Alkaloids from the flower of *Erythrina arborescens*†

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Phytochemical investigations on the flower of *Erythrina arborescens* resulted in the isolation of eight new *Erythrina* alkaloid, erytharborines A–H (1–8), together with 17 known alkaloids. Erytharborines A/B (1–2) and C (3) possessed an 2H-imidazole ring and a unique oxime moiety, respectively. The structures were elucidated on the basis of UV, IR, mass spectrometry and NMR spectroscopic data.

Introduction

The *Erythrina* and *Homoerythrina*-type alkaloids, derived from two tyrosine units via oxidative coupling and intramolecular rearrangement, consist of more than 200 alkaloids from *Erythrina* and *Cephalotaxus* genus.1,2 The erythrinan alkaloids are ubiquitous compounds in the *Erythrina* genus of family Leguminosae. Special attention has been received in this field mainly by their curare-like neuro muscular blocking activities,3 anxiolytic-like activity,4 induced sleep,5 anticonvulsant activity,6 anticataract7 and antifeedant8 activity etc. Particularly noteworthy, the star molecule, dihydro-β-erythroidine, was used as tool to characterize neuronal nicotinic acetyl-choline receptors.9 Thus, pharmaceutical chemists paid much more attention to this type natural products. The erythrinan alkaloids possessed 6/5/6/6 spirocycle systems with a stable 5S-chiral center, seemingly exhibiting a not so diverse and fascinating molecular architecture. Nevertheless, the spirocyclic and aromatic skeleton in erythrinan alkaloids became challenging polycyclic molecular architectures.10–12 Generally speaking, skeleton rearrangement served as the main pathway to structural diversity of natural products. Analogously, besides aromatic erythrinan alkaloid (erysotramidine13), this class compound also included nonaromatic alkaloid, e.g. six-membered lactone (β-erythroidine14) and pyridine ring D (erymelanthine15). Both molecules attracted many interests in total synthesis.1,16,17 Under considerable efforts of our research group devoted to the phytochemical investigations on *Erythrina* species, several novel dimeric and trimeric erythrinan alkaloids some of which showed cytotoxicity were obtained.18,19 As part of an ongoing research for structural newly erythrinan alkaloids, phytochemical investigation of the flowers of *Erythrina arborescens* Roxb. led to eight new alkaloids erytharborines A–H (1–8) (Fig. 1) together with seventeen known alkaloids. Their isolation and structure elucidation were described in this study.

Fig. 1 Structures of erytharborines A–H (1–8).
Results and discussion

The alkaloid fraction of *E. arborescens* was separated to yield a total of 25 compounds by a combination of chromatographic procedures as described in the Experimental section. All compounds might be alkaloids since they showed positive response with Dragendorff’s reagent on TLC.

The UV absorptions (202, 227, 289 and 322 nm) and IR spectrum (1710, 1629, 1479 cm⁻¹) of erytharborine A (I) indicated a good conjugated system. Presence of the typical conjugate olefinic signals (δH 6.81, 6.04, 5.96), two aromatic singlet protons (δH 7.57 and 7.27) and three methoxyl groups (δH 3.90, 3.81 and 3.20) in the ¹H NMR spectrum of 1, displayed the untapped A, B and D-rings of conjugated dienoid type erythrinan alkaloids. Two characteristic methylenes at δc 48.7 and 56.9 in the ¹³C NMR spectrum together with their HMBC correlations assigned themselves to C-4 and C-8, respectively. The untapped A, B and D-rings of 1 was further supported by its key correlations observed in the HMBC spectrum, δH 6.81 (H-1)/δc 76.8 (C-3) and 71.5 (C-5), δH 6.04 (H-2)/δc 48.7 (C-4), 140.4 (C-6), δH 5.96 (H-7)/δc 125.7 (C-1) and 71.5 (C-5), δH 7.27 (H-14)/δc 71.5 (C-5), 119.6 (C-12) and 149.9 (C-16), δH 7.57 (H-17)/δc 136.9 (C-13) and 152.4 (C-15) (Fig. 2). Its molecular formula C₂₀H₂₅NO₃Cl₂ of alkaloid (I) was deduced from HRESIMS at m/z = 380.1961 [M + H]+ (calcd. 380.1967), with three more carbons including two methyl groups (δc 25.0, 25.9) than general *Erythrina* alkaloid. In the HMBC spectrum, the correlations between H-17 and δc 155.4 (s) attributing the latter signal to C-11. Likewise, the correlations between H-8 (δH 4.56, 4.25) with δc 157.4 (s) attributing the latter signal to C-10. The HMBC correlations of δH 1.46 (3H) and 1.39 (3H) with δc 104.3 (s) established the linkage of the three carbons. Based on the molecular formula, 2H-imidazole ring was necessary in consideration of remainder unsaturation degrees of 1 (Fig. 2). In the ROESY spectrum, the NOE correlation of H-3/H-14 suggested H-3 was in β-orientation.

Erytharborine B (2) was obtained as pale yellow amorphous powder with similar UV and IR absorption to 1. Its molecular formula was confirmed to be C₁₇H₁₃NO₂ by HRESIMS at m/z = 364.1658 [M + H]+ (calcd. 364.1656), with 14 daltons more than 1. Comparing their closely resembled ³H and ¹³C NMR data value (Table 1), compound 2 must possess a methylenedioxy group (δH 6.12 and 6.09) at C-15 and C-16 in place with the two methoxyl groups (δH 3.90 and 3.81) in 1.

![Fig. 2 Key HMBC and 1H-1H COSY correlations of 1 and 3.](image)

The UV absorption of erytharborine C (3) at 204 and 289 nm indicated a tetrahydroisoquinoline chromophore. ²⁶ Meanwhile, its IR absorption bands at 3414 and 1611, 1513, and 1458 cm⁻¹ resulted from the hydroxyls and aromatic rings, which was consistent with the characteristic of *Erythrina* alkaloid. Its molecular formula was determined to be C₁₉H₂₅N₂O₄Cl₂ based on HRESIMS at m/z = 427.1919 [M + H]+, indicating nine degrees of unsaturation. The isotope peaks showed in the positive ESI-MS confirmed the presence of two chlorine atoms. The ¹H, ¹³C NMR and HSQC data for 3 indicated the presence of four methylenes, three methoxyls, three sp³ and three sp² methines, one sp³ and six sp² quaternary carbons. Above data further suggested 3 was similar to erhatridine (27) except for an additional carbon and nitrogen, and two chlorine atoms. In the HMBC spectrum, correlations from δH 6.71 (H-17) to δc 20.6 (C-11), δc 126.3 (C-13), and δc 147.8 (C-15), and from δH 6.28 (H-14) to δc 63.7 (C-5), δc 125.5 (C-12), and δc 146.1 (C-16) suggested D-ring was not changed. ²⁷ Its ¹H-¹H COSY correlations of H-11 (δH 2.98 and 2.49) to H-10 (δH 3.09 and 3.30), together with the correlation of H-10 with C-5 in the HMBC spectrum indicated C-ring was untapped. The coupling –CH₂ (δH 2.94 and 3.18) and –CH (δH 3.66) correlated with C-6 in the HMBC spectrum, respectively, also assigned them to H-8 and H-7 and suggested ring-B was substituted. The methine δH 5.86 was attributed to newly CH-19 based on its ¹H-¹H COSY correlation with H-7, which was supported by the HMBC correlations from H-19 to C-8. Downfield proton and carbon signal of CH-19 meant linkage with two Cl atoms, also consideration of its molecular formula. Finally, singlet signal H-1 (δH 7.07) showed HMBC correlations with C-7 and C-5 suggested a double bond at C-1/6. Correlations of H-3 (δH 4.14)/H-4 (δH 2.71 and 1.68) in the ¹H-¹H COSY spectrum together with the HMBC correlations between H-4 with δc 151.6 assigned the signal to C-2. The remainder of a nitrogen atom and degree of unsaturation suggested there should be an E-oxime moiety as shown in Fig. 2, which was supported by HMBC correlation of δH 11.20 (OH) with C-2.

The molecular formula C₂₀H₂₅NO₃Cl₂ of alkaloid (4) was established by HRESIMS ([M + H]+ at m/z 398.1281) and was consistent with the ¹³C NMR spectrum, which revealed 20 carbonic resonance signal. The 1D NMR spectroscopic data of compound 4 were similar to those of compound 3 except for the following differentiations: in the ¹H NMR spectrum, the signal displayed at δH 11.20 in 3 which was assigned to the active hydrogen in the oxime moiety was disappeared in compound 4. Correspondingly, the quaternary carbon signals at δC 151.6 (C-2) in compound 3 was replaced with a methylene (δc 33.1) in compound 4. Thus, compound 4 might be an analogue of 3 without the oxime moiety. The HMBC correlations of δH 2.05 (H-2)/δC 125.4 (C-1), δC 74.1 (C-3) and δC 140.5 (C-6) together with the HSQC data demonstrated directly that the methylene did belong to C-2. Relative configuration of H-3 in 3 and 4 was deduced as β from the coupling constants (J₃,4eq. = 5.0 Hz, J₃,4ax = 11.0 Hz) in the ¹H NMR spectrum. ²⁸ This presumption was confirmed by the obvious NOE correlations of H-3/H-14. Likewise, the correlations of H-7/H-17 in the ROESY spectrum showed H-7 in 3 and 4 was β-oriented, too. The oxime of C₂/N₁₈ in 3 was determined as E via NOE between OH and H-1.
Erytharborine E (5) was isolated as an amorphous solid. Its molecular formula was deduced as \( \text{C}_{19}\text{H}_{23}\text{N}_{2}\text{O}_{6} \) from the HRE-SIMS ([M + H]+ at 330.1699) and \(^{13}\text{C}\) NMR spectroscopic data, inferring nine degrees of unsaturation. In comparing with the \(^{13}\text{C}\) NMR data of erysotrine,\(^{13}\) compound 5 showed a oxygennated quaternary carbon \((\delta_{C} 71.2)\) and a oxygenated methine \((\delta_{C} 64.6)\) at up-field instead of olefinic signals of \(\delta_{C} 143.4 (\text{C}-6)\) and \(\delta_{C} 123.6 (\text{C}-7)\) of erysotrine, which suggested presence of an epoxide ring at C-6/7. The HMBC correlations of \(\delta_{H} 2.87 (\text{H}-8)/\delta_{C} 64.6 (\text{C}-7), \delta_{C} 71.2 (\text{C}-6), \delta_{H} 5.76 (\text{H}-1)/\delta_{C} 64.6 (\text{C}-7)\) and \(\delta_{H} 6.25 (\text{H}-2)/\delta_{C} 71.2 (\text{C}-6)\) confirmed this conclusion. The epoxide was assigned as \(\beta\)-orientation on the base of molecule model.

Erytharborine F (6) was obtained as a white amorphous powder. Its molecular formula was determined to be \( \text{C}_{19}\text{H}_{21}\text{N}_{2}\text{O}_{5} \) based on its HRESIMS at \( \text{m/z} \) 398.1212 ([M + Na]+) and NMR spectra. The \(^1\text{H}\) NMR spectra (Table 2) confirmed the presence of two aromatic singlet protons \((\delta_{H} 7.36 \text{ and } 7.18)\), one olefinic proton \((\delta_{H} 6.18)\) and three methyols \((\delta_{H} 3.93, 3.90 \text{ and } 3.20)\). Its \(^1\text{H}\) and \(^{13}\text{C}\) NMR data resembled those of (+)-10,11-dioxy-epierythrindine\(^{28}\) with exception for an additional hydroxyl group, which was deduced from its molecular formula. Substitution of hydroxyl group at C-7 was supported by the HMBC correlations of \(\delta_{H} 6.18 (\text{H}-1)/\delta_{C} 69.2 (\text{C}-7), \delta_{H} 4.31 (\text{H}-8)/\delta_{C} 69.2 (\text{C}-7)\) and \(\delta_{H} 4.98 (\text{H}-7)/\delta_{C} 144.8 (\text{C}-6)\). The signals at \(\delta_{H} 7.36 (\text{H}-17)\) showed correlation with the \(\delta_{C} 180.7 (\text{C}-11)\) in the HMBC spectrum, while the signals at \(\delta_{H} 4.31 (\text{H}-8)\) showed correlation with the \(\delta_{C} 152.4 (\text{C}-4)\), establishing dione at C-10/11. The hydroxyl group was determined to be attached at C-11 by HMBC correlations between \(\text{H}-8 (\delta_{H} 4.31)\) and \(\delta_{C} 172.6 (\text{C}=\text{O})\) assigned the carbonyl group to C-8 position. Its ROESY spectrum gave correlations of H-3/H-4, H-2/H-4 and H-7/H-14, which demonstrated the relative configuration of H-2, H-3 and H-7 were \(\beta\)-oriented.

| Table 1: \(^{13}\text{C}\) NMR spectroscopic data for 1–8 in acetone-\(d_6\) (\(\delta\) in ppm) |
|---|---|---|---|---|---|---|---|---|---|
| Entry | \(\delta_{C} (1)\) | \(\delta_{C} (2)\) | \(\delta_{C} (3)^{a}\) | \(\delta_{C} (4)^{a}\) | \(\delta_{C} (5)^{b}\) | \(\delta_{C} (6)^{a}\) | \(\delta_{C} (7)^{b}\) | \(\delta_{C} (8)^{b}\) |
| 1 | 125.7 d | 125.6 d | 114.5 d | 125.4 d | 128.6 d | 125.4 d | 123.8 d | 124.8 d |
| 2 | 132.6 d | 132.6 d | 151.6 s | 33.1 t | 136.9 d | 72.5 d | 62.8 d | 132.8 d |
| 3 | 76.8 d | 76.6 d | 73.4 d | 74.1 d | 76.5 d | 82.0 d | 76.9 d | 77.1 d |
| 4 | 48.7 t | 48.6 t | 42.9 t | 43.0 d | 46.5 t | 49.3 t | 35.6 t | 40.5 t |
| 5 | 71.5 s | 71.2 s | 63.7 s | 64.5 s | 66.1 s | 65.2 s | 62.3 s | 72.3 s |
| 6 | 140.4 s | 140.1 s | 150.1 s | 140.5 s | 71.2 s | 144.8 s | 140.7 s | 139.7 s |
| 7 | 120.9 d | 121.4 d | 51.3 s | 53.5 d | 64.6 d | 69.2 d | 70.7 d | 120.8 d |
| 8 | 56.9 t | 56.6 t | 49.9 t | 53.2 t | 56.4 t | 51.2 t | 172.6 s | 54.7 t |
| 9 | 157.5 s | 157.1 s | 39.8 t | 40.8 t | 51.6 t | 159.4 s | 35.8 t | 173.8 s |
| 10 | 155.4 s | 155.6 s | 20.6 t | 21.7 t | 29.4 t | 180.7 s | 25.9 t | 68.3 d |
| 11 | 119.6 s | 121.4 s | 25.3 s | 130.2 s | 130.9 s | 123.5 s | 126.8 s | 129.4 s |
| 12 | 136.9 s | 138.7 s | 126.3 s | 124.6 s | 141.2 s | 140.0 s | 131.9 s | 131.0 s |
| 13 | 108.2 d | 105.3 d | 110.9 d | 112.7 d | 110.1 d | 109.4 d | 110.3 d | 108.4 d |
| 14 | 152.4 s | 151.5 s | 147.8 s | 149.5 s | 148.0 s | 154.2 s | 147.9 s | 148.7 s |
| 15 | 149.9 s | 148.5 s | 146.1 s | 147.7 s | 148.8 s | 150.3 s | 149.9 s | 149.9 s |
| 16 | 109.9 d | 107.2 d | 112.7 d | 113.6 d | 112.7 d | 110.5 d | 114.3 d | 109.3 d |
| 17 | 25.0 q | 25.7 q | 25.9 q | 26.9 q | 74.3 d | | | |
| 18 | 104.3 s | 100.5 s | 58 q, 5 q | 56 q | 56.2 q | 56.0 q | 56.1 q | |
| 19 | 3-OCH\(_3\) | 56.2 q | 56.6 q | 56 q, 5 q | 55.9 q | 56.0 q | 56.2 q | 56.3 q |
| 20 | 15-OCH\(_3\) | 56.1 q | 55.4 q | 56.1 q | 56.3 q | 56.6 q | 56.2 q | 56.3 q |
| 21 | 16-OCH\(_3\) | 56.1 q | 55.3 q | 56.1 q | 56.4 q | 56.4 q | 56.3 q | 56.3 q |

\(^{a}\) \(^{13}\text{C}\) NMR recorded in 150 MHz. \(^{b}\) \(^{13}\text{C}\) NMR recorded in 125 MHz. Compound 3 was recorded in DMSO-\(d_6\).
Table 2  $^1$H NMR spectroscopic data for 1–8 in acetone-$d_6$ ($J$ in Hz)

| Entry | $\delta$H (1)$^a$ | $\delta$H (2)$^a$ | $\delta$H (3)$^a$ | $\delta$H (4)$^a$ | $\delta$H (5)$^b$ | $\delta$H (6)$^a$ | $\delta$H (7)$^a$ | $\delta$H (8)$^b$ |
|-------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 1     | 6.81 (dd, 10.2, 2.4) | 6.78 (dd, 10.3, 2.4) | 7.07 (s) | 5.97 (t, 3.7) | 5.76 (brd, 10.4) | 6.18 (d, 4.9) | 6.20 (br, s) | 6.75 (br, d, 10.3) |
| 2     | 6.04 (d, 10.2) | 6.01 (d, 10.2) | 2.88 (overlap), 2.05 (overlap) | 6.25 (brd, 10.4) | 4.38 (dd, 4.9, 4.2) | 4.60 (dd, 4.3, 3.2) | 6.04 (d, 10.3) |
| 3     | 3.76 (m) | 3.72 (m) | 4.14 (m) | 3.83 (m) | 3.79 (m) | 3.41 (dd, 12.0, 5.0) | 3.63 (dt, 11.9, 3.2) | 3.72 (dd, 11.5, 5.3) |
| 4     | 2.21 (dd, 11.3, 5.2), 1.95 (dd, 11.3, 10.2) | 2.21 (dd, 11.3, 5.3), 1.95 (dd, 11.3, 10.0) | 2.29 (dd, 11.0, 5.0), 1.68 (t, 11.0) | 2.15 (dd, 12.6, 5.0), 1.44 (t, 11.0) | 1.94 (dd, 12.6, 10.0) | 2.16 (t, 12.0), 2.08 (dd, 12.0, 5.0) | 2.13 (dd, 11.9, 3.2), 1.94 (t, 11.9) | 2.76 (dd, 11.5, 5.3), 1.86 (t, 11.5) |
| 7     | 5.96 (d, 2.4) | 5.97 (d, 2.8) | 3.66 (dd, 7.0, 3.5) | 3.27 (m) | 3.61 (overlap), 4.69 (dd, 7.8, 6.0), 4.36 (d, 6.0) | 5.86 (s) |
| 8     | 4.56 (dd, 15.8, 2.4), 4.25 (d, 15.8) | 4.55 (dd, 15.8, 2.8), 4.24 (d, 15.8) | 2.94 (dd, 10.0, 3.5), 3.18 (dd, 10.0, 7.0) | 2.60 (dd, 9.9, 6.7), 2.89 (d, 12.5) | 3.10 (t, 10.2), 2.16 (t, 12.0), 2.08 (dd, 12.0, 5.0) | 2.13 (dd, 11.9, 3.2), 1.94 (t, 11.9) | 2.76 (dd, 11.5, 5.3), 1.86 (t, 11.5) |
| 10    | 3.09 (m), 3.30 (overlap) | 3.01 (m), 2.54 (m) | 2.73 (m), 2.60 (m) | 3.13 (m), 2.44 (m) | 4.03 (m), 3.47 (m) | 3.02 (2H, overlap) | 5.38 (s) |
| 11    | 2.98 (m), 2.49 (m) | 7.15 (s) | 6.28 (s) | 6.55 (s) | 7.12 (s) | 7.18 (s) | 6.33 (s) | 7.02 (s) |
| 14    | 7.27 (s) | 7.15 (s) | 6.71 (s) | 6.78 (s) | 7.36 (s) | 6.81 (s) | 7.23 (s) |
| 17    | 7.57 (s) | 7.49 (s) | 6.71 (s) | 6.78 (s) | 7.36 (s) | 6.81 (s) | 7.23 (s) |
| 18    | 1.46 (3H, s) | 1.45 (3H, s) | 1.38 (3H, s) | 5.86 (d, 6.0) |
| 19    | 1.39 (3H, s) | 3.20 (3H, s) | 3.21 (3H, s) | 3.27 (3H, s), 3.22 (s) | 3.23 (3H, s) | 3.20 (3H, s) | 3.29 (3H, s) | 3.24 (3H, s) |
| 3-OCH$_3$ | 3.81 (3H, s) | 3.65 (3H, s) | 3.77 (s) | 3.79 (3H, s) | 3.93 (3H, s) | 3.81 (3H, s) | 3.84 (3H, s) |
| 15-OCH$_3$ | 3.90 (3H, s) | 3.75 (3H, s) | 3.72 (s) | 3.71 (3H, s) | 3.90 (3H, s) | 3.79 (3H, s) | 3.72 (3H, s) |
| OCH$_2$O | 6.12 (br, s), 6.09 (br, s) | 4.84 (d, 4.2), 3.59 (d, 4.3) | 4.98 (d, 6.0), 4.96 (d, 6.0) | 4.43 (s) |

$^a$ $^1$H NMR recorded in 600 MHz. $^b$ $^1$H NMR recorded in 400 MHz; compound 3 was recorded in DMSO-$d_6$. 

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erythrorines A-H, respectively. Additionally, all reported *Erythrina* and *Homoeorythrina*-type alkaloids have this configuration so far.

The known alkaloids were identified as, eyrharbicine (9), 8-oxoethraline epoxide (10), erysthatridine (11), erytathrine (12), 8-erysotramidine (13), 10,11-dioxyoerstotine (14), 11β-hydroxyerstotine (15), erytathrine (16), 8-erytathrine N-oxide (17), erysotrine (18), 8-oxoerytathrine (19), 8-oxoethraline (20), erytathrine (21), 8-erytathrine N-oxide (22), erythrine (23), erysotine (24), erysodine (25) on the basis of physical and spectroporamic comparison with published values.

**Conclusions**

To summary, twenty five erythrina alkaloids were isolated from the flowers of *E. arborescens* Roxb. and among them eight novel ones, erythorines A-H (1-8) have been elucidated. Alkaloids 1 and 2 were the first found erythrina alkaloids with 2H-imidazole ring. In addition, 3 was an alkaloid containing an oxime group. Other alkaloids (9-25) were first obtained from *E. Arborescens*. The discovery of compounds 1-8 is a further addition to the diverse of alkaloids belonging to the *Erythrina* genus.

**Experimental section**

**General experimental procedures**

Optical rotations were measured with a Jasco p-1020 digital polarimeter. UV spectra were recorded on a Shimadzu 2401PC spectrophotometer. IR spectra were obtained on a Bruker Tensor 27 infrared spectrophotometer with KBr pellets. 1H, 13C and 2D NMR spectra were obtained on Bruker AV-600, AVANCE III-500, and AVANCE III-400 MHz spectrometers with SiMe4 as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESI and HRMS data were recorded on a Bruker HCT/ESquire and a Shimadzu UPLC-IT-TOF spectrometer, respectively. Column chromatography (CC) was performed on either silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China) or RP-18 silica gel (20–45 μm, YMC Chemical Ltd., Japan). Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and spots were visualized with Dragendorff's reagent spray. MPLC was performed using a Buchi pump system coupled with RP-18 silica gel-packed glass columns (15 x 230 and 26 x 460 mm, respectively). HPLC was performed using Waters 1525EF pumps coupled with analytical semi-preparative or preparative Sunfire C18 columns (4.6 x 150 and 19 x 250 mm, respectively). The HPLC system employed a Waters 2998 photodiode array detector and a Waters fraction collector III.

**Plant material**

Flowers of *Erythrina arborescens* Roxb. Hort. Beng were collected in September 2014 in Yunnan Province, P. R. China, and identified by Dr Chun-Xia Zeng. A voucher specimen (no. Cai20140907) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and isolation**

The dried flowers of *E. Arborescens* (6.5 kg) were powdered and extracted three times with MeOH at room temperature. After removing the solvent, the residue was dissolved in 2% HCl soln and filtered. The acidic soln was washed with EtOAc three times. The aqueous layer was then adjusted to pH 8–9 with NH3·H2O and extracted with EtOAc to obtain crude alkaloid extract (62.5 g). The extract was subjected to column chromatography (CC) over silica gel and eluted with gradient CHCl3/MeOH (1 : 0–5 : 1) to afford seven fractions (I–VII).

Fraction II (10.4 g) was further chromatographed on a C18 MPLC column eluted with a gradient of MeOH–H2O (40 : 60–100 : 0 : v/v) to give the five subfractions II-1–II-5. Subfraction II-2 (2.5 g) was subjected to C18 MPLC column once again using MeOH–H2O (40 : 60–70 : 30 : v/v) as eluent to give the four subfractions (II-2-1–II-2-4). Fraction II-2-1 was further purified by a preparative column with a gradient flow from 40% to 55% aqueous methanol to give 19 (7 mg), 8 (50 mg), 5 (5 mg). Fraction II-2-2 was separated on a preparative C18 HPCL column with a gradient of MeOH–H2O (45 : 55–55 : 45, v/v) to afford 13 (4 mg) and 14 (7 mg). Fraction II-2-4 was purified by a preparative C18 HPCL column with a gradient of MeOH–H2O (50 : 50–65 : 35, v/v) to obtain 11 (20 mg) and 15 (10 mg). II-1 (7.5 mg) was separated using C18 MPLC column with a gradient of MeOH–H2O (30 : 70–60 : 40, v/v) to afford five subfractions (II-1-1–II-1-4). Alkaloid 21 (500 mg) was crystallized from II-1-2. Fraction II-1-3 was purified by a preparative C18 HPCL column with a gradient of MeOH–H2O (35 : 65–45 : 55, v/v) to obtain 22 (5 mg). II-2-5 was purified by a preparative C18 HPCL column with a gradient of MeOH–H2O (50 : 50–60 : 40, v/v) to obtain 20 (5 mg). Compounds 1 (2 mg), 2 (2 mg), 3 (1.6 mg), 4 (1 mg), 9 (3 mg), 10 (2 mg) and 17 (8 mg) were obtained from fraction II-1-4 using C18 MPLC column with a gradient of MeOH–H2O (40 : 60–70 : 30 : v/v), then followed by preparative HPLC with a gradient of MeOH–H2O (40 : 60–60 : 40, v/v).

Fraction III (0.9 g) was fractionated by C18 MPLC column with a gradient of MeOH–H2O (30 : 70–80 : 20, v/v) to give four subfractions (III-1–III-4). III-1 was subjected to a preparative C18 HPCL column with a gradient of MeCN–H2O (30 : 70–40 : 60, v/v) to afford 24 (20 mg). III-3 was further purified by a preparative C18 HPCL column with a gradient of MeCN–H2O (30 : 70–60 : 45, v/v) to afford 25 (18 mg).

Alkaloid 23 (1.5 g) was crystallized from fraction IV. The mother liquid of this fraction (3.0 g) was subjected to C18 MPLC column with a gradient of MeOH–H2O (20 : 80–70 : 30, v/v) to give the four subfractions (IV-1–IV-4). IV-2 was separated on a preparative C18 HPCL column with a gradient of MeCN–H2O (20 : 80–35 : 65, v/v) to afford 16 (12 mg), 17 (7 mg).

Fraction V (1.6 g) was chromatographed on a C18 MPLC column eluted with a gradient of MeOH–H2O (20 : 80–60 : 40, v/v) to give five subfractions V-1–V-5. V-1 (910 mg) was subjected to a C18 MPLC column once again with a gradient of MeOH–H2O (10 : 90–40 : 60, v/v) to give eight subfractions V-1-1–V-1-8.
Compound 6 (2 mg) and 7 (2 mg) was obtained from V-1-4 using a preparative C18 HPCL column with a gradient of MeOH–H2O (30 : 70–45 : 55, v/v). Compound 12 (2 mg) was obtained from V-1-6 using a preparative C18 HPCL column with a gradient of MeOH–H2O (40 : 60–50 : 50, v/v).

Erytharborine A (1)
Pale yellow amorphous powder; [α]D + 119.2 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 202 (4.03), 227 (3, 79), 289 (3.55), 322 (3.48) nm; IR (KBr) rmax 2927, 1710, 1629, 1479, 1383, 1252 cm⁻¹; for 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d₆), see Tables 1 and 2; positive HRESIMS m/z 380.1961 [M + H]⁺ (calcd. For C₂₂H₂₆N₆O₈, 380.1969).

Erytharborine B (2)
Pale yellow amorphous powder; [α]D + 377.3 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 201 (3.99), 230 (3.80), 288 (3.59), 327 (3.50), nm; IR (KBr) rmax 3429, 2930, 1722, 1633, 1594, 1508, 1479, 1392, 1252 cm⁻¹; for 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d₆), see Tables 1 and 2; positive HRESIMS m/z 364.1658 [M + H]⁺ (calcd. For C₂₁H₂₃N₃O₅Cl, 364.1656).

Erytharborine C (3)
White powder; [α]D + 112.2 (c = 0.25, CH₃OH); UV (CH₃OH) λmax (log ε) 204 (4.22) and 289 (3.46) nm; IR (KBr) rmax 3414, 2931, 1611, 1513, 1458, and 1256 cm⁻¹; for 1H (600 Hz) and 13C NMR (150 Hz) NMR data (DMSO-d₆), see Tables 1 and 2; positive ESIMS m/z 427 [M + H]⁺, HRESIMS m/z 427.1197 [M + H]⁺ (calcd. For C₂₀H₂₁N₃O₃, 427.1197).

Erytharborine D (4)
White powder; [α]D + 63.6 (c = 0.14, CH₃OH); UV (CH₃OH) λmax (log ε) 206 (3.68), 232 (3.08) and 283 (2.69) nm; 1H (600 Hz) and 13C (150 Hz) NMR data (acetone-d₆), see Tables 1 and 2; positive HRESIMS m/z 398 [M + H]⁺, HRESIMS m/z 398.1281 [M + H]⁺ (calcd. For C₂₀H₂₀N₂O₄Cl₂, 398.1284).

Erytharborine E (5)
Colorless oil; [α]D + 179.8 (c = 0.19, CH₃OH); UV (CH₃OH) λmax (log ε) 203 (3.93), 233 (3.42) and 283 (2.95) nm; 1H (600 Hz) and 13C (150 Hz) NMR data (acetone-d₆), Tables 1 and 2; positive ESIMS m/z 330 [M + H]⁺, HRESIMS m/z 330.1699 [M + H]⁺ (calcd. For C₁₉H₂₄N₂O₅, 330.1700).

Erytharborine F (6)
White powder; [α]D + 111.5 (c = 0.18, CH₃OH); UV (CH₃OH) λmax (log ε) 203 (3.63), 248 (3.33), 289 (3.13) and 352 (2.94) nm; 1H (600 Hz) and 13C (150 Hz) NMR data (acetone-d₆), Tables 1 and 2; positive ESIMS m/z 398 [M + Na]⁺, HRESIMS m/z 398.1212 [M + Na]⁺ (calcd. For C₁₉H₂₅NO₄Na, 398.1210).

Erytharborine G (7)
White powder; [α]D + 31.1 (c = 0.05, CH₃OH); UV (CH₃OH) λmax (log ε) 204 (4.33), 225 (3.80), and 283 (3.29) nm; 1H (600 Hz) and 13C (150 Hz) NMR data (acetone-d₆), Tables 1 and 2; positive ESIMS m/z 384 [M + Na]⁺, HRESIMS m/z 384.1417 [M + Na]⁺ (calcd. For C₁₉H₂₅NO₄Na, 384.1418).

Erytharborine H (8)
Colorless oil; [α]D + 161.1 (c = 0.25, CH₃OH); UV (CH₃OH) λmax (log ε) 204 (3.91), 241 (3.47) and 283 (2.88) nm; 1H (400 Hz) and 13C (125 Hz) NMR data (acetone-d₆), Tables 1 and 2; positive ESIMS m/z 366 [M + Na]⁺, HRESIMS m/z 366.1310 [M + Na]⁺ (calcd. For C₁₉H₂₅NO₄Na, 366.1312).

Conflicts of interest
There are no conflicts to declare.

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