New Flavonoid Glycosides and Cyanogenic Glycosides from *Dracocephalum peregrinum*

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Separation of ethyl acetate fractionation of *Dracocephalum peregrinum* afforded three new flavonoid glucosides (1—3), and a new cyanogenic glucoside (4). Their structures were elucidated based on HR-electron spray ionization (ESI)-MS, EI-MS, UV, IR, 1D-, and 2D-NMR data. 1—4 were tested *in vitro* for their antiinflammatory activity against the RAW 264.7, 293 cells. Among the compounds tested, 1—4 shown good antiinflammatory activity at 100 μg/ml by the measurement of nitric oxide (NO) in lipopolysaccharide (LPS) activated macrophages. But only 2 and 3 shown weak antiinflammatory activity at 100 μg/ml during the nuclear factor (NF)-κB activation assay.

Key words flavonoid glycoside; cyanogenic glycoside; *Dracocephalum peregrinum*; antiinflammatory

Genus *Dracocephalum* having more than 30 species, is an important member of the Lamiaceae family.[1)] Several species of *Dracocephalum* such as *D. peregrinum*, *D. heterophyllum*, *D. integrifolium*, *D. moldavicum*, *D. rupestre*, *D. ruyschiana* and *D. tanguticum* were used in Traditional Chinese Medicine treat icteritious hepatitis, lymphadenitis, throat-swelling diseases, flu fever, acute and chronic trachitis. So far, there are a few reports on researches of bioactivities and chemical constituents of this genus. Bioassays of *Dracocephalum* exhibited antibacterial, anti-hypoxia and antitussive activities,[2)] while Chemical investigation on these species resulted in the isolation of flavanoids,[3,4)] terpenoids,[5—7)] cinnamic acid derivatives[8)] and volatile oils.[9,10)] In our further phytochemical investigation on *Dracocephalum peregrinum*, we isolated three new flavonoid glucosides, Peregrinumin A (**1**), Peregrinumin B (**2**) and Peregrinumin C (**3**), and a new cyanogenic glucoside, Peregrinumcin A (**4**).

Results and Discussion

Peregrinumin A (**1**) was isolated as yellow amorphous powder, and its molecular formula was assigned to be $C_{32}H_{36}NaO_{16}$ by the peak in the HR-electron spray ionization (ESI)-MS at *m*/*z* 699.18948 ([M+Na]$^+$, $C_{32}H_{36}NaO_{16}$; Calcd 699.1901), indicating sixteen degrees of unsatruation. Its UV (MeOH) spectrum showed the presence of a flavonoid nucleus (269, 323 nm). The IR (KBr) spectrum showed the presence of hydroxy (3421, 2923, 2852 cm^{-1}) and carbonyl (1741 cm^{-1}). In the ^{1}H-NMR (dimethyl sulfoxide (DMSO)) spectrum of **1**, two groups of aromatic protons at δ: 6.94 (s, 1H), 6.43 (1H, d, J=1.8 Hz), 6.83 (1H, d, J=1.8 Hz) and 8.05 (d, J=8.4 Hz, 2×H), 7.12 (d, J=8.0 Hz, 2×H), a methoxyl protons at δ: 3.88 (3H, br s), and a hydroxyl protons at δ: 12.9 (1H, br s) were very similar to those of acacetin,[11)] and should, therefore, be assigned to H-3, H-6, H-8, H-2′, H-6′, H-3′, H-5′, 4′-OMe, and 5-OH. In the ^{13}C-NMR (DMSO) spectrum, the characteristic acetyl carbons at δ: 169.6, 20.6 and 169.4, 20.4 showed the presence of two acetyls. One group of carbon signals at δ: 99.4, 73.0, 76.2, 69.5, 75.5, 65.8 indicated the existence of glucose and the other group of carbon signals at δ: 97.0, 69.1, 71.5, 69.1, 68.3, 17.5 belonged to a moiety of rhamnose. The anomeric protons at δ: 5.16 (1H, d, J=7.8 Hz, H-1″) and 4.67 (1H, s, H-1‴) in the ^{1}H-NMR spectrum indicated the β-configuration of glucose and the α-configuration of rhamnose. In the ^{13}C-NMR spectrum of **1**, characteristic glycosylation shift (+11.2, +0.7 ppm) were observed for C-8 and C-6, suggesting that the glucose moiety was linked to C-7,[11)] and characteristic rhamnosylation shift (+3.4, −3.8 ppm) were observed for C-6″ and C-5″, suggesting that the rhamnose moiety was α-L-rhamnosyl-(1→6)-β-D-glucoside linkage.[12)] In the heteronuclear multiple bonding correlation (HMBC) spectrum (see Fig. 3), the long-range correlation from H-1″ (δ: 5.16) of glucose to C-7 (δ: 162.8) of aglycone and H-1‴ (δ: 4.67) of rhamnose to C-6″ (δ: 65.8) of glucose further confirmed the linkage of the glucose and rhamnose moieties. And the long-range correlation from H-2‴ (5.04) and H-3‴ (4.85) of rhamnose to two carbonyl carbons (δ: 169.6, 169.4) of ethanoyls concluded the acetylation of 2‴,3‴-position. Thus, the structure of **1** was established as acacetin-7-*O*-(2,3-

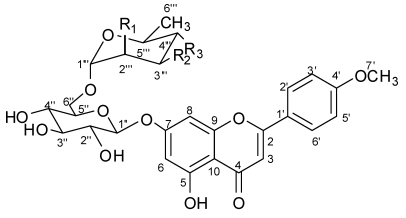

Fig. 1. The Structures of Peregrinumin A, B and C (**1**—**3**)

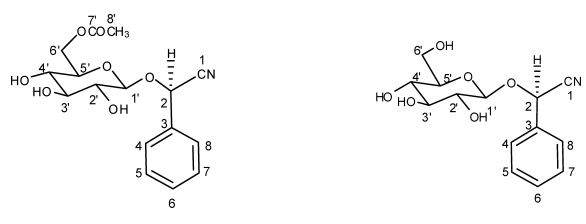

Fig. 2. The Structures of Peregrinumcin A (**4**) and 2*R*-Prunasin (**4a**)

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O-diacetyl-α-L-rhamnosyl)-(1→6)-β-D-glucoside, and named as Peregrinumin A.

Peregrinumin B (**2**) obtained as yellow amorphous powder. The molecular formula of was assigned to be $C_{32}H_{36}NaO_{16}$ based on ion peak at *m/z* 699.18856 ($[M+Na]^+$, $C_{32}H_{36}NaO_{16}$; Calcd 699.1901) in HR-ESI-MS. NMR spectrum of **2** also provided evidence for the presence of acacetin-7-*O*-α-L-rhamnosyl-(1→6)-β-D-glucoside as indicated by diagnostic signals similar to glucose and rhamnose portions and to the flavonoid portion of compound **1** (see Table 1 and discussion below). Although the difference in structure between **1** and **2** requires only aectylation position of sugar moiety, this change causes significant differences in spectral of C-2‴, C-3‴ and C-4‴ (see Table 1). And in the HMBC spectrum of **2** (see Fig. 3), the long-range correlation from H-2‴ (δ: 4.94) and H-4‴ (δ: 4.64) of rhamnose to two carbonyl carbons (δ: 169.8, 169.7) of acetyls could be explained with the acetylation of 2‴,4‴-position (see Fig. 1). Therefore, **2** was identified as acacetin-7-*O*-(2,4-*O*-diacetyl-α-L-rhamnosyl)-(1→6)-β-D-glucoside, named as Peregrinumin B.

Peregrinumin C (**3**), yellow amorphous powder, was assigned to be $C_{32}H_{36}NaO_{16}$ (HR-ESI-MS, *m/z* 699.18904). Analysis of NMR spectrum of **3**, **2** and **1** (see Table 1) revealed that the structures of these three compounds had the same skeleton except for acetylation position of rhamnose. The HMBC correlations of **3** between H-3‴ (δ: 4.91), H-4‴ (δ: 4.87) of rhamnose and carbonyl carbons (δ: 169.6, 169.6) of acetyls (see Fig. 3) suggested that the acetylation positions were C-3‴ and C-4‴. Based on the above evidences, the structure of **3** was elucidated as acacetin-7-*O*-(3,4-*O*-diacetyl-α-L-rhamnosyl)-(1→6)-β-D-glucoside, and named as Peregrinumin C.

Peregrinumcin A (**4**) was obtained as colourless needle crystal. Its molecular formula was assigned to be $C_{16}H_{19}NO_7$ by analysis of the HR-ESI-MS ($[M+Na]^+$, $C_{16}H_{19}NNaO_7$; Calcd 360.10592). Considering the aromatic UV absorption (268, 262 nm) and chemical shift distribution of 16 carbons observed in the ^{13}C-NMR (DMSO) spectra, a starting hypothesis for the structure elucidation of **4** was the presence of a prunasin/sambunigrin-type aromatic cyanogenic glycoside. Examination of both ^{1}H- and ^{13}C-NMR spectral data confirms that structure **4** was indeed prunasin derivate.[13] Compared NMR spectra of **4** to prunasin, the difference was one more typical acetyl signals (δ_C: 170.4, 20.7; δ_H: 2.05, s) detected in NMR. In the ^{13}C-NMR spectrum of **4**, glycosyla-

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Peregrinumin A, B and C (**1**—**3**) Observed at 600/125 MHz in DMSO; δ in ppm, *J* in Hz

No.	Peregrinumin A (**1**)		Peregrinumin B (**2**)		Peregrinumin C (**3**)	
	δ_C [a]	δ_H	δ_C [a]	δ_H	δ_C [a]	δ_H
2	163.8		163.9		163.8	
3	103.7	6.94 (s, 1H)	103.8	6.94 (s, 1H)	103.7	6.94 (s, 1H)
4	182.0		182.0		182.0	
5	161.1		161.1		161.1	
6	99.6	6.43 (d, *J*=1.8 Hz, 1H)	99.6	6.46 (d, *J*=1.8 Hz, 1H)	99.6	6.44 (d, *J*=1.8 Hz, 1H)
7	162.8		162.8		162.8	
8	94.4	6.83 (d, *J*=1.8 Hz, 1H)	94.9	6.84 (d, *J*=2.4 Hz, 1H)	94.7	6.83 (d, *J*=1.8 Hz, 1H)
9	156.7		156.9		157.0	
10	105.2		105.4		105.4	
1′	122.7		122.7		122.7	
2′	128.5	8.05 (d, *J*=8.4 Hz, 1H)	128.4	8.05 (d, *J*=9.0 Hz, 1H)	128.4	8.04 (d, *J*=8.0 Hz, 1H)
3′	114.6	7.12 (d, *J*=8.0 Hz, 1H)	114.6	7.12 (d, *J*=9.0 Hz, 1H)	114.6	7.11 (d, *J*=8.0 Hz, 1H)
4′	162.4		162.4		162.4	
5′	114.6	7.12 (d, *J*=8.0 Hz, 1H)	114.6	7.12 (d, *J*=9.0 Hz, 1H)	114.6	7.11 (d, *J*=8.0 Hz, 1H)
6′	128.5	8.05 (d, *J*=8.4 Hz, 1H)	128.4	8.05 (d, *J*=9.0 Hz, 1H)	128.4	8.04 (d, *J*=8.0 Hz, 1H)
7′	55.6	3.88 (br s, 3H)	55.6	3.85 (br s, 3H)	55.6	3.86 (br s, 3H)
5-OH		12.9 (br s, 1H)		12.9 (br s, 1H)		12.9 (br s, 1H)
1″	99.4	5.16 (d, *J*=7.8 Hz, 1H)	99.6	5.12 (d, *J*=7.2 Hz, 1H)	99.5	5.16 (d, *J*=7.8 Hz, 1H)
2″	73.0	3.28 (m, 1H)	73.1	3.27 (m, 1H)	73.0	3.28 (dd, *J*=7.2, 6.0 Hz, 1H)
3″	76.2	3.35 (m, 1H)	76.2	3.28 (m, 1H)	76.3	3.35 (m, 1H)
4″	69.5	3.19 (dd, *J*=8.4, 8.4 Hz, 1H)	69.3	3.19 (m, 1H)	69.5	3.19 (dd, *J*=8.4, 8.4 Hz, 1H)
5″	75.5	3.70 (dd, *J*=8.4, 7.8 Hz, 1H)	75.0	3.69 (dd, *J*=8.4, 7.8 Hz, 1H)	75.3	3.67 (dd, *J*=9.6, 6.0 Hz, 1H)
6″	65.8	3.85 (m, H-6″[b]), 3.60 (dd, *J*=3.6, 1.8 Hz, H-6″[a])	65.7	3.83 (m, H-6″[b]) 3.57 (dd, *J*=5.4, 11.4 Hz, H-6″[a])	65.9	3.84 (m, H-6″[b]) 3.60 (dd, *J*=12.0, 6.0 Hz, H-6″[a])
1‴	97.0	4.67 (s, 1H)	96.8	4.66 (s, 1H)	100.0	4.64 (s, 1H)
2‴	69.1	5.04 (dd, *J*=3.6, 1.8 Hz, 1H)	66.0	4.94 (dd, *J*=3.6, 1.2 Hz, 1H)	67.6	3.84 (m, 1H)
3‴	71.5	4.85 (dd, *J*=10.2, 3.6 Hz, 1H)	71.8	3.83 (dd, *J*=6.6, 3.6 Hz, 1H)	71.4	4.91 (m, 1H)
4‴	69.1	3.29 (m, 1H)	73.6	4.64 (dd, *J*=10.4, 8.0 Hz, 1H)	70.6	4.87 (dd, *J*=13.5, 3.6 Hz, 1H)
5‴	68.3	3.64 (dd, *J*=15.6, 6.0 Hz, 1H)	65.7	3.66 (dd, *J*=9.6, 3.6 Hz, 1H)	65.7	3.72 (dd, *J*=9.0, 6.0 Hz, 1H)
6‴	17.5	1.14 (m, 3H)	17.1	0.90 (d, *J*=6.6 Hz, 3H)	17.1	0.93 (d, *J*=6.6 Hz, 3H)
R1	169.6		169.8			
	20.6	1.99 (br s, 3H)	20.7	1.99 (br s, 3H)		
R2	169.4				169.6	
	20.4	1.82 (br s, 3H)			20.6	1.90 (br s, 3H)
R3			169.7		169.6	
			20.7	1.98 (br s, 3H)	20.6	1.80 (br s, 3H)

a) Chemical shifts and multiplicities based on HMQC and HMBC correlation peaks. *b*) C-atoms correlating with the corresponding H-atom.

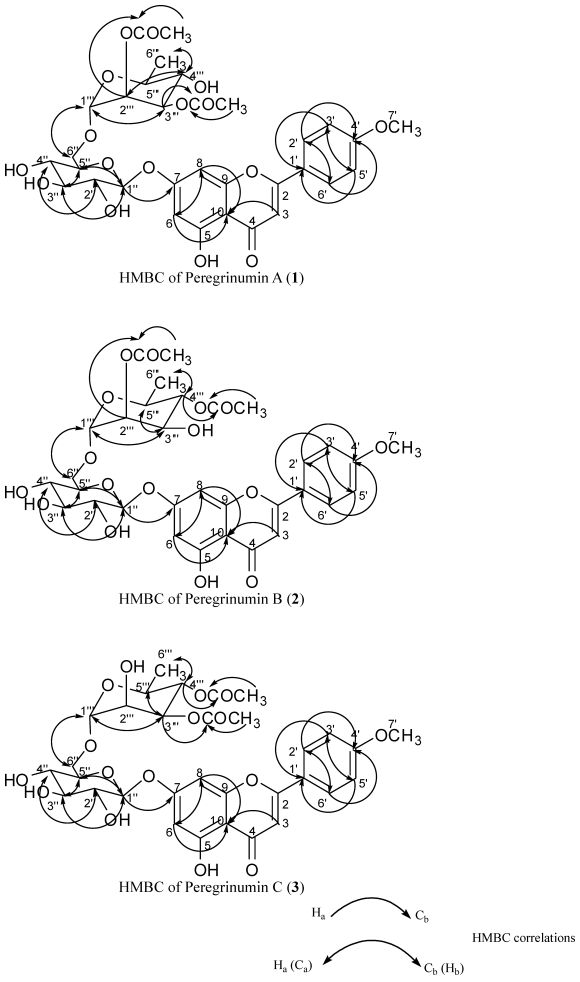

Fig. 3. HMBC of Peregrinumin A, B and C (**1**—**3**)

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data of Peregrinumcin A (**4**) Observed at 600/125 MHz in DMSO; δ in ppm, J in Hz

Position	δ_C [a]	δ_H	Key HMBC [b]
1	118.6		
2	67.9	5.90 (s, 1H)	1′, 1, 4, 8, 3
3	133.9		
4	129.0	7.46 (ddd, J=1.8, 1.8, 9.0 Hz, 1H)	2, 5, 7, 6, 3
5	127.3	7.55 (ddd, J=1.8, 8.4, 8.4 Hz, 1H)	2, 4, 8, 6, 3
6	130.0	7.43 (dddd, J=1.8, 1.8, 8.4, 8.4 Hz, 1H)	5, 7, 4, 8
7	127.3	7.55 (ddd, J=1.8, 8.4, 8.4 Hz, 1H)	2, 4, 8, 6, 3
8	129.0	7.46 (ddd, J=1.8, 1.8, 9.0 Hz, 1H)	2, 5, 7, 6, 3
1′	102.5	4.41 (d, J=7.8 Hz, 1H)	2, 2′, 5′, 3′
2′	73.1	3.10 (dd, J=7.8, 8.4 Hz, 1H)	3′, 1′
3′	76.3	3.17 (t, J=9.0 Hz, 1H)	4′, 5′, 3′
4′	70.0	3.14 (t, J=13.2 Hz, 1H)	6′, 4′, 5′, 3′
5′	73.9	3.42 (ddd, J=1.8, 6.6, 13.8 Hz, 1H)	6′, 4′, 3′, 1′
6′	63.5	4.26 (dd, J=12.0, 1.8 Hz, 1H)	4′, 5′, 1′, 7′
		4.16 (dd, J=12.0, 6.6 Hz, 1H)	4′, 5′, 7′
7′	170.4		
8′	20.7	2.05 (s)	7′

a) Chemical shifts and multiplicities based on HMQC and HMBC correlation peaks. *b*) C-atoms correlating with the corresponding H-atom.

tion shift was observed for C-2, suggesting that the glucose moiety was fused at C-2 and a acetylation shift (+0.7, −4.5 ppm) were observed for C-6′ and C-5′, suggesting that the 6′-acetylation of glucosyl, which was confirmed by HMBC spectrum correlations from H-6′ (δ: 4.26, 4.16) of glucose to the carbonyl carbons (δ: 170.4) of ethanoyl (see Table 2). The identification of β-D-glucose as the sugar moiety in **4** can be deduced from the following H, H-coupling pattern (see Table 2 for exact J and multiplicity values): H-1=d (7.8 Hz), H-2=dd (7.8, 8.4 Hz), H-3=t (9.0 Hz), H-4=t (13.2 Hz), H-5=ddd (1.8, 6.6, 13.8 Hz), H-6a=dd (1.8, 12.0 Hz), H-6b=dd (6.6, 12.0 Hz).[13] We make the new cyanogenic glycosides hydrolysised by NaOH (0.1 M) to give the known compoud **4a** (see structure in Fig. 2, ESI-MS, $C_{14}H_{18}O_6N$, $[M+H]^+$, m/z 296.12). Its NMR data was according to 2*R*-prunasin.[13] And the rotation results of **4** and **4a** were $[\alpha]_D^{25}=-35°$ (c=0.1950, CH_3OH) and −31° (c=0.2100, CH_3OH), therefore, the structure of **4** determined to be (2*R*)-β-D-(6-*O*-acetyl)-glucosyl-2-phenylacetonitrile, and named as Peregrinumcin A.

Table 3. Effect of **1**—**4** on NO Activities Induced by LPS

Group	Dosage (μg/ml)	NO concentration	Inhibition ratio (%)
DMSO [a]	0.5%		
LPS [a]	1.0	7.9±1.9	
Aminoguanidine [b]	50 mM	1.9±0.1**	75
1	100	3.5±0.3*	56
1	50	6.5±1.0	18
1	25	7.1±0.5	10
2	100	4.3±0.4*	45
2	50	5.7±0.3	28
2	25	7.2±0.1	9
3	100	3.6±0.5*	54
3	50	5.3±0.3	33
3	25	6.3±0.1	20
4	100	4.2±0.2*	49
4	50	5.4±0.5	32
4	25	6.4±0.2	20

a) Blank control. *b*) Pos. control. ** $p<0.01$, * $p<0.05$.

Table 4. Effect of **1**—**4** on NF-κB Activities Induced by LPS

Group	Dosage (μg/ml)	Mean ($\times 10^4$)	Inhibition ratio (%)
DMSO [a]	0.5%		
LPS [a]	10	35.1±0.6	
LGT [b]	10	9.7±1.8**	72
1	100	32.5±9.1	7.5
2	100	25.7±11.1	27
2	50	53.4±5.2	0
3	100	21.6±5.7	38
3	50	43.5±4.1	0
4	100	28.8±10.1	18

a) Blank control. *b*) Pos. control. ** $p<0.01$.

Compounds **1**—**4** were tested for effects of NO and nuclear factor (NF)-κB activity on RAW 264.7 and pNF-κB-luc-293 cells. Among the compounds tested, **1**—**4** were shown good inhibitory activities on nitric oxide (NO) production induced by LPS at dose of 100 μg/ml, and 50 μg/ml (Table 3). But only **2** and **3** were shown weak effects on NF-κB activity at dose of 100 μg/ml (Table 4).

Experimental

General Optical rotations: Perkin Elmer polarimeter 341 Polarimeter. IR: Bruker Vector 22. UV: Shimadzu UV-265. 1D- and 2D- (HMQC, HMBC, COSY, NOESY) NMR spectra: Bruker 600 NMR spectrometer. HR-ESI-MS: Q-TOF micro mass spectrometer. Silica gel: 200—300 mesh,

Yantai Chemical Plant, Yantai, P. R. China. Silica gel H: 10—40 μm, Yantai Chemical Plant, Yantai, P. R. China. Sephadex LH-20: Pharmacia. TCL: HSGF254 precoated silica gel plates, 10—40 μm, Yantai Chemical Plant, Yantai, P. R. China.

Plant Material Dried, whole plants of *Dracocephalum peregrinum* collected in Xinjiang autonomous region, P. R. China, in August 2007. The plant material was authenticated by Prof. Han-min Zhang. Voucher specimens (NO. DP070901) are deposited at the Department of Phytochemistry, School of Pharmacy, the Second Military Medical University, China.

Extraction and Isolation The air-dried and powdered whole plants of *Dracocephalum peregrinum* (10 kg) were refluxed with ethanol (75% v/v) three times, 2 h for each. After removal of the solvent under reduced pressure, the residue was partitioned sequentially with petroleum ether, chloroform, ethyl acetate, *n*-butanol to give four portions. The ethyl acetate portion (98 g) was subjected to silica gel column chromatography (200—300 mesh, 1.0 kg), eluting with the gradient $CHCl_3$: CH_3OH (30 : 1—15 : 1—10 : 1—5 : 1—2 : 1—1 : 1), and gave six fractions: I (10 g), II (11 g), III (12 g), IV (15 g), V (13 g), VI (11 g).

Fraction III (12 g) was subject to silica gel column chromatography (200—300 mesh, 100 g), eluting with the gradient $CHCl_3$: CH_3OH (30 : 1—15 : 1—10 : 1—5 : 1—2 : 1—1 : 1), to afford six subfractions (Ea1—Ea6), Ea4 was purified over silica gel column chromatography, eluting with $CHCl_3$: CH_3OH (20 : 1—10 : 1—5 : 1), to yield compound: **1** (12 mg). Fraction IV (15 g) was purified over silica gel column chromatography, eluting with $CHCl_3$: CH_3OH (15 : 1—8 : 1—4 : 1) to afford three subfractions (Eb1—Eb3), Eb2 subfraction purified over Sephadex LH-20 for two times, to yield compounds: **2** (15 mg) and **3** (13 mg). Fraction V (13 g) was purified over silica gel column chromatography, eluting with $CHCl_3$: CH_3OH (8 : 1—4 : 1—2 : 1—1 : 1) to afford four subfractions (Ec1—Ec4), and Ec3 was purified over Sephadex LH-20 several times, to yield compound: **4** (20 mg).

Peregrinumin A (=Aacacetin-7-*O*-(2,3-*O*-diacetyl-α-L-rhamnosyl)-(1→6)-β-D-glucoside, **1**): Yellow amorphous powder (CH_3OH). $[\alpha]_D^{25}=-78°$ (c=0.1250, CH_3OH). IR (KBr) cm^{-1}: 3421, 2923, 2852, 1741, 1656, 1605. UV λ_{max} (MeOH) nm (log ε): 205 (3.49), 269 (1.93), 323 (2.37). ^{1}H-NMR (600 MHz, DMSO) and ^{13}C-NMR (150 MHz, DMSO): Table 1. HR-ESI-MS m/z: 699.18948 ($[M+Na]^+$ $C_{32}H_{36}NaO_{16}$; Calcd 699.1901).

Peregrinumin B (=Acacetin-7-*O*-(2,4-*O*-diacetyl-α-L-rhamnosyl)-(1→6)-β-D-glucoside, **2**): Yellow amorphous powder (CH_3OH). $[\alpha]_D^{25}=-77°$ (c=0.1400, CH_3OH). IR (KBr) cm^{-1}: 3421, 2924, 1733, 1655, 1605. UV λ_{max} (MeOH) nm (log ε): 204 (3.64), 269 (1.93), 289 (2.37), 326 (2.34).^{1}H-NMR (600 MHz, DMSO) and ^{13}C-NMR (150 MHz, DMSO): Table 1. HR-ESI-MS m/z: 699.18856 ($[M+Na]^+$, $C_{32}H_{36}NaO_{16}$; Calcd 699.1901).

Peregrinumin C (=Acacetin-7-*O*-(3,4-*O*-diacetyl-α-L-rhamnosyl)-(1→6)-β-D-glucoside, **3**): Yellow amorphous powder (CH_3OH). $[\alpha]_D^{25}=-90°$ (c=0.1500, CH_3OH). IR (KBr) cm^{-1}: 3421, 2924, 1733, 1656, 1605. UV λ_{max} (MeOH) nm (log ε): 203 (4.87), 268 (4.19), 323 (4.65), 360 (2.18).^{1}H-NMR (600 MHz, DMSO) and ^{13}C-NMR (150 MHz, DMSO): Table 1. HR-ESI-MS m/z: 699.18904 ($[M+Na]^+$ $C_{32}H_{36}NaO_{16}$; Calcd 699.1901).

Peregrinumcin A (=(2*R*)-β-D-(6-*O*-Acetyl)-glucosyl-2-phenylacetonitrile, **4**): Colourless needle crystal (CH_3OH). $[\alpha]_D^{25}=-35°$ (c=0.1950, CH_3OH). IR (KBr) cm^{-1}: 3417, 3068, 3036, 2917, 1734, 1248, 1079. UV λ_{max} (MeOH) nm (log ε): 268 (0.91), 262 (1.02), 256 (1.14), 204 (4.03) nm. ^{1}H-NMR (600 MHz, DMSO) and ^{13}C-NMR (150 MHz, DMSO): Table 2. HR-ESI-MS m/z: 360.10552 ($[M+Na]^+$, $C_{16}H_{19}NNaO_7$; Calcd 360.10592).

Cell Lines Used RAW 264.7, 293, were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and maintained in media recommended by the suppliers supplemented with 10% fetal bovine serum (FBS) (Gibco, Paisley, U.K.), and streptomycin (100 mg/ml) in a humidified 5% CO_2 atmosphere at 37 °C.

Measurement of NO in LPS Activated Macrophages RAW 264.7 macrophages were seeded onto 24-well cell culture plates (10^5 cells/well). The cells were co-incubated with drugs and LPS (1 μg/ml) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the culture supernatants with Griess reagent in RAW 264.7. Aliquots of supernatants (100 μl) were incubated, in sequence, with 50 μl 1% sulphanilamide and 50 μl 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbances at 570 nm were read using a microtiter plate reader.

NF-κB Activation Assay NF-κB activation was assayed using stable pNF-κB-luc-293 cells (Baran *et al.*, 2007). The cells seeded in 96-well plate at 1.0×10^5 cells/well were pre-treated with test drugs for 15 min, and then incubated with 10 μg/ml recombinant human tumor necrosis factor (TNF-α) for 6 h. The cells were lysed, and luciferase activity was measured with a luciferase assay system (Promega, WI, U.S.A.).

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