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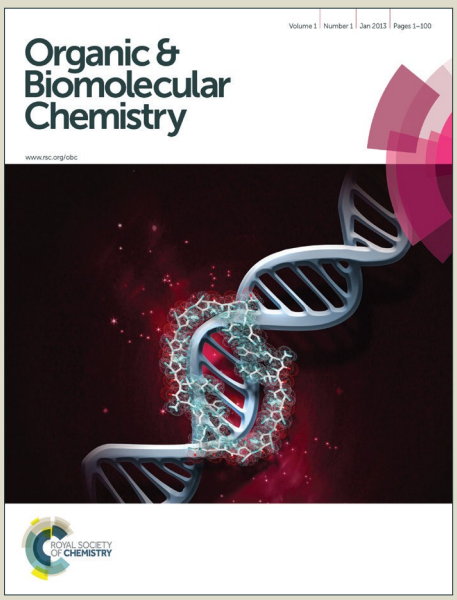

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PAPER

Antimalarial diterpenoid dimers of a new carbon skeleton from *Aphanamixis grandifolia*†

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Hua Zhang,[a,‡] Jia Liu,[a,‡] Li-She Gan,[b] Seema Dalal,[c] Maria B. Cassera[c] and Jian-Min Yue[a,*]

Chemical investigation into the minor constituents of *Aphanamixis grandifolia* yielded three new diterpenoid dimers, aphadilactones E–G (**1**–**3**) featuring a new carbon skeleton. Their structures and absolute configurations were fully established by comprehensive spectroscopic data analysis and ECD calculation. Discovery of another two new dimers (**4** and **5**) suggested the structure of recently reported aphanamene A to be re-investigated. Compounds **1**–**5** showed moderate antimalarial activities with low mocromolar IC_{50} values.

Introduction

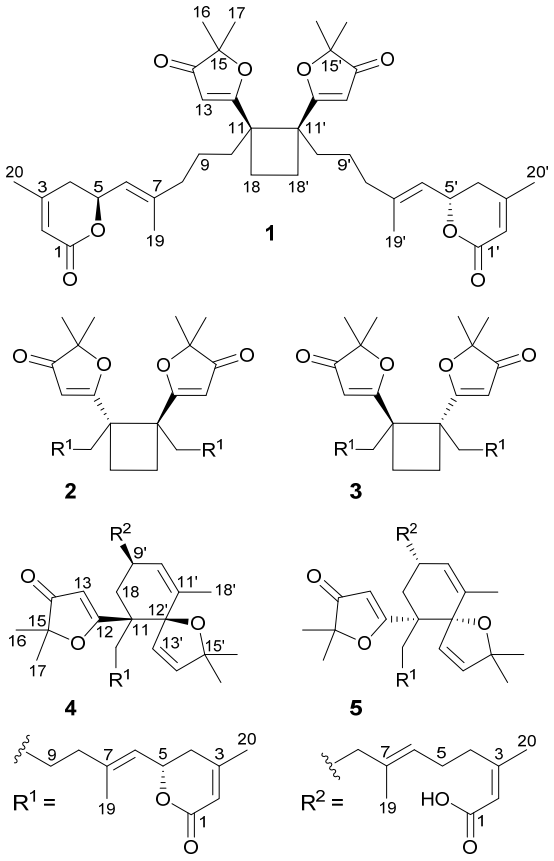
The genus *Aphanamixis* of Meliaceae family consists of *ca.* 25 species mainly growing in the tropical areas of Asia, and four *Aphanamixis* plants are distributed in the southern provinces of China such as Guangdong and Guangxi.[1] *A. grandifolia* is an oil plant with economic values in China, and its leaves and roots are also used by local residents as a folk medicine for the treatment of rheumatism and ache.[2] Although the first report on the secondary metabolites of *A. grandifolia* could be tracked back to 1978,[3] it was not until 2010[4-5] that natural product chemists started intensive investigation on this species. The predominant isolates from *A. grandifolia* were triterpenoids[4-6] and limonoids,[3,7] of which the former displayed insecticidal,[6e] antibacterial,[6j] anti-inflammatory,[8] and mainly cytotoxic[4-5,6c,6f-h] activities, while the latter also showed insecticidal[6g,7e] and cytotoxic[7a,7d] activities. Another interesting class of metabolites of this plant were a series of acyclic diterpenoids[6g,9] that had been discovered only since 2011.[9a] Albeit a small structure family, these diterpenes were reported to possess diverse biological properties such as antimalarial[9a] and antimicrobial[9c] activities. Recently, four novel dimers of the above-mentioned diterpene type, aphadilactones A–D, were obtained from *A. grandifolia* by our research group.[10] These dimers exhibited strong antimalarial and DGAT inhibitory activities which had immediately attracted attention from medicinal chemists with the total synthesis of aphadilactones A–D being reported by Yin et al. immediately after the publication of natural ones.[11] As an extension to that previous project, we have continued and accomplished the chemical investigation of this herb, and report herein a further five new dimeric members of this diterpenoid family, aphadilactones E–I (**1**–**5**). Dimers **1**–**3** bore a new carbon skeleton incorporating a 1,1,2,2-tetrasubstituted cyclobutane moiety. More excitingly, compounds **1**–**5** exhibited moderate antimalarial activities with low micromolar IC_{50} values. Details of the isolation, structure elucidation, and biological testing of these fascinating molecules are presented below.

Results and discussion

In an earlier study into the EtOAc partition from the EtOH extract of *A. grandifolia*, four new dimeric compounds, aphadilactones A–D, were isolated and identified as the major diterpenoid constituents.[10] Simultaneously detected in that previous investigation were a series of minor cometabolites that were further purified by normal and especially chiral HPLC to afford five new diterpenoid dimers, aphadilactones E–I (**1**–**5**). Absolute structures were assigned to **1**–**3** based on spectroscopic data, configurational analysis, biogenetic consideration and ECD calculation, while those of **4** and **5** were characterized via more methods including chemical degradation and fragment synthesis.

Compounds **1**–**3** were assigned the same molecular formula of $C_{40}H_{52}O_8$ by (+)-HRESIMS analysis ($[M + Na]^+$, Δmmu –0.6, –0.7, and 0.6 for **1**–**3**, respectively) and ^{13}C NMR data (Table 1), indicative of their isomeric nature. The IR spectra also showed the presence of lactone carbonyls (*ca.* 1700 and 1720 cm^{-1}) as their cometabolites aphadilactones A–D.[10] The NMR data (Table 1) of **1** exhibited 26 carbon resonances with 12 ones appearing in pairs [C-3(3′), C-5(5′), C-6(6′), C-7(7′), C-10(10′) and C-19(19′)], while those of **2** and **3** only revealed 20 carbons, all being comparable to the data of aphadilactones A–D.[10] The

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China. E-mail: jmyue@simm.ac.cn
[b] Institute of Modern Chinese Medicine, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, P. R. China
[c] Department of Biochemistry and the Virginia Tech Center for Drug Discovery, MC 0308, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, United States
‡ Equal contribution
† Electronic Supplementary Information (ESI) available: ECD calculations and full spectroscopic data including 1D & 2D NMR, IR and MS spectra for both natural and synthetic compounds. See DOI: 10.1039/x0xx00000x



aforementioned observations suggested that compounds **1**–**3** were also dimers likely derived from a common monomeric precursor as aphadilactones A–D.[10]

Analysis of ^{1}H–^{1}H COSY data (Fig. 1), taking **1** as an example, revealed spin systems of H_2-4(4′) to H-6(6′) and H_2-8(8′) to H_2-10(10′) that were connected via C-7(7′) by the HMBC correlations (Fig. 1) from H_3-19(19′) to C-6(6′), C-7(7′) and C-8(8′). The α,β-unsaturated δ-lactone moiety was established on the basis of the chemical shifts for C-1(1′) (δ_C 168.0) and C-5(5′) (δ_C 75.95/75.90),[10] together with the HMBC correlations from H-2(2′) to C-1(1′) and from H_3-20(20′) to C-2(2′), C-3(3′) and C-4(4′). In addition, the presence of a 2,2-dimethylfuran-3(2H)-one fragment was supported by the chemical shifts for C-12(12′) to C-15(15′) at δ_C 195.0, 103.7, 209.5 and 90.4, respectively,[10] as well as the HMBC correlations from H-16(16′)/17(17′) to C-14(14′) and C-15(15′) and from H-13(13′) to C-12(12′) and C-14(14′). The monomeric unit of **1** was then constructed by the HMBC correlations from H_2-10(10′) to C-11(11′), C-12(12′) and C-18(18′), being identical with that in aphadilactone A[10]. The above-mentioned analyses accounted for 14 unsaturation degrees of the 15 ones represented by the molecular formula of **1** with the remaining one requiring the existence of an extra ring, which suggested the formation of a four-membered ring linking the two monomeric fragments A and A′ (Fig. 1). The planar structure of **1**, which was likely a head-to-head and tail-to-tail [2+2]-cycloaddition adduct of two identical monomers, was finally confirmed as shown (Fig. 1) by the coupling patterns (multiplet peaks) of H_2-18(18′). Similarly, the planar structures of **2** and **3** were established to be the

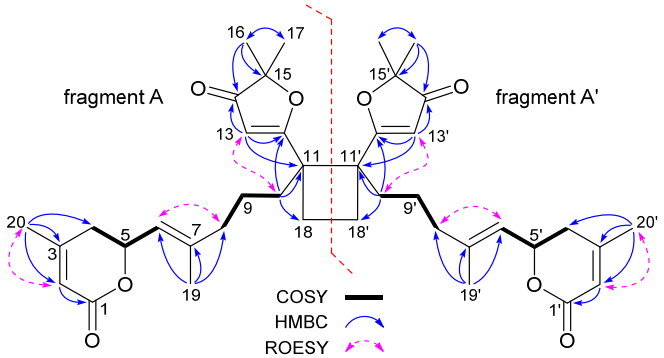


Fig. 1 Planar structure of **1** established by 2D NMR data.

same as that of **1**.

As with their cometabolites aphadilactones A–D,[10] both C-5 and C-5′ of **1**–**3** were assigned *S*-configurations from a biogenetic consideration, which is also the case for all monomeric members with an α,β-unsaturated δ-lactone fragment of this family of diterpenoids.[6g,9b,9c] The other two chiral centers of C-11 and C-11′ leads to four stereoisomers for this planar structure, whereas the number is reduced to three considering that two isomers with (11*R*, 11′*S*) and (11*S*, 11′*R*) configurations actually represent one compound. Therefore, the remaining three possible structures were assignable to **1**–**3**, respectively. Compound **1** was first distinguished from **2** and **3** based on the fact that **1** and a mixture of the latter two diastereoisomers were separated by routine achiral HPLC fractionation, while **2** and **3** could only be purified from each other via chiral HPLC. Indeed, the structural distinction and their different performance on normal and chiral HPLC columns of **1**–**3** were also reflected by their NMR data. The NMR signals for the fragments of CH_2-10(10′) to CH-13(13′) and CH_2-18(18′) nearby C-11(11′) in **1** were obviously differentiated from those in **2** and **3** (Table 1), while the corresponding data between **2** and **3** were almost identical [$\Delta\delta_C$ <0.15; $\Delta\delta_H$ <0.02 except for H_2-10(10′)]. The identification of **2** and **3** was very challenging owing to their incredibly close spectroscopic data, and actually in most cases they could have been identified as one pure isolate without proper chiral HPLC analysis. To eventually identify **2** and **3**, their experimental ECD spectra were acquired and compared with the theoretical data calculated by TDDFT computational chemistry method (for details, see ESI†). As shown in Fig. 2, the calculated ECD spectrum of the isomer with (11*S*, 11′*S*) configuration matched

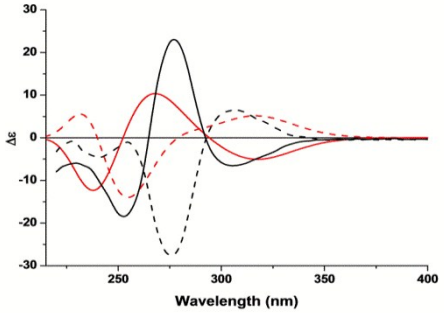


Fig. 2 Experimental (black color) and calculated (red color) ECD spectra for compounds **2** (solid line) and **3** (dashed line).

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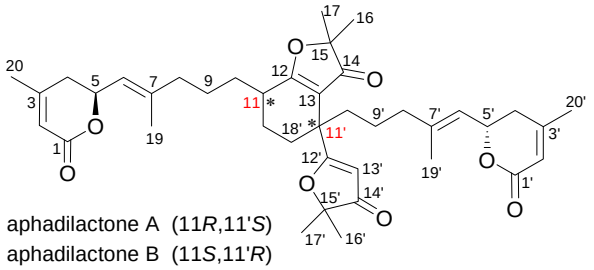
Table 1 NMR data for compounds **1**–**5** in CD_3OD

	1		**2**		**3**	
Position	δ_C	δ_H (multi., *J* in Hz)	δ_C	δ_H (multi., *J* in Hz)	δ_C	δ_H (multi., *J* in Hz)
1/1′	168.0		168.0		167.9	
2/2′	116.5	5.79 (m)	116.5	5.79 (m)	116.4	5.78 (m)
3/3′	161.2/161.1		161.1		161.2	
4/4′	35.7	2.35 (ddd, 18.2, 4.6, 2.4)	35.8	2.30 (dd, 18.1, 4.8)	35.8	2.29 (dd, 18.1, 4.7)
		2.42 (m)		2.37 (brdd, 18.1, 10.2)		2.37 (m)
5/5′	75.95/75.90	5.23 (ddd, 10.5, 8.5, 4.6)	75.7	5.17 (ddd, 10.2, 8.4, 4.8)	75.8	5.17 (ddd, 10.7, 8.4, 4.7)
6/6′	124.2/124.0	5.39 (brd, 8.5)	124.2	5.29 (brd, 8.4)	124.2	5.29 (brd, 8.4)
7/7′	143.5/143.3		143.5		143.1	
8/8′	40.3	2.14 (m, 2H)	40.2	2.08 (m, 2H)	40.2	2.07 (m, 2H)
9/9′	22.9	1.20 (m)	22.6	1.16 (m)	22.7	1.14 (m)
		1.39 (m)		1.44 (m)		1.44 (m)
10/10′	33.1/33.0	1.92 (m, 2H)	33.9	1.67 (m)	34.0	1.64 (m)
				1.77 (m)		1.83 (m)
11/11′	53.4		52.1		52.2	
12/12′	195.0		194.2		194.3	
13/13′	103.7	5.54 (s)	104.1	5.65 (s)	104.3	5.67 (s)
14/14′	209.5		209.5		209.6	
15/15′	90.4		90.5		90.7	
16/16′	23.5	1.30 (s)	23.4	1.38 (s)	23.5	1.38 (s)
17/17′	23.3	1.31 (s)	23.3	1.41 (s)	23.2	1.43 (s)
18/18′	26.1	2.06 (m)	24.4	1.96 (m)	24.6	1.98 (m)
		2.48 (m)		2.54 (m)		2.52 (m)
19/19′	16.6/16.5	1.73 (d, 1.2)/1.71 (d, 1.1)	16.4	1.69 (d, 1.3)	16.4	1.68 (d, 1.3)
20/20′	23.0	2.02 (brs)	23.0	2.03 (brs)	23.0	2.03 (brs)

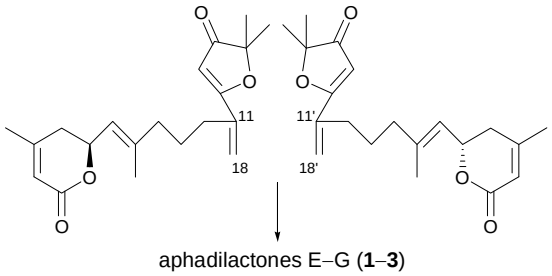
well with the experimental one of **2** except for a slight blue shift. Although not as good as the case of **2**, the curve trend of the calculated ECD spectrum of the isomer with (11*R*, 11′*R*) configuration was also consistent with the experimental one of **3**. The absolute structures of **1**–**3** were thus characterized featuring a new dimeric diterpenoid scaffold with a cyclobutane ring, and they were named aphadilactones E–G after the previously reported aphadilactones A–D.[10]

As with aphadilactones A–D (Fig 3),[10] compounds **1**–**3** should also be biosynthesized from the same monomeric intermediate (Scheme 1). This intermediate product could have been derived via dehydration of nemoralisins C[6g] or J[9b] found in the same species, and subsequent [2+2]-cycloaddition reaction in a way of head-to-head and tail-to-tail would yield the three diastereoisomeric dimers. Compared to **1**–**3**, the six previously reported dimers are all derived in a [4+2] way via



aphadilactone A (11*R*,11′*S*)
aphadilactone B (11*S*,11′*R*)
aphadilactone C (11*S*,11′*S*)
aphadilactone D (11*R*,11′R

Fig. 3 Structures of aphadilactones A–D.



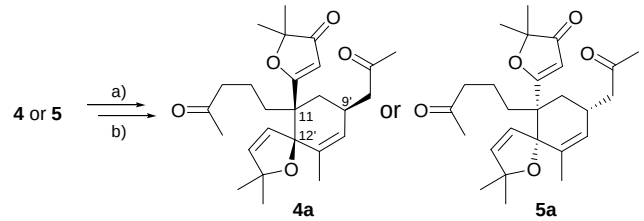
Scheme 1 Proposed biosynthetic pathway for dimers **1**–**3**.

Diels-Alder reaction and bear different backbones.[10,12] Given the rich double bond functionalities and their diverse regiochemistry in the monomeric precursors, formation of extra dimers with different ring systems is also possible.

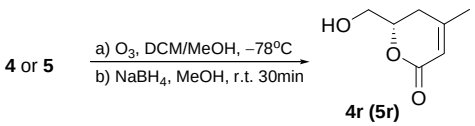
In addition to the three aforementioned compounds, chiral HPLC separation also yielded another two dimeric diastereoisomers (**4** and **5**). The absolute structures of **4** and **5** were characterized on the basis of NMR data particularly chemical degradation, fragment synthesis and calculated ECD data for their degradative products.

In brief, analysis of 2D NMR COSY and HMBC data (Fig. S31–S33 & S40–S42, ESI†) established their planar structures to be identical, and further examination of the NOESY spectra (Fig. S55 & S66, ESI†) of their degradative products (**4b**/**5b**) (Scheme S1, ESI†) confirmed that their cyclohexene core possessed the same relative configuration. Moreover, oxidative degradation of **4**/**5** also returned **4a**/**5a** (Scheme 2) that retained the original chiral centers and were identified to be a pair of enantiomers as supported by their identical NMR data but reversed $[\alpha]_D$ and ECD data. The two enantiomers were differentiated from each other by comparison of their experimental ECD spectra to the calculated ones (Fig. S4, ESI†). Finally, 5*S* configuration was assigned for both compounds by comparison of chiral HPLC chromatograms (Fig. S2, ESI†) of their reductive ozonolysis products (**4r**/**5r**, Scheme 3) to authentic synthetic samples. The structures of **4** and **5** with absolute configurations were thus characterized.

On reviewing the literature, compound **4** was found to have exactly the same structure as aphanamene A reported in



Scheme 2 Oxidative degradation of **4**/**5** [Reaction condition: a) 40 mol % $K_2OsO_4{\cdot}2H_2O$, 40 eq. $MeSO_2NH_2$, 120 eq. $K_3Fe(CN)_6$, 120 eq. K_2CO_3, ^{t}BuOH-H_2O (1:1), r.t.; b) $Pb(OAc)_4$, DCM, 0 °C].



Scheme 3 Reductive ozonolysis of **4**/**5**.

2013[12] and its NMR data also matched pretty well with those of the latter. However, the $[\alpha]_D$ and ECD data of both **4** and **5** were quite different from those of aphanamene A, plus the absolute configuration (5*S*,11*S*,9′*R*,12′*R*) of aphanamene A described in the text of that report did not match the drawn structure (5*S*,11*R*,9′*R*,12′*R*) in the figure. Considering the existence of highly similar diastereoisomers of this structure family and the necessity of chiral purification, the structure of aphanamene A and its physicochemical data should be re-checked.

All natural isolates were evaluated for their antimalarial activities with artemisinin as positive control as previously reported.[10] Compounds **1**–**5** exhibited remarkable to moderate inhibitory activities with IC_{50} values of 1.03±0.13, 2.86±0.47, ~20, 1.60±0.26, and 2.11±0.44 μM, respectively.

Conclusions

In summary, our further exploration of the minor chemical ingredients from *A. grandifolia* returned five new dimeric members of this rare diterpenoid family, and our bioassays have demonstrated them as mild antimalarial agents. The case of aphadilactones also indicates that many natural products may not be as optically pure as one has initially expected due to constraint of proper working conditions. As Prof. Williams has demonstrated in a recent review, even formation of enantiomeric natural products is not that uncommon.[13] Therefore, natural products chemists need to think and work 'more chirally' in their future researches.

Experimental

General

Optical rotations were measured on a Rudolph Autopol VI automatic polarimeter. UV spectra were acquired on a Shimadzu UV-2550 UV/visible spectrophotometer. IR spectra were obtained on a Perkin-Elmer 577 spectrometer. NMR experiments were performed on a Bruker Avance III 500 spectrometer and referenced to solvent peaks (δ_H 3.31 and δ_C 49.00 for CD_3OD). LR- and HR-ESIMS were carried out on a Bruker Daltonics esquire3000plus and a Waters LCT Premier XE spectrometers, respectively. Pre-coated silica gel GF254 plates (Yantai Huiyou Silica Gel Exploitation Company, Ltd., China) were used for TLC analyses. Silica gel H (300–400 mesh, Qingdao Haiyang Chemical Plant, Ltd., China), reversed-phase C18 silica gel (150–200 mesh, Merck, U.S.A.), and CHP20P MCI gel (75–150 μm, Mitsubishi Chemical Industries, Ltd., Japan) were used for column chromatography (CC). HPLC purifications were carried out on a Waters 1525 binary pump system equipped with a 2489 UV/visible detector and a YMC-Pack ODS-A (250×10 mm, S-5 μm) or a Daicel CHIRALPAK AD-H (250×10 mm, S-5 μm) columns. All solvents used for CC were of at least analytical grade (Shanghai Chemical Reagents Company, Ltd., China), and solvents used for UV, [α]D, and

NMR measurements were of suitable chromatographic grades from Merck or Sigma-Aldrich.

Plant material

The leaves of *Aphanamixis grandifolia* Bl. were collected in May 2010 from Sanya of Hainan Island, P. R. China, and were identified by Prof. Shi-Man Huang from Department of Biology, Hainan University. A voucher specimen has been deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, P. R. China (accession number: AP-2010-2Y).

Extraction and isolation

The air-dried powder of the leaves of *A. grandifolia* (5 kg) was extracted three times with 95% ethanol at room temperature to return a crude extract (350 g), which was then partitioned between EtOAc and water to yield an EtOAc-soluble fraction E (150 g). Fraction E was subjected to a column of MCI gel (MeOH-H_2O, 1:1 to 9:1) to give four fractions E1–E4, and fraction E2 (16.6 g) was further fractionated by silica gel H CC (petroleum ether-acetone, 10:1 to 1:1) to furnish six subfractions E2a–E2f. Fraction E2f (4.4 g) was fractionated on a reversed-phase C18 silica gel column (MeOH-H2O, 1:1 to 9:1) to afford another 10 elutions E2f1–E2f10 with E2f5 (151 mg) containing **4** and **5** and E2f8 (98 mg) containing **1–3**. E2f5 was first purified by silica gel H CC ($CHCl_3$-MeOH, 100:1 to 50:1) and then chiral HPLC (*n*-hexane-isopropanol, 7:3, 3.0 mL/min) to yield compounds **4** (20 mg) and **5** (13 mg). E2f8 was also fractionated by silica gel H CC ($CHCl_3$-MeOH, 100:1 to 50:1) and then normal HPLC (68% MeOH-H_2O, 3.0 mL/min) to yield compounds **1** (4.0 mg) and a mixture of **2** and **3** (8.5 mg). Subsequent chiral HPLC separation of the mixture (**2** and **3**) (n-hexane-isopropanol-ethanol, 7:1:2, 2.7 mL/min) returned **2** (2.0 mg) and **3** (3.0 mg) as single components.

Characterization of new compounds

Aphadilactone E (1). Pale gum; $[\alpha]_D^{22}$ –16.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 262 (4.47) nm; IR (KBr) ν_{max} 2984, 2931, 2867, 1703, 1646, 1574, 1435, 1383, 1362, 1277, 1247, 1175, 1075, 1042, 1014, 990, 850 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table 1; ESIMS *m/z* 661.5 $[M + H]^+$, 683.5$[M + Na]^+$; HRESIMS *m/z* 683.3554$[M + Na]^+$ (calcd for $C_{40}H_{52}O_8Na$, 683.3560).

Aphadilactone F (2). Pale gum;$[\alpha]_D^{22}$ –19.4 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 272 (4.46) nm; IR (KBr) ν_{max} 2978, 2930, 2867, 1720, 1700, 1641, 1578, 1399, 1384, 1362, 1277, 1247, 1173, 1074, 1043, 990, 848 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table 1; ESIMS *m/z* 661.5 $[M + H]^+$, 683.5 $[M + Na]^+$; HRESIMS *m/z* 683.3553 $[M + Na]^+$ (calcd for $C_{40}H_{52}O_8Na$, 683.3560).

Aphadilactone G (3). Pale gum;$[\alpha]_D^{22}$ –3.3 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 272 (4.49) nm; IR (KBr) ν_{max}2977, 2930, 2867, 1721, 1699, 1641, 1578, 1399, 1384, 1360, 1277, 1247, 1172, 1074, 1042, 991, 851 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table 1; ESIMS *m/z* 661.6 $[M+ H]^+$, 683.5$[M + Na]^+$; HRESIMS *m/z* 683.3566$[M + Na]^+$ (calcd for $C_{40}H_{52}O_8Na$, 683.3560).

Aphanamene H (4). Pale gum; $[\alpha]_D^{22}$ 61.8 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (logε) 270 (4.11), 248 (3.80) nm; IR (KBr) ν_{max} 3421, 2960, 2924, 1722, 1697, 1460, 1379, 1246, 1171, 999 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table S1, ESI†; ESIMS *m/z* 647.4 $[M + H]^+$, 669.3 $[M + Na]^+$, 645.7 $[M - H]^-$; HRESIMS *m/z* 669.3765 $[M + Na]^+$ (calcd for $C_{40}H_{54}O_7Na$, 669.3767).

Aphanamene I (5). Pale gum; $[\alpha]_D^{22}$ –76.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 272 (4.04), 248 (3.78) nm; IR (KBr) ν_{max} 3421, 2927, 2868, 1720, 1697, 1570, 1452, 1381, 1246, 1174, 1011 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table S1, ESI†; ESIMS *m/z* 647.5 $[M + H]^+$, 669.4 $[M + Na]^+$, 645.6 $[M - H]^-$; HRESIMS *m/z* 669.3792 $[M+H]^+$ (calcd for $C_{40}H_{54}O_7Na$, 669.3767).

Syntheses and Reactions

Oxidative degradation of 4 and 5. To a solution of compound **4** (3 mg, 0.0046 mmol) in ^tBuOH-H_2O (1.6 mL, 1:1), $K_2OsO_4 \cdot 2H_2O$ (0.684 mg, 0.0019 mmol), $K_3Fe(CN)_6$ (184 mg, 0.56 mmol), K_2CO_3 (77 mg, 0.56 mmol) and $MeSO_2NH_2$ (18 mg, 0.19 mmol) were added at room temperature.[14] After about 1.5 h (monitored by TLC), the reaction was quenched with 5 mL of saturated NaS_2O_3. The resulting aqueous mixture was then extracted with EtOAc (3 × 10 mL). The organic layer was combined, washed with brine (30 mL) and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed *in vacuo* at room temperature. The residue (a mixture of i and ii, Scheme S1, ESI†) was then dissolved in dried DCM (1.0 mL), and a fresh batch of $Pb(OAc)_4$ (1.0 mg) was slowly added at 0 °C[15] until the raw material was completely converted (monitored by TLC). After work-up, the resulting product was purified by semi-preparative HPLC (CH_3CN-H_2O, 6:4) to afford compounds **4a** (0.4 mg) and **4b** (1.0 mg). Compound **5** (3 mg) underwent the same procedure to produce compounds **5a** (0.6 mg) and **5b** (1.1 mg).

Compound 4a. $[\alpha]_D^{24}$ = 20.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 267 (3.81); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 263 (1.37), 218 (–1.28) nm; ^1H and ^{13}C NMR data, see Table S2, ESI†; ESIMS *m/z* 451.3 $[M + Na]^+$, 879.5 $[2M + Na]^+$; HRESIMS *m/z* 451.2466 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_5Na$, 451.2460).

Compound 4b. $[\alpha]_D^{24}$ = 60.2 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 270 (3.93); ^1H and ^{13}C NMR data, see Table S2, ESI†; ESIMS *m/z* 537.3 $[M + H]^+$, 1095.7 $[2M + Na]^+$; HRESIMS *m/z* 559.3040 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_6Na$, 559.3036).

Compound 5a. $[\alpha]_D^{24}$ = –27.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 268 (3.90); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 264 (–1.62), 217 (1.23) nm; ^1H and ^{13}C NMR data, see Table S2, ESI†; ESIMS *m/z* 451.3 $[M + Na]^+$, 879.6 $[2M + Na]^+$; HRESIMS *m/z* 451.2455 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_5Na$, 451.2460).

Compound 5b. $[\alpha]_D^{24}$ = –109.9 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 269 (4.02); ^1H and ^{13}C NMR data, see Table S2, ESI†; ESIMS *m/z* 537.3 $[M + H]^+$, 1095.7 $[2M + Na]^+$; HRESIMS *m/z* 559.3035 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_6Na$, 559.3036).

Syntheses of (*S*) and (*R*) forms of 6-(hydroxymethyl)-4-methyl-5,6-dihydro-2H-pyran-2-one. As previously reported.[10]

Reductive ozonolysis of 4 and 5. Compound **4** (1.0 mg, 0.0015 mmol) in DCM (1.0 mL) was ozonized at –78 °C and then treated with excess newly prepared methanolic solution of $NaBH_4$. The reaction was allowed to warm up to room temperature and kept stirring for 30 min. After work-up, the resulting product containing **4r** was directly subjected to chiral HPLC analysis with n-hexane/isopropanol (9:1, 3.0 mL/min) as mobile phase. Comparison of the chiral HPLC chromatogram

(Fig. S2, ESI†) with those of the above-described synthetic samples showed that **4r** was the *S*-form enantiomer. In the same way, the product **5r** from **5** was also identified to have a 5*S* configuration.

ECD calculations.

See experimental section, ESI†.

Bioassays

The antimalarial activities of compounds **1**–**5** were tested as previously reported.[10]

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