Supplemental Data

Title: Structure-activity relationships of the main bioactive constituents of *Euodia ruticarpa* on aryl hydrocarbon receptor activation and associated bile acid homeostasis

Authors: Youbo Zhang, Tingting Yan, Dongxue Sun, Cen Xie, Yiran Zheng, Lei Zhang, Tomoki Yagai, Kristopher W. Krausz, William H. Bisson, Xiuwei Yang, Frank J. Gonzalez

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Supplemental Table 1. List of qPCR mRNA primers

Gene	Forward primer sequence (5´~3´)	Reverse primer sequence (5´~3´)
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Ahr	AGCCGGTGCAGAAAACAGTAA	AGGCGGTCTAACTCTGTGTTC
Arnt	GACAGACCACAGGACAGTTCC	AGCATGGACAGCATTTCTTGAA
Cyp1a1	GACCCTTACAAGTATTTGGTCGT	GGTATCCAGAGCCAGTAACCT
Cyp1a2	AGTACATCTCCTTAGCCCCAG	GGTCCGGGTGGATTCTTCAG
Cyp1b1	CACCAGCCTTAGTGCAGACAG	GAGGACCACGGTTTCCGTTG
Cyp7a1	AACAACCTGCCAGTACTAGATAGC	GTGTAGAGTGAAGTCCTCCTTAGC
Lxr	CTCAATGCCTGATGTTTCTCCT	TCCAACCCTATCCCTAAAGCAA
Abcc2	GTGTGGATTCCCTTGGGCTTT	CACAACGAACACCTGCTTGG
Bsep	TCTGACTCAGTGATTCTTCGCA	GTGTAGAGTGAAGTCCTCCTTAGC
Fxr	TGGGCTCCGAATCCTCTTAGA	TGGTCCTCAAATAAGATCCTTGG
Shp	TCTGCAGGTCGTCCGACTATTC	AGGCAGTGGCTGTGAGATGC
Mdr1	CCCCCGAGATTGACAGCTAC	ACTCCACTAAATTGCACATTTCCTTC

Supplemental Methods

Serum Sample Processing

To each tube containing 80 µL of plasma, 300 µL of MeOH-ACN (2:1, v/v) containing IS (chlorpropamide, 20 µg/mL) was added. After vortexing for 1 min, the mixture was centrifuged at 15,000 g for 10 min at 4 °C. The upper layer was transferred to a clean tube and dried under vacuum. To each dried sample, 200 µL of MeOH-ACN (2:1, v/v) was added and the samples were vortexed for 1 min, and then centrifuged at 15,000 g for 10 min at 4 °C. The supernatants were injected into the UPLC–MS/MS system for analysis.

Preparation of Standards for Pharmacokinetic Analysis.

The solution of internal standard (IS) was prepared and diluted to 20 µg/mL using MeOH-ACN (2:1, v/v). The stock solutions of the authentic standards were prepared in IS solution. Working solutions of the analytes were prepared by appropriate dilution of the stock solutions in IS solution. To prepare quality control (QC) samples, each tube containing 80 µL of blank plasma was added with 20 µL of different concentrations of working solution (2:1, v/v) and 280 µL of IS solution. Processing was the same with that of the samples. The calibration standards at the concentrations were 20,000, 5,000, 1,000, 500, 100, 10, and 1 ng/mL for RUT and DHED, and 5,000, 2, 500, 1,000, 100, 10, 1, and 0.5 ng/mL for EOD. All the solutions were stored at 4 °C until analysis.

LC/MS/MS Analysis

The column was an Agilent SB-C18 (2.1 mm×50 mm, 1.8 µm) column equipped with a security guard (2.1 mm×15 mm, 1.8 µm) C18 column (Agilent, USA). The mobile phase was composed of acetonitrile (A) – 0.5 mM ammonium acetate aqueous solution (B) for RUT and EOD, and acetonitrile (A) – 0.1% (v/v) formic acid aqueous solution (B) for DHED. The gradient elution conditions were optimized as follows: linear gradient from 25 to 75% A (0–2.5 min), 75–95% A (2.5–3.5 min), and 25% A (3.5–6.5 min). The flow rate was 0.4 mL/min and the injection volume was 3 µL. The temperature of the column was set at 30 °C. The mass detection was in positive mode. The source parameters of mass spectrometer were as follows: drying gas (N2) flow rate, 10 L/min; heating gas flow rate, 10 L/min; nebulizing gas flow rate, 3.0 L/min; detector voltage, 1.8 kV; interface voltage, 3 kV; interface temperature, 300 °C; desolvation temperature, 250 °C and heat block temperature, 400 °C.