Cytotoxic and Leishmanicidal Aminoglycosteroids and Aminosteroids from *Holarrhena curtisii*

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The EtOH extract of the leaves of *Holarrhena curtisii* yielded five new steroidal alkaloids: 17-*epi*-holacurtine (**3**), 17-*epi*-*N*-demethylholacurtine (**4**), holacurtinol (**5**), 3α-amino-14β-hydroxypregnan-20-one (**7**), and 15α-hydroxyholamine (**8**), in addition to the known compounds, holacurtine (**1**), *N*-demethylholacurtine (**2**), and holamine (**6**). All eight compounds showed significant cytotoxic and leishmanicidal activities.

Plants belonging to the genus *Holarrhena* (Apocynaceae) are distributed over a wide area, from Africa, over the Indian subcontinent and extending to southeast Asia. The chemistry of some African and Indian species has been investigated, and these species are known to provide mainly steroidal alkaloids of the aminopregnane type.[1−19] The Indian species *H. antidysenterica* in particular has a long history of being used for medicinal purposes and as such represents one of the more well-studied species.[12−19] In view of the extensive use of *H. antidysenterica* in the Indian subcontinent for the treatment of dysentery,[18,20] we carried out preliminary screening for antileishmanial activity on the Malaysian species, *H. curtisii* King & Gamble, and found that both the chloroform and the ethanol extracts of the leaves showed significant activity when tested against *Leishmania donovani*. The activity was further traced to the basic fraction derived from these extracts. Subsequent fractionation of the leaf extract led to the isolation of three known (**1**, **2**, **6**) and five new (**3**−**5**, **7**, **8**) steroidal alkaloids from this plant, and the leishmanicidal activity detected in the preliminary screening could be traced to several of these constituents. Further bioassays, however, revealed that these compounds also showed cytotoxic activity. We report herein on the structure elucidation of these new steroidal alkaloids and their biological activities. A previous study of this species afforded the first aminoglycosteroid isolated from this genus, viz., holacurtine (**1**).[21]

Results and Discussion

The major compound obtained from the ethanol extract of the leaves was the known aminoglycosteroid holacurtine (**1**). Other known compounds obtained were *N*-demethylholacurtine (**2**)[22] and the aminosteroid, holamine (**6**).[4,6] In addition, five new steroidal alkaloids were obtained: 17-*epi*-holacurtine (**3**), 17-*epi*-*N*-demethylholacurtine (**4**), holacurtinol (15α-hydroxy-5,6-didehydro-*N*-demethylholacurtine) (**5**), 3α-amino-14β-hydroxypregnan-20-one (**7**), and 15α-hydroxyholamine (**8**). Compounds **1**−**5** all showed mass spectral data typical of aminoglycosteroids, displaying the pregnane aglycon fragment as the base peak. All five compounds gave HRMS data in agreement with the proposed structures (see Experimental Section), and their IR spectra showed absorptions for NH/OH and carbonyl functions. We have included NMR data in Tables 1 and 2 for the known compounds **1**, **2**, and **6**, because only low-resolution NMR data were determined previously. The spectra were assigned based on the application of standard 2D NMR methods (COSY, HMQC, HMBC, and NOE) and with reference to related compounds that have been previously reported.[23−27]

Compound **3** was obtained in amorphous form, $[\alpha]_D$ −13.3° ($CHCl_3$, *c* 0.68). The EIMS showed a molecular ion at *m*/*z* 491 and HRMS measurements gave the molecular formula, $C_{29}H_{49}NO_5$, which was identical to that of holacurtine (**1**). As in the case of **1**, the identity of the sugar moiety could be confirmed as 4-deoxy-4-methylamino-β-D-cymaropyranose by comparison of the NMR data with that of D-cymaropyranose.[28] In common with **1**, the anomeric proton and carbon signals were readily discerned in the downfield region at δ 4.81 and δ 95.0 in the ^{1}H and ^{13}C NMR spectra, respectively (Tables 1 and 2). In addition, the C-3 signal of the aglycon part has undergone the characteristic glycosylation shift to δ 76.6 when compared with the pregnane, 3β,16β-dihydroxy-5α-pregnan-20-one, confirming the attachment of the sugar moiety at C-3.[28,29] Further confirmation of the linkage position of the sugar unit was provided by NOE and HMBC data. Thus, irradiation of the anomeric-H signal (δ 4.81) caused an NOE enhancement of the aglycon H-3 signal (δ 3.64) and *vice versa*, and the HMBC spectrum showed correlations from C-3 to H-1′ and from C-1′ to H-3. The ^{1}H NMR spectrum of **3** was generally similar to that of **1** except for significant changes involving the H-17 signal, suggesting that **3** is the C-17 epimer of **1**. In holacurtine (**1**), H-17α appeared as a doublet of doublets at δ 2.90 with $J = 9$ and 4.5 Hz, indicative of an α-stereochemistry for H-17, which has been observed also for other pregnane glucosides,[27] whereas in compound **3**, H-17 was observed as a triplet with $J = 9$ Hz.[23] In addition, the effects of the change in configuration of C-17 in causing a downfield shift for C-12 and an upfield shift for C-18, previously noted for some oxypregnane-type compounds,[26] were also observed in the ^{13}C NMR spectrum of **3**. Furthermore, irradiation of the H-17 signal caused enhancement of the C-18 methyl signal and *vice versa*, which was not observed on similar irradiation of holacurtine (**1**). Compound **3** was therefore assigned

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**8**[a]

position	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[c]	**7**[c]	**8**[c]
1	0.90 m	0.89 m	0.95 td (13, 4)	0.95 m	1.10 m	1.5 m	1.2 m	1.5 m
	1.72 dt (13, 4)	1.72 dt (13, 4)	1.72 dt (13, 4)	1.72 dt (13, 4)	1.76 m	1.6 m	1.5 m	1.7 m
2	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m
	1.89 m	1.88 m	1.88 m	1.89 m	1.88 m	1.77 m	1.71 m	1.7 m
3	3.64 m	3.62 m	3.62 m	3.62 m	3.56 m	3.19 br s	3.22 br s	3.15 br s
4	1.3 m	1.2 m	1.24 m	1.26 m	2.16 m	1.86 m	1.2 m	1.92 m
	1.63 m	1.62 m	1.63 m	1.62 m	2.16 m	2.54 m	1.5 m	2.57 br d (14)
5	1.03 m	1.04 m	1.06 m	1.08 m			1.46 m	
6	1.3 m	1.2 m	1.24 m	1.26 m	5.42 br d (5)	5.37 br d (5)	1.2 m	5.38 br d (5)
	1.3 m	1.2 m	1.36 m	1.35 m			1.2 m	
7	1.03 m	1.04 m	1.06 m	1.08 m	2.16 m	1.5 m	1.01 m	1.7 m
	2.14 m	2.14 m	1.96 m	1.96 m	2.38 m	1.94 m	2.07 m	2.22 m
8	1.56 m	1.55 m	1.5 m	1.5 m	1.76 m	1.5 m	1.5 m	1.7 m
9	0.83 m	0.83 m	0.86 m	0.85 m	1.02 m	1.14 m	1.01 m	1.20 m
11	1.3 m	1.2 m	1.06 m	1.08 m	1.32 m	1.6 m	1.2 m	1.5 m
	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m	1.6 m	1.5 m	1.5 m
12	1.3 m	1.2 m	1.18 m	1.15 m	1.32 m	1.37 m	1.2 m	1.40 m
	1.5 m	1.5 m	1.18 m	1.15 m	1.5 m	2.03 m	1.5 m	1.92 m
14						1.14 m		1.20 m
15	1.77 m	1.77 m	1.5 m	1.5 m	4.37 d (6)	1.6 m	1.71 m	4.08 m
	2.09 m	2.09 m	2.03 m	2.03 m		1.6 m	2.07 m	
16	1.89 m	1.88 m	1.79 m	1.80 m	1.88 m	1.6 m	1.86 m	1.5 m
	1.99 m	1.99 m	2.11 m	2.10 m	2.38 m	2.15 m	1.94 m	2.80 m
17	2.90 dd (9, 4.5)	2.90 dd (9, 4.5)	3.25 t (9)	3.26 t (9)	3.13 dd (9, 4.5)	2.54 m	2.87 dd (9, 4.5)	2.80 m
18	0.96 s	0.96 s	1.21 s	1.21 s	0.96 s	0.63 s	0.93 s	0.63 s
19	0.79 s	0.79 s	0.79 s	0.79 s	0.96 s	0.99 s	0.74 s	0.99 s
21	2.23 s	2.23 s	2.14 s	2.15 s	2.24 s	2.11 s	2.20 s	2.11 s
1′	4.81 dd (9.5, 2)	4.83 dd (9.5, 2)	4.81 dd (9.5, 2)	4.82 dd (9.5, 2)	4.80 dd (9.5, 2)			
2′	1.5 m	1.5 m	1.5 m	1.5 m	1.5 m			
	2.19 m	2.18 m	2.18 m	2.18 m	2.16 m			
3′	3.78 m	3.67 m	3.77 m	3.62 m	3.56 m			
4′	2.26 m	2.61 dd (10,3)	2.22 m	2.57 dd (10, 3)	2.38 m			
5′	3.64 m	3.62 m	3.62 m	3.62 m	3.56 m			
6′	1.32 d (6)	1.31 d (6)	1.30 d (6)	1.30 d (6)	1.24 d (6)			
OMe	3.41 s	3.42 s	3.40 s	3.42 s	3.40 s			
NMe	2.45 s		2.43 s					
OH-14	4.35 brs	4.36 brs						

[a] $CDCl_3$. [b] 400 MHz. [c] 270 MHz.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**8** ($CDCl_3$, 67.5 MHz)

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	37.1	37.7	37.0	37.6	36.9	33.0	32.1	33.0
2	29.1	29.7	29.1	29.7	29.6	29.1	28.6	29.4
3	76.6	77.3	76.6	77.7	78.8	46.9	45.9	46.7
4	34.1	34.7	34.2	34.7	37.4	39.6	35.1	39.8
5	44.2	44.8	44.1	44.7	140.2	138.7	38.6	138.6
6	28.5	29.1	28.5	29.1	121.8	123.2	28.5	122.9
7	27.5	28.2	27.0	27.6	36.9	31.8	27.5	32.0
8	39.6	40.2	41.0	41.6	36.5	31.7	39.7	31.5
9	49.2	49.8	49.7	50.3	45.3	50.2	49.3	50.0
10	35.7	36.3	35.1	36.3	37.3	37.4	36.4	37.3
11	20.6	21.2	20.1	20.7	20.1	20.8	20.3	20.5
12	38.9	39.5	30.7	31.3	38.7	38.8	39.1	39.0
13	49.0	49.6	48.1	48.6	48.3	44.0	49.2	44.6
14	84.6	85.3	86.5	87.2	84.9	56.9	84.9	62.9
15	33.6	34.2	31.1	31.7	81.9	24.4	33.8	73.9
16	24.7	25.3	21.4	22.0	27.2	22.8	24.8	35.0
17	62.1	62.7	61.2	61.7	61.2	63.7	62.2	60.9
18	15.1	15.7	18.9	19.5	16.1	13.2	15.3	14.4
19	12.0	12.6	12.0	12.6	18.6	18.8	11.2	18.8
20	217.6	218.3	210.5	211.0	217.5	209.6	217.8	208.6
21	33.1	33.8	31.8	32.4	33.4	31.5	33.3	31.6
1′	95.0	95.7	95.1	95.9	95.5			
2′	33.8	35.1	33.8	34.9	34.7			
3′	73.0	78.8	73.2	78.3	77.2			
4′	63.7	56.6	63.8	56.5	56.3			
5′	70.3	71.9	70.5	71.4	71.8			
6′	19.1	19.1	19.2	19.2	18.7			
OMe	56.7	57.7	56.9	57.8	57.4			
NMe	33.8		33.9					

as 17-*epi*-holacurtine and was obtained as a natural product for the first time. Compound **3** was also readily obtained via base-promoted epimerization (heating in 2M KOH−EtOH) of **1**.[21]

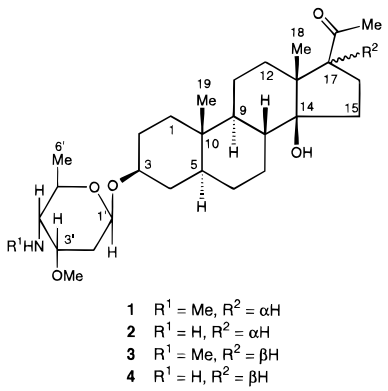
Compound **4**, $[\alpha]_D$ −15.6° ($CHCl_3$, *c* 0.17), had the formula $C_{28}H_{47}NO_5$ as determined by HRMS, differing from **3** by 14 mass units, suggesting replacement of a methyl group with H. This was supported by the NMR spectral data of **4**, which were similar to those of **3** except for the absence of an *N*-methyl resonance in both the ^{1}H and ^{13}C NMR spectra of **4**. The absence of the *N*-Me group has also caused a change in the H-4′ signal, which was observed as a doublet of doublets at δ 2.57 (J = 10, 3 Hz) and the C-4′ signal has also undergone an upfield shift from δ 63.8 in **3** to δ 56.5 in **4**. These values were identical to those of *N*-demethylholacurtine (**2**). Based on these observations, it could be concluded that compound **4** was 17-*epi*-*N*-demethylholacurtine.

Compound **5**, holacurtinol, $[\alpha]_D$ +30° ($CHCl_3$, *c* 0.05), was obtained in minute amounts. The EIMS showed a molecular ion at *m*/*z* 491 and HRMS gave the formula $C_{28}H_{45}NO_6$. The base peak observed at *m*/*z* 330 was due to the aglycon moiety. Other significant fragments noted at *m*/*z* 312 and 294, were attributable to successive losses of two molecules of H_2O and indicated the presence of two hydroxyl groups.[30] The IR spectrum showed, in addition to the NH/OH and C=O absorptions, a band at 1643 cm^{-1} due to C=C.[31] The NMR spectral data showed the presence of the same sugar unit as in **2** and **4**, that is, 4-deoxy-4-amino-β-D-cymaropyranose, while the pregnane aglycon portion was similar to that of holamine (**6**), in which a C(5)−C(6) double bond was present.[4,6] This was supported by the observation of the vinylic H-6 signal as a broad

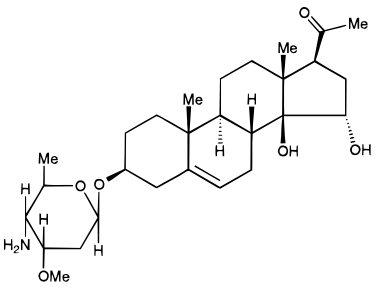
doublet at δ 5.42 (J = 5 Hz), which was similar to that of holamine (**6**). Comparison of the NMR spectra of **5** with the aminopregnanes **6** and **7** also indicated that the site of hydroxylation was in the five-membered ring of the aglycon moiety. The presence of an oxygenated quaternary carbon signal at δ 84.9 was characteristic of C-14 bearing a β-hydroxyl substituent, which was also a feature common in all the aminoglycosteroids **1**−**4**. The presence of another oxymethine at the equally downfield value of δ 81.9 would tend to suggest that this oxygenated carbon was adjacent to the hydroxylated C-14. This was supported by COSY and HMQC data, which confirmed the presence of a CH−CH_2−CH−OH fragment corresponding to C(17)−C(16)−C(15) as shown in structure **5**. The stereochemistry of the C-15 hydroxyl function was determined by NOE experiments in which irradiation of the H-15 oxymethine signal (δ 4.37, d, J = 6 Hz) resulted in enhancement of the C-18 methyl signal (δ 0.96) and *vice versa*. This is only possible if H-15 is β. The C-15 hydroxyl group therefore has α stereochemistry.

Compound **7**, $[\alpha]_D$ +23.3° ($CHCl_3$, *c* 0.26), showed a molecular ion at *m/z* 333, and HRMS gave the formula $C_{21}H_{35}NO_2$. The presence of the fragment ion at *m/z* 315, due to the loss of H_2O, indicated the presence of a hydroxyl function, while the base peak at *m/z* 56, due to the fragment $CH_2{=}CHCH{=}NH_2^+$, provided confirmation for the amino function at C-3.[32] The ^{1}H and ^{13}C NMR spectral data showed signals characteristic of a C_{21}-pregnane skeleton (Tables 1 and 2), with an amino group at C-3, a β-acetyl group at C-17 (δ H-17, 2.87, dd, J = 9, 4.5 Hz), and a β-OH substituent on the quaternary center at C-14 (δ C-14, 84.9). Determination of the α-orientation of the amino group was based on a consideration of the H-3 (δ 3.22, br s) and C-3 (δ 45.9) signals.[31] Compound **7** was therefore assigned as 3α-amino-14β-hydroxypregnan-20-one.

Compound **8**, $[\alpha]_D$ +47.2° ($CHCl_3$, *c* 0.23), was the most polar of the eight compounds isolated. The EIMS gave a molecular ion at *m/z* 331 and HRMS provided the formula $C_{21}H_{33}NO_2$. Other major fragments (*m/z* 313, 298, 275, 205, and 56) were typical of aminosteroids, and the fragment at *m/z* 313, due to the loss of H_2O, indicated the presence of a hydroxyl group. As in the case of holamine (**6**) but unlike that of **7**, the fragment at *m/z* 82, due to formation of a conjugated triene cation, was absent as a result of the presence of the C(5)−C(6) double bond.[33] The presence of the C(5)−C(6) double bond was also indicated by the 1643 cm^{-1} absorption in the IR spectrum, the vinylic H-6 signal (δ 5.38) in the ^{1}H NMR spectrum, and the olefinic carbon resonances (δ 138.6, 122.9) in the ^{13}C NMR spectrum. In addition, the presence of a secondary alcohol function was suggested by the oxymethine resonance at δ 73.9. The ^{1}H NMR spectral data of **8** were, in fact, similar to those of holamine (**6**), except for H-15 (δ 4.08), which has undergone a significant downfield shift when compared to **6**. Likewise, the ^{13}C NMR spectrum was generally similar to that of **6** except for the notable downfield shifts of the oxygenated C-15 and to a lesser extent, the adjacent C-14 and C-16 signals. Determination of the location of the hydroxyl function was also supported by COSY and HMQC data, which confirmed the CH−CH_2−CHOH−CH fragment corresponding to the partial structure of C(17)−C(16)−C(15)−C(14) as shown in structure **8**. The stereochemistry of the C(15)−OH group was deduced to be α from NOE experiments. Irradiation of the H-15 signal resulted in enhancement of the C-18 methyl signal and *vice versa*, indicating that H-15 is β. Compound **8** was therefore determined to be 15α-hydroxyholamine.



1 R^1 = Me, R^2 = αH
2 R^1 = H, R^2 = αH
3 R^1 = Me, R^2 = βH
4 R^1 = H, R^2 = βH



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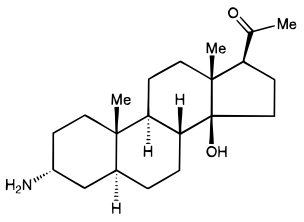

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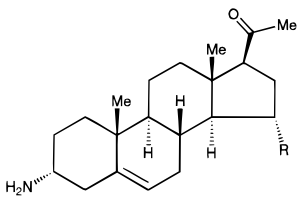

6 R = H
8 R = OH

Extracts of *H. curtisii* leaves showed positive activity against *L. donovani*, which was subsequently traced to the basic fraction. The results of the antileishmanial assays for compounds (**1**−**8**) are presented in Table 3 where it can be seen that all eight compounds were effective in the concentration ranges investigated, with holamine (**6**), holacurtine (**1**), *N*-demethylholacurtine (**2**), and 15α-hydroxyholamine (**8**) showing relatively higher activity than the other compounds. When these compounds were screened for cytotoxic activity using the HL-60 and P-388 cell lines (Table 3), it was observed that, whereas there was no significant activity against P-388 (including Adriamycin- and vincristine-resistant cell lines), these compounds showed marked inhibition against HL-60, particularly in the case of 17-*epi*-holacurtine (**3**). Although there appeared to be no correlation between the observed cytotoxic activity (HL-60) and antileishmanial activity in the case of the five aminoglycosteroids (**1**−**5**), such a correlation could be discerned for the three aminosteroids (**6**−**8**), suggesting in the case of these three compounds, that the observed leishmanicidal activity could be a consequence of the cytotoxic effects of these compounds. Among the aminoglycosteroids (**1**−**5**), it would appear that the C-17 α-acetyl epimers (**3** and **4**) displayed relatively greater potency against HL-60 when compared to the C-17 β-acetyl epimers (**1**, **2**, **5**). Particularly noteworthy is the marked increase in potency against HL-60 observed for 17-*epi*-holacurtine (**3**), especially, when compared to that of the closely related epimer **1** (IC_{50} 0.01 vs. 0.86 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. IR

Table 3. Cytotoxic and Leishmanicidal Activity of Compounds **1**−**8**

	IC_{50} (μg/mL)				
compound	HL-60	P-388	P-388/ADR[a]	P-388/VCR[b]	*L. donovani*
1	0.86	4.2	3.4	2.8	6.25 > IC_{50} > 1.56
2	2.6	4.0	11.5	3.5	6.25 > IC_{50} > 1.56
3	0.01	17	10.4	10.1	25.0 > IC_{50} > 6.25
4	0.21	10.5	13.5	10.2	25.0 > IC_{50} > 6.25
5	2.6	16	10.4	10	25.0 > IC_{50} > 6.25
6	0.2	0.91	0.85	0.83	1.56 > IC_{50} > 0.39
7	10.1	3.6	8.6	3.4	25.0 > IC_{50} > 6.25
8	1.2	4.1	3.6	1.4	6.25 > IC_{50} > 1.56

[a] Adriamycin-resistant P-388. [b] Vincristine-resistant P-388.

spectra were recorded on a Perkin−Elmer 1600 Series FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-GSX 270 spectrometer at 270 and 67.5 MHz, respectively, and on a JEOL JNM-LA 400 spectrometer at 400 MHz. MS were obtained on a VG ProSpec spectrometer.

Plant Material. Plant material was collected in Kedah, Malaysia (May 1995). Herbarium voucher specimens are deposited at the Herbarium of the Department of Chemistry, University of Malaya, Malaysia.

Extraction and Isolation. Extraction of the ground leaves (3.5 kg) was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere.[34,35] The alkaloids were isolated by initial column chromatography on Si gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were MeOH−$CHCl_3$, Et_2O−MeOH, and Et_2O−EtOH. The yields (g kg^{-1}) of the alkaloids (**1**−**8**) were as follows: **1** (0.079), **2** (0.017), **3** (0.019), **4** (0.0049), **5** (0.0014), **6** (0.0092), **7** (0.0074), and **8** (0.0067).

Holacurtine (1): amorphous; $[\alpha]_D$ 32°($CHCl_3$, *c* 0.98); IR (dry film) υ_{max} 3418 (NH/OH) and 1694 (C═O) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 491 $[M]^+$ (9), 416 (46), 317 (100), 299 (52), 177 (36); HREIMS *m*/*z* 491.3614 (calcd for $C_{29}H_{49}NO_5$, 491.3611).

***N*-Demethylholacurtine (2):** amorphous; $[\alpha]_D$ 11.2°($CHCl_3$, *c* 0.45); IR (dry film) υ_{max} 3387 (NH/OH) and 1694 (C═O) cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 477 $[M]^+$ (3), 373 (15), 317 (100), 299 (50), 205 (68); HREIMS *m*/*z* 477.3447 (calcd for $C_{28}H_{47}NO_5$, 477.3454).

17-*epi*-Holacurtine (3): amorphous; $[\alpha]_D$ −13.3°($CHCl_3$, *c* 0.68); IR (dry film) υ_{max} 3492 (NH/OH) and 1694 (C═O) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 491 $[M]^+$ (9), 416 (44), 317 (100), 299 (50), 177 (35); HREIMS *m*/*z* 491.3609 (calcd for $C_{29}H_{49}NO_5$, 491.3611).

17-*epi*-*N*-Demethylholacurtine (4): amorphous; $[\alpha]_D$ −15.6°($CHCl_3$, *c* 0.17); IR (dry film) υ_{max} 3380 (NH/OH) and 1693 (C═O) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 477 $[M]^+$ (3), 373 (19), 317 (100), 299 (56), 205 (73); HREIMS, MH^+ (self-protonated) *m*/*z* 478.3531 (calcd for $C_{28}H_{47}NO_5$ + H, 478.3532).

Holacurtinol (5): amorphous; $[\alpha]_D$ 30°($CHCl_3$, *c* 0.05); IR (dry film) υ_{max} 3392 (NH/OH), 1694 (C═O), and 1643 (C═C) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 491 $[M]^+$ (8), 330 (100), 312 (68), 294 (42); HREIMS *m*/*z* 491.3240 (calcd for $C_{28}H_{45}NO_6$, 491.3247).

Holamine (6): amorphous; $[\alpha]_D$ 12.4°($CHCl_3$, *c* 0.32); IR (dry film) υ_{max} 3387 (NH), 1701 (C═O), and 1659 (C═C) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 315 $[M]^+$ (16), 300 (12), 272(3), 259 (6), 56 (100).

3α-Amino-14β-hydroxypregnan-20-one (7): amorphous; $[\alpha]_D$ 23.3°($CHCl_3$, *c* 0.26); IR (dry film) υ_{max} 3404 (NH/OH) and 1692 (C═O) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 333 $[M]^+$ (9), 315 (42), 318 (5), 277 (10), 248 (24), 179 (30), 82 (36), 56 (100); HREIMS *m*/*z* 333.2665 (calcd for $C_{21}H_{35}NO_2$, 333.2668).

15α-Hydroxyholamine (8): amorphous; $[\alpha]_D$ 47.2°($CHCl_3$, *c* 0.23); IR (dry film) υ_{max} 3387 (NH/OH), 1702 (C═O), and 1643 (C═C) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 331 $[M]^+$ (22), 313 (28), 298 (38), 275 (16), 205 (60), 56 (100); HREIMS *m*/*z* 331.2508 (calcd for $C_{21}H_{33}NO_2$, 331.2511).

Conversion of Compound 1 to 3. Compound **1** (11 mg) was refluxed in ethanolic KOH (3 mL, 2M) for 3.5 h. After cooling, followed by addition of H_2O, the mixture was extracted with $CHCl_3$, and then chromatographed over SiO_2 (5% EtOH/Et_2O) to provide **3** (70%).

Cytotoxicity Assay.[36] Mouse lymphatic leukemia cells (P-388 and Adriamycin-resistant P-388 cells, P-388/ADR) and human leukemia HL-60 cells were maintained in minimum essential medium (MEM) supplemented with 10% fetal calf serum and kanamycin. Vincristine-resistant P-388 cells (P-388/VCR) were maintained in MEM supplemented with 10% fetal calf serum, kanamycin, and 0.5 μg/mL of vincristine. In a 96-well round-bottomed plate were placed 200 μL each of the cell suspension (20 000 cells/mL) followed by addition of 5 μL of each test solution. The initial concentration of the test solution was 25 μg/mL in a well, and eight different concentrations of each sample were examined. The cells were cultured for 3 days, and then the number of living cells were counted by the MTT staining method.[37] The experiments were repeated three times.

Leishmanicidal Assay.[38] *L. donovani* (promastigote) were maintained in 199 medium supplemented with 10% fetal calf serum and kanamycin. In a 96-well round-bottomed plate were placed 200 μL of each cell suspension (10 000 cells/mL) followed by addition of 5 μL of each test solution. The initial concentration of the test solution was 25 μg/mL in a well, and four different concentrations of each sample were examined. The activity was determined as described above.

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