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Development of improved inhibitors of wall teichoic acid biosynthesis with potent activity against *Staphylococcus aureus*

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ABSTRACT

A small molecule (1835F03) that inhibits *Staphylococcus aureus* wall teichoic acid biosynthesis, a proposed antibiotic target, has been discovered. Rapid, parallel, solution-phase synthesis was employed to generate a focused library of analogs, providing detailed information about structure–activity relationships and leading to the identification of targocil, a potent antibiotic.

More people now die from hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United States than from HIV/AIDs.[1] Until recently, vancomycin was known as the 'last line of defense' against these infections, but high-level vancomycin resistance has now begun to appear in *S. aureus*.[2–4] Although two new classes of antibiotics to treat *S. aureus* infections have been introduced in the past decade, clinical resistance to each has already appeared and can be expected to spread.[5–7] For these reasons, a deep pipeline of novel antibiotics is required to combat MRSA infections.[8]

One suggested but as yet unexploited antibiotic target in *S. aureus* is wall teichoic acid (WTA) biosynthesis.[9,10] WTAs are anionic, phosphate-rich, carbohydrate-based polymers that are synthesized on a lipid carrier inside the bacterial cell before being transported to the cell surface where they are covalently linked to peptidoglycan.[11] Their biological functions are diverse, ranging from cation homeostasis to critical roles in host colonization.[12] They are proposed virulence factors since deleting the biosynthetic pathway by knocking out the first gene (*tarO*) prevents infection. The downstream enzymes in this pathway are potential antibiotic targets since initiating flux into the biosynthetic pathway without completing it is deleterious to bacterial viability.[13]

We have discovered the first small molecule that specifically inhibits WTA biosynthesis using a cell-based, pathway-specific high-throughput screen that reports only on the antibiotic targets in the pathway. From a screen of 55,000 compounds, we identified **1835F03** (**1**) as a WTA-active antibiotic.[14] Compound **1** has good antibiotic activity (low μM) against all *S. aureus* strains tested, including both hospital- and community-acquired MRSA isolates. We identified the target of **1** as TarG, the transmembrane component of the two component ABC transporter that exports WTAs from the cytoplasm to the external surface of the bacterial membrane where they are attached to peptidoglycan (Fig. 1).[14] Two other compounds (**1856A19** and **1856K21**) that share a similar core with **1** were also identified as confirmed 'hits' from the screen, but their potencies were lower (Fig. 2). Moreover, commercial analogs of this class of inhibitors (**1856A19** and **1856K21**) appeared to have diminished target specificity relative to **1** since they showed partial growth inhibitory activity against the Δ*tarO* mutant strain (see Supplementary Table 1). Therefore we focused on **1** as a lead for optimization.

The commercially available analogs of compound **1** had limited structural variations and all lacked activity (see Supplementary Figs. 1 and 2). Therefore, we prepared a focused library of compound **1** analogs in order to identify sites on the scaffold that could be altered to improve potency without sacrificing selectivity for the target. The studies reported below provide key information about where this class of compounds is amenable to modification

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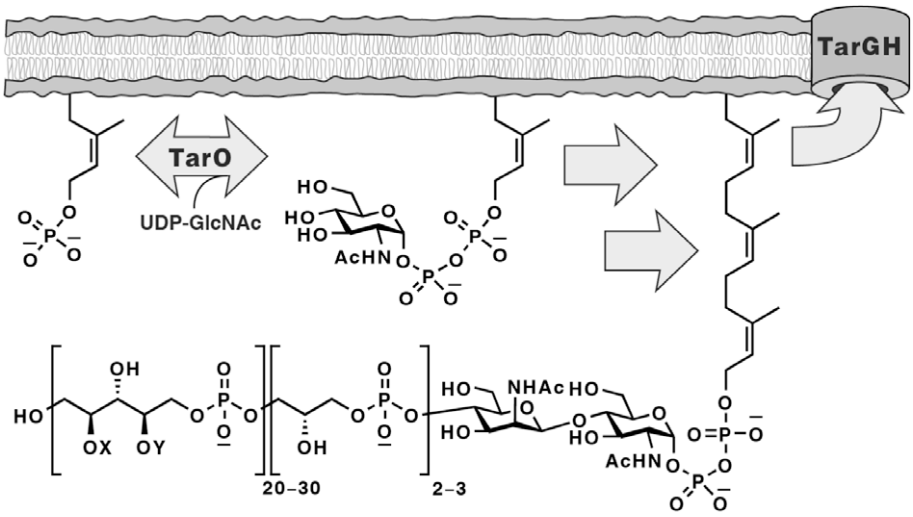


Figure 1. Poly(ribitol-phosphate) wall teichoic acids are constructed on a bactoprenol carrier lipid (C_{55}–P; partial structures shown here) embedded in the cytoplasmic membrane in *S. aureus*. TarGH is the ABC transporter that exports WTAs and is the target of the class of small molecule antibiotics described here. X and Y denote tailoring modifications of the WTA polymer (e.g., alanylation and glycosylation).

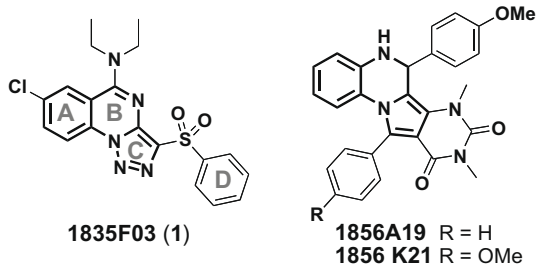
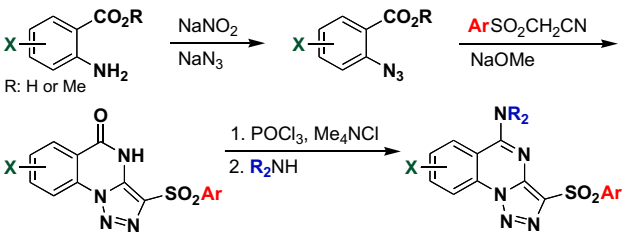


Figure 2. Chemical structures of the most active hits discovered in our high-throughput screen, with 'common' core structures shown in bold.

and have led to the identification of an analog 10 times more potent than **1** and is non-toxic in mice at doses of 75 mg/kg.

Based on the structure of **1** (**1835F03**), libraries of compounds with varying core substitution and side chains were prepared. The triazoloquinazoline core was synthesized following previously published methods (Scheme 1).[15] The 2-azidobenzoic acid or ester, prepared from the corresponding anthranilic acid derivative, was heated with an arylsulfonylacetonitrile in the presence of base to furnish the triazoloquinazolone framework in one step. This reaction is believed to proceed via 1,3-dipolar cycloaddition of the azide to the enolate form of the nitrile, followed by cyclocondensation of the resulting aminotriazole. The yields of the triazoloquinazolones varied depending on the substituents on the azidobenzoic acid. While the cyclization proceeded smoothly with electron-deficient ring systems, such as chloro- or nitro-substituted azidobenzoic acids, yields were low to moderate with electron-rich substrates.



Scheme 1. Synthesis of triazoloquinazoline analogs.

However, use of potassium carbonate in place of sodium methoxide (as previously published) resulted in moderate improvement. Chlorination of the resulting quinazolone using phosphorus oxychloride in the presence of tetramethylammonium chloride afforded the chloroquinazoline, which was converted in a straightforward manner to the corresponding aminoquinazoline derivatives. Whenever possible, the synthesis was carried out in a parallel format where the reactions were conducted in vials and the products were worked up by parallel solid phase extraction (SPE) using strong cation exchange (SCX) cartridges. Interestingly, attempts to remove the methyl ethers of **38** (in order to install solubilizing functional groups) utilizing boron tribromide caused ring opening of the triazole with concomitant loss of nitrogen, yielding inactive compounds (see SI for details).

All compounds were tested for antibacterial activity against a common laboratory strain of *S. aureus*, RN4220, and against the isogenic RN4220 Δ*tarO* strain, which lacks the first gene in the biosynthetic pathway and is thus not susceptible to WTA inhibitors. This latter strain allows for assessment of compound specificity since any toxic effects against it must be due to mechanisms other than WTA inhibition. The minimum inhibitory concentrations (MIC) of all compounds are reported against both strains in Tables 1 and 2 and Figure 3. Active compounds were subsequently tested against clinical *S. aureus* isolates (see Table 3).

The first analog library was designed to investigate the positions, sizes and electronics of the A-ring substituents. Commercial starting materials were chosen to probe the amenability of this area of the molecule to derivatization. The chloro group was placed at all possible locations on the A-ring (**2**–**4**), but activity was lost when this functionality was displayed anywhere other than C2. Therefore, we sought to optimize the substituent at this position. Modest changes at the C2 position (i.e., F, Me, Br, NO_2, CN; **5**–**9**) gave compounds with lower activity than the parent C2 chloro compound (**1**). Replacement of the chloro group with a methyl (**6**), bromo (**7**), or methoxy (**10**) substituent was tolerated, while a fluoro (**5**), nitro (**8**), or cyano (**9**) group abolished activity. From these data, it appears that size rather than electronics is the determining factor for activity. Likewise, substituents displayed at the C3 position resulted in inactive compounds **11**–**14**. Surprisingly, however, 2,3-dimethoxy substitution on the A-ring yielded a potent compound (**15**). Since both the mono-methoxy derivatives (**10** and **14**) are less active than the parent compound **1**, the effects of changing these positions were not additive. The benzodioxane