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# Synthesis and Antiviral Activities of Phenanthroindolizidine Alkaloids and Their Derivatives†

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Racemic phenanthroindolizidine alkaloids tylophorine, antofine, and deoxytylophorinine, and optically pure alkaloids *S*-(+)-tylophorine and *R*-(−)-tylophorine were synthesized and evaluated for their antiviral activities against tobacco mosaic virus (TMV). Further salinization modifications based on tylophorine increased stability and water solubility and improved the antiviral activity in application. The bioassay results showed that most of these synthesized compounds showed higher antiviral activity against TMV in vitro and in vivo than commercial Ningnanmycin. Especially, tylophorine salt derivatives **10**, **11**, **13**, **17**, and **22** emerged as potential inhibitors of plant virus. These findings demonstrate that these phenanthroindolizidine alkaloids and their salt derivatives represent a new template for antiviral studies and could be considered for novel therapy against plant virus infection.

**KEYWORDS: Phenanthroindolizidine alkaloid; racemic alkaloids; optically pure alkaloids; salt derivatives; 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; 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 mosic or leaf necrosis by harvesting time. Therefore, this plant virus has the name "plant cancer" and is difficult to control.

Ningnanmycin, a commercial antiviral agent, isolated from *Strepcomces noursei* var. *xichangensisn* by the Chengdu Institute of Biology, Chinese Academy of Sciences, is a kind of microbial pesticide. It is more effective in the treatment of plants against TMV than other existing commercial agents. However, the use of this agent for field trial is largely limited by its photosensitivity and water stickiness (*2*). Therefore, further research needs to be conducted in this area for the development of a highly efficient, novel, environmentally benign antiviral inhibitor.

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 (*3*). These compounds, commonly called tylophora alkaloids, have been targets of synthesis and modification for their significant cytotoxic activities (*4*). 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 (*5*). 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.

In our preliminary work, (−)-antofine was isolated from the aerial parts of *Cynanchum komarovii* and was first found to have good antiviral activity against TMV in vitro (*6*). However, the content of natural antofine is especially low. Antofine also has the drawbacks of being easily decomposed in light and having poor water solubility. All of these limited its application in plant protection. To extend our research work to tylophora alkaloids as a new class of antiviral agents against TMV, we designed and synthesized three representative racemic alkaloids (tylophorine, antofine, and deoxytylophorinine), two optically pure alkaloids (*S*-(+)-tylophorine and *R*-(−)-tylophorine), and a series of tylophorine salt derivates for antiviral activity evaluation.

## 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 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

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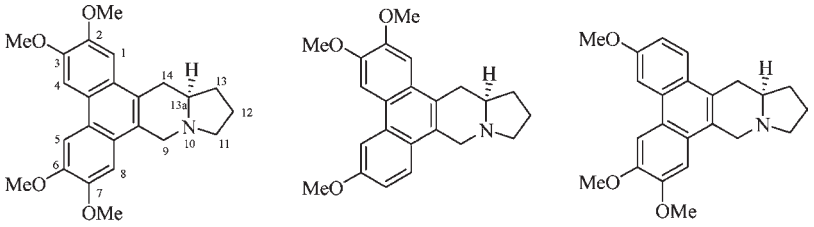



**Figure 1.** Chemical structures of tylophora alkaloids.

microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and are uncorrected. $^1$H NMR spectra were obtained at 300 MHz using a Bruker AC-P 300 and a Varain Mercury Plus 400 MHz spectrometer. Chemical shift values ($\delta$) are given in parts per million and were downfield from internal tetramethylsilane. High-resolution mass spectra (HRMS) were recorded on FT-ICR MS (Ionspec, 7.0T).

**Synthesis of 2,3-Bis(3,4-dimethoxyphenyl)acrylic Acid (3).** 3,4-Dimethoxybenzaldehyde (**1**) (30.0 g, 0.18 mol), 3,4-dimethoxyphenylacetic acid (**2**) (32.7 g, 0.17 mol), acetic anhydride (70 mL), and triethylamine (35 mL) were heated to reflux for 20 h with the exclusion of moisture. The solution was allowed to cool to room temperature, water (150 mL) was added, and the mixture was stirred for 1 h. The mixture was then poured into aqueous potassium carbonate (120.0 g in 300 mL water) and refluxed until nearly all of the gummy material had dissolved. The solution so obtained was cooled, extracted with ether (3 × 50 mL), and carefully acidified with concentrated hydrochloric acid (pH ≈1) to produce a white precipitate. The solid that separated was collected and washed from methanol (150 mL) to give **3** (47.0 g, 82.0%) as a mixture of *E*/*Z* isomers.

**Synthesis of 2,3,6,7-Tetramethoxyphenanthrene-9-carboxylic Acid (4).** To a stirred solution of the mixture of *E*-isomer **3** and *Z*-isomer **3** (24.1 g, 0.07 mol) in $CH_2Cl_2$ (500 mL) was added anhydrous $FeCl_3$ (1.71 g, 0.01 mol) under nitrogen at 0−5 °C, and a solution of *m*-CPBA (12.1 g, 0.07 mol) in $CH_2Cl_2$ (100 mL) was added to the mixture, warmed to room temperature for 6 h, and quenched with methanol (100 mL). The solvents were concentrated in vacuo, and the residue was washed with methanol (200 mL), filtered, and washed with methanol (3 × 30 mL) again to give acid **4** (21.6 g, 90.0%) as a light yellow solid: mp 285−287 °C (lit. (*7*) 285−287 °C); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.58 (s, 1H), 8.43(s, 1H), 8.03 (s, 1H), 7.99 (s, 1H), 7.54 (s, 1H), 4.08 (s, 3H), 4.07 (s, 3H), 3.94 (s, 3H), (s, 3H).

**Synthesis of 2,3,6,7-Tetramethoxy-9-(hydroxymethyl)phenanthrene (5).** To a mixture of $LiAlH_4$ (1.14 g, 0.03 mol) in 150 mL of THF at 0 °C was added **4** (6.84 g, 0.02 mol) in portions. The solution was refluxed for an additional 2 h, then brought back to 0 °C, at which point 10 mL of EtOAc was added dropwise, followed by 10 mL of 2 N HCl. The solution was stirred and filtered, and the solvent was removed by rotary evaporation to give 6.56 g (96.5%) of **5** as a white solid: mp 181−182 °C (lit. (*8*) 185 °C); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 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).

**Synthesis of (*S*)-(+)-*N*-[(2,3,6,7-Tetramethoxy-9-phenanthryl)-methyl]pyroglutamic Acid Methyl Ester (6).** Compound **5** (3.28 g, 10 mmol) was dissolved in 200 mL of $CHCl_3$ and cooled to 0 °C. A solution of $PBr_3$ (1.42 mL, 15 mmol) in 40 mL of $CHCl_3$ was added dropwise under nitrogen. 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 $Na_2SO_4$, filtered, and concentrated in vacuo to afford a white solid. The solid was then redissolved in 280 mL of DMF. L-Glutamic acid dimethyl ester hydrochloride (BMPAC, 3.0 g, 14.2 mmol) was added and allowed to stir for 20 min. $K_2CO_3$ (2.0 g, 14.4 mmol) was added, and the mixture was allowed to stir 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 dissolved in 50 mL of MeOH and 20 mL of AcOH 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 2.5 g (55.0%) of **6** as a white solid: mp 236−238 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.80 (s, 1H), 7.77 (s, 1H), 7.62 (s, 1H), 7.41 (s, 1H), 7.17 (s, 1H), 5.50 (d, $^2J_{HH}$ = 14.4 Hz, 1H), 4.40 (d, $^2J_{HH}$ = 14.4 Hz, 1H), 4.12 (s, 6H), 4.03 (s, 6H), 3.84−3.87 (m, 1H), 3.58 (s, 3H), 2.53−2.66 (m, 1H), 2.33−2.46 (m, 1H), 1.92−2.19 (m, 2H). HRMS (ESI) *m*/*z* calcd for $C_{25}H_{27}NO_7Na$ (M + Na)$^+$, 476.1680; found, 476.1680.

**Synthesis of (*S*)-(+)-*N*-[(2,3,6,7-Tetramethoxy-9-phenanthryl)-methyl]pyroglutamic Acid (7).** Compound **6** (1.81 g, 4.0 mmol) was stirred in a solution of 50 mL of dioxane, 40 mL of MeOH, and 30 mL of 2 N KOH for 3 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 **7** (1.73 g, 98.5%) as a white solid: mp > 300 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.01 (s, 1H), 7.97 (s, 1H), 7.47 (s, 1H), 7.44 (s, 1H), 7.36 (s, 1H), 5.40 (d, $^2J_{HH}$ = 14.8 Hz, 1H), 4.18 (d, $^2J_{HH}$ = 14.4 Hz, 1H), 4.01 (s, 6H), 3.89 (s, 3H), 3.85 (s, 3H), 3.65−3.70 (m, 1H), 2.27−2.45 (m, 2H), 2.06−2.20 (m, 1H), 1.81−1.94 (m, 1H).

**Synthesis of (*S*)-(+)-2,3,6,7-Tetramethoxyphenanthro[9,10-*b*]-11,14-indolizidinedione (8).** To a solution of **7** (1.1 g, 2.5 mmol) in 80 mL of $CH_2Cl_2$ was added freshly distilled oxalyl chloride (0.25 mL, 2.8 mmol) and 2 drops of DMF. The mixture was stirred for 2 h at room temperature and then warmed to reflux. $SnCl_4$ (1.25 mL, 5.2 mmol) in 20 mL of $CH_2Cl_2$ was added, and the mixture was refluxed for an additional 6 h. The solution was cooled to room temperature, and 15 mL of cold 2 N HCl was added slowly. The phases were separated, and the organic phase was dried over $Na_2SO_4$ and filtered. The solvent was removed by rotary evaporation, and the crude product was purified by flash column chromatography to give 0.99 g (94.0%) of **8** as a yellow solid: mp 225−227 °C; (lit. (*9*) 228−230 °C); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.10 (s, 1H), 7.78 (s, 1H), 7.76 (s, 1H), 7.28 (s, 1H), 5.72 (d, $^2J_{HH}$ = 18.0 Hz, 1H), 4.69 (d, $^2J_{HH}$ = 17.6 Hz, 1H), 4.38−4.49 (m, 1H), 2.92 (d, $^2J_{HH}$ = 29.2 Hz, 1H), 2.48−2.71 (m, 3H).

**Synthesis of (*S*)-(+)-2,3,6,7-Tetramethoxyphenanthro[9,10-*b*]-11-indolizidinone (9).** To a solution of **8** (0.84 g, 2.0 mmol) in ethanol (100 mL) was added sodium borohydride(0.15 g, 4.0 mmol) in three portions at 0 °C. The mixture was warmed to room temperature in a period of 6 h. Ice−water (10 mL) was added, followed by a saturated aqueous solution of ammounium chloride (15 mL). The mixture was extracted with dichloromethane. The extracts were dried with $MgSO_4$, filtered, and concentrated to obtain a white solid, which was used without further purification. To a solution of the solid in $CH_2Cl_2$ (20 mL) was added trifluoroacetic acid (10 mL) and triethylsilane (2 mL). The mixture was refluxed for 0.5 h, diluted with 15 mL of distilled water, and extracted with $CH_2Cl_2$ (50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by flash column chromatography on silica gel gave **9** (0.76 g, 94%) as a light yellow solid: mp 236−238 °C; (lit. (*9*) mp 238−240 °C); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83 (s, 1H), 7.82 (s, 1H), 7.27 (s, 1H), 7.15 (s, 1H), 5.31 (d, $^2J_{HH}$ = 17.4 Hz, 1H), 4.55 (d, $^2J_{HH}$ = 17.1 Hz, 1H), 4.12 (s, 6H), 4.06 (s, 3H), 4.04 (s, 3H), 3.87−4.00 (m, 1H), 3.43−3.50 (m, 1H), 2.77−2.91 (m, 1H), 2.48−2.64 (m, 3H), 1.96−2.09 (m, 1H).

**Synthesis of (*S*)-(+)-Tylophorine.** To a mixture of $LiAlH_4$ (0.08 g, 2.0 mmol) in 35 mL of THF at 0 °C was added dropwise a solution of **9** (0.41 g, 1.0 mmol) in 15 mL of THF. The mixture was refluxed for 6 h and then brought back to 0 °C, at which point EtOAc (10 mL) was added dropwise, followed by $H_2O$ (5 mL). The solution was filtered and dried over $MgSO_4$, and the solvent was removed by rotary evaporation to give 0.36 g (92.0%) of (*S*)-(+)-tylophorine with 92% enantiomeric excess (ee)

value: mp 282 °C dec; (lit. (*9*) mp 282−284 °C dec); $[\alpha]^{20}_{D}$ +100° ($CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.84 (s, 1H), 7.83 (s, 1H), 7.32 (s, 1H), 7.17 (s, 1H), 4.64 (d, $^2J_{HH}$ = 14.4 Hz, 1H), 4.12 (s, 6H), 4.06 (s, 6H), 3.68 (d, $^2J_{HH}$ = 14.4 Hz, 1H), 3.47−3.51 (m, 1H), 3.33−3.40 (m, 1H), 2.87−2.95 (m, 1H), 2.42−2.54 (m, 2H), 2.20−2.30 (m, 1H), 1.73−2.10 (m, 3H).

**Synthesis of (*R*)-(−)-Tylophorine.** Following the same procedure as for (*S*)-(+)-tylophorine gave optically pure alkaloid (*R*)-(−)-tylophorine with 96% ee value, $[\alpha]^{20}_{D}$ −93° ($CHCl_3$). The optical purity of the synthesized alkaloids was determined via HPLC using an Agilent 1100 instrument with an AD-H column and gave 92 and 96% ee values, respectively. Detection was conducted at 254 nm. *n*-Hexane/isopropanol/triethylamine (75:25:0.025) was used as mobile phase at a flow rate of 1.0 mL/min.

**General Procedure for the Preparation of Tylophorine Salt Derivatives 10−25.** To a solution of 1.0 mmol of inorganic acid or organic acid in 50 mL of $CHCl_3$ and 50 mL of $CH_3OH$ was added 1.0 mmol of racemic tylophorine in 50 mL of $CHCl_3$ slowly under nitrogen at 40−50 °C. The mixture was refluxed for 2 h and allowed to stand for 2 h; the solvents were removed partially by rotary evaporation and filtered to obtain the corresponding salt derivatives **10**−**25**.

*Data for* ***10****:* mp 258 °C dec; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.04 (s, 1H), 8.03 (s, 1H), 7.33 (s, 1H), 7.17 (s, 1H), 5.00−5.15 (m, 1H), 4.33−4.50 (m, 1H), 4.03 (s, 6H), 3.94 (s, 6H), 3.40−3.83 (m, 3H), 3.15−3.30 (m, 2H), 2.30−2.45 (m, 1H), 1.86−2.18 (m, 3H).

*Data for* ***11****:* mp 260 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.07 (s, 1H), 8.06 (s, 1H), 7.37 (s, 1H), 7.21 (s, 1H), 5.19−5.33 (m, 1H), 4.54−4.72 (m, 1H), 4.05 (s, 6H), 3.97(s, 6H), 3.64−3.91 (m, 3H), 3.15−3.48 (m, 2H), 2.53−2.64 (m, 1H), 1.86−2.31 (m, 3H).

*Data for* ***12****:* mp 240 °C dec; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.06 (s, 1H), 8.05 (s, 1H), 7.36 (s, 1H), 7.19 (s, 1H), 5.22−5.27 (m, 1H), 4.59−4.66 (m, 1H), 4.04 (s, 6H), 3.96 (s, 6H), 3.62−3.91 (m, 4H), 3.13−3.25 (m, 1H), 2.02−2.32 (m, 3H), 1.85−1.97 (m, 1H).

*Data for* ***13****:* mp 246 °C dec; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.02 (s, 1H), 8.00 (s, 1H), 7.82 (s, 2H), 7.45−7.51 (m, 1H), 7.30−7.40 (m, 3H), 7.14 (m, 1H), 4.86−4.91 (m, 1H), 4.12 (s, 3H), 4.11 (s, 3H), 4.06 (s, 3H), 4.04 (s, 3H), 3.97−4.00 (m, 1H), 3.65−3.76 (m, 1H), 3.38−3.48 (m, 1H), 3.17−3.27 (m, 1H), 3.00−3.12 (m, 1H), 2.77−2.90 (m, 1H), 2.31−2.42 (m, 1H), 1.90−2.20 (m, 3.H).

*Data for* ***14****:* mp 250 °C dec; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.80−7.85 (m, 2H), 7.21−7.26 (m, 1H), 7.02 (d, 1H), 5.37−5.57 (m, 1H), 4.78−4.93 (m, 1H), 4.44−4.60 (m, 1H), 4.04−4.15 (m, 12H), 3.55−3.65 (m, 1H), 3.32−3.44 (m, 1H), 3.08−3.20 (m, 2H), 2.66−2.69 (m, 1H), 2.48−2.56 (m, 1H), 2.37−2.46 (m, 1H), 2.13−2.30 (m, 2H), 1.89−1.91 (m, 1H), 1.64−1.80 (m, 4H), 1.46−1.56 (m, 1H), 1.02−1.11 (m, 1H), 0.88 (s, 3H), 0.62 (s, 3H).

*Data for* ***15****:* mp 225 °C dec; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.15 (br, 1H), 8.56 (s, 2H), 8.06 (s, 1H), 8.05 (s, 1H), 7.36 (s, 1H), 7.18 (s, 1H), 5.21−5.25 (m, 1H), 4.58−4.64 (m, 1H), 4.03 (s, 6H), 3.95 (s, 6H), 3.85−3.91 (s, 1H), 3.63−3.78 (m, 3H), 3.32−3.45 (m, 1H), 3.12−3.26 (m, 1H), 2.05−2.25 (m, 2H), 1.84−1.98 (m, 1H).

*Data for* ***16****:* mp 260 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.00 (s, 2H), 7.47 (d, $^3J_{HH}$ = 15.9 Hz, 1H), 7.19−7.32 (m, 4H), 7.05−7.09 (m, 1H), 6.79 (d, $^3J_{HH}$ = 8.1 Hz, 1H), 6.35 (d, $^3J_{HH}$ = 15.9 Hz, 1H), 4.56 (d, $^2J_{HH}$ = 15.6 Hz, 1H), 4.02 (s, 6H), 3.93 (s, 6H), 3.81 (s, 3H), 3.53 (d, $^2J_{HH}$ = 15.6 Hz, 1H), 3.32−3.37 (m, 2H), 2.71−2.89 (m, 1H), 2.30−2.40 (m, 2H), 2.10−2.24 (m, 1H), 1.58−1.95 (m, 3H).

*Data for* ***17****:* mp 260 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.03 (s, 2H), 7.35 (s, 1H), 7.20 (s, 1H), 4.75−4.81 (m, 1H), 4.20 (s, 4H), 4.07−4.14 (m, 1H), 4.03 (s, 6H), 3.94 (s, 6H), 3.48−3.54 (m, 2H), 2.67−3.00 (m, 3H), 2.21−2.35 (m, 1H), 1.67−2.03 (m, 3H).

*Data for* ***18****:* mp 241 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.06 (s, 2H), 7.66 (d, $^3J_{HH}$ = 7.5 Hz, 1H), 7.37 (s, 1H), 7.22 (s, 2H), 6.64−6.71 (m, 2H), 4.98−5.16 (m, 1H), 4.28−4.52 (m, 1H), 4.04 (s, 6H), 3.96 (s, 6H), 3.60−3.84 (m, 2H), 3.33−3.50 (m, 1H), 3.06−3.26 (m, 2H), 2.33−2.47 (m, 1H), 1.82−2.18 (m, 3H).

*Data for* ***19****:* mp 235 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.0 (br, 2H), 8.32 (s, 1H), 8.04 (s, 1H), 8.05 (s, 1H), 7.35 (s, 1H), 7.20 (s, 1H), 4.93 (d, $^2J_{HH}$ = 15.0 Hz, 1H), 4.12−4.29 (m, 1H), 4.04 (s, 6H), 3.95 (s, 6H), 3.50−3.70 (m, 2H), 2.90−3.27 (m, 3H), 2.58−2.67 (m, 4H), 2.27−2.44 (m, 1H), 1.73−2.13 (m, 3H).

*Data for* ***20****:* mp 240 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.02 (s, 2H), 7.34 (s, 1H), 7.20 (s, 1H), 4.73 (d, $^2J_{HH}$ = 15.3 Hz, 1H), 4.07−4.15 (m, 1H), 4.03 (s, 6H), 3.94 (s, 6H), 3.42−3.52 (m, 2H), 2.53−2.95 (m, 5H), 2.37−2.44 (m, 1H), 2.21−2.34 (m, 1H), 1.88−2.05 (m, 2H), 1.66−1.82 (m, 1H).

*Data for* ***21****:* mp 268 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.01 (s, 2H), 7.32 (s, 1H), 7.19 (s, 1H), 4.59 (d, $^2J_{HH}$ = 15.0 Hz, 1H), 4.02 (s, 6H), 3.93 (s, 6H), 3.61 (d, $^2J_{HH}$ = 15.3 Hz, 1H), 3.34−3.40 (m, 2H), 2.76−2.85 (m, 1H), 2.41 (s, 6H), 2.12−2.28 (m, 1H), 1.59−1.97 (m, 3H).

*Data for* ***22****:* mp 230 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.28 (s, 1H), 8.01 (s, 1H), 7.80 (s, 1H), 7.77 (s, 1H), 7.33 (s, 1H), 7.19 (s, 1H), 6.83 (s, 1H), 6.80 (s, 1H), 4.56 (d, $^2J_{HH}$ = 15.3 Hz, 1H), 4.02 (s, 6H), 3.93 (s, 6H), 5.50 (d, $^2J_{HH}$ = 14.7 Hz, 1H), 3.32−3.41 (m, 2H), 2.70−2.85 (m, 1H), 2.33−2.45 (m, 2H), 2.10−2.25 (m, 1H), 1.57−2.00 (m, 3H).

. *Data for* ***23****:* mp 230 °C dec; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.81 (s, 2H), 7.28 (s, 1H), 7.11 (s, 1H), 4.71 (d, $^2J_{HH}$ = 14.8 Hz, 1H), 4.16−4.22 (m, 1H), 4.12 (s, 6H), 4.05 (s, 6H), 3.50−4.00 (m, 3H), 3.45−3.50 (m, 1H), 3.36−3.40 (m, 1H), 3.00−3.07 (m, 1H), 2.50−2.80 (m, 2H), 2.24−2.36 (m, 1H), 1.80−2.16 (m, 3H), 1.18−1.26 (m, 1H).

*Data for* ***24****:* mp 241 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.07 (s, 2H), 7.20−7.37 (m, 4H), 6.39−6.42 (m, 2H), 5.00−5.37 (m, 2H), 4.44−4.70 (m, 1H), 4.05 (s, 6H), 3.97 (s, 6H), 3.50−3.80 (m, 3H), 3.08−3.23 (m, 1H), 1.80−2.30 (m, 4H).

*Data for* ***25****:* mp 204−206 °C dec; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.00−10.30 (br, 1H), 8.03−8.08 (m, 3H), 7.65−7.68 (m, 1H), 7.37 (s, 1H), 7.20 (s, 1H), 6.84 (d, $^3J_{HH}$ = 8.4 Hz, 1H), 5.24 (d, $^3J_{HH}$ = 15.6 Hz, 1H), 4.55−4.72 (m, 1H), 4.05 (s, 6H), 3.97 (s, 6H), 3.60−3.90 (m, 3H), 3.10−3.50 (m, 3H), 1.83−2.32 (m, 3H).

**Antiviral Biological Assay.** *Purification of TMV*. Using Gooding's method (*10*), 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.

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

*Antiviral Activity of Compounds against TMV in Vitro*. In vitro activity of the synthesized compounds against TMV was performed by the conventional half-leaf method (*11*). Fresh leaf of the 5−6 growth stage of tobacco inoculated by the juice-leaf rubbing method (concentration of TMV is 5.88 × $10^{-2}$ $\mu$g/mL) was cut into halves along the main vein. The halves were immersed into the solution of different concentrations (see **Table 1**) of the compounds and double distilled water for 20 min, respectively, and then cultured at 25 °C for 72 h. Every concentration for each compound was replicated at least three times.

*Protective Effect of Compounds against TMV in Vivo*. The compound solution was smeared on the left side and the solvent serving as 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 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 (*12*). 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 were recorded 3−4 days after inoculation (*12*). 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

**Table 1.** In Vitro Antiviral Activities against TMV

| compd | concn ($\mu$g/mL) | inhibition rate (%) | $EC_{50}$ ($\mu$g/mL) | compd | concn ($\mu$g/mL) | inhibition rate (%) | $EC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|---|---|---|
| **10** | 100 | 92.3 | 10.0 | **11** | 100 | 78.6 | 16.5 |
| | 50 | 88.2 | | | 50 | 52.2 | |
| | 25 | 75.4 | | | 25 | 47.4 | |
| | 5 | 30.2 | | | 5 | 42.6 | |
| **12** | 100 | 91.6 | <5.0 | **13** | 100 | 93.7 | 11.2 |
| | 50 | 89.9 | | | 50 | 70.0 | |
| | 25 | 76.8 | | | 25 | 48.0 | |
| | 5 | 62.1 | | | 5 | 44.4 | |
| **14** | 100 | 73.9 | 23.6 | **15** | 100 | 41.9 | >100 |
| | 50 | 60.6 | | | 50 | 36.4 | |
| | 25 | 42.4 | | | 25 | 28.6 | |
| | 5 | 32.3 | | | 5 | 7.1 | |
| **16** | 100 | 72.2 | 40.3 | **17** | 100 | 94.4 | 27.5 |
| | 50 | 68.5 | | | 50 | 64.0 | |
| | 25 | 18.5 | | | 25 | 35.7 | |
| | 5 | 15.7 | | | 5 | 6.7 | |
| **18** | 100 | 59.0 | 61.9 | **19** | 100 | 91.7 | 25.7 |
| | 50 | 54.6 | | | 50 | 77.8 | |
| | 25 | 24.3 | | | 40 | 65.2 | |
| | 5 | 23.7 | | | 25 | 48.9 | |
| **20** | 100 | 66.7 | <25.0 | **21** | 100 | 59.3 | 70.4 |
| | 50 | 61.5 | | | 50 | 51.9 | |
| | 40 | 69.1 | | | 25 | 17.1 | |
| | 25 | 65.7 | | | 5 | 11.1 | |
| **22** | 100 | 84.9 | 7.7 | **23** | 100 | 96.6 | 10.8 |
| | 50 | 70.0 | | | 50 | 87.9 | |
| | 25 | 54.7 | | | 25 | 62.9 | |
| | 5 | 50.0 | | | 5 | 32.8 | |
| **24** | 100 | 80.9 | 14.3 | **25** | 100 | 92.8 | 21.3 |
| | 50 | 74.3 | | | 50 | 79.9 | |
| | 25 | 58.8 | | | 25 | 35.7 | |
| | 5 | 31.4 | | | 5 | 15.6 | |
| **(±)-tylophorine** | 100 | 57.6 | 68.9 | **(±)-antofine** | 100 | 80.7 | <25.0 |
| | 50 | 42.5 | | | 50 | 69.7 | |
| | 25 | 41.2 | | | 25 | 68.8 | |
| | 5 | 34.0 | | | 5 | 56.7 | |
| **(±)-deoxytylophorinine** | 100 | 75.0 | <25.0 | ***S*-(+)-tylophorine** | 100 | 43.7 | >100 |
| | 50 | 66.7 | | | 50 | 35.2 | |
| | 40 | 79.2 | | | 25 | 50.6 | |
| | 25 | 65.0 | | | 5 | 41.7 | |
| ***R*-(−)-tylophorine** | 100 | 62.1 | 66.36 | **Ningnanmycin** | 500 | 59.3 | 440.0 |
| | 50 | 55.2 | | | 250 | 42.1 | |
| | 25 | 5.3 | | | 125 | 27.0 | |
| | 5 | 0 | | | 25 | 28.2 | |

counted and recorded 3−4 days after inoculation (*12*). 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).

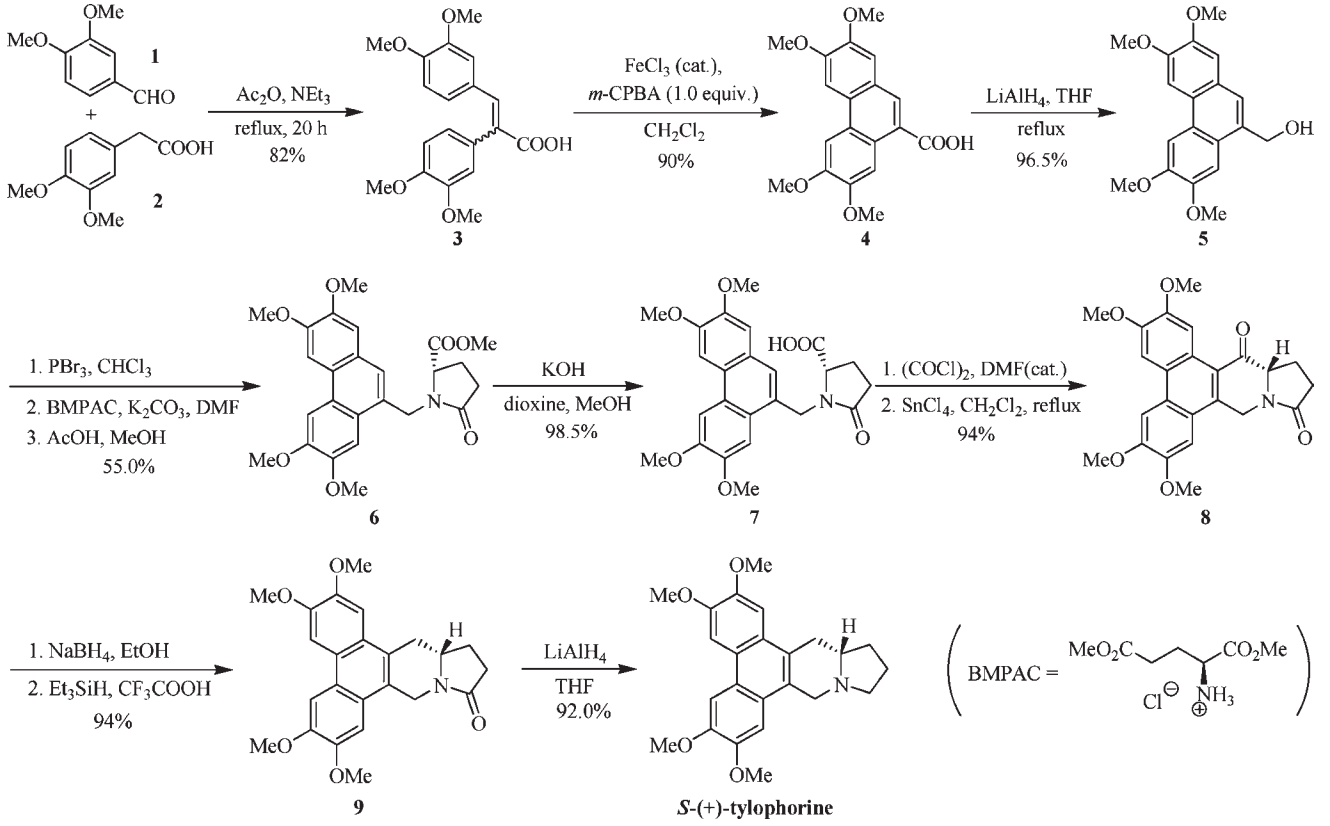
$$\text{inhibition rate (\%)} = [(\text{av local lesion no. of control} - \text{av local lesion no. of drug-treated})/\text{av local lesion no. of control}] \times 100\%$$

## RESULTS AND DISCUSSION

**Chemistry.** Racemic phenanthroindolizidine alkaloids tylophorine, antofine, and deoxytylophorinine were prepared in six steps with high overall yields, respectively, according to our reported procedure (*13*). This new and efficient strategy enjoys a number of advantages: the experimental procedure is simple under mild conditions, atom economy is very high without any protecting group, and starting materials are cheap or easily prepared. Hence, this short and practical method is applicable to large-scale production of tylophora alkaloids.

Optically pure alkaloids *S*-(+)-tylophorine and *R*-(−)-tylophorine were prepared from L-glutamic acid and D-glutamic acid, respectively, using the synthetic strategy shown in **Scheme 1**. *S*-(+)-Tylophorine was chosen as our initial target. The *E*- and *Z*-2,3-diphenylacrylic acids **3** are easily available by Perkin condensation of the appropriate aromatic aldehyde **1** with the corresponding benzeneacetic acid **2** (*13*, *14*). We found that such condensation invariably yielded a mixture of *E*-isomer as the main product and *Z*-isomer as byproduct. *E*-**3** and *Z*-**3** can be obtained, respectively, by precipitation at different pH values. The synthesis of the polymethoxy-substituted phenanthrene unit is the key step in the preparation of these alkaloids (*15*). Metal-based intramolecular oxidative couplings to yield phenanthrene ring by using thallium(III) trifluoroacetate (TTFA) (*16*), lead(IV) tetraacetate ($Pb(OAc)_4$) (*17*), vanadium oxytrifluoride ($VOF_3$) (*18*) or vanadium oxytrichloride ($VOCl_3$) (*14*), and iron(III) chloride ($FeCl_3$) (*13*, *19*) have been developed. However, these coupling reactions require large excess amounts of metal salts, at least stoichiometric; extensive application of these reagents has been limited by high toxicity, severe conditions, and low yields. Recently, we developed $FeCl_3$-catalyzed

**Scheme 1.** Synthesis of Optically Pure Alkaloids *S*-(+)-Tylophorine




intramolecular oxidative coupling for the direct construction of the phenanthrene ring using *m*-chloroperbenzoic acid (*m*-CPBA) as sole oxidant at room temperature with excellent yields (*7*). Interestingly, not only *E*-**3** but also *Z*-**3** gave the same oxidative coupling product **4** by using this method. In contrast to the other synthesis of phenanthrene, the reaction makes full use of the minor *Z*-**3**, which was a byproduct in other coupling reactions. This facile and efficient synthesis of the oxidative coupling product **4** has been applicable to large-scale production in 90% yield.

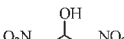
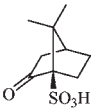
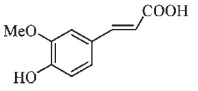
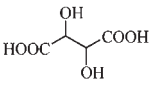
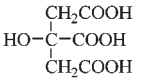
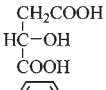
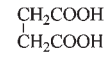
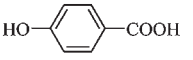
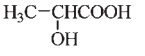
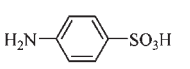
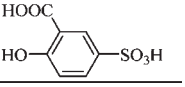
Lithium aluminum hydride reduction of carboxylic acid **4** gave alcohol **5** in 96.5% yield. Alcohol **5** was brominated by using $PBr_3$ to obtain bromide, which was used without further purification. Nucleophilic substitution reaction of bromide with L-glutamic acid dimethyl ester hydrochloride (BMPAC) and subsequent intramolecular acid-catalyzed cyclization gave a 55.0% yield of ester **6** over three steps. Ester **6** was hydrolyzed by potassium hydroxide rapidly to afford carboxylic acid **7** in 98.5% yield. Then we used intramolecular Friedel−Crafts reaction catalyzed by tin tetrachloride ($SnCl_4$) to construct indolizidine ring system **8** under mild conditions in 94% yield. Borohydride reduction of carbonyl function of **8** gave alcohol, which was subsequently reduced with $Et_3SiH/CF_3COOH$ to obtain amide **9** in 94% yield over two steps. Finally, lithium aluminum hydride reduction of **9** afforded optically pure alkaloid *S*-(+)-tylophorine in 92% yield with 92% ee value.

Having demonstrated the feasibility of the above synthetic pathway to *S*-(+)-tylophorine, we then turned our attention to the preparation of *R*-(−)-tylophorine from D-glutamic acid according to **Scheme 1** and obtained *R*-(−)-tylophorine with 96% ee value.

Tylophorine salt derivates (**10**−**25**, **Scheme 2**) were prepared from racemic tylophorine and the corresponding inorganic acids or organic acids. Only one paper reported the single crystal structure of natural (−)-antofine due to the instability of

**Scheme 2.** Synthesis of Tylophorine Salt Derivates **10**−**25**

(±)-Tylophorine + Acid ⟶ Salt

| compd | acid | compd | acid |
|---|---|---|---|
| **10** | HCl | **11** | HBr |
| **12** | HI | **13** |  |
| **14** |  | **15** |  |
| **16** |  | **17** |  |
| **18** |  | **19** |  |
| **20** |  | **21** |  |
| **22** |  | **23** |  |
| **24** |  | **25** |  |

tylophora alkaloids (*20*). Fortunately, we also gained a single crystal structure of tylophorine benzoate **13**, which further confirmed its construction (**Figure 2**).

**Antiviral Activity in Vitro.** To investigate the influence of chiral discrimination on antiplant virus, optically pure alkaloids *S*-(+)-tylophorine and *R*-(−)-tylophorine were synthesized (**Scheme 1**). A series of tylophorine salt derivates were synthesized to overcome the main drawbacks of easy decomposition and poor water solubility for the alkaloids in application (**10**−**25**, **Scheme 2**).

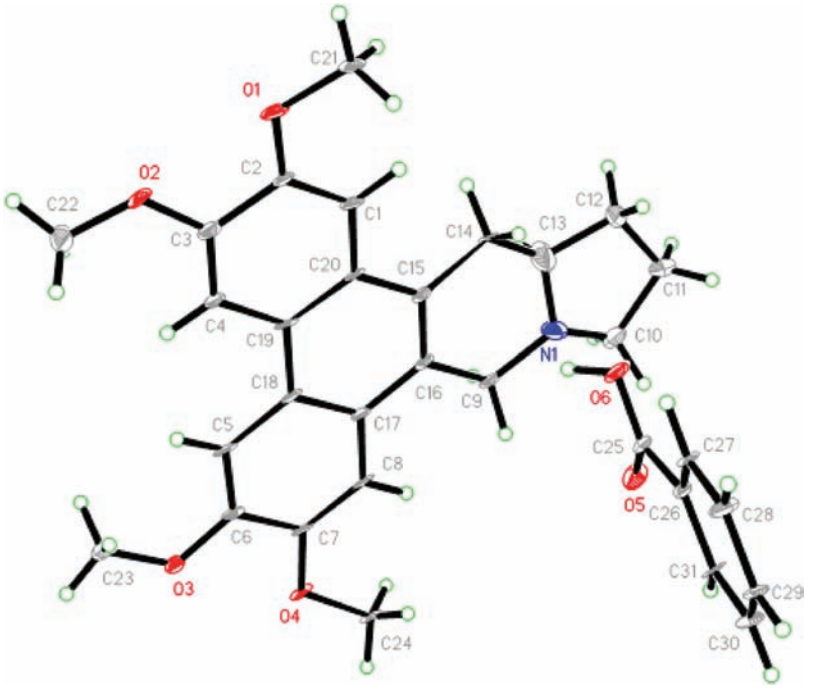



**Figure 2.** X-ray structure of tylophorine benzoate **13**.

To make a judgment of the antiviral potency of the synthesized compounds, the commercially available plant virucide Ningnanmycin (*21*), perhaps the most successful registered antiplant viral agent, was used as the control. The antiviral bioassay against TMV is assayed by the reported method (*11*), and the antiviral results of all the compounds against TMV using a half-leaf method in vitro are listed in **Table 1**. From the data in **Table 1**, we see that Ningnanmycin exhibited inhibition activity of 59.3% at 500 $\mu$g/mL, whereas most of the synthesized compounds, except for compounds **15**, **18**, and **21**, (±)-tylophorine, and *S*-(+)-tylophorine, exhibited higher antiviral activities than Ningnanmycin even at the concentration of 100 $\mu$g/mL. *R*-(−)-Tylophorine showed slight enhancement in anti-TMV activity (62.1%) compared to its enantiomer *S*-(+)-tylophorine (43.7%) at 100 $\mu$g/mL. Most of the tylophorine salt derivates showed higher antiviral activities than tylophorine alkaloid at 100 $\mu$g/mL (57.6%), which was probably caused by stability and water solubility. In addition, as shown in **Table 1**, most of the compounds were found to display excellent antiviral activities against TMV. Therefore, these compounds were bioassayed further to investigate their antiviral activities at different concentrations. The $EC_{50}$ value of Ningnanmycin on TMV was 440 $\mu$g/mL, whereas most of the compounds had $EC_{50}$ values of $< 100$ $\mu$g/mL.

**Antiviral Activity in Vivo.** The antiviral bioassay against TMV in vivo is assayed according to the reported method (*10*, *12*), and the antiviral results of all the synthesized compounds against TMV are listed in **Table 2**. All of the compounds exhibited protection activities of 26.7−78.4% at 100 $\mu$g/mL. Compounds **10**−**13**, **17**, **18**, and **22**, (±)-tylophorine, and (±)-deoxytylophorinine have higher protection activities (78.4, 66.7, 58.8, 76.5, 54.6, 56.8, 54.9, 63.3, and 60.0%, respectively) even at 100 $\mu$g/mL than the standard reference Ningnanmycin at 500 $\mu$g/mL (51.3%). In addition, compounds **14**−**16**, **19**−**21**, and **23**−**25**, (±)-antofine, *S*-(+)-tylophorine, and *R*-(−)-tylophorine showed 26.7−50.0% protection activities at 100 $\mu$g/mL. It is noteworthy that *S*-(+)-tylophorine and *R*-(−)-tylophorine exhibited the same protection activity (47.7%) at 100 $\mu$g/mL. From the data presented in **Table 2**, it can be observed that all of the compounds, except for compounds **12** and **25** and *S*-(+)-tylophorine, possess higher inactivation bioactivities (51.1−91.3%) even at 100 $\mu$g/mL than Ningnanmycin (50.5%) against TMV at 500 $\mu$g/mL. *R*-(−)-Tylophorine is more active than *S*-(+)-tylophorine, with inactivation rates of 62.2 and 24.4%, respectively. The data in **Table 2** also indicate that a change of the corresponding acid for tylophorine salt derivates might also affect the curative activity of the title compounds. Except for compound **16**, all of the compounds possess higher curative activities (26.1−72.4%) at 100 $\mu$g/mL than Ningnanmycin (23.0%) against TMV at 500 $\mu$g/mL. *R*-(−)-Tylophorine is appreciably more active than *S*-(+)-tylophorine, with curative rates of 55.2 and 48.3%, respectively.

**Table 2.** In Vivo Antiviral Activities against TMV

| compd | concn ($\mu$g/mL) | protection effect (%) | inactivation effect (%) | curative effect (%) |
|---|---|---|---|---|
| **10** | 100 | 78.4 | 60.9 | 34.7 |
| **11** | 100 | 66.7 | 56.5 | 44.4 |
| **12** | 100 | 58.8 | 39.1 | 37.8 |
| **13** | 100 | 76.5 | 69.6 | 44.9 |
| **14** | 100 | 47.7 | 55.6 | 65.5 |
| **15** | 100 | 45.5 | 57.8 | 51.7 |
| **16** | 100 | 45.5 | 51.1 | 13.8 |
| **17** | 100 | 54.6 | 64.4 | 44.8 |
| **18** | 100 | 56.8 | 60.0 | 72.4 |
| **19** | 100 | 50.0 | 82.6 | 39.1 |
| **20** | 100 | 50.0 | 80.4 | 42.1 |
| **21** | 100 | 25.0 | 71.1 | 44.8 |
| **22** | 100 | 54.9 | 60.9 | 42.9 |
| **23** | 100 | 37.3 | 91.3 | 38.3 |
| **24** | 100 | 38.6 | 75.6 | 34.5 |
| **25** | 100 | 38.6 | 42.2 | 62.1 |
| **(±)-tylophorine** | 100 | 63.3 | 73.9 | 43.5 |
| **(±)-antofine** | 100 | 26.7 | 66.3 | 54.4 |
| **(±)-deoxytylophorinine** | 100 | 60.0 | 67.4 | 26.1 |
| ***S*-(+)-tylophorine** | 100 | 47.7 | 24.4 | 48.3 |
| ***R*-(−)-tylophorine** | 100 | 47.7 | 62.2 | 55.2 |
| **Ningnanmycin** | 500 | 51.3 | 50.5 | 23.0 |

In summary, three racemic phenanthroindolizidine alkaloids have been prepared in large scale according to our reported

procedure. Using a $FeCl_3$ (cat.)/*m*-CPBA (1.0 equiv) oxidative coupling method as the key step, we also have accomplished the synthesis of two optically pure alkaloids from commercially available chemicals with 92 and 96% ee values, respectively. We presented structure optimization for tylophorine and synthesized a series of tylophorine salt derivatives, which generally had better stability and better water solubility in application than the alkaloid itself. The in vitro and in vivo antiviral bioassays showed that most of the synthesized compounds exhibited much higher inhibitory activity against TMV even at 100 $\mu$g/mL than commercial Ningnanmycin at 500 $\mu$g/mL. The influence of chiral discrimination on antiplant virus was not very obvious. In general, tylophorine salt derivatives exhibited higher inhibitory activity than alkaloids themselves and Ningnanmycin. **10**, **11**, **13**, **17**, and **22** emerged as potential inhibitors of plant virus. Therefore, the present work demonstrates that the antiviral activity of tylophora alkaloids was improved via the salinization of the alkaloids with inorganic or organic acid. Thus, the findings demonstrate that the synthesized alkaloids and their derivatives represent a new template for antiviral studies. Particularly, alkaloid salt derivatives could be considered for a new class of antiviral agents.

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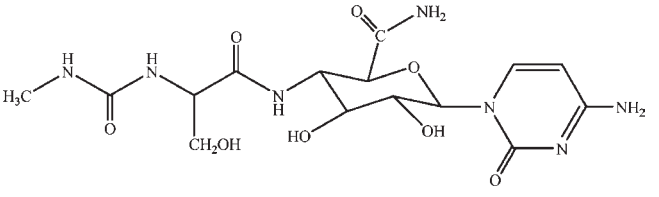
(21) Ningnanmycin is produced by *Strepcomces noursei* var. *xichangensisn*. Ningnanmycin is now commercially available in China as a registered plant virucide. However, its mechanism of action is not clear yet. Its structure is as follows:

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