Jorunnamycins A—C, New Stabilized Renieramycin-Type Bistetrahydroisoquinolines Isolated from the Thai Nudibranch *Jorunna funebris*

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Jorunnamycins A—C (1a—c), three stabilized renieramycin-type bistetrahydroisoquinolines, were isolated from the mantles, the visceral organs, and the egg ribbons of the Thai nudibranch *Jorunna funebris* that was pretreated with potassium cyanide (KCN), along with five known compounds, renieramycins M (2m), N (2n), O (2o), and Q (2q) and mimosamycin (3). The structures of 1a—c were elucidated from spectroscopic data and by chemical conversion of renieramycin M (2m) into 1c *via* 1a. The chemical stability and the oxidative degradation generating simple isoquinoline alkaloids of a carbinolamine analog 1d, which was easily prepared by reacting 1c with silver nitrate in aqueous acetonitrile, are discussed. The results of cytotoxicity studies are also presented.

Key words jorunnamycin; renieramycin; transformation; nudibranch; cytotoxicity

The nudibranch *Jorunna funebris* (Mollusca: Gastropoda: Opisthobranchia: Nudibranchia: Kentrodorididae) is one of the marine, slug-like invertebrates belonging to phylum Mollusca that lacks a protective hard shell.[1)] This nudibranch and other dorid nudibranchs can accumulate secondary metabolites that are derived from dietary organisms in order to protect themselves from predators.[2—4)] *J. funebris* is carnivorous and feeds mainly on sponges such as *Xestospongia* sp., *Haliclona* sp., *Euplacella cf. australis*,[5)] and *Oceanapia* sp.[6)] In 1988, de Silva and Gulavita isolated several simple isoquinolinequinones including renierol acetate (**4a**), renierol propionate (**4b**), *N*-formyl-1,2-dihydrorenierol acetate (**5a**), and *N*-formyl-1,2-dihydrorenierol propionate (**5b**) from *Xestospongia* sp. and its associated nudibranch *J. funebris*, but no bistetrahydroisoquinolines.[7—9)] An in-depth chemical analysis of the mantle and mucus of the Pacific nudibranch *J. funebris* by Fontana *et al.* resulted in the isolation of the bistetrahydroisoquinoline jorumycin (**6**), the primary assay of which revealed cytotoxicity against NIH 3T3 fibroblast cells and against some tumor cell lines, such as P388 mouse lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma, and MEL28 human melanoma, at very low concentrations.[10)] Compound **6** shows the promising antitumor properties: however, it is available only in very minute quantities, because **6** has relatively an unstable carbinolamine functional group that may be decomposed during isolation procedure. To obtain sufficient quantities of more stable derivatives with retention of bioactivity, we reported that ecteinascidin 770 was isolated from the Thai tunicate *Ecteinascidia thurstoni* pretreated with KCN in methanolic buffer solution.[11)] Furthermore, we have recently succeeded the isolation and structure elucidation of renieramycin M (**2m**) with gram-scale supply from a Thai sponge *Xestospongia* sp. that was also pretreated with KCN.[12)] As series of our search for new metabolites *via* the isolation and characterization of biologically active compounds from Thai marine animals, we found the Thai nudibranch *J. funebris* feeding on the blue sponge *Xestospongia* sp., growing around Sichang Island in the Gulf of Thailand. In this paper, we present the isolation, structure elucidation, and cytotoxicity of three stabilized bistetrahydroisoquinolines jorunnamycins A—C (**1a**—**c**) from the KCN-pretreated *J. funebris*. The preparation of a carbinolamine analog **1d** from **1c** and its chemical stability are also reported.

Results and Discussion

J. funebris (20 animals; 500 g, wet weight) was collected in the vicinity of Sichang Island at the depth of 3—5 m in March 2004 and its egg ribbons (23.2 g, wet weight) were separately obtained in November 2004. The animals were carefully dissected into two parts, the mantles and the visceral organs (the combined digestive glands and gonads). Then, the three parts were separately homogenized with phosphate buffer (pH 7). Aqueous KCN solution was added to each suspension, the mixture was macerated with methanol, and then the extract was filtered. The concentrated extract was partitioned between ethyl acetate and water, and the organic layer was concentrated *in vacuo* to give the residue (mantles, 700 mg; visceral organs, 980 mg; and egg ribbons, 270 mg). Each residue was separately subjected to flash column chromatography on silica gel to give isoquinoline-type compounds, as summarized in Table 1.

Three new compounds were isolated, and which NMR data were very characteristic of renieramycin-type marine natural products. Jorunnamycin A (**1a**) was obtained as a pale yellow amorphous solid. Its structure was identical to deangeloylrenieramycin M, based on NMR and MS data. High-resolution MS of **1a** revealed the molecular formula $C_{26}H_{27}N_3O_7$. All spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, and HR-EI-MS) were in complete agreement with those of the compound derived from semisynthetic methods from **2m**.[12—15)] This is the first report of discovering **1a** from the natural source.

Jorunnamycin B (**1b**) was isolated as a yellowish orange amorphous solid, $[\alpha]_D^{20}+117.6°$ ($c=0.11$, $CHCl_3$). The molecular formula of **1b** was determined to be $C_{26}H_{27}N_3O_8$ on

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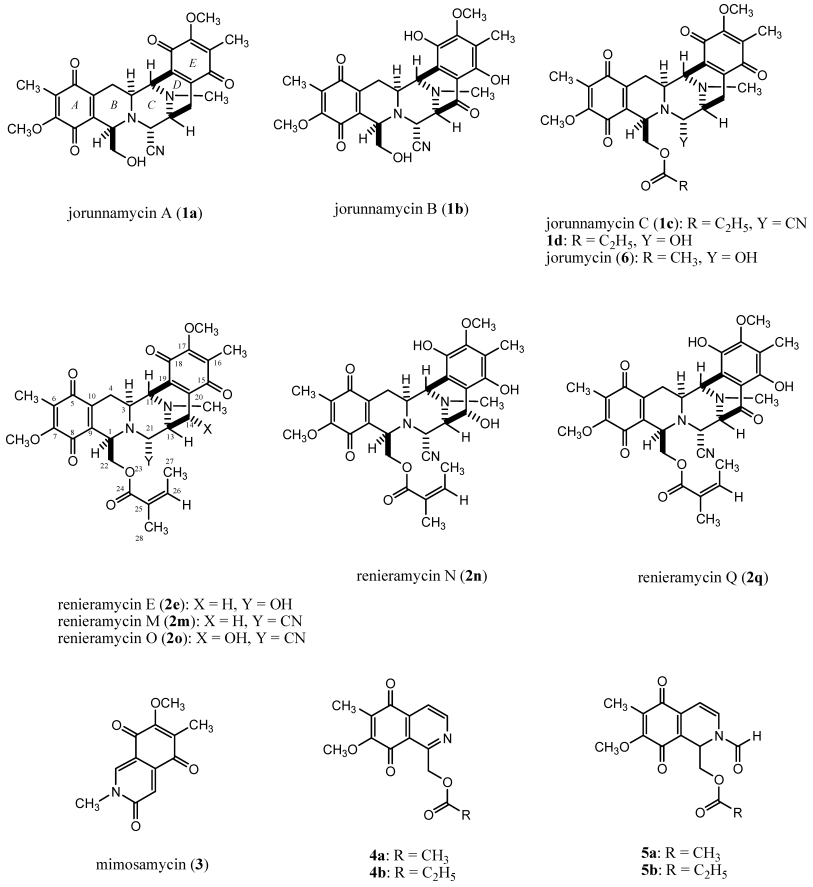


Fig. 1. Structure of Jorunnamycins and Related Marine Natural Products

Table 1. Isoquinoline Marine Natural Products from KCN-Pretreated *Jorunna funebris* (mg)

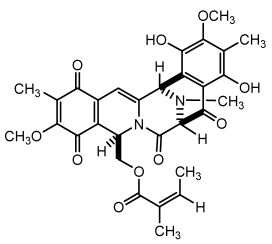
Parts	Residue	Jorunnamycins			Renieramycins				Mimosamycin
		A (**1a**)	B (**1b**)	C (**1c**)	M (**2m**)	N (**2n**)	O (**2o**)	Q (**2q**)	**3**
Mantles	700	7.5	0	0	25.3	0.5	6.4	0	1.0
Visceral organs	980	0	23.6	0	0	9.2	2.0	1.3	9.0
Egg ribbons	270	3.8	0	21.7	62.1	0	0	0	0

the basis of high-resolution MS, and it was 14 mass units more than that of **1a**. Characteristic D_2O exchangeable protons at δ 11.46 and 5.80 ppm, two carbonyl resonances (δ 185.3, 180.9 ppm) indicative of a quinone ring, and an unsaturated ketone carbonyl resonance (δ 197.4 ppm) were observed. For the purpose of discussion of the NMR spectra of **1b**, the nine protons (excluding the methyl groups and three hydroxyl groups) were made on the basis of ^1H–^1H COSY, HMQC, and HMBC (Table 2). The diagnostic homoallylic coupling (2.4 Hz) between 1-H and 4-Hβ through five bonds was confirmed. This coupling was negligible when the compound did not have quinone functionality at the E ring. These data revealed that one of the quinone rings of **1a** might have been reduced to form a hydroquinone with the pericarbonyl function (C-14), similar to renieramycin Q (**2q**)[13] and cribrostatin 4 (**7**).[16—18] In addition, characteristic angelic acid ester carbon signals were absent and methylene proton signals at C-22 were shifted upfield to δ 3.35 and 3.61 ppm compared with those of **2q** (δ 4.02, 4.09 ppm). Therefore, **1b** was surmised to be deangeloylrenieramycin Q.

Jorunnamycin C (**1c**) was isolated as a yellow amorphous solid, $[\alpha]_D^{20}-91.6°$ (c=0.1, $CHCl_3$). The molecular formula of **1c** was determined to be $C_{29}H_{31}N_3O_8$ on the basis of high-resolution MS, and it was 14 mass units higher than that of the jorumycin analog **8**, which was previously prepared from **1a** and acetyl chloride.[14] The ^1H- and ^{13}C-NMR spectral data of **1c** were very similar to those of **8** except for the presence of the propionic acid ester in **1c** as compared with the acetic acid ester in **8**. The characteristic NMR signals of the propionate at δ_C 173.4 ppm ($O\underline{C}OCH_2CH_3$); δ_H 2.11, 2.02 ppm and δ_C 27.4 ppm ($OCO\underline{CH_2}CH_3$); and δ_H 0.95 ppm and δ_C 8.9 ppm ($OCOCH_2\underline{CH_3}$). Treatment of **1a** with propionic anhydride in pyridine afforded **1c** in 71% yield, which was identical to the isolated compound in all respects. We also isolated known metabolites including renieramycins M (**2m**), N (**2n**), O (**2o**), and Q (**2q**) and mimosamycin (**3**), each of which was identified by comparing their ^1H-NMR data with those of authentic standards.

Table 2. ^{1}H- and ^{13}C-NMR Assignments for Jorunnamycin B (**1b**) in $CDCl_3$

Atom No.	^{13}C-NMR δ (multi.)	^{1}H-NMR δ (multi., integral, *J* in Hz)	HMBC correlation	NOE correlation
1	57.6 d	3.90 (1H, overlapped)		
3	53.7 d	3.35 (dt, 1H, 11.4, 2.7)	4-Hβ, 11-H	
4	24.0 t	α 3.08 (dd, 1H, 17.9, 2.5)		4-Hβ
		β 1.64 (ddd, 1H, 17.9, 11.4, 2.4)		4-Hα
5	185.3 s		4-Hα, 6-CH_3	
6	128.5 s		6-CH_3	
7	155.3 s		6-CH_3, 7-OCH_3	
8	180.9 s			
9	135.8 s		3-H, 4-Hα, 4-Hβ, 22-Ha	
10	141.1 s		4-Hα, 4-Hβ	
11	56.7 d	4.37 (br s, 1H)	NCH_3	3-H, NCH_3
13	65.9 d	3.44 (br s, 1H)	NCH_3	NCH_3
14	197.4 s		13-H, 15-OH	
15	155.0 s		15-OH	
16	119.3 s		16-CH_3, 15-OH	
17	154.0 s		16-CH_3, 17-OCH_3	
18	139.9 s			
19	116.4 s			
20	111.4 s		15-OH	
21	56.7 d	4.33 (d, 1H, 2.6)		1-H, 13-H, 22-Ha
22	63.5 t	a 3.61 (dd, 1H, 11.5, 3.0)		22-Hb
		b 3.35 (d, 1H, 11.5)		22-Ha
6-CH_3	8.9 q	1.90 (s, 3H)		
16-CH_3	9.1 q	2.17 (s, 3H)		17-OCH_3
7-OCH_3	61.1 q	3.96 (s, 3H)	6-CH_3	
17-OCH_3	61.5 q	3.85 (s, 3H)	16-CH_3	
NCH_3	42.7 q	2.44 (s, 3H)		
CN	115.4 s		21-H	
15-OH		11.46 (s, 1H)		
18-OH		5.80 (s, 1H)		



cribrostatin 4 (**7**)

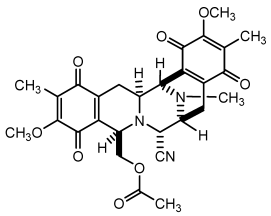


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Fig. 2. Structure of Cribrostatin **4** and Jorumycin Analog

Field observations during our collection trips around Sichang Island revealed that *J. funebris* fed on only the *Xestospongia* blue sponge. This suggested that the nudibranch could sequester secondary metabolites from the sponge. Many reports have indicated that the sequestered secondary metabolites demonstrate predator–prey relationships between nudibranchs and sponges, and nudibranchs surviving from predators.[19,20] In our case, **2m** was found to be the major component in both the mantles and egg ribbons of the nudibranch as well as in the sponge tissues. However, **2m** might exist as renieramycin E (**2e**) in nature, both of which showing strong cytotoxicity against tumor cell lines as well as antimicrobial activity. As the mantles and the egg ribbons are the most vulnerable to attack by predators and microorganisms, the nudibranch might be expected to sequester mainly **2e** from the *Xestospongia* sponge and to transfer it to these tissues to support its putative defensive mechanism against predators. Moreover, the fact that stabilized jorunnamycins A—C (**1a**—**c**) have not been isolated from *Xestospongia* sp. pretreated with KCN suggests that the nudibranch might hydrolyze the angelic acid ester into the corresponding carbinolamine analog. On the other hand, the corresponding carbinolamine analog of **1a** was further converted into the corresponding carbinolamine analog of **1c** in the nudibranch. Incubation of **1c** with aqueous 10% KCN solution in methanol at room temperature for one week and further HPLC analysis of the solution completely revealed the absence of **1a**. The result ruled out the possibility of **1a** as the hydrolysis product of **1c** during the extraction process. Compound **3** was also found mainly in the visceral organs. Thus, the chemical modification in the nudibranch might involve enzymatic transformation or oxidative degradation of bisisoquinolinequinones into simple isoquinolinequinones. Further studies are needed regarding the distribution of these compounds in the nudibranch.

Finally, we were very interested in the conversion of **1c** into the carbinolamine analog **1d**, because it might be a real natural product in *J. funebris*. Treatment of **1c** with aqueous silver nitrate in acetonitrile at 40 °C for 2 h afforded **1d** in quantitative yield. The NMR spectrum of the crude product appeared to be relatively clean and the ^{13}C-NMR spectral data of **1d** and **1c** revealed only two major differences: The C-21 carbon of **1d** was shifted downfield from δ 59.0 to 82.8 ppm, and the cyano carbon resonance at δ 116.9 ppm disappeared. An observable NOE between 14-Hβ (δ 2.22 ppm) and 21-H (δ 4.44 ppm) revealed the relative stereochemistry at C-21. We noted, however, that **1d** decomposed after NMR measurement in $CDCl_3$.[21] The next stage of the

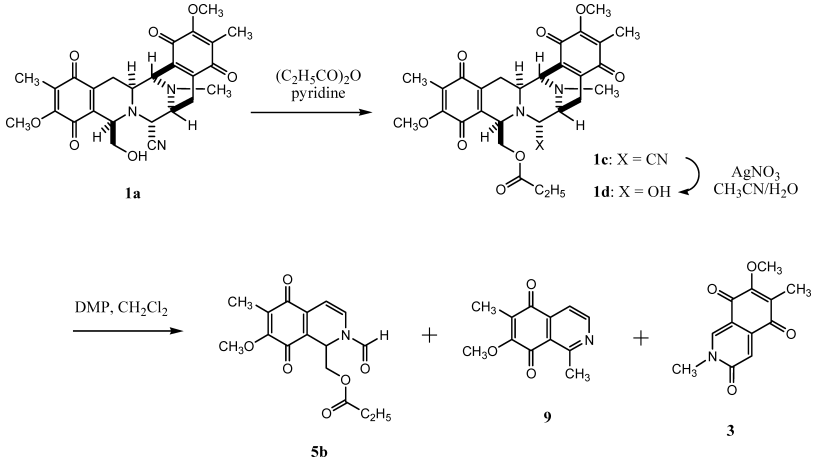

Chart 1. Transfornmation of **1a** to **1d** *via* **1c** Along with Oxidative Degradation Products

Table 3. Cytotoxicity of Jorunnamycins A—C to Various Cancer Cell Lines (IC_{50} nM)[a)]

Compound	HCT116	QG56	DU145
Jorunnamycin A (**1a**)	13.0	59.0	29.0
Jorunnamycin B (**1b**)	455.0	618.0	448.0
Jorunnamycin C (**1c**)	1.5	2.8	0.32
Renieramycin M (**2m**)	7.9	19.0	NT
Jorumycin (**6**)	0.57	0.76	0.49
Ecteinascidin 770	0.40	1.8	0.66

a) HCT116=human colon carcinoma; QG56=human lung carcinoma; DU145=human prostate carcinoma.

investigation involved generating simple isoquinolines from **1d**. Attempts under acidic conditions to treat **1d** were unsuccessful, but treatment of **1d** with Dess–Martin periodinane (DMP) in dichloromethane at 25 °C for 43 h gave **3** (16%), **5b**[22] (12%), and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**)[23—25] (36%). The mechanism of the oxidative degradation is not clear yet, although it is speculated that all the simple isoquinolines might be artifacts generated from bisisoquinolinequinones.

The cytotoxicity against three tumor cell lines of jorunnamycins A—C (**1a**—**c**) is summarized in Table 3. Jorunnamycin C (**1c**) displayed nanomolar inhibitory effect that is almost the same as those of jorumycin (**6**) and ecteinascidin 770. Jorunnamycin B (**1b**) with the oxygen function at C-14 was found to be much less cytotoxic than **1a**.

In summary, we have succeeded in isolating jorunnamycins A—C (**1a**—**c**) from the homogenized nudibranch *J. funebris* pretreated with KCN. Detailed studies of the biological activities of renieramycin analogs and the preparation of additional derivatives that are necessary for the clarification of the mechanism of action and the structure–cytotoxicity relationships are under way.[26]

Experimental

General Experimental Procedures Optical rotations were measured with a Horiba-SEPA polarimeter and a Perkin-Elmer 341 polarimeter. CD was obtained with a JASCO-J715 spectropolarimeter. IR spectra were obtained with a Hitachi 260-10 and a Perkin-Elmer spectrum 2000 FT-IR spectrophotometer. UV spectra were obtained on a Milton Roy Spectronic 3000 Diode Array spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, on a JEOL-JNM-LA 500 FT-NMR spectrometer and at 300 and 75 MHz, respectively, on a Bruker AVANCE DPX-300 FT-NMR spectrometer (ppm, *J* in Hz with TMS as internal standard). Mass spectra were recorded on a JMS 700 instrument with a direct inlet system operating at 70 eV.

Animal Material The nudibranch was identified by E. F. Kelaart 1858 as *Jorunna funebris*, a shell-less marine mollusc. The rhinophores are black with a white base. The body is white with irregular black rings and its mantle is covered in small spiculate papillae (caryophyllidia).[5)]

Extraction and Isolation *J. funebris* was collected by using SCUBA in the vicinity of Sichang Island at the depth of 3—5 m in March 2004. Its egg ribbons were obtained in November 2004 and the animals were frozen until used. Twenty animals (500 g, wet weight) were carefully dissected into two parts, the mantles and the visceral organs. The mantles, the visceral organs and the egg ribbons (23.2 g, wet weight) were separately homogenized with phosphate buffer and pH was adjusted to 7. Then, 10% KCN solution was added very slowly to the suspension, and the mixture was macerated three times with methanol at room temperature for 24 h. The combined methanol extracts were filtered, and the filtrate was concentrated *in vacuo* to give a syrupy residue that was partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated to dryness to give the crude extract of the mantles (*A*: 700 mg), the visceral organs (*B*: 980 mg), and the egg ribbons (*C*: 270 mg).

Part *A* (700 mg) was chromatographed on a silica gel column with a step gradient of hexane–ethyl acetate to give fifteen fractions *A*1—*A*15. Fraction *A*7 gave an orange precipitate of renieramycin M (**2m**, 25.3 mg). Combined fractions *A*8—*A*10 were further fractionated on a silica gel column (dichloromethane–ethyl acetate 6 : 1) to afford renieramycins N (**2n**, 0.5 mg) and O (**2o**, 6.4 mg), jorunnamycin A (**1a**, 7.5 mg), and mimosamycin (**3**, 1.0 mg). Compounds (**2m**, **2n**, **2o**, **3**) were identified by comparing their spectroscopic data with those of authentic standards.

Part *B* (980 mg) was separated on a silica gel column with ethyl acetate to give three fractions *B*1—*B*3. Fraction *B*1 was a yellow solid, the recrystallization from ethanol of which gave renieramycin N (**2n**, 9.2 mg). Combined mother liquor of **2n** was subjected to Sephadex LH-20 column chromatography with chloroform–ethyl acetate to give three fractions *B*′1—*B*′3. Reversed phase column chromatography of fraction *B*′2 was performed using methanol–H_2O 9 : 1, followed by silica gel column chromatography twice to provide renieramycins O (**2o**, 2.0 mg) and Q (**2q**, 1.3 mg), jorunnamycin B (**1b**, 23.6 mg), and mimosamycin (**3**, 9.0 mg).

The crude ethyl acetate extract (270 mg) of egg ribbons was suspended in methanol and extracted with hexane to give an orange precipitate (152.6 mg) that was filtered and subsequently separated on a silica gel column with hexane–ethyl acetate to give renieramycin M (**2m**, 62.1 mg) and jorunnamycin C (**1c**, 21.7 mg). The methanol extract was concentrated *in vacuo* to give a high-polarity slurry (83 mg), and this was subjected to silica gel column chromatography to give jorunnamycin A (**1a,** 3.8 mg).

Jorunnamycin A (**1a**): $[\alpha]_D^{25}$ −270.6° (*c*=1.0, $CHCl_3$); CD $\Delta\varepsilon$ nm (*c*=103.3 μM, methanol, 24 °C), −2.9 (352), −1.5 (300), −10.2 (280), +3.2 (257), −1.8 (230); IR ($CHCl_3$) 3631, 3368, 3015, 2945, 2840, 1656, 1449, 1375, 1311, 1189 cm^{-1}; UV λ_{max} (log ε) 269 (4.61), 370 (3.11) nm; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 4.15 (1H, d, *J*=2.4 Hz, 21-H), 4.07 (1H, d, *J*=2.6 Hz, 11-H), 4.03 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 3.89 (1H, ddd, *J*=3.7, 3.1, 2.4 Hz, 1-H), 3.71 (1H, dd, *J*=11.3, 3.1 Hz, 22-Ha), 3.48 (1H,

dd, J=11.3, 3.7 Hz, 22-Hb), 3.41 (1H, dd, J=7.6, 2.4 Hz, 13-H), 3.17 (1H, ddd, J=11.6, 2.6, 2.4 Hz, 3-H), 2.92 (1H, dd, J=17.4, 2.4 Hz, 4-Hα), 2.82 (1H, dd, J=21.1, 7.6 Hz, 14-Hα), 2.30 (3H, s, NCH_3), 2.27 (1H, d, J=21.1 Hz, 14-Hβ), 1.93 (6H, s, 6-CH_3, 16-CH_3), 1.42 (1H, ddd, J=17.4, 11.6, 2.4 Hz, 4-Hβ); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 186.3 (C-15), 185.5 (C-5), 182.3 (C-18), 181.4 (C-8), 155.7 (C-7), 155.4 (C-17), 141.7 (C-20), 141.4 (C-10), 136.1 (C-9), 135.6 (C-19), 128.8 (C-6), 128.6 (C-16), 116.9 (21-CN), 64.2 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 59.1 (C-21), 58.0 (C-1), 54.5 (C-13), 54.3 (C-3), 54.2 (C-11), 41.5 (NCH_3), 25.4 (C-4), 21.5 (C-14), 8.7 (6-CH_3), 8.7 (16-CH_3); HR-FAB-MS (Magic bullet) m/z 494.1910 [M^++H] (Calcd for $C_{26}H_{28}N_3O_7$, 494.1927).

Jorunnamycin B (**1b**): $[\alpha]_D^{20}$+117.6° (c=0.11, $CHCl_3$); CD $\Delta\varepsilon$ nm (c=86.4 μM, methanol, 25 °C), +3.4 (382), +1.6 (289), −6.7 (271), −4.7 (246), +1.2 (223), −10.7 (206); IR ($CHCl_3$) 3436, 2943, 1737, 1656, 1639, 1462, 1414, 1376, 1233, 755 cm^{-1}; UV λ_{max} (log ε) 254 (3.96), 275 (4.15), 371 (3.64) nm; ^{1}H- and ^{13}C-NMR spectral data: see Table 2; HR-FAB-MS m/z 510.1877 [M^++H] (Calcd for $C_{26}H_{28}N_3O_8$, 510.1876).

Jorunnamycin C (**1c**): $[\alpha]_D^{20}$−91.6° (c=0.1, $CHCl_3$); CD $\Delta\varepsilon$ nm (c=72.8 μM, methanol, 25 °C), +1.0 (438), −3.2 (353), −10.2 (280), +3.9 (255), −1.5 (227), +4.2 (208); IR ($CHCl_3$) 3453, 2943, 2851, 1736, 1655, 1617, 1450, 1411, 1374, 1235, 756 cm^{-1}; UV λ_{max} (log ε) 268 (4.25), 370 (3.14) nm; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 4.40 (1H, dd, J=11.6, 3.2 Hz, 22-Ha), 4.07 (1H, d, J=2.4 Hz, 21-H), 4.02 (1H, d, J=2.7 Hz, 11-H), 4.01 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 3.99 (1H, d, J=2.4 Hz, 1-H), 3.89 (1H, dd, J=11.6, 4.0 Hz, 22-Hb), 3.37 (1H, d, J=2.2 Hz, 13-H), 3.10 (1H, ddd, J=11.6, 2.7, 2.5 Hz, 3-H), 2.93 (1H, dd, J=17.1, 2.4 Hz, 4-Hα), 2.76 (1H, dd, J=21.1, 7.6 Hz, 14-Hα), 2.31 (1H, d, J=21.1 Hz, 14-Hβ), 2.29 (3H, s, NCH_3), 2.11 and 2.02 (1H each, dq, J=16.5, 7.6 Hz, $OCO\underline{CH_2}CH_3$), 1.95 (6H, s, 6-CH_3, 16-CH_3), 1.31 (1H, ddd, J=17.1, 11.6, 2.4 Hz, 4-Hβ), 0.95 (3H, t, J=7.6 Hz, $OCOCH_2\underline{CH_3}$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 186.2 (C-15), 185.4 (C-5), 182.4 (C-18), 180.9 (C-8), 173.4 ($O\underline{C}OCH_2CH_3$), 155.6 (C-7), 155.2 (C-17), 142.1 (C-20), 141.7 (C-10), 135.5 (C-9), 135.0 (C-19), 128.6 (C-6), 128.6 (C-16), 116.9 (21-CN), 63.6 (C-22), 61.1 (OCH_3), 61.0 (OCH_3), 59.0 (C-21), 55.9 (C-1), 54.6 (C-13), 54.5 (C-3), 54.3 (C-11), 41.5 (NCH_3), 27.4 ($OCO\underline{CH_2}CH_3$), 25.3 (C-4), 21.2 (C-14), 8.9 ($OCOCH_2\underline{CH_3}$), 8.8 (16-CH_3), 8.6 (6-CH_3); EI-MS m/z (%) 549 (M^+, 6), 462 (2), 435 (3), 221 (20), 220 (100), 219 (17), 218 (24), 204 (12); HR-EI-MS m/z 549.2112 [M^+] (Calcd for $C_{29}H_{31}N_3O_8$, 549.2111).

Transformation of 1a into 1c Propionic anhydride (64.1 μl, 0.5 mmol) was added to a stirred solution of jorunnamycin A (**1a**: 16.8 mg, 0.034 mmol) in pyridine (0.5 ml) at 0 °C, and the reaction mixture was stirred at 25 °C for 2.5 h. The reaction mixture was diluted with water (19 ml) and extracted with chloroform (10 ml×3). The combined organic extract was washed with brine (10 ml), dried, and concentrated *in vacuo* to give the residue (21.5 mg), the purification of which by silica gel column chromatography (hexane–ethyl acetate 5 : 1) afforded jorunnamycin C (**1c**: 13.3 mg, 71.1%) as a yellow amorphous solid, which gave spectral data that were in complete agreement with those of the natural product.

Transformation of 1c into 1d Jorunnamycin C (**1c**: 28.0 mg, 0.05 mmol) was dissolved in a mixture of acetonitrile and water [3 : 2 (v/v), 5 ml], and silver nitrate (216.6 mg, 1.28 mmol) was added. The mixture was stirred at 40 °C for 2 h. The reaction mixture was filtered and then washed with chloroform, and the combined filtrate was concentrated *in vacuo*. The residue was diluted with water (20 ml) and extracted with chloroform (20 ml×3). The combined organic extract was washed with brine (20 ml), dried, concentrated *in vacuo* to give the residue (**1d**: 28.0 mg, 100%) as a yellow solid, which gave only ^{1}H- and ^{13}C-NMR data. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 4.44 (1H, d, J=2.4 Hz, 21-H), 4.38 (1H, br s, 1-H), 4.38 (1H, dd, J=11.3, 3.4 Hz, 22-Ha), 4.00 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 3.91 (1H, dd, J=11.3, 4.0 Hz, 22-Hb), 3.84 (1H, d, J=2.7 Hz, 11-H), 3.19 (1H, app d, 13-H), 3.16 (1H, ddd, J=11.6, 3.1, 2.7 Hz, 3-H), 2.82 (1H, dd, J=16.8, 2.1 Hz, 4-Hα), 2.66 (1H, dd, J=21.1, 7.6 Hz, 14-Hα), 2.26 (3H, s, NCH_3), 2.22 (1H, d, J=21.1 Hz, 14-Hβ), 2.04 (2H, m, $OCO\underline{CH_2}CH_3$), 1.95 (3H, s, 6-CH_3), 1.93 (3H, s, 16-CH_3), 1.29 (1H, ddd, J=17.1, 11.6, 2.4 Hz, 4-Hβ), 0.90 (3H, t, J=7.3 Hz, $OCOCH_2\underline{CH_3}$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 186.5 (C-15), 185.8 (C-5), 182.5 (C-18), 181.3 (C-8), 173.5 ($O\underline{C}OCH_2CH_3$), 155.7 (C-7), 155.2 (C-17), 141.8 (C-20), 141.6 (C-10), 137.2 (C-9), 134.5 (C-19), 128.6 (C-6), 128.1 (C-16), 82.8 (C-21), 64.1 (C-22), 60.9 (OCH_3), 60.9 (OCH_3), 57.4 (C-13), 54.1 (C-11), 52.7 (C-1), 51.0 (C-3), 41.4 (NCH_3), 27.4 ($OCO\underline{CH_2}CH_3$), 25.6 (C-4), 20.5 (C-14), 8.9 ($OCOCH_2\underline{CH_3}$), 8.7 (6-CH_3), 8.6 (16-CH_3).

Oxidative Degradation of 1d Dess–Martin periodinane (DMP: 13.0 mg, 0.03 mmol) was added to a stirred solution of **1d** (11.1 mg, 0.021 mmol) in dichloromethane (5 ml) at 0 °C, and the mixture was stirred at 25 °C for 43 h. The reaction mixture was diluted with 10% aqueous $Na_2S_2O_3$ solution (20 ml) and extracted with dichloromethane (20 ml×3). The combined organic extracts were washed with brine (10 ml), dried, and concentrated *in vacuo* to give the residue (14.0 mg), the purification of which by silica gel column chromatography (ethyl acetate–hexane 6 : 1) afforded 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**[14]: 1.6 mg, 36%) and *N*-formyl-1,2-dihydrorenierol propionate (**5b**[7]: 0.8 mg, 12%). Further elution with ethyl acetate–hexane (3 : 1) gave mimosamycin (**3**[10]: 0.8 mg, 16%). The compounds were identified by comparing of their ^{1}H-NMR data with those of authentic standards.

Propionic Acid (2-Formyl-1,2,5,8-tetrahydro-7-methoxy-6-methyl-5,8-dioxo-1-isoquinolinyl)methyl Ester [*N*-Formyl-1,2-dihydrorenierol Propionate, 5b] Dark red amorphous powder, $[\alpha]_D^{21}$ −580° (c=0.07, $CHCl_3$) (lit.,[8] $[\alpha]_D$ − 551 (c=0.022, CH_2Cl_2); ^{1}H-NMR (300 MHz) major isomer δ: 8.42 (1H, s, CHO), 6.91 (1H, d, J=7.5 Hz, 3-H), 6.05 (1H, app d, J=7.5 Hz, 4-H), 5.94 (1H, dd, J=4.3, 3.7 Hz, 1-H), 4.27 (1H, dd, J=11.9, 4.7 Hz, 1-CH), 4.18 (1H, dd, J=11.9, 3.4 Hz, 1-CH), 4.07 (3H, s, OCH_3), 2.24 (2H, q, J=7.6 Hz, $OC\underline{H_2}CH_3$), 1.96 (3H, s, CH_3), 1.06 (3H, t, J=7.6 Hz, $OCH_2C\underline{H_3}$); minor isomer δ: 8.15 (1H, s, CHO), 7.43 (1H, d, J=7.3 Hz, 3-H), 6.23 (1H, app d, J=7.3 Hz, 4-H), 5.30 (1H, dd, J=10.1, 3.7 Hz, 1-H), 4.22 (1H, m, 1-CH), 4.06 (3H, s, OCH_3), 3.82 (1H, dd, J=11.9, 3.4 Hz, 1-CH), 2.35 (2H, q, J=7.3 Hz, $OC\underline{H_2}CH_3$), 1.98 (3H, s, CH_3), 1.14 (3H, t, J=7.3 Hz, $OCH_2C\underline{H_3}$); EI-MS m/z (%) 319 (M^+, 4), 234 (14), 233 (22), 232 (100), 205 (11), 204 (81). HR-EI-MS m/z 319.1052 [M^+] (Calcd for $C_{16}H_{17}NO_6$, 319.1056).

Assay for Cytotoxicity A single-cell suspension of each cell line (2×10^3 cells/well) was added to the serially diluted test compounds in a microplate. Then, the cells were cultured for 4 d. Cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50% compared with the untreated control.

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