Chinese Journal of Natural Medicines

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Citation: Hongwei CHEN, Meng DING, Jun LIN, Shuo YUAN, Kewu ZENG, Pengfei TU, Yong JIANG, NO inhibitory constituents from *Glycosmis craibii* var. glabra, Chinese Journal of Natural Medicines, 2024, 22(11), 1−7. doi: 10.1016/S1875−5364(24)60632−1.

View online: https://doi.org/10.1016/S1875−5364(24)60632−1

Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2024, **22**(11): 1040-1046
doi: 10.1016/S1875-5364(24)60632-1
Chinese Journal of Natural Medicines

•Original article•

NO inhibitory constituents from *Glycosmis craibii* var. *glabra*

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Available online 20 Nov., 2024

[ABSTRACT] Six novel compounds, comprising three quinolones (**1a**, **1b**, and **2**) and three flavanones (**3**–**5**), along with seven known analogs (**6**–**13**), were isolated from the 95% EtOH extract of the stems and leaves of *Glycosmis craibii* var. *glabra*. The structures of the new compounds were elucidated using HR-ESI-MS, UV, and 1D and 2D nuclear magnetic resonance (NMR) data analysis. The absolute configurations were determined through Mosher ester and electronic circular dichroism (ECD) spectral analysis. Compounds **2**, **6**, **9**, and **10** demonstrated inhibition of nitric oxide (NO) production stimulated by lipopolysaccharide in BV-2 microglial cells, with IC_{50} values ranging from 13.5 to 20.1 μmol·L^{-1}, comparable to the positive control, dexamethasone.

[KEY WORDS] *Glycosmis craibii* var. glabra; Quinolone; Flavanone; NO inhibition
[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2024)11-1040-07

Introduction

The Rutaceae family comprises approximately 50 species within the genus *Glycosmis*, predominantly found in tropical low-altitude forests. In China, 11 species and a variety of *Glycosmis* species exist, some of which have been utilized as herbal remedies for treating various ailments, including fever, cough, injuries, poor appetite, and pain [1-3]. These plants have been documented to contain coumarins, flavonoids, quinolones, carbazoles, acridones, sulfur-containing amides, and other compounds, exhibiting wound-healing, antitumor, and antibacterial activities [3-10]. However, with the exception of sulfur-containing amides [10], there is limited research on the chemical constituents with anti-inflammatory effects derived from *Glycosmis* species, which contribute significantly to their traditional efficacy. Consequently, investigating the anti-inflammatory constituents from *Glycosmis* species is of critical importance.

Glycosmis craibii var. *glabra* (Craib) Tanaka (syn. *Glycosmis ovoidea* Pierre) is a variety of *Glycosmis craibii* Tanaka, mainly distributed in the shrub or miscellaneous forest in Hainan Province, China [11]. Our team previously isolated acridones and coumarins from this plant, discovering their potent nitric oxide (NO) and 5-lipoxygenase (5-LOX) inhibitory activities [12-13]. In our ongoing investigation of new anti-inflammatory natural products from *G. craibii* var. glabra, we examined its 95% EtOH extract, leading to the identification of 13 compounds. Among these, compounds **1**–**7** are novel structures (Fig. 1). Their structures were elucidated using high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), UV, and 1D and 2D nuclear magnetic resonance (NMR) data analysis. We determined the absolute configurations through Mosher ester analysis, electronic circular dichroism (ECD) spectra, and comparison of specific rotation data with literature. Additionally, we evaluated the NO inhibitory activities of the isolated compounds.

Results and Discussion

(±)-Craiquinolone A (**1**) was isolated as a pale yellow oil. Its molecular formula was determined to be $C_{20}H_{27}NO_4$ based on HR-ESI-MS (*m/z* 346.2023 $[M + H]^+$, Calcd. for $C_{20}H_{28}NO_4$, 346.2018) and ^{13}C NMR data. The UV spectrum exhibited absorptions at 230, 279, and 320 nm, suggesting that **1** might possess a 2-quinolone skeleton [14]. The NMR data (Table 1) revealed four resonances in the aromatic region [δ_H 7.96 (1H, d, J = 8.0 Hz, H-5), 7.23 (1H, t, J = 8.0 Hz, H-6), 7.62 (1H, t, J = 8.0 Hz, H-7), 7.47 (1H, d, J = 8.0 Hz, H-8); δ_C 123.9 (C-5), 122.2 (C-6), 132.1 (C-7), 115.1 (C-8)], a conjugated olefinic singlet [δ_H 5.99 (1H, s, H-3); δ_C 98.0 (C-3)], an *N*-methyl [δ_H 3.63 (3H, s, NCH_3-1); δ_C 28.9 (NCH_3-1)], and a carbonyl [δ_C 163.6 (C-2)], indicating an *ortho*-disubstituted phenyl group corresponding to an *N*-methyl-quinolone skeleton. Further analysis of the ^1H and ^{13}C NMR data (Table 1) of **1** demonstrated a close structural

[Received on] 10-Feb.-2024
[Research funding] This work was supported by National Natural Science Foundation of China (Nos. 81973199, 82173949, and 81773864), and the Key Research and Development Project of Shandong Province (No. 2021CXGC010507).
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These authors have no conflict of interest to declare.

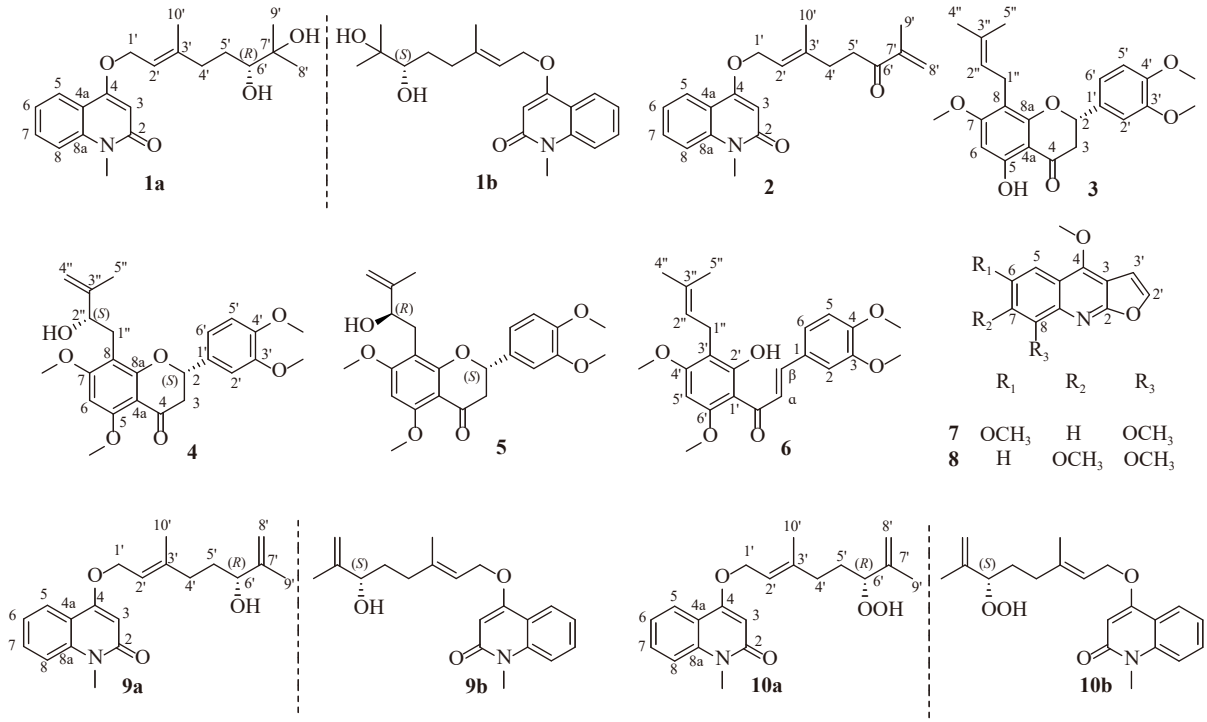

Fig. 1 Chemical structures of compounds 1–10.

similarity to glycopentanolone A [15], except for the geranyl group being replaced by a 6,7-dihydroxy-3,7-dimethyl-2,4-octenyl moiety [δ_H 4.79 (2H, d, J = 6.5 Hz, H-1'), 5.60 (1H, t, J = 6.5 Hz, H-2'), 2.38/2.18 (2H, m, H-4'), 1.78/1.43 (2H, m, H-5'), 3.30 (1H, m, H-6'), 1.13 (6H, s, H-8' and 9'), 1.82 (3H, s, H-10'); δ_C 66.4 (C-1'), 119.5 (C-2'), 143.2 (C-3'), 37.4 (C-4'), 30.4 (C-5'), 78.3 (C-6'), 72.8 (C-7'), 25.7 (C-8'), 25.2 (C-9'), 16.9 (C-10')] signals. The heteronuclear multiple bond correlations (HMBCs) from CH_2-1' to C-4, C-2', and C-3', and from H-3 to C-1' indicated the location of the geranyl moiety of **1** at C-4 (Fig. 2). The olefinic bond in compound **1** was determined to have an *E*-configuration due to the C-3' methyl group resonating at δ_C 16.8, a value distinct from the expected δ_C − 23 for a *Z*-configuration [16]. Consequently, the structure of **1** was proposed as shown and named craiquinolone A.

The zero specific rotation value indicated that **1** may exist as a pair of enantiomers. Consequently, compound **1** was isolated using chiral high-performance liquid chromatography (HPLC) to obtain the enantiomers **1a** and **1b** in a 1 : 1 ratio (Fig. 3). Their configurations were determined as 6'*S* for **1a** and 6'*R* for **1b** by comparing their specific rotation data ($[\alpha]_D^{25}$ −10 (*c* 0.1, MeOH) for **1a** and $[\alpha]_D^{25}$ + 10 (*c* 0.1, MeOH) for **1b**) with those of **9a** and **9b**, whose configurations were determined by the Mosher ester analysis (Fig. 4). Previous studies have also confirmed the correlation between the chirality of C-6' and the optical rotation of similar substituent groups [16-17].

Craiquinolone C (**2**) was isolated as a pale yellow oil. Its molecular formula was established as $C_{20}H_{23}NO_3$ based on HR-ESI-MS analysis (*m/z* 326.1754 $[M + H]^+$, Calcd. for $C_{20}H_{24}NO_3$, 326.1756) and ^{13}C NMR spectroscopic data. The UV spectrum exhibited absorptions at 230, 269, and 318 nm, indicating that **2** potentially possesses a 2-quinolone skeleton [14]. Comparison of ^1H and ^{13}C NMR data (Table 1) of **2** showed a close structural resemblance to glycopentanolone C [15], with the notable difference being the replacement of a hydroxy group at C-6' in glycopentanolone C by a carbonyl group [δ_C 201.1] in **2**. HMBCs from CH_2-1' to C-4, C-2', and C-3' indicated the positioning of the geranyl derivative moiety of **2** at C-4. Additionally, HMBCs from H-4' [δ_H 2.39 (2H, d, J = 7.6 Hz)], H-8' [δ_H 5.84, 6.11 (each 1H, br s)], and H-9' [δ_H 1.83 (3H, s)] to the carbonyl further confirmed the location of the carbonyl at C-6' (Fig. 2). Consequently, the structure of **2** was elucidated as depicted.

Craiflavanone A (**3**) was isolated as a pale yellow oil, with $[\alpha]_D^{25}$ −20 (*c* 0.1, MeOH). Its molecular formula was established as $C_{23}H_{26}O_6$ based on HR-ESI-MS (*m/z* 399.1811 $[M + H]^+$, Calcd. for $C_{23}H_{27}O_6$, 399.1808) and ^{13}C NMR data. The UV spectrum indicated that **3** possessed a flavanone skeleton[8]. The ^1H NMR data (Table 2) of **3** showed a group of signals for an ABX system [δ_H 6.89 (1H, d, J =8.2 Hz, H-5'), 6.97 (1H, dd, J = 8.2, 1.8 Hz, H-6'), 7.01 (1H, d, J = 1.8 Hz, H-2')], an aromatic singlet [δ_H 6.10 (1H, s, H-6)], an oxygenated aliphatic proton [δ_H 5.36 (1H, dd, J = 2.9, 12.7 Hz, H-2)], two aliphatic protons [δ_H 3.07 (1H, dd, J = 12.7, 17.0 Hz, H-3a), 2.83 (1H, dd, J = 2.9, 17.0 Hz, H-3b)], three methoxys [δ_H 3.86 (3H, s, OCH_3-7), 3.91 (3H, s, OCH_3-4'), 3.91 (3H, s, OCH_3-3')], and a group of isopentenyl [δ_H 3.23 (2H, d, J = 7.2 Hz, H-1''), 5.16 (1H, t, J = 7.2 Hz, H-2''), 1.64 (3H, s, H-4''), 1.63 (3H, s, H-5'')] signals. The above data indicated that **3** is a prenylated flavanone. The active hy-

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 1 and 2 in acetone-d_6 (*J* in Hz)

No.	1		2	
	^{1}H (*J* in Hz)	^{13}C, type	^{1}H (*J* in Hz)	^{13}C, type
2		163.6, C		163.4, C
3	5.99, s	98.0, CH	5.97, s	97.9, CH
4		162.2, C		162.1, C
4a		117.3, C		117.1, C
5	7.96, d (8.0)	123.9, CH	7.94, d (8.0)	123.9, CH
6	7.23, t (8.0)	122.2, CH	7.23, t (8.0)	122.1, CH
7	7.62, t (8.0)	132.1, CH	7.62, t (8.0)	132.0, CH
8	7.47, d (8.0)	115.1, CH	7.47, d (8.0)	115.1, CH
8a		141.0, C		140.9, C
1'	4.79, d (6.5)	66.4, CH_2	4.75, d (6.5)	66.2, CH_2
2'	5.60, t (6.5)	119.5, CH	5.62, t (6.5)	119.6, CH
3'		143.2, C		142.1, C
4'	2.38/2.18, m	37.4, CH_2	2.39, d (7.6)	34.6, CH_2
5'	1.78/1.43, m	30.4, CH_2	2.95, d (7.6)	36.2, CH_2
6'	3.30, m	78.3, CH		201.1, C
7'		72.8, C		145.2, C
8'	1.13, s	25.7, CH_3	5.84/6.11, br s	125.0, CH_2
9'	1.13, s	25.2, CH_3	1.83, s	17.8, CH_3
10'	1.82, s	16.9, CH_3	1.83, s	16.9, CH_3
N-CH_3	3.63, s	28.9, CH_3	3.60, s	28.9, CH_3

Assignments were based on HSQC and HMBC experiments

droxy signal at δ_H 12.14 (1H, br s) suggested the existence of 5-OH [8]. HMBCs of the three methoxy groups (δ_H 3.86, 3.91, 3.91) with C-7, C-3', and C-4'; δ_H 3.23 (2H, d, J = 7.2 Hz, H-1") with C-7, C-8, and C-8a, and OH-5 (δ_H 12.14) with C-6 (δ_C 92.6), C-5 (δ_C 162.7), and C-4a (δ_C 103.1) confirmed that the three methoxy groups are located at C-7, C-3', and C-4', respectively, while the isopentenyl and hydroxy groups were attached to C-8 and C-5, respectively (Fig. 2). The absolute configuration of C-2 was determined as *S* based on its negative optical rotation and a negative Cotton effect at 290 nm and a positive Cotton effect at 338 nm in its ECD spectrum (Fig. 5) [18, 19]. Consequently, the structure of **3** was elucidated as depicted.

Craiflavanone B (**4**) was obtained as a pale yellow oil, $[\alpha]_D^{25}$ −18 (*c* 0.1, MeOH). Its molecular formula was determined to be $C_{24}H_{28}O_7$ based on HR-ESI-MS (*m/z* 429.1915 $[M + H]^+$, Calcd. for $C_{24}H_{29}O_7$, 429.1913) and ^{13}C NMR data. The UV spectrum suggested that **4** possesses a flavanone skeleton [8]. Analysis of ^{1}H and ^{13}C NMR data (Table 2) of **4** revealed a close structural similarity to **3**, with the key difference being the replacement of 8-isopentenyl and hydroxy in 3 by 8-(2-hydroxy-3-methyl-3-butenyl) [δ_H 2.90 (1H, dd, J = 6.1, 12.9 Hz, H-1"a), 2.79 (1H, dd, J = 8.3, 12.9 Hz, H-1"b), 4.26 (1H, dd, J = 6.1, 8.3 Hz, H-2"), 4.61 (1H, br s, H-4"a), 4.49 (1H, br s, H-4"b), 1.61 (3H, s, H-5")] and a methoxy [δ_H 3.90 (3H, s)] in **4**. The HMBCs from CH_2-1" (δ_H 2.79 and 2.90) to C-7 (δ_C 166.0), C-8 (δ_C 108.3), and C-8a (δ_C 163.3) indicated that the modified isopentenyl is located at C-8 (Fig. 2). Similar to **3**, the absolute configuration of C-2 was determined to be *S* based on the negative optical rotation and ECD curve [18, 19] (Fig. 5). The absolute configuration of C-2" was established as *S* from the shifts of the ^{1}H NMR resonances observed in the Mosher ester analysis (Fig. 4). Consequently, the structure of **4** was proposed as shown.

Craiflavanone C (**5**) was obtained as a pale yellow oil, $[\alpha]_D^{25}$ −8 (*c* 0.1, MeOH). Its molecular formula was established as $C_{24}H_{28}O_7$ based on HR-ESI-MS analysis (*m/z* 429.1913 $[M + H]^+$, Calcd. for $C_{24}H_{29}O_7$, 429.1913) and ^{13}C NMR data. Examination of ^{1}H and ^{13}C NMR spectra (Table 2) revealed that **5** has a structural configuration closely resembling that of **4**, sharing an identical planar structure. The sole distinction between the compounds lies in the absolute configuration of C-2". The absolute configuration of C-2" in **5**

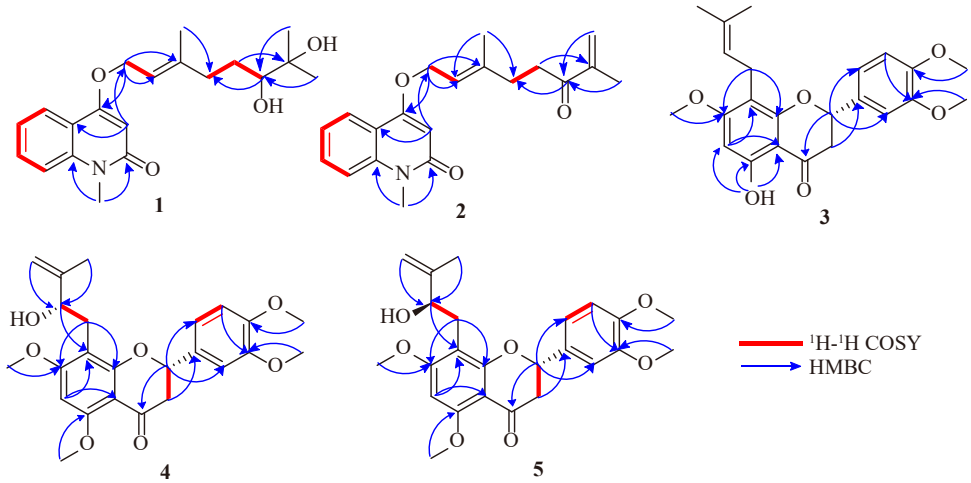

Fig. 2 Key ^{1}H-^{1}H COSY and HMBCs of compounds 1–5.

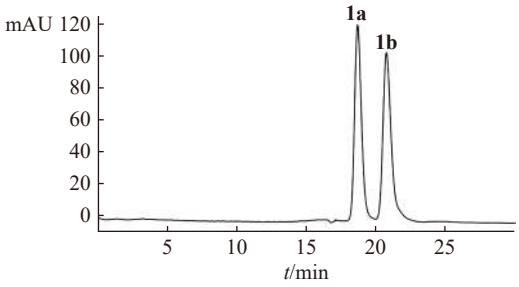

Fig. 3 The chiral HPLC separation of compounds 1a/1b.

was subsequently determined to be *R* based on the observed shifts in ^{1}H NMR resonances during Mosher ester analysis (Fig. 4). Consequently, the structure of **5** was elucidated as presented.

Through comparison of spectroscopic data with literature, the remaining seven known compounds were identified as 2'-dihydroxy-3,4,4',6'-quadrimethoxy-3'-(2'-isopentenyl)-chalcone [20], maculosidine (**7**) [21], skimmianine (**8**) [21], glycopentanolone C (**9**) [15], and glycopentanolone D (**10**) [15]. Chiral HPLC resolution of **9** yielded a pair of enantiomers, **9a** and **9b**, in a 1 : 1 ratio (Fig. S44). The 6'*R* and 6'*S* configurations of **9a** and **9b** were determined for the first time using Mosher ester analysis (Fig. 4). Similarly, **10a** and **10b** were isolated by chiral HPLC resolution in a 1 : 1 ratio (Fig. S44). The 6'*R* and 6'*S* configurations of **10a** and **10b** were determined for the first time by comparing their specific rotation data with those of **9a** and **9b**.

Given the traditional use of *Glycosmis* plants as anti-inflammatory agents and building upon our previous research [13], the isolated compounds (**1**–**10**) were evaluated for their inhibitory effects on NO production in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells. The results (Table 3) demonstrated that compounds **2**, **6**, **9**, and **10** exhibited inhibitory effects on NO production with IC_{50} values ranging from 13.5 to 20.1 μmol·L^{-1}, which were comparable to the positive control, dexamethasone (10.7 μmol·L^{-1}).

Experimental

General experimental procedures

Optical rotations were measured using a Rudolph Autopol IV automatic polarimeter (NJ, USA). IR spectra were recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer (MA, USA). UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer (Shimadzu Co., Tokyo, Japan). NMR spectra were acquired on a Varian INOVA-500 NMR spectrometer (Varian Medical Systems Inc., USA), utilizing acetone-d_6 or methanol-d_4 as solvents, with chemical shifts referenced to the deuterated solvent residual peak. HR-ESI-MS experiments were conducted on a Waters Xevo G2 Q-TOF mass spectrometer (Waters Co., Milford, MA, USA). The experimental ECD spectra were recorded on a J-810 spectrophotometer (JASCO, Japan).

Semipreparative HPLC was conducted using a Zorbax Eclipse XDB-C18 column (10 mm × 250 mm, 5 μm) on an Agilent 1260 series LC instrument equipped with a Diode Array Detector (DAD) (Agilent Technologies, Palo Alto, CA, USA). Preparative thin-layer chromatography (TLC) and TLC analyses were performed on pre-coated silica gel GF254 plates (Qingdao Marine Chemical Co., Ltd., China). Visualization of spots was achieved under UV light (254 and 365 nm) or by heating after spraying with 2% vanillin-H_2SO_4 solution. Column chromatography (CC) was executed on silica gel (100−200 mesh or 200−300 mesh, Qingdao Marine Chemical Co., Ltd., China), MCI gel (75−150 μm, Mitsubishi Chemical Ltd., Japan), Sephadex LH-20 (Amersham Biosciences, Sweden), and ODS (50 μm, YMC Co., Ltd., Japan).

All solvents utilized for HPLC were of HPLC grade (Fisher Chemical, USA), while solvents employed for isolation were of analytical grade (Beijing Chemical Works, China). Dexamethasone was obtained from Innochem (Beijing, China).

Plant material

The leaves and stems of *Glycosmis craibii* var. *glabra* (Craib) Tanaka were collected in Sanya, Hainan Province, China in March 2019. One of the authors (TU Pengfei) performed the botanical identification. A voucher specimen (No. GYS201903) has been deposited at the Herbarium of the Modern Research Center for Traditional Chinese Medicine of Peking University.

Extraction and isolation

Air-dried and finely powdered leaves and stems of *G. craibii* (9.6 kg) were refluxed thrice with 95% aqueous EtOH (100 L × 2 h) and concentrated under reduced pressure to yield a dry extract (650 g). The extract was suspended in H_2O and successively extracted with CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 extract (240 g) was subjected to silica gel CC and eluted with a stepwise gradient of petroleum ether–acetone (20 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, 1 : 1, and 0 : 1 gradient system, *V*/*V*) to obtain five fractions (A−E).

Fraction B (20.0 g) underwent separation using a Sepha-

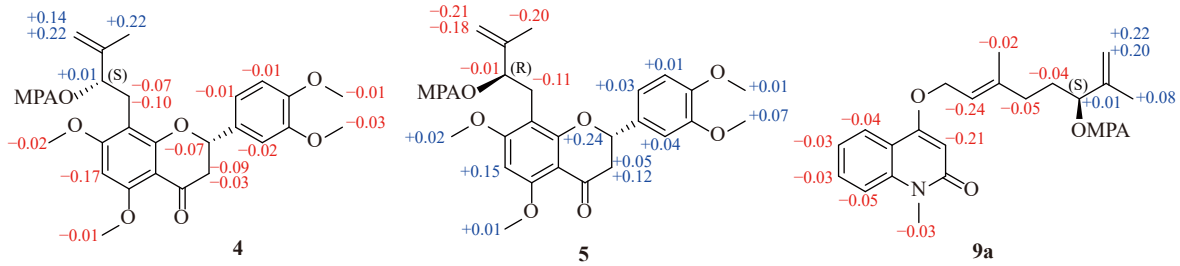

Fig. 4 Δ*δ* values (= $\delta_R - \delta_S$) obtained for (*R*)- and (*S*)-MPA esters of compounds 4, 5, and 9a. NMR data were obtained in $CDCl_3$.

Table 2 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 3–5 (*J* in Hz)

No.	3 (measured in $CDCl_3$)		4 (measured in methanol-d_6)		5 (measured in methanol-d_6)	
	^{1}H (*J* in Hz)	^{13}C, type	^{1}H (*J* in Hz)	^{13}C, type	^{1}H (*J* in Hz)	^{13}C, type
2	5.36, dd (2.9, 12.7)	78.8, CH	5.30, dd (2.9, 13.0)	80.2, CH	5.36, dd (2.8, 12.9)	80.1, CH
3	2.83, dd (2.9, 17.0)/ 3.07, dd (12.7, 17.0)	43.6, CH_2	2.71, dd (2.9, 16.4)/ 3.01, dd (13.0 16.4)	46.9, CH_2	2.75, dd (2.8, 16.4)/ 3.04, dd (12.9 16.4)	46.2, CH_2
4		196.6, C		192.9, C		192.9, C
4a		103.1, C		106.4, C		106.5, C
5		162.7, C		162.7, C		162.7, C
6	6.10, s	92.6, CH	6.31, s	89.9, CH	6.31, s	89.9, CH
7		165.8, C		166.0, C		166.2, C
8		109.1, C		108.3, C		108.3, C
8a		158.9, C		163.3, C		163.2, C
1'		131.6, C		133.6, C		133.2, C
2'	7.01, d (1.8)	109.3, CH	7.16, d (1.8)	112.7, CH	7.15, d (1.8)	112.7, CH
3'		149.2, C		150.6, C		150.6, C
4'		149.3, C		150.6, C		150.8, C
5'	6.89, d (8.2)	118.6, CH	6.98, d (8.2)	120.0, CH	6.98, d (8.3)	120.1, CH
6'	6.97, dd (1.8, 8.2)	111.1, CH	7.05, dd (1.8, 8.2)	111.1, CH	7.05, dd (1.8, 8.3)	111.2, CH
1"	3.23, d (7.2)	21.8, CH_2	2.90, dd (6.1, 12.9)/ 2.79, dd (8.3, 12.9)	29.8, CH_2	2.87, dd (6.2, 13.1)/ 2.82, dd (8.3, 13.1)	30.0, CH_2
2"	5.16, t (7.2)	122.6, CH	4.26, dd (6.1, 8.3)	76.6, CH	4.31, dd (6.2, 8.3)	76.3, CH
3"		131.6, C		148.5, C		148.7, C
4"	1.64, s	17.9, CH_3	4.61, br s 4.49, br s	111.1, CH_2	4.65, br s 4.62, br s	111.4, CH_2
5"	1.63, s	26.0, CH_3	1.61, s	17.2, CH_3	1.70, s	17.5, CH_3
OH-5	12.14, br s					
OCH_3-5			3.90, s	56.5, CH_3	3.90, s	56.5, CH_3
OCH_3-7	3.86, s	56.0, CH_3	3.93, s	56.5, CH_3	3.92, s	56.5, CH_3
OCH_3-3'	3.91, s	56.1, CH_3	3.85, s	56.2, CH_3	3.85, s	56.2, CH_3
OCH_3-4'	3.91, s	56.1, CH_3	3.87, s	56.3, CH_3	3.86, s	56.3, CH_3

Assignments were based on HSQC and HMBC experiments

dex LH-20 column eluted with MeOH−CH_2Cl_2 (1 : 1, *V/V*), yielding three subfractions (B1−B3). Subfraction B2 (5.2 g) was further divided into six fractions (B2a−B2f) utilizing an ODS column eluted with a stepwise gradient of MeOH−H_2O (35 : 65, 65 : 35, 85 : 15, and 100 : 0, *V/V*). Fraction B2b (130 mg) was subsequently purified by semipreparative HPLC [3.0 mL·min^{-1}, MeCN/H_2O (40 : 60, *V/V*)], resulting in **7** (11.0 mg, t_R 17.8 min) and **8** (17.0 mg, t_R 25.5 min). Subfraction B3 (12.2 g) underwent separation into six fractions (B3a−B3f) using an ODS column eluted with a stepwise gradient of MeOH−H_2O (50 : 50, 75 : 25, 90 : 10, and 100 : 0, *V/V*). Fraction B3d (220 mg) was purified by semipreparative HPLC [3.0 mL·min^{-1}, MeCN/H_2O (55 : 45, *V/V*)], yielding **1** (3.0 mg, t_R 11.5 min), **9** (21.0 mg, t_R 16.5 min), **10** (4.0 mg, t_R 21.5 min), and **2** (5.0 mg, t_R 25.5 min).

Fraction E (25.0 g) was fractionated into four components (E1−E4) utilizing an ODS column eluted with a stepwise gradient of MeOH−H_2O (30 : 70, 50 : 50, 70 : 30, 90 : 10, and 100 : 0, *V/V*). Fraction E4 (85 mg) underwent purification *via* semipreparative HPLC [3.0 mL·min^{-1}, MeCN/H_2O (55 : 45, *V/V*)] to yield **3** (1.5 mg, t_R 15.6 min), **4** (2.0 mg, t_R 20.3 min), **5** (6.0 mg, t_R 42.0 min), and **6** (15.0 mg, t_R 34.5 min).

The chiral separations of compounds **1**, **9**, and **10** were conducted using semipreparative normal-phase HPLC (NP-HPLC). The elution was performed with *n*-hexane-*i*PrOH in ratios of 80 : 20 (*V/V*), 78 : 22 (*V/V*), and 75 : 25 (*V/V*), respectively. The detection wavelength was set at 330 nm, and the flow rate was maintained at 1 mL·min^{-1}. The separation yielded the following compounds: **1a** (1.1 mg, t_R 18.5 min), **1b** (1.2 mg, t_R 21.0 min), **9a** (2.5 mg, t_R 20.6 min), **9b** (2.5 mg, t_R 22.1 min), **10a** (2.6 mg, t_R 19.1 min), and **10b** (2.7 mg, t_R 20.6 min).

Craiquinolone A (**1**): pale yellow oil; $[\alpha]_D^{25}$(*c* 0.1,

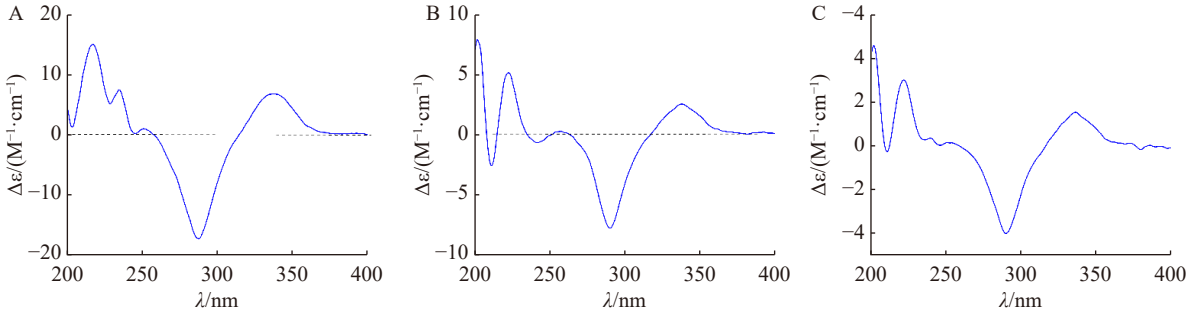

Fig. 5 Experimental ECD spectra of compounds 3–5 (A–C).

Table 3 Inhibitory effects of the isolates on LPS-activated NO production in BV-2 cells [a] (means ± SD, *n* = 3)

Compound	IC_{50} (μmol·L^{-1})
2	13.5 ± 0.4
6	15.8 ± 0.3
9	20.1 ± 0.4
10	14.5 ± 0.4
Dexamethasone [b]	10.7 ± 0.5

[a] Only compounds with observable inhibitory effects (IC_{50} < 40 μmol·L^{-1}) are listed. [b] Positive control.

MeOH); IR (KBr) ν_{max} 3302, 2936, 1633, 1583, 1232, 1153, 1118 cm^{-1}; UV (MeOH) λ_{max} (log ε) 230 (1.70), 279 (0.23), 320 (0.20) nm; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS *m/z* 346.2023 [M + H]$^+$ (Calcd. for $C_{20}H_{28}NO_4$, 346.2018).

(–)-Craiquinolone A (**1a**): pale yellow oil; $[\alpha]_D^{25}$ –10 (*c* 0.05, MeOH).

(+)-Craiquinolone A (**1b**): pale yellow oil; $[\alpha]_D^{25}$ +10 (*c* 0.05, MeOH).

Craiquinolone B (**2**): pale yellow oil; IR (KBr) ν_{max} 1633, 1583, 1232, 1117, 758 cm^{-1}; UV (MeOH) λ_{max} (log ε) 230 (1.31), 269 (0.20), 318 (0.17) nm; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS *m/z* 326.1754 [M + H]$^+$ (Calcd. for $C_{20}H_{24}NO_3$, 326.1756).

Craiflavanone A (**3**): pale yellow oil; $[\alpha]_D^{25}$ –20 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3490, 2936, 1659, 1595, 1513, 1270, 1107 cm^{-1}; UV (MeOH) λ_{max} (log ε) 230 (1.72), 290 (1.30), 325 (0.42) nm; ECD (MeOH) λ_{max} (Δ ε) 223 (+4.22), 292 (–5.50), 339 (+2.16) nm; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS *m/z* 399.1811 [M + H]$^+$ (Calcd. for $C_{23}H_{27}O_6$, 399.1808).

Craiflavanone B (**4**): pale yellow oil; $[\alpha]_D^{25}$ –18 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3402, 2926, 1664, 1599, 1464, 1265, 1116, 1022 cm^{-1}; UV (MeOH) λ_{max} (log ε) 225 (1.70), 290 (1.00), 319 (0.38) nm; ECD (MeOH) λ_{max} (Δ ε) 222 (+5.16), 290 (–7.80), 338 (+2.56) nm; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS *m/z* 429.1915 [M + H]$^+$ (Calcd. for $C_{24}H_{29}O_7$, 429.1913).

Craiflavanone C (**5**): pale yellow oil; $[\alpha]_D^{25}$ –8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3408, 2930, 1665, 1599, 1464, 1265, 1114, 1025 cm^{-1}; UV (MeOH) λ_{max} (log ε) 230 (1.68), 292 (1.30), 325 (0.40) nm; ECD (MeOH) λ_{max} (Δ ε) 222 (+3.00), 290 (–4.00), 336 (+1.53) nm; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS *m/z* 429.1913 [M + H]$^+$ (Calcd. for $C_{24}H_{29}O_7$, 429.1913).

(±)-glycopentanolone C (**9a/9b**): (–)-glycopentanolone C (**9a**): pale yellow oil; $[\alpha]_D^{25}$ –10 (*c* 0.05, MeOH); (+)-glycopentanolone C (**9b**): pale yellow oil; $[\alpha]_D^{25}$ +10 (*c* 0.05, MeOH).

(±)-glycopentanolone D (**10a/10b**): (–)-glycopentanolone D (**10a**): pale yellow oil; $[\alpha]_D^{25}$ –10 (*c* 0.05, MeOH); (+)-glycopentanolone D (**10b**): pale yellow oil; $[\alpha]_D^{25}$ +10 (*c* 0.05, MeOH).

Preparation of the (R)-and (S)-MPA esters of 4, 5, and 9a

Compound **4** (1.0 mg) was dissolved in 800 μL of $CDCl_3$, followed by the sequential addition of DCC (2 mg), DAMP (0.5 mg), and (*R*)-MPA (2 mg). The reaction mixture was stirred for 12 h at room temperature. The resulting crude products were separated by semipreparative HPLC [Zorbax Eclipse XDB-C_{18} column, 1.0 mL·min^{-1}, MeCN-H_2O (75 : 25)], yielding the (*R*)-MPA ester (**4r**, 0.6 mg) at 19.3 min. Using a similar procedure, the (*S*)-MPA ester (**4s**, 0.6 mg) was isolated from the reaction of **4** (1.0 mg) with (*S*)-MPA by semipreparative HPLC [Zorbax Eclipse XDB-C_{18} column, 1.0 mL·min^{-1}, MeCN-H_2O (75 : 25)] at 19.8 min. The (*R*)- and (*S*)-MPA esters of compounds **5** and **9a** were prepared using analogous methods.

Cell culture

The murine BV-2 microglial cells were obtained from Peking Union Medical College Cell Bank (Beijing, China). The experiment was structured into three groups: a control group (normal cultured cells), a model group (LPS concentration of 1.0 μg·mL^{-1}), and drug groups, with six wells allocated per group. Cell maintenance, experimental procedures, and data determination were conducted as previously described [22, 23]. Dexamethasone served as a positive control. To ascertain whether the inhibitory activities of these compounds were attributable to cytotoxicity, their effects on cell proliferation were assessed using the MTT method. None of the compounds exhibited significant cytotoxicity at a dosage of 40 μmol·L^{-1}.

Statistical analysis

All experimental results underwent tests for normality and homogeneity and are presented as means ± standard deviation (SD). Comparisons were conducted using independent

t-tests or one-way analysis of variance (ANOVA). Statistical analyses were performed using GraphPad Prism 7 Software, with a P value < 0.05 considered statistically significant.

Conclusion

This study reports the isolation and identification of six novel compounds and seven known analogs from the 95% ethanol extract of stems and leaves of *G. craibii* var. *glabra*. Compounds **1** and **2** represent new quinolones with oxygenated geranyl substitutions, while compounds **3**–**5** are novel prenylated flavanones. Additionally, two pairs of known quinolone enantiomers (**9a**/**9b** and **10a**/**10b**) were successfully separated *via* chiral HPLC for the first time. The absolute configurations of **1a**/**1b**, **3**, **4**, **5**, **9a**/**9b**, and **10a**/**10b** were determined using the Mosher method, CD spectroscopy, or by comparing their specific rotation data with literature values.

To identify and concentrate the anti-inflammatory components from *G. craibii* var. *glabra*, we assessed the inhibitory effects of all isolated compounds on NO production in BV-2 microglia. The results revealed that three quinolone derivatives exhibited superior inhibition activities, suggesting that quinolones are also among the anti-inflammatory active components of this genus of plants. Currently, numerous studies report on the antibacterial and anti-tumor activities of quinolones; however, their anti-inflammatory potential has received limited attention. This study represents the first report on the anti-inflammatory activity of quinolones, providing a foundation for future exploration of quinolone activity.

Appendix A. Supplementary data

Supplementary material related to this article can be obtained by contacting the corresponding authors *via* email.

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Cite this article as: CHEN Hongwei, DING Meng, LIN Jun, *et al*. NO inhibitory constituents from *Glycosmis craibii* var. *glabra* [J]. *Chin J Nat Med*, 2024, **22**(11): 1040-1046.