Alisiaquinones and Alisiaquinol, Dual Inhibitors of *Plasmodium falciparum* Enzyme Targets from a New Caledonian Deep Water Sponge

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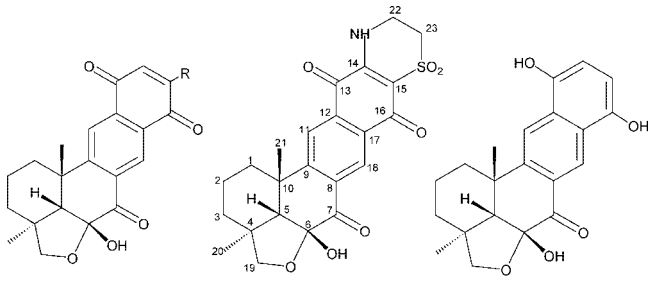
Four new meroterpenes, alisiaquinones A−C (**1**−**3**) and alisiaquinol (**4**), were isolated from a New Caledonian deep water sponge. Their structures and relative stereochemistry were elucidated by spectroscopic data analysis. They are related to xestoquinone, but showed unusual substitution on a tetrahydrofuran junction. They displayed micromolar range activity on two enzymatic targets of importance for the control of malaria, the plasmodial kinase Pfnek-1 and a protein farnesyl transferase, as well as on different chloroquine-sensitive and -resistant strains of *Plasmodium falciparum*. Alisiaquinone C displayed a submicromolar activity on *P. falciparum* and a competitive selectivity index on the different plasmodial strains.

During a reinvestigation of our extract library from New Caledonian marine organisms using high-throughput screening, the crude extracts of several sponges attracted our attention. One of these extracts inhibited a bovine protein farnesyl transferase (PFTase) and also displayed *in vitro* activity against *Plasmodium falciparum* and milder cytotoxicity on human cell lines. The dramatic effects of PFTase inhibitors on parasites have been documented,[1] but very few natural inhibitors of this target have been described. A few compounds of marine origin have been described as PFTase inhibitors: a cembranolide diterpene from the soft coral *Lobothytum cristagalli*,[2] heteronemine,[3] and more recently, the spongolactams.[4] The interest in PFTase inhibitors as anticancer agents has receded because of their toxicity in long-term chemotherapy. However, the possibility of using such inhibitors as antimalarial agents for short-term treatments should still be considered, in that there are very few existing treatments and that increasing drug resistance of this tropical disease kills 1−3 million people every year (mostly children in sub-Saharan Africa). We report here the isolation, structural determination, and biological activity of four new compounds, alisiaquinones A, B, and C and alisiaquinol (**1**−**4**).



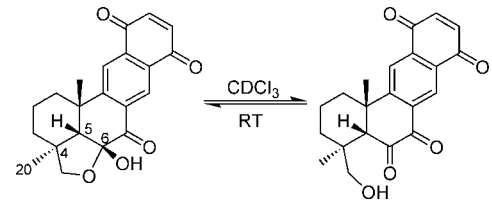


Figure 1. Ring chain equilibrium observed for **1**.

Results and Discussion

The sponge was collected by trawling on a deep sea mount of the Norfolk Rise (New Caledonia). Its freeze-dried powder was extracted with aqueous ethanol and the concentrated extract partitioned between water and methylene chloride. The biologically active organic extract was fractionated on Sephadex LH-20 followed by C_{18} semipreparative RP-HPLC to obtain alisiaquinone A (19 mg, 0.5% dry wt). Alisiaquinone B (3 mg, 0.05%) was further purified by silica gel SPE and preparative TLC. Alisiaquinone C (14 mg, 0.24%) and alisiaquinol (10 mg, 0.13%) were obtained by successive C_{18} semipreparative RP-HPLC.

Compound **1** was assigned the molecular formula $C_{21}H_{20}O_5$ (HRTOFMS). This was consistent with NMR data showing 10 quaternary carbons, five methines, four methylenes, and two methyls, accounting for $C_{21}H_{19}$. The UV spectrum displayed absorption bands at 213, 254, and 328 nm, suggesting a conjugated moiety, as seen in xestoquinone.[5] The IR spectrum displayed absorption bands at 1662 and 1696 cm^{-1}, indicating the presence of a quinone and carbonyls. ^1H and ^{13}C NMR data were also in agreement with the presence of the general skeleton of xestoquinone. Furthermore, NMR spectra revealed two equilibrium forms *a* and *b* for **1** in a ratio 80:20 displaying similar NMR signals. The major difference between these two forms concerned the quaternary C-6, which resonated at δ 97.9 in the first form and at δ 198.1 in the second. These signals were attributed to a hemiketal in form *a* and to a carbonyl in form *b*, suggesting a closed ring in form *a* and an open one in form *b* as shown in Figure 1. The coupling constant observed between the methylene protons at δ 3.56 and 3.79 (J_{gem} = 7.3 Hz) in form *a* confirmed that C-19 belonged to a ring, whereas in form *b* it did not. These protons appeared there at δ 3.12 and 3.34 with a wider coupling constant J = 11.7 Hz. These features represented a major difference from xestoquinone, which

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Table 1. NMR Data for Alisiaquinone A (**1**) in $CDCl_3$

	form *a*				form *b*			
#[a]	δ_C[b]		δ_H[c]	HMBC	δ_C[b]		δ_H[b]	HMBC
1	35.2	CH_2	1.62 m	2, 5, 9	35.2[d]	CH_2	1.51 m	3
			2.67 dd (10.8, 3.6)	3, 5, 9, 10			2.75 m	3
2	19.5	CH_2	1.60 m	1	18.2	CH_2	1.76 m	
			1.76 m	1			1.59 m	
3	34.2	CH_2	1.56 m	1, 2, 4, 5	35.0[d]	CH_2	1.81 m	1
							1.26 m	1
4	45.1	C			40.9	C		
5	58.6	CH	2.28 s	3, 4, 6, 7, 9,10, 19, 20, 21	61.5	CH	3.22 s	4, 6, 7, 9, 10, 19, 20
6	97.9	C			198.1	C		
7	193.7	C			179.6	C		
8	135.6	C			137.0	C		
9	156.3	C			155.6	C		
10	37.0	C			39.9	C		
11	122.3	CH	8.11 s	7, 8, 9, 10, 12, 17	123.0	CH	8.22 s	8, 10, 13, 17
12	135.6	C			135.5	C		
13	184.6	C			184.2	C		
14	139.4	CH	7.05 s	12, 13, 16, 17	138.9	CH	7.05 s	13, 16
15	138.7	CH	7.05 s	12, 13, 16, 17	139.0	CH	7.05 s	13, 16
16	183.6	C			183.3	C		
17	130.4	C			130.9	C		
18	127.8	CH	8.76 s	7, 9, 12, 16	129.0	CH	8.81 s	7, 9, 12, 16
19	79.2	CH_2	3.56 d (7.3)	4, 5, 6, 20	69.6	CH_2	3.12 d (11.7)	3, 5
			3.79 d (7.3)	3, 5, 20			3.34 d (11.7)	3
20	22.7	CH_3	0.42	3, 4, 5, 19	19.7	CH_3	0.28 s	3, 4, 5, 19
21	36.1	CH_3	1.35 s	1, 5, 9, 10	38.4	CH_3	1.31 s	1, 5, 9, 10

[a] The terpene numbering is adopted here. [b] 125 MHz. [c] 500 MHz;. [d] Signals may be interchanged.

presents a regular furanic ring and lacks the methyl group on C-4, as shown for **1** by 2D NMR analysis (Table 1). Alisiaquione A possessed two methyl groups, with the additional one at C-4 resonating at δ_H 0.42 and δ_C 22.7 (form *a*). The HMBC experiment revealed that this upfield H_3-20 signal at δ 0.42 (3H, s) correlated with a quaternary carbon, a methine, and two methylene signals resonating respectively at δ 34.2 (C-3), 45.1 (C-4), 58.6 (C-5), and 79.2 (C-19).

In the open form, the H-20 signal at δ 0.28 (3H, s) also showed correlations with a quaternary carbon at δ 40.9 (C-4), a methine at 61.5 (C-5), and two methylene signals resonating respectively at 35.0 (C-3) and 69.6 (C-19). The HMBC correlations of the H-5 proton at δ 2.28 (1H, s) confirmed the struture of alisiaquinone A, as it correlated with five quaternary carbons [δ 37.0 (C-10), 45.1 (C-4), 97.9 (C-6), 156.3 (C-9), and 193.7 (C-7)], two methylenic carbons [δ 34.2 (C-3) and 79.2 (C-19)], and two methyl carbons [δ 22.7 (C-20) and 36.1 (C-21)]. In the same way, the open form displayed a signal for H-5 at δ 3.22 (1H, s) correlating with five quaternary carbons [δ 39.9 (C-10), 40.9 (C-4), 155.6 (C-9), 179.6 (C-7), and 198.1 (C-6)], a methylenic carbon at δ 69.6 (C-19), and a methyl at δ 19.7 (C-20).

The NOESY NMR experiment gave insightful information about the relative configuration of the sesquiterpene moiety of alisiaquinone A. A key correlation was observed for both forms between H-5 and H-21, but none between H-5 and H-20, suggesting that H_3-21 and H-5 were not in the same plane as methyl 20, and led to the proposed relative stereochemistry for carbons 4, 5, and 10. Taking into account this relative stereochemistry deduced from the NOE experiment, the only possible ring closure leads to a β-hydroxyl group on C-6, corresponding to the lowest energy form. The equilibrium forms *a* and *b* were also found for alisiaquinones B and C and alisiaquinol with ratios for form *a*/form *b* determined by NMR as 80:20 ($CDCl_3$), 80:20 ($CDCl_3$/MeOH, 2:1) and 45:55 ($CDCl_3$/MeOH, 2:1) respectively.

Compound **2**, or alisiaquinone B ($C_{22}H_{22}O_6$, HRTOFMS), differed from alisiaquinone A with a methoxy group substituent on the quinone ring. The form *a* ^1H and ^{13}C NMR spectra displayed methoxy signals at δ_H 3.94 (3H, s) and δ_C 56.6, a quaternary carbon at δ 161.0, and methine signals at δ_H 6.26 (1H, s) and δ_C 110.2. The HMBC spectrum showed correlations between the methoxy group and its subtending quaternary carbon at 161.0 and between the signal at δ 6.26 and the signals at δ 135.5 (C-12) and 161.0; the latter proton signal was thus assigned to H-14, and the methoxy group positioned on C-15. For form *b*, these signals appeared respectively at δ 3.96 (3H, s) and δ 56.7 (O-Me), 6.31 (1H, s, H-14), and 110.4 (C-14), and 160.6 (C-15). In the HMBC spectrum of alisiaquinone B, form *b*, a correlation between the H-14 signal at δ 6.31 (1H, s) and the C-12 signal at δ 135.8 was also observed, and the structure **2** was assigned to alisiaquinone B.

Compound **3** ($C_{23}H_{23}NO_7S$, HRTOFMS), or alisiaquinone C, differed from alisiaquinone A by an additional heterocyle formed by a taurine substituent linked to the quinone. This was confirmed by the ^1H NMR spectrum, which lacked any downfield proton signals around δ 7.0, but had two methylene signals H-22 and H-23, respectively, at δ 4.02 (2H, m) and 3.33 (2H, m) for form *a* and at δ 4.04 (2H, m) and 3.36 (2H, m) for form *b*. The ^{13}C NMR signals of the quinone moiety of both forms were consistent with those observed for adociaquinone A,[6] the structure of which was confirmed by synthesis.[7] The H-22 protons were easily identified by their COSY correlations with the NH proton resonating at δ 6.92 (1H, s). The HMBC spectrum revealed correlations between the H-22 protons and the quaternary carbon at δ 146.4 (C-14) and between the H-23 protons and the quaternary carbon at δ 112.5 (C-15). These assigments were secured by an HMBC experiment optimized for long-range couplings (J_{CH} = 5 Hz), which showed for form *a* a small $^4J_{CH}$ correlation between the H-23 protons and C-16 at δ 174.8, this signal being assigned by its $^3J_{CH}$ HMBC correlation with H-18 at δ 8.75. For form *b*, a correlation between the H-22 and C-14 (δ 146.8) signals was observed. These experiment allowed us to assign structure **3** for alisiaquinone C.

Compound **4** ($C_{21}H_{22}O_5$, HRTOFMS), or alisiaquinol, showed a different IR spectrum, which revealed two large bands at 1677 and 3300 cm^{-1} but none around 1660 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups, but no quinone group. An A−B system was observed in the ^1H NMR spectra for the H-14 and H-15 protons resonating at δ 6.84 (1H, d) and 6.69 (1H, d) with a coupling constant of 8.0 Hz for the hemiketal form *a*. This A−B system was also observed in the open form with protons resonating at δ 6.91 (1H, d) and 6.74 (1H, d) and a coupling constant of 8.1 Hz. HMBC showed correlations between H-14 and three quaternary

Table 2. *In Vitro* Activity of Alisiaquinones A−C (**1**−**3**) and Alisiaquinol (**4**)

	CQ[c]		**1**		**2**		**3**		**4**	
	IC_{50}[a]	SI[b]	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI
PfFcMC29CQR[c]	0.60	20	8.5	4	2.6	<1	0.08	500	7.9	<1
Pf FcB1 CQR	0.18	60	7.4	5	8.4	<1	0.21	200	6.4	<1
Pf F32 CQS	0.06	200	9.1	4	7.1	<1	0.15	270	9.9	<1
MCF7	12		36		0.8		41		1.7	
PFTase	not tested		2.8		2.7		1.9		4.7	

[a] 50% Inhibitory concentration μM. [b] Selectivity index: IC_{50} on MCF7/IC_{50} on parasite. [c] CQ: chloroquine.

carbons resonating at δ 129.3 (C-12), 145.9 (C-13), and 148.5 (C-16) and correlations between H-15 and three quaternary carbons resonating at δ 124.6 (C-17), 145.9 (C-13), and 148.5 (C-16) for form *a*. These correlations were also observed for form *b* [H-14 to C-12 (δ 129.2), C-13 (146.2), and C-16 (148.7) and from H-15 to C-12 (δ 124.8), C-13 (δ 146.2), and C-16 (δ 148.7)]. These features revealed the presence of a quinol moiety for both forms and led us to propose the structure **4** for alisiaquinol.

Alisiaquinones (**1**−**3**) and alisiaquinol were isolated as new bioactive components of the sponge extract using assays for protein farnesyl transferase (PFTase) and *P. falciparum* (Table 2). They are related to xestoquinone,[5] methoxyxestoquinone,[8] adociaquinone,[6] and xestoquinol,[5] respectively, but differed with a unique substitution of the furanic ring. These new compounds displayed mild activity with micromolar range EC_{50} on PFTase as well as micromolar antiplasmodial activity for alisiaquinones A and B and alisioaquinol and submicromolar for alisiaquinone C on the different strains of *P. falciparum*. We showed previously that xestoquinone was an inhibitor of the plasmodial NIMA-like kinase Pfnek-1.[9,10] This prompted us to further investigate compounds **1**, **3**, and **4** on this latter enzyme of antimalarial interest since dual inhibitors of plasmodial targets are slower to develop resistance. The IC_{50} values were not determined on Pfnek-1; instead, the remaining enzyme activity (%) was measured in the presence of 50, 5, and 1 μM of the compounds. Pfnek-1 activity was completely inhibited with alisiaquinones A and alisiaquinol at 50 μM, whereas alisiaquinone C was a poor inhibitor of the kinase. At lower concentration (1 μM), alisiaquinone A and alisiaquinol displayed 41% and 49% inhibition, respectively, whereas there was almost no inhibition with alisiaquinone C. Hence, the IC_{50} on Pfnek-1 is close to 1 μM for alisiaquinone A and alisiaquinol, which is comparable to that observed previously with xestoquinone (IC_{50} = 1 μM).

Alisiaquinones A and B and alisiaquinol displayed similar activities *in vitro* on *P. falciparum*; however, alisiaquinone A was slightly less cytotoxic and had the same low selectivity index as xestoquinone. Alisiaquinone C, bearing the taurine substituent, showed a much better *in vitro* activity on *P. falciparum*, correlated with a better activity on PFTase, no activity on Pfnek-1, but a much higher selectivity index than the other compounds, especially on the chloroquine-resistant strain PfFcMC29. These results, especially the loss of activity of the hindered quinone system, suggest that the quinone/phenolic part of these compounds plays an important role in their mode of action on the ser/thr kinase Pfnek-1, but not on the PFTase, as all four compounds display micromolar range activity. However, the modification of the furan ring does not affect the activity very much, as shown by the comparison with xestoquinone.

The *in vivo* activity on rodent malaria was investigated for alisiaquinones A and C (Table 3); they reduced by 50% the parasitemia at 5 mg/kg and displayed a relatively high level of toxicity with 100% and 80% mortality at 20 mg/kg, thus precluding further antimalarial development.

This work highlights the interest of the substitution pattern of alisiaquinone C as a model for developing antimalarial drugs. It is also noteworthy that this substitution stabilized the compound, as it did not decompose as rapidly as the other compounds. It would be interesting to further investigate the mode of action of this compound and the possible oxidative processes involved.[11]

Table 3. *In Vivo* Activity of Alisiaquinones A (**1**) and C (**3**)

	parasitaemia inhibition (%) day 5	mortality (%) day 1	mortality (%) day 5	mortality (%) day 15
1: 5 mg/kg/day	63	0	0	40
1: 20 mg/kg/day	NM	100	100	100
3: 5 mg/kg/day	36	0	0	60
3: 20 mg/kg/day	42	60	80	100
CQ: 1 mg/kg/day	0	0	0	100
CQ: 10 mg/kg/day	100	0	0	0

From a chemotaxonomic point of view, the alisiaquinones might be biogenetic precursors of xestoquinone and related analogues. The stereochemistry of the C-5−C-6 bond and the substitution of C-4 by a methyl group block dehydration and further aromatization to lead to the furan as in the previously known compounds. The species identification of our specimens could not be achieved due to the lack of unambiguous morphological characters. A molecular phylogeny investigation will be undertaken on xestoquinone-related compounds containing *Xestospongia* and *Adocia* species in the near future, to provide linkages to the known sponges and to correlate chemotaxonomy and molecular systematics.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter in a 0.1 dm cuvette. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance DRX 500 spectrometer equipped with a 5 mm cryoprobe. ^{1}H and ^{13}C chemical shifts are referenced to the solvent $CDCl_3$ peak (δ 7.26 and 77.0 ppm, respectively). The UV spectra were recorded on a Beckman DU 640B spectrometer. The IR spectra were recorded on a Bruker Tensor 27 ATR. The high-resolution TOF-MS spectra were recorded on a Bruker Daltonics microTOF-LC mass spectrometer. Sephadex LH20 resin and Interchrom SI-S-1G/6 (Interchim) SPE cartridges were used to fractionate the CH_2Cl_2 extract. Purification of compounds was performed by Merck 20 × 20 cm preparative silica gel plates (6 mm thick) or by reversed-phase HPLC using a Hibar Lichrospher 100 RP-18 (5 μm) 25 × 250 mm column eluted with a gradient of MeOH/H_2O (50:50 to 80:20). Merck type Si60 F_{254} (250 μm thick) silica gel plates were used for analytical thin-layer chromatography. All solvents used for isolation were Merck HPLC grade.

Sponge Material. The sponge sample was collected in February 1990 with *R/V Alis* by trawling on a deep sea mount of the Norkfolk Rise, in the South of New Caledonia (L = 168°00′ E, l = 23°39′00 S) between 250 and 400 m deep. The sponge sample was immediately frozen on board until extract preparation. The voucher specimen (accessing number ORSTOM-R1514) is deposited at the Museum National d'Histoire Naturelle de Paris, France.

Extraction of the Sponge and Isolation of Alisiaquinones A, B, and C and Alisiaquinol. The frozen sponge was ground and freeze-dried. The sponge powder (75 g) was subsequently extracted once with 80% MeOH (500 mL) and twice with EtOH (2 × 500 mL). The extract was concentrated and partitioned between H_2O and CH_2Cl_2 (2:1). The CH_2Cl_2 extract was collected and then evaporated to dryness to yield 2.05 g of crude extract. A 100 mg aliquot was fractionated on a Sephadex LH20 with MeOH followed by C_{18} semipreparative RP-HPLC to obtain alisiaquinone A (19 mg, 5% dry weight). A 150 mg aliquot was fractionated by C_{18} semipreparative RP-HPLC to give alisiaquinone C (14 mg, 0.2%). Two parts of this fraction were grouped and then successively purified by silica gel SPE with MeOH as eluent and by preparative TLC with MeOH/CH_2Cl_2 (2:1) as eluent to give ali-

siaquinone B (3 mg, 0.05%). A 200 mg aliquot was fractioned and purified by C_{18} semipreparative RP-HPLC to give alisiaquinol (10 mg, 0.1%).

Alisiaquinone A (1): yellow-brown powder; $[\alpha]_D^{25}$ −40 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 213 (4.60), 254 (4.48), 328 (3.79) nm; IR (film) 1696, 1662, 1596 cm^{-1}; ^1H and ^{13}C NMR data in Table 1; HRTOFMS *m*/*z* 375.1206 ($M + Na^+$ calcd for $C_{21}H_{20}O_5Na$ 375.1203).

Alisiaquinone B (2): yellow-brown powder; $[\alpha]_D^{25}$ −27 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 214 (4.63), 273 (4.66), 338 (3.95) nm; IR (film) 1692, 1660, 1600 cm^{-1}; ^1H and ^{13}C NMR data in Supporting Information; HRTOFMS *m*/*z* 405.1321 ($M + Na^+$ calcd for $C_{22}H_{22}O_6Na$ 405.1309).

Alisiaquinone C (3): yellow powder; $[\alpha]_D^{25}$ −75 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 219 (4.42), 252 (sh, 4.25), 284 (4.47), 347 (sh, 3.72) nm; IR 1690, 1591, 1283 cm^{-1}; ^1H and ^{13}C NMR data in Supporting Information; HRTOFMS *m*/*z* 456.1111 ($M - H^-$ calcd for $C_{23}H_{22}NO_7S$ 456.1122).

Alisiaquinol (4): brown powder; $[\alpha]_D^{25}$ +90 (*c* 0.2, acetone); UV (EtOH) λ_{max} (log ε) 216 (4.33), 268 (4.14), 279 (4.14), 304 (sh, 3.92), 338 (sh, 3.59) nm; IR 3300, 1677, 1625 cm^{-1}; ^1H and ^{13}C NMR data in Supporting Information; HRTOFMS *m*/*z* 377.1366 ($M + Na^+$ calcd for $C_{21}H_{22}O_5Na$ 377.1359).

Plasmodium *in Vitro* Culture and Antiplasmodial Activity Evaluation. Parasites were cultured according to the method described by Trager and Jensen[12] with modifications described elsewhere.[13] The cultures were synchronized every 48 h by 5% D-sorbitol lysis.[14] The F32 strain was considered as a chloroquino-sensitive strain (chloroquine: CQ, IC_{50} = 60 nM), and FcB1 and FcM29 were considered as chloroquino-resistant strains (chloroquine, IC_{50} = 180 and 600 nM, respectively). *In vitro* antimalarial activity was evaluated by [^{3}H]-hypoxanthine (Amersham-France) incorporation as described by Desjardins et al.[15] with modifications.[16]

***In Vitro* Cytotoxicity Evaluation.** Human breast adenocarcinoma (MCF-7) cells were cultured in DMEM culture media containing 2 mM L-glutamine (Cambrex, Emerainville, France) supplemented with 10% fetal calf serum (FCS) (Cambrex) and incubated under standard conditions (37 °C, 5% CO_2). All experiments were carried out using cells in the exponential phase of growth. Cells were trypsinized, resuspended in DMEM containing 10% FCS, and seeded (200 000 cells/mL) in 96-well plates (100 μL/well). After 24 h the medium was replaced by fresh medium containing the molecules under evaluation at growing concentrations ranging from 0.01 to 100 μg/mL. Cell viability was evaluated by [^{3}H]-hypoxanthine incorporation. IC_{50} values were determined graphically from the dose−response curves.[17]

***In Vivo* Antiplasmodial Activity Testing.** To test the *in vivo* antimalarial activity, a 4-day suppressive *in vivo* assay was performed on CD female mice using *P. vinckei petteri*.[18] Mice (mean body weight: 20 ± 2 g) were infected with 10^6 parasitized red blood cells in RPMI on day 0. Groups of five mice were treated intraperitonally from day 0−3 with two doses (5 and 20 mg/kg) of alisiaquinones A and C. On day 4, Giemsa-stained smears were prepared for each mouse after tail blood sampling. Parasitaemia was estimated by visual counting of at least 5000 erythrocytes. Survival of the mice was followed for three additional weeks. Controls were mice treated with RPMI alone or chloroquine (1 and 10 mg/kg). Inhibition percentage was calculated with the following formula: (control parasitaemia − parasitaemia with drugs)/(control parasitaemia) × 100. Mice were maintained under institutional animal guidelines.

Protein Farnesyl Transferase Assay. Assays were carried out in 96-well microplates in a final volume of 50 μL containing 50 mM Tris/HCl pH = 7.5, 5 mM $MgCl_2$, 5 mM DTT, 0.1 mM $ZnCl_2$, 0.2% octyl-B-D-glucopyranoside, 10 μM GCVLS-dansyl substrate (Calbiochem, Darmstadt, Germany), 10 μM farnesyl pyrophosphate (Sigma, St Louis, MO), and chemical compounds to be studied and initiated by addition of 10 nM rat PFTase (Jena Biosciences, Jena, Germany). Stock solutions of alisiaquinones and alisiaquinol were prepared in 100% DMSO at a 10 mM final concentration and diluted in assay buffer to final concentrations ranging from 0.3 nM to 100 μM. Each compound was tested in triplicate, and final DMSO percentage was fixed to 1%. Reactions were incubated 30 min at 37 °C, and fluorescence was read on a Novostar (BMG Labtechnologies, Offenburg, Germany) plate reader at 340 nm excitation and 505 nm emission. IC_{50} values were determined by the sigmoidal dose−response calculation of the GraphPad Prism software.

Protein Kinase Pfnek-1 Assay. The assay was performed in a standard reaction (30 μL) containing 20 mM Tris/HCl pH = 7.5, 20 mM $MgCl_2$, 2 mM $MnCl_2$, 0.25 μCi 10 mM [γ-^{33}P] ATP, and 5 μg of myelin basic protein as an exogenous substrate. The reaction was stopped by addition of Laemmli buffer and loaded on an SDS gel. Following Coomassie blue staining, the gel was dried and exposed for autoradiography.

To test the effect of alisaquinones A and C and alisiaquinol, reactions were performed at three concentrations (50, 5, and 1 μM) of the different molecules (stock solutions were at 5 mM in DMSO). The final concentration of DMSO was identical in all reactions. The kinase assay was performed as described above, and the gel was additionally analyzed quantitatively by phosphorimaging.

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Supporting Information Available: NMR data tables and spectra for alisiaquinones A−C and alisiaquinol are available free of charge at http://pubs.acs.org.

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