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Domino Synthesis of Embelin Derivatives with Antibacterial Activity

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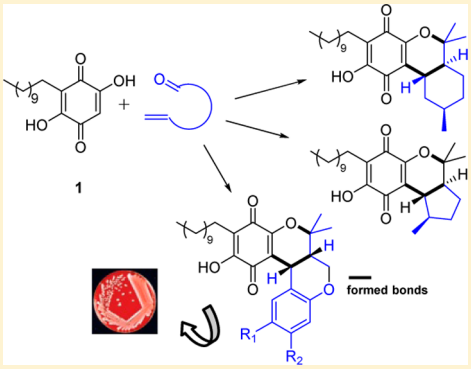
Supporting Information

ABSTRACT: A series of dihydropyran embelin derivatives was synthesized through a direct and highly efficient approach based on a domino Knoevenagel intramolecular hetero-Diels–Alder reaction from natural embelin (**1**), using unsaturated aldehydes in the presence of organocatalysts such as ethylendiamine diacetate or L-proline. The aliphatic aldehydes yielded exclusively *trans* adducts, while mixtures of *trans* and *cis* isomers were found in reactions with aromatic aldehydes, with the *cis* form always predominating. Some of the compounds obtained were active and selective against Gram-positive bacteria, including multiresistant *Staphylococcus aureus* clinical isolates.



Natural products are important sources for leads in drug discovery. More than 40% of today's prescription drugs, and even more of the anticancer agents in the clinic, can trace their origins or underlying synthetic design principles to the discovery of a natural product.[1,2] Embelin (**1**) is a natural hydroxybenzoquinone isolated as the active principle of the medicinal plant *Embelia ribes* Burm.f. (Myrsinaceae).[3]

This compound displays many biological activities such as anti-inflammmatory,[4−6] antibacterial,[7−9] antitumor,[10−12] anti-convulsant,[13] anxiolytic,[14] and antifertility[15] effects. All of these bioactivities make embelin (**1**) an interesting scaffold for synthesizing new and more selective therapeutic agents. On the other hand, 4*H*-pyrans and 4*H*-pyran-annulated heterocyclic moieties represent "privileged" structural motifs well distributed in naturally occurring compounds with a broad spectrum of significant biological activities.[16−18] Moreover, currently a number of drugs bearing the 4*H*-pyran moiety are in use in the treatment of various ailments, such as hypertension,[19] asthma,[20] ischemia,[21] and urinary incontinence.[22]

Taking into account all the information mentioned above, it was decided to prepare a set of diverse dihydropyran embelin derivatives as new potential bioactive compounds. Among the known procedures, one of the straightforward methods for the synthesis of this heterocyclic system is the domino Knoevenagel hetero-Diels–Alder reaction (DKHDA).[23−25] It consists of a Knoevenagel condensation of an aldehyde with a 1,3-dicarbonyl compound with formation of a 1,3-oxabutadiene as an intermediate, which undergoes a hetero-Diels–Alder reaction with either an enol ether or an electron-rich alkene. The DKHDA reaction can be performed as a two-, three-,or four-component transformation, with the stereoselectivity more pronounced in the case of two-component transformations.[26−30] Furthermore, in this last case, cascade polycyclization of unsaturated precursors has proven to be an extremely useful approach for the rapid and convergent construction of complex systems. This is of interest since the increase of molecular complexity is correlated with the probability of efficient binding with specific biological targets, and, in the case of embelin (**1**), the formation of more complex derivatives could lead to more selective compounds.[31,32]

Since embelin (**1**) contains a 2-hydroxy-1,4-quinone moiety that is a synthetic equivalent to a 1,3-dicarbonyl compound,[33−37] this molecule is an adequate substrate for DKHDA reactions. Herein are reported results on the preparation of complex and structurally diverse dihydropyran embelin derivatives through a domino Knoevenagel hetero-Diels–Alder reaction between embelin and aldehydes bearing a double bond. Some of the synthesized compounds were found to display antibacterial activity against Gram-positive bacteria, including multiresistant *Staphylococcus aureus* clinical isolates.

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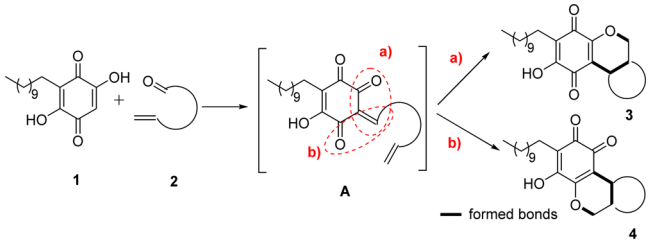
RESULTS AND DISCUSSION

The Knoevenagel condensation of embelin (**1**) with an aldehyde bearing a double bond at an appropriate distance (**2**) leads to a reactive intermediate (**A**), which undergoes an intramolecular hetero-Diels–Alder reaction with the dienophile moiety, yielding the corresponding dihydropyran embelin derivatives (Scheme 1). The polyfunctional intermediate (**A**) has two heterodiene systems that could lead to *ortho*- or *para*-benzoquinonic adducts.

Scheme 1. Reaction of Embelin (1) with Unsaturated Aldehydes (2)



Initially, the reaction of **1** and the commercial (+)-citronellal (**2a**) was examined using 1.5 equiv of aldehyde, a catalytic amount of ethylendiamine diacetate (EDDA, 5 mol %), and EtOH. Under these conditions, it was found that the reaction is regioselective since only the 1,4-benzoquinone adduct **3a** was obtained from the more electron-poor heterodiene (Table 1, entry 1). Two new fused rings next to the benzoquinone core and three σ bonds (two C–C σ bonds and one C–O σ bond) were formed in this one-pot reaction. Furthermore, the stereogenic center at the β-position to the carbonyl of (+)-citronellal produced high diastereoselectivity since only the *trans* adduct **3a** was detected. When the reaction was performed with EtOH at room temperature (5 days) or under reflux (2 h), in both cases only the *trans* adduct was obtained with a similar yield (70% vs 71%, Table 1, entry 1/columns 4 and 5). In the formation of *trans*-annulated products through intramolecular hetero-Diels–Alder reactions only the exo-*E*-anti transition state can be operative since the endo-*Z*-anti transition state is not possible for geometric reasons (Figure 1).[38]

Thus, the high diastereoselectivity can be explained by the conformational restrictions on the transition state leading to the cycloadduct due to a 1,3-allylic strain,[39] a sp^2-geminal effect,[40] and also, as a result of the asymmetric induction of the stereogenic center in the β-position, the carbonyl of the aldehyde.[24] The structure of the adduct **3a** was determined using 1D and 2D NMR spectra. In turn, the *trans* annelation of **3a** was determined by the NOE effect detected in the NOESY spectrum (Figure 2) and also by the coupling pattern for the H-10a proton, which resonated as a triplet of doublets at δ 2.26 (J = 3.0, 13.3 Hz). The anisotropic effect of the C=O bond caused a downfield shift for H-10a′, which appeared as a broad doublet at δ 2.82 (J = 12.5 Hz), while the hydrogen H-10b′ appeared at δ 0.68 as a double doublet (J = 14.0, 14.2 Hz).

When the reaction was carried out with 2,6-dimethylhept-5-enal, the formation of the two compounds **3b** and **3b′** was detected (Table 1, entry 2). Compound **3b** resulted as the expected *trans* adduct, while the structure of compound **3b′** was established as shown in Figure 3 after analysis of the COSY, HSQC, and HMBC spectra.

Compound **3b′** incorporates an ethoxy group and presumably is formed from the opening of the pyran ring of compound **3b** and subsequent attack of a molecule of EtOH. To avoid the formation of compound **3b′**, the reaction was carried out using CH_2Cl_2 instead of EtOH, and under these conditions only compound **3b** was obtained in high yield (97%) (Table 1, entry 2, column 6). The *trans* annelation of **3b** was again ratified by the coupling constant of the H-3b proton, which appeared at δ 1.69 as a double doublet (J = 9.2, 9.2 Hz) and by the NOEs detected in the ROESY spectrum. The reaction of (+)-citronellal was repeated with this aprotic solvent, and the yield was improved (85%).

Next, the unsaturated aromatic aldehydes **2c–2g** were employed in order to increase the structural complexity by forming tetracyclic adducts. The aromatic aldehydes **2c–2g** were prepared by the *O*-alkylation of the corresponding salicylaldehydes with 3,3-dimethylallyl bromide and Cs_2CO_3 in dry acetone.[41] The reaction of these aldehydes with embelin (**1**) predominantly yielded *cis*-annulated cycloadducts (entries 3–7). Different diastereomeric ratios were obtained depending of the nature of the aromatic aldehyde employed. Thus, the reaction of 4-methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde (entry 3) with **1** gave a mixture of *cis* and *trans* adducts (4:1), which could be separated in a facile manner using chromatography. A similar *cis*–*trans* ratio (3.7:1) was obtained when the reaction was performed in EtOH at room temperature, and the ratio was 6:1 when CH_2Cl_2 was employed. In the case of aldehyde **2d**, the presence of a bromine group led to a higher diastereoselectivity (8:1) when compared with the chlorinated aldehyde **2e** (6.7:1) or with the diisoprenylated aldehyde **2f** (4.5:1). These ratios were slightly improved when the reactions were carried out at room temperature in EtOH or CH_2Cl_2 (see entries 4–6, columns 5 and 6). With the 2-(3-methylbut-2-enyloxy)naphthalene-1-carbaldehyde (**2g**) only the *cis* adduct was formed. In this case and with the rest of the *cis* adducts, an endo-(*E*)-syn or an exo-(*Z*)-syn transition orientation can be assumed as transition structures (Figure 4).[42] In the *cis*-fused cycloadducts **3d–3g**, the ^{1}H NMR signal of the H-12b proton appeared around δ 4.20 as a doublet with J = 4.0–5.1 Hz, while in the *trans*-fused cycloadducts **4c–4f**, the signal of H-12b appeared less deshielded, at δ 3.62, with large coupling constants, J = 11.3–11.6 Hz.

Next, it was decided to use another organocatalyst in order to increase the diastereomeric ratio. Thus, L-proline was chosen since is an abundant and inexpensive amino acid capable of catalyzing diverse organic transformations, in both enantio- and nonenantioselective fashions, including domino processes such as Knoevenagel/hetero-Diels–Alder elimination reactions.[43] The efficacy of L-proline in diverse organic transformations is ascribable to the multiple catalytic roles it can play, such as an acid or a base or both simultaneously, as a nucleophile, and through its ability to produce enamine/iminium intermediates upon reaction with carbonyl/α,β-unsaturated carbonyl compounds. When the reactions were carried out in CH_2Cl_2 at room temperature, as shown in Table 1 (entries 3–6, column 7), the diastereomeric ratio increased in favor of the *cis*-diastereomer. Chiral HPLC analysis of adduct **3d** proved that this compound was almost racemic, and this demonstrated that the reaction takes place essentially in a nonenantioselective way.

On the basis of previous reports on the antibacterial activities of embelin (**1**) and its derivatives,[44] it was decided to test the

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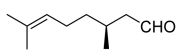
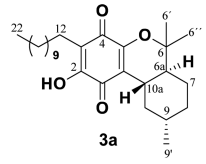
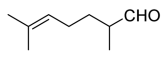
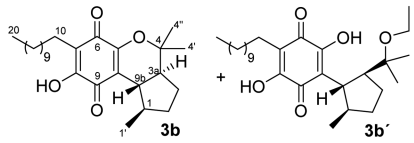
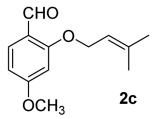
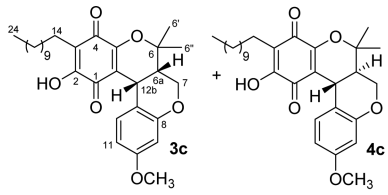
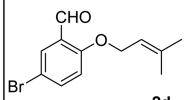
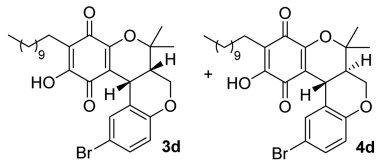
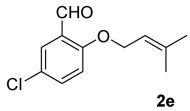
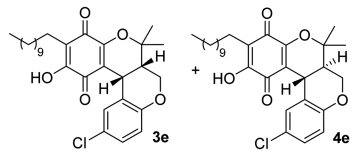
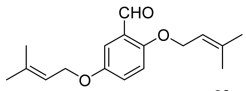
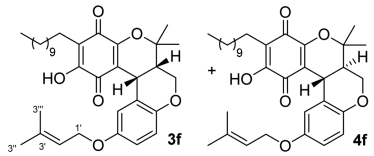
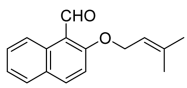
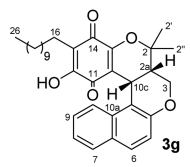
Table 1. Domino Synthesis of Dihydropyran Embelin Derivatives

entry	aldehyde	adducts	yield (%)[a] ratio	yield (%)[b] ratio	yield (%)[c] ratio	yield (%)[d] ratio
1	 (+)-2a	 3a	71	70	85	----
2	 (±)-2b	 3b + 3b′	80 (2.5:1)	69 (8.7:1)	97 (1:0)	----
3	 2c	 3c + 4c	85 (4:1)	44 (3.7:1)	50 (6:1)	75 (9.3:1)
4	 2d	 3d + 4d	78 (8:1)	66 (10.9:1)	64 (10:1)	67 (14:1)
5	 2e	 3e + 4e	69 (6.7:1)	80 (7.7:1)	63 (8:1)	50 (11:1)
6	 2f	 3f + 4f	77 (4.5:1)	37 (5:1)	52 (5.4:1)	67 (8:1)
7	 2g	 3g	71	92	---	---

[a]EDDA, EtOH, Δ. [b]EDDA, EtOH, rt. [c]EDDA, CH_2Cl_2, rt. [d]L-Proline, CH_2Cl_2, rt.

new compounds against two *Staphylococcus aureus* strains. This organism is the causal agent of most staphylococcal infections secondary to hospital admissions, and it often causes serious complications because of multiple antibiotic resistances. Therefore, there is an urgent need for novel antibiotics against *S. aureus*. All obtained adducts were tested for antibacterial activity against two reference and clinically relevant *S. aureus* strains, namely, vancomycin- and methicillin-sensitive *Staphylococcus aureus* ATCC25923 and vancomycin-intermediate and methicillin-resistant *S. aureus* NRS402 (Table 2).[45] The compounds had no effect on the growth of the two Gram-negative bacteria assayed, *Escherichia coli* and *Pseudomonas aeruginosa* (data not shown). Some of the synthesized adducts were not only active against the two *S. aureus* strains but also more active than embelin (**1**).

Good results were obtained from the *trans* tricyclic adducts **3a** and **3b**, synthesized from the aliphatic aldehydes, while the open compound **3b′** proved to be inactive. With respect to adducts obtained from aromatic aldehydes, it was determined that the nature of the substituent on the aromatic ring seems to

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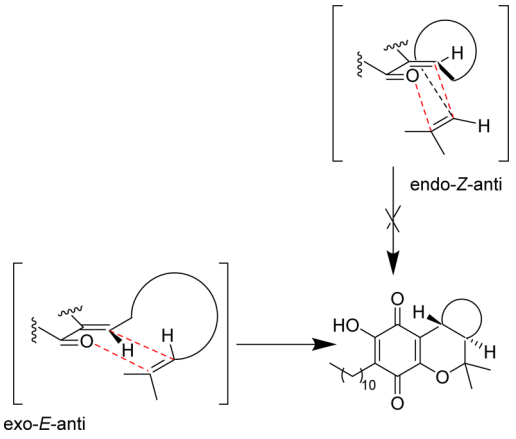

Figure 1. Exo-*E*-anti and endo-*Z*-anti transition states in the intramolecular DKHDA.

modulate the antibacterial activity. Thus, the presence of methoxy and chlorine groups led to the best results (Table 2, entries 5, 6, 9, and 10), while the occurrence of an isoprenyl chain or a fused aromatic ring produced inactivity (Table 2, entries 11, 12, and 13). This indicates that steric hindrance in this part of the molecule plays an important role in mediating the antibiotic activity against the two *Staphylococcus aureus* strains assayed.

In summary, a set of new dyhydropyran derivatives of embelin (**1**) were obtained through a Knoevenagel intramolecular hetero-Diels–Alder reaction from embelin (**1**) and aliphatic or aromatic aldehydes bearing a double bond in the presence of EDDA or L-proline. High diastereoselectivity was found in the case of aliphatic aldehydes since only *trans* adducts were obtained. When the reaction was carried out with aromatic aldehydes, *cis* adducts predominantly resulted, and the best *cis/trans* ratio was achieved using L-proline as catalyst. All synthesized compounds were tested against two important multiresistant *Staphylococcus aureus* clinical isolates, and the introduction of molecular complexity to the embelin (**1**) nucleus led to compounds more active and selective than this starting natural benzoquinone. The present results encourage

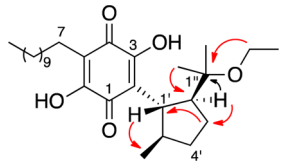

Figure 3. Key HMBC correlations for **3b′**.

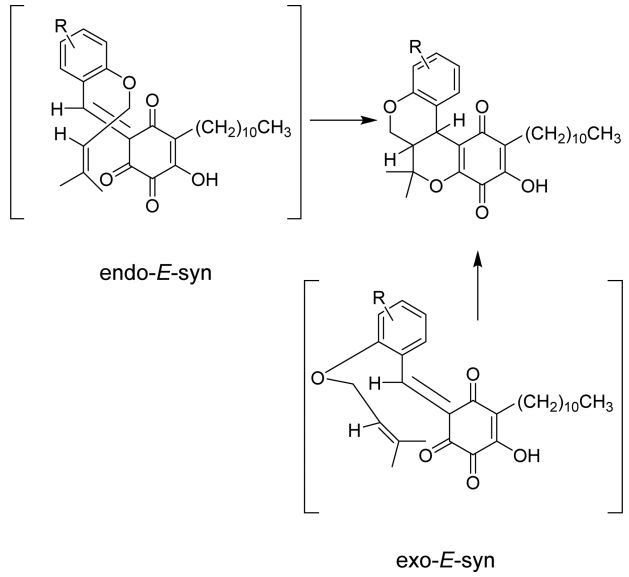

Figure 4. Endo-*E*-syn and exo-*E*-syn transition states in the intramolecular DKHDA.

further research with these compounds in order to develop novel antibiotic agents against Gram-positive bacteria.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 241 polarimeter. IR spectra were obtained using a Bruker IFS28/55 spectrophotometer. NMR spectra were recorded in $CDCl_3$ or C_6D_6 at 400, 500, or 600 MHz for ^1H NMR and 100 or 150 MHz for ^{13}C NMR. Chemical shifts (δ) are given in parts

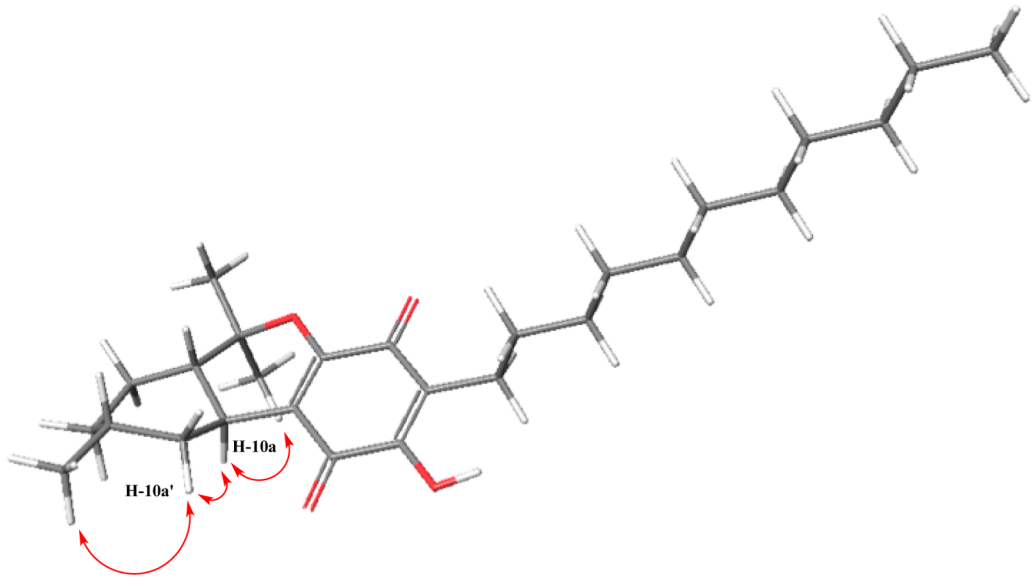

Figure 2. Structure of **3a** with the key NOEs.

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Table 2. MIC (μM) of Embelin Derivatives against Two Selected *Staphylococcus Aureus* Strains That Differ in Their Antibiotic Resistance Profile

entry	compound	*S. aureus* (NRS402)	*S. aureus* (ATCC25923)
1	**1**	16	32
2	**3a**	1.0	8.0
3	**3b**	4.0	64.0
4	**3b′**	>128	>128
5	**3c**	1.0	8.0
6	**4c**	4.0	64.0
7	**3d**	32.0	16.0
8	**4d**	32.0	16.0
9	**3e**	1.0	4.0
10	**4e**	2.0	4.0
11	**3f**	>128	>128
12	**4f**	>128	>128
13	**3g**	>128	>128
14	oxacillin[a]	>128	<1
15	vancomycin[a]	4.0	<1
16	mupirocin[a]	<1	<1

[a]MIC values are in μg/mL.

per million, and coupling constants (J) in hertz (Hz). ^{1}H and ^{13}C spectra were referenced using the solvent signal as internal standard. HREIMS were recorded using a high-resolution magnetic trisector (EBE) mass analyzer. Analytical thin-layer chromatography plates used were Polygram-Sil G/UV254. Preparative thin-layer chromatography was carried out with Analtech silica gel GF plates (20 × 20 cm, 1000 μm) using appropriate mixtures of ethyl acetate and hexanes. All solvents and reagents were purified by standard techniques reported[46] or used as supplied from commercial sources. The embelin (**1**) used in the reactions was obtained from *Oxalis erythrorhiza* Gill. ex Hook. (Oxalidaceae) following the procedure described in ref 7. Chiral HPLC analysis of compound **3d** was carried out with a Varian HPLC on a Chiralcel OD-H column using *n*-hexane−2-propanol (9:1) as the eluent at 280 nm. All compounds were named using the ACD40 Name-Pro program, which is based on IUPAC rules.

Representative Procedures for the Preparation of Dihydropyran Derivatives of Embelin (1). *Condition A.* A solution of 30 mg of embelin (**1**) (0.10 mmol) in 5 mL of EtOH was treated with 0.15 mmol of (+)-citronellal (27.7 μL) and 5 mol % of EDDA. The reaction mixture was refluxed until the disappearance of the starting benzoquinone occurred. The solvent was removed under vacuum, and the crude product was purified by preparative TLC (*n*-hexane−EtOAc, 7:3) to yield 30.5 mg (71%) of the dihydropyran derivative **3a**.

Condition B. A solution of embelin (**1**) (30 mg, 0.10 mmol) in 5 mL of EtOH was treated with 0.15 mmol of aldehyde **3g** (36.7 mg) and 5 mol % of EDDA. The reaction mixture was stirred at room temperature until the disappearance of the starting benzoquinone occurred. The solvent was removed under vacuum, and the crude product was purified by preparative TLC (*n*-hexane−EtOAc, 7:3) to yield 47.4 mg (92%) of the dihydropyran derivative **3g**.

Condition C. A solution of embelin (**1**) (30 mg, 0.10 mmol) in 5 mL of CH_2Cl_2 was treated with 0.15 mmol of 2,6-dimethylhep-5-enal (30.5 μL) and 5 mol % of EDDA. The reaction mixture was stirred at room temperature until the disappearance of the starting benzoquinone occurred. The solvent was removed under vacuum, and the crude product was purified by preparative TLC (*n*-hexane−EtOAc, 7:3) to yield 40.4 mg (97%) of the dihydropyran derivative **3b**.

Condition D. A solution of embelin (**1**) (30 mg, 0.10 mmol) in 5 mL of dry CH_2Cl_2 was treated wih 0.15 mmol (33.7 mg) of 4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (**2c**) and 5 mol % of L-proline. The reaction mixture was stirred at room temperature until the disappearance of the starting benzoquinone occurred (24 h). The solvent was removed under vacuum, and the crude product was purified by preparative TLC (*n*-hexane−EtOAc, 7:3) to yield 34.3 mg (68%) of **3c** and 3.7 mg (7%) of **4c**.

*2-Hydroxy-6,6,9-trimethyl-3-undecyl-6a,7,8,9,10,10a-hexahydro-1H-benzo[c]chromene-1,4(6H)-dione (**3a**).* Purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) yielded compound **3a** as an amorphous, brown solid [30.5 mg (71%) using condition A; 30.1 mg (70%) using condition B; 36.6 mg (85%) using condition C]; $[\alpha]^{20}_D$ +220 (c 0.01, $CHCl_3$); IR (film) ν_{max} 3336, 2922, 2854, 1656, 1634, 1595, 1458, 1333, 1275, 1209, 1170, 1138, 1110, 1085, 1011, 961, 932, 894, 861, 811, 763, 631 cm^{-1}; ^{1}H NMR δ 0.68 (1H, dd, J = 14.0, 14.2 Hz, H-10b′), 0.87 (3H, t, J = 7.1 Hz, H-22), 0.94 (3H, d, J = 6.5 Hz, H-9′), 1.06 (2H, m), 1.14 (3H, s, H-6′), 1.30 (16H, bs, H-13-H-20), 1.43 (2H, m), 1.48 (3H, s, H-6″), 1.60 (2H, m), 1.83 (2H, m), 2.26 (1H, td, J = 3.0, 13.3 Hz, H-10a), 2.37 (2H, td, J = 2.5, 7.7 Hz, H-12), 2.82 (1H, bd, J = 12.5 Hz, H-10a′); ^{13}C NMR δ 14.2 (CH_3, C-22), 19.7 (CH_3, C-9′), 22.3 (CH_3, C-6′), 22.5 (CH_2, C-12), 22.7 (CH_2, C-21), 27.0 (CH_3, C-6″), 27.4 (CH_2, C-7), 28.1 (CH_2, C-13), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 32.0 (CH_2, C-20), 32.5 (CH, C-10a), 33.6 (CH, C-9), 35.2 (CH_2, C-8), 38.4 (CH_2, C-10), 47.9 (CH, C-6a), 87.7 (C, C-6), 115.4 (C, C-11), 117.7 (C, C-3), 151.5 (C, C-2), 155.6 (C, C-5), 182.1 (C, C-1), 182.2 (C, C-4); EIMS m/z 430 [M$^+$] (100), 402 (8), 387 (6), 290 (19), 247 (15); HREIMS m/z 430.3094 (calcd for $C_{27}H_{42}O_4$, 430.3083).

*(±)-8-Hydroxy-1,4,4-trimethyl-7-undecyl-1,2,3,3a,4,9b-hexahydrocyclopenta[c]chromene-6,9-dione (**3b**).* Compound **3b** was obtained as an amorphous, brown solid after purification of the reaction mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) [23.7 mg (57%) using condition A; 26.6 mg (62%) using condition B, 40.4 mg (97%)]; IR (film) ν_{max} 3339, 2924, 2855, 2284, 1633, 1570, 1479, 1396, 1321, 1286, 1254, 1167, 1119, 1097, 1012, 965, 901, 851, 815, 764, 688, 644 cm^{-1}; ^{1}H NMR δ 0.87 (3H, t, J = 7.2 Hz, H-20), 1.24 (21H, bs, H-11-H-19, H-4′), 1.44 (5H, m), 1.50 (3H, s, H-4″), 1.66 (1H, m), 1.76 (1H, td, J = 5.4, 12.4 Hz, H-2′), 1.99 (1H, m, H-3a), 2.08 (2H, m), 2.37 (2H, t, J = 7.6 Hz, H-10), 7.44 (1H, bs, OH); ^{1}H NMR (C_6D_6) δ 0.80 (3H, s, H-4′), 0.90 (3H, t, J = 7.0 Hz, H-20), 1.13 (3H, s, H-4″), 1.24 (16H, bs, H-11-H-18), 1.37 (3H, m), 1.57 (3H, d, J = 6.3 Hz, H-1′), 1.65 (2H, m), 1.69 (1H, dd, J = 9.2, 9.2 Hz, H-3a), 1.78 (1H, m), 1.96 (1H, m, H-1), 2.62 (2H, t, J = 7.5 Hz, H-10), 7.47 (1H, bs, OH); ^{13}C NMR δ 14.1 (CH_3, C-20), 20.6 (CH_3, C-4′), 22.6 (CH_2, C-10), 22.7 (CH_2, C-19), 23.2 (CH_3, C-4″), 24.9 (CH_2, C-2), 28.1 (CH_2 + CH_3, C-11 + C-1′), 29.3 (CH_2), 29.4 (CH_2), 29.6 (4 × CH_2), 31.9 (CH_2, C-18), 34.2 (CH_2, C-3), 35.5 (CH, C-9b), 42.9 (CH, C-1), 52.8 (CH, C-3a), 84.0 (C, C-4), 116.0 (C, C-9a), 117.4 (C, C-7), 151.2 (C, C-8), 155.7 (C, C-5), 181.7 (C, C-9), 182.0 (C, C-6); EIMS m/z 416 [M$^+$] (100), 401 (53), 347 (11), 276 (17); HREIMS m/z 416.2938 (calcd for $C_{26}H_{40}O_4$, 416.2927).

*(±)-2-(2-(2-Ethoxypropan-2-yl)-5-methylcyclopentyl)-3,6-dihydroxy-5-undecylcyclohexa-2,5-diene-1,4-dione (**3b′**).* Compound **3b′** was obtained as an amorphous, brown solid after purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) [10.6 mg (23%) using condition A; 3.2 mg (7%) using condition B]; IR (film) ν_{max} 3318, 2924, 2855, 2361, 2336, 1615, 1502, 1465, 1340, 1316, 1264, 1167, 1118, 1066, 949, 816, 647 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J = 7.0 Hz, H-17), 0.92 (3H, t, J = 6.5 Hz, $OCH_2\underline{CH_3}$), 1.07 (6H, bs, H-1″ + H-1‴), 1.20 (1H, m, H-4′b), 1.25 (16H, bs, H-8-H-15), 1.51 (1H, m, H-3′b), 1.82 (1H, m, H-3′a), 1.87 (1H, m, H-4′b), 2.28 (1H, m, H-5′), 2.40 (2H, t, J = 7.5 Hz, H-7), 2.70 (2H, m, H-1′ + H-2′), 3.22 (2H, m, $O\underline{CH_2}CH_3$), 7.70 (1H, bs, OH); ^{13}C NMR δ 14.1 (CH_3, C-17), 15.9 (CH_3, $OCH_2\underline{CH_3}$), 18.8 (CH_3, C-1‴), 21.2 (CH_3, C-5″), 22.5 (CH_2), 22.7 (CH_2), 24.1 (CH_3), 27.1 (CH_2, C-4′), 28.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.6 (3 × CH_2), 29.7 (CH_2), 31.9 (CH_2), 34.1 (CH_2, C-3′), 39.1 (CH, C-5′), 44.4 (CH, C-1′), 51.5 (CH, C-2′), 56.0 (CH_2, $O\underline{CH_2}CH_3$), 77.0 (C, C-1⁗), 115.6 (C, C-5), 118.9 (C, C-2); EIMS m/z 462 [M$^+$] (1), 416 (13), 401 (13), 376 (12), 340 (8), 123 (10), 87 (100); HREIMS m/z 462.3318 (calcd for $C_{28}H_{46}O_5$, 462.3345).

(±)-2-Hydroxy-10-methoxy-6,6-dimethyl-3-undecyl-6,6a-dihydrochromeno[3,4-c]chromene-1,4(8H,12bH)-dione. Purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) yielded compounds **3c** and **4c** as amorphous, brown solids.

*cis Adduct **3c**:* 33.7 mg (68%) using condition A; 17.4 mg (35%) using condition B; 21.3 mg (43%) using condition C; 33.7 mg (68%)

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using condition D; IR (film) ν_{max} 3339, 2925, 2854, 2324, 1655, 1599, 1505, 1463, 1443, 1339, 1271, 1197, 1164, 1121, 1036, 995, 945, 831, 807 cm^{-1}; ^1H NMR δ 0.87 (3H, t, J = 7.0 Hz, H-24), 1.25 (16H, bs, H-16-H-23), 1.36 (3H, s, H-6′), 1.44 (2H, m, H-15), 1.55 (3H, s, H-6″), 2.12 (1H, m, H-6a), 2.40 (2H, t, J = 7.2 Hz, H-14), 3.74 (3H, s, 3H, OCH_3), 4.18 (1H, dd, J = 12.0, 6.0 Hz, H-7b), 4.16 (1H, d, J = 4.0 Hz, H-12b), 4.42 (1H, dd, J = 3.3, 11.7 Hz, H-7a), 6.29 (1H, d, J = 1.9 Hz, H-9), 6.47 (1H, bd, J = 8.0 Hz, H-12), 7.28 (1H, d, J = 8.4 Hz, H-11); ^{13}C NMR δ 14.1 (CH_3, C-24), 22.4 (CH_2), 22.6 (CH_2), 22.7 (CH_2), 24.3 (CH_3, C-6′), 27.1 (CH_3, C-6″), 27.6 (CH_2), 28.1 (CH, C-12b), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2, C-22), 38.2 (CH, C-6a), 55.2 (CH_3, OCH_3), 64.1 (CH_2, C-7), 81.1 (C, C-6), 100.9 (CH, C-9), 107.3 (CH, C-11), 112.4 (C, C-12a), 113.6 (C, C-13), 118.3 (C, C-3), 131.9 (CH, C-12), 151.5 (C, C-2), 154.1 (C, C-8), 154.7 (C, C-5), 158.8 (C, C-10), 181.5 (C, C-1), 183.2 (C, C-4); EIMS m/z 496 [M^+] (100), 476 (13), 411 (12), 356 (38), 298 (24), 203 (15); HREIMS m/z 496.2818 (calcd for $C_{30}H_{40}O_6$, 496.2825).

trans Adduct ***4c:*** 8.4 mg (17%) using condition A; 4.5 mg (9%) using condition B; 3.5 mg (7%) using condition C; 3.5 mg (7%) using condition D; IR (film) ν_{max} 3358, 2925, 2853, 1654, 1603, 1560, 1504, 1463, 1377, 1336, 1282, 1221, 1197, 1163, 1120, 1034, 986, 832 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J = 7.0 Hz, H-24), 1.26 (16H, bs, H-16-H-23), 1.29 (3H, s, H-6′), 1.49 (2H, m, H-15), 1.55 (3H, s, H-6″), 2.16 (1H, td, J = 5.6, 11.6 Hz, H-6a), 2.45 (2H, t, J = 7.9 Hz, H-14), 3.61 (1H, d, J = 11.5 Hz, H-12b), 3.75 (3H, s, OMe), 4.04 (1H, dd, J = 5.7, 9.8 Hz, H-7b), 4.38 (1H, d, J = 11.7, H-7a), 6.41 (1H, dd, J = 2.1, 8.5 Hz, H-11), 6.46 (1H, d, J = 2.1 Hz, H-9), 6.61 (1H, dd, J = 2.0, 8.7 Hz, H-12); ^{13}C NMR δ14.1 (CH_3, C-24), 19.5 (CH_3, C-6′), 22.6 (CH_2, C-23), 22.7 (CH_2, C-14), 27.0 (CH_2), 28.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2 × 2), 29.7 (CH_2), 30.7 (CH), 31.9 (CH_2, C-22), 45.8 (CH, C-6a), 55.4 (CH_3, OCH_3), 68.2 (CH_2, C-7), 80.4 (C-6), 102.8 (CH, C-9), 106.4 (CH, C-11), 113.9 (C, C-12a), 118.4 (C, C-13), 118.6 (C, C-3), 126.4 (CH, C-12), 151.5 (C, C-2), 154.8 (C, C-8), 156.7 (C, C-5), 159.7 (C, C-10), 181.8 (C, C-1), 182.5 (C, C-4); EIMS m/z 496 [M^+] (100), 481 (11), 411 (7), 356 (17), 298 (12), 203 (19); HREIMS m/z 496.2826 (calcd for $C_{30}H_{40}O_6$, 496.2825).

(±)-11-Bromo-2-hydroxy-6,6-dimethyl-3-undecyl-6,6a-dihydroisochromeno[3,4-c]chromene-1,4(8H,12bH)-dione. Purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) yielded compounds **3d** and **4d** as amorphous, brown solids.

cis Adduct ***3d.*** 37.5 mg (69%) using condition A; 32.6 mg (60%) using condition B; 31.6 mg (58%) using condition C; 34.3 mg (63%) using condition D; IR (film) ν_{max} 3353, 2928, 2857, 2335, 1657, 1600, 1485, 1394, 1339, 1278, 1257, 1179, 1124, 1089, 1012, 948, 889, 854, 820 cm^{-1}; ^1H NMR δ 0.87 (3H, t, J = 7.1 Hz, H-24), 1.24 (16H, bs, H-16-H-23), 1.31 (3H, s, H-6′), 1.45 (2H, m, H-15), 1.57 (3H, s, H-6″), 2.11 (1H, m, H-6a), 2.41 (2H, t, J = 7.4 Hz, H-14), 4.20 (1H, d, J = 5.1 Hz, H-12b), 4.24 (1H, dd, J = 5.1, 11.8 Hz, H-7b), 4.41 (1H, dd, J = 3.4, 11.8 Hz, H-7a), 6.63 (1H, d, J = 8.8 Hz, H-10), 7.20 (1H, d, J = 8.5 Hz, H-9), 7.44 (1H, s, H-12); ^{13}C NMR δ14.1 (CH_3, C-24), 22.7 (CH_2 × 2, C-14 + C-23), 24.0 (CH_3, C-6′), 27.2 (CH_3, C-6″), 28.1 (CH_2), 29.3 (CH, C-12b), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2, C-22), 37.9 (CH, C-6a), 64.3 (CH_2, C-7), 81.3 (C, C-6), 112.7 (C, C-12a), 112.7 (C-13), 118.1 (CH, C-9), 118.8 (C, C-3), 122.7 (C, C-11), 131.4 (CH, C-10), 133.4 (CH, C-12), 151.7 (C, C-2), 153.1 (C, C-8), 154.6 (C, C-5), 181.1 (C, C-1), 183.1 (C, C-4); EIMS m/z 544 [M^+] (100), 529 (7), 459 (16), 403 (22), 347 (8), 250 (11); HREIMS m/z 544.1840 (calcd for $C_{29}H_{37}O_5{}^{79}Br$, 544.1824), 546.1804 (calcd for $C_{29}H_{37}O_5{}^{81}Br$, 546.1804).

trans Adduct ***4d.*** 4.9 mg (9%) using condition A; 3.3 mg (6%) using condition B; 3.3 mg (6%) using condition C; 2.2 mg (4%) using condition D; IR (film) ν_{max} 3360, 2928, 2857, 2293, 1658, 1604, 1485, 1472, 1393, 1334, 1296, 1266, 1235, 1198, 1173, 1120, 1034, 986, 892, 861, 823 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J = 7.1 Hz, 3H, H-24), 1.26 (16H, bs, H-16-H-23), 1.30 (3H, s, H-6′), 1.51 (2H, m, H-15), 1.55 (3H, s, H-6″), 2.15 (1H, td, J = 6.1, 11.8 Hz, H-6a), 2.46 (2H, t, J = 7.7 Hz, H-14), 3.62 (1H, d, J = 11.6 Hz, H-12b), 4.04 (1H, dd, J = 10.1, 11.8 Hz, H-7b), 4.36 (1H, dd, J = 9.8, 9.8 Hz, H-7a), 6.80 (1H, d, J = 8.5 Hz, H-10), 6.79 (1H, d, J = 1.0 Hz, H-9), 7.25 (1H, m, H-12); ^{13}C NMR δ 14.1 (CH_3, C-24), 19.4 (CH_3, C-6′), 22.7 (CH_2 × 2, C-23 + C-14), 27.0 (CH_3, C-6″), 29.0 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.7 (2 × CH_2), 31.1 (CH, C-12b), 31.9 (CH_2, C-22), 45.5 (CH, C-6a), 68.2 (CH_2, C-7), 80.4 (C, C-6), 112.9 (C, C-13), 113.1 (C, C-3), 118.9 (CH, C-9), 119.0 (C, C-11), 128.7 (C, C-12a), 128.8 (CH, C-10), 131.0 (CH, C-12), 151.5 (C, C-2), 153.2 (C, C-8), 157.0 (C, C-5), 181.5 (C, C-1), 182.3 (C, C-4); EIMS m/z 545 [M^+] (100), 528 (22), 459 (16), 403 (12), 250 (21); HREIMS m/z 546.1821 (calcd for $C_{29}H_{37}O_5{}^{81}Br$, 546.1804), 544.1833 (calcd for $C_{29}H_{37}O_5{}^{79}Br$, 544.1824).

(±)-11-Chloro-2-hydroxy-6,6-dimethyl-3-undecyl-6a,7-dihydrochromeno[3,4-c]chromene-1,4(6H,12bH)-dione. Purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) yielded compounds **3e** and **4e** as amorphous, brown solids.

cis Adduct ***3e:*** 30.0 mg (60%) using condition A; 35.5 mg (71%) using condition B; 28.0 mg (56%) using condition C; 23.0 mg (46%) using condition D; IR (film) ν_{max} 3355, 2929, 2858, 2292, 1658, 1601, 1488, 1469, 1394, 1334, 1278, 1257, 1179, 1103, 1012, 949, 922, 890, 867, 821, 724 cm^{-1}; ^1H NMR δ 0.87 (3H, t, J = 6.8 Hz, H-24), 1.24 (16H, bs, H-16-H-23), 1.31 (3H, s, H-6′), 1.45 (2H, m, H-15), 1.58 (3H, s, H-6″), 2.13 (1H, m, H-6a), 2.41 (2H, t, J = 7.5 Hz, H-14), 4.20 (1H, d, J = 5.3 Hz, H-12b), 4.25 (1H, m, H-7b), 4.41 (1H, m, H-7a), 6.68 (1H, d, J = 8.3 Hz, H-9), 7.07 (1H, d, J = 8.3 Hz, H-10), 7.30 (1H, s, H-12); ^{13}C NMR δ 14.1 (CH_3, C-24), 22.7 (CH_2 × 2, C-14 + C-23), 23.9 (CH_3, C-6′), 27.2 (CH_3, C-6″), 28.0 (CH_2), 29.2 (CH_2), 29.3 (CH, C-12b), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2, C-22), 37.9 (CH, C-6a), 64.3 (CH_2, C-7), 81.4 (C, C-6), 112.7 (C, C-3), 117.6 (CH, C-9), 118.8 (C, C-13), 122.2 (C, C-12a), 125.4 (C, C-11), 128.5 (CH, C-12), 130.4 (CH, C-10), 151.7 (C, C-2), 152.6 (C, C-8), 154.8 (C, C-5), 181.1 (C, C-1), 183.3 (C, C-4); EIMS m/z 500.18 [M^+] (100), 485 (5), 413 (3), 360 (17), 301 (8); HREIMS m/z 500.2305 (calcd for $C_{29}H_{37}O_5Cl$, 500.2330).

trans Adduct ***4e:*** 4.5 mg (9%) using condition A; 4.5 mg (9%) using condition B; 3.5 mg (7%) using condition C; 2.0 mg (4%) using condition D; IR (film) ν_{max} 3492, 2960, 2925, 2854, 2384, 2349, 2293, 2167, 1661, 1605, 1445, 1375, 1237, 1125, 1098, 1036, 922, 818, 652 cm^{-1}; ^1H NMR δ 0.87 (3H, t, J = 7.0 Hz, H-24), 1.26 (16H, bs, H-16-H-23), 1.30 (3H, s, H-6′), 1.51 (2H, m, H-15), 1.56 (3H, s, H-6″), 2.15 (1H, m, H-6a), 2.46 (2H, t, J = 7.4 Hz, H-14), 3.62 (1H, d, J = 11.4 Hz, H-12b), 4.05 (1H, t, J = 10.7 Hz, H-7b), 4.37 (1H, dd, J = 9.8, 6.2 Hz, H-7a), 6.67 (1H, s, H-12), 6.82 (1H, d, J = 8.4 Hz, H-10), 7.12 (1H, dd, J = 1.7, 8.5 Hz, H-9); ^{13}C NMR δ 14.1 (CH_3, C-24), 19.4 (CH_3, C-6′), 22.7 (CH_2 × 2, C-14 + C-23), 27.0 (CH_3, C-6″), 27.2 (CH_2), 28.1 (CH_2, C-15), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.1 (CH, C-12b), 31.9 (CH_2, C-22), 45.4 (CH, C-6a), 68.1 (CH_2, C-7), 80.4 (C, C-6), 112.9 (C, C-3), 118.3 (CH, C-9), 119.0 (C, C-13), 125.7 (C, C-12a), 125.9 (CH, C-10), 128.0 (CH, C-12), 128.2 (C, C-11), 151.5 (C, C-2), 152.7 (C, C-8), 156.9 (C, C-5), 181.5 (C, C-1), 182.3 (C, C-4); EIMS m/z 500 [M^+] (100), 485 (17), 415 (10), 360 (13), 206 (22); HREIMS m/z 500.2351 (calcd for $C_{29}H_{37}O_5Cl$, 500.2330).

(±)-2-Hydroxy-6,6-dimethyl-11-((3-methylbut-2-en-1-yl)oxy)-3-undecyl-6,6a-dihydrochromeno[3,4-c]chromene-1,4(8H,12bH)-dione. Purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) yielded compounds **3f** and **4f** as amorphous, brown solids.

cis Adduct ***3f:*** 34.7 mg (63%) using condition A; 17.1 mg (31%) using condition B; 24.2 mg (44%) using condition C; 33.0 mg (60%) using condition D; IR (film) ν_{max} IR 3353, 2928, 2857, 1721, 1659, 1604, 1563, 1498, 1466, 1384, 1340, 1284, 1250, 1212, 1183, 1125, 1104, 1079, 1016, 990, 816, 764, 729, 691, 648, 614 cm^{-1}; ^1H NMR δ 0.87 (3H, t, J = 7.0 Hz, H-24), 1.25 (16H, bs, H-16-H-23), 1.28 (3H, s, H-6′), 1.45 (2H, m, H-15), 1.58 (3H, s, H-6″), 1.72 (3H, s, H-3″), 1.78 (3H, s, H-3‴), 2.12 (1H, m, H-6a), 2.41 (2H, t, J = 7.4 Hz, H-14), 4.25 (2H, dd, J = 12.5, 5.0 Hz, H-7b + H-1′b), 4.39 (1H, d, J = 4.0 Hz, H-12b), 4.42 (2H, m, H-7a + H-1′a), 5.47 (1H, t, J = 6.6 Hz, H-2′), 6.66 (1H, d, J = 9.0 Hz, H-9), 6.70 (1H, dd, J = 9.0, 2.5 Hz, H-10), 6.84 (1H, d, J = 2.5 Hz, H-12); ^{13}C NMR δ 14.1 (CH_3, C-24), 18.2

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(CH_3, C-3″), 22.7 (CH_2 × 2, C-14 + C-23), 23.8 (CH_3, C-6′), 25.8 (CH_3, C-3‴), 27.4 (CH_3, C-6″), 28.1 (CH_2, C-15), 29.3 (CH, C-12b), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2, C-22), 38.4 (CH, C-6a), 64.4 (CH_2, C-1′), 65.4 (CH_2, C-7), 81.7 (C, C-6), 113.2 (C, C-13), 114.4 (CH, C-9), 116.6 (CH, C-10), 116.7 (CH_2, C-12), 118.4 (C, C-3), 119.9 (CH, C-2′), 121.7 (C, C-3), 138.0 (C, C-12a), 148.1 (C, C-2), 152.7 (C, C-8), 154.8 (C, C-5), 181.3 (C, C-1), 183.4 (C, C-4); EIMS *m/z* 550 [M^+] (5), 482 (100), 395 (9), 341 (11), 284 (15), 189 (11); HREIMS *m/z* 550.3294 (calcd for $C_{34}H_{46}O_6$, 550.3294).

trans Adduct ***4f:*** 7.7 mg (14%) using condition A; 3.3 mg (6%) using condition B; 4.4 mg (8%) using condition C; 3.9 mg (7%) using condition D; IR (film) ν_{max} 3356, 2925, 2855, 2349, 1724, 1658, 1602, 1550, 1493, 1446, 1376, 1335, 1286, 1200, 1121, 1034, 816, 647; ^{1}H NMR δ 0.87 (3H, t, *J* = 7.0 Hz, H-24), 1.25 (16H, bs, H-16-H-23), 1.30 (3H, s, H-6′), 1.51 (3H, s, H-6″), 1.59 (2H, m, H-15), 1.70 (3H, s, H-3″), 1.77 (3H, s, Me-3‴), 2.12 (1H, m, H-6a), 2.45 (2H, t, *J* = 7.8 Hz, H-14), 3.62 (1H, d, *J* = 11.4 Hz, H-12b), 4.00 (2H, t, *J* = 11.0 Hz, H-7), 4.30 (1H, td, *J* = 7.0, 9.4 Hz, H-12b), 4.40 (2H, d, *J* = 6.8 Hz, H-1′), 5.45 (1H, t, *J* = 8.8 Hz, H-2′), 6.30 (1H, bs, H-12), 6.72 (1H, dd, *J* = 2.5, 8.8 Hz, H-10), 6.84 (1H, d, *J* = 8.6 Hz, H-9), 7.39 (1H, s, OH); ^{13}C NMR δ 14.1 (CH_3, H-24), 18.1 (CH_3, H-3″), 19.3 (CH_3, H-6′), 22.6 (CH_2, C-14), 22.7 (CH_2 C-23), 25.8 (CH_3, CH_3-3‴), 27.0 (CH_3, H-6″), 28.1 (CH_2, C-15), 29.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.7 (CH_2 × 2 C-20-C-21), 31.0 (CH, C-12b), 31.9 (CH_2, C-22), 46.2 (CH, C-6a), 65.3 (CH_2, C-1′), 67.9 (CH_2, C-7), 80.6 (C, C-6), 112.5 (CH, C-9), 113.4 (CH, C-10), 113.7 (C, C-13), 117.3 (CH, C-12), 118.7 (C, C-3), 119.8 (CH, C-2′), 128.6 (C, C-12a), 138.2 (C, C-3′), 148.0 (C, C-8), 151.5 (C, C-2), 153.1 (C, C-11), 156.8 (C, C-5), 181.7 (C, C-1), 182.3 (C, C-4); EIMS *m/z* 550 [M^+] (11), 482 (100), 397 (5), 342 (3), 284 (4), 189 (7); HREIMS *m/z* 550.3306 (calcd for $C_{34}H_{46}O_6$ [M^+] 550.3294).

*(±)-2-Hydroxy-6,6-dimethyl-3-undecyl-6a,7-dihydrobenzo[g]-chromeno[3,4-c]chromene-1,4(6H,14bH)-dione (**3g**).* Purification of the crude mixture by preparative TLC (*n*-hexane−EtOAc, 7:3) yielded compound **3g** as an amorphous, brown solid [37.4 mg (71%), using condition A; 47.4 mg (92%), using condition B]; IR (film) ν_{max} 3525, 3399, 2926, 2854, 1649, 1588, 1516, 1464, 1389, 1336, 1282, 1081, 951, 811, 743, 665 cm^{-1}; ^{1}H NMR δ 0.87 (3H, t, *J* = 7.0 Hz, 3H, H-26), 1.25 (16H, bs, H-18-H-25), 1.42 (2H, m, H-17), 1.60 (3H, s, H-2′), 1.62 (3H, s, H-2″), 2.25 (1H, m, H-10c), 2.36 (2H, t, *J* = 7.6 Hz, H-16), 4.08 (1H, t, *J* = 11.5 Hz, H-2a′), 4.45 (1H, m, H-3b), 4.70 (1H, d, *J* = 4.0 Hz, H-3a), 7.03 (1H, d, *J* = 9.0 Hz, H-5), 7.18 (1H, bs, OH), 7.41 (1H, t, *J* = 7.2 Hz, H-8), 7.56 (1H, t, *J* = 8.0 Hz, H-9), 7.70 (1H, d, *J* = 8.9 Hz, H-6), 7.81 (1H, d, *J* = 8.0 Hz, H-10), 8.01 (1H, d, *J* = 8.5 Hz, H-7); ^{13}C NMR δ 14.1 (CH_3, C-26), 22.5 (CH_2, C-16), 22.7 (CH_2, C-25), 25.6 (CH_3, C-2′), 25.9 (CH_3, C-2″), 27.1 (CH, C-10c), 28.0 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2, C-24), 37.9 (CH, C-2a), 62.9 (CH_2, C-3), 79.5 (C, C-2), 112.3 (C, C-13), 113.8 (C, C-5a), 118.0 (CH, C-10), 118.5 (CH, C-5), 123.5 (CH, C-6), 125.8 (CH, C-8), 128.5 (CH, C-9), 128.9 (C, C-10b), 129.6 (CH, C-7), 134.4 (C, C-10a), 151.5 (C, C-2), 151.6 (C, C-4a), 154.2 (C, C-15), 181.2 (C, C-11), 181.3 (C, C-14); EIMS *m/z* 516 [M^+] (100), 431 (11), 376 (30), 317 (14), 223 (12), 180 (30); HREIMS *m/z* 516.2877 (calcd for $C_{33}H_{40}O_5$, 516. 2876).

Biological Assays. Strains of methicillin-sensitive *Staphylococcus aureus* ATCC25923 (MSSA) and methicillin-resistant *Staphylococcus aureus* NRS402 were used *and* stored at −80 °C in brain heart infusion broth with 20% glycerol added. *S. aureus* ATCC25923 (MSSA) was obtained from the ATCC collection, and *S. aureus* NRS402 (also known as HIP12864 or NR-46074) was obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) collection. Strains were revived by plating on brain heart infusion broth and incubated at 37 °C overnight. 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 on performing 1:2 serial dilutions of each compound ranging from 1 to 128 μM. Aside from the test compounds, the antibiotics oxacillin, vancomycin, and mupirocin were included as controls. The inoculum size was 1 × 10^5 CFU/mL for all bacteria.[8,42,43]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b01038.

^{1}H and ^{13}C NMR spectra of new embelin derivatives (PDF)

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Notes

The authors declare no competing financial interest.

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