New Steroids and a New Alkaloid from the Gorgonian *Isis minorbrachyblasta:* Structures, Cytotoxicity, and Antilarval Activity

by **Shu-Hua Qi***[a]), **Li Miao**[b]), **Cheng-Hai Gao**[a]), **Ying Xu**[b]), **Si Zhang**[a]), and **Pei-Yuan Qian**[b])

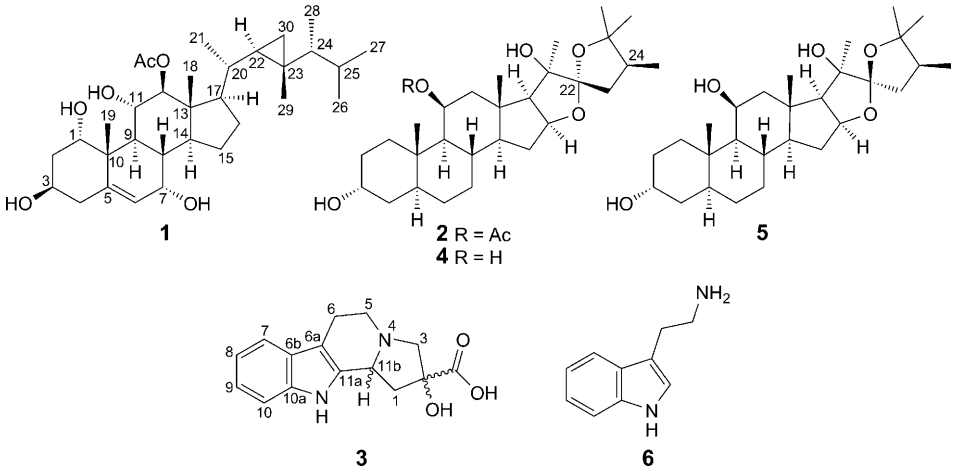
[a]) Key Laboratory of Marine Bio-resources Sustainable Utilization/Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, P. R. China (phone: +86-20-89023105; fax: +86-20-84458964; e-mail: shuhuaqi2001@yahoo.com)

[b]) Hong Kong University of Science and Technology/Department of Biology – CML/Clearwater Bay, KLN, Hong Kong, P. R. China

Two new polyoxygenated steroids, (1α,3β,7α,11α,12β)-gorgost-5-ene-1,3,7,11,12-pentol 12-acetate (**1**) and 11-*O*-acetyl-22-epihippuristanol (**2**), and a new alkaloid, 2,3,5,6,11,11b-hexahydro-2-hydroxy-1*H*-indolizino[8,7-*b*]indole-2-carboxylic acid (**3**), together with three known compounds, 22-epihippuristanol (**4**), hippuristanol (**5**), and tryptamine (**6**), were isolated from the EtOH/CH_2Cl_2 extracts of the South China Sea gorgonian *Isis minorbrachyblasta*. The structures of the new compounds were determined by spectroscopic methods. Compound **1** showed weak cytotoxicity against A549, HONE1, and HeLa cancer cell lines and strong antilarval activity towards *Bugula neritina* larvae with an EC_{50} value of 5.8 μg/ml. Compound **5** showed moderate cytotoxicity against A549, HONE1, and HeLa cell lines, and the epimer mixture **4**/**5** (weight ratio 3:2) exhibited potent cytotoxicity against A549 and HONE1 cell lines with IC_{50} values of 4.2 and 4.8 μg/ml, which indicated that epimers **4** and **5** might have a synergistic effect on their cytotoxicity against A549 and HONE1 cell lines.

Introduction. – Both *Isis minorbrachyblasta* and *I. hippuris* belong to the Isis genus, Isididae family of gorgonian corals. There are reports that *I. hippuris* is unique concerning its steroidal constituents that can be characterized as of the hippurin or hippuristanol type having a spiroketal [1–4], of the gorgosterol type possessing a cyclopropane [5–7], and of the hippuristerone type containing a 3-keto function [4][8]. These types also differ in the side-chain alkylation pattern. Some of these compounds have been reported to have significant biological activities, *e.g.*, hippuristanol and (2α)-2-hydroxyhippuristanol showed 50% inhibition (*in vitro*) of DBA/MC fibrosarcoma at 0.8 and 0.1 μg/ml [2], several hippuristanol-type steroids showed potent cytotoxicity against P-388, A549, HT29, and MEL28 cancer cell lines [4], and several gorgosterol-type steroids showed moderate activity against drug-resistant cells (KBC2) expressing P-glycoprotein [7]. However, there were no reports about the chemical constituents of *Isis minorbrachyblasta*. In our present investigation on the chemical constituents of the South China Sea gorgonian *I. minorbrachyblasta* ZOU 1991, two new steroids, (1α,3β,7α,11α,12β)-gorgost-5-ene-1,3,7,11,12-pentol 12-acetate (**1**) and 11-*O*-acetyl-22-epihippuristanol (**2**), and a new alkaloid, 2,3,5,6,11,11b-hexahydro-2-hydroxy-1*H*-indolizino[8,7-*b*]indole-2-carboxylic acid (**3**), were obtained, together with the three known compounds 22-epihippuristanol (**4**) [4], hippuristanol (**5**) [2][4], and tryptamine (**6**) [9]. The cytotoxicity of **1**, **5**, and the

epimer mixture **4**/**5** (weight ratio *ca.* 3:2) against human lung carcinoma A549, nasopharyngeal carcinoma HONE1, and cervical carcinoma HeLa cell lines, and antilarval activity of **1** and **5** against larval settlement of *Bugula neritina* larvae were evaluated. This article deals with the isolation and structural elucidation of **1**–**3** and biological activities of **1**, **5**, and the epimer mixture **4**/**5**.



Results and Discussion. – Compound **1** has the molecular formula $C_{32}H_{52}O_6$ as deduced from NMR spectra and HR-ESI-MS. The ^{13}C-NMR spectrum showed the presence of 32 C-atom signals: seven Me, five CH_2, and eight CH groups, three quaternary C-atoms, five O–CH groups (δ(C) 64.5, 65.7, 72.9, 74.1, and 86.2), a trisubstituted C=C bond (δ(C) 108.8 (*t*) and 116.0 (*d*)), and an AcO group (δ(C) 173.4 (*s*) and 21.2 (*q*)). The ^{1}H-NMR spectrum revealed the presence of four tertiary Me groups (δ(H) 0.78, 0.91, 1.08, and 2.10), four secondary Me groups (δ(H) 0.83, 0.88, 0.90, and 0.92), an olefinic H-atom (δ(H) 5.77 (*d*, J = 4.6 Hz)), five O–CH groups (δ(H) 4.71 (*d*, J = 8.6 Hz), 4.22 (br. *s*, 1 H), 3.90 (1 H, overlap), 3.86 (1 H, overlap), and 3.78 (br. *s*, 1 H)), and three characteristic high-field signals (δ(H) 0.43 (*dd*, J = 4.3 and 8.9 Hz, 1 H), −0.11 (*t*, J = 4.5 Hz, 1 H), and 0.21 (2 H, overlap)) due to the C(30) cyclopropane-ring H-atoms of a gorgosterol-type steroid [5–7]. These signals corresponded well to those observed in the ^{1}H- and ^{13}C-NMR data of (3β,7α,11α,12β)-gorgost-5-ene-3,7,11,12-tetrol 12-acetate and (1α,3β,5α,6β,11α,12β)-gorgostane-1,3,5,6,11,12-hexol 12-acetate that were isolated from gorgonian *I. hippuris* [6], and the only obvious difference between **1** and the former was a CH_2 group oxygenated to an O–CH group in **1**. The HMBCs (*Fig.*) δ(H) 1.08 (*s*, Me(19))/δ(C) 74.1 (*d*), 40.8 (*d*, C(9)), 43.6 (*s*, C(10)), and 142.9 (*s*, C(5)) suggested the assignation of δ(C) 74.1 to C(1) and oxygenation of C(1). Furthermore, OH–C(1) was determined to be in α-configuration by the observation that H–C(1) (δ(H) 4.22) appeared as a broad *s* and by the NOE correlations of H–C(1) with H_β–C(2) (δ(H) 1.82 (*m*)) and Me(19) in the NOESY plot. Based on the NMR-data comparison of **1** with (3β,7α,11α,12β)-gorgost-5-ene-3,7,11,12-tetrol 12-acetate and other analogues [6][7] and analysis of the

2D-NMR data of **1** (including HSQC, HMBC, and NOESY), the structure of **1** was elucidated as (1α,3β,7α,11α,12β)-gorgost-5-ene-1,3,7,11,12-pentol 12-acetate.

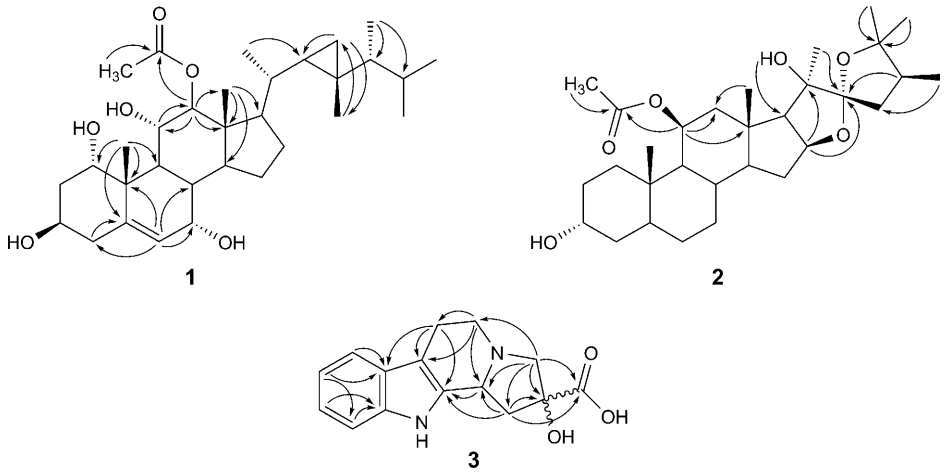


Figure. *Key HMBCs* (H → C) *of compounds* **1**–**3**

Compound **2** has the molecular formula $C_{30}H_{48}O_6$ as deduced from the NMR spectra. The ^{1}H-NMR spectrum revealed the presence of six tertiary Me groups (δ(H) 0.88, 0.97, 1.21, 1.27, 1.29 and 2.00), one secondary Me group (δ(H) 0.94), and three O–CH groups (δ(H) 5.32 (*d*, *J* = 3.1 Hz), 4.45 (*m*, 1 H), and 4.03 (br. *s*, 1 H)). The ^{13}C-NMR spectrum showed the presence of 30 C-atom signals, including 28 basic-skeleton C-atoms: six Me, eight CH_2, and six CH groups, two quaternary C-atoms, three O–CH (δ(C) 66.2, 69.6, and 79.0), three quaternary C-atoms (δ(C) 82.5, 84.2, and 118.6), and an AcO group (δ(C) 170.1 (*s*) and 21.9 (*q*)). These signals corresponded well to those observed in the ^{1}H- and ^{13}C-NMR spectra of 22-epihippuristanol (**4**) [4] and hippuristanol (**5**) [2][4], and the only obvious difference between **2** and **4** was the presence of an additional AcO group in **2**. The HMBCs (*Fig.*) of δ(H) 5.32 (*d*, *J* = 3.1 Hz, H–C(11)) and 2.00 (*s*, 3 H) with δ(C) 170.1 (*s*) suggested that the additional AcO group of **2** was attached to C(11). Furthermore, the NMR signals at δ(H) 4.45 (*m*, H–C(16)) and δ(C) 118.6 (*s*, C(22)) indicated the assignment of H_α–C(16) and (22*S*) configuration (usual data of the (22*S*) series: H–C(16) at δ(H) $\approx$ 4.4 and C(22) at δ(C) $\approx$ 118 usual data of the (22*R*) series: H–C(16) at δ(H) $\approx$ 4.3 and C(22) at δ(C) $\approx$ 115) [4]. Based on the NMR-data comparison of **2** and **4** [4] and analysis of the 2D-NMR data of **2** (including HSQC, HMBC, and NOESY), the structure of **2** was elucidated as (11β)-11-*O*-acetyl-22-epihippuristanol.

Compound **3** has the molecular formula $C_{15}H_{16}N_2O_3$ as deduced from the NMR spectra and HR-ESI-MS. Its negative-mode ESI-MS showed peaks at *m*/*z* 271 ([*M* – 1]$^-$), 211, 183, and 157, and its UV spectrum showed sharp absorptions at λ_{max} 223, 280, and 294 nm, both of which are typical for tetrahydro-β-carboline alkaloids [10][11]. Its ^{1}H-NMR spectrum displayed signals of a four-spin H-atom system at δ(H) 7.43 (*d*, *J* = 7.6 Hz, 1 H), 7.30 (*d*, *J* = 7.6 Hz, 1 H), 7.08 (*t*, *J* = 7.5 Hz, 1 H), and 7.00 (*t*, *J* = 7.5 Hz, 1 H). The ^{13}C-NMR spectrum showed the presence of four CH_2 (δ(C) 20.2,

43.7, 47.6 and 62.5) and one CH group (δ(C) 62.5), one quaternary C-atom (δ(C) 70.2), four low-field CH groups (δ(C) 111.9, 118.8, 119.8, and 122.3), four low-field quaternary C-atoms (δ(C) 107.7, 128.2, 134.5, and 138.2), and one C=O group (δ(C) 179.2). These data suggested that **1** was a 1,2,3,4-tetrahydro-β-carboline alkaloid [10][11]. Comparison of the NMR data between **3** and (11b*R*)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (trivial name harmicine) [11] showed that the only obvious difference between them was the presence of an additional C=O group and an oxygenated quaternary C-atom in **3** instead of a CH_2 group in harmicine, which suggested that **3** should have a harmicine structure. This suggestion was confirmed by the HMBC spectrum (*Fig.*). Furthermore, HMBCs of CH_2(1) (δ(H) 2.49 (br. *d*, J = 7.7 Hz) and CH_2(3) (δ(H) 3.43 and 3.13 (each *d*, J = 11.4 Hz, 1 H)) with, C(2) (δ(C) 70.1 (*s*)) and of H–C(3) with C(1) (δ(C) 43.7 (*t*)), C(5) (δ(C) 47.6 (*t*)), and C(11b) (δ(C) 58.5 (*d*)) suggested the assignment of C(2) (δ(C) 70.1 (*s*)). In addition, HMBCs of CH_2(1) and CH_2(3) with COOH (δ(C) 179.2 (*s*)) and C(2) indicated that the COOH group was attached to C(2) of the harmicine structure. The relative and absolute configurations of compound **3** remain to be determined. Based on the above data, the structure of **3** was elucidated to be 2,3,5,6,11,11b-hexahydro-2-hydroxy-1*H*-indolizino[8,7-*b*]indole-2-carboxylic acid (trivial name 2-hydroxyharmicine-2-carboxylic acid). Rarely, indole-type alkaloids were isolated from gorgonians, the only example being hicksoanes A–C from the gorgonian *Subergorgia hicksoni* [12].

The cytotoxicity of **1**, **5**, and an epimer mixture **4/5** (weight ratio *ca.* 3 : 2, as inferred from a NMR spectrum) against human-lung carcinoma A549, nasopharyngeal carcinoma HONE1, and cervical carcinoma HeLa was evaluated (*Table*). Compound **1** showed weak cytotoxicity against A549, HONE1, and HeLa cell lines, **5** showed moderate cytotoxicity against A549, HONE1, and HeLa cell lines with IC_{50} values of 20.3, 11.8, and 12.1 μg/ml, respectively, while the epimer mixture **4/5** showed potent cytotoxicity against A549 and HONE1 cell lines with IC_{50} values of 4.2 and 4.8 μg/ml and moderate cytotoxicity against HeLa cell line with an IC_{50} value of 10.5 μg/ml, which indicated that epimers **4** and **5** might have a synergistic effect on their cytotoxicity against A549 and HONE1 cell lines. The cytotoxicity IC_{50} value of **5** against A549 was *ca.* tenfold larger than the reported one [4], which might be caused by methodological errors and other factors.

Table. *Cytotoxicity of* **1**, **5**, *and an Epimer Mixture* **4/5** (weight ratio 3 : 2) *against Three Cancer Cell Lines, and Antilarval Settlement Activity of* **1** *and* **5** *against* Bugular neritina *Larvae*

	Cytotoxicity (IC_{50} [μg/ml])			Antilarval activity [μg/ml]	
	A549	HONE1	HeLa	EC_{50}	LC_{50}
1	47.5	49.4	98.3	4.8	> 100
5	20.3	11.8	12.1	42.6	> 100
4/5	4.2	4.8	10.5		

In addition, the antilarval activities of **1** and **5** were evaluated in settlement-inhibition assays with laboratory-reared *B. neritina* larvae. The results (*Table*) revealed that **1** showed strong antilarval activity and low toxicity towards *B. neritina* larvae with an EC_{50} value of 4.8 μg/ml and LC_{50} > 100 μg/ml, and **5** exhibited moderate antilarval

activity towards *B. neritina* larvae with EC_{50} 42.6 μg/ml. The EC_{50} value of **1** was lower than the standard requirement of an EC_{50} of 25 μg/ml established by the US Navy program as an efficacy level for natural antifoulants, indicating that **1** is a potential natural antifouling agent. The cytotoxicity and antilarval activity of compounds **2**–**4** and **6** were not tested because the available amounts were insufficient for a test.

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Experimental Part

General. TLC: silica gel GF_{254} (SiO_2; 200–300 mesh; *Qindao Marine Chemical Factory*, Qindao, P. R. China). Column chromatography (CC): SiO_2 (200–300 mesh; *Qindao Marine Chemical Factory*, Qindao, P. R. China). Optical rotations: *Horiba-SEAP-300* spectropolarimeter. UV Spectra: *Shimadzu-210A* double-beam spectrophotometer; MeOH soln.; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* IR spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AV-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *LCQDECA XP* HPLC/MS^n spectrometer; in m/z (rel. %).

Animal Material. The South China Sea gorgonian coral *Isis minorbrachyblasta* (2.0 kg, wet weight) was collected in Sanya, Hainan Province, China, in May 2007 and identified by *H. Huang*, the South China Sea Institute of Oceanology, Academia Sinica. A voucher specimen (No. 0701) was deposited with the South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

Extraction and Isolation. The frozen specimen was extracted with EtOH/CH_2Cl_2 2:1 (15 l), 3 × at r.t., and the soln. was evaporated. The residue was suspended in H_2O and extracted 3 × each with AcOEt and BuOH. The AcOEt and BuOH layers were concentrated to afford 25 g and 2 g of residues, resp. The AcOEt extract was subjected to CC (SiO_2, petroleum ether/AcOEt 10:0 → 0:10, TLC monitoring): *Fractions 1–9*. *Fr. 3* was subjected to CC (SiO_2 $CHCl_3$/acetone 8:2 → 7:3): **2** (2 mg), **4** (3 mg), **5** (10 mg), and **4**/**5** (weight ratio 3:2, 5 mg). *Fr. 8* was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1; then repeatedly SiO_2, $CHCl_3$/MeOH 8:2 → 7:3): **1** (8 mg), **3** (2.6 mg), and **6** (3 mg).

(1α,3β,7α,11α,12β)-Gorgost-5-ene-1,3,7,11,12-pentol 12-Acetate (**1**): White powder. $[\alpha]_D^{20} = -12$ (c = 0.1, $CHCl_3$). IR: 3418, 1729, 1250. ^1H-NMR (500 MHz, $CDCl_3$): 5.77 (*d*, J = 4.6, H–C(6)); 4.71 (*d*, J = 8.6, H–C(12)); 4.22 (br. *s*, H–C(1)); 3.90 (overlap, H–C(7)); 3.86 (overlap, H–C(11)); 3.78 (br. *s*, H–C(3)); 2.39 (br. *d*, J = 11.0, H_a–C(4)); 2.26 (*t*, J = 12.2, H_b–C(4)); 2.10 (*s*, Ac); 2.07 (overlap, H–C(9)); 2.07–2.04 (*m*, 1 H–C(2), 1 H–C(16)); 1.89–1.87 (*m*, 1 H–C(15)); 1.87–1.84 (*m*, H–C(14)); 1.86–1.82 (*m*, 1 H–C(2)); 1.63–1.60 (*m*, 1 H–C(16)); 1.59–1.56 (*m*, H–C(17)); 1.56–1.53 (*m*, H–C(25)); 1.39–1.36 (*m*, H–C(20)); 1.25–1.22 (*m*, H–C(8)); 1.19–1.13 (*m*, 1 H–C(15)); 1.08 (*s*, Me(19)); 0.92 (*d*, J = 7.0, Me(27)); 0.91 (*s*, Me(29)); 0.90 (*d*, J = 7.0, Me(21)); 0.88 (*d*, J = 7.0, Me(26)); 0.83 (*d*, J = 6.5, Me(28)); 0.78 (*s*, Me(18)); 0.43 (*dd*, J = 4.3, 8.9, 1 H–C(30)); 0.21 (overlap, H–C(22), H–C(24)); −0.11 (*t*, J = 4.5, 1 H–C(30)). ^{13}C-NMR (125 MHz, $CDCl_3$): 173.4 (*s*, Me*C*OO); 142.9 (*s*, C(5)); 126.7 (*d*, C(6)); 86.2 (*d*, C(12)); 74.1 (*d*, C(1)); 72.9 (*d*, C(11)); 65.7 (*d*, C(7)); 64.5 (*d*, C(3)); 57.5 (*d*, C(17)); 50.5 (*d*, C(24)); 47.8 (*d*, C(14)); 46.7 (*s*, C(13)); 43.6 (*s*, C(10)); 41.7 (*t*, C(4)); 40.8 (*d*, C(9)); 37.7 (*t*, C(2)); 36.9 (*d*, C(20)); 33.2 (*d*, C(8)); 32.1 (*d*, C(25)); 30.3 (*d*, C(22)); 27.9 (*t*, C(16)); 25.3 (*s*, C(23)); 23.5 (*t*, C(15)); 22.5 (*q*, C(27)); 22.2 (*q*, C(26)); 21.4 (*d*, C(30)); 21.2 (*q*, *Me*COO); 21.7 (*q*, C(21)); 17.6 (*q*, C(19)); 15.3 (*q*, C(29)); 13.8 (*q*, C(28)); 9.8 (*q*, C(18)). ESI-MS (pos.): 533 ($[M+H]^+$). HR-ESI-MS (pos.): 533.3759 ($[M+H]^+$, $C_{32}H_{53}O_6^+$; calc. 533.3842).

*(11β)-11-*O*-Acetyl-22-epihippuristanol (=(3α,5α,11β,22α,24*S*)-22,25-Epoxy-24-methylfurostan-3,11,20-triol 11-Acetate*; **2**): White powder. $[\alpha]_D^{20} = -20.1$ (c = 0.1, $CHCl_3$). IR: 3435, 1730, 1020. ^1H-NMR (500 MHz, $CDCl_3$): 5.32 (*d*, J = 3.1, H–C(11)); 4.46–4.42 (*m*, H–C(16)); 4.03 (br. *s*, H–C(3)); 2.29–2.26 (*m*, H–C(24)); 2.25 (*dd*, J = 12.2, 6.0, 1 H–C(23)); 2.17–2.15 (*m*, 1 H–C(12)); 2.09–2.06 (*m*,

1 H–C(15)); 2.00 (*s*, Ac); 1.98–1.96 (*m*, H–C(8)); 1.89–1.86 (*m*, 1 H–C(1)); 1.85–1.82 (*m*, 1 H–C(1)); 1.84–1.80 (*m*, 1 H–C(23)); 1.83–1.80 (*m*, 1 H–C(7)); 1.79 (*d*, *J* = 6.5, H–C(17)); 1.78–1.75 (*m*, 1 H–C(2)); 1.60–1.56 (*m*, 1 H–C(4)); 1.56–1.52 (*m*, 1 H–C(2)); 1.55–1.52 (*m*, H–C(5)); 1.50–1.46 (*m*, 1 H–C(15)); 1.45–1.42 (*m*, 1 H–C(12)); 1.37–1.34 (*m*, 1 H–C(6)); 1.35–1.32 (*m*, 1 H–C(4)); 1.29 (*s*, Me(18)); 1.27 (*s*, Me(27)); 1.22–1.19 (*m*, 1 H–C(6)); 1.21 (*s*, Me(21)); 1.08–1.04 (*m*, 1 H–C(7)); 1.05–1.02 (*m*, H–C(14)); 0.97 (*s*, Me(19)); 0.94 (*d*, *J* = 7.3, Me(28)); 0.90 (*dd*, *J* = 11.0, 3.0, H–C(9)); 0.88 (*s*, Me(26)). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.1 (*s*, Me*C*OO); 118.6 (*s*, C(22)); 84.2 (*s*, C(25)); 82.5 (*s*, C(20)); 79.0 (*d*, C(16)); 69.6 (*d*, C(11)); 66.2 (*d*, C(17)); 66.2 (*d*, C(3)); 58.0 (*d*, C(14)); 56.7 (*d*, C(9)); 44.6 (*t*, C(12)); 42.0 (*s*, C(13)); 41.0 (*d*, C(24)); 40.0 (*d*, C(5)); 39.9 (*t*, C(23)); 36.0 (*s*, C(10)); 35.2 (*t*, C(4)); 32.3 (*t*, C(1)); 31.9 (*t*, C(15)); 31.5 (*t*, C(7)); 30.8 (*d*, C(8)); 29.1 (*q*, C(26)); 28.6 (*t*, C(2)); 27.7 (*t*, C(6)); 25.6 (*q*, C(18)); 23.0 (*q*, C(27)); 21.9 (*q*, *Me*COO); 18.9 (*q*, C(21)); 14.7 (*q*, C(28)); 13.9 (*q*, C(19)). ESI-MS (pos.): 505 ($[M+H]^+$). HR-ESI-MS (pos.): 505.3446 ($[M+H]^+$, $C_{30}H_{49}O_6^+$; calc. 505.3529).

*2,3,5,6,11,11b-Hexahydro-2-hydroxy-1*H*-indolizino[8,7-*b*]indole-2-carboxylic Acid* (**3**): White powder. $[\alpha]_D^{20} = +124$ ($c = 0.1$, MeOH). IR: 3420, 1710, 1670, 1650, 1455. UV (MeOH): 223, 280, 294. ^{1}H-NMR (500 MHz, CD_3OD): 7.43 (*d*, *J* = 7.6, H–C(10)); 7.30 (*d*, *J* = 7.6, H–C(7)); 7.08 (*t*, *J* = 7.5, H–C(9)); 7.00 (*t*, *J* = 7.5, H–C(8)); 4.48 (*d*, *J* = 7.8, H–C(11b)); 3.43 (*d*, *J* = 11.4, H_a–C(3)); 3.41–3.38 (*m*, H_a–C(5)); 3.13 (*d*, *J* = 11.4, H_b–C(3)); 3.07–3.04 (*m*, H_b–C(5)); 2.92–2.89 (*m*, H_a–C(6)); 2.79–2.75 (*m*, H_b–C(6)); 2.49 (br. *d*, *J* = 7.7, CH_2(1)). ^{13}C-NMR (125 MHz, CD_3OD): 179.2 (*s*, COOH); 138.2 (*s*, C(10a)); 134.5 (*s*, C(11a)); 128.2 (*s*, C(6b)); 122.3 (*d*, C(8)); 119.8 (*d*, C(9)); 118.8 (*d*, C(10)); 111.9 (*d*, C(7)); 107.7 (*s*, C(6a)); 70.1 (*s*, C(2)); 62.5 (*t*, C(3)); 58.5 (*d*, C(11b)); 47.6 (*t*, C(5)); 43.7 (*t*, C(1)); 20.2 (*t*, C(6)). ESI-MS (neg.): 271 ($[M-H]^-$). HR-ESI-MS (neg.): 271.1154 ($[M-H]^-$, $C_{15}H_{15}N_2O_3^-$; calc. 271.1183).

Cytotoxicity Biossays. Human-lung carcinoma A549, nasopharyngeal carcinoma HONE1, and cervical carcinoma HeLa were purchased from the *American Type Culture Collection* (*ATCC*, Rockville, MD). Cytotoxicity assays were measured by the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method as described previously [13].

Larval-Settlement Bioassays. Antilarval activity of the compounds was evaluated in settlement-inhibition assays with laboratory-reared *Bugula neritina* larvae. The stock soln. of **1** and **5** in DMSO was diluted with autoclaved filtered sea water (FSW) to concentrations ranging from 0.5 to 100 µg/ml. About 20 competent larvae were added to each well in 1 ml of the test soln. Wells containing only FSW with DMSO served as the controls. The plates were incubated at 27° for 1 h. The percentage of larval settlement was determined by counting the settled, live individuals under a dissecting microscope and expressing the result as a proportion of the total number of larvae in the well. The procedures were the same as previously reported [14].

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