Electronic Supplementary Information (ESI)

Tyrosol and its metabolites as antioxidative and anti-inflammatory molecules in human endothelial cells

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ESI Materials and Methods

Synthesis of Tyr-GLU metabolite

To a solution of Tyr acetate **4** [1] (200 mg, 1.02 mmol) in anhydrous CH_2Cl_2 (6 mL) and trichloroacetimidate acetylate glucuronosyl donor **5** [2] (366 mg, 0.76 mmol) at −10 °C, $BF3{\cdot}OEt_2$ (25 μL, 0.19 mmol) was added drop wise. After 2 h, TLC (hexane-EtOAc 2:1) showed the formation of a new product and complete consumption of the glycosyl donor. The reaction was neutralized with NEt_3 and concentrated in vacuum. The resulting residue was purified by flash column chromatography (hexane-EtOAc from 3:1 to 1:1) to afford 2-[4'-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)phenyl] EtOAc, a solution of which (60 mg, 0.11 mmol) in MetOH (2 mL) was stirred at room temperature with a solution of Na_2CO_3 (22 mg, 0.204 mmol) in H_2O (0.5 mL). After 16 h, water (1 mL) was added, followed by addition of glacial acetic acid to adjust the pH to 6.2. The solvents were then removed and residue was purified by Sephadex G-25 eluting with H_2O-MeOH (9:1). Fractions containing the desired product were freeze-dried affording compound **2**, tyrosol 4'-*O*-β-D-glucuronide (Tyr-GLU) (25 mg, 98%); ^{1}H NMR (500.13 MHz, CD_3OD) δ: 7.13 (d, J = 8.36 Hz, H-2', H6'), 7.04 (d, J = 8.43 Hz, H-3', H-5'), 4.87 (d, J = 7.10 Hz, H-1"), 3.76 (bs, H-5"), 3.70 (t, J = 7.12 Hz, H-1), 3.57-3.45 (m, H-2", H-3", H-4"), 2.76 (t, J = 7.12 Hz, H-2); ^{13}C NMR (100.62 MHz, CD_3OD) δ: 176.6 (C-6"), 157.8 (C-4'), 134.3 (C-1'), 130.9 (C-2', C-6'), 118.0 (C-3', C-5'), 102.7 (C-1"), 77.8 (C-3"), 76.7 (C-5"), 74.8 (C-2"), 73.7 (C-4"), 64.4 (C-1), 39.5 (C-2). ESI-HRMS: Calcd for $C_{14}H_{18}O_8$ (M^-): 314.29. Found: 312.9.

Synthesis of Tyr-SUL metabolite

Candida antarctica lipase (Novozym 435®) (180 mg) was added to a mixture of Tyr (1 equiv) and the acylating agent methyl butyrate (20 equiv) in 45 mL of *t*-butyl methyl ether using a

dry round-bottom flask, and the mixture was stirred for 60 min at 40 °C. The enzyme was decanted and separated. The solvent was evaporated, and the product Tyr butyrate **6** was purified by flash column chromatography [1, 3]. Tyr butyrate **6** (70 mg, 0.33 mmol) and $SO_3 \cdot NMe_3$ (233 mg, 1.7 mmol) were subjected to sulphation conditions for 20 min. Microwave based sulphation reaction was performed using a microwave synthesizer in sealed reaction vessels [4]. TLC (ethyl acetate:MeOH; 10:1) showed the formation of a major product and complete consumption of the starting material. Solvents were removed and the crude extract was purified by using Sephadex LH-20 in a solvent mixture of CH_2Cl_2:MeOH (1:1) to afford triethylammonium, 4-(2-(butyryloxy)ethyl)phenyl sulphate salt, a solution of which (97 mg, 0.32 mmol) and potassium carbonate (K_2CO_3, 90 mg, 0.66 mmol) were prepared in MeOH (10 mL). The reaction mixture was stirred at room temperature for 24 h, neutralized with IR 120 H^+ resin, and the solvent was then removed in a vacuum. The crude extract was purified by column chromatography with RP-C18 silica gel eluting with H_2O:MeOH (from 100:0 to 70:30). Fractions containing the desired product were concentrated and freeze-dried affording compound **3**, tyrosol 4-sulfate (Tyr-SUL) (68 mg, 94%, white powder). ^{1}H-NMR (400 MHz, D_2O) δ: 7.18, 6.86 (2d, 4H, J = 8.4 Hz, H_{arom}), 3.78, 2.78 (2t, 4H, J = 6.7 Hz, CH_2OH, CH_2Ar); ^{13}C-NMR (75 MHz, D_2O) δ: 157 (Cq), 130.3 (2 × CH_{arom}), 129.8 (Cq), 117.1 (2 × CH_{arom}), 63.0 (CH_2OH), 36.9 (CH_2Ar). ESI-HRMS (ES^-) Calcd for $C_8H_9O_5S$ (M − H) 217.0171, Found: 217.0171.

SUPPLEMENTARY REFERENCES

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2. B. Fischer, A. Nudelman, M. Ruse, J. Herzig, H.E. Gottlieb and E. Keinan, A novel method for stereoselective glucuronidation, *J Org Chem*, 1984, **49**, 4988-93.

3. R. Lucas, F. Comelles, D. Alcantara, O. S. Maldonado, M. Curcuroze, J. L. Parra, and J. C. Morales, Surface-active properties of lipophilic antioxidants tyrosol and hydroxytyrosol fatty acid esters: a potential explanation for the nonlinear hypothesis of the antioxidant activity in oil-in-water emulsions, *J Agric Food Chem*, 2010, **58**, 8021-6.

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ESI Table 1. Sequences of primers for gene expression analysis.

Target	GenBank accession number	Direction	Sequence (5'→3')
GPX1	NM_000581	Forward Reverse	AGAATGTGGCGTCCCTCTGA ACCGTTCACCTCGCACTTCT
GCLC	NM_001498	Forward Reverse	TCCAGGTGACATTCCAAGCC GAAATCACTCCCCAGCGACA
HO-1	NM_002133	Forward Reverse	TCTTGGCTGGCTTCCTTACC GGATGTGCTTTTCGTTGGGG
E-selectin	NM_000450	Forward Reverse	AGCCCAGAGCCTTCAGTGTA AACTGGGATTTGCTGTGTCC
ICAM-1	NM_000201	Forward Reverse	CAGTCACCTATGGCAACGAC ATTCAGCGTCACCTTGGCTC
VCAM-1	NM_001078	Forward Reverse	TCCGTCTCATTGACTTGCAG CACCTGCATTCCTTTTTCCA
CCL2	NM_002982	Forward Reverse	CCCCAGTCACCTGCTGTTAT TGGAATCCTGAACCCACTTC
PTGS2	NM_000963	Forward Reverse	TGAGCATCTACGGTTTGCTG TGCTTGTCTGGAACAACTGC
GAPDH	NM_001289746	Forward Reverse	TCGACAATGGCAGCATCTAC ATCCGTCTCCACAGACAAGG
HPRT	NM_000194	Forward Reverse	ACCCCACGAAGTGTTGGATA AAGCAGATGGCCACAGAACT

ESI Table 2. Effects of Tyr, Tyr-GLU, and Tyr-SUL on hEC viability.

Concentration (μM)	Tyr	Tyr-GLU	Tyr-SUL
0	100 ± 3.1[a]	100 ± 4.4[a]	100 ± 10.7[a]
1	101 ± 2.7[a]	97 ± 5.6[a]	97 ± 4[a]
5	99 ± 4.8[a]	95 ± 6.2[a]	96 ± 4.8[a]
10	96 ± 4[a]	95 ± 3.8[a]	95 ± 6.5[a]
15	98 ± 2.9[a]	96 ± 7.8[a]	97 ± 4.7[a]
50	97 ± 2.5[a]	96 ± 2.4[a]	95 ± 2.9[a]
100	96 ± 6[a]	95 ± 9[a]	95 ± 5[a]
200	93 ± 4.3[a]	71 ± 2.3[b]	58 ± 11[b]

hECs were cultured in the presence of Tyr or Tyr metabolites (0-200 μM) for 48 h. Values are expressed in % of cells alive and are shown as mean ± SD of eight samples repeated in three separate experiments. Means within columns sharing the same letter are not significantly different from each other ($p < 0.05$).

ESI Table 3. Concentration of soluble forms of E-selectin, ICAM-1, and VCAM-1 in the medium of hECs.

Treatment	sE-selectin	sICAM-1	sVCAM-1
Control	2.9 ± 1.5^{a}	3.0 ± 0.7^{a}	3.2 ± 1.7^{a}
TNF-α	7.9 ± 1.0^{b}	7.4 ± 1.3^{b}	19.6 ± 3.0^{b}
Tyr + TNF-α	3.4 ± 1.8^{a}	5.9 ± 1.9^{a}	15.7 ± 5^{b}
Tyr-GLU + TNF-α	2.6 ± 2.5^{a}	3.1 ± 1.2^{a}	2.6 ± 2.5^{a}
Tyr-SUL + TNF-α	3.5 ± 2.8^{a}	2.9 ± 0.7^{a}	4.1 ± 2.8^{a}

hECs were untreated (control) or exposed to Tyr or its metabolites (100 μM) for 16 h and then with TNF-α (10 ng/mL) for additional 16 h. Values are expressed in pg/mL and are shown as mean ± SD of three independent experiments. Means within columns sharing the same letter are not significantly different from each other ($p < 0.05$).