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Antiplasmodial Chromanes and Chromenes from the Monotypic Plant Species *Koeberlinia spinosa*

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Abstract

Nine new compounds containing either a chromane or chromene ring moiety were isolated from the monotypic plant *Koeberlinia spinosa*. Compounds **1** – **4** are chromanes with all possible *E* and *Z* isomers of the isoprenoid side chain, with compound **5** a methylated derivative of **1**. Compounds **6** and **7** were assigned as diastereomeric cyclized derivatives of **2**, and were probably artifacts formed during the extraction or the isolation processes. Compounds **8** and **9** were characterized as new chromenes. Structure elucidation of **1** – **9** was conducted via 1D and 2D NMR spectroscopic data interpretation, and absolute configurations were determined by ECD spectroscopic analysis. Compounds **2**, **5**, **6**, and **7** had weak antiplasmodial activity, while none of the compounds exhibited antiproliferative activity. The isolation, structure elucidation, and biological evaluation of these compounds are presented.

Graphical Abstract

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Dedicated to Dr. Susan Band Horwitz, of Albert Einstein College of Medicine, Bronx, NY, for her pioneering work on bioactive natural products.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.005799.

¹H, ¹³C, gHMBC, gHSQC, selective NOESY, and UV and ECD spectra for compounds **1** – **9** (PDF)

Koeberlinia spinosa Zucc., also known as “crucifixion thorn”, is a monotypic species in the family Koeberliniaceae. The plant is a thorny, leafless, and xerophytic shrub found in North America and located primarily in the southwestern United States and northern Mexico.¹ In 2009 Lucero et al. identified 18 known volatile organic compounds from *Koeberlinia spinosa* via GC-MS analysis of an essential oil extracted from various plant parts by steam distillation, with the purpose of identifying known compounds to aid in biochemical ecology.² The extraction and analysis methods used by these investigators favored the identification of small volatile molecules, since larger molecules are not extracted or are not detected by GC-MS. In addition, the elevated temperatures of the distillation could have caused thermal decomposition of some larger and more complex compounds, and no phytochemical study of non-volatile compounds was conducted.

Over the past few years our group has been investigating plant extracts that display biological activity against the malaria parasite *Plasmodium falciparum* in the expectation of finding new antimalarial compounds, since the antimalarial drugs quinine and artemisinin are both plant-derived natural products. We are interested in this vector-borne disease due to its disproportionate effect in poor and underdeveloped countries without access to western medicine, and due to the appearance of artemisinin-resistant parasites in five countries and the high failure rates with four different artemisinin-based combination therapies in Cambodia.³ This growing resistance to artemisinin means that there is an urgent need for new and affordable antimalarial medicines. Therefore, when an extract of *K. spinosa* from the Natural Products Discovery Institute (NPDI) was found to have moderate activity (IC₅₀ value 5 μg/mL) against the Dd2 drug-resistant strain of *Plasmodium falciparum*, it was selected for bioassay-guided isolation. This is the first phytochemical investigation on a whole plant extract from this genus and species.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation

A 901 g sample of the whole plant of *Koeberlinia spinosa* was collected in 1997 and extracted with ethanol to yield 82 g of extract, of which 5.2 g were provided for this study. Liquid-liquid partition of 3.7 g of this extract between aqueous ethanol and dichloromethane afforded 2.3 g of a moderately active dichloromethane fraction with an IC₅₀ value of 5 μg/mL against *P. falciparum*. This fraction was loaded onto a polyamide column and eluted with methanol to produce 1.9 g of a detanninized fraction with an IC₅₀ value between 2.5 and 5 μg/mL. Further bioassay-guided fractionation utilizing reversed-phase (RP) solid-phase extraction (SPE), RP-HPLC, diol HPLC, and open silica gel columns afforded the seven new chromanes **1** – **7** and the two new chromenes **8** and **9**.

Compound **1** was isolated as a clear oil. Its chemical formula was determined to be C₂₂H₃₀O₄ by HRESIMS ([M+Na]⁺ *m/z* 381.2017, calcd for C₂₂H₃₀NaO₄⁺ *m/z* 381.2036) and ¹³C NMR spectroscopy. Summation of all integrals from the ¹H NMR spectrum in CD₃OD indicated the presence of 28 protons, consistent with the presence of two hydroxy groups (Table 1 and Table S.1, Supporting Information).

Further examination of the spectrum revealed two *meta*-coupled aromatic protons at δ_{H} 6.43 (1H, d, *J* = 2.9 Hz, H-7) and δ_{H} 6.35 (1H, d, *J* = 2.9 Hz, H-5), as well as an aromatic methyl group at δ_{H} 2.06 (3H, s, H-13'), indicating the presence of a highly substituted aromatic ring. A deshielded one-proton signal at δ_{H} 6.19 (1H, br s, H-3') was consistent with the presence of a vinyl proton α to a carbonyl group, and a methyl group with long-range allylic coupling at δ_{H} 2.12 (3H, d, *J* = 1.2 Hz, H-11') suggested the presence of a methyl group at the β position of an α,β -unsaturated carbonyl group. A broad vinyl triplet was observed at δ_{H} 5.36 (1H, br t, *J* = 6.7 Hz, H-7') and the presence of another methyl with long-range allylic coupling at δ_{H} 1.63 (3H, d, *J* = 1.4 Hz, H-10') indicated the presence of an isoprene unit. A singlet for deshielded methylene protons at δ_{H} 3.89 (2H, s, H-9') indicated that one of the hydroxy groups is attached to an isolated methylene unit. Two isolated and geminally coupled doublets at δ_{H} 2.82 (1H, d, *J* = 13.1 Hz, H-1'a) and δ_{H} 2.55 (1H, d, *J* = 13.1 Hz, H-1'b) indicated the presence of diastereotopic protons of a methylene unit next to a stereocenter and α to a carbonyl group. A benzylic methylene triplet was observed at δ_{H} 2.71 (2H, t, *J* = 6.9 Hz, H-4) and was coupled to two geminally coupled diastereotopic protons at δ_{H} 1.90 (1H, dt, *J* = 13.6, 6.9 Hz, H-3a) and δ_{H} 1.79 (1H, dt, *J* = 13.6, 6.9 Hz, H-3b). The remaining proton signals belonged to two overlapping methylene units at δ_{H} 2.20 (4H, m, H-5' and H-6') and a methyl group at δ_{H} 1.34 (3H, s, H-12').

The ¹³C NMR spectrum of **1** (Table 1) indicated the presence of 22 carbons, corresponding to the calculated molecular formula. The ¹³C NMR and HSQC spectra showed signals for an α,β -unsaturated ketone, aromatic carbons, two double bonds, five methylenes, one oxygen-bearing methylene, one oxygen-bearing quaternary carbon, and four methyl carbons.

The HMBC spectrum of **1** (Figure 1) exhibited correlations from the aromatic methyl at δ_{H} 2.06 (CH₃-13') to carbons at δ_{C} 127.8 (C-8), δ_{C} 145.7 (C-8a), and δ_{C} 116.8 (CH-7) and from the aromatic proton at δ_{H} 6.43 (1H, d, *J* = 2.9 Hz, H-7) to carbons at δ_{C} 127.8 (C-8), δ_{C} 150.8 (C-6), and δ_{C} 113.7 (CH-5). Correlations from the aromatic proton at δ_{H} 6.35 (1H, d, *J* = 2.9 Hz, H-5) to carbons at δ_{C} 150.8 (C-6), δ_{C} 145.7 (C-8a), δ_{C} 122.0 (C-4a), and δ_{C}

23.3 (CH₂-4) and from the benzylic-methylene protons at δ_{H} 2.71 (2H, t, J = 6.9 Hz, H-4) to carbons at δ_{C} 33.1 (CH₂-3) and δ_{C} 76.2 (C-2) were also observed, and confirm the presence of a 6,8-disubstituted chromane ring system. HMBC correlations from the methyl at δ_{H} 1.34 (CH₃-12') to δ_{C} 76.2 (C-2), δ_{C} 33.1 (CH₂-3), and δ_{C} 53.7 (CH₂-1') confirmed the attachment of this methyl group and the terpenoid side chain at C-2 on the chromane ring. These observations are consistent with the spectroscopic data of other reported chromanes.^{4,5}

The position of attachment of the terpenoid side chain was also confirmed by HMBC correlations from the diastereotopic methylene protons at δ_{H} 2.82 (H-1'a) and δ_{H} 2.55 (H-1'b) to the quaternary oxygen-bearing carbon at δ_{C} 76.2 (C-2), as well as to the carbonyl carbon at δ_{C} 201.7 (C-2'), the methyl carbon at δ_{C} 25.3 (C-12'), and the α -carbon at δ_{C} 126.2 (CH-3'). HMBC correlations from the methyl at δ_{H} 2.12 (H-11') to the quaternary carbon at δ_{C} 159.5 (C-4'), the α -carbon at δ_{C} 126.2 (CH-3'), and the methylene unit at δ_{C} 41.9 (CH₂-5') confirmed the locations of the β -carbon and the end of this isoprene unit. HMBC correlations from the vinyl proton at δ_{H} 5.36 (H-7') to the methylene carbons at δ_{C} 26.7 (CH₂-6') and δ_{C} 41.9 (CH₂-5') supported the attachment of the final isoprene unit. HMBC correlations from the oxygen-bearing methylene proton at δ_{H} 3.89 (H-9') with carbons at δ_{C} 137.0 (C-8'), δ_{C} 125.1 (CH-7'), and δ_{C} 13.7 (CH₃-10') confirmed that the isoprenoid chain has a terminal hydroxy group.

Selective NOE experiments were conducted on the protons of the C-3'/4' and C-7'/8' double bonds on the terpenoid side chain to determine their configurations (Figure 2). Saturation of the α -proton at δ_{H} 6.19 (H-3') enhanced the methylene signal at δ_{H} 2.19 (H-5'), and saturation of the vinyl proton at δ_{H} 5.36 (H-7') enhanced the methylene unit at δ_{H} 3.89 (H-9'). These observations confirm that the double bonds at C-3'/4' and C-7'/8' both have the *E* configuration.

The absolute configuration at the C-2 asymmetric center in **1** was determined to be *S* on the basis of an observed negative Cotton effect at 275 – 315 nm ($[\epsilon]_{296\text{ nm}} -3.96$) in the ECD spectrum, in agreement with ECD data on tocopherols and tocotrienols (Figure 3).^{5,6} Thus, **1** was determined to be the new compound (3*E*,7*E*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one.

Compound **2** was isolated as a colorless oil, and its molecular formula was determined to be C₂₂H₃₀O₄ by HRESIMS and ¹³C NMR spectroscopy. A side-by-side comparison of the NMR spectroscopic data with those of **1** showed them to be nearly identical except for the final isoprene unit on the side chain (Table 1). Examination of the HMBC spectrum for the vinyl proton at δ_{H} 5.22 (1H, br t, J = 7.2 Hz, H-7'), the methyl group at δ_{H} 1.74 (3H, d, J = 1.4 Hz, H-10'), and the methylene unit at δ_{H} 4.04 (2H, s, H-9') all confirmed the same connectivity as in **1**. The largest chemical shift difference was observed in the ¹³C NMR spectrum for the methyl at δ_{C} 21.5 (CH₃-10') compared to the C-10' methyl in **1** at δ_{C} 13.7.

Selective NOE experiments (Figures S2.6 and S2.7, Supporting Information) confirmed the configuration of the double bonds at C-3'/4' and C-7'/8' as *E* and *Z*, respectively. Thus, saturation of the α -proton at δ_{H} 6.18 (1H, d, J = 1.2 Hz, H-3') caused enhancement of the

methylene protons at δ_{H} 2.18 (2H, m, H-5') confirming that the double bond at C-3'/4' is *E*, while saturation of the vinyl proton at δ_{H} 5.22 (H-7') caused enhancement of the methyl protons at δ_{H} 1.74 (H-10') confirming that the double bond at C-7'/8' is in the *Z* configuration.

The absolute configuration of the chiral center at C-2 was assigned as *S* from examination of the ECD spectroscopic data (Figure 3). Thus, **2** was determined to be the new compound (3*E*,7*Z*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one.

Compound **3** was assigned the composition, C₂₂H₃₀O₄, by HRESIMS and ¹³C NMR spectroscopy. Comparison of the NMR spectroscopic data of **3** with those of **1** indicated a few significant differences in the ¹H NMR data (Table 1), with the C-11' methyl group resonating at δ_{H} 1.88 (1H, d, *J* = 1.4 Hz) instead of δ_{H} 2.1 in **1** and **2**. The H-5' methylene protons resonated at δ_{H} 2.62 (2H, m), as compared with about δ_{H} 2.2 in **1** and **2**. Differences were also observed in their ¹³C NMR data (Table 2), with chemical shifts at δ_{C} 25.2 (CH₃-11') and δ_{C} 34.6 (CH₂-5') as compared with δ_{C} 19.6 and 41.9 for **1**. These observations indicated that the compounds differ in the configuration of the double bond at C-3'/4'. A selective NOE experiment with saturation of H-3' at δ_{H} 6.19 (Figure S3.6, Supporting Information) displayed enhancement of the methyl group at δ_{H} 1.88 (H-11'), confirming the C-3'/4' double bond configuration to be *Z*. Thus, saturation of the vinyl proton at δ_{H} 5.44 (Figure S3.7, Supporting Information) displayed enhancement of the methylene at δ_{H} 3.90 (2H, s, H-9') confirming the double bond configuration at C-7'/8' to be *E*.

The absolute configuration of the asymmetric center at C-2 was confirmed as *S* from examination of the ECD spectroscopic data (Figure 3). Thus, **3** was determined to be the new compound (3*Z*,7*E*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one.

Compound **4** was assigned the composition C₂₂H₃₀O₄ by HRESIMS and ¹³C NMR spectroscopy. A side-by-side comparison of its NMR spectroscopic data with those of compounds **1** – **3** (Table 2) indicated that **4** is the C-7'/8' *Z* isomer of **3**. This observation was confirmed by selective NOE experiments (Figures S4.6 and S4.7, Supporting Information) that confirmed that the double bonds at both the C-3'/4' and C-7'/8' positions are in the *Z* configuration. The absolute configuration of the asymmetric center at C-2 was confirmed as *S* from examination of its ECD spectroscopic data (Figure 3). Thus, **4** was determined to be the new compound (3*Z*,7*Z*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one.

Compound **5** was determined to have the composition C₂₃H₃₂O₄ from HRESIMS and carbon NMR spectroscopy. The ¹H and ¹³C NMR spectra of **5** were extremely similar to those of **1** (Table 1), with the only major difference being signals for a new methoxy methyl group at δ_{H} 3.70 (3H, s, OCH₃-6). HMBC correlations from the methoxy methyl at δ_{H} 3.70 to the aromatic ring at δ_{C} 154.1 (C-6) showed that the methyl group is attached to the ring by an ether linkage. Selective NOE experiments (Figures S5.6 and S5.7, Supporting

Information) confirmed that the double bonds are both in the *E* configuration. The absolute configuration for the asymmetric center at C-2 was assigned as *S* from examination of ECD spectroscopic data (Figure 3). Thus, **5** was determined to be the new compound (3*E*,7*E*)-9-hydroxy-1-[(*S*)-6-methoxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one.

Compound **6** was isolated as a clear oil with a chemical composition of C₂₂H₃₀O₄ as determined by HRESIMS and ¹³C NMR data. Compound **6** has the same chemical composition as compounds **1** – **4** and the same chromane ring system, as confirmed by comparison of its ¹H and ¹³C NMR spectra with those of **1** – **4** (Tables 1 and 2). Major differences were, however, observed for the terpenoid side chain. In the first place, the ¹H NMR spectra obtained in CD₃OD for compounds **1** – **4** indicated the presence of two exchangeable protons, but the ¹H NMR spectrum of **6** indicated the presence of only one exchangeable proton. Another significant difference was the loss of the resonance at about δ_H 6.2 for C-3' and its replacement by a pair of one-proton doublets at δ_H 3.03 and 2.37. The ¹³C NMR spectrum of **6** had resonances at δ_C 52.9 and 78.7 assigned to C-3' and C-4', respectively, consistent with alkoxylation of the 3',4' double bond. HMBC correlations were observed from the geminally coupled diastereotopic protons at δ_H 3.00 (1H, d, *J* = 14.1 Hz, H-1'a) and δ_H 2.50 (1H, d, *J* = 14.1 Hz, H-1'b) to C-2', CH₂-3, CH₃-12', C-2 and also from the diastereotopic protons at δ_H 3.03 (1H, d, *J* = 13.7 Hz, H-3'a) and δ_H 2.37 (1H, d, *J* = 13.7 Hz, H-3'b) to C-2', C-4', CH₂-5', and CH₃-11' (Figure 3). These observations confirmed that the carbonyl carbon is still attached at the C-2' position on the side chain.

The fact that the diastereotopic protons at CH₂-3' have very different chemical shifts suggested that they are close to a chiral center, consistent with C-4' being the center involved. Since protons H-3'a, H-3'b, the methyl at δ_H 1.20 (3H, s, H-11'), and the diastereotopic methylene protons at δ_H 1.85 (1H, m, H-5'-a) and δ_H 1.76 (1H, m, H-5'-b) all gave HMBC correlations with the oxygen-bearing quaternary carbon at δ_C 78.7, this was assigned as the asymmetric quaternary carbon at C-4'. HSQC and HMBC correlations established the signals for the methylene units at C-5' and C-6'.

The diastereotopic protons at δ_H 1.85 (m, 1H, H-5'a) and δ_H 1.76 (m, 1H, H-5'b) are attached to the carbon at δ_C 40.2 (C-5') and gave HMBC correlations with C-3', C-4', C-11', another methylene at δ_C 23.96 (C-6') and a vinyl carbon at δ_C 125.8 (C-7'); these correlations were used to establish the carbon at δ_C 40.2 as C-5'. The diastereotopic protons at δ_H 2.18 (1H, m, H-6a') and δ_H 2.03 (1H, m, H-6b') are attached to the carbon at δ_C 23.96 and gave HMBC correlations with C-4', C-5', the vinyl carbon at δ_C 125.8 (C-7'), and a quaternary carbon at δ_C 137.1 (C-8'), which established the carbon at δ_C 23.96 as C-6'. The methyl group at δ_H 2.03 (3H, d, *J* = 2.1 Hz, H-10') is attached to the carbon at δ_C 21.4 (C-10') and showed HMBC correlations with the vinyl carbon at δ_C 125.8 (C-7'), the quaternary carbon at δ_C 137.1 (C-8'), and the oxygen-bearing carbon at δ_C 65.6 (C-9'). The vinyl proton at δ_H 5.36 (1H, m, H-7') had HMBC correlations with C-5', C-6', the methyl at δ_C 21.4 (C-10'), and the oxygen-bearing carbon at δ_C 65.6 (C-9'), when the experiment was optimized for a coupling of 12 Hz. The geminally coupled protons at δ_H 3.83 (1H, d, *J* = 16.6 Hz, H-9a') and δ_H 3.68 (1H, d, *J* = 16.6 Hz, H-9b') had HMBC correlations with the vinyl carbon at δ_C 125.8 (C-7'), the quaternary carbon at δ_C 137.1 (C-8'), and the methyl at δ_C 21.4 (C-10'). These correlations taken together were used to determine the end unit of

the side chain. Finally, the geminally coupled methylene protons at CH₂-9', which occurred as singlets in compounds **1** – **5**, had one additional HMBC correlation with the new oxygen-bearing quaternary carbon C-4'. This correlation confirmed that the α,β -unsaturated ketone of **1** underwent an internal cyclization with the hydroxy group at the end of the side chain to generate a cyclized seven-membered ring. A selective NOE experiment with saturation of the vinyl proton confirmed that the C-7'/8' double bond is in the *Z* configuration. The configuration of the chiral center at C-2 was confirmed as *S* from examination of the ECD spectroscopic data (Figure 3), but the configuration of the new chiral center at C-4' could not be determined. Compound **6** was thus assigned as either the new compound 1-[(*R*)-2,6-dimethyl-2,3,4,7-tetrahydrooxepin-2-yl]-3-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]propan-2-one or 1-[(*S*)-2,6-dimethyl-2,3,4,7-tetrahydrooxepin-2-yl]-3-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]propan-2-one. The difficulty of assigning the configuration of the tetrahydrooxepine ring has been noted by other investigators.^{7,8}

Compound **7** was isolated as a clear oil and was found to have the elemental composition C₂₂H₃₀O₄ from its HRESIMS, ¹³C NMR, and the HSQC spectroscopic data. Twenty-nine of the 30 protons were observed in its ¹H NMR spectrum obtained in CD₃OD, indicating the presence of only one hydroxy group, as in **6**. A side-by-side comparison of the ¹³C NMR spectra of **6** and **7** (Table 2) revealed them to be extremely similar. A similar comparison of the ¹H NMR spectra of **6** and **7** (Table 2) indicated that the major differences were for the protons in close proximity to the chiral center at C-4', and its HMBC correlations confirmed the same connectivity for the cyclized side chain as were observed for **6** (Figure 6).

Although **6** and **7** are very similar in almost every respect, they differ in HPLC retention times, with a *t_R* of 22.03 min for **6** and 23.67 min for **7** under standard conditions (Figures S6.9 and S7.12, Supporting Information). These findings indicated that **6** and **7** are diastereomers, and since the chiral center at C-2 was again confirmed as *S* from examination of its ECD spectroscopic data (Figure 3), **7** was concluded as being diastereomeric with **6** at C-4'. The ECD spectroscopic data and specific rotations of **6** and **7** were very similar, and could not be used to assign the configurations at C-4'. Thus, **7** was determined to be either the new compound 1-[(*S*)-2,6-dimethyl-2,3,4,7-tetrahydrooxepin-2-yl]-3-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]propan-2-one or 1-[(*R*)-2,6-dimethyl-2,3,4,7-tetrahydrooxepin-2-yl]-3-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]propan-2-one. The observation that compounds **6** and **7** are diastereomeric at C-4' suggests that the two compounds might be artifacts formed during extraction or isolation. A trace of acid could protonate the carbonyl group of **2** and activate the α,β -unsaturated ketone to Michael attack by the C-10' hydroxy group to generate the seven-membered ring seen in **6** and **7** (Scheme 1). Deprotonation and tautomerization would afford diastereomers **6** and **7**.

Compound **8** was isolated as a pale yellow oil and was found to have the molecular composition of C₂₂H₂₈O₄ as determined by its HRESIMS and ¹³C NMR data. Its ¹H NMR spectrum was similar to that of **1** (Tables 1 and 2), but it contained signals for two new deshielded doublets at δ_{H} 6.28 (1H, d, *J* = 9.0 Hz, H-4) and δ_{H} 5.71 (1H, d, *J* = 9.0 Hz, H-3). Examination of the aliphatic region revealed that the benzylic methylene protons and the diastereotopic protons at position C-3 were missing (Table 2). Integration of the ¹H NMR spectrum in CD₃OD revealed 26 protons, indicating the presence of two hydroxy groups.

Comparison of the ^{13}C NMR spectrum of **8** with that of **1** revealed that the chromane ring moiety is modified as a chromene ring moiety, and this conclusion was confirmed by HMBC correlations from the methyl at δ_{H} 1.47 (3H, s, H-12') to δ_{C} 78.3 (C-2), δ_{C} 130.7 (C-3), and δ_{C} 55.2 (C-1') and from one of the new signals at δ_{H} 6.28 (1H, d, $J = 9.0$ Hz, H-4) to δ_{C} 122.5 (C-4a) and δ_{C} 144.7 (C-8a). These assignments agreed with those of other reported chromenes.^{9,10} Comparison of the ^1H and ^{13}C NMR data for the side chain with those of **1** led to the same assignments, and the side-chain double bond configurations were determined as *E* by selective NOE experiments. The stereochemistry of the chiral center at C-2 was determined to be *R* by observation of a negative Cotton effect at 290 – 370 nm ($[\epsilon]_{321\text{ nm}} -6.00$) in the ECD spectrum (Figure 3). Thus, **8** was assigned as the new compound (3*E*, 7*E*)-9-hydroxy-1-[(*R*)-6-hydroxy-2,8-dimethyl-2*H*-chromen-2-yl]-4,8-dimethylnona-3,7-dien-2-one.

Compound **9** was also isolated as a pale yellow oil, with the elemental composition of $\text{C}_{22}\text{H}_{30}\text{O}_3$, as deduced from its HRESIMS and ^{13}C NMR data. Summation of the integration in CD_3OD only accounted for only 28 of the 30 protons, indicating the presence of two hydroxy groups. Comparison of the ^1H and ^{13}C NMR data of **8** with **9** (Table 2) revealed the presence of the same chromene ring moiety as well as the loss of the carbonyl oxygen at C-2'. Signals for a vinyl proton coupled to a new methylene unit were observed in the ^1H NMR spectrum at δ_{H} 5.14 (1H, m, H-3') and δ_{H} 2.12 (2H, m, H-2'), indicating the loss of the C-2' carbonyl group. This was confirmed by HMBC correlations from the methylene protons at δ_{H} 1.65 (2H, m, H-1') to δ_{C} 131.5 (C-3), δ_{C} 78.8 (C-2), δ_{C} 23.7 (C-2'), and δ_{C} 125.7 (C-3'). The rest of the side-chain chemical shifts matched those of **8**. The double bond configuration was determined by utilizing selective NOE experiments to confirm that both double bonds on the side chain are *E*. The configuration of the asymmetric center at C-2 was assigned as *R* by a negative Cotton effect in the ECD spectrum between 290 – 370 nm ($[\epsilon]_{321\text{ nm}} -5.27$). Thus, compound **9** was assigned as the new chromene (*R*)-2-[(3*E*,7*E*)-9-hydroxy-4,8-dimethylnona-3,7-dien-1-yl]-2,8-dimethyl-2*H*-chromen-6-ol.

Biological Activities

Chromane and chromene derivatives have been reported to possess potent antioxidant properties.¹¹ All compounds were evaluated against the chloroquine-resistant Dd2 strain of *P. falciparum* and the A2780 ovarian cancer cell line (Table 3).

Comparison of the antiparasmodial activities of the *E* and *Z* isomers **1** – **4** demonstrates that the configuration of both double bonds is crucial for this activity. Thus compound **2**, with an *EC*-3'/4' double bond and a *ZC*-7'/8' double bond, showed the most potent biological activity among these compounds. In addition **1** was inactive against *P. falciparum*, but its methylated derivative **5** proved to be moderately active, suggesting that the methoxy group is also important to the observed biological activity. The antiparasmodial activities of the cyclized compounds **6** and **7** also differ by a factor of 4.

A good antimalarial drug needs to be nontoxic to the individual taking the drug, so the isolated compounds were also tested for antiproliferative biological activity against the drug-sensitive A2780 ovarian cancer cell line as an approximate indication of toxicity to human

cells. None of the compounds tested were cytotoxic ($IC_{50} > 10 \mu M$) against the A2780 cell line under the conditions used.

Conclusions

Nine new compounds (**1** – **9**) were isolated from *K. spinosa*. All compounds were determined to contain either a chromane or chromene ring moiety with an isoprenoid side chain. Compounds **6** and **7** were determined to be diastereomers, but the absolute configuration at the new stereocenter could not be determined; the compounds are most likely artifacts generated during the extraction or isolation process. Bioassay results from testing against the Dd2 strain of *P. falciparum* indicated that both stereospecific cyclization of the side chain and an aromatic methoxy group modestly increased antiplasmodial activity.

EXPERIMENTAL SECTION

General Experimental Procedures

All chemicals used were purchased from either Sigma-Aldrich or the Fisher Scientific Company and were used without further purification. Optical rotations were measured on a JASCO P-2000 polarimeter. UV spectra and ECD analysis were obtained on a JASCO J-810 spectropolarimeter with a 1 cm cell in methanol or chloroform at room temperature with constant nitrogen flushing under the following conditions: speed 100 nm/min, time constant 1 s, bandwidth 1.0 nm. 1H and ^{13}C NMR spectra were obtained on a Bruker Avance 500 spectrometer. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS spectrometer. Semipreparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD-M10A diode array detector, a SCL-10A system controller, and a Phenomenex 5 μm , 100 Å, Luna C₁₈(2) (250 $\times$ 10 mm) column or a Kromasil 5 μm , 60 Å, Diol (250 $\times$ 10 mm) column. Solid-phase extractions were conducted with Thermo Scientific Hypersep C₁₈ 500 mg, 3 mL SPE cartridges, and all reverse phase TLC was conducted with Sorbtech C₁₈-W silica TLC plates, w/UV254 on an aluminum backing with a thickness of 150 μm .

Antimalarial Bioassay

Each fraction and isolated compound was tested against the chloroquine/mefloquine-resistant Dd2 strain of *Plasmodium falciparum* in a 72-hour treatment assay using the malaria SYBR green I-based fluorescence assay as described previously.^{12,13} The antimalarial drug artemisinin was used as a positive control, with IC_{50} values of 9.0 ± 3 nM.

Antiproliferative Bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Tech as previously reported.^{14,15} The A2780 cell line is a drug-sensitive ovarian cancer cell line.¹⁶

Plant Material

A sample of *Koeberlinia spinosa* was collected on April 25th, 1997 by D. E. Atha and Favio González of the New York Botanical Garden. Collection was in Pecos County, Texas between Sanderson and Fort Stockton on U.S. 285, about 35 miles south of East Dickinson

Blvd. at an altitude of 1067 m. The plant was a shrub, 1.3 to 2.0 m. tall, growing in a roadside habitat. Its NYBG barcode is 01159061: http://sweetgum.nybg.org/science/vh/specimen_details.php?irn=762431.

Extraction and Isolation

A dried and ground sample of stems, twigs, and root of *Koeberlinia spinosa* (901 g) was extracted with ethanol (ca. 5 L) to yield 82 g of extract, of which 5.2 g was supplied to Virginia Tech as 0038278-09B (X-4535) for isolation studies.

The crude plant extract (3.7 g) was dissolved in 60% aqueous ethanol (1 L) and extracted with 3×600 mL of dichloromethane to afford a dichloromethane fraction (2.3 g) with an IC_{50} of about $5 \mu\text{g/mL}$ against the Dd2 strain of *P. falciparum* and an inactive aqueous EtOH fraction. The active dichloromethane fraction was then loaded on a polyamide column, which was eluted with MeOH to remove tannins to give 1.9 g of a fraction with an IC_{50} value between 2.5 and $5 \mu\text{g/mL}$. The active detanninized fraction was then separated into the six fractions C1 – C6 by C_{18} solid-phase extraction (SPE) eluted with 50:50, 25:75, 10:90, 0:100 aqueous acetonitrile mixtures followed by a chloroform flush, which afforded the two active fractions C2 (939.9 mg) and C3 (331.2 mg) with IC_{50} values of between 2.5 and $5 \mu\text{g/mL}$ and $\ll 1.25 \mu\text{g/mL}$, respectively. SPE fraction C2 was separated further into five fractions (D1 – D5) by reversed-phase HPLC on a Phenomenex C_{18} (2), $5 \mu\text{m}$, 250×10 mm column with a flow rate of 3 mL/min and an aqueous acetonitrile mobile phase starting at 55:45 to 50.8:49.2 over 25 min then a 7 min continuous flow at 0:100 $\text{H}_2\text{O}-\text{CH}_3\text{CN}$. Fraction D3 (t_R 18 to 24 min, 353.3 mg) was weakly active with an IC_{50} value between 5 and $10 \mu\text{g/mL}$. Fraction D5 was the most active fraction, and its constituents were available in a much greater quantity in fraction C3. Fraction D3 was further separated into eight fractions (E1 – E8) by C_{18} HPLC with a gradient from 30:70 to 22.5:77.5 $\text{H}_2\text{O}-\text{MeOH}$ over 35 min. Four fractions (E2 (17.0 mg, t_R 16.83 min), E4 (82.0 mg, t_R 18.37 min), and E7 (10.6 mg, t_R 21.31 min) each contained one major component with minor impurities, and final purification was achieved on silica gel columns using 1:1 hexanes-EtOAc to afford compounds **8** (20.0 mg), **1** (78.3 mg), and **4** (9.5 mg) from E2, E4, and E7 respectively. Fraction E6 (50.5 mg, t_R 19.47 min) was found to be an isomeric mixture of two compounds. Final isolation was achieved on E6 with diol HPLC using isopropanol-hexanes (15:85) at 3 mL/min for seven minutes followed by gradient to isopropanol/hexanes (17:83) over 20 min to afford the two pure compounds **2** (5.2 mg, t_R 21.61 min) and **3** (13.5 mg, t_R 24.43 min). SPE fraction C3 (331.2 mg, IC_{50} value $\ll 1.25 \mu\text{g/mL}$) was separated into four subsequent fractions (F1 – F4) via C_{18} HPLC starting with a gradient from 55:45 to 50.8:49.2 $\text{H}_2\text{O}/\text{MeCN}$ over 25 min, then CH_3CN alone for 7 min. Fraction F4 (224.6 mg, IC_{50} value $\ll\ll 1.25 \mu\text{g/mL}$) was then separated further into twelve fractions (G1 – G12) via C_{18} HPLC with a gradient starting at 30:70 to 16.5:83.5 $\text{H}_2\text{O}-\text{MeOH}$ for 30 min at 3 mL/min, then MeOH alone for 7.5 min. Four semi-pure compounds G4 (8.4 mg, t_R 21.69 min), G6 (8.8 mg, t_R 23.20 min), G8 (10.1 mg, t_R 27.10 min), and G10 (16.2 mg, t_R 28.63 min) were obtained and were purified on silica gel open columns with 1:1 hexanes-EtOAc to afford compounds **7** (7.2 mg), **5** (5.7 mg), and **9** (6.5 mg) from fractions G6, G8, and G10, respectively. Fraction G4-1 (10.1 mg) was not homogeneous, and was finally purified via diol HPLC with a flow rate of 3 mL/min using an isocratic flow at 8:92 isopropanol-hexane

to afford compound **6** (4.1 mg, t_R 18.68 min). Fractions G7 (12.1 mg, t_R 24 to 27 min) and G12 (68.4 mg, t_R 31 to 42.5 min) were both complex mixtures and found to be highly active (IC_{50} value $\ll 1.25$ $\mu\text{g/mL}$). Fraction G7 was dropped after each subsequent fractionation (open silica gel column, then another round of C_{18} HPLC, and finally diol HPLC) led to a decrease in activity and insufficient material to continue isolating. Fraction G12 suffered from the same problems as G7 and was also dropped.

(3*E*,7*E*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one (**1**): clear oil; $[\alpha]_D^{23}$ 13.8 (c 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.25), 236 (2.97), 293 (2.57) nm; ECD (CH₃OH) $[\epsilon]_{296\text{ nm}}$ -3.96; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 381.2017 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{NaO}_4^+$, 381.2036).

(3*E*,7*Z*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one (**2**): clear oil; $[\alpha]_D^{22}$ 7.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.26), 238 (4.10) 293 (3.51) nm; ECD (CH₃OH) $[\epsilon]_{290\text{ nm}}$ -8.86; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 381.2037 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{NaO}_4^+$, 381.2036).

(3*Z*,7*E*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one (**3**): clear oil; $[\alpha]_D^{22}$ 7.5 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.28), 224 (4.06), 292 (3.52) nm; ECD (CH₃OH) $[\epsilon]_{294\text{ nm}}$ -8.36; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 381.2041 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{NaO}_4^+$, 381.2036).

(3*Z*,7*Z*)-9-hydroxy-1-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one (**4**): clear oil; $[\alpha]_D^{22}$ 6.3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.27), 224 (4.06), 293 (3.51) nm; ECD (CH₃OH) $[\epsilon]_{292\text{ nm}}$ -8.94; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 381.2020 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{NaO}_4^+$, 381.2036).

(3*E*,7*E*)-9-hydroxy-1-[(*S*)-6-methoxy-2,8-dimethylchroman-2-yl]-4,8-dimethylnona-3,7-dien-2-one (**5**): clear oil; $[\alpha]_D^{22}$ 10.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.26), 231 (4.06), 290 (3.45) nm; ECD (CH₃OH) $[\epsilon]_{292\text{ nm}}$ -7.78; ^1H and ^{13}C NMR spectra, see Table 1; HRESIMS m/z 395.2179 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{23}\text{H}_{32}\text{NaO}_4^+$, 395.2193).

1-[(*S* or *R*)-2,6-dimethyl-2,3,4,7-tetrahydrooxepin-2-yl]-3-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]propan-2-one (**6**): clear oil; $[\alpha]_D^{23}$ 4.3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.31), 293 (3.49) nm; ECD (CH₃OH) $[\epsilon]_{287\text{ nm}}$ -8.39; ^1H and ^{13}C NMR spectra, see Table 2; HRESIMS m/z 381.2049 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{NaO}_4^+$, 381.2036).

1-[(*R* or *S*)-2,6-dimethyl-2,3,4,7-tetrahydrooxepin-2-yl]-3-[(*S*)-6-hydroxy-2,8-dimethylchroman-2-yl]propan-2-one (**7**): clear oil; $[\alpha]_D^{23}$ 7.3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.30), 291 (3.47) nm; ECD (CH₃OH) $[\epsilon]_{289\text{ nm}}$ -8.67; ^1H and ^{13}C NMR spectra, see Table 2; HRESIMS m/z 381.2033 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{NaO}_4^+$, 381.2036).

(3*E*,7*E*)-9-hydroxy-1-[(*R*)-6-hydroxy-2,8-dimethyl-2*H*-chromen-2-yl]-4,8-dimethylnona-3,7-dien-2-one (**8**): light yellow oil; $[\alpha]_D^{23}$ 4.0 (c 0.1, CHCl₃); UV (CHCl₃)

$\lambda_{\max}$ (log ϵ) 248 (3.28), 328 (3.47) nm; ECD (CHCl₃) [ϵ]_{321 nm} -6.00; ¹H and ¹³C NMR spectra, see Table 2; HRESIMS m/z 379.1861 [M+Na]⁺ (calcd for C₂₂H₂₈NaO₄⁺ 379.1880).

(*R*)-2-[(3*E*, 7*E*)-9-hydroxy-4,8-dimethylnona-3,7-dien-1-yl]-2,8-dimethyl-2*H*-chromen-6-ol (**9**): light yellow oil; [α]_D²³ 10.0 (*c* 0.1, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ (log ϵ) 244 (4.14), 330 (3.57) nm. ECD (CHCl₃) [ϵ]_{325 nm} -5.27; ¹H and ¹³C NMR spectra, see Table 2; HRESIMS m/z 365.2052 [M+Na]⁺ (calcd for C₂₂H₃₀NaO₃⁺ 365.2087).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Selected HMBC correlations of **1**.

Figure 2.
NOE correlations for **1**.

Figure 3.

(A) ECD spectra of **1** – **7** in MeOH. (B) UV of **1** – **7** in MeOH. (C) ECD spectra of **8** and **9** in CHCl_3 at 1.0 mM. (D) UV spectra of **8** and **9** in CHCl_3 . All compounds except **1** were measured at 1.0 mM while **1** was at 0.5 mM.

Figure 3.
Selected HMBC correlations for **6** and **7**. Green correlations were obtained after the spectrum was optimized for a coupling of 12 Hz.

Scheme 1.

Proposed mechanism for the formation of 6 and 7 from 2.

Table 1

NMR Spectra of Compounds 1 – 5 in CD₃OD (*J* in Hz).^a

position	1		2		3		4		5	
	δ_H	δ_C , type	δ_H	δ_C , type	δ_H	δ_C , type	δ_H	δ_C , type	δ_H	δ_C , type
2		76.2, C		76.2, C		76.2, C		76.2, C		76.4, C
3	1.90, dt (13.6, 6.9) 1.79, dt (13.6, 6.9)	33.1, CH ₂	1.90, dt (13.6, 6.8) 1.79, dt (13.6, 6.8)	33.1, CH ₂	1.90 ^c , m 1.79, dt (13.6, 6.9)	33.2, CH ₂	1.91, dt (13.8, 6.9) 1.79, dt (13.8, 6.9)	33.2, CH ₂	1.92, dt (13.6, 6.7) 1.81, dt (13.6, 6.7)	33.0, CH ₂
4	2.71, t (6.9)	23.3, CH ₂	2.71, t (6.8)	23.3, CH ₂	2.7, t (6.9)	23.3, CH ₂	2.71, t (6.9)	23.3, CH ₂	2.76, t (6.7)	23.4, CH ₂
4a		122.0, C		122.0, C		122.0, C		122.0, C		121.9, C
5	6.35, d (2.9)	113.7, CH	6.35, d (2.9)	113.7, CH	6.34, d (2.9)	113.7, CH	6.34, d (2.9)	113.7, CH	6.47, d (2.9)	112.1, CH
6		150.8, C		150.8, C		150.8, C		150.8, C		154.1, C
7	6.43, d (2.9)	116.8, CH	6.43, d (2.9)	116.8, CH	6.43, d (2.9)	116.8, CH	6.43, d (2.9)	116.8, CH	6.55, d (2.9)	116.1, CH
8		127.8, C		127.9, C		127.9, C		127.9, C		128.0, C
8a		145.7, C		145.8, C		145.7, C		145.7, C		146.6, C
1'	2.82, d (13.1) 2.55, d (13.1)	53.7, CH ₂	2.81, d (13.1) 2.56, d (13.1)	53.7, CH ₂	2.81, d (13.0) 2.52, d (13.0)	53.8, CH ₂	2.81, d (13.1) 2.52, d (13.1)	53.8, CH ₂	2.82, d (13.3) 2.57, d (13.3)	53.7, CH ₂
2'		201.7, C		201.7, C		201.0, C		201.1, C		201.5, C
3'	6.19, brs	126.2, CH	6.18, brs	126.3, CH	6.19, brs	126.7, CH	6.19, brs	126.8, CH	6.18, brs	126.2, CH
4'		159.5, C		159.4, C		160.4, C		160.3, C		159.6, C
5'	2.19 ^b , m	41.9, CH ₂	2.18, m	42.4, CH ₂	2.62, m	34.6, CH ₂	2.59, m	35.1, CH ₂	2.19 ^d , m	41.9, CH ₂
6'	2.20 ^b , m	26.7, CH ₂	2.24, m	26.6, CH ₂	2.21, q (7.4)	27.4, CH ₂	2.22, q (7.8)	27.3, CH ₂	2.20 ^d , m	26.7, CH ₂
7'	5.36, brt (6.7)	125.1, CH	5.22, brt (7.2)	127.2, CH	5.44, brt (7.4)	125.9, CH	5.31, t (7.5)	127.86, CH	5.35, m	125.1, CH
8'		137.0, C		136.9, C		136.7, C		136.6, C		137.0, C
9'	3.89, s	68.7, CH ₂	4.04, s	61.3, CH ₂	3.9, s	68.9, CH ₂	4.09, s	61.3, CH ₂	3.89, s	68.7, CH ₂
10'	1.63, d (1.4)	13.7, CH ₃	1.74, d (1.3)	21.5, CH ₃	1.66, d (1.4)	13.7, CH ₃	1.75, d (1.4)	21.5, CH ₃	1.63, d (1.3)	13.7, CH ₃
11'	2.12, d (1.2)	19.6, CH ₃	2.11, d (1.2)	19.6, CH ₃	1.88, d (1.4)	25.9, CH ₃	1.87, d (1.4)	25.8, CH ₃	2.12, d (1.4)	19.6, CH ₃
12'	1.34, s	25.3, CH ₃	1.33, s	25.3, CH ₃	1.33, s	25.2, CH ₃	1.33, s	25.1, CH ₃	1.35, s	25.3, CH ₃
13'	2.06, s	16.5, CH ₃	2.06, s	16.5, CH ₃	2.06, s	16.4, CH ₃	2.06, s	16.4, CH ₃	2.09, s	16.6, CH ₃
OCH ₃									3.70, s	55.9, CH ₃

^a¹H NMR data obtained at 500 MHz; ¹³C NMR data acquired in CD₃OD at 125 MHz.

Overlapping signals. Chemical shifts determined from the HSQC spectrum.

Obscured signal.

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Table 2

NMR Spectra of Compounds 6 – 9 in CD₃OD (*J* in Hz).^a

position	6		7		8		9	
	δ_{H}	δ_{C} , type	δ_{H}	δ_{C} , type	δ_{H}	δ_{C} , type	δ_{H}	δ_{C} , type
2		76.1, C		76.0, C		78.3, C		78.8, C
3	1.94, dt (13.6, 6.0) 1.80 ^b , m	33.2, CH ₂	1.93 ^d , m 1.80 ^d , m	32.5, CH ₂	5.71, d (9.8)	130.7, CH	5.61, d (9.7)	131.5, CH
4	2.69, m	23.3, CH ₂	2.69 ^e , m	23.3, CH ₂	6.28 ^f , d (9.8)	124.3, CH	6.27 ^h , d (9.7)	124.2, CH
4a		122.3, C		122.2, C		122.5, C		122.7, C
5	6.36, d (2.9)	113.8, CH	6.34, d (2.9)	113.7, CH	6.29 ^f , s	111.4, CH	6.28 ^h , s	111.3, CH
6		151.1, C		150.9, C		151.8, C		151.3, C
7	6.45, d (2.9)	116.9, CH	6.43, d (2.9)	116.8, CH	6.44, d (2.9)	118.3, CH	6.43, d (2.9)	117.9, CH
8		127.8, C		127.9, C		127.1, C		126.8, C
8a		145.7, C		145.7, C		144.7, C		145.3, C
1'	3.00 ^c , d (14.1) 2.50, d (14.1)	52.6, CH ₂	2.84, d (14.8) 2.74, d (14.8)	53.9, CH ₂	2.79, d (13.4) 2.75, d (13.4)	55.2, CH ₂	1.65, m	41.9, CH ₂
2'		211.0,		210.2,		200.6, C	2.12 ^j , m	23.7, CH ₂
3'	3.03 ^c , d (13.7) 2.37, d (13.7)	52.9, CH ₂	2.94, d (15.0) 2.64, d (15.0)	53.6, CH ₂	6.18, brs	125.8, CH	5.14, brt (7.1)	125.7, CH
4'		78.7, C		78.4, C		159.8, CH		135.8, CH
5'	1.85 ^b , m 1.76 ^b , m	40.2, CH ₂	1.89 ^d , m 1.85 ^d , m	39.6, CH ₂	2.15 ^g , m	41.8, CH ₂	2.12 ^j , m	27.3, CH ₂
6'	2.18, m 2.03, m	23.96, CH ₂	2.11, m	24.1, CH ₂	2.17 ^g , m	26.7, CH ₂	2.00, t (7.6)	40.5, CH ₂
7'	5.36, m	125.8, CH	5.41, m	126.0, CH	5.35, brt (6.9)	125.1, CH	5.37, brt (7.2)	126.5, CH ₂
8'		137.1, C		137.2, C		136.9, C		135.9,
9'	3.83, d, (16.6) 3.68, d (16.6)	65.6, CH ₂	4.08, d (16.7) 3.98, d (16.7)	65.8, CH ₂	3.89, s	68.7, CH ₂	3.89, s	69.0, CH ₂
10'	1.51, d (2.1)	21.4, CH ₃	1.58, s	21.4, CH ₃	1.63, d (1.4)	13.7, CH ₃	1.62, s	13.7, CH ₃
11'	1.20, s	24.04, CH ₃	1.26, s	24.1, CH ₃	2.04, d (1.3)	19.6, CH ₃	1.58, d (1.4)	15.9, CH ₃
12'	1.35, s	25.7, CH ₃	1.34, s	25.0, CH ₃	1.47, s	27.0, CH ₃	1.33, s	26.3, CH ₃
13'	2.09, s	16.5, CH ₃	2.09, s	16.5, CH ₃	2.08, s	15.8, CH ₃	2.10, s	15.7, CH ₃

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¹H NMR data obtained at 500 MHz; ¹³C NMR data acquired in CD₃OD at 125 MHz.

b, c, d, f, g, h, i Overlapping signals. Chemical shifts determined from HSQC.

e Obscured signal.

Table 3IC₅₀ Values of Compounds **1** – **9** against *P. falciparum* Dd2 and A2780 Ovarian Cancer Cells

compound	<i>P. falciparum</i> Dd2 (μM)	A2780 (μM)
1	> 112	> 10
2	24 ± 3	> 10
3	> 112	> 10
4	> 112	> 10
5	24 ± 2	> 10
6	109 ± 8	> 10
7	23 ± 2	> 10
8	> 112	> 10
9	> 117	> 10
artemisinin	0.009 ± 0.003	NT ^a
paclitaxel	NT ^a	0.013 ± 0.001

^aNT = not tested.