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Total Synthesis of Auriculatin and Millexatin F and Discovery of Their Antibacterial Mechanism

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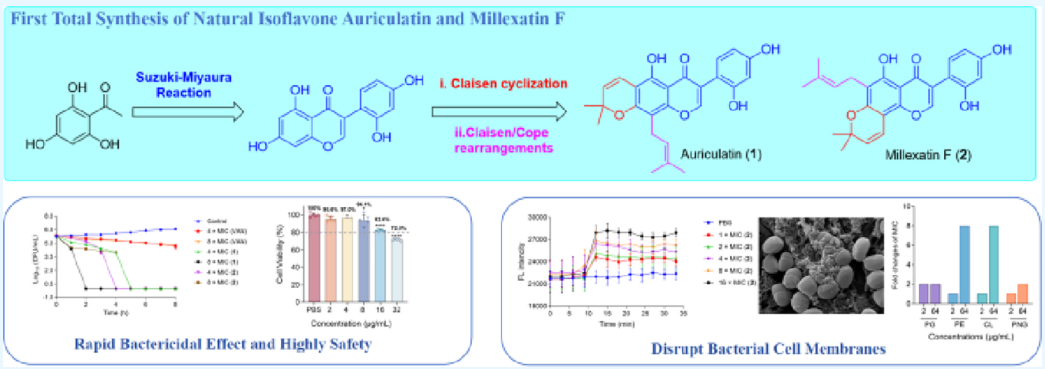

ABSTRACT: The emergence of multidrug-resistant (MDR) bacteria necessitates the urgent development of novel antibacterial agents. This study reported the first total synthesis of two antibacterial isoflavones, auriculatin (**1**) and millexatin F (**2**), derived from the tropical medicinal plant *Millettia extensa*. Through *in vitro* evaluations, both compounds **1** and **2** possessed significant antibacterial activities (MICs = 0.5−4 μg/mL) and rapid bactericidal properties against Gram-positive bacteria, along with high safety for mammalian cells. Mechanistic studies revealed that auriculatin (**1**) and millexatin F (**2**) interact with bacterial cell membranes, inducing alterations in bacterial morphology and membrane permeability and inducing a rise in the leakage of intracellular DNA and proteins, thereby leading to bacterial death. In addition, our studies indicated that millexatin F (**2**) could interact with phosphatidylethanolamine (PE) and cardiolipin (CL) of cytoplasmic membranes in both Gram-positive and Gram-negative bacteria. Furthermore, millexatin F (**2**) showed increased efficacy against Gram-negative bacteria when combined with a permeabilizer (polymyxin B nonapeptide), indicating potential for broader application. These findings underscore the therapeutic promise of auriculatin (**1**) and millexatin F (**2**) as lead candidates in the fight against bacterial infections.

INTRODUCTION

The discovery of antibiotics has provided an effective tool to treat bacterial infections. However, the widespread misuse of antibiotics has led to the emergence of multidrug-resistant (MDR) bacteria.[1] Today, MDR bacteria-induced infections now represent the second leading cause of death worldwide,[2] and the rapid emergence of these bacteria necessitates the development of novel and efficient antibacterial agents. Given their similar antimicrobial mechanisms, new antibacterial agents derived from approved antibiotic molecular frameworks often contribute to bacterial resistance.[3] Therefore, the development of a new generation of antibacterial agents with novel mechanisms of action is imperative.

Numerous strategies are currently being employed to discover novel antibacterial agents to address MDR bacterial infections. Among these strategies, natural products (NPs) and their chemically modified derivatives play a critical role.[4] From 1981 to 2019, approximately half of the antibacterial agents approved by the U.S. Food and Drug Administration (FDA) originated from or were derived from NPs.[5] Notably, clinically available antibacterial agents such as β-lactams (penicillin G) and quinolones (quinine) are NPs or their derivatives. Due to their rich and unique chemical diversity and established clinical efficacy, NPs represent a promising source of lead compounds and have generated considerable interest in the discovery of antibiotics.[6]

Flavonoids represent a large group of natural products derived from plants. Recent studies have indicated that certain prenylated flavonoids not only exhibit significant antimicrobial

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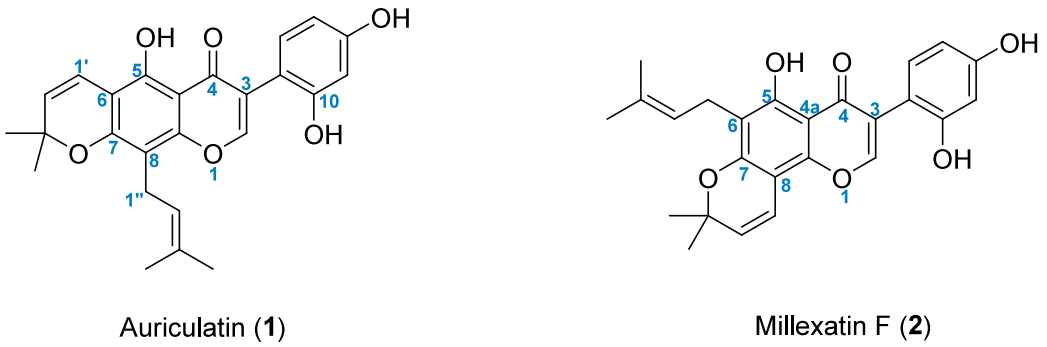
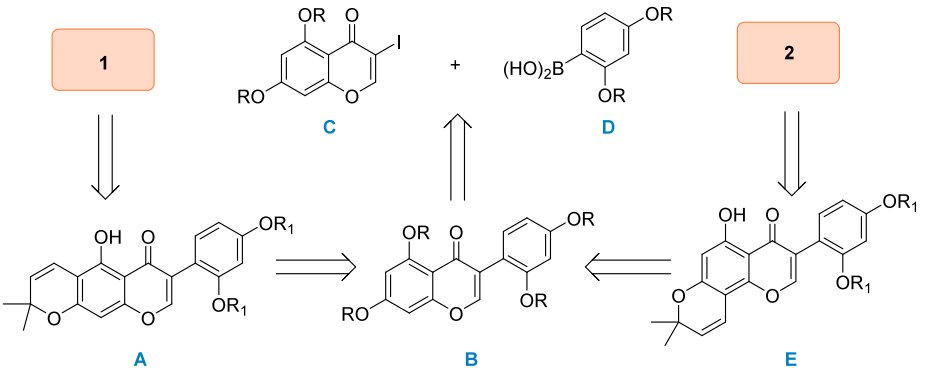
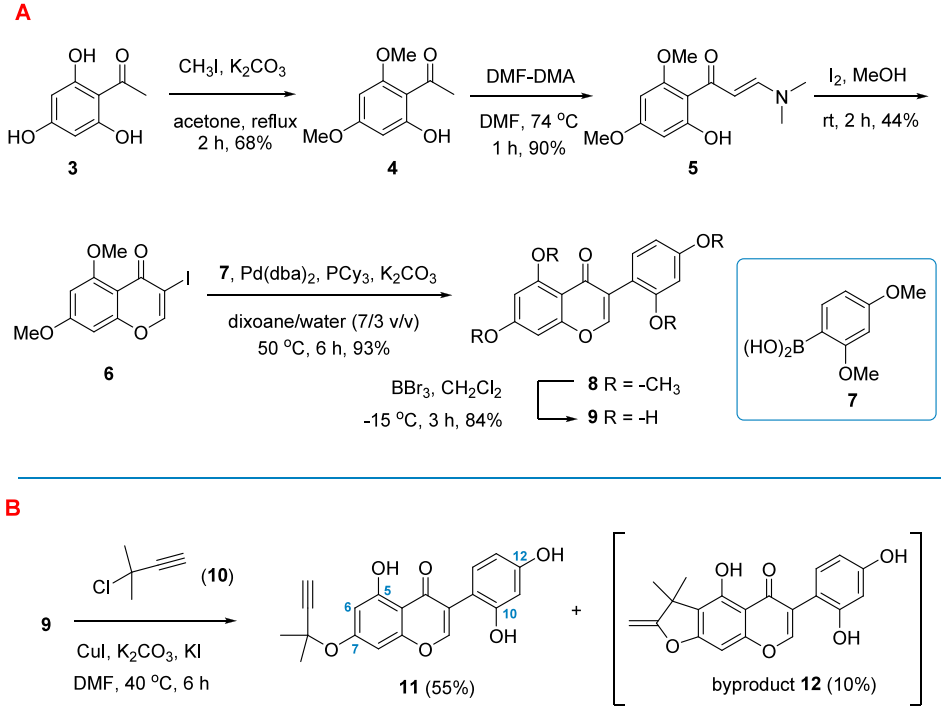


Figure 1. Chemical structures of auriculatin (**1**) and millexatin F (**2**).

Scheme 1. Retrosynthetic Analysis of Compounds 1 and 2



Scheme 2. (A) Synthesis of Intermediate 9 and (B) Synthesis of Intermediate 11



activity but also reduce virulence factors, reverse antibiotic resistance, and act synergistically with antibiotics.[7−11] For example, prenylated isoflavones, auriculatin (**1**) and millexatin F (**2**) (Figure 1), isolated from the tropical medicinal plant *Millettia extensa*,[12,13] demonstrated potent *in vitro* antibacterial activity against Gram-positive bacteria.[14] Prenylated isoflavones **1** and **2** are a pair of regioisomers characterized by the presence of an isopentenyl group and a pyran ring attached to the A ring. In auriculatin (**1**), the isopentenyl group is positioned at the C-8 position, while the pyran ring is attached at C-6/7. In contrast, in millexatin F (**2**), the isopentenyl group is positioned at the C-6 position, with the pyran ring attached at C-7/8. In 2018, Raksat et al. demonstrated that auriculatin (**1**) and millexatin F (**2**) exhibited significant antibacterial

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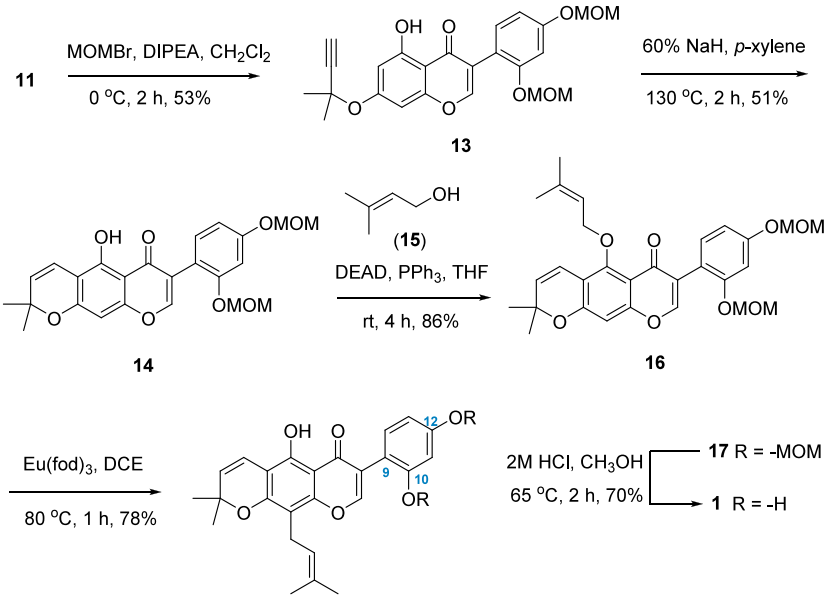
Scheme 3. Synthesis of Auriculatin (1)



activity against *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis*, and *Bacillus subtilis*, with minimum inhibitory concentration (MIC) values in the range of 2−4 μg/mL.[12] In 2021, Polbuppha et al. reported that natural products **1** and **2** demonstrated potent activity against *Enterococcus faecalis* (*E. faecalis*), *Micrococcus luteus*, *S. aureus*, *Streptococcus pyogenes*, *Candida tropicalis*, and Methicillin-resistant *S. aureus* (MRSA), with MIC values ranging from 2 to 4 μg/mL, which were comparable to those of vancomycin.[14]

However, obtaining sufficient quantities of natural isoflavones **1** and **2** for further pharmacological studies, such as investigating their antimicrobial mechanisms and *in vivo* activity, poses a challenge as they were isolated in poor yields from natural sources. Therefore, a suitable synthetic approach for both auriculatin (**1**) and millexatin F (**2**) is essential for antibacterial agent development and discovery. Given the remarkable antibacterial activities of auriculatin (**1**) and millexatin F (**2**) and our ongoing efforts in the synthesis of natural bioactive flavonoids,[15,16] we herein report the first total synthesis of both compounds, along with a discussion of their antimicrobial mechanisms.

RESULTS AND DISCUSSION

The retrosynthetic analyses for both compounds **1** and **2** are illustrated in Scheme 1. It was proposed that the target compound **1** could be synthesized by introducing an isopentenyl group at the C-8 position of the linear isoflavone **A**, using a Claisen/Cope rearrangement. Intermediate **A** could be derived from **B** through a regioselective cyclization reaction. The key intermediate **B** could be obtained from iodochromone **C** and commercially available phenylboronic acid **D** via a Suzuki−Miyaura cross-coupling reaction. Following a similar approach, the target compound **2** could also be synthesized from compounds **C** and **D**, with the angular isoflavone **E** as the key intermediate.

Our synthetic efforts began with commercially available 2,4,6-trihydroxyacetophenone (**3**). Compound **3** was converted into the known 3-iodochromone (**6**) following a reported protocol in three steps, with an overall yield of 27% (Scheme 2A).[17] Subsequently, the Suzuki−Miyaura coupling reaction of **6** with commercially available 2,4-dimethoxyphenylboronic acid (**7**), in the presence of K_2CO_3 and catalytic amounts of tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) and tricyclohexylphosphine (PCy_3) as a ligand, afforded the desired isoflavone (**8**) in good yield (84%).[16] Global demethylation of **8** with BBr_3 produced compound **9** in 83% yield. With the isoflavone core successfully synthesized, we then attempted the installation of the (2*H*)-pyran rings in both natural products **1** and **2**.

In order to achieve this, we exploited the distinct acidities of the hydroxyl groups at different positions, thereby enabling a regioselective propargylation of the hydroxyl group at C-7 without the necessity of protecting the other hydroxyl groups in compound **9**. This resulted in the formation of aryl propargyl ether (**11**) in 55% yield.[9,18] The modest yield was attributed to the formation of an unexpected byproduct (**12**), which was derived from the cyclization of the C-6 alkylation product of **9** with 3-chloro-3-methylbut-1-yne (**10**) (Scheme 2B).[19]

Treatment of **11** with methoxymethyl bromide (MOMBr) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) yielded the C-10/12 MOM-protected intermediate **13**, which was then subjected to NaH-catalyzed [3 + 3] Claisen rearrangement/cyclization conditions to generate linear isoflavone **14**.[20] Compound **14** underwent condensation with 3-methyl-2-buten-1-ol (**15**) under standard Mitsunobu conditions, including triphenylphosphine (PPh_3), diethyl azodicarboxylate (DEAD), and dry THF, to afford **16** in 86% yield.[11]

The 8-prenyl tetracyclic **17** was subsequently obtained from **16** through sequential Claisen/Cope rearrangements, catalyzed by europium(III)-tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate) [Eu(fod)$_3$], in a 78% yield.[11] Removing the MOM-protecting groups at C-10 and C-12 positions of **17** was achieved by stirring it in 2 moL/L HCl and CH_3OH for 4 h in reflux. After careful purification by column chromatography, auriculatin (**1**) was gotten in a yield of 70% (Scheme 3). The NMR spectroscopic data of the synthesized auriculatin (**1**) matched those reported for natural isolaxifolin.[21]

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Scheme 4. (A) Synthesis of Millexatin F (2), (B) Key HMBC Correlations of 14 and 18, and (C) Synthesis of Compound 20

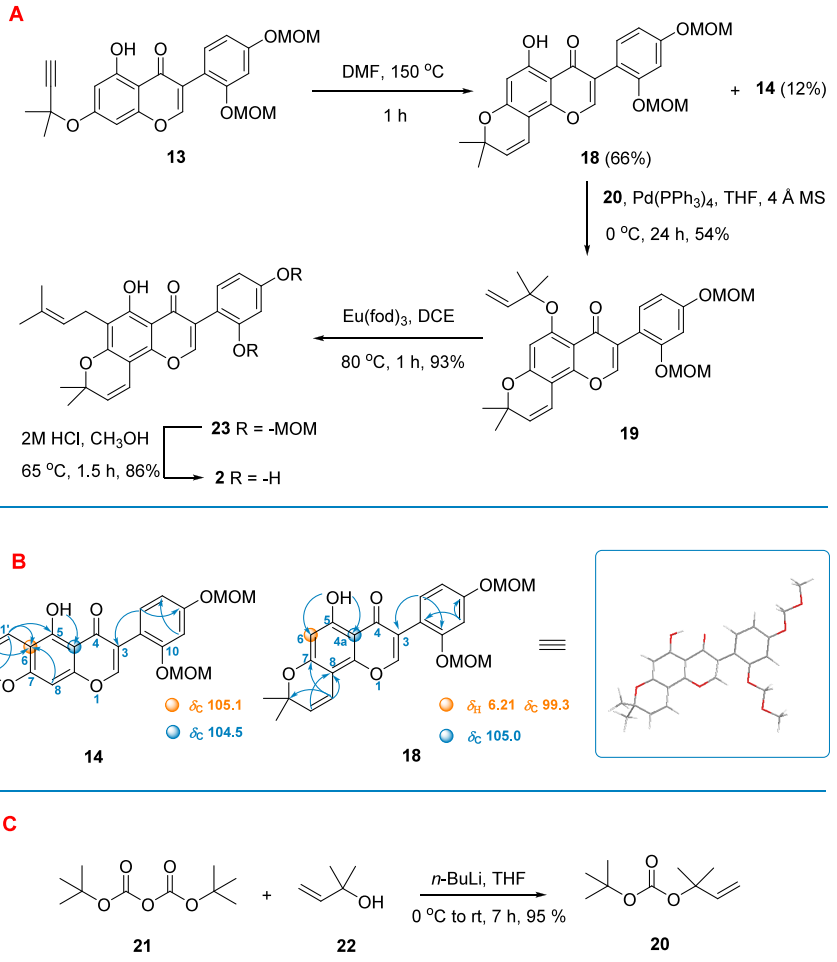

Table 1. MICs and MBCs of Compounds 1 and 2

	MIC/MBC (μg/mL)[a]					
com.	*S. aureus* ATCC29213	*S. aureus* ATCC25923	MRSA ATCC33591	*E. faecalis* ATCC29212	VRE ATCC51299	*E. coli* ATCC25922
1	4/32	2/32	1/16	1/32	1/32	>64/-[b]
2	1/8	2/32	0.5/2	2/8	1/16	>64/-[b]
VAN	0.5	0.25	0.5	2	64	>64
AMP	0.5	0.5	>64	0.5	8	4

[a]Each MIC or MBC was determined from at least three independent experiments. [b]Not tested.

Under an Ar atmosphere, compound **13** was heated in DMF at 150 °C, undergoing a [3 + 3] Claisen rearrangement/cyclization to form angular isoflavone **18** as the major product (66%), along with 12% of the linear product **14** (Scheme 4A).[16] To distinguish molecular structures of isomers **14** and **18**, we characterized them using 2D NMR spectroscopy (Scheme 4B). The HSQC data established all ^{1}J (^{1}H−^{13}C) connectivities (see the Supporting Information), and the key HMBC correlations of both **14** and **18** are shown in Scheme 4B. The correlation of 5-OH [δ_H 13.12] with C-6 [δ_C 105.1] and the absence of correlation between 5-OH and the tertiary carbon atom [C-8 (δ_H 6.26, δ_C 93.8)] indicated that **14** is a linear isomer. The HMBC correlations between 5-OH [δ_H 12.87] with C-4a [δ_C 105.0] and tertiary carbon C-6 [δ_H 6.21, δ_C 99.3] of compound **18** suggested that it is an angular isomer. Furthermore, the chemical structure of **18** was unambiguously corroborated by single X-ray crystallography data (Scheme 4B, single-crystal characterization data are available in the Supporting Information, CDCC 2400952).

After that, the synthesis of **19** was realized via a Pd-catalyzed allylation of isoflavone **18** with carbonic ester **20**, which was prepared from di-*tert*-butyl dicarbonate (**21**) and 2-methylbut-3-en-2-ol (**22**).[11] This was followed by a [Eu(fod)$_3$]-catalyzed aromatic *ortho*-Claisen rearrangement to afford compound **23** in a 93% yield.[16] Finally, deprotection of MOM ethers using dilute HCl in MeOH produced millexatin F (**2**) in 86% yield, with NMR data consistent with literature values.[12]

With chemically synthesized auriculatin (**1**) and millexatin F (**2**) prepared, we proceeded to evaluate their antibacterial effectiveness *in vitro*. Four susceptible strains (*S. aureus* ATCC29213, *S. aureus* ATCC25923, *E. faecalis* ATCC29212, and *Escherichia coli* (*E. coli*) ATCC25922) and two clinically relevant drug-resistant strains (MRSA ATCC33591 and VRE ATCC51299) were tested.[22] Their minimum inhibitory concentrations (MICs) and minimum bactericidal concen-

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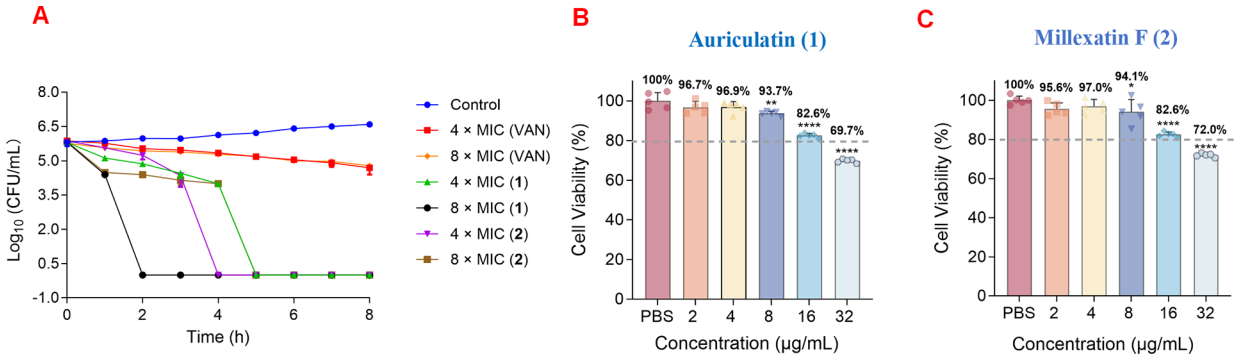

Figure 2. (A) Time-kill kinetics of **1**, **2**, and VAN against MRSA. (B) Toxicity of **1** against HUVECs (n = 5, mean ± SD). (C) Toxicity of **2** against HUVECs (n = 5, mean ± SD). *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 vs PBS.

trations (MBCs) are presented in Table 1. Vancomycin (VAN) and ampicillin (AMP), two approved antibiotics, were used as positive controls. Neither auriculatin (**1**) nor millexatin F (**2**) displayed inhibitory effects against the Gram-negative bacterium *E. coli*, whereas AMP exhibited substantial activity (MIC = 4 μg/mL). Compounds **1** and **2** demonstrated significant antibacterial activity against Gram-positive bacterial strains, with MIC values ranging from 0.5 to 4 μg/mL, comparable to AMP and VAN. Notably, compounds **1** and **2** displayed comparable or enhanced potency against the extensively drug-resistant strains MRSA ATCC33591 and VRE ATCC51299 in comparison to the drug-sensitive strains *S. aureus* ATCC29213, *S. aureus* ATCC25923, and *E. faecalis* ATCC29212. Compounds **1** and **2** exhibited similar activity (MICs = 0.5−1 μg/mL) to VAN (MIC = 0.5 μg/mL) and were substantially more effective than AMP (MIC > 64 μg/mL) against MRSA. Both compounds **1** and **2** exhibited greater antibacterial potency against VRE (MICs = 1 μg/mL) compared to VAN (MIC = 64 μg/mL) and AMP (MIC = 8 μg/mL). Bactericidal activities of compounds **1** and **2** were confirmed by evaluating their MBCs on the agar medium (Table 1). These results suggest that both compounds **1** and **2** possess potential as lead compounds for treating drug-resistant bacterial infections.

To investigate the bactericidal properties of compounds **1** and **2**, a time-kill kinetics assay against MRSA ATCC33591 was conducted.[23−25] As shown in Figure 2A, at a concentration of 8 × MIC (8 μg/mL), compound **1** eliminated all MRSA within 2 h, while compound **2** achieved complete eradication within 4−5 h. By contrast, the clinically used antibiotic vancomycin achieved only a 1.02 log reduction in bacterial count over 8 h at 8 × its MIC. Therefore, auriculatin (**1**) and millexatin F (**2**) exhibited rapid bactericidal effects against MRSA, potentially reducing the treatment duration for bacterial infections.

Given the rapid and efficient bactericidal effects of auriculatin (**1**) and millexatin F (**2**), their *in vitro* cytotoxicity was assessed against human umbilical vein endothelial cells (HUVECs) using the Cell Counting Kit-8 (CCK-8) assay.[26] As shown in Figure 2B,C after treatment with compounds **1** and **2** at a concentration of 16 μg/mL (16−32 times their MICs against MRSA), the HUVECs maintained over 80% cell viability, indicating that natural isoflavones **1** and **2** are highly safe for mammalian cells.

Given the promising antibacterial properties of auriculatin (**1**) and millexatin F (**2**) and their safety for mammalian cells, these compounds hold potential as lead candidates for drug-resistant bacteria treatment. Understanding their antibacterial mechanisms is essential; we thus conducted a series of experiments to elucidate their mechanisms of antibacterial action. Initially, the morphological changes of MRSA treated with compounds **1** and **2** were observed using scanning electron microscopy (SEM).[27] As shown in Figure 3A, the surface of untreated bacterial cells appeared smooth and intact. However, after treatment with compounds **1** or **2** (8 × MIC) for 2 h, the bacterial morphology exhibited irregular alterations. Some bacterial cells ruptured, with visible leakage of cellular contents, and cellular membranes displayed noticeable wrinkling, distortion, and blebbing. These results indicate that compounds **1** and **2** can induce bacterial membrane damage.

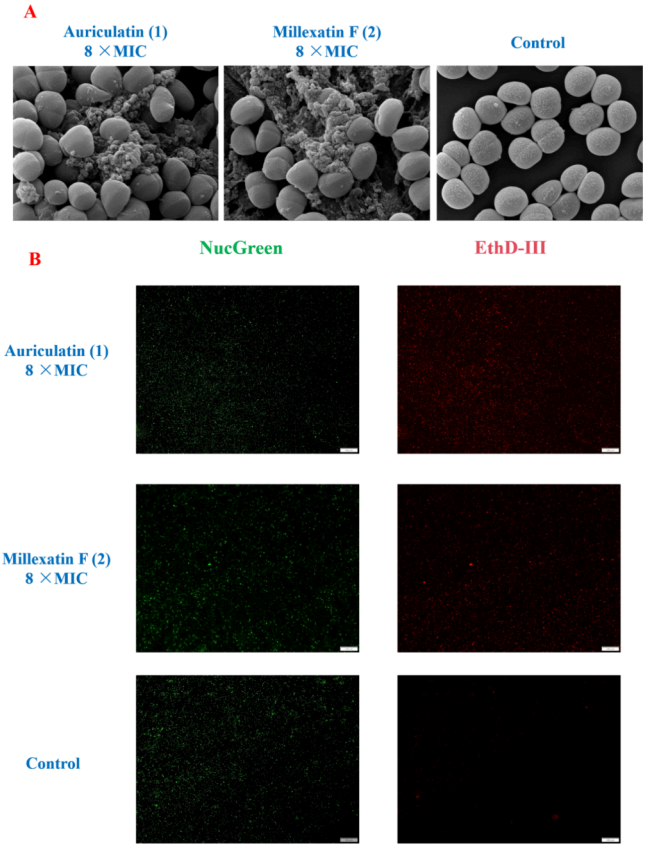

Figure 3. (A) SEM characterization on MRSA. Scale bar: 500 nm. (B) NucGreen and EthD-III staining of MRSA. Scale bar: 100 μm.

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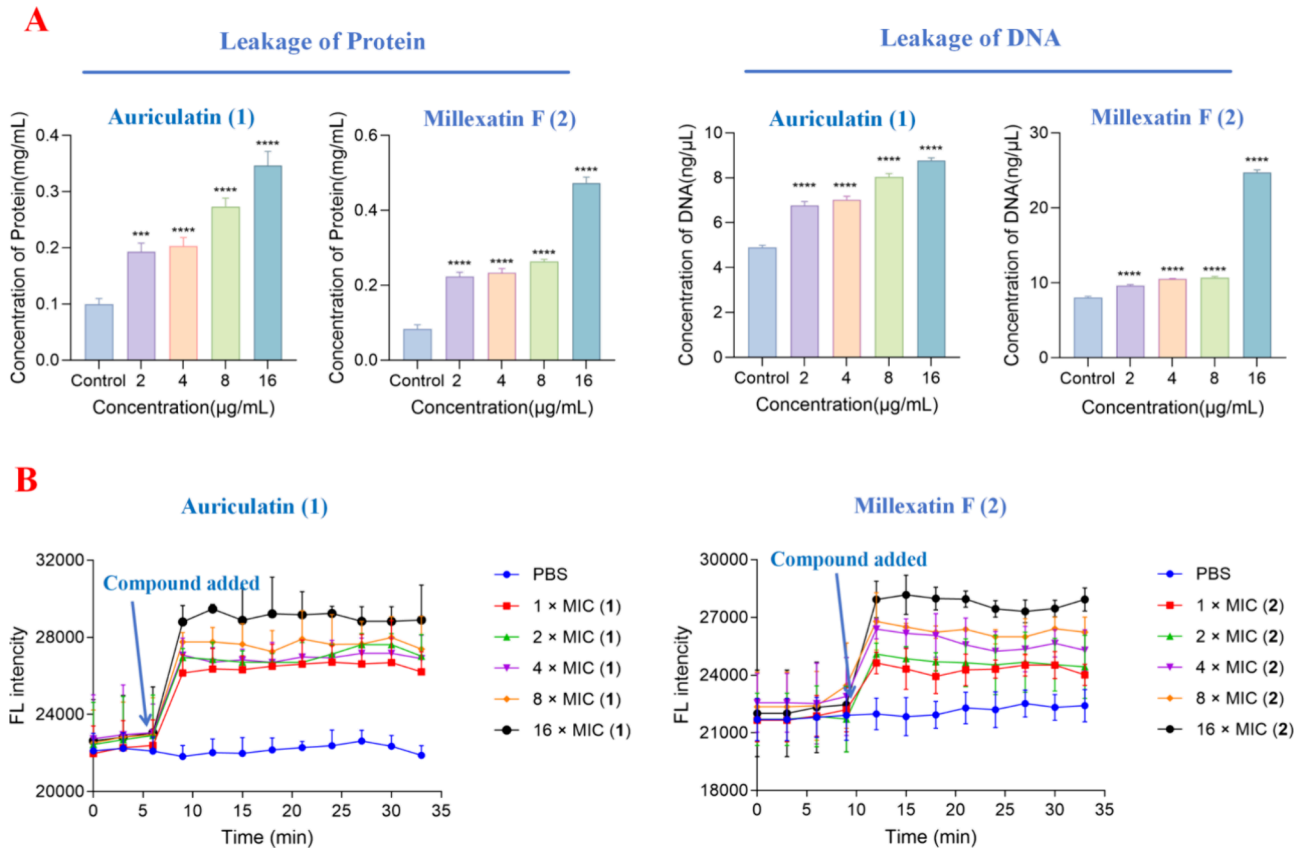

Figure 4. (A) Protein and DNA leakages after different concentrations of **1** or **2** treatment (n = 2, mean ± SD). (B) Membrane permeabilization ability of **1** or **2** using a SYTOX Green assay (n = 3, mean ± SD). *P < 0.05, ***P < 0.001, and ****P < 0.0001 vs PBS.

The mechanism of action of compounds **1** and **2** was further investigated using a LIVE/DEAD bacterial viability assay. The assay kit included two fluorescent nucleic acid stains: NucGreen and EthD-III.[11] NucGreen passes through both intact and impaired bacterial cell membranes and emits green fluorescence upon interacting with DNA, whereas EthD-III penetrates only damaged cell membranes, producing red fluorescence. As shown in Figure 3B, treatment with compounds **1** or **2** (8 × MIC) for 2 h resulted in both red and green fluorescence. In contrast, only green fluorescence was observed in the untreated control group. These results indicate that compounds **1** and **2** exert their bactericidal effect by disrupting bacterial cell membranes.

Disruption of bacterial membranes or alterations in their permeability typically result in the leakage of cytoplasmic contents, including nucleic acids and proteins. Consequently, to evaluate the extent of membrane damage, the concentrations of leaked nucleic acids and proteins were quantified after administering different concentrations of compounds **1** and **2**.[28,29] As illustrated in Figure 4A, auriculatin (**1**) induced dose-dependent leakage of intracellular nucleic acids and proteins into the bacterial supernatant. Compared to the control group, low concentrations of millexatin F (**2**) (2−8 μg/mL) induced minimal release of intracellular nucleic acids and proteins, whereas 16 μg/mL millexatin F (**2**) significantly increased the leakage rate. These findings indicate that both auriculatin (**1**) and millexatin F (**2**) can disrupt MRSA membranes, leading to leakage of nucleic acids and proteins.

We further conducted SYTOX Green uptake assays to evaluate the ability of auriculatin (**1**) and millexatin F (**2**) to permeabilize bacterial membranes. SYTOX Green can only penetrate impaired membranes, resulting in a sharply enhanced green fluorescence.[30,31] The extent of bacterial cell membrane damage can be indicated by the increased fluorescence intensity. Results shown in Figure 4B indicated that fluorescence intensity significantly increased after compounds **1** and **2** were added to SYTOX Green-treated MRSA, while the untreated control group remained unchanged. Additionally, the fluorescence intensity increased in direct proportion to the concentrations of compounds **1** and **2**, demonstrating that auriculatin (**1**) and millexatin F (**2**) can permeabilize MRSA membranes.

The cell envelope of Gram-positive bacteria comprises two distinct layers: the cytoplasmic membrane and the cell wall. The cytoplasmic membrane is composed of various phospholipids, including phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and cardiolipin (CL).[11,32] The cell wall of Gram-positive bacteria features a thick peptidoglycan (PGN) layer situated outside the cytoplasmic membrane. To further investigate the membrane-targeting mechanism of these compounds, millexatin F (**2**) was incubated with different concentrations of exogenous PG, PE, CL, and PGN, and the resulting changes in its MIC values against MRSA are illustrated in Figure 5A. The antibacterial activity of compound **2** significantly decreased, as evidenced by an 8-fold increase in the MIC, upon the addition of high concentrations (64 μg/mL) of PE and CL, while the addition of PGN and PG only doubled the MICs without significantly affecting antibacterial activity. These results indicate that isoflavone **2** can bind to PE and CL in the cytoplasmic membrane of Gram-positive

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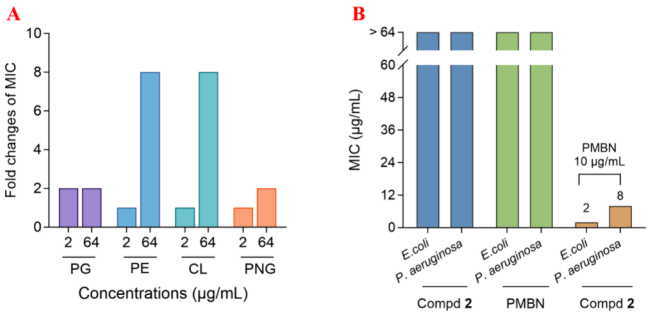

Figure 5. (A) Effects of exogenous addition of phospholipids and PGN on the antibacterial activity of compound **2**. (B) MIC (μg/mL) of **2** without or with PMBN against Gram-negative bacteria.

bacteria, thereby disrupting membrane integrity and leading to bacterial death.

As the components of phospholipids in both Gram-positive and Gram-negative cytoplasmic membranes are the same, we hypothesized that these isoflavones would be equally effective against the Gram-negative cytoplasmic membrane. However, Gram-negative bacteria possess more complex cell envelopes compared to those of Gram-positive bacteria. Only Gram-negative bacteria possess an outer membrane (OM), which consists of lipopolysaccharides, phospholipids, and proteins, situated outside the bacterial cell wall.[33]

We hypothesized that the OM of Gram-negative bacteria might act as a permeability barrier, preventing millexatin F (**2**) from attaching to the cytoplasmic membrane and thereby rendering it inactive. To elucidate the role of the OM, we determined the antibacterial activities of millexatin F (**2**) against two strains of Gram-negative bacteria, *E. coli* ATCC25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC27853, in the presence of a subinhibitory concentration (10 μg/mL) of the polymyxin B nonapeptide (PMBN).[10,33] PMBN is a known OM permeabilizer that enhances the OM permeability and has no intrinsic antibacterial activity.[34] As illustrated in Figure 5B, millexatin F (**2**) exhibited significant antibacterial activity against *E. coli* (MIC = 2 μg/mL) and *P. aeruginosa* (MIC = 8 μg/mL) in the presence of PMBN. In contrast, no antibacterial activity was observed for either millexatin F (**2**) or PMBN when tested individually (MICs > 64 μg/mL). The results indicate that millexatin F (**2**) can compromise the integrity of the cytoplasmic membrane in both Gram-positive and Gram-negative bacteria. Furthermore, the combination therapy of this isoflavone with PMBN presents a potential strategy for combating Gram-negative bacterial infections.

CONCLUSIONS

In summary, the synthesis of two natural isoflavones, auriculatin (**1**) and millexatin F (**2**), has been successfully achieved for the first time. The synthesis involved two key steps: a Suzuki−Miyaura coupling reaction to construct the isoflavone core followed by a Claisen/Cope rearrangement to introduce prenyl substituents. Both compounds **1** and **2** demonstrated significant antibacterial activity against five Gram-positive bacterial strains (including two kinds of MDR strains) with MIC values ranging from 0.5 to 4 μg/mL, which was superior to vancomycin in terms of their rapid bactericidal properties. The antibacterial action of both isoflavones **1** and **2** was found to involve disruption of the bacterial cell membranes, leading to significant leakage of vital intracellular components. Furthermore, millexatin F (**2**) was observed to interact with PE and CL of cytoplasmic membranes in both Gram-positive and Gram-negative bacteria, leading to membrane rupture and subsequent bacterial death. Overall, these results contributed valuable insights into the development of novel antibacterial agents derived from flavonoid structures.

METHODS

General Experimental Procedures. Melting points were recorded on a Büchi B-545 melting point apparatus (Büchi Corporation, Flawil, Switzerland). ^{1}H NMR, ^{13}C NMR, HMBC, and HSQC spectra were recorded on a Bruker Avance 400 spectrometer (Bruker Corporation, Billerica, USA) or a JEOL Eclipse-600 spectrometer (JEOL Com. Ltd., Tokyo, Japan), and tetramethylsilane (TMS) was used as the internal reference. The HRMS spectra were recorded by an Agilent 6545 LC/QTOF mass spectrometer (Agilent Technologies, Santa Clara, USA). The compounds were purified using manual silica gel (100−200 mesh) columns. Reagents and solvents were of analytical grade and purchased from Energy Chemical Company (Shanghai, China) or Shanghai Titan Scientific Co., Ltd.

*1-(2-Hydroxy-4,6-dimethoxyphenyl)ethan-1-one (**4**).* A mixture of 1-(2-hydroxy-4,6-dimethoxyphenyl)ethan-1-one (**3**) (1.00 g, 5.94 mmol), methyl iodide (2.10 g, 14.86 mmol), and potassium carbonate (2.05 g, 14.86 mmol) in 20 mL of acetone was stirred and heated under reflux for 2 h. The progress of the reaction was monitored by TLC, and the reaction was complete when compound **3** had been consumed. The reaction mixture was then poured into 30 mL of ice-cold water and extracted with 3 × 20 mL of ethyl acetate (EtOAc). The combined organic layers were dried over anhydrous sodium sulfate (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using a petroleum ether/ethyl acetate (PE/EtOAc, 9:1) eluent to yield compound **4** as a white solid (796 mg, 4.06 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.04 (s, 1H, −OH), 6.07 (d, J = 2.3 Hz, 1H, −Ph), 5.93 (d, J = 2.4 Hz, 1H, −Ph), 3.87 (s, 3H, $-OCH_3$), 3.83 (s, 3H, $-OCH_3$), 2.62 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.2, 167.6, 166.1, 162.9, 106.0, 93.5, 90.7, 55.5 (2 × C), 32.9. The spectroscopic data correspond to reported values.[35]

*(E)-3-(Dimethylamino)-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one (**5**).* Compound **4** (2.00 g, 10.20 mmol) was dissolved in anhydrous DMF (15 mL) and heated to 74 °C. Subsequently, DMF-DMA (6.7 mL, 51 mmol) was added dropwise to the solution. The reaction mixture was stirred for 1 h and then cooled to room temperature. H_2O (100 mL) was added to the reaction mixture with stirring, resulting in the precipitation of solids. The precipitate was collected by filtration to obtain the crude product **5**. The crude product was purified by chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 10:1) solvent system, yielding pure product **5** as a bright yellow solid (2.31 g, 9.18 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 15.65 (s, 1H, −OH), 7.92 (d, J = 12.3 Hz, 1H, $-CH{=}CHN(CH_3)_2$), 6.26 (d, J = 12.3 Hz, 1H, $-CH{=}CHN(CH_3)_2$), 6.07 (d, J = 2.4 Hz, 1H, −Ph), 5.92 (d, J = 2.4 Hz, 1H, −Ph), 3.84 (s, 3H, $-OCH_3$), 3.80 (s, 3H, $-OCH_3$), 3.14 (s, 3H, $-N(CH_3)_2$), 2.93 (s, 3H, $-N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.9, 167.9, 164.0, 161.7, 154.3, 105.4, 96.7, 94.0, 90.5, 55.6 (2 × C), 55.3 (2 × C). The spectroscopic data correspond to reported values.[35]

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*3-Iodo-5,7-dimethoxy-4H-chromen-4-one (**6**).* Compound **5** (2.00 g, 10.20 mmol) was dissolved in MeOH (25 mL) at room temperature. Iodine (3.87 g, 15.3 mmol) was added to the solution, and the mixture was stirred for 2 h. The solution was then washed with saturated $Na_2S_2O_3$ (15 mL). The aqueous layer was extracted three times with DCM (100 mL × 3). The combined organic layers were washed with brine (30 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified via flash chromatography (PE/EtOAc, 10:1) to afford **6** (2.17 g, 6.53 mmol, 64%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H, −OCH═C−), 6.38 (d, J = 2.3 Hz, 1H), 6.34 (d, J = 2.3 Hz, 1H, −Ph), 3.89 (s, 3H, $-OCH_3$), 3.85 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 164.2, 160.9, 159.7, 155.4, 107.4, 96.6, 92.4, 89.7, 56.4, 55.9. The spectroscopic data correspond to reported values.[36]

*3-(2,4-Dimethoxyphenyl)-5,7-dimethoxy-4H-chromen-4-one (**8**).* Compound **6** (2.40 g, 7.23 mmol) was dissolved in a mixture of 1,4-dioxane (35 mL) and H_2O (15 mL). K_2CO_3 (3.00 g, 21.69 mmol) and compound **7** (2.70 g, 14.46 mmol) were added to the solution. The mixture was purged with nitrogen for 10 min. PCy_3 (162 mg, 0.58 mmol) and $Pd(dba)_2$ (167 mg, 0.29 mmol) were then added to the mixture. The reaction mixture was warmed to 50 °C and stirred at this temperature for 6 h before being cooled to room temperature. A saturated aqueous solution of NH_4Cl (50 mL) was added to the mixture, which was then filtered. The filter cake was dissolved in ethyl acetate (EtOAc, 30 mL), poured into water (50 mL), and extracted with EtOAc three times. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 5:1) solvent system to yield compound **8** as a yellow solid (2.31 g, 6.72 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (s, 1H, −OCH═C−), 7.22 (d, J = 9.0 Hz, 1H, −Ph), 6.51 (dd, J = 5.9, 2.3 Hz, 2H, −Ph), 6.42 (d, J = 2.3 Hz, 1H, −Ph), 6.34 (d, J = 2.3 Hz, 1H, −Ph), 3.90 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.82 (s, 3H, $-OCH_3$), 3.74 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.3, 163.7, 161.4, 160.9, 160.07, 158.6, 151.7, 132.5, 123.2, 113.6, 110.1, 104.2, 98.9, 96.1, 92.6, 56.3, 55.7, 55.7, 55.4. The spectroscopic data correspond to reported values.[37]

*3-(2,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one (**9**).* Compound **8** (900 mg, 2.77 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL). A solution of 1 M BBr_3 in CH_2Cl_2 (25.0 mL, 24.9 mmol) was slowly added to the mixture at −15 °C under an Ar atmosphere. The reaction mixture was then stirred at room temperature overnight. Afterward, the mixture was poured into ice water (100 mL) and extracted with EtOAc (50 mL). The organic layer was washed with water (30 mL × 3) and brine (30 mL × 3). The aqueous layers were further extracted with EtOAc (30 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 2:1) solvent system to yield compound **9** as a yellow solid (666 mg, 2.33 mmol, 84% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.91 (s, 1H, −OH), 9.31 (s, 1H, −OH), 9.22 (s, 1H, −OH), 8.07 (s, 1H, −OCH═C−), 6.90 (d, J = 8.3 Hz, 1H, −Ph), 6.30 (dd, J = 4.1, 2.2 Hz, 2H, −Ph), 6.20 (dd, J = 8.3, 2.2 Hz, 1H, −Ph), 6.15 (d, J = 2.2 Hz, 1H, −Ph); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.3, 163.7, 161.4, 160.9, 160.0, 158.6, 151.7, 132.5, 123.2, 113.6, 110.1, 104.2, 98.9, 96.1, 92.6, 56.3, 55.7, 55.7, 55.4. The spectroscopic data correspond to reported values.[38]

*3-(2,4-Dihydroxyphenyl)-5-hydroxy-7-((2-methylbut-3-yn-2-yl)oxy)-4H-chromen-4-one (**11**).* Compound **9** (1.10 g, 3.84 mmol), CuI (72 mg, 0.38 mmol), K_2CO_3 (2.0 eq, 1.10 g, 7.68 mmol), and KI (1.5 eq, 1.00 g, 5.76 mmol) were suspended in DMF (20 mL). Then, compound **10** (1.2 equiv, 0.5 mL, 4.61 mmol) was added dropwise to the suspension. The reaction mixture was stirred at 40 °C for 2 h. The reaction was quenched by adding 1 mol/L aqueous HCl. The mixture was then poured into ice water (50 mL) and extracted with EtOAc (40 mL). The organic layer was washed with brine (30 mL × 3) and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 15:1) mixture as the eluent to yield compound **11** as a white solid (744 mg, 2.11 mmol, 55% yield) and compound **12** (135 mg, 0.38 mmol, 10% yield).

Compound **11** was a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.19 (s, 1H, −OH), 8.43 (s, 1H, −OH), 7.97 (s, 1H, −OCH═C−), 6.99 (d, J = 8.4 Hz, 1H, −Ph), 6.89 (d, J = 2.1 Hz, 1H, −Ph), 6.74 (d, J = 2.1 Hz, 1H, −Ph), 6.55 (d, J = 2.2 Hz, 1H, −Ph), 6.47 (dd, J = 8.4, 2.3 Hz, 1H, −Ph), 2.72 (s, 1H −C≡CH), 1.75 (s, 6H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.20, 162.81, 161.64, 158.31, 157.29, 156.95, 155.38, 130.83, 123.12, 112.06, 108.87, 106.31, 106.08, 103.23, 97.14, 84.43, 75.62, 73.06, 29.6 (2 × C), HRMS (ESI): m/z calcd for $C_{20}H_{17}O_6$ $[M + H]^+$, 353.1020; found, 353.1022. Compound **12** was a yellow solid (135 mg, 0.38 mmol, 10%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.45 (s, 1H, −OH), 9.40 (s, 1H, −OH), 9.31 (s, 1H, −OH), 8.26 (s, 1H, −OCH═C−), 6.97 (d, J = 8.3 Hz, 1H, −Ph), 6.77 (s, 1H, −Ph), 6.37 (d, J = 2.3 Hz, 1H, −Ph), 6.28 (dd, J = 8.3, 2.3 Hz, 1H, −Ph), 4.73 (d, J = 3.0 Hz, 1H, −C=CH_2), 4.52 (d, J = 3.0 Hz, 1H, −C=CH_2), 1.52 (s, 6H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.3, 171.0, 160.8, 158.7, 157.2, 156.6, 156.4, 155.8, 132.1, 120.7, 115.3, 108.3, 106.7, 106.2, 102.6, 89.4, 84.5, 42.6, 27.2, (2 × C); HRMS (ESI): m/z calcd for $C_{20}H_{17}O_6$ $[M + H]^+$, 353.1020; found, 353.1023.

*3-(2,4-Bis(methoxymethoxy)phenyl)-5-hydroxy-7-((2-methylbut-3-yn-2-yl)oxy)-4H-chromen-4-one (**13**).* Compound **11** (1.50 g, 4.26 mmol) was dissolved in CH_2Cl_2 (25 mL) under stirring. DIPEA (3.0 mL, 17 mmol) was added to the solution at room temperature, followed by the addition of MOMBr (1.00 mL, 12.8 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The mixture was then acidified with 1 mol/L aqueous HCl (4 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield crude product **13** as a red oil. The crude product was purified by silica gel column chromatography using a petroleum ether/ethyl acetate (PE/EtOAc, 30:1) solvent system to afford pure compound **13** as a yellow solid (878 mg, 1.99 mmol, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.78 (s, 1H, −OH), 7.84 (s, 1H, −OCH═C−), 7.20 (d, J = 8.4 Hz, 1H, −Ph), 6.92 (d, J = 2.3 Hz, 1H, −Ph), 6.81 (d, J = 2.2 Hz, 1H, −Ph), 6.78 (dd, J = 8.4, 2.2 Hz, 1H, −Ph), 6.69 (d, J = 2.2 Hz, 1H, −Ph), 5.19 (s, 2H, $-OCH_2OCH_3-$), 5.13 (s, 2H, $-OCH_2OCH_3-$), 3.49 (s, 3H, $-CH_3$), 3.44 (s, 3H, $-CH_3$), 2.69 (s, 1H, −C≡CH), 1.74 (s, 6H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.8, 162.0,

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161.9, 158.9, 157.4, 156.35, 154.3, 132.0, 121.2, 114.2, 109.0, 106.8, 104.1, 102.8, 97.4, 95.0, 94.5, 84.8, 75.2, 72.8, 56.3, 56.1, 29.6 (2 × C); HRMS (ESI): m/z calcd for $C_{24}H_{25}O_8$ $[M + H]^+$, 441.1544; found, 441.1547.

*7-(2,4-Bis(methoxymethoxy)phenyl)-5-hydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-g]chromen-6-one (**14**).* Under a nitrogen atmosphere, compound **13** (790 mg, 2.24 mmol) and 60% NaH (538 mg, 13.4 mmol) were dissolved in dry *p*-xylene (20 mL) at room temperature. The mixture was then stirred at 130 °C for 2 h. After the reaction was complete, the mixture was cooled and quenched with 3 mol/L aqueous HCl, followed by dilution with EtOAc. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 30:1) solvent system to yield compound **14** as a yellow solid (503 mg, 1.14 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.12 (s, 1H, −OH), 7.71 (s, 1H, −OCH═C−), 7.12 (d, J = 8.4 Hz, 1H, −Ph), 6.84 (d, J = 2.3 Hz, 1H, −Ph), 6.70 (dd, J = 8.4, 2.3 Hz, 1H, −Ph), 6.64 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 6.26 (s, 1H, −Ph), 5.54 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 5.11 (s, 2H, −OCH_2OCH_3−), 5.06 (s, 2H, −OCH_2OCH_3−), 3.41 (s, 3H, −CH_3), 3.36 (s, 3H, −CH_3), 1.40 (s, 6H, −CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.7 (C-4), 158.4 (C-7), 157.8 (C-8a), 156.3 (C-10), 155.8 (C-12), 155.3 (C-5), 153.0 (C-2), 131.0 (C-14), 127.1 (C-2'), 120.0 (C-3), 114.5 (C-1'), 113.1 (C-9), 108.0 (C-13), 105.1 (C-6), 104.5 (C-4a), 103.0 (C-11), 93.9, 93.9 (C-8), 93.3, 76.9 (C-3′), 55.2, 55.1, 27.2 (2 × C); HRMS (ESI): m/z calcd for $C_{24}H_{25}O_8$ $[M + H]^+$, 441.1544; found, 441.1546.

*7-(2,4-Bis(methoxymethoxy)phenyl)-2,2-dimethyl-5-((3-methylbut-2-en-1-yl)oxy)-2H,6H-pyrano[3,2-g]chromen-6-one (**16**).* Under a nitrogen atmosphere, a solution of compound **14** (174 mg, 0.40 mmol) in dry THF (10 mL) was prepared. DEAD (0.1 mL, 0.80 mmol, 2 equiv) and PPh_3 (210 mg, 0.62 mmol, 2 equiv) were added to the solution. Subsequently, a solution of compound **15** (0.2 mL, 0.80 mmol, 2 equiv) in dry THF (2 mL) was added to the mixture. The resulting solution was stirred at room temperature for 4 h. After confirming the completion of the reaction by TLC, the solvent was removed under reduced pressure. The resulting yellow viscous oil was purified by chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 10:1) solvent system. The desired product, compound **16**, was obtained as a yellow solid (150 mg, 0.29 mmol, 86%). Compound **16** was used immediately without further purification.

*7-(2,4-Bis(methoxymethoxy)phenyl)-5-hydroxy-2,2-dimethyl-10-(3-methylbut-2-en-1-yl)-2H,6H-pyrano[3,2-g]-chromen-6-one (**17**).* Compound **16** (150 mg, 0.30 mmol) was combined with $Eu(fod)_3$ (29 mg, 0.03 mmol) in dry DCE (10 mL) under a nitrogen atmosphere. The mixture was stirred at reflux for 1 h. The resulting orange oil was concentrated under vacuum and purified by chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 40:1) solvent system to yield compound **17** as a light yellow oil (120 mg, 0.24 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.48 (s, 1H, −OH), 8.59 (s, 1H, −OCH═C−), 8.00 (s, 1H, −CH═CHC$(CH_3)_2$), 7.03 (d, J = 8.4 Hz, 1H, −Ph), 6.75 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 6.56 (d, J = 2.5 Hz, 1H), 6.48 (dd, J = 8.4, 2.5 Hz, 1H), 5.66 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 5.58 (s, 1H, −Ph), 5.16 (t, J = 7.3 Hz, 1H, −CH═C$(CH_3)_2$), 3.42 (d, J = 7.3 Hz, 2H, −CH_2CH═C$(CH_3)_2$), 1.81 (s, 3H, −CH_3), 1.68 (s, 3H, −CH_3), 1.48 (s, 6H, −CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.1, 158.8, 156.8, 156.4, 154.9, 154.8, 153.9, 132.0, 131.7, 128.0, 122.1, 120.8, 115.9, 114.3, 109.0, 107.5, 105.9, 105.4, 104.1, 95.0, 94.5, 77.7, 56.3, 56.1, 28.2, 25.8, 21.3, 17.9 (2 × C); HRMS (ESI): m/z calcd for $C_{29}H_{33}O_8$ $[M + H]^+$, 509.2170; found, 509.2172.

*7-(2,4-Dihydroxyphenyl)-5-hydroxy-2,2-dimethyl-10-(3-methylbut-2-en-1-yl)-2H,6H-pyrano[3,2-g]chromen-6-one (**1**).* Compound **17** (114 mg, 0.23 mmol) was dissolved in MeOH (6 mL) and 2 mol/L aqueous HCl (2 mL). The solution was stirred at 65 °C for 2 h. After the reaction was completed (confirmed by TLC), the mixture was poured into ice-cold water. The resulting solution was neutralized to pH 7.0 using a saturated $NaHCO_3$ solution and then extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 10:1) solvent system to yield compound **1** as a yellow solid (68 mg, 0.26 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.48 (s, 1H, −OH), 8.59 (s, 1H, −OH), 8.00 (s, 1H, −OCH═C−), 7.03 (d, J = 8.4 Hz, 1H, −Ph), 6.75 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 6.56 (d, J = 2.5 Hz, 1H, −Ph), 6.48 (dd, J = 8.4, 2.5 Hz, 1H), 5.66 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 5.58 (s, 1H), 5.16 (t, J = 7.3 Hz, 1H, −CH═C$(CH_3)_2$), 3.42 (d, J = 7.3 Hz, 2H, −CH_2CH═C$(CH_3)_2$), 1.81 (s, 3H, −CH_3), 1.68 (s, 3H, −CH_3), 1.48 (s, 6H, −CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.4, 158.2, 157.8, 157.2, 154.9, 154.6, 154.4, 132.0, 130.7, 128.5, 122.7, 121.6, 115.7, 112.4, 108.7, 107.7, 106.4, 106.0, 105.3, 78.3, 28.3 (2 × C), 25.8, 21.3, 17.9; HRMS (ESI): m/z calcd for $C_{25}H_{25}O_6$ $[M + H]^+$, 421.1646; found, 421.1648.

*3-(2,4-Bis(methoxymethoxy)phenyl)-5-hydroxy-8,8-dimethyl-4H,8H-pyrano[2,3-f]chromen-4-one (**18**).* Compound **13** (1.03 g, 2.92 mmol) was dissolved in DMF (25 mL) and heated to 150 °C for 1 h. After the reaction was complete (confirmed by TLC), the mixture was poured into ice-cold water and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous Na_2SO_4. The solvents were then removed under reduced pressure. The residue was purified by column chromatography on silica gel using a petroleum ether/ethyl acetate (PE/EtOAc, 10:1) solvent system to yield compound **18** as a yellow solid (679 mg, 1.54 mmol, 66%) and compound **14** (123 mg, 0.28 mmol, 12%).

Compound **18** was a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.87 (s, 1H, −OH), 7.78 (s, 1H, −OCH═C−), 7.12 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 2.3 Hz, 1H, −Ph), 6.71 (dd, J = 8.4, 2.3 Hz, 1H, −Ph), 6.61 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 6.21 (s, 1H, −Ph), 5.51 (d, J = 10.0 Hz, 1H, −CH═CHC$(CH_3)_2$O−), 5.12 (s, 2H, −OCH_2OCH_3), 5.06 (s, 2H, −OCH_2OCH_3), 3.42 (s, 3H, −CH_3), 3.36 (s, 3H, −CH_3), 1.40 (s, 6H, −CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.7 (C═O), 161.2 (C-5), 158.4 (C-10), 157.9 (C-1a), 155.3 (C-12), 152.8 (C-2), 151.3 (C-7), 131.0 (C-14), 126.4 (C-2'), 120.1 (C-3), 113.7 (C-1'), 112.9 (C-9), 108.0 (C-13), 105.0 (C-5a), 103.0 (C-11), 100.16 (C-8), 99.23 (C-6), 93.93, 93.4, 77.0 (C-3′), 55.3 (−CH_3), 55.07

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($-CH_3$), 27.17 (2 × C); HRMS (ESI): m/z calcd for $C_{24}H_{25}O_8$ $[M + H]^+$, 441.1544; found, 441.1544.

*3-(2,4-Bis(methoxymethoxy)phenyl)-8,8-dimethyl-5-((2-methylbut-3-en-2-yl)oxy)-4H,8H-pyrano[2,3-f]chromen-4-one (**19**).* To a solution of 2-methylbut-3-en-2-ol (5.2 mL, 50 mmol) in THF (60 mL), *n*-BuLi (1.6 M in hexanes, 31 mL, 50 mmol) was added dropwise at 0 °C over 30 min. The clear solution was stirred at 0 °C for 20 min, followed by the addition of di-*tert*-butyl dicarbonate (12 mL, 50 mmol). The reaction mixture was then allowed to warm to 23 °C and stirred for an additional 8 h. The solvent was poured into ice-cold water, quenched with saturated aqueous $NaHCO_3$ solution, and extracted with EtOAc. The organic phase was washed with saturated aqueous NaCl (150 mL), dried over Na_2SO_4, and concentrated under reduced pressure to yield compound **20** as a clear, pale yellow liquid (6.03 g, 96%), which was used without further purification.

To a stirred suspension of compound **18** (600 mg, 1.36 mmol) and pulverized 4Å MS (1.0 g) in degassed THF (10 mL), compound **20** (7.50 g, 40.8 mmol) was added at room temperature. The mixture was cooled to 0 °C, and $Pd(PPh_3)_4$ (157 mg, 0.14 mmol) was added portion-wise. The reaction mixture was stirred for 24 h and then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a petroleum ether/ethyl acetate (PE/EtOAc, 3:1) solvent system to yield compound **19** as a colorless oil (373 mg, 0.73 mmol, 54%), which was used without further purification.

*3-(2,4-Bis(methoxymethoxy)phenyl)-5-hydroxy-8,8-dimethyl-6-(3-methylbut-2-en-1-yl)-4H,8H-pyrano[2,3-f]-chromen-4-one (**23**).* The method was identical to that described for the preparation of **17**. Compound **23** was a yellow oil (490 mg, 0.96 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.16 (s, 1H, −OH), 7.82 (s, 1H, −OCH═C−), 7.18 (d, J = 8.4 Hz, 1H, −Ph), 6.92 (d, J = 2.2 Hz, 1H, −Ph), 6.78 (dd, J = 8.4, 2.2 Hz, 1H, −Ph), 6.70 (d, J = 9.90 Hz, 1H, $-CH{=}CHC(CH_3)_2O-$), 5.58 (d, J = 9.90 Hz, 1H, $-CH{=}CHC(CH_3)_2O-$), 5.19 (s, 2H, $-OCH_2OCH_3$), 5.13 (s, 2H, $-OCH_2OCH_3$), 3.49 (s, 3H, $-CH_3$), 3.43 (s, 3H, $-CH_3$), 3.35 (d, J = 7.3 Hz, 2H, $-CH_2CH{=}C(CH_3)_2$), 1.81 (s, 3H, $-CH_3$), 1.68 (s, 3H, $-CH_3$), 1.48 (s, 6H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.8, 159.3, 158.8, 156.4, 153.6, 150.6, 132.03, 131.6, 127.1, 122.0, 121.0, 115.1, 114.3, 112.7, 109.0, 105.6, 104.1, 100.8, 94.9, 94.5, 77.8, 56.3, 56.1, 28.1 (2 × C), 27.9, 25.8, 21.3, 18.0; HRMS (ESI): m/z calcd for $C_{29}H_{33}O_8$ $[M + H]^+$, 509.2170; found, 509.2173.

*3-(2,4-Dihydroxyphenyl)-5-hydroxy-8,8-dimethyl-6-(3-methylbut-2-en-1-yl)-4H,8H-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 oil (110 mg, 0.26 mmol, 86%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.57 (s, 1H, −OH), 8.54 (s, 1H, −OH), 7.96 (s, 1H, −OCH═C−), 6.99 (d, J = 8.3 Hz, 1H, −Ph), 6.71 (d, J = 10.0 Hz, 1H, $-CH{=}CHC(CH_3)_2O-$), 6.55 (d, J = 2.2 Hz, 1H, −Ph), 6.47 (dd, J = 8.3, 2.2 Hz, 1H, −Ph), 5.62 (d, J = 10.0 Hz, 1H, $-CH{=}CHC(CH_3)_2O-$), 5.22 (t, J = 7.3 Hz, 1H, $-CH_2CH{=}C(CH_3)_2$), 3.36 (d, J = 7.3 Hz, 2H, $-CH_2CH{=}C(CH_3)_2$), 1.81 (s, 3H, $-CH_3$), 1.68 (s, 3H, $-CH_3$), 1.49 (s, 6H, $-CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 182.1, 158.9, 158.2, 157.2, 154.6 (2 × C), 150.4, 131.9, 130.7, 127.6, 123.0, 121.6, 114.7, 113.7, 112.5, 108.7, 106.4, 105.0, 100.8, 78.4, 28.2 (2 × C), 25.8, 21.4, 18.0; HRMS (ESI): m/z calcd for $C_{25}H_{25}O_6$ $[M + H]^+$, 421.1646; found, 421.1646.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c10340.

Antibacterial assay, time-killing study, cytotoxicity assay, morphology analysis with SEM, NucGreen and EthD-III fluorescence staining assays, DNA and protein leakage, SYTOX green assay, interaction with peptidoglycan and phospholipids, outer membrane permeability assay, NMR spectra of synthetic compounds, and X-ray data of compound **18** (PDF)

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Notes

The authors declare no competing financial interest.

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