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ORIGINAL ARTICLE

Six New 3,5-Dimethylcoumarins from *Chelonopsis praecox*, *Chelonopsis odontochila* and *Chelonopsis pseudobracteata*

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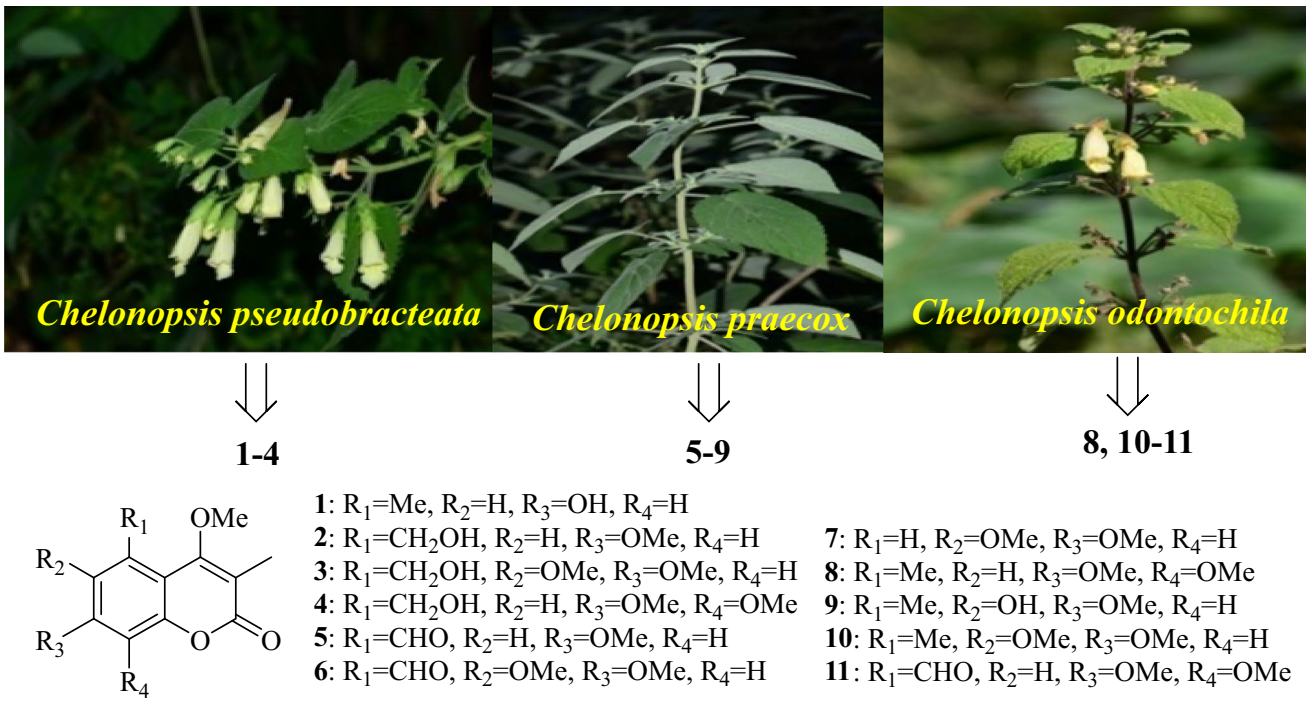
Abstract

Ten 3,5-dimethylcoumarins (**1**–**6** and **8**–**11**) involving six new ones (**1**–**6**), together with a known 3-methylcoumarin (**7**), were isolated from the aerial parts of three *Chelonopsis* plants, *C. praecox*, *C. odontochila*, and *C. pseudobracteata*. The structures of the new compounds were determined by extensive HRESIMS, 1D and 2D NMR spectroscopic analyses. According to the substitution at C-5, these coumarins were classified into 5-methyl, 5-hydroxymethyl, 5-formyl, and 5-*nor* types. All the isolates were assayed for their inhibition on α-glucosidase, protein tyrosine phosphatase 1B, and T-cell protein tyrosine phosphatase in vitro.

Graphic Abstract



Keywords 3,5-Dimethylcoumarins · 3-Methylcoumarin · *Chelonopsis* · Enzyme inhibition

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Extended author information available on the last page of the article

1 Introduction

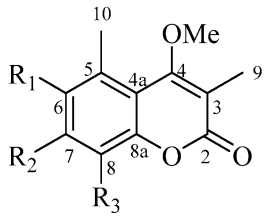
Coumarins with the benzo-α-pyrone core are widely distributed in plant kingdom and show a wide range of biological activities, including antimicrobial, antiviral, antidiabetic antiinflammatory, and antihypertensive activities, *etc*. [1]. Structurally, coumarins can be divided into simple coumarins, C-substituted coumarins, miscellaneous coumarins, biscoumarins, and triscoumarins. Besides hydroxy and methoxy groups, isopentenyl related C_5-groups are the most common substituents present in coumarins, which are generally located at C-3, C-6, or C-8 positions by C–C linkage [2–4]. The methyl substituent in coumarin is very unusual, and only limited coumarins with the methylation at C-3, C-5, or C-6 positions have been reported. Currently, tens of 3,5-dimethylcoumarins have been isolated from *Clutia lanceolata* [5], *Clutia abyssinica* [6], *Juniperus sabina* [7], *Leucas inflata* [8], and *Sideritis pullulans* [9], but never from *Chelonopsis* plants. Our previous investigation on *Chelonopsis* plants yielded a series of diterpenoids with α-glucosidase inhibitory activity, *i.e*., ten *ent*-kauranes from *C. praecox* [10], and 13 *ent*-labdanes and 11 *ent*-kauranes from *C. odontochila* [11]. As a continuous search for antidiabetic candidates from natural sources [12–16], ten 3,5-dimethylcoumarins (**1**–**6** and **8**–**11**) involving six new ones and one known 3-methylcoumarin (**7**) were first isolated from three *Chelonopsis* plants (Fig. 1). Herein, we report their isolation, structural elucidation, and enzymatic inhibition on α-glucosidase, protein tyrosine phosphatase 1B (PTP1B), and T-cell protein tyrosine phosphatase (TCPTP).

2 Results and Discussion

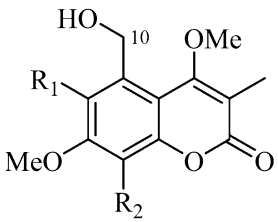
2.1 Structural Elucidation

Compound **1** had a chemical composition of $C_{12}H_{12}O_4$ deduced by the $[M+H]^+$ ion at *m/z* 221.0809, accounting for seven indices of hydrogen deficiency. The UV spectrum showed characteristic absorption at λ_{max} 321 nm for coumarins. The IR absorptions at 3204, 1681, 1610, and 1454 cm^{-1} were indicative for the presence of hydroxyl, carbonyl, and aromatic functionalities. In the ^1H NMR spectrum, two *meta*-coupled aromatic protons at δ_H 6.93 ($J=2.4$ Hz) and 6.87 ($J=2.4$ Hz), one methoxy at δ_H 3.64, and two singlet methyls at δ_H 2.56 and 2.10 were well recognized (Table 1). The ^{13}C NMR spectrum displayed 12 carbons comprising one carbonyl carbon, eight olefinic carbons, one methoxy, and two methyls. The ^1H and ^{13}C NMR data of **1** showed high resemblance with 6-hydroxy-3,5-dimethyl-4,7-dimethoxycoumarin (**9**) [5] except for the absence of a methoxy and an oxygenated methine in **9** being changed to be a methine in **1**. In the HMBC spectrum, the correlations from the methyl (δ_H 2.10) to C-2 (δ_C 164.0) and C-4 (δ_C 166.9), and from the methoxy (δ_H 3.64) to C-4 (δ_C 166.9) affirmed the 3-methyl and 4-methoxy substitution. Taking the ROESY correlations of Me-3/OMe-4/Me-5/H-6 into consideration, this compound was characterized to be 7-hydroxy-4-methoxy-3,5-dimethylcoumarin (**1**).

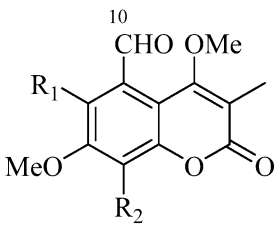
The molecular formula of **2** was assigned to be $C_{13}H_{14}O_5$ by the positive HRESIMS ion at *m/z* 251.0897 ($[M+H]^+$, calcd. for 251.0914). By comparing its ^1H and ^{13}C NMR data with those of **1**, the 5-methyl in **1** was changed to be a hydroxymethyl (δ_H 4.89, δ_C 56.2) in **2**, as well as an additional methoxyl group. The substitution of 5-hydroxymethyl and 7-methoxyl was confirmed by the HMBC correlations from H-10 (δ_H 4.89) to C-6 and C-4a, and from OMe-7 (δ_H 3.86) to C-7, as well as the ROESY correlations of H_3-9/OMe-4/H_2-10/H-6/



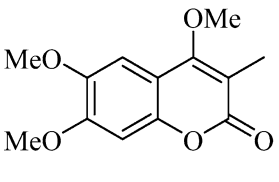
1: R_1=H, R_2=OH, R_3=H
8: R_1=H, R_2=OMe, R_3=OMe
9: R_1=OH, R_2=OMe, R_3=H
10: R_1=OMe, R_2=OMe, R_3=H



2: R_1=H, R_2=H
3: R_1=OMe, R_2=H
4: R_1=H, R_2=OMe



5: R_1=H, R_2=H
6: R_1=OMe, R_2=H
11: R_1=H, R_2=OMe



7

Fig. 1 Chemical structures of compounds **1**–**11**

Table 1 ^{1}H NMR data of compounds **1**–**7** (δ in ppm, J in Hz)

No.	1	2	3	4	5	6	7
5	–	–	–	–	–	–	7.07, s
6	6.87, d (2.4)	6.96, d (3.0)	–	6.99, s	7.24, d (2.7)	–	–
7	–	–	–	–	–	–	–
8	6.93, d (2.4)	6.77, d (3.0)	6.84, s	–	6.99, d (2.7)	6.89, s	6.85, s
3-Me	2.10, s	2.16, s	2.17, s	2.18, s	2.19, s	2.15, s	2.17, s
4-MeO	3.64, s	3.98, s	4.01, s	3.98, s	3.91, s	3.81, s	4.01, s
5-Me	2.56, s	–	–		–	–	–
5-$HOCH_2$	–	4.89, s	4.99, s	4.89, s	–	–	–
5-OHC	–	–	–	–	10.74, s	10.44, s	–
6-HO	–	–	–	–	–	–	–
6-MeO	–	–	3.86, s	–	–	3,82, s	3.95, s
7-MeO	–	3.86, s	3.93, s	3.97, s	3.89, s	3.93, s	3.94, s
8-MeO	–	–	–	3.95, s	–	–	–

Compound **1** was measured in pyridine-d_5, and **2**–**7** were measured in $CDCl_3$

OMe-7/H-8 (Fig. 2). Hence, compound **2** was defined as 5-hydroxymethyl-4,7-dimethoxy-3-methylcoumarin.

Compounds **3** and **4** were a pair of isomers with the same molecular formula of $C_{14}H_{16}O_6$, indicating an additional CH_2O moiety than **2**. In their ^{1}H and ^{13}C NMR spectra, three methoxy groups (δ_H 3.86, 3.93, 4.01 and δ_C 56.2, 61.9, 62.1 for **3**; δ_H 3.95, 3.97, 3.98 and δ_C 56.5, 61.67, 61.70 for **4**) were obviously recognized (Table 2), suggesting compounds **3** and **4** should be the methoxylated derivatives of **2**. The position of the additional methoxy in **3** and **4** were unambiguously determined by analyzing their ROESY experiments. In the ROESY spectrum of **3**, the correlation peaks of H_3-9/OMe-4/H_2-10/OMe-6 and OMe-7/H-8 revealed the methoxy at C-6 position. Similarly, the ROESY signals of H_3-9/OMe-4/H_2-10/H-6/OMe-7 in **4** supported the methoxy at C-8 position. Thus, compounds **3** and **4** were concluded as 5-hydroxymethyl-4,6,7-trimethoxy-3-methylcoumarin (**3**) and 5-hydroxymethyl-4,7,8-trimethoxy-3-methylcoumarin (**4**), respectively.

Compound **5** had a chemical composition of $C_{13}H_{12}O_5$ according to the protonated ion at m/z 249.0744 in the HRESIMS data. In the ^{1}H NMR spectrum, the presence of a formyl group at δ_H 10.74, two *meta*-coupled aromatic protons at δ_H 7.24 (J = 2.7 Hz) and 6.99 (J = 2.7 Hz), two methoxys at δ_H 3.91 and 3.89, and a methyl at δ_H 2.19 were easily recognized. By comparing with **2**, compound **5** had an additional formyl group at δ_H 10.74 and δ_C 192.3, but with the absence of a hydroxymethyl group (δ_H 4.89 and δ_C 64.6), indicating

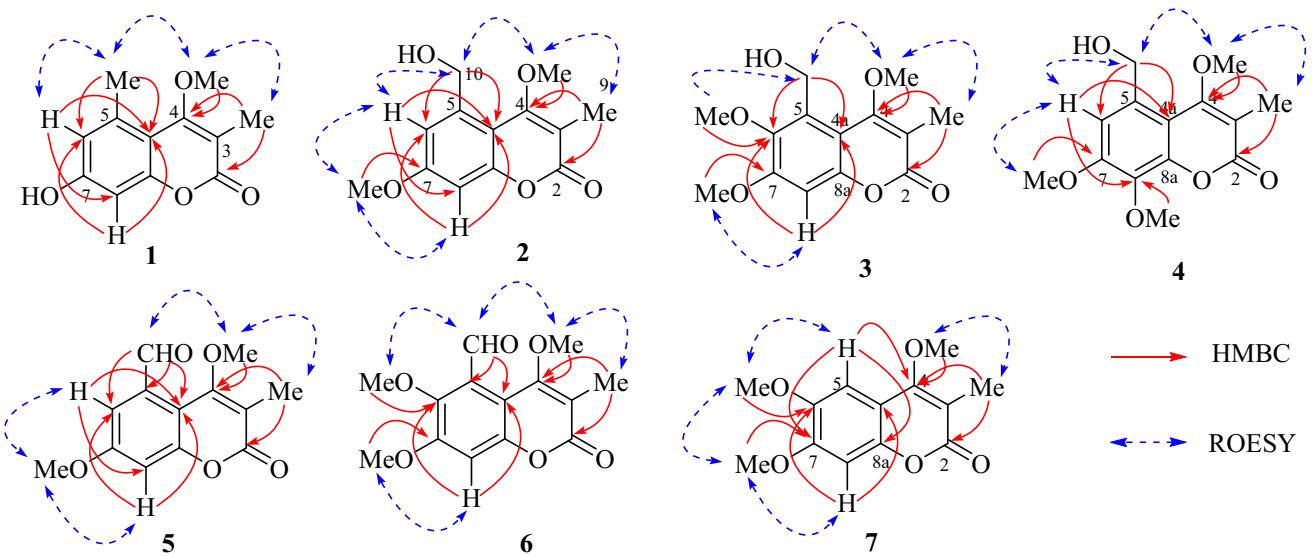


Fig. 2 Selected 2D NMR correlations of compounds **1**–**7**

Table 2 ^{13}C NMR data of compounds **1**–**7** (δ in ppm, J in Hz)

No.	1	2	3	4	5	6	7
2	164.0, C	164.4, C	164.1, C	163.7, C	163.6, C	164.1, C	165.2, C
3	108.9, C	110.4, C	111.5, C	111.2, C	111.9, C	110.0, C	109.6, C
4	166.9, C	165.6, C	165.3, C	165.2, C	164.5, C	163.7, C	164.2, C
4a	108.8, C	108.5, C	108.6, C	109.6, C	110.1, C	108.3, C	109.9, C
5	137.6, C	139.7, C	130.5, C	133.2, C	135.5, C	130.0, C	103.5, CH
6	117.2, CH	113.8, CH	145.0, C	110.0, CH	111.5, CH	143.2, C	146.5, C
7	161.2, C	161.9, C	155.6, C	154.2, C	161.5, C	156.0, C	152.5, C
8	101.3, CH	100.3, CH	100.5, CH	135.9, C	105.6, CH	101.5, CH	100.0, CH
8a	156.0, C	155.4, C	151.0, C	147.8, C	154.2, C	149.5, C	148.2, C
3-Me	10.6, CH_3	11.2, CH_3	11.0, CH_3	11.3, CH_3	10.9, CH_3	10.9, CH_3	10.9, CH_3
4-MeO	60.3, CH_3	61.6, CH_3	61.9, CH_3	61.7, CH_3	60.9, CH_3	60.7, CH_3	61.4, CH_3
5-Me	22.1, CH_3	–	–	–	–	–	–
5-$HOCH_2$	–	64.6, CH_2	56.2, CH_2	64.9, CH_2	–	–	–
5-OHC	–	–	–	–	192.3, CH	192.4, CH	–
6-OMe	–	–	62.1, CH_3	–	–	62.7, CH_3	56.6, CH_3
7-OMe	–	55.9, CH_3	56.2, CH_3	56.5, CH_3	56.1, CH_3	56.4, CH_3	56.6, CH_3
8-OMe	–	–	–	61.7, CH_3	–	–	–

Compound **1** was measured in pyridine-d_5, and **2**–**7** were measured in $CDCl_3$

the dehydrogenated derivative of **2**. The formyl group was assigned at C-5 by the ROESY correlations of H_3-9/OMe-4/H-10, and HMBC correlation from H-6 to C-10 and from H-10 to C-5, C-6 and C-4a. Consequently, compound **5** was defined as 5-formyl-4,7-dimethoxy-3-methylcoumarin.

The molecular formula of **6** was assigned as $C_{14}H_{14}O_6$ by the $[M+H]^+$ ion at *m/z* 279.0906 in positive HRESIMS spectrum. In the ^1H NMR spectrum, one formal at δ_H 10.44, one aromatic singlet at δ_H 6.89, three methoxy groups at δ_H 3.81, 3.82, and 3.93, and one methyl group at δ_H 2.15 were observed, showing an extra methoxy than **5**. The above deduction was consistent with that two *meta*-coupled protons at δ_H 7.24 and 6.99 in **5** was changed to be an aromatic singlet at δ_H 6.89 in **6**. By analyzing the ROESY experiment, the correlations of H_3-9/OMe-4/H-10/OMe-6 and OMe-7/H-8 demonstrated the structure of 5-formyl-4,6,7-trimethoxy-3-methylcoumarin (**6**).

Compound **7** was assigned with the chemical formula of $C_{13}H_{14}O_5$ by the $[M+H]^+$ ion at *m/z* 251.0898 in positive HRESIMS spectrum. In the ^1H NMR spectrum, two aromatic protons at δ_H 7.07 and 6.85, three methoxy groups at δ_H 4.01, 3.95, and 3.94, and a methyl group at δ_H 2.17, were recognized. Compared with 4,6,7-trimethoxy-3,5-dimethylcoumarin (**10**) [6], the 5-methyl in **10** was absent in **7** but with an extra aromatic singlet at δ_H 7.07. This proton (δ_H 7.07) was assigned to be H-5 by the HMBC correlation from H-5 to C-4, and ROESY correlations of H-5/OMe-6 and H-8/OMe-7. Thus, compound **7** was deduced as 4,6,7-trimethoxy-3-methylcoumarin, the demethylated derivative of **10**. Although this compound has been synthesized by methylation of 4-hydroxy-6,7-dimethoxy-3-methylcoumarin in 1949 [17], it is the first report of its natural occurrence and NMR spectroscopic data.

The known coumarins were determined to be 4,7,8-trimethoxy-3,5-dimethylcoumarin (**8**) [7], 6-hydroxy-4,7-dimethoxy-3,5-dimethylcoumarin (**9**) [5], 4,6,7-trimethoxy-3,5-dimethylcoumarin (**10**) [6], and 5-formyl-4,7,8-trimethoxy-3-methylcoumarin (**11**) [8] by comparing their ^1H and ^{13}C NMR data with those in the literatures.

In order to evaluate their antidiabetic potency, all the coumarins were assayed for their inhibitory activity on α-glucosidase, PTP1B, and TCPTP. As shown in Table 3, all the compounds showed only weak or no inhibition to three enzymes at the concentration of 200 μM. According to the previous study [5], this type of coumarins could enhance the glucose-triggered secretion of insulin from murine islets. Thus, further studies will be needed to reveal their targets and mechanisms in exerting hypoglycemic effects.

3 Experimental Section

3.1 General Experimental Procedures

A Jasco model 1020 digital polarimeter (Jasco Corp., Tokyo, Japan) was used to measure optical rotations. UV and IR data were obtained using a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan) and a Nicolet iS10 spectrometer (Thermo Fisher Scientific, Madison, WI, USA), respectively. A Waters AutoSpec Premier P776 mass spectrometer (Waters, Milford, MA, USA) or a

Table 3 Inhibitory rates of the isolates (200 μM) on α-glucosidase, PTP1B, and TCPTP

No.	Inhibition rates (%)		
	α-glucosidase	PTP1B	TCPTP
1	11.1 ± 4.1	26.2 ± 2.9	5.0 ± 1.3
2	22.3 ± 6.9	9.8 ± 2.5	− 4.9 ± 2.1
3	6.2 ± 4.6	6.8 ± 3.4	− 9.3 ± 3.2
4	26.4 ± 9.9	13.4 ± 5.9	− 9.1 ± 1.5
5	10.9 ± 9.1	19.0 ± 4.6	1.7 ± 0.5
6	0.8 ± 1.0	8.7 ± 3.5	− 3.2 ± 1.1
7	12.0 ± 3.7	13.6 ± 3.9	25.9 ± 2.4
8	− 4.5 ± 0.9	27.8 ± 7.9	7.1 ± 3.8
9	12.5 ± 3.9	9.5 ± 2.2	27.8 ± 4.1
10	11.2 ± 3.7	25.1 ± 1.9	− 3.1 ± 0.6
11	9.5 ± 2.3	35.7 ± 2.4	− 5.3 ± 2.0
Acarbose	87.9 ± 0.4	−	−
Na_3VO_4	−	69.7 ± 5.2	45.4 ± 2.1

Data were expressed as means ± SD (n = 3) from three independent experiments

Shimadzu LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan) was used to acquire the high-resolution mass spectra. ECD spectra were recorded on an Applied Photophysics Chirascan apparatus (Applied Photophysics, Surrey, UK). NMR spectra were obtained by using DRX-500, Avance III-600, and Ascend™ 800 MHz spectrometers (Bruker, Karlsruhe, Germany). TLC detection was run on silica gel plates (60 F254). Silica gel (200–300 mesh, Qingdao Makall group Co. Ltd., Qingdao, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography. A Dr-Flash II apparatus was applied to accomplish the MPLC separations. HPLC purifications were conducted on a Shimadzu LC-CBM-20 system (Shimadzu, Kyoto, Japan), equipped with an Agilent Eclipse XDB-C_{18} column (5 μm, 9.4 × 250 mm).

3.2 Plant Materials

The aerial parts of three *Chelonopsis* plants were collected in October 2016 from Lijiang, Yunnan Province of China, which were authenticated to be *Chelonopsis odontochila* Diels, *Chelonopsis pseudobracteata* C. Y. Wu et H. W. Li, and *Chelonopsis praecox* Weckerle and F. Huber by Dr. Chun-Lei Xiang. Voucher specimens (Nos. 2016102101, 2016102102, 2016102103) were deposited in the Laboratory of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences, China.

3.3 Extraction and Isolation

The air-dried plants of *C. pseudobracteata* (6 kg) were powdered and extracted three times with 90% aqueous EtOH (25 L × 3) at room temperature. The extract was evaporated under reduced pressure, and the residue was suspended in H_2O and partitioned with $CHCl_3$. The $CHCl_3$ extraction (95 g) was subjected to silica gel column chromatography (Si CC) and eluted with an acetone-petroleum ether solvent system (from 10:90 to 50:50, *v/v*) to afford seven fractions (A–G). Fraction C (19.8 g) was subjected over MCI gel CHP 20P column (H_2O–MeOH, 50:50–0:100) to provide five fractions, Frs. C1–C5. Fr. C_3 (3.4 g) was purified via Si CC (EtOAc-petroleum ether, 10:90–50:50) to give three fractions, Frs. C3-1–C3-3. Fr. C3-1 (600 mg) was purified by Sephadex LH-20 CC (MeOH–$CHCl_3$, 50:50) and semi-preparative HPLC (H_2O–MeCN, 36:64) to give compounds **1** (16 mg) and **3** (18 mg). Compounds **2** (25 mg) and **4** (38 mg) were obtained from Fr. C3-2 (750 mg) by Sephadex LH-20 CC (MeOH–$CHCl_3$, 50:50) and semi-preparative HPLC (H_2O–MeCN, 50:50).

The air-dried plants of *C. praecox* (25 kg) were powdered and extracted three times with 90% aqueous EtOH (100 L × 3) at room temperature. The combined EtOH extract was concentrated and partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extraction (380 g) was subjected to Si CC (4.0 kg, 30 × 100 cm), using a gradient elution of EtOAc-petroleum ether (from 10:90 to 100:0) to afford seven fractions (A–G). MPLC separation of Fr. D (46 g) by using a CHP20P MCI gel column (H_2O-MeOH, from 50:50 to 0:100) provided five fractions, Frs. D1–D5. Fr. D3 (1.3 g) was separated by Si CC (EtOAc-$CHCl_3$, 2:98–50:50) to afford five fractions, Frs. D3-1–D3-5. Fr. D3-1 (265 mg) was purified by Si CC (acetone-petroleum ether, 5:95), and semi-preparative HPLC (H_2O–MeCN, 42:58) to yield compounds **8** (25 mg), **5** (18 mg), and **6** (18 mg). Fr. E (17 g) was subjected to MPLC to give five fractions, Frs. E1–E5. Compounds **9** (5 mg) and **7** (25 mg) were obtained from Fr. E3 after repeated Si CC (acetone-petroleum ether, 10:90) and Sephadex LH-20 (MeOH–$CHCl_3$, 50:50), and semi-preparative HPLC (MeCN–H_2O, 40:60).

Air-dried and powdered plants of *C. odontochila* (8.0 kg) were extracted with 90% aqueous EtOH (35 L × 3) at room temperature. The combined EtOH extract was concentrated and partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extract (160 g) was chromatographed on a silica gel column (1.3 kg, 30 × 100 cm), and eluted with acetone-petroleum ether gradient (from 0:100 to 100:0) to yielded seven fractions, Frs. A–G. MPLC separation of Fr. C (15 g) with MCI gel CHP 20P column (H_2O–MeOH, from 50:50 to 0:100) gave rise to five fractions, Frs. C1–C5. Fraction C3 (2.8 g) was chromatographed on a silica gel column (acetone-$CHCl_3$, from 10:90 to 100:0) to provide four fractions, Frs. C3-1–C3-4.

Compound **11** (12 mg) was purified from Fr. C3-2 (500 mg) by Sephadex LH-20 CC (MeOH–$CHCl_3$, 50:50), and semi-preparative HPLC (H_2O–MeCN, 64:36). After repeated separation over Si CC (acetone-petroleum ether, 15:85), Sephadex LH-20 CC (MeOH–$CHCl_3$, 50:50), and semi-preparative HPLC (H_2O–MeCN, 60:40), compounds **10** (27 mg) and **8** (21 mg) were obtained from Fr. C3-3 (470 mg).

3.4 Spectroscopic Data of Compounds

3.4.1 7-Hydroxy-4-Methoxy-3,5-Dimethylcoumarin (1)

White amorphous powder; UV (MeOH) λ_{max} (log ε): 321 (3.99) nm; IR (KBr) ν_{max}: 3204, 1681, 1610, 1567, 1454, 1378, 1358, 1344, 1257, 1154, 1102, 1074, 1016 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 221.0809 $[M+H]^+$ (calcd. for $C_{12}H_{13}O_4$, 221.0808).

3.4.2 5-Hydroxymethyl-4,7-Dimethoxy-3-Methylcoumarin (2)

White amorphous powder; UV (MeOH) λ_{max} (log ε): 222 (3.91), 320 (3.90) nm; IR (KBr) ν_{max}: 3398, 1663, 1616, 1592, 1558, 1453, 1432, 1369, 1336, 1251, 1197, 1151, 1083, 1047, 1012, 946 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 251.0897 $[M+H]^+$ (calcd. for $C_{13}H_{15}O_5$, 251.0914).

3.4.3 5-Hydroxymethyl-4,6,7-Trimethoxy-3-Methylcoumarin (3)

White amorphous powder; UV (MeOH) λ_{max} (log ε): 294 (3.75), 328 (4.00) nm; IR (KBr) ν_{max}: 3434, 1683, 1603, 1562, 1452, 1418, 1366, 1331, 1262, 1224, 1161, 1130, 1081, 1061, 1004, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 281.1039 $[M+H]^+$ (calcd. for $C_{14}H_{17}O_6$, 281.1020).

3.4.4 5-Hydroxymethyl-4,7,8-Trimethoxy-3-Methylcoumarin (4)

White amorphous powder; UV (MeOH) λ_{max} (log ε): 318 (4.07) nm; IR (KBr) ν_{max}: 3435, 1687, 1595, 1571, 1457, 1421, 1337, 1274, 1136, 1096, 1052 1012 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 281.1014 $[M+H]^+$ (calcd. for $C_{14}H_{17}O_6$, 281.1020).

3.4.5 5-Formyl-4,7-Dimethoxy-3-Methylcoumarin (5)

White amorphous powder; UV (MeOH) λ_{max} (log ε): 218 (3.09), 291 (2.74), 328 (2.83) nm; IR (KBr) ν_{max}: 3426, 1725, 1688, 1605, 1448, 1367, 1336, 1258, 1168, 1089, 953 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 249.0744 $[M+H]^+$ (calcd. for $C_{13}H_{13}O_5$, 249.0758).

3.4.6 5-Formyl-4,6,7-Trimethoxy-3-Methylcoumarin (6)

White amorphous powder; UV (MeOH) λ_{max} (log ε): 207 (3.67), 224 (3.63), 290 (3.20), 329 (3.45) nm; IR (KBr) ν_{max}: 3420, 1721, 1693, 1603, 1455, 1388, 1369, 1266, 1226, 1078, 1006, 959 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 279.0906 $[M+H]^+$ (calcd. for $C_{14}H_{15}O_6$, 279.0863).

3.4.7 4,6,7-Trimethoxy-3-Methylcoumarin (7)

Colorless gum; UV (MeOH) λ_{max} (log ε): 208 (2.99), 222 (2.83), 287 (2.30), 333 (2.58) nm; IR (KBr) ν_{max}: 3431, 1709, 1620, 1580, 1453, 1372, 1337, 1249, 1215, 1162, 1025, 994 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 251.0898 $[M+H]^+$ (calcd. for $C_{13}H_{15}O_5$, 251.0914).

3.5 In Vitro Enzyme Inhibition Assays

In this study, three enzymes closely related to diabetes, namely α-glucosidase, PTP1B, and TCPTP, were applied to assess the antidiabetic potency of compounds. Enzyme inhibition was assayed in accordance with the previous reports [18, 19]. Acarbose (for α-glucosidase) and Na_3VO_4 (for PTP1B and TCPTP) were used as the positive controls.

4 Supporting Information

1D and 2D NMR, HRMS, UV and IR spectra of compounds **1**–**7**.

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Declarations

Conflict of interest The authors declare that they have no competing financial interest.

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