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New prenylated coumarins from the stems of *Toddalia asiatica*†

Wei Li,[a] Jun-Sheng Zhang,[a] Jia-Luo Huang,[a] Min-Hong Jiang,[a] You-Kai Xu,[b] Abrar Ahmed,[a] Sheng Yin[a] and Gui-Hua Tang *[a]

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Eight new prenylated coumarins (**1a**/**1b**, **2a**/**2b**, and **3**–**6**) including two pairs of enantiomers (**1a**/**1b** and **2a**/**2b**), a new phenolic acid derivative, methyl (*E*)-3,4-bis(4-hydroxyphenyl)-4-oxobut-2-enoate (**7**), and 33 known compounds (**8**–**40**) were isolated from the stems of *Toddalia asiatica*. Their structures were established from spectroscopic data and by chemical methods. The absolute configurations of two pairs of enantiomers (**1a**/**1b** and **2a**/**2b**) were determined by X-ray diffraction analysis together with ECD and specific optical rotation calculations. The inhibitory effects of selected compounds against phosphodiesterase-4 (PDE4) were evaluated, and compounds **12**, **19**, **21**–**23**, **26**, **34**, and **35** exhibited PDE4 inhibition activities with IC_{50} values less than 10 μM.

Introduction

Toddalia is a monotypic genus of the Rutaceae family containing the single species *Toddalia asiatica* (L.) Lam., which is a woody climber widely distributed in Africa, E, S, and SE Asia, Madagascar, and the Mascarene Islands.[1] In China, *T. asiatica*, well known as "Feilongzhangxue" (Chinese name), is a Traditional Chinese Medicine (TCM), whose roots and barks have been widely used for the treatment of many diseases including traumatic injury, rheumatism, stomach ache, dysmenorrhea, and pyogenic infections.[2] Previous phytochemistry investigations on the plants of *T. asiatica* have revealed the occurrence of the main chemical components including coumarins, alkaloids, triterpenoids, phenolic acids, flavonoids, and lignans.[3–5] These chemical components were found to have a wide range of bioactivities such as anti-inflammatory, anti-leukemic, anti-platelet aggregation, antibacterial, antifungal, antimalarial, and cytotoxic activities.[4,6–11]

Recently, our group have found a series of prenylated coumarins including an unusual group of phenylpropenoic acid-coupled prenylated coumarins with potent phosphodiesterase-4 (PDE4) inhibitory activities from the roots of *T. asiatica*.[11] In the course of our ongoing work for natural PDE4 inhibitors from this plant, the EtOH extract of the stems of *T. asiatica* were subjected to chromatographic procedures to yield eight new prenylated coumarins (**1a**/**1b**, **2a**/**2b**, and **3**–**6**) (Fig. 1) including two pairs of enantiomers (**1a**/**1b** and **2a**/**2b**), a new phenolic acid derivative (**7**) (Fig. 1), and 33 known compounds (**8**–**40**) (Fig. S1.1, see ESI†) including seven alkaloids (**34**–**40**). This paper focuses on the isolation, structural elucidation, and PDE4 inhibitory activities of these isolated compounds.

Results and discussion

Compound **1** was obtained as a colorless crystal, which had the molecular formula $C_{16}H_{16}O_6$ with nine degrees of unsaturation as determined by the HRESIMS and 1D NMR data. The characteristic absorption bands at ν_{max} 1727, 1600, 1500, and 1466 cm^{-1} in the IR spectrum of **1** suggested the presence of ester and aromatic moieties. The 1D NMR spectra of **1** displayed signals for a 5,7-dimethoxy-8-substituted coumarin unit [δ_H 7.94 (1H, d, J = 9.7 Hz, H-4), 6.29 (1H, s, H-6), 6.11 (1H, d, J = 9.7 Hz, H-3), 3.92 (3H, s, 5-OCH_3), and 3.91 (3H, s, 7-OCH_3); δ_C 161.4 (C, C-7), 160.6 (C, C-2), 157.4 (C, C-5), 154.9 (C, C-9), 138.8 (CH, C-4), 111.1 (CH, C-3), 107.0 (C, C-8), 103.9 (C, C-10), 90.4 (CH, C-6), 56.4 (CH_3, 7-OCH_3), and 56.1 (CH_3, 5-OCH_3)] as in co-isolated compounds **8**–**11**,[12–16] which was further confirmed by the HMBC correlations (Fig. 2). The remaining resonances in the 1D NMR data of **1** (Table 1) were consistent with one singlet methyl group, an oxygenated methylene, two oxygenated methines, and an oxygenated quaternary carbon, indicating the presence of a highly-oxidized isopentenyl derivative moiety. The structure of this highly-oxidized moiety was deduced to be 5-methyl-3,6-dioxabicyclo[3.1.0]hexan-2-yl by analysis of its 2D NMR data including ^1H–^1H COSY and HMBC correlations (Fig. 2) and the

[a]*School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, Guangdong 510006, China. E-mail: tanggh5@mail.sysu.edu.cn; Fax: +86-20-39943043; Tel: +86-20-39943043*

[b]*Key Laboratory of Tropical Plant Resource and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Yunnan 666303, China*

† Electronic supplementary information (ESI) available: Structures of known compounds, tables of the 1D NMR data assignments of all known compounds, Experimental section, 1D and 2D NMR, MS, HRMS, and IR spectra of new compounds, 1D NMR spectra of known ones. CCDC 1545037. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ra04794k

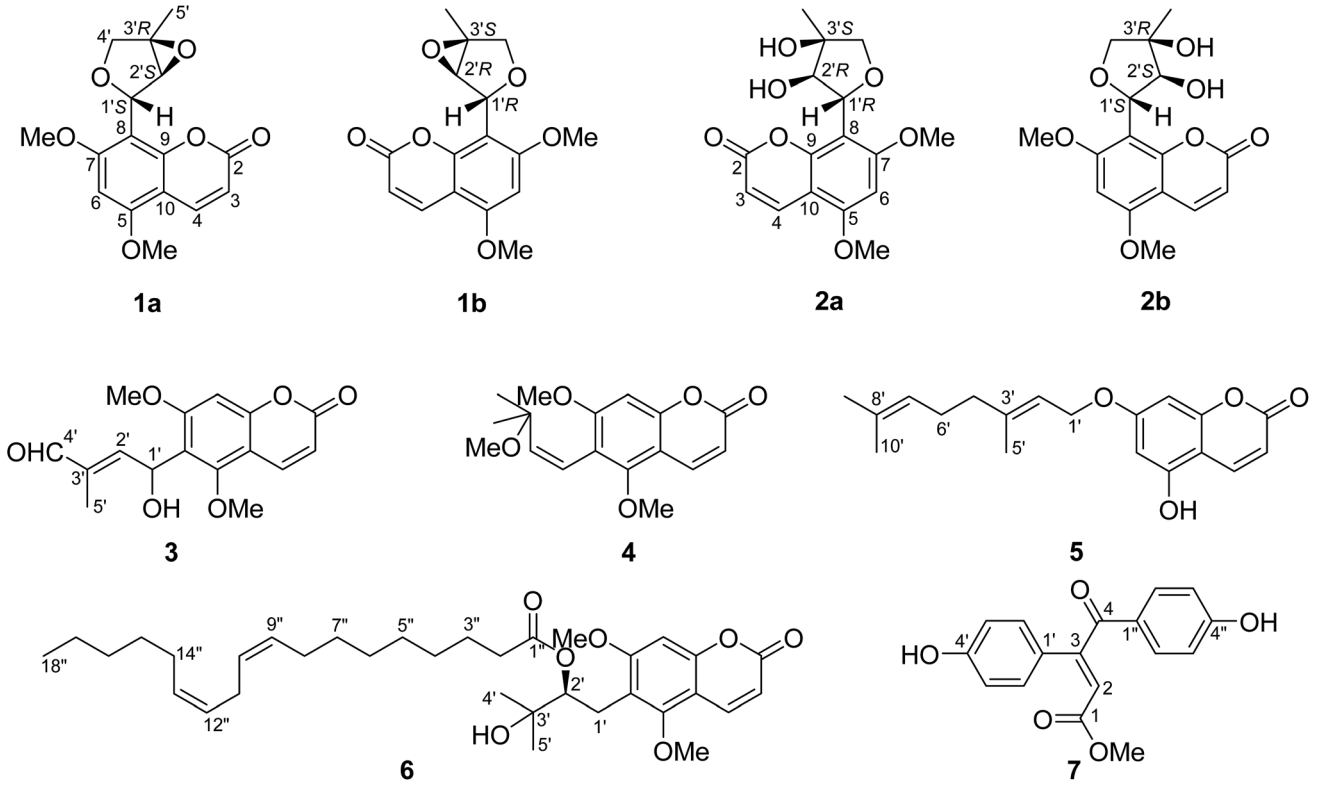

Fig. 1 Structures of new compounds **1–7** from *T. asiatica*.

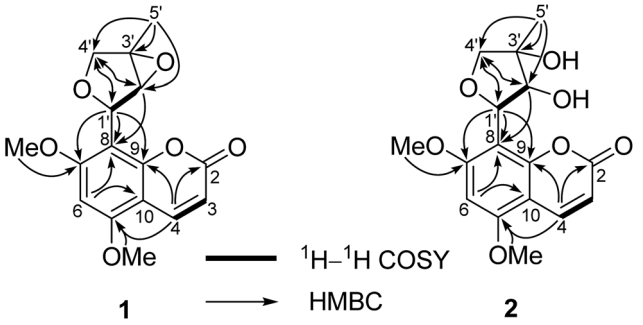

Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of **1** and **2**.

remaining two degrees of unsaturation (seven of nine degrees of unsaturation occupied by the coumarin unit). The HMBC correlations of the proton (δ_H 5.68, 1H, br. s) of the oxygenated CH-1′ with C-7, C-8, and C-9 confirmed that the moiety 5-methyl-3,6-dioxabicyclo[3.1.0]hexan-2-yl was linked at C-8 of the coumarin unit.

The small coupling constant of H-1′ (br. s) and H-2′ (br. s) in the ^{1}H NMR spectrum suggested that the dihedral angle between H-1′ and H-2′ was approximately 90°, and arbitrarily assigned H-1′ and H-2′ as β-axial and α-equitorial orientations, respectively.[17] The NOE correlation of H-2′/H_3-5′ in the NOESY spectrum indicated the same α-orientation of CH_3-5′ as H-2′. The single-crystal X-ray crystallographic analysis of **1** (Fig. 3) was performed with a high Flack parameter of 0.42 (19), which showed that **1** were a pair of enantiomers. So, the X-ray result only confirmed the relative configuration of **1** as the same as that deduced by the coupling constant and NOESY experiment.

Subsequently, compound **1** was subjected to HPLC with a chiral column to obtain the enantiomers **1a** and **1b**, which had opposite specific rotations ($[\alpha]_D^{20}$ = +61.0 for **1a** and $[\alpha]_D^{20}$ = −61.0 for **1b**) and mirror image-like ECD curves (Fig. 4). Unfortunately, no crystals of any of this pair of enantiomers were obtained. To determine the absolute configurations of the enantiomers, the experimental ECD spectra of **1a** and **1b** were compared with the calculated ECD spectra of (1′*S*,2′*S*,3′*R*)-**1** or (1′*R*,2′*R*,3′*S*)-**1** by the TDDFT method.

In Fig. 4, the experimental ECD spectrum of **1a** showed an ECD curve with three positive Cotton effects around 316 (+3.01), 261 (+1.40), and 211 (+16.7) nm, which matched the calculated ECD curve for (1′*S*,2′*S*,3′*R*)-**1**, indicating that **1a** possessed the same absolute configuration as (1′*S*,2′*S*,3′*R*)-**1**. The absolute configuration of **1b** was deduced to be the same as that of (1′*R*,2′*R*,3′*S*)-**1** on the basis of their matched ECD spectra. Thus, the enantiomers **1a** and **1b** were determined as shown and named (+)-toddalin E and (−)-toddalin E, respectively.

The molecular formula of **2** was determined as $C_{16}H_{18}O_7$ with 18 mass units more than that of **1** by its HRESIMS and 1D NMR data. Comparison of the ^{1}H and ^{13}C NMR data demonstrated that **2** displayed closely similarity with **1** (Table 1). The obvious differences were the chemical shifts of the C-8 substituent moiety [δ_C 76.8 (CH, C-1′), 79.8 (CH, C-2′), 78.0 (C, C-3′), 78.9 (CH_2, C-4′), and 21.9 (CH_3, C-5′) in **2**; δ_C 71.8 (CH, C-1′), 65.9 (CH, C-2′), 68.5 (C, C-3′), 71.9 (CH_2, C-4′), and 13.7 (CH_3,

Table 1 ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of **1–4** (J in Hz, δ in ppm)

	(±)-**1**[a]		(±)-**2**[b]		**3**[a]		**4**[a]	
Position	δ_H, multi. (J in Hz)	δ_C, type	δ_H, multi. (J in Hz)	δ_C, type	δ_H, multi. (J in Hz)	δ_C, type	δ_H, multi. (J in Hz)	δ_C, type
2		160.6, C		162.8, C		160.5, C		161.2, C
3	6.11, d (9.7)	111.1, CH	6.14, d (9.7)	111.1, CH	6.30, d (9.7)	113.7, CH	6.24, d (9.6)	112.8, CH
4	7.94, d (9.7)	138.8, CH	8.09, d (9.7)	140.8, CH	7.86, d (9.7)	138.5, CH	7.90, d (9.6)	139.0, CH
5		157.4, C		158.8, C		155.4, C		155.6, C
6	6.29, s	90.4, CH	6.60, s	92.5, CH		119.7, C		116.2, C
7		161.7, C		164.2, C		161.0, C		161.6, C
8		107.0, C		108.6, C	6.70, s	96.7, CH	6.62, s	95.6, CH
9		154.9, C		155.9, C		156.3, C		154.9, C
10		103.9, C		104.9, C		107.7, C		107.6, C
1′	5.68, br. s	71.8, CH	5.47, d (8.5)	76.8, CH	6.01, dd (10.4, 7.6)	64.4, CH	6.57, br. s	118.2, CH
2′	3.58, br. s	65.9, CH	4.45, d (8.5)	79.8, CH	6.85, dd (7.6, 1.4)	153.1, CH	6.57, br. s	140.8, CH
3′		68.5, C		78.0, C		138.9, C		75.8, C
4′	4.18, d (9.3), 3.92, d (9.3)	71.9, CH_2	4.20, d (9.2), 3.87, d (9.2)	79.0, CH_2	9.44, s	195.0, CH	1.38, s	26.0, CH_3
5′	1.75, s	13.7, CH_3	1.38, s	21.9, CH_3	1.78, d (1.4)	9.5, CH_3	1.38, s	26.0, CH_3
5-OMe	3.92, s	56.1, CH_3	3.99, s	56.8, CH_3	3.94, s	64.6, CH_3	3.78, s	62.1, CH_3
7-OMe	3.91, s	56.4, CH_3	3.97, s	57.0, CH_3	3.98, s	56.7, CH_3	3.89, s	56.3, CH_3
3′-OMe							3.24, s	50.7, CH_3
1′-OH					3.29, d (10.4)			

[a] In $CDCl_3$. [b] In CD_3OD.

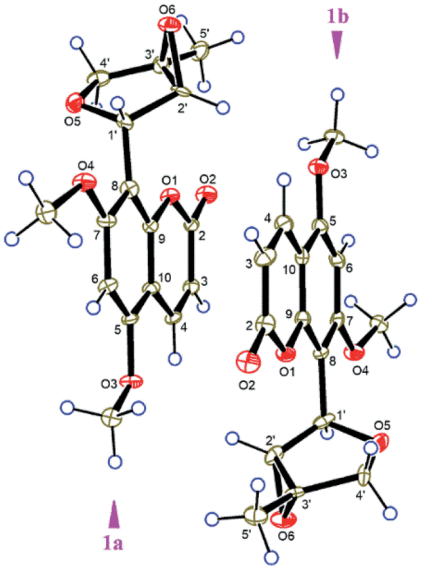

Fig. 3 Single-crystal X-ray structures of **1** (left: **1a**; right: **1b**).

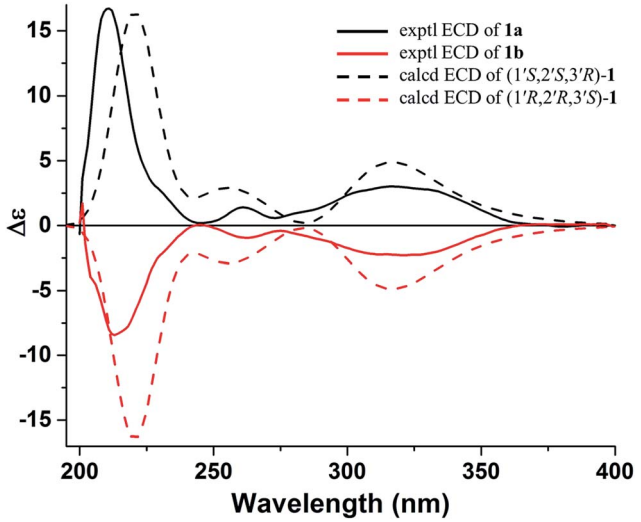

Fig. 4 Experimental ECD spectra of **1a** and **1b** and calculated ECD spectra (red shifted by 15 nm) of (1′*S*,2′*S*,3′*R*)-**1** and (1′*R*,2′*R*,3′*S*)-**1**.

C-5′) in **1**], which may be due to the open loop of the epoxy ring. The molecular formula of **2** with 18 mass units more than that of **1** and one degree of unsaturation less than that of **1** further confirmed the above proposed. Analysis of the 2D NMR data (Fig. 2) determined the gross structure of **2**. The NOE cross-peaks of H-2′/H_3-5′ and H-4′α indicated that the methyl group, H-2′, and H-4′α were cofacial, and were arbitrarily assigned α-orientation. The large ^{1}H–^{1}H coupling constant ($J_{1',2'}$ = 8.5 Hz) suggested the *trans*-relationship of H-1′ and H-2′.[18] Hence, the relative configuration of **2** was assigned to be the same as that of **1**. Compound **2** was also a racemic mixture because of its specific rotation approaching zero and no Cotton effect in its ECD spectrum. The enantiomers **2a** and **2b**, with opposite specific rotations ($[\alpha]_D^{20}$ = +116.2 for **2a** and $[\alpha]_D^{20}$ = −116.2 for **2b**) and mirror image-like ECD curves (Fig. 5), were separated by

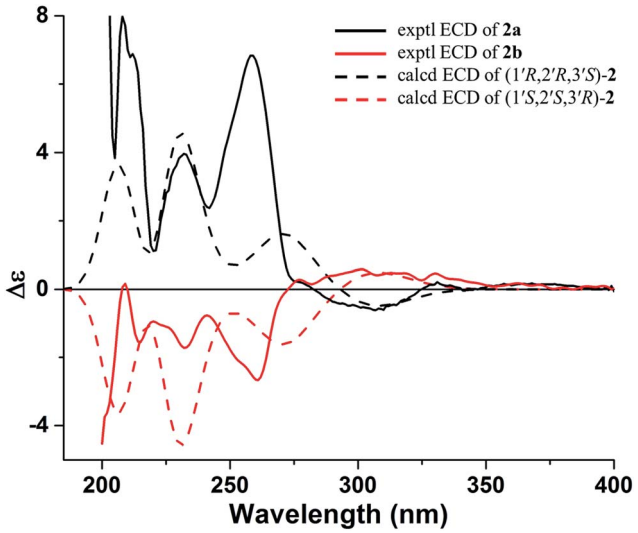

Fig. 5 Experimental ECD spectra of **2a** and **2b** and calculated ECD spectra (bule shifted by 30 nm) of (1′*S*,2′*S*,3′*R*)-**2** and (1′*R*,2′*R*,3′*S*)-**2**.

HPLC with a chiral column. The absolute configurations of **2a** and **2b** were determined as 1′*R*,2′*R*,3′*S* and 1′*S*,2′*S*,3′*R*, respectively, by using the same methods as described above (Fig. 5). Thus, the enantiomers **2a** and **2b** were given trivial names (+)-toddalin F and (−)-toddalin F, respectively.

As one may noticed, **1a** and **2a** both showed positive specific optical rotation, while their absolute configurations were opposite. In order to confirm the above assignments, calculations of the specific optical rotations for (1′*S*,2′*S*,3′*R*)-**1** and (1′*S*,2′*S*,3′*R*)-**2** were further carried out. The results showed that (1′*S*,2′*S*,3′*R*)-**1** exhibited a theoretical specific optical rotation of +95 (experimental value for **1a**: +61) while (1′*S*,2′*S*,3′*R*)-**2** exhibited a theoretical specific optical rotation of −117 (experimental value for **2b**: −116). The results of specific optical rotation calculations were in accordance with those of ECD calculations.

Compound **3** exhibited a pseudomolecular ion peak at *m*/*z* 305.1582 $[M + H]^+$, allowing the molecular formula $C_{16}H_{16}O_6$ to be assigned. Analysis the 1D and 2D NMR data suggested that **3** had a 5,7-dimethoxy-6-substituted coumarin unit as in co-isolated compounds **12–18**.[11,13,19,20] The remaining signals in the 1D NMR spectra of **3** (Table 1) were consistent with an OH group [δ_H 3.29 (1H, d, J = 10.4 Hz)], a conjugated aldehyde group [δ_H 9.44 (1H, s); δ_C 195.0 (CH)], a trisubstituted double bond [δ_H 6.85 (1H, dd, J = 7.6 and 1.4 Hz); δ_C 153.1 (CH) and 138.9 (C)], an oxygenated methine [δ_H 6.01 (1H, dd, J = 10.4 and 7.6 Hz); δ_C 64.4 (CH)], and a methyl group [δ_H 1.78 (1H, d, J = 1.4 Hz); δ_C 9.5 (CH_3)]. According to the ^{1}H–^{1}H COSY correlations of H-1′/H-2′ and 1′-OH and the key HMBC correlations of H-5′ to C-2′, C-3′, and C-4′, H-4′ to C-2′ and C-5′, H-2′ to C-4′ and C-5′, 1′-OH to C-6, and H-1′ to C-5 and C-7, the 6-substituent moiety was determined as shown (Fig. 6). The *E*-configuration of the double bond between C-2′ and C-3′ was deduced by the NOE correlation of H-4′/H-2′. This compound might be a racemoid because its specific rotation was zero. Thus, compound **3** was given a trivial name (±)-toddalin G.

Toddalin H (**4**) was assigned the molecular formula $C_{17}H_{20}O_5$ by its HRESIMS and 1D NMR data. The ^{1}H and ^{13}C NMR spectra of **4** were very similar to those of toddalenol (**15**),[13] except for the presence of an additional methoxy group instead of a hydroxy group at C-3′ and the different configuration of $\Delta^{1'}$. The protons at δ_H 6.57 (2H, br. s) assigned to carbons C-1′ and C-2′ suggested the double bond between C-1′ and C-2′ was *Z*-configuration. Analysis of the 2D NMR data of **4** (Fig. 6) further confirmed the structure of **4**.

Compound **5** possessed the molecular formula $C_{19}H_{22}O_4$ as determined by its HRESIMS and 1D NMR data. The ^{1}H and ^{13}C NMR data of **5** bore a high resemblance to those of 7-geranyloxy-5-methoxycoumarin[14] with the only difference being the absence of the methoxy group. This indicated that **5** was a demethoxy derivative of the known one, which was confirmed by its 2D NMR and MS data. Thus, compound **5** was named as 7-geranyloxy-5-hydroxycoumarin.

The molecular formula of compound **6** was established as $C_{34}H_{50}O_7$ on the basis of its HRESIMS and 1D NMR data. Comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of *ent*-toddalolactone (**16**)[11] showed the structural features of

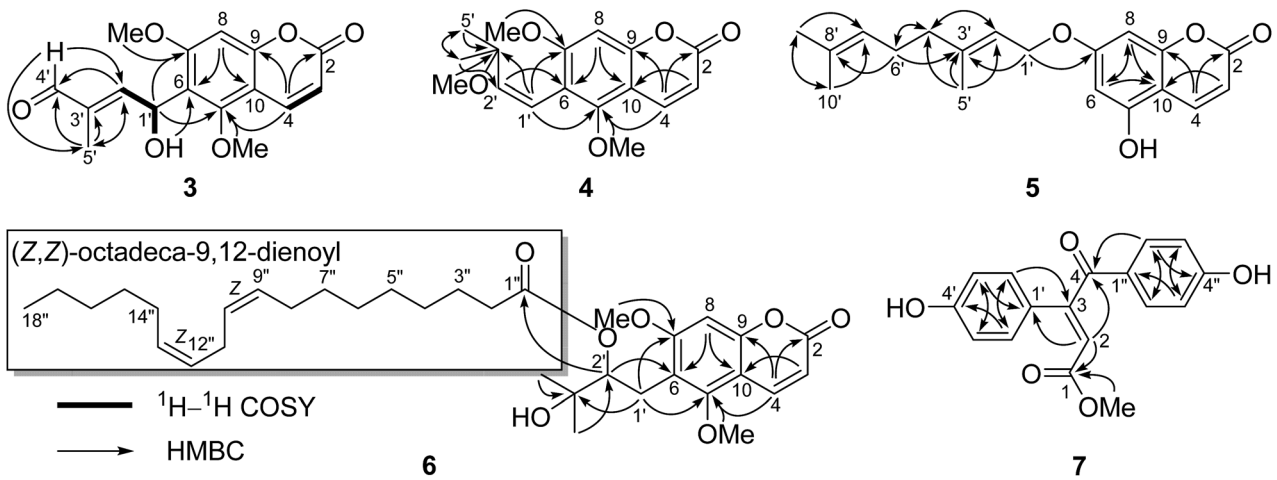

Fig. 6 Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **3**–**7**.

a prenylated coumarin identical to **16**, except for the presence of an additional fatty acid chain. The signals for the fatty acid chain in the 1D NMR spectra of **6** were almost identical to those of linoleic acid (9*Z*,12*Z*-octadecadieneoic acid),[21] which was linked to C-2′ by the HMBC correlation of H-2′ to the ester carbonyl (δ_C 173.2) (Fig. 6). The absolute configuration of **6** was confirmed by analysis of the specific optical rotation of the prenylated coumarin, an alkaline hydrolysis product of **6**, which showed a specific optical rotation of −64.0 (−69.0 for *ent*-toddalolactone). Thus, compound **6** was given a trivial name 2′-*O*-((*Z*,*Z*)-octadeca-9,12-dienoyl)-*ent*-toddalolactone.

The HRESIMS of **7** exhibited a molecular ion peak at *m*/*z* 299.0914 $[M + H]^+$ (calcd 299.0914), corresponding to the molecular formula $C_{17}H_{14}O_5$ with 11 degrees of unsaturation. The ^{1}H NMR spectrum of **7** showed the signals for two *p*-hydroxyphenyl groups [δ_H 7.97 (2H, d, J = 8.8 Hz), 7.50 (2H, d, J = 8.8 Hz), 6.87 (2H, d, J = 8.8 Hz), and 6.85 (2H, d, J = 8.8 Hz)], one olefinic proton [δ_H 7.48 (1H, s)], and a methoxy group [δ_H 3.91 (3H, s)]. The ^{13}C NMR spectrum, associated with DEPT experiments, resolved 17 carbon resonances attributable to two *p*-hydroxyphenyls [δ_C 126.1 (C, C-1′), 130.0 (CH × 2, C-2′ and C-6′), 117.0 (CH × 2, C-3′ and C-5′), 161.8 (C, C-4′), 130.3 (C, C-1″), 132.3 (CH × 2, C-2″ and C-6″), 116.7 (CH × 2, C-3″ and C-5″), and 164.9 (C, C-4″)], a conjugated ketone carbonyl [δ_C 188.9 (C, C-4)], an ester carbonyl [δ_C 172.1 (C, C-1)], one trisubstituted double bond [δ_C 118.9 (CH, C-2) and 148.8 (C, C-3)], and one methoxy [δ_C 53.0 (CH_3, 1-OMe)], which were assigned by the HSQC and HMBC correlations. The HMBC correlations of H-2 to C-1, C-4, and C-1′, H-2′ (6′) to C-3, and H-2″ (6″) to C-4 connected the two *p*-hydroxyphenyls, the conjugated ketone carbonyl, the double bond, and the ester carbonyl to construct the structure of **7** as shown (Fig. 6). The location of the methoxy group at C-1 was deduced by the HMBC correlation of protons at δ_H 3.91 to the ester carbonyl. The *E*-configuration of the double bond Δ^2 was determined by the NOE correlation of H-2″ (6″)/H-2. Thus, compound **7** was elucidated to be methyl (*E*)-3,4-bis(4-hydroxyphenyl)-4-oxobut-2-enoate.

The known compounds, gleinadiene (**8**),[12] toddalenone (**9**),[13] 8-geranyloxy-5,7-dimethoxycoumarin (**10**),[14] 5,7,8-trimethoxycoumarin (**11**),[14–16] toddaculin (**12**),[13] 6-(3-methyl-1,3-butadienyl)-5,7-dimethoxycoumarin (**13**),[19] toddanol (**14**),[13] toddalenol (**15**),[13] *ent*-toddalolactone (**16**),[11] (−)-toddalolactone 3′-*O*-β-D-glucopyranoside (**17**),[11] 6-formyllimettin (**18**),[20] 8-(3,3-dimethylallyl)-6,7-dimethoxycoumarin (**19**),[13] 6-(3-methyl-2-butenyl)-7-hydroxy-5-methoxycoumarin (**20**),[22] 6-geranyloxy-7-methoxycoumarin (**21**),[23] norbraylin (**22**),[24] braylin (**23**),[24] toddalins A–C (**24–26**),[11] toddalosin (**27**),[13,25] 5-*O*-(*E*)-feruloylquinic acid methyl ester (**28**),[26] hycandinic acid ester-1 (**29**),[27] 4-*O*-(*E*)-feruloylquinic acid methyl ester (**30**),[28,29] *trans*-*p*-coumaryl aldehyde (**31**),[30] ferulaldehyde (**32**),[31] *trans*-sinapaldehyde (**33**),[32] 8-acetonyldihydronitidine (**34**),[33] 8-acetonyldihydrochelerythrine (**35**),[34] decarine (**36**),[34] 4-methoxy-*N*-methyl-2-quinolone (**37**),[35] γ-fagarine (**38**),[35] haplopine (**39**),[36] and skimmianine (**40**),[36] were identified by comparison of their spectroscopic data with those reported in the literature. The ^{1}H and ^{13}C NMR data of all known compounds are provided in Tables S2.1–S2.10 (see ESI†).

Table 2 IC_{50} Values of active compounds against PDE4D2[a]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
12	9.98 ± 0.63[b]	**25**	16.65 ± 1.20[b]
19	6.64 ± 0.02	**26**	7.81 ± 0.40[b]
21	3.85 ± 0.34	**34**	5.14 ± 0.13
22	2.38 ± 0.14[b]	**35**	3.80 ± 0.18
23	0.96 ± 0.10[b]	Rolipram[c]	0.62 ± 0.03

[a] Compounds with IC_{50} > 50 μM were not listed. [b] Data collected from our previous work.[11] [c] Positive control.

The inhibitory activities of the isolates at an initial concentration of 10 μM against PDE4D2 were screened by using tritium-labelled adenosine 3′,5′-cyclic monophosphate ([^{3}H]-cAMP) as substrate (S3, see ESI†). The IC_{50} values of the active compounds with inhibition greater than 50% at 10 μM were listed in Table 2. Rolipram, a well-known PDE4 inhibitor, was used as positive control (IC_{50} = 0.62 μM). As show in Table 2, compounds **12**, **19**, **21–23**, **26**, **34**, and **35** exhibited moderate activities with IC_{50} values less than 10 μM toward PDE4D.

Experimental section

General experimental procedures

X-ray data were collected using an Agilent Xcalibur Nova X-ray diffractometer. Melting points were recorded on an X-4 melting instrument and were uncorrected. Optical rotations were detected on a Rudolph Autopol I automatic polarimeter, UV spectra on a Shimadzu UV-2450 spectrophotometer, ECD spectra on an Applied Photophysics Chirascan spectrometer, and IR spectra on PerkinElmer FT-IR C106150 and Bruker Tensor 37 infrared spectrophotometers. 1D and 2D NMR spectra were measured on Bruker AM-400 spectrometers at 25 °C. ESIMS was measured on a Finnigan LCQ Deca instrument, and HRESIMS was performed on a Waters Micromass Q-TOF spectrometer. Silica gel (100–200 and 300–400 mesh, Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 gel (Amersham Biosciences), reversed-phase C_{18} (RP-C_{18}) (12 nm, S-50 μm, YMC Co., Ltd.), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC). A Shimadzu LC-20 AT equipped with a SPD-M20A PDA detector was used for HPLC. An YMC-pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm) and a chiral column (Phenomenex Lux, cellulose-2, 250 × 10 mm, 5 μm) were used for semi-preparative HPLC separation. All solvents used were of analytical grade (Guangzhou Chemical Reagents Company, Ltd.). TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

Plant material

The stems of *T. asiatica* were collected from Xishuangbanna, Yunnan Province, P. R. China, in October 2012. This material was authenticated by one of the author (Y.-K. Xu) and a voucher specimen (FLZX201211) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

Extraction and isolation

The air-dried stems of *T. asiatica* (3.4 kg) were extracted with 95% EtOH (10 L × 3) at rt for three times. After evaporating the solvent, the residue (390 g) was suspended in H_2O (2 L) and extracted with EtOAc (2 L × 3) and *n*-BuOH (2 L × 3), respectively. The EtOAc partition (120 g) was chromatographed over silica gel CC (PE/EtOAc, 50 : 1 → 1 : 1) to give seven subfractions (I–VI). Each fraction was subjected to CC over RP-C_{18} column, silica gel, and Sephadex LH-20 and then further purified by semipreparative HPLC with a YMC-pack ODS-A column or a Phenomenex Lux chiral column to yield pure compounds. Fr. I yielded compound **12** (30 mg). Compounds **1a** (2 mg), **1b** (2 mg), **5** (9 mg), **10** (24 mg), **19** (315 mg), **20** (5 mg), and **21** (57 mg) were obtained from Fr. II. Fr. III afforded compounds **6** (29 mg), **8** (23 mg), **9** (10 mg), **22** (100 mg), **23** (16 mg), **31** (80 mg), **32** (10 mg), **33** (9 mg), and **36** (27 mg). Fr. IV gave compounds **4** (13 mg), **11** (3 mg), **13** (2 mg), **14** (95 mg), **18** (2 mg), **24** (22 mg), **26** (36 mg), and **37** (8 mg). Compounds **34** (52 mg), **35** (18 mg), **38** (14 mg), **39** (18 mg), and **40** (21 mg) were obtained from Fr. V. Fr. VI afforded compounds **2a** (1 mg), **2b** (2 mg), **3** (4 mg), **7** (2 mg), **15** (13 mg), **16** (83 mg), **17** (63 mg), **25** (28 mg), **27** (9 mg), **28** (32 mg), **29** (14 mg), and **30** (5 mg). The details on isolation of these compounds are provided in ESI.† Purity of the compounds was tested by using TLC and NMR spectra.

(+)-Toddalin E (1a). Colorless crystal; mp 183.3–185.0 °C; $[\alpha]_D^{20}$ +61.0 (*c* 0.069, MeOH); UV (MeOH) λ_{max} (log ε) 323 (3.71), 259 (3.52), 251 (3.52), 213 (3.96) nm; ECD (*c* 3.29 × 10^{-4} M, MeCN) λ_{max} ($\Delta\varepsilon$) 316 (+3.01), 261 (+1.40), 211 (+16.7) nm; IR (KBr) ν_{max} 1727, 1600, 1500, 1466, 1334, 1253, 1210, 1115 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 327.1 $[M + Na]^+$; HRESIMS *m*/*z* 303.0867 $[M - H]^-$ (calcd for $C_{16}H_{15}O_6^-$, 303.0874).

(−)-Toddalin E (1b). Colorless crystal; $[\alpha]_D^{20}$ −61.0 (*c* 0.068, MeOH); ECD (*c* 3.29 × 10^{-4} M, MeCN) λ_{max} ($\Delta\varepsilon$) 321 (−2.30), 262 (−0.95), 213 (−8.4) nm; melting point, UV, IR, NMR, MS, and HRESIMS are the same as those of **1a**.

(+)-Toddalin F (2a). Colorless oil; $[\alpha]_D^{20}$ +116.2 (*c* 0.087, MeOH); UV (MeOH) λ_{max} (log ε) 323 (3.60), 260 (3.43), 212 (3.91) nm; ECD (*c* 3.11 × 10^{-4} M, MeCN) λ_{max} ($\Delta\varepsilon$) 331 (+0.21), 257 (+6.69), 232 (+3.96), 208 (+7.98) nm; IR (KBr) ν_{max} 3426, 1719, 1604, 1504, 1472, 1434, 1335, 1259, 1222, 1142, 1118 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 323.2 $[M + H]^+$; HRESIMS *m*/*z* 345.0961 $[M + Na]^+$ (calcd for $C_{16}H_{18}O_7Na^+$, 345.0945).

(−)-Toddalin F (2b). Colorless oil; $[\alpha]_D^{20}$ −116.2 (*c* 0.170, MeOH); ECD (*c* 3.11 × 10^{-4} M, MeCN) λ_{max} ($\Delta\varepsilon$) 330 (−0.46), 261 (−2.67), 232 (−1.72), 215 (−1.55) nm; UV, IR, NMR, MS, and HRESIMS are the same as those of **2a**.

(±)-Toddalin G (3). Colorless oil; $[\alpha]_D^{20}$ 0 (*c* 0.248, MeOH); UV (MeOH) λ_{max} (log ε) 323 (4.05), 225 (4.24), 206 (4.26) nm; IR (KBr) ν_{max} 3359, 1722, 1654, 1602, 1385, 1353, 1205, 1139 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 305.0 $[M + H]^+$; HRESIMS *m*/*z* 303.0851 $[M - H]^-$ (calcd for $C_{16}H_{15}O_6^-$, 303.0874).

Toddalin H (4). Colorless oil; UV (MeOH) λ_{max} (log ε) 336 (3.54), 313 (3.53), 261 (3.95), 213 (3.78) nm; IR (KBr) ν_{max} 1725, 1597, 1563, 1451, 1419, 1376, 1360, 1203, 1124, 1088, 1070 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 287.1 $[M - H_2O + H]^+$, 305.0 $[M + H]^+$; HRESIMS *m*/*z* 327.1195 $[M + Na]^+$ (calcd for $C_{17}H_{20}O_5Na^+$, 327.1203).

7-Geranyloxy-5-hydroxycoumarin (5). Colorless oil; UV (MeOH) λ_{max} (log ε) 331 (3.52), 210 (3.82) nm; IR (KBr) ν_{max} 3420, 2966, 2927, 1694, 1654, 1611, 1568, 1458, 1428, 1372, 1137 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 6.16 (1H, d, *J* = 9.6 Hz, H-3), 8.01 (1H, d, *J* = 9.6 Hz, H-4), 6.41 (1H, s, H-6), 6.31 (1H, s, H-8), 4.55 (2H, d, *J* = 6.6 Hz, H-1′), 5.43 (1H, br. t, *J* = 6.8 Hz, H-2′), 2.09 (2H, m, H-4′), 1.73 (3H, s, H-5′), 2.11 (2H, m, H-6′), 5.07 (1H, br. t, *J* = 6.8 Hz, H-7′), 1.66 (3H, s, H-9′), 1.60 (3H, s, H-10′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 162.2 (C, C-2), 110.7 (CH, C-3), 139.4 (CH, C-4), 156.9 (C, C-5), 94.4 (CH, C-6), 162.9 (C, C-7), 99.0 (CH, C-8), 154.2 (C, C-9), 103.5 (C, C-10), 65.6 (CH_2, C-1′), 118.6 (CH, C-2′), 142.5 (C, C-3′), 39.7 (CH_2, C-4′), 16.9 (CH_3, C-5′), 26.4 (CH_2, C-6′), 123.8 (CH, C-7′), 132.1 (C, C-8′), 25.8 (CH_3, C-9′), 17.8 (CH_3, C-10′); ESIMS *m*/*z* 315.2 $[M + H]^+$; HRESIMS *m*/*z* 313.1439 $[M - H]^-$ (calcd for $C_{19}H_{21}O_4^-$, 313.1445).

2′-*O*-((*Z*,*Z*)-Octadeca-9,12-dienoyl)-*ent*-toddalolactone (6). Colorless oil; $[\alpha]_D^{20}$ −57.6 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 329 (3.87), 225 (4.03), 210 (4.20) nm; IR (KBr) ν_{max} 3459, 2929, 2855, 1736, 1610, 1565, 1462, 1382, 1205, 1134 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 6.23 (1H, d, *J* = 9.6 Hz, H-1), 7.82 (1H, d, *J* = 9.6 Hz, H-2), 6.59 (1H, s, H-8), 3.07 (1H, dd, *J* = 13.6 and 10.3 Hz, H-1′a), 2.90 (1H, dd, *J* = 13.6 and 3.1 Hz, H-1′b), 5.20 (1H, dd, *J* = 10.3 and 3.1 Hz, H-2′), 1.29 (3H, s, H-4′), 1.25 (3H, s, H-5′), 3.85 (3H, s, 5-OMe), 3.89 (3H, s, 7-OMe), 2.11 (1H, m, H-1‴a), 2.03 (1H, m, H-1‴b), 1.36 (2H, m, H-2‴), 1.08 (2H, m, H-3‴), 1.19 (2H, m, H-3‴), 1.28 (10H, m, H-6‴, H-7‴, H-15‴, H-16‴, and H-17‴), 2.02 (4H, m, H-8‴ and H-14‴), 5.32 (2H, m, H-9‴ and H-13‴), 5.30 (2H, m, H-10‴ and H-12‴), 2.76 (2H, t, *J* = 6.4 Hz, H-11‴), 0.88 (3H, t, *J* = 6.0 Hz, H-18‴); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 161.1 (C, C-2), 112.7 (CH, C-3), 138.8 (CH, C-4), 156.3 (C, C-5), 116.8 (C, C-6), 162.1 (C, C-7), 95.6 (CH, C-8), 155.4 (C, C-9), 107.1 (C, C-10), 23.9 (CH_2, C-1′), 77.9 (CH, C-2′), 72.6 (C, C-3′), 25.4 (CH_3, C-4′), 26.9 (CH_3, C-5′), 63.3 (CH_3, 5-OMe), 56.3 (CH_3, 7-OMe), 173.2 (C, C-1‴), 34.3 (CH_2, C-2‴), 25.0 (CH_2, C-3‴), 29.17, 29.18 (CH_2, C-4‴ and 5‴), 29.3 (CH_2, C-6‴), 29.7 (CH_2, C-7‴), 27.30, 27.34 (CH_2, C-8‴ and 14‴), 130.4 (CH, C-9‴), 128.1 (CH, C-10‴), 25.8 (CH_2, C-11‴), 128.2 (CH, C-12‴), 130.2 (CH, C-13‴), 29.5 (CH_2, C-15‴), 31.7 (CH_2, C-16‴), 22.7 (CH, C-17‴), 14.2 (CH_3, C-18‴); ESIMS *m*/*z* 571.4 $[M + H]^+$; HRESIMS *m*/*z* 593.3463 $[M + Na]^+$ (calcd for $C_{34}H_{50}O_7Na^+$, 593.3449).

Methyl (*E*)-3,4-bis(4-hydroxyphenyl)-4-oxobut-2-enoate (7). Colorless oil; UV (MeOH) λ_{max} (log ε) 349 (4.22), 232 (4.00), 206 (4.07), 194 (3.85) nm; IR (KBr) ν_{max} 3356, 1695, 1638, 1601, 1587, 1563, 1515, 1442, 1371, 1221, 1169 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 7.48 (1H, s, H-2), 7.50 (2H, d, *J* = 8.8 Hz, H-2′ and H-6′), 6.85 (2H, d, *J* = 8.8 Hz, H-3′ and H-5′), 7.97 (2H, d, *J* = 8.8 Hz, H-2″ and H-6″), 6.87 (2H, d, *J* = 8.8 Hz, H-3″ and H-5″), 3.91 (3H, s, 1-OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 172.1 (C, C-1), 118.9 (CH, C-2), 148.8 (C, C-3), 188.9 (C, C-4), 126.1 (C, C-1′), 130.0 (CH × 2, C-2′ and C-6′), 117.0 (CH × 2, C-3′ and C-5′), 161.8 (C, C-4′), 130.3 (C, C-1″), 132.3 (CH × 2, C-2″ and C-6″), 116.7 (CH × 2, C-3″ and C-5″), 164.9 (C, C-4″), 53.0 (CH_3, 1-OMe); ESIMS *m*/*z*

299.1 $[M + H]^+$; HRESIMS *m/z* 299.0914 $[M + H]^+$ (calcd for $C_{17}H_{15}O_5^+$, 299.0914).

X-ray crystallographic analysis of compound (1). $C_{16}H_{16}O_6$, $M = 304.30$, monoclinic, space group $P2_1$, $a = 8.9452$ (1) Å, $b = 7.6683$ (1) Å, $c = 19.8154$ (1) Å, $\beta = 91.4239$ (7)°, $\gamma = 1358.81$ (2) Å^3, $Z = 4$, $T =$ N/A K, μ (Cu Kα) = 0.963 mm^{-1}, $D_{calc} = 1.4874$ g cm^{-3}, 25 805 reflections measured ($4.46° \leq 2\theta \leq 144.7°$), 5326 unique ($R_{int} = 0.0276$, $R_{sigma} = 0.0180$) which were used in all calculations. The final R_1 was 0.0366 ($I \geq 2\sigma$ (I)) and wR_2 was 0.0979 (all data). Flack parameter = 0.42 (19). Crystallographic data for (**1**) has been deposited in the Cambridge Crystallographic Data Centre (CCDC number: 1545037†).

Hydrolysis of 2′-*O*-((*Z*,*Z*)-octadeca-9,12-dienoyl)-*ent*-toddalolactone (6). Compound **6** (4 mg) was treated with NaOH (1% in MeOH, 1 mL) at rt for 1 h. The mixture was then diluted with 10 mL of H_2O, followed by the extraction of EtOAc (3 × 20 mL). The organic layer was evaporated under vacuum to give a residue, which was purified on a flash silica gel column eluting with CH_2Cl_2 to afford a prenylated coumarin (1.1 mg). The structure of the prenylated coumarin was confirmed by comparison of its optical rotation data with that of the natural product.

Computational section

The details of the ECD and specific optical rotation calculations for compounds **1** and **2** are presented in ESI.†

PDE4D inhibitory screening assays

The screening assays for the inhibitory active compounds against PDE4D were performed as we described previously.[11,37] For more details about these experimental procedures, please see ESI.†

Conclusions

Forty-two compounds including nine new compounds (**1a**/**1b**, **2a**/**2b**, and **3**–**7**) were isolated from the stems of *T. asiatica*. The types of these compounds mainly involved coumarins (**1**–**6** and **8**–**27**), phenolic acid derivatives (**7** and **28**–**33**), and alkaloids (**34**–**40**). Two racemic mixtures, **1** and **2**, were subjected to HPLC with a chiral column to obtain two pairs of enantiomers **1a**/**1b** and **2a**/**2b**, whose absolute configurations were determined by X-ray diffraction analysis and ECD calculation. Interestingly, **1a** and **2a** both had positive specific optical rotations ($[\alpha]_D^{20}$ +61.0 for **1a**, +116.2 for **2a**), but they had opposite absolute configurations (1′*S*,2′*S*,3′*R* for **1a**, 1′*R*,2′*R*,3′*S* for **2a**) determined by ECD calculation. The assignments were further confirmed by specific optical rotation calculation ($[\alpha]_D^{20}$ +95 for (1′*S*,2′*S*,3′*R*)-**1**, +117 for (1′*R*,2′*R*,3′*S*)-**2**). The results of specific optical rotation calculations were in accordance with those of ECD calculations. The PDE4 inhibitory screening assays showed that compounds **12**, **19**, **21**–**23**, **26**, **34**, and **35** exhibited moderate activities with IC_{50} values less than 10 μM, which supported the structure–activity relationship summarized in previous study.[11]

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