Bioorganic & Medicinal Chemistry Letters 27 (2017) 5310–5321

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Compound design guidelines for evading the efflux and permeation barriers of *Escherichia coli* with the oxazolidinone class of antibacterials: Test case for a general approach to improving whole cell Gram-negative activity

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ARTICLE INFO

Article history:
Received 10 July 2017
Revised 26 September 2017
Accepted 7 October 2017
Available online 16 October 2017

Keywords:
Gram-negative
Outer membrane permeability
Oxazolidinones
Efflux pumps
Porins

ABSTRACT

Previously we reported the results from an effort to improve Gram-negative antibacterial activity in the oxazolidinone class of antibiotics via a systematic medicinal chemistry campaign focused entirely on C-ring modifications. In that series we set about testing if the efflux and permeation barriers intrinsic to the outer membrane of *Escherichia coli* could be rationally overcome by designing analogs to reside in specific property limits associated with Gram-negative activity: i) low MW (<400), ii) high polarity (clogD$_{7.4}$ <1), and iii) zwitterionic character at pH 7.4. Indeed, we observed that only analogs residing within these limits were able to overcome these barriers. Herein we report the results from a parallel effort where we explored structural changes throughout all three rings in the scaffold for the same purpose. Compounds were tested against a diagnostic MIC panel of *Escherichia coli* and *Staphylococcus aureus* strains to determine the impact of combining structural modifications in overcoming the OM barriers and in bridging the potency gap between the species. The results demonstrated that distributing the charge-carrying moieties across two rings was also beneficial for avoidance of the outer membrane barriers. Importantly, analysis of the structure-permeation relationship (SPR) obtained from this and the prior study indicated that in addition to MW, polarity, and zwitterionic character, having ≤4 rotatable bonds is also associated with evasion of the OM barriers. These combined results provide the medicinal chemist with a framework and strategy for overcoming the OM barriers in GNB in antibacterial drug discovery efforts.

In spite of the widespread and increasing prevalence of multidrug-resistant Gram-negative and Gram-positive bacteria (GNB and GPB), the number of new drugs being developed to treat bacterial infections is at its lowest point since the dawn of the antibiotic era.[1,2] Hence, there is an urgent need for new discovery platforms and approaches to develop new antibacterial drugs, and to stop the dangerous trends of increasing multidrug-resistance. The conventional target based high throughput assays have been demonstrated earlier as successful approaches to identify hits with micro- and nano-molar scales of activities in cell free assays, however poor membrane permeability and high susceptibility to active efflux have consistently frustrated efforts to evolve these "hits" into "leads" with promising whole cell activity.[3,4] The outer membrane of Gram-negative bacteria restricts penetration of hydrophobic compounds, and efflux pumps extrude any hydrophobic molecules that leak in through that barrier.[5,6] The net result is that hydrophobic compounds with potential antibacterial activity, as suggested by potent inhibition of their intended targets, are not active against these bacteria due to an inability to achieve sufficient concentrations at their sites of action. Extensive reviews of physicochemical properties of compounds that have shown good

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https://doi.org/10.1016/j.bmcl.2017.10.018
0960-894X/

activity against GNB provided a range of structural features that do not necessarily fall into the Lipinski's rule of five.[7,8]

The oxazolidinone class of antibacterials covers the important Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and penicillin-resistant *Streptococcus pneumoniae*.[9] Clinical resistance has emerged, albeit slowly, to this class of drugs, however structural modifications able to evade the mechanisms responsible have been reported.[10] Linezolid was the first oxazolidinone antibiotic to be approved by the US Food and Drug Administration (FDA) and has been used for the treatment of serious infections caused by Gram-positive strains such as MRSA and VRE since 2000 (Fig. 2). Favorable clinical results shown by linezolid prompted many pharmaceutical industries and academic institutions to explore the possibilities of expansion of antibacterial spectrum of this class leading to a broad SAR information available covering this scaffold (Fig. 1).[11–14] Many hundreds of oxazolidinone derivatives have been reported over the years since linezolid was discovered, but these compounds do not have structures that adhere to the guidelines we believe necessary for GNB activity. From our perspective, this explains why no derivative has yet been identified having a fully broadened spectrum of activity. In fact, linezolid is known to have much improved potency against *E. coli* and other common GNB when the efflux system is compromised.[15]

We recently reported the results from a focused medicinal chemistry campaign aimed at improving whole cell GNB activity in the oxazolidinone class of antibacterials.[16] Our approach was to design C-ring analogs with physicochemical and structural properties we believed to be in the correct range for GNB activity: i) low MW (preferably <400); ii) relatively high polarity (clog$D_{7.4}$ <1); and iii) charged character with bias to zwitterionic character at physiological pH.[16] From this effort we found that zwitterionic character in particular, when imparted onto the C-ring, resulted in significantly reducing the impact of efflux and the permeation barriers on activity against *E. coli*. This is best demonstrated by the lead compound, **DP-326**, identified in that effort (Fig. 2). Unfortunately, the modifications that led to minimization of the impact of the OM barriers were accompanied by a reduction in protein synthesis inhibitory potency compared to linezolid, as determined with a cell-free transcription-translation assay. This reduced biochemical potency appears reflected in the higher than desired antibacterial MIC values for **DP-326**.

In the present work we continued to leverage the known SAR in the oxazolidinone class of GPB antibacterials (Fig. 1) but expand our medicinal chemistry efforts to explore other regions on the oxazolidinone scaffold beyond the C-ring. Specifically, we investigated A- and B-ring regions that may be tolerant of modification, or where the available data suggest certain modifications have led to potency enhancements. Our goal was to identify analogs with distributed polar groups and multi-charge character that demonstrated reduced liability towards the OM barriers, but that avoided the undesired activity loss seen in our C-ring series. Each compound prepared was tested against a diagnostic MIC panel of wild-type and outer membrane compromised *E. coli* strains and a *Staphylococcus aureus* control strain to determine the impact of structural modifications on OM permeability and efflux liability, and to monitor our progress towards bridging the potency gap between the species caused by these barriers. We also analyzed our current overall SPR data gathered from our earlier and current studies in an effort to address the question of whether emphasizing appropriate physicochemical properties represents a useful medicinal chemistry approach for optimizing Gram-negative antibacterial activity in the oxazolidinones class of antibacterials.

In our previous study[16] we reported a rational and systematic medicinal chemistry approach, led by guidelines derived from data gathered on earlier GNB potent antibiotics, toward adding

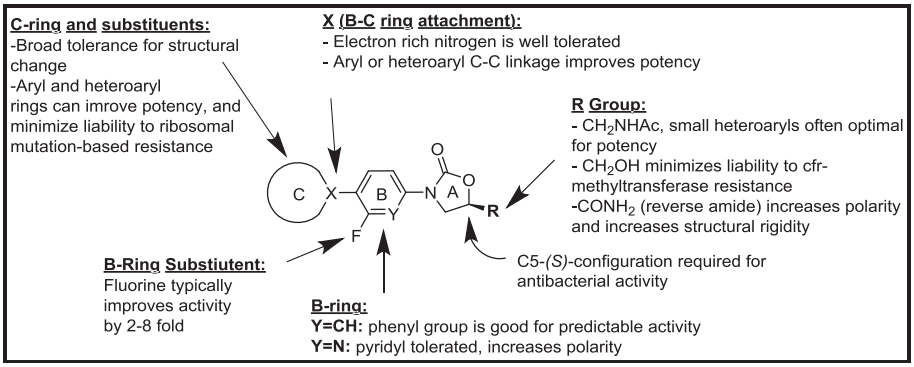

Fig. 1. Summary of known SAR in the oxazolidinone class of GPB antibacterials.

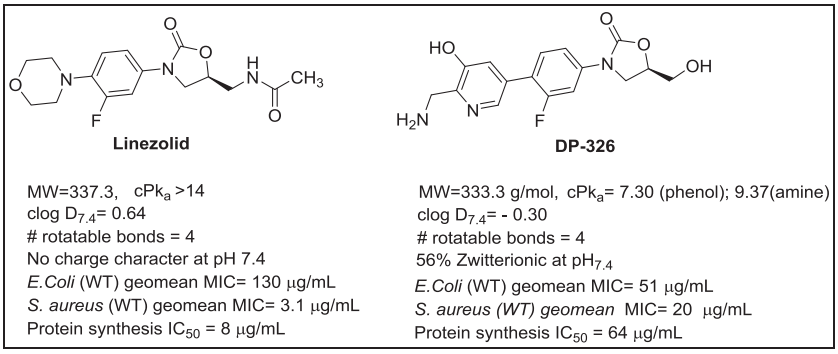

Fig. 2. Physicochemical properties and structures of Linezolid and DP-326.