# *β*-Carboline Alkaloids from *Stellaria dichotoma* L. var. *lanceolata* and Their Anti-inflammatory Bioactivity

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# Equal contribution

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**Abstract -** The investigation on chemical constituents of the roots of *Stellaria dichotoma* has resulted in the isolation of twenty one $\beta$-carboline alkaloids, including thirteen new compounds, stellarboline A-M (**1-13**), as well as eight known compounds. The structures of these new compounds were established on the basis of the spectroscopic analysis and the known compounds were identified by comparison of their spectroscopic and physical data with those reported in the literature. Among these isolated alkaloids, five compounds were examined for their anti-inflammatory potentials for the inhibition of NO production in LPS-treated RAW264.7 cells. All of the tested $\beta$-carbolines exhibited significant inhibition of NO production with the $IC_{50}$ values in the range between 11.3 and 19.3 μM.

Natural and synthetic β-carboline alkaloids exhibit a wide range of important medicinal bioactivities, particularly concerning the central nervous system.[1,2] Due to their unique rigid heterocyclic skeleton, many β-carbolines are known to bind with high affinity to benzodiazepine (BzR),[3] serotonin,[1,2] and dopamine[4] receptor sites and to inhibit monoamine oxidase A.[5,6] In the previous investigations for bioactive compounds, we have reported the synthesis of luzongerine A, characterized from *Illigera luzonensis*, which displayed significant *i*NOS inhibition activity.[7] Thus in the continuing course of our natural products research a relating to β-carbolines, *Stellaria dichotoma* L. var. *lanceolata* (Caryophyllaceae) was selected as a target of our integrated program aimed at new drug discovery since it was a rich source of alkaloids with basic skeleton of β-carboline.[8,9] *S. dichotoma* is distributed in Ningxia and neighboring provinces of China, and the roots of this plant are used as folk medicine for the treatment of fever.[10] Prior pharmalogical investigations showed that this species have various bioactivities such as cytotoxic,[11,12] antiallergic,[9] antifebrile,[10,13,14] and vasorelaxant activities.[15] The previous phytochemical studies reported that except β-carbolines, steroids, flavonoids, neolignans, phenylpropanoids, and cyclic peptides have been characterized from this plant.[10-17] In the present study, fractionation of methanol extract from the roots of *S. dichotoma* monitored with Dragendorff reagent led to the isolation of twenty one β-carboline alkaloids, including thirteen new constituents. Herein we wish to report the isolation and structural elucidation of these new alkaloids and their anti-inflammatory bioactivities.

**Results and Discussion**

Air-dried and powdered roots of *S. dichotoma* were extracted with hot MeOH and concentrated. The MeOH extract was suspended in $H_2O$ and partitioned with $CHCl_3$ and *n*-butanol successively, and afforded $CHCl_3$-, *n*-butanol-, and $H_2O$-solubles, respectively. Fractionation of the $CHCl_3$-solubles with silica gel column chromatography afforded eleven fractions and further monitoring with Dragendorff reagent displayed positive results for the fractions 4-7. The *n*-butanol- and $H_2O$-solubles were subjected into reversed phase Diaion HP-20 column chromatography and yielded eleven and ten subfractions, respectively. For the *n*-butanol-solubles, subfractions 6-10 also exhibited positive results against Dragendorff reagent examinations. However, there were not any subfractions of $H_2O$-solubles shown positive response to the alkaloid tests. Repeated purification of the alkaloid-containing fractions yielded totally twenty one *β*-carboline alkaloids, including thirteen new compounds, stellarboline A-M (**1-13**).

Stellarboline A (**1**) was isolated as yellow powder (mp > 280 ºC), and its molecular formula was determined as $C_{18}H_{15}N_3O_4$ established by HREIMS (*m/z* 337.1062 $[M]^+$). The UV spectrum of **1** exhibited characteristic absorption maxima of a β-carboline chromophore at 375, 298, 209 nm.[18] The infrared absorption bands at 3344 and 1695 $cm^{-1}$ were assignable to hydroxyl and carbonyl functionalities, respectively. In the $^1H$ NMR spectrum, a set of four mutually coupled symmetrical AA', BB' type aromatic protons 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), which were assignable to H-5, H-6, 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 exhibited NOESY correlation with H-5

was the characteristic proton signal for the H-4 of *β*-carboline alkaloids. 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 methoxyl group at δ 3.68 (3H, s) in the $^1$H NMR spectrum along with the carbon signals at δ 201.3, 167.9, 163.7 in the $^{13}$C NMR spectrum suggested the presence of one $COCH_3$ group (acetyl), one –CONHCH=CH– fragment, and one $COOCH_3$ group (methyl ester). In the HMBC long range proton-carbon correlation experiment, the $^2J$- and $^3J$-correlations of $\delta_H$ 6.15 (H-19) to $\delta_C$ 167.9 (C-20), $\delta_H$ 9.20 (H-4) to $\delta_C$ 163.7 (C-16), and $\delta_H$ 2.97 ($CH_3$-15) to $\delta_C$ 134.6 (C-1), proposed that the acetyl group was attached at C-1, and the –CONHCH=CHCOOCH$_3$ fragment was connected at C-3. It was further confirmed by the downfield proton signal at δ 9.20 for H-4 due to the electron-withdrawing effect of C-3 substituent. On the above basis of spectroscopic elucidation, the structure of **1** was fully established as shown in Figure 1 and was given the trivial name stellarboline A.

Compounds **2-13** were totally assigned as *β*-carboline derivatives since they all possessed the characteristic UV and IR spectral data as mentioned above and also displayed positive responses towards the Dragendorff reagent examinations. The major difference in the $^1$H NMR spectrum of **2** is the four mutually coupled aromatic proton signals in **1** were replaced by the set of three mutually coupled ABC type proton signals which was indicative of the 8-substitution. Moreover, one set of proton signals at δ 5.96 (1H, br s, *J* = 3.6 Hz, $D_2O$ exchangeable), 5.14 (1H, br s, *J* = 4.5 Hz, $D_2O$ exchangeable), 5.09 (1H, br s, *J* = 5.2 Hz, $D_2O$ exchangeable), 4.93 (1H, d, *J* = 7.3 Hz), 4.62 (1H, br t, *J* = 5.5 Hz, $D_2O$ exchangeable), 3.77 (1H, dd, *J* = 11.5, 5.3 Hz), 3.52

(1H, dd, *J* = 11.5, 5.9 Hz), 3.43-3.39 (2H, m), 3.32 (1H, m), and 3.25 (1H, m) was assignable to the presence of one glucose unit. It was further evidenced by the carbon signals at δ 102.8, 77.5, 75.9, 73.5, 70.0, and 60.9 in the $^{13}$C NMR spectrum. The connectivity of the glucose unit was β form suggested by the coupling constant of anomeric proton at δ 4.93 (7.3 Hz), and the sugar fragment was attached at C-8 through C−O linkage with the aid of $^{3}J$- HMBC long range correlation from the proton signal at δ 4.93 (H-1') to the carbon signal at δ 144.4 (C-8). Thus, the chemical structure of **2** was characterized as shown and given the trivial name stellarboline B. The $^{1}$H NMR and $^{13}$C NMR spectra of compound **3** were almost identical with **2** except the disappearance of the signals for 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 double bond at C-18−C-19 was *Z* and therefore the structure of **3** was confirmed as displayed in Fig. 1 and nomenclatured as stellarboline C. The $^{1}$H NMR and $^{13}$C NMR spectral characteristics of compound **4** were very similar with that of compound **3** and only the disappearance of the proton and carbon signals of the –CH=CHCOOCH$_3$ fragment was found while comparing their spectra. In addition, the molecular formula of **4** was determined as $C_{14}H_{11}N_3O_3$ from the molecular ion peak in the HREIMS spectral analysis (*m/z* 269.0801 $[M]^+$). It was also compatible with the assumption that the –CH=CHCOOCH$_3$ fragment was eliminated from the *β*-carboline basic skeleton of **3**. Thus, the structure of **4** was determined as shown and named trivially as stellarboline D.

Stellarboline E (**5**), purified as optically active yellow powder (mp 185-187 ºC, $[\alpha]_D^{25}$ −36.9), was exhibited to have the molecular formula as $C_{19}H_{19}N_3O_5$ from the molecular ion peak

at *m/z* 369.1328 in HREIMS analysis. The UV absorption maxima at 377, 287, and 219 nm and the IR absorption bands at 3350 and 1728 $cm^{-1}$ were compatible with the *β*-carboline alkaloid basic structure. Comparison of the $^1H$ NMR spectrum of **5** with dichotomide I,[9] the typical three mutually coupled proton signals at δ 8.63 (1H, br t, *J* = 6.2 Hz, $D_2O$ exchangeable), 3.87 (2H, dt, *J* = 6.2, 6.1 Hz), and 2.74 (2H, t, *J* = 6.1 Hz) for the side chain $–CH_2CH_2CO_2CH_3$ attached in the amide functional group in dichotomide I was replaced by the signals at δ 9.30 (1H, d, *J* = 10.0 Hz, $D_2O$ exchangeable), 5.65 (1H, dt, *J* = 10.0, 4.8 Hz), and 2.96 (2H, m). It was suggested that the C-18 was substituted with hydroxyl group which was also indicated by the downfield chemical shift of H-18. In addition, one more methyl group signal located at δ 3.28 which displayed HMBC $^3J$-correlation to the carbon signal at δ 77.7 (C-18). It was inferred that the amide N-17 was substituted by one methyl and one $–CHOHCH_2CO_2CH_3$ groups. The chemical structure of **5** was further confirmed by the NOESY and HMBC correlations and thus characterized as stellarboline E (Fig. 1).

Stellarboline F-H (**6-8**) were proposed as *β*-carboline derivatives since these three compounds possessed the UV and IR spectral characteristics similar with the *β*-carboline alkaloids and were also shown positive results against the Dragendorff reagent tests. The molecular formula of compound **6** was determined as $C_{21}H_{21}N_3O_6$ with the aid of HRESIMS analysis. In the $^1H$ NMR spectrum of **6**, a typical set of four mutually coupled symmetrical type 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, was indicative of the unsubstituted aromatic ring A as in **1**. A downfield singlet at δ 8.96 exhibited

NOESY correlation with H-5 was the characteristic proton signal for the H-4 of *β*-carboline alkaloids, and it also suggested that the C-3 was substituted with electron-withdrawing group. In addition, five mutually coupled protons at δ 4.73 (1H, dd, $J$ = 8.0, 4.5 Hz) and 2.35-2.24 (4H, m), and three methyl group at δ 3.81 (3H, s), 3.60 (3H, s), and 2.69 (3H, s) together with the carbon signals at δ 203.1, 180.9, 174.2, 167.1, and the HMBC long range correlation between δ 8.96 (H-4) and δ 167.1 (C-16) suggested the structure of **6** possessing the similar substitution pattern as in **1**, that is, C-1 with an acetyl group and C-3 with an amide function. However, the side chains attached with the nitrogen atom of amide group were different from **1** in **6**. Through the assistance of HMBC analysis, the $^{3}J$-correlations from δ 2.96 (H-19) to δ 180.9 (C-24), δ 3.60 (H-23) to δ 54.4 (C-18), and δ 3.81 (H-22) to δ 174.2 (C-21) constructed the presence of fragment of –N($CH_3$)CH($CO_2$H)$CH_2CH_2CO_2CH_3$. The stereochemistry at C-18 was determined as *S* by comparing the sign of optical rotation of **6** with (L)-glutamic acid,[19] and consequently the structure of **6** was established as stellarboline F drawn in Fig. 1. Compound **7** was also characterized as *β*-carboline derivatives and the minor spectral differences between **7** and **6** were disappearance of two methyl groups and occurrence of one 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) in **7**. The HMBC $^{3}J$-correlation through proton signal at δ 3.84 (H-1') to carbon signal at δ 173.7 (C-21) indicated the formation of ester linkage between the carbonyl group C-21 and the butyl fragment. The stereochemistry at C-18 was also determined to be *S* as in **7** and nomenclatured as stellarboline G (Fig. 1). Compound **8** was an isomer of **7** since the molecular formula was the same with **7** according to the HRESIMS analytical data. The UV, IR, $^{1}$H, and $^{13}$C NMR spectral characteristics of **8** were almost identical with that of **7**. However, the two-dimensional HMBC analysis of **8** exhibited

different proton-carbon long distance correlations as displayed in **7**. The HMBC $^{2}J$ ,$^{3}J$-correlations from H-18 (δ 4.38) to C-22 (δ 172.6) and from H-1' (δ 4.08) to C-22 inferred the ester formation between C-22 and the butyl residue. The absolute stereochemistry at C-18 of **8** was characterized as *S* since it also displayed positive optical rotation. Therefore, compound **8** was assigned the structure as shown in Fig. 1 and named as stellarboline H trivially.

The $^{1}$H NMR spectrum of compound **9** was very similar with that of **2** except the disappearance of side chain connected at amide group N-17 which was also supported by comparing the molecular formula of **9** ($C_{20}H_{21}N_3O_8$) with **2** ($C_{24}H_{25}N_3O_{10}$). The glucose unit was connected at C-8 through C−O linkage indicated with the $^{3}J$- long range HMBC correlation between H-1' (δ 4.92) and C-8 (δ 144.3). Therefore the structure of **9** could be assigned as stellarboline I shown in the figure. Compound **10** was determined as $C_{20}H_{21}N_3O_9$ according to the HRESIMS spectral data. Comparison of the $^{1}$H NMR and $^{13}$C NMR spectra of **9** and **10**, 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), one set of glucosyl protons at δ 5.93-3.25 (11H), and one acetyl methyl group at δ 2.81 (3H, s) could also be found in **10**, however, the downfield singlet representative for H-4 in **9** was disappeared and instead one more oxygenated quaternary carbon signal at δ 158.5 was appeared. It was inferred that the only difference between **9** and **10** was that the C-4 in **10** was hydroxylated and complete structural assignments were achieved with NOESY and HMBC experiments. Consequently, compound **10** was assigned to be stellarboline J as shown in Fig. 1. The molecular formula of **11** was determined as $C_{14}H_{11}N_3O_3$ from the molecular ion peak at *m/z* 269.0802 in HREIMS spectral analysis. Comparison of the molecular formula of

**11** with **10**, it could be found that the $C_6H_{10}O_6$ moiety was reduced in **11**. As considering the $^1H$ NMR spectrum of **11**, the only differences between **11** and **10** were the proton signals of glucosyl unit were disappeared and the mutually coupled aromatic protons was changed through ABC type to symmetrical AA', BB' type 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). Both of these spectral data suggested that the A-ring of **11** was changed to be unsubstituted and other substitution pattern was same as in **10**. The NOESY and HMBC experiments furnished the complete assignments of proton and carbon signals of **11** and its structure was established as stellarboline K (Fig. 1).

Stellarboline L (**12**) was isolated as optically active light yellow powder (mp 163-165 ºC, $[\alpha]_D^{25}$ −139.0) and the molecular formula was established as $C_{23}H_{23}N_3O_5$ by the HREIMS analysis (*m/z* 421.1634 $[M]^+$). The UV absorption maxima and the IR absorption bands were indicative of *β*-carboline alkaloid. In the $^1H$ NMR spectrum of **12**, there were proton signals for one 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); one downfield aromatic proton at δ 8.92 (1H, s); one 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); one –$CHCH_3$ fragment signals at δ 6.71 (1H, q, *J* = 6.6 Hz) and 2.06 (3H, d, *J* = 6.6 Hz); and one methyl group at δ 3.80 (3H, s). It was almost identical with the reported compound, dichotomide II ,[9] except the more 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 one methyl group at δ 1.92 (3H, s). In addition, there are five more carbon signals found at δ 168.9, 139.9, 127.3, 20.6, and 16.0 in the $^{13}C$ NMR spectrum. Through the 2D experimental analysis, it

displayed $^{3}J$-HMBC long range correlations from δ 1.92 (H-5') to δ 168.9 (C-1'), and δ 2.00 (H-4') to δ 127.3 (C-2'); and NOESY correlations of H-3' (δ 6.13)/H-4' and H-5'. These spectral evidences showed that compound **12** had one more angelic acid substituent and further confirmed to be attached at C-14 to form ester linkage by the downfield chemical shift of H-14 (δ 6.71). The absolute stereochemistry at C-14 was determined as *S* while compared the sign of optical rotation with that of dichotomide II ,[9] and consequently the structure was deduced as shown in Fig. 1.

Stellarboline M (**13**) was purified with the molecular formula as $C_{12}H_8N_2O_3$ according to the HREIMS analysis. It possessed the above mentioned spectral characteristics including UV, IR, and $^{1}$H NMR (four mutually coupled aromatic protons and one downfield aromatic proton singlet). In addition, in the $^{1}$H NMR and $^{13}$C NMR spectra of **13**, there were two $D_2O$ exchangeable hydroxyl group signals at δ 13.52 and 11.02, and one carboxylic acidic carbonyl carbon signal at δ 163.3. It suggested that **13** possessed one hydroxyl and one carboxylic acid groups which could be substituted at C-1 or C-3 inferred by the other spectral data. The correct substitution pattern of **13** could be deduced through 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. Conclusively, the structure of **13** was characterized as stellarboline M as shown in Fig. 1.

In addition to the thirteen new *β*-carboline derivatives **1**-**13**, eight known constituents, including stellarine A and B,[20] dichotomide I and II,[9] dichotomine A and B,[9] glucodichotomine B,[8] and 1-acetyl-3-methoxycarbonyl-*β*-carboline [21] were also characterized from the methanol

extracts of roots of *S. dichotoma*. These known metabolites were all identified by spectroscopic data comparison with the published values reported in the literature. Among these isolates, five *β*-carboline derivatives were subjected into the examination of their bioactivity of inhibition of NO production in LPS-treated RAW 264.7 cells according to the reported method.[22,23] All of them showed significant inhibitory effect on NO production and the results were shown in Fig. 2. Moreover, the $IC_{50}$ values of all the tested compounds were in the range between 11.3 and 19.3 μM and were listed in Table 1. The stellarboline L (**12**) is the most potent one with the $IC_{50}$ value of 11.31 μM~~, compared to the reference compound aminoguanidine ($IC_{50}$ of  μM).~~

## Experimental Section

**General Experimental Procedures.** Melting points were determined using 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. $^{1}$H- and $^{13}$C-NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on the 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 were reported in parts per million (ppm, δ). The low and high-resolution EI and ESI mass spectra 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 spraying Dragendorff reagents followed by heating at 110 °C. The alkaloid spots would display the colors of orange to

light yellow. Lipopolysaccharide (LPS) from *Escherichia coli* and Griess reagent were purchased from Sigma (USA).

**Plant Material.** The roots of *Stellaria dichotoma* L. var. *lanceolata* (Caryophyllaceae) was bought from the pharmaceutical market on 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) was deposited in the herbarium of Department of Chemistry, National Cheng Kung University, Tainan, Taiwan.

**Extraction and Isolation.** Powdered roots of *S. dichotoma* (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 $CHCl_3$-solubles (80 g), *n*-butanol solubles (220 g), and water solubles (900 g), respectively. Successively, the $CHCl_3$-solubles were subjected into column chromatography over silica gel, eluted using a step gradient of *n*-hexane/acetone (9:1 to 1:1), to afford eleven fractions based on TLC profile. Each fraction was concentrated *in vacuo* and monitored by spraying Dragendorff reagents on the plates. The fractions 4-7 displayed positive response toward Dragendorff reagents examinations were further purified with silica gel column chromatography. Fractions 4 was further column chromatographed over silica gel using a stepwise gradient of *n*-hexane /EtOAc (9:1 to 1:1) to afford stellarine B (20.0 mg). Further purification of fraction 5 by repeated column chromatography with *n*-hexane-diisopropylether gradient mixtures (from 10:1 to 1:1) and preparative TLC purification gave 1-acetyl-3-methoxycarbonyl-β-carboline (150.0 mg) and

**12** (3.0 mg). 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) followed by preparative TLC with *n*-hexane-acetone (3:1) to afford dichotomide I (2.0 mg), dichotomide II (4.0 mg), **1** (3.0 mg), **3** (2.0 mg), **5** (3.0 mg), and **11** (2.0 mg). Alkaloid-contained fraction 7 was further chromatographed over silica gel eluted with chloroform-acetone (from 20:1 to 1:1) to yield stellarine A (50.0 mg).

The *n*-butanol solubles were subjected into reversed phase Diaion HP-20 column chromatography and yielded eleven subfractions according to the TLC monitoring results. In addition, subfractions 6-10 exhibited positive results against Dragendorff reagent tests. Subfraction 6 was purified with silica gel column chromatography using a stepwise gradient of chloroform-methanol (from 5:1 to 1:1) to obtain dichotomine B (3.0 mg), glucodichotomine B (5.0 mg), and **9** (3.5 mg). The seventh subfraction was subjected into column chromatography with silica gel by mixing eluents of chloroform-methanol (5:1) and then afforded dichotomine A (3.5 mg) and **13** (2.0 mg). The subfraction 8 which also displayed positive results against Dragendorff reagents test was chromatographed with silica gel by a stepwise gradient of chloroform-methanol (from 5:1 to 1:1) to yield **6** (1.5 mg). Alkaloid-contained subfraction 9 was further subjected into silica gel column chromatography eluted with chloroform-methanol (from 9:1 to 1:1) and further purified by repeated silica gel column chromatography to afford **2** (2.0 mg), **4** (1.5 mg), **8** (3.0 mg), and **10** (1.6 mg). The last alkaloid-contained subfraction 10 was purified with the aid of silica gel column chromatography eluted with the step gradient of chloroform-methanol (9:1) and finally resulted in **7** (2.0 mg). The $H_2O$-solubles were also subjected into reversed phase Diaion HP-20 column chromatography and yielded ten

subfractions. However, further Dragendorff reagent examinations toward the subfractions of $H_2O$-solubles did not show any positive responses.

**Stellarboline A (1):** yellow powder; mp > 280 ºC (MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 375 (3.57), 298 (4.50), 209 (4.34) nm; IR (nujol) $\nu_{max}$ 3344, 3068, 2941, 1695, 1681, 1647, 1481, 1440 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 12.30 (1H, s, NH, $D_2O$ exchangeable), 11.05 (1H, d, *J* = 11.2 Hz, NH, $D_2O$ exchangeable), 9.20 (1H, s, H-4), 8.46 (1H, d, *J* = 7.8 Hz, H-5), 8.10 (1H, dd, *J* = 14.0, 11.2 Hz, H-18), 7.83 (1H, d, *J* = 8.0 Hz, H-8), 7.63 (1H, dd, *J* = 8.0, 7.5 Hz, H-7), 7.35 (1H, dd, *J* = 7.8, 7.5 Hz, H-6), 6.15 (1H, d, *J* = 14.0 Hz, H-19), 3.68 (3H, s, $CH_3$-21), 2.97 (3H, s, $CH_3$-15); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 201.3 (C-14), 167.9 (C-20), 163.7 (C-16), 142.6 (C-13), 138.6 (C-18), 136.8 (C-3), 135.6 (C-10), 134.6 (C-1), 132.3 (C-11), 129.8 (C-7), 122.6 (C-5), 121.4 (C-6), 120.4 (C-12), 119.6 (C-4), 113.6 (C-8), 102.0 (C-19), 51.3 (C-21), 26.5 (C-15); 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).

**Stellarboline B (2):** yellow powder; mp > 280 ºC (MeOH); $[\alpha]^{25}_D$ +94.0 (*c* 0.01, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 376 (3.12), 325 (3.65, sh), 297 (3.99), 230 (3.69, sh), 212 (3.92) nm; IR (nujol) $\nu_{max}$ 3360, 2934, 1711, 1639, 1506, 1445, 1381 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 11.51 (1H, br s, NH, $D_2O$ exchangeable), 11.09 (1H, d, *J* = 11.2 Hz, H-17), 9.25 (1H, s, H-4), 8.19 (1H, d, *J* = 7.8 Hz, H-5), 8.10 (1H, dd, *J* = 14.1, 11.2 Hz, H-18), 7.42 (1H, d, *J* = 7.9 Hz, H-7), 7.31 (1H, dd, *J* = 7.9, 7.8 Hz, H-6), 6.16 (1H, d, *J* = 14.1 Hz, H-19), 5.96 (1H, br d, *J* = 3.6 Hz, OH, $D_2O$ exchangeable), 5.14 (1H, br d, *J* = 4.5 Hz, OH, $D_2O$ exchangeable), 5.09 (1H, br d, *J* = 5.2 Hz, OH, $D_2O$ exchangeable), 4.93 (1H, d, *J* = 7.3 Hz, H-1'), 4.62 (1H, br t, *J* = 5.5 Hz,

OH, $D_2O$ exchangeable), 3.77 (1H, dd, *J* = 11.5, 5.3 Hz, H-6'), 3.69 (3H, s, $CH_3$-21), 3.52 (1H, dd, *J* = 11.5, 5.9 Hz, H-6'), 3.43-3.39 (2H, m, H-2' & H-5'), 3.32 (1H, m, H-3'), 3.25 (1H, m, H-4'), 3.00 (3H, s, $CH_3$-15); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 201.4 (C-14), 167.8 (C-20), 163.5 (C-16), 144.4 (C-8), 138.5 (C-3), 137.3 (C-18), 135.5 (C-10), 135.1 (C-1), 132.8 (C-11), 132.5 (C-13), 122.3 (C-6 & C-12), 120.1 (C-4), 116.7 (C-5), 115.8 (C-7), 102.8 (C-1'), 102.1 (C-19), 77.5 (C-5'), 75.9 (C-3'), 73.5 (C-2'), 70.0 (C-4'), 60.9 (C-6'), 51.3 (C-21), 26.6 (C-15); 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).

**Stellarboline C (3):** yellow powder; mp > 280 ºC (MeOH); UV (MeOH) $\lambda_{max}$ (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 (nujol) $\nu_{max}$ 3356, 3275, 2920, 1693, 1666, 1628, 1510, 1437, 1389 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 12.53 (1H, d, *J* = 12.4 Hz, NH, $D_2O$ exchangeable), 11.84 (1H, s, NH, $D_2O$ exchangeable), 10.14 (1H, s, OH, $D_2O$ exchangeable), 9.20 (1H, s, H-4), 7.96 (1H, d, *J* = 8.0 Hz, H-5), 7.74 (1H, dd, *J* = 12.4, 8.4 Hz, H-18), 7.22 (1H, dd, *J* = 8.0, 7.8 Hz, H-6), 7.04 (1H, d, *J* = 7.8 Hz, H-7), 5.35 (1H, d, *J* = 8.4 Hz, H-19), 3.73 (3H, s, $CH_3$-21), 2.92 (3H, s, $CH_3$-15); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 201.1 (C-14), 168.8 (C-20), 162.4 (C-16), 143.6 (C-8), 137.9 (C-18), 136.1 (C-3), 135.4 (C-10), 134.5 (C-1), 132.9 (C-11), 131.2 (C-13), 122.8 (C-12), 122.5 (C-6), 119.7 (C-4), 115.3 (C-7), 113.5 (C-5), 97.0 (C-19), 51.4 (C-21), 25.7 (C-15); 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).

**Stellarboline D (4):** yellow powder; mp > 280 ºC (MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 389 (3.50), 286 (4.17), 235 (4.15, sh), 212 (4.30) nm; IR (nujol) $\nu_{max}$ 3477, 3342, 3221, 2924, 1668,

1556, 1412, 1365 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 11.63 (1H, br s, NH, $D_2O$ exchangeable), 10.08 (1H, br s, NH, $D_2O$ exchangeable), 9.05 (1H, s, H-4), 8.21 (1H, br s, NH, $D_2O$ exchangeable), 7.90 (1H, d, *J* = 7.5 Hz, H-5), 7.66 (1H, br s, NH, $D_2O$ exchangeable), 7.20 (1H, dd, *J* = 7.8, 7.5 Hz, H-6), 7.02 (1H, d, *J* = 7.8 Hz, H-7), 2.90 (3H, s, $CH_3$-15); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 201.9 (C-14), 166.5 (C-16), 143.7 (C-8), 139.4 (C-3), 135.0 (C-10), 134.3 (C-1), 132.6 (C-11), 131.2 (C-13), 122.7 (C-12), 122.5 (C-6), 118.6 (C-4), 115.0 (C-7), 113.3 (C-5), 26.2 (C-15); 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).

**Stellarboline E (5):** yellow powder; mp 185-187 ºC (MeOH); $[\alpha]^{25}_D$ −36.9 (*c* 0.01, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 377 (3.32), 287 (4.15), 271 (3.96, sh), 219 (4.07) nm; IR (nujol) $\nu_{max}$ 3350, 2925, 2846, 1728, 1705, 1664, 1508, 1452 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 12.23 (1H, br s, NH, $D_2O$ exchangeable), 9.30 (1H, d, *J* = 10.0 Hz, OH, $D_2O$ exchangeable), 9.15 (1H, s, H-4), 8.45 (1H, d, *J* = 7.8 Hz, H-5), 7.83 (1H, d, *J* = 8.0 Hz, H-8), 7.62 (1H, dd, *J* = 8.0, 7.4 Hz, H-7), 7.34 (1H, dd, *J* = 7.8, 7.4 Hz, H-6), 5.65 (1H, dt, *J* = 10.0, 4.8 Hz, H-18), 3.65 (3H, s, $CH_3$-21), 3.28 (3H, s, $CH_3$-22), 2.96 (2H, m, $CH_2$-19), 2.93 (3H, s, $CH_3$-15); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 201.1 (C-14), 170.8 (C-20), 164.6 (C-16), 142.6 (C-13), 137.8 (C-3), 135.2 (C-10), 134.2 (C-1), 132.2 (C-11), 129.6 (C-7), 122.5 (C-5), 121.1 (C-6), 120.4 (C-12), 118.7 (C-4), 113.5 (C-8), 77.7 (C-18), 55.4 (C-21), 51.8 (C-22), 39.6 (C-19), 26.1 (C-15); 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).

**Stellarboline F (6):** yellow powder; mp > 280 ºC (MeOH); $[\alpha]^{25}_D$ +20.0 (*c* 0.04, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 378 (3.43), 287 (4.27), 268 (4.09, sh), 239 (3.79, sh), 220 (4.20) nm; IR

(nujol) $\nu_{max}$ 3032, 1734, 1647, 1537, 1456, 1232 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 300 MHz) δ 8.96 (1H, s, H-4), 8.25 (1H, d, *J* = 7.8 Hz, H-5), 7.73 (1H, d, *J* = 8.0 Hz, H-8), 7.62 (1H, dd, *J* = 8.0, 7.6 Hz, H-7), 7.35 (1H, dd, *J* = 7.8, 7.6 Hz, H-6), 4.73 (1H, dd, *J* = 8.0, 4.5 Hz, H-18), 3.81 (3H, s, $CH_3$-22), 3.60 (3H, s, $CH_3$-23), 2.96 (3H, s, $CH_3$-15), 2.35-2.24 (4H, m, H-19 & -20); $^{13}C$ NMR ($CD_3OD$, 75 MHz) δ 203.1 (C-14), 180.9 (C-24), 174.2 (C-21), 167.1 (C-16), 143.8 (C-13), 139.1 (C-3), 137.0 (C-10), 135.7 (C-1), 133.7 (C-11), 130.6 (C-7), 122.8 (C-5), 122.2 (C-6), 122.0 (C-12), 118.7 (C-4), 113.8 (C-8), 64.3 (C-23), 54.4 (C-18), 52.8 (C-22), 35.1 (C-20), 29.5 (C-19), 26.2 (C-15); 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).

**Stellarboline G (7):** yellow powder; mp 222-224 ºC (MeOH); $[\alpha]^{25}_D$ +48.0 (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 375 (3.50), 286 (4.35), 268 (4.16, sh), 220 (4.26) nm; IR (nujol) $\nu_{max}$ 3386, 2960, 1734, 1654, 1601, 1533, 1499, 1413 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 12.16 (1H, br s, NH, $D_2O$ exchangeable), 9.05 (1H, s, H-4), 8.93 (1H, br d, *J* = 6.4 Hz, NH, $D_2O$ exchangeable), 8.41 (1H, d, *J* = 7.8 Hz, H-5), 7.80 (1H, d, *J* = 8.0 Hz, H-8), 7.59 (1H, dd, *J* = 8.0, 7.3 Hz, H-7), 7.31 (1H, dd, *J* = 7.8, 7.3 Hz, H-6), 4.24 (1H, br d, *J* = 4.4 Hz, H-18), 3.84 (2H, t, *J* = 7.3 Hz, H-1'), 2.87 (3H, s, $CH_3$-15), 2.36 (2H, m, H-20), 2.22 (1H, m, H-19), 2.05 (1H, m, H-19), 1.39 (2H, m, H-2'), 1.19 (2H, m, H-3'), 0.73 (3H, t, *J* = 7.3 Hz, $CH_3$-4'); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 201.3 (C-14), 174.4 (C-22), 173.7 (C-21), 163.9 (C-16), 143.0 (C-13), 139.3 (C-3), 135.4 (C-10), 134.5 (C-1), 132.7 (C-11), 130.0 (C-7), 122.9 (C-5), 121.5 (C-6), 121.0 (C-12), 118.4 (C-4), 114.0 (C-8), 64.1 (C-1'), 53.9 (C-18), 30.9 (C-2'), 30.8 (C-20), 28.4 (C-19), 26.3 (C-15), 19.2 (C-3'), 14.1 (C-4'); 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).

**Stellarboline H (8):** yellow powder; mp 240-242 ºC (MeOH); $[\alpha]^{25}_{D}$ +8.0 (*c* 0.03, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 373 (3.70), 306 (3.95, sh), 286 (4.54), 268 (4.34, sh), 220 (4.43) nm; IR (nujol) $\nu_{max}$ 3200, 2959, 1734, 1635, 1527, 1458 $cm^{-1}$; $^{1}H$ NMR (DMSO-$d_6$, 400 MHz) δ 12.15 (1H, br s, NH, $D_2O$ exchangeable), 10.44 (1H, br d, *J* = 7.5 Hz, NH, $D_2O$ exchangeable), 9.02 (1H, s, H-4), 8.40 (1H, d, *J* = 7.8 Hz, H-5), 7.82 (1H, d, *J* = 8.1 Hz, H-8), 7.60 (1H, dd, *J* = 8.1, 7.6 Hz, H-7), 7.31 (1H, dd, *J* = 7.8, 7.6 Hz, H-6), 4.38 (1H, d, *J* = 5.3 Hz, H-18), 4.08 (2H, m, H-1'), 3.00 (3H, s, $CH_3$-15), 2.11-2.02 (4H, m, H-19 & -20), 1.56 (2H, m, H-2'), 1.34 (2H, m, H-3'), 0.86 (3H, t, *J* = 7.3 Hz, $CH_3$-4'); $^{13}C$ NMR (DMSO-$d_6$, 100 MHz) δ 202.0 (C-14), 175.8 (C-21), 172.6 (C-22), 164.9 (C-16), 142.5 (C-13), 138.9 (C-3), 135.0 (C-10), 134.4 (C-1), 131.9 (C-11), 129.4 (C-7), 122.4 (C-5), 120.9 (C-6), 120.5 (C-12), 118.1 (C-4), 113.5 (C-8), 64.0 (C-1'), 54.5 (C-18), 34.8 (C-20), 30.4 (C-2'), 27.4 (C-19), 26.7 (C-15), 18.8 (C-3'), 13.8 (C-4'); ESIMS *m/z* 478 $[M+K]^+$ (100), 462 $[M+Na]^+$ (72), 453 (48), 413 (34), 397 (35), 381 (83); HRESIMS *m/z* 462.1643 $[M+Na]^+$ (calcd for $C_{23}H_{25}N_3O_6Na$, 462.1641).

**Stellarboline I (9):** yellow powder; mp > 280 ºC (MeOH); $[\alpha]^{25}_{D}$ +40.0 (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 379 (3.71), 284 (4.47), 221 (4.48) nm; IR (nujol) $\nu_{max}$ 3300, 2924, 1670, 1578, 1397 $cm^{-1}$; $^{1}H$ NMR (DMSO-$d_6$, 400 MHz) δ 11.36 (1H, br s, NH, $D_2O$ exchangeable), 9.10 (1H, s, H-4), 8.23 (1H, br s, NH, $D_2O$ exchangeable), 8.12 (1H, d, *J* = 7.8 Hz, H-5), 7.69 (1H, br s, NH, $D_2O$ exchangeable), 7.40 (1H, d, *J* = 7.5 Hz, H-7), 7.28 (1H, dd, *J* = 7.8, 7.5 Hz, H-6), 5.95 (1H, br d, *J* = 3.9 Hz, OH, $D_2O$ exchangeable), 5.14 (1H, br d, *J* = 4.8 Hz, OH, $D_2O$ exchangeable), 5.09 (1H, br d, *J* = 5.3 Hz, OH, $D_2O$ exchangeable), 4.92 (1H, d, *J* = 7.8 Hz, H-1'), 4.62 (1H, br t, *J* = 5.8 Hz, OH, $D_2O$ exchangeable), 3.76 (1H, dd, *J* = 11.5, 5.6 Hz, H-6'), 3.50 (1H, dd, *J* = 11.5, 6.0 Hz, H-6'), 3.45-3.33 (3H, m, H-2', -3' & -5'), 3.24 (1H, m, H-4'), 2.90

(3H, s, $CH_3$-15); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 201.5 (C-14), 166.4 (C-16), 144.3 (C-8), 139.6 (C-3), 135.0 (C-1), 134.6 (C-10), 132.8 (C-11), 132.4 (C-13), 122.4 (C-6), 122.0 (C-12), 118.6 (C-4), 116.5 (C-5), 115.6 (C-7), 102.8 (C-1'), 77.6 (C-5'), 76.0 (C-3'), 73.5 (C-2'), 70.0 (C-4'), 61.0 (C-6'), 26.3 (C-15); 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).

**Stellarboline J (10):** yellow powder; mp > 280 ºC (MeOH); $[\alpha]^{25}_D$ +20.5 (*c* 0.02, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 367 (3.71), 297 (4.07, sh), 279 (4.31), 247 (4.05, sh) nm; IR (nujol) $\nu_{max}$ 3385, 2922, 1661, 1603, 1367, 1279 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 14.65 (1H, br s, OH, $D_2O$ exchangeable), 11.34 (1H, br s, NH, $D_2O$ exchangeable), 8.61 (1H, br s, NH, $D_2O$ exchangeable), 8.30 (1H, br s, NH, $D_2O$ exchangeable), 7.91 (1H, d, *J* = 7.2 Hz, H-5), 7.35 (1H, d, *J* = 7.2 Hz, H-7), 7.29 (1H, t, *J* = 7.2 Hz, H-6), 5.93 (1H, br d, *J* = 3.6 Hz, OH, $D_2O$ exchangeable), 5.12 (1H, br d, *J* = 4.4 Hz, OH, $D_2O$ exchangeable), 5.02 (1H, br d, *J* = 5.2 Hz, OH, $D_2O$ exchangeable), 4.92 (1H, d, *J* = 7.6 Hz, H-1'), 4.60 (1H, br t, *J* = 5.6 Hz, OH, $D_2O$ exchangeable), 3.76 (1H, dd, *J* = 10.4, 5.2 Hz, H-6'), 3.59 (1H, dd, *J* = 10.4, 6.0 Hz, H-6'), 3.50 (2H, m, H-2' & -5'), 3.32 (1H, m, H-3'), 3.25 (1H, m, H-4'), 2.81 (3H, s, $CH_3$-15); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 200.4 (C-14), 172.9 (C-16), 158.5 (C-4), 144.7 (C-8), 137.7 (C-10), 131.5 (C-13), 129.6 (C-1), 122.9 (C-6), 121.2 (C-12), 121.0 (C-3), 118.1 (C-11), 117.7 (C-5), 114.6 (C-7), 103.2 (C-1'), 78.0 (C-5'), 76.5 (C-3'), 74.0 (C-2'), 70.4 (C-4'), 61.4 (C-6'), 26.3 (C-15); 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).

**Stellarboline K (11):** yellow powder; mp 243-245 ºC (MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 363 (3.67), 303 (3.85, sh), 282 (4.31), 248 (3.99), 211 (4.19) nm; IR (nujol) $\nu_{max}$ 3474, 3329,

3261, 1675, 1601, 1448, 1377 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) δ 14.45 (1H, br s, OH, $D_2O$ exchangeable), 12.05 (1H, br s, NH, $D_2O$ exchangeable), 8.71 (1H, br s, NH, $D_2O$ exchangeable), 8.25 (1H, d, *J* = 7.3 Hz, H-5), 8.21 (1H, br s, NH, $D_2O$ exchangeable), 7.80 (1H, d, *J* = 8.0 Hz, H-8), 7.52 (1H, dd, *J* = 8.0, 7.0 Hz, H-7), 7.30 (1H, dd, *J* = 7.3, 7.0 Hz, H-6), 2.78 (3H, s, $CH_3$-15); 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).

**Stellarboline L (12):** yellow powder; mp 163-165 ºC ($CHCl_3$); $[\alpha]^{25}_D$ −139.0 (*c* 0.28, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 387 (1.32), 325 (4.03), 285 (4.25), 228 (4.15) nm; IR (nujol) $\nu_{max}$ 3371, 3053, 2935, 1697, 1629, 1479, 1444, 1379 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 12.52 (1H, br d, *J* = 12.4 Hz, NH, $D_2O$ exchangeable), 9.73 (1H, br s, NH, $D_2O$ exchangeable), 8.92 (1H, s, H-4), 8.16 (1H, d, *J* = 7.8 Hz, H-5), 7.72 (1H, dd, *J* = 12.4, 8.8 Hz, H-18), 7.60-7.55 (2H, m, H-7 & -8), 7.34 (1H, ddd, *J* = 7.9, 7.8, 1.9 Hz, H-6), 6.71 (1H, q, *J* = 6.6 Hz, H-14), 6.13 (1H, q, *J* = 7.0 Hz, H-3'), 5.26 (1H, d, *J* = 8.8 Hz, H-19), 3.80 (3H, s, $CH_3$-21), 2.06 (3H, d, *J* = 6.6 Hz, H-15), 2.00 (3H, d, *J* = 7.0 Hz, H-4'), 1.92 (3H, s, $CH_3$-5'); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 168.9 (C-1'), 168.7 (C-20), 163.8 (C-16), 140.6 (C-1 & -13), 139.9 (C-3'), 137.5 (C-3), 137.4 (C-18), 135.9 (C-10), 130.6 (C-11), 129.1 (C-7), 127.3 (C-2'), 121.9 (C-5 & -12), 121.1 (C-6), 115.9 (C-4), 112.1 (C-8), 97.0 (C-19), 69.8 (C-14), 51.1 (C-21), 20.6 (C-5'), 17.9 (C-15), 16.0 (C-4'); 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).

**Stellarboline M (13):** yellow powder; mp > 280 ºC (MeOH); UV (MeOH) $\lambda_{max}$ (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 (nujol) $\nu_{max}$ 3292, 3173, 1711, 1647, 1254 $cm^{-1}$; $^1H$ NMR

(DMSO-$d_6$, 400 MHz) δ 13.52 (1H, br s, OH, $D_2O$ exchangeable), 12.44 (1H, br s, NH, $D_2O$ exchangeable), 11.02 (1H, br s, OH, $D_2O$ exchangeable), 8.18 (1H, d, *J* = 7.8 Hz, H-5), 7.88 (1H, s, H-4), 7.55 (1H, d, *J* = 8.2 Hz, H-8), 7.46 (1H, dd, *J* = 8.2, 7.8 Hz, H-7), 7.24 (1H, dd, *J* = 7.8, 7.6 Hz, H-6); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 163.3 (C-14), 154.9 (C-1), 139.5 (C-13), 131.1 (C-10), 126.9 (C-7), 125.8 (C-3), 122.7 (C-11), 122.4 (C-12), 121.8 (C-5), 120.7 (C-6), 113.0 (C-8), 105.9 (C-4); 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 of LPS-stimulated RAW 264.7 cells.** RAW 264.7 cells ($1\times10^4$ cells/well) were seeded in 96 well plates with DMEM medium supplemented with 10% FBS and incubated for 12 h. The cells were individually pretreated with different concentration (6.25, 12.5, 18.75 μM) of eight compounds isolated from *Stellaria dichotoma* L. for 2 h. Then RAW cells were treated with 0.1 μg/ml of LPS, and incubated for 24 h. Control group was treated with LPS only. One hundred microliter of the medium was incubated with an equal volume of Griess reagent for 15 min at room temperature. Nitrite production was determined by the 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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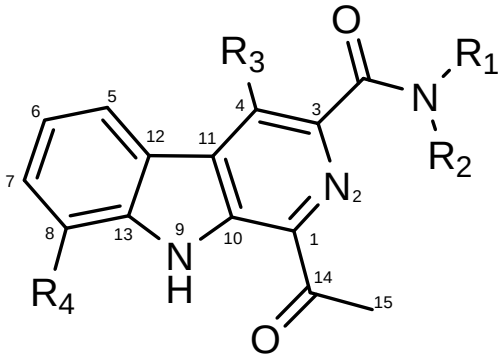
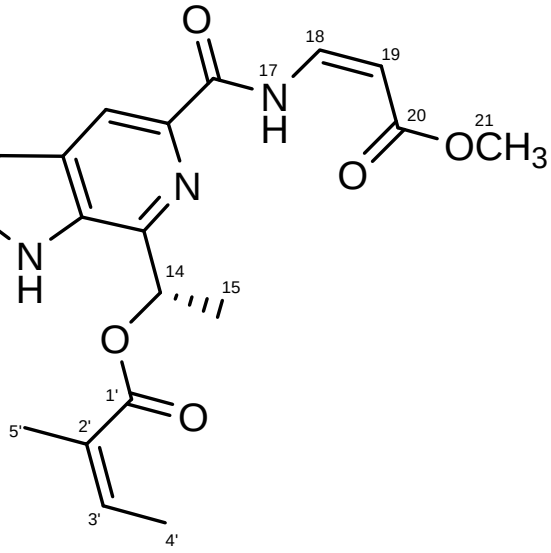
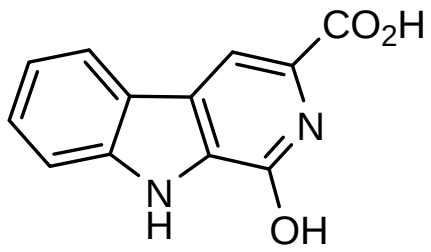
**Figure 1.** Chemical structures of *β*-carboline derivatives **1-13**




**1** $R_1$ = (E)-CH=CHCO$_2$CH$_3$, $R_2$ = H, $R_3$ = H, $R_4$ = H
**2** $R_1$ = (E)-CH=CHCO$_2$CH$_3$, $R_2$ = H, $R_3$ = H, $R_4$ = *O*-*β*-D-glc
**3** $R_1$ = (Z)-CH=CHCO$_2$CH$_3$, $R_2$ = H, $R_3$ = H, $R_4$ = OH
**4** $R_1$ = H, $R_2$ = H, $R_3$ = H, $R_4$ = OH
**5** $R_1$ = CHOHCH$_2$CO$_2$CH$_3$, $R_2$ = CH$_3$, $R_3$ = H, $R_4$ = H
**6** $R_1$ = CH(CO$_2$H)CH$_2$CH$_2$CO$_2$CH$_3$, $R_2$ = CH$_3$, $R_3$ = H, $R_4$ = H
**7** $R_1$ = CH(CO$_2$H)CH$_2$CH$_2$CO$_2$(CH$_2$)$_3$CH$_3$, $R_2$ = H, $R_3$ = H, $R_4$ = H
**8** $R_1$ = CH[CO$_2$(CH$_2$)$_3$CH$_3$]CH$_2$CH$_2$CO$_2$H, $R_2$ = H, $R_3$ = H, $R_4$ = H
**9** $R_1$ = H, $R_2$ = H, $R_3$ = H, $R_4$ = *O*-*β*-D-glc
**10** $R_1$ = H, $R_2$ = H, $R_3$ = OH, $R_4$ = *O*-*β*-D-glc
**11** $R_1$ = H, $R_2$ = H, $R_3$ = OH, $R_4$ = H




**12**




**13**

**Figure 2.** NO production after 24h-treatment of LPS-stimulated RAW 264.7 cells with eight compounds isolated from *S. dichotoma* at concentrations from 6.25 to 18.75 μM.

Data are the mean ± S.E. of three independent experiments. Statistically different when *$p$<0.05, **$p$<0.01, ***$p$<0.001 compared with control.

**Table 1.** $IC_{50}$ of isolated compounds from *S. dichotoma* on the inhibition of NO production after 24 h-treatment of LPS-stimulated RAW 264.7 cells.

| **Compound** | **$IC_{50}$ (μM)** |
|---|---|
| **1** | 17.3 |
| **12** | 11.3 |
| stellarine A | 19.3 |
| stellarine B | 18.6 |
| 1-acetyl-3-methoxycarbonyl-*β*-carboline | 17.9 |
| Aminoguanidine | |