

Supplementary Information

***Ilex kaushue* and Its Bioactive Component 3,5-Dicaffeoylquinic Acid Protected Mice from Lipopolysaccharide-Induced Acute Lung Injury.**

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Supplementary information Figure S1

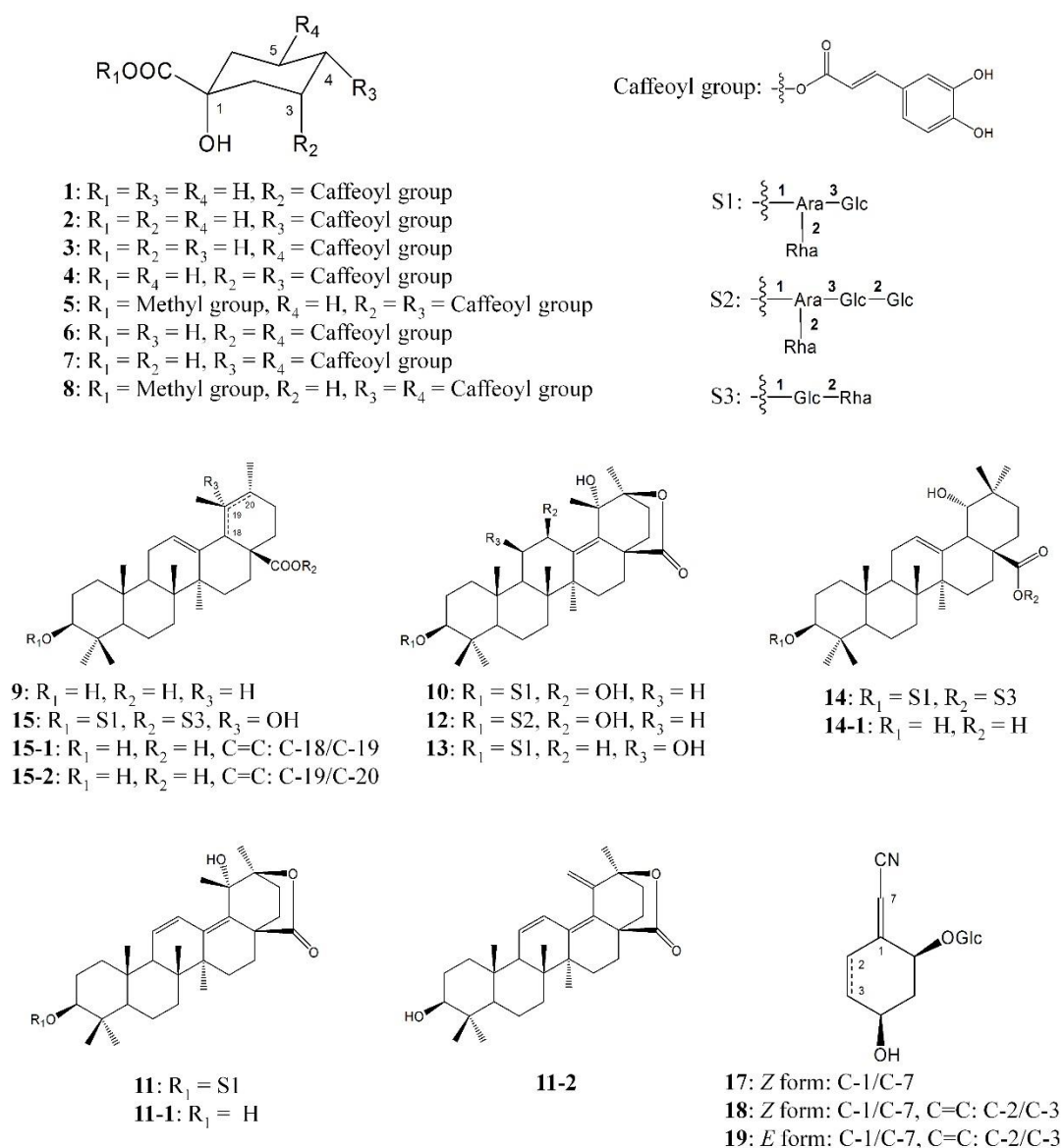


Fig. S1. Structures of isolatic and semi-synthetic compounds

Supplementary information Figure S2

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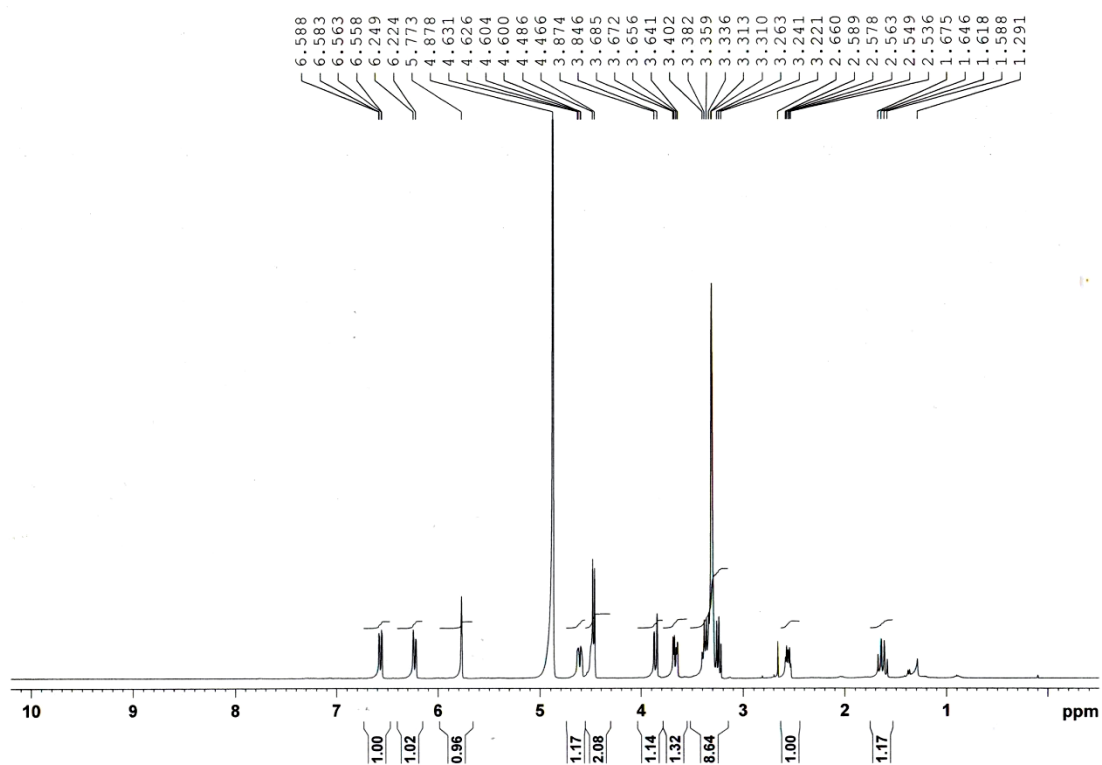


Fig. S2. ¹H NMR spectrum of menisdaurin F.

Supplementary infomration Figure S3

20150422 OP7

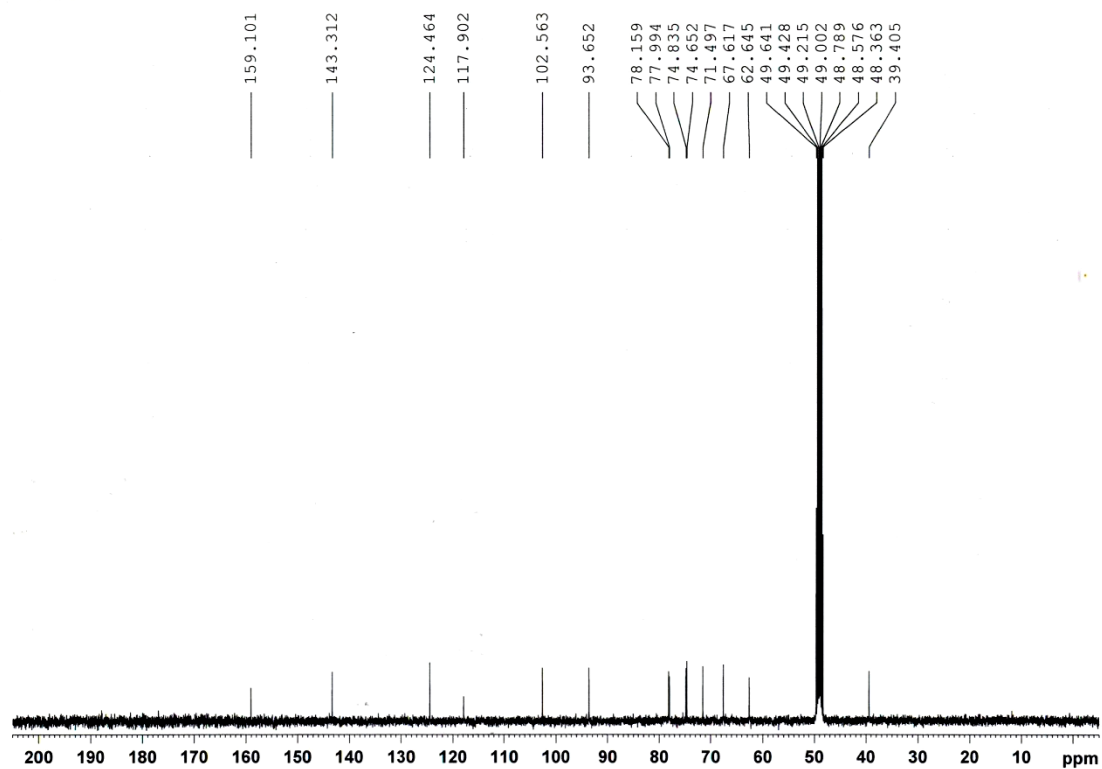


Fig. S3. ¹³C NMR spectrum of menisdaurin F.

Supplementary information Figure S4

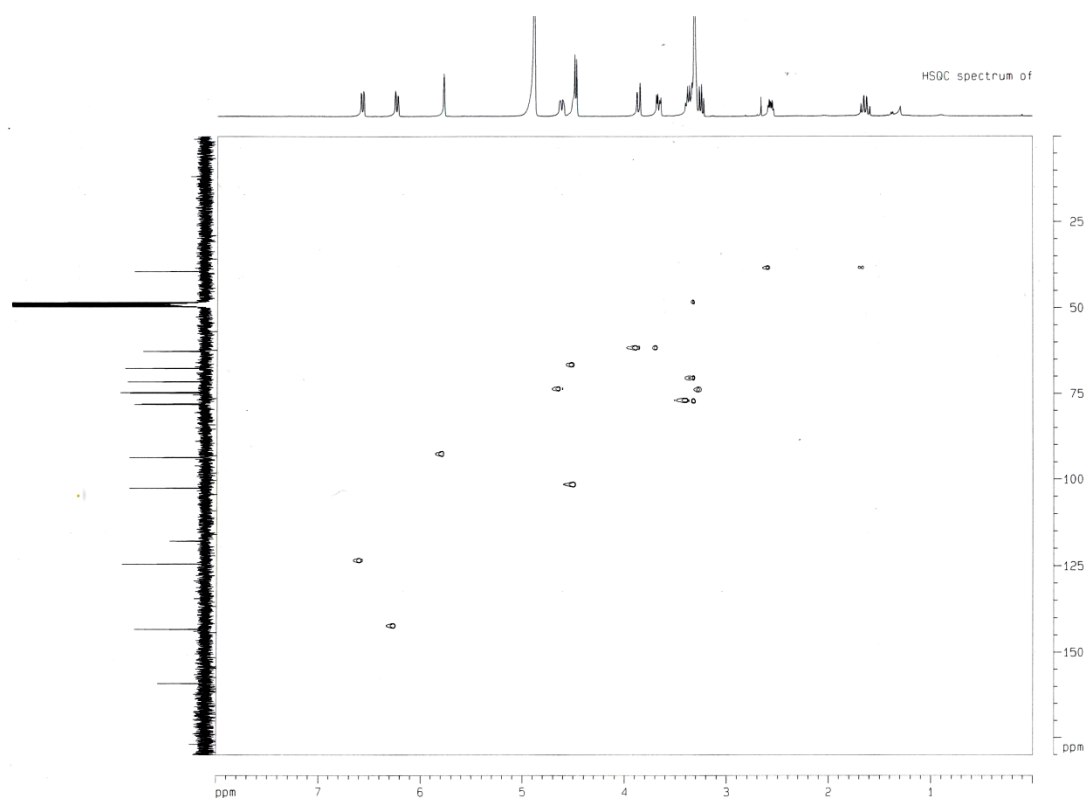


Fig. S4. HSQC spectrum of menisdaurin F.

Supplementary information Figure S5

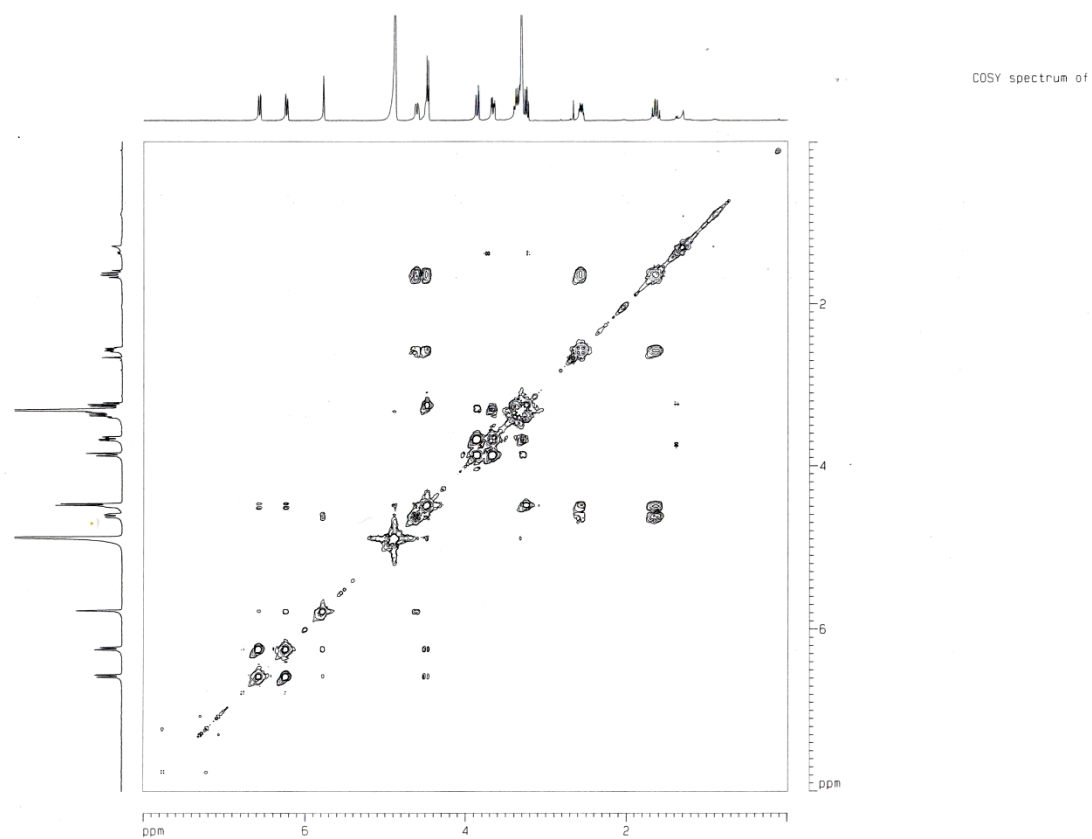


Fig. S5. COSY spectrum of menisdaurin F.

Supplementary information Figure S6

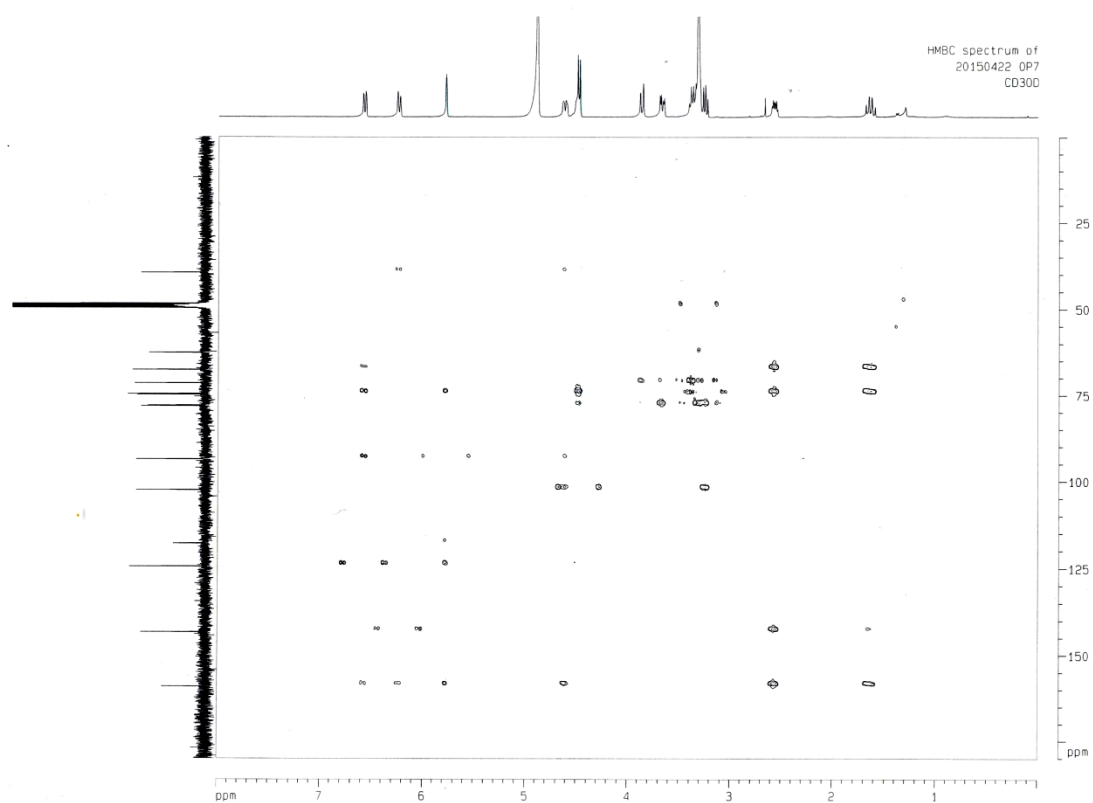


Fig. S6. HMBC spectrum of menisdaurin F.

Supplementary information Figure S7

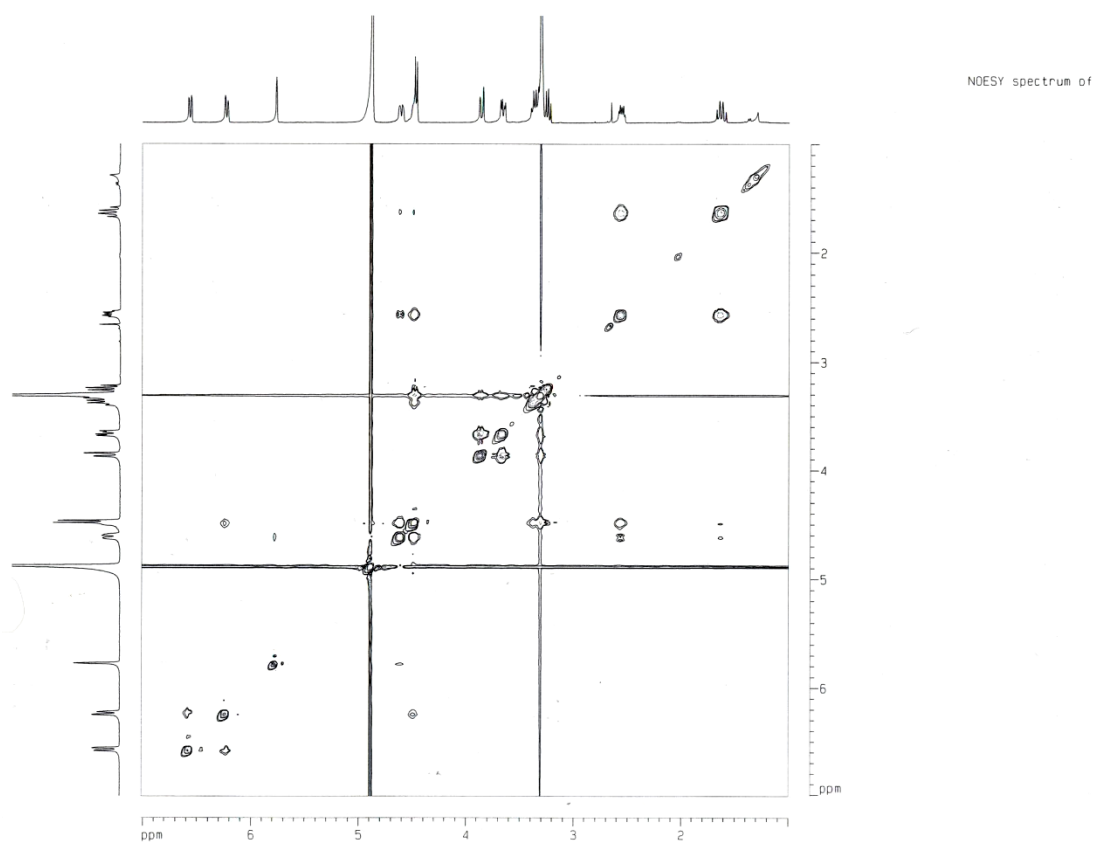


Fig. S7. NOESY spectrum of menisdaurin F.

Supplementary infomration Figure S8

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12/10/2015 10:02:59 AM

32-OP7-H#1-20 RT: 0.01-0.42 AV: 20

T: FTMS + p ESI Full ms [150.00-2000.00]

m/z= 308.7158-354.8541

Isotope	Min	Max
N-14	0	1
O-16	0	7
C-12	0	14
H-1	0	25
Na-23	0	1

Charge 1

Mass tolerance 140.00 ppm

Nitrogen rule not used

RDB equiv -1.00-100.00

max results 1

m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	Composition
336.1048	164920.2	100.00	336.1054	-1.85	C ₁₄ H ₁₉ O ₇ N Na

Fig. S8. HRESIMS spectrum of menisdaurin F.

Supplementary information Figure S9

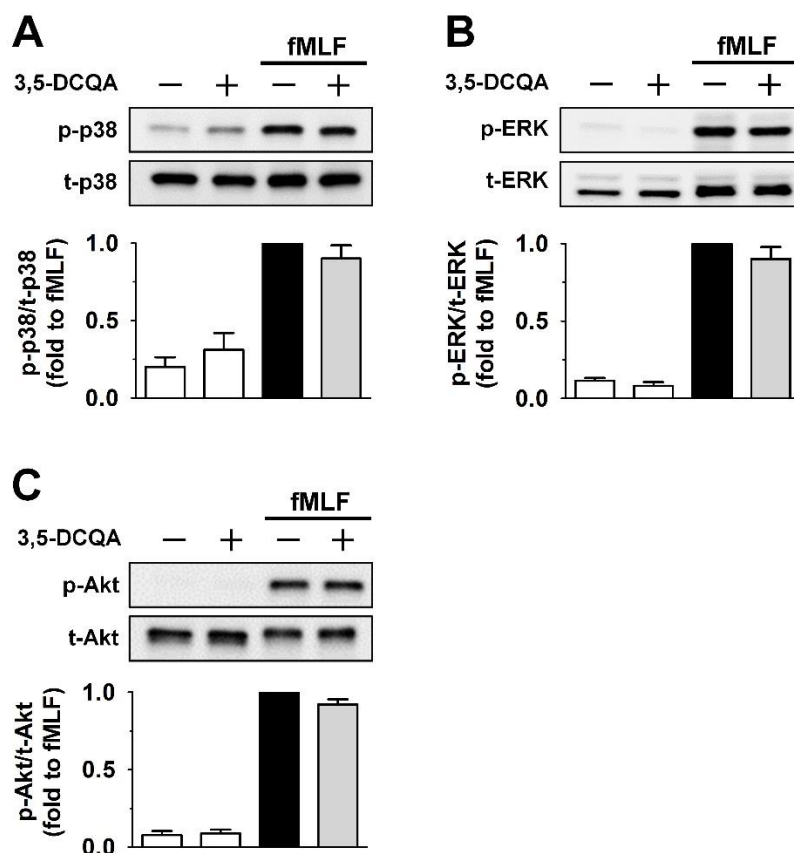


Fig. S9. 3,5-DCQA did not inhibited the phosphorylation of MAPKs and Akt in fMLF-activated human neutrophils. Human neutrophils were pre-incubated with dimethylsulfoxide (DMSO) or 3,5-DCQA (10 μ M) for 5 min before stimulation with or without fMLF (0.1 μ M) for another 0.5 min. All the Western blotting experiments were performed under the same condition. The targeted proteins were immunoblotted with its specific monoclonal antibody. (A) p38; (B) ERK; (C) Akt. Targeted bands were analyzed using a densitometer and normalized to the corresponding total protein or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The densitometric data were presented as mean $\pm$ S.E.M. (n = 3–4). Compared with fMLF group: # $p < 0.05$ and ## $p < 0.01$.

Supplementary information Figure S10

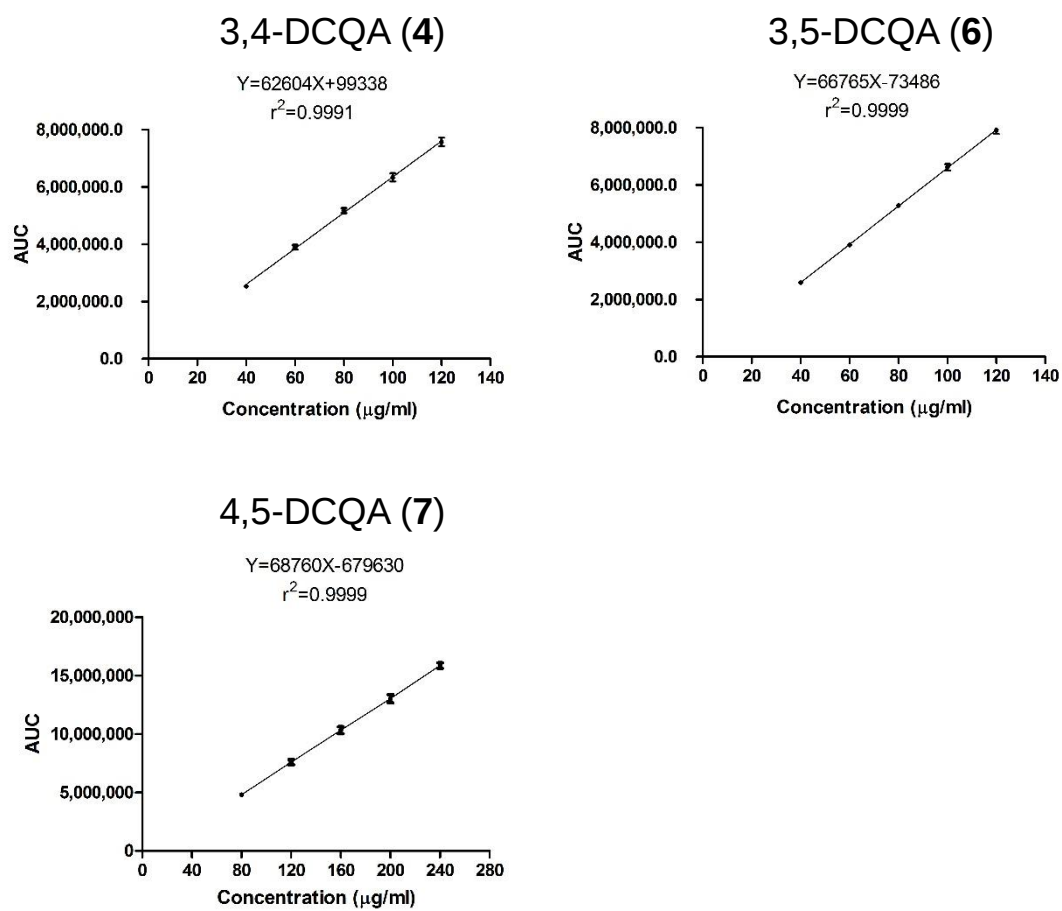


Fig. S10. Calibration curves of three chemical reference standards.

Supplementary information Figure S11

A

I. kaushue (IK)

KUD

NKUD



B

Batches	Sample (g)	Extracts (g)	Yield (%)	3,4-DCQA	3,5-DCQA	4,5-DCQA	HNE inhibition ^a
IKWE-1	20.0	7.33	36.65	3.70	4.78	9.09	11.37 ± 1.59
IKWE-2	21.1	7.96	37.74	3.34	4.45	8.58	10.40 ± 0.35
IKWE-3	20.8	8.41	40.43	4.25	4.87	8.89	9.73 ± 0.42
KUD-1	20.0	7.07	35.33	5.33	4.24	7.02	9.75 ± 1.09
NKUD-1	20.1	8.05	39.98	5.06	5.87	11.08	8.71 ± 0.15

Fig. S11. Quality control index of water extracts from different Kudingcha materials. (A) Three Kudingcha materials; (B) Index of QC. ^aConcentration necessary for 50 % inhibition at µg/ml. Data were presented as mean ± S.E.M. (n ≥ 3).

Supplementary information Figure S12

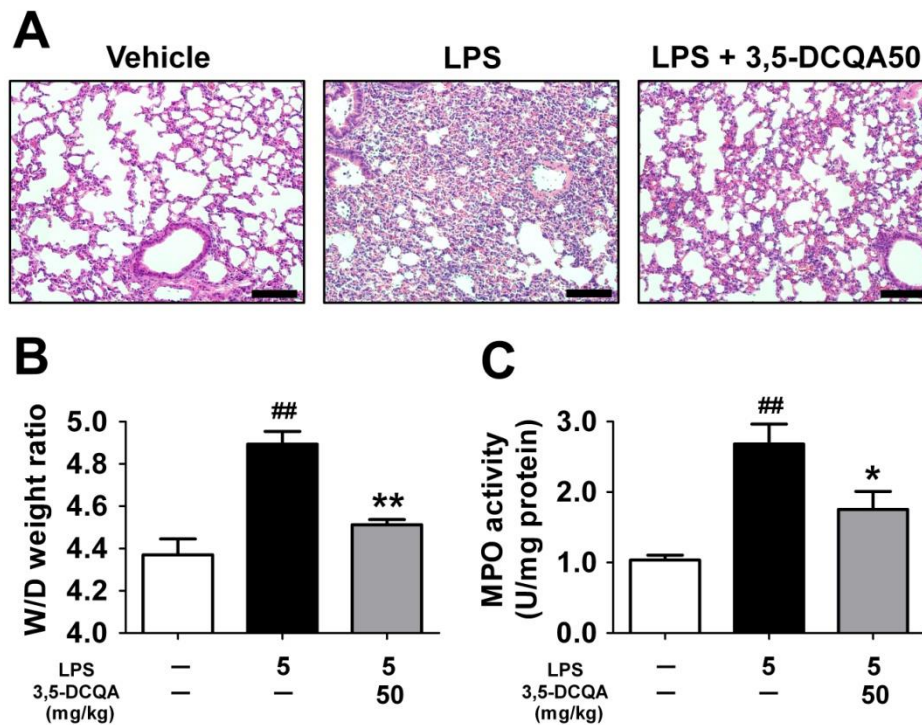
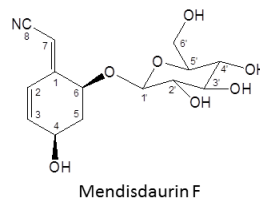
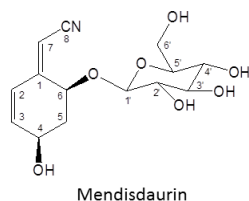


Fig. S12. The effects of post-treated 3,5-DCQA on LPS-induced ALI in mice. Mice received post-treatment of vehicle or 3,5-DCQA (50 mg/kg) intraperitoneally 1hour after intratracheal instillation of LPS. After 6 hours of LPS treatment, mice were anesthetized and their chests were opened. Whole lungs were obtained immediately. Left lobe was then dissected for histology or MPO activity. Right lobes were applied for W/D weight ratio. **(A)** Histological examination (scale bar = 50 μ M); **(B)** **(C)** Lung W/D weight ratio and MPO activity. Data were presented as mean $\pm$ S.E.M. (n = 3 for control; n = 6 for LPS or 3,5-DCQA groups). Compared with vehicle group: ^{##} $p < 0.01$; Compared with LPS group: ^{*} $p < 0.05$ and ^{**} $p < 0.01$.

Supplementary information Table S1

Table S1. ^1H and ^{13}C NMR data of menisdaurin and menisdaurin F



Position	Menisdaurine		Menisdaurine F	
	^1H	^{13}C	^1H	^{13}C
1		157.1, s		159.1, s
2	6.32, d, $J=10.0$ Hz	127.7, d	6.57, dd, $J=10.0, 2.0$ Hz	124.5, d
3	6.24, dd, $J=10.0, 3.2$ Hz	140.6, d	6.24, d, $J=10.0$ Hz	143.3, d
4	4.40, m	65.3, d	4.49, m	67.6, d
5	2.30, ddd, $J=13.2, 5.2, 4.4$ Hz 2.06, ddd, $J=13.2, 8.0, 6.2$ Hz	36.1, t	2.56, m 1.66, m	36.4, t
6	4.96, ddd, $J=8.0, 2.4, 1.2$ Hz	72.5, d	4.62, m	74.8, d
7	5.55, s	96.8, d	5.77, s	93.7, d
8		118.0, s		117.9, s
1'	4.58, d, $J=6.4$ Hz	101.5, d	4.48, d, $J=8.0$ Hz	102.6, d
2'	3.29~3.45, m	74.4, d	3.22~3.40, m	74.7, d
3'		77.9, d		78.0, d
4'		71.7, d		71.5, d
5'		78.1, d		78.2, d
6'	3.92, dd, $J=11.6, 2.0$ Hz 3.70, dd, $J=11.6, 6.0$ Hz	63.1, t	3.86, d, $J=11.6$ Hz 3.66, dd, $J=11.6, 5.2$ Hz	62.6, t

Supplementary information Table S2

Table S2. Effects of 3,5-DCQA on $O_2^{\bullet-}$ generation and elastase release in fMLF- or LTB4-induced human neutrophils

Inducers	IC ₅₀ (μ M)	
	$O_2^{\bullet-}$ generation	elastase release
fMLF	1.92 $\pm$ 0.54	12.02 $\pm$ 0.60
LTB4	—	2.90 $\pm$ 0.55

All data were presented as mean $\pm$ S.E.M. (n $\geq$ 3).

Supplementary information Table S3

Table S3. HNE inhibitory activities of caffeic acid analogs

Compounds	IC ₅₀ (μM)	Inh % ^a
1	> 30	-2.99 ± 1.35
2	> 30	-3.43 ± 4.85
3	> 30	-4.76 ± 3.36
4	> 30	9.91 ± 0.99
6	1.86 ± 0.06	90.53 ± 0.52
7	> 30	36.42 ± 2.25
CA	> 30	-4.61 ± 4.03
<i>p</i> -CA	> 30	-4.00 ± 3.42
3-HCA	> 30	-3.63 ± 3.91
FA	> 30	0.06 ± 4.79
RA	> 30	10.58 ± 4.03

^aHNE inhibition at 30 μM. CA, caffeic acid; *p*-CA, *p*-coumaric acid; 3-HCA, 3-hydroxycinnamic acid; FA, ferulic acid; RA, rosaminiric acid. All data were presented as mean ± S.E.M. (n ≥ 3). Data were presented as mean ± S.E.M. (n ≥ 3).

Supplementary information S1

General Experimental Procedures

Thin-layer chromatography (TLC) was used Merck 60 F254 silica gel aluminum backed plates. Flash column chromatography was performed using silica gel (Silicycle, 70-230 mesh or 230-400 mesh). The HPLC system was performed using Jasco PU-1580 intelligent HPLC pump, Jasco AS 1555-10 intelligent sampler, and Jasco UV-1575 UV-Vis detector. Semi-preparative (Develosil, C30-UG-5, 10 mm × 250 mm) or preparative column (Shiseido, CAPCELL PAK C18 AQ, 20 mm × 250 mm) were applied for compound purification. The MPLC-flash system was performed using Biotage[®] Isolera One with SNAP[®] KP-C18-HS 60 g cartridge column, and detection was conducted at 254 and 330 nm. The nuclear magnetic resonance (NMR) spectra using CD₃OD, DMSO-*d*₆ and C₅D₅N as the solvent were obtained on a Bruker AVANCE-400 MHz FT-NMR spectrometer. Low-resolution EI-MS were recorded on a Quattro GC/MS spectrometer having a direct inlet system, low-resolution and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer.

Bioactivity-guided fractionation

IKWE (5.50 g) was dissolved in ddH₂O and partitioned with ethyl acetate and *n*-butanol sequentially to generate the ethyl acetate (EA) (281.3 mg), *n*-BuOH (2.6 g), and H₂O (2.2 g) layers, respectively. The HNE activity assay was employed to monitor sub-fraction bioactivity. Ursolic acid (**9**) (26.6 mg) and β -sitosterol (**16**) (6.3 mg) were purified from the EA layer through repeated silica gel chromatography with the CHCl₃/MeOH (40/1, v/v) elution system.^{1,2} The *n*-BuOH layer (2.0 g) was divided into five fractions (Bu-1, 175.7 mg; Bu-2, 127.3 mg; Bu-3, 296.5 mg; Bu-4, 420.3 mg; Bu-5, 720.7 mg) using the gradient MPLC-Flash system (1500 mL H₂O/MeOH gradient elution from 100% H₂O to 100% MeOH). Menisdaurin F (**19**) (7.3 mg; rt, 44.8 min), menisdaurin D (**17**) (31.3 mg; rt, 51.2 min) and menisdaurin (**18**) (36.5 mg; rt, 56.1 min) were derived from fraction Bu-2 with 18% MeOH aqueous solution at 3 mL/min.³⁻⁶ Bu-4 was further divided into 3 fractions (Bu-4-1, 55.0 mg; Bu-4-2, 33.8 mg; Bu-4-3, 136.2 mg) with mobile phase consisting of 1% formic acid in 48% MeOH solution. 3,4-DCQA (**4**) (38.5 mg; rt, 72.0 min) and methyl 3,4-DCQA (**5**) (5.3 mg; rt, 68.8 min) were purified from Bu-4-1 with 1% formic acid in 40% MeOH solution at 3 mL/min.^{7,8} 3,5-DCQA (**6**) (26.0 mg; rt, 49.2 min) was obtained from Bu-4-2 with 1% formic acid in 44% MeOH solution at 3.5 mL/min.⁸ 4,5-DCQA (**7**) (55.3 mg; rt, 89.2 min) and methyl 4,5-DCQA (**8**) (11.8 mg; rt, 83.3 min) were

purified from Bu-4-3 with 1% formic acid in 43% MeOH solution at 3.0 mL/min.^{7,8}

Bu-5 was divided into two fractions (Bu-5-1, 480.6 mg; Bu-5-2, 135.2 mg) with 80% MeOH solution. Bu-5-1 was separated to give latifolioside H (**14**) (31.6mg; rt, 84.0 min) and latifolioside G (**15**) (85.0 mg; rt, 86.8 min) with 59% MeOH solution at 3 mL/min.⁹ Bu-5-2 was separated to provide kudinoside C (**12**) (27.0 mg; rt, 38.8 min), kudinoside A (**10**) (45.0 mg; 42.8 min), kudinoside D (**11**) (11.5 mg; 49.6 min) and kudinoside F (**13**) (20.6 mg; 51.7 min) with 75% MeOH solution at 3.5 mL/min.¹⁰⁻¹³ 3-CQA (**1**) (26.7 mg; rt, 9.1 min), 5-CQA (**3**) (57.3 mg; rt, 16.6 min) and 4-CQA (**2**) (33.1 mg; rt, 23.3 min) were purified directly with 25% MeOH mobile phase and 1% formic acid at 3.0 mL/min from IKWE solution (1.6 g in 20 mL of 25% MeOH solution).^{8,14,15} For acid hydrolysis, 30 mg of compounds **11**, **14** and **15** were heated in 2 mL of 1 M HCl (H₂O : *p*-dioxane = 1 : 1) solution at 95°C for 30 min. Reaction mixtures were partitioned with EA/H₂O three times. Aglycons were obtained from concentrated EA layers via HPLC purification: α -kudinlactone (**11-1**) (2.3 mg; rt, 7.6 min; 80% MeOH; 2.5 mL/min); kudinchagenin I (**11-2**) (1.5 mg; rt 16.1 min; 80% MeOH; 2.5 mL/min); siaresinolic acid (**14-1**) (2.7 mg; rt, 10.1 min; 90% MeOH; 2.5 mL/min); randialic acid B (**15-1**) (2.8 mg; rt, 22.3 min; 90% MeOH; 2.5 mL/min); sanguisorbigenin (**15-2**) (2.8 mg; rt, 25.4 min; 90% MeOH; 2.5 mL/min).^{9,11-13} Compound structures were determined based on 1D/2D NMR and physical properties,

in comparison with reference data.

Neochlorogenic acid (**1**)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -51.7° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm ($\log \epsilon$): 207 (3.92), 218 (3.98), 244 (3.83), 295 (3.91), 328 (4.02); IR ν_{max} (KBr) cm^{-1} : 3421, 1697, 1606, 1523, 1446, 983; Mp: 202-203 $^{\circ}$; ESIMS: m/z 353.1 $[\text{M}-\text{H}]^{+}$; ^1H NMR (CD_3OD , 400 MHz) δ : 7.58 (1H, d, $J = 16.0$ Hz, H-3'), 7.05 (1H, d, $J = 2.0$ Hz, H-5'), 6.94 (1H, dd, $J = 8.4, 2.0$ Hz, H-9'), 6.78 (1H, d, $J = 8.4$ Hz, H-8'), 6.31 (1H, d, $J = 16.0$ Hz, H-2'), 5.37 (1H, m, H-3), 4.17 (1H, m, H-5), 3.66 (1H, dd, $J = 8.4, 3.2$ Hz, H-4), 2.14~2.19 (3H, m, H-2a, H-6a, H-6b), 1.97 (1H, m, H-2b); ^{13}C NMR (CD_3OD , 100 MHz) δ : 178.3 (s, C-7), 169.0 (s, C-1'), 149.3 (s, C-7'), 146.8 (d, C-3'), 146.7 (s, C-6'), 127.9 (s, C-4'), 122.9 (d, C-9'), 116.5 (d, C-8'), 115.8 (d, C-2'), 115.1 (d, C-5'), 75.4 (s, C-1), 74.8 (d, C-4), 73.0 (d, C-3), 68.2 (d, C-5), 41.5 (t, C-6), 36.7 (t, C-2).

Cryptochlorogenic acid (**2**)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -82.0° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm ($\log \epsilon$): 207 (4.09), 218 (3.98), 244 (3.97), 295 (4.10), 328 (4.19); IR ν_{max} (KBr) cm^{-1} : 3414, 1695, 1606, 1520, 1447, 978; Mp: 205-206 $^{\circ}$; ESIMS: m/z 353.3 $[\text{M}-\text{H}]^{+}$; ^1H NMR (CD_3OD , 400 MHz) δ : 7.64 (1H, d, $J = 16.0$ Hz, H-3'), 7.07 (1H, d, $J = 1.6$ Hz, H-5'),

6.96 (1H, dd, $J = 8.0, 1.6$ Hz, H-9'), 6.79 (1H, d, $J = 8.0$ Hz, H-8'), 6.37 (1H, d, $J = 16.0$ Hz, H-2'), 4.81 (1H, dd, $J = 9.2, 2.4$ Hz, H-4), 4.32 (2H, m, H-3, H-5), 2.02~2.24 (4H, m, H-2a, H-2b, H-6a, H-6b). ^{13}C NMR (CD_3OD , 100 MHz) δ : 177.3 (s, C-7), 169.0 (s, C-1'), 149.4 (s, C-7'), 147.1 (d, C-3'), 146.7 (s, C-6'), 127.8 (s, C-4'), 123.0 (d, C-9'), 116.5 (d, C-8'), 115.2 (d, C-2'), 115.3 (d, C-5'), 79.2 (d, C-4), 76.6 (s, C-1), 69.6 (d, C-3), 65.5 (d, C-5), 42.6 (t, C-6), 38.4 (t, C-2).

Chlorogenic acid (**3**)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -60.3° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 206 (3.86), 218 (4.28), 244 (4.12), 297 (4.18), 328 (4.29); IR ν_{max} (KBr) cm^{-1} : 3409, 1697, 1604, 1521, 1447, 979; Mp: 210-202 $^\circ$; ESIMS: m/z 353.3 $[\text{M-H}]^+$; ^1H NMR (CD_3OD , 400 MHz) δ : 7.56 (1H, d, $J = 16.0$ Hz, H-3'), 7.06 (1H, s, H-5'), 6.95 (1H, d, $J = 8.0$ Hz, H-9'), 6.79 (1H, d, $J = 8.0$ Hz, H-8'), 6.26 (1H, d, $J = 16.0$ Hz, H-2'), 5.35 (1H, m, H-5), 4.19 (1H, m, H-3), 3.75 (1H, dd, $J = 8.4, 2.4$ Hz, H-4), 2.04~2.27 (4H, m, H-2a, H-2b, H-6a, H-6b); ^{13}C NMR (CD_3OD , 100 MHz) δ : 177.0 (s, C-7), 168.7 (s, C-1'), 149.3 (s, C-7'), 147.0 (d, C-3'), 146.5 (s, C-6'), 127.7 (s, C-4'), 123.0 (d, C-9'), 116.5 (d, C-8'), 115.2 (d, C-2'), 115.21 (d, C-5'), 76.1 (s, C-1), 73.5 (d, C-5), 71.8 (d, C-4), 71.3 (d, C-3), 38.8 (t, C-2), 38.1 (t, C-6).

3,4-Dicaffeoylquinic acid (4)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -220.0° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm ($\log \varepsilon$): 218 (4.56), 244 (4.40), 300 (4.53), 328 (4.62); IR ν_{max} (KBr) cm^{-1} : 3382, 1694, 1603, 1521, 1446, 979; Mp: 153-154 $^\circ$; ESIMS: m/z 515.2 $[\text{M}-\text{H}]^+$; ^1H NMR (CD_3OD , 400 MHz) δ : 7.58 (1H, d, $J = 16.0$ Hz, H-3'), 7.55 (1H, d, $J = 16.0$ Hz, H-3''), 7.04 (1H, d, $J = 1.2$ Hz, H-5'), 7.02 (1H, d, $J = 1.2$ Hz, H-5''), 6.92 (1H, dd, $J = 8.0, 1.2$ Hz, H-9'), 6.87 (1H, dd, $J = 8.0, 1.2$ Hz, H-9''), 6.77 (1H, d, $J = 8.0$ Hz, H-8'), 6.73 (1H, d, $J = 8.0$ Hz, H-8''), 6.29 (1H, d, $J = 16.0$ Hz, H-2''), 6.25 (1H, d, $J = 16.0$ Hz, H-2'), 5.65 (1H, m, H-3), 5.01 (1H, dd, $J = 8.8, 2.8$ Hz, H-4), 4.39 (1H, m, H-5), 2.14~2.39 (4H, m, H-2a, H-2b, H-6a, H-6b); ^{13}C NMR (CD_3OD , 100 MHz) δ : 178.0 (s, C-7), 168.6 (s, C-1'), 168.5 (s, C-1''), 149.3 (s, C-7'), 149.3 (s, C-7''), 147.3 (d, C-3'), 147.3 (d, C-3''), 146.6 (s, C-6'), 146.6 (s, C-6''), 127.7 (s, C-4''), 127.6 (s, C-4'), 123.2 (d, C-9''), 123.1 (s, C-9'), 116.5 (d, C-8''), 116.4 (s, C-8'), 115.2 (d, C-5'), 115.1 (s, C-5''), 115.0 (d, C-2'), 114.8 (d, C-2''), 76.4 (d, C-4), 75.2 (s, C-1), 70.1 (d, C-3), 65.8 (d, C-5), 41.8 (t, C-6), 36.9 (t, C-2).

Methyl 3,4-Dicaffeoylquinic acid (5)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -240.4° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm ($\log \varepsilon$): 207 (4.37), 218 (4.45), 244 (4.30), 296 (4.42), 328 (4.54); IR ν_{max} (KBr) cm^{-1} : 3392,

1691, 1604, 1521, 1446, 978; Mp: 159-160°; ESIMS: m/z 529.2 [M-H]⁺; ¹H NMR (CD₃OD, 400 MHz) δ : 7.56 (1H, d, J = 16.0 Hz, H-3'), 7.55 (1H, d, J = 16.0 Hz, H-3''), 7.03 (1H, d, J = 2.0 Hz, H-5'), 7.02 (1H, d, J = 2.0 Hz, H-5''), 6.91 (1H, dd, J = 8.4, 2.0 Hz, H-9'), 6.89 (1H, dd, J = 8.4, 2.0 Hz, H-9''), 6.76 (1H, d, J = 8.0 Hz, H-8'), 6.73 (1H, d, J = 8.0 Hz, H-8''), 6.27 (2H, d, J = 16.0 Hz, H-2', H-2''), 5.62 (1H, m, H-3), 5.03 (1H, dd, J = 8.4, 3.2 Hz, H-4), 4.31 (1H, m, H-5), 3.71 (s, -COOCH₃), 2.10~2.38 (4H, m, H-2a, H-2b, H-6a, H-6b); ¹³C NMR (CD₃OD, 100 MHz) δ : 176.1 (s, C-7), 168.5 (s, C-1'), 168.5 (s, C-1''), 149.6 (s, C-7'), 149.6 (s, C-7''), 147.4 (d, C-3'), 147.4 (d, C-3''), 146.8 (s, C-6'), 146.8 (s, C-6''), 127.8 (s, C-4'), 127.7 (s, C-4''), 123.2 (d, C-9'), 123.1 (s, C-9''), 116.5 (d, C-8'), 116.5 (s, C-8''), 115.2 (d, C-5'), 115.2 (s, C-5''), 115.1 (d, C-2'), 114.9 (d, C-2''), 75.5 (d, C-4), 75.2 (s, C-1), 69.8 (d, C-3), 66.0 (d, C-5), 52.9 (q, -COOCH₃), 41.3 (t, C-6), 36.8 (t, C-2).

3,5-Dicaffoylquinic acid (**6**)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -191.9° (c = 0.05, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 207 (4.30), 218 (4.39), 244 (4.24), 297 (4.37), 328 (4.50); IR ν_{max} (KBr) cm⁻¹: 3319, 1692, 1603, 1523, 1446, 977; Mp: 163-164°; ESIMS: m/z 515.2 [M-H]⁺; ¹H NMR (CD₃OD, 400 MHz) δ : 7.62 (1H, d, J = 16.0 Hz, H-3'), 7.58 (1H, d, J = 16.0 Hz, H-3''), 7.07 (2H, s, H-5', H-5''), 6.98 (1H, dd, J = 8.4, 2.0 Hz, H-9'), 6.96 (1H, dd, J =

8.4, 2.0 Hz, H-9"), 6.78 (2H, d, $J = 8.4$ Hz, H-8', H-8"), 6.36 (1H, d, $J = 16.0$ Hz, H-2"), 6.27 (1H, d, $J = 16.0$ Hz, H-2'), 5.43 (1H, m, H-3), 5.40 (1H, m, H-5), 3.98 (1H, dd, $J = 7.6, 3.2$ Hz, H-4), 2.13~2.34 (4H, m, H-2a, H-2b, H-6a, H-6b); ^{13}C NMR (CD₃OD, 100 MHz) δ : 177.5 (s, C-7), 168.9 (s, C-1'), 168.3 (s, C-1"), 149.6 (s, C-7'), 149.5 (s, C-7"), 147.3 (d, C-3'), 147.0 (d, C-3"), 146.8 (s, C-6'), 146.8 (s, C-6"), 127.9 (s, C-4'), 127.8 (s, C-4'), 123.1 (d, C-9'), 123.0 (s, C-9"), 116.4 (d, C-8'), 116.5 (s, C-8"), 115.6 (d, C-5'), 115.2 (s, C-5"), 115.1 (d, C-2'), 115.1 (d, C-2"), 74.7 (s, C-1), 72.5 (d, C-3), 72.1 (d, C-5), 70.6 (d, C-4), 37.7 (t, C-6), 36.0 (t, C-2).

4,5-Dicaffeoylquinic acid (7)

Light green powder; $[\alpha]^{22}_{\text{D}}$: -170.4° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 207 (4.37), 218 (4.43), 246 (4.28), 297 (4.40), 328 (4.51); IR ν_{max} (KBr) cm⁻¹: 3369, 1694, 1604, 1523, 1446, 980; Mp: 158-159°; ESIMS: m/z 515.2 [M-H]⁺; ^1H NMR (CD₃OD, 400 MHz) δ : 7.60 (1H, d, $J = 16.0$ Hz, H-3'), 7.52 (1H, d, $J = 16.0$ Hz, H-3"), 7.03 (1H, s, H-5'), 7.01 (1H, s, H-5"), 6.92 (1H, d, $J = 7.6$ Hz, H-9'), 6.90 (1H, d, $J = 7.6$ Hz, H-9"), 6.75 (2H, d, $J = 7.6$ Hz, H-8', H-8"), 6.29 (1H, d, $J = 16.0$ Hz, H-2"), 6.21 (1H, d, $J = 16.0$ Hz, H-2'), 5.63 (1H, m, H-5), 5.13 (1H, d, $J = 7.2$ Hz, H-4), 4.38 (1H, m, H-3), 2.03~2.27 (4H, m, H-2a, H-2b, H-6a, H-6b); ^{13}C NMR (CD₃OD, 100 MHz) δ : 176.8 (s, C-7), 168.5 (s, C-1'), 168.2 (s, C-1"), 149.6 (s, C-7'),

149.6 (s, C-7''), 147.7 (d, C-3'), 147.6 (d, C-3''), 146.7 (s, C-6'), 146.7 (s, C-6''), 127.6 (s, C-4'), 127.6 (s, C-4''), 123.2 (d, C-9'), 123.2 (s, C-9''), 116.4 (d, C-8'), 116.4 (s, C-8''), 115.1 (d, C-5'), 115.1 (s, C-5''), 114.7 (d, C-2'), 114.6 (d, C-2''), 76.0 (d, C-4), 75.6 (s, C-1), 69.3 (d, C-3), 69.0 (d, C-5), 39.3 (t, C-6), 38.3 (t, C-2).

Methyl 4,5-Dicaffeoylquinic acid (**8**)

Light green powder; $[\alpha]_D^{22}$: -162.7° ($c = 0.05$, MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 206 (4.32), 218 (4.41), 245 (4.27), 298 (4.39), 329 (4.52); IR $\nu_{\max}$ (KBr) cm^{-1} : 3424, 1693, 1604, 1522, 1445, 982; Mp: 156-157°; ESIMS: m/z 529.3 $[\text{M-H}]^+$; ^1H NMR (CD_3OD , 400 MHz) δ : 7.61 (1H, d, $J = 16.0$ Hz, H-3'), 7.51 (1H, d, $J = 16.0$ Hz, H-3''), 7.04 (1H, d, $J = 2.0$ Hz, H-5'), 7.01 (1H, d, $J = 2.0$ Hz, H-5''), 6.94 (1H, dd, $J = 8.0, 2.0$ Hz, H-9'), 6.92 (1H, dd, $J = 8.0, 2.0$ Hz, H-9''), 6.76 (2H, d, $J = 8.0$ Hz, H-8', H-8''), 6.30 (1H, d, $J = 16.0$ Hz, H-2''), 6.18 (1H, d, $J = 16.0$ Hz, H-2'), 5.53 (1H, m, H-5), 5.11 (1H, dd, $J = 8.0, 2.8$ Hz, H-4), 4.35 (1H, m, H-3), 2.34 (3H, s, $-\text{COOCH}_3$), 2.07~2.34 (4H, m, H-2a, H-2b, H-6a, H-6b); ^{13}C NMR (CD_3OD , 100 MHz) δ : 175.2 (s, C-7), 168.5 (s, C-1'), 167.9 (s, C-1''), 149.8 (s, C-7'), 149.7 (s, C-7''), 147.7 (d, C-3'), 147.7 (d, C-3''), 146.8 (s, C-6'), 146.8 (s, C-6''), 127.7 (s, C-4'), 127.5 (s, C-4''), 123.2 (d, C-9'), 123.1 (s, C-9''), 116.5 (d, C-8'), 116.5 (s, C-8''), 115.1 (d, C-5'), 115.1 (s, C-5''), 114.7 (d, C-2'), 114.5 (d, C-2''), 75.7 (d, C-4), 74.7 (s, C-1), 69.1 (d, C-3),

68.5 (d, C-5), 53.1 (q, -COOCH₃), 38.4 (t, C-2), 38.4 (t, C-6).

Ursolic acid (**9**)

White powder; $[\alpha]_D^{22}$: 87.4° (*c* = 0.05, MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 207 (3.59); IR $\nu_{\max}$ (KBr) cm⁻¹: 3421, 2927, 2856, 1691, 1459, 1387, 1091; Mp: 276-277°; *R_f* = 0.37 (CHCl₃ : MeOH = 40 : 1, v/v); ESIMS: *m/z* 455.4 [M-H]⁺; ¹H NMR (C₅D₅N, 400 MHz) δ : 5.50 (1H, br s, H-12), 3.46 (1H, dd, *J* = 9.6, 6.0 Hz, H-3), 2.64 (1H, d, *J* = 11.2 Hz, H-18), 1.25 (3H, s, H-23), 1.24 (3H, s, H-27), 1.06 (3H, s, H-26), 1.03 (3H, s, H-24), 1.02 (3H, d, *J* = 6.4 Hz, H-29), 0.97 (3H, d, *J* = 5.6 Hz, H-30); ¹³C NMR (C₅D₅N, 100 MHz) δ : 179.9 (s, C-28), 139.3 (s, C-13), 125.6 (d, C-12), 78.1 (d, C-3), 55.8 (d, C-5), 53.5 (d, C-18), 48.0 (d, C-9), 48.0 (s, C-17), 42.5 (s, C-14), 40.0 (s, C-8), 39.5 (d, C-20), 39.4 (s, C-4), 39.4 (d, C-19), 39.1 (t, C-1), 37.4 (t, C-22), 37.3 (s, C-10), 33.6 (t, C-7), 31.1 (t, C-21), 28.8 (q, C-23), 28.7 (t, C-15), 28.1 (t, C-2), 24.9 (t, C-16), 23.9 (q, C-27), 23.6 (t, C-11), 21.4 (q, C-30), 18.8 (t, C-6), 17.5 (q, C-29), 17.4 (q, C-26), 16.6 (q, C-24), 15.7 (q, C-25).

Kudinoside A (**10**)

White powder; $[\alpha]_D^{23}$: -126.6° (*c* = 0.05, MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 207 (4.09), 277 (3.85), 255 (3.69); IR $\nu_{\max}$ (KBr) cm⁻¹: 3398, 2942, 2875, 1732, 1641,

1451, 1377, 1075, 1055; Mp: 195-196°; ESIMS: m/z 949.5 $[M+Na]^+$; 1H NMR (C_5D_5N , 400 MHz) δ : 6.34 (1H, br s, Rha H-1"), 5.92 (1H, br s, H-12), 5.11 (1H, d, J = 8.0 Hz, Glc H-1"), 4.87 (1H, d, J = 5.6 Hz, Ara H-1'), 3.31 (1H, dd, J = 11.6, 4.0 Hz, H-3), 1.66 (3H, s, H-29), 1.64 (3H, d, J = 4.4 Hz, Rha H-6"), 1.63 (3H, s, H-27), 1.51 (3H, s, H-30), 1.23 (3H, s, H-23), 1.14 (3H, s, H-24), 0.91 (3H, s, H-26), 0.88 (3H, s, H-25); ^{13}C NMR (C_5D_5N , 100 MHz) δ : 175.5 (s, C-28), 146.4 (s, C-13), 137.5 (s, C-18), 104.8 (d, Ara C-1'), 104.7 (d, Glc C-1"), 101.9 (d, Rha C-1"), 88.3 (d, C-3), 85.6 (s, C-20), 82.2 (d, Ara C-3'), 78.6 (d, Glc C-5"), 78.2 (d, Glc C-3"), 75.0 (d, Glc C-2"), 74.7 (d, Ara C-2'), 74.3 (s, C-19), 73.9 (d, Rha C-4"), 72.5 (d, Rha C-3"), 72.4 (d, Rha C-2"), 71.4 (d, Glc C-4"), 70.0 (d, Rha C-5"), 68.2 (d, Ara C-4'), 66.0 (d, C-12), 64.9 (t, Ara C-5'), 62.5 (t, Glc C-6"), 56.2 (d, C-5), 44.9 (d, C-9), 44.1 (s, C-17), 43.9 (s, C-14), 41.7 (s, C-8), 39.6 (s, C-4), 39.2 (t, C-1), 37.0 (s, C-10), 35.5 (t, C-7), 32.8 (t, C-22), 28.8 (t, C-2), 28.8 (t, C-15), 28.4 (t, C-21), 28.0 (q, C-23), 26.7 (t, C-16), 26.2 (t, C-11), 25.2 (q, C-29), 23.5 (q, C-27), 19.5 (q, C-30), 18.6 (q, Rha C-6"), 18.5 (t, C-6), 18.2 (q, C-26), 16.9 (q, C-24), 16.7 (q, C-25).

Kudinoside D (**11**)

White powder; $[\alpha]^{23}_D$: -79.0° (c = 0.05, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 220 (3.77), 259 (4.26); IR ν_{max} (KBr) cm^{-1} : 3431, 2940, 1739, 1646, 1454, 1395, 1073; Mp:

201-202°; ESIMS: m/z 907.6 $[M-H]^+$; 1H NMR (C_5D_5N , 400 MHz) δ : 7.52 (1H, dd, J = 10.4, 2.0 Hz, H-11), 6.19 (1H, s, Rha H-1''), 5.78 (1H, d, J = 10.4, H-12), 5.13 (1H, d, J = 7.6, Glc H-1'''), 4.89 (1H, d, J = 5.6, Ara H-1'), 3.31 (1H, dd, J = 11.6, 4.0, H-3), 1.70 (3H, s, H-29), 1.66 (3H, d, J = 6.0, Rha H-1''), 1.54 (3H, s, H-30), 1.22 (3H, s, H-23), 1.11 (3H, s, H-24), 1.06 (3H, s, H-27), 0.89 (3H, s, H-26), 0.84 (3H, s, H-25); ^{13}C NMR (C_5D_5N , 100 MHz) δ : 175.2 (s, C-28), 140.7 (s, C-13), 135.5 (s, C-18), 128.4 (d, C-12), 127.2 (d, C-11), 104.9 (d, Ara C-1'), 104.8 (d, Glc C-1'''), 102.0 (d, Rha C-1''), 88.2 (d, C-3), 85.9 (s, C-20), 82.3 (d, Ara C-3'), 78.6 (d, Glc C-5'''), 78.3 (d, Glc C-3'''), 75.0 (d, Glc C-2'''), 74.8 (d, Ara C-2'), 74.1 (s, C-19), 73.9 (d, Rha C-4''), 72.5 (d, Rha C-2''), 72.5 (d, Rha C-3''), 71.5 (d, Glc C-4'''), 70.1 (d, Rha C-5''), 68.3 (d, Ara C-4'), 64.9 (t, Ara C-5'), 62.5 (t, Glc C-6'''), 55.3 (d, C-5), 54.5 (d, C-9), 43.8 (s, C-17), 42.2 (s, C-8), 42.2 (s, C-14), 39.7 (s, C-4), 38.4 (t, C-1), 36.6 (s, C-10), 32.9 (t, C-7), 32.9 (t, C-22), 28.6 (t, C-21), 27.7 (q, C-23), 26.5 (t, C-2), 26.3 (t, C-16), 25.9 (t, C-15), 23.7 (q, C-29), 19.5 (q, C-30), 18.7 (q, C-27), 18.6 (q, Rha C-6''), 18.5 (q, C-25), 18.4 (t, C-6), 16.5 (q, C-26), 16.4 (q, C-24).

Kudinoside C (**12**)

White powder; $[\alpha]^{23}_D$: -86.5° (c = 0.05, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 207 (3.88), 225 (3.67); IR ν_{max} (KBr) cm^{-1} : 3402, 2943, 2874, 1730, 1642, 1452, 1379,

1080; Mp: 193-194°; ESIMS: m/z 1087.7 $[M-H]^+$; 1H NMR (C_5D_5N , 400 MHz) δ : 6.40 (1H, br s, Rha H-1"), 5.92 (1H, br s, H-12), 5.27 (1H, d, $J = 7.6$ Hz, Glc H-1""), 5.16 (1H, d, $J = 7.6$ Hz, Glc H-1""), 4.76 (1H, m, Ara H-1'), 3.29 (1H, dd, $J = 11.6, 4.0$ Hz, H-3), 1.72 (3H, d, $J = 6.0$ Hz, Rha H-6"), 1.66 (3H, s, H-29), 1.62 (3H, s, H-27), 1.51 (3H, s, H-30), 1.25 (3H, s, H-23), 1.18 (3H, s, H-24), 0.90 (3H, s, H-26), 0.87 (3H, s, H-25); ^{13}C NMR (C_5D_5N , 100 MHz) δ : 175.4 (s, C-28), 146.3 (s, C-13), 137.4 (s, C-18), 106.4 (d, Glc C-1""), 105.1 (d, Ara C-1'), 103.1 (d, Glc C-1""), 100.9 (d, Rha C-1"), 88.4 (d, C-3), 85.7 (s, C-20), 84.8 (d, Glc C-2""), 83.0 (d, Ara C-3'), 78.8 (d, Glc-5""), 78.4 (d, Glc C-5""), 78.3 (d, Glc C-3""), 78.2 (d, Glc-3""), 76.1 (d, Glc C-2""), 74.3 (d, Ara C-2'), 74.2 (s, C-19), 73.9 (d, Rha C-4"), 72.5 (d, Rha C-3"), 72.4 (d, Rha C-2"), 70.8 (d, Glc C-4""), 70.4 (d, Glc C-4""), 69.8 (d, Rha C-5"), 69.4 (d, Ara C-4'), 66.0 (d, C-12), 65.8 (t, Ara C-5'), 62.3 (t, Glc C-6""), 61.8 (t, Glc-6""), 56.3 (d, C-5), 44.8 (d, C-9), 44.1 (s, C-17), 43.9 (s, C-14), 41.7 (s, C-8), 39.5 (s, C-4), 39.2 (t, C-1), 37.0 (s, C-10), 35.5 (t, C-7), 32.8 (t, C-22), 28.8 (t, C-2), 28.8 (t, C-15), 28.3 (t, C-21), 28.0 (q, C-23), 26.7 (t, C-16), 26.2 (t, C-11), 25.2 (q, C-29), 23.4 (q, C-27), 19.5 (q, C-30), 18.5 (t, C-6), 18.3 (q, Rha C-6"), 18.2 (q, C-26), 17.1 (q, C-24), 16.8 (q, C-25).

Kudinoside F (**13**)

White powder; $[\alpha]_D^{23}$: -104.4° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 214

(3.89), 227 (3.81); IR $\nu_{\max}$ (KBr) cm^{-1} : 3411, 2939, 1729, 1635, 1456, 1366, 1076, 1024; Mp: 261-262°; ESIMS: m/z 925.6 $[\text{M-H}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 6.22 (1H, br s, Rha H-1"), 5.13 (1H, d, $J = 7.6$ Hz, Glc H-1""), 4.95 (1H, d, $J = 7.6$ Hz, H-11), 4.86 (1H, d, $J = 5.6$ Hz, Ara H-1'), 3.28 (1H, dd, $J = 11.6, 4.0$ Hz, H-3), 1.81 (3H, s, H-29), 1.65 (3H, d, $J = 6.0$ Hz, Rha H-6"), 1.55 (3H, s, H-30), 1.27 (3H, s, H-27), 1.21 (3H, s, H-23), 1.10 (3H, s, H-24), 0.89 (3H, s, H-26), 0.82 (3H, s, H-25); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 175.4 (s, C-28), 143.5 (s, C-13), 135.6 (s, C-18), 104.9 (d, Ara C-1'), 104.8 (d, Glc C-1""), 101.9 (d, Rha C-1"), 88.0 (d, C-3), 85.3 (s, C-20), 82.4 (d, Ara C-3'), 78.6 (d, Glc C-5""), 78.2 (d, Glc C-3""), 74.9 (d, Glc C-2""), 74.6 (d, Ara C-2'), 73.9 (d, Rha C-4"), 73.2 (s, C-19), 72.5 (d, Rha C-2"), 72.5 (d, Rha C-3"), 71.5 (d, Glc C-4""), 71.4 (d, C-11), 70.0 (d, Rha C-5"), 68.3 (d, Ara C-4'), 65.0 (t, Ara C-5'), 62.5 (t, Glc C-6""), 55.9 (d, C-5), 50.1 (d, C-9), 46.2 (s, C-17), 45.5 (s, C-14), 42.7 (s, C-8), 39.5 (s, C-4), 39.0 (t, C-1), 37.1 (s, C-10), 35.1 (t, C-7), 33.5 (t, C-12), 32.4 (t, C-22), 29.2 (t, C-15), 29.2 (t, C-21), 27.9 (q, C-23), 26.9 (t, C-2), 26.6 (t, C-16), 26.5 (q, C-29), 21.5 (q, C-27), 20.2 (q, C-30), 18.6 (q, Rha C-6"), 18.4 (t, C-6), 17.1 (q, C-26), 16.9 (q, C-25), 16.8 (q, C-24).

Latifolioside H (**14**)

White powder; $[\alpha]_{\text{D}}^{23}$: -80.0° ($c = 0.05$, MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 207

(3.54); IR ν_{max} (KBr) cm^{-1} : 3414, 2937, 1739, 1635, 1454, 1386, 1073, 1030; Mp: 236-237°; ESIMS: m/z 1219.8 $[\text{M-H}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 6.72 (1H, br s, Rha H-1'''), 6.27 (1H, d, $J = 8.0$ Hz, Glc H-1'''), 6.23 (1H, br s, Rha H-1''), 5.55 (1H, m, H-12), 5.12 (1H, d, $J = 8.0$ Hz, Glc H-1'''), 4.87 (1H, m, Ara H-1'), 3.27 (1H, d, $J = 11.2$ Hz, H-3), 1.80 (3H, d, $J = 6.0$ Hz, Rha H-6''), 1.65 (3H, m, Rha H-6'''), 1.65 (3H, s, H-27), 1.16 (3H, s, H-23), 1.12 (3H, s, H-29), 1.10 (3H, s, H-24), 1.10 (3H, s, H-26), 0.87 (3H, s, H-25), 0.85 (3H, s, H-30); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 177.1 (s, C-28), 144.4 (s, C-13), 123.7 (d, C-12), 104.8 (d, Ara C-1'), 104.6 (d, Glc C-1'''), 101.9 (d, Rha C-1''), 101.4 (d, Rha C-1'''), 95.0 (d, Glc C-1'''), 88.2 (d, C-3), 82.3 (d, Ara C-3'), 81.1 (d, C-19), 79.8 (d, Glc C-3'''), 78.9 (d, Glc C-5'''), 78.5 (d, Glc-5'''), 78.2 (d, Glc C-3'''), 75.3 (d, Glc C-2'''), 74.9 (d, Glc C-2'''), 74.7 (d, Ara C-2'), 73.9 (d, Rha C-4''), 73.9 (d, Rha C-4'''), 72.5 (d, Rha C-3''), 72.4 (d, Rha C-4''), 72.4 (d, Rha C-3'''), 72.2 (d, Rha C-2'''), 71.3 (d, Glc C-4'''), 71.3 (d, Glc-4'''), 70.0 (d, Rha C-5''), 69.7 (d, Rha C-5'''), 68.3 (d, Ara C-4'), 64.9 (t, Ara C-5'), 62.4 (t, Glc C-6'''), 61.9 (d, Glc C-6'''), 56.1 (d, C-5), 48.2 (d, C-9), 46.6 (s, C-17), 44.9 (d, C-18), 42.3 (s, C-14), 40.1 (s, C-8), 39.5 (s, C-4), 39.0 (t, C-1), 37.1 (s, C-10), 35.4 (s, C-20), 33.3 (t, C-22), 32.6 (t, C-7), 29.5 (t, C-15), 28.9 (t, C-21), 28.7 (q, C-29), 28.0 (q, C-23), 27.8 (t, C-16), 26.6 (t, C-2), 24.6 (q, C-30), 24.5 (q, C-27), 24.1 (t, C-11), 18.6 (t, C-6), 18.6 (q, Rha C-6'''), 18.5 (q, Rha C-6''), 17.5 (q, C-26), 16.9 (q, C-24), 15.6 (q, C-25).

Latifolioside G (15)

White powder; $[\alpha]_D^{23}$: -113.7° ($c = 0.05$, MeOH); UV $\lambda_{\max}$ (MeOH) nm ($\log \varepsilon$): 206 (3.49); IR $\nu_{\max}$ (KBr) cm^{-1} : 3414, 2932, 1738, 1638, 1456, 1384, 1075, 1028; Mp: 203-204 $^\circ$; ESIMS: m/z 1219.8 $[\text{M}-\text{H}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 6.74 (1H, br s, Rha H-1'''), 6.19 (1H, br s, Rha H-1''), 6.18 (1H, d, $J = 8.4$ Hz, Glc H-1'''), 5.58 (1H, m, H-12), 5.11 (1H, d, $J = 7.6$ Hz, Glc H-1'''), 4.84 (1H, m, Ara H-1'), 3.25 (1H, dd, $J = 11.2, 2.8$ Hz, H-3), 1.78 (3H, d, $J = 6.0$ Hz, Rha H-6''), 1.72 (3H, s, H-27), 1.63 (3H, d, $J = 6.0$ Hz, Rha H-6'''), 1.42 (3H, s, H-29), 1.13 (3H, s, H-23), 1.13 (3H, s, H-26), 1.08 (3H, s, H-25), 1.08 (3H, s, H-30), 0.88 (3H, s, H-24); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 176.8 (s, C-28), 139.2 (s, C-13), 128.2 (d, C-12), 104.7 (d, Ara C-1'), 104.5 (d, Glc C-1'''), 101.8 (d, Rha C-1''), 101.3 (d, Rha C-1'''), 94.8 (d, Glc C-1'''), 88.2 (d, C-3), 82.2 (d, Ara C-3'), 80.0 (d, Glc C-3'''), 78.9 (d, Glc C-5'''), 78.4 (d, Glc-5''), 78.1 (d, Glc C-3''), 75.1 (d, Glc C-2'''), 74.8 (d, Glc C-2''), 74.6 (d, Ara C-2'), 73.8 (d, Rha C-4''), 73.7 (d, Rha C-4'''), 72.6 (d, Rha C-3''), 72.5 (d, Rha C-4''), 72.4 (d, Rha C-3'''), 72.2 (d, Rha C-2'''), 71.4 (d, Glc C-4'''), 71.3 (d, Glc-4''), 69.9 (d, Rha C-5''), 69.6 (d, Rha C-5'''), 68.2 (d, Ara C-4'), 64.8 (t, Ara C-5'), 62.4 (t, Glc C-6''), 62.2 (d, Glc C-6'''), 56.1 (d, C-5), 54.6 (d, C-18), 48.6 (s, C-17), 47.6 (d, C-9), 42.1 (s, C-14), 41.8 (d, C-20), 40.4 (s, C-8), 39.5 (s, C-4), 39.1 (t, C-1), 37.4 (t, C-22), 36.9 (s, C-10),

33.6 (t, C-7), 29.6 (t, C-15), 28.0 (q, C-23), 27.0 (q, C-29), 26.6 (t, C-16), 26.6 (t, C-21), 26.0 (t, C-2), 24.3 (q, C-27), 24.0 (t, C-11), 18.6 (t, C-6), 18.6 (q, Rha C-6'''), 18.5 (q, Rha C-6''), 17.3 (q, C-26), 16.9 (q, C-30), 16.6 (q, C-24), 15.7 (q, C-25).

β -sitosterol (**16**)

White powder; $[\alpha]_D^{23}$: -86.5° ($c = 0.05$, CHCl_3); UV λ_{max} (MeOH) nm (log ϵ): 245 (2.84); IR ν_{max} (KBr) cm^{-1} : 3427, 2936, 2868, 1463, 1380, 1051, 1023; Mp: 141-142°; $R_f = 0.55$ (CHCl_3 : MeOH = 40 : 1, v/v); ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 5.35 (1H, m, H-6), 3.53 (1H, m, H-3), 1.01 (3H, s, H-29), 0.92 (3H, d, $J = 6.4$ Hz, H-19), 0.85 (3H, t, $J = 7.2$ Hz, H-24), 0.84 (3H, d, $J = 7.2$ Hz, H-26), 0.81 (3H, d, $J = 7.2$ Hz, H-27), 0.69 (3H, s, H-28). ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 140.8 (s, C-5), 121.7 (d, C-6), 71.8 (d, C-3), 56.8 (d, C-14), 56.0 (d, C-17), 50.1 (d, C-9), 45.8 (d, C-22), 42.3 (t, C-4), 42.3 (d, C-13), 39.8 (t, C-12), 37.2 (t, C-1), 36.5 (s, C-10), 36.1 (d, C-18), 33.9 (t, C-20), 31.9 (t, C-7), 31.9 (d, C-8), 31.7 (t, C-2), 29.1 (d, C-25), 28.0 (t, C-16), 26.1 (t, C-21), 24.3 (t, C-15), 23.1 (t, C-23), 21.1 (t, C-11), 19.8 (q, C-19), 19.4 (q, C-26), 19.0 (q, C-27), 18.8 (q, C-28), 12.0 (q, C-24), 11.9 (q, C-29).

Menisdaurin D (**17**)

Colorless plates; $[\alpha]_D^{23}$: -141.0° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 214

(4.01); IR $\nu_{\max}$ (KBr) cm^{-1} : 3340, 2950, 2914, 2218, 1641, 1118, 1080, 1064, 1044; Mp: 165-166°; ESIMS: m/z 338.2 $[\text{M}+\text{Na}]^+$; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 5.50 (1H, s, H-7), 4.48 (1H, dd, $J = 8.4, 4.0$ Hz, H-6), 4.31 (1H, d, $J = 7.2$, H-1'), 3.73 (1H, m, H-4), 3.65 (1H, d, $J = 11.2$ Hz, H-6'a), 3.43 (1H, dd, $J = 11.2, 5.6$ Hz, H-6'b), 3.02~3.18 (4H, m, H-2'~H-5'), 2.48 (1H, m, H-2a), 2.11 (2H, m, H-2b, H5a), 1.77 (1H, m, H-3a), 1.60 (1H, m, H-5b), 1.41 (1H, m, H-3b). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 165.7 (s, C-1), 117.8 (s, C-8), 101.5 (d, C-1'), 93.5 (d, C-7), 78.0 (d, C-5'), 77.8 (d, C-3'), 75.0 (d, C-6), 74.0 (d, C-2'), 71.1 (d, C-4'), 66.3 (d, C-4), 62.2 (t, C-6'), 40.7 (t, C-5), 36.3 (t, C-3), 30.2 (t, C-2).

Menisdaurin (**18**)

Colorless plate; $[\alpha]^{23}_{\text{D}}$: -204.2° ($c = 0.05$, MeOH); UV $\lambda_{\max}$ (MeOH) nm ($\log \varepsilon$): 206 (3.71), 258 (4.39); IR $\nu_{\max}$ (KBr) cm^{-1} : 3343, 2914, 2211, 1648, 1627, 1074, 1059, 1020; Mp: 173-174°; ESIMS: m/z 336.3 $[\text{M}+\text{Na}]^+$; ^1H NMR (CD_3OD , 400 MHz) δ : 6.32 (1H, d, $J = 10.0$ Hz, H-2), 6.24 (1H, dd, $J = 10.0, 3.2$ Hz, H-3), 5.58 (1H, s, H-7), 4.96 (1H, ddd, $J = 8.0, 2.4, 1.2$ Hz, H-6), 4.58 (1H, d, $J = 6.4$ Hz, H-1'), 4.40 (1H, m, H-4), 3.92 (1H, dd, $J = 11.6, 2.0$ Hz, H-6'a), 3.70 (1H, dd, $J = 11.6, 6.0$ Hz, H-6'b), 3.29~3.45 (4H, m, H-2'~H-5'), 2.30 (1H, ddd, $J = 13.2, 5.2, 4.4$ Hz, H-5a), 2.06 (1H, ddd, $J = 13.2, 8.0, 6.2$ Hz, H-5b). ^{13}C NMR (CD_3OD , 100 MHz) δ : 157.1 (s, C-1),

140.6 (d, C-3), 127.7 (d, C-2), 118.0 (s, C-8), 101.5 (d, C-1'), 96.8 (d, C-7), 78.1 (d, C-5'), 77.9 (d, C-3'), 74.4 (d, C-2'), 72.4 (d, C-6), 71.7 (d, C-4'), 65.3 (d, C-4), 63.1 (t, C-6'), 36.1 (t, C-5).

α -Kudinlactone (**11-1**)

White powder; $[\alpha]_D^{23}$: 87.4° ($c = 0.05$, MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 220 (3.63), 259 (4.11); IR $\nu_{\max}$ (KBr) cm^{-1} : 3450, 2938, 2868, 1721, 1622, 1454, 1383, 1073, 1047; Mp: 231-232°; ESIMS: m/z 491.4 $[\text{M}+\text{Na}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 7.55 (1H, dd, $J = 10.4, 2.8$ Hz, H-11), 5.86 (1H, d, $J = 10.4$, H-12), 3.47 (1H, dd, $J = 10.4, 5.2$, H-3), 1.70 (3H, s, H-29), 1.54 (3H, s, H-30), 1.24 (3H, s, H-23), 1.05 (3H, s, H-27), 1.03 (3H, s, H-24), 0.96 (3H, s, H-25), 0.89 (3H, s, H-26); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 175.2 (s, C-28), 140.8 (s, C-13), 135.0 (s, C-18), 128.5 (d, C-12), 127.1 (d, C-11), 85.9 (s, C-20), 80.0 (d, C-3), 74.1 (s, C-19), 55.1 (d, C-5), 54.6 (d, C-9), 43.8 (s, C-17), 42.2 (s, C-8), 42.2 (s, C-14), 39.5 (s, C-4), 38.4 (t, C-1), 37.0 (s, C-10), 33.0 (t, C-7), 32.9 (t, C-22), 28.5 (t, C-21), 28.4 (q, C-23), 27.9 (t, C-2), 26.3 (t, C-16), 25.9 (t, C-15), 23.7 (q, C-29), 19.5 (q, C-30), 18.7 (q, C-27), 18.6 (t, C-6), 18.4 (q, C-25), 16.5 (q, C-26), 15.9 (q, C-24).

Kudinchenin I (**11-2**)

White powder; $[\alpha]^{23}_{\text{D}}$: 106.4° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 208 (4.45), 293 (4.64); IR ν_{max} (KBr) cm^{-1} : 3434, 2937, 2869, 1746, 1627, 1457, 1078, 1048; Mp: 244-245°; ESIMS: m/z 473.4 $[\text{M}+\text{Na}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 6.94 (1H, dd, $J = 10.4, 2.4$ Hz, H-11), 5.89 (1H, d, $J = 10.4$, H-12), 5.44 (1H, s, H-29a), 5.38 (1H, s, H-29b), 3.48 (1H, dd, $J = 9.2, 6.8$, H-3), 1.55 (3H, s, H-30), 1.24 (3H, s, H-23), 1.10 (3H, s, H-27), 1.02 (3H, s, H-24), 0.87 (3H, s, H-25), 0.76 (3H, s, H-26). ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 175.0 (s, C-28), 146.2 (s, C19), 141.3 (s, C-13), 131.5 (d, C-12), 129.2 (s, C-18), 125.8 (d, C-11), 111.7 (t, C-29), 83.2 (s, C-20), 78 (d, C-3), 55.1 (d, C-5), 54.1 (d, C-9), 45.3 (s, C-17), 42.8 (s, C-8), 41.9 (s, C-14), 39.5 (s, C-4), 38.4 (t, C-1), 36.9 (s, C-10), 35.5 (t, C-22), 32.9 (t, C-7), 31.4 (t, C-21), 28.4 (q, C-23), 27.9 (t, C-2), 25.6 (t, C-16), 25.4 (t, C-15), 21.7 (q, C-30), 20.2 (q, C-27), 18.5 (t, C-6), 18.3 (q, C-25), 16.7 (q, C-26), 16.0 (q, C-24).

Siaresinolic acid (**14-1**)

White powder; $[\alpha]^{23}_{\text{D}}$: 46.5° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 208 (4.02); IR ν_{max} (KBr) cm^{-1} : 3430, 2938, 2872, 1702, 1629, 1454, 1385, 1073, 1024; Mp: 271-272°; ESIMS: m/z 471.5 $[\text{M}-\text{H}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 5.60 (1H, m, H-12), 3.66 (2H, m, H-18, H-19), 3.46 (1H, dd, $J = 11.2, 5.2$ Hz, H-3), 1.68 (3H, s, H-27), 1.26 (3H, s, H-23), 1.21 (3H, s, H-29), 1.14 (3H, s, H-30), 1.10 (3H, s, H-26),

1.05 (3H, s, H-24), 0.94 (3H, s, H-25); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 180.9 (s, C-28), 144.9 (s, C-13), 123.5 (d, C-12), 81.3 (d, C-19), 78.1 (d, C-3), 55.9 (d, C-5), 48.4 (d, C-9), 46.1 (s, C-17), 44.8 (d, C-18), 42.1 (s, C-14), 40.0 (s, C-8), 39.4 (s, C-4), 38.8 (t, C-1), 37.5 (s, C-10), 35.7 (s, C-20), 33.7 (t, C-7), 33.4 (t, C-22), 29.2 (t, C-21), 29.2 (q, C-23), 28.9 (t, C-15), 28.8 (q, C-29), 28.4 (t, C-2), 28.1 (t, C-16), 24.9 (q, C-30), 24.8 (q, C-27), 24.2 (t, C-11), 19.0 (t, C-6), 17.5 (q, C-26), 16.5 (q, C-24), 15.5 (q, C-25).

Randialic acid B (**15-1**)

White powder; $[\alpha]^{23}_{\text{D}}$: 59.7° ($c = 0.05$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 224 (4.04); IR ν_{max} (KBr) cm^{-1} : 3440, 2932, 2868, 1696, 1456, 1384, 1276, 1084, 1033; Mp: 263-264°; ESIMS: m/z 453.4 $[\text{M}-\text{H}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 5.75 (1H, m, H-12), 3.49 (1H, dd, $J = 10.0, 6.0$ Hz, H-3), 1.89 (3H, s, H-29), 1.26 (3H, s, H-23), 1.16 (3H, s, H-27), 1.13 (3H, d, $J = 6.8$ Hz, H-30), 1.07 (3H, s, H-26), 1.05 (3H, s, H-24), 0.91 (3H, s, H-25); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 178.6 (s, C-28), 139.5 (s, C-13), 134.7 (s, C-19), 126.0 (d, C-12), 123.7 (s, C-18), 78.1 (d, C-3), 56.0 (d, C-5), 49.8 (s, C-17), 48.3 (d, C-9), 45.0 (s, C-14), 39.5 (s, C-4), 39.4 (t, C-1), 39.3 (s, C-8), 37.2 (s, C-10), 35.6 (t, C-7), 35.1 (t, C-22), 34.8 (d, C-20), 31.9 (t, C-21), 29.2 (t, C-15), 28.8 (q, C-23), 28.2 (t, C-2), 27.0 (t, C-16), 23.5 (t, C-11), 22.1 (q, C-27), 19.6

(q, C-29), 18.9 (q, C-30), 18.8 (t, C-6), 18.3 (q, C-26), 16.7 (q, C-24), 16.3 (q, C-25).

Sanguisorbigenin (15-2)

White powder; $[\alpha]_D^{23}$: 70.4° ($c = 0.05$, MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 208 (4.08), 250 (3.33); IR $\nu_{\max}$ (KBr) cm^{-1} : 3430, 2931, 1698, 1454, 1383, 1255, 1103, 1029; Mp: 251-252°; ESIMS: m/z 453.4 $[\text{M-H}]^+$; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) δ : 5.72 (1H, m, H-12), 3.67 (1H, br s, H-18), 3.48 (1H, dd, $J = 10.4, 6.0$ Hz, H-3), 1.72 (3H, s, H-29), 1.64 (3H, s, H-30), 1.22 (3H, s, H-23), 1.16 (3H, s, H-27), 1.05 (3H, s, H-26), 1.04 (3H, s, H-25), 0.92 (3H, s, H-24); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) δ : 179.8 (s, C-28), 138.5 (s, C-13), 129.0 (s, C-19), 127.3 (d, C-12), 123.9 (s, C-20), 78.1 (d, C-3), 56.0 (d, C-5), 50.7 (d, C-18), 48.2 (d, C-9), 47.0 (s, C-17), 43.8 (s, C-14), 39.6 (s, C-4), 39.4 (s, C-8), 39.3 (t, C-1), 37.3 (s, C-10), 34.3 (t, C-7), 33.5 (t, C-22), 28.8 (q, C-23), 28.7 (t, C-21), 28.7 (t, C-15), 28.2 (t, C-2), 24.0 (t, C-16), 23.7 (t, C-11), 22.2 (q, C-27), 20.5 (q, C-30), 18.7 (t, C-6), 18.0 (q, C-26), 17.4 (q, C-29), 16.7 (q, C-24), 16.1 (q, C-25).

Supplementary information S2

The enzyme inhibition assay modified according to the methods described before.¹⁶⁻¹⁷

In general, 25 µl of enzyme solution was added to 50 µl of drug solution in 96-well plate. After gentle shake for 5 min in the dark, 25 µl of substrate solution was add to the mixture and incubated until the chlorogenic or fluorogenic absorbance was measured by automatic microplate readers (Thermo Labsystems Multiskan Ascent and HIDEX Multilabel Reader). The blank solution consists of 50 µL of buffer and 50 µL of drug solution. Elaspol, CG inhibitor (CALBIOCHEM, 219372) and AEBSF (Enzo, ALX-270-022) were used as positive controls.¹⁹⁻²² Inhibition of enzyme activity (%) was determined by the following equation:

$$\frac{\text{Abs. diff. between control and blank} - \text{Abs. diff. between reaction solution and blank}}{\text{Abs. diff. between control and blank}} \times 100\%$$

The condition of enzymatic reactions such as substrates, buffers, detection wave and final working concentration were further described below.

Human neutrophil elastase

Human neutrophil elastase (Enzo, BML-SE284) and substrate (Enzo, BML-P213) were prepared in buffer A. Tested agents were prepared in buffer B. The final

concentrations of enzyme, substrate are 50 nM and 125 μ M, respectively. The absorption at 405 nm was measured 15 min after the enzymatic reaction began.

Human cathesin G

Human cathepsin G (Enzo, BML-SE283), substrate (Enzo, BML-P141) and tested agents were all prepared in prepared in buffer C. The final concentrations of enzyme, substrate are 100 nM and 200 μ M, respectively. The absorption at 405 nm was measured after the enzymatic reaction began at intervals of 30 min for 180 min.

Human proteinase 3

Human proteinase 3 (Merk, 539483), substrate (Enzo, BML-P303) and tested agents were all prepared in prepared in buffer D. The final concentrations of enzyme, substrate are 10 nM and 100 μ M, respectively. The absorption at 405 nm was measured 60 min after the enzymatic reaction began.

Human thrombin

Human thrombin (Sigma-aldrich, T7009-100KU) and substrate (Merck, 605211) were prepared in buffer A. Tested agents were prepared in buffer B. The final concentrations of enzyme, substrate are 10 nM and 25 μ M, respectively. The

excitation /emission waves at 340/460 nm was measured 30 min after the enzymatic reaction began.

Bovine chymotrypsin

Bovine chymotrypsin (Sigma-Aldrich, C4129) and substrate (Sigma-Aldrich, S9761) were prepared in buffer A. Tested agents were prepared in buffer B. The final concentrations of enzyme, substrate are 5 nM and 5 μ M, respectively. The ex/em waves at 340/460 nm was measured 30 min after the enzymatic reaction began.

Buffers

Buffer A: 20mM Tris-HCL, pH 7.4, 0.1% NaN₃

Buffer B: 20mM Tris-HCL, pH 7.4, 0.1% NaN₃, 5 mM CaCl₂

Buffer C: 100 mM Tris-HCl, 1.6 M NaCl, pH 7.5

Buffer D: 100 mM HEPES, pH 7.5, 500 mM NaCl, 10% DMSO, 170 μ M

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