Supplementary materials

Inhibition of microbial metabolites of Chinese dark tea on age-related neurodegenerative disorders in Senescence-accelerated mouse prone 8 (SAMP8) mice

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Extraction method and structural information of CDT-1 and CDT-2

1. 3,3’-azanediylbis (4- hydroxybenzoic acid) (CDT-1)

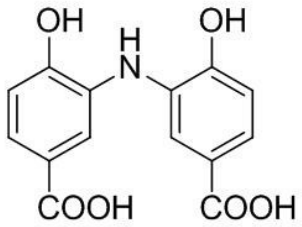

Fig. s1 Chemical structure of 3,3’-azanediylbis (4-hydroxybenzoic acid) (CDT-1)

(1) Extraction and isolation

Pu-erh tea (1.5 kg) was extracted with water (3×15 L) at 90℃for 30 min. The combined extract solution was filtered and concentrated in flash film concentrator to the filtered and concentrated in flash film concentrator to the volume of 1000 Ml, subsequently extracted with CHCl3 to remove purine alkaloid such as theobromine and caffeine, and then extracted with ethyl acetate and *n-Bu*OH successively. *n-Bu*OH extract was concentrated to dryness and subjected to column chromatography over silica gel, eluting with different rations of CHCl3-MeOH, then further reperted column chromatography over Sephadex LH-20 gave compound 1 (20 mg).

(2) Structure information

White powder; UV λ_{max} (CH_3OH): 230, 280 nm; IR (KBr); ν_{max} (solution in methanol): 3413, 1676, 1606, 1517, 1444, 1277, 1106, 971, 765 cm^{-1}; ^{1}HO NMR (500 MHz, DMSO-d6) and 13C NMR (125 MHz, DMSO-d6) see Table 1 below; positive-ion HRTOFMS m/z: 290.06534 $[M+H]^+$, calculated for $C_{14}H_{11}O_6N_{1.}$

Table s1 ^{1}H NMR and ^{13}C NMR data of compound CDT1 in DMSO-d_6

Position	^{1}H	^{13}C
1	-	131.3
2	7.73(*d*,1,*J*=1.8 Hz, Ar-H)	116.7
3	-	151.4
4	-	151.4
5	6.93(*d*,1,*J*=8.0 Hz, Ar-H)	114.7

6	7.40(*dd*,1,*J*=1.8,8.0 Hz,Ar-H)	123.1
7	-	168.1
1′	-	131.3
2′	7.73(*d*,1,*J*=1.8 Hz, Ar-H)	116.7
3′	-	151.4
4′	-	151.4
5′	6.93(*d*,1,*J*=1.8 Hz, Ar-H)	114.7
6′	7.40(*dd*,1,*J*=1.8,8.0 Hz,Ar-H)	123.1
7′	-	168.1
NH	6.72(s,1,NH)	

2. 8-C N-ethyl-2- pyrrolidinone substituted flavan-3-ols (CDT-2)

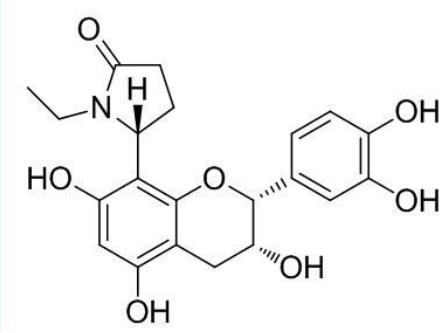

Fig. s2 Chemical structure of one of the 8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols (CDT-2)

The extraction preparation method and molecular structure information of CDT-2 have been described in details in our previous work (see reference s1). Here descripted it briefly. CDT-2 is the same compound as Puerins IV in the literature.

(1) Extraction and isolation

Pu-erh tea (1 kg) was extracted with ten times of water at 95℃ for 30 min, continuously extracted for three times. After removal of the water under wave membrane concentration, the aqueous solution afforded precipitates, which were removed by filtration. The filtrate was extracted with $CHCl_3$, ethyl acetate and n-BuOH in sequence. The ethyl acetate extract was separated into 10 fractions by silica gel column chromatography (6.5×35.0 cm) with gradient elution of chloroform and methanol from (1:0 to 0:1). The fraction 7 was subjected to Sephadex LH-20 column chromatography (4.0×50.0 cm) with H_2O containing

increasing proportions of acetone. The eluent which was obtained by elution of 30-40% acetone revealed the presence of special flavonal-3-ols derivatives by HPLC-DAD-ESI/MS analysis. This fraction was successively sub- jected to reversed phase C18 column by elution of 30% methanol to get the fraction containing compound 1-6. This fraction was subjected to preparative HPLC to yield compound 1 (5 mg), 2 (10 mg), 3 (5 mg) 4 (10 mg), 5 (3 mg), and 6 (8 mg), respectively. These compounds were used as chemical standards (purity of more than 95%) for identification of individual puerins in tea samples. Compound 1-6 were dissolved in methanol at the concentration of 0.1 mg/ml for HPLC-DAD-ESI/MS analysis. And the CDT-2 is the compound 4.

(2) Structure information

White amorphous powers; UV λ_{max} (MeOH): 230, 282 nm; IR (KBr) λmax (cm^{-1}): 3747, 3200, 2927, 1728, 1610, 1524, 1453, 1377, 1110, 1023, 996, 822, 768;.The detailed data of ^{1}H HMR (500 MHz, DMSO-d_6) and 13C NMR (125 MHz, DMSO-d_6) of CDT-2 see Table 2; positive-ion HRTOFMS m/z $[M+H]^+$: 402.15432, calculated for $C_{21}H_{23}O_7N_1$.

Table s2 ^{1}H NMR and ^{13}C NMR data of compound CDT-2 in DMSO-d_6

Position	^{1}H	^{13}C
2	4.73(1H, s)	78.6
3	3.97(1H, m)	64.9
4	2.72,2.50(each 1H, br d)	29.3
5	-	155.7
6	6.01(1H, s)	96.0
7	-	156.1
8	-	114.9
9	-	156.1
10	-	104.3
1′	-	131.0
2′	6.70(1H, br d)	114.9
3′	-	144.8
4′	-	144.8
5′	6.61(1H, s)	115.4
6′	6.69(1H, br s) 6.36(1H, s)	117.9
2′′	-	173.4
3′′	6.72 (s,1,NH)	31.3

4''	2.31,2.06(2H, m)	23.5
5''	5.26(1H, s)	51.3
6''	3.36(2H, m)	34.4
7''	0.86(3H,t,*J*=7.2)	12.8

References

[s1] W. N. Wang, L. Zhang, S. Wang, S. P. Shi, Y. Jiang, N. Li and P. F. Tu, 8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols as the marker compounds of Chinese dark teas formed in the post-fermentation process provide significant antioxidative activity, Food Chem., 2014, 152, 539-545.