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Design, Synthesis, Antiviral Activity, and Structure–Activity Relationships (SARs) of Two Types of Structurally Novel Phenanthroindo/quinolizidine Analogues

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Supporting Information

ABSTRACT: To investigate the influence of the variation of the original skeletons of natural phenanthroindo/quinolizidine alkaloids on antiviral activities, two types of structurally totally novel analogues **7a**, **7b**, **16a**, and **16b** were designed, synthesized, and evaluated against tobacco mosaic virus (TMV) for the first time. Bioassay results indicated that all four of the newly designed analogues showed good to excellent antiviral activities, among which analogue **16a** dispalyed comparable activity with that of ningnanmycin, perhaps one of the most successful commercial antiviral agents, thus emerging as a potential inhibitor of plant virus and serving as a new lead for further optimization. Further structure–activity relationships are also discussed, demonstrating for the first time that the same changes of the original skeletons of phenanthroindolizidine and phenanthroquinolizidine exihibted totally different antiviral activities results, providing some original and useful information about the preferential conformation for maintaining high activities.

KEYWORDS: *phenanthroindolizidine alkaloid, phenanthroquinolizidine alkaloid, antiviral activity, tobacco mosaic virus, structure–activity relationship, anti-TMV*

INTRODUCTION

Tobacco mosaic virus (TMV) is one of the most well-studied plant viruses worldwide, which can infect 9 plant families and at least 125 individual species, such as tobacco, pepper, cucumber, tomato, and many ornamental flowers.[1] TMV is known as "plant cancer" due to the fact that it is very difficult to control and has brought great disasters to agriculture. The economic loss caused by TMV is up to U.S. $100 million each year worldwide.[2]

Ningnanmycin (Figure 1) as a commercial plant-virus inhibitor is perhaps most effective against plant virus and displays 56.0% in vivo curative effect at 500 μg/mL. Another widely used antiplant viral agent is ribavirin (Figure 1), the inhibitory effect of which is always <50% at 500 μg/mL.

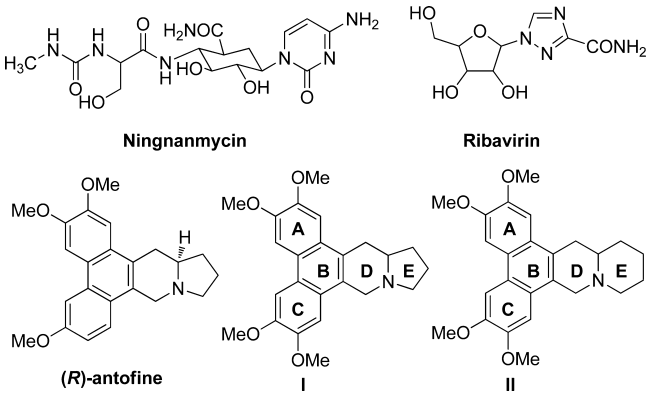

Figure 1. Chemical structures of ningnanmycin, ribavirin, and representative phenanthroindo/quinolizidine alkaloids.

Because of the great economic loss caused by TMV and the unsatisfactory inhibitory effects (usually 30–60%) of these antiviral agents, much effort has been made toward the development of novel and high effective plant virucides. Consequently, a number of chemicals, such as pyrazole derivatives,[3] nucleotides,[4] α-aminophosphonate derivatives,[5] 3-acetonyl-3-hydroxyoxindole,[6] triazolyl compounds,[7] oxidized polyamines,[8] and substituted phenylureas,[9] were reported to possess antiviral activities, few of which have been applied successfully in agriculture.

Compared with synthetic chemicals, natural product-based antiviral agents have many advantages, such as low mammalian toxicity, easy decomposition, friendly to environment, specific to targeted species, unique mode of action, and so on.[10,11] Phenanthroindo/quinolizdine alkaloids are a small family of natural products isolated mainly from the *Cynanchum*, *Pergularia*, and *Tylophora* species.[12] They exhibit diverse biological activities ranging from anticancer and anti-inflammatory to antiamoebic and antilupus effects.[13−17] In a program aimed at screening of plants for biologically active natural products as alternatives to conventional synthetic antiviral agents, our group first found that the alcohol extract of *Cynanchum komarovii* displayed moderate antiviral activity against TMV.[18] Further investigation demonstrated that the main active compound was (*R*)-antofine (Figure 1). Antiviral mechanism studies revealed that antofine has a favorable

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interaction with the origin of TMV RNA (oriRNA), exhibiting its virus inhibition by binding to oriRNA and interfering with virus assembly initiation.[19] Moreover, structure−activity relationship (SAR) studies showed that most compounds of the phenanthroindolizidine-based library with structural diversity exhibited antiviral effect against TMV.

In previous work, the SAR studies mainly focused on (1) substituted patterns of methoxyl group on the phenanthrene ring, (2) the number of methoxyl groups on the phenanthrene ring, (3) derivatation at the C-14 position, and (4) D ring opened derivatives.[20,21] However, there is no investigation on the variation of basic skeletons of phenanthroindo/quinolizines, which will largely change the conformation of the molecules. To study the influence of the variation of basic skeletons and conformations of these phenanthroindo/quinolizidine alkaloids, two types of structurally novel phenanthroindo/quinolizidine alkaloid analogues were designed, synthesized, and evaluated for their antiviral activity against TMV. The SAR study of these compounds against TMV is also discussed.

MATERIALS AND METHODS

Instruments. ^{1}H NMR spectra were obtained at 400 MHz using a Bruker AC-P 400. Chemical shift values (δ) were given in parts per million and were downfield from internal tetramethylsilane. High-resolution mass spectra (HRMS) were recorded on an FT-ICR MS (Ionspec, 7.0 T). Melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and were uncorrected. Reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified according to standard techniques just before use.

1-(2,3,6,7-Tetramethoxyphenanthren-9-yl)ethanone 3. To a solution of phenanthryl carboxylic acid (6.84 g, 20 mmol) in THF (100 mL) was added MeLi (28.8 mL, 1.6 M, 46 mmol) dropwise via a syringe at −78 °C under an atmosphere of nitrogen. The reaction mixture was stirred at this temperature for 30 min and then warmed to room temperature. Two hours later, saturated aqueous NH_4Cl (10 mL) was added to quench the reaction. After evaporation of THF, the aqueous layer was extracted with CH_2Cl_2 (80 mL × 3). The combined organic phase was washed sequentially with diluted aqueous HCl, water, and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Crude product was recrystallized from MeOH to give compound **3** (5.2 g, 77%) as a white solid: mp 214−215 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.44 (s, 1H), 8.41 (s, 1H), 7.98 (s, 1H), 7.95 (s, 1H), 7.53 (s, 1H), 4.07 (s, 3H), 4.04 (s, 3H), 3.94 (s, 3H), 3.89 (s, 3H), 2.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 201.0, 151.2, 148.9, 148.8, 148.8, 130.3, 129.5, 126.5, 124.9, 124.0, 122.4, 109.6, 106.8, 103.7, 103.3, 55.9, 55.7, 55.4, 55.1, 29.5; HRMS (ESI) calcd for $C_{20}H_{20}NO_5Na$ $(M + H)^+$ 363.1203, found 263.1205.

***tert*-Butyl 2-(2-Oxo-2-(2,3,6,7-tetramethoxyphenanthren-9-yl)ethyl)pyrrolidine-1-carboxylate 5a.** To a solution of compound **3** (0.68 g, 2.0 mmol) and i-Pr_2NEt (0.38 g, 3.0 mmol) in CH_2Cl_2 (50 mL) was added TMSOTf (0.53 g, 2.4 mmol) in CH_2Cl_2 (10 mL) dropwise. The reaction mixture was stirred at room temperature for 4 h and then quenched with water (30 mL). After separation, the organic phase was washed sequentially with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Crude product **4** was dissolved in CH_2Cl_2 (50 mL) without further purification, and then compound **2** (0.44 g, 2.18 mmol) was added. $BF_3{\cdot}Et_2O$ (3.0 mL, 30%) in CH_2Cl_2 (10 mL) was added dropwise at −78 °C. The reaction mixture was stirred at this temperature for 1 h, warmed to room temperature, and then quenched with diluted aqueous NaOH. After separation, the organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **5a** (0.73 g, 72%) as a light yellow solid: mp 147−149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.33 (s, 1H), 7.80 (s, 1H), 7.77 (s, 1H), 7.32 (s, 1H), 4.44 (br, 1H), 4.15 (s, 3H), 4.13(s, 3H), 4.06 (s, 6H), 3.86 (d, J = 14.8 Hz, 1H), 3.40 (br, 2H), 3.10−2.98 (m, 1H), 2.13 (br, 1H), 1.89 (br, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz) δ 199.2, 156.7, 152.2, 151.0, 150.4, 149.0, 130.3, 130.14 126.0, 125.1, 124.9, 123.5, 109.5, 107.2, 105.9, 102.6, 81.2, 56.1, 56.0, 55.9, 55.8, 46.0, 44.3, 33.1, 28.6, 24.9; HRMS (ESI) calcd for $C_{29}H_{36}NO_7$ $(M + H)^+$ 510.2486, found 510.2490.

2-(2-(2,3,6,7-Tetramethoxyphenanthren-9-yl)ethyl)-pyrrolidine 6a. To a solution of compound **5a** (0.37 g, 0.73 mmol) in EtOH (50 mL) was added $NaBH_4$ (0.1 g, 2.6 mmol). The reaction mixture was stirred at room temperature for 2 h and then quenched with water. After evaporation, the aqueous layer was extracted with CH_2Cl_2 (30 mL × 3). The combined organic phase was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Crude product was dissolved in CH_2Cl_2 (50 mL) without further purification, and then Et_3SiH (0.18 mL, 1.13 mmol) and CF_3COOH (0.33 mL, 4.4 mmol) were added. The reaction mixture was stirred at room temperature overnight and then quenched with diluted aqueous NaOH. After separation, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **6a** (0.27 g, 93%) as a white solid: mp 224−226 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.56 (s, 1H), 7.30 (s, 1H), 7.23 (s, 1H), 7.04 (s, 1H), 4.08 (s, 6H), 4.02 (s, 3H), 3.97 (s, 3H), 3.40−3.30 (m, 1H), 3.28−3.22 (m, 1H), 3.19−3.05 (m, 2H), 2.99−2.88 (m, 1H), 2.22−2.17 (m, 1H), 2.11−1.90 (m, 3H), 1.89−1.76 (m, 1H), 1.57−1.52 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 148.4, 148.3, 130.9, 125.9, 124.7, 124.5, 123.2, 122.9, 107.9, 104.1, 102.9, 102.2, 60.6, 56.2, 55.8, 55.8, 44.5, 31.9, 30.8, 30.3, 23.6; HRMS (ESI) calcd for $C_{24}H_{30}NO_4$ $(M + H)^+$ 396.2169, found 396.2168.

2,3,6,7-Tetramethoxy-11,12,13,13a,14,15-hexahydro-9*H*-phenanthro[9,10-e]pyrrolo[1,2-*a*]azepine 7a. To a solution of compound **6a** (0.2 g, 0.51 mmol) in toluene (20 mL) were added formaldehyde (1.0 mL, 30%) and CF_3COOH (0.5 mL, 6.7 mmol). A Dean−Stark trap topped with a reflux condenser was attached to the reaction vessel, and the reaction mixture was heated at reflux. Seven hours later, the solvent was evaporated under reduced pressure, and then aqueous NaOH was added to the residue. The aqueous solution was extracted with CH_2Cl_2 (30 mL × 3), and the combined organic phase was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **7a** (0.18 g, 87%) as a light yellow solid: mp 178−180 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 2H), 7.53 (s, 2H), 4.69 (s, 1H), 4.12 (s, 3H), 4.11 (s, 3H), 4.06 (s, 3H), 4.05(s, 3H), 3.88−3.83 (m, 1H), 3.65 (dd, J = 15.0, 7.5 Hz, 1H), 3.21 (s, 1H), 3.09 (t, J = 12.0 Hz, 1H), 2.69 (s, 2H), 2.22−2.10 (m, 1H), 2.00 (s, 1H), 1.78 (s, 2H), 1.57−1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.0, 147.8, 147.7, 147.5, 133.9, 127.4, 124.3, 123.8, 123.7, 122.9, 103.5, 103.2, 102.4, 102.3, 76.3, 76.0, 75.7, 55.0, 54.9, 54.8, 49.3, 30.2, 29.8, 28.7, 25.4, 19.9; HRMS (ESI) calcd for $C_{29}H_{30}NO_4$ $(M + H)^+$ 408.2169, found 408.2169.

2-(2,3,6,7-Tetramethoxyphenanthren-9-yl)acetonitrile 9. To a solution of compound **8** (7.2 g, 18.4 mmol) in DMF (150 mL) was added NaCN (1.0 g, 20.4 mmol). The reaction was stirred at room temperature for 5 h, and then solvent was evaporated under reduced pressure. Water and CH_2Cl_2 were added to the residue, and, after separation, the aqueous layer was extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give compound **9** (4.2 g, 68%) as a white solid: mp 198−200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.77 (s, 1H), 7.70 (s, 1H), 7.23 (s, 1H), 7.14 (s, 1H), 4.14 (s, 3H), 4.13 (s, 3H), 4.12 (s, 2H), 4.07 (s, 3H), 4.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 148.3, 148.1, 148.0, 124.6, 123.9, 123.6, 123.4, 122.7, 120.3, 116.7, 107.2, 102.6, 102.3, 101.6, 55.1, 55.0, 54.9, 21.3; HRMS (ESI) calcd for $C_{20}H_{23}N_2O_4$ $(M + NH_4)^+$ 499.2551, found499.2552.

2-(2,3,6,7-Tetramethoxyphenanthren-9-yl)ethanamine 10. To a solution of compound **9** (0.3 g, 0.89 mmol) in THF (30 mL) was added BH_3 (2.7 mL, 1 M in THF) at 0 °C under an atmosphere of

nitrogen. Then, the reaction was heated at reflux for 3 h. After the mixture had cooled to room temperature, diluted aqueous HCl was added to quench the reaction. The mixture was heated at reflux for an additional 0.5 h and then was cooled to room temperature. The mixture was extracted with EtOAc, and the aqueous layer was basified by aqueous NaOH to pH >7. The aqueous layer was extracted with EtOAc and then washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give compound **10** (0.25 g, 67%) as a white solid: mp 148−149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.75 (s, 1H), 7.44 (s, 1H), 7.38 (s, 1H), 7.17 (s, 1H), 4.11 (s, 3H), 4.09 (s, 3H), 4.05 (s, 3H), 4.02 (s, 3H), 3.21 (m, 4H), 2.2 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9, 148.8, 148.6, 130.8, 126.2, 125.3, 125.0, 124.6, 123.7, 107.9, 104.6, 103.4, 102.7, 56.1, 56.0, 55.9, 55.8, 41.8, 37.1; HRMS (ESI) calcd for $C_{20}H_{24}NO_4$ $(M + H)^+$ 342.1700, found 342.1702.

4-Oxo-4-((2-(2,3,6,7-tetramethoxyphenanthren-9-yl)ethyl)-amino)butanoic Acid 12a. To a solution of amine **10** (0.4 g, 1.17 mmol) in $CHCl_3$ (30 mL) was added dihydrofuran-2,5-dione (0.23 g, 2.34 mmol). The reaction was stirred at room temperature for 2 h, and then water (30 mL) was added and stirred for an additional 1 h. After separation, the aqueous layer was extracted with $CHCl_3$ (30 mL × 3). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **12a** (0.46 g, 89%) as a white solid: mp 203−204 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.16 (t, J = 5.2 Hz, 1H), 8.03 (s, 1H), 7.97 (s, 1H), 7.65 (s, 1H), 7.47 (s, 1H), 7.33 (s, 1H), 4.03 (s, 3H), 4.01 (s, 3H), 3.98 (s, 3H), 3.89 (s, 3H), 3.42−3.36 (m, 2H), 3.13 (t, J = 8 Hz, 2H), 2.46 (d, J = 6.4 Hz, 2H), 2.35 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO) δ 173.6, 171.1, 148.8, 148.7, 148.6, 148.5, 130.6, 125.8, 124.9, 124.5, 124.1, 123.3, 108.1, 105.0, 104.3, 103.7, 55.9, 55.8, 55.5, 55.3, 33.5, 30.1, 29.2; HRMS (ESI) calcd for $C_{24}H_{28}NO_7$ $(M + H)^+$ 442.1860, found 442.1866.

1-(2-(2,3,6,7-Tetramethoxyphenanthren-9-yl)ethyl)-pyrrolidine-2,5-dione 13a. To a solution of compound **12a** (0.46 g, 1.04 mmol) in Ac_2O (20 mL) was added AcONa (0.1 g). The reaction was stirred at 70 °C for 2 h and then cooled to room temperature. The reaction was quenched with water and stirred for an additional 1 h. After separation, the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **13a** (0.43 g, 98%) as a white solid: mp 262−263 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.76 (s, 1H), 7.75 (s, 1H), 7.50 (s, 1H), 7.17 (s, 1H), 4.18 (s, 3H), 4.13 (s, 3H), 4.11 (s, 3H), 4.02 (s, 3H), 3.83 (t, J = 8.6 Hz, 2H), 3.25 (t, J = 8.6 Hz, 2H), 2.74 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 149.02, 148.9, 129.4, 126.21, 125.2, 124.8, 124.0, 107.9, 104.7, 103.3, 102.8, 77.3, 77.0, 76.7, 56.2, 56.1, 56.0, 55.9, 39.2, 32.1, 28.2; HRMS (ESI) calcd for $C_{24}H_{26}NO_6$ $(M + H)^+$ 424.1755, found 424.1754.

5-Hydroxy-1-(2-(2,3,6,7-tetramethoxyphenanthren-9-yl)-ethyl)pyrrolidin-2-one 14a. To a solution of compound **13a** (0.48 g, 1.13 mmol) in CH_2Cl_2 (20 mL) was added $LiEt_3BH$ (1 M, 2.2 mL) at −60 °C. The reaction mixture was stirred for 2 h and then quenched with water. After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **14a** (0.44 g, 92%) as a white solid: mp 187−188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.75 (s, 1H), 7.68 (s, 1H), 7.44 (s, 1H), 7.13 (s, 1H), 4.99 (s, 1H), 4.12 (s, 3H), 4.11(s, 3H), 4.10 (s, 3H), 4.06−4.02 (m, 1H), 4.00 (s, 3H), 3.82−3.72 (m, 1H), 3.67−3.63 (m, 1H), 3.39−3.29 (m, 2H), 2.63−2.52 (m, 1H), 2.37−2.19 (m, 2H), 1.82−1.77 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 148.1, 148.0, 147.9, 129.7, 125.2, 123.7, 122.9, 106.8, 103.9, 102.1, 101.8, 83.2, 55.2, 55.1, 55.0, 54.9, 40.7, 31.4, 27.9, 27.8; HRMS (ESI) calcd for $C_{24}H_{28}NO_6$ $(M + H)^+$ 426.1911, found 426.1913.

2,3,12,13-Tetramethoxy-5,6,10,10a-tetrahydrodibenzo[*f*,*h*]-pyrrolo[2,1-*a*]isoquinolin-8(9*H*)-one 15a. To a solution of compound **14a** (0.4 g, 0.94 mmol) in CH_2Cl_2 (20 mL) was added TMSI (0.44 g, 2.1 mmol) at −55 °C. The reaction was stirred for 1 h and then quenched with diluted aqueous NaOH. After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **15a** (0.3 g, 80%) as a light yellow solid: mp 222−224 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.83 (s, 1H), 7.29 (s, 1H), 7.25 (s, 1H), 5.45 (br, 1H), 4.66 (d, J = 11.7 Hz, 1H), 4.13 (s, 6H), 4.06 (s, 3H), 4.05 (s, 3H), 3.18 (br, 2H), 3.14−2.98 (m, 2H), 2.71 (br, 1H), 2.48 (br, 1H), 1.80 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.1, 148.7, 148.6, 128.9, 125.9, 125.5, 124.3, 123.7, 123.2, 123.1, 104.6, 104.2, 103.7, 103.3, 56.2, 56.1, 56.0, 36.6, 29.7, 29.1, 26.6; HRMS (ESI) calcd for $C_{24}H_{26}NO_5$ $(M + H)^+$ 408.1805, found 408.1805.

2,3,12,13-Tetramethoxy-5,6,8,9,10,10a-hexahydrodibenzo-[*f*,*h*]pyrrolo[2,1-*a*]isoquinoline 16a. To a solution of compound **15a** (0.29 g, 0.71 mmol) in THF (30 mL) was added $LiAlH_4$ (50.0 mg, 1.3 mmol). The reaction mixture was heated at reflux for 2 h and then quenched with diluted aqueous NaOH after cooling to room temperature. After separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give compound **16a** (0.23 g, 82%) as a light yellow solid: mp 155−158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.83 (s, 1H), 7.35 (s, 1H), 7.30 (s, 1H), 4.66 (t, J = 8.1 Hz, 1H), 4.12 (s, 6H), 4.05 (s, 6H), 3.25−3.16 (m, 2H), 3.15−3.04 (m, 4H), 2.73−2.69 (m, 1H), 2.04−1.92 (m, 2H), 1.84−1.72 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8, 147.5, 147.3, 147.2, 129.3, 124.5, 124.2, 123.5, 122.9, 122.4, 104.1, 103.2, 102.4, 102.3, 58.8, 55.1, 55.0, 54.9, 54.8, 51.9, 44.2, 31.2, 23.4, 22.4; HRMS (ESI) calcd for $C_{24}H_{28}NO_4$ $(M + H)^+$ 394.2013, found 394.2018.

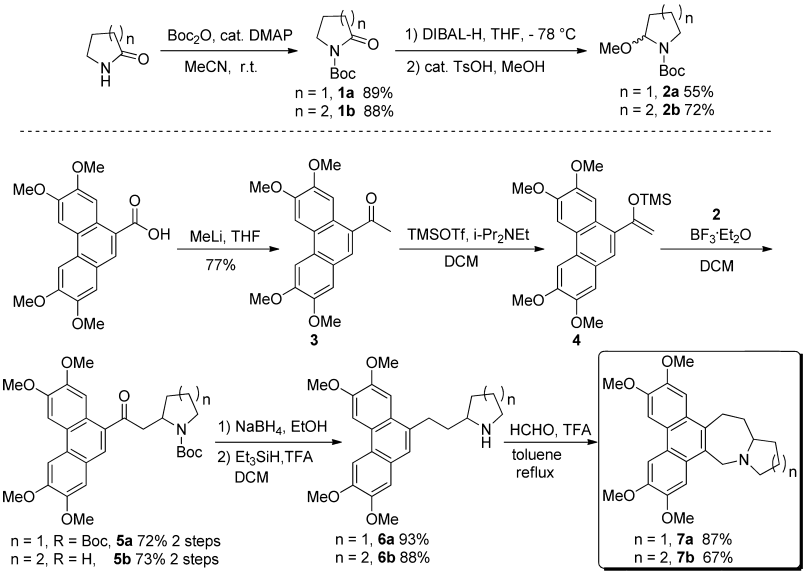
2-(Piperidin-2-yl)-1-(2,3,6,7-tetramethoxyphenanthren-9-yl)ethanone 5b. The synthetic procedure was similar to that of compound **5a**, and compound **5b** was obtained as a light yellow solid (73%): mp 203−205 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.55 (s, 1H), 8.45 (s, 1H), 8.07 (s, 1H), 8.03 (s, 1H), 7.64 (s, 1H), 4.09 (s, 3H), 4.06 (s, 3H), 3.95 (s, 3H), 3.90 (s, 3H), 3.85−3.77 (m, 1H), 3.69 (s, 1H), 3.57−3.55 (m, 1H), 3.26 (s, 2H), 2.97 (s, 1H), 1.94−1.91 (m, 1H), 1.77−1.75 (m, 4H); ^{13}C NMR (100 MHz, DMSO) δ 200.1, 151.5, 149.1, 149.0, 148.8, 130.0, 128.9, 126.7, 125.0, 123.9, 122.3, 109.7, 106.7, 103.8, 103.4, 56.0, 55.8, 55.5, 55.2, 52.6, 44.3, 28.9, 22.6, 22.2, 14.0; HRMS (ESI) calcd for $C_{25}H_{30}NO_5$ $(M + H)^+$ 424.2118, found 424.2123.

2-(2-(2,3,6,7-Tetramethoxyphenanthren-9-yl)ethyl)-piperidine 6b. The synthetic procedure was similar to that of compound **6a**, and compound **6b** was obtained as a white solid (88%): mp 175−176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.76 (s, 1H), 7.41 (s, 1H), 7.40 (s, 1H), 7.16 (s, 1H), 4.11 (s, 6H), 4.05 (s, 3H), 4.03 (s, 3H), 3.09 (t, J = 8.0 Hz, 3H), 2.71−2.59 (m, 2H), 1.89−1.82 (m, 4H), 1.64−1.61 (m, 2H), 1.52−1.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 148.5, 134.0, 126.4, 125.4, 124.9, 123.5, 107.9, 104.8, 103.4, 102.8, 77.3, 77.0, 76.7, 56.9, 56.1, 56.0, 55.9, 55.8, 47.2, 37.8, 33.0, 29.8, 26.7, 24.8; HRMS (ESI) calcd for $C_{25}H_{32}NO_4$ $(M + H)^+$ 410.2326, found 410.2331.

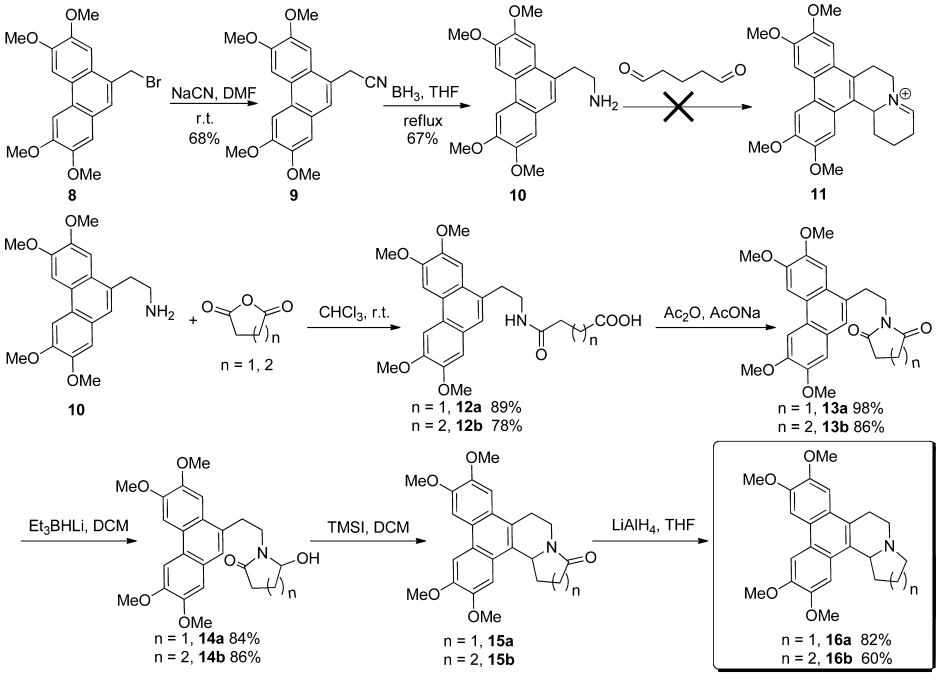
2,3,6,7-Tetramethoxy-9,11,12,13,14,14a,15,16-octahydrophenanthro[9,10-*e*]pyrido[1,2-*a*]azepine 7b. The synthetic procedure was similar to that of compound **7a**, and compound **7b** was obtained as a white solid (68%): mp 216−218 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 2H), 7.50 (s, 1H), 7.45 (s, 1H), 4.37 (d, J = 14.9 Hz, 1H), 4.16−4.02 (m, 13H), 3.56−3.53 (m, 1H), 3.30−3.19 (m, 1H), 3.06−3.04 (m, 1H), 2.60−2.41 (m, 2H), 2.01 (s, 1H), 1.68−1.60 (m, 5H), 1.49 (s, 1H), 1.34−1.31 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 148.6, 148.5, 148.3, 125.7, 124.2, 123.7, 104.6, 104.3, 103.4, 103.3, 94.5, 77.3, 77.0, 76.7, 56.8, 56.0, 55.9, 55.8, 55.7, 33.9, 33.5, 26.8, 26.5, 23.8; HRMS (ESI) calcd for $C_{26}H_{32}NO_4$ $(M + H)^+$ 422.2326, found 422.2332.

5-Oxo-5-((2-(2,3,6,7-tetramethoxyphenanthren-9-yl)ethyl)-amino)pentanoic acid 12b. The synthetic procedure was similar to that of compound **12a**, and compound **12b** was obtained as a white

Scheme 1. Synthetic Route for Compounds 7a and 7b



Scheme 2. Synthetic Route for Compounds 16a and 16b



solid (78%): mp 158−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.74 (s, 1H), 7.54 (s, 1H), 7.37 (s, 1H), 7.14 (s, 1H), 5.92 (s, 1H), 4.10 (s, 3H), 4.09 (s, 3H), 4.08 (s, 3H), 4.01 (s, 3H), 3.67 (d, J = 6.8 Hz, 2H), 3.25 (t, J = 6.8 Hz, 2H), 2.39 (t, J = 6.8 Hz, 2H), 2.25 (t, J = 7.2 Hz, 2H), 1.98−1.90 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 171.7, 147.9, 129.3, 123.5, 106.9, 103.8, 102.4, 101.8, 55.2, 55.1, 55.0, 54.9, 38.9, 34.3, 32.5, 28.9, 19.7, 15.3; HRMS (ESI) calcd for $C_{25}H_{30}NO_4(M + H)^+$ 456.2017, found 456.2018.

1-(2-(2,3,6,7-Tetramethoxyphenanthren-9-yl)ethyl)-piperidine-2,6-dione 13b. The synthetic procedure was similar to that of compound **13a**, and compound **13b** was obtained as a white solid (86%): mp 261−263 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.83 (s, 1H), 7.78 (s, 1H), 7.54 (s, 1H), 7.19 (s, 1H), 4.21 (s, 3H), 4.13−4.08 (m, 8H), 4.03 (s, 3H), 3.28−3.16 (m, 2H), 2.71 (t, J = 8.4 Hz, 4H), 2.04−1.93 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 148.0, 147.9, 147.8, 147.7, 129.4, 125.3, 124.6, 124.2, 123.7, 122.9, 106.9, 104.3, 102.1, 101.7, 55.3, 55.1, 55.0, 54.9, 39.5, 31.8, 31.5, 16.2; HRMS (ESI) calcd for calcd for $C_{25}H_{27}NO_6Na^+$ $(M + Na)^+$ 460.1731, found 460.1735.

6-Hydroxy-1-(2-(2,3,6,7-tetramethoxyphenanthren-9-yl)-ethyl)piperidin-2-one 14b. The synthetic procedure was similar to that of compound **14a**, and compound **14b** was obtained as a white solid (86%): mp 202−204 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.80 (s, 1H), 7.76 (s, 1H), 7.45 (s, 1H), 7.14 (s, 1H), 4.74 (s, 1H), 4.18−4.09 (m, 9H), 4.01 (s, 3H), 3.91−3.81 (m, 1H), 3.71−3.64 (m, 1H), 3.41−3.35 (m, 2H), 2.56−2.46 (m, 1H), 2.40−2.27 (m, 2H), 1.99−1.89 (m, 1H), 1.82−1.73 (m, 1H), 1.73−1.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 148.9, 148.9, 148.8, 148.8, 131.7, 126.3, 125.7, 124.8, 123.8, 107.8, 105.4, 103.2, 102.8, 81.1, 56.3, 56.1, 56.0, 55.9, 47.5, 32.6, 31.1, 15.9; HRMS (ESI) calcd for $C_{25}H_{30}NO_6^+$ $(M + H)^+$ 440.2068, found 440.2064.

2,3,13,14-Tetramethoxy-9,10,11,11a-tetrahydro-5*H*-dibenzo[*f*,*h*]pyrido[2,1-*a*]isoquinolin-8(6*H*)-one 15b. The synthetic procedure was similar to that of compound **15a**, and compound

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15b was obtained as a white solid (80%): mp 153−155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.82 (s, 1H), 7.32 (s, 1H), 7.21 (s, 1H), 5.35 (d, J = 10.8 Hz, 1H), 5.18 (dd, J = 12.4, 3.2 Hz, 1H), 4.13 (s, 6H), 4.05 (s, 3H), 4.04 (s, 3H), 3.19 (t, J = 16.0 Hz, 1H), 3.14−3.03 (m, 1H), 2.85−2.75 (m, 1H), 2.76−2.66 (m, 2H), 2.60−2.48 (m, 1H), 2.09−1.93 (m, 2H), 1.40−1.35 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 149.2, 148.6, 148.53, 128.2, 127.6, 125.5, 124.5, 123.5, 123.3, 104.2, 104.1, 103.8, 103.3, 56.2, 56.1, 56.1, 56.0, 56.9, 38.0, 32.1, 31.6, 26.8, 20.0, 14.2; HRMS (ESI) calcd for $C_{25}H_{27}NO_5$ $(M + H)^+$ 422.1962, found 422.1959.

2,3,13,14-Tetramethoxy-6,8,9,10,11,11a-hexahydro-5*H*-dibenzo[*f*,*h*]pyrido[2,1-*a*]isoquinoline 16b. The synthetic procedure was similar to that of compound **16a**, and compound **16b** was obtained as a white solid (60%): mp 89−92 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.82 (s, 1H),7.32 (s, 1H), 7.25 (s, 1H), 4.26 (d, J = 9.7 Hz, 1H), 4.11 (s, 6H), 4.04 (s, 3H), 4.03 (s, 3H), 3.47−3.39 (m, 1H), 3.28−3.25 (m, 1H), 3.21−3.13 (m, 2H), 2.93−2.87 (m, 1H), 2.21 (d, J = 11.9 Hz, 1H), 1.96−1.63 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 149.3, 148.8, 131.1, 126.6, 126.5, 124.7, 124.5, 124.2, 105.4, 104.8, 104.3, 103.9, 60.5, 56.8, 56.7, 56.6, 56.5, 46.3, 29.2, 28.5, 26.6, 22.5; HRMS (ESI) calcd for $C_{25}H_{30}NO_4$ $(M + H)^+$ 408.2169, found 408.2170.

Antiviral Biological Assay. The procedure of purifying TMV and the method to test the anti-TMV activity of the synthesized compounds were the same with those reported previously in the literature.[22]

RESULTS AND DISCUSSION

Chemistry. The synthesis of D-ring expanded phenanthroindo/quinolizidine alkaloid analogues **7a** and **7b** is shown in Scheme 1, which features a Mukaiyama−Aldol reaction and a Pictet−Spengler cyclization. The precursors **2** of the Mukaiyama−Aldol reaction were prepared from commercially available lactams through literature protocols.[23] Silylenol ether **4** was synthesized from acetophenanthrone **3**, which was obtained from readily available phenanthryl acid.[24] After extensive reaction screening, it was found that boron trifluoride was the optimal Lewis acid for the desired Mukaiyama−Aldol reactions, giving compounds **5** in good yields. Ketones **5** were reduced to alcohols by sodium borohydride, followed by deprotection and further reduction of the benzylic hydroxyl by triethylsilicane under acidic conditions, furnishing amines **6** efficiently. It is worth noting that the following Pictet−Spengler cyclization did not occur when concentrated hydrochloric acid was used as catalyst in ethanol, which may be due to the energy of the seven-membered ring transition state being too high. When the reaction was performed in a high boiling point solvent (toluene) using much stronger acid CF_3COOH as catalyst, the desired cyclized products **7** were obtained in good yields.

The synthesis of the isomerized phenanthroindo/quinolizidine analogues **16a** and **16b** is shown in Scheme 2, featuring a Bischler−Napieralski cyclization. Using readily available phenanthryl bromide **8** as starting material,[25] intermediate **10** could be easily be prepared via sequential nucleophilic substitution and reduction. With the phenanthrylethylamine **10** in hand, we initially attempted to construct the pentacyclic skeleton through Pictet−Spengler cyclization/condensation sequence. Unfortunately, although much effort was devoted to the screening of reaction conditions, including catalysts, solvents, and reaction temperature, all attempts to perform the desired Pictet−Spengler cyclization failed. The main difficulty was proposed to be the Pictet−Spengler cyclization, presumably because reactivity of the key intermediate formed through condensation of amine and aldehyde was so slow that self-condensation of the aldehyde occurred quickly. To improve the reactivity of the intermediate of the cyclization, we turned to an alternative Bischler−Napieralski cyclization approach, which employed highly reactive acyl iminium as key intermediate. Imides **13** prepared from amine **10** and corresponding anhydride were subjected to partial reduction, affording compounds **14**. The subsequent Bischler−Napieralski cyclization went smoothly when trimethyliodosilane was employed. After reduction, isomerized phenanthroindo/quinolizidine alkaloid analogues **16** were obtained in good yields.

Antiviral Activity. To make a judgment of the designed structurally novel phenanthroindo/quinolizidine alkaloid analogues **7a**, **7b**, **16a**, and **16b**, two commercially available antiviral agents were used as control. To further investigate the influence of the variation of the basic skeletons of the natural alkaloids on the antiviral effects, corresponding tylophorine **I** and phenanthroquinolizidine **II** (Figure 1) were also used for comparison, which were synthesized using methods reported previously by our group.[24] The in vitro and in vivo (protection, inactivation, and curative effect) antiviral results against TMV of phenanthroindo/quinolizidine alkaloids **I** and **II**, analogues **7a**, **7b**, **16a**, and **16b**, ribavirin, and ningnanmycin are listed in Table 1. All of the compounds were tested at both 500 and 100 μg/mL.

Table 1. In Vitro and in Vivo Antiviral Activity of Compounds 7a, 7b, 16a, and 16b against TMV

		inhibition rate (%)			
compd	concn (μg/mL)	in vitro effect	protection effect	inactivation effect	curative effect
I	500	38.5	40.4	45.2	42.6
	100	15.3	18.2	17.5	13.1
7a	500	43.8	46.5	47.3	42.5
	100	20	21.6	20.3	10.6
16a	500	60	54.1	55.8	58.6
	100	30.6	24.1	26.7	30
II	500	67.5	65.8	64.2	68.9
	100	32.4	32.1	30	34.7
7b	500	54.7	48.3	52.6	50.7
	100	27.6	26.9	28.9	21.7
16b	500	37.7	42.9	41.7	39.5
	100	10	17.5	18.3	12.4
ribavirin	500	41.3	38.9	37.2	39.4
	100	18.5	15.6	11.9	14.7
ningnanmycin	500	69.3	57.9	54.2	58.7
	100	26.8	38.4	20.0	23.1

The first in vitro anti-TMV bioassay demonstrated that all of the synthesized sturcturally novel analogues except for **16b** exhibted higher activity than ribavirin at both high (500 μg/mL) and low (100 μg/mL) concentrations. Especially, although compounds **16a** and **7b** showed only comparable inhibitory effect with that of ningnanmycin at 500 μg/mL, they exhibited higher antiviral activity at 100 μg/mL. Further in vivo anti-

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TMV bioassay indicated that all of the newly designed analogues displayed protection and inactivation effects superior to those of ribavirin at both concentrations, and all of them also showed preferable curative effects except for **7a** and **16b** at 100 μg/mL. Compared with ningnanmycin, perhaps the most effective commercial plant virucide, analogue **16a** can also exhibit higher inactivation effect at both concentrations, and **7b** and **16a** showed inactivation and curative effects superior to those of ningnanmycin at 100 μg/mL.

The SARs demonstrated that phenanthroindolizidine analogue **7a** with the six-membered D-ring of natural alkaloid expanded to seven-membered ring that showed higher in vitro, protection, and inactivation effects than tylophorine **I** at both concentrations. The isomerized analogue **16a** also displayed preferable antiviral effects both in vitro and in vivo relative to its precursor **I** at both high and low concentrations. Then, we can conclude that for natural phenanthroindolizidine alkaloid **I**, no matter how the six-membered D-ring was expanded to seven-membered or the fused pattern of D/E rings was changed, analogues **7a** and **16a** showed superior antiviral activity to that of the precursor **I**, which indicated that the original conformation of the phenanthroindolizidine was not optimal and deserved further optimization. However, no matter how the D-ring was expanded from six-membered to seven-membered or the fused pattern of D/E rings was changed, both phenanthroquinolizidine analogues **7b** and **16b** exhibited inferior inhibitory effects against TMV, indicating that the original conformation of the phenanthroquinolizidine was a relatively preferential conformation. Another interesting result was that although six-membered E ring compound **7b** showed higher activity than five-membered E ring compound **7a**, their corresponding counterparts **16b** and **16a** displayed reverse bioactive results, which suggested that the fused pattern of D/E rings of phenanthroquinolizidine alkaloid also has a great effect on its activity, and the original fused pattern is relatively optimal.

In summary, two types of structurally totally novel analogues of phenanthroindo/quinolizidines were synthesized and evaluated for their antiviral activities aganist TMV for the first time. The bioassay results indicated that most of the four designed structural analogues showed good to excellent in vivo anti-TMV activity, among which analogue **16a** dispalyed comparable activity with that of one of the most successful commercial antiviral agents, ningnanmycin, thus emerging as a potential inhibitor of plant virus. Further structure−activity relationships demonstrated for the first time that the original skeleton of the pentacyclic structure of phenanthroindolizidine is not optimal, and further optimization for preferential conformation is needed, whereas changes of the original skeleton of the phenanthroquinolizidine descreased its antiviral activity, indicating that the original conformation is a relative preferential conformation. Further investigations on structual optimization and mode of action are currently underway in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **3**−**7**, **9**, **10**, and **12**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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