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THREE NEW ANTI-ROTAVIRUS QUINOLINE ALKALOIDS FROM THE WHOLE PLANT OF *THALICTRUM GLANDULOSISSIMUM*

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Abstract – Three new quinoline alkaloids, 4-acetyl-6-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**1**), 4-acetyl-5-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**2**), and 4-acetyl-6-methyl-7-prenylquinolin-2(1*H*)-one (**3**) were isolated from the whole plants of *Thalictrum glandulosissimum*. Their structures were elucidated by spectroscopic methods, including extensive ^{1}H, ^{13}C, and 2D-NMR techniques. Compounds **1**-**3** were also tested for their anti-rotavirus activity. Compounds **1**-**3** exhibited potent anti-rotavirus activity with therapeutic index (TI) valves of 17.6, 15.0, and 23.6 respectively.

The genus *Thalictrum* belongs to the subfamily Thalictroideae of Ranunculaceae and consists of the subgenera *Thalictrum* and *Lecoyerium*.[1,2] This genus has around 200 species, which are distributed in Asia, Europe, Africa, North America, and South America. Around 67 species are recorded in the flora of China, and the majority of which are found in Southwest China. At least 43 species of this genus are used medicinally in China.[2,3] This genus is also well known for its diverse pharmacological activities, including anti-tumor, anti-virus, anti-microbial, anti-tuberculosis, anti-inflammatory, anti-malarial activities, and the like,[4] and the main active ingredients found in this genus are alkaloids,[5-7] flavonoids,[8,9] triterpenoid saponins,[10,11] lithospermoside,[12] and the like.

Thalictrum glandulosissimum is a herbaceous plant of *Thalictrum* genus with short rhizome and many thick fibrous roots. It distributed in Dali, Heqing, and Binchuan Prefecture, Yunnan Province, and grows on the hillside grassland at an altitude of 2500 meters.[2] The whole plants of *T. glandulosissimum* are used to treat some diseases, such as enteritis, dysentery, jaundice and throat by Bai nationality people in Yunnan Province of China, and the alkaloids are the main active components in this plants.[3,7,13,14] In the

course of identifying bioactive compounds from local plants, we now reinvestigated the chemical constituents of the whole plant of *T. glandulosissimum* collected in Heqing Prefecture, Yunnan Province. As a results, three new quinoline alkaloids (**1**-**3**) were isolated in this work. This paper describes the elucidation of the structures of these three compounds, and a preliminary evaluation of their antibacterial activity.

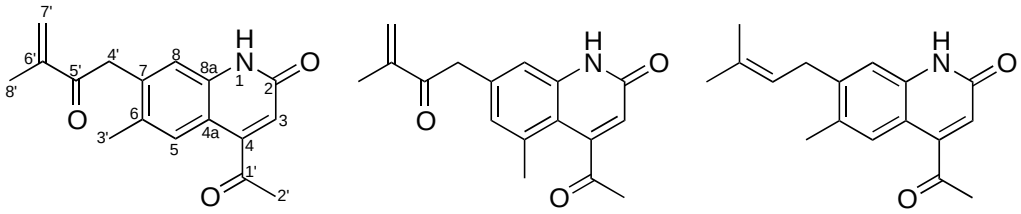

Figure 1. The new quinoline alkalids from *T. glandulosissimum*

A 95% aq. ethanol extract prepared from whole plants of *T. glandulosissimum* was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9.0 with saturated Na_2CO_3 aq. and extracted with EtOAc again. The EtOAc-soluble alkaloidal materials were subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford three new quinoline alkaloids, 4-acetyl-6-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**1**), 4-acetyl-5-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**2**), and 4-acetyl-6-methyl-7-prenylquinolin-2(1*H*)-one (**3**). The structures of compounds **1**-**3** were shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1**-**3** were listed in Table 1.

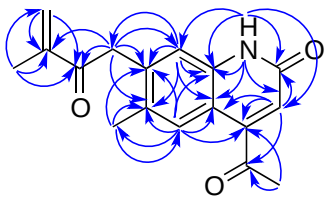

Figure 2. Key HMBC ( ) correlations of **1**

Compound **1** was obtained as a brown gum. Its (+) HRESIMS gave a quasimolecular ion at *m/z* 306.1112 $[M+Na]^+$. These data, established the molecular formula of **1** as $C_{17}H_{17}NO_3$, with ten degree of unsaturations. The IR spectrum of **1** exhibited absorption bands for amine (3276 cm^{-1}), carbonyl (1682, 1665, and 1650 cm^{-1}), and aromatic functionality (1615, 1536, and 1434 cm^{-1}). Its UV spectrum showed the maximum absorption at 342 and 235 nm was also supported the existences of the aromatic functionality. The ^{1}H, ^{13}C, and HSQC NMR data (Table 1) of **1** showed resonances due to a 1,2,4,5- tetrasubstituted benzene ring (C-5~C-8, C-4a and C-8a, H-5 and H-8), a 3-methyl-2-oxobut-3-enyl moiety [-CH_2-CO-C(=CH_2)Me, C-4'~8', H_2-4', H_2-7', and H_3-8'],[15] an acetyl group (C-1', C-2', and H_3-2'), a -NH-CO-CH-C- unit (C-2~4, H-1, and H-3).[16] In addition to the nine degree of unsaturations for the benzene ring, three carbonyl, and two double bond, the benzene ring and -NH-CO-CH-C- unit should be formed a quinolin-2(1*H*)-one system[16] to meet the ten degree of

unsaturations. These deductions were supported by the HMBC correlations (Figure. 2) from H-3 to C-2, C-4, C-4a, from NH to C-2, C-3, C-8, C-4a, C-8a, from H-8 to C-4a, C-8a, and from H-5 to C-4, C-4a, and C-8a.

Since the nucleus and the main substituent groups were determined, the substituent positions can also be determined by the further analysis of its HMBC correlations. The HMBC correlations of H-2′ with C-4, of H-3 with C-1′ indicated that the acetyl group was attached to C-4. The location of the 3-methyl-2-oxobut-3-enyl moiety was assigned to C-7 on the basis of HMBC correlations of the H_2-4′ with C-6, C-7, C-8, of H-8 with C-4′. Finally, the methyl group (C-3′) linked to C-6 was confirmed by the HMBC correlation of H_3-3′ with C-5, C-6 and C-7, of H-5 with C-3′. Accordingly, the structure of **1** was established as 4-acetyl-6-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one.

Table 1. ^{1}H and ^{13}C NMR Data of compounds **1-3** ($CDCl_3$, δ in ppm, *J* in Hz)

NO.	Compound **1**		Compound **2**		Compound **3**	
	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)
1		6.09 s		6.08 s		6.10 s
2	162.3 s		162.7 s		162.3 s	
3	120.7 d	6.67 s	121.1 d	6.63 s	120.1 d	6.62 s
4	151.9 s		151.5 s		151.7 s	
5	126.5 d	7.08 s	138.8 s		125.8 d	7.02 s
6	134.1 s		128.5 d	6.86 (d) 2.2	133.2 s	
7	135.2 s		135.5 s		136.3 s	
8	121.3 d	7.45 s	118.6 d	7.34 (d) 2.2	120.8 d	7.43 s
4a	124.5 s		126.9 s		122.7 s	
8a	135.8 s		136.6 s		134.6 s	
1'	190.6 s		190.9 s		190.9 s	
2'	28.6 q	2.38 s	28.2 q	2.33 s	28.3 q	2.36 s
3'	20.6 q	2.17 s	21.6 q	2.20 s	21.2 q	2.14 s
4'	44.2 t	4.36 s	48.7 t	4.45 s	27.6 t	3.35 (d) 6.8
5'	200.3 s		200.7 s		123.2 d	5.32 (t) 6.8
6'	144.7 s		144.3 s		133.9 s	
7'	123.3 t	5.87 s, 6.22 s	123.9 t	5.87 s, 6.24 s	17.6 q	1.76 s
8'	16.8 q	1.85 s	17.0 q	1.86 s	25.3 q	1.55 s

4-Acetyl-5-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**2**) was obtained as a brown gum and showed a quasi-molecular ion at *m/z* 306.1102 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 306.1106), corresponding to the molecular formula $C_{17}H_{17}NO_3$. The ^{1}H and ^{13}C NMR spectra of **2** were highly similar to those of **1**. These indicated that compounds **1** and **2** have very similar structures. The obvious chemical shift differences resulted from the proton signals on benzene ring. A pair of singlets at δ_H 7.08 s and 7.45 s in **1** were replaced by two doublets at δ_H 6.86 [(d) 2.2] and 7.34 [(d) 2.2] in **2**. These changes

revealed that **2** should be a 4,5,7-trisubstituted quinolin-2(1*H*)-one. In addition, the acetyl group located at C-4, the methyl group located at C-7, and the 3-methyl-2-oxobut-3-enyl moiety located at C-5, which were also supported by the HMBC correlations from H_3-2′ to C-4, from H_3-3′ to C-5, C-6, C-4a, from H-4′ to C-6, C-7, C-8. Therefore, the structure of **2** was established as shown.

4-Acetyl-6-methyl-7-prenylquinolin-2(1*H*)-one (**3**) was also obtained as brown gum with a molecular formula as $C_{17}H_{19}NO_2$, according to the ion peak of *m/z* 292.1318 ($[M+Na]^+$) in the HRESIMS. The UV and IR spectra of **3** were similar to those of **1**. The chemical shift differences resulted from the disappearance of a 3-methyl-2-oxobut-3-enyl moiety resonance and appearance of a prenyl resonance ($-CH_2CH=C(Me)_2$ C-4'~8', H_2-4', H-5', H_3-7', and H_3-8')[17] in **3**. These changes indicated that the 3-methyl-2-oxobut-3-enyl moiety at C-7 in **1** was converted into a prenyl group in **3**. The HMBC correlation from to H_2-4' to C-6, C-7, and C-8, from to H-5' to C-7 also supported the prenyl group located at C-7. In addition, the positions of the acetyl and methyl group can also be determined by further analysis of its HMBC correlations. The structure of **3** was therefore defined.

Since certain of the alkaloids from *Thalictrum* genus exhibit potential anti-viral activity,[13,18,19] compounds **1-3** were tested for their anti-rotavirus activity. Their ability to prevent the cytopathic effects of rotavirus in MA104 cells was tested according to our previous literatures,[20,21] and their effects were measured in parallel with the determination of antiviral activity using ribavirin as positive control. The results (Table 2) revealed that compounds **1-3** exhibited potent anti-rotavirus activity with therapeutic index (TI) valves of 17.6, 15.0, and 23.6 respectively.

Table 2. Anti-rotavirus activity of compounds **1-3**

No.	CC_{50} (μg/mL)	EC_{50} (μg/mL)	TI (CC_{50}/EC_{50})
1	238.2	13.5	17.6
2	212.6	14.2	15.0
3	263.8	11.2	23.6
Ribavirin	270.8	12.5	21.7

CC_{50}: mean (50%) value of cytotoxic concentration; EC_{50}: mean (50%) value of effective concentration; TI: therapeutic index, CC_{50}/EC_{50}.

EXPERIMENTAL

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-1900 spectrophotometer. A Bio-Rad FTS185 spectrophotometer was used for scanning IR spectra. ^1H, ^{13}C, and 2D-NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. ESIMS and HRESIMS analyses were measured on Agilent 1290 UPLC/6540 Q-TOF mass spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. Semi-preparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 mm × 25 cm) or Venusil MP C_{18} (2.0 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40 - 63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75 - 150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying

with 5% H_2SO_4 in ethanol and heating.

Plant Material. The whole plants of *Thalictrum glandulosissimum* (Finet & Gagnep.) W. T. Wang & S. H. Wang) were collected in Heqing Prefecture of Yunnan Province, People's Republic of China, in September 2019. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-19-09-199) has been deposited in Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University, P. R. China.

Extraction and Isolation. The air-dried and powdered whole plants of *T. glandulosissimum* (3.5 kg) were extracted with 95% aq. EtOH, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na_2CO_3 aq. and extracted with EtOAc again. The EtOAc-soluble alkaloidal materials (52.4 g) were applied to silica gel (200-300 mesh) column chromatography, eluting with $CHCl_3$/MeOH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. Further separation of fraction B (9:1, 6.22 g) by silica gel column chromatography, eluted with $CHCl_3$/Me_2CO (9:1-2:1), yielded mixtures B1–B7. Sub-fraction B1 (9:1, 1.46 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (68% MeOH/H_2O, flow rate 20 mL/min) to give **1** (10.8 mg), **2** (12.4 mg), and **3** (14.6 mg).

Anti-rotavirus assay. The human rotavirus Wa group was used to infect the cell culture MA104 *in vitro*, the 50% cytotoxicity concentration (CC_{50}) and half maximal effective concentration (EC_{50}) were evaluated, and the ribavirin was used as positive control.[20,21] MA-104 cells (1×10^5 cells *per* well) were grown in 96-well plates for 48 h. The media were removed and replaced by new media containing serial dilutions of compounds under test. After incubation for 72 h, the media were discarded, and 5 μL of MTT solution was added to each well. Plates were then incubated at 37 °C for 4 h. The solution was removed, and 100 μL of 0.04 mol/L HCl-isopropanol were added to each well to dissolve formazan crystals. Using a microplate reader, the absorbance of each well was measured at 540 nm. After subtracting the background absorbance at 655 nm, the 50% CC_{50} of each compound was estimated by regression analysis.

In the mixed treatment assay, each compound was mixed with a 0.01 multiplicity of infection (MOI) of the rotaviruses at various concentrations (1-160 μg/mL) and incubated at 4 °C for 1 h. The mixtures were inoculated in triplicates onto near confluent MA-104 cell monolayers (1×10^5 cells per well) for 1 h with occasional rocking. The solution was removed and the cells replaced with eagles minimum essential medium (EMEM) containing 1.0 μg/mL trypsin. The cells were incubated for 72 h at 37 °C under 5% CO_2 atmosphere until the cells in the control showed complete viral cytopathic effect (CPE) by light microscopy. EC_{50} was estimated by regression analysis.

4-Acetyl-6-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**1**) Obtained as brown gum; UV (MeOH) λ_{max} nm (log ε) 210 (4.25), 235 (3.64), and 342 (3.16); IR (KBr) ν_{max} 3276, 2928, 1682, 1665,

1650, 1615, 1536, 1434, 1146, 895, 764 cm^{-1}; positive ESIMS *m/z* 306 $[M+Na]^+$, positive HRESIMS *m/z* 306.1112 (calcd for $C_{17}H_{17}NNaO_3$, 306.1106).

4-Acetyl-5-methyl-7-(3-methyl-2-oxobut-3-enyl)quinolin-2(1*H*)-one (**2**) Obtained as brown gum; UV (MeOH) λ_{max} nm 210 (4.18), 232 (3.56), and 340 (3.22); IR (KBr) ν_{max} 3279, 2930, 1685, 1668, 1652, 1612, 1542, 1438, 1225, 1139, 869, 754 cm^{-1}; positive ESIMS *m/z* ESIMS *m/z* 306 $[M+Na]^+$, positive HRESIMS *m/z* 306.1102 (calcd for $C_{17}H_{17}NNaO_3$, 306.1106).

4-Acetyl-6-methyl-7-prenylquinolin-2(1*H*)-one (**3**) Obtained as brown gum; UV (MeOH) λ_{max} nm (log ε) 210 (4.22), 236 (3.61), and 345 (3.28); IR (KBr) ν_{max} 3275, 2934, 1685, 1668, 1654, 1610, 1530, 1432, 1239, 1142, 843, 768 cm^{-1}; positive ESIMS *m/z* 292 $[M+Na]^+$, positive HRESIMS *m/z* 292.1318 (calcd for $C_{17}H_{19}NNaO_2$, 292.1313).

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