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The Mosaic of Rottlerin: The Sequel

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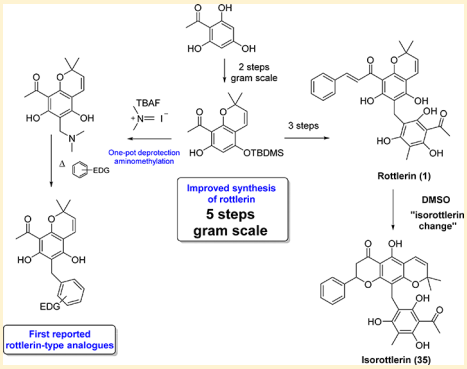
Supporting Information

ABSTRACT: Rottlerin (**1**) is a potent protein kinase C δ inhibitor that possesses a wide range of biological activities. However, the potential of this molecule to be developed as a drug has been restricted by its limited availability. We report herein a gram scale quantity synthesis of rottlerin in a five-step synthetic route that can be completed within 2 days. The methodology was extended by the reaction of the key aminochromene intermediate (**15**) with various electron-rich arenes, forming novel unsymmetrical methylene-bridged compounds. The X-ray crystal structure revealed the boomerang shape of this kind of molecule for the first time. The direct transformation of rottlerin (**1**) into the natural product, isorottlerin (**35**), was observed for the first time, and we named this transformation the "isorottlerin change". In addition, the antibacterial activities of rottlerin (**1**) and new rottlerin analogues **32**−**34** were examined against *Staphylococcus aureus*. The compounds showed MIC values as low as 2.0 μM, which were comparable to the clinically used antibiotic gentamicin.



Mallotus phillipinesis is an endangered medicinal plant that is found in India.[1] The fruit of the plant is covered with a red powder, known as kamala, within which can be found rottlerin (**1**) (Figure 1). First discovered by Thomas Anderson in 1855, rottlerin (**1**) has long intrigued researchers due to its broad spectrum of pharmacological activity. Rottlerin (**1**) is most well known for its inhibition of protein kinase C delta (PKCδ),[2] and extensive studies on the anticancer properties have been performed. Recently, Wang et al.[3] reported that rottlerin (**1**) drastically reduced the expression of Cdc20 oncoprotein in glioma cells, highlighting a potential new mechanism for the treatment of glioma. Yin et al.[4] also discovered the ability of rottlerin (**1**) to significantly inhibit S-phase kinase associated protein 2 (Skp2) expression in breast cancer cells, revealing a new therapeutic strategy for combating breast cancer.

While rottlerin (**1**) has been mostly studied for its anticancer activities against various cancer cells, its antibacterial activity is not as well studied despite a few recent studies showing that rottlerin exhibits an minimum inhibitory concentration (MIC) of 1.0 and 6.3 μM against multiple *Chlamydia* and *Helicobacter pylori* species.[5−7] It is also known that compounds similar to rottlerin (**1**), including drummondins A (**2**), B (**3**), C (**4**), and F (**5**) (Figure 1), from *Hypericum drummondii* possess potent antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis*.[8]

As rottlerin (**1**) is structurally similar to these compounds with the presence of a chromene moiety with a methylene bridge connected to a hydroxyacetophenone moiety, it would be interesting to see if rottlerin (**1**) and its analogues would possess any antibacterial activity against the methicillin-resistant *S. aureus*.

Although rottlerin (**1**) can be purchased in small quantities (10 mg) from several chemical suppliers, its limited availability and high price are obstacles that prevent more extensive research of this promising compound in various areas. Our research group has previously reported the first total synthesis of rottlerin (**1**) comprising a seven-step linear synthesis.[9] The previous synthesis (Scheme 1) began by using the commercially available trihydroxyacetophenone (**6**) to prepare the methoxymethyl (MOM)-protected chromene (**7**) using a three-step protocol. Subsequent aldol condensation afforded chromene (**8**), followed by acid deprotection to give 5-hydoxychromene (**9**) in 32% yield. Aminomethylation using Eschenmoser's salt gave chromene (**10**) in 88% yield. Finally, rottlerin (**1**) was synthesized in moderate yield by thermally reacting **10** with acetophenone (**11**). More recently, Wang et al.[3] described an eight-step linear synthesis of rottlerin (**1**).[10] However, both of these reported synthetic methodologies have drawbacks, including a long synthetic sequence, harsh and toxic reaction conditions such as demethylation using BBr_3, and extended production time.

We report herein an improved gram-scale, five-step synthesis of rottlerin (**1**) and the development of new rottlerin analogues. In addition, the potent antibacterial activities of rottlerin (**1**) are reported for the first time. It is anticipated that the efficient synthetic methodology reported herein will permit the preparation of large quantities of rottlerin (**1**) and its analogues to study their promising biological activities.

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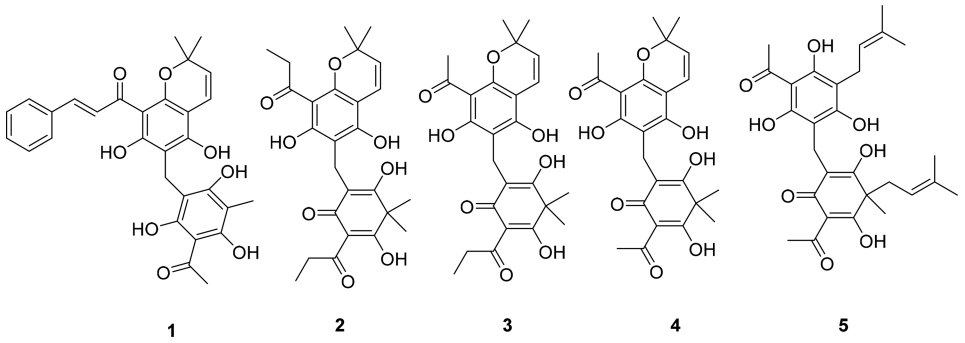
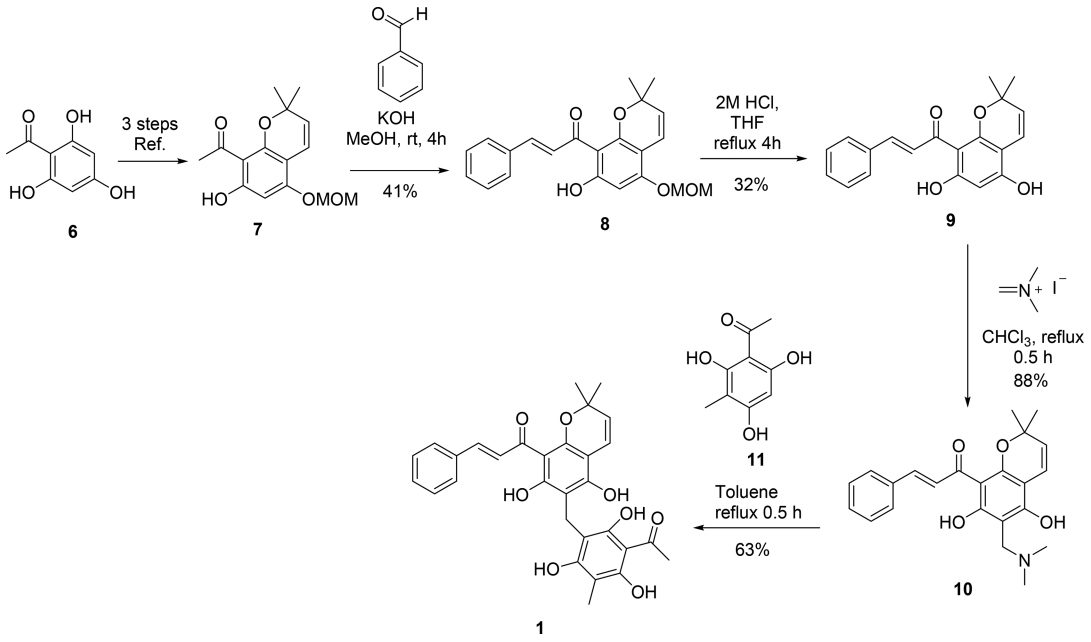
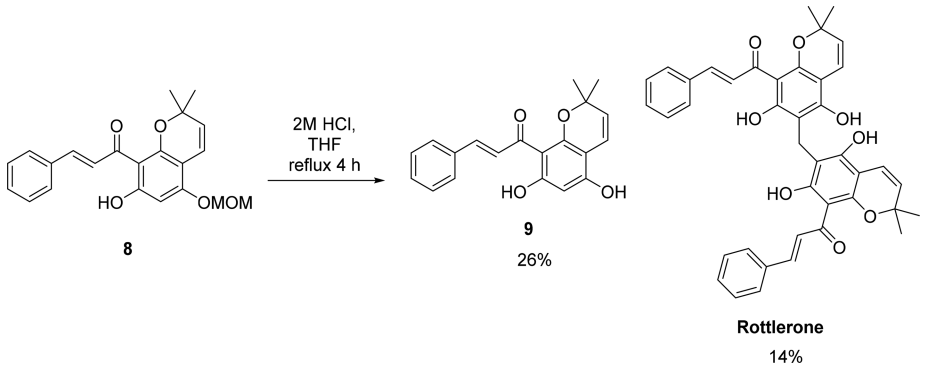


Figure 1. Structures of rottlerin (**1**) and drummondins A (**2**), B (**3**), C (**4**), and F (**5**).

Scheme 1. Original Synthesis of Rottlerin (1)[9]



Scheme 2. Identification of Products from the Acidic Deprotection of the MOM Group



RESULTS AND DISCUSSION

Scalable Synthesis of Rottlerin (1). The inspiration toward the improved synthesis began when investigating the cause of the low yield during the deprotection of the MOM group of chromene (**8**) (Scheme 1).[9] The reaction was reinvestigated in depth, and it was found that apart from the target polar 5-hydroxchromene (**9**) being formed in 26% yield (Scheme 2), a nonpolar compound with a similar R_f value to **8** was also formed.

This side product was purified by silica-gel chromatography, and it was identified to be the symmetrical homodimer rottlerone, in a yield of 14%. The formation of such symmetrical dimer was also reported by Dubois and Yu in their synthesis of methylenebis(chalcone)s and dimeric chromenes during the deprotection of MOM-protected chalcones under acidic conditions.[11,12]

To avoid the formation of rottlerone, the MOM protecting group was replaced with the *tert*-butyldimethylsilyl (TBDMS) protecting group, which does not produce formaldehyde upon

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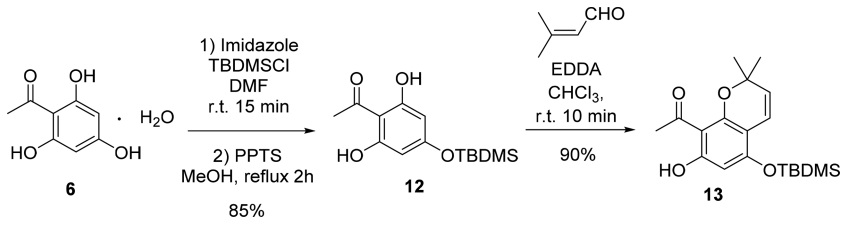
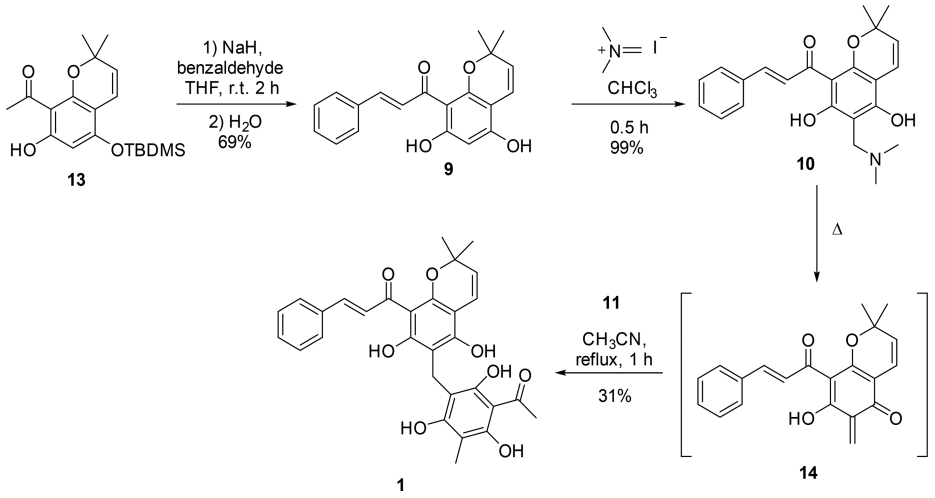
Scheme 3. Synthesis of TBDMS-Protected Chromene (13)



Scheme 4. An Improved Gram Scale Synthesis of Rottlerin (1)



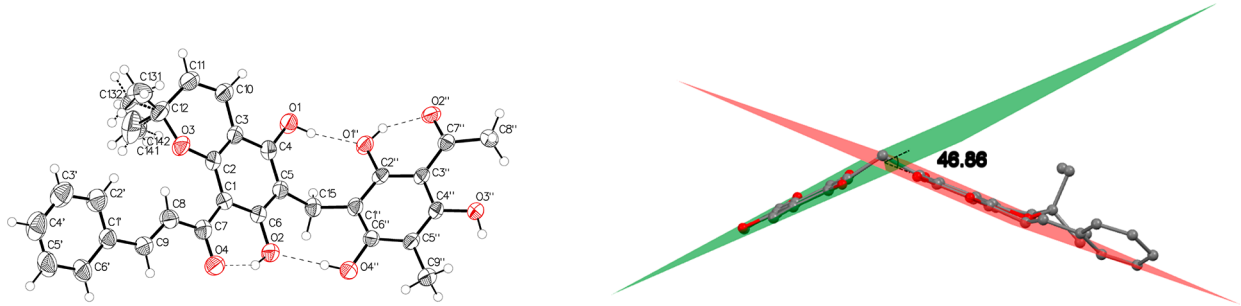


Figure 2. ORTEP diagram of rottlerin (**1**) and the measured angle around the methylene bridge.

deprotection. The synthesis of rottlerin (**1**) therefore began with the monoprotection of trihydroxyacetophenone monohydrate (**6**) using TBDMSCl to give the bis-protected acetophenone, which was not isolated. Addition of pyridinium *p*-toluenesulfonate (PPTS) to the reaction mixture and heating to reflux gave the monoprotected acetophenone (**12**) in 85% yield (Scheme 3).[13] Annulation was achieved by reacting acetophenone (**12**) with a 3-methyl-2-butenal in the presence of ethylenediamine diacetate (EDDA) at room temperature for 10 min, affording chromene (**13**) in 90% yield. Notably, this method is scalable and much more efficient than Adler's synthesis[14] of chromene (**13**) using microwave irradiation (300 W) for 1 h, which gave the product in 30% yield.

Reaction of chromene (**13**) with benzaldehyde under classical aldol conditions with base in aqueous EtOH did not afford the expected TBDMS-protected chalcone. Instead, cleavage of the TBDMS protecting group was observed, which could be due to the lability of TBDMS to NaOH. Therefore, the aldol condensation was performed using NaH as a base, and upon aqueous workup NaOH was generated *in situ*, which promoted the cleavage of TBDMS, forming the target chalcone (**9**) in a one-pot process in 69% yield (Scheme 4). Aminomethylation of chromene (**9**) using Eschenmoser's salt gave aminochromene (**10**) in quantitative yield. Heating aminochromene (**10**) at high temperature generated the putative *ortho*-quinone methide intermediate (**14**), which could be trapped with the acetophenone (**11**) in a 1,4-Michael addition reaction to provide rottlerin (**1**) in 31% yield after column chromatography and recrystallization from MeCN. While toluene was used as the solvent in the previous synthesis of rottlerin,[9] it was found that MeCN was a better solvent for the large-scale synthesis. Comparable yields were obtained after chromatography; however the drop in yield compared with the previous synthesis is mainly due to the product loss during recrystallization. Overall, the synthesis of rottlerin (**1**) was reduced from a seven-[9] to a five-step, gram-scale synthesis that could be completed in 2 days.

Crystallization of rottlerin (**1**) from EtOAc gave single crystals suitable for X-ray diffraction studies, thus allowing

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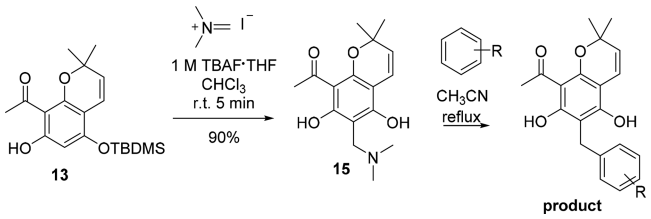
unambiguous structure determination (Figure 2). This is the first X-ray crystal structure of rottlerin (**1**).

In the solid state, rottlerin (**1**) exhibits an overall "boomerang-like" structure, with a 46.86° angle between the planes of the phloroglucinol and chromene moieties (Figure 2). Interestingly, two sets of intramolecular hydrogen bonds are observed between the phloroglucinol core and the chromene moiety. The hydrogen bonds of the 4-OH and 6-OH across the methylene bridge were shown to have distances of 1.878 and 1.891 Å, respectively. This could restrict rotation around the methylene bridge, thus accounting for the presence of only a single rotamer in solution, as observed by NMR spectroscopy. We postulate that by altering the substituents on the acetophenone moiety, it could be possible to engineer molecules with various angles to tailor their interactions with biological systems.

Synthetic Methodology and Substrate Scope Investigation. The scope of the 1,4-Michael addition reaction methodology was investigated by reacting the key chromene intermediate (**13**) with various arenes, to simpler rottlerin-type analogues lacking the chalcone moiety but bearing different substituents attached to the methylene group. The chromene intermediate (**13**) was treated with Eschenmoser's salt and tetrabutylammonium fluoride (TBAF) in $CHCl_3$ at room temperature for 5 min, allowing aminomethylation and deprotection of the TBDMS group in a one-pot reaction (Scheme 5) to give **15** in 90% yield.

Scheme 5. Synthesis of Aminochromene (15) and Novel Rottlerin-Type Analogues

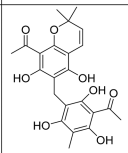
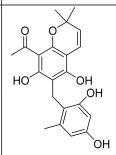
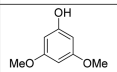
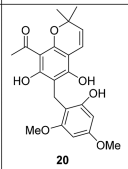
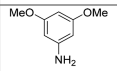
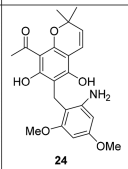
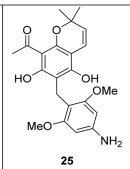
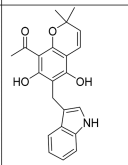
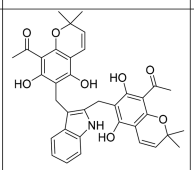
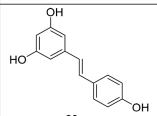
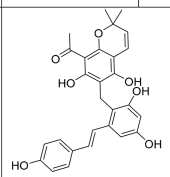


The reaction of aminochromene (**15**) with various electron-rich phenolic substrates (Scheme 5) such as acetophenone derivative **11**, orcinol (**17**), and dimethoxyphenol (**19**) provided new methylene-bridged compounds **16**, **18**, and **20**, respectively (Table 1, entries 1−3).

However, phenol (**21**) and the electron-deficient 3,5-difluorophenol (**22**) did not generate any products, and only starting material was recovered (Table 1, entries 4, 5). Probably the electron-deficient *ortho*-quinone methide intermediate[15] is favored to react with electron-rich arenes. Using the electron-rich 3,5-dimethoxyaniline (**23**) as substrate, two regioisomers, **24** and **25**, were produced in 14% and 20% yield, respectively (Table 1, entry 6).

The use of indole (**26**) as substrate afforded the new compounds **27** and **28** in 21% and 8% yield (Table 1, entry 7). Compound **27** was formed from the reaction of indole (**26**) at C-3 to one equivalent of chromene (**15**), whereas in compound **28**, indole **26** reacted at both C-2 and C-3 with two molecules of chromene (**15**) to form a dimeric species. The behavior of C-2 of indole acting as a nucleophilic center has also been reported by Sengul et al.[16] Recently, many research groups have reported that the structural modification of resveratrol (**29**) provides pronounced biological activities.[17−19] The reaction of resveratrol (**29**) using our methodology generated the highly oxygenated analogue **30** in 8% yield.

Table 1. List of Rottlerin-Type Analogues

Entry	Substrate	Product	Yield (%)
1	 **11**	 **16**	34
2	 **17**	 **18**	26
3	 **19**	 **20**	45
4	 **21**	N/A	0
5	 **22**	N/A	0
6	 **23**	 **24**  **25**	14 20
7	 **26**	 **27**  **28**	21 8
8	 **29**	 **30**	8

After multiple attempts of recrystallization, a single crystal was obtained for the orcinol-derived analogue (**18**) using MeCN as the solvent (Figure 3). The solid-state structure of **18** revealed only one intramolecular hydrogen bond across the methylene bridge, as opposed to the two hydrogen bonds observed for rottlerin (**1**). The measured distance of the hydrogen bond across the methylene bridge was 1.819 Å. The measured angle between the chromene and orcinol core of compound **18** was measured to be 69.96°, which is significantly greater than for rottlerin (**1**) (46.86°). As

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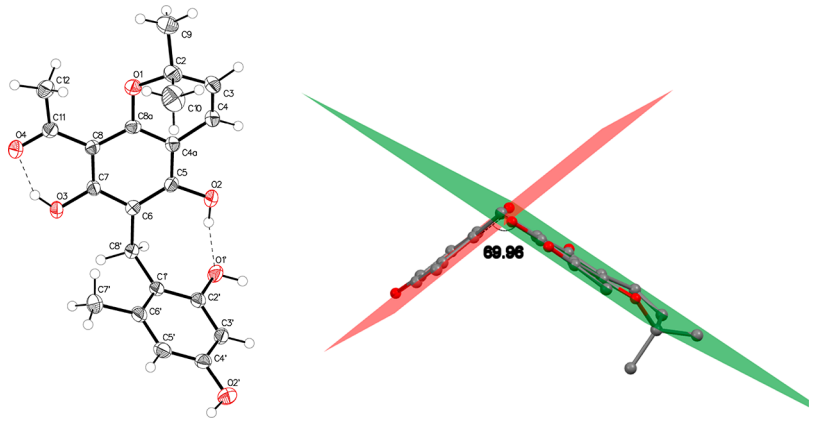
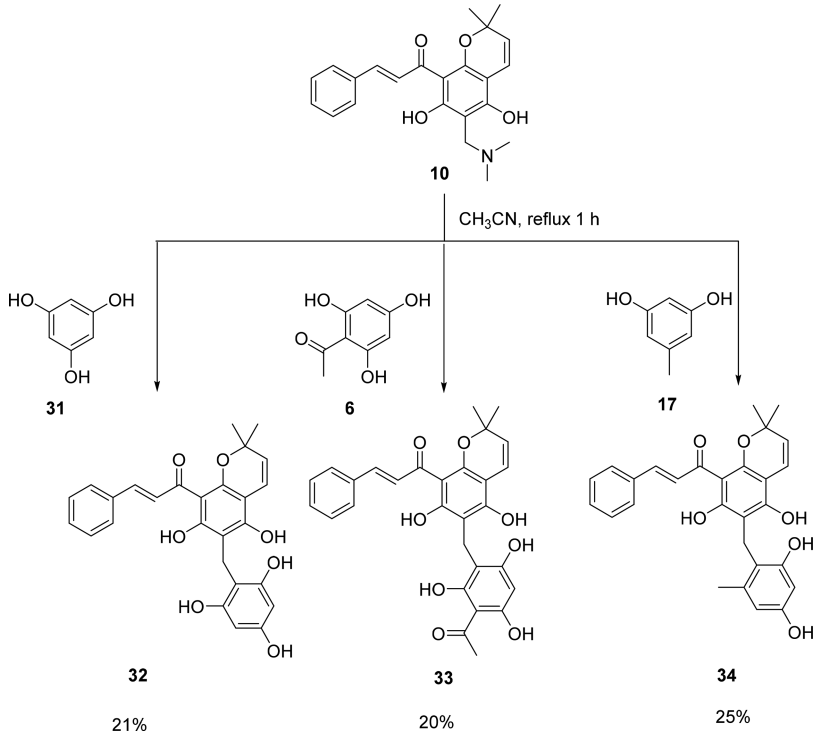


Figure 3. ORTEP diagram of compound **18** and the measured angle around the methylene bridge.

Scheme 6. Synthesis of New Rottlerin Analogues 32−34



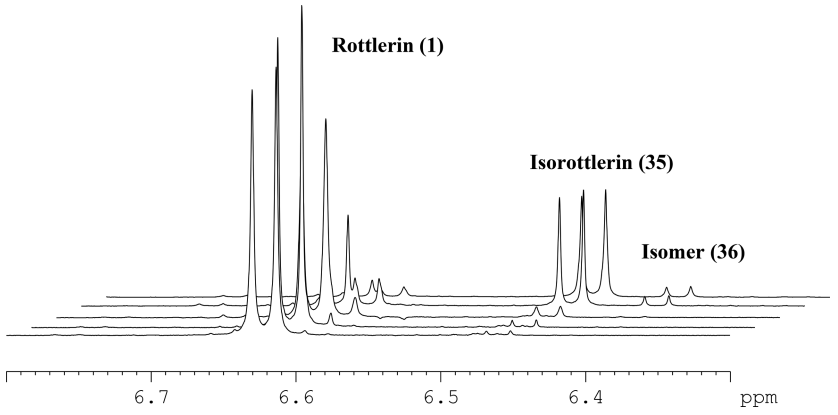


Figure 4. Stacked plot of ^{1}H NMR spectra of rottlerin (**1**) in DMSO-d_6 over two months showing the 6.30−6.80 ppm region.

observed, changing the number of hydrogen bonds can remarkably change the conformation of the molecule.

Overall, the methodology has shown the ability of generating unsymmetrical methylene-bridged compounds by utilizing the

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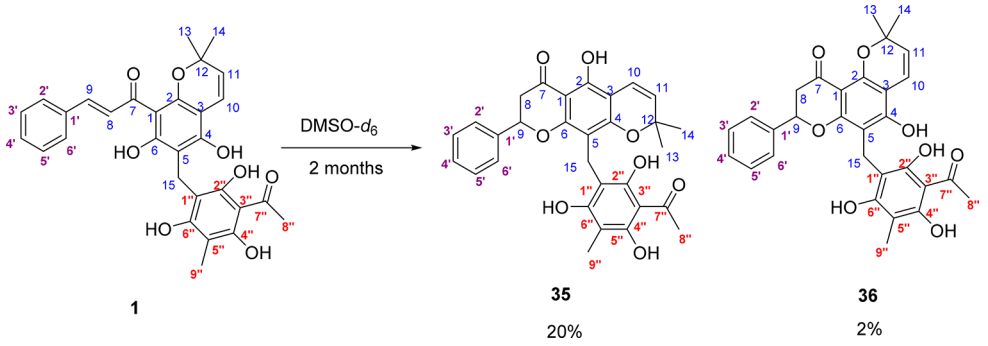
Scheme 7. Formation of Flavanones 35 and 36 from the Decomposition of Rottlerin (1)



generation of the *ortho*-quinone methide intermediate. However, due to the overall electrophilic nature of the *ortho*-quinone methide intermediate, only electron-rich arenes could undergo 1,4-addition to give methylene-bridged compounds.

Synthesis of Direct Rottlerin Analogues. With the scope of the methodology established, the 1,4-Michael addition reaction methodology was extended to direct rottlerin analogues bearing different substituents attached to the methylene bridge. Thus, the key aminochromene intermediate (**10**) was reacted with various phenolic derivatives such as phloroglucinol (**31**), phloroacetophenone (**6**), and orcinol (**17**) to generate the new rottlerin analogues **32**–**34** in moderate yields of 20–25% (Scheme 6). The low yields of these analogues were mainly caused by incomplete reactions and the loss of products through chromatography.

Stability of Rottlerin and the Isorottlerin Change. The stability of rottlerin (**1**) was investigated in DMSO, a commonly used solvent to dissolve compounds for biological testing. The stability of rottlerin (**1**) in DMSO-d_6 was monitored over two months at room temperature using NMR spectroscopy (Figure 4). The characteristic H-10 at 6.63 ppm (d, J = 10 Hz) of rottlerin (**1**) disappeared over time, while two new doublets at 6.46 and 6.42 ppm with the same coupling constant of 10 Hz appeared, suggesting that rottlerin (**1**) slowly reacted to form two distinct products in an approximately 7:1 ratio.

The mixture of the two products could not be separated by silica-gel chromatography due to their close R_f values. Therefore, preparative RP-HPLC was used to separate the two products. The products were identified as isorottlerin (**35**) and flavanone (**36**), which were isolated in yields of 20% and 2%, respectively, as racemic mixtures (Scheme 7).

The identities of compounds **35** and **36** were confirmed by phase-sensitive HSQC experiments. The presence of diastereotopic C-8 protons in the aliphatic region suggested the flavanone core in both molecules. Moreover, the appearance of a deshielded H-9 with three-bond correlations to both C-7 and C-6 in the HMBC spectrum (Figure 5) provided further evidence for the core structure of the molecules. The distinctive peak to distinguish between the two compounds was the presence of a four-bond HMBC correlation from the HO-2 to C-7 of isorottlerin (**37**), which is absent in compound **38**, as the OH group is at the 4-position and hence too far away from C-7.

As seen from the NMR stacked plot, the major isomer after incubating rottlerin (**1**) in DMSO-d_6 at room temperature for two months was isorottlerin (**35**), whereas the minor isomer was **36**. The conversion of rottlerin (**1**) into the two products could be accelerated by heating in DMSO-d_6 at 100 °C for 2 h,

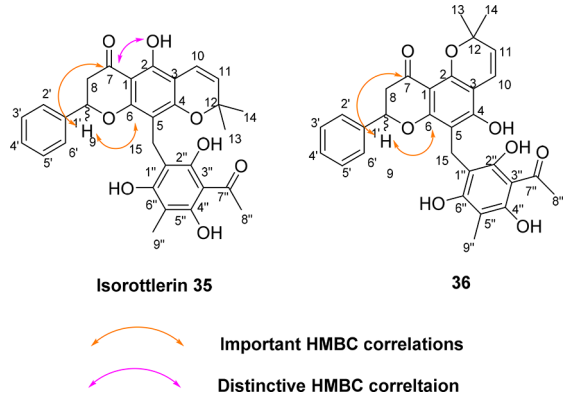


Figure 5. Important and distinctive HMBC correlations of the two isomers **35** and **36**.

which led to full conversion of rottlerin (**1**) into the two products in an approximately 1:1 ratio. The formation of the two products required the presence of DMSO, as the use of MeCN, toluene, acetone, and $CHCl_3$ as solvents did not lead to any changes in the NMR spectrum of rottlerin (**1**) even with intense heating. However, the exact mechanism of this reaction is yet to be determined. Nevertheless, the long-term storage of rottlerin (**1**) as a solid is recommended to prevent the conversion of rottlerin (**1**) to isorottlerin (**35**) and flavanone **36** in DMSO solution. If DMSO stock solutions are required, storage at low temperature, preferably at −20 °C or below, is recommended, along with regular NMR spectroscopic measurements to ensure that rottlerin (**1**) has not decomposed.

Isorottlerin (**35**) has not been synthesized before, and the transformation of rottlerin (**1**) to isorottlerin (**35**) was both unexpected and unprecedented. We named this transformation the "isorottlerin change", in analogy with the "rottlerone change".[20] The "rottlerone change" describing the conversion of rottlerin (**1**) to rottlerone under basic or acidic conditions (Scheme 8) has been reported and involves a disproportionation reaction.[21]

Antibacterial Activities of Compounds. Rottlerin (**1**), its analogues **32**–**34**, and its isomerization products **35** and **36** were tested for their antibacterial activities against *S. aureus*. The antimicrobial activity of the synthesized compounds was evaluated by determination of their MIC values. The clinically used antibiotic gentamicin was used as a positive control. The MIC values were also compared with drummondins A (**2**), B (**3**), C (**4**), and F (**5**), as they are structurally similar to rottlerin.[8] The results showed that synthesized rottlerin (**1**) showed excellent antibacterial activity against *S. aureus* with an

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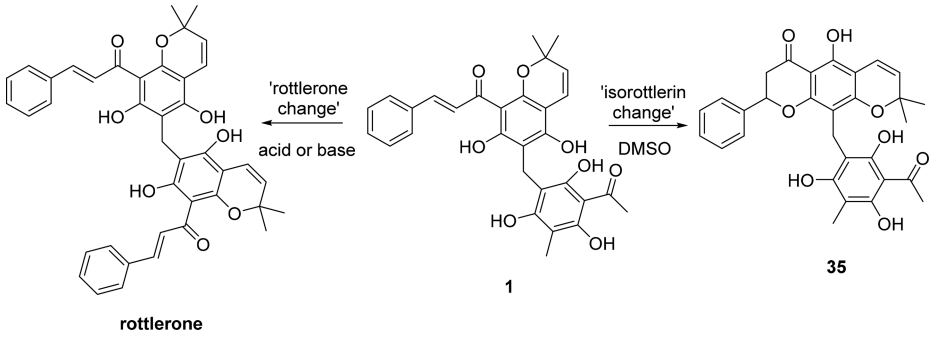
Scheme 8. "Rottlerone Change" and "Isorottlerin Change" of Rottlerin (1)



MIC value of 2.0 μM, making it comparable in potency with gentamicin and drummondins A (**2**), B (**3**), C (**4**), and F (**5**) (Table 2). On the other hand, the two isomers **35** and **36**

Table 2. MIC Values of Synthesized and Known Compounds against *S. aureus*

compound	MIC (μM)
gentamicin	2.0
rottlerin (**1**)	2.0
32	15.6
33	7.8
34	7.8
isorottlerin (**35**)	>125
36	>125
drummondins A (**2**)	1.56
drummondins B (**3**)	3.12
drummondins C (**4**)	3.12
drummondins F (**5**)	0.78

exhibited no antibacterial activity below 125 μM, suggesting that the chalcone moiety is essential for activity. Analogues **32**−**34** exhibited good antibacterial activity against *S. aureus*, with MICs of 15.6, 7.8, and 7.8 μM, respectively. The lower antibacterial activity of the analogues **32**−**34** compared to rottlerin (**1**) suggests that the nature of the arene attached to the methylene bridge is an important determinant of activity.

The underlying mechanism of rottlerin (**1**) against *S. aureus* is currently being investigated by our research group. Recent studies have suggested some possible mechanisms of the antiparasitic and antibacterial effects of rottlerin. For instance, rottlerin (**1**) has antiparasitic activity against *Toxoplasma gondii* through the inhibition of mTOR and elF2α phosphorylation, which accounts for their autophagy induction and inhibition of protein synthesis.[5] Rottlerin (**1**) also blocks ceramide trafficking from host Golgi apparatus to inhibit chlamydial growth by suppressing the ability of *Chlamydia* to acquire lipids.[6] The fact that rottlerin (**1**) targets various signaling pathways is of interest, and we are currently attempting to pinpoint the possible mechanism of its antibacterial activity against *S. aureus*.

In summary, the synthesis of rottlerin (**1**) has been simplified from the previous seven-step synthesis to a practical five-step gram-scale synthesis that can be achieved in 2 days. The key to the successful scalable synthesis of rottlerin (**1**) involves the use of the TBDMS protecting group to avoid the formation of rottlerone due to the deprotection of the MOM group under acidic conditions. The subsequent one-pot aldol condensation and deprotection is *de novo* and ultimately allowed rottlerin (**1**) to be synthesized in gram scale. The general substrate scope for the synthesis of methylene-bridged type compounds and rottlerin analogues was also investigated, revealing that electron-rich arenes were favored for 1,4-Michael addition reaction with the putative *ortho*-quinone methide intermediate. X-ray crystallography analysis of rottlerin (**1**) and its analogue also suggested that the conformation of the molecules in the solid state could be carefully tuned by altering the substituents on the arene group. Rottlerin (**1**) was shown to react with DMSO to undergo an unprecedented "isorottlerin change" to give the natural product isorottlerin (**35**). The mechanism for this transformation remains to be elucidated. The antibacterial activity of rottlerin and novel rottlerin analogues **32**−**36** was also tested against *S. aureus*. The results showed that rottlerin (**1**) was similarly potent to the clinically used antibiotic gentamicin, outlining the potential for its development as an antimicrobial agent.

EXPERIMENTAL SECTION

1-{4-[(*tert*-Butyldimethylsilyl)oxy]-2,6-dihydroxyphenyl}-ethan-1-one (12). To a solution of acetophenone **6** (4.0 g, 21.87 mmol) in dimethylformamide (DMF) (40 mL) at 0 °C were added imidazole (3.7 g, 53.72 mmol) and TBDMSCl (8.1 g, 53.72 mmol). The mixture was allowed to stir at room temperature for 15 min, then poured into water, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in MeOH (40 mL), and PPTS (6.7 g, 21.87 mmol) was added. The mixture was heated at reflux for 2 h, the solvent was removed *in vacuo*, water was added, and the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography on silica gel using *n*-hexane/EtOAc (19:1) to give **12** (6.5 g, 85%) as a white solid: mp 110−114 °C; lit.[14] 111−113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.1 (s, 2H, 2 × OH), 5.93 (s, 2H, H3, H5), 2.72 (s, 3H, $COCH_3$), 0.93 (s, 9H, $C(CH_3)_3$), 0.18 (s, 6H, $Si(CH_3)_2$).

1-{5-[(*tert*-Butyldimethylsilyl)oxy]-7-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl}ethan-1-one (13). To a solution of acetophenone **12** (6.4 g, 22.66 mmol) in $CHCl_3$ (50 mL) were added EDDA (4.1 g, 2.27 mmol) and 3-methyl-2-butenal (2.4 mL, 24.93 mmol). The reaction mixture was stirred for 10 min, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography on silica gel using *n*-hexane/EtOAc (40:1) to give **13** as a yellow solid (7.1 g, 90%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (d, J = 10.0 Hz, 1H, H4), 5.91 (s, 1H, H6), 5.39 (d, J = 10.0 Hz, 1H, H3), 2.64 (s, 3H, $COCH_3$), 1.47 (s, 6H, 2 × CH_3), 0.98 (s, 9H, $C(CH_3)_3$), 0.24 (s, 6H, $Si(CH_3)_2$).

(*E*)-1-(5,7-Dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one (9). Chromene **13** (7.0 g, 20.09 mmol) was dissolved in anhydrous tetrahydrofuran (THF) (40 mL) under an argon atmosphere. The solution was cooled to 0 °C, and 60% NaH in mineral oil (4.0 g, 100.45 mmol) was added in small portions over 5

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min. The mixture was allowed to stir for 5 min, and benzaldehyde (4.1 mL, 40.18 mmol) was added. The resulting mixture was allowed to stir for 2 h, poured into water (50 mL), and stirred for an additional 30 min. The mixture was then extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed with brine, dried using Na_2SO_4, and evaporated *in vacuo*. Flash chromatography on silica gel using *n*-hexane/EtOAc (8:2) gave **9** (4.5 g, 69%) as a red oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.23 (s, 1H, OH), 8.11 (d, J = 16 Hz, 1H, H_β), 7.76 (d, J = 16 Hz, 1H, H_α), 7.58−7.62 (m, 2H, ArH), 7.24−7.43 (m, 3H, ArH), 6.59 (d, J = 10 Hz, 1H, H4), 6.01 (s, 1H, H6), 5.48 (d, J = 10 Hz, 1H, H3), 1.55 (s, 6H, 2 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.2 (C═O), 166.5 (ArC), 158.8 (ArC), 156.9 (ArC), 142.6 (C_β), 135.7 (ArC), 130.3 (ArH), 129.1 (ArC), 128.4 ($C\alpha$), 127.5 (ArH), 124.9 (H3), 116.7 (H4), 106.6 (C8), 102.7 (C4a), 96.5 (C6), 78.4 (C2), 28.1 (2 × CH_3); HRMS (ESI) m/z calcd for $C_{20}H_{18}O_4$ $(M + H)^+$ 323.1278, found 323.1276.

(*E*)-1-{6-[(Dimethylamino)methyl]-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl}-3-phenylprop-2-en-1-one (10). To a solution of chromene **9** (4.50 g, 13.96 mmol) in $CHCl_3$ was added Eschenmoser's salt (0.057 g, 0.31 mmol). The mixture was heated at reflux for 0.5 h. After completion, water (50 mL) was added and the mixture extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were washed with brine, dried using Na_2SO_4, and evaporated *in vacuo*. A small amount of MeCN was added, and an orange precipitate was formed, which was filtered and dried to give **10** (4.66 g, 88%) as an orange powder: mp 110−114 °C; IR (neat) ν_{max} 2966, 2925, 1624, 1590, 1340, 1130, 972 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.14 (s, 1H, OH), 8.11 (d, J = 15.6 Hz, 1H, H_β), 7.81 (d, J = 15.6 Hz, 1H, H_α), 7.59−7.63 (m, 2H, ArH), 7.39−7.46 (m, 3H, ArH), 6.80 (d, J = 10.0 Hz, 1H, H4), 5.51 (d, J = 10.0 Hz, 1H, H3), 4.21 (s, 2H, NCH_2), 2.86 (s, 6H, $N(CH_3)_2$), 1.56 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.3 (C═O), 166.4 (ArC), 160.1 (ArC), 158.2 (ArC), 143.5 (C_β), 135.5 (ArC), 130.6 (ArCH), 129.2 (ArC), 128.5 (C_α), 127.1 (ArCH), 125.1 (C3), 117.5 (C4), 106.0 (ArC), 105.4 (ArC), 99.0 (ArC), 79.1 (C2), 51.0 (NCH_2), 42.1 ($N(CH_3)_2$), 28.5 (2 × CH_3); HRMS (ESI) m/z calcd for $C_{23}H_{25}NO_4$ $(M + H)^+$ 380.1856, found 380.1858.

1-{6-[(Dimethylamino)methyl]-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl}ethan-1-one (15). To a solution of **13** (1.0 g, 2.87 mmol) in $CHCl_3$ (20 mL) were added Eschenmoser's salt (0.80 g, 4.30 mmol) and 1 M TBAF in THF (2.87 mL, 2.87 mmol). The reaction was allowed to stir for 5 min. The solution was evaporated to dryness to give a crude yellow-orange solid. The residue was washed with hot *n*-hexane (3 × 20 mL) and filtered while hot. The filtrate was evaporated *in vacuo* to give **15** as a bright yellow powder (0.75 g, 90%): mp 146−150 °C; IR (neat) ν_{max} 3150, 2868, 1684, 1640, 1603, 1467, 1361 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.6 (s, 1H, OH), 6.79 (d, J = 10.0 Hz, 1H, H4), 5.45 (d, J = 10.0 Hz, 1H, H3), 4.22 (s, 2H, NCH_2) 2.84 (s, 3H, NCH_3), 2.82 (s, 3H, NCH_3), 1.48 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.8 (C═O), 165.4 (ArC), 159.8 (ArC), 125.0 (C3), 117.2 (C4), 104.6 (ArC), 98.1 (NCH_2), 78.4 (C2), 50.1 (NCH_2), 33.2 ($COCH_3$), 28.0 (2 × CH_3); HRMS (ESI) m/z calcd for $C_{16}H_{22}NO_4$ $(M + H)^+$ 292.1543, found 292.1544.

General Experimental Procedures for the Synthesis of Rottlerin (1) and Methylene-Bridged Chromene Compounds (16, 18, 20, 24, 25, 27, 28, 30, 32−34). Dimethylaminomethylchromene (**10** or **15**, 1.0 equiv) and arene (1.0 equiv) were added to MeCN (10 mL), and the mixture was heated at reflux for 2 h. The reaction was monitored by NMR by taking a small aliquot from the reaction mixture. The solvent was evaporated *in vacuo*, and the residue was purified by flash column chromatography to give the methylene-bridged chromene compound.

Rottlerin (1). Compound **1** was obtained (1.90 g, 31%) as a red-orange solid: mp 210−214 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 16.51 (s. 1H), 15.60 (bs, 1H, OH), 9.55 (bs, 1H, OH), 8.19 (d, J = 15.6 Hz, 1H, H8), 7.84 (d, J = 15.6 Hz, 1H, H9), 7.59−7.63 (m, 2H, H3′, H5′), 7.39−7.45 (m, 3H, H2′, H4′, H6′), 6.66 (d, J = 10.1 Hz, 1H, H10), 5.49 (d, J = 10.1 Hz, 1H, H11), 3.81 (s, 2H, H15), 2.71 (s, 3H, H8″), 2.08 (s, 3H, H9″), 1.53 (s, 6H, H13, H14); ^{13}C (150 MHz, $CDCl_3$) δ 204.2 (C7″), 193.0 (C7), 163.0 (C6), 160.8 (C6″), 159.7 (C2″), 158.9 (C4), 156.6 (C4″), 155.6 (C2), 143.5 (C9), 135.6 (C1′), 130.5 (C4′), 129.2 (C3′,C5′), 128.5 (C2′,C6′), 126.9 (C8), 125.2 (C11), 117.4 (C10), 106.6 (C5), 106.1 (C1″), 105.4 (C1), 104.6 (C3″), 103.9 (C3), 102.0 (C5″), 78.3 (C12), 32.7 (C8″), 28.2 (C13, C14), 16.0 (C15), 7.6 (C9″); HRMS (ESI) m/z calcd for $C_{30}H_{28}O_8$ $(M + H)^+$ 517.1857, found 517.1851.

1-[6-(3-Acetyl-2,4,6-trihydroxy-5-methylbenzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]ethan-1-one (16). The product was obtained as a white solid (25 mg, 34%): mp 186−190 °C; IR (neat) ν_{max} 3324, 2974, 2825, 1611, 1508, 1336, 1117 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 6.63 (d, J = 10.0 Hz, 1H, H4), 5.56 (d, J = 10.0 Hz, 1H, H3), 3.78 (s, 2H, CH_2), 2.69 (s, 3H, $COCH_3$), 2.68 (s, 3H, $COCH_3$), 2.07 (s, 3H, CH_3), 1.50 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, acetone-d_6) δ 205.1 (C═O), 204.6 (C═O), 162.2 (C7), 161.0 (C6′), 160.3 (C4′), 159.6 (C2′), 156.8 (C8a), 126.4 (C3), 117.6 (C4), 107.2 (C6), 106.0 (C1′), 105.4 (C5′), 104.2 (C4a), 79.0 (C2), 33.0 ($COCH_3$), 32.8 ($COCH_3$), 27.9 (2 × CH_3), 16.4 (CH_2), 8.4 (CH_3); HRMS (ESI) m/z calcd for $C_{23}H_{24}O_8$ $(M + Na)^+$ 451.1363, found 451.1359.

1-[6-(2,4-Dihydroxy-6-methylbenzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]ethan-1-one (18). The product was obtained as a white solid (15 mg, 26%): mp 172−176 °C; IR (neat) ν_{max} 3252, 2917, 1638, 1561, 1424, 1361, 1210, 1109 cm^{-1}; ^{1}H NMR (600 MHz, CD_3CN) δ 14.49 (s, 1H, OH), 6.55 (d, J = 10.0 Hz, 1H, H4), 6.25 (d, J = 2.4 Hz, 1H, H3′), 6.24 (d, J = 2.4 Hz, 1H, H5′), 5.48 (d, J = 10.0 Hz, 1H, H3), 3.79 (s, 2H, CH_2), 2.64 (s, 3H, $COCH_3$), 2.23 (s, 3H, CH_3), 1.45 (s, 6H, 2 × CH_3); ^{13}C NMR (100 MHz, CD_3CN) δ 204.7 (C═O), 164.0 (C7), 158.9 (C5), 156.4 (C8a), 154.5 (C2′), 126.2 (C3), 117.4 (C4), 116.9 (C1′), 111.5 (C5′), 107.0 (C6), 106.0 (C8), 103.1 (C4a), 100.6 (C6′), 100.4 (C3′), 78.8 (C2), 33.6 ($COCH_3$), 27.9 (2 × CH_3), 20.4 (CH_3), 18.5 (CH_2); HRMS (ESI) m/z calcd for $C_{21}H_{22}O_6$ $(M + Na)^+$ 393.1309, found 393.1306.

1-[5,7-Dihydroxy-6-(2-hydroxy-4,6-dimethoxybenzyl)-2,2-dimethyl-2*H*-chromen-8-yl]ethan-1-one (20). The product was obtained as a white solid (38 mg, 45%): mp 172−176 °C; UV (THF) λ_{max} 279 (ε 37 800 cm^{-1} M^{-1}) nm; IR (neat) ν_{max} 3289, 2924, 1636, 1599, 1516, 1467, 1365, 1268, 1137 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 6.60 (d, J = 10.0 Hz, 1H, H4), 6.22 (d, J = 2.4 Hz, 1H, H3′), 6.13 (d, J = 2.4 Hz, 1H, H5′), 5.57 (d, J = 10.0 Hz, 1H, H3), 4.00 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 2.67 (s, 3H, $COCH_3$), 1.50 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, acetone-d_6) δ 203.9 (C═O), 161.7 (C8), 160.2 (C4′), 157.9 (C5), 157.2 (C2′), 156.7 (C6′), 155.7 (C8a), 125.4 (C3), 116.3 (C4), 108.2 (C6), 106.0 (C1′), 102.7 (C4a), 95.2 (C3′), 91.0 (C5′), 78.2 (C2), 55.9 (OCH_3) 54.3 (OCH_3), 32.3 ($COCH_3$), 27.0 (2 × CH_3), 15.3 (CH_2); HRMS (ESI) m/z calcd for $C_{22}H_{24}O_7$ $(M + Na)^+$ 423.1414, found 423.1410.

1-[6-(2-Amino-4,6-dimethoxybenzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]ethan-1-one (24). The product was obtained as a yellow solid (26 mg, 14%): mp 170−174 °C; IR (neat) ν_{max} 3440, 3298, 2925, 1618, 1590, 1545, 1423, 1134, cm^{-1}; ^{1}H NMR (600 MHz, CD_3CN) δ 15.0 (s, 1H, OH), 6.57 (d, J = 10.0 Hz, 1H, H4), 6.02 (d, J = 2.4 Hz, 1H, H3′), 6.00 (d, J = 2.4 Hz, 1H, H5′), 5.52 (d, J = 10.0 Hz, 1H, H3), 3.99 (s, 3H, OCH_3), 3.73 (s, 2H, CH_2), 3.69 (s, 3H, OCH_3), 2.66 (s, 3H, $COCH_3$), 1.48 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3CN) δ 203.4 (C═O), 162.3 (C7), 160.2 (C4′), 158.5 (C5), 157.0 (C6′), 155.4 (C8a), 148.5 (C2′), 125.1 (C3), 116.5 (C4), 105.8 (C6), 104.7 (C8), 103.3 (C1′), 102.3 (C4a), 94.2 (C3′), 87.8 (C5′), 77.9 (C2), 55.8 (OCH_3), 54.4 (OCH_3), 32.4 ($COCH_3$), 27.0 (2 × CH_3), 16.0 (CH_2); HRMS (ESI) m/z calcd for $C_{22}H_{25}NO_6$ $(M + H)^+$ 400.1755, found 400.1753.

1-[6-(4-Amino-2,6-dimethoxybenzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]ethan-1-one (25). The product was obtained as yellow oil (26 mg, 14%): IR (neat) ν_{max} 3438, 3320, 2970, 1610, 1589, 1464, 1342, 1173, cm^{-1}; ^{1}H NMR (600 MHz, CD_3CN) δ 14.3 (s, 1H, OH), 8.65 (s, 1H, OH), 6.54 (d, J = 10 Hz, 1H, H4), 6.04 (s, 2H, H3′, H5′), 5.46 (d, J = 10 Hz, 1H, H3), 4.66 (bs, 2H, NH_2), 3.79 (s, 2H, CH_2), 3.78 (s, 6H, 2 × OCH_3), 2.63 (s, 3H,

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$COCH_3$), 1.47 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3CN) δ 203.8 (C═O), 164.8 (C7), 159.3 (C1′, C6′), 158.8 (C5), 155.8 (C8a), 149.8 (C4′), 125.2 (C3), 117.7 (C4), 107.7 (C6), 105.8 (C8), 104.5 (C1′), 92.2 (C3′, C5′), 78.4 (C2), 56.0 (2 × OCH_3), 33.6 ($COCH_3$), 28.0 (2 × CH_3), 16.0 (CH_2); HRMS (ESI) m/z calcd for $C_{22}H_{25}NO_6$ $(M + Na)^+$ 422.1574, found 422.1575.

1-{6-[(1*H*-Indol-3-yl)methyl]-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl}ethan-1-one (27). The product was obtained as a white solid (26 mg, 21%): mp 160−164 °C; IR (neat) ν_{max} 3540, 3220, 1611, 1338, 1203, 1111 cm^{-1}; ^1H NMR (600 MHz, CD_3CN) δ 14.20 (s, 1H, OH), 9.00 (s, 1H, OH), 7.61 (d, J = 7.9 Hz, 1H, H7′), 7.36 (d, J = 8.1 Hz, H4′), 7.11 (ddd, J = 1.0, 8.1, 15.1 Hz, 1H, H6′), 7.02 (ddd, J = 1.0, 8.1, 15.1 Hz, 1H, H5′), 6.89−6.91 (m, 1H, H2′), 6.54 (d, J = 9.9 Hz, 1H, H4), 5.54 (d, J = 9.9 Hz, 1H, H3), 4.00 (s, 2H, CH_2), 2.66 (s, 3H, $COCH_3$), 1.48 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3CN) δ 204.8 (C═O), 164.3 (C7), 158.0 (C5), 156.3 (C8a), 137.7 (C7′a), 128.3 (C3′a), 126.5 (C3), 123.5 (C2′), 122.5 (C6′), 119.9 (C7′), 119.7 (C5′), 117.3 (C4), 114.4 (C3′), 112.2 (C4′), 107.6 (C6), 106.5 (C8), 103.1 (C4a), 78.7 (C2), 33.7 ($COCH_3$), 27.8 (2 × CH_3), 18.8 (CH_2); HRMS (ESI) m/z calcd for $C_{22}H_{21}NO_4$ $(M + Na)^+$ 386.1363, found 386.1361.

1,1′-{[(1*H*-Indole-2,3-diyl)bis(methylene)]bis(5,7-dihydroxy-2,2-dimethyl-2*H*-chromene-6,8-diyl)}bis(ethan-1-one) (28). The product was obtained as a white solid (17 mg, 8%): mp 156−160 °C; IR (neat) ν_{max} 3552, 3260, 1609, 1554, 1460, 1209, 1105 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 14.4 (s, 1H, OH), 8.88 (s, 1H, OH), 8.05 (s, 1H, OH), 7.33 (d, J = 8.0 Hz, 1H, H7″), 7.20 (d, J = 8.0 Hz, 1H, H4″), 6.95 (ddd, J = 1.1, 8.1, 15.1 Hz, 1H, H6″), 6.85 (ddd, J = 1.1, 8.1, 15.1 Hz, 1H, H5″), 6.58 (d, J = 9.9 Hz, 1H, H4), 6.49 (d, J = 9.9 Hz, 1H, H4′), 5.50 (d, J = 9.9 Hz, 1H, H3), 5.44 (d, J = 9.9 Hz, 1H, H3′), 4.12 (s, 2H, CH_2), 4.09 (s, 2H, CH_2), 2.65 (s, 6H, 2 × $COCH_3$), 1.47 (s, 6H, 2 × CH_3), 1.44 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 203.0 (2 × C═O), 163.5 (ArC), 159.9 (ArC), 155.8 (ArC), 155.2 (ArC), 135.5 (ArC), 128.4 (ArC), 124.7 (ArCH), 124.6 (ArCH), 120.5 (ArCH), 120.4 (ArCH), 118.6 (ArCH), 118.4 (ArCH), 117.0 (ArCH), 116.9 (ArCH), 110.4 (ArCH), 108.0 (ArC), 105.9 (ArC), 104.8 (ArC), 103.2 (ArC), 102.8 (ArC), 77.5 (C2), 77.3 (C2′), 32.4 (2 × $COCH_3$), 26.8 (2 × CH_3), 26.7 (2 × CH_3), 19.1 (CH_2), 17.3 (CH_2); HRMS (ESI) m/z calcd for $C_{36}H_{35}NO_8$ $(M + Na)^+$ 632.2255, found 632.2257.

(*E*)-1-{6-[2,4-Dihydroxy-6-(4-hydroxystyryl)benzyl]-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl}ethan-1-one (30). The product was obtained as a white solid (16 mg, 8%): mp 178−182 °C; IR (neat) ν_{max} 3243, 2979, 1594, 1433, 1304, 1170 cm^{-1}; ^1H NMR (600 MHz, CD_3CN) δ 14.89 (s, 1H, OH), 7.62 (d, J = 16.0 Hz, 1H, H_α), 7.38 (d, J = 8.6 Hz, 2H, H2″, H6″), 6.85 (d, J = 16.0 Hz, 1H, H_β), 6.78 (d, J = 8.6 Hz, 2H, H3″, H5″), 6.71 (d, J = 2.1 Hz, H5′), 6.54 (d, J = 10.0 Hz, H4), 6.36 (d, J = 2.1 Hz, 1H, H3′), 5.46 (d, J = 10.0 Hz, 1H, H3), 3.94 (s, 2H, CH_2), 2.61 (s, 3H, $COCH_3$), 1.42 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3CN) δ 204.5 (C═O), 163.4 (C7), 159.7 (C5), 157.7 (C4″), 157.1 (C4′), 156.4 (C8a), 155.2 (C2′), 140.8 (C6′), 130.7 (C_β), 129.1 (C2″, C6″), 126.1 (C3), 125.2 (Cα), 117.5 (C4), 117.0 (C1′), 116.4 (C3″, C5″), 107.8 (C6), 105.8 (C8), 105.6 (C5′), 103.2 (C4a), 102.4 (C3′), 78.8 (C2), 33.5 ($COCH_3$), 27.9 (2 × CH_3), 18.0 (CH_2); HRMS (ESI) m/z calcd for $C_{28}H_{26}O_7$ $(M + Na)^+$ 497.1571, found 497.1567.

(*E*)-1-[5,7-Dihydroxy-2,2-dimethyl-6-(2,4,6-trihydroxybenzyl)-2*H*-chromen-8-yl]-3-phenylprop-2-en-1-one (32). The product was obtained as a red solid (20 mg, 21%): mp 248−252 °C; UV (THF) λ_{max} 285 (ε 121184 cm^{-1} M^{-1}), 349 (28376) nm; IR (neat) ν_{max} 3252, 2972, 1594, 1609, 1545, 1419, 1340, 1223, 1151 cm^{-1}; ^1H NMR (600 MHz, acetone-d_6) δ 8.28 (d, J = 15.6 Hz, 1H, H_β), 7.80 (d, J = 15.6 Hz, 1H, H_α), 7.71−7.73 (m, 2H, H3″, H5″), 7.43−7.51 (m, 3H, H2″, H4″, H6″), 6.67 (d, J = 10.0 Hz, 1H, H4), 5.98 (s, 2H, H3′, H5′), 5.53 (d, J = 10.0 Hz, 1H, H3), 3.74 (s, 2H, CH_2), 1.55 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, acetone-d_6) δ 192.2 (C═O), 163.3 (C7), 158.3 (C4′), 156.9 (C2′, C6′), 156.1 (C8a), 142.8 (C_α), 136.6 (C1″), 131.1 (4″), 130.0 (C3″, C5″), 129.1 (C2″, 6″), 128.1 (C_β), 125.3 (C3), 118.4 (C4), 108.9 (C6), 106.1 (C1′), 105.1 (C4a), 96.3 (C3′, C5′), 78.7 (C2), 28.2 (2 × CH_3), 16.9 (CH_2); HRMS (ESI) m/z calcd for $C_{27}H_{24}O_7$ $(M + H)^+$ 461.1595, found 461.1590.

(*E*)-1-[6-(3-Acetyl-2,4,6-trihydroxybenzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]-3-phenylprop-2-en-1-one (33). The product was obtained as a red solid (40 mg, 20%): mp 248−252 °C; UV (THF) λ_{max} 284 (ε 65916 cm^{-1} M^{-1}), 356 (47511) nm; IR (neat) ν_{max} 3224, 2978, 1649, 1539, 1481, 1345, 1217, cm^{-1}; ^1H NMR (600 MHz, acetone-d_6) δ 8.28 (d, J = 15.6 Hz, 1H, H_β), 7.82 (d, J = 15.6 Hz, 1H, H_β), 7.73−7.75 (m, 2H, H3″, H5″), 7.45−7.51 (m, 3H, H2″, H4″, H6″), 6.68 (d, J = 10.0 Hz, 1H, H4), 5.99 (s, 1H, H5′), 5.56 (d, J = 10.0 Hz, 1H, H3), 3.76 (s, 2H, CH_2), 2.66 (s, 3H, $COCH_3$), 1.56 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, acetone-d_6) δ 203.8 (C═O), 191.5 (C═O), 162.8 (C2′), 161.2 (C5′), 155.3 (C8a), 142.3 (C_α), 135.6 (C1″), 130.3 (C4″), 129.1 (C3″, C5″), 128.3 (C2″, C4″), 127.0 (C_β), 124.6 (C3), 117.3 (C4), 107.1 (C6), 105.7 (C1′), 104.7 (C3′), 104.1 (C4a), 95.6 (C5′), 78.0 (C2), 31.8 ($COCH_3$), 27.1 (2 × CH_3), 15.6 (CH_2); HRMS (ESI) m/z calcd for $C_{29}H_{28}O_8$ $(M + H)^+$ 504.1700, found 504.1693.

(*E*)-1-[6-(2,4-Dihydroxy-6-methylbenzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]-3-phenylprop-2-en-1-one (34). The product was obtained as a white solid (66 mg, 25%): mp 248−252 °C; UV (THF) λ_{max} 286 (ε 70 389 cm^{-1} M^{-1}), 351 (29 546) nm; IR (neat) ν_{max} 3244, 2978, 1600, 1508, 1471, 1345, 1113 cm^{-1}; ^1H NMR (600 MHz, CD_3CN) δ 14.82 (s, 1H, OH), 8.15 (d, J = 15.6 Hz, 1H, H_β), 7.74 (d, J = 15.6 Hz, 1H, H_α), 7.66−7.70 (m, 2H, H3″, H5″), 7.43−7.47 (m, 3H, H2″, H4″, H6″), 6.58 (d, J = 10.0 Hz, 1H, H4), 6.27 (d, J = 2.4 Hz, 1H, C3′), 6.25 (d, J = 2.4 Hz, 1H, C5′), 5.53 (d, J = 10.0 Hz, 1H, H3), 3.83 (s, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.51 (s, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3CN) δ 192.9 (C═O), 164.0 (C7), 158.9 (C5), 155.8 (C4′), 154.8 (C8a), 153.7 (C2′), 141.5 (C_α), 135.5 (C1″), 130.3 (C4″), 129.1 (C3″, C5″), 128.2 (C2″, C4″), 127.8 (C_α), 125.1 (C3), 116.7 (C4), 116.0 (C6′), 110.4 (C5′), 106.6 (C6), 102.6 (C4a), 99.7 (C3′), 78.1 (C2), 27.1 (2 × CH_3), 19.5 (CH_3), 17.7 (CH_2); HRMS (ESI) m/z calcd for $C_{28}H_{26}O_6$ $(M + Na)^+$ 481.1622, found 481.1619.

Isorottlerin (35). Rottlerin **1** (50 mg, 0.097 mmol) was dissolved in DMSO (1 mL) and heated at 100 °C for 2 h. The solution was cooled and the mixture purified directly by preparative HPLC using a 5 μM column (150 × 10 mm i.d.) at a flow rate of 7 mL min^{-1} with a gradient elution from 0 to 100% B (solvent A: 0.1% TFA/H_2O and solvent B: 0.1% TFA/CH_3CN) over 60 min, to afford isorottlerin (**35**) (10 mg, 20%) as a yellow solid: mp 118−122 °C; IR (neat) ν_{max} 3347, 2974, 1616, 1581, 1516, 1438, 1358, 1244, 1103 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 12.5 (s, 1H, OH), 11.8 (s, 1H, OH), 11.0 (s, 1H, OH), 9.00 (s, 1H, OH), 7.50−7.54 (m, 2H, H3′, H5′), 7.39−7.44 (m, 2H, H6′, H2′), 7.36−7.39 (m, 1H, H4′), 6.46 (d, J = 9.9 Hz, 1H, H4), 5.58 (dd, J = 2.9, 13.0 Hz, 1H, H8), 5.57 (d, J = 9.9 Hz, 1H, H3), 3.76 (d, J = 15.0 Hz, 1H, C$\underline{H}_2$), 3.69 (d, J = 15.0 Hz, 1H, C$\underline{H}_2$), 3.20 (dd, J = 13.0, 17.2 Hz, 1H, H7α), 2.89 (dd, J = 2.9, 17.2 Hz, 1H, H7β), 2.55 (s, 3H, $COCH_3$), 1.93 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.05 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 203.5 (C═O), 197.7 (C═O), 161.3 (C6″), 160.0 (C2″), 159.9 (C9a), 159.8 (C10a), 158.8 (C4″), 155.9 (C5), 139.5 (C1′), 129.0 (C2′, C6′), 128.8 (C4′), 127.3 (C3), 126.8 (C3′, C5′), 115.3 (C4), 108.5 (C10), 106.6 (C1″), 102.7 (C5″), 102.6 (C5a, C4a), 78.8 (C8), 78.1 (C2), 42.8 (C7), 33.3 ($COCH_3$), 27.8 (CH_3), 27.2 (CH_3), 16.9 (CH_2), 9.2 (CH_3); HRMS (ESI) m/z calcd for $C_{30}H_{28}O_8$ $(M + Na)^+$ 539.1676, found 539.1674.

6-(3-Acetyl-2,4,6-trihydroxy-5-methylbenzyl)-5-hydroxy-2,2-dimethyl-8-phenyl-8,9-dihydro-2*H*,10*H*-pyrano[2,3-*f*]-chromen-10-one (36). Rottlerin **1** (50 mg, 0.097 mmol) was dissolved in DMSO (1 mL) and heated at 100 °C for 2 h. The solution was cooled, and the mixture purified directly by preparative HPLC using a 5 μM column (150 × 10 mm i.d.) at a flow rate of 7 mL min^{-1} with a gradient elution from 0 to 100% B (solvent A: 0.1% TFA/H_2O and solvent B: 0.1% TFA/CH_3CN) over 60 min, to afford **38** (1.0 mg, 2%) as a yellow solid: mp 127−131 °C; IR (neat) ν_{max} 3262, 2973, 1606, 1553, 1471, 1344, 1284, 1129 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 12.8 (s, 1H, OH), 11.6 (s, 1H, OH), 11.3 (s, 1H, OH), 9.03 (s, 1H, OH), 7.51−7.54 (m, 2H, H2′, H6′), 7.41−7.46

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(m, 2H, H3′, H5′), 7.37−7.40 (m, 1H, H4′), 6.40 (d, J = 10.0 Hz, 1H, H4), 5.63 (dd, J = 3.0, 12.6 Hz, 1H, H8), 5.54 (d, J = 10.0 Hz, 1H, H3), 3.73 (d, J = 15.0 Hz, 1H, CH), 3.71 (d, J = 15.0 Hz, 1H, CH), 3.27−3.31 (m, 1H, H9α), 2.87 (dd, J = 3.0, 17.2 Hz, 1H, H9β), 2.61 (s, 3H, $COCH_3$), 1.94 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.08 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 203.6 (C═O), 197.4 (C10), 161.5 (C6″), 160.0 (C2″), 159.9 (C6a), 159.8 (C5), 159.0 (C4″), 154.9 (C10b), 139.2 (C1′), 129.1 (C3′, C5′), 129.0 (C4′), 127.5 (C3), 126.9 (C2′, C6′), 115.5 (C4), 109.1 (C6), 106.4 (C1″), 102.7 (C5″), 101.7 (C4a), 101.0 (C10a), 78.8 (C8), 78.0 (C2), 42.5 (C9), 33.3 ($COCH_3$), 27.6 (CH_3), 27.3 (CH_3), 16.2 (CH_2), 9.1 (CH_3); HRMS (ESI) m/z calcd for $C_{30}H_{28}O_8$ $(M + Na)^+$ 539.1676, found 539.1674.

Antibacterial Assay. The MIC of the active compounds was determined by following a previously published protocol.[22] The compounds were dissolved in sterilized DMSO and diluted in autoclaved Millipore water. A single colony of *S. aureus* was cultured overnight in tryptone soya broth (TSB) at 37 °C. The resulting bacteria were collected by centrifugation and resuspended in the same volume of TSB twice. The optical density (OD) of the resulting culture was adjusted to 0.1 at 600 nm (equivalent to 10^8 CFU/mL) in TSB, and the adjusted culture was further diluted to 10^5 CFU/mL in TSB. Then, 150 μL of the bacterial solution was added to wells of a 96-well plate containing 50 μL of serially diluted compound. Two controls were also prepared: one containing 150 μL of TSB media and 50 μL of sterile water and the other containing 150 μL of bacterial solution and 50 μL of sterilized water. The plates were incubated at 37 °C for 24 h, and the MIC was recorded by measuring the OD value at 600 nm using a Wallac Victor (PerkinElmer) microplate reader. The MIC value was determined as the lowest concentration of compound that inhibited the complete growth of the bacteria (i.e., OD similar to that of the control having no bacteria). Each experiment was performed in triplicate and was repeated in three independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00917.

Crystallographic data for rottlerin (**1**) and compound **18** (CIF)

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Notes

The authors declare no competing financial interest.

CCDC-1406109 contains supplementary crystallographic data for rottlerin (**1**). CCDC-1406110 contains supplementary crystallographic data for compound **18**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

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