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Bioactive 1,4-Dihydroxy-5-phenyl-2-pyridinone Alkaloids from *Septoria pistaciarum*

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Abstract

Four new 1,4-dihydroxy-5-phenyl-2-pyridinone alkaloids (**1–4**) were isolated from an EtOAc extract of a culture medium of *Septoria pistaciarum*. The structures of these compounds were determined by spectroscopic methods, and the absolute configuration of the major compound (**1**) by X-ray crystallographic analysis. Compound **1** exhibited moderate in vitro antiplasmodial (antimalarial) activity against chloroquine-sensitive (D6) and -resistant (W2) strains of *Plasmodium falciparum* and cytotoxic activity to Vero cells. Compound **2** was moderately active against both methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*.

Plant-like metabolic pathways found in the apicoplast,1 a chloroplast-like organelle of *Plasmodium* species, have been identified as suitable targets in malaria drug discovery. A number of herbicides and phytotoxins with known molecular targets in the plastid have been evaluated for antimalarial activity.2⁻4 Various phytotoxins released by plant pathogenic fungi are known 5⁻7 to inhibit metabolic pathways in the apicoplast.8 As part of our ongoing search for potential sources of new antimalarial compounds, we have screened a number of plant pathogenic fungi for antiplasmodial activity.

Septoria pistaciarum (Ascomycetes) is the causative agent of Septoria leaf and fruit spot disease in pistachio (*Pistachia vera*) in the US9 and Mediterranean countries.10 An EtOAc extract of this fungus showed potent herbicidal activity and moderate antiplasmodial activity against *Plasmodium falciparum* albeit with low selectivity index. No previous chemical work has been reported for this species. Bioassay-guided fractionation of the EtOAc extract led to the isolation of a new 1,4-dihydroxy-5-phenyl-2-pyridinone analogue as the active compound. Three additional new but inactive analogues of the same class were also isolated and identified. Several compounds of this class have previously been isolated from a number of fungal species and have shown antibacterial,11,12 antitumor,12 antifungal,11,13 and neurotrophic14 activities.

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Supporting Information available: NMR spectra of compounds **1**–**4**, single-crystal X- ray structure of compound, and cif files of the X-ray data for compound **1**.

Results and Discussion

Compound **1** was isolated as the major active constituent of the EtOAc extract of *S. pistaciarum*. The molecular formula of **1** was determined as $C_{22}H_{29}NO_4$ by HRESIMS. The ^{13}C NMR spectra confirmed the presence of 22 carbon atoms, which comprised five quaternary, 11 methine, two methylene, and four methyl carbons. The ^1H NMR and COSY spectra displayed six hydrogens in the aromatic region consisting of a one-proton singlet and an A_2B_2C system due to a monosubstituted phenyl group. NMR signals in the aromatic region showed close resemblance to those of the 1,4-dihydroxy-5-phenyl-2-pyridinone moiety of the antitumor agent, TMC-69-6H, and its analogues.12 IR absorptions (3109, 1642, and 1556 cm^{-1}) and UV maxima (207.1, 241.0, and 296.0 nm) of **1** were consistent with those reported for a compound with a 1,4-dihydroxy-2-pyridinone moiety.15 In its HMBC spectrum, H-2′ and H-6′ (δ_H 7.49) showed cross peaks with C-3′ and C-5′ (δ_C128.6), C-4′ (δ_C 127.9), C-1′ (δ_C 133.4), and C-5 (δ_C 114.2), and H-6 (δ_H 7.69) with C-2 (δ_C 158.0), C-4 (δ_C 161.4), C-5 (δ_C 114.2), and C-1′ (δ_C 133.4) supporting the partial structure of the substituted pyridinone ring moiety. In the aliphatic region, the ^1H NMR spectrum showed two doublets representing two oxymethine hydrogens, and four methyl resonances, comprising three doublets and a triplet. The ^1H NMR data additionally indicated the presence of a tetrahydropyrano ring attached to C-3 of the pyridinone ring similar to TMC-69-6H.12 The H-7 oxymethine doublet (δ_H 4.72) showed cross peaks in the HMBC spectrum with C-4 (δ_C 161.4), C-2 (δ_C 158.0), C-3 (δ_C 110.4), C-11 (δ_C 89.9), C-9 (δ_C 42.2), C-8 (δ_C 36.8), and C-15 (δ_C 17.5). The H-11 oxymethine doublet at δ_H 3.07 similarly displayed HMBC correlations with C-7 (δ_C 81.4), C-9 (δ_C 42.2), C-12 (δ_C 35.6), C-13 (δ_C 22.1), C-16 (δ_C 17.5), and C-17 (δ_C 17.2). The methyl triplet at δ_H 0.98 showed HMBC cross peaks with C-12 (δ_C 35.5) and C-13 (δ_C 22.0) confirming its ω location in the chain. These correlations strongly suggested the gross structure **1** depicted for this compound. The HMBC correlations of other proton resonances and COSY correlations further supported this structure (Figure 1).

The relative configuration of compound **1** was determined by the ^1H NMR coupling constants and ROESY data. A $^3J_{7,8}$ value of 10.2 Hz suggested that H-7 and H-8 were *trans*-diaxially oriented. A $^3J_{10,11}$ value of 10.2 Hz similarly indicated the *trans*-diaxial arrangement of H-10 and H-11. The lack of three-bond coupling of H-11 and H-12 may reflect a C-11 - C-12 conformation having H-11 and H-12 orthogonally oriented in order to minimize Van der Waals interaction between the C-10 and C-11 alkyl groups. In the ROESY spectrum H-7 displayed interactions with H-11 and CH_3-15 indicating the cofacial orientation of the three groups. Similarly, H-11 showed interactions with H-7 and CH_3-16 (Figure 1).

This compound afforded crystals that were suitable for crystallographic analysis. On the basis of CuKα X-ray diffraction data, the absolute configuration of compound **1** was assigned as 7*R*, 8*R*, 10*S*, 11*R*, 12*R*. The solid-state structure contains four independent molecules, one of which is illustrated in Figure 2. All four have an intramolecular OH...O hydrogen bond to the tetrahydropyran oxygen, with O...O distances in the range 2.577 (19) to 2.5930(19) Å. The four molecules differ only slightly in conformation, except for that about the bond joining the heterocyclic and phenyl rings, for which C-C-C-C torsion angles vary from −50.5(3) to +44.(3)º.

Compound **2** had a molecular formula of $C_{23}H_{31}NO_4$ as determined by HRESIMS. The ^1H and ^{13}C NMR spectra were similar to those of compound **1** except for the presence of an *O*-methyl resonance in the spectra of the former. This indicated that compound **2** was an *O*-methyl analogue of compound **1**. The COSY, HMQC, and HMBC spectra of compound **2** showed correlations identical to those observed for compound **1**. Additionally, the *O*-methyl

resonance showed no cross peaks in the HMBC spectrum whereas the OH proton (δ_H 9.69) correlated with C-3 (δ_C 111.8), C-4 (δ_C 161.8), and C-5 (δ_C 114.2) suggesting that the *O*-methyl group was located on the nitrogen. The up-field shift of H-6 of compound 2 relative to that of compound 1 is consistent with the structural modification. Identical coupling constants and ROESY correlations indicated that compounds **1** and **2** have the same relative configuration.

The molecular formula of compound **3** was established as $C_{23}H_{31}NO_5$ by HRESIMS. Its ^{1}H NMR spectrum was similar to compound **2**, the major differences being the replacement of the methyl triplet by two diastereotopic oxymethylene hydrogens in the spectrum of **3**. This suggested that compound **3** was the C-14 oxygenated analogue of **2**. Comparison of the ^{13}C NMR data of these compounds also confirmed the replacement of a methyl by an oxymethylene group in **3**. The COSY spectrum of compound **3** displayed cross peaks between the C-14 oxymethylene (δ_H 3.58, 3.73) and C-13 methylene protons (δ_H 1.23, 1.75). The COSY correlations for the rest of the hydrogen resonances were identical to those of compound **2**. In the HMBC spectrum the oxymethylene protons showed cross peaks with C-13 (δ_C 32.3) and C-12 (δ_C 30.1). The remaining HMBC correlations were identical to those observed for compound **2**. These observations suggested that compound **3** was the C-14 hydroxy analogue of **2**. ROESY correlations and coupling constants of H-7, H-9, and H-11 of compound **3** were identical to those observed for compound **1** indicating that these two compounds had the same relative configuration.

The molecular formula of compound **4** was determined by HRESIMS as $C_{25}H_{33}NO_6$. The ^{1}H and ^{13}C NMR spectra, COSY, and HMBC correlations of this compound were similar to those observed for compound **3**. Major differences involved the presence of an additional methyl singlet (δ_H 2.01) and two carbon signals [δ_C 21.2 (CH_3), 171.8 (CO)] in its 1H and 13C NMR spectra, respectively. The down-field shift of proton signals due to the C-14 oxymethylene group and HMBC correlations between these protons and the acetyl carbonyl carbon indicated that compound **4** was the 14-*O*-acetyl analogue of compound **3**.

Since the pyridinone analogues **1–4** presumably share a common biosynthetic origin involving an aromatic amino acid and an activated polyketide,16 analogues **2**, **3**, and **4** also possess 7*R*, 8*R*, 10*S*, 11*R*, 12*R* absolute configuration. Such an assumption is supported by the fact that all four compounds are strongly dextrorotatory.

The in vitro antiplasmodial activities of compounds **1–4** are summarized in Table 3. Compound **1** demonstrated moderate antiplasmodial activity whereas compounds **2–4** were inactive. The selectivity index of antiplasmodial activity was calculated based on their cytotoxicity to mammalian kidney fibroblasts (Vero cells) determined in parallel experiments. Compound **1** was cytotoxic to Vero cells, and its low selectivity indices preclude it as an antimalarial drug lead.

Compound **2** exhibited moderate activity against both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (Table 4). The cytotoxic potential of these compounds was further evaluated against a panel of solid tumor cell lines (SK-MEL, KB, BT- 549, SK-OV-3) and kidney epithelial cells (LLC-PK_{11}) (Table 5). Compound **1** showed moderate cytotoxicity against all the cell lines while compound **2** was inactive. The presence of an *N*-hydroxy group appears to be essential for the activity of these compounds. It is interesting to note that free an *N*-hydroxy group was also a prerequisite for the activity of cordypyridones, a group of 1,4-dihydroxy-2-pyridinone alkaloids, with moderate antiplasmodial activity previously reported from a fungus.15

Experimental Section

General Experimental Procedures

Melting points were measured with a Uni-melt, Thomas Hoover capillary melting point apparatus. Optical rotations were measured using a Rudolph Research Analytical Autopol IV automatic polarimeter model 589-546. UV and IR spectra were recorded on a Varian-50 Bio UV visible spectrophotometer and a Bruker-Tensor-27 infrared spectrophotometer, respectively. NMR spectra were recorded on a Varian-Mercury-plus-400 or Varian Unity-Inova-600 spectrometer using $CDCl_3$/methanol-d_4 as the solvent unless otherwise stated. MS analyses were performed on an Agilent Series 1100 SL equipped with an ESI source (Agilent Technologies, Palo Alto, CA, USA). Column chromatography was carried out on Merck silica gel 60 (230–400 mesh). Preparative TLC was carried out using silica gel GF plates (20 x 20 cm, thickness 0.25 mm).

Fermentation, Extraction, and Isolation

Septoria pistaciarum Caracc—(ATCC 22201) was obtained from the American Type Culture Collection, Manassas, VA and grown in potato dextrose agar plates at 27 ºC for 12 days. An Erlenmeyer flask containing 100 mL of potato dextrose broth was inoculated with small agar pieces (0.25” × 0.25”) and were incubated at 27 ºC for 7 days on a shaker (RPM = 100). This seed culture was used to inoculate 20 × 1L Erlenmeyer flasks each containing 500 mL of M-2 medium, (modified Czapex Dox medium: Czapex Dox 35.0 g, yeast extract 1.5 g, malt extract for 28 days on a shaker (RPM = 100). 1.5 g in 1L of distilled H_2O) and were incubated at 27 ºC The culture broth and mycelia were extracted with EtOAc (×3) and the organic layer was evaporated under reduced pressure to give an oily residue (5.2 g).

The oily extract (5.0 g) was chromatographed over Sephadex LH -20 and eluted with $CHCl_3$/MeOH (20:80) to give 12 fractions. Fractions 3, 4, 5, 6, and 7 showed antiplasmodial activity (IC_{50} < 529 ng/mL). Fractions 3, 4, and 5 were combined and chromatographed over a reversed phase C-18 column eluting with a gradient 10% to 100% MeOH/H_2O to yield five fractions. Subfraction 4 was further purified by crystallization from MeOH and CH_2Cl_2 to give compound **1** as white crystals (53.0 mg). Combined fractions 6 and 7 were chromatographed over a silica gel column using CH_2Cl_2/hexanes as the eluent to give 10 subfractions. Subfraction 7 was further separated on Sephadex LH-20 (MeOH) followed by preparative TLC (2% MeOH in CH_2Cl_2) to give compound **2** (20.0 mg) and compound **3** (12.0 mg). Subfraction 4 was chromatographed over reversed phase C-18 eluting with CH_3CN/H_2O (40:60) to give compound **4** (3.0 mg).

Compound 1: white crystals (CH_2Cl_2/MeOH); mp 220 0C; $[\alpha]^{26}_D$ +201 (*c*, 0.4, CH_3OH); UV (MeOH) λ_{max} (log ε) 207.1 (3.78), 241.0 (3.75), 296.0 (3.03) nm; IR ($CHCl_3$) ν_{max} 3109, 2963, 2927, 1642, 1556, 1454, 1225 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS $[M - H]^-$ *m/z* 370.2012 (calcd for $(C_{22}H_{29}NO_4 - H)^-$, 370.2018).

Compound 2: amorphous; $[\alpha]^{26}_D$ +15 (*c*, 0.375, MeOH); UV (MeOH) λ_{max} (log ε) 207.1 (2.15), 241.0 (1.96), 297.0 (0.88) nm; IR ($CHCl_3$) ν_{max} 3318, 2917, 1686, 1047 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS $[M + H]^+$ *m/z* 386.2423 (calcd for $(C_{23}H_{31}NO_4 + H)^+$, 386.2331).

Compound 3: amorphous; $[\alpha]^{26}_D$ +190 (*c*, 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 205.0 (3.61), 241.0 (3.46), 290.0 (2.72) nm; IR ($CHCl_3$) ν_{max} 3212, 2929, 2341, 1647, 1557, 1456, 1057 cm^{-1}; 1H and 13C NMR data (see Tables 1 and 2); HRESIMS [M + H]+ *m/z* 402.236 (calcd for $(C_{23}H_{31}NO_5 + H)^+$, 402.228).

Compound 4: amorphous: $[\alpha]^{26}_{D}$ +47 (*c*, 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 205.0 (2.56), 241.0 (2.69) nm; IR ($CHCl_3$) ν_{max} 3331, 2925, 2853, 1736, 1647, 1227, 1053 cm^{-1}; 1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS $[M + Na]^+$ *m/z* 466.2307 (calcd for $(C_{25}H_{33}NO_6 + Na)^+$, 466.2205).

Crystallographic Data for Compound 1

X-ray data were collected at low-temperature (90 K) on a Bruker Kappa Apex-II diffractometer equipped with graphite-monochromated CuKα (λ = 1.54178 Å) source, yielding 14,467 independent data to θ_{max} = 69.0º. Crystals were monoclinic, space group $P2_1$, with a = 11.1427(9), b = 13.0004(10), c = 28.073(2) Å, β = 90.430(5)º, Z = 8. Refinement of the model containing four independent molecules resulted in R = 0.038 for 1015 parameters. Resonant scattering, principally from the O atoms, resulted in a Flack17 parameter X = 0.01(9) and a Hooft18 parameter Y = 0.09(5) for 6739 Bijvoet pairs. This corresponds to a probability of 1.000 that the illustrated configuration is correct.

Antimicrobial Assay

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113 and *Aspergillus fumigatus* ATCC 90906 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI methods19,20 as described by Samoylenko et al.21 The drug controls ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included in each assay.

Assay for in vitro Antiplasmodial Activity

In vitro antiplasmodial activity was determined against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum* as described earlier.22 A 200 μL suspension of red blood cells, infected with *P. falciparum* (2% parasitemia and 2% hematocrit), in RPMI 1640 medium supplemented with 10% human serum and 60 μg/mL amikacin was added to the wells of a 96- well plate containing 10 μL of serially diluted samples. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2 and incubated at 37 ºC for 72 h in a modular incubation chamber (Billups-Rothenberg, CA). Parasitic lactate dehydrogenase (LDH) activity was determined by mixing 20 μL of the incubation mixture with 100 μL of Malstat reagent (Flow Inc., Portland, OR) and incubating at room temperature for 30 min. Twenty μL of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was added, and the plate further incubated in the dark for 1 h. The reaction was stopped by adding 100 μL of HOAc (5%). Plates were read at 650 nm. Artemisinin and chloroquine were included as the drug controls, and IC_{50} values were computed from the dose response curves.

Assay for Cytotoxicity to Mammalian Cells

In vitro cytotoxicity was determined against a panel of mammalian cells that included kidney fibroblast (Vero), kidney epithelial ($LLC\text{-}PK_{11}$), malignant melanoma (SK-MEL), oral epidermal carcinoma (KB), breast ductal carcinoma (BT-549), and ovary carcinoma (SK-OV-3) cells.23 The assay was performed in 96-well tissue culture-treated plates. Cells were seeded to the wells of a 96-well plate at a density of 25,000 cells/well and incubated for 24 h. Samples at different concentrations were added and plates were again incubated for 48 h. The number of viable cells was determined by using Neutral Red dye and IC_{50} values were obtained from dose response curves. Doxorubicin was used as a positive control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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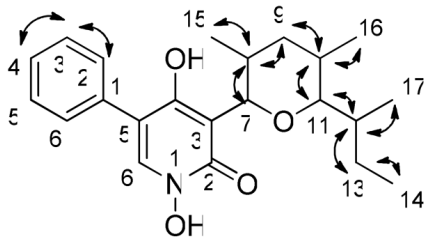
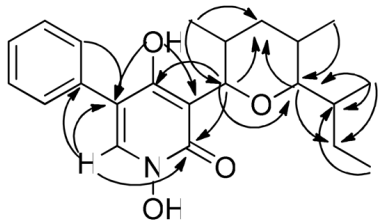


Key COSY ( ) correlations



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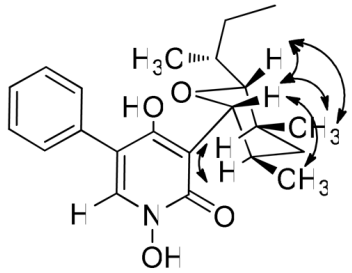

Key ROESY ( ) correlations

Figure 1.
COSY, HMBC, and ROESY correlations of compound **1**.

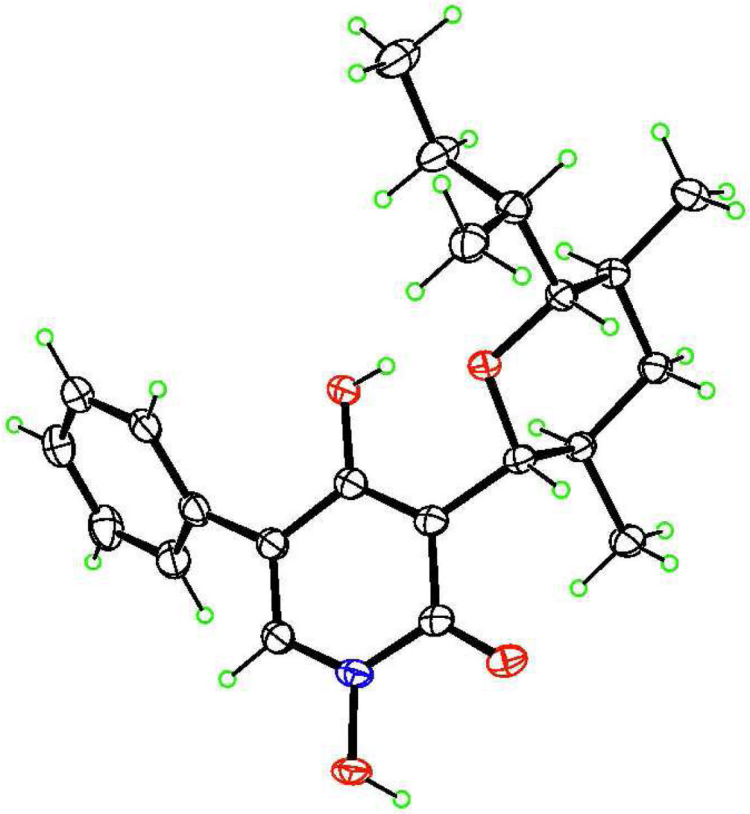

Figure 2.
Single-crystal X-ray structure of compound **1**, showing the absolute configuration of one of the four independent molecules.

Table 1

^{1}H NMR Data (δ_H (J in Hz) (400 MHz, $CDCl_3$/Methanol-d_4) for Compounds **1–4**.

	1		**2**		**3**		**4**	
Position	**δ_H (J in Hz)**	**HMBC**	**δ_H (J in Hz)**	**HMBC**	**δ_H (J in Hz)**	**HMBC**	**δ_H (J in Hz)**	**HMBC**
6	7.69, s	1′, 2, 4, 5	7.43, s	1′, 2, 4	7.42, s	1′, 2, 4, 5	7.44, s	1′, 2, 4, 5
7	4.72, d (10.2)	2, 3, 4, 8, 9, 11, 15	4.73, d (10.2)	2, 3, 4, 8, 9, 11	4.72, d (10.2)	2, 4, 8, 9, 11, 15	4.75, d (10.2)	2, 4, 8, 9, 11, 15
8	1.88, m	7, 15	1.87, m	7, 15	1.88, m	7, 15	1.88, m	7, 15
9	1.82, dt (13.2, 3.6) 1.12, q (12.6)	7, 8, 10, 15, 16	1.83, dt (13.2, 3.6) 1.14, q (12.6)	7, 8, 10, 11, 15, 16	1.82, dt (13.2, 3.6) 1.13, q (12.6)	7, 8, 10, 11, 15, 16	1.85 1.13, q (12.6)	7, 8, 10, 11, 15, 16
10	1.76, m	16	1.75, m	9. 16	1.76, m	11, 16	1.71, m	16
11	3.07, d (10.2)	7, 9, 12, 13, 16, 17	3.07, d (10.2)	7, 9, 12, 13, 16, 17	3.08, d (9.6)	7, 9, 12, 13, 16, 17	3.10, d (10.2)	7, 9, 12, 13, 16, 17
12	1.66, m	14, 17	1.65, m	14	1.96, m	13, 14, 17	1.61, m	14, 17
13	1.02, m 1.56, m	11, 12, 14, 17	1.00, m 1.54, m	12, 14, 17	1.23, m 1.74, m	11, 14, 17	1.21, m 1.61, m	12, 14, 17
14	0.90, t (7.2)	12, 13	0.88, t (7.2)	12, 13	3.56, brq (9.0) 3.71, m	12, 13	4.02, m 4.13, m	12, 13
15	0.73, d (6.6)	7, 8, 9	0.88, d (6.6)	7, 8, 9	0.86, d (6.6)	7, 8, 9	0.88, d (6.6)	7, 8, 9
16	0.84, d (6.6)	9, 10, 11	0.83, d (6.6)	9, 10, 11	0.84, d (6.6)	9, 10, 11	0.85, d (6.6)	9, 10, 11
17	0.98, d (6.6)	11, 12, 13	0.96, d (6.6)	11, 12, 13	0.97, d (7.2)	11, 12, 13	1.00, d (6.6)	11, 12, 13
2′,6′	7.49, d (7.8)	1′, 3′, 4′, 5, 5′	7.45, d (7.2)	1′, 3′, 4′, 5, 5′	7.42, d (7.8)	1′, 3′, 4′, 5, 5′	7.45, d (7.2)	1′, 3′, 4′, 5, 5′
3′,5′	7.43, t (7.8)	1′, 2′, 4′, 6′	7.40, t (7.8)	1′, 2′, 4′, 6′	7.38, t (7.8)	1′, 2′, 4′, 6′	7.40, t (7.2)	1′, 2′, 4′, 6′
4′	7.35, t (7.2)	2′, 3′, 5′, 6′	7.33, t (7.8)	2′, 3′, 5′, 6′	7.13, t (7.8)	2′, 3′, 5′, 6′	7.34, t (7.2)	2′, 3′, 5′, 6′
OH	9.72, s	3, 4, 5	9.69, s	3, 4, 5	9.66, s	3, 4, 5	9.49, s	3, 4, 5
OCH_3			4.01, s		4.03, s			
$COCH_3$							2.01, s	

Table 2

^{13}C NMR data (δ_C) (100 MHz, $CDCl_3$/Methanol-d_4) of Compounds **1–4**.

Position	1	2	3	4
2	158.0	158.0	158.0	158.0
3	110.4	111.8	111.7	111.7
4	161.4	161.8	161.8	161.7
5	114.2	114.3	114.4	114.3
6	131.8	132.9	133.0	133.4
7	81.4	81.3	81.4	81.5
8	36.8	36.9	36.8	36.8
9	42.2	42.2	42.1	42.0
10	32.6	32.6	32.6	32.7
11	89.9	89.9	89.8	89.6
12	35.6	35.6	30.1	30.4
13	22.1	22.1	32.3	28.4
14	12.8	12.8	61.0	63.0
15	17.5	17.8	17.7	17.8
16	17.5	17.5	17.6	17.3
17	17.2	17.1	17.4	17.6
1′	133.4	133.4	133.3	133.0
2′,6′	129.3	129.2	129.2	129.3
3′,5′	128.6	128.6	128.5	128.6
4′	127.9	127.8	127.8	128.0
OCH_3		65.0	65.0	65.1
$CO\underline{CH_3}$				21.1
$\underline{C}OCH3$				171.8

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Table 3

Antiplasmodial Activity of Compounds **1–4**

Compound	Chloroquine-sensitive (D6)-clone		Chloroquine-resistant (W2)-clone		Cytotoxicity to Vero cells
	IC_{50} ng/mL	**S.I.**	**IC_{50} ng/mL**	**S. I.**	**IC_{50} ng/mL**
1	330	5.0	200	4.8	1300
2	NA		NA		NC
3	NA		NA		NC
4	NA		NA		NC
chloroquine[a]	10		100		NC
artemisinin[a]	4.7		3.8		NC

[a]Positive controls

NC, not cytotoxic at the highest dose (4760 ng/mL) tested.

NA, not active at the highest dose (4760 ng/mL) tested.

S. I. (selectivity index) = IC_{50} for cytotoxicity/IC_{50} for antiplasmodial activity

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Table 4

Antifungal and Antibacterial Activity of Compounds **1–2**.

Compound	*Candida albicans*		*Aspergillus fumigatus*		*Staphylococcus aureus*		MRSA	
	IC_{50}	**MIC**	**IC_{50}**	**MIC**	**IC_{50}**	**MIC**	**IC_{50}**	**MIC**
1	12.07	NT	8.76	NT	NA	NA	NA	NA
2	NA	NA	NA	NA	2.79	5.00	2.99	5.00
amphotericin B	0.42	2.50	1.16	2.50	NT	NT	NT	NT
ciprofloxacin	NT	NT	NT	NT	0.09	0.25	0.05	0.25

IC_{50} and MIC (minimum inhibitory concentration, the lowest test concentration that allows no growth) values are in μg/mL.

NA: not active at the highest test concentration of 20 μg/mL.

NT: not tested.

Table 5

Cytotoxic Activity [IC_{50} (μg/mL)] of Compounds **1–2**

	SK-MEL	KB	BT-549	SK-OV-3	LLC-PK_{11}	Vero
1	1.5	5.0	1.65	2.6	1.65	1.4
2	NA	NA	NA	NA	NA	NA
doxorubicin[a]	0.6	1.35	0.8	1.2	0.6	>5.0

NA: not active at the highest test concentration of 10 μg/mL.

[a]Positive control.