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Aplysinellamides A−C, Bromotyrosine-Derived Metabolites from an Australian *Aplysinella* sp. Marine Sponge

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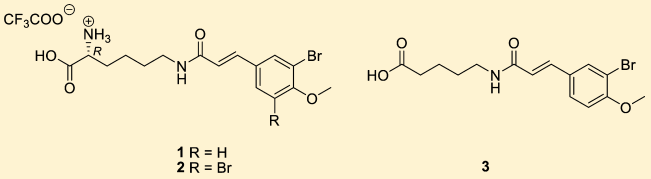
Supporting Information

ABSTRACT: Mass-directed fractionation of an extract from the Australian marine sponge *Aplysinella* sp., from the Great Barrier Reef, resulted in the isolation of four new bromotyrosine derivatives, aplysinellamides A−C (**1**−**3**) and aplysamine-1-*N*-oxide (**4**), along with six known compounds (**5**−**10**). The structure elucidation of compounds **1**−**4** was based on their 1D and 2D NMR and MS spectroscopic data. Aplysamine-1 (**6**) increased the apolipoprotein E secretion from human CCF-STTG1 astrocytoma cells by 2-fold at the concentration of 30 μM.



Alzheimer's disease (AD) has emerged as the most prevalent form of late-life mental disorder in humans and is typified by deposition of β-amyloid (Aβ) within the brain.[1] Aβ is a neurotoxic peptide, and the accumulation of Aβ leads to its deposition into plaques and the launching of a pathologic cascade that ultimately leads to neuronal death.[1] An impaired ability to clear Aβ peptides from the brain, rather than increasing production of Aβ peptides, is thought to underlie most cases of late-onset AD.[2]

Apolipoprotein E (ApoE) is the major apolipoprotein in the central nervous system and plays a central role in cholesterol transport.[3] In the brain, ApoE is mostly produced by astrocytes, and some of this ApoE binds to specific neuronal receptors for cholesterol uptake.[3] A previous study suggested that increased levels of highly lipidated ApoE could facilitate the clearance of Aβ peptides,[4,5] implying that the interference of ApoE secretion could potentially lead to the treatment of AD. Bexarotene is the only reported compound to date that activates ApoE expression.[6]

In our ongoing search for new lead compounds for neurodegenerative disorders, a drug discovery program was initiated to identify natural products as ApoE modulators. A high-throughput screening method was developed and used to screen a prefractionated natural product library of 102 432 fractions for their ability to modulate ApoE secretion from CCF-STTG1 cells. From 11 fractions derived from an extract of the Australian sponge *Aplysinella* sp., a single active fraction was identified with activity against ApoE. (+)-LRESIMS showed molecular ions at *m*/*z* 204/205/206, 212/213/214, 322/324/326, and 316/318 within the active fraction, and MS was used to guide large-scale isolation. Twenty grams of sponge material was then extracted by CH_2Cl_2 and MeOH. Mass-directed fractionation and purification of the combined CH_2Cl_2/MeOH extract led to the isolation of 10 bromotyrosine derivatives, including four new compounds, aplysinellamides A−C (**1**−**3**) and aplysamine-1-*N*-oxide (**4**), together with six known natural products, suberedamine A (**5**),[7] aplysamine-1 (**6**),[8] purealidine G (**7**),[9] 2,6-dibromo-4-[2-(dimethylamino)ethyl]phenol (**8**),[10] *N*,*N*,*N*-trimethyl-3-bromo-4-methoxytyrosine (**9**),[11] and 3,5-dibromo-4-methoxybenzoic acid (**10**).[12] Given solvents containing 0.1% TFA were used for the isolation, compounds **1**, **2**, **4**, **5**, **6**, **7**, **8**, and **9** were isolated as TFA salts. Herein we report the isolation and structure elucidation of aplysinellamides A−C (**1**−**3**) and aplysamine-1-*N*-oxide (**4**), as well as the ApoE modulation activities of compounds **1**−**10**.

RESULTS AND DISCUSSION

The freeze-dried and ground marine sponge (20.0 g) was sequentially extracted with *n*-hexane, CH_2Cl_2, and MeOH. The CH_2Cl_2 and MeOH extracts were combined and chromatographed using reverse phase C_{18}-bonded silica HPLC (gradient MeOH/H_2O/0.1% TFA) to yield 60 fractions. Fractions 22−27 contained the ions of interest (see above). Purification of these fractions using a Luna C_{18} semipreparative HPLC column (gradient MeOH/H_2O/0.1% TFA) yielded a new compound, aplysamine-1-*N*-oxide (**4**, 0.5 mg), and four known compounds, aplysamine-1 (**6**, 0.6 mg), purealidine G (**7**, 0.3 mg), 2,6-dibromo-4-[2-(dimethylamino)ethyl]phenol (**8**, 0.2 mg), and *N*,*N*,*N*-trimethyl-3-bromo-4-methoxytyrosine (**9**, 0.5 mg).

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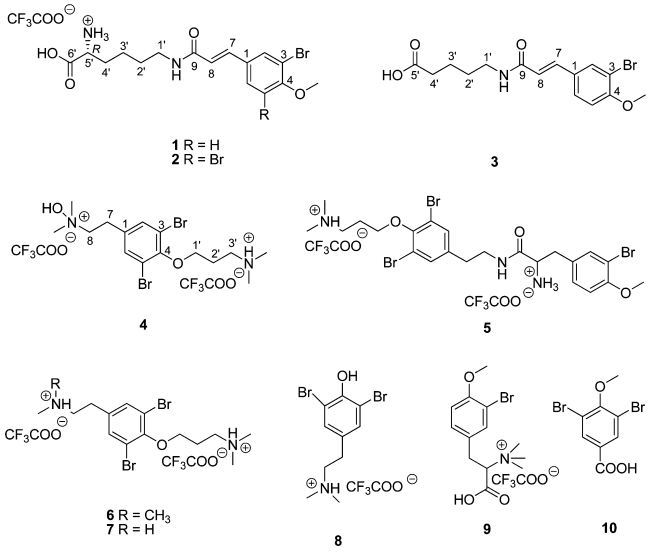

Mass analysis of other HPLC fractions indicated the presence of brominated metabolites. Further purifications of these fractions using a Luna C_{18} semipreparative HPLC column (gradient MeOH/H_2O/0.1% TFA) yielded three more new compounds, aplysinellamide A (**1**, 0.4 mg), aplysinellamide B (**2**, 0.3 mg), and aplysinellamide C (**3**, 0.5 mg), along with two known compounds, suberedamine A (**5**, 0.3 mg) and 3,5-dibromo-4-methoxybenzoic acid (**10**, 0.8 mg).

Aplysinellamide A (**1**) was obtained as a pale amorphous powder. The cluster of two isotopic ions at *m/z* 385/387 (1:1) in the (+)-LRESIMS spectrum indicated the presence of one bromine atom in the molecule. The molecular formula, $C_{16}H_{21}BrN_2O_4$, was deduced on the basis of HRESIMS measurement, with seven degrees of unsaturation. The ^{1}H and HSQC NMR data suggested that compound **1** contained three aromatic protons (δ_H 7.76, d, J = 2.1 Hz, 7.50, dd, J = 8.6, 2.1 Hz, and 7.05, d, J = 8.6 Hz), one isolated pair of *trans* olefins (δ_H 7.42, d, J = 15.7 Hz, and 6.46, d, J = 15.7 Hz), one methoxy (δ_H 3.91, s), one methine (δ_H 3.91, m), and several methylenes in the upfield region. The COSY correlation data established a 1,3,4-trisubstituted benzene moiety and a substituted aliphatic pentyl group. The proton and carbon chemical shifts of the methylene (δ_H 3.31 m, δ_C 38.5) and the methine (δ_H 3.91 m, δ_C 52.6) in the pentyl subunit suggested that a nitrogen was attached at the 1- and 5-positions of the subunit, respectively. HMBC correlations from the methylene (δ_H 1.97/1.90, H-4′) and methine (δ_H 3.91, H-5′) in the pentyl functionality to a carboxyl carbon (δ_C 170.6) suggested that the methine group in the pentyl group was connected to a carboxyl group. Thus, a lysine moiety was assigned. In addition, HMBC correlations from the olefinic proton (δ_H 7.42) to the aromatic carbons (δ_C 128.5, 128.6, and 131.6) and the carbonyl carbon (δ_C 167.3) (Figure 1) indicated that an α,β-unsaturated carbonyl group was linked to the 1,3,4-trisubstituted aromatic ring, thus forming a cinnamoyl-derived moiety. The lysine moiety was connected to the substituted cinnamoyl moiety through an amide bond by the HMBC correlations from the methylene protons (δ_H 3.31) in lysine to the carbonyl carbon C-9 (δ_C 167.3). Finally the substitution of a methoxy group on the benzene ring C-4 position was established by the HMBC correlations from the aromatic protons H-2/H-5/H-6 and the methoxy protons (δ_H 3.91) to the aromatic carbon (δ_C 157.3); thus the bromine atom was assigned to aromatic carbon C-3.

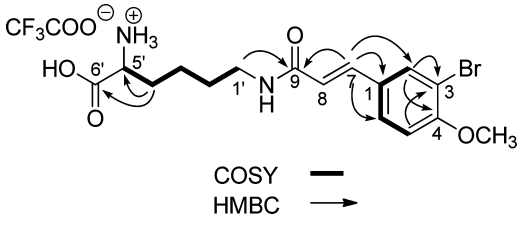

Figure 1. Key COSY and HMBC correlations for compound **1**.

The absolute configuration of the lysine moiety in aplysinellamide A (**1**) was determined by Marfey's method.[13] Acid hydrolysis of **1**, followed by derivatization with Marfey's reagent and LC-MS analysis, showed a peak with a retention time of 10.6 min, suggesting a D-lysine in the molecule (standard D-lysine derivative had a retention time of 10.5 min, while the L-lysine derivative eluted at 9.8 min). The structure of aplysinellamide A was therefore established as **1**.

Aplysinellamide B (**2**) exhibited a cluster of isotopic ions at *m/z* 463/465/467 (1:2:1) in the (+)-LRESIMS spectrum, indicating the presence of two bromine atoms in the molecule. The molecular formula, $C_{16}H_{20}Br_2N_2O_4$, was deduced by HRESIMS, with seven degrees of unsaturation. The ^{1}H, HSQC, and COSY NMR data of compound **2** were very similar to those of **1**, except that compound **2** had a two-proton singlet (δ_H 7.79, 2H), representing a symmetrical benzene moiety. Both the HSQC and HMBC correlations were observed from the aromatic proton (δ_H 7.79) to the carbon (δ_C 131.3), confirming a 3,5-dibromo-4-methoxybenzene ring system in the molecule. The structure of aplysinellamide B was therefore elucidated as **2**. Acid hydrolysis followed by derivatization with Marfey's reagent was carried out in an attempt to determine the absolute configuration of the lysine residue in **2**. However, LC-MS failed to detect any lysine derivative, maybe due to the low quantity of compound **2**. We propose that compound **2** has a D-lysine in the molecule based on its shared biogenesis with **1**.

Aplysinellamide C (**3**) was isolated as brown, amorphous powder. (+)-LRESIMS showed an isotopic ion cluster at *m/z* 356/358 (1:1), indicating the presence of one bromine atom in the molecule. The molecular formula of **3** was determined to be $C_{15}H_{18}BrNO_4$, on the basis of HRESIMS with seven degrees of unsaturation. The ^{1}H, HSQC, and COSY NMR data of compound **3** were also very similar to those of **1**; the difference was the absence of an amino methine in the aliphatic chain. HMBC correlations were observed from the methylene protons (δ_H 1.66 and 2.34) in the aliphatic chain to a carboxyl carbon (δ_C 176.0), indicating that the lysine functionality in **1** and **2** was replaced by a 5-aminopentanoic acid in **3**. Hence, aplysinellamide C was assigned to structure **3**.

Aplysamine-1-*N*-oxide (**4**) was isolated as a pale, amorphous powder. (+)-LRESIMS showed a cluster of isotopic ions at *m/z* 423/425/427 (1:2:1) and 212/213/214 (1:2:1), indicating the presence of two bromine atoms in the molecule. The molecular formula of **4** was determined to be $C_{15}H_{24}Br_2N_2O_2$ based on a HRESIMS measurement, with four degrees of unsaturation. ^{1}H, COSY, and HSQC NMR data suggested that compound **4** had similar structural feature to that of the known compound aplysamine-1 (**6**),[8] including a symmetrical tetrasubstituted benzene ring, two isolated *N*-methyl protons (δ 3.44, s, 6H and δ 2.81, s, 6H), and substituted ethyl and propyl groups. The obvious differences between **4** and **6** were that the proton chemical shifts of H-8 (δ 3.81, m, 2H) and one set of *N*-methyl groups (δ 3.44, m, 6H) in **4** were downfield shifted in

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comparison with corresponding signals in aplysamine-1 (**6**). These shifts were due to the formation of an *N*-oxide, thus the inductive effect of the positively charged nitrogen atom. This was supported by an uncounted oxygen atom in the molecule and the HMBC correlation from methyl protons (δ 3.44, s) to C-8 (δ 68.5). Hence, the structure of aplysamine-1-*N*-oxide was assigned as **4**.

Six known natural products, suberedamine A (**5**), aplysamine-1 (**6**), purealidine G (**7**), 2,6-dibromo-4-[2-(dimethylamino)ethyl]phenol (**8**), *N*,*N*,*N*-trimethyl-3-bromo-4-methoxytyrosine (**9**), and 3,5-dibromo-4-methoxybenzoic acid (**10**), were also isolated from the Australian sponge *Aplysinella* sp. Their ^{1}H and ^{13}C NMR data were identical to those reported in the literature.

Compounds **1**−**10** were evaluated for their ability to modulate ApoE secretion. Of the tested compounds, aplysamine-1 (**6**) increased ApoE secretion by 2-fold at the concentration of 30 μM (Figure 2). All of the other compounds showed no significant activity at concentrations of up to 40 μM (data not shown).

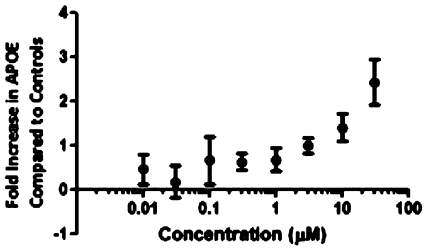

Figure 2. Activity of aplysamine-1 (**6**) in the ApoE modulation assay.

Marine sponges of the order Verongida have proven to be a remarkable source of chemically diverse bromotyrosine derivatives.[14] The chemical diversity of this structural class arises from the degree of bromination of the tyrosine moiety, as well as the subsequent oxidation, reduction, ring-opening, and rearrangement. Four sponge families from the order of Verongida, Aplysinidae, Aplysinellidae, Ianthellidae, and Pseudoceratinidae have been frequently reported to contain bromotyrosine derivatives.[15] Our study together with literature data suggests that this unique structural feature could be considered as a marker for chemotaxonomic identification of the Verongida sponges.

In conclusion, 10 bromotyrosine derivatives (**1**−**10**) were isolated from the Australian marine sponge *Aplysinella* sp., from the southern Great Barrier Reef, including the new compounds aplysinellamides A−C (**1**−**3**) and aplysamine-1-*N*-oxide (**4**). Aplysamine-1 (**6**) showed ApoE-modulating activity, increasing ApoE secretion from human astrocytoma cells by 2-fold at the concentration of 30 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a HORIBA SEPA-300 high-sensitivity polarimeter. UV spectra were recorded on a Jasco V650 UV/vis spectrophotometer. NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA spectrometer equipped with a triple resonance cryoprobe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for CD_3OD at δ_H 3.31 and δ_C 49.5 or DMSO-d_6 at δ_H 2.50 and δ_C 39.5. LRESIMS spectra were recorded on a Waters ZQ mass spectrometer. HRESIMS spectra were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. Alltech Davisil 40−60 μm Å C_{18} bonded silica was used for flash chromatography. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler was used for HPLC. A Thermo Scientific C_{18} Betasil 5 μm 143 Å column (21.2 mm × 150 mm) and a Phenomenex Luna C_{18} 5 μm 143 Å column (21.2 mm × 250 mm) were used for semipreparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered. A BIOLINE orbital shaker was used for the large-scale extraction of the sponge material.

Animal Material. The sponge *Aplysinella* sp. (family Aplysinellidae) was collected from Sykes Reef, Capricorn-Bunker Group, Great Barrier Reef, Queensland, Australia, latitude −23.41888889°, longitude 152.0505556°, depth 25 m. The sponge was kept frozen prior to freeze-drying and extraction. A voucher specimen (G307202) has been lodged at the Queensland Museum, Brisbane, Australia. A description and images of this species (as *Aplysinella* sp. OUT QM1194) are available from the following link (http://www.spongemaps.org/#!search-for-sponges-public/c16m4) using the search tool for Mudmap Number inserting the text "QM1194".

Detailed description of the species is as follow: it has a massive, digitate, encrusting growth form. In life its color is dark gray in the upper exterior, yellow in the lower exterior and with a yellow interior. The sponge is aerophobic such that when removed from seawater its pigments turn black. Oscules are large, on apex of digits, with some specimens known to have a large stalked central osculum and smaller oscules on the smaller digits. Sponge texture is firm and difficult to tear. The surface is conulose, with a tracery of minute conules in between large surface conules, superficially resembling species of *Dysidea*. The ectosome is membranous, heavily collagenous, and slightly arenaceous externally. The choanosomal skeleton consists of spongin fibers that are very heavy, concentrically stratified but lightly pithed, and form very wide meshes producing an irregular reticulation. The mesohyl collagen is also very heavy. There is no mineral skeleton.

Extraction and Isolation. The freeze-dried and ground sponge (20.0 g) was poured into a conical flask (1 L), *n*-hexane (500 mL) was added, and the flask was shaken at 200 rpm for 2 h. The *n*-hexane fraction was filtered under vacuum and then discarded. CH_2Cl_2/MeOH (4:1, 500 mL) was added to the defatted marine sample in the conical flask and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity and set aside. MeOH (500 mL) was added, and the MeOH/sponge mixture was shaken for a further 2 h at 200 rpm. Following filtration the sponge sample was extracted with another volume of MeOH (500 mL), while being shaken at 200 rpm for another 2 h. All CH_2Cl_2/MeOH extractions were combined and dried under vacuum to yield a dark brown solid (3.02 g). This material was preabsorbed onto C_{18}-bonded silica and then packed into a HPLC stainless steel guard cartridge (10 × 30 mm) that was subsequently attached to a C_{18} semipreparative HPLC column. A gradient HPLC condition from 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of 100% MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9.0 mL/min. Sixty fractions (60 × 1 min) were collected and then analyzed by LC-MS. Fractions 22/23 [(+)-LRESIMS, *m*/*z* 204/205/206], 24/25 [(+)-LRESIMS, *m*/*z* 212/213/214, *m*/*z* 322/324/326], and 26/27 [(+)-LRESIMS, *m*/*z* 316/318] contained the ions of interest. Further purification of these fractions with a Luna C_{18} semipreparative HPLC column yielded compounds **4** (0.5 mg, 0.0025% dry wt), **6** (0.6 mg, 0.003% dry wt), **7** (0.3 mg, 0.0015% dry wt), **8** (0.2 mg, 0.001% dry wt), and **9** (0.5 mg, 0.0025% dry wt). Further purification of the fractions 33, 34, 39, 40, 42, and 43 with a Luna C_{18} semipreparative column yielded compounds **1** (0.4 mg, 0.002% dry wt), **2** (0.3 mg, 0.0015% dry wt), **3** (0.5 mg, 0.0025% dry wt), **5** (0.3 mg, 0.0015% dry wt), and **10** (0.8 mg, 0.004% dry wt).

Aplysinellamide A (1): pale, amorphous powder; $[\alpha]_D^{23}$ −3.1 (*c* 0.007, MeOH); UV (MeOH), λ_{max} (log ε) 290 (3.76), and 218 (3.78) nm; IR (KBr) ν_{max} 2924, 2853, 1652, 1596, 1498, 1261, and 1052 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C NMR (CD_3OD, 150 MHz), see Table 2; (+)-LRESIMS *m*/*z* 385 (100%),

387 (100%); (+)-HRESIMS m/z 385.0755 $[M + H]^+$ (calcd for $C_{16}H_{22}{}^{79}BrN_2O_4$, 385.0765).

Table 1. ^{1}H NMR Data (600 MHz) for 1−4

	δ_H (J in Hz)			
position	1[a]	2[a]	3[a]	4[b]
2	7.76, d (2.1)	7.79, s	7.77, d (2.2)	7.66, s
5	7.05, d (8.6)		7.05, d (8.6)	
6	7.50, dd (8.6, 2.1)	7.79, s	7.50, dd (8.6, 2.1)	7.66, s
7	7.42, d (15.7)	7.38, d (15.7)	7.41, d (15.7)	3.10, t (8.4)
8	6.46, d (15.7)	6.55, d (15.7)	6.47, d (15.7)	3.81, t (8.4)
1′	3.31, m	3.32, m	3.33, m	3.98, t (5.8)
2′	1.62, m	1.62, m	1.60, m	2.14, m
3′	1.54, m	1.50, m	1.66, m	3.31, m
	1.49, m			
4′	1.97, m	1.94, m	2.34, t (7.1)	
	1.90, m	1.87, m		
5′	3.91, m	3.72, t (6.2)		
OCH_3	3.91, s	3.88, s	3.92, s	
8-NCH_3				3.44, s
3′-NCH_3				2.81, s

[a]In CD_3OD. [b]In DMSO-d_6.

Table 2. ^{13}C NMR Data (150 MHz) for 1−4

	δ_C, mult.			
position	1[a]	2[a]	3[a]	4[b]
1	128.6, C	133.9, C	128.8, C	136.6, C
2	131.6, CH	131.3, CH	131.8, CH	133.9, CH
3	111.5, C	118.0, C	111.6, C	118.0, C
4	157.3, C	154.9, C	151.7, C	151.5, C
5	111.9, CH	118.0, C	111.7, CH	118.0, C
6	128.5, CH	131.3, CH	128.5, CH	133.9, CH
7	138.7, CH	136.5, CH	138.5, CH	27.4, CH_2
8	119.2, CH	122.7, CH	119.3, CH	68.5, CH_2
9	167.3, C	166.6, C	167.0, C	
1′	38.5, CH_2	38.7, CH_2	38.6, CH_2	70.7, CH_2
2′	28.6, CH_2	28.7, CH_2	28.6, CH_2	25.3, CH_2
3′	21.9, CH_2	22.0, CH_2	21.9, CH_2	54.8, CH_2
4′	29.8, CH_2	30.1, CH_2	33.0, CH_2	
5′	52.6, CH	53.6, CH	176.0, C	
6′	170.6, C	171.6, C		
4-$OCHH_3$	55.4, CH_3	59.8, CH_3	55.3, CH_3	
8-NCH_3				56.2, CH_3
3′-NCH_3				42.9, CH_3

[a]CD_3OD. [b]DMSO-d_6.

Aplysinellamide B (2): brown, amorphous powder; $[\alpha]_D^{23}$ +0.29 (c 0.017, MeOH); UV (MeOH), λ_{max} (log ε) 280 (3.96), 224 (4.13), and 206 (4.18) nm; IR (KBr) ν_{max} 2925, 2854, 1635, 1420, 1261, and 1203 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C NMR (CD_3OD, 150 MHz), see Table 2; (+)-LRESIMS m/z 463 (50%), 465 (100%), 467 (50%); (+)-HRESIMS m/z 484.9682 $[M + Na]^+$ (calcd for $C_{16}H_{20}{}^{79}BrN_2O_4Na$, 484.9679).

Aplysinellamide C (3): brown, amorphous powder; UV (MeOH), λ_{max} (log ε) 290 (4.12), and 219 (4.09) nm; IR (KBr) ν_{max} 3422, 2938, 2880, 1649, 1497, 1293, and 1206 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz), see Table 1; ^{13}C NMR (CD_3OD, 150 MHz), see Table 2; (+)-LRESIMS m/z 356 (100%), 358 (100%); (+)-HRESIMS m/z 378.0322 $[M + Na]^+$ (calcd for $C_{15}H_{18}{}^{79}BrNO_4Na$, 378.0319).

Aplysamine-1-*N*-oxide (4): pale, amorphous powder; UV (MeOH), λ_{max} (log ε) 283 (1.97), and 217 (3.11) nm; IR (KBr) ν_{max} 3038, 2965, 1676, 1384, 1200, 1130, and 906 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 2; (+)-LRESIMS m/z 423 (50%), 425 (100%), 427 (50%); (+)-HRESIMS m/z 445.0097 $[M + Na]^+$ (calcd for $C_{15}H_{24}{}^{79}Br_2N_2O_2Na$, 445.0077).

Marfey's Derivatization and LC-MS Analysis of 1. Compound **1** (0.3 mg) was hydrolyzed with 6 N HCl (1 mL) at 110 °C for 20 h. After cooling, the reaction mixture was concentrated under the vacuum to dryness. The hydrolysate was added to 40 μL of 1 M $NaHCO_3$ and 200 μL of 1% 1-fluoro-2,4-dinitrophenyl-5-alaninamide in acetone. The solution was reacted at 40 °C for 1 h and subsequently analyzed by LC-MS [Waters ZQ-LCMS, Phenomenex Luna C_{18} (50 × 4.6 mm), 3.0 μm, gradient MeOH/H_2O/0.1% TFA; flow rate 1.0 mL/min]. LC-MS analysis showed a peak with a retention time of 10.6 min, while the standard D-lysine derivative had a retention time of 10.5 min. The L-lysine derivative had a retention time of 9.8 min.

ApoE Modulation Activity. The ApoE modulation assay was carried out on fractions and compounds as previously described.[16] Briefly, fractions or compounds were incubated with human CCF-STTG1 astrocytoma cells for 96 h. Supernatant samples were assayed for secreted ApoE using an ELISA assay detecting ApoE. The fold change over control cells was reported.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H, COSY, HSQC, and HMBC NMR spectra for aplysinellamide A−C (**1**−**3**) and aplysamine 1-*N*-oxide (**4**). LC-MS trace for Marfey's derivative of **1** and the two standards. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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