Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2019, **17**(5): 0394–0400
Chinese Journal of Natural Medicines

Six new monacolin analogs from red yeast rice

LIU Bing-Yu, XU Fei, BAI Jian, YAN Dao-Jiang, ZHANG Le, ZHANG Dan, HU You-Cai*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

Available online 20 May, 2019

[ABSTRACT] Six novel monacolin analogs, monacolins V_1–V_6 (**1**–**6**), together with seven known ones (**7**–**13**), were isolated from the ethyl acetate extract of red yeast rice. Their structures and absolute configurations were determined by spectroscopic methods, especially 2D NMR (^{1}H-^{1}HCOSY, HSQC, HMBC, and NOESY/ROESY) and CD spectroscopic analyses as well as chemical derivation. Monacolins V_2 (**2**) and V_3 (**3**) represent the first examples of monacolins with 3-hydroxybutyrate substitute. The anti-inflammatory inhibitory activities against the lipopolysaccharide (LPS) induced NO production in BV-2 cells as well as antioxidant activities against rat liver microsomal lipid peroxidation were evaluated.

[KEY WORDS] *Monascus purpureus*; Red yeast rice, Monacolins

[CLC Number] R284.1 **[Document code]** A **[Article ID]** 2095-6975(2019)05-0394-07

Introduction

Red yeast rice has been extensively used as food and traditional medicine for thousands of years in China [1]. Fermentation of rice with fungi of the genus *Monascus* is basic resource of red yeast rice. Many active constituents from *Monascus* have been previously reported, including pigments, monacolins, dimerumic acid, γ-aminobutyric acid (GABA), and γ-lactam [2–10]. The monacolin analogs showed inhibition against 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, mainly contributed by monacolin K [11–12]. Moreover, red yeast rice has also been reported to possess various bioactivities, such as cytotoxicity [13–14]. In our previous studies, four trace phenolic compounds were isolated from the ethyl acetate extract of red yeast rice (fermented by *Monascus purpureus*). As a continue research on bioactive secondary metabolites from *M. purpureus*, the ethyl acetate extract of red yeast rice was subsequently investigated and 13 monacolin analogs (**1**–**13**), including six new ones, monacolins V_1–V_6 (**1**–**6**) were obtained. Herein, we report the isolation, structure elucidation and bioactivity evaluation of these isolates.

Results and Discussion

Monacolin V_1 **(1)** was obtained as colourless oil, and its molecular formula was determined as $C_{22}H_{34}O_6$ by HR-ESI-MS at *m*/*z* 417.2252 $[M + Na]^+$, accounting for six degrees of unsaturation. The IR spectrum of **1** suggested the presence of hydroxy (3374 cm^{-1}) and carbonyl (1731 and 1666 cm^{-1}) groups. The ^{1}H NMR (Table 1) and HSQC spectra showed the presence of three oxygenated methines at δ_H 3.78 (1H, m, H-11), 4.23 (1H, m, H-13) and 5.28 (1H, dd, *J* = 6.0, 3.5 Hz, H-8); three methyls at δ_H 0.89 (3H, d, *J* = 7.0 Hz, H-17), 1.06 (3H, d, *J* = 7.0 Hz, H-16) and 1.96 (3H, s, H-2′); one methoxyl at δ_H 3.62 (3H, s, OMe-15); three olefinic protons at δ_H 5.78 (1H, dd, *J* = 9.5, 6.0 Hz, H-3), 5.96 (1H, d, *J* = 9.5 Hz, H-4), and 5.50 (1H, s, H-5); and partially overlapped aliphatic methylenes and/or methines between δ_H 1.30 and 2.47. The ^{13}C NMR spectrum (Table 2) revealed 22 carbon signals, comprising two ester carbonyls at δ_C 171.0 and 172.6; two double bonds at δ_C 129.3, 129.9, 133.2 and 134.1; three oxygenated carbons at δ_C 68.7, 68.9, and 71.3; one methoxyl group at δ_C 51.6; and three methyl groups at δ_C 14.2, 21.3 and 22.9. Thus, it was suggested that two additional rings were required to fulfil the remaining two of six degrees of unsaturation. The NMR spectra of **1** were very similar to those of methyl ester of hydroxyl acid form of monacolin L (**10**) [16], except the presence of an additional acetoxy group [δ_H 1.96

[Received on] 26-Mar.-2019
[Research funding] This work was supported financially by CAMS Initiative for Innovative Medicine (No. 2017-I2M-4-004) and the Drug Innovation Major Project (No. 2018ZX09711001-006).
[*Corresponding author] E-mail: huyoucai@imm.ac.cn
These authors have no conflict of interest to declare.

(1H, s, H-2′), δ_C 171.0 (C-1′), 21.3 (C-2′)]. The strong HMBC correlations from H_3-2′ to C-1′, and from H-8 to C-1′ together with the chemical shift of C-1′ (δ_C 171.0), clearly demonstrated the presence of an acetoxy unit attached at C-8. As a result, the planar structure of **1** was assigned (Fig. 1).

Table 1 ^{1}H NMR spectroscopic data of compounds 1–6, 1a, 4a and 5a (δ in ppm, *J* in Hz, 600 MHz)

No.	1	1a	2	3	4	4a	5	5a	6
1	1.75 m	1.60 m	1.72 m	1.72 m	1.66 m	1.54 m	1.87 m	1.87 m	2.05 m
2	2.41 m	2.37 m	2.36 m	2.35 m	2.35 m	2.34 m	2.36 m	2.40 m	2.45 m
3	5.78 dd (9.5, 6.0)	5.78 dd (9.5, 6.0)	5.79 dd (9.5, 5.0)	5.78 dd (9.5, 5.0)	5.69 dd (9.0, 5.7)	5.69 dd (9.0, 5.7)	5.73 dd (9.0, 5.0)	5.73 dd (9.0, 5.0)	/
4	5.96 d (9.5)	5.96 d (9.5)	5.98 d (9.5)	5.97 d (9.5)	5.92 d (9.0)	5.92 d (9.0)	5.93 d (9.0)	5.93 d (9.0)	5.68 s
4a	/	/	/	/	/	/	/	/	/
5	5.50 s	5.50 s	5.52 s	5.51 s	5.43 d (4.5)	5.43 d (4.5)	5.44 s	5.43 s	6.18 s
6	2.41 m	2.40 m	2.44 s	2.44 s	2.45 m	2.40 m	2.36 m	2.34 m	/
7*α*	1.98 m	1.93 m	2.02 m	1.99 m	1.74 m	1.73 m	1.87 m	1.80 m	2.68 m
7*β*	1.91 m		1.90m	1.92 m	1.66 m	1.64 m			2.42m
8	5.28 dd (6.0, 3.5)	5.28 dd (6.0, 3.5)	5.42 d (3.0)	5.46 dd (5.7, 3.4)	3.83 m	3.82 m	4.24 m	4.22 m	5.48 dd (5.8, 3.0)
8a	2.28 dd (12.0, 3.5)	2.28 dd (12.0, 3.5)	2.25 m	2.24 m	2.02 m	2.02 m	2.10 m	2.07 m	2.64 m
9*α*	1.54 m	1.48 m	1.62 m	1.61 m	2.10 m	2.08 m	1.87 m	1.80 m	1.66 m
9*β*	1.30 m	1.24 m			1.56 m	1.52 m	1.32 m	1.28 m	1.56 m
10*α*	1.54 m	1.48 m	1.56 m	1.53 m	1.56 m	1.53 m	1.53 m	1.55 m	1.56 m
10*β*	1.33 m	1.24 m	1.31 m	1.32 m	1.37 m	1.29 m	1.32 m	1.29 m	1.29 m
11	3.78 m	3.86 m	3.86 m	3.87 m	3.83 m	3.89 m	3.86 m	3.92 m	3.79 m
12*α*	1.61 m	1.70 m	1.56 m	1.61 m	1.56 m	1.63 m	1.66 m	1.60 m	1.66 m
12*β*		1.09 m		1.53 m		1.10 m	1.58 m	1.12 m	1.56 m
13	4.23 m	4.30 m	4.25 m	4.23 m	4.23 m	4.31 m	4.24 m	4.30 m	4.23 m
14	2.47 m	2.40 m	2.47 m	2.49 m	2.45 m	2.40 m	2.45 m	2.40 m	2.45 m
15	/	/	/	/	/	/	/	/	/
16	1.06 d (7.0)	1.06 d (7.0)	1.06 d (7.0)	1.06 d (7.0)	1.01 d (7.0)	1.01 d (7.0)	1.18 d (7.0)	1.17 d (7.0)	1.87 s
17	0.89 d (7.0)	0.87 d (7.0)	0.88 d (7.0)	0.87 d (7.0)	0.91 d (7.0)	0.91 d (7.0)	0.88 d (7.0)	0.88 d (7.0)	0.99 d (7.0)
OMe-15	3.62 s	3.62 s	3.71 s	3.71 s	3.62 s	3.62 s	3.62 s	3.62 s	3.62 s
1′	/	/	/	/	/	/	/	/	/
2′	1.96 s	1.96 s	2.35 m	2.40 m	/	/	/	/	2.29 m
3′	/	/	4.16 m	4.23 m	/	/	/	/	1.56 m/1.40 m
4′	/	/	1.21 d (7.0)	1.20 d (7.0)	/	/	/	/	0.84 d (7.0)
5′	/	/	/	/	/	/	/	/	1.04 d (7.0)
Me_1	/	1.26 s	/	/	/	1.26 s	/	1.26 s	/
Me_2	/	1.41 s	/	/	/	1.42 s	/	1.41 s	/

Notes: Data for compounds **1**, **4–6**, **1a**, **4a** and **5a** were recorded in CD_3COCD_3; Data for compounds **2** and **3** were recorded in $CDCl_3$

Table 2 ^{13}C NMR spectroscopic data (δ) of compounds 1–6, 1a, 4a and 5a (δ in ppm, 125 MHz)

No.	1	1a	2	3	4	4a	5	5a	6
1	37.1	37.3	36.2	36.3	44.1	44.0	36.7	36.8	38.3
2	31.6	31.6	30.8	30.8	33.3	33.3	31.6	31.6	43.1
3	134.1	134.1	133.6	133.6	134.3	134.2	133.7	133.6	202.7
4	129.3	129.3	128.3	128.3	129.3	129.3	129.9	129.9	123.4
4a	133.2	133.1	131.9	132.0	136.8	136.9	133.0	132.9	144.5
5	129.9	129.9	129.6	129.5	130.1	130.2	130.3	130.3	125.0

Continued

No.	1	1a	2	3	4	4a	5	5a	6
6	28.4	28.3	27.6	27.6	30.1	30.0	28.8	28.8	155.2
7	32.9	33.0	32.7	32.8	40.6	40.6	36.6	36.7	37.0
8	68.7	68.7	68.8	68.7	67.1	67.1	65.2	65.0	67.6
8a	37.8	37.8	37.5	37.4	43.6	43.5	39.2	39.4	39.7
9	24.4	24.1	23.0	22.9	26.9	26.5	24.2	24.2	24.6
10	35.5	34.3	34.4	34.3	36.8	35.3	35.5	34.2	35.2
11	71.3	69.5	71.2	71.2	71.9	69.7	71.4	69.6	71.4
12	44.3	37.4	42.4	42.3	44.3	37.5	44.4	37.6	44.3
13	68.9	66.8	69.0	69.0	69.0	66.9	68.9	66.9	68.8
14	43.2	41.9	41.7	41.7	43.2	41.9	43.1	41.9	43.2
15	172.6	171.6	173.1	173.1	172.6	171.6	172.5	171.6	172.5
16	22.9	22.9	22.9	22.8	21.7	21.7	23.5	23.6	24.0
17	14.2	14.1	14.0	14.1	14.0	14.0	14.4	14.3	10.6
OMe-15	51.6	51.6	52.0	52.0	51.5	51.6	51.5	51.6	51.6
1′	171.0	170.8	172.6	172.9	/	/	/	/	176.3
2′	21.3	21.3	44.1	43.9	/	/	/	/	42.0
3′	/	/	64.7	64.4	/	/	/	/	27.3
4′	/	/	23.0	22.8	/	/	/	/	11.9
5′	/	/	/	/	/	/	/	/	17.1
Me_1	/	30.5	/	/	/	30.6	/	30.5	/
Me_2	/	20.1	/	/	/	20.1	/	20.0	/

Notes: Data for compounds **1**, **4–6**, **1a**, **4a** and **5a** were recorded in CD_3COCD_3; Data for compounds **2** and **3** were recorded in $CDCl_3$.

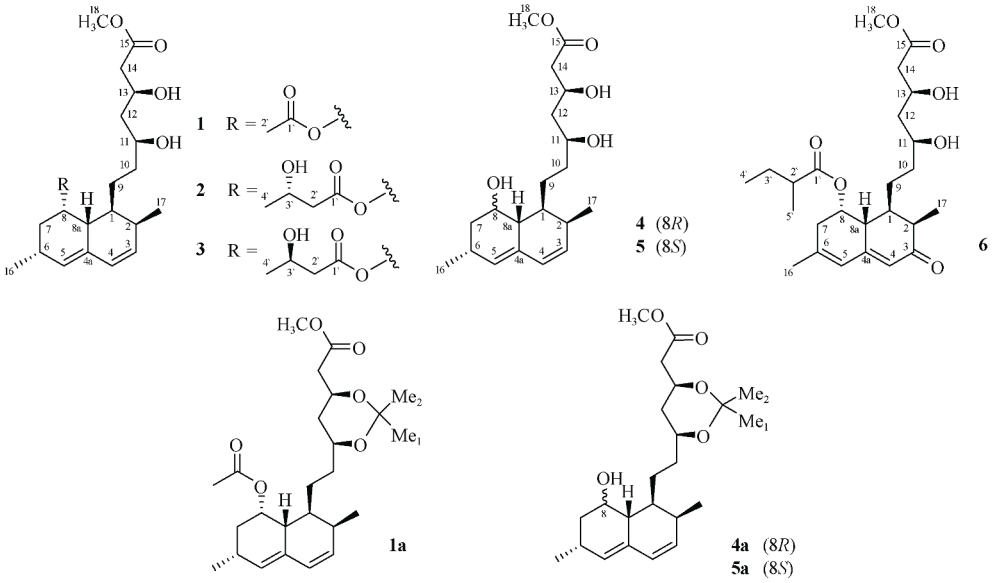

Fig. 1 Structures of compounds 1–6, 1a, 4a, and 5a

The relative stereochemistry of hydroxy groups at C-11 and C-13 was assigned on the basis of the chemical modification. Treatment of **1** with 2, 2-dimethoxypropane and pyridinium-*p*-toluenesulfonate afforded the acetone ketal **1a** (Fig. 1), resulting from ketal formation at the C-11 and C-13 hydroxy groups. It was reported that the ^{13}C NMR chemical shifts of acetonide methyl groups in a *syn*-acetonide are generally at δ_C 20.0 and 30.0, while the methyl signals of an *anti*-acetonide are both observed at δ_C 25 [19–20]. Comprehensive analyses of the 1D NMR and HSQC data of **1a**,

showed the presence of acetonide methyl groups at δ_C 20.1 and 30.5, respectively (Table 2). Therefore, the relative stereochemistry of hydroxy groups at C-11 and C-13 was defined as *syn* configuration. The relative stereochemistry of the A/B-rings system of **1** could be determined on the basis of the NOESY correlations (Fig. 2). The NOESY correlations between H-7*β*/H-8a, H-8a/H_3-17, indicated the *β*-configuration for H-8a and H_3-17. Similarly, the NOESY correlations between H_3-16 and H-7*α*, as well as between H-1 and H-2 suggested that H_3-16, H-1, and H-2 should be *α*-oriented. Based on the close NMR data and biogenesis of dehydromonacolin N [21] and **1**, the absolute configuration of monacolin V_1 (**1**) was deduced as 1*S*, 2*S*, 6*R*, 8*S*, 8a*R*, 11*S**, 13*S**.

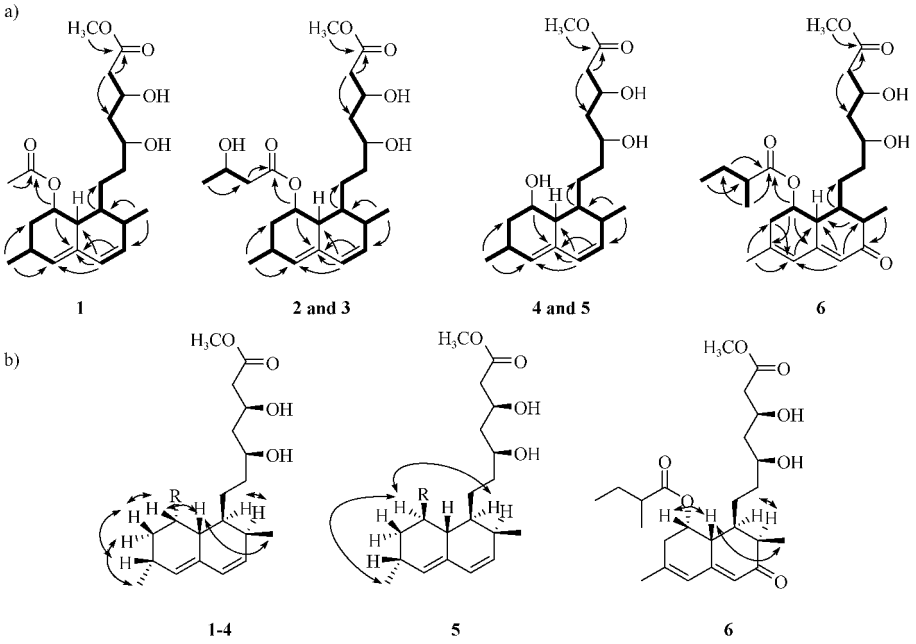

Fig. 2 a) ^{1}H-^{1}H COSY (bold lines), HMBC (arrows), and b) NOE (double arrows) correlations of compounds 1–6

Monacolins V_2 **(2)** and V_3 **(3)** were isolated as colorless oil and were assigned the same molecular formula of $C_{24}H_{38}O_7$ based on the HR-ESI-MS at *m/z* 461.2529 $[M + Na]^+$ and 461.2530 $[M + Na]^+$, respectively, with six degrees of unsaturation. The NMR spectra of **2** and **3** are very similar to those of **1**, expect the presence of an additional methyl group [δ_H 1.21/1.20 (3H, d, H-4′), δ_C 23.0/22.8], one methene group [δ_H 2.35/2.40 (2H, m, H-2′), δ_C 44.1/43.9] and one oxygenated methine group [δ_H 4.16/4.23 (1H, m, H-3′), δ_C 64.7/64.4] in **2** and **3**. These data clearly indicated that a 3-hydroxybutyrate unit was substituted at C-8 in **2** and **3** instead of the acetoxy unit in **1**, which was further confirmed by the ^{1}H-^{1}H COSY correlations of H_3-4′/H-3′/H_2-2′ and the long-range HMBC correlations of H_3-4′/C-2′ and H_2-2′/C-1′. NOESY data suggested that both of **2** and **3** had the same relative stereochemistry with compound **1**. From biogenetic considerations, the A/B ring moiety of monacolin analogs could be assumed to have the same absolute stereochemistry as in **1**. Compounds **2** and **3** showed very similar NMR spectra, except for the chemical shift of H-3′ (δ_H 4.16 for **2** and δ_H 4.23 for **3**). The absolute configuration of C-3′ in **2** and **3** was ensured by comparing the proton chemical shifts with those of known monacolins with similar side chain. As reported [22–23], configuration of C-3′ was assigned *S* when the chemical shifts of H-3′ was shifted downfield, and assigned *R* when the chemical shifts of H-3′ was shifted upfield. Therefore, the structures of **2** and **3** were deduced as 1*S*, 2*S*, 6*R*, 8*S*, 8a*R*, 11*S**, 13*S**, 3′*R* and 1*S*, 2*S*, 6*R*, 8*S*, 8a*R*, 11*S**, 13*S**, 3′*S*, respectively. This is the first report of monacolins substituted by 3-hydroxybutyrate at C-8.

Monacolins V_4 **(4)** and V_5 **(5)** were isolated as colorless oil. The same molecular formula of $C_{20}H_{32}O_5$ were assigned based on the HR-ESI-MS at *m/z* 375.2146 $[M + Na]^+$ and 375.2159 $[M + Na]^+$, respectively, with five degrees of unsaturation. The NMR spectra of **4** and **5** were very similar to those of **1**, expect for the absence of an acetoxy unit in **4** and **5**. Accordingly, H-8 in **4** and **5** were upfield shifted by $\Delta\delta_H$ 1.45 and 1.04 ppm, respectively, compared to that of **1**, which indicated a hydroxyl instead of acetoxy located at C-8 in **4**/**5**. The relative stereochemistry of hydroxy groups at C-11 and C-13 was also assigned by chemical modification where derivatives **4a** and **5a** were obtained. The chemical shifts of acetonide methyl groups at δ_C 20.1 and 30.5 (Table 2) in **4a** and **5a** suggested the relative stereochemistry of hydroxy groups at C-11 and C-13 were *syn* configuration. The NOESY correlations between H-8 and H_3-16/H-1 in **4**, indicated the *α*-orientation for H-8. Therefore, the absolute configuration of **4** was deduced as 1*S*, 2*S*, 6*R*, 8*R*, 8a*R*, 11*S**, 13*S**. While the stereochemistry of the A/B-rings of **5** was assigned as the same to that in **1**, based on the NOESY correlations and bio-

genetic considerations. Thus, the absolute configuration of **5** was deduced as 1*S*, 2*S*, 6*R*, 8*S*, 8a*R*, 11*S**, 13*S**.

Monacolin V_6 (**6**) was obtained as colorless oil, and its molecular formula was determined as $C_{25}H_{38}O_7$ by HR-ESI-MS at *m/z* 473.2503 $[M + Na]^+$, accounting for seven degrees of unsaturation. The 1D NMR (Table 1) and HSQC spectra of **6** suggested the presence of four methyls at δ_H 1.87 (3H, m, H-16), 1.04 (3H, d, *J* = 7.0 Hz, H-5′), 0.99 (3H, d, *J* = 7.0 Hz, H-17), and 0.84 (3H, d, J = 7.0 Hz, H-4′); one methoxyl at δ_H 3.62 (3H, s, 15-OMe); three oxygenated methines at δ_H 5.48 (1H, dd, *J* = 5.8, 3.0 Hz, H-8), 4.23 (1H, m, H-13), and 3.79 (1H, m, H-11); an *α*, *β*-unsaturated ketone [δ_H 5.68 (1H, s, H-4); δ_C 144.5, 123.4, and 202.7]; two ester carbonyls at δ_C 172.5 and 176.3; one olefinic proton at δ_H 6.18 (1H, s, H-5); and partially overlapped aliphatic methylenes and/or methines between δ_H 2.68 and 1.29. Comparison of the ^{13}C NMR spectra of **6** with those of monacolin O [20] revealed their similarity except for the presence of signals for a double bond in **6** instead of the signal for oxygenated methine, which was supported by the ^{13}C NMR spectrum at δ_C 125.0 (C-5).

Based on the very close chemical shifts of C-11 and C-13 in compounds **1** and **6**, the relative stereochemistry of hydroxy groups at C-11 and C-13 for **6** were also deduced as *syn* configuration. The ROESY correlations of H_2-9/H_3-17 and H-8a, and of H-8a/H-8 indicated that these protons were on the same face of the ring system and defined as β-configuration, whereas the H-1 and H-2 protons were on the other side of the ring and possessed *α*-configuration. Furthermore, the absolute configuration of **6** was determined by CD based on the octant rule for cyclohexanone [20]. A negative cotton effect at 336 nm (Δε-1.0) (Fig. 3) for n-π* clearly indicated (*R*)-configuration at C-8a [16, 24]. Therefore, 1*R*, 2*R*, 8*S*, 11*S**, 13*S** were assigned on the basis of their relative configurations determined by ROESY measurements.

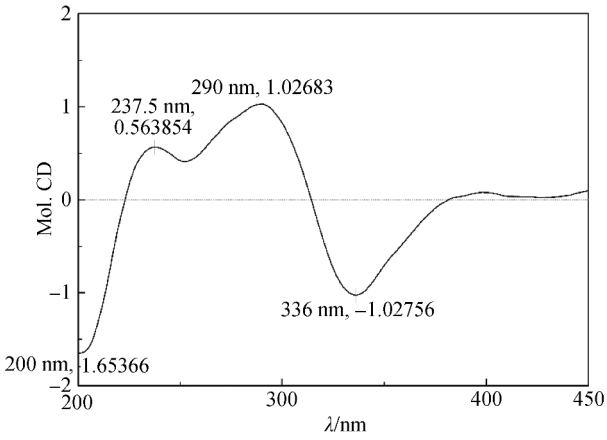

Fig. 3 CD spectrum of compound 6

The seven known compounds were identified as monacolin K (**7**) [12], monacolin K acid (**8**) [15], methyl ester of hydroxy acid form of monacolin K (**9**) [16], methyl ester of hydroxy acid form of monacolin L (**10**) [16], monacolin R (**11**) [17], monacolin S (**12**) [17–18], and methyl ester of hydroxy acid form of monacolin S (**13**) [18], by comparison of UV, ESI-MS and NMR with those reported.

Compounds **1**–**13** were assessed for their anti-inflammatory inhibitory activities against the lipopolysaccharide (LPS) induced NO production in BV-2 cells as well as their antioxidant activities against rat liver microsomal lipid peroxidation induced by Fe^{2+}-cystine *in vitro*. All of the tested compounds were inactive at a concentration of 10^{-5} mol·L^{-1} for anti-inflammatory activity and 10^{-4} mol·L^{-1} for antioxidant activity, respectively.

Experimental

General experimental procedures

Optical rotations were confirmed using a JASCO P-2000 polarimeter (Tokyo, Japan). IR spectra were taken on a Nicolet 5700 FT-IR spectrometer (Termo Electron Corporation, Madison, WI, USA). UV spectra were recorded on a JASCO V-650 spectrophotometer (JASCO, Tokyo, Japan). CD spectra were measured on a JASCO J-815 spectrometer (JASCO, Tokyo, Japan). NMR measurements were obtained on Bruker AV-III-500 and Bruker AV-III-600 HD spectrometers in CD_3COCD_3 or $CDCl_3$ with TMS as internal reference (Bruker, Fallanden, Switzerland). HR-ESI-MS were recorded on an Agilent Technologies 6520 Accurate Mass Q-TOF LC/MS spectrometer (Agilent Technologies, Santa Clara, USA). Column chromatography was performed with Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and MCI gel CHP 20P/P120 (Mitsubishi Chemical Corporation, Tokyo, Japan). TLC was performed on GF_{254} plates (Qingdao Marine Chemical Factory, Qingdao, China). Medium pressure liquid chromatography (MPLC) was carried out on a TELEDYNE ISCO CombiFlash Rf$^+$ [Universal Technology, Hong Kong, China]. Semipreparative HPLC was conducted using a SSI instrument with an Series 1500 photo diode array detector and YMC-Pack ODS-A column (250 mm × 10 mm, 5 μm).

Fungal material

Red yeast rice (fermented by *M. purpureus*) was purchased from the Zhejiang Sanhe Bio-tech Co., Ltd., Zhejiang, China. The sample was deposited at Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Beijing, China.

Extraction and isolation

The dried powder of red yeast rice (5.0 kg) was extracted three times with EtOAc (15.0 L × 3) at room temperature under sonication. After concentration under reduced pressure, the crude extract (105.9 g) was dissolved in MeOH, then extracted two times using petroleum ether to afford MeOH-soluble (68.5 g) and petroleum ether-soluble (34.7 g) fractions. The MeOH–soluble fraction was subjected to MCI with gradient MeOH–H_2O (20 : 80, 50 : 50, 90 : 10, 100 : 0) to give 4 fractions (A–D). Fraction C (5.1 g) was separated into 7 sub-

fractions (C1–C7) by MPLC eluted with a gradient of increasing MeOH (35%–100%) in H_2O. Sub-fraction C4 (915.8 mg) was applied to Sephadex LH-20 column chromatography eluted with MeOH to give 14 sub-fractions ($C4_a$–$C4_n$). Sub-fraction $C4_d$ (127.9 mg) was further purified by semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (35 : 65, *V*/*V*)] to give compound **6** (t_R 72.5 min, 1.7 mg). Sub-fraction $C4_f$ (96.8 mg) was further purified by semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (40 : 60, *V*/*V*)] to give compounds **12** (t_R 36.2 min, 8.7 mg) and **13** (t_R 42.7 min, 9.2 mg). Sub-fraction C5 (1.45 g) was chromatographed on a Sephadex LH-20 column using MeOH to give 14 sub-fractions ($C5_a$–$C5_n$), and then sub-fraction $C5_e$ (218.0 mg) was separated using semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (55 : 45, *V*/*V*)] to yield compounds **4** (t_R 19.5 min, 2.3 mg), **5** (t_R 21.0 min, 3.1 mg), **2** (t_R 24.0 min, 3.6 mg), **3** (t_R 25.0 min, 2.9 mg) and **1** (t_R 29.0 min, 9.8 mg). Sub-fraction $C5_g$ (109.8 mg) was further purified by semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (35 : 65, *V*/*V*)] to give compound **11** (t_R 51.3 min, 13.7 mg). Sub-fraction $C5_k$ (152.6 mg) was further purified by semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (50 : 50, *V*/*V*)] to give compounds **10** (t_R 12.4 min, 12.7 mg), **7** (t_R 22.7 min, 9.9 mg), and **9** (t_R 28.5 min, 7.6 mg). Fraction D (25.8 g) was separated into 10 subfractions (D1-D10) by MPLC eluted with a gradient of increasing MeOH (40%–100%) in H_2O. Sub-fraction D3 (1247.3 mg) was applied to Sephadex LH-20 column chromatography eluted by MeOH to give 14 sub-fractions ($D3_a$–$D3_n$). Sub-fraction $D3_c$ (119.7 mg) was further purified by semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (55 : 45, *V*/*V*)] to give compound **8** (t_R 42.8 min, 11.6 mg).

Monacolin V_1 (**1**), $C_{22}H_{34}O_6$; colorless oil; $[\alpha]_D^{20}$+36.8 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (logε): 229.6 (3.74), 221.8 (3.71), 202.2 (3.98) nm; IR ν_{max} 3374, 2954, 2919, 2851, 1731, 1666, 1618, 1437, 1380 cm^{-1}; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; HR-ESI-MS *m*/*z* 417.2252 $[M + Na]^+$ (Calcd. for $C_{22}H_{34}NaO_6$, 417.2248).

Monacolin V_2 (**2**), $C_{24}H_{38}O_7$; colorless oil; $[\alpha]_D^{20}$+37.3 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (logε): 236.8 (3.62), 229.8 (3.64), 202.6 (3.88) nm; IR ν_{max} 3365, 2920, 2851, 1723, 1666, 1615, 1380 cm^{-1}; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; HR-ESI-MS *m*/*z* 461.2529 $[M + Na]^+$ (Calcd. for $C_{24}H_{38}NaO_7$, 461.2510).

Monacolin V_3 (**3**), $C_{24}H_{38}O_7$; colorless oil; $[\alpha]_D^{20}$+116.9 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (logε): 237.4 (3.94), 230.0 (3.92), 201.8 (3.94) nm; IR ν_{max} 3343, 2954, 2918, 2850, 1731, 1664, 1624, 1468, 1383 cm^{-1}; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; HR-ESI-MS *m*/*z* 461.2530 $[M + Na]^+$ (Calcd. for $C_{24}H_{38}NaO_7$, 461.2510).

Monacolin V_4 (**4**), $C_{20}H_{32}O_5$; colorless oil; $[\alpha]_D^{20}$+146.8 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (logε): 236.4 (3.96), 229.4 (3.94), 202.2 (3.84) nm; IR ν_{max} 3341, 2921, 2852, 1721, 1665, 1434, 1373 cm^{-1}; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; HR-ESI-MS *m*/*z* 375.2146 $[M + Na]^+$ (Calcd. for $C_{20}H_{32}NaO_5$, 375.2142).

Monacolin V_5 (**5**), $C_{20}H_{32}O_5$; colorless oil; $[\alpha]_D^{20}$+113.4 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (logε): 238.4 (3.81), 231.4 (3.79), 202.6 (3.84) nm; IR ν_{max} 3341, 2920, 2851, 1725, 1663, 1438, 1379 cm^{-1}; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; HR-ESI-MS *m*/*z* 375.2159 $[M + Na]^+$ (Calcd. for $C_{20}H_{32}NaO_5$, 375.2142).

Monacolin V_6 (**6**), $C_{25}H_{38}O_7$; colorless oil; $[\alpha]_D^{20}$+28.8 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (logε): 352.0 (3.01), 290.4 (3.86), 229.2 (3.59) nm, 201.8 (3.82) nm; IR ν_{max} 3412, 3340, 2920, 2851, 1730, 1658, 1628, 1383 cm^{-1}; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; HR-ESI-MS *m*/*z* 473.2503 $[M + Na]^+$ (Calcd. for $C_{25}H_{38}NaO_7$, 473.2510).

Preparation of acetonide derivatives (1a, 4a, and 5a)

Compounds **1** (4 mg), **4** (2 mg), and **5** (3 mg) were treated with 2, 2-dimethyoxypropane (3 mL) and pyridinium-*p*-toluenesulfonate (5.6 mg) respectively, and stirred at room temperature for 6h, then quenched with 5% aqueous $NaHCO_3$, before extracted four times using CH_2Cl_2. The reaction solution was evaporated under reduced pressure and purified by semi-preparative HPLC [2.0 mL·min^{-1}; ACN–H_2O (75: 25, *V*/*V*)] to yield compounds **4a** (t_R 16.4 min, 0.8 mg), **5a** (t_R 19.1 min, 1.2 mg), and **1a** (t_R 23.0 min, 1.5 mg) respectively.

Acetonide Derivatives **1a**, $C_{25}H_{38}O_6$; colorless oil; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2; (+)-ESI-MS *m*/*z* 457 $[M + Na]^+$.

Acetonide derivatives **4a**/**5a**, $C_{23}H_{36}O_5$; colorless oil; ^{1}H- and ^{13}C NMR spectroscopic data see Tables 1 and 2, respectively; (+)-ESI-MS *m*/*z* 415 $[M + Na]^+$.

Anti-inflammatory activity assay

Compounds **1**–**13** were assessed for their anti-inflammatory inhibitory activities against the lipopolysaccharide (LPS) induced NO production in BV-2 cells using the Griess method.

Cell Culture and treatment: BV-2 cells (immortalized mouse microglia) were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum, 100 U·mL^{-1} penicillin, 100 μg·mL^{-1} streptomycin. Cells were initially grown in 96-well plate at a density of 2 × 10^5/mL in 96-well plate and pre-treated with compounds **1**–**13** at concentration of 10, 1 and 0.1 μmol·L^{-1}. One hour later cells were stimulated with LPS 300 ng·mL^{-1} and then incubated for 24 h.

NO Assay: The cell culture medium was assayed for the accumulation of nitrite (NO^{2-}), a stable breakdown product of NO. Briefly, a 100 μL aliquot of the sample was added to an equal volume of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% *N*-naphtyl-ethylenediamine dihydrochloride) in a 96-well plate, incubated at RT for 10 min. After incubation the absorbance of the resulting azo-dye product was determined spectrophotometrically at 540 nm. Fresh culture medium was used for blank-reading in all experiments. The amount of NO in the sample was calculated using a sodium

nitrite standard curve freshly prepared in culture medium.

TNF-α and IL-1β measurements: After the cells were incubated with compounds **1**–**13** and stimulated with LPS for 24 h, the cell culture medium were collected. IL-1β and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA) kit (R&D company) following the manufacturer's instructions. Determinations were performed in duplicate and the results were expressed as $pg \cdot mL^{-1}$.

Antioxidant assay

Measurement of lipid peroxidation product malondialdehyde (MDA): 10% liver microsomes was mixed with PBS ($0.1\ mol \cdot L^{-1}$) and cysteine ($10^{-3}\ mol \cdot L^{-1}$), and then the reaction system was mixed sufficiently for 15 min after the tested compound (10^{-4}, 10^{-5}, $10^{-6}\ mol \cdot L^{-1}$) was added. Ferrous sulfate ($10^{-2}\ mol \cdot L^{-1}$) was added and mixed for 15 min in a 37 °C water bath. The reaction was terminated by TCA, and then TBA was added and heated for 10 min at 100 °C. The supernatant was harvested and measured at the wavelength of 532 nm. The effect of the compounds on the production of MDA was calculated according to the *OD* value.

References

[1] Lin YL, Wang TH, Lee MH, *et al*. Biologically active components and nutraceuticals in the *Monascus*-fermented rice: a review [J]. *Appl Microbiol Biotechnol*, 2008, **77**(5): 965-973.

[2] Wei WD, Lin S, Chen MH, *et al*. Monascustin, an unusual γ-lactam from red yeast rice [J]. *J Nat Prod*, 2017, **80**(1): 201-204.

[3] Wild D, Toth G, Humpf HU. New *Monascus* metabolite isolated from red yeast rice (angkak, red koji) [J]. *J Agr Food Chem*, 2002, **50**(14): 3999-4002.

[4] Antonio B. Extracts of *Monascus purpureus* beyond stains--Profile of efficacy and safety of the use of extracts of *Monascus purpureus* [J]. *Chin J Integr Med*, 2005, **11**(4): 309-313.

[5] Liu BH, Wu TS, Su MC, *et al*. Evaluation of citrinin occurrence and cytotoxicity in *Monascus* fermentation products [J]. *J Agr Food Chem*, 2005, **53**(1): 170-175.

[6] Su NW, Lin YL, Lee MH, *et al*. Ankaflavin from *Monascus*-fermented red rice exhibits selective cytotoxic effect and induces cell death on Hep G2 cells [J]. *J Agr Food Chem*, 2005, **53**(6): 1949-1954.

[7] Lin CH, Lin TH, Pan TM. Alleviation of metabolic syndrome by monascin and ankaflavin: the perspective of *Monascus* function foods [J]. *Food Funct*, 2017, **8**(6): 2102-2109.

[8] Jůslová P, Martínková L, Křen V. Secondary metabolites of the fungus *Monascus*: A review [J]. *J Ind Microbiol*, 1996, **16**(3): 163-170.

[9] Huang ZB, Xu Y, Li LS, *et al*. Two new *Monacucus* metabolites with strong blue fluorescece isolated from red yeast rice [J]. *J Agr Food Chem*, 2008, **56**(1): 112-118.

[10] Ahn J, Jung J, Hyung W, *et al*. Enhancement of monascus pigment production by the culture of *Monascus* sp. J101 at low temperature [J]. *Biotechnol Prog*, 2006, **22**(1): 338-340.

[11] Li YG, Zhang F, Wang ZT, *et al*. Identification and chemical profiling of monacolins in red yeast rice using high-performance liquid chromatography with photodiode array detector and mass spectrometry [J], *J Pharm Biomed Anal*, 2004, **35**(5): 1101-1112.

[12] Endo A. Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species [J]. *J Antibiot*, 1979, **32**(8): 852-854.

[13] Lin WY, Hsu WY, Hish CH, *et al*. Proteome changes in Caco-2 cells treated with *Monascus*-fermented red mold rice extract [J]. *J Agr Food Chem*, 2007, **55**(22): 8987-8994.

[14] Ho BY, Pan TM. The *Monascus* metabolite monacolin K reduces tumor progression and metastasis of Lewis lung carcinoma cells [J]. *J Agr Food Chem*, 2009, **57**(18): 8258- 8265.

[15] Du QZ, Xia M, Ito Y. Preparation of mevinolinic acid from *Monascus purpureus* using high-speed countercurrent chromatography (HSCCC) [J]. *J Liq Chromatogr R T*, 2003, **26**(18): 3085-3092.

[16] Ma JY, Li YG, Ye Q, *et al*. Constituents of red yeast rice, a traditional Chinese food and medicine [J]. *J Agr Food Chem*, 2000, **48**(11): 5220-5225.

[17] Zhang ZH. Ali Z, Khan SI, *et al*. Cytotoxic monacolins from red yeast rice, a Chinese medicine and food [J]. *Food Chem*, 2016, **202**: 262-268.

[18] Jekkel A, Kónya A, Ilkőy E, *et al*. Microbial conversion of mevinolin [J]. *J Antibiot*, 1997, **50**(9): 750-754.

[19] Rychnovsky SD, Rogers BN, Richardson TI. Configurational assignment of polyene macrolide antibiotics using the [^{13}C] acetonide analysis [J]. *Acc Chem Res*, 1998, **31**(1): 9-17.

[20] Liu MT, Wang AL, Sun Z, *et al*. Cytotoxic monacolin analogs from *Monascus purpureus*-fermented rice [J]. *J Asian Nat Prod Res*, 2013, **15**(6): 600-609.

[21] Zhu L, Yau LF, Lu JG, *et al*. Cytotoxic dehydromonacolins from red yeast rice [J]. *J Agr Food Chem*, 2012, **60**(4): 934-939.

[22] Qiao LR, Xie D, Liu Q, *et al*. Microbial transformation of lovastatin by *Beauveria bassiana* [J]. *Acta Pharm Sin B*, 2012, **2**(3): 300-305.

[23] Vyas KP, Kari PH, Pitzenberger SM, *et al*. Biotransformation of lovastatin. I. Structure elucidation of *in vitro* and *in vivo* metabolites in the rat and mouse [J], *Drug Metab Dispos*, 1989, **18**(2): 203-211.

[24] Sorensen JL, Uclair K, Kennedy J, *et al*. Transformations of cyclic nonaketides by *Aspergillus terreus* mutants blocked for lovastatin biosynthesis at the *lovA* and *lovC* genes [J]. *Org Biomol Chem*, 2003, **1**(1): 50-59.

Cite this article as: LIU Bing-Yu, XU Fei, BAI Jian, YAN Dao-Jiang, ZHANG Le, ZHANG Dan, HU You-Cai. Six new monacolin analogs from red yeast rice [J]. *Chin J Nat Med*, 2019, **17**(5): 394-400.