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# A Novel Bromoindole Alkaloid from a Korean Colonial Tunicate *Didemnum* sp.

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**Abstract** – Chemical investigation on a colonial marine tunicate, *Didemnum* sp. led to the isolation of a series of indole alkaloids including a new (**1**) and two known metabolites (**2**-**3**). Based on the spectroscopic analysis including 1D and 2D NMR along with MS spectra, the structure of **1** (16-*epi*-18-acetyl herdmanine D) was elucidated as a new amino acid derivative. The absolute configuration of **1** was determined by comparison of specific rotation with the known compound. The structures of compounds **2** and **3** were also identified as bromoindole containing compounds *N*-(6-bromo-1*H*-indole-3-carbonyl)-L-arginine and (6-bromo-1*H*-indol-3-yl) oxoacetamide, respectively, based on $^{1}$H and $^{13}$C NMR data, MS data and specific rotation value. Their pharmacological potentials as antibacterial agents and FXR antagonists were investigated, but no significant activity was found. However, the structural similarity of compound **1** to compound **4** suggested the anti-inflammatory potential of compound **1**.
**Keywords** – *Didemnum* sp., Colonial tunicate, Bromoindole alkaloid

## Introduction

Marine ascidians are well-known sources of diverse bioactive natural products, and particularly rich in nitrogen-containing compounds ranging from peptides having cyclic structures with extensive modification to heterocyclic alkaloids derived from phenylalanine, tryptophan, tyrosine and lysine.[1] In particular, the marine ascidians in the genus *Didemnum* are widely known as the source of biologically active alkaloids such as the lamellarins (multi-drug resistance inhibitors and immunomodulators),[2-4] the ningalins (immunosuppressors),[5,6] ascididemin (an anti-neoplastic agent)[7] and the shishijimicins (anti-tumor agents).[8] A few representative brominated indole alkaloids are also found in this genus of marine invertebrates. In general, the bromine atom is frequently found on the C-6 position of indole unit and its C-3 position is attached to additional carbon, as shown in 6-bromotryptamine,[9] 2,2-bis(6'-bromo-3'-indolyl)ethylamine,[9] 2,5-bis(6'-bromo-3'-indolyl) piperazine,[9] didemnoline C,[10] 6-bromogranulatimide,[11] 3, 10-dibromofascaplysin,[12] 7,14-dibromoreticulatine,[12] and didemnidine B.[13]

Several bromoindole alkaloids have been isolated from the Korean marine invertebrates such as marine ascidian (herdmanine D from *Herdmania momus*)[14] and marine sponges in the genus of *Spongosorites* (bromodeoxy-topsentin and the spongotines).[15,16]

During the chemical investigation on the extract of Korean marine invertebrates, three indole-containing alkaloids are isolated. Throughout the spectroscopic data analysis, a major compound is identified as a new chemical entity related to the known compound, herdmanine D.[14] Two other compounds were revealed as previously reported compounds. Herein, we reports the isolation and structure elucidation of a new compound.

## Experimental

**General experimental procedures** – Optical rotation were recorded in MeOH using a 1.0 cm cell on a Rudolph Research Autopol III. The NMR spectra were recorded on a Bruker Avance DPX-500 or DPX-600 spectrometer using DMSO-$d_6$ as the solvent. Mass spectrometric data were obtained on JEOL JMS-AX505WA and JMS-600W instruments.

**Animal material** – A species of purple encrusting marine tunicate was collected by SCUBA near Hae-geumgang, Geoje in the South Sea of Korea. The sample

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for extraction was frozen after collection and stored at –20 °C before extraction. Voucher specimens (CMDD-HKK42) were anesthetized with 5% menthol in sterilized seawater for 2 h and stored in 10% formalin in sterilized seawater. The animal was taxonomically identified as *Didemnum* sp. by HKK42. The voucher was deposited at School of Earth and Environmental Sciences, Seoul National University.

**Extraction and isolation** – The frozen sample (7.2 kg, wet wt) was freeze-dried, and the dried sample (1.6 kg) was extracted with 50% MeOH in DCM for 24 h (5 L × 3 times). The combined extracts was dried under reduced pressure to yield 55 g of crude extract. The dried residues was suspended in MeOH (3 L) and partitioned with hexanes (3 L) to yield 7 g of hexanes-soluble materials. The MeOH soluble layer was dried, re-suspended in water (5 L) and washed with ethyl acetate three times to yield 16 g of ethyl acetate-solubles. To the residual water layer was added 1-buthanol (2.5 L × 3 times) and 1-butanol soluble materials (10 g) were extracted.

1-Butanol soluble fraction was subjected to LH-20 size exclusion chromatography with an eluent of 100% MeOH and separated into 8 sub-fractions. Fraction 7 was subsequently subjected to RP HPLC (Shisheido MGII 10 × 250). A gradient elution from 10% ACN in water to 70% ACN in water for 60 min, compound **1** (10.2 mg) and **3** (3.2 mg) were eluted at 31.2 and 35.4 min, respectively. Fractions 4 were combined and further fractionated by RP flash chromatography and followed by RH-HPLC (Shisheido MGII 10 × 250, a gradient elution from 10% ACN in water to 70% ACN in water for 60 min) and compound **2** (2.1 mg) was eluted at 20.5 min.

**16-*epi*-18-acetyl herdmanine D (1)** – amorphous solid; $[\alpha]_D^{31}$ +23.2 (*c* 0.2, MeOH); $^1$H NMR (DMSO-$d_6$, 500 MHz) and $^{13}$C NMR (DMSO-$d_6$, 125 MHz) see Table 1; ESI-MS *m/z* 461/463 $[M+H]^+$; HR-ESI-MS *m/z* 461.0345/463.0323 (calcd for $[M+H]^+$ $C_{20}H_{18}BrN_2O_6$, 461.0343/461.0322).

***N*-(6-bromo-1*H*-indole-3-carbonyl)-L-arginine (2)** – amorphous solid; $[\alpha]_D^{31}$ +4.2 (*c* 0.2, MeOH); $^1$H NMR ($CD_3OD$, 500 MHz) δ 7.96 (1H, brs, H-2), 7.98 (1H, d, *J* = 8.6 Hz, H-4), 7.26 (1H, dd, *J* = 8.6, 1.7 Hz, H-5), 7.57 (1H, d, *J* = 1.7 Hz, H-7), 4.52 (1H, dd, *J* = 8.4, 3.6 Hz, H-11), 1.86 (1H, m, H-12a), 2.02 (1H, m, H-12b), 1.70 (2H,

**Table 1.** NMR data of compound **1** in DMSO-$d_6$ at 500 MHz ($^1$H) and 125 MHz ($^{13}$C)

| position | $\delta_C$ | $\delta_H$, (*J in* Hz) | COSY | HMBC |
|---|---|---|---|---|
| 1-NH | | 12.63, brs | | |
| 2 | 134.5, CH | 8.14, s | | 3, 3a, 8 |
| 3 | 104.6, C | | | |
| 3a | 126.5, C | | | |
| 4 | 105.6, CH | 7.64, s | | 3, 5, 6, 7a |
| 5 | 149.5, C | | | |
| 5-OH | | 10.68, brs | | |
| 6 | 106.3, C | | | |
| 7 | 116.2, CH | 7.68, s | | 3a, 5, 6 |
| 7a | 131.2, C | | | |
| 8 | 162.7, C | | | |
| 9 | 148.7, C | | | |
| 10 | 121.2, CH | 7.04, d (7.4) | 11 | 9, 11, 12, 14 |
| 11 | 130.2, CH | 7.18, d (7.4) | 10 | 9, 10, 12 |
| 12 | 136.6, C | | | |
| 13 | 130.2, CH | 7.18, d (7.4) | 14 | 9, 12, 14 |
| 14 | 121.2, CH | 7.04, d (7.4) | 13 | 9, 10, 12, 13 |
| 15a | 37.1, $CH_2$ | 3.09, m | 15b, 16 | 11, 12, 13, 17 |
| 15b | | 2.91, m | 15a, 16 | 11, 12, 13, 17 |
| 16 | 55.7, CH | 4.09, brs | 15a, 15b, 18-NH | 17, 19 |
| 17 | 173.2, C | | | |
| 18-NH | | 7.31, brs | 16 | |
| 19 | 168.1, C | | | |
| 20 | 23.0, $CH_3$ | 1.79, s | | 19 |

m, H-13), 3.28 (1H, m, H-14a), 3.22 (1H, m, H-14b); $^{13}$C NMR ($CD_3OD$, 125 MHz) 130.1 (C-2), 111.8 (C-3), 138.6 (C-3a), 122.9 (C-4), 124.9 (C-5), 116.7 (C-6), 115.6 (C-7), 125.9 (C-7a), 167.4 (C-8), 180.1 (C-10), 55.5 (C-11), 31.0 (C-12), 26.3 (C-13), 42.1 (C-14), 158.9 (C-16); LR-ESI-MS *m/z* 396/398 $[M+H]^+$.

**(6-Bromo-1*H*-indol-3-yl) oxoacetamide (3)** – amorphous solid; $^1$H NMR (DMSO-$d_6$, 600 MHz) δ 8.65 (1H, brs, H-2), 8.08 (1H, d, $J$ = 8.5 Hz, H-4), 7.65 (1H, d, $J$ = 1.8 Hz, H-7), 7.26 (1H, dd, $J$ = 8.5, 1.8 Hz, H-5); $^{13}$C NMR (DMSO-$d_6$, 150 MHz) δ 183.1 (C-8), 166.4 (C-9), 137.4 (C-2), 136.3 (C-7a), 124.9 (C-3a), 123.9 (C-5), 122.8 (C-4), 114.8 (C-7), 114.7 (C-6), 114.0 (C-3); LR-ESI-MS *m/z* 267/269 $[M+H]^+$.

## Results and Discussion

**Structure elucidation of compound 1** – Compound **1** was isolated as dark yellow oil and the molecular formula of **1** was established as $C_{20}H_{17}BrN_2O_6$ by the interpretation of HRESIMS data ($[M+H]^+$ *m/z* 461.0345/463.0323). The $^1$H and $^{13}$C NMR spectrum of **1** in DMSO-$d_6$ showed three carbonyl carbons ($\delta_C$ 173.2, 168.1, and 162.7), two oxygenated carbons ($\delta_C$ 149.5, and 148.7) in the aromatic rings, three downfield shifted exchangeable protons ($\delta_H$ 12.63, 10.68, and 7.31), a downfield shifted singlet proton of heteroaromatic ring ($\delta_H$ 8.14), four protons of 1,4-disubstituted benzene ($\delta_H$ 7.18, and 7.04) and two singlet protons in aromatic ring systems ($\delta_H$ 7.68, and 7.64). Analysis of 1D and 2D NMR data led us to the assignment of a 3-carbonyl-6-bromo-5-hydroxy-indol-3-yl residue of **1** (Fig. 1, Table 1). The HMBC correlations from two aromatic methinyl protons ($\delta_H$ 7.68, H-7 and $\delta_H$ 7.64, H-4) to an oxygenated quaternary carbon ($\delta_C$ 149.6, C-5), a brominated quaternary carbon ($\delta_C$ 106.4, C-6), and two additional quaternary carbons at $\delta_C$ 126.5 (C-4a) and 131.2 (C-7a) and from a downfielded broad singlet methinyl proton ($\delta_H$ 8.14, H-2) to C-4a and C-7a as well as the presence of exchangeable proton ($\delta_H$ 12.63, 1-NH, brs) indicated the presence of indole structure with an hydroxy group at C-5 and a bromine atom at C-6. Additionally, H-2 showed an intense HMBC correlation to a carbonyl carbon at $\delta_C$ 162.7 (C-8), and the presence of a carbonyl group on C-3 of a modified indole was established. In addition, the presence of an oxygenated quaternary carbon on 1,4-disubstituted benzene is reminiscent of a tyrosine unit. The HMBC correlations from H-15a ($\delta_H$ 3.09) and H-15b ($\delta_H$ 2.91) to C-11/C-13, C-12, and C-17, from H-16 ($\delta_H$ 4.09) to two carbonyl carbons ($\delta_C$ 173.2, C-17, and 168.1, C-19), from H-16 to C-17, and C-19, and from a singlet methyl signal at $\delta_H$ 1.79 (H-20) to C-19 as well as a spin system for H-15a/15b, H-16, and 18-NH on COSY spectra supported the presence of a *N*-acteyl tyrosine residue. Finally, 3-carbonyl-6-bromo-5-hydroxy-indole and *N*-acteyl tyrosine residue were connected via ester linkage between a carbonyl group on C-3 and an hydroxy group on 1,4-disubstituted benezne. The upfield shifted carbon chemical shift of C-9 ($\delta_C$ 148.7) also support the presence of an ester bond rather than a phenolic hydroxy group on aromatic ring of *N*-acetyl tyrosine.

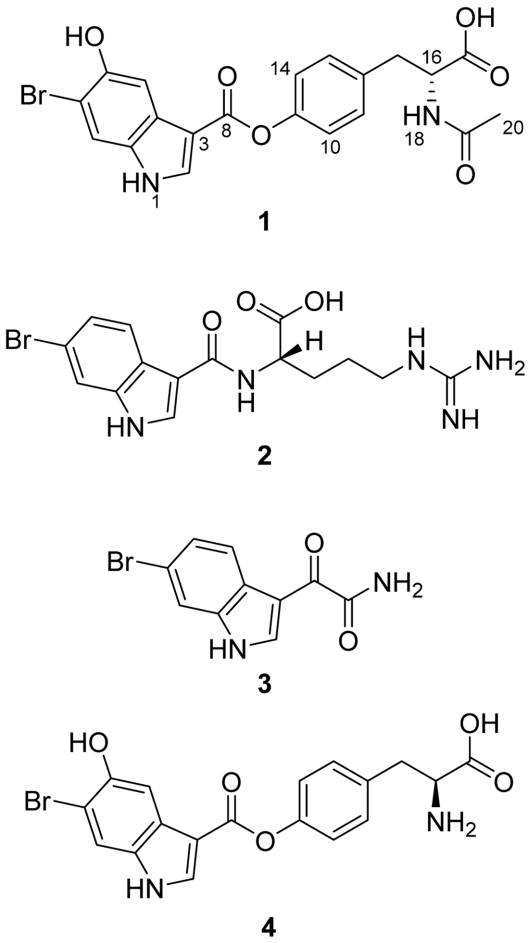


**Fig. 1.** Structures of compounds **1** - **3** from a marine colonial tunicate *Didemnum* sp. and herdmanine D (**4**).

The specific of compound **1** was evaluated as +23.2 degree, but the value of a known compound, herdmanine D (**4**), was reported as –4.7 degree.[14] Therefore, the absolute configuration of sterogenic center at C-16 derived from tyrosine residue in compoun **1** was determined as *R* by comparison of specific rotation value with the structurally related compounds (Fig. 1).

Along with compound **1**, two known brominated indole

alkaloids (**2**, **3**) were additionally obtained. Their structures were confirmed by comparison of $^{1}H$ and $^{13}C$ NMR data to the original data.[17,18]

The antibacterial and farnesoid-X-receptor antagonizing activities of compounds **1-3** were tested (data not shown), but they showed no significant activity on both bioassays. However, herdmanine D (**4**) was reported as an inhibitor of nitric oxide production and negative modulator of proinflammatory cytokine expressions such as TNF-α, IL-1β, and IL-6 in LPS-treated RAW 264.7 cells.[14] Due to the structural similarities between **1** and **4**, compound **1** is expected to have anti-inflammatory potential and need to be screened against inflammatory-related therapeutic targets.

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