4-Hydroxybenzoyl Derivative from the Aqueous Extract of the Hydroid *Campanularia* sp.

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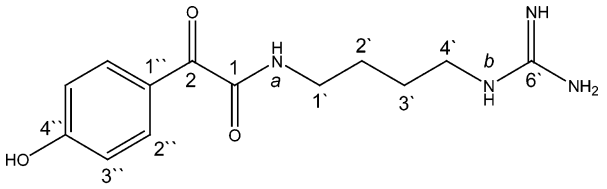
A new compound, *N*-(4-guanidinobutyl)-2-(4-hydroxyphenyl)-2-oxo-acetamide (**1**) was isolated from the aqueous extract of the hydroid *Campanularia* sp. Its structure was elucidated using NMR spectroscopic techniques and mass spectrometric analysis. The most stable conformation was determined using molecular modeling and the results of a NOESY experiment. Although compound **1** shows structural similarities to some highly potent histone deacetylase inhibitors (HDACi), e.g., suberoylanilide hydroxamic acid (SAHA) (**2**) and trichostatin A (TSA) (**3**), it does not inhibit the growth of ARP-1 cells at 100 μM concentration, a significant indication that it has no inhibitory activity to HDACs.

Marine invertebrates have drawn attention for their ability to produce an immense number and variety of bioactive secondary metabolites.[1] These metabolites may play a role in warding off predators, and perhaps they also repel fouling organisms.[1] In connection with our long-standing interest in the chemistry of marine invertebrates, we had the occasion to examine the hydroid *Campanularia* sp. collected from New Zealand's Fiordland National Park by Australian Institute of Marine Science scientists in 1991. We obtained its freeze-dried aqueous extract from the U.S. National Cancer Institute's Open Repository Program. Separations and purifications were carried out using column chromatographic techniques, including HPLC, and were guided by TLC and ^{1}H NMR to yield a new aromatic compound (**1**). The structure was elucidated by a combination of NMR techniques, exact mass spectral determination, and comparison with the NMR data of related compounds in the literature. The most stable conformation of **1** was determined using molecular modeling and the results of a NOESY experiment.



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The ^{13}C NMR and mass spectral data of **1** indicated it to have the molecular formula $C_{13}H_{18}N_4O_3$ and, thus, to have seven degrees of unsaturation. Further analysis of its ^{13}C NMR spectrum showed these elements of unsaturation to be present in the form of two carbonyls (δ_C 189.4, qC; 166.2, qC), an imine (δ_C 157.3, qC) group, and a 1,4-disubstituted benzene ring (δ_C 164.1, qC; 2 × 133.2, CH; 125.0, qC; 2 × 116.3, CH). The ^{13}C NMR spectrum also revealed the presence of two N-CH_2 (δ_C 41.0, CH_2; 38.5, CH_2) groups and two magnetically equivalent methylene groups (δ_C 26.6, CH_2). The presence of a guanidine moiety was confirmed by comparison of the characteristic carbon chemical shift value of C-6′ at δ_C 157.3 with those reported in the literature.[2−5]

The ^{1}H NMR data further supported the presence of 1,4-disubstituted benzene ring as the only cyclic element within the molecule [δ_H 7.8 (d, 2H, J = 8.8 Hz) and 6.8 (d, 2H, J = 8.8 Hz)] and also showed the presence of four methylene [δ_H 3.17 (d, 2H, J = 6.0 Hz), 3.06 (d, 2H, J = 6.0 Hz), 1.4 (t, 4H, J = 2.8 Hz)] groups, two N-H [δ_H 8.7 (t, 1H, J = 5.6 Hz), 7.4 (t, 1H, J = 5.6 Hz)] groups, and one hydroxyl [δ_H 10.8 (bs, 1H)] group.

After all protons had been assigned to their directly bonded carbon atoms, aided by an HSQC spectrum, it was possible to construct five substructures (Figure 1) by

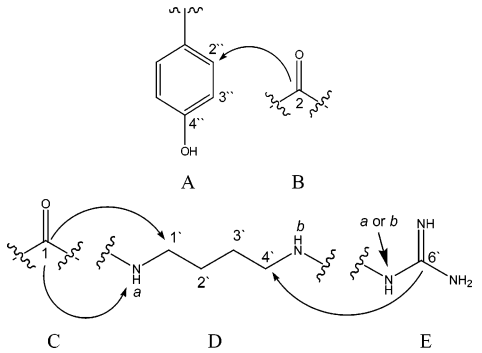

Figure 1. Substructures A−E of compound **1** and some key HMBC correlations.

interpretation of the ^{1}H−^{1}H COSY spectrum.

The connection between substructures A and B was established from the HMBC correlation observed between the signal at δ_C 189.4 assigned to C-2 and the aromatic protons at δ_H 7.8 (2H, d, 8.8) assigned to H-2″/H-6″. The signal at δ_C 166.2 (C-1) showed long-range correlations to the protons at δ_H 3.17 (2H, d, 6.0) and δ_H 8.70 (1H, t, 5.6) assigned as H-1′ and N-H*a*, respectively, showing substructure C to be connected to substructure D. Similarly, connection between substructure D and substructure E was assigned by a long-range correlation observed between δ_C 157.3 (C-6′) and the methylene group at δ_H 3.06 (2H, d, 6.0) assigned as H-4′.

The nature of the connection of substructure B to substructure C was confirmed by comparison of the ^{13}C NMR data with those reported in the literature for related compounds.[6]

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Table 1. 1D and 2D NMR Spectral Data for Compound **1** Obtained in DMSO-d_6 (δ in ppm)

no.	δ_C		δ_H (#H, m, *J*/Hz)	$^1H-^1H$ COSY	HMBC (δ_C to δ_H)	NOESY
1	166.2	qC			H-1′, N-H*a*	
2	189.4	qC			H-2″/H-6″	
1′	38.5	CH_2	3.17 (2H, d, 6.0)	H-2′/H-3′, N-H*a*	H-2′/H-3′, N-H*a*	H-2′/H-3′, N-H*a*
2′, 3′	26.6	CH_2	1.4 (4H, t, 2.8)	H-1′, H-4′	H-1′, H-4′	H-1′, H-4′, H-2″/H-6″
4′	41.0	CH_2	3.06 (2H, d, 6.0)	H-2′/H-3′, N-H*b*		H-2′/H-3′, N-H*b*
6′	157.3	qC			H-4′	
1″	125.0	qC			H-3″/H-5″	
2″, 6″	133.2	CH	7.8 (2H, d, 8.8)	H-3″/H-5″		
3″, 5″	116.3	CH	6.8 (2H, d, 8.8)	H-2″/H-6″	H-2″/H-6″	H-2″/H-6″, 4″-OH
4″	164.1	qC			H-2″/H-6″, H-3″/H-5″	
N-H*a*			8.7 (1H, t, 5.6)	H-1′		H-1′, H-2″/H-6″
N-H*b*			7.4 (1H, t, 5.6)	H4′		H-4′
4″-OH			10.8 (1H, bs)			H-3″/H-5″

The global minimum energy conformation of compound **1** in $CHCl_3$ (Figure 2) was deduced using a Monte Carlo

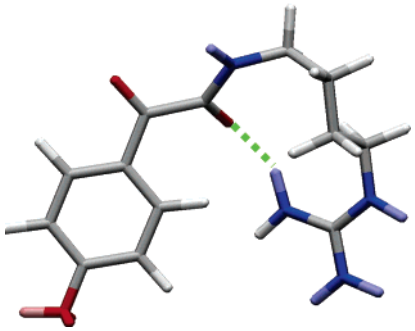
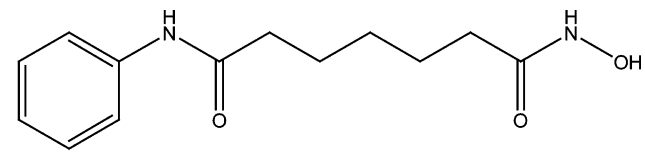


Figure 2. Global minimum energy conformation of compound **1** in $CHCl_3$ as determined by a Monte Carlo conformational search.

conformational search (Macromodel v6.5).[7] This search indicated the presence of a hydrogen bond between the carbonyl group at C-1 and the amino group of the guanidine moiety. This conformation was confirmed from the results of a NOESY experiment, which showed correlations between the methylene protons H-2′/H-3′ and the aromatic protons (H2″/H6″) and between the amide proton (N-H*a*) and the aromatic protons.

The biogenesis of compound **1** may be through the formation of agmatine (4-aminobutylguanidine) from arginine via arginine decarboxylase.[8] The phenolic ring with the attached two carbonyl groups are possibly formed from tyrosine by transamination, decarboxylation, and oxidation.

Compound **1** shows a structural resemblance to some histone deacetylase inhibitors (HDACi), e.g., suberoylanilide hydroxamic acid (SAHA) (**2**) and trichostatin A (TSA) (**3**). These agents cause cancer cell growth arrest and/or



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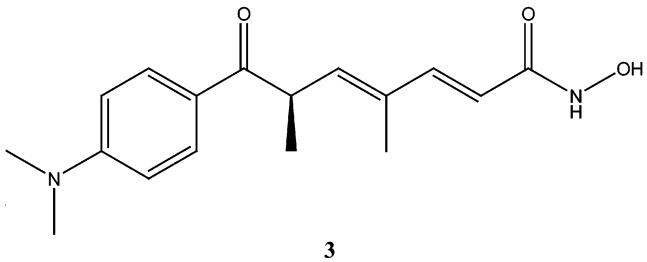

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apoptosis in vivo and in vitro.[9] Histone deacetylation by these compounds induces the expression of the *p21*^{WAF1} gene, which in turn arrests transformed cell growth.[9−13] In early 2004, Gui et al.[9] used a human myeloma cell line, ARP-1, to examine the changes in the *p21*^{WAF1} promotor caused by SAHA. This gene is expressed at low or almost undetectable levels in ARP-1 cells and is rapidly induced by SAHA with subsequent cell growth inhibition. In the present study, the same cells have been used to examine the possible HDAC inhibitory activity of compound **1**. Despite its similarity to SAHA and related compounds, compound **1**, up to 100 μM concentration, did not inhibit the growth of ARP-1 cells, a significant indication that it has no activity toward HDACs.

Experimental Section

General Experimental Procedures. UV and IR were measured on a Perkin-Elmer Lambda 15 UV/vis spectrometer and an Ati Mattson Genesis Series FTIR machine, respectively. ^1H, ^{13}C, and all NMR 2D experiments were recorded on a Varian Unity INOVA 400 MHz spectrophotometer, in DMSO-d_6. A low-resolution electrospray mass spectrum was obtained using a Perseptive Biosystems Mariner LC-MS, and high-resolution mass data were obtained on a Finnigan MAT 900 XLT. HPLC separations were carried out using a Phenomenex reversed-phase (C18, 10 × 250 mm) column and Spectraseries P100 isocratic pump and monitored using a Hewlett-Packard HP 1050 Series variable-wavelength UV detector.

Animal Material. A sample of *Campanularia* sp. (class Hydrozoa, order Leptothecatae, family Campanulariidae) was collected in 1991 from New Zealand's Fiordland National Park, at a depth of 20 m, by the Australian Institute of Marine Science scientists under contract with the National Cancer Institute. The sample was identified by John Hooper of the Queensland Museum, Australia. Collected material was stored at −20 °C until used. Voucher specimens (Q66C5373) are stored at the Smithsonian Institution, USA, and at the Queensland Museum in Brisbane, Australia.

Extraction and Isolation. The frozen sample was ground into a coarse powder and extracted with H_2O. Solid materials were removed by centrifugation, and the resulting aqueous solution was freeze-dried to provide 12 g of aqueous extract. The extract was shipped to our lab and stored at −20 °C until used. Desalting of the extract was carried out using a modification of the method described by West et al.[14,15] The extract was dissolved in the least amount (300 mL) of MeOH/H_2O (1:1) and passed through a column of DIAION HP20ss (25 × 1.5 cm) pre-equilibrated with MeOH/H_2O (1:1). The eluent was diluted with H_2O (500 mL) and passed back through the column. Finally, the eluent was diluted with H_2O (2 L) and passed back through the same column. The column was then washed with H_2O (1 L) and eluted with MeOH 100% (0.5 L). The methanolic eluent was concentrated under reduced

pressure and loaded on a Sephadex LH-20 column equilibrated with CH_2Cl_2/MeOH (1:1). Four fractions were collected. Fraction 3 gave interesting ^1H and ^{13}C NMR spectra. This fraction was purified by reversed-phase HPLC using a mixture of MeOH, water, and TFA (50:50:0.05) as eluent to yield 3.2 mg of **1**.

***N*-(4-Guanidinobutyl)-2-(4-hydroxyphenyl)-2-oxoacetamide (1):** colorless oil, 3.2 mg; UV (MeOH) λ_{max} (log ϵ) 295 (3.89), 209 (3.84) nm; IR ν_{max} 3186, 1674, 1601, 1584, 1202, 1167, 1137 cm^{-1}; NMR data (Table 1); LRESMS *m/z* 279.196 $[M + H]^+$, 147.009, 145.013; HRESMS *m/z* 279.1452 $[M + H]^+$ Δ 0.2 mmu calculated for $C_{13}H_{19}O_3N_4$.

Biological Assay. ARP-1 human multiple myeloma cells were maintained in RPMI medium 1640 as described.[16] Cells were grown in suspension and subcultured every 3−4 days in complete RPMI medium 1640 and seeded at a density of 2×10^5 cells per mL for cultures with compound **1**. Compound **1** was dissolved in DMSO and tested in various concentrations ranging up to 100 μM. Cell density and viability were determined as described.[17]

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Supporting Information Available: NMR spectra of **1**, including ^1H, ^{13}C, COSY, HSQC, and HMBC in DMSO-d_6, are available free of charge via the Internet at http://pubs.acs.org.

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