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# Structures and cytotoxicities of fascaplysin and related alkaloids from two marine phyla—*Fascaplysinopsis* sponges and *Didemnum* tunicates

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**Abstract**—The structural variations and bioactivity properties of the alkaloids in the fascaplysin (**1**) and the reticulatine (**3**) families were examined. Four organisms were analyzed consisting of two collections of the sponge *Fascaplysinopsis reticulata* and two collections of the tunicate *Didemnum* sp. Reported are the isolation of three new compounds: 3-bromofascaplysin (**2**), 14-bromoreticulatine (**4**), and 14-bromoreticulatate (**6**) along with reticulatate (**5**) previously known as a semi-synthetic product of **1**. Compounds **1** and **5** showed selectivity in a cell based cytotoxicity assay. 

The structural elements of fascaplysin (**1**) ($C_{18}H_{11}N_2O$), a planar fused-pentacyclic alkaloid, continue to provide inspiration for a variety of bioorganic chemistry studies. This compound was first isolated from a sponge, *Fascaplysinopsis* sp.[1] An X-ray diffraction analysis was used in the structure determination because the unfavorable ratio of H/CNO = 0.52 made NMR data ineffective to rule out other reasonable alternatives.[2] While two tautomeric forms are possible for **1** that shown here is favored both in the solid (X-ray) and solution (*N*H $\delta_H$ 11.5 DMSO-$d_6$[1]) state. It is extremely rare to observe an individual compound from multiple organisms in different phyla,[3] yet this circumstance is prominent in the literature for **1**. It has been isolated by five different laboratories from four distinct Thorectidae sponges—*F. reticulata*,[4] *F.* sp.,[1,5] *Hyrtios* cf. *erecta*,[6] and *Thorectandra* sp.;[7] and from two *Didemnum* tunicates.[8] In the course of these prior studies six other analogs were discovered and are divisible into separate structural classes: (i) fascaplysin, (ii) homofascaplysin, and (iii) reticulatine or secofascaplysin. There have been two other recent developments that deserve mention. The first was a biomimetic study[9] of cyclization of a ditryptophan by *N*-alkylation into a 12*H*-pyrido[1,2-*a*;3,4-*b*′]diindole of a fascaplysin or by *C*-alkylation into a 11,12-dihydro-11,12-diazaindeno[2,1-*a*]fluorene present in the tjipanazoles.[10] The second involved isolation of the bacterium *Pseudoalteromonas maricaloris* from *Fascaplysinopsis reticulata*, which in culture produced peptide pigments but not **1**.[11] We now contribute understanding on additional fascaplysin congeners and their properties through a collaborative study of sponges (by the UCSC group) and tunicates (by the OU group).

We used a Kupchan-type solvent partition to obtain fractions of the sponge, *F. reticulata* (UCSC coll. no. 89128 from Fiji and coll. no. 95604 from Indonesia) and tunicate *Didemnum* sp. (OU coll. no. 115T93 from Chuuk Atoll, Federated State of Micronesia and coll. no. 10N95 from Indonesia). Alkaloid containing fractions were quickly identified (LC–MS) whose molecular weights did not correspond to any known fascaplysin derivative. The MeOH (aq.) solvent partition fraction of the Indonesian sponge yielded 3-bromofascaplysin (**2**), 14-bromoreticulatine (**4**), and 14-bromoreticulatate (**6**), while the Fiji specimen gave a MeOH (aq.) solvent partition fraction, which eventually furnished reticulatate (**5**). These compounds proved to be brominated analogs of known reticulatine (**3**).[4] The $CH_2Cl_2$ fractions of the *Didemnum* collections both yielded fascaplysin (**1**) and 3-bromofascaplysin (**2**) (Fig. 1).

*Keywords*: alkaloid; *Didemnum*; fascaplysin; *Fascaplysinopsis*; sponge; tunicate.

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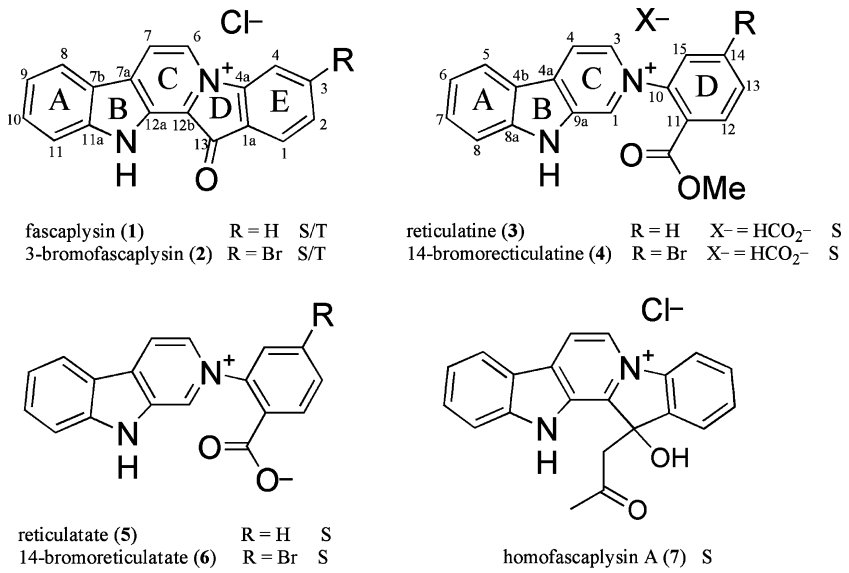


**Figure 1.** Structures of sponge (S) and tunicate (T) origin.

The known compound fascaplysin (**1**, LRFABMS peak at $m/z$ 271 [M$^+$]) was easily dereplicated by comparison of its spectral properties with those in the literature,[1,6] especially assignments recently proposed from extensive 2D NMR analysis.[6] Our treatment of **1** with sodium bicarbonate and acetone afforded a 3:1 mixture of (±)-homofascaplysin A (**7**)[4,6] and **1**. This further confirmed our characterization of **1** and provided additional information about its lability under basic conditions. Characterization of the first new compound obtained, 3-bromofascaplysin (**2**), was largely based on a comparison of its properties to those of **1**. Once the molecular formula of $C_{18}H_{10}BrN_2O$ was established, it was clear **2** differed from **1** only by the loss of H and the addition of Br. Comparison of the $^1$H and $^{13}$C NMR data in MeOH-$d_4$ of **1** (see Supporting Information) with **2**, shown in Table 1, revealed that the separate proton spin systems consisting of H6–H7 and H8–H11 matched well and the same was true for the carbon shifts at C1a, C4a, and C6–C11a. While the Br substituent had to be attached to the E-ring, the next step of the analysis was complicated by the circumstance that in MeOH-$d_4$ the E ring protons occurred as two singlets, $\delta_H$ 7.93 (2H) and 8.66 (1H). Fortunately, in DMSO-$d_6$ these signals were resolved into $\delta_H$ 8.00 ($J$=8.0 Hz, H1), $\delta_H$ 7.96 ($J$=7.9, 1.3 Hz, H2), and $\delta_H$ 8.95 ($J$=1.3 Hz, H4). A NOESY correlation between H4 and H6 along with HMBC data supported the unambiguous assignment of a Br at C3, thereby completing the structure of **2** with the counter ion assumed to be Cl$^-$.

Attention shifted next to the characterization of three sponge-derived reticulatine analogs. The first was 14-bromoreticulatine (**4**) whose molecular formula, $C_{19}H_{14}BrN_2O_2$, was established by a HRMS $m/z$ 381.0221 [M$^+$]. It was immediately clear that an aromatic ring H of reticulatine (**3**), previously isolated by our group,[4] was replaced in **4** by Br. The NMR data (Table 1) of both **3** and **4** were identical except for resonances of the D-ring, as the ABX pattern displayed only one large coupling. This meant the bromine was attached to either C13 or C14. The final assignment of Br at C14 was based on the key HMBC correlation from $\delta_H$ 8.21 ($J$=8.3 Hz, H12) to $\delta_C$ 163.2 (C16) and other expected correlations observed between H12–C10, H15–C10, and H3–C10. The usual chloride counter anion was replaced by a formate, likely derived by exchange during HPLC, as assigned by NMR signals, $\delta_H$ 8.53 bs, $\delta_C$ 166.2. Consistent with our careful approach, the carboxylate precursors to the reticulatines **3** and **4** were also isolated. They included reticulatate (**5**) of formula $C_{18}H_{13}N_2O_2$ based on a HRMS $m/z$ 289.0972 [MH]$^+$ and 14-bromoreticulatate (**6**) of formula $C_{18}H_{12}BrN_2O_2$ based on the HRMS cluster that included $m/z$ 367.0096 [MH]$^+$. Side-by-side comparison of the NMR resonances in Table 1 (assigned from $^1$H and $^{13}$C NMR, and HMBC data) for the *alkaloid* portion of two compound sets—**3** versus **5** and **4** versus **6**—clearly demonstrated that they were acid–ester congeners.

Fascaplysin (**1**) exhibits a broad range of bioactivities including antibacterial, antifungal, antiviral, HIV-1-RT, p56 tyrosine kinase, antimalarial, potency to numerous cancer cell lines,[12,13] specific inhibition of Cdk 4[14] and action as a DNA intercalator.[15] Thus, it was logical to conduct bioactivity screening on the compounds we isolated. Compounds **1**, **2**, **4**–**6** were screened in vitro for solid tumor selectivity against a panel of human and murine tumor cells.[16] The supporting data is shown in Table 2 (also see Table S1 in Supporting Information). Solid tumor selectivity is defined as a differential in kill zone units equal to or greater than 250 between any solid tumor cell line and either a normal or leukemia cell. Bengamide E, a known cytotoxic compound and analog of a compound involved in an anticancer clinical trial,[17] was included as a standard. As expected, fascaplysin (**1**) showed murine solid tumor selectivity and it was the most cytotoxic of the compounds tested.

**Table 1.** NMR data[a] of 13-bromofascaplysin (**2**), reticulatine (**3**), 14-bromoreticulatine (**4**), reticulatate (**5**), and 14-bromoreticulatate (**6**) in MeOH-$d_4$

| Position | **2** | | | | Position | **3** | | **4** | | | **5** | | | **6** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_H$ ($J$ in Hz)[g] | HMBC | | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | HMBC | $\delta_C$ | $\delta_H$ ($J$ in Hz) | HMBC | $\delta_C$ | $\delta_H$ ($J$ in Hz) | HMBC |
| 1a | 132.5 | | | | 1 | 130.5 | 9.37 dd (1.4, 0.6) | 130.0 | 9.41 bs | 10 | 130.0 | 9.21 dd (1.2, 0.6) | 3, 10 | 130.4 | 9.41 bs | 9a, 10 |
| 1 | 127.7 | 7.93 s | 8.00 d (8.1) | 1a, 3, 13 | 3 | 133.7 | 8.59 dd (6.3, 1.3) | 133.5 | 8.60 d (6.4) | 1, 4, 4a, 10 | 133.5 | 8.54 dd (6.4, 1.2) | 4, 4a, 10, 15 | 133.5 | 8.63 dd (6.4, 1.2) | 1, 4, 4a, 10 |
| 2 | 135.8 | 7.93 s | 7.96 dd (7.9, 1.3) | | 4 | 116.6 | 8.75 d (6.3) | 116.5 | 8.76 d (6.3) | 4a, 4b | 116.5 | 8.72 d (6.6) | 9a | 116.5 | 8.77 d (6.6) | 4b, 9a |
| 3 | 124.5 | | | | 4a | 145.3 | | 134.1 | | | 133.6 | | | 134.1 | | |
| 4 | 120.4 | 8.66 s | 8.95 d (1.1) | 1a, 3, 4a | 4b | 119.6 | | 119.4 | | | 119.5 | | | 119.5 | | |
| 4a | 149.5 | | | | 5 | 123.2 | 8.48 ddd (8.1, 1.0, 0.8) | 123.2 | 8.48 d (8.1) | 8a | 123.0 | 8.46 ddd (8.0, 0.9, 0.9) | 7, 8a | 123.2 | 8.50 ddd (8.1, 0.9, 0.9) | |
| 7, 8a | | | | | | | | | | | | | | | | |
| 6 | 127.9 | 9.34 d (6.0) | 9.68 d (6.2) | 4a, 7a, 12b | 6 | 122.1 | 7.51 ddd (8.1, 6.8, 1.4) | 122.1 | 7.51 t (7.4) | 4b, 8 | 121.8 | 7.49 ddd (8.1, 7.0, 1.0) | 4b, 8 | 122.1 | 7.53 ddd (8.1, 7.0, 1.0) | 4b, 8 |
| 7 | 121.0 | 8.93 d (6.0) | 9.22 d (6.2) | 7b, 12a | 7 | 128.0 | 7.80 m | 132.8 | 7.84 m | 5, 8a | 132.3 | 7.84 ddd (8.3, 7.1, 1.2) | 5, 8a | 132.8 | 7.88 ddd (8.4, 7.1, 1.3) | 5, 8a |
| 7a | 123.8 | | | | 8 | 112.8 | 7.80 m | 112.8 | 7.84 m | 4b, 6 | 112.5 | 7.78 ddd (8.5, 0.9, 0.9) | 4b, 6 | 112.7 | 7.82 ddd (8.4, 0.8, 0.8) | 4b, 6 |
| 7b | 121.2 | | | | 8a | 142.9 | | 145.3 | | | 144.8 | | | 145.2 | | |
| 8 | 125.3 | 8.45 d (7.5) | 8.58 d (8.0) | 11a | 9a | 134.5 | | 134.7 | | | 134.8 | | | 134.7 | | |
| 9 | 124.7 | 7.51 t (7.5) | 7.54 dt (7.6, 0.8) | 7b | 10 | 149.1 | | 143.6 | | | 141.6 | | | 143.8 | | |
| 10 | 136.1 | 7.87 t (7.5) | 7.91 dt (7.7, 0.7) | 11a | 11 | 125.9 | | 124.5 | | | 126.0[c] | | | 127.1[e] | | |
| 11 | 114.7 | 7.76 d (7.5) | 7.83 d (8.1) | 7b | 12 | 132.0 | 8.32 dd (8.4, 1.8) | 133.2 | 8.21 d (8.3) | 10, 14, 16 | 130.6 | 8.02 dd (7.4, 1.5) | 10, 16 | 133.5 | 8.25 d (8.4) | 10, 11, 14, 16 |
| 11a | 149.0 | | | | 13 | 131.5[b] | 7.90 m | 134.6 | 8.06 dd (8.4, 1.6) | 11, 14, 15 | 130.2[d] | 7.70 m | | 134.5 | 8.07 dd (8.5, 1.9) | 11, 14, 15 |
| 12a | 132.9 | | | | 14 | 134.1[b] | 7.90 m | 127.6 | | | 126.0[c] | 7.70 m | | 127.1[e] | | |
| 12b | 143.2 | | | | 15 | 132.8[b] | 7.90 m | 131.2 | 8.13 d (1.6) | 10, 11, 13, 14 | 130.2[d] | 7.70 m | | 131.0 | 8.12 d (1.7) | 10, 11, 13, 14 |
| 13 | 182.1 | | | | 16 | 164.0 | | 163.2 | | | 169.8[f] | | | 164.1 | | |
| | | | | | $OCH_3$ | 51.9 | 3.66 s | 51.9 | 3.66 s | 16 | | | | | | |

[a] Measured at 500 MHz ($^1$H) and 125 MHz ($^{13}$C), data for **3** from Ref. 4. [b,c,d,e] Assignments can be switched. [f] Shift from HMBC correlation. [g] Recorded in DMSO-$d_6$.

**Table 2.** Zone units differentials in the disk diffusion soft agar colony formation assay[a]

| Compound | Conc. (μg/disk) | Murine tumor selectivity | | Human tumor selectivity |
|---|---|---|---|---|
| | | $Z_{C38}$–$Z_{L1210}$ | $Z_{C38}$–$Z_{CFU(GM)}$ | $Z_{H116\backslash H125}$–$Z_{CEM}$ |
| Bengamide E (standard) | 7.5 | 250 | 150 | 50\300 |
| Fascaplysin (**1**) | 0.4 | 100 | 300 | 200\100 |
| 3-Bromofascaplysin (**2**) | 6.4 | −150 | 0 | 200\150 |
| 14-Bromoreticulatine (**4**) | 200 | 50 | 150 | –\– |
| Reticulatate (**5**) | 60 | 50 | 550 | 300\150 |
| 14-Bromoreticulatate (**6**) | 60 | 150 | – | –\– |

[a] Measured in zone units: 200 zone units = 6 mm. Murine cell lines: L1210 (lymphocytic leukemia), C38 (colon adenocarcinoma), CFU(GM) (colony-forming unit-granulocyte macrophage; normal hematopoietic); Human cell lines: H116 (colon), H125 (lung), CEM (leukemia), CFU(GM) (colony-forming unit-granulocyte macrophage; normal hematopoietic).

The analog, 3-bromofascapysin (**2**) was less cytotoxic and did not retain the selectivity observed for **1**. Another significant result of Table 2 is that reticulatate (**5**) demonstrated excellent specificity against both murine and human cell lines as seen in the selectivity differences for the murine C38 versus the CFU cell lines and for the human H116 versus the CEM cell lines.

A striking aspect of this study was our ability to conduct a parallel examination of sponge and tunicate specimens both possessing compounds **1** and **2**. Interestingly, none of the ring-opened compounds such as **3**–**6** isolated here from the sponges were observed in the tunicate crude extracts. These compounds **3**–**6** cannot be artifacts as they were observed by LC–MS runs of the crude extracts prior to chromatographic isolation. Current work is aimed at clarifying the biosynthetic factors responsible for these differences because base mediated ring opening of **1**, employing a 5 M NaOH/$CH_3OH$ solution to produce **5**,[18] can not operate in nature. Alternatively, a non-enzymatic condensation reaction involving **1** and a nucleophile may afford racemic homofascaplysin A (**7**), as noted above and indicated by a re-examination of a natural sample ($Cl^-$ anion) from our repository whose $[\alpha]_D^{20} = 0°$. We are currently investigating additional mono- and dibrominated compounds of tryptophan origin[19] from both tunicates and sponges available in our repositories. The halogenation pattern in the compounds reported here is quite distinct from those observed when **1** undergoes electrophilic halogenation.[20] These considerations as well as other bioactivity results will be reported in due course.

## Supporting Information

Supplementary material is available from the author upon request.

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