Novel Quinone Methides from *Salacia kraussii* with in Vitro Antimalarial Activity

Joaquim Neves Figueiredo,[†] Barbara Räz,[†,‡] and Urs Séquin*[,†]

Institut für Organische Chemie, Universität Basel, St. Johanns-Ring 19, CH-4056 Basel, Switzerland, and Schweizerisches Tropeninstitut, Socinstrasse 57, Postfach, CH-4002 Basel, Switzerland

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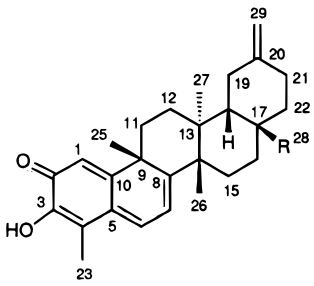
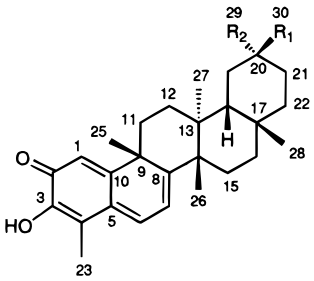
Three novel quinone methides, i.e., 28-*nor*-isoiguesterin-17-carbaldehyde (**1**), 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**), and 28-hydroxyisoiguesterin (**3**), together with the known celastrol (**5**), pristimerin (**6**), and isoiguesterol (**7**), were isolated from the roots of *Salacia kraussii* (Celastraceae) by bioassay-guided fractionation. The structures of the compounds were determined by DEPT and 2D NMR techniques. The isolates showed antimalarial activity 30−50-fold greater than their cytotoxicity (in HT-29 cells) in vitro, and they showed an additive effect when combined with each other. In vivo, **2** was found to be inactive against blood stages of *Plasmodium berghei* in mice after oral and parenteral administration, and the compound was toxic with increasing concentrations.

Salacia kraussii (Harv.) Harv. is a shrublet with annual shoots from a rhizome with a chrome yellow layer beneath the bark. It grows in dunes, bushy steppes, and open woods in the littoral of Mozambique and in the Province of Natal in South Africa.[1] In Mozambican traditional medicine, the plant is used against bilharziasis and dysentery. However, Celastraceae, the family in which Salacias belong, were also reported to possess antimalarial activity,[2] bioactivity inferred as due to the presence of celastroloids,[3−10] an important bioactive class of compounds comprising about 70 compounds.[2] However, despite their high bioactivity, the only pure quinone methide where antimalarial activity had been reported was pristimerin (**6**).[11] Thus, bioassay-guided fractionation using antimalarial activity testing was carried out with the root extracts from *Salacia kraussii*. Three novel quinone methides, i.e., 28-*nor*-isoiguesterin-17-carbaldehyde (**1**), 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**), and 28-hydroxyisoiguesterin (**3**), together with the known celastroloids celastrol (**5**), pristimerin (**6**), and isoiguesterol (**7**), could be isolated. The structures of the mentioned quinone methides, in particular of the novel dinortriterpenoids, were elucidated by use of COSY, DEPT, ^{1}H−^{13}C HETCOR, and long-range ^{1}H−^{13}C HETCOR. The antimalarial potential of these compounds and their interaction profiles were determined in vitro using two strains of *Plasmodium falciparum*; in vivo studies were carried out using *Plasmodium berghei* in mice. Cytotoxicity was measured using HT-29 cells. Since *Salacia kraussii* is a plant with unreported chemotaxonomy, it is noteworthy that, in addition to the quinone methides, two important chemotaxonomic markers for the Celastraceae,[12] dulcitol and gutta-percha (*trans*-polyisoprene), were isolated.



	R
1	CHO
2	COOMe
3	CH_2OH
4	CH_3



	R_1	R_2
5	Me	COOH
6	Me	COOMe
7	H	CH_2OH

Results and Discussion

The quinone methides were obtained as red amorphous solids from medium-pressure liquid chromatography (MPLC) and preparative HPLC fractionation of the combined petroleum ether and methylene chloride extracts, the latter after the removal of gutta-percha. The mass spectra of **1**−**3**, celastrol (**5**), pristimerin (**6**), and isoiguersterol (**7**) showed characteristic quinone methide signals at *m*/*z* M − 14, 241, 227, 202, 201, and 200.[13,14] Likewise, the well-known IR bands for the enol group hydrogen bonded to the neighboring carbonyl and for the conjugated carbonyl group of the quinone methide system were present at 3380 and 1607 cm^{-1}, respectively, in the spectra of all compounds. Furthermore, the quinone methide moiety was also evident from the characteristic ^{1}H and ^{13}C NMR resonances at δ 6.51−6.50 (s, H-1), 7.04−6.96 (br s, HO−C-3), 7.08−7.00

* To whom correspondence should be addressed. Tel.: 41-61-2671110. Fax: 41-61-2671103. E-mail: sequin@ubaclu.unibas.ch.
† Universität Basel.
‡ Schweizerisches Tropeninstitut.

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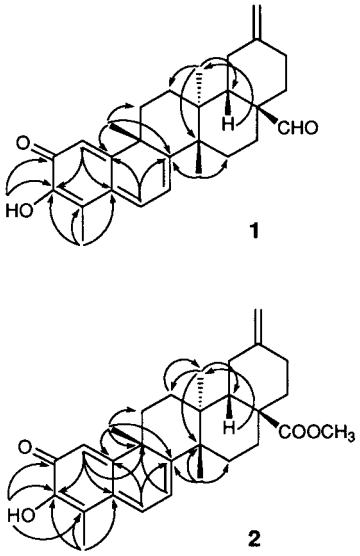

Figure 1. H to C correlations (→) observed in the long-range $^1H-^{13}C$ HETCOR spectra of **1** and **2** (optimized for $^nJ_{CH} = 7$ Hz with $n = 2$ or 3).

(d, H-6), 6.35−6.30 ppm (d, H-7), and at 120.4−119.4 (d, C-1), 178.4−178.4 (s, C-2), 147.0−146.0 (s, C-3), 120.4−117.0 (s, C-4), 127.7−127.3 (s, C-5), 135.4−133.3 (d, C-6), 118.3−117.8 (d, C-7), 172.6−168.1 (s, C-8), 43.1−42.7 (s, C-9), 165.0−164.4 (s, C-10).[6,15−17]

Celastrol (**5**), pristimerin (**6**), and isoiguesterol (**7**) were consequently readily identified by comparison with the published data.[2,6,17] The molecular formula of 28-*nor*-isoiguesterin-17-carbaldehyde (**1**) was found to be $C_{28}H_{34}O_3$ after accounting for the $[M]^+$ at *m/z* 418, for 4 × CH_3, 8 × CH_2, 5 × CH, and 11 × C (from DEPT and APT spectra), and for three oxygen atoms. Two of the latter belonged to the quinone methide moiety, and one to an aldehyde group as was evident from the proton singlet at 9.44 ppm, which was correlated to the carbonyl methine resonance at 205.2 ppm in the HETCOR. Likewise, the molecular formula of 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) was found to be $C_{29}H_{36}O_4$ using similar arguments: $[M]^+$ at *m/z* 448, 5 × CH_3, 8 × CH_2, 4 × CH, and 12 × C (from DEPT and APT spectra), and four oxygen atoms, two in the quinone methide moiety and two from a methyl ester group evident from the NMR spectra (three-proton singlet at 3.75 ppm and q at 52.2 ppm for the methoxy group, as well as s at 178.4 ppm for the carbonyl group). The molecular formula of the third dinortriterpene, 28-hydroxyisoiguesterin (**3**), was found to be $C_{28}H_{36}O_3$ from the $[M]^+$ at *m/z* 420, 4 × CH_3, 9 × CH_2, 4 × CH, and 11 × C (from DEPT and APT spectra), and three oxygen atoms, two in the quinone methide moiety and one from a hydroxymethyl group evident from the two one-proton doublets at 3.78 and 3.48 ppm, and the methylene carbon resonance at 69.4 ppm.

Additionally, an *exo*-methylene group was evident in all three compounds: two-proton signals at 4.64 ppm in **1**, 4.61 ppm in **2**, and 4.62 ppm in **3**, correlated in the HETCOR to the methylene carbons resonances at 109.5, 108.7, and 108.7 ppm, respectively. The only quinone methide possessing such features that has been described hitherto is isoiguesterin (**4**).[18] Comparison of the NMR data of **1**−**4** (Table 1) showed striking similarities indicating that the compounds, albeit different, possessed the same structural frame. In fact, as in isoiguesterin (**4**), compounds **1**−**3** showed the presence of 8 × CH_2, 4 × CH, and 11 × C. However, in contrast to isoiguesterin (**4**) with its five aliphatic methyl groups, **1**−**3** displayed four methyl groups. The missing methyl group was compensated by the presence of a CHO in **1**, $COOCH_3$ in **2**, and CH_2OH in **3**, leading to the conclusion that the specified groups replaced the (additional) methyl group in isoiguesterin (**4**). The long-range $^1H-^{13}C$ HETCOR (optimized for $^nJ_{CH} = 7$ Hz) of **1** and **2** (Figure 1, Table 2), as well as a two-bond coupling (19 Hz) between C-17 and the aldehyde proton observed in

Table 1. ^{13}C NMR Data of 28-*nor*-Isoiguesterin-17-carbaldehyde (**1**), 17-(Methoxycarbonyl)-28-*nor*-isoiguesterin (**2**), 28-Hydroxyisoiguesterin (**3**), and Isoiguesterin (**4**) ($CDCl_3$)

	compounds			
carbon no.	**1**	**2**	**3**	**4**[a]
1	119.7 d	119.6 d	119.6 d	119.6 d
2	178.0 s	178.3[b] s	178.4 s	178.3 s
3	146.0 s	146.0 s	146.0 s	146.0 s
4	117.0 s	117.0 s	117.1 s	117.1 s
5	127.7 s	127.5 s	127.5 s	127.4 s
6	133.4 d	133.6 d	133.9 d	133.9 d
7	118.0 d	117.8 d	118.0 d	117.9 d
8	168.1 s	168.7 s	169.8 s	170.1 s
9	42.8 s	42.7 s	43.0[b] s	42.9 s
10	164.6 s	164.8 s	165.0 s	165.0 s
11	33.6 t	33.7 t	33.8[c] t	33.9 t
12	29.1 t	29.4 t	29.7[c] t	29.7 t
13	40.1[b] s	40.4[c] s	41.0[b] s	41.3 s
14	44.1[b] s	44.2[c] s	44.6[b] s	44.8 s
15	27.4 t	28.0 t	27.9[c] t	28.4 t
16	26.6 t	31.7 t	29.9[c] t	36.0 t
17	47.7 s	45.7 s	36.1 s	31.6 s
18	37.8 d	39.3 d	38.8 d	44.9 d
19	31.9 t	32.0 t	32.6[c] t	30.4 t
20	146.0 s	146.6 s	147.2 s	147.9 s
21	31.5[c] t	31.8[d] t	30.3[c] t	30.5 t
22	31.1[c] t	32.8[d] t	30.1[c] t	36.9 t
23	10.2 q	10.2 q	10.3 q	10.2 q
25	38.7 q	38.9 q	38.6 q	38.9 q
26	21.0 q	21.0 q	21.1 q	21.3 q
27	20.2 q	19.4 q	20.4 q	19.7 q
28	205.2 d	178.4[b] s	69.4 t	31.1 q
29	109.5 d	108.7 d	108.7 d	108.2 t
30		52.2 q		

[a] Data from ref 18. [b−d] Entries with the same superscript are interchangeable.

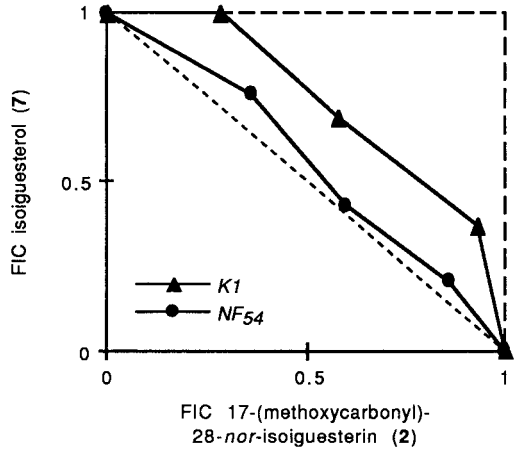

Figure 2. Isobolograms showing the effect of 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) on *P. falciparum* strains *K1* and NF_{54} in vitro when combined with isoiguesterol (**7**) at fixed ratios of their IC_{50} values.

Table 2. Long-Range $^1H-^{13}C$ Correlations of 17-(Methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) and 28-*nor*-Isoiguesterin-17-carbaldehyde (**1**)[a]

	$^1H-^{13}C$ correlations observed for carbon nos.[b]		
hydrogen no.	**2** (in $CDCl_3$)	**1** (in $CDCl_3$)	**1** (in C_{5D5N})
1	9, 5, 3, (1)	5, 3, (1)	5, 3, (1)
6 and HO-C-3[c]	10, 8, (6) and 4, (3), 2	10, 8, (6), (3), 2	10, 8, (6)
7	9, (7), 5	(7), 5	(7), 5
12	27	n.c.[d]	n.c.
18	27	27	27
23	5, 4, 3	(23), 5, 4, 3	(23), 5, 4, 3
25	11, 10, 9, 8	(25), 11, 10, 8	(25), 11, 10, 9, 8
26	15, 14, 8	15, 8	15, 8
27	18, 14, 12	18, 14, 12	18, 14, 12

[a] HETCOR optimized for $^nJ_{CH} = 7$ Hz, $n = 2$ or 3. [b] Numbers in parentheses: cross peaks observed for $^1J_{CH}$. [c] No correlations of HO-C-3 observed in C_5D_5N. [d] No cross peaks observed.

Table 3. Antiplasmodial Activity and Cytotoxicity of the Quinone Methides Isolated from the Roots of *Salacia kraussii*

	IC_{50} (ng/mL)		
compounds	P. falciparum K1[a]	P. falciparum NF_{54}[b]	HT-29 cells
28-*nor*-isoiguesterin-17-carbaldehyde (**1**)	94.0	79.9	n.t.[c]
17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**)	27.6	37.1	2380
28-hydroxyisoiguesterin (**3**)	114.4	140.2	6060
celastrol (**5**)	180.9	254.2	1300
pristimerin (**6**)	190.4	270.9	4600
isoiguesterol (**7**)	22.9	54.1	2430
chloroquine	61.1	4.6	58 000

[a] Chloroquine-resistant strain. [b] Chloroquine-sensitive strain. [c] Not tested.

the fully coupled ^{13}C NMR spectrum of **1**, corroborated these assignments. The position of the *exo*-methylene group could be inferred from the COSY spectrum, in which cross-peaks between the pairs H-18/H_2-19 and H_2-21/H_2-22 were the only peaks observed for the E ring, demonstrating that, like in isoiguesterin (**4**), no protons were available at C-20.

From the methylene chloride extract, gutta-percha (*trans*-polyisoprene) was isolated by precipitation in acetone, and from the methanolic extract, dulcitol was isolated. Both compounds were identified by comparison with published data (^1H, ^{13}C NMR, mp, IR and optical rotation).[19−24] Due to the presence of dulcitol, gutta-percha and celastroids, Salacias, formerly belonging to the Hippocrateaceae, were integrated into the Celastraceae.[12] Thus, the presence of these markers in *Salcia kraussii* reinforces the chemootaxonomical importance that they play in this family.

The in vitro activity (expressed as IC_{50} values) of the isolated quinone methides in comparison to chloroquine is shown in Table 3. The activity of all compounds except 28-*nor*-isoiguesterin-17-carbaldehyde (**1**) is slightly higher against the multidrug-resistant strain *K1* than against the drug-sensitive strain *NF_{54}* of *Plasmodium falciparum*. The best activities were found to be isoiguesterol (**7**) with 22.9 ng/mL against *K1* and 54.1 ng/mL against *NF_{54}* and 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) with 27.6 ng/mL against *K1* and 37.1 ng/mL against *NF_{54}*. The other four compounds showed a 3−9-fold lower activity than **2** and **7**, and all six compounds tested exhibited in vitro activity against the chloroquine-sensitive strain *NF_{54}* 10−50 times lower compared to the reference compound, chloroquine. In contrast, isoiguesterol (**7**) and 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) were two to three times more active than chloroquine against the multidrug-resistant strain *K1*. All six isolated quinone methides were found to be cytotoxic against the human adenocarcinoma cell line HT-29 in the range of 1300 ng/mL up to 6060 ng/mL. They displayed, however, a 10−100-fold higher activity against plasmodia than against HT-29 cells, thus indicating some selectivity.

Parenteral administration of 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) of 10 mg/kg body weight lead to death of mice after 1 day, whereas 5 mg/kg and 1 mg/kg parenteral as well as 30 mg/kg peroral of the latter could not cure mice nor reduce parasitaemia of *Plasmodium berghei* significantly.

In view of the fact that plant remedies from the Celastraceae family are used in the treatment of malaria by traditional healers, drug interaction studies were undertaken in vitro in order to detect synergistic, additive, or antagonistic effects within this group of compounds. The fractional inhibitory concentrations (FIC) were plotted onto isobolograms. Celastrol (**5**) and pristimerin (**6**) showed an additive effect. Similar effects were also observed when **5** and **6** were combined with the other isolated quinone methides. Only the most active compounds, 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) and isoiguesterol (**7**), showed a slightly antagonistic effect against strain *K1* (Figure 2). 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) also revealed a highly antagonistic effect to chloroquine against the chloroquine-sensitive strain *NF_{54}* and a slightly antagonistic effect against the multidrug-resistant strain *K1* (Figure 3).

In conclusion, quinone methides were active against both chloroquine-sensitive and multidrug-resistant *Plasmodium falciparum* strains in vitro. However, 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) was toxic and inactive in vivo. Therefore, bioavailability and toxicity of this group of compounds has to be taken into consideration for further development.

Experimental Section

General Experimental Procedures. The NMR spectra were measured on a Varian Gemini 300 and on

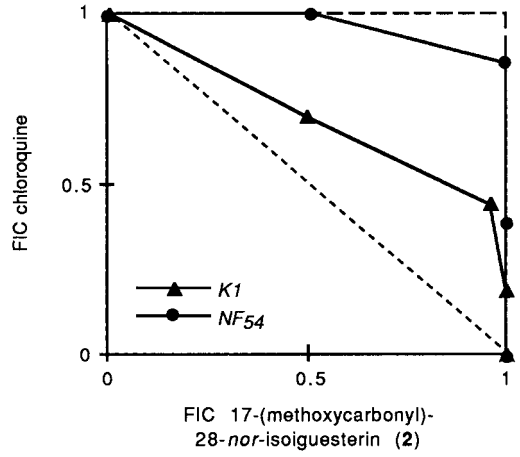

Figure 3. Isobolograms showing the effect of 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**2**) on *P. falciparum* strains *K1* and NF_{54} in vitro when combined with chloroquine at fixed ratios of their IC_{50} values.

a Varian VXR 400. The shifts are shown in ppm relative to tetramethylsilane (TMS). Multiplicities in ^{13}C NMR spectra were obtained from APT-, DEPT-, $^{1}H-^{13}C$ HETCOR-, or $^{1}H-^{13}C$-coupled spectra. The IR spectra were measured on a Perkin-Elmer 1600 series spectrometer. Mass spectra were recorded on a VG-70-250 spectrometer. The MPLC apparatus was a Büchi 681/687 coupled to a Kontron UV/vis spectrophotometer (Uvikon 735LC). Analytical HPLC was run on a Hewlett-Packard HP 1090 LC model and the preparative HPLC on a Waters 600E Millenium system coupled to a Waters diode-array detector model 991M.

MPLC method 1: (Büchi 681/687) Lichroprep Diol (Merck), 25−40 μm, 460 × 15 mm i.d.; eluent A, hexane; eluent B, tetrahydrofuran. Gradient: 100% A to 80% A in 20 min, 80% A to 50% A in 40 min, 50% A to 100% A in 20 min; flow rate = 15 mL/min, detection at 254 nm. **MPLC method 2:** (Büchi 681/687); column: Lichroprep Diol (Merck), 25−40 μm, 460 × 15 mm i.d.; eluent, 100% water to 100% methanol in 120 min; flow rate = 15 mL/min; detection at 254 nm. **HPLC method 1:** (Waters 600E) Eurospher 100 C18 (Knauer) 7 μm, 250 × 16.0 mm i.d.; eluent A, water; eluent B, tetrahydrofuran. Gradient: 60% A to 40% A in 20 min, 5 min 40% A, 40% A to 100% B in 10 min; flow rate = 12 mL/min. Detection with diode-array detector Waters 991M. **HPLC method 2:** (Waters 600E); column: Lichrospher 100 Diol (Knauer) 10 μm, 250 × 16.0 mm i.d. (preparative scale); column: Lichrospher 100 Diol (Knauer) 5 μm, 250 × 4.6 mm i.d. (analytical); eluent A, hexane; eluent B, tetrahydrofuran. Gradient: 10 min 100% A to 80% A, 80% A to 50% A in 20 min, 50% A to 100% B in 10 min; flow 12 mL/min (preparative), 1 mL/min (analytical). Detection with diode-array detector Waters 991M.

Plant Material. The roots of *S. kraussii* were collected in April 1994 in the Maputo Province, Mozambique. A voucher specimen identified by Mr. S. Bandeira, Maputo, was deposited in the Herbario do Departamento de Ciências Biologicas, Universidade Eduardo Mondlane, Maputo, Mozambique. A token herbarium was taken to Switzerland, reidentified by Dr. F. Haerdi, Allschwil, and deposted in the Herbarium generale of the Botanisches Institut der Universität Basel (BAS; voucher no. JF 394/01).

Extraction and Isolation. The ground roots of *S. kraussii* (800 g) were extracted consecutively with two 1 L portions (24 h per portion) of petroleum ether, methylene chloride, methanol, and water, at room temperature. A red solid (8.2 g) was obtained from the combined petroleum ether and methylene chloride extracts after the precipitation of *trans*-polyisoprene (18 g), attained by pouring the concentrated methylene chloride extract of *S. kraussii* into acetone. The red solid was submitted to preparative-scale chromatography: MPLC method 1, HPLC method 1, followed by HPLC method 2. **1** (185.7 mg), **2** (135.2 mg), **3** (6.7 mg), celastrol (**5**) (25.3 mg), pristimerin (**6**) (7.2 mg), and isoiguesterol (**7**) (44.9 mg), were thus obtained.

Additionally, from 5.81 g of the methanolic extract, 332 mg of pure dulcitol was obtained after recrystallization from water out of the first fraction of the MPLC Method 2.

Biological Tests. Stocks. Chloroquine-resistant *P. falciparum* (*K1*) was originally isolated from a patient in Thailand,[25] and the chloroquine-sensitive reference strain of *P. falciparum* (NF_{54}) was the one originally isolated at Amsterdam (NL) airport.[26] Cultures of both strains were maintained at Hoffmann-La Roche Ltd., Basel (courtesy of Dr. H. Matile).

Culture Conditions. Plasmodia were inoculated into human erythrocytes at a haematocrit of 6% in RPMI-1640 medium, supplemented with 32 mM $NaHCO_3$ and 35 mM HEPES. Heat-inactivated human plasma (10%) was added. Cultures were maintained with a parasitaemia below 10% at 37 °C in a humid atmosphere with 5% O_2, 5% CO_2, and 90% N_2.

Antimalarial Assay. The drug sensitivity assay was performed as previously described by Desjardins and co-workers.[27] Briefly, a suspension (200 μL) of *P. falciparum*-infected (with *K1*- and NF_{54}-strain, respectively) red blood cells (0.3% parasitaemia, 1.25% haematocrit) was inoculated into 96-well microtiter plates. The plasmodia were incubated in the presence of various drug concentrations (7−500 ng/mL). In addition, the known antimalarial drug, chloroquine, was tested in each experiment over a concentration of 0.3−250 ng/mL. After a preincubation of 50 h, 25 μL (0.5 μCi) of generally labeled [^{3}H]hypoxanthine (Amersham Int., Buckinghamshire, England) was added to each well and [^{3}H]hypoxanthine incorporation determined after a total incubation time of 68 h. Plasmodia were harvested on glass fiber filters (Filtermat A, Wallac). Dried filters were placed in plastic bags containing scintillation liquid (Beta Plate Scint, LKB Scintillation Products, England) and counted for 1 min in a liquid scintillation counter (Betaplate, Model 1205, Wallac). [^{3}H]Hypoxanthine incorporation specifically due to malaria parasites was obtained by subtracting the mean dpm of unparasitized red blood cells (negative contol) from all sample data, and the results were expressed as a percentage of uninhibited, parasite-specific [^{3}H]hypoxanthine incorporation (positive control). IC_{50} values were calculated by linear interpolation, selecting values above and below the 50% mark.[28] Minor deviations, if any, of the IC_{50} value calculated by linear interpolation from the IC_{50} value calculated by logit analysis were negligible.[29] All experiments were performed twice, with each drug in duplicate.

Drug Interaction. Drug interactions of the compounds isolated with each other were assessed according to Jaquet and co-workers.[30] Fixed ratios of starting concentrations (1 + 1, 1 + 3, 3 + 1) were prepared and analyzed in vitro and fractional inhibitory concentrations plotted onto isobolograms in order to visualize a potential synergistic, additive, or antagonistic effect.

Cell Line and Culture Conditions. HT-29 human adenocarcinoma cells were isolated from a primary tumor in 1964 and were obtained from the American Type Culture Collection (ATCC, reference no. HTB 38). HT-29 was cultivated in Minimum Essential Medium (MEM), supplemented with 10% heat-inactivated foetal bovine serum (Biological Industries, Israel).

Cytotoxicity Assay. The cytotoxicity assay was performed as previously described by Pagé and co-workers[31] with minor modifications. Briefly, cells were inoculated into 96-well flat-bottomed microtiter plates (Costar, USA) at a density of 5000 cells/100 μL culture medium. The cultures were incubated for 72 h in the presence of various drug concentrations (0.2−500 μg/mL; drug stock solutions: 30 mg/mL in DMSO). Background fluorescence of the drug-containing medium was determined for each drug dilution. Wells without drug served as controls (the influence of the DMSO vehicle was considered to be negligible under the assay conditions). Alamar Blue (10 μL) was added after 70 h and fluorescence development determined after a total drug incubation time of 72 h. Measurement of fluorescence was performed with the Millipore Cytofluor plate reader (Cytofluor 2300, Millipore Corp., Bedford, MA) at 530 nm excitation wavelength and 590 nm emission wavelength. IC_{50} values were calculated as described for the antimalarial assay above.

In Vivo Studies. In vivo studies were performed as described by Ridley and co-workers.[32] Compounds were administered as physiological salt solutions or suspensions containing 3% ethanol and 7% Tween 80. Groups of five mice were treated at day +3 after infection with *P. berghei* with a single dose either subcutaneous of 10, 5, and 1 mg/kg body weight or peroral with 30 mg/kg body weight. Antimalarial activity was expressed in terms of survival time compared to an untreated control group.

28-*nor*-Isoiguesterin-17-carbaldehyde (1): red solid; t_R 25.15 min (HPLC Method 2); UV/vis (MeOH) λ_{max} (log ϵ) 255, 424 (2.94) nm; IR (NaCl, film) ν_{max} 3390 w, 2944 s, 2866 m, 1706 s, 1583 s, 1506 s, 1444 s, 1283 m, 1222 m cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.44 (1H, s, H-28), 7.08 (1H, d, J = 7.1 Hz, H-6), 7.00 (1H, broadened s, HO−C-3), 6.54 (1H, s, H-1), 6.31 (1H, d, J = 7.1 Hz, H-7), 4.64 (2H, d, J = 1.7 Hz, H-29), 2.55 (1H, d, J = 15.2 Hz, H-19), 2.26 (3H, br d, H-18, H-21, H-22), 2.21 (3H, s, H-23), 2.12−1.60 (10H, m), 1.49 (3H, s, H-25), 1.37 (1H, m, H-16), 1.30 (3H, s, H-26), 0.77 (3H, s, H-27); ^{1}H NMR (C_5D_5N, 400 MHz) δ 10.78 (1H, broadened s, HO−C-3), 9.50 (1H, s, H-28), 6.91 (1H, d, J = 7.0 Hz, H-6), 6.73 (1H, s, H-1), 6.18 (d, J = 6.9 Hz, H-7), 4.76 (1H, s, H-29), 4.67 (1H, s, H-29), 2.55 (1H, d, J = 15.3 Hz, H-19), 2.33 (3H, s, H-23), 2.21 (3H, m, H-18, H-21, H-22), 2.09−2.08 (2H, m, H-19, H-11), 1.95−1.93 (2H, m, H-21, H-22), 1.85−1.63 (5H, m, H-11, H-12, H-15, H-16), 1.52−1.46 (1H, m, H-15), 1.39 (3H, s, H-25), 1.22 (1H, m, H-16), 1.18 (3H, s, H-26), 0.79 (3H, s, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz; * denotes interchangeable assignments) δ 205.2 (d, $^1J_{CH}$ = 170 Hz, C-28), 178.4 (s, C-2), 168.1 (s, C-8), 164.6 (s, C-10), 146.1* (s, C-3), 146.0* (s, C-20), 133.4 (d, $^1J_{CH}$ = 158 Hz, C-6), 127.7 (s, C-5), 119.7 (d, $^1J_{CH}$ = 161 Hz, C-1), 118.0 (d, $^1J_{CH}$ = 160 Hz, C-7), 117.0 (s, C-4), 109.5 (d, $^1J_{CH}$ = 155 Hz, C-29), 47.7 (br s, $^1J_{CH}$ = 19 Hz, C-17), 44.1* (s, C-14), 42.8 (s, C-9), 40.1* (s, C-13), 38.7 (q, $^1J_{CH}$ = 130 Hz, C-25), 37.8 (d, $^1J_{CH}$ = 127 Hz, C-18), 33.6 (t, $^1J_{CH}$ = 133 Hz, C-11), 31.9 (t, $^1J_{CH}$ = 123 Hz, C-19), 31.5* (t, $^1J_{CH}$ = 127 Hz, C-21), 31.1* (t, $^1J_{CH}$ = 128 Hz, C-22), 29.1 (t, $^1J_{CH}$ = 128 Hz, C-12), 27.4 (t, $^1J_{CH}$ = 128 Hz, C-15), 26.6 (t, $^1J_{CH}$ = 125 Hz, C-16), 21.0 (q, $^1J_{CH}$ = 127 Hz, C-26), 20.0 (q, $^1J_{CH}$ = 125 Hz, C-27), 10.2 (q, $^1J_{CH}$ = 128 Hz, C-23); ^{13}C NMR (C_5D_5N, 100 MHz; * denotes interchangeable assignments) δ 205.3 (d, C-28), 179.4 (s, C-2), 166.1 (s, C-8), 163.6 (s, C-10), 148.3 (s, C-3), 146.9 (s, C-20), 132.1 (d, C-6), 128.3 (s, C-5), 121.4 (d, C-1), 118.3 (d, C-7), 117.7 (s, C-4), 109.5 (d, C-29), 47.8 (s, C-17), 44.1* (s, C-14), 42.4 (s, C-9), 40.2* (s, C-13), 38.6 (q, C-25), 38.2 (d, C-18), 33.8 (t, C-11), 32.2 (t, C-19), 31.44* (t, C-21), 31.42* (t, C-22), 29.1 (t, C-12), 27.5 (t, C-15), 26.7 (t, C-16), 21.0 (q, C-26), 20.2 (q, C-27), 10.5 (q, C-23); EIMS (70 *e*V, ca. 230 °C) *m*/*z* 419 [M + 1]$^+$ (7), 418 [M]$^+$ (20), 404 [M − 14]$^+$ (3), 253 (6), 241 (10), 217 (22), 215 (13), 203 (17), 202 (100), 201 (67), 200 (24), 199 (15), 189 (23), 173 (12), 159 (15), 157 (12), 145 (10), 133 (18), 131 (10), 119 (15), 109 (7), 107 (12), 105 (21).

17-(Methoxycarbonyl)-28-*nor*-isoiguesterin (2): red solid; t_R 17.56 min (HPLC Method 2); UV/vis (MeOH) λ_{max} (log ϵ) 255, 424 (3.94) nm; IR (NaCl, film) ν_{max} 3392 w, 2950 m, 1722 s, 1596 s, 1558 w, 1517 m, 1436 s, 1286 m, 1210 s cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, broadened s, HO−C-3), 7.00 (1H, d, J = 6.8 Hz, H-6), 6.54 (1H, s, H-1), 6.30 (1H, d, J = 6.8 Hz, H-7), 4.61 (2H, s, H-29), 3.75 (3H, s, H-30), 2.62 (1H, d, J = 6.7 Hz, H-18), 2.49 (1H, d, J = 15.6 Hz, H-19), 2.30−2.06 (6H, m, H-11, H-16, H-19, H-21, H-22), 2.21 (3H, s, H-23), 2.04−1.94 (2H, m, H-11, H-22), 1.90−1.84 (2H, m, H-12), 1.82−1.72 (1H, m, H-15), 1.72−1.54 (2H, m, H-15, H-16), 1.48 (3H, s, H-25), 1.36 (3H, s, H-26), 0.74 (3H, s, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS (70 eV, ca. 240 °C) *m*/*z* 449 [M + 1]$^+$ (4), 448 [M]$^+$ (11), 434 [M − 14]$^+$ (4), 248 (8), 247 (41), 241 (8), 233 (7), 215 (16), 203 (6), 202 (40), 201 (37), 200 (11), 188 (15), 187 (100), 145 (17), 131 (18), 119 (19), 107 (8), 105 (17).

28-Hydroxyisoiguesterin (3): red solid; t_R 17.76 min (HPLC method 2); UV/Vis (MeOH) λ_{max} (log ϵ) 255, 424 (3.94) nm; IR (NaCl, film) ν_{max} 3391 w, 2949 m, 1590 s, 1512 m, 1437 s, 1286 m cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.02 (1H, d, J = 7.1 Hz, H-6), 6.97 (1H, broadened s, HO−C-3), 6.54 (1H, d, J = 1.4 Hz, H-1), 6.34 (1H, d, J = 7.2 Hz, H-7), 4.62 (2H, m, H-29), 3.78 (1H, d, J = 10.5 Hz, H-28), 3.48 (1H, d, J = 10.4 Hz, H-28), 2.45 (1H, d, J = 15.8 Hz, H-19), 2.22 (3H, s, H-23), 2.35−2.05 (3H, m), 2.05−1.9 (2H, dt, J_1 = 13.7 Hz and J_2 = 4.3 Hz), 1.9−1.7 (4H, m), 1.7−1.5 (4H, m), 1.48 (3H, s, H-25), 1.32 (3H, s, H-26), 1.4−1.2 (1H, m), 0.75 (3H, s, H-27), CH_2O*H* not observed; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS (70 eV, ca. 220 °C) *m*/*z* 421 [M + 1]$^+$ (4), 420 [M]$^+$ (12), 406 [M − 14]$^+$ (2), 267 (2), 253 (5), 241 (11), 215 (6), 214 (7), 203 (14),

202 (100), 201 (32), 200 (8), 187 (6), 145 (12), 109 (6), 107 (13), 105 (14).

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