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Synthesis and Antiviral Bioactivity of Novel 3-((2-((1*E*,4*E*)-3-Oxo-5-arylpenta-1,4-dien-1-yl)phenoxy)methyl)-4(3*H*)-quinazolinone Derivatives

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Supporting Information

ABSTRACT: A series of novel 3-((2-((1*E*,4*E*)-3-oxo-5-arylpenta-1,4-dien-1-yl)phenoxy)methyl)-4(3*H*)-quinazolinone derivatives were designed and synthesized. Antiviral bioassays indicated that a few of the compounds exhibited higher antiviral activities against tobacco mosaic virus (TMV) in vivo than the commercial agent ningnanmycin. In particular, compounds $\mathbf{A}_5$, $\mathbf{A}_{12}$, $\mathbf{A}_{25}$, and $\mathbf{A}_{27}$ possessed appreciable curative bioactivities on TMV in vivo, with 50% effective concentration values ranging from 132.25 to 156.10 μg/mL. These values are superior to that of ningnanmycin (281.22 μg/mL) and suggest that novel 4(3*H*)-quinazolinone derivatives containing 1,4-pentadien-3-one moiety can effectively control TMV. Evaluation of the antiviral properties in field studies and the mechanisms underlying the enhanced antiviral activities of these derivatives are an interesting topic for future investigation.

KEYWORDS: *4(3H)-quinazolinone derivatives, 1,4-pentadien-3-one moiety, antiviral activity, tobacco mosaic virus*

INTRODUCTION

Plants have evolved to produce secondary metabolites capable of antimicrobial activities that selectively suppress pathogens.[1] Natural product-based antiviral agents possess advantages over other general synthetic compounds for several reasons. First, natural products are sometimes specific to a target species and often exhibit a unique mode of action with low mammalian toxicity. Second, natural products possess the ability to decompose rapidly, thereby decreasing their harm to the environment.[2,3] Third, these products may have desirable biological activities. For instance, ningnanmycin, a successful anti-plant viral agent from a natural product, is a commercial antiviral agent isolated from *Streptomyces noursei* var.[4] Several triterpenoid glycosides,[5,6] *seco*-pregnane steroids,[7] and quassinoids,[8] which are isolated from plants, show excellent anti-tobacco mosaic virus (TMV) activities. Triterpene saponins,[9] eudesmanolides,[10] and limonoids[11] possess strong antiviral activities. Particular natural product based agrochemicals, such as phenanthrene-based antofine derivatives,[12] phenanthroindolizidine and its analogues,[13] 14-aminophenanthroindolizidines,[14] β-carboline alkaloid derivatives,[15] and phenanthroindo/quinolizidine analogues,[16] have great potential in further developing anti-TMV drugs. Wang et al. demonstrated the excellent antiviral capability of alkaloid antofine isolated from *Cynanchum komarovii*.[17,18] The alkaloid derivative NK-007, which has outstanding antiviral activity, was chosen to develop further a novel and potent anti-TMV agent.[19] Using natural products capable of anti-plant virus activities is indeed an effective method of controlling plant diseases.

Curcumin, which is isolated from turmeric, is a plant polyphenol compound that is a member of the ginger family. The curcumin analogue 1,4-pentadien-3-one is capable of numerous potential biological activities[20] and serves important functions in discovering new antiviral molecules. In our previous study, we designed and synthesized a series of 1,4-pentadien-3-one analogues containing pyrozole,[21] oxime esters, oxime ester,[22] and quinazoline group[23] with excellent antifungal and antiviral bioactivity against TMV and CMV. 4(3*H*)-Quinazolinone derivatives constitute an important class of pharmacophoric nitrogen-containing heterocyclic molecules that occupy a significant position in medicinal and pesticide chemistry. They present a wide range of biological properties, including antibacterial[24] and antiviral activities.[25] Various natural products with critical pharmacophores contain 4(3*H*)-quinazolinone group. For example, febrifugine is found in the herbal plant *Dichroa febrifuga*, which exhibits good antimalarial activity.[26] Ding et al. successfully synthesized 2-alkoxy-4(3*H*)-quinazolinone derivatives, which exhibit good fungicidal activities at a concentration of 50 mg/L.[27] In our previous work, we demonstrated the anti-TMV activity of a series of novel 4(3*H*)-quinazolinone derivatives of 2-aryl- or 2-methyl-3-(substituted-benzalamino)-4(3*H*)-quinazolinone[28] and 4(3*H*)-quinazolinone Schiff base.[29] Ouyang et al. have reported that 3-alkylquinazolin-4-one derivatives possess good antifungal activity.[30] Wang et al.[31] synthesized a series of (*E*)-3-[2-arylideneaminoethyl]-2-[4-(trifluoromethoxy)anilino]-4(3*H*)-quinazolinone derivatives, which exhibit strong antifungal and antibacterial activities, with 50% effective concentration (EC_{50}) values ranging from 45.96 to 93.31 μg/mL and from 20.09 to 21.33 μg/mL, respectively.

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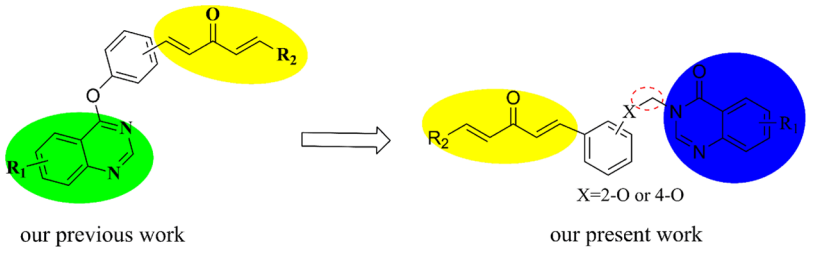
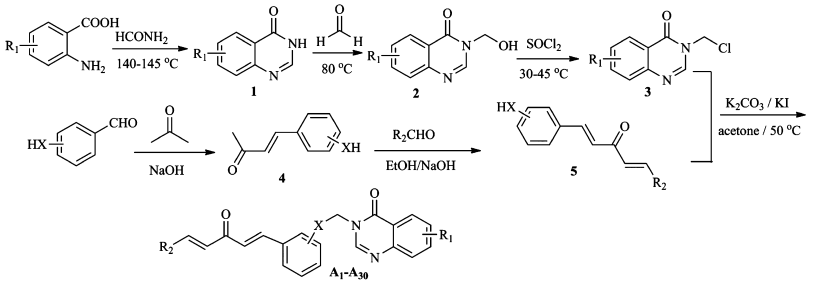


Figure 1. Design of the title compounds.



A_1:R_1=H R_2=Ph X=2-O; A_2:R_1=H R_2=4-Cl-Ph X=2-O; A_3:R_1=H R_2=2-F-Ph X=2-O;
A_4:R_1=8-CH3 R_2=Ph X=2-O; A_5:R_1=H R_2=2-OCH_3-Ph X=2-O; A_6:R_1=H R_2=4-C(CH_3)$_3$-Ph X=2-O;
A_7:R_1=H R_2=3-NO_2-Ph X=4-O; A_8:R_1=H R_2= 4-F-Ph X=2-O; A_9:R_1=H R_2=4-F-Ph X=4-O;
A_{10}:R_1=H R_2=furan-2-yl X=2-O; A_{11}:R_1=H R_2=4-CH_3-Ph X=2-O; A_{12}:R_1=8-CH_3 R_2=thiophen-2-yl X= 2-O;
A_{13}:R_1=8-CH_3 R_2=thiophen-2-yl X=4-O; A_{14}:R_1=H R_2=furan-2-yl X=4-O; A_{15}:R_1=8-CH_3 R_2=2-Cl-5-NO_2-Ph X=2-O;
A_{16}:R_1=H R_2=4-methylthiazol-5-yl X=2-O; A_{17}:R_1=H R_2=thiophen-2-yl X=2-O; A_{18}:R_1=H R_2=thiophen-2-yl X=4-O;
A_{19}:R_1=H R_2=4-OCH_3-Ph X=4-O; A_{20}:R_1=H R_2=2-CF_3-Ph X=2-O; A_{21}:R_1=H R_2=2,4-di-OCH_3-Ph X=4-O;
A_{22}:R_1=8-CH_3 R_2=2,4-di-OCH_3-Ph X=4-O; A_{23}:R_1=8-CH_3 R_2=pyrid-2-yl X=4-O; A_{24}:R_1=8-CH_3 R_2=2,4-di-OCH_3-Ph X=2-O;
A_{25}:R_1=8-CH_3 R_2=3,4-di-OCH_3-Ph X=4-O; A_{26}:R_1=H R_2=3,4-di-OCH_3-Ph X=4-O; A_{27}:R_1=H R_2=4-OCH_3-Ph X=2-O;
A_{28}:R_1=8-CH_3 R_2=2-OCH_3-Ph X=2-O; A_{29}:R_1=H R_2=3,4,5-tri-OCH_3-Ph X=4-O; A_{30}:R_1=H R_2=3,4,5-tri-OCH_3-Ph X=2-O.

Figure 2. Synthesis of the title compounds $\mathbf{A_1}$–$\mathbf{A_{30}}$.

Our research showed that 4(3*H*)-quinazolinone derivatives possess excellent anti-TMV activities. The third position of 4(3*H*)-quinazolinones is particularly an active site. Futhermore, 1,4-pentadien-3-one belongs to natural product derivatives, which exhibits outstanding antiviral bioactivity. However, no study has investigated 4(3*H*)-quinazolinone derivatives containing 1,4-pentadien-3-one moieties to date. In this study, we incorporated a 1,4-pentadien-3-one unit to the backbone of 4(3*H*)-quinazolinone, which may result in 4(3*H*)-quinazolinone derivatives with good antiviral activity. The 4(3*H*)-quinazolinone analogues $\mathbf{A_1}$ to $\mathbf{A_{30}}$ were designed and synthesized, and their antiviral activities against TMV were evaluated (Figure 1). The biological assay revealed that some of the title compounds displayed moderate to good antiviral activity. For example, the title compounds $\mathbf{A_5}$, $\mathbf{A_{12}}$, $\mathbf{A_{25}}$, and $\mathbf{A_{27}}$ showed superior curative activities against TMV in vivo relative to the commercial agricultural antiviral agent ningnanmycin. To the best of our knowledge, this study is the first to report the synthesis and antiviral activity of 3-((2-((1*E*,4*E*)-3-oxo-5-arylpenta-1,4-dien-1-yl)phenoxy)methyl)-4(3*H*)-quinazolinone derivatives.

MATERIALS AND METHODS

Equipment. The melting points of the products were determined under an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were left untouched. IR spectra were recorded on a Bruker VECTOR 22 spectrometer in a KBr disk. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) (solvent $CDCl_3$ or DMSO-d_6) spectra were obtained on a JEOL-ECX500 NMR spectrometer at room temperature using tetramethylsilane as an internal standard. Elemental analysis was performed through an Elementar Vario-III CHN analyzer. Analytical thin-layer chromatography (TLC) was conducted on silica gel GF254 (400 mesh). TLC purification was conducted using silica gel. All reagents and reactants were purchased from commercial suppliers and were analytical reagent grade or chemically pure. All solvents were dried, deoxygenated, and redistilled prior to use.

General Procedure for Preparation of the Key Intermediates. 3-Chloromethyl-4(3*H*)-quinazolinone and 3-chloromethyl-8-methyl-4(3*H*)-quinazolinone were prepared according to the literature.[32] (*E*)-4-(2-Hydroxyphenyl)-3-butylene-2-one and (*E*)-4-(4-hydroxyphenyl)-3-butylene-2-one were prepared according to a previous report.[33] Intermediate **5** was prepared following reported methods.[34]

General Procedure for Preparation of the Title Compounds $\mathbf{A_1}$ to $\mathbf{A_{30}}$. Figure 2 shows the synthesis of the target compounds $\mathbf{A_1}$ to $\mathbf{A_{30}}$. A 50 mL round-bottomed flask equipped with a magnetic stirrer was charged with intermediates **3** (2.62 mmol) and **5** (1.31 mmol), acetone (50 mL), and K_2CO_3 (5.24 mmol). The flask was stirred at room temperature for 10 min, after which KI (1.31 mmol) was added. The resulting mixture was refluxed and stirred at 56 °C for 5 to 10 h. TLC was used to monitor the progress of the reaction. After the reaction was completed (as indicated by TLC), the solvent was removed under depressurization, and the residue was dissolved with CH_2Cl_2. The crude product was then separated through TLC purification using silica gel. The solid was recrystallized from dichloromethane/petroleum ether (2:1, v/v) to obtain the title compounds $\mathbf{A_1}$ to $\mathbf{A_{30}}$ at 25% to 55% yields. The experimental protocols show the physical characteristics, IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis data for all the synthesized compounds. The data for compound $\mathbf{A_5}$ are shown below, and the data for compounds $\mathbf{A_1}$ to $\mathbf{A_{30}}$ are shown in the Supporting Information.

3-((2-((1E,4E)-5-(2-Methoxyphenyl)-3-oxopenta-1,4-dien-1-yl)-phenoxy)methyl)-4(3H)-quinazolinone ($\mathbf{A_5}$). Yellow solid: mp 170 to 173 °C; yield, 54.0%; IR (KBr, cm^{-1}) ν 3444.9 (amidic N–CH–N), 2948.8–2879.3 (C–H), 1660.7 (CH═CH–C═O), 1610.6 (C═O of Qu-ring), 1457.4–1598.2 (C═C and benzene and Qu-ring), 1298.1 (C–N), 1228.7 (C–O), 1159.4 (–O–CH_3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31(d, J = 8.05 Hz, 1H, Qu-5-H), 8.17 (s, 1H, Qu-2-H), 8.02 (d, J = 16.05 Hz, 2H, –O–Ph–CH═, ═CH–Ar), 7.76 (t, J_1

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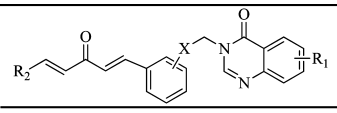
Table 1. Physical and Analytical Data of Synthesized Compounds A_1−A_{30}[a]

Compd.				Molecular Formula/M.W	M.p./°C	Yield [a]/%	Elemental Analysis/%: Found/Calcd.		
	R_1	R_2	X				C	H	N
A_1	H	Phenyl	2-O	$C_{26}H_{20}N_2O_3$ (408.4)	174-176	48.6	76.45/76.37	4.94/4.54	6.86/6.92
A_2	H	4-Clphenyl	2-O	$C_{26}H_{19}ClN_2O_3$ (442.9)	195-197	39.6	70.51/70.33	4.32/4.43	6.33/6.20
A_3	H	2-Fphenyl	2-O	$C_{26}H_{19}FN_2O_3$ (426.4)	142-144	44.7	73.23/73.00	4.49/4.67	6.57/6.07
A_4	8-CH_3	Phenyl	2-O	$C_{27}H_{22}N_2O_3$ (422.5)	162-164	38.7	76.76/76.63	5.25/5.20	6.63/6.98
A_5	H	2-OCH_3phenyl	2-O	$C_{27}H_{22}N_2O_4$ (438.5)	170-173	54.0	73.96/73.46	5.05/5.09	6.39/6.36
A_6	H	4-$C(CH_3)_3$phenyl	2-O	$C_{30}H_{28}N_2O_3$ (464.5)	158-160	47.6	77.56/77.19	6.08/5.83	6.03/6.05
A_7	H	3-NO_2phenyl	4-O	$C_{26}H_{19}N_3O_5$ (453.4)	223-225	26.9	68.87/68.45	4.22/4.00	9.27/9.64
A_8	H	4-Fphenyl	2-O	$C_{26}H_{19}FN_2O_3$ (426.1)	205-207	35.8	73.23/73.61	4.49/4.69	6.57/6.34
A_9	H	4-Fphenyl	4-O	$C_{26}H_{19}FN_2O_3$ (426.1)	211-213	32.3	73.23/73.52	4.49/4.66	6.57/6.36
A_{10}	H	2-furyl	2-O	$C_{24}H_{18}N_2O_4$ (398.4)	146-148	57.5	72.35/72.12	4.55/4.61	7.03/7.08
A_{11}	H	4-CH_3phenyl	2-O	$C_{27}H_{22}N_2O_3$ (422.2)	160-162	48.8	76.76/76.26	5.25/5.53	6.63/6.37
A_{12}	8-CH_3	2-thiophenyl	2-O	$C_{25}H_{20}N_2O_3S$(428.5)	151-153	51.6	70.07/70.38	4.70/4.56	6.54/6.29
A_{13}	8-CH_3	2-thiophenyl	4-O	$C_{25}H_{20}N_2O_3S$ (428.5)	194-195	42.0	70.07/70.29	4.70/4.68	6.54/6.39
A_{14}	H	2-furyl	4-O	$C_{24}H_{18}N_2O_4$ (398.4)	156-158	44.1	72.35/72.43	4.55/4.86	7.03/7.01
A_{15}	8-CH_3	2-Cl-5-NO_2phenyl	2-O	$C_{27}H_{20}ClN_3O_5$ (501.9)	207-209	27.7	64.61/64.29	4.02/4.37	8.37/8.21
A_{16}	H	4-methylthiazol-5-yl	2-O	$C_{24}H_{19}N_3O_3S$ (429.5)	198-200	53.3	67.12/67.02	4.46/4.50	9.78/9.80
A_{17}	H	2-thiophenyl	2-O	$C_{24}H_{18}N_2O_3S$ (414.5)	159-161	53.4	69.55/69.22	4.38/4.40	6.76/6.75
A_{18}	H	2-thopenyl	4-O	$C_{24}H_{18}N_2O_3S$ (414.5)	162-164	47.9	69.55/69.10	4.38/4.53	6.76/6.70
A_{19}	H	4-OCH_3phenyl	4-O	$C_{27}H_{22}N_2O_4$ (438.5)	219-221	43.5	73.96/73.53	5.06/5.08	6.39/6.41
A_{20}	H	2-CF_3phenyl	2-O	$C_{27}H_{19}F_3N_2O_3$ (476.4)	137-139	28.8	68.05/67.90	4.02/3.99	5.88/5.76
A_{21}	H	2,4-di-OCH_3phenyl	4-O	$C_{28}H_{24}N_2O_5$ (468.5)	165-167	44.9	71.78/71.68	5.16/5.47	5.98/6.02
A_{22}	8-CH_3	2,4-di-OCH_3phenyl	4-O	$C_{29}H_{26}N_2O_5$ (482.5)	185-187	40.7	72.18/72.50	5.43/5.76	5.81/5.47
A_{23}	8-CH_3	2-pyridyl	4-O	$C_{26}H_{21}N_3O_3$ (423.5)	194-196	42.5	73.74/73.44	5.00/4.59	9.92/10.03
A_{24}	8-CH_3	2,4-di-OCH_3phenyl	2-O	$C_{29}H_{26}N_2O_5$ (482.5)	190-192	57.7	72.18/72.45	5.43/5.08	5.81/5.59
A_{25}	8-CH_3	3,4-di-OCH_3phenyl	4-O	$C_{29}H_{26}N_2O_5$ (482.2)	142-143	49.2	72.18/72.53	5.43/5.27	5.81/5.39
A_{26}	H	3,4-di-OCH_3phenyl	4-O	$C_{28}H_{24}N_2O_5$ (482.5)	178-180	52.1	71.78/71.37	5.16/5.54	5.98/6.00
A_{27}	H	4-OCH_3phenyl	2-O	$C_{27}H_{22}N_2O_4$ (438.5)	154-156	50.5	73.96/73.57	5.06/5.39	6.39/6.53
A_{28}	8-CH_3	2-OCH_3phenyl	2-O	$C_{28}H_{24}N_2O_4$ (452.5)	142-144	48.8	74.32/73.78	5.35/5.56	6.19/6.48
A_{29}	H	3,4,5-tri-OCH_3phenyl	4-O	$C_{29}H_{26}N_2O_6$ (498.5)	180-182	49.0	69.87/69.63	5.26/5.67	5.62/5.27
A_{30}	H	3, 4,5-tri-OCH_3phenyl	2-O	$C_{29}H_{26}N_2O_6$ (498.5)	134-135	53.6	69.87/69.69	5.26/5.52	5.62/5.34

[a]The reaction was performed according to the general experimental program. X = 2-O or 4-O.

= 6.85 Hz, J2 = 8.60 Hz, 1H, Qu-7-H), 7.70 (d, J = 8.00 Hz, 1H, Ph−6−H), 7.66 (d, J = 8.00 Hz, 1H, Ar-6-H), 7.62 (d, J = 7.45 Hz, 1H, Qu-8-H), 7.50 (t, J_1 = 6.85 Hz, J_2 = 8.05 Hz, 1H, Qu−6−H), 7.39−7.36 (m, 2H, Ph-4-H, Ar-4-H), 7.23 (d, J = 8.05 Hz, 1H, Ph-3-H), 7.13−7.06 (m, 3H, −O−Ph−CH═CH−, −CH═CH−Ar, Ph-5-H), 6.99 (t, J_1 = J_2 = 7.45 Hz, 1H, Ar-5-H), 6.93 (d, 1H, J = 8.60 Hz, Ar-3-H), 6.06 (s, 2H, $-CH_2-$), 3.90 (s, 3H, OCH_3−2−Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.47, 160.61, 158.57, 154.58, 147.67, 145.10, 138.57, 136.79, 134.93, 131.75, 131.71, 128.81, 128.47, 127.77, 127.13, 126.97, 125.91, 125.84, 123.74, 123.48, 121.73, 120.74, 115.31, 111.11, 73.22, 55.49. Anal. Calcd for $C_{27}H_{22}N_2O_4$ (438.5): C, 73.46; H, 5.09; N, 6.36. Found: C, 73.96; H, 5.05; N, 6.39.

Antiviral Biology against TMV. *Purification of TMV.* Using the Gooding method,[35] the virus was multiplied in *N. tabacum* cv. K326 and purified. The absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer.

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

Half-Leaf Method Protective Effects of Title Compounds against TMV in Vivo. The compound solution was smeared on the left side of growing *N. tabacum* L. leaves of the same age. The solvent was smeared on the right side of the leaves to serve as the control. The leaves, which were previously scattered with silicon carbide, were inoculated with the virus after 12 h using a brush dipped in 6×10^{-3} mg/mL TMV and subsequently washed with water and rubbed softly along the nervature once or twice. The local lesions that appeared 3 to 4 d after the inoculation were counted.[36,37] Three replications were reproduced for each compound.

Inactivation Effects of Title Compounds on TMV in Vivo. The virus was inhibited after it was mixed with a compound solution of the same volume for 30 min. The right side of the *N. tabacum* L. leaves was then inoculated with the solvent and virus mixture for control. All of the leaves were previously scattered with silicon carbide. The number of local lesions was recorded 3 to 4 d after the inoculation.[37] Three replications were reproduced for each compound.

Curative Effects of Title Compounds against TMV in Vivo. Growing *N. tabacum* L. leaves of the same age were selected. The leaves were inoculated with TMV (concentration of 6×10^{-3} mg/mL) by dipping and brushing the whole leaves, which were previously scattered with silicon carbide. The leaves were then washed with water after inoculation for 0.5 h. The compound solution was smeared on the left side of the leaves, and the solvent was smeared on the right side for control. The number of local lesions was counted and recorded 3 to 4 d after the inoculation.[37] Three replications were reproduced for each compound. The inhibitory rate of the compound was calculated according to the following formula ("av" means average):

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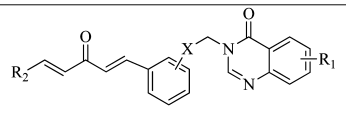
Table 2. Inhibitory Effect of the Title Compounds against TMV in Vivo at 500 μg/mL

Compd.				Curative activity (%)[a]	Inactivation activity (%)[a]	Protection activity (%)[a]
	R_1	R_2	X			
A_1	H	Ph	2-O	43.9±0.8	82.6±2.5	23.6±2.2
A_2	H	4-Cl-Ph	2-O	36.3±2.9	88.4±1.3	49.8±1.8
A_3	H	2-F-Ph	2-O	48.1±2.1	90.2±1.7	26.6±3.5
A_4	8-CH_3	Ph	2-O	46.5±1.6	82.2±2.5	49.2±2.4
A_5	H	2-OCH_3-Ph	2-O	64.3±1.1	82.0±1.6	54.4±2.1
A_6	H	4-$C(CH)_3$-Ph	2-O	28.8±1.0	87.5±0.1	56.5±0.7
A_7	H	3-NO_2-Ph	4-O	46.2±2.3	45.8±1.2	18.9±1.9
A_8	H	4-F-Ph	2-O	45.8±1.4	83.0±2.0	68.7±1.8
A_9	H	4-F-Ph	4-O	23.5±1.0	64.6±1.2	42.5±4.1
A_{10}	H	furan-2-yl	2-O	49.9±1.3	91.9±0.8	66.9±1.9
A_{11}	H	4-CH_3-Ph	2-O	29.6±2.8	81.5±3.1	54.2±2.6
A_{12}	8-CH_3	thiophen-2-yl	2-O	63.5±2.7	89.2±2.5	55.8±1.9
A_{13}	8-CH_3	thiophen-2-yl	4-O	26.5±3.4	75.1±3.6	59.1±0.9
A_{14}	H	furan-2-yl	4-O	12.7±2.0	85.1±2.1	63.1±2.4
A_{15}	8-CH_3	2-Cl-5-NO_2-Ph	2-O	43.6±1.5	72.9±4.1	39.0±0.4
A_{16}	H	4-methylthiazol-5-yl	2-O	50.1±1.2	89.3±0.2	71.7±3.0
A_{17}	H	thiophen-2-yl	2-O	34.0±3.1	83.7±1.2	64.6±2.1
A_{18}	H	thiophen-2-yl	4-O	34.6±1.5	50.0±4.8	54.7±2.4
A_{19}	H	4-OCH_3-Ph	4-O	21.9±1.9	78.3±0.8	20.0±2.3
A_{20}	H	2-CF_3-Ph	2-O	29.0±2.3	75.8±3.3	24.0±2.6
A_{21}	H	2, 4-di-OCH_3-Ph	4-O	35.0±2.8	83.2±2.0	47.9±3.9
A_{22}	8-CH_3	2, 4-di-OCH_3-Ph	4-O	48. 9±0.9	84.7±0.7	48.0±1.4
A_{23}	8-CH_3	pyrid-2-yl	4-O	51.9±1.1	81.4±0.2	24.1±0.8
A_{24}	8-CH_3	2, 4-di-OCH_3-Ph	2-O	36.2±0.8	86.1±1.8	30.3±0.6
A_{25}	8-CH_3	3, 4-di-OCH_3-Ph	4-O	69.0±0.3	64.6±4.8	66.5±0.8
A_{26}	H	3, 4-di-OCH_3-Ph	4-O	57.7±1.8	87.8±0.5	34.4±1.8
A_{27}	H	4-OCH_3-Ph	2-O	64.7±3.2	82.1±1.6	52.6±3.3
A_{28}	8-CH_3	2-OCH_3-Ph	2-O	59.4±2.4	90.0±2.5	47.0±2.1
A_{29}	H	3, 4, 5-tri-OCH_3-Ph	4-O	40.3±4.5	91.2±0.4	71.6±2.8
A_{30}	H	3, 4, 5-tri-OCH_3-Ph	2-O	57.0±3.4	47.0±4.1	51.7±1.6
Ningnanmycin[b]				58.4±0.9	95.8±1.4	68.4±2.2

[a]Average of three replicates. [b]The commercial, agricultural, and antiviral product ningnanmycin was used for comparison of activity. X = 2-O or 4-O.

$$\text{inhibition rate (\%)} = [(\text{av local lesion no. of control (not treated with compd)} - \text{av local lesion no. smeared with drugs})/\text{av local lesion no. of control (not treated with compd)}] \times 100\,\%$$

RESULTS AND DISCUSSION

Chemistry. Figure 2 shows the summary of the synthetic route designed for the 4(3*H*)-quinazolinone analogues. The starting materials were substituted with 2-aminobenzoic acid, formamide, salicylaldehyde, hydroxybenzaldehyde, and acetone. The substituted *o*-aminobenzoic acid was allowed to react with formamide for 4.5 h at 140 to 145 °C to obtain the intermediate 4(3*H*)-quinazolinone **1**, and then with 37% formaldehyde for 5 h at 80 °C to obtain the intermediate substituted 3-hydroxymethyl-4(3*H*)-quinazolinone **2**. The substituted 3-chloromethyl-4(3*H*)-quinazolinone **3** was prepared by chlorination reaction with $SOCl_2$ in 1,4-dioxane. Intermediates **5a** to **5m** were obtained through the condensation of the (*E*)-4-(2-hydroxyphenyl)-3-butene-2-ones and (*E*)-4-(4-hydroxyphenyl)-3-butene-2-ones with the substituted aldehydes in ethanol for 12 h at room temperature. The title compounds 4(3*H*)-quinazolinone derivatives (A_1−A_{30}) were synthesized by the etherification reaction of intermediates **5** and **3** with K_2CO_3/KI in acetone refluxed at 56 °C for 5 to 10 h. The final step of the reaction yielded some by-products. The purification compound obtained by the TLC and recrystallization methods involved the loss of some products. Thus, the yield of the title compounds is low. All compounds were confirmed by spectroscopic data and elemental analysis.

Table 1 shows the structures of all the compounds confirmed through IR, ^{1}H NMR, and ^{13}C NMR spectral and elemental analyses. The IR spectra of the title products A_1 to A_{30} exhibited characteristic absorption bands at 3421 to 3460 cm^{-1}, which indicate the presence of amidic N−CH−N. The stretching frequency at 1690 to 1650 cm^{-1} was assigned to C═O vibrations. The characteristic absorptions at 1491 to 1620

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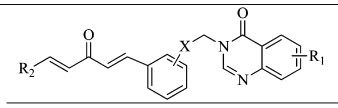
cm^{-1} and 1219 to 1316 cm^{-1} were attributed to the presence of C═C and C−O−C groups, respectively. ^1H NMR indicated that all of the phenyl protons had multiplets at 7.50 to 6.71 ppm. The main characteristic of the ^1H NMR spectra for the compound was the presence of a high-frequency downfield singlet δ_H 8.18−8.62 for N−C−H protons. N−CH_3 absorption peaks showed a singlet at 2.56 ppm to 3.87 ppm. The chemical shifts at nearly 188.00 ppm, 71.81 to 73.75 ppm, and 17.35 to 17.42 ppm in ^{13}C NMR also confirmed the presence of C═O, $-CH_2-$, and $-CH_3$, respectively.

Antiviral Activity Screening of Title Compounds against TMV in Vivo. The antiviral tests of $\mathbf{A_1}$ to $\mathbf{A_{30}}$ against TMV were conducted, and the results are summarized in Table 2. Most of the title compounds generally exhibited good antiviral activity against TMV in vivo, and some of them even showed higher antiviral activity than the control ningnanmycin. Our results indicated that compounds $\mathbf{A_5}$, $\mathbf{A_{12}}$, $\mathbf{A_{25}}$, and $\mathbf{A_{27}}$ exhibited higher curative activity than ningnanmycin, with values of 64.3%, 63.5%, 69.0%, and 64.7% at 500 μg/mL, respectively. The other compounds showed moderate curative activities at 500 mg/L. Among these compounds, $\mathbf{A_8}$, $\mathbf{A_{10}}$, $\mathbf{A_{14}}$, $\mathbf{A_{16}}$, $\mathbf{A_{17}}$, $\mathbf{A_{25}}$, and $\mathbf{A_{29}}$ exhibited significant protective effects against TMV and produced relatively high inhibitory activities with values of 68.7%, 66.9%, 63.1%, 71.7%, 64.6%, 66.5%, and 71.6% at 500 μg/mL, respectively. These values are similar to those of ningnanmycin (68.4%) against TMV at 500 μg/mL. Compounds $\mathbf{A_2}$, $\mathbf{A_3}$, $\mathbf{A_5}$, $\mathbf{A_6}$, $\mathbf{A_{10}}$, $\mathbf{A_{12}}$, $\mathbf{A_{14}}$, $\mathbf{A_{16}}$, $\mathbf{A_{24}}$, $\mathbf{A_{28}}$, and $\mathbf{A_{29}}$ inactivated efficacy at 88.4%, 90.2%, 87.5%, 91.9%, 89.2%, 85.1%, 89.3%, 86.1%, 87.8%, 90.0%, and 91.2%, respectively. These compounds exhibited slightly lower inactivation activities than ningnanmycin (95.8%) at 500 μg/mL.

Based on previous bioassays, all the test compounds showed moderate to excellent activity against plant viruses (TMV). Table 3 lists the EC_{50} values of some of the synthesized compounds. In short, the EC_{50} values of about 18 compounds with inhibition rate higher than 40% were obtained. Compounds $\mathbf{A_5}$, $\mathbf{A_{12}}$, $\mathbf{A_{25}}$, and $\mathbf{A_{27}}$ exhibited remarkable curative effects against TMV, with EC_{50} values of 132.25, 138.01, 156.10, and 135.89 μg/mL, respectively. These values indicate stronger curative activity against the tested virus of the compounds than those of the commercial agent ningnanmycin (EC_{50} = 281.22 μg/mL). However, the EC_{50} values of the others are higher than that of ningnanmycin. The results show that their anti-TMV activities are very good. These findings suggest that these compounds can be used as lead structures in discovering new antiviral agents.

Antiviral Activity and Structure Activity Relationship against TMV. Some of the compounds showed potential potency against TMV. Various 1,4-pentadien-3-one substituent groups were introduced into the 4(3*H*)-quinazolinone ring system to establish a structure−activity relationship based on the experimental data. Ningnanmycin, a commercially available plant virucide in China, was used as control. Most of the final compounds showed low to high antiviral activities against TMV at 500 μg/mL. Several title compounds showed comparably good activities relative to ningnanmycin. When R_1 was H and R_2 was substituted with 2-methoxyphenyl, 2-furyl, 4-methylthiazol-5-yl, or 3,4,5-trimethoxyphenyl groups, the corresponding target compounds exhibited excellent activity against TMV. The relationships of the antiviral activities to different R_1 and R_2 were deduced, as shown by the activity values in Table 2. The strongest curative activity against TMV was observed when R_1 was H and R_2 was changed to 2-methoxyphenyl, thiophen-2-yl, 3,4-dimethoxyphenyl, or 4-methoxyphenyl groups. Some of the title compounds were as potent as ningnanmycin. When R_1 was H and R_2 was substituted with 2-fluorophenyl, 4-methylthiazol-5-yl or 3,4,5-trimethoxyphenyl groups, the target compounds exhibited remarkable protective activities against TMV that surpassed those of ningnanmycin. Compounds $\mathbf{A_3}$, $\mathbf{A_{10}}$, $\mathbf{A_{28}}$, and $\mathbf{A_{29}}$ displayed good inactivation activities. The inactivation activities of these compounds were further investigated at various concentrations, using ningnanmycin as commercial control. The presence of 2-fluorophenyl, 2-furyl, 2-methoxyphenyl, or 3,4,5-trimethoxyphenyl groups in a compound effectively improved the antiviral activity of the compound more than that of other groups.

Table 3. EC_{50} Values of Some Compounds against TMV in Vivo

Compd.				EC_{50} (μg/mL)[a]
	R_1	R_2	X	
A_1	H	Ph	2-O	529.03±3.4
A_3	H	2-F-Ph	2-O	484.09±2.8
A_4	8-CH_3	Ph	2-O	496.40±1.7
A_5	H	2-OCH_3	2-O	132.25±2.5
A_7	H	3-NO_2-Ph	4-O	478.58±2.9
A_8	H	4-F-Ph	2-O	502.64±1.6
A_{10}	H	furan-2-yl	2-O	356.34±3.5
A_{12}	8-CH_3	thiophen-2-yl	2-O	138.01±2.3
A_{15}	8-CH_3	2-Cl-5-NO_2-Ph	2-O	509.87±2.1
A_{16}	H	4-methylthiazol-5-yl	2-O	323.76±4.8
A_{22}	8-CH_3	2, 4-di-OCH_3-Ph	4-O	345.15±5.3
A_{23}	8-CH_3	pyrid-2-yl	4-O	318.43±1.5
A_{25}	8-CH_3	3, 4-di-OCH_3-Ph	4-O	156.10±2.6
A_{26}	H	3, 4-di-OCH_3-Ph	4-O	298.44±3.2
A_{27}	8-CH_3	4-OCH_3-Ph	2-O	135.59±2.9
A_{28}	8-CH_3	2-OCH_3-Ph	2-O	289.35±1.4
A_{29}	H	3, 4, 5-tri-OCH_3-Ph	4-O	564.25±1.2
A_{30}	H	3, 4, 5-tri-OCH_3-Ph	2-O	300.18±3.1
Ningnanmycin[b]				281.22±2.0

[a]Average of three replicates. [b]The commercial, agricultural, and antiviral product ningnanmycin was used for comparison of activity.

In summary, 4(3*H*)-quinazolinone analogues containing a 1,4-pentadien-3-one moiety were prepared in moderate yield and evaluated for their antiviral activities against TMV. Some of the synthesized compounds exhibited moderate to excellent antiviral activity in vivo. The preliminary structure−activity relationships established the importance of the presence of a 1,4-pentadien-3-one moiety in the 4(3*H*)-quinazolinone component in obtaining title compounds with the desired antiviral activity. Compounds $\mathbf{A_5}$, $\mathbf{A_{12}}$, $\mathbf{A_{25}}$, and $\mathbf{A_{27}}$ possessed appreciable curative bioactivities against TMV in vivo, and such bioactivities were superior to those of ningnanmycin. Therefore, based on the antiviral studies on the biological efficacies, crop safety, and toxicities of these compounds, they can be considered for further development as a new class of tobacco protection agents.

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■ ASSOCIATED CONTENT

Supporting Information

Results of our physical analyses and the data on the synthesis and characterization of target compounds $\mathbf{A_1}$ to $\mathbf{A_{30}}$. 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.

■ ABBREVIATIONS USED

TLC, thin layer chromatography; EC_{50}, 50% effective concentration; ^{1}H NMR, ^{1}H nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance; TMV, tobacco mosaic virus; CMV, cucumber mosaic virus

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