**Vobatensines A–F, Cytotoxic Iboga-Vobasine Bisindoles from *Tabernaemontana corymbosa***

Dawn Su-Yin Sim,† Wuen-Yew Teoh,‡ Kae-Shin Sim,‡ Siew-Huah Lim,† Noel F. Thomas,† Yun-Yee Low,† and Toh-Seok Kam,§†

†Department of Chemistry and §Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

**ABSTRACT:** Six new bisindole alkaloids of the iboga-vobasine type, vobatensines A–F (1–6), in addition to four known bisindoles (8–11), were isolated from a stem bark extract of a Malayan *Tabernaemontana corymbosa*. The structures of these alkaloids were determined based on analysis of the spectroscopic data and in the case of vobatensines A (1), B (2), and 16-decarbomethoxyvocamine (8) also confirmed by partial syntheses. Nine of these alkaloids (1–5, 8–11) showed pronounced in vitro growth inhibitory activity against human KB, PC-3, LNCaP, HCT 116, HT-29, MCF7, MDA-MB-231, and A549 cancer cells.

The genus *Tabernaemontana* (Apocynaceae) comprises a large number of species that are distributed over the tropical and subtropical regions of the world, including parts of the Americas, Africa (including Madagascar), Asia, Oceania, and Australia.¹,² The large number of species, coupled with the wide geographical distribution, and the large number of synonyms have long presented a problem with respect to taxonomic classification. This long-standing issue has been addressed recently by a comprehensive review of this difficult genus (the Old World species), which has resulted in a significant reduction in the number of the Old World species.³ Plants of this genus are widely used in traditional medicine and are also prolific producers of indole and bisindole alkaloids including those with structurally novel molecular skeletons and useful biological activities.⁴,⁵ About 13 species are known to occur in Malaysia (Peninsular Malaya and Malaysian Borneo), with *T. corymbosa* being the most varied, as well as the most widely distributed, being encountered in a number of different locations.⁶,⁷ A number of the Malaysian *Tabernaemontana* species have been the subject of thorough chemical studies, with the discovery of many new and biologically active alkaloids.⁸–¹³ Herein are reported the isolation, structure determination, and biological activity of six new bisindole alkaloids (as well as the biological activity of four known bisindoles), isolated from a sample of *T. corymbosa* Roxb. ex Wall. collected near Taiping, in the state of Perak, Malaysia.¹⁴

**RESULTS AND DISCUSSION**

Compound 1 (vobatensine A, Chart 1) was obtained as a light yellowish oil with [α]_D^25 = 34 (c 0.72, CHCl₃). The IR spectrum showed bands due to NH (3395 cm⁻¹), OH (3248 cm⁻¹), and ester carbonyl functions (1720 cm⁻¹), while the UV spectrum showed characteristic indole absorption maxima at 228, 287, 295, and 308 nm.¹⁵ The ESIMS showed a [M + H]⁺ peak at m/z 663, and the ¹³C NMR and HRESIMS data established the molecular formula as C₃₅H₄₉NO₄. Examination of the ¹H, ¹³C, and 2-D NMR (COSY, HSQC, HMBC) data indicated a bisindole alkaloid, constituted from a union of vobasine and iboga moieties. Thus, the ¹H NMR spectrum of 1 (Table 1) showed the presence of two indole NH groups (δ 7.50, 7.69), an unsubstituted indole moiety (δ 7.01–7.53, 4H, vobasine), another indole ring substituted at C-10' and C-11' (δ 6.68, 6.90, iboga), an aromatic methoxy group (δ 3.99, iboga), a methyl ester group (δ 2.45, vobasine), a N-methyl (δ 2.57, vobasine), an ethylidene side chain (δ 1.66, 5.31; vobasine), and a hydroxyethyl side chain (δ 1.22, 3.83; iboga). The ¹³C NMR spectrum (Table 2) showed a total of 41 resonances, including those at δ 171.6 (ester carbonyl, vobasine), 49.9 (ester methyl, vobasine), 42.4 (NMe, vobasine), 118.6, 138.0 (olefinic C-19, C-20; vobasinyl), and 22.8, 71.6 (C-18’, C-19’; hydroxyethyl, iboga). The methyl ester group associated with the vobasinyl unit was notably shielded (δ 2.45), which was consistent with the configuration of C-16, possessing an ester function oriented within the shielding zone of the aromatic ring. The H-3 resonance of the vobasinyl unit was observed as a broad one-H doublet at δ 5.12 with J = 10.5 Hz, and since only one H-3 was present, the bisindole must be branched from C-3 of the vobasine half. The presence of two aromatic singlets (δ 6.68, 6.90) due to the indole moiety associated with the iboga half required the presence of two aromatic substituents. Since one is a methoxy group, the other position must correspond to the site of branching of the bisindole from the iboga moiety. The signal due to H-12' (δ 6.91, 6.93) of the iboga moiety generally lower field resonance was also readily confirmed by the observed NOE interaction between H-12’ and N1’-H.
Chart 1
The upfield shift of C-9' (δc 98.8) is a characteristic of adjacent (C-10') oxygenation and provided the first indication of aromatic methoxy substitution at C-10'. This conclusion derived further support from the observed H-9'/Ar-OMe NOE, and the observed three-bond correlation from MeO-10 derived further support from the observed H-9' aromatic methoxy substitution at C-10. This conclusion was determined readily to be R from the observed carbon shifts of C-15', corresponding to those of monomeric iboga alkaloids such as heynane or iboxigane, exemplifying the 19S series in iboga alkaloids with a hydroxethyl side chain (versus those of 19-epi-heynane or 19-epi-iboxigane, exemplifying the 19R series). The 19R compounds have their chemical shifts of C-15' at ca. δ 29 (i.e., deshielded by about 6 ppm when compared to the corresponding C-15' resonance in the 19S epimer), and C-21' at ca. δ 54.5 (i.e., shielded by about 5 ppm when compared to the 19S epimer). The geometry of the 19,20-double bond of the vobasinyl unit was established as E based on the reciprocal NOEs observed for H-18/H-15 and H-19/H-21.

The HMBC spectrum (Figure 1) shows the NOEs observed for H-18/H-15 and H-19/H-21 corresponding to those of monomeric iboga alkaloids such as heynane or iboxigane, exemplifying the 19S series in iboga alkaloids with a hydroxethyl side chain (versus those of 19-epi-heynane or 19-epi-iboxigane, exemplifying the 19R series). The 19R compounds have their chemical shifts of C-15' at ca. δ 29 (i.e., deshielded by about 6 ppm when compared to the corresponding C-15' resonance in the 19S epimer), and C-21' at ca. δ 54.5 (i.e., shielded by about 5 ppm when compared to the 19S epimer). The geometry of the 19,20-double bond of the vobasinyl unit was established as E based on the reciprocal NOEs observed for H-18/H-15 and H-19/H-21.
Table 2. $^{13}$C NMR Data ($\delta$) for 1–6 (CDCl$_3$)*

<table>
<thead>
<tr>
<th>C</th>
<th>1*</th>
<th>2*</th>
<th>3*</th>
<th>4*</th>
<th>5*</th>
<th>6*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>138.0</td>
<td>138.1</td>
<td>136.3</td>
<td>137.2</td>
<td>138.4</td>
<td>138.8</td>
</tr>
<tr>
<td>3</td>
<td>37.6</td>
<td>37.6</td>
<td>38.3</td>
<td>36.8</td>
<td>37.7</td>
<td>37.2</td>
</tr>
<tr>
<td>5</td>
<td>59.9</td>
<td>59.5</td>
<td>59.6</td>
<td>60.1</td>
<td>60.0</td>
<td>77.0</td>
</tr>
<tr>
<td>6</td>
<td>19.5</td>
<td>17.7</td>
<td>17.6</td>
<td>19.9</td>
<td>19.0</td>
<td>24.8</td>
</tr>
<tr>
<td>7</td>
<td>110.5</td>
<td>110.7</td>
<td>111.6</td>
<td>110.1</td>
<td>109.3</td>
<td>107.6</td>
</tr>
<tr>
<td>8</td>
<td>129.8</td>
<td>129.6</td>
<td>129.8</td>
<td>129.8</td>
<td>130.2</td>
<td>129.0</td>
</tr>
<tr>
<td>9</td>
<td>117.4</td>
<td>117.4</td>
<td>117.7</td>
<td>117.6</td>
<td>117.1</td>
<td>116.8</td>
</tr>
<tr>
<td>10</td>
<td>118.9</td>
<td>118.9</td>
<td>119.2</td>
<td>119.0</td>
<td>118.6</td>
<td>119.4</td>
</tr>
<tr>
<td>11</td>
<td>121.5</td>
<td>121.5</td>
<td>121.9</td>
<td>121.7</td>
<td>120.6</td>
<td>122.0</td>
</tr>
<tr>
<td>12</td>
<td>109.9</td>
<td>109.9</td>
<td>110.0</td>
<td>109.9</td>
<td>109.6</td>
<td>110.2</td>
</tr>
<tr>
<td>13</td>
<td>153.9</td>
<td>153.9</td>
<td>156.0</td>
<td>153.9</td>
<td>153.1</td>
<td>153.6</td>
</tr>
<tr>
<td>14</td>
<td>36.4</td>
<td>39.6</td>
<td>38.5</td>
<td>36.1</td>
<td>33.8</td>
<td>35.8</td>
</tr>
<tr>
<td>15</td>
<td>33.6</td>
<td>34.9</td>
<td>34.9</td>
<td>33.5</td>
<td>32.7</td>
<td>32.2</td>
</tr>
<tr>
<td>16</td>
<td>46.9</td>
<td>43.9</td>
<td>43.9</td>
<td>46.4</td>
<td>47.3</td>
<td>41.3</td>
</tr>
<tr>
<td>18</td>
<td>12.3</td>
<td>13.0</td>
<td>13.0</td>
<td>12.4</td>
<td>12.2</td>
<td>12.8</td>
</tr>
<tr>
<td>19</td>
<td>118.6</td>
<td>25.7</td>
<td>25.8</td>
<td>119.0</td>
<td>118.2</td>
<td>125.3</td>
</tr>
<tr>
<td>20</td>
<td>138.0</td>
<td>43.1</td>
<td>43.1</td>
<td>137.7</td>
<td>138.0</td>
<td>132.6</td>
</tr>
<tr>
<td>21</td>
<td>52.5</td>
<td>47.0</td>
<td>47.0</td>
<td>52.2</td>
<td>52.5</td>
<td>65.4</td>
</tr>
<tr>
<td>CO$_3$Me</td>
<td>49.9</td>
<td>49.9</td>
<td>49.9</td>
<td>50.0</td>
<td>49.9</td>
<td>50.0</td>
</tr>
<tr>
<td>CO$_2$Me</td>
<td>171.6</td>
<td>172.5</td>
<td>172.4</td>
<td>171.6</td>
<td>172.0</td>
<td>170.8</td>
</tr>
</tbody>
</table>

N(4)-Me 42.4 43.1 43.2 42.3 42.4 56.5

2’  1.24 1.48 1.68  1.09  1.09  1.25

3’  1.29 1.50 1.70  1.09  1.09  1.17

5’  1.08 1.35 1.48  1.09  1.09  1.17

6’  1.35 1.48 1.68  1.09  1.09  1.17

δ (CDCl$_3$) 100 MHz.

*Assignments based on HSQC and HMBC. *150 MHz. *100 MHz.

Figure 1. COSY and selected HMBCs of 1.

Figure 2. Selected NOEs of 1.

Figure 3. Selected NOEs of 2.

The hydroxyethyl substituent at C-20’ in the iboga half (corresponding to 19-epi-iboxigaine) is β-oriented (H-20’α) from the observed H-20’/H-16’ NOE, as is also the case in the other bisindoles 2–6.

Compound 2 (vobatensine B) was obtained as a light yellowish oil with [α]$_D^{20} + 29$ (c 0.40, CHC$_2$Cl$_2$). The IR spectrum showed bands due to NH (3394 cm$^{-1}$), OH (3267 cm$^{-1}$), and ester carbonyl functions (1720 cm$^{-1}$), while the UV spectrum showed characteristic indole absorption maxima at 227, 288, 296, and 307 nm. The ESIMS showed a [M + H]$^+$ peak at m/z 665, and the $^{13}$C NMR and HRESIMS data established the molecular formula as C$_{23}$H$_{27}$N$_4$O$_3$. Examination of the $^1$H, $^13$C, and 2-D NMR data indicated a general similarity to those of compound 1, indicating a similar constitution from the union of vobasin and iboga moieties. In common with 1, the $^1$H NMR spectrum of 2 (Table 1) also showed the presence of two indole NH ($\delta$ 7.35, 7.58), an unsubstituted indole moiety ($\delta$ 7.01–7.53, 4H, vobasinyl), another indole ring substituted at C-11’ ($\delta$ 6.70, 6.91, iboga), an aromatic methoxy group ($\delta$ 3.99, iboga), a methyl ester group ($\delta$ 2.44, vobasinyl), a N-methyl group ($\delta$ 2.54, vobasinyl), and a hydroxyethyl side chain ($\delta$ 1.24, 3.85; iboga). Similarly, the $^{13}$C NMR spectrum showed corresponding resonances at $\delta$ 172.5 (ester carbonyl, vobasinyl), 49.9 (ester methyl, vobasinyl), 43.1 (NMe, vobasinyl), and 22.8, 7.15 (C-18’, C-19’; hydroxyethyl, iboga). The configuration at C-16 of 2 was also similar to that in 1, with the shielded methyl ester group (δ 2.44) directed toward the aromatic ring. The major difference in the NMR data was the absence of signals due to the ethylidene side chain (vobasinyl), which were replaced instead by resonances due to an ethyl side chain ($\delta$ 0.95; 1.48, 1.68; vobasinyl) and the appearance of a one-H multiplet at $\delta$ 1.35 (H-20, vobasinyl). The configurations at C-3 and at C-19 remained unchanged from the similarity of the NMR data (vide supra). The configuration at C-20 was deduced from the observed H-20’/H-16’ NOE (Figure 3), which indicated that the C-20 ethyl substituent is β-oriented (H-20’α).
maxima at 228, 272 (sh), 284, 294, and 418 nm, indicative of indole and pseudoindoxyl chromophores,\(^1\) while the IR spectrum displayed bands due to NH (3350 cm\(^{-1}\)), ester (1725 cm\(^{-1}\)), and pseudoindoxyl carbonyl (1665 cm\(^{-1}\)) functions. The ESIMS showed a [M + H]\(^{+}\) peak at m/z 665, and the \(^{13}\)C NMR and HRESIMS data established the molecular formula as C\(_{41}\)H\(_{52}\)N\(_{4}\)O\(_{4}\). The \(^{1}H\) NMR data (Table 1) showed the presence of two indole groups, one at \(\delta 6.58\) (vobasyl), and the other as a broad singlet at \(\delta 4.09\), corresponding to the shielded NH of a pseudoindoxyl moiety (iboga),\(^19\) an unsubstituted indole chromophore from the presence of four aromatic resonances (\(\delta_H\) 7.07–7.53, vobasyl), another indole ring substituted at C-10’ and C-11’ (\(\delta 6.38, 6.99\), iboga), an aromatic methoxy group (\(\delta 3.88\), iboga), a methyl ester group (\(\delta 2.41\), vobasyl), a N-methyl group (\(\delta 2.52\), vobasyl), and two ethyl side chains (\(\delta 0.94; 1.46, 1.68\); vobasyl; 0.91; 1.47, 1.61; iboga). The \(^{13}\)C NMR spectrum (Table 2) showed a total of 41 carbon resonances comprising five methylyls, 10 methylenes, 15 methines, four tertiary carbons bonded to indolic nitrogen (corresponding to C-2, C-2’, C-13, C-13’), a methoxy-substituted aromatic carbon (C-10’), a ketocarbonyl (C-7’), an ester carbonyl (CO\(_2\)Me), and four quaternary carbon atoms. The observed C-2’ and C-7’ shifts of the iboga moiety are characteristic of a pseudoindoxyl moiety, and furthermore the C-2, C-2’ (\(\delta 48.5\)) resonances resembled those of iboluteine, suggesting that the spirocyclic C-2 in 3 has a similar configuration to that of C-2 in iboluteine.\(^{20,21}\) The corresponding shifts in iboga pseudoindoxyl alkaloids with opposite C-2 configuration (e.g., ervalutine) are found at \(\delta 72.7, 203.1, 41.0,\) and 53.2, for C-2’, C-7’, C-16’, and C-21’, respectively.\(^{16,21}\) This conclusion was also supported by the observed NH’/H-17/\(\beta\) reciprocal NOEs in 3 (in the case of iboga pseudoindoxyl alkaloids with the opposite C-2 configuration, e.g., ervalutine, the NH’/H-5/\(\alpha\), H-21’ NOEs are seen instead).\(^{16}\) In addition, the observed deshielding of H-21’ at \(\delta 3.54\) in 3 (versus \(\delta 2.43\) for H-21 in ervalutine) was a consequence of anisotropy from the proximate pseudoindoxyl carbonyl.

The similar connection of the monomeric indole halves from C-3 of the vobasine half to C-11’ of the iboga half (as in 1 and 2) was evident from the HMBC and NOE data (Figures 4 and 5, respectively), as are the configurations at C-3 and C-16, which are also similar to those of 1 and 2 from the similarity of the NMR data as described above for 1. The configuration at C-20 (vobasine) was inferred from the observed NOE for H-20/H-14 and H-19/H-16 (Figure 5), requiring H-20 to be \(\alpha\)-oriented (in the case of dregamine (7), which was also isolated, with H-20 being \(\beta\)-oriented, the H-20/H-16 and H-19/H-14 NOEs are seen instead).

Compound 4 (vobatensine D) was obtained as a light yellowish oil with \([\alpha]^{20}_D -39\) (c 0.33, CHCl\(_3\)). The UV spectrum showed absorption maxima at 227, 278, 286, 294, and 305 nm, indicative of indole and 7-hydroxyindolenine chromophores, while the IR spectrum indicated the presence of NH/OH (3200 cm\(^{-1}\)) and ester carbonyl functions (1723 cm\(^{-1}\)). The ESIMS showed a [M + H]\(^{+}\) peak at m/z 663, and \(^{13}\)C NMR and HRESIMS data established the molecular formula as C\(_{41}\)H\(_{52}\)N\(_{4}\)O\(_{4}\). The \(^{13}\)C NMR spectrum (Table 2) showed a total of 41 carbon resonances comprising five methylyls, nine methylenes, 15 methines, four tertiary carbons bonded to indolic nitrogen (corresponding to C-2, C-2’, C-13, C-13’), a methoxy-substituted aromatic carbon (C-10’), a hydroxy-substituted tertiary carbon (C-7’), an ester carbonyl (CO\(_2\)Me), and five quaternary carbon atoms.

Unlike the previous compounds 1–3, the \(^{1}H\) NMR spectrum of 4 (Table 1) showed the presence of only one indolic NH at \(\delta 7.73\) (vobasyl). Other signals in the \(^{1}H\) NMR spectrum included those of an unsubstituted indole chromophore (\(\delta_H\) 7.03–7.48, vobasyl), another indole ring substituted at C-10’ and C-11’ (\(\delta 6.88, 7.11\), iboga), an aromatic methoxy group (\(\delta 3.96\), iboga), a methyl ester group (\(\delta 2.42\), vobasyl), a N-methyl group (\(\delta 2.53\), vobasyl), an ethylidene side chain (\(\delta 1.63, 2.82\), vobasyl), and an ethyl side chain (\(\delta 0.91; 1.46, 1.51\); iboga). The observed NOE between the aromatic singlet at \(\delta 6.88\) (iboga) and H-6’ (\(\delta 1.83\), iboga) facilitated the attribution of this resonance to H-9’ and the aromatic singlet at \(\delta 7.11\) to H-12’. Aromatic methoxy substitution was at C-10’ from the observed ArOMe/H-9’ NOE (Figure 6). The absence of the indolic NH’ associated with the iboga unit, coupled with the observation of an imine carbon (\(\delta 192.1\)) and an oxygenated tertiary carbon (\(\delta 87.7, 7.7\)) associated with the iboga unit, indicated that the iboga moiety is a 7-hydroxyindolenine. As with the previous three compounds, the NMR data indicated similar branching of the bisindole from C-3 (\(\alpha\)-substitution) of the vobasyl half to C-11’ of the iboga

![Figure 4. COSY and selected HMBCs of 3.](image1)

![Figure 5. Selected NOEs of 3.](image2)

![Figure 6. Selected NOEs of 4.](image3)
half, a similar relative configuration at C-16 (δ 2.42, CO2Me-16), and similar E geometry of the 19,20-double bond (NOE: H-18/H-15 and H-19/H-21) (Figure 6). The configuration at C-7’ of the iboga moiety was assigned as S (C-7’-αOH), since the iboga half corresponds to the 7-hydroxyindolenine of ibogaine (which also occurs in the bark extract), for which the C-7’ configuration has been previously established.22

Compound 5 (vobatensine E) was obtained as a light yellowish oil with [α]_D^25 = –163 (c 0.67, CHCl_3). The UV spectrum showed indole absorption maxima at 229, 288, 294, and 307 nm, while the IR spectrum indicated the presence of NH (3409 cm⁻¹) and ester carbonyl functions (1723 cm⁻¹). The ESIMS showed a [M + H]⁺ peak at m/z 647, and the ¹³C NMR and HRESIMS data established the molecular formula as C₴₁H₅₀N₄O₄. The ¹³C NMR spectrum (Table 2) showed a total of 41 carbon resonances comprising five quaternary carbons, nine methylenes, 15 methines, four tertiary carbons bonded to indolic nitrogen (corresponding to C-2, C-2’, C-13, C-13’), a methoxy-substituted aromatic carbon (C-10’), an ester carbonyl (CO2Me), and six quaternary carbon atoms. The ¹H NMR spectrum (Table 1) showed the presence of two indolic NH (δ 7.44, 7.77), an unsubstituted indole ring (δ 6.99–7.52, vobasinyl), another indole ring substituted at C-9’ and C-10’ (δ 6.69, 7.06, iboga), an aromatic methoxy group (δ 3.04, iboga), a N-Me group (δ 2.61, vobasinyl), a methyl ester group (δ 2.42, vobasinyl), an ethyl side chain (δ 0.91; 1.46; 1.57; iboga), and an ethylidene side chain (δ 1.59, 5.30; vobasinyl).

The main difference compared with the previous four compounds was in the iboga moiety where a pair of AB doublets was observed at δ 6.69 and 7.06, with J = 8.5 Hz. An NOE was observed between the doublet at δ 7.06 and NH’, facilitating the assignment of this resonance to H-12’, and the doublet at δ 6.69 to H-11’. The observed NOE between H-11’ and Ar-OMe allowed placement of the methoxy substituent at C-10’, which, in turn, indicated that the bisindole is branched from the iboga C-9’ (Figure 7). This conclusion was also supported by the observed three-bond correlations from H-3 to C-8’ and C-10’, and from H-14 to C-9’, in the HMBC spectrum (Figure 7). Apart from this, the other features were similar to the previous bisindoles from the NMR data, viz., α-substitution at C-3 (H-3, δ 5.46, dd, J = 13, 2.5 Hz; NOE: H-3/N1-H), an E-configured 19,20-double bond (NOEs: H-18/H-15; H-19/H-21) (Figure 7), a methyl ester group directed toward indole ring (shielded CO2Me at δ 2.42), and a β-ethyl side chain at C-20’.

Compound 6 (vobatensine F) was the most polar of the bisindoles and was obtained as a light yellowish oil with [α]_D^25 = –57 (c 0.29, CHCl_3). The UV spectrum showed typical indole absorption maxima at 228, 286, 294, and 307 nm, while the IR spectrum indicated the presence of NH (3389 cm⁻¹) and ester carbonyl functions (1723 cm⁻¹). The ESIMS showed a [M + H]⁺ peak at m/z 663, and the ¹³C NMR and HRESIMS data established the molecular formula as C₄₁H₅₀N₄O₆. The ¹H and ¹³C NMR data (Tables 1 and 2, respectively) indicated a vobasine-iboga type bisindole alkaloid and, in fact, showed a similarity to those of the known bisindole, 16’-decarbamethoxyvobacamine (8),23 except for the notable downfield shifts of C-3, C-21, and NMe in the ¹³C NMR spectrum and of H-3, H-21, and NMe, in the ¹H NMR spectrum, features which are characteristic of alkaloid N-oxides. In addition, the observed molecular ion of 6 differed from that of 8 by 16 mass units. Compound 6 (vobatensine F) is therefore the N-4-oxide of 16’-decarbamethoxyvobacamine (8).

In addition to the six new bisindole alkaloids, four known bisindoles were also isolated: 16’-decarbamethoxyvobacamine (8),23 16’-decarbamethoxydihydrovobacamine (9),24 16’-decarbamethoxyvobacamine pseudoinodinyl (10),19 and vobaticrine (11).25 While alkaloids 8–10 are vobasinyl-iboga type bisindoles, vobaticrine (11) is a bisindole belonging to the vobasine-Styrenos group.25

The structures of several of the bisindoles isolated have also been confirmed by partial synthesis via acid-induced coupling of the appropriate vobasinyl and iboga moieties.26 The required vobasinyl cation precursors, vobasine (12) and tabernaemontaninol (13), were prepared by NaBH₄ reduction of vobasine (14) and tabernaemontanine (15), respectively. Reaction of vobasine (12) with 19-epi-iboxygaine (16) in the presence of 1.5% HCl in MeOH (reflux 1.5 h) resulted in the formation of vobasine A (1) as the sole product in 63% yield. Similar reaction of vobasine (12) with ibogaine (17) (reflux 3 h) gave 16’-decarbamethoxyvobacamine (8) as the sole product in 72% yield; none of the 3,9’-bisindole (vobasine, E, 5) was detected. Reaction of tabernaemontaninol (13) with 19-epi-iboxygaine (16) (reflux 1.5 h), on the other hand, yielded vobasine B (2) in 65% yield.

The bisindoles isolated were evaluated for cytotoxic effects against an array of human cancer cell lines including, KB, PC-3, LNCaP, HCT 116, HT-29, MCF7, MDA-MB-231, and A549 cells (Table 3). All the bisindoles tested (1–5, 8–11), showed growth inhibitory activity (IC₅₀ < 10 μM) against the cell lines tested with the exception of LNCaP (1–5, 8, 9, 11), KB (3–5, 11), MCF7 (4, 5), and A549 (4, 5, 9) (IC₅₀ > 10 μM). Notably pronounced effects were seen for all compounds against PC-3, HCT 116, and HT-29 cells (Table 3). Of the compounds tested, only compounds 4 and 5 were moderately effective in reversing multidrug resistance in vincristine-resistant KB cells (IC₅₀ KB/VJ300, ca. 30 μM; IC₅₀ KB/VJ300 + 0.1 μg/mL vincristine, ca. 9 μM).

**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. UV spectra were obtained on a Shimadzu UV-3100PC spectrophotometer. IR spectra were recorded on a PerkinElmer RX1 FT-IR spectrophotometer. ¹H and ¹³C NMR spectra were recorded in CDCl₃ using TMS as internal standard on JEOL 400 MHz and Bruker 600 MHz spectrometers. ESIMS and HRESIMS were obtained on Agilent 6530 Q-TOF and JEOL AccuTOF-DART mass spectrometers.

**Plant Material.** Plant material was collected near Taiping, Perak, Malaysia, and identification was confirmed by Dr. Richard C. K.
Table 3. Cytotoxic Effects of Compounds 1–S and 8–11a

<table>
<thead>
<tr>
<th>compound</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KB/S</td>
</tr>
<tr>
<td>vobatensine A (1)</td>
<td>8.2</td>
</tr>
<tr>
<td>vobatensine B (2)</td>
<td>8.0</td>
</tr>
<tr>
<td>vobatensine C (3)</td>
<td>&gt;10</td>
</tr>
<tr>
<td>vobatensine D (4)</td>
<td>&gt;10</td>
</tr>
<tr>
<td>vobatensine E (5)</td>
<td>&gt;10</td>
</tr>
<tr>
<td>16′-decarbomethoxyvocamine (8)</td>
<td>5.3</td>
</tr>
<tr>
<td>16′-decarbomethoxyhydrovocamine (9)</td>
<td>8.0</td>
</tr>
<tr>
<td>16′-deoxyvocamine pseudoiodoxygen (10)</td>
<td>8.3</td>
</tr>
<tr>
<td>vobatricine (11)</td>
<td>&gt;10</td>
</tr>
<tr>
<td>cinobufotaline</td>
<td>2.0</td>
</tr>
</tbody>
</table>

a IC50 (μM) 16′-Decarbomethoxyhydrovocamine (9): 1H NMR (CDCl3, 400 MHz) δ 0.86 (3H, t, J = 7 Hz, H-18), 0.94 (3H, t, J = 7.5 Hz, H-18), 1.16 (1H, br d, J = 13 Hz, H-15′), 1.33 (1H, m, H-20), 1.42 (1H, m, H-19′), 1.45 (1H, m, H-19), 1.50 (1H, m, H-19′), 1.50 (1H, m, H-20′), 1.52 (1H, m, H-17′), 1.67 (1H, m, H-19), 1.75 (1H, m, H-15), 1.78 (1H, m, H-14′), 1.94 (1H, m, H-17′), 2.01 (1H, m, H-14), 2.39 (1H, d, J = 13 Hz, H-21′), 2.43 (1H, s, CO2Me), 2.48 (1H, m, H-14), 2.53 (3H, s, N(4)-Me), 2.58 (1H, m, H-6′), 2.62 (1H, m, H-15), 2.78 (1H, m, H-16′), 2.78 (1H, m, H-21′), 2.87 (1H, t, J = 2.5 Hz, H-16), 2.94 (1H, m, H-3′), 2.97 (1H, m, H-21), 3.00 (1H, m, H-3′), 3.10 (1H, m, H-6′), 3.31 (1H, m, H-6′), 3.33 (1H, m, H-5′), 3.40 (1H, m, H-6′), 3.96 (1H, m, H-5), 3.96 (1H, s, OMe), 4.05 (1H, d, J = 10 Hz, H-3), 6.67 (1H, s, H-12), 6.91 (1H, s, H-9′), 7.00 (1H, m, H-12), 7.02 (1H, m, H-11), 7.03 (1H, m, H-10), 7.23 (1H, br s, N(1′)-H), 7.52 (1H, br d, J = 6 Hz, H-9′), 7.56 (1H, br s, N(1)-H), 13C NMR (CDCl3, 100 MHz) δ 120.1 (C-18′), 130.1 (C-18), 176.5 (C-6′), 209.5 (C-6′), 265.4 (C-19′), 279.4 (C-19), 321.9 (C-15′), 342.2 (C-17′), 350.8 (C-15), 376.2 (C-3′), 396.4 (C-14′), 415.3 (C-16′), 420.4 (C-20′), 431.3 (C-20′), 431.3 (N(4)-Me), 440.1 (C-16), 47.0 (C-21), 49.9 (CO2Me), 50.0 (C-3′), 54.3 (C-5′), 56.2 (10′-OMe), 57.7 (C-21′), 59.6 (C-5′), 98.7 (C-9′), 109.0 (C-7′), 109.9 (C-12), 110.9 (C-7′), 117.4 (C-9′), 118.8 (C-10), 121.4 (C-11), 128.3 (C-8′), 128.9 (C-12), 129.4 (C-13), 133.9 (C-13), 158.1 (C-2′), 130.0 (C-8), 142.6 (C-2′), 151.0 (C-10′), 172.6 (CO2Me).

NaBH4 Reduction of Vobasine (14) and Tabernaemontanae (15). NaBH4 (30 mg, 0.8 mmol) was added to a solution of 14 (35 mg, 0.1 mmol) in 2 mL of MeOH, and the mixture was stirred for 10 min at rt. An additional portion of NaBH4 (30 mg, 0.8 mmol) was added, and the mixture was stirred for another 10 min. Saturated NaHCO3 solution (5 mL) was added, and the mixture was extracted with CH2Cl2 (3 × 10 mL). The combined organic extract was washed with H2O (3 × 10 mL), dried (Na2SO4), concentrated in vacuo, and the residue purified by radial chromatography (SiO2, 100:2 Et2O/MEOH, NH4OH-saturated) to give vobasinol (12) (14 mg, 40%); HREMS m/z 355.2044 [M + H]+ (calcd for C30H28N5O4 + H, 355.2022). Following essentially the same procedure, NaNH4 (18 mg, 0.5 mmol) was added to a solution of 15 (30 mg, 0.085 mmol) in 2 mL of MeOH and the mixture refluxed for 10 min after which an additional portion of NaNH4 (18 mg, 0.48 mmol) was added and the mixture was refluxed for another 10 min. Workup as before gave tabernaemontanin (13) (16 mg, 53%); HREMS m/z 357.2170 [M + H]+ (calcd for C30H26N5O4 + H, 357.2176). The 1H NMR data of both compounds were identical with those of the natural materials.27,28

Partial Synthesis of Compounds 1, 2, and 8. The procedure is illustrated by the partial synthesis of vobatensine A (1). A solution of vobasinol (12, 10 mg, 0.03 mmol) and 19-epi-iboxagine (16, 10 mg, 0.03 mmol) in 1.5% methanolic HCl (5 mL) was heated under reflux.
for 1.5 h. The cooled reaction mixture was diluted with H2O, neutralized with K2CO3 and extracted with CH2Cl2. The extract was washed with H2O and dried over Na2SO4 and the solvent removed in vacuo to furnish a residue, which was purified by radial chromatography (SiO2, 100:2 to 100:7 CH2Cl2/MeOH, NH3-saturated) to give I (12 mg; 63%), [α]D20 = -51 (c 0.43, CHCl3), HRESIMS m/z 663.3895 [M + H]+ (calcd for C41H50N4O3 + H, 663.3910). Similar reaction of tabernaemontaninol (13) with 19-epi-ibogayxine (16) (reflux 1.5 h) yielded vobatensine B (2) in 65% yield, [α]D20 = +21 (c 0.69, CHCl3), HRESIMS m/z 665.4053 [M + H]+ (calcd for C41H52N4O4 + H, 665.4067). Reaction of vobasinol (12) with ibogaine (17) (reflux 3 h) gave 16-decarbomethoxyvoacorine (8) as the sole product in 72% yield, [α]D20 = -63 (c 0.33, CHCl3), HRESIMS m/z 647.3946 [M + H]+ (calcd for C41H50N4O4 + H, 647.3961). The 1H and 13C NMR data for the products were identical to those of the natural materials.

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that has been described previously.29 Vincristine and cisplatin were used as positive controls.

■ ASSOCIATED CONTENT

* Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b01117.

1H, 13C NMR, and selected HMBC and NOESY spectra for 1–6. (PDF)

■ AUTHOR INFORMATION

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

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of Malaya and MOHE Malaysia (HIR-005) for financial support.

■ REFERENCES