Identification of simple arylfluorosulfates as potent agents against resistant bacteria

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Sulfur fluoride exchange (SuFEx), a next generation of click chemistry, opens an avenue for drug discovery. We report here the discovery and structure–activity relationship studies of a series of arylfluorosulfates, synthesized via SuFEx, as antibacterial agents. Arylfluorosulfates 3, 81, and 101 showed potency to overcome multidrug resistance and were not susceptible to the generation of resistance. They exhibited rapid bactericidal potency and selectively killed gram-positive bacterial strains. These compounds also exhibited the ability to disrupt established bacterial biofilm and kill persisters derived from biofilm. Furthermore, arylfluorosulfate 3 had a synergistic effect with streptomycin and gentamicin. In addition, their anti-MRSA potency was evaluated and determined by the *Caenorhabditis elegans* model.

SuFEx | click chemistry | antibacterial agents | multidrug resistance | biofilm disruption

Antibiotic resistance is a tremendous threat to global health. Some of the most concerning multidrug-resistant pathogen strains include methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA, respectively), vancomycin-resistant *Enterococcus faecium* (VRE), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp (1–3). Antibiotic resistance is one of the biggest public health challenges and is a leading cause of death with at least 2.8 million infected cases and more than 35,000 deaths every year in the United States (4). Therefore, the development of novel antibacterial drugs with the ability to overcome drug-resistance is urgently needed. Furthermore, our general understanding of the role of our microbiomes (i.e., skin, oral, gut, etc.) in antibiotic resistance and responses is ever increasing (5–7). The next generation of antibacterial agents will require limited drug promiscuity to eliminate resistance and decrease unwanted off-target effects on our symbiotic commensal organisms and immunity (8, 9).

Sulfur fluoride exchange (SuFEx) (10), a new generation of click chemistry, has found diverse applications to chemical synthesis (11–16), materials science (17–22), chemical biology (23–28), and drug discovery (29, 30). In our previous studies, we demonstrated that SuFEx modification is a highly reliable approach for the late-stage functionalization of drugs and drug-like molecules to generate new compounds with improved properties (31, 32). Later on, Ravindar et al. reported the synthesis of arylfluorosulfate analogs and screened them for antimicrobial activity (33).

Here we report further screening studies on arylfluorosulfate derivatives (Ar-O-SO_2-F) in our laboratory and have found several simple molecules which are potent against methicillin- and vancomycin-resistant strains (Fig. 1 and *SI Appendix*, Figs. S1 and S2). Through structure and activity relationship (SAR) studies, we determined that the -OSO_2F moiety is essential for these compounds' antibacterial activities. Not only are they capable of inhibiting bacterial biofilm formation, but they are also able to disrupt established bacterial biofilm and induce the killing of persister cells. Significantly, these arylfluorosulfates are effective against MRSA infection in a *Caenorhabditis elegans*-based infection model. Our findings reported here thus may serve as the foundation toward the development of arylfluorosulfate-based antibacterial agents.

Results

In Vitro Phenotypic Screening of Arylfluorosulfates as Antibacterial Agents. To explore fluorosulfates as antibacterial agents, we prepared a library of arylfluorosulfates starting from phenols, polyphenols, and their structurally related analogs (synthetic route can be found in *SI Appendix*, Schemes S1–S3). The antimicrobial potencies of these arylfluorosulfate derivatives and the parent compounds were evaluated using a broth microdilution assay to determine the corresponding minimum inhibitory concentrations (MICs) against MRSA (American Type Culture Collection (ATCC) 43300). From this screening, we discovered several arylfluorosulfates possessing significantly enhanced activities against MRSA compared to their parent compounds **1**, **78**, and **99**, including **3**, **14**, **82**, **83**, **101**, and **102** (*SI Appendix*, Table S1). Among these arylfluorosulfates, **3** and **101** exhibited comparable MIC against MRSA as vancomycin (*SI Appendix*, Table S1). Likewise, in comparison to diethylstilbestrol (**112**),

Significance

Excessive use of antibiotics for patient therapy and livestock results in the selection of pathogenic bacteria resistant to multiple drugs. The spread of multidrug-resistant bacteria into the community is associated with increased morbidity, mortality, and financial burden of health care. We constructed a library of SuFEx-derived arylfluorosulfates that demonstrated potent activities in combating multidrug-resistant bacteria both in vitro and in vivo. Significantly, these arylfluorosulfate analogs show enhanced potency to disrupt bacterial biofilm as well as kill persisters and have a synergistic effect with clinical bactericidal drugs. This study suggests that arylfluorosulfates derived from SuFEx-based modification of phenols could serve as a convenient strategy to search for bactericidal candidates.

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The authors declare no competing interest.

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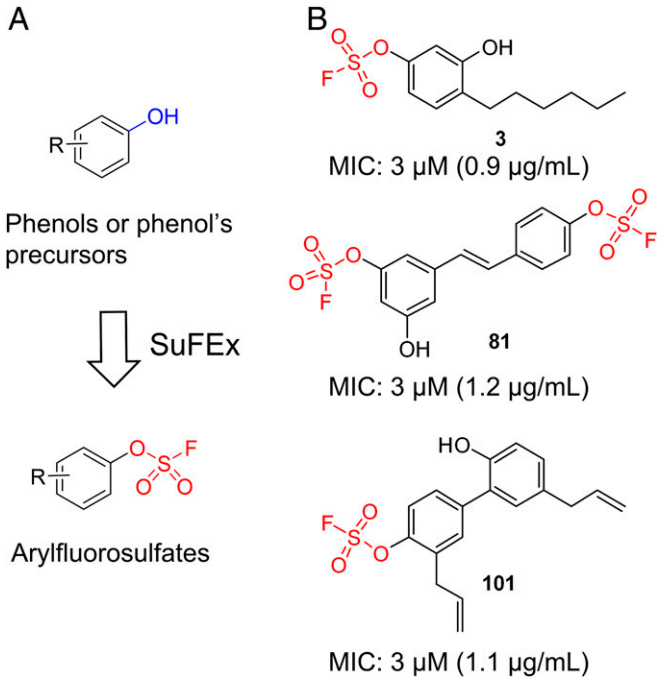

Fig. 1. (*A*) Arylfluorosulfates were derived from phenols or phenol's precursors. (*B*) The structures of antibacterial arylfluorosulfates **3**, **81**, and **101** and their MIC values against MRSA.

danthron (**121**), ethinylestradiol (**127**), tioxolone (**132**), nifuroxazide (**137**), estradiol (**142**), and phenolphthalein (**144**), their -OSO_2F substituted derivatives showed enhanced antimicrobial activities. In this screening, two arylfluorosulfates, **147** and **149**, were found to possess decreased potency from their parent compounds triclosan (**146**) and novobiocin (**148**).

The SAR Studies of Arylfluorosulfates. In order to evaluate the contribution of the -OSO_2F group to the activities of the hits discovered, we prepared corresponding -OMe, -OAc, or -OMs derivatives (*SI Appendix*, Schemes S1–S3) and performed SAR studies with a focus on hexylresorcinol, resveratrol, and honokiol derivatives.

As shown in *SI Appendix*, Table S2, parent hexylresorcinol **1** did not show any antibacterial activity. When -OSO_2F in the hit compound **3** was replaced by -OMe, -OAc, or -OMs, the resulting compounds **4** to **11** and **15** completely lost the capability of inhibiting the growth of *S. aureus* and MRSA, indicating the -OSO_2F moiety is essential for the observed antibacterial activity. In addition, upon -OSO_2F modification of the second -OH on hexylresorcinol to generate di-OSO_2F substituted derivative **2**, antibacterial activity was lost as well. When -OSO_2F moiety was transferred to the *ortho*-OH of the alkyl side chain to form compound **14**, a fourfold decrease of potency against *S. aureus* and MRSA was observed. Next, we examined the importance of the alkyl side chain. When the length of hydrophobic tail was increased from three carbons to six carbons, antibacterial activities increased gradually (**33** to **36** and **3**). However, further increasing the side chain length did not result in any enhancement of activity (**37** to **40**) with the MIC remaining at 3 μM. When an oxygen atom was introduced into the side chain to increase the compounds water solubility, compound **50** containing one oxygen atom exhibited fourfold decrease of potency against MRSA, while compounds **51** and **52** containing two oxygen atoms completely lost their anti-MRSA activities. When a carbonyl group was introduced into the side chain, compound **54** showed 32-fold decrease of potency compared with compound **3**. These observations implied that a hydrophobic side chain was critical for antibacterial activity. We then examined the role of the hydroxyl group of hit compound **3**. Compounds **56** and **57** lost activity completely when the hydroxyl group was removed or blocked by a methyl group. The position of the alkyl side chain of hit compound **3** also affected its antibacterial activity. When the side chain was shifted from the *para* position of -OSO_2F moiety to the *ortho*- or *meta* positions, both compounds **66** and **67** exhibited fourfold decrease of potency compared to hit compound **3**. When the benzene ring was replaced by a pyridine ring, the activity of compound **72** was reduced by 4 times. Another pyridylfluorosulfate **77** without a hydroxyl group showed abolished activity. These observations implied that the benzene ring from the hit compound **3** is important for activity.

To investigate the importance of the -OSO_2F group in resveratrol derivatives antibacterial activities, we tested anti-MRSA potency of resveratrol derivatives **79** to **98** as shown in *SI Appendix*, Table S3. The parent resveratrol **78** did not show any antibacterial activity. The -OMe, -OAc, or -OMs substituted derivatives **84** to **98** showed abolished activities compared to hit compounds **82** and **83**. This observation implied that the -OSO_2F group was essential. To our surprise, di-OSO_2F substituted compounds **80** and **81** showed enhanced potencies to inhibit MRSA with MIC values of 12.5 and 3.13 μM, which were a 50- and 200-fold increase of potency compared to parent compound resveratrol, respectively (*SI Appendix*, Tables S1 and S3).

A similar phenomenon was also exhibited with the honokiol derivatives, as shown in *SI Appendix*, Table S4. The parent honokiol **99** showed a decrease in antibacterial activity in comparison to **101**. When the -OSO_2F in the hit compounds **101** and **102** was replaced by -OMe, -OAc, or -OMs, the resulting compounds **103** to **111** produced diminished or abolished activities, indicating that the -OSO_2F moiety is essential. However, the di-OSO_2F substituted compound **100** did not show any activity. Similarly, in derivatives of diethylstilbestrol, danthron, ethinylestradiol, and tioxolone, the modification of the -OSO_2F moiety contributed to the antibacterial potency (*SI Appendix*, Table S5). By contrast, in the nifuroxazide derivatives, the -OSO_2F group was not essential, as all the -OMe, -OAc, and -OMs substituted derivatives **139** to **141** showed comparable activities compared to the -OSO_2F substituted compound **138** (*SI Appendix*, Table S5).

Spectrum of Activity of Arylfluorosufates. In addition to methicillin-sensitive *S. aureus* (MSSA) and MRSA, we further evaluated the potency of our arylfluorosulfates against a panel of

Table 1. Spectrum of activity of 3, 81, and 101

	MIC* (μM)					
Organism	**3**	**81**	**101**	Tet.	Cip.	Van.
S. aureus ATCC 6538	3.13	3.13	6.25	0.39	0.39	0.39
S. aureus clinical isolate 309-1	3.13	3.13	3.13	0.39	6.25	0.39
S. aureus clinical isolate 309-6	3.13	3.13	3.13	1.56	>100	0.39
S. aureus clinical isolate 309-8	3.13	3.13	3.13	100	100	0.39
S. aureus (MRSA) ATCC 43300	3.13	3.13	3.13	0.78	0.39	0.78
S. aureus (VRSA) VRS1	6.25	3.13	6.25	6.25	>100	>100
E. faecium (VRE) ATCC 700221	12.5	6.25	6.25	0.39	>100	>100
S. epidermidis (MRSE) ATCC 35983	6.25	6.25	6.25	3.13	0.78	1.56
Bacillus subtilis ATCC 6663	6.25	6.25	6.25	0.2	0.05	0.1
E. coli K-12	>100	>100	>100	1.56	0.10	100
K. pneumonia ATCC BAA-2470	>100	>100	>100	>100	100	>100

*Tetracycline (Tet.), ciprofloxacin (Cip.), and vancomycin (Van.) were used as positive controls. Values are expressed as the mean of triplicate experiments.

other bacterial strains, especially multidrug-resistant strains. As shown in Table 1 and *SI Appendix*, Table S6, arylfluorosulfates, notably compounds **3**, **81**, **101**, and **114**, were able to overcome multidrug resistance. They were potent against clinically isolated multidrug-resistant *S. aureus* strains, VRSA strain (VanA-type VRSA), vancomycin-resistant *E. faecium* strain (VanA-type VRE), and methicillin-resistant *Staphylococcus epidermidis* (MRSE). They also showed good potency against *Bacillus subtilis*. To our surprise, none of these arylfluorosulfates exhibited any potency on gram-negative bacteria strains, such as *Escherichia coli* and multidrug-resistant *K. pneumonia*.

Time-Kill Kinetics and Drug Resistance Studies. Since these arylfluorosulfates were bactericidal in nature, we investigated the rate of bactericidal action of arylfluorosulfates **3**, **81**, and **101** toward *S. aureus*. As shown in Fig. 2 *A–D*, all of them showed rapid bactericidal potency in a concentration-dependent manner. In contrast, vancomycin showed a much slower rate of killing, and there was no significant concentration dependence (34–36). At high concentrations, compounds **3**, **81**, and **101** killed *S. aureus* cells (10^6 c.f.u./mL) completely within 30 min. However, at relatively low concentrations, these arylfluorosulfates cannot eradicate *S. aureus* cells completely. The concentration of *S. aureus* cells is maintained at a low level (10^3 c.f.u./mL) (Fig. 2 *A–C*). For the colonies remaining on the plates under 4× MIC of compounds after time-kill kinetics assay, we picked three colonies randomly for MIC assay to investigate whether they were drug-resistant strains. The results showed that MIC values of corresponding compounds on these remaining colonies exhibited no change compared to wild-type *S. aureus*. This suggests that these colonies might be bacterial persisters (37, 38).

Next, we investigated the propensity of arylfluorosulfates **3**, **81**, and **101** to induce drug resistance by using the serial passage broth microdilution method (39, 40). As shown in Fig. 2*E*, serial passaging of *S. aureus* in the presence of sub-MIC of arylfluorosulfates **3**, **81**, or **101** over a period of 30 passages did not produce highly drug-resistant mutants. By contrast, the MIC value of ciprofloxacin, as a DNA gyrase inhibitor, showed 128-fold increase within 30 passages. These observations suggested that our arylfluorosulfates **3**, **81**, or **101** might be safe for long-term use without worrying about the problems caused by drug resistance.

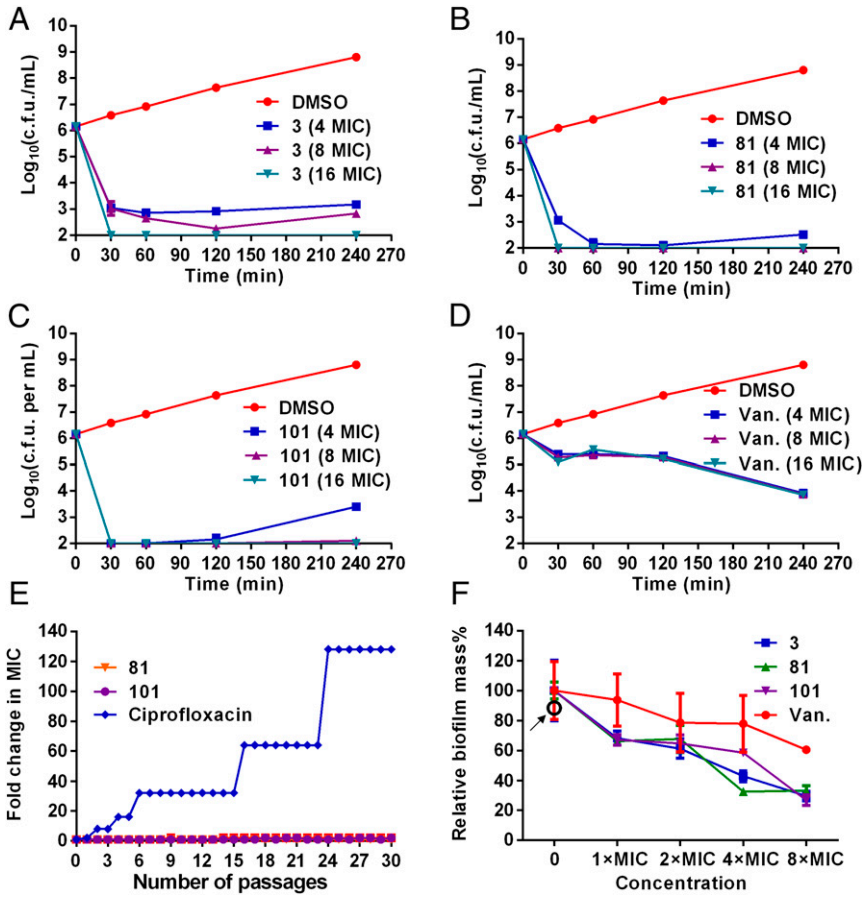

Fig. 2. Antimicrobial activity of arylfluorosulfates. (*A–D*) Time-kill kinetics of arylfluorosulfates **3**, **81**, and **101**. Exponential phase MRSA cells (ATCC 43300) were treated with arylfluorosulfates **3**, **81**, and **101** at concentrations of 4×, 8×, and 16× MIC, respectively. Vancomycin (Van.) was used as a positive control, while DMSO (0.1%) was used as a negative control. The density of bacteria was determined by serial dilution and plating on MHA plates. The detection limit of this method was 100 c.f.u./mL. (*E*) Resistance studies for arylfluorosulfates **3**, **81**, and **101**. After 30 passages of repeated exposure to 0.5× MIC of arylfluorosulfates **3**, **81**, or **101**, no highly drug-resistant *S. aureus* strain was obtained. Ciprofloxacin was used a control. (*F*) Arylfluorosulfates **3**, **81**, and **101** disrupted established biofilm. MRSA ATCC 43300 cells (100 μL/well, 10^7 c.f.u./mL) were transferred into a 96-well microplate and incubated for 24 h under stationary condition for biofilm formation. The supernatant was then discarded and established biofilms were exposed to arylfluorosulfates **3**, **81**, or **101** at various concentrations at 37 °C under stationary condition. After 24 h incubation, biofilm mass relative to negative control (0.1% DMSO) was measured using crystal violet staining. The black circle (black arrow) indicated the relative biofilm mass of initial established biofilm. Van. was used as a positive control. Values were expressed as the mean ± SEM of triplicate experiments.

Arylfluorosulfates Inhibit Biofilm Formation and Kill Persister Cells. Eighty percent of human microbial infections, such as periodontitis, endocarditis, and chronic lung infections, are accompanied by biofilm formation and bacteria present in biofilm. These are more resistant to conventional antibacterial agents than planktonic bacteria (41, 42). Therefore, biofilm poses a considerable impediment to antibacterial therapy (43). We explored the inhibitory effects of arylfluorosulfates **3**, **81**, and **101** on the biofilm formation of MRSA. As shown in *SI Appendix*, Fig. S3*A*, after 24 h incubation of MRSA bacteria (10^7 c.f.u./mL) with various concentrations of compounds under stationary conditions, the formation of biofilm was significantly inhibited. Under 2× MIC of compounds **81**, **101**, and vancomycin, or 4× MIC of compound **3**, the formation of biofilm was completely inhibited. The ideal antibacterial agents should not only inhibit biofilm formation but also destroy established biofilm (44). Next, we examined whether our compounds had ability to eradicate established biofilm. After incubation of matured biofilm (initial relative biofilm mass was 85%) with various concentrations of compounds **3**, **81**, and **101** for 24 h, the relative biofilm mass was quantified by crystal violet staining. As shown in Fig. 2*F*, all three of these compounds disrupted established biofilm in a concentration-dependent manner. Eight times MIC of arylfluorosulfates **3**, **81**, and **101** displayed ∼70% decrease in relative biofilm mass, while vancomycin only reduced the relative biofilm mass by 30%. Particularly, our arylfluorosulfates **3**, **81**, and **101** exhibited potency not only to inhibit the formation of biofilm but also to disrupt the established biofilm.

To assess bactericidal activity of arylfluorosulfates against persister cells, we exposed biofilms of MRSA to a high concentration of rifampicin (256× MIC) for 24 h to amass persister cells. This was achieved by following a similar procedure described previously (45); then the remaining persister cells were treated with 10× MIC of the arylfluorosulfates. As illustrated in *SI Appendix*, Fig. S3*B*, compounds **3**, **81**, and **101** killed >99.9% of persisters within 1 h.

Synergy of Arylfluorosulfate 3 with Conventional Antibiotics. The combination of multiple antibacterial drugs may help to increase the antibacterial potency, reduce the risk of drug resistance, and decrease the incidence of adverse drug reactions (46). We used the checkerboard method (47) to determine whether our arylfluorosulfates had synergism with conventional antibiotics. In this assay, twofold serial dilutions of compound A were combined with twofold serial dilutions of compound B to treat *S. aureus* (ATCC 6538) or MRSA (ATCC 43300) cells in a 96-well microplate. Then the fractional inhibitory concentration index (FICI) was calculated as the following formula: FICI = (MIC of compound A in combination)/(MIC of compound A alone) + (MIC of compound B in combination)/(MIC of compound B alone). Interaction between these two compounds was defined as synergy if FICI ≤ 0.5, no interaction if 0.5 < FICI ≤ 4, and antagonism if FICI > 4. The results showed that arylfluorosulfate **3** had synergistic effect with streptomycin and gentamicin (Fig. 3). FICI values between compound **3** and streptomycin were 0.5 on MSSA and MRSA (Fig. 3 *A* and *B*). Meanwhile, FICI values

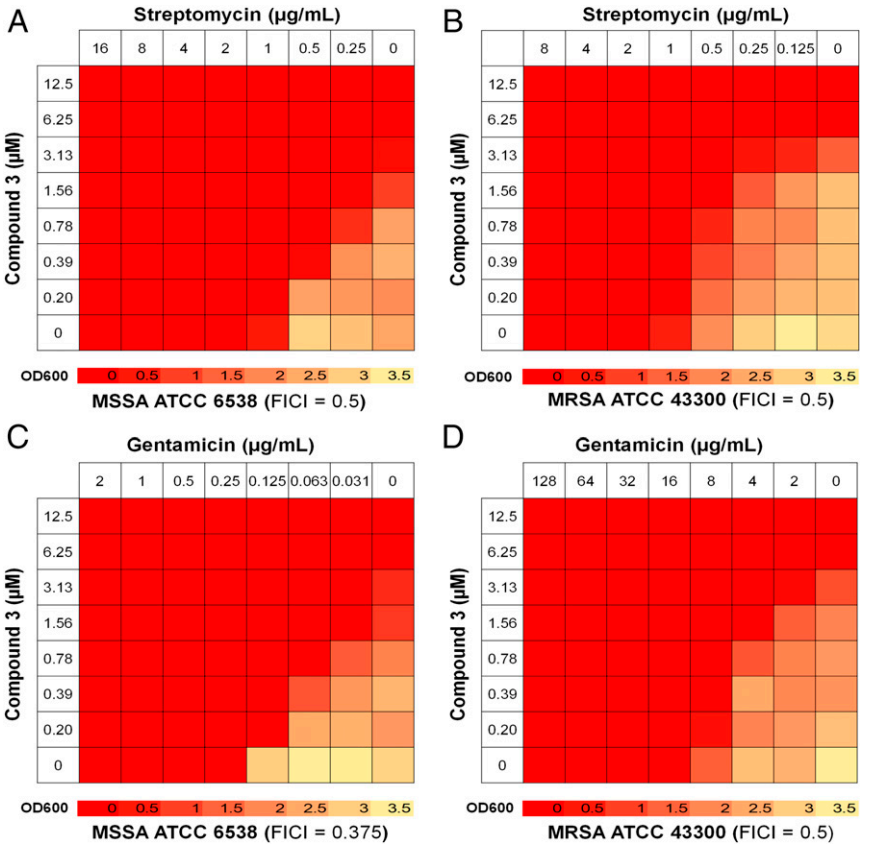

Fig. 3. Compound **3** showed synergistic effect with (*A* and *B*) streptomycin and (*C* and *D*) gentamicin. The synergisms against *S. aureus* (ATCC 6538) and MRSA (ATCC 43300) between compound **3** and conventional antibiotics were determined by the broth microdilution checkerboard method. The FICI = (MIC of compound A in combination)/(MIC of compound A alone) + (MIC of compound B in combination)/MIC of compound B alone). Synergy, FICI ≤ 0.5; no interaction, 0.5 < FICI ≤ 4; antagonism, FICI > 4.

between compound **3** and gentamicin were 0.375 and 0.5 on MSSA and MRSA, respectively (Fig. 3 *C* and *D*).

Mammalian Cytotoxicity and Hemolytic Activity of Arylfluorosulfates. Arylfluorosulfates, such as **3** and **81**, were hydrophobic compounds, and there was concern whether they showed an indiscriminate disruption on both bacterial and mammalian cells. Therefore, we evaluated their cytotoxicity on human cells and their hemolytic ability on human red blood cells, which were generally used to evaluate the toxicity of antibiotic agents (48). As shown in *SI Appendix*, Table S7, compounds **3**, **122**, and **133** did not show any inhibitory effect on the human HEK293T cells (human embryonic kidney cells). Meanwhile, compounds **81**, **101**, and **114** showed weak inhibitory effect on HEK293T cells with IC_{50} (compound concentration required to inhibit cell viability by 50%) values of 41, 85, and 67 μM, respectively. The cytotoxicity of compounds **128** and **138** were moderate with IC_{50} values of 22 and 20 μM, respectively. At the same time, the hemolytic ability of these arylfluorosulfates against human red blood cells was examined, with detergent Triton X-100 (2%) as a positive control. As shown in *SI Appendix*, Table S7, these compounds had HC_{50} (compound concentration causing 50% hemolysis of cells) values greater than 200 μM. The observations implied that these arylfluorosulfates possessed good selectivity on bacteria such as *S. aureus*. Hence, these arylfluorosulfates might be relatively safe.

Evaluation of In Vivo Anti-MRSA Activities of Arylfluorosulfates in a *C. elegans* Model. *C. elegans* is a cost-effective and time-saving whole-animal model for evaluating activities of antibacterial compounds. It is often used to assess a compound's in vivo potency against *S. aureus*, *E. faecalis*, *K. pneumonia*, and other bacteria strains (49–52). The generally used *C. elegans* strain for activity evaluation is *C. elegans* (glp-4; sek-1) mutant which contains two mutated sites. The glp-4 mutant is a temperature-sensitive sterile mutant. *C. elegans* containing this mutation is sterile at 25 °C but has the ability to produce progeny at 15 °C (53). Therefore, we can breed this mutant at 15 °C and then evaluate the compound's antibacterial potency on this mutant at 25 °C. The Sek-1 mutant has a mutation in the sek-1 gene in mitogen-activated protein kinase kinase (MAPKK) in the p38 MAPK pathway, and *C. elegans* containing this mutation is susceptible to multiple pathogens, which can reduce the assay time (54).

To investigate the in vivo antibacterial activity of arylfluorosulfates, we examined the potency of compounds **3**, **81**, and **101** using the *C. elegans* (glp-4; sek-1) model. As shown in Fig. 4, incubation with compounds **3** (Fig. 4*A*), **81** (Fig. 4*B*), **101** (Fig. 4*C*), and the positive control vancomycin (Fig. 4*D*) at concentrations of 10, 5, or 2.5 μg/mL prolonged the survival time of worms. Meanwhile, the worms incubated with negative control DMSO died within 5 d. Compounds **3** and **101** showed comparable activity to vancomycin, while compound **81** showed approximately a twofold increase in survival rate at 5 d under different concentrations in comparison to vancomycin. These results indicated that arylfluorosulfates **3**, **81**, and **101** were effective in the *C. elegans* model.

Discussion

Bacterial infections, especially the infection of drug-resistant bacteria strains, seriously endanger human health, so the development of new antibiotics is imperative and urgent (55, 56). The rapid development of SuFEx click chemistry provides a new strategy and a promising prospect for the discovery of novel antibacterial agents. $S^{(VI)}$-F groups, especially arylfluorosulfate, serve as versatile moieties to endow precursors with new or improved biological activities. For example, we previously reported that modification by the -OSO_2F group increased the activity of anticancer drugs, such as fulvestrant and combretastatin A4 (31). Additionally, there have been several accounts of arylfluorosulfate-containing compounds acting as a warhead for highly selective, covalent protein modifications (25, 26, 57).

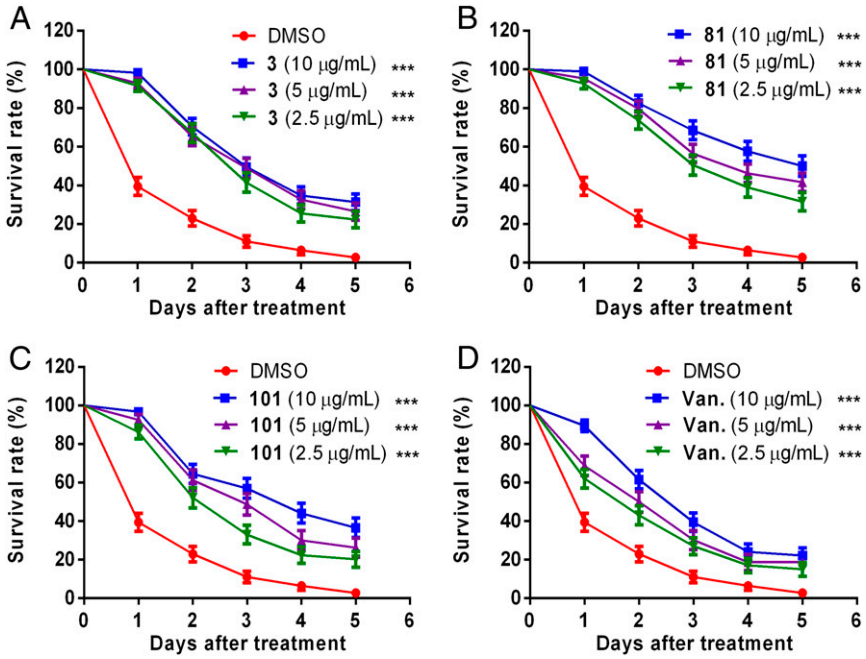

Fig. 4. In vivo antibacterial activity of arylfluorosulfates **3**, **81**, and **101** against MRSA infection in *C. elegans*. Worms were infected with MRSA for 24 h before being transferred into 96-well microplates, and tested compounds were then incubated with worms for 5 d. The worms were observed daily under microscope, and the Kaplan–Meier survival curves of MRSA-infected worms that were treated with (*A*) **3**, (*B*) **81**, (*C*) **101**, and (*D*) vancomycin are depicted. Error bars equal SEM. ***$P < 0.0001$ (determined by log-rank test) vs. negative control (DMSO).

In the current study, we discovered that the antibacterial activity of arylfluorosulfates, such as **3**, **81**, and **101**, requires the -OSO_2F moiety. These $S^{(VI)}$-F–containing compounds exhibited low cytotoxicity, almost complete suppression of the phenolic function's CYP-reactivity, and low hemolytic activity, and they selectively inhibited gram-positive bacterial strains. These characteristics might be derived from the intrinsic selectivity of arylfluorosulfates as covalent protein modifiers, which is currently under investigation in our laboratories. Furthermore, compound **3** exhibited a synergistic effect with gentamicin and streptomycin, and they exhibited potency against MRSA infection in *C. elegans*. Compounds **3**, **81**, and **101** were stable at physiological pH 7.4 at 37 °C (*SI Appendix*, Fig. S4) for 7 d, which was consistent with the stability of reported arylfluorosulfates (10, 58). These compounds were relatively simple and lipophilic, but they also fall within Lipinski's rule of 5 (*SI Appendix*, Table S8). Due to their lipophilic properties, we further examined whether their rapid bactericidal activities were associated with the permeabilization of bacterial membrane using propidium iodide influx assay (45). However, the incubation of MRSA with compounds **3**, **81**, and **101** at various concentrations did not cause an obvious increase of fluorescence over 60 min as shown in *SI Appendix*, Fig. S5. It implied that these arylfluorosulfates did not have any effect on the membrane permeability, and their rapid bactericidal activities should derive from other mechanisms.

In addition, although these parent compounds are widely used or evaluated clinically or in various animal models [for example, hexylresorcinol is widely used in the cosmetic, food, and pharmaceutical industries (59); resveratrol exists in many plant species and has been evaluated in many clinical trials and animal models (60); and honokiol is a component of traditional Chinese and Japanese herbal medicine (61)], these bioactive arylfluorosulfates exhibit relatively short half lives in vivo (e.g., $T_{1/2}$ of compound **3** in rats is 2.1 h). Further SAR optimization with multiple design iterations might help further improve their activities and PK/PD properties.

In the future, these arylfluorosulfates may act as lead compounds for further optimization to improve drug-like properties. As a result, it is expected that we might obtain ideal antibacterial agents with higher activities and better physicochemical properties. Continuing developments include efforts to decipher their mechanism of action.

Materials and Methods

The procedure of anti-MRSA phenotypic screening, SAR studies, spectrum of activity, cytotoxicity and hemolytic activity, chemical stability of arylfluorosulfates, and sterilizing ability testing of arylfluorosulfates are summarized in *SI Appendix, Materials and Methods*. The synthesis of derivatives, MIC assay, time-kill kinetic assay, cytotoxicity analysis, determination of hemolytic activity, antibiofilm assay, drug resistance study, synergism assay, and in vivo evaluation on the *C. elegans* model are also summarized in *SI Appendix, Materials and Methods*.

Data Availability. All study data are included in the article and/or *SI Appendix*.

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