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Isoflavone Dimers and Other Bioactive Constituents from the Figs of *Ficus mucuso*

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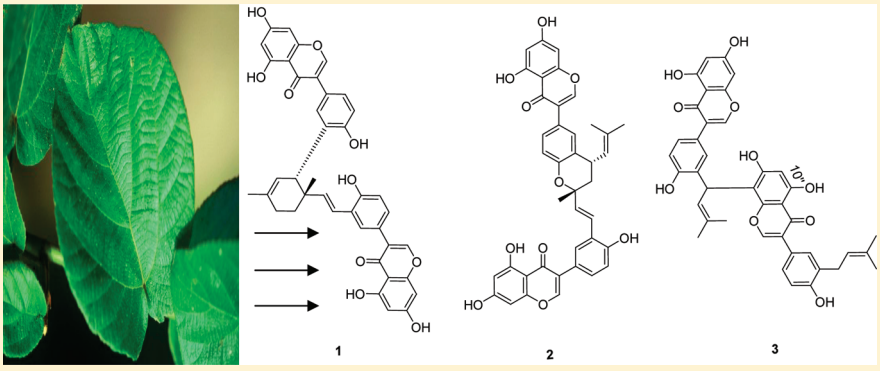
Supporting Information

ABSTRACT:



Phytochemical investigation of the figs of *Ficus mucuso* led to the isolation of three new isoflavone dimer derivatives, mucusisoflavones A−C (**1**−**3**), together with 16 known compounds. Some of the isolates were tested in vitro for their inhibitory properties toward β-glucuronidase and *Plasmodium falciparum* enoyl-ACP reductase (*Pf*ENR) enzymes. Compound **1** (IC_{50} 0.68 μM) showed inhibitory activity on β-glucuronidase enzyme, while **3** (IC_{50} 7.69 μM) exhibited a weak inhibitory activity against *P. falciparum* enoyl-ACP reductase (*Pf*ENR).

Ficus is a genus of about 150 plant species distributed worldwide.[1] In Cameroon, some plants of this genus are used for the treatment of several ailments. A mixture of powdered leaves of *Ficus mucuso* Welw. and palm oil is used to treat epilepsy in Fongo Tongo, a village of the western region of Cameroon.[2] A decoction of the stem bark is used by Baka pygmies to treat jaundice.[3] In a continuing search for bioactive compounds from traditional Cameroonian medicines, we have examined the MeOH extract of the figs of *F. mucuso*, and we report here the isolation and the structural elucidation of three new prenylated isoflavone dimer derivatives (**1**−**3**) together with the enzyme-inhibitory properties of some of the isolated compounds toward β-glucuronidase and *Plasmodium falciparum* enoyl-ACP reductase (*Pf*ENR) enzymes.

RESULTS AND DISCUSSION

The MeOH extract of the figs of *F. mucuso* was fractionated and purified on silica gel and Sephadex LH-20 columns, successively, to afford 19 compounds: mucusisoflavone A (**1**), mucusisoflavone B (**2**), mucusisoflavone C (**3**), β-sitosterol (**4**),[5] β-sitosterol 3-*O*-β-D-glucopyranoside (**5**),[6] lupeol acetate (**6**),[7] ursolic acid (**7**),[8] 3-*O*-*trans*-*p*-coumaroyltormentic acid (**8**),[9] 4-methoxybenzoic acid (**9**),[10] protocatechuic acid (**10**),[11] apigenin (**11**),[12] kaempferol (**12**),[13] 3-*O*-methylquercetin (**13**),[14] alpinumisoflavone

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Table 1. ^{13}C (125 MHz, methanol-d_4) and ^{1}H (500 MHz, methanol-d_4) NMR and HMBC Data for Compounds 1 and 2

	1				2			
position	δ_C	mult.	δ_H (J in Hz)	HMBC	δ_C	mult.	δ_H (J in Hz)	HMBC
2	154.6	CH	7.85, s	1′, 3, 4, 9, 10	154.8	CH	7.99, s	4, 9, 1′
3	124.5	qC			125.4	qC		
4	182.2	qC			182.2	qC		
5	163.7	qC			163.79	qC		
6	100.1	CH	6.15, d (2.0)	5, 8, 10	100.1	CH	6.21, d (2.0)	8
7	165.9	qC			166.0	qC		
8	94.9	CH	6.24, d (2.0)	4, 6, 10	94.8	CH	6.33, d (2.0)	
9	159.5	qC			159.67	qC		
10	106.2	qC			106.2	qC		
1′	123.4	qC			124.8	qC		
2′	132.7	CH	7.21, d (2.0)	3″, 3, 4′, 6′,	130.9	CH	7.22, d (2.0)	3, 4′
3′	131.2	qC			128.71	qC		
4′	156.7	qC			155.0	qC		
5′	115.6	CH	6.74, d (8.5)	1′, 3′, 3″	116.6	CH	6.84, d (8.0)	
6′	128.9	CH	7.12, dd (8.5, 2.0)	2′, 3, 4′, 5′	130.3	CH	7.23, dd (8.0, 2.0)	
1″	135.1	qC						
2″	125.6	CH	5.33, br d	3′, 3″, 4″, 6″, 7″,	77.8	qC		
3″a	45.1	CH	3.92, br d	1″, 2′, 2″, 3′, 4′, 4″	40.5	CH_2	2.02, dd (13.5, 6.0)	
3″b							1.75, dd (13.5, 11.5)	
4″	40.4	qC			33.2	CH	3.87, m	
5″a	34.9	CH_2	1.87, m	1″, 3″, 4″, 6″, 7″, 9″	128.68	CH	5.14, d (9.5)	
5″b			1.72, m	1″, 3″, 4″, 6″, 7″, 9″				
6″a	28.8	CH_2	2.26, m	1″, 2″, 4″, 5″, 7″	134.5	qC		
6″b			2.06, m	1″, 2″, 4″, 5″, 7″				
7″	23.8	CH_3	1.77, s	1″, 2″, 5″, 6″	18.1	CH_3	1.83, s	5″, 6″, 8″
8″	26.4	CH_3	1.22, s		26.0	CH_3	1.79, s	5″, 6″, 7″
9″	138.5	CH	6.48, d (16.5)	3″, 4″, 5″, 8″, 23″	24.3	CH_3	1.50, s	2″, 3″, 10″
10″	122.3	CH	6.40, d (16.5)	4″, 24″, 22″	136.4	CH	6.50, d (16.0)	2″, 11″
11″					123.8	CH	6.99, d (16.0)	2″, 10″
12″	154.5	CH	7.68, s	21″, 13″, 14″, 19″				
13″	122.5	qC			154.9	CH	8.06, s	22″, 15″, 20″
14″	182.1	qC			123.9	qC		
15″	163.6	qC			182.2	qC		
16″	100.0	CH	6.06, d (2.0)	15″, 18″, 20″	163.81	qC		
17″	165.7	qC			100.1	CH	6.20, d (2.0)	19″, 21″
18″	94.8	CH	6.12, d (2.0)	14″, 16″, 20″, 19″	166.0	qC		
19″	159.4	qC			94.8	CH	6.31, d (2.0)	17″, 21″
20″	106.1	qC			159.68	qC		
21″	124.9	qC			106.3	qC		
22″	128.1	CH	7.38, d (2.0)	24″, 26″, 21″, 13″	124.6	qC		
23″	127.2	qC			128.68	CH	7.58, d (2.0)	11″, 25″, 27″
24″	155.4	qC			126.0	qC		
25″	116.3	CH	6.72, d (8.5)	23″, 24″, 21″	156.3	qC		
26″	129.3	CH	7.11, dd (8.5, 2.0)	24″, 21″, 22″	118.3	CH	6.88, d (8.0)	24″, 22″
27″					129.4	CH	7.24, dd (8.0, 2.0)	25″, 23″

(**14**),[15] wighteone (**15**),[16] isoderrone (**16**),[17] isowighteone (**17**),[18] isowighteone hydrate (**18**),[18] and benjaminamide (**19**).[19]

Compound **1** was isolated as a yellowish, amorphous powder, $[\alpha]_D^{30}$ +33 (*c* 0.02, MeOH). It reacted positively with ferric chloride, indicating its phenolic nature. The molecular formula, $C_{40}H_{32}O_{10}$, implying 25 degrees of unsaturation, was deduced from NMR data and its positive HRFABMS, which showed a pseudomolecular ion peak $[M + H]^+$ at *m/z* 673.2243 (calcd 673.2074 for $C_{40}H_{33}O_{10}$). The UV spectrum in MeOH exhibited absorption bands at λ_{max} 222 and 261 nm, suggesting an isoflavone skeleton.[18] The IR spectrum of **1** showed absorption bands for hydroxy (3400 cm^{-1}), conjugated carbonyl (1653 cm^{-1}),

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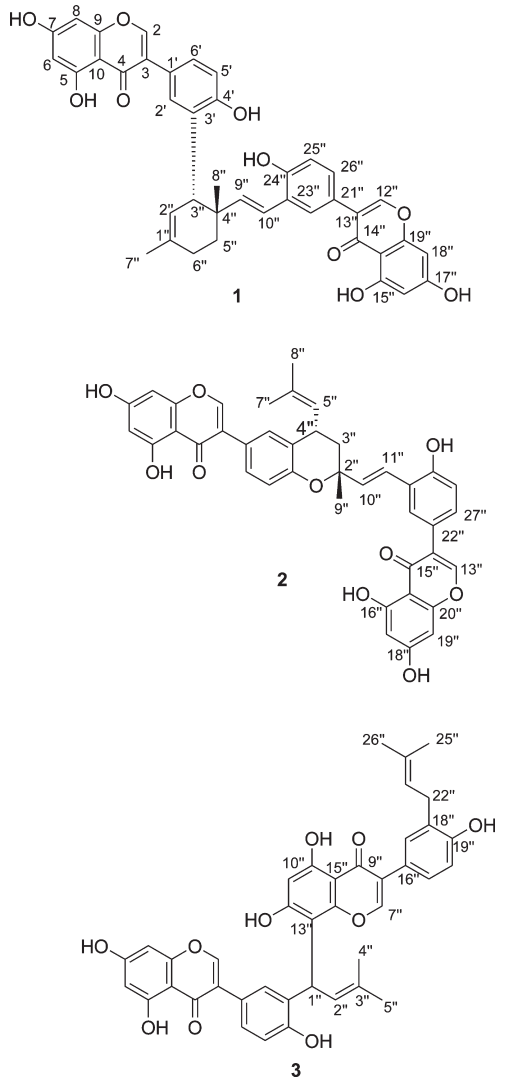

Figure 1. Structures of compounds **1**−**3** and **8**−**19**.

and aromatic system (1614 cm^{-1}) functionalities. The broad band decoupled ^{13}C NMR spectrum (Table 1) displayed 40 carbon signals, which were sorted by DEPT and HSQC techniques into 20 quaternary carbons [including two carbonyl functions at δ_C 182.2 (C-4) and 182.1 (C-14″)], 16 methine groups, two methylene groups at δ_C 28.8 and 34.9, and two methyl groups at δ_C 23.8 and 26.4. The ^1H NMR spectrum (Table 1) exhibited resonances for four *meta*-coupled aromatic protons at δ_H 6.15 (1H, d, J = 2 Hz, H-6), 6.06 (1H, d, J = 2 Hz, H-16″), 6.24 (1H, d, J = 2 Hz, H-8), and 6.12 (1H, d, J = 2 Hz, H-18″) and two sets of ABX aromatic protons at δ_H 7.21 (1H, d, J = 2.0 Hz, H-2′), 7.12 (1H, dd, J = 2.0, 8.5 Hz, H-6′) and 6.74 (1H, d, J = 8.5 Hz, H-5′) and δ_H 7.38 (1H, d, J = 2.0 Hz, H-22″), 7.11 (1H, dd, J = 2.0, 8.5 Hz, H-26″), and 6.72 (1H, d, J = 8.5 Hz, H-25″). The spectrum also exhibited two deshielded singlets, characteristic of H-2 of isoflavones at δ_H 7.85 (1H, s, H-2) and 7.68 (1H, s, H-12″), suggesting the presence of two isoflavone units in **1**.[20] The ^1H NMR data indicated that the isoflavone units of **1** were a 3′-substituted 4′,5,7-trihydroxyisoflavone system as in isowighteone **17**, an isoflavone isolated from the same extract. The monoterpene bridge linking the two isoflavone units was suggested by the significant fragment ion peak at m/z 336, obtained by a retro Diels−Alder-type fragmentation in the EIMS (Figure 2) of **1** and also confirming that the two isoflavone units were identical. The ^1H NMR spectrum showed that the C_{10} fragment contained resonances characteristic of conjugated olefinic protons of a *trans* double bond [δ_H 6.48 (1H, d, J = 16.5 Hz, H-9″) and 6.40 (1H, d, J = 16.5 Hz, H-10″)], another olefinic proton at δ_H 5.33 (1H, br d, H-2″), and one allylic methine proton at δ_H 3.92 (1H, br d, H-3″), bonded to an aromatic ring . In addition to these, there were resonances for one vinylic and one allylic methyl at δ_H 1.77 (3H, s, H-7″) and 1.22 (3H, s, H-8″), respectively, and four multiplets at δ_H 2.26 (1H, m, H-6″a), 2.06 (1H, m, H-6″b), 1.87 (1H, m, H-5″a), and 1.72 (1H, m, H-5″b), corresponding to two pairs of diastereotopic methylene protons. The $^1H-^1H$ COSY and HMBC spectra allowed the identification of the sequence $-CH-CH=C(CH_3)CH_2-CH_2-$, which could be extended to $-CH-CH=C(CH_3)CH_2-CH_2-C(CH_3)CH=CH-$ for the C_{10} fragment. In fact, couplings were observed in the $^1H-^1H$ COSY spectrum between the olefinic proton at δ_H 5.33 (br d) and the vinylic methyl group and the allylic methine [δ_H 3.92 (1H, br d)] and between the two methylene groups. An additional allylic coupling was observed between the vinylic methyl and the methylene protons at δ_H 2.26 and 2.06. In the HMBC spectrum, the proton at δ_H 6.48 (d, J = 16.5 Hz, H-9″) showed correlations with C-3″ (δ_C 45.1), C-4″ (40.4), C-5″ (34.9), and C-8″ (26.4). Thus, the terpene unit comprises a tetrasubstituted cyclohexene ring, with the *trans* double bond at C-4″, and two methyl groups at C-1″ and C-4″. Junctions between the two isoflavone units and the terpene bridge were made using HMBC correlations. Cross-peaks between the proton at δ_H 3.92 (br d, H-3″) and carbons at δ_C 132.7 (C-2′) and 156.7 (C-4′) suggested that C-3′ (δ_C 131.2) of the first isoflavone unit was linked to C-3″ (δ_C 45.1) of the terpene moiety. In addition, correlations between H-10″ of the terpene moiety [δ_H 6.40 (d, J = 16.5 Hz)] and C-22″ (δ_C 128.1) and C-24″ (δ_C 155.4) suggested that C-23″ (δ_C 127.2) of the second isoflavone unit was linked to C-10″ (δ_C 122.3) of the terpene moiety.

The relative configuration and conformation of the cyclohexene ring have been established by NOE studies. The NOEs between H-8″ and H-3″, H-8″ and H-10″, H-2″ and H-3″, H-2″ and H-7″, and H-2″ and H-2′ indicated their proximity. To establish the relative configuration at C-3″ and C-4″, the conformational flexibility of the cyclohexene ring had to be taken into account. In fact, studies have shown in the dimeric coumarin phebalin,[20] the xanthone garcilivin A,[21] and the synthetic 1,3,4-triphenyl-4-[(*E*)-styryl]-1-cyclohexene[22] (the latter two which both possess a cyclohexene system analogous to that of **1**) that the C-1″ styryl and C-2″ coumarin groups are in an axial−equatorial relationship, respectively, in phebalin. In garcilivin A and the synthetic cyclohexene derivative, the corresponding substituents occupy the equatorial and axial positions, respectively. With respect to the cyclohexene ring in compound **1**, the NOEs between H-8″ and H-3″, and between H-3″ and H-2″, clearly indicated that the C-4″ styryl and the C-3″ isoflavone units are in an axial−equatorial relationship, respectively (Figure 3), as in phebalin. Thus, compound **1** is the new isoflavone dimer derivative, named mucusisoflavone A. The fragmentation pattern of **1** (Figure 2) and HMBC correlations confirmed the structure assignment.

Compound **2** was isolated as yellowish crystals (*n*-hexane/EtOAc, 60:40), mp 210−212 °C, $[\alpha]_D^{30}$ +32 (*c* 0.03, MeOH). The molecular formula, $C_{40}H_{32}O_{10}$, with 25 degrees of

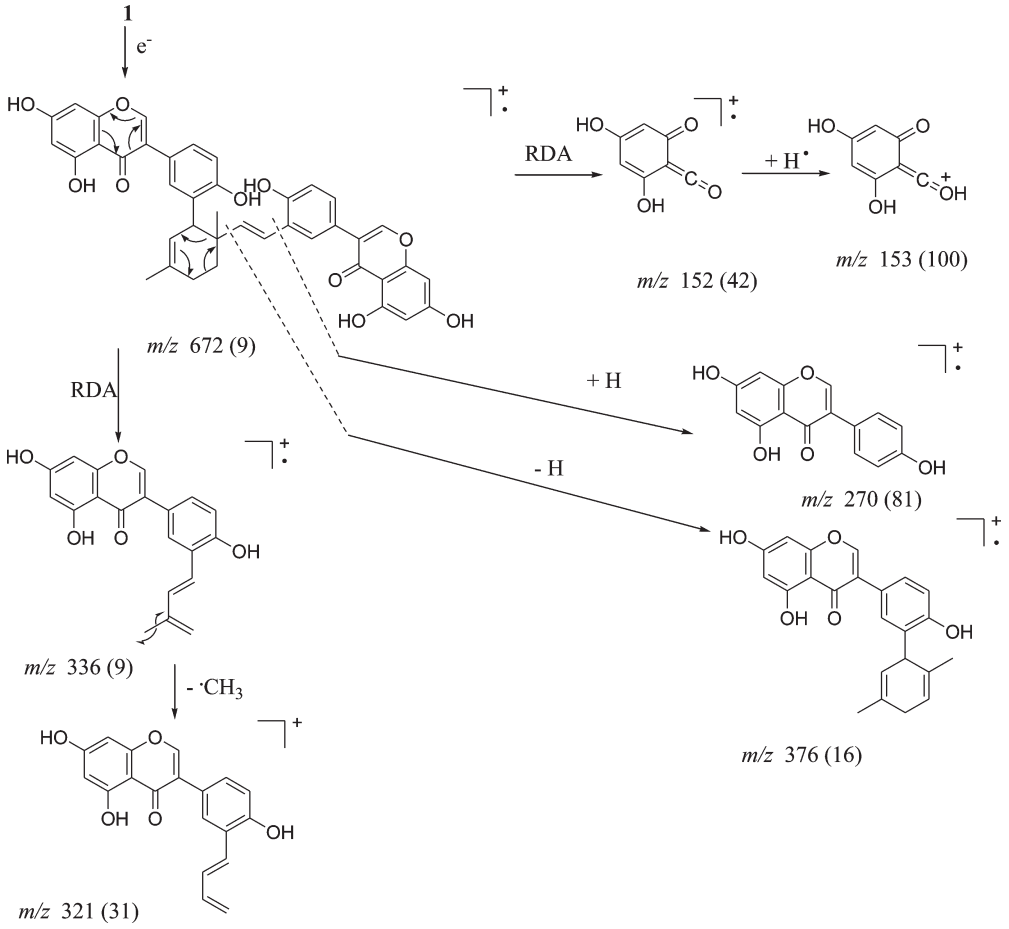

Figure 2. EI mass fragments of compound **1**.

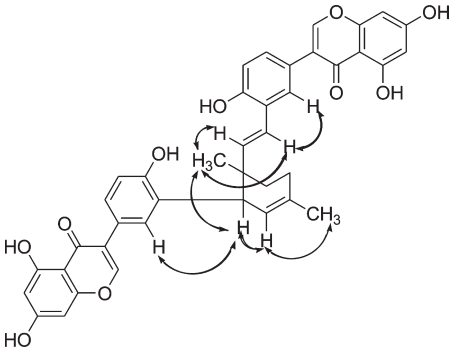

Figure 3. NOESY correlations observed in compound **1**.

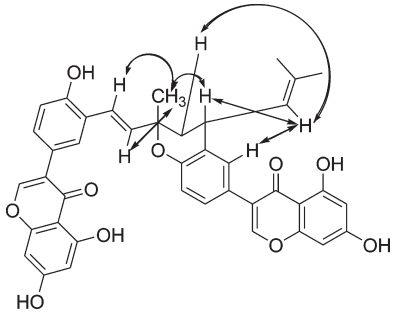

Figure 4. NOESY correlations observed in compound **2**.

unsaturation, was deduced from NMR data and its positive HRFABMS, which showed a pseudomolecular ion peak $[M + H]^+$ at m/z 673.2240 (calcd 673. 2074 for $C_{40}H_{33}O_{10}$). Like its isomer **1**, compound **2** also appeared to be an isoflavone dimer derivative based on its UV spectrum data in MeOH, which exhibited absorptions at λ_{max} 263 and 224 nm, and the two singlets in its ^{1}H NMR at δ_H 7.99 and 8.06, characteristic of two H-2 protons of isoflavones.[18] Its IR spectrum showed absorption bands at 3400, 1653, and 1616 cm^{-1}. The ^{1}H and ^{13}C NMR data (Table 1) of **2** were similar to those of mucusisoflavone A (**1**). The differences were proton resonances in the bridge linking the two isoflavone units. The cyclohexene ring system in **1** was replaced by a substituted dihydropyran ring in **2**, which showed, in addition to an oxygenated quaternary carbon (δ_C 77.8), two diastereotopic methylene protons [δ_H 2.02 (dd, J = 6.0, 13.5 Hz, H-3″a) and 1.75 (dd, J = 11.5, 13.5 Hz, H-3″b)], coupled to an allylic methine [δ_H 3.87 (m, H-4″)], vicinal to an aromatic ring. The ^{1}H NMR spectrum also displayed a characteristic 2,2-dimethylvinyl group [δ_H 1.83 (s, H-7″), 1.79 (s, H-8″), and 5.14 (d, J = 9.5 Hz, H-5″)], a methyl group (δ_H 1.5) and two *E*-configured olefinic protons (δ_H 6.50 and 6.99, J = 16 Hz). Furthermore, HMBC and COSY correlations established the fusion of the dihydropyran ring at C-3′ (δ_C 128.7) and C-4′ (δ_C 155.0) of the B-ring and also fixed the 2,2-dimethylvinyl and the allylic methyl groups at C-4″ (δ_C 33.2) and C-2″ (δ_C 77.8),

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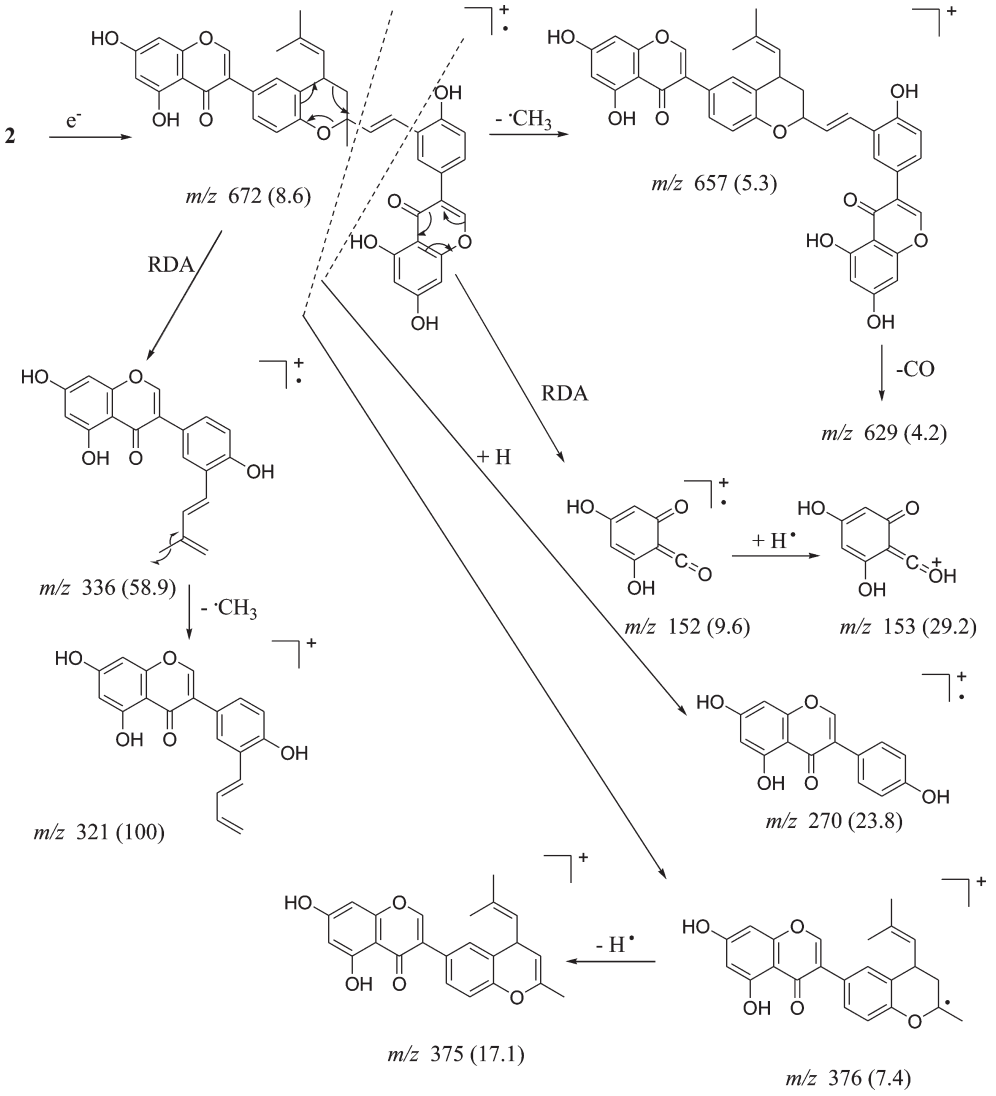

Figure 5. EI mass fragments of compound **2**.

respectively. Cross-peaks were observed between the two *E*-configured olefinic protons and C-2″, indicating the location of the *E* double bond at C-2″ of the pyran ring.

The relative configuration and conformation of the dihydropyran ring was assigned on the basis of NOE studies. In fact, the NOEs between H-9″ and H-3″b, H-4″, and H-11″ and the mutual enhancement of the allylic methyl and H-4″ are compatible only with axial positions for these groups. The vicinal coupling constants $J_{3''b4''} = 6.0$ and $J_{3''a4''} = 11.5$ Hz support the axial position of H-4″ and the preferred conformation of the dihydropyran ring as shown in Figure 4. Assignments for the terpene moiety are also in accordance with those of the structurally identical terpene bridge in garcilivin B.[21] On the basis of the above data, compound **2** was concluded to be a new isoflavone dimer, named mucusisoflavone B. The fragmentation pattern of **2** (Figure 5) and HMBC correlations confirmed the structure assignment.

Compound **3** was isolated as yellowish crystals (*n*-hexane/EtOAc mixture, 60:40), mp 206−207 °C, $[\alpha]_D^{30}$ +31 (*c* 0.03, MeOH). The molecular formula, $C_{40}H_{34}O_{10}$, was deduced from NMR data and its positive HRFABMS, which showed the pseudomolecular ion peak $[M + H]^+$ at *m/z* 675.2282 (calcd 675.2230 for $C_{40}H_{35}O_{10}$) corresponding to 24 degrees of unsaturation. The UV spectrum of compound **3** was similar to those of **1**, **2**, and isowighteone **17**, with absorption bands at λ_{max} 264 and 222 nm, characteristic of isoflavone derivatives.[18] The broad band decoupled ^{13}C NMR spectrum of **3** (Table 1) displayed 40 carbon resonances, which were sorted by DEPT and HSQC spectra into 21 quaternary carbons [including two carbonyl functions at δ_C 182.7 (C-9″) and 182.4 (C-4)], 14 methine groups, one methylene group at δ_C 29.3 (C-22″), and four primary sp^3 carbon atoms at δ_C 17.8 (C-4″), 18.2 (C-25″), 25.9 (C-26″), and 26.0 (C-5″). Its ^1H and ^{13}C NMR spectra were similar to those of **1** and **2** (Table 1), except for the absence of the terpene moiety and the appearance of two sets of signals, characteristic of two 3,3-dimethylallyl groups [(δ_H 1.70 (6H, s, H-25″ and H-26″), 3.32 (2H, overlapped dd, H-22″) and 5.34 (1H, br t, H-23″), and (δ_H 1.70 (3H, s, H-4″), 1.77 (3H, s, H-5″), 5.76 (d, J = 9 Hz, H-1″), and 6.00 (d, J = 9 Hz, H-2″)]. The presence of a substituted 3,3-dimethylallyl group was confirmed by the $^1H-^1H$ COSY spectrum, which revealed the existence of the fragment $-CH-CH=C(CH_3)_2$. Further comparison of the ^1H and ^{13}C NMR data of **3** with those of **17** led to

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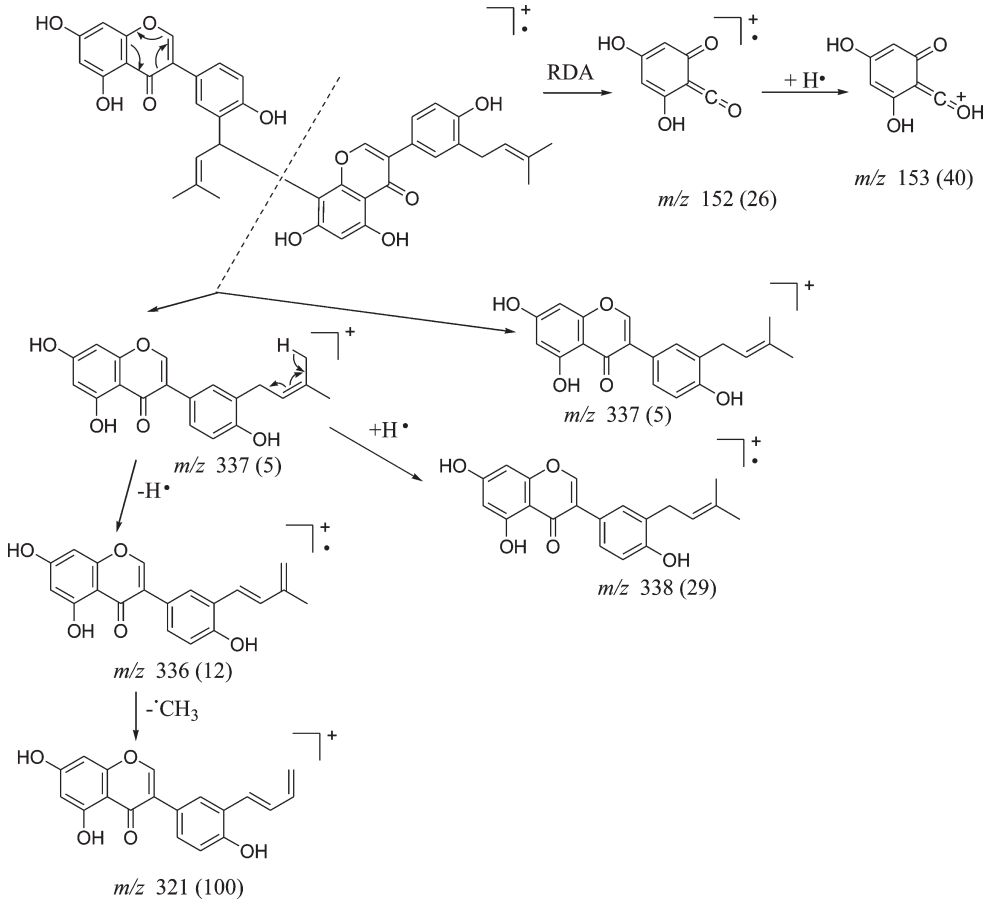

Figure 6. EI mass fragments of compound **3**.

the assumption that **3** is a dimer of isowighteone **17**. This was confirmed by the mass spectrum (Figure 6) of **3**, which showed the molecular ion peak $[M + H]^+$ at *m/z* 675.2282 and the prominent fragment ion $[M + H - 337]^+$ at *m/z* 338. The linkage of the two isowighteone units of **3** has been established by inspection of NMR data of **3** and **17** (Table 1). The absence in the ^{1}H NMR spectrum of **3** of the H-13″ resonance [(which appeared at δ_H 6.26 (d, J = 2 Hz, H-8)/ δ_C 94.5 in **17**], the presence of a quaternary carbon at δ_C 111.0 (C-13″), only one methylene resonance, and the appearance of the methine resonance at δ_H 5.76 (d, J = 9 Hz, H-1″)/δ_C 34.5 (C-1″) indicated that the two isowighteone units in **3** are linked by C-1″ and C-13″. The chemical shift of H-1″ (δ_H 5.76/ δ_C 34.5) is in accordance with its double benzylic and allylic nature. On the basis of the above data, compound **3** was concluded to be a new isoflavone dimer, named mucusisoflavone C.

Although a few dimeric isoflavones with a direct C−C bond or an ether linkage[23] between the two isoflavone units have been reported, compounds **1** and **2** appear to be the first examples of isoflavone dimers where the two isoflavone units are bonded via a C_{10} terpene moiety. Biogenetically, mucusisoflavones A (**1**) and B (**2**) are Diels−Alder adducts and would be synthesized through [2+4] reaction between two molecules of 3′-(3-methylbuta-1,3-dienyl)-4′,5,7-trihydroxyisoflavone, one as a dienophile and the other as a diene. This diene was not isolated from the plant extract, but compound **17** from which it can be derived has been isolated as one of the major compounds from the extract. Several Diels−Alder dimeric coumarins[20] or xanthones[21] are known and have been isolated from plants. All the dimeric coumarins are formed under *ortho* regioselective control[24] like **1**, whereas some dimeric xanthones are *meta* cycloaddition adducts like **2**. Many other natural Diels−Alder adducts of coumarins and xanthones were reported as optically inactive.[20,21]

Compounds **1**−**3**, **8**, **9**, and **12**−**19** were tested in vitro for their inhibitory effects toward the *Pf*ENR enzyme, which is a target in antimalarial drug research. As depicted in Table 3, results showed that, overall, all the pure compounds exhibited weaker *Pf*ENR-inhibitory activity compared to the reference drug, triclosan. The isolates showed IC_{50} values ranging from 7.69 μM (compound **3**) to 248.3 μM (compound **18**), whereas the IC_{50} value of the reference drug triclosan was 0.2 μM. However it is interesting to note that the presence of a 3-methylbut-2-enyl group at C-3 of the B-ring has shifted the IC_{50} from 58.95 μM (compound **17**) to 7.69 μM (compound **3**), indicating that this substitution may be important for activity. In contrast, an additional hydroxy group at C-3′ of the 3-methylbut-2-enyl leads to the loss of activity, as demonstrated by the IC_{50} value of compound **18** (IC_{50} 248.3 μM). Similarly, the presence of the 3-methylbut-2-enyl group at C-6 leads to the loss of activity as observed for compound **14**, **15**, and **16**.

Compounds **1**−**3** and **8**−**19** were also tested in vitro for their inhibitory effect toward the β-glucuronidase enzyme, which has been described as a pro-inflammatory enzyme.[25] As shown in Table 4, nine of the isolates exhibited promising inhibitory activities with IC_{50} values between 0.68 and 44.65 μM. Compound **1** (IC_{50} 0.68 μM) displayed the best potency and was more than 71 times more active than the reference drug.

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Table 2. ^{13}C (125 MHz, methanol-d_4) and ^{1}H (500 MHz, methanol-d_4) NMR and HMBC Data for Compounds 3 and 17

	3				17		
position	δ_C	mult.	δ_H (J in Hz)	HMBC	δ_C	mult.	δ_H (J in Hz)
2	154.6	CH	7.99, s	4, 9, 1′	154.5	CH	7.95, s
3	125.3	qC			123.9	qC	
4	182.4	qC			182.2	qC	
5	163.2	qC			163.8	qC	
6	100.1	CH	6.21, d (2.0)	8	100.7	CH	6.16, d (2.1)
7	165.9	qC			167.6	qC	
8	94.8	CH	6.32, d, (2.0)	6, 9	95.2	CH	6.26, d (2.1)
9	159.7	qC			159.9	qC	
10	106.5	qC			105.9	qC	
1′	122.6	qC			123.4	qC	
2′	131.8	CH	7.65, d (2.0)	4′	131.4	CH	7.19, d (1.8)
3′	131.3	qC			129.5	qC	
4′	156.4	qC			156.5	qC	
5′	115.7	CH	6.75, d (8.0)	3′	115.8	CH	6.79, d (8.1)
6′	128.4	CH	7.12, dd, (8.0, 2.0)		128.7	CH	7.15, dd (8.1, 1.8)
1″	34.5	CH	5.76, d (9.0)	2″	29.4	CH_2	3.33, d (7.2)
2″	126.1	CH	6.00, d (9.0)	1″	123.9	CH	5.34, m
3″	133.6	qC			133.1	qC	
4″	17.8	CH_3	1.70, s	2″, 3″, 5″	17.9	CH_3	1.72, s
5″	26.0	CH_3	1.77, s	2″, 3″, 4″	25.9	CH_3	1.72, s
7″	154.7	CH	8.17, s	9″, 14″, 16″			
8″	122.6	qC					
9″	182.7	qC					
10″	161.5	qC					
11″	99.9	CH	6.25, s	13″, 15″, 10″			
12″	163.9	qC					
13″	111.0	qC					
14″	157.1	qC					
15″	106.4	qC					
16″	124.3	qC					
17″	131.2	CH	7.19, d (2)	19″, 21″			
18″	129.5	qC					
19″	156.3	qC					
20″	115.9	CH	6.79, d (8.0)	18″			
21″	128.6	CH	7.18, dd (8.0, 2.0)	19″			
22″	29.3	CH_2	3.32, overlapped dd	18″, 23″, 24″			
23″	123.9	CH	5.34, br t				
24″	133.0	qC					
25″	18.2	CH_3	1.70, s	26″			
26″	25.9	CH_3	1.70, s	25″			

Interestingly, though possessing the same isoflavone units, compound **1** was more active than compounds **2** and **3**, indicating that the presence of a cyclohexene ring may significantly enhance the inhibitory activity of these isoflavone dimer derivatives. In addition, compound **15** (IC_{50} 4.03 μM) showed better activity than compound **17** (IC_{50} 17.60 μM), suggesting that the presence of a 3-methylbut-2-enyl group at C-6 (versus C-3′) increases the activity. For pyrano-isoflavones, the activity is negligible when the 2,2-dimethylpyrano ring is present on ring A, as shown in Table 3 for compound **16** (IC_{50} 13.13 μM).

Isoflavone metabolites are known to possess antifungal,[26] antiplasmodial, and antioxidant activities.[27] Consumption of isoflavones is also associated with human health benefits such as decreased risk of heart disease, reduced menopausal symptoms, and reduced risk of some hormone-related cancers.[28] This literature information supports the results of the in vitro enzyme-inhibitory evaluation of several of the compounds isolated from *F. mucuso* toward β-glucuronidase and *P. falciparum* enoyl-ACP reductase (*Pf*ENR) enzymes.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on a Gallenkamp melting point apparatus. Optical rotations were

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Table 3. In Vitro *Plasmodium falciparum* Enoyl-ACP Reductase Inhibiting Activities of Some Compounds from the Figs of *F. mucuso* and MeOH Extract

sample	concentration (mM)	% inhibition	$IC_{50} \pm SEM$ (μM)[a]
MeOH extract	0.250	2.5	ND
1	0.250	inactive	inactive
2	0.250	inactive	inactive
3	0.025	89.1	7.69 ± 0.8
8	0.250	3.9	ND
9	0.250	inactive	inactive
12	0.250	6.5	ND
13	0.100	88.2	35.2 ± 0.2
14	0.250	12.6	ND
15	0.150	42.6	ND
16	0.250	24.1	ND
17	0.075	66.9	58.95 ± 0.5
18	0.250	51.1	248.3 ± 0.7
19	0.250	inactive	inactive
triclosan[b]	0.050	99.5	0.2 ± 0.3

[a] IC_{50}: concentrations that inhibited 50% of enzymes relative to negative control; ND: not determined; SEM: standard mean error. [b] Standard used for the assay.

Table 4. In Vitro β-Glucuronidase-Inhibiting Activities of Some Compounds from the Figs of *F. mucuso* and MeOH Extract

sample	concentration (mM)	% inhibition	$IC_{50} \pm SEM$ (μM)[a]
MeOH extract	0.20	49.6	ND
1	0.20	98.7	0.68 ± 0.01
2	0.10	70.1	13.96 ± 1.23
3	0.10	94.0	3.08 ± 0.05
8	0.08	15.9	ND
9	0.20	66.2	124.0 ± 1.9
10	0.20	23.5	ND
11	0.20	15.7	ND
12	0.20	87.5	27.57 ± 0.69
13	0.20	85.2	44.65 ± 0.25
14	0.08	17.6	ND
15	0.20	97.3	4.03 ± 0.05
16	0.20	75.0	13.13 ± 0.05
17	0.10	92.7	17.60 ± 0.26
18	0.20	89.6	15.62 ± 0.07
19	0.08	1.7	ND
D-saccharic acid 1,4-lactone[b]	0.05	99.5	48.40 ± 1.25

[a] IC_{50}: concentrations that inhibited 50% of enzymes relative to negative control; ND: not determined; SEM: standard mean error. [b] Standard used for the assay.

measured with a JASCO DIP-360 polarimeter. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. A JASCO 320-A spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D and 2D NMR spectra were run on a Bruker spectrometer operating at 75, 100, 125, 150, 300, 400, 500, and 600 MHz, where chemical shifts (δ) were expressed in ppm with reference to the solvent signals. EIMS spectra were obtained on a Varian MAT 311A mass spectrometer. FABMS spectra were measured on a JEOL-HX 110 mass spectrometer. Column chromatography was performed on silica gel 230−400 mesh (Merck). Fractions were monitored by TLC using precoated aluminum-backed silica gel 60 F_{254} sheets. Spots were visualized under UV light (254 and 365 nm) or using ceric sulfate reagent.

Plant Material. The figs of *F. mucuso* were collected in June 2008 at Tongolo-Yaoundé, in the Central Region of Cameroon. The plant was identified by Mr. Nana Victor, botanist at the National Herbarium, where a voucher specimen (no. HNC 41204) is deposited.

Extraction and Isolation. The air-dried and powdered figs (4.6 kg) were extracted with MeOH (12 L) (72 h × 3) at room temperature. The extract was concentrated to dryness under reduced pressure at low temperature to give 325 g of a dark brownish crude extract. The extract was subjected to MPLC over silica gel 230−400 mesh (Merck) eluting with *n*-hexane, mixtures of *n*-hexane/EtOAc, EtOAc, and EtOAc/MeOH of increasing polarities. Subfractions (150) of 500 mL each were collected and combined according to their TLC profiles to afford seven fractions (F1−F7). Fraction F1 (65 g) was subjected to CC over silica gel 230−400 mesh (Merck), eluted with *n*-hexane/EtOAc mixtures (1:0−1:1), and yielded lupeol acetate (**6**) (4.02 g), β-sitosterol (**4**) (137 mg), and ursolic acid (**7**) (7 mg). Fraction F2 (22 g) was also subjected to CC over silica gel and eluted with *n*-hexane/EtOAc mixtures (1:9−0:1) to give alpinumisoflavone (**14**) (156 mg) and isoderrone (**16**) (1.02 g). Fraction F3 (27 g) was subjected to CC over silica gel with *n*-hexane/EtOAc mixtures (3:7−0:1) and yielded wighteone (**15**) (43 mg), isowighteone (**17**) (72 mg), and isowighteone hydrate (**18**). Fraction 4 (19 g) was subjected to CC over silica gel with *n*-hexane/EtOAc mixtures (3:7−0:1) and yielded apigenin (**11**) (47.5 mg), kaempferol (**12**) (13.2 mg), 3-*O*-methylquercetin (**13**) (8.3 mg), and 4-methoxybenzoic acid (**9**) (14 mg). Fraction 5 (32 g) was subjected to CC over silica gel with $CHCl_3$/MeOH mixtures (1:0−9:1). Subfractions were subjected to CC over Sephadex LH-20 using MeOH as solvent to yield mucusisoflavone A (**1**) (12 mg), mucusisoflavone B (**2**) (67 mg), mucusisoflavone C (**3**) (6 mg), and 3-*O*-*trans*-*p*-coumaroyltormentic acid (**8**) (5 mg). Fraction 6 (37 g) was subjected to CC over silica gel with $CHCl_3$/MeOH mixtures (1:0−7:3) to yield sitosterol 3-*O*-β-D-glucopyranoside (**5**) (69 mg) and benjaminamide (**19**) (145 mg), respectively. Fraction 7 (112 g) was subjected to silica gel with $CHCl_3$/MeOH mixtures (9.5:0.5−7:3) to yield protocatechuic acid (**10**) (23 mg).

*Mucusisoflavone A (**1**):* yellowish, amorphous powder; $[\alpha]_D^{30}$ +33 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 261 (3.08); 222 (2.85); IR ν_{max} 3400, 1653, 1614 cm^{-1}; ^1H (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4) (data see Table 1); EIMS *m*/*z* 672 $[M]^+$ (9), 376 (16), 321 (31), 270 (81), 241 (5), 153 (100), 69 (38), 55 (18); (+)-HRFABMS *m*/*z* 673.2243 $[M + H]^+$ (calcd for $C_{40}H_{33}O_{10}$, 673. 2074).

*Mucusisoflavone B (**2**):* yellowish crystals; mp 210−212 °C; $[\alpha]_D^{30}$ +32 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 261 (2.61); 222 (2.27); IR ν_{max} 3400, 1653, 1616 cm^{-1}; ^1H (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4) data (see Table 1); EIMS *m*/*z* 672 $[M]^+$ (8.6), 657 (5.3), 390 (7.4), 376 (7.4), 336 (58.9), 321 (100), 270 (23.8), 153 (29.2); (+)-HRFABMS *m*/*z* 673.2240 $[M + H]^+$ (calcd for $C_{40}H_{33}O_{10}$, 673. 2074).

*Mucusisoflavone C (**3**):* yellowish crystals; mp 206−207 °C; $[\alpha]_D^{30}$ +31 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 261 (2.73); 222 (2.52). IR ν_{max} 3400, 1653 cm^{-1}; ^1H (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4) data (see Table 1); EIMS *m*/*z* 338 (29), 321 (100), 283 (27), 253 (13), 213 (3), 185 (5), 169 (47), 153 (40), 115 (39), 96 (18), 69 (35), 55 (16); (+)-HRFABMS *m*/*z* $[M + H]^+$ 675.2282 (calcd 675.2230 for $C_{40}H_{35}O_{10}$).

***In Vitro P. falciparum* Enoyl-ACP Reductase Assay.** *P. falciparum* enoyl-ACP reductase was overexpressed, purified,[29] and assayed as described earlier.[30] The enzyme substrate crotonyl-CoA and NADH were purchased from Sigma, and the inhibitory activity

was measured at 340 nm following the manufacturer's instructions. All experiments were performed in 50 mM sodium phosphate buffer, pH 7.5, containing 100 mM NaCl. The standard assay mixture used as positive control contained 150 μM crotonyl-CoA, 100 μM NADH, 170 nM *Pf*ENR, and 5% DMSO in a total volume of 100 μL. The isolates as well as triclosan were dissolved in DMSO. The isolates were pipetted in 96-well UV-plates and serial diluted; then a mixture containing buffer, enzyme, and NADH was added. This mixture was preincubated for 5 min before initiating the reaction by adding the substrate crotonyl-CoA and then incubated at 25 °C for 10 min. The change in absorbance at 340 nm on a UV−vis spectrophotometer allowed the measurement of the inhibitory activity of compounds on the *Pf*ENR. The inhibitory activity was further converted by SOFTmax PRO software (Molecular Devices, Sunnyvale, CA) into percentage inhibition data using the following formula: % of inhibition = $[(E - S)/E] \times 100$, where E is the activity of enzyme without test material and S is the activity of enzyme with test material. The IC_{50} values (the concentration of compound that gave 50% reduction of the enzyme activity) were determined using EZ-Fit windows-based software (Perrella Scientific Inc. Amherst, NH). Percentage inhibition data were fitted to a log concentration curve of isolates using nonlinear regression.

***β*-Glucuronidase Enzyme Inhibition Assay.** Commercial reagents, solvents, β-glucuronidase enzyme (*Escherichia coli*), substrate (*p*-nitrophenyl-β-D-glucuronide), and standard inhibitor (D-saccharic acid 1,4-lactone) were purchased from Merck Germany, Sigma Chemical Co., or Fluka. Water used for buffer preparation and other reagents was deionized by Simplicity water purification system (Millipore). All the enzyme reactions were carried out in triplicates in microliter plates, using a Spectra Max-340 spectrophotometer (Molecular Devices). β-Glucuronidase activity was determined by measuring the absorbance at 405 nm using the spectrophotometric method of Collins[31] with the following modification. The total reaction volume was 250 μL. The reaction mixture containing 190 μL of 0.1 M acetate buffer, pH 7.0, 5 μL of enzyme (30 units), and 50 μL of 0.4 mM *p*-nitrophenyl-β-D-glucuronide was incubated at 37 °C for 30 min. The plate was read on a Spectra MAX-340 at 405 nm. The inhibitory activity was determined as described above, except 5 μL of each isolate dissolved in DMSO was mixed with the enzyme and incubated at 37 °C for 30 min before the addition of the substrates to initiate the reaction. The inhibitory activity (%) was calculated as described above. IC_{50} values (the concentration of isolate that inhibited the hydrolysis of *p*-nitrophenyl-β-D-glucuronide by 50%) were determined by monitoring the effect of increasing concentrations of compounds in the assay. The IC_{50} values were calculated using the EZ-Fit enzyme kinetic program (Perrella Scientific Inc., Amherst, NH).

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of compounds **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

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