Article

Synthesis and Antiviral Activity of Novel Myricetin Derivatives Containing a Ferulic Acid Amide Scaffolds

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Abstract: A variety of myricetin derivatives bearing ferulic acid amide scaffolds were designed and synthesized. The structures of all title compounds were determined by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and HRMS. Preliminary bioassays suggested that some of the target compounds exhibited remarkable antiviral activities. In particular, compound **4l** possessed significant protection activity against tobacco mosaic virus (TMV), with an half maximal effective concentration (EC_{50}) value of 196.11 μg/mL, which was better than commercial agent ningnamycin (447.92 μg/mL). Meanwhile, microscale thermophoresis (MST) indicated that compound **4l** have strong binding capability to tobacco mosaic virus coat protein (TMV-CP) with dissociation constant (K_d) values of 0.34 μmol/L, which was better than ningnamycin (0.52 μmol/L). These results suggest that novel myricetin derivatives bearing ferulic acid amide scaffolds may be considered as an activator for antiviral agents.

Keywords: myricetin; ferulic acid; antiviral activity; microscale thermophoresis; molecular docking

1. Introduction

Plant disease result in economic loss and decreases in the quality and quantity of agricultural products around the world, such as tobacco mosaic virus (TMV), it can easily infect economic crops, resulting in economic losses, people are obliged to spend millions of dollar to prevention and quarantines it [1]. Unfortunately, traditional pesticide, such as ningnanmycin and ribavirin due to its poor efficiency, high phytotoxicity, environment damage, pesticide residue and can even develop resistant from pesticide, have been eliminated and banned gradually [2, 3]. It is an urgent need to develop more greener and high-efficient promising pesticide to control and prevent plant disease.

Due to its low toxicity, easy decomposition, novel structure and environmental friendliness, natural products are devoted to synthesis new pesticides [4-6]. Myricetin is a kind of natural product which can extracted from several medicinal plant organs, vegetables and fruits [7], such as *myrica rubra Sieb* [8], *Abelmoschus manihot* [9] and *onions* [10]. Literature survey revealed that myricetin has

various biological activities, like antiviral [11, 12], antibacterial [13, 14], antioxidant [15], anticancer [16, 17] and so on. In our previous study, we have reported a series of myricetin derivatives with appreciable bioactivities against TMV [11].

Ferulic acid is a phenolic acid present in many plants, such as *Angelica sinensis*, *Cimicifuga heracleifolia* and *Lignsticum chuangxiong* [18]. According to reports, ferulic acid exhibits a wide range of bioactivities, such as antiviral [19], antibacterial [20], anticancer [21, 22] and attracted wide publicity in field of medicinal chemistry. In the further development of antiviral agents, a series of novel myricetin derivatives containing a 1,3,4-thiadiazole moiety was found to have excellent anti-TMV activity[12]. In this study, we aimed to use a ferulic acid amide to replace the 1,3,4-thiadiazole system to build novel myricetin derivatives containing a ferulic acid amide moiety for the development of antiviral agents. The preliminary bioassay results indicated that some of target compounds showed excellent antiviral activity, Among them, compound **4l** possessed significant protection activity against TMV. Meanwhile, MST and molecular docking indicated that compound **4l** have strong binding capability to TMV-CP. To the best of our knowledge, this is the first report on the synthesis and antiviral activity evaluation of myricetin derivatives containing a ferulic acid amide moiety (**Figure 1**).

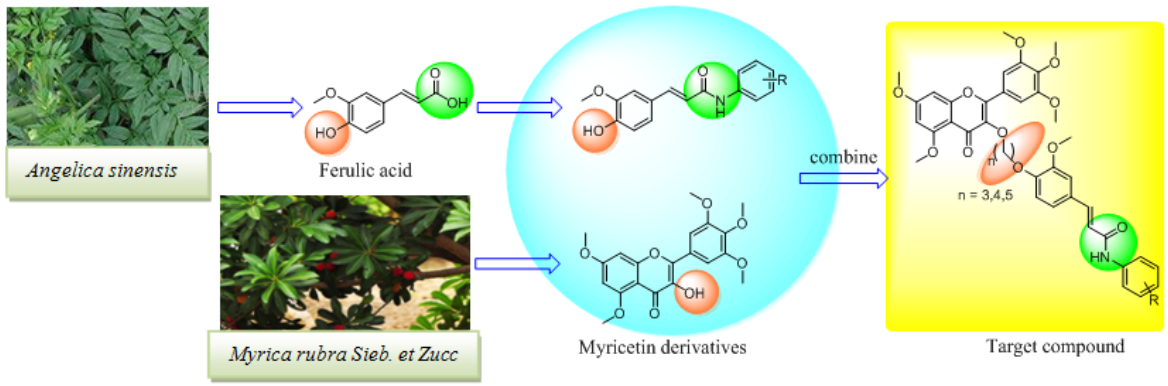


Figure 1. Design of novel myricetin derivatives containing ferulic acid amide scaffolds

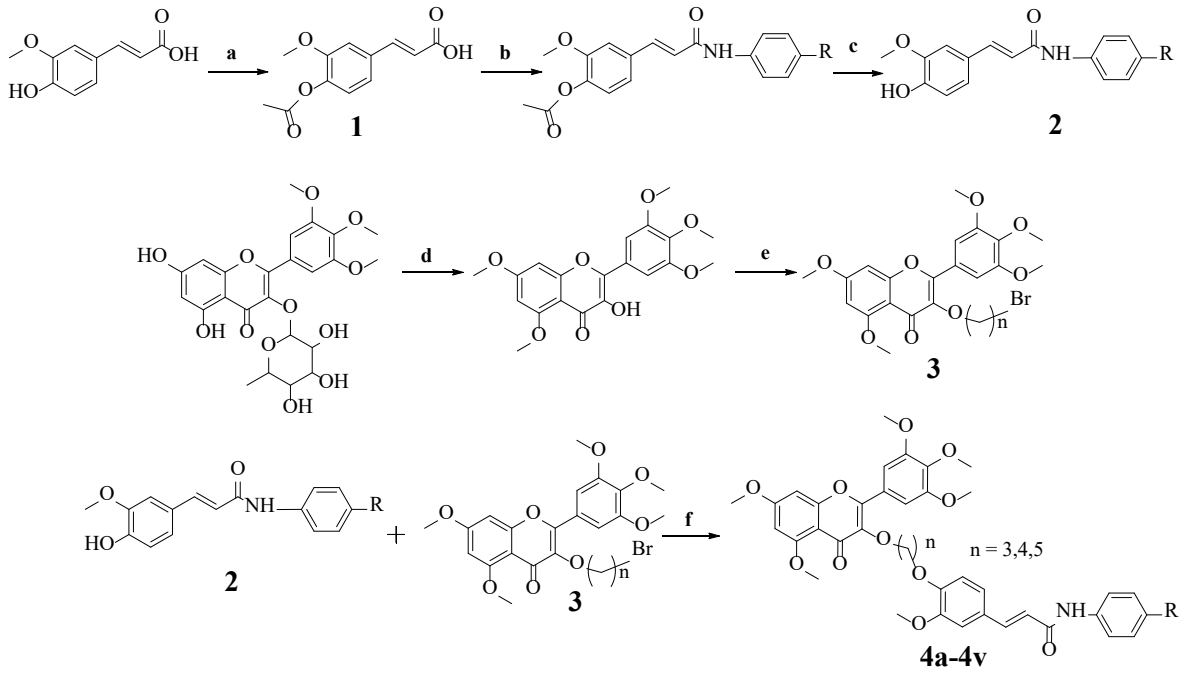
2. Results and Discussion

2.1. Chemistry

A synthetic route to myricetin derivatives containing ferulic acid amide scaffolds was designed and was shown in **Scheme 1**. According to previously reported methods [16, 23], (*E*)-3-(4-acetoxy -3-methoxyphenyl)acrylic acid (intermediate **1**) and 3-(bromomethoxy)-5,7-dimethoxy-2-(3,4,5-trimethoxy phenyl)-4*H*-chromen-4-one (intermediate **3**) could be obtained. The (*E*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-(substituted-phenyl) acryl amide intermediate **2** were prepared from substituted aniline and hydrazine hydrate by reported procedures [19, 24]. Finally, the title compounds **4a−4v** were synthesized by intermediates **2** and intermediates **3** in the K_2CO_3 and DMF at reflux for 5-7 h.

The structures of all title compounds were determined by ^{1}H NMR, ^{13}C NMR , ^{19}F NMR and HRMS, and the spectra data were shown in the Supplementary Materials. The data of **4a** was shown and discussed below. In the ^{1}H NMR, multiplet signals at δ 8.01–6.36 ppm revealed the presence of nitrogen hydrogen bond, protons in olefinic bonds and aromatic nuclei, and triplet

singlets at δ 4.23 and 4.18 ppm indicate the presence of $-CH_2-$ group. In addition, the four high-frequency single peaks and doublets peaks at 3.94-3.77 ppm revealed the presence of five $-OCH_3$, and double peak at δ 2.31 ppm indicate the presence of $-CH_3$ groups. Absorption signals at δ 174.07, 164.12 and 20.91 ppm in ^{13}C NMR spectra confirm the presences of –C=O– , –C=O–NH– and $-CH_3$ groups, respectively. The high-resolution mass spectrometry (HRMS) spectra of title compounds show characteristic absorption signals of $[M + H]^+$ ions, which is consistent with their molecular weight.



reation condition: **a** : acetic anhydride, 5%NaOH; **b** : R-PhNH$_2$, HOBt, EDCl; **c** :NH$_2$NH$_2$H$_2$O, CH$_3$CN; **d** : DMF, K$_2$CO$_3$, CH$_3$I, cone HCl; **e** :DMF, Br(CH$_2$)$_n$Br; **f** : DMF, K$_2$CO$_3$

4a : R = 4-CH$_3$, n = 3; **4b** : R = 4- OCH$_3$, n = 3; **4c** : R = 4-CH$_3$, n = 4; **4d** : R = 4-OCH$_3$, n = 4;
4e : R = 3-Cl, n = 3; **4f** : R = 3-Cl, n = 4; **4g** : R = 4-Cl, n = 4; **4h** : R = H, n = 4;
4i : R = H, n = 3; **4j** : R = 3,4-di-CH$_3$, n = 3; **4k** : R = 3,4-di-CH$_3$, n = 4; **4l** : R = 3,4-di-OCH$_3$, n = 3;
4m: R = 3,4-di-OCH$_3$, n = 4 **4n** : R = 4-Br, n = 3; **4o** : R = 4-Br, n = 4; **4p** : R = 3,4-di-Cl, n = 3;
4q : R = 3,4-di-Cl, n = 4; **4r** : R = 4-Cl, n = 5; **4s**: R = 3-Cl, n = 5; **4t** : R = 4-OCH$_3$, n = 5;
4u : R = 2-F, n = 3; **4v** : R = 2-F, n = 4

Scheme 1. Synthesis of the title compounds **4a–4v**.

2.2. Antiviral activity of title compounds against TMV in vivo

Using *N. tabacun L.* leaves under the same age as that of test subjects, the curative and protective activities against TMV (*in vivo*) at a concentration of 500 μg/mL were evaluated by the half-leaf blight spot methods [25, 26], and the obtained results were shown in **Table 1**. The preliminary bioassay results indicated that the inhibitory rates of target compounds (**4a−4v**) against TMV ranged from 15.8 to 55.5 % in terms of their curative activities, while their protective activities ranged from 5.3 to 62.1 %. Especially, compound **4n** showed 55.5 % curative effects at 500 μg/mL, which was better than that of myricetin (35.7 %) and ningnanmycin (53.2 %). In addition, compound **4l** exhibited significant protective activities against TMV at 500 μg/mL, the inhibition rate was 62.1 %, which was even better than that of myricetin (41.5 %) and ningnanmycin (55.7 %).

Table 1 Inhibition effect (%) of the compounds **4a–4v** against TMV [a]

Compounds	R	n	Curative Activity (%)	Protection Activity (%)
4a	4-CH_3	3	33.0	11.6
4b	4-OCH_3	3	42.1	35.6
4c	4-CH_3	4	39.6	21.2
4d	4-OCH_3	4	15.8	5.3
4e	3-Cl	3	37.5	51.6
4f	3-Cl	4	32.1	52.3
4g	4-Cl	4	38.1	40.3
4h	H	4	41.3	48.5
4i	H	3	43.5	31.2
4j	3,4-di-CH_3	3	39.4	46.4
4k	3,4-di-CH_3	4	21.1	25.9
4l	3,4-di-OCH_3	3	37.4	62.1
4m	3,4-di-OCH_3	4	31.2	13.5
4n	4-Br	3	55.5	53.3
4o	4-Br	4	28.4	19.5
4p	3,4-di-Cl	3	37.2	58.1
4q	3,4-di-Cl	4	34.2	43.9
4r	4-Cl	5	40.4	44.1
4s	3-Cl	5	43.2	37.8
4t	4-OCH_3	5	39.9	24.1
4u	2-F	3	37.0	41.1
4v	2-F	4	21.8	32.5
MY[b]	-	-	35.7	41.5
NNM[c]	-	-	53.2	55.7

[a] Average of three replicates; [b] The lead compound of myricetin; [c] The commercial antivirotics (NNM, ningnamycin) was used for comparison of antiviral activity.

To confirm the potential inhibitory capacity of these compounds against TMV, on the basis of our preliminary bioassay, we further evaluated the EC_{50} of some title compounds against TMV. As shown in **Table 2**, compounds **4l**, **4n** and **4p** exhibits excellent protection activities against TMV with the EC_{50} values of 196.1, 425.3 and 386.7 μg/mL respectively, which were superior to ningnamycin (447.9 μg/mL). Compound **4n** shows good curative activity against TMV the EC_{50} value is 472.4 μg/mL, which was near to ningnamycin (428.8 μg/mL).

Table 2 The EC_{50} values of **4l**, **4n** and **4p** against TMV [a]

	Compounds	R	n	Toxic regression equation	r	EC_{50} μg/mL
Curative Activity (%)	**4n**	4-Br	3	y=1.4582x+1.1002	0.9902	472.4
	NNM [b]	-	-	y=0.7650x+2.9863	0.9830	428.8
Protection Activity(%)	**4l**	3,4-di-OCH_3	3	y=2.0488x-0.3031	0.9891	196.1
	4n	4-Br	3	y=1.7099x+1.7002	0.9888	425.3
	4p	3,4-di-Cl	3	y=1.4133x+2.3311	0.9970	386.7
	NNM [b]	-	-	y=1.5482x+0.8954	0.9819	447.9

[a] Average of three replicates; [b] The commercial antivirotics (NNM, ningnamycin) was used for comparison of antiviral activity.

2.3. Structure activity relationship (SAR) of the title compounds against TMV

As indicated in **Tables 1** and **2**, the antiviral effects of target compounds were greatly affected by structural variations. Some structure–activity relationships (SAR) analyses were discussed as below. The presence of 4-Br, 3-Cl, 4-OCH_3 and H groups at the R position greatly increased the curative activities of the target compounds against TMV. For instance, the target compounds **4b**(4-OCH_3, n=3), **4i** (H, n=3), **4n** (4-Br, n=3) and **4s** (3-Cl, n=5) showed important antiviral activities against TMV, with inhibition rates of 42.1, 43.5, 55.5 and 43.2 %, respectively. Furthermore, when R was 3-Cl, 3,4-di-OCH_3 and 3,4-di-Cl groups, the protective activities of the relevant compounds **4f**, **4l** and **4p** at 500 μg/mL were 52.3, 62.1, and 58.1 %, respectively, which were superior to other substituent groups.

*2.4. Binding sites of **4l**, **4m**, myricetin and ningnanmycin to TMV-CP*

To further analyze the interactions between the compounds **4l**, **4m**, myricetin and ningnanmycin and TMV-CP, MST analysis was used [27-29]. The MST results as summarized in **Figure 2** and **Table 3** indicated that the binding of compounds **4l, 4m,** myricetin and ningnanmycin to TMV-CP protein yielded K_d values of 0.34 ± 0.09 μmol/L, 2.30 ± 0.77 μmol/L, 92.23 ± 47.54 μmol/L and 0.52 ± 0.25 μmol/L, respectively. As showed in MST, compound **4l** (K_d=0.34 ± 0.09 μmol/L) share strong affinity, which was better than that of controlled drug ningnanmycin (K_d=0.52 ± 0.25 μmol/L) and lead compound myricetin (K_d=92.23 ± 47.54 μmol/L). Based on anti-TMV activities and MST results, we can predict that the structural modification of the lead compound myricetin, such as the introduction of the active groups ferulic acid amide, could greatly improved the antiviral activities.

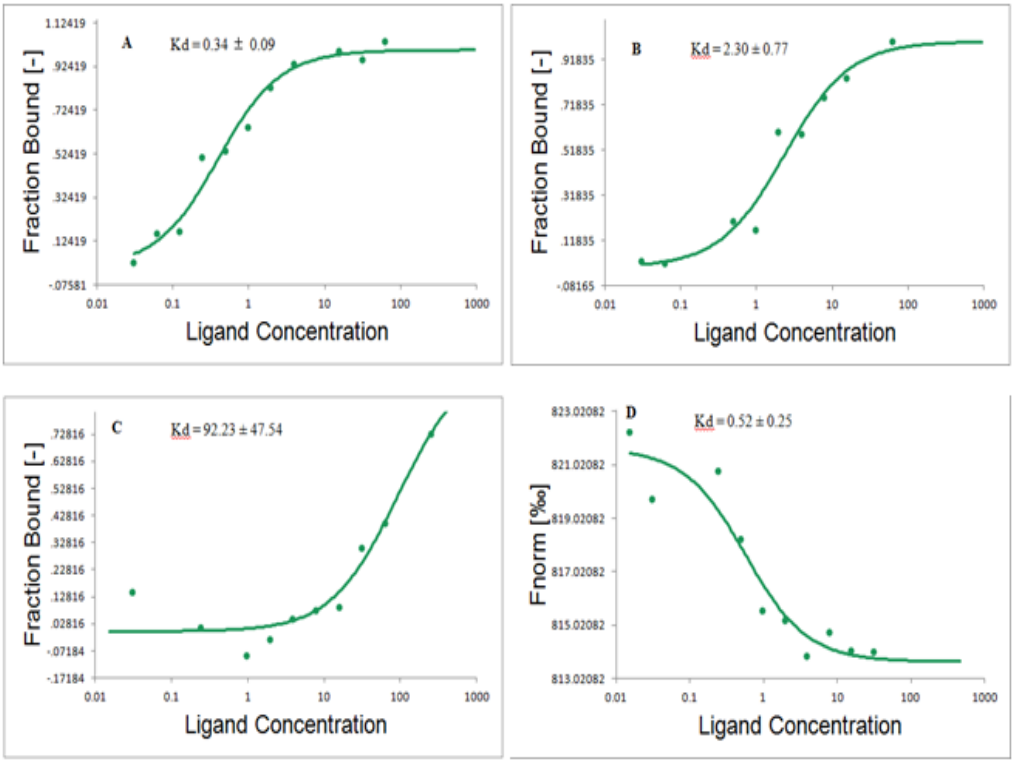


Figure 2. Microscale thermophoresis results of compounds **4l** (**A**), **4m** (**B**), myricetin (**C**) and ningnamycin (**D**)

Table 3. The dissociation constant of **4l**, **4m**, myricetin and ningnanmycin with TMV- CP.

Compounds	K_a (μmol/L)
4l	0.34 ± 0.09
4m	2.30 ± 0.77
myricetin	92.23 ± 47.54
ningnanmycin	0.52 ± 0.25

*2.5. Molecular docking of **4l** and myricetin with TMV-CP*

To identify the **4l** and myricetin recognition sites in TMV-CP (Protein Data Bank (PDB) code: 1EI7),we performed molecular docking using the gold method with 200 cycles [27, 29, 30]. As was shown in the **Figure 3**, the compound **4l** was well-embedded between the two subunits of TMV-CP. Previous reports have showed that these residues play key roles in the self-assembly of TMV particles[31]. The binding orientation of compound **4l** was clearly shown in **Figure 3**(A and B), it forms one hydrogen bond with ARG-46, with the highest docking score (1.909 Å) among the designed molecules. Besides, compound **4l** deep into the active pocket formed by amino-acid residue, including ARG-90, CLN-38 and THR-37. These interactions between small molecules and the TMV-CP may impair the interaction of twoTMV-CP subunits, hence preventing self-assembly of the TMV particle. As was shown in the **Figure 3**, The hydrogen bond strength of compound **4l** was stronger than that of myricetin (C and D). Based on molecular docking results of compound **4l** and myricetin, we can predict that the structural modification of the lead compound myricetin, could greatly improved the antiviral activities.

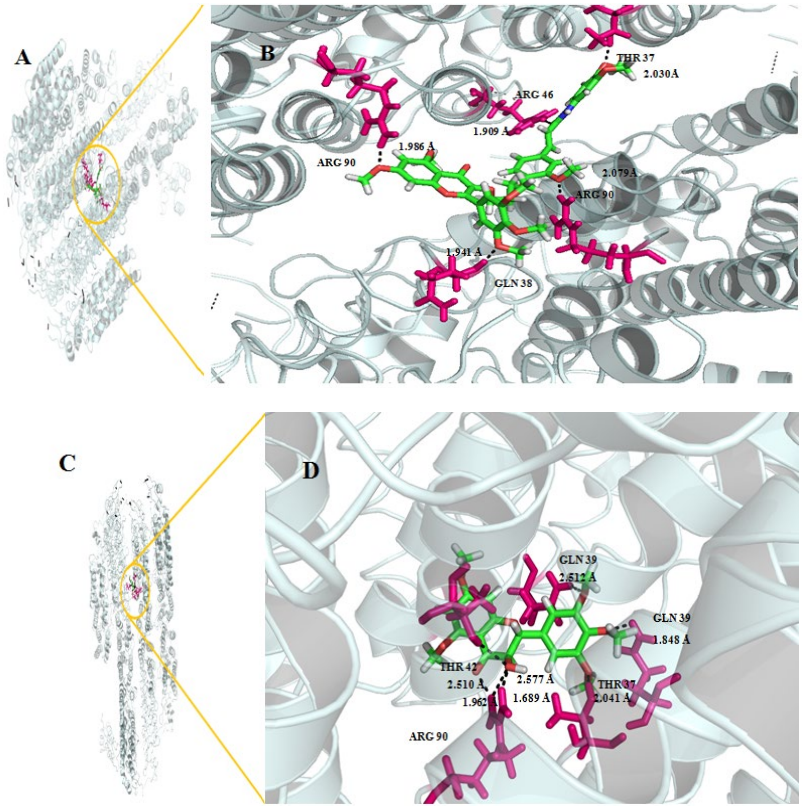


Figure 3 Molecular docking studies of compounds **4l** (**A**–**B**) and myricetin (**C**–**D**)

3. Experimental

The melting points were determined by X-4B microscopic melting point meter (Shanghai Yi Dian Physical Optics Instrument Co., Ltd. China); proton nuclear magnetic resonance (NMR) spectra were obtained on JEOL-ECX500 NMR spectrometer (JEOL, Tokyo, Japan) and Bruker Ascend-400 spectrometer (Bruker, Germany) with DMSO-d_6 or $CDCl_3$ as the solvent and TMS as the internal standard. High-resolution mass spectral (HRMS) data were performed with Thermo Scientific Q Exactive (Thermo Scientific, USA). The micro thermophoresis of the compound and TMV CP was determined by a micro thermophoresis instrument (NanoTemper Tchnologies GmbH, Germany); the fluorescence spectroscopy of the compound interacting with TMV CP was determined by FluoroMax-4 fluorescence spectrometer (HORIBA Scientific, France). All reagents (analytical grade) were purchased from commercial suppliers.

3.1. Chemistry

3.1.1. General synthesis procedure for intermediate **1**

Ferulic acid (3.01 g, 15.45 mmol) were added into bottom flask and dissolved it by 10 % NaOH (30 mL), then added acetic anhydride (1.97 g, 19.31 mmol). The mixture was stirred at room temperature for 1 h. Then added 200 mL H_2O to the reaction mixture and adjust pH to 4-5 by 10 % HCl, filtering the mixture and washing the precipitate by H_2O to obtained the intermediate **1** [16].

3.1.2. General synthesis procedure for intermediate **2**

Intermediate **1** (0.55 g, 2.33 mmol), 1-Hydroxybenzotriazole (0.38 g, 2.79 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.54 g, 2.79 mmol) were dropped into acetonitrile (20 mL), the mixture was stirred at room temperature for 3 h. Then acetonitrile (20 mL) containing substituted aniline (0.27 g, 2.56 mmol) was dropped slowly to the mixture, stirred and refluxed at 90 °C for 5 h until the reaction was completed (monitor by TLC: $V_{ethyl\ acetate}$: $V_{methanol}$ = 10:1). Then the reaction mixture was extracted by ethyl acetate and evaporated under reduced pressure. The product was dissolved in acetonitrile again, added hydrazine hydrate (0.24 g, 4.66 mmol), and stirred at room temperature for 2 h to obtained the intermediate **2** [19, 24].

3.1.3. General synthesis procedure for intermediate **3**

Preparation of the intermediate **3** has been previously described [23, 32]. The mixture of myricitrin (0.55 g, 5.01 mmol), CH_3I (2.02 g, 60.02 mmol), and K_2CO_3 (0.19 g, 6.13 mmol) was dissolved in *N*,*N*-dimethyl formamide (DMF; 30 mL), and stirred at 40 °C for 2 d until the reaction was complete (as indicated by TLC analysis). The reaction mixtures were then filtered, and the filtrate was dissolved in 50 mL water and finally extracted three times with dichloromethane (30 mL×3), combined the dichloromethane and concentrated under reduced pressure. The concentrated solution was diluted with 20 mL of absolute ethanol, stirred, and refluxed for 1 h. The concentrated hydrochloric acid (3 mL) was slowly added to the above obtained, for 2 h in reflux. The solid was precipitated from the clear solution. After cooling to room temperature, the reaction mixture was filtered, and the obtained solid product was dried at 40 °C for 2 h. Finally, dibromoalkanes and DMF were added reflux 6h to obtained intermediate **3**.

3.1.4. General synthesis procedure for target compound **4a–4v**.

A mixture of intermediate **2** (0.31 g, 1.08 mmol), anhydrous K_2CO_3 (0.41 g, 2.94 mmol) in DMF (30 mL) was stirred at 85 °C for 1 h, then DMF (20 mL) containing intermediate 3 (0.50 g, 0.98 mmol) was dropped slowly to the mixture and reacted at 105 °C for 6 h. After cooling to the room temperature, the reaction mixture was added about 200 mL H_2O and adjusted pH to 4-5 by 10 % HCl, filtered and washed by H_2O. Finally, compounds **4** was gained by re-crystallization from methanol.

(*E*)-3-(4-(3-((5,7-dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-3-yl)oxy)propoxy)-3-methoxyphenyl)-*N*-(p-tolyl)acrylamide (**4a**): gray solid, m. p. 215.4-215.5, yield: 58.68 %, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H, NH), 7.63 (t, *J* = 11.8 Hz, 1H, Ph-H), 7.57 (d, *J* = 7.1 Hz, 2H, Ph-2H), 7.32 (s, 2H, Ph-2H), 7.13 (d, *J* = 8.2 Hz, 2H, Ph-2H), 7.00 (d, *J* = 8.3 Hz, 1H, CO-CH=CH), 6.95 (s, 1H, Ph-H), 6.74 (d, *J* = 8.3 Hz, 1H, Ph-H), 6.51 (d, *J* = 2.1 Hz, 1H, Ph-H), 6.47 (s, 1H, CO-CH=), 6.36 (d, *J* = 2.2 Hz, 1H, Ph-H), 4.23 (t, *J* = 5.9 Hz, 2H, CH_2), 4.18 (t, *J* = 6.7 Hz, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.92 (d, *J* = 3.4 Hz, 6H, 2×OCH_3), 3.88 (s, 6H, 2×OCH_3), 3.77 (s, 3H, OCH_3), 2.31 (d, *J* = 9.9 Hz, 3H, CH_3), 2.25 (dd, *J* = 12.6, 6.3 Hz, 2H, CH_2), ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.07, 164.12, 163.82, 160.99, 158.83, 153.02, 152.77, 150.00, 149.22, 141.68, 140.56, 140.00, 129.51, 127.77, 125.94, 121.78, 119.88, 112.61, 110.41, 109.35, 105.91, 95.89, 92.49, 69.26, 66.00, 61.01, 56.38, 56.30, 55.86, 55.77, 30.13, 20.91, HRMS calcd for $C_{40}H_{41}NO_{11}[M+H]^+$: 712.2753, found 712.2752.

3.2. Antiviral activities in vitro

3.2.1. Purification of TMV

The upper leaves of *N. tabacum cv*. K_{326} were selected and inoculated with TMV, using previously reported methods for TMV purification[33].

3.2.2. Curative activity of the target 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 had previously been 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 as the control. The number of local lesions was counted and recorded 3–4 d after inoculation. Three replicates were set up for each[25, 26].

3.2.3. Protection activity of the target compounds against TMV *in vivo*

The compound solutions were smeared on the left side of the *N. tabacum L*. leaves, and the solvents were smeared on the right side as the control sample for growing *N. tabacum L*. leaves. After 12 h, crude TMV (concentration of 6×10^{-3} mg/mL) was inoculated on whole leaves at the same concentration on each side of the leaves, which were previously scattered with silicon carbide. After 0.5 h, the leaves were washed with water and then dried. The number of local lesions was recorded 3–4 d after inoculation[25, 26]. Three replicates were used for each compound. The inhibitory rate (*I* %) of the compound was calculated according to the following formula:

$$(I\ \%) = (C_{num} - T_{num}) / C_{num} \times 100\ \%$$

T_{num}: average local lesion number smeared with drugs

C_{num}: average local lesion number of control(not treated with compounds)

3.3 *Expression and purification of TMV-CP*

The expression vector, pET28a-TMV-CP, containing the full-length TMV-CP gene, was stored at -80 °C in our lab. A freshly transformed overnight culture of *Escherichia coli* strain *BL21(DE3)* containing the plasmid pET28a-TMV-CP was transferred to 1L Luria broth. The cells were grown at 37 °C in Luria-Bertani medium supplemented with 50 μg/mL kanamycin, and with an OD_{600} of 0.8. The cells were shaken at 200 rpm. Then protein expression was induced with 0.8 mmol IPTG at 16 °C overnight. The cells were harvested by centrifugation and then stored at -80 °C. When analyzed, the cells were resuspended in lysis buffer (20 mmol PB, 500 mmol NaCl, 30 mmol imidazole, 5 mmol β-mercaptoethanol and 5 % glycerol, pH=7.2) and then lysed at 4 °C by sonication. The lysate was clarified by centrifugation at 12, 000 g for 30 min at 4 °C, the soluble supernatants were loaded onto a 5 mL Ni-NTA column (GE Healthcare, USA), and the protein was eluted with a linear gradient of 30-350 mmol imidazole (pH=7.2). The crude protein was performed at 4 °C using a desalting column (GE Healthcare, USA) attached to an AKTA purifier protein liquid chromatography system (GE Healthcare, USA), and the fractions containing target protein with His-tags were pooled, concentrated to a suitable concentration by ultrafiltration (10 kDa cut-off). The dealt protein concentration was determined using a Genequant 100 (GE Healthcare, USA), and stored at -80 °C until further analysis [27-29].

3.4. *Interaction studies between **4l** or myricetin and TMV-CP*

The binding was calculated for MST Monolith NT. 115 (Nano Temper Technologies, Germany). A range of ligands from 0 to 5 μmol were incubated with 0.5 μmol of purified recombinant proteins for 5 min with a NT-647 dye (Nano Temper Technologies, Germany) and was used in the thermophoresis experiment at a final concentration of 20 *n*mol. A 16 point dilution series was made for selected compounds in DMSO. Each compound dilution series was subsequently transferred to protein solutions in 10 μmol Tris-HCl and 100 mmol sodium chloride pH=7.5, 0.05 % Tween-20. After a 15 min incubation of the labeled TMV-CP with each dilution point (1:1 mix) at room temperature, samples were filled into standard capillaries (NanoTemper Technologies, Germany). Measurements were taken on a Monolith NT.115 microscale thermophoresis system (NanoTemper Technologies, Germany) under a setting of 20 % LED and 40 % IR laser. Laser on time was set at 30 s, and laser-off time was set at 5 s. The K_d values were calculated from the duplicate reads of three separate experiments using the mass action equation in the Nano Temper software[28].

3.5. *Molecular docking*

The molecular docking was performed by using DS-CDocker implemented in Discovery Studio (version 4.5). The coat protein subunit amino acid sequence of tobacco mosaic virus (TMV) was searched by the UniProt database. The Protein BLAST server was used to search the template protein and the homologies of TMV-CP sequences were aligned. Homology modeling of TMV-CP was carried out using Create Homology Models, which is a module integrated in Discovery Studio. The obtained models were evaluated by Ramachandran plots. The 3D structures of the compounds were constructed using the Sketching module and optimized by the Full Minimization module. All parameters are default during the docking process[27, 29, 30].

4. Conclusions

A series of myricetin derivatives bearing ferulic acid amide scaffolds were designed and synthesized. Preliminary bioassays suggested that these compounds exhibited favorable curative and protective activities against TMV. Among them, compound **4l** showed remarkable protective activity against TMV, with the EC_{50} values of 196.11 μg/mL, which was superior to ningnamycin (447.92 μg/mL). Further the microscale thermophoresis studies revealed that compound **4l** have strong binding capability with TMV-CP, and the molecular docking studies were consistent with the experimental results. All these results support that the myricetin derivatives bearing ferulic acid amide scaffolds possess antiviral activities, and thus could be further studied as potential alternative templates in the search for novel antiviral agents.

Supplementary Materials: The following are available online. The data and spectrogram of compounds **4a–4v**.

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