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Salarins D–J, seven new nitrogenous macrolides from the madagascar sponge *Fascaplysinopsis* sp.

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ABSTRACT

Seven new nitrogenous macrolides, designated salarins D–J (**4**–**10**), closely related to salarins A–C (**1**–**3**), have been isolated from the Madagascar *Fascaplysinopsis* sp. sponge. The structure and relative stereochemistry of compounds **4**–**10** were elucidated by interpretation of MS, COSY, HSQC, HMBC, and NOESY spectra. Salarin B's structure (**2**), based on the structure of salarin J (**10**), is now amended to also incorporate the C-14 to -17 THF ring system. All compounds were evaluated for their cytotoxicity against K562 and UT-7 human leukemia cells. While salarins D, E, H, and J displayed dose and time dependent inhibition of proliferation, salarins F and I were not active in these assays.

1. Introduction

In continuation of our long-standing interest in the chemistry of marine sponges,[1,2] we have investigated the Madagascar *Fascaplysinopsis* sp. sponge collected in Salary Bay ca. 100 km north of Tuléar.[3] Recently we reported the isolation and structure elucidation of three unprecedented groups of cytotoxic sponge derived nitrogenous macrolides, i.e., salarins A–C (**1**–**3**),[4,5] tulearins A–C (**11**–**13**),[4,6] and taumycins A (**14**) and B (**15**).[7] Shortly thereafter, a fourth group, combining taumycin and salarin, designated tausalarin C (**16**) has likewise been isolated from a different batch of the *Fascaplysinopsis* sp. sponge[8]. All four groups are novel classes of marine natural compounds embodying rare unprecedented functional moieties. The structural similarity of the various sponge metabolites of the four groups (**1**–**16**) to microorganism and fungal metabolites (e.g., the cyanobacteria *Lyngbia bouillonii* metabolites madangolide and laingolide A)[9,10] suggested that these compounds originate from guest microorganisms rather than from the host sponge itself.[11–13] This notion is supported by the chemical content variations from one collection to the other.

The extracts of the Madagascar *Fascaplysinopsis* sp. were found to be active in the brine shrimp test as well as being cytotoxic to leukemia cells. All compounds were evaluated for their cytotoxicity against K562 and UT-7 human leukemia cells lines, using the colorimetric methylthiazole tetrazolium bromide (MTT) assay.[14–17] Salarin C, the most potent salarin, exhibited significant inhibitory activity against the leukemia cell lines, UT-7, and K562, and the murine pro B cell line Ba/F3 at concentrations of 0.0005–0.5 μg/ml.[5,17] In this paper, we report the isolation, structural elucidation as well as biological activity of the seven new salarins (**4**–**10**).

2. Result and discussion

Salarins D–J (**4**–**10**) were isolated from two collections of the Madagascar *Fascaplysinopsis* sp. sponge collected in Salary Bay, ca. 100 km north of Tuléar (Fig. 1). The $CHCl_3$/MeOH (1:1) extracts of the two collections were chromatographed on Sephadex LH-20, eluted with hexane/MeOH/$CHCl_3$ (2:1:1) to afford a complex cytotoxic mixture of compounds. From the latter mixture, we isolated upon repeated silica gel chromatographies (VLC) seven new salarins (D–J, compounds **4**–**10**; 0.002%–0.015%, dry weight) together with the known salarins A (**1**) and C (**3**). The following structure

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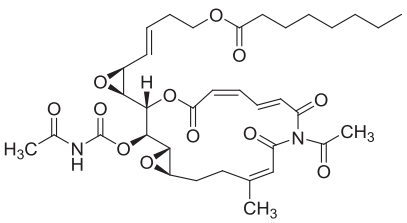
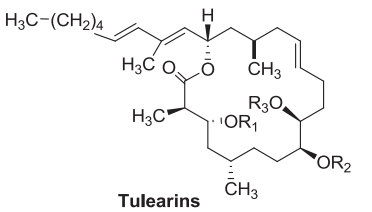
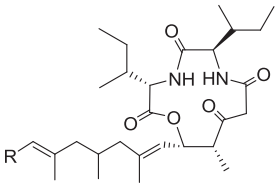


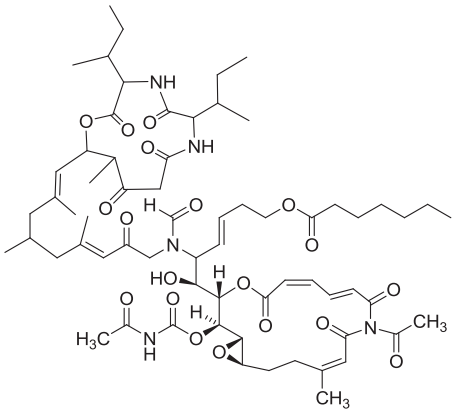
1 Salarin A



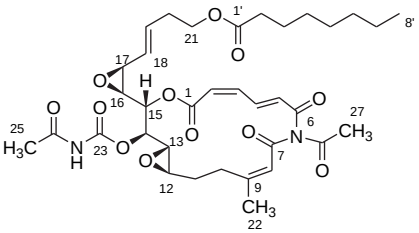
Tulearins
11 R_1,R_3= H R_2= $CONH_2$ (C-31)
12 R_1,R_2= $CONH_2$ R_3= H
13 R_1,R_2,R_3= H

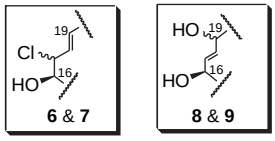


Taumucins
14 R=
15 R=COOH

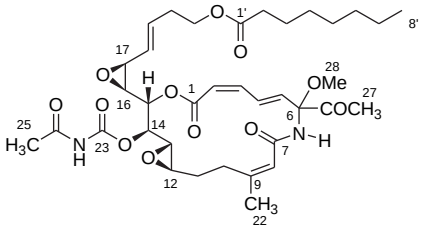


16 Tausalarin C

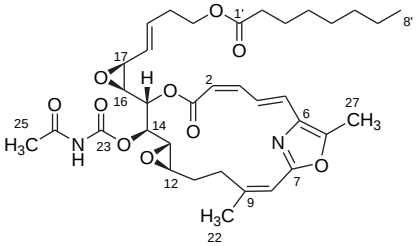




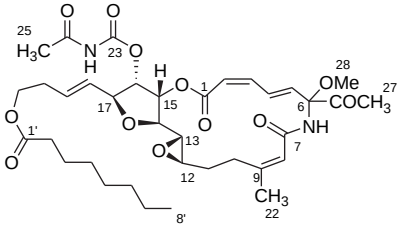
Salarin A (**1**)
Salarin G (**7**) 16-hydroxy-17-chloro
Salarin H (**8**) 16,19-dihydroxy-17-ene



Salarin D (**4**)
Salarin E (**5**) C(6)=O



Salarin C (**3**)
Salarin F (**6**) 16-hydroxy-17-chloro
Salarin I (**9**) 12,13,16,19-tetraol-17-ene



Salarin B (**2**)*
Salarin J (**10**) C(6)=O

*Amended structure for salarin B (see text).

Figure 1. Structures of known salarins A–C (**1**–**3**) and seven new congeners, salarins D–J (**4**–**10**).

elucidations of the seven compounds are grouped according to their similarity to salarins A–C (**1**–**3**).[4,5]

The molecular formula of salarin E (**5**), the first congener of salarin A, was established by HRESIMS to be $C_{33}H_{44}N_2O_{11}$; *m/z* 667.2825 $[M+Na]^+$ (calcd 677.2843) indicating 13 degrees of unsaturation. The ^1H, ^{13}C (Tables 1 and 3), COSY, HSQC, TOCSY, and HMBC spectra (Fig. 2), for **5**, revealed the presence of the following moieties: (a) two epoxides [δ_C 54.3 d and 54.4 d (*E*); δ 55.9 d and 57.1 d (*Z*)]; (b) an isolated double bond (δ_C 124.7 d and 133.8 d (*E*)); (c) another amide conjugated double bond (δ_C 120.3 d and 153.6 s (*Z*)); (d) an α,β,γ,δ-dienoate group (δ_C 166.1 s; 124.3 d, and 140.3 d (*Z*); and δ_C 137.8 d and 131.6 d (*E*)); (e) an octanoate ester (δ_C 173.7 s, 34.4 t, ca. 25.3 t additional five methylenes and a methyl group 14.0 q); (f) a diacylamine (δ_C 166.9 s and 164.5 s) and (g) an *N*-acetyl carbamate (δ_C 150.7 s, 171.5 s, and 25.0 q). The COSY experiment established three significant spin systems pointing to three sub-units, as depicted in Figure 2. The latter segments were correlated among themselves by a HMBC experiment. Comparison of the ^1H and ^{13}C NMR data of salarin E with those of salarin A (**1**)[4] indicated high similarity. The major distinction being the absence of one *N*-acetyl resonance signal, being replaced by a NH singlet at 7.96 ppm. CH-Correlations, derived from the HMBC experiment of **5** (Fig. 2), namely, correlations of H-4, H-5, and the NH with C-6 (δ_C 166.9 s) and of H-8 and NH with C-7 (δ_C 164.5 s), corroborated the *N*(6)-desacetyl structure for **5**, i.e., a diacylamine moiety in **5** instead of the unique triacylamine one present in **1**. The 2*Z*, 4*E*, 8*Z*, and 18*E* configurations of the double bonds ($J_{2,3}$=11.0 Hz, $J_{4,5}$=14.9 Hz, NOE between Me-22 and H-8, and $J_{18,19}$=15.7 Hz) and the 12*E* and 16*Z* configurations of the two epoxides ($J_{12,13}$=2.2 Hz and $J_{16,17}$=4.0 Hz), of compound **5**, were determined from *J*-values, NOEs and by comparison with the appropriate proton and carbon resonance counterpart values in compounds **1** and **3**.[4,5] The chirality of the six stereogenic carbon-atoms is assumed to be the same as in **1**.

The HRESIMS spectrum of salarin G (**7**) exhibited a pseudomolecular ion $[M+Na]^+$ at *m/z* 745.2727 suggesting, together with the ^{13}C spectrum (Table 3), the molecular formula $C_{35}H_{47}ClN_2O_{12}$, implying 13 degrees of unsaturation. The presence of one chlorine atom in the molecule was further confirmed by two dominant sodiated pseudomolecular ions $[M+Na]^+$ at *m/z* 745.3 and 747.3 with intensities of 1/0.33 in the ESIMS spectrum. Inspection of the ^1H and ^{13}C NMR features of **7** closely resembled those of **1** except for changes in the side chain. Namely, the 16(17)-epoxide of **1** and **5** is replaced by a 16-oxymethine-17-chloromethine functionality in **7** (δ_C 73.5 d & 64.5 d and δ_H 3.99 m & 4.57 dd for methines 16 and 17, respectively). Distinctive was the 64.5 d resonance of the chloromethine carbon-atom.[18] The 17-chloro-16-hydroxy-18-ene structure of **7** (and **6**) was confirmed from the COSY correlations from H-11 to H-21, as well as δ-values and coupling constants (Table 1), excluding a possible allylic rearrangement as was deduced for **8**, vide infra. Assuming salarin A (**1**) to be the precursor of **7** it can be suggested that the original epoxide oxygen atom keeps its configuration at C-16 while the configuration of the chlorinated allylic C-17 atom is unknown. Conformational mobility of the side chain prevented conclusions from the 15–18 segment coupling constants, leaving the chirality of C-17 unsolved as yet. To exclude the possibility that **6** and **7** are artifacts, products of HCl opening of the epoxide of salarin A or C, we treated **1** with traces of DCl in $CDCl_3$ and monitored the proton NMR. It was found that **1** is relatively stable under these mild acidic conditions for 48 h and then affords complex mixtures and not a single chlorohydrin.

The mass spectroscopic analysis, HRESIMS, of salarin H (**8**), the third congener of **1**, provided a pseudomolecular formula of $C_{35}H_{48}N_2O_{13}Na$, *m/z* 727.3050 for $[M+Na]^+$ (calcd 727.3054), indicating 13 degrees of unsaturation. The ^1H and ^{13}C NMR data (Tables 1 and 3) and careful analysis of the COSY and HMBC spectra (Fig. 2) led to the conclusion that **8** differs from salarins **A** and **G** due to the 16,19-dihydroxy-17-ene site of the side chain (δ_C 71.5 d, 128.0 d, 135.6 d and 68.7 d for C-16,17,18, and 19, respectively). An NOE between H-18 and H-15 and between H-17 and H-16 together with

Table 1
^1H NMR data for compounds **1**, **5**, **7**, **8**, and **10**[a]

Proton	1	5	7	8	10
2	6.08 d (11.4)	5.94 d (11.0)	6.15 d (11.3)	6.15 d (11.4)	5.91 d (11.3)
3	6.80 t (11.4)	6.71 t (11.0)	6.74 t (11.3)	6.79 t (11.4)	6.75 t (11.3)
4	8.23 dd (15.7,11.3)	8.13 dd (14.9, 11.0)	7.93 dd (15.7, 11.3)	8.14 dd (15.9, 11.4)	7.87 dd (16.2, 11.3)
5	6.52 d (15.7)	6.35 d (14.9)	6.44 d (15.7)	6.41 d (15.9)	6.34 d (16.2)
8	6.12 s	5.97 s	6.03 s	5.98 s	5.65 s
10	3.12 m	2.83 m (2H)	2.99 m	2.04 m	3.35 m
	2.30 m		2.29	1.72 m	1.63 m
11	2.12 m	1.96 m	1.93 m	1.72 m	1.85 m
	1.42 m	1.57 m	1.45 m	1.59 m	1.47 m
12	3.14 dd (7.1, 2.2)	2.98 m	3.00 dd (6.1, 2.5)	3.00 m	2.75 dt (8.7, 2.0)
13	3.32 dd (4.4, 2.2)	3.40 m	3.25 dd (3.7, 2.5)	3.25 dd (5.4, 2.1)	3.92 dd (7.8, 2.0)
14	5.06 t (4.4)	5.05 t (4.0)	5.20 t (3.7)	4.92 t (5.4)	3.43 t (7.8)
15	5.02 dd (7.3, 4.4)	5.15 dd (7.4, 4.0)	5.38 dd (7.4, 3.7)	5.34 dd (5.4, 4.2)	5.27 dd (7.8, 6.2)
16	3.26 dd (7.3, 3.8)	3.32 dd (7.4, 4.0)	3.99 m	4.84 brt (4.2)	5.04 dd (6.2, 4.9)
17	3.60 dd (6.5, 3.8)	3.56 dd (6.1, 4.0)	4.57dd (8.6, 4.4)	5.80 dd (16.0, 4.2)	4.45 brt (4.9)
18	5.60 dd (15.6, 6.5)	5.60 dd (15.7, 6.1)	5.71 dd (15.6, 8.6)	5.86 dd (16.0, 5.3)	5.61 dd (15.6, 4.9)
19	6.00 dt (15.6, 6.5)	5.98 dt (15.7, 7.0)	5.85 dt (15.6, 6.7)	4.24 m	5.86 dd (15.6, 6.9)
20	2.49 m (2H)	2.48 m (2H)	2.42 m (2H)	1.83 m (2H)	2.40 m (2H)
21	4.14 t (6.7) (2H)	4.12 t (6.8) (2H)	4.11 t (6.7) (2H)	4.29 dtt (11.3, 5.7, 2.3)	4.12 qd (6.6, 2.7) (2H)
				4.13 dtt (11.3, 5.7, 2.3)	
22	1.96 s	1.92 s	1.89 s	1.87 s	1.96 s
NH	8.54 s	8.36 s	8.30 s	8.25 s	7.81 s
		7.96 s			7.55 s
25	2.33 s	2.44 s	2.30 s	2.41 s	2.42 s
27	2.42 s		2.40 s	2.43 s	
2′	2.32 m (2H)	2.28 m (2H)	2.30 m (2H)	1.80 m (2H)	2.29 m (2H)
3′	1.60 m (2H)	1.53 m (2H)	1.60 m (2H)	1.60 m (2H)	1.60 m (2H)
4′	1.31 m (2H)	1.27 m (2H)	1.27 m (2H)	1.30 m (2H)	1.28 m (2H)
5′	1.30 m (2H)	1.28 m (2H)	1.27 m (2H)	1.30 m (2H)	1.28 m (2H)
6′	1.29 m (2H)	1.27 m (2H)	1.26 m (2H)	1.29 m (2H)	1.26 m (2H)
7′	1.30 m (2H)	1.27 m (2H)	1.25 m (2H)	1.28 m (2H)	1.26 m (2H)
8′	0.91 t (6.9)	0.86 t (6.9)	0.85 t (6.8)	0.88 t (6.7)	0.87 t (6.7)

[a] Data recorded in $CDCl_3$ on a Bruker Avance-500 MHz instrument, chemical shifts (δ) are in parts per million, *J*, in parenthesis, in Hz.

Table 2
^{1}H NMR data for compounds **2**–**4**, **6**, and **9**[a]

Proton	2	3	4	6	9
2	5.71 d (11.2)	6.18 d (11.3)	5.66 d (11.6)	5.63 m	5.62 d (11.9)
3	6.50 t (11.2)	7.05 t (11.3)	6.63 t (11.6)	6.84 t (11.9)	6.77 t (11.9)
4	7.71 dd (15.4, 11.2)	8.21 br t (13.8) [b]	7.20 dd (16.0, 11.6)	8.06 br t (12.8) [b]	8.19 br t (13.2)[b]
5	6.42 d (15.4)	6.58 d (15.7)	6.36 d (16.0)	6.70 d (14.9)	6.64 d (14.8)
8	5.33 s	6.00 s	5.73 s	6.01 s	5.99 s
10	2.10 m (2H)	2.48 m (2H)	2.78 dt (13.4, 8.5)	3.68 m	3.55 td (12.4, 3.6)
			2.15 m	2.23 m	1.87 m
11	1.96 m	2.04 m	2.16 m	2.23 m	2.57 dd (12.5 4.8)
	1.41 m	1.55 m	1.60 m	1.34 m	1.93 m
12	2.79 m	3.12 m	3.12 m	3.26 dd (9.5, 1.9)	3.74 td (9.5, 4.8)
13	3.08 dd (8.0, 1.9)	3.13 m	3.12 m	3.35 dd (8.8, 1.9)	3.94 br t (9.7)
14	3.63 t (8.0)	4.84 t (7.6)	4.71 t (5.4)	4.87 dd (8.8, 2.3)	5.03 dd (9.7, 3.0)
15	5.40 m	5.06 t (7.6)	4.84 dd (7.7, 5.4)	5.60 dd (9.3, 2.3)	5.74 dd (6.7, 3.0)
16	4.98 dd (6.4, 2.9)	3.34 dd (7.6, 4.0)	3.11 m	4.05 m	4.82 br s ($\Delta W_{1/2}$=7.1)
17	4.51 m	3.53 dd (6.8, 4.0)	3.49 dd (6.3, 4.0)	4.49 dd (7.4, 4.3)	5.87 ddd (16.0, 7.6, 4.0)
18	5.41 m	5.52 dd (15.6, 6.8)	5.48 dd (15.6, 6.3)	5.65 m	5.92 ddt (16.0, 5.7, 1.7)
19	5.89 dt (14.9, 6.8)	5.98 dt (15.6, 6.8)	5.88 dt (15.6, 6.8)	5.67 m	4.33 m
20	2.22 m (2H)	2.37 q (6.8) (2H)	2.40 m (2H)	2.45 m (2H)	1.85 m (2H)
21	4.09 m (2H)	4.08 t (6.8) (2H)	4.07 qd (6.7, 2.1) (2H)	3.98 t (6.8) (2H)	4.33 m
					4.19 dt (11.3, 5.6)
22	1.52 s	1.90 s	1.81 s	2.03 s	2.00 s
NH	7.89 s	7.38 s	8.03 s	7.36 s	7.60 s
			6.91 s		
25	2.26 s	2.30 s	2.37 s	2.42 s	2.41 s
27	1.90 s	2.39 s	2.28 s	2.36 s	2.35 s
28	3.18 s		3.16 s		
2′	2.28 m (2H)	2.28 m (2H)	2.26 m (2H)	2.23 m (2H)	2.27 m (2H)
3′	1.68 m (2H)	1.58 m (2H)	1.56 m (2H)	1.59 m (2H)	1.60 m (2H)
4′	1.29 m (2H)	1.30 m (2H)	1.25 m (2H)	1.26 m (2H)	1.30 m (2H)
5′	1.29 m (2H)	1.30 m (2H)	1.25 m (2H)	1.26 m (2H)	1.30 m (2H)
6′	1.28 m (2H)	1.28 m (2H)	1.22 m (2H)	1.25 m (2H)	1.28 m (2H)
7′	1.33 m (2H)	1.29 m (2H)	1.25 m (2H)	1.24 m (2H)	1.29 m (2H)
8′	0.97 t (6.8)	0.87 t (6.9)	0.85 (6.7)	0.87 t (6.8)	0.88 t (6.6)

[a] Data recorded in $CDCl_3$ on a Bruker Avance-500 MHz instrument, chemical shifts (δ) are in parts per million, J, in parenthesis, in Hz.
[b] Broad triplet due to small differences in the vicinal coupling constants.

a $J_{17,18}$=16.0 Hz coupling constant determined the 17*E* geometry of the double bond. Interestingly, the latter change of functionality brought about a change in the chemical shifts and coupling constant of H_2-21 i.e., δ_H 4.29 dtt, 4.13 dtt (2H) for **8** against δ_H 4.14 t (6.7) (2H) for compound **1**. A 16*S** configuration, on the same rationale as for **7**, is also suggested for **8**, whilst the configuration of C-19 remains unsolved. An acid catalyzed opening of the 16(17)-epoxide of **1**, followed by the allylic rearrangement of the initially obtained 18-ene-17-carbocation explains the production of the modified 16–19 site.

The HRESIMS mass spectrum of salarin D (**4**), a congener of salarin **B** (**2**), exhibited a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 725.3205 suggesting, together with the ^{13}C NMR data (Table 3) the molecular formula $C_{36}H_{50}N_2O_{12}$, implying 13 degrees of unsaturation. The 1D and 2D NMR spectra of **4** revealed a close relationship with salarin B vide infra, that is, **4** possessing the same methoxymethylketone lactam moiety (C6–C8 segment). The major difference being a 16(17)-epoxide in **4** (δ_C 56.3 d & 56.5 d and δ_H 3.11 m & δ_H 3.49 dd for CH-16 and -17, respectively), in addition to the 12(13)-epoxide as in compound **1**. On the basis of the $J_{16,17}$=4.0 Hz coupling constant a *Z*-configuration is suggested for the 16(17)-epoxide, as in compounds **1** and **3**.[4,7,19]

The ESIMS spectrum of salarin F (**6**), a congener of salarin C (**3**), exhibited two dominant sodiated pseudomolecular ions $[M+Na]^+$ at *m*/*z* 713.3 and 715.3 with intensities of 1/0.33, as in compound **7**, suggesting the presence of a chlorine atom in the molecule. The NMR data of **6** revealed a considerable similarity to the macrolide NMR data of salarin C (**3**); namely, **6** possesses the same derivatized macrolide fused to an oxazole-ring and carries an ester, but differs in methines CH-16 and CH-17 (δ_C 73.2 d & 65.3 d and δ_H 4.05 m & 4.49 dd, respectively) suggesting replacement of the 16(17)-epoxide of **3** by a 16-hydroxy-17-chloro functionality as in **7**.[18] The relative configurations of all stereocenters in **6**, except for C-17, are suggested, on the basis of NOE's, to be identical with those of **3** (Fig. 3).

Salarin I (**9**), a second congener of **3**, analyzed for $C_{35}H_{50}N_2O_{12}$ with 12 degrees unsaturation from the HRESIMS *m*/*z* 673.3338 $[M-H_2O+H]^+$ (calcd 673.3336). Loss of a molecule of water in the mass spectrometer, became evident from the ^{13}C NMR spectrum of **9** (Table 3) exhibiting six oxymethine resonances, i.e., δ_C 72.5 d, 71.9 d, 74.7 d, 70.9 d, 75.3 d, 68.9 d (four secondary alcohols and two ester carrying ones) and one oxymethylene 60.9 t.[20] The NMR data (Table 2 and 3) closely resembled those of salarin C (**3**) differing only in two sites. These being the replacement of the 12(13)-epoxide by a 12,13-diol (δ_C 72.5 d and 71.9 d) and the substitution of the 16(17)-epoxide of **3** by a 16,19-dihydroxy-17(*E*)-ene moiety in the side chain (δ_C 75.3 d, 125.1 d, 137.2 d, and 68.9 d, for C(16–19), respectively) as in **8**. An *E*-configuration of the 17(18)-double bond, as in salarin H (**8**), was established from the $J_{17,18}$=16.0 Hz coupling constant. As for the stereochemistry of the six stereogenic atoms of **9**, it is assumed that the chirality of C-14 and C-15 remains 14*R** and 15*S** as in salarin C (**3**) where it is the same as in salarin A (**1**) approved by X-ray analysis. Coupling constants of 9.7 Hz between H-13 and H-14 and between H-12 and H-13 point to dihedral angles of $\Phi_{12,13}=\Phi_{13,14}\approx 180$. Hence, H-13 has to be on the β-face of the molecule and H-12 on the α-face (H-14 is on the α-face), that is, chirality of 12*S** and 13*R**. Carbon-12 keeps the epoxide oxygen while C-13 is inverted. Coupling constant of ca. 7 Hz ($J_{15,16}$=6.7 Hz and $J_{16,17}$=7.6 Hz) point to a free rotation around the relevant bonds of the side chain.[21] Either C-16 or C-17 has to remain with the epoxide chirality, most likely C-16, as C-17 will create a stabilized vinyl carbocation. Therefore, C-16 will be 16*S** while the configuration of C-17 is still unknown.

The HRESIMS of salarin J (**10**) exhibited a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 667.2827, suggesting, together with the ^{13}C NMR

Table 3
^{13}C NMR data for compounds **1**–**10**[a]

No.	1	2	3	4	5	6	7	8	9	10
1	163.9 s	164.7 s	165.0 s	164.3 s	166.1 s	167.1 s	164.1 s	164.3 s	166.0 s	163.8 s
2	126.1 d	117.5 d	116.8 d	118.8 d	124.3 d	116.7 d	127.1 d	126.5 d	116.9 d	122.0 d
3	140.2 d	146.2 d	143.3 d	142.7 d	140.3 d	143.7 d	138.5 d	139.6 d	143.9 d	142.9 d
4	141.3 d	127.8 d	125.7 d	128.3 d	137.8 d	125.5 d	142.1 d	141.3 d	126.1 d	136.9 d
5	134.6 d	142.4 d	128.8 d	141.2 d	131.6 d	129.4 d	132.6 d	133.1 d	129.6 d	131.0 d
6	171.6 s	89.8 s	134.3 s	89.2 s	166.9 s	134.1 s	171.9 s	172.3 s	134.6 s	168.9 s
7	167.6 s	165.7 s	159.1 s	166.2 s	164.5 s	158.9 s	167.2 s	167.1 s	159.0 s	163.9 s
8	122.3 d	119.8 d	110.7 d	119.8 d	120.3 d	110.9 d	121.6 d	121.4 d	110.3 d	117.1 d
9	156.5 s	153.8 s	150.1 s	150.9 s	153.6 s	150.9 s	157.1 s	157.9 s	151.9 s	163.1 s
10	27.2 t	30.6 t	29.4 t	28.9 t	28.5 t	28.8 t	28.8 t	27.3 t	29.7 t	28.9 t
11	27.6 t	34.5 t	29.3 t	29.1 t	28.7 t	29.0 t	29.0 t	27.5 t	29.9 t	29.0 t
12	53.2 d	55.5 d	57.0 d	55.8 d	54.3 d	57.2 d	53.3 d	54.4 d	72.5 d	58.2 d
13	55.3 d	59.5 d	54.7 d	54.8 d	54.4 d	55.1 d	54.5 d	55.1 d	71.9 d	56.2 d
14	72.1 d	83.4 d	77.7 d	75.5 d	72.3 d	77.6 d	72.2 d	72.7 d	74.7 d	82.5 d
15	70.5 d	70.7 d	67.6 d	69.4 d	69.3 d	71.4 d	73.2 d	74.8 d	70.9 d	71.0 d
16	56.5 d	77.6 d	55.3 d	56.3 d	55.9 d	73.2 d	73.5 d	71.5 d	75.3 d	76.8 d
17	56.6 d	83.5 d	57.0 d	56.5 d	57.1 d	65.3 d	64.5 d	128.0 d	125.1 d	82.1 d
18	124.7 d	130.6 d	124.4 d	124.8 d	124.7 d	128.7 d	128.5 d	135.6 d	137.2 d	128.4 d
19	133.8 d	131.5 d	134.0 d	133.5 d	133.8 d	131.4 d	132.9 d	68.7 d	68.9 d	130.4 d
20	31.8 t	32.5 t	31.8 t	31.6 t	31.8 t	31.6 t	31.7 t	36.2 t	36.1 t	31.6 t
21	62.9 t	63.3 t	62.8 t	62.9 t	62.9 t	62.5 t	62.6 t	60.9 t	60.9 t	62.8 t
22	24.1 q	24.6 q	25.0 q	22.5 q	24.9 q	25.2 q	23.6 q	23.9 q	26.4 q	26.3 q
23	151.1 s	152.1 s	150.7 s	150.9 s	150.7 s	150.1 s	151.2 s	151.1 s	151.6 s	150.8 s
24	171.5 s	171.4 s	171.6 s	171.4 s	171.5 s	171.0 s	171.5 s	171.2 s	171.9 s	171.3 s
25	25.3 q	24.3 q	23.8 q	23.9 q	25.0 q	23.9 q	24.9 q	24.1 q	23.9 q	24.9 q
26	171.1 s	202.1 s	145.9 s	204.0 s		145.7 s	171.9 s	172.0 s	146.0 s	
27	24.0 q	24.0 q	10.1 q	24.9 q		10.2q	25.4 q	25.5 q	10.2 q	
28		51.0 q		50.9						
1′	173.7 s	173.6 s	173.7 s	173.7 s	173.7 s	173.1 s	173.9 s	174.1s	174.2 s	173.6 s
2′	34.2 t	34.8 t	34.1 t	34.2 t	34.4 t	34.2 t	34.2 t	34.4 t	34.2 t	34.3 t
3′	24.9 t	25.8 t	24.8 t	25.1 t	25.3 t	24.9 t	25.4 t	24.9 t	24.9 t	24.4 t
4′	26.1 t	29.9 t	28.8 t	29.4 t	28.6 t	29.6 t	29.9 t	29.1 t	29.1 t	29.6 t
5′	28.9 t	29.8 t	29.0 t	29.6 t	28.5 t	29.7 t	29.9 t	28.9 t	28.9 t	29.9 t
6′	31.6 t	32.4 t	31.6 t	31.8 t	31.6 t	31.5 t	31.6 t	31.6 t	31.6 t	31.6 t
7′	22.5 t	23.4 t	22.5 t	23.5 t	22.5 t	22.6 t	22.5 t	22.5 t	22.5 t	22.6 t
8′	14.0 q	14.2 q	13.9 q	14.6 q	14.0 q	14.0 q	14.0 q	13.9 q	14.0 q	14.0 q

[a] Data recorded in $CDCl_3$ on a Bruker Avance-400 MHz instrument (at 100 MHz), Chemical shifts (δ) are in parts per million; assignments were based on DEPT, ^1H–^1H COSY, HMQC, and HMBC experiments.

spectrum, the $C_{33}H_{44}N_2O_{11}$ molecular formula, indicating 13 degrees of unsaturation. Hence, compound **10** was implied to be a structural isomer of **5**, differing according to the NMR data only in the C(14–17) site. That is, the 16(17)-epoxide of **5**, is replaced in **10** by a 2,3-dioxygenated THF ring [an ethereal bridge between C-14 (δ_C 82.5 d) and C-17 (δ_C 82.1 d)]. The suggested disubstituted THF ring became evident from COSY, HMBC (Fig. 2) and NOE correlations (Fig. 3). NOE cross-peaks between H-14 and H-17 corroborated the presence of ethereal bridge (Fig. 3). Furthermore, C-16 was determined to carry the *N*-acetyl carbamate moiety according to $^3J_{CH}$ correlations (HMBC) from H-16 (δ_H 5.04 dd) to the carbamate C-23 atom (δ_C 150.8 s). The relative stereochemistry of C-12 to -16 was assumed to be the same as in the other salarins (12*R**, 13*S**, 14*R**, 15*S**, and 16*S**). The unchanged 16*S** configuration is suggested on the basis of an expected more stable vinyl C-17 carbocation while-opening the epoxide, vide infra. Additionally, a relative chirality of 17*S** is suggested, on the basis of an NOE between the cis oriental H-14 and -17.

Finally, the structure of the earlier reported salarin B (**2**)[4] is now suggested to be amended. The original structure implied one 12 (13)-epoxide and one 16,17-diol. Indeed, the FABMS (*m*/*z* 703.2) was shorter by 18 mu then the anticipated molecular peak for $C_{36}H_{52}N_2O_{13}$, rationalized by the loss of a molecule of water. Following the discovery of salarin J (**10**), vide supra, and the excellent NMR chemical shifts agreement of the C-12 to C-18 segment, in **10** and **2**, it is therefore suggested that salarin B also incorporates the same THF ring system as **10**. Repeating the mass analysis in the ESI mode afforded the pseudomolecular peak $[M+Na]^+$ at *m*/*z* 725.3260 (calcd for $C_{36}H_{50}N_2O_{12}$ 725.3261). Characteristically, as in **10**, were the chemical shifts of the ether carrying C-14 and C-17 atoms (δ_C 83.4 d and 83.5 d, respectively), which together with other NMR features, including HMBC correlations from H-16 (δ_H 4.98 dd) to C-14, C-15, C-17, and C-23 (83.4 d, 70.7 d, 83.5 d, and 152.1 s, respectively) evidenced the tetrahydrofuran ring. The relative configuration of the six asymmetric carbons of **2** was in good agreement with the chirality in **10**.

As for the biogenesis of salarins B (**2**) (similarity to the comparable sites, in salarin J (**10**) and D (**4**)) a 12,13-epoxy-14-hydroxy-15,16-epoxy carrying salarin possessing the 6-methoxy-6-methyl-ketone, is suggested to be the precursor. Carbamoylation of the 14-hydroxy group will lead to salarin D, whereas, acid catalyzed opening of the 15,16-epoxide with simultaneous ether formation to form the THF ring system by the 14-hydroxy group, followed by carbamoylation of the 16-hydroxy group will lead to salarin B (**2**) and J (**10**). The latter biogenesis suggested for all chiral centers of **2**, except for C-17, to be the same as in salarin A (**1**). An NOE between H-14 and H-17 requires both to be cis on the same α-face of the THF ring, hence, C-17 is of the *S** configuration.

Worth mentioning is a transannular NOE correlation between H-4 and H-13 observed for salarin C (**3**) and its congeners **6** and **9**, but not for salarins missing the oxazole-ring. The latter heterocycle diminishes the macrolide flexibility bringing H-4 spatially closer to H-13.

3. Conclusion

In conclusion, the study described here the isolation of seven new salarins together with two known ones from the Madagascar *Fascaplysinopsis* sp. sponge. The research expanded the library of the salarins and illuminated the variety of the metabolites of the

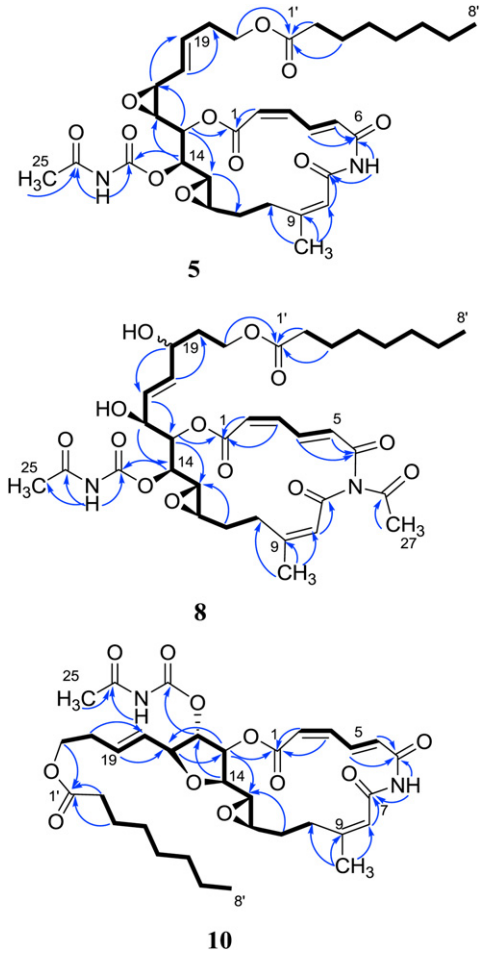


Figure 2. ^{1}H–^{1}H COSY (▬) and selected HMBC (⌒) correlations of compounds **5**, **8**, and **10**.

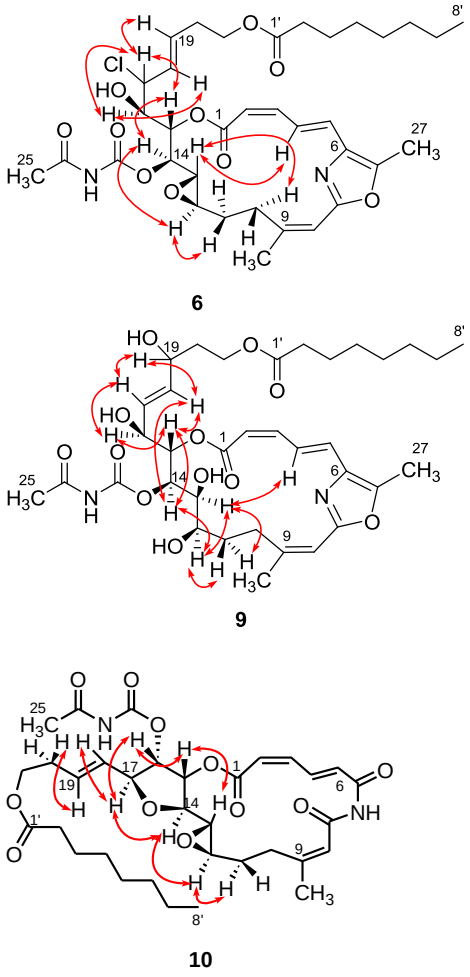


Figure 3. Key NOESY correlations and relative configurations assigned for **6**, **9**, and **10**.

sponge. In particular, the cytotoxicity assay showed that several of the salarins possess significant biological activity against K562 and UT-7 human leukemia cells. Salarins D, E, H, and J inhibited proliferation (30–50%) of K562 cells after 3 days in culture. Of these compounds, only salarin E inhibited proliferation (60%) of the UT7 cell line, while salarins F and I were not active in these assays.[17]

4. Experimental

4.1. General

Optical rotations were obtained with a Jasco P-1010 polarimeter. UV spectra were recorded on an Agilent model 8453 UV–visible spectrometer. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-400 and Avance-500 spectrometers. COSY, HMQC, NOESY, TOCSY, and HMBC were recorded using standard Bruker pulse sequences. FABMS measurements were recorded on a Fisons, Autospec Q instrument. Electrospray MS measurements were performed on Waters Micromass SYNAPT HDMS Mass spectrometer (TOF). ^{1}H and ^{13}C NMR data are presented in Tables 1–3.

4.2. Biological material

Fascaplysinopsis sp. was collected on two occasions from the west coast of Madagascar in Salary Bay ca. 100 km north of Tuléar in January 2007 and in February 2008 at a depth of 25–35 m. The identification of the spicule-less sponge genus was not straightforward. Its closest resemblance seems to be *Fascaplysinopsis* (Demospongiae, order Dictyoceratid, family Thorectidae) a genus described thus far only from Australia and Indonesia.

4.3. Extraction and isolation

The 2007 collection of the sponge (121 g) was homogenized and extracted twice with $CHCl_3$/MeOH (2:1) (250 mL). The combined organic phase was concentrated to yield a crude extract (2.6 g) that was subjected to partitioning by the Kupchan method.[22] Part of the dichloromethane fractional (210 mg) was chromatographed on a Sephadex LH-20 column, eluting with *n*-hexane/MeOH/$CHCl_3$ (2:1:1) to obtain 16 fractions of ca. 20 mL each. Fractions 8–12 (90 mg) were chromatographed over silica gel (VLC) using *n*-hexane with increasing proportions of ethyl acetate as eluent. Compound **3** (41 mg, 0.033 wt %) was afforded by elution with 30% ethyl acetate in hexane. Elution with 40% ethyl acetate in hexane afforded compound **1** (12 mg, 0.01 wt %) and **5** (9 mg, 0.009 wt %). Fractions 13–15 (58 mg) afforded compound **4** (7.6 mg 0.015 wt %) and compound **6**, (15 mg 0.012 wt %) with 50% ethyl acetate/hexane elution. The 2008 collection (115 g), resembled the isolation of the above salarins. The dichloromethane extract (180 mg) that was chromatographed on a Sephadex LH-20 column afforded 15 fractions of ca. 20 mL each. Fractions 8–10 (28 mg) were purified by VLC over silica gel, eluted with 55% ethyl acetate in hexane to afford pure **7** (6 mg 0.005 wt %), and compound **8** (2.1 mg 0.002 wt %). Fractions 11–12 (12 mg) afforded by elution with 60 % ethyl acetate/

hexane compound **10** (3.1 mg, 0.002 wt %) and compound **9** (7.6 mg, 0.007 wt %).

*4.3.1. Salarin D (**4**).* Yellow oil; $[\alpha]_D^{26}$ −29 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 256 (3.62); IR ($CHCl_3$) ν_{max} 3693, 3056, 2989, 1721, 1605, 1426, 1273 cm^{-1}; HRESIMS (TOF) *m*/*z* 725.3205 $[M+Na]^+$ (calcd for $C_{36}H_{50}N_2O_{12}Na$ 725.3255).

*4.3.2. Salarin E (**5**).* Yellow oil; $[\alpha]_D^{26}$ −98 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 228 (3.68); IR ($CHCl_3$) ν_{max} 3693, 3421, 3055, 2987, 1770, 1721, 1633, 1425 cm^{-1}; FABMS *m*/*z* 667.0 $[M+Na]^+$ (100), 645 $[M+H]^+$ (50); HRESIMS (TOF) *m*/*z* 677.2825 $[M+Na]^+$ (calcd for $C_{33}H_{44}N_2O_{11}Na$ 677.2843).

*4.3.3. Salarin F (**6**).* Bright orange oil; $[\alpha]_D^{26}$ −126 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 235 (3.65), 350 (3.13); IR ($CHCl_3$) ν_{max} 3693, 3420, 3056, 1728, 1602, 1370 cm^{-1}; FABMS *m*/*z* 655.2 $[M-HCl+H]^+$ (70), 691.1 $[M+H]^+$ (50), 713.1 $[M+Na]^+$ (100); HRESIMS (TOF) *m*/*z* 713.2827 $[M+Na]^+$ (calcd for $C_{35}H_{47}ClN_2O_{10}Na$ 713.2816).

*4.3.4. Salarin G (**7**).* Yellow oil; $[\alpha]_D^{26}$ −59 (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 244 (3.85); IR ($CHCl_3$) ν_{max} 3694, 3055, 2988, 1728, 1605, 1435 cm^{-1}; ESIMS (TOF) *m*/*z* 745.3 $[M+Na]^+$ (100), 709.3 $[M-HCl+Na]^+$ (45); HRESIMS (TOF) *m*/*z* 745.2727 $[M+Na]^+$ (calcd for $C_{35}H_{47}ClN_2O_{12}Na$ 745.2715).

*4.3.5. Salarin H (**8**).* Yellow oil; $[\alpha]_D^{26}$ −74 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 253 (3.55), 267 (3.54); IR ($CHCl_3$) ν_{max} 3673, 3348, 3057, 1756, 1720, 1626, 1426 cm^{-1}; FABMS *m*/*z* 727.1 $[M+Na]^+$ (100), 685.1 $[M-C_2H_2O+Na]^+$ (30); HRESIMS (TOF) *m*/*z* 727.3050 $[M+Na]^+$ (calcd for $C_{35}H_{48}N_2O_{13}Na$ 727.3054).

*4.3.6. Salarin I (**9**).* Bright orange oil; $[\alpha]_D^{26}$ +11 (*c* 0.55, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 226 (3.70), 271 (3.74), 350 (3.53); IR ($CHCl_3$) ν_{max} 3693, 3055, 2987, 1721, 1607, 1425, 1382, 1236, 1198, 1068, 923 cm^{-1}; ESIMS (TOF) *m*/*z* 673.3 $[M-H_2O+H]^+$ (35), 695.3 $[M-H_2O+Na]^+$ (100); HRESIMS (TOF) *m*/*z* 673.3338 $[M-H_2O+H]^+$ (calcd for $C_{35}H_{49}N_2O_{11}$ 673.3336).

*4.3.7. Salarin J (**10**).* Yellow oil; $[\alpha]_D^{26}$ −60 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 245 (3.72); IR ($CHCl_3$) ν_{max} 3654, 3342, 3055, 2989, 1722, 1606, 1422 cm^{-1}; HRESIMS (TOF) *m*/*z* 667.2827 $[M+Na]^+$ (calcd for $C_{33}H_{44}N_2O_{11}Na$ 667.2843).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2010.04.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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