Strychnan and Secoangustilobine A Type Alkaloids from *Alstonia spatulata.* Revision of the C-20 Configuration of Scholaricine

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Received August 6, 2010

A total of 25 alkaloids were isolated from the leaf and stem-bark extracts of *Alstonia spatulata*, of which five are new alkaloids of the strychnan type (alstolucines A−E, **1**−**5**) and the other, a new alkaloid of the secoangustilobine A type (alstolobine A, **6**). The structures of these alkaloids were established using NMR and MS analysis and, in the case of alstolucine B (**2**), also confirmed by X-ray diffraction analysis. A reinvestigation of the stereochemical assignment of scholaricine (**13**) by NMR and X-ray analyses indicated that the configuration at C-20 required revision. Alkaloids **1**, **2**, **6**, **7**, **9**, **10**, and **13** reversed multidrug resistance in vincristine-resistant KB cells.

Plants of the genus *Alstonia* (Apocynaceae), which are usually shrubs or trees, are distributed over the tropical parts of Central America, Africa, and Asia, with the center of diversity in the Malesian region.[1−3] About six species occur in Peninsular Malaysia and are mainly found in both the lowland and highland forests, as well as in swampy areas.[1,3] Several species have been reported to be used in the treatment of malaria and dysentery.[4,5] The genus represents a rich source of biologically active indole and bisindole alkaloids.[6−39] In continuation of our studies of the alkaloids of this genus,[9−15,20] in the wider context of our studies of the alkaloids of the Malaysian Apocynaceae,[6,7,40] we report the alkaloidal composition as well as the isolation of new biologically active alkaloids from *A. spatulata* Bl. There has been no previous phytochemical investigation of this species.

Results and Discussion

The EtOH extract of the leaves of *A. spatulata* provided a total of 19 alkaloids, including the new strychnan alkaloids alstolucines A−E (**1**−**5**) and the new secoangustilobine A type alkaloid, alstolobine A (**6**), in addition to 13 known alkaloids. The stem-bark extract provided a total of 11 known alkaloids. Of these, five are common to both the stem-bark and leaf extracts.

Alstolucine A (**1**) was obtained as a light yellowish oil, with $[\alpha]^{25}_{D}$ −438 ($CHCl_3$, *c* 0.12). The UV spectrum showed absorption maxima at 230, 298, and 328 nm, characteristic of a β-anilinoacrylate chromophore.[41] The IR spectrum (thin film) showed a broadened band at 3378 cm^{-1} due to the indolic NH function, a band at 1742 cm^{-1} due to a carbonate group (−OCO_2−), and a band at 1683 cm^{-1} due to an α,β-unsaturated ester function. The ESIMS of **1** showed a pseudomolecular ion peak at *m*/*z* 413, and HRESIMS measurements yielded the molecular formula $C_{23}H_{28}N_2O_5$ (DBE 11).

The ^{13}C NMR spectrum (Table 1) showed 23 carbon resonances, comprising three methyl, five methylene, eight methine, and seven quaternary carbons. The presence of conjugated ester and carbonate functionalities was supported by the observed quaternary carbon signals at δ 167.9 and 155.0, respectively, while the signals due to the two olefinic quaternary carbons at 167.9 (C-2) and 103.6 (C-16) are consistent with the presence of the β-anilinoacrylate moiety.

Table 1. ^{13}C NMR Data (δ) of **1**−**7**, **12**, and **13** (100 MHz, $CDCl_3$)[a]

C	1	2	3	4	5	6	7	12	13
2	167.9	172.2	166.8	171.8	168.5	132.9	168.7	132.9	172.1
3	58.9	60.6	74.2	60.4	58.5	41.7	58.6	44.3	60.7
5	53.5	54.0	68.2	53.6	52.7		53.1		53.7
6	45.6	43.4	38.6	43.0	44.8		45.2		43.1
7	58.1	56.7	52.7	57.1	58.9	101.6	58.3	101.7	57.4
8	135.3	135.4	132.9	136.4	136.1	127.7	135.0	127.8	136.7
9	120.8	119.6	119.6	111.3	112.4	120.5	120.8	120.6	110.9
10	120.9	121.1	121.8	122.2	122.2	120.2	121.2	120.1	122.3
11	127.8	127.6	128.8	115.8	116.0	122.5	128.0	122.5	115.6
12	109.6	109.7	110.4	141.7	141.8	110.8	109.8	110.9	142.0
13	144.1	144.2	143.6	132.2	132.2	136.2	144.2	136.5	131.9
14	27.4	31.7	27.3	31.5	26.4	23.2	26.6	25.0	30.8
15	27.0	30.8	29.6	30.7	27.4	47.7[b]	27.4	46.6	28.7
16	103.6	96.5	96.9	96.5	102.3	58.4	102.6	59.3	96.3
17						70.1		69.8	
18	17.2	29.2	29.3	29.3	29.4	115.1	29.4	115.1	19.6
19	76.5	208.5	206.9	208.5	209.8	140.0	210.0	141.2	68.4
20	41.2	50.0	45.5	49.6	49.3	83.5	49.5	82.8	45.7
21	47.6	45.6	60.4	45.4	46.5	47.6[b]	47.0	52.5	47.9
22	155.0					155.9			
23	63.8					61.4			
24	14.3					14.6			
CO_2Me	167.9	50.9	51.1	51.0	51.3	53.1	51.2	53.1	51.9
CO_2Me	51.0	167.2	167.8	167.5	167.8	173.5	168.0	174.1	169.1

[a] Assignments are based on HMQC and HMBC. [b] Assignments are interchangeable.

Two downfield signals at δ 76.5 and 63.8 are associated with the presence of oxymethine and oxymethylene moieties, respectively.

The ^{1}H NMR spectrum (Table 2) showed the presence of a 1,2-disubstituted aromatic moiety from the presence of signals due to the four contiguous aromatic hydrogens (δ 7.20, br d, J = 7.5 Hz, H-9; 6.90, br t, J = 7.5 Hz, H-10; 7.14, td, J = 7.5, 1.0 Hz, H-11; 6.82, br d, J = 7.5 Hz, H-12), an indolic NH as a broad singlet at δ 8.92, an oxymethine at δ 4.76, an oxymethylene at δ 4.21, and three methyl groups. The highest field methyl at δ 1.33 (t, J = 7.0 Hz) is associated with the oxymethylene at δ 4.21, constituting part of an ethoxy moiety, while the methyl at δ 1.34 (d, J = 6.0 Hz) is adjacent to the oxymethine at δ 4.76 (m), as shown by the COSY spectrum. The remaining methyl at δ 3.77 (s) is associated with the conjugated methyl ester function.

The COSY and HMQC data disclosed the following partial structures, viz., NCH_2CH_2, $NCHCH_2CHCHCH_2$, $CHCH_3$, and OCH_2CH_3, corresponding to the C-5−C-6, C-3−C-14−C-15−C-

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10.1021/np100552b © 2010 American Chemical Society and American Society of Pharmacognosy
Published on Web 11/02/2010

Table 2. ^{1}H NMR Data (δ) for **1**−**7** (400 MHz, $CDCl_3$)[a]

H	1	2	3	4	5	6	7
3α	4.04 m	3.87 br t (3.0)	4.29 m	3.96 m	4.08 m	2.86 m	4.05 m
3β						3.76 m	
5β	3.00 m	2.87 m	3.62 m	2.95 m	2.98 dt (11.5, 6.0)		2.96 ddd (11.0, 6.4, 5.5)
5α	3.20 ddd (11.4, 8.7, 6.6)	3.05 m	3.77 m	3.16 m	3.21 dt (11.5, 7.0)		3.16 dt (11.0, 7.0)
6α	2.00 m	1.83 m	2.12 dd (14.0, 8.0)	1.93 dd (12.8, 6.2)	2.04 dt (13.0, 6.0)		2.01 ddd (12.5, 6.5, 5.5)
6β	2.29 ddd (12.0, 8.0, 6.5)	3.04 m	2.69 m	3.13 m	2.35 dt (13.0, 7.0)		2.34 ddd (12.5, 7.5, 6.7)
7						6.37 br d (1.5)	
9	7.20 br d (7.5)	7.15 br d (8.0)	7.25 d (7.5)	6.73 d (8.0)	6.75 d (8.0)	7.54 br d (7.8)	7.19 br d (7.7)
10	6.90 br t (7.5)	6.90 td (8.0, 1.0)	6.96 t (7.5)	6.81 t (8.0)	6.78 t (8.0)	7.09 br t (7.8)	6.91 td (7.7, 1.0)
11	7.14 td (7.5, 1)	7.11 td (8.0, 1.0)	7.19 t (7.5)	6.67 d (8.0)	6.70 d (8.0)	7.18 td (7.8, 1.0)	7.15 td (7.7)
12	6.82 br d (7.5)	6.80 br d (8.0)	6.86 d (7.5)			7.31 br d (7.8)	6.84 br d (7.7)
14*R*	1.18 dt (13.6, 2.6)	1.47 dt (13.0, 3.0)	1.49 br d (13.5)	1.49 dt (13.5, 2.5)	1.21 br d (14.0)	1.32 m	1.19 dt (13.7, 2.4)
14*S*	2.24 dt (13.6, 3.5)	2.12 dt (13.0, 3.0)	2.73 m	2.16 dt (13.5, 3.0)	2.18 dt (14.0, 3.0)	1.32 m	2.18 dt (13.7, 3.3)
15α	3.09 m	3.47 m	3.62 m	3.51 d (2.5)	3.37 m	3.01 t (8.6)	3.38 m
17β						4.46 d (9.5)	
17α						4.79 m	
18a	1.34 d (6.0)	2.30 s	2.36 s	2.31 s	2.29 s	5.42 d (17.0)	2.26 s
18b						5.17 dd (10.8, 1.3)	
19	4.76 m					5.77 dd (17.0, 10.8)	
20	2.11 m	2.87 m	3.62 m	3.04 m	3.05 m		3.02 ddd (10.0, 6.0, 2.8)
21β	2.67 dd (14.0, 6.0)	2.64 t (12.0)	3.34 br d (13.0)	2.76 t (13.0)	2.84 dd (14.0, 6.0)	3.30 m (α)	2.81 dd (14.0, 6.0)
21α	3.03 dd (14.0, 11.8)	2.83 dd (12.0, 4.0)	3.66 m	2.91 m	3.36 dd (14.0, 11.0)	3.64 m (β)	3.28 dd (14.0, 10.0)
23	4.21 m					4.10 m	
	4.21 m					4.10 m	
24	1.33 t (7.0)					1.23 m	
CO_2Me	3.77 s	3.68 s	3.71 s	3.67 s	3.78 s	3.67 s	3.77 s
NH	8.92 br s	8.93 br s	8.88 br s	8.98 br s	8.88 br s	8.42 br s	8.82 br s

[a] Assignments are based on COSY and HMQC. *R*/*S* assignments of H-14 appear for compounds **1**−**5** and **7** only. α/β assignments of H-21 appear for compounds **1**−**5** and **7** only.

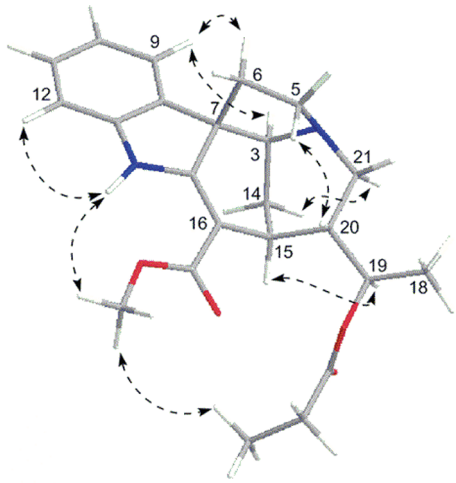


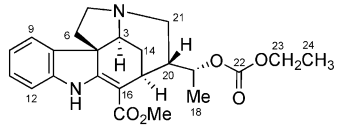
Figure 1. Selected NOEs of **1**.

20−C-21, C-19−C-18, and C-23−C-24 fragments, respectively. The NMR data and the partial structures from the COSY spectrum are suggestive of a strychnan skeleton as exemplified by akuammicine,[42] the main difference being that the ethylidene side chain has been replaced by an acylated hydroxyethyl group [i.e., $CH(OR)CH_3$ where R = $(CO)OCH_2CH_3$], as indicated by the NMR data.

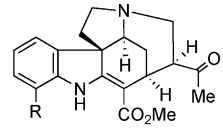
The relative configurations at the various stereogenic centers were established from NOEs (Figure 1) and analysis of the vicinal coupling constants. The reciprocal NOEs observed for H-9/H-3 determined the relative configurations at C-7 and C-3, which in turn allowed the orientation of H-15 to be assigned as α. The preferred boat conformation adopted by the piperidine ring D can be deduced from the observed H-14*S*/H-21α reciprocal NOEs (axial H-14 and H-21 NOEs)[43] as well as from analysis of the J_{20-21} vicinal coupling constants. Thus, the observation of H-21α as a doublet of doublets with coupling constants of 14 and 12 Hz (J_{21-21} = 14.0 Hz, $J_{20-21\alpha}$ = 12.0 Hz) requires H-20 to be β (i.e., *trans*-diaxial to H-21α). This is also consistent with the observation of reciprocal NOEs between H-20 and H-5β. The remaining configuration to be determined is that of the oxygenated C-19. This was achieved indirectly, by correlation with N(4)-demethylalstogustine (**9**), whose configuration was established by its relationship to alstogustine (**8**),[42] whose configuration had been in turn previously established by X-ray diffraction.[44] Thus treatment of alstolucine B (**2**) with NaOMe/MeOH gave a 2:1 mixture of **2** and its C-20 epimer, **7** (this step was necessary in view of the minor amount of natural **7** isolated). Reduction of **7** with $NaBH_4$ gave two products: the major product was identical to N(4)-demethylalstogustine (**9**), while the minor product was the corresponding C-19 epimer (**10**).[45] Subsequent treatment of **9** with ethyl chloroformate and triethylamine in CH_2Cl_2 gave an acylated derivative, which was identical ($[\alpha]_D$, ^{1}H and ^{13}C NMR, MS) with alstolucine A (**1**).

Alstolucine B (**2**) was isolated as colorless, block-shaped crystals, mp >160 °C (dec), with $[\alpha]^{25}_D$ −515 ($CHCl_3$, *c* 1.28). The UV spectrum (232, 295, and 326 nm) was similar to that of **1**, while the IR spectrum showed bands due to NH (3361 cm^{-1}), ketone (1704 cm^{-1}), and conjugated ester (1678 cm^{-1}) carbonyl functionalities. The ESIMS of **2** showed a pseudomolecular ion peak at *m*/*z* 339, and HRESIMS measurements yielded the molecular formula $C_{20}H_{22}N_2O_3$ (DBE 11). The ^{13}C NMR spectrum (Table 1) displayed a total of 20 carbon resonances, comprising two methyl, four methylene, seven methine, and seven quaternary carbons. The presence of carbonyl and conjugated ester functions was supported by the observed quaternary carbon signals at δ 208.5 and 167.2, respectively, while the signals at 172.2 (C-2) and 96.5 (C-16) are

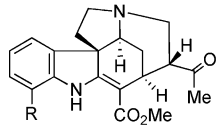
Chart 1



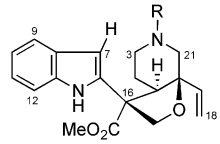
1



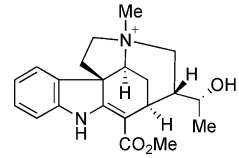
2 R = H
3 R = H, N(4)→O
4 R = OH



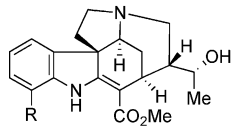
5 R = OH
7 R = H



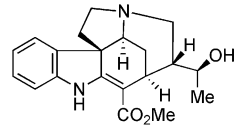
6 R = CO_2Et
12 R = H



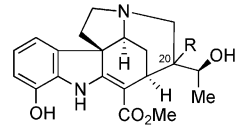
8



9 R = H
11 R = OMe



10



13 Scholaricine
R = α–H, 20*S* (revised)
R = β–H, 20*R* (previous assignment)

consistent with the presence of the β-anilinoacrylate moiety. The spectrum differs from that of **1** in that the signals due to the C-19 oxymethine and the associated ethyl carbonate group in the C-20 side chain have been replaced by the signal at δ 208.5 due to a keto-carbonyl group. The ^{1}H NMR spectrum (Table 2) was generally similar to that of **1** except that signals due to the C-19 oxymethine and the ethoxy group constituting part of the ethyl carbonate moiety of **1** were absent. Instead, a singlet at δ 2.30 due to an acetyl group was observed. These changes indicated that **2** possesses the same akuammicine-type ring system as that in **1**, except for replacement of the C-20 ethyl carbonate containing side chain by an acetyl group.

As with **1**, the relative configurations at the various stereogenic centers, C-3, C-7, and C-15, in **2**, were established from NOEs (Figure 2), which showed that these were similar to those in **1**. In contrast to **1** however, the preferred conformation adopted by the piperidine ring D in **2** is the chair conformation, as deduced from the presence of NOEs between H-21β and H-5β, H-6β and between H-14*S* and H-20α; the absence of NOEs between H-14*S* and H-21α; and the observed J_{20-21} vicinal coupling constants ($J_{20-21\beta}$ and $J_{20-21\alpha}$ values of 12 and 4 Hz, respectively), which are consistent with the dihedral angles resulting from a β-oriented or equatorially oriented C-20 acetyl group (H-21β and H-20α *trans*-diaxial, with the piperidine ring D in a chair conformation).[46,47] The structure and relative configuration of alstolucine B are therefore as shown in structure **2**, which was also confirmed by an X-ray diffraction analysis (Figure 3). Although alstolucine B (**2**) is isolated as an optically active alkaloid from a natural source for the first time, the racemic form is known as an intermediate compound in the synthesis of (±)-echitamidine.[45,48]

Alstolucine C (**3**) was obtained as a light yellowish oil with $[\alpha]^{25}_D$ −318 ($CHCl_3$, *c* 1.07). The ESIMS of **3** showed an $[M + H]^+$ at *m*/*z* 355, corresponding to the molecular formula $C_{20}H_{22}N_2O_4$, 16

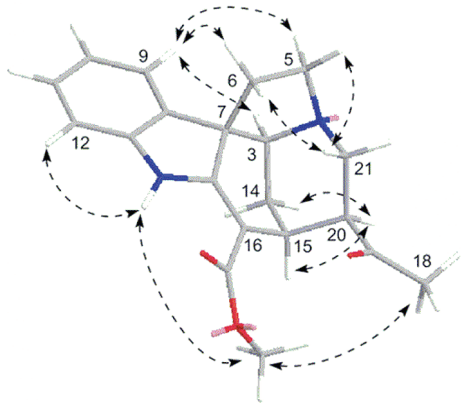


Figure 2. Selected NOEs of **2**.

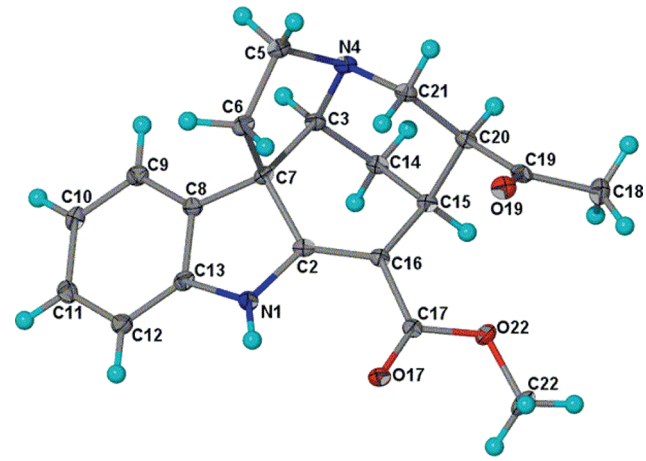


Figure 3. X-ray crystal structure of **2**. Thermal ellipsoids are shown at the 50% probability level.

mass units higher than that of alstolucine B (**2**). The UV and IR spectra were identical to those of **2**. Examination of the ^{1}H NMR spectrum (Table 2) showed downfield shifts for H-3, H-5, H-20, and H-21, while the ^{13}C NMR data (Table 1) showed downfield shifts involving C-3, C-5, and C-21, when compared to those of **2**. These features are characteristic of N-oxides, and this conclusion was further supported by conversion of alstolusine B into **3** by treatment with 3-chloroperbenzoic acid. Compound **3** is therefore the N-oxide of alstolucine B (**2**).

Alstolucine D (**4**) was obtained as a light reddish oil with $[\alpha]^{25}_D$ −313 ($CHCl_3$, *c* 0.25). The UV spectrum was similar to that of the other alstolucine compounds, while the IR spectrum indicated the presence of OH, NH, ester, and carbonyl functionalities. The ESIMS of **4** showed a pseudomolecular ion peak at *m*/*z* 355, which analyzed for $C_{20}H_{22}N_2O_4$, differing from alstolucine B (**2**) by 16 mass units, indicating replacement of H by OH. Examination of the NMR data (Tables 1 and 2) indicated a similarity with alstolucine B (**2**) except for differences in the aromatic region. The ^{1}H NMR spectrum (Table 2) showed the presence of three contiguous aromatic hydrogens. The observed H-3, H-6α/H-9 NOEs indicated substitution at C-12 by the hydroxy group, while the observed H-21*β*/H-5*β* and H-20/H-14*S* NOEs indicated an equatorially oriented C-20 acetyl side chain (H-20α) with ring D in the preferred chair conformation. The NMR and ROESY data were also similar to those obtained for alstolucine B (**2**), confirming the same relative configurations at the stereogenic centers, C-3, C-7, C-15, and C-20. Alstolucine D (**4**) is therefore the 12-hydroxy derivative of **2**.

Alstolucine E (**5**) was obtained as a light reddish oil with $[\alpha]^{25}_D$ −259 ($CHCl_3$, *c* 0.15). The UV, IR, and MS data were similar to those of alstolucine D (**4**), as were the NMR data (Tables 1 and 2), which were generally similar except for noticeable differences in the shifts of C-2, C-14, C-15, and C-16. Compound **5** possesses the same carbon skeleton as **4** (as indicated by the 2D NMR data) but differs in the configuration of the stereocenter at C-20. The observation of reciprocal NOEs for H-20/H-6*β* and H-14*S* and H-21α indicated a preferred boat conformation for the piperidine ring D with α-acetyl substitution at C-20 (H-20*β*). Alstolucine E (**5**) therefore possesses the opposite configuration at C-20 compared with alstolucines B, C, and D (**2**−**4**), with C-20 being *R* instead of *S* in **4**.

Alstolucine F (**7**) was previously reported from *A. scholaris*[36] but without any $[\alpha]_D$ value or NMR data. The racemic form is also known as an intermediate compound in the synthesis of (±)-alstogustine[45] and (±)-echitamidine.[45,48]

During the preparation of this article Morita et al. reported the results of their study of *A. pneumatophora* collected in Malaysia, which included isolation of the enantiomers of **3**, **4**, **5**, and **7**.[39] However the specific rotations of these purported enantiomers were significantly lower when compared with the strong negative values characteristic of this group of strychnan compounds,[27,42,43,49−53] e.g., $[\alpha]^{25}_D$ **3** −319 (present study) versus +25 (enantiomer[39]); **4** −326 (present study) versus +20 (enantiomer[39]); **5** −259 (present study) versus +21 (enantiomer[39]); **7** −371 (present study) versus +13 (enantiomer[39]).

In the course of the present study, the NMR data of a large number of strychnan alkaloids were compared.[39,42,43,45,48,49,51−53] It emerged from such a comparison that the configuration at C-20 attributed to the alkaloid scholaricine (**13**) required re-examination. Specifically, the resonances of C-2, C-14, and C-16 in the akuammicine-type alkaloids with C-20(*S*) are characteristically observed at *δ* 172, 31, and 96, respectively, while those with C-20(*R*) are usually found at *δ* 168, 27, and 103 (see Table 1 and refs 39, 42, 43, 45, 48, 49, and 51−53).[54] Scholaricine (**13**) was first reported by Atta-ur-Rahman and co-workers from *A. scholaris* but without any stereochemical assignments.[52] The configuration at C-20 was subsequently assigned as 20*R* by Yamauchi et al. from the observation that the same ketone product was obtained from the oxidation of scholaricine (**13**) and 19-epischolaricine,[43] following the same method used by Hesse for the assignment of the C-20 and C-19 configurations of 19-epialstogustine.[44] Comparison of the ^{13}C NMR data of **13** showed resonances for C-2, C-14, and C-16 at *δ* 172.1, 30.8, and 96.3, respectively, which correspond to the 20*S* series of these strychnan derivatives (Table 1). The 20*S* configuration was also supported by the observed H-21*β*/H-5*β*, H-6*β* and H-20/H-14*S* NOEs (axial H-20 in chair ring D). To secure unambiguous confirmation, an X-ray diffraction analysis was carried out (Figure 4) using a sample of **13** from our previous study of another *Alstonia* species,[12] which confirmed the configuration (C-20*S*) deduced from the NMR data (^{13}C NMR and NOEs).

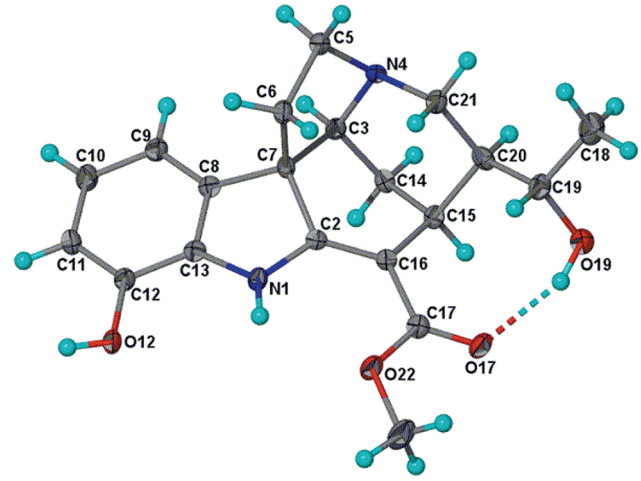


Figure 4. X-ray crystal structure of **13**. Thermal ellipsoids are shown at the 50% probability level.

Table 3. Cytotoxic Effects of Compounds

compound	IC$_{50}$, *μ*g/mL		
	KB/S[a]	KB/VJ300[a]	KB/VJ300(+)[b]
alstolucine A (**1**)	>25	>25	0.59
alstolucine B (**2**)	>25	>25	0.64
alstolucine C (**3**)	>25	>25	>25
alstolucine D (**4**)	>25	>25	10.33
alstolucine E (**5**)	>25	>25	19.22
alstolucine F (**7**)	>25	>25	2.61
N(4)-demethylalstogustine (**9**)	>25	>25	5.12
19-epi-*N*(4)-demethylalstogustine (**10**)	>25	>25	8.35
N(4)-demethyl-12-methoxyalstogustine (**11**)	>25	>25	10.56
scholaricine (**13**)	>25	13.35	1.15
akuammicine	>25	>25	12.29
vinervine	>25	>25	12.11
alstolobine A (**6**)	>25	>25	3.51
nor-6,7-secoangustilobine A (**12**)	>25	>25	>25
4,6-secoangustilobinal A	>25	>25	12.19
15-hydroxyangustilobine A	5.26	14.39	3.25
angustilobine B	6.99	8.12	0.84
undulifoline	>25	>25	>25

[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 *μ*g/mL (0.121 *μ*M), which did not affect the growth of the KB/VJ300 cells.

Alstolobine A (**6**) was isolated from the leaf extract of *A. spatulata* as a light yellowish oil, with $[\alpha]^{25}_D$ +40 ($CHCl_3$, *c* 0.10). The UV spectrum was characteristic of an indole chromophore with absorption maxima at 215 and 274 nm (log *ε* 4.67 and 4.13, respectively), while the IR spectrum showed bands due to OH (3320 cm^{-1}), ester (1734 cm^{-1}), and carbamate (1684 cm^{-1}) functions. The ESIMS of **6** showed a pseudomolecular ion peak at *m*/*z* 399, and HRESIMS measurements yielded the molecular formula $C_{22}H_{26}N_2O_5$, requiring 11 degrees of unsaturation. The ^{13}C NMR spectrum (Table 1) gave a total of 22 separate carbon resonances (two methyl, six methylene, seven methine, and seven quaternary

carbons), in agreement with the molecular formula. The ^{13}C NMR spectrum also indicated the presence of carbamate (δ 155.9) and ester (δ 173.5) functions and a vinyl side chain (δ 115.1, 140.0). Another sp^2 methine signal was observed at δ 101.6 in addition to the four due to the aromatic methines. The ^1H NMR spectrum of **6** (Table 2) showed the presence of a monosubstituted indole chromophore from the signals due to four adjacent aromatic hydrogens (δ 7.09−7.54), an indole NH (δ 8.42), and a methyl ester group (δ 3.67), in addition to the signals due to the vinyl side chain (δ 5.17, 5.42, 5.77). Other signals observed include an isolated aromatic broad singlet at δ 6.37, an aminomethylene at δ 3.30 and 3.64 (δ_C 47.6), and an oxymethylene at δ 4.46 and 4.79 (δ_C 70.1). Another oxymethylene at δ 4.10 was shown by the COSY spectrum to be coupled to the 3H multiplet at δ 1.23 and is therefore due to the presence of an ethoxy group. The isolated aromatic hydrogen at δ 6.37 is associated with the methine signal at δ 101.6. The COSY spectrum showed long-range coupling between this lone aromatic signal at δ 6.37 and the indolic NH, a feature that has been observed previously and that is reminiscent of the ring-opened secoangustilobine A compounds.[55] Furthermore, the isolated oxymethylene at δ 4.46 and 4.79 (δ_C 70.1) showed geminal coupling of 9.5 Hz, which is attributed to the methylene hydrogens (H_2C-17) α to the oxygen in a tetrahydrofuran ring, another common structural feature in secoangustilobine A compounds.[55]

The observed NOE between the indolic NH and the aromatic doublet at δ 7.31 allowed assignment of this resonance to H-12 and the lower field signal at δ 7.54 to H-9. The attachment of the vinylic side chain at C-20 was clear from the observed three-bond correlation from H-18 to C-20, while the observed three-bond correlation from H-9 to the aromatic methine signal at δ 101.6 confirmed that this signal was due to C-7 of a secoangustilobine A type alkaloid. In addition to the OCH_2CH_3 fragment, the COSY and HMQC data revealed partial structures that are consistent with a 6,7-secoangustilobine A type alkaloid, viz., NCH_2CH_2CH, CH_2CH, an isolated oxymethylene, and an isolated aminomethylene, corresponding to the C-3−C-14−C-15, C-18−C-19, C-17, and C-21 fragments, respectively. This left the ethoxy function, which must be part of an ethyl formate moiety linked to N-4. This conclusion is supported by the observed carbamate band at 1684 cm^{-1} in the IR spectrum and the carbamate carbonyl resonance at δ 155.9 in the ^{13}C NMR spectrum. The presence of the indolic NH ruled out attachment of the carbamate function on N-1. In addition, the presence of the ethyl formate group results in pronounced changes in the chemical shifts of H-3 and H-21 (shifted downfield) when compared with nor-6,7-secoangustilobine A (**12**), indicating that the site of substitution of the ethyl formate group is at N-4. Final proof was provided by chemical correlation with nor-6,7-secoangustilobine A (**12**): treatment of **12** with ethyl chloroformate and triethylamine in CH_2Cl_2 gave an N-acylated derivative, which was identical ($[\alpha]_D$, ^1H and ^{13}C NMR, MS) with alstolobine A (**6**). The relative configurations at the various stereogenic centers were established from the NOESY spectrum. The reciprocal NOEs observed for H-7/H-9, H-17β; H-17α/H-18a, CO_2Me; H-15/H-18a, CO_2Me; and *N*H/H-14, H-15, CO_2Me allowed assignment of the relative configurations at C-15, C-16, and C-20, as *R*, *S*, and *S*, respectively. These observations and the present chemical correlation between **12** and **6** establish the relative configuration for both alkaloids **12** and **6** (the relative configurations at the various stereogenic centers in **12** were not previously determined,[55] although those in several of the related angustilobine B derivatives were determined[12,56]). Since ^{13}C NMR data were not previously provided for nor-6,7-secoangustilobine A (**12**),[55] these are included in Table 1.

Alkaloids **1**, **2**, **6**, **7**, **9**, **10**, and **13** showed no appreciable cytotoxicity against drug-sensitive and vincristine-resistant KB cells (IC_{50} >25 μg/mL in all cases) but were found to reverse multidrug resistance in vincristine-resistant KB (VJ300) cells (Table 3), while alstolucine C (**3**), which is characterized by the presence of an N-oxide functionality, was found to be ineffective.[47] Angustilobine B and 15-hydroxyangustilobine A were cytotoxic to KB cells.

Experimental Section

General Experimental Procedures. Melting points were determined on a hot stage Leitz-Wetzlar melting point apparatus and were uncorrected. Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Perkin-Elmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ = 0.71073 Å), at 100 K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-97). ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer. All air- and moisture-sensitive reactions were carried out under N_2 in oven-dried glassware. MeOH was freshly distilled from magnesium turnings. CH_2Cl_2 was distilled from CaH_2, under N_2. All other reagents were used without further purification.

Plant Material. Plant material was collected in Johor, Malaysia, and identification was confirmed by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia. Herbarium voucher specimens (K674) are deposited at the Herbarium, University of Malaya.

Extraction and Isolation. Extraction of the leaf and stem-bark material and partitioning of the concentrated EtOH extracts with dilute acid were carried out as detailed elsewhere.[57] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of the appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal preparative TLC were Et_2O (NH_3-saturated), EtOAc (NH_3-saturated), EtOAc/hexanes (1:1; NH_3-saturated), EtOAc/hexanes (1:2; NH_3-saturated), EtOAc/MeOH (50:1; NH_3-saturated), EtOAc/MeOH (20:1; NH_3-saturated), $CHCl_3$ (NH_3-saturated), $CHCl_3$/hexanes (1:1; NH_3-saturated), $CHCl_3$/MeOH (50:1; NH_3-saturated), and $CHCl_3$/MeOH (20:1; NH_3-saturated). The yields (g kg^{-1}) of the alkaloids from the leaf extract were as follows: **1** (0.0003), **2** (0.0053), **3** (0.0021), **4** (0.0149), **5** (0.0028), **6** (0.00018), **7**[36] (0.00012), akuammicine[42] (0.0827), vinervine[58] (0.0058), *N*(4)-demethyl-12-methoxyalstogustine (**11**)[49] (0.0013), nor-6,7-secoangustilobine A (**12**)[55] (0.0035), 4,6-secoangustilobinal A[55] (0.0003), vincamine[59] (0.1368), 16-epivincamine[59,60] (0.0460), picrinine[61] (0.0295), undulifoline[31,62] (0.0052), vincaddiformine[63] (0.0058), 16*R*,19*E*-isositsirikine[64] (0.00057), and 20(*R*)-tubotaiwine[65] (0.0080). The yields (g kg^{-1}) of the alkaloids from the stem-bark extract were as follows: akuammicine (0.0023), vinervine (0.0050), *N*(4)-demethyl-12-methoxyalstogustine (0.0085), 15-hydroxyangustilobine A[55] (0.0072), angustilobine B (racemate)[55,66] (0.0011), 19,20-*E*-vallesamine[67] (0.0140), *N*(4)-demethylechitamine[43] (0.0265), undulifoline (0.0528), leuconoxine[68] (0.00065), 20(*R*)-tubotaiwine (0.0025), and 20(*S*)-tubotaiwine[43,69] (0.00073).

(−)-Alstolucine A (1): light yellowish oil; $[\alpha]^{25}_D$ −438 (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 230 (3.32), 298 (3.26), 328 (3.46) nm; IR (dry film) ν_{max} 3378, 1742, 1683 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1, respectively; ESIMS *m*/*z* 413 $[MH]^+$; HRESIMS *m*/*z* 413.2074 (calcd for $C_{23}H_{28}N_2O_5$ + H, 413.2071).

(−)-Alstolucine B (2): colorless crystals from $CHCl_3$; mp >160 °C (dec); $[\alpha]^{25}_D$ −515 (*c* 1.28, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 232 (3.85), 295 (3.75), 326 (3.91) nm; IR (dry film) ν_{max} 3361, 1704, 1678 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1, respectively; ESIMS *m*/*z* 339 $[MH]^+$; HRESIMS *m*/*z* 339.1714 $[MH]^+$ (calcd for $C_{20}H_{22}N_2O_3$ + H, 339.1703).

(−)-Alstolucine C (3): light yellowish oil; $[\alpha]^{25}_D$ −318 (*c* 1.07, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 228 (3.78), 291 (3.60), 328 (3.72) nm; IR (dry film) ν_{max} 3355, 1705, 1682 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1, respectively; ESIMS *m*/*z* 355 $[MH]^+$; HRESIMS *m*/*z* 355.1655 (calcd for $C_{20}H_{22}N_2O_4$ + H, 355.1652).

(−)-Alstolucine D (4): light reddish oil; $[\alpha]^{25}_D$ −313 (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (4.27), 234 (4.13), 288 (3.65), 336 (4.07) nm; IR (dry film) ν_{max} 3369, 1708, 1677 cm^{-1}; ^1H and ^{13}C NMR data,

Tables 2 and 1, respectively; ESIMS *m/z* 355 $[MH]^+$; HRESIMS *m/z* 355.1646 (calcd for $C_{20}H_{22}N_2O_4$ + H, 355.1652).

(−)-Alstolucine E (5): light reddish oil; $[\alpha]^{25}_D$ −259 (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 214 (3.69), 231 (3.60), 288 (3.19), 337 (3.60) nm; IR (dry film) ν_{max} 3379, 1709, 1681 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1, respectively; ESIMS *m/z* 355 $[MH]^+$; HRESIMS *m/z* 355.1657 (calcd for $C_{20}H_{22}N_2O_4$ + H, 355.1652).

(−)-Alstolucine F (7): light yellowish oil; $[\alpha]^{25}_D$ −371 (*c* 0.35, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 229 (3.32), 297 (3.21), 328 (3.38) nm; IR (dry film) ν_{max} 3364, 1704, 1678 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1, respectively; ESIMS *m/z* 339 $[MH]^+$; HRESIMS *m/z* 339.1710 (calcd for $C_{20}H_{22}N_2O_3$ + H, 339.1703).

Alstolobine A (6): light yellowish oil; $[\alpha]^{25}_D$ +40 (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 215 (4.67), 274 (4.13) nm; IR (dry film) ν_{max} 3320, 1734, 1684 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1, respectively; ESIMS *m/z* 399 $[MH]^+$; HRESIMS *m/z* 399.1981 (calcd for $C_{22}H_{26}N_2O_5$ + H, 399.1914).

Angustilobine B: colorless oil; $[\alpha]^{25}_D$ ±0 (*c* 0.20, $CHCl_3$) (lit.[55] $[\alpha]^{27}_D$ −136 (*c* 0.50, $CHCl_3$); lit.[66] $[\alpha]_D$ +46 (*c* 2, $CHCl_3$)).

Crystallographic data of alstolucine B (**2**): $C_{20}H_{22}N_2O_3$, M_r = 338.40, orthorhombic, space group $P2_12_12_1$, a = 7.8037(1) Å, b = 11.7086(2) Å, c = 18.3534(3) Å; V = 1676.96(5) $Å^3$, Z = 4, D_{calcd} = 1.340 g cm^{-3}, crystal size 0.14 × 0.26 × 0.55 mm^3, $F(000)$ = 720. The final R_1 value is 0.0350 (wR_2 = 0.0931) for 2069 reflections [$I > 2\sigma(I)$].

Crystallographic data of scholaricine (**13**): $2C_{20}H_{24}N_2O_4 \cdot C_4H_8O_2 \cdot H_2O$, Mr = 818.94, orthorhombic, space group $P2_12_12_1$, a = 10.4901(3) Å, b = 18.5736(5) Å, c = 21.1075(5) Å, V = 4112.56(19) $Å^3$, Z = 4, D_{calcd} = 1.323 g cm^{-3}, crystal size 0.10 × 0.28 × 0.30 mm^3, $F(000)$ = 1752. The final R_1 value is 0.0592 (wR_2 = 0.1641) for 3126 reflections [$I > 2\sigma(I)$].

Crystallographic data for the structures **2** and **13** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 787329 and 787330, respectively). 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).

Conversion of Alstolusine B (2) to Alstolusine C (3). *m*CPBA (2.3 mg, 0.013 mmol) was added to a stirred solution of **2** (3.0 mg, 0.009 mmol) in CH_2Cl_2 (3 mL) at 4 °C. After ca. 10 min, saturated Na_2CO_3 (5 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 10 mL). The extract was then dried (Na_2SO_4) and the solvent evaporated. Centrifugal TLC over SiO_2 (5% MeOH−$CHCl_3$) gave alstolusine C (**3**) (3.1 mg, 98%).

Epimerization of (−)-Alstolusine B (2) to Alstolusine F (7). To a solution of the ketone **2** (12 mg, 0.035 mmol) in 1 mL of MeOH was added a freshly prepared solution of Na (1.2 mg, 0.053 mmol) in 1 mL of MeOH at 0 °C. The mixture was allowed to stir at room temperature for 3 h. The solvent was evaporated in vacuo, and water was added. The product was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extract was dried (Na_2SO_4), filtered, and concentrated in vacuo, and the residue was purified by centrifugal preparative TLC (silica gel, 5% MeOH−$CHCl_3$) to afford 4.2 mg (35%) of the isomerized ketone **7** and 7.6 mg of the recovered starting material **2** (63%).

$NaBH_4$ Reduction of Alstolucine F (7). To a mixture of ketone **7** (8 mg, 0.024 mmol) in 2 mL of MeOH at 0 °C was added $NaBH_4$ (1.6 mg, 0.041 mmol). The solution was allowed to stir at room temperature for 1 h. Saturated $NaHCO_3$ (5 mL) solution was added, and the product was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo, and the residue was purified by centrifugal preparative TLC (silica gel, 10% MeOH−Et_2O) to afford 6.8 mg (85%) of *N*(4)-demethylalstogustine (**9**) and 0.8 mg of compound **10** (10%). *N*(4)-Demethylalstogustine (**9**): light yellowish oil; $[\alpha]^{25}_D$ −399 (*c* 0.33, $CHCl_3$) (lit.[43] $[\alpha]_D$ −442 (*c* 0.55, EtOH)); UV (EtOH) λ_{max} (log ε) 228 (3.88), 298 (3.82), 329 (3.98) nm; IR (dry film) ν_{max} 3373, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.52 (1H, br s, NH), 7.20 (1H, d, J = 7.5 Hz, H-9), 7.16 (1H, t, J = 7.5 Hz, H-11), 6.91 (1H, t, J = 7.5 Hz, H-10), 6.85 (1H, d, J = 7.5 Hz, H-12), 4.07 (1H, m, H-3), 3.82 (1H, s, CO_2Me), 3.62 (1H, m, H-19), 3.24 (1H, ddd, J = 11, 9, 6 Hz, H-5), 3.05 (1H, ddd, J = 11, 6, 4.5 Hz, H-5), 3.00 (1H, m, H-15), 2.94 (1H, t, J = 14 Hz, H-21), 2.66 (1H, dd, J = 14, 6 Hz, H-21), 2.32 (1H, m, H-6), 2.29 (1H, m, H-14*S*), 2.02 (1H, ddd, J = 12.4, 6, 5 Hz, H-6), 1.83 (1H, m, H-20), 1.22 (1H, dt, J = 13.7, 2.3 Hz, H-14*R*), 1.16 (1H, d, J = 6.2 Hz, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.9 (C, CO_2Me), 167.6 (C, C-2), 143.8 (C, C-13), 135.7 (C, C-8), 128.0 (CH, C-11), 121.2 (CH, C-10), 120.9 (CH, C-9), 109.7 (CH, C-12), 102.9 (C, C-16), 71.1 (CH, C-19), 59.1 (CH, C-3), 58.6 (C, C-7), 53.9 (CH_2, C-5), 51.5 (CH_3, CO_2Me), 48.4 (CH_2, C-21), 46.7 (CH_2, C-6), 45.5 (CH_2, C-20), 29.3 (CH, C-15), 27.4 (CH_2, C-14), 20.3 (CH_3, C-18); ESIMS *m/z* 341 $[MH]^+$; HRESIMS *m/z* 341.1866 (calcd for $C_{20}H_{25}N_2O_3$, 341.1860). Compound **10**: light yellowish oil; $[\alpha]^{25}_D$ −361 (*c* 0.18, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 226 (3.82), 298 (3.77), 329 (3.94) nm; IR (dry film) ν_{max} 3370, 1672 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.65 (1H, br s, NH), 7.20 (1H, br d, J = 7.5 Hz, H-9), 7.15 (1H, td, J = 7.5, 1 Hz, H-11), 6.91 (1H, br t, J = 7.5 Hz, H-10), 6.83 (1H, br d, J = 7.5 Hz, H-12), 4.04 (1H, m, H-3), 3.80 (1H, s, CO_2Me), 3.80 (1H, m, H-19), 3.18 (1H, ddd, J = 11, 9, 6.7 Hz, H-5), 3.09 (1H, dd, J = 14, 11 Hz, H-21), 2.99 (1H, ddd, J = 11, 6.7, 4 Hz, H-5), 2.95 (1H, m, H-15), 2.71 (1H, dd, J = 14, 6 Hz, H-21), 2.39 (1H, ddd, J = 12.8, 9, 7 Hz, H-6), 2.23 (1H, dt, J = 13.6, 3.4 Hz, H-14*S*), 2.03 (1H, m, H-20), 1.98 (1H, ddd, J = 12.8, 6, 4 Hz, H-6), 1.26 (1H, d, J = 6.2 Hz, H-18), 1.19 (1H, dt, J = 13.6, 2.7 Hz, H-14*R*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.1 (C, CO_2Me), 168.1 (C, C-2), 144.1 (C, C-13), 135.6 (C, C-8), 127.9 (CH, C-11), 121.1 (CH, C-10), 120.8 (CH, C-9), 109.7 (CH, C-12), 103.4 (C, C-16), 69.9 (CH, C-19), 59.2 (CH, C-3), 58.2 (C, C-7), 53.8 (CH_2, C-5), 51.3 (CH_3, CO_2Me), 47.4 (CH_2, C-21), 45.9 (CH_2, C-6), 43.4 (CH, C-20), 27.8 (CH, C-15), 27.6 (CH_2, C-14), 20.2 (CH_3, C-18); ESIMS *m/z* 341 $[MH]^+$; HRESIMS *m/z* 341.1867 (calcd for $C_{20}H_{25}N_2O_3$, 341.1860).

O-Acylation of *N*(4)-Demethylalstogustine (9). To a stirred solution of **9** (6.5 mg, 0.019 mmol), CH_2Cl_2 (5 mL), and TEA (13 μL, 0.095 mmol) was added dropwise ethyl chloroformate (9 μL, 0.095 mmol), and the mixture was stirred for 30 min at rt. The mixture was quenched with saturated NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), the solvent was evaporated in vacuo, and the residue was purified by centrifugal preparative TLC (SiO_2, 2% MeOH−$CHCl_3$) to give the *O*-carboethoxy derivative identical in all respects to **1** (5.4 mg, 69%) as a light yellowish oil.

Conversion of Nor-6,7-secoangustilobine A (12) to Alstolobine A (6). To a stirred solution of **12** (5.4 mg, 0.02 mmol), CH_2Cl_2 (5 mL), and TEA (11 μL, 0.10 mmol) was added dropwise ethyl chloroformate (11 μL, 0.10 mmol), and the mixture was stirred for 1 h at rt. The mixture was quenched with saturated NH_4Cl (15 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), the solvent was evaporated in vacuo, and the residue was purified by centrifugal preparative TLC (SiO_2, $CHCl_3$/NH_3-saturated) to give the *N*-carboethoxy derivative **6** (4.8 mg, 72%).

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that has been described in detail previously.[70,71]

Acknowledgment. We would like to thank the University of Malaya (UMRG) and MOHE Malaysia (FRGS) for financial support.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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NP100552B