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New chalcone derivatives: synthesis, antiviral activity and mechanism of action†

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In this work, twenty-eight chalcone derivatives containing a purine (sulfur) ether moiety were synthesized and their antiviral activities were evaluated. Biological results showed that compound **5d** exhibited outstanding inactive activity against tobacco mosaic virus (TMV) *in vivo* ($EC_{50} = 65.8 \mu\text{g mL}^{-1}$), which is significantly superior to that of ribavirin ($EC_{50} = 154.3 \mu\text{g mL}^{-1}$). Transmission electron microscopy indicated that compound **5d** can break the integrity of TMV particles. The results of microscale thermophoresis, fluorescence titration and molecular docking showed that compound **5d** had stronger combining affinity ($K_a = 1.02 \times 10^5 \text{ L mol}^{-1}$, $K_d = 13.4 \mu\text{mol L}^{-1}$) with TMV coat protein (TMV-CP), which is due to the formation of five hydrogen bonds between compound **5d** and the amino-acid residues of TMV-CP. These findings revealed that compound **5d** can effectively inhibit the infective ability of TMV. This work provides inspiration and reference for the discovery of new antiviral agents.

1. Introduction

As an important plant virus, tobacco mosaic virus (TMV) can cause serious plant diseases and huge economic losses to agricultural production. Statistics showed that the loss caused by TMV is up to \$ 100 million worldwide each year.¹ Owing to the absolute parasitic nature of plant virus to host cell and the lack of its complete immune system, and the diversity of virus transmission modes, the prevention and control of plant virus disease is still a pivotal problem in agricultural production. Few chemical agents for completely controlling the virus are currently unavailable.² Ribavirin and Ningnanmycin as two successful antiviral agents were widely used to prevent TMV disease, but only exhibit moderate field control effects.³ Therefore, it is an extremely urgent demand to develop more novel, high-efficient and low risk agents against TMV.

Natural products are always used as models or inspiration for the discovery of new agrochemicals with broad-spectrum biological activities.⁴ Chalcone, a kind of natural organic compound, has typical diaryl ketone scaffold with wide range of biological activities, such as anti-inflammatory,⁵ antimicrobial,^{6,7} insecticidal,⁸ antiviral^{9–11} and so on. Purine widely spread in nature, themselves and their derivatives also have a broad spectrum of biological activities, for example, antimicrobial,¹²

antiviral^{13,14} and antibacterial.¹⁵ Furthermore, we reported some compounds containing purine and chalcone moieties. Biological results indicated that the introduction of purine (sulfur) ether and the addition of diethyl malonate or nitromethane in the double bond are beneficial to the antiviral activity of the chalcone compounds.^{16–18}

TMV-CP plays an indispensable role in translation of mRNA, transcription of tRNA, extension, and self-assembly of TMV.^{19,20} It is always seen as a potential functional protein for development of antiviral drugs and exploration of the interaction of molecular–protein.²¹ In our previous works, we obtained some chalcone compounds with good anti-TMV activity based on TMV-CP.^{16,21,22}

To discover new antiviral agents with highly active and continue our research on chalcone antiviral drugs. We designed and synthesized a series of chalcone derivatives containing a purine (sulfur) ether moiety (Fig. 1), and evaluated their anti-TMV activities *in vivo*. Then, the preliminary interaction mechanism of the target compounds was performed *via* transmission electron microscopy (TEM), fluorescence titration (FT), microscale thermophoresis (MST) and molecular docking.

2. Experimental

2.1 General synthetic procedure of the intermediates 1a–1f

Firstly, aqueous sodium hydroxide solution (5% NaOH, 5.0 mmol) was added to a round-bottomed flask containing 1-(4-hydroxyphenyl)ethan-1-one (1.0 mmol) and differently substituted aldehydes (2.0 mmol). The mixture was stirred at room temperature for 24 h. After the reaction was completed, the mixture was poured into ice water and slowly dripped 5%

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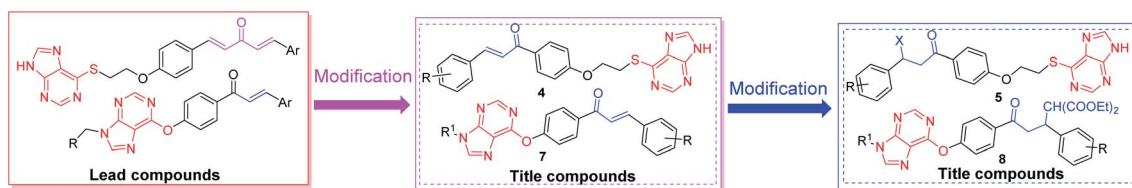


Fig. 1 Design strategy of the title compounds.

Table 1 Optimization of the reaction conditions for compound 5d

No.	Solvent	Catalyst	Temperature (°C)	Yield ^a (%)
1	EtOH	Et ₃ N	23	51
2	EtOH	K ₂ CO ₃	23	35
3	EtOH	KOH	23	92
4	MeOH	Et ₃ N	23	48
5	MeOH	K ₂ CO ₃	23	42
6	MeOH	KOH	23	85
7	EtOH	KOH	50	91
8	EtOH	KOH	76	60
9	MeOH	KOH	50	80
10	MeOH	KOH	76	64

^a The crude yield of the reaction.

HCl until a large amount of yellow solids were precipitated. Finally, the mixture was filtered under vacuum, and the residue was dried to yield the intermediates **1a–1f**.

2.2 General synthetic procedure of the intermediates 2a–2f

The intermediates **1a–1f** (1.0 mmol) and potassium carbonate (1.5 mmol) in acetonitrile (30 mL) were stirred for 1 h, and 1,2-dibromoethane (3.0 mmol) was slowly added, the reaction mixture was refluxed at 76 °C for 8 h. After completion of the reaction, the reaction system was poured into water, extracted with ethyl acetate, the solvent was removed under depressurization, and the residue was purified by column

chromatography on silica gel with a mixture of ethyl acetate/petroleum ether = 1 : 5 (v/v) to obtain the intermediates **2a–2f**.

2.3 General synthetic procedure of the intermediate 3

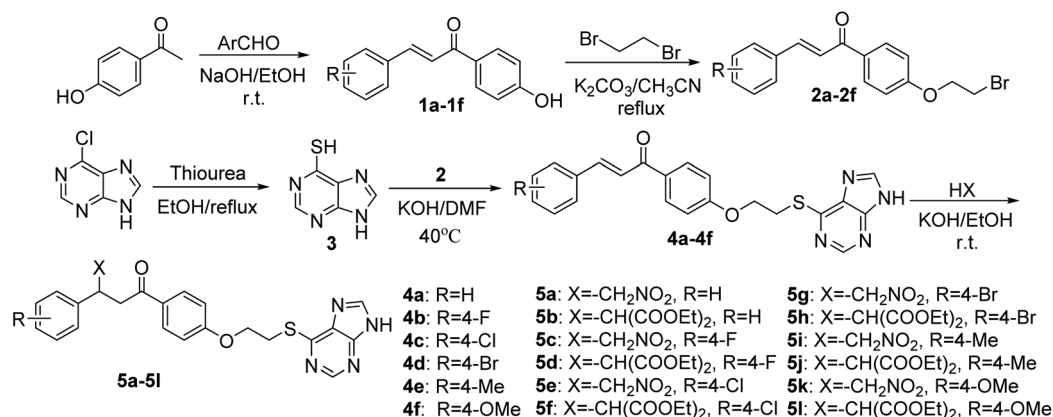
A solution of 6-chloro-9H-purine (1.0 mmol), thiourea (1.2 mmol) in ethanol (30 mL) was added to a reaction flask, and the mixture was refluxed for 3 h. The reaction system was poured into a saturated aqueous solution of sodium hydroxide, adjust the pH acidic with glacial acetic until a large amount of yellow solid appeared. The intermediate **3** was obtained by suction filtration and desiccation.

2.4 General synthetic procedure of the target compounds 4a–4f

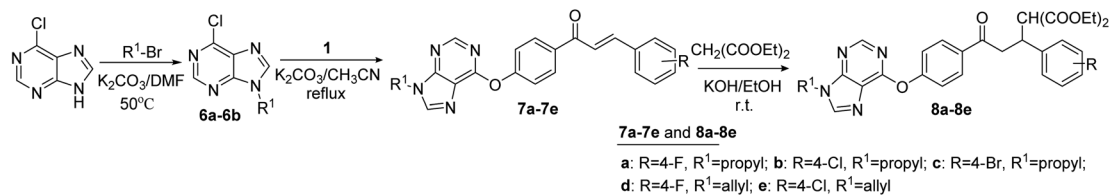
The intermediates **2a–2f** (0.5 mmol), potassium hydroxide (2.0 mmol) in DMF (10 mL) were stirred at room temperature for 1 h, the intermediate **3** (1.0 mmol) was added and the reaction system was stirred at 40 °C for 2–6 h. After the reaction was completed, the mixture was poured into saturated salt water. The precipitated solids were washed with small amount of CH₂Cl₂ and filtrated. The crude product was purified by recrystallization with EtOH or column chromatography on silica gel with a mixture of ethyl acetate/petroleum ether = 6 : 1 (v/v) to yield the target compounds **4a–4f**.

2.5 General synthetic procedure of the target compounds 5a–5l

A mixture of compounds **4a–4f** (1.0 mmol), nitromethane (20.0 mmol) or diethyl malonate (5.0 mmol) in ethanol was stirred for 0.5 h, potassium hydroxide (1.0 mmol/1.2 mmol) was dissolved



Scheme 1 The synthetic route of the target compounds **4a–4f** and **5a–5l**.



Scheme 2 The synthetic route of the target compounds 7a–7e and 8a–8e.

in ethanol, then added to the above mixture. The reaction system was stirred at room temperature for 2.0–4.0 h. After the reaction was completed, the reaction solvent was evaporated *in vacuo*, dichloromethane was added for extraction. The organic phase was dried (MgSO₄), filtrated and evaporated. The residue was purified by column chromatography on silica gel with a mixture of ethyl acetate/petroleum ether = 2 : 1 (v/v) or recrystallized with EtOH to obtain the target compounds 5a–5l.

2.6 General synthetic procedure of the intermediates 6a–6b

A solution of 6-chloro-9H-purine (1.0 mmol) and potassium carbonate (3.0 mmol) in DMF (10 mL) was stirred at room temperature for 1 h. Then, 1-bromopropane or 3-bromoprop-1-ene (1.1 mmol) was added slowly and the reaction was heated at 50 °C for 9 h. After the reaction was completed, the reaction system was poured into water, extracted with dichloromethane. The solvent was removed under depressurization, and the residue was purified by column chromatography on silica gel with a mixture of ethyl acetate/petroleum ether = 1 : 5 (v/v) to yield the intermediates 6a–6b.

2.7 General synthetic procedure of the target compounds 7a–7e

A solution of the intermediates 1a–1f (1.2 mmol), anhydrous potassium carbonate (3.0 mmol) in acetonitrile (30 mL) was stirred at room temperature for 1 h. Then the intermediates 6a–6b (1.0 mmol) was added, and the reaction was refluxed at 78 °C

for 9–12 h. After completion of the reaction, the reaction solvent was evaporated *in vacuo*, extracted with dichloromethane. The organic phase was dried (MgSO₄), filtrated and evaporated. The target compounds 7a–7e were obtained by column chromatography on silica gel with a mixture of ethyl acetate/petroleum ether = 1 : 3 (v/v).

2.8 General synthetic procedure of the target compounds 8a–8e

A mixture of compounds 7a–7e (1.0 mmol) and diethyl malonate (5.0 mmol) in ethanol was stirred for 30 min. Then, potassium hydroxide (1.0 mmol) was dissolved in ethanol, and added into the above mixture. The reaction system was stirred

Table 2 The antiviral activity of the target compounds against TMV at 500 μg mL⁻¹ *in vivo* (%)^a

Compd	Curative activity	Protective activity	Inactive activity
4a	29.2 ± 5.5	37.6 ± 2.4	40.2 ± 5.2
4b	29.0 ± 5.3	40.6 ± 2.8	40.6 ± 2.8
4c	32.0 ± 2.1	40.6 ± 1.3	51.5 ± 0.9
4d	41.0 ± 1.3	28.1 ± 0.9	54.1 ± 3.2
4e	32.8 ± 2.4	15.8 ± 3.7	47.0 ± 2.0
4f	35.2 ± 2.0	42.0 ± 2.9	54.3 ± 3.9
5a	34.9 ± 2.0	47.7 ± 1.8	52.2 ± 2.0
5b	45.4 ± 2.3	39.0 ± 2.1	43.3 ± 1.9
5c	47.0 ± 2.1	34.2 ± 2.5	62.7 ± 2.8
5d	41.3 ± 1.3	47.6 ± 3.3	89.5 ± 1.9
5e	29.6 ± 1.2	32.0 ± 1.3	56.0 ± 2.3
5f	47.2 ± 2.4	61.0 ± 0.4	80.9 ± 0.5
5g	43.3 ± 2.8	27.0 ± 2.5	55.8 ± 2.3
5h	39.2 ± 3.4	47.9 ± 2.0	84.0 ± 1.9
5i	39.0 ± 1.0	41.0 ± 1.8	47.3 ± 2.8
5j	47.8 ± 2.5	37.8 ± 1.2	51.0 ± 1.9
5k	30.0 ± 1.1	44.0 ± 3.5	35.5 ± 2.2
5l	32.5 ± 0.4	40.9 ± 2.6	50.3 ± 1.0
7a	51.1 ± 8.5	38.6 ± 8.3	65.9 ± 2.1
7b	39.1 ± 5.2	41.0 ± 4.6	75.3 ± 2.4
7c	38.4 ± 6.3	55.2 ± 1.6	60.8 ± 4.1
7d	36.3 ± 4.4	40.4 ± 2.5	67.4 ± 3.5
7e	30.8 ± 1.6	47.0 ± 5.1	66.2 ± 2.7
8a	45.0 ± 6.3	46.4 ± 2.6	81.7 ± 1.5
8b	48.8 ± 2.4	39.7 ± 2.1	82.7 ± 1.6
8c	44.0 ± 6.0	38.6 ± 0.2	87.4 ± 6.9
8d	41.8 ± 1.7	56.3 ± 2.1	72.4 ± 2.3
8e	38.4 ± 6.3	40.0 ± 3.5	74.2 ± 0.7
Ribavirin ^b	35.7 ± 4.9	54.7 ± 1.7	69.8 ± 1.3

^a Average of three replicates. ^b A commercial agricultural antiviral agent ribavirin as the positive control.

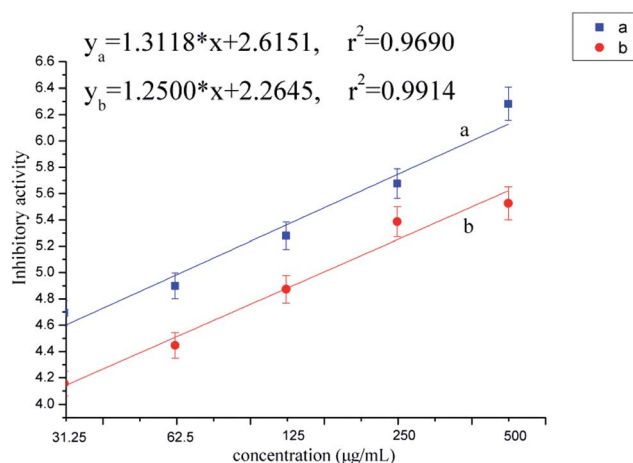


Fig. 2 The EC₅₀ curve of the compound 5d (a) and ribavirin (b), inhibitory activity = 5 + NORMSINV(inhibition ratio).

Table 3 The EC_{50} values of inactive activity of the addition compounds against TMV *in vivo*^a

Compd	EC_{50} ($\mu\text{g mL}^{-1}$)	Regression equation	r^2
5a	312.9 ± 3.0	$y = 1.2640x + 1.8458$	0.9718
5b	509.3 ± 4.0	$y = 1.2851x + 1.5212$	0.9619
5c	341.3 ± 3.0	$y = 1.3394x + 1.6071$	0.9940
5d	65.8 ± 2.9	$y = 1.3118x + 2.6151$	0.9690
5e	337.4 ± 5.0	$y = 1.2495x + 1.8411$	0.9659
5f	138.2 ± 3.4	$y = 1.2045x + 2.4217$	0.9299
5g	190.2 ± 2.0	$y = 1.0730x + 2.5545$	0.9834
5h	85.5 ± 1.1	$y = 1.1354x + 2.7897$	0.9553
5i	372.5 ± 4.4	$y = 1.4516x + 1.2678$	0.9332
5j	238.5 ± 5.1	$y = 1.3185x + 1.8651$	0.9039
5k	591.1 ± 6.0	$y = 1.2019x + 1.6687$	0.9979
5l	572.4 ± 6.4	$y = 1.3389x + 1.3077$	0.9618
8a	127.9 ± 2.9	$y = 1.2447x + 2.3777$	0.9516
8b	231.4 ± 3.5	$y = 1.8949x + 0.5196$	0.9417
8c	69.2 ± 4.0	$y = 1.1626x + 2.8607$	0.9515
8d	199.2 ± 5.1	$y = 1.4583x + 1.6469$	0.9839
8e	267.3 ± 5.9	$y = 1.5904x + 1.1400$	0.9393
Ribavirin ^b	154.3 ± 2.1	$y = 1.2500x + 2.2645$	0.9914

^a Average of three replicates. ^b A commercial antiviral agent ribavirin as the positive control.

at room temperature for 2.0–4.0 h. After the reaction was completed, the reaction solvent was evaporated *in vacuo*, dichloromethane was added for extraction. Then the organic phase was dried (MgSO_4), filtrated and evaporated. The residue was purified by column chromatography on silica gel with a mixture of ethyl acetate/petroleum ether = 2 : 1 (v/v) or recrystallized with EtOH to obtain the target compounds **8a–8e**.

3. Results and discussion

3.1 Synthesis

As shown in Schemes 1 and 2, firstly, the intermediates **1a–1f**, **2a–2f**, **3** and **6a–6b** were obtained according to previously reported methods.^{23,24} Then, the target compounds **4a–4f** and **7a–7e** were synthesized by removing the halogen hydride.^{17,24} Lastly, the target compounds **5a–5l** and **8a–8e** were obtained by

Michael addition reaction.^{23,24} In order to improve the yield of the target compounds, the reaction solvent, temperature and catalyst were optimized using the reaction of compound **5d** as the template. The molar ratio of **4b**: diethyl malonate: catalyst is 1.0 : 5.0 : 1.0, and reaction time is 4.0 h. The results showed that the reaction solvent has no significant effect on the yield, but the catalyst and temperature have great influence on it. When the reaction temperature was 23 °C (room temperature), with KOH as catalyst and EtOH as solvent, the yield of the compounds is highest. A good yield also can be obtained by appropriately raising the reaction temperature (50 °C). In addition, increasing the temperature to reflux will obviously decrease the yield (Table 1).

3.2 Spectral properties

¹H-NMR, ¹³C-NMR and HRMS spectra of all the target compounds are provided in the ESI.† For example, the analysis of the spectral data of **5d** is given below. In the ¹H NMR spectrum, 3H in the δ 13.58 ppm, δ 8.73 ppm and 8.47 ppm, can be attributed to the purine, whereas 8H appearing in the δ 7.86–6.98 ppm can be attributed to the benzene ring. Furthermore, 7H of δ 4.36 ppm, δ 4.21–4.07 ppm, 3.96 ppm and δ 3.59 ppm, can be attributed to the presence of $-\text{OCH}_2-$, $-\text{CO}-\text{CH}_2-$, $-\text{SCH}_2-$ and $\text{Ph}-\text{CH}-$ groups. Besides, 11H of δ 3.88–3.72 ppm, 3.28 ppm and 1.15 ppm can be attributed to the $-\text{CH}-(\text{COOEt})_2$ group. In the HRMS spectrum of the target compounds, the characteristic absorption signals of $[\text{M} + \text{Na}]^+$ ions were obtained, which were consistent with their molecular weights.

3.3 Antiviral activity

The purification of TMV, and anti-TMV activities *in vivo* of the target compounds were performed by the methods reported in the literature.^{25,26} The implementation details of bioassay methods are showed in the ESI.†

As shown in Table 2, some of the target compounds exhibited good anti-TMV activities *in vivo*. Of which, the curative effects of compounds **5c**, **5f**, **5j**, **7a** and **8b** are 47.0%, 47.2%, 47.8%, 51.1% and 48.8%, respectively, which are better than that of ribavirin (35.7%). The protective effects of compounds **5f**, **7c** and **8d** are 61.0%, 55.2% and 56.3%, respectively, similar

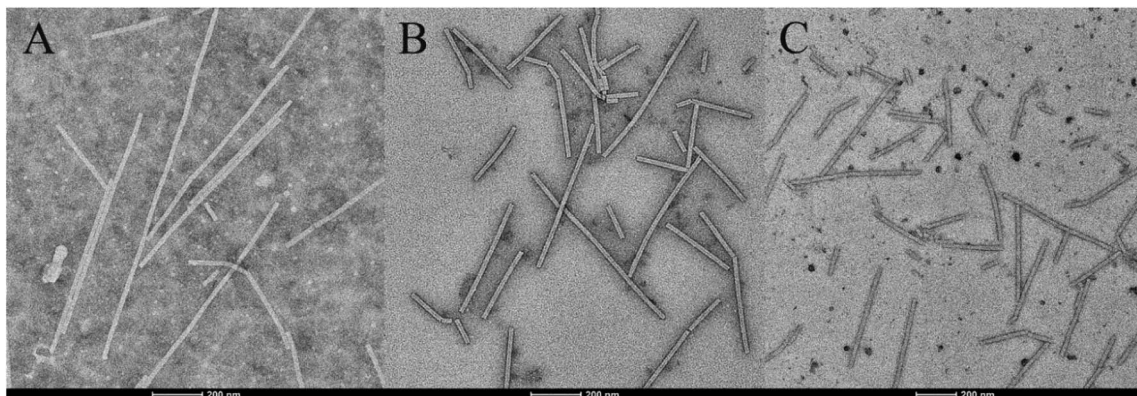


Fig. 3 The morphology changes of TMV particles treated with the compounds, CK (A), ribavirin + TMV (B) and **5d** + TMV (C).

to that of ribavirin (54.7%). Meanwhile, the compounds **5d**, **5f**, **5h**, **8a**, **8b** and **8c** have good inactive activity with the values of 89.5%, 80.9%, 84.0%, 81.7%, 82.7% and 87.4%, respectively, superior to the value of ribavirin (69.8%). The further anti-TMV activity results (Table 3, Fig. 2) showed that compounds **5d**, **5h** and **8c** possessed excellent inactive activity with the EC_{50} values of 65.8, 85.5 and 69.2 $\mu\text{g mL}^{-1}$, respectively, which are significantly superior to that of ribavirin ($EC_{50} = 154.3 \mu\text{g mL}^{-1}$).

3.4 Structure–activity relationships (SARs) of antiviral activities

The structure–activity relationships (SARs) showed that the addition compounds exhibited better antiviral activity than that of the corresponding precursor compounds, such as **5a** > **4a**, **5b** > **4b**, **8a** > **7a**, **8b** > **7b**, and so on. Among compounds **5**, electron-withdrawing groups of R can enhance the inactive activity, for example, **5c**, **5e**, **5g** > **5i**, **5k**; **5d**, **5f**, **5h** > **5j**, **5l**. Meanwhile, all of the others groups of R except H, the addition products with diethyl malonate have better inactive activity than the corresponding addition products with nitromethane, for instance, **5d** > **5c**, **5f** > **5e**, **5h** > **5g**, **5j** > **5i**, and **5l** > **5k**. Among compounds **8**, the propyl substituent compounds showed the higher inactive activity than the corresponding allyl substituent compounds, such as **8a** > **8d**, and **8b** > **8e**. In addition, ethoxy is favor to activity, such as **5d** > **8a**, **8d**; **5f** > **8b**, **8e**.

3.5 TEM study

The morphology of TMV particles after treatment with compounds was observed by TEM.²⁷ The normal morphology of TMV particles (CK) was nearly complete rod-like structures (Fig. 3A). However, compared to the positive control (ribavirin, Fig. 3B), compound **5d** caused TMV particles had significant

rupture and damage. The results revealed that compound **5d** can severely destroy the integrity of TMV particles (Fig. 3C), and probably affect the infectivity of TMV.

3.6 FT and MST

The binding affinities of **5d**, **5g**, **5k**, and ribavirin with TMV-CP were obtained *via* MST and FT.^{28,29} The details of assay methods are showed in the ESI.† As shown in Fig. 4, the association constant (K_a) between **5d** and TMV-CP was $1.02 \times 10^5 \text{ L mol}^{-1}$. The binding affinity of the compound **5d** with TMV-CP is stronger than that between ribavirin ($6.92 \times 10^3 \text{ L mol}^{-1}$), **5g** ($1.62 \times 10^3 \text{ L mol}^{-1}$), **5k** ($2.24 \times 10^2 \text{ L mol}^{-1}$) and TMV-CP. Then, the dissociation constant (K_d) of the interaction between compounds **5d**, **5g**, **5k** and ribavirin was measured by MST, with the values of 13.4, 33.2, 215.1 and 119.8 μM (Fig. 5). It suggested that the compound **5d** exhibited stronger combining capacity. These binding constants are consistent with the trend of inactive activity of the target compounds against TMV.

3.7 Molecular docking

Molecular docking was performed to further investigate the binding modes of the compounds and TMV-CP (PDB code: 1E17) by Discovery Studio 4.5.^{30,31} As shown in Fig. 6, the compound **5d** formed five conventional hydrogen bonds with different amino-acid residues of TMV-CP, including C=O...TYR139, 2.38 Å; C=O...ASN73, 2.21 Å; C=O...GLN257, 3.08 Å; NH...TRP217, 1.39 Å and NH...ASP219, 2.34 Å. Compound **5g** formed two hydrogen bonds with the amino-acid residues of TMV-CP, they are NH...ARG134 (2.58 Å) and NH...TRP217 (1.88 Å). Compound **5k** formed two hydrogen bonds with the amino-acid residues of TMV-CP, they are NH...SER147 (3.06 Å) and NH...ARG261 (2.46 Å). Finally, ribavirin formed three

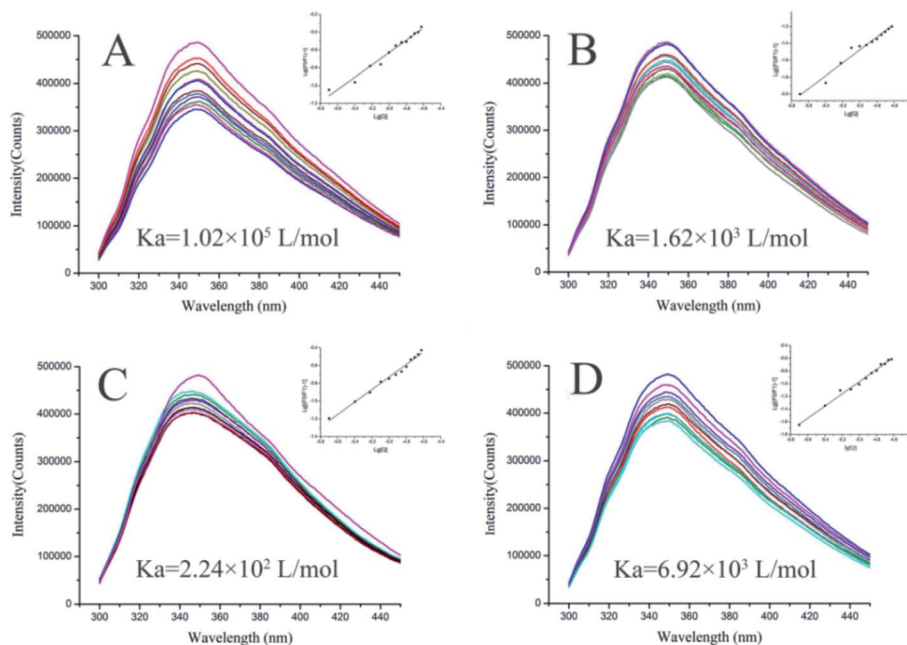


Fig. 4 The FT measurement results of compounds **5d** (A), **5g** (B), **5k** (C) and ribavirin (D) to TMV-CP.

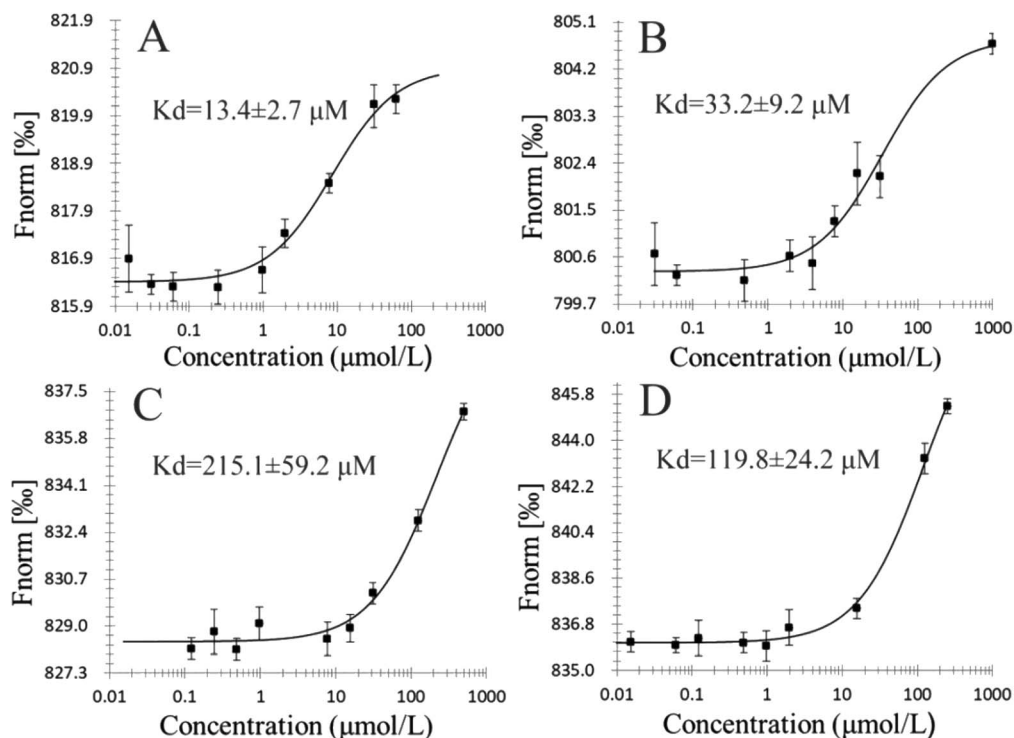


Fig. 5 The MST measurement results of compounds 5d (A), 5g (B), 5k (C) and ribavirin (D) to TMV-CP.

conventional hydrogen bonds with the amino-acid residues GLU222 (4.54 Å), ASP219 (3.93 Å), and ASP266 (5.50 Å) of TMV-CP. Moreover, we can learn from relevant research that the hydrogen bonds are beneficial to the stability of the drug-protein complexes.³² Noteworthy, the compounds 5d, 5g, 5k and

ribavirin all formed the pi-alkyl with the amino-acid residue LYS253 of TMV-CP, which may be a key binding site of the interaction between the compounds and TMV-CP. In addition, Libdock scores of the compounds 5d, 5g, 5k and ribavirin are 143.05, 136.24, 134.11 and 105.15, respectively. The binding

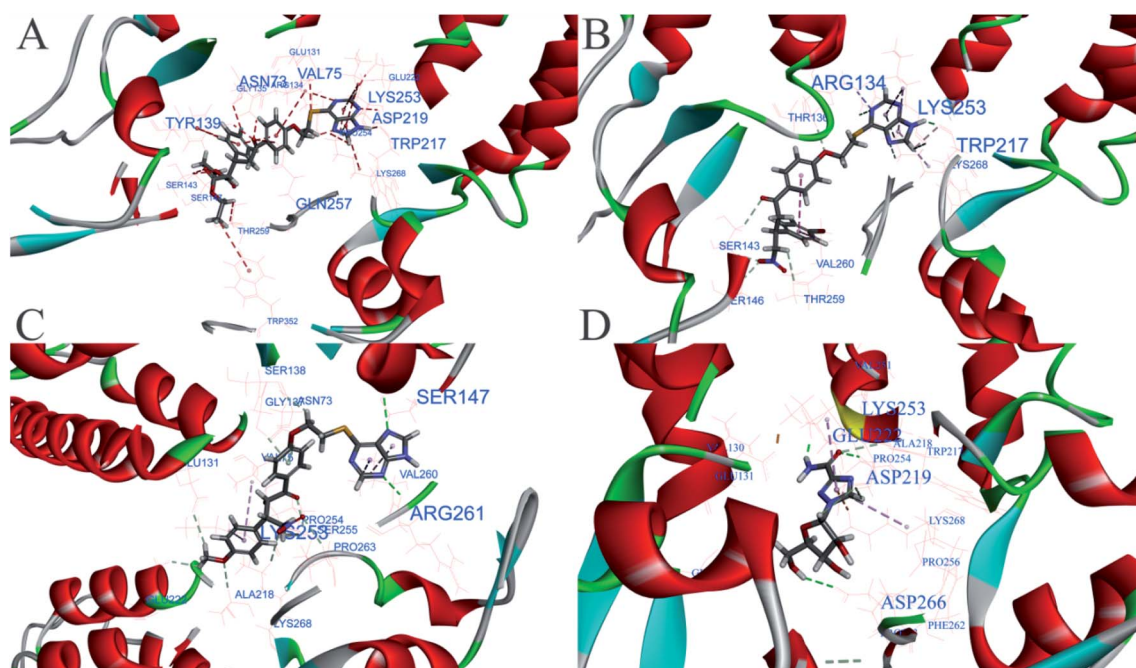


Fig. 6 The binding models of the compounds 5d (A), 5g (B), 5k (C) and ribavirin (D) to TMV-CP.

energy is 21.21, 36.03, 39.92 and 31.42 kcal mol⁻¹, which the low binding energy is more conducive to the binding of the receptor and ligand.³³ The above results manifested that the interaction between the compound **5d** and TMV-CP is stronger, and the complex formed by them was more stable. The interaction of the molecule-protein probably hindered the interaction between two subunits of TMV-CP, which play a negative role in the translation of mRNA, transcription of tRNA, extension, and self-assembly of TMV. It may be attributed to the inactive activity of the compounds against TMV.

4. Conclusions

In summary, twenty-eight chalcone derivatives containing a purine (sulfur) ether moiety were obtained, and the structures of the target compounds were characterized by NMR and HRMS. Bioassay results revealed that some target compounds displayed desirable antiviral activities against TMV *in vivo*. Of which, compound **5d** exhibited outstanding inactive activity against TMV with the EC₅₀ value of 65.8 μg mL⁻¹. The preliminary SARs manifested that the addition compounds with diethyl malonate exhibited better inactive activity. Furthermore, the preliminary antiviral mechanism was conducted. The results of TEM showed that compound **5d** can destroy the integrity of TMV particles, and probably affected the infectivity of TMV. The FT, MST and molecular docking results revealed that compound **5d** had stronger combining capacity with TMV-CP ($K_a = 1.02 \times 10^5$ L mol⁻¹, $K_d = 13.4$ μM), due to the formation of strong hydrogen bonding with the active sites of amino-acid residues of TMV-CP and the lower binding energy. These results suggested that the antiviral activity of the compound **5d** may depend on its stronger binding affinity with TMV-CP. This work provides beneficial reference for the design and development of new anti-TMV agents based on natural products.

Conflicts of interest

The authors declare that they have no competing interests.

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