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Structure and Antimicrobial Activity of Phloroglucinol Derivatives from *Achyrocline satureioides*

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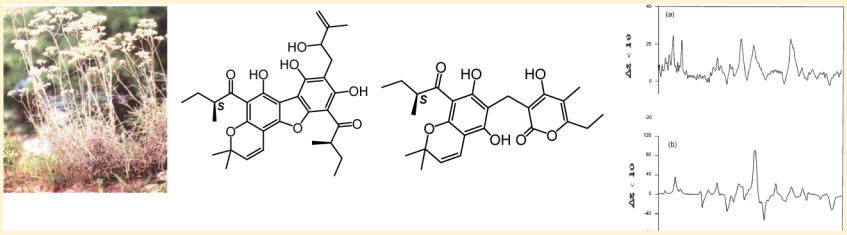
Supporting Information



ABSTRACT: The new prenylated phloroglucinol α-pyrones **1**−**3** and the new dibenzofuran **4**, together with the known 23-methyl-6-*O*-demethylauricepyrone (**5**), achyrofuran (**6**), and 5,7-dihydroxy-3,8-dimethoxyflavone (gnaphaliin A), were isolated from the aerial parts of *Achyrocline satureioides*. Their structures were determined by 1D and 2D NMR spectroscopic studies, while the absolute configuration of the sole stereogenic center of **1** was established by vibrational circular dichroism measurements in comparison to density functional theory calculated data. The same (*S*) absolute configuration of the α-methylbutyryl chain attached to the phloroglucinol nucleus was assumed for compounds **2**−**6** based on biogenetic considerations. Derivatives **7**−**16** were prepared from **1** and **5**, and the antimicrobial activities of the isolated metabolites and some of the semisynthetic derivatives against a selected panel of Gram-positive and Gram-negative bacteria, as well as a set of yeast molds, were determined.

Achyrocline satureioides (Lam.) DC (Asteraceae), a medium-sized aromatic annual herb, popularly known as "marcela" and "marcela blanca", is used in Argentinean folk medicine. This species is not cultivated in Argentina since the commercial plants are harvested from their natural habitat. Aqueous extracts of the aerial parts and/or flowers are frequently used for the treatment of several human ailments, particularly those related to gastrointestinal dysfunctions.[1] Moreover, this species is included in the preparation of some bitter beverages or aperitifs used for easing the digestion process.[2] Over the last decades, *A. satureioides* has been the subject of scientific research using in vitro and in vivo animal models, providing experimental evidence that plant extracts have antioxidant and free radical scavenger activities,[3−5] hepatoprotective effects and choleretic action,[6] vasodilatory[7] and blood glucose lowering properties,[8,9] analgesic and sedative effects,[10] and antiviral[11−13] and antibacterial action.[14] Furthermore, *A. satureioides* showed mutagenic activity in vitro against *Salmonella* and *Escherichia coli*, which could explain its popular use in dysentery, diarrhea, and intestinal infections.[15] Other biological activities reported for *A. satureioides* are insecticidal and trypanocydal,[16] cytotoxicity against human hepatocellular carcinoma cells,[17] and photoprotective action.[18] Animal data support the anti-inflammatory and immunomodulatory activities of plant extracts.[10,19−23] Toxicological studies of marcela aqueous extracts, performed in mice and rats, demonstrated low acute toxicity when administered intraperitoneally and no toxicity upon oral administration.[24] The high amount of flavonoids present in the polar extracts of this plant has been considered to be, at least in part, responsible for many of the reported biological activities.[10,25,26]

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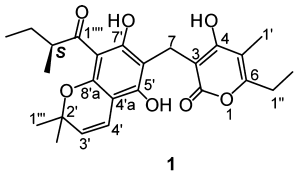
The limited number of phytochemical studies of *A. satureoides* has mainly been carried out using ethanol extracts of the aerial parts. Thus, germacrene D, caryophyllene, caryophyllene-1,10-epoxide, isognaphaliin (5,8-dihydroxy-3,7-dimethoxyflavone), 23-methyl-6-*O*-demethylauricepyrone, italidipyrone, kawapyrone, caffeoylcalleryanin, caffeic acid, protocatechuoylcalleryanin, galangin, and quercetin, including their 3-*O*-methyl derivatives, have been isolated.[27−29] The prenylated dibenzofuran achyrofuran (**6**) was also isolated from an ethanol extract of the aerial parts of *A. satureioides* after a bioassay-guided fractionation using the db/db mouse model for type 2 diabetes.[9]

Owing to the wide range of pharmacological and therapeutic properties of *A. satureioides*, the study of the *n*-hexane and dichloromethane extracts of the aerial parts of this species was carried out in search of potential antimicrobial metabolites. Herein the isolation and structural elucidation of prenylated phloroglucinol α-pyrones **1**−**3** and the new dibenzofuran **4** along with the identification of known aromatic compounds from the aerial parts of *A. satureioides* are reported. Owing to the significant yields of **1** and **5**, several transformations were carried out to obtain derivatives **7**−**16**. The structures of the isolated compounds and derivatives were determined by a combination of spectroscopic techniques, and most compounds were tested for antimicrobial activity against a selected panel of Gram-positive and Gram-negative bacteria, as well as a set of yeast molds. The results permit assignment of some structure−activity relationships.



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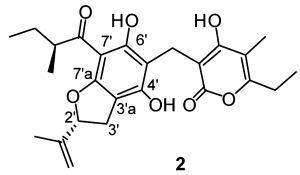

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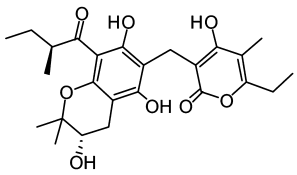

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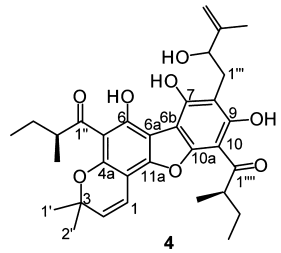

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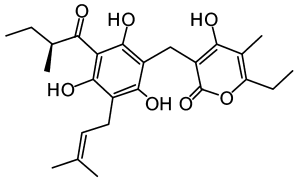

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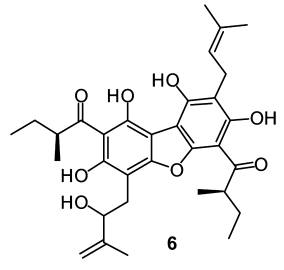

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RESULTS AND DISCUSSION

n-Hexane and dichloromethane extracts of the branches and flowers of *A. satureioides* were independently fractionated by column chromatography (CC) on silica. The obtained fractions were purified by successive column chromatography on Sephadex LH-20 and silica gel 60 G and also by preparative TLC to yield the new prenylated phloroglucinol α-pyrones **1**−**3**, the new dibenzofuran **4**, and the known 23-methyl-6-*O*-demethylauricepyrone (**5**),[27−29] achyrofuran (**6**),[9] and gnaphaliin A.[30] The known compounds were identified by comparison with published spectroscopic and other physical data.

Compound **1** was isolated as an amber viscous oil with the molecular formula $C_{25}H_{30}O_7$. Its IR spectrum showed absorption bands for hydroxy (3216 cm^{-1}) and conjugated carbonyl (1664 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1) displayed three singlets at δ 16.26, 10.61, and 9.94 for three protons interchangeable with D_2O, an AB system [δ 6.68 (1H, d, J = 10.0 Hz, H-4′) and 5.43 (1H, d, J = 10.0 Hz, H-3′] assigned to a double bond, a singlet attributable to an allylic methyl group at δ 1.94, an ethyl group [δ 2.55 (2H, q, J = 7.5 Hz, H-1‴), 1.18 (3H, t; J = 7.5 Hz, H-2‴)], and a *gem*-dimethyl singlet at δ 1.47 (6H). The ^1H NMR spectrum also showed signals characteristic of a methylene group flanked by two aromatic rings [δ 3.66 (1H, brs, H-7a) and 3.59 (1H, brs, H-7b)] and the signals of an α-methylbutyryl moiety [δ 3.77 (1H, sext, J = 6.7 Hz, H-2⁗′), 1.86 (1H, m, H-3a⁗′), 1.42 (1H, m, H-3b⁗′), 0.92 (3H, t, J = 7.2 Hz, H-4⁗), 1.16 (3H, d, J = 6.6 Hz, H-5⁗′)]. The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed a ketocarbonyl carbon at δ 210.9, an ester carbonyl carbon at δ 169.3, and five quaternary aromatic carbons at δ 167.6, 161.9, 161.2, 159.0, and 155.5. An oxygen-bearing carbon signal at δ 78.2 and two CH carbons at δ 124.8 and 117.3, attributable to vinyl carbon atoms characteristic of a 2,2-dimethyl-2*H*-pyran moiety, were also detected. All these data indicated that **1** is a phloroglucinol α-pyrone derivative related to 23-methyl-6-*O*-desmethylauricepyrone (**5**)[31] with a fused 2,2-dimethyl-2*H*-pyran ring. The location of the CH_3, CH_3CH_2−, and $CH_3CH_2CH(CH_3)CO$− substituents and of the fused pyran ring was deduced from the ^1H−^{13}C long-range correlations detected in the HMBC spectrum, as is summarized in Figure 1.

The absolute configuration of **1** was established by vibrational circular dichroism[32] (VCD) in the mid-IR spectral region by comparing the experimental and the density functional theory (DFT) level calculated spectra. It was anticipated that this task would be a rather laborious one, as was the recent absolute configuration determination of a thymol derivative,[33] due to the combination of several factors. Compound **1**, $C_{25}H_{30}O_7$, has 236 electrons and 180 vibrational frequency modes that are active in the IR and VCD spectra. The molecule is highly unsaturated, the sole stereogenic center is located two atoms away from a benzene ring on a conformational flexible chain, and the stereogenic center has two substituents with the minimum size difference (Me and Et groups) in a molecule that possesses additional conformational freedom. Thus, after in silico construction of the molecular model assuming the *S* absolute configuration, a Monte Carlo conformational search at the MMFF94 level of theory, using the Spartan'94 software (Wavefunction, Irvine, CA, USA), afforded 225 conformers in a 10 kcal/mol energy gap. To optimize the geometry of the Monte Carlo MM conformers, the 136 structures located in the initial 2 kcal/mol (the next conformer appeared 5.7 kcal/mol above the lowest energy conformer), accounting for 99.9% of the total conformational population, were submitted to single-point energy calculations using DFT, with the B3LYP functional and the 6-31G(d) basis set in the Spartan'04 suite, to provide 54 conformers in a 2.62 kcal/mol energy gap, again accounting for 99.9% of the conformational distribution. Complete geometry optimization at the DFT B3LYP/DGDZVP level of theory, employing the Gaussian'03 program (Gaussian Inc., Pittsburgh, PA, USA), provided 25 conformers appearing in an energy range of

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Table 1. NMR Data of 1−3 in $CDCl_3$

	1		2		3	
position	δ_H, J (Hz)[a]	δ_C[b]	δ_H, J (Hz)[a]	δ_C[b]	δ_H, J (Hz)[a]	δ_C[b]
2		169.3 C		169.2 C		169.5 C
3		102.0 C		102.4 C		102.1 C
4		167.6 C		166.5 C		167.3 C
OH-4	9.94 s		8.52 brs		9.08 s	
5		108.2 C		108.1 C		108.0 C
6		161.2 C		161.5 C		161.7 C
7	3.66 brs	17.4 CH_2	3.59 brs	18.2 CH_2	3.63 brs	17.8 CH_2
	3.59 brs					
2′		78.2 C	5.40 t (8.6)	89.7 CH		81.2 C
3′	5.43 d (10.0)	124.8 CH	3.00 dd (15.0, 8.0)	31.6 CH_2	3.88 t (5.0)	68.9 CH
			3.32 dd (15.0, 9.6)			
3′a				99.9 C		
4′	6.68 d (10.0)	117.3 CH		162.0 C	2.91 dd (13.7, 2.3)	25.8 CH_2
					2.69 m	
OH-4′			10.37 s			
4′a		104.0 C				102.1 C
5′		159.0 C		107.1 C		163.4 C
OH-5′	10.61 s				10.58 s	
6′		106.0 C		160. 0 C		106.1 C
OH-6′			14.0 s			
7′		161.9 C		103.4 C		158.1 C
OH-7′	16.26 s				14.22 s	
7′a				103.4 C		
8′		104.4 C				104.2 C
8′a		155.5 C				154.2 C
1″	1.94 s	9.4 CH_3	1.95 s	9.6 CH_3	1.96 s	9.6 CH_3
1‴	2.55 q (7.5)	24.3 CH_2	2.56 q (7.5)	24.5 CH_2	2.56 q (7.5)	24.5 CH_2
2‴	1.18 t (7.5)	11.6 CH_3	1.20 t (7.5)	11.7 CH_3	1.21 t (7.6)	11.7 CH_3
1⁗	1.47 s	27.8 CH_3		142.4 C	1.51 s	25.8 CH_3
2⁗	1.47 s	27.8 CH_3	5.14 s	114.2 CH_2	1.45 s	25.8 CH_3
			5.02 s			
3⁗			1.77 s	16.9 CH_3		
1⁗′		210.9 C		211.9 C		212.4 C
2⁗′	3.77 sext (6.7)	45.7 CH	3.93 sext (6.6)	46.0 CH	3.96 sext (6.6)	46.2 CH
3⁗′	1.86 m	26.7 CH_2	1.82 m	27.0 CH_2	1.82 m	27.0 CH_2
	1.42 m		1.40 m		1.40 m	
4⁗′	0.92 t (7.2)	11.9 CH_3	0.91 t (7.3)	12.1 CH_3	0.90 t (7.2)	12.1 CH_3
5⁗′	1.16 d (6.6)	16.7 CH_3	1.16 d (6.8)	16.8 CH_3	1.15 d (6.5)	16.9 CH_3

[a]Spectra recorded at 400 MHz. [b]Spectra recorded at 100 MHz. Data based on DEPT, HSQC, and HMBC experiments.

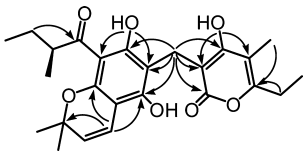

Figure 1. Key HMBC correlations of **1**.

1.19 kcal/mol to account for 91.4% of the total Boltzmann distribution with no imaginary harmonic vibrational frequencies calculated at the same level of theory. All these conformers were submitted to frequency analysis to complete the final IR and VCD spectra calculation. The conformational populations were obtained by means of the $\Delta G = -RT \ln K$ equation to generate the Boltzmann-averaged IR and VCD spectra. Computer comparison of the experimental and calculated VCD and IR spectra using the CompareVOA software[34] provided an anharmonicity factor of 0.975, which was used to prepare the spectra shown in Figure 2, thereby confirming the *S* absolute configuration for the sole stereogenic center of **1** assumed for the calculations.

Thus, the structure of **1** was established as (*S*)-3-[{5,7-dihydroxy-2,2-dimethyl-8-(2-methylbutanoyl)-2*H*-chromen-6-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2*H*-pyran-2-one.

Compound **2** was isolated as a viscous oil with the molecular formula $C_{25}H_{30}O_7$. Its spectroscopic data revealed a structure similar to **1**. Thus, its ^{1}H NMR spectrum (Table 1) exhibited signals characteristic of the same substituted phloroglucinol α-pyrone at δ 14.0 (1H, s, OH-6′), 10.37 (1H, s, OH-4′), 8.52 (brs, OH-4), 3.59 (2H, brs, H-7), 1.95 (3H, s, H-1″), 2.56 (2H, q, J = 7.5 Hz, H-1‴), 1.20 (3H, t, J = 7.5 Hz, H-2‴), 3.93 (1H, sext, J = 6.6 Hz, H-2⁗′), 1.82 (1H, m, H-3a⁗′), 1.40 (1H, m, H-3b⁗′), 0.91 (3H, t, J = 7.3 Hz, H-4⁗), and 1.16 (3H, d, J = 6.8 Hz, H-5⁗). The main difference between **2** and **1** was the absence of the AB system and the *gem*-dimethyl singlet characteristic of the 2,2-dimethyl-2*H*-pyran moiety. Instead, the presence of two double doublets at δ 3.00 (1H, J = 15.0, 8.0 Hz, H-3′a) and 3.32 (1H, J = 15.0, 9.6 Hz, H-3′b), a triplet at

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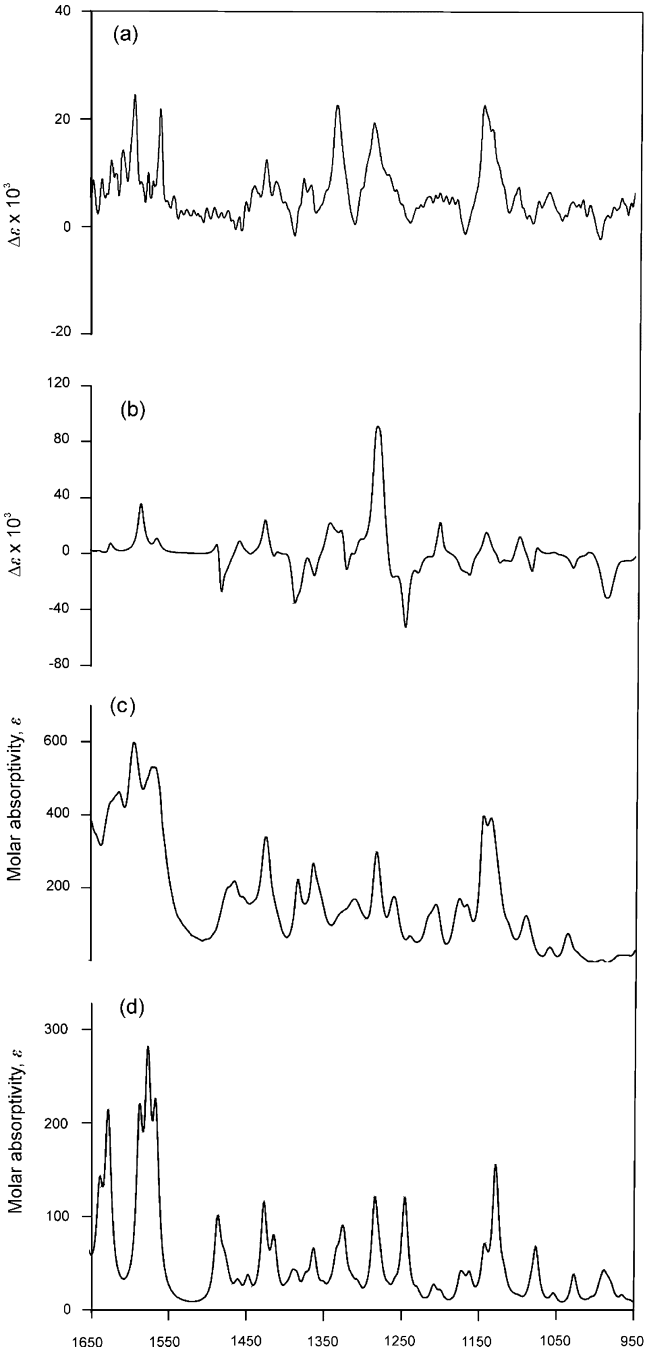

Figure 2. Comparison of the (a) experimental and (b) calculated VCD spectra and of the (c) calculated and (d) IR spectra of (*S*)-**1**.

δ 5.40 (1H, J = 8.6 Hz) corresponding to an oxymethine proton, and two singlets at δ 5.14 and 5.02 attributable to terminal vinyl protons indicated the presence of a 2,3-dihydro-2-(prop-1-en-2-yl)furan moiety. This fragment was verified by the ^{13}C NMR and DEPT signals at δ 31.6 (CH_2), 89.7 (CH), 142.4 (C), 114.2 (CH_2), and 16.9 (CH_3) and also by the observed HMBC correlations. The β-orientation of the hydrogen H-2′ was determined by analysis of the coupling constants.[35] Both **2** and **1** are derived from a combination of the acetate/malonate and isoprenoid biosynthetic pathways. The phloroglucinol and the α-pyrone rings are presumably formed via the corresponding polyketides (see Supporting Information).[36,37] Subsequent acylation with (*S*)-2-methylbutyryl CoA and alkylation with DMAPP would introduce the acyl and prenyl moieties. The phlorogluciol and the α-pyrone units will presumable be linked via the methylene bridge originating from formaldehyde. Thus, based on these biogenetic considerations the same *S* absolute configuration for the α-methylbutyryl moiety is assumed for **2** and also for **3**−**6**. Thus, the structure of **2** was elucidated as shown.

Compound **3** was isolated as a viscous, yellow oil with the molecular formula $C_{25}H_{32}O_8$. The ^{1}H and ^{13}C NMR data showed the signal pattern characteristic of phloroglucinol α-pyrones similar to those of **1**. The main difference was the absence of the C-3′−C-4′ double-bond doublets. The presence of a triplet at δ 3.88 (1H, J = 5.0 Hz, 3′), a doublet of doublets at 2.91 (1H, J = 13.7, 2.3, H-4′a), and a multiplet at 2.69 (1H, H-4′b) suggested the existence of a hydroxy group at C-3′ in the pyran ring fused to the phloroglucinol moiety. The location of the substituents on the pyrone and aromatic rings was also verified by the HMBC correlations. The axial α-orientation of the C-3′ hydroxy group was determined by analysis of the coupling constants of H-3′. Thus, the structure of **3** was established as 6-ethyl-4-hydroxy-5-methyl-3-[{3α,5,7-trihydroxy-2,2-dimethyl-8-(2-(*S*)-methylbutanoyl)chroman-6-yl}methyl]-2*H*-pyran-2-one.

Compound **4** was isolated as a yellow, viscous oil with the molecular formula $C_{32}H_{38}O_8$ and a positive specific rotation, $[\alpha]^{20}_D$ +12 (c 0.1, $CHCl_3$). The ^{1}H NMR spectrum (Table 2) showed signals attributable to two α-methylbutyryl groups at δ 3.85 sext (1H, J = 6.7 Hz, H-2‴′), 3.95 sext (1H, J = 6.5 Hz, H-2″), 1.47 (2H, m, H-3‴′a and H-3″a), 1.93 (2H, m, H-3‴′b and H-3″b), 1.25 (6H, m, H-5‴′ and H-5″), and 0.96 (6H, m, H-4‴′ and H-4″), as well as a 2-hydroxy-3-methyl-3-butenyl moiety at δ 3.14 dd (1H, J = 14.2, 4.0 Hz, H-1‴a), 3.10 dd (H, J = 14.2, 4.0 Hz, H-1‴b), 4.44 (1H, brs, H-2‴), 4.98 (1H, s, H-4‴a), 4.80 (1H, s, H-4‴b), and 1.89 (3H, s, H-5‴). The spectrum also showed three deshielded singlets attributable to hydroxy groups at δ 15.90, 14.44, and 10.06. The presence of an AB system at δ 6.70 (CH, J = 9.8 Hz, H-1) and 5.65 (CH, J = 9.8 Hz, H-2), together with two methyl singlets at δ 1.57 and 1.58, suggested the existence of a fused 2,2-dimethyl-2*H*-pyran ring as in **1**. The ^{13}C NMR, DEPT, and HREIMS data were consistent with two trisubstituted phloroglucinol units linked through a furan ring, establishing a dibenzofuran core as in achyrofuran (**6**) also isolated during this study. The ^{13}C NMR data of **4** (Table 2) displayed signals for 12 aromatic carbon atoms, from which five were assigned to the oxygenated carbons C-4a, C-6, C-7, C-9, C-10a, and C-11a at δ 154.4, 156.0, 159.6, 163.5, 152.5, and 156.3. The spectrum also showed resonances for two carbonyl carbon atoms at δ 212.3 and 212.7; two sp^2 carbons and a methyl group characteristic of an isopropenyl group at δ 147.3 (C), 110.8 (CH_2), and 18.3 (CH_3); and two vinyl carbons at δ 115.6 (C-1) and 127.2 (C-2). The HMBC correlations of OH-7 with C-8, C-10a, and C-6b; OH-9 with C-8, C-9, and C-10; OH-6 with C-5, C-11a, and C-6a; H-1‴ with C-2‴, C-3‴, and C-5‴; H-4‴ a with C-2‴, C-3‴, and C-5‴; H-3‴′a with C-2‴′, C-4‴′, and C-5‴′; H-4‴′ with C-3‴′; H-5‴′ with C-2‴′ and C-3‴′; H-1 with C-11b, C-4, C-2, and C-3; H-2 with C-11b, C-3, and C-4; H-1′ with C-2 and C-3; H-2″ with C-1″, C-3″, and C-4‴; H-4″ with C-2″; and finally H-5″ with C-2″ and C-3″ verified structure **4**.

Owing to the availabity of large amounts of **1** and **5**, several reactions at the hydroxy groups and the double bond of the dihydropyran ring of **1** (Scheme 1) and at the prenyl double bond of **5** (Scheme 2) were performed. Thus, when **1** was treated with diazomethane, compounds **7**−**9** were obtained. Compounds **7** and **9** have a 4*H*-pyran-4-one ring instead of the lactone ring.

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Table 2. NMR Data of 4 in $CDCl_3$

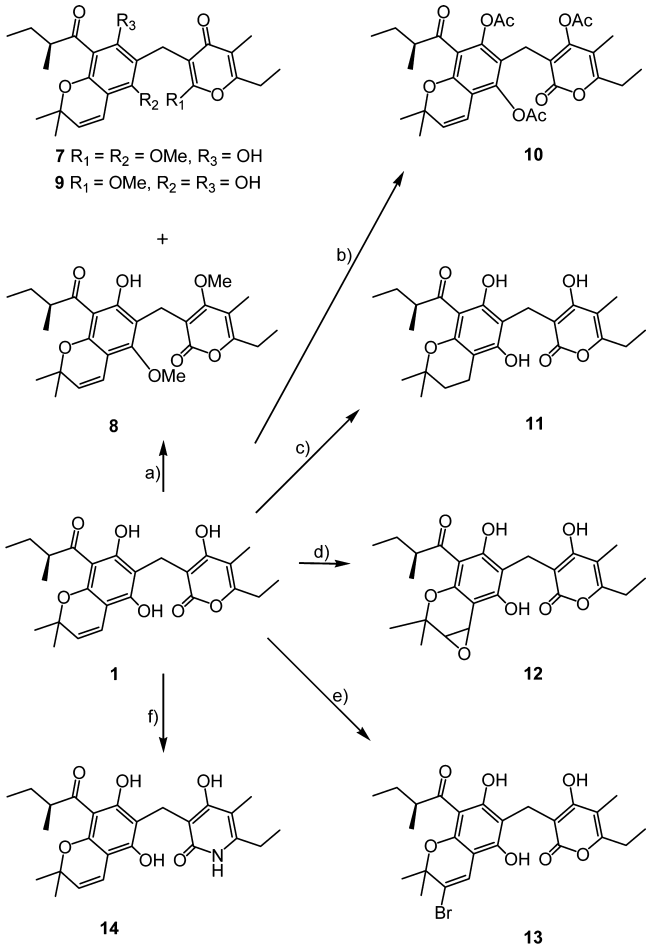
position	δ_H,[a] J (Hz)	δ_C[b]
1	6.70 d (9.8)	115.6 CH
2	5.65 d (9.8)	127.2 CH
3		79.4 C
4a		154.4 C
5		106.7 C
6		156.0 C
OH-6	15.90 s	
6a		106.9 C
6b		103.5 C
7		159.6 C
OH-7	10.06 s	
8		106.7 C
9		163.5 C
OH-9	14.44 s	
10		102.5 C
10a		152.5 C
11a		156.3 C
11b		100.6 C
1′	1.58 s	28.0 CH_3
2′	1.57 s	28.0 CH_3
1″		212.3 C
2″	3.95 sext (6.5)	46.5 CH
3″	1.47 m	27.1 CH_2
	1.93 m	
4″	0.96 m	12.0 CH_3
5″	1.25 m	16.8 CH_3
1‴	3.14 dd (14.2, 4.0)	30.1 CH_2
	3.10 dd (14.2, 7.9)	
2‴	4.44 brs	75.6 CH
3‴		147.3 C
4‴	4.98 s	110.8 CH_2
	4.80 s	
5‴	1.89 s	18.3 CH_3
1⁗		212.7 C
2⁗	3.85 sext (6.7)	46.2 CH
3⁗	1.47 m	26.9 CH_2
	1.93 m	
4⁗	0.96 m	12.0 CH_3
5⁗	1.25 m	16.8 CH_3

[a]Spectrum recorded at 400 MHz. [b]Spectrum recorded at 100 MHz. Data based on DEPT, HSQC, and HMBC experiments.

Acetylation of **1** with Ac_2O/pyridine yielded triacetate **10** (64%). Compound **11** was quantitatively obtained when **1** was hydrogenated in the presence of 10% Pd/C. Epoxidation of **1** with MCPBA afforded **12** (33%), while **13** was obtained in 55% yield after treatment with Br_2 in CH_2Cl_2. Transformation of the lactone moiety of **1** into lactam **14** was done using ammonium acetate in acetic acid. Compounds **15** (43%) and **3** (12%) were obtained by intramolecular cyclization of **5** with MCPBA, and **16** (82%) was obtained after hydrogenation of **5** (Scheme 2). Details for the transformations are given in the Experimental Section.

Compounds **1**–**16**, having a 2*S*-methylbutyryl moiety, were tested for inhibitory activity against a selected panel of Gram-positive and Gram-negative bacteria, as well as a set of yeast molds, the results being summarized in Table 3. As can be seen, achyrofuran (**6**) has strong in vitro antibacterial activity against Gram-positive bacteria, including the reference methicillin-resistant and vancomycin-intermediate *Staphyloccocus aureus* strain NRS402 (MIC 0.1 μM (0.07 mg/L)).[38] The dibenzofuran

Scheme 1. Preparation of 7–14 from 1[a]



[a]Reagents and conditions: (a) CH_2N_2/Et_2O, MeOH, rt, 8 h; (b) Ac_2O, DMAP, py, rt, 15 min; (c) 10% Pd/C, THF, H_2, 21 h; (d) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 72 h; (e) Br_2, CH_2Cl_2, rt, 12 h; (f) NH_4OAc, HOAc, reflux, 72 h.

4 showed inhibitory activity against *Staph. aureus* (both MSSA and VISA) and *Enteroccocus faecalis* strains at concentrations equal to and higher than 32 and 64 μM, respectively. Structural comparison of **4** and achyrofuran (**6**) shows that the presence of the dihydropyran ring fused to the phloroglucinol nucleus and the presence of a 2-hydroxy-3-methyl-3-butenyl fragment instead of the 3-methyl-2-butenyl fragment drastically reduces the activity. In turn, the *Staph. aureus* VISA strain was highly resistant to both commercial antibiotics as expected (MIC = 120 μM for ampicillin and MIC = 240 μM for kanamycin). Nevertheless, this isolate was still sensitive to **4** (MIC = 32 μM).

Compound **5** caused severe growth inhibition of *Staph. aureus* (MSSA and VISA) with MIC values of 8 μM for both strains. This result shows that **5** is clearly superior to ampicillin against multiresistant *Staph. aureus* (ampicillin MIC = 120 μM for VISA). The reduced prenyl double bond of **16** caused the same biological activity level, while intramolecular cyclization led to inactive **15** and **3**. The antimicrobial activity of **2** was moderate [MIC = 64 μM against *Staph. aureus* (VISA) and *E. faecalis* and MIC = 32 μM for *Staph. aureus* (MSSA)], while comparison of the activity of **2** vs **16** reveals that the presence of the isopropenyl group is important. Compound **1** was only marginally active [MIC = 64 μM against *Staph. aureus* (MSSA)], and considering the derivatives obtained from **1**, only the 22-bromo analogue **13**

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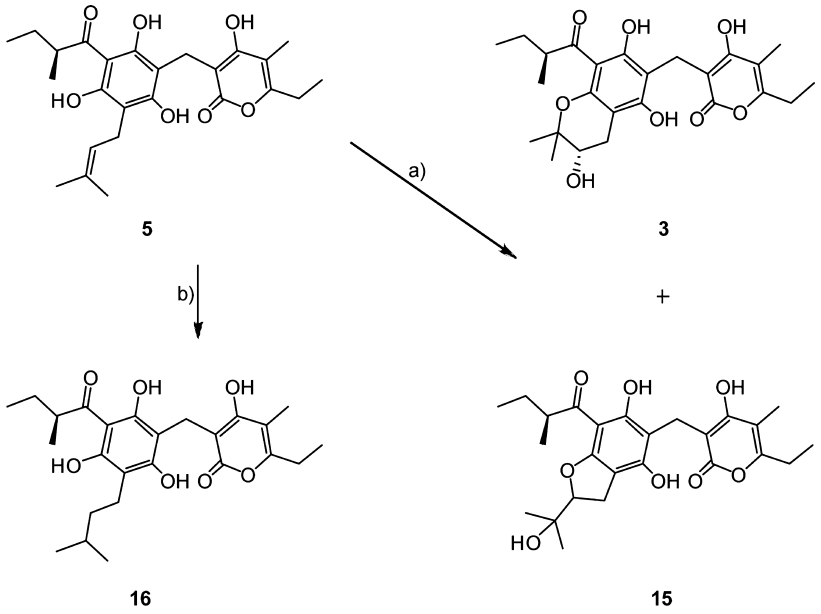
Scheme 2. Preparation of 3, 15, and 16 from 5[a]



[a]Reagents and conditions: (a) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 72 h. (b) 10% Pd/C, THF, H_2, 21 h.

Table 3. MIC of Metabolites 1−6 and Semisynthetic Derivatives 7−16 (μM)

	microbial strain[a]				
compound	*E. coli* (ATCC35218)	*Staph. aureus* (VISA)	*Staph. aureus* (MSSA)	*E. faecalis* (ATCC29212)	yeast sp.[b]
1	>128	64	64	>128	>128
2	>128	64	32	64	>128
3	>128	>128	>128	>128	>128
4	>128	32	32	64	>128
5	>128	8	8	64	>128
6	>128	<1	<1	2	>128
gnaphaliin A	>128	>128	>128	>128	>128
7	>128	>128	>128	>128	ND[c]
8	>128	>128	>128	>128	ND
9	>128	>128	>128	>128	ND
10	>128	>128	>128	>128	ND
11	>128	>128	>128	>128	ND
12	>128	>128	>128	>128	ND
13	>128	8	16	>128	ND
14	>128	32	32	>128	ND
15	>128	>128	>128	>128	ND
16	>128	4	4	32	ND
ampicillin	>128	120	<1	8.8	ND
kanamycin	>128	240	2.8	360	ND

[a]*Staphylococcus aureus* strains were ATCC25923 (as a MSSA representative) and NRS402 (as VISA representative). [b]Yeast species tested separately were *Saccharomyces cerevisiae* (BY4741), *Candida albicans* (ATCC10231), *Candida glabrata* (ATCC9984). [c]ND: not determined.

and lactam **14** showed moderate activity. Neither O-methylation nor O-acetylation increased the activity, and the isolated metabolites showed no activity against Gram-negative *E. coli* or yeast strains even at the highest tested concentrations.

In short, four new phloroglucinol derivatives (**1**−**4**) have been isolated from the aerial parts of *A. satureoides* and their structures determined by 1D and 2D NMR spectroscopic studies. The *S* absolute configuration of **1** was determined by vibrational circular dichroism in combination with DFT calculations. The established *S* absolute configuration of the α-methylbutyryl chain attached to the phloroglucinol nucleus was assumed for compounds **2**−**6** based on biogenetic considerations The antimicrobial activity of the natural products **1**−**6** as well as derivatives **7**−**16**, prepared from **1** and **5**, revealed the importance of the 3-methyl-2-butenyl chain attached to the phloroglucinol nucleus in the phloroglucinol α-pyrone series. Comparison of the activity of dibenzofurans **4** and **6** shows the presence of the dihydropyran ring fused to the phloroglucinol nucleus and the presence of a 2-hydroxy-3-methyl-3-butenyl group, instead of a 3-methyl-2-butenyl group, drastically reduced the activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 241 polarimeter. IR spectra were obtained

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using a Bruker IFS28/55 spectrophotometer. UV spectra were recorded in absolute MeOH on a JASCO V-560 spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer in $CDCl_3$, with TMS as the internal reference. 2D NMR experiments were conducted on a Bruker WP-500 SY NMR spectrometer at 500 MHz. Mass spectra were measured on a VG Autospec spectrometer. Column chromatography was carried out using silica gel (0.063–0.200 mm) and Sephadex LH-20. Silica gel 60 (Merck) was used on a Harrison Research 7924T Chromatotron. Macherey-Nagel polygram Sil G/UV254 and Analtech silica gel GF preparative layer with UV254 were used for TLC. All compounds were named using Autonom Program, which is based on IUPAC rules.

Plant Material. *Achyrocline satureioides* (Lam) D.C. (Asteraceae) was collected from Alpa Corral (Córdoba, Argentina) during August 2008. The plant material was identified by Dr. Margarita Grosso at the Systematical Botany Area of the Department of Natural Sciences, Universidad Nacional de Río Cuarto, Argentina. A voucher specimen is deposited in the herbarium of Natural Sciences (RCV 1921).

Extraction and Isolation. Branches (4.7 kg) and flowers (200 g) of *A. satureioides* were exhaustively extracted with *n*-hexane at room temperature. The extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness to provide residues of 130 and 7.5 g, respectively. The branches and flowers were subsequently extracted with CH_2Cl_2, affording 93 and 3.8 g of extracts, respectively. The *n*-hexane extract from the branches was fractionated initially by CC on silica gel. Elution with *n*-hexane/EtOAc mixtures of increasing polarity (100:0–0:100) and EtOAc/MeOH (100:0–19:1) afforded five fractions (I–V). Fractions III (35 g) and IV (70 g) were rechromatographed on Sephadex LH-20 CC eluting with *n*-hexane/CH_2Cl_2/MeOH (2:1:1). Some of the eluted products were purified by preparative TLC to yield **1** (275 mg), **2** (71 mg), **5** (7.8 g), and **6** (39 mg).

The DCM extract from the branches was subjected to silica gel CC with *n*-hexane/EtOAc (100:0–0:100) to provide 28 fractions. Fractions VIII (2.5 g), IX (1.13 g), and XII (6 g) were rechromatographed on Sephadex LH-20 with *n*-hexane/CH_2Cl_2/MeOH to give **1** (550 mg), **4** (71 mg), and achyrofuran (**6**) (160 mg). Gnaphaliin A (150 mg) was obtained as an amorphous solid from fractions XVI–XVIII (*n*-hexane–EtOAc, 1:1–1:4). The *n*-hexane extract from the flowers (7.5 g) was chromatographed on silica gel, yielding nine fractions (I–IX). Fractions V (970 mg), VI (630 mg), and VII (1.0 g) were subjected to Sephadex LH-20 CC (*n*-hexane/CH_2Cl_2/MeOH) and silica gel CC using *n*-hexane/EtOAc mixtures of increasing polarity. The subfractions were purified by radial chromatography to afford **1** (195 mg), **2** (84 mg), and 20 mg of **3**. The DCM extract from the flowers (3.8 g) was chromatographed on silica. Elution with *n*-hexane/EtOAc (19:1–0:1) afforded 10 fractions (I–X). Fraction III was rechromatographed on Sephadex LH-20 using CH_2Cl_2/MeOH (7:3) to give 98 mg of **1**.

*3-[{5,7-Dihydroxy-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-2H-chromen-6-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2H-pyran-2-one (**1**):* amber oil, $[\alpha]^{20}_D$ +9 (c 0.3, $CHCl_3$); UV (EtOH) λ_{max} 343 (2.05), 244 (2.47) nm; IR (film) ν_{max} 3216, 2972, 2935, 2876, 1776, 1726, 1664, 1593, 1463, 1424, 1360, 1137, 1035, 912, 783 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m*/*z* 442 $[M]^+$ (41), 276 (31), 261 (100), 231 (17), 219 (78), 155 (25); HREIMS 442.2006 (calcd for $C_{25}H_{30}O_7$, 442.1992).

*3-[{4,6-Dihydroxy-7-(2-(S)-methylbutanoyl)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2H-pyran-2-one (**2**):* amber oil, $[\alpha]^{20}_D$ +14 (c 0.1, $CHCl_3$); UV (EtOH) λ_{max} 346 (2.3), 243 (2.29) nm; IR (film) ν_{max} 3343, 2969, 2635, 2878, 1774, 1725, 1666, 1570, 1432, 1379, 1228, 1138, 898, 799 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m*/*z* 442 $[M]^+$ (12), 385 (41), 273 (25), 219 (99), 155 (36); HREIMS 442.1994 (calcd for $C_{25}H_{30}O_7$, 442.1992).

*6-Ethyl-4-hydroxy-5-methyl-3-[{3,5,7-trihydroxy-2,2-dimethyl-8-(2-(S)-methylbutanoylchroman-6-yl}methyl]-2H-pyran-2-one (**3**):* amber oil; $[\alpha]^{20}_D$ +6 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} 341 (2.52), 244 (2.51) nm; IR (film) ν_{max} 3441, 2968, 2934, 2877, 1721, 1662, 1624, 1570, 1461, 1425, 1374, 1175, 1126 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m*/*z* 460 $[M]^+$ (17), 385 (41), 373 (27), 293 (26), 237 (99), 219 (25), 155 (27); HREIMS 460.2077 (calcd for $C_{25}H_{32}O_8$, 460.2097).

*1′,1″-[6,7,9-Trihydroxy-8-(2-hydroxy-3-methylbut-3-en-1-yl)-3,3-dimethyl-3H-benzofuro[2,3-f]chromene-5,10-diyl]bis(2-(S)-methylbutan-1-one) (**4**):* amber oil; $[\alpha]^{20}_D$ +12 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} 341 (2.53), 239 (2.55), 203 (2.65) nm; IR (film) ν_{max} 2964, 2932, 2879, 1772, 1724, 1620, 1459, 1409, 1373, 1176, 1105, 1038, 950 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 2; EIMS *m*/*z* 550 $[M]^+$ (15), 479 (100), 465 (28), 442 (8), 303 (17), 219 (7); HREIMS 550.2607 (calcd for $C_{32}H_{38}O_8$, 550.2567).

*Reaction of **1** with Diazomethane.* A solution of **1** (55 mg, 0.13 mmol) in methanol (4 mL) was treated with a 2 M trimethylsilyldiazomethane solution in diethyl ether (2 mL). The reaction mixture was stirred at room temperature for 8 h, the solvent was evaporated under reduced pressure, and the residue was purified by preparative TLC (EtOAc/*n*-hexane, 3:17) to yield **7** (18.4 mg, 31%), **8** (6.6 mg, 11%), and **9** (4.5 mg, 8%).

*2-Ethyl-5-[{7-hydroxy-5-methoxy-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-2H-chromen-6-yl}methyl]-6-methoxy-3-methyl-4H-pyran-4-one (**7**):* amber oil; $[\alpha]^{20}_D$ +2 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 342 (2.37), 243 (2.36) nm; IR (film) ν_{max} 2971, 2935, 2875, 2624, 1775, 1724, 1670, 1662, 1589, 1465, 1429, 1380, 1344, 1266, 1156, 1129, 970 cm^{-1}; ^{1}H NMR δ 12.96 (1H, s, OH-7′), 6.5 (1H, d, *J* = 10,0 Hz, H-4′), 5.5 (1H, d, *J* =10.0 Hz, H-3′), 3.92 (3H, s, OMe), 3.80 (3H, s, OMe), 3.65 (2H, brs, H-7), 3.53 (1H, sext, *J* = 6,8 Hz, H-2‴′), 2.59 (2H, q, *J* = 7.5 Hz, H-1‴), 1.90 (3H, s, H-1″), 1.84 (1H, m, H-3‴′a), 1.43 (6H, s, H-24, H-2‴), 1.40 (1H, m, H-3‴′b), 1.21 (3H, t, *J* = 7.5 Hz, H-2‴), 1.15 (3H, d, *J* = 6.6 Hz, H-5‴′), 0.91 (3H, t, *J* = 7.0 Hz, H-4‴′); ^{13}C NMR δ 210.6 (C, C-1‴′), 181.2 (C, C-4), 162.8 (C, C-2), 161.7 (C, C-7′), 159.6 (C, C-6), 159.5 (C, C-5′), 153.3 (C, C-8′a), 125.9 (CH, C-3′), 117.9 (CH, C-4′), 117.8 (C, C-5), 113.4 (C, C-6′), 110.7 (C, C-8′), 106.6 (C, C-4′a), 103.4 (C, C-3), 77.4 (C, C-2′), 62.0 (CH_3, OMe), 55.7 (CH_3, OMe), 47.1 (CH, C-2‴′), 27.8 (CH_3, C-1‴′, C-2‴′), 26.4 (CH_2, C-3‴′), 24.3 (CH_2, C-1‴), 16.7 (CH_2, C-7), 16.4 (CH_3, C-5‴′), 11.9 (CH_3, C-4‴′), 11.3 (CH_3, C-2‴), 9.9 (CH_3, C-1″); EIMS *m*/*z* 470 $[M]^+$ (65), 455 (49), 438 (31), 289 (37), 181 (100), 113 (51); HREIMS 470.2305 (calcd for $C_{27}H_{34}O_7$, 470.2285).

*6-Ethyl-3-[{7-hydroxy-5-methoxy-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-2H-chromen-6-yl}methyl]-4-methoxy-5-methyl-2H-pyran-2-one (**8**):* amber oil; $[\alpha]^{20}_D$ +4.0 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} 243 (2.42) nm; IR (film) ν_{max} 2971, 2936, 2876, 1714, 1642, 1592, 1566, 1460, 1413, 1367, 1285, 1237, 1135 cm^{-1}; ^{1}H NMR δ 13.79 (1H, s, OH-7′), 6.47 (1H, d, *J* = 10.0 Hz, H-4′), 5.46 (1H, d, *J* = 10.0 Hz, H-3′), 3.79 (5H, s, OMe, H-7), 3.73 (1H, m, H-2‴′), 3.62 (3H, s, OMe), 2.50 (2H, q, *J* = 7.5 Hz, H-1), 1.90 (3H, s, H-1″); 1.84 (1H, m, H-3a‴′), 1.48 (6H, s, H-24, H-2‴), 1.41 (1H, m, H-3b‴′), 1.21 (3H, t, *J* = 7.5 Hz, H-2‴), 1.15 (3H, d, *J* = 6.6 Hz, H-5‴′), 0.90 (3H, t, *J* = 7.0 Hz, H-4‴′); ^{13}C NMR δ 211.4 (C, C-1‴′), 168.3 (C, C-2), 165.7 (C, C-4), 163.9 (C, C-7′), 160.9 (C, C-5′), 160.7 (C, C-6), 154.7 (C, C-8′a), 125.6 (CH, C-3′), 117.9 (CH, C-4′), 114.1 (C, C-10), 113.3 (C, C-6′), 108.6 (C, C-3), 107.9 (C, C-8′), 106.4 (C, C-4′a), 77.7 (C, C-2′), 62.1 (CH_3, OMe), 60.3 (CH_3, OMe), 46.5 (CH, C-2‴′), 27.7 (CH_3, C-1‴, C-2‴), 26.8 (CH_2, C-3‴′), 24.6 (CH_2, C-1‴), 18.7 (CH_2, C-7), 16.9 (CH_3, C-5‴′), 12.0 (CH_3, C-4‴′), 11.7 (CH_3, C-2‴), 10.1 (CH_3, C-1″); EIMS *m*/*z* 470 $[M]^+$ (86), 479 (100), 455 (64), 289 (100), 245 (89), 181 (93); HREIMS 470.2282 (calcd for $C_{27}H_{34}O_7$, 470.2305).

*3-[{5,7-Dihydroxy-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-2H-chromen-6-yl}methyl]-6-ethyl-2-methoxy-5-methyl-4H-pyran-4-one (**9**):* amber oil; $[\alpha]^{20}_D$ +9 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} 357 (2.43), 317 (2.41), 245 (2.42) nm; IR (film) ν_{max} 2969, 2931, 2875, 1776, 1729, 1660, 1599, 1543, 1466, 1431, 1379, 1283, 1188, 1161, 1130 cm^{-1}; ^{1}H NMR, δ 14.38 (1H, s, OH-7′), 12.13 (1H, s, OH-5′), 6.75 (1H, d, *J* = 10.0 Hz, H-4′), 5.37 (1H, d, *J* = 10.0 Hz, H-3′), 4.06 (3H, s, OMe), 3.76 (1H, sext, *J* = 6.8 Hz, H-2‴′), 3.64 (2H, brs, H-7), 2.64 (2H, q, *J* = 7.5 Hz, H-1‴), 1.95 (3H, s, H-1″), 1.84 (1H, m, H-3a‴′), 1.45 (6H, s, H-1‴, H-2‴), 1.39 (1H, m, H-3b‴′), 1.23 (3H, t, *J* = 7.6 Hz, H-2‴), 1.15 (3H, d, *J* = 6.7 Hz, H-5‴′), 0.90 (3H, t, *J* = 7.4 Hz, H-4‴′); ^{13}C NMR, δ 210.4 (C, C-1‴′), 183.1 (C, C-4), 164.9 (C, C-2), 164.0 (C, C-7′), 160.9 (C, C-6), 159.9 (C, C-5′), 155.2 (C, C-8′a), 123.8 (CH, C-3′), 118.3 (C, C-5), 117.7 (CH, C-4′), 106.5 (C, C-6′), 104.9 (C, C-8′), 103.7 (C, C-4′a), 103.4 (C, C-3), 77.7 (C, C-2′), 56.2

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(CH_3, OMe), 45.9 (CH, C-2‴′), 27.9 (CH_3, C-1‴, C-2‴), 27.0 (CH_2, C-3‴′), 24.4 (CH_2, C-1‴), 17.1 (CH_2, C-7), 16.4 (CH_3, C-5‴′), 12.1 (CH_3, C-4‴′), 11.3 (CH_3, C-2″), 9.8 (CH_3, C-1″); EIMS *m*/*z* 456 $[M]^+$ (41), 276 (31), 261 (100), 231 (17), 219 (78), 155 (25); HREIMS 456.2148 (calcd for $C_{26}H_{32}O_7$, 456.2133).

*6-[{4-Acetoxy-6-ethyl-5-methyl-2-oxo-2H-pyran-3-yl}methyl]-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-2H-chromene-5,7-diyl diacetate (**10**).* To a solution of **1** (46.5 mg, 0.11 mmol) in the minimum amount of pyridine were added a few crystals of DMAP and Ac_2O (29.8 μL, 0.32 mmol). The reaction mixture was stirred at room temperature for 15 min, the volatiles were removed under reduced pressure, and the residue was purified by preparative TLC (EtOAc/toluene, 3:17) to yield 38.2 mg (64%) of the title compound as an amber oil: $[\alpha]^{20}_D$ +3.3 (*c* 0.6, $CHCl_3$); UV (EtOH) λ_{max} 341 (1.55), 241 (1.57), 204 (1.66) nm; IR (film) ν_{max} 2975, 2938, 2877, 1775, 1713, 1647, 1578, 1460, 1430, 1369, 1287, 1190, 1124, 1105, 1067, 1046, 887 cm^{-1}; ^1H NMR δ 6.13 (1H, d, *J* = 10.0 Hz, H-4′), 5.63 (1H, d, *J* = 10.0 Hz, H-3′), 3.45 (2H, brs, H-7), 3.06 (1H, sext, *J* = 6.9 Hz, H-2‴′), 2.54 (2H, q, *J* = 7.5 Hz, H-1‴), 2.29 (6H, s, $OCOCH_3$), 2.18 (3H, s, $OCOCH_3$), 1.80 (1H, m, H-3a‴′), 1.75 (3H, s, H-1″), 1.44 (6H, s, H-1‴, H-2‴), 1.37 (1H, m, H-3b‴′), 1.21 (3H, t, *J* = 7.5 Hz, H-2‴), 1.12 (3H, d, *J* = 6.6 Hz, H-5‴′), 0.93 (3H, t, *J* = 7.0 Hz, H-4‴′); ^{13}C NMR δ 206.3 (C, C-1‴′), 168.7 (C, C-2), 168.3 (C, $OCOCH_3$), 167.2 (C, $OCOCH_3$), 164.1 (C, C-6), 161.4 (C, C-7′), 160.6 (C, C-4), 150.4 (C, C-8′a), 146.6 (C, C-5′), 131.2 (CH, C-3′), 121,4 (CH, C-4′a), 118.3 (C, C-4′a), 116.2 (C, C-5), 114.6 (C, C-6′), 113.4 (C, C-8′), 107.8 (C, C-3), 77.7 (C, C-2′), 48.3 (CH, C-2‴′), 28.1 (CH_3, C-1‴′, C-2‴′), 25.6 (CH_2, C-3‴′), 24.7 (CH_2, C-1‴), 20.7 (CH_3, $OCOCH_3$), 20.2 (CH_2, C-7); 18.6 (CH_3, C-5‴′), 11.9 (CH_3, C-4‴′, C-2‴), 10.1 (CH_3, C-1″); EIMS *m*/*z* 468 $[M]^+$ (17), 425 (100), 469 (48), 426 (56), 275 (59), 219 (45), 155 (52); HREIMS 468.2308 (calcd for $C_{31}H_{36}O_{10}$, 468.2295).

*3-[{5,7-Dihydroxy-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-chroman-6-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2H-pyran-2-one (**11**).* A solution of **1** (21 mg, 0.048 mmol) in THF (4 mL) was hydrogenated in the presence of a catalytic amount of 10% Pd/C under stirring for 21 h. The solution was filtered through Celite, and the solvent was removed under reduced pressure. The residue was purified by TLC (EtOAc/*n*-hexane, 3:17) to yield the title compound quantitatively as a reddish oil: $[\alpha]^{20}_D$ +12 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} 341 (2.10); 247 (2.50) nm; IR (film) ν_{max} 3171, 2972, 2936, 2876, 1777, 1727, 1665, 1621, 1597, 1570, 1461, 1424, 1370, 1277, 1256, 1159, 1140, 1117, 1037, 784, 741 cm^{-1}; ^1H NMR δ 16.40 (1H, s, OH-7′), 10.47 (1H, s, OH-5′), 10.14 (1H, s, OH-4), 3.75 (1H, sext, *J* = 6.8 Hz, H-2‴′), 3.65 (1H, brs, H-7a), 3.60 (1H, brs, H-7b), 2.65 (2H, m, H-4′), 2.55 (2H, q, *J* = 7.5 Hz, H-1‴), 1.94 (3H, s, H-1″), 1.84 (1H, m, H-3a‴′), 1.41 (1H, m, H-3b‴′), 1.37 (6H, s, H-1‴, H-2‴), 1.18 (8H, m, H-2‴, H-5‴′, H-3′), 0.91 (3H, t, *J* = 7.2 Hz, H-4‴′); ^{13}C NMR δ 210.9 (C, C-1‴′), 169.5 (C, C-2), 167.8 (C, C-4), 161.5 (C, C-7′), 161.3 (C, C-6), 161.2 (C, C-5′), 156.0 (C, C-8′a), 108.3 (C, C-5), 105.2 (C, C-6′), 104.6 (C, C-8′), 102.4 (C, C-4′a), 102.3 (C, C-3), 76.3 (C, C-2′), 45.8 (CH, C-2‴′), 31.8 (CH_2, C-3′), 26.8 (2CH_3, C-1‴′, C-2‴′), 26.8 (CH_2, C-3‴′), 24.4 (CH_2, C-1‴), 17.6 (CH_2, C-7), 17.1 (CH_2, C-4′), 16.8 (CH_3, C-5‴′), 12.0 (CH_3, C-4‴′), 11.8 (CH_3, C-2‴), 9.6 (CH_3, C-1″); EIMS *m*/*z* 444 $[M]^+$ (46), 387 (100), 233 (36), 221 (63), 155 (35); HREIMS 444.2148 (calcd for $C_{25}H_{32}O_7$, 444.2169).

*3-[{5,7-Dihydroxy-2,2-dimethyl-4-(2-(S)-methylbutanoyl)-2,7b-dihydro-1aH-oxireno[2,3-c]chromen-6-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2H-pyran-2-one (**12**).* To a solution of **1** (51 mg, 0.12 mmol) in CH_2Cl_2 (4 mL) were added 73 mg of MCPBA (3.6 equiv) in CH_2Cl_2 and 43.5 mg of $NaHCO_3$ (4.4 equiv). The reaction mixture was stirred for 72 h at room temperature, the solvent was evaporated, and the residue was purified by preparative TLC (Et_2O/*n*-hexane, 3:1) to afford **12** (17.4 mg, 33%) as an amber oil: $[\alpha]^{20}_D$ −10 (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} 359 (2.85), 241 (2.88), 206 (2,97) nm; IR (film) ν_{max} 3457, 3232, 2972, 2935, 2877, 2616, 1807, 1731, 1731, 1661, 1597, 1567, 1432, 1432, 1368, 1320, 1261, 1203, 1139, 737 cm^{-1}; ^1H NMR δ 11.30 (1H, s, OH-5′), 9.92 (1H, s, OH-4), 4.99 (1H, d, *J* = 4.7 Hz, H-4′), 3.78 (1H, m, H-3′), 3.73 (1H, m, H-2‴′), 3.65 (2H, brs, H-7), 2.57 (2H, q, *J* = 7.5 Hz, H-1‴), 1.96 (3H, s, H-1″), 1.82 (1H, m, H-3a‴′), 1.50 (3H, s, H-1‴or H-2‴), 1.36 (3H, s, H-1‴ or H-2‴), 1.20 (3H, m, H-2‴), 1.16 (3H, m, H-5‴′), 0.91 (3H, m, H-4‴′); ^{13}C NMR δ 211.5 (C, C-1‴′), 169.8 (C, C-2), 168.0 (C, C-4), 162.9 (C, C-7′), 162.1 (C, C-6), 161.6 (C, C-5′), 155.0 (C, C-8′a), 108.6 (C, C-5), 106.4 (C, C-6′), 104.5 (C, C-8′), 102.1 (C, C-4′a, C-3), 79.4 (C, C-2′), 70.2 (CH, C-3′), 62.5 (CH, C-4′), 46.0 (CH, C-2‴′), 26.8 (CH_2, C-3‴′), 24.4 (CH_2, C-1‴), 22.7 (CH_3, C-1‴′ or C-2‴′), 19.3 (CH_3, C-1‴′ or C-2‴′), 17.4 (CH_2, C-7), 16.8 (CH_3, C-5‴′), 11.9 (CH_3, C-4‴′), 11.8 (CH_3, C-2‴), 9.6 (CH_3, C-1″); EIMS *m*/*z* 458 $[M]^+$ (36), 401 (100), 247 (8), 235 (45), 167 (9), 155 (37); HREIMS 458.1941 (calcd for $C_{25}H_{30}O_8$, 458.1958).

*3-[{5,7-Dihydroxy-2,2-dimethyl-4-(2-(S)-methylbutanoyl)-2,7b-dihydro-1aH-oxireno[2,3-c]chromen-6-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2H-pyran-2-one (**13**).* To 35 mg of **1** (0.079 mmol) in 3 mL of CH_2Cl_2 was added 9 μL of Br_2 (2.2 equiv), and the reaction mixture stirred for 12 h at room temperature. The solvent was evaporated and the residue purified by TLC (EtOAc/*n*-hexane, 1:4) to yield 22.7 mg (55%) of the title compound as an amber oil: $[\alpha]^{20}_D$ +8 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} 355 (2.22), 248 (2.50) nm; IR (film) ν_{max} 3222, 2974, 2936, 2875, 2625, 1664, 1631, 1595, 1570, 1426, 1381, 1274, 1206, 1144, 1126, 933, 738 cm^{-1}; ^1H NMR δ 10.79 (1H, s, OH-5′), 9.81 (1H, s, OH-4), 7.06 (1H, s, H-4′), 3.70 (1H, sext, *J* = 6.7 Hz, H-2‴′), 3.60 (2H, brs, H-7), 2.56 (2H, q; *J* = 7.6 Hz, H-1‴), 1.95 (3H, s, H-1″); 1.85 (1H, m, H-3a‴′), 1.59 (6H, s, H-24, H-2‴), 1.43 (1H, m, H-3b‴′), 1.20 (3H, m, H-2‴), 1.16 (3H, m, H-5‴′), 0.92 (3H, t, *J* = 7.3 Hz, H-4‴′); ^{13}C NMR, δ 210.9 (C, C-1‴′), 169.5 (C, C-2), 167.7 (C, C-4), 162.3 (C, C-7′), 161.6 (C, C-6), 158.3 (C, C-5′), 153.9 (C, C-8′a), 120.9 (C, C-3′), 118.7 (CH, C-4′), 108.3 (C, C-5), 106.9 (C, C-6′), 105.4 (C, C-8′), 104.4 (C, C-4′a), 102.0 (C, C-3), 81.8 (C, C-2′), 45.9 (CH, C-2‴′), 26.8 (CH_2, C-3‴′), 26.2 (CH_3, C-1‴′, C-2‴′), 24.4 (CH_2, C-1‴), 17.6 (CH_2, C-7), 16.9 (CH_3, C-5‴′), 12.0 (CH_3, C-4‴′), 11.7 (CH_3, C-2‴), 9.6 (CH_3, C-1″); EIMS *m*/*z* 521 $[M]^+$ (58), 441 (88), 427 (24), 287 (100), 275 (55), 155 (28); HREIMS 521.4051 (calcd for $C_{25}H_{29}O_7Br$, 521.9940).

*3-[{5,7′-Dihydroxy-2,2-dimethyl-8-(2-(S)-methylbutanoyl)-2H-chromen-6-yl}methyl]-6-ethyl-4-hydroxy-5-methylpyridin-2(1H)-one (**14**).* A suspension of **1** (29.6 mg, 0.061 mmol) and 100 mg of NH_4OAc (20 equiv) in glacial acetic acid (5 mL) was heated under reflux for 27 h. After cooling to room temperature, a saturated $NaHCO_3$ solution was added, and the aqueous phase was extracted with DCM (3 × 15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated, and the resulting residue was purified by preparative TLC (EtOAc/*n*-hexane, 3:17) to yield compound **14** (8.0 mg, 27%) as an amber oil: $[\alpha]^{20}_D$ +9 (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} 359 (2.85), 241 (2.88), 206 (2.97) nm; IR (film) ν_{max} 3181, 2972, 2932, 1740, 1665, 1595, 1463, 1424, 1380, 1361; 1281; 1171, 1138; 1084 cm^{-1}; ^1H NMR, δ 16.25 (1H, s, OH-7′), 10.60 (1H, s, OH-5′), 9.95 (1H, s, OH-4), 6.71 (1H, d, *J* = 10.0 Hz, H-4′), 5.44 (1H, d, *J* = 10.0 Hz, H-3′), 3.78 (1H, sext, *J* = 6.8 Hz, H-2‴′), 3.66 (2H, brs, H-7), 2.55 (2H, q, *J* = 7.5 Hz, H-1‴), 1.95 (3H, s, H-1″), 1.87 (1H, m, H-3a‴′), 1.49 (6H, s, H-1‴′, H-2‴′), 1.41 (1H, m, H-3b‴′), 1.21 (3H, t, *J* = 7.5 Hz, H-2‴), 1.16 (3H, d, *J* = 6.6 Hz, H-5‴′), 0.93 (3H, t, *J* = 7.0 Hz, H-4‴′); ^{13}C NMR, δ 211.1 (C, C-1‴′), 169.5 (C, C-2), 167.7 (C, C-4), 162.0 (C, C-7′), 161.4 (C, C-6, 159.1 (C, C-5′), 155.7 (C, C-8′a), 124.9 (CH, C-3′), 117.5 (CH, C-4′), 108.3 (C, C-5), 106.1 (C, C-6′), 104.5 (C, C-8′), 104.1 (C, C-4′a), 102.2 (C, C-3), 78.4 (C, C-2′), 45.8 (CH, C-2‴′), 29.8 (CH_3, C-1‴′, C-2‴′), 26.9 (CH_2, C-3‴′), 24.4 (CH_2, C-1‴), 17.6 (CH_2, C-7), 16.8 (CH_3, C-5‴′), 12.1 (CH_3, C-4‴′), 10.8 (CH_3, C-2‴), 9.6 (CH_3, C-1″); EIMS *m*/*z* 442 $[M]^+$ (68), 288 (14), 275 (100), 261 (80), 219 (65); HREIMS 442.2230 (calcd for $C_{25}H_{32}NO_6$, 442.2208).

*3-[{4,6-Dihydroxy-2-(2-hydroxypropan-2-yl)-7-(2-(S)-methylbutanoyl)-2,3-dihydro benzofuran-5-yl}methyl]-6-ethyl-4-hydroxy-5-methyl-2H-pyran-2-one (**15**).* To a solution of **5** (99.5 mg, 0.23 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added 86 mg of MCPBA (0.35 mmol), and the reaction mixture was stirred for 1 h. The reaction was quenched with a 35% aqueous $NaHCO_3$ solution, the aqueous phase was extracted with DCM (3 × 20 mL), and the combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. After removal of solvent the residue was purified by preparative TLC (EtOAc/*n*-hexane, 1:4) to yield **3** (12.5 mg, 12%) and **15** (44.5 mg, 43%)

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as amber oils: $[\alpha]^{20}_{D}$ +3 (*c* 0.5, $CHCl_3$); UV (EtOH) λ_{max} 356 (2.4), 247 (2.35) nm; IR (film) ν_{max} 3345, 2973, 2935, 2876, 2618, 1665, 1568, 1434, 1382, 1136 cm^{-1}; ^1H NMR δ 4.83 (1H, t, *J* = 8.7 Hz, H-2‴′), 3.89 (1H, sext, *J* = 6.6 Hz, H- H-2‴), 3.56 (2H, m, H-7), 3.13 (1H, dd, *J* = 15.0, 9.5 Hz, H-1a‴′), 3.02 (1H, dd, *J* = 15.0, 8.3 Hz, H-1b‴′), 2.53 (2H, q, *J* = 7.5 Hz, H-1‴), 1.93 (3H, s, H-1″), 1.79 (1H, m, H-3a‴), 1.38 (1H, m, H-3b‴), 1.37 (3H, s, H-4‴′ or H-5‴′); 1.23 (3H, s, H-4‴′ or H-5‴′), 1.18 (3H, t, *J* = 7.5 Hz, H-2‴), 1.13 (3H, m, H-2‴), 0.90 (3H, m, H-4‴); ^{13}C NMR, δ 211.9 (C, C-1‴), 170.0 (C, C-2, C-4), 162.3 (C, C-1′), 161.5 (C, C-6), 160.1 (2C, C-3′, C-5′), 108.1 (C, C-5), 107.0 (C, C-6′), 103.8 (C, C-2′), 102.2 (C, C-3), 100.1(C, C-4′), 92.8 (CH, C-2‴′), 71.9 (C, C-3‴′), 45.9 (CH, C-2‴), 28.0 (CH_2, C-1‴′), 27.0 (CH_2, C-3‴), 26.1 (CH_3, C-4‴′ or C-5‴′), 24.4 (CH_2, C-1‴), 23.9 (CH_3, C-4‴′ or C-5‴′), 18.1 (CH_2, C-7), 16.8 (CH_3, C-5‴), 12.0 (CH_3, C-4‴), 11.6 (CH_3, C-2‴), 9.6 (CH_3, C-1″); EIMS *m*/*z* 460 $[M]^+$ (40), 403 (64), 320 (28), 294 (14), 280 (11), 237 (100), 176 (21), 155 (90); HREIMS 460.2097 (calcd for $C_{25}H_{32}O_8$, 460.2110).

*6-Ethyl-4-hydroxy-5-methyl-3-[2,4,6-trihydroxy-3-isopentyl-5-(2-(S)-methylbutanoyl)benzyl]-2H-pyran-2-one (**16**).* A solution of 61 mg (0.14 mmol) of **5** was hydrogenated following the procedure described for **1**, to afford, after purification by preparative TLC, 50.2 mg (82%) of the title compound as an amber oil: $[\alpha]^{20}_{D}$ +8 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} 335 (2.05), 244 (1.85) nm; IR (film) ν_{max} 3192, 2960, 2873, 2632, 1732, 1661, 1564, 1464, 1436, 1380, 1261, 1144, 932 cm^{-1}; ^1H NMR δ 3.81 (1H, sext, *J* = 6.6 Hz, 2‴), 3.59 (2H, brs, H-7), 2.55 (4H, m, H-1‴, H-1‴′), 1.94 (3H, s, H-1″), 1.83 (1H, m, H-3a‴), 1.63 (1H, m, H-3‴′), 1.43 (1H, m, H-3b‴), 1.36 (2H, m, 2‴′), 1.17 (6H, m, H-2‴, H-5‴), 0.96 (6H, d, *J* = 6.6 Hz, H-4‴′, H-5‴′), 0.92 (3H, t, *J* = 7.3 Hz, H-4‴); ^{13}C NMR δ 211.2 (C, C-1‴), 169.5 (C, C-2), 168.0 (C, C-4), 161.3 (2C, C-1′, C-6), 160.3 (2C, C-3′, C-5′), 108.4 (2C, C-4′, C-5), 106.2 (C, C-6′), 102.2 (2C, C-2′, C-3), 45.9 (CH, C-2‴), 38.2 (CH, C-3‴′), 28.5 (CH_2, C-2‴′), 27.0 (CH_2, C-3‴), 24.4 (CH_2, C-1‴), 22.6 ($2CH_3$, C-4‴′, C-5‴′), 17.9 (CH_2, C-7), 16.9 (CH_3, C-5‴), 12.1 (CH_3, C-4‴), 11.7 (CH_3, C-2‴), 9.6 (CH_3, C-1″); EIMS *m*/*z* 446 $[M]^+$ 446 (36), 389 (59), 279 (21), 223 (100), 155 (51); HREIMS 446.2305 (calcd for $C_{25}H_{34}O_7$, 446.2303).

Biological Assays. *Bacterial Strains.* The test organisms were a set of strains from collection strains of Gram-positive and Gram-negative bacteria: methicillin-sensitive *Staphylococcus aureus* ATCC25923 (MSSA); methicillin-resistant *Staph. aureus* NRS402, which is also intermediate resistant to vancomycin (VISA); *Enterococcus faecalis* ATCC29212 and *Escherichia coli* ATCC35218, as well as the yeast molds *Candida albicans* ATCC10231, *C. glabrata* ATCC9030, and *C. nivariensis* CBS 9984. Bacterial strains were stored at −80 °C in brain heart infusion broth with added 20% glycerol. Strains were revived by plating BHI broth (brain heart infusion) and incubated at 37 °C overnight. In the case of yeast species the same procedure was followed using Yeast Extract-Peptone-Dextrose (YPD) media.

MIC Determination. The antimicrobial activity was determined following the standard broth microdilution method described by the National Committee for Clinical Laboratory Standards. The MIC was determined by measuring bacterial growth after 24 h, performing 1:2 serial dilutions of each compound ranging from 1 to 128 μM. Aside from the tested compounds, antibiotics ampicillin (Sigma Chemical Co.) and kanamycin (Roche Applied Biosciences) were included as controls. The inoculum size was 1×10^5 CFU/mL for all bacteria.[38−40]

Vibrational Circular Dichroism Analysis. IR and VCD measurements were performed using a BioTools dual PEM ChiralIR FT spectrophotometer. A sample of 12.1 mg of **1** was dissolved in 150 μL of 100 atom % D $CDCl_3$ and placed in a BaF_2 cell with a path length of 100 μm, and data were acquired at a resolution of 4 cm^{-1} by averaging 1 h blocks for 7 h. The baseline was obtained by subtracting the spectrum of the solvent acquired under the same conditions while the sample stability was monitored by ^1H NMR measurements immediately prior to and after the VCD determination.

Computational Methods. In order to obtain the calculated IR and VCD spectra, a Monte Carlo search protocol was carried out using MMFF94 calculations and considering an energy cutoff of 10 kcal/mol. The single-point energy of each conformer was calculated with the DFT B3LYP/6-31G(d) level of theory in the Spartan'04 program. The structures were geometry optimized using DFT calculations at the B3LYP/DGDZVP level of theory employing the Gaussian 03W program to produce an accurate Boltzmann distribution. The calculated IR and VCD spectra were obtained considering a sum of Lorentzian bands with half-widths of 6 cm^{-1} from the final considered conformers. Molecular visualization was carried out with the GaussView 3.0 program. Geometry optimization and vibrational calculations required some 24 h computational time per conformer when using a desktop computer operating at 3 GHz with 8 Gb RAM.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of **1**−**4**. Schemes with plausible biosynthetic formation of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Retta, D.; Dellacassa, E.; Villamil, J.; Suárez, S. A.; Bandoni, A. L. *Ind. Crops Prod.* **2012**, *38*, 27−38.
(2) Ferraro, G.; Anesini, C.; Ouvina, A.; Retta, D.; Filip, R.; Gattuso, M.; Gattuso, S.; Hnatyszyn, O.; Bandoni, A. *Lat. Am. J. Pharm.* **2008**, *27*, 626−628.
(3) Arredondo, M. F.; Blasina, F.; Echeverry, C.; Morquio, A.; Ferreira, M.; Abin-Carriquiry, J. A.; Lafon, L.; Dajas, F. *J. Ethnopharmacol.* **2004**, *91*, 13−20.
(4) Gugliucci, A.; Menini, T. *Life Sci.* **2002**, *71*, 693−705.
(5) Desmarchelier, C.; Coussio, J.; Ciccia, G. *Braz. J. Med. Biol. Res.* **1998**, *31*, 1163−1170.
(6) Kadarian, C.; Broussalis, A. M.; Mino, J.; Lopez, P.; Gorzalczany, S.; Ferraro, G.; Acevedo, C. *Pharmacol. Res.* **2002**, *45*, 57−61.
(7) Hnatyszyn, O.; Moscatelli, V.; Rondina, R.; Costa, M.; Arranz, C.; Balaszczuk, A.; Coussio, J.; Ferraro, G. *Phytomedicine* **2004**, *11*, 366−369.
(8) Heng, S.; Harris, K. M.; Kantrowitz, E. R. *Eur. J. Med. Chem.* **2010**, *45*, 1478−1484.
(9) Carney, J. R.; Krenisky, J. M.; Williamson, R. T.; Luo, J. *J. Nat. Prod.* **2002**, *65*, 203−205.
(10) Simoes, C. M.; Schenkel, E. P.; Bauer, L.; Lengeloh, A. *J. Ethnopharmacol.* **1988**, *22*, 281−293.
(11) Bettega, J. M.; Teixeira, H.; Bassani, V. L.; Barardi, C. R.; Simoes, C. M. *Phytother. Res.* **2004**, *18*, 819−823.
(12) Zanon, S. M.; Ceriatti, F. S.; Rovera, M.; Sabini, L. J.; Ramos, B. A. *Rev. Lat. Microb.* **1999**, *41*, 59−62.
(13) Abdel-Malek, S.; Bastien, J.; Mahler, W.; Jia, Q.; Reinecke, M.; Robinson, W., Jr.; Shu, Y.; Zalles-Asin, J. *J. Ethnopharmacol.* **1996**, *50*, 157−22.
(14) Anesini, C.; Perez, C. *J. Ethnopharmacol.* **1993**, *39*, 119−128.
(15) Vargas, V. M. F.; Motta, V. E. P.; Leitão, A. C.; Henriques, J. A. P. *Mutat. Res.* **1990**, *240*, 13−18.

(16) Morquio, A.; Rivera-Megret, F.; Dajas, F. *Phytother. Res.* **2005**, *19*, 486−490.
(17) Ruffa, M. J.; Ferraro, G.; Wagner, M. L.; Calcagno, M. L.; Campos, R. H.; Cavallaro, L. *J. Ethnopharmacol.* **2002**, *79*, 335−339.
(18) Rojas de Arias, A.; Ferro, E.; Inchausti, A.; Ascurra, M.; Acosta, N.; Rodriguez, E.; Fournet, A. *J. Ethnopharmacol.* **1995**, *45*, 35−41.
(19) Cosentino, M.; Bombellia, R.; Carcano, E.; Luini, A.; Marino, F.; Cremab, F.; Dajas, F.; Lecchini, S. *J. Ethnopharmacol.* **2008**, *116*, 501−507.
(20) De Souza, K. C.; Bassani, V. L.; Schapoval, E. E. *Phytomedicine* **2007**, *14*, 102−108.
(21) Santos, A. L.; Ripoll, D.; Nardi, N.; Bassani, V. L. *Phytother. Res.* **1999**, *13*, 65−66.
(22) Puhlmann, J.; Knaus, U.; Tubaro, L.; Schaefer, W.; Wagner, H. *Phytochemistry* **1992**, *31*, 2617−2621.
(23) Wagner, H.; Proksch, A.; Riess-Maurer, I.; Vollmar, A.; Odenthal, S.; Stuppner, H.; Jurcic, K.; Le Turdu, M.; Fang, J. N. *Arzneim. Forsch.* **1985**, *35*, 1069−1075.
(24) Rivera, F.; Gervaz, E.; Sere, C.; Dajas, F. *J. Ethnopharmacol.* **2004**, *95*, 359−362.
(25) Lorenzo, D.; Tai-Seraffini, L.; Santos, A. C.; Frizzo, C. D. *Planta Med.* **2000**, *66*, 476−477.
(26) Middleton, E. *Int. J. Pharmacog.* **1996**, *34*, 344−348.
(27) Schmeda, G. *Rev. Latinoam. Quim.* **1984**, *15*, 134−135.
(28) Kaloga, M.; Haensel, R.; Cybulski, E. *Planta Med.* **1983**, *48*, 103−104.
(29) Ferraro, G. E.; Norbedo, C.; Coussio, J. D. *Phytochemistry* **1981**, *20*, 2053−2054.
(30) Joray, M. B.; Rollán, M. R.; Ruiz, G. M.; Palacios, S. M.; Carpinella, M. C. *Planta Med.* **2011**, *77*, 95−100.
(31) Rodríguez-Ramos, F.; Navarrete, A. *J. Nat. Prod.* **2009**, *72*, 1061−1064.
(32) García-Sánchez, E.; Ramírez-López, C. B.; Talavera-Alemán, A.; León-Hernández, A.; Martínez-Muñoz, R. E.; Martínez-Pacheco, M. M.; Gómez-Hurtado, M. A.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P.; del Río, R. E. *J. Nat. Prod.* **2014**, *77*, 1005−1012.
(33) Bustos-Brito, C.; Sánchez-Castellanos, M.; Esquivel, B.; Calderón, J. S.; Calzada, F.; Yepez-Mulia, L.; Hernández-Barragán, A.; Joseph-Nathan, P.; Cuevas, G.; Quijano, L. *J. Nat. Prod.* **2014**, *77*, 358−363.
(34) Debie, E.; Gussem, E. D.; Dukor, R. K.; Herrebout, W.; Nafie, L. A.; Bultinck, P. *ChemPhysChem* **2011**, *12*, 1542−1549.
(35) Amesty, A.; Burgueno-Tapia, E.; Joseph-Nathan, P.; Ravelo, A. G.; Estevez-Braun, A. *J. Nat. Prod.* **2011**, *74*, 1061−1065.
(36) Busch, B.; Fertweck, C. *Phytochemistry* **2009**, *70*, 1833−1840.
(37) Jihane, A.; Mo, X.; Huimin, Z.; Frost, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 5332−5333.
(38) Casero, C.; Estevez-Braun, A.; Ravelo, A. G.; Demo, M.; Mendez-Alvarez, S.; Machin, F. *Phytomedicine* **2013**, *20*, 133−138.
(39) Wallmann, J.; Bottner, A.; Goossens, L.; Hafez, H. M.; Hartmann, K.; Kaspar, H.; Kehrenberg, C.; Kietzmann, M.; Klarmann, D.; Klein, G.; Krabisch, P.; Kuhn, T.; Luhofer, G.; Richter, A.; Schulz, B.; Schwarz, S.; Sigge, C.; Traeder, W.; Waldmann, K. H.; Werckenthin, C.; Zschiesche, E. *Int. J. Antimicrob. Agents* **2006**, *27*, 482−490.
(40) Cos, P.; Hermans, N.; De Bruyne, T.; Apers, S.; Sindambiwe, J. B.; Vanden Berghe, D.; Pieters, L.; Vlietinck, A. J. *J. Ethnopharmacol.* **2002**, *79*, 155−163.