Supporting Information

A Novel Label-free Fluorescence Assay for Dipeptidyl Peptidase 4 Activity Detection Based on Supramolecular Self-Assembly

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1. Experimental section

1.1 Materials

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes, MW: 238.30, ≥99.5%, Sigma-Aldrich, USA), HCl (GR, MW: 36.46, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), Dimethyl Sulfoxide (DMSO, AR, MW: 78.13, Sinopharm Chemical Reagent Co.,Ltd Shanghai, China), Sodium hydroxide (NaOH, AR, Zhongxing, Zhejiang, China), Acetonitrile (CH3CN, Chromatographically pure, Merck KGaA, Germany), Formic acid (HCOOH, Chromatographically pure, Merck KGaA, Germany). 4-Sulfocalix[4]arene Hydrate (CX4, Tokyo chemical industry, Tokyo, Japan), 4-Sulfocalix[6]arene Hydrate (CX6, Tokyo chemical industry, Tokyo, Japan). Berberine Hydrochloride Hydrate (BE, Shanghai yuanye Bio-Technology Co., Ltd, Shanghai, China), 10,10'-Dimethyl-9,9'-biacridinium Dinitrate (LCG, Tokyo chemical industry, Tokyo, Japan). Saxagliptin (＞98.5%, MW:315.41, Dalian Meilun Biotechnology Co., Ltd, Dalian, China), Recombinant Human Dipeptidyl-Peptidase 4 (DPP4，> 95%, prospec, USA)，α-Glucosidase from Saccharomyces cerevisiae (Sigma-Aldrich, USA), Bovine serum albumin, fraction V, heat shock isolation (BSA, >98%, BBI Life Sciences, Shanghai, China), Lipase from porcine pancreas (Sigma-Aldrich, USA), α-chymotrypsin (Sigma, USA), Carboxypeptidase Y(worthington, USA), Trypsin (Gibco, USA).

1.2 Apparatus

AL104 electronic balance (METTLER TOLEDO, Switzerland), XS105 electronic balance (METTLER TOLED, Switzerland), pH meter (METTLER TOLEDO，Switzerland), Agilent 1100 LC system (Agilent Technologie, USA), Finnigan LCQ Deca XP^{plus} ion trap mass spectrometer with an ESI source (Thermo，USA), Reversed-phase Zorbax SB-C_{18} analytical column (4.6mm×100mm，1.8μm，Agilent, USA), ELGA purelab Milli-Q water system (ELGA, UK).

1.3 Methods

1.3.1 LC-MS for DPP4 recognition of substrate peptides

Three tetrapeptides were incubated with 100ng/mL DPP4 at concentration of 150μM in 5mM Hepes buffer at pH 7.0 for 2 hours. Then, the samples were being analyzed by LC-MS with other standard samples.

Finnigan LCQ Deca XPplus ion trap mass spectrometer (Thermo Finnigan, USA) was used for LC-MS analysis. The parameters of LC-MS analysis were as follows: nebulizing gas, high purity nitrogen (N_2); collosion gas, high-purity helium (He); ion spray voltage: -3kV; capillary temperature: 350℃; capillary voltage: −15 V; mass range: m/z 100−1500. The LC-MS method was applied with a reversed phase Zorbax SB-C18 analytical column (250 mm × 4.6 mm I.D., 5 μm, Agilent Technologies, USA) by a gradient elution using 0.05% (v/v) formic acid (A)-0.05% (v/v) formic acid acetonitrile (B) as the mobile phase 0 min (95% A) →30 min (57% A) → 35 min (5% A). The flow rate was 0.6 mL/min.

1.3.2 Optimization of assay

Ratio of CB7 and AO: Substrate peptide GPFG was incubated with different ratio of CB7/AO reporter pair: 20 μM/2 μM，30 μM /2 μM and 40 μM /2 μM, respectively in 5 mM Hepes buffer with pH 7.0. The fluorescent intensity was immediately measured by TECAN Infinite F200 Multi-function microplate.

Assay pH: DPP4 (100 ng/mL) was incubated with CB7/AO (20 μM/2 μM) and GPFG (150 μM) for 35 min at 37℃ in 5 mM Hepes buffer with pH 5.0, 6.5, 7.0, 7.5 and 9.0 respectively. The fluorescent

intensity was measured by TECAN Infinite F200 Multi-function microplate with the parameters: excitation wavelength 465 nm, emission wavelength 510 nm.

Concentration of Hepes buffer: DPP4 (100 ng/mL) was incubated with CB7/AO (20 μM/2 μM) and GPFG (150 μM) for 20 min at 37℃ in Hepes buffer with concentration of 1.0, 2.5, 5.0, 10.0 and 20.0 mM respectively. The fluorescent intensity was measured by TECAN Infinite F200 Multi-function microplate with the parameters: excitation wavelength 465 nm, emission wavelength 510 nm.

1.3.3 Dose-dependent DPP4 assay

In order to choose the appropriate concentration of DPP4 in the assay, GPFG (150 μM) was incubated with different concentrations of DPP4 (12.5, 25, 50, 75, 100, 125 and 175 ng/mL) at 37℃. The fluorescent intensity was measured by TECAN infinite F200 Multi-function microplate (excitation wavelength 465 nm, emission wavelength 510 nm).

1.3.4 Michaellis constant evaluation of DPP4

Different concentration of GPFG (0, 25, 30, 35, 45, 60, 80 and 150 μM) was incubated with DPP4 (100 ng/mL) at 37℃ in present with CB7/AO (20 μM/2 μM). The fluorescent intensity was measured by TECAN infinite F200 Multi-function microplate (excitation wavelength 465 nm, emission wavelength 510 nm).

1.3.5 Enzymatic specificity

Despite DPP4, other hydrolases were also investigated by present assay, bovine serum albumin (BSA), α-Glucosidase, Lipase, Carboxypeptidase, Chymotrypsin and Trypsin were chosen as the comparison samples. The fluorescent intensity was performed using TECAN infinite F200 multifunction microplate reader with an excitation wavelength of 320 nm and an emission wavelength of 450 nm.

1.3.6 Dose-dependent inhibition of DPP4 by hit compounds.

DPP4 inhibition of Salvianolic acid C, Herbacetin, Ellagic acid were further evaluated by using another DPP4 probe. Different concentrations of compounds were incubated with DPP4 (5 mU/mL) and TPE-KFPE (10 μM) for 30 min at 37℃. The PL intensity was measured in the condition of excitation wavelength 320nm and emission wavelength 450 nm on TECAN infinite M1000.

1.3.7 Human Serum and whole blood samples

The use of human serum and whole blood samples was approved by Zhejiang University. Human whole blood samples were obtained from Department of Neurology, Shanghai Fourth People's Hospital (Shanghai, China). Human serum samples were obtained from Department of Cardiology, the First People's Hospital of Xiaoshan District (Hanghzou, China). The study did not influence any diagnostic or therapeutic procedure. Blood samples were collected from both male and female patients with diabetes and healthy individuals. Blood samples were collected into glass test tubes without additives and stored at 4℃. The serum was separated by centrifugation at 3000 rpm for 30 min. Then, they were collected and stored at -80℃ until analysis.

1.3.8 Mice plasma

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996)

and were approved by the Institute Animal Care and Use Committee of Zhejiang University.

Six-eight weeks old male C57BL/6 mice were randomly divided into HFD group and control group. The HFD group mice were received high-fat diet (D12492, Open Sourse Diets, Huitebi Technology Co., Ltd, Beijing) for 12 days. Control group mice were received normal diet. The eyeballs of mice were peeled off and blood was collected. The plasma obtained 2 hours after centrifugation (15 min, 3000 rpm, at 4℃) and was stored at -80℃ until analysis.

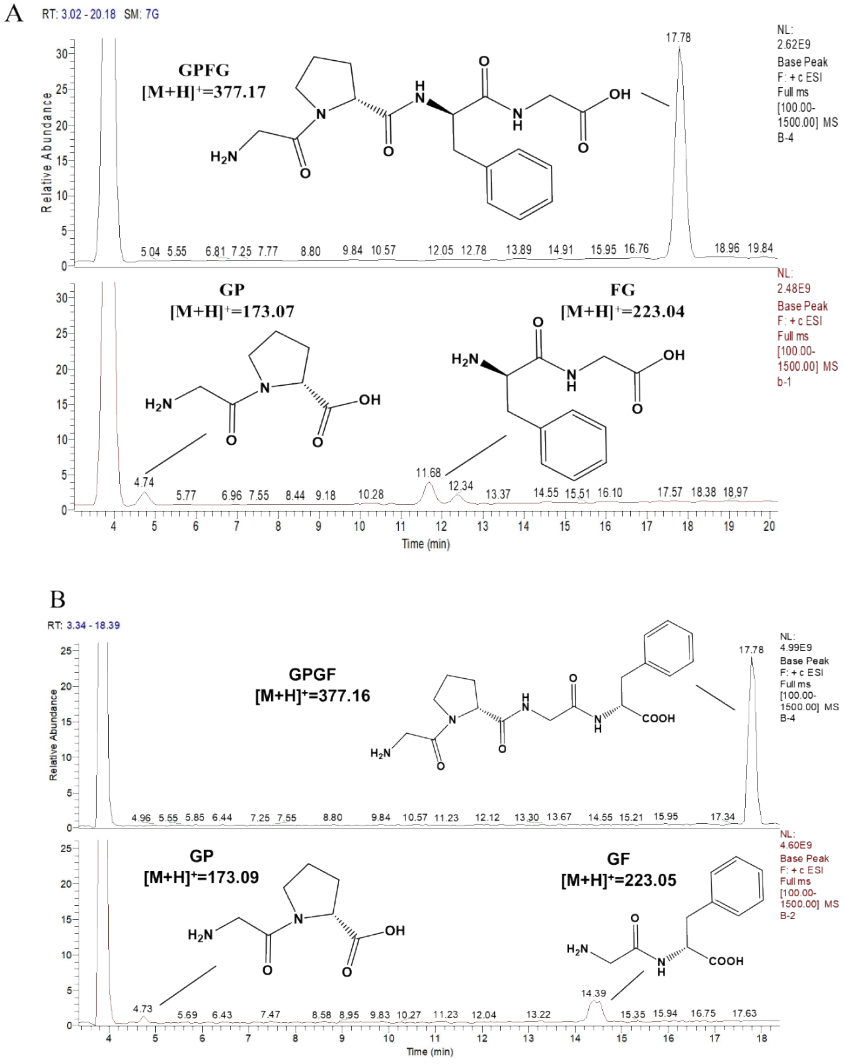
1.3.9 Data analysis.

All data and figures were analyzed and produced by GraphPad Prism 7.0. and Origin 8.5 software.

2. Results

2.1 DPP4 recognition of substrate peptides

DPP4 hydrolysis of substrate peptides GPFG, GPGG and GPGF were validated by LC-MS. All of the three peptides were able to be hydrolysed by DPP4.



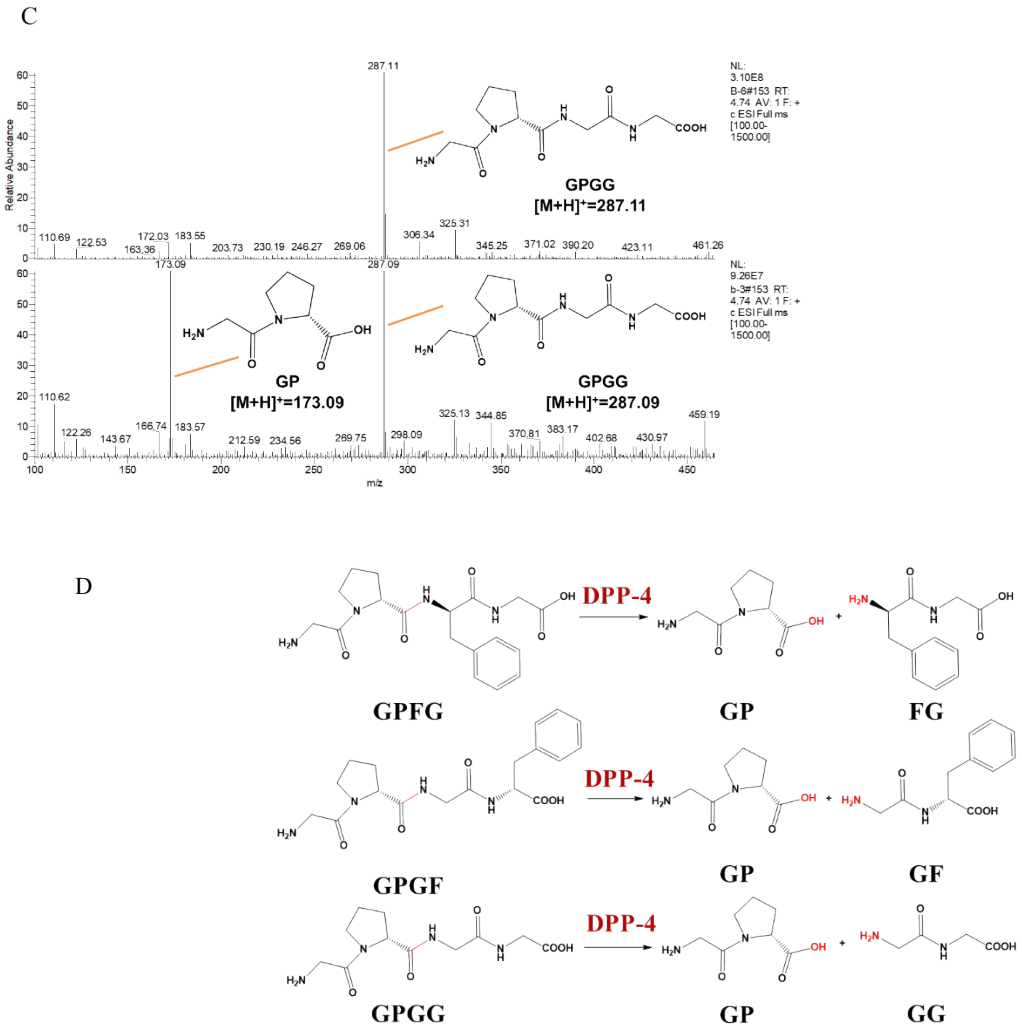
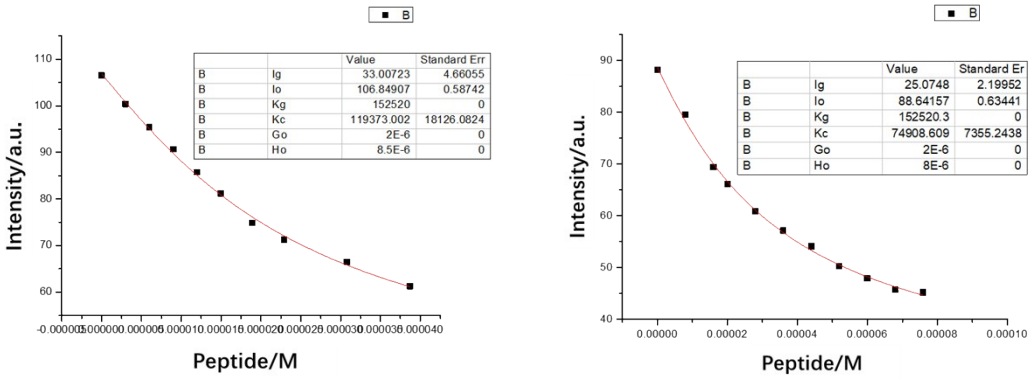


Fig. S-1 LC-MS result of DPP4 hydrolysis of substrate peptides (A) GPFG, (B) GPGF and (C) GPGG, (D) Molecular formula of the peptides

2.2 Competitive titration of peptide FG

The result of peptide FG competitive titration with CB[7]/AO (8 μM/2 μM) , CB[7]/AO (8.5 μM/2 μM) and CB[7]/AO (7 μM/2 μM), respectively. The association constant was calculated according to the average Kc value of 3 competitive titrations.



		Value	Standard Err
B	Ig	33.00723	4.66055
B	Io	106.84907	0.58742
B	Kg	152520	0
B	Kc	119373.002	18126.0824
B	Go	2E-6	0
B	Ho	8.5E-6	0

		Value	Standard Er
B	Ig	25.0748	2.19952
B	Io	88.64157	0.63441
B	Kg	152520.3	0
B	Kc	74908.609	7355.2438
B	Go	2E-6	0
B	Ho	8E-6	0

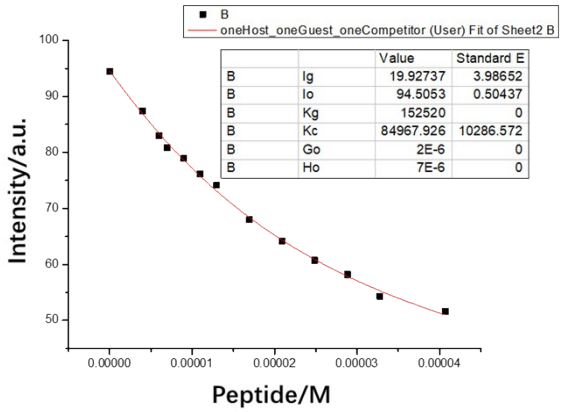


Fig. S-2 Competitive titrations of peptide FG with different ratio of CB[7] and AO.

2.3 Assay Optimization

In order to utilize the CB[7]-GPFG in 96-well plates or 384-well plates which obtains the fluorescence variance by fluorescence microplate reader. The fold change of the fluorescence is not big enough under such condition for microplate readers to detect. Therefore, we optimized the assay to meet the demand for being used by fluorescence microplate reader. CB7 and AO ratio was optimized from 8 μM/2 μM, 20 μM/2 μM to 30 μM/2 μM (Fig. S-2). CB7/AO (20 μM/2 μM) was chosen as the reporter. Then, the concentration and pH of Hepes buffer were optimized (Fig. S-3). Finally, CB7/AO (20 μM/2 μM) in 1.0 mM Hepes at pH 7.0 showed significant fluorescent decrease compared to other concentration and was selected as the optimized assay condition for further application.

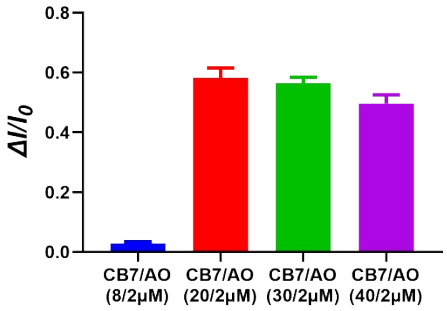


Fig. S-3. Optimization of CB7 and AO ratio in 100 μL reaction volume.

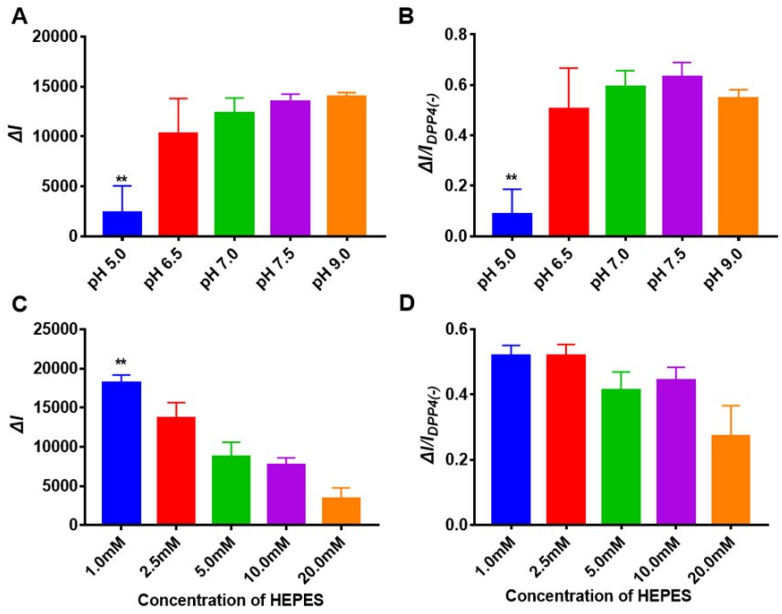


Fig. S-4. (A,B) Optimization of pH of Hepes buffer; (C,D) Optimization of concentration of Hepes buffer

2.4 Enzyme kinetic assay

Under optimized assay condition, only DPP4 together with GPFG caused a significant decrease of fluorescence. DPP4 or GPFG alone showed no significant influence of the fluorescence.

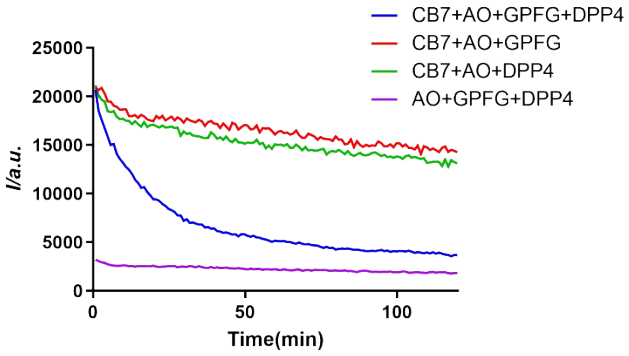


Fig. S-5. The evaluation of DPP4 kinetic assay

2.5 Detecting DPP4 activity and expression in whole blood sample from human

Whole blood samples from both healthy individuals and patients with type 2 diabetes were examined using CB[7]-GPFG assay and commercial ELISA kits. Result showed the correlation between DPP4 activity and expression.

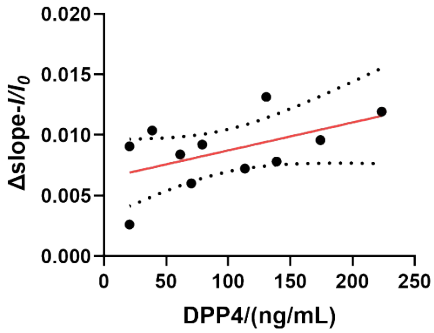


Fig. S-6 Correlation analysis between the hydrolytic rates of DPP4 using CB[7]-GPFG assay and protein expression levels in a panel of clinical patient's whole blood (n=11)

2.6 DPP4 inhibitor screening of a natural products library of 311 compounds

Table S1. DPP4 inhibitor screening by CB7-GPFG assay

No.	Name	CAS	Average Inhibition	SD
1	Herbacetin	527-95-7	85.01%	0.23
2	Ellagic acid	476-66-4	83.83%	0.26
3	Salvianolic acid C	115841-09-3	69.22%	0.11
4	Salvianolic acid A	96574-01-5	66.61%	0.35
5	Eriocitrin	13463-28-0	55.98%	0.23
6	Rhodionin	85571-15-9	53.18%	0.23
7	Taraxasterol acetate	6426-43-3	48.82%	0.07
8	Crocin I	N/A	48.30%	0.13
9	Rutin	153-18-4	48.16%	0.43
10	Isoliquiritin apioside	120926-46-7	46.95%	0.07
11	Aurantiamide acetate	N/A	46.92%	0.10
12	Kuromanin chloride	7084-24-4	46.66%	0.35
13	Cyanidin chloride	528-58-5	44.60%	0.04
14	Dimethyl lithospermate B	875313-64-7	44.41%	0.36
15	Crocin	42553-65-1	44.30%	0.29
16	Resibufogenin	465-39-4	44.11%	0.23
17	Schizandrin B	61281-37-6	43.51%	0.04
18	Isoliquiritigenin	961-29-5	40.46%	0.01
19	Aristolochic acid a(sh)	N/A	37.47%	0.24
20	Notoginsenoside R2	80418-25-3	37.41%	0.24
21	Evodiamine	518-17-2	37.15%	0.29
22	Chlorogenic acid	327-97-9	37.00%	0.34
23	15-hydroxyabieta-7,13-dien-18-oic acid	101821-23-2	36.56%	0.21
24	Pinocembrin	N/A	36.53%	0.04
25	Psoralidin	18642-23-4	35.95%	0.10
26	Irisflorentin	41743-73-1	35.66%	0.04

27	Geniposide	24512-63-8	34.60%	0.17
28	Tangeretin	481-53-8	33.54%	0.20
29	R-notoginsenoside R2	N/A	32.98%	0.21
30	Vitexia-glucoside	38950-94-6	32.21%	0.11
31	3-o-alpha-l-arabinopyranosylpomolic acid beta-d-glucopyranosyl ester	35286-58-9	31.26%	0.39
32	Ginsenoside F2	62025-49-4	31.09%	0.12
33	Ononin	486-62-4	29.43%	0.33
34	Bilobalide	33570-04-6	29.40%	0.10
35	Baicalin	21967-41-9	29.30%	0.09
36	Scutellarin	27740-01-8	28.98%	0.16
37	Sesamoside	117479-87-5	28.37%	0.18
38	Isorhamnetin-3-O-neohesperidoside	55033-90-4	28.05%	0.67
39	Amentoflavone	1617-53-4	27.15%	0.16
40	Paeonol	552-41-0	27.08%	0.23
41	Quercetin dihydrate	6151-25-3	26.94%	0.02
42	Neoandrographolide	27215-14-1	26.91%	0.29
43	Taraxasteryl acetate	6426-43-3	26.49%	0.18
44	10-gingerol	23513-15-7	26.45%	0.37
45	Oleandrin	465-16-7	26.09%	0.13
46	Crocin ii	55750-84-0	25.27%	0.27
47	Griffonilide	61371-55-9	25.12%	0.22
48	Astragalin	480-10-4	25.07%	0.03
49	L-epicatechin	490-46-0	25.05%	0.12
50	Resveratrol	501-36-0	24.65%	0.16
51	(-)-epigallocatechin gallate	989-51-5	24.54%	0.19
52	Aloe emodin	481-72-1	24.43%	0.20
53	Tiliroside(p)	N/A	24.32%	0.23
54	Usnic acid	125-46-2	24.23%	0.15
55	D-phenylalanine	673-06-3	24.12%	0.39
56	Cinobufagin	470-37-1	23.98%	0.29
57	Sauchinone	177931-17-8	23.81%	0.14
58	Ginsenosidero	34367-04-9	23.63%	0.22
59	Honokiol	35354-74-6	23.58%	0.28
60	(2β,3β,14r)-2,3-epoxygrayanotoxane-5,6β,10,14,16-pentol 14-acetate	37720-86-8	23.52%	0.16
61	Akebia saponin D	39524-08-8	22.48%	0.28
62	Cimicifugoside	66176-93-0	21.98%	0.13
63	Beta-d-glucopyranosiduronic acid	51059-44-0	21.78%	0.24
64	Curcurbitacin IIa	N/A	21.32%	0.34
65	Allantoin	97-59-6	21.05%	0.20
66	Anisodine hydrobromide	76822-34-9	20.74%	0.35
67	Dendrobine	2115-91-5	20.67%	0.04
68	Rhodojaponin III	26342-66-5	20.59%	0.08

69	Curcubitacin IIb	N/A	20.48%	0.12
70	Ginsenoside F1	53963-43-2	20.20%	0.17
71	Cinnamic acid	621-82-9	19.75%	0.11
72	Ginsenoside Rg2	52286-74-5	19.57%	0.35
73	Ginkgolide C	15291-76-6	19.40%	0.13
74	Eupatilin	22368-21-4	19.38%	0.16
75	Mangiferin	4773-96-0	19.14%	0.16
76	Scopoletin	92-61-5	19.06%	0.29
77	Salidroside	10338-51-9	18.92%	0.11
78	Desrhamnosyl isoacteoside	105471-98-5	18.89%	0.12
79	Apigenin-6-glucoside-8-arabinoside	51938-32-0	18.85%	0.21
80	Ginsenoside Rh1	63223-86-9	18.56%	0.27
81	Astragaloside II	84676-89-1	18.43%	0.33
82	Astragaloside III	84687-42-3	18.40%	0.24
83	Esculentoside A	65497-07-6	17.67%	0.15
84	Oxypaeoniflora	N/A	17.56%	0.18
85	Ursodeoxycholic acid	128-13-2	17.30%	0.15
86	Pseudoginsenoside rt5	98474-78-3	17.17%	0.10
87	Ginsenoside-Rg5	186763-78-0	17.14%	0.11
88	Azelaic acid	123-99-9	16.77%	0.21
89	Quinic acid	77-95-2	16.74%	0.24
90	Eupalinilide C	757202-11-2	16.74%	0.26
91	Salvianolic acid B	115939-25-8	16.46%	0.21
92	D-tetrahydropalmatine	6024-83-5	16.19%	0.31
93	Fraxinellon	28808-62-0	16.18%	0.08
94	Ginsenoside Rf	52286-58-5	16.10%	0.18
95	Ammonium glycyrrhizinate	1407-03-0	15.90%	0.06
96	Berberine hydrochloride	633-65-8	15.60%	0.14
97	Chonglou Saponin III	N/A	15.54%	0.29
98	Isochlorogenic acid C	57378-72-0	14.89%	0.26
99	4-hydroxybenzoic acid	99-96-7	14.82%	0.18
100	Ginsenoside Rk3	364779-15-7	14.66%	0.22
101	Rhein	478-43-3	14.64%	0.29
102	Paederosidic acid	18842-98-3	14.07%	0.16
103	Qingyangshengenin A	106644-33-1	14.00%	0.19
104	Rutaecarpine	84-26-4	13.82%	0.12
105	Tuberostemonine	6879-01-2	13.74%	0.34
106	Ophiopogonin D	945619-74-9	13.30%	0.14
107	Isochlorogenic acid B	14534-61-3	13.05%	0.33
108	Hesperetin	520-33-2	13.02%	0.14
109	Sec-O-glucosylhamaudol	80681-44-3	12.54%	0.39
110	Chrysophanic acid	481-74-3	12.32%	0.16
111	Eupalinilide D	757202-14-5	12.28%	0.28
112	Tauroursodeoxycholate	N/A	12.28%	0.15

113	Rosmarinic acid	537-15-5	12.27%	0.07
114	Leonurine hydrochloride	24697-74-3	12.22%	0.18
115	Isofraxidin	486-21-5	12.17%	0.38
116	Andrographolide	5508-58-7	12.13%	0.41
117	Safflomin A	78281-02-4	12.13%	0.23
118	Eleutheroside E	39432-56-9	12.01%	0.16
119	Trifolirhizin	6807-83-6	11.70%	0.26
120	Genistin	529-59-9	11.67%	0.24
121	(14r)-2β,3β-epoxygrayanotoxane-5,6β,10,14,16-pentol 6-acetate	26116-89-2	11.59%	0.20
122	2,3,5,4′-tetrahydroxy stilbene-2-O-β-d-glucoside	N/A	11.54%	0.05
123	Astragaloside A	83207-58-3	11.51%	0.07
124	L-phenylalanine	63-91-2	11.48%	0.52
125	Pectolinarin	28978-02-1	11.10%	0.20
126	Phloretin	60-82-2	11.04%	0.12
127	Chonglou saponin VI	N/A	10.98%	0.21
128	Myricetin	529-44-2	10.78%	0.25
129	Ginkgo biflavones	N/A	10.77%	0.16
130	Betulinic acid	472-15-1	10.77%	0.17
131	Sarsasapogenin 3-O-β-d-galactopyranosid	68422-00-4	10.39%	0.44
132	Ginsenoside Re	52286-59-6	9.85%	0.17
133	Paederoside	20547-45-9	9.59%	0.26
134	Obtusifolin	477-85-0	9.56%	0.17
135	Isoastragaloside IV	136033-55-1	9.26%	0.06
136	Eriodictyol	552-58-9	9.13%	0.15
137	Isorhamnetin	480-19-3	9.03%	0.07
138	4-O-beta-d-gulcosyl-5-O-methylvisamminol	84272-85-5	8.91%	0.26
139	Glycyrrhizic acid	1405-86-3	8.89%	0.33
140	Hyperoside	482-36-0	8.77%	0.07
141	2'-O-galloylhyperin	53209-27-1	8.53%	0.18
142	Ephedrine hydrochloride	50-98-6	8.52%	0.36
143	Coumarin	91-64-5	8.50%	0.29
144	Palmatine chloride	10605-02-4	8.20%	0.34
145	Ruscogenin	472-11-7	8.13%	0.06
146	Senecionine	130-01-8	7.74%	0.19
147	Emodin-8-glucoside	23313-21-5	7.49%	0.04
148	6-gingerol	23513-14-6	7.19%	0.27
149	Narirutin	14259-46-2	6.77%	0.24
150	Sophocarpine	6483-15-4	6.66%	0.24
151	Shanzhiside methyl ester	64421-28-9	6.21%	0.17
152	Isochlorogenic acid A	2450-53-5	6.11%	0.28

153	Magnolol	528-43-8	5.89%	0.10
154	Hesperidin	520-26-3	5.72%	0.23
155	Gallic acid	149-91-7	5.54%	0.17
156	Ginsenoside Rb1	41753-43-9	5.41%	0.06
157	8-O-acetyl shanzhiside methyl ester	57420-46-9	5.36%	0.24
158	3,4-dihydroxybenzoic acid	99-50-3	4.77%	0.07
159	Seneciphyllin	19776-79-5	4.76%	0.12
160	Tetrahydropalmatine hydrochloride	6024-85-7	4.48%	0.36
161	Ginsenoside Rb2	11021-13-9	4.21%	0.14
162	18alpha-glycyrrhetinic acid	1449-05-4	4.08%	0.17
163	(-)-catechin hydrate	18829-70-4	4.04%	0.15
164	Ginsenoside Rh3	105558-26-7	4.00%	0.42
165	Ginkgolide B	15291-77-7	3.89%	0.11
166	Eupalinilide B	757202-08-7	3.83%	0.21
167	Panaxadiol	19666-76-3	3.81%	0.08
168	Sodium danshensu	67920-52-9	3.79%	0.38
169	Helicid	80154-34-3	3.70%	0.24
170	Secologanic acid	60077-46-5	3.62%	0.18
171	Ginsenoside Rh4	174721-08-5	3.46%	0.44
172	Kaempferol	520-18-3	3.40%	0.25
173	Formononetin	485-72-3	2.79%	0.09
174	Sesamin	607-80-7	2.63%	0.44
175	Mangostin	6147-11-1	2.43%	0.22
176	Ginsenoside Rd	52705-93-8	2.34%	0.17
177	(-)-asarinin 97	133-05-1	2.17%	0.34
178	Eleutheroside B (syringin)	118-34-3	2.04%	0.14
179	Naringin	10236-47-2	1.99%	0.40
180	Rebaudioside A	58543-16-1	1.73%	0.08
181	Sinensetin(p)	N/A	1.70%	0.16
182	Isoginkgetin	548-19-6	1.51%	0.21
183	Emodin	518-82-1	1.44%	0.19
184	Qingyangshengenin	84745-94-8	1.18%	0.31
185	Gomisin A	58546-54-6	0.91%	0.24
186	Beta-sitosterol	83-46-5	0.75%	0.18
187	Corosolic acid	4547-24-4	0.29%	0.27
188	Dopac	102-32-9	0.01%	0.38
189	Ferulic acid	1135-24-6	-0.02%	0.16
190	Methyl paederosidate	122413-01-8	-0.03%	0.03
191	Neosperidin dihydrochalcone	20702-77-6	-0.16%	0.34
192	Tanshinone Ⅱa	N/A	-0.25%	0.24
193	4-hydroxycinnamic acid	501-98-4	-0.41%	0.17
194	Neochlorogenic acid	906-33-2	-0.43%	0.22
195	Asiaticoside	16830-15-2	-0.48%	0.20
196	Cynaroside	5373-11-5	-0.62%	0.23

197	Schisandrin A	61281-38-7	-1.75%	0.05
198	Gypenoside-XVII	80321-69-3	-1.87%	0.33
199	Protocatechualdehyde	139-85-5	-2.75%	0.31
200	Huperzine a	120786-18-7	-2.79%	0.17
201	Emodin-3-methyl ether	521-61-9	-2.90%	0.32
202	[(1as,1bα,5aα,6aβ)-1a,1b,2,5a,6,6a-hexahydro-6α-hydroxy-1aβ-(hydroxymethyl)oxireno[4,5]cyclopenta[1,2-c]pyran-2α-yl]6-O-α-d-galactopyranosyl-β-d-glucopyranoside	81720-05-0	-3.08%	0.13
203	Ginkgolide A	15291-75-5	-3.20%	0.26
204	Ursolic acid	77-52-1	-3.28%	0.08
205	Demethyleneberberine	25459-91-0	-3.39%	0.14
206	Betaine	107-43-7	-3.82%	0.43
207	Geniposidic acid	27741-01-1	-3.94%	0.32
208	6-acetyl-7-deacetylforskolin	64657-21-2	-4.13%	0.38
209	Salvianolic acid D	142998-47-8	-4.52%	0.16
210	6-(2-O-alpha-l-arabinopyranosyl-beta-d-glucopyranosyl)-2-[4-(beta-d-glucopyranosyloxy)phenyl]-5,7-dihydroxy-4h-1-benzopyran-4-one	53452-16-7	-4.93%	0.12
211	Genipin	6902-77-8	-5.10%	0.24
212	Chenodeoxycholic acid	474-25-9	-5.66%	0.23
213	Ginsenoside Rh2	78214-33-2	-5.66%	0.14
214	Nodakenin	495-31-8	-5.74%	0.22
215	Oxysophocarpine	26904-64-3	-6.09%	0.19
216	Cycloastragenol	84605-18-5	-6.45%	0.14
217	Epigoitrin	1072-93-1	-6.68%	0.24
218	Luteolin	491-70-3	-7.08%	0.12
219	(r)-(+)-corypalmine	13063-54-2	-7.10%	0.08
220	Vanillin	121-33-5	-7.63%	0.48
221	Syringic acid	530-57-4	-7.69%	0.26
222	Pachymic acid	29070-92-6	-7.86%	0.29
223	Caffeic acid	331-39-5	-7.93%	0.26
224	Didymin	14259-47-3	-8.85%	0.30
225	Schisantherin A	58546-56-8	-8.90%	0.41
226	Rhapontin	155-58-8	-8.91%	0.11
227	Columbamine	3621-36-1	-9.11%	0.11
228	Curcumin	458-37-7	-9.54%	0.06
229	Chonglou saponin VII	68124-04-9	-9.62%	0.35
230	Timosaponin a-Ⅲ	41059-79-4	-9.76%	0.41
231	P-hydroxybenzaldehyde	123-08-0	-9.78%	0.13
232	Ginsenoside F4	181225-33-2	-9.88%	0.20
233	Forsythin	487-41-2	-10.40%	0.17

234	Ophiopogonin B	38971-41-4	-10.44%	0.14
235	Puerarin	3681-99-0	-10.52%	0.35
236	Panaxatriol	32791-84-7	-10.71%	0.25
237	Macranthoidin B	136849-88-2	-11.18%	0.13
238	Phlorizin dihydrate	7061-54-3	-11.42%	0.06
239	Matrine	519-02-8	-11.71%	0.39
240	Phellodendrine	6873-13-8	-12.17%	0.35
241	1-butanol	71-36-3	-12.37%	0.25
242	Nsc150443	6681-18-1	-12.56%	0.14
243	Silibinin	22888-70-6	-12.78%	0.25
244	Stevia	N/A	-12.80%	0.21
245	Momordinic	96990-18-0	-12.82%	0.18
246	Rosavin(p)	84954-92-7	-13.03%	0.27
247	Schisandrin	7432-28-2	-13.03%	0.20
248	(20α,22r,25s)-spirosta-5-ene-3β-ol	512-06-1	-13.36%	0.36
249	Artemisinin	491-54-3	-13.51%	0.13
250	Gelsemine	509-15-9	-13.56%	0.22
251	Berberrubine	15401-69-1	-13.69%	0.44
252	Cryptochlorogenic acid	905-99-7	-14.17%	0.30
253	Coptisine chloride	6020-18-4	-14.25%	0.33
254	Dipsacoside B	33289-85-9	-14.57%	0.41
255	Atractylenolide III	73030-71-4	-14.59%	0.18
256	Aucubin	479-98-1	-14.77%	0.23
257	Lithospermic acid	28831-65-4	-14.78%	0.19
258	Nobiletin	478-01-3	-14.81%	0.26
259	Ginsenoside Rk1	N/A	-15.09%	0.33
260	Isovanillin	621-59-0	-16.03%	0.47
261	Amygdalin	29883-15-6	-16.32%	0.54
262	Cimigenol-3-O-β-d-xylpyranoside		-16.63%	0.04
263	Liquiritin(sh)	551-15-5	-18.12%	0.32
264	Jujuboside A	55466-04-1	-18.29%	0.07
265	(3b,6a,12b,24r)-20,24-epoxy-3,12,25-trihydroxydammaran-6-yl 2-O-(6-deoxy-alpha-l-mannopyranosyl)-beta-d-glucopyranoside	69884-00-0	-18.98%	0.82
266	Benzoic acid	65-85-0	-19.75%	0.26
267	Dehydroandrographolide	134418-28-3	-20.15%	0.02
268	Spinosin	72063-39-9	-21.23%	0.23
269	Rosarin	84954-93-8	-21.82%	0.24
270	Ginsenoside Rb3	68406-26-8	-22.49%	0.17
271	Dryocrassin	12777-70-7	-23.23%	0.23
272	2-atractylenolide	73069-14-4	-25.21%	0.17
273	Tetramethylpyrazine	1124-11-4	-25.34%	0.27
274	Diosgenin glucoside	14144-06-0	-26.07%	0.29

275	Danshensu	76822-21-4	-26.22%	0.20
276	Genipin 1-gentiobioside	29307-60-6	-26.48%	0.05
277	Seco-isolariciresinol diglucoside	148244-82-0	-26.76%	0.23
278	Isoimperatorin	482-45-1	-27.34%	0.13
279	Protopanaxadiol	N/A	-27.90%	0.07
280	Daidzein	486-66-8	-28.52%	0.15
281	20(s)-ginsenoside Ck	39262-14-1	-28.92%	0.17
282	Caulophylline	486-86-2	-29.16%	0.16
283	8-gingerol	30462-35-2	-29.51%	0.13
284	(-)-epigallocatechin	970-74-1	-29.65%	0.22
285	Allomatrine	641-39-4	-30.41%	0.36
286	Calycosin	CALYCOSIN	-30.56%	0.30
287	Ammothamnine	16837-52-8	-30.97%	0.36
288	Paeoniflorin	23180-57-6	-32.12%	0.29
289	Ginsenoside Rg1	22427-39-0	-33.08%	0.05
290	Lupeol	545-47-1	-33.34%	0.04
291	3-hydroxy-4-methoxycinnamic acid	537-73-5	-34.69%	0.37
292	Ginsenoside Rg3	14197-60-5	-34.75%	0.61
293	Stigmasterol	83-48-7	-36.21%	0.43
294	Gypenoside-XVIII	N/A	-36.91%	0.38
295	20(r)protopanaxdiol	N/A	-38.23%	0.09
296	Albiflorin	39011-90-0	-38.82%	0.33
297	Jatrorrhizine HCl(rg)	6681-15-8	-39.41%	0.34
298	3',5-dihydroxy-7-(β-d-glucopyranosyloxy)-4'-methoxyflavone	20126-59-4	-42.00%	0.30
299	Ginsenoside Rc	11021-14-0	-44.39%	0.17
300	Stachydrine hydrochloride	4136-37-2	-45.46%	0.50
301	Koumine	1358-76-5	-45.74%	0.16
302	Taxifolin 3-O-rhamnoside	29838-67-3	-45.99%	0.27
303	Atractylenolide-1	73069-13-3	-46.25%	0.30
304	Oleanic acid	508-02-1	-46.99%	0.23
305	Schisandrin C	61301-33-5	-50.02%	0.60
306	Benzoylpaeoniflorin	38642-49-8	-51.73%	0.22
307	Timosaponin BII	136656-07-0	-51.84%	0.20
308	(1s,2s)-(+)-pseudoephedrine hydrochloride	345-78-8	-56.84%	0.33
309	Beta-carotene	7235-40-7	-61.14%	0.28
310	20(r)-ginsenoside Rg3	38243-03-7	-61.77%	0.25
311	Agrimol B	55576-66-4	-67.71%	0.28