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Design, Synthesis, and Antiviral Activity of Novel Chalcone Derivatives Containing a Purine Moiety

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To find new antiviral agents, novel chalcone derivatives containing a purine moiety were designed and synthesized by combining bioactive substructures. The antiviral activities of the derivatives against tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) were evaluated. Results showed that most of the derivatives showed antiviral activities. Compounds **3n** and **3p** exhibited excellent curative, protective and inactivation activities against TMV, with the EC_{50} values of 452.4, 416.2, 241.2 and 438.7, 418.6, 261.7 μg•mL^{-1}, respectively, which were better than those of ribavirin (585.8, 436.0 and 268.7 μg•mL^{-1}). Compounds **3n** and **3p** showed remarkable curative and protective activities against CMV. Compound **3n** showed a moderate affinity to TMV coat protein, with binding constant K_a and K_d values of 1.5×10^4 L•mol^{-1} and 79.8 μmol•L^{-1}, respectively. These findings provided an important structural insight for further designs of highly active chalcone derivatives and a basis for further study on their mechanism of action.

Keywords chalcone, purine, antiviral activity, tobacco mosaic virus, cucumber mosaic virus

Introduction

Tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) are serious plant viruses that are difficult to control worldwide.[1] Traditional agrochemicals play a critical role in controlling these diseases. As a successfully registered plant viral inhibitor, ribavirin (**A**, Figure 1) is widely used to prevent plant virus diseases, but its inhibitory effect is less than 50% at 500 μg•mL^{-1}. To date, no chemical treatment can absolutely inhibit TMV and CMV once it has infected plants.[2] Meanwhile, excessive use of agrochemicals could cause adverse effects, such as environmental pollution and treatment resistance. Therefore, searching for novel, environmentally benign and ecologically safe antiviral agents with unique modes of action, remains a daunting task in pesticide research.

Given their low toxicity, easy decomposition, environmental friendliness, and unique mode of action, natural products[3,4] have attracted great attention for development into medicines and pesticides. Some natural products and their derivatives such as antofine and its derivative NK-007,[5,6] phenanthroindolizidine and its analogues,[7] and *β*-carboline alkaloid and its derivatives,[8] display significant inhibitory activities against TMV. So, it is a key pathway for the development of pesticides on the basis of the natural products.

As an important secondary metabolite in plants,[9-12] chalcone and its derivatives have a wide range of biological properties including anticancer,[13,14] antileishmanial,[15] tyrosinase inhibitory,[16] nematicidal,[17] and anti-TMV.[18,19] In our previous studies, a series of chalcone derivatives (**B**, Figure 1) with good bioactivities against TMV and CMV were reported.[20,21] Meanwhile, purines are endogenous inhibitory substances that widely exist in plants and animals; these compounds have been used as scaffolds for the development of biologically functional molecules in medicine. Acyclovir (**C**, Figure 1),[22] ganciclovir[23] and abacavir[24] derived from purine exhibit remarkable antiviral activities. We found that purine derivatives containing a 1,4-pentadien-3-one moiety (**D**, Figure 1) possess excellent antiviral activities against CMV,[25] but their application in controlling TMV has not been reported.

Basing on studies of purines, we found that *N*-9 substituted purine is a core process for biological and pharmaceutical activities,[26-29] while anti-HBV active purine-nucleoside analogs possess amido bonds.[30] To find new anti-TMV candidates, a series of novel chalcone derivatives containing a purine group (Figure 2)

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In Memory of Professor Enze Min.

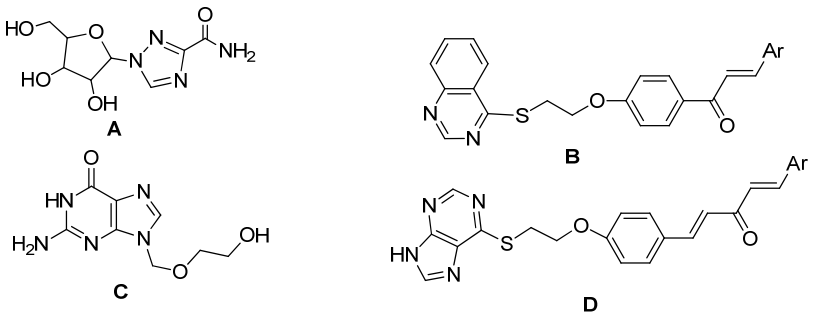


Figure 1 Chemical structures of known antiviral molecules.

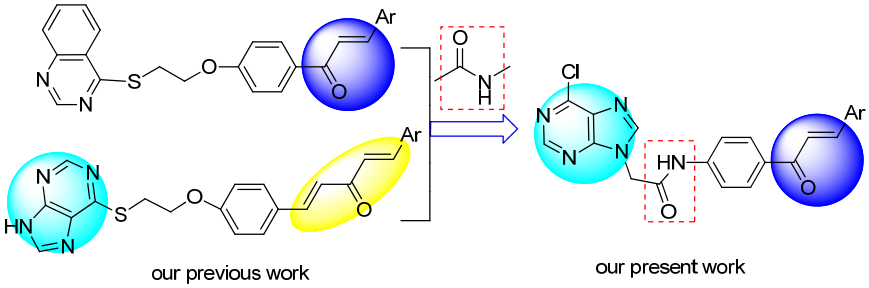
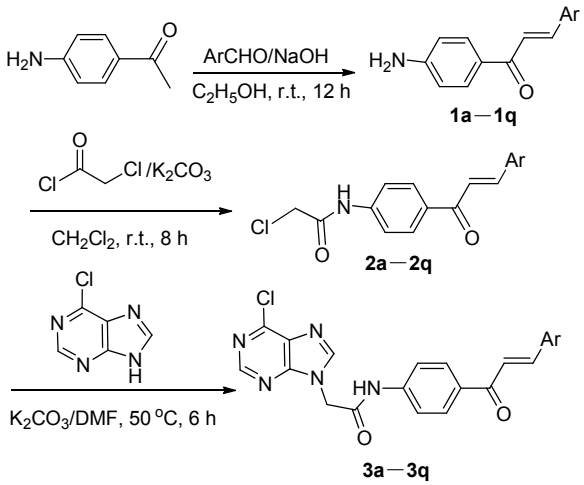


Figure 2 Design of target compounds.

were designed and synthesized through an amido link by combining bioactive substructures (Scheme 1). The anti-TMV and anti-CMV activities of these chalcone derivatives were evaluated. Then, the interaction of the most active compound with tobacco mosaic virus coat protein (TMV CP) was tested through fluorescence spectroscopy (FT) and microscale thermophoresis (MST).

Scheme 1 Synthetic routes of the target compounds



Ar =

a: 4-CH_3-C_6H_4; **b**: 4-OCH_3-C_6H_4; **c**: 2,4-$(OCH_3)_2$-C_6H_3; **d**: 3,4,5-$(OCH_3)_3$-C_6H_2; **e**: 3-F-C_6H_4; **f**: C_6H_5; **g**: 4-Cl-C_6H_4; **h**: 2-F-C_6H_4; **i**: 2,3-Cl_2-C_6H_3; **j**: 2-Br-C_6H_4; **k**: 4-Br-C_6H_4; **l**: 2,4-Cl_2-C_6H_3; **m**: 2-Cl-C_6H_4; **n**: 2,4-F_2-C_6H_3; **o**: 2,6-F_2-C_6H_3; **p**: 4-NO_2-C_6H_4; **q**: 4-F-C_6H_4

Experimental

Chemicals and instruments

Nuclear magnetic resonance (NMR) ^{1}H and ^{13}C spectra were determined on a JEOL-ECX 500 NMR spectrometer (JEOL Ltd., Japan) in DMSO-d_6 at 500 and 125 MHz, using TMS as an internal standard. High resolution mass spectrometer (HRMS) data were recorded on a Thermo Scientific Q Exactive (Thermo, USA). Melting points were recorded on an XT-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China), uncorrected. All solvents and reagents were of analytical reagent grade or chemically pure, and the solvents were dried in advance and distilled before use. Reaction progress was monitored by thin-layer chromatography on silica gel GF_{254}. Column chromatographic purification was carried out using silica gel (200—300 mesh).

General synthetic procedure

As shown in Scheme 1, intermediates **1a**—**1q** were prepared as previously reported,[31,32] and the corresponding intermediates **2a**—**2q** were obtained as previously described.[32] Then, anhydrous K_2CO_3 (0.2 g, 1.45 mmol) was added to a solution of 6-chloro-9*H*-purine (0.2 g, 1.29 mmol) in DMF (4 mL), and the mixture was stirred at room temperature for 1 h. Subsequently, a solution of corresponding **2a**—**2q** (1.29 mmol) in DMF (3 mL) was added to the mixture slowly, warmed to 50 ℃, and then stirred for 6 h. The mixture was cooled to room temperature and then poured into ice water. The solid was filtered, and washed with cold water. Then, the crude product was recrystallized from CH_2Cl_2/C_2H_5OH (V∶V=1∶2), filtered, washed, and dried to obtain the title compounds **3a**—**3q**.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(*p*-tolyl)-acryloyl)phenyl)acetamide (**3a**): Paly yellow solid, yield 87.2%; m.p. 201—202 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.93 (s, 1H, NH), 8.76 (s, 1H, purine-2-

H), 8.70 (s, 1H, purine-8-H), 8.14 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 7.86 (d, J=15.5 Hz, 1H, 3-H), 7.74 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.72 (d, J=8.5 Hz, 1H, 3-Ar-2,6-H), 7.65 (d, J=15.5 Hz, 1H, 2-H), 7.23 (d, J=8.0 Hz, 2H, 3-Ar-3,5-H), 5.30 (s, 2H, CH_2CO), 2.31 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.99, 165.72, 152.79, 152.31, 149.57, 148.98, 144.16, 143.20, 141.15, 133.38, 132.55, 131.06, 130.55, 130.55, 130.07, 130.07, 129.42, 129.42, 121.28, 119.14, 119.14, 46.98, 21.63; ESI-HRMS calcd for $C_{23}H_{19}ClN_5O_2$ $[M+H]^+$ 432.12273, found 432.12158.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (**3b**): Paly yellow solid, yield 85.1%; m.p. 208−209 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.92 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.13 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 7.81 (d, J=9.0 Hz, 2H, 1-Ar-3,5-H), 7.78 (d, J=15.5 Hz, 1H, 3-H), 7.71 (d, J=8.5 Hz, 1H, 3-Ar-2,6-H), 7.66 (d, J=15.5 Hz, 1H, 2-H), 6.98 (d, J=8.0 Hz, 2H, 3-Ar-3,5-H), 5.29 (s, 2H, CH_2CO), 3.78 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.90, 165.69, 161.84, 152.80, 152.32, 149.56, 148.99, 144.08, 143.07, 133.56, 131.29, 131.29, 131.06, 130.46, 130.46, 127.91, 119.83, 119.13, 119.13, 114.93, 114.93, 55.92, 46.98; ESI-HRMS calcd for $C_{23}H_{18}O_3N_5ClNa$ $[M+Na]^+$ 470.09904, found 470.09888.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2,4-dimethoxyphenyl)acryloyl)phenyl)acetamide (**3c**): Yellow solid, yield 80.5%; m.p. 190−192 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.90 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.09 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 7.94 (d, J=15.5 Hz, 1H, 3-H), 7.88 (d, J=9.0 Hz, 1H, 3-Ar-6-H), 7.73 (d, J=15.0 Hz, 1H, 2-H), 7.71 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 6.60 (d, J=2.5 Hz, 1H, 3-Ar-3-H), 6.59 (dd, J=8.5, 2.5 Hz, 1H, 3-Ar-5-H), 5.29 (s, 2H, CH_2CO), 3.87 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 188.03, 165.66, 163.58, 160.43, 152.80, 152.31, 149.56, 148.99, 142.94, 138.77, 133.75, 131.06, 130.52, 130.33, 130.33, 119.31, 119.14,119.14, 116.46, 106.86, 98.80, 56.36, 56.08, 46.98; ESI-HRMS calcd for $C_{24}H_{20}O_4N_5ClNa$ $[M+Na]^+$ 500.10960, found 500.10928.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (**3d**): Yellow solid, yield 88.4%; m.p. 169−171 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.17 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 7.87 (d, J=15.5 Hz, 1H, 3-H), 7.74 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.65 (d, J=15.5 Hz, 1H, 2-H), 7.19 (s, 2H, 3-Ar-2,6-H), 5.30 (s, 2H, CH_2CO), 3.83 (s, 6H, OCH_3), 3.67 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.93, 165.73, 153.62, 153.62, 152.79, 152.31, 149.57, 148.98, 144.63, 140.14, 133.38, 132.55, 131.06, 130.83, 130.60, 130.60, 121.47, 119.12, 119.12, 106.97, 106.97, 60.66, 56.64, 56.64, 46.98; ESI-HRMS calcd for $C_{25}H_{22}O_5N_5ClNa$ $[M+Na]^+$ 530.12017, found 530.12000.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(3-fluorophenyl)acryloyl)phenyl)acetamide (**3e**): Paly yellow solid, yield 80.6%; m.p.>250 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.17 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 7.98 (d, J=15.5 Hz, 1H, 3-H), 7.82 (d, J=10.5 Hz, 1H, 3-Ar-6-H), 7.73 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.68 (d, J=16.0 Hz, 1H, 2-H), 7.65 (s, 1H, 3-Ar-2-H), 7.48−7.37 (m, 1H, 3-Ar-5-H), 7.29−7.21 (m, 1H, 3-Ar-4-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.93, 165.76, 164.00, 162.06, 152.80, 152.31, 149.57, 148.97, 143.41, 142.59, 137.89, 133.12, 131.41, 131.06, 130.72, 130.72, 126.11, 123.83, 119.16, 119.16, 117.82, 115.22; ESI-HRMS calcd for $C_{22}H_{15}O_2N_5ClFNa$ $[M+Na]^+$ 458.07905, found 458.07849.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-cinnamoylphenyl)acetamide (**3f**): Paly yellow solid, yield 85.1%; m.p. 198−200 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.93 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.16 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 7.92 (d, J=15.5 Hz, 1H, 3-H), 7.88−7.82 (m, 2H, 3-Ar-2,6-H), 7.73 (d, J=9.0 Hz, 2H, 1-Ar-3,5-H), 7.69 (d, J=16.0 Hz, 1H, 2-H), 7.48−7.39 (m, 3H, 3-Ar-3,4,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 188.04, 165.73, 152.80, 152.32, 148.99, 144.09, 143.28, 135.27, 133.29, 131.09, 130.62, 130.62, 129.45, 129.45, 129.40, 129.40, 122.39, 119.17, 119.17, 46.99; ESI-HRMS calcd for $C_{22}H_{16}O_2N_5ClNa$ $[M+Na]^+$ 440.08847, found 440.08801.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(4-chlorophenyl)acryloyl)phenyl)acetamide (**3g**): Paly yellow solid, yield 89.5%; m.p. 188−190 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.16 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 7.95 (d, J=15.5 Hz, 1H, 3-H), 7.89 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.73 (d, J=8.5 Hz, 2H, 3-Ar-2,6-H), 7.68 (d, J=15.5 Hz, 1H, 2-H), 7.49 (d, J=8.0 Hz, 2H, 3-Ar-3,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.93, 165.74, 152.80, 152.31, 149.57, 148.98, 143.35, 142.60, 135.54, 134.27, 133.19, 131.08, 131.08, 130.66, 130.66, 129.48, 129.48, 123.15, 119.17, 119.17, 46.99; ESI-HRMS calcd for $C_{22}H_{16}O_2N_5Cl_2$ $[M+H]^+$ 452.06717, found 452.06756.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2-fluorophenyl)acryloyl)phenyl)acetamide (**3h**): Paly yellow solid, yield 90.3%; m.p.>250 ℃; ^1H NMR (500 MHz, DMSO-d_6) δ: 10.99 (s, 1H, NH), 8.81 (s, 1H, purine-2-H), 8.74 (s, 1H, purine-8-H), 8.19 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 8.14 (d, J=8.5, 2.0 Hz, 1H, 3-Ar-6-H), 8.01 (d, J=16.0 Hz, 1H, 3-H), 7.83 (d, J=15.5 Hz, 1H, 2-H), 7.78 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.55−7.48 (m, 1H, 3-Ar-3-H), 7.37−7.31 (m, 2H, 3-Ar-4,5-H), 5.35 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.87, 165.76, 162.43, 152.81, 152.31, 149.57, 148.98, 143.45, 135.15, 133.03, 131.06, 130.69, 130.69, 129.59, 125.49, 124.50, 122.84, 119.23, 119.23, 116.72, 116.55, 46.99; ESI-HRMS calcd for $C_{22}H_{15}O_2N_5ClFNa$ $[M+Na]^+$ 458.07905, found 458.07880.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2,3-dichlorophenyl)acryloyl)phenyl)acetamide (**3i**): Paly yellow solid, yield 88.3%; m.p. 154—156 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.96 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.17 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 8.14 (dd, J = 8.0, 1.0 Hz, 1H, 3-Ar-4-H), 7.98 (s, 2H, 2,3-H), 7.74 (d, J=9.0 Hz, 2H, 1-Ar-3,5-H), 7.69 (dd, J=8.0, 1.5 Hz, 1H, 3-Ar-6-H), 7.44 (t, J=8.0 Hz, 1H, 3-Ar-5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.71, 165.79, 152.79, 152.31, 149.57, 148.97, 143.60, 138.48, 135.47, 133.06, 132.82, 132.53, 132.46, 131.06, 130.86, 130.86, 128.97, 127.73, 126.52, 119.19, 119.19, 46.99; ESI-HRMS calcd for $C_{22}H_{15}O_2N_5Cl_3$ $[M+H]^+$ 486.02858, found 486.02817.

(*E*)-*N*-(4-(3-(2-Bromophenyl)acryloyl)phenyl)-2-(6-chloro-9*H*-purin-9-yl)acetamide (**3j**): Paly yellow solid, yield 89.2%; m.p. 198—200 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.17 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 8.16 (dd, J = 8.5, 2.0 Hz, 1H, 3-Ar-6-H), 7.94 (s, 2H, 2,3-H), 7.73 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.71 (d, J=8.0 Hz, 1H, 3-Ar-3-H), 7.47—7.41 (m, 1H, 3-Ar-5-H), 7.39—7.34 (m, 1H, 3-Ar-4-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.84, 165.77, 152.81, 152.31, 149.57, 148.98, 143.49, 141.35, 134.55, 133.83, 132.98, 132.66, 131.06, 130.78, 130.78, 129.27, 128.77, 125.87, 125.32, 119.20, 119.20, 47.00; ESI-HRMS calcd for $C_{22}H_{16}O_2N_5BrCl$ $[M+H]^+$ 496.01704, found 496.01706.

(*E*)-*N*-(4-(3-(4-Bromophenyl)acryloyl)phenyl)-2-(6-chloro-9*H*-purin-9-yl)acetamide (**3k**): Paly yellow solid, yield 75.2%; m.p. 176—178 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.15 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 7.95 (d, J=15.5 Hz, 1H, 3-H), 7.82 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.72 (d, J=8.5 Hz, 2H, 3-Ar-2,6-H), 7.66 (d, J=16.0 Hz, 1H, 2-H), 7.62 (d, J=8.5 Hz, 2H, 3-Ar-3,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.94, 165.75, 152.79, 152.32, 149.57, 148.98, 143.35, 142.71, 134.58, 133.17, 132.41, 132.41, 131.30, 131.30, 131.06, 130.67, 130.67, 124.44, 123.18, 119.17, 119.17, 46.98; ESI-HRMS calcd for $C_{22}H_{16}O_2N_5BrCl$ $[M+H]^+$ 496.01704, found 496.01675.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2,4-dichlorophenyl)acryloyl)phenyl)acetamide (**3l**): Paly yellow solid, yield 83.5%; m.p. 221—222 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.96 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.23 (d, J=8.5 Hz, 1H, 3-Ar-6-H), 8.17 (d, J=9.5 Hz, 2H, 1-Ar-2,6-H), 8.01 (d, J=15.5 Hz, 1H, 3-H), 7.91 (d, J=15.0 Hz, 1H, 2-H), 7.73 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.72 (d, J=1.5 Hz, 1H, 3-Ar-3-H), 7.52 (dd, J=8.5, 2.0 Hz, 1H, 3-Ar-5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.67, 165.79, 152.80, 152.31, 149.57, 148.98, 143.57, 137.32, 136.08, 135.65, 132.88, 131.94, 131.06, 130.83, 130.83, 130.33, 130.04, 128.48, 125.69, 119.17, 119.17, 46.99; ESI-HRMS calcd for $C_{22}H_{14}O_2N_5Cl_3Na$ $[M+Na]^+$ 508.01053, found 508.01031.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2-chlorophenyl)acryloyl)phenyl)acetamide (**3m**): Paly yellow solid, yield 81.0%; m.p. 186—188 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.69 (s, 1H, purine-8-H), 8.21—8.15 (m, 3H, 1-Ar-2,6-H, 3-Ar-6-H), 7.97 (s, 2H, 2,3-H), 7.73 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.53 (d, J=8.0 Hz, 1H, 3-Ar-3-H), 7.47—7.41 (m, 2H, 3-Ar-4,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.87, 165.77, 152.80, 152.31, 149.58, 148.97, 143.49, 138.60, 134.85, 132.99, 132.86, 132.49, 131.06, 130.77, 130.77, 130.57, 129.09, 128.22, 125.16, 119.21, 119.21, 46.99; ESI-HRMS calcd for $C_{22}H_{15}O_2N_5Cl_2Na$ $[M+Na]^+$ 474.04950, found 474.04929.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2,4-difluorophenyl)acryloyl)phenyl)acetamide (**3n**): Paly yellow solid, yield 85.4%; m.p. 191—193 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.96 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.69 (s, 1H, purine-8-H), 8.19 (d, J=8.5 Hz, 1H, 3-Ar-6-H), 8.14 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 7.94 (d, J=16.0 Hz, 1H, 3-H), 7.73 (d, J=8.5 Hz, 2H, 1-Ar-3,5-H), 7.72 (d, J=15.0 Hz, 1H, 2-H), 7.57—7.51 (m, 1H, 3-Ar-3-H), 7.39—7.34 (m, 1H, 3-Ar-5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.75, 165.77, 163.12, 160.65, 152.79, 152.31, 149.57, 148.97, 143.46, 134.22, 132.98, 131.21, 130.68, 130.68, 130.51, 124.20, 119.82, 119.21, 119.21, 113.17, 105.37, 46.98; ESI-HRMS calcd for $C_{22}H_{15}O_2N_5ClF_2$ $[M+H]^+$ 454.08769, found 454.08749.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(2,6-difluorophenyl)acryloyl)phenyl)acetamide (**3o**): Paly yellow solid, yield 80.2%; m.p.＞250 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.95 (s, 1H, NH), 8.76 (s, 1H, purine-2-H), 8.69 (s, 1H, purine-8-H), 8.04 (d, J=9.0 Hz, 2H, 1-Ar-2,6-H), 7.84 (d, J=16.0 Hz, 1H, 3-H), 7.74 (d, J=9.0 Hz, 2H, 1-Ar-3,5-H), 7.64 (d, J=16.0 Hz, 1H, 2-H), 7.57—7.51 (m, 1H, 3-Ar-4-H), 7.25—7.18 (m, 2H, 3-Ar-3,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.95, 165.77, 162.62, 160.55, 152.79, 152.30, 149.57, 148.96, 143.57, 133.05, 132.74, 131.05, 130.59, 130.59, 129.38, 127.82, 119.35, 119.35, 113.04, 112.84, 112.58, 46.99; ESI-HRMS calcd for $C_{22}H_{14}O_2N_5ClF_2Na$ $[M+Na]^+$ 476.06963, found 476.06918.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(4-nitrophenyl)acryloyl)phenyl)acetamide (**3p**): Paly yellow solid, yield 85.7%; m.p.＞250 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.96 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.25 (d, J=9.0 Hz, 2H, 3-Ar-3,5-H), 8.19 (d, J=8.5 Hz, 2H, 3-Ar-2,6-H), 8.14 (d, J=8.5 Hz, 2H, 1-Ar-2,6-H), 8.13 (d, J=16.0 Hz, 1H, 3-H), 7.77 (d, J=16.0 Hz, 1H, 2-H), 7.74 (d, J=9.0 Hz, 2H, 1-Ar-3,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.86, 165.79, 152.80, 152.32, 149.57, 148.98, 148.57, 143.60, 141.81,141.23, 132.92, 131.06, 130.86, 130.86, 130.38, 130.38, 126.48, 124.48, 124.48, 119.20, 119.20, 47.00; ESI-HRMS

calcd for $C_{22}H_{15}O_4N_6ClNa$ $[M + Na]^+$ 485.07355, found 485.07327.

(*E*)-2-(6-Chloro-9*H*-purin-9-yl)-*N*-(4-(3-(4-fluorophenyl)acryloyl)phenyl)acetamide (**3q**): Paly yellow solid, yield 87.2%; m.p. 181－182 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.92 (s, 1H, NH), 8.77 (s, 1H, purine-2-H), 8.70 (s, 1H, purine-8-H), 8.15 (d, J＝8.5 Hz, 2H, 3-Ar-3,5-H), 7.97－7.90 (m, 2H, 3-Ar-2,6-H), 7.89 (d, J＝15.5 Hz, 1H, 3-H), 7.73 (d, J＝8.5 Hz, 2H, 1-Ar-2,6-H), 7.69 (d, J＝15.5 Hz, 1H, 2-H), 7.29－7.24 (m, 2H, 1-Ar-3,5-H), 5.30 (s, 2H, CH_2CO); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 187.98, 165.72, 164.89, 162.91, 152.80, 152.30, 149.58, 148.96, 143.27, 142.84, 133.29, 131.98, 131.75, 131.06, 130.60, 130.60, 122.33, 119.18, 119.18, 116.54, 116.37, 47.00; ESI-HRMS calcd for $C_{22}H_{16}O_2N_5ClF$ $[M + H]^+$ 436.09711, found 436.09647.

Antiviral biological assay

Purification of TMV and CMV TMV and CMV were inoculated in *Nicotiana. tabacum* cv. K326, and purified by the Gooding method.[33] The concentration of virus was determined through an ultraviolet spectrophotometer at 260 nm.

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

Curative activities of compounds against TMV and CMV *in vivo* Growing leaves of *N. tabacum* L. of the same ages were selected. TMV and CMV (6 μg•mL^{-1}) were inoculated on the whole leaves with a brush, which were previously scattered with silicon carbide. After 1 h, the leaves were washed with water and dried. Then 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 recorded 3 to 4 d after inoculation.[25,34] Experiments for each compound were conducted in triplicate.

Protective activities of compounds against TMV and CMV *in vivo* The solution of the compound was smeared on the left side, whereas the solvent was smeared on the right side of the growing *N. tabacum* L. leaves and served as the control. The leaves, which were previously scattered with silicon carbide, were inoculated with 6 μg•mL^{-1} TMV and CMV after 12 h. Then, the leaves were washed with water. At 3－4 d after inoculation, the number of local lesions was counted.[25,34] All experiments for each compound were conducted in triplicate.

Inactivation activities of compounds against TMV and CMV *in vivo* The virus was inhibited by mixing with the compound solution at the same volume for 30 min and inoculated on the left side of *N. tabacum* L. leaves, and the solvent and virus mixture was smeared on the right side of the leaves as the control. The number of local lesions was recorded 3 to 4 d after inoculation.[25,34] Every experiment for each compound was conducted in triplicate.

The *in vivo* inhibiton rates of the compounds were calculated using the following formula ("av" means average). Inhibition rate＝[(av number of local lesionsin-control–av number of local lesions smeared with drugs)/ av number of local lesions of control]×100%.

Binding constant of compound 3n to TMV CP

In order to study the interactions of compound **3n** to TMV CP, the binding constant (K_a and K_d) of compound **3n** with TMV CP was measured through FT and MST. The TMV CP was cloned, expressed and purified as previously described[35] with a solution containing 50 mmol•L^{-1} phosphate at pH 7.4 for buffer. 500 μL 10 μmol•L^{-1} TMV-CP SEC buffer solution (20 mmol•L^{-1} PBS, 100 mmol•L^{-1} sodium chloride (pH 7.4)) was added to a 1.0 cm quartz cell. Then, the buffer solution was titrated with compound **3n** solution (1 mmol•L^{-1}). The ultimate antiviral **3n** concentrations were 0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0 and 20.0 μmol•L^{-1}. Fluorescence spectra were collected on a FluoroMax-4 spectrofluorometer at an excitation wavelength of 278 nm and the excitation and emission bandwidths were 5 nm. Then, the data emission spectra were recorded from 280 to 400 nm at 25 ℃. In addition, the K_d of compound **3n** to TMV CP labelled according to the recommended methods of the manufacturer, was determined on a NanoTemper Monolith NT.115 at 40% MST power and 90% LED. Compound **3n** (from 0.22 to 1000 μm) was diluted at 25 ℃ in the SEC buffer, with the ratio of **3n** and TMV CP at 1∶1 (V∶V).

Results and Discussion

Synthesis

As shown in Scheme 1, the compounds **1a**－**1q** were synthesized through Claisen-Schmidt condensation between 4-aminoacetophenone and aryl aldehyde. Then, **1a**－**1q** containing amide bond chalcones were obtained using chloroacetyl chloride. To obtain title compounds with a high yield, the reaction conditions (including reaction solvent, catalyst, and temperature) for the preparation of compound **3a** were optimized. Meanwhile, the molar ratio of 6-chloro-9*H*-purine/catalyst is 1∶1.15 and the reaction time is 6 h. The results showed that a maximum yield of **3a** up to 87.2% was achieved when the solvent, catalyst and temperature was DMF, K_2CO_3 and 50 ℃, respectively (Table 1). Other compounds with yield ranging from 75.2% to 90.3% were synthesized under these conditions.

Antiviral activity assay and structure-activity relationships

The antiviral activities of compounds **3a** － **3q** against TMV and CMV were assayed through the half leaf blight spot method, which was performed in triplicate (Tables 2 and 3). Ribavirin was used as a reference antiviral agent.

Table 1 Effect of different conditions for the synthesis of **3a**[a]

No.	Solvent	Catalyst	Temperature/℃	Yield[b]/%
1	acetone	Et_3N	25	0
2	THF	Et_3N	25	0
3	DMF	Et_3N	25	0
4	acetone	K_2CO_3	25	25.5
5	THF	K_2CO_3	25	30.2
6	CH_3CN	K_2CO_3	25	35.6
7	DMF	K_2CO_3	25	43.8
8	DMF	K_2CO_3	50	87.2

[a] General conditions: reactions were performed with the molar ratio of 6-chloro-9*H*-purine to catalyst 1∶1.15. [b] Yields of isolated products.

Table 2 Inhibition rates of the compounds against TMV *in vivo* at 500 μg•mL^{-1} (*I*/%)[a]

Compound	Curative	Protective	Inactivation
3a	41.2±1.9	43.8±1.5	80.8±1.9
3b	45.8±2.3	51.2±2.8	70.9±2.1
3c	42.3±2.2	44.3±2.1	56.3±3.3
3d	35.7±2.6	16.8±3.2	45.6±1.4
3e	42.5±3.3	59.4±1.3	73.5±2.6
3f	42.5±2.1	30.6±1.5	57.9±2.2
3g	53.7±2.2	45.9±1.9	75.1±1.8
3h	39.8±1.7	35.2±3.2	79.8±2.0
3i	43.8±1.9	53.9±3.1	85.1±3.7
3j	52.4±3.1	49.8±2.4	81.2±1.7
3k	46.3±1.2	21.4±1.1	45.8±2.3
3l	43.7±2.7	41.9±1.8	75.9±3.9
3m	42.5±2.9	47.5±2.3	71.3±2.4
3n	52.4±2.3	56.8±1.8	88.9±0.9
3o	48.9±2.1	55.1±1.7	80.5±3.1
3p	54.5±1.8	56.8±2.7	84.1±2.5
3q	53.9±2.5	52.8±1.4	79.5±2.3
Ribavirin	37.9±1.9	51.8±2.3	72.9±2.4

[a] All results are expressed as mean±SD; n=3 for all groups.

Table 3 Inhibition rates of the compounds against CMV *in vivo* at 500 μg•mL^{-1} (*I*/%)[a]

Compound	Curative	Protective	Inactivation
3a	24.5±2.5	52.6±1.9	31.9±3.1
3b	28.6±1.4	45.8±1.2	38.2±1.7
3c	34.8±2.7	51.2±2.5	38.9±2.9
3d	45.2±1.0	48.9±1.9	56.9±3.3
3e	35.7±1.6	49.8±2.7	54.5±1.9
3f	45.9±2.2	58.9±1.7	44.4±2.9
3g	48.6±2.3	57.3±2.8	54.8± 3.1
3h	35.1±1.9	23.5±2.3	34.9±1.5
3i	39.8±2.3	48.6±2.3	51.2±2.8
3j	31.2±1.8	41.5±3.7	42.5±2.3
3k	38.6±2.5	48.2±2.8	47.2±3.6
3l	43.5±1.1	52.1±2.4	51.9±1.7
3m	36.7±2.1	47.9±2.3	54.3±3.3
3n	47.8±2.7	52.8±2.5	65.1±1.9
3o	39.1±2.0	48.5±1.1	56.3±1.2
3p	44.8±1.9	55.9±1.7	59.0±1.8
3q	41.1±2.7	48.5±1.2	57.1±2.5
Ribavirin	41.2±1.6	52.1±3.5	68.2±2.4

[a] All results are expressed as mean±SD; n=3 for all groups.

As shown in Tables 2 and 3, most of the title compounds exhibited inhibitory activities against TMV *in vivo*. Compounds **3e**, **3i**, **3n**, **3o**, **3p**, and **3q** exhibited remarkable curative, protective and inactivation activities at 500 μg•mL^{-1}, with the values of 42.5%, 43.8%, 52.4%, 48.9%, 54.5%, 53.9%; 59.4%, 53.9%, 56.8%, 55.1%, 56.8%, 52.8% and 73.5%, 85.1%, 88.9%, 80.5%, 84.1%, 79.5%, respectively, which were better than those of ribavirin (37.9%, 51.8%, and 72.9%). Meanwhile, compounds **3a**−**3q** exhibited moderate antiviral activities against CMV. Compounds **3f**, **3g**, **3n**, **3p** showed better curative and protective activities with the values of 45.9%, 48.6%, 47.8%, 44.8% and 58.9%, 57.3%, 53.8%, 55.9%, respectively, than those of ribavirin (41.2% and 52.1%) at 500 μg•mL^{-1}. In order to confirm antiviral activities against TMV, the EC_{50} values of curative, protective and inactivation activities of compounds **3e**, **3i**, **3n**, **3o**, **3p**, and **3q** were investigated and summarized in Table 4, the results showed that compounds **3n** and **3p** exhibited remarkable curative, protective and inactivation activities against TMV, with EC_{50} values of 452.4, 416.2, 241.2 and 438.7, 418.6, 261.7 μg•mL^{-1}, respectively, which were better than those of ribavirin (585.8, 436.0 and 268.7 μg•mL^{-1}).

Table 4 EC_{50} values of the compounds against TMV *in vivo* (μg•mL^{-1})[a]

Compound	Curative	Protective	Inactivation
3e	612.1±3.5	405.8±3.3	312.5±8.6
3i	603.2±2.0	481.5±5.4	258.8±5.7
3n	452.4±3.7	416.2±3.9	241.2±4.9
3o	567.4±2.8	455.1±2.8	278.4±3.8
3p	438.7±3.5	418.6±3.3	261.7±7.5
3q	501.3±2.9	442.8±4.1	299.5±3.9
Ribavirin	585.8±4.1	436.0±4.3	268.7±5.1

[a] All results are expressed as mean±SD; n=3 for all groups.

On the basis of the antiviral activities against TMV, the preliminary structure-activity relationships were also

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observed. Electron-withdrawing groups favored antiviral activities at the same position, this finding is evidenced by the activity order of **3n** (Ar=2,4-F_2-C_6H_3)> **3c** [Ar=2,4-$(OCH_3)_2$-C_6H_3] and **3p** (Ar=4-NO_2-C_6H_4) >**3a** (4-CH_3-C_6H_4). Meanwhile, the bulky group of aromatic ring did not favor, for example **3b** (Ar= 4-OCH_3-C_6H_4) > **3c** (Ar = 2,4-$(OCH_3)_2$-C_6H_3) > **3d** [Ar=3,4,5-$(OCH_3)_3$-C_6H_2] and **3q** (Ar=4-F-C_6H_4)>**3g** (Ar = 4-Cl-C_6H_4) > **3k** (Ar = 4-Br-C_6H_4). However, when 2-substituted compound with different electron-withdrawing groups was introduced, the bulky group favored antiviral activities, for example, **3j** (Ar = 2-Br-C_6H_4) > **3m** (Ar = 2-Cl-C_6H_4) > **3h** (Ar = 2-F-C_6H_4). Notably, the 4-substituted compound with the same group showed better antiviral activities than the 2-substituted compound, such as **3q** (Ar=4-F-C_6H_4) >**3h** (Ar=2-F-C_6H_4), **3g** (Ar=4-Cl-C_6H_4)>**3m** (Ar= 2-Cl-C_6H_4), and **3k** (Ar = 4-Br-C_6H_4) > **3j** (Ar = 2-Br-C_6H_4). This finding indicates that appropriate compact electron-withdrawing groups favor antiviral activities against TMV, which is an important structural insight for further design of chalcone derivatives with highly active properties.

Binding constant of compound 3n to TMV CP

TMV CP, which is necessary for virus assembly initiation and elongation, is a key functional protein for TMV to infect the host.[36,37] Binding constant is an important parameter for studying the interaction between the ligand (molecule) and the receptor (protein). Under the optimal conditions, the SEC buffer is PBS (20 mmol•L^{-1} phosphate pH 7.4, 100 mmol•L^{-1} sodium chloride), the temperature is 25 ℃, the LED and MST power is 90% and 40% respectively. The K_a vaules of compound **3n** were determined through FT and MST. The binding constant of **3n** to TMV CP is listed in Table 5 and Figure 3. Compound **3n** showed a moderate affinity to TMV CP, with a K_a value of 1.5×10^4 L•mol^{-1}, which was better than that of ribavirin (2.5×10^3 L•mol^{-1}, indicating weak affinity). Meanwhile, the result of K_d through MST was similar to the FT finding, with a K_d between **3n** and TMV CP of 79.8 μmol•L^{-1}, which was better than that of ribavirin (112.5 μmol•L^{-1}). Thus, compound **3n** exhibited a moderate affinity to TMV CP, which is consistent with the inactivation activity. Therefore, the binding constant of the compound to TMV CP plays significant roles in antiviral capacity.

Table 5 Binding constant (K_a and K_d) of compound **3n**

Compound	K_a of FT/ (L•mol^{-1})	K_d of MST/ (μmol•L^{-1})	EC_{50} of inactivation/ (μg•mL^{-1})
3n	1.5×10^4	79.8±2.8	241.2±4.9
Ribavirin	2.5×10^3	112.5±3.7	268.7±5.1

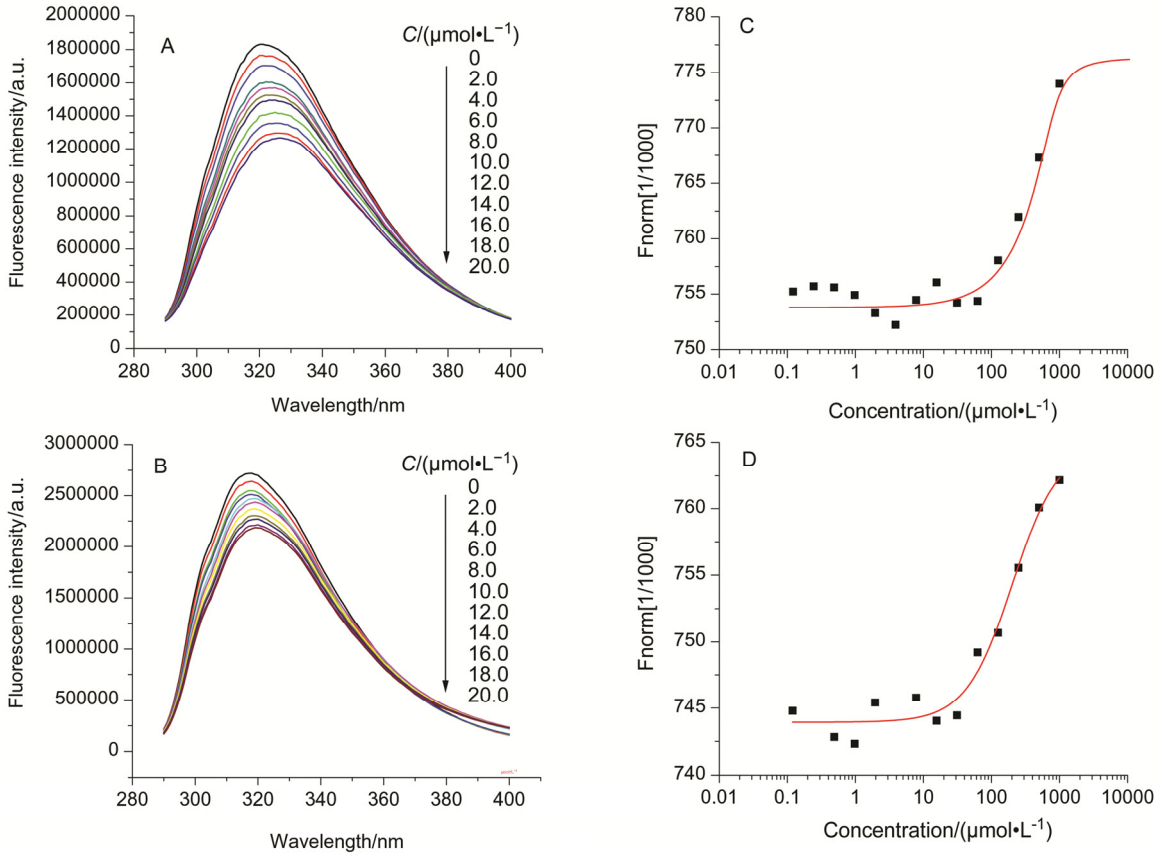

Figure 3 Binding affinity of **3n** and Ribavirin to TMV CP. (A) Fluorescence emission spectra of TMV CP in the presence of **3n**, (B) fluorescence emission spectra of TMV CP in the presence of Ribavirin, (C) binding of TMV-CP and **3n** through MST and (D) binding of TMV-CP and Ribavirin through MST.

Conclusions

A series of novel chalcone derivatives containing a purine moiety was designed and synthesized through an amido link by combining bioactive substructures. Their anti-TMV and anti-CMV activities were evaluated. Compounds **3n** and **3p** exhibited excellent curative, protective and inactivation activities against TMV and showed remarkable curative, and protective activities against CMV *in vivo*. The introduction of appropriate compact electron-withdrawing groups to the aromatic ring strengthened antiviral activities against TMV. Compound **3n** showed a moderate affinity to TMV CP, which is consistent with the inactivation activity. These findings provide an important structural insight for the design of highly active chalcone derivatives and serve as a basis for further study on their mechanisms of action.

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References

[1] Craeger, A. N.; Scholthof, K. B.; Citovsky, V.; Scholthof, H. B. *Plant Cell* **1999**, *11*, 301.
[2] Reichman, M.; Devash, Y.; Suhadolnik, R. J.; Sela, I. *Virology* **1983**, *128*, 240.
[3] Qian, X. H.; Lee, P. W.; Cao, S. *J. Agric. Food Chem.* **2010**, *58*, 2613.
[4] Seiber, J. N. *J. Agric. Food Chem.* **2011**, *59*, 1.
[5] An, T. Y.; Huang, R. Q.; Yang, Z.; Zhang, D. K.; Li, G. R.; Yao, Y. C.; Gao, J. *Phytochemistry* **2001**, *58*, 1267.
[6] Wang, Q. M.; Yao, Y. C.; Huang, R. Q.; Fan, Z. J.; Li, G. R.; Yu, X. S. *Agrochemicals* **2007**, *46*, 425.
[7] Wang, Z. W.; Wei, P.; Wang, L. Z.; Wang, Q. M. *J. Agric. Food Chem.* **2012**, *60*, 10212.
[8] Song, H. J.; Liu, Y. X.; Liu, Y. X.; Wang, L. Z.; Wang, Q. M. *J. Agric. Food Chem.* **2014**, *62*, 1010.
[9] Van Puyvelde, L.; De Kimpe, N.; Costa, J.; Munyjabo, V.; Nyirankuliza, S.; Hakizamungu, E.; Schamp, N. *J. Nat. Prod.* **1989**, *52*, 629.
[10] Haraguchi, H.; Ishikawa, H.; Mizutani, K.; Tamura, Y.; Kinoshita, T. *Bioorg. Med. Chem.* **1998**, *6*, 339.
[11] Vogel, S.; Heilmann, J. *J. Nat. Prod.* **2008**, *71*, 1237.
[12] Xu, H.; Wang, X. M.; Wei, X.; Li, J. Y.; Liu, K. *Chin. Chem. Lett.* **2009**, *20*, 576.
[13] Konieczny, M. T.; Konieczny, W.; Sabisz, M.; Skladanowski, A.; Augustynowicz-Kopec, E.; Wakiec, R.; Zwolska, Z. *Chem. Pharm. Bull.* **2007**, *55*, 817.
[14] Lin, Y. P.; Hu, C. Y.; Zheng, X.; Wang, X. L.; Wan, C. P.; Mao, Z. W. *Chin. J. Org. Chem.* **2017**, *37*, 237 (in Chinese).
[15] Zai, W. J.; Jiang, P. P.; Zhang, X. Y.; Loiseau, P. M.; Guo, S. H.; Fan, X. S. *Chin. J. Org. Chem.* **2015**, *35*, 1335 (in Chinese).
[16] Niu, C.; Li, G.; Aisa, H. A. *Chem. J. Chin. Univ.* **2014**, *35*, 1204 (in Chinese).
[17] Caboni, P.; Aissani, N.; Demurtas, M.; Ntalli, N.; Onnis, V. *Pest Manag. Sci.* **2016**, *72*, 125.
[18] French, C. J.; Elder, M.; Leggett, F.; Ibrahim, R. K.; Neil Towers, G. H. *Can. J. Plant Pathol.* **1991**, *13*, 1.
[19] Du, G.; Han, J. M.; Kong, W. S.; Zhao, W.; Yang, H. Y.; Yang, G. Y.; Gao, X. M.; Hu, Q. F. *Bull. Korean Chem. Soc.* **2013**, *34*, 1263.
[20] Song, B. A.; Xie, Y.; Hu, D. Y.; Xue, W.; Wu, F.; Wan, Z. H.; Li, X. Y.; Du, X. L. *CN 103755646 A*, **2014**.
[21] Wan, Z. H.; Hu, D. Y.; Li, P.; Xie, D. D.; Gan, X. H. *Molecules* **2015**, *20*, 11861.
[22] Rideout, J. E.; Krenitsky, T. A.; Elion, G. *EP 21293 A2*, **1981** [*Chem. Abstr.* **1981**, *95*, 43583].
[23] Verheyden, J. P.; Martin, J. C. *US 4355032 A*, **1982** [*Chem. Abstr.* **1982**, *100*, 126895].
[24] Daluge, S. M. *EP 434450 A2*, **1998** [*Chem. Abstr.* **1998**, *116*, 106698].
[25] Wu, F.; Li, P.; Hu, D. Y.; Song, B. A. *Res. Chem. Intermed.* **2016**, *42*, 7153.
[26] Li, J. J.; Gui, X. X. *Chin. Chem. Lett.* **2014**, *25*, 1341.
[27] Xu, H. B.; Ye, R. F.; Yang, S. Y.; Li, R.; Yang, X. *Chin. Chem. Lett.* **2014**, *25*, 29.
[28] Wang, J. H.; Wang, Q. D.; Zhang, L. R.; Fan, H. *Chin. J. Chem.* **2013**, *31*, 1181.
[29] D'hooghe, M.; Mollet, K.; De Vreese, R.; Jonckers, T. H. M.; Dams, G.; De Kimpe, N. *J. Med. Chem.* **2012**, *55*, 5637.
[30] Li, D. L.; Bao, H. L.; Tan, Q. T.; Ke, Y. P.; You, T. P. *Chin. J. Chem.* **2005**, *23*, 1659.
[31] Wu, J. Z.; Wang, C.; Cai, Y. P.; Peng, J.; Liang, D. L.; Zhao, Y. J.; Yang, S. L.; Li, X. K.; Wu, X. P.; Liang, J. *Med. Chem. Res.* **2012**, *21*, 444.
[32] Ren, J. L.; Zhang, X. Y.; Yu, B.; Wang, X. X.; Shao, K. P.; Zhu, X. G.; Liu, H. M. *Eur. J. Med. Chem.* **2015**, *93*, 321.
[33] Gooding, G. V. Jr.; Hebert, T. T. *Phytopathology* **1967**, *57*, 1285.
[34] Song, B. A.; Zhang, H. P.; Wang, H.; Yang, S.; Jin, L. H.; Hu, D. Y.; Pang, L. L.; Xue, W. *J. Agric. Food Chem.* **2005**, *53*, 7886.
[35] Li, X. Y.; Song, B. A.; Hu, D. Y.; Wang, Z. C.; Zeng, M. J.; Yu, D. D.; Chen, Z.; Jin, L. H.; Yang, S. *Virol. J.* **2012**, *9*, 279.
[36] Chen, M. H.; Hu, D. Y.; Li, X. Y.; Yang, S.; Zhang, W. Y.; Li, P.; Song, B. A. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3840.
[37] Ouyang, G. P.; Chen, Z.; Cai, X. J.; Song, B. A.; Bhadury, P. S.; Yang, S.; Jin, L. H.; Xue, W.; Hu, D. Y.; Zeng, S. *Bioorg. Med. Chem.* **2008**, *16*, 9699.

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