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Article

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# Diarylheptanoids from Rhizomes of *Alpinia officinarum* Inhibit Aggregation of Alpha-synuclein

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## ABSTRACT

Two new diarylheptanoids, alpinins A (**1**) and B (**2**), together with eighteen known diarylheptanoids (**3**–**20)**, were isolated from the rhizomes of *Alpinia officinarum*. Their structures were elucidated by comprehensive spectroscopic analysis including HRMS, IR, and 1D- and 2D-NMR. Structurally, alpinin A is a new member of the small family of oxa-bridged diarylheptanoids and contains the characteristic 2,6-*cis*-configured tetrahydropyran motif (C1-C5 oxa-bridge). The absolute configuration of alpinin A was confirmed by asymmetric total synthesis of the enantiomer (*ent*-**1**), corroborating the assignment of the molecular structure. The absolute configuration of alpinin B was determined on the basis of the analysis of the CD exciton chirality spectrum. We evaluated the inhibitory activity of all isolated diarylheptanoids against alpha-synuclein aggregation at 10 $\mu$M. Alpinins A and B significantly inhibited alpha-synuclein aggregation by 66% and 67%, respectively.


**KEYWORDS:** *Alpinia officinarum, diarylheptanoids, alpinin A, alpinin B, inhibit alpha-synuclein aggregation*

## INTRODUCTION

Parkinson's disease is the second most common progressive neurodegenerative disorder. The characteristic phenotypes of the disease are majorly the resting tremor and postural instability with cognitive and emotional disorders. Although the etiology and pathogenesis of Parkinson's disease are not fully understood, recent evidence suggests that environmental and genetic factors might account for the progression of disease.[1] Alpha-synuclein (α-synuclein), a protein expressed predominantly in neurons, especially at synaptic terminals, is the major component of Lewy bodies in Parkinson's disease patients,[2, 3] and is therefore implicated in the pathogenesis of the disease. Alpha-synuclein protein readily adopts various conformations[2, 3] it has a strong tendency to self-aggregate into oligomers, followed by fibrils that are deposited as Lewy bodies and other similar pathologies. Therefore, small organic molecules that can inhibit α-synuclein aggregation might provide a potentially effective treatment of Parkinson's disease. Herein, we report the isolation and/or synthesis of new natural diarylheptanoid products and the preliminary evaluation of their inhibition activity against the α-synuclein aggregation.

*Alpinia officinarum* (Hance) is a perennial herb from Zingiberaceae family that is ubiquitous in tropical and subtropical Asian regions, especially Southern China. The rhizome of *Alpinia officinarum* is used as a traditional Chinese medicine for strengthening the circulatory system, treating stomach ache, cold and swelling symptoms.[4] Besides its medicinal use, *A. officinarum* is also a valuable dietary material. Phytochemical studies have revealed that diarylheptanoids,[5-7] flavonoids,[8] and volatile oils are the major characteristic compounds of *A. officinarum*. It is noteworthy that many diarylheptanoid natural products present in *Alpinia* species possess cytotoxic, antioxidant, anti-inflammatory, antiplatelet and antiproliferative activities.[9-12] We were intrigued by the finding that the total extract of *A. officinarum* inhibited the aggregation of α-

synuclein and exhibits a neuroprotective effect. In this article, we describe the isolation and structural elucidation of two new diarylheptanoids and 18 known ones (Figure 1). We also investigated their inhibitory effects on α-synuclein aggregation and accomplished the first asymmetric total synthesis of the enantiomer of compound **1**.

## MATERIALS AND METHODS

**General Experimental Procedures.** Solvents (Merck; A.R.) for isolation were used as received unless stated otherwise. Silica gel (Qingdao Marine Chemical Incorporation; 200–300 mesh), MCI Gel CHP20P (Supelco; 63–150 μm) and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. Fractions were monitored by thin-layer chromatography (TLC) on precoated silica gel 60 $GF_{254}$ aluminum plates (Merck), and the signals were observed on heated silica gel plates after spraying 10% $H_2SO_4$ in EtOH. Preparative HPLC was performed using a Waters 2996 photodiode array detector (220 nm) equipped with a preparative OBD column (150 × 19 mm, 5.0 μm, flow rate 15.0 mL/min; Waters Xbridge). Optical rotation was obtained by a Jasco P-2000 polarimeter. Spectral data on UV, CD, IR, and NMR were gained from a Perkin-Elmer Lambda 900 UV/VIS/NIR spectrophotometer, a Chirascan Applied Photophysics spectropolarimeter, a Perkin-Elmer 577 spectrometer (KBr discs), and a Bruker AM-400 spectrometer, respectively. High-resolution mass spectrometry (HRMS) was recorded using a Waters Xevo G2-XS QTOF in an electrospray-ionization mode (ESI).

**Plant Material.** The dried rhizomes of *Alpinia officinarum* Hance were procured in August 2009 from Zhanjiang, Guangdong, China, authenticated by one of the authors (Guang-miao Fu), and preserved as a voucher specimen (TCM-40) in the room CYT5014 at HKUST, Hong Kong SAR, China.

**Extraction and Isolation.** A schematic flowchart of the details of extraction and isolation is provided in the *Supporting Information*. The dried grounded rhizomes (8.0 kg) of *A. officinarum* were extracted with 70% EtOH (40.0 L × 3, 2 h each), and the pooled extract was then concentrated *in vacuo* to give a brown paste (700.0 g), which was successively partitioned between chloroform (1.0 L × 3), and *n*-BuOH (1.0 L × 3) with water (1.0 L) to yield fractions of chloroform (120.0 g), *n*-BuOH (150.0 g), and water (420.0 g), respectively.

The *n*-BuOH fraction (80.0 g) was fractionated by silica gel column chromatography using a stepwise gradient elution of $CH_2Cl_2$:MeOH (v/v, 100:0, 50:1, 20:1, 15:1, 10:1, 5:1 and 1:1) to yield seven fractions designated Fr. 1–7. Purification of Fr. 2 (9.7 g) was performed with an MCI Gel CHP20P column elution with a gradient of MeOH:$H_2O$ (v/v, 2:8, 4;6, 5:5, 6:4, 8:2 and 9:1), Sephadex LH-20 column elution with a mixture of $CHCl_3$:MeOH (v/v, 1:1), and finally preparative HPLC using ACN:$H_2O$:TFA (v/v/v, 20:80:0.025, in 20 min). This provided the new, analytically pure compounds **1** (39 mg, alpinin A) and **2** (28 mg, alpinin B), as well as the previously known diarylheptanoid **3** (28 mg). The separation of Fr. 3 (6.8 g) with Sephadex LH-20 elution with $CHCl_3$/$CH_3OH$ (v/v, 1:1), and preparative HPLC elution with ACN:$H_2O$:TFA (v/v/v, 10:90:0.025, in 40 min) yielded three known diarylheptanoid compounds: **6** (15 mg), **7** (13 mg), and **8** (20 mg). Fr. 4 (10.6 g) was chromatographed over silica gel with gradient eluting solvents $CHCl_3$:MeOH (v/v, 10:0, 9:1, 8:2, 7:3, 6:4 and 1:1) to give three subfractions designated Fr.4-1–Fr.4-3, which were categorized according to their polarity estimated by TLC. Compound **11** (17 mg) was obtained from Fr. 4-1 by Sephadex LH-20 column chromatography elution with $CHCl_3$:MeOH (v/v, 1:1), and compound **9** (8 mg) was found in Fr. 4-2 through silica gel column chromatography elution with a gradient of $CHCl_3$:MeOH (v/v, 20:1, 10:1, 5:1).

The chloroform fraction (68.0 g) was separated by a silica gel column chromatography using gradient elution with petroleum ether (60-80°C):EtOAc (v/v, 10:0, 9:1, 8:2; 7:3, 6:4, 5:5 and 0:10) afforded six subfractions designated Fr. A–Fr. F, which were categorized according to their polarity estimated by TLC. Separation of Fr.B (9.6 g) on a silica gel column chromatography elution with a gradient of petroleum ether (60-80°C):EtOAc (v/v, 30:1, 20:1, 10:1, 5:1, and 1:1) yielded two pure compounds **17** (35 mg), and 1**9** (28 mg), as well as a mixture of **18** and **20,** which was efficiently separated by Sephadex LH-20 column chromatography elution with $CHCl_3$:MeOH (v/v, 1:1) (**18,** 38 mg; **20,** 25 mg). Fr. C (7.5 g) was further fractionated on a silica gel column eluted with petroleum ether (60-80°C):EtOAc (v/v, 15:1) to give **12** (20 mg) and three subfractions designated Fr.C-1–Fr.C-3. Compound **13** (22 mg) and **14** (8 mg) from Fr. C-2, **15** (16 mg) from Fr. C-3 were then obtained through Sephadex LH-20 column chromatography elution with $CHCl_3$:MeOH (v/v, 1:1). Similarly, compounds **10** (11 mg) and **16** (25 mg) were isolated through sequential separation of Fr. D (5.5 g) by Sephadex LH-20 (eluted with $CHCl_3$:MeOH, v/v, 1:1) and silica gel column chromatography (eluted with petroleum ether (60-80°C):EtOAc, v/v, 20:1–10:1). Fr. E (8.8 g) was further fractionated by Sephadex LH-20 (eluted with $CHCl_3$:MeOH, v/v, 1:1), and preparative HPLC (eluted with ACN:$H_2O$:TFA, v/v/v, 10:90:0.025, in 40 min) to give compounds **4** (17 mg) and **5** (9 mg).

**Methylation of 1.** $K_2CO_3$ (41.0 mg) was added to an acetone solution of compound **1** (13.1 mg in 2 mL) at room temperature, and stirred under a nitrogen atmosphere for 10 min. Iodomethane (0.6 mL) was then added and mixed for 1.5 h at room temperature, followed by heating under reflux at 60°C overnight (~16 h). Upon cooling to room temperature, the reaction mix was worked up and organic residue was purified by column chromatography on silica gel ($CH_2Cl_2$:MeOH, v/v, 40:1) to give methylated analogue of alpinin A (**1a**, 12.0 mg).

**Methylation of 2.** Following the same procedure as described for synthesis of **1a** from alpinin A, the methylated analogue (**2a,** 11.8 mg) of alpinin B was obtained from 12.8 mg alpinin B (**2**).

**Bis-*p*-bromobenzoate (2b) of 2a.** $Et_3N$ (32 μL), 4-dimethylaminopyridine (DMAP, 15 mg), and *p*-bromobenzoyl chloride (40 mg) were added sequentially to a solution of **2a** (10 mg) in anhydrous $CH_2Cl_2$ (0.6 mL). The reaction mixture was heated to 60°C and stirred for 9 h. Upon cooling to room temperature, the reaction was quenched by $H_2O$ (20 mL). The mixture was then extracted with EtOAc (10 mL× 3), and the combined organic fractions were concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography ($CH_2Cl_2$:MeOH, v/v, 9.5:0.5) to afford **2b** (6 mg).

*Alpinin A (**1**)*: yellowish oil. $[\alpha]_D^{25}$ +51.1° (*c* 0.42, MeOH). UV (MeOH, nm): $\lambda_{max}$ (log ε), 281 (1.05). IR (KBr, $cm^{-1}$): $\nu_{max}$ 3358, 2941, 2856, 2361, 2032, 1674, 1607, 1527, 1455, 1340, 1284, 1203, 1090, 990, 958, 800. See Table 1 for the $^1$H- and $^{13}$C-NMR data. HRMS(ESI): *m/z* 399.1447 $[M+Na]^+$ (calcd. for $C_{20}H_{24}O_7Na$, 399.1420), *m/z* 377.1607 $[M+H]^+$ (calcd. for $C_{20}H_{25}O_7$, 377.1600).

*(1R,3S,5R)-1,5-epoxy-3-hydroxy-1-(3,4,5-trimethoxy-phenyl)-7-(3,4-dimethoxy-phenyl)heptane (**1a**):* amorphous powder. $[\alpha]_D^{25}$ −3.2° (*c* 0.10, MeOH). UV (MeOH, nm): $\lambda_{max}$ (log ε) 230 (2.54), 280 (2.02). IR (KBr, $cm^{-1}$): $\nu_{max}$ 3418, 3073, 2999, 2933, 2853, 2837, 1591, 1516, 1464, 1453, 1418, 1260, 1235, 1154, 1139, 1070, 1027, 807. $^1$H-NMR ($CD_3OD$, 400 MHz) *δ*: 4.31 (1H, brd, *J* = 11.1 Hz, H-1), 1.95, 1.15 (each 1H, m, H-2), 3.77 (1H, m, H-3), 2.01, 1.33 (each 1H, m, H-4), 3.35 (1H, m, H-5), 1.90, 1.80 (each 1H, m, H-6), 2.70 (2H, m, H-7), 6.70 (2H, s, H-2′ and H-6′), 6.84 (1H, d, *J* = 2.0 Hz, H-2″), 6.79 (1H, d, *J* = 2.0, 8.0 Hz, H-5″), 6.70 (1H, d, *J* = 8.0 Hz, H-6″), 3.84 (6H, s, H-3′ and H-5′), 3.78 (3H, s, H-4′), 3.75 (6H, s, H-3″ and H-4″). See Table 1 for the

$^{13}$C-NMR data ($CD_3OD$, 100 MHz). HRMS(ESI): *m/z* 455.2047 $[M+Na]^+$ (calcd. for $C_{24}H_{32}O_7Na$, 455.2046).

*Alpinin* ***B*** ***(2)***: yellowish oil. $[\alpha]_D^{25}$ +14.6° (*c* 0.22, MeOH); UV (MeOH, nm): $\lambda_{max}$ (log ε) 281 (2.11). IR (KBr, $cm^{-1}$): $\nu_{max}$ 3375, 2942, 2856, 2361, 2034, 1609, 1528, 1455, 1342, 1285, 1203, 1090, 991, 960, 800. See Table 1 for the $^1$H-NMR ($CD_3OD$, 400 MHz) and $^{13}$C-NMR ($CD_3OD$, 100 MHz) data. HRMS(ESI): *m/z* 401.1584 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_7Na$, 401.1576), *m/z* 379.1752 $[M+H]^+$ (calcd. for $C_{20}H_{27}O_7$, 379.1757).

(3*R*,5*R*)-3,5-dihydroxy-1-(3,4,5-trimethoxy-phenyl)-7-(3,4-dimethoxy-phenyl)-heptane (**2a**): amorphous powder. $[\alpha]_D^{25}$ +6.5° (*c* 0.10, MeOH). UV (MeOH, nm): $\lambda_{max}$ (log ε) 230 (2.66), 280 (2.25). IR (KBr, $cm^{-1}$): $\nu_{max}$ 3418, 3000, 2934, 2853, 1591, 1516, 1465, 1453, 1419, 1261, 1235, 1155, 1140, 1070, 1028, 807, 764. $^1$H NMR ($CD_3OD$, 400 MHz): $\delta$ 2.69 (2H, m, H-1), 1.75 (2H, m, H-2), 3.70 (1H, m, H-3), 1.55 (2H, m, H-4), 3.70 (1H, m, H-5), 1.75 (2H, m, H-6), 2.63 (2H, m, H-7), 6.50 (2H, s, H-2′, H-6′), 6.82 (1H, d, *J* = 2.0 Hz, H-2″), 6.79 (1H, d, *J* = 2.0, 8.0 Hz, H-5″), 6.70 (1H, d, *J* = 8.0 Hz, H-6″), 3.80 (6H, s, H-3′, H-5′), 3.78 (3H, s, H-4′), 3.74 (6H, s, H-3″, H-4″). See Table 1 for the $^{13}$C-NMR ($CD_3OD$, 100 MHz) data. HRMS(ESI): *m/z* 457.2198 $[M+Na]^+$ (calcd. for $C_{24}H_{34}O_7Na$, 457.2202).

**Total synthesis of *ent*-1.**

*(S)-1-(3',4'-Dibenzyloxyphenyl)hex-5-ene-3-ol (**23**)* [13]

Compound **22** (336 mg, 1.7 mmol) was added to a solution of (1*R*)-(−)-10-camphorsulfonic acid (CSA, 13 mg, 0.06 mmol) and aldehyde **21** (200 mg, 0.6 mmol) in $CH_2Cl_2$ (0.096 mL) under a nitrogen atmosphere. After stirring at room temperature for 5 days, the reaction mixture was diluted with 20 mL $CH_2Cl_2$, quenched with 10 mL of saturated $NaHCO_3$ solution, and extracted with $CH_2Cl_2$ (3 × 5 mL). The combined organic phase was washed with brine, dried over $Na_2SO_4$,

filtered, and concentrated under reduced pressure. The resultant residue was purified by silica gel chromatography (hexane:EtOAc = 5:1) to give compound **23** as a white solid (130 mg, 58%). [α] $^{25}_{D}$ −13.5° (*c* 1.0, MeOH) *ee* = 92.5 %, $t_R$ (*R*) = 20.8 min, $t_R$ (*S*) = 18.7 min, hexane:*i*-PrOH = 90:10. $^1$H-NMR ($CDCl_3$, 400 MHz) *δ:* 1.63 (s, 1H,OH), 1.68–1.74 (m, 2H), 2.15 (dt, *J* = 14.8 Hz, *J* = 8.0 Hz, 1H), 2.28 (dt, *J* = 14.0 Hz, *J* = 5.6 Hz, 1H), 2.60 (dt, *J* = 16.4 Hz, *J* = 8.4 Hz, 1H), 2.70 (dt, *J* = 14.0 Hz, *J* = 7.6 Hz, 1H), 3.55–3.63 (m, 1H), 5.10–5.20 (m, 6H), 5.73–5.90 (m, 1H), 6.72 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 6.81 (d, *J* = 1.6 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 7.27–7.49 (m, 10H). $^{13}$C-NMR ($CDCl_3$, 100 MHz,) *δ:* 31.5, 38.4, 42.1, 69.7, 71.3, 71.6, 115.4, 115.7, 118.3, 121.2, 127.36, 127.39, 127.74, 127.77, 128.5, 134.7, 135.5, 137.5, 137.6, 147.2, 148.9.

*(1S, 3R, 5S)-1,5-epoxy-3-acetoxy-1-(3,4-diacetoxy-5-methoxy-phenyl)-7-(3,4-dibenzyloxy-phenyl) heptane (**25**)*

$BF_3$-$OEt_2$ (0.077 mL, 0.62 mmol) was added to a stirred solution of aldehyde **24** (78 mg, 0.31 mmol), homoallylic alcohol **23** (120 mg, 0.31 mmol), TMSOAc (204 mg, 1.55 mmol), and AcOH (0.12 mL, 2.17 mmol) in dry $CH_2Cl_2$ (5 mL) at 0°C under a nitrogen atmosphere. The reaction mixture was warmed to room temperature and was subsequently stirred for 2 h. When the reaction was completed, the mixture was diluted with $CH_2Cl_2$ and quenched by saturated aqueous $NaHCO_3$ solution (3 mL). While the separated aqueous phase was extracted with $CH_2Cl_2$ (3 × 5 mL), the combined organic extracts were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (hexane:EtOAc = 5:1) to afford two colorless oils, **25** (80 mg, 38%) and its 3-fluoro derivative (62 mg, 31%).

$[\alpha]_D^{25}$ −15.8° (*c* 1.0, $CHCl_3$). IR (KBr, $cm^{-1}$): $\nu_{max}$ 2954, 2919, 2849, 1769, 1728, 1604, 1505, 1455, 1424, 1367, 1324, 1240, 1240, 1207, 1180, 1136, 1087, 1012, 891, 850, 750, 696. $^1$H-

NMR ($CDCl_3$, 400 MHz) *δ:* 1.38 (q, *J* = 11.6 Hz, 1H), 1.50 (q, *J* = 11.6 Hz, 1H), 1.66−1.79 (m, 1H), 1.85−1.95 (m, 1H), 1.95−2.02 (m, 1H), 2.04 (s, 3H), 2.20−2.27 (m, 1H), 2.28 (s, 3H), 2.30 (s, 3H), 2.56−2.76 (m, 2H), 3.37−3.49 (m, 1H), 3.83 (s, 3H), 4.32 (d, *J* = 11.2 Hz, 1H), 4.91−5.03 (m, 1H), 5.13 (d, *J* = 3.6 Hz, H), 6.65−6.75 (m, 1H), 6.75−6.85 (m, 2H), 6.85−6.90 (m, 2H), 7.22−7.39 (m, 6H), 7.39−7.50 (m, 4H). $^{13}$C-NMR ($CDCl_3$, 100 MHz) *δ:* 20.3, 20.6, 21.3, 31.1, 37.0, 37.5, 39.1, 56.3, 70.4, 71.3, 71.6, 74.5, 76.3, 107.3, 112.4, 115.4, 115.7, 121.2, 127.3, 127.4, 127.7, 128.4, 131.0, 135.3, 137.4, 137.6, 140.6, 143.2, 147.2, 148.9, 152.2, 167.9, 168.3, 170.5. HRMS(ESI): *m/z* 682.2781 $[M]^+$ (calcd. for $C_{40}H_{42}O_{10}$, 682.2778).

*(1S, 3R, 5S)-1,5-epoxy-3-hydroxy-1-(3,4-dihydroxy-5-methoxy-phenyl)-7-(3,4-dihydroxy-phenyl) heptane (ent-**1**)*

Concentrated HCl (0.2 mL) was added to a solution of **25** (80 mg, 0.12 mmol) in MeOH (2 mL) at room temperature, followed by stir mixing at room temperature for 12 h. The solvent was removed under vacuum, the crude product was then re-dissolved in MeOH (2 mL), and 10% Pd/C (10 mg) was carefully added. The mixture was then degassed and filled with nitrogen, degassed again, and refilled with hydrogen (1 atm). The reaction was stirred at room temperature for another 12 h. When the starting material was completely consumed, the reaction mixture was filtered through celite and the filtrate was concentrated under vacuum. The resultant residue was purified by silica column chromatography (hexane:EtOAc = 1:1) to afford *ent*-alpinin A (*ent*-**1**) as a white solid (32 mg, 73%). $[\alpha]_D^{25}$ –40.5° (*c* 1.0, MeOH). IR (KBr, $cm^{-1}$): $\nu_{max}$ 3364, 2922, 2852, 1632, 1526, 1462, 1365, 1288, 1237, 1203, 1077, 785, 651. $^1$H-NMR ($CDCl_3$, 400 MHz) *δ*: 1.23 (q, *J* = 11.6 Hz, 1H), 1.43 (q, *J* = 11.6 Hz, 1H), 1.65–1.80 (m, 1H), 1.80–1.92 (m, 1H), 1.93–2.02 (m, 1H), 2.04–2.14 (m, 1H), 2.48–2.70 (m, 2H), 3.40–3.50 (m, 1H), 3.77–3.85 (m, 1H), 3.86 (s, 3H), 4.22 (d, *J* = 11.2 Hz, 1H), 4.63 (br, 1H), 6.50–6.60 (m, 3H), 6.60–6.75 (m, 2H).

$^{13}$C-NMR ($CDCl_3$, 100 MHz) $\delta$: 30.6, 37.7, 40.4, 42.2, 55.3, 67.6, 74.9, 77.6, 101.5, 106.6, 114.9, 115.2, 119.3, 133.1, 133.2, 133.6, 142.8, 144.7, 144.9, 148.1.

**Activity Bioassay**. A filter trap assay was performed and aggregated α-synuclein was detected using western blot analysis as described previously with slight modification.[14] Recombinant human α-synuclein (GenWay Biotech., CA, USA) was diluted in 1× Tris-buffered saline (20 mM Tris, 500 mM NaCl, pH 7.5) and incubated with extracts or compounds at room temperature for 7 days. After incubation, the mixtures were loaded onto a Bio-Dot SF Microfiltration Apparatus (Bio-Rad, CA, USA) sandwiched with a 0.45 μm nitrocellulose membrane (Bio-Rad). After filtration and washing twice with Tris-buffered saline, the amount of trapped α-synuclein was determined by western blot analysis using an anti-α-synuclein antibody (1:2000, BD Bioscience, CA, USA), and an anti-mouse secondary antibody (1:1000, Cell Signaling Technology, MA, USA) and visualized by an ECL detection kit (GE Healthcare, Buckinghamshire, UK). The band intensity was quantified using ImageJ software (https://imagej.nih.gov/ij/features.html). DMSO and Congo red served as the solvent and positive control, respectively. We diluted the DMSO stock solution (0.02%) with Tris-buffered saline to obtain the working concentrations for the assay. Data are expressed as the mean ± SEM of three individual experiments (n = 3 per treatment group).

## RESULTS AND DISCUSSION

The molecular formula of Alpinin A (**1**, yellowish oil) is determined as $C_{20}H_{24}O_7$ based on positive-ion HRMS(ESI) spectrum, the pseudomolecular ion peaks at *m*/*z* 399.1447 for $[M+Na]^+$ and *m*/*z* 377.1607 for $[M+H]^+$. The IR spectrum of **1** displayed absorptions for hydroxyl (broad, 3359 $cm^{-1}$) and substituted benzene groups (1674, 1608 and 1527 $cm^{-1}$). In the $^1$H-NMR

spectrum (Table 1), five low-field resonances ($\delta_H$: 6.48–6.62 ppm) demonstrated the presence 1,3,4,5-tetrasubstituted benzene [$\delta_H$: 6.51 (1H, s, H-2') and 6.52 (1H, s, H-6')] and 1,3,4-trisubstituted benzene [$\delta_H$: 6.62 (1H, d, $J$ = 8.1 Hz, H-5"), 6.64 (1H, d, $J$ = 1.8 Hz, H-2"), and 6.48 (1H, dd, $J$ = 8.1, 1.8 Hz, H-6")], and four signals ($\delta_H$: 4.18, 3.78, 3.43, 3.81 ppm) suggested that there were four hydrogens on the carbons with oxygen substitution. The $^{13}$C-NMR and DEPT spectra (Table 1) revealed that this molecule consisted of 20 carbons, 12 of which were aromatic ($sp^2$ hybridized; $\delta_C$ 103–150 ppm) and 8 of which were aliphatic ($sp^3$ hybridized; $\delta_C$ 32–79 ppm). This is concordant with two aromatic rings [1,3,4,5-tetrasubstituted benzene; $\delta_C$ 134.5 (C-1'), 102.8 (C-2'), 149.4 (C-3'), 134.5 (C-4'), 146.3 (C-5'), 108.0 (C-6') ppm, as well as a 1,3,4-trisubstituted benzene: $\delta_C$ 135.0 (C-1"), 116.3 (C-2"), 146.1 (C-3"), 144.2 (C-4"), 116.6 (C-5"), 120.7 (C-6") ppm] and heptane moiety with three oxygenated carbons [$\delta_C$ 78.9 (C-1), 76.3 (C-3 with a hydroxyl group), and 69.0 (C-5) ppm] plus methoxy group ($\delta_C$ 56.7 ppm). Analysis of the DEPT and $^{13}$C-NMR spectra suggested one methoxy, four methylenes, eight methines including three oxymethines, and seven quaternary carbons, which are consistent with cyclic ether substituted with two aromatic rings as represented in compound **1**. The cyclic ether ring was proposed to be a tetrahydropyran (THP) on the basis of comprehensive analysis of $^1$H-$^1$H-COSY, HSQC, HMBC, and NOESY (NOE correlation between H-5 and H-1), which also allowed us to confirm one methoxy group at the aromatic ring (see *Supporting Information*). The spectral analysis results consistently indicated that alpinin A (**1**) is a THP-containing diarylheptanoid. A literature search also indicated that our proposed structure of **1** is similar to two known THP-containing diarylheptanoids 5-[4-hydroxy-6-(4-hydroxyphenethyl)-tetrahydro-2*H*-pyran-2-yl]-3-methoxybenzene-1,2-diol isolated from the rhizomes of *Zingiber officinale*[15] and 1,5-epoxy-3-hydroxy-1-(3,4-dihydroxy-5-methoxy-phenyl)-7-(3,4-dihydroxy-

phenyl)heptane.[16] Comparison of the NMR data of these three diarylheptanoids revealed obvious differences at either the aromatic or THP region, which indicate that **1** is a new member of THP-containing diarylheptanoids; its relative structure of **1** was assigned as shown in Figure 1. However, its absolute configuration could not be established unambiguously with Mosher's ester method, because contrary shielding effects were observed for the protons on the same side. Therefore, in order to fully establish the relative and absolute configurations of alpinin A and provide sustainable expedited access to the THP-containing diarylheptanoids, we initiated the asymmetric total synthesis of alpinin A (Scheme 1).

The synthesis of *ent*-**1** commenced with asymmetric allylation of aldehyde **21**[13] by using Loh's allyl transfer method,[17] which provided the homoallylic alcohol **23** with a yield of 58% and 92.5% ee. The Prins cyclization of homoallylic alcohol **23** and the known aldehyde **24**[18] afforded tetrahydropyran **25** with a yield of 38%, along with a 3-fluoro derivative (31%).[18] The 2-step global deprotection (deacetylation with HCl/MeOH and Pd/C-catalyzed benzylation) was carried out to furnish alpinin A with a 73% overall yield for two steps. The NMR and MS spectroscopic data of our synthetic material were identical to those derived from natural alpinin A isolated from *A. officinarum*, which confirmed the molecular structure of our proposed compound **1**. However, the opposite sign of the specific rotation of the synthetic and natural materials [synthetic: $[\alpha]_D^{25}$ −40.0° (*c* 1.0, MeOH), natural: $[\alpha]_D^{25}$ +51.1° (*c* 0.42, MeOH)] indicated that the synthetic alpinin A (*ent*-**1**) was the enantiomer of **1**, which in turn confirmed the absolute configuration of the natural (+)-alpinin A. The total synthesis was not only a conclusive and powerful way to confirm the relative and absolute configurations of alpinin A, but also provided efficient (only 4 steps) and expedited access to the alpinin A and related THP-containing diarylheptanoids.

The molecular formula of Alpinin B (**2**, yellowish oil) is determined as $C_{20}H_{26}O_7$ based on HRMS(ESI) spectrum, pseudomolecular ion peaks *m*/z at 379.1751 for $[M+H]^+$ and *m*/*z* at 401.1558 for $[M+Na]^+$. The carbon number of this formula was verified by the $^{13}$C-NMR spectrum. Similar to the IR spectrum of **1**, the IR spectrum of **2** also indicated the presence of hydroxyl (3376 $cm^{-1}$) and substituted benzene (1609 and 1527 $cm^{-1}$) groups, which was consistent with a maximum absorption in the UV spectrum (281.4 nm). Careful analysis of its $^1$H- and $^{13}$C-NMR data indicated that the compound has the same 1,3,4,5-tetrasubstituted and 1,3,4-trisubstituted aromatic rings as compound **1**. Additionally, seven carbons of the heptane-3,5-diol moiety were also observed at $\delta_C$ 32.9 (C-1), 41.4 (C-2), 68.7 (C-3), 45.6 (C-4), 68.7 (C-5), 41.3 (C-6) and 32.4 (C-7) in the $^{13}$C-NMR spectrum, and was further confirmed by the HSQC and $^1$H-$^1$H COSY spectral data. Furthermore, the $sp^3$-hybridized aliphatic hydrogen and carbon resonances in the NMR spectra were nearly identical to those of the previously reported linear diarylheptanoid derived from the rhizomes of *Tacca chantrieri*.[19] However, these two compounds differ mainly with respect to the chemical shifts derived from their aromatic rings. Compound **2** resonates at $\delta_C$ 104.8 (C-2'), 149.5 (C-3'), 132.9 (C-4'), 146.3 (C-5'), 109.8 (C-6'), and $\delta_H$ 6.32. 6.33 (each 1H, s, H-2', 6'), while the known diarylheptanoid has the corresponding signals at $\delta_C$ 116.5 (C-2'), 146.0 (C-3'), 144.1 (C-4'), 116.2 (C-5'), 120.6 (C-6'), and $\delta_H$ 6.65 (1H, d, $J$ = 8.1 Hz, H-5'), 6.62 (1H, d, $J$ = 2.0 Hz, H-2'), 6.50 (1H, dd, $J$ = 8.1, 2.0 Hz, H-6').[19] This indicates that C-3' was substituted by a methoxy group, whose assignment was unequivocally substantiated by long-range correlation (HMBC) analysis: protons of the methoxy group at $\delta_H$ 3.81 were correlated with carbon C-3' at $\delta_C$ 149.5. The HMBC correlations between $\delta_H$ 6.32 (H-2') to $\delta_C$ 32.9 (C-1) as well as between $\delta_H$ 6.63 (H-2") and $\delta_H$ 6.51 (H-6") to $\delta_C$ 32.4 (C-7), indicated that the two benzene rings were located at two ends of the heptane chain. Based on the

above evidence, the planar structure of **2** was proposed to be a linear diarylheptanoid (Figure 1) and named alpinin B.

In order to determine the absolute configuration of alpinin B (**2**), the two hydroxyl groups were derivatized to *p*-bromobenzoate for the CD spectrum (using the CD exciton chirality method).[16] First, the phenolic hydroxyl groups of 4'-OH, 5'-OH, 3''-OH and 4''-OH were alkylated by iodomethane to provide the corresponding tetramethyl ether (**2a)**; then, the compound was reacted with *p*-bromobenzoyl chloride to give the dibenzoate **2b**. The CD spectrum of **2b** exhibited positive (238.5 nm, $\Delta\varepsilon$ +15.1) and negative (253.0 nm, $\Delta\varepsilon$ -13.4) Cotton effects, which confirmed the absolute configuration as 3*R* and 5*R*.[19,20] Therefore, the absolute configuration of **2** was proposed to be (3*R*,5*R*)-alpinin B.

The structures of compounds **3**–**20** were elucidated on the basis of careful comparison of the NMR data with those in the literature, and their molecular structures are shown as **3–5**,[19] **6**,[21] **7–8**,[19] **9**,[22] **10**,[23] **11**,[24] **12**,[5] **13**,[25] **14**,[26] **15**,[27] **16**,[28] **17–18**,[24] **19**,[29] and **20**[26] in Figure 1.

The inhibitory effects of compounds **1–10**, **12–18** and **20** on α-synuclein aggregation were evaluated (Table 2). A filter trap assay was performed, and aggregated α-synuclein was detected by western blot analysis. Compounds **1–3** and **5–7**, which featured the presence of 3,4-dihydroxy substitution on the benzene ring, exhibited the most potent inhibitory activity against α-synuclein aggregation, which suggests that the presence of the 3,4-dihydroxy group on the benzene ring is crucial to their inhibitory activity. In addition, when comparing the inhibitory activity of compounds **3-4**, with that of **6-8**, the more phenolic hydroxyl groups on the benzene exhibited a greater activity, which suggests that the inhibition of α-synuclein aggregation is strongly correlated with the number of hydroxyl groups on the aromatic ring. Furthermore, **1a**, a tetramethylated analogue of compound **1**, showed no inhibitory activity, further supporting the

355 importance of hydroxyl groups in the observed inhibition. Finally, the results show that the
356 methoxy substitution on C-3 of the benzene ring is not essential for inhibitory activity, because
357 no significant activity difference was observed for compounds **4** and **8** as well as compounds **2**
358 and **5**.

359

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367 **Notes**

368 The authors declare no competing financial interests.

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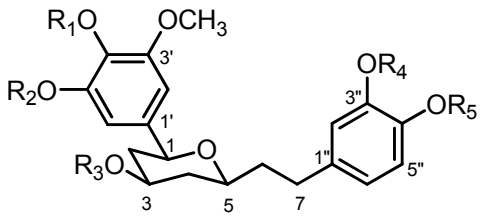


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| No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | H | H | H | H | H |
| **1a** | $CH_3$ | $CH_3$ | H | $CH_3$ | $CH_3$ |

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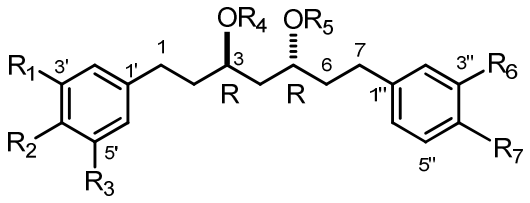


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| No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ |
|---|---|---|---|---|---|---|---|
| **2** | $OCH_3$ | OH | OH | H | H | OH | OH |
| **2a** | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | H | $OCH_3$ | $OCH_3$ |
| **2b** | $OCH_3$ | $OCH_3$ | $OCH_3$ | *p*-Br-Bz | *p*-Br-Bz | $OCH_3$ | $OCH_3$ |
| **3** | $OCH_3$ | OH | H | H | H | OH | OH |
| **4** | $OCH_3$ | OH | H | H | H | H | OH |
| **5** | OH | OH | H | H | H | OH | OH |
| **6** | $OCH_3$ | OH | OH | H | H | H | OH |
| **7** | OH | OH | H | H | H | H | OH |
| **8** | H | OH | H | H | H | H | OH |

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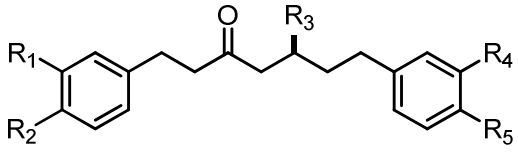


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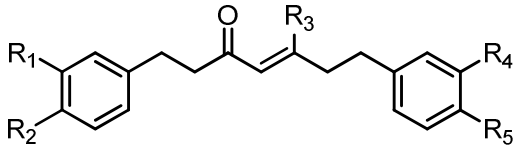
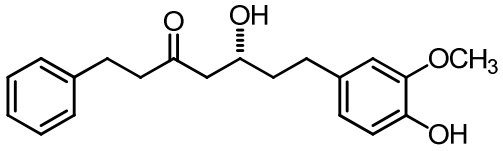
| No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **9** | $OCH_3$ | OH | OH | OH | OH |
| **10** | H | OH | OH | H | OH |
| **12** | H | H | OH | H | OH |
| **13** | H | H | OH | H | H |
| **14** | H | H | $OCH_3$ | H | H |




| No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **15** | $OCH_3$ | OH | H | OH | OH |
| **16** | OH | OH | H | H | OH |
| **17** | H | H | H | H | OH |
| **18** | H | H | H | H | H |
| **20** | H | H | OH | H | H |

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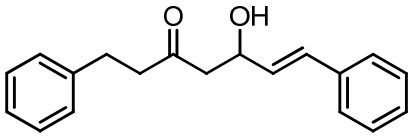


**19**

**Figure 1** Chemical structure of natural diarylheptanoids **1–20** and their derivatives **1a**, **2a**, **2b**.

**Scheme 1** The enantioselective synthesis of proposed structure of compound **1**

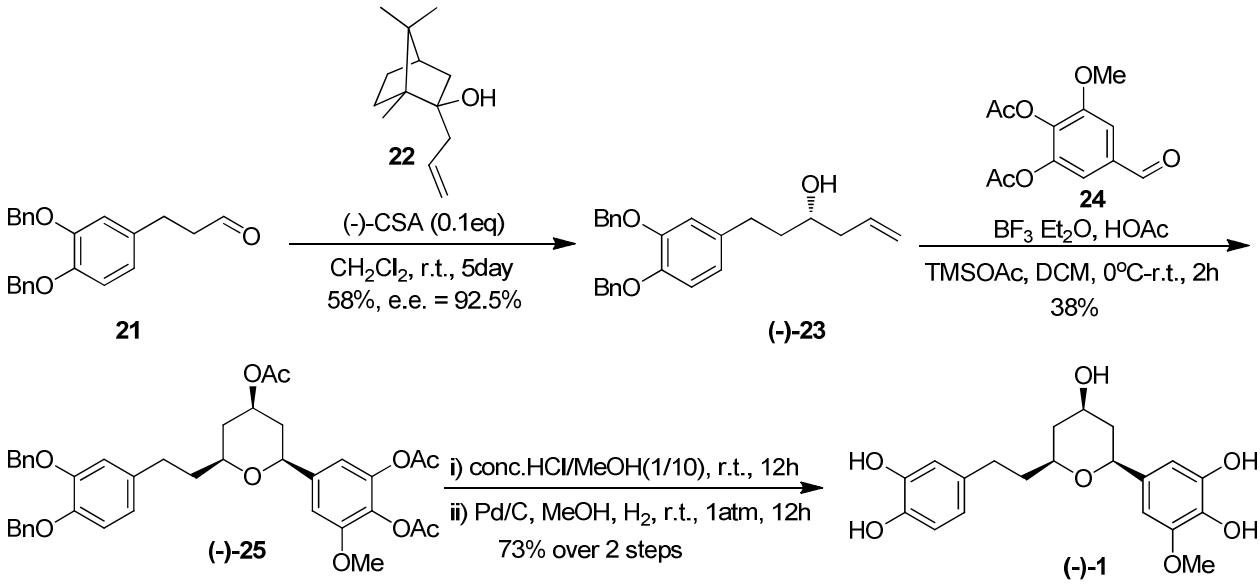

**Table 1.** NMR Data for Compounds **1**–**2, 1a** and **2a** [400 MHz ($^1$H NMR) and 100 MHz ($^{13}$C NMR) in $CD_3OD$]

| Position | 1 | | 1a | 2 | | 2a |
|---|---|---|---|---|---|---|
| | $\delta_H$ ($J$ in Hz) | $\delta_C$ type | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ type | $\delta_C$ |
| 1 | 4.18, brd (11.1) | 78.9 CH | 79.0 | 2.59, m | 32.9 $CH_2$ | 33.4 |
| 2 | 2.08, m | 39.1 $CH_2$ | 39.0 | 1.68, m | 41.4 $CH_2$ | 41.2 |
| | 1.41, dd (5.80, 11.7) | | | | | |
| 3 | 3.78, m | 76.3 CH | 76.3 | 3.84, m | 68.7 CH | 68.6 |
| 4 | 1.95, m | 43.6 $CH_2$ | 44.0 | 1.54, m | 45.6 $CH_2$ | 45.7 |
| | 1.21, m | | | | | |
| 5 | 3.43, m | 69.0 CH | 68.9 | 3.84, m | 68.7 CH | 68.5 |
| 6 | 1.84, m | 41.8 $CH_2$ | 41.9 | 1.68, m | 41.3 $CH_2$ | 41.1 |
| | 1.75, m | | | | | |
| 7 | 2.60, m | 32.0 $CH_2$ | 32.4 | 2.52, m | 32.4 $CH_2$ | 32.6 |
| 1′ | | 134.5 C | 136.4 | | 134.5 C | 136.6 |
| 2′ | 6.51, s | 102.8 CH | 104.4 | 6.32, s | 104.8 CH | 106.7 |
| 3′ | | 149.4 C | 154.4 | | 149.5 C | 154.3 |
| 4′ | | 134.5 C | 140.1 | | 132.9 C | 139.9 |
| 5′ | | 146.3 C | 154.4 | | 146.3 C | 154.3 |
| 6′ | 6.52, s | 108.0 CH | 104.4 | 6.33, s | 109.8 CH | 106.7 |
| 1″ | | 135.0 C | 138.2 | | 135.3 C | 136.7 |
| 2″ | 6.64, d (2.0) | 116.3 CH | 113.6 | 6.63, d (2.0) | 116.5 CH | 113.5 |
| 3″ | | 146.1 C | 150.3 | | 146.0 C | 150.3 |
| 4″ | | 144.2 C | 148.6 | | 144.1 C | 148.5 |
| 5″ | 6.62, d (8.0) | 116.6 CH | 113.2 | 6.66, d (8.0) | 116.2 CH | 113.1 |
| 6″ | 6.48, dd (2.0, 8.0) | 120.8 CH | 121.6 | 6.51 dd (2.0, 8.0) | 120.6 CH | 121.6 |
| 3′-$OCH_3$ | 3.81, s | 56.6 $CH_3$ | 56.6 | 3.81, s | 56.5 $CH_3$ | 56.6 |
| 4′-$OCH_3$ | | | 61.2 | | | 61.1 |
| 5′-$OCH_3$ | | | 56.6 | | | 56.6 |
| 3″-$OCH_3$ | | | 56.5 | | | 56.5 |

| | | |
|---|---|---|
| 4″-$OCH_3$ | 56.4 | 56.4 |

**Table 2**. Effects of the isolated compounds in inhibiting alpha-synuclein aggregation

| Compound | Inhibition (%) | Compound | Inhibition (%) | Compound | Inhibition (%) |
|---|---|---|---|---|---|
| **1** | 66.1±4.4 | **8** | 32.2±11.7 | **16** | 0.0 |
| **2** | 67.3±6.2 | **9** | 0.0 | **17** | 0.0 |
| **3** | 72.4±4.4 | **10** | 10.6±3.8 | **18** | 40.4±17.2 |
| **4** | 16.6±10.6 | **12** | 40.3±14.9 | **20** | 30.3±5.5 |
| **5** | 65.9±9.8 | **13** | 20.1±14.6 | **DMSO** | 0.0 |
| **6** | 76.0±3.5 | **14** | 0.0 | **Congo red**[a] | 64.5±7.6 |
| **7** | 61.3±6.3 | **15** | 59.1±11.2 | | |

[a]Congo red and the isolated compounds at the concentration of 10 μM (Inhibition percentage compared to the control DMSO)

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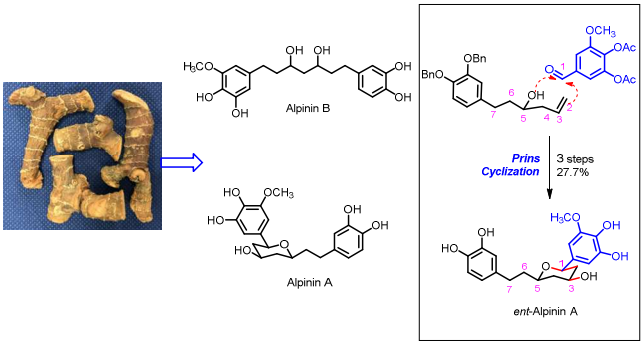
TOC Graphic

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