**Abstract:** A series of new oxadiazole sulfone derivatives containing an amide moiety was synthesized based on fragment virtual screening to screen high-efficiency antibacterial agents for rice bacterial diseases. All target compounds showed greater bactericidal activity than commercial bactericides. 3-(4-fluorophenyl)-N-((5-(methylsulfonyl)-1,3,4-oxadiazol-2-yl)methyl)acrylamide (10) showed excellent antibacterial activity against *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola*, with EC₅₀ values of 0.36 and 0.53 mg/L, respectively, which were superior to thiodiazole copper (113.38 and 131.54 mg/L) and bismethiazol (83.07 and 105.90 mg/L). The protective activity of compound 10 against rice bacterial leaf blight and rice bacterial leaf streak was 43.2% and 53.6%, respectively, which was superior to that of JHXJZ (34.1% and 26.4%) and thiodiazole copper (33.0% and 30.2%). The curative activity of compound 10 against rice bacterial leaf blight and rice bacterial leaf streak was 44.5% and 51.7%, respectively, which was superior to that of JHXJZ (32.6% and 24.4%) and thiodiazole copper (27.1% and 28.6%). Moreover, compound 10 might inhibit the growth of *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* by affecting the extracellular polysaccharides, destroying cell membranes, and inhibiting the enzyme activity of dihydrolipoamide S-succinytransfase.

**Keywords:** antibacterial activity; fragment; virtual screening; DLST inhibitors

**1. Introduction**

Rice bacterial diseases threaten global food security due to their high frequency, serious damage, and difficulty to prevent and control [1–3]. Among them, rice bacterial leaf blight (RBLB) and rice bacterial leaf streak (RBLS), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv. *oryzicola* (Xoc), are two extremely destructive bacterial diseases that can cause 20–50% loss of agricultural production [4,5]. Today, traditional commercial bactericides, such as bismethiazol and thiodiazole copper, are used to control RBLB and RBLS [6]. However, the long-term repeated use of traditional commercial bactericides has caused the control effect of bacteria to decrease and serious pollution to the environment to increase year by year [7–9], hence there is an urgent need for their replacement with new bactericides [10]. Therefore, the discovery of novel antibacterial agents is presently an urgent problem.

The research and development of new drugs is a long and complicated process, which requires significant resources and time costs. According to the report of the Tufts Center for the Study of Drug Development, the development of a new drug requires an average of 10–15 years, USD 1.4 billion in resource costs, and USD 1.16 billion in time costs [11,12]. As a mature drug design method, virtual screening to find candidate compounds has become the main method of drug design [13]. Fragment-based drug discovery (FBDD) could explore a larger chemical space through a smaller number of fragment compounds,
discover molecules with higher binding efficiency, reduce the cost of drug development, and improve the efficiency of drug development [14,15]. Therefore, the fragment-based virtual screening guided discovery of new drugs is a research hotspot in the field of plant protection.

In our previous work, we found the sulfone candidate antibacterial agent JHXJZ [16]. Dihydrolipoamide S-succinyltransferase (DLST) was verified to be the target of JHXJZ by the click chemistry and quantitative chemical proteomic approach [17,18]. In this work, based on DLST, a model was established by rapidly identifying the non-effect fragments, thereby selectively replacing and optimizing the affinity of the drugs. A series of compounds was designed and synthesized, and some candidate drugs were selected. Then, their antibacterial activities against rice bacterial diseases were evaluated, and their mechanism of action was initially explored.

2. Results

2.1. Molecular Design

JHXJZ was dismantled into fragments and their ligand efficiency (LE) was evaluated. Fragment a was chosen due to its better binding free energy (ΔG). Because changing the number of carbon atoms in the sulfone had a significant impact on the affinity, we chose compounds a1 and a2 (core 1 and core 2) as promising starting core fragments for lead compound generation. Structure-based fragment virtual screening was conducted on core 1 and 2 in combination with DLST. Soon afterwards, the top 10 candidates with favorable ΔG values of the two core fragments were obtained. According to the feasibility of the synthesis and purification of the fragments, we considered whether it was possible to further synthesize and optimize. The six fragments were synthesized out together and the result showed that most of the fragments had excellent bactericidal activity except for the pyridine fragments. We believed that this type of structure had further enhanced value in the later stage, for instance, fragments 119 and 612. Finally, fragments were determined according to the feasibility of the synthesis and derivatization of the fragments. Additionally, based on the combination mode of the fragments, we performed a single-replacement optimization scan and further optimized multiple-replacement optimization for the derivation of new compounds. From the later experimental results, compounds 24, 10, and 16, which showed better bactericidal activity, had become the candidates. The construction process of this model based on DLST is shown in Figure 1.

![Deconstruction Analysis](image1)

**Figure 1.** The fragment-based virtual screening: the structural optimization of JHXJZ.

DLST was verified as the target of JHXJZ by the click chemistry and quantitative chemical proteomic approach. Therefore, we performed the fragment-based virtual screening based on JHXJZ (Figure 2). We found that the binding mode of JHXJZ was important
to the structure-based lead optimization, and we predicted the binding mode of JHXJZ with DLST through molecular docking (Figure 2). Then, we analyzed the binding mode of JHXJZ to identify a prioritized pharmacophore. The stability of the binding mode was confirmed by a 10 ns molecular dynamic simulation. For DLST and JHXJZ, the binding mode was in an extended conformation and located in a hydrophobic pocket. Moreover, JHXJZ could form a hydrogen bond with ARG407, ALA410, and LYS178, and then form a halogen bond with GLU33. The JHXJZ bound away from the hydrophobic region that consisted of Arg180, Arg407, and Arg636.

**Figure 2.** The binding mode of JHXJZ.

Based on the binding mode of JHXJZ, we deconstructed JHXJZ into fragments and evaluated their ligand efficiency (LE). The binding free energy (ΔG) was determined using the Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA) (Table 1). Fragment a showed the lowest binding free energy and the highest ligand efficiency (ΔG = −16.79 kcal/mol, LE = 1.87). Fragment c showed the lowest ligand efficiency and the highest binding free energy (ΔG = −6.55 kcal/mol, LE = 0.81). Hence, fragment c was a portion incapable of contributing binding free energy, and the compound showed modification potential for structural evolution. The structural modification was performed on fragment a to optimize binding affinity. Typically, the addition of non-bond interactions and hydrophobic interactions can effectively improve the affinity (Table 2). It was noticed that changing the number of carbon atoms in the sulfone had a significant impact on the affinity (a1, ΔG = −19.11 kcal/mol, LE = 1.47; a2, ΔG = −21.31 kcal/mol, LE = 1.52). Therefore, we chose compounds a1 and a2 (core 1 and core 2) as promising starting core fragments for lead compound generation.

**Table 1.** The deconstruction analysis of JHXJZ.

| Compound | Structure | ΔG     | LE  |
|----------|-----------|--------|-----|
| JHXJZ    |           | −25.68 | 1.60|
| a        |           | −16.79 | 1.87|
| b        |           | −10.33 | 0.86|
AILDE web server. A series of compounds was generated based on compounds that had the potential for further optimization. To further optimize the binding free energy of the target compounds, we tried to synthesize the target compounds. The six fragments were synthesized out to further synthesize and optimize. In the process of screening the fragments, we considered whether it was possible to further synthesize and optimize. In the process of screening the fragments, we tried to synthesize the target compounds. The six fragments were synthesized out to further synthesize and optimize. In the process of screening the fragments, we considered whether it was possible to further synthesize and optimize. In the process of screening the fragments, we considered whether it was possible to further synthesize and optimize. In the process of screening the fragments, we considered whether it was possible to further synthesize and optimize. In the process of screening the fragments, we considered whether it was possible to further synthesize and optimize. In the process of screening the fragments, we considered whether it was possible to further synthesize and optimize.

Table 1. Cont.

| Compound | Structure | ΔG  | LE  |
|----------|-----------|-----|-----|
| c        | ![Structure Image](image1) | −6.55 | 0.81 |
| d        | ![Structure Image](image2) | −7.67 | 1.53 |

Table 2. The optimization analysis of compound a.

| Compound | Structure | ΔG  | LE  |
|----------|-----------|-----|-----|
| a        | ![Structure Image](image3) | −16.79 | 1.87 |
| a1       | ![Structure Image](image4) | −19.11 | 1.47 |
| a2       | ![Structure Image](image5) | −21.31 | 1.52 |
| a3       | ![Structure Image](image6) | −16.11 | 1.34 |
| a4       | ![Structure Image](image7) | −17.54 | 1.35 |
| a5       | ![Structure Image](image8) | −17.91 | 1.19 |

Structure-based fragment virtual screening was conducted on core 1 and 2 in combination with DLST. All linked fragments were refined. The top 10 candidates with favorable ΔG values of the two core fragments were obtained (Table 3). According to the feasibility of the synthesis and purification of the fragments, we considered whether it was possible to further synthesize and optimize. In the process of screening the fragments, we tried to synthesize the target compounds. The six fragments were synthesized out together and the result showed that most of fragments had excellent bactericidal activity except for the pyridine fragments (Table 4). Among them, according to the feasibility of the synthesis and derivatization of the fragments, we believed that fragments 119 and 612 had the potential for further optimization. To further optimize the binding free energy of compounds 9 and 11, the ligand-directing evolution strategy was performed using the AILDE web server. A series of compounds was generated based on compounds 9 and 11, and the top 10 candidates with favorable ΔΔG values are shown in the Supplementary Materials. In total, 26 compounds were synthesized by ranking the binding energy and synthetic accessibility, and the antibacterial activity of all the target compounds was evaluated (Tables 4, S1 and S2).
formed hydrogen bonds with TYR195 and TRY422, and formed π stacking with TYR195.

shown in Figure 3. Compared to the binding mode of JHXJZ, compounds
activity. The binding modes of JHXJZ and compounds

Table 3. The fragment structure generated from core1 and 2 via ACFIS webserver.

| Fragment | Structure | ΔG   | Fragment | Structure | ΔG   |
|----------|-----------|------|----------|-----------|------|
| core1    | ![core1](image) | −19.11 | core2    | ![core2](image) | −21.31 |
| 858      | ![858](image) | −29.69 | 677      | ![677](image) | −31.70 |
| 677      | ![677](image) | −28.15 | 1247     | ![1247](image) | −30.59 |
| 612      | ![612](image) | −27.48 | 288      | ![288](image) | −29.36 |
| 1247     | ![1247](image) | −26.55 | 858      | ![858](image) | −29.19 |
| 119      | ![119](image) | −26.44 | 1347     | ![1347](image) | −28.85 |
| 708      | ![708](image) | −26.15 | 119      | ![119](image) | −28.76 |
| 1347     | ![1347](image) | −26.02 | 612      | ![612](image) | −28.71 |
| 1523     | ![1523](image) | −26.01 | 260      | ![260](image) | −28.65 |
| 1549     | ![1549](image) | −24.94 | 254      | ![254](image) | −27.81 |
| 266      | ![266](image) | −24.58 | 1549     | ![1549](image) | −27.47 |

Table 4. The six screened fragments.

| Number | Compound | Fragment | n | ΔG   | EC₅₀  |
|--------|----------|----------|---|------|-------|
| 1      | 1        | ![1](image) | 0 | −29.69 | 0.68  |
| 2      | 2        | ![2](image) | 1 | −29.19 | 0.72  |
| 3      | 3        | ![3](image) | 0 | −28.15 | 0.53  |
| 4      | 4        | ![4](image) | 1 | −31.70 | 0.45  |
| 5      | 5        | ![5](image) | 0 | −26.55 | 0.48  |
| 6      | 6        | ![6](image) | 1 | −30.59 | –     |
| 7      | 7        | ![7](image) | 0 | −24.94 | 3.49  |
| 8      | 8        | ![8](image) | 1 | −27.47 | 7.45  |
| 9      | 9        | ![9](image) | 0 | −27.48 | 0.73  |
| 10     | 10       | ![10](image) | 1 | −28.71 | 0.89  |
| 11     | 11       | ![11](image) | 0 | −26.44 | –     |
| 12     | 12       | ![12](image) | 1 | −28.76 | –     |
Among target compounds 1–26, compounds 24, 10, and 16 showed better bactericidal activity. The binding modes of JHXJZ and compounds 24, 10, and 16 against DLST are shown in Figure 3. Compared to the binding mode of JHXJZ, compounds 24, 10, and 16 formed hydrogen bonds with TYR195 and TRY422, and formed π stacking with TYR195. In addition, a stronger hydrophobic interaction was formed due to molecular collisions with Arg180/407/636. We believe that this computational framework might be used to eliminate non-effect fragments and optimize binding affinity.

Figure 3. The binding modes of JHXJZ and compounds 24, 10, and 16.

2.2. Chemicals

The different substituted oxadiazole thioethers (intermediates 1a–2a) were prepared by glycine ethyl ester hydrochloride, di-tert-butyl carbonate, N₂H₄·H₂O, CS₂, bromide, and trifluoroacetic acid. Different acids containing aromatic groups were added to SOCl₂ and refluxed to obtain intermediates 1b–17b. The intermediates 1c–26c were oxidized by (NH₄)₆Mo₇O₂₄·4H₂O and hydrogen peroxide solution to obtain the target compounds 1–26 (Figure 4). The details of ¹H NMR, ¹³C NMR, physicochemical property, melting point, yield, and HRMS are provided in the Supplementary Materials.

Figure 4. Synthesis route of compounds 1–26.

2.3. Antimicrobial Activity In Vitro Test

All the target compounds showed excellent antibacterial activity against Xoo at 50 and 10 mg/L (Table S3). In addition to compounds 2 and 7, the antibacterial activity of all target compounds against Xoo was greater than 90% at 10 mg/L. Interestingly, the antibacterial activity of compound 9 was 97.6% at 50 mg/L. However, when the concentration was reduced to 10 mg/L, the antibacterial activity of compound 9 was 100%. This difference may be related to the solubility of compound 9. When the antibacterial activity was tested, we found that the solution with a concentration of 50 mg/L of compound 9 was turbid. However, the solution of compound 9 was transparent at 10 mg/L. The EC₅₀ values of compounds 1–26 against Xoo were 0.36–7.45 mg/L, which were superior to those of bismerthiazol (83.07 mg/L) and thiodiazole copper (113.38 mg/L). Among them,
24 compounds showed EC_50 values less than 1.0 mg/L. For example, the EC_50 values of compounds 4, 10, 14, 15, 16, and 24 against Xoo were 0.45, 0.36, 0.43, 0.43, 0.42, and 0.40 mg/L, respectively.

Meanwhile, all the target compounds showed good antibacterial activity against Xoc at 50 and 10 mg/L (Table S4). The antibacterial activity of compounds 1–26 was greater than 90% at 50 mg/L. In addition, the EC_50 values of compounds 1–26 against Xoc were 0.53–10.77 mg/L, which were superior to those of bismertoniazol (105.90 mg/L) and thiodiazole copper (131.54 mg/L). Among them, 10 compounds showed EC_50 values less than 1.0 mg/L. For example, the EC_50 values of compounds 8, 9, 10, 12, and 14 against Xoc were 0.70, 0.78, 0.53, 0.64, and 0.61 mg/L, respectively.

2.4. Antibacterial Activity In Vivo Test

The antibacterial activity of compound 10 against RBLB and RBLS was evaluated (Figure S1 and Table 5). The protective activity of compound 10 against RBLB and RBLS was 43.2% and 53.6%, respectively, which was superior to that of JHXJZ (34.1% and 26.4%) and thiodiazole copper (33.0% and 30.2%). The curative activity of compound 10 against RBLB and RBLS was 44.5% and 51.7%, respectively, which was superior to that of JHXJZ (32.6% and 24.4%) and thiodiazole copper (27.1% and 28.6%).

Table 5. Protective and curative activities of compound 10 against two rice bacterial diseases at 200 mg/L.

| Treatment Group | Protective Activity (%) | Curative Activity (%) | Protective Activity (%) | Curative Activity (%) |
|-----------------|-------------------------|-----------------------|-------------------------|-----------------------|
| 10              | 43.2 ± 5.8              | 44.5 ± 2.7            | 53.6 ± 1.8              | 51.7 ± 3.5            |
| JHXJZ           | 34.1 ± 1.6              | 32.6 ± 1.5            | 26.4 ± 5.2              | 24.4 ± 2.2            |
| BT              | 39.8 ± 1.6              | 38.0 ± 2.7            | 47.4 ± 3.5              | 45.2 ± 3.3            |
| TC              | 33.0 ± 3.2              | 27.1 ± 3.1            | 30.2 ± 4.4              | 28.6 ± 5.0            |

2.5. Enzyme Activity Detection of DLST

DLST was found as the main target of JHXJZ in Xoo. JHXJZ may affect the cells by regulating the lysine succinyl modification level energy metabolism process [17,18]. The inhibitory activity of compound 10 on DLST was evaluated (Figure 5). After compound 10 treated Xoo and Xoc, the relative inhibition rates of DLST were 49.5% and 59.1% at 10 mg/L, respectively. However, when the concentration was reduced to 1 mg/L, the relative inhibition rates of DLST were 15.5% and 20.7%, respectively.

Figure 5. DLST activity of Xoo and Xoc at 10, 5, and 1 mg/L.
2.6. Biofilm Formation

The inhibitory activity of compound 10 on the biofilm formation of Xoo and Xoc was evaluated (Figure 6). At the concentrations of 10, 5, and 1 mg/L, the inhibition rates of biofilm formation were 72.0%, 64.0%, and 27.2%, and 71.5%, 51.7%, and 25.2%, respectively. Therefore, compound 10 might affect the activity of bacteria through inhibiting the formation of biofilm.

![Figure 6. Effects of Xoo and Xoc on the biofilm formation (a), extracellular polysaccharide (EPS) production (b), and membrane permeability (c,d) at 10, 5, and 1 mg/L.](image)

2.7. Extracellular Polysaccharide Production

The inhibitory activity of compound 10 on the extracellular polysaccharide production of Xoo and Xoc was evaluated (Figure 6). The inhibition rates of extracellular polysaccharide production were 98.0%, 95.9%, and 70.4%, and 95.9%, 92.9%, and 62.2% at 10, 5, and 1 mg/L, respectively. Therefore, compound 10 might destroy the normal reproductive cycle of bacteria by inhibiting the production of extracellular polysaccharides.

2.8. Membrane Permeability

The effects of compound 10 on the cell membrane permeability of Xoo and Xoc at 10, 5, and 1 mg/L were determined (Figure 6). After 30 min, the cell membrane permeability increased as the treatment time increased. The cell membrane permeability of the different treatment groups and the negative control group did not differ before 180 min. However, after 180 min, the different treatment groups and the cell membrane permeability of the negative control group showed differential effects and had concentration dependence.

2.9. Morphological Change in Bacteria

The morphological changes of Xoo and Xoc were observed by the scanning electron microscope (Figure 7). The cell shapes of the negative control groups of Xoo and Xoc were full and there were no obvious wrinkles on the surface. At the concentrations of 1, 5, and
10 mg/L of compound 10, wrinkles and deformations appeared on the cell surface, and the degree of wrinkles and deformation gradually increased as the concentration increased.

![Figure 7. Changes in bacterial morphology of Xoo (a–d) and Xoc (e–h) at 10, 5, and 1 mg/L.](image)

3. Discussion

In summary, via fragment-based virtual screening, a series of new oxadiazole sulfone derivatives was synthesized to screen high-efficiency antibacterial agents for rice bacterial diseases. Interestingly, all synthetic target compounds showed excellent antibacterial activities against Xoo and Xoc. Compound 10 showed good antibacterial activity in vivo against RBLB and RBLS. Compound 10 showed good inhibitory activity against DLST, which indicated that DLST might be the target of compound 10 in Xoo and Xoc. In addition, compound 10 might suppress the growth of Xoo and Xoc by inhibiting the formation of biofilm and the production of extracellular polysaccharides and changing the cell membrane permeability and cell surface morphology. The method of compounds' structural design based on fragment virtual screening can improve the efficiency of finding highly active compounds. In addition, compound 10 can be studied as a potential antibacterial agent in the future.

4. Materials and Methods

4.1. Molecular Design

4.1.1. Molecular Docking and Dynamics Simulation

Molecular docking was performed using AutoDock 4.2. The protein crystal was generated on BLAST in Uniprot (A0A0K0GL90) and built by Swiss–Model [19,20]. The homology modeling template 1C4T showed 65.8% sequence identity [21]. The protein structures were prepared by removing the water molecules and then adding hydrogen atoms and repairing the side chains. JHXJZ was docked into DLST, and 10 poses were exported for further analysis. The molecular dynamic was performed using AMBER 16 [22]. The dominant conformation was confirmed by 10 ns of dynamic trajectory analysis, and the binding energy calculation was conducted via the MM–PBSA method [23].

4.1.2. Fragment-Based Virtual Screening

Fragment-based virtual screening was performed using the ACFIS web server (http://chemyang.ccnu.edu.cn/ccb/server/ACFIS/ 11 January 2021). The selected compound, in combination with DLST as the starting structure, was linked to the refined database containing over 1500 fragments. The ΔG value of each newly generated compound was calculated by the MM–PBSA method after a minimization.
4.1.3. Ligand-Directing Evolution

The ligand-directing evolution was performed using the AILDE web server (http://chemyang.ccnu.edu.cn/ccb/server/AILD 15 January 2021) based on the result of the fragment virtual screening. Every hydrogen atom of the selected favored fragment was replaced by the 10 most used substituents (−CH₃, −OH, −F, −Cl, −Br, −CONH₂, −CF₃, −NH₂, −NO₂, and −OCH₃) to generate the potential compounds in each snapshot. We used the MD simulation to refine the newly generated receptor–ligand complex to obtain the final structure. The binding free energy (ΔG) of the refined complex structures was evaluated using the MM–PBSA method.

4.2. Chemicals

The synthesis processes of the intermediates and target compounds were supervised through thin-layer chromatography (TLC). Using an XT-4 binocular microscope (Beijing Tech Instrument Co., Beijing, China), the melting points were measured. ¹³C and ¹H NMR spectra were obtained by a Bruker Ascend–400 spectrometer (Bruker, Karlsruhe, Germany). The HRMS data were acquired by a Thermo Scientific Q Exactive (Thermo Scientific, Waltham, MA, USA).

General Procedures for the Preparation of Compounds 1–26. The different oxadiazoole intermediates 1a–2a were synthesized according to known methods [23]. Different acids containing aromatic groups were added to SOCl₂ (5–8 mL) and refluxed for 5–8 h to obtain intermediates 1b–17b. Next, the triethylamine (3.8 mmol) and intermediates 1a–2a (2.5 mmol) were added to CH₂Cl₂ in ice–water bath conditions. The intermediates 1b–17b were added and stirred for 2.5–5 h. Then, the intermediates 1c–26c were obtained by silica gel column chromatography. Finally, the intermediates 1c–26c (1.5 mmol), (NH₄)₆Mo₇O₂₄·4H₂O (0.3 mmol), and hydrogen peroxide solution (30%, 15 mmol) were admixed and stirred at 25 °C for 3–8 h. The saturated ice saltwater was added to the wash, and the target compounds 1–26 were obtained with a yield of 52–89% (Figure 4). The details of compounds 1–26 can be found in the Supplementary Materials.

4.3. Antibacterial Activity In Vitro Test

The antibacterial activities in vitro of compounds 1–26 against Xoo and Xoc were evaluated according to our previously reported method [24]. The compounds were dissolved in DMSO, and the solutions were diluted with 0.1% Tween 20 and nutrient broth (NB, 1%) media to prepare different concentrations of the solutions. After shaking the bacteria at 28 °C for 1–2 d, the inhibition rates were calculated—by comparing differences of the OD₅₉₅ values between the treatment group and negative controls by a microplate spectrophotometer. The test was repeated three times.

4.4. Antibacterial Activity In Vivo Test

The antibacterial activities of compound 10 against RBLB and RBLS were evaluated at 200 mg/L using the leaf-cutting and needleless injector method according to the reported method [25]. The NB (1%) media containing Xoo and Xoc at the logarithmic growth period were inoculated on rice leaves. In the protective activity, the agent was sprayed onto rice blades, and bacteria were inoculated after 24 h. In the curative activity, the bacteria were inoculated into the rice leaves, and the test compounds were sprayed after 24 h. The antibacterial activities were calculated by the disease index and lesion length of rice leaves at 2 weeks post-spraying.

4.5. Enzyme Activity Detection of DLST

The inhibitory activity of compound 10 against DLST was tested according to the previously reported method and the instructions of the enzyme activity kit [17,18]. The Xoo and Xoc bacteria solutions at the logarithmic growth period were added to the NB medium (5 mL) and shaken at 28 °C until the OD₆₀₀ value reached 0.3. Compound 10 and JHXJZ, at the final concentrations of 10, 5, and 1 mg/L, were mixed into the bacterial suspension,
and the mixture was continuously shaken for 12 h. The solution without the compound was used as the negative control in the same experimental conditions. Xoo and Xoc were cultivated to a logarithmic growth phase (the negative control). Then, the bacterial solution was collected by centrifugation. The DLST proteins of Xoo and Xoc were extracted by the method described in the kit. Soon afterward, the enzyme activity of DLST was tested by a microplate spectrophotometer (Shanghai Enzyme Link Biotechnology Co., Ltd. Suzhou, China) using the enzyme assays kits.

4.6. Biofilm Formation

The effects of compound 10 on the biofilm formation of Xoo and Xoc were determined according to the previously reported method [26]. Compound 10 and bacteria with a logarithmic growth phase were mixed to prepare solutions of 10, 5, and 1 mg/L, respectively. The bacteria were poured out after standing for 5 d at 28 °C. Next, the crystalline violet (0.1%, w/v) was added. The ethanol (2 mL) was added to dissolve the crystal violet on the bottle wall. The inhibition activities were calculated by the difference in the OD_{590} values.

4.7. Membrane Permeability

The effects of compound 10 on the membrane permeability of Xoo and Xoc were tested according to the previously reported works [27,28]. Xoo and Xoc, both with a logarithmic growth phase, were collected by centrifugation. Compound 10 was prepared as solutions of 10, 5 and 1 mg/L, respectively, and the conductivities were measured at 0–300 min.

4.8. Extracellular Polysaccharide Production

According to our previously reported work, the effects of compound 10 on the extracellular polysaccharide (EPS) production of Xoo and Xoc were tested [29]. After shaking at 28 °C for 3 d, the bacteria were centrifuged, and anhydrous ethanol was added to obtain the deposit after 12 h. The EPS production was acquired by centrifugation, drying, and weighing.

4.9. Morphological Change in Bacteria

According to the previously reported method [30–32], the changes in bacterial cell morphology were observed via scanning electron microscopy (FEI, Hillsboro, OR, USA). The details of the assay were adequately described in the published previous paper.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms222312953/s1.

Author Contributions: J.C., C.W. and J.H. conceived and designed the experiments; the calculation part was performed by J.H.; the experiments were performed by C.W., Y.C., X.L. and Y.W.; J.C., Z.W., S.W. and C.W. analyzed and interpreted the data; C.W. wrote the paper; J.C. critically revised the paper with regard to important intellectual content. All authors have read and approved the final version of the manuscript.

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Data Availability Statement: Characterizations, physical, analytical, and bactericidal activity data of target compounds 1–26 were showed in Supplementary Materials.

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Conflicts of Interest: The authors declare no conflict of interest.
Abbreviations

NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectrometry; MM/PBSA, molecular mechanics/Poisson–Boltzmann surface area; LE, ligand efficiency; FBDD, fragment-based drug discovery.

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