Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

The oxadiazole antibacterials

Jeshina Janardhanan, Mayland Chang and Shahriar Mobashery

The oxadiazoles are a class of antibacterials discovered by *in silico* docking and scoring of compounds against the X-ray structure of a penicillin-binding protein. These antibacterials exhibit activity against Gram-positive bacteria, including against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). They show *in vivo* efficacy in murine models of peritonitis/sepsis and neutropenic thigh MRSA infection. They are bactericidal and orally bioavailable. The oxadiazoles show promise in treatment of MRSA infection.

Address
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, United States

Corresponding authors: Chang, Mayland (mchang@nd.edu) and Mobashery, Shahriar (mobashery@nd.edu)

Current Opinion in Microbiology 2016, **33**:13–17

This review comes from a themed issue on **Antimicrobials**

Edited by **Michael J Pucci** and **Thomas J Dougherty**

For a complete overview see the Issue and the Editorial

Available online 27th May 2016

http://dx.doi.org/10.1016/j.mib.2016.05.009

Introduction

The modern pharmaceutical industry had its beginnings in the development of the first penicillins, β-lactam antibiotics isolated from the mold *Penicillium notatum* that inhibit penicillin-binding proteins (PBPs) involved in bacterial cell-wall synthesis. This success created the impetus in exploration of natural products for discoveries of the early classes of antibiotics [1]. The heyday of this period came to be known as the Golden Age of Antibiotics, spanning the 1950s through 1970s. The success was so profound that it created a perception of glut in the field in the subsequent years. Many pharmaceutical companies abandoned research on antibiotics, for reasons that have been elaborated elsewhere and will not be repeated here [2,3]. The departure of Big Pharma created a void that smaller companies and universities tried to fill. Notwithstanding the return from this exodus of a handful of companies in the past few years, the field moved in new directions, largely away from natural products. A number of new strategies have been applied to antibiotic discovery, including continued screening of natural products [4], high-throughput screening of synthetic compound libraries [5], target-directed rational design [6] and *in silico* docking and scoring [7,8] of critical targets. The discovery of the oxadiazole class of antibacterials, the subject of this report, came out of the *in silico* search with a penicillin-binding protein (PBP) as the target [9••].

We docked and scored a 1.2-million ZINC library of drug-like compounds with the X-ray structure of PBP2a of methicillin-resistant *Staphylococcus aureus* (MRSA). The *mecA* gene of the *mec* operon encodes PBP2a. When the first strains of *S. aureus* that came to be known as MRSA were identified in the early 1960s [10], it was noted that they became broadly resistant to the entire class of β-lactam antibiotics [11]. The resistance profile for MRSA often has an inducible phenotype. It involves detection of the β-lactam antibiotic in the growth medium, an information that is transduced to the cytoplasm [12–14]. The ensuing transcriptional derepression of a set of genes leads to the expression of PBP2a. This enables the organism to survive in the face of the challenge by β-lactam antibiotics, as PBP2a can perform the critical cell-wall crosslinking reaction that other PBPs are incapable of, as they would be inhibited by the antibiotic [15,16]. This selection pressure has been important for effective global dissemination of MRSA over the previous half a century. The organisms that are collectively referred to as MRSA remain a major clinical problem to the present day [16].

We reasoned that the clinical success of β-lactam antibiotics over the previous several decades [17–19] warranted exploration of other inhibitor classes for PBPs. The argument was that the cell wall, and specifically PBPs as biosynthetic enzymes for it, remain important targets for antibiotics. The point is underscored as the cell wall is a critically important macromolecular entity in bacteria, which is absent in mammalian organisms. Inhibition of the crosslinking event, performed by certain PBPs (transpeptidases), could not be sustained by the bacterium and would lead to lethal consequences. It was in this light that the list of the docked and scored compounds for binding to PBP2a was scrutinized to identify molecules that had the potential as antibacterial candidates.

The docking and scoring procedure ranked the library compounds and we selected over 100 of the top-ranking molecules based on the pragmatic consideration of ease of synthesis for further study. These compounds were examined for their antibacterial activity at this stage with living bacteria, as opposed to their inhibitory properties with the recombinant purified PBP2a. If a compound did not exhibit antibacterial activity with living bacteria, we abandoned it. The bar was set high to weed out molecules

early in discovery. We performed the antibacterial testing with the ESKAPE panel of bacteria, comprised of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* species (the underlined first letters for the names of the genera makes the acronym), which account for the majority of the nosocomial resistant organisms [20,21]. These studies identified oxadiazole **1** as a hit (Figure 1), with modest minimal-inhibitory concentrations (MICs) of $\geq$64 μg/mL against the Gram-positive organisms (*E. faecium* and *S. aureus*). Whereas the poor activity could have been the basis for abandoning this hit, the activity was reproducible and was of interest since no antibacterial activity had been attributed to this molecular scaffolding previously and the compound class was amenable to facile synthesis. These considerations prompted us to explore the oxadiazoles further. It was fortunate that merely the third oxadiazole that was synthesized in our labs improved the MIC to 2 μg/mL (compound **2**, Figure 1) against the tested *S. aureus* strain, which justified additional efforts on this molecular template [9••].

Structure–activity relationship and the mode of action

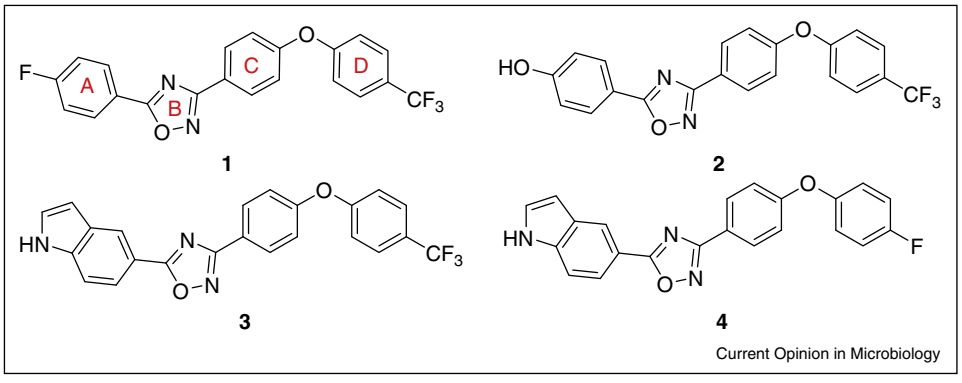
We showed that antibacterial **2** inhibited cell-wall biosynthesis and not replication, transcription or translation using macromolecular synthesis assays. In this assay, the incorporation of radiolabeled precursors — [methyl-^{3}H]-thymidine, [5-^{3}H]-uridine, L-[4,5-^{3}H]-leucine or D-[2,3-^{3}H]-alanine into the DNA, RNA, protein or peptidoglycan, respectively, of *S. aureus* strains in the log phase of growth, was monitored in the presence of sub-MIC concentrations of the antibacterial. Antibiotics such as ciprofloxacin, rifampicin, tetracycline and fosfomycin, known to inhibit the respective pathway, were used as positive controls [9••]. Antibacterial **2** inhibited PBP2a, which was consistent with the search paradigm. The compound was tested against a broader panel of Gram-positive bacteria and displayed MIC values ranging from 1 to 2 μg/mL against an extended panel, including against MRSA and VRE, with the exception of Streptococci (Table 1) [9••].

Meanwhile, we synthesized a collection of oxadiazoles in efforts to define the structure–activity relationship for this class. Modifications of ring A (see the chemical structure **1**, Figure 1) generated a series of active antibacterials. Loss of activity was observed when the hydrogen-bond donor at the 4-position of ring A was replaced with halogens or other hydrogen-bond accepting moieties, as did several hetero-aromatic systems and aliphatic heterocycles. Although compounds with pyrazoles at ring A showed very good *in vitro* antibacterial activity, they exhibited some toxicity to mammalian cells. Antibacterial **3** (also designated as ND-421 in some publications), is a lead molecule of this series.

Isomeric arrangements of heteroatoms in ring B retained activity. Other five-membered ring species, such as pyrazoles and isoxazoles produced antibacterial activity. Several structural variations in ring C were well tolerated. Replacing the bridging oxygen with a sulfide or an amine did not affect the antibacterial activity. Fused C and D rings were mostly inactive or had a higher MIC values of 8 μg/mL [22]. Replacing the trifluoromethyl at the 4-position of the diphenyl ether moiety with a fluorine (antibacterial **4**) did not affect activity. A number of substitutions in ring D were tolerated [22].

It is known that β-lactam antibiotics often target more than one PBP for inhibition, as each bacterium has several closely related PBPs [23]. This multiplicity of targeting is actually an aspect of the success of β-lactams [18]. Hence, it is likely that oxadiazoles would target more than a single PBP in various organisms. As such the MIC that is measured is a composite of the inhibition profile for the collection of PBPs that are inhibited. To gain insight into the structural attributes that impart antibacterial activity, a three-dimensional quantitative structure–activity relationship (3D-QSAR) model was developed based on the

Figure 1



Oxadiazole compounds with antibacterial activity synthesized based on an *in silico* search with a penicillin-binding protein (PBP) as the target.