Synthesis and Biological Activities of Hydroxytyrosol Ester Derivatives

[1,2]Hao Zang, [1,2]Qian Xu, [1-3]Luyun Zhang, [1-3]Guangqing Xia*, [3]Jiaming Sun* and [1,2]Junyi Zhu
[1]*Tonghua Normal University, School of Pharmacy and Medicine, Tonghua 134002, China.*
[2]*Tonghua Normal University, Green Medicinal Chemistry Laboratory, Tonghua 134002, China.*
[3]*Changchun University of Chinese Medicine, Jilin Ginseng Academy, Changchun 130117, China*.
qingguangx@163.com*; sun_jiaming2000@163.com*

(Received on 5th November 2018, accepted in revised form 17th April 2019)

Summary: A series of hydroxytyrosol (HT) derivatives were synthesized by modification of alcohol hydroxyl group of HT, twenty-five target compounds were obtained and characterized by NMR and HRMS. The antioxidant activities of those compounds were evaluated in three different assays. Except **3e** and **3y**, all other compounds demonstrated significant 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical cation scavenging activity ranging from IC_{50} 3.4 to 24.4 μM, which were more potent than L-ascorbic acid (IC_{50}=24.8 μM). Compounds **3b-3d**, **3f-3k**, **3m-3x** were better than Trolox (18.3 μM). Moreover, the ferric reducing antioxidant power (FRAP) of all compounds were discovered to be more potent than L-ascorbic acid (40.7 mmol/g), except **3e**, all other compounds (141.5-202.1 mmol/g) were better than Trolox (94.7 mmol/g). Compounds **3a-3d**, **3f-3j**, **3l-3m**, **3o, 3q**, **3t**, **3v-3y** exhibited more potent hydroxyl radical scavenging activity (IC_{50}=245.1-475.1 μM) than L-ascorbic acid (554.4 μM) and Trolox (500.4 μM). Compounds **3q**, **3t** and **3y** exhibited more potent α-Glucosidase inhibition activity (39.1-52.4 μM) than Acarbose (60.9 μM). Compounds **3a**, **3d**, **3f-3m**, **3s-3t**, **3v-3y** showed some acetylcholinesterase inhibition activities, compounds **3a**, **3d**, **3f-3j**, **3l-3m**, **3o-3p**, **3s-3t**, **3w** showed some butyrylcholinesterase inhibition activities.

Keywords: Hydroxytyrosol; Ester derivatives; Synthesis; Biological activity.

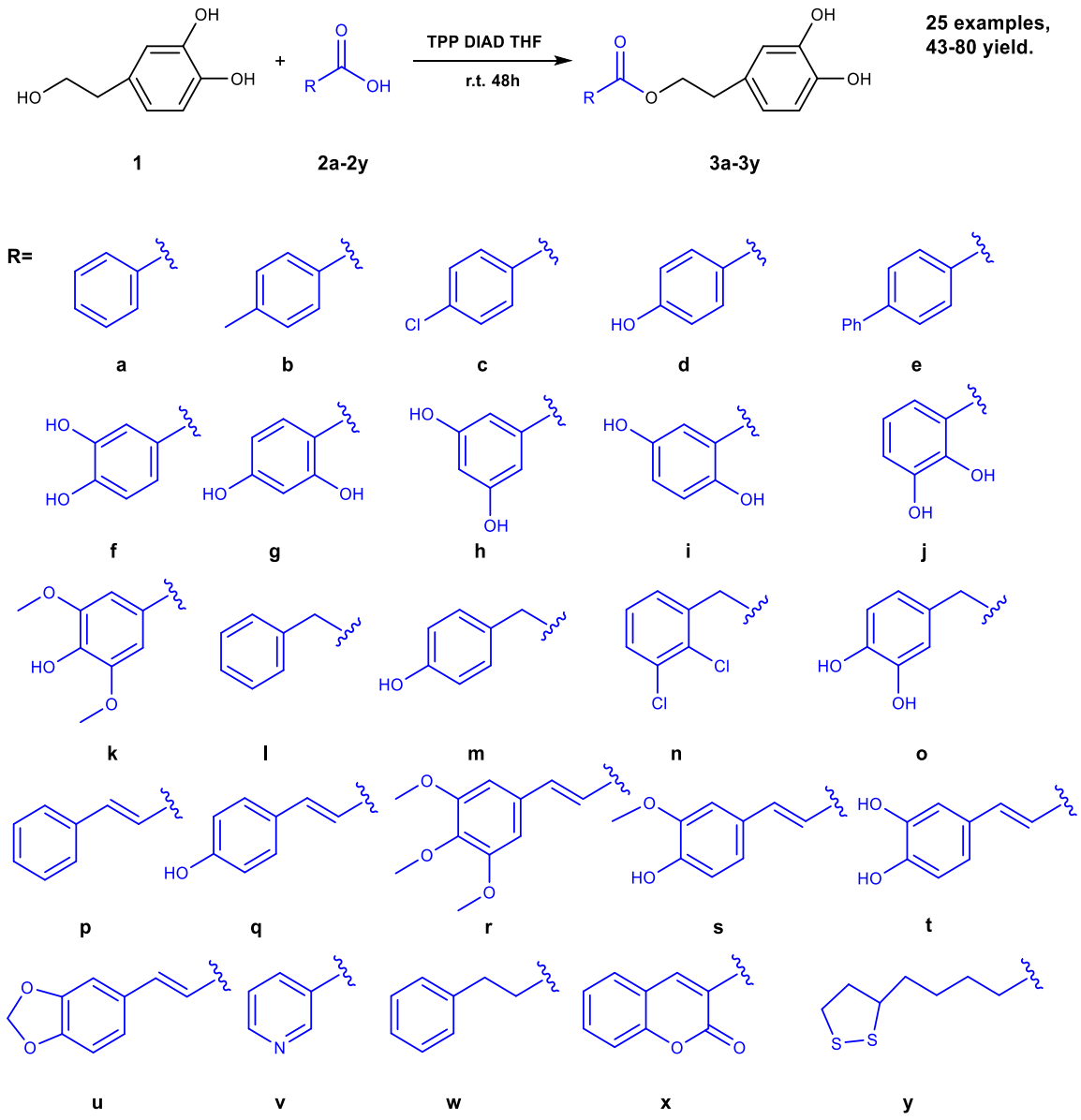
Introduction

Mediterranean diet is important for human health and has been extensively studied. This diet is characterized by an abundant intake of cereals, vegetables and fruits, olive oil, nuts and beans, a small amount of meat, fish, etc. It is well known that the Mediterranean diet is very effective against cardiovascular disease, diabetes, inflammation, cancer and aging. Many benefits of the diet are due to the presence of large amounts of antioxidants and anti-inflammatory substances in several components of the diet. There is much evidence that the health benefits of the Mediterranean diet are mainly attributed to the consumption of olive oil. Evidence suggests that phenols, minor constituents in olive oil, might provide health benefits, hydroxytyrosol (HT) is the most abundant natural phenol in olive oil.

Among various phenols on the market, HT is the only substance approved by the European Food Safety Authority as a novel food [1], it is recommended to be added to fish oil and vegetable oil. The safety profile of HT appears to be excellent, there was no genotoxicity and mutagenicity [2], no adverse effects were observed in toxicological evaluation [3]. HT exhibits a wide range of biological activities which attribute to the ortho-diphenolic moiety. The main biological activities of HT is antimicrobial activity [4-6], to prevent damages caused by various free radicals [7,8], to prevent and treat diabetes and increase the sensitivity of adipose tissue to insulin [9,10], to reduce the extent of atherosclerotic lesions and prevent myocardial ischemia/reperfusion injury [11-12], to improve the symptoms of Parkinson's disease [13] and Alzheimer's disease [14-16], to inhibit multiple tumor proliferation [17-23] and so on. In view of these, to develop HT derivatives may provide promising antioxidant, hypoglycemic agent and cholinesterase inhibitor.

However, the highly polar nature of HT reduces its solubility in lipids; therefore, we make efforts to synthesize HT ester derivatives (Scheme 1) to enhance hydrophilic/lipophilic balance. Taking into account the biological activities of HT, we assayed the antioxidant activities (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical cation scavenging activity assay, ferric reducing antioxidant power (FRAP) assay and hydroxyl radical scavenging activity assay), α-Glucosidase inhibition activity, cholinesterase inhibition activities (acetylcholinesterase inhibition activities assay and butyrylcholinesterase inhibition activities assay).

***To whom all correspondence should be addressed.**



Scheme-1: Synthesis of HT esters **3a-3y**.

Experimental

General Information

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), S-Butyrylthiocholine chloride were purchased from the supplier (Sigma-Aldrich). Dicyclohexylcarbodiimide (DCC, 98%), 4-Dimethylaminopyridine (DMAP, 98%), Triphenylphosphine (TPP, 98%), Diisopropyl azodicarboxylate (DIAD, 97%), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, 98%), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ, 98%), Acetylthiocholine iodide (ATCI, 98%) were purchased from the supplier (Energy Chemical). Acarbose 98% from Ark Pharm. p-Nitrophenyl-α-D-glucopyranoside (pNPG, 99%) from acros. Donepezil hydrochloride 98% 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%)

5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB, 99%) were purchased from the supplier (Alfa Aesar).

Sephadex LH-20 (GE) were used for column chromatography, methanol 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-NMR and ^{13}C-NMR spectra were measured on an AV-600 Spectrometer (Bruker, Germany) using tetramethylsilane as an internal standard. Electrospray ionization mass spectrometry (ESI-MS) was performed on an Aglient 6520 Q-TOF (Agilent, USA) in positive ionization mode. Melting points were determined in open capillary tubes and the temperature was uncorrected.

Synthesis

General experimental procedures for the synthesis of HT ester derivatives (**3a-3y**)

HT (0.4 mmol, 1.0 equiv), organic acids **2** (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.

3,4-dihydroxyphenethyl benzoate (**3a**): a gray solid, yield 69%. m.p. 103-105 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.77 (s, 1H, -OH), 8.69 (s, 1H, -OH), 7.93 (d, *J* = 7.1 Hz, 2H), 7.65 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 6.68 (d, *J* = 2.0 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.54 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.38 (t, *J* = 6.8 Hz, 2H), 2.84 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.7, 145.1, 143.7, 133.3, 129.8, 129.0, 128.7, 128.6, 119.5, 116.2, 115.5, 65.6, 33.8. HRMS calculated for C15H14O4Na 281.0790, found 281.0684.

3,4-dihydroxyphenethyl 4-methylbenzoate (**3b**): a yellow solid, yield 79%. m.p. 128-130 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.84 (s, 1H, -OH), 8.76 (s, 1H, -OH), 7.82 (d, *J* = 7.8 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 6.77–6.61 (m, 2H), 6.52 (d, *J* = 7.4 Hz, 1H), 4.35 (t, *J* = 6.6 Hz, 2H), 2.83 (t, *J* = 6.6 Hz, 2H), 2.37 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.6, 145.1, 143.8, 143.5, 129.3, 129.1, 128.6, 127.1, 119.5, 116.3, 115.6, 65.4, 33.8, 21.1. HRMS calculated for C16H16O4Na 295.0947, found 295.0812.

3,4-dihydroxyphenethyl 4-chlorobenzoate (**3c**): a brown solid, yield 70%. m.p. 125-126 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.77 (s, 1H, -OH), 8.69 (s, 1H, -OH), 7.92 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 6.67 (d, *J* = 1.6 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.53 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.38 (t, *J* = 6.8 Hz, 2H), 2.84 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): 164.8, 145.1, 143.8, 138.2, 130.9, 128.9, 128.6, 128.5, 119.5, 116.2, 115.5, 65.8, 33.7. HRMS calculated for C15H13ClO4Na 315.0400, found 315.0250.

3,4-dihydroxyphenethyl 4-hydroxybenzoate (**3d**): a light gray solid, yield 60%. m.p. 130-132 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.31 (s, 1H, -OH), 8.74 (s, 2H, -OH), 7.78 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.72–6.59 (m, 2H), 6.52 (d, *J* = 6.9 Hz, 1H), 4.30 (t, *J* = 6.8 Hz, 2H), 2.81 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.5, 161.9, 145.1, 143.7, 131.3, 128.8, 120.4, 119.5, 116.2, 115.5, 115.3, 65.0, 33.9. HRMS calculated for C15H14O5Na 297.0739, found 297.0595.

3,4-dihydroxyphenethyl [1,1'-biphenyl]-4-carboxylate (**3e**): a yellow solid, yield 69%. m.p. 134-135 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.78(s，1H, -OH)，8.70(s，1H, -OH)，8.01 (s, 1H), 8.00 (s, 1H), 7.82 (d, *J* = 7.1 Hz, 2H), 7.74 (d, *J* = 6.5 Hz, 2H), 7.50 (s, 2H), 7.44 (d, *J* = 6.3 Hz, 1H), 6.70 (s, 1H), 6.66 (d, *J* = 7.4 Hz, 1H), 6.55 (d, *J* = 6.9 Hz, 1H), 4.41 (s, 2H), 2.86 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ165.5, 145.1, 144.7, 143.8, 138.8, 129.7, 129.1, 128.6, 128.4, 127.0, 126.9, 119.5, 116.2, 115.5, 65.6, 33.8. HRMS calculated for C21H18O4Na 357.1103, found 357.0910.

3,4-dihydroxyphenethyl 3,4-dihydroxybenzoate (**3f**): a brown solid, yield 43%. m.p. 144-146 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.75 (s, 1H, -OH), 9.35 (s, 1H, -OH), 8.72 (s, 2H, -OH), 7.33 (d, *J* = 2.1 Hz, 1H), 7.29 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.65 (dd, *J* = 8.7, 5.0 Hz, 2H), 6.52 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.28 (t, *J* = 6.9 Hz, 2H), 2.80 (t, *J* = 6.9 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.6, 150.4, 145.1, 145.0, 143.7, 128.8, 121.7, 120.7, 119.5, 116.3, 116.2, 115.5, 115.3, 65.0, 33.9. HRMS calculated for C15H14O6Na 313.0688, found 313.0535. Spectroscopic data were accordant with those previously reported[24].

3,4-dihydroxyphenethyl 2,4-dihydroxybenzoate (**3g**): a light gray solid, yield 65%.

m.p. 160-161 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.71 (s, 1H, -OH), 10.46 (s, 1H, -OH), 8.77 (s, 1H, -OH), 8.70 (s, 1H, -OH), 7.60 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 2.0 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.52 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.36 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.28 (d, *J* = 2.3 Hz, 1H), 4.37 (t, *J* = 6.9 Hz, 2H), 2.83 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 169.1, 164.2, 162.8, 145.1, 143.8, 131.5, 128.5, 119.5, 116.2, 115.5, 108.3, 103.9, 102.4, 65.5, 33.7. HRMS calculated for C15H14O6Na 313.0688, found 313.0557.

3,4-dihydroxyphenethyl 3,5-dihydroxybenzoate (**3h**): a gray solid, yield 68%. m.p. 128-130 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.61 (s, 2H, -OH), 8.73 (s, 2H, -OH), 6.79 (d, *J* = 2.2 Hz, 2H), 6.65 (d, *J* = 8.0 Hz, 2H), 6.52 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.43 (t, *J* = 2.1 Hz, 1H), 4.31 (t, *J* = 6.9 Hz, 2H), 2.80 (t, *J* = 6.9 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.7, 158.5, 145.1, 143.7, 131.5, 128.7, 119.5, 116.2, 115.5, 107.2, 107.1, 65.4, 33.8. HRMS calculated for C15H14O6Na 313.0688, found 313.0551.

3,4-dihydroxyphenethyl 2,5-dihydroxybenzoate (**3i**): a white solid, yield 75%. m.p. 160-161 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.95 (s, 1H, -OH), 9.21 (s, 1H, -OH), 8.77 (s, 1H, -OH), 8.73 (s, 1H, -OH), 7.12 (d, *J* = 2.8 Hz, 1H), 6.97 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 1H), 6.72–6.63 (m, 2H), 6.54 (d, *J* = 7.8 Hz, 1H), 4.41 (t, *J* = 6.8 Hz, 2H), 2.85 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 168.9, 153.4, 149.6, 145.2, 143.8, 128.4, 124.0, 119.6, 118.2, 116.2, 115.6, 114.1, 112.4, 66.0, 33.6. HRMS calculated for C15H14O6Na 313.0688, found 313.0552.

3,4-dihydroxyphenethyl 2,3-dihydroxybenzoate (**3j**): a brown solid, yield 56%. m.p. 136-138 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.43 (s, 1H, -OH), 9.39 (s, 1H, -OH), 8.77 (s, 1H, -OH), 8.70 (s, 1H, -OH), 7.20 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.02 (dd, *J* = 7.8, 1.4 Hz, 1H), 6.74 (t, *J* = 7.9 Hz, 1H), 6.66 (dd, *J* = 18.5, 5.0 Hz, 2H), 6.53 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.41 (t, *J* = 6.8 Hz, 2H), 2.85 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 169.4, 149.6, 146.1, 145.1, 143.8, 128.4, 120.7, 119.5, 119.5, 118.9, 116.2, 115.5, 113.0, 66.0, 33.6. HRMS calculated for C15H14O6Na 313.0688, found 313.0555.

3,4-dihydroxyphenethyl 4-hydroxy-3,5-dimethoxybenzoate (**3k**): a light gray solid, yield 50%. m.p. 140-142 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.30 (s, 1H, -OH), 8.74 (s, 1H, -OH), 8.70 (s, 1H, -OH), 7.16 (s, 2H), 6.68 (d, *J* = 1.8 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.54 (dd, *J* = 8.0, 1.9 Hz, 1H), 4.32 (t, *J* = 6.8 Hz, 2H), 3.80 (s, 6H), 2.82 (t, *J* = 6.8 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.5, 147.5, 145.1, 143.7, 140.6, 128.8, 119.6, 119.4, 116.4, 115.5, 106.7, 65.3, 56.0, 33.9. HRMS calculated for C17H18O7Na 357.0951, found 357.0761.

3,4-dihydroxyphenethyl 2-phenylacetate (**3l**): a brown oil, yield 76%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.76 (s, 1H, -OH), 8.70 (s, 1H, -OH), 7.30 (t, *J* = 7.3 Hz, 2H), 7.27–7.18 (m, 3H), 6.62 (dd, *J* = 8.1, 5.0 Hz, 2H), 6.43 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.14 (t, *J* = 7.0 Hz, 2H), 3.63 (s, 2H), 2.69 (t, *J* = 7.0 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 171.1, 145.1, 143.7, 134.3, 129.3, 128.4, 128.3, 126.7, 119.5, 116.2, 115.5, 65.1, 40.3, 33.7. HRMS calculated for C16H16O4Na 295.0947, found 295.0830.

3,4-dihydroxyphenethyl 2-(4-hydroxyphenyl)acetate (**3m**): a brown solid, yield 56%. m.p. 119-121 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.29 (s, 1H, -OH), 8.76 (s, 1H, -OH), 8.68 (s, 1H, -OH), 7.00 (d, *J* = 8.3 Hz, 2H), 6.68 (d, *J* = 8.3 Hz, 2H), 6.62 (dd, *J* = 10.0, 4.7 Hz, 2H), 6.43 (dd, *J* = 7.9, 1.5 Hz, 1H), 4.12 (t, *J* = 7.0 Hz, 2H), 3.49 (s, 2H), 2.68 (t, *J* = 7.0 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 171.5, 156.2, 145.1, 143.7, 130.2, 128.5, 124.4, 119.5, 116.2, 115.5, 115.1, 65.0, 39.1, 33.7. HRMS calculated for C16H16O5Na 311.0896, found 311.0756.

3,4-dihydroxyphenethyl 2-(2,3-dichlorophenyl)acetate (**3n**): a brown solid, yield 80%. m.p. 108-109 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.74 (s, 1H, -OH), 8.68 (s, 1H, -OH), 7.57 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.37 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 6.61 (dd, *J* = 10.6, 5.0 Hz, 2H), 6.41 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.17 (t, *J* = 6.9 Hz, 2H), 3.86 (s, 2H), 2.69 (t, *J* = 6.9 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 169.6, 145.0, 143.7, 135.3, 131.8, 131.6, 130.8, 129.4, 128.4, 128.0, 119.5, 116.2, 115.5, 65.4, 39.3, 33.7. HRMS calculated for C16H14Cl2O4Na 363.0167, found 362.9984.

3,4-dihydroxyphenethyl 2-(3,4-dihydroxyphenyl)acetate (**3o**): a brown oil, yield 57%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.75 (s, 4H, -OH), 6.62 (td, *J* = 7.7, 3.8 Hz, 4H), 6.45 (ddd, *J* = 13.2, 8.0, 2.0 Hz, 2H), 4.11 (t, *J* = 7.1 Hz, 2H), 3.41 (s, 2H), 2.67 (t, *J* = 7.1 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 171.4, 145.1, 145.0, 144.1, 143.7, 128.4, 124.9, 120.0, 119.5, 116.6, 116.2,

115.5, 115.4, 65.0, 39.2, 33.8. HRMS calculated for C16H16O6Na 327.0845, found 327.0678.

3,4-dihydroxyphenethyl cinnamate (**3p**): a gray solid, yield 78%. m.p. 102-105 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.77 (s, 1H, -OH), 8.69 (s, 1H, -OH), 7.71 (dd, *J* = 6.6, 2.8 Hz, 2H), 7.63 (d, *J* = 16.0 Hz, 1H), 7.42 (dd, *J* = 5.0, 1.7 Hz, 3H), 6.67–6.63 (m, 2H), 6.61 (d, *J* = 16.0 Hz, 1H), 6.51 (dd, *J* = 8.0, 1.9 Hz, 1H), 4.26 (t, *J* = 7.0 Hz, 2H), 2.78 (t, *J* = 7.0 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.1, 145.1, 144.5, 143.8, 134.0, 130.5, 128.9, 128.5, 128.4, 119.5, 118.0, 116.2, 115.5, 64.9, 33.8. HRMS calculated for C17H16O4Na 307.0947, found 307.0821.

3,4-dihydroxyphenethyl (E)-3-(4-hydroxyphenyl)acrylate (**3q**): a light gray solid, yield 54%. m.p. 148-150 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.00 (s, 1H, -OH), 8.73 (s, 2H, -OH), 7.54 (s, 3H), 6.79 (s, 2H), 6.64 (s, 2H), 6.50 (s, 1H), 6.35 (d, *J* = 15.5 Hz, 1H), 4.22 (s, 2H), 2.75 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.5, 159.8, 145.1, 144.7, 143.7, 130.3, 128.6, 125.0, 119.5, 116.2, 115.7, 115.5, 114.1, 64.7, 33.9. HRMS calculated for C17H16O5Na 323.0896, found 323.0727.

3,4-dihydroxyphenethyl (E)-3-(3,4,5-trimethoxyphenyl)acrylate (**3r**): a brown solid, yield 77%. m.p. 134-135 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.76 (s, 1H, -OH), 8.70 (s, 1H, -OH), 7.57 (d, *J* = 15.9 Hz, 1H), 7.06 (s, 2H), 6.67–6.60 (m, 3H), 6.51 (dd, *J* = 8.0, 1.5 Hz, 1H), 4.26 (t, *J* = 7.0 Hz, 2H), 3.81 (s, 6H), 3.69 (s, 3H), 2.78 (t, *J* = 7.0 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.3, 153.0, 145.1, 144.7, 143.7, 139.5, 129.6, 128.5, 119.4, 117.3, 116.2, 115.5, 106.0, 64.8, 60.1, 56.0, 33.8. HRMS calculated for C20H23O7 375.1444, found 375.1242.

3,4-dihydroxyphenethyl (E)-3-(4-hydroxy-3-methoxyphenyl)acrylate (**3s**): a light gray solid, yield 53%. m.p. 109-111 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.57 (s, 1H, -OH), 8.74 (s, 2H, -OH), 7.53 (d, *J* = 15.6 Hz, 1H), 7.31 (s, 1H), 7.10 (s, 1H), 6.79 (d, *J* = 7.0 Hz, 1H), 6.65 (s, 2H), 6.51 (d, *J* = 6.1 Hz, 1H), 6.44 (d, *J* = 15.6 Hz, 1H), 4.24 (s, 2H), 3.81 (s, 3H), 2.76 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.6, 149.3, 147.9, 145.1, 143.7, 128.6, 125.5, 123.2, 119.5, 116.2, 115.5, 115.5, 114.4, 111.2, 64.6, 55.7, 33.9. HRMS calculated for C18H18O6Na 353.1001, found 353.0852.

3,4-dihydroxyphenethyl (E)-3-(3,4-dihydroxyphenyl)acrylate (**3t**): a off-white solid, yield 43%. m.p. 92-94 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.75 (s, 4H, -OH), 7.45 (d, *J* = 15.9 Hz, 1H), 7.03 (s, 1H), 6.99 (d, *J* = 8.2 Hz, 1H), 6.76 (d, *J* = 8.2 Hz, 1H), 6.68–6.60 (m, 2H), 6.54–6.46 (m, 1H), 6.23 (d, *J* = 15.9 Hz, 1H), 4.22 (t, *J* = 7.0 Hz, 2H), 2.75 (t, *J* = 7.0 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.5, 148.4, 145.6, 145.1, 143.7, 128.6, 125.5, 121.3, 119.5, 116.2, 115.7, 115.5, 114.8, 113.9, 64.7, 33.9. HRMS calculated for C17H16ONa 339.0845, found 339.0703. Spectroscopic data were accordant with those previously reported [24].

3,4-dihydroxyphenethyl (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylate (**3u**): a brown solid, yield 69%. m.p. 141-142 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.76 (s, 1H, -OH), 8.69 (s, 1H, -OH), 7.54 (d, *J* = 15.9 Hz, 1H), 7.40 (d, *J* = 1.5 Hz, 1H), 7.18 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 6.64 (dd, *J* = 5.0, 3.0 Hz, 2H), 6.50 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.47 (d, *J* = 15.9 Hz, 1H), 6.07 (s, 2H), 4.24 (t, *J* = 7.0 Hz, 2H), 2.76 (t, *J* = 7.0 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.4, 149.3, 148.0, 145.1, 144.4, 143.7, 128.6, 128.4, 125.0, 119.5, 116.2, 115.9, 115.5, 108.4, 106.7, 101.6, 64.8, 33.8. HRMS calculated for C18H16O6Na 351.0845, found 351.0669.

3,4-dihydroxyphenethyl nicotinate (**3v**): a yellow solid, yield 65%. m.p. 152-154 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.05 (s, 1H, -OH), 8.81 (s, 1H, -OH), 8.78 (s, 1H), 8.69 (s, 1H), 8.25 (d, *J* = 6.5 Hz, 1H), 7.57 (s, 1H), 6.72–6.60 (m, 2H), 6.54 (d, *J* = 6.6 Hz, 1H), 4.42 (s, 2H), 2.86 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 164.6, 153.6, 149.9, 145.1, 143.8, 136.8, 128.5, 125.7, 123.9, 119.5, 116.2, 115.5, 66.0, 33.7. HRMS calculated for C14H13NO4Na 282.0743, found 282.0613.

3,4-dihydroxyphenethyl 3-phenylpropanoate (**3w**): a brown oil, yield 77%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.76 (s, 1H, -OH), 8.68 (s, 1H, -OH), 7.26 (t, *J* = 7.6 Hz, 2H), 7.21–7.15 (m, 3H), 6.62 (dd, *J* = 15.0, 4.9 Hz, 2H), 6.44 (dd, *J* = 8.0, 1.8 Hz, 1H), 4.11 (t, *J* = 7.0 Hz, 2H), 2.82 (t, *J* = 7.6 Hz, 2H), 2.66 (t, *J* = 7.0 Hz, 2H), 2.59 (t, *J* = 7.6 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 172.1, 145.1, 143.7, 140.4, 128.5, 128.3, 128.2, 126.0, 119.4, 116.2, 115.5, 64.8, 35.1, 33.7, 30.2. HRMS calculated for C17H18O4Na 309.1103, found 309.0954.

3,4-dihydroxyphenethyl 2-oxo-2H-chromene-3-carboxylate (**3x**): a yellow solid, yield 70%. m.p. 65-68 °C.^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.78 (s, 1H, -OH), 8.71 (s, 1H, -OH), 8.68 (s, 1H), 7.90 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.78–7.71 (m, 1H), 7.47–7.38 (m, 2H), 6.67 (dd, *J* = 14.0, 4.9 Hz, 2H),

6.55 (dd, *J* = 8.0, 1.9 Hz, 1H), 4.35 (t, *J* = 7.1 Hz, 2H), 2.83 (t, *J* = 7.1 Hz, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 162.4, 155.9, 154.5, 148.7, 145.1, 143.8, 134.6, 130.3, 128.3, 124.9, 119.6, 117.8, 117.6, 116.3, 116.2, 115.6, 66.0, 33.7. HRMS calculated for C18H14O6Na 349.0688, found 349.0500.

3,4-dihydroxyphenethyl 5-(1,2-dithiolan-3-yl)pentanoate (**3y**): a light gray oil, yield 47%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.74 (s, 1H, -OH), 8.68 (s, 1H, -OH), 6.63 (d, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 6.46 (dd, *J* = 8.0, 1.9 Hz, 1H), 4.12 (t, *J* = 7.0 Hz, 2H), 3.59 (dd, *J* = 8.7, 6.0 Hz, 1H), 3.17 (s, 1H), 3.12 (s, 1H), 2.68 (t, *J* = 7.0 Hz, 2H), 2.40 (dd, *J* = 12.3, 6.1 Hz, 1H), 2.27 (t, *J* = 7.2 Hz, 2H), 1.85 (dd, *J* = 12.9, 6.6 Hz, 1H), 1.51 (d, *J* = 4.7 Hz, 4H), 1.36–1.30 (m, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 172.7, 145.0, 143.7, 128.5, 119.4, 116.1, 115.4, 64.6, 56.0, 40.0, 38.1, 34.0, 33.8, 33.4, 28.0, 24.2. HRMS calculated for C16H22O4S2Na 365.0858, found 365.0705. Spectroscopic data were accordant with those previously reported [25].

Biological Activities Assay

Partition Coefficient Determination (Log P)

Log P values, which express the partitioning of the compounds in an n-octanol-water system, were calculated using Crippen's fragmentation through the ChemBioDraw Pro (14.0 Ultra, Cambridge Soft Company) program. In this program, the contribution of each atom to molar hydrophobicity was evaluated within a molecular database of experimental partitioning values, using a constrained least-squares technique.

ABTS Radical Cation Scavenging Assay

The ABTS radical cation (ABTS•$^+$) scavenging activity of the compounds was valuated following the procedures as previously described with some modifications [26]. Briefly, 1ml 2.6 mM of potassium persulfate was added to 1ml 7 mM of ABTS•$^+$, and the mixture was allowed to stand in the dark at room temperature for 12-16 h before using. The ABTS•$^+$ solution was diluted with methanol to provide an absorbance of 0.70 ± 0.02 at 734 nm. The diluted ABTS•$^+$ solution (190 µl) was added to sample fractions (10 µl) in DMSO at different concentrations (from 31.25 µM to 2 mM). A standard curve was constructed by measuring the reduction in absorbance of the ABTS•$^+$ solution at different concentrations of Trolox (0-4 mM). Each treatment was conducted in triplicate. The plates were incubated at room temperature for 20 min in the dark, the absorbance in each well was read at 734 nm on a microplate spectrophotometer (BioTek). L-ascorbic acid and Trolox were used as a positive control. The inhibition rates of ABTS•$^+$ were calculated according to the following formula: ABTS scavenging rate (%) = [1−(absorbance of compound−absorbance of blank)/absorbance of control]×100. IC_{50} values were calculated and expressed as means ± the standard deviation (SD) in millimolar.

FRAP Assay

Ferric reducing ability of the compounds was conducted according to the procedures as previously described with slight modifications[26]. FRAP reagent was made freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid, and 20 mM aqueous ferric chloride ($FeCl_3$) solution in a 10:1:1 (v/v) ratio. The TPTZ solution was prepared in the same day. Each sample in DMSO solution (1 mM , 20 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. Ferrous sulfate ($FeSO_4$) at ten different concentrations (from 0 to 8 mM) 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 as previously described with slight modifications [27]. Each sample in DMSO solution (50 µl) (from 0.5 to 10 mM) was treated with 3 mM $FeSO_4$ solution (50 µl), 3 mM H_2O_2 solution (50 µl) were vortexed in 96-well plates, stand for 10 min and added 6 mM salicylic acid solution (50 µl), vortexed and the plates were 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 (the concentrations required to scavenge 50% hydroxyl radicals present in the test solution) were calculated and expressed as means ± SD in micromolar.

α-Glucosidase Inhibition Assay

α-Glucosidase was assayed according to the method as described previously with slight

modifications [28]. Each sample in DMSO solution(20 µl) (from 50 µM to 10 mM) 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 p-nitrophenyl-α-D-glucopyranoside (pNPG) solution (pH 6.9, 5 mM, in 0.1 M phosphate buffer) was added to each well, the reaction mixtures were incubated at 25°C for 5 min. Before incubating as well as after that, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Acarbose was used as a positive reference. The α-Glucosidase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

Acetylcholinesterase Inhibition Assay

Acetylcholinesterase (AChE) inhibition activities were measured according to the method as previously described with little modifications[29]. Each sample in 10 % DMSO solution(20 µl) (from 0.5 to 10 mM) 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 acetylthiocholine iodide (ATCI) solution (pH 8.0, 1.78 mM, in 0.1 M phosphate buffer) and 20 µl 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, the reaction mixtures were incubated at 25 °C for 5 min. Before incubating as well as after that, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Donepezil was used as a positive reference. The acetylcholinesterase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

Butyrylcholinesterase Inhibition Assay

Butyrylcholinesterase (BChE) inhibition activities were measured according to the method as described previously with little modifications [29]. Each sample in 10 % DMSO solution (20 µl) (from 0.5 to 10 mM) 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 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) solution (pH 8.0, 1.25 mM, in 0.1 M phosphate buffer) were added to each well, the reaction mixtures were incubated at 25°C for 5 min. Before incubating as well as after that, the absorbance was recorded at 405 nm on a microplate spectrophotometer (BioTek). Donepezil was used as a positive reference. The butyrylcholinesterase inhibition activity was expressed as % inhibition and was calculated as follows:

$$\%inhibition = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{control}}\right) \times 100\%$$

Statistical Analysis

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

Results and Discussion

Synthesis

The desired HT esters **3a**-**3y** were obtained in moderate to excellent yields (Scheme 1). In the experiment, the target compound **3f** was synthesized by using HT and compound **2f** as an example. The synthetic conditions of compound **3f** were discussed. Initially, we used DCC and DMAP as a condensing agent and found that there was serious side effect and the yield was low. It is considered that HT had an alcoholic hydroxyl group and othro-diphenolic hydroxyl group, the phenolic acids also had othro-diphenolic hydroxyl group, all of them were involved in the esterification reaction, and the selectivity of the reaction was poor. When we used TPP and DIAD according to the reference [30], the side effect was significantly reduced and the yield was significantly improved accordingly. However, the reaction still produced a by-product and its polarity was close to the target compound, the use of silica gel column was difficult to purify, so we used Sephadex LH-20 gel to purify and obtain the target compound. We applied the method successfully in synthesizing other target compounds. The advantages of that method lay in short steps, good selectivity and high yield.

Biological Activities

Partition Coefficient Determination (Log P)

The Log P values of HT and compounds **3a**-**3y** were calculated. The values calculated are shown in Table 1. The Log P values of compounds **3a**-**3y** (1.75-4.76) were increased compared to HT (0.96), the results showed that through structural

modification, hydrophilic/lipophilic balance of HT ester derivatives has enhanced.

ABTS Radical Cation Scavenging Assay

The ABTS•$^{+}$ assay is a method widely used for measuring radical-scavenging activity of antioxidants. The radical scavenging activity of compounds **3a-3y** in comparison with HT, L-ascorbic acid and Trolox determined by ABTS•$^{+}$ assays are shown in Table 1. Except **3e** and **3y**, all other compounds showed higher antioxidant capacity ranging from IC_{50} 3.4 to 24.4 μM than L-ascorbic acid (24.8 μM), compounds **3b-3d**, **3f-3k**, **3m-3x** were better than Trolox (18.3 μM). As seen in Table 1, compounds **3f** (3.4 μM) and **3i** (4.2 μM) were the most effective ABTS•$^{+}$ scavengers. The experimental results of compounds **3a-3u** showed substituent is beneficial, **3e** and **3r** showed the poor antioxidant capacity because of steric hindrance. The experimental results of compounds **3f-3k** showed that two hydroxyl groups are essential to elicit the antioxidant activity. Compared to **3q**, **3s** and **3t** showed good activities probably due to the presence of a methoxy or hydroxyl group in the ortho position of its hydroxyl group. Compared to **3a**, antioxidant activity of **3v** was obviously enhanced.

FRAP Assay

Results of the reducing power of compounds **3a-3y** evaluated by the FRAP assay (expressed as in millimoles of Fe (II) per gram) in comparison with HT, L-ascorbic acid and Trolox are summarized in Table 1. All compounds (70.0-202.1 mmol/g) were discovered to be more potent than L-ascorbic acid (40.7 mmol/g), Except **3e**, all other compounds (141.5-202.1 mmol/g) were better than Trolox (94.7 mmol/g). As seen in Table 1, the reducing power of compounds **3i** (202.1 mmol/g) and **3o** (197.3 mmol/g) are strongest. The experimental results showed that **3e** has the poorest antioxidant capacity due to steric hindrance. The experimental results showed that two hydroxyl groups are important for antioxidant activity; moreover, othro-dihydroxy is more effective. compared to **3q**, **3s** and **3t** showed good activities probably due to the presence of a methoxy or hydroxyl group in the ortho position of its hydroxyl group.

Hydroxyl Radical Scavenging Assay

The radical scavenging activity of compounds **3a-3y** in comparison with HT, L-ascorbic acid and Trolox determined by hydroxyl radical scavenging assays are shown in Table 1. Compounds **3a-3d**, **3f-3j**, **3l-3m**, **3o**, **3q**, **3t**, **3v-3y** exhibited more potent hydroxyl radical scavenging activity (IC_{50}=245.1-475.1 μM) than L-ascorbic acid (554.4 μM) and Trolox (500.4 μM). As seen in Table-1, compounds **3o** were the most effective hydroxyl radical scavenger (245.1 μM). We found the conclusion similar to experimental results of ABTS assay.

Table-1: Log P_{calcd}, Antioxidant activity of compounds **3a-3y.**

Compound	$LogP_{calcd}$	ABTS IC_{50} (μM)	FRAP (mmol/g)	·OH IC_{50} (μM)
3a	**3.09**	**24.4±1.6**	**165.5±0.3**	**475.1±0.8**
3b	**3.57**	**15.2±0.4**	**160.0±0.4**	**469.8±2.0**
3c	**3.65**	**17.5±0.5**	**168.5±1.6**	**337.1±9.9**
3d	**2.70**	**16.5±0.2**	**156.6±1.9**	**441.4±11.8**
3e	**4.76**	**32.5±0.2**	**70.0±1.6**	**1319.3±5.4**
3f	**2.31**	**3.4±0.0**	**151.4±0.6**	**343.2±7.3**
3g	**2.31**	**14.1±0.0**	**159.3±1.1**	**449.0±2.4**
3h	**2.31**	**4.9±0.1**	**149.2±2.6**	**467.3±2.6**
3i	**2.31**	**4.2±0.1**	**202.1±0.1**	**457.1±0.8**
3j	**2.31**	**4.9±0.1**	**193.5±1.0**	**406.6±3.1**
3k	**2.45**	**14.1±0.1**	**156.4±1.8**	**768.5±6.0**
3l	**3.03**	**23.8±0.3**	**158.5±1.6**	**409.1±1.1**
3m	**2.64**	**16.3±0.2**	**186.2±0.8**	**399.5±0.3**
3n	**4.15**	**14.5±0.1**	**161.5±0.4**	**600.5±1.4**
3o	**2.25**	**6.7±0.1**	**197.3±0.4**	**245.1±13.6**
3p	**3.43**	**17.7±0.0**	**167.6±0.3**	**608.8±4.5**
3q	**3.04**	**14.7±0.0**	**159.2±2.0**	**403.9±5.5**
3r	**3.05**	**17.8±0.2**	**164.6±0.8**	**648.0±9.6**
3s	**2.91**	**8.0±0.1**	**171.7±0.3**	**602.6±12.1**
3t	**2.65**	**12.2±0.7**	**189.0±0.7**	**377.6±2.3**
3u	**3.21**	**15.2±0.5**	**170.0±1.9**	**590.5±13.7**
3v	**1.75**	**10.9±0.2**	**167.5±0.4**	**426.3±9.8**
3w	**3.45**	**17.4±0.1**	**164.7±1.9**	**369.1±1.6**
3x	**2.74**	**16.6±0.3**	**155.5±0.5**	**430.0±5.8**
3y	**3.58**	**28.9±0.0**	**141.5±0.7**	**400.7±4.3**
HT	**0.96**	**4.7±0.0**	**146.5±2.0**	**544.8±9.3**
L-ascorbic acid	**None**	**24.8±0.3**	**40.7±1.7**	**554.4±3.9**
Trolox	**None**	**18.3±0.2**	**94.7±0.6**	**500.4±13.8**

α-Glucosidase Inhibition Assay

Results of the α-Glucosidase inhibition activity of compounds **3a-3y** evaluated by the α-Glucosidase Inhibition assay in comparison with HT and Acarbose are summarized in Table 2. Compounds **3q**, **3t** and **3y** (39.1-52.4 μM) showed inhibition potency than Acarbose (60.9 μM). As seen in Table 2, compounds **3q** (39.1 μM) and **3t** (43.2 μM) were the most effective α-Glucosidase inhibitors. The experimental results showed that **3d**, **3f**, **3g**, **3q**, **3t** exhibited good inhibition activity, we found a common feature from their structures: para-hydroxyl substitution, this may explain the inhibition activity.

Acetylcholinesterase Inhibition Assay

The acetylcholinesterase activity of compounds **3a-3y** in comparison with HT and Donepezil determined by acetylcholinesterase inhibition assays are shown in Table-2. Compounds **3a**, **3d**, **3f-3m**, **3s-3t**, **3v-3y** showed weaker AChE inhibition activities than Donepezil.

Butyrylcholinesterase Inhibition Assay

The butyrylcholinesterase activity of compounds **3a-3y** in comparison with HT and Donepezil determined by butyrylcholinesterase inhibition assays are shown in Table 2. Compounds **3a**, **3d**, **3f-3j**, **3l-3m**, **3o-3p**, **3s-3t**, **3w** showed weaker BChE inhibition activities than Donepezil.

Table-2: Hypoglycemic activity, anticholinesterase activity of compounds **3a-3y.**

Compound	α-Glucosidase IC_{50} (μM)	AchE IC_{50} (μM)	BchE IC_{50} (μM)
3a	**469.1±6.3**	**611.5±5.7**	**813.1±14.3**
3b	**774.9±2.8**	**>1000**	**>1000**
3c	**351.7±4.8**	**>1000**	**>1000**
3d	**62.4±1.4**	**755.0±7.9**	**422.4±8.5**
3e	**276.3±3.1**	**>1000**	**>1000**
3f	**70.6±1.1**	**401.6±2.8**	**375.6±11.1**
3g	**73.0±0.9**	**520.4±3.1**	**210.0±8.3**
3h	**141.8±2.1**	**509.7±4.7**	**338.8±9.1**
3i	**221.5±2.9**	**496.0±7.1**	**155.5±2.8**
3j	**109.0±1.5**	**631.9±6.3**	**237.0±1.4**
3k	**319.0±4.1**	**753.7±8.7**	**>1000**
3l	**600.3±7.1**	**638.0±6.1**	**315.1±6.3**
3m	**244.7±3.6**	**783.7±4.8**	**301.7±9.6**
3n	**115.2±2.1**	**>1000**	**>1000**
3o	**136.6±1.4**	**>1000**	**491.6±4.2**
3p	**207.0±3.2**	**>1000**	**977.0±8.2**
3q	**39.1±0.5**	**>1000**	**>1000**
3r	**729.7±8.5**	**>1000**	**>1000**
3s	**158.5±2.1**	**851.3±3.2**	**727.5±12.1**
3t	**43.2±1.5**	**549.4±5.3**	**159.1±3.3**
3u	**108.3±0.8**	**>1000**	**>1000**
3v	**>800**	**531.2±4.3**	**>1000**
3w	**312.6±1.5**	**491.8±5.0**	**935.6±6.6**
3x	**367.6±3.7**	**955.4±8.4**	**>1000**
3y	**52.4±0.5**	**731.1±8.6**	**>1000**
HT	**>800**	**>1000**	**636.4±12.5**
Acarbose	**60.9±1.0**	**None**	**None**
Donepezil	**None**	**0.1±0.0**	**3.6±0.1**

Conclusion

In conclusion, a series of HT ester derivatives that have been synthesized from natural olive oil phenol HT, all compounds showed high antioxidant activity, and compounds **3f**, **3i** and **3o** showed higher antioxidant activity, determined by ABTS, FRAP and hydroxyl radical assays. Compounds **3q**, **3t** and **3y** showed higher α-Glucosidase inhibition activity than Acarbose, some compounds showed weak inhibition activities on both cholinesterases. This study indicates that HT ester derivatives with functional potential are beneficial for human health and which are worthy of further investigation.

Acknowledgements

This work was financially supported by the National Science Foundation of China (31570347) and the Science and Technology Development Funds of Jilin Province(20160520044JH).

Supplementary Information

Supplementary Materials (^{1}H and ^{13}C-NMR spectra and HRMS of compounds **3a-3y)**.

References

1. Nutrition and Allergies. EFSA Panel on Dietetic Products, D. Turck, J. Bresson, B. Burlingame, T. Dean, S. Fairweather-Tait, M. Heinonen, K. I. Hirsch-Ernst, I. Mangelsdorf, H. J. McArdle, A. Naska, M. Neuhauser-Berthold, G. Nowicka, K. Pentieva, Y. Sanz, A. Siani, A. Sjodin, M. Stern, D. Tome, M. Vinceti, P. Willatts, K. Engel, R. Marchelli, A. Poting, M. Poulsen, J. Schlatter, E. Turla and H. Loveren, Safety of hydroxytyrosol as a novel food pursuant to Regulation (EC) No 258/97, *EFSA. Journal*., **15**, 4728 (2017).
2. D. Aunon-Calles, E. Giordano, S. Bohnenberger and F. Visioli, Hydroxytyrosol is not genotoxic in vitro, *Pharmacol. Res.*, **74**, 87 (2013).
3. D. Aunon-Calles, L. Canut and F. Visioli, Toxicological evaluation of pure hydroxytyrosol, *Food. Chem. Toxicol.*, **55**, 498 (2013).
4. S. Lee-Huang, P. L. Huang, D. Zhang, J. W. Lee, J. Bao, Y. Sun, Y. T. Chang, J. Zhang and P. L. Huang, Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: Part I. fusion inhibition, *Biochem. Biophys. Res. Commun.*, **354**, 872 (2007).
5. N. Zoric, I. Horvat, N. Kopjar, A. Vucemilovic, D. Kremer, S. Tomic and I. Kosalec,

Hydroxytyrosol expresses antifungal activity in vitro, *Curr. Drug. Targets*., **14**, 992 (2013).
6. G. Bisignano, A. Tomaino, R. Lo Cascio, G. Crisafi, N. Uccella and A. Saija, On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol, *J. Pharm. Pharmacol*., **51**, 971 (1999).
7. F. Visioli, G. Bellomo and C. Galli, Free radical-scavenging properties of olive oil polyphenols, *Biochem. Biophys. Res. Commun*., **247**, 60 (1998).
8. T. Hashimoto, M. Ibi, K. Matsuno, S. Nakashima, T. Tanigawa, T. Yoshikawa and C. Yabe-Nishimura, An endogenous metabolite of dopamine, 3,4-dihydroxyphenylethanol, acts as a unique cytoprotective agent against oxidative stress-induced injury, *Free. Radic. Biol. Med.*, **36**, 555 (2004).
9. Z. Bahadoran, P. Mirmiran and F. Azizi, Dietary polyphenols as potential nutraceuticals in management of diabetes: a review, *J. Diabetes. Metab. Disord.*, **12**, 43 (2013).
10. F. Hadrich, Z. Bouallagui, H. Junkyu, H. Isoda and S. Sayadi, The α-glucosidase and α-amylase enzyme inhibitory of hydroxytyrosol and oleuropein, *J. Oleo. Sci*., **64**, 835 (2015).
11. M. Gonzalez-Santiago, E. Martin-Bautista, J. J. Carrero, J. Fonolla, L. Baro, M. V. Bartolome, P. Gil-Loyzaga and E. Lopez-Huertas, One-month administration of hydroxytyrosol a phenolic antioxidant present in olive oil to hyperlipemic rabbits improves blood lipid profile antioxidant status and reduces atherosclerosis development, *Atherosclerosis*, **188**, 35 (2006).
12. U. Catalan, M. C. Lopez de Las Hazas, L. Rubio, S. Fernandez-Castillejo, A. Pedret, R. de la Torre, M. J. Motilva and R. Sola, Protective effect of hydroxytyrosol and its predominant plasmatic human metabolites against endothelial dysfunction in human aortic endothelial cells, *Mol. Nutr. Food. Res*., **59**, 2523 (2015).
13. G. Yu, A. Deng, W. Tang, J. Ma, C. Yuan and J. Ma, Hydroxytyrosol induces phase II detoxifying enzyme expression and effectively protects dopaminergic cells against dopamine- and 6-hydroxydopamine induced cytotoxicity, *Neurochem. Int*., **96**, 113 (2016).
14. C. St-Laurent-Thibault, M. Arseneault, F. Longpre and C. Ramassamy, Tyrosol and hydroxytyrosol two main components of olive oil, protect N2a cells against amyloid-β-induced toxicity. involvement of the NF-κB signaling, *Curr. Alzheimer. Res.*, **8**, 543 (2011).
15. M. C. Crespo, J. Tome-Carneiro, C. Pintado, A. Davalos, F. Visioli and E. Burgos-Ramos, Hydroxytyrosol restores proper insulin signaling in an astrocytic model of Alzheimer's disease, *Biofactors*, **43**, 540 (2017).
16. Y. Peng, C. Hou, Z. Yang, C. Li, L. Jia, J. Liu, Y. Tang, L. Shi, Y. Li, J. Long and J. Liu, Hydroxytyrosol mildly improve cognitive function independent of APP processing in APP/PS1 mice, *Mol. Nutr. Food. Res*., **60**, 2331 (2016).
17. M. C. Lopez de Las Hazas, C. Pinol, A. Macia and M. J. Motilva, Hydroxytyrosol and the colonic metabolites derived from virgin olive oil intake induce cell cycle arrest and apoptosis in colon cancer cells, *J. Agric. Food. Chem.*, **65**, 6467 (2017).
18. B. Zhao, Y. Ma, Z. Xu, J. Wang, F. Wang, D. Wang, S. Pan, Y. Wu, H. Pan, D. Xu, L. Liu and H. Jiang, Hydroxytyrosol, a natural molecule from olive oil, suppresses the growth of human hepatocellular carcinoma cells via inactivating AKT and nuclear factor-kappa B pathways, *Cancer. Lett*., **347**, 79 (2014).
19. H. Zubair, A. Bhardwaj, A. Ahmad, S. K. Srivastava, M. A. Khan, G. K. Patel, S. Singh and A. P. Singh, Hydroxytyrosol induces apoptosis and cell cycle arrest and suppresses multiple oncogenic signaling pathways in prostate cancer cells, *Nutr. Cancer.*, **69**, 932 (2017).
20. S. Li, Z. Han, Y. Ma, R. Song, T. Pei, T. Zheng, J. Wang, D. Xu, X. Fang, H. Jiang and L. Liu, Hydroxytyrosol inhibits cholangiocarcinoma tumor growth: An in vivo and in vitro study, *Oncol. Rep*., **31**, 145 (2014).
21. G. Toteda, S. Lupinacci, D. Vizza, R. Bonofiglio, E. Perri, M. Bonofiglio, D. Lofaro, A. La Russa, F. Leone, P. Gigliotti, R. A. Cifarelli and A. Perri, High doses of hydroxytyrosol induce apoptosis in papillary and follicular thyroid cancer cells, *J. Endocrinol. Invest*., **40**, 153 (2017).
22. E. H. Sarsour, M. Goswami, A. L. Kalen, J. T. Lafin and P. C. Goswami, Hydroxytyrosol inhibits chemokine C-C motif ligand 5 mediated aged quiescent fibroblast-induced stimulation of breast cancer cell proliferation, *AGE*., **36**, 9645 (2014).
23. N. El-Azem, M. Pulido-Moran, C. L. Ramirez-Tortosa, J. L. Quiles, F. E. Cara, P. Sanchez-Rovira, S. Granados-Principal and M. Ramirez-Tortosa, Modulation by hydroxytyrosol of oxidative stress and antitumor activities of paclitaxel in breast cancer, *Eur. J. Nutr*., **57**, 1 (2018).
24. L. Chapado, P. J. Linares-Palomino, S. Salido, J. Altarejos, J. A. Rosado and G. M. Salido, Synthesis and evaluation of the platelet

antiaggregant properties of phenolic antioxidants structurally related to rosmarinic acid, *Bioorg. Chem*., **38**, 108 (2010).
25. R. Bernini, F. Crisante, N. Merendino, R. Molinari, M. C. Soldatelli and F. Velotti, Synthesis of a novel ester of hydroxytyrosol and α-lipoic acid exhibiting an antiproliferative effect on human colon cancer HT-29 cells, *Eur. J. Med. Chem*., **46**, 439 (2011).
26. L. M. Dong, X. C. Jia, Q. W. Luo, Q. Zhang, B. Luo, W. B. Liu, X. Zhang, Q. L. Xu and J. W. Tan, Phenolics from *Mikania micrantha* and their antioxidant activity, *Molecules*, **22**, 1140 (2017).
27. Z. Guo, D. Lin, J. Guo, Y. Zhang and B. Zheng, In vitro antioxidant activity and in vivo anti-fatigue effect of sea horse (Hippocampus) peptides, *Molecules*, **22**, 482 (2017).
28. T. Yuan, C. Wan, K. Liu and N. P. Seeram, New maplexins F–I and phenolic glycosides from red maple (*Acer rubrum*) bark, *Tetrahedron*, **68**, 959 (2012).
29. M. Ozturk, U. Kolak, G. Topcu, S. Oksuz and M. I. Choudhary, Antioxidant and anticholinesterase active constituents from *Micromeria cilicica* by radical-scavenging activity-guided fractionation, *Food. Chem*., **126**, 31 (2011).
30. G. Appendino, A. Minassi, N. Daddario, F. Bianchi and G. C. Tron, Chemoselective esterification of phenolic acids and alcohols, *Org. Lett*., **4**, 3839 (2002).