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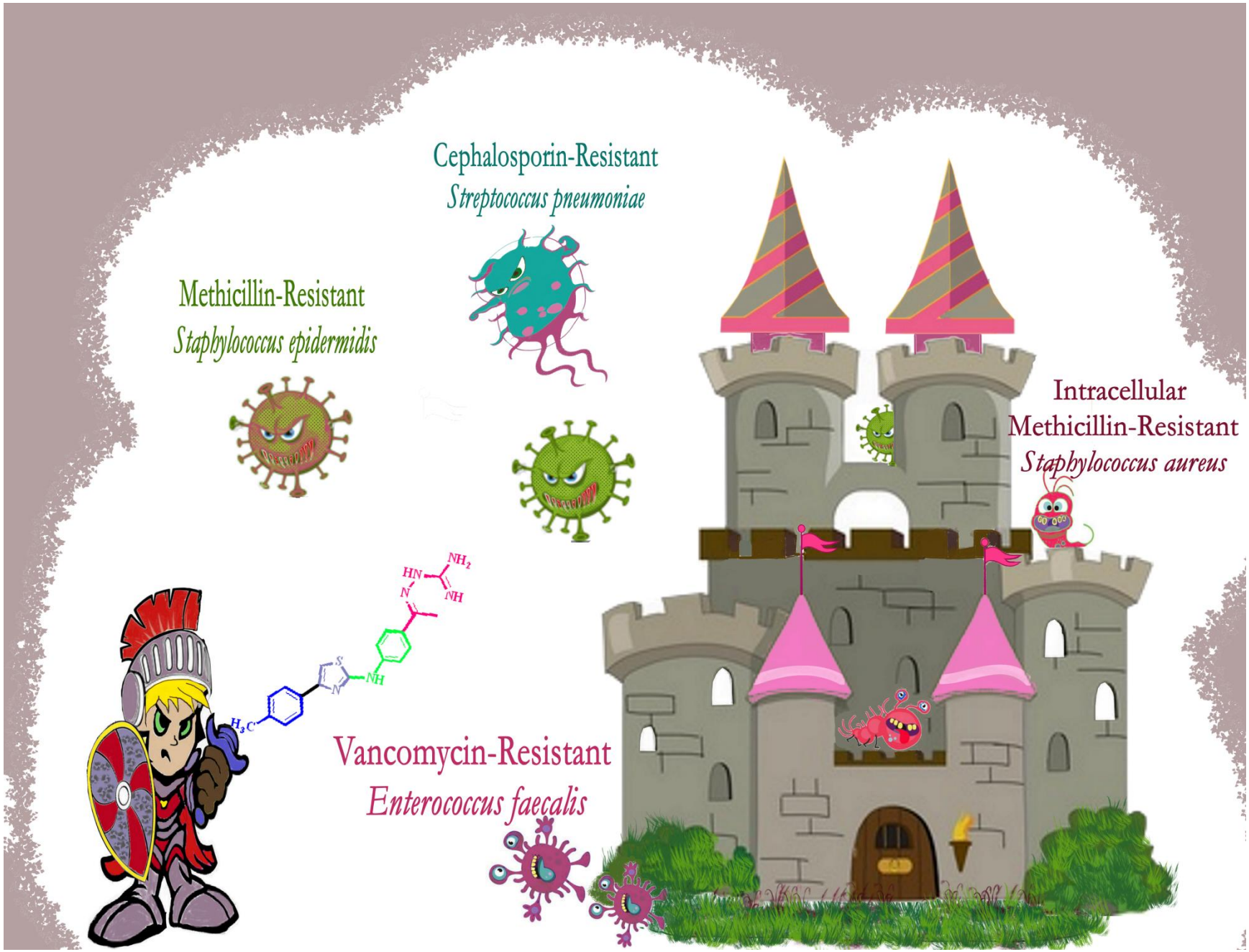
Cephalosporin-Resistant
Streptococcus pneumoniae
Methicillin-Resistant
Staphylococcus epidermidis
Intracellular
Methicillin-Resistant
Staphylococcus aureus
Vancomycin-Resistant
Enterococcus faecalis

Evaluation of *N*-Phenyl-2-aminothiazoles for Treatment of Multi-Drug Resistant and Intracellular *Staphylococcus aureus* Infections

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Abstract. The increasing emergence of antibiotic-resistant bacterial pathogens calls for additional urgency in the development of new antibacterial candidates. *N*-Phenyl-2-aminothiazoles are promising candidates that possess potent anti-MRSA activity and could potentially replenish the MRSA antibiotic pipeline. The initial screen of a series of compounds in this novel class against several bacterial strains revealed that the aminoguanidine analogues possessed promising activities and superior safety profiles. The determined MICs of these compounds were comparable to, if not better than, those of the control drugs (linezolid and vancomycin). Remarkably, compounds **3a**, **3b,** and **3e** possessed potent activities against multidrug resistant staphylococcal isolates and several clinically important pathogens, such as vancomycin-resistant enterococci (VRE) and *Streptococcus pneumoniae*. In addition, the compounds were superior to vancomycin in the rapid killing of MRSA and the longer post-antibiotic effects. Furthermore, low concentrations of compounds **3a**, **3b,** and **3e** reduced the intracellular burden of MRSA by greater than 90%. Initial *in vitro* PK/toxicity assessments revealed that compound **3e** was highly tolerable and possessed a low metabolic clearance rate and a highly acceptable half-life.

Key words: Intracellular bacteria, post-antibiotic effect, methicillin-resistant *Staphylococcus aureus*, bacterial resistance, vancomycin-resistant *Enterococci.*

1. Introduction. Based on the rapid emergence of global antimicrobial resistance (AMR), the decreasing efficacy of current antibiotics, and their extensive effects on public health and the global economy, immediate action is urgently needed to identify and develop new therapeutic agents that will refresh the declining antibiotic pipeline. Over $20 billion are estimated to be spent annually in the United States healthcare system to treat antibiotic-resistant infections. Additionally, drug-resistant bacteria are infecting more than 7,500 people daily and cause more than 35,000 deaths each year in the United States.[1] Most worrisome, resistance has been reported to every known class of antibiotics.[2]

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a growing threat human health as a global nosocomial endemic due to the development of concomitant resistance to the most commonly used antibiotics, such as penicillins, aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides.[3] Moreover, due to the emerging resistance to both vancomycin and linezolid, the drugs of last resort for the treatment of staphylococcal infections, the morbidity and mortality of MRSA have significantly increased, resulting in a substantial economic burden and severe clinical consequences [4].

The World Health Organization (WHO) has declared both methicillin-resistant and vancomycin-intermediate or -resistant *Staphylococcus aureus* (MRSA, VISA and VRSA, respectively) as high priorities in the priority pathogen list of antibiotic-resistant bacteria [5]. According to the Centers for Disease Control and Prevention (CDC), MRSA is categorized as a serious threat pathogen. Approximately 323,700 patients with MRSA infections were hospitalized in 2017 in the United States, and these infections were associated with approximately 10,600 deaths. [1] Moreover, up to 53 million people globally may be potential carriers of MRSA, posing a high risk of infection to themselves and others. [6] Overall, *S. aureus*

is deemed the main culprit behind healthcare-associated infections (HAIs) in Europe and the United States, most of which are caused by MRSA.[7-10] Thus, this highly evolving superbug requires swift action to combat its infections.

In 2014, our group launched a project aiming to discover and develop new compounds with antibacterial activity against multidrug-resistant bacteria. A highly active novel phenylthiazole scaffold **I** (Figure 1) was discovered.[11] The main features that actively play a role in the antibacterial activity were postulated to include a thiazole nucleus flanked by two main structural components: a lipophilic moiety at C2 and a guanidine group at C5. [11] Despite its anti-MRSA activity, the scaffold suffered from extensive hepatic metabolism. A pyrimidine junction was inserted to conjoin the aminoguanidine tail with the thiazole core **II** in an attempt to further improve the pharmacokinetic (PK) performance of the lead compound **I** (Figure 1).[12] This pyrimidine ring enhanced the PK properties but conversely led to a decrease in its aqueous solubility, therefore limiting its oral bioavailability and excluding it from the drug-like category.[12] Accordingly, the next study aimed at increasing the topological polar surface area (tPSA)/lipophilicity ratio by substituting the pyrimidine ring with the more hydrophilic oxadiazole ring **III**.[13] The oxadiazole ring fulfilled the required physicochemical target, but proved to be less active against MRSA than its pyrimidine analogue. Based on this information, a new strategy was devised to increase the antibacterial activity without compromising the augmented oral bioavailability. The hetero ring junction, a key element required to balance the metabolic stability and the physicochemical properties, was further modulated into a thiazole ring **IV** (Figure 1).[14] This modification yielded compounds with potent anti-MRSA activity and hepatic metabolic stability, and most importantly, they eradicated intracellular MRSA.

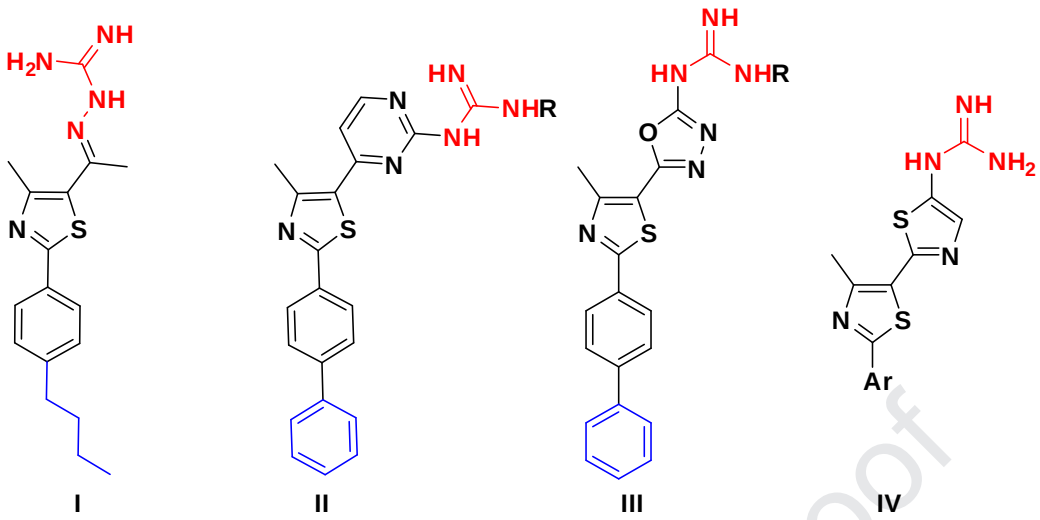


Figure 1. The transitional stages in optimizing the 'drug-likeness' of the phenyl thiazole anti-MRSA nucleus. Lipophilic component colored blue and cationic part colored red for the sake of clarity.

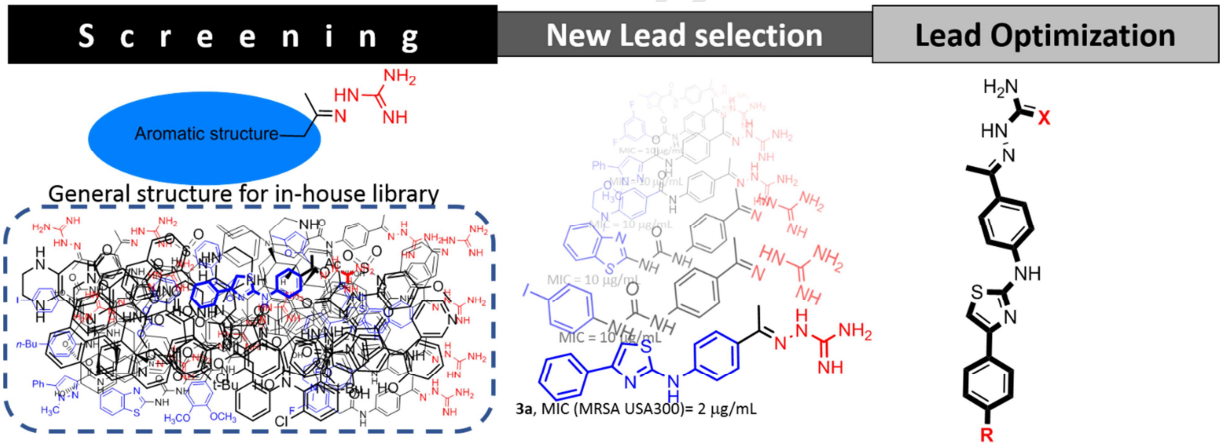


Figure 2. Progress in development of novel antimicrobials and the aim of the present work.

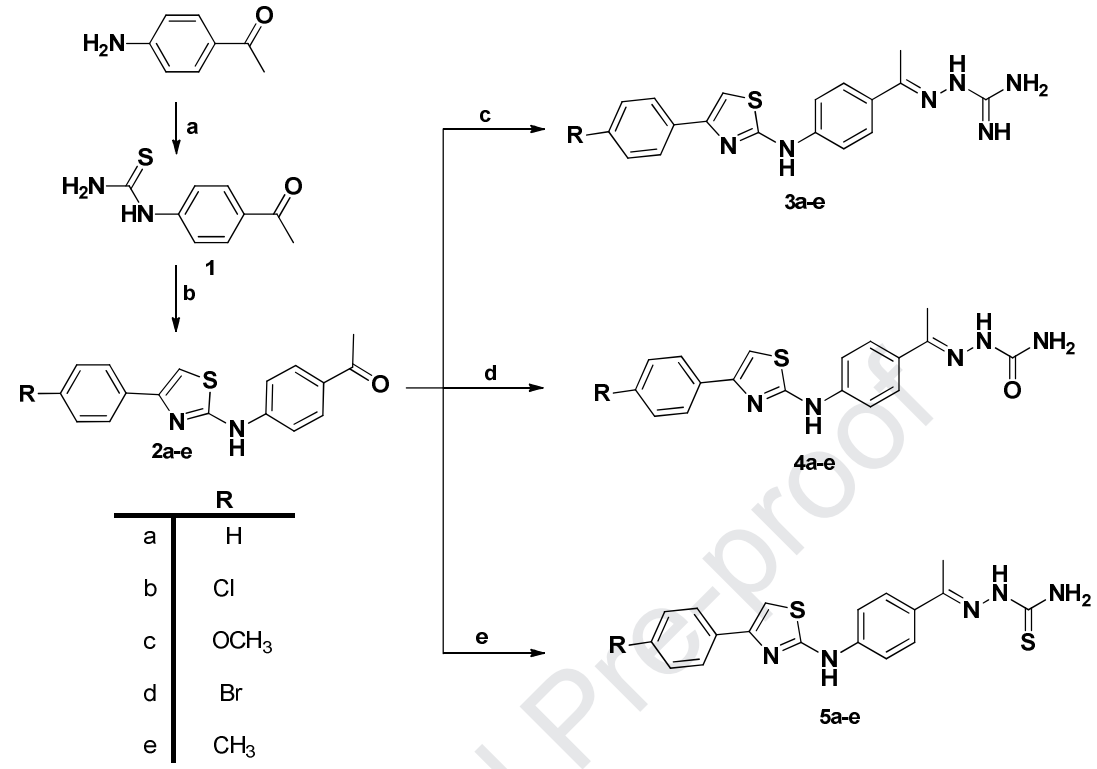
In addition to the phenylthiazole nucleus, screening of an in-house library of aminoguanidines connected with diverse aromatic structures provided a limited number of novel structures (Figure 2).[15] Among the newly obtained leads, the *N*-phenyl-2-aminothiazole **3a** was one of the most potent compounds of those tested against MRSA USA300 (MIC = 2 µg/mL). Other compounds showed modest activities (MICs ranged from 8 to 16 µg/mL) (Figure 2).[15] Therefore, in the present study, the SAR of this novel lead compound **3a** was studied by focusing on the two sides of the structure. First, the aminoguanidine moiety was replaced with

the highly structurally related semicarbazide and its thio analogues. Second, the substitution of the terminal phenyl with electronically different groups was achieved. The new compounds were initially screened against MRSA and *E. coli* strains, and the metabolic stability of the most promising analogue was investigated. Additionally, selected potent derivatives were further subjected to bacteriological profiling.

2. Results and Discussion

2.1. Chemistry. The acetyl phenyl thiourea **1** was obtained by reacting 4-amino acetophenone with potassium thiocyanate in presence of water that had been acidified with hydrochloric acid. The base-promoted cyclisation of compound **1** with phenacyl bromides produced the thiazole derivatives **2a-e,** which are the main intermediates, from which the final products were afforded. As shown in **Scheme 1**, the acetyl moiety in compounds **2a-e** was replaced with various cationic tails, such as aminoguanidine, semicarbazide, and thiosemicarbazide, and furnished the desired final compounds **3a-e**, **4a-e,** and **5a-e**, respectively.

Scheme 1



Reagents and conditions: (a) KSCN, conc. HCl, H_2O, reflux 6h; (b) phenacyl bromide derivative, anhydrous sodium acetate, THF, reflux 5h; (c) aminoguanidine HCl, conc. HCl, C_2H_5OH, reflux 2 h; (d) semicarbazide HCl, conc. HCl, C_2H_5OH, reflux 10 h; (e) thiosemicarbazide HCl, conc. HCl, C_2H_5OH, reflux 10h.

The structures of compounds **2a-e**, **3a-e**, **4a-e,** and **5a-e** were supported by the results of elemental analyses and spectroscopic data: IR, ^{1}H NMR and ^{13}C NMR. The structures of compounds **2a-e** were confirmed by the disappearance of the exchangeable signal of the NH_2 protons at δ 3.40 ppm and the appearance of the singlet signal corresponding to the CH of thiazolyl moiety in the range between δ 7.35-8.13 ppm, unlike its precursor, compound **1**.

The IR spectra of target compounds **3a-e** were characterized by the disappearance of the C=O band, while the ^{1}H NMR spectra showed the appearance of new exchangeable singlet signals of NH at δ 7.34-11.38 ppm and NH_2 at δ 7.78-11.49 ppm. The IR spectra of compounds **4a-e** showed new C=O bands at 1654-1693 cm^{-1}, which were also confirmed by the presence of signals in the ^{13}C NMR spectra at δ 158.1-162.8 ppm. The ^{1}H NMR spectra revealed new

exchangeable NH singlet signals at δ 8.86-10.66 ppm and NH_2 at δ 4.29-6.55 ppm. Finally, the structures of compounds **5a-e** were confirmed by the appearance of new C=S bands in IR spectra at 1083-1296 cm^{-1} and by ^{13}C NMR peaks at δ 178.8 -197.9 ppm. The ^{1}H NMR spectra also supported the attachment of the thiosemicarbazide moiety because a new exchangeable NH_2 signal appeared at δ 4.50-8.15 ppm.

2.2. Biological results and discussion

2.2.1. Antimicrobial activity

2.2.1.1. Initial screening against methicillin-resistant S. aureus, and *E. coli strains*. The initial screening results revealed that the most active derivatives belonged to the aminoguanidine series **3a-e**. Compounds **3a**, **3b**, and **3e** exhibited the most potent activity against MRSA USA300, with MIC values ranging from 2 to 4 µg/mL. Additionally, they exhibited one-fold higher MIC values against *tolC*-mutant *E. coli* (deficient in *tolC*, an essential component of the AcrAB-TolC efflux pump). They also exhibited a lower antibacterial activity against the wild-type *E. coli* (MICs = 8-16 µg/mL). Thus, the lower activity of the compounds against *E. coli* may be attributed to their efflux out of the bacterial cell. These 3 compounds were selected for further investigations.

Table 1. Initial screening of all the synthesized compounds against methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* isolates.

Compounds/ Control antibiotics	**MICs (µg/mL)**		
	MRSA NRS384 (USA300)	***E. coli* JW55031 (*tolC*-mutant)**	***E. coli* BW25113 (wild-type strain)**
2a	>64	>64	>64
2b	>64	>64	>64
2c	>64	>64	>64
2d	>64	>64	>64
2e	>64	64	>64
3a	2	4	8
3b	4	8	8
3c	32	2	4
3d	16	2	4

Compounds/ Control antibiotics	**MICs (µg/mL)**		
	MRSA NRS384 (USA300)	***E. coli* JW55031 (*tolC*-mutant)**	***E. coli* BW25113 (wild-type strain)**
3e	2	4	16
4a	>64	>64	>64
4b	>64	>64	>64
4c	>64	>64	>64
4d	>64	>64	>64
4e	32	32	>64
5a	>64	>64	>64
5b	32	64	>64
5c	>64	>64	>64
5d	>64	>64	>64
5e	>64	>64	>64
Vancomycin	1	NT[1]	NT
Gentamicin	NT	0.25	0.25

[1]NT: not tested

*2.2.1.2. Antibacterial activity of compounds **3a**, **3b** and **3e** against a wide panel of clinical staphylococcal isolates.* After the initial screen, we investigated the activity of compounds **3a, 3b** and **3e** against clinical isolates of methicillin-sensitive, methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* (MSSA, MRSA, and VRSA). As presented in Table 2, the compounds maintained their potent activity against the tested MSSA, MRSA, and VRSA strains, inhibiting their growth at concentrations ranging from 1 to 2 µg/mL. These compounds were equipotent to vancomycin (MICs ranged from 1 to 2 µg/mL against MSSA and MRSA) and linezolid (MICs = 1-2 µg/mL against MSSA and MRSA, except for MRSA NRS107). Interestingly, they maintained their potency against linezolid-resistant and vancomycin-resistant staphylococcal strains. Furthermore, compounds **3a**, **3b,** and **3e** displayed bactericidal activity against the tested strains, as their MBC values were equal to or one- to three-fold higher than their corresponding MICs. Thus, these compounds were superior to linezolid, a frontline drug used to treat staphylococcal infections that exhibits a bacteriostatic activity. As previously

established, chlorophenylthiazoles exert a profound effect on the antibacterial activity.[16] Analogously, compound **3b** emerged as the most potent analogue against the tested staphylococcal strains.

Table 2. MICs and MBCs in µg/mL of compounds **3a**, **3b** and **3e** against *Staphylococcus aureus* clinical isolates.

Bacterial strains	**Compounds**/control antibiotics									
	3a		**3b**		**3e**		Linezolid		Vancomycin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MSSA ATCC 6538	2	2	1	1	2	4	1	16	1	2
MSSA NRS 107	1	4	1	1	2	2	1	64	2	2
MRSA NRS119	2	4	1	1	2	4	64	> 64	1	2
MRSA NRS123 (USA400)	2	2	1	1	2	2	1	32	1	1
MRSA NRS 385 (USA500)	2	2	1	2	2	2	1	32	1	1
MRSA NRS 386 (USA700)	2	8	1	4	2	2	2	64	1	1
VRSA 10	2	2	1	1	2	2	1	> 64	64	> 64
VRSA 12	2	4	1	1	2	2	1	64	64	64

*2.2.1.3. Spectrum of antibacterial activity***.** Furthermore, the spectra of antibacterial activity of the three most potent derivatives was examined against a panel of clinically relevant Gram-positive bacterial pathogens. Consistent with the results presented in **Table 2**, the compounds maintained their notable antibacterial activity against different known Gram-positive pathogens by inhibiting their growth at concentrations of 1 to 2 µg/mL (**Table 3**). Interestingly, they exhibited potent activity against *S. epidermidis*, a common cause of skin infections, and implanted medical device infections. Due to formation of strongly attached biofilms, *S. epidermidis* infections are difficult to treat.[17, 18] Furthermore, the compounds retained their superiority to vancomycin when screened against vancomycin-resistant enterococci (VRE), a leading cause of nosocomial infections that causes 20-30% of VRE infections in the USA.[19] In

addition, the potent activities of compounds **3a**, **3b** and **3e** were further extended to include *Listeria monocytogenes* and *Streptococcus pneumoniae.* The MBCs of the tested compounds were equal to or one- to three-fold higher than their corresponding MICs, indicating that the compounds exhibited bactericidal activity against the tested strains.

Table 3. MICs and MBCs (µg/mL) of compounds **3a**, **3b** and **3e** against clinically important Gram-positive bacterial pathogens

Bacterial Strains	**Compounds**/control antibiotics									
	3a		**3b**		**3e**		Linezolid		Vancomycin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Methicillin-resistant *Staphylococcus epidermidis* NRS101	1	1	1	1	1	1	1	16	1	2
Vancomycin-resistant *Enterococcus faecalis* ATCC 51299	2	4	1	4	2	4	1	16	32	64
Vancomycin-resistant *Enterococcus faecium* ATCC 700221	2	4	1	2	2	4	1	16	>64	>64
***Listeria monocytogenes* ATCC 19111**	1	4	1	2	1	2	≤0.5	16	1	1
Cephalosporin-resistant *Streptococcus pneumoniae* ATCC 51916	2	4	2	4	2	4	1	32	1	2
Methicillin-resistant *Streptococcus pneumoniae* ATCC 700677	2	8	2	4	2	8	1	16	2	2

2.2.1.4 Time to kill assay. One of the most fundamental parameters to consider when designing a therapeutic is the killing kinetics of the drug. Fast-acting drugs hold a significant advantage over the slow-acting drugs because they are more efficacious in eradicating the infection. Although vancomycin is the drug of choice for treating MRSA infections, its clinical efficiency is affected by its slow bactericidal mode of action, which leads to difficulty in eradicating MRSA. A time to kill assay was conducted with 5 × MIC against MRSA USA 400 to confirm the bactericidal properties of the three most promising compounds (Figure 3). As mentioned above, vancomycin exhibited a slow bactericidal activity. Vancomycin reduced the bacterial count by 3-$\log_{10}$ after 12 hours and completely eradicated it after 24 hours. Remarkably, the tested compounds exhibited

rapid bactericidal activity and required only two hours to reduce the bacterial count by 3-$\log_{10}$. Compound **3b** exhibited the most efficient bactericidal activity by completely eradicating the high MRSA count within two hours. However, compounds **3a** and **3e** completely eliminated the high inoculum after eight hours.

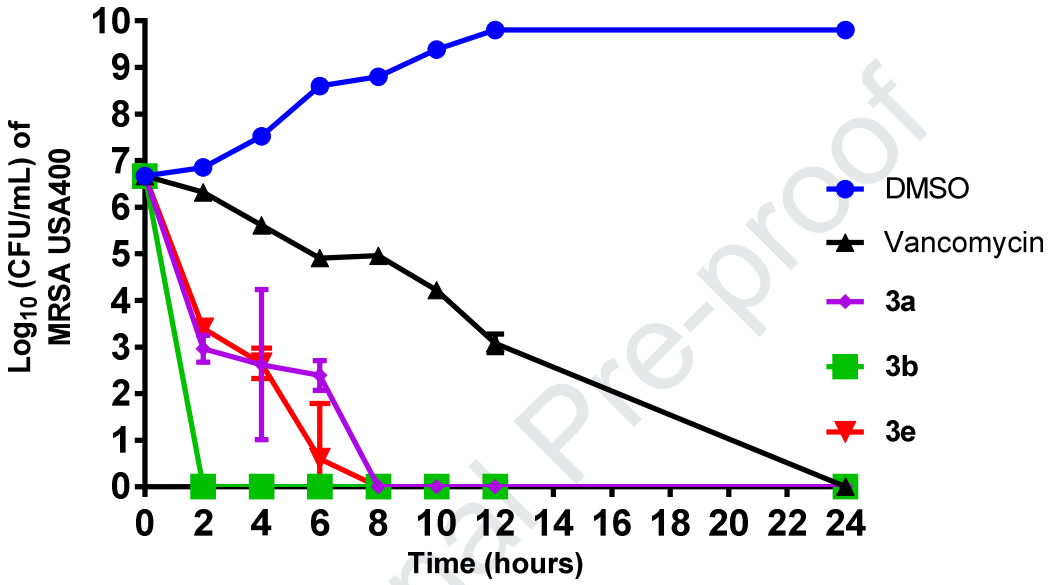


Figure 3. Killing kinetics of **3a**, **3b** and **3e** (tested in triplicates at 5 × MIC) against methicillin-resistant *Staphylococcus aureus* NRSA 123 (MRSA USA400) over a 24-h incubation period at 37°C. DMSO (solvent for the compounds) served as a negative control and vancomycin served as a control drug. The error bars represent standard deviation values obtained from triplicate samples used for each compound/antibiotic studied.

2.2.2. Post-antibiotic effect. The rapid bactericidal activity of *N*-phenyl-2-aminothiazoles encouraged us to investigate the post-antibiotic effect (PAE) of these compounds. Drugs exhibiting a long PAE are considered advantageous, as these agents may require fewer doses in the clinic.[20] The PAEs of compounds **3b** and **3e** were tested against MRSA USA400. The time required for bacteria exposed to each test agent to increase by 1-$\log_{10}$ relative to the untreated control was determined. As shown in Table 4, vancomycin possessed a shorter PAE of 2 hours,

consistent with previous reports.[21, 22] and **3a** showed the same PAE as vancomycin. Conversely, compound **3e** exhibited a long PAE of 6 hours, while compound **3b** exhibited a shorter PAE of 4 hours, exceeding the PAE of vancomycin.

Table 4. Post-antibiotic effect (PAE) of **3b**, **3e** and vancomycin (tested at 5 × MIC) against methicillin-resistant *Staphylococcus aureus* NRS 123 (USA400).

PAE (hours)			
3a	**3b**	**3e**	Vancomycin
2	4	6	2

2.2.3. *Toxicity evaluation.* The development of a safe antimicrobial agent is a top priority in any research endeavor, where minimizing toxicity to host tissues must be carefully assessed. Consequently, the three most auspicious compounds (**3a**, **3b,** and **3e**) were thoroughly examined for cytotoxicity toward human colorectal adenocarcinoma (Caco-2) cells and human keratinocyte (HaCaT) cells (Figures 4 & 5).

2.2.3.1 In vitro cytotoxicity assay using Caco-2 cells. The compounds displayed an acceptable tolerability to Caco-2 cells at high concentrations. Compounds **3a** and **3b** were nontoxic to Caco-2 cells at a concentration of 32 µg/mL, with approximately 100% cell viability recorded. Nevertheless, compound **3e** out-performed all the other compounds, as approximately 100% of the cells were still viable after treatment with a concentration as high as 64 µg/mL (Figure 4). These concentrations represent 16 to 64 times their MIC values against MRSA strains, providing them a broad safety window.

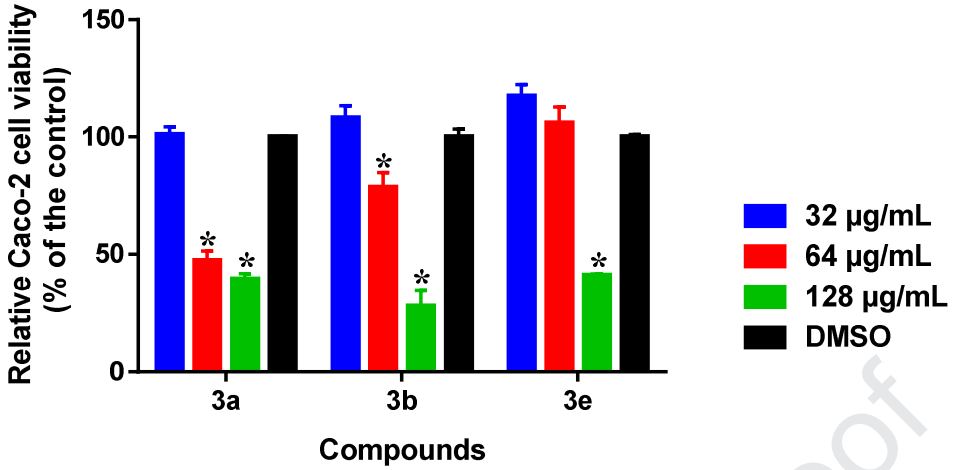


Figure 4. Analyzing the toxicity of compounds **3a**, **3b** and **3e** (tested in triplicates at 32, 64 and 128 µg/mL) against human colorectal cells (Caco-2) using the MTS assay. Results are presented as percent viable cells relative to DMSO (negative control to determine a baseline measure for the cytotoxic impact of each compound). The absorbance values represent an average of three samples analyzed for each compound. Error bars represent standard deviation values. Data were analyzed via a two-way ANOVA with post hoc Dunnett's test for multiple comparisons. * denotes a statistical difference ($P<0.05$) between values obtained for the compounds and the DMSO.

2.2.3.2. In vitro cytotoxicity assay using HaCaT cells. Similar to the results obtained with Caco-2 cells, the tested compounds exhibited a good toxicity profile at high concentrations when evaluated against HaCaT cells. All three compounds were nontoxic to HaCaT cells at a concentration of 32 µg/mL, as approximately 100% of the cells were viable (Figure 5).

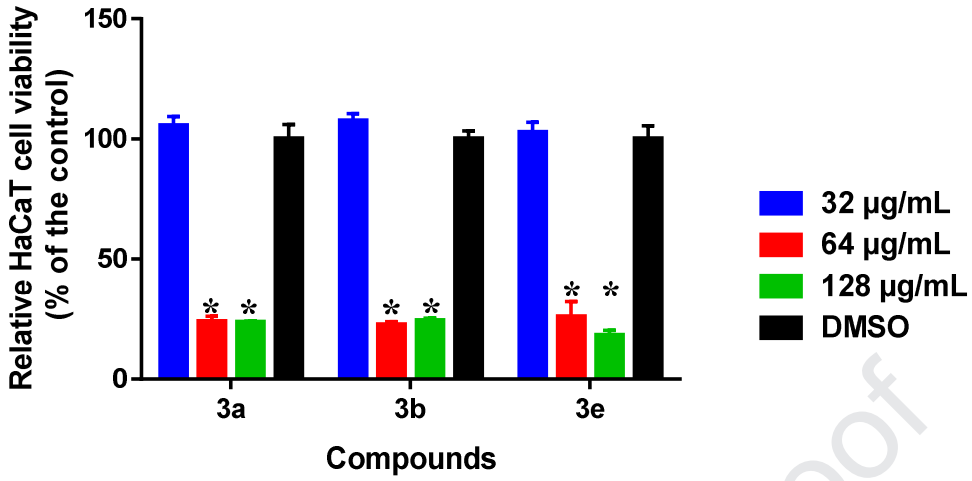


Figure 5. Analyzing the toxicity of compounds **3a**, **3b** and **3e** (tested in triplicates at 32, 64 and 128 µg/mL) against human keratinocyte cells (HaCaT) using the MTS assay. Results are presented as percent viable cells relative to DMSO (negative control to determine a baseline measure for the cytotoxic impact of each compound). The absorbance values represent an average of three samples analyzed for each compound. Error bars represent standard deviation values. Data were analyzed via a two-way ANOVA with post hoc Dunnett's test for multiple comparisons. * denotes a statistical difference ($P<0.05$) between values obtained for the compounds and the DMSO.

*2.2.4. Intracellular infection of J774 cells with MRSA and treatment with compounds **3a**, **3b** and **3e***. The rapid and potent bactericidal activity of *N*-phenyl-2-aminothiazoles, as well as the longer post-antibiotic effect prompted us to investigate whether these compounds penetrate macrophages infected with MRSA and kill the bacteria intracellularly. MRSA is capable of multiplying within the phagolysosome after being taken up by macrophages, which allows the bacteria to survive and reinfect the host.[23] Most antibiotics are inactive against intracellular MRSA, including the frontline drugs vancomycin and linezolid.[24, 25] The progenitor phenylthiazoles exhibited significant intracellular clearance activity.[26] This finding prompted us to investigate the intracellular clearance activity of the newly synthesized compounds. First, the compounds were tested for their cytotoxicity toward murine macrophages (J774).

Compounds **3b** and **3e** were nontoxic to J774 cells at concentrations up to 32 µg/mL, whereas compound **3a** was nontoxic at the concentration of 16 µg/mL, with 100% cell viability observed (Figure 6A). Based on the results of the cytotoxicity assay, the compounds were tested for their intracellular clearance activity at their nontoxic concentrations. As depicted in Figure 6B, vancomycin was incapable of reducing the intracellular MRSA burden, consistent with previous reports. Remarkably, compound **3e** exhibited the highest intracellular clearance activity, reducing the intracellular MRSA burden by approximately 2.49-$\log_{10}$, which is equivalent to a 99% reduction at 4× MIC (8 µg/mL). Even at a lower concentration (2× MIC), compound **3e** still managed to reduce the intracellular infection by 1.19-$\log_{10}$, which is equivalent to 93% reduction. Compound **3a** generated a 1.19-$\log_{10}$ reduction (93% reduction in CFU) at 4× MIC (8 µg/mL). This value was reduced to an approximately 1.07-$\log_{10}$ reduction (88% reduction) when the concentration was decreased by one-fold (2× MIC). Compound **3b** generated a 1.56-$\log_{10}$ reduction (an approximately 97% reduction) and 0.51-$\log_{10}$ reduction (an approximately 69% reduction) at 4× and 2× MIC, respectively. Consequently, these three analogues not only exhibited a more rapid bactericidal activity against MRSA than vancomycin but also surpassed vancomycin in the ability to reduce the intracellular MRSA burden inside infected macrophages.

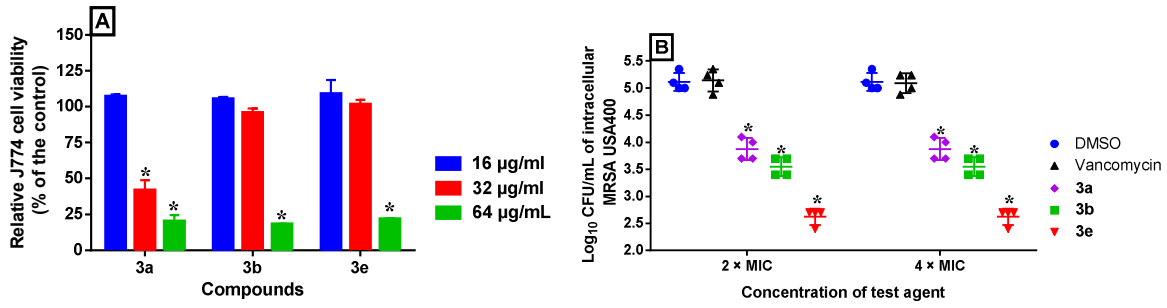


Figure 6A. *In vitro* analysis of the cytotoxicity of compounds **3a**, **3b** and **3e** toward murine macrophage (J774) cells using the MTS assay. Results are presented as average percentages of viable mammalian cells relative to DMSO. Data were analyzed using a two-way ANOVA with

Dunnett's post hoc test for multiple comparisons. * denotes a statistically significant difference ($P<0.05$) between values obtained for the compounds and DMSO.

Figure 6B. Examination of the activity of compounds **3a**, **3b** and **3e** toward the clearance of intracellular MRSA present inside murine macrophage (J774) cells. Data are presented as $\log_{10}$ CFU/mL of intracellular MRSA USA400 inside infected murine macrophages after treatment with 2 × MIC and 4 × MIC of compound **3a**, **3b**, **3e** or vancomycin (tested in quadruplicate) for 24 h. Data were analyzed using a two-way ANOVA, followed by Dunnett's post hoc test for multiple comparisons ($P < 0.05$) utilizing GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). Asterisks (*) denote statistically significant differences between treatment with compound **3a**, **3b** or **3e** and vancomycin.

Multi-step resistance study against MRSA. Having demonstrated that *N*-phenyl-2-aminothiazoles compounds could reduce the burden of MRSA inside macrophages, we proceeded to evaluate the ability of MRSA to develop resistance against these compounds. A multi-step resistance selection test was conducted against MRSA USA400. Interestingly, the MIC of **3e** changed by 1-fold only after the thirteenth passage without additional increase thereafter (Figure 7). The MIC values of **3b** increased by one-fold after the seventh passage and by three folds after the fourteenth passage. Moreover, the MIC values of **3a** increased one-fold after the fifth passage and another 3-fold-increase was observed after the eleventh passage. These results indicate that MRSA is unlikely to form rapid resistance to *N*-Phenyl-2-aminothiazoles, even after exposure to them for 14 passages. In contrast, MICs of ciprofloxacin, one of the antibiotics used in the treatment of MRSA infections, rapidly increased. MIC of ciprofloxacin increased by one-fold after the second passage and continued to increase up to a 127-fold increase by passage 14.

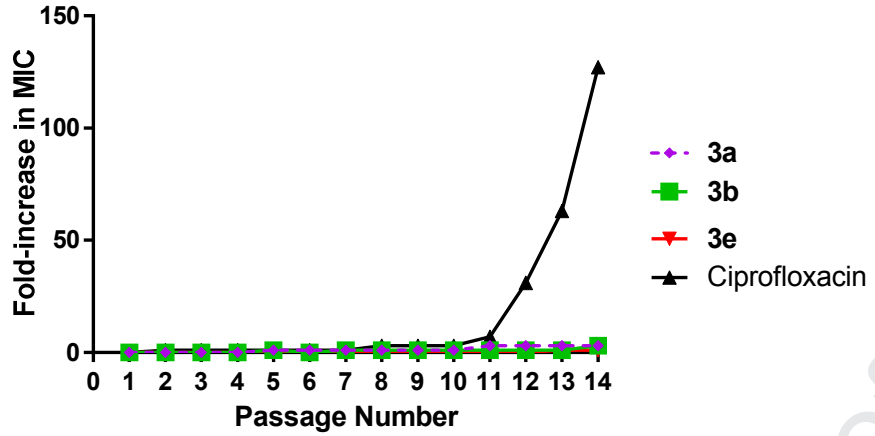


Figure 7. Multi-step resistance selection of *N*-Phenyl-2-aminothiazoles **3a, 3b** and **3e** and ciprofloxacin against methicillin-resistant *S. aureus* USA400 (NRS123). Bacteria were serially passaged over a 14-day period and the broth microdilution assay was used to determine the MIC of each test agent after each successive passage. A four-fold shift in MIC would be indicative of bacterial resistance to the test agent.

2.2.5 Initial assessment of the physicochemical properties and pharmacokinetic parameters. One of the most critical characters of candidate drugs that must be evaluated early in the drug development process is the physicochemical properties. The physicochemical properties of the most potent analogue, **3e**, were explored. First, the aqueous solubility was analyzed using a turbidometric solubility assay in phosphate-buffered saline. Solubility directly affects the pharmacokinetic profiles of compounds, routes of administration and the formulation of compounds into suitable dosage forms.[27] Compound **3e** showed moderate aqueous solubility compared with two FDA-approved drugs, rifampicin and tamoxifen (Table 5).

Table 5. Evaluation of solubility in phosphate-buffered saline (PBS)

Compound/Drug	Solubility limit (μM)	cLogP	Note
3e	26.7	4.12	
Rifampicin	183.3		Reference with good solubility
Tamoxifen	2.1		Reference with limited solubility

The solubility limit corresponds to the highest concentration of test compound at which no precipitate was detected (OD_{540})

The antibacterial activity of the synthesized compounds is not the sole factor for them to be considered successful candidates. The compounds must possess a set of drug-like properties. For instance, compounds should possess an acceptable metabolic clearance rate to guarantee the survival of the candidate compounds through phase 1 clinical trials in humans. The metabolic stability and clearance rate of the most potent derivative, **3e**, were evaluated through an incubation with human liver microsomes to address these needs (Table 6). The intrinsic half-life ($t_{1/2}$) of compound **3e** in the presence of NADPH was approximately 2 hours, which is comparable to propranolol and higher than imipramine, drugs with a low clearance rate. Despite the increase in its half-life in the absence of NADPH suggesting that the compound is mainly metabolized by the co-factor CYP-450, compound **3e** exhibited pronounced stability to hepatic metabolism, with a clearance rate of less than 60 μL/min-mg. This result endows the new series of *N*-phenyl-2-aminothiazoles an advantage over the old generation of phenylthiazoles that were rapidly metabolized with a $t_{1/2}$ of less than half an hour.[11]

Table 6. Analysis of the metabolic stability of compound **3e** in human liver microsomes

Test Article	Test Concentration (μM)	Mean CL_{int}[1] (μL/min-mg)	Mean $t_{1/2}$ (minutes)	Notes
Terfenadine	0.1	578.3	8.95	High clearance control
Verapamil	0.1	211.1	24.20	High clearance control
Propranolol	0.1	48.2	143.80	Low clearance control
Imipramine	0.1	79.5	87.20	Low clearance control
3e (with NADPH)	0.1	58.9	117.60	Stable to metabolism
3e (without NADPH)	0.1	27.3	253.85	Stable to metabolism

[1]CL_{int}, intrinsic clearance rate; $t_{1/2}$, half-life

3. Conclusions. The rapid spread of antimicrobial resistance has wreaked havoc in the community, particularly with the associated lack of new antibiotics being introduced to the market. MRSA represents one of the challenges in the healthcare system due to its increased resistance to a wide range of antibiotics. Faced with this challenge, the mission of medicinal chemists is to rejuvenate the current antibiotic armamentarium with new members that are less susceptible to the ever-growing bacterial resistance. Therefore, the SAR of a series of *N*-phenyl-2-aminothiazole derivatives as novel antibiotics was studied from both sides of the molecule: the lipophilic motif and the nitrogenous head. Interestingly, the aminoguanidine series displayed the best efficacy against MRSA. The three most active aminoguanidine derivatives, **3a**, **3b** and **3e,** displayed excellent activity against a larger panel of Gram-positive bacteria, including MSSA, MRSA, VRSA, *S. epidermidis*, VRE, *S. pneumoniae* and *L. monocytogenes*. Moreover, the compounds exhibited a rapid bactericidal activity and a longer PAE than vancomycin against MRSA. These compounds also displayed an excellent tolerability in Caco-2 and HaCaT cells. Furthermore, these compounds surpassed vancomycin in killing MRSA intracellularly. Collectively, the *N*-phenyl-2-aminothiazole derivatives are promising anti-MRSA candidates. Further investigations are warranted to clearly elucidate their antibacterial mechanisms of action and to evaluate their activities in a suitable animal model.

4. Experimental Procedures

4.1. Chemistry

4.1.1. General. Melting points were obtained using a Stuart melting point apparatus and were uncorrected. Microanalyses for C, H and N were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University. IR spectra were recorded on Shimadzu IR 435 spectrophotometer (Shimadzu Corp., Kyoto, Japan) at the Faculty of Pharmacy, Cairo

University, Cairo, Egypt and values are presented in cm^{-1}. ^1H NMR spectra were recorded using a Bruker 400 MHz (Bruker Corp., Billerica, MA, USA) spectrophotometer at the Faculty of Pharmacy, Cairo University, Cairo, Egypt, and Varian Mercury-300BB 300 MHz (Varian Corp., Palo Alto, CA, USA) spectrophotometer at the Cairo University and Faculty of Science, Cairo University, Cairo, Egypt. Tetramethylsilane (TMS) was used as an internal-standard, and chemical shifts were recorded in ppm on the δ scale and coupling constants (J) were reported in Hz. ^{13}C NMR spectra were recorded using a Bruker 100 MHz spectrophotometer at the Faculty of Pharmacy, Cairo University, Cairo, Egypt and Varian Mercury-300BB 75 MHz at the Faculty of Science, Cairo University, Cairo, Egypt. The progress of the reactions was monitored with TLC using precoated aluminum sheet silica gel MERCK 60F 254. The spots were visualized using a UV lamp. The solvent system used for this assay was ethyl acetate: hexane [7:3]. Compounds **1** and **2a** were prepared as described in the literature.[28-30]

4.1.2. Synthesis of 1-(4-((4-phenylthiazol-2-yl)amino)phenyl)ethanone derivatives (**2b-e**). Anhydrous sodium acetate (0.33 g, 4 mmol) was added to a heated solution of compound **1** (0.39 g, 2 mmol) in THF (25 mL), followed by the addition of a solution of the appropriate phenacyl bromides (4 mmol) in THF. This mixture was heated under reflux for 5-6 h and the progress of the reaction was monitored using TLC. Then, the reaction mixture was poured on 50 mL of ice water, and the formed precipitate was filtered and crystallized from ethanol.

4.1.2.1. 1-(4-((4-(4-Chlorophenyl)thiazol-2-yl)amino)phenyl)ethanone (**2b**). Yellowish brown crystals (0.54 g, 64.3%), m.p.198-200 °C; IR (KBr, *ν* cm^{-1}): 3275 (NH) and 1677 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.50 (s, 3H, CH_3C=O), 7.50 (s, 1H, thiazolyl), 7.52 (d, 2H, *J* = 8.0 Hz, ArH), 7.83 (d, 2H, *J* = 8.0 Hz, ArH), 7.96-7.99 (dd, 4H, *J* = 3.2 Hz, 8.0 Hz, ArH), 10.76

(s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 26.7 ($\underline{C}H_3C=O$), 105.5, 116.4, 127.8, 129.1, 130.2, 130.4, 132.6, 133.6, 145.5, 149.4 162.8(ArCs), 196.4 (C=O).

4.1.2.2. 1-(4-((4-(4-Methoxyphenyl)thiazol-2-yl)amino)phenyl)ethanone (**2c**). Yellow crystals (0.8 g, 95.8 %), m.p. 212-214 °C; IR (KBr, *v* cm^{-1}): 3271 (NH) and 1654 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.50 (s, 3H, $CH_3C=O$), 3.94 (s, 3H, OCH_3), 7.19 (d, 1H, *J* = 8.6 Hz, ArH), 7.64 (d, 2H, *J* = 8.6 Hz, ArH), 7.89 (d, 2H, *J* = 8.6 Hz, ArH), 7.95-7.98 (dd, 1H, *J* = 2.12, 8.6 Hz, ArH), 8.07 (d, 1H, *J* = 2.12 Hz, ArH), 8.10 (s, 1H, thiazolyl), 8.17 (d, 1H, *J* = 8.6 Hz, ArH), 10.02 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 26.7 ($\underline{C}H_3C=O$), 56.8 (CH_3O), 104.0, 114.5, 126.9, 129.0, 129.5, 130.4, 132.4, 144.3, 150.6, 159.4, 162.5 (ArCs), 196.5 (C=O).

4.1.4.3. 1-(4-((4-(4-Bromophenyl)thiazol-2-yl)amino)phenyl)ethanone (**2d).** Yellow solid (1.6 g, 83.2 %), m.p. 224-226 °C; IR (KBr, *v* cm^{-1}): 3417 (NH) and 1678 (C=O); ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.50 (s, 3H, $CH_3C=O$), 7.67 (d, 2H, *J* = 8.0 Hz, ArH), 7.74 (d, 2H, *J* = 8.0 Hz, ArH), 7.86 (d, 2H, *J* = 8.0 Hz, ArH), 7.91 (d, 2H, *J* = 8.0 Hz, ArH), 8.13 (s, 1H, thiazolyl), 9.97 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,75 MHz) δ: 26.3 ($\underline{C}H_3C=O$), 120.9, 127.1, 127.2, 128.9, 130.0, 131.6, 131.9, 132.0, 133.1, 143.8, 181.1 (ArCs), 196.4 (C=O).

4.1.2.4. 1-(4-((4-(p-Tolyl)thiazol-2-yl)amino)phenyl)ethanone (**2e**). Yellow solid (1 g, 63.3 %), m.p. 170-172 °C; IR (KBr, *v* cm^{-1}): 3271 (NH) and 1654 (C=O); ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.33 (s, 3H, CH_3), 2.50 (s, 3H, $CH_3C=O$), 7.24 (d, 2H, *J* = 8.0 Hz, ArH), 7.35 (s, 1H, thiazolyl), 7.81-7.85 (dd, 4H, *J* = 2.1Hz, 8.0 Hz, ArH), 7.97 (d, 2H, *J* = 8.0 Hz, ArH), 10.69 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,75 MHz) δ: 20.8 (CH_3), 26.2 ($\underline{C}H_3C=O$), 103.3, 115.8, 125.6, 129.2, 129.7, 129.9, 131.6, 137.0, 145.2, 150.3, 162.1 (ArCs), 196.0 (C=O).

4.1.3. General procedure for synthesis of 2-(1-(4-((4-phenylthiazol-2-yl)amino)phenyl) ethylidene)hydrazinecarboximidamide derivatives (**3a-e**) To a solution of the key intermediates **2a-e** (1 mmol) in absolute ethanol (25 mL), 1 mL of concentrated HCl was added, followed by dry aminoguanidine HCl (0.22g, 2 mmol). This mixture was heated under reflux for 20-24 h, the progress of the reaction was monitored by TLC. The reaction mixture was rinsed with sodium bicarbonate solution, the solid formed is filtered giving the pure compounds **3a-e**.

4.1.3.1. 2-(1-(4-((4-Phenylthiazol-2-yl)amino)phenyl)ethylidene)hydrazinecarboximidamide (**3a**). Yellow solid (0.2 g, 60.8 %), m.p. 249-251 °C; IR (KBr, ν cm^{-1}): 3545, 3410, 3244 (NH/NH_2), 1670, 1620, 1566 (C=N); ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.41 (s, 3H, CH_3), 7.87 (s, 1H, NH, D_2O exchangeable), 7.88-7.92 (m, 5H, ArH), 8.15 (s, 1H, thiazolyl), 8.22 (d, 2H, ArH), 8.26 (d, 2H, ArH), 11.38-11.49 (2s, 4H, NH, NH, NH_2, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,75 MHz) δ: 14.6 (CH_3), 120.7, 121.9, 124.9, 127.2, 127.5, 137.7, 140.2, 144.1, 147.8, 150.1, 150.6, 156.0, 156.1 (ArCs + C=N); Anal. Calcd. for $C_{18}H_{18}N_6S$ (350.44): C, 61.69; H, 5.18; N, 23.98; found: C, 61.74; H, 5.20; N, 23.89.

4.1.3.2. 2-(1-(4-((4-(4-Chlorophenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboximidamide (**3b**). Yellow solid (0.3 g, 67.7 %), m.p. 274-276 °C; IR (KBr, ν cm^{-1}): 3444, 3248, 3170 (NH/NH_2), 1670, 1600, 1543 (C=N); ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.34 (s, 3H, CH_3), 7.43 (s, 1H, thiazolyl), 7.45 (d, 2H, J = 8.0 Hz, ArH), 7.78 (s, 1H, NH, D_2O exchangeable), 7.81 (d, 2H, J = 8.0 Hz, ArH), 7.92-7.99 (dd, 4H, J = 3.6 Hz, 8.0 Hz, ArH), 10.69 (s, 2H, NH_2, D_2O exchangeable), 11.14 (s, 1H, NH, D_2O exchangeable), 11.15 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,75 MHz) δ: 14.2 (CH_3), 104.4, 116.2, 127.3, 127.7, 128.6, 129.4, 132.0, 133.3, 142.4, 148.8, 151.4, 155.9, 162.7 (ArCs+C=N); Anal. Calcd. for $C_{18}H_{17}ClN_6S$ (384.89): C, 56.17; H, 4.45; N, 21.84; found: C, 56.20; H, 4.65; N, 21.66.

4.1.3.3. 2-(1-(4-((4-(4-Methoxyphenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboximidamide (**3c**). Yellow solid (0.2 g, 63.15 %), m.p. 205-207 °C; IR (KBr, *v* cm^{-1}): 3437, 3363, 3286 (NH/NH_2), 1685, 1624, 1597 (C=N); ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.34 (s, 3H, CH_3), 3.94 (s, 3H, OCH_3), 7.34 (s, 1H, NH, D_2O exchangeable), 7.39 (d, 2H, *J* = 7.5 Hz, ArH), 7.44 (d, 2H, *J* = 7.5 Hz, ArH), 7.92 (d, 2H, *J* = 7.5 Hz, ArH), 7.95 (d, 2H, *J* = 7.5 Hz, ArH), 8.16 (s, 1H, thiazolyl), 10.67 (s, 2H, NH_2, D_2O exchangeable), 11.20 (s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,75 MHz) δ: 14.3 (CH_3), 59.5 (OCH_3), 103.6, 106.9, 116.1, 125.6, 127.7, 128.6, 129.3, 134.4, 142.4, 150.0, 151.3, 156.0, 162.5 (ArCs+ C=N); Anal. Calcd. for $C_{19}H_{20}N_6OS$ (380.47): C, 59.98; H, 5.30; N, 22.09; found: C, 59.87; H, 5.23; N, 22.14.

4.1.3.4. 2-(1-(4-((4-(4-Bromophenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboximidamide (**3d**). Yellow solid (0.3 g, 79.2 %), m.p. 172-174 °C; IR (KBr, *v* cm^{-1}): 3375, 3228, 3155 (NH/NH_2), 1685, 1627, 1585 (C=N); ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.43 (s, 3H, CH_3), 7.83 (d, 2H, *J* = 8.0 Hz, ArH), 7.92 (d, 2H, *J* = 8.0 Hz, ArH), 7.95 (s, 2H, NH_2, D_2O exchangeable), 8.10 (s, 1H, thiazolyl), 8.13 (d, 2H, *J* = 8.0 Hz, ArH), 8.26 (d, 2H, *J* = 8.0 Hz, ArH), 11.26 (s, 1H, NH, D_2O exchangeable), 11.29 (s, 1H, NH, D_2O exchangeable), 11.35 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 14.8 (CH_3), 122.5, 123.0, 125.5, 127.7, 128.1, 128.4, 131.6, 138.2, 140.8, 144.6, 148.3, 150.6, 156.5 (ArCs + C=N); Anal. Calcd. for $C_{18}H_{17}BrN_6S$ (429.34): C, 50.36; H, 3.99; N, 19.57; found: C, 50.42; H, 3.73; N, 19.79.

4.1.3.5. 2-(1-(4-((4-(p-Tolyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboximidamide (**3e**). Yellow solid (0.3 g, 68.7 %), m.p. 205-207 °C; IR (KBr, *v* cm^{-1}): 3385, 3240, 3159 (NH/NH_2), 1674, 1612, 1566 (C=N); ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.33 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.23 (d, 2H, *J* = 8.0 Hz, ArH), 7.30 (s, 1H, thiazolyl), 7.78 (s, 2H, NH_2, D_2O

exchangeable), 7.80-7.82 (dd, 4H, *J* = 2.7 Hz, 8.0 Hz, ArH), 7.97 (d, 2H, *J* = 8.0 Hz, ArH), 10.59 (s, 1H, NH, D_2O exchangeable), 11.13 (s, 2H, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,75 MHz) δ: 14.2 (CH_3), 20.8 (CH_3), 102.7, 116.1, 120.6, 125.5, 127.7, 129.2, 131.8, 136.9, 142.5, 150.1, 151.4, 155.9, 162.4 (ArCs+ C=N); Anal. Calcd. for $C_{19}H_{20}N_6S$ (364.47): C, 62.61; H, 5.53; N, 23.06; found: C, 62.96; H, 5.30; N, 23.15.

4.1.4. General procedure for synthesis of 2-(1-(4-((4-phenylthiazol-2-yl)amino)phenyl) ethylidene)hydrazinecarboxamide derivatives (**4a-e**). To a solution of **2a-e** (1 mmol) in absolute ethanol (25 mL), 1 mL of concentrated HCl was added, followed by dry semicarbazide HCl (0.22g, 2 mmol). This mixture was heated under reflux for 20-24 h, the progress of the reaction was monitored by TLC. The reaction mixture was rinsed with sodium bicarbonate solution, the solid formed is filtered giving the pure compounds **4a-e**.

4.1.4.1. 2-(1-(4-((4-Phenylthiazol-2-yl)amino)phenyl)ethylidene)hydrazinecarboxamide (**4a**). Yellow solid (0.1 g, 37 %), m.p. 216-218 °C; IR (KBr, *v* cm^{-1}): 3464, 3390, 3275 (NH/NH_2), 1678 (C=O), 1604, 1566 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.17 (s, 3H, CH_3), 4.29 (s, 2H, NH_2, by D_2O exchangeable), 7.31-7.34 (m, 1H, ArH), 7.38 (s, 1H, thiazolyl), 7.42-7.46 (m, 2H, ArH), 7.76 (d, 2H, *J* = 8.0 Hz, ArH), 7.86 (d, 2H, *J* = 8.0 Hz, ArH), 7.94 (d, 2H, *J* = 8.0 Hz, ArH), 9.24 (s, 1H, NH, D_2O exchangeable), 10.51 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 13.5 (CH_3), 104.8, 116.3, 126.1, 127.3, 128.2, 129.1, 130.1, 134.7, 141.9, 145.7, 150.6, 158.2 (ArCs+ C=N), 162.6 (C=O); Anal. Calcd. for $C_{18}H_{17}N_5OS$ (351.43): C, 61.52; H, 4.88; N, 19.93; found: C, 61.76; H, 4.62; N, 19.62.

4.1.4.2. 2-(1-(4-((4-(4-Chlorophenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboxamide (**4b**). Yellow solid (0.1 g, 25.9 %), m.p. 140-142 °C; IR (KBr, *v* cm^{-1}): 3464, 3406, 3282 (NH/NH_2), 1654 (C=O), 1593, 1535 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.18 (s, 3H,

CH_3), 6.30 (s, 2H, NH_2, D_2O exchangeable), 7.44 (s, 1H, thiazolyl), 7.48 (d, 1H, *J* = 8.0 Hz, ArH), 7.51 (d, 1H, *J* = 8.0 Hz, ArH), 7.76 (d, 2H, *J* = 8.0 Hz, ArH), 7.85 (d, 1H, *J* = 8.0 Hz, ArH), 7.88 (d, 1H, *J* = 8.0 Hz, ArH), 7.94-7.98 (m, 2H, ArH), 9.97 (s, 1H, NH, D_2O exchangeable), 10.66 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 13.7 (CH_3), 116.8, 127.8, 127.8, 129.1, 130.1, 131.3, 133.6, 141.9, 145.7, 149.2, 158.0, 158.2 (ArCs+ C=N), 162.8 (C=O); Anal. Calcd. for $C_{18}H_{16}ClN_5OS$ (385.87): C, 56.03; H, 4.18; N, 18.15; found: C, 56.25; H, 4.03; N, 18.34.

4.1.4.3. 2-(1-(4-((4-(4-Methoxyphenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboxamide (**4c**). Yellow solid (0.2 g, 70.8 %), m.p. 225-227 °C; IR (KBr, *v* cm^{-1}): 3433, 3309, 3255 (NH/NH_2), 1685 (C=O), 1585,1531(C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.14 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.47 (s, 2H, NH_2, D_2O exchangeable), 6.48-6.51 (m, 2H, ArH), 7.07 (d, 1H, *J* = 8.0 Hz, ArH), 7.49 (d, 1H, *J* = 8.0 Hz, ArH), 7.76-7.80 (m, 3H, ArH), 8.10 (d, 1H, ArH), 9.27 (s, 1H, thiazolyl), 9.28 (s, 1H, NH, D_2O exchangeable), 9.99 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 13.5 (CH_3), 56.2 (CH_3O), 106.5, 116.4, 116.7, 122.3, 126.8, 127.1, 125.9, 134.0, 135.5, 143.7, 146.2, 158.2 (ArCs+ C=N) , 163.4 (C=O). Anal. Calcd. for $C_{19}H_{19}N_5O_2S$ (381.45): C, 59.82; H, 5.02; N, 18.36; found: C, 59.63; H, 5.36; N, 18.59.

4.1.4.4. 2-(1-(4-((4-(4-Bromophenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carboxamide (**4d**). Yellow solid (0.2 g, 37.2 %), m.p. 157-159 °C; IR (KBr, *v* cm^{-1}): 3479, 3425, 3248 (NH/NH_2), 1681 (C=O), 1581, 1523 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.16 (s, 3H, CH_3), 6.55 (s, 2H, NH_2, D_2O exchangeable), 7.24-7.32(m, 1H, ArH), 7.38 (s, 1H, thiazolyl), 7.51-7.54 (m, 4H, ArH), 7.79 (d, 2H, *J* = 8.0 Hz, ArH), 7.90 (d, 1H, *J* = 8.0 Hz, ArH), 8.86 (s, 1H, NH, D_2O exchangeable), 9.42 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100

MHz) δ: 13.6 (CH_3), 113.9, 114.3, 117.0, 122.3, 127.7, 128.5, 130.6, 131.5, 135.1, 137.8, 143.7, 157.7 (ArCs+ C=N), 158.1 (C=O); Anal. Calcd. for $C_{18}H_{16}BrN_5OS$ (430.32): C, 50.24; H, 3.75; N, 16.27; found: C, 50.52; H, 3.95; N, 16.38.

4.1.4.5. 2-(1-(4-((4-(p-Tolyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazinecarboxamide (**4e**).Yellow solid (0.2 g, 43.8 %), m.p. 208-210 °C; IR (KBr, *v* cm^{-1}): 3464, 3421, 3232 (NH/NH_2), 1693 (C=O), 1597, 1566 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.18 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.40 (s, 2H, NH_2, D_2O exchangeable), 7.24 (d, 2H, *J* = 8.0 Hz, ArH), 7.29 (s, 1H, thiazolyl), 7.75 (d, 2H, *J* = 8.0 Hz, ArH), 7.82 (d, 2H, *J* = 8.0 Hz, ArH), 7.86 (d, 2H, *J* = 8.0 Hz, ArH), 9.25 (s, 1H, NH, D_2O exchangeable), 10.53 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 13.5 (CH_3), 21.2 (CH_3), 103.9, 116.3, 116.7, 126.0, 127.2, 129.7, 130.0, 131.3, 137.4, 145.0, 150.5, 158.1 (ArCs+ C=N) , 162.5 (C=O); Anal. Calcd. for $C_{19}H_{19}N_5OS$ (365.45): C, 62.44; H, 5.24; N, 19.16; found: C, 62.77; H, 5.03; N, 19.39.

4.1.5. General procedure for synthesis of 2-(1-(4-((4-phenylthiazol-2-yl)amino)phenyl) ethylidene)hydrazinecarbothioamide (**5a-e**). To a solution of **2a-e** (1 mmol) dissolved in absolute ethanol (25 mL), 1 mL of concentrated HCl was added, followed by dry thiosemicarbazide HCl (0.25g, 2 mmol). This mixture was heated under reflux for 20-24 h, the progress of the reaction was monitored by TLC. The reaction mixture was rinsed with sodium bicarbonate solution, the solid formed is filtered giving the pure compounds **5a-e**.

4.1.5.1. 2-(1-(4-((4-Phenylthiazol-2-yl)amino) phenyl) ethylidene) hydrazine carbothioamide (**5a**). Yellow solid (0.3 g, 49.6 %), m.p. 229-231 °C; IR (KBr, *v* cm^{-1}): 3417, 3317, 3228 (NH/NH_2), 1589, 1562 (C=N), 1249, 1087 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.51 (s, 3H, CH_3), 4.72 (s, 2H, NH_2, D_2O exchangeable), 7.43 (s, 1H, thiazolyl), 7.77-7.90 (m, 5H, ArH), 7.94 (d, 2H, *J* = 8.0 Hz, ArH), 7.98 (d, 2H, *J* = 8.0 Hz, ArH), 10.96 (s, 1H, NH, D_2O

exchangeable), 11.11 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 14.1(CH_3), 103.9, 116.6, 126.2, 128.0, 128.1, 129.1, 130.6, 134.9, 142.5, 148.1, 150.6, 163.0 (ArCs+ C=N), 179.0 (C=S); Anal. Calcd. for $C_{18}H_{17}N_5S_2$ (367.49): C, 58.83; H, 4.66; N, 19.06; found: C, 58.65; H, 4.93; N, 19.46.

4.1.5.2. 2-(1-(4-((4-(4-Chlorophenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carbothioamide (**5b**). Yellow solid (0.2 g, 44.9 %), m.p. 221-223 °C; IR (KBr, *v* cm^{-1}): 3348, 3240, 3159 (NH/NH_2), 1592, 1535 (C=N), 1249, 1091 (C=S); ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.30 (s, 3H, CH_3), 4.50 (s, 2H, NH_2, D_2O exchangeable), 7.49-8.29 (m, 9H, 8 ArH and 1H, thiazolyl), 10.76 (s, 1H, NH, D_2O exchangeable), 11.09 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 17.0 (CH_3), 116.9, 117.3, 127.8, 128.7, 129.1, 130.1, 131.0, 133.6, 144.8, 150.4, 151.4, 163.0 (ArCs+ C=N) , 196.5 (C=S); Anal. Calcd. for $C_{18}H_{16}ClN_5S_2$ (401.94): C, 53.79; H, 4.01; N, 17.42; found: C, 53.97; H, 4.21; N, 17.15.

4.1.5.3. 2-(1-(4-((4-(4-Methoxyphenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carbothioamide (**5c**). Yellow solid (0.1 g, 32.7 %), m.p. 202-204 °C; IR (KBr, *v* cm^{-1}): 3429, 3360, 3143 (NH/NH_2), 1589, 1492 (C=N), 1269, 1095 (C=S),; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.26 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.08 (d, 1H, *J* = 8.0 Hz, ArH), 7.52 (d, 1H, *J* = 8.0 Hz, ArH), 7.85-7.90 (m, 4H, ArH), 8.06 (s, 2H, NH_2, D_2O exchangeable), 8.15-8.23 (m, 2H, ArH), 8.24 (s, 1H, thiazolyl), 10.13 (s, 1H, NH, D_2O exchangeable), 10.17 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 14.3 (CH_3), 55.6 (CH_3O), 111.4, 112.3, 114.4, 121.9, 127.3, 128.0, 131.2, 131.9, 133.3, 147.0, 156.5, 179.1(ArCs+ C=N) , 181.4 (C=S); Anal. Calcd. for $C_{19}H_{19}N_5OS_2$ (397.52): C, 57.41; H, 4.82; N, 17.62; found: C, 57.60; H, 4.55; N, 17.46.

4.1.5.4. 2-(1-(4-((4-(4-Bromophenyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazine carbothioamide (**5d**). Yellow solid (0.1 g, 31.3 %), m.p. 174-176 °C; IR (KBr, *v* cm^{-1}): 3410, 3232, 3140 (NH/NH_2), 1589, 1504 (C=N)),1296, 1083 (C=S); ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.28 (s, 3H, CH_3), 7.33 (s, 1H, NH, D_2O exchangeable), 7.45 (s, 2H, NH_2, D_2O exchangeable), 7.55 (d, 2H, *J* = 8.0 Hz, ArH), 7.56-7.58 (m, 1H, ArH), 7.70 (s, 1H, thiazolyl), 7.74-7.76 (m, 1H, ArH), 7.90 (d, 2H, *J* = 8.0 Hz, ArH), 8.00-8.02 (m, 1H, ArH), 8.30-8.32 (m, 1H, ArH), 10.26 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 14.0 (CH_3), 123.2, 127.7, 129.0, 129.5, 130.6, 131.6, 131.9, 132.2, 136.1, 137.1, 147.4, 179.3 (ArCs+ C=N), 197.9 (C=S); Anal. Calcd. for $C_{18}H_{16}BrN_5S_2$ (446.39): C, 48.43; H, 3.61; N, 15.69; found: C, 48.70; H, 3.45; N, 15.41.

4.1.5.5. 2-(1-(4-((4-(p-Tolyl)thiazol-2-yl)amino)phenyl)ethylidene)hydrazinecarbothioamide (**5e**). Yellow solid (0.3 g, 65.6 %), m.p. 224-226 °C; IR (KBr, *v* cm^{-1}): 3414, 3348, 3236 (NH/NH_2), 1597, 1566 (C=N), 1257, 1091 (C=S); ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 7.25 (d, 2H, *J* = 8.0 Hz, ArH), 7.31 (s, 1H, thiazolyl), 7.77 (d, 2H, *J* = 8.0 Hz, ArH), 7.82 (d, 2H, *J* = 8.0 Hz, ArH), 7.92 (m, 1H, ArH), 7.95 (d, 1H, *J* = 8.0 Hz, ArH), 8.15 (s, 2H, NH_2, D_2O exchangeable), 10.13 (s, 1H, NH, D_2O exchangeable), 10.55 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6,100 MHz) δ: 14.0 (CH_3), 21.2 (CH_3), 116.7, 126.0, 128.0, 129.0, 129.7, 130.4, 132.1, 137.5, 142.5, 145.7, 150.5, 163.0 (ArCs+ C=N), 178.8 (C=S); Anal. Calcd. for $C_{19}H_{19}N_5S_2$ (381.52): C, 59.81; H, 5.02; N, 18.36; found: C, 59.57; H, 4.94; N, 18.55.

4.2. Biological assays

4.2.1. Bacterial strains, cell lines, media and reagents. The 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). *E. coli* BW25113 and JW25113 strains were obtained from The Coli Genetic Stock Center (CGSC), Yale University. The human colorectal adenocarcinoma (Caco-2) cell line, human keratinocyte cell line (HaCaT), and murine macrophage (J774) cells were purchased from the American Type Culture Collection (ATCC). Linezolid, gentamicin sulfate and vancomycin hydrochloride were purchased from commercial vendors and dissolved either in sterile water or in DMSO to prepare stock solutions. Cation-adjusted Mueller Hinton broth (CA-MHB), tryptic soy agar (TSA), tryptic soy broth (TSB), phosphate-buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and 96-well plates were all purchased from commercial sources. Synthesized compounds were prepared in DMSO at stock concentrations of 10 mg/mL.

Antimicrobial assay

4.2.2. Determination of MICs and MBCs against the tested bacterial pathogens. MICs of the tested compounds and control antibiotics were determined using the broth microdilution method according to the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI).[31] MBCs of these compounds were tested by plating 4 µL from wells with no growth onto Tryptic soy agar plates. Plates were incubated at 37 °C for 18-20 h before recording the MBC. The MBC was categorized as the lowest concentration that reduced bacterial growth by 99.9%.[32]

Bacterial strains were grown aerobically overnight on tryptone soy agar plates at 37°C. Afterwards, a bacterial solution equivalent to 0.5 McFarland standard was prepared and diluted in cation-adjusted Mueller-Hinton broth (CAMHB) (for staphylococcal and *E. coli* strains) or tryptone soya broth (TSB) (for *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pneumonia* and *Listeria monocytogenes* strains) to achieve a bacterial concentration of

approximately 5 × 10^5 CFU/mL and seeded in 96-well plates. Compounds and control drugs were added in the first row of the 96-well plates and serially diluted along the plates. Plates were then incubated aerobically at 37 °C for 18-20 h. MICs are defined as the minimum concentrations of the compounds and control drugs that completely inhibited the visual growth of bacteria.

4.2.3. Time-kill assay against MRSA. A time to kill assay was performed against MRSA USA 400 to confirm the bactericidal activity of the tested compounds, as described previously.[13, 33] MRSA USA400 cells in the logarithmic growth phase were diluted to 4.68 × 10^6 colony-forming units (CFU/mL) and exposed to concentrations equivalent to 5 × MIC (in triplicate) of compounds **3a**, **3b** and **3e** and vancomycin in tryptic soy broth. Aliquots (100 µL) were collected from each treatment at the indicated time points, subsequently serially diluted in PBS and plated on tryptic soy agar plates. Plates were incubated at 37°C for 18-20 h before the viable CFU/mL was determined.

4.2.4. *Post-antibiotic effect (PAE) of N*-phenyl-*2-aminothiazoles against MRSA.* The PAE compound **3b**, compound **3e** and vancomycin was determined using the methods described in previous studies [34].

4.2.5. In vitro analysis of the cytotoxicity toward Caco-2, HaCaT and J774 cells. As described in previous reports.[35] , the tested compounds were incubated with human colorectal cells (Caco-2), human keratinocytes (HaCaT) and murine macrophages (J774) to determine the potential toxicity toward mammalian cells.

*4.2.6. Intracellular infection of J774 cells with MRSA and treatment with compounds **3a**, **3b** and **3e***. The ability of the compounds to reduce the burden of intracellular MRSA was evaluated using previously described methods.[36]

4.2.6. Multi-step resistant study against MRSA. The potential for MRSA to develop resistance to **3a, 3b**, **3e** and ciprofloxacin was evaluated using the multistep resistance selection method, as previously described.[37] Briefly, the broth microdilution assay was utilized to determine the MICs of **3a, 3b**, **3e** and ciprofloxacin against MRSA USA400. Bacteria were passaged for 14 consecutive passages. Resistance was said to have occurred when the MIC increased more than 4-fold from the initial MIC. After the end of experiment, resistant mutants developed were aseptically got rid of, to prevent their spread to the environment.

4.2.7. PBS Solubility Screening. The solubility screen was conducted as described previously.[12]

*4.2.8. Analysis of the Metabolic Stability of Compound **3e**.* The metabolic stability of compound 3e was analyzed as described in a previous study.[35]

Supporting information. The supporting information is available free of charge on the journal website. Scanned copies from ^{1}H and ^{13}C NMR spectra of all new described compounds, protocol used for MIC determination and details about PAE, cytotoxicity, PBS solubility screen and metabolic stability analyses.

Conflicts of interest

There are no conflicts to declare.

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Abbreviations. AMR, antimicrobial Resistance; CFU, colony forming unit; Caco-2, human colorectal adenocarcinoma cells; HaCaT, human keratinocyte cells; MIC, minimum inhibitory concentration; MBC; minimum bactericidal concentration; PAE, post-antibiotic effect; CL_{int}, intrinsic clearance rate; $t_{1/2}$, half-life,

References

[1] Centers for Disease Control and Prevention. Antibiotic/ antimicrobial resistance. Biggest threats and data. 2019 https://www.cdc.gov/drugresistance/biggest-threats.html, in.

[2] G. Kapoor, S. Saigal, A. Elongavan, Action and resistance mechanisms of antibiotics: A guide for clinicians, J Anaesthesiol Clin Pharmacol, 33 (2017) 300-305.

[3] D.C. Kaur, S.S. Chate, Study of Antibiotic Resistance Pattern in Methicillin Resistant Staphylococcus Aureus with Special Reference to Newer Antibiotic, J Glob Infect Dis, 7 (2015) 78-84.

[4] S. Tsiodras, H.S. Gold, G. Sakoulas, G.M. Eliopoulos, C. Wennersten, L. Venkataraman, R.C. Moellering Jr, M.J.J.T.L. Ferraro, Linezolid resistance in a clinical isolate of Staphylococcus aureus, 358 (2001) 207-208.

[5] B. Tornimbene, S. Eremin, M. Escher, J. Griskeviciene, S. Manglani, C.L. Pessoa-Silva, WHO global antimicrobial resistance surveillance system early implementation 2016–17, The Lancet Infectious Diseases, 18 (2018) 241-242.

[6] S.J. Peacock, G.K. Paterson, Mechanisms of methicillin resistance in Staphylococcus aureus, Annual review of biochemistry, 84 (2015).

[7] I. Gould, Costs of hospital-acquired methicillin-resistant Staphylococcus aureus (MRSA) and its control, International journal of antimicrobial agents, 28 (2006) 379-384.

[8] F.R. DeLeo, H.F. Chambers, Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era, The Journal of clinical investigation, 119 (2009) 2464-2474.

[9] I. Gould, J. Reilly, D. Bunyan, A. Walker, Costs of healthcare-associated methicillin-resistant Staphylococcus aureus and its control, Clinical Microbiology and Infection, 16 (2010) 1721-1728.

[10] I.M. Gould, M.Z. David, S. Esposito, J. Garau, G. Lina, T. Mazzei, G. Peters, New insights into meticillin-resistant Staphylococcus aureus (MRSA) pathogenesis, treatment and resistance, International journal of antimicrobial agents, 39 (2012) 96-104.

[11] H. Mohammad, A.S. Mayhoub, A. Ghafoor, M. Soofi, R.A. Alajlouni, M. Cushman, M.N. Seleem, Discovery and characterization of potent thiazoles versus methicillin- and vancomycin-resistant Staphylococcus aureus, J Med Chem, 57 (2014) 1609-1615.

[12] M.A. Seleem, A.M. Disouky, H. Mohammad, T.M. Abdelghany, A.S. Mancy, S.A. Bayoumi, A. Elshafeey, A. El-Morsy, M.N. Seleem, A.S. Mayhoub, Second-generation phenylthiazole antibiotics with enhanced pharmacokinetic properties, Journal of medicinal chemistry, 59 (2016) 4900-4912.

[13] M. Hagras, N.S. Abutaleb, A.O. Ali, J.A. Abdel-Aleem, M.M. Elsebaei, M.N. Seleem, A.S. Mayhoub, Naphthylthiazoles: Targeting Multidrug-Resistant and Intracellular Staphylococcus aureus with Biofilm Disruption Activity, ACS Infect Dis, (2018).

[14] I. Eid, M.M. Elsebaei, H. Mohammad, M. Hagras, C.E. Peters, Y.A. Hegazy, B. Cooper, J. Pogliano, K. Pogliano, H.S. Abulkhair, Arylthiazole antibiotics targeting intracellular methicillin-resistant Staphylococcus aureus (MRSA) that interfere with bacterial cell wall synthesis, European journal of medicinal chemistry, 139 (2017) 665-673.

[15] I.H. Eissa, H. Mohammad, O.A. Qassem, W. Younis, T.M. Abdelghany, A. Elshafeey, M.M. Abd Rabo Moustafa, M.N. Seleem, A.S. Mayhoub, Diphenylurea derivatives for combating methicillin- and vancomycin-resistant Staphylococcus aureus, Eur J Med Chem, 130 (2017) 73-85.

[16] P. Gahtori, S.K. Ghosh, Design, synthesis and SAR exploration of hybrid 4-chlorophenylthiazolyl-s-triazine as potential antimicrobial agents, Journal of enzyme inhibition and medicinal chemistry, 27 (2012) 281-293.

[17] J.W. Costerton, P.S. Stewart, E.P. Greenberg, Bacterial biofilms: a common cause of persistent infections, science, 284 (1999) 1318-1322.

[18] M. Otto, Staphylococcus epidermidis—the'accidental'pathogen, Nature reviews microbiology, 7 (2009) 555.

[19] H.A. Khan, A. Ahmad, R. Mehboob, Nosocomial infections and their control strategies, Asian pacific journal of tropical biomedicine, 5 (2015) 509-514.

[20] S. Gudmundsson, S. Einarsson, H. Erlendsdottir, J. Moffat, W. Bayer, W.A. Craig, The post-antibiotic effect of antimicrobial combinations in a neutropenic murine thigh infection model, J Antimicrob Chemother, 31 Suppl D (1993) 177-191.

[21] K. Totsuka, M. Shiseki, K. Kikuchi, Y. Matsui, Combined effects of vancomycin and imipenem against methicillin-resistant Staphylococcus aureus (MRSA) in vitro and in vivo, J Antimicrob Chemother, 44 (1999) 455-460.

[22] M.T. Suller, D. Lloyd, The antibacterial activity of vancomycin towards Staphylococcus aureus under aerobic and anaerobic conditions, J Appl Microbiol, 92 (2002) 866-872.

[23] R.S. Flannagan, B. Heit, D.E. Heinrichs, Intracellular replication of Staphylococcus aureus in mature phagolysosomes in macrophages precedes host cell death, and bacterial escape and dissemination, Cell Microbiol, 18 (2016) 514-535.

[24] M.M. Elsebaei, N.S. Abutaleb, A.A. Mahgoub, D. Li, M. Hagras, H. Mohammad, M.N. Seleem, A.S. Mayhoub, Phenylthiazoles with nitrogenous side chain: An approach to overcome molecular obesity, Eur J Med Chem, 182 (2019) 111593.

[25] R.G. Wunderink, J. Rello, S.K. Cammarata, R.V. Croos-Dabrera, M.H. Kollef, Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant Staphylococcus aureus nosocomial pneumonia, Chest, 124 (2003) 1789-1797.

[26] Y. Hosny, N.S. Abutaleb, M. Omara, M. Alhashimi, M.M. Elsebaei, H.S. Elzahabi, M.N. Seleem, A.S. Mayhoub, Modifying the lipophilic part of phenylthiazole antibiotics to control their drug-likeness, Eur J Med Chem, 185 (2020) 111830.

[27] K.T. Savjani, A.K. Gajjar, J.K. Savjani, Drug solubility: importance and enhancement techniques, ISRN Pharm, 2012 (2012) 195727.

[28] D.P. Mahajan, R.S. Bendre, Green synthesis and characterization of some 4-substituted-N-aryl-1, 3-thiazole-2-amine derivative, Asian Journal of Biochemical and Pharmaceutical Research, 2 (2014) 103-108.

[29] Dipesh P. Mahajan, J.D. Bhosale, R.S. Bendre, Synthesis, Characterization and Plant Growth Regulator Activity of Some Substituted 2-Amino Benzothiazole Derivatives, Journal of Applicable Chemistry, 2 (2013) 765-771.

[30] S. Demirci, Synthesis of Thiazole Derivatives as Antimicrobial Agents by Green Chemistry Techniques, Journal of the Turkish Chemical Society, Section A: Chemistry, 5 (2018) 393-414.

[31] Clinical and Laboratory Standards Institute, CLSI. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.* M7-A9. 9th ed., in, Wayne, PA, 2012.

[32] A. Kotb, N.S. Abutaleb, M.A. Seleem, M. Hagras, H. Mohammad, A. Bayoumi, A. Ghiaty, M.N. Seleem, A.S. Mayhoub, Phenylthiazoles with tert-Butyl side chain: Metabolically stable with anti-biofilm activity, Eur J Med Chem, 151 (2018) 110-120.

[33] H. Mohammad, A. AbdelKhalek, N.S. Abutaleb, M.N. Seleem, Repurposing niclosamide for intestinal decolonization of vancomycin-resistant enterococci, International journal of antimicrobial agents, 51 (2018) 897-904.

[34] T. Tomozawa, C. Sugihara, M. Kakuta, K. Sugihara, T. Koga, In vitro postantibiotic effects of tomopenem (CS-023) against Staphylococcus aureus and Pseudomonas aeruginosa, J Med Microbiol, 59 (2010) 438-441.

[35] A. Hammad, N.S. Abutaleb, M.M. Elsebaei, A.B. Norvil, M. Alswah, A.O. Ali, J.A. Abdel-Aleem, A. Alattar, S.A. Bayoumi, H. Gowher, M.N. Seleem, A.S. Mayhoub, From Phenylthiazoles to Phenylpyrazoles: Broadening the Antibacterial Spectrum toward Carbapenem-Resistant Bacteria, J Med Chem, 62 (2019) 7998-8010.

[36] M.M. Elsebaei, H. Mohammad, A. Samir, N.S. Abutaleb, A.B. Norvil, A.R. Michie, M.M. Moustafa, H. Samy, H. Gowher, M.N. Seleem, A.S. Mayhoub, Lipophilic efficient phenylthiazoles with potent undecaprenyl pyrophosphatase inhibitory activity, Eur J Med Chem, 175 (2019) 49-62.

[37] A. Mancy, N.S. Abutaleb, M.M. Elsebaei, A.Y. Saad, A. Kotb, A.O. Ali, J.A. Abdel-Aleem, H. Mohammad, M.N. Seleem, A.S. Mayhoub, Balancing Physicochemical Properties of Phenylthiazole Compounds with Antibacterial Potency by Modifying the Lipophilic Side Chain, ACS Infect Dis, 6 (2020) 80-90.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: