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Design, Synthesis, and Antiviral Activity Evaluation of Phenanthrene-Based Antofine Derivatives

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Supporting Information

ABSTRACT: On the basis of our previous structure−activity relationship (SAR) and antiviral mechanism studies, a series of phenanthrene-based antofine derivatives (**1**−**12** and **18**−**50**) 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 **19** and **27** displayed higher activity than commercial Ribavirin, thus emerging as potential inhibitors of plant virus. The novel concise structure provides another new template for antiviral studies.

KEYWORDS: *Phenanthrene-based antofine derivatives, antiviral activity, tobacco mosaic virus, structure−activity relationship, TMV, SAR*

INTRODUCTION

As one of the most well-studied viruses, tobacco mosaic virus (TMV) is known to infect members of 9 plant families and at least 125 individual species, including tobacco, tomato, pepper, cucumbers, and a number of ornamental flowers. The amount of loss can vary from 5 to 90% depending upon the strain of TMV, the total time of infection by TMV, the temperature during disease development, and the presence of other diseases. It is found that, in certain fields, 90−100% of the plants show mosic or leaf necrosis by harvesting time. Therefore, this plant virus has the name "plant cancer" and is difficult to control.[1]

As a successfully registered plantviral inhibitor, Ribavirin (Figure 1) is widely used to prevent TMV disease.[2] However, the inhibitory effects of Ribavirin are less than 50% at 500 μg/mL. In fact, there are no super chemical treatments that can absolutely inhibit TMV once it has infected the plants. Because of the unsatisfactory cure rate (30−60%) of common antiviral agents (Ribavirin, Ningnanmycin, Virus A, etc.) and the economic loss of tobacco, many efforts have been performed to develop novel, potent, and structure concise antiviral agents. Some chemicals, such as triazolyl compounds,[3] thiadiazoles,[4,5] pyrazole derivatives,[6,7] cyanoacrylate derivatives,[8,9] α-aminophosphonate derivatives,[10,11] *N*-(pyrimidin-5-yl)-*N*′-phenylureas,[12] and some natural products,[13,14] have been found to possess antiviral activity. However, there are only a few reports on economically viable antiviral chemicals available for application in agriculture;[15] thus, there still lies a great deal of scope 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 toxicity in mammals. Another benefit is their ability to decompose rapidly, thereby reducing their risk to the environment.[16,17] An additional advantage is that natural products can be a candidate that possesses the desirable biological activities.

Natural phenanthroindolizidine alkaloid antofine (Figure 1, **Ia**) and its analogues [e.g., tylophorine (**Ib**) and deoxytylophorinine (**Ic**)] have been isolated primarily from the genera *Cynanchum*, *Pergularia*, and *Tylophora* in the Asclepiadaceae family.[18] These compounds, commonly called tylophora alkaloids, have been targets of synthesis and modification for their significant cytotoxic activities.[19]

In the process of developing new potent plant virus inhibitors, our research group first found that the extract from the aerial parts of *Cyanchum komarovii* showed excellent antiviral activity against TMV. Using a bioassay-directed fractionation approach, the main active substances in *C. komarovii* were determined as tylophorine alkaloids, in which antofine (**Ia**) presents a high level.[20] The other four alkaloids (Figure 2), 6-hydroxyl-2,3-dimethoxyphenanthroindolizidine (**Id**), 7-demethoxytylophorine *N*-oxide (**Ie**), 14-hydroxyantofine *N*-oxide (**If**), and 2,3-dimethoxy-6-(3-oxobutyl)-7,9,10,11,11a,12-hexahydrobenzo-[*f*]pyrrolo[1,2-*b*]isoquinoline (**Ig**) were obtained at a lower level. The bioassay results showed that antofine (**Ia**) and 6-hydroxyl-2,3-dimethoxyphenanthroindolizidine (**Id**) displayed excellent antiviral activity.[21] 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 50% inhibition at 500 μg/mL, whereas antofine (**Ia**) and 6-hydroxyl-2,3-dimethoxyphenanthroindolizidine (**Id**) has 63 and 70% inhibitory activity, respectively, even at the concentration of 1.0 μg/mL, which was 10−100 times more active than any reported plant virus inhibitors.[21,22] Moreover, the structure−activity relationship (SAR) studies showed that the presence of free nitrogen in tertiary amine and phenanthrene ring are essential for high antiviral activity.[23−25]

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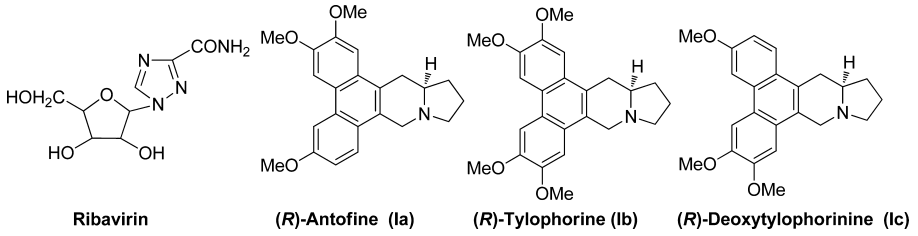

Figure 1. Chemical structures of Ribavirin and tylophora alkaloids **Ia**–**Ic**.

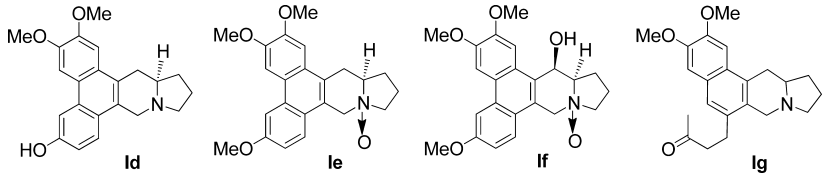

Figure 2. Chemical structures of tylophora alkaloids **Id**–**Ig**.

Further antiviral mechanism studies revealed that antofine has 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.[26]

On the basis of the above findings, a series of phenanthrene-based antofine derivatives (**1**–**12** and **18**–**50**) were designed targeting TMV RNA and synthesized and systematically evaluated for their antiviral activity against TMV. Herein, we report the recent research results about this work.

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 ultraviolet (UV). Melting points were determined using an X-4 binocular microscope melting point (mp) apparatus (Beijing Tech Instruments Co., Beijing, China), and the thermometer was uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were obtained using Bruker AV 400, Bruker AV300, and a Varian Mercury Plus 400 MHz spectrometer. Chemical shifts (δ) were given in parts per million (ppm) and were measured downfield from internal tetramethylsilane. ^{13}C NMR spectra were recorded using Bruker AV 400 (100 MHz) and Bruker AV300 (75 MHz) with $CDCl_3$ or dimethylsulfoxide (DMSO)-d_6 as a solvent. Chemical shifts (δ) were reported in ppm using the solvent peak. Elemental analyses were determined on a Yanaco C, H, N Corder MT-3 elemental analyzer. High-resolution mass spectra were obtained with a Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) spectrometer (Ionspec, 7.0 T).

Synthesis of Piperidin-1-yl(2,3,6,7-tetramethoxyphenanthren-9-yl)methanone (2). To acid **1** (4.0 g, 11.7 mmol) was added dropwise freshly distilled oxalyl chloride (50 mL) and dimethylformamide (two drops) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h and refluxed for 3 h. The excess of oxalyl chloride was removed under reduced pressure, and acyl chloride was used in the next reaction without further purification.

The above acyl chloride was dissolved in CH_2Cl_2 (50 mL) and added dropwise to a solution of piperidine (1.2 g, 14.0 mmol) and triethylamine (2.8 g, 27.7 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was warmed to room temperature, and stirring was continued for 10 h. The organic phase was washed successively with 10% aqueous hydrochloric acid and water, then dried over Na_2SO_4 anhydrous, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel to give compound **2** (4.5 g, 94% yield) as a white powder. mp = 203–204 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (s, 1H, Ar-*H*), 7.77 (s, 1H, Ar-*H*), 7.48 (s, 1H, Ar-*H*), 7.20 (s, 1H, Ar-*H*), 7.18 (s, 1H, Ar-*H*), 4.12 (s, 6H, OCH_3), 4.02 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 3.95 (m, 1H, NC*H*), 3.83 (m, 1H, NC*H*), 3.20 (m, 2H, NC*H*), 1.64–1.83 (m, 4H, NCH_2CH_2), 1.35–1.48 (m, 2H, $NCH_2CH_2CH_2$). Anal. Calcd for $C_{24}H_{27}NO_5$: C, 70.40; H, 6.65; N, 3.42. Found: C, 70.41; H, 6.85; N, 3.46.

Synthesis of (*S*)-1-(2,3,6,7-Tetramethoxyphenanthrene-9-carbonyl)pyrrolidine-2-carboxylic Acid (4). To the solution of ester **3** (6.0 g, 13.2 mmol) in methanol (150 mL) was added 4 N NaOH solution (100 mL). The reaction mixture was refluxed for 2 h and then concentrated *in vacuo* to remove methanol. The residue was acidified to a pH of 2 with 10% HCl at 0 °C and filtered to afford acid **4** (5.7 g, 98% yield) as a white powder. mp = 248–250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.86 (brs, 1H, CO_2H), 8.07 (s, 1H, Ar-*H*), 8.04 (s, 1H, Ar-*H*), 7.66 (s, 1H, Ar-*H*), 7.59 (s, 1H, Ar-*H*), 7.46 (s, 1H, Ar-*H*), 4.59 (dd, J = 4.9, 13.6 Hz, 1H, NC*H*), 4.06 (s, 6H, OCH_3), 3.91 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.12–3.23 (m, 2H, NCH_2), 2.25–2.40 (m, 1H, $NCHCH_2$), 1.91–2.03 (m, 1H, $NCHCH_2$), 1.75–1.86 (m, 2H, NCH_2CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.7, 168.4, 149.8, 149.3, 149.1, 148.9, 131.3, 124.9, 124.3, 121.9, 121.4, 108.8, 105.7, 104.1, 103.7, 58.2, 56.0, 55.9, 55.6, 55.5, 48.3, 29.2, 24.5. Anal. Calcd for $C_{24}H_{25}NO_7$: C, 65.59; H, 5.73; N, 3.19. Found: C, 65.82; H, 5.96; N, 3.40.

Synthesis of (*S*)-1-(2,3,6,7-Tetramethoxyphenanthrene-9-carbonyl)pyrrolidine-2-carboxamide (5). To a stirred solution of acid **4** (3.5 g, 8.0 mmol) and Et_3N (0.8 g, 8.0 mmol) in tetrahydrofuran (THF) (120 mL) was added ethyl chloroformate (2.0 g, 18.4 mmol) at −15 °C. The mixture was stirred at −15 °C for 30 min, and then 25% solution of $NH_3{\cdot}H_2O$ in H_2O (5 mL, 32.0 mmol) was added dropwise. Another 1 h later, the mixture was warmed to room temperature, stirred for 12 h, and then concentrated *in vacuo*. The residue was taken into CH_2Cl_2 (200 mL), washed with saturated aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), and brine (100 mL), then dried over $MgSO_4$ anhydrous, and concentrated *in vacuo* to afford compound **5** (2.5 g, 72%) as a white powder. mp = 206–208 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (s, 2H, Ar-*H*), 7.58 (s, 2H, Ar-*H*), 7.21 (s, 1H, Ar-*H*), 6.07 (s, 1H, NH_2), 4.87 (s, 1H, NH_2), 4.12 (s, 6H, OCH_3), 4.04 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 3.90–4.00 (m, 1H, NC*H*), 3.15–3.45 (m, 2H, NCH_2), 1.70–2.35 (m, 4H, $NCH_2CH_2CH_2$). Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.82; H, 6.25; N, 6.63.

Synthesis of (*S*)-1-(2,3,6,7-Tetramethoxyphenanthrene-9-carbonyl)pyrrolidine-2-carbonitrile (6). To a stirred solution of compound **5** (2.5 g, 5.7 mmol) and Et_3N (2.6 g, 25.7 mmol) in CH_2Cl_2 (80 mL) was added trifluoroacetic anhydride (2.4 g, 11.4 mmol) at 0 °C under N_2. The mixture was warmed to room temperature for 10 h, then washed with saturated aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), and brine (100 mL), dried over $MgSO_4$ anhydrous, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford compound **6** (2.1 g, 88% yield)

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as a white powder. mp = 163−165 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (s, 1H, Ar-*H*), 7.78 (s, 1H, Ar-*H*), 7.57 (s, 1H, Ar-*H*), 7.29 (s, 1H, Ar-*H*), 7.20 (s, 1H, Ar-*H*), 5.11 (dd, *J* = 2.7, 7.3 Hz, 1H, NC*H*), 4.14 (s, 3H, OCH_3), 4.13 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 3.26−4.02 (m, 2H, NCH_2), 2.83−2.45 (m, 2H, NCHCH_2), 1.96−2.22 (m, 2H, NCH$_2$CH_2). Anal. Calcd for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.72; H, 5.96; N, 6.93.

Synthesis of (*S*)-1-(2,3,6,7-Tetramethoxyphenanthrene-9-carbonyl)pyrrolidine-2-carbaldehyde (8). To a solution of oxalyl chloride (0.8 g, 6.3 mmol) in CH_2Cl_2 (15 mL) was added dropwise the solution of DMSO (1.3 g, 13.8 mmol) in CH_2Cl_2 (5 mL) at −78 °C. The mixture was stirred for 15 min, and then the solution of alcohol **7** (2.0 g, 4.7 mmol) in CH_2Cl_2 (15 mL) was added dropwise. After stirring for 2 h, the solution of Et_3N (2.4 g, 23.8 mmol) in CH_2Cl_2 (5 mL) was added. The mixture was warmed to room temperature, then washed with 5% aqueous HCl solution (50 mL), H_2O (50 mL), and brine (50 mL), dried over $MgSO_4$ anhydrous, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford compound **8** (1.4 g, 72% yield) as a white powder. mp = 121−123 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.83 (s, 1H, *CHO*), 7.78 (d, *J* = 3.0 Hz, 1H, Ar-*H*), 7.76 (d, *J* = 2.5 Hz, 1H, Ar-*H*), 7.63 (s, 1H, Ar-*H*), 7.56 (d, *J* = 2.6 Hz, 1H, Ar-*H*), 7.17 (d, *J* = 3.2 Hz, 1H, Ar-*H*), 5.00 (dd, *J* = 2.4, 7.0 Hz, 1H, NC*H*), 4.12 (s, 6H, OCH_3), 4.10 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 3.27−3.38 (m, 2H, NCH_2), 2.07−2.32 (m, 2H, NCHCH_2), 1.77−1.93 (m, 2H, NCH$_2$CH_2). Anal. Calcd for $C_{24}H_{25}NO_6$: C, 68.07; H, 5.95; N, 3.31. Found: C, 68.22; H, 6.17; N, 3.53.

Synthesis of (*S*)-Methyl-1-(2,3-bis(3,4-dimethoxyphenyl)-acryloyl)pyrrolidine-2-carboxylate (11). To acid **10** (12.0 g, 34.9 mmol) was added freshly distilled oxalyl chloride (100 mL) at room temperature. The reaction mixture was then stirred for 4 h. Then, the excess of oxalyl chloride was removed under reduced pressure, and acyl chloride was used in the next reaction without further purification.

The above acyl chloride was dissolved in CH_2Cl_2 (50 mL) and added dropwise to a stirred solution of methyl L-prolinate hydrochloride (5.6 g, 33.8 mmol), Et_3N (6.8 g, 67.3 mmol), and 4-dimethylaminopyridine (DMAP) (0.43 g, 3.5 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The reaction mixture was warmed to room temperature, and stirring was continued for 4 h. The organic phase was washed successively with 10% aqueous hydrochloric acid and water, then dried over Na_2SO_4 anhydrous, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel to give compound **11** (11.9 g, 75% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.94 (s, 1H, Ar-*H*), 6.91 (s, 2H, Ar-*H*), 6.83 (s, 1H, Ar-*H*), 6.80 (s, 1H, Ar-*H*), 6.74 (s, 1H, Ar-*H*), 6.70 (s, 1H, Ar-*H*), 4.57 (dd, *J* = 7.5, 5.3 Hz, 1H, NC*H*), 3.88 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.57 (s, 3H, CO_2CH_3), 3.23−3.38 (m, 2H, NCH_2), 1.73−2.30 (m, 4H, NCH$_2$CH_2CH_2). High-resolution mass spectrometry (HRMS) [electrospray ionization (ESI)] calcd for $C_{25}H_{30}NO_7$ $(M + H)^+$ 456.2017, found 456.2013.

Synthesis of (*S*)-2,3-Bis(3,4-dimethoxyphenyl)-1-(2-(hydroxymethyl)pyrrolidin-1-yl)prop-2-en-1-one (12). To acid **10** (12.0 g, 34.9 mmol) was added freshly distilled oxalyl chloride (100 mL) at room temperature. The reaction mixture was then stirred for 4 h. Then, the excess of oxalyl chloride was removed under reduced pressure, and acyl chloride was used in the next reaction without further purification.

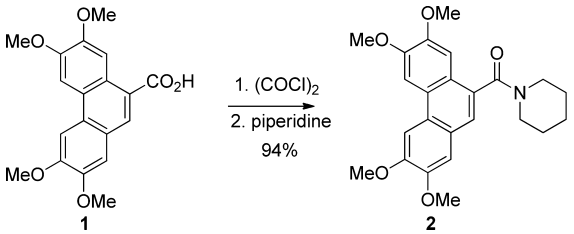
The above acyl chloride was dissolved in CH_2Cl_2 (50 mL) and added dropwise to a stirred solution of methyl L-prolinol (4.2 g, 41.6 mmol) and Et_3N (4.2 g, 41.6 mmol) in CH_2Cl_2 (100 mL) at −78 °C. The reaction mixture was stirred for 3 h, then warmed to room temperature, washed successively with 10% aqueous hydrochloric acid and water, then dried over Na_2SO_4 anhydous, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel to give compound **12** (13.2 g, 89% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.65−7.40 (m, 7H, Ar-*H*, Ar-*CH*), 4.06−4.38 (m, 1H, NC*H*), 3.88 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 3.64−3.73 (m, 2H, OCH_2), 3.10−3.60 (m, 2H, NCH_2), 1.50−2.18 (m, 4H, NCH$_2$CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.6, 149.1, 148.9, 148.8, 148.2, 136.1, 130.7, 128.0, 127.9, 123.0, 121.8, 112.4, 112.2, 111.4, 110.7, 66.7, 61.1, 55.9, 55.7, 55.4, 49.7, 28.2, 24.6. HRMS (ESI) calcd for $C_{24}H_{30}NO_6$ $(M + H)^+$ 428.2068, found 428.2064.

Synthesis of (*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carboxylic Acid (14). To a stirred solution of L-proline (30.0 g, 0.26 mol) and Et_3N (48 mL, 0.35 mol) in CH_2Cl_2 (600 mL) was added Boc_2O at 0 °C. The reaction mixture was warmed to room temperature and stirred for 12 h, and then 10% aqueous HCl solution (80 mL) was added. The organic phase was separated, washed with brine (200 mL), then dried over Na_2SO_4 anhydrous, filtered, and concentrated *in vacuo*. The residue was recrystallized using ethyl acetate and petroleum ether to afford acid **14** (52.3 g, 93% yield) as a white powder. mp = 134−135 °C (literature[27] mp = 135−137 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.20 (brs, 1H, CO_2H), 4.23−4.35 (m, 1H, 2-*H*), 3.34−3.54 (m, 2H, 5-*H*), 2.04−2.27 (m, 2H, 3-*H*), 1.89−1.96 (m, 2H, 4-*H*), 1.44 [d, *J* = 22.6 Hz, 9H, C(CH_3)$_3$].

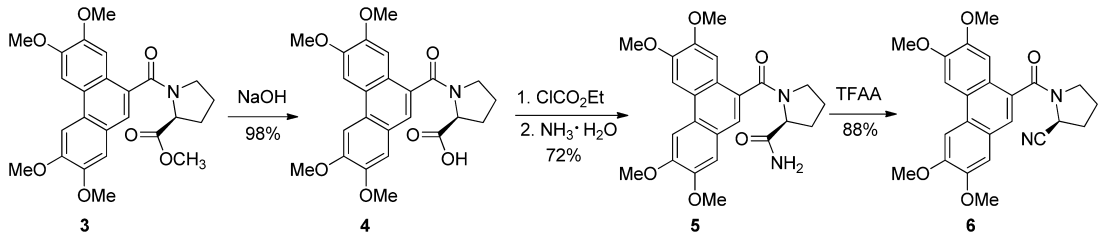
Synthesis of (*S*)-*tert*-Butyl-2-carbamoylpyrrolidine-1-carboxylate (15). To a stirred solution of acid **14** (35.0 g, 0.16 mol) and Et_3N (16.5 g, 0.16 mol) in THF (300 mL) was added ethyl chloroformate (40.0 g, 0.37 mol) at −10 °C. The mixture was stirred at −10 °C for 30 min, and then 25% solution of $NH_3{\cdot}H_2O$ in H_2O (62 mL) was added dropwise. Another 1 h later, the mixture was warmed to room temperature, stirred for 12 h, and then concentrated *in vacuo*. The residue was taken into CH_2Cl_2 (600 mL), washed with saturated aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), and brine (100 mL), then dried over $MgSO_4$ anhydrous, and concentrated *in vacuo*. The residue was recrystallized using ether to afford compound **15** (33.5 g, 96%) as a white powder. mp = 106−107 °C (literature[28] mp = 103.6−107.7 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.83 (s, 1H, *NH*), 6.13 (s, 1H, *NH*), 4.81−4.89 (m, 1H, 2-*H*), 4.18−4.29 (m, 1H, 5-*H*), 3.43−3.48 (m, 1H, 5-*H*), 1.87−2.30 (m, 4H, 3,4-*H*), 1.43 [s, 9H, C(CH_3)$_3$].

Synthesis of (*S*)-*tert*-Butyl-2-cyanopyrrolidine-1-carboxylate (16). To a stirred solution of compound **15** (20.0 g, 93.5 mmol) and Et_3N (42.0 g, 0.42 mol) in CH_2Cl_2 (300 mL) was added trifluoroacetic anhydride (39.0 g, 0.19 mol) at 0 °C under N_2. The mixture was warmed

Scheme 1. Synthesis of Compound 2



Scheme 2. Synthesis of Compounds 4−6



to room temperature for 10 h, then washed with saturated aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), and brine (100 mL), dried over $MgSO_4$ anhydrous, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel to afford compound **16** (17.7 g, 97% yield) as a thick oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.16−4.38 (m, 1H, 2-*H*), 3.34−3.58 (m, 2H, 5-*H*), 1.87−2.24 (m, 4H, 3,4-*H*), 1.49 [s, 9H, C(CH_3)$_3$].

Synthesis of (*S*)-*tert*-Butyl-1-((10-bromo-2,3,6,7-tetramethoxyphenanthren-9-yl)methyl)pyrrolidine-2-carbimidate (19). The solution of compound **16** (1.4 g, 7 mmol) in TFA was stirred for 2 h at 0 °C and then concentrated *in vacuo* to give crude product **17**, which was used in the next reaction without further purification.

The above crude product **17** was taken into acetonitrile (180 mL). Then, to the solution was added K_2CO_3 (3.5 g, 25.5 mmol) and bromide **18** (3.0 g, 6.4 mmol). The stirred solution was refluxed for 8 h, then cooled to room temperature, and concentrated *in vacuo*. The residue was taken into CH_2Cl_2 (200 mL), washed with saturated aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), and brine (100 mL), then dried over $MgSO_4$ anhydrous, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel to give compound **19** (2.1 g, 68% yield) as a slight yellow powder. mp = 135−137 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (s, 1H, Ar-*H*), 7.75 (s, 1H, Ar-*H*), 7.71 (s, 1H, Ar-*H*), 7.61 (s, 1H, Ar-*H*), 6.59 (s, 1H, *NH*), 4.61 (d, *J* = 13.4 Hz, 1H, Ar-CH_2), 4.38 (d, *J* = 13.4 Hz, 1H, Ar-CH_2), 4.11 (s, 6H, OCH_3), 4.06 (s, 6H, OCH_3), 3.56 (dd, *J* = 2.8, 10.0 Hz, 1H, NC*H*), 3.37 (t, *J* = 7.8 Hz, 1H, NCH_2), 2.87−2.93 (m, 1H, NCH_2), 2.22−2.32 (m, 1H, NCHCH_2), 1.92−1.97 (m, 1H, NCHCH_2), 1.83−1.88 (m, 1H, NCH_2CH_2), 1.65−1.75 (m, 1H, NCH_2CH_2), 0.55 [s, 9H, C(CH_3)$_3$]. Anal. Calcd for $C_{28}H_{35}BrN_2O_5$: C, 60.11; H, 6.31; N, 5.01. Found: C, 60.20; H, 6.45; N, 5.23.

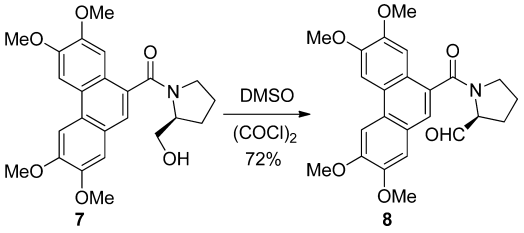
Antiviral Biological Assay. The anti-TMV activity of the synthesized compounds was tested using our previously reported method.[23]

Antiviral Activity of Compounds against TMV in Vitro. Fresh leaf of the 5−6 growth stage of tobacco inoculated by the juice-leaf rubbing method (concentration of TMV is 5.88×10^{-2} μg/mL) was cut into halves along the main vein. The halves were immersed into the solution of 500 μg/mL of the compounds and double-distilled water for 20 min, respectively, and then cultured at 25 °C for 72 h. Each compound was replicated at least 3 times.

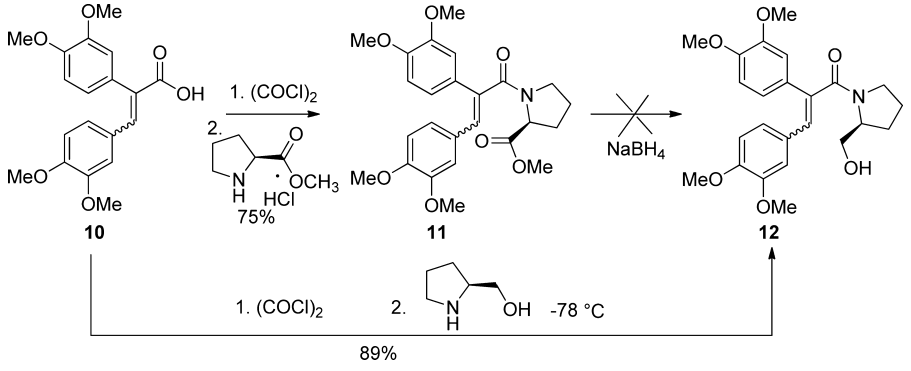
Protective Effect of Compounds against TMV in Vivo. The compound solution was smeared on the left side, and the solvent served as a control on the right side of growing *Nicotiana tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in TMV of 6×10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3−4 days after inoculation were counted. There are three replicates for each compound.

Inactivation Effect of Compounds against TMV in Vivo. The virus was inhibited by mixing with the compound solution at the same volume for 30 min. The mixture was then inoculated on the left side of the leaves of *N. tabacum* L., whereas the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local lesion numbers

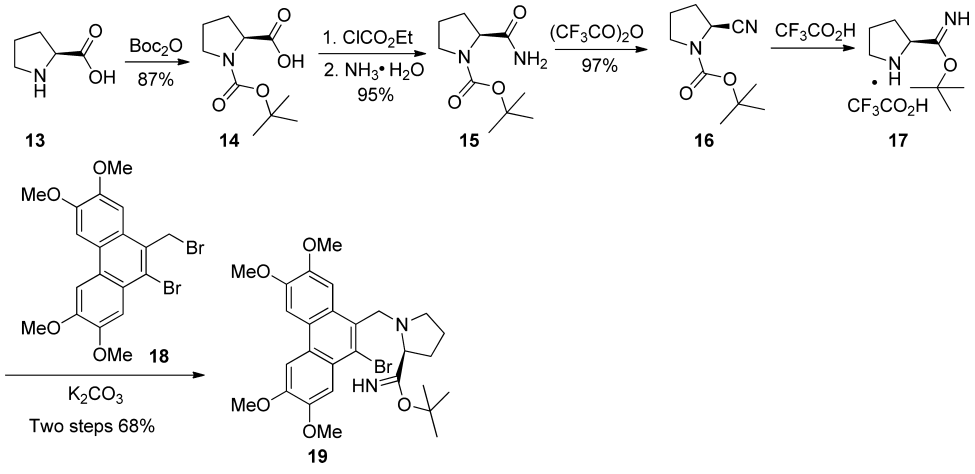
Scheme 3. Synthesis of Compound 8



Scheme 4. Synthesis of Compounds 11 and 12



Scheme 5. Synthesis of Compound 19



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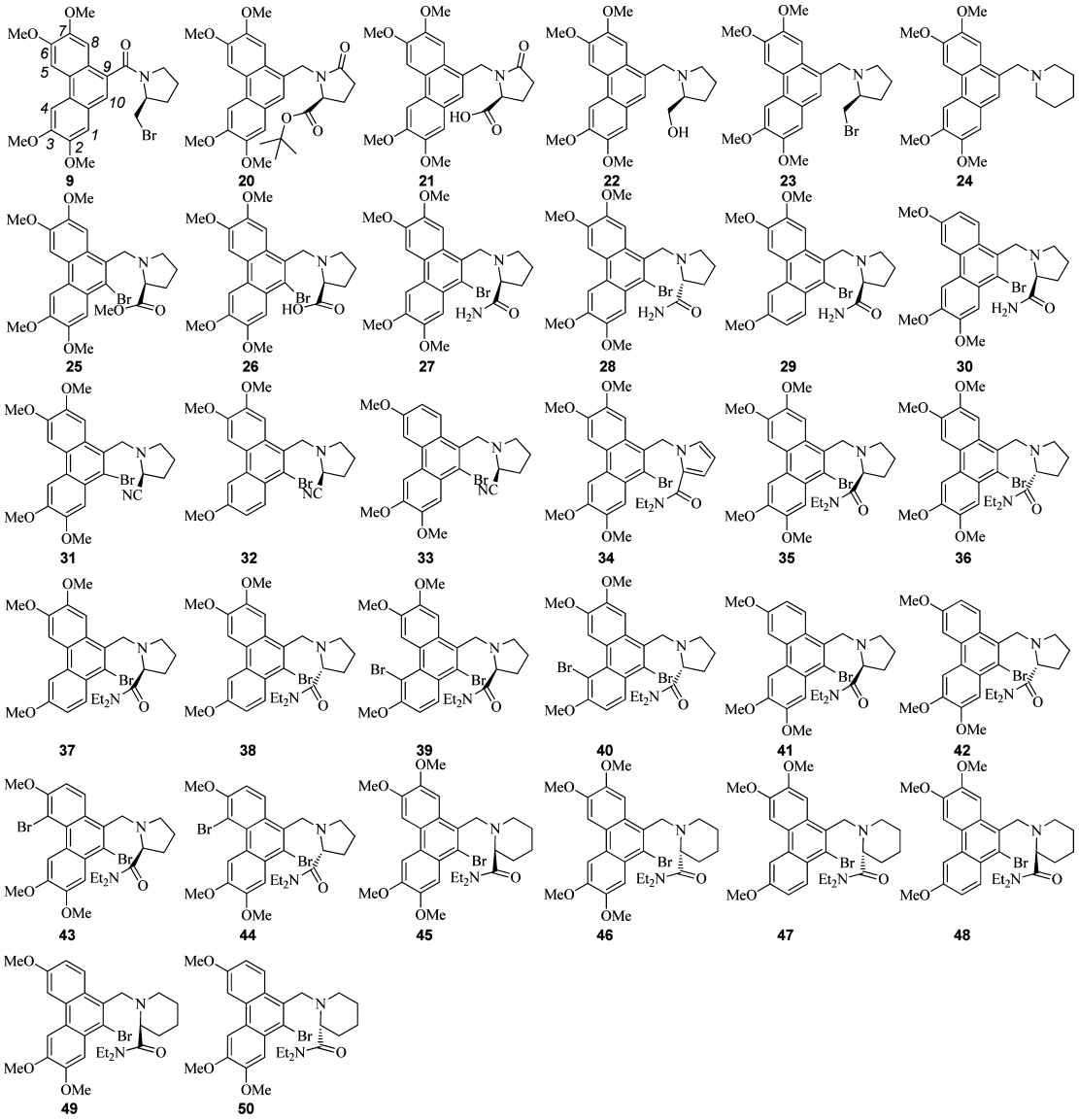

Figure 3. Chemical structures of compounds **9** and **20**−**50**.

were recorded 3−4 days after inoculation. There are three replicates for each compound.

Curative Effect of Compounds against TMV in Vivo. Growing leaves of *N. tabacum* L. of the same ages were selected. TMV (concentration of 6.0×10^{-3} mg/mL) was dipped and inoculated on the whole leaves. Then, the leaves were washed with water and dried. The compound solution was smeared on the left side, and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3−4 days after inoculation. There are three replicates for each compound.

The *in vitro* and *in vivo* inhibition rates of the compound were then calculated according to the following formula ("av" means average, and controls were not treated with compound): inhibition rate (%) = [(av local lesion number of control − av local lesion number of drug-treated)/av local lesion number of control] × 100%.

RESULTS AND DISCUSSION

Chemistry. As shown in Scheme 1, condensation of the 9-phenanthrenecarbonyl chloride (prepared by chlorination of 9-phenanthrenecarboxylic acid **1**[23] with oxalyl chloride) with piperidine in the presence of Et_3N gave compound **2** in 94% yield. As depicted in Scheme 2, hydrolysis of ester **3**[29] gave acid **4** in 98% yield. Treatment of compound **4** with ethyl chloroformate and $NH_3 \cdot H_2O$ afforded amide **5**. Dehydration of compound **5** with trifluoroacetic anhydride (TFAA) gave nitrile **6** in 88% yield. Oxidation of alcohol **7**[29] gave the aldehyde **8** in 72% yield (Scheme 3). To test the effect of the phenanthrene ring on the anti-TMV activity, compounds **11** and **12** were designed and synthesized. As shown in Scheme 4, condensation of 2,3-bis(3,4-dimethoxyphenyl)acryloyl chloride (prepared by chlorination of acid **10**[30] with oxalyl chloride) with L-proline methyl ester hydrochloride in the presence of Et_3N gave compound **11** in 75% yield. The next reduction of ester **11** was carried out in various conditions, but only the complex result was obtained. At last, condensation of 2,3-bis(3,4-dimethoxyphenyl)-acryloyl chloride with L-prolinol at −78 °C afforded alcohol **12** in 89% yield. During preparation of nitrile **31**, the imidate ester **19** was obtained. As shown in Scheme 5, *N*-Boc protection of L-proline (**13**) gave acid **14**. Treatment of compound **14** with ethyl chloroformate and $NH_3 \cdot H_2O$ afforded amide **15**. The amide **15** was conveniently dehydrated to nitrile **16** by action of TFAA. The next deprotection in TFA gave a new structural rearranging com-xpound **17**, which coupling with bromide **18** afforded imidate ester **19**. Other phenanthrene-based antofine derivatives **9** and

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20–**50** (Figure 3) were prepared according to our previously published literature.[29−33]

Antiviral Activity. To make a judgment of the antiviral potency of the phenanthrene-based antofine derivatives (**1**–**12** and **18**–**50**), the commercial plant virucide Ribavirin was used as the control.

The first *in vitro* anti-TMV bioassay indicated that most of the tested compounds displayed good antiviral activity, of which compounds **19**, **27**, **44**, and **47** exhibited higher inhibition than Ribavirin (Table 1). Therefore, these compounds were bioassayed further to investigate their antiviral activity *in vivo*.

Table 1. Antiviral Activity of Compounds 1−12 and 18−50 against TMV at 500 μg/mL

		in vivo		
compound	*in vitro* inhibition rate (%)	inactivation effect (%)	curative effect (%)	protection effect (%)
1	0	4.2	3.6	4.8
2	32.5	24.5	28.9	30.6
3	31.6	29.1	22.8	29.5
4	33.3	27.2	28.3	30
5	27	25.3	23.5	24.3
6	12.5	17.6	15.6	10.3
7	32.8	29.5	30.2	25.6
8	22.2	15.2	20.3	21.4
9	21.9	27.6	26.7	36.9
10	2.3	4.1	2.7	1.9
11	17.5	21.1	17.2	23.6
12	11.7	20.9	18.1	19.3
18	6.1	4.3	1.8	5.2
19	39.7	36.6	40.3	42.2
20	14.2	27.1	16.8	23.5
21	32.4	33.7	25.6	35.3
22	15.9	17.4	23.5	25.2
23	32.1	20.3	16.3	21.1
24	38.4	30	35.2	35.9
25	19.6	21.4	27.8	33.3
26	20.4	17	13.5	10.2
27	44.3	42.1	36.6	39.5
28	10.5	0	12.2	15.3
29	35.5	12.3	30.7	32.4
30	0	14.5	5.6	0
31	24.7	10.3	18.2	33.3
32	20.9	27.7	18.9	24.5
33	22.7	12.6	20.5	15.3
34	18.2	20	10.6	13.1
35	17.2	22	21.6	25.3
36	33.3	21.3	30.2	27.6
37	12.5	23.7	8.9	16.8
38	35.6	26.9	31.6	27.1
39	19.6	17.8	22.3	15.3
40	22.4	10.4	13.3	20.3
41	23.5	15.7	14.2	18.9
42	35.7	27.6	31.4	33.1
43	32.5	20.7	23.5	26.1
44	42.6	30.2	35.8	39.2
45	19.7	21.5	26.3	28.5
46	23.5	13.7	14.2	21.7
47	39.2	28.6	31.5	37.2
48	21.6	23.7	14.9	19.6
49	30.7	25.2	26.4	34.3
50	12.1	15.4	17.6	21.3
Ribavirin	38.5	32.1	34.5	34.2

As shown in Table 1, the synthesized compounds also exhibited good to excellent *in vivo* anti-TMV activity, especially for compounds **19** and **27**, which displayed higher antiviral activity than Ribavirin, thus emerging as new lead compounds.

*Aryl Amides **2**–**9**, **11**, and **12** and Acids **1** and **10***. Aryl amides **2**, **4**, **5**, **7**, and **9** exhibited almost similar antiviral activity, which is slightly lower than Ribavirin. The difference between compounds **3**–**9** lies in the substituents on the proline side. The ester **3**, nitrile **6**, and aldehyde **8** showed relatively lower antiviral activity, which indicated that the proline side of the designed compounds may serve as a hydrogen donor. The bromide **9** displayed similar antiviral activity to alcohol **7**. It seemed to be that the bromide **9** serves as a prodrug of alcohol **7**. To test the effect of the phenanthrene ring on the antiviral activity, compounds **11** and **12** were prepared. The bioassay results indicated that the phenanthrene ring is essential for high antiviral activity (antiviral effect: **3** > **11** and **7** > **12**). A similar result was also reported in our previous work.[25] It ought to be mentioned that the amino substituents are also important for maintaining high antiviral activity. For instance, the acids **1** and **10** without amino groups almost exhibited no antiviral activity.

*Arylmethylamines **19**–**50** and Bromide **18***. In comparison to corresponding aryl amides, the arylmethylamines displayed lower or similar antiviral activity. However, most of the arylmethylamines showed good to excellent anti-TMV activity, of which compounds **19** and **27** exhibited higher activity than Ribavirin and emerged as potential inhibitors of plant virus.

The mainly difference between compounds **19**–**23** and **25**–**27** lies in the substituents on the proline side. The imidate ester **19**, acid **21**, and amide **27** displayed relatively higher antiviral activity than the others, which further indicated that the proline side of the designed compounds may serve as a hydrogen donor. As the enantiomer of amide **27**, compound **28** showed a significantly lower antiviral activity, which indicated that the stereo configuration plays an important role for keeping high activity. The methoxy substituent on the phenanthrene unit is important for maintaining high activity. Removing the methoxyl at the 7 position of phenanthrene led to a significant decrease in antiviral activity (antiviral effect: **30** < **27**). Changing the amide group to the nitrile group decreased the antiviral activity (antiviral effect: **27** > **31** and **29** > **32**), except for compound **30** (antiviral effect: **30** < **33**). Replacement of the amino group by the diethylamino group decreased the antiviral activity for compounds **27** and **29** and increased the antiviral activity for compounds **28** and **30**. The antiviral activity of compound **34** is lower than that of compounds **35** and **36**, which indicated that the saturated pentaheterocycle is favorable for maintaining high activity. Introduction of the bromine atom at the 4 position of the phenanthrene ring increased the antiviral activity for compound **37** and decreased antiviral activity for compound **38**. Introduction of the bromine atom at the 5 position of the phenanthrene ring increased the antiviral activity (antiviral effect: **41** < **43** and **42** < **44**). Replacement of the saturated pentaheterocycle by the saturated hexaheterocycle decreased the antiviral activity for the *R* configuration (antiviral effect: **36** > **46**, **38** > **48**, and **42** > **50**) and increased the antiviral activity for the *S* configuration (antiviral effect: **41** < **49**, **35** < **45**, and **37** < **47**). For saturated pentaheterocycle compounds, the favorable antiviral configuration is *R*, except for compound **27**. However, for saturated hexaheterocycle compounds, the favorable antiviral configuration is *S*. The bromide **18** without the amino group also exhibited no antiviral activity.

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In summary, on the basis of our previous SAR and antiviral mechanism studies, a series of phenanthrene-based antofine derivatives (**1**−**12** and **18**−**50**) 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 **19** and **27** displayed higher activity than commercial Ribavirin, thus emerging as potential inhibitors of plant virus. The novel concise structure provides another new template for antiviral studies, which may have different mechanisms of action. Further studies on structural optimization and mode of action are currently underway in our laboratories.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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