β-Carboline Alkaloids from *Stellaria dichotoma* var. *lanceolata* and Their Anti-inflammatory Activity

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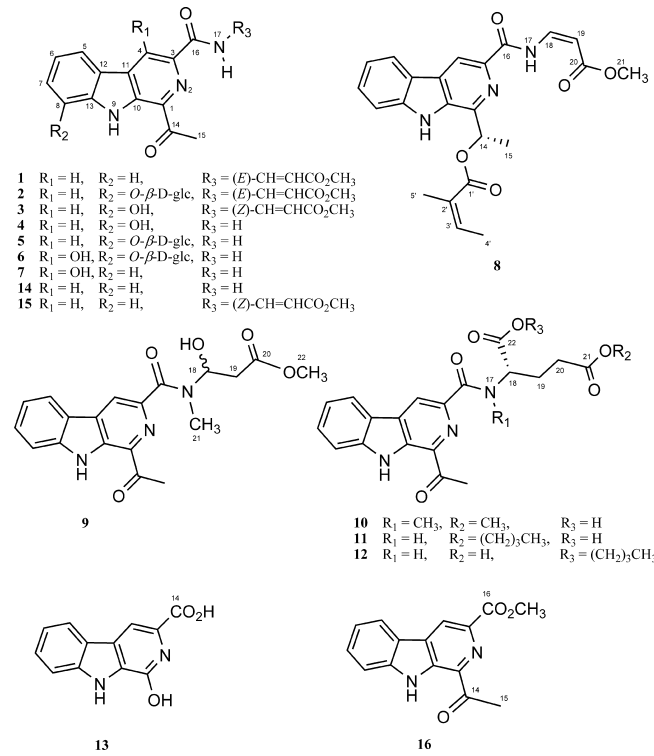
The present investigation on the chemical constituents of the roots of *Stellaria dichotoma* var. *lanceolata* has resulted in the isolation of 21 *β*-carboline alkaloids, including 13 new compounds, dichotomides III−XIV (**1**−**12**) and dichotomine E (**13**), and eight known compounds. The structures of the new compounds were established on the basis of spectroscopic data analysis. Among these isolated alkaloids, five compounds were examined for their anti-inflammatory potential for the inhibition of NO production in LPS-treated RAW 264.7 cells. All compounds tested exhibited significant inhibition of NO production, with IC_{50} values in the range of 11.3 to 19.3 μM.

In a previous investigation for bioactive compounds of potential therapeutic value, we have reported the synthesis of luzongerine A, a *β*-carboline alkaloid characterized from *Illigera luzonensis*, which displayed significant *i*NOS inhibitory activity.[1] Thus, in the continuing course of a natural products research program relating to *β*-carbolines, *Stellaria dichotoma* L. var. *lanceolata* Bunge (Caryophyllaceae) was selected as a target plant aimed at new drug discovery, since it is known to produce *β*-carboline alkaloids.[2,3] This species is distributed in Ningxia and neighboring provinces of mainland China, and the roots are used as a folk medicine for the treatment of fever in the late stage of febrile diseases.[4] Prior investigations have shown that this species and its constituents exhibit various bioactivities such as on cytotoxic,[5,6] antiallergic,[7] antifebrile,[4,7,8] and vasorelaxant effects.[9] Previous phytochemical studies have reported that, in addition to *β*-carbolines, cyclic peptides, flavonoids, neolignans, phenylpropanoids, and sterols have also been characterized from this plant.[4−11] The traditional medicinal use of *S. dichotoma* var. *lanceolata* is for eliminating heat from blood, and the mechanism of action is related to anti-inflammatory activity. In the present study, fractionation of a methanol extract from the roots of *S. dichotoma* var. *lanceolata* led to the isolation of 21 *β*-carboline alkaloids, including 13 new constituents (**1**−**13**). Herein, we wish to report the isolation and structural elucidation of these new alkaloids as well as the evaluation of some of the isolates obtained for their anti-inflammatory potential in an *i*NOS inhibition assay.



Results and Discussion

The air-dried and powdered roots of *S. dichotoma* var. *lanceolata* were extracted with hot MeOH and concentrated. The MeOH extract was suspended in H_2O and partitioned with $CHCl_3$ and *n*-butanol successively, affording $CHCl_3$-, *n*-butanol-, and H_2O-soluble extracts, respectively. Fractionation of the $CHCl_3$ extract using silica gel column chromatography afforded 11 fractions, of which fractions 4−7 were found to contain alkaloids. The *n*-butanol-soluble extract was also found to be alkaloid-positive. Repeated purification of the alkaloid-containing fractions with conventional chromatographic techniques yielded totally 21 *β*-carboline alkaloids, including 13 new compounds, dichotomides III−XIV (**1**−**12**) and dichotomine E (**13**).

Dichotomide III (**1**) was isolated as a yellow powder (mp >280 °C), and its molecular formula was determined as $C_{18}H_{15}N_3O_4$ by HREIMS (*m*/*z* 337.1062 $[M]^+$). The UV spectrum of **1** exhibited characteristic absorption maxima of a *β*-carboline chromophore at 375, 298, and 209 nm.[12] The IR bands at 3344 and 1695 cm^{-1} were assignable to hydroxy and carbonyl functionalities, respectively. In the ^1H NMR spectrum, a set of four mutually coupled symmetrical-type aromatic protons that resonated at δ 8.46 (1H, d, $J = 7.8$ Hz), 7.35 (1H, dd, $J = 7.8$, 7.5 Hz), 7.63 (1H, dd, $J = 8.0$, 7.5 Hz), and 7.83 (1H, d, $J = 8.0$ Hz), assignable to H-5, H-6,

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H-7, and H-8, respectively, was indicative of the unsubstituted aromatic ring A of a β-carboline basic skeleton. A downfield singlet at δ 9.20 that exhibited a NOESY correlation with H-5 was assigned as the characteristic H-4 proton signal of a β-carboline alkaloid. In addition, three mutually coupled protons at δ 11.05 (1H, d, J = 11.2 Hz, D_2O-exchangeable), 8.10 (1H, dd, J = 14.0, 11.2 Hz), and 6.15 (1H, d, J = 14.0 Hz), a methyl group at δ 2.97 (3H, s), and a methoxy group at δ 3.68 (3H, s) in the ^{1}H NMR spectrum, along with the signals at δ 201.4, 167.9, and 163.7 in the ^{13}C NMR spectrum, suggested the presence of a (*E*)-CONHCH=CH fragment, an acetyl group, and a methyl ester group, respectively. In a HMBC experiment, the ^2J and ^3J correlations of δ_H 6.15 (H-19) to δ_C 167.9 (C-20), δ_H 9.20 (H-4) to δ_C 163.7 (C-16), and δ_H 2.97 (CH_3-15) to δ_C 134.6 (C-1) were used to propose that the acetyl group is attached to C-1 and the $-$CONHCH=CHCOOCH$_3$ fragment is connected to C-3. The latter assignment was confirmed by the downfield proton signal at δ 9.20 for H-4 due to the electron-withdrawing effect of this C-3 substituent. On the basis of the above information, the structure of **1** was fully established as shown. This compound was accorded the trivial name dichotomide III, proposed following a previous convention.[2,3]

Compounds **2**−**13** were assigned as β-carboline derivatives since they all were found to possess characteristic UV and IR spectroscopic data as mentioned for **1** and also displayed positive responses toward the Dragendorff reagent. The major difference in the ^{1}H NMR spectrum of **2** was that the four mutually coupled aromatic proton signals in **1** were replaced by a set of three mutually coupled ABX-type proton signals [δ 8.19 (1H, d, J = 7.8 Hz), 7.42 (1H, d, J = 7.9 Hz), and 7.31 (1H, dd, J = 7.9, 7.8 Hz)], which was indicative of substitution at C-5 or C-8. Moreover, a set of proton signals at δ 5.96 (1H), 5.14 (1H), 5.09 (1H), 4.93 (1H), 4.62 (1H), 3.77 (1H), 3.52 (1H), 3.43−3.39 (2H), 3.32 (1H), and 3.25 (1H) was assignable to the presence of a glucose unit. This was evidenced further by the corresponding signals at δ 102.8, 77.5, 75.9, 73.5, 70.0, and 60.9 in the ^{13}C NMR spectrum. The glucose unit was found to be in the β form, as suggested by the coupling constant of the anomeric proton at δ 4.93 (7.3 Hz), and the sugar unit was attached at C-8 through a C−O linkage as indicated by the ^3J-HMBC correlation from H-1′ (δ 4.93) to C-8 (δ 144.4). Thus, the chemical structure of **2** (dichotomide IV) was characterized as shown. The ^{1}H NMR and ^{13}C NMR spectra of compound **3** were almost identical with those of **2**, except for the absence of the signals for a glucose moiety. Another marked difference in **3** was that the coupling constant between H-18 and H-19 was reduced from 14.1 Hz in **2** to 8.4 Hz. It was proposed that the configuration of the double bond at C-18−C-19 is *Z*, with the complete assignments of the ^{1}H and ^{13}C NMR spectroscopic data accomplished using the COSY, NOESY, HMQC, and HMBC spectra. Therefore, the structure of **3** (dichotomide V) was assigned as shown. In turn, the ^{1}H NMR and ^{13}C NMR spectroscopic characteristics of compound **4** were very similar to those of compound **3**, but with the absence of signals of a $-$CH=CHCOOCH$_3$ fragment evident for **4**, when comparing their NMR spectra. The molecular formula of **4** was determined as $C_{14}H_{11}N_3O_3$ from the molecular ion peak in the HREIMS (*m*/*z* 269.0801 $[M]^+$) and was compatible with the elimination of a $-$CH=CHCOOCH$_3$ fragment from the β-carboline basic skeleton. Thus, structure **4** was assigned to dichotomide VI.

The ^{1}H NMR spectrum of dichotomide VII (**5**) was found to be very similar to that of **2** except for the absence of a side chain unit connected at the N-17 amide group. This was supported by comparing the molecular formulas of **5** ($C_{20}H_{21}N_3O_8$) and **2** ($C_{24}H_{25}N_3O_{10}$). A glucose unit in **5** was connected at C-8 through a C−O linkage, as indicated by the ^3J-HMBC correlation between H-1′ (δ 4.92) and C-8 (δ 144.3). Therefore, the structure of dichotomide VII was established as **5**. Compound **6** (dichotomide VIII) was determined as $C_{20}H_{21}N_3O_9$ according to the HRESIMS data obtained. In the ^{1}H NMR spectrum of **6**, signals for three mutually coupled aromatic protons at δ 7.91 (1H, d, J = 7.2 Hz), 7.35 (1H, d, J = 7.2 Hz), and 7.29 (1H, t, J = 7.2 Hz), a set of glucosyl protons at δ 4.92−3.25 (11H), and an acetyl methyl group at δ 2.81 (3H, s) were evident. However, the downfield H-4 singlet in **5** was absent, and instead an additional oxygenated quaternary carbon signal at δ 158.5 appeared in the ^{13}C NMR spectrum of **6**. It was inferred that the only difference between **5** and **6** is that the C-4 in the latter compound is hydroxylated. Accordingly, the structure of dichotomide VIII was assigned as **6**. The molecular formula of **7** was established as $C_{14}H_{11}N_3O_3$ from the molecular ion peak at *m*/*z* 269.0802 in the HREIMS. On comparison of the molecular formulas of **7** and **6**, a $C_6H_{10}O_6$ moiety was indicated as being absent in **7**. In the ^{1}H NMR spectrum of **7**, the proton signals for a glucosyl unit were absent and the aromatic protons changed from an ABC pattern in **6** to four mutually coupled protons at δ 8.25 (1H, d, J = 7.3 Hz), 7.30 (1H, dd, J = 7.3, 7.0 Hz), 7.52 (1H, dd, J = 8.0, 7.0 Hz), and 7.80 (1H, d, J = 8.0 Hz). Accordingly, it could be determined that the A ring of **7** is unsubstituted. The structure of dichotomide IX (**7**) was established as shown.

Dichotomide X (**8**) was purified as an optically active light yellow powder (mp 163−165 °C, $[\alpha]^{25}_D$ −139.0), and the molecular formula was established as $C_{23}H_{23}N_3O_5$ from the HREIMS (*m*/*z* 421.1634 $[M]^+$). In the ^{1}H NMR spectrum of **8**, there were signals observed for a set of four aromatic protons at δ 8.16 (1H, d, J = 7.8 Hz), 7.60−7.55 (2H, m), and 7.34 (1H, ddd, J = 7.9, 7.8, 1.9 Hz), a downfield aromatic proton at δ 8.92 (1H, s), a set of three mutually coupled protons at δ 12.52 (1H, d, J = 12.4 Hz, D_2O exchangeable), 7.72 (1H, dd, J = 12.4, 8.8 Hz), and 5.26 (1H, d, J = 8.8 Hz), signals for a $-$CHCH$_3$ group at δ 6.71 (1H, q, J = 6.6 Hz) and 2.06 (3H, d, J = 6.6 Hz), and a singlet for a methyl group at δ 3.80 (3H). This ^{1}H NMR spectrum was very similar to that of the previously reported compound dichotomide II,[3] except for the additional appearance of two coupled proton signals at δ 6.13 (1H, q, J = 7.0 Hz) and 2.00 (3H, d, J = 7.0 Hz) and resonances for a methyl group at δ 1.92 (3H, s). In addition, there were five more carbon signals found at δ 168.9, 139.9, 127.3, 20.6, and 16.0 in the ^{13}C NMR spectrum of **8**. This compound displayed ^3J-HMBC correlations from δ 1.92 (CH_3-5′) to δ 168.9 (C-1′), and δ 2.00 (CH_3-4′) to δ 127.3 (C-2′), and NOESY correlations from H-3′ (δ 6.13) to H-4′ and H-5′. These data provided evidence that compound **8** has an additional angelic acid substituent when compared with dichotomide II. This was confirmed as being attached at C-14 to form an ester linkage, from the downfield chemical shift of H-14 (δ 6.71). The absolute configuration at C-14 was proposed as *S* by comparison of the sign of the specific rotation of **8** with that of dichotomide II.[9] Consequently, the structure of dichotomide X (**8**) was deduced as shown.

Dichotomide XI (**9**), purified as an optically active yellow powder (mp 185−187 °C, $[\alpha]^{25}_D$ −36.9), was determined to have a molecular formula of $C_{19}H_{19}N_3O_5$ by HREIMS (*m*/*z* 369.1328 $[M]^+$). On comparison of the ^{1}H NMR spectrum of **9** with that of dichotomide I,[3] the three mutually coupled proton signals at δ 3.87 (2H, dt, J = 6.2, 6.1 Hz), 2.74 (2H, t, J = 6.1 Hz), and 3.75 (3H, s), for the side chain $-$CH$_2$CH$_2$CO$_2$CH$_3$ attached to the amide functional group in the latter compound, were replaced by signals at δ 9.30 (1H, d, J = 10.0 Hz, D_2O-exchangeable), 5.65 (1H, dt, J = 10.0, 4.8 Hz), 2.96 (2H, m), and 3.65 (3H, s). The chemical shift of H-18 (δ 5.65) indicated that C-18 is substituted with a hydroxy group. In addition, when compared with dichotomide I, an additional methyl group signal in **9** appeared at δ 3.28, and this displayed a HMBC ^3J-correlation to the carbon signal at δ 77.7 (C-18). It was inferred that the amide at N-17 is substituted by a methyl and a $-$CHOHCH$_2$CO$_2$CH$_3$ group. The full assignments of ^{1}H and ^{13}C NMR signals of **9** were determined from the NOESY and HMBC spectra (Figure 1). Therefore, the chemical structure of dichotomide XI was characterized as **9**. Due to the limited

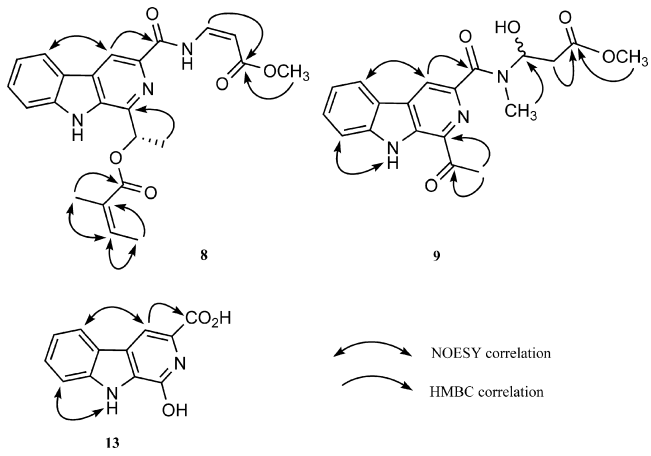

Figure 1. HMBC and NOESY correlations of **8**, **9**, and **13**.

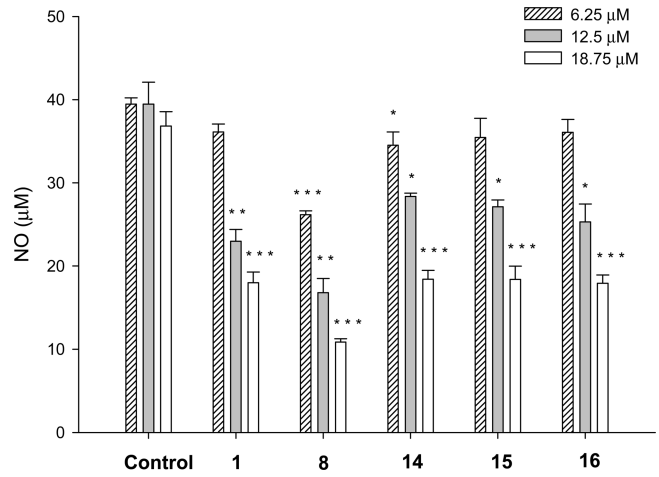

Figure 2. NO production after 24 h treatment of LPS-stimulated RAW 264.7 cells for five compounds (**1**, **8**, and **14**−**16**) isolated from *S. dichotoma* var. *lanceolata*, at concentrations from 6.25 to 18.75 μM. Data are the means ± SE of three independent experiments. Statistically different when $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared with the control.

quantity of this purified compound obtained, the absolute configuration at C-18 could not be determined and remains unknown.

The molecular formula of compound **10** was established as $C_{21}H_{21}N_3O_6$, with the aid of HRESIMS analysis. In the ^{1}H NMR spectrum, resonances for four mutually coupled aromatic protons at δ 8.25 (1H, d, J = 7.8 Hz), 7.35 (1H, dd, J = 7.8, 7.6 Hz), 7.62 (1H, dd, J = 8.0, 7.6 Hz), and 7.73 (1H, d, J = 8.0 Hz), assignable to H-5, H-6, H-7, and H-8, respectively, were indicative of the presence of an unsubstituted aromatic ring A, as in **1**. A downfield singlet at δ 8.96 (1H) that exhibited a NOESY correlation with H-5 was assigned to H-4 of a β-carboline alkaloid, and this suggested that C-3 is substituted with an electron-withdrawing group in **10**. In addition, a methyl group that resonated at δ 2.96 (3H, s), together with the signals at δ 203.1 and 26.3, indicated the presence of an acetyl group. The HMBC long-range correlation between δ 8.96 (H-4) and δ 167.1 (C-16) demonstrated that the structure of **10** possesses a similar substitution pattern to that of **1**, with C-1 attached to an acetyl group and C-3 to an amide function. However, in compound **10**, the side chains attached to the nitrogen atom (N-17) were different from those in **1**. In the ^{1}H NMR spectrum, five mutually coupled protons at δ 4.73 (1H, dd, J = 8.0, 4.5 Hz) and 2.35−2.24 (4H, m), a methyl group at δ 3.81 (3H, s) together with the carbon signal at δ 174.2, and a carboxylic acid carbonyl at δ 180.9 in the ^{13}C NMR spectrum suggested the presence of a $-CHCH_2CH_2-$ moiety, a methyl ester group, and a carboxylic acid unit, respectively. Through the use of the HMBC spectrum, the ^3J-correlations from δ 2.35−2.24 (H-19) to δ 180.9 (C-22) and from δ 3.81 (H-24) to δ 174.2 (C-21) could be used to construct the fragment $-CH(CO_2H)CH_2CH_2CO_2CH_3$. Moreover, the methyl group signal at δ 3.60 (CH_3-23) displayed a ^3J-HMBC correlation with the resonance at δ 54.4 (C-18), confirming that the amide (N-17) is substituted with a methyl and the $-CH(CO_2H)CH_2$-$CH_2CO_2CH_3$ fragment. The relative configuration at C-18 was determined as *S*, by comparing the sign of specific rotation of **10** with L-glutamic acid,[13] and consequently, the structure of dichotomide XII was established as shown. Compound **11** was also characterized as a β-carboline derivative, and the only minor spectroscopic differences between **11** and **10** were the disappearance of two methyl group signals in **11** and the occurrence of resonances for a butyl group at δ 3.84 (2H, t, J = 7.3 Hz), 1.39 (2H, m), 1.19 (2H, m), and 0.73 (3H, t, J = 7.3 Hz). The HMBC ^3J-correlation of the signal at δ 3.84 (H-1′) to the signal at δ 173.7 (C-21) indicated the formation of an ester linkage between the C-21 carbonyl group and the butyl substituent. The relative configuration at C-18 was also determined as *S* as in **10**, and, therefore, the structure of compound **11** (dichotomide XIII) was established as shown. Compound **12** was recognized as being an isomer of **11**, since the same molecular formula was assigned by HRESIMS. The UV, IR, and ^{1}H, and ^{13}C NMR spectroscopic characteristics of **12** were almost identical with those of **11**. However, the HMBC spectrum of **12** exhibited different proton−carbon correlations. Thus, the ^2J, ^3J-HMBC correlations of H-18 (δ 4.38)/C-22 (δ 172.6) and H-1′ (δ 4.08)/C-22 provided evidence for ester formation between C-22 and the butoxy group present. The absolute stereochemistry at C-18 of **12** was proposed as *S* since it also displayed a positive optical rotation as did **10**. Therefore, dichotomide XIV was assigned structure **12**.

Compound **13** was determined to have the molecular formula $C_{12}H_8N_2O_3$, according to the HREIMS. It was found to possess UV and IR spectroscopic characteristics for a β-carboline alkaloid. In the ^{1}H NMR spectrum, four mutually coupled aromatic protons at δ 8.18 (1H, d, J = 7.8 Hz, H-5), 7.55 (1H, d, J = 8.2 Hz, H-8), 7.46 (1H, dd, J = 8.2, 7.8 Hz, H-7), and 7.24 (1H, dd, J = 7.8, 7.6 Hz, H-6) and a downfield aromatic proton singlet at δ 7.88 (1H, s, H-4) were characteristic of unsubstituted ring A and a substituent at C-3. In addition, in the ^{1}H NMR and ^{13}C NMR spectra of **13**, there were two D_2O-exchangeable hydroxy group signals observed at δ 13.52 and 11.02 and a carboxylic acidic carbonyl carbon signal at δ 163.3. It was considered that **13** possesses a hydroxy and a carboxylic acid group substituted at C-1 and C-3, from the analysis of all of the spectroscopic data observed. The correct substitution pattern of **13** was deduced as a result of the long-range HMBC correlation between H-4 (δ 7.88) and C-14 (δ 163.3) so that the COOH was attached at C-3 and then OH was located at C-1. Accordingly, the structure of **13** was characterized as shown, and this was named dichotomine E, following a previous convention.[2,3]

In addition to the 13 new β-carboline alkaloids **1**−**13**, eight known compounds, comprising stellarines A (**14**) and B (**15**),[14] dichotomides I and II,[3] dichotomines A and B,[3] glucodichotomine B,[2] and 1-acetyl-3-methoxycarbonyl-β-carboline (**16**),[15] were also characterized from the methanol extract of the roots of *S. dichotoma* var. *lanceolata*. These known metabolites were all identified by spectroscopic data comparison with the published values reported in the literature. The newly characterized compounds **9**−**12** might be artifacts of the extraction procedure used, but no corresponding carboxylic acid derivatives were evident in the crude extracts investigated.

Five of the β-carboline derivatives isolated were subjected to an examination of their ability to inhibit NO production in LPS-treated RAW 264.7 cells, based on a reported method.[16,17] All of the compounds tested displayed significant inhibitory effects on NO production, and the results are shown in Figure 2. The IC_{50}

Table 1. ^{1}H NMR Spectroscopic Data for the Isolated β-Carboline Derivatives **1**−**13** [DMSO-d_6, 400 MHz, δ ppm (J = Hz)]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**[a]	**9**	**10**[b]	**11**	**12**	**13**
4	9.20 (s)	9.25 (s)	9.20 (s)	9.04 (s)	9.10 (s)			8.92 (s)	9.15 (s)	8.96 (s)	9.05 (s)	9.02 (s)	7.88 (s)
5	8.46 (d, 7.8)	8.19 (d, 7.8)	7.96 (d, 8.0)	7.90 (d, 7.5)	8.12 (d, 7.8)	7.91 (d, 7.2)	8.25 (d, 7.3)	8.16 (d, 7.8)	8.45 (d, 7.8)	8.25 (d, 7.8)	8.41 (d, 7.8)	8.40 (d, 7.8)	8.18 (d, 7.8)
6	7.35 (dd, 7.8, 7.5)	7.31 (dd, 7.9, 7.8)	7.22 (dd, 8.0, 7.8)	7.20 (dd, 7.8, 7.5)	7.28 (dd, 7.8, 7.5)	7.29 (t, 7.2)	7.30 (dd, 7.3, 7.0)	7.34 (ddd, 7.9, 7.8, 1.9)	7.34 (dd, 7.8, 7.4)	7.35 (dd, 7.8, 7.6)	7.31 (dd, 7.8, 7.3)	7.31 (dd, 7.8, 7.6)	7.24 (dd, 7.8, 7.6)
7	7.63 (dd, 8.0, 7.5)	7.42 (d, 7.9)	7.04 (d, 7.8)	7.02 (d, 7.8)	7.40 (d, 7.5)	7.35 (d, 7.2)	7.52 (dd, 8.0, 7.0)	7.58 (m)	7.62 (dd, 8.0, 7.4)	7.62 (dd, 8.0, 7.6)	7.59 (dd, 8.0, 7.3)	7.60 (dd, 8.1, 7.6)	7.46 (dd, 8.2, 7.8)
8	7.83 (d, 8.0)						7.80 (d, 8.0)	7.58 (m)	7.83 (d, 8.0)	7.73 (d, 8.0)	7.80 (d, 8.0)	7.82 (d, 8.1)	7.55 (d, 8.2)
9	12.30 (s)	11.51 (brs)	11.84 (s)	11.63 (brs)	11.36 (brs)	11.34 (brs)	12.05 (brs)	9.73 (brs)	12.23 (brs)		12.16 (brs)	12.15 (brs)	12.44 (brs)
14								6.71 (q, 6.6)					13.52 (brs)
15	2.97 (s)	3.00 (s)	2.92 (s)	2.90 (s)	2.90 (s)	2.81 (s)	2.78 (s)	2.06 (d, 6.6)	2.93 (s)	2.96 (s)	2.87 (s)	3.00 (s)	
17	11.05 (d, 11.2)	11.09 (d, 11.2)	12.53 (d, 12.4)	8.21 (brs) 7.66 (brs)	8.23 (brs) 7.69 (brs)	8.59 (brs) 8.30 (brs)	8.66 (brs) 8.21 (brs)	12.52 (brd, 12.4)			8.93 (brd, 6.4)	10.44 (brd, 7.5)	
18	8.10 (dd, 14.0, 11.2)	8.10 (dd, 14.1, 11.2)	7.74 (dd, 12.4, 8.4)					7.72 (dd, 12.4, 8.8)	5.65 (dt, 10.0, 4.8)	4.73 (dd, 8.0, 4.5)	4.24 (brd, 4.4)	4.38 (d, 5.3)	
19	6.15 (d, 14.0)	6.16 (d, 14.1)	5.35 (d, 8.4)					5.26 (d, 8.8)	2.96 (m)	2.30 (m)	2.22 (m) 2.05 (m)	2.07 (m)	
20										2.30 (m)	2.36 (m)	2.07 (m)	
21	3.68 (s)	3.69 (s)	3.73 (s)					3.80 (s)	3.28 (s)				
22									3.65 (s)				
23										3.60 (s)			
24										3.81 (s)			
1′		4.93 (d, 7.3)			4.92 (d, 7.8)	4.92 (d, 7.6)					3.84 (t, 7.3)	4.08 (m)	
2′		3.41 (m)			3.39 (m)	3.50 (m)					1.39 (m)	1.56 (m)	
3′		3.32 (m)			3.39 (m)	3.32 (m)		6.13 (q, 7.0)			1.19 (m)	1.34 (m)	
4′		3.25 (m)			3.24 (m)	3.25 (m)		2.00 (d, 7.0)			0.73 (t, 7.3)	0.86 (t, 7.3)	
5′		3.41 (m)			3.39 (m)	3.50 (m)		1.92 (s)					
6′		3.77 (dd, 11.5, 5.3)			3.76 (dd, 11.5, 5.6)	3.76 (dd, 10.4, 5.2)							
		3.52 (dd, 11.5, 5.9)			3.50 (dd, 11.5, 6.0)	3.59 (dd, 10.4, 6.0)							

[a] $CDCl_3$, 400 MHz. [b] CD_3OD, 300 MHz.

Table 2. ^{13}C NMR Spectroscopic Data for the Isolated β-Carboline Derivatives **1**−**13** (DMSO-d_6, 100 MHz,)

carbon	1	2	3	4	5	6	7[a]	8[b]	9	10[c]	11	12	13
1	134.6	135.1	134.5	134.3	135.0	129.6	140.6	140.6	134.2	135.7	134.5	134.4	154.9
3	136.8	138.5	136.1	139.4	139.6	121.0	127.4	137.5	137.8	139.1	139.3	138.9	125.8
4	119.6	120.1	119.7	118.6	118.6	158.5	164.8	115.9	118.7	118.7	118.4	118.1	105.9
5	122.6	116.7	113.5	113.3	116.5	117.7	122.8	121.9	122.5	122.8	122.9	122.4	121.8
6	121.4	122.3	122.5	122.5	122.4	122.9	121.1	121.1	121.1	122.6	121.5	120.9	120.7
7	129.8	115.8	115.3	115.0	115.6	114.6	138.0	129.1	129.6	130.6	130.0	129.4	126.9
8	113.6	144.4	143.6	143.7	144.3	144.7	113.3	112.1	113.5	113.8	114.0	113.5	113.0
10	135.6	135.5	135.4	135.0	134.6	137.7		135.9	135.2	137.0	135.4	135.0	131.1
11	132.3	132.8	132.9	132.6	132.8	118.1		130.6	132.2	133.7	132.7	131.9	122.7
12	120.4	122.3	122.8	122.7	122.0	121.2		121.9	120.4	122.0	121.0	120.5	122.4
13	142.6	132.5	131.2	131.2	132.4	131.5		140.6	142.6	143.8	143.0	142.5	139.5
14	201.4	201.4	201.1	201.9	201.5	200.4	199.4	69.8	201.1	203.1	201.3	202.0	163.3
15	26.5	26.6	25.7	26.2	26.3	26.3	25.8	17.9	26.1	26.2	26.3	26.7	
16	163.7	163.5	162.4	166.5	166.4	172.9	172.1	163.8	164.6	167.1	163.9	164.9	
18	138.6	137.3	137.9					137.4	77.7	54.4	53.9	54.5	
19	102.0	102.1	97.0					97.0	39.6	29.5	28.4	27.4	
20	167.9	167.8	168.8					168.7	170.8	35.1	30.8	34.8	
21	51.3	51.3	51.4					51.1	51.8	174.2	173.7	175.8	
22									55.4	180.9	174.4	172.6	
23										64.3			
24										52.8			
1′		102.8			102.8	103.2		168.9			64.1	64.0	
2′		73.5			73.5	74.0		127.3			30.9	30.4	
3′		75.9			76.0	76.5		139.9			19.2	18.8	
4′		70.0			70.0	70.4		16.0			14.1	13.8	
5′		77.5			77.6	78.0		20.6					
6′		60.9			61.0	61.4							

[a] Some carbon signals were not detectable. [b] $CDCl_3$, 100 MHz. [c] CD_3OD, 75 MHz.

values of the tested compounds **1**, **8**, and **14**−**16** were 17.3, 11.3, 19.3, 18.6, and 17.9 μM, respectively, and were compared to a reference compound, aminoguanidine (IC_{50} value of 4.6 μM).

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto MP-S3 micro melting point apparatus without correction. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FTIR-8501 spectrophotometer. ^1H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on Bruker AC-200, AVANCE-300, AMX-400, and Varian UNITY plus 400 NMR spectrometers, using tetramethylsilane (TMS) as the internal standard. Standard pulse sequences and parameters were used for the NMR experiments, and all chemical shifts are reported in parts per million (ppm, δ). The low- and high-resolution EIMS and ESIMS were obtained on VG 70-250S and Bruker APEX II mass spectrometers, respectively. TLC was conducted on precoated Kieselgel 60 F 254 plates (Merck), and the spots were detected by examining the plates sprayed with Dragendorff reagent followed by heating at 110 °C. The alkaloid spots displayed colors of orange to light yellow. All chemicals including lipopolysaccharide (LPS) from *Escherichia coli* and Griess reagent were purchased from Sigma (St. Louis, MO).

Plant Material. The roots of *Stellaria dichotoma* var. *lanceolata* (Caryophyllaceae) were purchased from a pharmaceutical market in August, 1999, in Tainan, Taiwan, and the plant material was identified and authenticated by Prof. C. S. Kuoh, Department of Bioscience, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (TSWu 199900105) has been deposited in the herbarium of the Department of Chemistry, National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. Powdered roots of *S. dichotoma* var. *lanceolata* (5.8 kg) were extracted with MeOH six times (6 × 10 L) under reflux for 8 h and concentrated to give a brown syrup (1.2 kg). The extract was suspended in H_2O and partitioned with $CHCl_3$ and *n*-butanol to afford $CHCl_3$ (80 g), *n*-butanol (220 g), and water extracts (900 g), respectively. Of these, the $CHCl_3$ extract was subjected to column chromatography over silica gel, eluted using a step gradient of *n*-hexane−acetone (9:1 to 1:1), to afford 11 pooled fractions. Fractions 4−7 were Dragendorff-positive and were further purified using column chromatography. Fraction 4 was chromatographed further over silica gel using a stepwise gradient of *n*-hexane−EtOAc (9:1 to 1:1) to afford **15** (20 mg). Further purification of fraction 5 by repeated column chromatography with *n*-hexane−diisopropyl ether gradient mixtures (from 10:1 to 1:1) afforded **16** (150 mg), and preparative TLC purification (*n*-hexane−diisopropyl ether, 5:1) gave **8** (3.0 mg, R_f = 0.3). The sixth fraction was purified by repeated silica gel column chromatography using a stepwise gradient of *n*-hexane−EtOAc (from 4:1 to 1:1) and yielded **1** (3.0 mg), **3** (2.0 mg), **9** (3.0 mg), and dichotomide II (4.0 mg), respectively. Preparative TLC purification of the minor subfraction with *n*-hexane−acetone (3:1) afforded **7** (2.0 mg, R_f = 0.4) and dichotomide I (2.0 mg, R_f = 0.1). The alkaloid-containing fraction 7 was further chromatographed over silica gel eluted with chloroform−acetone (from 20:1 to 1:1) to yield **14** (50 mg).

The *n*-butanol-soluble extract was subjected to reversed-phase Diaion HP-20 column chromatography and yielded 11 pooled subfractions. Of these, subfractions 6−10 exhibited positive results against Dragendorff's reagent. Subfraction 6 was purified with silica gel column chromatography using a stepwise gradient of chloroform−methanol (from 5:1 to 1:1) to obtain **5** (3.5 mg), dichotomine B (3.0 mg), and glucodichotomine B (5.0 mg). The seventh subfraction was subjected to column chromatography over silica gel with chloroform−methanol (5:1) as eluent and afforded **13** (2.0 mg) and dichotomine A (3.5 mg). Subfraction 8, which also displayed positive results against Dragendorff's reagent, was chromatographed over silica gel, using a stepwise gradient of chloroform−methanol (from 5:1 to 1:1), to yield **10** (1.5 mg). The alkaloid-containing subfraction 9 was subjected to silica gel column chromatography eluted with chloroform−methanol (from 9:1 to 1:1) and further purified by repeated silica gel column chromatography using the same solvent system to afford **2** (2.0 mg), **4** (1.5 mg), **6** (1.6 mg), and **12** (3.0 mg). The final alkaloid-containing subfraction 10 was purified with the aid of silica gel column chromatography eluted with chloroform−methanol (9:1) to afford **11** (2.0 mg).

The H_2O-soluble extract was subjected also to reversed-phase Diaion HP-20 column chromatography and yielded 10 subfractions. However, none of these were found to be alkaloid-positive.

Dichotomide III (1): yellow powder; mp >280 °C (MeOH); UV (MeOH) λ_{max} (log ε) 375 (3.57), 298 (4.50), 209 (4.34) nm; IR (KBr) ν_{max} 3344, 3068, 2941, 1695, 1681, 1647, 1481, 1440 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; EIMS *m/z* 337 $[M]^+$ (49), 278 (69), 237 (48), 209 (100), 181 (23); HREIMS *m/z* 337.1062 $[M]^+$ (calcd for $C_{18}H_{15}N_3O_4$, 337.1059).

Dichotomide IV (2): yellow powder; mp >280 °C (MeOH); $[\alpha]^{25}_D$ +94.0 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 376 (3.12), 325 (3.65, sh), 297 (3.99), 230 (3.69, sh), 212 (3.92) nm; IR (KBr) ν_{max} 3360, 2934, 1711, 1639, 1506, 1445, 1381 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2;

ESIMS *m/z* 538 $[M + Na]^+$ (45), 469 (100), 463 (90); HRESIMS *m/z* 538.1440 $[M + Na]^+$ (calcd for $C_{24}H_{25}N_3O_{10}Na$, 538.1438).

Dichotomide V (3): yellow powder; mp >280 °C (MeOH); UV (MeOH) λ_{max} (log ε) 388 (3.07), 332 (3.49, sh), 304 (3.98, sh), 289 (4.03), 275 (3.96, sh), 237 (3.83), 214 (4.00) nm; IR (KBr) ν_{max} 3356, 3275, 2920, 1693, 1666, 1628, 1510, 1437, 1389 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; EIMS *m/z* 353 $[M]^+$ (85), 294 (77), 253 (58), 225 (100), 197 (15); HREIMS *m/z* 353.1012 $[M]^+$ (calcd for $C_{18}H_{15}N_3O_5$, 353.1008).

Dichotomide VI (4): yellow powder; mp >280 °C (MeOH); UV (MeOH) λ_{max} (log ε) 389 (3.50), 286 (4.17), 235 (4.15, sh), 212 (4.30) nm; IR (KBr) ν_{max} 3477, 3342, 3221, 2924, 1668, 1556, 1412, 1365 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; EIMS *m/z* 269 $[M]^+$ (100), 252 (39), 224 (74); HREIMS *m/z* 269.0801 $[M]^+$ (calcd for $C_{14}H_{11}N_3O_3$, 269.0798).

Dichotomide VII (5): yellow powder; mp >280 °C (MeOH); $[\alpha]^{25}_D$ +40.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 379 (3.71), 284 (4.47), 221 (4.48) nm; IR (KBr) ν_{max} 3300, 2924, 1670, 1578, 1397 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; ESIMS *m/z* 454 $[M + Na]^+$ (100), 440 (58), 381 (54); HRESIMS *m/z* 454.1225 $[M + Na]^+$ (calcd for $C_{20}H_{21}N_3O_8Na$, 454.1226).

Dichotomide VIII (6): yellow powder; mp >280 °C (MeOH); $[\alpha]^{25}_D$ +20.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 367 (3.71), 297 (4.07, sh), 279 (4.31), 247 (4.05, sh) nm; IR (KBr) ν_{max} 3385, 2922, 1661, 1603, 1367, 1279 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; ESIMS *m/z* 470 $[M + Na]^+$ (76), 437 (19), 413 (51), 381 (100), 353 (26); HRESIMS *m/z* 470.1178 $[M + Na]^+$ (calcd for $C_{20}H_{21}N_3O_9Na$, 470.1175).

Dichotomide IX (7): yellow powder; mp 243−245 °C (MeOH); UV (MeOH) λ_{max} (log ε) 363 (3.67), 303 (3.85, sh), 282 (4.31), 248 (3.99), 211 (4.19) nm; IR (KBr) ν_{max} 3474, 3329, 3261, 1675, 1601, 1448, 1377 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; EIMS *m/z* 269 $[M]^+$ (100), 252 (31), 224 (90), 209 (27); HREIMS *m/z* 269.0802 $[M + H]^+$ (calcd for $C_{14}H_{11}N_3O_3$, 269.0798).

Dichotomide X (8): yellow powder; mp 163−165 °C ($CHCl_3$); $[\alpha]^{25}_D$ −139.0 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 387 (1.32), 325 (4.03), 285 (4.25), 228 (4.15) nm; IR (KBr) ν_{max} 3371, 3053, 2935, 1697, 1629, 1479, 1444, 1379 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 421 $[M]^+$ (31), 362 (19), 338 (100), 321 (23), 262 (45), 237 (43), 195 (43), 193 (54); HREIMS *m/z* 421.1634 $[M]^+$ (calcd for $C_{23}H_{23}N_3O_5$, 421.1632).

Dichotomide XI (9): yellow powder; mp 185−187 °C (MeOH); $[\alpha]^{25}_D$ −36.9 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 377 (3.32), 287 (4.15), 271 (3.96, sh), 219 (4.07) nm; IR (KBr) ν_{max} 3350, 2925, 2846, 1728, 1705, 1664, 1508, 1452 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; EIMS *m/z* 369 $[M]^+$ (41), 338 (19), 296 (22), 237 (100), 209 (95), 181 (30), 154 (18), 132 (87); HREIMS *m/z* 369.1328 $[M]^+$ (calcd for $C_{19}H_{19}N_3O_5$, 369.1320).

Dichotomide XII (10): yellow powder; mp >280 °C (MeOH); $[\alpha]^{25}_D$ +20.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 378 (3.43), 287 (4.27), 268 (4.09, sh), 239 (3.79, sh), 220 (4.20) nm; IR (KBr) ν_{max} 3032, 1734, 1647, 1537, 1456, 1232 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz), see Table 1; ^{13}C NMR (CD_3OD, 75 MHz), see Table 2; ESIMS *m/z* 434 $[M + Na]^+$ (100); HRESIMS *m/z* 434.1326 $[M + Na]^+$ (calcd for $C_{21}H_{21}N_3O_6Na$, 434.1328).

Dichotomide XIII (11): yellow powder; mp 222−224 °C (MeOH); $[\alpha]^{25}_D$ +48.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 375 (3.50), 286 (4.35), 268 (4.16, sh), 220 (4.26) nm; IR (KBr) ν_{max} 3386, 2960, 1734, 1654, 1601, 1533, 1499, 1413 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; ESIMS *m/z* 462 $[M + Na]^+$ (100), 447 (56), 437 (46), 418 (34), 381 (84); HRESIMS *m/z* 462.1644 $[M + Na]^+$ (calcd for $C_{23}H_{25}N_3O_6Na$, 462.1641).

Dichotomide XIV (12): yellow powder; mp 240−242 °C (MeOH); $[\alpha]^{25}_D$ +8.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 373 (3.70), 306 (3.95, sh), 286 (4.54), 268 (4.34, sh), 220 (4.43) nm; IR (KBr) ν_{max} 3200, 2959, 1734, 1635, 1527, 1458 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; HRESIMS *m/z* 462.1643 $[M + Na]^+$ (calcd for $C_{23}H_{25}N_3O_6Na$, 462.1641).

Dichotomine E (13): yellow powder; mp >280 °C (MeOH); UV (MeOH) λ_{max} (log ε) 347 (3.98), 332 (4.05), 319 (3.96), 306 (3.82, sh), 281 (4.28), 273 (4.22), 260 (4.12), 239 (4.35), 232 (4.29, sh), 222 (4.15, sh) nm; IR (KBr) ν_{max} 3292, 3173, 1711, 1647, 1254 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; EIMS *m/z* 228 $[M]^+$ (40), 182 (30), 167 (34), 149 (46), 129 (50), 91 (100); HREIMS *m/z* 228.0537 $[M]^+$ (calcd for $C_{12}H_8N_2O_3$, 228.0533).

Inhibition of NO Production in LPS-Stimulated RAW 264.7 Cells. The effects of the isolated compounds on the inhibition of NO production was based on a reported method.[16,17] RAW 264.7 cells (1×10^4 cells/well) were seeded in 96-well plates with DMEM medium supplemented with 10% FBS and incubated for 12 h. The cells were pretreated individually with different concentrations (6.25, 12.5, 18.75 μM) of five compounds isolated from *S. dichotoma* var. *lanceolata* for 2 h. Then, RAW cells were treated with 0.1 μg/mL of LPS and incubated for 24 h. A control group was treated with LPS only. One hundred microliters of the medium was incubated with an equal volume of Griess reagent for 15 min at room temperature. Nitrite production was determined using an ELISA reader at 570 nm. The amount of NO in the sample was measured by standard curve, generated with diluted $NaNO_2$.

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Supporting Information Available: NMR spectra for compounds **1**−**13**. This information is available free of charge via the Internet at http://pubs.acs.org.

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