Bioorganic & Medicinal Chemistry Letters 24 (2014) 905–910

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Designing analogs of ticlopidine, a wall teichoic acid inhibitor, to avoid formation of its oxidative metabolites

Maya A. Farha [†], Kalinka Koteva [†], Robert T. Gale, Edward W. Sewell, Gerard D. Wright [*], Eric D. Brown [*]

M.G. DeGroote Institute for Infectious Disease Research and Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main St. W., Hamilton, Ontario L8N 3Z5, Canada

ARTICLE INFO

Article history:
Received 27 November 2013
Revised 17 December 2013
Accepted 17 December 2013
Available online 23 December 2013

Keywords:
Wall teichoic acids
MRSA
Ticlopidine
Clopidogrel
Structure–activity relationship

ABSTRACT

The thienopyridine antiplatelet agent, ticlopidine and its analog, clopidogrel, have been shown to potentiate the action of β-lactam antibiotics, reversing the methicillin-resistance phenotype of methicillin-resistant *Staphylococcus aureus* (MRSA), in vitro. Interestingly, these thienopyridines inhibit the action of TarO, the first enzyme in the synthesis of wall teichoic acid, an important cell wall polymer in Gram-positive bacteria. In the human body, both ticlopidine and clopidogrel undergo a rapid P450-dependent oxidation into their respective antiplatelet-active metabolites, resulting in very low plasma concentrations of intact drug. Herein, a series of analogs of ticlopidine and clopidogrel that would avoid oxidative metabolism were designed, prepared and evaluated as inhibitors of TarO. Specifically, we replaced the P450-labile thiophene ring of ticlopidine and clopidogrel to a more stable phenyl group to generate 2-(2-chlorobenzyl)-1,2,3,4-tetrahydro-isoquinoline) (**6**) and (2-chloro-phenyl)-(3,4-dihydro-1*H*-isoquinolin-2-yl)-acetic acid methyl ester (**22**), respectively. The latter molecules displayed inhibitory activity against TarO and formed the basis of a library of analogs. Most synthesized compounds exhibited comparable efficacy to ticlopidine and clopidogrel. So far, it was introduction of a trifluoromethyl group to compound **6**, to generate 2-(2-trifluoromethyl-benzyl)-1,2,3,4-tetrahydro-isoquinoline (**13**) that exhibited enhanced activity against TarO. Compound **13** represents a novel stable inhibitor of TarO with synergistic impact on β-lactam antibiotics against MRSA and low potential for P-450 metabolism.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen and the cause of serious infections both in the hospital and community settings. Although new agents have recently been introduced to treat resistant *S. aureus* infection,[1] MRSA remains a challenging adversary. Indeed, resistance to the limited spectrum of antibiotics available to treat MRSA infections has emerged.[2–4] There is thus an urgent need for new strategies to overcome resistance.

Novel approaches that aim to reverse resistance of MRSA to well-established antibiotics, specifically β-lactams, through the use of adjuvant molecules, have recently been explored.[5–8] In this approach, the adjuvant targets cellular processes that underpin the resistance phenotype, restoring sensitivity to β-lactam antibiotics. Interestingly, several cellular processes have been found to play critical roles in the methicillin resistance phenotype of MRSA,[7,9,10] including the synthesis of wall teichoic acids (WTAs). We and others have shown that inhibition of WTA synthesis can render MRSA sensitive to the action of β-lactam antibiotics.[5,6,11]

WTAs are anionic polymers found on the cell surface of Gram-positive bacteria. Like that of peptidoglycan (PG), their synthesis begins on an undecaprenyl phosphate carrier lipid followed by elongation into long polymers containing ribitol phosphate repeats by the consecutive action of Tar enzymes. Unlike PG, however, WTA polymers are not essential for viability. Nevertheless, the genes encoding WTA synthesis display idiosyncratic patterns of dispensability; late-acting genes are essential while early steps, encoded by *tarO* and *tarA*, are not.[12] Further, the late-acting genes are conditionally essential; they become dispensable on deletion of an early step gene.[12]

We recently discovered a novel inhibitor of TarO, the first step in the synthesis of WTAs, through a high-throughput combination screen looking for molecules that potentiated the β-lactam cefuroxime against MRSA.[6] In that effort, we screened a collection of previously approved drugs with well-established pharmacology and toxicology profiles.[13] Several non-antibiotic drugs were found to potentiate cefuroxime against MRSA. A counterscreen of these for loss of synergy with cefuroxime in a *tarO* deletion background led to the discovery that ticlopidine, an antiplatelet drug, had impacted wall teichoic acid synthesis. The combination of ticlopidine, which has no antibacterial activity on its own, and cefuroxime resulted in a highly synergistic interaction against the problematic

* Corresponding authors. Tel.: +1 905 525 9140.
E-mail addresses: wrightge@mcmaster.ca (G.D. Wright), ebrown@mcmaster.ca (E.D. Brown).
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.12.069

community-acquired (CA-) strain of MRSA, USA300. This potentiation was also observed with the commercially available analog of ticlopidine, clopidogrel. Ticlopidine displayed expected signature interactions with late-acting WTA genes; ticlopidine was antagonistic with genetic blocks in late steps and similarly, antagonized the inhibition by targocil,[14] an inhibitor of the late-acting enzyme, TarG. In vitro, ticlopidine also inhibited the function of recombinant TarO.

Ticlopidine (Fig. 1) and its analog clopidogrel (Fig. 1) are thienopyridine pro-drugs that inhibit adenosine-5′-diphosphate (ADP) mediated platelet aggregation.[15] Both molecules are converted to thiol-containing active metabolites through a thiolactone moiety by cytochromes P450 in the liver[16] (Fig. 2). Indeed, studies of the distribution and elimination of ticlopidine and clopidogrel reveal that both drugs are rapidly and extensively metabolized such that plasma concentrations of intact molecules are relatively very low.[16,17]

Knowledge of the metabolic pathways of ticlopidine and clopidogrel inspired the rational design and synthesis of 25 varying analogs described herein. Indeed, our main objective was to design a TarO inhibitor that, in principle, would evade the metabolism described above. As such, we can presume that target therapeutic plasma concentrations of the intact antibacterial agent can be achieved.

Synthesized compounds were tested for their ability to (1) synergize with the β-lactam cefuroxime against CA-MRSA USA300, (2) antagonize the TarG inhibitor, targocil, against CA-MRSA USA300, and (3) inhibit TarO in vitro. Here, we measure drug–drug interactions via the widely used FIC index (ΣFIC).[18] We define synergy as having a ΣFIC $\leqslant$0.5, additivity as >0.5–4.0 and antagonism as $\geqslant$4.0. A representative checkerboard analysis is shown in Supplementary Figure 1. Our in vitro assay for TarO activity follows the incorporation of radiolabelled GlcNAc onto undecaprenyl-P-P on a membrane system to generate the product of TarO, undecapreny-P-P-GlcNAc.[6] Given the labor- and resource-intensive nature of the latter assay, we tested representative analogs from each series.

We began our SAR study by investigating the requirement for either the AB-ring or BC-ring system of ticlopidine (Fig. 1) for activity, with compounds **2** and **3**, respectively (Table 1). Both compounds failed to exhibit the drug interactions expected of a TarO inhibitor, resulting in additivity (ΣFIC = 2.0) when combined with either cefuroxime or targocil. Additionally, compounds **2** and **3** displayed very weak inhibitory activities against TarO in vitro (IC_{50} = 4.3 mM and 1.1 mM, respectively) compared to ticlopidine (IC_{50} = 0.15 mM). Further changes to the B-ring of compound **3** (compounds **4** and **5**) failed to generate activity (Table 1). Thus there was a strict requirement for all ring systems present in ticlopidine for its TarO activity.

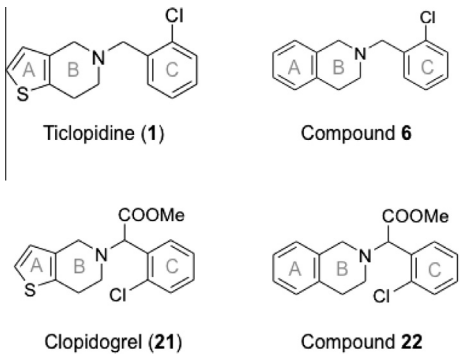

Figure 1. Chemical structures of lead candidate TarO inhibitors and their phenyl derivatives.

More importantly, to eliminate the possibility of conversion of ticlopidine to its active antiplatelet metabolite, we replaced its P450-labile thiophene ring (A-ring) to a more stable phenyl group to generate compound **6** (Fig. 1). As such, oxidation to the thiolactone intermediate would not be possible. Compound **6** was synthesized via coupling of 1,2,3,4-tetrahydroisoquinoline with 2-chlorobenzyl chloride (Scheme 1a).

Compound **6** retained activity in all of our assays, sharing relatively similar FIC indices (ΣFIC = 0.13 with cefuroxime, 8.1 with targocil) and inhibition constant (IC_{50} = 0.52 mM) with ticlopidine (Table 2). Thus, this phenyl derivative of ticlopidine formed the basis of the first library of analogs we explored. We first investigated all possible positions of the chloro (–Cl) group originally found at the *ortho* position in the C-ring of **6**. Compounds with *meta*- (**7**) and *para*-chloro (**8**) substitutions were no longer capable of potentiating the activity of cefuroxime (ΣFIC = 2.0) but did, however, antagonize the activity of targocil (ΣFIC = 8.1) (Table 2). Importantly, in vitro, **7** led to an IC_{50} of 0.27 mM. This suggests that the analogs are in fact inhibitors of TarO, hence antagonizing the late-step inhibitor targocil, but are perhaps not potent enough to reverse β-lactam resistance and potentiate the activity of cefuroxime. Indeed, an intricate mechanism is involved in re-sensitizing MRSA to β-lactams upon inhibition of WTA synthesis.[6] This observation also implies that our assay for combination with targocil may be a more sensitive indicator of cell-based TarO activity than potentiation of cefuroxime. Finally, removal of the chloro group (**9**) decreased activity in our cell-based assays, yielding additive ΣFIC's of 0.63 with cefuroxime and 1.3 with targocil (Table 2). Compound **9** however exhibited equipotent activity as compound **6** in vitro (IC_{50} = 0.27 mM). These data suggest that the chlorine atom of the parent compound (**6**) plays a key role in its cell-based antibacterial activity. Its relatively strong electronegativity likely influences the hydrophilic–hydrophobic balance of the molecule and is needed for cellular activity but can be spared for its in vitro activity against TarO.

Next, given the importance of functionalization at the C2 position of the C-ring of **6**, we prepared a series of compounds (**10**–**13**) with varying substitutions at this position, namely methyl ($-CH_3$) (**10**) fluoro (–F) (**11**), nitro ($-NO_2$) (**12**) and trifluoromethyl ($-CF_3$) (**13**) groups (Table 2). All four compounds were found to be active, potentiating the activity of cefuroxime and antagonizing targocil to similar degrees (Table 2) and to levels comparable to the activity of the parent compound **6**. Importantly, substitution of the C2-chloro group of **6** to a trifluoromethyl (**13**), yielded a more potent compound than **6**, lowering the ΣFIC to 0.094 for synergy with cefuroxime and increasing the ΣFIC to 8.3 for antagonism with targocil (Table 2). Here, the presence of the trifluoromethyl group likely increases the stability and lipophilicity of the molecule[19] and thus its in vivo uptake. In fact, compound **13** yielded a comparable inhibition constant in vitro against TarO (IC_{50} = 0.35 mM) to that of parent compound **6** and ticlopidine. Additional modifications to compound **6** included conversion of its C-ring to a pyridyl group (**14**) (Table 2). Introduction of the nitrogen heteroatom completely abolished activity, leading to additivity with cefuroxime and targocil (ΣFIC's = 2.0). This suggests that decreasing lipophilicity is detrimental, especially for the cell-based assays. Finally, substitution of the C-ring of **6** to a naphthalene group gave compound **15** (Table 2). The latter exhibited signature interactions of a TarO inhibitor but with a small loss of activity with cefuroxime (ΣFIC = 0.31) but not targocil antagonism (ΣFIC = 8.1). In vitro, the napthyl derivative caused little change in activity relative to compound **6** (IC_{50} = 0.22 mM). This finding was surprising because **15** bears a different conformation and thus occupies a different area of space relative to **6**. Here, addition of the bulky second ring may be compensating for the lack of substitution at the *ortho* position, which is crucial for activity. This