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RESEARCH ARTICLE

# Biocatalytic synthesis of diaryl disulphides and their bio-evaluation as potent inhibitors of drug-resistant *Staphylococcus aureus*

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## Abstract

*Staphylococcus aureus* is a WHO Priority II pathogen for its capability to cause acute to chronic infections and to resist antibiotics, thus severely impacting healthcare systems worldwide. In this context, it is urgently desired to discover novel molecules to thwart the continuing emergence of antimicrobial resistance. Disulphide containing small molecules has gained prominence as antibacterials. As their conventional synthesis requires tedious synthetic procedure and sometimes toxic reagents, a green and environmentally benign protocol for their synthesis has been developed through which a series of molecules were obtained and evaluated for antibacterial activity against ESKAPE pathogen panel. The hit compound was tested for cytotoxicity against Vero cells to determine its selectivity index and time-kill kinetics was determined. The activity of hit was determined against a panel of *S. aureus* multi-drug resistant clinical isolates. Also, its ability to synergize with FDA approved drugs was tested as was its ability to reduce biofilm. We identified bis(2-bromophenyl) disulphide (**2t**) as possessing equipotent antimicrobial activity against *S. aureus* including MRSA and VRSA strains. Further, **2t** exhibited a selectivity index of 25 with concentration-dependent bactericidal activity, synergized with all drugs tested and significantly reduced preformed biofilm. Taken together, **2t** exhibits all properties to be positioned as novel scaffold for anti-staphylococcal therapy.

KEYWORDS

antibacterial, green, MRSA

## 1 | INTRODUCTION

*Staphylococcus aureus*, a gram-positive coccus listed as a WHO Priority two pathogen, is one of the most common causes of acute and complicated skin and skin structure infections to device-related bacteremia and infective endocarditis (Baker & Alvi, 2004). It is typically a treatable infection but with continuing emergence of drug resistance, especially to Vancomycin, there is an urgent and unmet requirement to discover and develop novel antibiotics targeting *S. aureus* with a new mode of action, thus escaping existing antimicrobial resistance mechanisms. According to CDC, *S. aureus* accounts for 80,461 infections and 11,285 deaths per year in the United States alone, which is more than HIV and TB combined, thus necessitating a dedicated drug discovery effort (Baud et al., 2012).

Small molecules and their analogues have been used for the treatment of a vast array of diseases including microbial infections

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(Bornscheuer & Kazlauskas, 2006; Carlqvist et al., 2005; Centers for Disease Control and Prevention, 2013; Chauhan, Kumar, & Srinivas, 2003). In this context, Psammaplin A, a naturally occurring small molecule derived from marine natural product, is known for wide array of biological activities including antimicrobial activities (Clinical and Laboratory Standards Institute, 2007; Das et al., 2016; Denard, Hartwig, & Zhao, 2013; Fuente, Sonawane, Arumainayagam, & Verkman, 2006; García et al., 2011). However, limitations such as low percentage in natural resources and tedious total synthesis have generally restrained its detailed biological activity studies. Nicolaou et al. in 2001 developed an elegant synthetic protocol towards generation of a library of hetero and homodimeric analogue molecules containing disulphide bond (García et al., 2011). Moreover, some disulphides (such as pyritinol and diallyldisulphide) also display antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Gogoi, Hazarika, Rao, & Dutta, 2006; Guan, Fu, & He, 2012). Despite the fact that there are many useful synthetic protocols for oxidative coupling of thiols (—SH) into disulphide (S—S) bond, there remain some limitations such as (a) possibilities of over-oxidation of disulphide into sulphoxide/sulphone in case of metal catalyzed oxidative coupling; (b) the atom economy is rather poor; (c) use of metals, or harsh reagents, and (d) poor or no recyclability of catalyst (Hancock, 1997; Hindmarch, Coleston, & Kerr, 1990; Hong et al., 2015; Hung & Stanbury, 2005; Infectious Diseases Society of America, 2010). Thus it is of great importance to develop an economical and green protocol for the synthesis of disulphide compounds, which can then be evaluated for their antibacterial activity.

Biocatalysis has emerged as an elegant approach for many challenging chemical transformations including tandem reaction (Kang et al., 2014; Kumar, Sharma, Sharma, Shard, & Sinha, 2012; Lai, Zheng, Chai, Zhang, & Chen, 2010; MacNeil et al., 2001; Nicolaou, Hughes, Pfefferkorn, Barluenga, & Roecker, 2001). Among various biocatalysts, Porcine pancreas lipase (PPL-triacylglycerol lipase EC 3.1.1.3) is an inexpensive enzyme, which catalyzes the hydrolysis of ester bonds in triglyceride as its native activity. It can also catalyzes many heteroatom bond forming reactions (C—O and C—N) as well as C—C bond formation, where some of these unnatural reactions occur in aqueous-organic medium with pH maintenance (O'ara, Hill, & Maslin, 2000; Saima, Equbal, Lavekar, & Sinha, 2016; Saima, Lavekar, Kumar, & Sinha, 2015; Schrittwieser, Sattler, Resch, Mutti, & Kroutil, 2011; Shard, Kumar, Saima, Sharma, & Sinha, 2014). Thus, we decided to explore PPL as a biocatalyst for synthesis of targeted small molecule (2) containing S—S bond via oxidative coupling of thiophenol (1) using water as a reaction media (Table 1), thus avoiding use of any toxic solvent or acidic/basic/metal catalysts (Sharma et al., 2011; Sharma, Sharma, Kumar, Kumar, & Sinha, 2009; Sharma, Sharma, Kumar, & Sinha, 2013; Tambe, Sampath, & Modak, 2001; Twentyman & Luscombe, 1987). A series of disulfides were synthesized using PPL as catalyst in water at room temp (Table 1) and were screened for their in vitro antimicrobial activities against ESKAPE pathogen panel (consisting of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* BAA-1705, *Acinetobacter baumannii* BAA-1605, and *Pseudomonas aeruginosa* ATCC 27853), wherein based upon the structure–activity relationship (SAR), bis(2-dibromophenyl) disulphide (**2t**), one of the smallest disulphide molecule (Table 2) exhibiting MIC of 1 mg/L, was identified as a potent inhibitor of highly drug-resistant clinical strains of *S. aureus* including MRSA and VRSA strains with improved selectivity index (25) and concentration dependent bactericidal activity (with a 10 $\log_{10}$ CFU/mL reduction), which was also comparable to Vancomycin.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals

All the starting materials were reagent grade. The aromatic/heteroaromatic thiols were obtained from commercial sources (Alfa Aesar or Sigma–Aldrich). The solvents used for isolation/purification of compounds were obtained from commercial sources (Merck) and used without further purification. Column chromatography was performed using silica gel (Merck, 60–120 mesh size). The chromatographic solvents are mentioned as v/v ratio. All the synthesized compounds were fully characterized by $^{1}H$ and $^{13}C$ Nuclear magnetic resonance (NMR) using two-dimensional NMR spectra recorded on a Bruker Avance-400 MHz spectrometer. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet. The $^{13}C$ NMR spectra were proton decoupled. The melting points were determined on a digital Barnsted Electrothermal 9,100 apparatus and were uncorrected. High resolution electrospray ionization mass spectrometry (HRMS-ESI) spectra were determined using micromass Q-TOF Ultima spectrometer and reported as mass/charge (m/z) (relative intensity) (Supporting Information).

### 2.2 | Bacterial strains, growth media and reagents

All strains including *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* BAA-1705, *Acinetobacter baumannii* BAA-1605, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were obtained from the American type culture collection (ATCC, Manassas, VA). The clinical drug-resistant *S. aureus*, *E. faecalis*, and *E. faecium* strains were procured from Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA)/biodefense and emerging infectious disease (BEI), Manassas, VA. All the strains were routinely grown on Mueller-Hinton cation supplemented broth II (MHBII), Mueller-Hinton agar (MHA) and Trypticase soy broth (TSB; Becton-Dickinson, NJ). All the other chemicals and antibiotics were procured from Sigma–Aldrich (St. Louis, MO). Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Lonza (Lonza). Before starting the experiment, single colony was picked from MHA plate and was inoculated in liquid medium and incubated at 37 °C for overnight to get the starter culture.

### 2.3 | Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out by broth microdilution assay as per Clinical & Laboratory Standards Institute (CLSI) guidelines (Weber, Stecher, & Faber, 1995). The synthesized compounds were solubilized in Dimethyl sulfoxide (DMSO) as 10 mg/mL stock solutions and were stored in −20 °C. Bacterial cultures were inoculated in

**TABLE 1** MIC of synthesized disulphides against ESKAPE pathogen panel

| | Minimum inhibitory concentration (mg/L) | | | | |
|---|---|---|---|---|---|
| **Comp code** | ***S. aureus* ATCC 29213** | ***E. coli* ATCC 25922** | ***K. pneumoniae* BAA-1705** | ***P. aeruginosa* ATCC 25923** | ***A. baumannii* BAA-1605** |
| **2a** | >64 | >64 | >64 | >64 | >64 |
| **2b** | >64 | >64 | >64 | >64 | >64 |
| **2c** | >64 | >64 | >64 | >64 | >64 |
| **2d** | 8 | >64 | >64 | >64 | 64 |
| **2e** | 32 | >64 | >64 | >64 | >64 |
| **2f** | >64 | >64 | >64 | >64 | >64 |
| **2g** | >64 | >64 | >64 | >64 | >64 |
| **2h** | >64 | >64 | >64 | >64 | >64 |
| **2i** | 4 | >64 | >64 | >64 | >64 |
| **2j** | >64 | >64 | >64 | >64 | >64 |
| **2k** | >64 | >64 | >64 | >64 | >64 |
| **2l** | 16 | >64 | >64 | >64 | >64 |
| **2m** | >64 | >64 | >64 | >64 | >64 |
| **2n** | >64 | >64 | >64 | >64 | >64 |
| **2o** | >64 | >64 | >64 | >64 | >64 |
| **2p** | >64 | >64 | >64 | >64 | >64 |
| **2q** | >64 | >64 | >64 | >64 | >64 |
| **2r** | >64 | >64 | >64 | >64 | >64 |
| **2s** | 8 | 64 | >64 | 32 | >64 |
| **2t** | 1 | >64 | >64 | >64 | >64 |
| **2u** | >64 | >64 | >64 | >64 | >64 |
| **2v** | >64 | >64 | >64 | >64 | >64 |
| **2w** | >64 | >64 | >64 | >64 | >64 |
| **2x** | >64 | >64 | >64 | >64 | >64 |
| Levofloxacin | <0.5 | <0.5 | 64 | 4 | 8 |

MHBII, optical density (OD) of the cultures was measured at the wavelength of 600 nm followed by dilution to ~$10^6$ cfu/mL. The concentrations of test compounds ranged from 64–0.5 mg/L in serially diluted fashion and 2.5 μL of each concentration was added to 96-well microtiter plate (Corning). Later, 97.5 μL of bacterial suspension was added to each well containing the test compound. Controls were included that is, cells alone and media alone (without compound + cells, DMSO control) and plates were incubated at 37 °C for 16–18 hr. Minimum Inhibitory concentration (MIC) values were determined by the absence or presence of visible growth. For each compound, MIC determinations were carried independently three times using duplicate samples.

## 2.4 | Cytotoxicity assay of 2t

To test the effect of **2 t** on the growth of mammalian cells, a cytotoxicity assay was performed (Xiang, Liu, Chen, Wu, & Lin, 2013). In brief, ~5,000 Vero cells (African green monkey kidney cell line, ATCC CCL-81) were seeded in a 96-well plate and grown for 24 hr at 37 °C in a humidified atmosphere of 5% $CO_2$ in DMEM medium supplemented with 10% fetal bovine serum, 1.5 g/L sodium bicarbonate, 100 mg/L Penicillin and 10 mg/L Streptomycin. Next day, different concentrations of **2 t** were diluted in media in triplicate. Following 72 hr of incubation, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added to a final concentration of 1 mg/mL for 2 hr. At the end of the incubation period, the media was removed by gently inverting the plate and 0.1 mL DMSO was added to the wells to solubilize the formazan crystals. The plates were gently vortexed for 15 min at room temperature to dissolve the precipitate and absorbance was measured at 595 nm. The mean values of absorbance of treated vs untreated cells were compared, and percentage survival vs concentration of compound was plotted to determine the $CC_{50}$ concentration.

## 2.5 | Bacterial time kill study with 2t

The bactericidal activity was assessed by the time-kill method. *S. aureus* ATCC 29213 cells were diluted up to ~$10^5$ cfu/mL, treated with 1× and 10× MIC of **2t** and Vancomycin and incubated at 37 °C. 0.1 mL samples were withdrawn after time intervals of 0, 1, 6, and 24 hr, serially diluted and plated on MHA followed by incubation at 37 °C for 18–20 hr. Kill curves were constructed by counting the colonies from plates and plotting the cfu/mL of surviving bacteria at each time point in the presence and absence of compound. Bactericidal activity was defined as a reduction of at least ≥1 $\log_{10}$ of the total count of CFU/mL in the original inoculum.

## 2.6 | Synergy of 2t with approved drugs

Determination of interaction with standard drugs was tested by the checkerboard method against *S. aureus* ATCC 29213. Serial twofold dilutions of each drug were freshly prepared before testing. **2t** was

**TABLE 2** Determination of MIC of **2t** against MDR *S. aureus* and *Enterococcus* clinical strain panel

| | | MIC (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Strains** | | **2t** | **Methicillin** | **Ceftriaxone** | **Meropenem** | **Gentamicin** | **Linezolid** | **Vancomycin** | **Teicoplanin** |
| MSSA | *S. aureus* ATCC 29213 | 1 | >64 | 16 | <0.5 | 1 | 0.5 | 1 | 1 |
| MRSA | NR 119 | 1 | >64 | >64 | 64 | 32 | 32 | 1 | 1 |
| | NR 100 | 1 | >64 | >64 | 16 | 1 | 2 | 1 | 1 |
| | NR 10129 | 1 | >64 | >64 | 16 | 0.25 | 2 | 1 | 1 |
| | NR 10198 | 1 | >64 | >64 | 64 | 0.25 | 1 | 1 | 1 |
| | NR 10192 | 1 | >64 | >64 | 64 | 0.5 | 2 | 1 | 1 |
| | NR 10191 | 1 | >64 | >64 | 64 | 0.5 | 1 | 1 | 1 |
| | NR 10193 | 2 | >64 | >64 | >64 | 0.25 | 1 | 1 | 1 |
| | NR 10186 | 1 | >64 | >64 | 16 | 0.5 | 2 | 1 | 1 |
| | NR 10194 | 1 | 32 | 32 | 1 | 0.25 | 1 | 1 | 0.5 |
| VRSA | VRS 1 | 1 | >64 | >64 | 64 | 32 | 4 | >64 | 64 |
| | VRS 4 | 1 | >64 | >64 | 32 | 1 | 4 | >64 | >64 |
| | VRS 12 | 1 | >64 | >64 | 8 | 4 | 4 | >64 | 32 |
| Enterococcus | *E. faecalis* NR 31884 | >64 | 64 | NT | NT | NT | NT | 2 | NT |
| | *E. faecalis* NR 31885 | >64 | >64 | NT | NT | NT | NT | 2 | NT |
| | *E. faecalis* NR 31886 | >64 | >64 | NT | NT | NT | NT | 2 | NT |
| | *E. faecalis* NR 31887 | >64 | 16 | NT | NT | NT | NT | 1 | NT |
| | *E. faecalis* NR 31888 | >64 | >64 | NT | NT | NT | NT | 1 | NT |
| | *E. faecium* NR 31903 | >64 | >64 | NT | NT | NT | NT | >64 | NT |
| | *E. faecium* NR 31909 | >64 | >64 | NT | NT | NT | NT | >64 | NT |
| | *E. faecium* NR 31912 | >64 | >64 | NT | NT | NT | NT | >64 | NT |

twofold diluted along the ordinate while the antibiotics were serially diluted along the abscissa in 96 well microtiter plates. 97.5 μL of ~$10^6$ CFU/mL was added to each well and plates were incubated at 37 °C for 18–24 hr. After the incubation period was over, Fractional inhibitory concentrations (ΣFICs) were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone and FIC B is the MIC of drug B in the combination/MIC of drug B alone. The combination is considered synergistic when the ∑FIC is ≤0.5, indifferent when the ∑FIC is >0.5 to 4, and antagonistic when the ∑FIC is >4 (Guan et al., 2012).

### 2.7 | Activity of 2t against *S. aureus* biofilm

Biofilm assay was performed as mentioned previously (Hancock, 1997). Briefly, *S. aureus* ATCC 29213 strain was cultured overnight in TSB supplemented with 1% glucose on rotary shaker at 37 °C, 180 RPM. The culture was diluted 1:100 and diluted culture (0.2 mL/well) was transferred into 96 well flat bottom tissue culture plates. The plates were covered with adhesive lid to maintain low oxygen conditions to increase biofilm formation and incubated for 48 hr at 37 °C. Subsequently, media was decanted, plates were rinsed gently thrice with 1× PBS (pH 7.2) to remove the planktonic bacteria. The plates were refilled with TSB containing different drug concentrations and incubated for 24 hr at 37 °C. Post drug treatment media was once again decanted and wells were rinsed with thrice with PBS. The plates were incubated at 60 °C for 1 hr for biofilm fixation and stained by 0.06% crystal violet for 10 min. Wells were rinsed with PBS and biofilm bound crystal violet was eluted by 30% acetic acid (0.2 mL each) and quantified by measuring the absorbance at 600 nm.

### 2.8 | Resistance studies with 2t

After MIC was determined, serial passaging was initiated by harvesting bacterial cells growing at the highest concentration of the compound with one-half MIC of the MIC and inoculating into fresh media. This inoculum was subjected to another MIC assay. After 24 hr incubation period, cells growing in the highest concentration of the compound from the previous passage were once again harvested and assayed for the MIC. The process was repeated for 35 passages. The MIC value of the compound was plotted against the number of passages and the fold increase in MIC was determined.

### 2.9 | Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). Comparison between three or more groups was analyzed using one-way ANOVA, with posthoc Tukey's multiple comparisons test. *p*-values of <.05 were considered to be significant.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Synthesis of diaryl disulphide derivatives

We first started the reaction for synthesis of disulphides using PPL as biocatalyst in water. Thus, PPL provided a series of disulphides in good to high yield (**2a-2w**) from corresponding thiophenol (**1a-1w**). Interestingly, the aliphatic thiol (**1x**) had also undergone oxidation towards formation of desired product allicin (**2x**), albeit in low yield using PPL in water whereas other lipase such as *Candida antarctica* (CAL-B), *Candida cylindracea* lipase

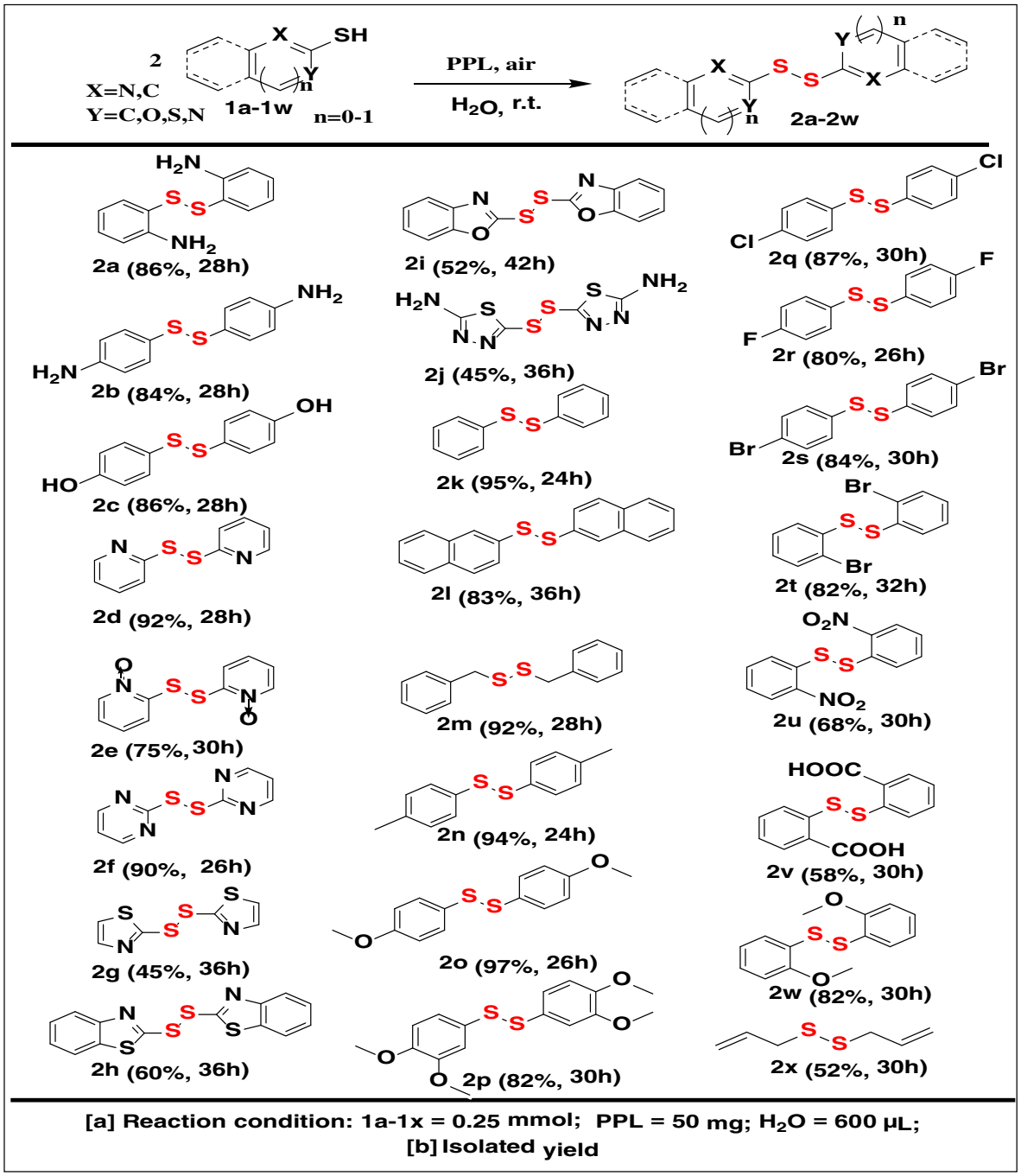


**FIGURE 1** Synthesis of disulphide containing small molecules (**2a-2x**) using PPL-water catalytic system[a,b] [Color figure can be viewed at wileyonlinelibrary.com]

(CCL) did not provide the corresponding disulphides. Moreover, when oxidation of **1a** was conducted in neat water (without PPL), the yield of **2a** drastically reduced which clearly suggested the crucial role of PPL towards efficient oxidative coupling of **1a** into disulphide (**2a**).

A mixture of thiophenol (**1a**, 0.25 mmol) and PPL (50 mg) in deionized water (0.6 mL) was stirred at room temperature for 28 hr (or till completion of reaction as monitored by thin layer chromatography (TLC) towards formation of desired disulphide (**2a**). 2 mL of water was added to crude reaction mixture and was extracted with ethyl acetate (3 mL × 2). The combined organic part was dried over sodium sulfate filtered, concentrated on rotavapor and chromatographed over silica gel by hexane/ethyl acetate (99:1 to 95:5). The same procedure was followed for the compounds (**2a-2x**). The products were further recrystallized with methanol and characterized by comparing the $^{1}$H NMR and $^{13}$C NMR data with those reported in the literature (Hindmarch et al., 1990; Hong et al., 2015; Hung & Stanbury, 2005). Their structures and yields were reported in Figure 1 and detailed $^{1}$HNMR and $^{13}$CNMR data were listed in Supporting Information.

## 3.2 | Evaluation of antibacterial activity of diaryl disulphide derivatives

The antibacterial activity of diaryl disulphides were evaluated against an ESKAPE pathogen panel and the results were tabulated in Table 1.

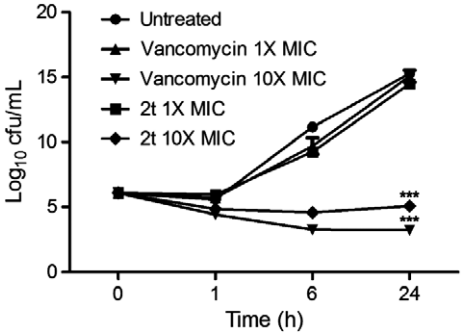


**FIGURE 2** Bacterial killing kinetics with **2t**. *S. aureus* ATCC 29213 was incubated with 1× and 10× MIC of **2t** and Vancomycin for 24 hr, samples were plated and CFU was determined

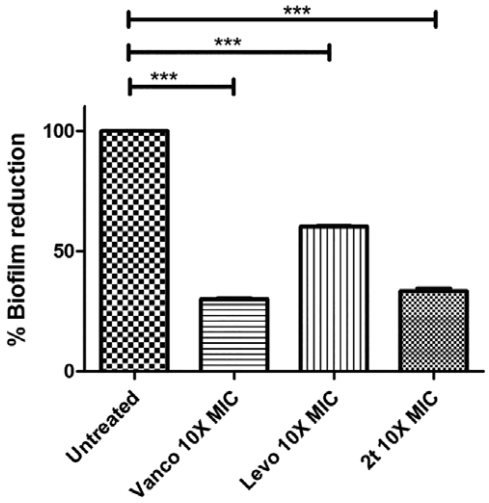


**FIGURE 3** Activity of **2t** against *S. aureus* biofilm. *S. aureus* biofilm was treated with 10× MIC of **2t**, Vancomycin and levofloxacin and stained to enumerate the reduction in biofilm. The percentage reduction is plotted

Interestingly, bromo substitution at para (**2s**) and ortho-position **(2t**) an aromatic ring of disulphide exhibited MIC values of 1 mg/L **(2t**) and 8 mg/L (**2s**) respectively whereas other halogen counterpart such as chloro (**2q**) or fluoro (**2r**) did not show any activity. Thus, **2t** was found to be most potent compound out of series targeting *S. aureus*. The lack of similar activity against gram-negative bacteria is most likely due to its inability to penetrate the outer membrane, a well-known resistance mechanism (Zheng, Shi, Du, Cao, & Zhang, 2013).

Furthermore, the antimicrobial activity of **2t** was determined against an extended strain panel consisting of *S. aureus*, *Enterococcus faecium* and *Enterococcus faecalis* clinical strains with well-defined resistance patterns including strains resistant to Methicillin, Vancomycin and other clinically utilized antibiotics. As tabulated in Table 2, **2t** was equally potent against multiple drug-resistant strains including Methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *S. aureus* (VRSA) but not *E. faecium* and *E. faecalis*, thus indicating a potentially new mechanism of action and lack of cross-resistance with existing resistance mechanisms.

### 3.3 | Evaluation of cell cytotoxicity of diaryl disulphide against Vero cells

The $CC_{50}$ of **2t** was determined against Vero cells to be 25 mg/L. Thus the selectivity index ($CC_{50}$/MIC) was calculated to be 25, which is an acceptable starting point from drug discovery perspective.

**TABLE 3** FIC of **2t** with other approved drugs

| Drug | Fractional inhibitory concentration (FIC) | Inference |
|---|---|---|
| Ceftazidime | 0.05 | Synergy |
| Daptomycin | 0.033 | Synergy |
| Erythromycin | 0.035 | Synergy |
| Gentamycin | 0.035 | Synergy |
| Linezolid | 0.035 | Synergy |
| Levofloxacin | 0.033 | Synergy |
| Meropenem | 0.033 | Synergy |
| Minocycline | 0.033 | Synergy |
| Oxytetracycline | 0.035 | Synergy |
| Rifampicin | 0.033 | Synergy |
| Vancomycin | 0.5 | Synergy |

### 3.4 | Time-kill assay of 2t

To determine the time kill kinetics of **2t**, *S. aureus* ATCC 29213 cells were incubated with 1-10× MIC for 24 hr and plated at different time points. After 24 hr, **2t** caused a decrease of 10 $\log_{10}$ CFU/mL in the presence of 10× MIC while Vancomycin at 10× MIC reduced the CFU by 12 $\log_{10}$ CFU/mL (Figure 2). Taken together, **2t** exhibits concentration-dependent bactericidal activity, which is comparable to Vancomycin.

### 3.5 | Synergy of 2t with FDA approved drugs

The ability of **2t** to synergize with FDA approved compounds was determined using the chequerboard method. As can be seen in Table 3, **2t** exhibited an FIC of <0.5 with all the antibiotics tested, indicating synergy. This indicates its potential to be utilized in combination therapy for treatment of serious *S. aureus* infections.

### 3.6 | Activity of 2t against *S. aureus* biofilm

Since biofilms are a hallmark of recurrent and severe *S. aureus* infection, in this context, the ability of **2t** to inhibit biofilm formation was determined. As can be seen in Figure 2–3**t** at 10× MIC caused ~70% reduction in biofilm, which was comparable to Vancomycin.

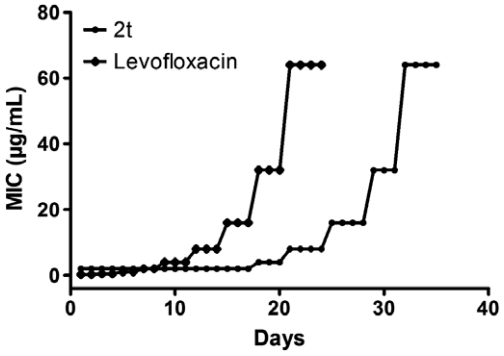


**FIGURE 4** Resistance studies with **2t**. MIC values of levofloxacin and **2t** against *S. aureus* ATCC 29213 after the number of serial passages indicated

Levofloxacin, on the other hand, was less effective against *S. aureus* biofilm and caused only a ~40% reduction.

### 3.7 | Resistance studies with 2t

The propensity of bacteria to generate resistance to antimicrobial agents was evaluated using serial exposure (Tambe et al., 2001). As can be seen in Figure 4, *S. aureus* develops resistance to **2t** when exposed to sub-inhibitory concentrations after at least 25 days of exposure with complete resistance (MIC >64 mg/L, 64-fold) achieved in 34 days. In comparison, *S. aureus* develops resistance to levofloxacin within 14 days of exposure with high level resistance (MIC >64 mg/L, 256-fold) within 24 days. Thus, the propensity to generate resistance in 2 t is lower as compared to levofloxacin although it is present.

In conclusion, a series of diaryl disulphide derivatives were synthesized and evaluated for their antibacterial efficacy against an ESKAPE pathogen panel, which identified **2t** as being highly potent against *S. aureus* (MIC = 1 mg/L). **2t** was equipotent against MRSA and VRSA drug-resistant clinical isolates and exhibited concentration-dependent bactericidal activity along with significantly low cell cytotoxicity profile. Additionally, it synergized will all antibiotics tested as well as significantly reduced preformed biofilm. In comparison to levofloxacin, its propensity to generate resistant *S. aureus* was lower. Further studies relating to structural optimization as well as determination of its mechanism of action of this lead are currently underway in our laboratory.

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## CONFLICT OF INTEREST

The authors declare no competing financial interests or other conflict of interest.

## TRANSPARENCY DECLARATIONS

None to declare.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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