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Research Article

Synthesis and Antiviral Activity of Hydrogenated Ferulic Acid Derivatives

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A series of hydrogenated ferulic acid amide derivatives **4** were synthesized. The molecular structures of the synthesized compounds were analyzed by ^{1}H NMR and HRMS. The biological activity study showed that some of them displayed excellent protection activity and curative activity against TMV at 500 μg/mL.

1. Introduction

Viruses are among the most agriculturally important groups of plant pathogens, causing serious economic losses in many major crops by reducing yield and quality [1]. Although plant viruses are genetically rather simple, they are difficult to prevent or control and have devastating impact on crop growth. So, plant virus is often referred to as "plant cancer" for several reasons. It is reported that three class pesticides are discovered. For example, nucleoside medicines as ribavirin, tiazofurin, selenazofurin, and benzamide riboside, nonnucleosides such as dufulin and mycophenolic acid, and natural product as ningnanmycin can inhibit virus replication effectively and suppress virus symptoms.

Ferulic acid is isolated from many staple foods, such as fruits, vegetables, cereals, and coffee. It and its derivatives exhibited diversity activity, such as anticancer [2, 3], antiatherogenic [4], anticarcinogenic [5], and antibacterial agents [6], as well as anti-inflammatory activity [7, 8]. In view of these facts mentioned earlier, and also as a part of our work on the synthesis of bioactive lead compounds for drug discovery, the title compounds were designed. We herein report a series of hydrogenated ferulic acid amide derivatives **4** that have been found to possess potent antiviral activities.

2. Results and Discussion

2.1. Chemistry. All of the hydrogenated ferulic acid amide derivatives were prepared from a key intermediate hydrogenated ethyl ferulate, which was easily prepared by catalytic hydrogenation of the ferulic acid **1** using Pd/C and H_2 (1 atm), as shown in Scheme 1. Traditional methods of synthesis of amide suffer from disadvantages, such as long reaction time and inconvenience of handling. In this paper, an eco-friendly and high-yielding method for hydrogenated ferulic acid amides **3** from hydrogenated ethyl ferulate **2** and substituted 2-amino-1-phenylethanol was applied. It can be obtained under microwave irradiation at 130°C in free-solvent conditions for 30 min in generally good yields. As was mentioned earlier, alkylation of the compounds **3** yielded the compounds **4** followed by alkylation with bromoalkane to give the N-(2-alkoxy-2-phenylethyl) hydrogenated ferulic acid amide derivatives **4**.

2.2. Spectrum. In the ^{1}H NMR spectra of title compounds, the NH proton signals of title compounds were appeared at δ 5.81 ~ 6.02 ppm. All the alkyl or aryl groups showed the normal location. All the title compounds of HRMS matched with the theoretical values.

Table 1: Antiviral activities of the title compounds at 500 μg/mL.

Compound	R_1	R_2	Anti-TMV activities	
			Protection effect	Curative effect
4a	*m*-MeO	Benzyl	0	24.2
4b	*m*-NO_2	Benzyl	10.4	0
4c	*m*-NO_2	Propynyl	0	0
4d	*p*-NO_2	Benzyl	0	0
4e	*p*-benzyloxy	Benzyl	0	12.3
4f	*p*-benzyloxy	Propynyl	15.2	28.9
4g	*p*-benzyloxy	Allyl	22.8	20.7
Ribavirin			32.6	38.5

2.3. Antiviral Activity. To make a judgment of the antiviral potency of the title compounds, the commercially available plant virucide ribavirin was used as the control. The *in vivo* antiviral results of all the title compounds against TMV are listed in Table 1. The results showed that title compounds exhibited varying degrees of activities against TMV. Compound **4g** displayed good protection activity (22.8%) at 500 μg/mL, which is the same of ribavirin at 500 μg/mL (32.6%). Compounds **4a** and **4f** showed the same curative activity level (24.2% and 28.9%, resp.) as ribavirin at 500 μg/mL (32.6%).

3. Experimental

3.1. Materials and Methods. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument using TMS as an internal standard and $CDCl_3$ as solvent. Melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and were uncorrected. HRMS were recorded on an IonSpec 7.0 T Fourier Transform Ion Cyclotron Resonance (FTICR) Mass Spectrometer. All chemicals or reagents were purchased from standard commercial suppliers.

3.2. Synthesis. All of the hydrogenated ferulic acid amide derivatives were prepared as shown in Scheme 1.

To a solution of 3-(3,4-dimethoxyphenyl)acrylic acid (15 g, 72 mmol) in ethyl acetate (150 mL) and ethanol (150 mL), Pd/C (1.5 g, 10%) was added under the N_2 atmosphere. The mixture was refluxed for 72 h. After the reaction was completed, the Pd/C was filtered, and the solvent was evaporated. The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated to afford white solid, yield 92%, m.p. 92–94°C (ref. 96–97°C).

To a solution of 3-(3,4-dimethoxyphenyl)propanoic acid (1.44 g, 6.84 mmol) in THF under −20°C, isobutyl chloroformate (0.89 g, 6.84 mmol) and NMM (0.7 g, 7.18 mmol) were added. The mixture was stirred for 30 min. A solution of 2-amino-1-(4-nitrophenyl)ethanol (7.18 mmol) in THF was dropwise in the mixture, then it was stirred for 12 h at room temperature. After the reaction was completed, the THF was evaporated, and the residue was diluted with water and extracted several times with dichloromethane. The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. The remainder was purified by chromatography on silica gel using petroleum ether (60–90°C) and ethyl acetate as the eluent to afford compound **3**.

3-(3,4-Dimethoxyphenyl)-N-(2-hydroxy-2-(3-methoxyphenyl) ethyl)propanamide ***3a***. Yellow solid, yield 85%, m.p. 112–113°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (d, J = 7.7 Hz, 2H, CH_2CH_2), 2.91 (t, J = 7.7 Hz, 2H, CH_2CH_2), 3.32 (s, 1H, $ArCHCH_2$), 3.69 (s, 1H, $ArCHCH_2$), 3.83 (s, 9H, *p*-CH_3O + *p*-CH_3O + *p*-CH_3O), 4.77 (s, 1H, ArCH), 5.80 (br, 1H, CONH), 7.01–6.67 (m, 6H, Ar-H).

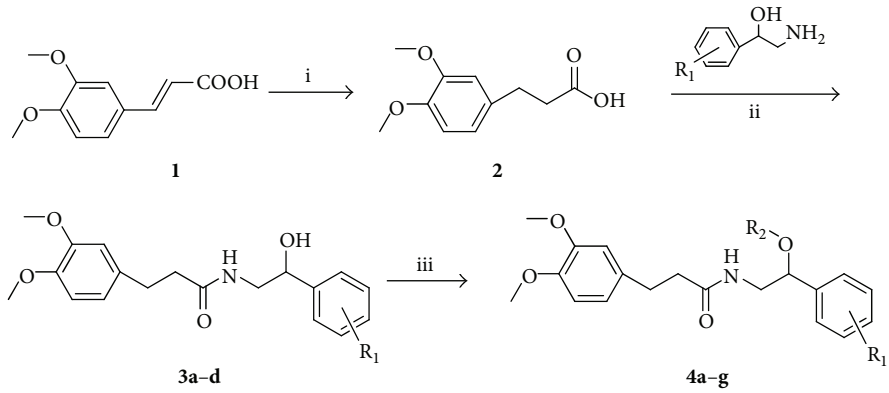
3-(3,4-Dimethoxyphenyl)-N-(2-hydroxy-2-(3-nitrophenyl) ethyl)propanamide ***3b***. Yellow oil, yield 82%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (t, J = 7.6 Hz, 2H, CH_2CH_2), 2.90 (t, J = 7.6 Hz, 2H, CH_2CH_2), 3.30–3.36 (m, 1H, CH_2CH), 3.63–3.68 (m, 1H, CH_2CH), 3.85 (s, 6H, *p*-CH_3O + *o*-CH_3O), 4.90 (dd, J = 2.4 Hz, J = 7.2 Hz, 1H, CH_2CH), 6.03 (br, 1H, CONH), 6.70–6.79 (m, 3H, Ar-H), 7.63–8.20 (m, 4H, Ar-H).

3-(3,4-Dimethoxyphenyl)-N-(2-hydroxy-2-(4-nitrophenyl) ethyl)propanamide ***3c***. White solid, yield 51%, m.p. 133–135°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47–2.52 (m, 2H, CH_2CH_2), 2.92 (t, J = 7.2 Hz, 2H, CH_2CH_2), 3.26–3.32 (m, 1H, CH_2CH), 3.66–3.71 (m, 1H, CH_2CH), 3.87 (s, 6H, *p*-CH_3O + *o*-CH_3O), 4.91 (dd, J = 2.4 Hz, J = 6.8 Hz, 1H, CH_2CH), 5.73 (s, 1H, CONH), 6.71–6.81 (m, 3H, Ar-H), 7.45 (d, J = 8.8 Hz, 2H, Ar-H), 8.17 (d, J = 8.8 Hz, 1H, Ar-H).

N-(2-(4-(Benzyloxy)phenyl)-2-hydroxyethyl)-3-(3,4-dimethoxyphenyl)propanamide ***3d***. Yellow solid, yield 67%, m.p. 132–133°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (t, J = 7.5 Hz, 2H, CH_2CH_2), 2.91 (t, J = 7.4 Hz, 2H, CH_2CH_2), 3.24 (t, J = 11.2 Hz, 1H, $ArCHCH_2$), 3.80–3.66 (m, 1H, $ArCHCH_2$), 3.88 (s, 6H, *p*-CH_3O + *o*-CH_3O), 4.22 (d, J = 11.5 Hz, 1H, $ArCHCH_2$), 4.37 (d, J = 8.7 Hz, 1H, $ArCH_2$), 4.48 (d, J = 11.6 Hz, 1H, $ArCHCH_2$), 5.10 (s, 2H, $ArCH_2$), 5.86 (br, 1H, CONH), 6.84–6.69 (m, 3H, Ar-H), 7.53–7.22 (m, 9H, Ar-H).

RX (7.56 mmol) was added to a mixture of **3** (3.78 mmol), 30% aqueous sodium hydroxide solution (18.9 mmol), and catalytic amount of hexadecyl trimethyl ammonium bromide (0.4 mmol) in 6 mL of CH_2Cl_2 at room temperature. The reaction mixture was heated at reflux for 8 h. Subsequently, the mixture was diluted with water and extracted several times with dichloromethane. The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. The remainder was purified by chromatography on silica gel using petroleum ether (60–90°C) and ethyl acetate as the eluent to afford the title compounds **4a**–**4g**.

N-(2-(Benzyloxy)-2-(3-methoxyphenyl)ethyl)-3-(3,4-dimethoxyphenyl)propanamide ***4a***. Yellow oil, yield 85%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42 (t, J = 7.7 Hz, 2H, CH_2CH_2), 2.88 (t, J = 7.7 Hz, 2H, CH_2CH_2), 3.25–3.16 (m, 1H, $ArCHCH_2$), 3.77–3.69 (m, 1H, $ArCHCH_2$), 3.87–3.80 (m, 9H, *p*-CH_3O + *p*-CH_3O + *o*-CH_3O), 4.22 (d, J = 11.6 Hz, 1H, ArCH),



Scheme 1: Reagents and conditions: (i) 10% Pd/C, H_2, EtOH, EA; (ii) isobutyl chloroformate, NMM; (iii) R_1Br, TBAB, NaOH, 65°C.

4.38 (dd, J = 8.8 Hz, J = 3.7 Hz, 1H, $ArCHCH_2$), 4.49 (d, J = 11.6 Hz, 1H, ArCH), 5.81 (s, 1H, CONH), 6.80–6.70 (m, 3H, Ar-H), 6.87–6.81 (m, 3H, Ar-H), 7.38–7.24 (m, 5H, Ar-H). HRMS (ESI) m/z Calcd for $C_{27}H_{31}NO_5Na^+$ $[M+Na]^+$ 472.2094, found 472.2091.

N-(2-(Benzyloxy)-2-(3-nitrophenyl)ethyl)-3-(3,4-dimethoxyphenyl)propanamide ***4b***. Yellow oil, yield 82%, ^1H NMR (400 MHz, $CDCl_3$) δ 2.44 (t, J = 7.6 Hz, 2H, CH_2CH_2), 2.88 (t, J = 7.6 Hz, 2H, CH_2CH_2), 3.28–3.22 (m, 1H, CH_2CH), 3.75–3.72 (m, 1H, CH_2CH), 3.86 (s, 3H, o-CH_3O), 3.88 (s, 3H, p-$ArOCH_3$), 4.28 (d, J = 11.5 Hz, 1H, Ar-CH), 4.51 (d, J = 11.5 Hz, 1H, Ar-CH), 4.56 (d, J = 8.0 Hz, 1H, CH_2CH), 5.81 (br, 1H, CONH), 6.84–6.72 (m, 3H, Ar-H), 7.43–7.32 (m, 4H, Ar-H), 7.57 (t, J = 8.0 Hz, 1H, Ar-H), 7.67 (d, J = 8.0 Hz, 1H Ar-H), 8.21 (d, J = 8.0 Hz, 1H, Ar-H), 8.27 (s, 1H, Ar-H). HRMS (ESI) m/z Calcd for $C_{26}H_{28}N_2O_6Na^+$ $[M+Na]^+$ 487.1840, found 487.1835.

3-(3,4-Dimethoxyphenyl)-N-(2-(3-nitrophenyl)-2-(prop-2-yn-1-yloxy)ethyl)propanamide ***4c***. Yellow oil, yield 95%, ^1H NMR (400 MHz, $CDCl_3$) δ 2.40 (m, 3H, CH_2CH_2+ C≡CH), 2.80 (d, J = 7.6 Hz, 2H, CH_2CH_2), 3.22–3.08 (m, 1H, $ArCHCH_2$), 3.66 (m, 1H, $ArCHCH_2$), 3.88–3.72 (m, 7H, p-CH_3O + o-CH_3O + OCH_2CH), 4.09 (m, 1H, OCH_2CH), 4.64 (dd, J = 3.0 Hz, J = 8.0 Hz, 1H, $ArCHCH_2$), 6.02 (br, 1H, CONH), 6.78–6.61 (m, 3H, Ar-H), 7.46 (s, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 8.24–8.03 (m, 2H, Ar-H). HRMS (ESI) m/z Calcd for $C_{22}H_{24}N_2O_6Na^+$ $[M+Na]^+$ 435.1527, found 435.1526.

N-(2-(Benzyloxy)-2-(4-nitrophenyl)ethyl)-3-(3,4-dimethoxyphenyl)propanamide ***4d***. Yellow solid, yield 76.8%, m.p. 133–135°C, ^1H NMR (400 MHz, $CDCl_3$) δ 2.43 (t, J = 7.6 Hz, 2H, CH_2CH_2), 2.87 (t, J = 7.6 Hz, 2H, CH_2CH_2), 3.19–3.26 (m, 1H, CH_2CH), 3.67–3.73 (m, 1H, CH_2CH), 3.83 (s, 3H, o-$ArOCH_3$), 3.85 (s, 3H, p-$ArOCH_3$), 4.26 (d, J = 11.6 Hz, 1H, Ar-CH), 4.47 (d, J = 11.6 Hz, 1H, Ar-CH), 4.54 (dd, J = 4 Hz, J = 8 Hz, 1H, CH_2CH), 5.86 (br, 1H, CONH), 6.71–6.78 (m, 3H, Ar-H), 7.25–7.38 (m, 5H, Ar-H), 7.50 (d, J = 8.4 Hz, 2H, Ar-H), 8.21 (d, J = 8.4 Hz, 2H,Ar-H). HRMS (ESI) m/z Calcd for $C_{26}H_{28}N_2O_6Na^+$ $[M+Na]^+$ 487.1840, found 487.1836.

N-(2-(Benzyloxy)-2-(4-(benzyloxy)phenyl)ethyl)-3-(3,4-dimethoxyphenyl)propanamide ***4e***. White solid, yield 68%, m.p. 107-108°C, ^1H NMR (400 MHz, $CDCl_3$) δ 2.45 (t, J = 8.0 Hz, 2H, CH_2CH_2), 2.92 (t, J = 8.0 Hz, 2H, CH_2CH_2), 3.30–3.21 (m, 1H, $ArCHCH_2$), 3.77–3.69 (m, 1H, $ArCHCH_2$), 3.87 (s, 3H, o-CH_3O), 3.89 (s, 3H, p-CH_3O), 4.23 (d, J = 11.6 Hz, 1H, $ArCH_2$), 4.38 (dd, J = 8.8 Hz, J = 3.8 Hz, 1H, $ArCHCH_2$), 4.48 (d, J = 11.6 Hz, 1H, $ArCH_2$), 5.11 (s, 2H, $ArCH_2$), 5.83 (br, 1H, CONH), 6.84–6.74 (m, 3H, Ar-H), 7.02 (d, J = 8.4 Hz, 2H, Ar-H), 7.46–7.25 (m, 8H, Ar-H), 7.48 (d, J = 7.4 Hz, 2H, Ar-H). HRMS (ESI) m/z Calcd for $C_{33}H_{35}NO_5Na^+$ $[M+Na]^+$ 548.2407, found 548.2405.

N-(2-(4-(Benzyloxy)phenyl)-2-(prop-2-yn-1-yloxy)ethyl)-3-(3,4-dimethoxyphenyl)propanamide ***4f***. White solid, yield 68%, m.p. 93-94°C, ^1H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 1H, C≡CH), 2.48 (t, J = 7.6 Hz, 2H, CH_2CH_2), 2.92 (t, J = 7.6 Hz, 2H, CH_2CH_2), 3.13–3.20 (m, 1H, CH_2CH), 3.70–3.78 (m, 1H, CH_2CH), 3.82 (d, J = 2.0 Hz, 1H, OCH_2), 3.86 (s, 3H, o-$ArOCH_3$), 3.88 (s, 3H, p-$ArOCH_3$), 4.08 (d, J = 2.0 Hz, 1H, OCH_2), 4.47 (dd, J = 7.6 Hz, J = 9.2 Hz, 1H, CH_2CH), 5.06 (s, 2H, p-$ArOCH_2Ph$), 5.87 (br, 1H, NH), 6.74–6.81 (m, 3H, Ar-H), 6.96 (d, J = 8.8 Hz, 2H, Ar-H), 7.21 (d, J = 8.8 Hz, 2H, Ar-H), 7.32–7.44 (m, 5H, Ar-CH). HRMS (ESI) m/z Calcd for $C_{29}H_{31}NO_5Na^+$ $[M+Na]^+$ 496.2094, found 496.2090.

N-(2-(Allyloxy)-2-(4-(benzyloxy)phenyl)ethyl)-3-(3,4-dimethoxyphenyl)propanamide ***4g***. White solid, yield 65%, m.p. 68-69°C, ^1H NMR (400 MHz, $CDCl_3$) δ 2.57–2.41 (m, 2H, CH_2CH_2), 2.92 (d, J = 7.1 Hz, 2H, CH_2CH_2), 3.38–3.15 (m, 2H, CH_2CH), 3.78–3.58 (m, 2H, CH_2CH), 3.97–3.81 (m, 6H, p-CH_3O + o-CH_3O), 4.74 (d, J = 7.8 Hz, 1H, CH_2CH), 5.07 (s, 1H, CH_2CH), 5.20 (dd, J = 21.0 Hz, J = 13.9 Hz, 1H, CHPh), 5.85 (br, 1H, CONH), 6.87–6.70 (m, 3H, $CH=CH_2$), 6.96 (s, 1H, Ar-H), 7.53–7.16 (m, 7H, Ar-H). HRMS (ESI) m/z Calcd for $C_{29}H_{33}NO_5Na^+$ $[M+Na]^+$ 498.2251, found 498.2258.

3.3. Antiviral Activity

3.3.1. Purification of TMV. Using Gooding's method [9], the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000 g, treated with PEG twice, and centrifuged again. The whole experiment was processed at 4°C. Absorbance value was estimated at 260 nm by ultraviolet spectrophotometer. Consider

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

3.3.2. Preparation of Medicaments. Tested compounds and ribavirin used as a reference antiviral agent were first dissolved in minimum volume of N,N-dimethylformamide (DMF) and then diluted with distilled water containing 1% Tween 20 at 500 μg/mL concentration.

3.4. Protection Effect of Compounds against TMV In Vivo. The compound solution was smeared on the left side and the solvent serving as control on the right side of growing *N. tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in TMV of 6×10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3-4 days after inoculation were counted [10]. There are three replicates for each compound.

3.5. Curative Effect of Compounds against TMV In Vivo. The growing leaves of *Nicotiana tabacum* L. of the same ages were selected. The tobacco mosaic virus (concentration of 6×10^{-3} mg/mL) was dipped and inoculated on the whole leaves. The leaves were then washed with water and dried. The compound solution was smeared on the left side, and the solvent was kept on the right side for control. The local lesion numbers were then recorded 3-4 days after inoculation [11]. For each compound, three repetitions were conducted to ensure the reliability of the results, which were measured according to the following formula:

$$\text{inhibition rate (\%)} = \frac{\text{av local lesion numbers of control (not treated with compound) av local lesion numbers smeared with drugs}}{\text{av local lesion numbers without drugs}}. \quad (2)$$

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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