

REAGENTS

ELISA kit for CXCL15 (ml098438), TNF (ml002095), and IL-11 (ml063115) were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd.. ELISA kit for CXCL2 (EK2142), IL-1 β (EK201B), SPP1 (EK2135), CSF3(EK269) and IL-23(EK223) were purchased from Multi Sciences (Lianke) Biotech Co., Ltd.. ALOX12 (49874S) and BMP7 (4693S) were purchased from Cell Signaling Technology Co., Ltd.. COL19A3 (ABIN6746559) was purchased from antibodies-online GmbH. PTGS2 (12375-1-AP), FAP (11779-1-AP), CD11b (21851-1-AP) and Gr-1 (65140-1-Ig) were purchased from proteintech Co.,Ltd..

QUALITATIVE ANALYSIS OF YA-HAN-LU-DUAN

Instruments

Agilent 1290 Infinity II Ultra-Performance Liquid Chromatography (UPLC) system (Agilent, United States); high-resolution mass spectrometer (Q-TOF G6545, Agilent, USA); chromatographic column: Agilent EclipsePlus C18 RRHD (1.8 μ m, 2.1 mm $\times$ 100 mm).

Ya-Han-Lu-Duan extraction and serum preparation containing Ya-Han-Lu-Duan

Ya-Han-Lu-Duan (YHLD) was obtained from the Dai Hospital of Xishuangbanna Dai Autonomous Prefecture. One gram of YHLD was weighed and extracted with 10 mL of methanol by ultrasonication for 30 min. The mixture was then centrifuged at 3000 rpm for 10 min. The supernatant was filtered through a 0.22 μ m microporous membrane, and the filtrate was transferred into an UPLC vial for analysis.

Mice were administered YHLD by intragastric gavage at a high dose for 3 consecutive days. Plasma was collected at 1 h and 2 h after the last administration and mixed uniformly. Methanol was added to the plasma at a volume ratio of 1:3 (plasma:methanol), vortexed for 30 s, and allowed to stand at 4 $^{\circ}$ C for 20 min. The mixture was then centrifuged at 12,000 $\times$ g for 15 min, and the supernatant was collected and vacuum-dried. The residue was reconstituted in 100 μ L methanol,

centrifuged at $12,000 \times g$ for 10 min, and the supernatant was collected. This supernatant was again vacuum-dried, reconstituted in 100 μL methanol, and centrifuged at $12,000 \times g$ for 10 min. The final supernatant was transferred to an autosampler vial for subsequent instrumental analysis.

Chromatographic conditions

Chromatographic separation was performed on an Agilent 1290 Infinity II UPLC system equipped with an Agilent EclipsePlus C18 column (2.1 mm $\times$ 100 mm, 1.8 μm).

In positive ion mode, the mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, using a gradient elution.

In negative ion mode, the mobile phase consisted of (A) 0.01% formic acid in water and (B) 0.01% formic acid in acetonitrile, also under gradient elution.

The gradient program was as follows: 0–5.0 min, 2% B; 5.0–20.0 min, 2% $\rightarrow$ 100% B; 20.0–25.0 min, 100% B; 25.0–30.0 min, 100% $\rightarrow$ 2% B; 30.0–33.0 min, 2% B.

Column temperature: 40 $^{\circ}\text{C}$; flow rate: 0.4 mL/min; injection volume: 5 μL .

Mass spectrometric conditions

An electrospray ionization (ESI) source was used for data acquisition in both positive and negative ion modes. Drying gas temperature: 320 $^{\circ}\text{C}$; drying gas flow: 8 L min^{-1} ; Sheath gas temperature: 350 $^{\circ}\text{C}$; sheath gas flow: 11 L min^{-1} ; Nebulizer pressure: 35 psi; Nozzle voltage: 3.5 kV; Capillary voltage: 1000 V; Mass scan range: m/z 100–1000; Collision energy: 10, 20, and 40 eV (stepped); High-purity nitrogen was used as the nebulizer gas, drying gas, and sheath gas.

Statistical analysis

The MS data was processed by Agilent MassHunter Workstation Qualitative Analysis 10.0 (Agilent, USA) with the steps of raw data introduction, peak extraction and adduct deconvolution. The identification was finally determined by in consideration of retention time error of reference substance, mass error of mother ion, match degree

of daughter ions, isotope distribution and peak area after searching the reference substance database (Agilent- Natural Standard NPs PCDL, NatureStandard Shanghai Standard Technology Co., Ltd.) and theoretical database constructed by literature and public databases.

Results

Total ion chromatogram of YHLD extraction and plasma samples can be found in Figure 1. Detailed information of the main compounds in YHLD and its prototype compounds in plasma can be found in Supplementary Tables 1 and 2.

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Supplementary Table 1 Detailed information of main compounds in Ya-Han-Lu-Duan

Number	Identification	Formula	Adducts	Theoretical <i>m/z</i>	Mass error (ppm)	RT (minutes)
1	[Z,E]-4,8,12-Trimethyl-3,7,11-tridecatrienoate	C ₁₄ H ₂₂ O ₂	(M-H)-	221.1544	-1.26	13.52
2	1-Formyl-3-hydroxyneogrifolin	C ₂₃ H ₃₂ O ₄	(M-H)-[-H ₂ O]	353.2122	0.1	17.447
3	1-Formylneogrifolin	C ₂₃ H ₃₂ O ₃	(M-H)-[-H ₂ O]	337.2172	-0.33	16.945
4	1-Methyl-2-[(4Z,7Z)-4,7-tridecadienyl]-4(1H)-quinolone	C ₂₃ H ₃₁ NO	(M+CH ₃ COO)-	396.2537	-2.1	16.423
5	1-Methyl-4-methylethenylcyclohexene	C ₁₀ H ₁₆	(M+HCOO)-[-H ₂ O]	0	-0.41	26.306
6	2-Isopropyl-5-methylanisole	C ₁₁ H ₁₆ O	(M-H)-	163.1131	1.32	18.845
7	3-Hydroxyneogrifolin	C ₂₂ H ₃₂ O ₃	(M-H)-[-H ₂ O]	325.217	-0.7	18.489
8	4,8,12-Trimethyl tridecanoic acid	C ₁₆ H ₃₂ O ₂	(M-H)-	255.2329	-0.22	19.664
9	5beta-Cyprinol	C ₂₇ H ₄₈ O ₅	(M+Cl)-	487.3202	2.41	19.768
10	Asarumin B	C ₁₃ H ₁₆ O ₄	M-	236.1053	-0.43	13.52
11	Citraurin beta	C ₃₀ H ₄₀ O ₂	(M+CH ₃ COO)-[-H ₂ O]	473.3052	-2.26	19.806
12	Cordyceptide A	C ₂₇ H ₄₁ N ₃ O ₅	M-	487.305	-4.93	26.097

13	Deoxycapillartemisin	C ₁₉ H ₂₆ O ₃	(M-H)-[-H ₂ O]	283.17	-1.39	17.114
14	Dulcoside A	C ₃₈ H ₆₀ O ₁₇	(M-H)-[-H ₂ O]	769.3631	-2.7	18.92
15	Ganoderic acid DM	C ₃₀ H ₄₄ O ₄	(M+Cl)-[-H ₂ O]	485.2819	-1.79	18.822
16	Isomangiferolic acid	C ₂₉ H ₄₆ O ₃	(M-H)-[-H ₂ O]	423.3265	-0.96	20.473
17	Kleinioxanthrone 3	C ₃₁ H ₄₂ O ₅	(M-H)-[-H ₂ O]	475.2851	-0.79	19.662
18	Lup-20(29)-ene-3alpha-acetoxy-24-oic acid	C ₃₂ H ₅₀ O ₄	(M-H)-[-H ₂ O]	479.3523	-1.7	20.614
19	Neotriptophenolide	C ₂₁ H ₂₆ O ₄	(M-3H)-3[-H ₂ O]	107.0504	1.48	17.505
20	Pentahydroxybufostane	C ₂₈ H ₅₀ O ₅	(M + Cl)-	501.3368	4.05	19.976
21	Protostemonine	C ₂₃ H ₃₁ NO ₆	(M+HCOO)-[-H ₂ O]	444.2033	-0.44	18.417
22	Ricinoleic acid	C ₁₈ H ₃₄ O ₃	(M-H)-[-H ₂ O]	279.2326	-1.12	19.02
23	(-)-Jasmonic acid	C ₁₂ H ₁₈ O ₃	(M+2(NH ₄))+2[- H ₂ O]	114.0917	3.57	19.684
24	1-Indanone	C ₉ H ₈ O	(M+NH ₄)+[-H ₂ O]	132.081	2.04	6.96
25	Benzoic Acid	C ₇ H ₆ O ₂	(M+H)+	123.0442	0.91	8.323
26	Brevilin A	C ₂₀ H ₂₆ O ₅	(M+4(NH ₄))+4[- H ₂ O]	100.0758	1.7	19.073
27	Clematoside B	C ₈₇ H ₁₄₂ O ₄₈	M+2	977.4359	2.87	19.221

28	Gentianamine	C ₁₁ H ₁₁ NO ₃	(M+H)+[-H ₂ O]	188.0708	1.15	6.936
29	Psammosilenin A	C ₅₁ H ₆₄ N ₈ O ₈	(M+2H)+2[-H ₂ O]	450.2436	-1.11	9.952
30	Sodium tauropythocholate	C ₂₆ H ₄₄ NNaO ₇ S	(M+Na)+	560.2616	-3.39	22.069

Supplementary Table 2 Detailed information of Ya-Han-Lu-Duan prototype compounds in plasma

Number	Identification	Formula	Adducts	Theoretical <i>m/z</i>	Mass error (ppm)	RT (minutes)
1	[Z,E]-4,8,12-Trimethyl-3,7,11-tridecatrienoate	C ₁₄ H ₂₂ O ₂	(M-H)-	221.1546	-0.55	13.515
2	1-Formyl-3-hydroxyneogrifolin	C ₂₃ H ₃₂ O ₄	(M-H)-[-H ₂ O]	353.2121	-0.33	17.459
3	1-Formylneogrifolin	C ₂₃ H ₃₂ O ₃	(M-H)-[-H ₂ O]	337.2173	-0.18	16.946
4	1-Methyl-2-[(4Z,7Z)-4,7-tridecadienyl]-4(1H)-quinolone	C ₂₃ H ₃₁ NO	(M+CH ₃ COO)-	396.2538	-1.78	16.421
5	1-Methyl-4-methylethenylcyclohexene	C ₁₀ H ₁₆	(M+HCOO)-[-H ₂ O]	0	-0.07	26.315
6	2-Isopropyl-5-methylanisole	C ₁₁ H ₁₆ O	(M-H)-	163.1131	1.65	18.844
7	3-Hydroxyneogrifolin	C ₂₂ H ₃₂ O ₃	(M-H)-[-H ₂ O]	325.217	-1.14	18.504

8	4,8,12-Trimethyl tridecanoic acid	C ₁₆ H ₃₂ O ₂	(M-H)-	0	0.58	19.652
9	5beta-Cyprinol	C ₂₇ H ₄₈ O ₅	(M+Cl)-	487.3205	3.19	19.762
10	Asarumin B	C ₁₃ H ₁₆ O ₄	M-	236.1054	0.19	13.514
11	Citraurin beta	C ₃₀ H ₄₀ O ₂	(M+HCOO)-[- H ₂ O]	473.305	-2.51	19.654
12	Cordyceptide A	C ₂₇ H ₄₁ N ₃ O ₅	M-	487.3046	-4.93	26.106
13	Deoxycapillartemisin	C ₁₉ H ₂₆ O ₃	(M-H)-[-H ₂ O]	283.1702	-0.56	17.119
14	Dulcoside A	C ₃₈ H ₆₀ O ₁₇	(M-H)-[-H ₂ O]	769.3627	-3.13	18.935
15	Ganoderic acid DM	C ₃₀ H ₄₄ O ₄	(M+Cl)-[-H ₂ O]	485.2824	-0.22	18.945
16	Isomangiferolic acid	C ₂₉ H ₄₆ O ₃	(M-H)-[-H ₂ O]	423.3264	-1.23	20.467
17	Kleinioxanthrone 3	C ₃₁ H ₄₂ O ₅	(M-H)-[-H ₂ O]	475.2848	-1.28	19.658
18	Lup-20(29)-ene-3alpha-acetoxy-24-oic acid	C ₃₂ H ₅₀ O ₄	(M-H)-[-H ₂ O]	479.3521	-2.17	20.615
19	Neotriptophenolide	C ₂₁ H ₂₆ O ₄	(M-3H)-3[-H ₂ O]	107.0503	0.34	17.705
20	Pentahydroxybufostane	C ₂₈ H ₅₀ O ₅	(M+Cl)-	501.3365	3.49	20.377
21	Protostemonine	C ₂₃ H ₃₁ NO ₆	(M+HCOO)-[- H ₂ O]	444.2029	-0.84	18.434
22	Ricinoleic acid	C ₁₈ H ₃₄ O ₃	(M-H)-[-H ₂ O]	279.2328	-0.58	19.037

23	(-)-Jasmonic acid	C ₁₂ H ₁₈ O ₃	(M+2(NH ₄))+2[- H ₂ O]	114.0912	-1.73	20.234
24	1-Indanone	C ₉ H ₈ O	(M+NH ₄)+[-H ₂ O]	132.0808	0.39	6.638
25	Benzoic Acid	C ₇ H ₆ O ₂	(M+H)+[-H ₂ O]	105.0334	-1.16	7.737
26	Brevilin A	C ₂₀ H ₂₆ O ₅	(M+4(NH ₄))+4[- H ₂ O]	100.0753	-4.92	19.664
27	Clematoside B	C ₈₇ H ₁₄₂ O ₄₈	M+2	977.4354	2.39	19.245
28	Gentianamine	C ₁₁ H ₁₁ NO ₃	(M+H)+[-H ₂ O]	188.0707	0.37	6.638
29	Psammosilenin A	C ₅₁ H ₆₄ N ₈ O ₈	(M +2H)+2[-H ₂ O]	450.2435	-1.74	9.94
30	Sodium tauropythocholate	C ₂₆ H ₄₄ NNaO ₇	(M+H)+	560.2619	-3.36	22.041
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Supplementary Table 3 Affinity of Ya-Han-Lu-Duan prototype compounds with PTGS2 and ALOX12

Number	Identification	Affinity (kcal/mol)	
		PTGS2	ALOX12
1	[Z,E]-4,8,12-Trimethyl-3,7,11-tridecatrienoate	-4.5	-4.5
2	1-Formyl-3-hydroxyneogrifolin	-7.5	-6.2
3	1-Formylneogrifolin	-7.2	-6.5
4	1-Methyl-2-[(4Z,7Z)-4,7-tridecadienyl]-4(1H)-quinolone	-6.5	-6.9
5	1-Methyl-4-methylethenylcyclohexene	-6.6	-6.5
6	2-Isopropyl-5-methylanisole	-5.8	-5.5
7	3-Hydroxyneogrifolin	-5.8	-5.6
8	4,8,12-Trimethyl tridecanoic acid	-4.8	-4.7
9	5beta-Cyprinol	-8.8	-7.1
10	Asarumin B	-6.2	-5.6
11	Citraurin beta	-6.8	-6.6
12	Cordyceptide A	-8.7	-7.4
13	Deoxycapillartemisin	-8	-7.2
14	Dulcoside A	-9.5	-8.6
15	Ganoderic acid DM	-9.1	-7.7
16	Isomangiferolic acid	-8.3	-8.1
17	Kleinioxanthrone 3	-7.1	-6.1
18	Lup-20(29)-ene-3alpha-acetoxy-24-oic acid	-8	-8.2
19	Neotriptophenolide	-8.5	-7.2
20	Pentahydroxybufostane	-10.3	-8.3
21	Protostemonine	-9.1	-8.5
22	Ricinoleic acid	-4.8	-4.8

23	(-)-Jasmonic acid	-6.1	-5.8
24	1-Indanone	-6.4	-5.6
25	Benzoic Acid	-5.7	-5.7
26	Brevilin A	-8.1	-6.7
27	Clematoside B	-10.2	-8.6
28	Gentianamine	-6.7	-6.2
29	Psammosilenin A	-9.7	-9.1
30	Sodium tauropyrrocholate	-9	-7.7
