (c = 0.102 g/L, methanol).

(1*S*,3*R*)-5′,7-Dichloro-6-fluoro-3-methyl-2,3,4,9-tetrahydrospiro-[*β*-carboline-1,3′-indol]-2′(1′*H*)-one (19b). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.70 (s, 1H), 10.51 (s, 1H), 7.44 (d, J = 9.7 Hz, 1H), 7.34 (dd, J = 8.6, 2.0 Hz, 1H), 7.27 (d, J = 6.0 Hz, 1H), 7.05 (d, J = 2.0 Hz, 1H), 6.94 (d, J = 8.5 Hz, 1H), 3.91 (m, 1H), 3.13 (bd, J = 6.2 Hz, 1H), 2.77 (dd, J = 15.0, 3.5 Hz, 1H), 2.39 (dd, J = 15.5, 10.5 Hz, 1H), 1.17 (d, J = 6.0 Hz, 3H). MS (ESI) m/z 392.0 $(M + 2H)^+$; $[\alpha]_D^{25}$ = −238.9° (c = 0.134 g/L, methanol).

Acknowledgment. We thank Hui Qing Ang, Kang Min Low, Augustin Notedji, William Tan, Maria Tan, Jeanette Wu, Nicole Ye, and Peiting Zeng for pharmacology support and Christophe Bury for chiral separation support. This work was supported by a grant from the Medicines for Malaria Venture and a translational research grant (WT078285) from the Wellcome Trust to the Novartis Institute for Tropical Diseases, the Genomics Institute of the Novartis Research Foundation and the Swiss Tropical and Public Health Institute.

Supporting Information Available: Additional experimental details for in vitro and in vivo pharmacokinetic studies, antimalarial assays, and *P. berghei* mouse efficacy studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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