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Discovery of potent wall teichoic acid early stage inhibitors

Marc A. Labroli *, John P. Caldwell *, Christine Yang, Sang Ho Lee, Hao Wang, Sandra Koseoglu, Paul Mann, Shu-Wei Yang, Jing Xiao, Charles G. Garlisi, Christopher Tan, Terry Roemer, Jing Su

Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

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ABSTRACT

The widespread emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has dramatically eroded the efficacy of current β-lactam antibiotics and created an urgent need for novel treatment options. Using an *S. aureus* phenotypic screening strategy, we have identified small molecule early stage wall teichoic acid (WTA) pathway-specific inhibitors predicted to be chemically synergistic with β-lactams. These previously disclosed inhibitors, termed tarocins, demonstrate by genetic and biochemical means inhibition of TarO, the first step in WTA biosynthesis. Tarocins demonstrate potent bactericidal synergy in combination with broad spectrum β-lactam antibiotics across diverse clinical isolates of methicillin-resistant *Staphylococci*. The synthesis and structure–activity relationships (SAR) of a tarocin series will be detailed. Tarocins and other WTA inhibitors may provide a rational strategy to develop Gram-positive bactericidal β-lactam combination agents active against methicillin-resistant *Staphylococci*.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are a major cause of bloodstream infections in the hospital and in the community[1–4] with MRSA being reported as the second leading cause of mortality by drug-resistant bacterial pathogens in the United States. Alarmingly, the rise in MRSA and MRSE infections has coincided with an decline in the efficacy of β-lactam antibiotics.[1–4] Numerous reports, including our previous disclosures, detail a β-lactam combination agent strategy applied to methicillin resistant *Staphylococci* which was contingent upon the identification of new targets and pathways that normally buffer the drug resistant pathogen from the effects of β-lactams (either directly or indirectly) and that if inactivated by genetic or small molecule inhibition, result in restored sensitization to this important class of antibiotics.[5–10] A series of reports has shown that inactivation or alteration of wall teichoic acid (WTA) production causes MRSA to become highly susceptible to β-lactam antibiotics both in vitro as well as in a murine infection and offers an opportunity to test our β-lactam combination agent strategy.[5,11–16]

WTA is a Gram-positive specific anionic glycophosphate polymer comprising nearly 50% of the dry weight of the *S. aureus* cell wall that is cross-linked to the peptidoglycan and has critical roles in cell growth, division, morphology and in vivo virulence.[12,17,18] The WTA biosynthetic pathway is categorized into two phenotypically distinct groups; the non-essential WTA early-stage genes *tarO*, *tarA* and *mnaA*, and the conditionally essential WTA late stage genes which are responsible for WTA synthesis and eventual transport to the extracellular environment.[17–24] Although *Staphylococci* without WTA are viable, inactivation of late-stage WTA biosynthetic steps results in a growth inhibitory bacteriostatic effect.[14,17,21,23] The essentiality of the late-stage genes can be suppressed by concurrent inactivation of one of the non-essential WTA early stage genes (Fig. 1). This finding is an example of the essential gene paradox.[20,21,23–25,27]

We used the essential gene paradox to identify late-stage WTA inhibitors whose antibacterial activity reproduces the conditional essentiality of late-stage WTA genes in a phenotypic screen.[17,26] These compounds should inhibit wild-type *S. aureus* growth yet their activity is suppressed if an early non-essential step in WTA biosynthesis is also inactivated. In this way, multiple classes of late stage WTA inhibitors were identified, including targocil and L-638, which both inhibit polymer synthesis by targeting the WTA integral membrane transporter subunit, TarG[17,26,29,30] (Fig. 1). Recently, we have disclosed a phenotypic screening strategy which takes advantage of the essential gene paradox in order to identify early-stage inhibitors of wall teichoic acid production.[29] Furthermore, inhibition of TarO should sensitize MRSA to β-lactam antibiotics.[29]

Compounds from this phenotypic screen were prioritized for follow up studies provided that they: (i) were synergistic in

* Corresponding authors.
E-mail addresses: marc.labroli@merck.com (M.A. Labroli), john.caldwell@merck.com (J.P. Caldwell).

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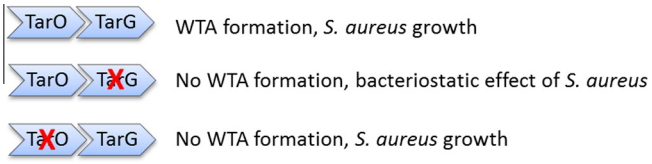
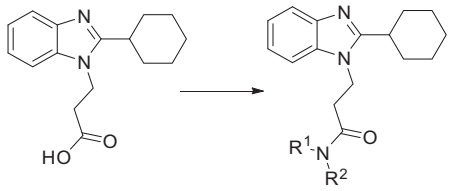


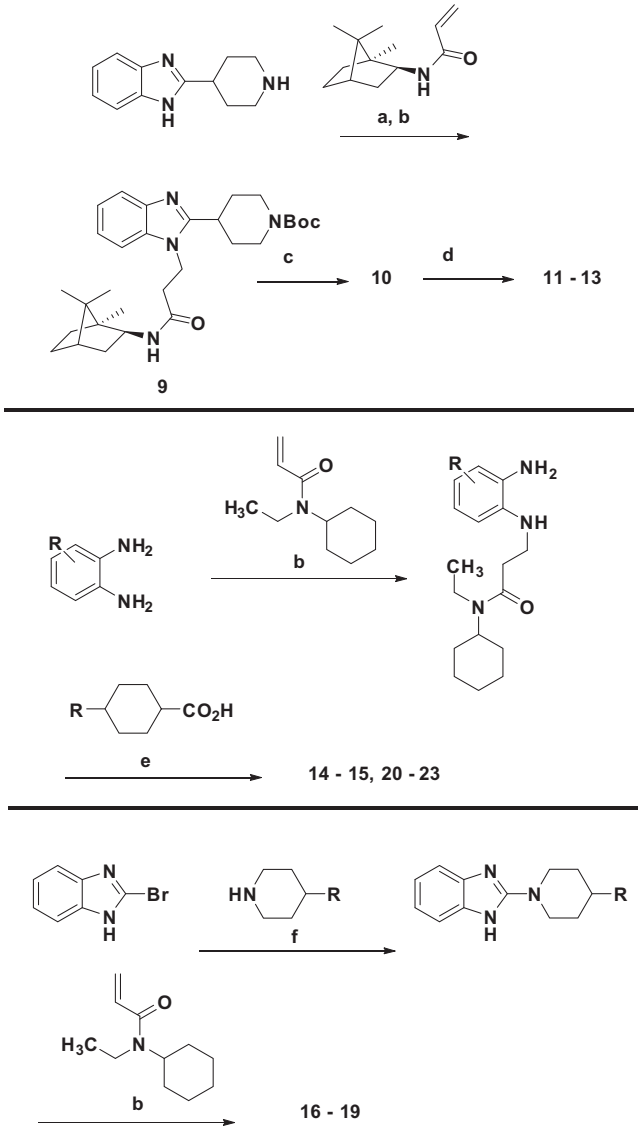
Figure 1. Effect of TarO and TarG inhibition on wall teichoic acid biosynthesis and *S. aureus* growth.



Scheme 1. Reagents and conditions: HATU, DIEA, amine, DMF, room temperature.

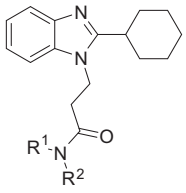
combination with imipenem below its clinical breakpoint (4 μg/mL); (ii) conferred resistance to cell lysis mediated by the *Staphylococcal*-specific lytic bacteriophage ØK ('PhageK') that utilizes WTA as its receptor for bacteriophage entry into the cell;[27] and (iii) demonstrated a dose-dependent suppression of the late-stage inhibitor L-638.[28] From our compound collection of several million compounds we found two compounds, an oxazolidinone (named 'tarocin A') and a benzimidazole (named 'tarocin B', **1a**),[29] that met all pathway-specific criteria. The SAR investigation of the 'tarocin A' series will be disclosed in due course. We investigated the SAR around **1a** in an effort to improve potency, physicochemical properties and potentiation with a β-lactam.

In the cellular assays, the initial lead **1a** when combined with 4 μg/mL of imipenem (IPM) exhibited synergistic effects with an $MITC_{95}$ = 1.56 μM,[31] conferred resistance to PhageK-mediated cell lysis (EC_{50} = 7.9 μM), and suppressed the inhibition of targocil (EC_{50} = 2.4 μM). When tested without imipenem, **1a** was inactive in these cellular assays; therefore, potentiating the effect of imipenem as expected from a WTA early-stage inhibitor. Initially we investigated the SAR of the amide region to explore the structural requirements to retain the potency of **1a**. Analogs were prepared in a parallel fashion using standard amidation conditions as shown in Scheme 1 from the carboxylic acid precursor. Select analogs are



Scheme 2. Reagents and conditions: (a) Boc_2O, CH_2Cl_2; (b) Michael acceptor, K_2CO_3, DMF, 120 °C; (c) TFA, CH_2Cl_2; (d) *N*-derivatization; (e) PPA, 200 °C; (f) amine, DMF, 140 °C.

Table 1
WTA profile of amide analogs



#	R^1	R^2	COL $MITC_{95}$ + IPM (μM)	EC_{50} PhageK (μM)	EC_{50} targocil reversal (μM)	*c*Log*P*
1a	(*R*)-(−)-Isonorbornyl	–H	1.6	7.9	2.4	6.9
1b	(*R*)-(+)-Bornyl	–H	16.7	178.5	57.7	6.9
2	–Ph	–Et	50	200	200	5.7
3	–*c*-heptyl	–Et	4.7	200	200	6.6
4	–*c*-hexyl	–Et	1.9	10.1	6.2	6.0
5	–*c*-pentyl	–Et	12.5	200	29.7	5.5
6	–*c*-hexyl	–^{n}Pr	6.2	200	7.8	6.6
7	–*c*-hexyl	–CH_3	4.7	25.0	11.4	5.5
8	–*c*-hexyl	–H	25	200	138.3	5.1