JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Natural Berberine-Hybridized Benzimidazoles as Novel Unique Bactericides against *Staphylococcus aureus*

Hang Sun, Mohammad Fawad Ansari, Bo Fang,* and Cheng-He Zhou*

Cite This: *J. Agric. Food Chem.* 2021, 69, 7831−7840 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Natural berberine-hybridized benzimidazoles as potential antibacterial agents were constructed to treat *Staphylococcus aureus* infection in the livestock industry. Bioassay showed that some new berberine-benzimidazole hybrids exhibited potent antibacterial efficacies, especially, the 2,4-dichlorobenzyl derivative **7d** not only showed strong activity against *S. aureus* ATCC 29213 with the MIC value of 0.006 mM but also effectively eradicated bacterial biofilm and exhibited low toxicity toward mammalian cells. The drug combination experiments showed that compound **7d** together with norfloxacin could enhance the antibacterial efficacy. Moreover, the 2,4-dichlorobenzyl derivative **7d** did not show obvious propensity to develop bacterial resistance. Preliminary mechanism studies revealed that the active molecule **7d** could damage the membrane integrity, stimulate ROS generation, and bind with DNA as well as *S. aureus* sortase A, thus exerting powerful antibacterial ability. In light of these facts, berberine-benzimidazole hybrid **7d** showed a large potentiality as a new bactericide for treating *S. aureus* in the livestock industry.

KEYWORDS: *berberine, benzimidazole, antibacterial, resistance, Staphylococcus aureus*

INTRODUCTION

Antibiotics play a crucial role in ensuring the safe supply of food-producing animal in sustainable animal production systems.[1,2] However, extensive and uncontrolled exposure of various antibiotics has caused an unprecedented risk from bacterial resistance.[3,4] In particular, *Staphylococcus aureus* has shown resistance to most currently approved antibiotics, such as penicillin and tetracycline.[5,6] Therefore, it is imperative to exploit new effective and innovative antibacterial agents to defend the invasion caused by drug-resistant *Staphylococcus aureus* strains.

Natural products were significant resources of alternative classes of antibiotics with new antibacterial scaffolds.[7] Berberine, a natural isoquinoline alkaloid isolated from several medicinal herbs such as *Berberis vulgaris* and *Rhizoma coptidis*, has been used as a nonprescription medicine to treat infectious diseases (such as bacillary dysentery, acute gastroenteritis, and cholera) in China for 2000 years.[8−10] Currently, various clinical applications of berberine have been discovered, especially in antibacterial usage, which revealed that berberine with a quaternary nitrogen, polycyclic, and planar system could helpfully increase membrane permeability and strengthen the bind affinities with amino acids in biomolecules.[11−15] Therefore, the unique and favorable structural features endowed natural berberine superiorities to be used as a novel promising scaffold for further antibacterial drug developments.

The successful applications of antibacterial azoles greatly hindered the invasion of bacteria in humans, terrestrial and aquatic animals, as well as plants.[16−19] In particular, benzimidazoles have attracted considerable attention because of the purine-like structure in benzimidazole ring that enabled benzimidazole derivatives to block the biosynthesis of proteins and nucleic acids.[20,21] A variety of bactericides containing benzimidazole fragment have been launched into the market, such as carbendazim and thiabendazole,[22] demonstrating the enormous potentiality in the antibacterial aspect.

Herein, by incorporating benzimidazoles into the C-12 position of berberine backbone, a series of berberine-hybridized benzimidazoles were constructed in this work, which were expected to overcome drug resistance (Figure 1). Moreover, a variety of substituents including chlorine and bromine atoms, methyl and methoxy groups, aliphatic chains, halogen-containing phenyl moieties, as well as amide and ester groups were introduced to adjust the physicochemical properties and to investigate the effect on bioactivities. Antibacterial evaluation, bactericidal kinetic assay, drug combination study, resistance development evaluation, and cytotoxicity assay were conducted to verify the antibacterial potentiality of new berberine-hybridized benzimidazoles. Finally, the preliminary explorations of antibacterial mechanism were performed by experiments including membrane depolarization and permeabilization assays, reactive oxygen species (ROS) generation, glutathione (GSH) activity determination, docking study, and interaction with DNA.

MATERIALS AND METHODS

Instruments and Chemicals. NMR spectra were recorded on Bruker AVANCE III 10 600 spectrometers (600 and 400 MHz). High-resolution mass spectra (HRMS) were recorded on a Bruker

Received: April 29, 2021
Revised: June 23, 2021
Accepted: June 24, 2021
Published: July 6, 2021

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

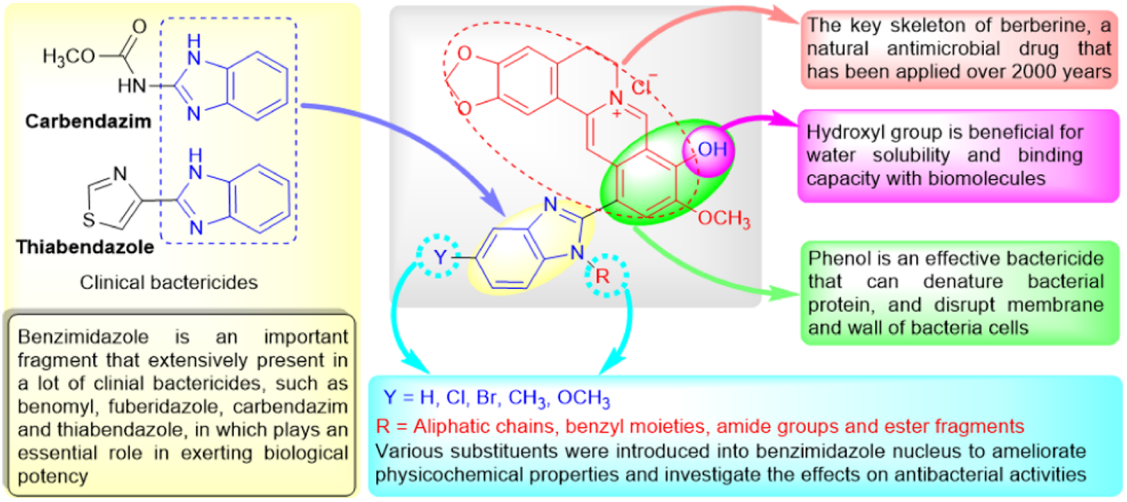


Figure 1. Design concept of novel berberine-hybridized benzimidazoles as new potential bactericides.

impact II 10200 spectrometer. Melting points of new compounds were measured by an X-6 melting point apparatus (Beijing Focus Instrument Co., Ltd., China). A TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd., Beijing, China) and an F-7000 spectrofluorometer (5J1-0004 model, Hitachi, Tokyo, Japan) were utilized to record UV spectra and fluorescence spectra, respectively. The tested bacterial strains were provided by Sichuan Provincial People's Hospital, Chengdu, China. All reagents and solvents were commercially available.

Chemistry. *Synthesis of Berberrubine 1.* Compound **1** was synthesized according to the reported procedure.[23]

Synthesis of Compound 2. The mixture of berberrubine **1** (1.10 mmol) and hexamethylenetetramine (5.50 mmol) in trifluoroacetic acid (10 mL) was refluxed for 8 h. The reaction system was cooled to room temperature, and then the diluted hydrochloric acid (10%, 5 mL) was added with stirring for 20 min. A saturated solution of sodium hydrogen carbonate was added to neutralize the mixture, and the solid was precipitated. The resulting precipitation was purified by column chromatography on silica gel using dichloromethane/methanol (V/V, 15/1, Rf = 0.5) as an eluent to produce target compound **2** with a yield of 55.4%. The corresponding data are provided in the Supporting Information.

Synthesis of Compounds 3a–e. The mixture of intermediate **2** (0.65 mmol), corresponding *o*-phenylenediamines (1.30 mmol), and sodium pyrosulfite (1.30 mmol) in *N*,*N*-dimethyl formamide (10 mL) was heated at 110 °C for 6 h. After the reaction was completed, water (20 mL) was added and the solid was precipitated. The residue was dissolved in chloroform (30 mL) and washed with saturated sodium chloride solution (3 × 30 mL). The organic phase was dried over anhydrous sodium sulfate and then evaporated. Crude product was purified by silica gel column chromatography using dichloromethane/methanol (V/V, 15/1, Rf = 0.2–0.4) as an eluent to give pure target compounds **3a–e** with yields of 37.6–80.2%. The corresponding data are provided in the Supporting Information.

Synthesis of Compounds 4a–f, 5a–d, 6a–c, 7a–e, 8a–b, 9a–b, and 10. Berberine-hybridized benzimidazoles **4–10** were prepared according to the procedure described for **3a** using intermediate **2** (0.65 mmol) and the corresponding mono-substituted *o*-phenylenediamines **11–17** (1.30 mmol) as starting materials in the presence of sodium pyrosulfite (1.30 mmol). Pure target compounds **4–10** were obtained with yields from 20.2 to 87.5%. The corresponding data are provided in the Supporting Information.

Synthesis of Intermediates 11–17. Mono-substituted *o*-phenylenediamine derivatives **11–17** were synthesized according to the reported procedure.[24]

Antibacterial Activity Assay. The prepared intermediate **2**, berberine-hybridized benzimidazoles **3–10**, berberine, and norfloxacin determined the in vitro antibacterial activities against Gram-positive bacteria (methicillin-resistant *S. aureus* N315, *S. aureus*, *S. aureus* ATCC 29213) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Escherichia coli*), and detailed procedure is given in the Supporting Information.

Biofilm Disruption Evaluation. The biofilm disruption activity of active compound **7d** against *S. aureus* ATCC 29213 was measured using crystal violet staining assay,[25] and the detailed procedure is provided in the Supporting Information.

Metabolic Activity Evaluation. The preformed *S. aureus* ATCC 29213 biofilm was incubated with increasing concentration of compound **7d** for 24 h at 37 °C. Resazurin solution (5 μg/mL, 25 μL) was added into control and treated cells to stain the biofilm for 40 min at 37 °C, and the absorbance was measured at 571 nm.

Bactericidal Activity Evaluation. An overnight culture of *S. aureus* ATCC 29213 was diluted in a Mueller-Hinton broth medium and incubated at 37 °C with aeration at 225 rpm for 2 h (early exponential) or 5 h (late exponential). The bacteria were then treated with compound **7d** at 6 × MIC and 8 × MIC in culture tubes at 37 °C and 225 rpm. At different intervals, the bacteria solution (100 μL) was moved to a 96-well plate, centrifuged at 4000 rpm for 5 min, and resuspended in sterile phosphate-buffered saline (100 μL). Tenfold serially diluted suspensions were plated on Mueller-Hinton agar plates and incubated at 37 °C overnight. Colonies were counted, and CFU per mL was calculated.

Cytotoxicity Evaluation. MTT assay was conducted to evaluate the cytotoxicity of active compound **7d** and berberine against human embryonic kidney (Hek) 293T and human fibroblast (HFC) normal cells,[14] and the hemolysis was also evaluated. The detailed procedure is depicted in the Supporting Information.

Drug Combination. The drug combination study of compound **7d** and norfloxacin was determined by the checkerboard titration method,[26] and the detailed procedure is provided in the Supporting Information.

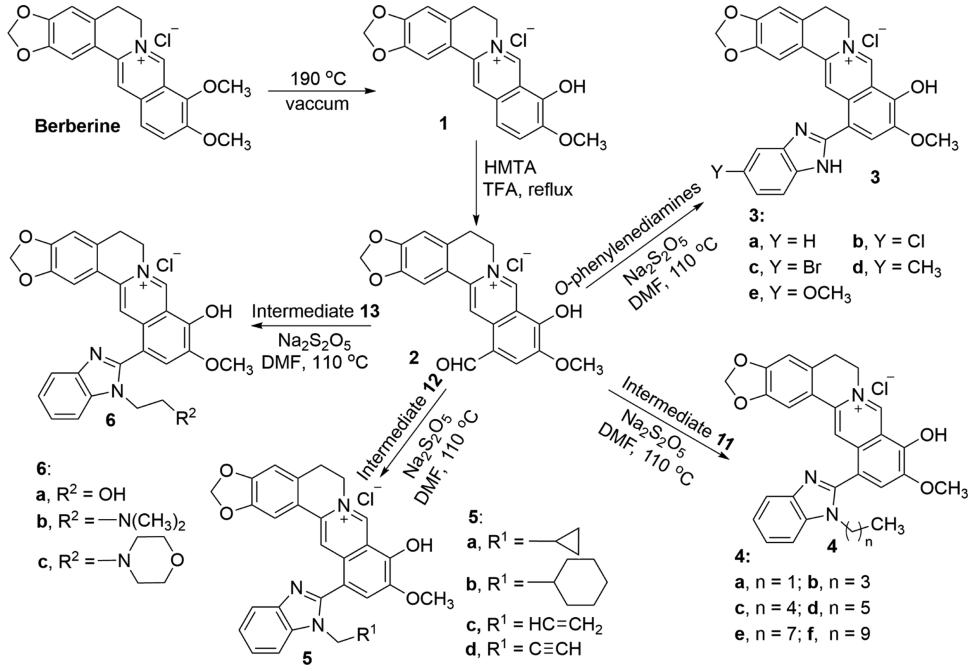
Propensity to Develop Bacterial Resistance. The propensity of compound **7d**, norfloxacin, and drug combination to induce resistance in *S. aureus* ATCC 29213 was evaluated, and the detailed procedure is depicted in the Supporting Information.

Membrane Depolarization Assay. The membrane depolarization ability of compound **7d** against *S. aureus* ATCC 29213 was tested using 3,3′-dipropylthiadicarbocyanine iodide (diSC35) as fluorescence probe, and the detailed procedure is provided in the Supporting Information.

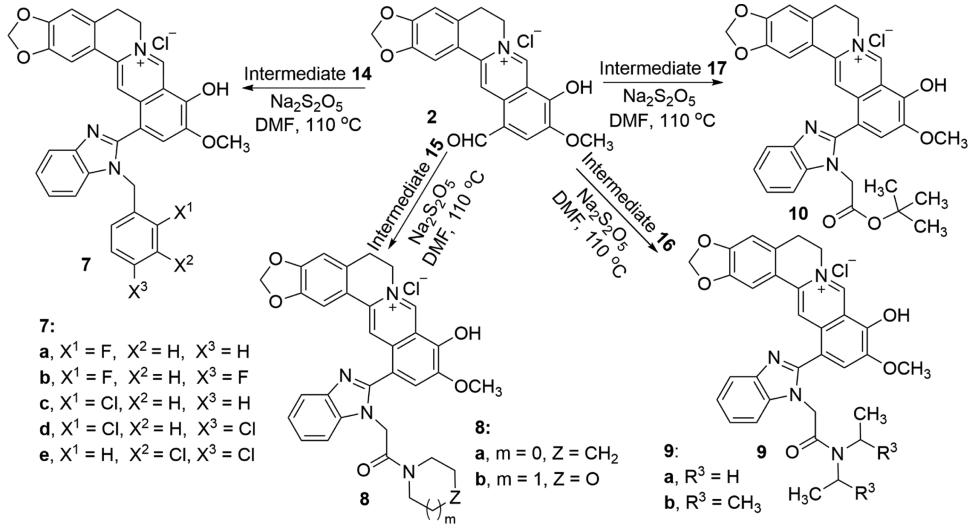
Membrane Permeabilization Assay. The membrane permeabilization ability of compound **7d** against *S. aureus* ATCC 29213 was tested using propidium iodide (PI) as fluorescence probe, and the detailed procedure is provided in the Supporting Information.

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831–7840

Scheme 1. Synthetic Route for Target Berberine-Hybridized Benzimidazoles 3−6



Scheme 2. Synthetic Route for Target Berberine-Hybridized Benzimidazoles 7−10



Molecular Simulation. The docking investigation between compound **7d** and *S. aureus* sortase A (PDB code: 1T2W) was conducted by Sybyl-x 2.0.

Supramolecular Interaction of Active Molecule 7d and Calf Thymus DNA. The interaction mode of active compound **7d** with DNA was explored through UV−vis and fluorescence spectra using acridine orange (AO) as spectral probe, and the detailed procedure is provided in the Supporting Information.

Determination of the Intracellular Reactive Oxygen Species (ROS). The intracellular ROS of *S. aureus* ATCC 29213 was measured using the standard 2,7-dichlorofluorosceindiacetate (DCFH-DA) assay,[27] and the detailed procedure is provided in the Supporting Information.

Determination of Intracellular Glutathione (GSH) Activity. The activity of intracellular GSH in *S. aureus* ATCC 29213 was determined by the standard Ellman's assay,[27] and the detailed procedure is provided in the Supporting Information.

RESULTS AND DISCUSSION

Chemistry. Synthetic routes to prepare berberine-hybridized benzimidazoles are outlined in Schemes 1, 2, and S1. Commercially available berberine underwent demethylation at 190 °C under vacuum to generate berberrubine **1**, which was further reacted with hexamethylenetetramine in trifluoroacetic acid to afford berberrubine-12-carbaldehyde **2** in 55.4% yield. The cyclization reaction of formyl derivative **2** and the corresponding *o*-phenylenediamines produced target berberine-hybridized benzimidazoles **3a−e** in the yields of 37.6−80.2%.

In a similar method to that described for *N*-1-alkylated benzimidazoles, the synthesis of compound **4a** was initially attempted by direct nucleophilic substitution of compound **3a** with bromoethane using potassium carbonate as base and tetrabutylammonium bromide as a phase transfer catalyst. However, no desired compound was obtained. Variations of

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

Table 1. Antibacterial Activities of Intermediate 2, Berberine-Hybridized Benzimidazoles 3−10, and Reference Drugs (MIC, mM)

	Gram-positive bacteria			Gram-negative bacteria			
compds	MRSA N315	*S. aureus*	*S. aureus* ATCC 29213	*Klebsiella pneumoniae*	*E. coli*	*Pseudomonas aeruginosa*	*A. baumannii*
2	0.166	0.166	0.664	0.332	0.664	0.166	0.664
3a	0.068	0.135	0.068	0.540	0.270	0.270	0.270
3b	0.031	0.252	0.504	0.252	0.504	0.252	0.252
3c	0.247	0.247	0.247	0.495	0.247	0.124	0.247
3d	0.033	0.131	0.016	0.131	0.262	0.131	0.033
3e	0.254	0.254	0.127	0.508	0.254	0.127	0.254
4a	0.016	0.127	0.008	0.255	0.510	0.127	0.127
4b	0.121	0.121	0.015	0.241	0.483	0.241	0.121
4c	0.059	0.059	0.029	0.235	0.471	0.235	0.118
4d	0.115	0.007	0.229	0.029	0.029	0.014	0.029
4e	0.218	0.218	0.055	0.437	0.437	0.218	0.218
4f	0.208	0.104	0.208	0.208	0.417	0.104	0.104
5a	0.242	0.015	0.030	0.121	0.015	0.004	0.015
5b	0.225	0.028	0.056	0.112	0.112	0.056	0.056
5c	0.249	0.031	0.498	0.249	0.249	0.249	0.249
5d	0.250	0.031	0.250	0.250	0.250	0.250	0.250
6a	0.247	0.247	0.124	0.494	0.494	0.124	0.247
6b	0.470	0.470	0.235	0.235	0.470	0.235	0.470
6c	0.218	0.218	0.436	0.436	0.436	0.218	0.436
7a	0.014	0.027	0.055	0.110	0.220	0.055	0.055
7b	0.107	0.053	0.007	0.107	0.107	0.107	0.107
7c	0.214	0.003	0.214	0.107	0.428	0.107	0.027
7d	0.006	0.013	0.006	0.101	0.202	0.051	0.013
7e	0.101	0.025	0.025	0.202	0.202	0.101	0.101
8a	0.219	0.219	0.219	0.219	0.219	0.219	0.219
8b	0.213	0.213	0.213	0.426	0.426	0.213	0.213
9c	0.218	0.218	0.218	0.436	0.055	0.218	0.218
9b	0.208	0.208	0.208	0.208	0.416	0.208	0.208
10	0.218	0.218	0.218	0.218	0.218	0.218	0.218
berberine	0.689	0.086	0.172	0.172	0.086	1.377	0.172
norfloxacin	0.025	0.025	0.006	0.013	0.050	0.006	0.025

reaction conditions (temperature, bases, solvents, and catalysts) were tried, but the reaction failed. This situation resulted in an alternative strategy from the reaction of mono-substituted *o*-phenylenediamines with compound **2** to prepare target molecules. Commercially available *o*-phenylenediamine was reacted with corresponding chlorinated or brominated compounds together with potassium carbonate to smoothly afford intermediates **11**−**17** (Scheme S1), which were further reacted with compound **2** in the presence of sodium pyrosulfite in *N*,*N*-dimethyl formamide at 110 °C to successfully access target products **4**−**10** (Schemes 1 and 2). The structures of all new berberine-hybridized benzimidazoles were confirmed through NMR and HRMS spectra, and the detailed synthetic processes and spectral data are provided in the Supporting Information.

Antibacterial Activity Assay. Table 1 shows that some target berberine-hybridized benzimidazoles exhibited more potent antibacterial effects in comparison to berberine and norfloxacin against the tested bacterial strains. This indicated that the introduction of benzimidazoles to berberine skeleton was greatly helpful for the antibacterial activities. Berberrubine-12-formaldehyde **2** displayed high inhibitory concentration toward the tested bacterial strains, while MIC values decreased when the formyl group was cyclized to afford benzimidazole derivative **3a**, suggesting the great contribution of benzimidazole in exerting antibacterial activity. Compound **3a** could obviously inhibit the growth of MRSA and *S. aureus* ATCC 29213 in a low concentration of 0.068 mM, which was much lower than berberine. The inhibition concentration of **3b** against MRSA N315 strain was shown to be comparable to norfloxacin and exhibited a profound effect of 0.031 mM. Significantly, compound **3d** bearing electron-donating methyl group exhibited profound antibacterial effect with low MIC values (0.016−0.033 mM) toward MRSA, *S. aureus* ATCC 29213, and *Acinetobacter baumannii*. However, compound **3e** with methoxy group abolished the antibacterial activity partially or completely.

Alkyl chains were known to exert advantageous effects on biological activity via regulating lipid−water distribution coefficient and binding affinity to targets.[28,29] Therefore, compounds **4a**−**f** were synthesized to investigate the spacer lengths of alkyl chains on the antibacterial activities. Ethyl derivative **4a** could effectively inhibit the growth of *S. aureus* 29213 and MRSA with low MIC values of 0.008 and 0.016 mM, respectively. Pentyl derivative **4c** exhibited good potencies with MIC values ranging from 0.029 to 0.059 mM against MRSA, *S. aureus* and *S. aureus* 29213. Particularly, compound **4d** exhibited excellent inhibitory effects (MIC = 0.029 mM) against *K. pneumoniae*, *E. coli*, and *A. baumannii*. Meanwhile, hexyl derivative **4d** was active toward *S. aureus* with a low MIC value of 0.007 mM that was 12 and 4 times higher than those for berberine and norfloxacin, respectively.

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

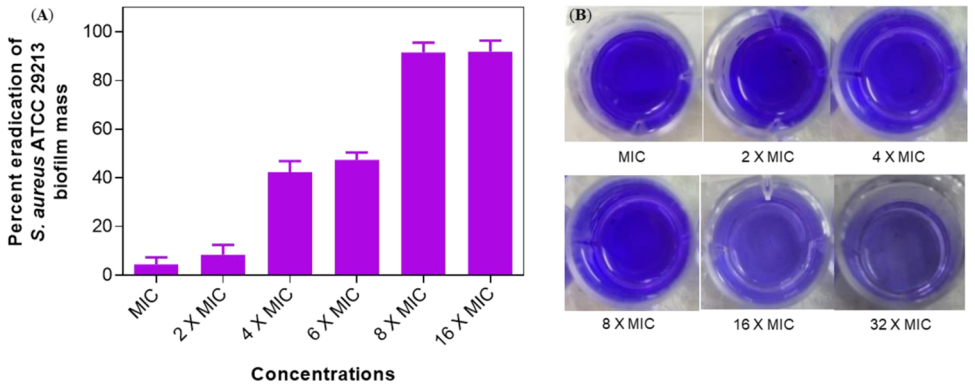


Figure 2. (A) Eradication of the preformed *S. aureus* ATCC 29213 biofilms by compound **7d**. (B) Images of *S. aureus* ATCC 29213 treated with **7d** after staining by crystal violet.

When the number of carbon atoms increased, octyl and decyl compounds **4e** and **4f** were essentially inactive toward the tested bacteria, which might result from large lipophilicity. The data strongly indicated that the lengths of the alkyl chains significantly affected antibacterial activity and moderate lengths might be helpful for improving antibacterial activities. Inspired by the improved biological potentiality by incorporating cyclanes,[25] compounds **5a** and **5b** were synthesized and tested for their antibacterial potencies, where cyclopropyl derivative **5a** was active toward the tested bacteria except for MRSA and *K. pneumoniae*. It was highly sensitive to *S. aureus*, *A. baumannii*, *E. coli*, and *S. aureus* ATCC 29213 at low concentrations (0.015−0.030 mM). Moreover, this compound exerted comparable activity (MIC = 0.004 mM) to norfloxacin (MIC = 0.006 mM) for *P. aeruginosa*. When cyclopropane was expanded to cyclohexane, the antibacterial activity of compound **5b** significantly reduced.

Compounds **5c** and **5d** bearing allyl and propargyl groups were prepared to investigate the effects of unsaturated bonds on the antibacterial effect, respectively. Molecules **5c** and **5d** both showed a 3-fold decrease in anti-*S. aureus* with a low MIC value (0.031 mM) in comparison to berberine (0.081 mM). To explore the effect of hydrophilic groups on the antibacterial activity of berberine derivatives, compounds **6a−c** were prepared. Both of them were inactive toward the tested strains, suggesting that the increase of water solubility was deleterious to the antibacterial activities.

The structure and activity relationship (SAR) investigations were further moved onto the effect of halogen-containing benzyl groups, which were helpful for increasing lipid solubility and membrane penetrability,[30,31] and compounds **7a−e** were synthesized. Fluorobenzyl derivative **7a** gave relatively low MIC values (0.014−0.055 mM) against MRSA, *S. aureus*, *S. aureus* ATCC 29213, *P. aeruginosa*, and *A. baumannii*, which were comparable to or even higher than norfloxacin. When the number of fluorine atom was increased, the antibacterial potency of compound **7b** dropped obviously except that the inhibitory concentration toward *S. aureus* ATCC 29213 was decreased by 8-fold up to 0.007 mM compared to the fluorobenzyl molecule **7a** (MIC = 0.055 mM). It seemed that the displacement of the fluorine atom (**7a**) by a chlorine atom (**7c**) reduced the activity of the molecule, but surprisingly enhanced its effect against *S aureus* (8 times in comparison to norfloxacin) and *A. baumannii* (the same as for norfloxacin). The introduction of more chlorine atoms resulted in compound **7d**, which could effectively suppress *S. aureus* ATCC 29213 growth with a low concentration of 0.006 mM. Furthermore, this molecule possessed 114- and 4-fold antibacterial potency than berberine and norfloxacin in inhibiting MRSA growth, respectively. Additionally, compound **7d** gave a low MIC value (0.013 mM) toward *S. aureus* and *E. coil*, which was more than 2-fold higher than norfloxacin. Moreover, it showed relatively good inhibitory effect on *P. aeruginosa* with a MIC value of 0.051 mM, which was 27 times more potent than berberine. However, the 3,4-dichlorobenzyl derivative **7e** only exhibited good inhibitory effect (MIC = 0.025 mM) against *S. aureus* and *S. aureus* ATCC 29213, which suggested that the number and location of halogen atoms on benzyl group would result in different effects on bioactivities.

Encouraged by the enhanced antimicrobial activity through the incorporation of amino derivatives,[32] compounds **8** and **9** were synthesized to study the impact of amide groups on biological activities. Unfortunately, all of them abolished the activity completely or partially, indicating that the introduction of amides at the *N*-1 position of benzimidazole nucleus was not favorable for antibacterial potency. Stimulated by the lipophilic ester group, tert-butyl ester derivative **10** was synthesized and tested the antibacterial activity; however, it was not active toward the tested strains.

Biofilm Disruption Evaluation. The biofilm formation of *S. aureus* made conventional antibiotics hard to adequately attack and destroy infectious biofilm populations.[33−35] Thus, new antibacterial agents inhibiting biofilm formation were imperative to confront *Staphylococcus* infection. The ability of compound **7d** to disrupt the preformed biofilm of *S. aureus* ATCC 29213 was evaluated. As shown in Figure 2, the 2,4-dichlorobenzyl derivative **7d** could eradicate more than 40% biofilm of *S. aureus* ATCC 29213 at concentrations of 4 × and 8 × MIC. Especially, when its concentration was increased to 16 × and 32 × MIC, the disruption of *S. aureus* ATCC 29213 biofilm exceeded 90%. These results demonstrated that compound **7d** could effectively disrupt bacterial biofilm to exert antibacterial activity.

Metabolic Activity Evaluation. The alamar blue assay was conducted to determine the viability of *S. aureus* ATCC 29213 within the matured biofilm. As shown in Figure S1, the cell viability of *S. aureus* ATCC 29213 within biofilm reduced about 47% in the presence of compound **7d** at the concentration of 8 × MIC, which indicated that active molecule **7d** possessed certain ability to disrupt matured biofilm and then inhibit the viability of *S. aureus* ATCC 29213 cells.

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

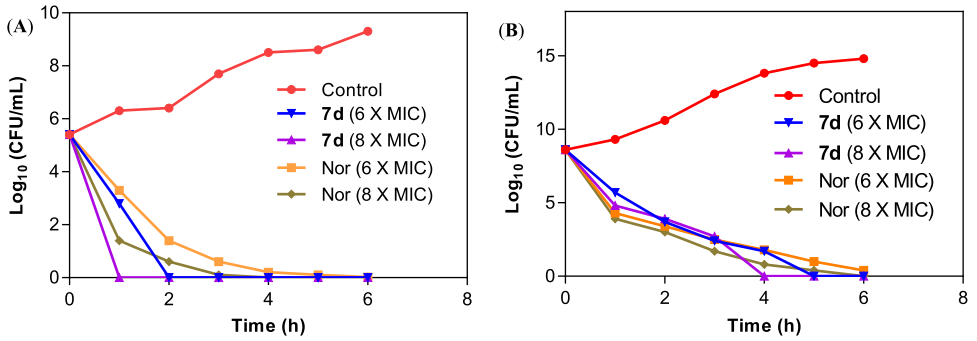


Figure 3. Time-dependent killing of **7d** (6 × MIC and 8 × MIC) against early (**A**) and late (**B**) phase exponential *S. aureus* ATCC 29213.

Bactericidal Activity Evaluation. Efficient bactericidal activity is one of the most important features of an effective antibacterial agent.[36] The bactericidal performance of active molecule **7d** (6 × MIC and 8 × MIC) against *S. aureus* ATCC 29213 (early and late exponential phases) was evaluated, and the results are recorded in Figure 3. Compound **7d** could rapidly kill early exponential phase *S. aureus* ATCC 29213 (2 h, 6 × MIC; 1 h, 8 × MIC), and the late exponential phase bacteria were also killed in the presence of molecule **7d** (5 h, 6 × MIC; 3 h, 8 × MIC). In addition, it could be clearly observed in Figure 3 that the bactericidal rate of compound **7d** was more rapid than norfloxacin against *S. aureus* ATCC 29213. These results demonstrated that compound **7d** possessed strong bactericidal potency against *S. aureus* ATCC 29213, which would effectively reduce the probability of bacterial resistance development.

Cytotoxicity Evaluation. Excellent safety of antibacterial agents would reinforce their potential applications in the agricultural industry.[37] Therefore, the cytotoxicity of highly active compound **7d** against Hek 293T, HFC, and red blood cells was evaluated with berberine as the positive control. As shown in Figure S2, the viabilities of Hek 293T and HFC cells still maintained 77.3 ± 7.0 and 52.3 ± 1.7% in the presence of compound **7d** with the concentration of 50 μM (8 × MIC), suggesting that compound **7d** exhibited relatively low cytotoxicity toward the mammalian cells. In addition, no hemolysis was observed with compound **7d** at concentrations ≤512 μg/mL, indicating that compound **7d** possessed good selectivity for *S. aureus* ATCC 29213 over mammalian cells. Moreover, a higher cell viability was observed for compound **7d** than berberine, which indicated the advantage of this modification of chemical structure.

Drug Combination. Combination therapy has been considered as an alternative method to enhance the antibacterial efficacy, reduce the usage doses, and combat serious drug resistance.[38] Therefore, drug interaction assays were conducted to determine the synergistic effects of active compound **7d** with norfloxacin. It could be clearly observed that the treated bacteria with drug combination were more susceptible in contrast to their individual use (Table S1), which suggested that the tolerance of norfloxacin might be reduced by compound **7d**. The most efficient potentiation was obtained on the co-administration of norfloxacin with compound **7d** against *E. coli* with outstanding synergism (FIC = 0.0157). Moreover, the MIC values of compound **7d** toward MRSA, *S. aureus*, and *S. aureus* ATCC 29213 were reduced to 0.0004 mM. The synergy of target berberine-hybridized benzimidazole **7d** provided a promising strategy for searching how the limited therapeutic range of antibacterial drugs could be broadened to more effectively address bacterial infections. Furthermore, the availability of this type of synergistic use needs further studies to optimize the antibacterial potencies, and deep mechanism investigation is indispensable to explain the drug combination.

Propensity to Develop Bacterial Resistance. The slow propensity to develop bacterial resistance is a crucial feature of antibacterial agents.[39,40] Therefore, the probability of compound **7d** or combination use to induce resistance in *S. aureus* ATCC 29213 was conducted with norfloxacin as positive control. The results in Figure 4 showed that the susceptibility

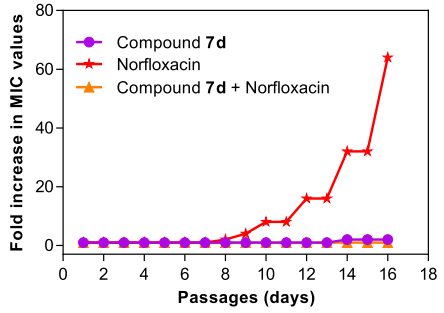


Figure 4. Propensity of *S. aureus* ATCC 29213 resistance development treated by compound **7d**, norfloxacin, and drug combination.

of *S. aureus* ATCC 29213 to the 2,4-dichlorobenzyl derivative **7d** was nearly unchanged after 15 passages, while the MIC values for norfloxacin were obviously increased by 32-fold after 14 passages, suggesting that compound **7d** was hard to develop resistance in *S. aureus* ATCC 29213. Moreover, the emergence of resistance in norfloxacin was effectively reversed upon combination with compound **7d**, which was consistent with the drug interaction that the 2,4-dichlorobenzyl derivative **7d** could potentially elevate bacterial inhibitory efficacy.

Membrane Depolarization Assay. The similar chemical properties and action mechanisms of berberine to quaternary ammonium antiseptics make it target cytoplasmic membrane and DNA.[41] To explore whether benzimidazole-hybridized berberines target bacterial membrane or not, a membrane depolarization study was performed using the DiSC35 fluorescence dye, which can be accumulated within the bacterial membrane, causing fluorescence quenching.[42] Fluorescence intensity could be sharply increased when the membrane was depolarized. As shown in Figure S3A, when *S. aureus* ATCC 29213 was treated with the 2,4-dichlorobenzyl derivative **7d** at increasing concentrations, a growing trend in fluorescence intensity was observed in a concentration-dependent manner, which demonstrated that compound **7d**

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

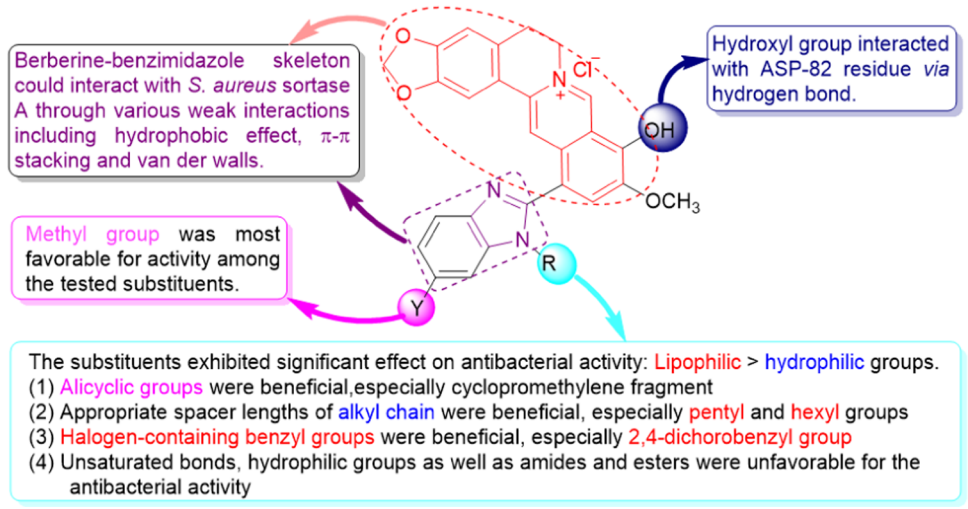


Figure 5. Preliminary summary of structure and activity relationships of berberine-hybridized benzimidazoles.

could interact with bacterial membrane and cause membrane depolarization.

Membrane Permeabilization Assay. Prodium iodide (PI) is a membrane-permeable dye that can only bind to nucleic acids by penetrating incomplete bacterial membranes, resulting in significant fluorescence changes.[43,44] When *S. aureus* ATCC 29213 was treated with active molecule **7d**, a remarkable concentration-dependent increase of the PI fluorescence was found (Figure S3B), which indicated that compound **7d** could promote the inner membrane permeability of *S. aureus* ATCC 29213, thereby breaking the bacterial membrane integrity and resulting in the bacterial cell death. Moreover, the concentration-dependent effect of this compound on membrane permeabilization was correlated with its dose-dependent bactericidal behavior, suggesting that the rapid bactericidal activity of compound **7d** might be attributed to its membrane-disrupting activity.

Docking Simulation. *S. aureus* sortase A (PDB ID: 1T2W) as an attractive target in anti-*S. aureus* drug exploitation was utilized in a flexible ligand-receptor docking investigation to further rationalize the observed antibacterial activity of active molecule **7d**. As shown in Figure S4, active molecule **7d** could well match the active pocket of sortase A through various weak interactions including the hydrophobic effect, $\pi-\pi$ stacking, and van der Waals. Noticeably, the hydroxyl group at the C-9 position of berberine skeleton could pick up a hydrogen bond with the oxygen atom of the carbonyl group in ASP-82 residue with a distance of 2.1 Å, and a binding energy was as low as −6.79 kJ/mol (total score, 5.25), which suggested the great contribution of the hydroxyl group in exerting antibacterial activity. In addition, the alkyl interactions between the chlorine atoms of benzyl group and various residues (ASN-127, PRO-126, PRO-79, and ARG-125) were observed. These interactions might promote the stabilization of compound−enzyme complex, which might be responsible for the excellent antibacterial activity of compound **7d**.

Supramolecular Interactions between Active Molecule 7d and Calf Thymus DNA. DNA has been considered as a potent drug target and is widely utilized for the design and development of new efficient antibacterial drugs.[45−47] Therefore, the interaction mode of compound **7d** and DNA was explored using AO spectral probe. The absorption spectra of DNA−AO complex varying with compound **7d** concentrations are recorded in Figure S5A. It could be clearly observed that the absorption spectra of the DNA−AO complex (499.5 nm) did not show redshift and hypochromic effect with the addition of compound **7d**, which indicated that the interaction of active molecule **7d** with DNA was not the typical intercalative mode.

Fluorescence quenching experiment was further performed to explore the target mode of compound **7d** toward DNA. As the concentration of compound **7d** increased, the fluorescence intensity of DNA−AO complex was significantly quenched (Figure S5B); however, no blueshift or redshift was observed. Meanwhile, the calculated fluorescence quenching data in the Supporting Information indicated that compound **7d** could quench the DNA−AO complex via a static quenching mechanism. The decrease in the fluorescence intensity of the DNA−AO complex might be attributed to the result of the substitution of AO by compound **7d** or the formation of a **7d**−DNA−AO complex. The results in absorption spectrum showed that the binding of compound **7d** to DNA−AO was not a typical intercalative action, so compound **7d** was likely to interact with DNA via groove binding action.

Chloride quenching experiments were also conducted to further confirm the binding of compound **7d** with DNA. As shown in Figure S6C, the fluorescence intensity of compound **7d**−DNA complex remained basically unchanged with the addition of sodium chloride, which suggested that the electrostatic effect was not the interaction mode of compound **7d** and DNA. These results suggested that compound **7d** was sufficient to interact with DNA to form a supramolecular complex via groove binding action, thus exerting good antibacterial activity.

Intracellular ROS Determination Assay. ROS may cause disruption of membrane integrity and increase membrane permeability when the dose produced exceeds the antioxidant capacity of protective enzymes.[48] To test whether active compound **7d** possessed potential in perturbing the bacterial metabolism process through facilitating ROS generation, the intracellular ROS level of *S. aureus* ATCC 29213 was detected when treated by active compound **7d** using DCFH-DA dye. The results showed that the treatment of *S. aureus* ATCC 29213 cells by 2,4-dichlorobenzyl derivative **7d** led to significant intracellular ROS production (Figure S6A). The increasing concentration of compound **7d** was found to be highly correlated with the extent of ROS generation. There

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

were 3-, 5-, and 7-fold increases of ROS production in *S. aureus* ATCC 29213 treated with compound **7d** at the concentrations of MIC, 2-fold and 4-fold MIC values, respectively.

Determination of GSH Activity. To further investigate the role of oxidative stress in interfering with the normal function of bacterial cells, the activity of GSH was determined. As shown in Figure S6B, when bacteria were treated with compound **7d**, a gradual reduction of GSH activity in *S. aureus* ATCC 29213 was observed, which might be attributed to the ROS-dependent oxidation. These results showed that the increase of oxidative stress in *S. aureus* ATCC 29213 might also be responsible for the good antibacterial activity of 2,4-dichlorobenzyl derivative **7d**.

In summary, a unique type of novel berberine-hybridized benzimidazoles was developed that exhibited potent antibacterial effects. Particularly, the 2,4-dichlorobenzyl derivative **7d** showed excellent inhibitory effects with a low MIC value of 0.006 mM against *S. aureus* ATCC 29213. The SARs indicated that the substituents on benzimidazole nucleus were important factors on antibacterial activities and the introduction of hydrophobic groups was favorable for antibacterial activity (Figure 5). The combination use of active compound **7d** with norfloxacin was found to be synergistic against the tested bacteria, and resistance study revealed that compound **7d** exhibited lower propensity to develop *S. aureus* ATCC 29213 resistance than norfloxacin. Preliminary mechanism studies demonstrated that compound **7d** with low toxicity might damage membrane integrity, increase oxidative stress, and bind with DNA as well as *S. aureus* sortase A, empowering favorable biofilm disruption ability and rapid bactericidal activity. These results provided powerful information for further development of compound **7d** as an alternative agricultural available agent to confront the growing challenges in *S. aureus* infections.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c02545.

Results of metabolic activity, drug combination, cytotoxicity, membrane depolarization and permeabilization, docking simulation, supramolecular interaction between active compound **7d** and calf thymus DNA, intracellular ROS generation and intracellular glutathione oxidation; synthetic procedures and characterization data of all new compounds; specific operation steps of in vitro antibacterial activity, biofilm disruption, bactericidal activity, membrane depolarization, membrane permeabilization, supramolecular interaction between active compound **7d** and calf thymus DNA, intracellular ROS generation, intracellular glutathione oxidation and drug interaction, as well as resistance development and cytotoxicity assay; and NMR and HRMS spectra of some representative compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Bo Fang − *College of Pharmacy, National & Local Joint Engineering Research Center of Targeted and Innovative Therapeutics, Chongqing Key Laboratory of Kinase Modulators as Innovative Medicine, Chongqing University of Arts and Sciences, Chongqing 402160, China*; Email: fangbo2100@163.com

Cheng-He Zhou − *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China*; orcid.org/0000-0003-3233-5465; Email: zhouch@swu.edu.cn

Authors

Hang Sun − *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China*

Mohammad Fawad Ansari − *Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.1c02545

Funding

This research was supported in part by grants from the National Natural Science Foundation of China (21971212, 21901028), Postdoctoral Science Foundation Project of Chongqing Science and Technology Bureau (cstc2019jcyj-bshX0124), China Postdoctoral Science Foundation (2019M653821XB), Natural Science Foundation of Chongqing (cstc2019jcyj-msxmX0202), and Chongqing Special Foundation for Postdoctoral Research Proposal (No. XmT2018082).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

MRSA N315, methicillin-resistant *Staphylococcus aureus* N315; S. aureus, *Staphylococcus aureus*; S. aureus ATCC 29213, *Staphylococcus aureus* ATCC 29213; P. aeruginosa, *Pseudomonas aeruginosa*; K. pneumoniae, *Klebsiella pneumoniae*; A. baumannii, *Acinetobacter baumannii*; E. coli, *Escherichia coli*; MIC, minimum inhibitory concentration; SARs, structure−activity relationships; CLSI, Clinical and Laboratory Standards Institute; diSC35, 3,3′-dipropylthiadicarbocyanine iodide; PI, propidium iodide; ROS, reactive oxygen species; GSH, glutathione; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; AO, acridine orange; DNA, deoxyribonucleic acid; FIC, fractional inhibitory concentration

■ REFERENCES

(1) Chen, J. M.; Sun, R. X.; Pan, C. G.; Sun, Y.; Mai, B. X.; Li, Q. X. Antibiotics and Food Safety in Aquaculture. *J. Agric. Food Chem.* **2020**, *68*, 11908−11919.

(2) Hassan, Y. I.; Kosir, V.; Yin, X.; Ross, K.; Diarra, M. S. Grape Pomace as A Promising Antimicrobial Alternative in Feed: A Critical Review. *J. Agric. Food. Chem.* **2019**, *67*, 9705−9718.

(3) Van Boeckel, T. P.; Pires, J.; Silvester, R.; Zhao, C.; Song, J.; Criscuolo, N. G.; Gilbert, M.; Bonhoeffer, S.; Laxminarayan, R. Global Trends in Antimicrobial Resistance in Animals in Low- and Middle-Income Countries. *Science* **2019**, *365*, No. eaaw1944.

(4) Jiang, S.; Su, S. J.; Chen, M.; Peng, F.; Zhou, Q.; Liu, T. T.; Liu, L. W.; Xue, W. Antibacterial Activities of Novel Dithiocarbamate-Containing 4H-Chromen-4-one Derivatives. *J. Agric. Food Chem.* **2020**, *68*, 5641−5647.
(5) Troeman, D. P. R.; Van Hout, D.; Kluytmans, J. A. J. W. Antimicrobial Approaches in the Prevention of *Staphylococcus aureus* Infections: A Review. *J. Antimicrob. Chemother.* **2019**, *74*, 281−294.
(6) Lin, S.; Liu, J.; Li, H.; Liu, Y.; Chen, Y.; Luo, J.; Liu, S. Development of Highly Potent Carbazole Amphiphiles as Membrane-Targeting Antimicrobials for Treating Gram-Positive Bacterial Infections. *J. Med. Chem.* **2020**, *63*, 9284−9299.
(7) Hobson, C.; Chan, A. N.; Wright, G. D. The Antibiotic Resistome: A Guide for the Discovery of Natural Products as Antimicrobial Agents. *Chem. Rev.* **2021**, *121*, 3464−3494.
(8) Porras, G.; Chassagne, F.; Lyles, J. T.; Marquez, L.; Dettweiler, M.; Salam, A. M.; Samarakoon, T.; Shabih, S.; Farrokhi, D. R.; Quave, C. L. Ethnobotany and the Role of Plant Natural Products in Antibiotic Drug Discovery. *Chem. Rev.* **2021**, *121*, 3495−3560.
(9) Sun, H.; Ansari, M. F.; Battini, N.; Bheemanaboina, R. R. Y.; Zhou, C. H. Novel Potential Artificial MRSA DNA Intercalators: Synthesis and Biological Evaluation of Berberine-Derived Thiazolidinediones. *Org. Chem. Front.* **2019**, *6*, 319−334.
(10) Xu, B.; Jiang, X.; Xiong, J.; Lan, J.; Tian, Y.; Zhong, L.; Wang, X.; Xu, N.; Cao, H.; Zhang, W.; Zhang, H.; Hong, X.; Zhan, Y. Y.; Zhang, Y.; Hu, T. Structure−Activity Relationship Study Enables the Discovery of A Novel Berberine Analogue as the RXRα Activator to Inhibit Colon Cancer. *J. Med. Chem.* **2020**, *63*, 5841−5855.
(11) Li, T.; Wang, P.; Guo, W.; Huang, X.; Tian, X.; Wu, G.; Xu, B.; Li, F.; Yan, C.; Liang, X. J.; Lei, H. Natural Berberine-Based Chinese Herb Medicine Assembled Nanostructures with Modified Antibacterial Application. *ACS Nano* **2019**, *13*, 6770−6781.
(12) Zhang, G.-B.; Maddili, S. K.; Tangadanchu, V. K. R.; Gopala, L.; Gao, W. W.; Cai, G. X.; Zhou, C. H. Discovery of Natural Berberine-Derived Nitroimidazoles as Potentially Multi-Targeting Agents against Drug-Resistant *Escherichia coli*. *Sci. China Chem.* **2018**, *61*, 557−568.
(13) Gao, W.-W.; Gopala, L.; Bheemanaboina, R. R. Y.; Zhang, G. B.; Li, S.; Zhou, C. H. Discovery of 2-Aminothiazolyl Berberine Derivatives as Effectively Antibacterial Agents toward Clinically Drug-Resistant Gram-Negative *Acinetobacter baumanii*. *Eur. J. Med. Chem.* **2018**, *146*, 15−37.
(14) Gaba, S.; Saini, A.; Singh, G.; Monga, V. An Insight into the Medicinal Attributes of Berberine Derivatives: A Review. *Bioorg. Med. Chem.* **2021**, *38*, No. 116143.
(15) Jeyakkumar, P.; Zhang, L.; Avula, S. R.; Zhou, C. H. Design, Synthesis and Biological Evaluation of Berberine-Benzimidazole Hybrids as New Type of Potentially DNA-Targeting Antimicrobial Agents. *Eur. J. Med. Chem.* **2016**, *122*, 205−215.
(16) Zhang, L.; Peng, X. M.; Damu, G. L. V.; Geng, R. X.; Zhou, C. H. Comprehensive Review in Current Developments of Imidazole-Based Medicinal Chemistry. *Med. Res. Rev.* **2014**, *34*, 340−437.
(17) Gao, W. W.; Zhou, C. H. Antimicrobial 2-Aminothiazolyl Quinolones: What is Their Potential in the Clinic? *Future Med. Chem.* **2017**, *9*, 1461−1464.
(18) Wang, J.; Ansari, M. F.; Zhou, C. H. Identification of Unique Quinazolone Thiazoles as Novel Structural Scaffolds for Potential Gram-Negative Bacterial Conquerors. *J. Med. Chem.* **2021**, *64*, 7630−7645.
(19) Wang, J.; Ansari, M. F.; Lin, J. M.; Zhou, C. H. Design and Synthesis of Sulfanilamide Aminophosphonates as Novel Antibacterial Agents towards *Escherichia coli*. *Chin. J. Chem.* **2021**, *39*, 2251−2263.
(20) Morcoss, M. M.; Abdelhafez, E. S. M. N.; Ibrahem, R. A.; Abdel-Rahman, H. M.; Abdel-Aziz, M.; Abou El-Ella, D. A. Design, Synthesis, Mechanistic Studies and in Silico ADME Predictions of Benzimidazole Derivatives as Novel Antifungal Agents. *Bioorg. Chem.* **2020**, *101*, No. 103956.
(21) Wang, Y.-N.; Bheemanaboina, R. R. Y.; Cai, G. X.; Zhou, C. H. Novel Purine Benzimidazoles as Antimicrobial Agents by Regulating ROS Generation and Targeting Clinically Resistant *Staphylococcus aureus* DNA Groove. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 1621−1628.
(22) Liu, H.-B.; Gao, W. W.; Tangadanchu, V. K. R.; Zhou, C. H.; Geng, R. X. Novel Aminopyrimidinyl Benzimidazoles as Potentially Antimicrobial Agents: Design, Synthesis and Biological Evaluation. *Eur. J. Med. Chem.* **2018**, *143*, 66−84.
(23) Jeyakkumar, P.; Liu, H. B.; Gopala, L.; Cheng, Y.; Peng, X. M.; Geng, R. X.; Zhou, C. H. Novel Benzimidazolyl Tetrahydroprotoberberines: Design, Synthesis, Antimicrobial Evaluation and Multi-Targeting Exploration. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1737−1743.
(24) Wang, Y.-N.; Bheemanaboina, R. R. Y.; Gao, W. W.; Kang, J.; Cai, G. X.; Zhou, C. H. Discovery of Benzimidazole-Quinolone Hybrids as New Cleaving Agents toward Drug-Resistant *Pseudomonas aeruginosa* DNA. *ChemMedChem* **2018**, *13*, 1004−1017.
(25) Zhang, E.; Bai, P. Y.; Cui, D. Y.; Chu, W. C.; Hua, Y. G.; Liu, Q.; Yin, H. Y.; Zhang, Y. J.; Qin, S.; Liu, H. M. Synthesis and Bioactivities Study of New Antibacterial Peptide Mimics: The Dialkyl Cationic Amphiphiles. *Eur. J. Med. Chem.* **2018**, *143*, 1489−1509.
(26) Yong, J.; Zu, R.; Huang, X.; Ge, Y.; Li, Y. Synergistic Effect of Berberine Hydrochloride and Fluconazole against *Candida albicans* Resistant Isolate. *Front. Microbiol.* **2020**, *11*, No. 1498.
(27) Roy, S.; Mondal, A.; Yadav, V.; Sarkar, A.; Banerjee, R.; Sanpui, P.; Jaiswal, A. Mechanistic Insight into the Antibacterial Activity of Chitosan Exfoliated MoS_2 Nanosheets: Membrane Damage, Metabolic Inactivation, and Oxidative Stress. *ACS Appl. Bio. Mater.* **2019**, *2*, 2738−2755.
(28) Shi, Y.-G.; Zhang, R. R.; Zhu, C. M.; Xu, M. F.; Gu, Q.; Ettelaie, R.; Lin, S.; Wang, Y. F.; Leng, X. Y. Antimicrobial Mechanism of Alkyl Gallates against *Escherichia coli* and *Staphylococcus aureus* and Its Combined Effect with Electrospun Nanofibers on Chinese *Taihu icefish* Preservation. *Food Chem.* **2021**, *346*, No. 128949.
(29) Wang, J.; Battini, N.; Ansari, M. F.; Zhou, C. H. Synthesis and Biological Evaluation of Quinazolonethiazoles as New Potential Conquerors towards *Pseudomonas aeruginosa*. *Chin. J. Chem.* **2021**, *39*, 1093−1103.
(30) Wang, Y.-X.; Pang, W. Q.; Zeng, Q. X.; Deng, Z. S.; Fan, T. Y.; Jiang, J. D.; Deng, H. B.; Song, D. Q. Synthesis and Biological Evaluation of New Berberine Derivatives as Cancer Immunotherapy Agents through Targeting IDO1. *Eur. J. Med. Chem.* **2018**, *143*, 1858−1868.
(31) Wang, L. L.; Battini, N.; Bheemanaboina, R. R. Y.; Zhang, S. L.; Zhou, C. H. Design and Synthesis of Aminothiazolyl Norfloxacin Analogues as Potential Antimicrobial Agents and Their Biological Evaluation. *Eur. J. Med. Chem.* **2019**, *167*, 105−123.
(32) Xie, Y. P.; Ansari, M. F.; Zhang, S. L.; Zhou, C. H. Novel Carbazole-Oxadiazoles as Potential *Staphylococcus aureus* Germicides. *Pestic. Biochem. Physiol.* **2021**, *175*, No. 104849.
(33) Li, Z.-Z.; Tangadanchu, V. K. R.; Battini, N.; Bheemanaboina, R. R. Y.; Zang, Z. L.; Zhang, S. L.; Zhou, C. H. Indole-Nitroimidazole Conjugates as Efficient Manipulators to Decrease the Genes Expression of Methicillin-Resistant *Staphylococcus aureus*. *Eur. J. Med. Chem.* **2019**, *179*, 723−735.
(34) Farha, A. K.; Yang, Q. Q.; Kim, G.; Zhang, D.; Mavumengwana, V.; Habimana, O.; Li, H. B.; Corke, H.; Gan, R. Y. Inhibition of Multidrug-Resistant Foodborne *Staphylococcus aureus* Biofilms by A Natural Terpenoid (+)-Nootkatone and Related Molecular Mechanism. *Food Control* **2020**, *112*, No. 107154.
(35) Zhang, J.; Chen, C.; Chen, J.; Zhou, S.; Zhao, Y.; Xu, M.; Xu, H. Dual Mode of Anti-Biofilm Action of G3 against *Streptococcus mutans*. *ACS Appl. Mater. Interfaces* **2020**, *12*, 27866−27875.
(36) Chandankere, R.; Ravikumar, Y.; Zabed, H. M.; Sabapathy, P. C.; Yun, J. H.; Zhang, G. Y.; Qi, X. H. Conversion of Agroindustrial Wastes to Rhamnolipid by *Enterobacter* sp. UJS-RC and Its Role against Biofilm-Forming Foodborne Pathogens. *J. Agric. Food Chem.* **2020**, *68*, 15478−15489.
(37) Li, H.; Liu, J. Y.; Liu, C. F.; Li, H. Z.; Luo, J. C.; Fang, S. F.; Chen, Y. Z.; Zhong, R. C.; Liu, S. P.; Lin S, M. Design, Synthesis, and Biological Evaluation of Membrane-Active Bakuchiol Derivatives as

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840

Effective Broad-Spectrum Antibacterial Agents. *J. Med. Chem.* **2021**, *64*, 5603−5619.
(38) Kim, W.; Zou, G.; Hari, T. P. A.; Wilt, I. K.; Zhu, W.; Galle, N.; Faizi, H. A.; Hendricks, G. L.; Tori, K.; Pan, W.; Huang, X.; Steele, A. D.; Csatary, E. E.; Dekarske, M. M.; Rosen, J. L.; Ribeiro, N. dQ.; Lee, K.; Port, J.; Fuchs, B. B.; Vlahovska, P. M.; Wuest, W. M.; Gao, H.; Ausubel, F. M.; Mylonakis, E. A Selective Membrane-Targeting Repurposed Antibiotic with Activity against Persistent Methicillin-Resistant *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 16529.
(39) Tang, J.; Tang, G.; Niu, J. F.; Yang, J. L.; Zhou, Z. Y.; Gao, Y. B.; Chen, X.; Tian, Y. Y.; Li, Y.; Li, J. Q.; Cao, Y. S. Preparation of A Porphyrin Metal−Organic Framework with Desirable Photodynamic Antimicrobial Activity for Sustainable Plant Disease Management. *J. Agric. Food Chem.* **2021**, *69*, 2382−2391.
(40) Zhang, Y.; Tangadanchu, V. K. R.; Bheemanaboina, R. R. Y.; Cheng, Y.; Zhou, C. H. Novel Carbazole-Triazole Conjugates as DNA-Targeting Membrane Active Potentiators against Clinical Isolated Fungi. *Eur. J. Med. Chem.* **2018**, *155*, 579−589.
(41) Liu, Y.; Zhu, K. X.; Cao, L.; Xie, Z. F.; Gu, M.; Lü, W.; Li, J. Y.; Nan, F. J. Berberine Derivatives with A Long Alkyl Chain Branched by Hydroxyl Group and Methoxycarbonyl Group at 9-Position Show Improved Anti-Proliferation Activity and Membrane Permeability in A549 cells. *Acta Pharmacol. Sin.* **2020**, *41*, 813−824.
(42) Wang, M.; Gao, R. X.; Zheng, M. M.; Sang, P.; Li, C. P.; Zhang, E.; Li, Q.; Cai, J. F. Development of Bis-cyclic Imidazolidine-4-one Derivatives as Potent Antibacterial Agents. *J. Med. Chem.* **2020**, *63*, 15591−15602.
(43) Cui, S.-F.; Addla, D.; Zhou, C. H. Novel 2-Amino-thiazolquinolones: Design, Synthesis, Bioactive Evaluation, SARs, and Preliminary Antibacterial Mechanism. *J. Med. Chem.* **2016**, *59*, 4488−4510.
(44) Kaur, J.; Cao, X.; Abutaleb, N. S.; Elkashif, A.; Graboski, A. L.; Krabill, A. D.; AbdelKhalek, A. H.; An, W.; Bhardwaj, A.; Seleem, M. N.; Flaherty, D. P. Optimization of Acetazolamide-Based Scaffold as Potent Inhibitors of Vancomycin-Resistant *Enterococcus*. *J. Med. Chem.* **2020**, *63*, 9540−9562.
(45) Sui, Y.-F.; Ansari, M. F.; Zhang, S. L.; Zhou, C. H. Discovery of Novel Purinylthiazolylethanone Derivatives as Anti-*Candida albicans* Agents through Possible Multifaceted Mechanisms. *Eur. J. Med. Chem.* **2021**, *221*, No. 113557.
(46) Chen, J.-P.; Battini, N.; Ansari, M. F.; Zhou, C. H. Membrane Active 7-Thiazoxime Quinolones as Novel DNA Binding Agents to Decrease the Genes Expression and Exert Potent Anti-Methicillin-Resistant *Staphylococcus aureus* Activity. *Eur. J. Med. Chem.* **2021**, *217*, No. 113340.
(47) Basarab, G. S.; Doig, P.; Eyermann, C. J.; Galullo, V.; Kern, G.; Kimzey, A.; Kutschke, A.; Morningstar, M.; Schuck, V.; Vishwanathan, K.; Zhou, F.; Gowravaram, M.; Hauck, S. Antibacterial Spiropyrimidinetriones with *N*-Linked Azole Substituents on a Benzisoxazole Scaffold Targeting DNA Gyrase. *J. Med. Chem.* **2020**, *63*, 11882−11901.
(48) Borisov, V. B.; Siletsky, S. A.; Nastasi, M. R.; Forte, E. ROS Defense Systems and Terminal Oxidases in Bacteria. *Antioxidants* **2021**, *10*, 839.

https://doi.org/10.1021/acs.jafc.1c02545
J. Agric. Food Chem. 2021, 69, 7831−7840