Supporting Information

Pharmacokinetic comparisons of Paeoniflorin and Paeoniflorin-6'O-benzene sulfonate in rats via different routes of administration

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Plant material

The roots of *Paeonia lactiflora* were collected from plants cultivated in Bozhou (China).

A voucher specimen was deposited at the Herbarium of Anhui Medical University (No. 08274). The voucher specimen was identified by Professor Lumin Pan in the College of Pharmacy, Anhui University of Chinese Medicine. Pae [$C_{23}H_{28}O_{11}$, MW: 480.45] that was extracted from the roots of *Paeonia lactiflora* was purified by solvent extraction and column chromatography in the Chemistry Lab of the Institute of Clinical Pharmacology of Anhui Medical University (Hefei, Anhui Province, China). The dried and powdered roots of *Paeonia lactiflora* were extracted with 70% ethanol under reflux, and then the concentrated extract was dissolved in water and passed in tandem through a macroporous resin column. The concentration of 40% eluate under reduced pressure gave the TGP. Subsequently, TGP was subjected to a silica gel column (column dimensions: 3 cm $\times$ 1.2 m; silica gel diameter: 74 μ m) and then eluted with ethyl acetate/methanol (20/1). Pae was acquired after the concentration of the collected eluate ([Chen et al., 2012](#); [Wang et al., 2012](#)). The purity of Pae was greater than 95% as determined by the HPLC assay.

References

- Chen JY, Wu HX, Chen Y, Zhang LL, Wang QT, Sun WY, Wei W. (2012). Paeoniflorin inhibits proliferation of fibroblast-like synoviocytes through suppressing G-protein-coupled receptor kinase 2. *Planta Med* 78, 665-671.
- Wang C, Yuan J, Yang ZY, et al. (2012). Pharmacokinetics of paeoniflorin microemulsion after repeated dosing in rats with adjuvant arthritis. *Pharmazie* 67: 997-1001.

Fig.1S. CP-25 was synthesized by conventional acidylated reaction in help of catalyst (DMAP), acid binding agent (pyridine) and esterification reagent (benzenesulfonyl chloride). A: reaction liquids; B: The products in chemical reactions were detected by TLC on plate GF254 with developing agent consisting of chloroform/ methanol (8/1, v/v). The standard substances were labeled by rightward arrows; C: CP-25 was separated and purified by silica gel column chromatography (silica gel H: 100~200 mesh, eluant: dichloromethane/methanol=30/1, v/v); D: CP-25, the white crystalline powder

Fig.2S. The HPLC chromatograms of CP-25. The purity of CP-25 in present study was 98.8% based on the results of area normalization method. The retention time of CP-25 was 8.7 min approximately. (mobile

phase: methanol/water =53/47; flow rate:0.8 mL·min⁻¹; wavelength:230 nm; column temperature : 30°C;
Agilent HC-C18 column: (4.6mm×250mm,5μm)

Fig. 3S The mass spectrum (MS) of CP-25. CP-25 was ionized in the negative ESI-MS mode.

Fig. 4S The ^{13}C -NMR spectra of the CP-25 dissolved in DMSO- d_6 . A ^{13}C -NMR, AV-400; B DEPT-135°

Fig. 5S The ^1H - ^1H COSY spectra of CP-25 dissolved in DMSO- d_6 . The correlation peaks in the COSY were formed by two hydrogenous groups that were directly connected, such as peaks produced by $\text{H}_{5'}$ with $\text{H}_{4'}/\text{H}_{6'}$ (red line), and the peaks formed by $\text{H}_{2'}$ with $(\text{OH})_{2'}$, $\text{H}_{1'}$ or $\text{H}_{3'}$ (green line). Finally, the hydrogen signals in Tab. 1 were further confirmed and supported by the results of ^1H - ^1H COSY.

Fig. 6S The ^1H - ^{13}C HSQC spectrum of CP-25 dissolved in DMSO- d_6 . The correlation peaks in HSQC were formed by carbon and hydrogen that were directly connected, such as peaks produced by $\text{H}_{6'}$ with $\text{C}_{6'}$ (green line) and the peak formed by $\text{H}_{3'}$ with $\text{C}_{3'}$ (red line). Consequently, the carbon signals in Tab. 1 were further verified by confirmed hydrogen signals and the correlation peaks in HSQC.

Fig7S. Infrared Spectroscopy of CP-25

Fig.8S. Standard curves of CP-25 and Pae in plasma of rat ($\bar{x} \pm s, n=4$)

Table.1S. Standard curve of Pae in plasma of rat ($\bar{x} \pm s$, n=4)

Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)		Peak area				$\bar{x} \pm s$	RSD%
0.05	7.5	8.2	7.3	6.1		7.28 $\pm$ 0.59	8.1%
0.10	9.78	14.1	11.6	9.75		11.31 $\pm$ 1.54	13.6%
0.25	27.3	21.3	22.7	25.2		24.12 $\pm$ 2.13	8.8%
0.50	58.7	45.5	59.7	58.9		55.70 $\pm$ 5.10	9.1%
1.00	83.8	89.2	87.4	100.6		90.25 $\pm$ 5.18	5.7%
2.50	217.5	221.6	204.7	227.7		217.88 $\pm$ 6.78	3.1%
5.00	411.2	448.5	472.3	424.8		439.20 $\pm$ 21.20	4.8%
20.00	1672	1540	1668	1724		1651.00 $\pm$ 55.50	5.7%

Table.2S. Standard curve of CP-25 in plasma of rat ($\bar{x} \pm s$, n=4)

Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)		Peak area				$\bar{x} \pm s$	RSD%
0.05	5.0	5.5	6.3	5.7		5.63 $\pm$ 0.38	6.7%
0.10	9.29	15.2	10.7	9.88		11.27 $\pm$ 1.47	13.0%
0.25	27.9	27.5	23.1	24.1		25.65 $\pm$ 2.05	7.9%
0.50	51.6	42.3	45.2	54.7		48.45 $\pm$ 4.70	9.7%
1.00	90.2	91.6	95.3	101.1		94.55 $\pm$ 3.65	3.9%
2.50	217.7	217.7	204.3	217		214.18 $\pm$ 4.94	2.3%
5.00	410.8	438.3	450.5	439.6		434.80 $\pm$ 12.00	2.8%
10.00	789.0	832.0	814.0	875.0		827.50 $\pm$ 26.00	3.1%

Table.3S. Recovery of Pae and CP-25 in plasma of rats ($\bar{x} \pm s, n=4$)

	Concentration		Peak area						Recoveries
	($\mu\text{g}\cdot\text{mL}^{-1}$)	Control group			Sample group				
Pae	0.05	6.52	6.27	6.43	6.47	6.03	5.93	(95.9±2.4)%	
	0.40	35.2	34.7	31.4	36.9	32.6	33.5	(101.8±5.2)%	
	0.80	80.3	78.9	79.3	77.5	77.6	78.6	(98.0±0.9)%	
CP-25	0.20	18.9	19.3	19.4	19.8	18.8	17.5	(97.4±4.9)%	
	0.50	51.2	50.8	49.8	50.3	48.6	49.4	(97.7±1.4)%	
	1.00	99.2	97.1	97.4	95.0	98.7	92.7	(97.5±2.7)%	

Table.4S. Precision of Pae and CP-25 in plasma of rats ($\bar{x} \pm s, n=4$)

	Concentration		Intra-day				Inter-day			
	($\mu\text{g} \cdot \text{mL}^{-1}$)		Peak area		RSD%		Peak area		RSD%	
Pae	0.05	7.1	6.4	6.2	5.4%	7.8	7.4	7.6	1.8%	
	0.40	34.6	32.8	30.5	4.4%	33.7	36.2	35.9	3.0%	
	0.80	79.1	80.5	80.8	0.9%	81.7	83.4	76.7	3.2%	
CP-25	0.20	18.1	19.3	21.1	5.5%	19.2	18.3	19.9	2.9%	
	0.50	55.6	54.2	47.1	6.6%	52.4	51.3	54.7	2.4%	
	1.00	99.1	104.0	100.9	1.7%	97.6	102.2	99.8	1.6%	

Table.5S. Stability of Pae and CP-25 in plasma of rats ($\bar{x} \pm s$, n=4)

	Concentrations ($\mu\text{g}\cdot\text{mL}^{-1}$)	0(h)	2(h) at room temperature	Freezing and thawing at day7	Freezing and thawing at day30	RSD (%)
Pae	0.05	7.8	6.9	8.1	7.4	5.3%
	0.40	31.7	30.8	32.3	31.5	1.3%
	0.80	82.4	79.2	81.7	80.9	1.2%
CP-25	0.20	17.3	16.8	17.9	17.6	2.0%
	0.50	50.2	48.7	49.5	50.3	1.2%
	1.00	97.8	96.5	97.3	95.4	0.8%