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Suberitine A–D, Four New Cytotoxic Dimeric Aaptamine Alkaloids from the Marine Sponge *Aaptos suberitoides*

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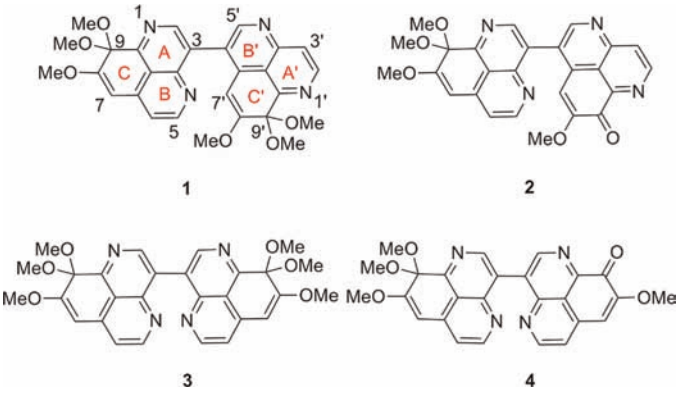
ABSTRACT



Suberitine A–D (1–4), four new bis-aaptamine alkaloids with two aaptamine skeleton units, 8,9,9-trimethoxy-9*H*-benzo[*de*][1,6]-naphthyridine and demethyl(oxy)-aaptamine, linked through a rare C-3–C-3′ or C-3–C-6′ σ-bond between the 1,6-naphthyridine rings, together with two known monomers 5 and 6, were isolated from the marine sponge *Aaptos suberitoides*. Their structures were elucidated using NMR spectroscopy. Compounds 2 and 4 showed potent cytotoxicity against P388 cell lines, with IC_{50} values of 1.8 and 3.5 μM, respectively.

Marine sponges represent an important and vast resource for the discovery of marine alkaloids. Such alkaloids have generated much interest as a result of their varied, often unusual, pharmacological activities, and because of the challenging problems presented by their structure elucidation and synthesis.[1] The aaptamines, which have an unusual benzo[*de*][1,6]-naphthyridine framework, are a class of typical marine alkaloids from sponges. The first and most representative member of this family, reported in 1982 by Nakamura et al.,[2] was obtained from the marine sponge *Aaptos aaptos*, collected off the shores of Okinawa, Japan. To date, this type of alkaloid has only been found in members of the Demospongiae class of sponges, including the genus *Xestospongia* (Haplosclerida, Petrosiidae), *Suberites* (Hadromerida, Suberitidae), *Luffariella* (Dictyoceratida, Thorectidae), *Hymeniacidon* (Halichondrida, Halichondriidae), and *Aaptos* (Hadromerida, Suberitidae).[3] In particular, the genus *Aaptos* has been found to be an abundant source of aaptamine alkaloids. Moreover, most aaptamines exhibit prominent and diverse bioactivities such as antiviral,[4,5]

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antimicrobial,[6] cytotoxic,[6,7] sortase A inhibitory,[8] and antidepressant activities.[9]

During our search for new bioactive compounds from marine organisms, we encountered the marine sponge *Aaptos suberitoides*, collected from the Xisha islands in the South China Sea (NaiHai). A combination of bioassay- and structure-guided isolation yielded four new dimeric aaptamine alkaloids, suberitine A−D (**1**−**4**), together with the two known monomers **5** and **6**.[6,10] These unusual compounds, structurally linked two aaptamine units through a rare C-3−C-3′ or C-3−C-6′ σ-bond between the aromatic 1,6-naphthyridine rings, were the first examples of aaptamine dimers obtained in nature and represent new members of the aaptamine family, although dozens of aaptamine alkaloids had been found in marine sponge in past studies.[3] So far, only two analogs, lihouidine from the marine sponge *Suberea* n. sp. (Aplysinellidae, Verongida)[3,11] and 4,4′-binecatorone from fruiting bodies of *Lectarius necator*,[12] which contain two modified aaptamine structural units joined by benzene ring moieties, have been reported. In this paper, we describe the isolation, structure elucidation, and cytotoxicities of the new compounds.

A frozen specimen (2.0 kg, wet weight) was homogenized and then extracted with MeOH. The crude methanolic extract was subjected to silica gel column chromatography eluted with a gradient of petroleum ether/acetone (from 10:0 to 0:10, v/v), giving 10 fractions (P-1−P-10). Using a combination of bio- and structure-guided isolation methods, each fraction was detected by TLC and HPLC and tested for cytotoxicity against P388, HeLa, and K562 cell lines. The aaptamine alkaloids were in the most bioactive fractions, P-6 and P-7 (Supporting Information). The P-7 fraction was subsequently separated by silica gel column chromatography, ODS column chromatography, and semipreparative HPLC to afford four new bis-aaptamine alkaloids **1**−**4**. Two known monomeric aaptamines, 8,9,9-trimethoxy-9*H*-benzo[*de*][1,6]-naphthyridine (**5**) and demethyl(oxy)-aaptamine (**6**), were also obtained from the P-6 fraction.

Suberitine A (**1**),[13] was obtained as a bright yellow gum. The molecular formula was determined as $C_{28}H_{26}N_4O_6$ by HRESIMS at m/z 515.1922 $[M + H]^+$ (calcd 515.1931), requiring eighteen degrees of unsaturation. The IR absorption bands at 1640, 1563, 1543, 1273, and 1199 cm^{-1}, together with the UV absorptions at 206, 234, and 384 nm, were strongly characteristic of the spectra of aaptamine alkaloids.[6]

In the ^1H NMR spectra of **1** (Table 1), four singlet proton signals at δ_H 9.02, 8.99, 6.57, and 6.05, two sets of coupled doublet proton signals at δ_H 8.92 (d, J = 4.4 Hz) and 7.49 (d, J = 4.4 Hz), and 8.97 (d, J = 5.5 Hz) and 8.05 (d, J = 5.5 Hz), and six methoxy group proton signals at δ_H 4.05, 3.68, 3.35, 3.26, 3.23, and 3.18 were observed, implying the presence of two benzo[*de*][1,6]-naphthyridine skeleton units in **1**. The ^{13}C NMR (DEPT) spectrum (Table 1) with 28 carbon resonances, including six methoxy signals, eight methine signals, and fourteen quaternary carbon signals, further confirmed this arrangement.

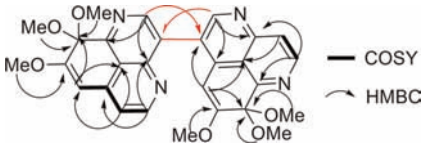

Figure 1. Key COSY and HMBC correlations for suberitine A (**1**).

A detailed examination of the ^1H NMR spectra of **5** and **6** revealed that the coupling constants of H-2 and H-3 in the A ring were usually around 5.5 Hz, whereas they were around 4.4 Hz for H-5 and H-6 in the B ring (Table 1 and Supporting Information). The linkage of the two benzo[*de*][1,6]-naphthyridine skeleton units in **1** was therefore deduced to occur at rings A and B, respectively, in two aaptamine units, and both the aaptamine units in **1** were assigned as 8,9,9-trimethoxy-9*H*-benzo[*de*][1,6]-naphthyridine (**5**), because of the occurrence of six methoxy groups, and the HMBC (Table 1 and Supporting Information) correlations from 9-OMe (δ_H 3.26, 3.35) to C-9 (δ_C 97.7) and from 9′-OMe (δ_H 3.18, 3.23) to C-9′ (δ_C 97.6). Finally, the ^3J correlations in the HMBC of H-2 (δ_H 8.99) to C-6′ (δ_C 126.5) and H-5′ (δ_H 9.02) to C-3 (δ_C 130.7) unambiguously established the structure of **1**, as shown in Figure 1. In addition, the absence of optical activity for **1** excluded the possibility of axial chirality.

Suberitine B (**2**)[14] is a yellow gum. Its molecular formula $C_{26}H_{20}N_4O_5$ was obtained from HRESIMS of the protonated molecular ion $[M + H]^+$ at m/z 469.1495. The IR and UV spectra of compound **2** were similar to those of aaptamine alkaloids.[6] The ^1H NMR spectra of **2** (Table 1 and Supporting Information) exhibited the unique features of aaptamine dimers with four singlet proton signals and two sets of coupled doublet proton signals, indicating that **2** should possess a carbon skeleton similar to that of **1**. However, the carbon resonance at δ_C 177.8 (C-9′) revealed the presence of one carbonyl group in **2**, which is obviously

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(13) Suberitine A (**1**): bright yellow gum; UV (MeOH) λ_{max} (log ε) 206 (3.87), 234 (4.03), 384 (3.14) nm; IR (KBr) ν_{max} 1640, 1563, 1543, 1273, and 1199 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 515 ($[M + H]^+$); HRESIMS m/z 515.1922 ($[M + H]^+$) (calcd for $C_{28}H_{27}N_4O_6$, 515.1931).

(14) Suberitine B (**2**): yellow gum; UV (MeOH) λ_{max} (log ε) 209 (3.88), 232 (3.75), 373 (3.11) nm; IR (KBr) ν_{max} 2949, 2838, 1688, 1648, 1387, 1257, and 1098 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 469 ($[M + H]^+$); HRESIMS m/z 469.1495 ($[M + H]^+$) (calcd for $C_{26}H_{21}N_4O_5$, 469.1506).

Table 1. NMR Data for Suberitine A–D (**1**–**4**)

	1[a,c]		**2**[b,d]		**3**[a,c]		**4**[b,d]	
no.	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	149.4	8.99 s	148.7	9.05 s	149.3	8.98 s	149.4	9.13 s
3	130.7		128.6		130.9		128.4	
3a	149.1		148.0		149.6		148.4	
5	158.2	8.92 d (4.4)	156.6	8.96 d (4.4)	157.6	8.78 d (4.4)	156.1	8.85 d (4.4)
6	120.1	7.49 d (4.4)	118.4	7.29 d (4.4)	119.9	7.45 d (4.4)	117.9	7.20 d (4.4)
6a	141.8		139.7		141.7		139.2	
7	101.5	6.57 s	99.3	6.23 s	101.9	6.59 s	99.6	6.21 s
8	163.2		162.5		162.9		161.9	
9	97.7		97.0		98.1		96.4	
9a	158.2		157.1		157.7		155.9	
9b	118.1		116.3		118.3		116.5	
OMe-8	56.8	4.05 s	56.2	4.06 s	56.8	4.05 s	56.2	4.03 s
OMe-9	52.6	3.26 s	52.3	3.40 s	52.5	3.28 s	52.2	3.41 s
	52.7	3.35 s	52.3	3.52 s	52.5	3.28 s	52.2	3.41 s
2′	148.5	8.97 d (5.5)	149.5	9.24 d (5.5)	149.3	8.98 s	151.7	9.35 s
3′	123.5	8.05 d (5.5)	127.0	8.26 d (5.5)	130.9		134.2	
3a′	149.9		149.1		149.6		147.2	
5′	159.8	9.02 s	159.2	9.17 s	157.6	8.78 d (4.4)	156.3	9.01 d (4.4)
6′	126.5		129.4		119.9	7.45 d (4.4)	121.5	7.48 d (4.4)
6a′	139.5		134.6		141.7		136.8	
7′	99.6	6.05 s	106.6	6.58 s	101.9	6.59 s	108.3	6.76 s
8′	163.3		156.1		162.9		156.3	
9′	97.6		177.8		98.1		177.8	
9a′	157.2		158.2		157.7		148.4	
9b′	118.3		118.0		118.3		118.2	
OMe-8′	56.4	3.68 s	56.0	3.72 s	56.8	4.05 s	56.2	4.04 s
OMe-9′	52.5	3.18 s			52.5	3.28 s		
	52.6	3.23 s			52.5	3.28 s		

[a] Measured at 600 MHz (^{1}H) in CD_3OD. [b] Measured at 600 MHz (^{1}H) in $CDCl_3$. [c] Measured at 150 MHz (^{13}C) in CD_3OD. [d] Measured at 150 MHz (^{13}C) in $CDCl_3$.

different from **1**. In further 1D and 2D NMR spectral analyses, the HMBC correlations from 9-OMe (δ_H 3.40, 3.52), two of the four methoxy groups, to C-9 (δ_C 97.0), from H-7 (δ_H 6.23) to C-9, and from H-7′ (δ_H 6.58) to C-9′ (δ_C177.8) suggested that **2** is comprised of 8,9,9-trimethoxy-9*H*-benzo[*de*][1,6]-naphthyridine (**5**) and demethyl(oxy)-aaptamine (**6**). The remaining problem, the junction of the two aaptamine residues, was determined to be through a C-3–C-6′ σ-bond as in **1**, based on the key HMBC correlations from the characteristic singlet imino proton H-2 (δ_H 9.05) to the quaternary carbon C-6′ (δ_C 129.4) and from H-5′ (δ_H 9.17) to the quaternary carbon C-3 (δ_C 128.6).

Suberitine C (**3**)[15] is a bright yellow gum, and HRESIMS gave the same molecular formula, $C_{28}H_{26}N_4O_6$, as that of **1**. The ^{1}H and ^{13}C NMR signals of **3** (Table 1) were very similar to those of **5**, indicating **3** to be a symmetrically dimeric structure of two 8,9,9-trimethoxy-9*H*-benzo[*de*]-[1,6]-naphthyridine units (**5**). Furthermore, the two imino singlet proton signals at δ_H 8.98 (H-2 and H-2′), and the coupling constant values of two sets of coupled doublet proton signals at δ_H 8.78 (2H, d, J = 4.4 Hz, H-5 and H-5′) and 7.45 (2H, d, J = 4.4 Hz, H-6 and H-6′), also showed that the two aaptamine residues were linked by a C-3–C-3′ σ-bond, unlike compounds **1** and **2**. HRESIMS showed that suberitine D (**4**),[16] a yellow gum, had the same molecular formula, $C_{26}H_{20}N_4O_5$, as **2**. The 1D NMR data were almost identical to those for **2**, suggesting **4** was an isomer of **2**. In the ^{1}H NMR spectra of **4** (Table 1), two imino singlet proton signals at δ_H 9.13 (H-2) and δ_H 9.35 (H-2′) and two sets of coupled doublet proton signals at δ_H 8.85 (d, J = 4.4 Hz) and 7.20 (d, J = 4.4 Hz), 9.01 (d, J = 4.4 Hz) and 7.48 (d, J = 4.4 Hz), respectively, as well as the crucial HMBC correlations from H-2 (δ_H 9.13) to C-3′ (δ_C 134.2) and from H-2′ (δ_H 9.35) to C-3 (δ_C 128.4), could reasonably establish the structure of **4**.

Suberitine **A**–**D** (**1**–**4**) were evaluated for their cytotoxicities against P388, HeLa, and K562 cell lines using the MTT method.[17] Compounds **2** and **4**, with a carbonyl

(15) Suberitine C (**3**): bright yellow gum; UV (MeOH) λ_{max} (log ε) 204 (3.85), 233 (4.05), 389 (3.05) nm; IR (KBr) ν_{max} 1664, 1580, 1565, 1293, 1220, and 1185 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 515 ([M + H]$^+$); HRESIMS *m*/*z* 515.1938 ([M + H]$^+$) (calcd for $C_{28}H_{27}N_4O_6$, 515.1931).

(16) Suberitine D (**4**): yellow gum; UV (MeOH) λ_{max} (log ε) 205 (3.89), 230 (3.77), 367 (3.13) nm; IR (KBr) ν_{max} 2955, 2839, 1698, 1650, 1457, 1398, 1268, and 1017 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 469 ([M + H]$^+$); HRESIMS *m*/*z* 469.1504 ([M + H]$^+$) (calcd for $C_{26}H_{21}N_4O_5$, 469.1506).

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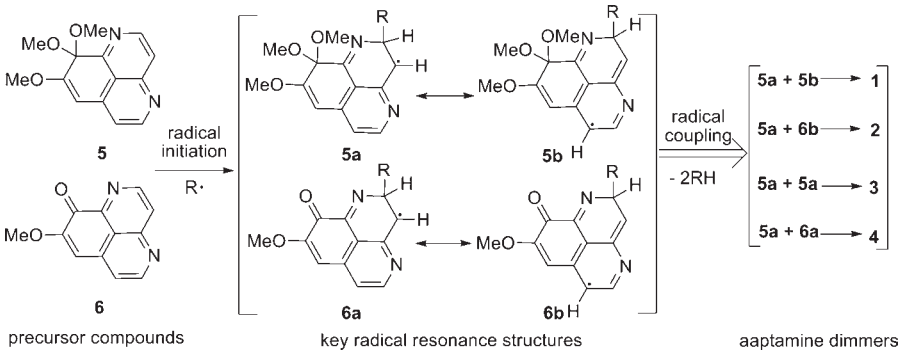

Figure 2. Proposed biogenesis pathway of dimeric aaptamine alkaloids, suberitine A–D (**1**–**4**).

group at C-9 or C-9′, showed more potent cytotoxicities than the monomers **5** and **6** did against the selected P388 cell line, with IC_{50} values of 1.8 and 3.5 μM, respectively (Supporting Information). Compounds **1** and **3** were inactive against the same cell line (inhibiton rate < 50% at 10 μM). None of the dimers (**1**–**4**) or their monomers (**5** and **6**) showed cytotoxicity activities against the HeLa and K562 cell lines (inhibiton rate < 50% at 10 μM).

As there was some doubt as to whether these dimeric compounds were natural or artificial products, a mixture of 8,9,9-trimethoxy-9*H*-benzo[*de*][1,6]-naphthyridine (**5**) and demethyl(oxy)-aaptamine (**6**) was dissolved in MeOH with the presence of silica gel and the mixture was stirred at 40 °C for 48 h (Supporting Information). HPLC did not identify the presence of any dimeric compounds as reaction products, suggesting that the four dimers are natural rather than artificial products.

An interesting topic worthy of further consideration in this context is why coupling only occurred at C-3 or C-6 in the aaptamine monomers. From a chemical viewpoint, this is easy to understand because the stable resonance structures of the aaptamine monomers are consistent with these coupling positions. It was deduced that a plausible biogenetic pathway for these unusual aaptamine dimers in vivo is a free-radical coupling mechanism, as shown in Figure 2.

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Supporting Information Available. Experimental procedure; HRESIMS, 1D NMR, 2D NMR spectra of **1**–**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.