Bromomethylthioindole Inspired Carbazole Hybrids as Promising Class of Anti-MRSA Agents

Chia-Yi Cheng,[†] Chun-Ping Chang,[†] Tsai-Ling Yang,[‡] Lauderdale,[‡] Guann-Yi Yu,[‡] Jinq-Chyi Lee,[†] Yi-Wun Jhang,[†] Chien-Huang Wu,[†] Yi-Yu Ke,[†] Amit A. Sadani,[†] Ching-Fang Yeh,[†] I-Wen Huang,[‡] Yi-Ping Kuo,[‡] De-Jiun Tsai,[‡] Teng-Kuang Yeh,[†] Chen-Tso Tseng,[†] Jen-Shin Song,[†] Yu-Wei Liu,[†] Lun K. Tsou,^{*,†} and Kak-Shan Shia^{*,†}

[†]Institute of Biotechnology and Pharmaceutical Research and [‡]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County 35053, Taiwan, R.O.C.

S Supporting Information

ABSTRACT: Series of *N*-substituted carbazole analogues bearing an indole ring were synthesized as anti-methicillin-resistant *Staphylococcus aureus* (MRSA) agents from a molecular hybridization approach. The representative compound **19** showed an MIC = 1 $\mu\text{g/mL}$ against a panel of MRSA clinical isolates as it possessed comparable *in vitro* activities to that of vancomycin. Moreover, compound **19** also exhibited MIC = 1 $\mu\text{g/mL}$ activities against a recent identified Z172 MRSA strain (vancomycin-intermediate and daptomycin-nonsusceptible phenotype) and the vancomycin-resistant *Enterococcus faecalis* (VRE) strain. In a mouse model with lethal infection of MRSA (4N216), a 75% survival rate was observed after a single dose of compound **19** was intravenously administered at 20 mg/kg. In light of their equipotent activities against different MRSA isolates and VRE strain, the data underscore the importance of designed hybrid series for the development of new *N*-substituted carbazoles as potential anti-MRSA agents.

KEYWORDS: Methicillin-resistant *Staphylococcus aureus*, vancomycin, carbazole derivatives, molecular hybridization, resistant bacteria

The great discovery and deployment of antibiotics had succumbed to the steady emergence of multidrug-resistant bacterial strains and new microbial pathogens, which placed tremendous pressure on the race to develop novel antimicrobial agents.^{1–5} *Staphylococcus aureus* is the leading Gram-positive pathogen in clinical setting responsible for a wide spectrum of diseases ranging from mild skin infections to severe life threatening pneumonia and bacteremia.^{6–10} Methicillin was introduced in early 1960s but methicillin-resistant *S. aureus* (MRSA) strains emerged soon after. The prevalence of MRSA increased steadily starting in the 1980s, and MRSA has become resistant to many marketed antibiotics due to the extensive selective pressure from utility of these drugs in the hospital setting.^{2,6} Decreased efforts in the development of other classes of antibiotics further complicated the limited treatment options.^{2,3,7}

MRSA arises when methicillin-susceptible *S. aureus* (MSSA) acquires a large mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) through horizontal gene transfer.^{11,12} Incorporation of the SCC*mec* leads to the expression of an altered penicillin-binding protein PBP2' (PBP2a), which has >100-fold lower binding affinity for nearly all available β -lactam antibiotics, rendering them ineffective

against MRSA.¹ Glycopeptide-based antibiotics, especially vancomycin, have been the main drug of choice for treating severe MRSA infections.^{13,14} However, a decrease in efficacy of vancomycin has attracted great attention due to the emergence of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) that was associated with increasing treatment failures.¹⁵ In addition, reports of vancomycin-resistant *S. aureus* (VRSA) are alarming due to the presence of transferable vancomycin resistance plasmids among the infection strains.¹⁶ In the clinical setting, coinfection of MRSA and vancomycin-resistant enterococci (VRE) after surgical procedures has gradually but surely reduced the effectiveness of glycopeptide-based antibiotics over the past decade, leading to yet another challenge in the fight against bacterial infection.^{17,18} Moreover, the increasing prevalence of MRSA in hospitals and emergence of community-associated MRSA (CA-MRSA) strains have become a leading cause of serious complications in patient treatments.^{19–21} It has been noted that the over millions of hospitalizations associated with

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these infections yearly would place a tremendous economic burden on many healthcare systems worldwide.^{22,23}

With the shift in MRSA susceptibility and an increase in CA-MRSA infections,^{19–21,24} there is an urgent medical need for the development of new antimicrobial agents.¹⁴ To bolster the dwindling antibiotic arsenal, our research group has conducted an in-house screening program, leading to the identification of a novel hit (**1**) possessing a *N*-substituted bromoindole scaffold with a moderate anti-MRSA (ATCC 43300) activity at MIC = 8 $\mu\text{g/mL}$ (Figure 1, Table S1).

Figure 1. Strategy to generate novel antibiotic agents based on molecular hybridization.

Interestingly, a series of marine natural products known as bromomethylthioindoles were isolated from red algae *Laurencia brongniartii* by Y. Kamei et al. and identified to possess potent anti-MRSA activities (Figure 1).²⁵ The regioselective synthesis of these alkylthio- and arylthioindoles was recently reported by Suzuki et al.²⁶ Meanwhile, it was noted that naturally occurring carbazoles (Figure 1) possessed moderate antibacterial activities against both Gram-positive and Gram-negative bacteria.^{27–29} In particular, numerous series of *N*-substituted carbazole derivatives were synthesized and have been reported to exhibit diverse biological activities, such as antimicrobial, antitumor, antioxidant, and anti-inflammatory properties.^{30–32} Moreover, carbazole-containing drug, carvedilol, was approved for the treatment of mild to severe congestive heart failure and of high blood pressure.³³ Yet, reported *N*-substituted carbazoles or different carbazole cores only exhibited moderate activities against MSSA or MRSA with the MIC ranging from 4 to 16 $\mu\text{g/mL}$.^{29,34} In view of the above-reported bioactivities, it was foreseen to amalgamate the two anti-MRSA pharmacophores in one molecular unit to generate a new scaffold for anti-MRSA evaluation. In particular, we envisioned that *N*-alkylation of carbazole with halogenated indole analogues would provide a platform for generation of anti-MRSA derivatives. We have designed a facile synthetic route to generate a series of hybrids, several of which have been shown potent activities against MSSA, MRSA, and VRE. Herein, we wish to disclose our findings, including (1) a simple and efficient synthetic route to obtain potent *N*-substituted carbazole derivatives based on molecular hybridization; (2) the structure–activity relationship analysis; (3) a broad spectrum of antimicrobial activities against clinical MRSA and VRSA isolates, and (4) an *in vivo* study of compound **19** in the MRSA (4N216) systemic infection mouse model.

To validate whether the in-house hit is a false positive, hit compound **1** and its analogue **2** were resynthesized according to a two-step synthetic sequence in Scheme 1, involving *N*-substitution with epibromohydrin under basic conditions (78%)

followed by coupling with an aryl amine under refluxing ethanol (80%).

Scheme 1. Synthesis of *N*-Substituted Indole Derivatives **1** and **2**^a

^aReagents and conditions: (a) KOH, DMF, 0 °C, 30 min then epibromohydrin, rt, 16 h, 78%; (b) aryl amine, EtOH, reflux, 80%.

In general, carbazole-based derivatives with a β -hydroxy amine moiety were readily prepared through a two-step synthetic sequence as shown in Scheme 2. Accordingly, carbazole-based

Scheme 2. Synthesis of *N*-Substituted Carbazole Derivatives **3–24** with a β -Hydroxyl Amine^a

^aReagents and conditions: (a) KOH, DMF, 0 °C, 30 min then epibromohydrin, rt, 16 h, 78–81%; (b) aryl amines, EtOH, reflux, 68–75%.

derivatives **3–24**, containing dichloro-, dibromo-, and monobromo substituents at C-3 and C-6 positions of the carbazole ring, were first deprotonated with potassium hydroxide (1.4 equiv), and the resulting anion was allowed to react with epibromohydrin (2.5 equiv) to provide epoxide intermediates **B–E** in 78–81% yields. These intermediates were further coupled with various aryl amines to furnish the corresponding products **3–24**, respectively, in 68–75% yields.

For comparison purposes, compounds **25** and **26** without containing a β -hydroxyl amine group were also synthesized according to Scheme 3. 9*H*-Carbazole substrate, containing dichloro substituents at C-3 and C-6 positions, was first treated

Scheme 3. Synthesis of *N*-Substituted Carbazole Derivatives **25–26** without a β -Hydroxy Amine^a

^aReagents and conditions: (a) KOH, 2-(3-bromopropoxy)-tetrahydro-2*H*-pyran, DMF, 0 °C to rt, 12 h; (b) cat. PTSA, MeOH, rt, 12 h; (c) Dess–Martin periodinane (DMP), CH_2Cl_2 , 0 °C to rt, 1 h, 72% over three steps; (d) indole amines, MeOH/THF (1:1), 0 °C to rt, 1 h then NaBH_4 , 0 °C to rt, 12 h, 80–82%.

Table 1. Antibacterial Activity of Compounds 11, 12, 19, 22, and 24 against Clinical MSSA and MRSA Strains

compd	minimum inhibitory concentration ($\mu\text{g/mL}$)							
	MSSA ^a		MRSA ^b					
	M056	M013	N043	C217	Y069	L166	Z234	4N216
11	1–2	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	0.5
22	1	1	1	1	1	1	1	1
24	1	4	4	4	4	4	4	0.5–1
vancomycin	2	≤ 0.5	1	1	1	2	1	1

^aClinical MSSA strain M056. ^bClinical MRSA strains M013, N043, C217, Y069, L166, Z234, and 4N216.

with potassium hydroxide and 2-(3-bromopropoxy)-tetrahydro-2H-pyran followed by acidic hydrolysis and Dess–Martin periodinane oxidation, giving rise to aldehyde **F** in 72% yields over three steps. Intermediate **F** was further reacted with 2-(6-chloro-1H-indol-3-yl)ethan-1-amine or 2-(6-fluoro-1H-indol-3-yl)ethan-1-amine followed by reduction with NaBH_4 to afford compounds **25** and **26**, respectively, in 80 and 82% yields through a reductive amidation process.

However, to investigate whether stereoisomers arising at the β -hydroxylamine position within these derivatives affect the anti-MRSA activities, we further synthesized enantiomeric pairs (+)-**13** and (–)-**14** as well as (+)-**20** and (–)-**21** to compare the MIC values with their respective racemic counterparts ($\pm$)-**12** and ($\pm$)-**19**. The synthesis was achieved by using commercially available (–)-glycidol ($[\alpha]_{\text{D}}^{20} -15$; neat) and (+)-glycidol ($[\alpha]_{\text{D}}^{20} +15$; neat) as enantioenriched source and through a three-step synthetic sequence reported in the literature,³⁵ including tosylation of the hydroxyl group and 9H-carbazole-*N* alkylation, followed by opening the epoxide ring with an indole amine.

Derivatives hybridized with carbazole and indole units were first evaluated for bacterial growth inhibitions using standard techniques against a methicillin-susceptible *S. aureus* (MSSA) strain ATCC 29213 and a methicillin-resistant *S. aureus* (MRSA) strain ATCC 43300 (Table S1 in Supporting Information). From initial screening of our in-house compounds, the discovery of *N*-substituted carbazoles with anti-MRSA activities had prompted us to synthesize a series of compounds (Schemes 1–3) and investigate their structure–activity relationships as listed in Table S1. Hit **1**, an upper *N*-substituted bromoindole connecting to a lower 2-(3-fluoro-phenyl)ethylamine fragment with a 2-hydroxypropyl linker, was resynthesized and exhibited both anti-MSSA and anti-MRSA activities at a moderate concentration of MIC = 16–32 and 8 $\mu\text{g/mL}$, respectively. Along with hit **1**, the corresponding 3-halogenated **2** was also prepared and showed an improvement in activity against both MSSA and MRSA by 2- to 8-fold, respectively. These results had prompted us to further modify these hits to obtain a novel series of *N*-substituted carbazole derivatives incorporating an indole functionality. Concurrently, structurally related *N*-substituted carbazole derivatives, referred to as DCAP analogues, were reported to be antibacterial against a panel of clinically isolated BSL-2 pathogenic strains.³⁶ Inspired by the structural similarity, we then symmetrized the upper *N*-substituted bromoindole to the *N*-substituted dibromo-carbazole ring to afford compound **3**,

resulting in a remarkable 8-fold increase in both anti-MSSA and anti-MRSA activities relative to hit **1**. Encouraged by these findings, we started to systematically modify the peripheral functional groups of the carbazole ring with particular emphasis on the NH-substitution fixed with a 2-hydroxypropyl linker. Carbazole derivatives **4–24** (Table S1) with structural modifications extending from the tail of the 2-hydroxypropyl linker were then synthesized according to Schemes 1–3. As illustrated with compounds **3** and **4**, substitution of a hydroxyl or halogen group on the terminal phenyl ring was first examined. As a result, activities (MIC = 1–2 $\mu\text{g/mL}$) against tested ATCC 29213 and 43300 strains were retained. However, increasing the hydrophilicity met with little success as seen in compound **5** with a dramatic decrease in anti-MSSA and anti-MRSA activity by 8-fold (MIC = 16 $\mu\text{g/mL}$). Replacing the phenyl unit with an imidazole ring at the tail portion gave rise to compound **6** showing less activity (MIC = 4 $\mu\text{g/mL}$) against tested strains. We then prepared compounds **7–11** to address the importance of the size and length of the lower projected functionalities. With the triazole group lacking the carbon extension from the 2-hydroxypropyl moiety in compound **7**, both anti-MSSA and anti-MRSA activities were significantly reduced to MIC = 16 $\mu\text{g/mL}$. Despite incorporating a larger functional group at the terminal portion as indicated in compounds **8** and **9** (MIC > 32 $\mu\text{g/mL}$), both anti-MSSA and anti-MRSA activities could not be restored. This observation appeared to reveal that flexibility and the extension of the lower accessory groups could influence the anti-MSSA and anti-MRSA activities as seen with compound **10** (MIC = 2 $\mu\text{g/mL}$). To test this hypothesis, we readily introduced different aliphatic chain length at the lower portion of the indole ring. The choice of the indole unit was inspired by compound **10** and the bromomethylthioindoles isolated from red algae that possessed potent anti-MRSA activities.^{25,26} Consistent with the regiochemistry of the isolated bromomethylthioindoles, we connected the carbazole moiety via the C-3 position on the indole ring while maintaining a halogen group on the phenyl ring. Compound **11** thus obtained had a substantial (>16 fold) improvement in both anti-MSSA and anti-MRSA activities as compared to compounds **8** and **9**, suggesting that the flexibility of the functional group extended from 2-hydroxypropyl linker might allow for a favorable conformation fitting into the target protein. However, there was no substantial difference in activities between the methylene ($-\text{CH}_2-$) and ethylene ($-\text{CH}_2-\text{CH}_2-$) carbon extensions as seen with compounds **10** (MIC = 2 $\mu\text{g/mL}$) and **11** (MIC = 1 $\mu\text{g/mL}$). When comparing compound **12** with

Table 2. Antibacterial Activity of Compounds 11, 12, 19, 22, and 24 against Z172, VRE and Several Gram Negative Bacteria

compd	minimum inhibitory concentration (μg/mL)					
	Z172 ^a	ATCC 51299 (VRE)	ATCC 25922 (<i>E. coli</i>)	ATCC 27853 (<i>P. aeruginosa</i>)	ATCC 700603 (<i>K. pneumoniae</i>)	ATCC 19606 (<i>A. baumannii</i>)
11	2	2	8–16	>64	>64	16
12	2	1	>64	>64	>64	>64
19	1	1	4	>64	>64	8
22	1	2	>64	>64	>64	>64
24	4	2	>64	>64	>64	>64
vancomycin	4	8–16	N.A.	N.A.	N.A.	N.A.

^aZ172 is a hospital-associated MRSA strain in Taiwan that exhibits vancomycin-intermediate and daptomycin-nonsusceptible phenotypes.

Table 3. Pharmacokinetic Studies of Compound 19 and Vancomycin in Male ICR Mice^a

compd	route	dose (mg/kg)	C_{max} (ng/mL)	$T_{1/2}$ (h)	CL (mL/min/kg)	V_{ss} (L/kg)	AUC _(0–24h) (ng/mL·h)
19	i.v.	10	29667	10.3	6.9	4.9	20546
vancomycin	i.v.	10	58500	0.6	11.7	0.3	13715

^aValues indicate mean ($n = 3$, per time point). Abbreviations: half-life ($T_{1/2}$); total body clearance (CL); volume of distribution at steady-state (V_{ss}); area under the curve (AUC). C_{max} was taken at $t = 20$ min.

11, varying the halogen substituent and its position on the indole ring had limited effects on their activities against tested strains. Compound 15 containing a weak electron-donating group on the indole ring accounted for a marginal 2-fold decrease in potency. More importantly, racemic 12 and its enantiomers (S)-13 and (R)-14 showed equipotent activities against tested strains at a MIC value of 1 $\mu\text{g/mL}$, suggesting that inhibitory activities are little influenced by the stereochemistry of the secondary hydroxyl group in the linker. A similar phenomenon was observed for racemic 19 and its enantiomers (S)-20 and (R)-21 with MIC values around 0.5–1 $\mu\text{g/mL}$. These results imply that antimicrobial activities of this series of compounds might arise from binding to bacterial membranes rather than inhibiting an enzyme or protein. To examine the significance of the halogen atom around the carbazole ring, we found that the dibromo decoration was advantageous. As compared to compound 16 (MIC = 4 $\mu\text{g/mL}$) without any halogen substitution, compound 11 (MIC = 1 $\mu\text{g/mL}$) bearing a bromo atom at C-3 and C-6 positions exhibited a 4-fold increase in potency, suggesting that an electron-withdrawing group(s) on the carbazole ring could be a structural requirement for showing anti-MRSA and anti-MSSA activity. Indeed, irrespective of the halogen substituent on the indole ring, compounds 17 and 18, merely containing a bromo atom on the carbazole ring, possessed anti-MRSA activities as potent as its dibromo counterpart 11 (MIC = 1 $\mu\text{g/mL}$). Further structural modifications were carried out to remove the hydroxyl moiety of the linker. As a result, the inhibitory activities of compounds 25 and 26 were retained as compared to their hydroxyl analogue 19 (MIC = 1 $\mu\text{g/mL}$), verifying that the hydroxyl group was structurally redundant.

The antibacterial spectra of potent carbazole derivatives, such as compounds 11, 12, 19, 22, and 24, were further evaluated against a panel of clinical isolates of MSSA and MRSA, results of which were compiled in Table 1. M056 is a MSSA clinical isolate and the above five compounds have exhibited comparable potent antibacterial activities to that of vancomycin. Moreover, these compounds also possessed a broad spectrum of inhibitory activities against multiple clinically isolated MRSA strains, including M013, N043, C217, Y069, L166, Z234, and 4N216, at MIC values as low as 0.5–1 $\mu\text{g/mL}$. In contrast to the indole-containing N-substituted carbazoles (11, 12, 19), compound 24 showed a higher MIC value ranging from 1 to 4 $\mu\text{g/mL}$ against

majority of the clinical MRSA strains tested. Structural comparison between compounds 19 and 24 suggests that the indole moiety, with the corresponding linker length, plays a vital role in the growth inhibition of the clinical MRSA strains. Interestingly, shortening of the linker length in compound 22 has restored the potent anti-MRSA activities. Taken together, these data strongly supported the structural hybridization with enhanced anti-MRSA activities and provided a new lead structural motif.

Recently, the emergence of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) has been reported from healthcare system around the world. VISA was associated with increasing treatment failures.¹⁵ Alternatively, daptomycin has been employed for the management of serious infections caused by MRSA with reduced susceptibility to vancomycin, such as VISA.³⁷ In addition, MRSA and VRE have emerged as the two most common Gram-positive multidrug-resistant pathogens associated with many health care facilities. Z172, a hospital-associated MRSA strain in Taiwan, exhibited a vancomycin-intermediate and daptomycin-nonsusceptible phenotype.³⁸ Even though the occurrence of MRSA strains with reduced susceptibility to vancomycin and daptomycin along with VRE strains resistant to daptomycin are still not prevalent, their rising appearances in the hospital settings have necessitated the development of new class of anti-MRSA agents in combating these bacteria. In particular, compounds 19 exhibited potent inhibitory activities (MIC = 1 $\mu\text{g/mL}$) against Z172 and VRE (Table 2). Indeed, compound 19 showed an increase of 4–16-fold in the inhibitory activities in comparison to those of vancomycin (Table 2). Of note, the carbazole-based compounds 12 and 19 are about 4–18 times more potent than the recently reported coumarin-based inhibitors against VRE (ATCC 51299).³⁹ To assess the antibacterial spectrum across other genera, several Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, were further selected for evaluation. Nevertheless, we observed a significant loss of activities against various Gram-negative bacterial strains as mentioned above. Thus, the carbazole-based series are tentatively designated as Gram-positive-specific inhibitors. Interestingly, comparing compound 11 to 12, substitution of halogens on the indole ring provided a 4-fold increase in inhibitory activities against *E. coli* and *A.*

baumannii. These results suggested that carbazole-based inhibitors might hit a different target(s) compared to vancomycin as compounds **12** and **19** exerted potent activities against both VRE (ATCC 51299) and a vancomycin-intermediate and daptomycin-nonsusceptible MRSA strain (Z172).

Of the synthesized carbazole-based inhibitors, compound **19** has a broader spectrum of antimicrobial activities particularly for resistant strains VRE (ATCC 51299) and MRSA (Z172); thus, its pharmacokinetic studies were conducted prior to *in vivo* efficacy studies. As compiled in Table 3, compound **19** (10 mg/kg, iv) displays a longer half-life ($t_{1/2}$ = 10.3 vs 0.6 h) and larger blood exposure (AUC = 20546 vs 13715 ng/mL) than vancomycin (10 mg/kg, iv), which served as a positive control in animal studies.

To evaluate the *in vivo* tolerability of the carbazole series, compounds **11** or **19** were intravenously administered at a dose of 100 mg/kg into ICR mice. Body weight was monitored for 2 weeks after the injection, and survival with increased body weight was observed in all animals with the treatment (Figure S1 in Supporting Information). These results, together with the pharmacokinetic properties provide strong evidence that these novel anti-MRSA carbazole series are well-tolerated *in vivo*. Encouraged by these promising data, compound **19** was then subjected to a MRSA (4N216) mouse systemic infection model. Mice were infected via intraperitoneal injection (ip) at 3×10^6 CFU/0.2 mL of MRSA strain 4N216 per mouse. Survival rate was calculated at 7 days postinfection. As depicted in Figure 2,

Figure 2. Survival rate of **19** (20 mg/kg, iv) in a MRSA (methicillin-resistant *Staphylococcus aureus*) mouse systemic infection model. Five-week-old ICR female mice were infected via intraperitoneal injection (ip) at 3×10^6 CFU/0.2 mL of MRSA strain 4N216 per mouse ($n = 8$ mice/group). Survival rate was calculated at 7 days postinfection. Arrow denotes time of dosing at 30 min postinfection.

following a single intravenous injection at a dose of 20 mg/kg (five times less than the amount used in the *in vivo* toxicity experiment) at 30 min postinfection, compound **19** could neutralize MRSA (4N216) infection in an effective manner, resulting in a 75% survival rate compared to vancomycin (100% survival rate) as a reference under the same treatment conditions. These results indicate that the newly developed hybrid series might have great potential to become a new generation of antimicrobial agents by further structural modifications especially using **19** as a template.

We have designed and synthesized a series of novel anti-MRSA agents through a molecular hybridization approach by combining a carbazole scaffold with a halogenated indole moiety. Among them, the representative compound **19** not only exhibited potent *in vitro* activities against various MSSA and MRSA clinical isolates, and VRE (ATCC 51299) strain with a MIC value as low as 0.5–1 μ g/mL, but also maintained a 75% survival rate in a

MRSA (4N216) mouse systemic infection model at a dose of 20 mg/kg following intravenous administration. Originally inspired by marine natural products possessing similar *in vitro* anti-MRSA activities to the marketed vancomycin, current findings provide a good starting point for further lead optimization. Although the precise mechanism of the newly developed hybrids remains unclear, however, they might possess a novel mechanism of action different from any known classes of antibiotics in light of their equipotent activities (MIC = 1 μ g/mL) against a broad spectrum of tested MSSA, MRSA, and VRE strains.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.6b00289.

Synthetic procedures, spectral data, protocols of bioassay, and ^1H and ^{13}C NMR spectra of key compounds **19**, **25** and **26**; structure–activity relationships of compounds **1**–**26** against ATCC 29213 and ATCC 43300 strains; acute toxicity studies of compounds **11** and **19** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +886-37-246-166, ext. 35769. Fax: + 886-37-586-456. E-mail: kelvintsou@nhri.org.tw.

*Tel: +886-37-246-166, ext. 35709. Fax: + 886-37-586-456. E-mail: ksshia@nhri.org.tw.

Notes

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■ ABBREVIATIONS

MRSA, Methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MSSA, methicillin-susceptible *S. aureus*; MIC, minimal inhibitory concentration; SAR, structure–activity relationships; AUC, area under the curve

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