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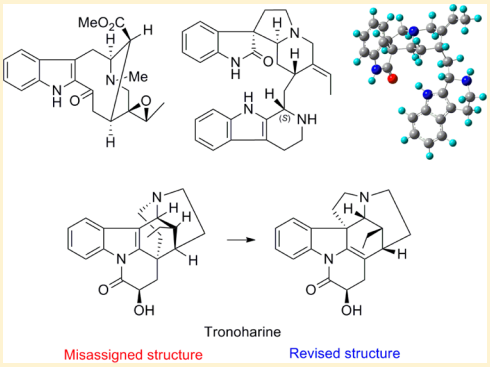
Cytotoxic Vobasine, Tacaman, and Corynanthe-Tryptamine Bisindole Alkaloids from *Tabernaemontana* and Structure Revision of Tronoharine

Dawn Su-Yin Sim,[†] Kam-Weng Chong,[†] Choy-Eng Nge,[†] Yun-Yee Low,[†] Kae-Shin Sim,[‡] and Toh-Seok Kam*[,†]

[†]Department of Chemistry and [‡]Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

Supporting Information

ABSTRACT: Seven new indole alkaloids (**1**−**7**) comprising four vobasine, two tacaman, and one corynanthe-tryptamine bisindole alkaloid were isolated from the stem-bark extract of a Malayan *Tabernaemontana*. Two of the new vobasine alkaloids (**1**, **3**), as well as 16-epivobasine (**15**) and 16-epivobasenal (**17**), showed appreciable cytotoxicity toward KB cells (IC_{50} ca. 5 μg/mL). The structure of the known *Tabernaemontana* alkaloid tronoharine (**8**) was revised based on newly acquired NMR data, as well as X-ray diffraction analysis.



The genus *Tabernaemontana* (Apocynaceae) incorporates a large number of species, which are distributed over the tropical and subtropical regions of the world, including parts of the Americas, Africa including Madagascar, Asia, Oceana, and Australia.[1] The taxonomical aspects associated with this genus have long presented a formidable challenge to botanists, due in part to the large number of species, the wide geographical distribution, and the large number of synonyms. The latest and most comprehensive review of this difficult genus (the Old World species) is that by Leeuwenberg, whose revision has resulted in a significant reduction in the number of the Old World species.[1] Plants of this genus are prolific producers of alkaloids, in particular indole and bisindole alkaloids, and are well known to elaborate alkaloids with structurally novel molecular skeletons and useful biological activities.[2−5] About 13 species are known to occur in Malaysia (Peninsular Malaya and Malaysian Borneo), with *T. corymbosa* being the most widely distributed, being encountered in a number of different locations.[1] A number of the Malaysian *Tabernaemontana* have been the subject of thorough chemical studies, with the discovery of many new and biologically active alkaloids.[4−11] We now report the isolation, structure determination, and biological activity of seven new alkaloids, comprising vobasine, tacaman, and a heterodimeric alkaloid, as well as the structure revision of the known hexacyclic alkaloid tronoharine.[12]

RESULTS AND DISCUSSION

Compound **1** (vobasidine A) was obtained as a light yellowish oil with $[\alpha]^{25}_{D}$ −35 ($CHCl_3$, *c* 0.2). The IR spectrum showed bands due to NH (3311 cm^{-1}), ester, and conjugated carbonyl functions (1727 and 1647 cm^{-1}), while the UV spectrum showed absorption maxima at 207, 226, 238, and 314 nm, indicative of a 2-acylindole chromophore.[13] The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 369, and ^{13}C NMR and HRESIMS data established the molecular formula as $C_{21}H_{24}N_2O_4$.

The ^1H and ^{13}C NMR data (Tables 1 and 2) showed the presence of an unsubstituted indole chromophore from the presence of four aromatic resonances (δ_H 7.17−7.73), an indolic NH (δ_H 8.94), a methyl ester group (δ_H 2.66; δ_C 170.9, 50.4), an N4-Me (δ_H 2.68; δ_C 43.1), and a conjugated carbonyl as part of a 2-acylindole (δ_C 188.9) moiety. The ^{13}C NMR data (Table 2) showed a total of 21 carbon resonances, including three methyl, three methylene, eight methine, and two quaternary carbon atoms. The COSY spectrum showed, in addition to the four aromatic hydrogens and an isolated aminomethylene (C-21, δ_C 46.8), a $CH_3CH{-}O$ and a $CH_2CHCHCHCH_2$ fragment (Figure 1). One of the two methylenes was deduced to be α to the ketocarbonyl function from its ^{13}C NMR resonance at δ 42.5. The shifts for the two contiguous carbons at δ_C 58.7 and 62.8 suggested the presence of an epoxide functionality. On the basis of these observations and analysis of the HMBC data (Figure 1), a vobasine-type alkaloid incorporating an epoxide moiety at C-19, C-20 was indicated, as shown in structure **1**. The chemical shift of the ester methyl attached to C-16 was relatively shielded at δ 2.66, which was consistent with a C-16 configuration that places the

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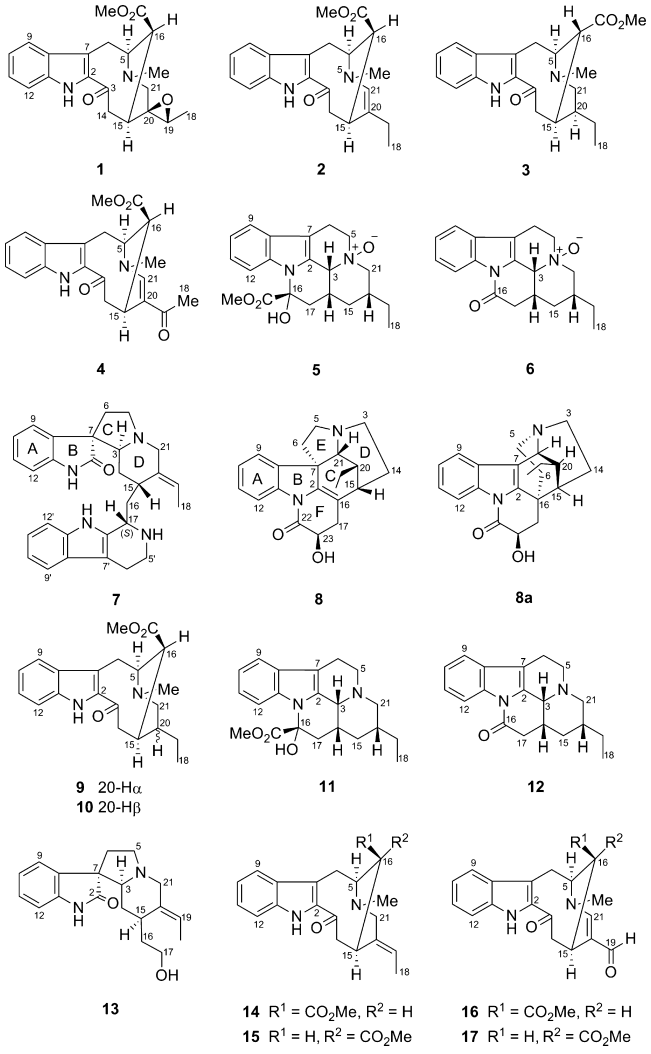

methyl ester function within the shielding zone of the indole moiety. The C-16 hydrogen was observed at δ 3.27, and its relatively downfield shift was probably a result of paramagnetic deshielding due to proximity to the epoxide moiety (H-16 in a 1,3-diaxial relationship with the epoxide oxygen).

There are four possibilities as regards the orientation of the epoxide moiety and the corresponding relative configurations at C-19 and C-20 as shown in Figure 2a–d. The stereostructures depicted in b and d with Me-18 directed toward C-15 were ruled out from the NOEs observed for H-19/H-15, H-14β and Me-18/H-21β (Figure 3). The β-orientation of the epoxide moiety was deduced (Figure 2a) from the downfield shift of H-16 (*vide supra*) and from the H-19/H-15, H-14β NOEs (Figure 3). In the case of an α-oriented epoxide (Figure 2c), the H-16 signal would be expected in the normal region of ca. δ 2.8–3.0, while it is the β-oriented H-14 that should be deshielded instead, due to its proximity to the epoxide oxygen. This was clearly not the case, as H-14β was observed at δ 2.82 (H-14α was assigned from its NOE with H-21α, Figure 3). In addition, in the case of an α-oriented epoxide (c), the expected NOEs would be for H-19/H-16, H-15, which was not the case. On the basis of the preceding discussion, the relative configuration of the epoxide moiety is therefore 19*R*, 20*S*.

Compound **2** (vobasidine B) was obtained as a light yellowish oil with $[\alpha]^{25}_{D}$ −142 ($CHCl_3$, *c* 0.1). As in **1**, the IR spectrum of **2** showed bands due to NH (3312 cm^{-1}), ester, and conjugated ketocarbonyl (1727 and 1638 cm^{-1}) functions, while the UV spectrum showed absorption maxima at 212, 237, and 313 nm, indicative of a 2-acylindole chromophore. The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 353, and HRESIMS and ^{13}C NMR data established the molecular formula as $C_{21}H_{24}N_2O_3$.

The ^1H and ^{13}C NMR data (Tables 1 and 2) showed a number of features that were common with those of **1** such as the presence of four aromatic hydrogens (δ_H 7.16–7.76), an indolic NH (δ_H 9.11), a methyl ester group (δ_H 2.82; δ_C 171.3, 50.6), an N4-Me (δ_H 2.91; δ_C 40.6), and a conjugated carbonyl as part of a 2-acylindole (δ_C 190.2). The ^{13}C NMR data (Table 2) showed a total of 21 carbon resonances, including three methyl, three methylene, eight methine, and three quaternary carbon atoms. The resonance of the ester methyl linked to C-16 at δ 2.82 indicated a similar C-16 configuration as that in compound **1**, with the ester group located within the shielding zone of the aromatic moiety. There were, however, a number of notable differences in the NMR data of **1** and **2**. First, the signals attributed to an epoxide function in **1** were not observed in **2**. Instead of an epoxide moiety, an ethyl side chain was present in compound **2** from the 3H triplet at δ_H 1.06 (δ_C 13.4) and the 2H multiplet at δ_H 2.10 (δ_C 25.6). In addition, C-21, which was a methylene in **1**, is an olefinic methine in **2** (δ_H 5.66, δ_C 128.4), with the adjacent C-20 observed as a quaternary olefinic carbon at δ 114.1, the C-20–C-21 double bond forming part of an enamine moiety in **2**. This was also consistent with the observation that C-20, being the β-carbon of an enamine moiety, was the more shielded (δ_C 114.1) despite being a quaternary center, compared to the methine C-21 (δ_C 128.4). Compound **2** is therefore the 20,21-dehydro derivative of tabernaemontanine (**9**) or dregamine (**10**).

Compound **3** (vobasidine C) was obtained as a light yellowish oil with $[\alpha]^{25}_{D}$ −117 ($CHCl_3$, *c* 0.2). In common with **1** and **2**, the IR spectrum of **3** showed bands due to NH (3313 cm^{-1}), ester, and conjugated ketocarbonyl functionalities (1729 and 1632 cm^{-1}), while the UV spectrum showed absorption maxima at 208, 238, and 319 nm for a 2-acylindole chromophore. The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 355, and ^{13}C NMR and HRESIMS data established the molecular formula as $C_{21}H_{26}N_2O_3$, two mass units more than **2**, suggesting a hydrogenated derivative of **2**, as in tabernaemontanine (**9**) or dregamine (**10**), an inference that was also supported by comparison of the NMR data of **3** with those of **9** and **10**.[14] The configuration at C-20 in **3** could be inferred by comparison of the NMR data with the NMR data of tabernaemontanine (**9**, H-20α, β-oriented Et) and dregamine (**10**, H-20β, α-oriented Et) (Table 1). In the case of dregamine (**10**), H-21α was observed as a triplet with J = 12.5 Hz, requiring H-21α to be *trans* diaxial to H-20, which in turn required H-20 to be β-oriented ($J_{21\alpha-21\beta} = J_{20\beta-21\alpha}$ = 12.5 Hz). In the case of tabernaemontanine (**9**), however, H-21α was observed as a doublet of doublets with J = 13 and 3 Hz, which indicated that H-20 had an α-orientation ($J_{21\alpha-20\alpha} = J_{ax-eq}$ = 3 Hz). Since H-21α in compound **3** was observed as a doublet of doublets with J = 13 and 4 Hz (resembling the case of tabernaemontanine **9**), it can, therefore, be concluded that the configuration of C-20 in **3** is *R* (H-20α, β-oriented Et). This conclusion was also in agreement with the NOEs, viz., H-20α/H-14α,β, H-15, H-21α,β; H-19/H-21β; and H-18/H-15 (Figure 4). Another major difference between the NMR data of **3** versus **1** and **2** (as well as **9**, **10**) was the resonance of the C-16 ester methyl, which in **3** was relatively deshielded at δ

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Table 1. ^{1}H NMR Data (δ) for 1−6 and 8−10 ($CDCl_3$)

H	1[a]	2[b]	3[b]	4[b]	5[a]	6[b]	8[b]	9[b]	10[b]
3					4.70 br s	4.63 br s	2.42 td (13, 5)		
							2.99 ddd (13, 5, 1.2)		
5	4.06 ddd (10, 8, 3)	4.03 t (7.5)	3.78 td (9, 3)	4.26 m	3.95 m	3.86 m	2.84 dd (12, 7.8)	3.96 td (9, 3.5)	4.00 ddd (10, 8, 2.5)
					3.98 m	3.88 m	3.01 dd (12, 7.8)		
6	3.39 dd (15, 10) (α)	2.87 m	3.44 dd (9, 3) (α)	2.96 t	3.10 m	3.04 m	1.70 ddd (14, 7.8, 1.2)	3.30 dd (15, 9)	3.32 dd (15, 10)
	3.46 dd (15, 8) (β)	3.65 dd (14, 7.5)	3.44 dd (9, 3) (β)	3.91 m	3.10 m	3.04 m	2.69 ddd (14, 12, 7.8)	3.44 dd (15, 9)	3.39 dd (15, 8)
9	7.73 dd (8, 0.8)	7.76 br d (8)	7.71 dd (8, 0.6)	7.74 d (7.5)	7.50 d (7.5)	7.44 d (8)	7.20 br d (7.5)	7.70 br d (8)	7.70 dd (8, 0.5)
10	7.17 ddd (8, 5, 2.5)	7.16 ddd (8, 6, 2)	7.16 ddd (8, 6, 2)	7.18 t (7.5)	7.19 m	7.32 t (8)	7.12 td (7.5, 1.4)	7.15 ddd (8, 5, 2)	7.16 ddd (8, 5, 3)
11	7.34 m	7.36 m	7.36 m	7.36 t (7.5)	7.21 m	7.38 t (8)	7.24 td (7.5, 1.4)	7.33 m	7.33 m
12	7.34 m	7.36 m	7.37 m	7.39 d (7.5)	7.16 dd (7.5, 0.8)	8.38 d (8)	8.07 br d (7.5)	7.33 m	7.33 m
14	2.82 dd (13, 7) (β)	2.67 m	2.73 dd (12, 7) (β)	2.60 m	3.18 m	3.24 m	1.75 m	2.76 dd (12, 6.5)	2.68 dd (12, 7)
	3.34 t (13) (α)	3.31 m	3.48 t (12) (α)	3.57 m			1.85 tdd (13, 8, 4)	3.40 t (12)	3.11 t (12)
15	2.28 ddd (13, 7, 3)	3.20 m	2.87 m	3.93 m	1.23 q (13)	0.61 q (13)	2.32 m	2.70 m	2.90 m
					1.80 br d (13.5)	1.77 br d (13.5)			
16	3.27 t (3)	2.66 m	2.67 br s	2.58 m				3.03 t (3)	2.87 br s
17					2.32 dd (15, 3)	2.73 dd (17, 5)	2.18 dd (17, 13)		
					2.58 dd (15, 4)	2.95 dd (17, 5)	2.73 dd (17, 7.8)		
18	1.38 d (5.5)	1.06 t (7)	0.94 t (7)	2.24 s	0.88 t (7.5)	0.85 t (7.5)	0.70 t (7)	0.97 t (7)	1.02 t (7.5)
19	3.08 q (5.5)	2.10 m	1.41 m		1.11 m	1.07 m	0.80 m	1.54 m	1.35 m
		2.10 m	1.52 m		1.16 m	1.14 m	0.80 m	1.73 m	1.35 m
20			1.39 m		2.45 m	2.51 m	1.98 tdd (7, 2.7, 1.4)	1.54 m	1.91 m
21	2.42 d (13.5) (β)	5.66 br s	2.45 d (13) (β)	7.32 br s	2.88 t (12)	2.80 t (11.5)	3.73 dd (2.7, 1.4)	2.50 d (13)	2.61 m
	3.57 d (13.5) (α)		3.17 dd (13, 4) (α)		3.16 m	3.04 m		3.19 dd (13, 3)	2.80 t (12.5)
23							4.49 dd (13, 7.8)		
CO_2Me	2.66 s	2.82 s	3.58 s	2.85 s	3.83 s			2.61 s	2.64 s
NMe	2.68 s	2.91 s	2.57 s	3.35 s				2.57 s	2.64 s
NH	8.94 br s	9.11 br s	9.09 br s	9.21 br s				9.09 br s	8.91 br s

[a]600 MHz. [b]400 MHz.

3.58 compared to those in **1** (δ 2.66), **2** (δ 2.82), **9** (δ 2.61), and **10** (δ 2.64), as a result of the change in configuration of C-16 in **3** (16*R*), which results in the ester function directed away from the anisotropic influence of the indole moiety.

Compound **4** (vobasidine D) was obtained as a light yellowish oil with $[\alpha]^{25}_D$ −70 ($CHCl_3$, *c* 0.3). The IR spectrum and UV data were similar to those of the previous three compounds, indicating the presence of similar functionalities and chromophores. The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 367, and ^{13}C NMR and HRESIMS data established the molecular formula as $C_{21}H_{22}N_2O_4$. The ^{13}C NMR data (Table 2) showed a total of 21 carbon resonances, including three methyl, two methylene, eight methine, and three quaternary carbon atoms. The ^{1}H and ^{13}C NMR data indicated a vobasine-type alkaloid and showed some similarity to those of **2**. Features in common with **2** include the presence of an unsubstituted indole moiety, an indolic NH, a methyl ester group, an N4-Me, and a conjugated carbonyl, which was part of a 2-acylindole. The C-16 ester methyl resonance was at δ 2.85, which indicated a similar C-16 configuration as in compounds **1** and **2**. In common with **2**, a trisubstituted double bond was present, corresponding to C-20═C-21. However, unlike **2**, an acetyl side chain was attached to C-20 in **4**, instead of an ethyl group in **2**. This was evident from the replacement of signals of an ethyl group with those of an acetyl group (δ_H 2.24; δ_C 24.2, 192.1). The presence of an acetyl side chain at C-20 was also consistent with the deshielding of the β-olefinic carbon C-21 (δ_C 145.9) versus that of C-20 (114.1) as well as the C-19 carbonyl resonance at δ_C 192.1, indicating conjugation with the C-20−C-21 double bond.

Compound **5** was obtained as a light yellowish oil, with $[\alpha]^{25}_D$ −6 ($CHCl_3$, *c* 0.2). The UV spectrum showed absorption maxima at 222, 273, 279, and 290 nm characteristic of an indole chromophore, while the IR spectrum indicated the presence of an OH and ester functions (3199 and 1746 cm^{-1}). The ESIMS of **5** showed an $[M + H]^+$ peak at *m*/*z* 371, which analyzed for $C_{21}H_{26}N_2O_4$ (16 mass units higher than that of tacamine). The ^{1}H NMR data showed the presence of four

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Table 2. ^{13}C NMR Data (δ) for 1−6 and 8 ($CDCl_3$)[a]

C	1[b]	2[c]	3[c]	4[c]	5[b]	6[c]	8[c]
2	133.9	135.0	134.9	135.5	127.2	128.4	142.0
3	188.9	190.2	191.3	189.8	69.0	68.0	45.8
5	56.7	55.0	53.6	55.3	67.8	68.0	53.8
6	20.1	25.3	19.2	28.0	19.9	20.2	43.2
7	120.5	120.5	121.4	117.4	104.9	112.0	50.2
8	128.5	128.3	128.4	127.8	127.1	127.9	137.9
9	120.9	121.0	120.9	120.5	118.9	118.6	120.5
10	120.6	120.4	120.5	120.6	121.1	124.6	125.3
11	127.0	126.9	127.0	126.8	123.3	125.9	127.5
12	111.8	111.9	112.1	112.1	111.2	116.7	115.5
13	136.4	136.5	136.4	136.4	135.6	135.4	138.7
14	42.5	47.3	45.6	46.6	28.1	30.6	27.2
15	37.6	29.6	30.8	26.0	29.2	30.3	35.3
16	45.1	45.5	41.6	44.6	81.3	166.2	113.2
17					39.2	38.5	34.6
18	13.3	13.4	12.8	24.2	11.1	11.0	11.7
19	58.7	25.6	26.2	192.1	25.8	25.9	23.7
20	62.8	114.1	42.2	114.1	32.0	31.6	42.5
21	46.8	128.4	46.8	145.9	62.7	63.1	65.8
22							169.0
23							67.7
CO_2Me	50.4	50.6	51.6	50.7	54.5		
CO_2Me	170.9	171.3	174.4	169.9	173.6		
NMe	43.1	40.6	43.2	42.0			

[a]Assignments based on HSQC and HMBC. [b]150 MHz. [c]100 MHz.

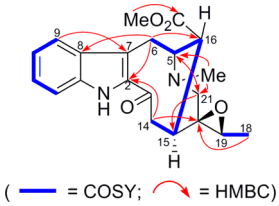

Figure 1. COSY and selected HMBCs of 1.

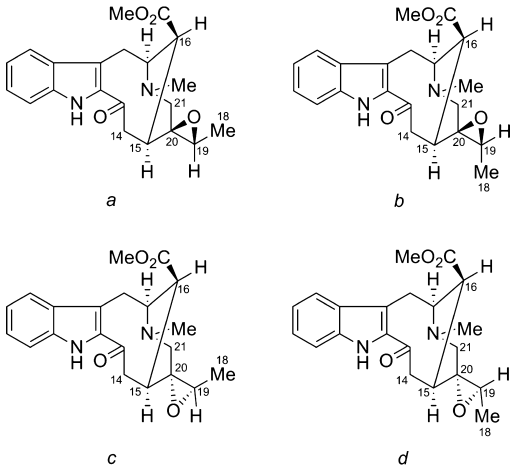

Figure 2. Four possible 19,20 configurations of 1.

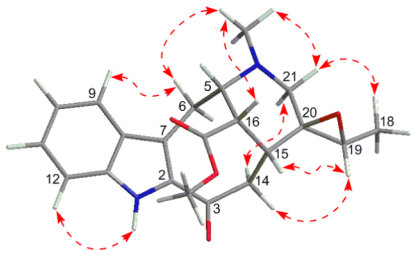

Figure 3. Selected NOEs of 1.

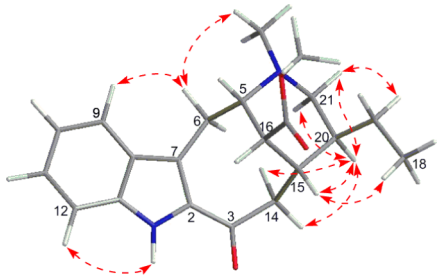

Figure 4. Selected NOEs of 3.

aromatic hydrogens (δ 7.16−7.50), a methyl ester (δ 3.83), and an ethyl side chain (δ 0.88; 1.11, 1.16). The ethyl side chain (corresponding to C-18−C-19) was attached to C-20, identified as a methine from the COSY spectrum. Another distinct feature was the presence of a signal at δ 4.70, characteristic of H-3 in tacaman-type alkaloids, but noticeably deshielded in the present instance. The ^{13}C NMR spectrum accounted for all 21 carbons (including two methyl, six methylene, seven methine, and two quaternary carbon atoms). Another notable feature was the presence of a low-field resonance at δ 81.3 due to a carbon linked to OH and CO_2Me groups and characteristic of C-16 in tacaman- and vincamine-type alkaloids. Comparison of the NMR data of **5** with those of tacamine (**11**)[15] revealed a close correspondence, except for the characteristic downfield shifts of C-3, C-5, and C-21 and of H-3, H-5, and H-21, in the ^{13}C and ^{1}H NMR spectra, respectively,

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of **5** compared with those of **11**. Compound **5** is therefore the N4-oxide of tacamine (**11**).

Compound **6** was obtained as a light yellowish oil, with $[\alpha]^{25}_{D}$ +25 ($CHCl_3$, *c* 0.3). The UV spectrum showed absorption maxima at 205, 239, 263, 292, and 300 nm, characteristic of an *N*-acyl indole chromophore, while the IR spectrum showed the presence of a lactam carbonyl at 1711 cm^{-1}. The presence of the lactam carbonyl function was also supported by the resonance at δ 166.2 in the ^{13}C NMR spectrum. The ESIMS of **6** showed an $[M + H]^+$ peak at *m*/*z* 311, which analyzed for $C_{19}H_{22}N_2O_2$ (16 mass units higher than that of tacamonine). The ^1H NMR spectrum showed a general similarity with those of **5** and **11**, with some notable differences. The ethyl side chain, attached to the methine C-20 as well as the characteristically deshielded H-3 (δ 4.63), were still observed in the ^1H NMR spectrum of **6**. However, the signals of the methyl ester group present in **5** were absent in the NMR spectra of **6**. This was due to the change in C-16, which in **6** is a lactam carbonyl. The ^1H and ^{13}C NMR data of **6** were in fact similar to those of tacamonine (**12**),[16] except for the deshielding of H-3, H-5, and H-21 and C-3, C-5, and C-21 observed in the ^1H and ^{13}C NMR spectra, respectively, of **6** compared to those of **12**. Compound **6** is therefore the N4-oxide of tacamonine (**12**).

Compound **7** (taipinisine) was obtained as a light yellowish oil with $[\alpha]_D$ −29 ($CHCl_3$, *c* 0.3). The UV spectrum showed absorption maxima at 209, 224, 252, 262, 283, and 291 nm, indicating the presence of oxindole and indole chromophores, while the IR spectrum showed bands due to NH (3293 cm^{-1}) and γ-lactam carbonyl (1702 cm^{-1}) functions. The ESIMS showed an $[M + H]^+$ peak at *m*/*z* 453, which in conjunction with ^{13}C NMR data analyzed for $C_{29}H_{32}N_4O$. The ^{13}C NMR data of **7** (Table 3) gave a total of 29 carbon resonances (including one methyl, seven methylene, 12 methine, and five quaternary carbons). The MS and ^{13}C NMR data suggested a heterodimeric alkaloid. Examination of the ^1H NMR data (Table 3) showed the presence of eight aromatic resonances (δ_H 6.85−7.43) corresponding to two unsubstituted indole moieties, two indolic NH, and an ethylidene side chain. The resonances at δ 182.4 and 56.7 in the ^{13}C NMR data were readily attributed to the C-2 lactam carbonyl and the spirocyclic C-7, respectively, of the oxindole moiety.

The COSY spectrum showed the presence of two NCH_2CH_2, $NCHCH_2CHCH_2CH$, an aminomethylene (NCH_2), and an ethylidene side chain (Figure 5). These partial structures suggested that the oxindole half constituting the heterodimer was a tetracyclic corynanthean oxindole with an ethylidene side chain, while the other tryptamine-derived partner was a tetrahydro-β-carboline. Indeed, the carbon resonances of the oxindole and indole halves could be assigned on the basis of comparison with those of other tetracyclic oxindoles [e.g., (7*S*)-geissoschizol oxindole][17] and with heterodimeric alkaloids incorporating a tetrahydro-β-carboline unit (e.g., buchtienine).[18] These assignments were supported by the HMBC spectrum (Figure 5). The three-bond correlations from H-6 to the oxindole C-8 and C-2 indicated the connection of one of the NCH_2CH_2 fragments to C-7, while the three-bond correlations from H-3 to C-2, C-8, and C-21 indicated the branching of the $CHCH_2CHCH_2CH$ fragment from N-4 (Figure 5). The three-bond correlations from H-21 to C-19 and C-15 were consistent with the usual attachment of the ethylidene side chain at C-20, while the correlation from H-3 to C-15 completed the assembly of ring D. The connection of

Table 3. ^1H and ^{13}C NMR Data for 7 (600 MHz, $CDCl_3$)[a]

	δ_C	δ_H		δ_C	δ_H
2	182.4		2′	136.3	
3	67.4	2.92 m	5β′	41.4	2.91 m
5α	54.2	2.54 q (9)	5α′		3.13 m
5β		3.34 td (9, 2)	6′	22.7	2.63 m
6β	35.4	2.12 m			2.63 m
6α		2.46 m	7′	108.4	
7	56.7		8′	127.4	
8	133.5		9′	117.8	7.43 d (7.5)
9	125.4	7.41 d (7)	10′	119.1	7.05 t (7.5)
10	122.7	7.02 t (7)	11′	121.3	7.09 t (7.5)
11	127.7	7.16 td (7, 5)	12′	111.0	7.28 dd (7.5, 4)
12	109.5	6.85 d (7)	13′	135.8	
13	140.0		N(1)-H′		8.43 br s
14β	27.7	1.19 td (13, 5)			
14α		1.34 d (13)			
15	29.3	3.08 m			
16a	35.9	1.37 m			
16b		2.12 m			
17	49.7	3.88 br d (10)			
18	12.5	1.50 d (7)			
19	119.6	5.28 t (7)			
20	137.6				
21α	58.7	2.94 m			
21β		3.37 d (11)			
N(1)-H		8.72 br s			

[a]Assignments based on COSY, HSQC, HMBC, and NOESY.

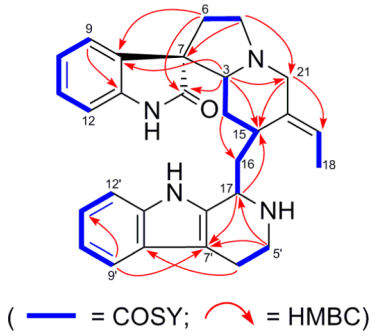

Figure 5. COSY and selected HMBCs of **7**.

the tetracyclic oxindole half to the tetrahydro-β-carboline half was via the methylene bridge corresponding to C-16 and the correlations from the H-14 to C-16 and from H-17 to C-15. The remaining correlations were consistent with a tetrahydro-β-carboline unit linked to the oxindole half at C-17 (Figure 5). The structure was also consistent with the NMR data of the related alkaloid strychnofoline from the African *Strychnos usambarensis*.[19] The structure of strychnofoline was confirmed by X-ray analysis as well as by a recent synthesis.[20]

The observation of Wenkert−Bohlmann bands in the IR spectrum of **7** indicated a *trans*-C/D ring junction. The resonance of H-3 at δ 2.92 (upfield of ca. δ 4.0) was consistent with H-3 adopting an α-orientation (3*S*).[5] The relative configuration at the spirocyclic center can be assigned as 7*S* by analogy with those of the (7*S*)- and (7*R*)-geissoschizol oxindoles.[17,21] This assignment was also supported by the observation of the H-9 resonance at δ 7.41, due to paramagnetic deshielding as a result of proximity of H-9 with the β-oriented *N*-4 lone pair.[22] The geometry of the C-19−C-

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20 double bond was assigned as *E* from the NOEs for H-18/H-15 and H-19/H21β (Figure 6). The relative configuration at C-

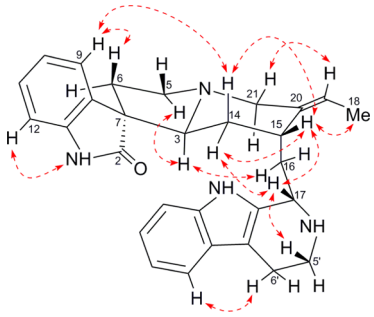

Figure 6. Selected NOEs of **7**.

15 was assigned as 15*R* (H-15β) from several observations. First, reciprocal NOEs were observed between H-15 and both H-14 and H-18 (Figure 6), which were consistent with a β-oriented H-15 (an α-oriented H-15 would be expected to experience a strong NOE with only H-14α). Second, examination of the vicinal coupling data for H-14 provided incontrovertible evidence for H-15 having a β-orientation. The H-14 resonances at δ 1.19 and 1.34 can be distinguished by the NOE between the former resonance and H-9, which in turn led to the respective assignment of H-14β (δ 1.19) and H-14α (δ 1.34). The H-14β resonance was observed as a triplet of doublets with J = 13 and 5 Hz, which required H-14β to be *trans*-diaxial with H-3α. On the other hand, H-14α was observed as a doublet with J = 13 Hz. This indicated an absence of vicinal coupling with both H-3α and H-15β, a situation that was possible only when both the H-14α/H-3α and H-14α/H15β dihedral angles approach 90°. Examination of models showed that this was indeed the case. Furthermore, comparison with (7*S*)-geissoschizoloxindole (**13**)[17] would prove instructive, as in this instance H-3 and H-15 are both α-oriented. In **13**, H-14β was a triplet of doublets with J = 12 and 5.5 Hz, while H-14α was a doublet of triplets with J = 12 and 3 Hz. This coupling behavior was entirely consistent with H-15 having an α-orientation ($J_{3\alpha-14\alpha} = J_{14\alpha-15\alpha}$ = 3 Hz).

The remaining configuration to be assigned was that at C-17. Density functional theory (DFT) calculations (performed at the B3LYP/6-31G(d) level of theory using Gaussian 09 software)[23] indicated that, of the two possible stereoisomers, the one with a 17*S* (H-17β) configuration represents the lower energy epimer when compared to the alternative (17*R*) (H-17α) epimer, by about 5.8 kJ mol^{-1}. The DFT results also indicated that for the 17*S* epimer the heterodimeric alkaloid adopts a preferred (energy minimized) conformation in which the tetrahydro-β-carboline half is in a plane approximately orthogonal to the approximate plane defined by the oxindole moiety, in addition to indicating the presence of H-bonding between the β-carboline indolic NH and the oxindole carbonyl oxygen (Figure 7). This preferred conformation is also consistent with the NOE observed between H-17 and H-14α, H-15 (Figure 6, the H-17/14α NOE was much stronger than the H-17/H-15 interaction, as shown by both 1D and 2D NOESY). In the case of the 17*R* epimer, the DFT results showed that the preferred conformation is one where the β-carboline moiety is directed away from the oxindole unit (Figure 8), in which case an NOE between H-17 and H-14α would not have been possible. On the basis of the above considerations, the relative configuration of C-17 can be

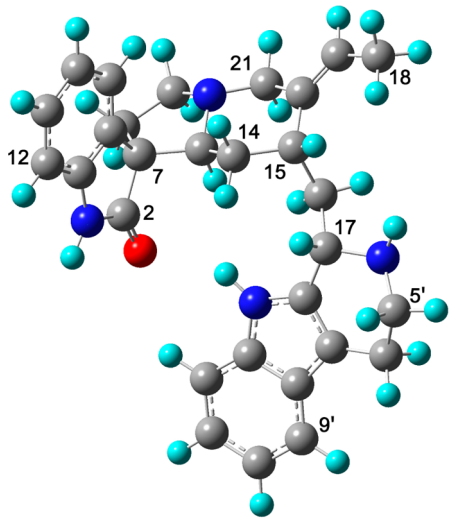

Figure 7. DFT energy minimized conformation of **7** (17*S*).

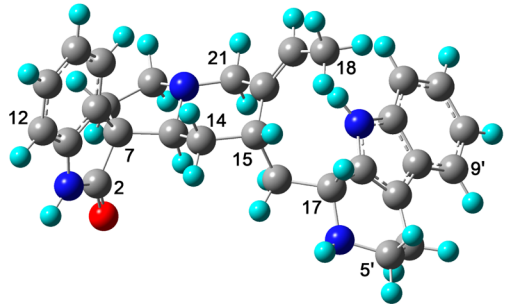

Figure 8. DFT energy minimized conformation of **7** (17*R*).

provisionally assigned as 17*S*. Compound **7** represents the first isolation of a heterodimeric alkaloid of the corynanthe-tryptamine type, constituted from the union of a corynanthean oxindole with a tetrahydro-β-carboline, in the genus *Tabernaemontana*.

We previously isolated tronoharine as a minor alkaloid from another sample of *T. corymbosa* collected from a different location.[12] The proposed structure **8a** was based on analysis of the MS and NMR data. We have now obtained tronoharine from the present sample of *T. corymbosa*, this time in sufficient amount, which has enabled us to reevaluate and confirm the previously proposed structure. The spectroscopic data of the present sample were virtually in complete agreement with the previous data except for the HMBC data (Figure 9), where two key three-bond correlations (H-9 to C-7, and H-6 to C-8) were observed in the present instance, which were not detected in the previously obtained HMBC spectrum due to the limitations imposed by the small quantity of the sample. In view of the present observation of these two key correlations, an

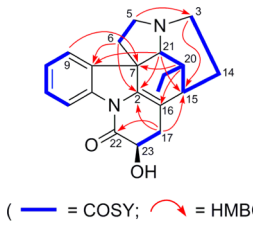

Figure 9. COSY and selected HMBCs of **8**.

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interchange in the assignment of C-7 and C-16 was indicated, which had important ramifications as regards the final structure deduced for this alkaloid. As can be seen, the interchange in the assignment of C-7 and C-16, necessitated by the previous misassignment of C-7 and C-16, and now reassigned as C-16 and C-7, respectively, required a corresponding change in the connection of C-6 to C-7 and the shift of the double bond from C-2−C-7 to C-2−C-16. The revised structure is as shown in **8**, which represents a new hexacyclic alkaloid of the aspidospermatan group (stemmadenine subtype), which has incorporated an additional six-membered, hydroxy-lactam ring (ring F), linked from the indolic nitrogen to C-16. To secure unambiguous confirmation of the structure, X-ray diffraction analysis was carried out, which provided final verification of the revised structure (Figure 10).

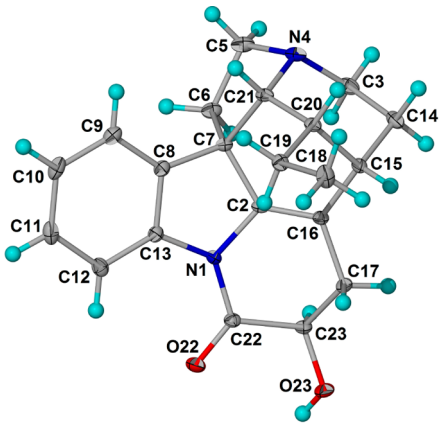

Figure 10. X-ray crystal structure of **8**.

Alkaloids **1**, **3**, **15**, and **17** showed appreciable cytotoxicity toward KB cells (IC_{50} ca. 5 μg/mL), while **3** and **7** showed moderate effects in reversing MDR in vincristine-resistant KB (KB/VJ300) cells (IC_{50} ca. 9 μg/mL in the presence of 0.1 μg/mL vincristine) (Table 4). Among the cytotoxic alkaloids, a distinct structure−activity correlation was observed. It can be seen that for a pair of vobasine alkaloids that are epimeric at C-16 it was invariably the C-16 epimer, which has the methyl ester group directed away from the indole moiety, that was found to be cytotoxic toward KB cells (IC_{50} ca. 5 μg/mL), whereas the corresponding epimer with the methyl ester group directed toward the indole moiety was invariably found to be ineffective (IC_{50} > 25 μg/mL). This was evident when comparing 16-epitabernaemontanine (**3**) versus tabernaemontanine (**9**); 16-epivobasine (**15**) versus vobasine (**14**); and 16-epivobasenal (**17**) versus vobasenal (**16**).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a PerkinElmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on JEOL and Bruker 400 MHz and 600 MHz spectrometers. HRESIMS data were obtained on an Agilent 6530 Q-TOF mass spectrometer.

Plant Material. Plant material (*T. corymbosa* Roxb. ex Wall) was collected near Taiping, Perak, Malaysia, and identification was confirmed by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia. Herbarium voucher specimens (K693) are deposited at the herbarium at the University of Malaya.

Extraction and Isolation. Extraction of alkaloids from the ground stem-bark material was carried out in the usual standard manner by partitioning the concentrated EtOH extract with 3% tartaric acid. The alkaloids were isolated by initial column chromatography (SiO_2, MeOH−CH_2Cl_2, with increasing percentage of MeOH). This was followed by rechromatography of the appropriate partially resolved fractions using CC or centrifugal preparative TLC. Solvent systems used for centrifugal preparative TLC were Et_2O−hexanes (1:3), Et_2O−hexanes (1:1), Et_2O, 1−5% MeOH−Et_2O, EtOAc−hexanes (1:1), EtOAc−hexanes (3:1), EtOAc−MeOH (20:1), 20−80% CH_2Cl_2−hexanes, and 2−5% MeOH−CH_2Cl_2. The yields (mg kg^{-1}) of the alkaloids were as follows: **1** (0.52), **2** (0.15), **3** (1.3), **4** (0.22), **5** (0.22), **6** (0.14), **7** (0.25), **8** (0.18), **9** (144), **10** (10.7), **11** (0.26), **12** (0.64), **14** (129), **15** (2.06), **16** (6.5), **17** (0.91).

*Vobasidine A (**1**):* light yellowish oil; $[\alpha]^{25}_D$ −35 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 (4.18), 226 (4.06), 238 (4.02), 314 (4.05) nm; IR (dry film) ν_{max} 3311, 1727, 1647 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 369.1808 $[M + H]^+$ (calcd for $C_{21}H_{24}N_2O_4$ + H, 369.1809).

*Vobasidine B (**2**):* light yellowish oil; $[\alpha]^{25}_D$ −142 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 212 (4.18), 237 (4.07), 313 (3.94) nm; IR (dry film) ν_{max} 3312, 1727, 1638 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 353.1857 $[M + H]^+$ (calcd for $C_{21}H_{24}N_2O_3$ + H, 353.1860).

Table 4. Cytotoxic Effects of Compounds

	IC_{50}, μg/mL (μM)		
compound	KB/S[a]	KB/VJ300[a]	KB/VJ300(+)[b]
vobasidine A (**1**)	5.7 (15.48)	>25	>25
vobasidine B (**2**)	18.9 (53.67)	>25	>25
vobasidine C (**3**)	5.5 (15.53)	>25	9.4 (26.54)
vobasidine D (**4**)	>25	>25	>25
taipinisine (**7**)	20.0 (44.22)	>25	8.4 (18.57)
tabernaemontanine (**9**)	>25	>25	>25
dreagamine (**10**)	>25	>25	>25
vobasine (**14**)	>25	>25	>25
16-epivobasine (**15**)	5.6 (15.90)	22 (62.47)	13.5 (38.33)
vobasenal (**16**)	>25	>25	>25
16-epivobasenal (**17**)	5.1 (14.48)	>25	>25
vincristine	0.007 (0.008)	5.4 (6.55)	

[a]KB/S and KB/VJ300 are vincristine-sensitive and vincristine-resistant human oral epidermoid carcinoma cell lines, respectively. [b]With added vincristine, 0.1 μg/mL (0.121 μM), which did not affect the growth of the KB/VJ300 cells.

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*Vobasidine C (**3**):* light yellowish oil; $[\alpha]^{25}_{D}$ −117 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 208 (4.36), 238 (4.15), 319 (4.21) nm; IR (dry film) ν_{max} 3313, 1729, 1632 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 355.2013 $[M + H]^+$ (calcd for $C_{21}H_{26}N_2O_3$ + H, 355.2016).

*Vobasidine D (**4**):* light yellowish oil; $[\alpha]^{25}_{D}$ −70 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 (4.04), 236 (3.84), 297 (4.13) nm; IR (dry film) ν_{max} 3304, 1727, 1639 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 367.1654 $[M + H]^+$ (calcd for $C_{21}H_{22}N_2O_4$ + H, 367.1652).

*Tacamine-N-oxide (**5**):* light yellowish oil; $[\alpha]^{25}_{D}$ −6 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 222 (4.56), 273 (3.95), 279 (3.93), 290 (3.81) nm; IR (dry film) ν_{max} 3199, 1746 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 371.1967 $[M + H]^+$ (calcd for $C_{21}H_{26}N_2O_4$ + H, 371.1965).

*Tacamonine-N-oxide (**6**):* light yellowish oil; $[\alpha]^{25}_{D}$ +25 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 205 (4.07), 239 (4.03), 263 (3.73), 292 (3.54), 300 (3.55) nm; IR (dry film) ν_{max} 1711 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 311.1762 $[M + H]^+$ (calcd for $C_{19}H_{22}N_2O_2$ + H, 311.1754).

*Taipinisine (**7**):* light yellowish oil; $[\alpha]^{25}_{D}$ −29 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (4.25), 224 (4.16), 252 (3.66), 262 (3.63), 283 (3.58), 291 (3.53) nm; IR (dry film) ν_{max} 3293, 2806, 2745, 1702 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 453.2650 $[M + H]^+$ (calcd for $C_{29}H_{32}N_4O$ + H, 453.2649).

*Tronoharine (**8**):* white, amorphous, and subsequently colorless prisms from CH_2Cl_2−EtOH; mp 222−226 °C; $[\alpha]^{25}_{D}$ +141 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 (4.35), 251 (3.85), 273 (3.83), 295 (3.51) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 337.1914 $[M + H]^+$ (calcd for $C_{21}H_{24}N_2O_2$ + H, 337.1911).

Computational Method. Structures corresponding to the two stereoisomers of compound **7**, i.e., (17*S*)-**7** and (17*R*)-**7**, were optimized with DFT using a restricted B3LYP functional with the 6-31G(d) basis set.[23] Structures were initially built using GaussView 5 and then optimized at the semiempirical level of theory (PM6). These structures were then imported into Gaussian 09 for DFT-level geometry optimization to obtain the energy-minimized conformations.

X-ray Crystallographic Analysis of 8. X-ray diffraction analysis was carried out on an Agilent Technologies SuperNova Dual CCD area detector system equipped with mirror monochromator and using Mo Kα radiation (λ = 0.710 73 Å), at 100 K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-2014). All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with relative isotropic parameters. Crystallographic data for compound **8** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

*Crystallographic data of **8**:* colorless prisms, $C_{21}H_{24}N_2O_2$. M_r = 336.42, orthorhombic, space group $P2_12_12_1$, a = 9.1808(3) Å, b = 11.2481(3) Å, c = 16.7513(5) Å, Z = 4, D_{calc} = 1.292 g cm^{-3}, crystal size 0.2 × 0.2 × 0.1 mm^3, $F(000)$ = 720, T = 100 K. The final R_1 value is 0.0327 (wR_2 = 0.0868) for 4565 reflections [$I > 2\sigma(I)$]. CCDC number: 1015007.

Cytotoxicity Assays. Cytotoxicity assays were carried out following the published procedures.[24]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR for compounds **1**−**8**. Calculated free energies and Cartesian coordinates for compound **7** [(17*S*)-**7** and (17*R*)-**7**]. X-ray crystallographic data in CIF format for compound **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 603-79674266. Fax: 603-79674193. E-mail: tskam@um.edu.my.

Notes

The authors declare no competing financial interest.

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