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Total synthesis of phenanthroindolizidine alkaloids (\pm)-antofine, (\pm)-deoxypergularinine, and their dehydro congeners and evaluation of their cytotoxic activity

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Abstract

Due to their limited natural abundance and significant biochemical effects, we synthesized the alkaloids (\pm)-antofine (**1a**), (\pm)-deoxypergularinine (**1b**), and their dehydro congeners (**2** and **3**) starting from the corresponding phenanthrene-9-carboxaldehydes. We also evaluated their in vitro cytotoxic activity. Compounds **1a** and **1b** showed significant potency against various human tumor cell lines, including a drug-resistant variant, with EC₅₀ values ranging from 0.16–16 ng/mL. Structure–activity correlations of these alkaloids and some of their synthetic intermediates were also ascertained. The non-planar structure between the two major moieties, phenanthrene and indolizidine, plays a crucial role in the cytotoxic activity of phenanthroindolizidines. Increasing the planarity and rigidity of the indolizidine moiety significantly reduced potency. A methoxy group at the 2-position (**1a**) was more favorable for cytotoxic activity than a hydrogen atom (**1b**).

Introduction

Phenanthroindolizidine alkaloids are pentacyclic natural products produced by various plants of the Asclepiadaceae family, including *Cryptocarya* and *Ficus* species. Biological activities of these compounds include antiamebic, antibacterial, anti-inflammatory, and antifungal effects, as well as significant cytotoxic activity against various cancer cell lines.^{1,2} Prototype compounds, such as antofine (**1a**), not only exhibit IC₅₀ values in the low nanomolar range, but are also equally effective against drug-sensitive and multidrug-resistant (MDR) human cancer cell lines.^{3,4} Alkaloid **1a** exerts its cytotoxicity by inhibiting protein and nucleic acid synthesis. Recent mechanism studies by Lee et al. indicated that **1a** exhibits inhibitory activity on cell proliferation by arresting the G2/M phase of the cell cycle.⁵ Compound **1a** also interacts with the bulged regions of DNA and RNA.^{6,7}

Due to low natural abundance of this compound type, various syntheses of **1a** and other phenanthroindolizidine alkaloids, in both racemic and optically active forms, have been reported in an effort to further investigate the biochemical and pharmaceutical effects.^{8–14} The most commonly reported synthetic methodology toward the highly condensed natural

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alkaloids (\pm)-**1a** and its geometric isomer (\pm)-deoxypergularinine (**1b**) involves formation of the biaryl bond of the phenanthrene ring system in the final step, after coupling of the aromatic residues with the appropriate heterocycle. The other common sequence to these alkaloids uses differentially substituted phenanthrenes as starting materials, which are coupled with the appropriate heterocycle through a halomethyl moiety. Diverse strategies have been employed for attaching the pyrrolidine ring system. These methods include strategies based on the alkylation of pyrrole, alkylation of proline, use of a pyrrole-substituted Wittig-like reagent, and ortho metalation. Other strategies include a pyridine-based strategy and a cyclohexadienone photorearrangement approach. Enantioselective approaches based on alkene metathesis have recently been reported.

Chuang et al.¹⁵ previously published an efficient synthesis of the compound type, preparing both unsubstituted phenanthroindolizine and the racemic alkaloid tylophorine (2,3,6,7-tetramethoxyphenanthroindolizine). Herein, we describe the total synthesis of **1a** and **1b**, which are 2,3,6- and 3,6,7-trimethoxy-substituted phenanthroindolizidine isomers, respectively, using a similar synthetic protocol. Their dehydroiminium chlorides (**2**) and bromides (**3**) were also prepared. We evaluated in vitro cytotoxic activity and report structure–activity relationships (SAR) of these natural alkaloids, their salts, and certain synthetic intermediates.

Results and Discussion

Our retrosynthetic analysis of **1a** and **1b** was identical to that described and shown in reference 15. The starting materials for our synthesis were the known phenanthryl aldehydes **10a** and **10b**, which were synthesized from the commercially available acids **4a** and **4b** and aldehydes **5a** and **5b** via a conventional five-step sequence, according to a previously reported procedure, as shown in Scheme 1.¹⁵

As shown in Scheme 2, aldehydes **10a** and **10b** were converted first to carboxylic acids **11a** and **11b** and then to acyl azides **12a** and **12b** through the reported sequence¹⁵ of Wittig reaction with (carboethoxymethylene)triphenyl phosphorane, hydrolysis to give the carboxylic acid, and treatment with oxalyl chloride and sodium azide to give the azide. These relatively unstable azides were used directly in the next reaction after purification by silica gel chromatography. A sequence involving a thermal Curtius rearrangement, an electrocyclic reaction, and hydrogen shift in boiling *o*-dichlorobenzene in the presence of I₂, rather than Hg(OAc)₂ as in reference 15, gave good yields of the isoquinolinones **13a** and **13b** from **12a** and **12b**, respectively. The pyrrolidine ring was added by the established methodology (*N*-alkylation with 1-bromo-3-chloropropane, conversion of bromide to iodide with excess sodium iodide in CH₃CN, radical cyclization with *n*-Bu₃SnH/AIBN in refluxing toluene)¹⁵ to afford phenanthroindolizidinones **14a** and **14b**. Finally, reduction of **14a** and **14b** with sodium bis(2-methoxyethoxy)aluminum hydride in refluxing dioxane gave racemic antofine **1a** and deoxypergularinine **1b**, respectively. Dehydroiminium chlorides **2a** and **2b** were prepared by exposing CHCl₃ solutions of **1a** and **1b**, respectively to light and air,¹⁷ while the corresponding dehydroiminium bromides **3a** and **3b** were obtained from oxidation of **1a** and **1b** by *N*-bromosuccinimide in CHCl₃.¹⁸

Biological Results

In order to explore their cytotoxic spectra and critical drug-resistance profiles, the target compounds and several synthetic intermediates were screened against four human tumor cell lines, A549 (lung), MCF-7 (breast), KB (nasopharyngeal) and KB-VIN (multidrug-resistant KB subline).¹⁹ The results are shown in Table 1. Compound **1a** exhibited the best activity against all four cell lines, with EC₅₀ values ranging from 0.16 to 4.5 ng/mL. Compound **1b** also showed pronounced activity with EC₅₀ values from 0.011 to 0.043 μ g/mL; however, it

was less potent (ca. 10- to 70-fold) than **1a**. Both compounds showed no significant loss in potency against the drug-resistant KV-VIN cell line as compared with the parental KB cell line. Compounds **2a**, **2b**, **3a**, and **3b**, which have a dehydroiminium skeleton, showed reduced potency against all cell lines with EC₅₀ values ranging from 0.2 to 8.26 μg/mL. Interestingly, **14b** with an amide functionality also had moderate potency against all four cell lines, with EC₅₀ values from 1.15 to 2.14 μg/mL. The remaining tested intermediates **11a–13a** were inactive in this assay.

Our data confirmed and extended SAR findings reported previously for this compound class.^{4,15,20} Introduction of double bonds adjacent to the phenanthrene nucleus reduced activity substantially. Compounds **1a/1b** (non-planar) showed greater potency than their respective dehydro-counterparts, **2a/2b** and **3a/3b** (planar). The dehydro compounds have a charged pyridinium ring, which could decrease cellular permeability. In addition, compounds **1a/1b** were much more potent than **14a/14b**. The ketone at position-9 in the latter compounds changes the tertiary amine in the indolizidine nucleus to an amide, and increases the rigidity and planarity. The five-ring system is essential for cytotoxic activity; isoquinoline **13b** (four-ring) and phenanthrenes **11a** and **12a** (three-ring) were inactive in all cell lines. Recently, several groups have reported that cytotoxic potency of phenanthroindolizidine is influenced by the substitution types and patterns on the phenanthrene ring.^{4,20} For example, Fu et al. found that changing the 2-methoxy of **1a** to a bulkier isopropoxy or more polar hydroxy group decreased cytotoxic activity.⁴ In our study, we further found that the absence of the 2-methoxy as in **1b** also decreases potency. Specifically, decreased cytotoxicity (10- to 70-fold) was observed for **1b** relative to **1a**, indicating that the C-2 methoxy group on the phenanthrene ring is important for optimal activity.

In summary, total syntheses of two natural phenanthroindolizidine alkaloids antofine (**1a**) and deoxypergularinine (**1b**) and their dehydroiminium chlorides (**2a** and **2b**) and bromides (**3a** and **3b**) were accomplished using a methodology based on thermal and radical cyclizations and a Curtius rearrangement. Alkaloids **1a** and **1b** showed profound cytotoxic activity against four cancer cell lines, with comparable potency in KB and KB-VIN cell lines. From activity results, we demonstrated that a non-planar five-ring structure and a methoxy group at the 2-position are important and favorable for potent activity of phenanthroindolizidine alkaloids.

Experimental Section

General Methods

Melting points were determined on a Yanaco MP-S3 melting point apparatus and are uncorrected. IR spectra were determined as KBr discs on a Shimadzu FT-IR Prestige 21. ¹H and ¹³C-NMR spectra were recorded on Bruker Avance NMR 300 and AV 500 spectrometers, using tetramethylsilane (TMS) as an internal standard; all chemical shifts were reported in ppm (δ). All MS and HRMS spectra (EI) were obtained on a VG-70-250S mass spectrometer. FAB and HR-FAB mass spectra were obtained on a Joel JMS-700 spectrometer. Tetrahydrofuran was distilled over sodium, and dichloromethane was distilled over CaH₂ unless otherwise stated. All other solvents and chemicals were used as purchased unless otherwise stated. Reactions were performed under Ar atmosphere in oven-dried flasks unless otherwise indicated.

Preparation of stilbene acids **6a** and **6b**

(E)-2-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)acrylic acid 6a—A solution of homoveratric acid (2.78 g, 20 mmol), *p*-anisaldehyde (3.96 g, 20 mmol), acetic anhydride (5.7 mL) and triethylamine (2.1 mL) was refluxed with stirring under Ar for 10 h. The resulting solution was cooled to room temperature, 10% K₂CO₃ solution (100 mL) was added, and then

the resulting mixture was stirred for 2 h at 60 °C. The reaction mixture was cooled to room temperature and neutralized with concentrated HCl (pH = 4). The yellow stilbene acid obtained was collected by filtration and recrystallized from CH₃OH to yield pure **6a** (4.96 g, 79%): yellow needles, mp 217~218 °C, IR (KBr) cm⁻¹: 3536, 2959, 1679, 1601; ¹H-NMR (300 MHz, CDCl₃) δ: 7.87 (1H, s, H-3''), 7.06 (2H, d, *J* = 8.4 Hz, H-2' and H-6'), 6.91 (1H, d, *J* = 8.0 Hz, H-5), 6.82 (1H, d, *J* = 8.0 Hz, H-6), 6.76 (1H, s, H-2), 6.71 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 3.93 (3H, s, OCH₃-4), 3.81 (3H, s, OCH₃-3), 3.93 (3H, s, OCH₃-4'); ¹³C-NMR (75 MHz, CDCl₃) δ: 172.5, 160.6, 149.2, 148.7, 142.1, 132.7, 128.6, 128.1, 127.0, 122.1, 113.8, 113.8, 112.8, 111.5, 55.9, 55.8, 55.2; EIMS *m/z*: 314 ([M⁺], 100), 178 (9), 137(17); anal. calcd for C₁₈H₁₈O₅: C, 68.78; H, 5.77. Found: C, 68.68; H, 5.73.

(E)-3-(3,4-Dimethoxyphenyl)-2-(4-methoxyphenyl)acrylic acid 6b—Homoanisic acid **4b** and 3,4-dimethoxybenzaldehyde **5b** were used as starting materials in an analogous procedure as for the preparation of **6a** to give **6b** (4.75 g, 76%): yellow needles, mp 218~219 °C, IR (KBr) cm⁻¹: 3536, 2983, 1663, 1595; ¹H-NMR (300 MHz, CDCl₃) δ: 7.86 (1H, s, H-3''), 7.20 (2H, d, *J* = 8.5 Hz, H-2 and H-6), 6.95 (2H, d, *J* = 8.0 Hz, H-3 and H-5), 6.81 (1H, dd, *J* = 8.3, 1.5 Hz, H-6'), 6.73 (1H, d, *J* = 8.3 Hz, H-5'), 6.53 (1H, d, *J* = 1.5 Hz, H-2'), 3.84 (3H, s, OCH₃-4'), 3.81 (3H, s, OCH₃-3'), 3.46 (3H, s, OCH₃-4); ¹³C-NMR (75 MHz, CDCl₃) δ: 173.1, 159.3, 150.3, 148.2, 142.2, 131.2×2, 128.0, 127.3, 125.8, 114.3×2, 112.5, 110.5, 55.8, 55.3, 55.2; EIMS *m/z*: 314 ([M⁺], 100), 178 (9), 137(17); anal. calcd for C₁₈H₁₈O₅: C, 68.78; H, 5.77. Found: C, 68.58; H, 5.77.

Preparation of stilbene methyl esters **7a** and **7b**

(E)-2-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)acrylic acid methyl ester 7a—Stilbene acid **6a** (6.28g, 20 mmol) was dissolved in 120 mL water containing 8 mL concentrated H₂SO₄, and the resulting solution was refluxed for 4 h. The solvent was evaporated under reduced pressure, and the crude residue was purified by silica gel column chromatography (*n*-hexane–CHCl₃ = 1:2) to afford **7a** (4.21 g, 63.1%): yellow crystals, mp 111~112 °C, IR (KBr) cm⁻¹: 3000, 2951, 1708, 1602; ¹H-NMR (300 MHz, CDCl₃) δ: 7.77 (1H, s, H-3''), 7.02 (2H, d, *J* = 8.8 Hz, H-2' and H-6'), 6.90 (1H, d, *J* = 8.1 Hz, H-5), 6.78 (1H, dd, *J* = 8.1, 1.2 Hz, H-6), 6.74 (1H, d, *J* = 1.2 Hz, H-2), 6.70 (2H, d, *J* = 8.8 Hz, H-3' and H-5'), 3.92 (3H, s, OCH₃-4), 3.80 (3H, s, OCH₃-3), 3.79 (3H, s, OCH₃-1''), 3.76 (3H, s, OCH₃-4'); ¹³C-NMR (75 MHz, CDCl₃) δ: 168.7, 160.3, 149.1, 148.5, 140.2, 132.4×2, 129.6, 128.6, 127.3, 122.1, 113.7×2, 112.8, 111.4, 55.8, 55.7, 55.2, 52.3; EIMS *m/z*: 328 ([M⁺], 100), 269 (10), 151(58); anal. calcd for C₁₉H₂₀O₅: C, 69.50; H, 6.14. Found: C, 69.63; H, 6.11.

(E)-3-(3,4-Dimethoxyphenyl)-2-(4-methoxyphenyl)acrylic acid methyl ester 7b—Esterification of **6b** according to the above procedure afforded **7b** (4.21 g, 63.1%): yellow crystals, mp 72~73 °C, IR (KBr) cm⁻¹: 3005, 2950, 2838, 1700, 1604; ¹H-NMR (300 MHz, CDCl₃) δ: 7.76 (1H, s, H-3''), 7.16 (2H, d, *J* = 8.5 Hz, H-2 and H-6), 7.04 (2H, d, *J* = 8.5 Hz, H-3 and H-5), 6.84 (1H, dd, *J* = 8.4, 2.2 Hz, H-6'), 6.71 (1H, d, *J* = 8.4 Hz, H-5'), 6.47 (1H, s, H-2'), 3.82 (3H, s, OCH₃-4'), 3.80 (3H, s, OCH₃-3'), 3.76 (3H, s, OCH₃-1''), 3.44 (3H, s, OCH₃-4); ¹³C-NMR (75 MHz, CDCl₃) δ: 168.5, 159.0, 149.8, 148.0, 140.2, 131.0×2, 129.5, 128.4, 127.4, 125.2, 114.2×2, 112.3, 110.4, 55.6, 55.1, 55.0, 52.1; EIMS *m/z*: 328 ([M⁺], 100), 121 (34); anal. calcd for C₁₉H₂₀O₅: C, 69.50; H, 6.14. Found: C, 69.48; H, 6.05.

Preparation of phenanthrene esters **8a** and **8b**

3,6,7-Trimethoxyphenanthrene-9-carboxylic acid methyl ester 8a—A solution composed of stilbene ester **7a** (6.56 g, 20 mmol) and 1 mL of trifluoroacetic acid anhydride in 60 mL of dry CH₂Cl₂ was added dropwise to a solution of vanadium oxytrifluoride (4.96 g, 40 mmol) in 60 mL CH₂Cl₂ and 30 mL EtOAc containing 4 mL of trifluoroacetic acid and 1 mL of trifluoroacetic anhydride. During the addition (60 min), the reaction mixture was cooled

in an ice-salt bath. The dark brown mixture was stirred for an additional 0.5 h in an ice-salt bath, then poured onto crushed ice, and was extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane– $\text{CHCl}_3 = 1:4$) to afford **8a** (4.82 g, 73.9%): colorless crystals, mp 150–151 °C, IR (KBr) cm^{-1} : 3116, 2943, 1713, 1613; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 8.64 (1H, s, H-8), 8.42 (1H, s, H-10), 7.84 (1H, d, $J = 8.6$ Hz, H-1), 7.83 (1H, s, H-5), 7.78 (1H, d, $J = 2.4$ Hz, H-4), 7.20 (1H, dd, $J = 8.6, 2.4$ Hz, H-2), 4.10 (3H, s, OCH_3 -6), 4.08 (3H, s, OCH_3 -7), 4.02 (3H, s, OCH_3 -3), 4.00 (3H, s, OCH_3 -11); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 168.2, 160.1, 149.8, 148.8, 133.3, 131.8, 131.2, 125.1, 124.9, 124.2, 121.6, 116.0, 106.9, 103.6, 103.1, 55.8 $\times 2$, 55.5, 52.0; EIMS m/z : 326 ($[\text{M}^+]$, 100), 283 (14); anal. calcd for $\text{C}_{19}\text{H}_{18}\text{O}_5$: C, 69.93; H, 5.56. Found: C, 69.70; H, 5.53.

2,3,6-Trimethoxyphenanthrene-9-carboxylic acid methyl ester 8b—Phenanthrene ester **8b** (4.73 g, 72.5%) was prepared from **7b** according to the above procedure: colorless crystals, mp 154–155 °C, IR (KBr) cm^{-1} : 3001, 2950, 1708, 1617; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 8.91 (1H, d, $J = 9.2$ Hz, H-8), 8.23 (1H, s, H-10), 7.79 (1H, s, H-5), 7.74 (1H, s, H-4), 7.24 (1H, d, $J = 9.2$ Hz, H-7), 7.18 (1H, s, H-1), 4.08 (3H, s, OCH_3 -6), 4.00 (9H, s, OCH_3 -2,3, and 6); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 168.1, 157.9, 159.8, 149.5, 131.5, 129.4, 128.2, 126.6, 125.4, 123.6, 123.1, 115.6, 109.1, 104.2, 103.0, 55.9, 55.8, 55.4, 52.0; EIMS m/z : 326 ($[\text{M}^+]$, 100), 181 (20); anal. calcd for $\text{C}_{19}\text{H}_{18}\text{O}_5$: C, 69.93; H, 5.56. Found: C, 69.61; H, 5.52.

Preparation of phenanthrene alcohols **9a** and **9b**

(3,6,7-Trimethoxyphenanthrene-9-yl)methanol 9a—To a suspension of LiAlH_4 (2.4 g, 20 mmol) in 70 mL of dry THF was added a solution of **8a** (6.52 g, 20 mmol) in 100 mL of dry THF. The reaction mixture was stirred under Ar for 3 h and quenched by sequential addition of 4 mL of water, 4 mL of 15% NaOH, and 8 mL of water. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CHCl_3 , and the solution was washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl_3) to afford **9a** (4.96 g, 83.2%): colorless crystals, mp 155–156 °C, IR (KBr) cm^{-1} : 3386, 2933, 1610, 1525; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 7.83 (1H, s, H-5), 7.77 (1H, d, $J = 1.6$ Hz, H-4), 7.73 (1H, d, $J = 8.6$ Hz, H-1), 7.55 (1H, s, H-10), 7.49 (1H, s, H-8), 7.17 (1H, dd, $J = 8.6, 1.6$ Hz, H-2), 5.05 (2H, d, $J = 4.1$ Hz, H-11), 4.08 (3H, s, OCH_3 -6), 4.03 (3H, s, OCH_3 -7), 4.00 (3H, s, OCH_3 -3), 1.87 (1H, br s, D_2O -exchangeable, OH-11); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 158.3, 149.4, 148.8, 131.3, 131.2, 130.2, 125.7, 125.5, 124.8, 124.5, 115.4, 104.8, 103.9, 103.7, 64.7, 55.9 $\times 2$, 55.5; EIMS m/z : 298 ($[\text{M}^+]$, 100), 152 (21); anal. calcd for $\text{C}_{18}\text{H}_{18}\text{O}_4$: C, 72.47; H, 6.08. Found: C, 72.37; H, 6.06.

(2,3,6-Trimethoxyphenanthrene-9-yl)methanol 9b—Phenanthrene alcohol **9b** (4.97 g, 83.5%) was obtained using **8b** according to the above procedure: colorless crystals, mp 185–186 °C, IR (KBr) cm^{-1} : 3217, 2951, 1611, 1512; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 8.10 (1H, d, $J = 9.0$ Hz, H-9), 7.88 (1H, d, $J = 2.2$ Hz, H-5), 7.85 (1H, s, H-4), 7.55 (1H, s, H-10), 7.25 (1H, dd, $J = 9.0, 2.2$ Hz, H-7), 7.19 (1H, s, H-1), 5.13 (2H, s, H-11), 4.10 (3H, s, OCH_3 -6), 4.03 (6H, s, OCH_3 -2 and 3), 4.00 (3H, s, OCH_3 -3); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 158.1, 149.6, 149.4, 132.8, 131.7, 127.0, 126.1, 124.4, 124.1, 123.2, 115.2, 108.4, 104.7, 103.4, 64.3, 55.1, 55.9, 55.5; EIMS m/z : 298 ($[\text{M}^+]$, 100), 283 (6); anal. calcd for $\text{C}_{18}\text{H}_{18}\text{O}_4$: C, 72.47; H, 6.08. Found: C, 72.43; H, 6.06.

Preparation of phenanthrene aldehydes **10a** and **10b**

3,6,7-Trimethoxyphenanthrene-9-carbaldehyde 10a—To a solution of **9a** (5.96 g, 20 mmol) in 150 mL CH_2Cl_2 was added pyridinium chlorochromate (5.17 g, 20 mmol), and the

reaction mixture was stirred under Ar at room temperature for 2.5 h. Aqueous 100 mL NaHCO₃ was added, the mixture was filtered on Celite, and the filtrate was extracted with CH₂Cl₂. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl₃) to afford **10a** [4.98 g (84.1%)]: colorless crystals, mp 169~170 °C, IR (KBr) cm⁻¹: 2997, 2936, 1672, 1615, 1518; ¹H-NMR (300 MHz, CDCl₃) δ: 10.06 (1H, s, CHO-9), 8.77 (1H, s, H-8), 7.80 (1H, s, H-10), 7.73 (1H, d, *J* = 8.7 Hz, H-1), 7.55 (2H, s, H-4 and H-5), 7.13 (1H, dd, *J* = 8.7, 1.6 Hz, H-2), 4.03 (3H, s, OCH₃-6), 4.00 (3H, s, OCH₃-7), 3.97 (3H, s, OCH₃-3); ¹³C-NMR (75 MHz, CDCl₃) δ: 193.6, 160.9, 150.3, 148.9, 140.0, 133.9, 131.9, 127.2, 124.5, 124.0, 123.6, 115.9, 106.0, 103.7, 102.7, 55.8, 55.6, 55.4; EIMS *m/z*: 296 ([M⁺], 100), 253 (20); anal. cacl'd for C₁₈H₁₆O₄: C, 72.96; H, 5.44. Found: C, 72.88; H, 5.38.

2,3,6,-Trimethoxyphenanthren-9-carbaldehyde 10b—Phenanthrene aldehyde **10b** (4.89 g, 82.6%) was prepared according to the above procedure: colorless crystals, mp 183~184 °C, IR (KBr) cm⁻¹: 3011, 2960, 2938, 1679, 1616, 1515; ¹H-NMR (300 MHz, CDCl₃) δ: 10.25 (1H, s, CHO-9), 9.31 (1H, d, *J* = 9.2 Hz, H-8), 7.97 (1H, s, H-10), 7.83 (1H, d, *J* = 2.2 Hz, H-5), 7.80 (1H, s, H-4), 7.30 (1H, dd, *J* = 9.2, 2.2 Hz, H-7), 7.29 (1H, s, H-1), 4.12 (3H, s, OCH₃-6), 4.05 (3H, s, OCH₃-3), 4.02 (3H, s, OCH₃-2); ¹³C-NMR (75 MHz, CDCl₃) δ: 193.5, 158.6, 151.9, 149.8, 138.3, 131.7, 128.8, 127.7×2, 125.6, 122.2, 116.1, 109.5, 104.6, 103.3, 56.1×2, 55.5; EIMS *m/z*: 296 ([M⁺], 100), 281 (6); anal. cacl'd for C₁₈H₁₆O₄: C, 72.96; H, 5.44. Found: C, 72.99; H, 5.42.

Preparation of phenanthrene acrylic acids **11a** and **11b**

(E)-3-(3,6,7-Trimethoxyphenanthren-9-yl)acrylic acid 11a—A mixture of **10a** (2.96 g, 10 mmol) and (carboethoxymethylene)triphenylphosphorane (4.76 g, 13 mmol) in 80 mL toluene was refluxed under Ar for 5 h. After cooling, the resulting solution was directly purified by silica gel column chromatography (*n*-hexane-CHCl₃ = 1:4) and afforded the ethyl ester (3.19 g, 87.2%). Then, a solution of 30 mL 1 N KOH was added to a solution of the ethyl ester (3.19g, 8.7 mmol) in 60 mL EtOH, and the reaction mixture was heated under reflux for 3 h. After cooling, the solvent was evaporated under reduced pressure. The residue was dissolved in 30 mL water and acidified with 10% HCl. The yellow solid was filtered and recrystallized from CH₃OH to afford pure **11a** (2.68 g, 91.2%): yellow crystals, mp 258~259 °C, IR (KBr) cm⁻¹: 3530, 2946, 1679, 1605, 1519; ¹H-NMR (300 MHz, CDCl₃) δ: 12.50 (1H, br s, D₂O-exchangeable, OH), 8.34 (1H, d, *J* = 15.7 Hz, H-11), 8.12 (1H, s, H-8), 8.10 (1H, s, H-10), 8.06 (1H, s, H-4), 7.92 (1H, d, *J* = 8.8 Hz, H-1), 7.47 (1H, s, H-5), 7.24 (1H, d, *J* = 8.8 Hz, H-2), 6.61 (1H, d, *J* = 15.7 Hz, H-12), 4.04 (3H, s, OCH₃-7), 4.00 (3H, s, OCH₃-6), 3.97 (3H, s, OCH₃-3); ¹³C-NMR (75 MHz, CDCl₃) δ: 167.5, 158.9, 149.5, 149.0, 141.4, 131.6, 130.9, 126.7, 124.9×2, 124.5, 124.1, 120.9, 116.5, 104.7, 104.1×2, 55.9, 55.6, 55.4; EIMS *m/z*: 338 ([M⁺], 100), 295 (37), 277(62); anal. cacl'd for C₂₀H₁₈O₅: C, 70.99; H, 5.36. Found: C, 70.82; H, 5.35.

(E)-3-(2,3,6-Trimethoxyphenanthren-9-yl)acrylic acid 11b—Phenanthrene acrylic acid **11b** (2.75 g, 92.5%) was prepared according to the above procedure: yellow crystals, mp 205~206 °C, IR (KBr) cm⁻¹: 3530, 2953, 2937, 1689, 1616, 1512; ¹H-NMR (300 MHz, CDCl₃) δ: 12.40 (1H, br s, D₂O-exchangeable, OH), 8.34 (1H, d, *J* = 15.6 Hz, H-11), 8.12 (1H, d, *J* = 2.0 Hz, H-5), 8.10 (1H, d, *J* = 9.2 Hz, H-8), 8.09 (1H, s, H-10), 8.06 (1H, s, H-4), 7.49 (1H, s, H-4), 7.29 (1H, dd, *J* = 9.2, 2.0 Hz, H-7), 6.57 (1H, d, *J* = 15.6 Hz, H-12), 4.04 (3H, s, OCH₃-6), 4.00 (3H, s, OCH₃-3), 3.92 (3H, s, OCH₃-2); ¹³C-NMR (75 MHz, CDCl₃) δ: 169.5, 158.0, 150.0, 149.6, 141.2, 131.1, 127.5, 126.4, 125.3, 125.0, 123.4×2, 120.8, 116.1, 109.1, 104.9, 104.1, 56.0, 55.6, 55.5; EIMS *m/z*: 338 ([M⁺], 100), 293 (8); anal. cacl'd for C₂₀H₁₈O₅: C, 70.99; H, 5.36. Found: C, 70.95; H, 5.34.

Preparation of phenanthrene acryloyl azides **12a** and **12b**

(E)-3-(3,6,7-Trimethoxyphenanthren-9-yl)acryloyl azide 12a—A mixture of **11a** (1.69 g, 5 mmol) and oxalyl chloride (1.27 g, 10 mmol) in 70 mL toluene was heated for 5 h at 80 °C. After cooling, the resulting mixture was concentrated under reduced pressure to afford the acyl chloride quantitatively. The acyl chloride was added immediately to a suspension of NaN₃ (0.98 g, 15 mmol) in 60 mL dry acetone in an ice bath. The reaction mixture was stirred for 2 h at room temperature and filtered. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (CHCl₃) to afford **12a** (1.67 g, 92.1%): yellow crystals, mp 121~122 °C, IR (KBr) cm⁻¹: 2933, 2140, 1674, 1604, 1509; ¹H-NMR (300 MHz, CDCl₃) δ: 8.45 (1H, d, *J* = 15.5 Hz, H-11), 7.84 (2H, s, H-8 and H-10), 7.78 (1H, d, *J* = 8.6 Hz, H-1), 7.76 (1H, d, *J* = 1.6 Hz, H-4), 7.38 (1H, s, H-5), 7.20 (1H, dd, *J* = 8.6, 1.6 Hz, H-2), 6.54 (1H, d, *J* = 15.5, H-12), 4.10 (3H, s, OCH₃-7), 4.07 (3H, s, OCH₃-7), 3.92 (3H, s, OCH₃-3); ¹³C-NMR (75 MHz, CDCl₃) δ: 172.0, 159.4, 149.8, 149.2, 144.5, 132.4, 131.2, 126.6, 125.5, 125.4, 125.1, 124.5, 120.4, 116.1, 104.0, 103.8, 103.7, 56.0×2, 55.5; EIMS *m/z*: 335 ([M⁺-N₂], 100); anal. calcd for C₂₀H₁₇N₃O₄: C, 66.11; H, 4.72; N, 11.56. Found: C, 66.82; H, 4.80; N, 11.07.

(E)-3-(2,3,6-Trimethoxyphenanthren-9-yl)acryloyl azide 12b—Phenanthrene acryloyl azide **12b** (1.64 g, 90.3%) was obtained using the above procedure: yellow crystals, mp 130~131 °C, IR (KBr) cm⁻¹: 3008, 2938, 2834, 2141, 1678, 1613, 1513; ¹H-NMR (300 MHz, CDCl₃) δ: 8.51 (1H, d, *J* = 15.5 Hz, H-11), 8.07 (1H, d, *J* = 9.0 Hz, H-8), 7.83 (1H, s, H-5), 7.75 (1H, s, H-10), 7.24 (1H, d, *J* = 9.0 Hz, H-7), 7.16 (1H, s, H-1), 6.53 (1H, d, *J* = 15.5 Hz, H-12), 4.10 (3H, s, OCH₃-6), 4.03 (3H, s, OCH₃-3), 4.02 (3H, s, OCH₃-2); ¹³C-NMR (75 MHz, CDCl₃) δ: 172.0, 158.3, 150.3, 149.7, 144.4, 131.3, 127.8, 126.5, 125.8, 125.5, 124.1, 123.8, 120.5, 115.6, 108.7, 104.6, 103.1, 56.0, 55.9, 55.5; EIMS *m/z*: 335 ([M⁺-N₂], 100), 320 (11); anal. calcd for C₂₀H₁₇N₃O₄: C, 66.11; H, 4.72; N, 11.56. Found: C, 66.46; H, 4.82; N, 11.08.

Preparation of isoquinolinones **13a** and **13b**

6,7,10-Trimethoxy-2H-2-azatriphenylene-1-one 13a—A mixture of azide **12a** (1.1 g, 3 mmol) and I₂ (0.02 g, catalytic amount) in 30 mL *o*-dichlorobenzene was refluxed for 2 h. After cooling, compound **13a** was isolated by filtration and washed with *n*-hexane. The crude product was recrystallized from a CH₃OH-CHCl₃ mixture to afford pure isoquinolinone **13a** (839 mg, 83.5%): pale yellow crystals, mp 269~270 °C, IR (KBr) cm⁻¹: 3272, 2939, 2890, 1630, 1614, 1555, 1503; ¹H-NMR (300 MHz, DMSO-*d*₆) δ: 11.70 (1H, br s, D₂O-exchangeable, NH), 10.30 (1H, d, *J* = 9.5 Hz, H-12), 8.10 (2H, s, H-8 and H-9), 7.93 (1H, s, H-5), 7.47 (2H, m, H-3 and H-4), 7.27 (1H, d, *J* = 9.5 Hz, H-11), 4.07 (3H, s, OCH₃-7), 4.01 (3H, s, OCH₃-6), 3.99 (3H, s, OCH₃-10); ¹³C-NMR (75 MHz, DMSO-*d*₆) δ: 162.6, 157.7, 150.9, 149.6, 136.3, 130.3, 130.1, 129.0, 126.2, 123.2, 122.1, 117.0, 115.0, 106.1, 105.0, 104.7, 101.0, 55.9, 55.8, 55.3; EIMS *m/z*: 335 ([M⁺], 100), 292 (17); anal. calcd for C₂₀H₁₇NO₄: C, 71.63; H, 5.11; N, 4.18. Found: C, 71.22; H, 5.17; N, 4.03.

7,10,11-Trimethoxy-2H-2-azatriphenylene-1-one 13b—Isoquinolinone **13b** (820 mg, 81.6%) was prepared according to the above procedure: pale yellow crystals, mp 274~275 °C, IR (KBr) cm⁻¹: 3267, 3219, 2957, 1627, 1554, 1517; ¹H-NMR (300 MHz, DMSO-*d*₆) δ: 11.70 (1H, br s, D₂O-exchangeable, NH), 10.04 (1H, s, H-12), 8.56 (1H, d, *J* = 9.2 Hz, H-5), 8.11 (1H, d, *J* = 2.0 Hz, H-8), 8.10 (1H, s, H-9), 7.44 (1H, m, H-4), 7.43 (1H, m, H-3), 7.29 (1H, dd, *J* = 9.2, 2.0 Hz, H-6), 4.04 (3H, s, OCH₃-11), 4.03 (3H, s, OCH₃-10), 3.92 (3H, s, OCH₃-7); ¹³C-NMR (75 MHz, DMSO-*d*₆) δ: 162.7, 160.1, 149.0, 148.4, 137.3, 133.2, 130.4, 127.3, 125.0, 123.3, 120.6, 116.2, 115.7, 108.7, 105.1, 104.5, 100.6, 55.7, 55.6, 55.2; EIMS *m/z*: 335 ([M⁺], 100), 320 (12), 293 (17); anal. calcd for C₂₀H₁₇NO₄: C, 71.63; H, 5.11; N, 4.18. Found: C, 71.78; H, 5.13; N, 4.22.

Preparation of phenanthroindolizidiones 14a and 14b

2-(3-Chloropropyl)-6,7,10-trimethoxy-2H-2-azatriphenylen-1-one—To a suspension of NaH (60% dispersion in oil, 80 mg, 2 mmol) in DMF (5 mL) cooled in an ice bath, a solution of **13a** (335 mg, 1 mmol) in DMF (15 mL) was added with stirring. After the addition was completed, the mixture was added dropwise to a solution of 1-bromo-3-chloropropane (628 mg, 4 mmol) in 5 mL DMF. The mixture was stirred at room temperature overnight. The solvent was evaporated *in vacuo*, and water was then added. The mixture was extracted with CHCl₃, washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane–CHCl₃ = 1:4) to afford the chloropropyl isoquinolinone of **13a** (292 mg, 71.2%): colorless crystals, mp 183–184 °C, IR (KBr) cm⁻¹: 2988, 2934, 1644, 1598, 1528; ¹H-NMR (300 MHz, CDCl₃) δ: 10.31 (1H, d, *J* = 9.5 Hz, H-12), 7.93 (2H, s, H-8 and H-9), 7.73 (1H, s, H-5), 7.48 (1H, d, *J* = 7.3 Hz, H-3), 7.32 (1H, d, *J* = 9.5 Hz, H-11), 7.21 (1H, d, *J* = 7.3 Hz, H-4), 4.32 (2H, t, *J* = 6.4 Hz, H-1'), 4.14 (3H, s, OCH₃-7), 4.11 (3H, s, OCH₃-6), 4.03 (3H, s, OCH₃-10), 3.64 (2H, t, *J* = 5.9 Hz, H-3'), 2.40 (2H, tt, *J* = 6.4, 5.9 Hz, H-2'); ¹³C-NMR (75 MHz, CDCl₃) δ: 162.1, 158.2, 151.0, 149.6, 135.6, 133.0, 131.1, 130.0, 127.0, 123.8, 122.3, 118.3, 114.5, 105.4, 105.2, 104.0, 101.4, 56.0×2, 55.5, 47.9, 42.2, 31.1; EIMS *m/z*: 413 ([M+2], 42), 411 ([M⁺], 80), 376 (46); anal. cacl'd for C₂₃H₂₂ClNO₄: C, 67.07; H, 5.38; N, 3.40. Found: C, 67.79; H, 5.40; N, 3.39.

2,3,6-Trimethoxy-11,12,12a,13-tetrahydro-10H-9α-azacyclopenta[β]

triphenylen-9-one 14a—A solution of chloropropyl isoquinolinone (251 mg, 0.5 mmol) and NaI (375 mg, 2.5 mmol) in dry CH₃CN (15 mL) was placed in a sealed tube and then heated at 125 °C for 12 h. After cooling, the reaction mixture was filtered, and concentrated under reduced pressure to yield the iodopropyl isoquinolinone quantitatively. Subsequently, a solution of AIBN (33 mg, 0.2 mmol) in 2.4 mL toluene was added dropwise (syringe pump) to a degassed solution of iodopropyl isoquinolinone (251 mg, 0.5 mmol) and *n*-Bu₃SnH (175 mg, 0.6 mmol) in 25 mL of toluene at refluxing temperature for 6 h. The reaction mixture was then cooled and the solvent was removed under reduced pressure. The residue was washed with *n*-hexane, purified by silica gel column chromatography (CHCl₃) to give phenanthroindolizidione **14a** (140 mg, 74.6%): colorless crystals, mp 252–253 °C, IR (KBr) cm⁻¹: 2947, 1631, 1614, 1518; ¹H-NMR (300 MHz, CDCl₃) δ: 9.27 (1H, d, *J* = 9.3 Hz, H-8), 7.82 (2H, s, H-4 and H-5), 7.81 (1H, d, *J* = 9.3 Hz, H-7), 7.22 (1H, s, H-1), 4.09 (3H, s, OCH₃-3), 4.01 (3H, s, OCH₃-2), 3.99 (3H, s, OCH₃-6), 3.86 (1H, m, H-11), 3.83 (1H, m, H-13), 3.80 (1H, m, H-11), 3.46 (1H, dd, *J* = 15.6, 3.9 Hz, H-14), 2.80 (1H, dd, *J* = 15.6, 12.0 Hz, H-14), 2.39 (1H, m, H-13), 2.12 (1H, m, H-12), 1.89 (1H, m, H-12), 1.82 (1H, m, H-13); ¹³C-NMR (75 MHz, CDCl₃) δ: 164.3, 157.6, 149.9, 149.4, 132.5, 130.8, 129.5×2, 126.3, 124.2, 123.4, 115.1, 104.7, 104.0, 103.7, 55.9, 55.8, 55.4, 55.1, 45.2, 33.8, 32.5, 23.5; EIMS *m/z*: 377 ([M⁺], 100), 308 (57), 280 (45); anal. cacl'd for C₂₃H₂₃NO₄: C, 73.19; H, 6.14; N, 3.71. Found: C, 73.09; H, 6.40; N, 3.59.

2-(3-Chloropropyl)-7,10,11-trimethoxy-2H-2-azatriphenylen-1-one

—The chloropropyl isoquinolinone of **13b** (297 mg, 72.2%) was prepared according to the above procedure: colorless crystals, mp 184–185 °C, IR (KBr) cm⁻¹: 3119, 2957, 1644, 1614, 1599, 1519; ¹H-NMR (300 MHz, CDCl₃) δ: 10.05 (1H, s, H-12), 8.37 (1H, d, *J* = 9.2 Hz, H-5), 7.91 (1H, s, H-9), 7.90 (1H, d, *J* = 2.6 Hz, H-8), 7.47 (1H, d, *J* = 7.4 Hz, H-3), 7.29 (1H, d, *J* = 7.4 Hz, H-4), 7.24 (1H, dd, *J* = 9.2, 2.6 Hz, H-6), 4.31 (2H, t, *J* = 6.2 Hz, H-1'), 4.15 (3H, s, OCH₃-11), 4.12 (3H, s, OCH₃-10), 4.04 (3H, s, OCH₃-7), 3.63 (2H, t, *J* = 6.2 Hz, H-3'), 2.40 (2H, p, *J* = 6.2 Hz, H-2'); ¹³C-NMR (75 MHz, CDCl₃) δ: 162.6, 160.3, 149.6, 148.6, 136.8, 133.7, 133.2, 126.6, 125.6, 124.1, 120.9, 117.2, 115.1, 109.1, 104.9, 103.4, 101.5, 56.0, 55.8, 55.5, 47.7, 42.1, 31.2; EIMS *m/z*: 413 ([M+2], 37), 411 ([M⁺], 100), 376 (28); anal. cacl'd for C₂₃H₂₂ClNO₄: C, 67.07; H, 5.38; N, 3.40. Found: C, 67.07; H, 5.36; N, 3.37.

3,6,7-Trimethoxy-11,12,12a,13-tetrahydro-10H-9 α -azacyclopenta[β]

triphenylen-9-one 14b—Phenanthroindolizidinone **14b** (143 mg, 75.8%) was obtained using the above procedure: colorless crystals, mp 200~201 °C, IR (KBr) cm^{-1} : 2959, 2937, 1638, 1615, 1516; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 9.02 (1H, s, H-8), 8.00 (1H, d, $J = 9.0$ Hz, H-1), 7.88 (1H, d, $J = 2.2$ Hz, H-4), 7.86 (1H, s, H-5), 7.22 (1H, dd, $J = 9.0, 2.2$ Hz, H-2), 4.11 (3H, s, OCH_3 -7), 4.09 (3H, s, OCH_3 -6), 4.04 (3H, s, OCH_3 -3), 3.87 (1H, m, H-11), 3.83 (1H, m, H-13), 3.79 (1H, m, H-11), 3.66 (1H, dd, $J = 15.6, 4.2$ Hz, H-14), 2.93 (1H, dd, $J = 15.6, 13.6$ Hz, H-14), 2.42 (1H, m, H-13), 2.15 (1H, m, H-12), 1.93 (1H, m, H-12), 1.90 (1H, m, H-13); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 164.6, 159.3, 149.6, 148.6, 134.2, 133.1, 126.7, 125.3, 124.3, 123.0, 122.0, 115.5, 108.1, 104.4, 102.9, 55.9 \times 2, 55.5, 55.3, 45.4, 33.9, 32.3, 23.5; EIMS m/z : 377 ($[\text{M}^+]$, 100), 308 (38), 280 (28); anal. cacl'd for $\text{C}_{23}\text{H}_{23}\text{NO}_4$: C, 73.19; H, 6.14; N, 3.71. Found: C, 73.42; H, 6.17; N, 3.86.

Preparation of phenanthroindolizidines 1a and 1b

Antofine 1a—To a solution of **15a** (39.7 mg, 1 mmol) in dry 5 mL dioxane was added a 3.5 M solution of sodium bis(2-methoxyethoxy)aluminium hydride in toluene (0.4 mL, 1.4 mmol) and the mixture was refluxed for 2 h in the dark. After evaporation of the solvents, the residue was diluted with 10 mL H_2O and then basified with 10 mL 10% aqueous NaOH. The mixture was extracted with CHCl_3 , dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl_3 -MeOH = 19:1) to yield **1a** (32.8 mg, 90.3%): colorless crystals, mp 211~212 °C, IR (KBr) cm^{-1} : 2914, 1615, 1529, 1512; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 7.90 (1H, s, H-4), 7.89 (1H, d, $J = 2.1$ Hz, H-5), 7.79 (1H, d, $J = 9.0$ Hz, H-8), 7.28 (1H, s, H-1), 7.19 (1H, dd, $J = 9.0, 2.1$ Hz, H-7), 4.66 (1H, d, $J = 14.9$ Hz, H-9), 4.09 (3H, s, OCH_3 -3), 4.05 (3H, s, OCH_3 -2), 4.00 (3H, s, OCH_3 -6), 3.66 (1H, d, $J = 14.9$ Hz, H-9), 3.45 (1H, td, $J = 8.8, 2.0$ Hz, H-11), 3.30 (1H, dd, $J = 15.7, 2.3$ Hz, H-14), 2.87 (1H, dd, $J = 15.7, 10.5$ Hz, H-14), 2.44 (1H, m, H-13a), 2.43 (1H, q, $J = 8.8$ Hz, H-11), 2.19 (1H, m, H-13), 1.99 (1H, m, H-12), 1.88 (1H, m, H-12), 1.72 (1H, m, H-13); $^{13}\text{C-NMR}$ (100 MHz) δ : 157.4 (C-8), 149.3 (C-3), 148.3 (C-2), 130.1 (C-4b), 127.0 (C-14b), 126.6 (C-8b), 125.5 (C-14a), 124.2 (C-8), 124.1 (C-8a), 123.5 (C-4a), 114.8 (C-7), 104.6 (C-5), 103.9 (C-1), 103.8 (C-4), 60.2 (C-13a), 56.0 (OCH_3 -3), 55.8 (OCH_3 -2), 55.5 (OCH_3 -6), 55.0 (C-11), 53.8 (C-9), 33.6 (C-14), 31.2 (C-13), 21.5 (C-12); EIMS m/z : 363 ($[\text{M}^+]$, 30), 294 (100); anal. cacl'd for $\text{C}_{23}\text{H}_{25}\text{NO}_3$: C, 76.01; H, 6.93; N, 3.85. Found: C, 75.95; H, 6.80; N, 3.98.

Deoxypergularinine 1b—Deoxypergularinine **1b** (33.4 mg, 92.0%) was prepared according to the above procedure: colorless crystals, 209~210 °C, IR (KBr) cm^{-1} : 2959, 2912, 1612, 1512; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 7.91 (1H, d, $J = 9.2$ Hz, H-1), 7.88 (1H, s, H-5), 7.86 (1H, d, $J = 2.5$ Hz, H-4), 7.21 (1H, dd, $J = 9.2, 2.5$ Hz, H-2), 7.09 (1H, s, H-8), 4.62 (1H, d, $J = 14.5$ Hz, H-9), 4.09 (3H, s, OCH_3 -7), 4.03 (3H, s, OCH_3 -6), 4.01 (3H, s, OCH_3 -3), 3.72 (1H, d, $J = 14.5$ Hz, H-9), 3.47 (1H, td, $J = 8.1, 2.0$ Hz, H-11), 3.40 (1H, dd, $J = 16.5, 3.0$ Hz, H-14), 3.00 (1H, dd, $J = 16.5, 10.5$ Hz, H-14), 2.59 (1H, m, H-13a), 2.58 (1H, dd, $J = 8.1, 8.1$ Hz, H-11), 2.25 (1H, m, H-13), 2.06 (1H, m, H-12), 1.96 (1H, m, H-12), 1.82 (1H, m, H-13); $^{13}\text{C-NMR}$ (100 MHz) δ : 157.7 (C-3), 149.4 (C-7), 148.3 (C-6), 130.4 (C-4b), 126.6 (C-8b), 125.3 (C-14b), 125.2 (C-14a), 125.1 (C-1), 124.2 (C-8a), 123.4 (C-4a), 114.9 (C-2), 104.6 (C-4), 103.8 (C-5), 102.9 (C-8), 60.2 (C-13a), 56.0 (OCH_3 -7), 55.9 (OCH_3 -6), 55.5 (OCH_3 -3), 55.4 (C-11), 53.1 (C-9), 32.6 (C-14), 30.9 (C-13), 21.5 (C-12); EIMS m/z : 363 ($[\text{M}^+]$, 40), 294 (100); anal. cacl'd for $\text{C}_{23}\text{H}_{25}\text{NO}_3$: C, 76.01; H, 6.93; N, 3.85. Found: C, 75.85; H, 6.81; N, 3.97.

Preparation of dehydrophenanthroindolizidine chlorides 2a and 2b

Dehydroantofine chloride 2a—A solution of antofine **1a** (36.3 mg, 1 mmol) in 10 mL CHCl_3 was exposed to light. After one day, a yellow product had formed, and was filtered and recrystallized from CH_3OH to afford pure **2a** (28.3 mg, 71.6%): yellow crystals, >290 °C, IR

(KBr) cm^{-1} : 2968, 1609, 1519; $^1\text{H-NMR}$ (300 MHz, CD_3OD) δ : 9.89 (1H, s, H-9), 8.74 (1H, s, H-14), 8.42 (1H, d, $J = 8.9\text{ Hz}$, H-8), 7.77 (1H, s, H-1), 7.52 (1H, d, $J = 2.2\text{ Hz}$, H-5), 7.50 (1H, s, H-1), 7.24 (1H, dd, $J = 8.9, 2.2\text{ Hz}$, H-7), 4.95 (2H, t, $J = 7.4\text{ Hz}$, H-11), 4.05 (6H, s, OCH_3 -2 and OCH_3), 4.01 (3H, s, OCH_3 -6), 3.60 (2H, t, $J = 7.4\text{ Hz}$, H-13), 2.63 (2H, quin, $J = 7.4\text{ Hz}$, H-12); $^{13}\text{C-NMR}$ (100 MHz) δ : 161.8, 154.3, 151.6, 151.3, 139.9, 137.9, 132.6, 128.9, 125.7 \times 2, 120.9, 119.6, 117.3, 117.0, 107.0, 106.6, 105.4, 59.5, 56.6, 56.4, 56.0, 32.1, 22.8; EIMS m/z : 360 ($[\text{M}^+ - \text{Cl}]$, 46), 154 (100); anal. calcd for $\text{C}_{23}\text{H}_{22}\text{ClNO}_3$: C, 69.78; H, 5.60; N, 3.54. Found: C, 69.92; H, 5.45; N, 3.36.

Deoxypergularinine chloride 2b—Deoxypergularinine chloride **2b** (27.8 mg, 70.3%) was prepared according to the above procedure: yellow crystals, $>290\text{ }^\circ\text{C}$, IR (KBr) cm^{-1} : 2964, 1608, 1517; $^1\text{H-NMR}$ (300 MHz, CD_3OD) δ : 9.51 (1H, s, H-9), 8.30 (1H, s, H-14), 8.09 (1H, d, $J = 9.0\text{ Hz}$, H-1), 7.13 (1H, s, H-8), 6.94 (1H, dd, $J = 9.0, 1.5\text{ Hz}$, H-2), 6.68 (1H, d, $J = 1.5\text{ Hz}$, H-4), 6.61 (1H, s, H-5), 4.74 (2H, t, $J = 7.2\text{ Hz}$, H-11), 3.89 (3H, s, OCH_3 -7), 3.86 (3H, s, OCH_3 -6), 3.80 (3H, s, OCH_3 -3), 3.41 (2H, t, $J = 7.2\text{ Hz}$, H-13), 2.54 (2H, p, $J = 7.2\text{ Hz}$, H-12); $^{13}\text{C-NMR}$ (75 MHz) δ : 162.5, 151.1, 150.9, 150.8, 140.0, 137.7, 134.6, 128.0, 124.3, 124.1, 120.2, 118.5, 116.7, 116.0, 106.1, 104.1 \times 2, 58.6, 57.3, 56.4, 56.3, 31.4, 22.2; EIMS m/z : 360 ($[\text{M}^+ - \text{Cl}]$, 43), 154 (100); anal. calcd for $\text{C}_{23}\text{H}_{22}\text{ClNO}_3$: C, 69.78; H, 5.60; N, 3.54. Found: C, 69.68; H, 5.55; N, 3.48.

Preparation of dehydrophenanthroindolizidine bromides 3a and 3b

Dehydroantofine bromide 3a—To a solution of antofine **1a** (36.3 mg, 1 mmol) in 10 mL CHCl_3 was added NBS (150 mg, 0.4 mmol) in small portions with stirring. After stirring for 1 h at room temperature, the orange solid formed was collected by filtration. The crude product was purified by aluminum oxide gel chromatography (CHCl_3 – $\text{MeOH} = 9:1$) to provide **3a** (23.1 mg, 52.6%): yellow crystals, $>290\text{ }^\circ\text{C}$, IR (KBr) cm^{-1} : 2970, 1637, 1610, 1521; $^1\text{H-NMR}$ (300 MHz, CD_3OD) δ : 9.91 (1H, s, H-9), 8.76 (1H, s, H-14), 8.43 (1H, d, $J = 9.0\text{ Hz}$, H-8), 7.79 (1H, s, H-1), 7.53 (1H, d, $J = 2.1\text{ Hz}$, H-5), 7.52 (1H, s, H-1), 7.26 (1H, dd, $J = 9.0, 2.1\text{ Hz}$, H-7), 4.96 (2H, t, $J = 7.4\text{ Hz}$, H-11), 4.07 (6H, s, OCH_3 -2 and OCH_3), 4.02 (3H, s, OCH_3 -6), 3.62 (2H, t, $J = 7.4\text{ Hz}$, H-13), 2.65 (2H, quin, $J = 7.4\text{ Hz}$, H-12); $^{13}\text{C-NMR}$ (100 MHz) δ : 162.3, 154.8, 151.9, 151.8, 140.3, 138.3, 133.0, 129.3, 126.2 \times 2, 121.2, 120.1, 117.7, 117.5, 107.4, 107.0, 105.9, 59.7, 57.0, 56.9, 56.3, 32.5, 23.1; EIMS m/z : 360 ($[\text{M}^+ - \text{Cl}]$, 67), 154 (100); anal. calcd for $\text{C}_{23}\text{H}_{22}\text{BrNO}_3$: C, 76.64; H, 6.15; N, 3.89. Found: C, 76.91; H, 6.25; N, 3.96.

Deoxypergularinine bromide 3b—Deoxypergularinine bromide **3b** (24.2 mg, 55.1%) was prepared according to the above procedure: yellow crystals, $>290\text{ }^\circ\text{C}$, IR (KBr) cm^{-1} : 2965, 1639, 1608, 1516; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ : 9.51 (1H, s, H-9), 8.31 (1H, s, H-14), 8.10 (1H, d, $J = 9.1\text{ Hz}$, H-1), 7.13 (1H, s, H-8), 6.95 (1H, dd, $J = 9.1, 1.6\text{ Hz}$, H-2), 6.68 (1H, d, $J = 1.6\text{ Hz}$, H-4), 6.63 (1H, s, H-5), 4.75 (2H, t, $J = 7.1\text{ Hz}$, H-11), 3.90 (3H, s, OCH_3 -7), 3.86 (3H, s, OCH_3 -6), 3.81 (3H, s, OCH_3 -3), 3.43 (2H, t, $J = 7.1\text{ Hz}$, H-13), 2.55 (2H, quin, $J = 7.1\text{ Hz}$, H-12); $^{13}\text{C-NMR}$ (100 MHz) δ : 163.3, 151.4, 151.2, 151.0, 140.2, 138.0, 134.8, 128.4, 124.4, 124.1, 120.8, 118.8, 116.8, 116.4, 106.1, 104.9 \times 2, 59.4, 57.4, 56.5, 56.3, 32.2, 23.0; EIMS m/z : 360 ($[\text{M}^+ - \text{Cl}]$, 55), 154 (100); anal. calcd for $\text{C}_{23}\text{H}_{22}\text{BrNO}_3$: C, 76.64; H, 6.15; N, 3.89. Found: C, 76.32; H, 6.00; N, 3.72.

In Vitro Cytotoxicity Assay—The sulforhodamine B assay was used according to the procedures developed and validated at NCI.¹⁸ Doxorubicin was used as the positive control antitumor drug. The in vitro anticancer activities are expressed as EC_{50} values, which is the test compound concentration ($\mu\text{g/mL}$) that reduced the cell number by 50% after 72 h of continuous treatment. The values were interpolated from dose-response data. Each test was performed in triplicate with variation less than 5%. The EC_{50} values determined in each of

independent tests varied less than 10%. Compound stock solutions were prepared in DMSO with the final solvent concentration $\leq 1\%$ DMSO (v/v), a concentration without effect on cell replication. The cells were cultured at 37 °C in RPMI-1640 supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2% (w/v) sodium bicarbonate, 10% (v/v) fetal bovine serum, and 100 $\mu\text{g/mL}$ kanamycin in a humidified atmosphere containing 5% CO_2 .

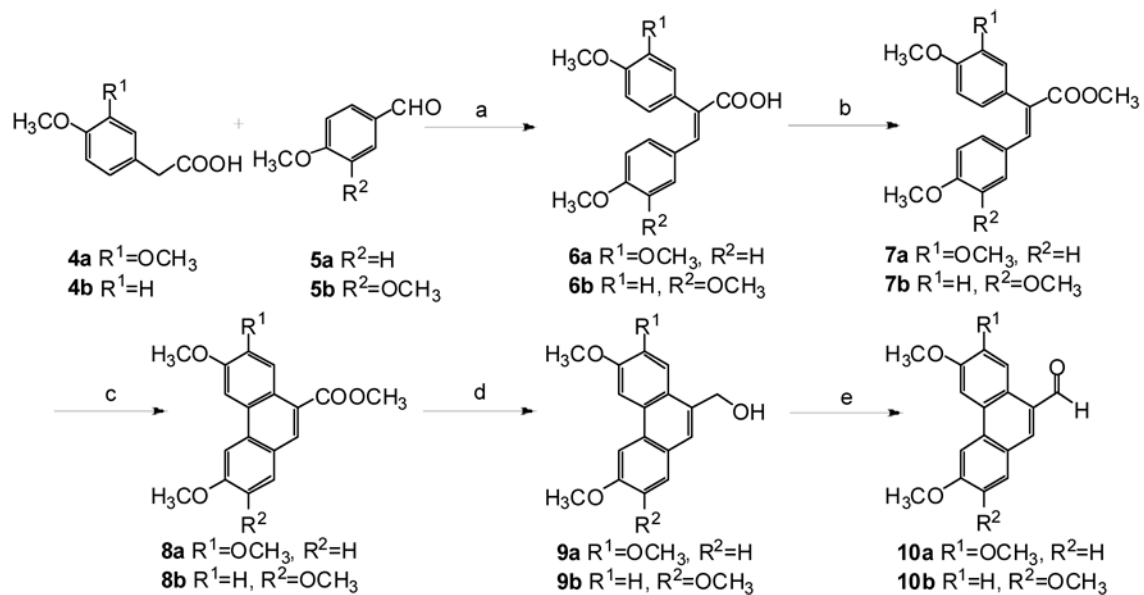
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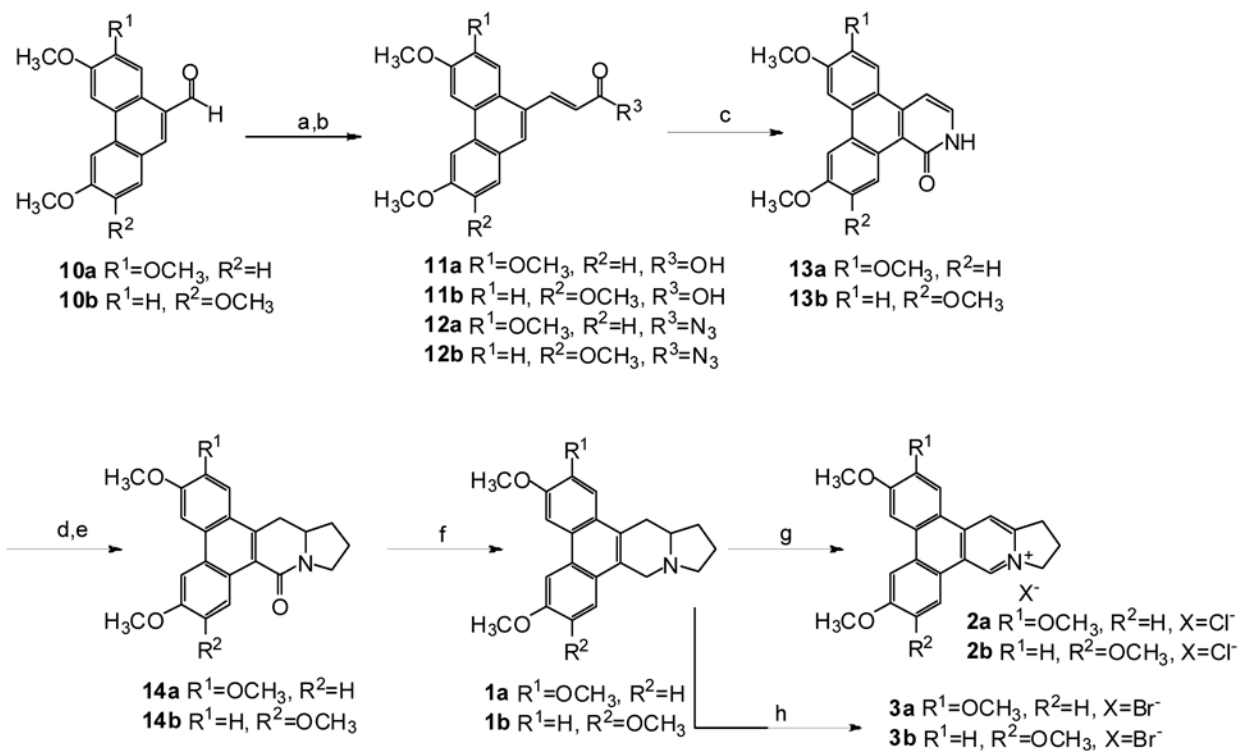
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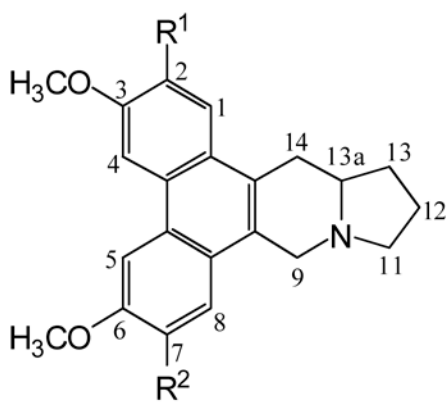
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**Scheme 1.**

(a) (i) Ac_2O , Et_3N , reflux, 10 h; (ii) $\text{K}_2\text{CO}_3(\text{aq})$, 2 h; (b) H_2SO_4 , CH_3OH , reflux, 4 h; (c) VOF_3 , CH_2Cl_2 , CF_3COOH , $(\text{CF}_3\text{CO})_2$, ice-salt bath, 1 h; (d) LiAlH_4 , THF , rt, 3 h; (e) PCC , CH_2Cl_2 , rt, 4 h.

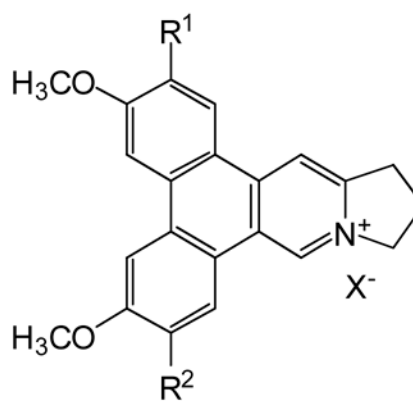
**Scheme 2.**

(a) (i) $\text{Ph}_3\text{P} = \text{CHCO}_2\text{Et}$, toluene, reflux, 4 h; (ii) KOH , $\text{EtOH-H}_2\text{O}$, reflux, 3 h; (b) (i) $(\text{COCl})_2$, toluene, 80°C , 5 h; (ii) NaN_3 , acetone, rt, 2 h; (c) I_2 , *o*-dichlorobenzene, reflux, 1 h; (d) (i) $\text{Br}(\text{CH}_2)_3\text{Cl}$, DMF, rt, overnight; (ii) NaI , CH_3CN , reflux, overnight; (e) AIBN , *n*- Bu_3SnH , toluene, reflux, 6 h; (f) $\text{NaAl}(\text{OCH}_2\text{CH}_2\text{OMe})_2\text{H}_2$, dioxane, 2 h; (g) light, CHCl_3 ; (h) NBS , CHCl_3 , rt, 1 h.



1a R¹=OCH₃, R²=H

1b R¹=H, R²=OCH₃



2a R¹=OCH₃, R²=H, X=Cl⁻

2b R¹=H, R²=OCH₃, X=Cl⁻

3a R¹=OCH₃, R²=H, X=Br⁻

3b R¹=H, R²=OCH₃, X=Br⁻

Table 1
Effect of **1–3**, **11a–13a**, and **15b** against Tumor Cell Line Replication

compound	EC ₅₀ (μg/mL)			
	A549	MCF-7	KB	KB-VIN
1a	0.00016	0.0045	0.00043	0.00083
1b	0.011	0.043	0.023	0.016
2a	0.73	1.72	0.93	0.33
2b	1.06	1.49	0.95	5.66
3a	0.49	1.29	5.37	5.14
3b	0.32	1.18	0.29	8.26
11a	NA	NA	NA	14.72
12a	NA	8.29	13.52	9.36
13a	NA	NA	NA	7.39
14b	1.15	2.14	1.39	1.16

^a Cell line: A549 = lung; MCF-7 = breast; KB = nasopharynx; KB-VIN = nasopharynx MDR