ABSTRACT: Easily accessible 3,3′-diindolylmethanes (DIMs) were utilized to generate a focused library of indolo[2,3-b]quinolines (2), chromeno[2,3-b]indoles (3), and 3-alkenyl-oxindoles (4) under 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)-mediated oxidative conditions. DIMs with ortho-NHTosyl (NHTs) phenyl group afforded indolo[2,3-b]quinolines (2), whereas DIMs with ortho-hydroxy phenyl groups yielded chromeno[2,3-b]indoles (3) and 3-alkenyl-oxindoles (4). The mild conditions and excellent yields of the products make this method a good choice to access a diverse library of bioactive molecules from a common starting material. Two optimized compounds 2a and 2n displayed excellent activity against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Compound 2a showed the minimum inhibitory concentration values in the concentration between 1 and 4 μg/mL, whereas compound 2n revealed the values of 1–2 μg/mL. Furthermore, both the compounds were highly bactericidal and capable to kill the MRSA completely within 360 min. Collectively, the results suggested that both compounds 2a and 2n possess enormous potential to be developed as anti-MRSA agents.

INTRODUCTION

3,3′-Diindolylmethane (DIM), renowned for its anticancer properties, is a metabolite product of indole-3-carbinol (I3C), which is a glucosinolate conjugate in various cruciferous vegetables. 1 Formation of DIM under the physiological conditions is considered as a prerequisite for the I3C-induced anticarcinogenesis. 2 Given its biological significance, several methodologies for the synthesis of DIMs have been reported. 3

DIMs were also utilized as substrates in synthetic transformations to access diverse molecular scaffolds that include synthesis of indolo[2,3-b]quinolines by a SnCl₂·H₂O-mediated reductive intramolecular C–N bond formation, 4 tetrahydroindolocarbazoles under acid-mediated conditions, 5 and bis-indole alkaloid analogues by palladium and gold catalysis. 6 In addition, DIMs were utilized as key intermediates in the synthesis of fluorene, 7 azeppinoindole, 8 and tetrahydrocarbazoles. 9 The simplicity in the generation of DIMs and also our continuous interest toward the development of DIM conjugates of pharmacological relevance 10 encouraged us to investigate its synthetic utility as a promising substrate for indole-rich diverse molecular motifs. A synthetic methodology that can build a diverse array of molecules from the readily available starting materials or easily assembled substrates is the need of the hour for generating a rapid library of compounds in the drug discovery endeavors. In the present study, we have explored DDQ-mediated oxidative transformations of 3,3′-diindolylphenylmethanes (DIPMs, 1) generated from N-Ts-2-amino-benzaldehyde and salicylaldehyde with various indoles. DDQ, which is readily available, cheap, and ecofriendly reagent, mediated various intramolecular cyclizations by means of C–C, 11 C–N, 12 C–O, 13 and C–S 14 bond formations. We have readily transformed DIPMs under DDQ-mediated oxidative conditions to indolo[2,3-b]quinolines 2 (Scheme 1), chromeno[2,3-b]indoles 3 (Scheme 2), and 3-alkenyl-oxindoles 4 (Scheme 3), which are found as the core structures in natural products and drug targets (Figure 1).

Indolo[2,3-b]quinoline is a core structure in perophoramide and communesin alkaloids. 15 6H-Indolo[2,3-b]quinoline...
and its methylated analogue (neocryptolepine) are natural products, known for their DNA intercalation and topoisomerase II inhibition properties. Recently, several neocryptolepine derivatives were synthesized and their antimicrobial and antiproliferative activities were explored. In general, most of the analogues were active against Gram-positive bacteria and inactive against Gram-negative bacteria. Similar antibacterial activity trend was observed for other reported indolo[2,3-
quinoline derivatives.\textsuperscript{18} Owing to their biological significance in medicinal chemistry research, several efficient methods were reported for the synthesis of indolo[2,3-\textit{b}]quinolines.\textsuperscript{16,19} The present method, however, is a highly efficient metal-free method under oxidative condition, which facilitates the synthesis of a library of indolo[2,3-\textit{b}]quinolines under mild conditions from the easily accessible DIPMs.

Chromeno[2,3-\textit{b}]indole is a core structure in hyrtimomine and goniomedine alkaloids.\textsuperscript{20} The current available methods for the synthesis of chromeno[2,3-\textit{b}]indole tetracycles include the intramolecular cyclizations of Knoevenagel adducts and 2-phenoxy-3-indolecarboxylates, further facilitated by POCl\textsubscript{3}.\textsuperscript{21} Recently, palladium-catalyzed conditions were employed in a cascade reaction between 2-bromoindole and salicylaldehyde.\textsuperscript{22} Owing to their limited synthetic approaches, the biological evaluation of these molecules is limited to in vitro antiproliferative activity.\textsuperscript{21a} Our present method can afford chromeno[2,3-\textit{b}]indole under mild conditions with a high yield.

The focused library of these molecules was evaluated for their selective anti-methicillin-resistant \textit{Staphylococcus aureus} (anti-MRSA) activity by screening against a range of Gram-positive and Gram-negative bacteria. The zone of inhibition assay suggested that indolo[2,3-\textit{b}]quinolines 2\textit{a} and 2\textit{n} were highly active against \textit{S. aureus}, which causes a wide range of clinical infections. We have evaluated the anti-MRSA efficacy of these two optimized compounds by performing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) experiments. We also performed the time-kill kinetics to understand the rate at which compounds 2\textit{a} and 2\textit{n} are capable to kill MRSA.
Scheme 3. Scope of DDQ-Induced Oxidation of DIPM 1C to 3-Alkenyl-oxindoles 4

**RESULTS AND DISCUSSIONS**

**Synthesis.** Our initial attempt of treating DIPM 1A, prepared by the facile condensation of ortho-NHTs benzaldehyde and N-methylindole, with DDQ (1.5 equiv) using DCM as the solvent afforded the expected indolo[2,3-b]quinoline 2b (Scheme 1) by the C2–N bond formation, but complete conversion of the starting material was not observed. To our delight, an increase in DDQ to 3 equiv afforded the desired product 2b in 100% yield (quantitative) either by using DCM or DMF solvents (entries 1 and 2, Table 1). This reaction was unsuccessful in water, whereas a trace amount of 2b was observed even in the absence of DDQ at 110 °C (entries 3 and 4, Table 1). The product formation was observed in the presence of other oxidants, namely, phenyliodine diacetate (PIDA) and ceric ammonium nitrate (CAN) (entries 5–7, Table 1), albeit less yielding than DDQ.

We extended the scope of the present method using DIPM 1A with differently substituted 1H-indoles and N-methylindoles (Scheme 1). DIPMs synthesized using 1H-indole and N-phenylindole underwent facile transformation to the corresponding indolo[2,3-b]quinolines 2a and 2c in 89 and 92% yields, respectively. The DIPMs with varied halogen and electron withdrawing group (EWG)- or electron donating group (EDG)-substituted indoles underwent DDQ-mediated...
transformation to furnish the respective products 2d–m and 2o–p in good to excellent yields. The DIPM with an OMe substitution in the phenyl ring afforded indolo[2,3-b]quinoline 2n in 90% yield. Most of the indolo[2,3-b]quinoline derivatives that were screened for antimicrobial activity possess a simple methyl substitution at the 11 position.17,18 In this context, the indolyl group at the 11 position appears as a spectator substituent in the formation of indolo[2,3-b]quinoline 2; however, its presence in conjugation with quinoline can bear far-stretched implications in the biological activity.

The aforementioned results encouraged us to further explore the utility of DIPM 1B with ortho-hydroxy substitution in the phenyl ring and 1H-indole under DDQ-mediated optimized condition using DMF as the solvent (entry 2, Table 1), as DIPM 1B is insoluble in DCM. As expected, we observed the formation of chromeno[2,3-b]indole 3a in 87% yield, confirmed by NMR and single-crystal X-ray analyses (Scheme 2).23 DIPM 1B with differently substituted 1H-indoles afforded the desired chromeno[2,3-b]indole 3b–h in excellent yields (Scheme 2). DIPM 1B with mono- and disubstituted phenyl groups also afforded the desired products 3f and 3g in 87 and 76% yields, respectively.

DIPM 1C generated from N-methylindole and salicylaldehyde under DDQ conditions, however, afforded an inseparable mixture of products. The protection of hydroxyl group of the resulting crude mixture with silyl protection (TBDMS) facilitated the separation of the mixture, over column chromatography, which exhibited same mass values as observed by the high-resolution mass spectrometry. Further, NMR and single-crystal X-ray analyses confirmed the formation of a geometrical mixture of 3-alkenyl-oxindoles 4 (Scheme 3).23 3-Alkenyl-oxindole is also a biologically relevant molecule present in pharmaceuticals.24 The present method of DDQ-mediated oxidative transformation of DIPMs to 3-alkenyl-oxindoles can serve as an alternative to metal-mediated intramolecular cyclization strategies.25 Various substituted DIPMs 1C were treated under DDQ-mediated oxidative conditions, which furnished 3-alkenyl-oxindole products 4a, 4a′ to 4e, 4e′ and 4f in good to excellent yields over two steps (Scheme 3). Based on the X-ray analysis of 4f and 4b′ to 4e, 4e′ and 4f are separately mentioned in the Supporting Information.

Antibacterial Activity. Infections associated with multi-drug-resistant bacteria are increasing at an alarming rate, and these are untreatable in some cases. To tackle this problem, chemical entities with new structures and new targets are urgently required.26 S. aureus-based infections are increasingly difficult to treat due to the emergence of methicillin-resistant S. aureus (MRSA), which is resistant toward many β-lactam antibiotics.27 Natural products with novel scaffolds are undergoing clinical evaluation for producing novel antibiotics.

| s. no. | oxidant (equiv) | solvent | 2b (%) |
|-------|----------------|---------|--------|
| 1     | DDQ (3.0)      | DCM     | 100    |
| 2     | DDQ (3.0)      | DMF     | 100    |
| 3     | DDQ (3.0)      | H2O     | n.r.   |
| 4     | DDQ (3.0)      | DMF     | trace  |
| 5     | PIDA (1.0)     | HFIP    | 30     |
| 6     | PIDA (2.0)     | HFIP/DCM (1:5) | 40 |
| 7     | CAN (3.0)      | DCM     | 80     |

All of the reactions were conducted using 0.08 mmol of DIPM 1A in 1.5 mL of undistilled solvents at room temperature. 1Isolated yields. Reaction temperature = 110 °C. PIDA = phenyliodine diacetate, CAN = ceric ammonium nitrate, HFIP = 1,1,1,3,3,3-hexafluoro-2-propanol, n.r. = no reaction.
in the drug discovery endeavors. Therefore, our aim is to identify such agents that will display selective anti-MRSA activity without showing much activity against other bacteria. Selective antibacterial agents will create lesser resistance developing pressure in the bacterial, thereby holding the antibacterial efficacy with sufficient longevity. To achieve this, we set our goal to screen all of the synthesized compounds, namely, indolo[2,3-b]quinolines (2) and chromeno[2,3-b]-indoles (3), whose core structures are present in natural products, along with 3-alkenyl-oxindoles (4), against an array of Gram-positive and Gram-negative bacteria. The preliminary antibacterial efficacy of compounds 2a--p, 3a--h and 4a--e', 4a'--e', and 4f were investigated by performing the zone of inhibition experiment against both Gram-positive and Gram-negative bacteria (Table 2). The results suggested that indolo[2,3-b]quinolines (2) and chromeno[2,3-b]-indoles (3) were not effective against the inhibition of Pseudomonas aeruginosa and Salmonella typhi and 3-alkenyl-oxindoles (4) were not inhibitory against any bacteria. Even though chromeno[2,3-b]indoles 3a--c and 3f exhibited a broad-spectrum antibacterial activity, indolo[2,3-b]quinolines 2a and 2n displayed more selectivity toward inhibiting S. aureus over other bacteria. The other compounds in this class, such as 2b--d, 2g, 2i, 2k, 2m (with N-methylindoles or N-phenylindoles), 2e, 2f, 2h, 2j, 2l, and 2o (derived from differently substituted 1H-indoles), showed no inhibitory activity against S. aureus. The mechanism of action of compounds 2a and 2n is yet to be explored; however, it was quite clear that the 6-OMe group present in compound 2n probably does not significantly affect binding to the target site, therefore displaying similar zone of inhibition as that of compound 2a. In contrast, the other compounds in this series with diverse functionalities probably prevent binding to its target, leading to an inactiveness toward S. aureus, except compound 2p, which showed a moderate zone of inhibition.

Table 2. Zone of Inhibition (Diameter in mm) Studies of Indolo[2,3-b]quinolines 2, Chromeno[2,3-b]indoles 3, and 3-Alkenyl-oxindoles 4

|       | Bacillus cereus | S. aureus | Staphylococcus epidermidis | Staphylococcus simulans | Escherichia coli | Klebsiella pneumoniae | Proteus mirabilis |
|-------|----------------|-----------|---------------------------|------------------------|-----------------|----------------------|------------------|
| 2a    | 0              | 18 ± 0    | 0                         | 6 ± 0                  | 0               | 5 ± 0                | 0                |
| 2b    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2c    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2d    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2e    | 0              | 0         | 9 ± 1                     | 9.66 ± 0.57            | 9.33 ± 0.57     | 10.33 ± 0.57         | 0                |
| 2f    | 0              | 0         | 0                         | 0                      | 0               | 17 ± 0               | 0                |
| 2g    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2h    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2i    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2j    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2k    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2l    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2m    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 2n    | 0              | 15 ± 0    | 0                         | 0                      | 0               | 0                    | 0                |
| 2o    | 9.66 ± 0.57    | 0         | 0                         | 0                      | 8 ± 1           | 0                    | 0                |
| 2p    | 0              | 7±1       | 7.66 ± 0.57               | 0                      | 0               | 0                    | 0                |
| 3a    | 0              | 15 ± 0    | 7 ± 0                     | 8 ± 0                  | 17 ± 0          | 11 ± 0               | 0                |
| 3b    | 8 ± 0          | 8 ± 0     | 6 ± 0                     | 10 ± 0                 | 7 ± 0           | 0                    | 17 ± 0           |
| 3c    | 0              | 10 ± 0    | 18 ± 0                    | 12 ± 0                 | 18 ± 0          | 7 ± 0                | 0                |
| 3d    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 3e    | 0              | 0         | 6 ± 0                     | 0                      | 7 ± 0           | 11 ± 0               | 0                |
| 3f    | 0              | 8 ± 0     | 15 ± 0                    | 9 ± 0                  | 18 ± 0          | 5 ± 0                | 0                |
| 3g    | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |
| 3h    | 0              | 10 ± 0    | 0                         | 0                      | 8 ± 0           | 0                    | 0                |
| 4a−f  | 0              | 0         | 0                         | 0                      | 0               | 0                    | 0                |

**Zone of inhibition was an average of three replications ± standard deviation.

Table 3. Antibacterial Activity of Compounds 2a and 2n

|       | MIC in μg/mL (μM) | MBC in μg/mL (μM) | methicillin | methicillin |
|-------|------------------|-------------------|-------------|-------------|
|       | 2a               | 2n                | 2a          | 2n          | 2a          | 2n            |
| S. aureus-MTCC737 | 1 (3) | 1 (2.8) | 1 (2.5) | 4 (12) | 2 (5.5) | 4 (10) |
| MRSA-ATCC35591   | 2 (6) | 2 (5.5) | >64 (>159) | 4 (12) | 4 (11) | >64 (>159) |
| MRSA-R3545      | 2 (6) | 1 (2.8) | 32 (80) | 4 (12) | 2 (5.5) | >64 (>159) |
| MRSA-R3889      | 2 (6) | 1 (2.8) | 32 (80) | 8 (24) | 4 (11) | >64 (>159) |
| MRSA-R3890      | 4 (12) | 1 (2.8) | >64 (>159) | 8 (24) | 8 (22) | >64 (>159) |
| E. coli-MTCC443  | >64 (>192) | >64 (>176) | ND* | >64 (>176) | >64 (>176) | ND |
| A. baumannii-MTCC1425 | >64 (>192) | >64 (>176) | ND* | >64 (>176) | >64 (>176) | ND |
| K. pneumoniae-ATCC700603 | >64 (>192) | >64 (>176) | ND* | >64 (>176) | >64 (>176) | ND |

*ND stands for not determined.
To investigate the anti-MRSA activity of the most effective compounds $2a$ and $2n$, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined against four different methicillin-resistant *S. aureus* strains (three of them were clinically isolated strains). One methicillin-susceptible *S. aureus* strain was also included in this study (Table 3). We have not included any compound from chromeno[2,3-\(b\)]indole 3 series, as they displayed better efficacy against the other bacteria over *S. aureus*, and herein the goal was to identify selective anti-MRSA agents. Additionally, various Gram-negative bacteria (such as *E. coli*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*) were also included to verify selective activity toward MRSA. To understand the efficacy of these new compounds as anti-MRSA agents, the activity was compared with the conventional antibiotic methicillin. The results suggested that both compounds $2a$ and $2n$ displayed excellent activity against both methicillin-susceptible and methicillin-resistant *S. aureus*. The antibiotic methicillin was active against the susceptible bacterial strain with an MIC value of 1 \(\mu\)g/mL, whereas it displayed very high values against all of the MRSA strains (concentration ranging from 32 to >64 \(\mu\)g/mL), indicating a high level of methicillin resistance in them. In comparison, compounds $2a$ and $2n$ displayed similar activity against both drug-susceptible and drug-resistant bacterial strains. Both compounds displayed the MIC value of 1 \(\mu\)g/mL against the methicillin-susceptible strain. For the MRSA strains, the MIC values varied in the concentration of 1–4 \(\mu\)g/mL depending on the bacterial strains. Compounds $2a$ and $2n$ displayed the MBC values in the concentration between 2 and 8 \(\mu\)g/mL, where methicillin displayed the value of >64 \(\mu\)g/mL against all of the MRSA tested. More importantly, both compounds were inactive against the Gram-negative bacteria and did not display any activity against them even at 64 \(\mu\)g/mL. Collectively, the results suggested that both compounds $2a$ and $2n$ are selective anti-MRSA agents that displayed excellent activity toward MRSA over other bacteria.

**Bactericidal Kinetics against MRSA.** Further, anti-MRSA efficacy of the compounds was investigated by performing the time-kill kinetics. To understand the rate at which compounds $2a$ and $2n$ are capable to kill MRSA, the experiment was performed at three different compound concentrations (4, 8, and 16 \(\mu\)g/mL). As shown in Figure 2, both compounds displayed bactericidal activity (>3 log reduction in cell viability). The rate of MRSA killing was highly dependent on the compound concentration. Although, at a lower concentration (4 \(\mu\)g/mL), both compounds displayed relatively slower kinetics; faster kinetics was observed at a higher concentration of 16 \(\mu\)g/mL. At this concentration, both compounds $2a$ and $2n$ were capable of killing MRSA completely within 360 min.

## CONCLUSIONS

In conclusion, we demonstrated the broad utility of DIPM substrates under DDQ-mediated oxidative conditions to produce biologically relevant molecules. DIPMs generated from *N*-methylindolyl or 1H-indole appended to ortho-NHTs phenyl group afforded indolo[2,3-\(b\)]quinolines 2 in the presence of DDQ. DIPMs with 1H-indole appended to ortho-hydroxy phenyl group afforded chromeno[2,3-\(b\)]indoles 3, whereas DIPMs with *N*-methylindolyl afforded 3-alkenylindoles 4 under the DDQ-mediated conditions. All of the reactions were conducted in an expeditious manner at room temperature under mild conditions, affording the products in excellent yields. The antibacterial screening through the zone of inhibition experiment resulted in two potent and selective anti-MRSA agents. Further detailed studies suggested that compounds $2a$ and $2n$ were highly bactericidal and displayed excellent MIC and MBC values at a lower concentration. Taken together, this study paves the way to identify the lead compounds that possess a high potential to be developed as anti-MRSA agents.

### EXPERIMENTAL SECTION

**General Information.** All of the chemicals and solvents were purchased as reagent grade and used without further purification and distillation. The reactions were monitored by TLC and the spots were visualized by short/long wavelength UV lamp. \(^1\)H and \(^13\)C NMR were recorded at 500 and 125 MHz, respectively, using CDCl\(_3\)/dimethyl sulfoxide (DMSO)-\(d_6\) as solvents and chemical shifts were given in parts per million. Flash column chromatography was performed using silica gel 100–200 mesh. High-resolution electrospray ionization mass spectrometry analysis was performed using an orbitrap analyzer and the ions are given in m/z.

**General Procedure for DDQ-Mediated Synthesis of Indolo[2,3-\(b\)]quinolines 2 and Chromeno[2,3-\(b\)]indoles 3.** To a solution of DIPM 1A/1B (0.1–0.5 mmol, 0.07 M) in DCM or DMF solvent was added DDQ (3 equiv) at room temperature. After the completion of the starting material, as indicated by TLC (<5 min), the reaction mixture was quenched with a saturated sodium bicarbonate/10 M NaOH solution and extracted in a DCM solvent (1 × 3). The combined organic layers were dried over anhydrous Na\(_2\)SO\(_4\) and the solvent was evaporated in vacuum. The crude mixture was purified by flash column chromatography using EtOAc/hexane (1:4) or DCM/hexane (1:1 to 1:0) solvent system to afford the desired products.

**General Procedure for the Synthesis of 3-Alkenylindoles 4.** DIPM 1C was treated with DDQ according to the above-mentioned DDQ-mediated oxidation procedure, and the resulting crude mixture was treated with imidazole (5 equiv) and TBDMSI (5 equiv) in DCM solvent. After the completion of the reaction, as indicated by TLC, the reaction mixture was quenched with saturated NaHCO\(_3\) and extracted in...
DCM (1 × 3). The combined DCM layers were dried over anhydrous Na₂SO₄, and the solvent was evaporated on a rotavap. The crude mixture was purified by flash column chromatography using EtOAc/hexane (1:4) solvent to afford the TBS-protected 3-alkenyl-oxindole products.

**Test Bacterial Pathogens.** Compounds 2a–p, 3a–h, and 4a–e, 4a′–e′, and 4f were screened for their antibacterial activity against four Gram-positive bacteria, namely, *B. cereus* MTCC 1305, *S. aureus* MTCC 902, *S. epidermidis* MTCC 435, and *S. simulans* MTCC 3610, and five Gram-negative bacteria, namely, *E. coli* MTCC 2622, *K. pneumoniae* MTCC 109, *P. mirabilis* MTCC 425, *P. aeruginosa* MTCC 2642, and *S. typhi* MTCC 3216. All of the bacterial pathogens were procured from Microbial Type Culture Collection and Gene Bank, CSIR-MTCC, Chandigarh, India, and maintained on nutrient slants. The MIC and MBC experiments of the optimized compounds 2a and 2n were performed against one methicillin-susceptible *S. aureus* MTCC737, four MRSA strains, *E. coli* MTCC443, *A. baumannii* MTCC1425, and *K. pneumoniae* ATCC700603. Methicillin-resistant *S. aureus* (MRSA) ATCC3591 and *K. pneumoniae* ATCC700603 were obtained from the American Type Culture Collection (ATCC). Clinically isolated MRSA strains, MRSA-R3545, MRSA-R3889, and MRSA-R3990, were obtained from the Department of Neuromicrobiology, National Institute of Mental Health and Neuro Sciences, Hosur Road, Bangalore 560029, India. All of the MTCC bacterial strains were purchased from MTCC (Chandigarh, India).

**Antibacterial Activity. Zone of Inhibition Assay.** The preliminary antibacterial screening was performed by the disk diffusion method against the test bacteria (CLSI, 2012). The test cultures maintained in the nutrient agar slants at 4 °C were subcultured in a nutrient broth to obtain the working cultures approximately containing 1 × 10⁸ cfu/mL. Mueller–Hinton (MH) agar plates were swabbed with each bacterial strain. Each compound (30 μg) in 30 μL of DMSO was incorporated in a sterile disk of 6 mm diameter and DMSO served as the control. Zone of inhibition was measured after 24 h of incubation at 37 °C.

**MIC Assay.** The experiment was performed by following the reported protocol.²⁹ The required working concentrations of the compounds were prepared in Millipore water from 10 mg/mL stock solution in DMSO; 100 μL of the compounds was then serially diluted by twofold in a 96-well plate in an autoclaved Millipore water. After that, the mid-log phase (6 h grown) bacterial culture (~10⁹ cfu/mL) was diluted to ~10⁸ cfu/mL in the nutrient broth. These bacterial suspensions (150 μL) were then added to the wells containing 50 μL of the compound solution. After that, the plate was incubated with shaking for 24 h at 37 °C. At the end, the optical density (OD) of the plates was measured at 600 nm using TECAN Plate Reader (Infinite series, M200 pro). The MIC value was considered as the lowest concentration of the compound at which the OD was similar to that of the media control. The experiment was performed in triplicate.

**MBC Assay.** The same experimental protocol was followed as outlined for the MIC assay. Here, instead of the OD measurement, the solution from the subcultivated agar wells was directly drop plated (3 μL) on the agar plate and again incubated for 24 h. Finally, the MBC values were determined as the minimum concentration of the compounds at which no bacterial colony was observed.

**Time-Kill Kinetics.** The time-kill kinetics of compounds 2a and 2n were performed against MRSA-ATCC3591. The experiment was performed at three different concentrations (4, 8, and 16 μg/mL) of both the compounds by following the reported protocol.²⁹e The required concentration of the compounds was prepared in Millipore water from 10 mg/mL stock solution in DMSO and 50 μL was added to the wells of the 96-well plate. After that, the mid-log phase (6 h grown) MRSA culture was diluted to ~10⁸ cfu/mL in the nutrient broth and 150 μL was added to the wells containing the compound and allowed to incubate under shaking at 37 °C. A control experiment was performed in which the same volume of the autoclaved Millipore water was added instead of the compound. However, at different time intervals, 20 μL of aliquots was serially diluted by 10-fold in 0.9% saline, and 20 μL of these serially diluted solutions were spot plated on the agar plates. The plates were then incubated for 24 h at 37 °C and viable bacterial colonies counted. The bacterial cell viability was determined and the results were presented in the logarithmic scale.

# ASSOCIATED CONTENT

## Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00840.

Plausible mechanism, complete spectroscopic characterization details of compounds 2, 3, and 4 including copies of ¹H NMR and ¹³C NMR (PDF)

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**Notes**

The authors declare no competing financial interest.

# ACKNOWLEDGMENTS

Financial support from The Kerala State Council for Science, Technology & Environment (KSCSTE), India, grant no. 1422/2014/KSCSTE is gratefully acknowledged.

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