DOI 10.1007/s10600-019-02889-z
Chemistry of Natural Compounds, Vol. 55, No. 6, November, 2019

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF TYROSOL PHENOLIC ACID ESTER DERIVATIVES

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Sixteen tyrosol derivatives were synthesized and characterized by NMR and HR-MS. The antioxidant activity of those compounds was evaluated using four different assays. The results showed that some target compounds displayed better antioxidant activity than L-ascorbic acid and Trolox. Five target compounds exhibited more potent α-glucosidase inhibition activity (18.1–56.7 μM) than acarbose (60.9 μM). Eight target compounds showed some anticholinesterase activities.

Keywords: tyrosol, phenolic acid, ester derivatives, synthesis, biological activity.

Epidemiological studies in Mediterranean countries, where the traditional diet is rich in olive oil and unsaturated fatty acids, have shown that there is a low incidence of degenerative diseases, such as heart disease and tumors [1–3]. Tyrosol is one of the major natural phenolic antioxidant contained in olive oil [4] and has been reported to possess various physiological activities via its potent antioxidant activity [5]. Tyrosol shows antigenotoxic activity and prevents apoptosis in keratinocytes [6]. It also prevents Alzheimer′s disease and Parkinson′s disease by antagonizing β-amyloid (Aβ) and inhibiting apoptosis of dopaminergic neurons [7, 8]. Tyrosol can also effectively increase lifespan [9], protect the heart [10], and prevent cancer [11]. Despite these remarkable properties, tyrosol is still seldom used as dietary supplement or stabilizer in foods and cosmetics.

Phenolic acids have attracted widespread research attention because of their broad range of biological activities and their potential health benefits. Our previous work [12] found that the phenolic hydroxyl group is vital to the activity of the compound, so we study some compounds that contain tyrosol linked with phenolic acids through the ester bond. Most of the phenolic acids we selected are famous natural products such as gallic acid, caffeic acid, sinapic acid, and so on. The list also contains a well-known antioxidant such as Trolox. The antioxidant activity of the target compounds may be higher than tyrosol and phenolic acids themselves. Based on the antioxidant activity of tyrosol as an antioxidant, this study reports the synthesis of 16 tyrosol ester derivatives (**3a**–**3p**). All of the compounds were analyzed for their antioxidant activity, hypoglycemic activity, and anticholinesterase activity.

The desired tyrosol esters **3a**–**3p** were obtained in moderate to good yields (Scheme 1). In the experiment, the target compound **3a** was synthesized using compounds **1** and **2a** as an example. The synthetic conditions of compound **3a** were discussed. Initially, we use DCC and DMAP as a condensing agent and found that there is serious side effect and the yield is low. It is considered that tyrosol has an alcoholic hydroxyl group and a phenolic hydroxyl group. The phenolic acids also have a phenolic hydroxyl group, all of them are involved in the esterification reaction, and the selectivity of the reaction is poor. When we use TPP and DIAD according to the reference [13], the side effect was significantly reduced, and the yield was significantly improved; however, the reaction still produces a by-product, and its polarity is close to the target compound. The use of a silica gel column to purify is difficult, so we used Sephadex LH-20 gel to obtain the target compound.

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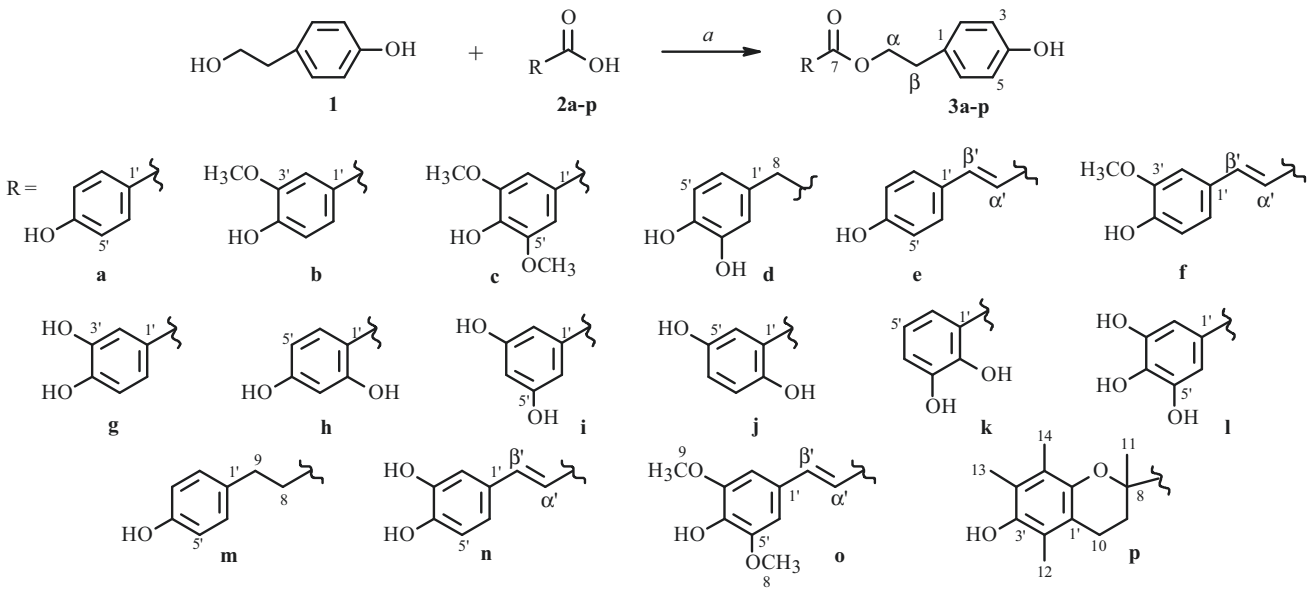
TABLE 1. Antioxidant Activity of Compounds **3a–3p** (IC_{50}, μM)

Compound	DPPH	ABTS	FRAP, mmol/g	·OH
3a	> 100	89.66 ± 1.38	8.21 ± 0.06	> 2500
3b	> 100	44.32 ± 0.56	8.53 ± 0.06	> 2500
3c	> 100	20.93 ± 1.95	11.23 ± 0.13	> 2500
3d	8.75 ± 0.11	8.81 ± 0.27	65.30 ± 0.03	404.1 ± 5.3
3e	> 100	39.15 ± 5.00	8.21 ± 0.03	> 2500
3f	37.91 ± 0.19	12.78 ± 2.95	18.89 ± 0.29	> 2500
3g	1.81 ± 0.17	6.54 ± 0.95	58.27 ± 0.16	797.3 ± 5.6
3h	> 100	> 200	7.83 ± 0.03	> 2500
3i	> 100	11.81 ± 0.44	7.96 ± 0.03	> 2500
3j	22.65 ± 0.04	9.17 ± 2.20	43.80 ± 0.19	1352.8 ± 0.8
3k	1.66 ± 0.13	8.29 ± 1.56	52.65 ± 0.38	675.1 ± 8.3
3l	1.13 ± 0.03	4.51 ± 0.20	52.67 ± 0.60	530.5 ± 18.6
3m	> 100	15.68 ± 1.83	8.54 ± 0.10	> 2500
3n	5.02 ± 0.14	14.32 ± 1.10	55.31 ± 0.79	486.7 ± 11.6
3o	55.92 ± 0.15	26.12 ± 0.32	24.39 ± 0.13	1505.8 ± 5.1
3p	8.39 ± 0.35	15.56 ± 1.20	22.76 ± 0.33	705.3 ± 1.3
1	> 100	28.49 ± 3.20	8.78 ± 0.10	> 2500
L-Ascorbic acid	9.86 ± 0.33	27.34 ± 3.63	17.29 ± 0.24	–
Trolox	2.50 ± 0.02	16.71 ± 3.07	32.46 ± 0.03	652.4 ± 7.5



a. TPP-DIAD-THF, r.t., 48 h

Scheme 1

We applied the method to successfully synthesized other target compounds. The method has the advantages of short steps, good selectivity, and high yield.

The radical scavenging activity of tyrosol esters (**3a–3p**) in comparison with compound **1**, L-ascorbic acid, and Trolox determined by the DPPH assays is shown in Table 1. The antioxidant activity of **3a–3p** varied depending on the number of free hydroxyl groups contained in the molecule, showing a direct relationship with the antioxidant power. In particular, compounds containing the *ortho*-diphenolic structure are more active than L-ascorbic acid in antioxidant activity, such as **3d**, **3g**, **3k**, **3l**, and **3n**. Compound **3p** is also better than L-ascorbic acid because it bears the Trolox moieties. Compounds **3g**, **3k**, and **3l** are better than Trolox. Compounds **3h–3j** all contain two non-adjacent phenolic hydroxyl groups, but they did not perform well. Finally, other tyrosol derivatives (**3a**, **3b**, **3c**, **3e**, **3f**, **3m**, and **3o**) showed lower radical scavenging activities than L-ascorbic acid. So the *ortho*-diphenolic structure is crucial for antioxidant activity. Compound **1** showed lower activity in DPPH assays.

TABLE 2. α-Glucosidase Inhibition Activity of Compounds **3a–3p**

Compound	IC_{50}, μM	Compound	IC_{50}, μM
3a	42.6 ± 0.3	**3j**	245.8 ± 3.8
3b	439.1 ± 8.6	**3k**	138.5 ± 1.9
3d	384.2 ± 5.0	**3l**	88.8 ± 0.5
3e	19.0 ± 0.3	**3m**	100.2 ± 4.8
3f	146.8 ± 2.2	**3n**	18.1 ± 0.1
3g	56.7 ± 0.9	**3o**	729.6 ± 6.1
3h	48.9 ± 0.5	**3p**	250.9 ± 3.4
3i	203.6 ± 1.3	Acarbose	60.9 ± 1.0

TABLE 3. Anticholinesterase Activity of Compounds **3a–3p** (IC_{50}, μM)

Compound	AChE	BChE	Compound	AChE	BChE
3b	828.0 ± 17.7	912.1 ± 8.8	**3j**	205.4 ± 6.6	511.6 ± 5.9
3d	> 1000	363.3 ± 7.6	**3k**	> 1000	865.0 ± 6.5
3f	846.7 ± 15.5	> 1000	**3l**	634.2 ± 9.8	586.7 ± 4.6
3g	219.7 ± 10.6	690.2 ± 7.9	Donepezil	0.1 ± 0.0	3.6 ± 0.1
3h	535.0 ± 12.8	> 1000			

The $ABTS^{\bullet+}$ assay is a method widely used for measuring the radical-scavenging activity of antioxidants. The radical scavenging activity of tyrosol esters (**3a–3p**) in comparison with compound **1**, L-ascorbic acid, and Trolox determined by $ABTS^{\bullet+}$ assays is shown in Table 1. Compounds **3c**, **3d**, **3f**, **3g**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, **3o**, and **3p** showed higher antioxidant capacity, ranging from IC_{50} = 4.51 to 26.12 μM, than L-ascorbic acid (IC_{50} = 27.34 μM). Compounds **3d**, **3f**, **3g**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n**, and **3p** are better than Trolox (16.71 μM). Compared to L-ascorbic acid, compound **1** showed similar activity in ABTS assays.

Results of the reducing power of tyrosol esters (**3a–3p**) evaluated by the FRAP assay (expressed in millimoles of Fe(II) per gram) in comparison with compound **1**, L-ascorbic acid, and Trolox are summarized in Table 1. Compounds **3d, 3f**, **3g**, **3j**, **3k**, **3l**, **3n**, **3o**, and **3p** (18.89–65.30 mmol/g) showed higher antioxidant potency than L-ascorbic acid (17.29 mmol/g), and **3d**, **3g**, **3j**, **3k**, **3l**, and **3n** are better than Trolox (32.46 mmol/g). Compound **1** showed lower activity in FRAP assays.

The radical scavenging activity of tyrosol esters (**3a–3p**) in comparison with compound **1** and Trolox determined by hydroxyl radical scavenging assays is shown in Table 1. Compounds **3d**, **3l**, and **3n** exhibited more potent hydroxyl radical scavenging activity (IC_{50} = 404.1–530.5 μM) than Trolox (652.4 μM). Compound **1** showed lower activity in hydroxyl radical scavenging assays.

Results of the α-glucosidase inhibition activity of tyrosol esters (**3a–3p**) evaluated by the α-glucosidase inhibition assay in comparison with compound **1** and acarbose are summarized in Table 2. Compounds **3a**, **3e**, **3g**, **3h**, and **3n** (18.1–56.7 μM) showed higher inhibition potency than acarbose (60.9 μM). Compounds **1** and **3c** (IC_{50} > 800 μM) showed lower inhibition activity.

The anticholinesterase activity of tyrosol esters (**3a–3p**) in comparison with compound **1** and donepezil determined by cholinesterase inhibition assays is shown in Table 3. Compounds **3b**, **3f**, **3g**, **3h**, **3j**, and **3l** showed weaker AChE inhibitory activities, ranging from IC_{50} = 205.4 to 846.7 μM, than donepezil (IC_{50} = 0.1 μM). Compounds **3b**, **3d**, **3g**, **3j**, **3k**, and **3l** showed weaker BChE inhibitory activities, ranging from IC_{50} = 363.3 to 912.1 μM, than donepezil (IC_{50} = 3.6 μM). Compounds **3b**, **3g**, **3j**, and **3l** showed inhibitory effects on both cholinesterases, and compounds **1**, **3a**, **3c**, **3e**, **3i**, and **3m–3p** (IC_{50} > 1000 μM) showed lower activity in cholinesterase inhibition assays.

Generally, free radicals play an important role in a number of biological processes. Many of these are necessary for life. ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis [14]. However, because of their reactivity, these same free radicals can participate in unwanted side reactions, resulting in cell damage. Excessive amounts of these free radicals can lead to cell injury and death, which is recognized as a leading cause of a variety of chronic diseases such as atherosclerosis, diabetes, and dyslipidemia [15]. It is well known that

antioxidants can scavenge free radicals and ROS and protect the body from oxidative damage. Dietary antioxidants have been investigated for potential effects on neurodegenerative diseases such as Alzheimer′s disease [16] and Parkinson′s disease [17]. To some extent, this study indicates that tyrosol ester derivatives are all potent antioxidants; they also have hypoglycemic activity and anticholinesterase activity, which are potential functional chemicals beneficial for human health worthy of further investigation.

EXPERIMENTAL

General. All manipulations were conducted with a standard Schlenk tube under N_2. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Yeast α-glucosidase (EC 3.2.1.20), electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7), horse serum butyrylcholinesterase (BChE, EC 3.1.1.8), and *s*-butyrylthiocholine chloride were purchased from the supplier (Sigma-Aldrich). Tyrosol, 98%, triphenylphosphine (TPP, 98%), diisopropyl azodicarboxylate (DIAD, 97%), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium (ABTS, 98%), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ, 98%), and acetylthiocholine iodide (ATCI, 98%) were purchased from the supplier (Energy Chemical). Acarbose (98%) was from Ark Pharm. *p*-Nitrophenyl-α-D-glucopyranoside (pNPG, 99%) were from ACROS. Donepezil hydrochloride (98%) was from Adamas. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 98%) was purchased from the supplier (TCI). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, 95%) and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, 99%) were purchased from the supplier (Alfa Aesar).

Sephadex LH-20 (GE) was used for column chromatography, with MeOH as eluent. Chemical reactions were monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} plates. Developed plates were visualized by ultraviolet light (254 nm). ^{1}H and ^{13}C NMR spectra were measured on an AV-600 Spectrometer (Bruker, Germany) using tetramethylsilane (TMS) as internal standard. High-Resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on an Aglient 6520 Q-TOF spectrometer (Agilent, USA). Melting points were determined in open capillary tubes and the temperature was uncorrected.

Synthesis of the Target Compounds. The synthesis of compounds **3a–3f** was reported in our previous work [12].

Synthesis of Target Compounds 3g–3p. Tyrosol (**1**, 0.4 mmol, 1.0 equiv), organic acids **2g–2p** (0.4 mmol, 1.0 equiv), and TPP (0.4 mmol, 1.0 equiv) were placed in a dry standard Schlenk tube under N_2. Dry THF (1.0 mL) was added, followed by the addition of DIAD (0.4 mmol, 1.0 equiv) at 0°C. The reaction mixture was stirred at room temperature for 48 h, and the reaction was monitored with TLC. The crude reaction mixture was purified by column chromatography on Sephadex LH-20 to afford the corresponding product.

4-Hydroxyphenethyl 3,4-Dihydroxybenzoate (3g). $C_{15}H_{14}O_5$, white solid, yield 69%; mp 178–179°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.44 (3H, s, 3′, 4′, 4-OH), 7.34 (1H, d, J = 2.0, H-2′), 7.29 (1H, dd, J = 8.2, 2.0, H-6′), 7.08 (2H, d, J = 8.4, H-2, 6), 6.80 (1H, d, J = 8.2, H-5′), 6.70 (2H, d, J = 8.4, H-3, 5), 4.30 (2H, t, J = 6.8, H-α), 2.86 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 165.6 (C-7), 155.8 (C-4), 150.4 (C-4′), 145.0 (C-3′), 129.8 (C-2, 6), 128.1 (C-1), 121.8 (C-1′), 120.7 (C-6′), 116.3 (C-5′), 115.3 (C-2′), 115.2 (C-3, 5), 65.0 (C-α), 33.7 (C-β). HR-ESI-MS *m/z* 273.0821 $[M - H]^-$.

4-Hydroxyphenethyl 2,4-Dihydroxybenzoate (3h). $C_{15}H_{14}O_5$, white solid, yield 67%; mp 100–102°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 10.72 (1H, s, 2′-OH), 10.45 (1H, s, 4′-OH), 9.24 (1H, s, 4-OH), 7.59 (1H, d, J = 8.8, H-6′), 7.08 (2H, d, J = 8.4, H-2, 6), 6.70 (2H, d, J = 8.4, H-3, 5), 6.37 (1H, dd, J = 8.8, 2.4, H-5′), 6.29 (1H, d, J = 2.4, H-3′), 4.38 (2H, t, J = 6.8, H-α), 2.89 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 169.1 (C-7), 164.3 (C-4′), 162.8 (C-2′), 155.9 (C-4), 131.5 (C-6′), 129.8 (C-2, 6), 127.8 (C-1), 115.2 (C-3, 5), 108.4 (C-5′), 103.9 (C-1′), 102.5 (C-3′), 65.5 (C-α), 33.5 (C-β). HR-ESI-MS *m/z* 273.0786 $[M - H]^-$.

4-Hydroxyphenethyl 3,5-Dihydroxybenzoate (3i). $C_{15}H_{14}O_5$, white solid, yield 78%; mp 125–127°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.61 (2H, s, 3′, 5′-OH), 9.21 (1H, s, 4-OH), 7.08 (2H, d, J = 7.4, H-2, 6), 6.79 (2H, s, H-2′, 6′), 6.69 (2H, d, J = 7.4, H-3, 5), 6.43 (1H, s, H-4′), 4.33 (2H, s, H-α), 2.87 (2H, s, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 165.7 (C-7), 158.5 (C-3′, 5′), 155.8 (C-4), 131.5 (C-1), 129.8 (C-2, 6), 128.0 (C-1′), 115.2 (C-3, 5), 107.2 (C-4′), 107.1 (C-2′, 6′), 65.4 (C-α), 33.6 (C-β). HR-ESI-MS *m/z* 297.0411 $[M + Na]^+$.

4-Hydroxyphenethyl 2,5-Dihydroxybenzoate (3j). $C_{15}H_{14}O_5$, white solid, yield 82%; mp 91–92°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.94 (1H, s, 2′-OH), 9.23 (1H, s, 5′-OH), 9.22 (1H, s, 4-OH), 7.12 (1H, d, J = 3.0, H-6′), 7.10 (2H, d, J = 8.4, H-2, 6), 6.97 (1H, dd, J = 8.9, 3.0, H-4′), 6.81 (1H, d, J = 8.9, H-3′), 6.70 (2H, d, J = 8.4, H-3, 5), 4.42 (2H, t, J = 6.8, H-α), 2.91 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 168.8 (C-7),

155.9 (C-4), 153.3 (C-2′), 149.5 (C-5′), 129.8 (C-2, 6), 127.7 (C-1), 123.9 (C-6′), 118.1 (C-4′), 115.2 (C-3, 5), 114.0 (C-3′), 112.3 (C-1′), 65.9 (C-α), 33.4 (C-β). HR-ESI-MS *m/z* 297.0411 $[M + Na]^+$.

4-Hydroxyphenethyl 2,3-Dihydroxybenzoate (3k). $C_{15}H_{14}O_5$, white solid, yield 86%; mp 110–111°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 10.42 (1H, s, 2′-OH), 9.39 (1H, s, 3′-OH), 9.22 (1H, s, 4-OH), 7.19 (1H, d, J = 7.9, H-6′), 7.09 (2H, d, J = 8.2, H-2, 6), 7.02 (1H, d, J = 7.9, H-4′), 6.74 (1H, t, J = 7.9, H-5′), 6.69 (2H, d, J = 8.2, H-3, 5), 4.43 (2H, t, J = 6.8, H-α), 2.92 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 169.4 (C-7), 155.9 (C-4), 149.6 (C-2′), 146.1 (C-3′), 129.8 (C-2, 6), 127.7 (C-1), 120.7 (C-4′), 119.4 (C-6′), 118.9 (C-5′), 115.2 (C-3, 5), 113.0 (C-1′), 66.0 (C-α), 33.4 (C-β). HR-ESI-MS *m/z* 297.0409 $[M + Na]^+$.

4-Hydroxyphenethyl 3,4,5-Trihydroxybenzoate (3l). $C_{15}H_{14}O_6$, white solid, yield 40%; mp 207–209°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.21 (3H, s, 3′, 5′, 4-OH), 8.92 (1H, s, 4′-OH), 7.07 (2H, d, J = 8.4, H-2, 6), 6.92 (2H, s, H-2′, 6′), 6.69 (2H, d, J = 8.4, H-3, 5), 4.28 (2H, t, J = 6.8, H-α), 2.85 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 165.8 (C-7), 155.8 (C-4), 145.5 (C-3′, 5′), 138.4 (C-4′), 129.8 (C-2, 6), 128.1 (C-1), 119.4 (C-1′), 115.1 (C-3, 5), 108.5 (C-2′, 6′), 65.0 (C-α), 33.7 (C-β). HR-ESI-MS *m/z* 313.0544 $[M + Na]^+$.

4-Hydroxyphenethyl 3-(4-Hydroxyphenyl)propanoate (3m). $C_{17}H_{18}O_4$, white solid, yield 85%; mp 122–123°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.19 (1H, s, 4-OH), 9.15 (1H, s, 4′-OH), 6.99 (2H, d, J = 8.0, H-2, 6), 6.95 (2H, d, J = 8.1, H-2′, 6′), 6.68 (2H, d, J = 8.0, H-3, 5), 6.65 (2H, d, J = 8.1, H-3′, 5′), 4.11 (2H, t, J = 6.8, H-α), 2.77–2.65 (4H, m, H-β, 9), 2.50 (2H, t, J = 7.1, H-8). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 172.2 (C-7), 155.8 (C-4), 155.6 (C-4′), 130.5 (C-1′), 129.7 (C-2, 6), 129.1 (C-2′, 6′), 127.9 (C-1), 115.1 (C-3, 5, 3′, 5′), 64.7 (C-α), 35.6 (C-8), 33.5 (C-β), 29.5 (C-9). HR-ESI-MS *m/z* 309.0756 $[M + Na]^+$.

4-Hydroxyphenethyl (*E*)-3-(3,4-Dihydroxyphenyl)-acrylate (3n). $C_{17}H_{16}O_5$, white solid, yield 59%; mp 173–174°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.23 (3H, s, 3′, 4′, 4-OH), 7.45 (1H, d, J = 15.9, H-β'), 7.06 (2H, d, J = 8.2, H-2, 6), 7.04 (1H, d, J = 1.2, H-2′), 6.99 (1H, d, J = 8.1, 1.2, H-6′), 6.76 (1H, d, J = 8.1, H-5′), 6.69 (2H, d, J = 8.2, H-3, 5), 6.23 (1H, d, J = 15.9, H-α'), 4.24 (2H, t, J = 6.8, H-α), 2.82 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 166.5 (C-7), 155.8 (C-4), 148.4 (C-4′), 145.6 (C-3′), 145.1 (C-β'), 129.8 (C-2, 6), 128.0 (C-1), 125.5 (C-1′), 121.3 (C-6′), 115.7 (C-5′), 115.1 (C-3, 5), 114.8 (C-α'), 113.9 (C-2′), 64.7 (C-α), 33.7 (C-β). HR-ESI-MS *m/z* 323.0539 $[M + Na]^+$.

4-Hydroxyphenethyl (*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)acrylate (3o). $C_{19}H_{20}O_6$, colorless oil, yield 46%. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.20 (1H, s, 4-OH), 8.95 (1H, s, 4′-OH), 7.53 (1H, d, J = 15.9, H-β'), 7.07 (2H, d, J = 8.4, H-2, 6), 7.02 (2H, s, H-2′, 6′), 6.70 (2H, d, J = 8.4, H-3, 5), 6.50 (1H, d, J = 15.9, H-α'), 4.27 (2H, t, J = 6.8, H-α), 3.80 (6H, s, H-8, 9), 2.83 (2H, t, J = 6.8, H-β). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 166.5 (C-7), 155.8 (C-4), 148.0 (C-3′, 5′), 145.4 (C-β'), 138.3 (C-4′), 129.7 (C-2, 6), 127.9 (C-1), 124.3 (C-1′), 115.1 (C-3, 5), 114.8 (C-α'), 106.2 (C-2′, 6′), 64.6 (C-α), 56.1 (C-8, 9), 33.7 (C-β). HR-ESI-MS *m/z* 367.0749 $[M + Na]^+$.

4-Hydroxyphenethyl 6-Hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylate (3p). $C_{22}H_{26}O_5$, white solid, yield 91%; mp 137–139°C. ^{1}H NMR spectrum (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.20 (1H, s, 4-OH), 7.45 (1H, s, 3′-OH), 6.94 (2H, d, J = 8.4, H-2, 6), 6.66 (2H, d, J = 8.4, H-3, 5), 4.23–4.08 (2H, m, H-α), 2.75–2.62 (2H, m, H-β), 2.49–2.43 (1H, m, H-10a), 2.26–2.23 (1H, m, H-10b), 2.22–2.14 (1H, m, H-9a), 2.07 (3H, s, H-14), 2.03 (3H, s, H-12), 1.96 (3H, s, H-13), 1.76–1.71 (1H, m, H-9b), 1.45 (3H, s, H-11). ^{13}C NMR spectrum (150 MHz, DMSO-d_6, δ, ppm): 172.8 (C-7), 155.9 (C-4), 145.7 (C-6′), 144.7 (C-3′), 129.7 (C-2, 6), 127.9 (C-1), 122.6 (C-5′), 120.9 (C-4′), 120.2 (C-2′), 116.4 (C-1′), 115.1 (C-3, 5), 76.4 (C-8), 65.5 (C-α), 33.5 (C-β), 30.2 (C-10), 25.2 (C-9), 20.3 (C-11), 12.7 (C-14), 11.8 (C-12), 11.7 (C-13). HR-ESI-MS *m/z* 763.2607 $[2M + Na]^+$.

DPPH Assay. DPPH assay was performed according to the method as previously described with slight modifications [18, 19]. Each sample in DMSO solution (100 μL) was added to 100 μL of DPPH methanol solution (50 μM). The solution was vortexed in 96-well plates for 10 s and then left at room temperature for 20 min in the dark. The absorbance of the resulting solution was measured at 492 nm on a microplate spectrophotometer (BioTek). L-Ascorbic acid and Trolox were used as positive references. IC_{50} values (concentration required to scavenge 50% DPPH radicals present in the test solution) were calculated and expressed as means ± SD in micromoles.

ABTS Free Radical Cation ($ABTS^{\bullet+}$) Assay. The $ABTS^{\bullet+}$ scavenging activity of the sample was assayed following procedures previously described with slight modifications [19]. $ABTS^{\bullet+}$ solution was produced by reacting 7 mM ABTS water solution (10 mL) with 2.6 mM potassium persulfate (10 mL). The mixture was allowed to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was diluted with methanol to provide an absorbance of 0.70 ± 0.02 at 734 nm. Then each sample in DMSO solution (5 μL) was added to 200 μL of diluted $ABTS^{\bullet+}$ solution. The solution was vortexed in

96-well plates for 10 s and then left at room temperature for 20 min in the dark. The absorbance of the resulting solution was measured at 734 nm on a microplate spectrophotometer (BioTek). L-Ascorbic acid and Trolox were used as positive references. IC_{50} values were calculated and expressed as means ± SD in micromoles.

Ferric Reducing Antioxidant Power (FRAP) Assay. Ferric reducing ability of the sample was conducted according to procedures previously described with slight modifications [19, 20]. FRAP reagent was made freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM aqueous $FeCl_3$ solution in a 10:1:1 (v/v) ratio. Each sample in DMSO solution (5 μL) was added to 180 μL of FRAP reagent and vortexed in 96-well plates for 10 s and then incubated at 37°C for 30 min in the dark. The absorbance was determined at 595 nm using a microplate spectrophotometer (BioTek). L-Ascorbic acid and Trolox were used as positive references. $FeSO_4$ was used for a calibration curve. FRAP values were calculated and expressed as means ± SD in millimoles of Fe(II) per gram.

Hydroxyl Radical (·OH) Assay. Hydroxyl radical scavenging activity was assayed according to the method previously described with slight modifications [21]. Each sample in DMSO solution (50 μL) was treated with 3 mM $FeSO_4$ solution (50 μL) and 3 mM H_2O_2 solution (50 μL), vortexed in 96-well plates, and left to stand for 10 min; 6 mM salicylic acid solution (50 μL) was added. The reaction mixtures were vortexed and the plates incubated at room temperature for 30 min in the dark. The absorbance of the resulting solution was measured at 492 nm on a microplate spectrophotometer (BioTek). Trolox was used as a positive reference. IC_{50} values (concentration required to scavenge 50% hydroxyl radicals present in the test solution) were calculated and expressed as means ± SD in micromoles.

***α*-Glucosidase Inhibition Assay**. *α*-Glucosidase was assayed according to the method previously described with slight modifications [22]. Each sample in DMSO solution (20 μL) was added to 100 μL of *α*-glucosidase solution (pH 6.9, 0.1 U/mL, in 0.1 M phosphate buffer). The reaction mixtures were incubated at 25°C for 10 min. Then 50 μL pNPG solution (pH 6.9, 5 mM, in 0.1 M phosphate buffer) was added to each well, and the reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Acarbose was used as a positive reference.

Acetylcholinesterase Inhibition Assay. Acetylcholinesterase (AChE) inhibitory activities were measured according to the method previously described with slight modifications [23]. Each sample in 10% DMSO solution (20 μL) was added to 120 μL phosphate buffer (pH 8.0, 0.1 M) and 20 μL of acetylcholinesterase solution (pH 8.0, 0.8 U/mL, in 0.1 M phosphate buffer). The reaction mixtures were incubated at 25°C for 15 min. Then 20 μL ATCI solution (pH 8.0, 1.78 mM, in 0.1 M phosphate buffer) and 20 μL DTNB solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, and the reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Donepezil was used as a positive reference.

Butyrylcholinesterase Inhibition Assay. Butyrylcholinesterase (BChE) inhibitory activities were measured according to the method previously described with slight modifications [23]. Each sample in 10% DMSO solution (20 μL) was added to 120 μL phosphate buffer (pH 8.0, 0.1 M) and 20 μL of butyrylcholinesterase solution (pH 8.0, 0.8 U/mL, in 0.1 M phosphate buffer). The reaction mixtures were incubated at 25°C for 15 min. Then 20 μL butyrylthiocholine chloride solution (pH 8.0, 0.4 mM, in 0.1 M phosphate buffer) and 20 μL DTNB solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, and the reaction mixtures incubated at 25°C for 5 min. Before and after incubation, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Donepezil was used as a positive reference.

The *α*-glucosidase, acetylcholinesterase, and butyrylcholinesterase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\%\text{Inhibition} = (1 - \Delta A_{sample}/\Delta A_{control}) \times 100\%.$$

All the experiments were carried out in triplicate, and the data were analyzed using SPSS software (Version 22.0) and Origin software (Version 8.0).

ACKNOWLEDGMENT

This work was supported by the Science and Technology Development Funds of Jilin Province (No. 20160520044JH) and the Science and Technology Projects of Administration of Traditional Chinese Medicine of Jilin Province (No. 2017110). In addition, Hao Zang and Peng Shen contributed equally to this work.

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