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Synthesis and Antiviral Activities of Antofine Analogues with Different C-6 Substituent Groups

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Supporting Information

ABSTRACT: On the basis of previous structure−activity relationship (SAR) and antiviral mechanism studies, antofine analogues with different substituent groups at the C-6 position targeting tobacco mosaic virus (TMV) RNA were synthesized for the first time. The antofine analogues **1a**−**8a** and **1b**−**9b** were evaluated for their antiviral activity against TMV. The SAR study of antofine analogues is discussed. Most of the compounds were found to exhibit higher antiviral activity than commercial Ningnanmycin in vitro and in vivo. The groups with hydrogen donor or electron-withdrawing groups at the C-6 position were found to be favorable for antiviral activity.

KEYWORDS: *antofine analogues, antiviral activity, TMV, SAR*

INTRODUCTION

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. Therefore, this plant virus has the name "plant cancer" and is difficult to control. Every year, the loss caused by TMV is up to U.S. $100 million worldwide.[1] Ningnanmycin, a commercial antiviral agent, 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] In fact, there are no chemical treatments that can absolutely inhibit TMV once it does infect plants. Therefore, the development of a highly efficient, novel, environmentally benign antiviral agent is needed.

Phenanthroindolizidine alkaloids are a small group of alkaloids isolated mainly from *Cynanchum*, *Pergularia*, *Tylophora*, and some other genera of the Asclepiadaceae family.[3] Biological activities of these compounds include antiarthritis,[4] antilupus,[5] antiamoebic,[6] and anti-inflammatory effects,[7] as well as a most notable antitumor activity.[8−15] (*R*)-6-*O*-Desmethylantofine (**2a**, Figure 1) was found to exhibit excellent antitumor activity, which was higher than that of (*R*)-antofine (**1a**, Figure 1) due to the hydroxyl group at the C-6 position.[15−17] However, unlike the well-studied antitumor activity, the antiviral activity study of phenanthroindolizidine alkaloids is very limited. In our previous work, (*R*)-antofine and (*R*)-6-*O*-desmethylantofine were isolated from *Cynanchum komarovii*, and both were found to possess excellent antiviral activity against TMV.[18,19] The antiviral mechanism study revealed that antofine could interact selectively and tightly with target sites by embedding in the pockets created by different structures of TMV RNA. The study also indicated that antofine has a favorable interaction with the origin of TMV RNA (oriRNA), exerting its virus inhibition by binding to oriRNA and interfering with virus assembly initiation.[20] Further study confirmed that antofine analogues bound selectively with TMV RNA bulged structures and disrupted in vitro virus assembly through small molecule−RNA interactions and therefore disrupted interaction between TMV RNA and TMV coat protein (CP).[21]

In a previous work, the modification of antofine was focused on the position and number of methoxy groups on the phenanthrene ring. However, there is no study about the C-6 substituted antofine derivatives. To study the influence of the substituent group at the C-6 position on antiviral activity and optimize phenanthroindolizidine alkaloids as antiviral agents, the C-6-substituted antofine analogues **1a**−**8a** and **1b**−**9b** targeting TMV RNA were designed, synthesized, and evaluated for their antiviral activity against TMV. The structure−activity relationship study of the antofine analogues against TMV is discussed.

EXPERIMENTAL PROCEDURES

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

The phenanthroindolizidine alkaloids **1a**, **1b**, **2a**, and **2b** (Figure 1) were prepared as described previously.[22,23] The synthetic route of derivatives **3a**−**8a** and **3b**−**9b** is shown in Scheme 1.

General Procedure for the Synthesis of 3a−7a and 3b−7b. To a solution of (*R*)- or (*S*)-6-*O*-desmethylantofine (**2a** or **2b**, 0.81 g,

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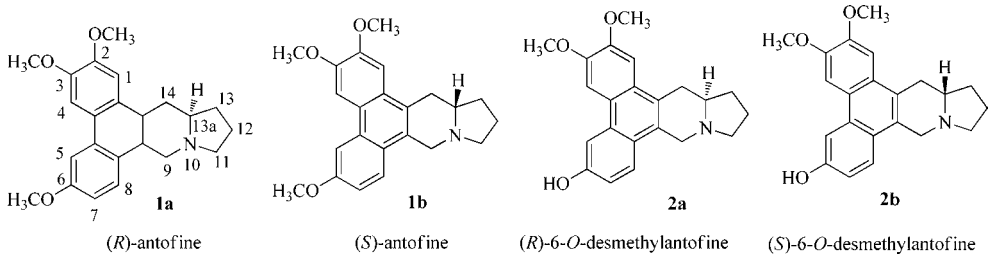
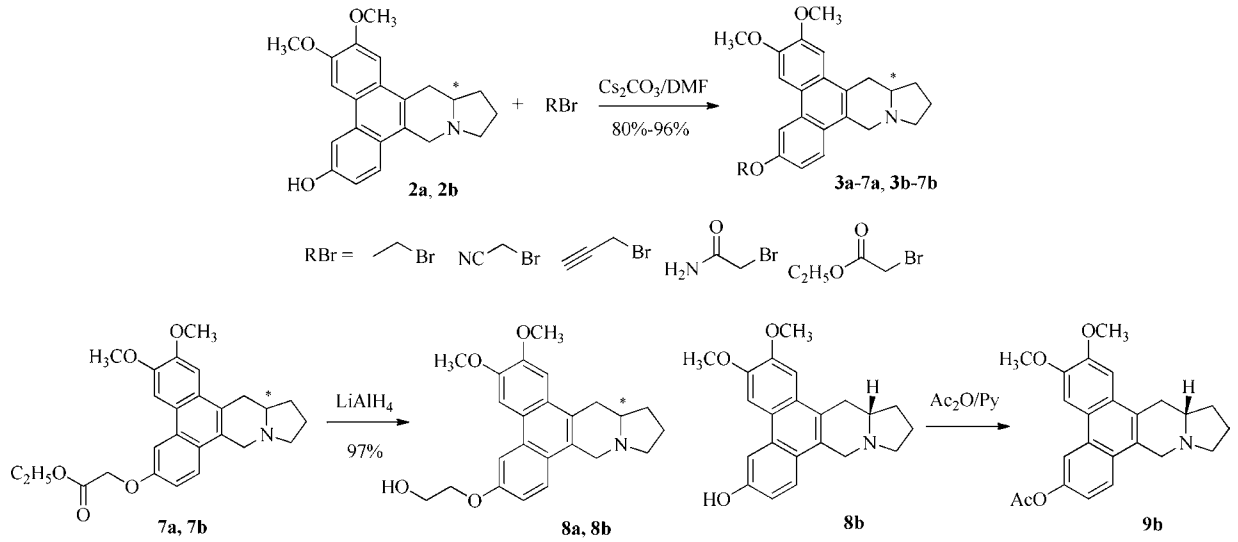


Figure 1. Chemical structures of phenanthroindolizidine alkaloids **1a**, **1b**, **2a**, and **2b**.

Scheme 1. Synthesis of Antofine Analogues 3a−8a and 3b−9b



2.33 mmol) in *N*,*N*-dimethylformamide (15 mL) was added Cs_2CO_3 (0.91 g, 2.79 mmol). The mixture was stirred at room temperature for 0.5 h and then brought to 0 °C; then a solution of α-bromo compounds (bromoacetonitrile, 3-bromopropyne, 2-bromoacetamide, ethyl bromoacetate, or bromoethane) (2.79 mmol) in *N*,*N*-dimethylformamide (10 mL) was added slowly. The mixture was warmed to room temperature and stirred until TLC showing the reaction was finished. EtOAc (30 mL) and water (30 mL) were added, and the organic layer was separated; then the water phase was extracted with EtOAc. The organic phases were combined and dried over sodium sulfate and filtered. Solvent was then evaporated and the crude product purified by column chromatography (EtOAc as eluent) to give the products **3a**−**7a** and **3b**−**7b**.

*Data for (R)-6-ethoxy-2,3-dimethoxyphenanthro[9,10-b]-indolizidine (**3a**):* white solid, yield 88%; mp 211−213 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 2H), 7.76 (d, $^3J_{HH}$ = 8.8 Hz, 1H), 7.25 (s, 1H), 7.16 (d, $^3J_{HH}$ = 9.2 Hz, 1H), 4.64 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 4.21 (q, $^3J_{HH}$ = 6.8 Hz, 2H), 4.07 (s, 3H), 4.03 (s, 3H), 3.62 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 3.43 (t, $^3J_{HH}$ = 8.4 Hz, 1H), 3.66 (d, $^2J_{HH}$ = 15.6 Hz, 1H), 2.80−2.87 (m, 1H), 2.36−2.43 (m, 2H), 2.15−2.25 (m, 1H), 1.98−2.03 (m, 1H), 1.83−1.90 (m, 1H), 1.67−1.75 (m, 1H), 1.51 (t, $^3J_{HH}$ = 6.8 Hz, 1H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 148.2, 147.2, 129.1, 126.8, 126.0, 125.6, 124.8, 124.4, 123.1, 123.0, 122.4, 114.0, 104.5, 102.8, 102.7, 62.6, 59.1, 54.9, 54.8, 54.0, 52.8, 32.6, 30.2, 20.5, 14.0. HRMS (ESI) calcd for $C_{24}H_{28}NO_3$ $[M + H]^+$, 378.2064; found, 378.2069.

*Data for (S)-6-ethoxy-2,3-dimethoxyphenanthro[9,10-b]-indolizidine (**3b**):* white solid, yield 89%; mp 212−214 °C. ^{1}H NMR, ^{13}C NMR, and HRMS (ESI) are the same as for compound **3a**.

*Data for (R)-6-propargyloxy-2,3-dimethoxyphenanthro[9,10-b]-indolizidine (**4a**):* white solid, yield, 85%; mp 197−199 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (br, 1 H), 7.91 (s, 1 H), 7.83 (d, $^3J_{HH}$ = 9.2 Hz,1 H), 7.31 (s, 1 H), 7.24−7.26 (m, 1 H), 4.90 (s, 2 H), 4.69 (d, $^2J_{HH}$ = 14.8 Hz, 1 H), 4.10 (s, 3 H), 4.06 (s, 3 H), 3.69 (d, $^2J_{HH}$ = 14.4 Hz, 1 H), 3.47 (t, $^3J_{HH}$ = 8.0 Hz, 1 H), 3.35 (d, $^2J_{HH}$ = 15.6, 1 H), 2.87−2.93 (m, 1 H), 2.59 (s, 1 H), 2.44−2.52 (m, 2 H), 2.20−2.29 (m, 1 H), 2.00−2.06 (m, 1 H), 1.90−1.97 (m, 1 H), 1.74−1.82 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 149.5, 148.4, 130.1, 127.1, 126.6, 126.1, 124.7, 124.3, 123.5, 115.1, 106.6, 104.0, 103.8, 78.7, 75.8, 60.2, 56.3, 56.0, 55.9, 55.1, 53.9, 33.8, 31.3, 21.6. HRMS (ESI) calcd for $C_{25}H_{26}NO_3$ $[M + H]^+$, 388.1907; found, 388.1909.

*Data for (S)-6-propargyloxy-2,3-dimethoxyphenanthro[9,10-b]-indolizidine (**4b**):* white solid, yield, 85%; mp 195−197 °C;. ^{1}H NMR, ^{13}C NMR, and HRMS (ESI) are the same as for compound **4a**.

*Data for (R)-6-cyanomethyloxy-2,3-dimethoxyphenanthro[9,10-b]indolizidine (**5a**):* yellow solid, yield, 93%; mp 228−230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (d, $^4J_{HH}$ = 2.0 Hz, 1H), 7.88 (s, 1H), 7.87 (d, $^3J_{HH}$ = 7.6 Hz, 1H), 7.32 (s, 1H), 7.23−7.26 (m, 1H), 4.96 (s, 2H), 4.69 (d, $^2J_{HH}$ = 14.8 Hz, 1H), 4.12 (s, 3H), 4.07 (s, 3H), 3.70 (d, $^2J_{HH}$ = 14.8 Hz, 1H), 3.47 (t, $^3J_{HH}$ = 8.0 Hz, 1H), 3.34−3.38 (m, 1H), 2.88−2.96 (m, 2H), 2.42−2.51 (m, 1H), 2.21−2.29 (m, 1H), 2.01−2.07 (m, 1H), 1.91−1.97 (m, 1H), 1.75−1.82 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.6, 149.8, 148.7, 130.2, 127.3, 127.1, 126.7, 125.7, 124.9, 123.5, 115.5, 114.9, 107.3, 104.1, 103.8, 60.3, 56.1, 56.0, 55.2, 54.5, 53.9, 33.9, 31.4, 21.7. HRMS (ESI) calcd for $C_{24}H_{25}N_2O_3$ $[M + H]^+$, 389.1860; found, 389.1858.

*Data for (S)-6-cyanomethyloxy-2,3-dimethoxyphenanthro[9,10-b]indolizidin (**5b**):* yellow solid, yield, 92%; mp 227−229 °C. ^{1}H NMR, ^{13}C NMR, and HRMS (ESI) are the same as for compound **5a**.

*Data for (R)-6-carbamoylmethyloxy-2,3-dimethoxyphenanthro-[9,10-b]indolizidin (**6a**):* light yellow solid, yield, 92%; mp 232−234 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, $^4J_{HH}$ = 2.4 Hz, 1H), 8.06 (s, 1H), 7.84 (d, $^3J_{HH}$ = 9.2 Hz, 1H), 7.66 (s, 1H), 7.50 (s, 1H), 7.34 (s, 1H), 7.21 (dd, $^3J_{HH}$ = 9.2 Hz, $^4J_{HH}$ = 2.4 Hz, 1H), 4.69 (s, 2H), 4.60 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 4.02 (s, 3H), 3.95 (s, 3H), 3.60 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 3.34−3.37 (m, 2H), 2.75−2.82 (m, 1H), 2.38−2.47 (m, 2H), 2.14−2.18 (m, 1H), 1.82−1.88 (m, 2H), 1.63−1.68 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 155.8, 149.3, 148.4,

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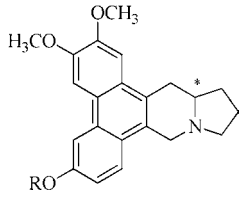
Table 1. In Vitro and in Vivo Antiviral Activities of Antofine Analogues 1a−8a and 1b−9b against TMV[a]



Compd	R	C-13a (*R*/*S*)	Conc. (μg/mL)	Inhibition rate (%)[a]			
				In Vitro effect	Protection effect	Inactivation effect	Curative effect
1a	CH_3	*R*	500	65.8	55.7	58	62.5
			100	30.4	32.3	29.4	33.4
1b	CH_3	*S*	500	60.2	51.1	55.5	56.4
			100	30.5	37.2	26.7	28.4
2a	H	*R*	500	70.5	60.4	65.3	72.4
			100	40	35.4	33.5	42
2b	H	*S*	500	52.2	43.2	46.2	49.6
			100	21.3	19.5	17.8	23.2
3a	CH_3CH_2	*R*	500	50	40.2	43.7	49.7
			100	32.5	10.4	28.5	27.2
3b	CH_3CH_2	*S*	500	56.2	48	53.2	51.1
			100	25.6	28.5	23	24.3
4a	$HC{\equiv}CH_2$	*R*	500	61.2	58.6	52.8	59.3
			100	43.7	39.7	38.9	36.1
4b	$HC{\equiv}CH_2$	*S*	500	75	71.6	70	74.9
			100	48.6	40.9	42.4	45.5
5a	$CNCH_2$	*R*	500	79.3	74.5	72.3	75.1
			100	40	42.3	38.7	44.1
5b	$CNCH_2$	*S*	500	60	54.3	58.2	57.8
			100	31.5	26.3	27.9	30.4
6a	$H_2N(C{=}O)CH_2$	*R*	500	78.5	72.7	71.2	77.1
			100	49.2	40	44.6	46.8
6b	$H_2N(C{=}O)CH_2$	*S*	500	52.3	48.4	46.1	45.2
			100	20.9	21.2	14.3	17
7a	$C_2H_5O(C{=}O)CH_2$	*R*	500	74.6	66.1	67.6	70
			100	42.5	34.5	35.1	38.9
7b	$C_2H_5O(C{=}O)CH_2$	*S*	500	50.4	49.8	45.2	40.6
			100	24.3	22.4	18.1	17.5
8a	$OHCH_2CH_2$	*R*	500	78.2	71.6	70.4	74.2
			100	47.5	39.5	40.9	42.8
8b	$OHCH_2CH_2$	*S*	500	43.9	42.3	40.5	44
			100	10	10.5	12	18.6
9b	CH_3CO	*S*	500	68.7	54.2	58.5	59.4
			100	30.6	16.2	19.3	22.4
Ningnanmycin	—	—	500	69.3	57.9	54.2	58.7
			100	26.8	38.4	25	23.1

[a]The deviation of values was ±5%.

129.7, 126.3, 125.7, 124.3, 123.6, 123.0, 116.0, 105.9, 104.5, 104.2, 67.2, 59.9, 55.8, 55.4, 54.4, 53.1, 32.8, 30.8, 21.2. HRMS (ESI) calcd for $C_{24}H_{27}N_2O_4$ $[M + H]^+$, 407.1965; found, 407.1959.

*Data for (S)-6-carbamoylmethyloxy-2,3-dimethoxyphenanthro-[9,10-b]indolizidin (**6b**):* light yellow solid, yield, 92%; mp 230−232 °C. ^1H NMR, ^{13}C NMR, and HRMS (ESI) are the same as for compound **6a**.

*Data for (R)-6-carbethoxymethyloxy-2,3-dimethoxyphenanthro-[9,10-b]indolizidin (**7a**):* white solid, yield, 96%; mp 174−176 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.89 (s, 1H), 7.83 (d, $^3J_{HH}$ = 8.8 Hz, 1H), 7.32 (s, 1H), 7.21 (dd, $^4J_{HH}$ = 1.6 Hz, $^3J_{HH}$ = 8.8 Hz, 1H), 4.82 (s, 2H), 4.67 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 4.32 (q, $^3J_{HH}$ = 7.2 Hz, 2H), 4.11 (s, 3H), 4.07 (s, 3H), 3.69 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 3.46 (t, $^3J_{HH}$ = 8.0 Hz, 1H), 3.33−3.38 (m, 1H), 2.86−2.93 (m, 1H), 2.42−2.50 (m, 2H), 2.21−2.29 (m, 1H), 1.99−2.06 (m, 1H), 1.87−1.95 (m, 1H), 1.74−1.82 (m, 1H), 1.32 (t, $^3J_{HH}$ = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 155.8, 149.7, 148.6, 130.3, 127.3, 126.8, 126.3, 125.0, 124.6, 123.6, 114.8, 106.7, 104.2, 104.0, 66.1, 61.6, 60.4, 56.2, 56.1, 55.2, 54.0, 34.0, 31.5, 21.8, 14.4. HRMS (ESI) calcd for $C_{26}H_{30}NO_5$ $[M + H]^+$, 436.2118; found, 436.2124.

*Data for (S)-6-carbethoxymethyloxy-2,3-dimethoxyphenanthro-[9,10-b]indolizidin (**7b**):* white solid, yield, 95%; mp 173−175 °C. ^1H NMR, ^{13}C NMR, and HRMS (ESI) are the same as for compound **7a**.

General Procedure for the Synthesis of 8a and 8b. To a solution of **7a** or **7b** (0.42 g, 0.97 mmol) in THF (15 mL) at 0 °C was added lithium aluminum hydride (0.11 g, 2.90 mmol). The mixture

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was stirred at room temperature for 1 h and then was brought back to 0 °C; water was added dropwise. The mixture was extracted with dichloromethane, and then the combined organic phase was dried over sodium sulfate and filtered. The solvent was removed by evaporation to give **8a** or **8b** as a white solid.

*Data for (R)-6-(2-hydroxyethyloxy)-2,3-dimethoxyphenanthro-[9,10-b]indolizidin (**8a**):* yield, 97%; mp 214−216 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, $^4J_{HH}$ = 1.6 Hz, 1H), 8.07 (s, 1H), 7.81 (d, $^3J_{HH}$ = 8.8 Hz, 1H), 7.32 (s, 1H), 7.23 (dd, $^4J_{HH}$ = 1.6, $^3J_{HH}$ = 8.8 Hz, 1H), 4.55 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 4.24 (t, $^3J_{HH}$ = 4.8 Hz, 2H), 4.02 (s, 3H), 3.94 (s, 3H), 3.82 (q, $^3J_{HH}$ = 4.8 Hz, 1H), 3.52 (d, $^2J_{HH}$ = 14.8 Hz, 1H), 3.27−3.33 (m, 2H), 2.72−2.78 (m, 1H), 2.28−2.39 (m, 2H), 2.11−2.19 (m, 1H), 1.79−1.90 (m, 2H), 1.61−1.68 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 149.3, 148.3, 129.8, 126.4, 126.4, 125.5, 124.2, 123.3, 123.0, 115.7, 105.6, 104.5, 104.1, 69.8, 59.9, 59.8, 55.8, 55.4, 54.5, 53.3, 33.1, 30.9, 21.2. HRMS (ESI) calcd for $C_{24}H_{28}NO_4$ $[M + H]^+$, 394.2013; found, 394.2010.

*Data for (S)-6-(2-hydroxyethyloxy)-2,3-dimethoxyphenanthro-[9,10-b]indolizidin (**8b**):* yield, 97%; mp 216−218 °C. ^{1}H NMR, ^{13}C NMR, and HRMS (ESI) are the same as for compound **8a**.

Procedure for the Synthesis of (*S*)-6-Acetoxy-2,3-dimethoxyphenanthro[9,10-*b*]indolizidin (9b). A mixture of (*S*)-6-*O*-desmethylantofine (0.32 g, 0.92 mmol), pyridine (15 mL), and acetic anhydride (12 mL) was stirred at room temperature for 3 h. The solvent was removed by rotary evaporation, and the crude product was washed with ether to give **9b** (0.35 g, 97%) as a white solid: mp 232−234 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, $^4J_{HH}$ = 2.0 Hz, 1H), 7.89 (d, $^3J_{HH}$ = 9.2 Hz, 1H), 7.87 (s, 1H), 7.32 (s, 1H), 7.30 (dd, $^3J_{HH}$ = 9.2 Hz, $^4J_{HH}$ = 2.0 Hz, 1H), 4.70 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 4.11 (s, 3H), 4.07 (s, 3H), 3.71 (d, $^2J_{HH}$ = 15.2 Hz, 1H), 3.45−3.49 (m, 1H), 3.36 (dd, $^2J_{HH}$ = 15.6 Hz, $^4J_{HH}$ = 2.4 Hz, 1H), 2.88−2.95 (m, 1H), 2.44−2.50 (m, 2H), 2.21−2.29 (m, 1H), 2.01−2.07 (m, 1H), 1.90−1.97 (m, 1H), 1.74−1.82 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 149.7, 148.7, 148.5, 129.9, 128.0, 127.5, 127.0, 126.7, 124.3, 123.8, 120.1, 114.5, 104.0, 103.9, 60.2, 56.1, 56.0, 55.2, 54.0, 34.0, 31.4, 21.7, 21.4. HRMS (ESI) calcd for $C_{24}H_{26}NO_4$ $[M + H]^+$, 392.1856; found, 392.1858.

Antiviral Biological Assay. The procedure of purifying TMV, the method to test the antiviral activity of compounds against TMV in vitro, and the method to test the protective effect, the inactivation effect, and the curative effect of compounds against TMV in vivo were described in the literature.[24]

Antiviral Activity of Compounds against TMV in Vitro. A fresh leaf of the 5−6 growth stage of tobacco (*Nicotiana tabacum* var. Xanthi nc) inoculated by the juice-leaf rubbing method (concentration of TMV is 5.88×10^{-2} μg/mL) was cut in half along the main vein. The halves were immersed into the solution of 500 μg/mL (or 100 μg/mL) of the compounds and double-distilled water for 20 min and then cultured at 25 °C for 72 h. 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 served as a control on the right side for growing *N. tabacum* var. Xanthi nc 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 were 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 sides of the leaves of *N. tabacum* var. Xanthi nc, whereas the right sides of the leaves were inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3−4 days after inoculation. There were three replicates for each compound.

Curative Effect of Compounds against TMV in Vivo. Growing leaves of *N. tabacum* var. Xanthi nc 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 were 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, "no." means number, 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

Antiviral Activity of Antofine Analogues. The in vitro (half-leaf method using picked leaves) and in vivo (protection, inactivation, and curative effect assays using whole plants) antiviral results against TMV of antofine analogues **1a**−**8a** and **1b**−**9b** are listed in Table 1. The antiviral activity of compounds (*R*)-antofine (**1a**) and (*S*)-antofine (**1b**) against TMV has been reported in our previous work.[25] Compounds (*R*)- and (*S*)-desmethoxyantofine **2a** and **2b** and Ningnanmycin were used as the controls. The compounds were tested at both 500 and 100 μg/mL.

Generally, all of the antofine analogues exhibited good antiviral activity against TMV both in vitro and in vivo, and some of them showed even higher antiviral activity than Ningnanmycin. Our results indicated that compounds **4b**, **5a**, **6a**, **7a**, and **8a** exhibited higher in vitro activity than Ningnanmycin at two concentrations, and more compounds, **1a**, **1b**, **2a**, **3a**, **4a**, and **5b**, exhibited higher in vitro activity than Ningnanmycin at the lower concentration (100 μg/mL). Ningnanmycin showed excellent protection activity, and only compounds **4b**, **5a**, and **6a** exhibited higher protection activity than Ningnanmycin at two concentrations, whereas compounds **2a**, **7a**, and **8a** exhibited higher protection activity than Ningnanmycin at the higher concentration (500 μg/mL). For the inactivation effect and curative effect, more antofine analogues exhibited higher activity than Ningnanmycin. Compounds **1a**, **2a**, **3a**, **4b**, **5a**, **5b**, **6a**, **7a**, **8a**, and **9b** showed higher inactivation activity than Ningnanmycin at two concentrations. Compounds **1a**, **2a**, **4b**, **5a**, **6a**, **7a**, and **8a** showed higher curative activity than Ningnanmycin at two concentrations, and compounds **1b**, **3a**, **4a**, and **5b** showed higher curative activity than Ningnanmycin at 100 μg/mL.

The structure−activity relationships showed the same tendency in the four test modes. In other words, compounds that exhibited higher in vitro activity (such as **4b**, **5a**, **6a**, **7a**, and **8a**) also exhibited higher protection, inactivation, and curative activities. However, the active tendency was different for *R*-configuration compounds **1a**−**8a** and *S*-configuration compounds **1b**−**9b**. In other words, the influence of the C-6 substituent on the antiviral activity was also affected by the stereochemistry of C-13a. For the compounds with *R*-configuration, the antiviral activity reduced obviously when the hydroxyl group at the C-6 position was changed to methoxyl (**1a**) or ethoxyl (**3a**) (inhibition rate: **3a** < **1a** < **2a**). This indicated that a hydrogen donor at the C-6 position was important for antiviral activity and that a linear alkyl at the C-6 position was unfavorable for antiviral activity. When another hydrogen donor group (amide or alcoholic hydroxyl) or electron-withdrawing group (cyano group or ethoxycarbonyl-methoxyl) was introduced at the C-6 position, the antiviral

activity distinctly increased (**5a**, **6a**, **7a**, **8a** > **2a**) in all four test modes. For the compounds with *S*-configuration, when the hydroxy group at the C-6 position was changed to methoxyl, ethoxyl, propargyloxy, or cyanomethyloxy, the antiviral activity increased (**1b**, **3b**, **4b**, **5b** > **2b**). When amide or ethoxycarbonylmethoxy was introduced at the C-6 position, **6b** and **7b** showed the same activity level as **2b**. When the hydroxyl group at the C-6 position was changed to 2-hydroxyethoxyl, the antiviral activity reduced obviously (**8b** < **2b**). Acetylation of the hydroxyl group at the C-6 position led to increased activity (**9b** > **2b**).

In summary, antofine analogues with different C-6 substituent groups were prepared in excellent yield and evaluated for their antiviral activities against TMV. Although the mechanism study indicated that the substituent groups on the phenanthrene ring had little effect on drug−RNA interaction,[21] this work suggested that both the stereochemistry of C-13a and the substituent at the C-6 position affected the antiviral activity. In general, (*R*)-antofine analogues have better antiviral activity than (*S*)-antofine analogues, and the groups with hydrogen donor or electron-withdrawing groups at the C-6 position are favorable for antiviral activity. Among these compounds, (*R*)-6-*O*-desmethylantofine (**2a**), (*S*)-6-*O*-propargyl derivative **4b**, (*R*)-6-*O*-cyanomethyl derivative **5a**, (*R*)-6-*O*-carbamoylmethyl derivative **6a**, (*R*)-6-*O*-carbethoxymethyl derivative **7a**, and (*R*)-6-*O*-(2-hydroxyethyl) derivative **8a** exhibited excellent antiviral activity, which were better than that of Ningnanmycin both in vitro and in vivo. Thus, antofine analogues with different C-6 substituent groups could be considered as a new class of antiviral agents.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR and HRMS spectra of compounds **3a**, **4b**, **5a**, **6a**, **7a**, **8a**, and **9b**.This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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