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Short communication

Antiproliferative activity of arborescidine alkaloids and derivatives

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ABSTRACT

Current issues in cancer research involve searching for novel anticancer compounds that can be used to regulate the cell cycle and lead to more effective treatments of tumors. In this study, it was hypothesized that possessing a cyclic alkaloid similar to harmine, arborescidines can disrupt the proliferative state of cancer cells and block the activity of topoisomerases. The antiproliferative activity of arborescidines A–C and their derivatives was evaluated in vitro against four human tumor cell lines: gastric adenocarcinoma, lung cancer, bladder carcinoma and leukemia. Assuming the mechanism of action by topoisomerase II binding model, the compounds possessing the greatest activity had nonpolar side-chain into hydrophobic binding region on the DNA/topo II complex.

1. Introduction

Current issues in cancer research involve searching for novel anticancer compounds that can be used to regulate the cell cycle and lead to more effective treatments of tumors. In vitro studies have shown the ability of many β-carbolines to retain the cell cycle progression of tumor cells [1,2]. Although no information is available concerning the biological effects of chiral arborescidines [3], it was hypothesized that possessing a cyclic alkaloid similar to harmine, arborescidine A can disrupt the proliferative state of cancer cells and block the activity of topoisomerases [2]. The aim of this work was to assess the in vitro antiproliferative activity of arborescidines A–C and their derivatives against four human tumor cell lines: gastric adenocarcinoma, lung cancer, bladder carcinoma and leukemia. Human normal lung fibroblasts were used as controls.

Structurally, the arborescidines comprise a tetracyclic framework featuring a common β-carboline core. Although demonstrated as a useful synthetic method, this asymmetric reduction remains to be fully explored in the arena of total synthesis of alkaloid natural products [4–8].

2. Chemistry

The basic framework of arborescidine alkaloids consists of a tetracyclic moiety and was demonstrated previously that an alternative to their synthesis to be the Noyori asymmetric hydrogen-transfer reaction as a key step. For the present work, an efficient synthesis of 6-bromotryptamine (**4a**), which was a key precursor to β-carbolines **7** and **8** (Scheme 1), was required. Schumaker and Davidson methodology showed to be the most efficient for the synthesis of **4a**. The precursor to tryptamine **4a** is 6-bromoindole, which was easily prepared by a modified Batcho–Leimgruber indole synthesis [9]. Thus, reaction of 6-bromoindole with 1-(dimethylamino)-2-nitroethylene (DMANE) [10] in the presence of TFA afforded 3-[(*E*)-2-nitroethenyl]-6-bromoindole in 96% yield. Reduction with in situ generated borane proceeded smoothly to provide **4a** in 73% yield.

Then, arborescidine A (**1a**) and desbromoarborescidine A (**1b**) were obtained following the sequence depicted in Scheme 1. Thus, compounds **4a**,**b** and glutaric anhydride in CH_2Cl_2 at room temperature formed the corresponding amide carboxylic acids, which upon treatment with $SOCl_2$/MeOH afforded the corresponding methyl esters in yields around 83% (two steps). Treatment with $POCl_3$ promoted the Bischler–Napieralsky cyclization producing imines **7a**,**b** in yields of 86%. β-Carboline imines **7a**,**b** were hydrogenated by the Noyori method using preformed (*S*,*S*)-TsDPEN–Ru(II) complex in DMF and a HCO_2H–Et_3N mixture, which

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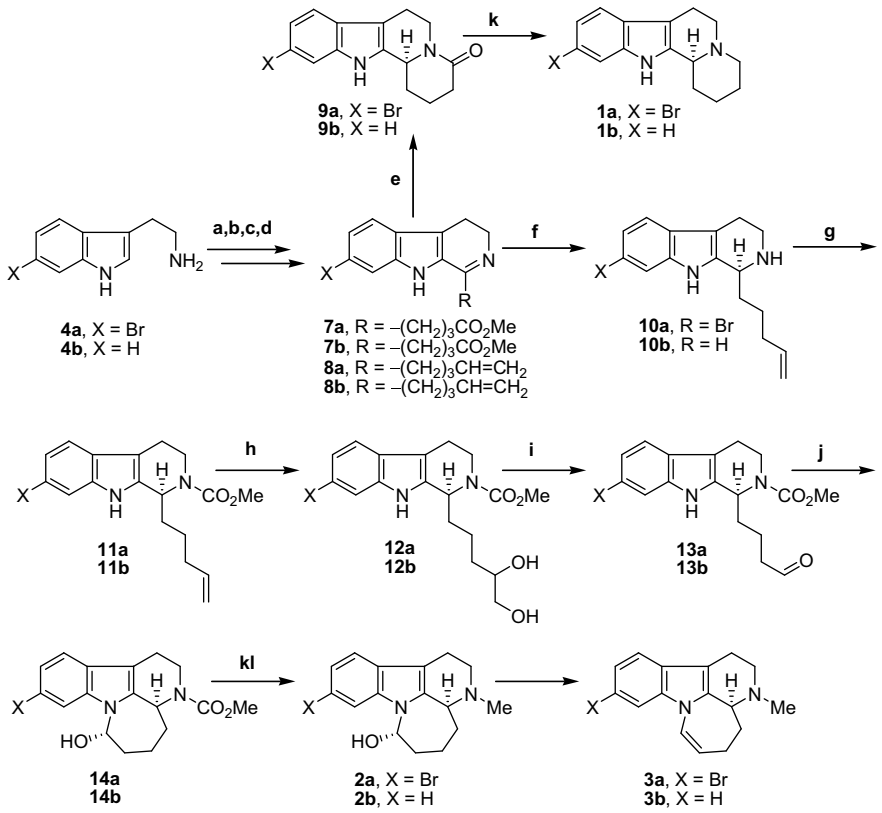


Scheme 1. Reagents and conditions: (a) **4a**/**4b**, glutaric anhydride, CH_2Cl_2, rt, 10 min. (b) $SOCl_2$, MeOH, 0 °C to rt, 3 h. (c) **4a**/**4b**, EDC, HOBt, 5-hexenoic acid, CH_2Cl_2, rt, 12 h. (d) $POCl_3$, benzene, reflux, 2–3 h. (e) (*R*,*R*)-TsDPEN–Ru(II) complex (or (*S*,*S*)-TsDPEN–Ru(II) complex), Et_3N:HCO_2H (5:2, v/v), DMF, rt, 12 h. (f) (*R*,*R*)-TsDPEN–Ru(II) complex (or (*S*,*S*)-TsDPEN–Ru(II) complex), Et_3N:HCO_2H (5:2, v/v), MeCN, rt, 12 h. (g) MeOCOCl, Et_3N, CH_2Cl_2, 0 °C to rt, 12 h. (h) OsO_4/*t*-BuOH, NMO, THF–H_2O, 0 °C to rt, 10 h. (i) $NaIO_4$, THF:H_2O (1:2). (j) TFA, THF, 1 h (>20:1 *trans*/*cis*). (k) AlH_3, THF, rt, 15 min. (l) $MeO_2CNSO_2NEt_3$, benzene, 8 h.

afforded lactams (*R*)-**9a**,**b** in 89% (96% ee) and 91% (>95% ee) yields, respectively for **9a** and **9b**. The enantiomeric excesses were determined by HPLC analysis using a ChiralPack OD column. The (*S*)-**9a**,**b** were obtained by changing the (*S*,*S*)-TsDPEN for (*R*,*R*)-TsDPEN chiral ligand of ruthenium catalyst giving similar yields and ee%. Reduction of lactams **9a**,**b** using Brown's procedure with AlH_3 [11,12] afforded (+)-**1a** in 78% and (+)-**1b** [13] in 89% yields, respectively.

Next, treatment of **4a**,**b** with 5-hexenoic acid [14] in the presence of EDC/HOBt [15] gave amides **8a**,**b** in quantitative yields (Scheme 1). Bischler–Napieralsky reaction in MeCN afforded imines **8a**,**b** in 90% and 88% yields, respectively from **4a**,**b**. Noyori asymmetric hydrogenation reaction of imines **8a**,**b** in DMF afforded the respective amines **10a** (96% yield) and **10b** (95% yield). Free amines **10a**,**b** were converted to the corresponding methyl carbamates **11a**,**b**. Then, dihydroxylation of carbamates **11a**,**b** employing OsO_4 and NMO gave **12a**,**b** in 88% and 89% yields, respectively. The obtained diols were treated with $NaIO_4$ to give unstable aldehydes **13a**,**b** in excellent yields (90%). Treatment of crude aldehydes **13a**,**b** with aqueous trifluoracetic acid in THF afforded a >20:1 mixture of *trans*/*cis*-**14a**,**b** (90–95% yields). Finally, after reduction of **14a**,**b** with AlH_3, amines **2a**,**b** were obtained in 92–96% yields. Then, conversion of **2a**,**b** to arborescidine B and desbromoarborescidine B required dehydration of the alcohols by using the Burgess reagent in benzene [16] that gave **3a** in 84% and **3b** in 86% yields, respectively.

The enantioselective syntheses of desbromoarborescidine A (**1b**), desbromoarborescidine B (**2b**), and desbromoarborescidine C (**3b**) were obtained via routes that proceeded in high yields and few steps. Arborescidines were obtained following previously described procedure in five steps and 50% overall yield (**1a**), eight steps and 61% overall yield (**2a**), and nine steps and 51% overall yield (**3a**), respectively, from 6-bromotryptamine. The syntheses feature the use of the Noyori catalytic asymmetric hydrogen-transfer reaction to introduce chirality in dihydro-β-carbolines as **4**. On the basis of an ample precedent from Noyori's work, the reduction produces dihydro-β-carbolines, and ultimately products, possessing the *R* or *S* absolute configuration, depending on Noyori's catalyst employed. The synthetic arborescidines and derivatives displayed optical rotations that were in accordance with those of the natural products, thereby supporting the *S* configuration for natural arborescidines.

Thus, the activity of these synthesized compounds and intermediates to in vitro antiproliferative activity against four human tumor cell lines were analyzed as described below.

3. Antiproliferative activity assay

Human cell lines were purchased from ATCC (VA, USA). They included normal MRC-5 lung fibroblasts (CCL-171), AGS gastric adenocarcinoma (CRL-1739), HL-60 leukemia cells (CCL-240), lung cancer (SK-MES-1) and J82 bladder carcinoma (HTB-1). Cells were plated at a density of 50,000 cells/mL in 96 well plates. One day after seeding cells were treated with medium containing the compounds at concentrations ranging from 0 up to 100 μM for 3 days and finally the MTT reduction assay was carried out [17]. Untreated cells were used as controls. Etoposide (inhibitor of topoisomerase II) was used as reference compound. Normal human lung fibroblasts were used in order to assess the selectivity of the compounds against cancer cells. Results are expressed as IC_{50}

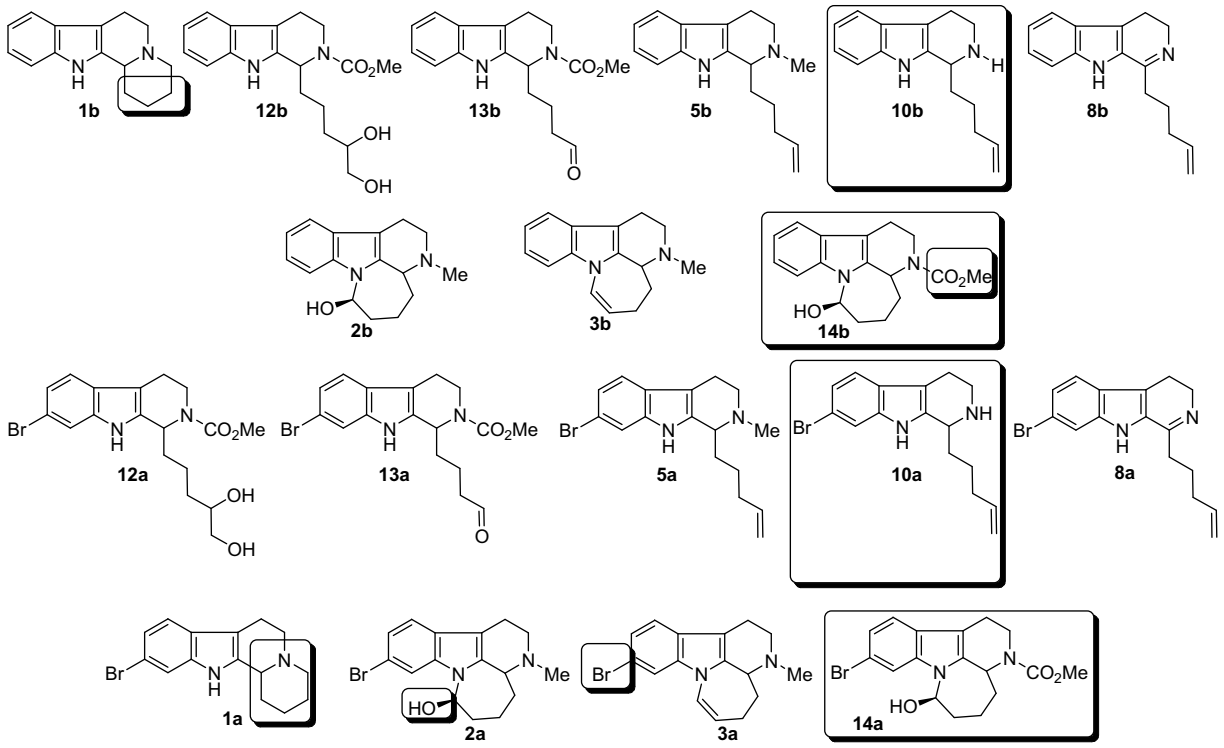


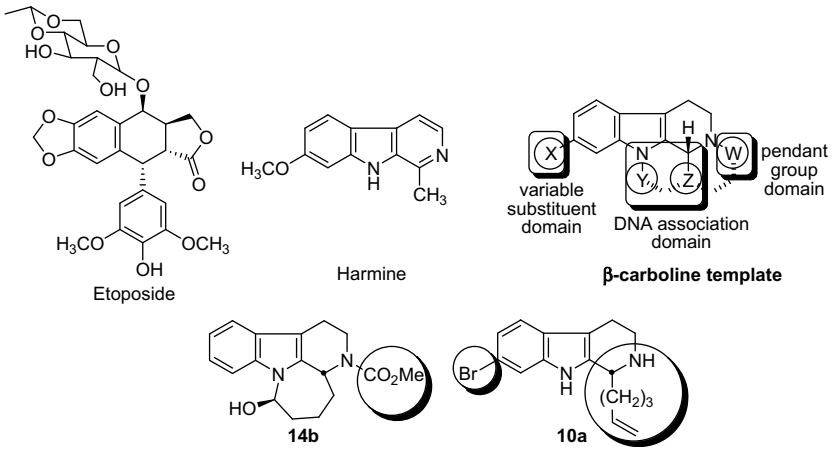
Fig. 1. Arborescidine alkaloids (**1**–**3**) and β-carboline derivatives tested towards cytotoxicity to human lung fibroblasts and different human tumor cell lines.

values. All tested compounds were synthesized as described above or according to previous work in good yields [3] (Fig. 1).

4. Results and discussion

Approaches to the construction of this heterocyclic target system by other groups have included the diastereoselective vinylogous Mannich reaction [18], Fischer indole synthesis [19], Bischler–Napieralski reactions [3,20,21], and the asymmetric Pictet–Spengler reaction [22]. Using our approach in the target synthesis of complex indole alkaloids and their synthetic analogues, we undertook the synthesis of a simple indole alkaloid, (*S*)-(−)-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine **1b** (desbromoarborescidine A), the main constituent of *Dracontomelum mangiferum* B1 [23,24].

All the compounds tested possess the *S* and *R* absolute stereochemistry, and no significant differences in the IC_{50} among *R* and *S* compounds were observed. There was no significative improvement in the IC_{50} when natural arborescidines (*S* absolute stereochemistry) and opposite configuration were tested. The better results obtained for gastric, fibroblast and lung cells were presented by amine compound **10a** ($IC_{50} \cong 8.8$, 18.1 and 12.7 μM, respectively); for bladder was showed by carbamate **14b** ($IC_{50} \cong 18.1$ μM); and for leukemia arborescidine A, **1a** ($IC_{50} \cong 34.5$ μM). Arborescidine alkaloids are known to be active against oral carcinoma (KB, 100% of activity at 30 μM). The most active compound in that screening was Arborescidine D with an IC_{50} of 9.0 μM against KB cells [25]. In our approach testing different cell lines the best result was obtained with bromo compound **10a**. Most of the compounds did not show antiproliferative activity towards leukemia cells at concentrations up to 100 μM, however product **1a** displayed



Scheme 2. Topoisomerase II binding model [2]: the compounds possessing the greatest activity (**10a** and **14b**) incorporate either nonpolar side-chain into hydrophobic binding region on the DNA/topo II complex (Y/Z) that might enhance activity against topo II.

a selective inhibition against this cell line. At 100 μM compounds **12a**, **3a** and **14b** were active against two or more cancer cell lines but were not toxic towards normal fibroblasts. Chbani [25] has reported a moderate activity of the brominated indole alkaloid arborescidine D against the growth of KB human oral carcinoma cells with an IC_{50} value of 3 μg/mL.

Assuming the mechanism of action by topoisomerase II binding model [1], the compounds possessing the greatest activity (**14b** and **10a**, IC_{50} from 16.8 to 8.8 μM) incorporate either nonpolar side-chain into hydrophobic binding region on the DNA/topo II complex (Y/Z) that might enhance activity against topo II when occupied by a compound as depicted in Scheme 2. Derivatives substituted with NH functionalities in the pendant group domain (W) as well as Br (X) in the pharmocophore showed to be more potent toxins. Data from other derivatives further support this hypothesis.

5. Conclusion

The three parent compounds showed antiproliferative activity values ranging from 30 up to >100 μM, arborescidine C being the most active product. Assuming the mechanism of action by topoisomerase II binding model [1,2], the compounds possessing the greatest activity (**14b** and **10a**, IC_{50} from 16.8 to 8.8 μM) incorporate either nonpolar side-chain into hydrophobic binding region on the DNA/topo II complex (Y/Z) that enhances activity against topo II when occupied by a compound. Derivatives substituted with NH functionalities in the pendant group domain (W) as well as Br (X) in the pharmocophore are more potent toxins. Data from other derivatives further support this hypothesis. Studies are ongoing in order to obtain more selective and active alkaloid derivatives as well as to corroborate their mechanism(s) of action (Table 1).

6. Experimental protocols

6.1. Chemistry

6.1.1. General methods

Purchased chemical reagents were used without further purification. THF was freshly distilled from sodium benzophenone ketyl under nitrogen prior to use. Dichloromethane was distilled under CaH_2 prior to use. DMF was pre-dried by P_2O_5, then collected from CaH_2 prior to use. Methanol was freshly distilled from magnesium prior to use. Solvents for extraction and column chromatography were distilled prior to use. Merck silica gel (230–400 mesh) was used as stationary phase for flash chromatography. Melting points: m.p. (uncorrected) were determined using an Electrothermal 9100 apparatus. IR spectroscopy: FT-IR Nicolet Nexus 470 equipment and KCl cell. Other known compounds used in this research were purchased from standard chemical suppliers and were dried and/or purified by usual methods. Column chromatography was performed using silica gel Merck 230–400 mesh. TLC analyses were performed with silica gel plates Merck using iodine, $KMnO_4$ and UV-lamp for visualization. Mass spectrometry experiments were performed on a high-resolution high accuracy hybrid double quadrupole (Qq) and orthogonal time-of-flight (Tof) mass spectrometer (QTof, Micromass UK). The temperature of the nebulizer was 50 °C. The ESI source and the mass spectrometer were operated in the positive-ion mode. The cone and extractor potential were set to 40 and 10 V, respectively. NMR spectroscopy: NMR spectra were recorded on Bruker (400 MHz for ^1H and 100 MHz for ^{13}C) with TMS as internal.

6.1.1.1. N1-[2-(1H-3-Indolyl)ethyl]-5-hexenamide. To a solution of tryptamine (1.19 mmol) and 5-hexenoic acid (0.136 g, 1.19 mmol) in CH_2Cl_2 (12.0 mL) at 0 °C were added HOBt (0.177 g, 1.31 mmol) and EDC (0.251 g, 1.31 mmol). The reaction mixture was stirred at room temperature for 10 h, then washed with 5% aqueous HCl (3 × 15.0 mL), 5% aqueous $NaHCO_3$ (20.0 mL), H_2O (20.0 mL), and brine (20.0 mL), and dried (Na_2SO_4). Purification by flash chromatography (CH_3Cl/MeOH, 10%, $R_f = 0.43$) afforded amidoalkene in 99% yield as a brown oil. FT-IR (KBr film) cm^{-1}: 3045, 3284, 3077, 2973, 2929, 2859, 1648, 1537, 1546, 1434, 1340, 1253, 1228, 1099, 914, 742. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.68 (2H, quint, *J* 7.8), 2.03 (2H, q, *J* 7.2), 2.10 (2H, t, *J* 7.8), 2.96 (2H, t, *J* 6.7), 3.58 (2H, q, *J* 6.1), 4.94 (1H, dd, *J* 10.2, 3.0), 4.96 (1H, dd, *J* 17.6, 3.0), 5.68 (1H, br s), 5.73 (1H, ddt, *J* 17.6, 10.2, 6.1), 6.99 (1H, d, *J* 1.5), 7.10 (1H, dt, *J* 7.8, 0.7), 7.19 (1H, dt, *J* 7.8, 0.7), 7.35 (1H, d, *J* 8.7), 7.58 (1H, d, *J* 8.7), 8.48 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.7, 25.3, 33.0, 35.9, 39.7, 11.3, 112.7, 115.2, 118.6, 119.3, 122.0, 122.1, 127.3, 136.4, 137.8, 173.0.

*6.1.1.2. 1-(4-Pentenyl)-4,9-dihydro-3H-β-carboline (**8b**).* A solution of N1-[2-(1*H*-3-indolyl)ethyl]-5-hexenamide (1.65 g, 4.94 mmol) and 3.21 mL of $POCl_3$ in 123 mL of dry MeCN was heated to reflux for 3 h, cooled to room temperature, and then concentrated. The

Table 1
Cytotoxic activity (IC_{50}, μM) of synthesized compounds (table shows *S*-configuration data) towards human lung fibroblasts (MRC-5), human gastric adenocarcinoma (AGS), human lung cancer (SK-MES-1), human bladder carcinoma (J82) and human leukemia (HL-60) cells. Both (*S*)- and (*R*)-derivative compounds were assayed without statistically differences in IC_{50}.

Compound	Lung fibroblasts	Gastric adenocarcinoma	Bladder carcinoma	Lung cancer	Leukemia
1a	71.6	65.3	>100	>100	34.5
1b	82.5	50.4	90.2	65.1	>100
2a	31.9	30.9	>100	>100	>100
2b	>100	>100	>100	>100	>100
3a	>100	>100	45.5	91.3	>100
3b	62.6	41.7	29.5	32.3	>100
5a	78.9	69.9	78.8	75.8	>100
5b	42.2	34.5	76.4	58.6	>100
8a	40.1	26.9	39.3	39.7	>100
8b	44.6	40.3	47.6	36.1	>100
10a	18.1	8.8	18.9	12.7	>100
10b	20.1	14.3	25.7	19.1	69.3
11b	>100	>100	>100	>100	>100
12a	>100	>100	>100	>100	>100
12b	>100	63.1	>100	88.3	>100
13a	53.6	21.4	74.9	48.2	99.4
14a	60.0	22.8	16.4	18.1	>100
14b	35.7	16.8	72.8	25.1	96.6
Etoposide	3.93	0.36	2.5	2.8	0.80

Values represent the means of three experiments in quadruplicate.

resulting orange viscous oil was purified by flash chromatography (CH_3Cl/MeOH, 10%, $R_f = 0.71$) to afford a yellow oil in 86% yield. The spectroscopic properties of the product were in accordance with imine **8b**. FT-IR (KBr film) cm^{-1}: 3070, 2935, 2877, 2836, 2768, 2730, 1639, 1602, 1546, 1444, 1373, 1319, 1250, 1218, 998, 914, 746. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.83 (2H, quint, *J* 7.7), 2.09 (2H, q, *J* 7.7), 2.76 (2H, t, *J* 8.9), 2.88 (2H, t, *J* 8.5), 3.88 (2H, t, *J* 8.2), 4.89 (1H, dd, *J* 12.0, 1.6), 4.94 (1H, dd, *J* 15.7, 3.1), 5.73 (1H, ddt, *J* 15.7, 12.0, 8.5), 7.12 (1H, dt, *J* 7.1, 0.7), 7.24 (1H, dt, *J* 7.1, 0.7), 7.40 (1H, d, *J* 8.3), 7.59 (1H, d, *J* 8.3), 10.20 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.3, 26.1, 33.2, 34.6, 47.4 112.2, 115.0, 117.1, 119.9, 124.5, 125.0, 125.3, 128.6, 137.2, 137.8, 162.2. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{16}H_{18}N_2 + H]^+$ 239.1548, found 239.1542.

*6.1.1.3. 1-(4-Pentenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (**10b**).* The preformed catalyst solution was added to a mixture of imine **8b** (2.37 mmol) in 24 mL of DMF, followed by a mixture of HCO_2H–Et_3N (5:2 v/v, 1.22 mL) at room temperature. After the resulting solution was stirred at room temperature for 12 h, the DMF was distilled off under a high vacuum, and the crude purified by flash chromatography ($CHCl_3$/MeOH, 10%, $R_f = 0.52$) to afford in 95% the amine **10b** as a brown solid. (*R*)-**10b**, $[\alpha]_D$ −25 ($c = 1$, $CHCl_3$); (*S*)-**10b**, $[\alpha]_D$ +23 ($c = 1$, $CHCl_3$). FT-IR (KBr film) cm^{-1}: 3409, 3218, 3062, 2929, 2844, 2744, 1641, 1562, 1452, 1343, 1317, 1288, 1155, 1108, 1002, 909, 744. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.52–1.70 (3H, m), 1.84–1.91 (1H, m), 2.09–2.19 (2H, m), 2.68–2.80 (1H, m), 2.75 (1H, dq, *J* 8.0, 1.9), 3.03 (1H, ddd, *J* 15.5, 8.0, 5.5), 3.34 (1H, dt, *J* 14.5, 4.5), 4.07 (1H, br s), 4.98 (1H, br d, *J* 10.2), 5.03 (1H, dd, *J* 17.1, 1.6), 5.80 (1H, ddt, *J* 17.1, 10.2, 6.7), 7.09 (1H, dt, *J* 7.6, 0.7), 7.14 (1H, dt, *J* 7.6, 0.7), 7.30 (1H, d, *J* 7.8), 7,47 (1H, d, *J* 7.8), 7.84 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.6, 25.0, 33.7, 34.3, 42.5, 52.5, 109.0, 110.7, 115.0, 118.0, 119.3, 121.5, 127.5, 135.6, 136.1, 138.3. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{16}H_{20}N_2 + H]^+$ 241.1705, found 241.1701.

*6.1.1.4. Methyl 1-(4-pentenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-2-carboxylate (**11b**).* To a cold solution of amine **10b** (5.67 mmol) and triethylamine (0.861 g, 8.50 mmol) in dry CH_2Cl_2 (94.0 mL) kept at 0 °C was added dropwise a solution of methyl chloroformate (1.07 g, 11.3 mmol) in CH_2Cl_2 (10 mL). After 1 h, the reaction mixture was diluted with water (60.0 mL), followed by saturated aqueous NH_4Cl solution (100 mL) and extracted with CH_2Cl_2. The organic layers were washed with saturated aq $NaHCO_3$ solution (100 mL) and water (100 mL) and dried. The solvent was removed and the residue purified by flash chromatography to give the methyl 1-(4-pentenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-2-carboxylate as a brown solid in 99%. (*R*)-**11b**, $[\alpha]_D$ −2.0 ($c = 1$, $CHCl_3$); (*S*)-**11b**, $[\alpha]_D$ +3.0 ($c = 1$, $CHCl_3$). ^1H NMR (400 MHz, d^6-DMSO) δ: 1.44–1.55 (2H, m), 1.73–1.81 (1H, m), 1.87–1.94 (1H, m), 2.10 (2H, quint, *J* 7.6), 2.61–2.67 (2H, m), 3.15 (2H, s), 3.15–3.20 (1H, m), 3.65 (3H, s), 4.35 (1H, br t d, *J* 12.6), 4.95 (1H, d, *J* 8.2), 5.17 (1H, br d, *J* 4.4), 5.20 (1H, dt, *J* 17.2, 15.0), 5.83 (1H, ddt, *J* 17.2, 8.2, 5.5), 6.95 (1H, dt, *J* 7.6, 0.7), 7.04 (1H, dt, *J* 7.8, 0.7), 7.29 (1H, d, *J* 8.1), 7.36 (1H, d, *J* 8.1), 10.69 (1H, br s, NH). ^{13}C NMR (100 MHz, d^6-DMSO), δ: 20.6, 24.8, 32.7, 33.3, 37.7, 50.9, 52.1, 106.2, 110.7, 114.5, 117.3, 118.2, 120.5, 126.2, 134.6, 135.7, 138.2, 155.3. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{18}H_{22}N_2O_2 + H]^+$ 299.1760, found 299.1762.

*6.1.1.5. 2-Methyl-1-(4-pentenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (**5b**).* To a solution of methyl 1-(4-pentenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-2-carboxylate (**11b**, 0.502 mmol) in dry THF (6.0 mL) was added a solution of AlH_3 in THF (1.55 M, 1.94 mL, 3.01 mmol) at room temperature. After 10 min, the reaction was quenched with saturated aq sodium sulphate solution and filtered. The solids were washed with CH_2Cl_2 (200 mL), dried with Na_2SO_4, and evaporated in vacuo. Purification by chromatography eluting with EtOAc/Et3N (5%) afforded a white solid in 92% yield. (*R*)-**5b**, $[\alpha]_D$ −1.2 ($c = 1$, $CHCl_3$); (*S*)-**5b**, $[\alpha]_D$ +1.5 ($c = 1$, $CHCl_3$). FT-IR (film, KBr) cm^{-1}: 3411, 3301, 3059, 2938, 2839, 2779, 1690, 1646, 1464, 1376, 1321, 1299, 1178, 903, 749. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.28–1.34 (1H, m), 1.41–1.49 (1H, m), 1.57–1.66 (1H, m), 1.70–1.79 (1H, m), 1.95 (2H, q, *J* 7.0), 2.35 (3H, s), 2.55–2.71 (3H, m), 3.00–3.08 (1H, m), 3.37 (1H, t, *J* 5.2), 4.84 (1H, dt, *J* 10.2, 0.8), 4.89 (1H, dd, *J* 17.1, 1.7), 5.66 (1H, ddt, *J* 17.1, 10.2, 6.6), 6.97 (1H, dt, *J* 6.8, 0.9), 7.01 (1H, dt, *J* 6.8, 0.9), 7.15 (1H, d, *J* 7.5), 7.36 (1H, d, *J* 7.5), 7.96 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.9, 24.3, 32.0, 33.8, 41.7, 49.5, 59.7, 107.8, 110.6, 114.6, 117.8, 119.0, 121.0, 127.1, 134.8, 135.8, 138.5. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{17}H_{22}N_2 + H]^+$ 255.1861, found 255.1855.

*6.1.1.6. Methyl 1-(4,5-dihydroxipentil)-2,3,4,9-tetrahydro-1*H*-β-carboline-2-carboxylate (**12b**).* Osmium tetroxide (67.0 μL of a freshly prepared 0.039 M solution in *t*-BuOH) was added to a solution of methyl 1-(4-pentenyl)-2,3,4,9-tetrahydro-1H-β-carboline-2-carboxylate (**11b**, 0.775 mmol) and *N*-methylmorpholine *N*-oxide (0.256 mL, 50% v/v in water) in a 9:1 THF–H_2O solution (9.70 mL) at 0 °C. After 12 h at room temperature, the mixture was treated with Florisil (0.350 g) and $NaHSO_3$ (0.111 g), stirred for 1 h, filtered, and concentrated. The residue was diluted with EtOAc, and the organic layer was washed with 5% H_3PO_4 and brine, dried, and concentrated. Purification by flash chromatography gave a mixture of diols **12b** in 89% yield. ^1H NMR (400 MHz, d^6-DMSO, 353 K) δ: 1.18–1.54 (4H, m), 1.78–1.88 (2H, m), 2.64–2.68 (2H, m), 3.18–3.21 (1H, m), 3.28 (1H, d, *J* 5.5), 3.43–3.46 (1H, m), 3.66 (3H, s), 4.26 (1H, dd, *J* 12.3, 4.4), 5.16 (1H, dd, *J* 8.9, 4.4), 6.95 (1H, t, *J* 7.3), 7.03 (1H, t, *J* 7.3), 7.30 (1H, d, *J* 8.0), 7.36 (1H, d, *J* 8.0), 10.57 (1H, br s). ^{13}C NMR (100 MHz, d^6-DMSO, 353 K) δ: 20.5, 21.4, 32.9, 34.0, 37.7, 51.0, 51.8, 65.6, 70.8, 106.1, 110.6, 117.1, 118.0, 120.3, 126.1, 134.7, 135.7, 155.4. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{18}H_{24}N_2O_4 + H]^+$ 333.1814, found 333.1821.

*6.1.1.7. Methyl 1-(3-formylpropyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-2-carboxylate (**13b**).* A solution of **12b** (0.584 mmol) in 58.4 mL of THF–H_2O (1:2) was treated at 0 °C with a solution of 0.131 g (0.613 mmol) of sodium metaperiodate ($NaIO_4$) in 6 mL of water. After the resulting solution was stirred for 1 h at 0 °C, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with brine, dried, and concentrated in vacuo to give aldehyde **13b** as a colorless solid in 90% yield. (*R*)-**13b**, $[\alpha]_D$ −5.1 ($c = 1$, $CHCl_3$); (*S*)-**13b**, $[\alpha]_D$ +6.0 ($c = 1$, $CHCl_3$). FT-IR (film, KBr) cm^{-1}: 3371, 3054, 3010, 2949, 2848, 2723, 1701, 1675, 1471, 1448, 1409, 1228, 1112, 1018, 744. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.55–1.93 (4H, m), 2.39–2.52 (2H, m), 2.71 (1H, dd, *J* 15.3, 3.5), 2,84 (1H, br d, *J* 5.0), 3.19 (1H, br q, *J* 9.7), 3.76–3.79 (3H, br s), 4.35/4.52 (1H, br d, *J* 9.4), 5.20/5.35 (1H, br s), 7.10 (1H, dt, *J* 7.2, 1.2), 7.15 (1H, dt, *J* 7.2, 1.2), 7.29 (1H, d, *J* 7.9), 7.47 (1H, d, *J* 7.9), 9.66/9.74 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.4, 25.5, 38.4, 43.2, 51.1, 52.8, 67.8, 107.8, 110.9, 117.9, 119.2, 121.6, 126.6, 134.0, 136.0, 156.7, 202.4. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{17}H_{20}N_2O_3 + H]^+$ 301.1552, found 301.1548.

*6.1.1.8. Desbromoarborescidine C (**2b**).* To a solution of **14b** (0.256 mmol) in dry THF (4.3 mL) was added a solution of AlH_3 in THF (1.55 M, 0.330 mL, 0.512 mmol) at room temperature. After 10 min, the reaction was quenched with saturated aq sodium sulphate solution and filtered. The solids were washed with CH_2Cl_2 (50 mL), and the filtrate was dried with Na_2SO_4, evaporated, and concentrated in vacuo. Purification of the residue by column chromatography afforded a white solid in 92% yield, which was characterized as desbromoarborescidine C. (*R*)-**2b**, $[\alpha]_D$ −3.7 ($c = 1$, $CHCl_3$); (*S*)-**2b**, $[\alpha]_D$ +3.3 ($c = 1$, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ:

1.58–1.75 (4H, m), 1.85–1.92 (2H, m), 2.27 (1H, q, *J* 13.2), 2.41 (1H, dd, *J* 11.5, 2.1), 2.81 (3H, s), 2.93–2.98 (1H, m), 3.05–3.07 (1H, m), 3.14–3.20 (1H, m), 3.38–3.42 (1H, m), 6.28 (1H, d, *J* 3.4), 7.16 (1H, t, *J* 6.0), 7.27 (1H, t, *J* 6.0), 7.40 (1H, d, *J* 6.6), 7.48 (1H, d, *J* 6.6). HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{16}H_{20}N_2O + H]^+$ 257.1654, found 257.1650.

*6.1.1.9. Desbromoarborescidine B (**3b**).* A solution of **2b** (0.0896 mmol) and Burgess reagent (0.043 g, 0.179 mmol) in dry benzene (9.0 mL) was heated to reflux for 8 h under nitrogen atmosphere. The reaction solution was cooled, diluted with EtOAc (9.0 mL), washed with brine (4 × 9.0 mL), dried, and evaporated. Column chromatography of the residue over silica gel gave **3b** as colorless oil in 86% yield. (*R*)-**3b**, $[\alpha]_D$ −68 (*c* = 1, $CHCl_3$); (*S*)-**3b**, $[\alpha]_D$ + 61 (*c* = 1, $CHCl_3$). FT-IR (KBr film) cm^{-1}: 3047, 2933, 2902, 2840, 2784, 1673, 1569, 1558, 1470, 1437, 1382, 1316, 1222, 1052, 997, 898, 744. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 1.89 (H, dq, *J* 10.2, 3.6), 2.37 (1H, br d, *J* 13.0), 2.43 (1H, br d, *J* 13.0), 2.51–2.60 (1H, m), 2.55 (3H, s), 2.71–2.76 (2H, m), 2.94 (1H, dt, *J* 7.1, 1.8), 3.15 (1H, dd, *J* 5.1, 1.7), 3.43 (1H, d, *J* 10.1), 5.06 (1H, q, *J* 4.2), 6.93 (1H, dt, *J* 10.0), 7.14 (1H, t, *J* 7.3), 7.20 (1H, t, *J* 7.3), 7.33 (1H, d, *J* 8.2), 7.47 (1H, d, *J* 8.2). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 20.6, 28.0, 30.0, 42.4, 52.8, 62.5, 108.1, 109.1, 109.2, 110.0, 118.2, 120.2, 121.8, 122.0, 127.2, 136.1. HRMS, ESI(+)-MS: *m*/*z* calcd. for $[C_{16}H_{18}N_2 + H]^+$ 239.1548, found 239.1552.

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