

# Spongian Diterpenes with Thyrotropin Releasing Hormone Receptor 2 Binding Affinity from *Spongia* sp.

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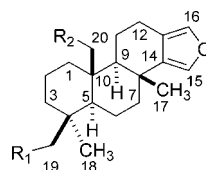
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High-throughput screening of a plant and marine invertebrate extract library to find natural products with rat thyrotropin releasing hormone (TRH) receptor 2 binding affinity led to the isolation of four new (**1–4**) and one known (**5**) spongian diterpene from the sponge *Spongia* sp. The structures were assigned from interpretation of 2D NMR and high-resolution ESIMS data. The absolute configurations of **1–4** were proposed on the basis of analysis of their CD spectra. Diterpenes **1–5** showed rat TRH receptor 2 binding affinity with IC<sub>50</sub> values of 23  $\mu$ M, 70  $\mu$ M, 400  $\mu$ M, 600  $\mu$ M, and 1 mM, respectively.

Thyrotropin releasing hormone (TRH) is a tripeptide that has been proposed to play an important role in neurotransmitter signaling.<sup>1</sup> TRH binds specifically to G-protein coupled receptors and TRH receptors, and binding sites have been found in the brain, pituitary, and dorsal and ventral horns of the spinal cord, suggesting a potential role for TRH in pain control.<sup>2</sup> Two subtypes of the TRH receptor, TRH-R1 and TRH-R2, are found in rat brain tissues, and their distribution is remarkably complementary, with TRH-R1 being highly expressed in neuroendocrine brain regions and TRH-R2 being expressed in brain regions that are important for the transmission of somatosensory signals and higher brain function.<sup>3–8</sup> Agonists and antagonists of TRH binding therefore show potential therapeutic value in regulating endocrine function, in controlling pain, and in the treatment of spinal cord injury.

We undertook a high-throughput screening campaign of 20 000 Queensland plants and marine invertebrates to find natural products that show TRH-R2 binding affinity and have already reported the discovery of two bioactive acylphloroglucinols, corymbones A and B, from the flowers of *Corymbia peltata*.<sup>9</sup> This paper reports on the purification, structure determination, and biological activity of four new and one known spongian diterpene isolated from a TRH-R2 bioactive extract from the Great Barrier Reef sponge *Spongia* sp.

The CH<sub>2</sub>Cl<sub>2</sub>, MeOH, and H<sub>2</sub>O extracts of *Spongia* sp. were screened for TRH-R2 binding affinity, and only the CH<sub>2</sub>Cl<sub>2</sub> extract displaced tritiated 3-methyl-TRH from rat TRH-R2. Bioassay-guided purification of the CH<sub>2</sub>Cl<sub>2</sub> extract by centrifugal partition chromatography (CPC) with a solvent system consisting of heptane/CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN (10:3:7) eluting first with the upper phase in ascending mode and then switching to descending mode with the lower phase as mobile phase generated four bioactive fractions. A late eluting fraction was the most bioactive, but it consisted of a mixture of two monoacetates, **1** and **2**. This fraction was further purified by HPLC on diol-bonded Si gel, eluting with hexane/CH<sub>2</sub>Cl<sub>2</sub> (45:55) to yield compound **1** (3.2 mg, 0.32%) and compound **2** (1.0 mg, 0.10%). Two fractions that eluted before the CPC mode was switched from ascending to descending and one fraction that eluted just after it was switched were pure weakly bioactive compounds, **5** (8.6 mg, 0.86%), **3** (4.4 mg, 0.44%), and **4** (8.6 mg, 0.86%), respectively. Compound **5** was identified as the known compound spongia-13(16),14-diene from 2D NMR analysis and by comparison with literature data.<sup>10</sup>



- 1: R<sub>1</sub> = OH, R<sub>2</sub> = OAc
- 2: R<sub>1</sub> = OAc, R<sub>2</sub> = OH
- 3: R<sub>1</sub> = OAc, R<sub>2</sub> = OAc
- 4: R<sub>1</sub> = OH, R<sub>2</sub> = OH
- 5: R<sub>1</sub> = H, R<sub>2</sub> = H

Compound **1** was isolated as an optically active colorless gum. It displayed a [M + Na]<sup>+</sup> ion at *m/z* 383.2207 in the (+) HRESIFTMS, allowing a molecular formula of C<sub>22</sub>H<sub>32</sub>O<sub>4</sub> to be assigned. Infrared absorption bands at 3411 and 1738 cm<sup>-1</sup> suggested **1** contained alcohol and ester functionalities. The <sup>1</sup>H NMR spectrum (Table 1) exhibited signals for a 3,4-disubstituted furan ( $\delta_{\text{H}}$  6.93, H-16 and 7.00, H-15), three quaternary methyl groups ( $\delta_{\text{H}}$  1.64, 1.17, and 0.85, 20-Ac, H<sub>3</sub>-17, and H<sub>3</sub>-18, respectively), two oxygenated methylenes ( $\delta_{\text{H}}$  4.17 and 4.52 H<sub>2</sub>-20, and  $\delta_{\text{H}}$  3.15 and 3.22, H<sub>2</sub>-19) and a further 14 proton multiplets which resonated upfield of  $\delta_{\text{H}}$  2.60. Analysis of the gHMQC spectrum of **1** indicated that the molecule contained seven aliphatic methylenes ( $\delta_{\text{C}}$  18.3, 19.4, 21.0, 22.6, 35.6, 35.8, 42.3, C-6, C-2, C-11, C-12, C-1, C-3, and C-7, respectively), two oxygenated methylenes ( $\delta_{\text{C}}$  64.5, C-20 and 65.5, C-19), two bridgehead methines ( $\delta_{\text{C}}$  57.2, C-5 and 57.6, C-9), two furan methines ( $\delta_{\text{C}}$  135.7, C-15 and 136.9, C-16), and three methyls ( $\delta_{\text{C}}$  20.4, 25.8, and 27.4 (20-Ac, C-17, and C-18, respectively)). Correlations observed in the gCOSY spectrum revealed that **1** had a –CH<sub>2</sub>–CH<sub>2</sub>CH<sub>2</sub>– and two –CH<sub>2</sub>CH<sub>2</sub>CH– partial structures. HMBC correlations from the methyl signal  $\delta_{\text{H}}$  0.85 (H-18) to  $\delta_{\text{C}}$  35.8 (C-3), 38.2 (C-4), and 57.2 (C-5), from the second methyl signal  $\delta_{\text{H}}$  1.17 (H-17) to  $\delta_{\text{C}}$  42.3 (C-7), 34.8 (C-8), and 57.6 (C-9), and from the oxygenated methylene proton  $\delta_{\text{H}}$  4.52 (H-20b) to  $\delta_{\text{C}}$  35.6 (C-1), 57.2 (C-5), 57.6 (C-9), and 41.7 (C-10) provided evidence that the three propyl partial structures were joined to form a decalin. Additional HMBC correlations from  $\delta_{\text{H}}$  6.93 (H-16), 7.00 (H-15), 2.56 (H-12 $\alpha$ ), and 2.18 (H-12 $\beta$ ) to  $\delta_{\text{C}}$  120.0 (C-13) and from  $\delta_{\text{H}}$  1.17 (H-17) to  $\delta_{\text{C}}$  137.5 (C-14) established that **1** possessed a spongian skeleton. The second oxygenated methylene was attached to C-4 since HMBC correlations were observed between  $\delta_{\text{H}}$  0.85 (H-18) and  $\delta_{\text{C}}$  65.5 (C-19). HMBC correlations from the methyl proton  $\delta_{\text{H}}$  1.64 and the oxygenated methylene proton  $\delta_{\text{H}}$  4.52 (H-20b) to a carbonyl carbon at  $\delta_{\text{C}}$  170.0 indicated that an acetate was attached to C-20. The orientation of the hydroxymethylene C-19 was concluded to be  $\beta$  (axial) since the methyl (C-18) that was also attached at C-4 resonated at  $\delta_{\text{C}}$  27.4, and this was indicative of an equatorial ( $\alpha$ ) methyl group.<sup>11</sup> ROESY correlations between H-19a and H-19b to H-20a and H-20b confirmed that the two oxygenated methylenes were 1,3-diaxial. Additional ROESY

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**Table 1.** NMR Data for Compounds **1–4** (600 MHz) in C<sub>6</sub>D<sub>6</sub>

	<b>1</b>		<b>2</b>		<b>3</b>		<b>4</b>	
	$\delta_C^a$	$\delta_H$ (J, Hz)	$\delta_C^a$	$\delta_H$ (J, Hz)	$\delta_C^a$	$\delta_H$ (J, Hz)	$\delta_C^a$	$\delta_H$ (J, Hz)
1	35.6	0.54, ddd (3.6; 13.2; 13.8) 1.99, ddd (5.2; 5.2; 13.2)	35.1	0.49, ddd (3.6; 13.2; 13.8) 2.07, dd (5.2; 13.2)	35.6	0.53, ddd (3.6; 13.2; 13.8) 1.94, dd (5.2; 13.2)	36.2	0.56, ddd (3.6; 13.2; 13.8) 1.88, m
2	19.4	1.31, m 1.52, dddd (3.6; 3.6; 13.8; 13.8; 13.8)	19.9	1.35, m 1.61, dddd (3.6; 3.6; 13.8; 13.8; 13.8)	18.9	1.41, m 1.62, m	19.9	1.35, m 1.58, m
3	35.8	0.78, ddd (4.2; 13.8; 13.8) 1.70, m	36.3	0.85, m 1.67, m	36.2	0.81, m 1.65, m	36.5	0.85, m 1.59, m
4	38.2	—	36.9	—	36.4	—	38.9	—
5	57.2	0.89, dd (5.0; 12)	56.8	0.85, m	57.0	0.85, m	56.9	0.88, m
6	18.3	1.25, m 1.44, m	18.8	1.38, m 1.44, m	18.7	1.28, m 1.46, m	19.1	1.27, m 1.47, m
7	42.3	1.34, m 1.83, m	42.7	1.31, m 1.82, m	42.0	1.29, m 1.78, m	42.5	1.34 m 1.89 m
8	34.8	—	34.8	—	34.7	—	34.8	—
9	57.6	1.06, d (12)	57.3	1.00, m	57.8	1.03, m	57.7	1.10 m
10	41.7	—	40.3	—	40.9	—	42.4	—
11	21.0	1.82, m	20.5	1.67, m	21.2	1.67, m	20.4	1.70 m
12	22.6	2.18, dddd (1.8; 6.6; 13.2; 15.6) 2.56, dd (5.4; 15.6)	22.4	2.17, ddd (1.8; 6.6; 13.8; 16.8) 2.58, dd (4.8; 16.8)	22.2	2.14, ddd (6.6; 13.2; 15.6) 2.54, dd (5.4; 15.6)	22.2	2.18, ddd (6.6, 13.2, 15.6) 2.59, bd (15.6)
13	120.0	—	120.0	—	119.9	—	120.0	—
14	137.5	—	137.8	—	136.9	—	138.0	—
15	135.7	7.00, ddd (0.6; 0.6; 1.8)	135.5	7.01, bd (1.5)	135.7	7.03, bd (1.5)	135.6	7.02, bd (1.5)
16	136.9	6.93 bd (1.8)	136.7	6.90, q (1.5)	136.9	6.98, q (1.5)	136.7	6.91, bd (1.5)
17	25.8	1.17, s	26.0	1.20, s	25.7	1.12, s	25.7	1.29, s
18	27.4	0.85, s	27.5	0.90, s	28.1	0.84, s	28.3	0.85, s
19	65.5	3.15, dd (4.2; 10.2) 3.22, dd (4.2; 10.2) 0.59, t (4.2)	66.9	3.84, d (10.8) 4.20, d (10.8)	66.6	3.91, d (10.2) 4.21, d (10.2)	66.7	3.25, d (10.2) 3.32, d (10.2)
19-OH	—	—	—	—	—	—	—	—
20	64.5	4.17, dd (1.2; 12.0) 4.52, d (12.0)	62.4	3.44, d (11.4) 3.50, d (11.4) 0.38, m	64.3	4.16, d (12.0) 4.47, d (12.0)	63.2	3.52, s
20-OH	—	—	—	—	—	—	—	—
19-Ac	—	—	170.1	—	169.9	—	—	—
19-Ac	—	—	20.5	1.67, s	20.5	1.67, s	—	—
20-Ac	170.0	—	—	—	170.1	—	—	—
20-Ac	20.4	1.64, s	—	—	20.3	1.67, s	—	—

<sup>a</sup> <sup>13</sup>C NMR chemical shifts were assigned from HMQC and HMBC correlations.

correlations were observed between H-17 and H-20a and H-20b, while H-18 correlated to H-5 and H-3 $\alpha$  and H3 $\beta$ . The structure of **1** was established as 20-acetoxy-19-hydroxyspongia-13(16),14-diene. The CD spectrum of **1** showed positive Cotton effects at  $[\theta]_{272} = 0.57 \times 10^4$  and  $[\theta]_{231} = 1.24 \times 10^4$ , which was consistent with the molecule possessing a 4*S*,5*R*,8*R*,9*R*,10*S*-configuration.<sup>12–15</sup>

Accurate mass measurement of the  $[M + Na]^+$  ion at  $m/z$  383.2200 in the (+) HRESIFTMS allowed a molecular formula of C<sub>22</sub>H<sub>32</sub>O<sub>4</sub> to be assigned to **2**. Inspection of the <sup>1</sup>H NMR spectrum of **2** (Table 1) clearly indicated that **2** was isomeric with **1** since all but the signals associated with the two oxygenated methylenes were almost identical in chemical shift. The acetate was attached to C-19 since HMBC correlations were observed from H-18 ( $\delta_H$  0.90) to C-19 ( $\delta_C$  66.9) and from the methylene proton at  $\delta_H$  4.20 (H-19b) and the methyl singlet at 1.67 to an ester carbonyl at  $\delta_C$  170.1. Compound **2** was therefore 20-hydroxy-19-acetoxyspongia-13(16),14-diene. A CD spectrum very similar to **1** ( $[\theta]_{273} = 0.71 \times 10^4$  and  $[\theta]_{229} = 1.25 \times 10^4$ ) suggested that **2** also possessed a 4*S*,5*R*,8*R*,9*R*,10*S*-configuration.

The LRESIMS of compound **3** had a  $[M + Na]^+$  ion at  $m/z$  425, which in combination with NMR data allowed a molecular formula of C<sub>24</sub>H<sub>34</sub>O<sub>5</sub> to be assigned to the structure. Compound **3** decomposed before an accurate mass measurement could be obtained. The <sup>1</sup>H NMR spectrum of **3** (Table 1) was also very similar to that of **1**, the only major differences being the addition of a methyl singlet at  $\delta_H$  1.67 and the downfield shift of H-19a and H-19b to  $\delta_H$  3.91 and 4.21. These data were consistent with an additional acetate being attached to C-19 in compound **3**. In fact the chemical shifts for the protons attached to C-19 were almost identical with those observed in **2**. Analysis of 2D NMR spectra (gCOSY, gHMQC, and gHMBC) acquired for **3** confirmed this assignment. Compound **3** was therefore 19,20-diac-

etoxyspongia-13(16),14-diene. Positive Cotton effects ( $[\theta]_{276} = 0.57 \times 10^4$  and  $[\theta]_{231} = 0.90 \times 10^4$ ) indicated that **3** also possessed a 4*S*,5*R*,8*R*,9*R*,10*S*-configuration.

The (+) HRESIFTMS of compound **4** displayed a  $[M + H]^+$  ion peak at  $m/z$  319.2281, allowing a molecular formula of C<sub>30</sub>H<sub>30</sub>O<sub>3</sub> to be assigned. The infrared spectrum lacked an absorption at 1720 cm<sup>-1</sup>, suggesting that **4** did not contain acetates. The lack of acetate methyl signals and the upfield shift of the oxygenated methylene protons H-20a and H-20b, while all other signals were almost identical compared to **1** in the <sup>1</sup>H NMR spectrum of **4** (Table 1), indicated that **4** was 19,20-hydroxyspongia-13(16),14-diene. The CD spectrum for **4** contained positive Cotton effects at  $[\theta]_{277} = 0.63 \times 10^4$  and  $[\theta]_{228} = 2.45 \times 10^4$ , confirming that **4** possessed the same absolute configuration as **1**.

Although over 25 spongian diterpenes have been reported in the literature, compounds **1–4** are the first to be oxygenated at C-20.

Compound **1** was the most active of the five compounds in the TRH-R2 receptor binding assay, exhibiting an IC<sub>50</sub> of 23  $\mu$ M. Compounds **2**, **3**, **4**, and **5** were only weakly active, displaying IC<sub>50</sub>'s of 70  $\mu$ M, 400  $\mu$ M, 600  $\mu$ M, and 1 mM, respectively. The reference compound TRH had an IC<sub>50</sub> of 23 nM.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-1020 polarimeter (10 cm cell). UV spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer, and IR spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Varian Inova 600 MHz NMR spectrometer. Samples were dissolved in benzene, and chemical shifts were calculated relative to the proto-deutero solvent peak (C<sub>6</sub>D<sub>6</sub>) at  $\delta_H$  7.10 and  $\delta_C$  128.0. 2D NMR spectra were recorded at 30 °C using standard Varian pulse

sequences gCOSY, gHMQC, gHMBC, and ROESY. HRESIMS were recorded on a Bruker Daltonics APEX III 4.7e FT mass spectrometer equipped with an Apollo API source. HPLC separations were achieved using a Rainin Microsorb diol-bonded Si gel semipreparative column (3  $\mu$ m, 10 mm  $\times$  50 mm). CPC separation was performed on a SANKI LLB-M high-performance CPC system. All solvents used were Omnisolv HPLC grade, and H<sub>2</sub>O used was Millipore Milli-Q PF filtered.

**Animal Material.** The sponge *Spongia* sp. was collected from the northwest side of West Islet, Wreck Reef, Coral Sea, by scuba at a depth of 20 m in January 1996. A voucher specimen, G306622, is deposited at the Queensland Museum.

This species corresponds to Burton's 1934 "*Spongia officinalis* Linnaeus", a commercial bath sponge from the northern hemisphere, which he excessively grouped with specimens from all over the world. The southeastern Pacific species possibly represents a new species that is morphologically very similar to *S. officinalis*.

The sponge has a lobate, massive growth form, with short tapering fistules on the apex. It is dark brown to blackish alive with dark brown exterior and beige interior. It contains single, conspicuous, discrete oscules of moderate size, on apexes of short tapering fistules, with slightly raised membranous lips. The sponge has a soft, spongy, compressible texture with an opaque, membranous, optically smooth, uneven, microconulose surface ornamentation. The ectosomal skeleton is membranous, darkly pigmented and may be pushed up into microconules by ascending choanosomal primary fibers. The surface is unarmoured. The choanosomal skeleton has regular reticulations of mainly secondary fibers and a reduced number of ascending primary fibers that are relatively small in diameter within the choanosome and slightly larger at the surface. Primary fibers are lightly cored with sand and spicule detritus. Fibers are nonlaminated and are not pithed. Mesohyl collagen is light and slightly granular.

**Extraction and Isolation.** The freeze-dried ground sponge *Spongia* sp. (1 g) was extracted exhaustively with CH<sub>2</sub>Cl<sub>2</sub> (200 mL), yielding a colorless gum (123 mg).

The CH<sub>2</sub>Cl<sub>2</sub> extract was subjected to centrifugal partition chromatography with a solvent system consisting of heptane/CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN (10:3:7) elution in ascending mode with the upper phase. Nine fractions were collected. The mode was switched to descending mode with the lower phase as mobile phase, and a further five fractions were collected. Fraction 13 (21.4 mg) was the most bioactive fraction. Fractions 9 and 12 were less active but were pure compounds, **3** (4.4 mg) and **4** (8.6 mg). Fraction 3 was compound **5** (8.6 mg). Fraction 13 was purified by HPLC on diol-bonded Si gel eluting with *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub> (45:55), yielding compounds **2** (1.0 mg) and **1** (3.2 mg).

**20-Acetoxy-19-hydroxyspongia-13(16),14-diene (1):** colorless gum;  $[\alpha]^{17.9}_D -141.3$  (*c* 0.15, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 221 (4.19) nm; CD  $\lambda_{ext}([\theta])$  231 (+12 400), 272 (+5720) nm; IR (KBr)  $\nu_{max}$  3411 br, 2933, 2852, 1738, 1370, 1240, 1038, 755 cm<sup>-1</sup>; <sup>1</sup>H (600 MHz, C<sub>6</sub>D<sub>6</sub>) and <sup>13</sup>C NMR (150 MHz, C<sub>6</sub>D<sub>6</sub>), Table 1; (+)-HRESIMS *m/z* 383.2207 [M + Na]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>32</sub>O<sub>4</sub>Na, 383.2193).

**19-Acetoxy-20-hydroxyspongia-13(16),14-diene (2):** colorless gum;  $[\alpha]^{17.9}_D -263.0$  (*c* 0.08, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 224 (4.20) nm; CD  $\lambda_{ext}([\theta])$  229 (+12 480), 273 (+7140) nm; IR (KBr)  $\nu_{max}$  3412 br, 2932, 1725, 1381, 1244, 1036, 755 cm<sup>-1</sup>; <sup>1</sup>H (600 MHz, C<sub>6</sub>D<sub>6</sub>) and <sup>13</sup>C NMR (150 MHz, C<sub>6</sub>D<sub>6</sub>), Table 1; (+)-HRESIMS *m/z* 383.2200 [M + Na]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>32</sub>O<sub>4</sub>Na, 383.2193).

**19,20-Diacetoxyspongia-13(16),14-diene (3):** colorless gum;  $[\alpha]^{17.9}_D -160.4$  (*c* 0.36, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 217 (4.20) nm; CD  $\lambda_{ext}([\theta])$  231 (+8900), 276 (+5690) nm; IR (KBr)  $\nu_{max}$  3409 br, 2930, 2871, 1738, 1727, 1379, 1357, 1242, 1032 cm<sup>-1</sup>; <sup>1</sup>H (600 MHz, C<sub>6</sub>D<sub>6</sub>) and <sup>13</sup>C NMR (150 MHz, C<sub>6</sub>D<sub>6</sub>), Table 1; (+)-LRESIMS *m/z* 425 [M + Na]<sup>+</sup>.

**19,20-Dihydroxyspongia-13(16),14-diene (4):** colorless gum;  $[\alpha]^{17.9}_D -25.6$  (*c* 0.17, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 222 (4.18) nm; CD  $\lambda_{ext}([\theta])$  228 (+24 500), 277 (+6280) nm; IR (KBr)  $\nu_{max}$  3378 br, 2930, 1380, 1244, 1024, 755 cm<sup>-1</sup>; <sup>1</sup>H (600 MHz, C<sub>6</sub>D<sub>6</sub>) and <sup>13</sup>C NMR (150 MHz, C<sub>6</sub>D<sub>6</sub>), Table 1; (+)-HRESIMS *m/z* 319.2281 [M + H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>31</sub>O<sub>3</sub>, 319.2268).

**TRHR-2 Receptor Binding Assay.** Assays were performed in 50 mM Tris buffer containing 3 mM MgCl<sub>2</sub> and 1 mg/mL BSA, pH 7.4, with HEK2935 cell membranes expressing recombinant rat TRH receptor 2 (supplied by AstraZeneca R&D Montreal) (~10  $\mu$ g of protein as determined by the Pierce BCA method) and [<sup>3</sup>H]3-methylhistidyl-TRH (1 nM equivalent to 50 000 dpm) in a total volume of 210  $\mu$ L. Controls included 3  $\mu$ M TRH for nonspecific binding. Compounds were tested at a final concentration of 2% DMSO. Reactions were initiated by the addition of membranes and then continuously mixed for 90 min at 23 °C prior to rapid filtration and washing over GF/B filtermats (Tomtec 96 Mach 2). Mats were dried and counted for 1 min per assay by liquid scintillometry (Betaplate, Wallac). IC<sub>50</sub> values for the isolated compounds were obtained by testing three wells per concentration.

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**Supporting Information Available:** Photograph of the sponge *Spongia* sp. This material is available free of charge via the Internet at <http://pubs.asc.org>.

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