JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Design, Synthesis, Antiviral Activity, and SARs of 14-Aminophenanthroindolizidines

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Supporting Information

ABSTRACT: Based on our previous structure−activity relationship and antiviral mechanism studies, a series of 14-aminophenanthroindolizidines (**1a−i**, **2**, and **3**) were designed, targeting tobacco mosaic virus (TMV) RNA, and synthesized and systematically evaluated for their antiviral activity against TMV. The bioassay results showed that most of these compounds exhibited good to excellent in vivo anti-TMV activity, of which compounds **1d** and **1h** displayed significantly higher activity than commercial ningnanmycin, and thus emerged as potential inhibitors of plant virus. The introduction of amino groups at the 14-position of phenanthroindolizidines, which is proposed to interact with arginine residues around the TMV RNA, increased anti-TMV activity.

KEYWORDS: *14-aminophenanthroindolizidines, antiviral activity, tobacco mosaic virus, TMV, structure−activity relationship studies, SARs*

INTRODUCTION

Plant viruses cause a variety of detrimental effects, and usually the infected plants are more susceptible to damage by pests and pathogens. Viruses and viroids are responsible for a wide variety of plant diseases which cause significant and costly crop losses annually.[1] As one of the most well-studied viruses, tobacco mosaic virus (TMV) is known to infect more than 400 plant species belonging to 36 families, such as tobacco, tomato, potato, and cucumber.[2] However, there are no chemical treatments that can absolutely inhibit TMV once it does infect the plants. Because of the unsatisfactory cure rate (30−60%) by common antiviral agents (e.g., ningnanmycin, virus A, and ribavirin) and economic loss of tobacco, much effort has been directed toward the development of novel, potent, and structurally concise antiviral agents. Some chemicals, such as pyrazole derivatives,[3] nucleotides,[4] α-aminophosphonate derivatives,[5] 3-acetonyl-3-hydroxyoxindole,[6] triazolyl compounds,[7] oxidized polyamines,[8] substituted phenylureas,[9] and some natural products,[10−12] have been found to possess antiviral activity. However, because there are only a few reports on economically viable antiviral chemicals available for application in agriculture,[13] there is great potential for further research in this field.

Natural product-based agrochemicals offer advantages that they can sometimes be specific to a target species and have a unique mode of action with low mammalian toxicity. Another benefit is their ability to decompose rapidly, thereby reducing their risk to the environment.[14,15] An additional advantage is that natural products can be candidates that possess desirable biological activities.

In a program aimed at screening of plants for biologically active natural products as alternatives to conventional synthetic agrochemicals, we found that the alcohol extract of *Cynanchum komarovii* displayed moderate antiviral activity against TMV. Using a bioassay-directed fractionation approach, we found that the alkaloids are the main active components, and antofine (Figure 1, **I**) is a major component of the alkaloids.[16]

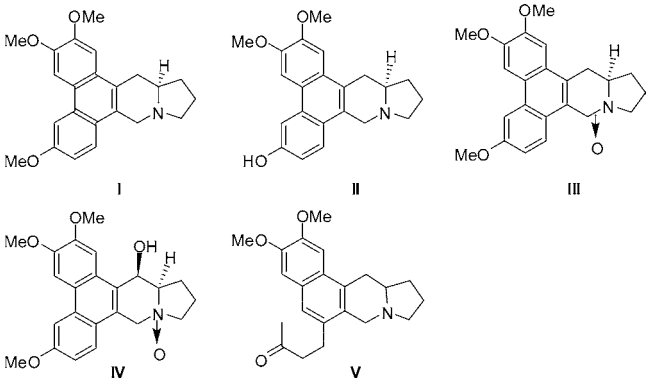


Figure 1. Chemical structures of tylophora alkaloids **I**−**V**.

Moreover, another four alkaloids (Figure 1), 6-hydroxyl-2,3-dimethoxyphenanthroindolizidine (**II**), 7-demethoxytylophorine *N*-oxide (**III**), 14-hydroxyantofine *N*-oxide (**IV**), and 2,3-dimethoxy-6-(3-oxobutyl)-7,9,10,11,11a,12-hexahydrobenzo-[*f*]pyrrolo[1,2-*b*]isoquinoline (**V**), were obtained at lower levels. The bioassay results showed that antofine (**I**) and 6-hydroxyl-2,3-dimethoxyphenanthroindolizidine (**II**) displayed excellent antiviral activity.[17] For example, the commercial antiviral agents 2,4-dioxohexahydro-1,3,5-triazine (DHT) and 1,5-diacetyl-2,4-dioxohexahydro-1,3,5-triazine (DADHT) and moroxydine hydroxychloride copper acetate (virus A) showed

Received: March 28, 2012
Revised: May 22, 2012
Accepted: May 24, 2012
Published: June 4, 2012

50% inhibition at 500 μg/mL, whereas antofine (**I**) and 6-hydroxyl-2,3-dimethoxyphenanthroindolizidine (**II**) have 63% and 70% inhibitory activity, respectively, even at a concentration of 1.0 μg/mL, which was 10−100 times more active than any reported plant virus inhibitor.[18,19] Moreover, the structure−activity relationship studies showed that most compounds of the antofine-based library with structural diversity exhibited inhibitory activity against TMV higher than that of commercial antiviral agents,[19,20] and the presence of free nitrogen in the tertiary amine and phenanthrene ring were essential for high antiviral activity.[21]

Further antiviral mechanism studies revealed that, antofine-based alkaloids have a favorable interaction with the origin of TMV RNA (oriRNA), likely exerting its virus inhibition by binding to oriRNA and interfering with virus assembly initiation.[22]

Based on the above findings, a series of 14-amino-phenanthroindolizidines (**1a−i**, **2**, and **3**) were designed, targeting TMV RNA, and synthesized. We propose that the 14-amino groups might sufficiently interact with arginine residues around the TMV RNA, thus increasing the binding effect of phenanthroindolizidines to TMV RNA. The obtained compounds were characterized and systematically evaluated for their antiviral activity against TMV.

MATERIALS AND METHODS

Synthetic Procedures. Reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified by standard techniques just before use. Reaction progress was monitored by thin-layer chromatography on silica gel GF_{254} with detection by UV. Melting points were determined using an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China), and the thermometer was uncorrected. ^{1}H NMR spectra were obtained by using Bruker AV 400, Bruker AV300, and a Varian Mercury Plus 400 MHz spectrometers. Chemical shifts (δ) were given in parts per million (ppm) and were measured downfield from internal tetramethylsilane. ^{13}C NMR spectra were recorded by using Bruker AV 400 (100 MHz) and Bruker AV300 (75 MHz) with $CDCl_3$ or DMSO-d_6 as a solvent. The chemical shifts (δ) were reported in parts per million using the solvent peak. The enantiomer excess values were determined by HPLC (SPD-20AT, Shimadzu, Japan) on a chiralcel column. Optical rotations were recorded on a Perkin-Elmer 341 MC polarimeter. High-resolution mass spectra were obtained with an FT-ICR MS spectrometer (Ionspec, 7.0 T).

General Procedure for the Preparation of Esters 7-*S* and 7-*R*. To a solution of compound **4** (3.0 g, 10.1 mmol) in $CHCl_3$ (200 mL) was added dropwise a solution of PBr_3 (4.1 g, 15.1 mmol) in $CHCl_3$ (20 mL) under nitrogen at 0 °C. The solution was then stirred at room temperature for 4 h and poured over ice, and the two layers were separated. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford compound **5** as a white powder. The powder was then redissolved in DMF (280 mL), and D/L-glutamic acid dimethyl ester hydrochloride (BMPAC, 3.0 g, 14.2 mmol) was added. The solution was stirred for 20 min, K_2CO_3 (2.0 g, 14.4 mmol) was added, and the mixture was stirred at room temperature overnight. The solution was then concentrated using a rotary evaporator, and the product was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to obtain a crude product, **6-*R*/*S***. The crude product, **6-*R*/*S***, was dissolved in MeOH (50 mL) and AcOH (20 mL) and stirred for 3 h at 45 °C. The solution was then evaporated, and the crude product was purified by flash column chromatography to give compound **7-*R*/*S*** as a white powder.

10-(Bromomethyl)-2,3,6-trimethoxyphenanthrene (5). Mp: 175−176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.80 (s, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.67 (s, 1H), 7.51 (s, 1H), 7.18 (d, J = 8.8 Hz, 1H), 4.95 (s, 2H), 4.11 (s, 3H), 4.09 (s, 3H), 4.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 149.4, 149.1, 131.8, 130.5, 128.1, 127.0, 125.3, 125.2, 125.1, 115.7, 105.0, 104.0, 103.9, 56.1, 56.0, 55.6, 33.9; HRMS (ESI) calcd for $C_{18}H_{17}O_3$ $(M - Br)^+$ 281.1178, found 281.1184.

(*S*)-*N*-[(2,3,6-Trimethoxy-10-phenanthryl)methyl]-pyroglutamic Acid Methyl Ester (7-*S*). Yield: 54%; mp: 191−193 °C; $[\alpha]_D^{20}$ = +71.0 (c = 1, $CHCl_3$); >99% ee [amylose-2 chiralcel column (Phenomenex), flow rate 0.5 mL/min, acetonitrile, 254 nm UV detector, t_R = 9.17 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.84 (s, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.62 (s, 1H), 7.42 (s, 1H), 7.20 (d, J = 8.8 Hz, 1H), 5.49 (d, J = 14.8 Hz, 1H), 4.40 (d, J = 14.8 Hz, 1H), 4.11 (s, 3H), 4.04 (s, 3H), 4.02 (s, 3H), 3.83 (d, J = 8.8 Hz, 1H), 3.57 (s, 3H), 2.64−2.55 (m, 1H), 2.44−2.34 (m, 1H), 2.17−2.06 (m, 1H), 2.00−1.94 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 172.3, 158.6, 149.8, 149.0, 131.5, 130.1, 127.6, 126.4, 126.0, 125.3, 124.7, 115.5, 105.4, 104.0, 103.7, 58.5, 56.4, 56.0, 55.6, 52.2, 44.8, 29.9, 22.8; HRMS (ESI) calcd for $C_{24}H_{26}NO_6$ $(M + H)^+$ 424.1755, found 424.1760.

(*R*)-*N*-[(2,3,6-Trimethoxy-10-phenanthryl)methyl]-pyroglutamic Acid Methyl Ester (7-*R*). Yield: 56%; mp: 190−191 °C; $[\alpha]_D^{20}$ = −73.2 (c = 1, $CHCl_3$); >99% ee [amylose-2 chiralcel column (Phenomenex), flow rate 0.5 mL/min, acetonitrile, 254 nm UV detector, t_R = 8.59 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.84 (d, J = 1.6 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.62 (s, 1H), 7.43 (s, 1H), 7.20 (dd, J = 8.8, 2.0 Hz, 1H), 5.49 (d, J = 14.4 Hz, 1H), 4.41 (d, J = 14.4 Hz, 1H), 4.11 (s, 3H), 4.05 (s, 3H), 4.02 (s, 3H), 3.83 (dd, J = 9.2, 3.2 Hz, 1H), 3.57 (s, 3H), 2.65−2.54 (m, 1H), 2.43−2.35 (m, 1H), 2.17−2.06 (m, 1H), 2.02−1.95 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 172.3, 158.6, 149.8, 149.1, 131.5, 130.1, 127.6, 126.4, 126.0, 125.3, 124.7, 115.5, 105.4, 104.0, 103.7, 58.5, 56.4, 56.0, 55.6, 52.2, 44.7, 29.9, 22.8; HRMS (ESI) calcd for $C_{24}H_{26}NO_6$ $(M + H)^+$ 424.1755, found 424.1760.

General Procedure for the Preparation of Acids 8-*S* and 8-*R*. To the solution of ester **7-*R*/*S*** (1.5 g, 3.6 mmol) in methanol (30 mL) and dioxane (30 mL) was added 2 N KOH solution (20 mL). The reaction mixture was stirred for 3 h at room temperature and then concentrated in vacuo. The residue was taken into H_2O (50 mL) and acidified to a pH of 2 with 10% HCl at 0 °C and then filtered to afford acid **8-*R*/*S*** as a white powder.

(*S*)-*N*-[(2,3,6-Trimethoxy-10-phenanthryl)methyl]-pyroglutamic Acid (8-*S*). Yield: 95%; mp: 271−272 °C; $[\alpha]_D^{20}$ = +63.0 (c = 1, DMSO); >99% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 15:85 and 0.1% trifluoroacetic acid, 254 nm UV detector, t_R = 4.64 min]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 (s, 1H), 8.11 (s, 1H), 8.07 (s, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.53 (s, 1H), 7.49 (s, 1H), 7.23 (d, J = 8.8 Hz, 1H), 5.42 (d, J = 14.8 Hz, 1H), 4.24 (d, J = 14.8 Hz, 1H), 4.05 (s, 3H), 4.01 (s, 3H), 3.90 (s, 3H), 3.71 (d, J = 7.2 Hz, 1H), 2.49−2.31 (m, 2H), 2.23−2.10 (m, 1H), 1.93−1.87 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 173.2, 158.2, 149.3, 148.8, 134.0, 129.9, 126.4, 126.3, 125.3, 124.8, 124.4, 115.9, 104.9, 104.6, 104.1, 57.9, 55.8, 55.5, 55.4, 43.6, 29.2, 22.3; HRMS (ESI) calcd for $C_{23}H_{24}NO_6$ $(M + H)^+$ 410.1598, found 410.1604.

(*R*)-*N*-[(2,3,6-Trimethoxy-10-phenanthryl)methyl]-pyroglutamic Acid (8-*R*). Yield: 96%; mp: 273−274 °C; $[\alpha]_D^{20}$ = −76.1 (c = 1, DMSO); >99% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 15:85 and 0.1% trifluoroacetic acid, 254 nm UV detector, t_R = 3.75 min]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.09 (s, 1H), 8.12 (s, 1H), 8.07 (s, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.53 (s, 1H), 7.49 (s, 1H), 7.22 (d, J = 8.0 Hz, 1H), 5.42 (d, J = 14.8 Hz, 1H), 4.24 (d, J = 14.8 Hz, 1H), 4.04 (s, 3H), 4.00 (s, 3H), 3.90 (s, 3H), 3.69 (d, J = 8.8 Hz, 1H), 2.46−2.31 (m, 2H), 2.21−2.08 (m, 1H), 1.94−1.87 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 173.2, 158.2, 149.3, 148.8, 131.0, 129.9, 126.5, 126.3, 125.4, 124.8, 124.4, 115.9, 104.9, 104.6, 104.1, 57.9, 55.8, 55.5, 55.4, 43.6, 29.2, 22.3; HRMS (ESI) calcd for $C_{23}H_{24}NO_6$ $(M + H)^+$ 410.1598, found 410.1604.

General Procedure for the Preparation of Ketones 9-*S* and 9-*R*. To a solution of acid **8-*R*/*S*** (1.5 g, 3.6 mmol) in CH_2Cl_2 (100

mL) were added freshly distilled oxalyl chloride (0.54 g, 4.3 mmol) and 2 drops of DMF. The mixture was stirred for 3 h at room temperature and then warmed to reflux. $SnCl_4$ (1.21 g, 4.6 mmol) in CH_2Cl_2 (20 mL) was added, and the mixture was warmed to 30 °C for an additional 3 h. The solution was cooled to 0 °C, and cold H_2O (50 mL) was added slowly. The phases were separated, and the organic phase was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed by rotary evaporator, and the crude product was recrystallized using ethyl acetate to give **9-*R*/*S*** as a yellow powder.

(*S*)-3,6,7-Trimethoxyphenanthro[9,10-*b*]-11,14-indolizidinedione (9-*S*). Yield: 85%; mp: 259−261 °C; $[\alpha]_D^{20}$ = +169.2 (*c* = 1, $CHCl_3$); 98% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 10:90 and 0.1% triethylamine, 254 nm UV detector, t_R = 6.25 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (d, *J* = 9.2 Hz, 1H), 7.77 (s, 1H), 7.76 (d, *J* = 2.4 Hz, 1H), 7.26 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.18 (s, 1H), 5.60 (d, *J* = 18.0 Hz, 1H), 4.53 (d, *J* = 18.0 Hz, 1H), 4.36 (t, *J* = 6.8 Hz, 1H), 4.12 (s, 3H), 4.06 (s, 3H), 4.00 (s, 3H), 2.62−2.50 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 174.1, 158.2, 151.7, 149.9, 137.0, 131.1, 129.2, 127.7, 123.1, 122.7, 122.3, 115.9, 104.6, 104.3, 103.7, 61.2, 56.2, 56.1, 55.5, 40.6, 30.1, 20.8; HRMS (ESI) calcd for $C_{23}H_{22}NO_5$ $(M + H)^+$ 392.1492, found 392.1499.

(*R*)-3,6,7-Trimethoxyphenanthro[9,10-*b*]-11,14-indolizidinedione (9-*R*). Yield: 86%; mp: 258−260 °C; $[\alpha]_D^{20}$ = −171.4 (*c* = 1, $CHCl_3$); 94% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 10:90 and 0.1% triethylamine, 254 nm UV detector, t_R = 3.65 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (d, *J* = 9.2 Hz, 1H), 7.83 (s, 1H), 7.82 (s, 1H), 7.31 (d, *J* = 9.2 Hz, 1H), 7.24 (s, 1H), 5.66 (d, *J* = 18.0 Hz, 1H), 4.60 (d, *J* = 18.0 Hz, 1H), 4.41 (t, *J* = 6.4 Hz, 1H), 4.16 (s, 3H), 4.09 (s, 3H), 4.03 (s, 3H), 2.59 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 174.0, 157.9, 151.4, 149.7, 136.8, 130.9, 129.0, 127.4, 122.7, 122.5, 122.1, 115.7, 104.4, 104.1, 103.4, 61.0, 56.1, 56.0, 55.4, 40.4, 30.1, 20.8; HRMS (ESI) calcd for $C_{23}H_{22}NO_5$ $(M + H)^+$ 392.1492, found 392.1499.

General Procedure for the Preparation of 2-(13a*S*,14*S*), 2-(13a*S*,14*R*), 2-(13a*R*,14*R*) and 2-(13a*R*,14*S*). To a solution of **9-*S*/*R*** (2.0 g, 5.1 mmol) and *n*-butylamine (1.9 g, 25.6 mmol) in CH_2Cl_2 (70 mL) was added dropwise a solution of $TiCl_4$ (1.0 g, 5.1 mmol) in CH_2Cl_2 (10 mL) at −78 °C under N_2. The mixture was stirred at room temperature for 10 h, MeOH (20 mL) and $NaBH_3CN$ (0.6 g, 10.2 mmol) were added, and the mixture was stirred for an extra 30 min. A saturated solution of ammonium chloride (10 mL) was then added, and the mixture was filtered on Celite and washed with CH_2Cl_2 (3 × 30 mL). The organic phase was washed successively with 10% aqueous Na_2CO_3 (50 mL), water (100 mL), and brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel to afford compounds [**2-(13a*S*,14*S*)** and **2-(13a*S*,14*R*)**]/[**2-(13a*R*,14*R*)** and **2-(13a*R*,14*S*)**] as a yellow powder.

(13a*S*,14*S*)-14-(Butylamino)-3,6,7-trimethoxy-12,13,13a,14-tetrahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-11(9*H*)-one [2-(13a*S*,14*S*)]. Yield: 63%; mp: 101−103 °C; $[\alpha]_D^{20}$ = +160.2 (*c* = 1, $CHCl_3$); 96% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 10:90 and 0.1% triethylamine, 254 nm UV detector, t_R = 5.88 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 9.1 Hz, 1H), 7.90 (s, 1), 7.89 (s, 1), 7.25 (s, 1H), 7.17 (s, 1H), 5.29 (d, *J* = 17.6 Hz, 1H), 4.54 (d, *J* = 17.6 Hz, 1H), 4.37 (s, 1H), 4.10 (s, 3H), 4.03 (s, 6H), 3.91 (m, 1H), 2.88−2.84 (m, 1H), 2.78−2.67 (m, 2H), 2.58−2.42 (m, 2H), 2.29−2.19 (m, 1H), 1.64 (s, 1H), 1.37−1.22 (m, 4H), 0.81 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.67, 157.91, 149.87, 149.10, 131.01, 129.98, 125.26, 124.66, 124.19, 123.84, 122.33, 115.23, 104.77, 103.78, 103.22, 58.92, 56.17, 56.03, 55.55, 54.19, 49.91, 41.08, 33.23, 31.03, 20.79, 20.30, 13.94; HRMS (ESI) calcd for $C_{27}H_{32}N_2O_4$ $(M + Na)^+$ 471.2254, found 471.2251.

(13a*S*,14*R*)-14-(Butylamino)-3,6,7-trimethoxy-12,13,13a,14-tetrahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-11(9*H*)-one [2-(13a*S*,14*R*)]. Yield: 28%; mp: 110−111 °C; $[\alpha]_D^{20}$ = +133.6 (*c* = 1, $CHCl_3$); 95% ee [AD-RH chiralcel column (Daicel), flow rate 1.0 mL/min, 2-propanol/acetonitrile 40:60 and 0.1% triethylamine, 254 nm UV detector, t_R = 4.63 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 9.2 Hz, 1H), 7.93 (s, 2H), 7.28 (s, 1H), 7.22 (s, 1H), 5.33 (d, *J* = 17.6 Hz, 1H), 4.60 (d, *J* = 17.6 Hz, 1H), 4.40 (s, 1H), 4.12 (s, 3H), 4.06 (s, 3H), 4.04 (s, 3H), 3.97−3.92 (m, 1H), 2.90−2.84 (m, 1H), 2.79−2.68 (m, 2H), 2.60−2.52 (m, 1H), 2.50−2.42 (m, 1H), 2.35−2.21 (m, 1H), 1.40−1.21 (m, 5H), 0.82 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.66, 157.91, 149.86, 149.10, 131.02, 130.00, 125.26, 124.67, 124.20, 123.84, 122.33, 115.24, 104.76, 103.77, 103.21, 58.93, 56.18, 56.03, 55.56, 54.21, 49.95, 41.08, 33.25, 31.05, 20.81, 20.32, 13.96; HRMS (ESI) calcd for $C_{27}H_{32}N_2O_4$ $(M + Na)^+$ 471.2254, found 471.2252.

(13a*R*,14*R*)-14-(Butylamino)-3,6,7-trimethoxy-12,13,13a,14-tetrahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-11(9*H*)-one [2-(13a*R*,14*R*)]. Yield: 59%; mp: 104−105 °C; $[\alpha]_D^{20}$ = −145.9 (*c* = 1, $CHCl_3$); 97% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 10:90 and 0.1% triethylamine, 254 nm UV detector, t_R = 5.05 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 9.2 Hz, 1H), 7.92 (s, 2H), 7.29−7.25 (m, 1H), 7.20 (s, 1H), 5.31 (d, *J* = 17.6 Hz, 1H), 4.58 (d, *J* = 17.6 Hz, 1H), 4.41 (s, 1H), 4.11 (s, 3H), 4.04 (s, 3H), 4.03 (s, 3H), 3.94−3.92 (m, 1H), 2.87−2.83 (m, 1H), 2.79−2.67 (m, 2H), 2.59−2.43 (m, 2H), 2.29−2.25 (m, 1H), 1.45−1.17 (m, 5H), 0.81 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 158.0, 150.0, 149.2, 131.0, 125.3, 124.7, 124.2, 123.9, 122.4, 115.3, 104.8, 103.9, 103.3, 58.9, 56.2, 56.1, 55.6, 54.2, 49.8, 41.1, 33.2, 31.0, 20.8, 20.3, 13.9; HRMS (ESI) calcd for $C_{27}H_{33}N_2O_4$ $(M + H)^+$ 449.2435, found 449.2439.

(13a*R*,14*S*)-14-(Butylamino)-3,6,7-trimethoxy-12,13,13a,14-tetrahydrodibenzo[*f*,*h*]pyrrolo[1,2-b]isoquinolin-11(9*H*)-one [2-(13a*R*,14*S*)]. Yield: 25%; mp: 107−108 °C; $[\alpha]_D^{20}$ = −129.6 (*c* = 1, $CHCl_3$); 90% ee [AD-RH chiralcel column (Daicel), flow rate 1.0 mL/min, 2-propanol/acetonitrile 40:60 and 0.1% triethylamine, 254 nm UV detector, t_R = 5.46 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 9.2 Hz, 1H), 7.89 (s, 2H), 7.22 (m, 2H), 5.42 (d, *J* = 16.4 Hz, 1H), 4.41 (d, *J* = 6.0 Hz, 1H), 4.35 (d, *J* = 16.4 Hz, 1H), 4.11 (s, 3H), 4.06 (s, 3H), 4.02 (s, 3H), 3.99 (m, 1H), 2.70−2.65(m, 2H), 2.56−2.53 (m, 3H), 2.19−2.08 (m, 1H), 1.46−1.14 (m, 5H), 0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 157.7, 149.9, 149.2, 131.5, 126.1, 125.7, 124.6, 124.0, 123.9, 115.1, 104.9, 103.8, 103.5, 60.4, 59.9, 56.1, 56.1, 55.5, 46.7, 39.9, 32.5, 30.6, 27.0, 20.4, 13.9; HRMS (ESI) calcd for $C_{23}H_{22}NO_4$ $(M - C_4H_9NH_2)^+$ 376.1549, found 376.1542.

General Procedure for the Preparation of 3-(13a*S*,14*S*), 3-(13a*S*,14*R*), 3-(13a*R*,14*R*), and 3-(13a*R*,14*S*). A solution of [**2-(13a*S*,14*S*)**]/[**2-(13a*S*,14*R*)**]/[**2-(13a*R*,14*R*)**]/[**2-(13a*R*,14*S*)**] (0.7 mmol) and $LiAlH_4$ (3.3 mmol) in THF was refluxed for 2 h under N_2 and then cooled to 0 °C, and H_2O (10 mL) was added. The mixture was concentrated in vacuo, and then H_2O (50 mL) and CH_2Cl_2 (60 mL) were added. The organic phase was separated and dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was taken into 10% aq HCl solution (80 mL) and filtered. The filtrate was cooled, made alkaline with aqueous NaOH to pH ≈ 12, and then filtered to afford compound [**3-(13a*S*,14*S*)**]/[**3-(13a*S*,14*R*)**]/[**3-(13a*R*,14*R*)**]/[**3-(13a*R*,14*S*)**] as a yellow powder.

(13a*S*,14*S*)-*N*-Butyl-3,6,7-trimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-14-amine [3-(13a*S*,14*S*)]. Yield: 70%; mp: 86−87 °C; $[\alpha]$ = +58.6 (*c* = 1, $CHCl_3$); 98% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 5:95 and 0.1% triethylamine, 254 nm UV detector, t_R = 5.22 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 9.2 Hz, 1H), 7.89−7.88 (m, 2H), 7.26− 7.23 (m, 1H), 7.14 (s, 1H), 4.62 (d, *J* = 15.2 Hz, 1H), 4.21 (s, 1H), 4.08 (s, 3H), 4.03 (s, 3H), 4.01 (s, 3H), 3.50−4.47 (m, 2H), 3.03−2.95 (m, 1H), 2.93−2.85 (m, 1H), 2.50−2.44 (m, 1H), 2.41−2.35 (m, 1H), 2.22−2.13 (m, 1H), 2.00−1.85 (m, 3H), 1.52−1.40 (m, 2H), 1.35−1.23 (m, 3H), 0.86 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 149.5, 148.7, 130.9, 130.1, 126.3, 125.6, 125.1, 123.9, 115.0, 104.8, 103.8, 103.5, 64.9, 56.0, 55.9, 55.5, 55.3, 54.6, 54.3, 51.0, 32.9, 26.1, 21.8, 20.5, 14.0; HRMS (ESI) calcd for $C_{27}H_{35}N_2O_3$ $(M + H)^+$ 435.2648, found 435.2642.

(13a*S*,14*R*)-*N*-Butyl-3,6,7-trimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-14-amine [3-

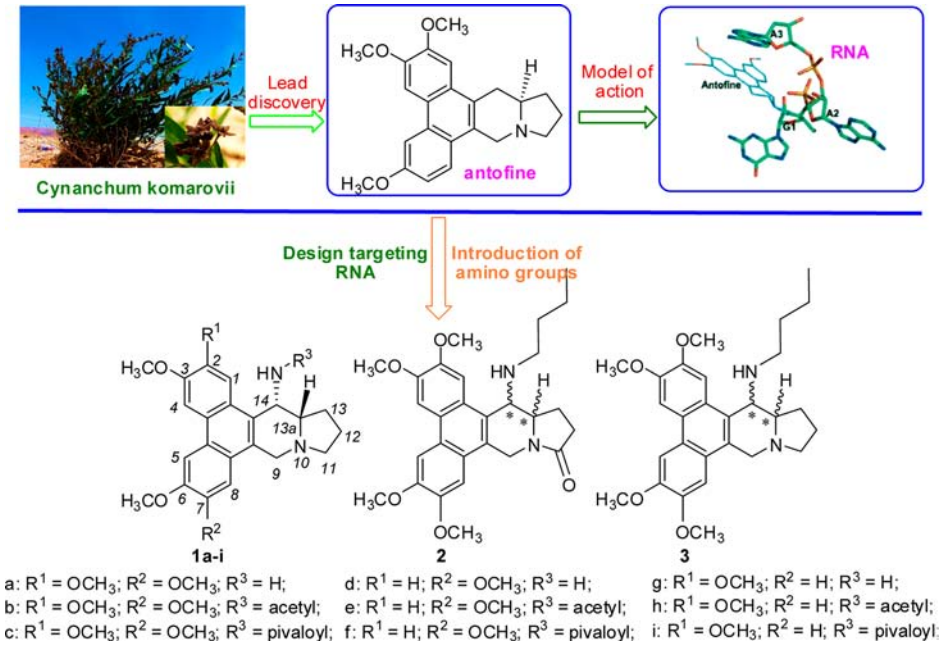
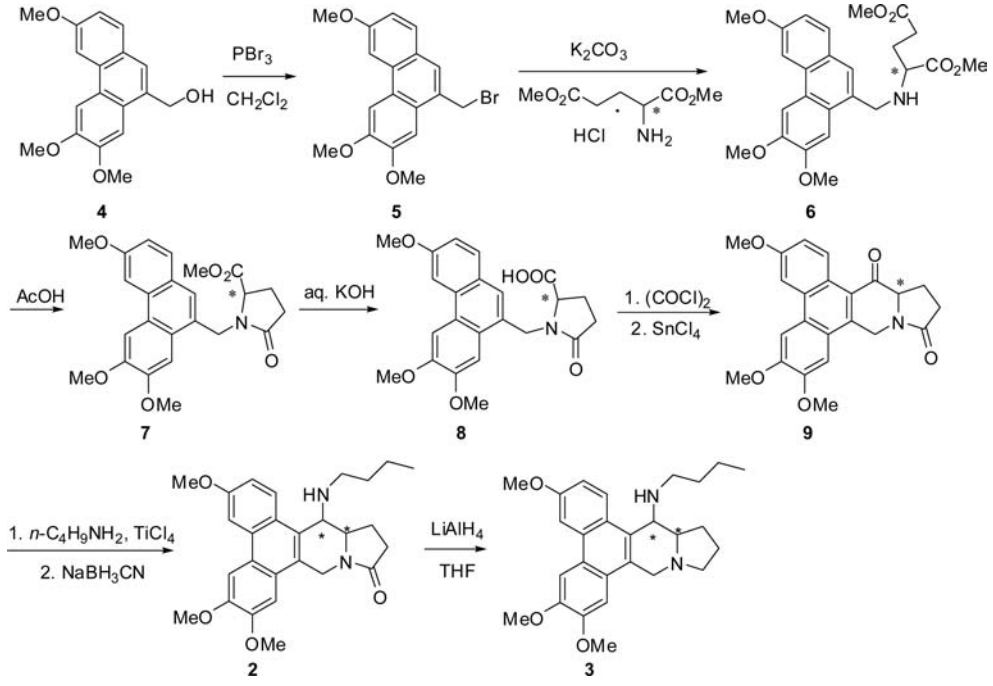


Figure 2. Design of compounds **1a−i**, **2**, and **3**.

Scheme 1. Synthesis of Compounds 2 and 3



(13a*S*,14*R*)]. Yield: 92%; mp: 65−66 °C; $[\alpha]_D^{20}$ = +74.2 (*c* = 1, $CHCl_3$); >99% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 5:95 and 0.1% triethylamine, 254 nm UV detector, t_R = 6.18 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 9.2 Hz, 1H), 7.89 (s, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.20 (m, 1H), 7.15 (s, 1H), 4.45 (d, *J* = 14.4 Hz, 1H), 4.38 (d, *J* = 6.8 Hz, 1H), 4.10 (s, 3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.76−3.72 (m, 1H), 3.33−3.28 (m, 1H), 2.75−2.50 (m, 4H), 2.47−2.40 (m, 1H), 2.05−1.81 (m, 4H), 1.41−1.34 (m, 2H), 1.30−1.25 (m, 2H), 0.85 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 149.5, 148.7, 131.3, 129.7, 126.3, 125.4, 124.7, 123.9, 114.7, 104.7, 103.9, 103.5, 68.0, 56.0, 56.0, 55.5, 54.5, 47.3, 32.8, 32.0, 22.2, 20.5, 14.0; HRMS (ESI) calcd for $C_{27}H_{35}N_2O_3$ $(M + H)^+$ 435.2648, found 435.2640.

(13a*R*,14*R*)-*N*-Butyl-3,6,7-trimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-14-amine [3-(13a*R*,14*R*)]. Yield: 67%; mp: 82−84 °C; $[\alpha]_D^{20}$ = −50.8 (*c* = 1, $CHCl_3$); 98% ee [Cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 5:95 and 0.1% triethylamine, 254 nm UV detector, t_R = 5.45 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.8 Hz, 1H), 7.88 (s, 2H), 7.20 (m, 2H), 4.61 (d, *J* = 15.2 Hz, 1H), 4.15 (d, *J* = 10.4 Hz, 1H), 4.08 (s, 3H), 4.02 (s, 3H), 4.00 (s, 3H), 3.47 (d, *J* = 15.2 Hz, 1H), 3.02−3.00 (m, 1H), 2.90−2.70 (m, 1H), 2.51−2.30 (m, 2H), 2.18−2.15 (m, 1H), 2.05−1.80 (m, 3H), 1.56−1.20 (m, 6H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 148.4, 147.7, 129.9, 129.3, 125.2, 124.5, 124.1, 124.1, 122.8, 113.9, 103.7, 102.8, 102.5, 63.9, 54.9, 54.9, 54.5, 54.3, 53.7, 53.3,

dx.doi.org/10.1021/jf3013376 | *J. Agric. Food Chem.* 2012, 60, 5825−5831

Table 1. In Vitro Antiviral Activity of Compounds 1a−i, 2, and 3 against TMV

compd	concn (μg/mL)	inhibitory effect (%)	compd	concn (μg/mL)	inhibitory effect (%)
1a	500	12.9	**2-(13a*R*,14*R*)**	500	38.2
	100	0		100	17.5
1b	500	34.7	**2-(13a*S*,14*R*)**	500	45.7
	100	21.1		100	20.0
1c	500	42.4	**2-(13a*R*,14*S*)**	500	44.1
	100	23.1		100	20.4
1d	500	72.4	**3-(13a*S*,14*S*)**	500	62.3
	100	45.0		100	28.4
1e	500	23.5	**3-(13a*R*,14*R*)**	500	47.6
	100	7.4		100	24.3
1f	500	49.1	**3-(13a*S*,14*R*)**	500	72.3
	100	30.0		100	41.2
1g	500	48.2	**3-(13a*R*,14*S*)**	500	51.4
	100	32.5		100	19.9
1h	500	75.1	ribavirin	500	41.0
	100	55.0		100	19.6
1i	500	48.3	(±)-tylophorine	500	51.8
	100	28.7		100	27.6
2-(13a*S*,14*S*)	500	52.5	ningnanmycin	500	70.2
	100	30.0		100	23.9

50.2, 32.0, 25.1, 20.8, 19.5, 13.0; HRMS (ESI) calcd for $C_{27}H_{35}N_2O_3$ $(M + H)^+$ 435.2643, found 435.2642.

(13a*R*,14*S*)-*N*-Butyl-3,6,7-trimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-14-amine [3-(13a*R*,14*S*)]. Yield: 87%; mp: 62−64 °C; $[\alpha]_D^{20} = -64.2$ (c = 1, $CHCl_3$); >99% ee [cellulose-1 chiralcel column (Phenomenex), flow rate 1.0 mL/min, 2-propanol/acetonitrile 5:95 and 0.1% triethylamine, 254 nm UV detector, t_R = 6.42 min]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.4 Hz, 1H), 7.89 (s, 1H), 7.88 (s, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.15 (s, 1H), 4.45 (d, J = 14.0 Hz, 1H), 4.37 (d, J = 5.6 Hz, 1H), 4.10 (s, 3H), 4.05 (s, 3H), 4.01 (s, 3H), 3.69 (d, J = 14.0 Hz, 1H), 3.33 (s, 1H), 2.76−2.70 (m, 1H), 2.65−2.60 (m, 1H), 2.59−2.37 (m, 3H), 2.00−1.92 (m, 3H), 1.39−1.36 (m, 2H), 1.28−1.26 (m, 2H), 1.22−1.19 (m, 1H), 0.86 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3, 149.5, 148.7, 131.3, 129.8, 128.0, 126.4, 125.4, 124.7, 123.8, 114.6, 104.7, 103.8, 103.6, 68.3, 61.0, 56.0, 55.9, 55.4, 54.5, 53.6, 47.3, 32.8, 32.1, 22.2, 20.4, 14.0; HRMS (ESI) calcd for $C_{27}H_{35}N_2O_3$ $(M + H)^+$ 435.2629, found 435.2642.

Antiviral Biological Assay. The anti-TMV activity of the synthesized compounds was tested using our previously reported method.[20]

RESULTS AND DISCUSSION

Chemistry. The 14-aminophenanthroindolizidines **1a−i** (Figure 2) were prepared according to Wang et al.[23] The synthetic route for compounds **2** and **3** is depicted in Scheme 1: treatment of alcohol **4** with PBr_3 afforded bromide **5**, coupling of **5** with BMPAC gave diester **6-(*R*/*S*)**, which was treated with AcOH to afford ester **7-(*R*/*S*)**. Hydrolysis of **7-(*R*/*S*)** gave acid **8-(*R*/*S*)**. The next Friedel−Crafts reaction of acid **8** was carried out under a variety of conditions; we found that the pH value of solution and the reaction temperature significantly affected the ee value of ketone **9-(*R*/*S*)**. The optimal conditions are as follows: carrying out the reaction at 30 °C and treating the reaction using cold H_2O instead of 10% aq HCl solution. Reductive amination of ketone **9-(*R*/*S*)** gave amides **2-[(13a*R*,14*R*) and (13a*R*,14*S*)]/[(13a*S*,14*S*) and (13a*S*,14*R*)]**, which can be easily converted to compound **3-(13a*R*,14*R*)/(13a*R*,14*S*)/(13a*S*,14*S*)/(13a*S*,14*R*)** using $LiAlH_4$ as reductant.

Antiviral Activity. To make a judgment of the antiviral potency of 14-aminophenanthroindolizidines (**1a−i**, **2**, and **3**), the commercial plant virucide ningnanmycin, perhaps the most successful registered antiplant viral agent, ribavirin, and (±)-tylophorine[20] were used as the controls.

The first in vitro anti-TMV bioassay indicated that ribavirin exhibited an inhibitory effect of 41.0% at 500 μg/mL, whereas most of the synthesized compounds except for **1a**, **1b**, **1e**, and **2-(13a*R*,14*R*)**, exhibited antiviral activity higher than that of ribavirin even at a concentration of 100 μg/mL (Table 1). Compounds **1d**, **1h**, and **3-(13a*S*,14*R*)** exhibited an inhibitory effect significantly higher than that of ningnanmycin and their parent compound (±)-tylophorine. The removal of a methoxyl at the 2-position or 7-position on the phenanthrene unit increased the inhibitory effect of the 14-aminophenanthroindolizidines except for **1e** (inhibitory effect: **1e** < **1b**). Among 14-amino-substituted compounds **1a**, **1d**, and **1e**, the methoxyl at 2-position was removed for **1d**, and **1d** displayed the best inhibitory effect. However, among the 14-acetamino-substituted compounds **1b**, **1e**, and **1h**, the methoxyl at 7-position was removed for **1h**, and **1h** showed the best inhibitory effect. The 14-pivalamide group-substituted compounds **1c**, **1f**, and **1i** exhibited about similar moderate inhibitory effects. The data suggest that the methoxy substituents on the phenanthrene unit and the amino groups at the 14-position of the phenanthroindolizidines are important for maintaining high antiviral activity. In order to investigate the effect of the optical configuration on antiviral activity, 14-*n*-butylamino-substituted compounds **2** and **3** were prepared and evaluated for their antiviral activity. The compounds **2**, containing a ketone group at the 11-position, displayed about similar moderate inhibitory effects and the activity is relatively lower than that for compounds **3**. For compounds **3**, the (13a*S*,14*R*) configuration is confirmed to be the optimal antiviral configuration.

As shown in Table 1, most of the compounds were found to display excellent antiviral activity against TMV. Therefore, these compounds were bioassayed further to investigate their antiviral activity in vivo.

dx.doi.org/10.1021/jf3013376 | J. Agric. Food Chem. 2012, 60, 5825−5831

As shown in Table 2, most of the synthesized compounds also exhibited good to excellent in vivo anti-TMV activity, of

Table 2. In Vivo Anti-TMV Activity of Compounds 1a−i, 2, and 3

compd	concn (μg/mL)	inactivation effect (%)	curative effect (%)	protection effect (%)
1a	500	17.6	15.5	18.4
	100	11.2	0	7.3
1b	500	46.4	37.4	41.5
	100	31.1	25.3	29.3
1c	500	28.5	38.5	38.9
	100	16.9	26.3	22.0
1d	500	70.3	68.0	71.6
	100	32.8	48.2	38.9
1e	500	38.1	25.2	28.6
	100	16.7	0	15.3
1f	500	31.9	45.0	43.1
	100	15.4	27.7	23.6
1g	500	41.9	42.3	44.4
	100	32.1	29.6	30.1
1h	500	72.2	69.6	72.7
	100	40.4	50.8	55.3
1i	500	37.2	45.6	52.6
	100	23.6	31.4	24.7
2-(13a*S*,14*S*)	500	47.2	47.5	50.3
	100	25.6	20.3	26.2
2-(13a*R*,14*R*)	500	49.4	41.7	48.2
	100	25.4	23.6	20.5
2-(13a*S*,14*R*)	500	50.0	52.5	54.8
	100	27.6	24.3	28.6
2-(13a*R*,14*S*)	500	43.3	36.2	46.8
	100	19.8	14.3	17.6
3-(13a*S*,14*S*)	500	58.9	60.2	65.5
	100	30.0	25.9	34.9
3-(13a*R*,14*R*)	500	53.5	58.2	57.6
	100	23.7	20.5	27.4
3-(13a*S*,14*R*)	500	64.2	66.6	69.5
	100	32.6	30.4	36.8
3-(13a*R*,14*S*)	500	42.3	50.2	54.2
	100	15.2	16.7	20.0
ribavirin	500	33.8	36.7	38.9
	100	9.8	8.2	7.5
(±)-tylophorine	500	46.5	52.0	56.6
	100	33.3	30.7	31.5
ningnanmycin	500	68.5	56.0	66.6
	100	37.7	18.9	25.2

which compounds **1d** and **1h** displayed significantly higher activity than commercial ningnanmycin, thus emerging as new lead compounds.

14-Aminophenanthroindolizidines 1a−i. Compounds **1a−i** exhibited an inactivation effect of 17.6−72.2% at 500 μg/mL and 11.2−40.4% at 100 μg/mL. The inactivation effect of compounds **1b**, **1c**, **1e**, **1f**, **1g**, and **1i** (46.4%, 28.5%, 38.1%, 31.9%, 41.9%, and 37.2%, respectively) are equivalent to ribavirin (33.8%) and lower than ningnanmycin (68.5%) at 500 μg/mL. From the data presented in Table 2, it can be observed that compounds **1b**, **1c**, **1d**, **1f**, **1g**, **1h**, and **1i** possess excellent curative bioactivities (37.4%, 38.5%, 68.0%, 45.0%, 42.3%, 69.6%, and 45.6%, respectively) and protection bioactivities (41.5%, 38.9%, 71.6%, 43.1%, 44.4%, 72.7%, and 52.6%, respectively) at 500 μg/mL. Among the compounds, **1d** and **1h** showed excellent inactivation activity (70.3% and 72.2%, respectively), curative activities (68.0% and 69.6%, respectively), and protection activities (71.6% and 72.7%, respectively) at 500 μg/mL. When the concentration was lowered to 100 μg/mL, the curative activity (50.8%) and the protection activity (55.3%) of compound **1h** are significantly higher than that of ningnanmycin (18.9% and 25.2%). As well as the in vitro SARs, the methoxy substituents on the phenanthrene unit and the amino groups at 14-position of phenanthroindolizidines play an important role for maintaining high in vivo antiviral activity. Among 14-amino-substituted compounds **1a**, **1d**, and **1e**, the methoxyl was removed at 2-position for **1d**, and **1d** displayed the best in vivo antiviral activity. However, among the 14-acetamino-substituted compounds **1b**, **1e**, and **1h**, the methoxyl was removed at 7-position for **1h**, and **1h** showed the best in vivo antiviral activity. The 14-pivalamide group-substituted compounds **1c**, **1f**, and **1i** exhibited about similar moderate in vivo antiviral activity.

Optical Pure Compounds 2 and 3. The optical pure compounds **2** and **3** were prepared to investigate the effect of configuration on antiviral activity. As shown in Table 2, all the compounds **2** and **3** displayed higher in vivo antiviral activity than ribavirin. In addition, the antiviral activity of the compounds is similar to or higher than ningnanmycin. Compounds **2** displayed about similar inhibitory effects and the effects relatively lower than that of compounds **3**, which indicated that the saturated indolizidine ring is favorable for antiviral activity. For compounds **3**, the (13a*S*,14*R*) configuration is confirmed to be the optimal in vivo antiviral configuration.

In summary, based on our previous work and the reported structure−activity relationship, a series of 14-aminophenanthroindolizidines (**1a−i**, **2**, and **3**) were prepared and systematically evaluated for their antiviral activity against TMV. The bioassay results indicated that most of these compounds exhibited good to excellent in vivo anti-TMV activity, of which compounds **1d** and **1h** displayed significantly higher activity than that of commercial ningnanmycin, thus emerging as potential inhibitors of plant virus. The methoxy substituents on the phenanthrene unit and the amino groups at the 14-position of phenanthroindolizidines play an important role for maintaining high antiviral activity. The (13a*S*,14*R*) configuration is confirmed to be the optimal antiviral configuration. Further studies on structural optimization and mode of action are currently underway in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra and HPLC chrmatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Funding Sources

We gratefully acknowledge assistance from the National Key Project for Basic Research (2010CB126100), the National Natural Science Foundation of China (21132003, 21121002), the China Postdoctoral Science Foundation (2011M500519), the National Key Technology Research and Development

Program (2011BAE06B02-17), and the Tianjin Natural Science Foundation (No. 11JCZDJC20500).

Notes

The authors declare no competing financial interest.

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