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Cytotoxic Isoquinoline Quinones from the Thai Sponge *Cribrochalina*

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Abstract

Three isoquinoline quinones, (+)-*N*-formyl-1,2-dihydrorenierone (**1**), *O*-demethylrenierone (**2**), and mimosamycin (**3**), were isolated from the Thai purplish-blue sponge, *Cribrochalina* sp. The cytotoxicity against various cancer cell lines of the three compounds, determined employing the SRB method, was first reported here. It was found that all three were highly active, especially against MCF-7 breast carcinoma and HeLa cervical cancer cell lines. Also, this report is the first isolation of (+)-enantiomer of *N*-formyl-1,2-dihydrorenierone from a naturally occurring source.

Keywords: *Cribrochalina*, sponge, isoquinoline quinones, *N*-formyl-1,2-dihydrorenierone, *O*-demethylrenierone, mimosamycin, cytotoxicity.

Introduction

Isoquinoline quinones are among the most prominent groups of marine alkaloids that exhibit several interesting bioactivities, including antimicrobial, cytotoxic, and insecticidal activities. Found only in three distant major producers, actinomycetes of the *Streptomyces*, sponges of the *Xestospongia* and *Cribrochalina*, and tunicates of the *Ecteinascidia*, their structures are varied from alkaloids bearing single unit of isoquinoline skeleton to *bis*- and *tris*-isoquinoline quinones (for examples, see Fukumi et al., 1977; McIntyre et al., 1979; Frincke & Faulkner, 1982; McKee & Ireland, 1987; Wright et al., 1990; Rinehart et al., 1990; Pettit et al., 2000). Among these, the ecteinascidins, *tris*-isoquinoline quinones from *Ecteinascidia turbinata*, are possibly the most promising marine natural products being advanced toward clinical use, with ectienascidin 743 currently in phase II clinical trials (Munro et al., 1999). Here, we report the isolation of three single-unit isoquinoline quinones, *N*-formyl-1,2-dihydrorenierone (**1**), *O*-demethylrenierone (**2**), and mimosamycin (**3**), from the Thai purplish-blue sponge *Cribrochalina* sp. (Figure 1). The cytotoxicity of these three alkaloids against various cancer cell lines is first reported here.

Materials and methods

General experimental methods

UV spectra were obtained with a Hewlett Packard 8452A spectrometer. IR spectra were recorded on a Jasco IR-810 spectrometer. NMR experiments were performed on a Varian Unity Inova 500 spectrometer (500 MHz for ^{1}H NMR), referring to the signals of operating solvent $CDCl_3$ (7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C NMR spectra). Mass spectra were recorded in ESI mode on a Micromass LCT module spectrometer. The optical rotation was determined employing an Atago 15,536 polarimeter. The HPLC instrument was a Waters 600E solvent delivery system equipped with a Waters 484 tunable absorbance detector.

Extraction and isolation

The colonies of purplish-blue sponge, later identified to belong to the genus *Cribrochalina* (family Niphatidae) by Mr. Somchai Bussarawit of Phuket Marine Biological Center (PMBC), Phuket, Thailand, were collected in June 2000 and in April 2001 at the depth of 3–6 m in the vicinity of Koh-Tao, Surat-Thani province, Thailand. Once collected, the animal material was immediately preserved under ice then at −20 °C until extraction. The voucher specimens from both collections were deposited at the Marine Biodiversity Research Unit, PMBC, Phuket, Thailand.

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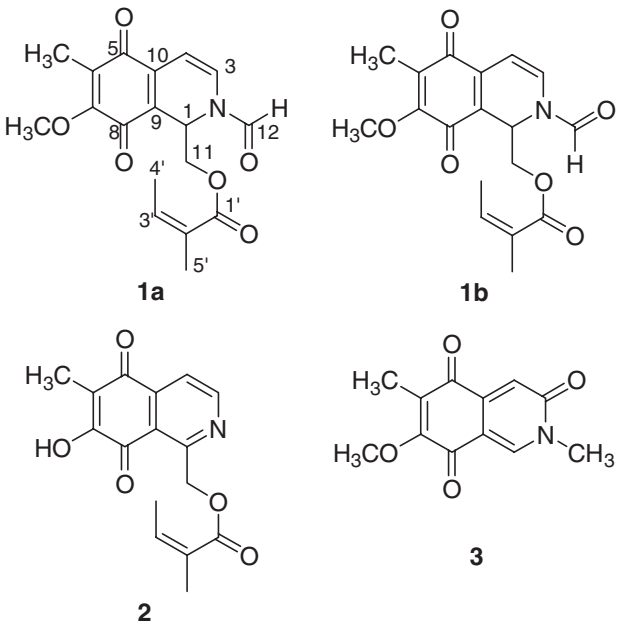

Figure 1. Structures of compounds **1**, **2** and **3**.

The freeze-dried sponge (112 g) was extracted in MeOH then partitioned to yield the cytotoxic CH_2Cl_2-soluble material. This CH_2Cl_2 extract was subjected to a SiO_2 column (3% MeOH in CH_2Cl_2) to afford four main fractions. The first three major fractions were then separately fractionated using Sephadex LH20 (MeOH) then C-18 reverse phase HPLC (Hypersil, 4.0 × 250 mm; gradient aqueous MeCN from 25 to 70%). Compounds **1** (4.2 mg) and **2** (4.4 mg) were obtained as dark red and orange solids from fractions 1 and 2, and from fraction 3, respectively. The last major fraction was also fractionated using Sephadex LH20 (MeOH), and compound **3** (4.4 mg) was recrystalized from a CH_2Cl_2-MeOH mixture as yellow crystal.

N-Formyl-1,2-dihydrorenierone (1)

Dark red solid. UV (MeOH) λ_{max} (log ε) 218 (4.35), 268 (3.96), 344 (3.53) nm; IR (thin film) ν 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) major component 8.45 (1H, s; H-12), 6.93 (1H, d, J = 7.8 Hz; H-3), 6.07 (1H, qq, J = 7.3, 1.4 Hz; H-3′), 6.04 (1H, d, J = 7.8 Hz; H-4), 6.00 (1H, dd, J = 4.6, 3.2 Hz; H-1), 4.36 (1H, dd, J = 11.9, 4.6 Hz; H-11a), 4.21 (1H, dd, J = 11.9, 3.2 Hz; H-11b), 4.08 (3H, s; 7-OCH_3), 1.95 (3H, s; 6-CH_3), 1.92 (3H, dq, J = 7.3, 1.4 Hz; H-4′), 1.78 (3H, quintet, J = 1.4 Hz; H-5′); minor component 8.24 (1H, s; H-12), 7.45 (1H, d, J = 7.8 Hz; H-3), 6.25 (1H, d, J = 7.8 Hz; H-4), 6.16 (1H, qq, J = 7.3, 1.4 Hz; H-3′), 5.37 (1H, dd, J = 10.5, 2.7 Hz; H-1), 4.36 (1H, dd, J = 11.9, 10.5 Hz; H-11a), 4.07 (3H, s; 7-OCH_3), 3.91 (1H, dd, J = 11.9, 2.4 Hz; H-11b), 1.99 (3H, dq, J = 7.3, 1.4 Hz; H-4′), 1.98 (3H, s; 6-CH_3), 1.88 (3H, quintet, J = 1.4 Hz; H-5′); ^{13}C NMR ($CDCl_3$; 125 MHz) major component 184.9 (s; C-5), 180.2 (s; C-8), 167.4 (s; C-1′), 162.5 (d; C-12), 156.3 (s; C-7), 139.8 (d; C-3′), 135.4 (s; C-10), 133.1 (d; C-3), 127.1 (s; C-6), 126.9 (s; C-2′), 124.0 (s; C-9), 101.0 (d; C-4), 63.1 (t; C-11), 61.1 (q; 7-OCH_3), 47.4 (d; C-1), 20.6 (q; C-5′), 15.7 (q; C-4′), 8.6 (q; 6-CH_3); minor component 184.7 (s; C-5), 180.2 (s; C-8), 166.7 (s; C-1′), 161.3 (d; C-12), 156.0 (s; C-7), 140.8 (d; C-3′), 136.2 (s; C-10), 129.4 (d; C-3), 128.0 (s; C-6), 126.5 (s; C-2′), 123.1 (s; C-9), 102.9 (d; C-4), 61.3 (q; 7-OCH_3), 60.9 (t; C-11), 49.5 (d; C-1), 20.5 (q; C-5′), 15.9 (q; C-4′), 8.8 (q; 6-CH_3); ESI-MS *m/z* (% relative intensity) 368 ($[M+Na]^+$; 100), 281 (10), 258 (15), 246 (19); $[\alpha]_D$ (MeOH; c = 0.04) +230°.

O-Demethylrenierone (2)

Orange solid. UV (MeOH) λ_{max} (log ε) 228 (3.92), 298 (3.31), 488 (2.22) nm; IR (thin film) ν 3300, 1680 cm^{-1}; ^1H NMR ($CDCl_3$; 500 MHz); 8.99 (1H, d, J = 4.8 Hz; H-3), 7.96 (1H, d, J = 4.8 Hz; H-4), 6.14 (1H, qq, J = 7.4, 1.4 Hz; H-3′), 5.81 (1H, s; H-11), 2.02 (3H, dq, J = 7.4, 1.4 Hz; H-4′), 1.98 (3H, s; 6-CH_3), 1.98 (3H, quintet, J = 1.4 Hz; H-5′); ^{13}C NMR ($CDCl_3$; 125 MHz) 183.6 (s; C-5), 181.3 (s; C-8), 167.8 (s; C-1′), 157.1 (s; C-1), 155.3 (d; C-3), 154.0 (s; C-7), 139.9 (d; C-3′), 138.4 (s; C-10), 127.8 (s; C-2′), 120.5 (s; C-6), 120.3 (s; C-9), 119.1 (d; C-4), 65.3 (t; C-11), 20.6 (q; C-5′), 15.8 (q; C-4′), 9.1 (q; 6-CH_3); ESI-MS *m/z* (% relative intensity) 302 (MH^+; 5), 216 (100).

Mimosamycin (3)

Yellow crystal from CH_2Cl_2-MeOH mixture. UV (MeOH) λ_{max} (log ε) 212 (4.32), 318 (3.96), 384 (3.33) nm; IR (thin film) ν 1640 cm^{-1}; ^1H NMR ($CDCl_3$; 500 MHz) 8.28 (1H, s; H-1), 7.13 (1H, s; H-4), 4.18 (3H, s; 7-OCH_3), 3.67 (3H, s; 2-CH_3), 2.07 (3H, s; 6-CH_3); ^{13}C NMR ($CDCl_3$; 125 MHz) 183.5 (s; C-5), 177.3 (s; C-8), 162.8 (s; C-3), 159.5 (s; C-7), 142.1 (d; C-1), 138.9 (s; C-10), 133.9 (s; C-9), 133.1 (s; C-6), 116.7 (d; C-4), 61.3 (q; 7-OCH_3), 38.4 (q; 2-CH_3), 9.5 (q; 6-CH_3); ESI-MS *m/z* (% relative intensity) 234 (MH^+; 100).

Cytotoxic activity

The procedure of cytotoxicity determination was employed using sulphorhodamine B (SRB) assay as described by Skehan et al. (1990). Here, four cancer cell lines, MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (human cervical cancer) and HT-29 (colon cancer) were used. In brief, monolayered cultures of each cell line in 96-well microtiter plates (2×10^3 cells/well) were treated with a serial dilution of each sample in suitable culture medium. The plate was incubated according to the reported condition for 7 days, at the midway of which time the medium was refreshed once (exposure time 72 h). Survival percentage was measured colorimetrically using the SRB assay method as reported, with a detection at 492 nm (Power Wave X plate reader). Mortality percentages were calculated then converted into IC_{50} values as reported.

Results and discussion

The isolation of the purplish-blue sponge, later identified as a *Cribrichalina* sp., led to the isolation of three known isoquinoline quinones, *N*-formyl-1,2-dihydrorenierone (**1**), *O*-demethylrenierone (**2**), and mimosamycin (**3**). The structure elucidation of all three isolated compounds was achieved mainly by the NMR spectral analysis, and strongly supported by comparison with the previously reported spectral data (Frincke & Faulkner, 1982, for **1** and **2**; Kitahara et al., 1985, for **1**; Fukumi et al., 1977, for **3**). The presence of two rotamers of compound **1** as reported earlier by Frincke and Faulkner (1982) was also observed here and was unambiguously confirmed by means of a series of nOe-ds experiments.

Among the physical properties of the three compounds, the most interesting feature was the optical activity of **1**. While most of its spectral data were identical to those previously reported, here we found that our isolated compound is in fact the enantiomer of the one of Frincke and Faulkner (1982). With some slight deviation due to the instrumental difference, the specific rotation reported here is +230°, as compared to that reported earlier of −227°. Thus, we propose here the first report of the positive enantiomer of **1**. Besides the generic difference of the producing organisms, the opposite enantiomeric identity could also be related to collecting locations of the two organisms. The aspect of different collecting sites that could lead to different types of compounds isolated even from the same animal species has already been observed in other groups of marine invertebrates (for examples, see Bontemps et al., 1994).

Although several members of the isoquinoline quinones have already been reported as cytotoxic, this activity for the three compounds isolated here has never been mentioned. The cytotoxicity of the three isolated compounds was determined using the SRB method, targeting four solid-tumor cell lines; KB, HT-29, MCF-7 and HeLa. It was found that all the three alkaloids were potently cytotoxic, especially against MCF-7 and HeLa cell lines, with IC_{50} at the magnitude of $10^{-2} - 10^{-1}$ µg/mL (Table 1). More interestingly, while these agents were highly active against MCF-7 and HeLa, they exhibited high selectivity. Their activity significantly dropped to completely inactive at the concentration of 0.2 µg/mL in KB and HT-29 cell lines.

Among the three compounds, **1** seems the most interesting with keen selectivity. The compound lost its activity by more than 10-fold when the target cell lines changed from MCF-7 to HeLa, and were considerably less active against KB and HT-29. On the other hand, compound **3**, which was comparatively as active as **1** in MCF-7, was less discriminative, maintaining its potency at the same magnitude of 10^{-2} µg/mL in both susceptible cell lines. Considering the similarity in core structures, this evidently different potency could result from the presence of long hydrophobic, and possibly electrophilic, angelate side chain.

Table 1. Cytotoxicity of compounds **1**, **2**, and **3** against MCF-7 and HeLa cell lines.[a]

	IC_{50} (µg/mL)	
compound	MCF-7	HeLa
1	$3.0 \times 10^{-2} \pm 1.8 \times 10^{-3}$	$9.1 \times 10^{-1} \pm 1.6 \times 10^{-1}$
2	$2.8 \times 10^{-2} \pm 0.9 \times 10^{-3}$	NA[b]
3	$4.2 \times 10^{-2} \pm 0.7 \times 10^{-3}$	$2.6 \times 10^{-2} \pm 1.9 \times 10^{-3}$

Notes: [a] All three compounds were completely inactive in KB and HT-29 at a concentration as low as 0.2 µg/mL. [b] Unable to obtain IC_{50} due to sharp drop in activity profile.

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