Bioactive 5α-Pregnane-Type Steroidal Alkaloids from *Sarcococca hookeriana*

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The bioassay-guided phytochemical investigation of *Sarcococca hookeriana* with respect to cholinesterase inhibitory properties has yielded two new 5α-pregnane-type steroidal alkaloids, hookerianamides J (**1**) and K (**2**), along with eight known compounds (**3**−**10**). The structures of **1** and **2** were elucidated by spectroscopic methods. These compounds displayed good to moderate activities in vitro against the enzymes acetylcholinesterase (IC_{50} 8.1−48.5 μM) and butyrylcholinesterase (IC_{50} 0.4−4.0 μM). Compounds **1**−**10** were also tested in vitro for their leishmanicidal activity against *Leishmania major* and for their antibacterial activities against *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus faecalis*, and *Pseudomonas pallida*.

Sarcococca hookeriana (Baill.) Hook. (Buxaceae) is an evergreen shrub, distributed from Eastern to Western Nepal, Northern Assam, Southern Tibet, and Bhutan.[1] Rural communities in Nepal have used the root extracts of this plant against gout.[2] Previous studies on plants from the genus *Sarcococca* have reported the isolation and microbial transformation of several bioactive steroidal alkaloids.[3−11] A number of studies have reported biological properties of steroidal alkaloids isolated from *Sarcococca* species such as antibacterial,[12] antileishmanial,[11] antiplasmodial,[13] cholinesterase inhibition,[3−10] and cytotoxic[14] activities. In continuation of our ongoing study to search for bioactive constituents from this genus, we have investigated the plant *S. hookeriana* and report herein the isolation and structure elucidation of two new steroidal alkaloids, hookerianamides J (**1**) and K (**2**), as well as eight known compounds (**3**−**10**). An evaluation was made with respect to the cholinesterase inhibitory, leishmanicidal, and antibacterial activities of the isolated compounds.

Hookerianamide J (**1**) was obtained as a white, amorphous solid from an alkaline fraction of the CH_2Cl_2 extract of *S. hookeriana*. The specific rotation of **1**, $[\alpha]_D^{20}$ +78 (*c* 0.02, MeOH), indicated the presence of chirality in the molecule. The IR spectrum of compound **1** showed intense absorptions at 3327 (OH), 3223 (NH), 2922 (CH), 1666 (amidic C═O), and 1622 (C═C) cm^{-1}. The HREIMS of **1** showed the molecular ion $[M^+]$ at *m*/*z* 442.3559, supporting the formula $C_{28}H_{46}N_2O_2$ (calcd 442.3538) and indicating seven double-bond equivalents. The NMR spectroscopic data of compound **1** (Table 1) suggested a pregnane-type steroidal skeleton.[7,9,13] In the ^1H NMR spectrum, two signals, resonating as sharp singlets at δ 0.81 and 0.85, were assigned to the methyl groups C-18 and C-19, which are attached to a tertiary carbon. A doublet appearing at δ 1.07 ($J_{21,20}$ = 6.9 Hz) was assigned to the methyl group C-21 attached to a secondary carbon. Two other singlets at δ 1.83 and 2.13 corresponded to the C-5′ and C-4′ methyl protons of a *N*-senecoyl moiety linked at C-3. A singlet integrating for 6H and resonating at δ 2.19 was due to Me_2N_b protons. A quartet at δ 2.80 ($J_{20,21}$ = 6.8 Hz) was assigned to the C-20 methine proton, which supported the presence of a double bond between C-16/C-17.[7] A double doublet resonating at δ 3.61 ($J_{4eq,3ax}$ = 3.9 Hz, $J_{4eq,5ax}$ = 3.9 Hz) was assigned to the C-4eq proton suggesting, the axial (β) orientation of the C-4 OH group.[7] A multiplet at δ 4.27 was assigned to C-3ax methine proton, while a doublet at δ 5.62 ($J_{3ax,NH}$ = 7.4 Hz) was assigned to the amidic N_aH. A broad singlet at δ 5.50 and a singlet at δ 5.66 were assigned to C-16 and C-2′ olefinic protons, respectively. The broadband decoupled ^{13}C NMR spectrum of compound **1** showed resonances for all 28 carbons in the molecule, which were sorted by DEPT and HSQC techniques into seven methyl, seven methylene, nine methine, and five quaternary carbons. The signals at δ 169.0 (C-1′), 118.0 (C-2′), and 152.3 (C-3′) and the methyl signals at δ 27.4 (C-4′) and 19.7 (C-5′) were consistent with the presence of a senecoylamino moiety in the molecule.[7,9] Similarly, the signals at δ 156.1 (C-17) and 123.2 (C-

Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Hookerianamides J (**1**) and K (**2**)[a]

	hookerianamide J (**1**)		hookerianamide K (**2**)	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	34.5, CH_2	1.75, 1.81, m	32.9, CH_2	1.67, 1.55, m
2	31.4, CH_2	1.43, 1.52, m	31.8, CH_2	1.38, 1.75, m
3	50.3, CH	4.27, m	56.5, CH	2.82, ddd (12.2, 4.9, 4.2)
4	71.4, CH	3.61, dd (3.9, 3.9)	123.7, CH	5.26, d (4.9)
5	47.4, CH	1.21, m	157.2, $_qC$	
6	20.5, CH_2	1.31, 1.46, m	20.7, CH_2	1.22, 1.49, m
7	25.0, CH_2	1.59, 1.66, m	27.6, CH_2	1.19, 1.24, m
8	33.5, CH	1.65, m	35.4, CH	1.36, m
9	57.6, CH	1.35, m	53.9, CH	1.35, m
10	37.3, $_qC$		39.1, $_qC$	
11	22.0, CH_2	1.76, 1.68, m	24.0, CH_2	1.63, 1.07, m
12	32.6, CH_2	1.65, 1.72, m	39.7, CH_2	1.55, 1.08, m
13	46.5, $_qC$		40.3, $_qC$	
14	55.1, CH	1.26, m	158.7, $_qC$	
15	30.0, CH_2	1.28, 1.54, m	120.7, CH	5.48, br s
16	123.2, CH	5.50, br s	28.7, CH_2	1.48, 1.87, m
17	156.1, $_qC$		54.8, CH	1.72, m
18	16.0, CH_3	0.81, s	10.1, CH_3	0.60, s
19	12.8, CH_3	0.85, s	12.0, CH_3	0.78, s
20	59.1, CH	2.80, d (6.8)	61.8, CH	2.39, m
21	16.2, CH_3	1.07, d (6.9)	12.3, CH_3	0.83, d (6.4)
Me_2N_a			39.4, CH_3[b]	2.19, s
Me_2N_b	42.4, CH_3[b]	2.19, s	39.7, CH_3[b]	2.14, s
1′	169.0, $_qC$			
2′	118.0, CH	5.66, s		
3′	152.3, $_qC$			
4′	27.4, CH_3	2.13, s		
5′	19.7, CH_3	1.83, s		
N_a-H		5.62, d (7.4)		

[a] All chemical shift assignments were done on the basis of $^1H-^1H$ COSY, HSQC, HMBC, and DEPT NMR techniques. [b] Two methyl groups attached on the nitrogen atom.

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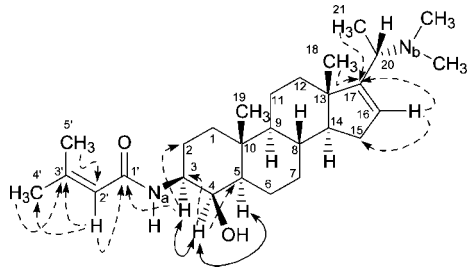


Figure 1. Selected HMBC (dashed arrows) and NOESY (solid arrows) interactions for **1**.

16) indicated the presence of a trisubstituted C=C bond, while the signal at δ 71.4 suggested the presence of a hydroxyl moiety in compound **1**.[7] The HMBC correlations of H-3 to C-1′, C-4 (δ 71.4), and C-2 (δ 31.4); H-4 to C-3 (δ 50.3); H-5 (δ 1.21) to C-4 (δ 71.4); H-16 to C-15 (δ 30.0) and C-17; H-20 (δ 2.80) and H-18 to C-17; and H-2′ to C-3′ and C-4′ supported the position of the assigned functionalities in compound **1** (Figure 1). In the NOESY experiment, the H-4 (δ 3.61) signal showed a connectivity with both H-3 (δ 4.27) and H-5 (δ 1.21), indicating the β-orientation of the C-4 hydroxyl group (Figure 1). The assigned relative configuration was supported by a NOESY experiment and by chemical shift/coupling constant comparison with reported data of hookerianamides A−G.[7,9] The absolute configuration of compound **1** was proposed on biogenetic grounds, as the pregnane-type steroidal alkaloids were biosynthesized from cholesterol via pregnenolone.[15] The proposed structure was further supported by the mass fragmentation pattern [*m/z:* 442 (M^+, 4), 427 (100), 409 (66), 98 (14), 83 (32), 72 (36), 55 (12)] observed in the EIMS. The above-mentioned spectroscopic observations and comparative literature analysis suggest the structure (20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-senecoylamino)-5α-pregn-16-en-4β-ol for **1**, which was given the trivial name hookerianamide J.

Hookerianamide K (**2**) was obtained as a white, amorphous solid with $[\alpha]_D^{25}$ +19 (*c* 0.3, MeOH). The IR absorptions of compound **2** at 1632 cm^{-1} suggested the presence of olefinic bonds. The HREIMS supported the formula $C_{25}H_{42}N_2$ from its molecular ion at *m/z* 370.3287 (calcd 370.3348), indicating six double-bond equivalents. The NMR spectroscopic data of compound **2** (Table 1) were consistent with a pregnane-type steroidal skeleton.[7,9] In the ^{1}H NMR spectrum, a singlet at δ 2.19 (6H) was assigned to the N_a-methyl protons attached to C-3, whereas another singlet at δ 2.14 (6H) was assigned to the N_b-methyl protons attached to C-20. A proton resonating at δ 2.82 (ddd, J = 12.2, 4.9, and 4.2 Hz) was assigned as the C-3 methine proton. Two signals, one doublet at δ 5.26 (1H, J = 4.9 Hz) and one broad singlet at δ 5.48, were assigned to the olefinic protons H-4 and H-15, respectively. The broadband decoupled ^{13}C NMR spectrum of compound **2** displayed 25 carbon signals, which were sorted by DEPT and HSQC techniques into seven methyl groups, seven methylene groups, seven methine groups, and four quaternary carbons. The HMBC correlations of H-19 (δ 0.78) to C-5 (δ 157.2); H-4 to C-3 (δ 56.5); H-18 (δ 0.60) and H-17 (δ 1.72) to C-14 (δ 158.7); and H-15 to C-16 (δ 28.7) supported the position of the assigned functionalities in compound **2**. The configuration of compound **2** was assigned by analogy to compound **1**. On the basis of above observations, compound **2** was proposed as (20*S*)-20-(*N*-dimethylamino)-3β-(*N*-dimethylamino)-pregn-4,14-diene and was given the trivial name hookerianamide K.

In addition to compounds **1** and **2**, eight known pregnane-type steroidal alkaloids, hookerianamides H (**3**) and I (**4**),[13] chonemorphine (**5**),[16] *N*-methypachysamine A (**6**),[17,18] epipachysamine-*E*-5-en-4-one (**7**),[19] vagenine A (**8**),[20] 2,3-dehydrosarsalignone (**9**),[8] and sarcovagine C (**10**),[21] were also isolated and characterized.

In order to identify novel potent cholinesterase inhibitors for rational drug design and discovery of mechanism-based inhibitors to be applied to the treatment of Alzheimer's disease and related dementias, compounds **1**, **2**, **5**−**7**, and **10** were tested in vitro for their inhibitory properties toward AChE and BChE (Table 2). Such inhibitory activity had previously been published for compounds **3**, **4**, **8**, and **9**.[4,9,13] The cholinesterase inhibitory activity was also determined for the crude MeOH/H_2O extract (1.0 mg/mL), which exhibited 81% and 87% inhibition of AChE and BChE, respectively. Similarly, alkaline and neutral dichloromethane fractions (1.0 mg/mL) showed 88% and 97% inhibition for AChE and 85% and 96% inhibition for BChE, respectively. All the steroidal alkaloids displayed moderate to potent cholinesterase inhibition toward AChE. Interestingly, all compounds exhibited more potent activity toward BChE than AChE. In particular, compounds **1**, **5**, **7**, and **10** were found to be 10 times more potent than the standard compound galanthamine. All the known cholinesterase-inhibiting drugs used

Table 2. In Vitro Cholinesterase Inhibitory Activities of Compounds **1**−**10**

	IC_{50} (μM) ± SEM[a]	
compound	AChE	BChE
1	48.5 ± 0.10	0.8 ± 0.02
2	24.2 ± 0.10	4.0 ± 0.2
5	28.0 ± 0.6	0.5 ± 0.001
6	22.1 ± 0.01	1.6 ± 0.01
7	9.9 ± 0.12	0.6 ± 0.005
10	8.1 ± 0.22	0.4 ± 0.03
galanthamine[b]	0.5 ± 0.01	8.5 ± 0.001

[a] Standard error of the mean of five assays. [b] Positive control used in the assays.

Table 3. In Vitro Leishmanicidal Activities of Compounds **1**−**10**

	IC_{50}	
compound	μg/mL	μM
1	5.0 ± 0.03	11.3
2	2.5 ± 0.05	6.8
3	8.6 ± 0.13	22.4
4	4.0 ± 0.06	8.8
7	n.t.[c]	n.t.[c]
8	1.6 ± 0.05	3.3
9	9.3 ± 0.02	21.3
10	0.7 ± 0.05	1.5
amphotericinB[a]	0.5 ± 0.02	0.5
pentamidine[b]	2.6 ± 0.09	7.5

[a] Positive control used in the assay. [b] Positive control used in the assay. [c] n.t. = not tested.

Table 4. Antimicrobial Zone of Inhibition (in mm) of Fractions and Compounds **1**−**10**

	ZOI			
extracts and compounds[a]	*B. subtilis*	*M. luteus*	*S. faecalis*	*P. pallida*
CH_2Cl_2 alkaline extract	28.0	25.0	17.5	23.5
CH_2Cl_2 neutral extract	29.0	32.0	15.5	21.5
CH_2Cl_2 acidic extract	21.5	22.0	10.0	13.0
1	15.0	n.a.[d]	n.a.[d]	12.0
2	13.0	9.5	10.0	10.5
3	11.5	11.0	n.a.[d]	n.a.[d]
4	17.5	18.0	8.00	18.5
5	16.5	11.5	10.0	14.0
6	19.0	11.0	8.0	14.5
7	9.0	n.a.[d]	n.a.[d]	n.a.[d]
8	17.0	n.a.[d]	10.0	13.5
9	20.0	12.5	10.0	16.5
10	20.0	13.5	12.0	17.5
ampicillin[b]	29.0	26.0	25.0	n.t.[e]
gentamicin[c]	n.t.[e]	n.t.[e]	n.t.[e]	30.0

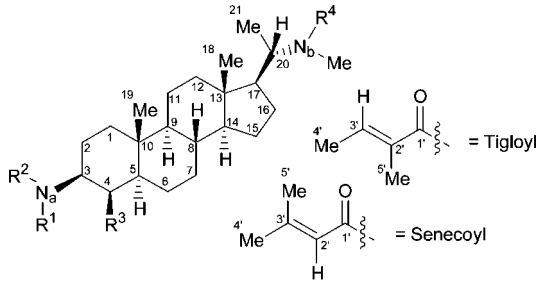
[a] Tested at 100.0, 1.0, and 0.25 mg/mL for extracts, compounds, and standards, respectively. [b] Positive control in this assay, used for Gram-positive bacteria. [c] Positive control in this assay, used for Gram-negative bacteria. [d] n.a. = not active. [e] n.t. = not tested.

Table 5. MIC Values for Compounds **1**−**10**

bacteria[a]	MIC values of compounds (μM)										standards[b]
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
B. subtilis	141.4	337.8	323.8	69.4	90.3	83.5	568.1	64.3	35.6	64.3	89.7
M. luteus	n.t.[c]	650.6	323.8	34.7	90.3	167.1	n.t.[c]	n.t.[c]	142.6	64.3	5.6
P. pallida	70.7	168.9	n.t.[c]	69.4	180.6	167.1	n.t.[c]	64.3	35.6	32.1	1.3

[a] *S. faecalis* was not tested for MIC due to its low activity as presented in Table 4. [b] Positive control used for *B. subtilis* and *M. luteus* is ampicillin, and that for *P. pallida* is gentamicin. [c] n.t. = not tested due to their inactivity in measurement of ZOI as presented in Table 4.

Scheme 1. Chemical Structures for Compounds **1**−**10**



	R^1	R^2	R^3	R^4	Unsaturation
1	H	Senecoyl	OH	Me	$\Delta^{16,17}$
2	Me	Me	H	Me	$\Delta^{4,5,\ \&\ 14,15}$
3	H	CHO	O	Me	$\Delta^{2,3}$
4	Me	COPh	H	H	-
5	H	H	H	Me	-
6	Me	Me	H	Me	-
7	H	Senecoyl	O	H	$\Delta^{5,6}$
8	H	Senecoyl	OAc	H	-
9	H	Tigloyl	O	H	$\Delta^{2,3,\ \&\ 5,6}$
10	H	Tigloyl	OAc	H	-

in the treatment of Alzheimer's disease suffer from several drawbacks such as high toxicity, short duration of biological action, low bioavailability, and narrow therapeutic windows.[22] Hence, further cholinesterase inhibitory studies on compounds of *Sarcococca* alkaloids may reveal other lead candidates for the discovery of clinically useful agents against various nervous system disorders.

Compounds **1**−**4** and **8**−**10** were also tested in vitro with respect to their leishmanicidal activity against *Leishmania major* (Table 3). Such activity had previously been published for compounds **6** and **7**.[11] All compounds displayed moderate to potent leishmanicidal activity, with compound **10** showing the best potency (IC_{50} 1.5 μM), comparable to the positive controls amphotericin B (IC_{50} 0.5 μM) and pentamidine (IC_{50} 7.5 μM).

The antibacterial property of extracts and compounds **1**−**10** with respect to the measurement of zone of inhibition (ZOI) was also studied (Table 4). The extracts and most of the compounds displayed moderate to good antibacterial properties against *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus faecalis*, and *Pseudomonas pallida*. The minimum inhibitory concentration (MIC) values of compounds **1**−**10** against *B. subtilis*, *M. luteus*, and *P. palida* were also determined (Table 5). The MIC values of compounds **4**, **5**, and **8**−**10** against *B. subtilis* were found in the range of 35.6−90.3 μM, comparable to that of ampicillin (MIC = 89.7 μM), whereas the MIC values against *M. luteus* and *P. pallida* were found to be only moderate.

Experimental Section

General Experimental Procedures. Optical rotations were measured in methanol solution on a JASCO digital polarimeter (model DIP-3600). The specific rotation is given in deg cm^2 g^{-1}. IR spectra were recorded in $CHCl_3$ on a JASCO A-302 IR spectrophotometer. The ^1H, ^{13}C, and 2D-NMR spectra were recorded on a Bruker AMX-500 spectrometer using $CDCl_3$ as solvent. Homonuclear $^1H-^1H$ connectivities were determined by using COSY 45° experiment. One-bond $^1H-^{13}C$ connectivities were determined by HSQC gradient pulse factor selection. Two- and three-bond $^1H-^{13}C$ connectivities were determined by HMBC experiment. Proton chemical shifts were reported in δ (ppm) with reference to the residual $CDCl_3$ signal at δ 7.26, and ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ 77.0. Coupling constants (*J*) were measured in Hz. The EIMS were recorded on a double-focusing mass spectrometer (Varian MAT 311A). HREIMS were recorded on a JEOL HX 110 mass spectrometer. Column chromatography was carried out on silica gel 60 (70−230 and 240−300 mesh sizes, E. Merck). Precoated silica gel TLC plates (E. Merck, F_{254}) were used to check the purity of compounds, and Dragendorff's spray reagent was used for the visualization of compounds by TLC.

Plant Material. Whole plants of *S. hookeriana* were collected from Phulchowki (8000 ft.), Lalitpur District, Nepal, in April 2002. The plant was identified by Dr. M. K. Adhikari, Senior Scientific Officer, at the National Herbarium and Plant Laboratories Section, Lalitpur, Nepal, where a voucher specimen (no. 101/2002) has been deposited.

Extraction and Isolation. Air-dried whole plants (31.0 kg) of *S. hookeriana* were extracted with 80% methanol/water (120 L). The concentrated methanolic aqueous extract (2.8 kg) was dissolved in cold distilled water (12 L) and defatted with petroleum ether (30 L) (304.3 g). The aqueous layer was extracted with dichloromethane (30 L) to obtain a neutral fraction (107.8 g). The aqueous fraction was then acidified with acetic acid to pH 3−4 and extracted with dichloromethane (30 L) to obtain an acidic fraction (85.0 g). The aqueous acidic fraction was then made alkaline by adding ammonia solution (pH 9−10) and extracted with dichloromethane (30 L) to obtain the alkaline fraction (24.7 g). On the basis of the cholinesterase inhibitory activity, the alkaline fraction was initially selected for bioassay-guided fractionation through repeated column chromatography and was adsorbed on 28.0 g of silica gel and eluted with different gradients of petroleum ether, acetone, and ethanol. Elution with 15−25% acetone in petroleum ether afforded subfraction E (2.1 g), which was adsorbed on 4.0 g of silica gel and chromatographed on a column to afford 15 subfractions, E_1-E_{15}, of 250 mL each. Subfractions E_4-E_8 were found to exhibit significant cholinesterase inhibition activity. Compounds **1** and **2** were obtained after elution of subfraction E_8 (305.0 mg) with petroleum ether/acetone/diethylamine mixture of 65:32:3 and 70:27:3, respectively. Compound **3** (11.5 mg) was obtained by column chromatography of subfraction E_5 (70.0 mg) upon elution with petroleum ether/acetone/diethylamine (80:18:2). Compounds **4** (18.3 mg), **9** (15.4 mg), and **10** (13.2 mg) were obtained after elution of subfraction E_7 (190.0 mg) with petroleum ether/acetone/diethylamine mixture of 72:25:3, 68:29:3, and 70:27:3, respectively. Similarly, compounds **5** (13.6 mg), **6** (19.3 mg), and **7** (14.7 mg) were isolated from subfraction E_4 (224.0 mg) with a mixture of petroleum ether/acetone/diethylamine of 88:9:3, 85:12:3, and 75:22:3, respectively. The neutral fraction was also fractionated by eluting with petroleum ether and acetone, and 20 fractions were obtained, N_1-N_{20}, of 200 mL each. On the basis of the TLC analysis, fractions N_3 and N_4 were combined and separated by column chromatography. Upon elution with 15−25% of acetone/petroleum ether, three subfractions, NS_1-NS_3, of 200 mL each were obtained. Compound **8** (10.3 mg) was isolated from subfraction NS_3 (80.0 mg) upon elution with a petroleum ether/acetone/diethylamine mixture of 70:27:3.

Hookerianamide J (1): white, amorphous solid; $[\alpha]_D^{20}$ +78 (*c* 0.02, MeOH); IR ($CHCl_3$) ν_{max} 3327, 3223, 2922, 1666, 1622 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), Table 1; EIMS *m/z* 442 $[M]^+$ (4), 427 (100), 409 (66), 98 (14), 83 (32), 72 (36), 55 (12); HREIMS *m/z* 442.3559 (calcd for $C_{28}H_{46}N_2O_2$, 442.3538).

Hookerianamide K (2): white, amorphous solid; $[\alpha]_D^{25}$ +19 (*c* 0.3, MeOH); IR ($CHCl_3$) ν_{max} 1632 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz)

and ^{13}C NMR ($CDCl_3$, 125 MHz), Table 1; EIMS *m/z* 370 $[M]^+$ (9), 355 (20), 303 (21), 110 (13), 84 (64), 72 (100), 58 (12); HREIMS *m/z* 370.3287 (calcd for $C_{25}H_{42}N_2$, 370.3348).

In Vitro Cholinesterase Inhibition Assay and Determination of IC_{50} Value. Cholinesterase inhibitory activity was determined as previously described.[7,9]

In Vitro Antileishmanial Assay. The antileishmanial activity was determined as previously described.[11]

Antibacterial Assay. In vitro antibacterial activity tests of the extracts and pure compounds were determined by the agar well diffusion method as described in previous reports.[23,24]

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References and Notes

(1) Shrestha, B. P. *Flora of Kathmandu Valley*, Bulletin of Department of Medicinal Plants, No. 11, His Majesty's Government of Nepal, Ministry of Forests and Soil Conservation, Department of Medicinal Plants, Kathmandu, Nepal, 1986; p 633.
(2) Rajbhandari, K. R. *Ethnobotany of Nepal*; Ethnobotanical Society of Nepal, Central Department of Botany, Tribhuvan University: Kathmandu, Nepal, 2002; p 22.
(3) Kalauni, S. K.; Choudhary, M. I.; Shaheen, F.; Manandhar, M. D.; Atta-ur-Rahman; Gewali, M. B.; Khalid, A. *J. Nat. Prod.* **2001**, *64*, 842–844.
(4) Kalauni, S. K.; Choudhary, M. I.; Khalid, A.; Manandhar, M. D.; Shaheen, F.; Atta-ur-Rahman; Gewali, M. B. *Chem. Pharm. Bull.* **2002**, *50*, 1423–1426.
(5) Atta-ur-Rahman; Zaheer-ul-Haq; Khalid, A.; Anjum, S.; Khan, M. R.; Choudhary, M. I. *Helv. Chim. Acta* **2002**, *85*, 678–688.
(6) Atta-ur-Rahman; Zaheer-ul-Haq; Feroz, F.; Khalid, A.; Nawaz, S. A.; Khan, M. R.; Choudhary, M. I. *Helv. Chim. Acta* **2004**, *87*, 439–448.
(7) Choudhary, M. I.; Devkota, K. P.; Nawaz, S. A.; Shaheen, F.; Atta-ur-Rahman, *Helv. Chim. Acta* **2004**, *87*, 1099–1108.
(8) Atta-ur-Rahman; Zaheer-ul-Haq; Feroz, F.; Khalid, A.; Nawaz, S. A.; Khan, M. R.; Choudhary, M. I. *Steroids* **2004**, *69*, 735–741.
(9) Choudhary, M. I.; Devkota, K. P.; Nawaz, S. A.; Ranjit, R.; Atta-ur-Rahman, *Steroids* **2005**, *70*, 295–303.
(10) Devkota, K. P.; Choudhary, M. I.; Nawaz, S. A.; Lannang, A. M.; Lenta, B. N.; Fokou, P. A.; Sewald, N. *Chem. Pharm. Bull.* **2007**, *55*, 682–684.
(11) Devkota, K. P.; Choudhary, M. I.; Ranjit, R.; Samreen; Sewald, N. *Nat. Prod. Res.* **2007**, *21*, 292–297.
(12) Atta-ur-Rahman; Anjum, S.; Farooq, A.; Khan, M. R.; Parveen, Z.; Choudhary, M. I. *J. Nat. Prod* **1998**, *61*, 202–206.
(13) Devkota, K. P.; Lenta, B. N.; Choudhary, M. I.; Naz, Q.; Boyom, F. F.; Rosenthal, P. J.; Sewald, N. *Chem. Pharm. Bull.* **2007**, *55*, 1397–1401.
(14) Zou, Z. M.; Li, L. J.; Yang, M.; Yu, S. S.; Cong, P. Z.; Yu, D. Q. *Phytochemistry* **1997**, *46*, 1091–1093.
(15) Devkota, K. P.; Lenta, B. N.; Fokou, P. A.; Sewald, N. *Nat. Prod. Rep.* **2008**, *25*, 612–630.
(16) Chatterjee, A.; Das, B. *Indian J. Chem.* **1967**, *5*, 146–150.
(17) Kikuchi, T.; Uyeo, S.; Ando, M.; Yamamoto, A. *Tetrahedron Lett.* **1964**, 1817–1823.
(18) Tomita, M.; Uyeo, S.; Kikuchi, T. *Tetrahedron Lett.* **1964**, 1641–1644.
(19) Jayasinghe, U. L. B.; Nadeem, M.; Atta-ur-Rahman; Choudhary, M. I.; Ratnayake, H. D.; Zareen, A. *Nat. Prod. Lett.* **1998**, *12*, 103–109.
(20) Atta-ur-Rahman; Anjum, S.; Farooq, A.; Khan, M. R.; Choudhary, M. I. *Phytochemisty* **1997**, *46*, 771–775.
(21) Yu, S. S.; Zou, Z. M.; Jie, Z.; Yu, D. Q.; Cong, P. Z. *Chin. Chem. Lett.* **1997**, *8*, 511–514.
(22) Guillozet, A. L.; Smiley, J. F.; Mash, D. C.; Mesulam, M. M. *Ann. Neurol.* **1997**, *42*, 909–918.
(23) Perez, C.; Paul, M.; Bazerque, P. *Acta Biol. Med. Exp.* **1990**, *15*, 113–115.
(24) Parekh, J.; Chanda, S. *Turk. J. Biol.* **2007**, *31*, 3–58.

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