Tetrahedron 67 (2011) 2591–2595

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Fascioquinols A–F: bioactive meroterpenes from a deep-water southern Australian marine sponge, *Fasciospongia* sp.

Hua Zhang, Zeinab G. Khalil, Robert J. Capon *

Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland 4072, Australia

ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form 19 January 2011
Accepted 7 February 2011
Available online 12 February 2011

Keywords:
Fascioquinol
Fasciospongia
Meroterpene
Cytotoxicity
Antibacterial

ABSTRACT

Chemical investigation of a southern Australian deep-water marine sponge, *Fasciospongia* sp., returned the new meroterpene sulfate fascioquinol A (**1**) together with a series of acid mediated hydrolysis/cyclization products, fascioquinols B (**2**), C (**3**) and D (**4**), and strongylophorine-22 (**5**). Additional co-metabolites include the new meroterpenes fascioquinol E (**6**) and fascioquinol F (**8**), together with the known sponge metabolite geranylgeranyl 1,4-hydroquinone (**7**). Structures were assigned to **1**–**8** on the basis of detailed spectroscopic analysis, chemical interconversion, mechanistic and biosynthetic considerations, and literature comparisons. The known 1,4-hydroquinone **7** was identified as the dominant cytotoxic principle in the *Fasciospongia* sp. extract, with selective inhibitory activity against gastric adenocarcinoma (AGS, IC_{50} 8 μM) and neuroblastoma (SH-SY5Y, IC_{50} 4 μM) cell lines. By contrast, while the fascioquinols displayed little or no inhibitory activity towards human cell lines, **1** and **2** displayed promising Gram-positive selective antibacterial activity towards *Staphylococcus aureus* (IC_{50} 0.9–2.5 μM) and *Bacillus subtilis* (IC_{50} 0.3–7.0 μM).

1. Introduction

During an investigation aimed at discovering new anticancer agents from marine organisms we screened a collection of ~2600 southern Australian and Antarctic marine invertebrates and algae for cytotoxic properties against colon (HT-29), lung (A549), skin (MM96L and SK-MEL-28), prostate (DU145), ovarian (JAM and C180-13S) and breast (MDA-MB-231) human cancer cell lines. This screening strategy identified >120 promising cytotoxic extracts, with early investigations leading to the discovery of the terpenyl-taurine phorbasins,[1] terpenyl-pyrrolizidine bistellettazines,[2] indolo-imidazole trachycladindoles[3] and polyketide-phosphodiester franklinolides.[4] This current report describes our investigations into another extract from this priority list, a deep-water *Fasciospongia* sp. (CMB-02028) collected during scientific trawling operations (−100 m) off the southern coast of Australia. Metabolites recovered from this specimen include a new meroterpene sulfate fascioquinol A (**1**) together with a series of new acid mediated hydrolysis/cyclization products, fascioquinols B (**2**), C (**3**) and D (**4**), and the known sponge metabolite strongylophorine-22 (**5**).[5] Other biosynthetically related co-metabolites include the new meroterpenes fascioquinol E (**6**) and fascioquinol F (**8**), together with the known sponge metabolite geranylgeranyl 1,4-hydroquinone (**7**).[6] What follows is an account of the isolation and structure elucidation of **1**–**8**, together with consideration of the biosynthetic and chemical relationships between these metabolites, and an assessment of cytotoxic and antibiotic properties.

2. Results and discussion

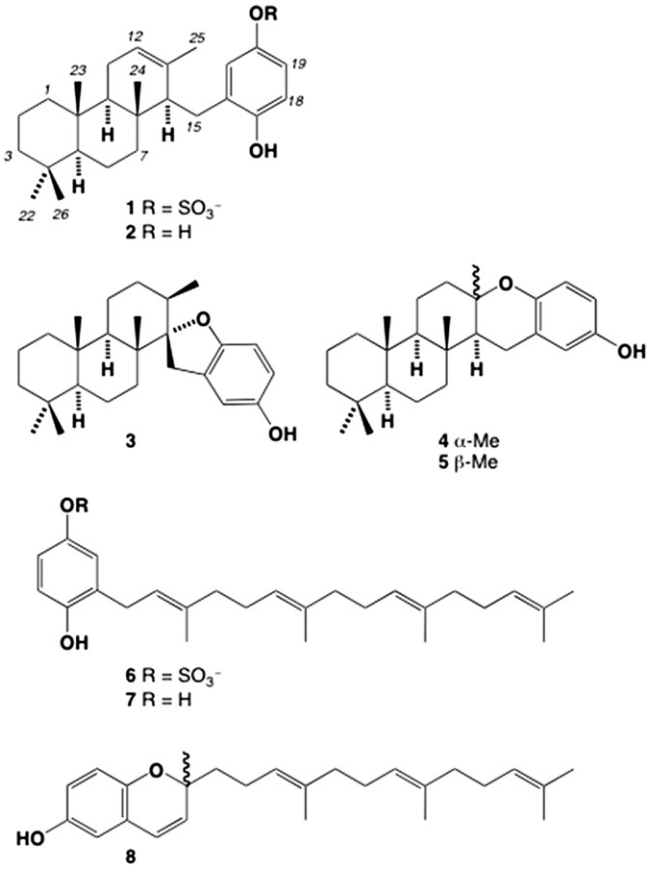
A portion of the aqueous ethanol extract obtained from *Fasciospongia* sp. (CMB-02028) was concentrated in vacuo and subjected to sequential solvent partitioning and trituration, followed by gel (Sephadex LH-20), normal (silica) and reverse phase (C8 and C18) column chromatography, to yield **1**–**8**.

HRESI(−)MS analysis of fascioquinol A (**1**) revealed a highest mass ion consistent with an anionic molecular formula ($C_{26}H_{37}O_5S^-$, Δmmu 0.6) suggestive of eight double bond equivalents (DBE) (assuming an S^{II} oxidation state). The NMR (methanol-d_4) data for **1** (Table 1) revealed resonances consistent with a trisubstituted double bond (δ_H 5.30, br s, H-12; δ_C 122.7, C-12; 137.0, C-13) bearing an olefinic methyl (δ_H 1.44, br s, H_3-25; δ_C 22.6, C-25), and a 1,2,4-trisubstituted benzene (δ_H 6.66, d, 8.7 Hz, H-18; 6.91, dd, 8.7 and 2.7 Hz, H-19; 7.14, d, 2.7 Hz, H-21; δ_C 132.2, C-16; 153.4, C-17; 116.0, C-18; 120.5, C-19; 146.6, C-20; 124.0, C-21). These observations accounted for five DBE and suggested that **1** incorporated at least three additional rings.

Analysis of COSY NMR (methanol-d_4) data for **1** (Table 1) revealed structure subunits as indicated in Fig. 1, comprising (i) H_2-1 through H_2-2 to H_2-3, (ii) H-5 through H_2-6 to H_2-7, (iii) H-9

* Corresponding author. Tel.: +61 7 3346 2979; fax: +61 7 3346 2090; e-mail address: r.capon@uq.edu.au (R.J. Capon).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.02.015



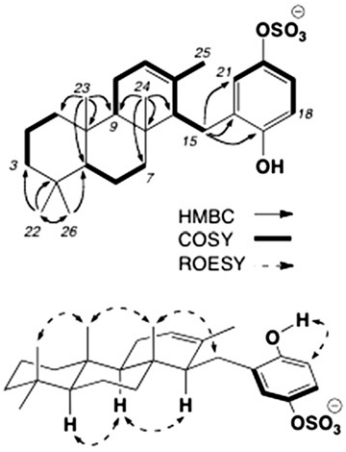


Fig. 1. Key 2D NMR correlations for **1**.

through H_2-11, H-12, H_3-25, H-14 to H_2-15 and (iv) H-18 through H-19 to H-21. Consideration of the HMBC NMR (methanol-d_4) data for **1** revealed key correlations that defined (a) geminal dimethyls (δ_H 0.89, s, H_3-22; 0.85, s, H_3-26; δ_C 34.1, C-22; 22.3, C-26) with correlations to C-3, C-4 and C-5, (b) a tertiary methyl (δ_H 0.95, s, H_3-23; δ_C 16.4, C-23) with correlations to C-1, C-5, C-9 and C-10, and (c) a tertiary methyl (δ_H 0.89, s, H_3-24; δ_C 15.4, C-24) with correlations to C-7, C-8, C-9 and C-14. The NMR (DMSO-d_6) data for **1** (Supplementary data, Table S1) revealed an exchangeable phenol resonance (δ_H 9.00, s) displaying a ROESY correlation to H-18 (δ_H 6.61, d, 8.6 Hz), consistent with a 17-OH moiety. The structure arguments presented above accounted for all but the elements of OSO_3 and supported positioning of a sulfate moiety at C-20. Taken with HMBC correlations from H_2-15 to C-16, C-17 and C-21 (Table 1), the analysis presented above defined the planar structure for **1** as indicated. Consideration of the ROESY NMR (methanol-d_4)

Table 1
NMR data (methanol-d_4, 600 MHz) for fascioquinol A (**1**)

No.	δ_C	δ_H (mult., J (Hz))	COSY	ROESY	HMBC (^1H–^{13}C)
1	41.4	β 1.64–1.69[A] (m)	1α,	11α	2, 3, 5, 10
		α 0.83–0.89[B] (m)	1β, 2β	3α, 5, 9	23,
2	19.8	β 1.60–1.66[A] (m)	2α, 3α	23, 26	3
		α 1.38–1.43[C] (m)	1α, 2β		1, 4
3	43.3	β 1.35–1.40[C] (m)	3α,	22, 26	1, 5, 26
		α 1.19 (ddd, 14.0, 13.0, 4.1)	2β, 3β	22	2, 4, 22, 26
4	34.2				
5	57.6	0.91 (dd, 12.0, 1.9)	6β	1α, 7α, 9	4, 6, 22, 23, 26
6	20.2	β 1.40–1.45[C] (m)	5, 6α, 7α	7β, 23, 26	10
		α 1.56–1.61 (m)	6β, 7α, 7β	7, 22	7, 8, 10
7	42.4	β 2.03 (ddd, 12.8, 3.1, 3.1)	6α, 7α	15b, 6α, 6β, 24	5, 9
		α 1.30 (ddd, 13.3, 12.8, 3.9)	6α, 6β, 7β	5, 6α, 14	6, 8, 14, 24
8	38.3				
9	56.9	1.25 (dd, 10.1, 7.0)	11	1α, 5, 14	1, 8, 10, 11, 14, 23, 24
10	38.6				
11	24.0	1.89–1.94 (2H, m)	9, 12, 14, 25	1β, 12, 23, 24	12, 13
12	122.7	5.30 (br s)	11, 14, 25	11, 25	
13	137.0				
14	56.2	2.39 (br d, 9.7)	11, 12, 15a	7α, 9, 21	13, 16
15	27.1	a 2.68 (dd, 15.3, 9.7)	14, 15b	21, 24, 25	13, 14, 16, 17, 21
		b 2.53 (dd, 15.3, 2.4)	15a	7β, 21, 24	8, 13, 14, 16, 17, 21
16	132.2				
17	153.4				
18	116.0	6.66 (d, 8.7)	19		16, 17, 20
19	120.5	6.91 (dd, 8.7, 2.7)	18, 21		17, 20, 21
20	146.6				
21	124.0	7.14 (d, 2.7)	19	14, 15a, 15b	15, 17, 19, 20
22	34.1	0.89 (s)		3α, 3β, 6α	3, 4, 5, 26
23	16.4	0.95 (s)		2β, 6β, 11β, 24, 26	1, 5, 9, 10
24	15.4	0.89 (s)		7β, 11β, 15a, 15b, 23	7, 8, 9, 14
25	22.6	1.44 (br s)	11, 12	12, 15a	12, 13, 14
26	22.3	0.85[B] (s)		2β, 3β, 6β, 23	3, 4, 5, 22

[A–D]Overlapping resonances within the column.

data for **1** revealed key correlations that defined the complete relative configuration. More specifically, ROESY correlations (Fig. 1) established that H-5, H-9 and H-14 were positioned one (α) face, while H_3-26, H_3-23, H_3-24 and H_2-15 were positioned on the opposite (β) face of the fused tricyclic system. Thus the complete relative stereostructure for fascioquinol A (**1**) could be assigned as indicated.

During HPLC fractionation of **1** it proved advantageous to employ an H_2O/MeCN gradient, with a constant 0.01% TFA modifier to enhance peak shape and improve resolution. The benefits of a TFA modifier notwithstanding, during concentration in vacuo (freeze drying) samples of **1** underwent acid mediated degradation to yield **3**–**5** (structure assignments detailed below). The proposition that the cyclic ethers **3**–**5** were handling artifacts was further supported by acid hydrolysis studies (see Experimental section), and the fact that formation could be avoided by either neutralizing HPLC fractions with sodium bicarbonate prior to concentration in vacuo, or by avoiding HPLC in favour of gel chromatography (Sephadex LH-20) in neutral solvent (MeOH).

HRESI(−)MS analysis of fascioquinol B (**2**) revealed a pseudo-molecular ion $(M-H)^-$ attributed to a molecular formula ($C_{26}H_{38}O_2$, Δmmu −0.5) consistent with a desulfated analogue of **1**. Supportive of such a structure assignment, the ^{1}H NMR (methanol-d_4) data for **2** (Supplementary data, Table S2) was almost identical to that for **1**, with the significant exception being an ~0.5 ppm shielding of resonances for H-19 and H-21, which now flanked a phenol rather than a sulfate functionality. Likewise, diagnostic ^{13}C NMR chemical shift differences between **1** and **2** (C-17 $\Delta\delta_C$ −4.4, C-19 $\Delta\delta_C$ −6.9, C-20 $\Delta\delta_C$+4.6, C-21 $\Delta\delta_C$ −6.9) could be attributed to hydrolysis of the C-20 sulfate moiety to a phenol.

HRESI(±)MS analysis of the acid degradation products **3**–**5** revealed pseudo-molecular ions consistent with molecular formulae ($C_{26}H_{38}O_2$, Δmmu −0.6, −1.1 and 0.6, respectively) isomeric with **2**. Detailed analysis of the NMR (methanol-d_4) data for **3**–**5** (Supplementary data, Tables S3–S5) revealed a loss of the $\Delta^{12,13}$ moiety with concomitant ring closure to yield cyclic ethers. For example, analysis of the NMR data for fascioquinol C (**3**) revealed diagnostic resonances for a secondary aliphatic H_3-25 methyl (δ_H 0.68, d, 6.5 Hz), a quaternary oxygenated C-14 (δ_C 96.2), and an isolated H_2-15 benzylic spin system (δ_H 2.73, d, 16.6 Hz, H_b-15; 3.19, d, 16.6 Hz, H_a-15), with strong ROESY correlations observed between H_b-15 and H_3-25, and between H_a-15 and H_3-24—consistent with the five member cyclic ether as indicated (with the C-13 and C-14 relative stereochemistry assigned on mechanistic grounds as indicated below). Similar analysis of the NMR data for **4** and **5** confirmed retention of H-14 (**4** δ_H 1.26, d, 8.2 Hz; **5** δ_H 1.61, m), and the appearance of quaternary oxygenated C-13 (**4** δ_C 75.3; **5** δ_C 76.8) and tertiary aliphatic H_3-25 methyl (**4** δ_H 1.11, s; **5** δ_H 1.15, s) resonances, consistent with the C-13 epimeric six member cyclic ethers as indicated. On reviewing the scientific literature **5** proved to be identical in all respects, including absolute configuration, with the known marine sponge metabolite strongylophorine-22 first reported in 2003 by Miyaoka et al. from an Okinawan specimen of *Petrosia* (*Strongylophora*) *corticata*.[5] Based on this comparison, fascioquinol D (**4**) was assigned as the C-13 epimer as indicated.

HRESI(−)MS analysis of fascioquinol E (**6**) revealed a highest mass ion isomeric with **1**, whereas the NMR (methanol-d_4) data (Supplementary data, Table S6a) disclosed a simplified *E*,*E*,*E*-geranylgeranyl side chain. Consistent with this interpretation, acid mediated hydrolysis of **6** yielded the known sponge metabolite geranylgeranyl 1,4-hydroquinone (**7**).[6] Comparison of NMR shifts between **6** and **7** (as discussed above for **1** and **2**) permitted structure assignment of the meroterpene sulfate fascioquinol E (**6**) as indicated. Literature reports on **6** are limited to a single occurrence in a 1989 patent as a synthetic reverse transcriptase inhibitor,[7] with the current report being the first account of **6** as a natural product.

HRESI(−)MS analysis of fascioquinol F (**8**) revealed a pseudo-molecular ion $(M-H)^-$ consistent with a molecular formula $C_{26}H_{36}O_2$ (Δmmu −0.2), while the NMR ($CDCl_3$) data (Supplementary data, Table S8) disclosed resonances (δ_H 5.58, d, 9.8 Hz, H-1′; 6.25, d, 9.8 Hz, H-2′; 1.35, s, H_3-17′) suggestive of a chromene moiety. The planar structure assigned to **8** was confirmed by spectroscopic comparison to the closely related marine brown alga metabolite dictyochromenol[8] and the sponge metabolite 2-(hexaprenylmethyl)-2-methylchromenol.[9] As the latter two natural products exhibited small but positive $[\alpha]_D$ measurements (+4 and +3.6 (*c* 1, $CHCl_3$), respectively), the near zero $[\alpha]_D^{22}$ + 0.06 (*c* 0.58, $CHCl_3$) value for **8** was taken as evidence of a near racemic mixture. Literature reports of **8** are limited to a single occurrence in a 1989 patent as a synthetic anti-ulcer agent,[10] with the current report being the first account of **8** as a natural product.

In an attempt to explain the mechanism for the acid mediated transformations noted above we propose (Fig. 2) that following hydrolysis of the sulfate **1** to the phenol **2**, cyclization of **2** to **3**–**5** proceeds via initial protonation of $\Delta^{12,13}$ to yield a C-13 tertiary carbocation. This intermediate carbocation then undergoes either (i) pro *R* facial S_N1 addition by the adjacent 17-OH to yield **4**, or (ii) pro *S* facial S_N1 addition to yield **5**, or (iii) stereocontrolled intramolecular 1,2-hydride transfer (across the α face) to yield a C-14 tertiary carbocation which in turn undergoes S_N1 addition by the adjacent 17-OH from the less sterically hindered α face. This latter stereocontrolled 1,2-hydride transfer and α facial attack suggest assignment of the C-13/C-14 relative configuration to fascioquinol C (**3**) as indicated. Furthermore, given the proposed mechanistic relationship to **5**, a sponge metabolite of known absolute configuration,[5] we propose that **1**–**4** belong to the same antipodal series.

The natural product meroterpenes **1** and **6**–**8** were assessed in cytotoxicity and cell proliferation assays against human gastric (AGS)

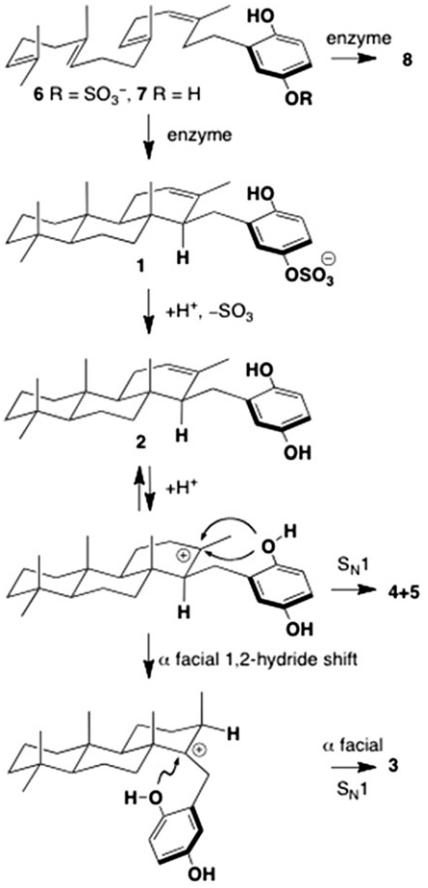


Fig. 2. Biosynthetic and acid mediated relationships of **1**–**8**.

and colourectal (HT-29) adenocarcinoma, neuroblastoma (SH-SY5Y) and human foreskin fibroblast (HFF-1) cell lines. These assays demonstrated that while the fascioquinols A (**1**), E (**6**) and F (**8**) were inactive (IC_{50} >30 μM), the geranylgeranyl 1,4-hydroquinone **7** was selectively cytotoxic to gastric adenocarcinoma (AGS, IC_{50} 8 μM) and neuroblastoma (SH-SY5Y, IC_{50} 4 μM) cell lines. The complete set of meroterpenes **1**–**8** was also tested in antimicrobial assays against two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, and a fungus (*Candida albicans*). These assays (Table 2 and Supplementary data) confirmed that fascioquinols A (**1**) and B (**2**) displayed promising Gram-positive selective antibacterial properties towards *S. aureus* (IC_{50} 0.9–2.5 μM) and *B. subtilis* (IC_{50} 0.3–7.0 μM).

Table 2
Antibacterial screening data (IC_{50} μM) for **1**–**8**

No.	*S. aureus*[a]	*S. aureus*[b]	*B. subtilis*[c]	*B. subtilis*[d]
1	2.4	2.5	0.85	1.8
2	1.0	0.95	0.30	7.0
3	5.4	5.6	1.4	1.7
4	25	>30	2.3	16
5	7.8	9.5	2.8	1.6
6	4.1	5.1	3.1	5.4
7	8.0	10	>30	23
8	13	16	>30	>30

Bacterial strains are
[a] ATCC 25923.
[b] ATCC 9144.
[c] ATCC 6051.
[d] ATCC 6633.

3. Experimental section

3.1. General experimental procedures

See Supplementary data.

3.2. Animal materials (collection and taxonomy)

See Supplementary data.

3.3. Bioassays

See Supplementary data.

3.4. Extraction and isolation

A portion (200 mL) of the aqueous EtOH extract of the *Fasciospongia* sp. was decanted and concentrated in vacuo to return a black solid (2.24 g), which was subsequently partitioned into *n*-BuOH (672 mg) and H_2O (1.5 g) soluble fractions. Bioassay established that cytotoxic agents were concentrated in the *n*-BuOH soluble partition, which was subsequently defatted by sequential trituration to yield *n*-hexane (87 mg) and CH_2Cl_2 (565 mg) soluble fractions.

A portion of the CH_2Cl_2 soluble material (133 mg) was fractionated on an Alltech C_{18} SPE cartridge (10% stepwise gradient elution from 50% H_2O/MeCN to 100% MeCN with a constant 0.1% TFA) to return six fractions (F1–F6), which were concentrated in vacuo at 40 °C. A portion (32/72 mg) of F2 was resolved by HPLC (Zorbax Eclipse C_8 semi-preparative column, gradient elution at 4 mL/min 30% H_2O/MeOH to 10% H_2O/MeOH with 10% isocratic MeCN over 20 min) to yield strongylophorine-22 (**5**, 6.4 mg), fascioquinol D (**4**, 4.1 mg) and fascioquinol C (**3**, 2.1 mg).

A second portion of the CH_2Cl_2 solubles (21 mg) was subjected to an HPLC (Zorbax Eclipse C_8 semi-preparative column, gradient elution at 4 mL/min 50% H_2O/MeCN to 100% MeCN over 20 min with a constant 0.02% TFA) to yield a pure sample of fascioquinol A (**1**), which underwent conversion to a mixture of **3**–**5** (6.1 mg) during concentration in vacuo (freeze drying). Similarly, a pure sample of fascioquinol E (**6**) underwent conversion to geranylgeranyl 1,4-hydroquinone **7** (4.0 mg).

A third portion of the CH_2Cl_2 solubles (17 mg) was fractionated using the same HPLC method as described above for the second portion, however, eluted samples of fascioquinol A (**1**) and fascioquinol E (**6**) were neutralized to pH ~7 by the addition of sodium bicarbonate and back extraction into $CHCl_3$ prior to concentration in vacuo. This process returned **1** (5.5 mg) and **6** (4.3 mg).

A fourth portion of the CH_2Cl_2 solubles (382 mg) was fractionated by a Sephadex LH-20 column (90×2.2 cm, MeOH) to return six mixed fractions (F1–F6) and two pure compounds, fascioquinol A (**1**, 143.8 mg) and fascioquinol E (**6**, 30.4 mg). Fraction F5 (65 mg) was subjected to normal phase isocratic silica gel column chromatography (28×2.2 cm, 6:1 *n*-hexane/acetone) to return geranylgeranyl 1,4-hydroquinone (**7**, 37.4 mg) and a mixture that was further separated by HPLC (Zorbax StableBond C_{18} semi-preparative column, gradient elution at 4 mL/min 20% H_2O/MeCN to 100% MeCN over 15 min) to yield fascioquinol F (**8**, 5.8 mg).

The yields of natural products in the *Fasciospongia* sp. extract, based on an estimated total weight to weight % from the crude aqueous EtOH extract are; fascioquinol A (**1**, 9.5%), fascioquinol E (**6**, 2.0%), geranylgeranyl 1,4-hydroquinone (**7**, 2.5%) and fascioquinol F (**8**, 0.4%).

*3.4.1. Fascioquinol A (**1**).* Off-white solid; $[\alpha]_D^{22}$ +24.6 (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (sh) (3.95), 281 (3.44) nm; ^1H and ^{13}C NMR (600 MHz, methanol-d_4) Table 1 (600 MHz, DMSO-d_6) Table S1; ESI (−)MS *m*/*z* 461 M^-; HRESI(−)MS *m*/*z* 461.2361 M^- (calcd for $C_{26}H_{37}O_5S$, 461.2367).

*3.4.2. Fascioquinol B (**2**).* White solid; $[\alpha]_D^{22}$ +39.6 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (sh) (3.94), 296 (3.70) nm; ^1H and ^{13}C NMR (600 MHz, methanol-d_4) Table S2 (see Supplementary data); ESI(±)MS *m*/*z* 383 $[M+H]^+$, 381 $[M-H]^-$; HRESI(−)MS *m*/*z* 381.2794 $[M-H]^-$ (calcd for $C_{26}H_{37}O_2$, 381.2799).

*3.4.3. Fascioquinol C (**3**).* White solid; $[\alpha]_D^{22}$ −13.0 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 219 (sh) (3.67), 230 (3.59), 306 (3.43) nm; ^1H and ^{13}C NMR (600 MHz, $CDCl_3$) Table S3 (see Supplementary data); ESI(±)MS *m*/*z* 383 $[M+H]^+$, 381 $[M-H]^-$; HRESI(−)MS *m*/*z* 381.2793 $[M-H]^-$ (calcd for $C_{26}H_{37}O_2$, 381.2799).

*3.4.4. Fascioquinol D (**4**).* White solid; $[\alpha]_D^{22}$ +13.4 (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (sh) (3.79), 230 (sh) (3.65), 298 (3.48) nm; ^1H and ^{13}C NMR (600 MHz, $CDCl_3$) Table S4 (see Supplementary data); ESI(±)MS *m*/*z* 383 $[M+H]^+$, 381 $[M-H]^-$; HRESI(−)MS *m*/*z* 381.2788 $[M-H]^-$ (calcd for $C_{26}H_{37}O_2$, 381.2799).

*3.4.5. Strongylophorine-22 (**5**).* White solid; $[\alpha]_D^{22}$ −44.7 (*c* 0.64, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 221 (sh) (3.76), 230 (sh) (3.67), 298 (3.52) nm; ESI(±)MS *m*/*z* 383 $[M+H]^+$, 381 $[M-H]^-$; ^1H and ^{13}C NMR (600 MHz, $CDCl_3$) Table S5 (see Supplementary data); HRESI (+)MS *m*/*z* 787.5642 $[2M+Na]^+$ (calcd for $C_{52}H_{76}NaO_4$, 787.5636).

*3.4.6. Fascioquinol E (**6**).* Off-white solid; UV (MeOH) λ_{max} (log ϵ) 281 (3.45) nm; ^1H and ^{13}C NMR (600 MHz, methanol-d_4) Table S6a (see Supplementary data); ^1H and ^{13}C NMR (600 MHz, DMSO-d_6) Table S6b (see Supplementary data); ESI(−)MS *m*/*z* 461 $^-$; HRESI(−) MS *m*/*z* 461.2363 M^- (calcd for $C_{26}H_{37}O_5S$, 461.2367).

*3.4.7. Geranylgeranyl 1,4-hydroquinone (**7**).* Pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 294 (3.66) nm; ^1H and ^{13}C NMR (600 MHz, $CDCl_3$) Table S7 (see Supplementary data), compares favourably

with literature data; ESI(±)MS m/z 383 $[M+H]^+$, 381 $[M-H]^-$; HRESI(−)MS m/z 381.2801 (calcd for $C_{26}H_{37}O_2$, 381.2799).

*3.4.8. Fascioquinol F (**8**).* Pale yellow oil; $[\alpha]_D^{22}+0.06$ (c 0.58, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (sh) (4.31), 264 (3.67), 331 (3.60) nm; ^1H and ^{13}C NMR (600 MHz, $CDCl_3$) Table S8 (see Supplementary data); ESI(±)MS m/z 381 $[M+H]^+$, 403 $[M+Na]^+$, 379 $[M-H]^-$; HRESI(−) MS m/z 379.2641 (calcd for $C_{26}H_{35}O_2$, 379.2643).

3.5. Preparative acid hydrolysis of fascioquinol A (1)

A sealed solution of **1** (6.5 mg) in MeOH (650 μL) and TFA (20 μL) was stirred at 40 °C overnight. HPLC fractionation of the reaction mixture (Zorbax Eclipse C_8 semi-preparative column, gradient elution at 4 mL/min, 35% H_2O/MeCN to 100% MeCN over 15 min) yielded fascioquinol B (**2**, 2.4 mg, 47%).

3.6. Analytical acid hydrolysis/cyclization study of fascioquinol A (1)

Three sealed solutions of **1** (1.0 mg) in 20:1, 10:1 and 5:1 ratios of MeOH/TFA (200 μL) were stirred at 40 °C overnight. HPLC analysis (Zorbax Eclipse C_8 analytical column, gradient elution at 1.0 mL/min 30% H_2O/MeCN to 100% MeCN with a constant 0.01% TFA over 15 min) detected only the desulfated analogue **2** in all the three reaction mixtures. Two sealed solutions of **1** (1.0 mg) in 1:1 MeOH/TFA (200 μL) and 1:1:2 MeCN/H_2O/TFA (200 μL) were stirred at room temperature overnight. HPLC analysis (as above) detected only the ring cyclization products **3**–**5**.

Acknowledgements

We thank CSIRO Marine Research and the crew of the RV Franklin for assistance in sponge collection, L. Goudie (Museum Victoria) for sponge taxonomy, C. Cuevas and colleagues (PharmaMar) for preliminary in vitro anticancer screening, M.M. Conte (UQ) for in house in vitro cytotoxicity and cell proliferation screening, and A.M. Piggott (UQ) for the acquisition of HRESIMS data. This work was funded partially by the Australian Research Council, with additional support from PharmaMar (Madrid, Spain).

Supplementary data

These data include general experimental procedures, animal materials (collection and taxonomy), preliminary cytotoxic screening results on crude *n*-BuOH partition, MTT cytotoxicity and cell proliferation, and antimicrobial assays for pure compounds, tabulated NMR data and ^1H NMR spectra for all compounds **1**–**8**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.02.015. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Zhang, H.; Capon, R. J. *Org. Lett.* **2008**, *10*, 1959–1962.
2. El-Naggar, M.; Piggott, A. M.; Capon, R. J. *Org. Lett.* **2008**, *10*, 4247–4250.
3. Capon, R. J.; Peng, C.; Dooms, C. *Org. Biomol. Chem.* **2008**, *6*, 2765–2771.
4. Zhang, H.; Conte, M. M.; Capon, R. J. *Angew. Chem., Int. Ed.* **2010**, *49*, 9904–9906.
5. Hoshino, A.; Mitome, H.; Miyaoka, H.; Shintani, A.; Yamada, Y.; van Soest, R. W. M *J. Nat. Prod.* **2003**, *66*, 1600–1605.
6. Vidari, G.; Vita-Finzi, P.; Zanocchi, A. M.; Noy, G. P. *J. Nat. Prod.* **1995**, *58*, 893–896.
7. Hiroichi, Y.; Kenji, S. Jpn. Kokai Tokkyo Koho. JP 01294,660, 1989.
8. Dave, M.-N.; Kusumi, T.; Ishitsuka, M.; Iwashita, T.; Kakisawa, H. *Heterocycles* **1984**, *22*, 2301–2307.
9. Venkateswarlu, Y.; Reddy, M. V. R. *J. Nat. Prod.* **1994**, *57*, 1286–1289.
10. Kazuhiko, Y.; Yoshiyuki, T.; Masashi, T.; Osamu, I.; Noriyuki, M. Eur. Pat. Appl. EP 304842, 1989.