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Aaptamine-Related Alkaloid from the Marine Sponge *Aaptos aaptos*

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Abstract

A new aaptamine-related alkaloid, 1,3-dioxolo [4,5-*d*] benzo [*de*]-1,6-naphthyridine (methylenedioxyaaptamine, **1**), was isolated from the organic extracts of the Bornean marine sponge *Aaptos aaptos*, together with a known aaptamine derivative, 8,9,9-trimethoxy-9*H*-benzo [*de*]-1,6-naphthyridine (**2**). The structure of compound **1** was elucidated by interpretation of its spectroscopic data. Two compounds were tested for their cytotoxic potentials against adult T-cell leukemia (ATL) cells, and compound **1** showed moderate cytotoxic potential.

Keywords

Aaptos aaptos, cytotoxic compound, aaptamine derivatives, marine sponge

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The aaptamines are interesting group of biologically active marine alkaloids having rare 1*H*-benzo[*de*]-1,6-naphthyridine skeleton.[1] Various aaptamine derivatives have been isolated from marine sponges belonging to the genus mainly *Aaptos* moreover the genus *Hymeniacidon*, *Luffariella*, *Suberea*, *Suberites,* and *Xestospongia*.[2-5] Their biological activities, such as cytotoxic activity,[6] antiviral activity[7], and antimicrobial activity,[8] have been revealed. Therefore, aaptamine derivatives are the intriguing focus for the natural product and bioactivity study. In the course of our search for cytotoxic compounds from marine invertebrates, we isolated a new aaptamine-related alkaloid, 1,3-dioxolo [4,5-*d*] benzo [*de*]-1,6-naphthyridine (**1**), together with a known 8,9,9-trimethoxy-9*H*-benzo [*de*]-1,6-naphthyridine(**2**)[9] from *A. aaptos*. In this article, we describe the isolation and structural determination of compound **1**.

Compound **1** was obtained as green waxy solid. It had a molecular formula of $C_{12}H_8N_2O_2$, which was suggested by high resolution fast-atom bombardment mass spectrometry (HRFABMS) [*m/z* 213.0665 $(M + H)^+$, Δ + 0.1 mmu]. The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra of **1** (Table 1) were almost identical with those of aaptamine (Table 1).[1] In the ^{1}H-^{1}H correlation spectroscopy, two spin systems were determined based on the correlations of H-2/H-3 and H-5/H-6 (Figure 1). The combination of the key heteronuclear multiple bond (HMBC) correlation (Figure 1) facilitated the aaptamine-type structure of **1**. The location of an additional methylene group [δ_H 6.25 (2H, s) and δ_C 105.9] in **1** was established by the HMBC correlation of methylene protons to the oxygenated aromatic carbon signals at δ 133.2 (C-8) and δ 155.8 (C-9). Thus, **1** was identified as 1,3-dioxolo [4,5-*d*] benzo [*de*]-1,6-naphthyridine (methylenedioxyaaptamine, Figure 1). This is the first report of the presence of compound **1** in the natural source, although compound **1** was previously reported as a HCl salt of the synthetic product.[10]

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Table 1. Comparison of NMR Spectral Data of **1** With Those of Aaptamine.[1]

	Compound 1[a]		Aaptamine[b]	
Position	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)
1				
2	7.67 (d, 5.4)	143.0 (d)	7.90 (brd, 6.5)	141.4(d)
3	6.21 (d, 5.4)	99.5 (d)	6.52 (d, 6.5)	98.3(d)
3a		152.7 (s)		149.4(s)
5	7.20 (d, 6.9)	130.0 (d)	7.45 (d, 7.3)	129.2(d)
6	6.87 (d, 6.9)	116.4 (d)	6.93 (d, 7.3)	101.3(d)
6a		135.4 (s)		132.6(s)
7	6.94 (s)	100.4 (d)		113.5(d)
8		133.2 (s)		157.1(s)
9		155.8 (s)		131.2(s)
9a		125.3 (s)		133.2(s)
9b		120.7 (s)		115.9(s)
10	6.25(s)	105.9 (t)	4.03(s)	57.0(q)
			3.86(s)	61.1(q)

[a]Measured at 600 (^{1}H) and 150 (^{13}C) MHz at 300K in CD_3OD.
[b]Measured in D_2O.

Cytotoxic effects of compounds **1** and **2** against ATL cells were investigated by WST-8 assay. Compound **1** showed a moderate cytotoxic effect against the ATL-related leukemia cell line, S1T, with IC_{50} value of 0.29 μM, while compound **2** did not show cytotoxicity (>10 μg/mL). This indicates that these aaptamine-related compounds showed inhibitory effects on cell proliferation, warranting further investigation with the aim of developing novel anti-ATL drugs.

Experimental

General Procedures

Optical rotation was measured at 25°C on a JASCO DIP-370S polarimeter. NMR spectra were recorded with JEOL ECX400 and ECX600 spectrometers, and UV and IR spectra on a UV-210 and a JASCO FT/IR 5300. FAB mass spectra were obtained using a JEOL JMS-700 Mstation. Column chromatography was performed with silica gel 60 (Merck, 70-230 μm). Silica gel 60F plates (Merck, 0.25 mm thick) were used for thin-layer chromatography. High-performance liquid chromatography (HPLC) was performed using a Waters 501 HPLC pump with a Shodex UV-41 detector. A C_{18} column (4.6 mmϕ x 250 mm) was used for HPLC.

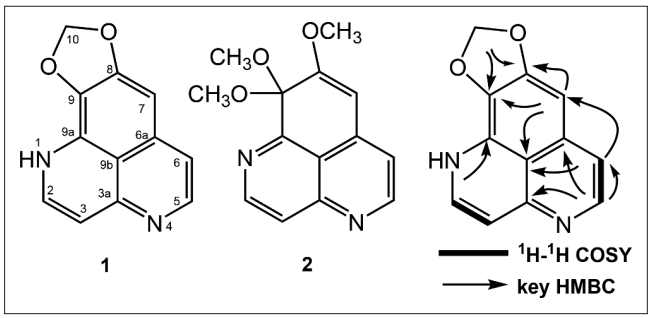

Figure 1. Chemical structures of compounds **1** and **2**, and ^{1}H-^{1}H COSY and key HMBC correlations of **1**.

Biological Materials

The marine sponge was collected at a depth of 15 m at Sepanggar Island, Sabah (6°03'N, 116°04'E), Malaysia, on November 24, 2015. The voucher specimen is deposited in the collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (BORNEENSIS).

Extraction and Isolation

The sample (930 g, wet weight) was chopped into small pieces and extracted with MeOH (3 L) at room temperature for 1-2 weeks. Extracts were concentrated under reduced pressure at 40-45°C and the residue (19.4 g) was partitioned between AcOEt (3 L) and H_2O (1 L). The AcOEt extract (336 mg) was subjected to silica gel flash chromatography to give 17 fractions. The active 8th fraction (37 mg) was further separated by reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, 4.6 × 250 mm) with 35% MeOH to furnish compound **2** (2.7 mg). The active 11th fraction was purified by reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, 4.6 × 250 mm) with 50% MeOH to furnish compound **1** (1.1 mg).

Cell Lines and Cultures

The ATL cell line S1T was maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin,

100 µg/mL streptomycin, and 2 mM L-glutamate. Generally, cell cultures were split every 2-3 days, and used for in vitro assays during the log phase of growth.

Cytotoxicity

The assay was performed according to previous described procedures.[11,12] The cells were cultured at a density of 1×10^4 cells per well in at least triplicates in the absence or presence of a test sample in 10-fold dilutions for 72 hours in flat bottom 96-well plates at 37°C in a humidified water-jacketed CO_2 incubator. The inhibition of cell proliferation was determined using a 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium monosodium salt (WST-8) assay kit (Dojindo, Japan). The viable cells convert the WST-8 tetrazolium salt into a water-soluble formazan. The concentration at which cell proliferation is inhibited by 50% compared with untreated control cells is expressed as the IC_{50}.

1,3-Dioxolo [4,5-*d*] Benzo [*De*]-1,6-Naphthyridine (1)

Green waxy solid.
IR (film): 2917, 2849, 1733, 1665, 1629, 1606, 1592, 1553, 1542, 1496, 1464, 1443, 1388, 1323, 1302, 1226, 1102, 1061, 1042, 1023, 903, 845, 805, 779, 757, 643 cm^{-1}.
UV λ_{max} (MeOH): 236 nm (logε =4.29), 258 nm (4.38), 318 nm (3.64), 401 nm (3.71).
^1H and ^{13}C NMR (CD_3OD): Table 1.
HRFABMS *m/z*: 213.0665 $[M + H]^+$ (calcd for $C_{12}H_9N_2O_2$ 213.0664, + 0.1 mmu).

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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