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Balancing Physicochemical Properties of Phenylthiazole Compounds with Antibacterial Potency by Modifying the Lipophilic Side Chain

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Bacterial resistance to antibiotics is presently one of the most pressing healthcare challenges and necessitates discovering new antibacterials with unique chemical scaffolds. However, determining the optimal balance between structural requirements for pharmacological action and pharmacokinetic properties of novel antibacterial compounds is a significant challenge in drug development. The incorporation of lipophilic moieties within a compound's core structure can enhance biological activity but have a deleterious effect on drug-like properties. In this article, the lipophilicity of alkynylphenylthiazoles, previously identified as novel antibacterial agents, was reduced by introducing cyclic amines to the lipophilic side chain. In this regard, substitution with methylpiperidine (compounds **14-16**) and thiomorpholine (compound **19**) substituents significantly enhanced the aqueous solubility profile of the new compounds more than 150-fold compared to the first-generation lead compound **1b**. Consequently, the pharmacokinetic profile of compound **15** was significantly enhanced with a notable improvement both in half-life and the time the compound's plasma concentration remained above its minimum inhibitory concentration (MIC) against methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, compounds **14-16** and **19** were found to exert a bactericidal mode of action against MRSA and were not susceptible to resistance formation after 14 serial passages. Moreover, these compounds (at 2 × MIC) were superior to the antibiotic vancomycin in disrupting mature MRSA biofilm. The modifications to the alkynylphenylthiazoles reported herein successfully improved the pharmacokinetic profile of this new series while maintaining the compounds' biological activity against MRSA.

Key words: antibiotic resistance; MRSA; VRSA; Suzuki coupling; anti-biofilm.

Antibiotics are a cornerstone of medical treatment for various bacterial infections and are prescribed at a rate that exceeds the limit of 800 per 1000 people[1]. Therefore, the emergence of bacterial resistance to antibiotics is a notable threat to modern medicine. Though more than 40 antibacterial agents are currently undergoing investigation in clinical trials, many are derivatives of existing antibiotic classes. The low rate of success (~20%) for infectious disease agents to receive regulatory approval necessitates the continuous discovery and development of new antibiotic scaffolds.

The antimicrobial activity of thiazole-based scaffolds was very well-reported.[2-8] In an intensive effort to identify new antibacterial agents, our group focused on studying the antibacterial activity of phenylthiazoles as a subclass of thiazoles antimicrobials. These compounds inhibited growth of multidrug-resistant bacterial species of clinical interest, including methicillin-resistant *S. aureus*, including inhibition of staphylococcal biofilm formation, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant enterococci; they exhibited excellent antivirulent properties[9-18]. More importantly, bacterial mutants exhibiting resistance to these phenylthiazoles could not be isolated using multiple approaches. However, a drawback of the first-generation compounds was their poor physicochemical profile (in particular limited aqueous solubility and rapid hepatic metabolism) which hindered their investigation in appropriate pre-clinical animal models of bacterial infection.

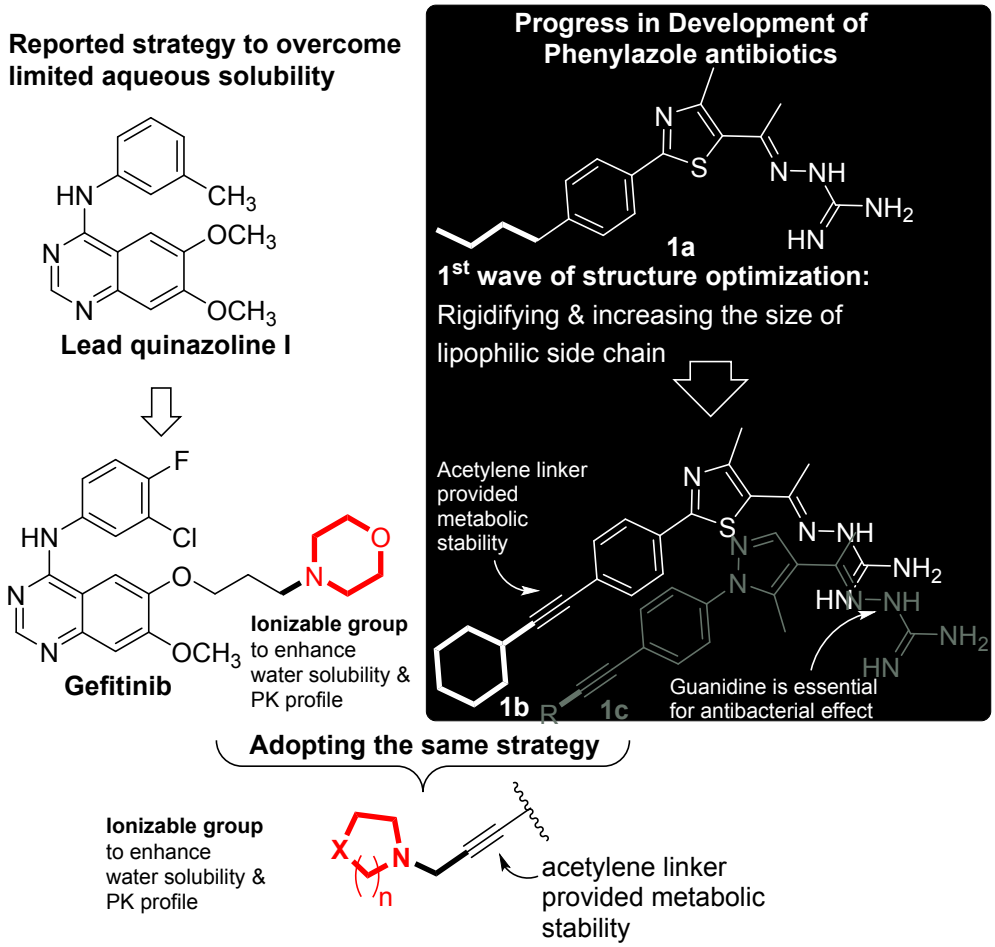


Figure 1. Reported chemical optimization to improve physicochemical properties of the quinoline epidermal growth factor receptor inhibitor gefitinib[19] and schematic representation of the aim of the current report with the phenylthiazole compounds.

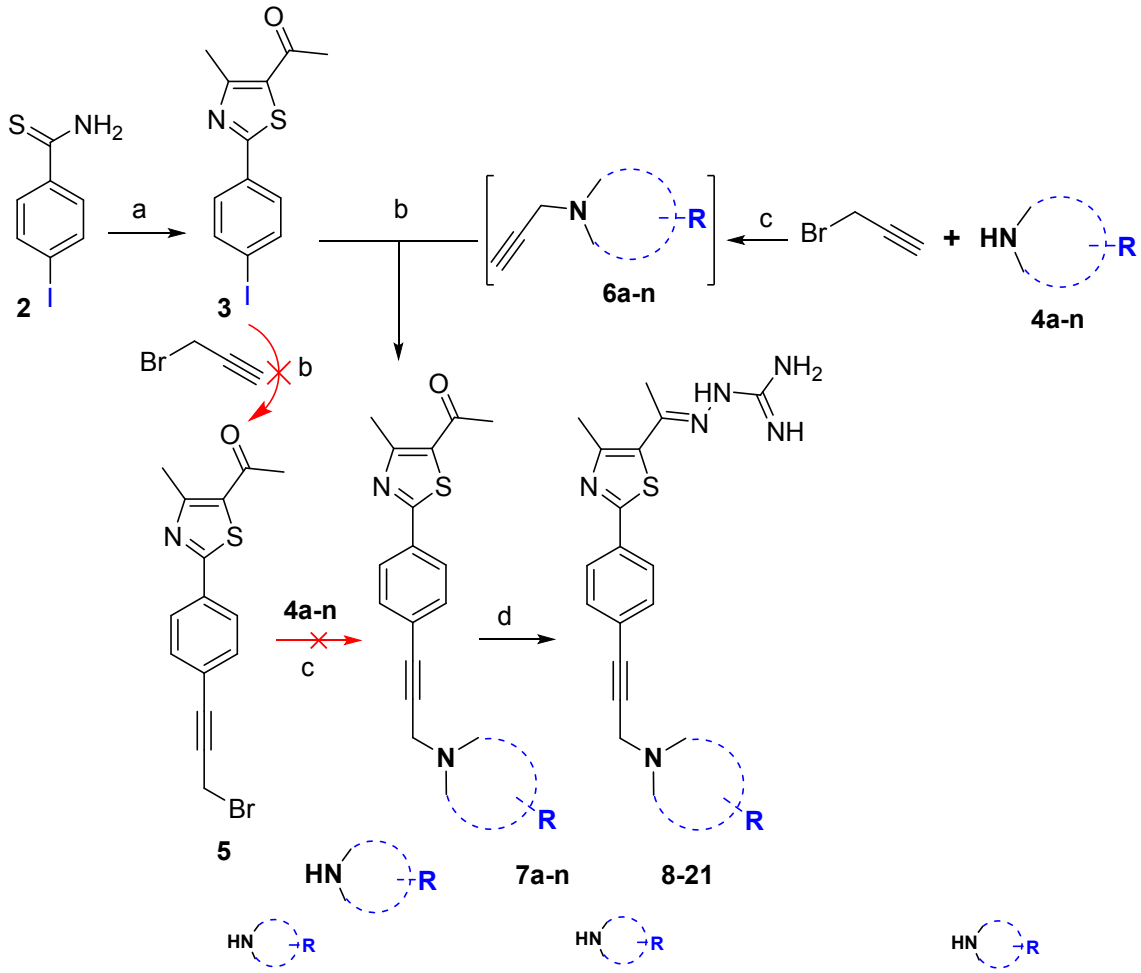
Identifying the correct balance between excellent physicochemical properties and the desired pharmacological effect for small molecules is a consistent challenge in drug discovery. In most cases, the structural motifs required to enhance the biological activity of a compound have a negative effect on its physicochemical characteristics. In the quest to improve the antibacterial potency of the first phenylthiazole lead compound **1a**, lipophilic moieties were added to the para-position of the phenyl ring (Figure 1). This tactic successfully enhanced the antibacterial potency of the second-generation compounds and resulted in derivatives with similar *in vitro* potency against MRSA to frontline therapeutics such as vancomycin and linezolid. However, the

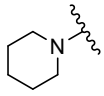
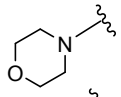
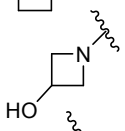
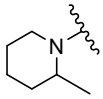
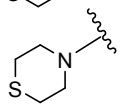
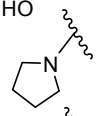
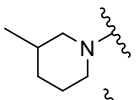
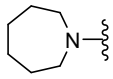
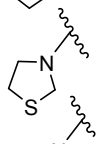
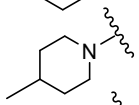
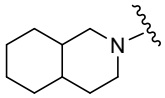
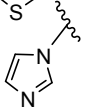
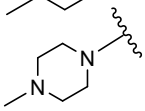
modifications made had a profoundly deleterious effect on the physicochemical properties, especially aqueous solubility, of these second-generation compounds.[11, 20] This problem was partially solved by replacing the central thiazole nucleus with a more polar pyrazole (structure **1c**, Figure 1).[21] In this report, an alternative strategy was adopted in which we kept the essential structural elements (guanidine head and acetylene linker) tethered to the phenylthiazole core to maintain the positive attributes previously studied and inserted a cyclic nitrogen group within the lipophilic side chain (Figure 1). The addition of an ionizable/polar moiety to the active scaffold has been used by medicinal chemists to enhance the overall aqueous solubility and pharmacokinetic profile of promising small molecules.[19] A successful example of this strategy is the synthesis of the anticancer drug gefitinib, from the lead quinazoline I,[19] highlighted in Figure 1.

RESULTS AND DISCUSSION

Chemistry. Thioamide starting material **2** was prepared as previously reported.[13] A shorter synthetic pathway to tether the acetylenic part was first tried by allowing compound **3** to react with propargyl bromide under standard Sonogashira conditions (Scheme 1). However, the desired compound **5** could not be isolated from the massively produced by-products. Alternatively, *N*-propargylcyclic amines **6a-n** were prepared first and then allowed to react with iodophenylthiazole intermediate **3** under the same standard Sonogashira conditions to yield compounds **7a-n**. Subsequent reaction with aminoguanidine in the presence of a catalytic amount of hydrochloric acid provided the desired final products **8-21** (Scheme 1).

Scheme 1



4a, 6a, 7a, 8		**4f, 6f, 7f, 13**		**4k, 6k, 7k, 18**	
4b, 6b, 7b, 9		**4g, 6g, 7g, 14**		**4l, 6l, 7l, 19**	
4c, 6c, 7c, 10		**4h, 6h, 7h, 15**		**4m, 6m, 7m, 20**	
4d, 6d, 7d, 11		**4i, 6i, 7i, 16**		**4n, 6n, 7n, 21**	
4e, 6e, 7e, 12		**4j, 6j, 7j, 17**			

Reagents and conditions: (a) Absolute EtOH, heat to reflux, 6 h, (b) $PdCl_2(PPh_3)_2$ (5% mol), CuI (7.5% mol), Et_3N, DME, heat at 50°C for 24 h in sealed flask; (c) anhydrous K_2CO_3, DMF, heat at 110°C for 4 h; (d) aminoguanidine HCl, EtOH, conc. HCl, heat to reflux, 3 h.

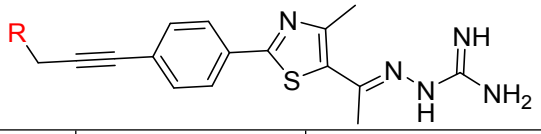
Biological Results and Discussion.

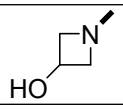
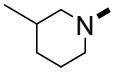
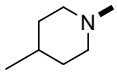
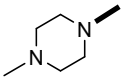
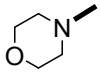
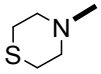
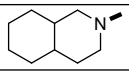
Antibacterial evaluation of new derivatives against MRSA. The newly-synthesized phenylthiazole derivatives were initially screened against MRSA USA300, which is a significant source of MRSA skin and soft tissue infections (SSTIs) worldwide.[22] The phenylthiazole derivatives were also screened against an *Escherichia coli* strain (JW55031) deficient in the AcrAB-TolC efflux pump, to investigate the compounds' activity against Gram-negative bacteria.

The initial screening against MRSA USA300 revealed that smaller nitrogenous rings resulted in compounds with weaker antibacterial activity (Table 1). This activity was slightly improved upon using pyrrolidine (compound **10**, MIC = 32 μg/mL) and was significantly enhanced with 6-membered nitrogenous rings. In this vein, the piperidine-containing derivative **13** (MIC = 8 μg/mL) was four-times more potent than the pyrrolidine-containing derivative **10**. Methylation of the piperidine ring in position-2, -3 or -4 had a positive impact on anti-MRSA activity as the MIC value of compounds **14-16** were one-fold lower than the corresponding unsubstituted piperidine-containing derivative **13**. Interestingly, our attempts to further enhance the polarity of the side chain by embedding a second polar atom within the terminal side chain ended up with two inactive compounds; one containing a methylpiperazine side chain (compound **17**, MIC = 64 μg/mL) and the second consisting of a morpholine substituent (compound **18**, MIC > 64 μg/mL). Unlike the morpholine ring, the thiomorpholine ring resulted in a compound (**19**, MIC = 4 μg/mL) with similar potency to the first-generation lead **1a**. Finally, the side chain consisting of a 6-membered ring provided the optimum bulkiness for anti-MRSA activity as compounds with a smaller ring size (compounds **8-12**) or larger ring size (compounds **20** and **21**) were all either less active or inactive against MRSA USA300 (Table 1). The MIC for the control antibiotic linezolid and vancomycin was 1 μg/mL against MRSA USA300. All reported compounds inhibited growth

of *E. coli* JW55031 at concentrations identical to or one-fold above/below their MIC against MRSA USA300 (Table 1). Additionally, all compounds were inactive against *E. coli* wild-type strain, which means that this class of antibiotic is efficient only for treatment of Gram-positive infections.

Table 1. Initial screening results (MIC in μg/mL) of the new series of phenylthiazoles against methicillin-resistant *S. aureus* USA300, *E. coli* JW55031 and *E. coli* BW25113.



Compounds/ Control drugs	R	MRSA USA300	*E. coli* JW55031 (TolC mutant)	*E. coli* BW25113 (wild-type strain)
8		64	64	> 64
9		64	32	> 64
10		32	32	> 64
11		> 64	> 64	> 64
12		> 64	64	> 64
13		8	16	> 64
14		4	8	64
15		4	4	64
16		4	4	64
17		64	64	> 64
18		> 64	> 64	> 64
19		4	8	> 64
20		64	> 64	> 64
21		64	> 64	> 64

1a		4	NT	> 64
1b		1	NT	> 64
Linezolid		1	8	NT[1]
Vancomycin		1	NT[1]	NT[1]
Gentamicin		NT	0.5	0.5

[1]NT: Not tested

Assessment of physicochemical properties and pharmacokinetic profile. After investigating the antibacterial activity of the compounds against MRSA USA300 and *E. coli* JW55031, we next assessed the physicochemical properties of the most promising derivatives. First, the aqueous solubility of the compounds was analyzed as solubility directly impacts the pharmacokinetic profile of bioactive compounds and is highly-dependent on a molecule's polarity/lipophilicity ratio[23-24]. Moreover, excessive lipophilic properties can negatively impact the formulation of compounds into suitable dosage forms.[25] It has been shown that compounds with a high logP value (≥ 4) are challenging to formulate in an oral dosage form.[26] To investigate whether our hypothesis of inserting a nitrogen atom within the lipophilic side chain will positively impact the aqueous solubility of the phenylthiazole compounds, the four most potent derivatives against MRSA (**14-16** and **19**) were evaluated via a turbidometric solubility assay. The methylpiperidine-containing analogues (**14-16**) were 150-times more soluble than the lead compound **1b** (Table 2). Compound **15** was the most soluble derivative (solubility limit exceeded 500 μM) from this new series and exhibited a solubility profile similar to the high-solubility control drug verapamil. Compound **19**, containing the thiomorpholine ring, also provided sufficient enhancement in this regard as its aqueous solubility limit was almost 0.3 μM (Table 2). This value represents a 107-fold improvement over the solubility limit of the first-generation lead compound **1b**.

Table 2. Evaluation of aqueous solubility limit of phenylthiazole compounds **1a**, **1b**, **14-16**, **19**, and control drugs.

Compound/Drug	Solubility limit (μM)[1]
1a	65
1b	2.7
14	427
15	> 500
16	375
19	292
Tamoxifen	15.6
Verapamil	> 500

[1]Solubility limit corresponds to the highest concentration of test compound where no precipitate was detected (OD_{540}).

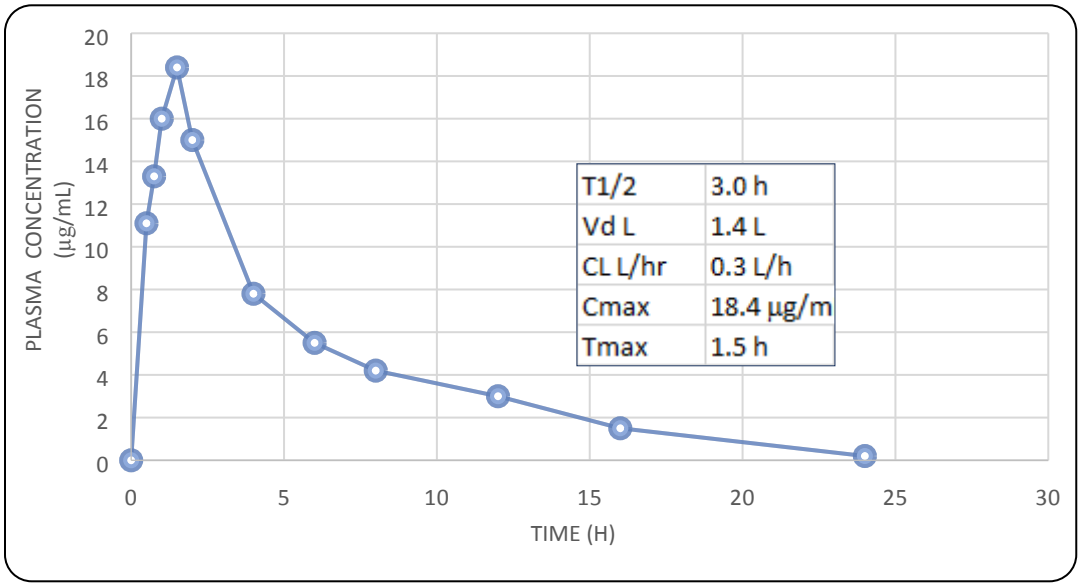


Figure 2. Pharmacokinetic profile of compound **15** after a single 50 mg/kg oral dose in rats (n = 3).

The initial series of phenylthiazole compounds synthesized could not be administered orally either due to metabolic instability (resulting in rapid elimination) or poor aqueous solubility that precluded formulation of the compounds in a proper oral dosage form.[17] The current series of phenylthiazole analogues were designed with an acetylene linker to block the metabolic soft spot present in compound **1a.**[20] To test the impact of decreasing the overall lipophilicity and increasing the hydrophilicity of the new set of alkynylphenylthiazoles on the compounds' pharmacokinetic profile, compound **15** was administered orally to rats. Blood samples were collected from each rat

over a 24 hour period (Figure 2). Compound **15** was selected as it exhibited the best aqueous solubility profile *in vitro*. The pharmacokinetic profile of **15** confirmed a notable improvement in the compound's stability to metabolism (half-life of three hours). Compound **15**'s half-life exceeded the half-life of the first-generation lead compound **1a** by more than six times.[13] Furthermore, the maximum plasma concentration (C_{max}) of compound **15** was 18.4 μg/mL which was more than three-fold higher than its MIC against MRSA USA300. Moreover compound **15** reached a plasma concentration equal to its MIC (4 μg/mL) against MRSA USA300 within 30 minutes and remained at or above this concentration for nearly eight hours.

Antibacterial activity of active analogues against a panel of *S. aureus* isolates. After confirming the notable improvement in aqueous solubility, metabolic stability, and pharmacokinetic profile of this new series of phenylthiazole antibacterial agents, we tested their activity against additional strains of methicillin-sensitive, methicillin-resistant, vancomycin-intermediate, and vancomycin-resistant staphylococci (MSSA, MRSA, VISA, and VRSA).

Table 3. The minimum inhibitory concentration (MIC in μg/mL) and minimum bactericidal concentration (MBC in μg/mL) of compounds **14-16** and **19** against 25 strains of methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA).

Bacterial Strain	**Compounds/Control antibiotics**											
	14		**15**		**16**		**19**		Linezolid		Vancomycin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MSSA[1] ATCC 6538	16	32	4	16	4	16	8	16	1	16	1	2
MSSA NRS107	16	16	8	16	8	8	8	32	1	64	2	2
MRSA[2] NRS108	16	16	8	32	8	16	16	32	1	16	1	2
MRSA NRS194	16	64	8	32	8	32	16	64	1	16	1	2
MRSA NRS119	8	16	8	8	8	8	16	16	64	> 64	1	1
MRSA NRS123 (USA400)	16	64	8	32	8	32	16	64	1	64	1	1

Bacterial Strain	Compounds/Control antibiotics											
	14		**15**		**16**		**19**		Linezolid		Vancomycin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MRSA NRS382 (USA100)	16	64	8	32	8	16	16	32	1	16	2	2
MRSA NRS383 (USA200)	16	32	8	32	8	32	16	32	1	64	1	1
MRSA NRS384 (USA300)	8	16	4	8	4	8	8	16	1	32	1	1
MRSA NRS385 (USA500)	16	64	4	16	8	16	16	16	1	32	1	1
MRSA NRS386 (USA700)	16	64	8	32	8	32	16	64	2	64	1	1
MRSA NRS387 (USA800)	16	32	8	32	8	32	8	32	1	64	1	1
MRSA NRS483 (USA1000)	16	64	8	32	8	32	16	32	2	>64	1	1
MRSA NRS484 (USA1100)	16	32	4	16	8	16	16	64	1	>64	2	2
VISA[3] NRS1	16	32	8	32	8	32	16	64	0.5	8	4	4
VISA NRS19	16	32	16	64	8	32	16	64	1	> 64	4	4
VISA NRS37	16	64	16	32	8	32	16	64	1	16	4	8
VRS2[4]	16	64	8	64	8	32	8	64	2	32	64	64
VRS5	16	32	16	64	8	32	16	32	1	64	> 64	> 64
VRS6	8	16	8	32	4	8	16	64	1	32	> 64	> 64
VRS7	16	16	8	16	8	16	16	32	1	> 64	> 64	> 64
VRS9	16	32	8	32	8	16	16	64	1	16	> 64	> 64
VRS10	16	64	8	32	8	32	8	64	1	> 64	64	> 64
VRS11a	16	16	8	16	8	16	16	64	0.5	16	> 64	> 64
VRS12	16	64	16	16	8	32	16	32	1	64	1	64

[1]MSSA, Methicillin-sensitive *Staphylococcus aureus*; [2]MRSA, Methicillin-resistant *Staphylococcus aureus*; [3]VISA, Vancomycin-intermediate *Staphylococcus aureus*; [4]VRS, Vancomycin-resistant *Staphylococcus aureus*.

Compounds **14-16** and **19** inhibited growth of all methicillin-sensitive, methicillin-resistant, vancomycin-intermediate and vancomycin-resistant *S. aureus* (MSSA, MRSA, VISA and VRSA) strains tested at concentrations ranging from 4 to 16 μg/mL (Table 3). Compounds **15** and **16** were the two most potent compounds with MIC values ranging primarily between 4 to 8

μg/mL. The compounds also inhibited growth of *S. aureus* strains exhibiting resistance to the two frontline treatment options for MRSA infections (linezolid and vancomycin).

To ascertain if the phenylthiazole compounds were bacteriostatic or bactericidal agents against the tested staphylococcal strains, the MBC was determined. The MBC values were not more than three-fold higher than the compounds' MIC values against all *S. aureus* strains tested indicating they are bactericidal agents. To confirm the bactericidal activity of the compounds, a time-kill assay (Figure 3) was performed against MRSA USA400. This is a highly-virulent strain of MRSA associated with many cases of SSTIs as well as invasive infections such as necrotizing pneumonia, pulmonary abscesses, and sepsis.[27-29]

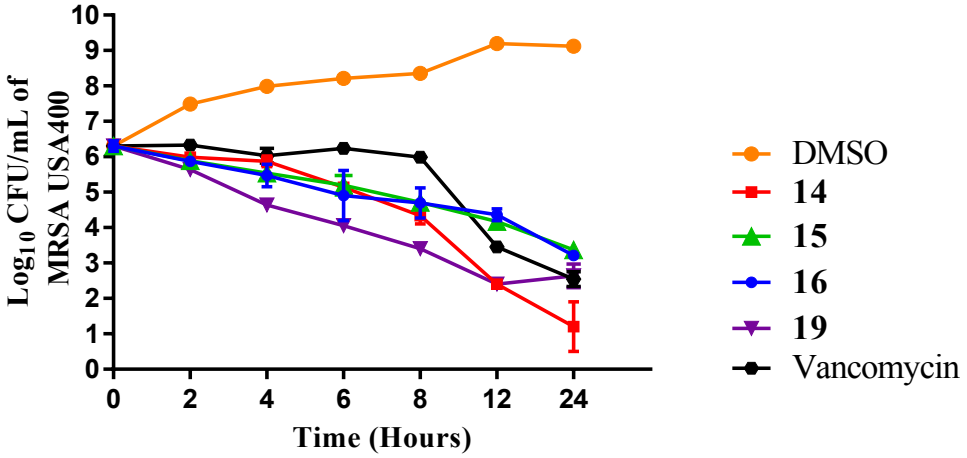


Figure 3. Time-kill assay for compounds **14-16**, **19** and vancomycin (tested in triplicates at 4 × MIC) against methicillin-resistant *Staphylococcus aureus* (MRSA) USA400. DMSO (solvent for the compounds) served as a negative control. The error bars represent standard deviation values.

Compounds **15** and **16** were as effective as vancomycin, the drug of choice for treatment of MRSA infections, as all three agents reduced the burden of MRSA by 3-$\log_{10}$ within 24 hours. The 2-methylpiperidine **14** and thiomorpholine-containing derivative **19** achieved the same effect more rapidly, within 12 hours.

Selectivity towards prokaryotic cells was evaluated using two cell lines; one carcinogenic and one non-carcinogenic that are human colorectal adenocarcinoma (Caco-2) and fibroblast-like monkey kidney cell line (VERO cell) to determine the potential toxic effect *in vitro*. As summarized in Figures 1S and 2S, all four tested compounds **14**, **15**, **16** and **19** were highly tolerable to both cell lines at concentration as high as 32 µg/mL.

Multi-step resistance study against MRSA. As reported previously, MRSA was unable to develop resistance to the initial series of phenylthiazole compounds[21,30-31]. To test whether the modifications made to the new compounds affected this feature, a multi-step resistance selection study was conducted (Figure 4). The MIC values of compounds **15** and **19** remained consistent and did not change throughout the 14 passages. The MIC values for compounds **14** and **16** increased one-fold after the tenth and ninth passage respectively, but they remained stable thereafter. The results from the multi-step resistance study indicate that MRSA was unable to develop rapid resistance to the four phenylthiazole compounds tested.

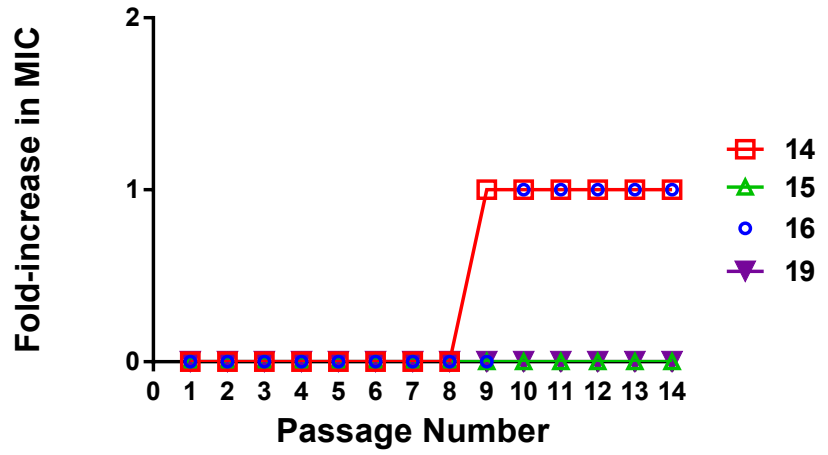


Figure 4. Multi-step resistance study of compounds **14-16** and **19** against methicillin-resistant *S. aureus* USA400. Bacteria were serially passaged over a 14-day period, and the broth microdilution assay was used to determine the MIC of each compound after each successive passage. A four-fold increase in the MIC would be indicative of bacterial resistance forming to the test agent.

MRSA biofilm eradication assessment. *S. aureus* is one of the most important bacterial species capable of forming biofilms. Biofilms can form on medical devices such as indwelling catheters, implanted artificial bones, knees and hips as well as prosthetic heart valves.[32-33] This increases the chance of developing chronic, recurrent infections.[34] Staphylococcal biofilm-related infections are difficult to eradicate and typically must be treated with antibiotic combinations. Most drugs of choice for treatment of staphylococcal infections like glycopeptides (vancomycin) have very limited activity on bacteria embedded within biofilms.[18, 35] As a consequence, infected medical devices have to be surgically extracted and replaced, which can lead to serious risks and complications.[36] Hence, there is a need to develop drugs capable of disrupting staphylococcal biofilms.

Compounds **14-16** and **19** were examined for their ability to disrupt pre-formed, mature MRSA USA300 biofilm. Advantageously, the new phenylthiazoles were superior to vancomycin in MRSA biofilm eradication. Due to its large molecular structure and polar nature, vancomycin, the drug of choice for treatment of invasive MRSA infections, does not penetrate biofilms effectively.[37-38] At 2 × MIC, vancomycin only disrupted 12.8% of MRSA biofilm mass (Figure 5). In contrast, compound **19** (at 2 × MIC) disrupted ~42% of MRSA300 biofilm mass. Compounds **14** and **15** exhibited similar activity as they disrupted ~39% of the pre-formed MRSA300 biofilm. Compound **16** disrupted ~31% of MRSA300 biofilm mass at the same concentration (2 × MIC).

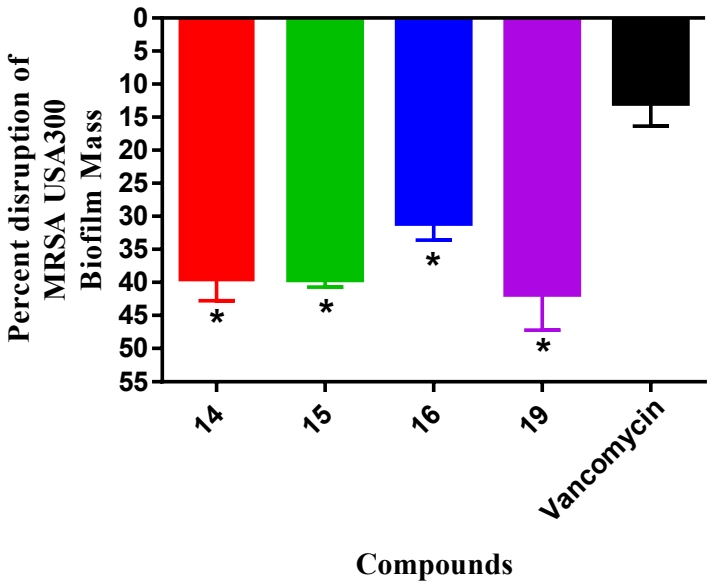


Figure 5. Disruption of mature MRSA USA300 biofilm by compounds **14-16** and **19** and vancomycin (all at 2 × MIC). Data are presented as percent disruption of MRSA USA300 mature biofilm in relation to DMSO (the solvent for the compounds that served as a negative control). The values represent an average of four samples analyzed for each compound/antibiotic. Error bars represent standard deviation values. An asterisk (*) denotes statistical significance ($P < 0.05$) between results for compounds **14-16** and **19** relative to vancomycin as analyzed via an unpaired t-test.

Conclusion. During the process of developing phenylthiazole antibiotics, the structure-activity-relationships led us to an "obese molecule" in which increasing the lipophilicity improved the compounds' antibacterial activity. However, this had a deleterious effect on the compounds' physicochemical profile. This article tackled this problem by maintaining the essential structural elements of the phenylthiazole core and inserting a nitrogen atom within the lipophilic side chain. A six-membered cyclic amine with methyl substituent or sulfur atom resulted in derivatives with the most-balanced antibacterial and physicochemical properties. Compound **15** demonstrated a highly acceptable *in vivo* pharmacokinetic profile in terms of oral absorbability, metabolic stability and plasma concentration above the compound's MIC values against MRSA. The notable improvement in the pharmacokinetic behavior was accompanied with a slight decrease in

antibacterial activity as the MIC values reported here were 2-4 times higher than the previously tested alkynylphenylthiazoles.

Methods

General Chemistry: The purity of all biologically-tested compounds were confirmed to be not less than 95% using elemental analysis. All starting material chemicals and solvents were obtained from commercial sources. The masses were weighed on a microbalance with a resolution of 0.0001 g. Visualization on TLC was acquired using UV light (254 nm) and also staining with iodine. ^{1}H NMR spectra were done at 400 MHz and ^{13}C spectra were determined at 100 MHz in deuterated dimethyl sulfoxide (DMSO-d_6) on a Varian Mercury VX-400 NMR spectrometer. Chemical shifts are given in parts per million (ppm) on the delta (δ) scale. Chemical shifts were calibrated relative to those of the solvents. Flash chromatography was performed on 230-400 mesh silica. The progress of reactions was monitored with Merck silica gel IB2-F plates (0.25 mm thickness). Mass spectra were recorded at 70 eV. High resolution mass spectra for all ionization techniques were obtained from a FinniganMAT XL95. Melting points were determined using capillary tubes with a Stuart SMP30 apparatus and are uncorrected. All yields reported refer to isolated yields.

Compounds 7a-n. *General procedure*: to dry DMF (5 mL) in a round flask, appropriate *Sec*. amines **4a-n** (200 mg, 1.5-2 mmole), propargyl bromide (476 mg, 300 μL, 3-4 mmol, 2 equiv.), and anhydrous potassium carbonate (830 mg, 4.5-6 mmole, 3 equiv.) were heated at 110 ^{o}C and stirred for 4 h. Next, the reaction mixture was taken without purification and added to a 75-mL sealed tube charged with compound **3** (300 mg, 0.87 mmol) in dry DME (10 mL) and triethylamine (2 mL). After the reaction mixture was purged with dry nitrogen gas for 15 min, dichlorobis(triphenylphosphine)palladium (II) (46 mg, 0.065 mmol) and copper(I) iodide (33 mg,

0.17 mmol) were added. The sealed tube was then maintained at 50 °C for 24 h and reaction progress was monitored by TLC. After completion of the reaction, the reaction mixture was passed through a pad of silica gel with DCM (50 mL). Organic materials were then collected, concentrated under vacuum and purified using silica gel chromatography (DCM/ methanol 98:2). Physical properties, yields, and characterization data of isolated products are listed below:

1-(2-(4-(3-(Azitidin-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethan-1-one (7a). Orange oil (225 mg, 83%); ^{1}H NMR (DMSO-d_6) δ: 8.21 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 4.33 (t, J = 8.2 Hz, 4H), 3.77 (s, 2H), 2.62 (s, 3H), 2.59 (s, 3H), 2.52 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 191.55, 168.38, 158.69, 133.23, 132.94, 127.22, 125.93, 92.81, 83.74, 50.03, 49.66, 30.61, 18.58, 17.88; MS (*m/z*); 310 (M^+, 14.7%).

1-(2-(4-(3-(3-Hydroxyazitidin-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethan-1-one (7b). Yellowish oil (235 mg, 82.4%); ^{1}H NMR (DMSO-d_6) δ: 7.61 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 4.40 (brs, 1H), 3.71 (m, 1H), 3.41 (s, 2H), 2.62 (s, 3H), 2.59 (dd, J = 11.2, 3.4 Hz, 2H), 2.57 (dd, J = 11.1, 6.2 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 191.95, 168.14, 158.66, 132.73, 132.44, 129.27, 127.19, 94.51, 84.64, 62.74, 48.23, 44.25, 30.66, 18.54; MS (*m/z*); 326 (M^+, 100%).

1-(4-Methyl-2-(4-(3-(pyrrolidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7c). Dark brown oil (170 mg, 60%); ^{1}H NMR (DMSO-d_6) δ: 7.88 (d, J = 8.8 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 3.63 (s, 2H), 2.61 (s, 3H), 2.49 (m, 4H), 2.31 (s, 3H), 1.73 (m, 4H); ^{13}C NMR (DMSO-d_6) δ: 190.45, 160.34, 148.33, 142.74, 136.33, 132.93, 126.62, 123.91, 89.12, 84.45, 53.66, 43.39, 23.86, 18.55, 16.61; MS (*m/z*); 324 (M^+, 19.51%).

1-(4-Methyl-2-(4-(3-(thiazolidin-3-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7d). Dark yellow oil (163 mg, 54%); ^{1}H NMR (DMSO-d_6) δ: 8.02 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.1

Hz, 2H), 4.69 (s, 2H), 3.86 (s, 2H), 3.14 (t, *J* = 6.4 Hz, 2H), 2.77 (t, *J* = 6.4 Hz, 2H), 2.61 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 190.66, 163.16, 158.83, 136.13, 132.64, 131.96, 127.17, 124.31, 93.40, 84.45, 60.06, 53.44, 43.39, 24.11, 18.45, 16.71; MS (*m/z*); 342 (M^+, 37.73%).

1-(2-(4-(3-(1*H*-Imidazol-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethan-1-one (7e). Dark brown oil (215 mg, 76%); ^{1}H NMR (DMSO-d_6) δ: 7.91 (d, *J* = 8.4 Hz, 2H), 7.78 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 6.8 Hz, 1H), 6.96 (d, *J* = 6.8 Hz, 1H), 5.22 (s, 2H), 2.68 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 192.15, 166.34, 163.73, 136.93, 133.64, 132.72, 131.91, 129.83, 126.73, 124.96, 121.33, 87.32, 84.55, 43.39, 29.86, 16.45; MS (*m/z*); 321 (M^+, 32.29%).

1-(4-Methyl-2-(4-(piperidin-1-yl)prop-1-yn-1-yl)phenyl)-4-thiazol-5-yl)ethan-1-one (7f). Yellow oil (233 mg, 78.9%); ^{1}H NMR (DMSO-d_6) δ: 7.99 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 3.51 (s, 2H), 2.71 (s, 3H), 2.57 (s, 3H), 2.49 (m, 4H), 1.51 (m, 4H), 1.39 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 191.35, 167.92, 158.56, 133.83, 132.54, 132.26, 127.25, 125.61, 89.58, 84.63, 53.10, 48.06, 30.64, 25.94, 24.04, 18.45; MS (*m/z*); 338 (M^+, 29.17%).

1-(4-Methyl-2-(4-(3-(2-methylpiperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7g). Brown oil (214 mg, 69.7%); ^{1}H NMR (DMSO-d_6) δ: 7.88 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 7.2 Hz, 2H), 4.51 (s, 2H), 3.73 (m, 1H), 3.51 (m, 2H), 2.69 (s, 3H), 2.52 (s, 3H), 1.60-1.22 (m, 6H), 1.05 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 191.15, 168.12, 162.97, 158.83, 132.39, 132.26, 127.34, 126.08, 88.70, 84.88, 54.66, 53.39, 43.87, 34.62, 30.69, 26.34, 24.76, 20.41, 18.31; MS (*m/z*); 352 (M^+, 49.21%).

1-(4-Methyl-2-(4-(3-(3-methylpiperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7h). Yellow oil (250 mg, 81.4%); ^{1}H NMR (DMSO-d_6) δ: 8.00 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 2H), 3.51 (s, 2H), 2.77 (m, 2H), 2.71 (s, 3H), 2.58 (s, 3H), 214-1.46 (m, 7H), 0.87 (d, *J* =

6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 191.14, 168.02, 158.69, 133.53, 133.22, 132.24, 126.91, 125.92, 89.49, 84.43, 60.35, 52.40, 48.01, 32.62, 30.99, 25.64, 18.94, 17.34; MS (*m/z*); 352 (M^+, 49.33%).

1-(4-Methyl-2-(4-(3-(4-methylpiperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7i). Light-yellow oil (270 mg, 87.9%); ^{1}H NMR (DMSO-d_6) δ: 7.88 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 3.51 (s, 2H), 2.84 (m, 4H), 2.59 (s, 3H), 2.31 (s, 3H), 2.20 (m, 4H), 1.61-1.11 (m, 1H), 0.90 (d, J = 6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 191.33, 161.84, 160.13, 136.33, 133.30, 132.44, 126.31, 124.52, 88.59, 84.93, 52.75, 47.60, 34.71, 30.29, 22.24, 18.81, 16.84; MS (*m/z*); 352 (M^+, 49.31%).

1-(4-Methyl-2-(4-(3-(4-methylpiperazin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7j). Orange oil (234 mg, 75.9%); ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 3.55 (s, 2H), 2.72 (s, 3H), 2.59 (s, 3H), 2.52 (s, 8H), 2.16 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 191.18, 167.73, 158.63, 132.62, 132.43, 127.41, 126.15, 89.25, 84.83, 54.15, 51.15, 47.42, 46.01, 31.04, 18.74; MS (*m/z*); 353 (M^+, 9.14%).

1-(4-Methyl-2-(4-(3-(4-morpholinoprop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7k). Brown oil (263 mg, 88%); ^{1}H NMR (DMSO-d_6) δ: 7.99 (d, J = 7.2 Hz, 2H), 7.58 (d, J = 7.2 Hz, 2H), 3.98 (s, 2H), 3.62 (t, J = 6.4 Hz, 4H), 2.71 (s, 3H), 2.57 (s, 3H), 2.53 (t, J = 6.4 Hz, 4H); ^{13}C NMR (DMSO-d_6) δ: 191.23, 161.94, 158.61, 136.43, 133.21, 132.04, 127.56, 125.72, 88.69, 84.53, 62.35, 54.40, 45.41, 18.24, 16.31; MS (*m/z*); 340 (M^+, 100%).

1-(4-Methyl-2-(4-(3-(4-thiomorpholinoprop-1-yn-1-yl)phenyl)thiazol-5-yl)ethan-1-one (7l). Yellow oil (212 mg, 68%); ^{1}H NMR (DMSO-d_6) δ: 8.19 (d, J = 7.2 Hz, 2H), 7.48 (d, J = 7.2 Hz, 2H), 4.61 (s, 2H), 4.02 (t, J = 6.4 Hz, 4H), 2.69 (s, 3H), 2.53 (t, J = 6.4 Hz, 4H), 2.39 (s, 3H); ^{13}C

NMR (DMSO-d_6) δ: 191.63, 163.04, 158.91, 136.43, 133.88, 133.18, 127.16, 124.62, 88.79, 82.43, 54.15, 46.41, 29.45, 18.78, 16.17; MS (*m/z*); 356 (M^+, 9.42%).

1-(2-(4-(3-(Azepan-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethan-1-one (7m). Dark-brown oil (223 mg, 72%); ^{1}H NMR (DMSO-d_6) δ: 8.06 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 4.29 (t, J = 5.4 Hz, 4H), 4.06 (s, 2H), 2.61 (s, 3H), 2.46 (s, 3H), 2.31-1.46 (m, 8H); ^{13}C NMR (DMSO-d_6) δ: 191.35, 167.92, 158.16, 143.83, 133.54, 133.26, 127.25, 125.83, 88.58, 84.63, 54.10, 44.06, 30.64, 25.94, 24.44, 18.45; MS (*m/z*); 352 (M^+, 8.17%).

1-(4-Methyl-2-(4-(3-(octahydroisoquinolin-2(1*H*)-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl) ethan-1-one (7n). Dark-brown oil (273 mg, 79.8%); ^{1}H NMR (DMSO-d_6) δ: 7.88 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 3.51 (s, 2H), 2.87 (m, 2H), 2.73 (m, 2H), 2.59 (s, 3H), 2.31 (s, 3H), 2.20-0.89 (m, 12H); ^{13}C NMR (DMSO-d_6) δ: 191.15, 160.42, 148.46, 133.53, 132.64, 129.46, 126.22, 124.40, 91.28, 84.83, 59.64, 53.38, 47.01, 42.38, 33.12, 30.74, 27.14, 26.34, 24.04, 19.01, 16.65; MS (*m/z*); 392 (M^+, 20.60%).

2-(1-(2-(4-(3-(Substituted)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethylidene)-1-carboximidamide (8-21). *General procedure*: Thiazole derivatives **7a-n** (0.57-0.64 mmol) were dissolved in absolute ethanol (15 mL). Concentrated hydrochloric acid (1 mL) and aminoguanidine hydrochloride (355 mg, 3.2 mmol, 5 equiv.) were subsequently added. The reaction mixture was heated at reflux for 3 h. After completion of the reaction, as monitored by TLC, the solvent was concentrated under reduced pressure, poured onto crushed ice, and neutralized with sodium carbonate to pH 7-8. The formed precipitated was collected by filtration and washed with copious amount of water. Crystallization from absolute ethanol afforded the desired products as solids. Physical properties, yields, and characterization data of isolated final products are listed below:

2-(1-(2-(4-(3-(Azetidin-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethylidene) hydrazine-1-carboximidamide (8). Yellow solid (160 mg, 68%); mp = 240-241 °C. ^{1}H NMR (DMSO -d_6) δ: 8.00 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 5.71 (brs, 2H), 4.92 (brs, 2H), 4.35 (t, J = 5.2 Hz, 4H), 3.77 (s, 2H), 2.71 (s, 3H), 2.55 (s, 3H), 2.43 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 168.38, 163.42, 158.69, 153.21, 132.61, 132.18, 127.31, 125.97, 93.21, 83.64, 51.03, 49.16, 30.51, 18.39, 17.77; HRMS (EI) *m/z* 366.1621 M^+, calc. for $C_{19}H_{22}N_6S$ 366.1627; Anal. Calc. for: $C_{19}H_{22}N_6S$ (366): C, 62.27; H, 6.05; N, 22.93%; Found: C, 62.29; H, 6.07; N, 22.96%.

2-(1-(2-(4-(3-Hydroxyazetidin-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl) ethylidene) hydrazine-1-carboximidamide (9). Orange solid (167 mg, 71%); mp = 249-251 °C. ^{1}H NMR (DMSO -d_6) δ: 7.66 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 5.69 (brs, 4H), 3.86 (m, 1H), 3.53 (brs, 1H), 3.47 (s, 2H), 2.61 (s, 3H), 2.49 (s, 3H), 2.47 (dd, J = 10.2, 3.4 Hz, 2H), 2.45 (dd, J = 10.1, 6.2 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ: 168.67, 161.72, 158.53, 152.72, 145.69, 133.05, 132.88, 127.93, 125.17, 94.25, 83.54, 62.35, 53.53, 49.86, 30.81, 18.39; HRMS (EI) *m/z* 382.1590 M^+, calc. for $C_{19}H_{22}N_6OS$ 382.1576; Anal. Calc. for: $C_{19}H_{22}N_6OS$ (382): C, 59.66; H, 5.80; N, 21.97%; Found: C, 59.61; H, 5.78; N, 21.94%.

2-(1-(4-Methyl-2-(4-(3-(pyrrolidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethylidene) hydrazine-1-carboximidamide (10). Brown solid (174 mg, 74%); mp = 245-247 °C. ^{1}H NMR (DMSO -d_6) δ: 7.87 (d, J = 7.2 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 5.76 (brs, 2H), 5.66 (brs, 2H), 3.76 (s, 2H), 2.59 (m, 4H), 2.57 (s, 3H), 2.31 (s, 3H), 1.73 (m, 4H); ^{13}C NMR (DMSO-d_6) δ: 161.57, 160.24, 148.44, 142.99, 136.48, 133.36, 132.27, 126.22, 124.14, 88.99, 84.49, 52.43, 43.89, 23.77, 18.83, 16.55; HRMS (EI) *m/z* 380.1793 M^+, calc. for $C_{20}H_{24}N_6S$ 380.1783; Anal. Calc. for: $C_{20}H_{24}N_6S$ (380): C, 63.13; H, 6.36; N, 22.09%; Found: C, 63.16; H, 6.37; N, 22.11%.

2-(1-(4-Methyl-2-(4-(3-(thiazolidin-3-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethylidene) hydrazine-1-carboximidamide (11). Brown solid (174 mg, 74%); mp = 245-247 °C. ^{1}H NMR (DMSO -d_6) δ: 7.96 (d, J = 8.4 Hz, 2H), 7.81 (brs, 4H), 7.62 (d, J = 8.4 Hz, 2H), 4.69 (s, 2H), 4.04 (s, 2H), 3.13 (t, J = 6.4 Hz, 2H), 2.87 (t, J = 6.4 Hz, 2H), 2.62 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 163.54, 158.54, 147.69, 143.56, 136.18, 132.16, 124.52, 121.64, 89.69, 84.69, 61.13, 53.45, 42.68, 29.27, 18.43, 16.65; HRMS (EI) *m/z* 398.1362 M^+, calc. for $C_{19}H_{22}N_6S_2$ 398.1347; Anal. Calc. for: $C_{19}H_{22}N_6S_2$ (398): C, 57.26; H, 5.56; N, 21.09%; Found: C, 57.29; H, 5.58; N, 21.10%.

2-(1-(2-(4-(3-(1*H*-Imidazol-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethylidene) hydrazine-1-carboximidamide (12). Dark-brown solid (148 mg, 63%); mp = 205-207 °C. ^{1}H NMR (DMSO -d_6) δ: 7.91 (d, J = 8.4 Hz, 2H), 7.78 (s, 1H), 7.57 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 7.2 Hz, 1H), 6.96 (d, J = 7.2 Hz, 1H), 5.76 (brs, 2H), 5.66 (brs, 2H), 5.22 (s, 2H), 2.59 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 165.25, 163.63, 155.63, 147.65, 136.43, 134.74, 133.52, 132.94, 131.91, 127.53, 124.43, 122.65, 94.52, 84.55, 43.39, 29.96, 18.45; HRMS (EI) *m/z* 377.1411 M^+, calc. for $C_{19}H_{19}N_7S$ 377.1423; Anal. Calc. for: $C_{19}H_{19}N_7S$ (377): C, 60.46; H, 5.07; N, 25.98%; Found: C, 60.42; H, 5.08; N, 26.00%.

2-(1-(4-Methyl-2-(4-(3-(piperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl)ethylidene) hydrazine-1-carboximidamide (13). Yellow solid (196 mg, 84%); mp = 255-257 °C. ^{1}H NMR (DMSO -d_6) δ: 11.53 (brs, 1H), 11.06 (brs, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.80 (brs, 2H), 7.67 (d, J = 8.4 Hz, 2H), 4.32 (s, 2H), 3.51 (m, 4H), 3.02 (m, 4H), 2.63 (s, 3H), 2.45 (s, 3H), 1.84 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 167.64, 161.53, 158.62, 147.29, 136.46, 132.90, 132.07, 127.33, 125.94, 89.57, 84.63, 53.17, 48.14, 30.90, 25.93, 24.91, 18.55; HRMS (EI) *m/z* 394.1938 M^+, calc. for

$C_{21}H_{26}N_6S$ 394.1940; Anal. Calc. for: $C_{21}H_{26}N_6S$ (394): C, 63.93; H, 6.64; N, 21.30%; Found: C, 63.96; H, 6.65; N, 21.34%.

2-(1-(4-Methyl-2-(4-(3-(2-methylpiperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl) ethylidene)hydrazine-1-carboximidamide (14). Yellow solid (196 mg, 84%); mp = 255-257 °C. ^{1}H NMR (DMSO -d_6) δ: 7.88 (d, J = 7.2 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 5.77 (brs, 4H), 3.78 (s, 2H), 3.55 (m, 2H), 3.51 (m, 1H), 2.59 (s, 3H), 2.31 (s, 3H), 1.61-1.19 (m, 6H), 1.06 (d, J = 4.8 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 161.69, 160.15, 148.55, 136.43, 133.09, 132.58, 126.33, 124.31, 87.68, 85.23, 54.72, 53.26, 43.91, 34.73, 26.32, 24.71, 20.33, 18.70, 16.49; HRMS (EI) *m/z* 408.2118 M^+, calc. for $C_{22}H_{28}N_6S$ 408.2096; Anal. Calc. for: $C_{22}H_{28}N_6S$ (408): C, 64.68; H, 6.91; N, 20.57%; Found: C, 64.70; H, 6.95; N, 20.54%.

2-(1-(4-Methyl-2-(4-(3-(3-methylpiperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl) ethylidene)hydrazine-1-carboximidamide (15). Yellow-white solid (203 mg, 87%); mp = 259-261 °C. ^{1}H NMR (DMSO -d_6) δ: 11.37 (brs, 1H), 10.92 (brs, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.72 (brs, 2H), 7.70 (d, J = 8.4 Hz, 2H), 4.34 (s, 2H), 3.57 (d, J = 9.6 Hz, 2H), 3.47 (m, 2H), 2.63 (s, 3H), 2.45 (s, 3H), 1.86 (m, 1H), 1.12-103 (m, 4H), 0.95 (d, J = 6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 163.51, 161.25, 158.12, 147.83, 136.74, 132.78, 132.11, 127.53, 124.97, 94.38, 86.43, 54.72, 53.26, 42.81, 33.73, 28.32, 24.71, 23.12, 18.53, 16.69; HRMS (EI) *m/z* 408.2105 M^+, calc. for $C_{22}H_{28}N_6S$ 408.2096; Anal. Calc. for: $C_{22}H_{28}N_6S$ (408): C, 64.68; H, 6.91; N, 20.57%; Found: C, 64.71; H, 6.94; N, 20.55%.

2-(1-(4-Methyl-2-(4-(3-(4-methylpiperidin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl) ethylidene)hydrazine-1-carboximidamide (16). Yellow solid (212 mg, 90.9%); mp = 263-265 °C. ^{1}H NMR (DMSO -d_6) δ: 7.88 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 5.74 (brs, 4H), 3.51 (s, 2H), 2.84 (t, J = 12.2 Hz, 4H), 2.59 (s, 3H), 2.31 (s, 3H), 2.17 (t, J = 10.8 Hz, 4H), 1.17 (m,

1H), 0.91 (d, J = 6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ: 161.61, 160.19, 148.52, 143.11, 136.38, 133.30, 132.52, 126.30, 124.27, 88.60, 84.82, 52.52, 47.43, 34.35, 30.36, 22.24, 18.65, 16.49; HRMS (EI) *m/z* 408.2096 M^+, calc. for $C_{22}H_{28}N_6S$ 408.2122; Anal. Calc. for: $C_{22}H_{28}N_6S$ (408): C, 64.68; H, 6.91; N, 20.57%; Found: C, 64.71; H, 6.94; N, 20.55%.

2-(1-(4-Methyl-2-(4-(3-(4-methylpiperazin-1-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl) ethylidene)hydrazine-1-carboximidamide (17). Orange solid (166 mg, 71.5%); mp = 253-255 °C. ^{1}H NMR (DMSO -d_6) δ: 8.01 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 5.75 (brs, 4H), 3.55 (s, 2H), 2.84 (m, 4H), 2.72 (s, 3H), 2.58 (s, 3H), 2.52 (m, 4H), 2.16 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 168.18, 165.19, 158.55, 142.31, 132.98, 131.95, 127.21, 124.93, 89.17, 84.12, 55.01, 52.03, 47.33, 46.34, 30.08, 18.55; HRMS (EI) *m/z* 409.2051 M^+, calc. for $C_{21}H_{27}N_7S$ 409.2049; Anal. Calc. for: $C_{21}H_{27}N_7S$ (409): C, 61.59; H, 6.65; N, 23.94%; Found: C, 61.61; H, 6.64; N, 23.97%.

2-(1-(4-Methyl-2-(4-(3-morpholinoprop-1-yn-1-yl)phenyl)thiazol-5-yl)ethylidene)hydrazine -1-carboximidamide (18). Dark-brown solid (192 mg, 82.4%); mp = 253-255 °C. ^{1}H NMR (DMSO -d_6) δ: 7.99 (d, J = 7.2 Hz, 2H), 7.58 (d, J = 7.2 Hz, 2H), 5.65 (brs, 2H), 5.48 (brs, 2H), 3.62 (s, 2H), 3.56 (m, 4H), 2.71 (s, 3H), 2.57 (s, 3H), 2.53 (m, 4H); ^{13}C NMR (DMSO-d_6) δ: 167.28, 165.49, 157.75, 141.37, 133.98, 132.45, 132.05, 126.21, 124.95, 89.17, 83.72, 58.01, 50.23, 44.13, 21.28, 18.55; HRMS (EI) *m/z* 396.1721 M^+, calc. for $C_{20}H_{24}N_6OS$ 396.1732; Anal. Calc. for: $C_{20}H_{24}N_6OS$ (396.5): C, 60.58; H, 6.10; N, 21.20%; Found: C, 60.61; H, 6.14; N, 21.17%.

2-(1-(4-Methyl-2-(4-(3-thiomorpholinoprop-1-yn-1-yl)phenyl)thiazol-5-yl)ethyl-idene) hydrazine-1-carboximidamide (19). Yellow solid (172 mg, 74.4%); mp = 263-264 °C. ^{1}H NMR (DMSO -d_6) δ: 7.96 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 5.65 (brs, 4H), 4.53 (s, 2H), 4.26 (m, 4H), 2.83 (m, 4H), 2.59 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6) δ: 167.11, 165.39, 158.75,

142.37, 133.78, 132.35, 126.11, 124.85, 89.17, 85.72, 58.34, 48.53, 43.13, 30.51, 18.98, 16.55; HRMS (EI) *m/z* 412.1488 M^+, calc. for $C_{20}H_{24}N_6S_2$ 412.1504; Anal. Calc. for: $C_{20}H_{24}N_6S_2$ (412): C, 58.22; H, 5.86; N, 20.37%; Found: C, 58.21; H, 5.84; N, 20.35%.

2-(1-(2-(4-(3-(Azepan-1-yl)prop-1-yn-1-yl)phenyl)-4-methylthiazol-5-yl)ethylidene) hydrazine-1-carboximidamide (20). Brown solid (196 mg, 84%); mp = 275-277 °C. ^{1}H NMR (DMSO -d_6) δ: 8.02 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 5.49 (brs, 2H), 5.48 (brs, 2H), 4.41 (t, *J* = 6.8 Hz, 4H), 4.02 (s, 2H), 2.57 (s, 3H), 2.34 (s, 3H), 1.95-1.05 (m, 8H); ^{13}C NMR (DMSO-d_6) δ: 165.49, 161.15, 157.86, 147.45, 136.43, 133.19, 132.48, 126.23, 124.41, 89.68, 84.93, 59.76, 44.41, 34.71, 27.33, 23.70, 18.49; HRMS (EI) *m/z* 408.2081 M^+, calc. for $C_{22}H_{28}N_6S$ 408.2096; Anal. Calc. for: $C_{22}H_{28}N_6S$ (408): C, 64.68; H, 6.91; N, 20.57%; Found: C, 64.70; H, 6.95; N, 20.54%.

2-(1-(4-Methyl-2-(4-(3-(octahydroisoquinolin-2(1*H*)-yl)prop-1-yn-1-yl)phenyl)thiazol-5-yl) ethylidene)hydrazine-1-carboximidamide (21). Yellow solid (183 mg, 80%); mp = 253-255 °C. ^{1}H NMR (DMSO -d_6) δ: 7.88 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 5.76 (brs, 2H), 5.66 (brs, 2H), 3.51 (s, 2H), 2.87 (t, *J* = 10.8 Hz, 2H), 2.73 (d, *J* = 8.8 Hz, 2H), 2.59 (s, 3H), 2.31 (s, 3H), 1.86-0.92 (m, 12H); ^{13}C NMR (DMSO-d_6) δ: 160.51, 158.90, 148.96, 143.81, 136.58, 132.50, 128.63, 126.39, 124.17, 88.58, 84.92, 59.12, 52.89, 47.69, 41.95, 41.44, 32.35, 29.91, 26.60, 26.17, 18.55, 16.47; HRMS (EI) *m/z* 448.2417 M^+, calc. for $C_{25}H_{32}N_6S$ 448.2409; Anal. Calc. for: $C_{25}H_{32}N_6S$ (448.6): C, 66.93; H, 7.19; N, 18.73%; Found: C, 66.91; H, 7.16; N, 18.75%.

Microbiological assays:

Bacterial strains, mammalian cell lines and antibiotics. Bacterial strains used in this study were obtained from the Biodefense and Emerging Infections Research Resources Repository (BEI Resources) and the American Type Culture Collection (ATCC). Human colorectal

adenocarcinoma (Caco-2) cell line, human keratinocyte cell line (HaCaT) and murine macrophage (J774) cells were purchased from American Type Culture Collection (ATCC). Linezolid (Chem-Impex International, Wood Dale, IL, USA) and vancomycin hydrochloride (Gold Biotechnology, St. Louis, MO, USA), were purchased from commercial vendors. Phenylthiazole compounds were prepared as a stock concentration of 10 mg/mL in DMSO.

Determination of MICs and MBCs of the new phenylthiazole compound series against *Staphylococcus aureus* clinical isolates. The broth microdilution method was utilized to test the antibacterial activity of the new set of phenylthiazole compounds against a panel of clinically-important *S. aureus* strains according to the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI).[39]

Time-kill assay against MRSA. The test was performed against MRSA USA400, as described previously[10]. Briefly, MRSA USA400 cells in logarithmic growth phase were diluted and compounds were added at 4 × MIC (using triplicate samples for each test agent). At the corresponding time intervals, samples were collected, diluted and plated onto Tryptic soy agar plates. Plates were incubated aerobically at 37 ℃ for at least 18 hours before determining the number of viable colony forming units (CFU)/mL.

Multi-step resistance study against MRSA. The ability of MRSA USA400 to develop resistance to the new set of phenylthiazoles was investigated via a multi-step resistance study, as described previously[11]. Resistance was defined as a greater than four-fold increase from the initial MIC[40].

MRSA biofilm eradication assessment. Compounds **14-16** and **19** were examined for their ability to disrupt a well-established, mature MRSA USA300 biofilm using the microtiter plate biofilm formation assay, following a procedure described previously.[18]

***In vivo* pharmacokinetic study.** Compound **15**'s pharmacokinetic profile was determined following Institutional Animal Care and Use Committee guidelines, as described in a previous report.[17]

ASSOCIATED CONTENTS

Supporting information. The supporting information is available free of charge on the ACS publication website. The strains used in the manuscript and their corresponding sites of isolation, ^{1}H and ^{13}C NMR spectra of all new described compounds.

Abbreviations. PK, pharmacokinetic; $t_{1/2}$, half-life; CL, clearance; C_{max}, maximum plasma concentration; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; VISA, vancomycin-intermediate *Staphylococcus aureus*; VRSA, vancomycin-resistant *Staphylococcus aureus*.

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