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Synthesis of a potent new antimalarial through natural products conjugation

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Abstract

Three natural products have been assembled to obtain a new antimalarial hit. (+)-Usnic acid was used as scaffold to design and synthesize new products, that were tested on asexual development for *P. falciparum* and *P. berghei*. Among them, the ester of (+)-usnic acid-4-aminobutyric acid **14** with dihydroartemisinin shows considerable in vivo antimalarial activity against *P. berghei* in mice, similar to the synthetic drug artesunate. Compound **14** behaves as a delivery system for dihydroartemisinin and combine the effects of the endoperoxide with the redox properties of the phenolic portions of (+)-usnic acid.

Keywords

antiprotozoal agents; drug discovery; Mannich bases; medicinal chemistry; usnic acid

Introduction

About 40% of the world's population live at risks of malaria, with current estimates of 800,000 deaths per year.^[1,2] Although expanded malaria control programs are under way in terms of preventive drug treatment and mosquito control through insecticide-treated bed nets, treating ill patients, including children and pregnant women, is insufficient, as a result of drug resistance and recrudescence. Most of the available drugs against *P. falciparum* parasite are directed to the erythrocytic stage, the phase that causes disease (fever) and death. A number of old and new drugs, from quinine to chloroquine, to antibiotics, to artemisinins have been demonstrated in clearing asexual parasites from the blood.^[2-4] In spite of this, malaria transmission remains unabated with resistance to the leading compound, artesunate (dihydroartemisinin succinate) steadily growing.^[2,5,6]

A major priority of the malaria eradication research agenda are research activities aimed at generating new strategies to fight the disease. Antimalarial drug discovery thus run convergent ways to fight malaria infection by developing pathways that combine the control

of the parasitemia at the blood stage with interventions that block the parasite life cycle at transmission stages (host to vector or vice-versa).^[7]

These steps can reasonably be obtained by combining drugs that act at erythrocytic and exoerythrocytic stages of parasite development, or, with provisional success, by combining the two effects in one entity. “Covalent bitherapy” involves linking two or more molecules with independent intrinsic activities into a single agent, where one of the components could also act as a prodrug or a redox sensitizer for target specific endpoints.^[8]

Our strategy involves the study of simple derivatives of natural products that possess intrinsic anti-parasitic action, and are endowed with specific redox properties.^[9] In fact, parasite development depends on a sophisticated and balanced redox system, where it activates enzymes for self-protection against pro-oxidant metabolic products (i.e. hemozoin) or against drugs that produce ROS by themselves leading to parasite death.^[10]

During the course of discovering new antimalarial drugs candidates, we analyzed natural products that are accessible from Nature in high quantities or are available through an economical synthetic approach. We paid particular attention to determine the antimalarial action of natural acylphloroglucinols,^[9,11] since it was previously shown that some products belonging to this class of compounds possess putative anti-plasmodial activity.^[9,11]

(+)-Usnic acid, a dibenzo-furandione acylphloroglucinol **1**, is a yellow pigment widely spread in lichens (*Cladonia*, *Usnea*, *Ramalina*), representing up to 6% of the vegetable material in some species, is easily recovered from the natural source and is now commercially available.^[12] Usnic acid, is a weak antimalarial agent for blood stage parasites, effective only at micromolar concentrations (IC₅₀ 5.26 µg/mL).^[13] It is highly lipophilic due to the presence of strong intramolecular hydrogen bonding, and has been classified as a membrane disrupter.^[14,15] The acid enol proton is considered to be the major actor, the -triketone moiety in (+)-usnic acid being apparently responsible for its activity/toxicity while the enol oxygen is prevented from alkylation.^[16] (+)-Usnic acid also possesses two phenolic hydroxyl groups with weak acidity. They can be alkylated only after long reaction times,^[16] with no effects on the enol portion. Indeed these two phenols contribute to usnic acid's redox potentials, in particular the one in para-position to the aromatic methyl ketone.^[17a] Usnic acid has the property to decrease lipid peroxidation and to increase the activities of antioxidant enzymes such as glutathione and superoxide dismutase.^[17b] Indeed, it behaves as a bi-functional anti-oxidant-pro-oxidant depending on its concentration, due to the formation of transient monoelectronic species.

In line with this argument, we hypothesized that modifying the triketone moiety should result in a loss of toxicity, but maintain the redox properties of the molecule. Modifications also increase water solubility and could improve the anti-parasitic action. The compounds were prepared from commercial (+)-usnic acid that was condensed with appropriate amine derivatives, chosen because they present interesting features, such as an easy supply of diversity, inferring solubility or affinity for nucleic acids. A potential increase of activity in various fields and specifically as antimalarial agents is also expected when they are conjugated to drugs.^[18] (compounds **2-8**).

Usnic acid reactivity is mainly due to the presence of the acyclic methyl ketone that easily reacts with nucleophilic nitrogens to give the corresponding enamines because of the extended conjugation with the enolized -diketone group.^[18] Of the three hydroxyl groups, the enolic hydroxyl at position 3 has the strongest acidic character due to the inductive effect of the keto group, whereas the hydroxyl group at positions 8 and 10 are less acidic.^[19]

The chemical reactivity with amines and aminoacids, also exploited by others,^[20,21] was further confirmed through the preparation of new derivatives. The general procedure consisted in treating a solution of (+)-usnic acid in ethanol or THF with an appropriate amine followed by heating to reflux under nitrogen atmosphere for several hours. With amine derivatives and amino acids, these reactions proceeded only in alkaline medium, in the pH range of 9-10. In this pH range, usnic acid appeared to be in its partial salt form, where the enolic 3-OH is not involved in intramolecular hydrogen bond and consequently this increases its ethanol solubility, while at pH < 9 no condensation occurred.

Trans-enamines are the only products of condensation because of the involvement of the nitrogen linked proton in a strong hydrogen bond with the 3-keto group of (+)-usnic acid, as evidenced by the downfield shift of the enamine proton around 13 ppm (t, $J=5.2$ Hz, in the γ -aminobutyric acid (GABA)-usnic derivative), leaving unaltered the remaining hydrogen bonds between the phenol group in position 10 and the keto group in position 1 and between the phenol group in position 8 and the acetophenone in position 17.

Hydrazones **9-11** were obtained by reaction of (+)-usnic acid with hydrazides, as confirmed by IR measurements that showed the presence of C=N stretching at 1622 cm⁻¹. Compounds **11-13** were prepared by linking (+)-usnic acid to, respectively, 7-chloro-4-hydrazino-quinoline, 7-chloro-4-(aminoethyl)amino-quinoline and 6-chloro-9-(aminoethyl)amino-2-methoxyacridine. Compound **14** was obtained through the esterification of an activated γ -aminobutyric-usnic derivative with dihydroartemisinin, where the *trans*-enamine derived from the condensation of (+)-usnic acid with γ -aminobutyric acid, to obtain stereoselectively the ester in *trans* configuration, as reported in Scheme 1.

Compound **15** was synthesized by condensing 4-amino-1-butanol to usnic acid, followed by BF₃ catalyzed condensation with dihydroartemisinin (Scheme 2). A mixture (3:2 v/v) of anomers was obtained, which was directly submitted to antimalarial assays,^[22] although an analytical sample was purified through preparative HPLC for the characterization.

The compounds were tested for their *in vitro* antimalarial activity against *P. falciparum* K 1 strain and for cytotoxicity using the rat skeletal myoblast cell line L6. Table 1 summarizes the *in vitro* assay results. Compounds showing good *in vitro* activity were tested in the *P. berghei* mouse model. The *in vivo* tests results are summarized in Table 2.

The overall reactivity and activity/toxicity of usnic acid is mainly related to the triketone portion. Our first aim was to synthesize a number of enamines at the aliphatic methylketone, that produced compounds that could avoid or lessen the intrinsic toxicity of the compound (see Table 1).

The appropriate primary amines or aminoderivatives, chosen as a representative of substituents affecting physico-chemical characteristics, were condensed with usnic acid. Some amino acids (compounds **4-8**) were chosen because of their ability to modulate polarity and solubility and as mimics of tryptophan, phenylalanine, histidine; these are known to interfere with the normal functions of the surface proteins, which are rich in these amino acids and mediate adhesion and invasion processes.^[23] Aroylhydrazones **9-11** were prepared because of their potential activity as iron chelators.^[24]

We observed that compounds **7, 9, 10** (Table 1) possess minor *in vitro* activities relative to usnic acid (>5 μ g/mL) against asexual parasites, while the other compounds were found to be more active, with relevance to the products conjugated with known antimalarial scaffolds such as 4-amino-quinoline^[25,26] **11** and **12**, and acridine **13**,^[27] as well as dihydroartemisinin **14** and **15**.^[28]

Compound **11**, where the 7-chloro-4-(aminoethyl)amino-quinoline forms a hydrazone with (+)-usnic acid, showed weak activity (IC_{50} 1.1 μ g/mL), the 4-(2-aminoethyl)amino-7-chloroquinoline derivative **12** (chloroquine mimic) showed good *in vitro* activity (IC_{50} 0.167 μ g/mL), but due its limited therapeutic index we focused on the more active 9-chloro-6-aminoethylamino-2-methoxy-acridine **13** (IC_{50} 0.03 μ g/mL) and dihydroartemisinin derivatives **14** and **15** (IC_{50} 1 ng/mL and 8 ng/mL respectively). Intraperitoneal administration of the three compounds resulted in the complete loss of activity for **13**, while the positive results given by the dihydroartemisinin derivative **14** were also confirmed by p.o administration. Compound **14** achieved a complete cure when given i.p. and a delay of parasitemia of 29 days when given p.o., close to the efficacious dose of artesunate.

Compound **14** is a dihydroartemisinin ester like artesunate,^[29] where the esterifying portion is a natural phloroglucinol linked to -aminobutyric acid as a spacer. Since the enamine bonding is considered very stable in hydrolytic conditions, the potency of compound **14** could be due to the nature of the ester linkage that degrades in the presence of hydrolytic enzymes. To avoid doubts about chemical instability in the medium, compound **14** was left in a buffer solution at pH 7.4 for 5 days at room temperature and we found no evidence of degradation. When incubated with mouse liver microsomes, it releases the conjugated usnic-4-aminobutyric acid **16** and after 30 min ester **14** is completely hydrolyzed as monitored through HPLC analyses (see supporting information). Dihydroartemisinin can be anticipated to remain circulating for a protracted period, possibly due to a slow release that limits massive drug excretion.

A remarkable result is the oral efficacy of **14** that excludes a rapid excretion by conjugation of the phenol groups. Compound **14** behaves as a delivery system for dihydroartemisinin, the compound responsible of killing the parasite at the erythrocyte level.^[30] In addition, one molecule of **14** contains about 40% of the active principle dihydroartemisinin and this offers the opportunity to modulate drug administration resulting in less toxic doses. Usnic-4-aminobutyric acid **16** is apparently a “neutral” molecule. Indeed, it shows no activity at the erythrocyte stage and no cellular toxicity. These data suggest that the development of new antimalarials against the asexual blood stages could benefit from carriage by a highly functionalized molecule with pro-oxidant and antioxidant properties. Artesunate is also considered a delivery system for dihydroartemisinin, however, the succinate moiety only contributes to an increase in its solubility.

To further support our hypothesis, the corresponding ether derivative with dihydroartemisinin, compound **15**, was prepared and tested using the same conditions and dosages. Compound **15** demonstrated marked *in vitro* activity, but was also more toxic. The mice died between day 5 and 6 post infection with a reduced parasitaemia. Apparently, compound **15** does not release dihydroartemisinin *in vivo* with the appropriate kinetics to be effective against *Plasmodium*. In contrast, the usnic-aminobutanol portion (compound **17**) demonstrated weak anti-parasitic activity and less toxicity.

The lack of *in vivo* activity for **13** should be taken into consideration by research groups that are exploring the utility of acridine based drugs,^[31] since the blockage of the amino portion results in complete *in vivo* inactivity. Thus, in spite of the reported promising *in vitro* results, studies should first consider the mechanisms of action before conceiving new drug designs, since a number of factors contribute to the maintenance of *in vivo* activity.

In conclusion, we have reported a possible approach to design new antimalarial drugs that could interfere with different steps of *Plasmodium* life cycle. The opportunity to assemble natural products seems promising because of the versatility of these molecules that bear functionalities involved in cell-to-cell communication, which can also be critical to parasite

survival. An approach alternative to the current artemisinin combination therapy (ACT), that can also open new opportunities to study complex life cycles in pathogens.^[32]

Experimental Section

Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. Optical rotation was recorded on a JASCO P-1030 Polarimeter, using a 1 dm cell at the sodium D line ($\lambda = 589$ nm).

Infrared spectra were obtained on a FT/IR-4100 JASCO Spectrometer. NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^1H NMR, 100 MHz for ^{13}C). The artemisinin derivative **14** was chosen as representative compound for total assignments of carbon and proton resonances through 2D-NMR experiments (^1H , ^1H COSY, ^1H , ^{13}C HSQC, and ^1H , ^{13}C HMBC experiments) using sequences available in Bruker software. Data for ^1H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, etc.), integration and coupling constant (Hz), whereas ^{13}C NMR analyses are reported in terms of chemical shift.

Elemental analyses were performed with a Perkin-Elmer series II CHNS/O Analyzer 2400. Mass spectra were recorded on a LCQ Advantage Thermo Fisher Mass Spectrometer equipped with a ESI source (spray voltage: 4.5 kV, capillary temperature: 275.90 °C, sheath gas flow rate: 15 arbitrary units). LC-MS analysis was performed using the following equipment: HPLC Agilent 1100 with quaternary pump, diode array detector, autosampler, thermostated column holder, Bruker ion-trap Esquire 3000+ Mass Spectrometer with ESI; method: column Supelco Ascentis-Express 50 $\times$ 4.6 mm, 2.7 μm ; phase A: Milli-Q water containing 0.05% (v/v) TFA; phase B: Acetonitrile (LC-MS grade) containing 0.05% TFA; gradient: from 40% B to 100% B in 6 min, washing at 100% B for 1 min, equilibration at 5% B in the next; flow: 1 mL min⁻¹, temperature: 40°C; UV Detection at 220 and 254 nm with reference at 500 nm (40 nm bandwidth); ESI+ detection in the 50-2000 m/z range with alternating MS/MS. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063-0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040-0.063 mm; Merck). Reverse phase chromatography was performed on a Biotage SP1 instrument (pressure limit: 7 bar, range of flow rate: 1-100 mL min⁻¹) equipped with a UV detector (220 nm and 254 nm) and a Windows XP operating system control. Yields are given after purification, unless differently stated. When reactions were performed under anhydrous conditions, the mixtures were maintained under nitrogen. All reagents were purchased from Sigma-Aldrich and Fluka and used without further purification. The reactions were monitored by analytical TLC using silica gel 60 F₂₅₄ precoated glass plates (0.25 mm thickness) and silica gel reverse phase RP-18 F₂₅₄ precoated glass plates (0.25 mm thickness). Visualization was accomplished by irradiation with a UV lamp and staining with molybdic acid or vanillin solution.

Synthetic procedure to enamines and hydrazones

Commercially available (+)-usnic acid was refluxed in ethanol or ethanol-tetrahydrofuran and the eventual addition of triethylamine, with the appropriate amine, hydrazine or aminoacid (1 equiv/mol) until disappearance of the starting material. The reaction mixture was taken to dryness and the crude material crystallized or purified by chromatography, to give the appropriate derivative. Detailed procedures are described in the supporting information.

Biological Methods

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^3H -hypoxanthine incorporation assay,^[38,39] using the chloroquine and pyrimethamine resistant K1 strain that originate from Thailand^[40] and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg mL⁻¹ and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g L⁻¹), NaHCO₃ (2.1 g L⁻¹), neomycin (100 U mL⁻¹), Albumax^R (5 g L⁻¹) and washed human red cells A⁺ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg mL⁻¹ were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO₂, 3% O₂, 93% N₂. After 48 h 50 µL of ^3H -hypoxanthine (=0.5 µCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate^{TM} cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate^{TM} liquid scintillation counter (Wallac, Zurich, Switzerland). IC₅₀ values were calculated from sigmoidal inhibition curves by linear regression using Microsoft Excel.^[41]

In vitro cytotoxicity with L-6 cells. Assays were performed in 96-well microtiter plates, each well containing 100 µL of RPMI 1640 medium supplemented with 1% L-glutamine (200mM) and 10% fetal bovine serum, and 4000 L-6 cells (a primary cell line derived from rat skeletal myoblasts).^[42,43] Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg mL⁻¹ were prepared. After 70 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10µl of Alamar Blue was then added to each well and the plates incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC₅₀ values were calculated by linear regression from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).⁹

In vivo antimalarial efficacy studies, 4 days treatment: In vivo antimalarial activity was assessed basically as previously described (Peters, 1987). Groups of three female NMRI mice (20–22 g) intravenously infected with 2×10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA.^[44] Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water for intraperitoneal (i.p.) administration and in 0.5% hydroxypropyl methyl cellulose (HPMC), 0.5% benzylalcohol, 0.4% Tween 80 for oral (p.o.) administration. Compounds were administered in a volume of 10 mL kg⁻¹ on four consecutive days (4, 24, 48 and 72 h post infection). Parasitemia was determined on day 4 post infection (24h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean per cent parasitemia for the control (n = 5 mice) and treated groups expressed as a per cent relative to the control group. The survival time in days was also recorded up to 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites. All protocols and procedures used in the current study were reviewed and approved by the local veterinary authorities of the Canton Basel-Stadt. Test compounds were formulated in hydroxypropyl methyl cellulose (HPMC) and administered po (3 mice per group).

Microsomal Incubation

A typical incubation medium contained 1 mg of mouse liver microsomes pooled from male (Sigma Aldrich), 100 mM potassium phosphate (pH 7.4) buffer, 10 mM MgCl₂, and a NADPH-generating system (10 mM G6P, 0.5 mM NADP and 1 unit G6PDH) in a final

volume of 1 mL. Incubation mixtures were preincubated in the presence of a NADPH-generating system at 37 °C for 15 min. The reaction was initiated by the addition of the compound solution in buffer to a final concentration of 1 µM. Incubations were performed in air at 37 °C in a shaking bath. Reactions were stopped by adding 1 mL of CH₃CN, centrifuged, filtered and the supernatant analyzed by HPLC (300 nm) using CH₃CN/H₂O + CF₃COOH 0.05% gradient and a Purospher RP18 5 µm column on a Hitachi Elite Lachrom Instrument equipped with a DAD detector.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kappe SHI, Vaughan AM, Boddey JA, Cowman AF. *Science*. 2010; 328:862–865. [PubMed: 20466924]
2. Wells TNC, Alonso PL, Gutteridge WE. *Nat Rev Drug Discovery*. 2009; 8:879–891.
3. Iskra J. *Antimalar Drugs*. 2009;141–182.
4. World Malaria Report. WHO; 2009. p. 1-78.
5. a) Dondorp AM, Yeung S, White L, Nguon C, Day NPJ, Socheat D, von Seidlein L. *Nat Rev Microbiol*. 2010; 8:272–280. [PubMed: 20208550] b) Saralamba S, Pan-Ngum W, Maude RJ, Lee SJ, Tarning J, Lindegårdh N, Chotivanich K, Nosten F, Day NPJ, Socheat D, White NJ, Dondorp AM, White L. *Proc Natl Acad Sci U S A*. 2011; 108(1):397–402. [PubMed: 21173254]
6. Maude RJ, Woodrow CJ. *Drug Dev Res*. 2010; 71(1):12–19. [PubMed: 21399699]
7. Burrows JN, Leroy D, Lotharius J, Waterson D. *Future Med Chem*. 2011; 3(11):1401–1412. [PubMed: 21879844]
8. Muregi FW, Ishih A. *Drug Dev Res*. 2010; 71:20–32. [PubMed: 21399701]
9. Verotta L. *Phytochem Rev*. 2003; 1:389–407.
10. a) Pal C, Bandyopadhyay U. *Antioxid Redox Signaling*. 2012;1–55.b) Vega-Rodríguez J, Franke-Fayard B, Dinglasan RR, Janse CJ, Pastrana-Mena R, Waters AP, Coppens I, Rodríguez-Orengo JF, Jacobs-Lorena M, Serrano AE. *PLoS Pathog*. 2009; 5(2):e1000302. [PubMed: 19229315]
11. Singh IP, Sidana J, Bansal P, Foley WJ. *Expert Opin Ther Pat*. 2009; 19(6):847–866. [PubMed: 19473105]
12. a) Cocchiello M, Skert N, Nimis P, Sava GA. *Naturwissenschaften*. 2002; 89:137–146. [PubMed: 12061397] b) Ingólfssdóttir K. *Phytochemistry*. 2002; 61:729–736. [PubMed: 12453567]
13. Verotta L, Appendino G, Bombardelli E, Brun R. *Bioorg Med Chem Lett*. 2007; 17(6):1544–1548. [PubMed: 17234416]
14. Frankos, VH. NTP nomination for usnic acid and *Usnea barbata*. 2004. available at <http://ntp-server.niehs.nih.gov/>
15. Guo L, Shi Q, Fang J-L, Mei N, Ali AA, Lewis SM, Leakey JEA, Frankos VH. *J Environ Sci Health, Part C*. 2008; 26:317–338.
16. a) Kutney JP, Sanchez IH. *Can J Chem*. 1977; 55:1085–1090.b) Einarssdóttir E, Groeneweg J, Björnsdóttir GG, Harðardóttir G, Omarsdóttir S, Ingólfssdóttir K, Ögmundsdóttir HM. *Planta Med*. 2010; 76:969–974. [PubMed: 20143294]

17. a) Jorge SMA, Pontinha ADR, Marques MPM, Oliveira-Brett AM. *Anal Lett.* 2010; 43:1713–1722. b) Kolhardt-Floehr C, Boehm F, Troppens S, Lademann J, Truscott TG. *J Photochem Photobiol B.* 2010; 101:97–102. [PubMed: 20656501]
18. Tomasi S, Picard S, Lainé C, Babonneau V, Goujeon A, Boustie J, Uriac P. *J Comb Chem.* 2006; 8:11–14. [PubMed: 16398547]
19. Green TK, Lane CA. *J Chem Educ.* 2006; 83(7):1046–1048.
20. Luzina OA, Polovinka MP, Salakhutdinov NF, Tolstikov GA. *Russ Chem Bull Int Ed.* 2007; 56(6): 1249–1251.
21. Bazin M-A, Le Lamer A-C, Delcros J-G, Rouaud I, Uriac P, Boustie J, Corbel J-C, Tomasi S. *Bioorg Med Chem.* 2008; 16(14):6860–6866. [PubMed: 18558490]
22. The mixture of anomers was submitted to *in vitro*, *in vivo* assays, as reported by other authors. Miller MJ, Walz AJ, Zhu H, Wu C, Morasky G, Möllmann U, Tristani EM, Crumbliss AL, Ferdig MT, Checkly L, Edwards RL, Boshoff HI. *J Am Chem Soc.* 2011; 133:2076–2079. [PubMed: 21275374]
23. Rowe JA, Claessens A, Corrigan RA, Arman M. *Expert Rev Mol Med.* 2009; 11:1–29.
24. Walcourt A, Loyevsky M, Lovejoy DB, Gordeuk VR, Richardson DR. *Int J Biochem Cell Biol.* 2004; 36(3):401–407. [PubMed: 14687919]
25. Vippagunta SR, Dorn A, Matile H, Bhattacharjee AK, Karle JM, Ellis WY, Ridley RG, Vennerstrom JL. *J Med Chem.* 1999; 42(22):630–9.
26. Pussard E, Verdier F. *Fundam Clin Pharmacol.* 1994; 8(1):1–17. [PubMed: 8181791]
27. Singh T, Stein RG, Biel JH. *J Med Chem.* 1973; 16(1):89–90. [PubMed: 4565562]
28. Verotta L, Monti D. *PCT Int Appl.* 2010 WO 2010034512.
29. Lin AJ, Zikry AB, Kyle DE. *J Med Chem.* 1997; 40(9):1396–1400. [PubMed: 9135037]
30. Haynes RK, Chan W-C, Wong H-N, Li K-Y, Wu W-K, Fan K-M, Sung HHY, Williams ID, Prosperi D, Melato S, Coghi P, Monti D. *ChemMedChem.* 2010; 5:1–19.
31. a) Guetzoian L, Yu X-M, Ramiandrasoa F, Pethe S, Rogier C, Pradines B, Cresteil T, Perrée-Fauvet M, Mahy J-P. *Bioorg Med Chem.* 2009; 17(23):8032–8039. [PubMed: 19879150] b) Valdes AF-C. *Open Med Chem J.* 2001; 5:11–20. [PubMed: 21673977]
32. Delves M, Plouffe D, Scheuer C, Meister S, Wittlin S, Winzler EA, Sinden RE, Leroy D. *PloS Med.* 2012; 9(2):e1001169. [PubMed: 22363211]
33. Luzina OA, Polovinka MP, Salakhutdinov NF, Tolstikov GA. *Russ Chem Bull.* 2007; 56(6):1249–1251.
34. Melato S, Coghi P, Basilico N, Prosperi D, Monti D. *Eur J Org Chem.* 2007; 36:6118–6123.
35. Sunduru N, Sharma M, Srivastava K, Rajakumar S, Puri S, Saxena JK, Chauhan PMS. *Bioorg Med Chem.* 2009; 17(17):6451–6462. [PubMed: 19665899]
36. Guetzoian L, Yu X-M, Ramiandrasoa F, Pethe S, Rogier C, Pradines B, Cresteil T, Perree-Fauvet M, Mahy J-P. *Bioorg Med Chem.* 2009; 17(23):8032–8039. [PubMed: 19879150]
37. Jones M, Mercer AE, Stocks PA, La Pensee LJI, Cosstick R, Park BK, Kennedy ME, Piantanida I, Ward SA, Davies J, Bray PG, Rawe SL, Baird J, Charidza T, Janneh O, O'Neill PM. *Bioorg Med Chem.* 2009; 19(7):2033–2037.
38. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. *Antimicrob Agents Chemother.* 2009; 16:710–718. [PubMed: 394674]
39. Matile, H.; Pink, JRL. *Immunological Methods.* Lefkovits, I.; Pernis, B., editors. Academic Press; San Diego: 1990.
40. Thaithong S, Beale GH, Chutmongkonkul M. *Trans R Soc Trop Med Hyg.* 1983; 77:228–231. [PubMed: 6346591]
41. Huber W, Koella JC. *Acta Trop.* 1993; 55:257–261. [PubMed: 8147282]
42. Page C, Page M, Noel C. *J Oncol.* 1993; 3:473–476.
43. Ahmed SA, Gogal RM, Walsh JE. *J Immunol Methods.* 1994; 170:211–224. [PubMed: 8157999]
44. Peters, W. *Chemotherapy and drug resistance in malaria.* Vol. 1. Academic Press, Inc.; New York: 1987. p. 145-273.

Figure 1.

Chemical formulas of the synthesized compounds **2-15**. Non IUPAC numbering for usnic acid was used, which allowed comparison with spectroscopic assignments in the literature.

Scheme 1.

Synthesis of compound **14**. Reaction conditions: a: γ -aminobutyric acid, EtOH, reflux, N_2 , 5 h, 73%; b: dihydroartemisinin, 2-chloro-4,6-dimethoxy-1,3,5-triazine/ *N*-methylmorpholine, rt, N_2 , 24 h, 60%.

Scheme 2.

Synthesis of compound **15**. Reaction conditions: a: 4-amino-1-butanol, EtOH, reflux, N₂, 5 h, 66%; b: dihydroartemisinin, BF₃·Et₂O, CH₂Cl₂, 0°C to rt, N₂, 2 h, 75% (/ 3:2).

Table 1

In vitro activity against *P. falciparum*^[a] and L6 cells.^[b]

Compound	P. f. K1		Cytotox. L6		P. f. K1		Cytotox. L6	
	IC ₅₀ ^[c]	IC ₅₀ ^[c]	IC ₅₀ ^[c]	IC ₅₀ ^[c]	Compound	IC ₅₀ ^[c]	IC ₅₀ ^[c]	IC ₅₀ ^[c]
1	15.28	11.63	[d]		10	>10.5	66.97	
2	4.68	>208			11	2.12	9.75	
3	3.06	23.94			12	0.305	8.29	
4	7.45	165.3			13	0.048	9.60	
5	9.60	34.62			14	0.0014	0.816	
6	1.61	157.1			15	0.0117	0.015	
7	>10	23.51			16	12.67	39.82	
8	>10	67.36			17	5.61	13.58	
9	9.08	21.06						

[a] Positive controls (*P. falciparum*): chloroquine, IC₅₀ 0.222 μmol/mL and artemisinin IC₅₀ 0.0035 μmol/mL

[b] Positive control (L6 cells): podophyllotoxin IC₅₀ 0.169 μmol/mL

[c] The IC₅₀ values in μmol/mL are the means of two independent assays; the individual values vary less than a factor 2

[d] from ref. 16b.

Table 2*In vivo* antimalarial activity of in vitro active compounds in *P. berghei* infected mice.

Compound	Day × dose (mg/kg)	Route	% inhibition of parasitemia	Mean survival days (days)
control				7
artesunate	4 × 100	po	99.94	27
13	4 × 50	ip	0	7
14	4 × 50	ip	99.71	>30
14	4 × 3	po	81.88	7
14	4 × 10	po	99.8	10
14	4 × 30	po	99.62	16
14	4 × 100	po	99.43	29.3
15 ^[a]	4 × 50	ip	99.62	5.3
15 ^[a]	4 × 3	po	0	4
15 ^[a]	4 × 10	po	51.17	9.7
15 ^[a]	4 × 30	po	99.89	17
15 ^[a]	4 × 100	po	99.81	23.3

^[a] compound not soluble, administered as suspension