Leuconicines A−G and (−)-Eburnamaline, Biologically Active Strychnan and Eburnan Alkaloids from *Leuconotis*

Chew-Yan Gan,[†] Yun-Yee Low,[†] Tadahiro Etoh,[‡] Masahiko Hayashi,[‡] Kanki Komiyama,[§] and Toh-Seok Kam*,[†]

Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia, Faculty of Pharmacy, Iwaki Meisei University, 5-5-1 Iino, Chuo-dai, Iwaki, Fukushima 970-8551, Japan, and Center for Basic Research, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo, 108-8642, Japan

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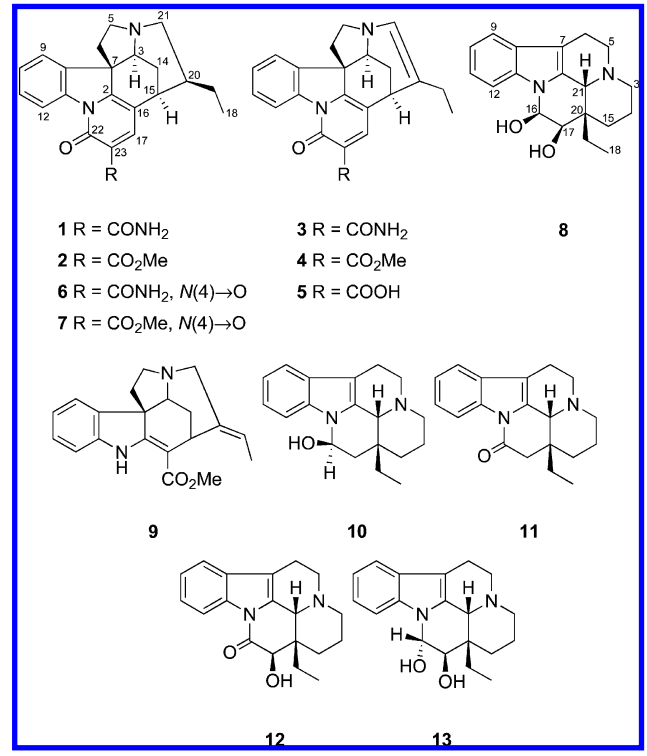
Seven new indole alkaloids of the *Strychnos* type, leuconicines A−G (**1**−**7**), and a new eburnan alkaloid, (−)-eburnamaline (**8**), were isolated from the stem-bark extract of two Malayan *Leuconotis* species. The structures of these alkaloids were established using NMR and MS analysis and in the case of **8** also by partial synthesis. Alkaloids **1**−**5** reversed multidrug resistance in vincristine-resistant KB cells.

Plants of the genus *Leuconotis* (Apocynaceae) are usually woody climbers and occur in Indonesia and Peninsular Malaysia.[1,2] The genus comprises a small group of 10 species, of which three (*L. griffithii* Hook, *L. maingayi* Dyer, and *L. eugenifolia* D. C.) are found in Peninsular Malaysia.[1−3] The latex from *L. eugenifolia* has been used for the treatment of yaws and worm infections.[4] Previous studies of Malayan *L. griffithii* and *L. eugenifolia* provided, in addition to the ring-opened alkaloids leuconolam and rhazinilam and their derivatives, various strychnan, kopsan, and eburnan derivatives,[5,6] while several yohimbines and the pentacyclic diazaspiro alkaloid leuconoxine were subsequently reported from *L. eugenifolia* occurring in Indonesia.[7] The alkaloidal composition bears a striking similarity to plants of the genus *Kopsia*.[8] In continuation of our studies of biologically active alkaloids from Malaysian Apocynaceae,[8−27] we undertook an investigation of the alkaloids of *Leuconotis* including *L. griffithii* and *L. maingayi* (the latter species has not been previously investigated) and reported the isolation of the new tetracyclic ring-opened oxindole alkaloid leucolusine[28] and the cytotoxic bisindole alkaloid leucophyllidine[29] from *L. griffithii*. We now report the isolation, structure, and biological activity of new strychnan (**1**−**7**) and eburnan (**8**) alkaloids from the stem-bark extract of *L. griffithii* and *L. maingayi*.

Results and Discussion

Leuconicines A (**1**) and B (**2**) were isolated from *L. maingayi*, while leuconicines A−G (**1**−**7**) and eburnamaline (**8**) were obtained from *L. griffithii*. Leuconicine A (**1**) was obtained as a light yellowish oil, with $[\alpha]^{25}_{D}$ −473 ($CHCl_3$, *c* 0.24). The UV spectrum showed absorption maxima at 211, 282, and 369 nm, characteristic of a methyleneindoline chromophore.[30] The IR spectrum (thin film) showed a broadened band at 3361 cm^{-1} due to an amide NH_2 function and another broad band at 1672 cm^{-1}, due probably to overlap of conjugated lactam carbonyl and the amide I (carbonyl) band, while the amide II (NH) band was observed at 1614 cm^{-1}. In $CHCl_3$ solution, the two primary amide NH stretching (symmetric and asymmetric) bands were both detected. The H-bonded NH stretching absorptions were seen at 3479 and 3299 cm^{-1}, while the free NH stretching vibrations were observed at 3684 and 3620 cm^{-1}. The lactam carbonyl and amide II signals were unchanged at 1674 and 1613 cm^{-1}. The EIMS of **1** showed a molecular ion at *m/z* 361, which was also the base peak, the odd mass indicating the presence of a third nitrogen atom. This was confirmed by HREIMS, which yielded the molecular formula $C_{22}H_{23}N_3O_2$ requiring 13 double-bond equivalents. Other notable fragment peaks were observed at *m/z* 332 (M − Et) and 288 (M − Et − $CONH_2$).



The ^{13}C NMR spectrum (Table 1) accounted for all of the 22 carbon resonances, comprising one methyl, five methylene, eight methine, and eight quaternary carbons. The presence of conjugated lactam and amide carbonyl functionalities was supported by the observed quaternary carbon signals at δ 161.1 and 165.8, respectively. The ^1H NMR spectrum (Table 2) showed the presence of an unsubstituted aromatic moiety (δ 7.40, dd, J = 8, 1.3 Hz, H-9; 7.30, td, J = 8, 1.3 Hz, H-10; 7.38, td, J = 8, 1.3 Hz, H-11; 8.50, dd, J = 8, 1.3 Hz, H-12), an olefinic singlet at δ 8.29, and an ethyl side chain (δ 1.06, t, J = 7.3 Hz, H-18; 1.28, sept., J = 7.3 Hz, H-19; 1.51, sept., J = 7.3 Hz, H-19). In addition, the signals due to the hydrogens of a primary amide group were observed as two distinct one-H doublets (J = 4 Hz) at δ 9.48 and 5.92. The two signals are mutually coupled, as shown by homonuclear decoupling, and both slowly exchanged on addition of D_2O (complete exchange required more than 24 h). The nonequivalence of these amide hydrogens is likely a consequence of a substantial barrier to rotation

* To whom correspondence should be addressed. Tel: 603-79674266. Fax: 603-79674193. E-mail: tskam@um.edu.my.
[†] University of Malaya.
[‡] Iwaki Meisei University.
[§] Kitasato University.

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Table 1. ^{13}C NMR Data (δ) of **1**−**8** (100 MHz, $CDCl_3$)[a]

C	1	2	3	4	5	6	7	8
2	161.6	162.0	161.2	159.3	159.7	161.3	158.5	131.5
3	62.2	62.1	59.9	59.9	60.0	74.7	75.0	44.8
5	54.4	54.3	53.5	53.5	53.6	67.4	68.1	50.9
6	44.8	44.7	46.0	46.0	46.3	39.8	39.9	16.7
7	55.5	55.5	56.5	56.7	57.1	51.5	51.7	105.6
8	139.9	139.3	139.8	139.4	139.8	137.6	137.3	128.7
9	120.2	119.9	120.5	120.3	120.8	120.5	120.0	118.0
10	127.0	126.6	127.0	126.7	128.0	127.6	127.3	120.2
11	128.1	128.2	128.3	128.4	128.8	129.5	129.6	121.3
12	117.5	117.7	117.8	118.1	118.4	117.8	118.0	112.3
13	140.6	140.8	140.5	140.7	139.9	139.9	140.2	137.2
14	31.3	31.2	30.8	30.8	30.9	27.1	27.2	20.0
15	36.3	35.9	33.6	33.6	33.9	34.4	34.5	21.9
16	115.7	113.9	119.3	118.7	116.1	114.7	113.1	77.0
17	145.4	145.9	142.9	143.7	143.9	144.9	145.2	71.7
18	11.5	11.4	12.8	12.8	12.8	11.0	11.1	6.9
19	26.4	26.3	27.4	27.5	27.5	24.9	25.0	22.9
20	38.7	38.5	122.8	122.6	122.7	34.4	34.4	40.8
21	51.4	51.3	129.8	129.9	130.2	64.9	65.2	55.8
22	161.1	158.8	158.2	158.6	163.1	156.9	157.8	
23	120.2	120.0	120.8	120.3	122.4	122.0	122.0	
CO_2Me		52.4		52.2			52.6	
CO_2Me		165.9		166.0			165.1	
$CONH_2$	165.8		166.1			165.3		
CO_2H					166.0			

[a] Assignments based on HMQC and HMBC.

Table 2. ^1H NMR Data (δ) for **1**−**8** (400 MHz, $CDCl_3$)[a]

H	1	2	3	4	5	6	7	8
3	4.11 m	4.18 m	4.21 m	4.19 m	4.25 m	4.50 m	4.45 m	2.35 m 2.53 br d (13)
5	2.90 m	2.95 m	3.18 dd (12, 6.4)	3.18 dd (12, 6.4)	3.22 dd (12, 6.5)	3.65 dd (12, 8)	3.65 dd (12, 8)	3.14 ddd (14, 12, 6)
	3.17 m	3.23 m	3.30 td (12, 4.4)	3.29 td (12, 4.5)	3.33 td (12, 4)	3.86 td (12, 9)	3.84 td (12, 9)	3.22 dd (14, 6)
6	2.02 m	2.01 m	1.86 dd (12, 4.4)	1.85 dd (12, 4.5)	1.90 dd (12, 4)	2.29 dd (15, 8)	2.28 dd (15, 8)	2.42 ddd (16, 6, 2)
	2.94 m	2.92 m	2.28 td (12, 6.4)	2.29 m	2.31 dd (12, 6.5)	2.58 m	2.51 m	2.88 dddd (16, 12, 6, 2)
9	7.40 dd (8, 1.3)	7.39 dd (8, 1)	7.49 dd (8, 1)	7.44 dd (7.5, 1)	7.52 dd (8, 1)	7.52 dd (8, 1)	7.45 dd (8, 1)	7.45 dd (7, 1)
10	7.30 td (8, 1.3)	7.28 td (8, 1)	7.34 td (8, 1)	7.30 td (7.5, 1)	7.40 td (8, 1)	7.37 td (8, 1)	7.34 td (8, 1)	7.13 td (7, 1)
11	7.38 td (8, 1.3)	7.36 td (8, 1)	7.42 td (8, 1)	7.41 td (7.5, 1)	7.47 td (8, 1)	7.46 td (8, 1)	7.44 td (8, 1)	7.17 td (7, 1)
12	8.50 dd (8, 1.3)	8.56 dd (8, 1)	8.57 dd (8, 1)	8.65 dd (7.5, 1)	8.55 dd (8, 1)	8.50 dd (8, 1)	8.55 dd (8, 1)	7.79 dd (7, 1)
14*R*	1.38 dt (13, 3)	1.43 dt (13, 3)	1.33 dt (13, 3)	1.35 m	1.36 dt (13, 3)	1.45 ddd (14, 4, 3)	1.46 m	1.29 m
14*S*	2.21 dt (13, 3)	2.24 dt (13, 3)	2.28 m	2.29 m	2.34 m	2.85 dt (14, 3)	2.84 ddd (14, 3, 2)	1.70 dt (13, 3.6)
15α	2.92 m	2.88 m	3.12 m	3.05 m	3.16 m	3.07 m	3.03 m	0.66 td (13, 3.6)
15*β*								1.37 br d (13)
16								5.54 d (3)
17	8.29 s	7.91 s	8.48 s	8.12 s	8.44 s	8.31 s	7.90 s	3.90 d (3)
18	1.06 t (7.3)	1.07 t (7.3)	1.07 t (7.3)	1.77 t (7.4)	1.08 t (7.3)	1.11 t (7.3)	1.11 t (7.3)	0.89 t (7.7)
19	1.28 sept. (7.3)	1.29 sept. (7.3)	2.14 q (7.3)	2.14 q (7.4)	2.15 q (7.3)	1.28 sept. (7.3)	1.29 sept. (7.3)	1.79 dq (14.5, 7.7)
	1.51 sept. (7.3)	1.47 sept. (7.3)	2.14 q (7.3)	2.14 q (7.4)	2.15 q (7.3)	1.51 sept. (7.3)	1.47 sept. (7.3)	2.30 dq (14.5, 7.7)
20	1.88 m	1.93 m				2.52 m	2.57 m	
21*β*	1.95 t (11)	2.01 t (11)	5.55 s	5.55 s	5.58 s	3.02 t (13)	3.03 t (12)	4.02 br s
21α	3.03 dd (11, 4)	3.09 dd (11, 4)				3.56 dd (13, 4.4)	3.55 dd (12, 5)	
OMe		3.95 s		3.95 s			3.96 s	
NH	5.92 d (4)		6.30 d (4.4)			6.32 d (4.4)		
	9.48 d (4)		9.51 d (4.4)			9.33 d (4.4)		
OH					14.31 br s			

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

of the amide C−N bond (due to partial double-bond character) as well as H-bonding, a phenomenon that has been encountered previously.[31,32]

The NMR data were suggestive of a strychnan derivative, such as akuammicine (**9**), except for the presence of an ethyl in place of an ethylidene side chain, and the incorporation of an additional ring. The COSY and HMQC data disclosed partial structures that are consistent with the presence of an akuammicine-type skeleton, viz., NCH_2CH_2 and NCH_2CH, corresponding to the C-5−C-6 and C-21−C-20 units, respectively. The COSY spectrum also showed the presence of an $NCHCH_2$ fragment. This partial structure should in fact be part of the N−C-3−C-14−C-15 fragment, but extensive overlap allowed only the N−C-3−C-14 fragment to be discerned. The presence of the C-15 methine as part of the N−C-3−C-14−C-15 fragment linked from C-15 to C-20 can, however, be deduced from the three-bond correlations observed from H-3 and H-21 to C-15 in the HMBC spectrum. Additional elements to be accounted for include a conjugated lactam carbonyl (δ_C 161.1), a trisubstituted alkene (δ_C 120.2, 145.4; olefinic H, δ_H 8.29), and a primary amide function. The lactam carbonyl must be attached to the indolic nitrogen N(1) from the observed deshielding of the aromatic H-12. The observed reciprocal NOEs between H-9 (δ 7.40) and H-3 (δ 4.11) allowed the assignment of the aromatic signal at δ 8.50 as H-12. Insertion of the trisubstituted double bond between this lactam carbonyl and C-16 results in the formation of the sixth ring, a 2-pyridone, and completes the assembly of the molecule. Attachment of the C-17 olefinic methine is to C-16, from the observed three-bond correlations from this hydrogen (H-17) to C-2, C-15, and C-22, while the substitution of the amide function at C-23 is supported by the three-bond correlation from H-17 to the amide carbonyl as seen in the HMBC spectrum (Figure 1).

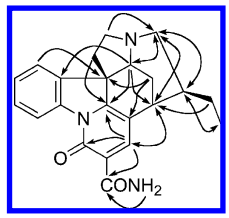

Figure 1. Selected HMBCs of **1**.

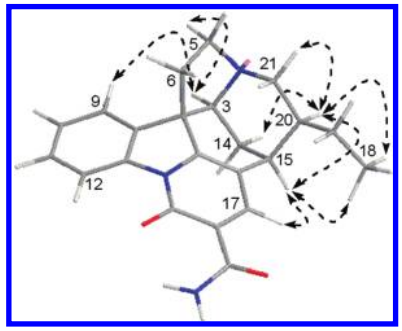

Figure 2. Selected NOEs of **1**.

The relative configurations at the various stereogenic centers were established from NOEs (Figure 2) 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, which is also in accord with the observed NOE between H-15 and H-17. The preferred chair conformation adopted by the piperidine ring D can be deduced from the absence of NOEs between H-14*S* and H-21α,[33] as well as from analysis of the J_{20-21} vicinal coupling constants. Thus, the observed $J_{20-21\beta}$ and $J_{20-21\alpha}$ values of 11 and 4 Hz, respectively, are consistent with the dihedral angles resulting from a β- or equatorially oriented C-20 ethyl group (H-21β and H-20α *trans*-diaxial with the piperidine ring D in a chair conformation).[34] In addition, the NOEs observed for H-15/H-20, H-18 and H-20/H-14*S*, H21α are consistent only with β-ethyl substitution or an equatorially oriented ethyl at C-20. The structure and relative configuration of leuconicine A are therefore as shown in structure **1**.

Leuconicine B (**2**) was obtained as a light yellowish oil with $[\alpha]^{25}_D$ +527 ($CHCl_3$, *c* 0.48). The UV spectrum (203, 279, 374 nm) was similar to that of **1**, while the IR spectrum showed bands due to conjugated ester (1737 cm^{-1}) and lactam (1703 cm^{-1}) carbonyl functionalities. The mass spectrum of **2** showed a molecular ion at *m/z* 376 (base peak), which analyzed for $C_{23}H_{24}N_2O_3$, requiring 13 degrees of unsaturation. The ^{13}C NMR spectrum of **2** showed a total of 23 carbon resonances including two methyl, five methylene, eight methine, and eight quaternary carbons. The ^1H and ^{13}C NMR data of **2** (Tables 2 and 1) were generally similar to those of **1**, indicating the presence of an unsubstituted aromatic moiety, an olefinic singlet, a conjugated lactam function, and an ethyl side chain. Notable differences include the absence of the signals due to the amide hydrogens and the presence instead of signals due to a methyl ester (δ_C 165.9, 52.4; δ_H 3.95), suggesting the replacement of the amide function in **1** by a methyl ester moiety at C-23 in **2**. The HMBC data also indicated incorporation of a 2-pyridone ring branched from the indolic nitrogen and in addition confirmed the substitution at C-23 by a methyl ester group. Thus, the resonance at δ 158.8 was assigned to the C-22 lactam carbonyl carbon from the observed three-bond correlation from the olefinic H-17 to C-22, while the other carbonyl resonance was assigned to the ester carbonyl attached to C-23, from the observed three-bond correlations from H-17 and the ester methyl hydrogens to the carbonyl resonance at δ 165.9. The relative configurations at the various stereogenic centers were established from the observed NOEs (which were similar to those seen in **1**, Figure 2) as well as from analysis of the coupling constants (Table 2), which showed that the configurations at the stereogenic centers in **2** are similar to those in **1**. An alkaloid (alkaloid 376) with similar NMR (low-field) data was previously isolated from *L. griffithii* and *L. eugenifolia*, but with incorrect assignments of some of the ^{13}C NMR signals and without establishment of configuration at the various stereogenic centers.[6]

Compound **3**, leuconicine C, was isolated as a light yellowish oil, $[\alpha]^{25}_D$ −374 ($CHCl_3$, *c* 0.22). The UV and IR spectra were similar to those of **1**, suggesting an akuammicine-type compound with similar functionalities, such as the presence of a primary amide group. The EIMS of **3** showed a molecular ion at *m/z* 359, and HRMS measurements established the molecular formula as $C_{22}H_{21}N_3O_2$ with 14 double-bond equivalents. The ^1H and ^{13}C NMR data of **3** (Tables 2 and 1, respectively) were generally similar to those of **1**. However, the ^{13}C NMR spectrum of **3** indicated the presence of an additional double bond from the resonances observed at δ 122.8 and 129.8, corresponding to olefinic quaternary and methine carbons, respectively. Comparison of the ^{13}C NMR spectrum with that of **1** showed that while the chemical shifts of the other carbons were essentially unchanged, those of C-20 and C-21 have undergone substantial shifts to the lower field sp^2 region, indicating that these carbons correspond to the site of the unsaturation. This was also supported by the observed two-bond correlation from H-21 to C-20 and the three-bond correlations from H-3 and H-15 to C-21 in the HMBC spectrum. The signal due to H-20 seen in the spectrum of **1** (δ 1.88) was absent in that of **3**. Instead, an olefinic H signal corresponding to H-21 was observed as a singlet at δ 5.55 in the ^1H NMR spectrum of **3**, in place of the two H-21 signals previously seen for **1** at δ 1.95 and 3.03. Corresponding changes have also occurred in the signals due to H-19, which in **3** are seen as a quartet (J = 7.3 Hz) at δ 2.14. These features are all consistent with the presence of a C-20−C-21 double bond. Catalytic hydrogenation (H_2, Pd/C) of **3** yielded **1** as the sole product, furnishing additional proof for the structure of leuconicine C (**3**).

Leuconicine D (**4**) was obtained as a light yellowish oil, with $[\alpha]^{25}_D$ −501 ($CHCl_3$, *c* 0.42). The UV and IR spectra of **4** were similar to those of **2**, indicating the presence of similar functionalities. The EIMS of **4** showed a molecular ion peak at *m/z* 374, and HREIMS measurements gave the molecular formula $C_{23}H_{22}N_2O_3$, requiring 14 double-bond equivalents. As in the case of **3** and **1**, comparison of the ^1H and ^{13}C NMR spectroscopic data of **4** and **2** (Tables 2 and 1) indicated that **4** is the 20,21-dehydro congener of **2**. The C-20 and C-21 olefinic signals of **4** were seen at δ 122.6 and 129.9, respectively, and the vinylic H-21 signal was seen as a singlet at δ 5.55. The assignment of the vinylic singlet to H-21 was consistent with the observed two-bond correlation from H-21 to C-20 and the three-bond correlation from H-3 to C-21 in the HMBC spectrum. As in the case of **3**, catalytic hydrogenation of **4** proceeded smoothly to furnish **2** as the sole product. These facile hydrogenations (**3** to **1** and **4** to **2**) provided additional support for the configurational assignments of **1** and **2**, as hydrogenation was seen to proceed from the less hindered concave or α-face.

Leuconicine E (**5**) was isolated as a light yellowish oil, with $[\alpha]^{25}_D$ −193 ($CHCl_3$, *c* 0.04). The EIMS showed, in addition to the M^+ ion peak at *m/z* 360, a fragment peak due to M − CO_2 at *m/z* 316. HREIMS measurements established the molecular formula as $C_{22}H_{20}N_2O_3$. The UV spectrum of **5** was similar to those of **1**−**4**, while the IR spectrum showed broadened bands at 3457 and 1728 cm^{-1}, due to OH and acid/lactam carbonyl functionalities, respectively. The ^1H and ^{13}C NMR spectra of **5** were also generally similar to those of **3** and **4**, indicating the presence of a 1,2-disubstituted aromatic moiety (δ 7.52, dd, J = 8, 1 Hz, H-9; 7.40, td, J = 8, 1 Hz, H-10; 7.47, td, J = 8, 1 Hz, H-11; 8.55, dd, J = 8, 1 Hz, H-12), two isolated olefinic hydrogens (singlets at δ 5.58 and 8.44),

two trisubstituted double bonds (δ 122.4, 143.9; 122.7, 130.2), and an ethyl side chain (δ 1.08, t, $J = 7.3$ Hz, H-18; 2.15, q, $J = 7.3$ Hz, H-19). Two downfield quaternary carbon signals were observed at δ 159.7 and 166.0, of which the former was assigned to the lactam carbonyl C-22 from the observed three-bond correlations from H-17 to this carbon.

A notable difference in the ^{1}H NMR data of **5** compared with those of compounds **1**−**4** was the absence of signals due to either the methyl ester CH_3 singlet or the amide NH_2 doublets, which were observed in the previous four compounds. In their place however, a low-field one-hydrogen broad singlet (exchanged with D_2O) was observed at δ 14.31, suggesting the presence of an acid function. Since the carbonyl resonance observed at δ 166.0 was not associated with ester or amide groups, it must be associated with the carboxylic acid group. The observed three-bond correlation from H-17 to the carboxyl carbon confirmed the substitution of C-23 by the carboxylic acid group.

Leuconicine F (**6**) was obtained as a light yellowish oil, with $[\alpha]^{25}_D$ −353 ($CHCl_3$, *c* 0.19). The EIMS showed the highest mass fragment at *m*/*z* 361, which corresponds to the fragment ion from loss of oxygen, a behavior characteristic of alkaloid *N*-oxides. The molecular ion could be detected by ESIMS, which showed an $[M + H]^+$ peak at *m*/*z* 378. HRESIMS measurements gave the formula $C_{22}H_{24}N_3O_3$, 16 mass units higher than that of **1**. The UV and IR spectra were similar to those of **1**, while the NMR data of **6** showed a close resemblance to those of **1**, except for the downfield shifts of the carbon resonances for C-3, C-5, and C-21. Similarly the H-3, H-5, and H-21 signals have undergone downfield shifts compared to those in **1**. Compound **6** was therefore readily identified as the *N*(4)-oxide of **1**.

Leuconicine G (**7**) was obtained as a light yellowish oil, with $[\alpha]^{25}_D$ −265 ($CHCl_3$, *c* 0.06) and UV and IR spectra that were similar to those of **2**. As in the case of **6**, compound **7** was readily identified as the *N*(4)-oxide of **2** on the basis of the ESIMS data ($[M + H]^+$ *m*/*z* 393, $C_{23}H_{25}N_2O_4$) and the characteristic downfield shifts of the C-3, C-5, and C-21 resonances and the corresponding H-3, H-5, and H-21 resonances in the ^{13}C and ^{1}H NMR spectra, respectively, when compared to those of **2**.

Eburnamaline (**8**) was obtained as a light yellowish oil, with $[\alpha]^{25}_D$ −49 ($CHCl_3$, *c* 0.21). The UV spectrum (230 and 280 nm) was typical of an indole chromophore, while the IR spectrum indicated the presence of hydroxy groups at 3370 cm^{-1}. The EIMS of **8** showed a molecular ion at *m*/*z* 312, which analyzed for $C_{19}H_{24}N_2O_2$ (requiring nine double-bond equivalents, 16 mass units higher than (−)-eburnamine (**10**)), with a prominent fragment peak due to loss of H_2O at *m*/*z* 294. The NMR data (Tables 1 and 2) showed a close resemblance to those of (−)-eburnamine (**10**),[6,35,36] except for some notable differences associated with changes involving ring E. First, compared with **10**, a doublet was observed at δ 3.90 (δ_C 71.7), which indicated the presence of an oxymethine. This doublet was coupled to the other oxymethine hydrogen (H-16), which required it to be vicinal to C-16. Eburnamaline (**8**) is therefore the 17-hydroxy congener of (−)-eburnamine (**10**). This conclusion is consistent with the absence of the H-17 signals seen in **10** and the presence of a CHCH fragment in **8**, in place of the $CHCH_2$ fragment seen in the COSY spectrum of **10**. Since this eburnan alkaloid co-occurs with (−)-eburnamine, (+)-eburnamonine, (+)-isoeburnamine, and (+)-eburnamenine in the stem-bark extract (see Experimental Section), and all these alkaloids belong to the 20β, 21β (20*R*, 21*R*) enantiomeric group, it is reasonable to suppose that **8** also belongs to the same series with 20*R*, 21*R* configuration.[11,15,37]

The configuration at C-16 in the 16-hydroxy-substituted eburnan alkaloids can be deduced from the presence or absence of paramagnetic deshielding exerted by the oxygen of the C-16 hydroxy substituent.[24,38−40] Thus, in (−)-eburnamine (β-OH at C-16), the H-15 resonances are found upfield at ca. δ 0.69 and 1.16 (absence of paramagnetic deshielding by OH), whereas in (+)-isoeburnamine, the α-oriented OH at C-16 causes a downfield shift of the H-15 resonances to δ 1.55 and 1.64, as a result of paramagnetic deshielding by the hydroxy oxygen.[36,37] The observed upfield resonances for H-15 in eburnamaline (**8**) at δ 0.66 and 1.37, coupled with the observed H-16/H-15α NOE, provided strong support for the presence of a β-oriented OH at C-16. The relative configuration at the hydroxy-substituted C-17 was deduced to be *R* (β-OH) on the basis of the following grounds. First, the reciprocal NOEs observed between H-16/H-17, H-17/H-15β, and H-17/H-18 are consistent only with a β-oriented OH group at C-17 (H-17α). Second, the observed J_{16-17} of 3 Hz is in agreement with an equatorially disposed H-17 (an axial or β-oriented H-17 would result in H-17 and H-16 being *trans*-diaxial). Third, the resonances for H-21 and H-19 were shifted downfield (δ 4.02; 1.79, 2.30) when compared to those of (−)-eburnamine (**10**) (δ 3.48; 1.27, 1.89), as a result of paramagnetic deshielding exerted by the proximate oxygen of the β-oriented OH group at C-17.

Finally, proof of the C-16 and C-17 configurations in **8** was achieved by partial synthesis from (+)-eburnamonine (**11**). Treatment of **11** by LDA in THF in the presence of molecular oxygen at 0 °C gave (+)-17β-hydroxyeburnamonine (**12**) as the major product in modest yield (26%).[41] The yield was significantly improved by the use of (+)-camphorsulfonyl oxaziridine in the enolate-mediated stereoselective oxidation.[42] Thus, treatment of **11** by LDA in THF, in the presence of (+)-camphorsulfonyl oxaziridine at 0 °C, provided **12** as the major product with an improved yield of 83%. The assignment of the configuration at C-17 in **12** was based on the observed downfield shift of the H-21 and H-19 signals (compared to (+)-eburnamonine **11**)[36,43] as a result of paramagnetic deshielding by the β-oriented OH group at C-17, the observed H-17/H-15β, H-18 NOEs, and its subsequent conversion to **8**. Thus, $LiAlH_4$ reduction of **12** gave the two epimeric reduction products **8** (54%) and **13** (39%).[36] The major product showed $[\alpha]_D$, TLC R_f, ESIMS, and ^{1}H and ^{13}C NMR data identical with those of **8**. The minor product was assigned as the 16α-OH epimer **13** on the basis of the observed downfield shift of H-15 compared to those of **8** due to paramagnetic deshielding, as well as the virtual absence of H-16−H-17 vicinal coupling ($J_{16\beta-17\alpha} \approx 0$), in agreement with the required H-16−H-17 dihedral angle of ca. 90^o in **13**.

Table 3. Cytotoxic Effects of Compounds **1**−**8**

compound	IC_{50}, μg/mL (μM) KB/S[a]	KB/VJ300[a]	KB/VJ300(+)[b]
leuconicine A (**1**)	>25	>25	2.57 (7.12)
leuconicine B (**2**)	>25	>25	1.98 (5.27)
leuconicine C (**3**)	>25	>25	3.86 (10.75)
leuconicine D (**4**)	>25	>25	4.62 (12.35)
leuconicine E (**5**)	>25	>25	13.52 (37.56)
leuconicine F (**6**)	>25	>25	>25
leuconicine G (**7**)	>25	>25	>25
(−)-eburnamaline (**8**)	>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 μg/mL (0.121 μM), which did not affect the growth of the KB/VJ300 cells.

Leuconicines A−G (**1**−**7**) and eburnamaline (**8**) showed no significant cytotoxicity against drug-sensitive and vincristine-resistant KB cells (IC_{50} >25 μg/mL in all cases), but leuconicines A−E (**1**−**5**) were found to reverse multidrug resistance in vincristine-resistant KB (VJ300) cells (Table 3). In the strychnan series, neither the presence or absence of an additional amide nitrogen in the side chain nor the presence or absence of unsaturation across C-20 and C-21 appears to affect the biological activity. The presence of an *N*-oxide functionality, however, has the effect of abolishing the biological activity altogether.

Experimental Section

General Experimental Procedures. Melting points were determined on a hot stage Leitz-Wetzlar melting point apparatus and are 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. ^{1}H 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. EIMS and HREIMS were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer. All air- and moisture-sensitive reactions were carried out under argon in oven-dried glassware. THF was freshly distilled from Na/benzophenone, and CH_2Cl_2 from CaH_2, under N_2.

Plant Material. Plant material was collected in Selangor and Pahang, Malaysia, and identification was confirmed by Dr. Richard C. K. Chung, Forest Research Institute, Malaysia. Herbarium voucher specimens (GK381, K672) are deposited at the Herbarium, University of Malaya.

Extraction and Isolation. Extraction of the ground bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid, as has been described in detail elsewhere.[44] 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−hexanes (1:4), Et_2O−hexanes (1:3), Et_2O−hexanes (1:2), Et_2O−hexanes (3:1), Et_2O, EtOAc−hexanes (1:4), EtOAc−hexanes (1:3), EtOAc−hexanes (1:2), EtOAc−hexanes (1:1), EtOAc, $CHCl_3$, $CHCl_3$−MeOH (100:7), and $CHCl_3$−MeOH (10:1). The yields (g kg^{-1}) of the alkaloids from *L. maingayi* were **1** (0.0030), **2** (0.0049), **10** (0.0353), (+)-isoeburnamine (0.0085), and (+)-eburnamenine (0.0005). The yields (g kg^{-1}) of the alkaloids from *L. griffithii* were **1** (0.0297), **2** (0.0379), **3** (0.0034), **4** (0.0033), **5** (0.0034), **6** (0.0016), **7** (0.0007), **8** (0.0003), **10** (0.0107), **11** (0.0015), (+)-isoeburnamine (0.0100), and (+)-eburnamenine (0.0023).

Leuconicine A (1): light yellowish oil and subsequently light yellowish crystals from MeOH/CH_2Cl_2; mp >220 °C (dec); $[\alpha]^{25}_D$ −473 (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (4.28), 282 (3.55), 369 (3.82) nm; IR (dry film) ν_{max} 3361, 1672, 1614 cm^{-1}; IR ($CHCl_3$ solution) ν_{max} 3684, 3620, 3479, 3299, 1674, 1613 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 361 [M]$^+$ (100), 344 (8), 332 (13), 316 (7), 288 (18), 277 (33), 260 (44), 246 (63), 218 (17), 204 (40), 190 (28), 166 (7), 135 (4), 123 (68), 94 (22); HREIMS *m/z* 361.1791 (calcd for $C_{22}H_{23}N_3O_2$, 361.1790).

Leuconicine B (2): light yellowish oil; $[\alpha]^{25}_D$ +527 (*c* 0.48, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 203 (4.22), 279 (3.09), 374 (3.76) nm; IR (dry film) ν_{max} 1737, 1703 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 376 [M]$^+$ (100), 361 (7), 347 (19), 317 (6), 305 (9), 292 (40), 274 (15), 260 (59), 246 (79), 218 (24), 204 (51), 190 (35), 167 (8), 135 (5), 123 (91), 110 (21); HREIMS *m/z* 376.1784 (calcd for $C_{23}H_{24}N_2O_3$, 376.1787).

Leuconicine C (3): light yellowish oil; $[\alpha]^{25}_D$ −374 (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 205 (4.36), 286 (3.34), 371 (3.84) nm; IR (dry film) ν_{max} 3357, 1674, 1616 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 359 [M]$^+$ (100), 341 (15), 330 (5), 313 (11), 289 (12), 275 (16), 257 (7), 246 (10), 204 (8), 170 (14), 156 (6), 121 (7); HREIMS *m/z* 359.1629 (calcd for $C_{22}H_{21}N_3O_2$, 359.1634).

Leuconicine D (4): light yellowish oil; $[\alpha]^{25}_D$ −501 (*c* 0.42, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (4.08), 286 (2.96), 376 (3.72) nm; IR (dry film) ν_{max} 1737, 1698 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 374 [M]$^+$ (100), 359 (9), 331 (5), 290 (17), 272 (7), 247 (14), 231 (11), 217 (7), 190 (7), 171 (3), 121 (5), 108 (2); HREIMS *m/z* 374.1632 (calcd for $C_{23}H_{22}N_2O_3$, 374.1630).

Leuconicine E (5): light yellowish oil; $[\alpha]^{25}_D$ −193 (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (5.87), 281 (2.81), 365 (3.29) nm; IR (dry film) ν_{max} 3457, 1728 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 360 [M]$^+$ (100), 345 (4), 316 (29), 287 (25), 259 (12), 232 (29), 217 (13), 204 (22), 191 (13), 163 (4), 121 (10), 108 (7); HREIMS *m/z* 360.1473 (calcd for $C_{22}H_{20}N_2O_3$, 360.1474).

Leuconicine F (6): light yellowish oil; $[\alpha]^{25}_D$ −353 (*c* 0.19, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (4.44), 283 (3.39), 367 (3.99) nm; IR (dry film) ν_{max} 3379, 1673, 1618 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 361 [M − O]$^+$ (100), 344 (7), 332 (12), 316 (7), 289 (15), 277 (30), 260 (33), 246 (50), 232 (11), 204 (21), 190 (15), 167 (4), 123 (34), 110 (10), 94 (8), 69 (7), 41 (5); ESIMS *m/z* 378 [MH]$^+$; HRESIMS *m/z* 378.1825 (calcd for $C_{22}H_{24}N_3O_3$, 378.1812).

Leuconicine G (7): light yellowish oil; $[\alpha]^{25}_D$ −265 (*c* 0.06, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (3.88), 287 (2.79), 367 (3.37) nm; IR (dry film) ν_{max} 1733, 1701 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 376 [M − O]$^+$ (100), 361 (6), 347 (17), 322 (10), 292 (36), 260 (42), 246 (58), 232 (18), 204 (27), 190 (19), 167 (7), 151 (4), 123 (53), 94 (13), 69 (9), 41 (6); ESIMS *m/z* 393 [MH]$^+$; HRESIMS *m/z* 393.1815 (calcd for $C_{23}H_{25}N_2O_4$, 393.1809).

(−)-Eburnamaline (8): light yellowish oil; $[\alpha]^{25}_D$ −49 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 230 (3.76), 280 (3.16) nm; IR (dry film) ν_{max} 3370 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Tables 2 and 1, respectively; EIMS *m/z* 312 [M]$^+$ (100), 294 (23), 283 (20), 265 (76), 242 (26), 224 (38), 208 (18), 196 (12), 180 (8), 144 (5); HREIMS *m/z* 312.1827 (calcd for $C_{19}H_{24}N_2O_2$, 312.1838); ESIMS *m/z* 313 [MH]$^+$; HRESIMS *m/z* 313.1926 (calcd for $C_{19}H_{25}N_2O_2$, 313.1911).

Catalytic Hydrogenation of Leuconicine C (3). Leuconicine C (**3**) (10.2 mg, 0.028 mmol) was dissolved in CH_2Cl_2 (15 mL) and then stirred over 10% Pd/C (5.8 mg) under a hydrogen atmosphere at room temperature for 2 h. The catalyst was removed by filtration over silica gel. Evaporation of the solvent in vacuo, followed by chromatography of the resulting residue (silica gel, MeOH−$CHCl_3$) provided **1** (7.8 mg, 76%).

Catalytic Hydrogenation of Leuconicine D (4). Catalytic hydrogenation of leuconicine D (**4**) (10.5 mg, 0.028 mmol) in the same manner provided leuconicine B (**2**) (7.5 mg, 71%).

Oxidation of (+)-Eburnamonine (11). Method A. A solution of (+)-eburnamonine (**11**) (100 mg, 0.34 mmol) in anhydrous THF (5 mL) was added to a solution of LDA (0.42 mL, 2 M in THF) in dry THF (10 mL) at 0 °C, and the resulting mixture was stirred for 30 min. Dry O_2 was then bubbled into the solution for 10 min. Na_2SO_3 solution (1 M, 5 mL) was added and the mixture extracted with EtOAc (3 × 15 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting residue was purified by centrifugal preparative TLC (silica gel, 2% MeOH−$CHCl_3$) to afford **12** (27.5 mg, 26%).

Method B. A solution of (+)-eburnamonine (**11**) (46 mg, 0.16 mmol) in anhydrous THF (2 mL) was added to a solution of LDA (0.2 mL, 2 M in THF) in dry THF (2 mL) at 0 °C, and the resulting mixture was stirred for 30 min. A solution of (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine (90 mg, 0.4 mmol) in THF (1 mL) was added and the mixture stirred for 20 min. The reaction was quenched by addition of a saturated solution of NH_4Cl (2 mL). The mixture was poured into brine (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extract was dried (Na_2SO_4), filtered, and concentrated in vacuo, and the resulting residue was purified by centrifugal preparative TLC (silica gel, 2% MeOH−$CHCl_3$) to afford **12** (40 mg, 83%).

(+)-17β-Hydroxyeburnamonine (12): light yellowish oil; $[\alpha]^{25}_D$ +126 (*c* 0.62, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.15), 282 (3.56) nm; IR (dry film) ν_{max} 3382, 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (1H, dd, J = 7, 1 Hz, H-12), 7.40 (1H, dd, J = 7, 1 Hz, H-9), 7.29 (1H, m, H-11), 7.26 (1H, m, H-10), 4.78 (1H, br s, OH-17), 4.17 (1H, br s, H-21), 4.15 (1H, s, H-17), 3.30 (1H, m, H-5), 3.26 (1H, m, H-5), 2.83 (1H, dddd, J = 16, 14, 6, 2 Hz, H-6), 2.62 (1H, br d, J = 11 Hz, H-3), 2.40 (1H, dd, J = 16, 6 Hz, H-6), 2.35 (1H, m, H-3), 2.22 (1H, dq, J = 14.6, 7.4 Hz, H-19), 1.96 (1H, dq, J = 14.6, 7.4 Hz, H-19), 1.77 (1H, dt, J = 13.5, 3.5 Hz, H-14), 1.51 (1H, br d, J = 13.7 Hz, H-15β), 1.37 (1H, br d, J = 13.5 Hz, H-14), 0.89 (3H, t, J = 7.4 Hz, H-18), 0.70 (1H, td, J = 13.7, 3.5 Hz, H-15α); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.0 (C, C-16), 134.6 (C, C-13), 131.6 (C, C-2), 130.5 (C, C-8), 124.4 (CH, C-11), 124.2 (CH, C-10), 118.4 (CH, C-9), 116.4 (CH, C-12), 113.0 (C, C-7), 75.1 (CH, C-17), 55.6 (CH, C-21), 50.3 (CH_2, C-5), 44.7 (CH_2, C-3), 41.9 (C, C-20), 23.0 (CH_2, C-15), 21.2 (CH_2, C-19), 20.2 (CH_2, C-14), 16.6 (CH_2, C-6), 7.1 (CH_3, C-18); ESIMS *m/z* 311 [MH]$^+$; HRESIMS *m/z* 311.1760 (calcd for $C_{19}H_{23}N_2O_2$, 311.1760).

$LiAlH_4$ Reduction of (+)-17β-Hydroxyeburnamonine (12). To a solution of **12** (72 mg, 0.23 mmol) in dry THF (10 mL) at 0 °C was added $LiAlH_4$ (23 mg, 0.6 mmol), and the mixture was refluxed for 2 h. The mixture was cooled to 0 °C, after which H_2O (0.1 mL), then NaOH (3 M, 0.1 mL), and water (0.3 mL) were added. The mixture was stirred for 3 h at room temperature and then filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the resulting residue was purified by centrifugal preparative TLC (silica gel, 5%

MeOH−CH_2Cl_2, NH_3-saturated) to afford **8** (39 mg, 54%) and **13** (28 mg, 39%).[45] **Compound 13:** white, amorphous solid and subsequently colorless crystals from CH_2Cl_2; mp 190−193 °C; $[\alpha]^{25}_D$ −44 (*c* 0.62, MeOH); UV (EtOH) λ_{max} (log ϵ) 229 (4.41), 281 (3.82) nm; IR (dry film) ν_{max} 3448 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.47 (1H, br d, J = 7.5 Hz, H-12), 7.34 (1H, br d, J = 7.5 Hz, H-9), 7.20 (1H, td, J = 7.5, 1 Hz, H-10), 7.16 (1H, td, J = 7.5, 1 Hz, H-11), 5.74 (1H, br s, H-16), 4.04 (1H, br s, H-21), 3.84 (1H, br s, H-17), 3.23 (1H, m, H-5), 3.14 (1H, m, H-5), 2.85 (1H, m, H-6), 2.66 (1H, m, H-6), 2.55 (2H, m, H-3), 2.29 (1H, dq, J = 14.5, 7.3 Hz, H-19), 1.71 (1H, dq, J = 14.5, 7.3 Hz, H-19), 1.47 (1H, td, J = 14, 3.5 Hz, H-15α), 1.37 (1H, m, H-15β), 1.33 (2H, m, H-14), 0.92 (3H, t, J = 7.3 Hz, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.4 (C, C-13), 130.5 (C, C-2), 129.1 (C, C-8), 121.4 (CH, C-10), 120.3 (CH, C-11), 118.7 (CH, C-12), 110.2 (CH, C-9), 105.5 (C, C-7), 79.9 (CH, C-16), 73.4 (CH, C-17), 56.4 (CH, C-21), 51.5 (CH_2, C-5), 45.2 (CH_2, C-3), 39.0 (C, C-20), 23.1 (CH_2, C-19), 22.3 (CH_2, C-15), 20.4 (CH_2, C-14), 16.6 (CH_2, C-6), 7.1 (CH_3, C-18); ESIMS *m*/*z* 313 $[MH]^+$; HRESIMS *m*/*z* 313.1915 (calcd for $C_{19}H_{25}N_2O_2$, 313.1916).

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that has been described in detail previously.[46,47]

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

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