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Design, Synthesis, and Antiviral Evaluation of Phenanthrene-Based Tylophorine Derivatives as Potential Antiviral Agents

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A series of C9-substituted phenanthrene-based tylophorine derivatives (PBTs) were designed, synthesized, and first evaluated for their antiviral activities against tobacco mosaic virus (TMV). These compounds contain a phenanthrene core structure and can be synthesized some efficiently with excellent yields compared with tylophorine alkaloid. The bioassay results show that some of these compounds exhibited higher antiviral activity against TMV in vivo than tylophorine and commercial Ningnanmycin. Especially, compounds **3**, **4**, **9**, **13**, and **16** emerged as potential inhibitors of plant virus. These new findings demonstrate that these phenanthrene-based tylophorine derivatives (PBTs) represent another new template for antiviral studies and could be considered for novel therapy against plant virus infection.

KEYWORDS: Phenanthrene-based tylophorine derivatives; synthesis; antiviral activities; tobacco mosaic virus; inhibitors

INTRODUCTION

Plant viruses are unique in the deceptive simplicity of their structure. However, this simplicity leads to a greater dependence on the host, and a highly intricate relationship exists between the two which complicates the strategic designs to control plant viruses and the losses caused by them (*1*). The plant disease caused by tobacco mosaic virus (TMV) is found worldwide. 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 on 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 mosaic or leaf necrosis by harvesting time. So, plant virus has the name of "plant cancer" and is difficult to control.

Natural phenanthroindolizidine alkaloid tylophorine (**Figure 1**) and its analogues (e.g., antofine and deoxytylophorinine) have been isolated primarily from the genera *Cynanchum*, *Pergularia*, and *Tylophora* in the Asclepiadaceae family (*2*). These compounds, commonly called tylophora alkaloids, have been targets of synthesis and modification for their significant cytotoxic activities (*3*). Evaluation of tylophora alkaloids in the National Cancer Institute's antitumor screen showed a uniform and potent growth inhibitory effect ($GI_{50} \cong 10^{-8}$ M) against all 60 cell lines, with notable selectivity toward several refractory cell lines, including melanoma and lung tumor cell lines (*4*). Recently, polar phenanthrene-based tylophorine derivatives (PBTs) were synthesized and evaluated as potential antitumor agents (*5*−*7*). To date, most of the studies have been focused on anticancer activity in medicinal formulation. However, relatively little is known about the antiviral activity of tylophora alkaloids in pesticide formulation, and the antiviral activity of PBTs in pesticide formulation is virtually unknown.

In our preliminary work, (−)-antofine was isolated from the aerial parts of *Cynanchum komarovii* and was found to have good antiviral activity against TMV in vitro (*8*). However, the content of natural antofine is especially low. Antofine also has the drawbacks of being easily decomposed in light and poor water solubility. All of these limited its application in plant protection. To extend our research work on tylophora alkaloids as antiviral agents against TMV, we designed and synthesized three representative racemic alkaloids (tylophorine, antofine and deoxytylophorinine) (*9*), two optically pure alkaloids ((*S*)-(+)-tylophorine and (*R*)-(−)-tylophorine) and a series of tylophorine salt derivates for antiviral activity evaluation (*10*). Most of these compounds exhibited good to excellent antiviral activity against TMV, and tylophorine salt derivatives also had better stability and better water solubility in application than alkaloid itself. These findings encouraged us to further study the optimal structure of the natural product. In this article, we report our recent research results in the design and synthesis of C9 substituted PBTs. Several N-containing cyclic and acyclic moieties were introduced at the C9 position in order to explore and optimize the activity profiles of C9 substituted PBTs. In addition, we extended our in vivo antiviral evaluation against TMV.

MATERIALS AND METHODS

Synthetic Procedures. Reagents were purchased from commercial sources and were used as received. All anhydrous solvent were dried and

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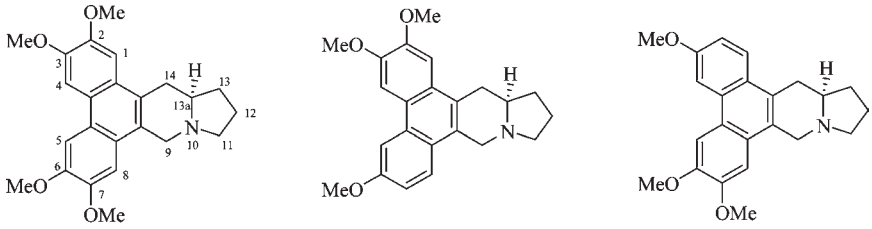


Figure 1. Chemical structures of tylophora alkaloids.

purified by standard techniques just befor use. Reaction progress was monitored by thin-layer chromatography on silica gel GF-254 with detection by UV. Melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and are uncorrected. ^{1}H NMR spectra were obtained at 400 MHz using a Bruker AC-P 400 Chemical shift values (δ) are given in ppm and were downfield from internal tetramethylsilane. High-resolution mass spectra (HRMS) were recorded on FT-ICR MS (Ionspec, 7.0 T).

General Procedure for the Synthesis of Methyl Esters of Amino Acid Hydrochlorides 2, 7, and 14. To a solution of amino acid (L-proline, piperidine-2-carboxylic acid, or 6-aminohexanoic acid) (4 mmol) in dry MeOH (4 mL) was added dropwise $SOCl_2$ (0.4 mL) at −30 °C. The reaction mixture was warmed to room temperature and heated to reflux for 1 h. Then the solvent was removed in vacuo, and the product was used in the next reaction without further purification.

General Procedure for the Synthesis of *N*-(2,3,6,7-Tetramethoxy-9-phenanthrylcarbonyl) Substituted Amino Acid Methyl Esters 3, 8, and 15. To acid **1** (1.71 g, 0.005 mol) was added dropwise freshly distilled oxalyl chloride (12.5 mL, 0.145 mol) 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 (30 mL) and added dropwise to a solution of the methyl esters of amino acid hydrochlorides (0.005 mol) and triethylamine (1.21 g, 0.012 mol) in CH_2Cl_2 (20 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, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/petroleum ether as eluant.

*Data for **3**:* yield, 89.6%; mp 203−204 °C (lit. (*12*) 205−208 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.8 (s 1H), 7.8 (s, 1H), 7.47 (s, 1H), 7.58 (s, 1H), 7.2 (s, 1H), 4.83−4.85 (m, 1H), 4.13 (s, 6H), 4.10 (s, 3H), 4.03 (s, 3H), 3.84 (s, 3H), 3.39−3.41 (m, 1H), 2.24−2.30 (m, 1H), 2.32−2.42 (m, 1H), 2.08−2.10 (m, 1H),1.86−1.98 (m, 2H); HRMS (ESI) *m*/*z* calcd for $C_{25}H_{28}NO_7$ (M + H), 454.1860; found, 454.1862.

*Data for **8**:* yield, 86.8%; mp 212−213 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.79 (s, 1H), 7.61 (s, 1H), 7.54 (s, 1H), 7.21 (s, 1H), 5.76−5.80 (m, 1H), 4.14 (s, 6H), 4.11 (s, 3H), 4.04 (s, 3H), 3.82 (s, 3H), 3.70−3.75 (m, 1H), 3.36−3.45 (m, 1H), 3.16−3.26 (m, 1H), 2.37−2.50 (m, 1H), 1.72−1.92 (m, 2H), 1.20−1.30 (m, 2H); HRMS (ESI) *m*/*z* calcd for $C_{26}H_{29}NO_7Na$ (M + Na), 490.1836; found, 490.1829.

*Data for **15**:* yield, 85.6%; mp 158−159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.79 (s, 1H), 7.76 (s, 1H), 7.71 (s, 1H), 7.22 (s, 1H), 6.15 (t, $^3J_{HH}$ = 5.6 Hz, 1H), 4.13 (s, 3H), 4.12 (s, 3H), 4.04 (s, 3H), 4.03 (s, 3H), 3.67 (s, 3H), 3.55−3.60 (m, 2H), 2.37 (t, $^3J_{HH}$ = 7.6 Hz, 2H), 1.68−1.79 (m, 4H), 1.45−1.55 (m, 2H); HRMS (ESI) *m*/*z* calcd for $C_{26}H_{32}NO_7$ (M + H), 470.2173; found, 470.2169.

General Procedure for the Synthesis of *N*-(2,3,6,7-Tetramethoxy-9-phenanthrylmethyl) Substituted Amino Acid Methyl Esters 4 and 9. A solution of 0.01 mol of compound **3** or **8** and triethyloxonium fluoroborate (0.011 mol) in 20 mL of dry CH_2Cl_2 was stirred for 20 h at room temperature. The solvent was removed in vacuum, and the residue was dissolved in 20 mL of ethanol. Sodium borohydride (0.95 g, 0.025 mol) was added in small portions to the stirred solution at 0 °C and then stirred at room temperature for 12 h. The solution was poured into 150 mL of water, and the solid that formed was filtered.

*Data for **4**:* yield, 91.8%; mp 165−167 °C (lit. (*12*) 160−163 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s 1H), 7.80 (s, 1H), 7.78 (s, 1H), 7.47 (s, 1H), 7.19 (s, 1H), 4.57 (d, $^2J_{HH}$ = 12.4 Hz, 1H), 4.12 (s, 9H), 4.02 (s, 3H), 3.69 (s, 3H), 3.60 (d, $^2J_{HH}$ = 12.4 Hz, 1H), 3.27 (t, $^3J_{HH}$ = 7.6 Hz, 1H), 2.80−2.90 (m, 1H), 2.29−2.39 (m, 1H), 2.12−2.25 (m, 1H), 1.90−2.05 (m, 1H), 1.68−1.87 (m, 2H); HRMS (ESI) *m*/*z* calcd for $C_{25}H_{30}NO_6$ (M + H) 440.2068; found, 440.2065.

*Data for **9**:* yield, 92.6%; mp 155−157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.79 (s, 1H), 7.78 (s, 1H), 7.44 (s, 1H), 7.18 (s, 1H), 4.34 (d, $^2J_{HH}$ = 12.4 Hz, 1H), 4.12 (s, 9H), 4.03 (s, 3H), 3.75 (s, 3H), 3.57-(d, $^2J_{HH}$ = 12.4 Hz, 1H), 3.10−3.19 (m, 1H), 2.81−2.94 (m, 1H), 2.06−2.17 (m, 1H), 1.73−1.93 (m, 2H), 1.56−1.69 (m, 1H), 1.32−1.52 (m, 3H); HRMS (ESI) *m*/*z* calcd for $C_{26}H_{32}NO_6$ (M + H), 454.2224; found, 454.2226.

General Procedure for the Synthesis of *N*-(2,3,6,7-Tetramethoxy-9-phenanthrylmethyl) Substituted Amino Acids 5 and 10. A solution of ester **4** or **9**, 4 M NaOH, and MeOH (1:1) was refluxed for 4 h. The solvents were concentrated, and 10 mL of water was added. The solution was cooled to 0 °C and acidified with concentrated hydrochloric acid (pH ≈1) to produce a white precipitate. The solid that separated was collected to give **5** or **10** as a white solid.

*Data for **5**:* yield, 96.8%; mp 201−203 °C (lit. (*12*) 203−205 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s 1H), 7.68 (s, 1H), 7.67 (s, 1H), 7.54 (s, 1H), 7.20 (s, 1H), 5.12 (d, $^2J_{HH}$ = 13.2 Hz, 1H), 4.65 (d, $^2J_{HH}$ = 13.2 Hz, 1H), 4.46−4.56 (m, 1H), 4.14 (s, 3H), 4.12 (s, 3H), 4.08 (s, 3H), 4.03 (s, 3H), 3.23−3.42 (m, 2H), 2.57−2.71 (m, 2H), 2.30−2.44 (m, 2H), 1.86−2.15 (m, 3H); HRMS (ESI) *m*/*z* calcd for $C_{24}H_{28}NO_6$ (M + H), 426.1911; found, 426.1911.

*Data for **10**:* yield, 96.5%; mp 186−188 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.23 (s, 1H), 7.96 (s, 1H), 7.95 (s, 1H), 7.51 (s, 1H), 7.35 (s, 1H), 5.11 (s, 2H), 4.37−4.42 (m, 1H), 4.02 (s, 6H), 3.96 (s, 3H), 3.90 (s, 3H), 3.02−3.08 (m, 1H), 2.73−4.77 (m, 1H), 2.09−2.04 (m, 1H), 1.82−1.88 (m, 1H), 1.30−1.82 (m, 4H); HRMS (ESI) *m*/*z* calcd for $C_{25}H_{30}NO_6$ (M + H), 440.2068; found, 440.2065.

General Procedure for the Synthesis of 6, 11, 12, and 16. To a suspension of **5** (**10**, **1**, or **15**) (1 mmol) in 15 mL of dry THF was added $LiAlH_4$ (1 g) in portions at 0 °C. After addition, the reaction mixture was refluxed for 2 h and then cooled to 0 °C. The reaction mixture was quenched with water carefully and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated to give the target compounds.

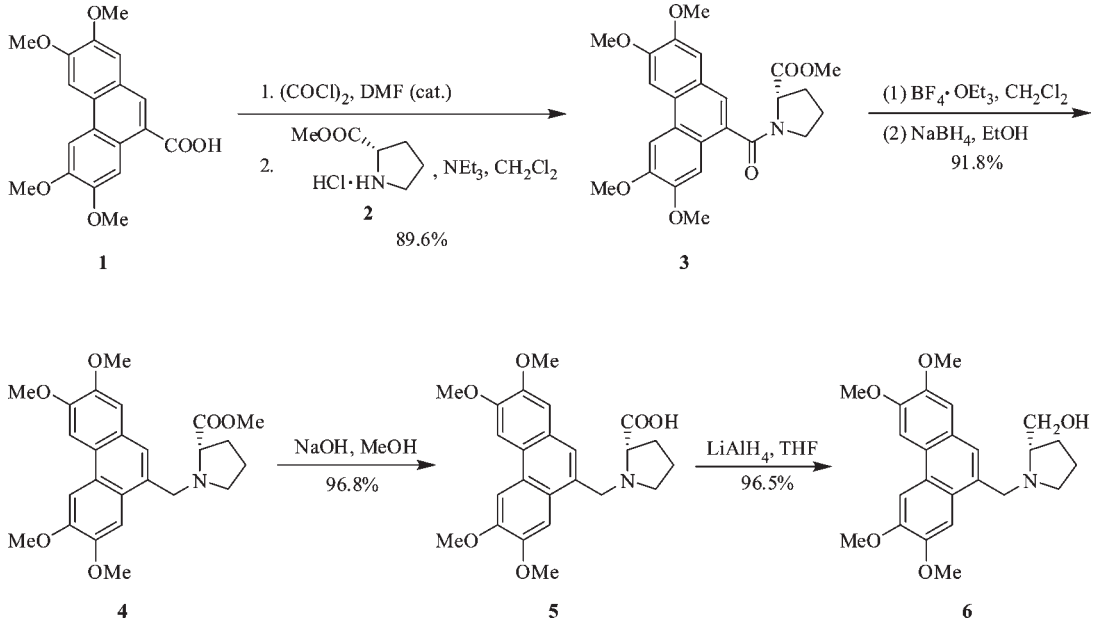
*Data for **6**:* yield, 96.5%; mp 221−222 °C (lit. (*13*) 223−225 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.78 (s, 1H), 7.60 (s, 1H), 7.54 (s, 1H), 7.20 (s, 1H), 4.50 (d, 1H), 4.12 (s, 6H), 4.09 (s, 3H), 4.04 (s, 3H),-3.45−3.58 (m, 1H), 2.85−3.05 (m, 1H), 2.37−2.57 (m, 1H), 1.59−2.08 (m, 1H); HRMS (ESI) *m*/*z* calcd for $C_{24}H_{30}NO_5$ (M + H), 412.2118; found, 412.2122.

*Data for **11**:* yield, 92.5%; mp 219−221 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.78 (s, 1H), 7.73 (s, 1H), 7.52 (s, 1H), 7.20 (s, 1H), 4.50 (d, $^2J_{HH}$ = 12.8 Hz, 1H), 4.12 (s, 6H), 4.05 (s, 3H), 4.04 (s, 3H), 3.60−3.90 (m, 3H), 2.75−2.90 (m, 1H), 2.59−2.73 (m, 1H), 2.24−2.40 (m, 1H), 1.77−1.90 (m, 1H), 1.28−1.75 (m, 6H); HRMS (ESI) *m*/*z* calcd for $C_{25}H_{32}NO_5$ (M + H), 426.2275; found, 426.2276.

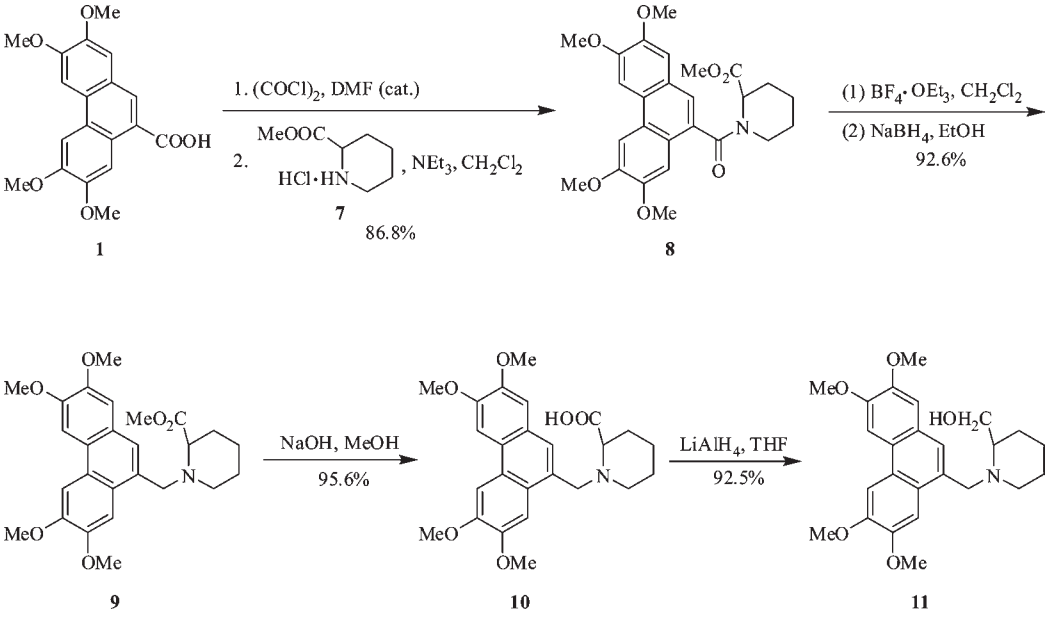
*Data for **12**:* yield, 96.5%; mp 181−182 °C (lit. (*13*) 185 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.75 (s, 1H), 7.56 (s, 1H), 7.54 (s, 1H), 7.18 (s, 1H), 5.11 (s, 2H), 4.13 (s, 3H), 4.12 (s, 3H), 4.06 (s, 3H), 4.02 (s, 3H).

*Data for **16**:* yield, 87.3%; mp 128−130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.79 (s, 1H), 7.76 (s, 1H), 7.70 (s, 1H), 7.21 (s, 1H),

Scheme 1. Synthesis of PBTs Starting from L-Proline



Scheme 2. Synthesis of PBTs Starting from Piperidine-2-carboxylic Acid



6.13 (br, 1H), 4.13 (s, 6H), 4.03 (s, 6H), 3.68 (t, $^2J_{HH}$ = 6.0 Hz, 2H), 3.54−3.62 (m, 2H), 1.67−1.77 (m, 2H), 1.55−1.66 (m, 2H),1.42−1.60 (m, 4H); HRMS (ESI) *m*/*z* calcd for $C_{25}H_{32}NO_6$ (M + H), 441.2146; found, 441.2143.

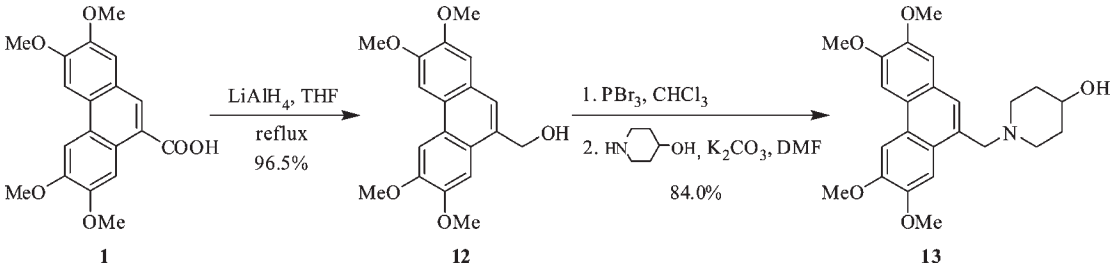
Synthesis of *N*-(2,3,6,7-Tetramethoxy-9-phenanthrylmethyl)-piperidin-4-ol (13). Compound **12** (1.64 g, 5 mmol) was dissolved in 100 mL of $CHCl_3$ and cooled to 0 °C. A solution of PBr_3 (0.71 mL, 7.5 mmol) in 20 mL of $CHCl_3$ was added dropwise under nitrogen. The solution was then stirred at room temperature for 4 h, then poured over ice, and the two layers were separated. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford a white solid. The solid was then redissolved in 120 mL of DMF. 4-Hydroxypiperidine (0.71 g, 7.0 mmol) was added and stirred for 20 min. K_2CO_3 (1.0 g, 7.2 mmol) was added, and the mixture was stirred at room temperature overnight. The solution was then rotary evaporated, and the product was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated to obtain a crude product. The crude product was purified by flash column chromatography to give 1.73 g (84.0%) of **13** as a white solid: mp 201−203 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s 1H), 7.81(s, 1H), 7.79 (s, 1H), 7.47(s, 1H), 7.20 (s, 1H), 4.13 (s, 6H), 4.05 (s, 3 H), 4.04 (s, 3 H), 3.88 (s, 2H), 3.69−3.78 (m, 1H), 2.80−2.90 (m, 2H), 2.25 (t, $^3J_{HH}$ = 10.0 Hz, 2H), 1.84−1.94 (m, 2H), 1.50−1.63 (m, 4H); HRMS (ESI) *m*/*z* calcd for $C_{24}H_{30}NO_5$ (M + H), 412.2118; found, 412.2122.

Antiviral Biological Assay. *Purification of Tobacco Mosaic Virus.* Using Gooding's method (*14*), the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000*g*, treated with PEG twice, and centrifuged again. The whole experiment was processed at 4 °C. Absorbance value was estimated at 260 nm by ultraviolet spectrophotometer.

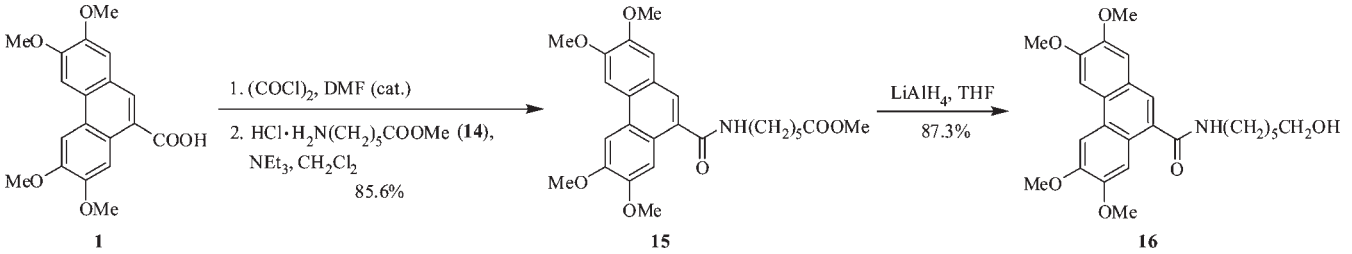
$$\text{virus concn} = (A_{260} \times \text{dilution ratio})/E_{1\text{cm}}^{0.1\%,\,260\text{nm}}$$

Protective Effect of Compounds against TMV in Vivo. The compound solution was smeared on the left side while the solvent served as the control on the right side of growing *N. tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in tobacco mosaic virus of 6×10^{-3} mg/mL to inoculate the leaves, which

Scheme 3. Synthesis of PBTs Starting from 4-Hydroxypiperidine



Scheme 4. Synthesis of PBTs Starting from 6-Aminohexanoic Acid



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 (*15*). 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., while the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3−4 days after inoculation (*15*). 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. The tobacco mosaic virus (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 (*15*). There are three replicates for each compound. The in vivo inhibition rate of the compound was then calculated according to the following formula (av means average, and controls were not treated with compound).

$$\text{inhibition rate (\%)} = [(\text{av local lesion no. of control} - \text{av local lesion no. of drug-treated})/\text{av local lesion no. of control}] \times 100\%$$

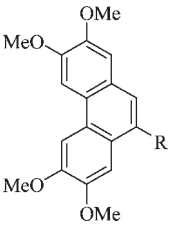
RESULTS AND DISCUSSION

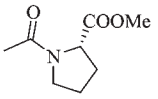
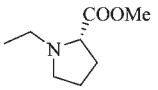
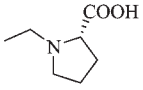
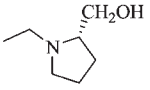
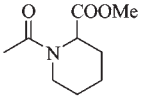
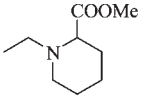
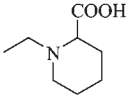
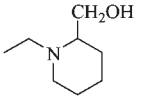
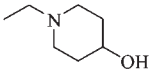
Chemistry. The PBTs can be efficiently synthesized by different methods as shown in **Schemes 1**, **2**, **3**, and **4**. Condensation of the 9-phenanthrenecarbonyl chloride (prepared by chlorination of 9-phenanthrenecarboxylic acid **1** (*11*, *16*) with oxalyl chloride) with amino acid methyl ester hydrochlorides (**2**, **7**, **14**) in the presence of triethylamine gave the amidoesters (**3**, **8**, **15**) in good yields (**Schemes 1**, **2**, and **4**). The Borch reduction of **3** and **8**, consisting of an *O*-alkylation of the tertiary amide with triethyloxonium fluoroborate and later sodium borohydride reduction, led to the aminoesters **4** and **9** in high yields. Hydrolysis of **4** and **9** to the corresponding sodium salts with sodium hydroxide and acidification with hydrochloric acid gave amino acids **5** and **10** in almost quantitative yields (**Schemes 1** and **2**). The reduction of **5**, **10**, **1**, and **15** with lithium aluminum hydride led to the alcohols **6**, **11**, **12**, and **16** in excellent yields. Lithium aluminum hydride reduction of carboxylic acid **1** gave alcohol **12** in 96.5% yield. Alcohol **12** was brominated by using PBr_3 to obtain bromide, which was used without further purification. Nucleophilic substitution reaction of bromide with 4-hydroxypiperidine gave **13** in 84.0% yield (**Scheme 3**).

Antiviral Activity in Vivo. To make a judgment of the antiviral potency of the C9-substituted phenanthrene-based tylophorine derivatives (PBTs), the commercially available plant virucide Ningnanmycin (*17*), perhaps the most successful registered antiplant viral agent, and tylophorine alkaloid were used as the controls. The antiviral bioassay against TMV in vivo is assayed by the reported method (*14*, *15*), and the antiviral results of all the PBTs against TMV are listed in **Table 1**. Compounds **3**, **8**, **9**, **13**, and **15**, containing five-membered ring, six-membered ring, and straight-chain structures, exhibited higher protection activity (56.7%, 56.3%, 64.6%, 70.8%, and 54.6%, respectively) at 500 μg/mL than tylophorine and Ningnanmycin at 500 μg/mL (53.3% and 48.7%, respectively). Compound **4**, containing a five-membered ring structure, showed the same protection activity level (38.3%) as tylophorine at 100 μg/mL (37.9%). Compounds **4**, **6**, **10**, and **13**, containing five-membered ring and six-membered ring structures, showed higher inactivation activity (80.0%, 74.2%, 68.9%, and 71.1%, respectively) at 500 μg/mL than tylophorine and Ningnanmycin at 500 μg/mL (62.6% and 68.4%, respectively). Compound **16**, containing a straight-chain structure, showed the same inactivation activity level (60.9%) as tylophorine and Ningnanmycin at 500 μg/mL. In addition, compounds **4** and **9** showed higher inactivation activities (54.7% and 50.2%, respectively) than Ningnanmycin (48.0%) at 100 μg/mL. Compound **16** also showed higher inactivation activity (57.8%) than tylophorine and Ningnanmycin (56.4% and 48.0%, respectively) at 100 μg/mL. For curative effect, compounds **3**, **5**, **15**, and **16**, containing five-membered ring and straight-chain structures, exhibited higher curative activity (64.6%, 54.1%, 65.9%, and 65.0%, respectively) at 500 μg/mL than Ningnanmycin at 500 μg/mL (47.9%). In addition, compounds **3**, **15**, and **16**, containing simple five-membered ring structure and straight-chain structures, exhibited the same curative activity (64.6%, 65.9%, and 65.0%, respectively) as tylophorine (65.9%) at 500 μg/mL. At 100 μg/mL, compounds **15** and **16** also exhibited higher curative activity (48.0% and 49.6%, respectively) than Ningnanmycin (39.0%).

In summary, a series of C9-substituted phenanthrene-based tylophorine derivatives (PBTs) have been synthesized efficiently

Table 1. In Vivo Antiviral Activities against TMV



compd	R	conc. (μg/mL)	Protection effect (%)	Inactivation effect (%)	Curative effect (%)
3		500	56.7	8.4	64.6
		100	19.2	8.0	10.6
4		500	47.1	80.0	33.7
		100	38.3	54.7	2.9
5		500	35.4	39.6	54.1
		100	23.3	21.3	14.2
6		500	19.2	74.2	34.6
		100	10.4	36.9	13.4
8		500	56.3	17.3	17.9
		100	12.5	4.4	4.9
9		500	64.6	59.1	11.8
		100	27.1	50.2	1.2
10		500	12.9	68.9	45.1
		100	10.0	38.7	28.9
11		500	21.3	8.4	46.8
		100	4.6	5.2	36.2
13		500	70.8	71.1	37.4
		100	11.3	9.3	33.7
15	—CONH$(CH_2)_5$COOMe	500	54.6	6.7	65.9
		100	14.2	5.8	48.0
16	—CONH$(CH_2)_5CH_2$OH	500	41.7	60.9	65.0
		100	22.5	57.8	49.6
Tylophorine		500	53.3	62.6	65.9
		100	37.9	56.4	57.7
Ningnanmycin		500	48.7	68.4	47.9
		100	47.5	48.0	39.0

in excellent yield compared with tylophorine alkaloid itself. The in vivo antiviral bioassay showed that most of the PBTs exhibited good to excellent inhibitory activity against TMV compared to commercial Ningnanmycin. Especially, compounds **3**, **4**, **8**, **9**, **13**, and **15** exhibited excellent protection activities. Compounds **4**, **6**, **9**, **10**, **13**, and **16** exhibited excellent inactivation activities. Compounds **3**, **5**, **15**, and **16** exhibited excellent curative activities. So, compounds **3**, **4**, **9**, **13**, and **16**, containing five-membered ring, six-membered ring, and straight-chain structures, respectively, emerged as potential inhibitors of plant virus. Therefore, the present work demonstrates that the antiviral activity against TMV was maintained via the simplification of tylophorine alkaloid

structure. Thus, the findings demonstrate that the synthesized PBTs represent a new template for antiviral studies.

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