Bioorganic & Medicinal Chemistry Letters 23 (2013) 3905–3909

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Anthraquinone derivatives from *Rumex* plants and endophytic *Aspergillus fumigatus* and their effects on diabetic nephropathy

Yang Yang [a,c,†], Yong-Ming Yan [a,†], Wei Wei [d,†], Jie Luo [a], Lan-Sheng Zhang [a], Xiao-Jiang Zhou [d], Peng-Cheng Wang [a], Yong-Xun Yang [b], Yong-Xian Cheng [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China
[b] College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, People's Republic of China
[c] University of Chinese Academy of Sciences, Beijing 100039, People's Republic of China
[d] College of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, People's Republic of China

ARTICLE INFO

Article history:
Received 14 January 2013
Revised 26 March 2013
Accepted 23 April 2013
Available online 30 April 2013

Keywords:
Anthraquinone
Oxanthrone C-glycoside
Diabetic nephropathy
Rumex
Endophytic *Aspergillus fumigatus*

ABSTRACT

Two new oxanthrone C-glycosides, patientosides A (**14**) and B (**15**), together with three known ones (**11**–**13**), were isolated from *Rumex patientia*. Their structures were identified on the basis of spectroscopic methods. The absolute configuration for **14** and **15** were deduced by analysis of their CD spectra and comparison with those of known similar compounds. Compounds **11**–**15**, and 14 known anthraquinones (**1**–**4**, **6**–**10**, **16**–**20**) previously isolated from *Rumex nepalensis*, *Rumex hastatus*, and endophytic *Aspergillus fumigatus*, respectively, as well as a commercially available compound rhein (**5**) were evaluated for their inhibitory effects on IL-6 and extracellular matrix production in mesangial cells.

Diabetic nephropathy (DN) is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. DN is also a major complication of diabetes and a leading cause of end-stage renal disease (ESRD).[1,2] Increasing clinical evidences shows that chronic inflammation, overproduction of extracellular matrix and reactive oxygen species in renal cells were implicated in the progression of DN. Therefore, research targeting on these pathogenic factors may contribute to the intervention of DN.

The genus *Rumex* (Polygonaceae) comprises approximately 200 species which were widely distributed in the world, and China has 26 species.[3] *Rumex* plants contain anthraquinones,[4–10] which showed various pharmacological properties, such as antitumor,[11] antimutagenicity,[12] and antioxidant activities.[7] As our continuous study on the DN prevention and therapy.[13,14] The root of *Rumex patientia* was investigated which led to the isolation of two new oxanthrone C-glycoside, patientosides A (**14**) and B (**15**), together with three known ones (**11**–**13**) (Fig. 1). These five oxanthrone C-glycosides (**11**–**15**) and commercially available rhein (**5**), as well as previously isolated anthraquinone derivatives from *Rumex nepalensis* (**1**–**4**, **6**–**10**), *Rumex hastatus* (**1**, **2**, **6**, **8**, **9**), and endophytic *Aspergillus fumigatus* isolated from the leaves of *Trifolium repens* (**16**–**20**) were evaluated for their potential on DN.

Compound **14**[15] was isolated[16] as a yellow amorphous solid. Its molecular formula was assigned as $C_{22}H_{24}O_{10}$ by HR-EI-MS peak at *m*/*z* 448.1362 ([M]$^+$, calcd for 448.1369). The UV spectra of **14** showed maximum absorptions at 211, 252, 275, and 366 nm, characteristic of a highly conjugated system.[17] The IR absorptions indicated the presence of hydroxyl (3441, 3425 cm^{-1}) and carbonyl (1639 cm^{-1}) groups. The ^{1}H NMR spectra showed two *meta*-coupled aromatic proton spin systems: δ_H 6.73 (1H, br s, H-2), 7.23 (1H, br s, H-4), 7.07 (1H, d, J = 2.4 Hz, H-5), and 6.41 (1H, d, J = 2.4 Hz, H-7). Analysis of the ^{13}C NMR and DEPT spectrum of **14** showed 22 carbon resonances, including two methyls (one methyl at δ_C 22.2 and one methoxyl at δ_C 56.1), one oxymethylene (δ_C 63.1), four aromatic methines, five sp^3 oxymethines, and ten quaternary carbons, of which one was carbonyl (δ_C 192.5). The ^{1}H and ^{13}C NMR spectroscopic data of **14** (Table 1) were similar to those of rumejaposide E,[5] an oxanthrone C-glycoside, except for the presence of an additional methoxyl group (δ_C 56.1). The methoxyl group was located at C-6 by the HMBC correlations from protons (δ_H 3.91) to C-6 (Fig. 2). The Cotton effects of **14** in the range of 195–400 nm (Fig. 3) were similar to those of mayoside (10*R*) and opposite to

* Corresponding author. Tel./fax: +86 871 65223048.
E-mail address: yxcheng@mail.kib.ac.cn (Y.-X. Cheng).
† These authors contributed equally to this work.

0960-894X
http://dx.doi.org/10.1016/j.bmcl.2013.04.059

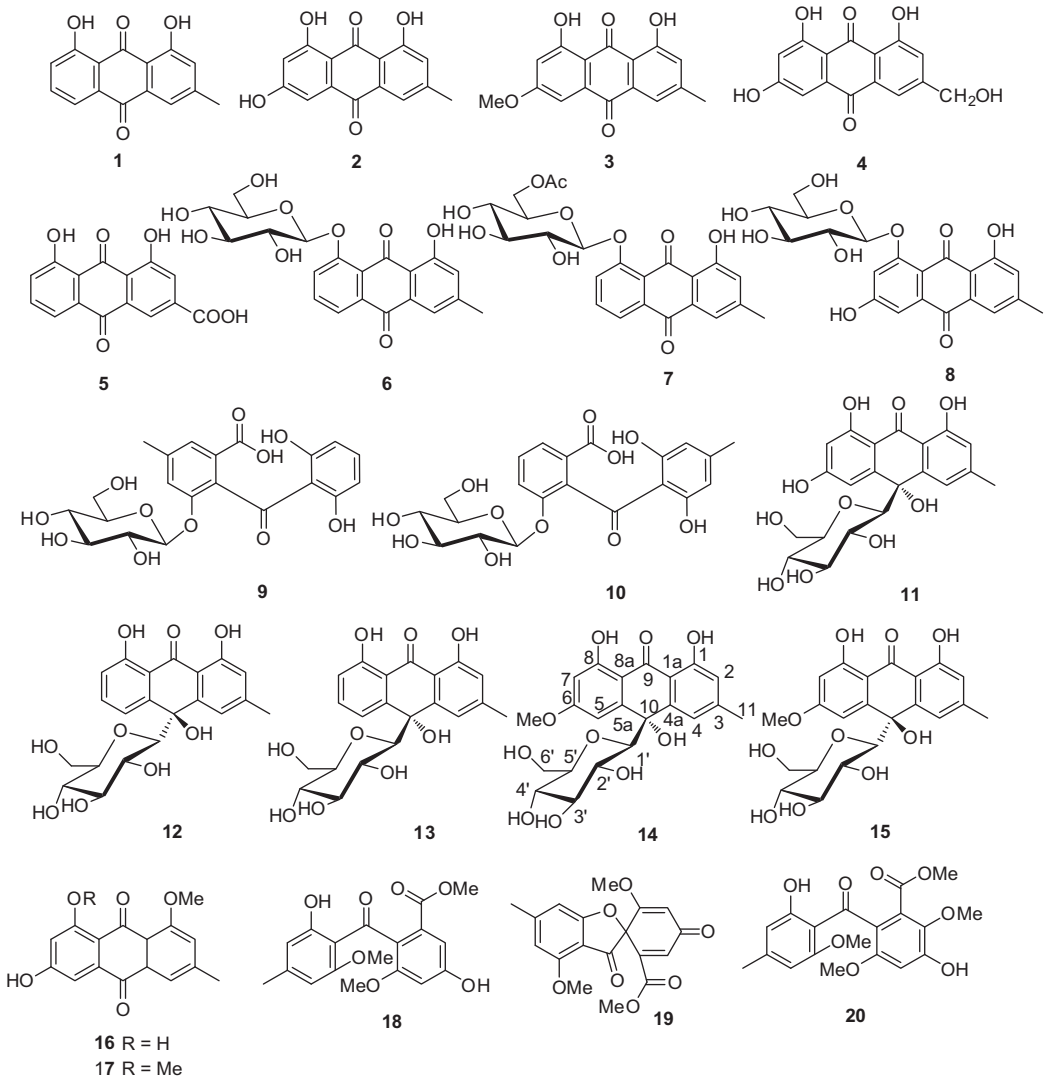

Figure 1. The structure of compounds **1**–**20**.

Table 1
^{1}H and ^{13}C NMR data of compound **14**[a] and **15**[a] in CD_3COCD_3

No.	**14**		**15**	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1		162.6		162.1
1a		114.8		114.7
2	6.73, br s	117.8	6.72, br s	117.6
3		147.2		148.0
4	7.23, br s	119.9	7.23, br s	118.7
4a		150.9		148.0
5	7.07, d, 2.4	106.2	6.95, d, 2.2	107.5
5a		146.0		148.4
6		167.0		166.4
7	6.41, d, 2.4	100.7	6.44, d, 2.2	100.6
8		164.9		165.4
8a		111.0		111.1
9		192.5		192.5
10		76.5		76.7
11	2.39, s	22.2	2.38, s	22.4
1′	3.29, d, 9.5	84.6	2.26, d, 9.4	84.4
2′	3.06, dd, 9.1, 9.1	72.7	3.13, m	72.6
3′	3.35, overlap	79.2	3.35, m	79.2
4′	2.93, m	71.6	2.93, m	71.6
5′	2.99, m	81.1	2.95, d, 5.2	81.0
6′	3.52, br d, 11.7	63.1	3.50, br d, 10.2	63.1

Table 1 (*continued*)

No.	**14**		**15**	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
	3.36, overlap		3.40, m	
OMe	3.91, s	56.1	3.92, s	56.1
1-OH	11.9, br s		11.9, br s	
8-OH	12.2, br s		12.2, br s	

[a] ^{1}H NMR recorded in 500 MHz, ^{13}C NMR recorded in 125 MHz.

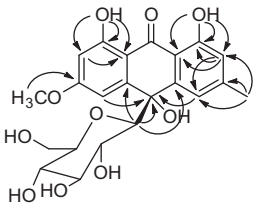

Figure 2. The key HMBC correlations of compound **14**.

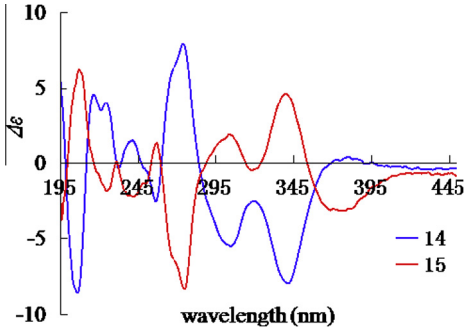

Figure 3. The CD spectrum of compounds **14** and **15**.

those of saroside (10*S*),[18] indicating the 10*R* configuration of **14**. Therefore, the structure of **14** was determined to be (10*R*)10-*C*-β-glucopyranosyl-1,8-dihydroxy-3-methyl-6-methoxy-9(10*H*)-anthracenone, named patientoside A.

Compound **15**[19] was also isolated as a yellow amorphous solid, and has the molecular formula of $C_{22}H_{24}O_{10}$ deduced from its HR-EI-MS (*m*/*z* 448.1363 ([M]$^+$, calcd for 448.1369), which was the same as compound **14**. The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) of compounds **14** and **15** were highly similar, except for C-4a and C-5a, suggesting the difference of the configuration at C-10. The Cotton effects in the range of 195–400 nm of **15** are opposite to those of patientoside A, which further confirmed our above conclusion. Thus, compound **15** was determined to be (10*S*)10-C-*β*-glucopyranosyl-1,8-dihydroxy-3-methyl-6-methoxy-9(10*H*)-anthracenone, named patientoside B.

The known compounds from *R. patientia* were each identified as rumejaposide E (**11**),[5] cassialoin (**12**),[20] and rumejaposide I (**13**)[20] by comparison their spectroscopic data and the Cotton effects with those in the literatures.

Rumex plants were traditionally used for the treatment of renal and urogenital disorders, the isolated compounds from *Rumex* plants and *A. fumigatus* were therefore screened for their inhibition on the secretion of IL-6 and overproduction of extracellular matrix production in high-glucose-induced mesangial cells. The results showed that compounds **1**–**4**, **6**–**9**, **14**–**20** could significantly inhibit the secretion of IL-6 at 10 μM (Fig. 4),[21–24] whereas, rhein is inactive even at the concentration of 35 μM (data not shown). In contrast, rhein has been proved effective in the treatment of experimental DN, but it is active in vitro assay at a relatively high concentration (88 μM).[25] The difference of potency between rhein and the isolates implied that the carboxylic acid group in the structure of rhein is unfavorable for keeping the activity, and these isolates may be more potent than rhein against DN. The overproduction of extracellular matrix production is implicated in the pathogenic process of DN and chronic kidney diseases. Our investigation on extracellular matrix production inhibition showed that compounds **1**–**3**, **9**, **11**, **14**, **17**–**20** could significantly decrease collagen IV and fibronectin production at 10 μM (Figs. 5 and 6). In addition, cell viability assay[26] showed that all the compounds are not cytotoxic at 10 μM (data not shown). The above data suggested that anthraquinone derivatives are of great value for anti-DN drug optimization.

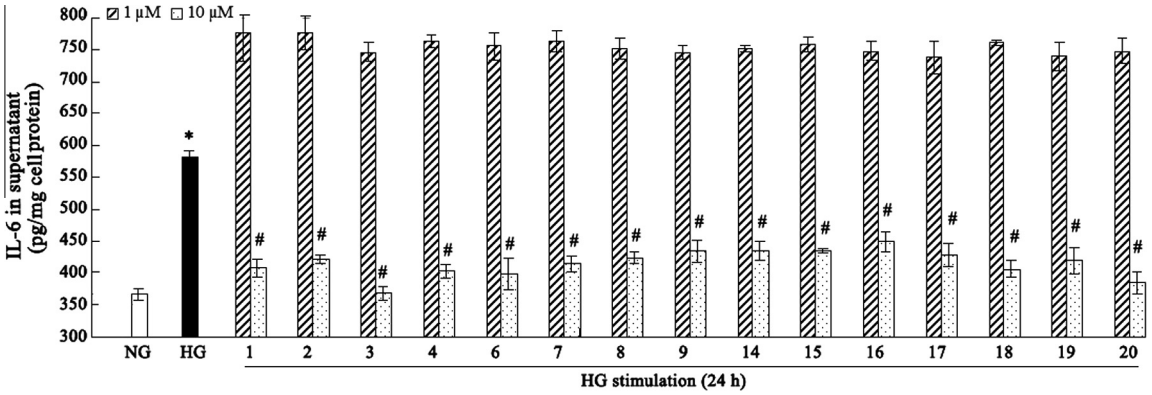

Figure 4. Inhibitory effect of the compounds on IL-6. $^*P$ <0.05 versus normal glucose; $^\#P$ <0.05 versus high glucose. chrysophanol (**1**), emodin (**2**), physcion (**3**), aloe-emodin (**4**), chrysophanol-8-O-β-D-glucopyranoside (**6**), emodin-8-O-β-D-(6)-O-acetyl)glucopyranoside (**7**), emodin-8-O-β-D-glucopyranoside (**8**), nepalenside A (**9**), nepalenside B (**10**), 1-O-methylemodin (**16**), questin (**17**), 1,2-seco-trypacidin (**18**), trypacidin (**19**), and asperfumin (**20**). Rhein (**5**) was from a commercial source.

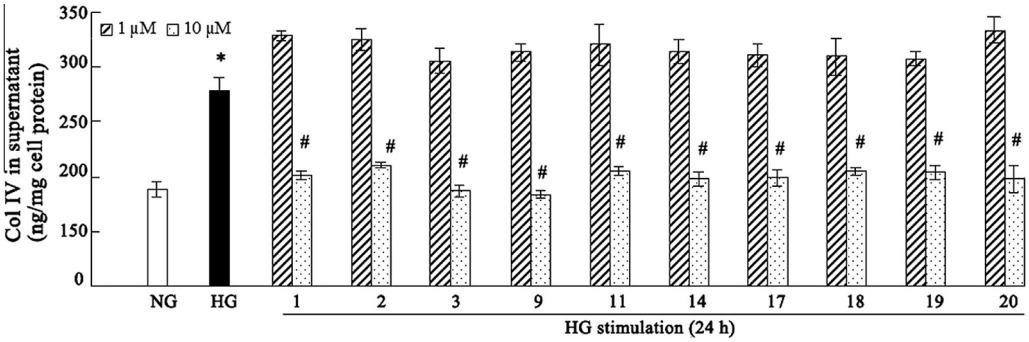

Figure 5. Inhibitory effect of the compounds on collagen IV. $^{*}P$ <0.05 versus normal glucose; $^{\#}P$ <0.05 versus high glucose.

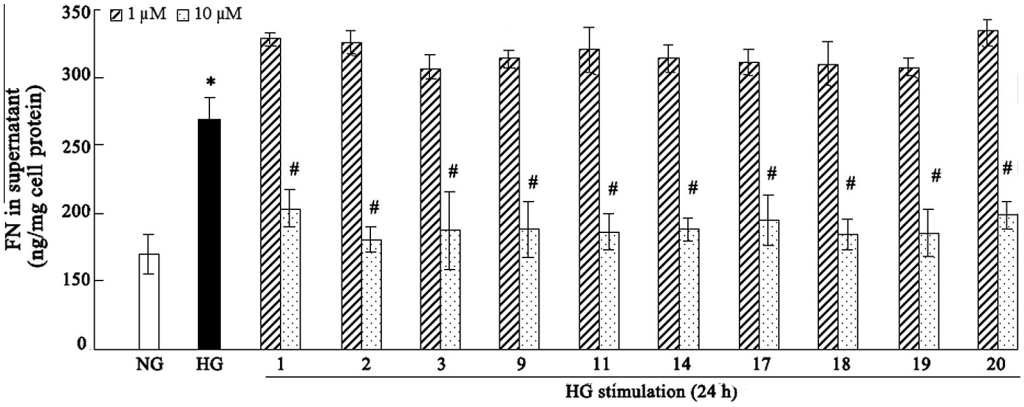

Figure 6. Inhibitory effect of the compounds on fibronectin. $^{*}P$ <0.05 versus normal glucose; $^{\#}P$ <0.05 versus high glucose.

Acknowledgements

This work was financially supported by the following grants: National Natural Science Foundation of China (20972165), Key Project for Drug Innovation (2012ZX09103-201-052) from the Ministry of Science and Technology of China, the Open Research Fund of State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources, the Key Research Program of the Chinese Academy of Sciences (Grant No. KSZD-EW-Z-004) and Project of Natural Compound Library Construction from Chinese Academy of Sciences (KSCX2-EW-R-15).

References and notes

1. Berkman, J.; Rifkin, H. *Metabolism* **1973**, *22*, 715.
2. Giunti, S.; Barit, D.; Cooper, M. E. *Minerva Med.* **2006**, *97*, 241.
3. Zhang, H.; Guo, Z.; Wu, N.; Xu, W.; Han, L.; Li, N.; Han, Y. *Molecules* **2012**, *17*, 843.
4. Khetwal, K. S.; Manral, K.; Pathak, R. P. *Ind. Drugs* **1987**, *24*, 328.
5. Jiang, L.; Zhang, S.; Xuan, L. *Phytochemistry* **2007**, *68*, 2444.
6. Wang, Z. Y.; Zhao, H. P.; Zuo, Y. M.; Wang, Z. Q.; Tang, X. M. *Chin. Chem. Lett.* **2009**, *20*, 839.
7. Demirezer, L. O.; Kuruüzüm-Uz, A.; Bergere, I.; Schiewe, H. J.; Zeeck, A. *Phytochemistry* **2001**, *58*, 1213.
8. Mei, R. Q.; Liang, H. X.; Wang, J. F.; Zeng, L. H.; Lu, Q.; Cheng, Y. X. *Planta Med.* **2009**, *75*, 1162.
9. Zhang, L. S.; Li, Z.; Mei, R. Q.; Liu, G. M.; Long, C. L.; Wang, Y. H.; Cheng, Y. X. *Helv. Chim. Acta* **2009**, *92*, 774.
10. Liang, H. X.; Dai, H. Q.; Fu, H. A.; Dong, X. P.; Adebayo, A. H.; Zhang, L. X.; Cheng, Y. X. *Phytochem. Lett.* **2010**, *3*, 181.
11. Kimura, Y.; Sumiyoshi, M.; Taniguchi, M.; Baba, K. *Cancer Sci.* **2008**, *99*, 2337.
12. Lee, N. J.; Choi, J. H.; Koo, B. S.; Ryu, S. Y.; Han, Y. H.; Lee, S. I.; Lee, D. U. *Biol. Pharm. Bull.* **2005**, *28*, 2158.
13. Tong, X. G.; Zhou, L. L.; Wang, Y. H.; Xia, C. F.; Wang, Y.; Liang, M.; Hou, F. F.; Cheng, Y. X. *Org. Lett.* **1844**, *2010*, 12.
14. Cheng, Y. X.; Zhou, L. L.; Yan, Y. M.; Chen, K. X.; Hou, F. F. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7434.
15. Patientoside A (**14**): yellow amorphous solid; $[\alpha]^{22.5}_{D}$ −56.6 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 367 (3.46), 276 (3.17), 252 (3.11), 211 (3.75) nm; CD (*c* 0.1, MeOH) $\Delta\varepsilon_{206}$ −7.91, $\Delta\varepsilon_{274}$ +7.47, $\Delta\varepsilon_{305}$ −5.16, $\Delta\varepsilon_{341}$ −7.48; IR (KBr) ν_{max} 3441, 3425, 2921, 2852, 1639, 1619, 1482, 1378, 1290, 1267, 1216, 1160, 1085, 1019 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EI-MS: *m*/*z* 448 $[M]^{+}$; HR-EI-MS: *m*/*z* 448.1362 $[M]^{+}$ (calcd for $C_{22}H_{24}O_{10}$, 448.1369).
16. Isolation: The air-dried roots of *R. patientia* (19 kg) were ground into powders, and extracted with 95% EtOH (40 L × 4) at room temperature to give a residue (2.4 kg) after removal of solvent under reduced pressure. The combined extracts were suspended in H_2O and followed by successive partition with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extracts (245 g) were divided into seven fractions by silica gel column chromatography eluted with increasing MeOH in $CHCl_3$ to afford to fractions A–G. Fraction D (7.0 g) was subjected to a MCI gel CHP 20P column eluted with gradient aqueous MeOH (30–100%) to yield fractions D1–D6. Fraction D3 (700 mg) was passed through a Sephadex LH-20 column (MeOH) to afford fractions D3a–D3d, D3c (260 mg) was separated on silica gel column with $CHCl_3$–MeOH (9:110:1) as solvents to afford compounds **11** (9 mg), **14** (7 mg) and 15 (10 mg). Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatograph, the column used was a 250 mm 4.6 mm i.d., 5 μm, Daicel Chiralpak IC. And further purification via semi-preparative HPLC (hexane/EtOH, 6:94, flow rate: 1 mL/min, detection at 230 nm) gave **12** (2.5 mg, t_R = 38.1 min) and **13** (3.2 mg, t_R = 42.4 min).
17. Harding, W. W.; Henry, G. E.; Lewis, P. A.; Jacobs, H.; McLean, S.; Reynolds, W. F. *J. Nat. Prod.* **1999**, *62*, 98.
18. Hernandez-Medel, M. D. R.; Ramirez-Corzas, C. O.; Rivera-Dominguez, M. N.; Ramirez-Mendez, J.; Santillan, R.; Rojas-Lima, S. *Phytochemistry* **1999**, *50*, 1379.
19. Patientoside B (**15**): yellow amorphous solid; $[\alpha]^{25}_{D}$ +1.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 367 (3.43), 276 (3.16), 253 (3.11), 211 (3.72) nm; CD (*c* 0.1, MeOH) $\Delta\varepsilon_{207}$ +6.32, $\Delta\varepsilon_{275}$ −8.40, $\Delta\varepsilon_{304}$ +1.93, $\Delta\varepsilon_{340}$ +4.69; IR (KBr) ν_{max} 3441, 2924, 2855, 1620, 1482, 1378, 1306, 1258, 1160, 1093, 985 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EI-MS: *m*/*z* 448 $[M]^{+}$; HR-EI-MS: *m*/*z* 448.1363 $[M]^{+}$ (calcd for $C_{22}H_{24}O_{10}$, 448.1369)
20. Zhu, J. J.; Zhang, C. F.; Zhang, M.; Annie Bligh, S. W.; Yang, L.; Wang, Z. M.; Wang, Z. T. *J. Chromatogr. A* **2012**, *1217*, 5383.
21. Cell culture and reagents: The established rat mesangial cell line (RMC) HBZY-1 was obtained from The Chinese Center for Type Culture Collection (Wuhan, China). The cells were cultured in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in an atmosphere containing 5% CO_2. RMC between passages 3 and 10

were used for experiments. After pre-incubation in DMEM supplemented with 0.1% fetal calf serum for 24 h, cells were then treated with normal glucose group (NG, 5.6 mM glucose), high glucose group (HG, 25 mM glucose) or mannitol group (MN, 5.6 mM glucose and 19.4 mM mannitol). DMEM (5.6 mM or 25 mM glucose) was purchased from GIBCO BRL (GIBCO/Invitrogen Corp., Carlsbad, CA, USA). Mannitol were from Sigma (St. Louis, MO, USA).

22. Inhibition of fibronectin, collagen IV, and IL-6 secretion: Rat mesangial cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) containing 5.6 mM D-glucose (pH 7.4; Sigma Chemical Co., St. Louis, MO), supplemented with 20% fetal calf serum (FCS; Invitrogen), 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES. After the mesangial cells reached 80% confluence, their growth was arrested in 0.5% FCS for 24 h. Exposure of the mesangial cells to medium containing high-concentration glucose induced the overproduction of fibronectin (FN), collagen IV (Col IV), and IL-6, as described in the previous reports.[23,24] To determine whether the compounds inhibited the FN, collagen IV, and IL-6 overproduction triggered by high glucose, the mesangial cells were pretreated with 1 or 10 μM of each compound for 1 h and then stimulated with high glucose for 24 h. The levels of supernatant FN, collagen IV, and IL-6 were measured with a solid-phase quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit for FN, collagen IV, and IL-6 (Uscn Life Science Inc.). The concentration in the culture supernatant was normalized to the total amount of cell protein, quantified with the BCA method.[25] Statistical analysis: The differences were tested using ANOVA. All values are expressed as mean ± SD, and statistical significance was defined as $P < 0.05$.

23. Min, D. Q.; Lyons, J. G.; Bonner, J.; Twigg, S. M.; Yue, D. K.; McLennan, S. V. *Am. J. Physiol. Renal Physiol.* **2009**, *297*, F1229.
24. Xia, L.; Wang, H.; Goldverg, H. J.; Munk, S.; Fantus, I. G.; Whiteside, C. I. *Am. J. Physiol. Renal Physiol.* **2006**, *290*, F345.
25. Zheng, J. M.; Zhu, J. M.; Li, L. S.; Liu, Z. H. *Br. J. Pharmacol.* **2008**, *153*, 1456.

26. MTT assay: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure cell viability, Briefly, cells were seeded at 10^4 cells/well in 96-well plates. Before experiments, the medium was removed and replaced with serum-free medium for 24 h incubation. Cells were incubated in presence or absence of compounds (10 μM) for 48 h. Then 50 μL of MTT (5 mg/mL) was added to each well and incubation continued at 37 °C for additional 4 h. The medium was then carefully removed, so as not to disturb the formazan crystals formed. Dimethyl sulphoxide (DMSO; 150 μL), which solubilized the formazan crystals, was added to each well and the absorbance of solubilized blue formazan read at wavelength of 570 nm using a microplate reader. The reduction in optical density was used as a measurement of cell proliferation, normalized to cells incubated in a control medium.