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Phloroglucinols from the Roots of *Garcinia dauphinensis* and their Antiproliferative and Antiplasmodial Activities¹

Rolly G. Fuentes^{†,‡}, Kirk C. Pearce[†], Yongle Du[†], Andriamalala Rakotondrafara[§], Ana L. Valenciano[⊥], Maria B. Cassera[⊥], Vincent E. Rasamison^{||}, T. Daniel Crawford[†], David G. I. Kingston^{†,*}

[†]Department of Chemistry and the Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061, United States

[‡]Division of Natural Sciences and Mathematics, University of the Philippines Visayas Tacloban College, 6500 Tacloban City, Philippines

[§]Département d'Ethnobotanique et de Botanique, Centre National d'Application des Recherches Pharmaceutiques, Ambodivoanjo, BP 702, Antananarivo 101, Madagascar

[⊥]Department of Biochemistry and Molecular Biology, Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia, Athens, GA 30602, United States

^{||} Centre National d'Application des Recherches Pharmaceutiques, B.P 702, 101 Antananarivo, Madagascar

Abstract

Garcinia dauphinensis is a previously uninvestigated endemic plant species of Madagascar. The new phloroglucinols dauphinols A-F and 3'-methylhyperjovoinol B (**1-7**) and six known phloroglucinols (**8-13**) together with tocotrienol **14** and three triterpenoids **15-17** were isolated from an ethanolic extract of *G. dauphinensis* roots using various chromatographic techniques. The structures of the isolated compounds were elucidated by NMR, MS, optical rotation, and ECD data. Theoretical ECD spectra and specific rotations for **2** were calculated and compared to experimental data in order to assign its absolute configuration. Among the compounds tested, **1** showed the most promising growth inhibitory activity against A2870 ovarian cancer cells, with $IC_{50} = 4.5 \pm 0.9 \mu M$, while **2** had good antiplasmodial activity against the Dd2 drug-resistant strain of *Plasmodium falciparum*, with $IC_{50} = 0.8 \pm 0.1 \mu M$.

¹Dedicated to Dr. Rachel Mata, National Autonomous University of Mexico, Mexico City, Mexico and Dr. Barbara N. Timmermann, University of Kansas, for their pioneering work on bioactive natural products.

*Corresponding Author dkingston@vt.edu Tel: +1 540 231 6570. Fax: +1-540-231-3255.

ASSOCIATED CONTENT

Supporting Information

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¹H and ¹³C NMR spectra of compounds **1-7**; NOESY spectrum for compound **2**; experimental ECD spectra of compounds **1-4**, **6**, **7**, and **11**; calculated weighted average internuclear distances and experimental NOESY intensities for **2a-2d** and their enantiomers; comparison of calculated and experimental ECD spectra for all eight stereoisomers of **2**; calculated specific rotations for all eight stereoisomers of **2**; table of relative free energies and room-temperature Boltzmann population for conformers of enantiomerically unique stereoisomers of **2**; .jdx files for compounds **1-7**.

The authors declare no competing financial interest.

Graphical Abstract

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Garcinia dauphinensis P. Sweeney and Z. S. Rogers
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The genus *Garcinia*, family Clusiaceae, is native to Asia and Africa and includes more than 250 species.¹ Plants of this genus are utilized in many communities as traditional medicines to treat common illnesses,²⁻⁴ and thus this genus has been extensively studied for its chemical constituents and pharmacological properties. Phytochemical studies of different *Garcinia* sp. have led to the isolation of xanthenes, phloroglucinols, flavonoids, benzophenones, triterpenoids, and lactones.⁴⁻⁶ α-Mangostin, a xanthone isolated from *G. mangostana*, has various pharmacological properties such as antioxidant, anticancer, anti-inflammatory, anti-allergy, and analgesic activities.⁷ (–)-Hydroxycitric acid from *G. cambogia* was shown to block fat accumulation and suppress appetite.^{4,8} *G. subelliptica* and *G. purpurea* have been reported to contain benzophenone derivatives which inhibit proliferation of methicillin-resistant *Staphylococcus aureus* (MRSA),⁹ and phloroglucinols isolated from *G. parvifolia* and *G. subelliptica* have been reported to possess inhibitory activities against oxidation processes and proliferation of cancer cells.^{5,10}

G. dauphinensis P. Sweeney & Z.S. Rogers is endemic to Madagascar,¹¹ and its constituents and their biological activities have not previously been investigated. In this study, we report the seven new phloroglucinols dauphinols A-F and 3'-methylhyperjovoinol B (**1-7**), six known phloroglucinols (**8-13**), one tocotrienol (**14**), and three known triterpenoids (**15-17**). Isolated compounds were tested for their antiproliferative activity against the A2780 human ovarian cancer cell line and for their antiplasmodial activity against the drug-resistant Dd2 strain of *Plasmodium falciparum*.

RESULTS AND DISCUSSION

The dried roots of *G. dauphinensis* were extracted with EtOH and the resulting extract was partitioned successively between aqueous MeOH and hexanes and then aqueous MeOH and EtOAc. A combination of column chromatography and HPLC yielded the seven new phloroglucinols **1-7**, the six known phloroglucinols **8-13**, tocotrienol **14**, and the three known triterpenoids **15-17** (Figure 1).

Compound **1** was isolated as a brownish, amorphous compound and had the molecular formula C₂₆H₃₈O₄ based on its negative mode HRESIMS, which showed an intense peak at *m/z* 413.2699 [M-H][–], corresponding to C₂₆H₃₇O₄[–] (calc. 413.2697, 0.5 ppm). The peaks

at δ_C 160.2 (C-1), 161.2 (C-5), and 163.0 (C-3) in its ^{13}C NMR spectrum indicated a phloroglucinol skeleton (Table 1).

Substitution at C-2 and C-4 on the phloroglucinol moiety was indicated by the shifts at δ_C 104.6 and 104.7, respectively. A singlet at δ_H 5.82 which correlated with a signal at δ_C 95.5 indicating that the phloroglucinol unit contains a single methine hydrogen, and its location at C-6 was deduced by the observation of HMBC correlations from H-6 to C-1 and C-5. An isolated methylene group which gave an AB quartet at δ_H 3.46 was assigned to C-1', based on cross peaks in the HMBC data from H₂-1' to C-5 (δ_C 161.2), C-4 (δ_C 104.7), C-3 (δ_C 163.0), C-3' (δ_C 134.3), C-7' (δ_C 26.0), and C-8' (δ_C 19.8) (Figure 2). C-4' and C-7' were shown by COSY and HMBC correlations (Figure 2) to be part of a cyclohexene ring substituted with the C-8' methyl group, a prenyl group, and gem-dimethyl groups. The presence of the prenyl group (C-1'''-C-5''') was indicated by ^{13}C NMR signals for two vinyl methyls (δ_C 25.9, δ_C 18.0), a methine (δ_C 125.1), a methylene (δ_C 29.8) and a quaternary carbon (δ_C 130.7). It was placed at C-4' based on the COSY correlations between H₂-1''' (δ_H 2.20 and 2.07) and H-4' (δ_H 1.66) (Figure 2). The protons of both methyl groups on the cyclohexene ring, H₃-9' (δ_H 0.84) and H₃-10' (δ_H 0.89), showed cross peaks in the HMBC spectrum to C-4', C-5', and C-6', indicating that they are geminal and located at C-5'. The presence of a 3H triplet at δ_H 0.91, a 3H doublet at δ_H 1.15, and a 1H sextet at δ_H 3.73 indicated the presence of a 2-methylbutanoyl group. With all the locations accounted for, this group was placed at C-2. The configurations at C-4' and C-2'' were tentatively assigned by analogy with those of compound **2**, as described below, and thus dauphinol A (**1**) was identified as (*S*)-2-methyl-1-(2,4,6-trihydroxy-3-[(*S*)-2,4,4-trimethyl-3-(3-methylbut-2-en-1-yl)cyclohex-1-en-1-yl]methyl}phenyl)butan-1-one.

Compound **2** was also isolated as a brownish, amorphous solid. Its HRESIMS established its molecular formula as C₂₆H₃₈O₄ (found 413.2684 [M-H]⁻, calcd. C₂₆H₃₇O₄⁻ 413.2697, 3.1 ppm), isomeric with **1**. Its NMR data (Table 1) were similar to those of **1**, with the major differences being the signals for C-2', C-3', C-8', and H-8'. The signals at δ_C 127.4, 134.3, 19.8 and δ_H 1.91 (3H, s) in **1** appeared at δ_C 40.0, 152.3, 108.8, and δ_H 4.83 and 4.64 (1H each, s) in **2**. In addition the 1H resonance of the C-1' methylene bridge is shifted from δ_H 3.46 in **1** to two doublets of doublets at 2.72 and 2.61 in **2** for the AB part of a spin system with one adjacent proton. These changes indicated that C-1' is next to a methine group (C-2'), and that the endocyclic double bond in **1** is replaced by an exocyclic double bond in **2**. This conclusion was confirmed by COSY and HMBC correlations (Figure 2). Thus, dauphinol B (**2**) was identified as 4-[[5,5-dimethyl-4-(3-methylbut-2-en-1-yl)-3-methylenecyclohexyl]methyl]-2-(2''-methylbutanoyl)phloroglucinol.

The relative configuration at the 2' and 4' positions of **2** was determined by a NOESY spectrum, which only showed a correlation between H-4' and H-8a', with no detectable correlations between H-2' and H-4' (Table 2); the weighted average internuclear distances for the four diastereomers with the 2''*S* configuration were calculated as described below. The best fit is for structures **2c** and **2d** (Figure 3), and the *anti* relative configuration at C-2' and C-4' is thus assigned for the left-hand side of compound **2**.

There remained the question of the absolute configuration of **2**, which could be any of the possible configurations **2c** – **2f** (Figure 4) based on the present evidence.

Determination of relative and absolute configurations has previously been accomplished through the simulation of ECD spectra and specific rotations followed by subsequent comparison with their respective experimental data for caged prenylxanthenes from *G. bracteata*,⁶ prenylated phloroglucinols from *G. nuntasaenii*,¹² and sesquiterpenoid lactones from *Trichospira verticillata*,¹³ among others. Thus, ring conformations of **2** were generated based on proposed stable conformations of methylenecyclohexane reported by Taskinen¹⁴ and Li.¹⁵ Additional side chain conformations of each unique ring conformer were identified by systematically rotating torsional angles along rotatable bonds to produce all diverse low-energy conformers. This conformer generation process was conducted for each of the four enantiomerically unique stereoisomers of **2**, resulting in 2093 total conformers. Geometries of all conformers were optimized, and conformers whose absolute energies matched to within 10^{-6} Hartree were considered to be identical. Of the 2093 total conformers, 934 were unique (Table S6). Boltzmann populations were also computed for each of the 934 unique conformers in order to determine individual contributions to the simulated weighted average ECD spectra and specific rotations. Conformers with a Boltzmann population less than 1% were deemed insignificant and were not included in subsequent calculations. Of the 934 unique conformers, 80 had significant Boltzmann populations. For each significant conformer, individual ECD spectra, specific rotations, and internuclear distances were calculated and combined (according to their respective Boltzmann populations) in order to generate the associated data for the eight unique stereoisomers. Data for the four “*trans*” isomers **2c** – **2f** are shown in Figure 5 and Table 3.

These data indicated that the best fit between experimental and calculated data, both for the calculated ECD spectrum and the calculated specific rotation, was for the *RSS* isomer. The agreement is not perfect, but it is clearly better than any of the alternate configurations. Thus **2** was identified as (2'*R*,4'*S*,2''*S*)-4-([5',5'-dimethyl-4'-(3-methylbut-2-en-1-yl)-3'-methylenecyclohexyl]methyl)-2-(2''-methylbutanoyl)phloroglucinol.

As noted above, similar calculations were not performed for the other isolated phloroglucinols with a 2-methylbutanoyl side chain because of computational complexity. Nevertheless, it is likely based on biosynthetic reasoning that these side chains also have the *S* configuration. In addition, the ECD spectra of **1** – **4** and **6** – **7** all showed positive Cotton effects at about 285 nm: An ECD spectrum was not obtained for **5**. This is consistent with a UV absorption of 292 nm for the acylphloroglucinol chromophore,¹⁶ suggesting that this effect is associated with the 2-methylbutanoylphloroglucinol chromophore. These considerations suggest that compounds **1** – **7** all have the 2''*S* configuration, and this is thus indicated in Figure 1.

The molecular formula of **3** was determined to be C₂₆H₃₈O₄ based on its HRESIMS data with an [M-H]⁻ peak at *m/z* 413.2685 (calcd. for C₂₆H₃₇O₄⁻ 413.2692, -2.9 ppm). The ¹H NMR data indicated that **3** is also a phloroglucinol derivative (Table 1), and the presence of a 2-methylbutanoyl group was confirmed by comparison of its ¹H NMR signals with those of compounds **1** and **2**. Two vinyl protons at δ_H 5.18 and 4.81 both had cross peaks to the same

methylene protons (H_2-5' , δ_H 2.39 m and 2.55 m) in a COSY spectrum. COSY data also showed correlations between $H-4'$ and H_3-10' and between $H-6'$ and H_3-8'/H_3-9' . These correlations established the structure of the side chain from $C-3' - C-10'$. The presence of a vinyl methylene group (H_2-4''') was deduced from singlet signals at δ_H 4.69 and 4.66 which showed allylic coupling (4J) with H_3-5''' in the COSY experiment. Based on a COSY correlation from $H-2'$ to H_2-1''' , the group $C-1'''-C-5'''$ was located at $C-2'$ of the side chain. The 1H and ^{13}C NMR data of the isoprenoid side chain of **3** were essentially identical to those of the same side chain of the phloroglucinol goudotianone **2** from *Garcinia goudotiana*.¹⁷ Thus, dauphinol C (**3**) was identified as 4-[2'-(3-methylbut-3-enyl)-3',7'-dimethylocta-3',6'-dienyl]-2-(2-methylbutanoyl)phloroglucinol.

Compound **4** gave a protonated molecular ion peak at m/z 415.2829 in its HRESIMS, corresponding to $C_{26}H_{39}O_4$ $[M+H]^+$ (calcd. 415.2843, 3.3 ppm), isomeric with **3**. The 1H NMR data of **4** showed similar resonances to those of **3** except that a one-proton triplet at δ_H 5.10 in **4** replaced the signals for the vinyl methylene protons at δ_H 4.69 and 4.66 (Table 1). Together with the COSY data, they suggest that a dimethylallyl group is located at $C-2''$ instead of the isopentenyl group of **3**. In confirmation, the reported compound goudotianone **1**¹⁷ has 1H NMR data similar to those of **4** except that signals for a benzoyl group at $C-2$ replaced those of a 2-methylbutanoyl moiety. Dauphinol D (**4**) was thus identified as 4-[3',7'-dimethyl-2'-(3-methylbut-2-en-1-yl)-octa-3',6'-dien-1'-yl]-2-(2-methylbutanoyl)phloroglucinol.

Compound **5** was isolated as an amorphous colorless solid. Its molecular formula was determined to be $C_{26}H_{40}O_5$ based on HRESIMS (found 433.2964 $C_{26}H_{41}O_5$ $[M+H]^+$ calc. 433.2954, 2.3 ppm), 18 mass units more than compounds **4** and **5**. Its 1H NMR spectrum was similar to those of **4** and **5**, but lacked the signals for two vinylic protons at δ_H 5.16 and 4.80 in **3** (Table 1). Two peaks for methyl groups at δ_H 1.22 and 1.23 were observed, suggesting that they are located on an oxygenated carbon. These data together with the COSY results indicated that **5** is a hydrated derivative of **3** and **4**. Hence, dauphinol E (**5**) is identified as 4-[3',7'-dimethyl-2'-(3-hydroxy-3-methylbutyl)octa-3',6'-dien-1'-yl]-2-(2-methylbutanoyl)phloroglucinol. It is possible that **5** is an artefact formed by hydration of **3** or **4** during extraction or fractionation, but this is considered unlikely because no other equally plausible potential hydration products of **1 - 4** were isolated.

Compound **6** was isolated as amorphous colorless solid. It gave an intense $[M+Na]^+$ peak at m/z 387.2147 in its HRESI mass spectrum, corresponding to $C_{21}H_{32}O_5Na$ (calcd. 387.2142, 1.2 ppm) and a composition of $C_{21}H_{32}O_5$. The presence of a 2-methylbutanoyl group was indicated by similar resonances to those of the aforementioned compounds (Table 1), and a comparison of its 1H and ^{13}C NMR spectra with those of the known compound **8**¹⁸ showed that the two compounds differed only in that **6** has a 2-methylbutanoyl group at $C-2$ instead of a 2-methylpropanoyl group. Thus, dauphinol F (**6**) was identified as 4-(3'-hydroxy-3',7'-dimethyloct-6'-en-1'-yl)-2-(2-methylbutanoyl)-phloroglucinol.

Compound **7** was isolated as an amorphous colorless solid. It was determined to have the molecular formula $C_{21}H_{30}O_4$ by HRESIMS (found 347.2219 $[M+H]^+$, calcd. 347.2217, 0.5 ppm). Its 1H and ^{13}C NMR data in $CDCl_3$ were similar to the NMR data in C_6D_6 of the

known compound hyperjovinol B (Table 4).¹⁸ The only significant difference was that the ¹H NMR spectrum of **7** had signals for a 2-methylbutanoyl group at C-2, as indicated by a sextet at δ_{H} 3.73, in place of the 2-methylpropanoyl group in hyperjovinol B. Compound **7** also differs from the known compound empetriferdinin B¹⁹ in having its 2-methylbutanoyl group at C-2 instead of C-4. Compound **7**, 3'-methylhyperjovinol B, was thus identified as 1-[(8a*R**,10a*R**)-1,3-dihydroxy-8,8,10a-trimethyl-5,6,7,8a,9,10a-hexahydro-1H-xanthen-2-yl]-2-methylbutan-1-one. Its relative configurations at C-8a and C-10a were established as *R** and *R** by comparison with the ¹H NMR spectrum of hyperjovinol B.

The other isolated compounds were identified as hyperjovinol A (**8**),¹⁸ 4-geranyl-2-(2'-methylbutyryl)phloroglucinol (**9**),^{20,21} 4-geranyl-2-(2'-methylpropionyl)phloroglucinol (**10**),^{20,21} empetrifarinol B (**11**),¹⁹ 2-methyl-2-(4'-methylpent-3'-en-1'-yl)-6-(2"-methylbutyryl)chroman-5,7-diol (**12**),¹⁹ empetrifranzinan C (**13**),¹⁹ δ -tocotrienol (**14**),²² lupeol (**15**),²³ and a mixture of α -amyrin (**16**)²⁴ and β -amyrin (**17**)²⁴ by comparison of their ¹H NMR spectra with those of the known compounds.

Compounds available in sufficient amount were screened for their antiproliferative activity to the A2780 human ovarian cancer cell line, and for antiparasmodial activity against the drug-resistant Dd2 strain of *Plasmodium falciparum* (Table 5).

Among the isolated compounds tested, **1** showed the lowest IC₅₀ (4.5 ± 0.9 μ M) for antiproliferative activity, while **2** had the most potent antiparasmodial activity, with an IC₅₀ of 0.8 μ M (Table 3). Significantly, **2** had one of the highest IC₅₀ values against the A2780 cell line, so it is almost 20-fold more potent against *P. falciparum* than it is against ovarian cancer cells. Phloroglucinols have been isolated from different natural sources and are known to possess various biological activities.²⁵ As one example, the dimeric phloroglucinols isolated from *Mallotus oppositifolius* had IC₅₀ values ranging from 1.1 – 6.3 μ M for their antiproliferative activity against A2780 and 0.14 – 0.75 μ M for their antiparasmodial activity,²⁶ in comparison with 4.5 – 19.7 μ M and 4.7 – 14.1 μ M respectively for all of the monomeric phloroglucinols isolated from *G. dauphinensis* except for **2**. The monomeric compounds, except for **2**, are thus about an order of magnitude less potent than the dimeric compounds against *P. falciparum*, but are also significantly less potent against human cancer cells. Compound **2** thus emerges as an interesting lead compound, with antiparasmodial activity comparable to that of the dimeric phloroglucinols from *M. oppositifolius*, but with reduced antiproliferative activity.

In summary, this is the first study to report constituents of the roots of *G. dauphinensis*. Most of the isolated compounds are phloroglucinol derivatives, consistent with previous studies of other *Garcinia* species. The anticancer and antiparasmodial activities of the isolated compounds are good to moderate, and support the concept that phloroglucinols have antiparasmodial potential.

EXPERIMENTAL SECTION

General Experimental Procedures.

Optical rotations were obtained using a JASCO P-2000 polarimeter. ECD spectra were acquired on JASCO J-815 spectrometer. UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. NMR spectra were recorded on a Bruker Avance 500 spectrometer with deuterated solvent as indicated. High resolution electrospray ionization mass spectra (HRESIMS) were obtained on an Agilent 6220 LC-TOF-MS in the positive ion mode. Unless otherwise stated, HPLC was carried out on a Phenomenex Luna 5 μ C₁₈ 4.6 x 250 mm column at a flow rate of 1 mL/min.

Plant Material.

Roots of *Garcinia dauphinensis* P. Sweeney & Z.S. Rogers were collected at Antsiranana, Diana, Madagascar coordinates 13°07'12"S 049°14'02"E (−13.1200000, 49.2338889) on January 22, 2007 as RLL 489, MG4225. The plant was a shrub 3 m high, with yellow resin and white flowers. A voucher specimen of this plant is deposited at the herbaria of the Missouri Botanical Garden (MO), of the Centre National d'Application des Recherches Pharmaceutiques (CNARP), of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar (TAN), and of the Muséum National d'Histoire Naturelle in Paris, France (P)

Extraction and Isolation.

Dried roots of *G. dauphinensis* (271 g) were extracted with EtOH to yield 31.3 g of extract, a portion of which was shipped to Virginia Tech. One gram of this extract was resuspended in 10% MeOH (15 mL) and extracted with hexanes (3 x 15 mL) to give 0.32 g hexanes extract. The MeOH layer was dried in vacuo, resuspended in water (15 mL) and extracted with EtOAc to provide the EtOAc extract (0.56 g). The hexanes extract was subjected to silica gel column chromatography (30 x 180 mm) using the hexanes:EtOAc gradient (95:5-8:2) to give fractions 1A-1K. Fraction 1F (72.8 mg) which was eluted using hexanes:EtOAc (9:1) was subjected to ODS column chromatography (15 x 110 mm) using MeCN (70-100%) and afforded a mixture of compounds **16** and **17** (18.6 mg), **15** (12.7 mg), and **14** (2.2 mg). Fraction 1D (8.5 mg) isolated using hexanes:EtOAc (95:5) was subjected to ODS column chromatography (10 x 130 mm) to give fractions 3A-3C. Fraction 3A (3.5 mg) eluted with 80% MeOH was subjected to solid phase extraction (HyperSep C₁₈ 2.8 mL) using 100% MeOH as the eluent. The eluted fraction was further purified by HPLC with elution with MeOH:H₂O, 80:20, and yielded **13** (0.61 mg, *t_R* 32.8 min). Fraction 1E (13.1 mg) which was eluted from a hexanes:EtOAc gradient (95:5) was dissolved in 80% MeOH, subjected to SPE (HyperSep C₁₈), and washed with 80-100% MeOH to give subfractions 1E-1 to 1E-4. Fraction 1E-3 (3.8 mg) was subjected to HPLC using a solvent gradient from MeOH:H₂O, 80:20 to 90:10 from 0 to 60 min, to 100:0 from 60 to 65 min and ending with 100% MeOH wash from 65 to 70 min. This process afforded compounds **12** (0.17 mg; *t_R* 38.0 min) and **7** (0.58 mg; *t_R* 35.5 min). The EtOAc extract was subjected to silica column chromatography (35 x 300 mm) using the CHCl₃:MeOH gradient (1:0-0:1) to give fractions 4A-4M. Fraction 4B (27.5 mg) eluted using CHCl₃:MeOH (98:2) was resuspended in 70% MeCN, subjected to SPE (HyperSep C₁₈) and eluted with 70-100% MeCN to afford subfractions 4B-1 to

4B-6. Fraction 4B-1 (4.31 mg) eluted with 70% MeCN was subjected to HPLC using a solvent gradient from MeCN:H₂O, 65:35 to 70:30 from 0 to 10 min, to 75:25 from 10 to 30 min, to 100:0 from 30 to 35 min and ending with a 100% MeCN wash from 35 to 45 min. The process afforded compounds **9** (1.37 mg; *t_R* 27.2 min) and **10** (0.33 mg; *t_R* 22.4 min). Fraction 4B-3 (3.06 mg) eluted with 80% MeCN was also subjected to HPLC using a solvent gradient from MeCN:H₂O, 75:25 to 80:20 from 0 to 5 min, to 80:20 from 5 to 35 min, to 90:10 from 35 to 36 min and ending with a 100% MeCN wash from 36 to 45 min and yielded **1** (0.68 mg; *t_R* 21.5 min), **2** (0.28 mg; *t_R* 19.5 min), **3** (0.37 mg; *t_R* 17.0 min), and **4** (0.22 mg; *t_R* 16.0 min). Fraction 4E (16.1 mg) eluted using CHCl₃:MeOH (9:1) was resuspended in 60% MeCN, subjected to SPE using 60-100% MeCN and final elution with 100% MeOH to give subfractions 4E-1 to 4E-5. Fraction 4E-1 (3.2 mg) eluted with 60% MeCN was subjected to HPLC using a solvent gradient from MeCN:H₂O, 55:45 to 60:40 from 0 to 10 min, to 70:30 from 10 to 35 min, to 100:0 from 35 to 36 min and ending with a 100% MeCN wash from 36 to 45 min. This led to the isolation of **8** (0.50 mg; *t_R* 12.9 min) and **6** (1.6 mg; *t_R* 16.3 min). Fraction 4C (9.0 mg) eluted with CHCl₃:MeOH (9:1) was resuspended in 60% MeCN, subjected to SPE using 60% MeCN and eluted with 100% MeCN and 100% MeOH to provide subfractions 4C-1 to 4C-4. Subfraction 4C-2 (2.35 mg) was subjected to HPLC using a solvent gradient from MeCN:H₂O, 65:35 to 70:30 from 0 to 10 min, to 72:28 from 10 to 15 min, to 100:0 from 15 to 16 min and ending with a 100% MeCN wash from 16 to 20 min. This process yielded **11** (0.26 mg; *t_R* 9.8 min). Fraction 4F (5.0 mg) eluted with CHCl₃:MeOH (9:1) was subjected to SPE. It was resuspended in 3 mL 60% MeCN then transferred to a HyperSep C₁₈ column. Elution with 60% MeOH followed by 100% MeCN and 100% MeOH gave subfractions 4F-1 to 4F-5. Subfraction 4F-2 (2.00 mg) eluted with 60% MeCN was then subjected to HPLC using a solvent gradient from MeCN:H₂O, 60:40 to 70:30 from 0 to 30 min, to 70:30 from 30 to 40 min, to 80:20 from 40 to 45 min and ending with a 100% MeCN wash from 45 to 55 min to yield compound **5** (0.57 mg; *t_R* 40.0 min).

Compound 1: amorphous brown solid; $[\alpha]_D^{20} -29$ (c. 0.1 MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 291 (4.1) 231 (4.1) nm; ECD spectrum, see Figure S2, Supporting Information; ¹H and ¹³C NMR spectra, see Table 1; HRESIMS *m/z* 413.2699 [M-H]⁻ (calc. for C₂₆H₃₇O₄ 413.2697, +0.31 ppm).

Compound 2: amorphous brown solid; $[\alpha]_D^{22} -16$ (c. 0.05 MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 291 (4.3) 227 (4.2) 200 (4.4); ECD spectrum, see Figure S2, Supporting Information; ¹H and ¹³C NMR spectra, see Table 1; HRESIMS *m/z* 413.2684 [M-H]⁻ (calc. for C₂₆H₃₇O₄ 413.2697, -3.15 ppm).

Compound 3: amorphous colorless solid; $[\alpha]_D^{21} -3.3$ (c. 0.07 MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 292 (4.0) 214 (4.1) nm; ECD spectrum, see Figure S2, Supporting Information; ¹H and ¹³C NMR spectra, see Table 1; HRESIMS *m/z* 415.2835 [M+H]⁺ (calc. for C₂₆H₃₉O₄ 415.2842, -1.9 ppm).

Compound 4: amorphous colorless solid; $[\alpha]_D^{21} +5.3$ (c. 0.03 MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 292 (3.8) 206 (4.1) nm; ECD spectrum, see Figure S2, Supporting Information; ¹H and

^{13}C NMR spectra, see Table 1; HRESIMS m/z 415.2829 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{26}\text{H}_{39}\text{O}_4$ 415.2843, -3.4 ppm).

Compound 5: amorphous colorless solid; $[\alpha]_{\text{D}}^{22} -13.9$ (c. 0.03 MeOH); UV (MeOH) λ_{max} (log ϵ) 293 (4.2) 200 (4.2) nm; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 433.2964 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{26}\text{H}_{41}\text{O}_5$ 433.2954, $+2.3$ ppm).

Compound 6: amorphous colorless solid; $[\alpha]_{\text{D}}^{22} +18.2$ (c. 0.16 MeOH); UV (MeOH) λ_{max} (log ϵ) 292 (4.2) 200 (4.2) nm; ECD spectrum, see Figure S2, Supporting Information; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 387.2147 $[\text{M}+\text{Na}]^+$ (calc. for $\text{C}_{21}\text{H}_{32}\text{O}_5\text{Na}$ 387.2142, $+1.2$ ppm).

Compound 7: amorphous colorless solid; $[\alpha]_{\text{D}}^{23} +11$ (c. 0.04 MeOH); UV (MeOH) λ_{max} (log ϵ) 295 (4.0) 217 (3.9) nm; ^1H and ^{13}C NMR spectra, see Table 2; see Figure S2, Supporting Information; HRESIMS m/z 347.2219 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{21}\text{H}_{31}\text{O}_4$ 347.2217, $+0.54$ ppm).

Computational Details.

Side chain conformational searches for each ring conformer were generated using the Open Babel software²⁷ in conjunction with the MMFF94 force field²⁸ and the Confab systematic rotor conformer generator.²⁹ An energy cutoff of 10 kcal/mol was employed such that only side chain conformers with a relative energy less than the cutoff were kept. Geometries were optimized at the DFT/B3LYP/6-31G* level of theory³⁰⁻³³ within a MeOH solvent simulated using the polarizable continuum model (PCM).³⁴ Harmonic vibrational frequencies were also computed at the same level of theory to ensure that no imaginary values were present, thus confirming that all of the structures were minima on their respective potential energy surfaces. Thermal Gibbs free energies were obtained using partition functions computed within the harmonic oscillator/rigid rotor approximations,^{35,36} permitting the calculation of room-temperature equilibrium Boltzmann populations. Excitation energies and rotatory strengths for each transition (in the velocity representation) were calculated for the first 40 electronic excited states at the TDDFT/CAM-B3LYP/aug-cc-pVDZ level of theory,³⁷⁻³⁹ again including the PCM description of the MeOH solvent. All calculations were performed using the Gaussian 09 electronic structure package.⁴⁰ The ECD spectra were simulated by overlapping Gaussian functions for each transition according to⁴¹

$$\Delta\epsilon(\tilde{\nu}) = \frac{1}{(2.296 \times 10^{-39})\sqrt{\pi}\sigma} \sum_a \tilde{\nu}_{0a} R_{0a} \exp\left[-\left(\frac{\tilde{\nu} - \tilde{\nu}_{0a}}{\sigma}\right)^2\right]$$

where σ is defined as half the bandwidth at 1/e peak height and $\tilde{\nu}_{0a}$ and R_{0a} are the excitation energy (in wavenumbers) and rotatory strength for transition $0 \rightarrow a$, respectively. The σ value is an empirical parameter, and we chose a value of 0.50 eV in agreement with the resolution of the experimental ECD bandwidths.

Individual conformer proton distances were calculated and averaged according to their Boltzmann populations in order to compare with experimental NOESY data. In the case of

methyl hydrogens, simple averages of the three individual Boltzmann-averaged distances are reported.

Weighted average ECD spectra, specific rotations, and internuclear distances for all possible stereoisomers of **2** are included in the Supporting Information. Additionally, relative thermal Gibbs free energies and associated room-temperature equilibrium Boltzmann populations of all unique conformers are reported as well.

Antiproliferative Activity.

The methods reported previously was employed in determining the inhibitory activity of the isolated compounds against A2780.²¹ A2780 cells (5×10^4 cells/well) were seeded in 96-well plate and incubated at 5% CO₂ and 37 °C. After 3 h, they were treated with the compounds for 48 h. Paclitaxel was used as the positive control. Then, the medium with the compound was removed and 200 µl of 1% Alamar blue was added to each well for 3 h. Fluorescence was measured to calculate the cell viability. The assay was conducted in two independent trials with each trial having three replications.

Antiplasmodial Activity.

The antiplasmodial assay was conducted using the methods described previously.²⁶ Briefly, ring stage parasite cultures at 1% parasitemia, 1% hematocrit were treated with the samples for 72 h under a 5% CO₂, 5% O₂, and 90% N₂ gas mixture at 37 °C. The parasite viability was determined by DNA quantitation using SYBR Green I (25 µL of lysis buffer with SYBR Green I at 0.33 µL of SYBR Green I/mL of lysis buffer). GraphPad Prism 6 software using a nonlinear regression curve fitting was used to calculate half-maximum inhibitory concentration (IC₅₀). IC₅₀ values are presented as mean ± SD of three independent experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of compounds **1-17**.

Figure 2.
Key COSY and HMBC correlations for **1** and **2**.

Figure 3.
Structures of compounds **2a** – **2d**.

Figure 4. Structures of compounds **2c** – **2f**. The stereochemical descriptors refer to the 2', 4', and 2'' positions, respectively.

Figure 5. Comparison of Experimental and Calculated ECD Spectra for Stereoisomers **2c** – **2f**. The Stereochemical Descriptors Refer to the 2',4', and 2'' Positions Respectively.

Table 1.

¹H and ¹³C NMR Data of Compounds 1–6 in CDCl₃

	1		2		3		4		5		6	
C/H	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C} ^a	δ_{H} mult (<i>J</i> in Hz)	δ_{C} ^a	δ_{H} mult (<i>J</i> in Hz)	δ_{C}
1		161.2		160.1		159.3		159.7		160.0		nd
2		104.6		106.5		104.7		106.9		106.7		nd
3		163.0 ^a		163.2		163.4		162.8		nd		nd
4		104.7		107.1		106.9		104.8		104.0		nd
5		161.3		160.1		160.2		160.1		160.0		nd
6	5.82 s	95.5	5.81 s	95.5	5.79 s	95.2	5.81 s	95.4	5.80 s	95.4	5.84 s	95.8
1'	3.46 ABq (16.0)	26.3	2.72 dd (14.3, 5.0) 2.61 dd (14.3, 9.3)	25.9	2.65 dd (14.3, 5.9) 2.53 dd (14.3, 8.4)	27.0	2.62 dd (14.6, 5.3) 2.55 dd (14.6, 8.4)	26.3	2.66 dd (14.2, 5.6) 2.57 over- lapped	26.7	2.67 qdd	15.9
2'		127.4	2.27 m	40.0	2.82 m	39.0	2.83 m	40.2	2.86 m	39.4	1.75 t (6.7)	39.9
3'		134.3		152.3		136.6		137.0		136.0		74.9
4'	1.66 bt	52.2	1.98 dd (11.0, 3.5)	54.1	5.18 t (7.3)	127.4	4.81 bt	127.0	5.16 t (7.2/7.1)	127.6	1.57 m	42.1
5'		32.6		35.6	2.55 m 2.39 m	26.5	2.55 m 2.36 m	26.5	2.56 over- lapped 2.38 m	26.7	2.06 q (15.4, 7.6)	23.1
6'	1.52 m 1.12 m	31.6	1.55 m 1.30 m	35.9	4.81 t (7.3)	123.5	5.18 bt	123.4	4.80 t (6.9/7.1)	123.7	5.12 t (7.2)	124.0
7'	1.89 m	26.0	1.38 m	29.2		131.5		131.5		131.2		132.9
8'	1.91 s	19.8	4.83 s 4.64 s	108.8	1.52 s	17.7	1.71 s	17.7	1.52 s	18.0	1.69 s	17.9
9'	0.84 s	27.6	0.84 s	28.9	1.62 s	25.8	1.62 s	25.8	1.61 s	26.1	1.62 s	25.9
10'	0.89 s	28.1	0.96 s	26.0	1.70 s	18.6	1.51 s	18.7	1.69 s	18.8	1.24 s	26.9
1''		210.5		210.7		210.4		210.4		210.3		nd
2''	3.73 sext (6.7)	46.2	3.72 sext (6.6)	46.5	3.71 sext (6.5)	46.2	3.71 sext (6.5)	46.2	3.72 sext (6.6)	46.4	3.77 sext (6.6)	46.0
3''	1.83 m 1.40 dq (14.5, 7.3)	27.0	1.84 m 1.40 m	27.3	1.82 m 1.38 m	27.1	1.82 m 1.38 m	27.1	1.82 m 1.39 m	27.4	1.83 m 1.39 m	27.1
4''	0.91 t (7.4)	12.1	0.91 t (7.4)	12.4	0.91 t (7.4)	12.1	0.90 t (7.4)	12.1	0.90 t (7.4)	12.4	0.91 t (7.4)	12.1
5''	1.15 d (6.8)	16.9	1.16 d (6.6)	17.1	1.16 d (6.8)	16.7	1.15 d (6.7)	16.7	1.15 d (6.7)	16.9	1.16 d (6.7)	16.9
1'''	2.20 m 2.07 m	29.8	2.19 m 2.05 m		1.59 m	30.6	2.17 m 2.08 m	31.3	1.52 m	29.4		
2'''	5.13 bt	125.1	5.01 t (6.6)		1.92 m	35.9	5.10 bt	123.2	1.35 m	40.8		
3'''		130.7				146.4		133.1		71.5		
4'''	1.69 s	25.9	1.68 s		4.67 d (14.8)	109.8	1.63 s	18.1	1.22 s	29.7		
5'''	1.62 s	18.0	1.61 s		1.70 s	22.6	1.71 s	25.9	1.23 s	29.7		

	1		2		3		4		5		6	
C/H	δ_{H} mult(<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}	δ_{H} mult (<i>J</i> in Hz)	δ_{C} ^{<i>a</i>}	δ_{H} mult (<i>J</i> in Hz)	δ_{C} ^{<i>a</i>}	δ_{H} mult (<i>J</i> in Hz)	δ_{C}
OH	5.10 bs		5.18 bs		5.33 bs		5.28 s		1.64 bs		7.54 bs	
OH	nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}		12.46 s	
OH	nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}		nd ^{<i>b</i>}	
OH									nd ^{<i>b</i>}		nd ^{<i>b</i>}	

^{*a*}Data generated from HMBC and HSQC correlations

^{*b*}nd = not detected

Table 2.

Calculated Weighted Average Internuclear Distances (in Å) and Observed NOE Effects

Atom pair	NOE observed	2a	2b	2c	2d
H-2' – H-4'	No	2.4	2.4	3.8	3.8
H-2' – H-1''' ^a	Yes	4.7	4.7	2.5	2.7

^aThe shorter of the two H-2' – H-1''' distances is reported

Table 3.Comparison of experimental and calculated specific rotations for 2c – 2f^a

Experiment	<i>RSS</i>	<i>SRS</i>	<i>RSR</i>	<i>SRR</i>
–16	–47.8	+147.8	–147.8	+47.8

^aThe stereochemical descriptors refer to the 2',4', and 2'' positions, respectively.

Table 4.Comparison of ^1H and ^{13}C NMR Data of 7 and Hyperjovanol B

7 (in CDCl_3)		hyperjovanol B (in C_6D_6)	
δ_{H} mult (J in Hz)	δ_{C}	δ_{H} mult (J in Hz)	δ_{C}
1	nd		165.4
2	103.5		103.8
3	157.5		157.9
4 5.46 s	95.7	5.48 s	95.5
4a	159.6		159.9
5ax 1.02 td (13.2, 4.0)	41.6	0.99 td (13.3, 4.4)	41.5
5eq 1.18 m		1.15 ddd (13.3, 4.8, 3.4)	
6 1.31 m	19.9	1.29 m	19.9
7 1.57 td (13.4, 4.0) 1.91 m	39.8	1.56 td (12.6, 4.1) 1.89 ddd (12.6, 4.8, 3.4)	39.5
8	33.7		33.4
8a 1.45 m	47.8	1.45 dd (13.3, 4.9)	47.8
9 2.29 m 2.89 dt (4.5)	17.3	2.28 dd (16.6, 13.3) 2.87 dd (16.6, 4.9)	17.7
9a	103.5		103.8
10a	79.3		79.0
11 0.76 s	32.2	0.76 s	31.9
12 1.08	20.8	0.62 s	20.5
13 0.63 s	20.0	1.07 s	19.9
1'	210.1		210.3
2' 3.78 sext (6.7)	46.0	3.89 sept (6.8)	39.1
3' 1.45 m 2.00 m	27.1	1.23 d (6.8)	19.6
4' 0.95 t (7.4)	12.2	1.22 d (6.8)	19.5
5' 1.26 d (6.7)	16.9		
OH 4.63 bs			
OH 14.83 bs			

Table 5.Antiproliferative and Antiplasmodial Activities of Compounds Isolated from *G. dauphinensis*

compound	Antiproliferative activity against the A2780 ovarian cancer cell line IC ₅₀ (μM) ^a	Antiplasmodial activity against <i>P. falciparum</i> Dd2 strain IC ₅₀ (μM) ^b
1	4.5 ± 0.9	4.7 ± 0.3
2	15.2 ± 0.3	0.8 ± 0.1
3	7.0 ± 2.4	12.0 ± 0.7
5	ND	8.3 ± 0.6
6	6.4 ± 0.2	5.4 ± 0.2
7	ND	14.1 ± 0.1
9	12.4 ± 2.3	ND
10	16.4 ± 0.6	12.5 ± 0.7
11	10.0 ± 1.6	ND
14	19.7 ± 2.5	5.3 ± 0.1
Paclitaxel	0.013 ± 0.001	NT
Artemisinin	NT	0.0085 ± 0.0003

^aData are presented as mean ± SEM (n = 3)^bData are presented as mean ± SD (n = 3).