

Article

Total Syntheses and Antibacterial Studies of Natural Isoflavones: Scandenone, Osajin, and 6,8-Diprenylgenistein

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Abstract: Isoflavones are a class of natural products that exhibit a wide range of interesting biological properties, including antioxidant, hepatoprotective, antimicrobial, and anti-inflammatory activities. Scandenone (1), osajin (2), and 6,8-diprenylgenistein (3) are natural prenylated isoflavones that share the same polyphenol framework. In this research, the key intermediate 15 was used for the synthesis of the natural isoflavones 1–3, establishing a stereoselective synthetic method for both linear and angular pyran isoflavones. The antibacterial activities of 1–3 were also evaluated, and all of them displayed good antibacterial activity against Gram-positive bacteria. Among them, 2 was the most potent one against MRSA, with a MIC value of 2 µg/mL, and the SEM assay indicated that the bacterial cell membranes of both MRSA and *E. faecalis* could be disrupted by 2. These findings suggest that this type of isoflavone could serve as a lead for the development of novel antibacterial agents for the treatment of Gram-positive bacterial infections.

Keywords: isoflavones; natural products; total synthesis; antibacterial activity



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1. Introduction

Isoflavones are plant secondary metabolites characterized by a B-ring attached to the C-3 position of their C-ring (Figure 1) [1,2]. They exhibit a range of biological properties, including antioxidant [3], hepatoprotective [4], antimicrobial [5], and anti-inflammatory [6] activities. Prenylated isoflavones in particular have pronounced pharmacological effects due to their higher lipophilicity and affinity for biological cell membranes. Scandenone (1) is a natural prenylated isoflavone that has been mainly isolated from the Fabaceae and Moraceae plant families [7,8]. Till now, the biological activities of scandenone (1) have been extensively studied. It is a powerful protein kinase inhibitor and displays potent anti-inflammatory activity [9]. The antiviral [7], antibacterial [10], and insecticidal properties [11,12] of scandenone (1) have also been discussed in previously published papers. In 2006, Toker reported that scandenone (1) from the fruits of *Maclura pomifera* (Rafin.) *Schnider* (Moraceae) possessed good antibacterial activity against both Gram-positive (*Staphylococcus aureus* MIC = 0.5 µg/mL, *Enterococcus faecalis* MIC = 0.5 µg/mL) and Gram-negative (*Escherichia coli*, MIC = 2 µg/mL) bacterial strains [10]. In 2018, Raksat et al. found that scandenone (1) from *Milletia extensa* displayed promising antibacterial activity against *S. aureus* TISTR 1466, *S. epidermidis* ATCC 12228, and *B. subtilis* TISTR 008 with the same MIC value of 2 µg/mL [13]. However, Ramasami et al. found that scandenone (1) from *Erythrina addisoniae* did not exhibit significant antibacterial activity against *S. aureus* or *E. coli* with MIC values > 64 µg/mL [14]. These inconsistencies may be due to

the different levels of purity of **1** that were exacted from various natural sources, and they suggest that rigorous antibacterial evaluation is needed to determine its authentic activity.

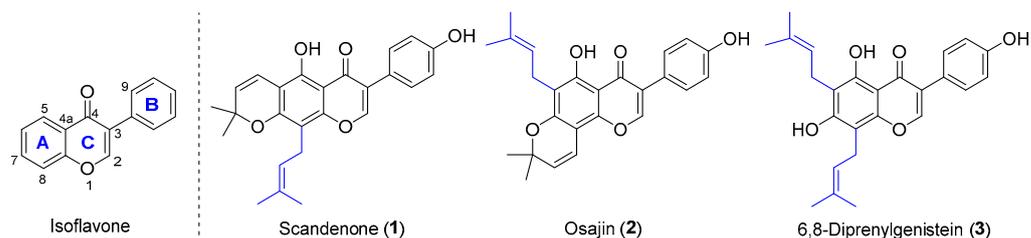
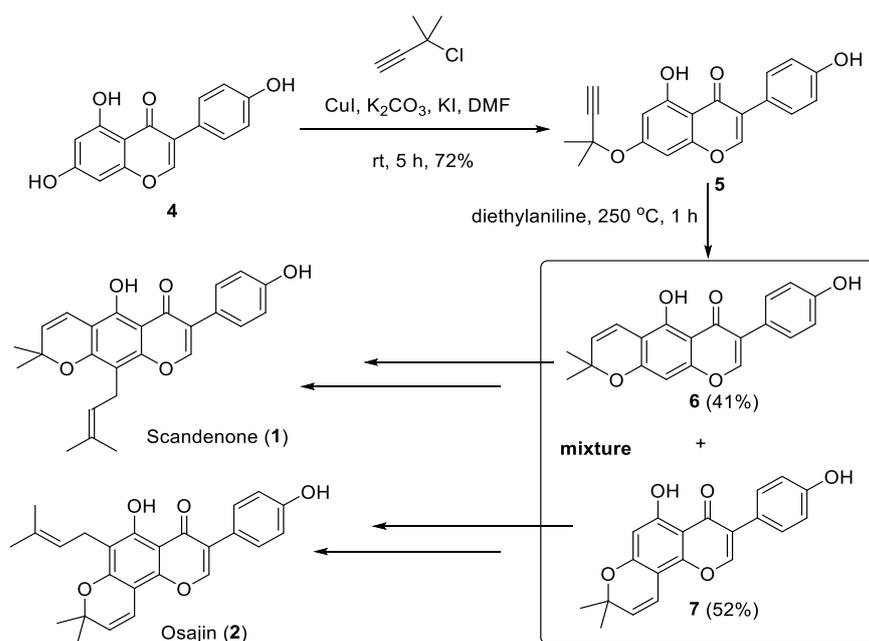


Figure 1. The Structures of Isoflavone and Compounds **1–3**.

Osajin (**2**) and 6,8-diprenylgenistein (**3**) (Figure 1) are natural prenylated isoflavones, that contain very close chemical structures to scandenone (**1**) and have been investigated over the years for their anticancer [15], anti-inflammatory [16], and antioxidant properties [17]. In particular, 6,8-diprenylgenistein (**3**) isolated from the roots of *Glycyrrhiza uralensis* has been reported to display remarkable antibacterial activity against *Streptococcus mutans* (MIC = 2 µg/mL) and MRSA (MIC = 8 µg/mL) [18]. We have recently become interested in exploring the pharmacological potential of flavonoids [19–22]. However, isoflavones **1–3** could only be obtained from the respective natural sources in low yields, insufficient for further detailed investigation. We therefore initiated a program to synthesize isoflavones **1–3** to investigate their antibacterial properties.

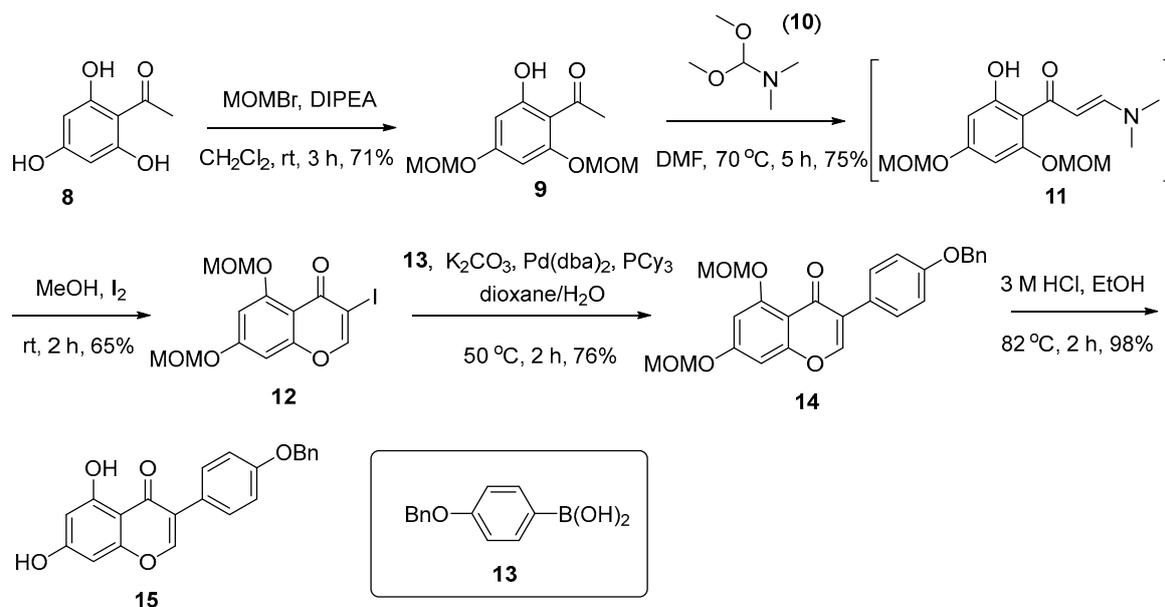
2. Results and Discussions

To our surprise, it was not until recently that the first total synthesis of Scandenone (**1**) and Osajin (**2**) was reported. As demonstrated in Scheme 1, Wang et al. [23] reported a chemoselective propargylation followed by a Claisen rearrangement to construct the crucial pyran isoflavone cores (compounds **6** and **7**) of isoflavones **1** and **2**. However, in our hands, the chemoselective propargylation only gave about 15% yield of pure **5**, and the separation of the highly polar compounds **6** and **7** by chromatography was very difficult. To our knowledge, no synthetic approach to 6,8-diprenylgenistein (**3**) has been reported. The above-mentioned reasons were the impetus for our current efforts to synthesize the prenylated isoflavones **1–3** and to compare their antibacterial activities.



Scheme 1. Reported Synthetic Approaches for Isoflavones **1** and **2**.

As shown in Scheme 2, we started our synthesis to afford natural compounds 1–3 by making a known intermediate, 3-iodochromone 12 [24]. Using previously published procedures, the selective protection of two hydroxyl groups in 8 with methoxymethyl bromide (MOMBr) and *N,N*-Diisopropylethylamine (DIPEA) provided MOM ether 9 (71%) [25], which was treated with dimethylacetal (10) in DMF to furnish enamino ketone 11 (75%). Enaminone 11 was subsequently subjected to intramolecular cyclization using I₂ in MeOH at rt to give 3-iodochromone 12 (65%). We then utilized a palladium-catalyzed Suzuki reaction with 12 and phenylboronic acid 13 to give the desired isoflavone 14 in 76% yield [26,27]. Removal of the MOM groups of 14 provided the desired 5,7-dihydroxyl-isoflavone derivative 15 in 98% yield [25].



Scheme 2. Synthesis of the Key Intermediate 15.

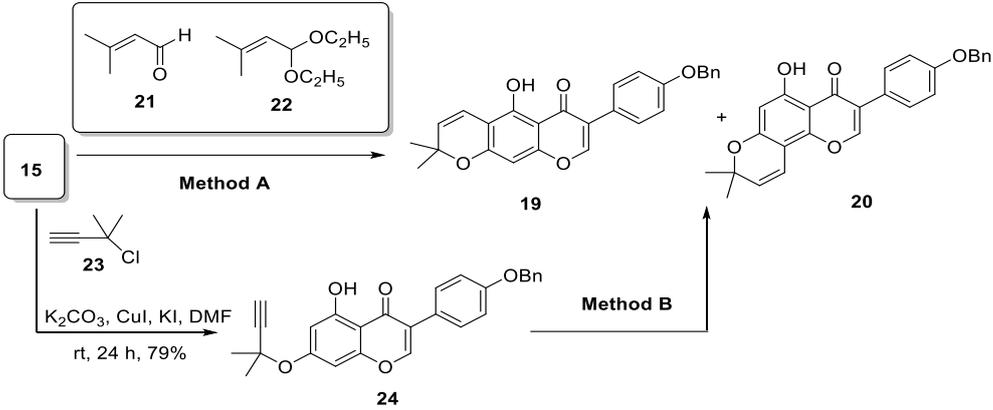
With the key intermediate 15 in hand, we next investigated the di-prenylation of 15 with 1-bromo-3-methylbut-2-ene (17) or 3-methylbut-2-en-1-ol (18) to get compound 16. As shown in Table 1, we first tested previously reported conditions that used 17 as a reagent, the mixture of CH₂Cl₂ and H₂O as a solvent, and KOH as a base (entry 1) [28]. However, when we applied these conditions to get 16, an inseparable mixture of products was obtained, and no 16 could be isolated. Replacing KOH with a weaker base such as K₂CO₃ and DBU [29,30], only afforded the desired product 16 in low yields (entry 2, 3). The unsuccessful attempts are likely attributable to the simultaneous occurrence of C- and O-prenylations during the reaction. To avoid the O-prenylation, we then turned to imply 3-methylbut-2-en-1-ol (18) as a reagent and appropriate organic acids as catalysts in hydrophobic solvents to obtain 16. After a few attempts, we found that the use of *p*-toluenesulfonic acid in CH₂Cl₂ could generate 16 in 14% yield (entry 4). Finally, 16 could be obtained in a satisfactory isolated yield (72%) using acidic alumina as a catalyst and 1,2-dichloroethane (DCE) as a solvent (entry 5) [31–33]. Compound 16 was treated with newly prepared sodium dodecane-1-thiolate in reflux DMF to generate natural product 3 in 78% yield [19].

Table 1. Synthesis of Isoflavone 3.

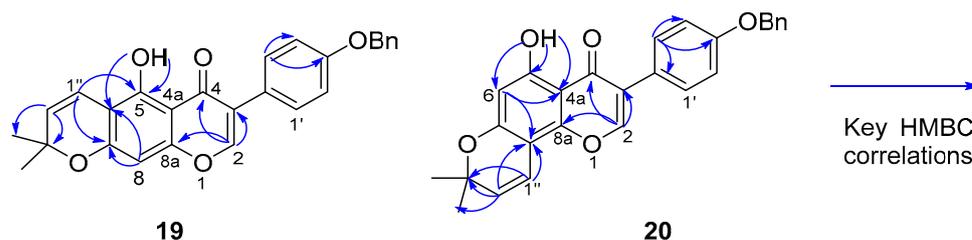
Entry	Conditions	Yields of Isolated 16 (%)
1	compound 17, KOH, H ₂ O, 0 °C to rt	-
2	compound 17, K ₂ CO ₃ , acetone, rt	7
3	compound 17, DBU, THF, 50 °C	20
4	compound 18, <i>p</i> -toluenesulfonic acid, CH ₂ Cl ₂ , rt	14
5	compound 18, acidic alumina, DCE, 80 °C	72

As shown in Table 2, the synthesis of scandenone (1) and osajin (2) commenced with the stereocontrolled preparations of tetracyclic isoflavens 19 and 20, which possessed a linear or angular pyran attached to the A-ring, respectively. Our first attempt was to synthesize 19 and 20 in a one-step pyran annulation of 15. As depicted in Table 2 (entry 1 and 2), electrocyclization of 15 with α,β -unsaturated aldehyde (21) [34] or 1,1-diethoxy-3-methylbut-2-ene (22) [25] could only provide a mixture of linear and angular isomers (19 and 20). To overcome this problem, the Claisen rearrangement/cyclization reaction was explored for the stereoselective construction of the pyran rings of both 19 and 20. Treatment of 15 with 3-chloro-3-methylbut-1-yne (23) and K₂CO₃ in DMF in the presence of a catalytic amount of KI afforded C-7 propargyl ether 24 in a good yield (79%). As shown in Table 2, an intensive screening of reaction conditions was carried out to identify suitable reaction conditions that would lead to the regioselective Claisen rearrangement or cyclization of 24. It was observed that the solvent of the reaction significantly affected the regioselectivity. Aromatic Claisen rearrangement or cyclization in DMF afforded 20 as the main product (77%, entry 3), while in xylene, 19 was isolated as the main product in a yield of 55% (entry 4). We then found that the addition of KOH to xylene could effectively control the regioselectivity, and pure 19 was obtained in 93% yield (entry 5) [33,35], whereas the reported Claisen rearrangement/cyclization conditions of 15 only yielded a roughly 1:1 mixture of 19 and 20 under harsh conditions (entry 6) [23].

Since the NMR data of 3-(4-(benzyloxy)phenyl)-5-hydroxy-8,8-dimethyl-4*H*,8*H*-pyrano chromen-4-one (19) and 4-(Benzyloxy)phenyl)-5-hydroxy-8,8-dimethyl-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (20) have not been reported yet; the distinction between the two compounds was challenging and may lead to misassignment due to their structural similarity. We therefore employed 2D NMR spectroscopy to unequivocally confirm the structures of 19 and 20. The HSQC data established all 1J (^1H - ^{13}C) connectivities (see the Supplementary Materials), and the key HMBC correlations are shown in Figure 2. The HMBC spectra of 19 showed a correlation of 5-OH [δ_{H} 13.18] with C-6 [δ_{C} 105.0], while there was no correlation of 5-OH with the tertiary carbon atom [C-8 (δ_{H} 6.34, δ_{C} 93.8)], suggesting that 19 is a linear isomer. The HMBC correlations between 5-OH [δ_{H} 12.95] with C-4a [δ_{C} 105.0] and tertiary carbon C-6 [δ_{H} 6.30, δ_{C} 99.3] of 20 indicated that it was an angular isomer.

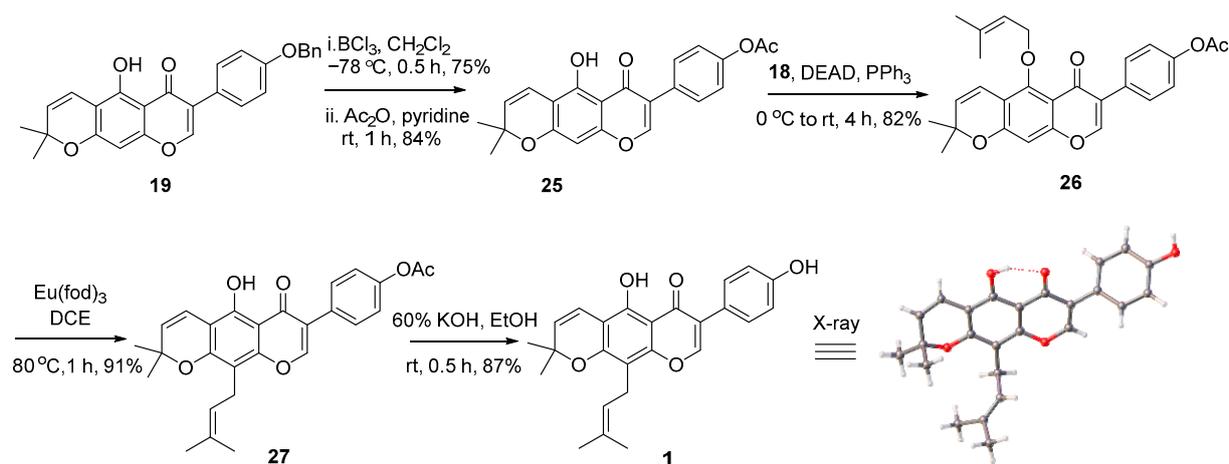
Table 2. Optimization of Reaction Conditions for Compounds **19** and **20**.


Entry	Method	Conditions	19, Yield ¹ (%)	20, Yield ¹ (%)
1	Method A	21 , Ca(OH) ₂ , CH ₃ OH, 0 °C to rt	27	22
2	Method A	22 , 3-picoline, toluene, 110 °C	25	27
3	Method B	DMF, 130 °C	9	77
4	Method B	xylene, 130 °C	55	33
5	Method B	xylene, KOH, 130 °C	93	-
6	Method B	diethylaniline, 250 °C	41	40

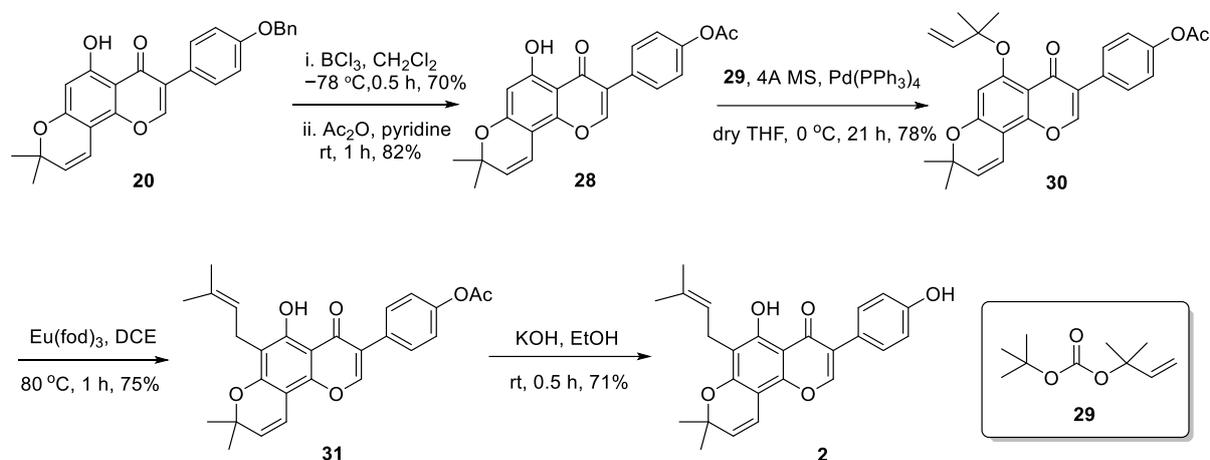
¹ Isolated yield.**Figure 2.** Key HMBC correlations of **19** and **20**.

Since selective debenzoylation is difficult in the presence of both pyran and isopentene groups, the benzyl group in **19** was removed and replaced by the acetyl protecting group to give compound **25**, which was then subjected to condensation with **18** under typical Mitsunobu conditions using triphenylphosphine (PPh₃) and diethyl azodicarboxylate (DEAD) to obtain **26** (Scheme 3). Compound **26** was subjected to a europium(III)-tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate) [Eu(fod)₃]-catalyzed aromatic *para*-Claisen-Cope rearrangement conditions to afford **27** (91%) [34]. Deprotection of the -Ac group with 60% KOH in EtOH completed the synthesis of scandenone (**1**) (87%), the structure of which was confirmed by single-crystal X-ray analysis (see the Supplementary Materials). Our X-ray analysis data was in line with previously published results [14].

In the same manner, compound **20** was smoothly translated into Ac-protected compound **28**, and compound **30** was obtained by Pd-catalyzed allylation of **28** with *tert*-butyl (2-methylbut-3-en-2-yl) carbonate (**29**), in the presence of Pd(PPh₃)₄ and 4 Å molecular sieves in nitrogen atmosphere in 78% yield (Scheme 4) [34]. Subsequently, an [Eu(fod)₃]-catalyzed aromatic *ortho*-Claisen rearrangement was taken to afford **31** in a yield of 84%. Finally, treatment of **31** with a KOH aqueous solution provided natural isoflavone **2** in 71% yield.



Scheme 3. Synthesis of Scandenone (1).



Scheme 4. Synthesis of Osajin (2).

The target compounds (1–3) were evaluated for their *in vitro* antibacterial activities against three Gram-positive (G+) bacterial strains (*S. aureus* ATCC29213, MRSA ATCC33591, and *Enterococcus faecalis* ATCC29212,) and one Gram-negative (G-) bacterial strain (*E. coli* ATCC25922) using minimum inhibitory concentration (MIC) values. Ampicillin and vancomycin were used as the positive controls [33].

As shown in Table 3, the anti-bacterial activity data showed that 1–3 were active against three G+ bacteria, including a multidrug-resistant strain (MRSA ATCC33591), but not for G-strain *E. coli* ATCC25922, which is consistent with the results reported by Raksat et al. [13] and Nkengfack et al. [18]. Among these compounds, natural isoflavone 2 exhibited the most potent antibacterial activity against G+ bacteria, with MIC values ranging from 2 to 8 $\mu\text{g}/\text{mL}$. The MIC of natural compound 2 against *S. aureus* ATCC29213 was 2 $\mu\text{g}/\text{mL}$, which was 16-fold lower compared with the positive control, ampicillin. Remarkably, these natural flavones (1–3) gave low MIC values (2–4 $\mu\text{g}/\text{mL}$) against MRSA, which were much better than ampicillin. Previously published work indicated that the antibacterial activity of natural flavonoids against G+ bacteria depends on balanced lipophilicity [36]. Compared to the parent isoflavone, prenylated isoflavones 1–3 have a higher lipophilicity, which may endow them with a higher antibacterial activity against G+ bacteria. Therefore, compound 2 displayed the most potent antibacterial activity due to its well-balanced lipophilicity [37].

Table 3. MICs of compounds 1–3.

Compound	MIC ($\mu\text{g/mL}$)			
	<i>S. aureus</i> ATCC29213	<i>E. faecalis</i> ATCC29212	MRSA ATCC33591	<i>E. coli</i> ATCC25922
1	16	16	4	>128
2	2	8	2	>128
3	8	16	4	>128
Ampicillin	32	1	>128	4
Vancomycin	2	2	1	128

To further confirm the antibacterial activity of Osajin (**2**), we subsequently performed a scanning electron microscopy (SEM) assay [32,33], to image both MRSA ATCC33591 and *E. faecalis* ATCC29212 after incubation with **2** for 2 h. As shown in Figure 3, the untreated control groups (Figure 3A,C) had an intact cell structure and surface morphology, while treatment of MRSA ATCC33591 and *E. faecalis* ATCC29212 with **2** at a concentration of $8 \times$ its MIC resulted in a rough and wrinkled cell membrane surface (Figure 3B,D), indicating that the bacterial cell membrane was disrupted by **2**. These findings suggested that Osajin (**2**) could inhibit bacterial growth by disrupting the integrity of the cell membrane.

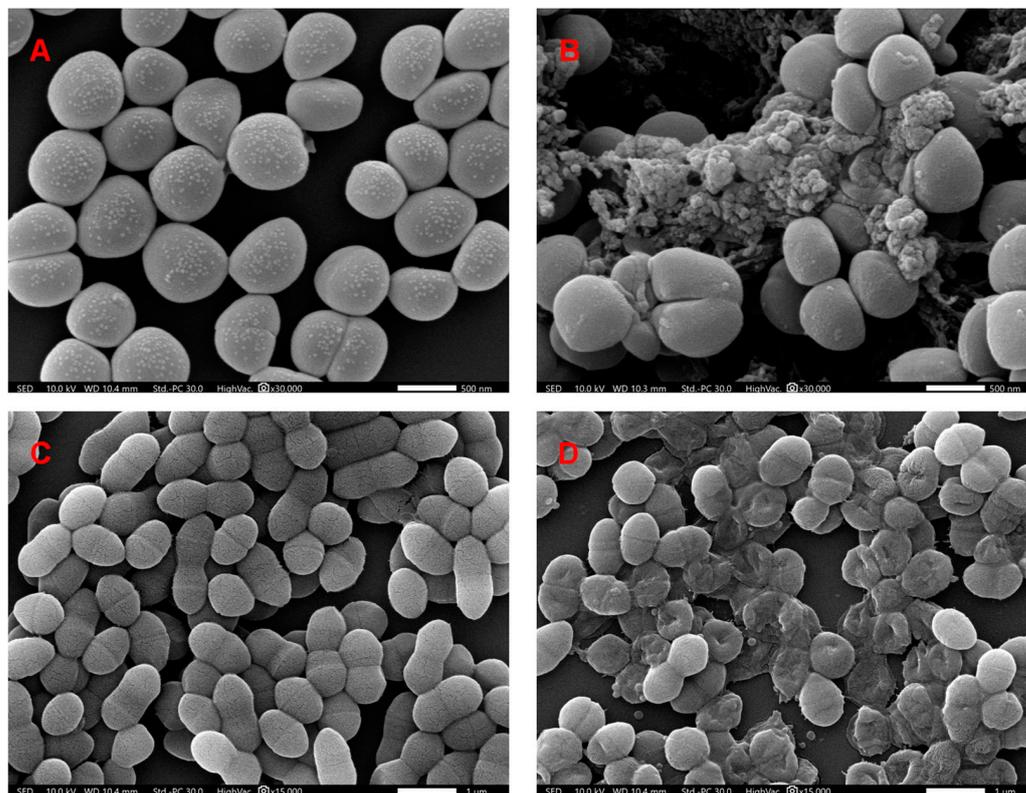


Figure 3. SEM images of MRSA ATCC33591 and *E. faecalis* ATCC29212 bacterial cells. (A) MRSA ATCC33591 cells without treatment; scale bar: 500 nm. (B) MRSA ATCC33591 cells treated with $8 \times$ MIC of compound **2**, scale bar: 500 nm. (C) *E. faecalis* ATCC29212 cells without treatment; scale bar: 1 μm . (D) *E. faecalis* ATCC29212 cells treated with $8 \times$ MIC of compound **2**, scale bar: 1 μm .

3. Materials and Methods

3.1. General Experimental Procedures

Melting points were recorded on a Büchi B-545 melting point apparatus (Sigma-Aldrich, St. Louis, MO, USA). Infrared (IR) spectra were recorded on a Thermo Scientific Nicolet iS5 FT-IR spectrometer (Waltham, MA, USA). ^1H NMR, ^{13}C NMR, HMBC, and HSQC spectra were recorded on a Bruker Avance 400 spectrometer (Billerica, MA, USA) or

a JEOL Eclips-600 spectrometer (Akishima, Japan), and tetramethylsilane (TMS) was used as the internal reference. The HR-MS spectra were recorded by Thermo QExactive (Thermo Scientific) and Agilent 6545 LC/QTOF mass spectrometers (Santa Clara, CA, USA). The crystal structure was analyzed with Oxford X-calibur E four-circle X-ray diffractometry (Oxford Instruments, Oxford, UK). Column chromatography was performed on silica gel (100–200 mesh). Reagents were purchased from commercial sources and used as received, unless mentioned otherwise. The solvents were of analytical grade.

3.2. Synthesis and Characterization of the Compounds

3.2.1. 1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)ethan-1-one (**9**)

To a stirred solution of 2,4,6-trihydroxyacetophenone (5.00 g, 29.76 mmol) in CH_2Cl_2 (50 mL), DIPEA (10.4 mL, 59.52 mmol) was added at room temperature, and then MOMBr (5.0 mL, 59.52 mmol) was added to the reaction mixture at 0 °C. The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was acidified with 10% HCl (aq.) (15 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layer and extracts were combined, dried, and evaporated to give a red oil, which was chromatographed on silica gel (petroleum ether/EtOAc = 30/1) to give **9** (5.47 g, 21.36 mmol, 71%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 6.26–6.18 (m, 2H, –Ph), 5.23 (s, 2H, – OCH_2OCH_3), 5.14 (s, 2H, – OCH_2OCH_3), 3.49 (s, 3H, – OCH_3), 3.44 (s, 3H, – OCH_3), 2.62 (s, 3H, – COCH_3); ^{13}C NMR (150 MHz, CDCl_3) δ 203.3 (C=O), 166.9, 163.5, 160.4, 106.9, 97.2, 94.5, 94.0 (2 × C), 56.7 (– OCH_3), 56.5 (– OCH_3), 33.0 (– CH_3). The spectroscopic data corresponds to reported values [24].

3.2.2. (*E*)-3-(Dimethylamino)-1-(2-hydroxy-4,6-bis(methoxymethoxy)phenyl)prop-2-en-1-one (**11**)

Compound **9** (5.47 g, 21.36 mmol) was dissolved in dry DMF (25 mL) and heated to 74 °C. After that, compound **10** (14.2 mL, 106.8 mmol) was added dropwise, and the reaction mixture was stirred for 3 h and then cooled to rt. H_2O (150 mL) was added to the reaction solution with stirring, and solids precipitated out. The precipitate was filtered off to afford crude product **11**. The crude material was purified by flash chromatography (PE/EtOAc = 10/1) to give **11** (4.99 g, 16.06 mmol, 75%) as a bright yellow solid: ^1H NMR (400 MHz, CDCl_3) δ 15.10 (s, 1H, –OH), 7.85 (d, J = 12.4 Hz, 1H, = $\text{CHN}(\text{CH}_3)_2$), 6.22 (d, J = 12.4 Hz, 1H, – $\text{CH}=\text{CHN}(\text{CH}_3)_2$), 6.19 (d, J = 2.4 Hz, 1H, –Ph), 6.10 (d, J = 2.4 Hz, 1H, –Ph), 5.14 (s, 2H, – OCH_2OCH_3), 5.08 (s, 2H, – OCH_2OCH_3), 3.45 (s, 3H, – OCH_3), 3.39 (s, 3H, – OCH_3), 3.09 (s, 3H, – $\text{N}(\text{CH}_3)_2$), 2.85 (s, 3H, – $\text{N}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3) δ 190.0 (C=O), 166.9, 161.3, 158.9, 154.5, 107.0, 97.9, 96.9, 95.2, 94.4, 94.1, 56.7 (– OCH_3), 56.3 (– OCH_3), 45.3 (– CH_3), 37.3 (– CH_3). The spectroscopic data corresponds to reported values [24].

3.2.3. 3-Iodo-5,7-bis(methoxymethoxy)-4*H*-chromen-4-one (**12**)

Iodine (6.12 g, 24.09 mmol) was added to propenone **11** (4.99 g, 16.06 mmol) in MeOH (50 mL), and the solution was stirred at room temperature for 2 h. The mixture was washed with saturated $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL), and the aqueous layer was extracted with EtOAc (3 × 50 mL) and dried with Na_2SO_4 . The crude material was purified by flash chromatography (PE/EtOAc = 30/1) to give **12** (4.10 g, 10.46 mmol, 65%), light-yellowish solids: ^1H NMR (400 MHz, CDCl_3) δ 8.03 (s, 1H, – $\text{OCH}=\text{C}$ –), 6.68 (d, J = 2.3 Hz, 1H, –Ph), 6.65 (d, J = 2.3 Hz, 1H, –Ph), 5.23 (s, 2H, – OCH_2OCH_3), 5.16 (s, 2H, – OCH_2OCH_3), 3.48 (s, 3H, – OCH_3), 3.43 (s, 3H, – OCH_3); ^{13}C NMR (100 MHz, CDCl_3) δ 171.3 (C=O), 161.6, 159.3, 158.2, 155.7, 108.8, 102.1, 96.8, 95.5, 94.4, 89.5, 56.8 (– CH_3), 56.6 (– CH_3). The spectroscopic data corresponds to reported values [24].

3.2.4. 3-(4-Hydroxyphenyl)-5,7-bis(methoxymethoxy)-4*H*-chromen-4-one (**14**)

To a solution of the appropriate **12** (4.10 g, 10.46 mmol) in a mixture of 1,4-dioxane (35 mL) and water (15 mL), K_2CO_3 (4.34 g, 31.38 mmol) and **13** (2.89 g, 20.92 mmol) were

added. The mixture was purged with nitrogen for 10 min. To the mixture were then added PCy_3 (235 mg, 0.84 mmol) and $\text{Pd}(\text{dba})_2$ (241 mg, 0.43 mmol). The mixture was warmed to 50 °C and then stirred at this temperature for 2 h. It was then cooled to ambient temperature. An aq. saturated solution of NH_4Cl (50 mL) was added to the mixture, and the mixture was filtered. The filter cake was dissolved in ethyl acetate (EtOAc, 30 mL), poured into water (50 mL), and extracted with EtOAc. The organic phase was combined, washed with brine, and dried with anhydrous Na_2SO_4 . The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica using a PE/EtOAc mixture (3:2 (*v/v*)) as the eluent to afford compound **14** (3.61 g, 10.08 mmol, 76%) as a colorless oil: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.22 (s, 1H, $-\text{OCH}=\text{C}-$), 7.50–7.29 (m, 7H, $-\text{OBn}$ and $-\text{Ph}$), 7.04 (d, $J = 8.8$ Hz, 2H, $-\text{Ph}$), 6.81 (d, $J = 2.2$ Hz, 1H, $-\text{Ph}$), 6.69 (d, $J = 2.2$ Hz, 1H, $-\text{Ph}$), 5.32 (s, 2H, $-\text{OCH}_2\text{OCH}_3$), 5.26 (s, 2H, $-\text{OCH}_2\text{OCH}_3$), 5.14 (s, 2H, $-\text{OCH}_2\text{Ph}$), 3.43 (s, 3H, $-\text{OCH}_3$), 3.42 (s, 3H, $-\text{OCH}_3$); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 174.3, 161.1, 159.2, 158.5, 158.4, 151.9, 137.6, 130.8 (2 \times C), 128.9 (2 \times C), 128.3, 128.1 (2 \times C), 125.0, 124.9, 114.9 (2 \times C), 110.9, 101.9, 97.2, 95.7, 94.6, 69.6, 56.7, 56.6; HRMS (ESI) calculated for $\text{C}_{26}\text{H}_{25}\text{O}_7^+$ [$\text{M} + \text{H}$] $^+$ 449.1595, found 449.1598.

3.2.5. 3-(4-(Benzyloxy)phenyl)-5,7-dihydroxy-4H-chromen-4-one (15)

A solution of **14** (3.06 g, 6.84 mmol) and 3 M HCl (70 mL) in EtOH (150 mL) was stirred at 82 °C for 2 h. After the completion of the reaction, the mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with a saturated NaCl solution and dried with anhydrous Na_2SO_4 , and the solvents were removed in vacuum. The residue obtained was purified by flash column chromatography with PE/EtOAc (10:1, *v/v*) as the eluent to give **15** (2.43 g, 6.75 mmol, 98%) as a yellow solid: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.92 (s, 1H, $-\text{OH}$), 11.05 (s, 1H, $-\text{OH}$), 8.37 (s, 1H, $-\text{OCH}=\text{C}-$), 7.52–7.29 (m, 7H, $-\text{OBn}$ and $-\text{Ph}$), 7.07 (d, $J = 8.5$ Hz, 2H, $-\text{Ph}$), 6.43 (d, $J = 1.8$ Hz, 1H, $-\text{Ph}$), 6.26 (d, $J = 1.8$ Hz, 1H, $-\text{Ph}$), 5.15 (s, 2H, $-\text{OCH}_2\text{Ph}$); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 180.5 (C=O), 164.9, 162.4, 158.6, 158.0, 154.8, 137.5, 130.6 (2 \times C), 128.9 (2 \times C), 128.3, 128.1 (2 \times C), 123.6, 122.3, 115.1 (2 \times C), 104.9, 99.5, 94.2, 69.6. HRMS (ESI) calculated for $\text{C}_{22}\text{H}_{17}\text{O}_5^+$ [$\text{M} + \text{H}$] $^+$ 361.1071, found 361.1070.

3.2.6. 3-(4-(Benzyloxy)phenyl)-5,7-dihydroxy-6,8-bis(3-methylbut-2-en-1-yl)-4H-chromen-4-one (16)

Under a N_2 atmosphere, to a suspension of compound **15** (3.20 g, 8.88 mmol), acidic Al_2O_3 (17.80 g), and 4 Å molecular sieves (1.00 g) in dry DCE (160 mL) was added **18** (10 eq, 9.6 mL, 88.80 mmol). The mixture was stirred at 80 °C for 24 h. After the reaction was completed (detected by TLC), it was cooled to ambient temperature and filtered through a Celite. The filter cake was washed with EtOAc, and the filtrate was concentrated in vacuum to obtain a residue, which was further purified by silica gel column chromatography (PE/EtOAc (25:1, *v/v*)) to give the pure compound **16** (3.17 g, 72%) as a yellow solid: ^1H NMR (400 MHz, CDCl_3) δ 13.20 (s, 1H, $-\text{OH}$), 7.90 (s, 1H, $-\text{OCH}=\text{C}-$), 7.51–7.30 (m, 7H, $-\text{Ph}$ and $-\text{OBn}$), 7.05 (d, $J = 8.7$ Hz, 2H, $-\text{Ph}$), 6.38 (s, 1H, $-\text{OH}$), 5.33–5.18 (m, 2H, $-\text{CH}=\text{C}(\text{CH}_3)_2$), 5.11 (s, 2H, $-\text{OCH}_2\text{Ph}$), 3.48 (t, $J = 6.9$ Hz, 4H, $-\text{CH}_2-$), 1.85 (d, $J = 4.5$ Hz, 6H, $-\text{CH}_3$), 1.76 (d, $J = 10.9$ Hz, 6H, $-\text{CH}_3$); ^{13}C NMR (100 MHz, CDCl_3) δ 180.1 (C=O), 158.5, 157.8, 156.5, 152.2, 151.4, 135.8, 134.5, 133.05, 129.1 (2 \times C), 127.5 (2 \times C), 126.9, 126.4 (2 \times C), 122.5, 121.9, 120.5, 120.3, 113.9 (2 \times C), 109.1, 104.7, 104.3, 68.99, 24.8, 24.7, 20.6 (2 \times C), 16.9, 16.8. HRMS (ESI) calculated for $\text{C}_{32}\text{H}_{33}\text{O}_5^+$ [$\text{M} + \text{H}$] $^+$ 497.2323, found 497.2322.

3.2.7. 5,7-Dihydroxy-3-(4-hydroxyphenyl)-6,8-bis(3-methylbut-2-en-1-yl)-4H-chromen-4-one (3)

Under a N_2 atmosphere, a solution of 30% $\text{CH}_3\text{ONa}/\text{CH}_3\text{OH}$ aqueous solution (1.8 mL, 9.68 mmol) and dodecyl mercaptan (3 eq, 2.1 mL) in DMF (35 mL) was stirred at rt for 15 min. Then **16** (1.20 g, 2.42 mmol) was added to the reaction solution with stirring and refluxing at 120 °C for 24 h. After the reaction was completed (detected by TLC), 3 M HCl was added to adjust the pH to 7, and the mixture was extracted with EtOAc.

The organic layers were combined, dried over anhydrous Na₂SO₄, and evaporated. The residue obtained was purified over flash column chromatography with PE/EtOAc (25:1, *v/v*) as the eluent to give the compound **3** (767 mg, 78%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 13.11 (s, 1H, -OH), 7.89 (s, 1H, -OCH=C-), 7.33 (d, *J* = 8.1 Hz, 2H, -Ph), 6.82 (d, *J* = 8.1 Hz, 2H, -Ph), 6.40 (s, 1H, -OH), 5.32–5.18 (m, 2H, -CH=C(CH₃)₂), 3.47 (t, *J* = 6.3 Hz, 4H, -CH₂-), 1.84 (d, *J* = 4.9 Hz, 6H, -CH₃), 1.75 (d, *J* = 10.9 Hz, 6H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C=O), 158.6, 156.4, 155.1, 152.3, 151.7, 134.5, 133.1, 129.2 (2 × C), 122.2, 121.7, 120.4, 120.2, 114.7 (2 × C), 109.2, 104.7, 104.4, 24.8, 24.7, 20.6 (2 × C), 16.9, 16.8. HRMS (ESI) calculated for C₂₅H₂₇O₅⁺ [M + H]⁺ 407.1853, found 407.1855.

3.2.8. 3-(4-(Benzyloxy)phenyl)-5-hydroxy-7-((2-methylbut-3-yn-2-yl)oxy)-4H-chromen-4-one (**24**)

Compound **15** (2.20 g, 6.11 mmol), CuI (700 mg, 1.94 mmol), K₂CO₃ (1.1 eq, 300 mg, 2.13 mmol), and KI (1.2 eq, 390 mg, 7.33 mmol) were suspended in DMF (20 mL). Then **23** (1.2 eq, 240 mg, 2.33 mmol) was added dropwise, and the resulting reaction mixture was stirred for 24 h at rt. The reaction mixture was quenched by the addition of 1 mol/L aqueous HCl. The organic layer was separated and then washed with a saturated aqueous NaCl solution and dried with anhydrous Na₂SO₄, and the solvents were removed in vacuum. The residue obtained was purified over flash column chromatography with PE/EtOAc (40:1, *v/v*) as the eluent to give compound **24** (550 mg, 79%): ¹H NMR (400 MHz, CDCl₃) δ 12.69 (s, 1H, -OH), 7.78 (s, 1H, -OCH=C-), 7.40–7.22 (m, 7H -OBn and -Ph), 6.97 (d, *J* = 8.8 Hz, 2H, -Ph), 6.72 (d, *J* = 2.2 Hz, 1H, -Ph), 6.62 (d, *J* = 2.2 Hz, 1H, -Ph), 5.02 (s, 2H, -OCH₂Ph), 2.61 (s, 1H, -C≡CH), 1.66 (s, 6H, -CH₃); ¹³C NMR (150 MHz, CDCl₃) δ 181.0 (C=O), δ 162.1, 162.2, 159.1, 157.4, 152.9, 136.9, 130.2 (2 × C), 128.7 (2 × C), 128.1, 127.5 (2 × C), 123.7, 123.3, 115.1 (2 × C), 106.9, 102.8, 97.3, 84.8, 75.3, 72.8, 70.1, 29.6 (2 × C). HRMS (ESI) calculated for C₂₇H₂₃O₅⁺ [M + H]⁺ 427.1540, found 427.1540.

3.2.9. 3-(4-(Benzyloxy)phenyl)-5-hydroxy-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**19**)

Under a N₂ atmosphere, compound **24** (500 mg, 1.17 mmol) and KOH (391.61 mg, 6.98 mmol) were dissolved in dry *p*-xylene (25 mL) at rt, and the resulting mixture was stirred for 1 h at 130 °C. After the reaction was completed, the mixture was cooled, quenched with 3 mol/L HCl, and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated in a vacuum. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 80:1) to afford compound **19** (463 mg, 93%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 13.18 (s, 1H, 5-OH), 7.82 (s, 1H, H-2), 7.45 (d, *J* = 8.6 Hz, 4H, H-1', H-5' and -Bn), 7.40 (t, *J* = 7.3 Hz, 2H, -Bn), 7.35 (d, *J* = 7.1 Hz, 1H, -Bn), 7.05 (d, *J* = 8.7 Hz, 2H, H-2' and H-4'), 6.73 (d, *J* = 10.0 Hz, 1H, H-1''), 6.34 (s, 1H, H-8), 5.63 (d, *J* = 10.0 Hz, 1H, H-2''), 5.11 (s, 2H, -OBn), 1.48 (s, 6H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 179.8 (C-4), 158.5 (C-7), 157.9 (C-3'), 156.2 (C-8a), 155.9 (C-5), 151.5 (C-2), 135.8 (-Bn), 129.1 (2 × C, C-1' and C-5'), 127.6 (2 × C, -Bn), 127.1 (C-2''), 127.0 (-Bn), 126.4 (2 × C, -Bn), 122.4 (C-6'), 122.2 (C-3), 114.4 (C-1''), 114.0 (2 × C, C-2' and C-4'), 105.0 (C-6), 104.5 (C-4a), 93.8 (C-8), 77.0 (C-3''), 69.0 (-OCH₂Ph), 27.3 (2 × C). HRMS (ESI) calculated for C₂₇H₂₃O₅⁺ [M + H]⁺ 427.1540, found 427.1541.

3.2.10. (4-(Benzyloxy)phenyl)-5-hydroxy-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**20**)

A solution of **24** (500 mg, 1.17 mmol) in DMF (25 mL) was heated to 130 °C for 1 h. After completion of the reaction (detected by TLC), the mixture was poured into ice-cold water and extracted with EtOAc. The organic layer was washed with a saturated aqueous NaCl solution and dried with anhydrous Na₂SO₄, and the solvents were removed in vacuum. The residue obtained was purified over flash column chromatography with PE as the eluent to give **20** (416 mg, 77%) and **19** (49 mg, 9%) that could also be obtained. Compound **20** was a yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 12.95 (s, 1H, 5-OH), 7.88

(s, 1H, H-2), 7.45 (d, $J = 8.7$ Hz, 4H, H-1', H-5' and -Bn), 7.40 (t, $J = 7.4$ Hz, 2H, -Bn), 7.35 (dd, 1H, -Bn), 7.05 (d, $J = 8.3$ Hz, 2H, H-2' and H-4'), 6.68 (d, $J = 10.0$ Hz, 1H, H-1''), 6.30 (s, 1H, H-6), 5.59 (d, $J = 10.0$ Hz, 1H, H-2''), 5.11 (s, 2H, -OCH₂Bn), 1.48 (s, 6H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 179.9 (C-4), 161.2 (C-7), 158.5 (C-5), 157.9 (C-3'), 151.4 (C-2), 151.1 (C-8a), 135.8 (-Bn), 129.1 (2 \times C, C-1' and C-5'), 127.6 (2 \times C, -Bn), 127.0 (-Bn), 126.4 (C-2''), 126.4 (2 \times C, -Bn), 122.5 (C-6'), 122.09 (C-3), 113.9 (2 \times C, C-2' and C-4'), 113.5 (C-1''), 105.0 (C-4a), 100.1 (C-8a), 99.3 (C-6), 77.0 (C-3'), 69.0 (-OCH₂Bn), 27.2 (2 \times C, -CH₃). The spectroscopic data corresponds to reported values [35].

3.2.11. 4-Hydroxy-7-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-pyrano[3,2-g]chromen-6-one (6)

Under a N₂ atmosphere, compound **19** (900 mg, 2.11 mmol) was dissolved in CH₂Cl₂ (30 mL), and the mixture was cooled to -78 °C. To this solution was added BCl₃ (2.6 mL, 1.0 M solution in toluene, 2.53 mmol), and the reaction mixture was stirred for 0.5 h at the same temperature. The reaction mixture was quenched with a mixture of saturated aqueous NaHCO₃ solution and MeOH ($v/v = 1:1$) and diluted with EtOAc (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated in a vacuum. The obtained residue was purified on a flash silica gel (PE/EtOAc, 25:1) to afford **6** (530 mg, 75%) as a light yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.33 (s, 1H, -OH), 9.59 (s, 1H, -OH), 8.31 (s, 1H, -OCH=C-), 7.35 (d, $J = 8.6$ Hz, 2H, -Ph), 6.79 (d, $J = 8.6$ Hz, 2H, -Ph), 6.57 (d, $J = 10.1$ Hz, 1H, -CH=CHC(CH₃)₂O-), 6.43 (s, 1H, -Ph), 5.75 (d, $J = 10.1$ Hz, 1H, -CH=CHC(CH₃)₂O-), 1.39 (s, 6H, -CH₃); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 180.4 (C=O), 158.7, 157.4, 156.6, 155.9, 154.1, 130.1 (2 \times C), 128.9, 122.3, 120.9, 115.0 (2 \times C), 114.4, 105.3, 104.6, 94.5, 78.0, 27.8 (2 \times C). The spectroscopic data correspond to reported values [38].

3.2.12. 4-(5-Hydroxy-2,2-dimethyl-6-oxo-2H,6H-pyrano[3,2-g]chromen-7-yl)phenyl acetate (25)

Under a N₂ atmosphere, to a solution of compound **6** (800 mg, 2.38 mmol) in pyridine (10 mL), Ac₂O (0.31 mL, 3.57 mmol, 1.5 eq) was added. The mixture was stirred at rt for 1 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was quenched by dilution with 50 mL of H₂O and extracted with CH₂Cl₂. The organic layer dried over anhydrous Na₂SO₄, and the solvent was evaporated in vacuum. The obtained residue was purified on a flash chromatography silica gel (PE/EtOAc, 25:1) to obtain the product **25** (756 mg, 84%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 13.07 (s, 1H, -OH), 7.84 (s, 1H, -OCH=C-), 7.54 (d, $J = 8.5$ Hz, 2H, -Ph), 7.17 (d, $J = 8.5$ Hz, 2H, -Ph), 6.72 (d, $J = 10.1$ Hz, 1H, -CH=CHC(CH₃)₂O-), 6.33 (s, 1H, -Ph), 5.72 (d, $J = 10.1$ Hz, 1H, -CH=CHC(CH₃)₂O-), 2.32 (s, 3H, -CH₃), 1.47 (s, 6H, -CH₃); ¹³C NMR (150 MHz, CDCl₃) δ 180.6 (C=O), 169.5, 159.7, 157.33, 156.9, 153.1, 150.8, 130.1 (2 \times C), 128.5, 128., 123.1, 121.9, 115.5 (2 \times C), 106.1, 105.8, 95.0, 78.2, 28.4 (2 \times C), 21.2. The spectroscopic data correspond to reported values [39].

3.2.13. 4-(8,8-Dimethyl-5-((3-methylbut-2-en-1-yl)oxy)-4-oxo-4H,8H-pyrano[2,3-f]chromen-3-yl)phenyl acetate (26)

Under a N₂ atmosphere, to a solution of **25** (720 mg, 1.92 mmol), DEAD (0.6 mL, 3.84 mmol, 2 eq) and PPh₃ (1.00 g, 3.84 mmol, 2 eq) in dry THF (25 mL), was added a solution of compound **18** (332 mg, 3.84 mmol, 2 eq) in 4 mL dry THF. The resulting mixture was stirred for 4 h at rt. After the reaction was completed (TLC), the solvent was removed under reduced pressure, and the yellow viscous oil was dissolved in EtOAc and subjected to flash chromatography on silica gel (EtOAc/PE 1:50 to 1:20) to give **26** (703 mg, 82%) as a yellow solid. Compound **26** was used immediately, without further purification.

3.2.14. 4-(5-Hydroxy-2,2-dimethyl-10-(3-methylbut-2-en-1-yl)-6-oxo-2*H*,6*H*-pyrano[3,2-*g*]chromen-7-yl)phenyl acetate (**27**)

A suspension of **26** (800 mg, 1.79 mmol) and Eu(fod)₃ (160 mg, 0.18 mmol, 0.1 eq) in dry DCE (25 mL) was stirred at reflux for 1 h under a N₂ atmosphere. The resulting orange oil was concentrated in vacuum and subjected to flash chromatography (EtOAc/PE 1:50 to 1:20) to furnish **27** (730 mg, 91%) as a light yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 13.02 (s, 1H, -OH), 7.93 (s, 1H, -OCH=C-), 7.56 (d, *J* = 8.5 Hz, 2H, -Ph), 7.18 (d, *J* = 8.5 Hz, 2H, -Ph), 6.74 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 5.63 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 5.18 (t, *J* = 7.1 Hz, 1H, -CH=C(CH₃)₂), 3.40 (d, *J* = 7.1 Hz, 2H, -CH₂-), 2.33 (s, 3H, -Ac), 1.82 (s, 3H, -CH₃), 1.69 (s, 3H, -CH₃), 1.47 (s, 6H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 180.9 (C=O), 169.5, 157.1, 154.9, 154.6, 153.1, 150.7, 131.8, 130.1 (2 × C), 128.7, 128.1, 122.7, 121.9, 121.8 (2 × C), 115.8, 107.6, 105.9, 105.6, 77.9, 28.2 (2 × C), 25.8, 21.3, 21.2, 17.9. The spectroscopic data correspond to reported values [40].

3.2.15. 5-Hydroxy-7-(4-hydroxyphenyl)-2,2-dimethyl-10-(3-methylbut-2-en-1-yl)-2*H*,6*H*-pyrano[3,2-*g*]chromen-6-one (**1**)

Compound **27** (400 mg, 0.89 mmol) was dissolved in 10 mL of EtOH and 0.17 mL of a 60% KOH aqueous solution (1.78 mmol). After stirring for 30 min, the mixture was acidified with 3 mol/L HCl to pH 6 and extracted with EtOAc. The organic phase was washed with brine, dried over Na₂SO₄, and evaporated. The residue was submitted to flash column chromatography (silica gel, EtOAc/PE 1:50 to 1:20) to afford target **1** (316 mg, 87%) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 13.03 (s, 1H, -OH), 7.89 (s, 1H, -OCH=C-), 7.33 (d, *J* = 7.2 Hz, 2H, -Ph), 6.81 (d, *J* = 7.2 Hz, 2H, -Ph), 6.74 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 5.59 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 5.24 (t, *J* = 7.2 Hz, 1H, -CH=C(CH₃)₂), 3.35 (d, *J* = 7.2 Hz, 2H, -CH₂-), 1.81 (s, 3H, -CH₃), 1.68 (s, 3H, -CH₃), 1.48 (s, 6H, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 180.4 (C=O), 155.9, 155.0, 153.7 (2 × C), 151.7, 130.7, 129.2 (2 × C), 127.0, 122.3, 121.7, 120.8, 114.7, 114.7 (2 × C), 106.5, 104.8, 104.4, 76.8, 27.1 (2 × C), 24.7, 20.2, 16.8. HRMS (ESI) calculated for C₂₅H₂₅O₅⁺ [M + H]⁺ 405.1697, found 405.1697.

3.2.16. 5-Hydroxy-3-((4-hydroxyphenyl)-8,8dimethyl-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**7**)

The method was identical to that described for the preparation of **6**. Compound **7** (1.75 g, 70%) was a light yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.07 (s, 1H, -OH), 9.64 (s, 1H, -OH), 8.38 (s, 1H, -OCH=C-), 7.37 (d, *J* = 8.6 Hz, 2H, -Ph), 6.82 (d, *J* = 8.6 Hz, 2H, -Ph), 6.64 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 6.23 (s, 1H, -Ph), 5.75 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 1.42 (s, 6H, -CH₃); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 181.1 (C=O), 162.0, 159.3, 158.1, 154.6, 152.1, 130.8 (2 × C), 128.7, 123.1, 121.5, 115.6 (2 × C), 114.4, 105.9, 101.2, 99.9, 78.7, 28.3 (2 × C). The spectroscopic data correspond to reported values [41].

3.2.17. 3-(4-(Benzyloxy)phenyl)-5-hydroxy-7-((2-methylbut-3-yn-2-yl)oxy)-4*H*-chromen-4-one (**28**)

The method was identical to that described for the preparation of **25**. Compound **28** (744 mg, 82%) was a white solid. ¹H NMR (400 MHz, CDCl₃) δ 12.84 (s, 1H, -OH), 7.91 (s, 1H, -OCH=C-), 7.56–7.55 (m, 2H, -Ph), 7.17 (d, *J* = 8.6 Hz, 2H, -Ph), 6.68 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 6.29 (s, 1H, -Ph), 5.59 (d, *J* = 10.0 Hz, 1H, -CH=CHC(CH₃)₂O-), 2.32 (s, 3H, -Ac), 1.47 (s, 6H, -CH₃); ¹³C NMR (150 MHz, CDCl₃) δ 180.7 (C=O), 169.6, 162.3, 159.8, 153.0, 152.2, 150.9, 130.1 (2 × C), 128.5, 127.7, 123.3, 121.9, 114.6 (2 × C), 106.1, 101.3, 100.6, 78.3, 28.3 (2 × C), 21.3. HRMS (ESI) calculated for C₂₂H₁₉O₆⁺ [M + H]⁺ 379.1176, found 379.1176.

3.2.18. 4-(8,8-Dimethyl-5-((2-methylbut-3-en-2-yl)oxy)-4-oxo-4*H*,8*H*-pyrano[2,3-*f*]chromen-3-yl)phenyl acetate (**30**)

To a solution of 2-methylbut-3-en-2-ol (3.5 mL, 33.48 mmol, 1.0 eq) in THF (60 mL), *n*-BuLi was added in hexanes (2.5 M, 13.2 mL, 33.48 mmol, 1.0 eq) at 0 °C over the course of 10 min. The clear solution was stirred at 0 °C for 20 min and then added *di-tert*-butyl dicarbonate (7.32 g, 33.48 mmol, 1.0 eq). The clear solution was allowed to heat to 23 °C and further stirred for 4 h. The solvent was poured into ice-cold water, quenched with a saturated aqueous NaHCO₃ solution, and extracted with EtOAc. The organic phase was separated, washed with sat. aq. NaCl (150 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give **29** as a clear, pale, light yellow liquid (6.03 g, 96%), which was used without further purification.

To a stirred suspension of **28** (200 mg, 0.53 mmol) and pulverized 4Å molecular sieves (100 mg) in degassed THF (5 mL), **29** (500 mg, 2.65 mmol) was added at room temperature. After cooling to −20 °C, Pd(PPh₃)₄ (61 mg, 0.05 mmol) was added portionwise to the mixture. After stirring for 21 h, the reaction mixture was filtered through a pad of Celite and the filtrate was concentrated in a vacuum. The residue was purified by silica gel column chromatography (PE/EtOAc = 30:1) to give **30** (178 mg, 78%) as a colorless oil, which was used without further purification.

3.2.19. 4-(5-Hydroxy-8,8-dimethyl-6-(3-methylbut-2-en-1-yl)-4-oxo-4*H*,8*H*-pyrano[2,3-*f*]chromen-3-yl)phenyl acetate (**31**)

The method was identical to that described for the preparation of **27**. Compound **31** was a yellow solid (420 mg, 75%). ¹H NMR (400 MHz, CDCl₃) δ 13.07 (s, 1H, −OH), 7.89 (s, 1H, −OCH=C−), 7.54 (d, *J* = 8.3 Hz, 2H, −Ph), 7.17 (d, *J* = 8.3 Hz, 2H, −Ph), 6.69 (d, *J* = 10.0 Hz, 1H, −CH=CHC(CH₃)₂O−), 5.59 (d, *J* = 10.0 Hz, 1H, −CH=CHC(CH₃)₂O−), 5.23 (t, *J* = 6.6 Hz, 1H, −CH=C(CH₃)₂), 3.35 (d, *J* = 6.6 Hz, 2H, −CH₂−), 2.33 (s, 3H, −Ac), 1.81 (s, 3H, −CH₃), 1.68 (s, 3H, −CH₃), 1.48 (s, 6H, −CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 180.6 (C=O), 169.5, 159.4, 157.3, 152.7, 150.7, 150.5, 131.7, 130.1 (2 × C), 128.7, 127.3, 122.9, 121.9, 121.8 (2 × C), 114.9, 113.0, 105.6, 100.8, 77.9, 28.2 (2 × C), 25.8, 21.3, 21.2, 17.9. HRMS (ESI) calculated for C₂₇H₂₇O₆⁺ [M + H]⁺ 447.1802, found 447.1803.

3.2.20. 5-Hydroxy-3-(4-hydroxyphenyl)-8,8-dimethyl-6-(3-methylbut-2-en-1-yl)-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**2**)

The method was identical to that described for the preparation of **1**. Compound **2** was a yellow solid (320 mg, 71%). ¹H NMR (400 MHz, CDCl₃) δ 13.07 (s, 1H, −OH), 7.85 (s, 1H, −OCH=C−), 7.31 (d, *J* = 7.3 Hz, 2H, −Ph), 6.81 (d, *J* = 7.3 Hz, 2H, −Ph), 6.70 (d, *J* = 9.9 Hz, 1H, −CH=CHC(CH₃)₂O−), 5.59 (d, *J* = 9.9 Hz, 1H, −CH=CHC(CH₃)₂O−), 5.24 (t, *J* = 7.3 Hz, 1H, −CH=C(CH₃)₂), 3.35 (d, *J* = 7.3 Hz, 2H, −CH₂−), 1.81 (s, 3H, −CH₃), 1.68 (s, 3H, −CH₃), 1.48 (s, 6H, −CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 180.1 (C=O), 158.2, 156.2, 155.0, 151.3, 149.5, 130.6, 129.3 (2 × C), 126.1, 122.5, 121.8, 120.8, 114.7 (2 × C), 113.9, 111.8, 104.5, 99.7, 76.8, 27.1 (2 × C), 24.7, 20.2, 16.9. HRMS (ESI) calculated for C₂₅H₂₅O₅⁺ [M + H]⁺ 405.1697, found 405.1696.

3.2.21. MICs Tests

S. aureus ATCC29213, *E. faecalis* ATCC29212, MRSA ATCC33591, and *E. coli* ATCC 25922 were selected to evaluate the target compounds **1–3**. The bacterial strains were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and kept in our laboratory. The MICs were evaluated according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The experiment was performed as reported in the literature [32,33].

3.2.22. Scanning Electron Microscopy (SEM) Characterization

A single colony of *S. aureus* ATCC29213 or *E. faecalis* ATCC29212 was added to LB broth (1.0 mL) and incubated in a shaker (200 rpm, 37 °C) for 12 h. Then the solution was diluted to a concentration of 1 × 10⁹ CFU/mL in PBS. After that, compound **2** (8 × MIC)

was added, and the resulting mixture was incubated at 37 °C for 2 h. Subsequently, the bacterial cells were washed twice with PBS and added to 2.5% glutaraldehyde overnight at 4 °C. The bacterial cells were osmicated in 1% Osmium tetroxide for 1 h at 4 °C to further fix and stain them, then gradually eluted by an ascending graded series of EtOH, dried, and plated with gold at the critical point. Finally, the cells were visualized under SEM (Hitachi S-3400 N, Tokyo, Japan). The negative control was a bacterial suspension without compound 2 treatment [32,33].

4. Conclusions

In conclusion, the efficient syntheses of three natural isoflavones, scandenone (1), osajin (2), and 6,8-diprenylgenistein (3), were accomplished with overall yields of 10%, 6%, and 14%, respectively. Notably, natural isoflavone 3 was synthesized for the first time. The presently described synthesis of compounds 1 and 2 features a stereoselective construction of the pyran ring, and di-prenylation of key intermediate 15 is the key step to obtain isoflavone 3. The antibacterial activities of the synthetic isoflavones 1–3 against *S. aureus* ATCC29213, *E. faecalis* ATCC29212, MRSA ATCC33591, and *E. coli* ATCC 25922 were assessed, and it was shown that all of the natural isoflavones displayed good antibacterial activity against Gram-positive bacteria. Furthermore, the SEM assay indicated that the bacterial cell membranes of Gram-positive bacteria could be disrupted by this kind of natural isoflavone. Taken together, these results demonstrate that natural prenylated isoflavones have tremendous potential to be developed as lead compounds for further optimization to address the problem of bacterial resistance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29112574/s1>. ¹H NMR and ¹³C NMR spectra for the synthesized compounds and HRMS spectra for new compounds. Key crystal data for compound 1, and LCMS reports for compounds 1–3 (PDF).

Author Contributions: W.D. and T.S. conceived and designed this research; H.D. synthesized compounds 1 and 2; Y.C. synthesized compound 3; Y.Z. evaluated the antibacterial activity of 1–3 and performed the scanning electron microscopy (SEM) assay; X.Z. and J.L. Writing—original draft; J.W. Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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