JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Rotenoids, Flavonoids, and Chalcones from the Root Bark of *Millettia usaramensis*

Tsegaye Deyou,[†,‡] Ivan Gumula,[†,‡] Fangfang Pang,[§] Amra Gruhonjic,[‡,⊥] Michael Mumo,[†] John Holleran,[∥] Sandra Duffy,[∥] Paul A. Fitzpatrick,[⊥] Matthias Heydenreich,[▽] Göran Landberg,[⊥] Solomon Derese,[†] Vicky Avery,[∥] Kari Rissanen,[§] Máté Erdélyi,[*,‡,#] and Abiy Yenesew[*,†]

[†]Department of Chemistry, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya

[‡]Department of Chemistry and Molecular Biology, [⊥]Sahlgrenska Cancer Centre, and [#]Swedish NMR Center, University of Gothenburg, SE-40530, Gothenburg, Sweden

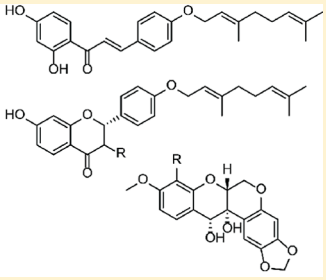
[§]Department of Chemistry, Nanoscience Center, University of Jyvaskyla, P.O. Box. 35, FI-40014 Jyvaskyla, Finland

[∥]Discovery Biology, Eskitis Institute for Drug Discovery, Griffith University, Nathan Qld 4111 Australia

[▽]Institut für Chemie, Universität Potsdam, Karl-Liebknecht-Straße 24-25, D-1146, Potsdam, Germany

Supporting Information

ABSTRACT: Five new compounds, 4-*O*-geranylisoliquiritigenin (**1**), 12-dihydrousararotenoid B (**2**), 12-dihydrousararotenoid C (**3**), 4′-*O*-geranyl-7-hydroxyflavanone (**4**), and 4′-*O*-geranyl-7-hydroxydihydroflavanol (**5**), along with 12 known natural products (**6**−**17**) were isolated from the CH_2Cl_2/MeOH (1:1) extract of the root bark of *Millettia usaramensis* ssp. *usaramensis* by chromatographic separation. The purified metabolites were identified by NMR spectroscopic and mass spectrometric analyses, whereas their absolute configurations were established on the basis of chiroptical data and in some cases also by X-ray crystallography. The crude extract was moderately active (IC_{50} = 11.63 μg/mL) against the ER-negative MDB-MB-231 human breast cancer cell line, and accordingly compounds **6**, **8**, **9**, **10**, **12**, and **16** also showed moderate to low cytotoxic activities (IC_{50} 25.7−207.2 μM). The new natural product **1** exhibited antiplasmodial activity with IC_{50} values of 3.7 and 5.3 μM against the chloroquine-sensitive 3D7 and the chloroquine-resistant Dd2 *Plasmodium falciparum* strains, respectively, and was also cytotoxic to the HEK293 cell line.



The genus *Millettia* (Leguminoseae, subfamily: Papilionoideae) consists of more than 200 species that are native in the tropical and subtropical regions of Africa, Asia, and Australia.[1,2] Of these, 139 species are endemic to Africa. *Millettia usaramensis* ssp. *usaramensis*, a shrub or tree that can grow up to 10 m high, is one of the six *Millettia* species that are found in Kenya.[3] In traditional medicine, its roots are used as antidote against snake bite.[4] Whereas the roots of this plant have not yet been phytochemically analyzed, previous investigations of its stem bark yielded unique 12a-hydroxyrotenoids with the unusual *trans*-B/C ring junction as well as chalcones and isoflavones.[5,6] These compound groups have lately been recognized as emerging leads for antimalarial[7,8] and anticancer[9,10] therapy. Herein, the isolation and identification of a new chalcone (**1**), two new 12-dihydrorotenoids (**2**, **3**), a new flavanone (**4**), a new dihydroflavonol (**5**), and 12 known secondary metabolites (**6**−**17**) are reported. The antiplasmodial activity of compound **1** and the cytotoxic activities of some of the compounds are also presented.

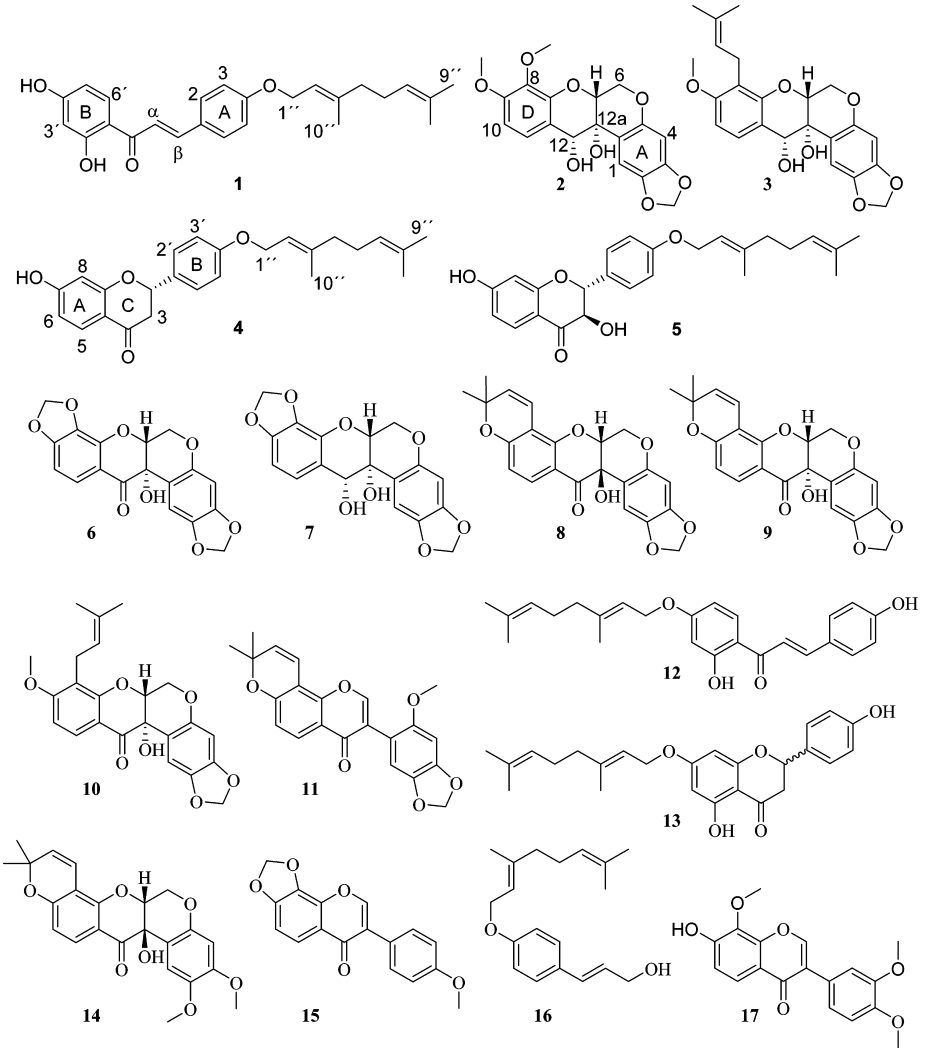
RESULTS AND DISCUSSION

Column chromatographic separation of the CH_2Cl_2/MeOH (1:1) extract of the dried and ground root bark of *M. usaramensis* ssp. *usaramensis*, followed by gel filtration over Sephadex LH-20, and further purification by MPLC and RP-HPLC afforded five new secondary metabolites (**1**−**5**) and the 12 known compounds usararotenoid A (**6**),[5] 12-dihydrousararotenoid A (**7**),[5] millettosin (**8**),[11] 12a-epimillettosin (**9**),[5] usararotenoid C (**10**),[6] jamaicin (**11**),[12] 4′-*O*-geranylisoliquiritigenin (**12**),[13] 7-*O*-geranyl-5-hydroxyflavanone (**13**),[14] tephrosin (**14**),[15] maximaisoflavone H (**15**),[16] colenemol (**16**),[17] and 7-hydroxy-8,3′,4′-trimethoxyisoflavone (**17**).[18] Compounds **6**−**17** were previously reported from the stem bark of the plant,[5,6] from the seeds of *Millettia dura*,[11] and from the roots of *Tephrosia villosa*.[14] The identities of **6**−**17** were confirmed by comparison of their spectroscopic and physical data to those previously published.

Received: July 1, 2015

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

Chart 1



As part of the structural work, the X-ray structures of usararotenoid A[5] (**6**, Figure 1), 12-dihydrousararotenoid A[11]

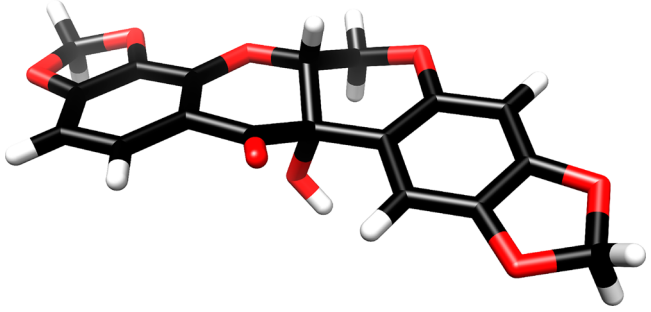


Figure 1. X-ray crystal structure of usararotenoid A (**6**).

(**7**, Figure 2), and 12a-epimillettosin[5] (**9**, Figure 3) were obtained. The latter solid-state structure was accomplished for the first time, confirming the 6a*R*,12a*S* absolute configuration of the B/C ring junction of **9**, which was previously proposed based on an $[\alpha]_D^{20}$ value of +230.4 and the positive and negative Cotton effects at 348 and 324 nm, respectively, in the electronic circular dischroism (ECD) spectrum.

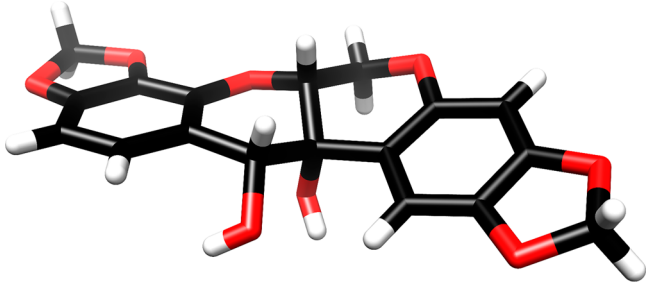


Figure 2. X-ray crystal structure of 12-dihydrousararotenoid A (**7**).

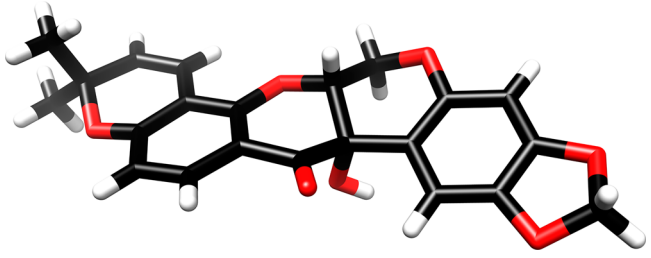


Figure 3. X-ray crystal structure of 12a-epimillettosin (**9**).

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

Compound **1** was isolated as a yellow solid. Its HREIMS molecular ion at *m/z* 392.1968 and ^{13}C NMR data are consistent with the molecular formula $C_{25}H_{28}O_4$ (calcd 392.1988). Its UV absorbance at λ_{max} 299 and 370 nm along with the characteristic *trans*-olefinic doublets H-α (δ_H 7.39) and H-β (δ_H 7.80) with ^3J = 15.4 Hz, carbonyl (δ_C 192.2), C-α (δ_C 118.9), and C-β (δ_C 144.7) observed by NMR spectroscopy (Table 1) is indicative of a chalcone core skeleton.[19,20] A broad

Table 1. ^1H and ^{13}C NMR Spectroscopic Data for 4-*O*-Geranylisoliquiritigenin (1) Acquired in CD_2Cl_2 (δ_H, Multiplicity (*J* in Hz))

position	δ_C, type	δ_H, m (*J* in Hz)	HMBC (H→C)
1	127.4, C		
2/6	130.5, CH	7.54, AA′	β, 4, 6
3/5	115.3, CH	6.91, XX′	1, 4
4	161.3, C		
C=O	192.2, C		
C-α	118.9, CH	7.39, d (15.4)	1, β
C-β	144.7, CH	7.80, d (15.4)	2, 6
1′	114.2, C		
2′	163.6, C		
OH-2′		13.64, br s	1′, 3′, 4′
3′	103.7, CH	6.45, m	1′, 2′, 4′, 5′
4′	166.2, C		
5′	108.3, CH	6.47, m	1′, 3′
6′	132.0, CH	7.82, d (8.0)	2′, 4′, C=O
1″	65.2, CH_2	4.60, d (7.2)	4, 2″, 3″
2″	117.6, CH	5.50, t (7.2)	
3″	142.0, C		
4″	39.6, CH_2	2.10−2.17, m	2″, 3″
5″	25.8, CH_2	2.10−2.17, m	3″, 6″
6″	123.8, CH	5.12, t (7.2)	5″, 8″, 9″
7″	131.9, C		
8″	26.3, CH_3	1.71, s	6″, 7″, 9″
9″	17.8, CH_3	1.63, s	6″, 7″, 9″
10″	16.8, CH_3	1.77, s	2″, 3″, 4″

singlet at δ_H 13.64 suggests a hydrogen-bonded hydroxy group (OH-2′), whereas three mutually coupled aromatic protons, H-3′ (δ_H 6.45), H-5′ (δ_H 6.47), and H-6′ (δ_H 7.82), indicate a trisubstituted, dioxygenated B ring. The exclusive *ortho*-coupling (*J* = 8.0 Hz) of H-6′ (δ_H 7.82) is compatible with a 2′,4′-dioxygenation of this trisubstituted ring, which is corroborated by the NOESY and the HMBC cross-peak pattern of **1** (Table 1, Figures S5 and S7, Supporting Information). The AA′XX′ spin system (δ_H 6.91, 7.54) of the A ring indicates 1,4-disubstitution, whereas the chemical shift of C-4 (δ_C 161.3) reveals oxygenation at this position. Connection of ring A to C-β of the olefinic moiety is revealed by the HMBC cross-peaks between H-2/6 (δ_H 7.54) and C-β (δ_C 144.7) as well as between H-β (δ_H 7.80) and C-2/6 (δ_C 130.5). Three methyl (δ_H 1.63, 1.71, and 1.77), one oxymethylene (δ_H 4.60, d, *J* = 7.2 Hz), two methylene (δ_H 2.10−2.17, m), and two methine olefinic protons (δ_H 5.12, t, *J* = 7.2 Hz and δ_H 5.50, t, *J* = 7.2 Hz) connected by COSY and TOCSY cross-peaks (Figures S3 and S4, Supporting Information) suggest the presence of a geranyloxy or a neryloxy substituent. The chemical shifts of C-4″ (δ_C 39.6) and C-10″ (δ_C 16.8) are in better agreement with a geranyloxy rather than a neryloxy group, whose corresponding carbons would be expected to give rise to signals at approximately δ_C 32 and δ_C 23, respectively.[21] This conclusion is further corroborated by the NOE between H-1″ and CH_3-10″. The NOE between the oxymethylene H-1″ (δ_H 4.60) of the geranyloxy side chain and the H-3/5 (δ_H 6.91) of ring A reveals the position of geranyloxy substitution at C-4, which was further supported by the HMBC correlation of H-1″ (δ_H 4.60) and C-4 (δ_C 161.3). The *E*-configuration of the 2″-double bond was confirmed by the observation of an NOE between H-2″ (δ_H 5.50) and H-4″ (δ_H 2.10−2.17) and the absence of an NOE between H-2″ (δ_H 5.50) and CH_3-10″ (δ_H 1.77, Supporting Information). On the basis of the above spectroscopic data **1** was characterized as (*E*)-1-(2,4-dihydroxyphenyl)-3-(4-[{(*E*)-3,7-dimethylocta-2,6-dien-1-yl}oxy]-phenyl)prop-2-en-1-one and was assigned the trivial name 4-*O*-geranylisoliquiritigenin.

Compound **2** was obtained as a colorless, amorphous solid and was assigned the molecular formula $C_{19}H_{18}O_8$ based on HREIMS analysis (M^+ obs *m/z* 374.0999, calcd 374.1002) and ^{13}C NMR data (Table 2). Its ^1H and ^{13}C NMR data are compatible with a 12-dihydro-12a-hydroxyrotenoid derivative.[5] The ABC spin system of H-6a (δ_H 4.28, Table 2), H-6α (δ_H 4.34), and H-6β (δ_H 4.39) supports that **2** is a 12a-hydroxyrotenoid derivative.[5] In contrast to common rotenoids possessing a C-12 carbonyl group, C-12 of **2** is an oxymethine functionality, as revealed by its chemical shift of δ_C 70.6. The C-12 hydroxylation is indicated by the coupling (d, *J* = 11.4 Hz) of H-12 (δ_H 4.89) and OH-12 (δ_H 2.78); upon addition of D_2O to the solution, the signal of OH-12 disappeared and the doublet of H-12 collapsed into a singlet. In ring D, the locations of CH_3O-8 (δ_H 3.79) and CH_3O-9 (δ_H 3.84) are revealed by their HMBC cross-peaks to C-8 (δ_C 136.5) and C-9 (δ_H 153.3), respectively, and by the HMBC cross-peaks of the *ortho*-coupled (*J* = 9.0 Hz) H-10 (δ_C 6.68) to C-8 and H-11 (δ_C 7.27) to C-9, indicating that ring D of **2** is *ortho*-dioxygenated. The proposed di-*ortho*-substitution is corroborated by the deshielding of OCH_3-8 (δ_C 60.9).[22] Ring A possesses two isolated aromatic protons, i.e., H-1 (δ_H 7.77) and H-4 (δ_H 6.39), and a methylenedioxy substituent (δ_H 5.92, δ_C 101.9), whose substitution pattern has been previously reported for the rotenoids of this plant.[5] The *trans*-orientation of the B/C ring junction is indicated by a deshielding of H-1.[6,23−25] Moreover, the NOE of H-6a and H-12 indicates their 1,3-diaxial relationship and, hence, their β-orientations. H-6a and H-6α are in a 1,2-*trans*-diaxial orientation, as revealed by their large scalar coupling (*J* = 10.8 Hz). The similar chemical shifts of rings A−C and the coupling constants of the H-6α, H-6β, and H-6a ABX spin system of **2** (Table 2) to those of 12-dihydrousararotenoid A (**7**, $J_{H6\alpha,6a}$ = 9.9 Hz, $J_{H6\beta,6a}$ = 4.6 Hz), whose configuration[5] was previously established by X-ray crystallography[6] and confirmed in this investigation (Figure 2), further support its proposed relative configuration. Compound **2** gave a high positive specific rotation, $[\alpha]_D^{20}$ +134, and a positive ECD Cotton effect at ca. 295 nm, similar to **7**, indicating that their absolute configurations should be the same. On the basis of the above spectroscopic evidence, compound **2** was characterized as (6a*R*,12*R*,12a*R*)-8,9-dimethoxy-6,6a-dihydrochromeno[2,3-*c*][1,3]dioxolo[4,5-*g*]chromene-12,12a-(12*H*)-diol and was given the trivial name 12-dihydrousararotenoid B.

Compound **3** was isolated as a colorless, amorphous solid and was assigned the molecular formula $C_{23}H_{24}O_7$ based on HREIMS analysis ($[M]^+$ obs *m/z* 412.1520, calcd 412.1522) and ^{13}C NMR data (Table 2). Its NMR spectra showed similarities to those of **2**, suggesting a close structural

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for 12-Dihydrousararotenoid B (2) and 12-Dihydrousararotenoid C (3) Acquired in CD_2Cl_2 and Acetone-d_6, Respectively (δ_H, Multiplicity (J in Hz))

	2			3		
position	δ_C, type	δ_H, m (J in Hz)	HMBC(H→C)	δ_C, type	δ_H, m (J in Hz)	HMBC (H→C)
1	107.2, CH	7.77, s	2, 3, 4a, 12a, 12b	107.2, CH	7.81, s	2, 3, 4, 4a, 12b
2	142.7, C			142.5, C		
3	147.4, C			149.1, C		
4	98.4, CH	6.39, s	2, 4a, 12b	98.3, CH	6.45, s	1, 2, 3, 4a
4a	149.4, C			149.6, C		
6α	62.6, CH_2	4.34, dd (10.8, 9.6)	12, 12a	62.3, CH_2	4.34, dd (13.8, 10.2)	4a, 12, 12a
6β		4.39, dd (9.6, 4.8)			4.37, dd (10.2, 3.6)	
6a	73.4, CH	4.28, dd (10.8, 4.8)	12a	70.7, CH	4.26, dd (10.8, 4.8)	4a, 12a
7a	149.9, C			151.2, C		
8	136.5, C			118.0, C		
9	153.3, C			157.8, C		
10	106.6, CH	6.68, d (9.0)	8, 9, 11a	105.3, CH	6.67, d (9.0)	7a, 8, 9, 11a
11	123.6, CH	7.27, d (9.0)	9, 12	126.9, CH	7.42, d (9.0)	7a, 9, 10, 11a
11a	119.7, C			117.0, C		
12	70.6, CH	4.89, d (11.4)	11, 11a, 12b	73.0, CH	4.91, d (10.8)	7a, 11a, 12b
12a	64.6, C			64.4, CH	2.78, d (10.8)	
12b	115.7, C			115.1, C		
1′				22.3, CH_2	3.36, m	7a, 8, 9, 2′, 3′
2′				122.2, CH	5.25, t (7.2)	1′, 4′, 5′
3′				131.5, C		
4′				17.8, CH_3	1.68, s	2′, 3′
5′				25.8, CH_3	1.78, s	2′, 3′
OMe-8	60.9, CH_3	3.79, s	8			
OMe-9	56.3, CH_3	3.84, s	9	55.8, CH_3	3.85, s	9
−OCH_2O-10/11	101.9, CH_2	5.92, d (1.2)	2, 3	101.4, CH_2	5.93, d (1.2)	2′, 3′
		5.93, d (1.2)			5.95, d (1.2	
OH-12		2.78, d (11.4)	12, 12a		2.78, d (10.8)	
OH-12a		2.50, s	6a, 12, 12a, 12b			

resemblance. Hence, for ring A of **3**, two aromatic singlets H-1 (δ_H 7.81) and H-4 (δ_H 6.45) and a C-2/C-3 methylenedioxy group (δ_H 5.94, δ_C 101.4) were evident. Its B ring possesses an ABC spin system, H-6a (δ_H 4.26), H-6α (δ_H 4.34), and H-6β (δ_H 4.37), typical for 12a-hydroxyrotenoids.[5] Similar to **2**, compound **3** is also a 12-dihydrorotenoid derivative, evidenced by the presence of the signals of a C-12 (δ_C 73.0) oxymethine carrying a hydroxy group (δ_H 2.78). The latter proton couples (J = 10.8 Hz) to H-12 (δ_H 4.91), and its signal along with its coupling to H-12 disappears upon addition of D_2O, confirming its exchangeable nature. Further similarities of **2** and **3** are confirmed by observation of a methylenedioxy at C-2/C-3 on ring A, which has a pair of *ortho*-coupled aromatic protons, H-10 (δ_H 6.67, d, J = 9.0 Hz) and H-11 (δ_H 7.42, d, J = 9.0 Hz), and a methoxy functionality (δ_H 3.85, δ_C 55.8) at C-9 (δ_C 157.8) of ring A, whose placement is confirmed by the H-10 (δ_H 6.67) to CH_3O-9 (δ_H 3.85) NOE. However, the C-8 methoxy of **2** is replaced with a C-prenyl unit in **3**, as revealed by the signals H-1′ (δ_H 3.36, m), H-2′ (δ_H 5.25, t, J = 7.2 Hz), H-4′ (δ_H 1.68, s), and H-5′ (1.78, s), showing the corresponding COSY, NOESY, and HMBC cross-peak patterns (Table 2, Figures S19−S22, Supporting Information). The placement of the 3,3-dimethylallyl group at C-8 is confirmed by the NOEs observed between CH_3O-9 (δ_H 3.85) and H-1′ (δ_H 3.36) as well as H-2′ (δ_H 5.25, Figure S20, Supporting Information) and by the HMBC cross-peaks of H-1′ (δ_H 3.36) to C-7a (δ_C 151.2), C-8 (δ_C 118.0), and C-9 (δ_C 157.8, Table 2). Similar to **2**, the *trans*-geometry of the B/C ring junction of **3** was derived from the strong deshielding of H-1 (δ_H 7.81) and the NOEs and J-couplings corresponding to that explained above for **2**. Moreover, its comparable chemical shifts and coupling constants to those of **7**, whose configuration was confirmed by X-ray crystallography,[6] support the proposed relative configuration at C-6a, C-12a, and C-12. Once again the high positive specific rotation $[\alpha]_D^{20}$ +96 and positive ECD Cotton effect at ca. 295 nm are consistent with the identical configurations of the 12-dihydrorotenoids (**2** and **7**) of this plant. On the basis of the above spectroscopic evidence, this new compound (**3**) was characterized as (6a*R*,12*R*,12a*R*)-9-methoxy-8-(3-methylbut-2-en-1-yl)-6,6a-dihydrochromeno-[2,3-*c*][1,3]dioxolo[4,5-*g*]chromene-12,12a(12*H*)-diol and was given the trivial name 12-dihydrousararotenoid C.

Compound **4** was obtained as a white solid. Its molecular formula was determined to be $C_{25}H_{28}O_4$ on the basis of HRMS (EI: $[M]^+$ obs m/z 392.1968, calcd 392.1987; ESI: $[M + H]^+$ obs m/z 393.2068, calcd 393.2066) and ^{13}C NMR (Table 3). Analysis of its NMR data identified four $^{1}H/^{1}H$ spin systems: the aromatic ABX system of ring A with H-5 (δ_H 7.79, Table 3), H-6 (δ_H 6.57), and H-8 (δ_H 6.48), the aliphatic ABX spin system of ring C with H-3a (δ_H 2.78), H-3b (δ_H 3.06), and H-2 (δ_H 5.40), the AA′XX′ system of ring B [(H-2′ (δ_H 7.37) and H-3′ (δ_H 6.93)], and the spin system of the H-1″−H-10″ geranyloxy moiety[13] (Table 3). The above data along with the observation of the ^{13}C NMR resonances 4-C═O (δ_C 191.9), C-2 (δ_C 79.9), and C-3 (δ_C 44.1) indicated a flavanone core skeleton. Ring A is expected to be oxygenated at C-7 (δ_C 164.2), based on biogenetic considerations,[23] while oxygenation at C-4′ of ring B is revealed by its high chemical shift (δ_C

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data for (2*S*)-4′-*O*-Geranyl-7-hydroxyflavanone (**4**) and (2*R*,3*R*)-4′-*O*-Geranyl-7-hydroxyflavonol (**5**) Acquired in CD_2Cl_2 and DMSO-d_6, Respectively (δ_H, Multiplicity (*J* in Hz))

	4			5		
position	δ_C, type	δ_H, m (*J* in Hz)	HMBC (H→C)	δ_C, type	δ_H, m (*J* in Hz)	HMBC (H→C)
2	79.9, CH	5.40, dd (2.4, 13.2)	3, 1′, 2′, 6′	83.1, CH	5.09, d (11.6)	3, 4, 8a, 2′/6′
3	44.1, CH_2	2.78, dd (2.4, 16.8) 3.06, dd (13.2, 16.8)	2, 1′	72.5, CH	4.50, dd (2.5, 11.6)	2, 4, 1′
3-OH					5.52, d (2.5)	
4	191.9, C=O			192.3, C=O		
4a	114.9, C			111.9, C		
5	129.4, CH	7.79, d (8.4)	7, 8a	128.6, CH	7.63, d (8.7)	4, 7, 8a
6	110.9, CH	6.57, dd (2.4, 8.4)	7, 8, 4a	111.0, CH	6.52, dd (2.2, 8.7)	4a, 8
7	164.2, C			165.2, C		
8	103.6, CH	6.48, d (2.4)	6, 7, 4a, 8a	102.4, CH	6.28, d (2.2)	4a, 6, 7, 8a
8a	164.0, C			162.8, C		
1′	130.9, C			129.5, C		
2′	128.0, CH	7.37, m	2, 3′, 4′, 6′	129.3, CH	7.42, d (8.6)	2, 1′, 4′
3′	115.7, CH	6.93, m	1′, 4′, 5′	114.2, CH	6.95, d (8.6)	1′, 4′, 3′/5′
4′	159.5, C			158.6, C		
1″	65.3, CH_2	4.56, d (6.6)	4′, 2″, 3″	64.4, CH_2	4.56, d (6.6)	4′, 2″, 3″
2″	119.6, CH	5.46, t (6.0)	4″, 10″	119.7, CH	5.43, t (6.6)	4″, 10″
3″	141.6, C			140.2, CH		
4″	39.8, CH_2	2.11, m	2″, 6″	39.0, CH_2	2.06, m	2″, 3″, 5″, 10″
5″	26.5, CH_2	2.11, m	4″, 6″, 7″	25.8, CH_2	2.08, m	3″, 4″, 6″, 7″
6″	124.0, CH	5.10, m	8″, 9″	123.8, CH	5.08, m	8″, 9″
7″	132.0, C			131.0, C		
8″	17.7, CH_3	1.60, s		25.5, CH_3	1.64, s	6″, 7″, C9″
9″	25.7, CH_3	1.67, s		17.6, CH_3	1.57, s	6″, 7″, 8″
10″	16.6, CH_3	1.73, s		16.3, CH_3	1.71, s	2″, 3″, 4″

159.5). The HMBC correlation of CH_2-1″ to C-4′ indicates the connection of the geranyloxy group to C-4′ of ring B through an ether linkage, which conclusion is corroborated by the NOE correlation observed between CH_2-1″ and H-3′/H-5′. The *E*-configuration of the 2″-double bond was confirmed by the observation of an NOE between H-2″ (δ_H 5.46) and H-4″ (δ_H 2.11) and the absence of an NOE between H-2″ (δ_H 5.46) and CH_3-10″ (δ_H 1.73, Supporting Information). The ECD spectrum of **4** displayed positive and negative Cotton effects at 332 and 302 nm, respectively, consistent with a 2*S*-configuration.[25] This new compound was identified as (*S*)-*E*-2-(4-[{3,7-dimethylocta-2,6-dien-1-yl}oxy]phenyl)-7-hydroxychroman-4-one and was given the semisystematic name (*S*)-4′-*O*-geranyl-7-hydroxyflavanone.

The molecular formula of compound **5**, isolated as a white, amorphous solid, was determined to be $C_{25}H_{28}O_5$ on the basis of HRESIMS ($[M + H]^+$ obs *m*/*z* 409.2020, calcd 409.2015) and ^{13}C NMR. Its NMR spectroscopic features were similar to those of compound **4** except that those of **5** were typical of a dihydroflavonol. Thus, ring A of **5** exhibits an AMX spin system (Table 3), i.e., H-5 (δ_H 7.63, d, *J* = 8.7 Hz), H-6 (δ_H 6.52, dd, *J* = 8.7, 2.2 Hz), and H-8 (δ_H 6.28, d, *J* = 2.2 Hz), compatible with C-7 (δ_C 165.2) oxygenation, similar to **4**. The large scalar coupling constant (*J* = 11.6 Hz) of H-2 (δ_H 5.09) and H-3 (δ_H 4.50) of ring C, which is hydroxylated (δ_H 5.52) at C-3 (δ_C 72.5), indicates the diaxial orientation of these protons. The 4′-*O*-geranyl substitution of ring B of **5** corresponds to that of **4**, as revealed by their similar NMR data. Accordingly, the *E*-configuration of the 2″-double bond was confirmed by the observation of an NOE between H-2″ (δ_H 5.43) and H-4″ (δ_H 2.06), whereas no NOE was observed between H-2″ (δ_H 5.43) and CH_3-10″ (δ_H 1.71, Supporting Information). The sequential positive and negative Cotton effects at 334 and 300 nm are consistent with the 2*R*,3*R* absolute configuration.[25] On the basis of the above data, the structure of the new compound was characterized as (2*R*,3*R*)-2-(4-[{(*E*)-3,7-dimethylocta-2,6-dien-1-yl}oxy]phenyl)-3,7-dihydroxychroman-4-one and was given the trivial name (2*R*,3*R*)-4′-*O*-geranyl-7-hydroxydihydroflavonol.

The crude extract of the root bark of *M. usaramensis* ssp. *usaramensis* and some of its constituents were tested for cytotoxicity against the MDB-MB-231 human breast cancer and against the HEK293 human embryonic kidney cell lines (Table 4). The cytotoxicity of the crude extract (IC_{50} 11.63 μg/mL) on MDB-MB-231 cells is comparable to that of **10**, whereas all other tested constituents show lower toxicities. The antiplasmodial activity of 4′-*O*-geranylisoliquiritigenin (**12**) against chloroquine-sensitive (D6) and chloroquine-resistant (W2) *Plasmodium falciparum* has been previously reported (IC_{50} 10.6 and 8.7 μM, respectively).[6] Its isomer, the new geranylated chalcone **1**, shows moderate antiplasmodial activity against the chloroquine-sensitive 3D7 (IC_{50} 3.7 μM) and the chloroquine-resistant Dd2 (IC_{50} 5.3 μM) *P. falciparum* strains. Compound **1** also shows toxicity against HEK-293 cells (100% inhibition at 40 μM; see the Experimental Section for details), demonstrating no selectivity for the malaria parasite and limiting its development as an antimalarial lead compound. The major rotenoids of this plant (Table 4) were also tested for antiplasmodial activities against the two strains but were only moderately active.

In conclusion, a new chalcone (**1**), two new 12-dihydrorotenoids (**2** and **3**), a new flavanone (**4**), a new dihydroflavonol (**5**), and 12 known natural products (**6**–**17**) were isolated from the root bark of *M. usaramensis* ssp.

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 4. Cytotoxic Activities of the *M. usaramensis* ssp. *usaramensis* Crude Root Extract and of Some of Its Isolated Constituents against MDB-MB-231 Cells

sample	IC_{50}[a,b]		
	MDB-MB-231	3D7	Dd2
M. usaramensis crude root extract[a]	11.63		
4-*O*-geranylisoliquiritigenin (**1**)		3.67	6.97
usararotenoid A (**6**)	87.3	99%[c]	90%[c]
12-dihydrousararotenoid A (**7**)	>279.3		
millettosin (**8**)	61.7		
12a-epimillettosin (**9**)	100.7	28%[c]	29%[c]
usararotenoid C (**10**)	25.7		
jamaicin (**11**)		94%[c]	81%[c]
4′-*O*-geranylisoliquiritigenin (**12**)	125.5		
tephrosin (**14**)		13.27	12.05
colenemol (**16**)	207.2		

[a]IC_{50} is given in μg/mL for crude and in μM for pure compounds; 95% confidence intervals are given in the Supporting Information (S74). [b]As positive controls pyrmethamine (IC_{50} = 6.1 ± 5.1 nM (3D7), 62% at 40 μM (Dd2), 75% at 40 μM (HEK293)), chloroquine (IC_{50} = 4.3 ± 0.3 nM (3D7), IC_{50} = 69.9 ± 34.5 nM (Dd2), 51% at 40 μM (HEK293)), pyronaridine (IC_{50} = 10.7 ± 10.0 nM (3D7), IC_{50} = 12.6 ± 7.2 nM (Dd2), IC_{50} = 2.71 ± 1.3 μM (HEK293)), puromycin (IC_{50} = 43.7 ± 29.7 nM (3D7), IC_{50} = 54.3 ± 12.8 nM (Dd2), IC_{50} = 0.46 ± 1.41 μM (HEK293)), artesunate (IC_{50} = 1.6 ± 1.5 nM (3D7), IC_{50} = 0.8 ± 0.5 nM (Dd2), 73% at 20 μM (HEK293)), and dihydroartemisinin (IC_{50} = 0.4 ± 0.5 nM (3D7), IC_{50} = 0.4 ± 0.3 nM (Dd2), 54% at 40 μM (HEK293)) were used. Details are given in the Supporting Information. [c]The largest percentage inhibition observed at 100 μM concentration is given, where IC_{50} could not be accurately determined. The inhibitory activities are given as the mean value of at least two independent measurements.

usaramensis. Among the compounds tested for cytotoxicity, usararotenoid C (**10**) showed the highest activity. 4-*O*-Geranylisoliquiritigenin (**1**) showed moderate antiplasmodial activity with marginal selectivity index.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on a B-545 Switzerland Büchi melting point apparatus, optical rotations were measured on a PerkinElmer 341-LC polarimeter, whereas ECD experiments were run on a Jasco J-715 spectropolarimeter. UV spectra were recorded on a Specord S600 (Analytik Jena AG) spectrophotometer. NMR spectra were acquired at 400, 500, 600, and 800 MHz (^{1}H NMR) on Varian MR-400, Varian VNMR-S 500, Bruker Avance 600, and Bruker Avance III HD 800 spectrometers, using the residual solvent peaks as reference. The spectra were processed using MestReNova 10.0. EIMS spectra were obtained on a Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK), using direct inlet and 70 eV ionization voltage. LC-ESIMS were acquired on a PerkinElmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source connected to a Gemini 5 mm RPC_{18} 110 Å column and applying a H_2O/MeCN (80:20−20:80) gradient with a separation time of 8 min. TLC was carried out on Merck precoated silica gel 60 F254 plates. Column chromatography and MPLC were run on silica gel 60 (70−230 mesh). Gel filtration was done on Sephadex LH-20. Preparative HPLC was carried out on a Waters 600E instrument using the Chromulan (Pikron Ltd.) software and an RP C_8 Kromasil (250 mm × 55 mm) column with a H_2O/MeOH solvent system. X-ray data were obtained using an Agilent SuperNova Dual diffractometer with Atlas detector at T = 123.0(1) K using mirror-monochromatized Cu Kα radiation (λ = 1.541 84 Å).

Plant Material. The root bark *of M. usaramensis* ssp. *usaramensis* was collected from the Jadini forest, at the Coast Province, Kenya, in February 2008. The plant material was identified by Mr. S. G. Mathenge of the Herbarium, School of Biological Sciences, University of Nairobi, where the voucher specimen (Mathenge 2008/374) was deposited.

Extraction and Isolation. The dried and ground root bark of *M. usaramensis* ssp. *usaramensis* (1 kg) was extracted using 3 × 3 L of CH_2Cl_2/MeOH (1:1), for 24 h in each case, yielding 110 g of a brown-orange crude extract following concentration using a rotary evaporator. Approximately 100 g of the crude extract was subjected to column chromatography on silica gel (500 g) eluting with *n*-hexane containing increasing percentages of EtOAc. The fractions eluting with 2% EtOAc in *n*-hexane gave colenemol (**16**, 110 mg) and millettosin (**8**, 12.1 mg). The fractions eluted with 3% EtOAc in *n*-hexane were purified by crystallization from MeOH to yield 12a-epimillettosin (**9**, 140.4 mg). The mother liquid of the crystallization was subjected to column chromatography on silica gel eluting with *n*-hexane and increasing amounts of EtOAc and subsequently on Sephadex LH-20 using CH_2Cl_2/MeOH (1:1) eluent, yielding 12-dihydrousararotenoid C (**3**, 19 mg). The fractions eluting with 4% EtOAc in *n*-hexane were further purified by crystallization from MeOH, giving usararotenoid A (**6**, 86.0 mg). The mother liquid of this fraction was further separated by MPLC with *n*-hexane and increasing amounts of CH_2Cl_2 to give 4′-*O*-geranylisoliquiritigenin (**12**, 95.0 mg), 4-*O*-geranylisoliquiritigenin (**1**, 94.7 mg), and an additional mixture of two compounds. The latter mixture was separated by preparative HPLC, using the MeOH/H_2O gradient elution in decreasing polarity to give usararotenoid C (**10**, 9.4 mg) and 12a-epimillettosin (**9**, 6.0 mg). Another portion of this fraction (4% EtOAc in *n*-hexane) was purified on Sephadex, using CH_2Cl_2/MeOH (1:1) as an eluent, and gave maximaisoflavone H (**15**, 4.0 mg) and an additional 7 mg of usararotenoid A (**6**). The fractions eluted by 5% EtOAc in *n*-hexane were combined and subjected to column chromatography, using CH_2Cl_2/*n*-hexane (8:2), to afford three subfractions. One of these was purified by preparative HPLC to give 7-*O*-geranyl-5-hydroxyflavanone (**13**, 2.6 mg). Tephrosin (**14**, 2.5 mg) was obtained from the fractions eluted with 6% EtOAc in *n*-hexane, by purification on Sephadex with CH_2Cl_2/MeOH (1:1) and subsequently by preparative RP-HPLC, using MeOH/H_2O gradient elution. The fraction eluted with 7% EtOAc in *n*-hexane was subjected to column chromatography over silica gel using a 0−70% CH_2Cl_2 in *n*-hexane eluent mixture to afford jamaicin (**11**, 27.6 mg), 4′-*O*-geranyl-7-hydroxydihydroflavonol (**5**, 2.8 mg), and 4′-*O*-geranyl-7-hydroxyflavanone (**4**, 207.4 mg). 12-Dihydrousararotenoid B (**2**, 15.0 mg) was isolated from the fraction eluted with 8% EtOAc in *n*-hexane by purification on Sephadex with a CH_2Cl_2/MeOH (1:1) eluent mixture. The fractions eluted with 11−15% EtOAc in *n*-hexane were combined, concentrated, and crystallized from MeOH to give 12-dihydrousararotenoid A (**7**, 157.8 mg). Following crystallization, the mother liquid was subjected to preparative TLC (eluted with 4% acetone in CH_2Cl_2), yielding 7-hydroxy-8,3′,4′-trimethoxyisoflavone (**17**, 2.1 mg).

4-O-Geranylisoliquiritigenin (1): yellow solid; mp 95−97 °C; UV (MeOH) λ_{max} (log ε) 299 (4.2), 370 (3.5) nm; ^{1}H and ^{13}C NMR data, see Table 1 and Figures S1−S7; HREIMS m/z 392.1968 (calcd for $C_{25}H_{28}O_4$ 392.1988).

12-Dihydrousararotenoid B (2): colorless, amorphous solid; $[\alpha]_D^{20}$ +134 (c 0.0001, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 301 (4.2) nm; ECD (MeOH) 295 (+2.1); ^{1}H and ^{13}C NMR data, see Table 2 and Figures S9−S14; HREIMS m/z 374.0999 (calcd for $C_{19}H_{18}O_8$ 374.1002).

12-Dihydrousararotenoid C (3): colorless, amorphous solid; $[\alpha]_D^{20}$ +96 (c 0.0001, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 299 (4.3) nm; ECD (MeOH) 295 (+2.4); ^{1}H and ^{13}C NMR data, see Table 2 and Figures S17−S22; EIMS m/z 412 $[M]^+$ (18), 389 (34), 256 (40), 192 (26), 163 (25), 150 (27), 137 (42), 107 (43), 81 (37), 69 (100); HREIMS m/z 412.1520 (calcd for $C_{23}H_{24}O_7$ 412.1522).

(S)-4′-O-Geranyl-7-hydroxyflavanone (4): colorless, sticky oil; $[\alpha]_D^{20}$ −28 (c 0.0002, MeOH); UV (MeOH) λ_{max} (log ε) 275 (4.2), 310 (3.7) nm; ECD (MeOH) 332 (+4.3), 302 (−8.7); ^{1}H and ^{13}C NMR data, see Table 3 and Figures S25−S29; HREIMS m/z 392.1968 (calcd for $C_{25}H_{28}O_4$ 392.1988).

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

*(2R,3R)-4′-O-Geranyl-7-hydroxydihydroflavonol (**5**):* colorless, amorphous solid; UV (MeOH) λ_{max} 279, 305 nm; ECD (MeOH) 334 (+0.8), 300 (−3.3); ^{1}H and ^{13}C NMR data, see Table 3 and Figures S31−S36; HRESIMS *m/z* 409.2020 $[M + H]^+$ (calcd for $C_{25}H_{28}O_5$ 409.1937).

*12a-Epimillettosin (**9**):* needles (MeOH); mp 256−258 °C; $[\alpha]_D^{20}$ +230 (*c* 0.0013, CH_2Cl_2); ECD (MeOH): 324 (−10.2), 348 (+31.9); UV (MeOH) λ_{max} (log ε) 235 (4.54), 240 (2.38), 276 (4.38), 312 (3.99); ^{1}H and ^{13}C NMR data, see Figures S67−S75; ESIMS *m/z* 395.0 $[M + H - H_2O]^+$, 377.3 $[M]^+$.

Cytotoxicity Assays. MDB-MB-231 human breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density (10 000 cells per well) to ensure exponential growth for the duration of the assay. After a 24 h preincubation growth, the medium was replaced with experimental medium containing the appropriate drug concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). After 72 h incubation, cell viability was measured using Alamar Blue reagent (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± standard error for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least six times. Statistical analyses were performed using a two-tailed Student's *t* test. $P < 0.05$ was considered to be statistically significant (Supporting Information, S74).

To assess the cytotoxicity of compounds on HEK-293 cells in dose response, a resazurin-based viability assay was used. In brief, HEK293 cells were grown in DMEM medium (Life Technologies), containing 10% fetal calf serum (FCS; Gibco), trypsinised, counted, and seeded at 2000 cells per well in 45 μL of media into TC-treated 384-well plates (Greiner) and left to adhere overnight at 37 °C, 5% CO_2, and 60% humidity. Test compounds were prepared by diluting compounds 1 in 25 in sterile H_2O and then another 1 in 10 dilution, to give a final test concentration of 40 μM, 0.4% DMSO. Plates were incubated for 72 h at 37 °C, 5% CO_2, and 95% humidity, and then the media was removed and replaced by 35 μL of 44 μM resazurin in DMEM without FCS. The plates were incubated for another 4−6 h at 37 °C, 5% CO_2, and 95% humidity, before reading on an EnVision plate reader (PerkinElmer) using fluorescence excitation/emission settings of 530 nm/595 nm. The percent growth was standardized to controls (5 μM puromycin as positive and 0.4% DMSO as negative control) using Microsoft Excel 2013. A statistical analysis including IC_{50} determination and graphical output was performed in GraphPad Prism 6 using nonlinear regression variable slope curve fitting.

***P. falciparum* Culture.** In vitro parasite cultures of the *P. falciparum* strains 3D7 and Dd2 were maintained in RPMI with 10 mM Hepes (Life Technologies), 50 μg/mL hypoxanthine (Sigma), and 5% human serum from male AB plasma and 2.5 mg/mL AlbuMAX II (Life Technologies). Human 0+ erythrocytes were obtained from the Australian Red Cross Blood Service (Agreement No. 13-04QLD-09). The parasites were maintained at 2−8% parasitemia (% P) at 5% hematocrit (% H) and incubated at 37 °C, 5% CO_2, 5% O_2, 90% N_2, and 95% humidity.

***P. falciparum* Growth Inhibition Assay.** A well-established asexual *P. falciparum* imaging assay was used to determine parasite growth inhibition.[26] In brief, sorbitol (5% w/v) synchronization was performed twice, approximately 8 h apart, on each synchronization day for two consecutive ring cycles, i.e., on days 1 and 3 of assay preparation. On day 2, the culture was split to approximately 2% trophozoite parasitemia. On day 4, the culture was split to 1−1.5% trophozoite parasitemia, which yielded approximately an 8% ring parasitemia after 48 h on day 5, the day of the assay. Compound stocks (10 mM in 100% DMSO) were diluted 1 in 25 in H_2O, just prior to use. An additional 1 in 10 dilution was performed, resulting in a 1:250 overall compound dilution and a final DMSO concentration of 0.4%. For dose−response curves, a three-step logarithmic serial dilution was prepared at 40 μM top concentration for test compounds for the asexual assay and 2 μM for the positive control artemisinin. A 5 μL portion of the diluted test compound or control solutions (2 μM artemisinin as positive and 0.4% DMSO as negative control) was added to 384-well CellCarrier imaging plates (PerkinElmer). Parasite cultures were added to a final concentration of 2% parasitemia and 0.3% hematocrit. Plates were incubated for 72 h at 37 °C, 5% CO_2, and 95% humidity. On day 8, the permeabilization and nuclear staining buffer was prepared in PBS containing 10 μg/mL saponin, 0.01% Triton X, 5 mM EDTA (all Sigma), and 0.5 μg/mL 4′,6-diamidino-2-phenylindole (DAPI; Life Technologies).[26]

Prior to imaging on an Opera Confocal Imager (PerkinElmer) at 405 nm excitation with a 20× water, objective plates were incubated overnight at room temperature. An automated primary image analysis was performed concurrent with image acquisition, utilizing an Acapella software (PerkinElmer) script to determine the number of parasites based on object size and fluorescence intensity.[26] Determination of the percent growth compared to controls (2 μM artemisinin as positive and 0.4% DMSO as negative control) was performed in Microsoft Excel 2013. Statistical analysis including IC_{50} determination and graphical output was performed in GraphPad Prism 6 using nonlinear regression variable slope curve fitting. As positive controls pyrimethamine (3D7: 2.5 nM, Dd2: 50% at 40 μM, HEK270: 4.22 nM), chloroquine (3D7: 4.5 nM, Dd2: 45.5 nM, HEK270: 60% at 40 μM), pyrionaridine (3D7: 3.6 nM, Dd2: 7.51 nM, HEK270: 1.79 nM), puromycin (3D7: 22.7 nM, Dd2: 45.2 nM, HEK270: 361 nM), artesunate (3D7: 0.5 nM, Dd2: 0.443 nM, HEK270: 75% at 20 μM), and DHA (3D7: 0.1 nM, Dd2: 0.146 nM, HEK270: 50% at 20 μM) were used. Statistical data are given in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00581.

1D and 2D NMR, MS, UV, and CD spectra and data for X-ray crystallography, cytotoxicity, and antiplasmodial assays (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +46 31 786 9033. E-mail: mate@chem.gu.se (M. Erdélyi).

*Tel: +254 733 832 576. Fax: +254 20 444 6138. E-mail: ayenesew@uonbi.ac.ke (A. Yenesew)

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Mr. S. G. Mathenge of the Herbarium, Botany Department, University of Nairobi, for the identification of the plant species. T.D. thanks DAAD-NAPRECA for a Ph.D. scholarship; I.G. thanks the Swedish Institute for a Kenyan-Swedish sandwich Ph.D. scholarship. The International Science Program (ISP Sweden, grant KEN-02), the Swedish Research Council (2012-6124), and the Australian Research Council (grant LP120200557 to V.M.A.) are thankfully acknowledged for funding support. We thank the Australian Red Cross Blood Service for the provision of human blood.

■ REFERENCES

(1) Geesink, R. *Tephrosiae*; The Royal Botanic Gardens: Kew, 1981.
(2) Banzouzi, J. T.; Prost, A.; Rajemiariaho, M.; Ongoka, P. *Int. J. Bot.* **2008**, *4*, 406−420.

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX

(3) Beentje, H. *Kenya Trees, Shrubs and Lianas*; National Museums of Kenya: Nairobi, Kenya, 1994.
(4) Kokwaro, J. O. *Medicinal Plants of East Africa*, 2nd ed.; Kenya Literature Bureau: Nairobi, Kenya, 1993.
(5) Yenesew, A.; Midiwo, J. O.; Waterman, P. G. *Phytochemistry* **1998**, *47*, 295−300.
(6) Yenesew, A.; Derese, S.; Midiwo, J. O.; Oketch-Rabah, H. A.; Lisgarten, J.; Palmer, R.; Heydenreich, M.; Peter, M. G.; Akala, H.; Wangui, J.; Liyala, P.; Waters, N. C. *Phytochemistry* **2003**, *64*, 773−779.
(7) Sinha, S.; Medhi, B.; Sehgal, R. *J. Mod. Med. Chem.* **2013**, *1*, 64−77.
(8) Ntie-Kang, F.; Onguene, P. A.; Lifongo, L. L.; Ndom, J. C.; Sippl, W.; Mbaze, L. M. *Malar. J.* **2014**, *13*, 81.
(9) Ravishankar, D.; Rajora, A. K.; Greco, F.; Osborn, H. M. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 2821−2831.
(10) Park, E. J.; Pezzuto, J. M. *Anti-Cancer Agents Med. Chem.* **2012**, *12*, 836−851.
(11) Ollis, W. D.; Rhodes, C. A.; Sutherland, I. O. *Tetrahedron* **1967**, *23*, 4741−4760.
(12) Dagne, E.; Bekele, A.; Waterman, P. G. *Phytochemistry* **1989**, *28*, 1897−1900.
(13) Dagne, E.; Bekele, A.; Noguchi, H.; Shibuya, M.; Sankawa, U. *Phytochemistry* **1990**, *29*, 2671−2673.
(14) Madhusudhana, J.; Reddy, R. V.; Reddy, B. A.; Reddy, M. V.; Gunasekar, D.; Deville, A.; Bodo, B. *Nat. Prod. Res.* **2010**, *24*, 743−749.
(15) Carlson, D. G.; Weislede, D.; Tallent, W. H. *Tetrahedron* **1973**, *29*, 2731−2741.
(16) Dagne, E.; Mammo, W.; Bekele, A. *Bull. Chem. Soc. Ethiop.* **1991**, *5*, 81−86.
(17) Brader, G.; Bacher, M.; Hofer, O.; Greger, H. *Phytochemistry* **1997**, *45*, 1207−1212.
(18) Puebla, P.; Oshima-Franco, Y.; Franco, L. M.; Santos, M. G.; Silva, R. V.; Rubem-Mauro, L.; Feliciano, A. S. *Molecules* **2010**, *15*, 8193−8204.
(19) Vijayakumar, S.; Arulkumaran, R.; Sundararajan, R.; Sakthinathan, S. P.; Suresh, R.; Kamalakkannan, D.; Ranganathan, K.; Sathiyamoorthy, K.; Mala, V.; Vanangamudi, G.; Thirunarayanan, G. *Int. Lett. Chem., Phys. Astron.* **2013**, *14*, 68−86.
(20) Abegaz, B. M.; Ngadjui, B. T.; Dongo, E.; Ngameni, B.; Nindi, M. N.; Bezabih, M. *Phytochemistry* **2002**, *59*, 877−883.
(21) Kozawa, M.; Morita, N.; Baba, K.; Hata, K. *Chem. Pharm. Bull.* **1977**, *25*, 515−516.
(22) Oberholzer, M. E.; Rall, G. J. H.; Roux, D. G. *Tetrahedron Lett.* **1974**, *15*, 2211−2214.
(23) Dewick, P. M. Isoflavonoids. In *The Flavonoids: Advances in Research Since 1986*; Chapman and Hall: London, 1994.
(24) Yenesew, A.; Midiwo, J. O.; Waterman, P. G. *J. Nat. Prod.* **1997**, *60*, 806−807.
(25) Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177−2215.
(26) Duffy, S.; Avery, V. M. *Am. J. Trop. Med. Hyg.* **2012**, *86*, 84−92.

DOI: 10.1021/acs.jnatprod.5b00581
J. Nat. Prod. XXXX, XXX, XXX−XXX