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Brief Article

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Discovery and Characterization of Potent Thiazoles versus Methicillin- and Vancomycin-Resistant *Staphylococcus aureus*

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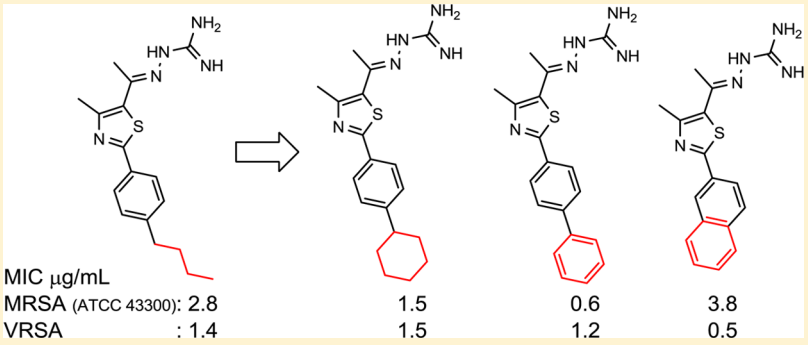
Supporting Information



ABSTRACT: Methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA) infections are growing global health concerns. Structure−activity relationships of phenylthiazoles as a new antimicrobial class have been addressed. We present 10 thiazole derivatives that exhibit strong activity against 18 clinical strains of MRSA and VRSA with acceptable PK profile. Three derivatives revealed an advantage over vancomycin by rapidly eliminating MRSA growth within 6 h, and no derivatives are toxic to HeLa cells at 11 μg/mL.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a rapidly expanding global health concern. It is currently the most common pathogen linked to patients with skin and soft-tissue infections.[1] Apart from the high mortality and rapid transmission rates, MRSA infections result in an estimated $3 billion to $4 billion of additional health care costs per year.[2] Resistance has also emerged to therapeutic agents once deemed to be the drugs of choice in treating MRSA infections, such as vancomycin[3] and linezolid.[4] Researchers and clinical-care providers are thus facing an increasingly difficult challenge trying to construct novel antimicrobials and new therapeutic options to treat MRSA-related infections.

The thiazole ring is a key structural component for a wide spectrum of therapeutic agents including anticonvulsants,[5] anticancer,[6,7] antiviral,[8] and antibacterial agents.[9,10] In this study, whole-cell screening assays of libraries of substituted thiazoles and thiadiazoles identified a novel lead compound that displayed notable antibacterial activity against MRSA. The lead **1a** (Figure 1) consists of a thiazole central ring connected to two unique structural features: a cationic element at the C5 position and a lipophilic moiety at the C2 position. These two structural components have been hypothesized to contribute to the antibacterial activity of the lead compound. Structural optimizations were focused on the lipophilic side chain at thiazole-C2 of the lead compound in an attempt to enhance the antimicrobial activity of the lead compound against MRSA and VRSA. Chemical modifications reported here involved building

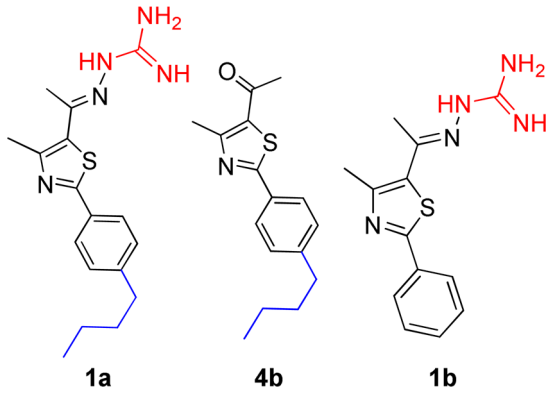


Figure 1. Chemical structures of lead **1a** and **4b** (removal of the cationic moiety) and **1b** (removal of the lipophilic alkane side chain).

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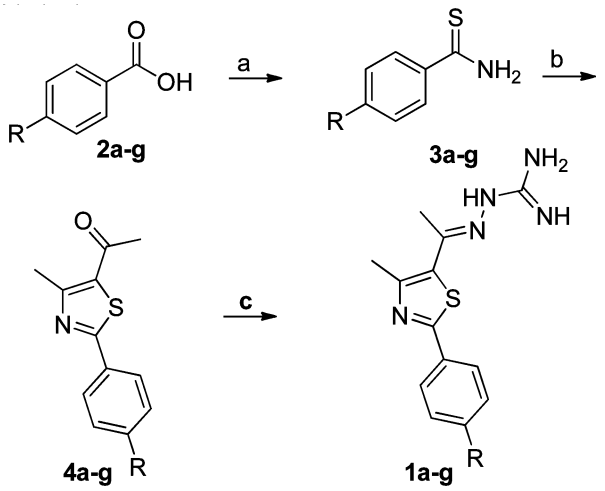
a focused library of phenylthiazoles with different lipophilic moieties at the phenyl para position to define the structure–activity relationships (SARs) at the thiazole-C2 position in a rigorous way. Our objectives were to investigate the antimicrobial activities of the thiazole derivatives against MRSA and VRSA, ascertain the killing kinetics of MRSA in vitro by the lead compound and two derivatives, determine the cytotoxic impact of the derivatives on mammalian cells in vitro, and investigate the pharmacokinetics (namely, solubility, permeability, and metabolic stability) of the thiazole compounds.

CHEMISTRY

Thiazole ethyl ketone derivatives **4a–g** were prepared in moderate yields by heating thioamides **3a–g**, obtained by treatment of the corresponding amides with Lawesson's reagent in dry THF, with 3-chloropentane-2,4-dione in absolute ethanol (Scheme 1). The methyl ketones **4a–g** were gently

Scheme 1. Preparation of 1a–g[a]



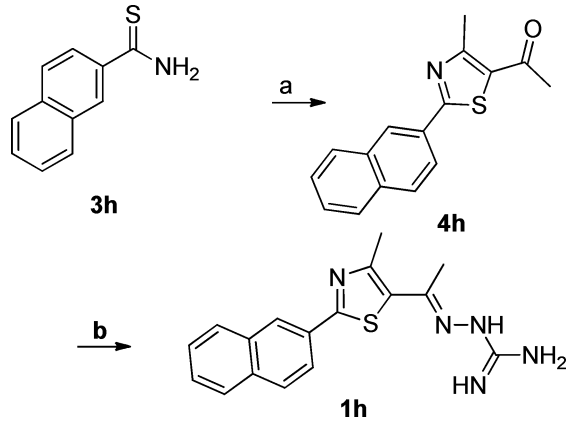
[a]Reagents and conditions: (a) (i) $SOCl_2$, heat to reflux, 2 h, (ii) NH_4OH, 0–23 °C, 2–5 h, (iii) Lawesson's reagent, dry THF, 50–60 °C, 5–24 h; (b) absolute ethanol, 3-chloropentane-2,4-dione, heat to reflux, 12 h, 63%; (c) aminoguanidine hydrochloride, absolute EtOH, heat to reflux, 24 h.

heated with aminoguanidine hydrochloride in the presence of lithium chloride as a catalyst to afford hydrazinecarboximidamide derivatives **1a–g** (Scheme 1). Similarly, the final products **1h**, **7**, **8**, and **12** were obtained using a similar synthetic protocol (Schemes 2–4).

BIOLOGICAL RESULTS AND DISCUSSION

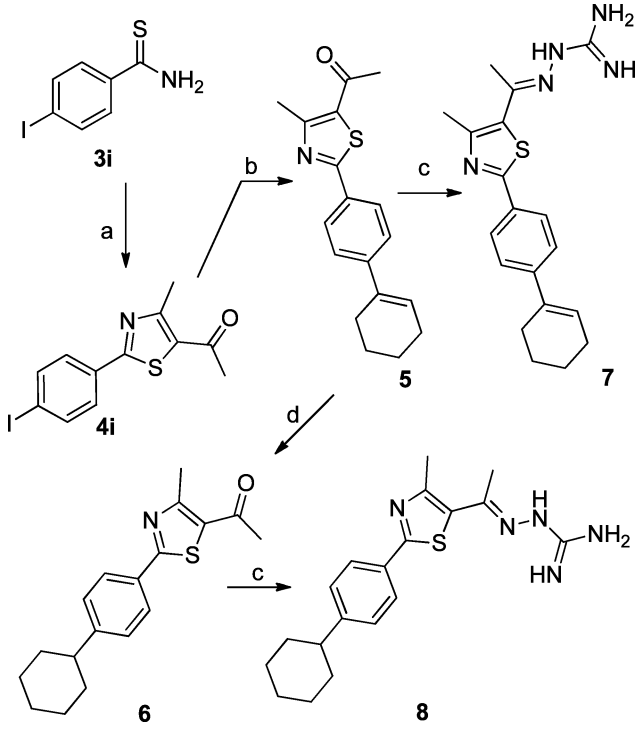
The 10 substituted thiazole compounds we synthesized inhibited growth of 18 different strains of MRSA and VRSA at 0.4–5.5 μg/mL (Table 1). The lead **1a** inhibited the growth of MRSA strains at 1.4–5.5 μg/mL. Subsequently synthesized derivatives demonstrated a 2- to 5-fold improvement in the MIC values. Initially, the effect of increasing the length of the alkyl side chain, through insertion of methylene units, was explored. As the length of the alkyl side chain increased from

Scheme 2. Preparation of 1h[a]



[a]Reagents and conditions: (a) absolute ethanol, 3-chloropentane-2,4-dione, heat to reflux, 12 h, 67%; (b) aminoguanidine hydrochloride, absolute ethanol, heat to reflux, 24 h, 40%.

Scheme 3. Preparation of 7 and 8[a]



[a]Reagents and conditions: (a) absolute ethanol, 3-chloropentane-2,4-dione, heat to reflux, 12 h, 58%; (b) cyclohexene, $PdAcO_2$, Et_3N, DMF, 80 °C, 5 h, 39%; (c) aminoguanidine hydrochloride, absolute ethanol, heat to reflux, 24 h; (d) H_2, Pd/C, methanol, 23 °C, 24 h, 38–42%.

two (**1c**) to three (**1a**) to four (**1d**) methylene units, there was a consistent improvement in the MIC values observed against all MRSA strains tested. However, additional lengthening of the alkyl side chain appeared to nullify the improvement observed in the antimicrobial activity, as the MIC for **1e** (containing six methylene units) nearly matched or exceeded the values obtained for **1d**. This result held true, as an increase to eight methylene units (**1f**) resulted in an MIC that nearly matched or exceeded the MIC attained for **1a**. Altogether this indicates that an alkyl side chain with four methylene units exhibits the

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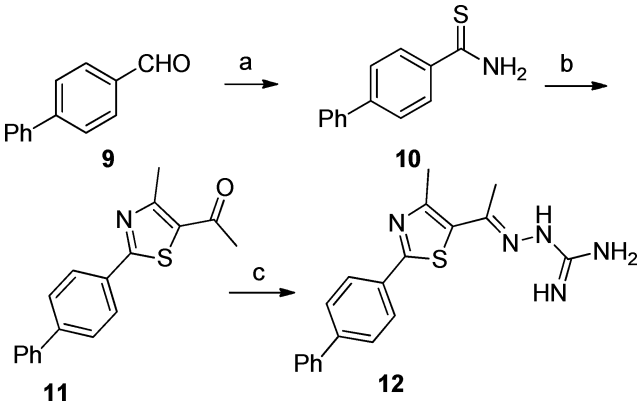
Scheme 4. Preparation of 12[a]



[a]Reagents and conditions: (a) (i) H_2NOH HCl, DMSO, 100 °C, 20 min; (ii) NaOH, H_2O_2, 12 h; (iii) Lawesson's reagent, THF, 23 °C, 12 h; (b) absolute ethanol, 3-chloropentane-2,4-dione, heat to reflux, 12 h, 49%; (c) aminoguanidine hydrochloride, absolute ethanol, heat to reflux, 24 h, 45%.

optimum potency against MRSA and addition of methylene units to the alkyl side beyond four units will not significantly enhance the antimicrobial activity of the lead compound.

Replacement of the linear alkyl side chain with a branched alkane (**1g**) produced mixed results. There was a modest improvement in the MIC values against six MRSA strains (1.0 μg/mL for **1g** compared to 1.4 μg/mL for **1a**) and a nearly 2- to 5-fold enhancement in the MIC for five additional strains. Substitution of the alkyl side chain with a more bulky fused ring system (**1h**) did not significantly enhance the activity of the derivative against the MRSA strains tested. However, replacement of the alkyl side chain with conformationally restricted analogues (**7**, **8**, and **12**) demonstrated the most consistent, significant improvement in the MIC obtained relative to the lead **1a** (2- to 4-fold improvement against 16 MRSA strains tested).

The MIC values obtained for **7**, **8**, and **12** on multiple occasions matched or were lower than the values of antibiotic vancomycin against the MRSA strains tested. Furthermore, all 10 thiazole compounds proved to be more potent than vancomycin in inhibiting growth of VISA and VRSA strains. Compounds **7**, **8**, and **12** also proved to be more effective at eliminating growth of MRSA NRS119, a strain resistant to linezolid (a drug of last resort in the treatment of MRSA infections), and several strains resistant to multiple antibiotic classes including lincosamides, aminoglycosides, fluoroquinolones, and macrolides (USA100, USA200, and USA500). In addition to this, all 10 compounds exhibited excellent activity against MRSA USA300, a strain responsible for most cases of community-acquired MRSA (CA-MRSA) and MRSA skin and soft tissue infections (SSTIs) in the U.S.[11,12]

A drawback of several commercial antimicrobials used to treat MRSA infections, including vancomycin and linezolid, is that they are only capable of inhibiting bacterial growth (but do not kill the bacteria) or that they exhibit a very slow bactericidal effect resulting in difficulty in clearing the infection.[13,14] Thus, a compound that demonstrates the ability to rapidly kill MRSA is highly desirable, since it limits the possibility of developing bacterial resistance/tolerance. We studied the rate at which the compounds were able to eliminate MRSA (USA300) in vitro in a time-kill assay. The results (Figure S1) indicate that at 3.0 × MIC, **1a**, **1d** (derivative that contains one more methylene unit in the alkyl side chain), and **8** (derivative that replaces the alkyl side chain with a cyclohexane ring) are bactericidal. However, the rate of clearance of MRSA (USA300) varies among the three compounds. Compound **1d** mimics the action of **1a**, rapidly eliminating MRSA completely within 2 h. This would appear logical, as **1a** and **1d** are similar in structure, the major difference resulting from the number of methylene units contained in the alkyl side chain. Compound **8** requires more than double the time (6 h) to logarithmically reduce MRSA colony forming units (CFUs) to zero. Though **8** appears to be more potent compared to **1a** and **1d** (when comparing MIC values), the last two appear capable of clearing MRSA colonies (albeit at a higher concentration) more rapidly. Vancomycin was not able to reduce the number of CFU by 3 $\log_{10}$ within a 12 h window. Collectively, this indicates that the thiazole compounds possess a selective advantage over vancomycin in terms of rate of elimination of MRSA cells. This information is clinically

Table 1. Antimicrobial Activities (μg/mL) of Modified Thiazole Compounds Screened against *Staphylococcus aureus*

	MIC ± SD (μg/mL) of thiazole compounds and vancomycin against *S. aureus*										
strain	**1a**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**7**	**8**	**12**	VAN
MRSA ATCC 43300	2.8 ± 0	1.2 ± 0	0.8 ± 0	3.0 ± 0	2.2 ± 0	1.9 ± 0	3.8 ± 0	1.2 ± 0	1.5 ± 0	0.6 ± 0	0.7 ± 0
VISA ATCC 700699	1.4 ± 0	1.9 ± 0.7	0.5 ± 0.2	0.7 ± 0	1.1 ± 0	1.6 ± 0.6	3.2 ± 1.1	0.5 ± 0.2	0.7 ± 0	0.6 ± 0	2.9 ± 0
VISA HIP07256	1.4 ± 0	1.9 ± 0.7	0.7 ± 0.3	1.5 ± 0	2.9 ± 1.3	1.9 ± 0	2.6 ± 1.1	0.8 ± 0.3	0.7 ± 0	0.5 ± 0.2	2.9 ± 0
VISA LIM 3	1.4 ± 0	1.6 ± 0.7	0.5 ± 0	0.7 ± 0	1.1 ± 0	1.6 ± 0.6	1.9 ± 0	0.8 ± 0.3	0.5 ± 0.2	0.6 ± 0	2.9 ± 0
MRSA NRS107	1.4 ± 0	1.2 ± 0	0.5 ± 0	1.0 ± 0.4	1.1 ± 0	1.0 ± 0	1.9 ± 0	0.6 ± 0	0.6 ± 0.2	1.2 ± 0	0.7 ± 0
MRSA NRS108	1.4 ± 0	1.2 ± 0	0.9 ± 0.3	3.0 ± 0	4.4 ± 0	1.9 ± 0	1.9 ± 0	1.7 ± 1.0	1.5 ± 0	2.0 ± 0.7	0.7 ± 0
MRSA NRS119	1.4 ± 0	1.9 ± 0.7	0.9 ± 0.3	1.5 ± 0	2.2 ± 0	1.9 ± 0	3.8 ± 0	0.6 ± 0	0.7 ± 0	0.6 ± 0	1.2 ± 0.4
MRSA USA400	1.4 ± 0	1.2 ± 0	0.9 ± 0.3	1.5 ± 0	4.4 ± 0	1.9 ± 0	1.9 ± 0	0.6 ± 0	0.7 ± 0	0.8 ± 0.3	0.7 ± 0
MRSA NRS194	1.4 ± 0	1.9 ± 0.7	1.1 ± 0	3.0 ± 0	4.4 ± 0	2.6 ± 1.1	1.9 ± 0	0.8 ± 0.3	0.7 ± 0	1.4 ± 0.9	0.7 ± 0
MRSA USA100	5.5 ± 0	1.6 ± 0.7	1.1 ± 0	3.0 ± 2.6	2.2 ± 0	1.3 ± 0.6	1.9 ± 0	1.0 ± 0.3	0.7 ± 0	2.0 ± 0.7	1.2 ± 0.4
MRSA USA200	2.8 ± 0	1.9 ± 0.7	1.1 ± 0	1.2 ± 0.4	2.2 ± 0	2.6 ± 1.1	2.6 ± 1.1	1.0 ± 0.3	1.2 ± 0.4	0.6 ± 0	0.4 ± 0
MRSA USA300	1.4 ± 0	1.2 ± 0	1.1 ± 0	2.5 ± 0.9	2.2 ± 0	1.3 ± 0.6	1.9 ± 0	1.2 ± 0	0.7 ± 0	1.0 ± 0.3	0.6 ± 0.2
MRSA USA500	1.4 ± 0	1.9 ± 0.7	1.1 ± 0	3.0 ± 0	3.7 ± 1.3	1.3 ± 0.6	2.6 ± 1.1	1.0 ± 0.3	0.9 ± 0.6	1.6 ± 0.7	1.0 ± 0.4
MRSA USA700	1.8 ± 0.8	1.2 ± 0	0.9 ± 0.3	4.0 ± 1.7	1.8 ± 0.6	1.0 ± 0	2.6 ± 1.1	1.2 ± 0	1.5 ± 0	0.8 ± 0.3	1.0 ± 0.4
MRSA USA800	2.3 ± 0.8	1.9 ± 0.7	0.7 ± 0.3	1.5 ± 0	1.8 ± 0.6	1.3 ± 0.6	1.9 ± 0	0.8 ± 0.3	0.7 ± 0	1.2 ± 0	0.7 ± 0
MRSA USA1000	1.4 ± 0	1.6 ± 0.7	1.1 ± 0	2.5 ± 0.9	2.2 ± 0	1.0 ± 0	1.9 ± 0	0.8 ± 0.3	0.9 ± 0.6	0.8 ± 0.3	0.6 ± 0.2
MRSA USA1100	2.8 ± 0	1.6 ± 0.7	1.1 ± 0	1.5 ± 0	2.2 ± 0	1.9 ± 0	1.9 ± 0	0.6 ± 0	0.7 ± 0	0.6 ± 0	0.7 ± 0
VRSA	1.4 ± 0	1.2 ± 0	1.1 ± 0	1.5 ± 0	2.2 ± 0	1.6 ± 0.6	1.9 ± 0	1.2 ± 0	1.5 ± 0	0.6 ± 0	185.5 ± 0

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significant, as it would impact the size and timing of the dose given to patients with an infection caused by MRSA.

In addition to this, combination therapy using multiple antibiotics to treat MRSA infections is commonly used in clinical practice. Antibiotics that are bacteriostatic or exhibit a slow bactericidal effect (such as vancomycin)[13] are often paired with antibiotics exhibiting a rapid bactericidal effect (such as rifampin) to limit the emergence of bacterial strains with reduced susceptibility to vancomycin.[13] As the thiazole compounds presented here exhibit a rapid bactericidal effect against MRSA, analysis of synergy between the thiazole compounds and commercial antimicrobials (such as vancomycin and linezolid) for potential use in combination therapy would be an interesting avenue to further explore.

The cytotoxicity assay (Figure S2) confirmed that all of the compounds are selective for bacterial cell inhibition over mammalian cells. All compounds tested were not toxic to the HeLa human tumor cell line up to 11 μg/mL; this concentration is more than 20-fold higher than the MIC for the most potent thiazole derivatives (**8** and **12**). Irrespective of the modification made to the alkane side chain of the lead compound (addition of methylene units or substitution with a cyclic moiety), the subsequent derivatives maintained a good toxicity profile when tested against HeLa cells.

Physicochemical properties, including solubility and permeability, of potential therapeutic agents are critical factors that need to be explored early in drug development. Though a compound proves potent against a target organism during in vitro studies and exhibits limited toxicity to cultured mammalian cells, the drug candidate can fail in animal and human studies if the drug is poorly soluble in aqueous solutions or is incapable of passing through cellular barriers. Analysis of the hydrogen bonding potential and lipophilicity of a compound can lend valuable insight into potential solubility and permeability issues. After documentation of the strong antimicrobial activity of the thiazole compounds and determination of their limited toxicity against mammalian cells, it was critical to establish whether the compounds possess potential solubility and permeability issues. By use of Lipinski's rule of 5 and topological polar surface area (TPSA) as guidelines, the results in Table S2 demonstrate that all 10 thiazole compounds possess clogP and TPSA values that are associated with good solubility and permeability qualities. Two derivatives (**1e** and **1f**) violate one parameter of the rule of 5 with each derivative possessing a clogP above 5. These derivatives contain the longest linear alkyl chain (six and eight methylene units for **1e** and **1f**, respectively) connected to the phenylthiazole nucleus. This result supports the notion that an ideal thiazole side chain should have four methylene units, as compounds possessing an alkyl side chain with more than four methylene units exhibit a decrease in the antimicrobial activity against MRSA and pose potential solubility issues.

To confirm if the thiazole compounds possess good physicochemical properties as predicted, lead **1a** was analyzed using the Caco-2 permeability assay, MDCK-MDR1 permeability assay, and a solubility screen utilizing phosphate buffered saline (PBS). The solubility screen was used to determine the highest concentration of lead **1a** in comparison with three control drugs that was able to dissolve in PBS before precipitate formed. The PBS solubility screen indicates that **1a** possesses acceptable solubility compared to the reference tested drugs. When compared to drugs with poor aqueous solubility, **1a** was soluble at twice the concentration of the antihypertensive drug reserpine (31.3 μM) and nearly 4 times the concentration of the cancer drug tamoxifen (15.6 μM) as presented in Table S3. Taking into consideration that both drugs are administered orally, the solubility properties of phenylthiazoles are highly promising.

The Caco-2 permeability assay revealed the lead **1a** surprisingly exhibited very limited permeability across the membrane from the apical (A) to basolateral (B) direction as demonstrated in Table S4, maybe because of the slight acidic experimental conditions. Compound **1a** exhibits a higher apparent permeability coefficient (P_{app}) in the basolateral to apical direction (P_{app} = 2.2 × 10^{-6} cm/s) which mimics the result obtained with the control drug ranitidine (P_{app} = 1.2 × 10^{-6} cm/s in the B−A direction compared to 0.2 × 10^{-6} cm/s in the A−B direction). Transporters in the membranes can enhance or reduce the permeability of a compound. The presence of efflux transporters on the apical surface of epithelial cells in the intestine may play a role in preventing the absorption of the thiazole compounds and passage through Caco-2 cells. The efflux ratio of >2 for the lead thiazole compound supports the notion that the compound may be a substrate for an efflux transporter (possibly P-glycoprotein which is a potential reason for the higher rate of transfer of compound from the B to A direction). To confirm if this was the case, **1a** was analyzed using Madin−Darby canine kidney (MDCK) cells transfected with a gene overexpressing multidrug resistance protein 1 (MDR1), also referred to as P-glycoprotein 1 (Pgp). As presented in Table S5, a higher rate of transfer of **1a** is observed in the B to A direction (P_{app} = 3.6 × 10^{-6} cm/s) compared to the A to B direction (P_{app} = 0.7 × 10^{-6} cm/s), consistent with what is observed with the Caco-2 permeability assay. The efflux ratio determined from the MDCK-MDR1 permeability assay for **1a** is 5.0, indicating that the compound may be subject to the effect of Pgp. An increasing number of hydrogen bond acceptors (oxygen and nitrogen atoms) has been shown to increase the likelihood of Pgp efflux of drugs.[15] Thus, constructing derivatives of the lead thiazole compound focusing on modifications to the cationic head (where the hydrogen bond acceptor groups are present) is one mechanism to enhance permeability. A delicate balance between addition or substitution of functional groups would need to be achieved to ensure that permeability is enhanced without reducing the solubility profile of the thiazole compounds. Another method to enhance passage of the thiazole compounds across the intestinal membrane is to use a higher concentration of the lead compound, especially if the compound is a substrate for efflux transporters. The Caco-2 assay utilized a low concentration (10 μM) of the lead compound. The concentration of a drug in the gastrointestinal lumen after an oral dose is typically 50−100 μM.[15] Thus, testing the lead compound at a higher concentration is necessary to confirm if the poor permeability observed in Caco-2 assay is potentially due to the low concentration of compound used in the assay. A higher concentration may help the thiazole compound to effectively cross the intestinal barrier, as efflux transporters will eventually become saturated in a concentration-dependent manner permitting compound that has passively diffused across the membrane to reach the portal circulation. Taken collectively, the permeability profile of the thiazole compounds is initially promising and can be potentially improved by modifying the structure of the lead compound or increasing the concentration of the compound used.

In addition to testing the solubility and permeability characteristics of the lead **1a**, the metabolic stability of **1a**

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was investigated using human liver microsomes. As Table S6 demonstrates, **1a** is subject to metabolism by the liver with a microsomal intrinsic clearance of 80.3 μL min^{-1} mg^{-1} and a half-life of 28.8 min. These values align with the values obtained with verapamil (the metabolized control drug) rather than for warfarin (nonmetabolized control drug). Removing the cofactor NADPH significantly reduced the metabolism of **1a**, indicating that these metabolic processes are NADPH-dependent.

In conclusion, the identification of novel antimicrobial agents to treat an array of infections caused by methicillin-resistant and vancomycin-resistant *S. aureus* requires a multifold approach from whole-cell screening of chemical libraries to rational drug design. We present the exciting discovery of a lead antimicrobial compound, identified from whole-cell screening of a library of thiazole and thiadiazole compounds, that is capable of inhibiting growth of 18 strains of MRSA and VRSA. The lead compound consists of a thiazole central ring connected to two structural elements critical for activity, namely, a cationic element at the C5 position and a lipophilic moiety at the C2 position. A focused library of derivatives containing modifications to the lipophilic moiety was constructed to enhance the antimicrobial activity against MRSA and VRSA. The lead compound and nine derivatives are capable of inhibiting growth of 18 different clinical isolates of MRSA and VRSA at 0.5−3.0 μg/mL. The antibacterial spectrum of some analogues surpassed that of several known antibiotics in eliminating the growth of highly resistant strains such as MRSA NRS119, a strain resistant to linezolid (a drug of last resort in treatment of MRSA infections), and several other strains resistant to multiple antibiotic classes including vancomycin, lincosamides, aminoglycosides, fluoroquinolones, and macrolides (VRSA, USA100, USA200, and USA500). Furthermore, the lead compound and two derivatives exhibit a rapid bactericidal effect, eliminating MRSA growth in vitro within 6 h. In addition to this, six derivatives, including the three most potent compounds against MRSA, are not toxic. The 10 thiazole compounds were predicted to have good solubility and permeability characteristics based on the criteria set forth by Lipinski's rule of 5. However, analysis of permeability of the lead **1a** via the Caco-2 and MDCK-MDR1 assays indicated that the compound had poor permeability from the apical to basolateral surface of the membrane (possibly due to the effect of the Pgp efflux transporter). We confirmed that the lead compound does not target the integrity of the bacterial cell wall or cytoplasmic membrane (data not published); further explanation of the molecular target of the thiazole compounds will be presented in a future study. The characterization of the novel thiazole compounds presents an intriguing step in the development of a novel class of therapeutic agents effective for treating MRSA and VRSA infections.

EXPERIMENTAL SECTION

Chemistry. Detailed procedures are in Supporting Information. The purities of tested compound are ≥95% (HPLC). HPLC analyses were performed on a Waters binary HPLC system (model 1525, 20 μL injection loop) equipped with a Waters dual wavelength absorbance UV detector (model 2487) set for 254 nm and using a 5 μm C-18 reverse phase column.

Preparation of Thioamides 3a−g. General Procedure. Thioamides **3a−g** (1−5 mmol), which were obtained by treatment of their corresponding carboxylic acids **2a−g** with thionyl chloride followed by gradual addition to ammonia solution, and Lawesson's reagent (1.2 equiv) were added to dry THF (15−40 mL). The reaction mixtures were stirred at room temperature for 5−12 h. The solvent was evaporated under reduced pressure, and the residues were partitioned between aqueous $NaHCO_3$ (2 M, 25−50 mL) and ethyl acetate (25−75 mL). The organic solvent was separated and dried over anhydrous $MgSO_4$. After solvent evaporation, the crude products were further purified by silica gel flash chromatography, using hexane−ethyl acetate (4:1), to yield the corresponding thioamides as yellow solids (55−57%) in the desired purity degree. Characterizations of **3a−g** are listed in Supporting Information.

Preparation of Methyl Ketones 4a−i. General Procedure. Thiobenzamides **3a−i** (2−10 mmol) and 3-chloropentane-2,4-dione (1.4 equiv) were added to absolute ethanol (10−30 mL). The mixtures were heated at reflux for 12−24 h. After evaporation of solvent under reduced pressure, the brown residues were collected and purified by silica gel flash chromatography, using hexane−ethyl acetate (9:1), to yield **4a−i** in the desired purity. Compounds **4a**[16] and **4b**[17] are previously reported. Characterizations of **4c−i** are listed in Supporting Information.

Preparation of Hydrazinecarboximidamides 1a−h, 7, 8, and 12. General Procedure. The ketone derivative **4a−h**, **5**, **6**, or **11** (1−10 mmol) was dissolved in absolute ethanol (10−50 mL). Aminoguanidine hydrochloride (1 equiv) and a catalytic amount of LiCl (5−20 mg) were added. The mixtures were heated at reflux for 24 h. The solvent was evaporated under reduced pressure. The crude product was purified by crystallization from 70% methanol and then recrystallized from absolute methanol to afford the desired compounds as solids. Compound **1a**[16] is previously reported. Physical properties and spectroscopic data of hydrazinecarboximidamides **1a−h**, **7**, **8**, and **12** are listed below.

2-[1-(4-Methyl-2-phenylthiazol-5-yl)ethylidene]hydrazinecarboximidamide (1b). Yellowish white solid (124 mg, 61%): mp 195−196 °C. ^{1}H NMR (DMSO-d_6) δ 11.65 (brs, 1 H), 8.88 (brs, 1 H), 7.91−7.88 (m, 4 H), 7.48 (m, 3 H) 2.60 (s, 3 H), 2.43 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 165.79, 160.11, 156.96, 153.33, 147.96, 133.51, 131.53, 130.24, 126.90, 19.27, 19.03; ESIMS m/z (rel intensity) 274 (MH$^+$, 100); HRESIMS, m/z 274.1128 MH$^+$, calcd for $C_{13}H_{16}N_5S$ 274.1126; HPLC purity (methanol/water, 1:1), 95.44%.

2-{1-[4-Methyl-2-(4-propylphenyl)thiazol-5-yl]ethylidene}-hydrazinecarboximidamide (1c). Yellowish white solid (100 mg, 55%): mp 256−257 °C. ^{1}H NMR (DMSO-d_6) δ 11.47 (brs, 1 H), 7.80 (d, J = 8.1 Hz, 2 H), 7.76 (brs, 3 H), 7.29 (d, J = 8.1 Hz, 2 H), 2.58 (s, 3 H), 2.55 (t, J = 7.8 Hz, 2 H), 2.41 (s, 3 H), 1.60 (m, 2 H), 0.88 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 165.01, 156.80, 153.28, 148.14, 146.06, 131.21, 130.14, 126.95, 126.88, 37.94, 24.71, 19.14, 19.05, 14.54; ESIMS m/z (rel intensity) 316 (MH$^+$, 100); HRESIMS, m/z 316.1590 MH$^+$, calcd for $C_{16}H_{22}N_5S$ 316.1596; HPLC purity (methanol/water, 1:1), 97.09%.

2-{1-[4-Methyl-2-(4-pentylphenyl)thiazol-5-yl]ethylidene-hydrazinecarboximidamide (1d). Yellow solid (54 mg, 50%): mp 210 °C. ^{1}H NMR (DMSO-d_6) δ 11.41 (brs, 1 H), 7.81 (d, J = 7.8 Hz, 2 H), 7.78 (brs, 3 H), 7.31 (d, J = 7.8 Hz, 2 H), 2.62 (m, 5 H), 2.41 (s, 3 H), 1.57 (m, 2 H), 1.27 (m, 4 H), 0.84 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 165.43, 156.16, 152.71, 147.59, 145.73, 130.57, 130.47, 129.51, 126.49, 35.25, 31.21, 30.66, 22.26, 18.52, 18.45, 14.24; ESIMS m/z (rel intensity) 344 (MH$^+$, 100); HRESIMS, m/z 344.1913 MH$^+$, calcd for $C_{18}H_{26}N_5S$ 244.1909; HPLC purity (methanol/water, 1:1), 95.12%.

2-{1-[4-Methyl-2-(4-heptylphenyl)thiazol-5-yl]ethylidene-hydrazinecarboximidamide (1e). Yellow solid (151 mg, 53%): mp 233−235 °C. ^{1}H NMR (DMSO-d_6) δ 11.43 (brs, 1 H), 7.80 (m, 5 H), 7.30 (d, J = 8.1 Hz, 2 H), 2.61 (m, 5 H), 2.42 (s, 3 H), 1.55 (t, J = 6.6 Hz, 2 H), 1.24 (m, 8 H), 0.82 (t, J = 6.6 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 165.98, 156.86, 153.25, 148.09, 146.28, 131.17, 131.06, 130.07, 126.89, 35.88, 32.15, 31.57, 29.54, 29.43, 23.00, 19.19, 19.05, 14.86; ESIMS m/z (rel intensity) 372 (MH$^+$, 100); HRESIMS, m/z 372.2228 MH$^+$, calcd for $C_{20}H_{30}N_5S$ 372.2222; HPLC purity (methanol/water, 1:1), 99.31%.

2-{1-[4-Methyl-2-(4-nonylphenyl)thiazol-5-yl]ethylidene-hydrazinecarboximidamide (1f). Yellow solid (133 mg, 55%): mp 203−206 °C. ^{1}H NMR (DMSO-d_6) δ 11.32 (brs, 1 H), 7.79 (d, J = 8.1 Hz, 2 H), 7.60 (brs, 3 H), 7.28 (d, J = 8.1 Hz, 2 H), 2.58 (m, 5 H),

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2.41 (s, 3 H), 1.55 (m, 2 H), 1.22 (m, 12 H), 0.82 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (DMSO-d_6) δ 165.80, 156.87, 153.45, 147.99, 146.28, 131.60, 131.19, 129.44, 126.25, 36.34, 35.29, 31.60, 30.98, 29.28, 29.19, 28.99, 22.42, 18.46, 18.39, 14.27; ESIMS m/z (rel intensity) 400 (MH^+, 100); HRESIMS, m/z 400.2540 MH^+, calcd for $C_{22}H_{34}N_5S$ 400.2535; HPLC purity (methanol/water, 1:1), 95.40%.

2-{1-[2-(4-(*tert*-Butyl)phenyl)-4-methylthiazol-5-yl]-ethylidene}hydrazinecarboximidamide (1g). Off-white solid (155 mg, 67%): mp 252−253 °C. ^{1}H NMR (DMSO-d_6) δ 11.41 (brs, 1 H), 7.83 (d, J = 8.4 Hz, 2 H), 7.65 (brs, 3 H), 7.51 (d, J = 8.4 Hz, 2 H), 2.59 (s, 3 H), 2.41 (s, 3 H), 1.29 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 165.90, 156.75, 154.40, 153.34, 148.22, 131.09, 130.94, 127.02, 126.76, 35.60, 31.81, 19.13, 19.05; ESIMS m/z (rel intensity) 330 (MH^+, 100); HRESIMS, m/z 330.1755 MH^+, calcd for $C_{17}H_{24}N_5S$ 330.1752; HPLC purity (methanol/water, 1:1), 96.86%.

2-{1-[4-Methyl-2-(naphthalen-2-yl)thiazol-5-yl]ethylidene}-hydrazinecarboximidamide (1h). Yellow solid (80 mg, 40%): mp 288−290 °C. ^{1}H NMR (DMSO-d_6) δ 11.39 (brs, 1 H), 8.50 (s, 1 H), 8.08−7.94 (m, 4 H), 7.73 (brs, 3 H), 7.58 (m, 2 H), 2.64 (s, 3 H), 2.44 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 165.86, 156.73, 153.56, 148.19, 134.70, 133.75, 131.77, 130.94, 129.91, 129.59, 128.71, 128.41, 128.07, 126.44, 124.20, 19.06, 17.18; ESIMS m/z (rel intensity) 324 (MH^+, 100); HRESIMS, m/z 324.1179 MH^+, calcd for $C_{17}H_{18}N_5S$ 324.1283; HPLC purity (methanol/water, 1:1), 95.99%.

2-{1-[2-(4-(1-Cyclohexenyl)phenyl)-4-methylthiazol-5-yl]-ethylidene}hydrazinecarboximidamide (7). Yellow solid (58 mg, 42%): mp 213−215 °C. ^{1}H NMR (DMSO-d_6) δ 11.29 (brs, 1 H), 7.82 (d, J = 8.1 Hz, 2 H), 7.37 (d, J = 8.1 Hz, 2 H), 7.05 (brs, 3 H), 5.74 (m, 2 H), 2.80 (m, 1 H), 2.58 (s, 3 H), 2.37 (s, 3 H), 2.20−2.06 (m, 3 H), 1.82−1.71 (m, 3 H); ^{13}C NMR (DMSO-d_6) δ 164.46, 157.32, 151.22, 149.83, 146.15, 132.04, 131.07, 128.02, 127.11, 126.79, 126.31, 42.01, 32.85, 29.43, 25.59, 18.49, 17.77; ESIMS m/z (rel intensity) 354 (MH^+, 100); HRESIMS, m/z 354.1759 MH^+, calcd for $C_{19}H_{24}N_5S$ 354.1752; HPLC purity (methanol/water, 1:1), 97.50%.

2-{1-[2-(4-Cyclohexylphenyl)-4-methylthiazol-5-yl]-ethylidene}hydrazinecarboximidamide (8). Yellow solid (42 mg, 38%): mp 273−276 °C. ^{1}H NMR (DMSO-d_6) δ 11.27 (brs, 1 H), 7.80 (d, J = 8.4 Hz, 2 H), 7.50 (brs, 3 H), 7.33 (d, J = 8.4 Hz, 2 H), 2.58 (s, 3 H), 2.57 (m, 1 H), 2.40 (s, 3 H), 1.76 (m, 5 H), 1.37 (m, 4 H); ^{13}C NMR (DMSO-d_6) δ 166.88, 156.35, 152.19, 150.97, 147.98, 130.33, 130.20, 127.13, 126.04, 44.34, 33.86, 26.35, 25.62, 16.70, 16.20; ESIMS m/z (rel intensity) 356 (MH^+, 100); HRESIMS, m/z 356.1912 MH^+, calcd for $C_{19}H_{26}N_5S$ 356.1909; HPLC purity (methanol/water, 1:1), 98.09%.

2-{1-[2-([1,1′-Biphenyl]-4-yl)-4-methylthiazol-5-yl]-ethylidene}hydrazinecarboximidamide (12). Yellow solid (104 mg, 45%): mp 278−280 °C. ^{1}H NMR (CD_3OD) δ 8.00 (d, J = 9.0 Hz, 2 H), 7.73 (d, J = 9.0 Hz, 2 H), 7.66 (d, J = 9.0 Hz, 2 H), 7.45 (t, J = 9.0 Hz, 2 H), 7.36 (t, J = 9.0 Hz, 1 H), 2.66 (s, 3 H), 2.42 (s, 3 H); ^{13}C NMR (CD_3OD) δ 166.40, 155.92, 152.80, 148.24, 143.22, 139.60, 131.49, 130.29, 128.59, 127.16, 127.04, 126.47, 126.34, 16.74, 16.26; ESIMS m/z (rel intensity) 350 (MH^+, 100); HRESIMS, m/z 350.1435 MH^+, calcd for $C_{19}H_{20}N_5S$ 350.1439; HPLC purity (methanol/water, 1:1), 95.96%.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures, characterization, yields, and physical and spectral data of **3f**, **4c−i**, **5−8**, **11**, and **12** and biological tests. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MIC, minimum inhibitory concentration; CFU, colony forming unit; PK, pharmacokinetics

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