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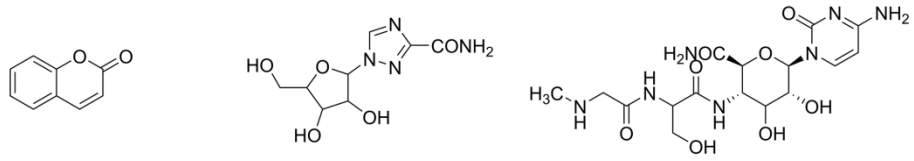


Figure 1. Chemical structures of Cumarin, Ribavirin and Ningnanmycin.

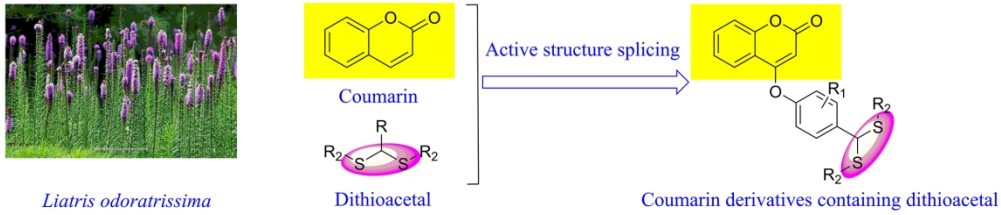


Figure 2. Design of title compounds

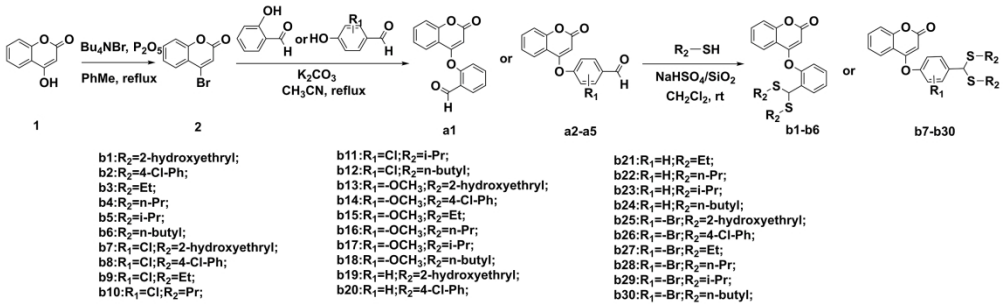


Figure 3. Synthetic route for title compounds b1-b30.

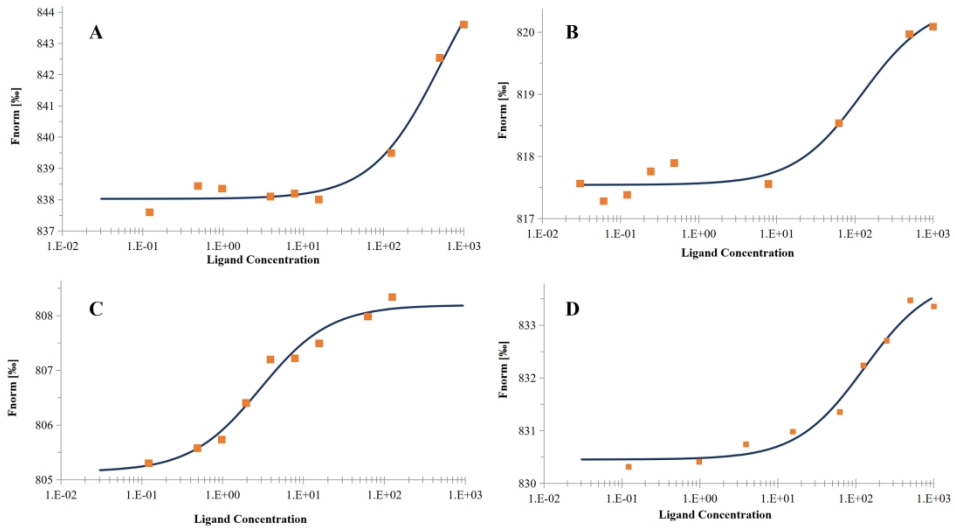


Figure 4. Microscale thermophoresis (MST) test results of compounds b20 (A), b24 (B), b21 (C), and Ribavirin (D) with TMV CP.

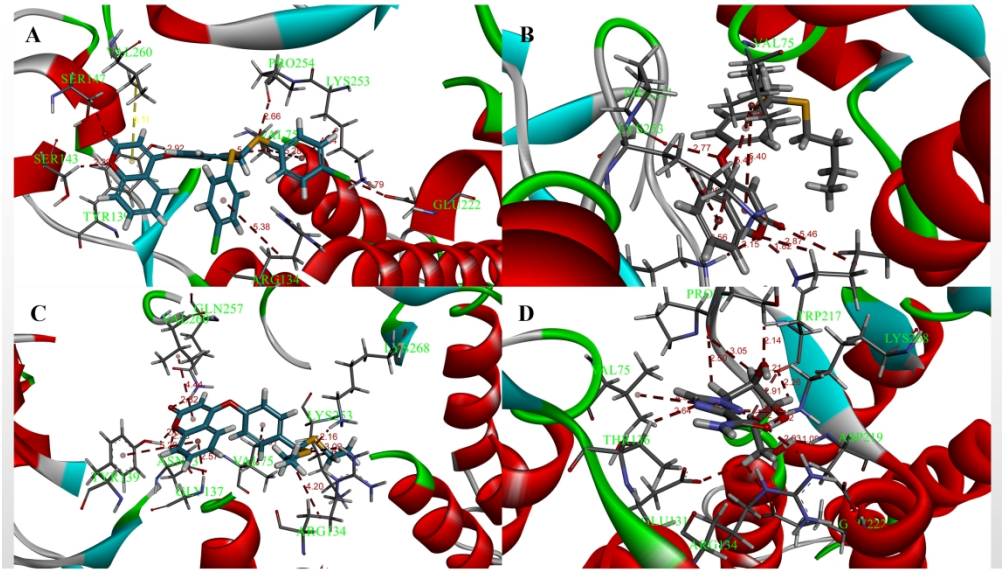


Figure 5. Molecule docking results of compounds b20 (A), b24 (B), b21 (C), and Ribavirin (D) with TMV CP

219x128mm (300 x 300 DPI)

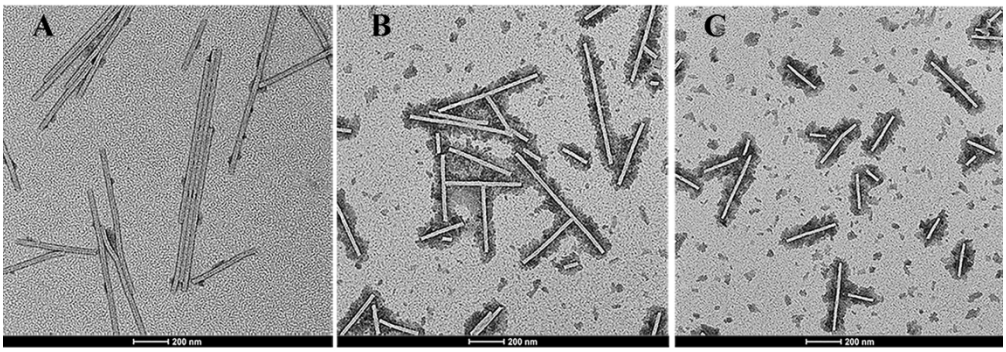
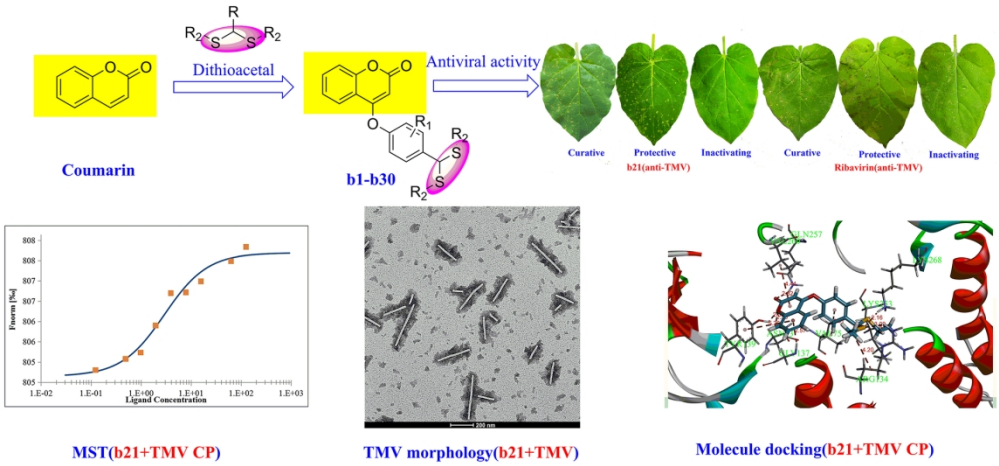


Figure 6. The effect on the morphology of TMV particles of compounds CK (A), Ribavirin (B) and b21 (C).

216x80mm (300 x 300 DPI)



TOC Graphic

84x43mm (600 x 600 DPI)

Design, Synthesis and Antiviral Activity of Coumarin Derivatives Containing Dithioacetal Structures

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ABSTRACT: In this study, a series of coumarin derivatives containing dithioacetals were synthesized, characterized, and assessed for their anti-tobacco mosaic virus (TMV) activity. Biological tests showed that most of the title compounds exhibited significant anti-TMV biological activity; in particular, compound **b21** showed good inactivating activity anti-TMV, with an EC_{50} of 54.2 mg/L, superior to that of ribavirin (134.2 mg/L). Transmission electron microscopy analyses showed that compound **21** severely ruptured TMV particles. The interaction of compound **b21** with TMV coat protein (TMV CP) was investigated using microscale thermophoresis (MST) and molecular docking. Compound **b21** exhibited a strong binding ability (Kd) to TMV CP, with a value of 2.9 μM, superior to ribavirin.

KEYWORDS: *coumarin, dithioacetal, tobacco mosaic virus, transmission electron microscopy, molecular docking*

INTRODUCTION

Tobacco mosaic virus (TMV) is known as plant cancer and is widely distributed in nature. TMV is the most persistent plant virus, being able to survive in dry plants for up to 100 years, and more than 885 species in 165 plant families can be infected by the virus.[1] It is difficult to effectively control TMV once a plant is infected by. Ribavirin and ningnanmycin (Figure 1) are two current antiviral agents used to control plant viral diseases, but the former has a poor effect, while the latter has an unsatisfactory field control effect.[2,3] Therefore, new, broad-spectrum, and highly effective anti-TMV agents need to be developed.

Figure 1

Natural products have been increasingly used in the creation of new pesticides in recent years because of their unique safety.[4] Coumarin (Figure 1) are an important organic heterocyclic compound that has pyranone as a parent ring structure. Natural coumarins are present in many high-altitude plants in nature such as *Umbelliferae*, *Rutaceae*, *Leguminosae*, *Compositae*, *Orchidaceae*, etc. Furthermore, coumarins have a variety of unique physiological and biological activities, such as anti-cancer,[5] anti-HIV,[6,7] antibacterial,[8,9] antioxidant and anti-inflammatory,[10,11] anti-influenza,[12] anti-tuberculosis,[13] anti-Alzheimer's disease,[14,15] antiviral[16,17,18] and other biological and medicinal activities.

Our group has reported a series of vanillin derivatives containing dithioacetal (Figure 2) which are very active against cucumber mosaic virus (CMV) and potato mosaic virus (PVY).[19] By further derivatization, our group synthesized a series of

dithioacetal derivatives with anti-CMV, TMV, PVY and tomato chlorosis virus (ToCV) activities.[20,21] However, no compounds with good curative, protective and passivation activity against TMV have been obtained, especially compounds without very good inactivating activity against TMV. Therefore, in the present study, a series of coumarin derivatives containing dithioacetal (Figure 2) were designed and synthesized, and their anti-TMV protective, curative and inactivating activities were tested. The results of biological activity assays indicate that most of these compounds exhibit excellent anti-TMV activity.

Figure 2

MATERIALS AND METHODS

Chemicals and Instruments. Reagents and solvents were purchased from commercial sources. Reaction progress was monitored by thin-layer chromatography (TLC) on silica GF_{254}, and the melting points of compounds were confirmed using a WRX-4 monocular microscope (Shanghai Yice Apparatus & Equipment Co., Ltd, China). ^{1}H NMR and ^{13}C NMR were conducted on a Bruker Ascend-400 spectrometer (Bruker, Germany) with deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d_6) as the solvent, while tetramethyl silane (TMS) was used as an internal standard. High-resolution mass spectrometry (HRMS) data were obtained using a Thermo Scientific Q Exactive instrument (Thermo, Missouri, USA).

General Procedure for the preparation of intermediates 2 and a1-a5. The synthetic route of coumarin derivatives containing dithioacetal is shown in Figure 3. Substituted 4-bromo-2H-chromen-2-one (intermediate **2**) was synthesized according

to Balalas's method,[22] where 4-hydroxycoumarin (1 g, 6.16 mmol) was added to toluene (12.4 mL, 0.5 M), under Ar atmosphere and heated to 100 ℃. Subsequently, Bu_4NBr (2.98 g, 9.26 mmol) was added, heating was continued until the Bu_4NBr was dissolved, and then P_2O_5 (1.75 g, 12.34 mmol) was added, and the mixture was further refluxed for 3 hours. The hot organic layer was then poured out, and the lower layer was extracted with hot toluene (2×20 mL). The combined toluene layer was then successively washed with 5% $NaHCO_3$ (2 × 30 mL), water (50 mL) and saturated brine (50 mL). Subsequently, the organic layer was dried with anhydrous Na_2SO_4 (10 g) and concentrated in vacuum to obtain the intermediate **2** (1.34 g, 96%), m.p. 87-89 ℃.

Intermediates a1-a5 were prepared as described in the literature.[23] K_2CO_3 (4 mmol) was added to a solution containing intermediate **2** (2 mmol) and salicylaldehyde (2 mmol) or substituted 4-hydroxybenzaldehyde (2 mmol) in acetonitrile, after which the mixture was heated at 80 ℃, and the reaction was monitored by TLC until the reaction was completed. The mixture then filtered and concentrated before being recrystallized from ethyl acetate to afford intermediates **a1-a5**. The representative data for compound **a1** are shown below.

*2-((2-oxo-2H-chromen-4-yl)oxy)benzaldehyde (**a1**)*, light yellow solid; yield, 62%; m.p. 145-147 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.16 (s, 1H, -CHO), 8.08-8.01 (m, 2H, Ar-H), 7.77-7.73 (m, 1H, Ar-H), 7.68-7.63 (m, 1H, Ar-H), 7.52 (t, J = 7.6 Hz, 1H, Ar-H), 7.46-7.33 (m, 2H, Ar-H), 7.32-7.25 (m, 1H, Ar-H), 5.37 (s, 1H, -CH-); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 187.54 (1C), 166.40 (1C), 162.16 (1C), 153.97 (1C),

153.78 (1C), 136.32 (1C), 133.39 (1C), 130.88 (1C), 128.31 (1C), 127.64 (1C), 124.55 (1C), 123.03 (1C), 122.85 (1C), 117.16 (1C), 114.87 (1C), 94.48 (1C); HRMS (ESI) m/z for $C_{16}H_{10}O_4Na$ $[M+Na]^+$ calcd: 289.0471, found: 289.0466.

Figure 3

General Synthesis Procedure for the Coumarin Derivatives Containing Dithioacetal Moiety b1-b30. The synthetic route of the title compounds **b1-b30** is shown in Figure 3. The title compounds were synthesized from the intermediate **a** and various types of thiols according to the methods described in the literature.[24] Compounds **a1-a5** (1 mmol) and thiol (2 mmol) were dissolved in dichloromethane at room temperature, followed by the addition of the catalyst $NaHSO_4/SiO_2$ (0.01 mmol). The reaction was monitored by TLC. After the material was completely converted, the solid SiO_2 was removed by filtration, and the filtrate was concentrated in vacuo to obtain the crude product, which was recrystallized from ethyl acetate to afford title compounds **b1-b30** (Figure 3). The representative data for compound **b1** are shown below.

4-(2-(bis((2-hydroxyethyl)thio)methyl)phenoxy)-2H-chromen-2-one ***(b1),*** white solid, yield, 76.8%; m.p. 107-109 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11-8.01 (m, 1H, Ar-H), 7.83-7.81 (m, 1H, Ar-H), 7.68-7.61 (m, 1H, Ar-H), 7.42-7.36 (m, 4H, Ar-H), 7.14-7.12 (m, 1H, Ar-H), 5.44 (s, 1H, -CH-), 5.27 (s, 1H, -SCHS-), 3.66-3.63 (m, 4H, $-SCH_2-$), 2.83-2.76 (m, 2H, $-CH_2-$), 2.65-2.59 (m, 2H, $-CH_2-$), 2.45 (s, 2H, -OH); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.89 (1C), 162.69 (1C), 153.71 (1C), 149.14 (1C), 133.25 (1C), 132.92 (1C), 130.10 (1C), 129.99 (1C), 127.62 (1C), 124.56 (1C),

123.07 (1C), 121.95 (1C), 117.13 (1C), 115.12 (1C), 94.01 (1C), 61.57 (1C), 46.49 (2C), 35.69 (2C); HRMS (ESI) *m/z* for $C_{20}H_{20}O_5NaS_2$ $[M+Na]^+$ calcd: 427.0644, found: 427.0644.

Antiviral activity assay. *Extraction of TMV*[25]. The TMV viruses were propagated in *Nicotiana. tabacum* cv. K326, ground in phosphate buffer and filtered through multilayer gauze. The extract was centrifuged at 10,000×g for 5 min, treated twice with polyethylene glycol 6000, centrifuged again, and the extract was stored at 4 ℃. The absorbance values were tested using an ultraviolet spectrophotometer at 260 nm.

Curative mode Anti-TMV[26] *activities of title compounds in vivo. Nicotiana. tabacum* L. plants were used to evaluate the anti-TMV activities. The whole leaf was sprinkled with silicon carbide, inoculated with TMV virus at a concentration of 6×10^{-3} mg mL^{-1}, rinsed with water after half an hour, and then air dried. The drug solution was evenly applied to the right side of the leaves, while the solvent was applied to the left side of the leaves as a control. The plants were incubated at 28 ℃ in a greenhouse under an illumination of 10 000 lx at 28±2 ℃. After 3-4 days, the number of local lesions was counted. Measurements were performed in triplicate.

Protective mode Anti-TMV activities of title compounds in vivo. The drug solution was evenly applied to the right side of the leaves, while the solvent was applied to the left side of the leaves as a control. After air drying, the plants were placed in a greenhouse for 24 hours. The whole leaves were sprinkled with diamond, inoculated with TMV virus, and incubated for half an hour, and then rinsed with water and air dried. The plants were incubated at 28 ℃ in a greenhouse under an illumination of 10

000 lx at 28±2 ℃. After 3-4 days, the number of local lesions was counted. Measurements were performed in triplicate.

Inactivating mode Anti-TMV activities of title compounds in vivo. Nicotiana. tabacum L. plants were used to evaluate the anti-TMV activities. After the whole leaf was sprinkled with silicon carbide, the drug and TMV virus solutions were mixed for 30 min and then inoculated on the left leaf, while the right leaf was inoculated with the TMV virus solution as a control. After incubating for half an hour, the leaves were rinsed with water and incubated at 28 ℃ in a greenhouse under an illumination of 10 000 lx at 28±2 ℃. After 3-4 days, the number of local lesions was counted. Measurements were performed in triplicate.

Effect of compounds on the morphology of TMV particles.[27,28] After mixing the compound solution with an equal volume of the TMV virus solution and incubating for 30 min, the mixture was adsorbed on a 200-mesh copper mesh carbon support membrane and counterstained with 1% phosphotungstic acid with a pH of 7.4. for 30 s. After fully drying, the morphologies of the TMV particles were observed via transmission electron microscope (TEM) with an FEI Talos F200C instrument at 200 kV.

Molecular docking. The interaction model of compounds **b20**, **b21** and **b24** with TMVCP (PDB code: 1EI7) was studied using Discovery Studio 4.5.[29,30] The molecular docking results were processed according to a previously described method.[31]

Determination of binding capacity. The interaction of compounds with TMV CP

177 was studied through MST. The Kd values of compounds with TMV CP were assessed
178 through MST using Monolith NT.115 software (Nano Temper Technologies, Germany)
179 at 25 ℃ .[30,32]

180 **RESULTS AND DISCUSSION**

181 **Chemistry.** The synthetic route of the coumarin derivatives containing dithioacetal
182 is shown in Figure 3. 4-Bromocoumarin (**2**) was synthesized by Appel Reaction,
183 acetonitrile was used as a solvent and K_2CO_3 was used as a catalyst. The intermediate
184 **2** was refluxed with salicylaldehyde or the correspondingly substituted
185 4-hydroxybenzaldehyde for 5-10 hours to produce the intermediates **a1-a5**, and the
186 title compounds **b1-b30** were synthesized using $NaHSO_4/SiO_2$ as a catalyst by
187 condensation of **a1-a5** and a substituted thiol (Figure 3). The structures of
188 intermediates **a1-a5** and title compounds **b1-b30** have been identified by ^{1}H NMR,
189 ^{13}C NMR and HRMS (Supporting Information).

190 **Antiviral Activity.** *Anti-TMV Activity in Vivo.* The anti-TMV activities of the target
191 compounds **b1-b30** at a concentration of 500 mg/L were evaluated by the half-leaf
192 method, and ribavirin was used as a reference drug.[33] The anti-TMV activity results
193 for these compounds are shown in Table 1. The target compounds showed excellent
194 activity against TMV. Compounds **b15**, **b21** and **b24** showed good curative effects
195 with values of 65.8, 62.1 and 67.3%, respectively, which were superior to ribavirin
196 (40.3%). Compounds **b6**, **b16** and **b18** showed good protective effects with values of
197 61.9, 68.5 and 64.6%, respectively, which were superior to ribavirin (51.2%).
198 Moreover, compounds **b15**, **b21** and **b24** showed good inactivating effects with values

of 81.7, 94.2 and 84.2%, respectively, which were superior to that of ribavirin (71.2%). In addition, the EC_{50} of inactivating activity of compounds **b15**, **b21** and **b24** (68.4, 54.2, and 65.3 mg/L, respectively) were superior to that of ribavirin (134.2 mg/L). Interestingly, compounds **b21 and b24** showed excellent activity in all three modes (curative, protective, and inactivating), with compound **b21** showing the best inactivating activity.

Structure-activity relationships (SARs). SARs were determined based on the preliminary results. The type and position of the substituent of the title compounds greatly influenced their anti-TMV curative, protective and inactivating activities. When R_2 was an aliphatic group (Et, *n*-Pr and *n*-butyl), it increases the anti-TMV activity of the compound was increased. For example, in terms of anti-TMV curative activity, **b15**, **b16** and **b18** > **b14**; **b21** and **b24** > **b20**; and **b27**, **b20** and **b30** > **b26**. However, this was not followed by all the compounds. For example, **b2** > **b3**, **b4** and **b6**. In terms of protective activity, **b3**, **b4** and **b6** > **b2**; **b9** and **b12** > **b8**; **b16** and **b18** > **b14**; **b21**, **b22** and **b24** > **b20**; and **b27**, **b28** and **b30** > **b26**. However, this effect was not observed in compound **b10**. When R_2 was aliphatic, there was no branching (Et, n-Pr and n-butyl group), which is better than *i*-Pr. For example, in terms of protective activity, **b16** >**b17**; **b22** > **b23**; and b28 > b29, with the exception of **b10** < **b11**. In terms of inactivating activity, **b10** > **b11**; **b22** > **b23**; and **b28** > **b29**. However, **b17** did not follow this rule.

Determination of binding capacity. TMV CP plays a very important role in tRNA synthesis and mRNA translation in the self-assembly of anti-TMV agents.[34] Since the

target compound exhibited significant inactivating activity, with compound **b21** showing the best inactivating activity, we speculated that compound **b21** was highly active against TMV CP. Therefore, the binding affinities of **b20**, **b21**, **b24** and ribavirin toward TMV CP were measured via MST. The results of the MST analysis are shown in Table 2 and Figure 4. The results showed that compared with ribavirin (128.7±34.7 μM), compound **b21** and TMV CP had stronger binding activity, with a dissociation constant (Kd) of 2.9±0.8 μM, which was better than that of **b20** (527.7±210.9 μM) and **b24** (123.4±54.7 μM). The results of the MST analysis were consistent with the previously observed inactivating abilities of the compounds.

Figure 4

Molecular docking. To further investigate the combination modes of the target compounds and TMV CP, we used Discovery Studio 4.5 for molecular docking analysis. Compounds **b20**, **b24** and **b21** were selected for molecular docking with TMV CP (PDB code: 1EI7).[32] The docking results are shown in Figure 5, and the binding activity of these compounds with TMV-CP can be explained by hydrogen bonding and nonhydrogen bonding interactions. As shown in Figure 5A, Compound **b20** forms one conventional hydrogen bond with the active site of TYR139 at a distance of 2.92 Å. In addition, it forms one halogen (2.79 Å) with GLU222, one sulfur-X (2.66 Å) with PRO254, and VAL75 (5.21 Å & 5.28 Å), LYS253 (4.64 Å), VAL260 (5.11 Å) and ARF134 (5.38 Å) form five pi-alkyl interactions. Compound **b24** forms two conventional hydrogen bonds with amino acids LYS268 (2.68 Å) and ARG134 (1.82 Å), as shown in Figure 5B. In addition, it forms an unfavorable

acceptor-acceptor interaction with PRO254 (2.77 Å), forming three pi-alkyl interactions are formed with LYS253 (4.49 Å) and VAL75 (5.40 Å & 5.28 Å). Compound **b21** forms five conventional hydrogen bonds with amino acids GLN257 (2.82 Å), ASN73 (2.38 Å), TYR139 (2.05 Å), LYS268 (2.16 Å) and ARG134 (3.09 Å), one pi-donor hydrogen bond with GLY137 (2.57 Å), and form four alkyl or pi-Alkyl bonds with amino acids VAL260 (4.44 Å), VAL75 (4.81 Å) and LYS253 (6.26 Å), as shown in Figure 5C. Molecular docking results of ribavirin with TMV CP showed that ribavirin forms three conventional hydrogen bonds with amino acids LYS268 (2.48 and 1.72 Å) and ARG134 (2.03 Å), one pi-alkyl interaction with VAL75 (4.54 Å), and one unfavorable donor-donor interaction with ASP219 (1.08 Å), as shown in Figure 5D. The docking results indicate that compound **b21** and TMV-CP have five conventional hydrogen bonds, which are more than ribavirin, compound b20 and compound **b24**, suggesting that hydrogen bonding between the compound and TMV-CP may play a critical role in their interaction.

Figure 5

Effect of the compounds on the morphology of TMV particles. Transmission electron microscope (TEM) studies of the effects of **b21** and ribavirin on the morphology of TMV granules (Figure 6). Normal TMV particles are linear, rod-like structures (Figure 6A) with almost no breaks. After 30 min of interaction between ribavirin and TMV, partial fragmentation of TMV particles was observed (Figure 6B). When **b21** and TMV interacted for 30 min, the TMV particles were observably broken, with different lengths and short rod-like structures (Figure 6C).

Figure 6

In summary, in this study, we synthesized a series of coumarin derivatives containing dithioacetal moieties and tested their anti-TMV activities. The results of the biological activity assay showed that the title compound exhibited good curative, protective and inactivation activities. In particular, compounds **b15**, **b21** and **b24** exhibited excellent anti-TMV inactivation activity with EC_{50} values of 68.4, 54.2 and 65.3 mg/L, respectively. SAR analysis results showed that the introduction of unbranched aliphatic groups (Et, *n*-Pr and *n*-butyl) into the compound enhances their anti-TMV activity. With respect to the title compound, it has good anti-TMV inactivation activity, and compound **b21** exhibited good binding ability toward TMV CP through molecular docking analysis. The results of MST experiments showed that compound **b21** has strong binding ability with TMV CP, which is consistent with the results of molecular docking experiments. The TEM results showed that the morphology of the TMV particles was severely disrupted and ruptured in the presence of compound **b21**. These findings indicate that compound **b21** has a strong binding ability toward TMV and can disrupt its structure and cause its rupture, thereby inactivating the virus and inhibiting the infection of plants. The results of the current study provide reliable support for the use of the dithioacetal-containing coumarin structure as a potential lead compound for anti-TMV virus agents.

CONTENT ASSOCIATED

Supporting Information

The synthesis, physical analysis, ^{1}H NMR spectra, ^{13}C NMR spectra and

high-resolution mass spectrum (HRMS) of compounds **a1-a5** and **b1-b30** can be found in the supplementary data.

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Notes

The authors declare no competing financial interest.

ABRREVIATIONS USED

EC_{50}, half-maximal effective concentration; TMS, tetramethylsilane; ^{1}H NMR, ^{1}H nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance; HRMS, high resolution mass spectrum; CMV, cucumber mosaic virus; TMV, tobacco mosaic virus; PVY, potato mosaic virus; ToCV, tomato chlorosis virus; THF, tetrahydrofuran; TEM, transmission electron microscopy; MST, microscale thermophoresis; SARs, Structure-activity relationships; Kd, dissociation constant.

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Information of FIGURE

Figure 1. Chemical structures of Cumarin, Ribavirin and Ningnanmycin.

Figure 2. Design of title compounds.

Figure 3. Synthetic route for title compounds **b1-b30**.

Figure 4. Microscale thermophoresis (MST) test results of compounds **b20** (A), **b24** (B), **b21** (C), and **Ribavirin** (D) with TMV CP.

Figure 5. Molecule docking results of compounds **b20** (A), **b24** (B), **b21** (C), and **Ribavirin** (D) with TMV CP

Figure 6. The effect on the morphology of TMV particles of compounds **CK** (A), **Ribavirin** (B) and **b21** (C).

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437 **Table 1. Antiviral activities of target compounds b1-b30 against TMV *in vivo* at**

438 **500 mg/L**

Compound	Curative activity[a] (%)	Protective activity[a] (%)	Inactivating activity[a] (%)	EC_{50} for TMV inactivating activity[a] (mg/L)
b1	43.7±1.7	47.6±1.2	60.5±4.8	242.7±3.6
b2	56.9±1.8	42.7±2.0	67.9±1.9	183.2±4.3
b3	45.6±1.3	50.5±3.3	73.3±4.5	117.8±4.6
b4	34.2±3.7	45.1±2.5	63.2±3.6	262.4±3.3
b5	49.3±2.8	43.7±3.5	64.2±4.9	220.5±7.8
b6	47.2±2.2	61.9±1.4	78.3±3.2	75.3±2.7
b7	44.2±1.9	39.4±1.9	53.2±4.2	363.7±3.2
b8	44.1±4.3	31.5±4.4	62.8±4.4	248.9±2.8
b9	46.0±3.3	45.6±1.2	77.4±4.9	98.2±4.5
b10	44.1±4.4	25.5±1.6	76.2±4.2	104.3±4.3
b11	41.4±5.0	43.1±2.5	69.7±3.2	162.3±5.3
b12	42.2±1.1	55.5±1.5	78.4±4.2	102.2±2.8
b13	24.1±4.0	49.6±5.0	74.5±4.0	132.4±6.4
b14	38.2±4.5	50.8±3.8	63.5±5.1	216.4±3.1
b15	65.8±2.3	49.4±3.1	81.7±2.0	68.4±4.4
b16	47.3±4.3	68.2±3.7	74.1±1.5	125.5±3.5
b17	45.3±2.5	25.7±3.2	79.0±4.5	84.4±2.0
b18	49.7±2.1	64.6±1.0	68.2±3.8	194.2±1.9

b19	28.2 ±4.3	22.7 ±2.9	45.4 ±1.9	421.5 ±7.6
b20	44.1 ±2.4	30.0 ±4.3	61.6 ±1.7	232.9 ±2.6
b21	62.1 ±3.1	54.5 ±1.9	94.2 ±1.1	54.2 ±2.8
b22	40.2 ±2.1	42.9 ±1.2	78.2 ±4.1	83.6 ±4.3
b23	33.1 ±1.3	26.6 ±3.7	66.4 ±1.1	173.4 ±3.4
b24	67.3 ±2.1	55.5 ±1.8	84.2 ±2.1	65.3 ±1.8
b25	53.2 ±1.6	49.1 ±2.2	78.2 ±3.1	94.6 ±3.7
b26	39.5 ±2.8	28.9 ±4.3	63.3 ±2.3	224.1 ±3.1
b27	45.3 ±2.8	49.2 ±3.1	68.1 ±1.9	159.7 ±2.6
b28	44.0 ±4.3	57.6 ±1.1	73.3 ±3.1	133. ±2.5
b29	32.2 ±2.3	42.1 ±2.2	58.2 ±3.4	322.6 ±2.0
b30	52.1 ±3.0	49.4 ±3.2	78.1 ±1.3	107.5 ±3.7
Ribavirin[b]	40.3 ±2.6	51.2 ±3.3	71.2 ±2.4	134.2 ±4.6

439 [a] Average of three replicates.
440 [b] Ribavirin was used as the control.
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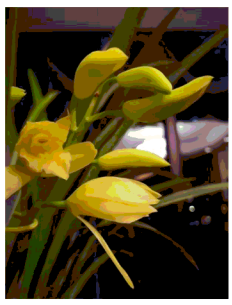
446 **Table 2 Interaction results of target compounds with TMV CP**

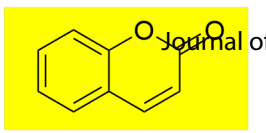
Compound	**b20**	**b21**	**b24**	Ribavirin[b]
Kd[a] (μM)	527.7±210.9	2.9±0.8	123.4±54.7	128.7±34.7
EC_{50} (mg/L)	232.9±2.6	54.2±2.8	65.3±1.8	134.2±4.6

447 [a] Average of three replicates.

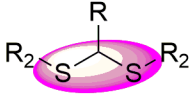
448 [b] Ribavirin was used as the control.



Cymbidium

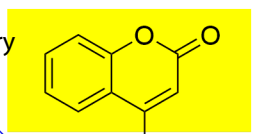


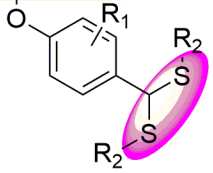
Coumarin



Dithioacetal

Active structure splicing





Coumarin derivatives containing dithioacetal