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Synthesis, Structure–Activity Relationship Studies, and Antibacterial Evaluation of 4-Chromanones and Chalcones, as Well as Olympicin A and Derivatives

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Supporting Information



ABSTRACT: On the basis of recently reported abyssinone II and olympicin A, a series of chemically modified flavonoid phytochemicals were synthesized and evaluated against *Mycobacterium tuberculosis* and a panel of Gram-positive and -negative bacterial pathogens. Some of the synthesized compounds exhibited good antibacterial activities against Gram-positive pathogens including methicillin resistant *Staphylococcus aureus* with minimum inhibitory concentration as low as 0.39 μ g/mL. SAR analysis revealed that the 2-hydrophobic substituent and the 4-hydrogen bond donor/acceptor of the 4-chromanone scaffold together with the hydroxy groups at 5- and 7-positions enhanced antibacterial activities; the 2',4'-dihydroxylated A ring and the lipophilic substituted B ring of chalcone derivatives were pharmacophoric elements for antibacterial activities. Mode of action studies performed on selected compounds revealed that they dissipated the bacterial membrane potential, resulting in the inhibition of macromolecular biosynthesis; further studies showed that selected compounds inhibited DNA topoisomerase IV, suggesting complex mechanisms of actions for compounds in this series.

INTRODUCTION

Because of the emergence and spread of multidrug resistant microorganisms and pathogenic bacterial infections, novel chemotype antibacterial agents demonstrating distinct modes of action from existing antibiotics are urgently needed. Natural products are known as rich sources of bioactive molecules and chemical diversity and have thus provided invaluable chemical scaffolds as well as served as an inspiration toward antibacterial drug discovery and development.^{1–4} In this context, synthesis and evaluation of natural-product-inspired compound libraries represent an attractive approach for discovering novel antibacterial agents.⁵

Flavonoids are a large family of polyphenolic phytochemicals, which widely exist in the plant kingdom.⁶ As such, flavonoids

have been the focus of numerous basic biomedical research as well as clinical investigation.^{7,8} As examples, high dietary intake of flavonoids may offer potential to reduce the risk of various cancers according to a number of epidemiological studies.^{9–13} In addition, flavonoids have been reported to display a broad spectrum of pharmacological activities, such as antimicrobial,^{14–16} anti-inflammatory,^{17,18} cancer preventive^{19,20} and anticancer,^{21,22} and antioxidant activities.^{23,24} It is also noteworthy that some widely investigated flavonoids, such as flavone acetic acid (FAA),²⁵ flavopiridol,^{26–28} silibinin (silybin),^{29,30} and quercetin³¹ and its derivatives³² (Figure 1), have progressed

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Figure 1. Skeleton structures of chalcones, 4-chromanones, and representative structures of naturally occurring flavonoids including abyssinone II and olympicin A.

to various stages of clinical trials.³³ In this regard, plant-derived phytochemicals including chemically modified flavonoids and derivatives continue to attract great interest in the development of novel antibiotics.³⁴

Furthermore, chalcones (1,3-diaryl-2-propen-1-ones), one subclass of structural analogues of flavonoids, have been reported to exhibit diverse biological activities,^{35–38} in which the enone functional group and the 2'-hydroxy group constitute important structural motifs for antibiotic activity. From a chemistry point of view, chalcones and 4-chromanones are structurally related, and 2'-hydroxychalcones serve as important synthetic precursors for the synthesis of 4-chromanones following an intramolecular conjugate addition of the phenol on the α , β -unsaturated system.³⁹ Notably, the 4-chromanone derivatives containing an aromatic substituent at the 2-position, so-called flavanones, have been identified as an important class of bioactive heterocycles.^{40–42}

As a result of our longstanding interest in developing naturalproduct-inspired new antibacterial agents, we recently reported the identification of abyssinone II as a promising antibacterial lead by screening a focused flavonoid and resveratrol library.⁴ In addition, olympicin A, a member of the natural acylphloroglucinol chemical class, was recently isolated from the plant Hypericum olympicum and reported to exhibit potent antibacterial activity against a panel of multidrug-resistant (MDR) strains of clinically relevant Staphylococcus aureus, with minimum inhibitory concentration (MIC) values ranging from 0.5 to 1 μ g/mL.^{44,45} Very recently, we have shown that synthetic olympicin A also exhibited good activity against Clostridium difficile (MIC = $1-2 \ \mu g/mL$).⁴⁶ Inspired by the antibacterial activity of the natural products abyssinone II and olympicin A, in this work we employed the 4-chromanone and chalcone structural scaffolds as chemical starting points to design and synthesize chemically modified flavonoid analogues. Subsequently, several series of structurally related flavonoids were synthesized and evaluated in vitro against a broad set of bacterial pathogens and a detailed structure–activity relationship (SAR) has been obtained. Furthermore, the antibacterial basis of promising lead compounds and their ability to inhibit bacterial topoisomerases such as DNA gyrase or topo IV have also been examined.

RESULTS AND DISCUSSION

Synthesis of Olympicin A and Derivatives. The isolation and chemical synthesis of olympicin A (2a) was originally reported by Shiu et al., and its synthesis involved a four-step reaction sequence. However, the overall yield was only 3.3% from 1a.44 To improve the reaction efficiency and develop a modular synthesis toward olympicin A and derivatives, we evaluated diverse protecting schemes including the tertbutyldimethylsilyl (TBDMS), the base-stable methoxymethyl (MOM), and the *p*-toluenesulfonyl (Ts) groups and developed an improved synthesis of 2a by using the Ts protecting strategy, improving the overall yield to 40% from 1a (Scheme 1). In particular, we found that the reported low yield may be largely due to the instability of the TBDMS protecting group under basic reaction conditions (K₂CO₃, 80 °C) when introducing the geranyl group. To address this, the base-stable MOM group was next applied instead of TBDMS. Following the O-geranylation reaction, it was found that 2a decomposed during the deprotection of the MOM groups because of the instability of O-geranyl group under the acidic condition, and the deprotected 1a was recovered from the reaction. Subsequently the Ts group was used to protect the hydroxy group,⁴⁷ and only the tris(tosylate) 1b was obtained as the major product because of the lack of selectivity of the Ts group under the reaction condition. Nevertheless, we found that the tosylate group at the 2-position of 1b was very labile, and the geranyl group could be selectively introduced with sodium hydride as the base. Final removal of the Ts group was performed with excess sodium methoxide in methanol under reflux⁴⁸ to afford the chiral olympicin A (2a). The spectroscopic Scheme 1. Improved Synthesis of Olympicin A $(2a)^{a}$



^aReagents and conditions: (i) SOCl₂, 80 °C, 2 h; (ii) AlCl₃, CS₂, PhNO₂, 0.5 h; (iii) TsCl, K₂CO₃, acetone, 1 h, 73%; (iv) geranyl bromide, NaH, DMF, 1 h; (v) CH₃ONa, MeOH, reflux, 8 h, 55% (two-step overall yield); 52% for 2b (two-step overall yield).

data of our synthetic olympicin A were in good agreement with those of the natural product.⁴⁴ For comparison, the racemic olympicin A (2b) was also synthesized under the same conditions to study the potential effect of stereochemistry on antibacterial property.

Next, to further expand the chemical diversity and investigate the influence of 2-substitution on antibacterial activity in the scaffold, an array of racemic olympicin derivatives (2c-e) and enantiomeric form **2f** were designed and synthesized (Scheme 2). Compounds 1a' and 1a were first protected by reacting with MOMCl in the presence of diisopropylethylamine (DIPEA) to provide 1c and 1c' in moderate yields. The O-alkylation reaction was subsequently carried out with appropriate alkyl bromide using sodium hydride as a base, after which the MOM groups were removed with hydrochloric acid to give 2c-f in moderate to high yields (66–93%).

Synthesis of 2-Substituted 4-Chromanone and Derivatives. 4-Chromanone derivatives bearing an aryl substituent in the 2-position are normally synthesized by reacting an acetophenone with an arylaldehyde under strong basic or acidic conditions.^{49,50} However, because of the side reaction of self-condensation of the aliphatic aldehyde,⁵¹ these conditions are not ideal for the synthesis of 2-alkyl substituted 4-chromanones. Several different synthetic methods to prepare such 4-chromanone derivatives have been reported, 51-53 either involving microwave irradiation or requiring very long reaction times. Here, a sealed pressure tube was introduced as a reaction vessel for the preparation of 2-alkyl substituted 4-chromanones. On the basis of the scaffold, several modification strategies (Figure 2) were applied to develop chemical diversity and further evaluate the SAR.



Figure 2. Modifications based on the 4-chromanone scaffold.

As shown in Scheme 3, a series of 2-alkyl-4-chromanone derivatives 3a-f were synthesized by reacting 2,4-dihydroxyacetophenone (1d) with an appropriate aliphatic aldehyde in the presence of pyrrolidine in ethanol. This reaction was performed in a sealed pressure tube at 150 °C for 1 h, obtaining yields over 30%. Furthermore, the reaction time was shortened significantly. In contrast, a lower yield (12%) was afforded despite a longer reaction time (72 h) for the synthesis of 3b under the conditions (ethanol, 60 °C, pyrrolidine). To optimize the reaction conditions, a panel of different amine bases was screened. We noted that under the same conditions, no product was detected using DIPEA and only traces of product were formed when using morpholine as a base monitored by HPLC, respectively. To investigate the potential effects of the 4-carbonyl group as a hydrogen bond acceptor, the 4-chromanones 3a-d were further reduced by NaBH4 in methanol to provide 4-chromanol derivatives 4a-d in moderate to good yields (64 - 81%).⁵⁴

Accordingly, when the bicyclic (-)-myrtenal (1f) was used as the substrate under these reaction conditions (Scheme 3, step i), no product was obtained. Subsequently, a lower temperature (75 °C) was applied (Scheme 4) to optimize the reaction conditions. The corresponding chalcone intermediate 3g was obtained in low yield (9.5%). To improve the reaction yield, 1d was first regioselectively protected by reacting with MOMCl in the presence of DIPEA to provide the MOMprotected acetophenone 1e in 86% yield. Again, when the reaction was performed at 150 °C, only traces of 3h were detected by HPLC. In contrast, a much higher yield (48%) was obtained when the reaction temperature was reduced to 75 °C. The MOM-protected 3h can be further cyclized in ethanol in the presence of sodium acetate to yield 4-chromanone 3i as a mixture of two diastereomers.⁵⁵ Final deprotection of the O-MOM group was performed in methanol using concentrated HCl at room temperature to give 3j in 90% yield.

Next, on the basis of the promising antibacterial activity of 3f, a focused set of 4-chromanone analogues (Scheme 5) were subsequently designed and synthesized to investigate the antibacterial effect of the phenol free hydroxy group at different positions. Compound 3f was resynthesized from the MOM-protected 1e by using diethylamine (DEA) as a base in

> **2f**, $R = CH_2CH=CH_2$ (S-isomer from 1c' 90%)



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chiral starting material, 65%

Scheme 3. Synthesis of 2-Alkylated 4-Chromanones and Derivatives^a



^aReagents and conditions: (i) pyrrolidine, EtOH, 150 °C, pressure tube, 1 h; (ii) NaBH₄, MeOH, rt, 24 h.

Scheme 4. Reactions of Acetophenone and (-)-Myrtenal^a



"Reagents and conditions: (i) DIPEA, MOMCl, 0 °C, 1 h; (ii) pyrrolidine, EtOH, 75 °C, pressure tube, 1 h; (iii) NaOAc, EtOH, reflux, 24 h; (iv) concentrated HCl, MeOH, rt, overnight.





^{*a*}Reagents and conditions: (i) DEA, EtOH, 150 °C, pressure tube, 1 h; (ii) concentrated HCl, MeOH, rt, overnight.

an overall 58% yield following *O*-MOM deprotection. Accordingly, **3k**,**l** with the 5- or 6-hydroxy group were also synthesized using appropriate MOM-protected **1g**,**h** in moderate yields (58–75%).

To further expand the SAR and evaluate the influence of the 5-hydroxy group, an array of 5,7-dihydroxy-4-chromanones were synthesized. Accordingly, 2,4,6-trihydroxyacetophenone (1j) was used to prepare 4-chromanone derivatives 5 (Scheme 6). Unfortunately, no product was obtained under the reaction conditions of pyrrolidine in ethanol at 150 °C in a pressure tube. Therefore, the bis-MOM-protected acetophenone 1k was next prepared in 79% yield.⁵⁶ The corresponding MOM-protected 4-chromanones 5 were obtained by reacting 1k with an appropriate aldehyde in the presence of DEA in ethanol in a pressure tube at 150 °C in moderate yields (55–70%).





^aReagents and conditions: (i) DIPEA, MOMCl, 0 °C, 1 h, 79%; (ii) DEA, EtOH, 150 °C, pressure tube, 1 h; (iii) concentrated HCl, MeOH, rt, 24 h; (iv) pyridine, EtOH, rt, 26–72 h. ^bWithout isolating the MOM-protected intermediate.



^aReagents and conditions: (i) DEA, EtOH, 150 °C, pressure tube, 4 h; (ii) HCl, MeOH, rt, 16 h; (iii) HCl, EtOH, microwave irradiation, 150 °C, 0.5 h.





^aReagents and conditions: (i) pyrrolidine, EtOH, 150 °C, pressure tube, 2–16 h; (ii) HCl, rt, overnight.

Subsequent removal of the MOM groups using concentrated HCl in methanol at room temperature afforded the corresponding 4-chromanones in 75–94% yields. Next, to evaluate the role of the carbonyl group at the 4-position and further explore the chemical diversity of the 4-chromanone scaffold, a panel of 4-oximinochromanes **6a**–**f** were produced by reacting the corresponding **5** with an appropriate hydroxylamine in ethanol in the presence of pyridine in high yields (80–90%). Notably, the reaction time in this series differed from 26 to 72 h, and the electron donating group such as MeO in **6b** greatly facilitated the reaction and reduced the reaction time (26 h).

As illustrated in Scheme 7, to synthesize the bicyclic compound **5n** bearing the myrtenal motif, the reaction time needed to be extended to 4 h under the reaction conditions, and the chalcone **5m** was obtained in overall two-step 34% yield after *O*-MOM deprotection. Subsequent intramolecular conjugate addition of the phenol on the α,β -unsaturated system was performed under microwave irradiation in the presence of catalytic amounts of concentrated HCl to give **5n** in 72% yield as a mixture of two diastereomers.⁴⁹ We also noted that the reaction was completed in 0.5 h, which was a great improvement in comparison to the condition of refluxing with sodium acetate in ethanol in Scheme 4. Next, 2-spiro-4-chromanones $(7\mathbf{a}-\mathbf{c})$ were synthesized by reacting $1\mathbf{e}$ with an appropriate cycloketone in a pressure tube at 150 °C for 2–16 h in the presence of pyrrolidine (Scheme 8). The following *O*-MOM deprotection was performed in one pot with excess concentrated HCl. The spiro compounds $7\mathbf{a}-\mathbf{c}$ were obtained in moderate to high yields, with $7\mathbf{c}$ giving the lowest yield (53%) probably due to the steric hindrance of the sevenmembered ring.

Synthesis of 2-Substituted Aromatic Chalcone and Flavanone Derivatives. To systematically investigate the SAR of the chalcone and 4-chromanone scaffolds on antibacterial activities, diverse aromatic aldehydes were introduced to synthesize a series of 2-aryl chalcone and flavanone derivatives (Scheme 9). Accordingly, the Claisen-Schmidt aldol condensations were performed at room temperature with the addition of 60% KOH aqueous solution to the mixture of appropriate MOM-protected acetophenone and aldehyde in methanol.⁵⁵ Subsequent removal of the MOM-protecting group with concentrated HCl afforded the chalcone derivatives 8a-h. Chalcone 8f was synthesized as a comparison under the same conditions to verify the potential effect of 2'-hydroxy group on antibacterial activity. Additionally, in comparison to 8a with the ortho-substituted allyloxy group, regioisomers 8g and 8h (with the allyloxy group at the meta- and para-position, respectively) were next synthesized to investigate the influence of the substitution at the R₂ position in the scaffold. Subsequent intramolecular conjugate additions of the phenol on the $\alpha_{i}\beta$ -unsaturated system of 8a-e were carried out under microwave irradiation in the presence of catalytic amounts of concentrated HCl to yield corresponding flavanones 8i-m in 50-75% yields.

All the synthesized compounds were characterized by ¹H and ¹³C NMR spectroscopy and high-resolution mass spectrometry (HRMS), and purity was analyzed by reverse phase HPLC. The structures of **3g** and **8a** were confirmed by X-ray crystallography,





"Reagents and conditions: (i) 60% KOH aq, MeOH, rt, 60 h; (ii) concentrated HCl, rt, overnight; (iii) microwave, HCl, EtOH, 150 °C, 1.5 h. ^bWithout 2'-OH function for **8f**.

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Table 1. Antibacterial Activities (MIC, μ g/mL) of Olympicin A and Derivatives^{*a*}

Compound	Chemical structure	<i>M. tuberculosis</i> (H37Rv) ^b	E. faecalis (ATCC 33186)	S. aureus (ATCC 29213) (MSSA)	S. aureus (NRS 70) (MRSA)	<i>E. coli</i> (K12)	E. coli (∆tolC)
2a (olympicin A)		200	1.56	1.56	1.56	>200	>200
2b		200	1.56	1.56	3.13	>200	>200
2c	HO CH C	100	12.5	12.5	6.25	>200	3.13
2d		100	1.56	1.56	1.56	>200	3.13
2e	но	100	0.78	1.56	1.56	>200	>200
2f	HO	100	12.5	12.5	12.5	>200	3.13
ampicillin		nd ^c	0.78	1.56	12.5	3.13	1.56
kanamycin		nd	25	1.56	50	3.13	< 0.2
vancomycin		nd	0.78	0.39	<0.2	100	100
chloramphenicol		nd	0.78	3.13	1.56	3.13	0.39

^aNo test compounds were active (>200 μ g/mL) against other Gram-negative bacteria including *K. pneumonia* (ATCC 33495) and *P. aeruginosa* (PAO1). ^bPositive controls isoniazid and rifampin inhibited *M. tuberculosis* at 0.03 and 0.05 μ g/mL, respectively. ^cnd = not determined.

and their ORTEP drawings are shown in Figure S1 (Supporting Information).

All the synthesized compounds were evaluated against *Mycobacterium tuberculosis* (H37Rv) and a wide set of clinically relevant Gram-positive and -negative bacterial pathogens including *Enterococcus faecalis* (ATCC 33186), *Staphylococcus aureus* (ATCC 29213 and NRS 70), *Escherichia coli* (K12 and $\Delta tolC$), *Klebsiella pneumoniae* (ATCC 33495), and *Pseudomonas aeruginosa* (PAO1). Their antitubercular and antibacterial activities are summarized in Tables 1–3.

Olympicin A Series. In the olympicin A series, olympicin A (2a) and analogues (2b-f) showed weak antitubercular activity with MICs of 100–200 μ g/mL (Table 1). The observed weak antituberculosis activity may be attributed to the general polar nature of this chemical series and decreased membrane penetration. In contrast, the olympicin derivatives with geranyloxy (2a and 2b), n-hexyloxy (2d), and n-octyloxy (2e) groups showed good to potent anti-Gram-positive activity against *E. faecalis* and *S. aureus* strains (MIC = $0.78-3.13 \ \mu g/mL$). However, the less lipophilic olympicin derivatives 2c and 2f with a shorter allyloxy chain exhibited about 8- to 16-fold decrease of antibacterial activity (MIC = $6.25-12.5 \ \mu g/mL$). In terms of stereochemistry effect, the racemic olympicin A $(\mathbf{2b})$ and allyloxy derivative (2c) showed largely the same antituberculosis and anti-Gram-positive activity compared to their corresponding chiral S-isomers 2a and 2f, respectively. Notably, the anti S. aureus activity (1.56 μ g/mL) of our synthetic sample (2a) of olympicin A is consistent with the previously reported anti S. aureus activity $(0.5-1 \ \mu g/mL)$ of natural olympicin A.44 In addition, none of these olympicin analogues were active against Gram-negative microorganisms except for the reengineered E. coli strain ($\Delta tolC$) with deficient efflux activity.

4-Chromanone and Chalcone Series. Antituberculosis Activity. In the 4-chromanone and chalcone chemical series (Tables 2 and 3), the majority of compounds exhibited weak antituberculosis activity. The 2-propyl-4-chromanol 4a showed the most potent activity in the entire series with a MIC of 12.5 μ g/mL. The reduced 4-chromanol variants 4a and 4c (4-OH, 12.5 and 25 μ g/mL, respectively) showed more potent antituberculosis activity than their corresponding 4-chromanones 3a (200 μ g/mL) and 3c (50 μ g/mL). In addition, the 4-oximinochromane 6a (=NOH, 100 μ g/mL) displayed greater potency than 6b (=NOMe, 200 μ g/mL) and 6c (=NOBn, >200 μ g/mL). Taken together, these results indicate that the small, polar, and hydrophilic groups (e.g., –OH and =NOH) are more favorable at the 4-position of the flavonoid scaffold for antituberculosis activity, suggesting these polar groups may function as hydrogen bond donors when interacting with potential biological cellular target.

Different lengths of the 2-alkyl side chains in the scaffold displayed an important relationship with antituberculosis activity as well. Compared to 3a (three-carbon, 200 μ g/mL), **3b** (six-carbon, 50 μ g/mL), and **3c** (seven-carbon, 50 μ g/mL), compound 3d with a nine-carbon linear chain was inactive, suggesting the nine-carbon alkyl group is too long and bulky. To investigate the impact of the substituent at the 5-position in the scaffold, a hydrogen bond donor and/or acceptor (hydroxy group) was introduced. These data showed that the compounds bearing 5,7-dihydroxy functionalities (5b, 5d, and 5f) exhibited similar MIC values to the sole 7-hydroxychromanones 3a-c. However, **5h** (5,7-di-OH, nine-carbon chain, 100 μ g/mL) was more potent than the corresponding 3d (7-OH, nine-carbon chain, >200 μ g/mL). In addition, the position of the sole hydroxy group in the scaffold also played a notable role in their antituberculosis activity by comparing 3f (7-OH, 100 μ g/mL) to 3k (6-OH, 200 μ g/mL) and 3l (5-OH, >200 μ g/mL), with **3f** bearing the 7-hydroxy function being the most potent.

In general, 2-aryl substituted compounds together with 2-spiro derivatives possessing a cyclic ring system were less

Table 2. Antibacterial Activities (MIC, μ g/mL) of 4-Chromanone Derivatives^{*a*}



Compound	Chemical structure	<i>M. tuberculosis</i> (H37Rv)	E. faecalis (ATCC 33186)	S. aureus (ATCC 29213) (MSSA)	S. aureus (NRS 70) (MRSA)	<i>E. coli</i> (K12)	E. coli (∆tolC)
3a	HOLOC	200	>200	200	>200	>200	50
3b	но	50	>200	>200	>200	>200	3.13
3c	HOLO	50	>200	>200	>200	>200	1.56
3d	HO	>200	200	>200	>200	>200	>200
3e	HOLOCO	100	>200	50	50	>200	12.5
3f	HO	100	12.5	6.25	3.13	>200	1.56
3k	HO	200	6.25	6.25	12.5	>200	3.13
31		>200	>200	>200	>200	>200	>200
3i	момо	100	>200	>200	>200	>200	6.25
3j	HOTO	>200	>200	200	200	>200	6.25
4a	HO	12.5	100	50	50	>200	50
4b	HO	50	50	>200	200	>200	3.13
4c	HO	25	25	12.5	12.5	>200	6.25
4d	HO	>200	50	50	25	>200	12.5
5a	момо	100	>200	>200	>200	>200	100
5b	HO	200	200	100	100	>200	50
5c	момо	50	>200	>200	>200	>200	6.25
5d	HO O O	50	100	6.25	6.25	>200	3.13
5e	момо	50	>200	>200	>200	>200	12.5
5f	HO HO	50	200	12.5	3.13	>200	1.56
5g	момо	100	>200	>200	>200	>200	>200

Table 2. continued

Compound	Chemical structure	<i>M. tuberculosis</i> (H37Rv)	E. faecalis (ATCC 33186)	S. aureus (ATCC 29213) (MSSA)	S. aureus (NRS 70) (MRSA)	<i>E. coli</i> (K12)	E. coli (∆tolC)
5h	HOLOGO	100	100	100	3.13	>200	12.5
5i	момо	>200	>200	>200	>200	>200	>200
5j	HO	200	6.25	3.13	3.13	>200	1.56
5k	HOHO	200	50	100	50	>200	6.25
51	HO	100	25	25	25	>200	6.25
5n	HOLOG	200	12.5	12.5	6.25	>200	3.13
6a	он мон	100	6.25	12.5	12.5	>200	>200
6b	HO O O	200	>200	100	25	>200	>200
6c	OH NOBn	>200	50	>200	>200	>200	>200
6d	HO O	100	100	100	50	>200	25
6e	HO O O	100	12.5	6.25	6.25	>200	3.13
6f	HO HO	100	6.25	3.13	3.13	>200	3.13
7a	HOTOC	200	>200	>200	>200	>200	100
7b	HOLOC	100	>200	200	200	>200	50
7c	нобоб	100	200	50	50	>200	12.5
8i	носто	100	50	25	25	>200	12.5
8j	носто	>200	50	1.56	1.56	>200	1.56
8k	носто	>200	1.56	12.5	3.13	>200	>200
81	HOTON	>200	200	>200	>200	>200	12.5
8m	HOTOG	>200	50	25	12.5	>200	6.25

"No test compounds were active (>200 μ g/mL) against other Gram-negative bacteria including *K. pneumonia* (ATCC 33495) and *P. aeruginosa* (PAO1). The MIC values of control antibiotics used in this study are shown in Table 1.

active against *M. tuberculosis* than 2-alkylated derivatives in the entire series. Moreover, the MOM protected derivatives exhibited

comparable or more potent antitubercular activity than their corresponding free phenol parent molecules. The SAR analysis

Table 3. Antibacterial Activities (MIC, μ g/mL) of Chalcones and Derivatives^{*a*}

$\mathbf{R}_{1} \stackrel{\parallel}{\underset{4}{\parallel}} \stackrel{\scriptstyle}{\underset{2}{\parallel}} \stackrel{\scriptstyle}{\underset{\mathbf{R}_{2}}{\parallel}} \mathbf{R}_{2}$							
Compound	Chemical structure	<i>M. tuberculosis</i> (H37Rv)	E. faecalis (ATCC 33186)	S. aureus (ATCC 29213) (MSSA)	S. aureus (NRS 70) (MRSA)	<i>E. coli</i> (K12)	E. coli (∆tolC)
3g	но он	100	3.13	1.56	1.56	>200	1.56
3h	момо	100	>200	>200	>200	>200	25
5m	но он о	>200	25	>200	200	>200	6.25
8a	HOUTOH	200	12.5	6.25	0.39	>200	3.13
8b	нотон	>200	1.56	3.13	0.78	>200	>200
8c	носон	>200	1.56	25	6.25	>200	>200
8d	HOCH	100	25	3.13	3.13	>200	12.5
8e	HO OH O	>200	50	>200	25	>200	12.5
8f	но	200	100	12.5	6.25	>200	6.25
8g	ностон	50	>200	6.25	12.5	>200	3.13
8h	но он	50	100	25	>200	>200	>200

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above demonstrates that the 2-alkyl hydrophobic substituents as well as the small and polar functionalities at the 4-position (e.g., hydrogen bond donor groups OH and ==NOH) play important roles in antituberculosis activities in the 4-chromanone scaffold.

General Antibacterial Spectra. In addition to antituberculosis evaluation, antimicrobial assessment against representative clinical pathogens revealed that the majority of compounds from these series exhibited notable anti-Gram-positive bacteria activities including against *E. faecalis* and *S. aureus* (MSSA and MRSA) and poor activity against Gram-negative bacteria including *E. coli, K. pneumoniae*, and *P. aeruginosa*.

Compared with the 4-chromanone flavonoid series, the chalcone series generally exhibited more potent anti-Gram-positive activity than their corresponding cyclized derivatives. The 2',4'-di-OH chalcone compound **8a** having an appended 2-allyloxy group exhibited the best activity (MIC of $0.39-6.25 \ \mu g/mL$) against MSSA and MRSA, and the *o*-hydroxy group appeared to have a beneficial effect on anti-Gram-positive bacterial activity

by comparing 8a (2',4'-di-OH, 2-allyloxy, 0.39–12.5 μ g/mL) to 8f (4'-OH, 2-allyloxy, 6.25–100 μ g/mL) (Table 3). Notably, this observation regarding the importance of the o-hydroxy group in the chalcone scaffold is also in agreement with that found in the 4-chromanone flavonoid series, whereas the corresponding hydroxy group at the 5-position of the 4-chromanone scaffold enhanced the antibacterial activity. However, the 2',4',6'-tri-OH chalcone compounds possessing an additional 6'-hydroxy group 5m (25 to >200 μ g/mL) and 8e (25 to >200 μ g/mL) showed significantly decreased activities against Gram-positive microorganisms compared to the 2',4'-di-OH chalcones 3g (1.56–3.13 μ g/mL) and 8a $(0.39-12.5 \ \mu g/mL)$, respectively. The notable decreased activity for trihydroxy compounds is likely due to increased hydrophilicity and polarity properties and thus decreased bacterial membrane penetration. Taken together, these data suggest that two free phenol hydroxy groups on the 4-chromanone and chalcone scaffolds are optimal for Gram-positive antibacterial activity.

Furthermore, in the chalcone series (Table 3), the lipophilic *O*-alkyl substituent at the 2-position of aromatic chalcones also

^aNo test compounds were active (>200 μ g/mL) against other Gram-negative bacteria including K. pneumonia (ATCC 33495) and P. aeruginosa (PAO1). The MIC values of control antibiotics used in this study are shown in Table 1.

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had a great impact on antibacterial activities, since the chalcones bearing diverse side chains such as the allyloxy (**8a**), *n*-hexyloxy (**8b**), and *n*-octyloxy (**8c**) exhibited notable differences in their activities; compound **8b** bearing the *n*-hexyloxy group showed optimal antibacterial activity against *E. faecalis*, MSSA, and MRSA (1.56, 3.13, and 0.78 μ g/mL, respectively). Compound **8a** bearing the 2-allyloxy function demonstrated 2-fold more potent activity against MRSA and an 8-fold reduction in activity against *E. faecalis* than **8b**.

In the flavonoid series, the length of 2-alkyl substitutions in the 4-chromanone scaffold plays an important role in determining antibacterial activities. The 5,7-dihydroxy-4-chromanones with long aliphatic alkyl chains 5d, 5f, 5h, and 5j (six to ninecarbon chain, $3.13-6.25 \ \mu g/mL$) showed better activity against MRSA than the shorter chain derivative 5b (three-carbon chain, 100 µg/mL), and notably, the 2-(2,6-dimethyl-5heptenyl) substituted 5i with a branched and unsaturated alkyl chain displayed the best potency among these five 2-alkylated derivatives against three Gram-positive bacteria tested (E. faecalis, MSSA, and MRSA; 6.25, 3.13, and 3.13 µg/mL, respectively). These results suggest that the branched unsaturated substitution in 5j may play an important role in enhancing interactions and binding affinity with cellular biological target because of its favorable lipophilicity and high conformational flexibility, as previously noted for the prenylated derivatives.^{57,58} We also observed that the introduction of an additional 5-OH group to the 7-hydroxy-4-chromanones 5b (100 μ g/mL), 5d (6.25 μ g/mL), 5f (3.13 μ g/mL), 5h (3.13 μ g/mL), and 5n (6.25 μ g/mL) significantly improved antibacterial activity against MRSA, compared to their corresponding 7-OH-4chromanones 3a-d (>200 μ g/mL) and 3j (200 μ g/mL) except that 5j maintained anti-MRSA activity (3.13 μ g/mL) compared to 3f. These data demonstrate the importance of the 5-OH group in the 7-OH-4-chromanone scaffold for antibacterial activity.

In addition, the reduced variants 4-chromanols 4a-d (in particular 2-*n*-heptyl-7-OH-4-chromanol 4c, 12.5–25 μ g/mL; and 2-*n*-nonyl-7-OH-4-chromanol 4d, 25–50 μ g/mL) also displayed improved antibacterial activities compared to their

corresponding 4-chromanones 3a-d, which were not active against Gram-positive bacteria tested. In contrast, the 4-oximinochromanes (6a and 6d-f: =NOH at the 4-position) showed more potent activity against E. faecalis and MSSA and comparable activity against MRSA than their corresponding 4-chromanones (5h, 5b, 5d, and 5f), suggesting the free oxime =NOH functionality is preferred compared to the carbonyl group. Furthermore, by comparison of **6a** with the free 4-oxime functionality (=NOH, 12.5 μ g/mL), compounds **6b** (=NOMe, 25–100 μ g/mL) and 6c (=NOBn, >200 μ g/mL) showed decreased antibacterial activity against S. aureus. This observation is also consistent with the trend toward the antituberculosis activity found in this series, indicating that the small, polar, and hydrogen bond donor functionalities (e.g., -OH and =NOH groups) at the 4-position may be more favorable for antibacterial properties, together with the results based upon 4-chromanols (4a-d).

To further evaluate the importance of the 5-, 6-, and 7-OH functionality, a set of 4-chromanone derivatives 3f (7-OH), 3k (6-OH), and 3l (5-OH) were subsequently synthesized. Biological evaluation revealed that the 6- or 7-hydroxy group also proved to be a determining factor for antibacterial activities, since 3f (7-OH, 3.13-12.5 μ g/mL) and 3k (6-OH, 6.25–12.5 μ g/mL) demonstrated almost equally potent antibacterial activities against Gram-positive bacteria tested. However, their regioisomer 31 with the 5-OH substitution was not active. The lack of antibacterial activity of 31 may be due to the presence of intramolecular hydrogen bonding between the carbonyl group at the 4-position and the hydroxy group at the 5-position. It should be noted that all the MOM protected derivatives (3h, 3i, 5a, 5c, 5e, 5g, and 5i) completely lost anti-Gram-positive bacterial activities against E. faecalis and S. aureus, demonstrating the importance of the free phenol hydroxy functionality and its weakly acidic nature in the scaffold. This observation is consistent with our previous report.⁴³ Additionally, among the chemical series bearing a cyclic/bicyclic ring system at the 2-position (5k, 2-cyclopentyl; 5l, 2-cyclohexyl; 5n, 2-myrtenyl; and the 2-spiro compounds 7a-c), compound 5n bearing the myrtenyl motif showed



Figure 3. General SAR of 4-chromanone and chalcone derivatives.

Table 4	 Cytotoxicity 	[,] and Solubility	Profiles of	f Selected	Lead	Compounds"
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compd	molecular weight (g/mol)	lipophilicity (cLogP) ^b	solubility (µg/mL)	cytotoxicity IC_{50} $(\mu g/mL)^c$	MIC against MRSA $(\mu g/mL)$	selectivity index (SI) ^d
3f	288.2	3.77	>200	28.8 ± 1.8	3.13	9.2
3g	284.1	3.19	>200	6.9 ± 0.3	1.56	4.4
5f	278.2	3.20	150.0	33.0 ± 2.1	3.13	10.5
5h	306.2	4.03	>200	8.6 ± 2.7	3.13	2.8
5j	304.2	3.38	>200	27.0 ± 2.8	3.13	8.6
6a	321.2	4.42	37.5 ± 17.7	10.1 ± 1.9	12.5	0.8
6f	293.2	3.59	>200	9.6 ± 0.1	3.13	3.1
8a	296.1	3.37	>200	21.2 ± 5.4	0.39	54.4
8b	340.2	4.76	>200	7.2 ± 0.4	0.78	9.2
8d	323.2	3.82	100.0	16.7 ± 0.1	3.13	5.3
8j	340.2	4.36	>200	24.5 ± 2.1	1.56	15.7
8k	368.2	5.19	>200	17.0 ± 0.0	3.13	5.4
thioridazine	nd ^e	nd	nd	5.3 ± 1.3	nd	nd
verapamil	nd	nd	nd	57.3 ± 3.2	nd	nd
$DMSO^{f}$	nd	nd	nd	177.5 ± 10.6	nd	nd

^{*a*}DMEM/FBS: Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). ^{*b*}The cLogP values of compounds were calculated using ChemBioOffice Ultra, version 12.0, from CambridgeSoft Corporation. ^{*c*}Cytotoxicity IC₅₀, concentration that reduces viability of Vero kidney cells by 50%. ^{*d*}Selectivity index = (cytotoxic IC₅₀)/(MIC against MRSA). ^{*e*}nd = not determined. ^{*f*}Carrier effect.

relatively good antibacterial activity ($6.25-12.5 \ \mu g/mL$) against Gram-positive bacteria tested. In contrast, the 7-OH and 2-myrtenyl substituted flavonoid **3j** was largely inactive. Interestingly, its corresponding ring-opened chalcone derivative **3g** demonstrated very good anti-Gram-positive activity ($1.56-3.13 \ \mu g/mL$).

A detailed SAR of the 4-chromanone and chalcone series is summarized in Figure 3.

Solubility and Cytotoxicity Determination. Cytotoxicity against mammalian (Vero epithelial) cells and solubility in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) were evaluated for a selected panel of 4-chromanone and chalcone lead compounds, and the results are shown in Table 4. Overall, all the tested compounds had good solubility ($\geq 100 \, \mu g/mL$) in the DMEM/ FBS medium used in the cytotoxicity assay except for the 4-oxime derivative 6a (37.5 \pm 17.7 μ g/mL). The selectivity indices (SI) for the compounds were calculated as the ratio of the IC₅₀ value of cytotoxicity against Vero monkey kidney cell line and the MIC value against tested MRSA. Notably, 4-chromanone derivatives 5f(SI = 10.5) and 8j(SI = 15.7) and the chalcone derivative 8a (SI = 54.4) possessed a more favorable selectivity index (SI > 10). On the basis of these promising antibacterial lead structures, advanced medicinal chemistry will be applied to produce compounds with improved potency and decreased cytotoxicity.

Time Kill Experiments and Mutation Selection. The bactericidal activities of compounds 3g, 5j, 8a, and 8d were examined against *S. aureus* Newman (Figure 4). The most effective compound was the chalcone derivative 3g, which killed more than 6 log of cells in just 2 h at 4× its MIC, but at its MIC (1.56 μ g/mL against *S. aureus* Newman) up to 24 h was required to achieve a 3 log reduction in cells. Compound 3g thus exhibited concentration-dependent killing. Compound 8a (MIC = 6.25 μ g/mL) was also rapidly bactericidal at 4× its MIC and achieved a 6 log reduction in 6 h. Interestingly, compound 8d (6.25 μ g/mL) was entirely bacteriostatic and failed to kill more than 3 log of culture at 4× its MIC, even up to 24 h of exposure. At concentrations between 1× and 4× their MICs, spontaneous mutants of *S. aureus* Newman could not be selected with 8a, 3g, and 5j, but mutants arose to 8d at a frequency of 10⁻¹⁰. The



Figure 4. Time kill studies against *S. aureus* Newman exposed to $4 \times$ MIC of compounds. Each point represents the average of two biological replicates.

controls mupirocin and vancomycin were either bacteriostatic or slowly bactericidal over a 24 h period, and mutants could only be selected with mupirocin at frequencies of $10^{-7}-10^{-9}$.

Effects on Macromolecular Synthesis and Membrane Potential. Several key biosynthetic processes were simultaneously inhibited in S. aureus Newman exposed to the compounds 2a, 5j, 8a, 3g, and 8d (Figure 5). These effects are consistent with the bacterial membrane being the primary target site of action, resulting in multiple nonspecific cellular effects. Surprisingly, the bacteriostatic compound 8d displayed the same time-dependent effects on macromolecular synthesis as the bactericidal compounds. To determine if the compounds dissipated the membrane potential of S. aureus, the fluorescent probe DiOC2(3) was used. Compounds 2a, 5j, 3g, and 8d all dissipated the membrane potential of S. aureus Newman in a concentration-dependent manner. Maximum dissipation occurred at 4× their MICs and was similar to the control CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) (Figure 6); as expected, vancomycin (at 4× MIC) failed to affect the membrane potential in S. aureus.



Figure 5. Effects of **2a**, **5j**, **3g**, **8a**, and **8d** and indicated positive controls at 4× their MICs on macromolecular synthesis in *S. aureus* Newman. The standard error of the mean (SEM) is shown for three biological replicates. ERY = erythromycin (MIC = $0.78 \ \mu g/mL$); RMP = rifampicin ($0.12 \ \mu g/mL$); CIP = ciprofloxacin ($0.25 \ \mu g/mL$).



Figure 6. Dissipation of the staphylococcal membrane potential by 2a, 5j, 3g, and 8d. A representative of three biological replicates is shown. Vancomycin (MIC = $0.8 \ \mu g/mL$) and CCCP (MIC = $6.25 \ \mu g/mL$) were used as negative and positive controls.

Inhibition of Bacterial Topoisomerase IV and DNA Gyrase. Clinically, DNA gyrase and topo IV are validated and attractive antibacterial targets for fluoroquinolone and novobiocin antibiotics. However, because of the emergence of target-based bacterial resistance with fluoroquinolone class and safety concerns of novobiocin, novel DNA gyrase and topoisomerase inhibitors are urgently needed for the treatment of pathogenic and resistant bacterial infections.⁵⁹ Thus, development of new chemotype bacterial topo inhibitors has attracted great interest in the scientific community, and recent examples include bisbenzimidazoles,⁶⁰ anziaic acid, and its analogues^{61,62} as bacterial topo IA inhibitors, and pyridylureas⁶³ and pyrrolamides⁵⁹ as topo II inhibitors. Previously, flavonoids have been identified as bacterial topoisomerase inhibitors,⁶⁴⁻⁶⁶ and we next tested to see if these promising antibacterial compounds from olympicin A, 4-chromanone, and chalcone series inhibited E. coli topo I, topo IV, and DNA gyrase. The results are shown in Table 5 and Figure S2 (Supporting Information). The E. coli topo I assay (relaxation of negatively supercoiled

Table 5. *E. coli* Topoisomerases and DNA Gyrase Inhibition of Selected Analogues

compd	topo Ι IC ₅₀ (μM)	DNA gyrase IC ₅₀ (µM)	topo IV IC ₅₀ (μM)
2a	>500	500-1000	30-60
2e	>500	>1000	30-60
3g	>500	250-500	60-120
5f	>500	>1000	120-250
8a	>500	500-1000	250-500
8b	>500	>1000	60-120
8d	>500	>1000	500-1000
8j	>500	>1000	>1000
8k	>500	>1000	>1000
known inhibite	ors 14–19 ^{<i>a</i>}	$0.2^{b}_{,b} 100^{c}_{,c}$	$2.1,^{b} 806^{c}$

 $^{a}\mathrm{IC}_{50}$ of known inhibitor for assay: anziaic acid^{61} for Topo I. $^{b}\mathrm{IC}_{50}$ of known inhibitor for assay: ciprofloxacin 67 for DNA gyrase and topo IV. $^{c}\mathrm{IC}_{50}$ of known inhibitor for assay: nalidixic acid^{68} for DNA gyrase and topo IV.

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plasmid DNA) was performed at both 0.5 and 5 mM magnesium chloride concentrations, and no significant inhibition was observed against topo I at 0.5 mM compound concentration. The assay against E. coli gyrase (supercoiling of relaxed plasmid DNA) and E. coli topo IV (decatenation of catenated kinetoplast DNA) was subsequently performed. Again for DNA gyrase, inhibition was not observed or was weak with 8a, 3g, and 2a (MIC values of 0.39-6.25 μ g/mL against S. aureus) exhibiting IC₅₀ values of >0.25 mM. However, the E. coli topo IV showed more significant sensitivity toward 2a and 2e (MIC values of 0.78–1.56 μ g/mL against *E. faecalis* and S. aureus) with IC₅₀ values of 30-60 μ M (Table 5 and Figure S2, Supporting Information). These results showed that the olympicin A (2a) and its analogue 2e may serve as a promising and novel scaffold for topo IV inhibitors. Interestingly, olympicin A was also recently reported as a moderate ATP-dependent Mycobacterium tuberculosis MurE ligase inhibitor with an IC_{50} value of 75 $\mu\mathrm{M.}^{45}$ Studies to determine if the olympicin A analogues 2c-f inhibit the MurE pathway in M. tuberculosis remain to be performed. Finally, the antibacterial mechanism of the 4-chromanone flavanone compounds 8k and 8j (MIC values of 1.56-12.5 µg/mL against S. aureus) may not be likely to involve topoisomerase inhibition, as they were inactive in these topo enzyme inhibition assays (Table 5). Collectively, no correlations between whole-cell-based activity and topoisomerase inhibition were observed for selected compounds, suggesting that the membrane is likely the primary biological target responsible for antibacterial activity and topo IV is a secondary or alternative target. Further mechanistic studies are warranted to define the exact mechanism of antibacterial action of these chemically modified flavonoid and polyphenol compounds.

CONCLUSIONS

In summary, 58 olympicin A, 4-chromanone, and chalcone derivatives containing various functionalities were synthesized and evaluated against Mycobacterium tuberculosis and a panel of clinically relevant Gram-positive and -negative bacterial pathogens. Bacterial evaluation showed that this class of compounds generally exhibited good activities against Grampositive bacteria tested. Systematic SAR study revealed that the phenol hydroxy groups at the 5- and 7-position of the 4-chromanone scaffold were essential for antibacterial activities. Additionally, the hydrogen bond donor/acceptor functionality at the 4-position together with the lipophilic 2-alkyl moiety in the scaffold also played important roles in antibacterial activities. The flavanone derivatives bearing the lipophilic substituent on the 2-phenyl ring showed good antibacterial properties as well. In the chalcone chemical series, both hydroxy groups at 2'- and 4'-position, as well as the bicyclic myrtenyl motif and the 2-alkyloxy substitution on the aromatic ring, favored anti-Gram-positive bacterial activities. The selected compounds generally possessed favorable solubility, and 5f, 8a, and 8j had more desirable selectivity indices ranging from 10.5 to 54.4. In addition, compounds 2a, 5j, 3g, and 8d were found to disrupt bacterial membrane potential and have secondary inhibitory effect on macromolecular biosynthesis of DNA, RNA, and protein. Further evaluation of selected compounds against bacterial topoisomerases and DNA gyrase revealed that 2a and 2e inhibited topoisomerase IV (IC₅₀ = 30-60 μ M). Taken together, the antibacterial agents identified from this study provide chemically modified flavonoid phytochemicals as promising antibacterial leads for further medicinal chemistry

optimization in an effort to identify advanced experimental candidates with antimicrobial therapeutic potential.

EXPERIMENTAL SECTION

Chemical Synthesis. General Procedures. Solvents and reagents were supplied from Aldrich, Acros, or Fisher and used without further purification. NMR spectra were recorded on a Bruker AM-400 (400 MHz) spectrometer. High-resolution mass spectra were obtained on an Agilent 6530 Accurate Mass Q-TOF LC/MS instrument. Reactions in pressure tube were carried out using a Q-Tube reactor from Q Labtech. Microwave reactions were conducted using a Biotage Initiator reactor. All reactions were monitored by either TLC or HPLC. Compounds were purified by flash chromatography on silica gel on a Biotage Isolera One system. The purity of compounds was determined by analytical HPLC (Shimadzu LC-20A series) using a Gemini, 3 μ m, C18, 110 Å column (50 mm × 4.6 mm, Phenomenex) and flow rate of 1 mL/min. Gradient conditions were the following: solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile), 0-2.0 min 100% A, 2.0-7.0 min 0-100% B (linear gradient), 7.0-8.0 min 100% B, UV detection at 254 and 220 nm. All the tested compounds were obtained with ≥95% purity by HPLC. NMR standards used are as follows. ¹H NMR: CDCl₃, 7.26 ppm; CD₃OD, 3.31 ppm; DMSO-d₆, 2.50 ppm. ¹³C NMR: CDCl₃, 77.16 ppm; CD₃OD, 49.00 ppm; DMSO- d_6 , 39.52 ppm. MOM-protected acetophenones were synthesized as described in the literature.^{56,69,70}

(S)-2-(2-Methylbutanoyl)benzene-1,3,5-triyl Tris(4-methylbenzenesulfonate) (1b). To a solution of 1a (150 mg, 0.715 mmol) in acetone (11 mL) were added p-toluenesulfonic chloride (408 mg, 2.14 mmol) and K₂CO₃ (845 mg, 6.15 mmol) successively. The resulting mixture was stirred under reflux for 1 h, and then acetone was removed under reduced pressure. The residue was diluted with water and DCM, and the organic layer was washed with 1 M HCl aq, water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (first 85/15, then 75/25) to give the product (350 mg, 0.52 mmol, 73%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.69 (d, J = 8.3 Hz, 2H), 7.62 (d, J = 8.4 Hz, 4H), 7.36 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.3 Hz, 4H), 7.0 (s, 2H), 2.64-2.59 (m, 1H), 2.44 (s, 9H), 1.56-1.50 (m, 1H), 1.24-1.14 (m, 1H), 0.90 (d, J = 7.0 Hz, 3H), 0.76 (t, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 201.2, 149.8, 146.8, 146.5, 146.4, 131.7, 131.3, 130.2, 130.0, 128.5, 127.1, 114.3, 48.6, 24.5, 21.80, 21.79, 14.2, 11.2. HRMS calculated for $C_{32}H_{32}O_{10}S_3\ (M+H)^+\ 673.1230,$ found $(M+H)^+$ 673.1229. HPLC purity: 98.6% (254 nm), *t*_R = 8.17 min; 99.5% (220 nm), $t_{\rm R} = 8.17$ min.

1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)-2-methylbutan-1-one (1c). To a suspension of 1a' (1.12 g, 5.33 mmol) in DCM (11 mL) at 0 $^\circ C$ was added DIPEA (2.78 mL, 15.9 mmol) carefully. After stirring for 10 min, MOMCl (1.21 mL, 15.9 mmol) was added to the solution dropwise. The resulting mixture was stirred for 1 h. Afterward, the solution was poured into sat. NH₄Cl aq, and then water and DCM were added into the mixture. The organic layer was washed with water, brine and dried over Na2SO4. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/ EtOAc (90/10) to give the product (1.02 g, 3.42 mmol, 64%) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 13.74 (s, 1H), 6.27 (s, 2H), 5.24 (s, 2H), 5.16 (br s, 2H), 3.65-3.60 (m, 1H), 3.51 (s, 3H), 3.46 (s, 3H), 1.87-1.80 (m, 1H), 1.43-1.36 (m, 1H), 1.16 (d, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.3, 167.2, 163.2, 160.1, 106.7, 97.6, 94.9, 94.4, 94.1, 56.9, 56.6, 46.6, 27.1, 16.7, 12.2. HRMS calculated for C15H22O6 (M + H)⁺ 299.1489, found (M + H)⁺ 299.1483. HPLC purity: 99.8% (254 nm), $t_{\rm R}$ = 7.42 min; 99.8% (220 nm), $t_{\rm R}$ = 7.42 min.

(S)-1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)-2-methylbutan-1-one (1c'). Synthesis and purification were performed as described in compound 1c. Product: 1.03 g, 3.46 mmol, 65%. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 13.74 (s, 1H), 6.27 (s, 2H), 5.24 (s, 2H), 5.16 (br s, 2H), 3.65–3.60 (m, 1H), 3.52 (s, 3H), 3.46 (s, 3H), 1.87–1.80 (m, 1H), 1.43–1.36 (m, 1H), 1.16 (d, J = 6.8 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.3, 167.2, 163.2, 160.1, 106.7, 97.6, 94.9, 94.4, 94.1, 56.8, 56.6, 46.6, 27.1, 16.7, 12.1. HRMS calculated for C₁₅H₂₂O₆ (M + H)⁺ 299.1489, found (M + H)⁺ 299.1475. HPLC purity: 99.9% (254 nm), *t*_R = 7.38 min; 100% (220 nm), *t*_R = 7.38 min.

General Procedure for the Synthesis of 2a,b. To a solution of 1b (410 mg, 0.61 mmol) in DMF (4 mL) at 0 °C was added NaH (49 mg, 1.21 mmol) carefully. After stirring for 30 min, geranyl bromide (198 mg, 0.91 mmol) in DMF (1 mL) was added dropwise. The resulting mixture was stirred at room temperature for 1 h. Afterward, the reaction was quenched with water and extracted with EtOAc twice. The organic layer was washed with water, brine and dried over Na2SO4. The solvent was removed under reduced pressure, and the residue was dissolved in MeOH (10 mL), followed by the careful addition of sodium methoxide (659 mg, 12.2 mmol). The resulting mixture was stirred under reflux for 8 h, and then MeOH was removed. The residue was diluted with 1 M HCl aq and extracted with EtOAc twice. The organic layer was washed with water, brine and dried over Na2SO4. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to give the product.

(*S*,*E*)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (Olympicin A, **2a**). Purified with hexane/EtOAc (90/10) to give the product (116 mg, 0.34 mmol, 55%, two-step overall yield) as pale yellow oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 5.98 (d, *J* = 2.2 Hz, 1H), 5.91 (d, *J* = 2.2 Hz, 1H), 5.54 (br, 1H), 5.52–5.49 (m, 1H), 5.11–5.09 (m, 1H), 4.56 (d, *J* = 6.6 Hz, 2H), 3.67–3.63 (m, 1H), 2.14–2.08 (m, 4H), 1.81–1.78 (m, 1H), 1.74 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.40–1.33 (m, 1H), 1.12 (d, *J* = 6.7 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.8, 167.3, 163.1, 162.8, 142.6, 132.1, 123.7, 118.3, 105.8, 96.6, 92.1, 65.8, 46.1, 39.6, 27.0, 26.4, 25.8, 17.8, 16.73, 16.67, 12.0. HRMS calculated for C₂₁H₃₀O₄ (M + H)⁺ 347.2217, found (M + H)⁺ 347.2210. HPLC purity: 99.7% (254 nm), *t*_R = 8.18 min; 99.6% (220 nm), *t*_R = 8.18 min.

(E)-1-(2-((3,7-Dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (**2b**). Purified with hexane/EtOAc (90/10) to give the product (110 mg, 0.32 mmol, 52% over two steps) as pale yellow oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 5.98 (d, *J* = 2.2 Hz, 1H), 5.91 (d, *J* = 2.2 Hz, 1H), 5.54 (br, 1H), 5.52–5.49 (m, 1H), 5.11–5.09 (m, 1H), 4.56 (d, *J* = 6.6 Hz, 2H), 3.67–3.63 (m, 1H), 2.14–2.08 (m, 4H), 1.81–1.78 (m, 1H), 1.74 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.40–1.33 (m, 1H), 1.12 (d, *J* = 6.7 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.8, 167.3, 163.1, 162.8, 142.6, 132.1, 123.7, 118.3, 105.8, 96.6, 92.1, 65.8, 46.1, 39.6, 27.0, 26.4, 25.8, 17.8, 16.74, 16.68, 12.0. HRMS calculated for C₂₁H₃₀O₄ (M + H)⁺ 347.2217, found (M + H)⁺ 347.2214. HPLC purity: 99.7% (254 nm), $t_{\rm R}$ = 8.17 min; 99.6% (220 nm), $t_{\rm R}$ = 8.17 min.

General Procedure for the Synthesis of 2c–f. To a solution of 1c (200 mg, 0.67 mmol) in DMF (4 mL) at 0 °C was added NaH (54 mg, 1.34 mmol) carefully. After stirring for 30 min, appropriate alkyl bromide (1 mmol) in DMF (1 mL) was added dropwise. The resulting mixture was stirred at room temperature until the complete consumption of 1c (1–7 h). Afterward, the reaction was quenched with water and extracted with EtOAc twice. The organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue was dissolved in MeOH (10 mL), followed by the addition of concentrated HCl (510 μ L, 6 mmol) carefully. The resulting mixture was stirred at room temperature overnight, and the solvent was removed under reduced pressure. The crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to give the product.

1-(2-(*Allyloxy*)-4,6-*dihydroxyphenyl*)-2-*methylbutan*-1-*one* (2*c*). Purified with hexane/EtOAc (85/15) to give the product (157 mg, 0.63 mmol, 93% over two steps) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.11–6.02 (m, 1H), 6.00 (d, J = 2.1 Hz, 1H), 5.91 (d, J = 2.1 Hz, 1H), 5.86 (br, 1H), 5.42 (d, J = 17.2 Hz, 1H), 5.35 (d, J = 10.4 Hz, 1H), 4.57 (d, J = 5.6 Hz, 2H), 3.69–3.64 (m, 1H), 1.84–1.79 (m, 1H), 1.42–1.35 (m, 1H), 1.13 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.9, 167.2, 163.1, 162.3, 132.1, 119.2, 105.7, 96.9, 92.3, 70.0, 46.1, 27.0, 16.7, 11.9. HRMS calculated for $C_{14}H_{18}O_4$ (M – H)⁻ 249.1132, found (M – H)⁻ 249.1102. HPLC purity: 99.7% (254 nm), t_R = 7.16 min; 99.9% (220 nm), t_R = 7.16 min.

1-(2-(Hexyloxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (**2d**). Purified with reverse phase C18 silica gel chromatography with H₂O/MeCN (45/55) to give the product (130 mg, 0.44 mmol, 66% over two steps) as pale solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.01 (s, 1H), 5.92 (s, 1H), 3.96 (t, *J* = 6.5 Hz, 2H), 3.74–3.69 (m, 1H), 1.85–1.75 (m, 3H), 1.46–1.33 (m, 7H), 1.14 (d, *J* = 6.8 Hz, 3H), 0.92–0.87 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.8, 167.3, 163.2, 163.0, 105.6, 96.6, 91.9, 69.2, 46.0, 31.6, 29.1, 26.8, 26.1, 22.7, 17.0, 14.1, 11.9. HRMS calculated for C₁₇H₂₆O₄ (M – H)⁻ 293.1758, found (M – H)⁻ 293.1713. HPLC purity: 99.2% (254 nm), *t*_R = 7.93 min; 99.9% (220 nm), *t*_R = 7.92 min.

1-(2,4-Dihydroxy-6-(octyloxy)phenyl)-2-methylbutan-1-one (**2e**). Purified with reverse phase C18 silica gel chromatography with H₂O/MeCN (40/60) to give the product (151 mg, 0.47 mmol, 70% over two steps) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.33 (br, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.91 (d, *J* = 1.5 Hz, 1H), 3.97 (t, *J* = 6.5 Hz, 2H), 3.74–3.69 (m, 1H), 1.85–1.77 (m, 3H), 1.46–1.28 (m, 11H), 1.14 (d, *J* = 6.8 Hz, 3H), 0.90–0.87 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.7, 167.3, 163.2, 163.0, 105.6, 96.6, 91.9, 69.2, 46.1, 31.9, 29.4, 29.3, 29.1, 26.8, 26.4, 22.8, 17.0, 14.2, 11.9. HRMS calculated for C₁₉H₃₀O₄ (M + H)⁺ 323.2217, found (M + H)⁺ 323.2213. HPLC purity: 98.8% (254 nm), *t*_R = 8.31 min; 99.9% (220 nm), *t*_R = 8.31 min.

(S)-1-(2-(Allyloxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (**2f**). Purified with hexane/EtOAc (85/15) to give the product (152 mg, 0.61 mmol, 90% over two steps) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.11–6.02 (m, 1H), 6.00 (d, J = 2.1 Hz, 1H), 5.91 (d, J = 2.1 Hz, 1H), 5.86 (br, 1H), 5.42 (d, J = 17.2 Hz, 1H), 5.35 (d, J = 10.4 Hz, 1H), 4.57 (d, J = 5.6 Hz, 2H), 3.69–3.64 (m, 1H), 1.84–1.79 (m, 1H), 1.42–1.35 (m, 1H), 1.13 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 210.8, 167.3, 163.0, 162.3, 132.1, 119.3, 105.7, 96.9, 92.3, 70.1, 46.2, 27.0, 16.7, 11.9. HRMS calculated for C₁₄H₁₈O₄ (M – H)⁻ 249.1132, found (M – H)⁻ 249.1104. HPLC purity: 99.1% (254 nm), $t_{\rm R} = 7.17$ min; 99.6% (220 nm), $t_{\rm R} = 7.17$ min.

General Procedure for the Synthesis of 3a-e. To a solution of 1d (152 mg, 1 mmol) in ethanol (2.5 mL) were added pyrrolidine (220 mg, 3.1 mmol) and corresponding aldehyde (6 mmol) successively. The resulting mixture was stirred at 150 °C in a pressure tube for 1 h. Afterward, the solution was diluted with ethyl acetate and the organic layer was washed with 10% HCl, water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to give the product.

7-Hydroxy-2-propylchroman-4-one (*3a*). Purified with hexane/ EtOAc (87/13) to give the product (85 mg, 0.41 mmol, 41%) as light brown solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.13 (br, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H), 6.43 (s, 1H), 4.42 (br, 1H), 2.67–2.63 (m, 2H), 1.85–1.80 (m, 1H), 1.68–1.44 (m, 3H), 0.99–0.95 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.0, 164.32, 164.29, 129.5, 114.6, 110.8, 103.4, 78.0, 42.6, 37.1, 18.3, 14.0. HRMS calculated for C₁₂H₁₄O₃ (M – H)⁻ 205.0870, found (M – H)⁻ 205.0863. HPLC purity: 97.3% (254 nm), *t*_R = 6.31 min; 98.6% (220 nm), *t*_R = 6.31 min.

2-Hexyl-7-hydroxychroman-4-one (**3b**). Purified with hexane/ EtOAc (90/10) to give the product (92 mg, 0.37 mmol, 37%) as light brown solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.78 (d, J =8.7 Hz, 1H), 6.55 (dd, J = 8.6, 1.7 Hz, 1H), 6.44 (d, J = 1.7 Hz, 1H), 4.43–4.39 (m, 1H), 2.66–2.63 (m, 2H), 1.87–1.82 (m, 1H), 1.67– 1.64 (m, 1H), 1.53–1.30 (m, 8H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.1, 164.34, 164.30, 129.4, 114.6, 110.8, 103.4, 78.3, 42.6, 35.0, 31.8, 29.2, 25.0, 22.7, 14.2. HRMS calculated for C₁₅H₂₀O₃ (M – H)[–] 247.1340, found (M – H)[–] 247.1331. HPLC purity: 98.4% (254 nm), $t_{\rm R} =$ 7.06 min; 99.1% (220 nm), $t_{\rm R} =$ 7.06 min.

2-Heptyl-7-hydroxychroman-4-one (3c). Purified with hexane/ EtOAc (90/10) to give the product (91 mg, 0.35 mmol, 35%) as light brown solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.78 (d, J = 8.7 Hz, 1H), 6.55 (dd, J = 8.7, 2.3 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 4.43-4.39 (m, 1H), 2.69-2.60 (m, 2H), 1.88-1.80 (m, 1H), 1.70-1.63 (m, 1H), 1.53–1.25 (m, 10H), 0.89–0.86 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.0, 164.4, 164.3, 129.4, 114.6, 110.9, 103.4, 78.3, 42.6, 35.0, 31.9, 29.5, 29.3, 25.0, 22.8, 14.2. HRMS calculated for $C_{16}H_{22}O_3$ (M - H)⁻ 261.1496, found (M - H)⁻ 261.1479. HPLC purity: 95.0% (254 nm), *t*_R = 7.28 min; 96.1% (220 nm), *t*_R = 7.28 min. 7-Hydroxy-2-nonylchroman-4-one (3d). Purified with hexane/ EtOAc (90/10) to give the product (98 mg, 0.33 mmol, 33%) as light brown solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.23 (br, 1H), 7.79 (d, J = 8.7 Hz, 1H), 6.56 (dd, J = 8.7, 2.1 Hz, 1H), 6.43 (d, J = 2.1 Hz, 1H), 4.45-4.38 (m, 1H), 2.70-2.61 (m, 2H), 1.88-1.80 (m, 1H), 1.70-1.63 (m, 1H), 1.55-1.25 (m, 14H), 0.89-0.86 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.1, 164.4, 129.5, 114.6, 110.9, 103.4, 78.3, 42.5, 35.0, 32.0, 29.7, 29.6, 29.5, 29.4, 25.0, 22.8, 14.2. HRMS calculated for $C_{18}H_{26}O_3 (M - H)^-$ 289.1809, found $(M - H)^-$ 289.1787. HPLC purity: 99.1% (254 nm), $t_{\rm R} = 7.71$ min; 99.3% (220 nm),

 $t_{\rm R}$ = 7.71 min. 7-Hydroxy-2-phenethylchroman-4-one (**3e**). Purified with reverse phase C18 silica gel chromatography with H₂O/MeCN (45/55) to give the product (54 mg, 0.20 mmol, 20%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.79 (d, *J* = 8.6 Hz, 1H), 7.27–7.25 (m, 2H), 7.20–7.16 (m, 3H), 6.53 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.45 (d, *J* = 1.7 Hz, 1H), 4.43–4.37 (m, 1H), 2.91–2.76 (m, 2H), 2.71–2.59 (m, 2H), 2.22–2.14 (m, 1H), 2.01–1.94 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.7, 164.3, 164.2, 141.0, 129.5, 128.7, 128.6, 126.3, 114.6, 111.0, 103.4, 77.1, 42.6, 36.6, 31.2. HRMS calculated for C₁₇H₁₆O₃ (M – H)⁻ 267.1027, found (M – H)⁻ 267.1012. HPLC purity: 100% (254 nm), *t*_R = 6.69 min; 99.5% (220 nm), *t*_R = 6.69 min.

General Procedure for the Synthesis of 3f, 3k, and 3l. To a solution of corresponding MOM-protected acetophenone (196 mg, 1 mmol) in ethanol (2.5 mL) was added DEA (154 mg, 2.1 mmol) and (\pm)-citronellal (309 mg, 2 mmol) successively. The resulting mixture was stirred at 150 °C in a pressure tube for 1 h. The resulting solution was cooled to room temperature and diluted with EtOAc. The organic layer was washed with 10% HCl aq, water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the residue was dissolved in methanol (14 mL), followed by the careful addition of concentrated HCl (340 μ L, 4 mmol), and the resulting mixture was stirred at room temperature overnight. Afterward, the solution was concentrated and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/ EtOAc to give the product.

2-(2,6-Dimethylhept-5-en-1-yl)-7-hydroxychroman-4-one (**3f**). Purified with hexane/EtOAc (90/10) to obtain a mixture of two diastereomers (167 mg, 0.58 mmol, 58% over two steps) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.79 (d, *J* = 8.6 Hz, 2H), 6.55 (d, *J* = 8.7 Hz, 2H), 6.43 (s, 2H), 5.98 (br, 2H), 5.11–5.08 (m, 2H), 4.55–4.50 (m, 2H), 2.69–2.56 (m, 4H), 2.04–1.90 (m, 6H), 1.82–1.69 (m, 2H), 1.68 (s, 6H), 1.60 (s, 6H), 1.44–1.32 (m, 4H), 1.26–1.19 (m, 2H), 0.97–0.94 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.95, 192.86, 164.4, 164.32, 164.28, 131.7, 131.6, 129.4, 124.6, 124.5, 114.6, 110.9, 103.4, 76.2, 43.2, 42.9, 42.3, 42.2, 37.5, 36.9, 28.9, 28.5, 25.9, 25.5, 25.4, 20.0, 19.4, 17.8. HRMS calculated for C₁₈H₂₄O₃ (M – H)⁻ 287.1653, found (M – H)⁻ 287.1632. HPLC purity: 97.8% (254 nm), *t*_R = 7.32 min; 98.0% (220 nm), *t*_p = 7.32 min.

2-(2,6-Dimethylhept-5-en-1-yl)-6-hydroxychroman-4-one (**3k**). Purified with hexane/EtOAc (92/8) to obtain a mixture of two diastereomers (216 mg, 0.75 mmol, 75% over two steps) as yellow oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.40 (d, J = 3.1 Hz, 2H), 7.07 (dd, J = 8.9, 3.1 Hz, 2H), 6.91 (br, 2H), 6.87 (dd, J = 8.9, 2.6 Hz, 2H), 5.11–5.08 (m, 2H), 4.51–4.45 (m, 2H), 2.71–2.58 (m, 4H), 2.04–1.72 (m, 8H), 1.68 (s, 6H), 1.60 (s, 6H), 1.45–1.17 (m, 6H), 0.97–0.94 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 194.2, 194.1, 156.44, 156.38, 150.5, 131.61, 131.56, 125.3, 124.61, 124.56, 121.0,

119.4, 119.3, 111.1, 76.6, 76.1, 43.7, 43.4, 42.30, 42.26, 37.5, 36.9, 28.9, 28.6, 25.8, 25.5, 25.4, 20.0, 19.4, 17.8. HRMS calculated for $C_{18}H_{24}O_3$ (M – H)⁻ 287.1653, found (M – H)⁻ 287.1613. HPLC purity: 99.8% (254 nm), $t_{\rm R}$ = 7.40 min; 99.7% (220 nm), $t_{\rm R}$ = 7.38 min.

2-(2,6-Dimethylhept-5-en-1-yl)-5-hydroxychroman-4-one (31). Purified by reverse phase C18 silica gel chromatography with H₂O/MeCN (30/70) to obtain a mixture of two diastereomers (170 mg, 0.59 mmol, 59% over two steps) as pale yellow oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 11.69 (s, 2H), 7.33 (t, *J* = 8.3 Hz, 2H), 6.48 (dd, *J* = 8.3, 0.8 Hz, 2H), 6.41 (ddd, *J* = 8.3, 2.2, 0.8 Hz, 2H), 5.11–5.07 (m, 2H), 4.52–4.48 (m, 2H), 2.77–2.62 (m, 4H), 2.07–1.63 (m, 8H), 1.68 (s, 6H), 1.61 (s, 6H), 1.47–1.18 (m, 6H), 0.98–0.95 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 198.8, 198.7, 162.2, 161.84, 161.79, 138.24, 138.23, 131.7, 131.6, 124.54, 124.48, 109.2, 108.4, 107.48, 107.47, 76.2, 75.6, 43.0, 42.7, 42.21, 42.15, 37.5, 36.8, 28.9, 28.6, 25.9, 25.5, 25.4, 20.0, 19.4, 17.8. HRMS calculated for C₁₈H₂₄O₃ (M – H)⁻ 287.1653, found (M – H)⁻ 287.1604. HPLC purity: 99.9% (254 nm), *t*_R = 8.00 min; 100% (220 nm), *t*_R = 7.99 min.

(E)-3-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)-1-(2-hydroxy-4-(methoxymethoxy)phenyl)prop-2-en-1-one (3h). To a solution of 1e (196 mg, 1 mmol) in ethanol (2.5 mL) were added pyrrolidine (149 mg, 2.1 mmol) and (-)-myrtenal (300 mg, 2 mmol) successively. The resulting mixture was stirred at 75 °C in a pressure tube for 1 h. Afterward, the solution was diluted with ethyl acetate, and the organic layer was washed with 10% HCl, water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (97/3) to give compound 3h (160 mg, 0.48 mmol, 48%) as yellow oil. ¹H NMR (400 MHz, $CDCl_3$: δ (ppm) 13.37 (s, 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.52 (d, J =15.1 Hz, 1H), 6.88 (d, J = 15.1 Hz, 1H), 6.61 (d, J = 2.4 Hz, 1H), 6.54 (dd, J = 8.9, 2.4 Hz, 1H), 6.21 (m, 1H), 5.20 (s, 2H), 3.47 (s, 3H),2.68-2.67 (m, 1H), 2.55-2.41 (m, 3H), 2.19-2.18 (m, 1H), 1.39 (s, 3H), 1.18 (d, J = 9.0 Hz, 1H), 0.80 (s, 3H). ¹³C NMR (100 MHz, $CDCl_3$): δ (ppm) 192.9, 166.2, 163.5, 146.5, 145.6, 137.3, 131.3, 116.7, 115.2, 108.1, 104.0, 94.2, 56.5, 41.6, 40.8, 38.1, 33.1, 31.3, 26.3, 21.0. HRMS calculated for $C_{20}H_{24}O_4$ (M + H)⁺ 329.1747, found $(M + H)^+$ 329.1735. HPLC purity: 98.8% (254 nm), $t_R = 7.98$ min; 98.5% (220 nm), $t_{\rm R} = 7.98$ min.

(E)-1-(2,4-Dihydroxyphenyl)-3-(6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)prop-2-en-1-one (3g). To a solution of 3h (130 mg, 0.396 mmol) in methanol (6 mL) was added concentrated HCl (154 μ L, 1.78 mmol) dropwise. The resulting mixture was stirred at room temperature for 24 h, after which the solvent was removed. The crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (90/10) to obtain compound 3g (100 mg, 0.35 mmol, 89%) as yellow solid. ¹H NMR (400 MHz, $CDCl_3$: δ (ppm) 13.56 (s, 1H), 7.72 (d, J = 9.5 Hz, 1H), 7.51 (d, J =15.1 Hz, 1H), 6.87 (d, J = 15.2 Hz, 1H), 6.43–6.41 (m, 2H), 6.20 (m, 1H), 2.67 (t, J = 5.4 Hz, 1H), 2.54–2.41 (m, 3H), 2.18 (br, 1H), 1.38 (s, 3H), 1.18 (d, J = 9.0 Hz, 1H), 0.79 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.0, 166.2, 163.1, 146.5, 145.8, 137.5, 132.1, 116.6, 114.5, 108.0, 103.8, 41.5, 40.8, 38.1, 33.1, 31.3, 26.3, 21.0. HRMS calculated for $C_{18}H_{20}O_3 (M - H)^- 283.1340$, found $(M - H)^-$ 283.1313. HPLC purity: 99.3% (254 nm), $t_{\rm R}$ = 7.48 min; 99.2% (220 nm), $t_{\rm R} = 7.48$ min.

2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)-7-(methoxymethoxy)chroman-4-one (3i). To a solution of **3h** (60 mg, 0.183 mmol) in ethanol (3 mL) was added sodium acetate (300 mg, 3.6 mmol). The resulting mixture was refluxed for 24 h, after which the solvent was removed. The residue was dissolved in ethyl acetate, and the organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (95/5) to obtain a mixture of two diastereomers **3i** (28 mg, 0.085 mmol, 47%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.82–7.79 (m, 2H), 6.66–6.62 (m, 2H), 6.61 (s, 2H), 5.67–5.64 (m, 2H), 5.19 (s, 2H), 5.18 (s, 2H), 4.88–4.77 (m, 2H), 3.47 (s, 3H), 3.46 (s, 3H), 2.82–2.72 (m, 2H), 2.66–2.53 (m, 2H), 2.51–2.38 (m, 3H), 2.34–2.23 (m, SH), 2.12 (br, 2H), 1.32–1.30 (m, 6H), 1.28–1.14 (m, 2H), 0.87 (s, 3H), 0.74 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 191.6, 191.5, 163.59, 163.58, 163.50, 163.48, 145.4, 145.3, 128.72, 128.67, 122.1, 120.7, 115.9, 115.8, 111.0, 103.63, 103.58, 94.15, 94.14, 80.3, 79.8, 77.4, 56.5, 56.4, 42.6, 42.2, 41.0, 40.9, 40.8, 38.2, 38.0, 31.8, 31.7, 31.38, 31.36, 26.24. 26.20, 21.40, 21.35. HRMS calculated for C₂₀H₂₄O₄ (M + H)⁺ 329.1747, found (M + H)⁺ 329.1736. HPLC purity: 99.6% (49.0% + 50.6%) (254 nm), $t_{\rm R}$ = 7.66 and 7.68 min; 98.8% (220 nm), $t_{\rm R}$ = 7.66 min. The split HPLC product peaks further supported the product is a mixture of two diastereomers (see Supporting Information for the details of HPLC trace).

2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)-7-hydroxychroman-4-one (3j). To a solution of 3i (38 mg, 0.116 mmol) in methanol (2 mL) was added concentrated HCl (45 µL, 0.52 mmol) carefully. The resulting mixture was stirred at room temperature for 24 h, after which the solvent was removed. The crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (85/15) to obtain a mixture of two diastereomers 3i (30 mg, 0.106 mmol, 90%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.79–7.76 (m, 2H), 7.67 (br, 2H), 6.54–6.51 (m, 2H), 6.43-6.42 (m, 2H), 5.65-5.64 (m, 2H), 4.87-4.77 (m, 2H), 2.84-2.75 (m, 2H), 2.67-2.54 (m, 2H), 2.49-2.22 (m, 8H), 2.11 (br, 2H), 2.00 (br, 2H), 1.31-1.29 (m, 6H), 1.26-1.12 (m, 2H), 0.85 (s, 3H), 0.73 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.39, 192.36, 164.0, 163.7, 145.3, 145.2, 129.4, 129.3, 122.2, 120.9, 114.89, 114.86, 110.62, 110.61, 103.5, 80.1, 79.6, 77.4, 77.1, 42.6, 42.2, 40.9, 40.83, 40.79, 40.7, 38.2, 38.0, 31.8, 31.7, 31.39, 31.36, 26.24, 26.20, 21.38, 21.36. HRMS calculated for C₁₈H₂₀O₃ (M - H)⁻ 283.1340, found (M – H)⁻ 283.1310. HPLC purity: 99.4% (49.7% + 49.7%) (254 nm), $t_{\rm R}$ = 7.09 and 7.11 min; 99.6% (50.6% + 49.0%) (220 nm), $t_{\rm R}$ = 7.09 and 7.11 min. The split HPLC product peaks further supported the product is a mixture of two diastereomers (see Supporting Information for the details of HPLC trace).

General Procedure for the Synthesis of 4a–d. To a solution of appropriate 4-chromanone 3a-d (0.22 mmol) in methanol (3 mL) was added sodium borohydride (16.6 mg, 0.44 mmol) every 1.5 h for a total of 9 h. The resulting mixture was allowed to stir at room temperature overnight. The mixture was cooled to 0 °C, quenched with saturated NH₄Cl aq carefully, and extracted with EtOAc twice. The combined organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to give the product.

2-Propylchroman-4,7-diol (4a). Purified with hexane/EtOAc (85/15) to give the product (29 mg, 0.14 mmol, 64%) as light brown oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.19 (d, J = 8.4 Hz, 1H), 6.64 (br, 1H), 6.34 (dd, J = 8.4, 2.4 Hz, 1H), 6.24 (d, J = 2.4 Hz, 1H), 4.83–4.79 (m, 1H), 4.04–4.98 (m, 1H), 2.68 (br, 1H), 2.33 (br, 1H), 2.23–2.17 (m, 1H), 1.74–1.62 (m, 2H), 1.59–1.39 (m, 3H), 0.94 (t, J = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 156.6, 155.8, 128.3, 118.3, 108.6, 103.1, 75.2, 65.5, 38.0, 37.6, 18.4, 14.1. HRMS calculated for C₁₂H₁₆O₃ (M – H₂O + H)⁺ 191.1067, found (M – H₂O + H)⁺ 191.1058. HPLC purity: 95.3% (220 nm), $t_{\rm R} = 5.91$ min.

2-Hexylchroman-4,7-diol (4b). Purified with hexane/EtOAc (85/15) to give the product (42 mg, 0.17 mmol, 77%) as light brown oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.18 (d, *J* = 8.4 Hz, 1H), 6.72 (br, 1H), 6.34 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.25 (d, *J* = 2.4 Hz, 1H), 4.82–4.80 (m, 1H), 4.03–3.97 (m, 1H), 2.75–2.73 (m, 1H), 2.43 (br, 1H), 2.24–2.19 (m, 1H), 1.76–1.52 (m, 3H), 1.50–1.30 (m, 7H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 156.5, 155.8, 128.3, 118.3, 108.6, 103.1, 75.5, 65.6, 38.0, 35.5, 31.9, 29.3, 25.2, 22.7, 14.2. HRMS calculated for C₁₅H₂₂O₃ (M – H₂O + H)⁺ 233.1536, found (M – H₂O + H)⁺ 233.1534. HPLC purity: 97.8% (220 nm), $t_{\rm R}$ = 6.73 min.

2-Heptylchroman-4,7-diol (4c). Purified with hexane/EtOAc (85/15) to give the product (39 mg, 0.15 mmol, 68%) as light brown oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.25 (d, J = 8.4 Hz, 1H), 6.39 (dd, J = 8.4, 2.5 Hz, 1H), 6.26 (d, J = 2.4 Hz, 1H), 5.82 (br, 1H), 4.88–4.82 (m, 1H), 4.07–4.01 (m, 1H), 2.29–2.23 (m, 1H), 2.11–2.09 (m, 1H), 1.92 (br, 1H), 1.78–1.38 (m, 5H), 1.33–1.28 (m,

9H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 156.6, 155.9, 128.3, 118.5, 108.5, 103.1, 75.5, 65.6, 38.2, 35.6, 32.0, 29.7, 29.4, 25.2, 22.8, 14.2. HRMS calculated for C₁₆H₂₄O₃ (M – H)⁻ 263.1653, found (M – H)⁻ 263.1632. HPLC purity: 95.7% (220 nm), $t_{\rm R}$ = 6.97 min.

2-Nonylchroman-4,7-diol (4d). Purified with hexane/EtOAc (85/15) to give the product (52 mg, 0.18 mmol, 81%) as light brown oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.19 (d, J = 8.4 Hz, 1H), 6.54 (br, 1H), 6.34 (dd, J = 8.4, 2.4 Hz, 1H), 6.25 (d, J = 2.4 Hz, 1H), 4.83–4.81 (m, 1H), 4.02–3.99 (m, 1H), 2.61–2.59 (m, 1H), 2.32 (br, 1H), 2.25–2.20 (m, 1H), 1.75–1.53 (m, 2H), 1.49–1.27 (m, 14H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 156.5, 155.8, 128.3, 118.3, 108.6, 103.1, 75.5, 65.6, 38.1, 35.5, 32.0, 29.72, 29.70, 29.5, 25.2, 22.8, 14.2. HRMS calculated for C₁₈H₂₈O₃ (M – H)⁻ 291.1966, found (M – H)⁻ 291.1926. HPLC purity: 97.7% (220 nm), $t_{\rm p} = 7.41$ min.

General Procedure for the Synthesis of 5a–l. To a solution of 1h (256 mg, 1 mmol) in ethanol (2.5 mL) were added DEA (154 mg, 2.1 mmol) and corresponding aldehyde (2 mmol) successively. The resulting mixture was stirred at 150 °C in a pressure tube for 1 h. Afterward, the solution was diluted with ethyl acetate and the organic layer was washed with 10% HCl, water, brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to give the MOM-protected product.

To a solution of appropriate MOM-protected compound (0.22 mmol) in methanol (4 mL) was added concentrated HCl (172 μ L, 1.98 mmol) at room temperature, and the resulting mixture was stirred overnight. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to give the corresponding deprotected product.

5,7-Bis(methoxymethoxy)-2-propylchroman-4-one (5a). Purified with hexane/EtOAc (87/13) to give the product (195 mg, 0.63 mmol, 63%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.37 (s, 1H), 6.29 (s, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.39–4.35 (m, 1H), 3.51 (s, 3H), 3.46 (s, 3H), 2.64–2.53 (m, 2H), 1.83–1.79 (m, 1H), 1.66–1.43 (m, 3H), 0.96 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 190.1, 164.8, 163.2, 159.5, 107.5, 97.8, 97.2, 95.1, 94.1, 77.3, 56.64, 56.57, 44.3, 37.0, 18.3, 14.0. HRMS calculated for C₁₆H₂₂O₆ (M + H)⁺ 311.1489, found (M + H)⁺ 311.1481. HPLC purity: 98.0% (254 nm), $t_{\rm R}$ = 6.78 min; 96.3% (220 nm), $t_{\rm R}$ = 6.78 min.

5,7-Dihydroxy-2-propylchroman-4-one (5b). Purified with hexane/ EtOAc (85/15) to give the product (46 mg, 0.207 mmol, 94%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 12.03 (s, 1H), 7.24 (br, 1H), 5.97 (s, 1H), 5.93 (s, 1H), 4.41–4.36 (m, 1H), 2.73– 2.57 (m, 2H), 1.86–1.79 (m, 1H), 1.69–1.42 (m, 3H), 0.97 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 197.0, 165.3, 164.2, 163.7, 103.2, 96.5, 95.5, 41.6, 36.9, 18.2, 14.0. HRMS calculated for C₁₂H₁₄O₄ (M – H)⁻ 221.0819, found (M – H)⁻ 221.0802. HPLC purity: 96.7% (254 nm), $t_{\rm R}$ = 6.54 min; 100% (220 nm), $t_{\rm R}$ = 6.54 min.

2-Hexyl-5,7-bis(methoxymethoxy)chroman-4-one (5c). Purified with hexane/EtOAc (90/10) to give the product (220 mg, 0.63 mmol, 63%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.37 (s, 1H), 6.30 (s, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.39–4.32 (m, 1H), 3.51 (s, 3H), 3.47 (s, 3H), 2.65–2.53 (m, 2H), 1.84–1.77 (m, 1H), 1.67–1.60 (m, 1H), 1.55–1.30 (m, 8H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 190.0, 164.8, 163.2, 159.5, 107.5, 97.8, 97.3, 95.1, 94.1, 77.6, 56.6, 56.5, 44.3, 34.9, 31.8, 29.2, 24.9, 22.7, 14.2. HRMS calculated for C₁₉H₂₈O₆ (M + H)⁺ 353.1959, found (M + H)⁺ 353.1954. HPLC purity: 99.5% (254 nm), $t_{\rm R}$ = 7.49 min; 99.3% (220 nm), $t_{\rm R}$ = 7.49 min.

2-Hexyl-5,7-dihydroxychroman-4-one (5d). Purified with hexane/ EtOAc (85/15) to give the product (44 mg, 0.165 mmol, 75%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 12.02 (s, 1H), 6.99 (br, 1H), 5.96 (d, *J* = 1.9 Hz, 1H), 5.93 (d, *J* = 1.9 Hz, 1H), 4.40– 4.33 (m, 1H), 2.72–2.57 (m, 2H), 1.86–1.78 (m, 1H), 1.69–1.62 (m, 1H), 1.54–1.30 (m, 8H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 197.0, 165.3, 164.2, 163.7, 103.2, 96.5, 95.5, 77.7, 41.6, 34.9, 31.8, 29.1, 24.9, 22.7, 14.2. HRMS calculated for C₁₅H₂₀O₄

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 $(M - H)^{-}$ 263.1289, found $(M - H)^{-}$ 263.1264. HPLC purity: 97.9% (254 nm), $t_{\rm R} = 7.26$ min; 99.1% (220 nm), $t_{\rm R} = 7.26$ min.

2-Heptyl-5,7-bis(methoxymethoxy)chroman-4-one (**5e**). Purified with hexane/EtOAc (90/10) to give the product (220 mg, 0.60 mmol, 60%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.37 (s, 1H), 6.29 (s, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.38–4.31 (m, 1H), 3.51 (s, 3H), 3.46 (s, 3H), 2.64–2.52 (m, 2H), 1.85–1.76 (m, 1H), 1.68–1.59 (m, 1H), 1.54–1.27 (m, 10H), 0.89–0.86 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 190.0, 164.8, 163.2, 159.6, 107.5, 97.8, 97.3, 95.1, 94.2, 77.6, 56.64, 56.57, 44.3, 34.9, 31.9, 29.5, 29.3, 25.0, 22.8, 14.2. HRMS calculated for C₂₀H₃₀O₆ (M + H)⁺ 367.2115, found (M + H)⁺ 367.2114. HPLC purity: 99.6% (254 nm), $t_{\rm R}$ = 7.69 min; 98.8% (220 nm), $t_{\rm R}$ = 7.69 min.

2-Heptyl-5,7-dihydroxychroman-4-one (5f). Purified with hexane/ EtOAc (90/10) to give the product (49 mg, 0.176 mmol, 80%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 12.03 (s, 1H), 6.57 (br, 1H), 5.96 (s, 1H), 5.93 (s, 1H), 4.39–4.34 (m, 1H), 2.72– 2.57 (m, 2H), 1.86–1.79 (m, 1H), 1.69–1.62 (m, 1H), 1.54–1.26 (m, 10H), 0.90–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 196.9, 165.0, 164.3, 163.6, 103.3, 96.5, 95.4, 77.8, 41.7, 34.9, 31.9, 29.4, 29.3, 25.0, 22.8, 14.2. HRMS calculated for C₁₆H₂₂O₄ (M – H)⁻ 277.1445, found (M – H)⁻ 277.1420. HPLC purity: 98.6% (254 nm), $t_{\rm R}$ = 7.47 min; 98.8% (220 nm), $t_{\rm R}$ = 7.47 min.

5,7-Bis(methoxymethoxy)-2-nonylchroman-4-one (5g). Purified with hexane/EtOAc (90/10) to give the product (218 mg, 0.55 mmol, 55%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.37 (s, 1H), 6.29 (s, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.37–4.31 (m, 1H), 3.51 (s, 3H), 3.47 (s, 3H), 2.64–2.53 (m, 2H), 1.81–1.76 (m, 1H), 1.67–1.59 (m, 1H), 1.54–1.26 (m, 14H), 0.89–0.85 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 190.1, 164.8, 163.2, 159.6, 107.5, 97.8, 97.3, 95.1, 94.2, 77.6, 56.64, 56.57, 44.3, 34.9, 32.0, 29.63, 29.63, 29.5, 29.4, 25.0, 22.8, 14.2. HRMS calculated for C₂₂H₃₄O₆ (M + H)⁺ 395.2428, found (M + H)⁺ 395.2407. HPLC purity: 99.9% (254 nm), $t_{\rm R}$ = 8.09 min; 99.5% (220 nm), $t_{\rm R}$ = 8.09 min.

5,7-Dihydroxy-2-nonylchroman-4-one (5h). Purified with hexane/ EtOAc (90/10) to give the product (55 mg, 0.18 mmol, 82%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 12.05 (s, 1H), 5.95 (s, 1H), 5.92 (s, 1H), 5.44 (br, 1H), 4.40–4.35 (m, 1H), 2.72–2.58 (m, 2H), 1.86–1.80 (m, 1H), 1.69–1.61 (m, 1H), 1.54–1.26 (m, 14H), 0.90–0.88 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 197.1, 165.4, 164.2, 163.7, 103.2, 96.5, 95.5, 77.7, 41.6, 34.8, 32.0, 29.62, 29.58, 29.5, 29.4, 24.9, 22.8, 14.2. HRMS calculated for C₁₈H₂₆O₄ (M – H)⁻ 305.1758, found (M – H)⁻ 305.1719. HPLC purity: 99.7% (254 nm), $t_{\rm R}$ = 7.87 min; 99.2% (220 nm), $t_{\rm R}$ = 7.87 min.

2-(2,6-Dimethylhept-5-en-1-yl)-5,7-bis(methoxymethoxy)chroman-4-one (5i). Purified with hexane/EtOAc (90/10) to obtain a mixture of two diastereomers (275 mg, 0.70 mmol, 70%) as clear oil. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.35 (s, 2H), 6.27 (s, 2H), 5.22 (s, 4H), 5.14 (s, 4H), 5.07-5.05 (m, 2H), 4.46-4.41 (m, 2H), 3.49 (s, 6H), 3.44 (s, 6H), 2.61-2.45 (m, 4H), 1.99-1.68 (m, 8H), 1.65 (s, 6H), 1.57 (s, 6H), 1.41–1.14 (m, 6H), 0.93–0.91 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 189.9, 189.8, 164.72, 164.67, 163.1, 159.49, 159.48, 131.5, 131.4, 124.54, 124.48, 107.5, 97.8, 97.22, 97.21, 95.0, 94.1, 76.1, 75.5, 56.53, 56.46, 44.9, 44.5, 42.11, 42.06, 37.4, 36.8, 28.7, 28.4, 25.8, 25.4, 25.3, 19.9, 19.4, 17.7. HRMS calculated for $C_{22}H_{32}O_6 (M + H)^+$ 393.2272, found $(M + H)^+$ 393.2263. HPLC purity: 98.8% (254 nm), $t_{\rm R}$ = 7.71 min; 98.4% (220 nm), $t_{\rm R}$ = 7.71 min. 2-(2,6-Dimethylhept-5-en-1-yl)-5,7-dihydroxychroman-4-one (5j). Purified with hexane/EtOAc (90/10) to obtain a mixture of two diastereomers (54 mg, 0.176 mmol, 80%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 12.02 (s, 2H), 7.25 (br, 2H), 5.97 (s, 2H), 5.94 (s, 2H), 5.09-5.08 (m, 2H), 4.51-4.46 (m, 2H), 2.73-2.56 (m, 4H), 2.04-1.70 (m, 8H), 1.68 (s, 6H), 1.60 (s, 6H), 1.53-1.32 (m, 4H), 1.25-1.18 (m, 2H), 0.98-0.94 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 197.1, 197.0, 165.4, 164.2, 163.67, 163.65, 131.72, 131.67, 124.54, 124.48, 103.2, 96.6, 95.62, 95.60, 76.3, 75.7, 42.3, 42.14, 42.08, 41.9, 37.5, 36.8, 28.9, 28.5, 25.8, 25.5, 25.4, 20.0, 19.4, 17.8. HRMS calculated for $C_{18}H_{24}O_4$ (M – H)⁻ 303.1602, found $(M - H)^{-}$ 303.1564. HPLC purity: 98.4% (254 nm), t_{R} = 7.50 min; 98.4% (220 nm), $t_{\rm R} = 7.50$ min.

2-Cyclopentyl-5,7-dihydroxychroman-4-one (5k). Purified with hexane/EtOAc (85/15) to give the product (174 mg, 0.70 mmol, 70% over two steps) as white solid. ¹H NMR (400 MHz, DMSO- d_6): δ (ppm) 12.12 (s, 1H), 10.73 (s, 1H), 5.83 (s, 2H), 4.26–4.20 (m, 1H), 2.80–2.73 (m, 1H), 2.61–2.56 (m, 1H), 2.18–2.12 (m, 1H), 1.84–1.78 (m, 1H), 1.72–1.42 (m, 6H), 1.32–1.27 (m, 1H). ¹³C NMR (100 MHz, DMSO- d_6): δ (ppm) 196.4, 166.5, 163.4, 163.0, 101.8, 95.6, 94.8, 80.7, 43.3, 40.1, 28.3, 27.7, 25.0, 24.9. HRMS calculated for C₁₄H₁₆O₄ (M – H)⁻ 247.0976, found (M – H)⁻ 247.0954. HPLC purity: 99.6% (254 nm), $t_{\rm R} = 6.84$ min.

2-Cyclohexyl-5,7-dihydroxychroman-4-one (5l). Purified with hexane/EtOAc (85/15) to give the product (168 mg, 0.64 mmol, 64% over two steps) as white solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 12.12 (s, 1H), 10.74 (s, 1H), 5.84 (s, 2H), 4.24–4.18 (m, 1H), 2.85–2.77 (m, 1H), 2.55–2.51 (m, 1H), 1.91–1.88 (m, 1H), 1.75–1.63 (m, 5H), 1.28–1.01 (m, 5H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ (ppm) 196.7, 166.5, 163.4, 163.0, 101.8, 95.6, 94.7, 80.9, 40.9, 38.3, 27.58, 27.56, 25.9, 25.43, 25.36. HRMS calculated for C₁₅H₁₈O₄ (M – H)⁻ 261.1132, found (M – H)⁻ 261.1106. HPLC purity: 99.1% (254 nm), *t*_R = 7.05 min; 99.5% (220 nm), *t*_R = 7.05 min. (E)-3-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)-1-(2,4,6-

trihydroxyphenyl)prop-2-en-1-one (5m). To a solution of 1h (256 mg, 1 mmol) in ethanol (2.5 mL) were added DEA (154 mg, 2.1 mmol) and (-)-myrtenal (300 mg, 2 mmol) successively. The resulting mixture was stirred at 150 °C in a pressure tube for 4 h. Afterward, the solution was diluted with ethyl acetate and the organic layer was washed with 10% HCl, water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue was dissolved in methanol (15 mL), followed by the addition of concentrated HCl (765 μ L, 9 mmol) dropwise. The resulting mixture was stirred at room temperature for 16 h, after which the solvent was removed. The crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (75/25) to obtain compound 5m (102 mg, 0.34 mmol, 34%) as yellow solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 7.55 (d, J = 15.4 Hz, 1H), 7.38 (d, J = 15.4 Hz, 1H), 6.11 (s, 1H), 5.81 (s, 2H), 2.65-2.64 (m, 1H),2.57–2.47 (m, 3H), 2.18–2.17 (m, 1H), 1.38 (s, 3H), 1.18 (d, J = 9.0 Hz, 1H), 0.82 (s, 3H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 194.6, 166.2, 165.9, 148.3, 144.2, 135.5, 125.5, 105.9, 96.0, 42.9, 42.0, 38.7, 33.7, 32.0, 26.6, 21.2. HRMS calculated for C₁₈H₂₀O₄ (M – H)⁻ 299.1289, found (M - H)⁻ 299.1240. HPLC purity: 99.6% (254 nm), $t_{\rm R}$ = 7.29 min; 99.9% (220 nm), $t_{\rm R}$ = 7.29 min.

2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)-5,7-dihydroxychroman-4-one (5n). To a suspension of 5m (57 mg, 0.19 mmol) in ethanol (2.5 mL) was added catalytic amount of concentrated HCl. The resulting mixture was stirred at 150 °C under microwave irradiation for 30 min. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc (75/25) to obtain a mixture of two diastereomers 5n (41 mg, 0.136 mmol, 72%) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 12.05 (br, 2H), 5.98-5.94 (m, 4H), 5.68-5.67 (m, 2H), 4.86-4.75 (m, 2H), 2.89-2.80 (m, 2H), 2.69-2.55 (m, 2H), 2.53-2.45 (m, 2H), 2.43-2.26 (m, 6H), 2.16-2.15 (m, 2H), 1.34-1.33 (m, 6H), 1.29-1.16 (m, 2H), 0.88 (s, 3H), 0.78 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 196.82, 196.80, 165.2, 164.2, 163.5, 145.0, 144.8, 122.4, 121.2, 103.21, 103.18, 96.55, 96.54, 95.7, 79.5, 79.0, 42.5, 42.2, 40.9, 40.8, 39.8, 39.7, 38.2, 38.0, 31.7, 31.6, 31.4, 31.3, 26.20, 26.16, 21.4, 21.3. HRMS calculated for $C_{18}H_{20}O_4 (M - H)^-$ 299.1289, found $(M - H)^-$ 299.1247. HPLC purity: 99.7% (254 nm), $t_{\rm R}$ = 7.53 min; 99.9% (220 nm), $t_{\rm R}$ = 7.52 min.

General Procedure for the Synthesis of 6a–f. To a suspension of corresponding hydroxyamine (0.8 mmol) in ethanol (1 mL) was added pyridine (64 mg, 0.8 mmol). After stirring at room temperature for 15 min, the solution of appropriate 4-chromanone (0.1 mmol) in ethanol (2 mL) was added. The resulting mixture was stirred for 26–72 h at room temperature, after which the solvent was removed. EtOAc was added to the residue, and the organic layer was washed with water, brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/ EtOAc to give oxime compound.

5,7-Dihydroxy-2-nonylchroman-4-one oxime (**6a**). Purified with hexane/EtOAc (85/15) to give the product (29 mg, 0.09 mmol, 90%) as white solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 5.89 (d, J = 2.3 Hz, 1H), 5.85 (d, J = 2.3 Hz, 1H), 3.99–3.93 (m, 1H), 3.24 (dd, J = 17.1, 3.5 Hz, 1H), 2.32 (dd, J = 17.1, 11.4 Hz, 1H), 1.79–1.70 (m, 1H), 1.67–1.59 (m, 1H), 1.57–1.30 (m, 14H), 0.92–0.88 (m, 3H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 161.5, 160.7, 159.5, 154.7, 98.8, 97.3, 96.2, 76.2, 36.1, 33.1, 30.69, 30.68, 30.62, 30.5, 29.0, 26.2, 23.7, 14.5. HRMS calculated for C₁₈H₂₇NO₄ (M – H)⁻ 320.1818. HPLC purity: 99.9% (254 nm), $t_{\rm R} = 7.62$ min; 99.5% (220 nm), $t_{\rm R} = 7.62$ min.

5,7-Dihydroxy-2-nonylchroman-4-one O-Methyloxime (**6b**). Purified with hexane/EtOAc (90/10) to give the product (27 mg, 0.081 mmol, 81%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 10.96 (s, 1H), 6.04 (d, *J* = 2.3 Hz, 1H), 5.96 (d, *J* = 2.3 Hz, 1H), 5.60 (br, 1H), 4.03–3.98 (m, 1H), 3.94 (s, 3H), 3.18 (dd, *J* = 17.2, 3.0 Hz, 1H), 2.36 (dd, *J* = 17.2, 11.6 Hz, 1H), 1.82–1.73 (m, 1H), 1.66–1.57 (m, 1H), 1.54–1.27 (m, 14H), 0.90–0.86 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 159.7, 159.1, 158.7, 154.1, 98.3, 97.0, 95.8, 75.1, 62.5, 35.0, 32.0, 29.67, 29.66, 29.59, 29.5, 28.6, 25.2, 22.8, 14.3. HRMS calculated for $C_{19}H_{29}NO_4$ (M – H)[–] 334.2024, found (M – H)[–] 334.1977. HPLC purity: 99.8% (254 nm), t_R = 8.13 min; 99.1% (220 nm), t_R = 8.13 min.

5,7-Dihydroxy-2-nonylchroman-4-one O-Benzyloxime (**6***c*). Purified with hexane/EtOAc (92/8) to give the product (34 mg, 0.083 mmol, 83%) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 10.89 (s, 1H), 7.39–7.31 (m, 5H), 6.01 (d, *J* = 2.4 Hz, 1H), 5.94 (d, *J* = 2.3 Hz, 1H), 5.45 (br, 1H), 5.12 (s, 2H), 4.02–3.96 (m, 1H), 3.22 (dd, *J* = 17.2, 3.0 Hz, 1H), 2.40 (dd, *J* = 17.2, 11.6 Hz, 1H), 1.80–1.72 (m, 1H), 1.65–1.57 (m, 1H), 1.54–1.27 (m, 14H), 0.91–0.87 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 159.7, 159.1, 158.7, 154.5, 137.0, 128.72, 128.68, 128.5, 98.4, 96.9, 95.8, 76.7, 75.0, 35.0, 32.0, 29.67, 29.65, 29.58, 29.4, 28.9, 25.1, 22.8, 14.3. HRMS calculated for C₂₅H₃₃NO₄ (M + H)⁺ 412.2482, found (M + H)⁺ 412.2472. HPLC purity: 99.1% (254 nm), *t*_R = 8.36 min; 98.7% (220 nm), *t*_R = 8.37 min.

5,7-Dihydroxy-2-propylchroman-4-one Oxime (6d). Purified with hexane/EtOAc (83/17) to give the product (20 mg, 0.084 mmol, 84%) as white solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 5.90 (s, 1H), 5.85 (s, 1H), 3.98 (br, 1H), 3.24 (d, *J* = 17.0, 1H), 2.33 (dd, *J* = 17.0, 11.4 Hz, 1H), 1.75–1.72 (m, 1H), 1.63–1.47 (m, 3H), 1.0–0.96 (m, 3H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 161.5, 160.7, 159.5, 154.8, 98.8, 97.3, 96.2, 76.0, 38.2, 28.0, 19.4, 14.3. HRMS calculated for C₁₂H₁₅NO₄ (M – H)⁻ 236.0928, found (M – H)⁻ 236.0908. HPLC purity: 99.9% (254 nm), *t*_R = 6.35 min; 100% (220 nm), *t*_R = 6.34 min.

2-Hexyl-5,7-dihydroxychroman-4-one Oxime (**6e**). Purified with hexane/EtOAc (86/14) to give the product (22 mg, 0.08 mmol, 80%) as white solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 5.90 (d, J = 2.3 Hz, 1H), 5.85 (d, J = 2.3 Hz, 1H), 3.97–3.91 (m, 1H), 3.24 (dd, J = 17.1, 3.1 Hz, 1H), 2.32 (dd, J = 17.1, 11.4 Hz, 1H), 1.78–1.69 (m, 1H), 1.65–1.49 (m, 2H), 1.45–1.32 (m, 7H), 0.92–0.89 (m, 3H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 161.4, 160.6, 159.5, 154.7, 98.8, 97.3, 96.2, 76.2, 36.0, 32.9, 30.3, 29.0, 26.2, 23.6, 14.4. HRMS calculated for C₁₅H₂₁NO₄ (M – H)⁻ 278.1398, found (M – H)⁻ 278.1354. HPLC purity: 99.8% (254 nm), $t_{\rm R} = 7.02$ min; 99.9% (220 nm), $t_{\rm R} = 7.01$ min.

2-Heptyl-5,7-dihydroxychroman-4-one Oxime (6f). Purified with hexane/EtOAc (90/10) to give the product (25 mg, 0.085 mmol, 85%) as white solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 5.90 (s, 1H), 5.85 (s, 1H), 3.95 (br, 1H), 3.24 (d, J = 17.0 Hz, 1H), 2.32 (dd, J = 17.0, 11.4 Hz, 1H), 1.75–1.73 (m, 1H), 1.63–1.33 (m, 11H), 0.90 (br, 3H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 161.5, 160.7, 159.5, 154.7, 98.8, 97.3, 96.2, 76.2, 36.0, 33.0, 30.6, 30.3, 29.0, 26.2, 23.7, 14.4. HRMS calculated for C₁₆H₂₃NO₄ (M – H)⁻ 292.1554, found (M – H)⁻ 292.1512. HPLC purity: 99.9% (254 nm), $t_{\rm R} = 7.22$ min; 99.8% (220 nm), $t_{\rm R} = 7.22$ min.

General Procedure for the Synthesis of 7a–c. To a solution of 1e (196 mg, 1 mmol) in ethanol (2.5 mL) were added pyrrolidine (149 mg, 2.1 mmol) and corresponding cycloketone (2 mmol) successively. The resulting mixture was stirred at 150 °C in a pressure tube for 2–16 h. The resulting solution was cooled to room temperature, and concentrated HCl (1.02 mL, 12 mmol) was added. The resulting mixture was then stirred at room temperature overnight. Afterward, the solution was diluted with EtOAc, and the organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/ EtOAc to give the product.

7-Hydroxyspiro[chroman-2, 1'-cyclopentan]-4-one (**7a**). Purified with hexane/EtOAc (90/10) to give the product (170 mg, 0.78 mmol, 78% over two steps) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.78 (d, J = 8.6 Hz, 1H), 7.12 (br, 1H), 6.49 (dd, J = 8.6, 2.3 Hz, 1H), 6.36 (d, J = 2.3 Hz, 1H), 2.78 (s, 2H), 2.09–2.04 (m, 2H), 1.87–1.83 (m, 2H), 1.72–1.59 (m, 4H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.5, 163.7, 162.8, 129.2, 114.9, 110.1, 104.0, 90.5, 46.8, 37.7, 24.0. HRMS calculated for C₁₃H₁₄O₃ (M – H)⁻ 217.0870, found (M – H)⁻ 217.0854. HPLC purity: 99.1% (254 nm), $t_{\rm R} = 6.26$ min; 98.7% (220 nm), $t_{\rm R} = 6.26$ min.

7-Hydroxyspiro[*chroman-2*, 1'*-cyclohexan*]-4-*one* (**7b**). Purified with hexane/EtOAc (90/10) to give the product (207 mg, 0.89 mmol, 89% over two steps) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.77 (d, *J* = 8.6 Hz, 1H), 7.45 (br, 1H), 6.50 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.42 (d, *J* = 2.2 Hz, 1H), 2.66 (s, 2H), 1.99–1.96 (m, 2H), 1.72–1.59 (m, 3H), 1.50–1.44 (m, 4H), 1.34–1.25 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.6, 164.0, 162.2, 129.0, 114.6, 110.1, 103.9, 80.5, 47.9, 35.0, 25.3, 21.6. HRMS calculated for C₁₄H₁₆O₃ (M – H)⁻ 231.1027, found (M – H)⁻ 231.1007. HPLC purity: 98.2% (254 nm), *t*_R = 6.47 min; 98.8% (220 nm), *t*_R = 6.47 min.

7-Hydroxyspiro[chroman-2,1'-cycloheptan]-4-one (7c). Purified by reverse phase C18 silica gel chromatography with H₂O/MeCN (65/35) to give the product (130 mg, 0.53 mmol, 53% over two steps) as white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.26 (br, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 6.51 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.40 (d, *J* = 2.2 Hz, 1H), 2.70 (s, 2H), 2.09–2.03 (m, 2H), 1.77–1.61 (m, 6H), 1.57– 1.50 (m, 2H), 1.43–1.36 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.1, 164.6, 162.5, 128.9, 114.3, 110.2, 103.9, 84.7, 48.5, 38.4, 29.4, 22.1. HRMS calculated for C₁₅H₁₈O₃ (M – H)[–] 245.1183, found (M – H)[–] 245.1154. HPLC purity: 97.4% (254 nm), *t*_R = 6.67 min; 97.0% (220 nm), *t*_R = 6.67 min.

General Procedure for the Synthesis of 8a–h. To a solution of corresponding acetophenone (1 mmol) and appropriate benzaldehyde (1.5 mmol) in methanol (10 mL) was added 60% KOH aqueous solution (1.5 mL) dropwise at room temperature. The resulting solution was stirred for 60 h at room temperature, after which the reaction mixture was neutralized with 10% HCl aq and extracted with ethyl acetate twice. The organic layer was washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue was dissolved in methanol (14 mL), followed by the careful addition of concentrated HCl (340 μ L, 4 mmol). The obtained mixture was stirred at room temperature until the MOM-protected chalcone was gone, and then the mixture was concentrated. The crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to afford the chalcones.

(E)-3-(2-(*Allyloxy*)*phenyl*)-1-(2,4-*dihydroxyphenyl*)*prop*-2-*en*-1*one* (**8***a*). Purified with hexane/EtOAc (90/10) to give the product (175 mg, 0.59 mmol, 59% over two steps) as yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 13.49 (s, 1H), 8.18 (d, *J* = 15.6 Hz, 1H), 7.81 (d, *J* = 9.4 Hz, 1H), 7.74 (d, *J* = 15.6 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 8.4 Hz, 1H), 7.0 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.43–6.41 (m, 2H), 6.22 (s, 1H), 6.16–6.09 (m, 1H), 5.47 (d, *J* = 17.2 Hz, 1H), 5.35 (d, *J* = 10.6 Hz, 1H), 4.65 (d, *J* = 5.3 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.8, 166.5, 162.9, 158.1, 140.5, 133.0, 132.2, 132.0, 130.1, 124.2, 121.4, 121.2, 118.4, 114.8, 112.8, 107.9, 103.9, 69.5. HRMS calculated for C₁₈H₁₆O₄ (M – H)⁻ 295.0976, found (M – H)⁻ 295.0955. HPLC purity: 100% (254 nm), *t*_R = 7.20 min; 99.9% (220 nm), *t*_R = 7.20 min. (*E*)-1-(2,4-*Dihydroxyphenyl*)-3-(2-(*hexyloxy*)*phenyl*)*prop*-2-*en*-1*one* (*8b*). Purified by reverse phase C18 silica gel chromatography with H₂O/MeCN (43/57) to give the product (170 mg, 0.50 mmol, 50% over two steps) as yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 13.61 (s, 1H), 8.11 (d, *J* = 15.6 Hz, 1H), 7.81–7.77 (m, 2H), 7.57 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 6.99–6.92 (m, 3H), 6.45–6.43 (m, 2H), 4.08–4.04 (m, 2H), 1.93–1.86 (m, 2H), 1.56–1.49 (m, 2H), 1.39–1.32 (m, 4H), 0.92–0.88 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.0, 166.4, 163.2, 158.8, 141.1, 132.14, 132.06, 130.7, 123.8, 121.3, 120.7, 114.6, 112.3, 108.1, 103.8, 68.7, 31.7, 29.4, 26.1, 22.7, 14.1. HRMS calculated for C₂₁H₂₄O₄ (M – H)⁻ 339.1602, found (M – H)⁻ 339.1550. HPLC purity: 99.6% (254 nm), $t_{\rm R}$ = 8.00 min; 98.9% (220 nm), $t_{\rm R}$ = 8.01 min.

(E)-1-(2,4-Dihydroxyphenyl)-3-(2-(octyloxy)phenyl)prop-2-en-1one (**8***c*). Purified by reverse phase C18 silica gel chromatography with H₂O/MeCN (45/55) to give the product (195 mg, 0.53 mmol, 53% over two steps) as yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 13.64 (s, 1H), 8.12 (d, *J* = 15.6 Hz, 1H), 7.80 (s, 1H), 7.77 (d, *J* = 6.2 Hz, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.35 (t, *J* = 8.2 Hz, 1H), 7.14 (br, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.46–6.44 (m, 2H), 4.06–4.03 (m, 2H), 1.92–1.85 (m, 2H), 1.55–1.48 (m, 2H), 1.39–1.27 (m, 8H), 0.88–0.85 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 193.0, 166.3, 163.3, 158.8, 141.1, 132.2, 132.1, 130.6, 123.8, 121.2, 120.7, 114.6, 112.3, 108.2, 103.8, 68.7, 31.9, 29.5, 29.4, 29.3, 26.4, 22.8, 14.2. HRMS calculated for C₂₃H₂₈O₄ (M + H)⁺ 369.2060, found (M + H)⁺ 369.2057. HPLC purity: 99.5% (254 nm), *t*_R = 8.36 min; 99.4% (220 nm), *t*_R = 8.36 min.

(E)-1-(2,4-Dihydroxyphenyl)-3-(4-(piperidin-1-yl)phenyl)prop-2en-1-one (**8d**). Purification was performed without chromatography because of the poor solubility of product. The crude solid was washed with small amount of methanol three times to give the product (210 mg, 0.65 mmol, 65% over two steps) as yellow solid. ¹H NMR (400 MHz, DMSO- d_6): δ (ppm) 8.19 (d, J = 9.0 Hz, 1H), 7.98–7.92 (m, 3H), 7.77 (d, J = 15.4 Hz, 1H), 7.67 (br, 2H), 6.45 (dd, J = 8.9, 2.2Hz, 1H), 6.33 (d, J = 2.2 Hz, 1H), 3.49–3.46 (m, 4H), 1.88 (br, 4H), 1.64 (br, 2H). ¹³C NMR (100 MHz, DMSO- d_6): δ (ppm) 191.7, 166.3, 165.8, 133.5, 131.0, 113.5, 108.8, 103.1, 24.0. HRMS calculated for C₂₀H₂₁NO₃ (M – H)⁻ 322.1449, found (M – H)⁻ 322.1407. HPLC purity: 99.0% (254 nm), $t_R = 5.99$ min; 98.8% (220 nm), $t_R =$ 5.98 min.

(E)-3-(2-(Allyloxy)phenyl)-1-(2,4,6-trihydroxyphenyl)prop-2-en-1one (**8e**). Purified with hexane/EtOAc (70/30) to give the product (106 mg, 0.34 mmol, 34% over two steps) as yellow solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.25 (d, *J* = 15.8 Hz, 1H), 8.10 (d, *J* = 15.8 Hz, 1H), 7.61 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.34–7.30 (m, 1H), 7.0– 6.94 (m, 2H), 6.16–6.09 (m, 1H), 5.86 (s, 2H), 5.45 (dd, *J* = 17.3, 1.6 Hz, 1H), 5.29 (dd, *J* = 10.6, 1.4 Hz, 1H), 4.63 (d, *J* = 5.2 Hz, 2H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 194.5, 166.4, 166.1, 158.9, 138.2, 134.6, 132.4, 129.5, 129.1, 126.0, 122.0, 117.8, 113.9, 106.0, 96.0, 70.3. HRMS calculated for C₁₈H₁₆O₅ (M – H)⁻ 311.0925, found (M – H)⁻ 311.0877. HPLC purity: 97.5% (254 nm), *t*_R = 6.93 min; 97.2% (220 nm), *t*_R = 6.93 min.

(E)-3-(2-(Ållyloxy)phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**8f**). Purified with hexane/EtOAc (80/20) to give the product (205 mg, 0.73 mmol, 73% over two steps) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.42 (br, 1H), 8.16 (d, *J* = 15.8 Hz, 1H), 8.01–7.99 (m, 2H), 7.70 (d, *J* = 15.8 Hz, 1H), 7.61 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.36–7.31 (m, 1H), 7.03–6.96 (m, 3H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.11–6.02 (m, 1H), 5.43 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.29 (dd, *J* = 10.6, 1.3 Hz, 1H), 4.61–4.59 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 190.8, 161.6, 158.0, 140.6, 132.9, 131.8, 131.5, 130.5, 129.6, 124.3, 122.8, 121.1, 118.1, 115.9, 112.7, 69.3. HRMS calculated for C₁₈H₁₆O₃ (M – H)⁻ 279.1027, found (M – H)⁻ 279.0988. HPLC purity: 99.6% (254 nm), $t_{\rm R}$ = 6.99 min.

(E)-3-(3-(Allyloxy)phenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1one (**8g**). Purified with hexane/EtOAc (90/10) to give the product (113 mg, 0.38 mmol, 38% over two steps) as yellow solid. ¹H NMR (400 MHz, DMSO- d_6): δ (ppm) 13.40 (s, 1H), 10.77 (br, 1H), 8.23 (d, J = 9.0 Hz, 1H), 7.98 (d, J = 15.4 Hz, 1H), 7.76 (d, J = 15.4 Hz, 1H), 7.53 (s, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.05 (dd, J = 8.0, 2.0 Hz, 1H), 6.43 (dd, J = 8.9, 2.3 Hz, 1H), 6.30 (d, J = 2.2 Hz, 1H), 6.11–6.03 (m, 1H), 5.44 (dd, J = 17.3, 1.4 Hz, 1H), 5.29 (d, J = 10.5 Hz, 1H), 4.65 (d, J = 5.2 Hz, 2H). ¹³C NMR (100 MHz, DMSO- d_6): δ (ppm) 191.5, 165.9, 165.3, 158.6, 143.7, 136.0, 133.6, 133.3, 130.0, 122.2, 121.5, 117.7, 117.4, 114.1, 113.0, 108.3, 102.6, 68.4. HRMS calculated for C₁₈H₁₆O₄ (M + H)⁺ 297.1121, found (M + H)⁺ 297.1107. HPLC purity: 99.7% (254 nm), $t_R = 7.22$ min; 99.6% (220 nm), $t_R = 7.22$ min.

(E)-3-(4-(Allyloxy)phenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1one (**8h**). Purified with hexane/EtOAc (90/10) to give the product (130 mg, 0.44 mmol, 44% over two steps) as yellow solid. ¹H NMR (400 MHz, DMSO- d_6): δ (ppm) 13.54 (s, 1H), 10.70 (br, 1H), 8.19 (d, *J* = 9.0 Hz, 1H), 7.87–7.75 (m, 4H), 7.04 (d, *J* = 8.7 Hz, 2H), 6.41 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.29 (d, *J* = 2.3 Hz, 1H), 6.10–6.01 (m, 1H), 5.42 (dd, *J* = 17.3, 1.4 Hz, 1H), 5.28 (d, *J* = 10.5 Hz, 1H), 4.65 (d, *J* = 5.2 Hz, 2H). ¹³C NMR (100 MHz, DMSO- d_6): δ (ppm) 191.5, 165.8, 165.1, 160.4, 143.7, 133.4, 133.0, 131.0, 127.4, 118.6, 117.8, 115.1, 113.0, 108.2, 102.6, 68.4. HRMS calculated for C₁₈H₁₆O₄ (M + H)⁺ 297.1121, found (M + H)⁺ 297.1108. HPLC purity: 99.8% (254 nm), t_R = 7.18 min; 99.6% (220 nm), t_R = 7.18 min.

General Procedure for the Synthesis of 8i–m. The obtained appropriate chalcone (0.2 mmol) was dissolved in ethanol (2.5 mL) with a catalytic amount of concentrated HCl and then heated at 150 $^{\circ}$ C under microwave irradiation for 1.5 h. The solvent was removed under reduced pressure and the crude material was adsorbed onto silica gel and subjected to silica gel chromatography with hexane/EtOAc to afford the corresponding flavanones.

2-(2-(*A*llyloxy)*phenyl*)-7-*hydroxychroman-4-one* (**8***i*). Purified with hexane/EtOAc (90/10) to give the product (30 mg, 0.1 mmol, 50%) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.85 (d, *J* = 8.7 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.54 (br, 1H), 7.30 (t, *J* = 8.5 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.58 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.51 (d, *J* = 2.1 Hz, 1H), 6.03–5.95 (m, 1H), 5.87 (dd, *J* = 11.3, 5.0 Hz, 1H), 5.34 (d, *J* = 17.2 Hz, 1H), 5.24 (d, *J* = 10.6 Hz, 1H), 4.61–4.51 (m, 2H), 2.97–2.86 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.6, 164.5, 163.9, 154.9, 133.0, 129.6, 129.5, 127.8, 126.7, 121.2, 117.8, 114.8, 112.0, 111.0, 103.6, 75.0, 69.0, 43.4. HRMS calculated for C₁₈H₁₆O₄ (M – H)⁻ 295.0976, found (M – H)⁻ 295.0949. HPLC purity: 98.0% (254 nm), *t*_R = 6.76 min.

2-(2-(Hexyloxy)phenyl)-7-hydroxychroman-4-one (**8***j*). Purified by reverse phase C18 silica gel chromatography with H₂O/MeCN (50/50) to give the product (35 mg, 0.1 mmol, 52%) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.86 (d, *J* = 8.7 Hz, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.31 (t, *J* = 8.5 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.59 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 5.86–5.82 (m, 1H), 4.03–3.93 (m, 2H), 2.96–2.87 (m, 2H), 1.79–1.72 (m, 2H), 1.45–1.37 (m, 2H), 1.31–1.27 (m, 4H), 0.87–0.84 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.8, 164.5, 164.1, 155.5, 129.52, 129.50, 127.5, 126.5, 120.7, 114.7, 111.5, 111.0, 103.6, 75.1, 68.3, 43.3, 31.6, 29.2, 25.9, 22.6, 14.1. HRMS calculated for C₂₁H₂₄O₄ (M − H)[−] 339.1602, found (M − H)[−] 339.1563. HPLC purity: 99.9% (254 nm), *t*_R = 7.65 min; 99.6% (220 nm), *t*_R = 7.65 min.

7-Hydroxy-2-(2-(octyloxy)phenyl)chroman-4-one (**8***k*). Purified by reverse phase C18 silica gel chromatography with H₂O/MeCN (42/58) to give the product (37 mg, 0.1 mmol, 50%) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.86 (d, *J* = 8.7 Hz, 1H), 7.58 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.60 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.53 (d, *J* = 2.2 Hz, 1H), 5.86–5.82 (m, 1H), 4.03–3.93 (m, 2H), 2.97–2.87 (m, 2H), 1.79–1.72 (m, 2H), 1.44–1.37 (m, 2H), 1.32–1.24 (m, 8H), 0.87–0.83 (m, 3H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 192.9, 164.6, 164.2, 155.5, 129.53, 129.49, 127.5, 126.5, 120.7, 114.6, 111.5, 111.0, 103.6, 75.1, 68.3, 43.3, 31.9, 29.4, 29.3, 29.2, 26.2, 22.7, 14.2. HRMS calculated for C₂₃H₂₈O₄ (M + H)⁺ 369.2060, found (M + H)⁺ 369.2056. HPLC purity: 99.9% (254 nm), *t*_R = 8.05 min.

7-Hydroxy-2-(4-(piperidin-1-yl)phenyl)chroman-4-one (81). Purified with hexane/EtOAc (84/16) to give the product (37 mg, 0.11 mmol, 57%) as pale yellow solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 7.73 (d, J = 8.7 Hz, 1H), 7.37–7.35 (m, 2H), 7.02–6.99 (m, 2H), 6.50 (dd, J = 8.7, 2.2 Hz, 1H), 6.36 (d, J = 2.2 Hz, 1H), 5.39 (dd, J = 12.9, 2.9 Hz, 1H), 3.19–3.16 (m, 4H), 3.06 (dd, J = 16.9, 12.9 Hz, 1H), 2.70 (dd, J = 16.9, 3.0 Hz, 1H), 1.75–1.69 (m, 4H), 1.63–1.59 (m, 2H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 193.6, 166.8, 165.6, 153.8, 131.2, 129.9, 128.5, 117.7, 115.1, 115.0, 111.7, 103.8, 81.0, 51.9, 44.8, 26.8, 25.4. HRMS calculated for C₂₀H₂₁NO₃ (M – H)⁻ 322.1449, found (M – H)⁻ 322.1401. HPLC purity: 97.4% (254 nm), $t_{\rm R} = 5.22$ min; 98.7% (220 nm), $t_{\rm R} = 5.22$ min.

2-(2-(Allyloxy)phenyl)-5,7-dihydroxychroman-4-one (**8***m*). Purified with hexane/EtOAc (85/15) to give the product (47 mg, 0.15 mmol, 75%) as white solid. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 7.54 (d, *J* = 7.6 Hz, 1H), 7.30 (td, *J* = 7.9, 1.6 Hz, 1H), 7.03–6.99 (m, 2H), 6.11–6.01 (m, 1H), 5.95 (d, *J* = 2.1 Hz, 1H), 5.90 (d, *J* = 2.1 Hz, 1H), 5.73 (dd, *J* = 12.7, 3.2 Hz, 1H), 5.38 (dd, *J* = 17.3, 1.6 Hz, 1H), 5.25 (dd, *J* = 10.6, 1.4 Hz, 1H), 4.65–4.56 (m, 2H), 2.99–2.91 (m, 1H), 2.83–2.78 (m, 1H). ¹³C NMR (100 MHz, CD₃OD): δ (ppm) 197.7, 168.3, 165.5, 165.0, 156.4, 134.6, 130.5, 128.8, 127.5, 122.0, 117.6, 113.2, 103.3, 97.2, 96.2, 75.7, 70.0, 43.1. HRMS calculated for C₁₈H₁₆O₅ (M – H)⁻ 311.0925, found (M – H)⁻ 311.0877. HPLC purity: 99.8% (254 nm), *t*_R = 7.14 min; 99.6% (220 nm), *t*_R = 7.14 min.

MIC Determination. MIC values were determined against *M. tuberculosis* (H37Rv) and other bacteria using the standard microbroth dilution method exactly as previously described,⁷¹ which is based on the methods by the Clinical and Laboratory Standards Institute.^{72,73} The maximum test concentration used was 200 μ g/mL.

Cytotoxicity Assays. Cytotoxicity was assessed in vitro using Vero cells (kidney epithelial cells, ATCC CCL-81) as described previously.⁷⁴ In brief, monolayers of cells cultured in Dulbecco's modified Eagle medium (DMEM)/10% fetal bovine serum (FBS) were trypsinized, seeded at approximately 10% confluence in white-walled 96-well plates, and incubated overnight to allow adherence. Medium was replaced with DMEM/FBS containing 2-fold serial dilutions of test compounds. Detection was performed using MTT (CellTiter96, Promega) with overnight solubilization according to the manufacturer's instructions.

Approximation of Solubility in Cell Culture Media. The 2-fold serial dilutions in clear, round-bottom 96-well plates were prepared in DMEM with 5% FBS. Plates were incubated at 37 °C overnight. The highest compound concentration that did not result in visible precipitation of compound was used to approximate the solubility limit in cell culture medium.

Time Kill Experiments. The ability of compounds to kill *S. aureus* Newman was evaluated using standard approaches.⁷⁵ Overnight cultures were diluted 1:25 in fresh Mueller–Hinton broth and grown to $OD_{600nm} \approx 0.3$ before being exposed to compounds at various increments of their MICs. The number of viable bacteria over time was then determined.

Macromolecular Synthesis. The effects of compounds on key macromolecular processes were determined as described,⁷⁶ using radiolabeled precursors from American Radiolabeled Chemicals, Inc.: [methyl-³H]thymidine, [5,6-³H]uridine, and [4,5-³H]leucine for DNA, RNA, and protein, respectively. Experiments were performed on three independent mid-logarithmic cultures (OD_{600nm} \approx 0.3). The antibiotics ciprofloxacin (DNA, from Sigma-Aldrich), rifampicin (RNA, from TCI America), and erythromycin (protein, from Calbiochem) were used as positive controls.

Analysis of the Membrane Potential. The effects of compounds on the membrane potential of *S. aureus* were evaluated by flow cytometry using the fluorescent probe diethyloxacarbocyanine dye DiOC₂(3). The emission of red fluorescence of DiOC₂(3) from cells is dependent on the membrane potential, while green fluorescence emission is independent of the membrane potential. Therefore, the ratio of red to green fluorescence provides a measure of the effects of compounds on the membrane potential. These experiments were performed as previously described,⁷⁶ using *S. aureus* Newman grown to OD_{600nm} \approx 0.3 in Mueller–Hinton II. Samples were analyzed using BD LSR II flow cytometer and the effects of compounds on the membrane potential evaluated using the software FlowJo X 10.0.7 as described.⁷⁶ Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP; Sigma-Aldrich) and vancomycin were used as positive and negative controls, respectively.

Assay of Relaxation Activity of *E. coli* Topoisomerase I. Recombinant *E. coli* topoisomerase I (EcTopI) was purified with published procedures.⁷⁷ CsCl gradient purified plasmid DNA (160 ng) was added to relaxation reaction buffer containing 10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.1 mg/mL gelatin, and 0.5 mM MgCl₂. The resulting solution was divided into 9.5 μ L aliquots, to which 0.5 μ L of the 20 mM compounds dissolved in DMSO was added for final compound concentrations of 500 μ M. The compound control reaction received only DMSO. EcTopI (20 ng) in 10 μ L of the relaxation reaction buffer was then added to each DNA/compound reaction mixture. The reaction mixtures were then incubated at 37 °C for 30 min before the reaction was terminated, and the mixtures were loaded onto a 1% agarose gel for electrophoretic analysis in TAE buffer (40 mM Tris–acetate, pH 8.1, 2 mM EDTA) as described previously.⁷⁸

E. coli DNA Gyrase Supercoiling Assay. The *E. coli* DNA gyrase supercoiling assay was performed according to the manufacturer's protocol. Briefly, 1.25 U of *E. coli* DNA gyrase (New England Biolabs) was added to 20 μ L of DNA gyrase reaction mixture (35 mM Tris-HCl, pH 7.5, 24 mM KCl, 4 mM MgCl₂, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/mL BSA, 6.5% glycerol) containing 300 ng of relaxed plasmid DNA substrate (New England Biolabs) in the presence of indicated concentration of compound. After incubation at 37 °C for 30 min, the reactions were stopped and analyzed by agarose gel electrophoresis similarly as the topoisomerase I relaxation assay.⁷⁸ IC₅₀ values (compound concentrations at which only 50% of the input DNA was converted to supercoiled form) were calculated by spot densitometry analysis with AlphaView, AlphaImager Mini (Protein Simple). The IC₅₀ values represent the average from at least two experiments performed separately.

E. coli Topoisomerase IV Decatenation Assay. For the assay of decatenation activity of topoisomerase IV,⁷⁹ 0.25 U of *E. coli* topoisomerase IV (Topogen) was added to 20 μL of decatenation reaction mixture (40 mM HEPES-KOH, pH 8.0, 100 mM potassium glutamate, 10 mM magnesium acetate, 10 mM dithiothreitol, 50 μ g of BSA/mL) containing 290 ng of kinetoplast DNA substrate (Topogen) and 1 mM ATP in the presence of indicated concentration of compound. After incubation at 37 °C for 30 min, the reactions were stopped by the addition of stop buffer (50 mM EDTA, 50% glycerol, and 0.5% (v/v) bromophenol blue). The DNA samples were electrophoresed in a 1% agarose gel containing 0.5 μ g/mL ethidium bromide in TAE buffer also containing 0.5 μ g/mL ethidium bromide. Visualization of the bands was carried out by exposure to UV light. IC₅₀ values (compound concentrations at which only 50% of kinetoplast DNA was converted into monomeric products) were calculated by spot densitometry analysis with AlphaView, AlphaImager Mini (Protein Simple). The IC₅₀ values represent the average from at least two experiments performed separately.

ASSOCIATED CONTENT

Supporting Information

NMR and HRMS spectra and HPLC profiles of all synthetic compounds; ORTEP views of **3g** and **8a**; inhibition data. This material is available free of charge via the Internet at http:// pubs.acs.org.

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The authors declare no competing financial interest.

Notes

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ABBREVIATIONS USED

ATCC, American Type Culture Collection; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Cip, ciprofloxacin; DEA, diethylamine; DIPEA, diisopropylethylamine; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; MIC, minimum inhibitory concentration; MOM, methoxymethyl; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; NMR, nuclear magnetic resonance; SAR, structure—activity relationship; SEM, standard error of mean; SI, selectivity index; Topo, topoisomerase; TBDMS, *tert*-butyldimethylsilyl; Ts, *p*-toluene-sulfonyl

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