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NOTE

New vasorelaxant indole alkaloids, taberniacins A and B, from *Tabernaemontana divaricata*

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Abstract
Taberniacins A (**1**) and B (**2**), new indole alkaloids, were isolated from the stems of *Tabernaemontana divaricata* (Apocynaceae). Structure elucidation of **1** and **2** was based on spectroscopic methods and total synthesis. Each alkaloid showed vasorelaxant activity against phenylephrine-induced contraction of isolated rat aorta.

Keywords Indole alkaloid · Taberniacin A · Taberniacin B · *Tabernaemontana divaricata* · Vasorelaxant

Introduction

Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult., belonging to the family Apocynaceae, is widely distributed in the tropical and subtropical areas of Asia and Australia [1]. It is mainly known as a garden tree, but in China and Thailand it is traditionally used for the treatment of fever and pain [1, 2]. *T. divaricata* is known for the production of a wide variety of indole alkaloids [3–10]. According to van Beek et al.[11], these alkaloids are classified into 11 classes: Vincosan, Corynanthean, Vallesiachotaman, Strychnan, Aspidospermatan, Plumeran, Eburan, Ibogan, Tacaman, Bis-indole and miscellaneous. Recently, we isolated a new type of tetrakis-indole alkaloid as alasmontamine A from *T. elegans* [12], and new yohimbine-related alkaloids from *T. corymbosa* [13]. In our search for structurally and biogenetically interesting natural products [14–18], we have isolated two new indoles, taberniacins A (**1**) and B (**2**) (Fig. 1).

In this paper we would like to describe the structures of taberniacins A (**1**) and B (**2**) proposed on the basis of their spectral data and total synthesis, and their vasorelaxant activity.

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Results and discussion

Taberniacin A (**1**), a yellow amorphous solid, has the molecular formula $C_{17}H_{14}N_4O$ by HRESITOFMS [*m*/*z* 291.1269 $(M+H)^+$, $\Delta+2.3$ mmu] compatible with 13° of unsaturation. IR spectrum suggested the presence of NH (3334 cm^{-1}) and amide carbonyl (1672 cm^{-1}) groups. The ^{13}C NMR (Table 1) spectrum of **1** disclosed seventeen carbon signals due to one amide carbonyl (δ_C 168.8), seven sp^2 quaternary carbons (δ_C 157.9, 149.6, 138.1, 133.0, 127.1, 125.1 and 119.2), seven sp^2 methines (δ_C 151.5, 135.4, 129.9, 125.4, 120.6, 120.2, and 112.8), two sp^3 methylenes (δ_C 48.6 and 19.4). ^1H and ^{13}C NMR signals for **1** were assigned by detail analysis of the HSQC spectrum. The ^1H–^1H COSY spectrum revealed the connectivity of C-5–C-6 and C-9 to C-12 (Fig. 2). HMBC correlations of H_2-5 (δ_H 4.05) to C-3 (δ_C 157.9) and C-7 (δ_C 119.2), and H_2-6 (δ_H 3.04) to C-2 (δ_C 127.1) and C-8 (δ_C 125.1), and H-9 (δ_H 7.65) to C-7, C-8, and C-13 (δ_C 138.1), and H-11 (δ_H 7.33) to C-13, and H-12 (δ_H 7.45) to C-8 indicated the presence of a 3,4-dihydro-β-carboline skeleton. The signals at δ_H 9.11 and δ_H 9.06 appeared as broadened singlets, while the signal δ_H 8.50 appeared as a triplet ($J=2.4$ Hz) were attributed to H-15, H-17 and H-19, respectively, which associated with a 3,5-disubstituted pyridine system. The presence of primary amide was deduced from the HMBC correlations of H-17 (δ_H 9.11) to C-18 (δ_C 129.9) and H-19 (δ_H 9.06) to C-20 (δ_C 168.8) and mass spectrum. The connectivity of 3,4-dihydro-β-carboline and nicotinamide was confirmed by the HMBC

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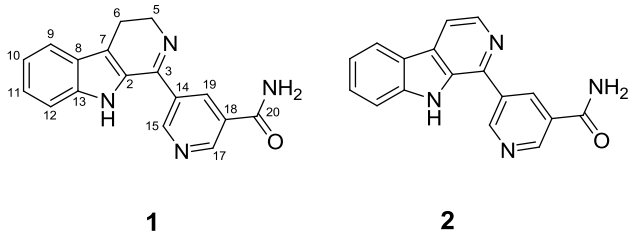

Fig. 1 Structures of isolated compounds (**1** and **2**) from *Tabernaemontana divaricata*

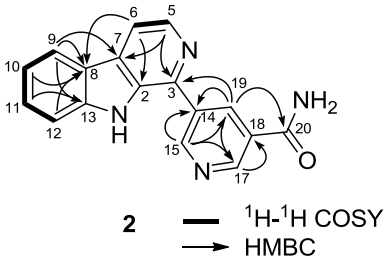

Fig. 3 Selected 2D NMR correlations for taberniacin B (**2**)

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** and **2** in $CDCl_3/CD_3OD$

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
2		127.1		134.9
3		157.9		138.6
5	4.05 (2H, m)	48.6	8.48 (1H, d, 5.2)	139.0
6	3.04 (2H, t, 8.4)	19.4	8.12 (1H, d, 5.2)	115.7
7		119.2		131.6
8		125.1		121.7
9	7.65 (1H, d, 7.8)	120.2	8.21 (1H, d, 7.8)	122.2
10	7.17 (1H, dd, 7.8, 7.8)	120.6	7.32 (1H, dd, 7.8, 7.8)	120.8
11	7.33 (1H, dd, 7.8, 7.8)	125.4	7.60 (1H, dd, 7.8, 7.8)	129.6
12	7.45 (1H, d, 7.8)	112.8	7.63 (1H, d, 7.8)	112.7
13		138.1		142.4
14		133.0		134.9
15	8.50 (1H, t, 2.4)	135.4	8.78 (1H, t, 2.1)	136.1
17	9.11 (1H, brs)	129.9	9.13 (1H, d, 1.8)	130.6
18		149.6		148.5
19	9.06 (1H, brs)	151.5	9.29 (1H, d, 1.8)	152.2
20		168.8		169.5

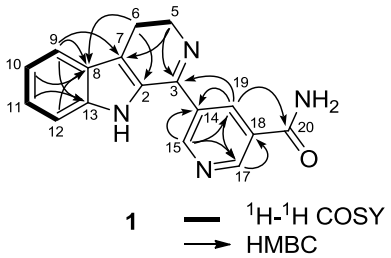

Fig. 2 Selected 2D NMR correlations for taberniacin A (**1**)

correlations of H-19 to C-3 and C-14 (δ_C 133.0). Therefore, taberniacin A could be assigned as **1**. While a related alkaloid, naucledine, which possessing methyl nicotinate, was isolated from *Nauclea diderrichii* and synthesized by Mclean et al. [19, 20], this is the first report for the isolation of a β-carboline alkaloid attaching nicotinamide.

Taberniacin B (**2**) was obtained as a white powder. The HRESITOFMS revealed a pseudo-molecular ion peak at [*m/z* 289.1102 $(M+H)^+$, $\Delta+1.3$ mmu], compatible with the molecular formula $C_{17}H_{12}N_4O$ and 14° of unsaturation. The molecular formula of taberniacin B (**2**) was less than that of taberniacin A (**1**) by 2 mass units. ^{1}H and ^{13}C NMR data (Table 1) of taberniacin B (**2**) indicated that a double bond took the place of a single bond between C-5 and C-6 of taberniacin A (**1**). The structure of taberniacin B (**2**) was finally established by 2D NMR correlations (Fig. 3) and taberniacin B (**2**) was indicated the dehydrogenated derivative of taberniacin A (**1**).

In previous papers, we have reported vasorelaxant activities of β-carboline alkaloids [21, 22]. Taberniacins A (**1**) and B (**2**) were also expected to show strong vasoleraxant activity. Thus, to evaluate their biological activity and confirm the proposed structures, we synthesized **1** and **2**. We have attempted the preparation of β-carboline skeleton and primary amide moiety with the combination of Bischler–Napieralski procedure [23] and hydrolysis of a cyano group.

Tryptamine was reacted with acid chloride of 5-bromo-3-pyridinecarboxylic acid to afford the corresponding amide **3** in 62% yield. For preparation of aromatic nitrile **4** from **3**, we initially investigated two methods, a Rosemund–von Braun reaction [24] and a Cu-catalyzed cyanation [25], which gave only recovery of starting materials or decomposition. In the other procedures for the synthesis of nitriles, application of the Weissman protocol [26] ($K_4[Fe(CN)_6]$/$Pd(OAc)_2/Na_2CO_3$) in *N*,*N*-dimethylacetamide (DMAc) gave the best result, 80% yield of **4**. Bischler–Napieralski cyclization of amide **4** in the presence of $POCl_3$ resulted in the formation of the desired 3,4-dihydro-β-carboline **5**. Subsequent transformation of nitriles into primary amides in mild conditions using the urea-hydrogen peroxide adduct (UHP) [27] gave **1** in good yield (Fig. 4).

Direct conversion of **1** into **2** by dehydrogenation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) ended in a low yield of **2** with the recovery of **1**. We next chose to synthesize β-carboline **6**, which could be then converted to amide easily, from 3,4-dihydro-β-carboline **5** by dehydrogenation with DDQ. This attempt generated the desired

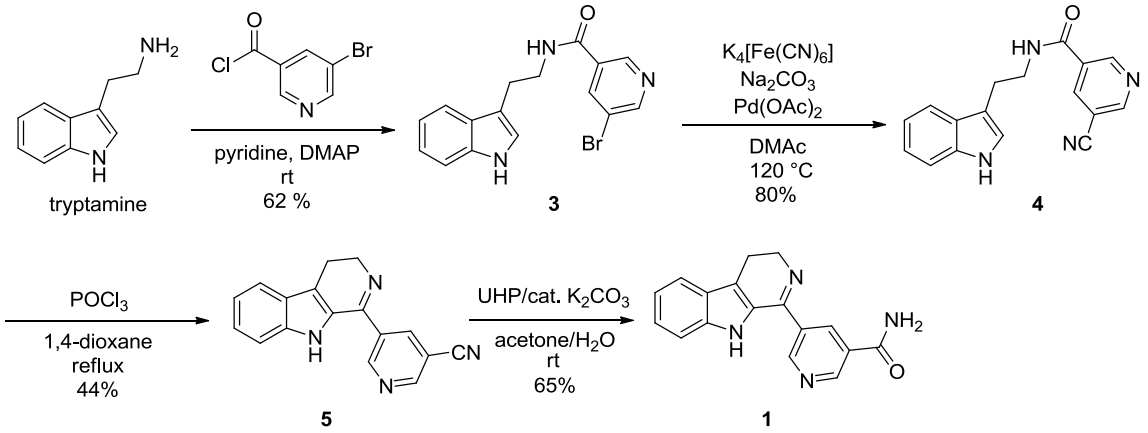

Fig. 4 Total synthesis of taberniacin A (**1**)

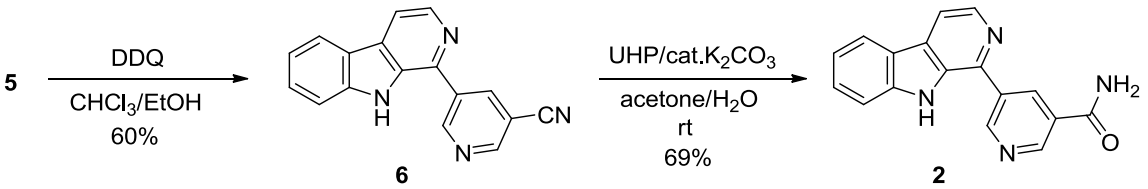

Fig. 5 Total synthesis of taberniacin B (**2**)

β-carboline **6** in good yield. The primary amide was formed by the same hydrolysis method in 69% yield to afford taberniacin B (**2**) (Fig. 5). The spectroscopic data and retention time of the synthetic compounds **1** and **2** were identical to those of the natural products.

Vasorelaxant activities of synthesized taberniacins A (**1**) and B (**2**) were evaluated. After a maximal response was achieved when applying phenylephrine (PE, 3×10^{-7} M) to thoracic aortic rings with endothelium, we added taberniacins A (**1**) and B (**2**). Both alkaloids showed a moderate vasorelaxant activity on isolated rat aorta in a concentration-dependent manner, as shown in Fig. 6. The IC_{50} values of taberniacins A (**1**) and B (**2**) were 2.86 μM and 580 nM, respectively. The mode of action of both alkaloids on vasorelaxant activity is under investigation.

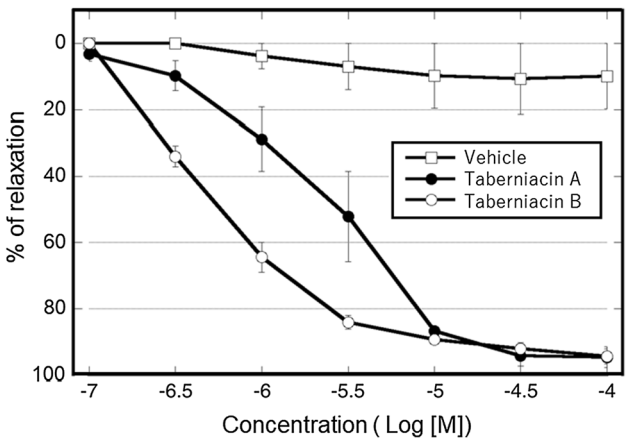

Fig. 6 Relaxation effect of taberniacins A (**1**) and B (**2**) on rat aortic rings precontracted with phenylephrine 3×10^{-7} M

Experimental section

General Experimental Procedures Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO FT/IR-4100 spectrophotometer and PerkinElmer FT-IR spectrometer, spectrum RX 1. High-resolution ESI MS were obtained on a LTQ Orbitrap XL (Thermo Scientific) and LC MS were obtained on a Shimadzu LCMS- IT- TOF (Shimadzu). ^{1}H and 2D NMR spectra were recorded on a Bruker AV 400 spectrometer, and chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0 for $CDCl_3$) and TMS for $CDCl_3$/CD_3OD. Standard pulse sequences were employed for the 2D NMR experiments.

Material The stems of *T. divaricata* were collected at Okinawa, Japan, in 2015. The botanical identification was made by Dr. Yusuke Hirasawa, Faculty of Pharmaceutical Sciences, Hoshi University. The voucher specimen

(Herbarium No. HO_3) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan.

Vasodilation Assay The vasorelaxant activities of alkaloids **1** and **2** were tested using the same procedure as reported previously by Mukhtar et al. [28]. The animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

Extraction and Isolation Dried stems of *T. divaricata* (2.5 kg) were defatted with hexane, and the plant material was dried and then soaked in 25% NH_4OH for 2 h. They were then soaked and macerated with $CHCl_3$ twice over a period of 3 days. The supernatant obtained was concentrated to give crude alkaloids (3.5 g). The crude alkaloids (3.0 g) were subjected to a SiO_2 column ($CHCl_3$/MeOH, 1:0 → 0:1). Further purification of the 7th and 8th fractions was done by a preparative thin layer chromatography ($CHCl_3$/MeOH, 85:15, saturated with NH_4OH) to get a mixture of taberniacins A (**1**) and B (**2**). This mixture was further purified by ODS HPLC (CAPCELL PAK C18 MG-II, 5 µm, 10 × 250 mm; eluent, 40% MeOH/0.1% TFA aq.; flow rate, 2.0 mL/min; UV detection at 254 nm) to afford taberniacins A (**1**, 0.5 mg, 0.000025%) and B (**2**, 0.5 mg, 0.000025%).

Taberniacin A (1): yellow amorphous solid, IR (Zn–Se) ν_{max} 3334, 1672 cm^{-1}; UV (MeOH) λ_{max} (log ε) 201 (4.21), 327 (3.54) nm; ESIMS (pos.) *m/z* 291 $(M+H)^+$; HRESITOFMS *m/z* 291.1269 [$(M+H)^+$; calcd. for $C_{17}H_{15}N_4O$, 291.1246].

Taberniacin B (2): white powder, IR (Zn–Se) ν_{max} 3322, 1678 cm^{-1}; UV (MeOH) λ_{max} (log ε) 219 (4.42), 240 (sh, 4.21), 290 (4.03), 362 (3.64) nm; ESIMS (pos.) *m/z* 289 $(M+H)^+$; HRESITOFMS *m/z* 289.1102 [$(M+H)^+$; calcd. for $C_{17}H_{13}N_4O$, 289.1089].

***N*-(2-(1*H*-indol-3-yl)ethyl)-5-bromonicotinamide (3)** To a solution of 5-bromonicotinic acid (6.0 mmol, 1.2 g) in toluene (10 mL), we added thionyl chloride (10 mL) at room temperature. The mixture was refluxed for 2 h and concentrated under vacuum to give crude acyl chloride (1.3 g), which, without further purification, was used in the next reaction. To a solution of tryptamine (6.0 mmol, 1.0 g) and *N*,*N*-dimethyl-4-aminopyridine (DMAP, 1.2 mmol, 146 mg) in pyridine (10 mL), we added the acyl chloride at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was diluted with $CHCl_3$ and successively washed with water and brine, dried (Na_2SO_4), concentrated, and purified by column chromatography on NH–SiO_2. Elution with $CHCl_3$/MeOH (20:1, v/v) gave **3** (1.26 g, 62%) as a yellow crystal; IR (Zn–Se) ν_{max} 3272, 1644 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.72 (d, 1H, J = 2.0 Hz), 8.66 (d, 1H, J = 2.0 Hz), 8.18 (brs, 1H), 8.15 (t, 1H, J = 2.0 Hz), 7.62 (d, 1H, J = 8.0 Hz), 7.39 (d, 1H, J = 8.0 Hz), 7.23 (dd, 1H, J = 7.2, 7.2 Hz), 7.14 (dd, 1H, J = 7.2, 7.2 Hz), 7.07 (d, 1H, J = 1.6 Hz), 6.29 (brs, 1H), 3.80 (q, 2H, J = 6.4 Hz), 3.11 (t, 2H, J = 6.4 Hz); ^{13}C-NMR ($CDCl_3$) δ 164.3, 152.9, 145.8, 137.8, 136.5, 131.7, 127.3, 122.3, 122.2, 120.9, 119.5, 118.5, 112.5, 111.5, 40.7, 25.1; HRESIMS *m/z* 368.0179 [calcd. for $C_{16}H_{14}BrN_3NaO$ $(M+Na)^+$, 368.0198].

***N*-(2-(1*H*-indol-3-yl)ethyl)-5-cyanonicotinamide (4)** A round flask was charged with the aryl bromide **3** (3.5 mmol, 1.2 g), DMAc (6 mL), $K_4[Fe(CN)_6]$ (3.9 mmol, 1.65 g), Na_2CO_3 (17.5 mmol, 1.9 g), and $Pd(OAc)_2$ (0.5 mol%, 30 mg). The flask was filled with nitrogen and heated to 120 °C for 14 h. The reaction mixture was cooled to room temperature and diluted with 20 mL of $CHCl_3$. The resulting slurry was filtered through Celite and the filtrate washed with water and brine, dried (Na_2SO_4), concentrated, and purified by column chromatography on SiO_2. Elution with $CHCl_3$/MeOH (20:1, v/v) gave **4** (810 mg, 80%) as a yellow solid; IR (Zn–Se) ν_{max} 3308, 2237, 1652 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.97 (d, 1H, J = 2.0 Hz), 8.89 (d, 1H, J = 2.0 Hz), 8.24 (brs, 1H), 8.23 (t, 1H, J = 2.0 Hz), 7.60 (d, 1H, J = 7.6 Hz), 7.39 (d, 1H, J = 8.0 Hz), 7.22 (ddd, 1H, J = 7.2, 7.2, 1.2 Hz), 7.13 (ddd, 1H, J = 7.2, 7.2, 0.8 Hz), 7.08 (d, 1H, J = 2.0 Hz), 6.48 (brs, 1H), 3.81 (q, 2H, J = 6.4 Hz), 3.12 (t, 2H, J = 6.4 Hz); ^{13}C-NMR ($CDCl_3$) δ 163.4, 153.9, 150.1, 138.4, 136.4, 130.3, 127.2, 122.4, 122.3, 119.6, 118.5, 115.9, 112.5, 111.5, 109.9, 40.8, 25.0; HRESIMS *m/z* 313.1070 [calcd. for $C_{17}H_{14}N_4NaO$ $(M+Na)^+$, 313.1065].

5-(4,9-dihydro-3*H*-pyrido[3,4-*b*]indol-1-yl)nicotinonitrile (5) To a solution of amide **4** (2.8 mmol, 800 mg) in 1,4-dioxane (5 mL) at 0 °C, we added $POCl_3$ (8.6 mmol, 800 µL) dropwise. The mixture was refluxed for 2 h followed by cooling to room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (25 mL) and partitioned with $CHCl_3$. The combined organic layers were dried (Na_2SO_4), concentrated, and purified by column chromatography on NH–SiO_2. Elution with $CHCl_3$/MeOH (20:1, v/v) gave **5** (330 mg, 44%) as a red solid; IR (Zn–Se) ν_{max} 2236 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 9.23 (d, 1H, J = 2.0 Hz), 9.00 (d, 1H, J = 2.0 Hz), 8.42 (t, 1H, J = 2.0 Hz), 8.06 (brs, 1H), 7.67 (d, 1H, J = 7.6 Hz), 7.43 (d, 1H, J = 8.4 Hz), 7.36 (ddd, 1H, J = 8.4, 8.4, 1.2 Hz), 7.23 (ddd, 1H, J = 8.0, 8.0, 1.2 Hz), 4.10 (dd, 2H, J = 8.8, 8.4 Hz), 3.01 (dd, 2H, J = 8.8, 8.4 Hz); ^{13}C-NMR ($CDCl_3$) δ 155.1, 152.8, 152.0, 138.8, 137.2, 133.7, 126.5, 125.6, 125.4, 121.0, 120.3, 119.6, 116.0, 112.3, 110.3, 49.2, 19.2; HRESIMS *m/z* 273.1113 [calcd. for $C_{17}H_{13}N_4$ $(M+H)^+$, 273.1140].

1-(pyridin-3-yl)-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (1) (Taberniacin A) To a solution of nitrile **5** (0.037 mmol, 10 mg) in acetone–water (1:1, 2 mL) at room temperature, we added UHP (0.15 mmol, 13.8 mg) and anhydrous K_2CO_3 (1 mg). The mixture was stirred for 1 h and acetone was removed under vacuum. H_2O was added to the residue and the precipitate was filtered off. The product was purified by washing with H_2O and $CHCl_3$ to afford **1** (6.9 mg, 65%) as a yellow amorphous solid; All spectroscopic data corresponded to those of natural product **1**.

5-(9*H*-pyrido[3,4-*b*]indol-1-yl)nicotinonitrile (6) To a solution of amide **5** (0.037 mmol, 10 mg) in $CHCl_3$:EtOH (1:1, 2 mL) at room temperature, we added DDQ (0.074 mmol, 16.7 mg). The mixture was stirred for 12 h and concentrated and purified by column chromatography on ODS. Elution with MeOH/H_2O (6:4, v/v) gave **6** (6 mg, 60%) as a red amorphous solid; IR (Zn–Se) ν_{max} 2361 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 9.50 (d, 1H, J = 2.4 Hz), 8.97 (d, 1H, J = 2.0 Hz), 8.81 (brs, 1H), 8.67 (t, 1H, J = 2.0 Hz), 8.62 (d, 1H, J = 5.2 Hz), 8.19 (d, 1H, J = 8.0 Hz), 8.05 (d, 1H, J = 5.2 Hz), 7.61 (ddd, 1H, J = 8.4, 7.2, 1.2 Hz), 7.58 (brd, 1H, J = 8.0 Hz), 7.37 (ddd, 1H, J = 8.0, 6.8, 1.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 153.8, 152.3, 144.2, 141.3, 136.8, 135.3, 133.9, 133.0, 131.5, 129.8, 123.3, 122.2, 121.9, 117.2, 114.1, 113.5, 111.8; HRESIMS *m/z* 271.0948 [calcd. for $C_{17}H_{11}N_4$ $(M+H)^+$, 271.0984].

1-(pyridin-3-yl)-9*H*-pyrido[3,4-*b*]indole (2) (Taberniacin B) Nitrile **6** (0.019 mmol, 5 mg) was converted to **2** (3.7 mg, 69%) as a white powder using the same procedure as taberniacin A (**1**). All spectroscopic data corresponded to those of natural product **2**.

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