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FULL PAPER

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Three Previously Undescribed Chlorophenyl Glycosides from the Bulbs of *Lilium regale*

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Three previously undescribed chlorophenyl glycosides, (2,4,6-trichloro-3-hydroxy-5-methoxyphenyl)methyl β-D-glucopyranoside (**1**), (2,4-dichloro-3,5-dimethoxyphenyl)methyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (**2**) and 4-chloro-3-methoxy-5-methylphenyl 6-*O*-(6-deoxy-β-L-mannopyranosyl)-β-D-glucopyranoside (**3**) were obtained from *Lilium regale*. The absolute configurations of these new finds were elucidated by comprehensive analyses of spectroscopic data combined with acid hydrolysis derivatization. (2,4-dichloro-3,5-dimethoxyphenyl) methyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (**2**) can inhibit the proliferation of lung carcinoma A549 cells with an IC_{50} value of 29 μM.

Keywords: *Lilium regale*, chlorophenyl glycosides, structural elucidation, anti-proliferative activity.

Introduction

Lilium is a large genus of Liliaceae widely distributed in the northern hemisphere.[1] In China, several *Lilium* species have been used as traditional medicine for the treatment of lung ailments.[2] For example, Baihe Wuyao decoction (BWD), a prescription of Traditional Chinese Medicines, composed of Lilii Bulbus and Linderae Radix, has been used to treat epigastric pain and superficial gastritis for hundreds of years in China.[3] Previous phytochemical investigations indicated that *Lilium* contains several steroidal saponins, phenylpropanoid glycosides as well as phenyl glycosides.[4–8]

To search for new bioactive constitutes of *Lilium*. *Lilium regale* Wilson was selected and three new chlorophenyl glycosides (**1**-**3**) were obtained from its bulb subsequently (*Figure 1*). The structures of these new findings were elucidated by comprehensive spectroscopic analyses. The configurations of monosaccharide moiety were confirmed by HPLC analyses after an acid hydrolysis and derivatization reaction combined with NMR data. This article described the isolation, structure elucidation as well as anti-proliferative activity of these previously undescribed compounds.

Results and Discussion

Compound **1** was obtained as a white powder. Its molecular formula was assigned as HR-ESI-MS by the pseudo-molecular ion peak at *m/z* 440.9898 $[M+Na]^+$ (calculated for 440.9882, $C_{14}H_{17}Cl_3O_8$). Besides, the isotopic molecular ion peaks at *m/z* 442.9870, 444.9843 indicated that compound **1** contains three chlorine atoms. The ^{1}H-NMR spectrum indicated the presence of two oxy-methylene protons at δ 5.15 (1H, d, $J=11.3$) and 4.97 (1H, d, $J=11.3$), the anomeric proton at δ 4.39 (1H, d, $J=7.7$) and an oxy-methyl protons at δ 3.85 (3H, s). The ^{13}C-NMR spectrum of compound **1** indicated the presence of a methoxy carbon at δ 61.0 (3-OMe), an oxy-

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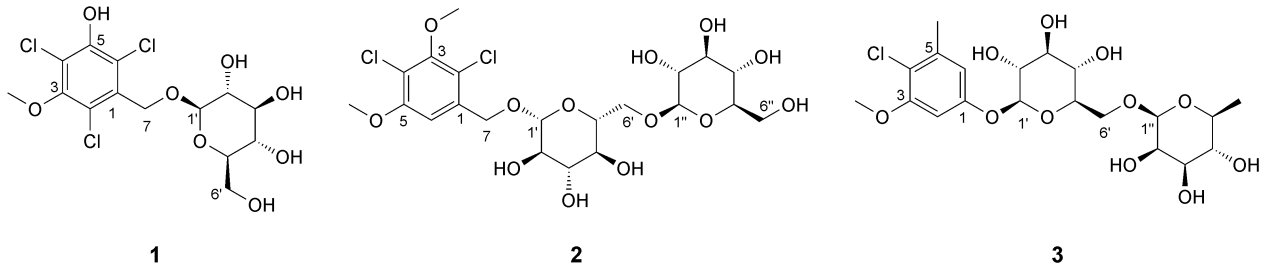

Figure 1. Structures of compounds **1 – 3**.

methylene carbon at δ 67.2 (C-7), a set of one glucosyl moiety at δ 62.6 (C-6′), 71.4 (C-4′), 75.0 (C-2′), 78.0 (C-5′), 78.1 (C-3′), 104.1 (C-1′), three chlor-bearing aromatic carbons at δ 119.2 (C-4), 121.3 (C-2), 122.8 (C-6) and two oxy-bearing carbons at δ 150.8, 153.0. Besides, The HMBCs from δ 5.15 (Ha-7), 4.97 (Hb-7) to 121.3 (C-2), 122.8 (C-6), 133.8 (C-1) indicated the aglycon should be 2,4,6-trichloro-3-hydroxy-5-methoxyphenylethanol. The sugar obtained from the hydrolysate was identified as D-glucose (*Figure 2*). The coupling constant of the anomeric proton (d, $J=7.7$) indicated the β-configuration for the D-glucopyranosyl after an acid hydrolysis and derivatization reaction. The HMBCs from δ 5.15 (H-7), 4.97 (H-7) to δ 104.1 as well as from δ 4.39 (H-1′) to δ 67.2 indicated the glucosyl moiety was connected to C-7 of the aglycon. Finally, compound **1** was elucidated as (2,4,6-trichloro-3-hydroxy-5-methoxyphenyl)methyl β-D-glucopyranoside.

Compound **2** was obtained as faint yellow powder. Its molecular formula was established by HR-ESI-MS at *m/z* 583.0975 $[M+Na]^+$ (calculated for 583.0956, $C_{21}H_{30}Cl_2O_{13}$). The isotopic molecular ion peak at *m/z*: 585.0950 also indicated that **2** contains two chlorine atoms. The ^{1}H-NMR spectrum of **2** suggested the presence of an aromatic proton at δ 7.39 (H-6), two oxymethylene protons at δ 4.82 (1H, d, $J=14.7$, H-7) and 4.72 (1H, d, $J=14.7$, H-7), two methoxy groups at δ 3.88 (5-OMe) and 3.80 (3-OMe). The sugar components also were further identified as D-glucose by HPLC analysis after acid hydrolysis and derivatization of **2**. The anomeric protons at δ 4.28 (1H, d, $J=7.7$, H-1″) and 4.27(1H, d, $J=7.8$, H-1′) indicated both of two glucosyl moieties were β-configured. The ^{13}C-NMR spectrum for the aglycon moiety (*Table 1*) of **2** was in good agreement with those of curculigine H.[9] The aglycon moiety was also confirmed by the HMBCs from 4.82, 4.72 to 136.3 (C-

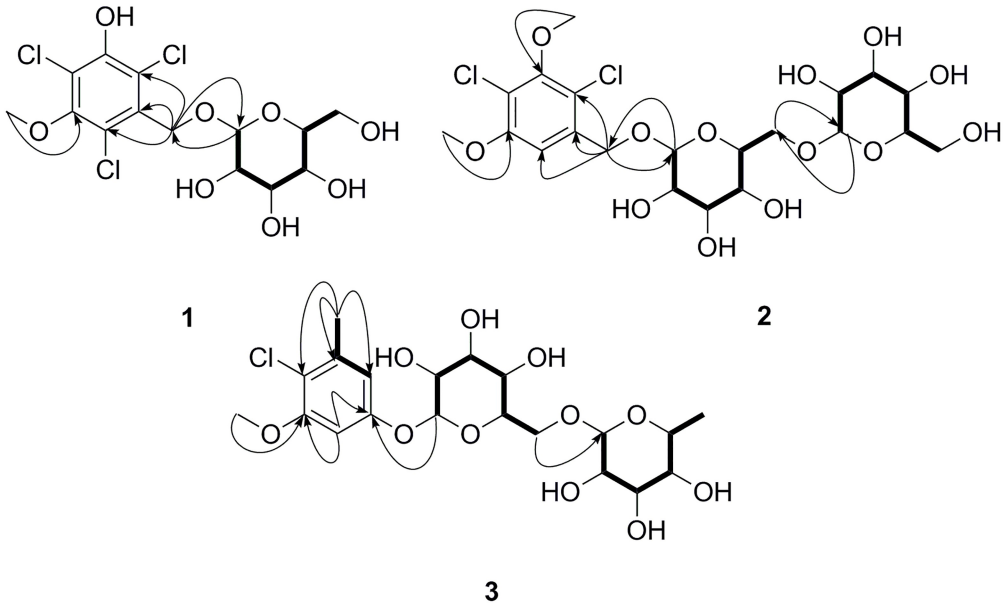

Figure 2. Key HMBC (H→C) and ^{1}H,^{1}H-COSY (–) correlations of **1 – 3**.

Table 1. ^{1}H-NMR and ^{13}C-NMR data of **1**–**3**[a].

No.	**1**[b]		**2**[b]		**3**[b]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	–	133.8	–	136.3	–	155.0
2	–	121.3	–	117.4	6.68 (d, *J* = 1.7)	110.8
3	–	153.0	–	152.2	–	157.1
4	–	119.2	–	115.1	–	139.0
5	–	150.8	–	154.4	–	110.1
6	–	122.8	7.39 (s)	107.8	6.59 (d, *J* = 1.6)	108.1
7	5.15 (d, *J* = 11.3) 4.97 (d, *J* = 11.3)	67.2	4.82 (d, *J* = 14.7) 4.72 (d, *J* = 14.7)	66.4		
3-OMe	3.85 (s)	61.0	3.80 (s)	60.4	3.84 (s)	56.7
5-OMe			3.88 (s)	56.6		
5-Me					2.34 (s)	22.1
1′	4.39 (d, *J* = 7.7)	104.1	4.27 (d, *J* = 7.8)	102.3	4.90 (d, *J* = 7.7)	102.3
2′	3.17–3.20 (m)	75.0	3.16–3.19 (m)	76.4	3.49–3.52 (m)	74.8
3′	3.32–3.38 (m)	78.1	3.02–3.08 (m)	76.9	3.43–3.47 (m)	78.1
4′	3.32–3.38 (m)	71.4	3.09–3.17 (m)	73.4	3.34–3.36 (m)	71.4
5′	3.26–3.32 (m)	78.0	3.35–3.37 (m)	75.9	3.54–3.61 (m)	77.0
6′	3.72 (dd, *J* = 11.9, 5.3) 3.84–3.88 (m)	62.6	4.00 (dd, *J* = 11.6, 1.7) 3.61 (dd, *J* = 11.6, 5.2)	68.3	4.01 (d, *J* = 9.4) 3.55–3.60 (m)	67.8
1″			4.28 (d, *J* = 7.7)	103.3	4.69 (d, *J* = 1.7)	102.1
2″			2.96 (t, 8.4)	73.5	3.81–3.83 (m)	72.1
3″			3.10–3.15 (m)	76.7	3.66–3.70 (m)	72.3
4″			3.02–3.10 (m)	70.0	3.33–3.36 (m)	74.1
5″			3.10–3.15 (m)	69.8	3.60–3.65 (m)	69.8
6″			3.66 (dd, *J* = 11.6, 1.9) 3.41–3.43 (m)	61.0	1.21 (d, *J* = 6.3)	17.9

[a] ^{1}H (700 MHz) and ^{13}C (175 MHz) spectra were taken on a 700 MHz NMR spectrometer. [b] **1** and **3** were measured in CD_3OD and **2** was measured in (D_6)DMSO.

1), 117.4 (C-2), 107.8 (C-6), from7.39, 3.88 to 154.4 (C-5), and from 3.80 to 152.2 (C-3). The HMBCs from 4.82, 4.72 to 102.3 as well as from 4.00 (H-1′), 3.61 (H-1′) to 103.3 (C-1″) suggested that the structure of **2** should be (2,4-dichloro-3,5-dimethoxyphenyl)methyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

Compound **3** was obtained as a white powder too. The molecular formula of **3** was assigned based on its HR-ESI-MS ion peak as *m/z* 503.1309 $[M+Na]^+$: (calculated for 503.1291, $C_{20}H_{29}ClO_{11}$). The ^{1}H-NMR spectrum of **3** exhibited signals for two aromatic protons at δ 6.68 (d, *J* = 1.7, H-2) and 6.59 (d, *J* = 1.7, H-6), one methoxy protons at δ 3.84 (3H, s, 3-OMe), one methyl protons at δ 2.34 (3H, s) as well as two anomeric protons at δ 4.90 (1H, d, *J* = 7.7, H-1′) and 4.69 (1H, d, *J* = 1.7, H-1″). The ^{13}C-NMR spectrum of **3** showed the 20 carbon signals. With the help of HSQC spectrum, these carbon signals can be assigned as six aromatic carbons at δ 157.1 (C-3), 155.0 (C-1), 139.0 (C-4), 110.8 (C-2), 110.1 (C-5), 108.1 (C-6), ten oxy-methine carbons at δ 102.3 (C-1′), 102.1 (C-1″), 78.1 (C-3′), 77.0 (C-5′), 74.8 (C-2′), 74.1 (C-4″), 72.3 (C-3″), 72.1 (C-2″), 71.4 (C-4′), 69.8 (C-5″), one oxy-methylene at δ 67.8 (C-6′), one methoxy carbon at δ 56.7 (3-OMe) and two methyl carbons at δ 22.1 (5-Me) and 17.9 (C-6″). Subsequent HPLC analysis after acid hydrolysis and derivatization of **3** indicated the absolute configurations of monosaccharide moieties should be a D-glucose and an L-rhamnose. The ^{1}H,^{1}H-COSY correlations also indicated the presence of a β-configured glucosyl moiety (δ_H: 4.90 (H-1′, *J* = 7.7), 3.49-3.52 (H-2′), 3.43-3.47 (H-3′), 3.34-3.36 (H-4′), 3.54-3.61 (H-5′), 4.01 and 3.55-3.60 (H-6′) and an α-rhamnosyl moiety (δ_H: 4.69 (H-1″ *J* = 1.7), 3.81-3.83 (H-2″), 3.66-3.68 (H-3″), 3.33-3.36 (H-4″), 3.60-3.65 (H-5″), 1.21 (H-6″)). The HMBCs from 6.68 to 139.0, 155.0, from 3.84 to157.1, from 6.59 to 139.0, from 2.34 to 108.1, 110.1, 139.0, from 4.90 to 155.0, as well as from 4.69 to 67.8 indicated the structure of compound **3** should be 4-chloro-3-methoxy-5-methylphenyl 6-*O*-(6-deoxy-β-L-mannopyranosyl)-β-D-glucopyranoside.

Subsequent MTT assay indicated that compound **2** can inhibit the proliferation of lung carcinoma A549 cells with an IC_{50} value of 29 μM.

Conclusions

Three new chlorophenyl glycosides had been isolated from the bulk of *L. regale*. The absolute configurations of new compounds were elucidated by interpretation of spectroscopic spectra including NMR and HR-Orbitrap-IMS, as well as the results of hydrolysis reactions. Amount of these new compounds, **2** exhibited moderate growth inhibition against lung carcinoma A549 cell lines with an IC_{50} value of 29 μM.

Experimental Section

General

The optical rotations were recorded on a perkin-Elmer-241 polarimeter (PerkinElmer, Inc., Waltham, MA, USA). The IR spectra were recorded on a Cary 600 Series FT-IR (KBr) spectrometer (PerkinElmer, Inc., Waltham, MA, USA). The HR-ESI-MS data were obtained using a Q Exactive UHMR Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, MA, USA). The 1D and 2D NMR data were obtained using a Bruker Bruker-Ascend-700-MHz spectrometer (Bruker Corporation, Billerica, MA, USA). The semi-preparative HPLC was conducted by an NP7000 serials instrument with a U3000 serial UV detector (Hanbon Sci. & Tech, Jiangsu, China) using a YMC-Pack ODS-A column (10×250 mm, 5 μm) (YMC Co., Ltd. Kyoto, Japan). The analytic HPLC was performed on an Ultimate 3000 series pump (Thermo Scientific, Waltham, MA USA) equipped with a Hypersil GOLD™ column (4.6×250 nm, 5 μm) (Thermo Fisher Scientific, MA, USA) using an Ultimate 3000 DAD detector (Thermo Scientific, Waltham, MA USA). Column chromatography was performed using AB-8 macroporous resin (Changfeng Chemical Co., Ltd, Shifang, China) and reversed-phase silica gel (20–40 μm, 100 A; Welch Materials, Inc., Shanghai, China). Thiazolyl blue was purchased from Sigma-Aldrich (USA). L-rhamnose and D-glucose were purchased from Energy Chemical (Chengdu, China). L-cysteine methyl ester hydrochloride was purchased from Chroma-Biotechnology Co. Ltd. (Chengdu, China). Phenyl isothiocyanate was purchased from Aladdin (Chengdu, China). All solvents used were of analytical grade.

Plant Material

L. regale Wilson was collected from Chengdu (Sichuan Province, China) in August 2020 and identified by Prof. Pei Jin, Chengdu University of TCM. A voucher specimen (cd20200808) was deposited in the State Key Laboratory of Southwestern Chinese Medicine Resources, College of Pharmacy, Chengdu University of TCM.

Extraction and Isolation

The air-dried and powdered of *L. regale* (5.00 kg) were extracted with 90% EtOH (10 L, 4 times) under reflux. The extract (0.59 kg) was further suspended in water (2 L) and extracted by AcOEt (2 L, 3 times). The AcOEt extract was passed through an AB-8 macroporous resin (100 mm ×800 mm) eluted with H_2O (fr. 1a), MeOH-H_2O (1:1) (fr. 1b). Fr. 1b (171 g) was applied to a reversed-phase silica gel column (70 mm ×600 mm) and eluted with MeOH-H_2O (46:56, 700 mL/min) and yield five fractions (fr. 1b1-fr. 1b5, each fraction was collected with the guide of a UV detector (under 205 nm)). Fr. 1b4 (12.7 g) was applied to the reversed-phase column using an ODS-packed column (YMC-Pack ODS-A, 10×250 mm, S-5 μm) and eluted with acetonitrile-H_2O (22:78, 4 mL/min) to yield compound **1** (13.2 mg), **2** (17.8 mg) and **3** (22.5 mg).

Acid Hydrolysis and Derivatization of **1–3**

Firstly, compounds **1–3** (each 1.00 mg) were heated with trifluoroacetic acid (TFA) (2 M) for 6 h at 100 °C. The mixture was cooled and partitioned between CH_2Cl_2 (2 mL) and water layer was dried under reduced pressure. Then, L-cysteine methyl ester hydrochloride (1.5 mg) and the residue of the aqueous phase were dissolved in pyridine (0.5 mL) and heated at 60 °C for 1 h, and phenyl isothiocyanate (0.5 mL) was added to the mixture and heated at 60 °C for 1 h. The solvent was finally analyzed by HPLC (25% CH_3CN/H_2O, flow rate = 0.8 mL/min) equipped with a diode array detector (DAD) detector (under 254 nm) using a C-18 column. The absolute configuration of the monosaccharides was confirmed by comparing the retention time with those of L-rhamnopyranose (t_R = 11.53 min), and D-glucose (t_R = 14.64 min). The detailed process was described previously.[10]

(2,4,6-Trichloro-3-hydroxy-5-methoxyphenyl) methyl β-D-glucopyranoside (**1**). a white powder, $[\alpha]_D^{20} = -150$ (c = 0.01, MeOH); IR (KBr) ν_{max} 3420.1, 1636.6, 1457.3, 1402.6, 1075.3, 1039.7, 759.4 cm^{-1}; UV λ_{max} 209 (3.30) nm; HRMS: *m/z* 440.9898 $[M+Na]^+$, calc. for 440.9882. ^{1}H- and ^{13}C-NMR data: see *Table 1*.

(2,4-Dichloro-3,5-dimethoxyphenyl)methyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (**2**). a faint yellow powder, $[\alpha]_D^{20} = -45$ (c = 0.01, MeOH); IR (KBr)

ν_{max} 3400.6, 1649.6, 1026.0, 999.7, 827.2, 766.2 cm^{-1}; UV λ_{max} 201 (3.72) nm; HRMS: *m/z* 583.0975 $[M+Na]^+$, calc. for 583.0956. ^{1}H- and ^{13}C-NMR data: see *Table 1*.

4-Chloro-3-methoxy-5-methylphenyl 6-*O*-(6-deoxy-β-L-mannopyranosyl)-β-D-glucopyranoside (**3**). a white powder, $[\alpha]_D^{20} = -15.0$ (c = 0.01, MeOH); IR (KBr) ν_{max} 3420.3, 2925.5, 1649.6, 1593.8, 1457.4, 1065.3, 670.0 cm^{-1}; UV λ_{max} 202 (3.71) nm; HRMS: *m/z* 503.1309 $[M+Na]^+$, calc. for 503.1291. ^{1}H- and ^{13}C-NMR data: see *Table 1*.

Cytotoxic Activity Assay

Lung carcinoma A549 cells were used in the cytotoxicity assay. A549 cells were cultured in DEME medium supplemented with 10% fetal bovine serum in 5% CO_2 at 37 °C in a 96-well plate, each well was a plate with 10×10^5 cells. After cell attachment overnight and removed the medium, A549 cells were exposed to the test compounds at concentrations of 3.125, 6.25, 12.5, 25, 50 μM in triplicate for 72 h, and the positive control Taxol (antiproliferative activity against A549 cell lines with an IC_{50} value of 0.015 ± 0.005 μM). The cytotoxicity assay was performed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After the addition of a 20 μL MTT solution (5 mg/mL), cells were incubated for 4 h. After adding 100 mL DMSO, cells were shaken for 15 min to mix thoroughly. The absorbance of each cell was measured at 490 nm in a Multiscan photometer. The detailed process was described in our previous work.[11–13]

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Author Contribution Statement

All the authors have contributed significantly to the submitted work. J. Y., D.G., X.F., W.G., L.Z., and H.Z. performed the experiments, analyzed the data, and wrote the article. J. P. and Y.D. gave guidance and suggestions for the experiment and the article. C.P. and D.L. conceived and designed the experiments.

References

[1] J. Zhou, R. F. An, X. F. Huang, 'Genus Lilium: A review on traditional use, phytochemistry and pharmacology', *J. Ethnopharmacol.* **2021**, *270*, 113852.
[2] L. M. Luo, L. Fu, J. H. Zhan, X. G. Pei, X. J. Zhou, N.H Chen, 'Study on effects of total saponins from Lilii Bulbus on proliferation apoptosis, invasion and metastasis of lung cancer cells and its preliminary mechanism', *China J. Chin. Mater. Med.* **2018**, *43*, 4498–4505.
[3] Y. Chen, R. Li, N. Hu, C. P. Yu, H. Y. Song, Y. D. Li, Y. J. Dai, Z. Guo, M. Li, Y. Zheng, Z. Y. Guo, Y. J. Qi, 'Baihe Wuyao decoction ameliorates CCl_4-induced chronic liver injury and liver fibrosis in mice through blocking TGF-β1/Smad2/3 signaling, anti-inflammation and anti-oxidation effects', *J. Ethnopharmacol.* **2020**, *263*, 113227.
[4] A. S. Gur'eva, P. K. Kintya, N. E. Mashchenko, A. S. Shashkov, 'Glycosides of the bulbs of *Lilium regale*', *Chem. Nat. Compd.* **1996**, *32*, 384–385.
[5] Y. Mimaki, Y. Sashida, O. Nakamura, T. Nikaido, T. Ohmoto, 'Steroidal saponins from the bulbs of *Lilium regale* and L. henryi', *Phytochemistry.* **1993**, *33*, 675–682.
[6] A. F. Murray, K. Palatini, S. Komarnytsky, T. J. Gianfagna Jr., J. P. Munafo, 'Phenylpropanoid glycerol glucosides attenuate glucose production in hepatocytes', *ACS Omega.* **2019**, *4*, 10670–10676.
[7] L. Jin, Y. L. Zhang, L. M. Yan, Y. L. Guo, L. X. Niu, 'Phenolic compounds and antioxidant activity of bulb extracts of six Lilium species native to China', *Molecules* **2012**, *17*, 9361–9378.
[8] X. X. Hong, J. G. Luo, L. Y. Kong, 'Two new chlorophenyl glycosides from the bulbs of *Lilium brownie* var. *viridulum*', *J. Asian. Nat. Prod. Res.* **2012**, *14*, 769–775.
[9] Z. H. Wang, J. Huang, X. C. Ma, G. Y. Li, Y. P. Ma, N. Li, J. H. Wang, 'Phenolic glycosides from *Curculigo orchioides* Gaertn', *Fitoterapia.* **2013**, *86*, 64–69.
[10] J. F. Chen, L. Tan, F. Ju, Q. X. Kuang, T. L. Yang, F. Deng, Y. C. Gu, .L. S. Jiang, Y. Deng, D. L. Guo, 'Phenolic glycosides from Sanguisorba officinalis and their anti-inflammatory effects', *Nat. Prod. Res.* **2020**, *18*, 1–8.
[11] D. L. Guo, L. Qiu, D. Feng, X. He, X. H. Li, Z. X. Cao, Y. C. Gu, L. Mei, F. Deng, Y. Deng, 'Three new α-pyrone derivatives induced by chemical epigenetic manipulation of *Penicillium herquei*, an endophytic fungus isolated from *Cordyceps sinensis*', *Nat. Prod. Res.* **2020**, *34*, 958–964.
[12] Y. M. Cao, D. L. Guo, M. Y. Jin, L. Tan, T. L. Yang, F. Deng, Y. C. Gu, X. H. Li, Z. X. Cao, Y. Deng, 'Two new norsesquiterpenoids from *Fusarium tricinctum*, an endophytic fungus isolated from *Ligusticum chuanxiong*', *Nat. Prod. Res.* **2020**, *24*, 1–5.
[13] G. Y. Liu, L. Tan, L.i Cheng, L. S. Ding, Y. Zhou, Y. Deng, Y. Q. He, D. L. Guo, S. J. Xiao, 'Dendrobine-type alkaloids and bibenzyl derivatives from Dendrobium findlayanum', *Fitoterapia* **2020**, *142*, 104497.

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