Notes

Bisnicalaterine A, a Vobasine-Vobasine Bisindole Alkaloid from Hunteria zeylanica

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A new bisindole alkaloid, bisnicalaterine A (1), consisting of two vobasine-type skeletons, and 3-epivobasinol (2) and 3-O-methylepivobasinol (3), with vobasine-type skeletons, were isolated from the leaves of *Hunteria zeylanica*, and their structures were elucidated on the basis of spectroscopic data and chemical correlation. Bisnicalaterine A showed moderate cytotoxicity against various human cancer cell lines.

Hunteria zeylanica (Retz.) Gardner ex Thwaites is a member of the Apocynaceae family in Malaysia, found mostly in Pahang and Selangor.¹ Traditionally the latex has been used for smearing on the sores caused by yaws.² The bark and leaves have been known to produce various skeletal alkaloids depending on the area where the plants were distributed.^{3–8} Some pharmacological actions such as antinociceptive, antipyretic, and anti-inflammatory activities have also been reported recently.9-15 In our search for structurally and biogenetically interesting alkaloids from tropical plants in Malaysia, bisnicalaterine A (1), a new bisindole alkaloid consisting of two vobasine-type skeletons, and two new indole alkaloids, 3-epivobasinol (2) and 3-O-methylepivobasinol (3), with a vobasine-type skeleton, have been isolated from the leaves of H. zeylanica together with perivine $(4)^{16}$ and vobasine $(5)^{17}$ In this paper, we describe the isolation and structure elucidation of 1-3 and cytotoxicity of 1 against various human cancer cell lines.

Leaves of *H. zeylanica* were extracted with MeOH, and a part of the extract was partitioned between EtOAc and 3% aqueous tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na₂CO₃, were extracted with CHCl₃. The CHCl₃soluble materials were subjected to an amino silica gel column (*n*hexane/EtOAc, 4:1 \rightarrow 1:1; *n*-hexane/CHCl₃, 1:1; and then CHCl₃/ MeOH, 1:0 \rightarrow 0:1) followed by a Sephadex LH-20 column, C₁₈ HPLC (CH₃CN/H₂O, 3:7, containing 0.1% TFA) and then an amino silica gel column (*n*-hexane/EtOAc, 8:1 \rightarrow 2:1) to give **1** (7.9 mg, 0.004%), **2** (58.0 mg, 0.03%), and **3** (19.9 mg, 0.01%) together with known alkaloids perivine (**4**)¹⁶ and vobasine (**5**).¹⁷

Compound 1, $[\alpha]_{D}^{2^{2}}$ -93 (*c* 1.0, MeOH), showed a pseudomolecular ion peak at m/z 691 (M + H)⁺ in the ESIMS, and the molecular formula C₄₂H₅₀N₄O₅ was established by HRESIMS [m/z691.3900 (M + H)⁺, Δ +4.1 mmu]. IR absorptions implied the presence of hydroxy (3400 cm⁻¹) and ester carbonyl (1720 cm⁻¹) functionalities. Analysis of the ¹H and ¹³C NMR data (Table 1) and the HMQC spectrum of 1 revealed the presence of eight sp³ methine, six sp³ methylene, six methyl, 10 sp² methine, and 12 sp² quaternary carbons.

The gross structure of **1** was deduced from analyses of the 2D NMR data, including the ${}^{1}H{}-{}^{1}H$ COSY, HMQC, and HMBC



spectra in methanol- d_4 (Figure 1). The ¹H⁻¹H COSY and HSQC spectra revealed connectivities of six partial structures **a**-**f** and was classified into two units, A and B, as shown in Figure 1.

In unit A, the HMBC cross-peaks of H-3 to C-2 and C-7 and of H-6 to C-2, C-7, and C-8 revealed the attachment of partial structure **a** to the indole moiety, while the HMBC cross-peaks of H-19 to C-15, C-20, and C-21, H₂-21 to C-20, and H-15 to C-20 revealed the connectivity of partial structures **a**, **b**, and C-21 through C-20. In addition, the HMBC cross-peaks of the N-2-methyl protons to C-21 and C-5 established the connections between C-5 and C-21 through a nitrogen atom (N-2). Another partial structure, **c**, and the presence of a methyl carboxylate moiety at C-16 were analyzed by the HMBC correlations as shown in Figure 1. These data suggested that unit A possessed a vobasine-type skeleton as in deoxovobasine.¹⁸ The structure of unit B was also analyzed using the HMBC correlations in Figure 1, showing similar correlations as in unit A. The HMBC cross-peaks of H-6' to C-2', C-7', and C-8' revealed the attachment of partial structure **d** to the indole

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Table 1. ¹H NMR Data (J, Hz) of Bisnicalaterine A (1), 3-Epivobasinol (2), and 3-O-Methylepivobasinol (3) at 300 K^a

	1	2	3
3	6.51 (1H, dd, 13.0, 2.3)	5.09 (1H, dd, 12.3, 3.8)	5.03 (1H, dd, 12.5, 3.6)
5	4.13 (1H, m)	3.55 (1H, m)	3.63 (1H, m)
6a	3.72 (1H, m)	3.24 (1H, m)	3.35 (1H, m)
6b	3.52 (1H, m)	3.06 (1H, m)	3.17 (1H, m)
9	7.61 (1H, d, 7.2)	7.47 (1H, d, 7.8)	7.55 (1H, d, 7.9)
10	7.04 (1H, m)	7.02 (1H, t, 7.3)	7.08 (1H, td, 7.0, 1.0)
11	7.04 (1H, m)	7.09 (1H, t, 7.2)	7.16 (1H, td, 7.0, 1.1)
12	7.08 (1H, d, 7.0)	7.17 (1H, d, 7.9)	7.24 (1H, dd, 7.9, 0.9)
14a	3.30 (1H, m)	2.56 (1H, m)	2.66 (1H, m)
14b	2.48 (1H, m)	2.04 (1H, m)	2.17 (1H, m)
15	3.91 (1H, m)	3.84 (1H, m)	3.95 (1H, m)
16	2.80 (1H, br.s)	2.56 (1H, m)	2.66 (1H, m)
18	1.79 (3H, d, 6.5)	1.65 (3H, d, 6.7)	1.71 (3H, dd, 6.8, 1.8)
19	5.48 (1H, q, 6.5)	5.34 (1H, q, 6.7)	5.41 (1H, qd, 6.8, 1.2)
21a	3.90 (1H, m)	3.64 (1H, m)	3.74 (1H, m)
21b	3.12 (1H, s)	2.85 (1H, d, 13.9)	2.95 (1H, m)
NMe	2.70 (3H, s)	2.47 (3H, s)	2.57 (3H, s)
COOMe	2.60 (3H, s)	2.37 (3H, s)	2.44 (3H, s)
OMe			3.30 (3H, s)
3'	5.49 (1H, dd, 12.0, 4.0)		
5'	4.00 (1H, m)		
6'a	3.56 (1H, m)		
б'b	3.28 (1H, m)		
9'	7.52 (1H, d, 6.9)		
10'	6.94 (1H, m)		
11'	6.94 (1H, m)		
12'	7.23 (1H, d, 7.2)		
14'a	2.92 (1H, m)		
14'b	2.28 (1H, m)		
15'	3.77 (1H, m)		
16'	2.63 (1H, br.s)		
18'	1.78 (3H, d, 6.5)		
19'	5.54 (1H, q, 6.5)		
21'a	3.90 (1H, m)		
21'b	3.12 (1H, s)		
NMe'	2.63 (3H, s)		
COO <u>Me</u> '	2.41 (3H, s)		

^{*a*} δ in ppm.



Figure 1. Selected 2D NMR correlations for bisnicalaterine A (1).

moiety, and those of H-19' to C-15', C-20', and C-21' revealed the connectivity of partial structures **d**, **e**, and C-21' through C-20'. The HMBC cross-peaks of the *N*-4-methyl protons to C-21' and C-5' established the connections between C-5' and C-21' through a nitrogen atom (N-4). The partial structure **f** and the presence of a methyl carboxylate moiety at C-16' were analyzed by the HMBC correlations as shown in Figure 1, thus revealing a vobasine-type skeleton as in vobasinol.¹⁹ Finally, the linkage of units A and B through a C-3–N-3 bond was provided by the HMBC correlation

of H-3 to C-13'. Thus, the gross structure of bisnicalaterine A was assigned as **1**, shown in Figure 1.

The relative configuration of **1** was assigned using ¹H NMR chemical shifts, ¹H $^{-1}$ H coupling values, and NOESY correlations. The NOESY correlations of H-15 to H₃-18 and of H-15' to H₃-18' established the *E*-configuration of the ethylidene side chains as shown in Figure 2. The configurations of C-16 and C-16' were proven to be *S** by the highly shielded chemical shifts ($\delta_{\rm H}$ 2.60 and 2.41) of methoxy carbonyl groups at C-16 and C-16', which can be explained by the anisotropic effect of the indole ring. The β -orientation of H-3 and H-3' was elucidated by the coupling constant of H-3 (dd, *J* = 13.0, 2.3 Hz) and H-3' (dd, *J* = 12.0, 4.0 Hz) and NOESY correlations of H-3/H-15 and H-3'/H-15'. The conformation of **1** through the N-3–C-3 bond was assigned by the NOESY correlations of H-3/H-3', H-14a/16'-COOMe, and H-14b/H-12' as shown in a computer-generated 3D drawing (Figure 3).

The absolute configuration of **1** was elucidated by applying the exciton chirality CD method.²⁰ The sign of the first Cotton effect $[\lambda_{\text{max}} 237 \text{ nm} (\Delta \epsilon + 21.80)]$ was positive, while that of the second one $[\lambda_{\text{max}} 222 \text{ nm} (-30.44)]$ was negative (Figure 4), indicating that the chirality between the two indole chromophores in **1** was a right-handed screw, as shown in Figure 3.

Compound **2**, $[\alpha]_D^{22} - 10$ (*c* 1.0, MeOH), showed a pseudomolecular ion peak at m/z 355 (M + H)⁺ in the ESIMS. The molecular formula C₂₁H₂₆N₂O₃ was established by HRESIMS [m/z 355.2002 (M + H)⁺, $\Delta - 2.0$ mmu]. IR absorptions implied the presence of hydroxy (3400 cm⁻¹) and ester carbonyl (1730 cm⁻¹) functionalities. The ¹³C NMR data revealed the presence of four sp³ methine, three sp³ methylene, three methyl, five sp² methine, and six sp² quaternary carbons (Table 2).



unit A

unit B

Figure 2. Selected NOESY correlations for units A and B in bisnicalaterine A (1).



Figure 3. Stereostructure of bisnicalaterine A (1) with selected NOESY correlations and arrows denoting two electric transition dipoles.

The gross structure of **2** was deduced from the ¹³C NMR data, which are highly similar to those of vobasinol.¹⁹ The noticeable difference of the chemical shifts of the C-14 (δ_C 36.9), C-15 (δ_C 30.6), and ester carbonyl carbon (δ_C 171.6) can be explained by the α -orientation of the C-3 hydroxy group. Lack of an intramolecular hydrogen bond between this hydroxy group and the C-16 ester carbonyl carbon causes an upfield shift of the ester carbonyl carbon compared to that (δ_C 174.3) of vobasinol, with a β -oriented hydroxy group.¹⁹ The fact that the hydroxy group in 3-epivobasinol is α -oriented, instead of β as in vobasinol, is also supported by the coupling constant of H-3 (dd, J = 12.4, 3.9 Hz). Spectroscopic data including specific rotation of an oxidative derivative prepared from **2** with CrO₃ were identical with those of vobasine.¹⁷ Thus, the structure of **2** was elucidated to be 3-epivobasinol.

Compound 3, $[\alpha]_D^{22} - 13$ (*c* 1.0, MeOH), showed a pseudomolecular ion peak at m/z 369 (M + H)⁺ in the ESIMS, and the molecular formula $C_{22}H_{28}N_2O_3$ was established by HRESIMS [m/z369.2161, (M + H)⁺, $\Delta - 1.7$ mmu]. IR absorptions implied the presence of an ester carbonyl (1730 cm⁻¹) functionality. The ¹³C NMR data of **3** revealed the presence of four sp^3 methine, three sp^3 methylene, four methyl, five sp^2 methine, and six sp^2 quaternary carbons (Table 2).

The ¹H and ¹³C NMR spectra of **3** were very similar to those of **2** except for the addition of an *O*-methyl group ($\delta_{\rm H}$ 3.30; $\delta_{\rm C}$ 53.4). The downfield shift of C-3 ($\delta_{\rm C}$ 74.6) and upfield shift of C-14 ($\delta_{\rm C}$ 31.7) compared to those of **2** in the ¹³C NMR spectrum and demethoxy fragment ion peak [m/z 337, (M – CH₃OH)⁺] were observed in the ESIMS spectrum. Thus, **3** was elucidated to be 3-*O*-methylepivobasinol. 3-*O*-Methylepivobasinol (**3**) could not be obtained by methanolysis of 3-epivobasinol (**2**).

Bisnicalaterine A (1) showed a moderate cytotoxicity against five cancer cell lines (IC₅₀ 16.2 μ g/mL against HL-60; 31.3 μ g/mL against RPMI-8226; 28.1 μ g/mL against NCI-H226; 21.9 μ g/mL against HCT-116; 38.0 μ g/mL against MCF-7), but the others did not.

So far, two C-3–N-1' vobasine–vobasine bisindole alkaloids, hazuntamine²² and vobasonidine,²³ have been isolated from *Hazunta modesta* var. *methuenii* and *Tabernaemontana corymbosa*,



Figure 4. CD and UV spectra of bisnicalaterine A (1).

respectively. A new bisindole alkaloid, bisnicalaterine A (1), consisting of two vobasine-type skeletons, and 3-epivobasinol (2) and 3-O-methylepivobasinol (3), with vobasine-type skeletons, were isolated from the leaves of *Hunteria zeylanica*, Biogenetically, bisnicalaterine A (1), consisting of two vobasine-type skeletons, might be generated through *N*-oxidation of a sarpagine-type indole alkaloid by Polonovski-type fragmentation or C–N bond cleavage of a quaternary alkaloid⁵ followed by introduction of another vobasine-type alkaloid as a nucleophile.

Experimental Section

General Experimental Procedures. ¹H and 2D NMR spectra were recorded on a JEOL ECA600 and a Bruker AV 400 spectrometer, and chemical shifts were referenced to the residual solvent peaks ($\delta_{\rm H}$ 3.31 and $\delta_{\rm C}$ 49.0 for methanol- d_4). Standard pulse sequences were employed for the 2D NMR experiments. ¹H-¹H COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY spectra in the phasesensitive mode were measured with a mixing time of 800 ms. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long-range C-H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

Material. Leaves of *H. zeylanica* were collected in Kampung Padang, Malaysia, in 1994. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. A voucher specimen (Herbarium No. KL 4345) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation. The leaves of *H. zeylanica* was extracted with MeOH, and part (50 g) of the extract was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na₂CO₃(aq) to pH 10 and extracted with CHCl₃ to give an alkaloidal fraction (2.06 g). The alkaloidal fraction was subjected to an amino silica gel column (*n*-hexane/EtOAc, 4:1 \rightarrow 1:1; *n*-hexane/CHCl₃, 1:1; CHCl₃/MeOH, 1:0 \rightarrow 0:1) to give 16 fractions. Fraction 6 was subjected to a silica gel column (CHCl₃/MeOH, 1:0 \rightarrow 4:1) to give vobasine (5)¹⁷ and 3-*O*-methylepivobasinol (3, 19.9 mg,

Table 2. ¹³C NMR Data of Bisnicalaterine A (1), 3-Epivobasinol (2), and 3-*O*-Methylepivobasinol (3) at 300 K^{α}

	1	2	3
2	134.4	135.7	135.9
3	52.8	66.8	74.6
5	60.4	58.8	58.8
6	20.5	19.0	19.1
7	111.0	109.5	112.2
8	130.3	128.9	129.0
9	118.9	118.0	118.1
10	119.9	118.8	119.0
11	123.3	122.1	122.5
12	111.2	110.3	110.2
13	137.5	136.9	137.3
14	34.1	36.9	31.7
15	32.9	30.6	30.6
16	47.7	46.1	46.6
18	12.5	12.1	12.2
19	121.6	119.1	119.1
20	138.0	135.9	133.2
21	52.9	52.0	52.2
NMe	42.2	41.9	42.1
COOMe	51.1	49.8	49.9
	172.4	171.6	171.7
OMe			53.4
2'	137.5		
3'	66.7		
5'	60.1		
6'	20.4		
7'	109.3		
8'	130.4		
9'	119.3		
10'	120.2		
11'	123.2		
12'	111.8		
13'	136.6		
14'	38.7		
15'	32.0		
16'	47.5		
18'	12.4		
19'	121.4		
20'	138.0		
21'	53.2		
NMe'	42.0		
COO <u>Me</u> '	50.3		
	172.7		

^{*a*} δ in ppm.

0.01%). Fractions 9 and 10 were further separated using a Sephadex LH-20 column to give four fractions. The first eluted fraction was subjected to ODS HPLC (H₂O/CH₃CN, 7:3; TFA 0.1%) followed by an amino silica gel column (*n*-hexane/EtOAc, $8:1 \rightarrow 2:1$) to give bisnicalaterine A (1, 7.9 mg, 0.004%), while the third eluted fraction was subjected to a silica gel column (CHCl₃/MeOH, 20:1 \rightarrow 10:1) to give perivine (4).¹⁶ Fraction 12 was further separated by using a silica gel column (CHCl₃/MeOH, 20:1 \rightarrow 0:1) to give 3-epivobasinol (2, 58.0 mg, 0.03%).

Bisnicalaterine A (1): brown, amorphous solid; $[\alpha]_D^{22} - 93$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} 205 (ϵ 41 400), 225 (ϵ 35 600), and 285 (ϵ 11 600); CD (MeOH) λ_{max} 203 ($\Delta \epsilon$ 9.88), 222 ($\Delta \epsilon$ -30.44), 230 ($\Delta \epsilon$ 0), 237 ($\Delta \epsilon$ 21.80); IR (KBr) ν_{max} 3400 and 1720 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 691 (M + H)⁺; HRESIMS *m*/*z* 691.3900 [(M + H)⁺] (calcd for C₄₂H₅₁N₄O₅, 691.3859).

3-Epivobasinol (2): brown, amorphous solid; $[\alpha]_D^{22} - 10$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} 205 (ϵ 10 800), 225 (ϵ 9500), and 285 nm (ϵ 3000); CD (MeOH) λ_{max} 204 ($\Delta\epsilon$ 7.49) and 229 ($\Delta\epsilon$ -3.49); IR (KBr) ν_{max} 3400 and 1730 cm⁻¹; ¹H and ¹³C NMR, see Tables 1 and 2; ESIMS *m*/*z* 355 (M + H)⁺; HRESIMS *m*/*z* 355.2002 [(M + H)⁺] (calcd for C₂₁H₂₇N₂O₃, 355.2022).

3-O-Methylepivobasinol (3): brown, amorphous solid; $[\alpha]_D^{22} - 13$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} 205 (ϵ 22 800), 225 (ϵ 20 400), and 285 nm (ϵ 6600); CD (MeOH) λ_{max} 204 ($\Delta \epsilon$ 10.46) and 234 ($\Delta \epsilon - 4.91$); IR (KBr) ν_{max} 1730 cm⁻¹; ¹H and ¹³C NMR, see Tables 1 and 2; ESIMS *m*/*z* 369 (M + H)⁺; HRESIMS *m*/*z* 369.2161 [(M + H)⁺] (calcd for C₂₂H₂₉N₂O₃, 369.2178).

Conversion of 3-Epivobasinol to Vobasine. To a solution of 3-epivobasinol (2, 2.0 mg) in pyridine (0.3 mL) was added CrO₃ (1.0 mg), and the mixture was kept at rt for 8 h. After evaporation, the residue was applied to a silica gel column (CHCl₃/MeOH, 9:1) to give a compound (1.6 mg) whose spectroscopic data and $[\alpha]_D$ value were identical with those of vobasine.¹⁷

Cytotoxic Activity. Each cell line [HL-60 (human blood premyelocytic leukemia), RPMI8226 (multiple myeloma), NCI-H226 (nonsmall cell lung carcinoma), HCT-116 (human colon cancer), and MCF-7 (human breast adenocarcinoma) cells] was seeded onto 96-well microtiter plates at 1×10^4 and 5×10^3 cells per well for HL-60, RPMI8226, NCI-H226, HCT-116, and MCF-7, respectively. Cells were preincubated for 24 h at 37 $^{\circ}\mathrm{C}$ in a humidified atmosphere of 5% CO_2. Different concentrations of each compound $(10 \,\mu\text{L})$ were added to the cultures, and then the cells were incubated at 37 °C for 48 h. On the third day, 15 µL of MTT solution (5 mg/mL) was added into each well of the cultured medium. After a further 2 h of incubation, $100 \,\mu\text{L}$ of 10% SDS/0.01 N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipet. The optical density measurements were made using a micropipet reader (Benchmark Plus microplate spectrometer, BIO-RAD) equipped with a two-wavelengths system (550 and 700 nm). In each experiment, three replicates of wells were prepared for each sample. The ratio of the living cells was determined on the basis of the difference of the absorbance between those of samples and controls. These differences are expressed in percentage, and cytotoxic activity was indicated as an IC₅₀ value.

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Supporting Information Available: ¹H and ¹³C NMR spectra of 1-3 are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

- (1) Whitmore, T. C. Tree Flora Malaysia; Longman: FRIM, 1973.
- (2) Perry, L. M. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses; The MIT Press: Cambridge, MA, 1980.

- (3) Lavaud, C.; Massiot, G.; Vercauteren, J.; Le Menolivier, L. Phytochemistry 1982, 21, 445–447.
- (4) Subhadhirasakul, S.; Aimi, N.; Takayama, H.; Ponglux, D.; Sakai, S. Chem. Pharm. Bull. 1994, 42, 991–993.
- (5) Subhadhirasakul, S.; Takayama, H.; Miybe, Y.; Kitajima, M.; Ponglux, D.; Sakai, S.; Aimi, N. *Heterocycles* **1995**, *41*, 2049–2056.
- (6) Subhadhirasakul, S.; Takayama, H.; Miyabe, Y.; Aimi, N.; Ponglux, D.; Sakai, S. Chem. Pharm. Bull. 1994, 42, 2645–2646.
- (7) Takayama, H.; Subhadhirasakul, S.; Mizuki, J.; Kitajima, M.; Aimi, N.; Ponglux, D.; Sakai, S. *Chem. Pharm. Bull.* **1994**, *42*, 1957–1959.
- (8) Takayama, H.; Subhadhirasakul, S.; Ohmori, O.; Kitajima, M.; Ponglux, D.; Aimi, N. *Heterocyles* 1998, 47, 87–90.
- (9) Leewanich, P.; Tohda, M.; Matsumoto, K.; Subhadhirasakul, S.; Takayama, H.; Aimi, N.; Watanabe, H. *Eur. J. Pharmacol.* **1997**, *332*, 321–326.
- (10) Leewanich, P.; Tohda, M.; Matsumoto, K.; Subhadhirasakul, S.; Takayama, H.; Aimi, N.; Watanabe, H. *Eur. J. Pharmacol.* **1998**, *348*, 271–277.
- (11) Leewanich, P.; Tohda, M.; Matsumoto, K.; Subhadhirasakul, S.; Takayama, H.; Watanabe, H. *Biol. Pharm. Bull.* **1996**, *19*, 394–399.
- (12) Reanmongkol, W.; Matsumoto, K.; Watanabe, H.; Subhadhirasakul, S.; Sakai, S. *Biol. Pharm. Bull.* **1994**, *17*, 1345–1350.
- (13) Reanmongkol, W.; Tohda, M.; Matsumoto, K.; Subhadhirasakul, S.; Takayama, H.; Sakai, S.; Watanabe, H. *Biol. Pharm. Bull.* **1995**, *18*, 910–912.
- (14) Reanmongkol, W.; Matsumoto, K.; Watanabe, H.; Subhadhirasakul, S.; Takayama, H.; Sakai, S. *Biol. Pharm. Bull.* **1995**, *18*, 33–36.
- (15) Mohamad, K.; Suzuki, T.; Baba, Y.; Zaima, K.; Matsuno, Y.; Hirasawa, Y.; Mukhtar, M. R.; Awang, K.; Hadi, A. H. A.; Morita, H. *Heterocycles* **2007**, *74*, 969–976.
- (16) Svoboda, G. H. J. Am. Pharm. Assoc. 1958, 47, 834.
- (17) Renner, U. Experientia 1959, 15, 185–186.
- (18) Jaeggi, K. A.; Renner, U. Helv. Chim. Acta 1972, 55, 446-449.
- (19) Renner, U.; Prins, D. A. Experientia 1961, 17, 106.
- (20) Harada, N.; Nakanishi, K. J. Am. Chem. Soc. 1969, 91, 3989-3991.
- (21) Bui, A.; Das, B. C.; Guittet, E.; Stoven, V. *Heterocycles* **1994**, *38*, 1025–1032.
- (22) Kam, T.; Sim, K. Helv. Chim. Acta 2002, 85, 1027-1032.
- (23) Grierson, D. Org. React. 1990, 39, 85-295.

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