Aplysamine 6, an Alkaloidal Inhibitor of Isoprenylcysteine Carboxyl Methyltransferase from the Sponge *Pseudoceratina* sp.

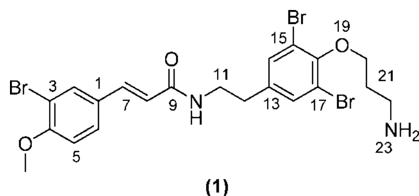
Malcolm S. Buchanan,† Anthony R. Carroll,† Gregory A. Fechner,† Anthony Boyle,† Moana Simpson,† Rama Addepalli,† Vicky M. Avery,† John N. A. Hooper,‡ Tony Cheung,§ Huwei Chen,§ and Ronald J. Quinn*,†

Eskitis Institute, Griffith University, Brisbane, Queensland 4111, Australia, Queensland Centre for Biodiversity, Queensland Museum, South Brisbane, Queensland 4101, Australia, and AstraZeneca R & D Boston, 35 Gatehouse Drive, Waltham, Massachusetts 02451

Received November 19, 2007

The anticancer target isoprenylcysteine carboxyl methyltransferase (Icmt) was the focus of a natural product high-throughput screening campaign. The Australian marine sponge *Pseudoceratina* sp. yielded aplysamine 6, a new bromotyrosine derivative with an α,β-unsaturated amide linkage, as the bioactive constituent. Its structure was determined by 1D and 2D NMR spectroscopy.

Isoprenylcysteine carboxyl methyltransferase (Icmt) methylates the carboxyl-terminal isoprenylcysteine of CAAX proteins.[1,2] Recent studies provided strong evidence that blocking Icmt activity significantly mislocalizes RAS regulatory proteins, and tumorigenesis is markedly impaired in cells that lack Icmt. The development of Icmt inhibitors is a new approach to finding anticancer drugs. Previously we reported the isolation of the alkaloid spermatinamine from *Pseudoceratina* sp., as an Icmt inhibitor.[3] Another extract studied during our campaign to discover Icmt inhibitors was from another *Pseudoceratina* sp. (Pseudoceratinidae). Bioassay-guided purification of this extract afforded the new bromotyrosine derivative aplysamine 6 (**1**), which is a class of bromotyrosine derivative containing one bromotyrosine unit and one bromomethoxycinnamoyl unit. Marine sponges of the order Verongida are characterized by chemotaxonomic markers, bromotyrosine-derived metabolites, many of which possess potent antimicrobial and cytotoxic activities.[4] Chemical modification occurs in both the side chain and the aromatic ring of the brominated tyrosine precursors, giving rise to a broad range of biosynthetically related compounds. Psammaplysene bromotyrosine derivatives, which possess an α,β-unsaturated amide linkage similar to **1**, have been reported as FOXO1a nuclear export inhibitors.[5] Aplysamine 3 (purpuramine H), the closest reported analogue to **1**,[6,7] has an α,β-unsaturated double bond, while aplysamine 3 has an oxime unit. This paper reports the isolation, structure elucidation, and Icmt inhibitory activity of aplysamine 6 (**1**).



(1)

The HRESIMS of optically inactive aplysamine 6 (**1**) exhibited a 1:2:2:1 ion cluster at *m/z* 589/591/593/595, indicating the presence of three bromine atoms and established the molecular formula as $C_{21}H_{24}Br_3N_2O_3$. Its ^1H NMR spectrum (Table 1) displayed one amide proton [δ 8.09 (t, 6.5 Hz)], a protonated amino group [δ 7.88 (br m, 3H)], five aromatic protons ascribable to the presence of two benzene rings [δ 7.78, 7.14, 7.55 (3H)], and a *trans* α,β-unsaturated carbonyl group [δ 7.32 (d, 16.0 Hz); 6.52 (d, 16.0 Hz)]. The ^1H NMR spectrum also displayed signals for five methylenes [δ 3.41 (q, 6.5 Hz, 2H); 2.75 (q, 6.5 Hz, 2H); 4.00 (t, 6.5 Hz, 2H); 2.08 (br quin., 6.5 Hz, 2H); 3.07 (m, 2H)]. The ^{13}C NMR spectrum (Table 1) showed resonances for 21 carbons. The foregoing spectroscopic data clearly revealed that compound **1** was a bromotyrosine metabolite.

Table 1. ^1H (600 MHz), ^{13}C (125 MHz), gCOSY, and gHMBC NMR Data for Aplysamine 6 (**1**) in DMSO-d_6

position	δ_C	δ_H (mult., *J* Hz)	COSY (H no.)	$^{2,3}J_{CH}$ HMBC (C no.)
1	129.0 qC			
2	131.6 CH	7.78 (d, 1.8)	6	3, 4, 6, 7
3	111.1 qC			
4	156.1 qC			
5	112.9 CH	7.14 (d, 8.4)	6	1, 3, 4
6	128.4 CH	7.55 (dd, 8.4, 1.8)	2, 6	2, 4, 7
4-OMe	56.4 CH_3	3.88 (s, 3H)		4
7	136.8 CH	7.32 (d, 16.0)	8	1, 2, 6, 8, 9
8	121.1 CH	6.52 (d, 16.0)	7	1, 9
9	165.0 qC			
10 (N)		8.09 (t, 6.5)	11	9, 11
11	39.7 CH_2[a]	3.41 (q, 6.5, 2H)	10, 12	9, 12, 13
12	33.5 CH_2	2.75 (t, 6.5, 2H)	11	11, 13, 14, 18
13	139.3 qC			
14	133.0 CH	7.55 (s)		12, 15, 16 18
15	117.2 qC			
16	150.4 qC			
17	117.2 qC			
18	133.0 CH	7.55 (s)		12, 14, 16, 17
20	70.3 CH_2	4.00 (t, 6.5, 2H)	21	16, 21, 22
21	27.7 CH_2	2.08 (br quin., 6.5, 2H)	20, 22	20, 22
22	36.5 CH_2	3.07 (m, 2H)	21	21
23 (N)		7.88 (br m, 3H)	22	

[a] Chemical shift obtained from DEPT as signal obscured by DMSO peak.

The connectivity of the methylene groups was achieved from $^1H-^1H$ correlations in a gCOSY experiment (Table 1). Thus, the signal at δ 2.08 showed correlations with an oxygen-bearing methylene at δ 4.00 and a nitrogen-bearing methylene at δ 3.07. The latter methylene also showed correlations to the amino protons (δ 7.88), forming a $O-CH_2-CH_2-CH_2-NH_2$ unit. The methylene at δ 3.41 coupled to the methylene at δ 2.75 and the amide proton at δ 8.09, giving a $NH-CH_2-CH_2$ unit. The coupling pattern in proton signals at δ 7.78 (d, 1.8), 7.14 (d, 8.4), and 7.55 (dd, 8.4, 1.8) indicated the presence of a 1,3,4-trisubstituted aromatic moiety. The position of the methoxyl [δ 3.88 (s, 3H)] at C-4 in this ring was confirmed by its ROESY correlation with the aromatic proton at δ 7.14 (C-5). The gHMBC spectrum (Table 1) showed a proton

* To whom correspondence should be addressed. Tel: 61 7 3875 6000. Fax: 61 7 3875 6001. E-mail: r.quinn@griffith.edu.au.
† Griffith University.
‡ Queensland Museum.
§ AstraZeneca.

10.1021/np0706623 CCC: $40.75 © 2008 American Chemical Society and American Society of Pharmacognosy
Published on Web 04/05/2008

signal at δ 7.55 (s, 2H) correlated to C-12, C-15/C-17, C-16, and C-14/C-18, revealing a symmetrical tetrasubstituted aromatic ring.

Completion of the structure by linking the units together was established from gHMBC correlations. Thus, in the gHMBC spectrum the C-20 methylene protons at δ 4.00 showed a correlation with the quaternary aromatic carbon at δ_C 150.4 (C-16), attaching the $O-CH_2-CH_2-CH_2-NH_2$ group to the tetrasubstituted aromatic ring. The olefinic protons δ 7.32 and 6.52 showed correlations to the quaternary aromatic carbon at δ_C 129.0 (C-1), linking the α,β-unsaturated carbonyl group to the trisubstituted aromatic ring. A gHMBC correlation between δ 3.41 (H-11) and δ_C 165.0 (C-9) confirmed an amide bond. All that remained was for the three bromine atoms to be attached to the remaining positions on the two aromatic rings. The ^1H and ^{13}C NMR chemical shifts were consistent with similar brominated aromatic rings in previously reported bromotyrosines.[6,7] The foregoing spectroscopic data indicated that the structure of aplysamine 6 was **1**.

Aplysamine 6 (**1**) showed inhibition of Icmt at an IC_{50} of 14 μM (assay performed in duplicate on four independent days). It showed no activity in an assay designed to detect artifacts up to 100 μM, ruling out assay technology interference. Aplysamine 6 (**1**) adds to the small list of inhibitors of the Icmt cancer target.

Experimental Section

General Experimental Procedures. UV and FTIR spectra were recorded on an Agilent 8453 UV/vis spectrophotometer and a Bruker Tensor 27 FTIR spectrophotometer, respectively. NMR spectra were recorded at 30 °C on Varian Inova 500 and 600 MHz NMR spectrometers. Samples were dissolved in DMSO-d_6 (residual ^1H δ 2.50 and ^{13}C δ 39.5 ppm). Multiplicity was determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3). Standard parameters were used for the 2D experiments, which included gradient COSY, HSQC ($^1J_{CH}$ = 140 Hz), and HMBC ($^nJ_{CH}$ = 8.3 Hz). HRESIMS were measured on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer, fitted with an Apollo API source. A Betasil C_{18} 5 μm (21.2 mm × 150 mm i.d.) was used for semipreparative HPLC. A Waters 600 pump fitted with a 996 photodiode array detector and 717 plus autosampler was used for the semipreparative separations. C_{18} was 04K-4348 Sepra C_{18} end-capped silica. Water was Millipore Milli-Q PF filtered, while all other solvents used were Laboratory-Scan HPLC grade. Isoprenylcysteine carboxyl methyltransferase (Icmt) and Biotin-*S*-farnesyl-L-cysteine (BFC) were provided by AstraZeneca (Boston, MA). *S*-Adenosylhomocysteine (SAH) was purchased from Fluka (Buchs, Switzerland). *S*-Adenosylmethionine (SAM), magnesium chloride hexahydrate ($MgCl_2$), tartrazine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), dimethyl sulfoxide (DMSO), and dithiotreitol (DTT) were from Sigma (St. Louis, MO). [3H] *S*-Adenosylmethionine, [3H] biotin, and streptavidin PVT SPA beads were purchased from Amersham (GE Healthcare, Cardiff, UK). Assays were carried out in white, clear flat-bottomed 384-well microtiter plates from BD Bioscience (CA).

Animal Material. The sponge sample *Pseudoceratina* sp. 1247 (phylum Porifera, class Demospongiae, order Verongida, family Pseudoceratinidae) was collected by scuba diving at a depth of 21 m at North Halls off Sunshine Coast, Queensland, Australia, in October 1999. Description: yellow, aerophobic, cavernous. A voucher sample, G315753, was lodged at the Queensland Museum, South Brisbane, Queensland, Australia.

Animal Description: This species was thought to be new to science, so far known only from moderately deeper reefs from the Gold Coast up to Rainbow Beach, South East Queensland. The growth form was massive, bulbous, subspherical to globular, cavernous internally, and up to 10 cm in diameter. When alive, the color was yellow externally and internally. Aerophobic pigments darken to blue-black in air. The oscules were massive on the upper surface (>5 mm diameter) and lie in pits surrounded by surface conules, opening into huge cavernous subectosomal chambers. It had a tough texture, collagenous, and slightly mucous. All surfaces were covered with large surface conules, ridges, pits and bumps, with conules up to 10 mm high, producing a highly ridged and mounded appearance. The ectosomal skeleton was smooth, opaque, densely pigmented, highly collagenous, lacking any foreign inclusions, and thick (up to 400 μm thick). The subectosomal region was less densely pigmented, but highly collagenous, with some granular cellular inclusions and sparse scattering of foreign debris. Occasional fibers projected into the peripheral region. A choanosomal skeleton had sparse fibers, predominantly dendritic and occasionally reticulating. The fibers had only pith and no bark elements and sometimes incorporated detritus. Large sand grains and other detritus were embedded in mesohyl and surrounding aquiferous canals. Neither megascleres or microscleres were present.

Extraction and Isolation. The material was ground (10.8 g) and extracted sequentially with *n*-hexane, CH_2Cl_2/MeOH (4:1), and finally MeOH. The CH_2Cl_2/MeOH (4:1) extract (0.67 g) was further purified by being preabsorbed on C_{18} and loaded into a refillable preparative guard column (30 mm × 10 mm i.d.), in line with a semipreparative C_{18} HPLC column. The following solvent conditions were used: H_2O/1% TFA to MeOH/1% TFA in 103 min, then isocratic for 17 min (flow 5 mL/min); 60 fractions were collected. Fraction 41 contained aplysamine 6 (**1**) (33.5 mg, 0.3% dry wt) and eluted with a retention time of 82 min. Compound **1** was isolated as its trifluoroacetate salt.

Bioassays. The principle, procedure, and method for the Icmt and artifact assays have been described in detail previously.[3]

Aplysamine 6 (1), (2*E*)-*N*-{2-[4-(3-aminopropoxy)-3,5-dibromophenyl]ethyl}-3-(3-bromo-4-methoxyphenyl)acrylamide: gum; UV λ_{max} (log ϵ) 302 sh (4.02), 291 (4.10), 207 (4.44) nm; IR ν_{max} (film) 3411, 3273, 3062, 1682, 1541, 1497, 1458, 1261, 1202, 1136, 1053, 1017, 722 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; positive-LRESIMS *m/z* 588.98 $[C_{21}H_{23}Br_3{}^{79}N_2O_3+H]^+$ (28), 590.98 $[C_{21}H_{23}Br_2{}^{79}Br^{81}N_2O_3+H]^+$ (98), 592.98 $[C_{21}H_{23}Br^{79}Br_2{}^{81}N_2O_3+H]^+$ (100), 594.98 $[C_{21}H_{23}Br_3{}^{81}N_2O_3+H]^+$ (48); positive-HRESIMS *m/z* 590.9303 $[C_{21}H_{23}Br_2{}^{79}Br^{81}N_2O_3+H]^+$ (calcd 590.9311, Δ 1.4 ppm).

Acknowledgment. We thank AstraZeneca for financial support. We are indebted to J. Mitchell, Griffith University, Brisbane, for HRESIMS analyses.

Supporting Information Available: Photograph of the investigated sponge is available free of charge via the Internet at http://pubs.acs.org/jnp.

References and Notes

(1) Winter-Vann, A. M.; Casey, P. J. *Nat. Rev. Cancer* **2005**, *5*, 405–412.
(2) Winter-Vann, A. M.; Baron, R. A.; Wong, W.; Dela Cruz, J.; York, J. D.; Gooden, D. M.; Bergo, M. O.; Young, S. G.; Toone, E. J.; Casey, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4336–4341.
(3) Buchanan, M. S.; Carroll, A. R.; Fechner, G. A.; Boyle, A.; Simpson, M.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Su, N.; ChenH.; Quinn, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6860–6863.
(4) Tilvi, S.; Rodrigues, C.; Naik, C. G.; Parameswaran, P. S.; Wahidhulla, S. *Tetrahedron* **2004**, *60*, 10207–10215.
(5) Schroeder, F. C.; Kau, T. R.; Silver, P. A.; Clardy, J. *J. Nat. Prod.* **2005**, *68*, 574–576.
(6) Jurek, J.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **1993**, *56*, 1609–1612.
(7) Yagi, H.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 3749–3754.

NP0706623