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New bioactive halenaquinone derivatives from South Pacific marine sponges of the genus *Xestospongia*

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ABSTRACT

Bioassay-directed fractionation of South Pacific marine sponges of the genus *Xestospongia* has led to the isolation of a number of halenaquinone-type polyketides, including two new derivatives named xestosaprol C methylacetal **7** and orhalquinone **8**. Chemical characterization of these two new compounds was achieved by extensive 1D and 2D NMR spectroscopic studies. Evaluation of anti-phospholipase A_2, anti-farnesyltransferase and antiplasmodial activities of this series is presented and structure/activity relationships are discussed. Orhalquinone **8** displayed a significant inhibition of both human and yeast farnesyltransferase enzymes, with IC_{50} value of 0.40 μM and was a moderate growth inhibitor of *Plasmodium falciparum*.

1. Introduction

Marine sponges of the genus *Xestospongia* (Desmospongia Class, Haplosclerida Order, Petrosiidae Family) are an extremely rich source of secondary metabolites with more than 300 examples reported to date encompassing terpenoids, alkaloids, pentacyclic polyketides, and ene–yne tetrahydrofurans. A number of these natural products exhibit biological activities such as antimicrobial,[1] antifungal,[2] cardiotonic,[3] antitumoral,[4] inhibition of histamine release,[5] and antimalarial[6] activities. In comparison, few studies have been carried out on the marine sponge *Xestospongia testudinaria* yielding acetylenic acids,[7] a brominated bisacetylenic acid,[8] esters of brominated acetylenic fatty acids,[9] and bromopolyacetylenes.[10]

In our ongoing search for anti-phospholipase A_2 (PLA_2) compounds within the frame of the program CRISP (Coral Reef Initiative in the South Pacific), three specimens of marine sponges of the genus *Xestospongia* were selected for their significant in vitro activity: one sample of *X. testudinaria* from the Solomon Islands and two samples of unidentified species from the Fiji Islands. The crude extracts of these sponges also displayed anti-farnesyltransferase and antiplasmodial activities.

Chemical investigations of the three crude extracts led to the isolation of a series of halenaquinone-type compounds. From the sponge *X. testudinaria* collected in the Solomon Islands six halenaquinone-type derivatives were isolated: a new analogue named xestosaprol C methylacetal **7**, and five previously reported metabolites namely 3-ketoadociaquinone A **2** and B **3**,[11,12] tetrahydrohalenaquinone A **4**[11,13] and B **5**,[12–14] and halenaquinol sulfate **6**.[15] Both samples of *Xestospongia* sp. from Fiji Islands yielded the known pentacyclic polyketide halenaquinone **1**[16] as a major constituent, and halenaquinol sulfate **6**. One of these specimens also furnished both new compounds xestosaprol C methylacetal **7** and orhalquinone **8**, whereas the other sample revealed the presence of halenaquinol (Fig. 1).

The current report describes the isolation of the halenaquinone-type derivatives, the structural elucidation of both new derivatives **7** and **8** and results of the evaluation of anti-phospholipase A_2, anti-farnesyltransferase, antiplasmodial and cytotoxic activities of the series.

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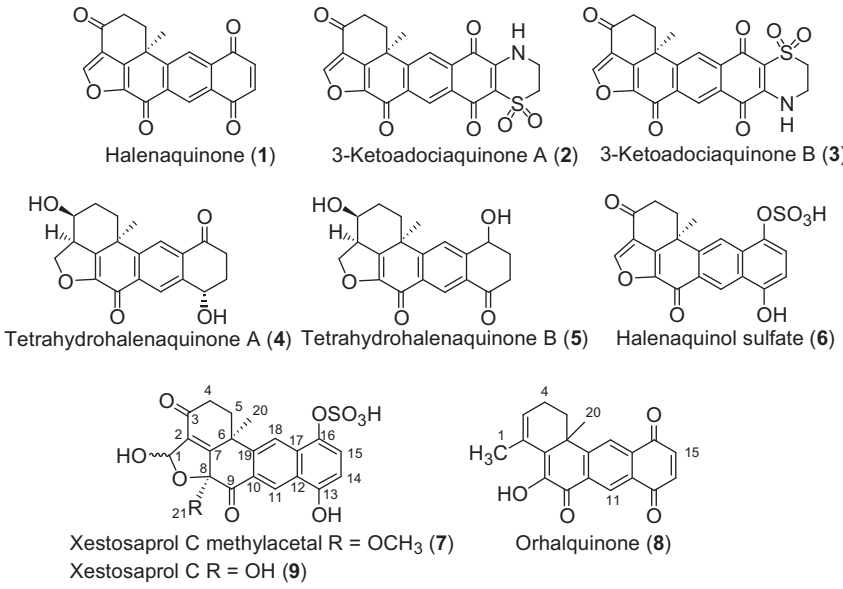

Figure 1. Structures of compounds **1**–**8** isolated from marine sponges of the genus *Xestospongia* collected in the South Pacific and structure of xestosaprol C (**9**). The stereochemistry of **7** is depicted as relative.

2. Results and discussion

Repeated chromatographic fractionation of the CH_2Cl_2 and/or CH_2Cl_2/MeOH sponge crude extracts using silica gel led to the isolation of eight biological halenaquinone derivatives. The structures of the known compounds **1**–**6** were rapidly determined by standard dereplication protocols. The structures of xestosaprol C methylacetal **7** and orhalquinone **8** were obtained through detailed examinations of mass data and extensive 1D and 2D NMR studies.

2.1. Structure of xestosaprol C methylacetal 7

Compound **7** was isolated as an orange amorphous solid, $[\alpha]_D^{20}$ = +193 (*c* 0.01, MeOH). A molecular formula of $C_{21}H_{18}O_{10}S$ was deduced for **7** from analysis of the pseudomolecular ion peak $[M+H]^+$ observed at 463.0689 (*m*/*z* 463.0699, Δ −1.0 mmu) in the ESI mass spectrum. The UV spectrum of **7**, with absorption maxima (EtOH) at 219 nm (ε 14,056) and 275 nm (ε 11,928), suggested the presence of a polycyclic ring system while the IR spectrum showed hydroxyl (3402 cm^{-1}) and conjugated carbonyl (1678, 1604 cm^{-1}) absorption bands.

The ^1H and ^{13}C NMR spectra of **7** recorded in CD_3OD revealed characteristic signals of the halenaquinone family of polycyclic polyketides (Table 1). Many of the resonances were observed as pairs in a 2:1 ratio. The presence of resonances attributable to two pairs of aromatic proton singlets at δ_H 8.93/8.97 and 8.38/8.39, a pair of mutually coupled aromatic doublets at δ_H 6.80 (d, *J* = 8.3 Hz) and 7.50 (d, *J* = 8.3 Hz), four methylene protons at δ_H 2.99, 2.55, 2.79 and 2.25, and a pair of methyl singlets at δ_H 1.95/1.89, also in a 2:1 ratio showed similarities with xestosaprol C (**9**), a hemi-acetal analogue of halenaquinone previously isolated from a Japanese collection of *Xestospongia sapra*.[17] Indeed, these observations combined with two pairs of singlets (δ_H 6.13/6.38 (1H) and δ_H 3.42/3.31 (3H)) each in a ratio of 2:1 and their respective two pairs of quaternary carbons resonances at δ_C 100.6/102.4 and 106.0/107.5 suggested the presence of a methylacetal moiety in **7**. Correlations observed in the HMBC NMR spectrum of **7** between resonances of the acetal methine (δ_H 6.13/6.38) and methoxy groups (δ_H 3.42/3.31) and C-8 (δ_C 106.0/107.5) established connectivity of the methoxyl group to C-8 (Fig. 2). Therefore, compound **7** was deduced to be a mixture of unseparable diastereoisomers, epimeric in C-1 (δ_H 6.13 for the major isomer and δ_H 6.38 for the minor). NOESY correlations were observed between the methoxyl protons at δ_H 3.42 (CH_3-21) and methyl protons at δ_H 1.95 (CH_3-20) for the major epimer and between δ_H 3.31 (CH_3-21) and

Table 1
NMR spectroscopic data of xestosaprol C methylacetal **7** recorded in CD_3OD (400 MHz for ^1H NMR data and 100 MHz for ^{13}C NMR data)

No.	δ_C	δ_H (mult., *J*)	HMBC (H→C)
1	100.6, 102.4[a]	6.13 (s), 6.38[a] (s)	7, 8
2	137.5		
3	195.7		
4	37.3	2.99 (m)	2, 3, 5, 6
		2.55 (m)	2, 3, 5, 6
5	40.4	2.79 (m)	3, 4, 6, 7, 20
		2.25 (m)	3, 4, 6, 19, 20
6	38.9		
7	161.6		
8	106.0, 107.5[a]		
9	191.7		
10	128.0		
11	126.4, 126.5[a]	8.93, 8.97 (s)[a]	9, 13, 17, 19
12	125.3		
13	153.9		
14	108.8	6.80 (d, 8.3)	12, 13, 16
15	123.4	7.50 (d, 8.3)	13, 16, 17
16	141.9		
17	133.4		
18	122.1[b]	8.38 (s), 8.39 (s)[a]	6, 9, 10, 12, 13, 16
19	145.0		
20	26.4, 26.0[a]	1.95 (s), 1.89 (s)[a]	5, 6, 7, 19
21	52.0 51.3[a]	3.42 (s), 3.31 (s)[a]	8

[a] Signals due to a minor epimer.
[b] Identical ^{13}C resonance for the two epimers.

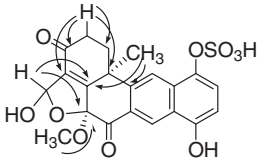

Figure 2. Selected HMBC correlations of xestosaprol C methylacetal **7**.

methyl protons at δ_H 1.89 (CH_3-20) for the minor epimer, establishing a *syn*-relationship between CH_3-21 and CH_3-20 for both compounds. No NOE correlations were observed that allowed assignment of the relative configuration at C-1. Compound **7** is thus xestosaprol C methylacetal. In our study, in addition to **7**, other mixtures of acetals were detected in the ^{1}H NMR spectra of different column chromatography fractions but they were present in too minor amounts to be isolated. Given that MeOH was used in the isolation of **7**, it was possible that this compound was an artifact. To remove any ambiguity, we attempted to purify a fraction containing **7** under the same conditions but using CH_2Cl_2/EtOH solvent mixtures. Characteristic peaks assigned to methoxyl groups at δ_H 3.42/3.31 were observed in the ^{1}H NMR spectrum, confirming its isolation as a natural compound.

2.2. Structure of orhalquinone 8

Compound **8** was isolated as an optically active, orange amorphous powder: $[\alpha]_D^{20}$ = +122 (*c* 0.01, MeOH). A molecular formula of $C_{20}H_{16}O_4$ was deduced from the ESI mass spectrum, which exhibited a protonated molecular ion $[M+H]^+$ at *m*/*z* 321.1131 (calcd 321.1121, Δ 0.96 mmu), indicating the presence of 13 degrees of unsaturation in the molecule. The UV spectrum, with absorption maxima (EtOH) at 218 nm (ε 13,868), 245 nm (ε 13,113) and 334 nm (ε 4622), suggested the presence of a polycyclic ring system. The IR spectrum showed hydroxyl (3370 cm^{-1}) and conjugated carbonyl (1675, 1650, and 1625 cm^{-1}) absorption bands.

The ^{1}H and ^{13}C NMR spectra of **8** recorded in $CDCl_3$ showed similarities with those observed for halenaquinone (**1**), namely a 6,7-fused naphthoquinone ring (see Table 2). Furthermore, the ^{1}H NMR spectrum revealed additional resonances attributable to two pairs of mutually coupled methylene protons at δ_H 2.55/2.42 (H_2-4) and 2.49/1.70 (H_2-5), a methyl substituted olefin at δ_H 2.29 (CH_3-1), 5.90 (H-3) and an exchangeable proton at δ_H 7.09 (OH). Twenty signals were observed in the ^{13}C NMR spectrum consisting of three carbonylgroups, one tetrasubstituted quaternary carbon, seven quaternary olefinic resonances, five olefinic methines, two sp^3 methylene carbons and two methyl groups (see Table 2). Analysis of correlations observed in HSQC and HMBC 2D NMR experiments established a molecular scaffold similar to halenaquinone, but resonances associated with an α-keto furan ring were absent in the spectra observed for **8**. Instead resonances consistent with the presence of a tri-substituted olefin bearing a methyl group were observed. A ^{1}H–^{1}H COSY NMR spectrum exhibited correlations between the olefinic methine proton at δ_H 5.90 (H-3) and both the methyl protons at δ_H 2.29 (CH_3-1) and the diastereotopic methylene proton resonances observed at δ_H 2.55/2.42 (H_2-4), which in turn correlated with a second pair of alkyl methylene protons at δ_H 2.49/1.70 (H_2-5) establishing a CH_2–CH_2–CH=C(CH_3) spin system. HMBC correlations observed between the axial methyl protons (δ_H 1.48) and C-5 (δ_C 33.6) and between the olefinic methine proton at H-3 (δ_H 5.90) and the allylic methyl carbon C-1 (δ_C 23.3) and the methylene resonance C-5 (δ_C 33.6) confirmed connectivity of C-1 through C-7. The remaining exchangeable proton resonance at δ_H 7.09 was assigned as an enolic group located at C-8 by virtue of observing HMBC correlations between it and C-6 (weak), C-7, C-8, and C-9 (Fig. 3). NOESY correlations observed between the allylic methyl (CH_3-1), enolic and olefinic methine (H-3) resonances and from this latter proton with the methylenic protons at δ 2.55/2.42 (H_2-4) and the axial methyl at δ_H 1.48 (CH_3-20) completed the structural assignment of the new metabolite, named orhalquinone **8**.

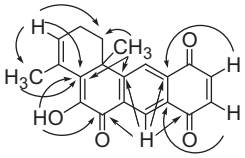

Figure 3. Selected HMBC correlations observed for orhalquinone **8**.

2.3. Bioactivity of compounds 1–8

Compounds **1**–**8** were evaluated for activity against bee venom phospholipase A_2, yeast (*Saccharomyces cerevisiae*) and human protein farnesyltransferases, FcB1 and 3D7 strains of *Plasmodium falciparum* and VERO cells. The results from the in vitro assays and preliminary SAR are presented in Tables 3–5.

Halenaquinone **1** appeared as the most PLA_2 inhibitor of the series with an IC_{50} of 3.7 μM (Table 3). A 30- to 40-fold decrease in potency was observed between the most active pentacyclic polyketide compounds and those incorporating a dioxothiazine unit (**2** and **3**). Among pentacyclic polyketide compounds, the presence of a secondary alcohol at C-3 rather than a ketone abrogated PLA_2 inhibition (**4** and **5**). These results highlight the importance of the C-3 oxidation state and the presence of quinone ring E for the observed anti-PLA_2 activity. Similar conclusions have been reported previously regarding the structure–activity relationship of protein tyrosine kinase inhibition exhibited by halenaquinone.[18]

Table 2
NMR spectroscopic data of orhalquinone **8** recorded in $CDCl_3$ (600 MHz for ^{1}H data and 150 MHz for ^{13}C data)

No.	δ_C	δ_H (mult., *J*)	HMBC (H→C)
1	23.3	2.29 (br s)	3, 7
2	131.9[a]		
3	131.8	5.90 (br s)	1, 5, 7
4	23.5	2.55 (m)	3
		2.42 (br dm, 19.2)	3, 6
5	33.6	2.49 (dd, 13.0, 6.3)	3, 4, 6, 7, 8, 19, 20
		1.70 (ddd, 12.9, 11.8, 6.3)	4, 6, 7, 19, 20
6	39.5		
7	133.1		
8	142.5		
9	179.3		
10	132.0[a]		
11	126.0	8.92 (s)	9, 13, 17, 19
12	130.2		
13	183.7		
14	138.9	7.05 (ABq, 10.4)	12, 15, 16
15	139.5	7.07 (ABq, 10.4)	13, 14, 17
16	184.5		
17	133.7		
18	125.1	8.34 (s)	6, 9, 10, 12, 16
19	156.3		
20	27.4	1.48 (s)	5, 6, 7, 19
OH	—	7.09 (s)	6, 7, 8, 9

[a] Assignments may be interchanged.

Table 3
Inhibitory activities of compounds **1**–**8** on bee venom PLA_2

Compound	IC_{50}[a] (μM)
Halenaquinone **1**	3.7 ± 0.3
3-Ketoadociaquinone A **2**	140 ± 10.7
3-Ketoadociaquinone B **3**	105 ± 8.4
Tetrahydrohalenaquinone A **4**	600 ± 36.5
Tetrahydrohalenaquinone B **5**	1000 ± 63.8
Halenaquinol sulfate **6**	1000 ± 48.5
Xestosaprol C methylacetal **7**	570 ± 28.3
Orhalquinone **8**	1570 ± 92.6
Manoalide	0.5 ± 0.05

[a] IC_{50} values (μM ± SEM; *n* = 2).

Table 4
Inhibitory activities of compounds **1–8** on FTases from the yeast *Saccharomyces cerevisiae* (FL) and from human (FH)

Compound	IC_{50} FL[a] (μM)	IC_{50} FH[a] (μM)
Halenaquinone **1**	1.57 ± 0.15	0.93 ± 0.18
3-Ketoadociaquinone A **2**	1.48 ± 0.14	4.19 ± 0.62
3-Ketoadociaquinone B **3**	3.75 ± 0.21	9.27 ± 0.91
Tetrahydrohalenaquinone A **4**	>30	>30
Tetrahydrohalenaquinone B **5**	>30	>30
Halenaquinol sulfate **6**	16.11 ± 1.20	21.51 ± 2.16
Xestosaprol C methylacetal **7**	6.71 ± 1.12	4.34 ± 0.36
Orhalquinone **8**	0.40 ± 0.01	0.41 ± 0.03

[a] IC_{50} values (μM ± SEM; $n = 4$).

Concerning the protein farnesyltransferase (FTase) bioassays, there was a high degree of correlation between results on FTases from both yeast and human origin (Table 4). The presence of dioxothiazine substitution (**1**vs **2** and **3**) led to little variation in activity. Interestingly no detectable inhibition was observed for tetrahydrohalenaquinones A (**4**) and B (**5**). Furthermore, quinol sulfates **6** and **7** exhibited modest activity suggesting that the presence of a quinone moiety is essential for activity. Sub-micromolar inhibition of both yeast and mammalian farnesyltransferase enzymes was observed for orhalquinone **8** (IC_{50} 0.40 μM), highlighting this scaffold as a significant modification for enhancing activity. In order to validate this finding, synthesis of analogues is in progress.

Antiplasmodial activity data revealed that compounds **2**, **3**, and **8** were the most active of the series (Table 5). A lack of detectable cytotoxicity towards the VERO cell line for the three natural products indicated modest (5–40) levels of selectivity. The antiplasmodial activity did not depend on the chloroquine sensitivity of the strain tested since no difference was observed for the IC_{50} between strains FcB1 and 3D7. Unlike the PLA_2 assay results, the presence of a dioxothiazine moiety enhanced the antiplasmodial activity (**2** and **3** vs **1**). The three active molecules (**2**, **3**, and **8**) were also active in the FTase assays, which could suggest correlations on their mode of action against *P. falciparum*.[19,20]

3. Conclusions

In conclusion, our search for new inhibitors of phospholipase A_2, human and yeast farnesyltransferases and antimalarial natural products has led to an investigation of the chemistry of *Xestospongia* sp. sponges. In addition to a number of known examples, two new members (**7** and **8**) belonging to the halenaquinone polyketide family of secondary metabolites were isolated and characterized. Screening of the resultant focused library against the target assays identified halenaquinone **1** as the most active inhibitor of PLA_2—further mechanism of action studies are warranted to determine the influence of the 1,4-quinone or α-keto furan moieties on this observed activity. The new natural product orhalquinone **8** was identified as a sub-micromolar inhibitor of yeast and human farnesyltransferase enzymes as well as being a modest growth inhibitor of *P. falciparum*. Due to the novelty of this tetracyclic ring structure, orhalquinone **8** constitutes a promising scaffold from which more potent and selective inhibitors of farnesyltransferase could be developed.

4. Experimental

4.1. General experimental procedures

Optical rotations were recorded on a Perkin–Elmer 341 polarimeter. IR spectra were recorded on a FT-IR Shimadzu 8400 S spectrometer. UV spectra were recorded on a UVIKON 930 spectrometer. Mass spectra were recorded on an API Q-STAR PULSAR I of Applied Biosystem. NMR spectra were obtained on either a Bruker AC300, AVANCE 400 or 600 spectrometer using standard pulse sequences. The acquisition of HMBC spectra were optimized for either 7 or 8.3 Hz. Silica gel column chromatography was carried out using Kieselgel 60 (230–400 mesh, Merck). Fractions were analyzed by TLC using aluminum-backed sheets (Si gel 60 F254) and visualized under UV (254 nm) and Lieberman spray reagent. Preparative TLC used glass plates coated with Si gel 60 F254, 0.25 mm thick.

4.2. Animal material

Specimens of *Xestospongia* (Order Dictyoceratida, Family Dysideidae) were collected in the South Pacific and identified by John Hooper, Queensland Museum Brisbane, where a voucher specimen is available for each specimen under the accessing numbers G324370 (*X. testudinaria* R3176), 324624 (*Xestospongia* sp. R3242), 324686 (*Xestospongia* sp. R3304).

4.3. Extraction and isolation

Lyophilized sponge specimen of *X. testudinaria* (R 3176) (70 g) was successively extracted at room temperature for 3 days with CH_2Cl_2 (1 L) and 1:1 CH_2Cl_2/MeOH (1 L). Each extract was filtered,

Table 5
Antiplasmodial activities against FcB1 and 3D7 *Plasmodium falciparum* strains and cytotoxic activities of compounds **1–8**

Compound	IC_{50} FcB1[a] (μM)[b]	IC_{50} 3D7[a] (μM)[b]	IC_{50} VERO[c] (μM) [b]
Halenaquinone **1**	>30	>30	>60
3-Ketoadociaquinone A **2**	1.08 ± 0.07	1.67 ± 0.18	>45
3-Ketoadociaquinone B **3**	3.89 ± 0.16	4.12 ± 0.25	>45
Tetrahydrohalenaquinone A **4**	>29	>29	>58
Tetrahydrohalenaquinone B **5**	>29	>29	>58
Halenaquinol sulfate **6**	>24	>24	>48
Xestosaprol C methylacetal **7**	>21	>21	>42
Orhalquinone **8**	9.22 ± 0.44	10.94 ± 0.28	>62
Chloroquine[d]	0.15	0.04	NT[e]
Doxorubicin[f]	NT	NT	5.12 ± 0.17

[a] *Plasmodium falciparum* strains, FcB1 being chloroquine-resistant and 3D7 being chloroquine sensitive, highest concentration tested: 10 μg/mL.
[b] When lower than the highest concentration tested, values are presented as the mean ± SEM ($n = 3$).
[c] VERO cells were used for the evaluation of cytotoxicity, highest concentration tested: 20 μg/mL.
[d] Chloroquine was the positive control for *P. falciparum*.
[e] NT: not tested.
[f] Doxorubicin was the positive control for VERO cells.

evaporated under vacuum. The CH_2Cl_2 crude extract (2 g) was fractionated on a silica gel column chromatography using a linear gradient of acetone in dichloromethane as eluent and yielded 6 fractions with three active fractions (F2–F4) as determined by anti-PLA_2 bioassay. Fractions F2 and F3 eluted with 10% and 20% acetone, respectively, afforded 3-ketoadociaquinone A **2** (3.5 mg, 0.005% sponge dry weight) and 3-ketoadociaquinone B **3** (2.1 mg, 0.003%) by crystallization from MeOH. Fraction F4 eluted with 30% of acetone yielded tetrahydrohalenaquinone A **4** (3 mg, 0.004%) and tetrahydrohalenaquinone B **5** (1.5 mg, 0.002%), after purification by preparative TLC (AcOEt 100%).

The CH_2Cl_2/MeOH crude extract of *X. testudinaria* (12 g) was fractionated by silica gel chromatography using a linear gradient of methanol in dichloromethane as eluent. The fraction F2 eluted with 20% MeOH furnished halenaquinol sulfate **6** (6.6 mg, 0.009%) and xestosaprol C methylacetal **7** (4.8 mg, 0.006%), which were obtained after purification on preparative TLC (CH_2Cl_2/MeOH 8:2).

One of the lyophilized sponge specimens of *Xestospongia* sp. (R 3304) (50 g) was successively extracted at room temperature for 2 days with CH_2Cl_2 (1 L) then 1:1 CH_2Cl_2/MeOH (1 L). After solvent evaporation under reduced pressure, the residue was dried in vacuo. The crude extract (7.7 g) then obtained was separated into two. One portion of the crude extract (3 g) was chromatographed on a silica gel column, using a linear gradient of methanol in dichloromethane as eluent. Out of the four fractions obtained, the fractions eluted with 100% CH_2Cl_2 and 5% MeOH/CH_2Cl_2 were combined and chromatographed on a further silica gel column in dichloromethane with increasing amounts of acetone. The CH_2Cl_2 (100%) subfraction afforded after purification on preparative TLC (CH_2Cl_2/hexane 8:2) orhalquinone **8**. The 4% acetone subfraction afforded pure halenaquinone **1**. The fraction eluted with 20% MeOH/CH_2Cl_2 furnished halenaquinol sulfate **6**, whereas xestosaprol C methylacetal **7** was obtained with 40% MeOH. The same procedure was repeated on the remaining crude extract (4.7 g) to ultimately yield halenaquinone **1** (745 mg, 1.5% dry weight), halenaquinol sulfate **6** (300 mg, 0.6%), xestosaprol C methylacetal **7** (5 mg, 0.01%) and orhalquinone **8** (10.3 mg, 0.02%).

The other sample of lyophilized *Xestospongia* sp. sponge (R3242) (2 g) was extracted at room temperature overnight with MeOH (100 mL). The solvent was filtered, and concentrated under reduced pressure yielding 0.42 g of crude extract, which was subjected to a C18 flash chromatography column (gradient MeOH/H_2O/formic acid 0.1%). Halenaquinol sulfate was obtained from the fraction eluted with 40% MeOH, while halenaquinol and halenaquinone were isolated from the fraction eluted with 60% MeOH.

4.3.1. Halenaquinone 1

Orange/yellow solid, $[\alpha]_D^{20}$ = +24 (*c* 0.05, $CHCl_3$) (lit.[16] +62.1 (*c* 0.066, CH_2Cl_2)).

4.3.2. 3-Ketoadociaquinone A 2

Yellow solid, $[\alpha]_D$ = +19 (*c* 0.05, MeOH) (lit.[11] $[\alpha]_D$ = +65.4 (concentration and solvent not reported)).

4.3.3. 3-Ketoadociaquinone B 3

Yellow solid, $[\alpha]_D$ = +21 (*c* 0.05, MeOH) (lit.[12] $[\alpha]_D$ = +13 (*c* 0.12, MeOH)).

4.3.4. Tetrahydrohalenaquinone A 4

Yellow solid, $[\alpha]_D$ = −24 (*c* 0.05, MeOH), (lit.[13] $[\alpha]_D$ = +12 (concentration and solvent not reported)).

4.3.5. Tetrahydrohalenaquinone B 5

Yellow solid, $[\alpha]_D$ = −14 (*c* 0.05, MeOH) (lit.[14] $[\alpha]_D$ = −42 (*c* 0.35, MeOH) for xestosaprol A and lit.[13] $[\alpha]_D$ = +24 (concentration and solvent not reported)).

4.3.6. Halenaquinol sulfate 6

Yellow solid, $[\alpha]_D$ = +38 (*c* 0.025, MeOH) (lit.[15] $[\alpha]_{577}$ = +106 in MeOH (concentration not reported)).

4.3.7. Xestosaprol C methylacetal 7

Orange powder, $[\alpha]_D^{20}$ = +193 (*c* 0.01, MeOH). UV (EtOH) λ_{max} nm (log ε) 219 (14,056), 275 (11,928); IR cm^{-1} 3402, 1678, 1604, 1373, 1346, 1238; ESIMS showed peaks at *m*/*z* 445.0583 corresponding to $[MH^+-H_2O]$ (calcd 445.0593, Δ −1.0 mmu), at *m*/*z* 463.0689 for $[M+H]^+$ (*m*/*z* 463. 0699, Δ −1.0 mmu) for $C_{21}H_{19}O_{10}S$, at *m*/*z* 485.0514 for $[MNa+H]^+$ (*m*/*z* 485.0518, Δ −0.4 mmu), at *m*/*z* 507.0343 for $[MNa+Na]^+$ (*m*/*z* 507.0338, Δ +0.5 mmu); for ^1H and ^{13}C NMR data, see Table 1.

4.3.8. Orhalquinone 8

Orange powder; $[\alpha]_D^{20}$ = +122 (*c* 0.01, MeOH); UV (EtOH) λ_{max} nm (log ε) 218 (13,868), 245 (13,113), 334 (4622); IR 3370, 1675, 1650, 1625, 1450, 1380, 1360, 1280, 1225, 1130, 1050 cm^{-1}; ESIMS $[M+H]^+$ at *m*/*z* 321.1131 (calcd 321.1121, Δ 0.96 mmu) for $C_{20}H_{17}O_4$; ^1H NMR ($CDCl_3$, 600 MHz) see Table 2. ^{13}C NMR ($CDCl_3$, 150 MHz) see Table 2. ^1H NMR (DMSO-d_6, 400 MHz) δ 8.94 (1H, s, OH), 8.57 (1H, s, H-11), 8.34 (1H, s, H-18), 7.16 (2H, s, H-14 and H-15), 5.86 (1H, s, H-3), 2.50/1.54 (2H, m, H_2-5), 2.49/2.34 (2H, m, H_2-4), 2.19 (3H, s, Me-1), 1.41 (3H, s, Me-20); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.1 (C-1), 131.4 (C-3), 22.8 (C-4), 32.9 (C-5), 39.1 (C-6), 133.5 (C-7), 143.6 (C-8), 178.8 (C-9), 132.0 (C-10), 124.0 (C-11), 130.1 (C-12), 183.8 (C-13), 139.2 (C-14), 139.2 (C-15), 184.5 (C-16), 133.6 (C-17), 124.8 (C-18), 156.0 (C-19), 26.6 (C-20). The resonance for C-2 was either not observed or was obscured.

4.4. PLA_2 inhibition assay

Bioassay-guided fractionation was based on a colorimetric bioassay.[21,22] Each extract or fraction (100 μg dissolved in DMSO (10 μL) were incubated in 96 well plates for 1 h at 25 °C, with *Apis mellifera* venom PLA_2 (Sigma, 2 μL of a 1 mg/mL DMSO stock solution). Substrate solution (198 μL) containing L-α-phosphatidylcholine (L-α-lecithin, 3.5 mM) and red phenol (0.055 mM), NaCl (100 mM), $CaCl_2$ (10 mM) and Triton (7 mM) at pH 7.6 were added. Manoalide was used as a positive control. Colorimetric measurements were made as duplicates at time 0 and after 5 min and read at 550 nm on a Ceres 900 spectrophotometer.

4.5. Yeast FTase inhibition assay[23–25]

Assays were performed in 96 well plates, prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read on Wallac Victor fluorimeter. To each well, farnesyl pyrophosphate (20 μL of a 10 μM stock solution) was added to 180 μL of a solution containing 2 μL of varied concentrations of potential inhibitors (dissolved in DMSO) and 178 μL of a solution composed of 0.1 mL of partially purified recombinant yeast FTase (2.2 mg/mL) and 7.0 mL of dansyl-GCVLS peptide (in the following buffer: 5.8 mM DTT, 6 mM $MgCl_2$, 12 μM $ZnCl_2$, and 0.09% (w/v) CHAPS, 53 mM Tris/HCl, pH 7.5). Then the fluorescence development was recorded for 15 min (0.7 s per well, 20 repeats) at 30 °C with an excitation filter at 340 nm and an emission filter at 486 nm. Each measurement was performed twice as duplicates.

4.6. Human FTase inhibition assay

Assays were performed in 96 well plates, as described for yeast FTase but octyl-D-glucopyranoside (0.18%) was used instead of CHAPS and the solution contains 5 μL of partially purified human FTase (1.5 mg/mL) in 1 mL peptide solution. Each measurement was performed twice as duplicates.

4.7. Antiplasmodial and cytotoxicity assays

Assays were performed in 96 well plates with *P. falciparum* in vitro culture at ring stage after synchronization by combination of magnetic purification and D-sorbitol lysis.[26,27] In parallel, the cytotoxicity was evaluated on VERO cells.[28] After dilution at 10 mg/mL in DMSO (mother solution) the compounds were serially diluted in culture medium and incubated for 48 h with either parasitized erythrocytes (hematocrit: 2%, parasitaemia: 1%) or with VERO cells (10^5 cells/well). The growth inhibition was followed by ^{3}H-hypoxanthine incorporation (9.25 kBq/well) as described.[28] IC_{50} was determined graphically on inhibition versus concentration graphs. Every experiment was performed in triplicate and at three independent times. Chloroquine and doxorubicin were used as positive controls for growth inhibition.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.066.

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