Brief Articles

Antimalarial and Antiplasmodial Activities of Norneolignans. Syntheses and SAR

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A systematic change of the substituents and side chain of the norneolignan hinokiresinol afforded a 10 fold improvement of the IC_{50} value toward inhibition of the growth of *Plasmodium falciparum*. The more potent compounds controlled the parasitemia in mice infected with *Plasmodium berghei*.

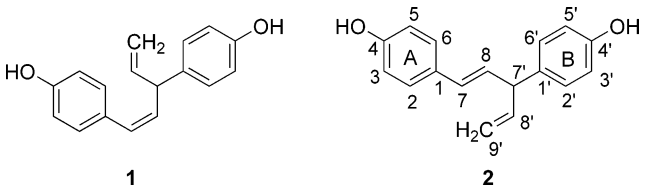
Introduction

Malaria is one of the most devastating diseases in the world killing 1−3 million people and infecting 300−500 million each year.[1] The fight against malaria is complicated by the ability of the pathogenic *Plasmodium* parasites to develop resistance to existing drugs.[1−3] Attempt to control the epidemic in the future could be based on a combination of continued development of new drugs, prevention of infection, and development of a vaccine.[2] In the absence of an efficient vaccine the only options are prevention and development of new drugs. In the past the most efficient method for developing new drugs against malaria has been design of drugs using antiplasmodial natural products as templates.[4] During our continued search for appropriate natural products we noticed that the norneolignan (+)-nyasol (**1**, Chart 1) possesses a modest antiplasmodial activity with an IC_{50} value of 12 μM against a chloroquine-susceptible strain of *P. falciparum* as well as a chloroquine-resistant strain.[5] The *E*-isomer of nyasol, hinokiresinol (**2**), is also naturally occurring[5,6] and was found to possesses some antiplasmodial activity with an IC_{50} value of about 50 μM.[7] Hinokiresinol was believed to be useful as a template for development of new antiplasmodial compounds.

Hinokiresinol and nyasol both displays a number of other biological activities. (+)-Hinokiresinol and (−)- and (+)-nyasol all show binding affinity for the bovine uterine estrogen receptor, but (+)-nyasol is found to bind approximately seven times stronger than (−)-nyasol and (+)-hinokiresinol only has a poor affinity.[8] The compounds stimulate the growth of estrogen dependent human breast cancer cells (T47D) indicating that they are estrogen agonists. In addition (−)-nyasol increases hexobarbital sleeping time in mice,[9] and nyasol possesses antifungal activity[10] and inhibits testosterone 5α-reductase.[11]

The antiplasmodial activity of hinokiresinol[7] has encouraged us to use this compound as a template for developing new antimalarial drugs. The choice of a synthetic protocol using chalcones as starting materials[12] enabled us to take advantage of a library of chalcones prepared as potential antiparasitic drugs.[13]

Chart 1



Results and Discussions

Chemistry. Analogues, in which the substituents of the aromatic rings and the side chain were changed in a systematic way, were prepared (Table 1). Variations of the substitution pattern were made to represent different combinations of electron-donating and electron-withdrawing groups on the aromatic rings, that is donating−donating (**2** and **a**), donating−withdrawing (**b**, **c**, and **d**), withdrawing−donating (**e**, **f**, **g**, and **h**), and withdrawing−withdrawing (**i** and **j**) (Table 1).

The synthetic procedure was based on the method developed for synthesis of hinokiresinol using chalcones as starting materials.[7] The 1,4-addition of alkylmagnesium halide and reduction with sodium borohydride to give a mixture of the two epimeric alcohols (**7a**−**j**, **8a**−**j**, **9a**) succeeded in all attempted cases (Scheme 1).

The final step in the synthesis of **2** was an elimination of water provoked by heating a methanolic solution of **7** ($R^2 = R^3 = R^{2'} = R^{3'} = R^{5'} =$ H, $R^4 = R^{4'}$ = tetrahydropyranyloxy) with hydrochloric acid.[7] In all the cases **7a**, **7d**, and **7e**, this procedure afforded substitution of the hydroxyl group with a methoxy group. Replacement of the hydrochloric acid with *p*-toluenesulfonic acid in toluene under reflux afforded the desired products in the cases of compounds **10a**, **11a**, **10c**, **11c**, **10d**, **10j**, and **11j** in yields ranging from 30% to 85% (Scheme 1). Substitution of *p*-toluenesulfonic acid with acidic ion-exchange resin Dowex 50WX8−100 enabled the elimination to give compounds **10a**, **11a**, **10b**, **11b**, **10g**, **11h**, **10i**, and **11i**, yields ranging from 28% to 87%. The compounds **11d**−**g**, **10e**, and **10h** were not formed under acidic conditions. Application of Martin's sulfurane dehydrating agent[15,16] under basic conditions successfully enabled synthesis of these compounds. In two cases

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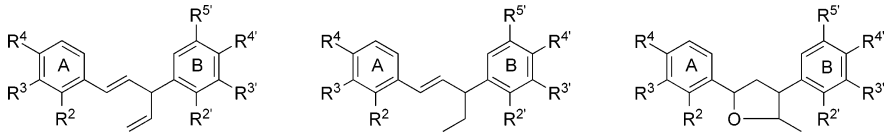
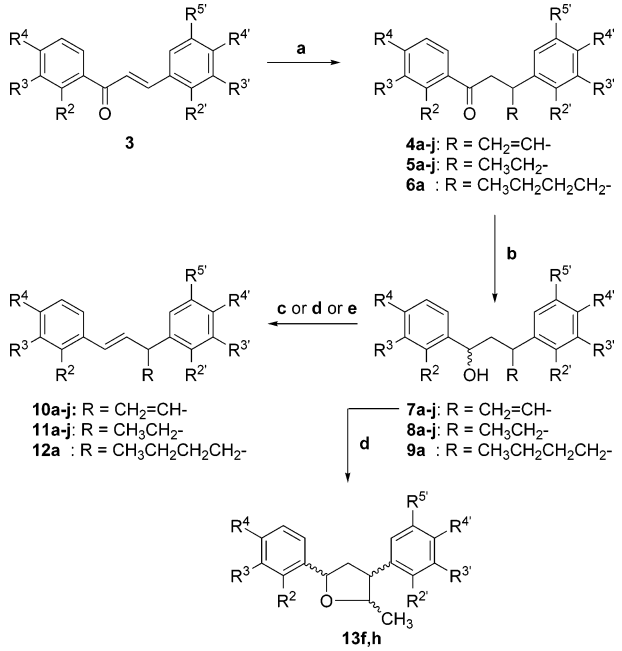
Table 1. Structure and Antiplasmodial Activities for Compounds **10**, **11**, and **13**[a]



compd	ring A	ring B	**10** $IC_{50} \pm SD$ (μg/mL)	**11** $IC_{50} \pm SD$ (μg/mL)	**13** $IC_{50} \pm SD$ (μg/mL)
rac-**2**	4-OH	4′-OH	13 ± 3	-	-
(+)-**2**	4-OH	4′-OH	14 ± 3	-	-
(−)-**2**	4-OH	4′-OH	20 ± 6	-	-
a	4-$OCH_2CH{=}CH_2$	2′,4′-$(OCH_3)_2$	7 ± 2	4 ± 1	-
b	2,4-$(OCH_3)_2$	2′,4′-$(Cl)_2$	7 ± 1	6 ± 2	-
c	2-$CH_2N(CH_3)_2$	2′,4′-$(Cl)_2$	3 ± 1	4.1 ± 0.6	-
d	2,3,4-$(OCH_3)_3$	3′-F	6 ± 2	3.9 ± 0.7	-
e	2-F	3′,5′-$(OCH_3)_2$	6 ± 1	6 ± 2	-
f	2-Br	2′,4′-$(OCH_3)_2$	8.8 ± 0.3	5.4 ± 0.2	3 ± 1
g	2,4-$(F)_2$	2′,4′-$(OCH_3)_2$	7 ± 2	3.9 ± 0.1	-
h	2,4-$(Cl)_2$	2′,4′-$(OCH_3)_2$	1.5 ± 0.1	4.2 ± 0.1	6.9 ± 0.7
i	4-F	4′−F	10 ± 1	13 ± 4	-
j	2-CF_3	2′,4′-$(Cl)_2$	8.2 ± 0.6	6.0 ± 0.6	-

[a] The antiplasmodial assay was performed as previously described.[14]

Scheme 1[a]

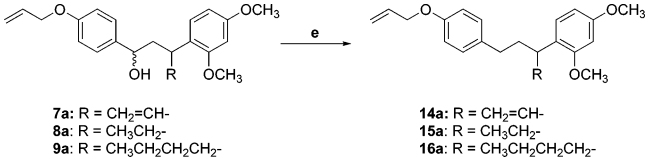


[a] Reagents and conditions: (a) Alkyl Grignard reagent, CuI, THF, 0 °C, 2 h; (b) $NaBH_4$, EtOH, rt overnight; (c) *p*-TsOH, toluene, reflux, 1−48 h; (d) Dowex 50WX8−100 ion-exchange resin, reflux, 2 h to 6 days; (e) Martin's sulfurane, Et_3N, toluene, reflux, 1−48 h. The substituents R^2−$R^{5'}$ are given in Table 1.

(**7f** and **7h**) the reaction of alcohols with ion-exchange resin resulted in formation of a mixture of the diastereomeric tetrahydrofurans **13f** and **13h**. In general, the compounds with electron-withdrawing substituents in the A-ring seem less willing to eliminate water under acidic conditions while the substitution pattern on the B-ring appears to affect the reactivity less.

The importance of the side chain and the double bond for the antiparasitic effect was investigated by reduction of the double bonds and extension or removal of the side chain using compound **10a** as a template. (Scheme 2). Reduction of the alcohols **7a**, **8a**, and **9a** with triethylsilane and trifluoroacetic acid afforded **14a**, **15a**, and **16a**, respectively. Prolonged reduction of the chalcone **3a** afforded the diphenylpropane **17a** (Table 2).

Scheme 2[a]



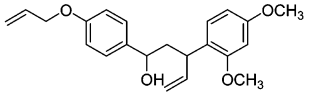
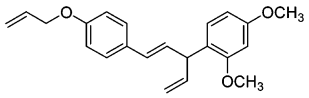
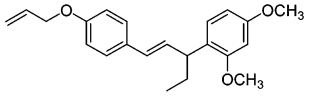
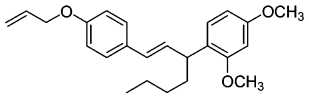
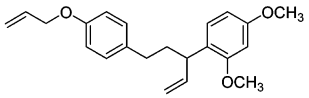
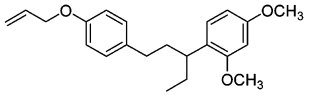
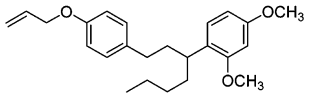
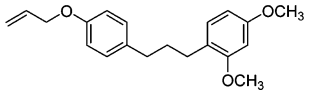
[a] Reagents and conditions: (e) Et_3SiH, TFA, CH_2Cl_2, rt, 1−48 h.

In Vitro Antiplasmodial Activity. The antiplasmodial activities of the synthesized compounds were tested in an in vitro malaria assay against a chloroquine-sensitive strain of *P. falciparum* parasites, 3D7, according to a previously described protocol.[14] Introduction of different substituents on the aromatic rings enabled investigation of the relationships between the substitution pattern and the antiplasmodial activity. The results are displayed in Table 1 and Table 2. All tests were performed three times each time in triplicate. All the analogues display better antiplasmodial activity than hinokiresinol. Introduction of electron-withdrawing groups in either ring A (**10e**, **10f**, **10g**, **10h**) or ring B (**10b**, **10c**, **10d**) increases the activity, whereas substitution with electron-withdrawing fluorine atoms in both rings (**10i**) reduces the potency to the level of hinokiresinol. All compounds were synthesized as racemic mixtures. No attempts were made to separate the enantiomers of the analogues since the enantiomers of hinokiresinol (**2**) showed similar activities.[7]

Reduction of the terminal double bond only slightly affects the activities since the reduced compounds in general are as potent as or a little more potent than the parent compounds (compare series **10** with series **11**, Table 1). A noticeable exception, however, is compounds **10h** and **11h** where the unsaturated compound (**10h**) is almost three times as active as the analogue with saturated side chain (**11h**). The poor importance of the double bonds reveals that no specific electrostatic interactions between the binding site and the double bonds are determining for the affinities.

A series of analogues of compound **10a** was prepared for further investigation of the importance of lipophilicity and electrostatic interactions (Table 2). Reduction of the central alkene without affecting the terminal alkene (**14a**) and reduction of both alkenes (**15a**) gradually increases the activity. This clearly indicates that lipophilic interactions rather than electrostatic interactions are responsible for the increase in potency.

Table 2. Antiplasmodial Activities for 5-(4-(Allyloxy)Phenyl)-3-(2,4-dimethoxyphenyl)Propanes Modified at C-7 and C-7′

Compound		IC_{50} ± SD (μg/mL)
7a		16 ± 7
10a		7 ± 2
11a		4 ± 1
12a		2.7 ± 0.2
14a		5.2 ± 0.9
15a		3 ± 1
16a		2.2 ± 0.4
17a		9.5 ± 0.2

The antiplasmodial assay was performed according to Ziegler et al.[14]

Replacement of the ethyl group with a butyl group in the 7′-position (**12a**) more than doubles the potency further supporting the importance of lipophilic interactions. The even higher potency of **16a**, in which a butyl group has been introduced and the internal double bond has been reduced, also substantiates the importance of lipophilic interactions. Removal of the substituent at C-7′ (**17a**) decreases the activity and the analogue, in which a polar hydroxyl group (**7a**) is introduced, is even less potent. These findings confirm that the antiplasmodial activity of the compounds in general correlates to the lipohilicity of the aliphatic part of the molecules.

In accordance with the above observation the tetrahydrofuran analogues (**13f** and **13h**) were active constituting a novel and interesting class of antiplasmodial compounds. The limited flexibility of these compounds restricts the number of allowed conformations. The significant activities of the tetrahydrofurans therefore might indicate that one or more of these isomers represent one of the active conformations of the norneolignans. Since no protocol exists for reliable synthesis of these derivatives and since they in both cases were formed as a complex mixture of up to eight stereoisomers, which could not be separated even by HPLC, this path was not followed.

The reported SERCA inhibitory activities of the antiplasmodial drugs artemisinin[17] and curcumin[18] and the structural resemblance of especially curcumin and norneolignans encouraged us to screen two of the most potent compounds, **10h** and **16a** for rabbit muscle SERCA inhibitory activity, but no inhibition was observed up to a concentration of about 3.5 μg/mL.

Table 3. In Vivo Activities for Compounds **10h** and **16a**[a]

compd	dose (mg/kg)	% parasitemia mean	% parasitemia SD	% inhibition
control	0	34.6	2.1	0
chloroquine	12.4	1.1	0.2	97
10h	25	14.7	3.6	57
16a	100	14.0	2.5	59
	50	17.6	4.6	49
	25	25.8	1.1	25

[a] Mice were infected with *Plasmodium berghei* at day 0, treated with the test substances **10h** and **16a** at day 4 and 5 and the parasitemia counted at day 5.

In Vivo Antimalarial Activity. The two most active compounds, **16a** and **10h**, were tested for in vivo antimalarial activity in mice infected with *Plasmodium berghei* using chloroquine as a positive control. The mice were infected with parasites at day 0. Treatment with the test compounds started at day 4 and the parasitemia was determined after 2 days of treatment (day 5). Both compounds significantly decreased the parasitemia in the mice at day 5, with **10h** as the most active almost bisecting the parasitemia on day 5 by a dose of 25 mg/kg (Table 3). Limited solubility prevented injection of a larger dose. No apparent toxicity was observed.

Conclusion

Methods for synthesis of hinokiresinol analogues have been developed. The potencies of the hinokiresinol analogues were successfully improved approximately 10 times from an IC_{50} value of 13 μg/mL (hinokiresinol) to an IC_{50} value of 1.5 μg/mL (**10h**) by changing the substitution pattern on the rings. The in vitro antiplasmodial activity of hinokiresinol is determined by lipophilic interactions rather than specific electrostatic interactions. Further optimization based on this observation enabled an almost seven time improvement of the IC_{50} value of **7a** from 16 μg/mL to an IC_{50} value of **16a** of 2.2 μg/mL. The most active compounds, **10h** and **16a**, control an infection of *P. berghei* in mice. Consequently, the norneolignan skeleton is a promising template for development of new drugs against malaria.

Experimental Section

General. (*E*)-1-(4-(allyloxy)phenyl)-3-(2,4-dimethoxyphenyl)-prop-2-en-1-one (**3a**),[13] (*E*)-3-(2,4-dichlorophenyl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (**3b**),[19] (*E*)-3-(3-fluorophenyl)-1-(2,3,4-trimethoxyphenyl)prop-2-en-1-one (**3d**),[13] (*E*)-1-(2-fluorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (**3e**),[13] (*E*)-1-(2-bromophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**3f**),[13] (*E*)-1-(2,4-difluorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**3g**), (*E*)-1-(2,4-dichlorophenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**3h**),[19] (*E*)-1,3-bis(4-fluorophenyl)prop-2-en-1-one (**3i**),[13] and (*E*)-3-(2,4-dichlorophenyl)-1-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (**3j**)[20] were prepared according to literature methods.

Chalcones (3). General Procedure. To a solution of sodium hydroxide (3.20 mmol, 0.128 g) in EtOH (30 mL) were added equimolar amounts of the appropriate benzaldehyde (12.8 mmol) and the appropriate acetophenone (12.8 mmol), and the reaction mixture was stirred at room-temperature overnight. If the product had precipitated, the reaction mixture was filtered, and the precipitate was washed with water and recrystallized from EtOH or EtOH−water. If no precipitation had occurred, the reaction mixture was diluted with water (30 mL), neutralized with 1 M HCl and extracted four times with EtOAc (40 mL). The combined

organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by column chromatography.

1,3-Diphenylpent-4-en-1-ones (4a−j), 1,3-diphenylpentan-1-ones (5a−j) and 1,3-Diphenylheptan-1-ones (6a). General procedure A. Under nitrogen, a solution of vinylmagnesium bromide (1 M solution in THF, 2.2 equiv, 7.2 mmol, 7.2 mL) was added to a suspension of cuprous iodide (0.1 equiv, 0.33 mmol, 0.063 g) in THF (10 mL) and cooled to 0 °C. The reaction mixture was stirred for 15 min at 0 °C. A solution of the appropriate chalcone (**3**) (1 equiv, 3.3 mmol) in THF (10 mL) was added to the reaction mixture. Stirring was continued at 0 °C for 2 h. The reaction mixture was quenched with saturated NH_4Cl solution. The reaction mixture was diluted with Et_2O (75 mL) and was successively washed with water (2 × 75 mL) and brine (75 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

General Procedure B Under nitrogen, a solution of ethylmagnesium chloride (25% w/w solution in THF, 2.2 equiv, 7.2 mmol, 2.49 mL) was added to a suspension of cuprous iodide (0.1 equiv, 0.33 mmol, 0.063 g) in THF (10 mL) and cooled to 0 °C. The reaction mixture was stirred for 15 min at 0 °C. A solution of the appropriate chalcone (**3**) (1 equiv, 3.3 mmol) in THF (10 mL) was added to the reaction mixture. Stirring was continued at 0 °C for 2 h. The reaction was quenched with saturated NH_4Cl solution. The reaction mixture was diluted with Et_2O (75 mL) and was successively washed with water (2 × 75 mL) and brine (75 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

General Procedure C. Under nitrogen, a solution of *n*-butylmagnesium chloride (20% w/w solution in THF/toluene, 2.2 equiv, 6.8 mmol, 3.96 g) was added to a suspension of cuprous iodide (0.1 equiv, 0.31 mmol, 0.059 g) in THF (10 mL) and cooled to 0 °C. The reaction mixture was stirred for 15 min at 0 °C. A solution of the appropriate chalcone (**3**) (1 equiv, 3.1 mmol) in THF (10 mL) was added to the reaction mixture. Stirring was continued at 0 °C for 2 h. The reaction was quenched with saturated NH_4Cl solution. The reaction mixture was diluted with Et_2O (75 mL) and was successively washed with water (2×75 mL) and brine (75 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

1,3-Diphenylpent-4-en-1-ols (7a−j), 1,3-Diphenylpentan-1-ols (8a−j), and 1,3-Diphenylheptan-1-ols (9a). General Procedure. The appropriate 1,3-diphenylpent-4-en-1-one (**4**, 1 equiv, 2.5 mmol) or 1,3-diphenylpentan-1-one (**5**, 1 equiv, 2.5 mmol) or 1,3-diphenylheptan-1-one (**6**, 1 equiv, 2.5 mmol) was dissolved in EtOH (20 mL). $NaBH_4$ (5 equiv, 12.4 mmol, 0.469 g) was added. After stirring overnight at room temperature 5% NaOH solution (100 mL) was added, and the mixture was extracted with Et_2O (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

(*E*)-1,3-Diphenylpenta-1,4-dienes (10a−j), (*E*)-1,3-Diphenylpent-1-enes (11a), (*E*)-1,3-Diphenylhept-1-enes (12a), and 5-Phenyl-tetrahydro-3-phenyl-2-methylfurans (13f and 13h). General Procedure D for Synthesis of 10a, 10c, 10d, 10j, 11a, 11c, 11j. The appropriate alcohol (**7**, **8**, or **9**) (1 equiv, 0.42 mmol) was dissolved in toluene (6 mL). *p*-Toluenesulfonic acid monohydrate (0.1 equiv, 0.04 mmol, 0.008 g) was added. The reaction flask, equipped with Dean−Stark trap, was heated to reflux, and the reaction mixture was stirred under reflux for 1 h to 48 h. The reaction mixture was cooled and diluted with Et_2O (15 mL). The mixture was washed successively with water (2 × 15 mL) and brine (15 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

General Procedure E for Synthesis of 10a, 10b, 10g, 10i, 11a, 11b, 11h, 11i, 12a, 13f, 13h. Alcohol (**7**, **8**, or **9**) (1 equiv, 0.36 mmol) was dissolved in toluene (10 mL). Dowex 50WX8−100 ion-exchange resin (7 mL), washed with toluene, was added, and the reaction mixture was stirred under reflux for 2 h to 6 days. The reaction mixture was cooled, filtered, and concentrated in vacuo. The product was purified by column chromatography.

General Procedure F for Synthesis of 10e, 10f, 10h, 11d, 11e, 11f, 11g. The appropriate alcohol (**7**, **8** or **9**) (1 equiv, 0.26 mmol) was dissolved in toluene (50 mL). Et_3N (5 mL) was added, and the reaction mixture was heated to reflux. Martin's sulfurane (2.5 equiv to 5 equiv, 0.66 mmol to 1.32 mmol, 0.44 g to 0.89 g) was added, and the reaction mixture was stirred under reflux for 1 h to 48 h. The reaction mixture was cooled, and saturated $NaHCO_3$ solution (50 mL) was added. The layers were separated, and the aqueous layer was extracted with Et_2O (2 × 25 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

3,5-Diphenylpent-1-ene (14a), 1,3-Diphenylpentane (15a), 1,3-Diphenylheptane (16a), and 1,3-Diphenylpropane (17a). General Procedure G. Ketone or alcohol (0.42 mmol) was dissolved in TFA (3 mL) under nitrogen. Et_3SiH (2.2 equiv, 0.93 mmol, 0.15 mL) was added, and the reaction mixture was stirred at room temperature for 1.5 to 4.5 h. The reaction mixture was concentrated in vacuo and was diluted with EtOAc (15 mL). The mixture was successively washed with 1 M NaOH solution (15 mL) and brine (15 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by column chromatography.

General Procedure H. As procedure G, but using CH_2Cl_2 as solvent (4 mL), catalytic amount of TFA (0,1 mL) and 20 equiv of Et_3SiH (8.5 mmol, 1.35 mL). Stirring at room temperature for 20 h to 48 h.

In Vitro Antiplasmodial Screening. A Desjardin's radioisotope method for measuring the growth of a chloroquine sensitive strain of *Plasmodium falciparum* (3D7) modified according to Ziegler et al. was adopted.[14] In shortly 50 μL of growth medium (RPMI 1640 with HEPES and supplemented with 0.45% Albumax II, 1.42 mM L-glutamine, 133 μM hypoxanthine and 44 mg of gentamicine sulfate/mL) containing test substances added from a dimethyl sulfoxide (DMSO) stock was mixed with 50 μL of a suspension of parasitized erythrocytes (blood type 0, hematocrit 5%; parasitemia 2 to 3%). The maximum final DMSO concentration in the growth medium was 0.5%. The test samples were incubated for 24 h at 37 °C in an atmosphere consisting of 93% of nitrogen, 5% of carbon dioxide, and 2% of oxygen. The incubation was continued for additional 24 h after addition of a solution of [^{3}H]phenylalanine (25 μL, 1.6 MBq/mL). The cells were harvested on a filter plate, and the incorporated [^{3}H]phenylalanine counted in a scintillation counter. Each compound was tested three times each time in triplicate.

In Vivo Antimalarial Screening. Thirty NMRI mice were allocated into six groups of each five mice. At day 0 red blood cells (1×10^6) infected with *Plasmodium berghei* K173 and suspended in 0.2 mL of inoculation solution (0.9% saline solution) were injected in each mouse. At day 4, when the parasitemia was 2−9%, five of the groups of mice were injected ip with the dose given in Table 3 dissolved in 0.9% of saline added 10% of Tween 80, pH adjusted to 4.5. In the control group two mice were not treated at all, whereas the remaining three mice were injected with the vehicle. The parasitemia was determined on day 5 in a blood sample taken from the tail vein. One drop was placed on an object glass and stained with Giesma, and the number of infected blood cells were counted under a microscope.

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Supporting Information Available: Detailed experimental procedures for the syntheses and NMR and HRMS spectra for the new compounds are described. This material is available free of charge via the Internet at http://pubs.acs.org.

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