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Synthesis and antibacterial evaluation of a novel series of synthetic phenylthiazole compounds against methicillin-resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* infections are a significant global health challenge in part due to the emergence of strains exhibiting resistance to nearly all classes of antibiotics. This underscores the urgent need for the rapid development of novel antimicrobials to circumvent this burgeoning problem. Previously, whole-cell screening of a library of 2,5-disubstituted thiazole compounds revealed a lead compound exhibiting potent antimicrobial activity against MRSA. The present study, conducting a more rigorous analysis of the structure–activity relationship of this compound, reveals a nonpolar, hydrophobic functional group is favored at thiazole-C2 and an ethylidenehydrazine-1-carboximidamide moiety is necessary at C5 for the compound to possess activity against MRSA. Furthermore, the MTS assay confirmed analogs **5**, **22d**, and **25** exhibited an improved toxicity profile (not toxic up to 40 μg/mL to mammalian cells) over the lead **1**. Analysis with human liver microsomes revealed compound **5** was more metabolically stable compared to the lead compound (greater than eight-fold improvement in the half-life in human liver microsomes). Collectively the results presented demonstrate the novel thiazole derivatives synthesized warrant further exploration for potential use as future antimicrobial agents for the treatment of multidrug-resistant *S. aureus* infections.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections remain a significant public health challenge globally. Though reports have indicated the incidence of healthcare-associated MRSA (HA-MRSA) infections have diminished [1,2], transmission of community-associated MRSA (CA-MRSA) infections, primarily strains USA300 and USA400 [3], has continued to present major problems amongst a diverse population including healthcare workers [4], prison inmates [5,6], military service personnel [6], contact sport athletes [7,8], homeless individuals [9], intravenous drug users [9,10], tattoo recipients [11], neonates [12], and young children [13,14]. Moreover, CA-MRSA infections are typically associated with more severe morbidity and mortality than their HA-MRSA counterparts [15]. While CA-MRSA is a leading cause of skin and soft-tissue infections [16,17], MRSA has also been associated with more complicated medical diseases including necrotizing pneumonia [18], osteomyelitis [19], and sepsis [20], leading to over 11,000 deaths annually [21].

A recent study has estimated the total annual burden upon society for treatment of CA-MRSA infections alone may exceed US$13 billion [22]. Part of the associated cost is due to failure of current antimicrobials to treat certain clinical isolates of MRSA that have developed resistance to these therapeutic agents. Indeed, clinical isolates of both CA-MRSA and HA-MRSA have been documented that exhibit resistance to an array of different antibiotic classes including the β-lactams [23], macrolides [24], quinolones [25,26], tetracyclines [27], and lincosamides [27]. Further exacerbating the problem, strains have emerged which exhibit resistance to first-line

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antibiotics (such as mupirocin [27,28] for the treatment of MRSA skin infections) and drugs deemed agents of last resort (such as linezolid [29,30] and vancomycin [31]). Prudent use and development of effective antimicrobials is a critical step to alleviate complications and costs associated with MRSA infections. Therefore there is an urgent need for the development of novel therapeutic agents and treatment strategies to circumvent this significant global health issue.

Utilizing whole-cell screening of a library of substituted thiazoles, our research group identified a novel lead thiazole compound that possesses potent antimicrobial activity against clinically relevant isolates of MRSA, vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA) [32]. The basic structure of the lead **1** consists of a central thiazole ring connected to two distinct moieties – a lipophilic side chain at C2 and a cationic amino group at C5. The objectives of the present study were to construct a series of analogs to the lead **1** (Table 1) with modifications to the functional groups at both the thiazole-C2 and C5 positions to more rigorously ascertain the structure–activity relationships of these compounds against a diverse array of HA-MRSA and CA-MRSA isolates, identify new derivatives exhibiting an improved toxicity profile against mammalian cells, and to enhance the metabolic stability profile of the lead **1**.

2. Chemistry

The detailed synthetic protocols and spectral data of the lead **1** (Fig. 1) in addition to all intermediates have been reported elsewhere [32,33]. All thiazole compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma–Aldrich, St. Louis, MO, USA) to achieve a stock 10 mM solution.

The (4-iodophenyl)thiazole derivative **3** was prepared by heating a mixture of the commercially available 4-iodothiobenzamide **2** and 3-chloro-2,4-pentanedione in absolute ethanol, as illustrated in Scheme 1. The phenylthiazolyl methyl ketone derivatives **4** and **6** were prepared via the Sonogashira cross coupling of the (4-iodophenyl)thiazole derivative **3** with commercially available 1-hexyne and 1-nonyne, respectively, in DMF using a bis(-triphenylphosphine)palladium(II) dichloride catalyst, copper(I) iodide co-catalyst, and caesium carbonate base (Scheme 1). The hydrazinecarboximidamide derivatives **5** and **7** were synthesized by treatment of the phenylthiazolyl methyl ketone derivatives **4** and **6**, respectively, with aminoguanidine hydrochloride in the presence of a catalytic amount of lithium chloride in absolute ethanol (Scheme 1).

Table 1
Minimum inhibitory concentration (MIC) of thiazole compounds against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300.

Analog	MIC (μg/mL)
1 (lead)	1.3
5	1.4
7	12.6
9	>35.1
10	>45.8
11	>42.4
12	44.5
13	44.2
15	>37.0
16	>38.3
19	>36.9
21b	>39.8
21c	>46.2
22a	>46.7
22b	5.9
22c	3.3
22d	6.3
24	>43.9
25	1.6

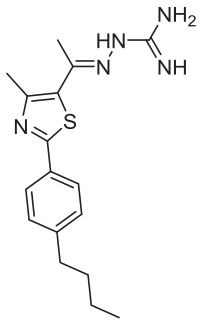

Fig. 1. Chemical structure of the lead compound **1**.

The amide derivatives **10**–**13** were prepared in quantitative yields by reacting the 4-butylphenylthiazole acid chloride intermediate **8** [34] with the appropriate amines in THF, as illustrated in Scheme 2. Compound **16** was synthesized in three steps, starting with the formation of the amide derivative **9** by way of reacting the acid chloride intermediate **8** with ammonium hydroxide in THF at room temperature. The amide intermediate **9** was then heated in thionyl chloride to give the nitrile intermediate **14**, which upon subsequent treatment with NaN_3 in the presence of iodine gave the tetrazole-containing thiazole derivative **16** as shown in Scheme 2. The nitrile intermediate **14** was also treated with hydroxylamine hydrochloride in absolute ethanol with a catalytic amount of potassium carbonate to afford the thiazole derivative **15**.

The phenylthiazolyl methyl ketone derivative **18** was prepared by treatment of the commercially available 4-aminothiobenzamide **17** with 3-chloro-2,4-pentanedione in absolute ethanol. Synthesis of the hydrazinecarboximidamide derivative **19** was achieved by treatment of the phenylthiazolyl methyl ketone derivative **18** with aminoguanidine hydrochloride in the presence of a catalytic amount of lithium chloride (Scheme 3).

Phenylthiazole methylketone derivatives **21a**–**d** and **24** were prepared via the Suzuki-Miyaura cross coupling of the (4-iodophenyl)thiazole derivative **3** with the commercially available phenylboronic acid derivatives **20a**–**d** and **23**, respectively, in the presence of a catalytic quantity of palladium(II) acetate and (2-biphenyl)dicyclohexylphosphine ligand, as shown in Scheme 4. Synthesis of the hydrazinecarboximidamide derivatives **22a**–**d** and **25** was achieved by treatment of phenylthiazole methylketone derivatives **21a**–**d** and **24**, respectively, with aminoguanidine hydrochloride in the presence of lithium chloride as a catalyst (Scheme 4).

3. Biological results and discussion

3.1. Antibacterial activity of thiazole compounds and vancomycin against MRSA, VISA, and VRSA

To ascertain the structure–activity relationships of the lead thiazole compound more thoroughly, derivatives were initially constructed with modifications to the thiazole-C5 cationic moiety (keeping the lipophilic alkane side chain at thiazole-C2 intact). Substitution of the ethylidenehydrazine-1-carboximidamide of the lead **1** with moieties such as a tetrazole (**16**), an amide derivative (**9**–**13**), or a hydroxamidine (**15**) results in complete abolishment of antimicrobial activity against MRSA (minimum inhibitory concentration (MIC) > 35.1 μg/mL (Table 1). This trend continues when the amino moiety is replaced with a ketone in derivatives **21b**–**c** and