Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2019, **17**(9): 0698–0706
Chinese Journal of Natural Medicines

New aporphine alkaloids with selective cytotoxicity against glioma stem cells from *Thalictrum foetidum*

DING Cai-Feng[1, 5], DAI Zhi[1], YU Hao-Fei[1, 4], ZHAO Xu-Dong[2*], LUO Xiao-Dong[1, 3*]

[1] *Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;*
[2] *Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Science/Key Laboratory of Bioactive Peptides of Yunnan Province, Kunming Institute of Zoology, Kunming 650223, China;*
[3] *Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education and Yunnan Province, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China;*
[4] *School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650500, China;*
[5] *University of Chinese Academy of Sciences, Beijing 100049, China*

Available online 20 Sep., 2019

[ABSTRACT] Seven new isoquinoline alkaloids, 9-(2′-formyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy dehydroaporphine (**1**), 9-(2′-formyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy oxoaporphine (**2**), 3-methoxy-2′-formyl oxohernandalin (**3**), (–)-9-(2′-methoxycarbonyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy aporphine (**4**), (–)-2′-methoxycarbonyl thaliadin (**5**), (–)-9-(2′-methoxyethyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy aporphine (**6**), (–)-3-methoxy hydroxyhernandalinol (**7**), together with six known isoquinoline alkaloids (**8**–**13**) were isolated from the roots of *Thalictrum foetidum*. Their structures were elucidated by extensive spectroscopic measurements. Compounds **1** and **2** showed significant selective cytotoxicity against glioma stem cells (GSC-3[#] and GSC-18[#]) with IC_{50} values ranging from 2.36 to 5.37 μg·mL^{-1}.

[KEY WORDS] *Thalictrum foetidum*; Aporphine alkaloids; Selective cytotoxicity; Glioma stem cells

[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2019)09-0698-09

Introduction

Isoquinoline alkaloids are a large family of natural compounds found in the plants of the family Papareraceae, Berberidaceae, and Ranunculaceae. They are basically derived from the precursor dopamine via various inter-molecular reactions, and possess a broad range of biological activities, including analgesic [1], antiamoebic [2], antigout [3], antimicrobial [4], and antitumor effects [5]. Many important molecules of isoquinoline alkaloids type such as berberine, morphine, codeine, emetine as well as papaverine have been used extensively in clinical medicine. This indicates that isoquinoline alkaloids have remarkable medicinal relevance and are therefore of great interest, especially when searching for novel natural products [6-18] as drug candidate.

Previous investigation on *Thalictrum* (Ranunculaceae) indicated the presence of different classes of isoquinoline alkaloids, some of which showed anti-infectious, antitumor, anti-parasite and platelet aggregation effects [19]. *Thalictrum foetidum* is a tall perennial rigid herb indigenous to China (Yunnan, Sichuan and Tibet). The extracts of its roots have been used to cure dysentery, sore throat, and enteritis in folk medicine [20]. The use of the plant to cure cancer can be traced back to Tang, but research on chemical compositions of *T. foetidum* is limited [21-23]. Thus, based on the background of the structure and the biological activities of the genus, a phytochemical investigation of the total alkaloid of the roots of *T. foetidum* was carried out in order to isolate new active alkaloids. This investigation led to the isolation of seven new isoquinoline alkaloids (**1**–**7**) and six known analogues (**8**–**13**) namely 3-methoxydehydrohernandaline (**8**), thaliadine (**9**), 6-

[Received on] 22-Apr.-2019
[Research funding] This work was supported by the Natural Science Foundation of China (U1802281, 31770388).
[*Corresponding author] E-mail: xdluo@mail.kib.ac.cn (LUO Xiao-Dong); zhaoxudong@mail.kiz.ac.cn (ZHAO Xu-Dong)
These authors have no conflict of interest to declare.

(1, 3-dioxolo[4, 5-*g*]isoquinolin-5-ylcarbonyl)-2, 3-dimethoxy-benzoicacidmethyl ester (**10**), *O*-methylflavinantine (**11**), 8-oxyberberine (**12**), and berberine (**13**) [24-25] (Fig. 1). Furthermore, compounds **1** and **2** exhibited significant selective cytotoxicity against glioma stem cells (GSCs) with IC_{50} values ranging from 2.36 to 5.37 μg·mL^{-1}.

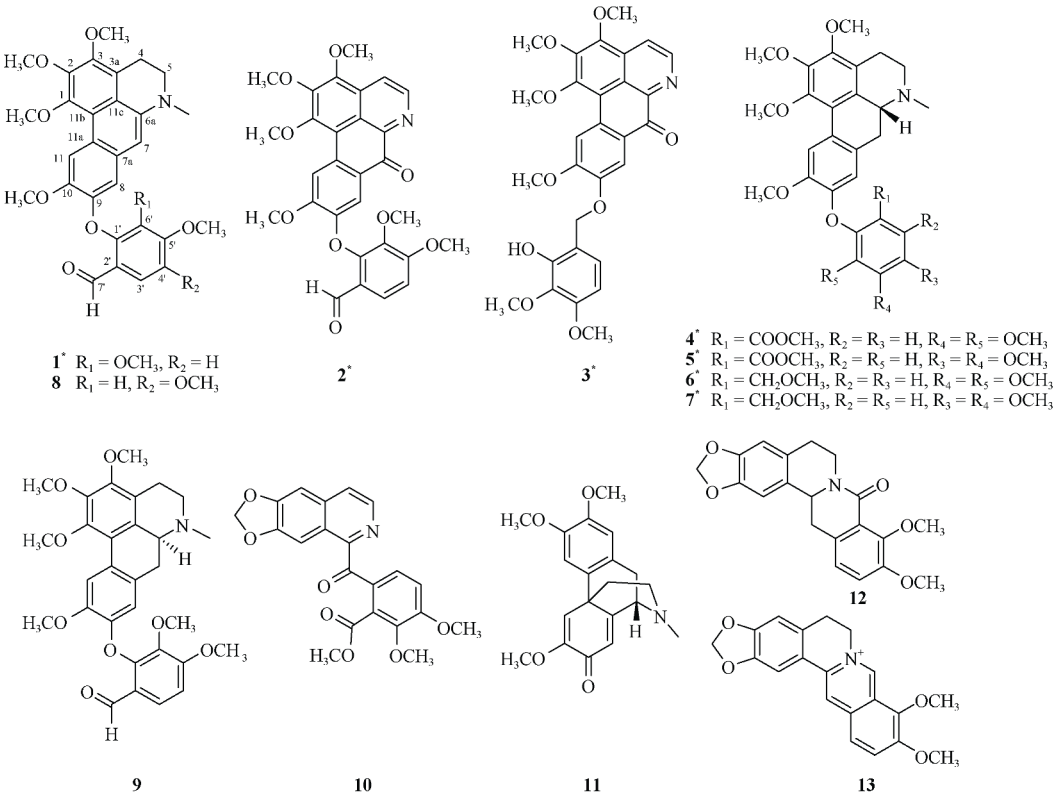

Fig. 1 Structures of compounds 1–13 (* new compounds)

Results and Discussion

Compounds **1**–**7** were obtained as amorphous powder, positive to Dragendorff′s reagent.

Compound **1** gave a molecular ion peak at *m/z* 556.1942 $[M + Na]^+$ (Calcd. for $C_{30}H_{31}NO_8$ $[M + Na]^+$ 556.1942) in HRESIMS, consistent with the molecular formula $C_{30}H_{31}NO_8$, indicating 16 indices of hydrogen deficiency. The IR spectrum indicated the presence of conjugated carbonyl (1637 cm^{-1}) functional group and aromatic rings (1594, 1543, and 1459 cm^{-1}). Its UV (CH_3OH) spectrum showed characteristic absorptions for an aporphine-type isoquinoline alkaloid [9] at λ_{max} of 274 and 244 nm. The ^{13}C NMR spectrum (Table 1) of **1** in combination with the DEPT and HSQC data, showed 30 carbon resonances, classified as 20 aromatic carbons signals, one aldehydic carbonyl group, six methoxyl groups, one *N*-methyl group, and two sp^3 methylenes carbon signals. The ^1H and ^{13}C NMR spectra were similar to those of 2-[6a, 7-didehydro-1, 2, 3, 10-tetramethoxyaporphin]-9-oxy-4, 5-dimethoxy-benzaldehyde [26], except for the substituents pattern of ring E. The appearance of coupled *ortho*-protons at δ_H 6.93 (1H, d, J = 9.0Hz) and 7.77 (1H, d, J = 9.0Hz) in **1,** instead of the two isolated protons at δ_H 7.02 (1H, s) and 7.41 (1H, s) in 2-[(6a, 7-didehydro-1, 2, 3, 10-tetramethoxyaporphin)-9-oxy-4, 5-dimethoxy-benzaldehyde, suggested E is a 1, 2, 3, 4-tetrasubtituted benzene ring in **1**. The presence of the substituents known as 2′-CHO, 5′-OCH_3, 6′-OCH_3 was further supported by the correlations of the methoxy groups (δ_H 3.73 and 3.93) with δ_C 141.7 (C-6′) and 159.4 (C-5′), δ_H10.18 (H-7′) with δ_C 123.7 (C-2′) and 188.6 (C-7′), δ_H7.77 (H-3′) with δ_C 151.9 (C-1′), 159.4 (C-5′), and 188.6 (C-7′), then δ_H 6.93 (H-4′) with δ_C 123.7 (C-2′) and 141.7 (C-6′) in the HMBC spectrum of **1** (Fig. 2), along with the correlations of δ_H 3.73 (6′-OCH_3)/3.97 (5′-OCH_3), δ_H 3.97 (5′-OCH_3)/6.93 (H-4′), δ_H 10.18 (H-7′)/6.72 (H-8), and δ_H 10.18 (H-7′)/7.77 (H-3′) in its ROESY spectrum (Fig. 2). Therefore, the structure of **1** was elucidated as 9-(2′-formyl-5′, 6′-dimethoxy-phenoxy)-1, 2, 3, 10-tetramethoxy dehydroaporphine.

The molecular formula of compound **2** was assigned as $C_{29}H_{25}NO_9$ based on the obtained positive HRESIMS ion at *m/z* 554.1421 $[M + Na]^+$ (Calcd. for $C_{29}H_{25}NO_9$ $[M + Na]^+$ 554.1422). Its IR spectrum indicated a conjugated carbonyl (1647 cm^{-1}) functional group and aromatic rings (1593, 1503, and 1460 cm^{-1}). Its UV (CH_3OH) spectrum showed an extend conjugation system at 247.0, 277.0, 362.0 and 383.0 nm, which resembled to an oxoaporphinechromophore [27]. The ^1H and ^{13}C NMR data (Table 1) of **2** also showed characteristic oxoaporphine chemical shifts of one pair of AB spin system

[δ_H 8.16 (1H, d, *J* = 3.6 Hz, H-4) and 8.91 (1H, d, *J* = 3.6 Hz, H-5)], and one typical conjugated ketonic carbon at δ_C 180.9 (C-7). In addition to the oxoaporphine scaffold, the remaining signals indicated a trisubstituted benzaldehyde with two *O*-methyls. Comparison of NMR spectra of **2** and the related compounds in the literatures, revealed similarities with those of 1, 2, 3, 10-tetramethoxy-9-(4, 5-dimethoxy-2-formylphenoxy)oxoaporphine [28]. The most obvious difference between them was the substituents models of ring E. The structure of E ring in compound **2** like that in compound **1** was easily established based on its 2D NMR spectra (Fig. 3). Thus compound **2** was elucidated as 9-(2′-formyl- 5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy oxoaporphine.

Compound **3** had the molecular formula of $C_{29}H_{27}NO_9$, consistent with its positive HRESIMS ion at *m/z* 556.1578 $[M + Na]^+$ (calcd for $C_{29}H_{27}NO_9$ $[M + Na]^+$ 556.1578). Its IR spectrum indicated characteristic absorption of hydroxyl group at (3432 cm^{-1}), a conjugated carbonyl group (1647 cm^{-1}), and aromatic rings (1595, 1503, and 1459 cm^{-1}). Its UV (CH_3OH) spectrum also showed an oxoaporphine chromophore conjugation system at 242.0, 258.0, 360.0 and 385.0 nm. Comparison of the ^1H and ^{13}C NMR data (Table 1)

Table 1 ^1H and ^{13}C NMR data of 1–3 in $CDCl_3$ (δ in ppm, *J* in Hz)

No.	**1**[a]		**2**[b]		**3**[b]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1		150.1		155.7		155.5
2		145.8		147.0		147.3
3		147.6		148.4		148.2
3a		121.5		131.0		131.0
4	3.19, t, *J* = 5.4 Hz	24.2	8.16, d, *J* = 3.6 Hz	118.9	8.17, d, *J* = 3.6 Hz	118.8
5	3.23, t, *J* = 5.4 Hz	49.9	8.91, d, *J* = 3.6 Hz	144.5	8.92, d, *J* = 3.6 Hz	144.5
6a		142.2		145.5		145.6
7	6.38, s	102.5		180.9		180.9
7a		120.7		126.1		126.1
8	6.72, s	111.0	7.74, s	114.5	7.69, s	113.8
9		148.3		148.4		147.9
10		145.6		153.9		153.9
11	9.11, s	109.6	8.86, s	110.5	8.85, s	110.4
11a		120.0		131.2		130.8
11b		129.1		115.4		115.7
11c		121.6		122.3		122.3
1′		151.9		150.9		127.0
2′		123.7		123.2		146.0
3′	7.77, d, *J* = 9.0 Hz	123.9	7.76, d, *J* = 5.8 Hz	124.9		141.7
4′	6.93, d, *J* = 9.0 Hz	108.9	6.95, d, *J* = 5.8 Hz	109.3		153.8
5′		159.4		159.4	6.85, d, *J* = 5.6 Hz	109.4
6′		141.7		141.2	7.17, d, *J* = 5.6 Hz	123.8
7′	10.18, s	188.6	10.16, s	188.1	4.61, s	61.1
1-OCH_3	3.97, s	60.5	4.11, s	61.1	4.12, s	61.1
2-OCH_3	4.06, s	61.3	4.10, s	61.5	4.12, s	61.5
3-OCH_3	3.92, s	60.8	4.16, s	61.8	4.17, s	61.8
10-OCH_3	4.10, s	56.2	4.14, s	56.2	4.15, s	56.2
3′-OCH_3					3.91, s	60.8
4′-OCH_3					3.72, s	56.1
5′-OCH_3	3.97, s	56.3	3.97, s	56.3		
6′-OCH_3	3.73, s	61.1	3.69, s	60.9		
N-CH3	2.94, s	40.5				

[a] ^1H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively; [b] ^1H and ^{13}C NMR data were recorded at 400 and 150 MHz, respectively

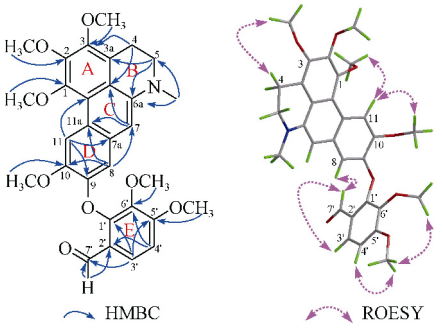

Fig. 2 Key HMBC and ROESY correlations for compound 1

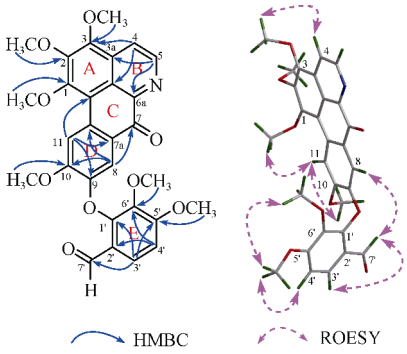

Fig. 3 Key HMBC and ROESY correlations for compound 2

of **2** and **3** indicated the same oxoaporphine scaffold rings A–D. Besides, the HMBC spectrum showed the correlations of the methylene signal at δ_H 4.61 (2H, s) and δ_C 123.8 (C-6′), 127.0 (C-1′), 146.0 (C-2′), and 147.9 (C-9). The coupled aromatic protons of δ_H 6.85 (1H, d, J = 5.6 Hz, H-5′) with δ_C 127.0 (C-1′), and 141.7 (C-3′), along with δ_H 7.17 (1H, d, J = 5.6 Hz, H-6′) with δ_C 146.0 (C-2′), and 153.8 (C-4′) (Fig. 4). These data indicated that the 2′, 3′, 4′-trisubstituted benzyloxy portion was assigned to C-9 of the oxoaporphine skeleton. Those substituents were 2′-OH, 3′-OCH_3, 4′-OCH_3 were respectively confirmed by further analysis of the HMBC and ROESY data of **3** (see Fig. 4). Hence, the structure of **3** was elucidated as 3-methoxy-2′-formyl oxohernandalin.

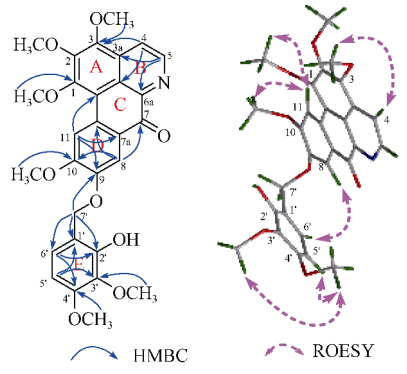

Fig. 4 Key HMBC and ROESY correlations for compound 3

Compound **4** was assigned the molecular formula of $C_{31}H_{35}NO_9$ on the basis of its HRESIMS ion at *m/z* 566.2386 $[M + H]^+$ (Calcd. for $C_{31}H_{35}NO_9$ $[M + H]^+$ 566.2385). Its IR spectrum indicated conjugated carbonyl functional group (1728 cm^{-1}), and aromatic rings (1596, 1506, and 1457 cm^{-1}). Its UV (CH_3OH) spectrum showed the absorptions at λ_{max} of 217.0, 277.0, 292.0 and 301.0 nm. The ^1H NMR spectrum of **4** displayed signals for a 1, 2, 4, 5-tetrasubstituted aromatic protons [δ_H 6.28 and 8.02 (each 1H, s, H-8 and H-11)], and a 1, 2, 3, 4-tetrasubstituted benzene ring [δ_H 7.76 (1H, d, J= 6.0 Hz, H-3′), 6.83 (1H, d, J= 6.0 Hz, H-4′)], one *N*-methyl signal at δ_H 2.44 (3H, s), seven methoxy singlets at δ_H 3.69, 3.72, 3.73, 3.85, 3.98 (each 3H, s) and 3.93 (6H, s), and four sp3 signals for three methylenes and on methane protons. The ^{13}C NMR data (Table 2) showed 31 carbon resonances, including 18 aromatic carbon signals, one ester carbonyl group, seven methoxy groups, one *N*-methyl group at δ_C 44.0, one methine at δ_C 62.5, and three methylenes at δ_C 23.7, 33.7, and 52.9. On the basis of the above data, **4** was deduced as a typical 5, 6, 6a, 7-tetrahydro-6-methyl-4*H*-dibenzoquinolin [29] alkaloid, similar to thaliadine [30], except for the acetate group in compound **4** replacing one aldehydic carbon in thaliadine. The acetate group was placed at C-2′ based on the HMBC correlations from δ_H 3.69 (*O*-methyl) and 7.76 (H-3′) to δ_C 165.5 (C-7′), along with the ROESY correlation of δ_H 3.69/ 7.76 (H-3′) (Figure S2). Thus, compound **4** was elucidated as (–)-9-(2′-methoxycarbonyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy aporphine.

Compound **5** possessed the same molecular formula of $C_{31}H_{35}NO_9$ as **4**, consistent with its positive HRESIMS at *m/z* 566.2384 $[M + H]^+$ (Calcd. for $C_{31}H_{35}NO_9$ $[M + H]^+$ 566.2385). The ^1H and ^{13}C NMR spectral data of **5** were similar to those of **4**, except for the substituted modes of methoxy in ring E. Compared with **4**, the appearance of the two isolated aromatic protons at δ_H 7.47 (1H, s) and 6.59 (1H, s) replacing the coupled AB spin system at δ_H 7.76 (1H, d, J = 6.0 Hz) and 6.83 (1H, d, J = 6.0 Hz) suggested that E is a 1, 2, 4, 5-tetrasubstituted benzene ring. The HMBC correlations of the methoxy groups (δ_H 3.92 and 3.81) with the carbons at δ_C 145.2 (C-4′) and 153.4 (C-5′), δ_H 7.47 (H-3′) with δ_C 150.9 (C-1′)/ 153.4 (C-5′)/165.6 (C-7′) and δ_H 6.59 (H-6′) with δ_C 114.2 (C-2′)/145.2 (C-4′), suggested that it is 4′-OCH_3, 5′-OCH_3 in **5** instead of 5′-OCH_3, 6′-OCH_3 observed in **4**. Therefore, the structure of **5** was elucidated as (–)-2′-methoxycarbonyl thaliadin.

The molecular formula of compound **6** was determined to be $C_{31}H_{37}NO_8$ in reference to its HRESIMS ion at *m/z* 552.2590 $[M + H]^+$ (Calcd. for $C_{31}H_{37}NO_8$ $[M + H]^+$ 552.2592). The ^1H and ^{13}C NMR data (Table 2) for **6** were similar to those of **4**, except for the presence of one methylene group attached to oxygen [δ_H 4.40 (2H, d, J = 1.6 Hz) and δ_C 69.0] in **6** replacing one ester carbonyl group (δ_C 165.5) in **4**. This was indicated by the correlations of δ_H 4.40 (H_2-7′) with δ_C 58.3 (*O*-CH_3)/123.5 (C-5′)/125.0 (C-2′)/153.3 (C-1′) and δ_H 3.30 (*O*-CH_3) with δ_C 69.0 in its HMBC spectrum, along

with the ROESY correlations of δ_H 3.30 (*O*-CH_3)/7.17 (H-3′) and δ_H 4.40 (H-7′)/6.37 (H-8). Then **6** was elucidated to be (–)-9-(2′-methoxyethyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy aporphine.

Table 2 ^{1}H and ^{13}C NMR data of 4–7 in $CDCl_3$ (δ in ppm, *J* in Hz)

No.	4		5		6		7	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1		149.4		149.4		149.4		149.4
2		145.2		145.2		145.4		145.5
3		149.7		149.9		149.7		149.9
3a		123.0		123.1		122.1		121.8
4	2.85, m 2.79, d, *J* = 2.4Hz	23.7	2.81–2.87, m	23.5	2.87, d, *J* = 4.0Hz 2.83, t, *J* = 6.4Hz	23.6	2.89, m 2.84, m	23.6
5	3.02, m 2.36, m	52.9	3.01–3.05, m 2.38, m	52.9	2.99-3.02, dd, *J* = 10.8, 4.8Hz 2.36, m	52.9	2.92, m 2.38, d, *J* = 3.6Hz	52.9
6a	2.91, m	62.5	2.94, dd, *J*= 9.2, 4.0Hz	62.6	2.90, br s	62.6	3.02, dd, *J* = 10.8, 4.0 Hz	62.7
7	2.75, dd, *J* = 2.8, 9.2 Hz 2.41, m	33.7	2.43, br s 2.81, m	33.9	2.75, d, *J* = 4.0Hz 2.40, m	33.8	2.80, m 2.41, m	33.8
7a		128.5		128.9		125.7		128.9
8	6.28, s	113.6	6.45, s	115.7	6.37, s	114.2	6.47, s	115.7
9		146.8		146.4		147.4		146.6
10		147.4		148.2		146.6		148.0
11	8.02, s	112.0	8.02, s	112.3	8.03, s	112.2	8.02, s	112.2
11a		125.7		126.6		125.0		126.4
11b		122.6		122.4		122.6		122.3
11c		130.7		130.8		130.2		130.8
1′		149.0		150.9		153.3		150.8
2′		117.8		114.2		125.0		121.8
3′	7.76, d, *J* = 6.0 Hz	127.5	7.47, s	113.3	7.17, d, *J* = 8.8 Hz	123.5	7.00, s	111.2
4′	6.83, d, *J* = 6.0 Hz	108.1		145.2	6.82, d, *J* = 8.8 Hz	108.7		146.3
5′		157.4		153.4		153.3		149.0
6′		142.6	6.59, s	105.5		141.9	6.56, s	104.7
7′		165.5		165.6	4.40, d, *J* = 1.6 Hz	69.0	4.45, d, *J* = 2.4 Hz	68.6
1-OCH_3	3.72, s	60.6	3.74, s	60.6	3.88, s	60.7	3.76, s	60.7
2-OCH_3	3.93, s	61.0	3.93, s	61.0	3.93, s	61.0	3.94, s	61.0
3-OCH_3	3.85, s	60.3	3.86, s	60.3	3.73, s	60.4	3.87, s	60.4
10-OCH_3	3.98, s	56.3	3.94, s	56.3	3.98, s	56.4	3.94, s	56.3
4′-OCH3			3.92, s	56.2			3.90, s	56.2
5′-OCH3	3.93, s	56.1	3.81, s	56.4	3.86, s	56.0	3.78, s	56.1
6′-OCH3	3.78, s	61.2			3.75, s	61.0		
7′-OCH3	3.69, s	51.9	3.74, s	52.0	3.30, s	58.3	3.35, s	58.3
N-CH_3	2.44, s	44.0	2.45, s	44.0	2.43, s	44.0	2.45, s	44.2

^{1}H and ^{13}C NMR spectra were recorded at 400 and 150 MHz, respectively

Compound **7** possessed the same molecular formula of $C_{31}H_{37}NO_8$ as **6** based on its positive HRESIMS at *m/z* 552.2591 $[M + H]^+$ (Calcd. for $C_{31}H_{37}NO_8$ $[M + H]^+$ 552.2592). The ^{1}H and ^{13}C NMR spectral data of **7** were similar to those of **6**, except for the substituted pattern of methoxy in ring E. Like compound **5**, compound **7** has a 1, 2,

4, 5-tetrasubstitued ring E, with 2′-methoxyethyl, 4′-methoxyl and 5′-methoxyl being the substituents supported by its 2D NMR spectra. Thus, compound **7** was established as (–)-3-methoxy hydroxyhernandalinol.

The structures of compounds **4**–**7** have been established and all the signals were assigned by 2D NMR spectrum (see Figures S1 and S2). Furthermore, compounds **4**–**7** have the same sign of optical rotation (6a*R*) as those of glaucine (–118.4°, *c* 0.89, $CHCl_3$) and isocorytuberine (–181.0°, *c* 0.50, CH_3OH)[31].

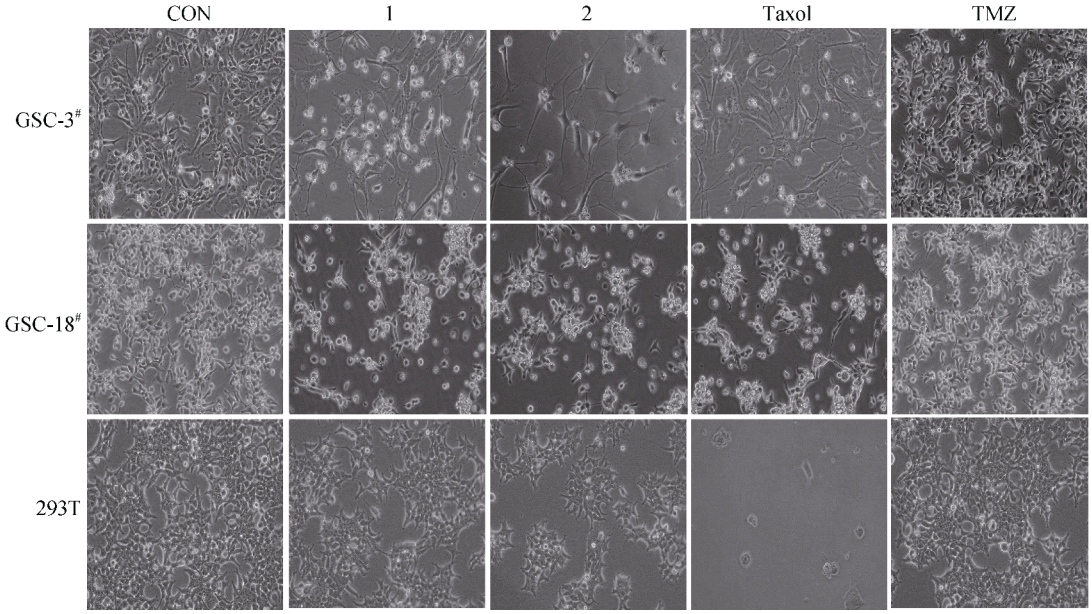

Fig. 5 Cytotoxicity test of 1 and 2 against GSCs (GSC-3# and GSC-18#) and human normal cell (293T) at 10 μg·mL^{-1} concentration by phenotypic screening

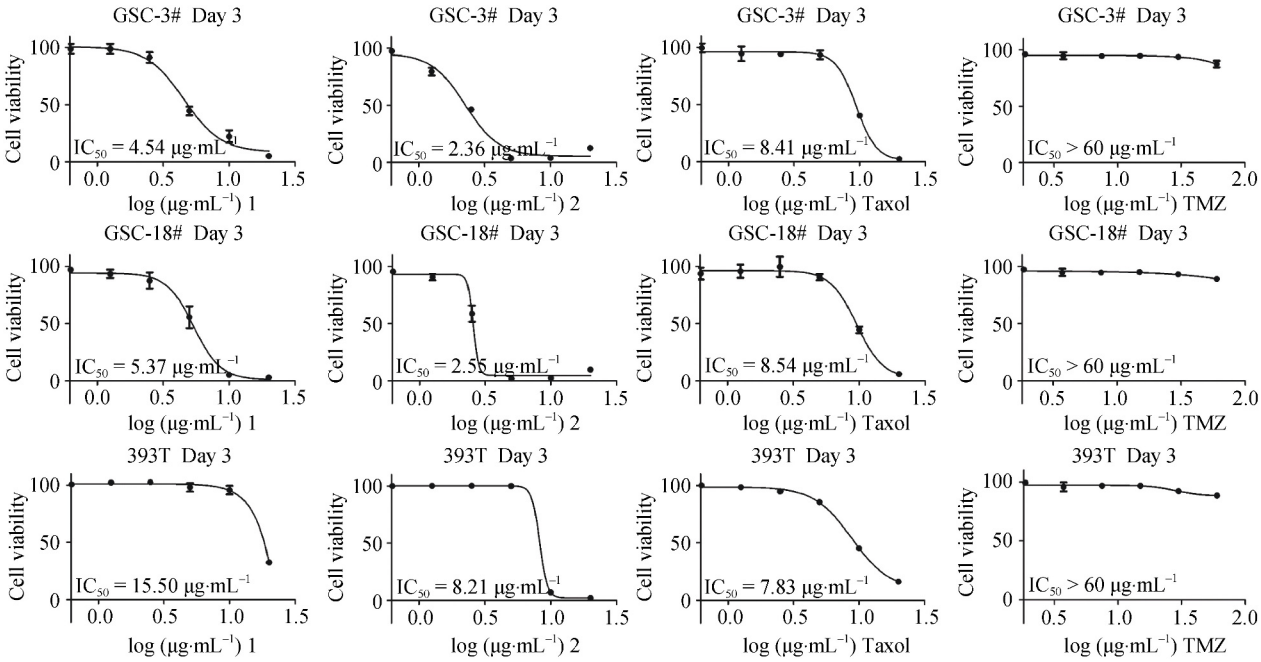

Fig. 6 The IC$_{50}$ values of 1 and 2 against GSC-3#, GSC-18# and 293T

All the isolated compounds were evaluated for their anti-cancer activity against human glioma stem cell lines (GSC-3# and GSC-18#) and for cytotoxicity against human normal embryonic kidney (293T) by phenotypic screening. The results showed that compounds **1** and **2** were able to significantly inhibit the growth of GSCs (GSC-3# and GSC-18#) (Fig. 5). Further cell viability assay using the MTS method showed that compounds **2** and **1** respectively exhibited IC$_{50}$ values of 2.36 and 4.54 μg·mL^{-1} against GSC-3#, 2.55 and 5.37 μg·mL^{-1} against GSC-18#, while in contrast the well

known anti-tumor drug taxol (positive control) showed IC_{50} values of 8.41 and 8.54 $\mu g \cdot mL^{-1}$ against GSC-3# and GSC-18# respectively. These two new compounds showed much better activities compared to the primary glioblastoma chemotherapy drug temozolomide (TMZ) which presented an IC_{50} of 60 $\mu g \cdot mL^{-1}$ against both cell lines (Fig. 6). It is worth noted that the IC_{50} of **1** and **2** against human normal cell line (293T) were 15.5 and 8.2 $\mu g \cdot mL^{-1}$, indicating that compounds **1** and **2** showed 3-times selective antitumor activities against GSCs.

Experimental

General

An Agilent 1290 UPLC/6540 Q-TOF spectrometer was used to measure HRESIMS and ESIMS spectra. UV spectra were detected on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained using a Bruker Tensor-27 infrared spectrometer and a KBr disk. Optical rotations were determined on a JASCO P-1020 digital polarimeter. NMR spectra were recorded on a Bruker AVANCE 400 and 600 spectrometers with TMS as internal standard. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), sephadex LH-20 (20–150μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) and RP-C_{18} (40–63 μm, Fuji). Thin layer chromatography (TLC) was performed on silica gel plates (GF254, Qingdao Marine Chemical Co., Ltd., Qingdao, China) and RP-C18 GF254 (Merck). Fractions were monitored by TLC and spots were visualized by spraying with Dragendorff's reagent. Methanol, hydrochloric, ethyl acetate, chloroform, ammonia solution and acetone were purchased from Tianjin Chemical Reagents Co. (Tianjin, China).

Plant materials

The roots of *Thalictrum foetidum* were harvested at Yunnan Province in March 2017. Botanical identification was done by Mr. Jun Zhang at the Kunming Plant Classification Biotechnology Co. Ltd., where a voucher specimen was kept under the reference number (No. 20170301).

Extraction and isolation

Air-dried and powdered roots (7.2 kg) of *Thalictrum foetidum* were extracted with methanol (2 h × 3) under reflux conditions. The solvent was evaporated *in vacuo* to give a residue, which was dissolved in 0.5% hydrochloric acid and then adjusted to a pH of 2. The solution was filtered and adjusted to a pH of 10 with 10% ammonia, and the basified solution was then partitioned with EtOAc to afford the alkaloidal extract (90.0 g). The extract was chromatographed on a silica gel column (chloroform-methanol, 100 : 0 to 0 : 100 *V/V*) to afford fractions I-IX. Fraction I (180.0 mg) was subjected to a silica gel column (Petroleum ether-acetone, 6 : 1 to 2 : 1 *V/V*) to afford fractions I-1 to I-5. Subfraction I-4 (140.0 mg) was further separated on sephadex-LH20 and purified by semi-preparative HPLC (Acetonitrile–H_2O, 90 : 10, *V/V*) to yield compound **1** (1.0 mg) and compound **8** (1.2 mg). Fraction III (1.6 g) was subjected to a preparative reversed- phase RP-C_{18} column with a gradient elution of 0–100% (*V/V*) methanol–water and further purified by semi-preparative HPLC (Methanol–H_2O, 90 : 10, *V/V*) to yield compound **4** (43.0 mg), **5** (20.0 mg), **6** (2.0 mg), **7** (1.5 mg) and **9** (8.0 mg). Fraction IV (2.2 g) was subjected to a preparative reversed-phase RP-C_{18} column with a gradient elution of 0–100% (*V/V*) methanol-water to yield subfractions IV-1 to IV-6. Subfraction IV-2 (750.0 mg) was further purified on sephadex-LH20 to get compound **12** (5.0 mg). Subfraction IV-3 (205.0 mg) was further separated on silica gel column (chloroform–acetone, 8 : 1 to 6 : 1 *V/V*) and purified by semi-preparative HPLC (Acetonitrile–H_2O, 70 : 30, *V/V*) to yield compound **2** (2.5 mg). Subfraction IV-4 (1.3 g) was further chromatographed over a sephadex-LH20, then submitted to silica gel column (petroleum ether–ethyl acetate, 3 : 1 to 0 : 1 *V/V*) to yield compound **3** (14.0 mg), compound **10** (13.0 mg), compound **11** (8.0 mg) and compound **13** (1.0 g).

9-(2′-formyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy dehydroaporphine (**1**): Amorphous powder, $C_{30}H_{31}NO_8$; UV (MeOH) λ_{max} (log ε) 274.0 (2.88), 244.0 (2.66) and 195.0 (3.34) nm; IR (KBr) ν_{max} 2923, 2851, 1637, 1594, 1543, 1459, 1384, 1057, 876 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; ESIMS *m/z* 556 $[M + Na]^+$; HRESIMS *m/z* 556.1942 $[M + Na]^+$ (Calcd. for $C_{30}H_{31}NO_8$ 556.1942 $[M + Na]^+$).

9-(2′-formyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy oxoaporphine (**2**): Amorphous powder, $C_{29}H_{25}NO_9$; UV (MeOH) λ_{max}(log ε) 383.0 (2.77), 362.0 (2.73), 277.0 (3.57) and 247.0 (3.36) nm; IR (KBr) ν_{max} 2923, 2851, 1647, 1593, 1503, 1460, 1391, 1091, 892 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; ESIMS *m/z* 532 $[M + H]^+$, *m/z* 554 $[M + Na]^+$; HRESIMS *m/z* 554.1421 $[M + Na]^+$ (Calcd. for $C_{29}H_{25}NO_9$ 554.1422 $[M + Na]^+$).

3-methoxy-2′-formyl oxohernandalin (**3**): Amorphous powder, $C_{29}H_{27}NO_9$; UV (MeOH) λ_{max}(log ε) 385.0 (3.39), 360.0 (3.32), 258.0 (3.93) and 242.0 (3.97) nm; IR (KBr) ν_{max}3432, 2939, 2853, 1647, 1595, 1503, 1459, 1392, 1092, 1007, 898 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 1; ESIMS *m/z* 534 $[M + H]^+$, *m/z* 556 $[M + Na]^+$; HRESIMS *m/z* 556.1578 $[M + Na]^+$ (Calcd. for $C_{29}H_{27}NO_9$ 556.1578 $[M + Na]^+$).

(–)-9-(2′-methoxycarbonyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy aporphine (**4**): Amorphous powder, $C_{31}H_{35}NO_9$; $[\alpha]_D^{20}$ −68.6 (*c* 0.07, CH_3OH); UV (MeOH) λ_{max}(log ε) 301.0 (3.87), 292.0 (3.86), 277.0 (3.95) and 217.0 (4.37) nm; IR (KBr) ν_{max}1728, 1596, 1506, 1457, 1395, 1091, 1019, 878 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 2; ESIMS *m/z* 566 $[M + H]^+$; HRESIMS *m/z* 566.2386 $[M + H]^+$ (Calcd. for $C_{31}H_{35}NO_9$ 566.2385 $[M + H]^+$).

(–)-2′-methoxycarbonyl thaliadin (**5**): Amorphous powder, $C_{31}H_{35}NO_9$; $[\alpha]_D^{20}$ −139.5 (*c* 0.04, CH_3OH); UV (MeOH) λ_{max}(log ε) 302.0 (3.99), 290.0 (3.96), 280.0 (4.01), 252.0 (3.90) and 221.0 (4.42) nm; IR (KBr) ν_{max}1726, 1610, 1508,

1462, 1265, 1209, 1082, 778 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 2; ESIMS *m/z* 566 $[M + H]^+$; HRESIMS *m/z* 566.2384 $[M + H]^+$ (Calcd. for $C_{31}H_{35}NO_9$ 566.2385 $[M + H]^+$).

(–)-9-(2′-methoxyethyl-5′, 6′-dimethoxyphenoxy)-1, 2, 3, 10-tetramethoxy aporphine (**6**): Amorphous powder, $C_{31}H_{37}NO_8$; $[\alpha]_D^{20}$ −58.6 (*c* 0.05, CH_3OH); UV (MeOH) λ_{max}(log ε) 301.0 (3.61), 293.0 (3.59), 280.0 (3.71), 254.0 (4.06) and 219.0 (4.06) nm; IR (KBr) ν_{max}1633, 1499, 1425, 1226, 1091, 878 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 2; ESIMS *m/z* 552 $[M + H]^+$, *m/z* 574 $[M + Na]^+$; HRESIMS *m/z* 552.2590 $[M + H]^+$ (Calcd. for $C_{31}H_{37}NO_8$ 552.2592 $[M + H]^+$).

(–)-3-methoxy hydroxyhernandalinol (**7**): Amorphous powder, $C_{31}H_{37}NO_8$; $[\alpha]_D^{20}$ −30.0 (*c* 0.16, CH_3OH); UV (MeOH) λ_{max} (log ε) 280.0 (2.45), 255.0 (2.25) and 196.0 (3.13) nm; IR (KBr) ν_{max}1614, 1508, 1463, 1194, 1085, 881 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data see Table 2; ESIMS *m/z* 552 $[M + H]^+$, *m/z* 574 $[M + Na]^+$; HRESIMS *m/z* 552.2591 $[M + H]^+$ (Calcd. for $C_{31}H_{37}NO_8$ 552.2592 $[M + H]^+$).

Cytotoxicity

Cell lines and cultures

GSC-3# and GSC-18# glioma stem cell lines were established from different human glioblastoma multiforme samples at Kunming Institute of Zoology. These cell lines were cultured in DMEM F12 supplemented with 1 × B27 (Life Technologies, 12, 587–010) and bFGF (Peprotech, AF-100-18B). GSCs were cultured in laminin (Gibco, 1, 725, 712) pre-coated dishes. Human normal cell lines 293T were cultured in DMEM complete medium.

Cell viability assay by MTS method

GSC-3#, GSC-18# and the human normal cell 293T were seeded on laminin pre-coated 96-well-plate with 20, 000 cells/well. The compounds **1** and **2** were added with a serial dilution (60, 30, 15, 7.5, 3.75, 1.875, 0.937, 0.468 μg·mL^{-1}) and cultured in cell incubator for 72 h. MTS reagent was diluted 1: 5 with fresh medium and subsequently the fresh medium was added with 100 μL/well. The cells were incubated for 1.5 h. Absorbance was measured by Hybrid Reader (Bio-Tek Synergy H1) at 490 nm. The half-maximal inhibitory concentration (IC_{50}) was measured and calculated by Graph Pad Prism 5 software.

Acknowledgments

The authors would like to thank Dr. Guy Sedar Singor Njateng (University of Dschang) for polishing English language.

References

[1] Proudfit HK, Anderson EG. Morphine analgesia: blockade by raphe magnus lesions [J]. *Brain Res*, 1975, **98**(3): 612-618.

[2] Chatterjee DK, Iyer N, Ganguli BN. Antiamoebic activity of chonemorphine, a steroidal alkaloid, in experimental models [J]. *Parasitol Res*, 1987, **74**(1): 30-33.

[3] Singh HP, Utreja P, Tiwary AK, *et al*. Elastic liposomal formulation for sustained delivery of colchicine: *in vitro* characterization and *in vivo* evaluation of anti-gout activity [J]. *Aaps Journal*, 2009, **11**(1): 54-64.

[4] Qian C, Zhu F. Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease [J]. *BMC Neurosci*, 2006, **7**(1): 78-86.

[5] Lei QF, Zhao XL, Xu LJ, *et al*, Chemical constituents of plants from tribe chelidonieae and their bioactivities [J]. *Chin Herb Med*, 2014, **6**(1): 1-21.

[6] Yu HF, Qin XJ, Ding CF, *et al*. Nepenthe-like indole alkaloids with antimicrobial activity from *Ervatamia chinensis* [J]. *Org Lett*, 2018, **20**(13): 4116-4120.

[7] Yu HF, Huang WY, Ding CF, *et al*. Cage-like monoterpenoid indole alkaloids with antimicrobial activity from *Alstonia scholaris* [J]. *Tetrahedron Lett*, 2018, **59**(31): 2975-2978.

[8] Wei X, Yang J, Ma HX, *et al*. Antimicrobial indole alkaloids with adductive C9 aromatic unit from *Gelsemium elegans* [J]. *Tetrahedron Lett*, 2018, **59**(21): 2066-2070.

[9] Wang B, Zhao YL, Liu YP, *et al*. Anti-inflammatory isoquinoline with bis-seco-aporphine skeleton from *Dactylicapnos scandens* [J]. *Org Lett*, 2018, **20**(6): 1647-1650.

[10] Ding CF, Ma HX, Yang J, *et al*. Antibacterial indole alkaloids with complex heterocycles from *Voacanga Africana* [J]. *Org Lett*, 2018, **20**(9): 2702-2706.

[11] Pan ZQ, Qin XJ, Liu YP, *et al*. Alstoscholarisines H-J, indole alkaloids from *Alstonia scholaris*: structural evaluation and bioinspired synthesis of alstoscholarisine H [J]. *Org Lett*, 2016, **18**(4): 654-657.

[12] Cheng GG, Li D, Hou B, *et al*. Melokhanines A-J, bioactive monoterpenoid indole alkaloids with diverse skeletons from *Melodinus khasianus* [J]. *J Nat Prod*, 2016, **79**(9): 2158-2166.

[13] Chen YY, Yang J, Yang XW, *et al*. Alstorisine A, a nor-monoterpenoid indole alkaloid from cecidogenous leaves of *Alstonia scholaris* [J]. *Tetrahedron Lett*, 2016, **57**(16): 1754-1757.

[14] Wei X, Jiang LP, Guo Y, *et al*. Indole alkaloids inhibiting neural stem cell from *Uncaria rhynchophylla* [J]. *Nat Prod Bioprospect*, 2017, **7**(5): 413-419.

[15] Yang XW, Yang CP, Jiang LP, *et al*. Indole alkaloids with new skeleton activating neural stem cells [J]. *Org Lett*, 2014, **16**(21): 5808-5811.

[16] Cai XH, Bao MF, Zhang Y, *et al*. A new type of monoterpenoid indole alkaloid precursor from *Alstonia rostrata* [J]. *Org Lett*, 2011, **13**(14): 3568-3571.

[17] Feng T, Li Y, Liu YP, *et al*. Melotenine A, a cytotoxic monoterpenoid indole alkaloid from *Melodinus tenuicaudatus* [J]. *Org Lett*, 2010, **12**(5): 968-971.

[18] Feng T, Cai XH, Li Y, *et al*. Melohenines A and B, two unprecedented alkaloids from *Melodinus henryi* [J]. *Org Lett*, 2009, **11**(21): 4834-4837.

[19] Sheng GL, Yu QS, Dong KM, *et al*. Research progress on chemical constituents and pharmacological activities of alkaloids from plants in *Thalictrum L* [J]. *Drugs & Clinic*, 2014, **29**(3): 312-321.

[20] Tang YL, Tan L, Dai W, *et al*. The application of the extracts of

Thalictrum foliolosum in the preparation of antitumor drugs [P]. CN 101306094A. 2008.

[21] Abdizhabbarova S, Ismailov ZF, Yunusov SY. Alkaloids of the roots of *Thalictrum foetidum* [J]. *Chem Nat Comp*, 1968, **4**(5): 281-281.

[22] Ganenko TV, Isaev MI, Lutskii VI, *et al*. Triterpene glycosides and their genins from *Thalictrum foetidum* III. The structure of cyclofoetoside A [J]. *Chem Nat Compd*, 1986, **22**(1): 61-65.

[23] Mukhamedova S, Maekh CK, Yunusov SY. Alkaloids of *Thalictrum foetidum* [J]. *Chem Nat Compd*, 1983, **19**(3): 376-377.

[24] Wang L, Zhang SY, Chen L, *et al*. New enantiomeric isoquinoline alkaloids from *Coptis chinensis* [J]. *Phytochemistry Lett*, 2014, **7**(1): 89-92.

[25] Wang W, Zhang QW, Ye WC, *et al*. Isoquinoline alkaloids from the rhizoma of *Coptis chinensis* [J]. *Chin J Nat Med*, 2007, **5**(5): 348-350.

[26] Ran XY, Yuan L, Zhou M, *et al*. Study on the chemical constituents in *Thalictrum elegans* (Ⅱ) [J]. *J Guizhou Normal College,* 2015, **31**: 16-17.

[27] Protais P, Arbaoui J, Bakkali EH, *et al*. Effects of various isoquinoline alkaloids on *in vitro* 3H-dopamine uptake by rat striatal synaptosomes [J]. *J Nat Prod*, 1995, **58**(10): 1475-1484.

[28] Liang ZY, Yang XS, Wang Y, *et al*. Two new oxoaporphine alkaloids from *Thalictrum elegans* [J]. *Chin J Nat Med*, 2005, **23**(7): 895-897.

[29] Wright CW, Marshall SJ, Russell PF, *et al*. *In vitro* antiplasmodial, antiamoebic, and cytotoxic activities of some monomeric isoquinoline alkaloids [J]. *J Nat Prod*, 2000, **63**(12): 1638-1640.

[30] Kupchan SM, Liepa AJ, Kameswaran V, *et al*. Tumor inhibitors LXXXVII, total synthesis of the tumor-inhibitory alkaloid thalicarpine [J]. *J Amer Chem Soc*, 1973, **95**(9): 2995-3000.

[31] Czarnocki Z, Mieczkowski JB, Ziolkowski M. Enantioselective synthesis of (*R*)-(–)-laudanosine and (*R*)-(–)-glaucine from L-ascorbic acid [J]. *Tetrahedron*, 1996, **7**(9): 2711-2720.

Cite this article as: DING Cai-Feng, DAI Zhi, YU Hao-Fei, ZHAO Xu-Dong, LUO Xiao-Dong. New aporphine alkaloids with selective cytotoxicity against glioma stem cells from *Thalictrum foetidum* [J]. *Chin J Nat Med*, 2019, **17**(9): 698-706.