Bioorganic Chemistry 99 (2020) 103820

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Seven pairs of new enantiomeric sesquiterpenoids from *Curcuma phaeocaulis*

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ARTICLE INFO

Keywords:
Curcuma phaeocaulis
Sesquiterpenoids
Enantiomers
Chiral separation
Vasorelaxant activity
Anti-platelet aggregative activity

ABSTRACT

Seven pairs of new enantiomeric sesquiterpenoids, (+)/(−)-phaeocauline A − G [(+)/(−)-**1**–**7**], were isolated from the rhizomes of *Curcuma phaeocaulis* by chiral HPLC separation. Their structures, including absolute configurations, were determined by spectroscopic analyses and ECD data. The isolates were assessed for vasorelaxant, anti-platelet aggregative, and neuroprotective activities. Enantiomers (+)-**1** and (−)-**1** showed similar activity against abnormal platelet aggregation induced by arachidonic acid, while their C-4 epimers (+)-**2** and (−)-**2** were inactive, which indicated that those effects were stereoselective, but not enantioselective. Compounds (+)/(−)-**3**–**5** exhibited vasorelaxant effects against KCl-induced contraction of rat aortic rings.

1. Introduction

Chirality is one of the most important properties of both macroscopic as well as microscopic objects in nature, and it is defined by the presence of one or more asymmetric carbon atoms in an organic molecule. Enantiomers have the same planar chemical structure, but they are mirror images of each other; such molecules exhibit similar physical and chemical properties, except for their optical activities in opposite directions [1]. As for their biological and or pharmaceutical activity, some enantiomers exhibit the same activity, while others exhibit a large difference or even opposite activity [2–4]. Therefore, it is very important to assess the biological activities of each enantiomer of a chiral molecule. Furthermore, the increasing interest in exploring the physiological activity and pharmacological action of enantiomers of chiral drugs has resulted in an increased demand for enantioseparation. At present, HPLC using the chiral stationary phase is one of the most effective methods to isolate and purify a small amount of a chiral substance [5].

In recent years, sesquiterpenoids have become the focus of research due to their diverse skeletal types and rich activities [6]. With the development of research methods and techniques, many more enantiomers of sesquiterpenoids have been discovered [7–10]. *Curcuma phaeocaulis* Val. (*Peng Ezhu* in Chinese) has been shown to contain a large number of sesquiterpenoids [11–13], some of which are enantiomers [14]. Its rhizomes, known as *Rhizoma curcumae*, have been widely used to promote blood circulation and remove blood stasis in China [15]. Recent studies have demonstrated that many of these sesquiterpenoids in *C. phaeocaulis* have anti-inflammatory [14,16], antioxidative [17], cytotoxic [18], anti-platelet aggregative [19], and hepatoprotective effects [20]. However, there are very limited studies on the bioactivities of enantiomeric sesquiterpenoids from the genus *Curcuma.* In this study, seven pairs of new enantiomeric sesquiterpenoids [(+)/(−)-**1**–**7**] were obtained by chiral HPLC separation (Fig. 1). According to the traditional functions of *C. phaeocaulis*, their blood-activating activities, including vasorelaxant and anti-platelet aggregative effects, were evaluated.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on an Anton Paar MCP 200 polarimeter (Anton Paar GmbH, Austria). ECD spectra were recorded using an Applied Photophysics Chirascan and Chirascan-plus circular dichroism spectrometer (Applied Photophysics Ltd., Leatherhead, England). An Agilent Cary 600 FT-IR microscope instrument (Agilent Technologies Inc., CA, USA) was used to measure IR spectra. NMR data were obtained by a Bruker-AVIIIHD-600 spectrometer (Bruker Corporation, MA, USA) or an Agilent-NMR-vnmrs 600 spectrometer (Agilent Technologies Inc., CA, USA) with the solvent peaks used as

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https://doi.org/10.1016/j.bioorg.2020.103820
Received 24 February 2020; Accepted 2 April 2020
Available online 07 April 2020

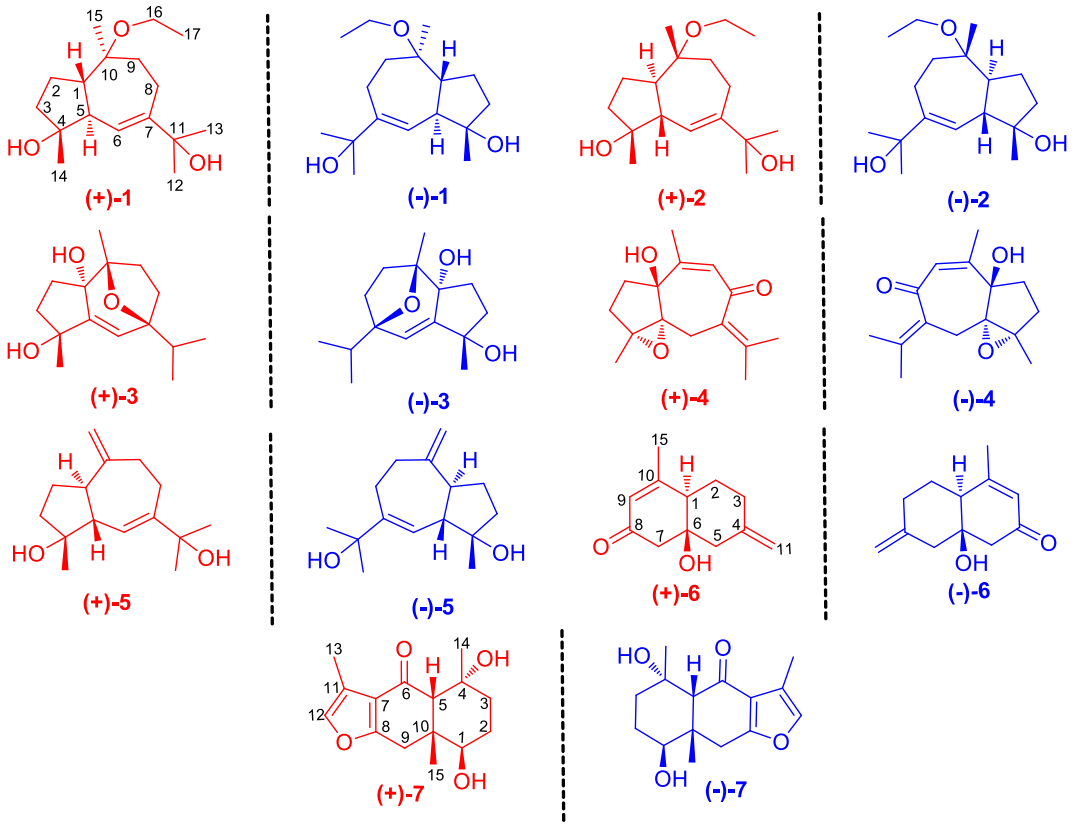

Fig. 1. Structures of compounds (+)/(−)-**1**–**7**.

references. HRESIMS data were acquired on a Waters Synapt G_2 HDMS instrument (Waters Corporation Milford, MA, USA). Column chromatography was performed using silica gel (200–300 mesh; Yantai Institute of Chemical Technology, Yantai, China) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was performed using glass precoated silica gel GF_{254} plates (Qingdao Marine Chemical Inc., Qingdao, China). HPLC separation was carried out using an Agilent 1100 instrument with a Kromasil semi-preparative C_{18} column (250 × 10 mm^2, 5 μm) for reversed-phase semi-preparative HPLC and a Daicel Chiralpak AD-H column (250 × 4.6 mm^2, 5 μm) for normal-phase enantioseparation. Vasorelaxant activity assays were conducted with a PL3508B6/C-V Panlab 8 Chamber Organ Bath System (including stimulating electrodes, Panlab Eight-Chamber Organ Baths, organ chambers, tissue hooks, Labchart Pro software). Anti-platelet aggregation assays were measured on an SC-2000 Platelet aggregometer (Beijing Success Technology Development Co., Ltd., Beijing, China). Healthy New Zealand rabbits of either sex weighing 2.2–2.5 kg were purchased from Dashuo Experimental Animal Co., Ltd (Chengdu, China). PC12 cells were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Arachidonic acid (AA) and adenosine diphosphate (ADP) were purchased from Dalian Meilun Biotechnology Co., Ltd. Fetal bovine serum (FBS) was purchased from Zhejiang Tianhang Biotechnology Co., Ltd, and dulbecco's modified Eagle's medium (DMEM) was purchased from Hyclone (Logan, UT, USA).

2.2. Plant material

C. phaeocaulis was collected in Sanjiang Town, Chongzhou City, Sichuan Province, China, in March 2018. The sample was identified by Dr. Jihai Gao and deposited in the School of Pharmacy in Chengdu University of TCM, Chengdu, China (voucher specimen: CP-20180303).

2.3. Extraction and isolation

The dry rhizomes of *C. phaeocaulis* (50 kg) were extracted with 95% EtOH (3 × 50 L) under reflux three times (3 h each time). The EtOH extract was evaporated under reduced pressure to yield a residue, which was dispersed in H_2O and partitioned with EtOAc. The EtOAc soluble fraction (300 g) was separated on a silica gel (200–300 mesh) column and eluted with a gradient of petroleum ether/EtOAc (5:1–0:1) and EtOAc/MeOH (1:0–0:1) into 11 fractions (A–K).

Fraction D (6 g) was applied to a Sephadex LH-20 column (CH_2Cl_2/MeOH, 1:1) to afford four subfractions (D_1–D_4). Subfraction D_2 (3.6 g) was further separated by RP-MPLC with MeOH/H_2O (20:80–0:100) to afford eight fractions ($D_{2\text{-}1}$ − $D_{2\text{-}8}$). $D_{2\text{-}2}$ (500 mg) was purified by a Sephadex LH-20 column (petroleum ether/CH_2Cl_2/MeOH, 5:5:1) and a silica gel column (CH_2Cl_2/ Me_2CO, 50:1–0:1) successively to get $D_{2\text{-}2\text{-}2\text{-}2}$ (15 mg). Compound **3** (2 mg, t_R = 51.3 min) was obtained from $D_{2\text{-}2\text{-}2\text{-}2}$ by RP semi-preparative HPLC (50% MeOH in H_2O).

Fraction E (14 g) was subjected to Sephadex LH-20 column chromatography (petroleum ether/CH_2Cl_2/MeOH, 5:5:1) to obtain $E_{2\text{-}3}$ (4.3 g), which was further separated by RP-MPLC with MeOH/H_2O (10:90–0:100) to afford 10 fractions ($E_{2\text{-}3\text{-}1}$ − $E_{2\text{-}3\text{-}10}$). $E_{2\text{-}3\text{-}1}$ (303 mg) was further purified by column chromatography on silica gel (CH_2Cl_2/ EtOAc, 50:1–1:1), followed by preparative TLC (CH_2Cl_2/ Me_2CO, 9:1) to yield $E_{2\text{-}3\text{-}1\text{-}2\text{-}3}$ (23 mg) and $E_{2\text{-}3\text{-}1\text{-}2\text{-}4}$ (28 mg). Compounds **4** (3 mg, t_R = 10.3 min) and **6** (2 mg, t_R = 10.2 min) were obtained from $E_{2\text{-}3\text{-}1\text{-}2\text{-}3}$ and $E_{2\text{-}3\text{-}1\text{-}2\text{-}4}$ by HPLC (30% MeOH in H_2O and 15% acetonitrile in H_2O), respectively. Separation of $E_{2\text{-}3\text{-}10}$ (98 mg) by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:1–1:1) and HPLC (60% MeOH in H_2O) successively yielded compound **5** (2.5 mg, t_R = 11.6 min).

Fraction F (36 g) was separated into 19 fractions (F_1 − F_{19}) by RP-MPLC with MeOH/H_2O (10:90–0:100). Subfractions F_5 (1.4 g), F_{11} (459 mg), and F_{15} (385 mg) were separated by Sephadex LH-20

Table 1
Chiral separation of racemic mixtures **1–7**.

Compound		Chiral phase	Mobile phase	Flow rate (mL/min)	Retention time (min)	Amount (mg)	Peak area ratio [(+)/(−)]
1	(+)-**1**	AD-H	*n*-hexane/ethanol, 25:1	1.0	12.0	0.51	1.02:1
	(−)-**1**				13.2	0.50	
2	(+)-**2**	AD-H	*n*-hexane/ethanol, 10:1	1.0	7.0	0.45	1.05:1
	(−)-**2**				5.4	0.43	
3	(+)-**3**	AD-H	*n*-hexane/ethanol, 50:1	1.0	11.8	0.80	1.05:1
	(−)-**3**				15.9	0.76	
4	(+)-**4**	AD-H	*n*-hexane/ isopropyl alcohol, 10:1	1.0	12.9	1.26	1.15:1
	(−)-**4**				15.3	1.10	
5	(+)-**5**	AD-H	*n*-hexane/ isopropyl alcohol, 10:1	1.0	7.8	1.18	0.99:1
	(−)-**5**				13.9	1.20	
6	(+)-**6**	AD-H	*n*-hexane/ethanol, 10:1	1.0	26.0	0.74	1.06:1
	(−)-**6**				39.3	0.70	
7	(+)-**7**	AD-H	*n*-hexane/ethanol, 10:1	1.0	10.7	0.57	1.14:1
	(−)-**7**				12.2	0.50	

columns (petroleum ether/CH_2Cl_2/MeOH, 5:5:1) followed by silica gel chromatography columns (CH_2Cl_2/ Me_2CO, 20:1–0:1) to afford $F_{5\text{-}6\text{-}1}$ (22 mg), $F_{11\text{-}2\text{-}1}$ (11 mg), and $F_{15\text{-}2\text{-}2}$ (14 mg), respectively. Then, compounds **7** (1.3 mg, t_R = 20.2 min), **1** (1.2 mg, t_R = 47.8 min), and **2** (1 mg, t_R = 53.7 min) were obtained from $F_{5\text{-}6\text{-}1}$, $F_{11\text{-}2\text{-}1}$, and $F_{15\text{-}2\text{-}2}$ via RP semi-preparative HPLC (50%, 55%, and 64% MeOH in H_2O), respectively. Finally, Racemic compounds **1–7** were submitted to chiral separation on a Daicel Chiralpak AD-H column to afford their enantiomers (Table 1).

(+)-Phaeocauline A and (−)-phaeocauline A [(+)-**1** and (−)-**1**]: Colorless oil; $\{[\alpha]_D^{20}$ + 75.5 (*c* 0.08, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 191 (−8.6) nm; (+)-**1**$\}$;$\{[\alpha]_D^{20}$ −78.2 (*c* 0.06, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 190 (+8.8) nm; (−)-**1**$\}$; UV (MeCN) λ_{max} (log ε) 193 (3.76) nm; IR ν_{max} 3362, 2971, 2932, 1670, 1452, 1378, 1299, 1219, 1183, 1134, 1093, 1064, 952, 857, 786, 711, 653 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 305.2098 $[M+Na]^+$ (calcd for $C_{17}H_{30}O_3Na$, 305.2093).

(+)-Phaeocauline B and (−)-phaeocauline B [(+)-**2** and (−)-**2**]: Colorless oil; $\{[\alpha]_D^{20}$ + 13.6 (*c* 0.06, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 190 (+8.8), 208 (−6.0) nm; (+)-**2**$\}$;$\{[\alpha]_D^{20}$ −15.8 (*c* 0.05, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 191 (−8.6), 208 (+8.6) nm; (−)-**2**$\}$; UV (MeCN) λ_{max} (log ε) 193 (3.66) nm; IR ν_{max} 3340, 3277, 2969, 2932, 1446, 1405, 1374, 1280, 1228, 1181, 1140, 1094, 1062, 959, 858, 741 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 305.2090 $[M+Na]^+$ (calcd for $C_{17}H_{30}O_3Na$, 305.2093).

(+)-Phaeocauline C and (−)-phaeocauline C [(+)-**3** and (−)-**3**]:

Table 2
^{1}H NMR (600 MHz) data of compounds 1–7 (δ in ppm, *J* in Hz).

Position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**5**[b]	**6**[a]	**7**[a]
1	2.01, m	2.27, ddd (10.7, 10.7, 5.6)			2.52, m	2.92, ddd (11.3, 8.0, 8.0)	2.67, d (13.0)	3.63, ddd (4.1, 3.4, 3.4)
2	1.73, m	1.80, m	1.74, m	1.72, m	1.84, m	1.99, m	2.07, m	2.33, m
	1.57, m	1.66, m	1.59, m	1.46, ddd (13.0, 10.5, 8.2)	1.84, m	1.89, m	1.66, m	1.64, m
3	1.55, m	1.70, m	2.05, m	1.92, m	1.76, m	2.08, m	2.44, m	1.98, ddd (13.7, 13.7, 3.9)
	1.55, m	1.53, m	1.73, m	1.84, dd (13.5, 8.2)	1.76, m	2.08, m	2.20, m	1.40, m
5	2.25, ddd (11.6, 2.5, 2.5)	2.03, dd (10.7, 3.9)			1.95, m	2.24, dd (11.3, 3.4)	2.40, m	2.19, s
							2.26, dd (13.5, 1.9)	
6	5.92, d (3.1)	6.05, d (3.9)	5.79, s	3.25, d (16.8) 2.33, d (16.8)	6.12, brs	6.81, brs		
7							2.55, d (15.8) 2.36, d (15.8)	
8	2.35, m	2.39, dd (15.7, 7.8)	1.64, m		2.52, m	2.82, dd (14.7, 7.5)		
	2.02, m	1.99, m	1.64, m		1.97, m	2.27, m		
9	1.73, m	1.65, m	1.82, m	5.73, s	2.48, m	2.64, dd (13.0, 7.5)	5.79, brs	3.41, d (16.4)
	1.58, m	1.65, m	1.57, m		1.93, m	2.14, m		2.24, d (16.4)
11			1.85, m				4.78, brs 4.67, brs	
12	1.26, s	1.28, s	0.94, d (6.8)	1.74, s	1.27, s	1.56, s		7.21, s
13	1.26, s	1.28, s	0.97, d (7.0)	1.79, s	1.29, s	1.56, s		2.13, brs
14	1.13, s	1.27, s	1.42, s	1.38, s	1.25, s	1.50, s		1.24, s
15	1.18, s	1.18, s	1.29, s	1.89, s	4.70, m	4.91, m	1.90, brs	1.07, s
16	3.36. q (7.0)	3.37, q (7.0)						
17	1.05, t (7.0)	1.06, t (7.0)						
OH	3.50, s (OH-4)	3.15, s (OH-4)	2.58, s (OH-1)	4.45, s (OH-1)	3.30, s (OH-4)		3.16, s (OH-6)	3.76, d (4.1, OH-1)
	3.31, s (OH-11)	3.31, s (OH-11)	3.49, s (OH-4)		3.35, s (OH-11)			3.17, s (OH-4)

[a] Data were measured in acetone-d_6.
[b] Data were measured in pyridine-d_5.

Table 3
^{13}C NMR (150 MHz) data of compounds 1–7 in acetone-d_6 (δ in ppm).

Position	1	2	3	4	5	6	7
1	48.7	50.1	81.0	82.9	48.9	47.4	73.2
2	22.5	23.6	30.0	34.3	25.9	25.5	26.1
3	41.3	40.9	40.9	30.3	40.5	35.4	34.7
4	79.9	80.3	77.4	70.6	80.1	145.5	71.5
5	50.7	50.6	153.6	69.6	56.0	48.2	60.4
6	123.0	122.9	122.1	24.4	122.6	74.2	196.8
7	150.4	150.9	87.0	133.8	151.6	52.6	120.9
8	23.6	24.1	36.9	195.8	29.7	197.1	167.8
9	37.4	37.5	32.1	129.8	38.6	127.4	34.0
10	79.3	79.7	85.3	148.4	155.9	160.6	42.1
11	73.2	73.2	34.6	139.2	73.3	111.2	119.6
12	29.3	29.2	18.2	21.7	29.2		139.6
13	29.4	29.2	18.2	22.4	29.2		9.3
14	22.8	26.4	29.8	15.8	27.4		30.5
15	18.9	19.0	21.2	20.0	106.5	22.0	25.9
16	56.2	55.8					
17	16.7	16.7					

Colorless oil; {[α]$_D^{20}$ + 26.6 (*c* 0.03, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 197 (−61.2), 221 (+12.6) nm; (+)-**3**};{[α]$_D^{20}$ −23.0 (*c* 0.04, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 197 (+55.4), 221 (−11.5) nm; (−)-**3**}; UV (MeCN) λ_{max} (log ε) 185 (4.03) nm; IR ν_{max} 3483, 3439, 1699, 1465, 1448, 1363, 1342, 1298, 1221, 1184, 1144, 1114, 1067, 1041, 1020, 963, 906, 867, 848, 830, 758, 675 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 275.1629 [M+Na]$^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1623).

(+)-Phaeocauline D and (−)-phaeocauline D [(+)-**4** and (−)-**4**]: Colorless oil; {[α]$_D^{20}$ + 89.2 (*c* 0.04, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 238 (+8.4), 270 (−5.8), 342 (−2.5) nm; (+)-**4**}; {[α]$_D^{20}$ −78.6 (*c* 0.06, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 238 (−8.6), 270 (+5.9), 342 (+2.5) nm; (−)-**4**}; UV (MeCN) λ_{max} (log ε) 263 (3.12), 241 (3.09) nm; IR ν_{max} 3348, 2979, 2943, 1649, 1590, 1432, 1416, 1372, 1302, 1288, 1274, 1221, 1176, 1149, 1109, 1083, 1064, 1030, 1000, 970, 944, 909, 882, 852, 793, 707, 650 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 271.1311 [M+Na]$^+$ (calcd for $C_{15}H_{20}O_3Na$, 271.1310).

(+)-Phaeocauline E and (−)-phaeocauline E [(+)-**5** and (−)-**5**]: Colorless oil; {[α]$_D^{20}$ + 28.7 (*c* 0.03, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 191 (−39.1), 208 (+4.6), 218 (−3.6) nm; (+)-**5**}; {[α]$_D^{20}$ −26.6 (*c* 0.03, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 192 (+39.1), 208 (−3.8), 218 (+3.7) nm; (−)-**5**}; UV (MeCN) λ_{max} (log ε) 196 (4.01) nm; IR ν_{max} 3393, 3183, 2918, 2848, 1644, 1468, 1419, 1371, 877, 816, 720, 644 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6 or pyridine-d_5) and ^{13}C NMR (150 MHz, acetone-d_6) and data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 259.1679 [M+Na]$^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

(+)-Phaeocauline F and (−)-phaeocauline F [(+)-**6** and (−)-**6**]: Colorless oil; {[α]$_D^{20}$ + 47.1 (*c* 0.03, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 213 (+9.3), 239 (−18.3) nm; (+)-**6**}; {[α]$_D^{20}$ −42.5 (*c* 0.04, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 213 (−9.3), 239 (+18.9) nm; (−)-**6**}; UV (MeCN) λ_{max} (log ε) 234 (3.37), 190 (3.49) nm; IR ν_{max} 3344, 2952, 2829, 1657, 1438, 1401, 1380, 1344, 1285, 1245, 1150, 1118, 1096, 1074, 1002, 976, 877, 866, 851, 828 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 215.1050 [M+Na]$^+$ (calcd for $C_{12}H_{16}O_2Na$, 215.1048).

(+)-Phaeocauline G and (−)-phaeocauline G [(+)-**7** and (−)-**7**]: Colorless oil; {[α]$_D^{20}$ + 64.5 (*c* 0.05, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 274 (+0.9), 312 (−2.5) nm; (+)-**7**}; {[α]$_D^{20}$ −59.5 (*c* 0.05, MeOH); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 273 (−0.9), 312 (+2.5) nm; (−)-**7**}; UV (MeCN) λ_{max} (log ε) 205 (3.28), 269 (2.50) nm; IR ν_{max} 3391, 3183, 2920, 2853, 1643, 1562, 1460, 1422, 1252, 1200, 1113, 1064, 1029, 962, 810, 763, 720 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 2 and 3, respectively; (+)-HRESIMS *m/z* 287.1263 [M+Na]$^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

2.4. Vasorelaxant activity assay

The vasorelaxant activity of the isolates against KCl-induced contractions of rat aorta rings was measured as described previously [21]. The experimental details are provided in the Supplementary Data.

2.5. Anti-platelet aggregative activity assay

The anti-platelet aggregative effects of the isolates were evaluated by suppressing ADP- or AA-induced platelet aggregation in rabbit platelets [22]. The experimental details are provided in the Supplementary Data.

2.6. Neuroprotective activity assay

The protection of the isolates on glutamate-induced cytotoxicity in PC12 cells was studied using the MTT method [23]. The experimental details are provided in the Supplementary Data.

3. Results and discussion

Compound **1** was assigned with the molecular formula $C_{17}H_{30}O_3$ by an ion peak at *m/z* 305.2098 [M+Na]$^+$ in the HRESIMS. The ^{1}H NMR data (Table 2) of **1** showed signals of four tertiary methyl groups, one ethoxy group, one olefinic proton, and several aliphatic methylenes and methines. A total of 17 carbon signals, corresponding to five methyl groups, five methylenes, three methines (one olefinic), and four quaternary carbons (three oxygenated and one olefinic), were revealed by its ^{13}C NMR spectrum (Table 3) and DEPT analysis. These data indicated that compound **1** was a guaiane sesquiterpenoid similar to 4α,10β,11-trihydroxy-1,5-trans-guaiane-6,7-ene [24], with the exception of the replacement of a hydroxy group with an ethoxy group in **1** [δ_H 3.36 (2H, q, J = 7.0 Hz), 1.05 (3H, t, J = 7.0 Hz); δ_C 56.2 (t), 16.7 (q)]. Three oxygenated quaternary carbons were confirmed to be C-4 (δ_C 79.9), C-10 (δ_C 79.3), and C-11 (δ_C 73.2) according to the HMBC correlations (Fig. 2) of H$_3$-14 with C-3, C-4, and C-5; of H$_3$-12 with C-7, C-11, and C-13; and of H$_3$-15 with C-1, C-9, and C-10, while the ethoxy group was located at C-10 based on the HMBC correlation (Fig. 2) from H$_2$-16 to C-10. The NOE correlations of H-1 with H$_3$-14 and H-16 and of H-5 with OH-4 and H$_3$-15 (Fig. 3) indicated that compound **1** has the same relative configuration as that of 4α,10β,11-trihydroxy-1,5-trans-guaiane-6,7-ene. Consequently, the structure of **1** was identified as (1β,5α)-10β-ethoxyguaia-6(7)-en-4α,11-diol.

The HRESIMS data of **2** indicated that it possesses the same molecular formula as **1**. Comparison of the ^{1}H, ^{13}C, and 2D NMR data of **2** and **1** suggested that the two compounds have the same planar structure. Compound **2** was identified as the C-4 epimer of compound **1**

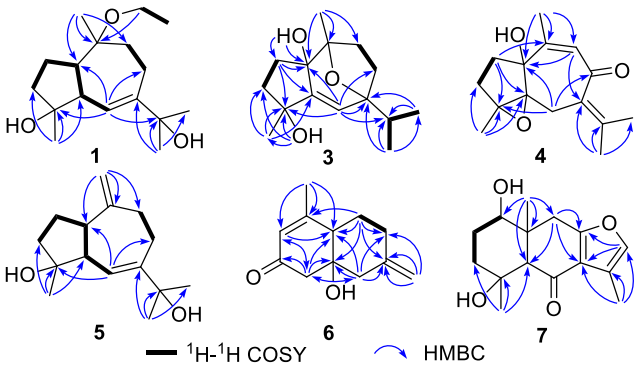

Fig. 2. Key ^{1}H−^{1}H COSY and HMBC correlations of **1** and **3**–**7**.

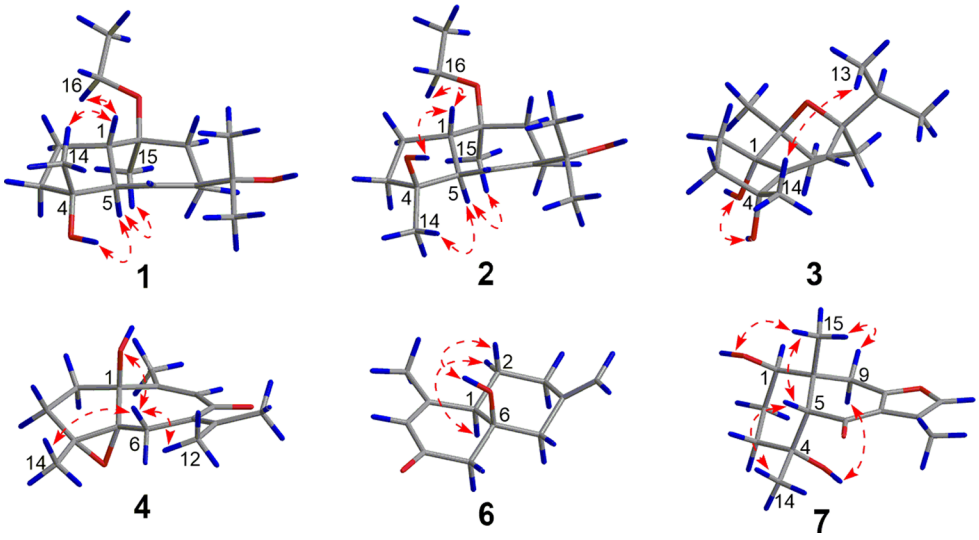

Fig. 3. Key NOE correlations of **1**, **2**, **4**, **6**, and **7** and ROESY correlations of **3**.

according to the NOE correlations (Fig. 3) of H-1 with OH-4 and H-16 and of H-5 with H_3-14 and H_3-15. Thus, compound **2** was determined to be (1*α*,5*β*)-10*α*-ethoxyguaia-6(7)-en-4*α*,11-diol.

Compound **3** was obtained as a colorless oil. Its molecular formula was elucidated as $C_{15}H_{24}O_3$ by the positive HRESIMS data (*m/z* 275.1629 $[M+Na]^+$; calcd. for $C_{15}H_{24}O_3Na$, 275.1623). The ^1H NMR data (Table 2) of **3** displayed signals for two tertiary methyl groups (δ_H 1.29 and 1.42), two secondary methyl groups [δ_H 0.94 (d, J = 6.8 Hz) and 0.97 (d, J = 7.0 Hz)], and one olefinic proton at 5.79 (s). A total of 15 carbon signals (Table 3) attributed to 4 × CH_3, 4 × CH_2, 2 × CH (one olefinic methine), and 5 × C (one olefinic carbon and four oxygenated carbons) were revealed in the ^{13}C NMR and DEPT spectra. The above data indicated that **3** is very similar to a known 7,10-epoxy guaiane sesquiterpenoid, pubinernoid B [25], with the exception of an additional hydroxy group at C-1 (δ_C 81.0) in **3**. This assignment was confirmed by $^1H-^1H$ COSY correlations (Fig. 2) and the HMBC correlations of H_3-15 with C-1, C-9, and C-10; of OH-1 with C-1 and C-2; and of OH-4 with C-3, C-4, and C-14 (Fig. 2). The relative configuration was established by analysis of ROESY data. Correlations of H_3-14 with H_3-13 and of OH-1 with OH-4 (Fig. 3) demonstrated that H_3-14, isopropyl-7, and 7-*O*-10 bridge were cofacial, whereas OH-1 and OH-4 occupied the opposite face. Thus, the structure of compound **3** was determined as 7*β*,10*β*-epoxyguaia-5(6)-en-1*α*,4*α*-diol.

The molecular formula ($C_{15}H_{20}O_3$) of compound **4** was determined from its $[M+Na]^+$ ion peak at *m/z* 271.1311 (calcd. 271.1310) in its HRESIMS. The ^1H, ^{13}C, and DEPT NMR data (Tables 2 and 3) of **4** suggested that it is also a guaiane type sesquiterpene similar to 1-epiaerugidiol [26]. The presence of the 4,5-epoxide ring in **4** was determined by the molecular formula ($C_{15}H_{20}O_3$), the up-field shift of C-4 (δ_C 70.6), and the down-field shift of C-5 (δ_C 69.6), as well as the absence of the proton signal of H-5. The planar structure of **4** is supported by $^1H-^1H$ COSY and HMBC data, especially the correlations from H_3-14 to C-3, C-4, and C-5; from H_3-12 to C-7, C-11, and C-13; from H_3-15 to C-1, C-9, and C-10; and from H-6 to C-1, C-4, and C-8 (Fig. 2). In the 1D NOE experiment of **4**, H_3-14 and OH-1 were enhanced when H-6a (δ_H 3.25) was irradiated (Fig. 3), which indicated that OH-1, H-6a (δ_H 3.25), and H_3-14 are cofacial. Thus, the structure of compound **4** was established as 1*β*-hydroxy-4*α*,5*α*-epoxyguaia-7(11),9(10)-dien-8-one.

The HRESIMS, ^1H and ^{13}C NMR data (Tables 2 and 3) of compound **5** showed that it is an isomer of 4*β*,12-dihydroxyguaian-6,10-diene, a known guaiane-type sesquiterpene isolated from the rhizomes of *Alisma orientale* [27]. Detailed analysis of the $^1H-^1H$ COSY, HSQC, and HMBC data confirmed that they have the same planar structure. The relative configuration of **5** was elucidated by $^1H-^1H$ coupling constants, NOE data, and the empirical pyridine-induced deshielding effect. The large coupling constant between H-1 and H-5 (J = 11.3 Hz) showed the presence of the trans-guaiane skeleton [28]. The NOE correlation of H_3-14 with H-5 revealed the anti-orientation of OH-4 and H-5 (Fig. 3). To further verify the relative configuration using the empirical pyridine-induced deshielding effect [29,30], the ^1H NMR data of **5** were collected in acetone-d_6 and pyridine-d_5, respectively. The downfield shift ($\Delta\delta$ + 0.40) was observed for H-1 in pyridine-d_5, which confirmed that OH-4 and H-1 are cofacial. Therefore, the structure of **5** was defined as (1*α*,5*β*)-guaia-6(7),10(15)-dien-4*α*,11-diol.

Compound **6** has the molecular formula of $C_{12}H_{16}O_2$ with five indices of hydrogen deficiency, as indicated by the HRESIMS and ^{13}C NMR data. The ^1H NMR data (Table 2) of **6** showed proton signals attributed to an allylic methyl group (δ_H 1.90), a terminal double bond (δ_H 4.67 and 4.78), and a conjugated trisubstituted double bond (δ_H 5.79). The ^{13}C NMR and DEPT data (Table 3) revealed the presence of 12 carbons, including one methyl, five methylenes (one olefinic), two methines (one olefinic), two olefinic quaternary carbons, one carbonyl carbon, and one oxygen-bearing quaternary carbon. The above functionalities accounted for three out of the five indices of hydrogen deficiency, and the remaining two indices suggested **6** to be bicyclic. Finally, compound **6** was assigned to be a degraded cadinene by the $^1H-^1H$ COSY correlations of H-1/H_2-2/H_2-3 and the HMBC correlations of H_3-15 with C-1, C-9, and C-10; of H-2 with C-1, C-3, C-4, C-6, and C-10; of H-11 with C-3, C-4, and C-5; of H-5 with C-1, C-3, C-4, C-6, and C-7; of H-7 with C-1, C-5, C-6, C-8, and C-9; and of H-9 with C-1 and C-7 (Fig. 2). The NOE correlations of H-1 with H-2a (δ_H 2.07) and of OH-6 with H-2b (δ_H 1.66) suggested that the two six-membered rings are trans-fused (Fig. 3). Thus, the structure of **6** was elucidated as (1*α*)-6*β*-hydroxy-12,13,14-trinorcadinane-4(11),9(10)-dien-8-one.

The molecular formula of compound **7** was assigned as $C_{15}H_{20}O_4$, which is the same as that of an eudesmane-type sesquiterpene, curcolonol [31]. A comparison of the ^1H and ^{13}C NMR data of **7** (Tables 2 and 3) and curcolonol suggested that the two compounds are structurally quite similar. Compound **7** was identified as the C-5 epimer of curcolonol, according to the $^1H-^1H$ COSY and HMBC data (Fig. 2) combined with the NOE correlations from H_3-15 to OH-1, H-5, and H-9b (δ_H 2.24); from H_3-14 to H-5; and from H-9a (δ_H 3.41) to OH-4 (Fig. 3). Thus, compound **7** was identified to be (5*β*)-1*β*,4*α*-dihydroxy-8,12-epoxyeudsma-7(8),11(12)-dien-6-one.

Interestingly, although compounds **1**–**7** all contain multiple chiral centers, their specific optical rotations are close to zero and no cotton effects were observed in their ECD spectra. It suggested that they may be isolated as racemic mixtures. Subsequently, compounds **1**–**7** were

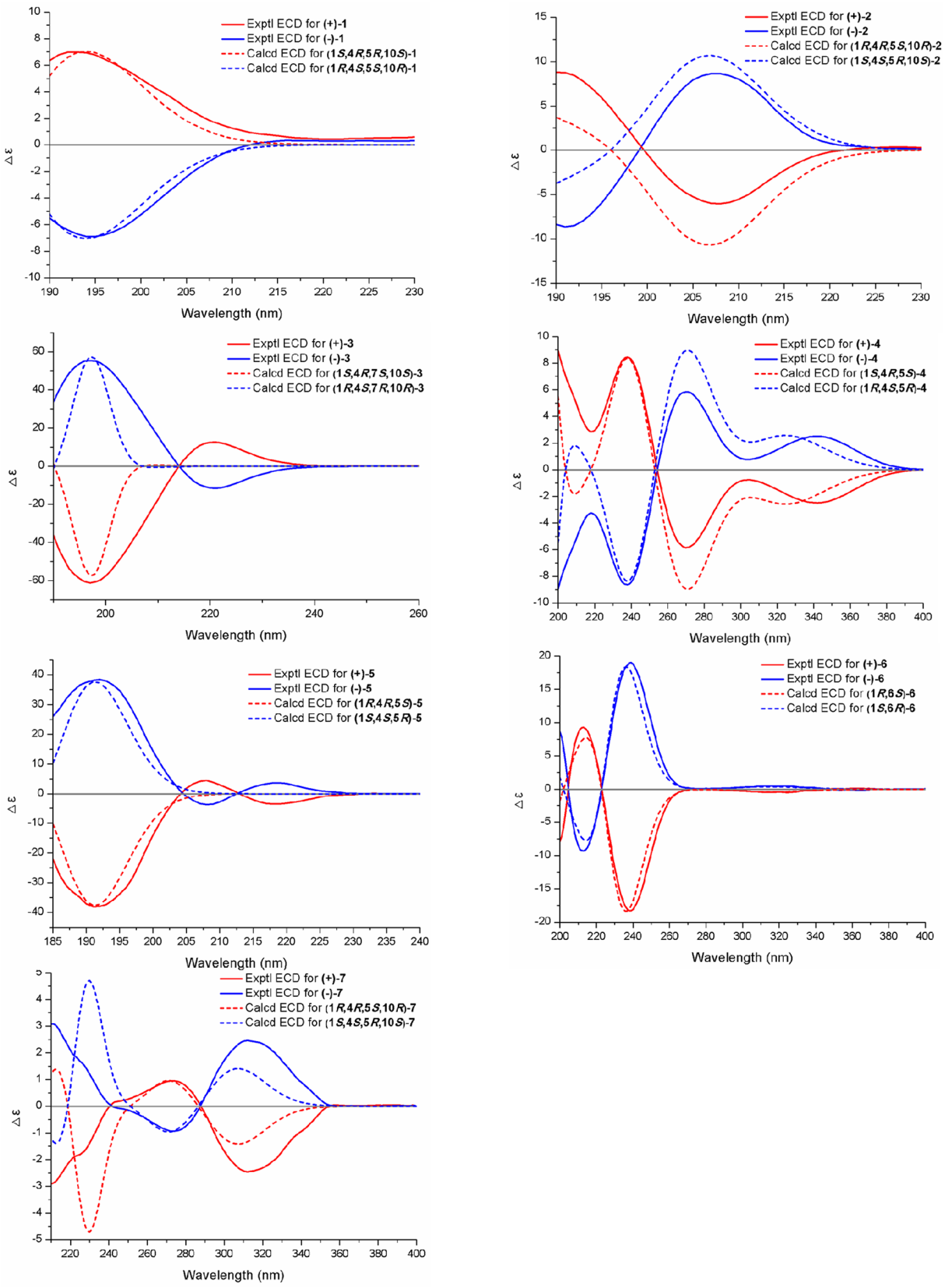

Fig. 4. The experimental and calculated ECD spectra of **1–7**. The TDDFT calculations of ECD spectra of **1–7** were conducted at the CAM-B3LYP/DGDZVP level.

subjected to chiral HPLC separation on a chiral column, yielding (+)-**1**/(−)-**1**, (+)-**2**/(−)-**2**, (+)-**3**/(−)-**3**, (+)-**4**/(−)-**4**, (+)-**5**/(−)-**5**, (+)-**6**/(−)-**6**, and (+)-**7**/(−)-**7**, respectively (Table 1). Each pair of enantiomers showed opposite ECD curves and specific optical rotations.

Since the relative configurations of **1–7** have been determined as mentioned above, ECD calculations were performed to define the absolute configurations [32–34] (Supplementary Data, Experimental Section). Consequently, the seven pairs of enantiomers were

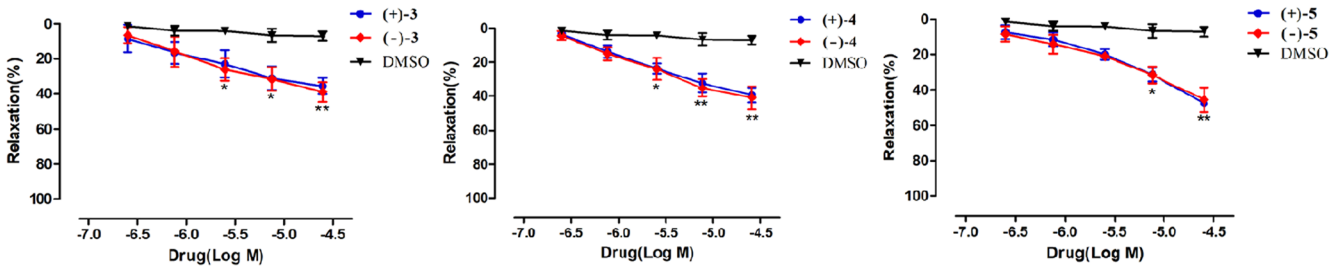

Fig. 5. Vasorelaxant activities of **3–5** against KCl-induced contraction of rat aorta rings. The data are presented as the means ± S.E.M., n = 5. (One-way ANOVA followed by Dunnett's post hoc test, *p < 0.05 and **p < 0.01 vs. control group).

determined to be (+)-(1*S*,4*R*,5*R*,10*S*)-10-ethoxyguaia-6(7)-en-4,11-diol [(+)-phaeocauline A, (+)-**1**], (−)-(1*R*,4*S*,5*S*,10*R*)-10-ethoxyguaia-6(7)-en-4,11-diol [(−)-phaeocauline A, (−)-**1**], (+)-(1*R*,4*R*,5*S*,10*R*)-10-ethoxyguaia-6(7)-en-4,11-diol [(+)-phaeocauline B, (+)-**2**], (−)-(1*S*,4*S*,5*R*,10*S*)-10-ethoxyguaia-6(7)-en-4,11-diol [(−)-phaeocauline B, (−)-**2**], (+)-(1*S*,4*R*,7*S*,10*S*)-7,10-epoxyguaia-5(6)-en-1,4-diol [(+)-phaeocauline C, (+)-**3**], (−)-(1*R*,4*S*,7*R*,10*R*)-7,10-epoxyguaia-5(6)-en-1,4-diol [(−)-phaeocauline C, (−)-**3**], (+)-(1*S*,4*R*,5*S*)-1-hydroxy-4,5-epoxyguaia-7(11),9(10)-dien-8-one [(+)-phaeocauline D, (+)-**4**], (−)-(1*R*,4*S*,5*R*)-1-hydroxy-4,5-epoxyguaia-7(11),9(10)-dien-8-one [(−)-phaeocauline D, (−)-**4**], (+)-(1*R*,4*R*,5*S*)-guaia-6(7),10(15)-dien-4,11-diol [(+)-phaeocauline E, (+)-**5**], (−)-(1*S*,4*S*,5*R*)-guaia-6(7),10(15)-dien-4,11-diol [(−)-phaeocauline E, (−)-**5**], (+)-(1*R*,6*S*)-6-hydroxy-12,13,14-trinorcadinane-4(11),9(10)-dien-8-one [(+)-phaeocauline F, (+)-**6**], (−)-(1*S*,6*R*)-6-hydroxy-12,13,14-trinorcadinane-4(11),9(10)-dien-8-one [(−)-phaeocauline F, (−)-**6**], (+)-(1*R*,4*R*,5*S*,10*R*)-1,4-dihydroxy-8,12-epoxyeudsma-7(8),11(12)-dien-6-one [(+)-phaeocauline G, (+)-**7**], and (−)-(1*S*,4*S*,5*R*,10*S*)-1,4-dihydroxy-8,12-epoxyeudsma-7(8),11(12)-dien-6-one [(−)-phaeocauline G, (−)-**7**] by comparison of the calculated ECD spectra with the experimental ECD spectra (Fig. 4).

Since the rhizomes of *C. phaeocaulis* are a traditional Chinese medicine for promoting blood circulation and removing stasis, the isolates were investigated for anti-platelet aggregative and vasorelaxant activities. Enantiomers (+)-**1** and (−)-**1** showed similar activity against abnormal platelet aggregation induced by arachidonic acid (AA) with inhibition rates of 27.78 ± 4.36% and 31.63 ± 7.10%, respectively, while their C-4 epimers (+)-**2** and (−)-**2** were inactive at 0.1 mM (the inhibition of aspirin was 72.89 ± 7.65% at 0.1 mM). Compounds (+)/(−)-**3–5** exhibited vasorelaxant effects against KCl-induced contraction of rat aortic rings with maximal vasorelaxation (E_{max}) of 35.51 ± 3.65%/38.96 ± 3.26%, 39.42 ± 4.63%/40.93 ± 5.68%, and 47.71 ± 4.35%/45.64 ± 6.85%, respectively (Fig. 5), and there were no significant differences in the activities of each pair of the enantiomers. In addition, the isolates were investigated for their neuroprotective activities based on the results of the previous neuroprotective studies of similar sesquiterpenoids from *C. phaeocaulis* [14,32].[14,29] However, they did not show significant activity at 50 μM.

4. Conclusion

In summary, seven pairs of new enantiomeric sesquiterpenoids, [(+)/(−)-**1–7**], were obtained from the rhizomes of *Curcuma phaeocaulis* by chiral HPLC separation, and their structures were determined by comprehensive spectroscopic analyses and ECD data. (+)/(−)-**1** showed moderate activity against abnormal platelet aggregation induced by arachidonic acid (AA), while (+)/(−)-**3–5** exhibited vasorelaxant effects against KCl-induced contraction of rat aortic rings. The difference in anti-platelet aggregative activity between (+)/(−)-**1** and (+)/(−)-**2** indicated that those effects were stereoselective, but not enantioselective. Moreover, a comparison of the vasorelaxant effects of compounds (+)/(−)-**2** and (+)/(−)-**5** suggested that the substituents at C-10 are closely related to this activity. This study not only enriched the enantiomeric sesquiterpenoids in the genus *Curcuma*, but also partly revealed the material basis of *Curcuma phaeocaulis* for activating blood circulation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Support was received from the National Natural Science Foundation of China (Grant No. 81903777), the Postdoctoral Science Foundation of China (Grant No. 2019M653362), the Youth Science and Technology Innovation Research Team Program of Sichuan (Grant No. 2017TD0001), the Scientific Research Innovation Team Construction in Sichuan Provincial University) (Grant No. 18TD0017), and the "Xing lin Scholar" Plan of Chengdu University of Traditional Chinese Medicine (Grant Nos. YXRC2018005 and BSH2018009).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103820.

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