

Pulicatin A–E, Neuroactive Thiazoline Metabolites from Cone Snail-Associated Bacteria

Zhenjian Lin,[†] Rowena R. Antemano,^{†,‡} Ronald W. Hughen,[§] Ma. Diarey B. Tianero,^{†,‡} Olivier Peraud,[†] Margo G. Haygood,[△] Gisela P. Concepcion,[‡] Baldomero M. Olivera,^{||} Alan Light,[§] and Eric W. Schmidt^{*,†,||}

Departments of Medicinal Chemistry, Anesthesiology, and Biology, University of Utah, Salt Lake City, Utah 84112, United States, Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines, and Department of Environmental and Biomolecular Systems, OGI School of Science & Engineering, Oregon Health & Science University, Beaverton, Oregon 97006, United States

Received August 19, 2010

The cone snail *Conus pulicarius* from the Philippines provides a specific habitat for actinomycetes and other bacteria. A phenotypic screen using primary cultures of mouse dorsal root ganglion neurons revealed that one *C. pulicarius* associate, *Streptomyces* sp. CP32, produces a series of natural products that enhance or diminish whole-cell Ca²⁺ flux. These compounds include known thiazoline compounds and a series of new derivatives, pulicatin A–E (**6–10**). Individual compounds were shown to bind to a series of human receptors, with selective binding to the human serotonin 5-HT_{2B} receptor. Here, we report the structure elucidation of the new compounds and results of the neurological assays.

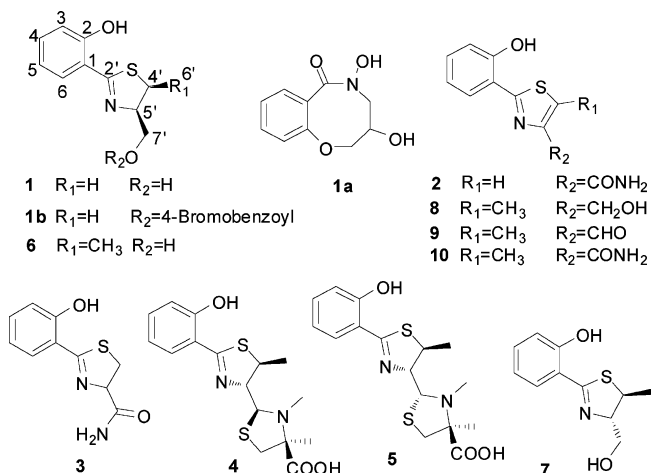
Symbiotic bacteria living with animals have been implicated in the synthesis of defensive and other natural products of importance to drug discovery.^{1–5} In the marine environment, most studies of symbiont-derived natural products have focused on soft-bodied, benthic invertebrates that are otherwise vulnerable to predation. By contrast, cone snail mollusks are well defended by their shells and their arsenal of extremely diverse, snail-derived peptide toxins that act on channels and receptors.⁶ We sought to investigate whether this type of well-defended organism would also house symbiotic bacteria involved in the synthesis of allelochemicals, potentially including venom components, antibacterial defenses, and other small-molecule natural products. In addition to investigating this basic question, we reasoned that exploration of an untapped bacterial niche could lead to the discovery of new bioactive natural products.

We recently reported that at least some cone snails are associated with diverse actinomycetes and other bacteria and that extracts of some of these bacteria exhibit neurological activity.⁷ In order to maximize the discovery of new active bacterial metabolites from these new niches, we applied a broad-scope assay involving primary cultures of mouse dorsal root ganglion (DRG) neurons.^{7–9} The dorsal horn contains many types of neurons, largely involved in pain and other sensory perception, which express different types and combinations of channels and receptors. In this phenotypic assay different responses are observed in individual cells representing different types of neurons when active compounds or extracts are applied. Using this broadly targeted biological assay as a primary screening strategy, we tested extracts from a total of 254 cone snail-associated bacteria, which yielded 29 isolates that either increase or decrease Ca²⁺ influx into DRG cells. One of the active isolates, *Streptomyces* sp. CP32 from *C. pulicarius*, was investigated by assay-guided fractionation, leading to the isolation of five known analogues (**1–5**) together with five new analogues, pulicatin A–E (**6–10**).

Results and Discussion

Extracts of pilot-scale cultures of strain CP32 were strongly active in the DRG assay, resulting in K⁺-stimulated Ca²⁺ influx. CP32

was fermented in 2.8 L Fernbach flasks for eight days. In an assay-guided procedure, the culture broth was centrifuged and subjected to HP20 adsorption chromatography, followed by C₁₈ flash chromatography and HPLC to yield compounds **1–10**.



A known compound, aerugine (**1**), was isolated as a pale yellow solid.^{10,11} The NMR and low-resolution MS data also exactly matched a siderophore compound (**1a**) isolated from a bacterium associated with a marine sponge,¹² leading us to initially misassign the structures of this compound series. High-resolution electrospray mass spectrometry (HRESIMS) revealed ions at *m/z* 210.0583 ([M + H]⁺), indicating a molecular formula of C₁₀H₁₁NO₂S, allowing us to reassign the structure as **1**. The difference between the reported structures of these two molecules is the presence of a thiazoline group in **1** or a hydroxamic acid group in **1a**. However, in the structure elucidation of the siderophore compound, no evidence was provided for either the phenolic ether bond or the hydroxamic acid group. Comparison of the spectroscopic data of **1** and **1a** suggests that they may be identical. The 4-bromobenzoyl derivative **1b** was synthesized from **1**, and a shift of C-7' from 3.85 to 4.58 ppm indicated a primary alcohol group at C-7'. All of these data, as well as the HMBC correlations (Figure 1), were compatible with the structure for aerugine. Other known compounds, pulicatin F (**2**) and G (**3**), have been synthesized but are not previously known as natural products.^{13,14} They are included in this series because of their structural similarities with the remainder of the compounds. Watasemycin A (**4**) and B (**5**) were identified by comparison of NMR and MS data with literature values.¹⁵

* Corresponding author. Mailing address: College of Pharmacy, Medicinal Chemistry, 307 Skaggs Hall, 30 S. 2000 E., Rm. 201, Salt Lake City, UT 84112-5820. Tel: (801) 585-5234. Fax: (801) 585-9119. E-mail: ewsl@utah.edu.

[†] Department of Medicinal Chemistry, University of Utah.

[‡] University of the Philippines.

[§] Department of Anesthesiology, University of Utah.

[△] Oregon Health & Science University.

^{||} Department of Biology, University of Utah.

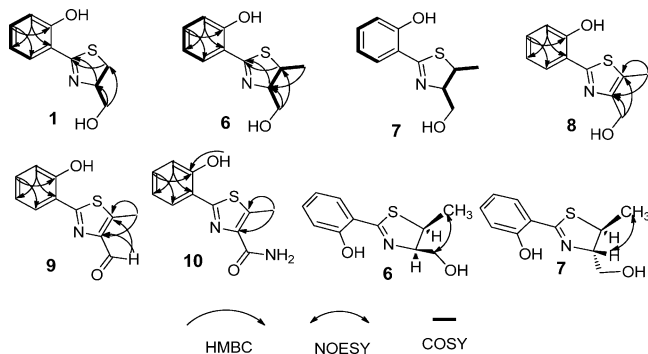


Figure 1. Key HMBC, ^1H - ^1H COSY, and NOESY correlations of compounds **1** and **6**-**10**.

Pulicatin A (**6**) was isolated as a pale yellow solid and assigned the molecular formula $\text{C}_{11}\text{H}_{13}\text{NO}_2\text{S}$ on the basis of HRESIMS analysis (m/z 224.0741 for $[\text{M} + \text{H}]^+$) and NMR experiments (Table 1). Compound **6** had a higher mass than **1** by 14 Da, indicating that an additional CH_2 was present. The ^1H NMR and ^{13}C NMR spectra of **6** were very similar to those of **1** except that they have signals corresponding to a methyl group at δ_{H} 1.35. Compound **6** contained a methine group (δ_{H} 4.04 m; δ_{C} 45.9 CH) instead of the methylene group in **1**. A COSY experiment (Figure 1) led to the identification of the $\text{CH}_3\text{CHCHCH}_2\text{OH}$ fragment. The HMBC (Figure 1) cross-peaks observed from both H-4' and H-5' to C-2' confirmed the planar structure of **6**. The NOESY correlations between H₃-6' and H₂-7' indicated a 4'S*,5'R* relative configuration of **6**.

Pulicatin B (**7**) was isolated as a pale yellow solid. Its HRESIMS analysis (m/z 224.0741 for $[\text{M} + \text{H}]^+$) indicated a molecular formula of $\text{C}_{11}\text{H}_{13}\text{NO}_2\text{S}$, which was identical to that of **6**. Its MS and NMR data (Table 1) were almost identical to those of **6** except that the chemical shifts of C-5', C-6', and C-7' were observed slightly downfield of those in **6** (Table 1), indicating it was isomeric with **6**. The NOESY correlations (Figure 2) between H₃-6' and H-5' indicated **7** was a diastereoisomer of **6** with a 4'S*,5'S* relative configuration.

To determine the absolute configurations of **6** and **7**, we employed the method of Yamaguchi and Yasuhara for determination of the absolute configurations of primary alcohols with the asymmetric center at the 2-position.¹⁶ In this approach, an acyclic alcohol is converted to the corresponding (*R*)-(+)-MTPA and (*S*)-(–)-MTPA esters. The magnitude of lanthanoid-induced shift (LIS) by $\text{Eu}(\text{fod})_3$ for the methoxy group of the (*R*)-(+)-MTPA ester is larger than that of the (*S*)-(–)-MTPA ester when the asymmetric center is in *R*-configuration. Sugimoto applied this method to a

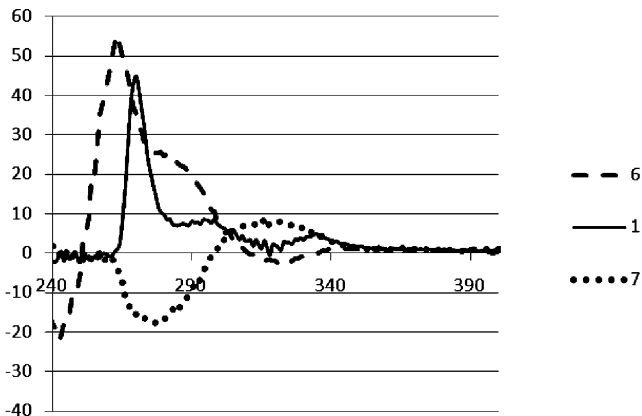


Figure 2. CD spectra of compounds **1**, **6**, and **7**.

Table 2. LIS of Mosher Esters of **1**, **6**, and **7**

MTPA esters	$\Delta\delta_{\text{OCH}_3}$		configuration of C5'
	(<i>S</i>)-	(<i>R</i>)-	
1	+0.01	+0.05	<i>R</i>
6	+0.05	+0.34	<i>R</i>
7	+0.16	+0.13	<i>S</i>

primary alcohol in which a hydroxymethyl group is situated on the asymmetric ring carbon atom of the carbocycle.¹⁷ Len et al. assumed that complexation of europium salts with both oxygens of the ester and methoxy groups induced the existence of different conformers.¹⁸ Compounds **1**, **6**, and **7** were transformed to the corresponding (*R*)-(+)-MTPA and (*S*)-(–)-MTPA esters. The LIS values of the methoxy groups are given in Table 2. For **1** and **6**, the LIS values for (*R*)-(+)-MTPA esters were larger than (*S*)-(–)-MTPA esters, indicating that the asymmetric centers at C-5' of **1** and **6** were in the *R*-configurations. Since this is the known configuration of (+)-aerugine (**1**), this method is in good agreement with the literature.¹⁹ Compound **7** showed larger LIS values for the (*S*)-(–)-MTPA ester, indicating that the asymmetric center at C-5' of **7** was in an *S*-configuration. These conclusions were consistent with the circular dichroism spectra of **1**, **6**, and **7**: the CD spectra of **1** and **6** were very similar, but opposite that of **7** (Figure 2).

Another pale yellow solid compound, pulicatin C (**8**), was assigned the molecular formula $\text{C}_{11}\text{H}_{10}\text{NO}_2\text{S}$ on the basis of HRESIMS analysis (m/z 222.0584 $[\text{M} + \text{H}]^+$) and NMR experiments. In contrast to **6**, **8** has NMR signals for a tetrasubstituted double bond (δ_{C} 128.6 C, 149.5 C) instead of the two sp^3 -hybridized carbons in **6**. A singlet was assigned to a vinylic methyl (δ_{H} 2.52 s,

Table 1. NMR Spectroscopic Data (500 MHz for ^1H , 125 MHz for ^{13}C , CDCl_3) for Pulicatin A–E (**6**–**10**)

position	pulicatin A (6)		pulicatin B (7)		pulicatin C (8)		pulicatin D (9)		pulicatin E (10)	
	δ_{C} , mult	δ_{H} (<i>J</i> in Hz)	δ_{C} , mult	δ_{H} (<i>J</i> in Hz)	δ_{C} , mult	δ_{H} (<i>J</i> in Hz)	δ_{C} , mult	δ_{H} (<i>J</i> in Hz)	δ_{C} , mult	δ_{H} (<i>J</i> in Hz)
1	116.6, C		116.3, C		115.4, C		116.3, C		116.5, C	
2	159.2, C		159.2, C		156.6, C		157.1, C		156.5, C	
2-OH		12.25, brs		12.4, brs		11.90, brs		11.64, brs		11.16, s
3	133.3, CH	7.44, d (9.0)	133.1, CH	7.39, d (8.3)	126.8, CH	7.53, d (8.5)	127.6, CH	7.51, d (8.5)	127.8, CH	7.55, d (7.0)
4	119.1, CH	6.88, dd (9.0, 8.5)	118.9, CH	6.88, dd (8.3, 8.2)	119.2, CH	6.89, dd (8.5, 7.3)	119.9, CH	6.92, dd (8.5, 7.5)	120.1, CH	6.94, dd (7.7, 7.5)
5	130.8, CH	7.36, dd (8.5, 8.5)	130.6, CH	7.36, dd (8.3, 8.2)	131.2, CH	7.29, dd (8.2, 7.3)	132.5, CH	7.35, dd (8.5, 7.3)	132.4, CH	7.35, dd (7.4, 7.4)
6	117.3, CH	7.00, d (8.5)	117.1, CH	6.99, d (8.3)	117.5, CH	7.03, d (8.2)	118.2, CH	7.07, d (8.3)	117.9, CH	7.06, d (8.1)
2'	173.0, C		173.0, C		166.3, C		166.3, C		165.5, C	
4'	45.9, CH	4.04, m	45.2, CH	3.96, dq (6.8, 6.8)	128.6, C		143.5, C		121.6, C	
5'	79.0, CH	4.56, ddd (6.8, 6.8, 6.7)	84.2, CH	4.45, ddd (6.8, 5.5, 4.8)	149.5, C		147.3, C		141.3, C	
6'	16.7, CH_3	1.35, d (7.1)	21.7, CH_3	1.49, d (6.8)	10.9, CH_3	2.52, s	12.5, CH_3	2.86, s	13.1, CH_3	2.88, s
7'	61.7, CH_2	4.04 m, 4.19, dd (10.4, 6.1)	63.2, CH_2	3.85, dd (11.2, 5.5); 3.78, dd (11.2, 4.8)	58.5, CH_2	4.73, s	185.4, C	10.15, s	164.9, C	

Table 3. Ligand Displacement Activity at the 5-HT_{2B} Receptor

compound	<i>K_i</i> (nM) vs 5-HT _{2B}
1	1360 ± 110
2	1031 ± 76
3	1260 ± 99
4	3922 ± 465
5	4695 ± 262
6	505 ± 29
9	1541 ± 134

δ_C 10.9 CH₃). These data indicated that **8** was the 4',5'-dehydro derivative of **6**. This was also supported by the HMBC correlations (Figure 1) from H₃-6' to C-4' and C-5' and from H₂-7' to C-4' and C-5'.

Pulicatin D (**9**) was isolated as a white solid. The molecular formula for **9**, C₁₁H₈NO₂S, was derived from NMR data and the HRESIMS ion at *m/z* 220.0427 ([M + H]⁺). The NMR data were very similar to those of **8**, except that **9** had an aldehyde group (δ_C 185.4 CH) that was assigned to C-7. The chemical shift of the aldehyde carbon indicated it was conjugated with the C-4'-C-5' double bond. This was also supported by the HMBC correlations (Figure 1) from H₃-6' to C-4' and C-5' and from H₂-7' to C-4' and C-5'.

Pulicatin E (**10**) was isolated as a pale yellow solid. It was assigned the molecular formula C₁₁H₁₀N₂O₂S on the basis of HRESIMS analysis (*m/z* 235.0546 [M + H]⁺) and NMR experiments. Analysis of the NMR spectra of **10** showed this compound to have a carbonyl instead of the aldehyde group present in **9**. On the basis of the molecular formula, the carbonyl was assigned to a carboxamide group located at C-5'.

In the DRG assay of purified compounds, two metabolites (**1** and **3**) observably increased Ca²⁺ influx at 20 μ g/mL and were responsible for the activity of the extract of CP32, while compound **6** showed a decreased Ca²⁺ influx (see Supporting Information). This was of particular interest, as **1** and **6** differ solely by a methyl group, yet they exhibit opposite effects on whole DRG cells. Although the activity seems modest, in our experience an observable effect in these neurons requires a relatively high dose that almost completely blocks the target. Therefore, to determine the potential molecular targets and potencies underlying these observed phenotypes, we further screened **1**–**5**, **6**, and **9** at the National Institute of Mental Health's Psychoactive Drug Screening Program (PD-SP).²⁰ The remaining compounds were isolated in insufficient amounts for testing. A primary assay was performed binding 46 targets at a final concentration of 10 μ M of each compound. Initial hits were obtained in radioligand displacement assays. For targets where significant binding activity was detected, secondary binding assays were performed, and *K_i* values were calculated using radioligand displacement with test compound concentrations from 1 to 10 000 nM. *K_i* values from secondary assays were >5000 nM, with the exception of results reported below.

All of the seven tested compounds showed binding activity to the 5-HT_{2B} receptor, with *K_i* values from 505 to 4695 nM (Table 3). Compound **6** exhibited the strongest effect, while bisthiazolines **4** and **5** were weak in comparison to the single-thiazol(in)e compounds. Compounds were relatively selective for 5-HT_{2B} versus other serotonin receptors, for which no significant binding was detected at 10 μ M, indicating that **6** has at least 20-fold selectivity for 5-HT_{2B} over related receptors. Only **2** and **3** showed selectivity for the histamine H₁ receptor, at 517 ± 28 and 678 ± 46 nM, respectively, indicating the potential importance of the amide functionality for H₁ receptor affinity. Only **3** exhibited significant binding to the kappa opioid receptor (3708 ± 145 nM).

The G protein-coupled receptor 5-HT_{2B} has been extensively explored in drug discovery because of its diverse physiological effects, but undesired cardiac activity has limited development of drugs that target this receptor.²¹ Recently 5-HT_{2B} has been shown to play a direct role in modulating neuropathic pain in the dorsal

horn of rats, where selective 5-HT_{2B} antagonism enhanced evoked potentials under certain conditions.²² However, the PDSP results and their connection to the DRG assay need to be interpreted with caution, especially because binding assays do not necessarily reflect physiological function.

Pulicatin is a member of a large family of benzyl thiazole and thiazoline natural products, including aerugine,^{11,19} aeruginic acid,²³ ulbactins,^{19,24} desferrithiocin,²⁵ pyochelin,²⁶ yersiniabactin,²⁷ vibriobactin,²⁸ micacocidins,^{29,30} thiazostatins,³¹ watasemycins,¹³ and other unnamed small molecules.³² Gene clusters for new compounds in this group are commonly found in bacteria.^{33,34} However, the 4'-methyl group is a feature that has only been found previously in **9** and **10** among natural products in this series and rarely elsewhere.

Neurological activity has not been previously reported for compounds in this series. Compounds from the aerugine family exhibited antimicrobial³⁵ and anti-inflammatory activity³⁶ at mid-micromolar levels. Aeruginic acid, which is somewhat similar to compounds **2** and **8**–**10**, exhibited antihypertensive activity,³⁷ also in the micromolar range.

A remaining question is whether the pulicatin is important to symbiosis or to the survival of *C. pulicarius*. Strain CP32 has a 16S gene that is very similar to genes identified in the uncultivated pool in this organism and is in fact identical to the published CP5 sequence (500 bp) and to the sequence from uncultivated bacteria associated with the cone snails. In addition, actinobacteria were identified in the foot margin and in the hepatopancreas by *in situ* hybridization.⁷ These results are at best suggestive of a role for the pulicatin and require further investigation. Finally, **6** was also identified as an active component of *Streptomyces* sp. strain CT8, cultivated from the hepatopancreas of the cone snail *C. tribblei*, possibly indicating a habitat-related function for the compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-370 polarimeter. UV spectra were obtained using a Perkin-Elmer Lambda2 UV/vis spectrometer. Circular dichroism spectra were obtained on a Jasco J720A spectropolarograph. NMR data were collected using either a Varian INOVA 500 (¹H 500 MHz, ¹³C 125 MHz) NMR spectrometer with a 3 mm Nalorac MDBG probe or a Varian INOVA 600 (¹H 600 MHz, ¹³C 150 MHz) NMR spectrometer equipped with a 5 mm ¹H[¹³C,¹⁵N] triple resonance cold probe with a z-axis gradient, utilizing residual solvent signals for referencing. High-resolution mass spectra (HRMS) were obtained using a Bruker (Billerica, MA) APEXII FTICR mass spectrometer equipped with an actively shielded 9.4 T superconducting magnet (MagneX Scientific Ltd., UK), an external Bruker APOLLO ESI source, and a Synrad 50W CO₂ CW laser.

Bacterial Material. *Streptomyces* sp. CP32 was cultivated from *C. pulicarius* obtained by professional collectors near Mactan Island, Cebu, Philippines, as previously described.⁷ The strain was cultured from dissected foot and body tissue and purified, and later the strain was recovered from a glycerol stock and used for further chemical analysis. The 16S gene was cloned using primers 27f and 1492r and submitted to GenBank, accession number HQ337089.

Fermentation and Extraction. *Streptomyces* sp. CP32 was cultured at 30 °C with shaking at 200 rpm in 8 2.8 L Fernbach flasks each containing 1 L of the medium ISP2 (0.2% yeast extract, 1% malt extract, 0.2% glucose, 2% NaCl). After 8 days, the broth was centrifuged and the supernatant was extracted with HP-20 resin for 4 h. The resin was filtered through cheesecloth and washed with H₂O to remove salts. The filtered resin was eluted with MeOH to yield an extract, which was further purified by bioassay-guided (DRG) fractionation.

Purification. The extract (400 mg) was separated into seven fractions (Fr 1–Fr 7) on a C₁₈ column using step-gradient elution of MeOH in H₂O (20%, 40%, 50%, 60%, 70%, 80%, 100%). Fr 3, eluting in 50% MeOH, was further purified by C₁₈ HPLC using 45% CH₃CN in H₂O to obtain compound **3** (1.5 mg). Fr 4, eluting in 60% MeOH, was further purified by C₁₈ HPLC using 65% MeOH in H₂O to obtain compounds **1** (3.0 mg), **6** (1.0 mg), and **7** (0.4 mg). Fr 5, eluting in 70% MeOH, was further purified by C₁₈ HPLC using 68% CH₃CN in H₂O with

0.1% TFA to obtain compound **9** (5.0 mg) and subfraction Fr 5-1. Fr 5-1 was further purified by C₁₈ HPLC using 48% CH₃CN in H₂O with 0.1% TFA to obtain compounds **8** (1.0 mg), **10** (0.3 mg), **2** (1.5 mg), **4** (10.0 mg), and **5** (2.0 mg).

Preparation of (S)- and (R)-MTPA Esters. Compounds **1** (100 μg), **6** (500 μg), and **7** (100 μg) were separately transferred into clean reaction bottles and dried completely under vacuum. Pyridine (0.5 mL) and α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (1 equiv) were added into the reaction bottle quickly under a N₂ gas stream and then stirred for 12 h at room temperature. The organic layer was then washed with H₂O and concentrated under reduced pressure to obtain the ester. Final purification was achieved by HPLC (90% MeOH, 4.0 mL/min).

Enantioselective Analysis with Eu(fod)₃. Each MTPA ester of **1**, **6**, and **7** was added to an NMR tube in CDCl₃, and an equimolar mixture of Eu(fod)₃ was added to each tube. The difference in chemical shift with and without the chiral reagent was recorded: Δδ_{OCH₃} (ppm) = Δδ_{OCH₃}(with Eu(fod)₃) - Δδ_{OCH₃}(without Eu(fod)₃).

Preparation of p-Bromobenzoate (1a). To a stirred suspension of **1** (1.0 mg) in dry CH₂Cl₂ (2 mL) were added Et₃N (1 mL) and p-BrC₆H₄COCl (3 mg) at room temperature. Four hours later the reaction was quenched by adding H₂O (2 mL). The mixture was extracted with EtOAc (3 × 5 mL), and the EtOAc solution was dried on anhydrous Na₂SO₄ and evaporated at reduced pressure. The residue was subjected to C₁₈ HPLC (95% MeOH/H₂O) to give **1a** (1.1 mg): ¹H NMR (CDCl₃, 400 MHz) δ 7.36–7.99 (8H, m, ArH), 4.92 (1H, m, H-4'), 4.35, 4.28 (each 1H, m, H-7'), 3.46, 3.24 (each 1H, m, H-4'); ESIMS m/z 392.0, 394.0 [M + H]⁺.

DRG Assay. For details of the isolation and culture of DRG neurons see Light et al.⁸ Briefly, dorsal root ganglia cells from cervical and lumbar regions were obtained from mice and used in an assay with bacterial culture extracts and pure compounds. DRG cells were suspended in medium with additives and loaded with Fura-2 AM (Molecular Probes), a fluorescent dye used to measure intracellular calcium levels. Experiments were performed at room temperature (20 to 25 °C) in a 24-well plate format using fluorescence microscopy. Individual cells were treated as single samples, so that the individual responses of diverse neuron subtypes from the DRG could be examined. After baseline measurements, the cells were treated with 25 mM KCl solution and then washed. After return to baseline, bacterial extracts, fractions, or pure compounds were applied. This solution was then later replaced with 25 mM KCl solution. The use of KCl permitted observation of direct, modulatory, as well as inhibitory and excitatory effects of the extracts.

Receptor Affinity Screen. Assays for the following receptors were performed by the PDSP/NIMH: (1) muscarinic receptors: M1, M2, M3, M4, M5; (2) serotonin receptors: 5ht1a, 5ht1b, 5ht1d, 5ht1e, 5ht2a, 5ht2b, 5ht2c, 5ht3, 5ht4, 5ht5a, 5ht6, 5ht7; (3) GABA receptors: BZP (rat brain site), GABA A, GABA B; (4) histaminergic receptors: H1, H2, H3, H4; (5) dopamine receptors: D1, D2, D3, D4, D5; (6) transporters: NET, SERT, DAT; (7) opiate receptors: DOR, KOR, MOR; (8) adrenergic receptors: Alpha1A, Alpha1B, Alpha1D, Alpha2A, Alpha2B, Alpha2C, Beta1, Beta2, Beta3; (9) others: Sigma 1, Sigma 2, Ca+Channel. Detailed online protocols are available for all assays at the PDSP/NIMH Web site (<http://pdsp.med.unc.edu/>). The serotonin and histamine receptor protocols have been described.^{38–40}

Pulicatin A (6): pale yellow solid (MeOH); [α]_D²⁵ -53 (c 0.1, CHCl₃); UV (MeOH) λ_{max} (log ε) 211 (4.12), 250 (3.71), 312 (3.50) nm; CD (CHCl₃) λ_{max} (Δε) 263 (+52), 292 (+17), 327 (-1.3) nm; IR (film) ν_{max} 2925, 1700, 1670, 1565, 1535, 1510, 1310, 1265, 760 cm⁻¹; ¹H and ¹³C NMR (see Table 1); HRESIMS m/z 224.0741 [M + H]⁺ (calcd for C₁₁H₁₃NO₂S, 224.0740).

Pulicatin B (7): pale yellow solid (MeOH); [α]_D²⁵ -25 (c 0.1, CHCl₃); UV (MeOH) λ_{max} (log ε) 211 (4.11), 250 (3.69), 312 (3.47) nm; CD (CHCl₃) λ_{max} (Δε) 276 (-17), 324 (7.6) nm; IR (film) ν_{max} 2909, 1685, 1520, 1457, 1415, 1340, 1310, 1265, 770 cm⁻¹; ¹H and ¹³C NMR (see Table 1); HRESIMS m/z 224.0741 [M + H]⁺ (calcd for C₁₁H₁₃NO₂S, 224.0740).

Pulicatin C (8): pale yellow solid (MeOH); UV (MeOH) λ_{max} 204 (4.08), 296 (3.82), 326 (3.79) nm; IR (film) ν_{max} 2925, 1700, 1625, 1505, 1415, 1280, 1000, 775 cm⁻¹; ¹H and ¹³C NMR (see Table 1); HRESIMS m/z 222.0584 [M + H]⁺ (calcd for C₁₁H₁₁NO₂S, 222.0583).

Pulicatin D (9): white solid (CHCl₃); UV (MeOH) λ_{max} 219 (4.10), 289 (3.84), 321 (3.77) nm; IR (film) ν_{max} 2835, 1700, 1670, 1535, 1415, 1430, 1295, 1250, 745 cm⁻¹; ¹H and ¹³C NMR (see Table 1); HRESIMS m/z 220.0427 [M + H]⁺ (calcd for C₁₁H₉NO₂S, 220.0427).

Pulicatin E (10): pale yellow solid (MeOH); UV (MeOH) λ_{max} 212 (4.11), 279 (3.87), 321 (3.79) nm; IR (film) ν_{max} 2924, 1670, 1595, 1490, 1475, 1415, 1360, 1150, 750 cm⁻¹; ¹H and ¹³C NMR (see Table 1); HRESIMS m/z 235.0546 [M + H]⁺ (calcd for C₁₁H₁₀N₂O₂S, 235.0536).

Acknowledgment. This work was funded by ICBG grant U01TW008163 from Fogarty (NIH). We thank the government of the Philippines and the community of Mactan Island for permission to conduct this study. Binding assay data was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by B. L. Roth MD, PhD, at the University of North Carolina at Chapel Hill and Project Officer J. Driscoll at NIMH, Bethesda, MD, USA.

Supporting Information Available: NMR data for pulicatin A–E (**6**–**10**), DRG assay results, application of LIS, ¹H NMR of MTPA esters for compounds **1**, **6**, and **7**, and HRESIMS data of compounds **1** and **6**–**10**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Schmidt, E. W. *Nat. Chem. Biol.* **2008**, *4*, 466–473.
- Piel, J. *Nat. Prod. Rep.* **2009**, *26*, 338–362.
- Piel, J. *Nat. Prod. Rep.* **2004**, *21*, 519–538.
- White, J. F.; Torres, M. S. *Defensive Mutualism in Microbial Symbiosis*; CRC Press: New York, 2009.
- Gil-Turnes, M. S.; Hay, M. E.; Fenical, W. *Science* **1989**, *246*, 116–118.
- Terlau, H.; Olivera, B. M. *Physiol. Rev.* **2004**, *84*, 41–68.
- Peraud, O.; Biggs, J. S.; Hughen, R. W.; Light, A. R.; Concepcion, G. P.; Olivera, B. M.; Schmidt, E. W. *Appl. Environ. Microbiol.* **2009**, *75*, 6820–6826.
- Light, A. R.; Hughen, R. W.; Zhang, J.; Rainier, J.; Liu, Z.; Lee, J. *J. Neurophysiol.* **2008**, *100*, 1184–1201.
- Martin, D. J.; McClelland, D.; Herd, M. B.; Sutton, K. G.; Hall, M. D.; Lee, K.; Pinnock, R. D.; Scott, R. H. *Neuropharmacology* **2002**, *42*, 353–366.
- Zamri, A.; Abdallah, M. A. *Tetrahedron* **1999**, *56*, 249–256.
- Zunnundzhanov, A.; Bessonova, I. A.; Abdullaev, N. D.; Ogai, D. K. *Khim. Prir. Soedin.* **1987**, *553*, 558.
- You, M.-X.; Zhang, H.-P.; Hu, C.-Q. *Chin. J. Chem.* **2008**, *26*, 1332–1334.
- Mathur, K. B.; Iyer, R. N.; Dhar, M. L. *J. Sci. Ind. Res., Sect. B* **1962**, *21B*, 34–37.
- Istanbulu, I.; Safak, C.; Sahin, M. F. *Hacettepe Univ. Eczacilik Fak. Derg.* **1986**, *6*, 21–28.
- Sasaki, O.; Igarashi, Y.; Saito, N.; Furumai, T. *J. Antibiot. (Tokyo)* **2002**, *55*, 249–255.
- Yasuhara, F.; Yamaguchi, S. *Tetrahedron Lett.* **1977**, *47*, 4085–4088.
- Sugimoto, Y.; Tsuyuki, T.; Moriyama, Y.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 3723–3724.
- Len, C.; Pilard, S.; Lipka, E.; Vaccher, C.; Dubois, M.-P.; Shrinška, Y.; Tran, V.; Rabiller, C. *Tetrahedron* **2005**, *61*, 10583–10595.
- Kikuchi, K.; Chen, C.; Adachi, K.; Nishijima, M.; Araki, M.; Sano, H. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1996**, *38th*, 427–432.
- <http://pdsp.med.unc.edu/>.
- Brea, J.; Castro-Palomino, J.; Yeste, S.; Cubero, E.; Parraga, A.; Dominguez, E.; Loza, M. I. *Curr. Top. Med. Chem.* **2010**, *5*, 493–503.
- Aira, Z.; Buesa, I.; Salgueiro, M.; Bilbao, J.; Aguilera, L.; Zimmermann, M.; Azkue, J. *J. Neuroscience* **2010**, *168*, 831–841.
- Yamada, Y.; Seki, N.; Kitahara, T.; Takahashi, M.; Matsui, M. *Agric. Biol. Chem.* **1970**, *34*, 780–783.
- Kikuchi, K.; Chen, Y.; Adachi, K.; Nishijima, M.; Nishida, A.; Takatera, T.; Sano, H. *Jpn. Kokai Tokkyo Koho* **1998**, *6 pp*.
- Naegeli, H. U.; Zaehner, H. *Helv. Chim. Acta* **1980**, *63*, 1400–1406.
- Cox, C. D.; Rinehart, K. L., Jr.; Moore, M. L.; Cook, J. C., Jr. *Proc. Natl. Acad. Sci. U. S. A.* **1981**, *78*, 4256–4260.
- Drechsel, H.; Stephan, H.; Lotz, R.; Haag, H.; Zaehner, H.; Hantke, K.; Jung, G. *Liebigs Ann.* **1995**, *10*, 1727–1733.
- Griffiths, G. L.; Sigel, S. P.; Payne, S. M.; Neilands, J. B. *J. Biol. Chem.* **1984**, *259*, 383–385.
- Ino, A.; Kobayashi, S.; Hidaka, S.; Kawamura, Y.; Ozaki, M.; Hayase, Y.; Takeda, R.; Murabayashi, A. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1996**, *38*, 121–126.

- (30) Kobayashi, S.; Hidaka, S.; Kawamura, Y.; Ozaki, M.; Kayase, Y. *J. Antibiot.* **1998**, *51*, 323–327.
- (31) Shindo, K.; Takenaka, A.; Noguchi, T.; Hayakawa, Y.; Seto, H. *J. Antibiot.* **1989**, *42*, 1526–1529.
- (32) Bukovits, G. J.; Mohr, N.; Budzikiewicz, H.; Korth, H.; Pulverer, G. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1982**, *37B*, 877–880.
- (33) Waterfield, N. R.; Sanchez-Contreras, M.; Eleftherianos, I.; Dowling, A.; Wilkinson, P.; Parkhill, J.; Thomson, N.; Reynolds, S. E.; Bode, H. B.; Dorus, S.; ffrench-Constant, R. H. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 15967–15972.
- (34) Udvary, D. W.; Zeigler, L.; Asolkar, R. N.; Singan, V.; Lapidus, A.; Fenical, W.; Jensen, P. R.; Moore, B. S. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10376–10381.
- (35) Carmi, R.; Carmeli, S. *J. Nat. Prod.* **1994**, *57*, 1200–1205.
- (36) Matsui, M.; Yamada, Y.; Takahashi, M.; Seki, N. *Ger. Offen. DE 2045818 A 19710408*, 1971.
- (37) Imai, T.; Takahashi, M.; Seki, N.; Irie, Y. *Ger. Offen. DE 2045819 A 19710408*, 1971.
- (38) Roth, B. L.; Nakaki, T.; D M Chuang, D. M.; Costa, E. *J. Pharmacol. Exp. Ther.* **1986**, *238*, 480–485.
- (39) Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1403–1410.
- (40) Moguelevsky, N.; Varsalona, F.; Noyer, M.; Gillard, M.; Guillaume, J. P.; Lida Garcia, L.; Szpirer, C.; Szpirer, J.; Bollen, A. *Eur. J. Biochem.* **1994**, *224*, 489–495.

NP100588C