Bioorganic & Medicinal Chemistry Letters 18 (2008) 6179–6183

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design at the atomic level: Generation of novel hybrid biaryloxazolidinones as promising new antibiotics

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ARTICLE INFO

Article history:
Received 12 May 2008
Revised 30 September 2008
Accepted 2 October 2008
Available online 7 October 2008

Keywords:
Oxazolidinone
Biaryloxazolidinone
Structure-based drug design
Linezolid
Sparsomycin
Gram-negative bacteria
X-ray crystal structure
Ribosome
Oral antibiotics
Hybrid antibiotics

ABSTRACT

From the X-ray crystal structures of linezolid and the non-selective antibiotic sparsomycin, we have derived a new family of hybrid oxazolidinones. From this initial compound set we have developed a new biaryloxazolidinone scaffold that shows both potent antimicrobial activity as well as selective inhibition of ribosomal translation. The synthesis of these compounds is outlined.

Bacterial resistance to antibiotics has increased in part because of an extended 'drought' in antibacterial drug development. This 38-year innovation gap ended in 2000 with the introduction of linezolid (currently marketed under the tradename Zyvox).[1] Linezolid represents a new chemical class of oxazolidinone-containing antibacterials.

Unfortunately, it took less than a year after its introduction for case reports to appear detailing instances of vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* showing cross-resistance to linezolid.[2,3]

The design of new antibiotics that have a broader and more potent spectrum of antibacterial activity is an important objective. For ribosomal inhibitors this objective can be accomplished by boosting intrinsic affinity for the ribosome, thereby overcoming resistance and enhancing the spectrum against both linezolid-susceptible and -resistant Gram-positive organisms and important Gram-negative community respiratory pathogens such as *Moraxella catarrahalis* and *Haemophilus influenzae*. Our discovery program began with a detailed X-ray structural knowledge of the provocative juxtaposition of the linezolid-binding site[4] with that of the non-selective antibiotic sparsomycin within the 50S ribosomal subunit (Fig. 1A and B). In Figure 1A we show sparsomycin (pink) and linezolid (blue) as space-filling representations within the RNA bases (gray) of the peptidyl transferase center. In this figure the overlapping region of these two antibiotics is highlighted by the dotted circle. Figure 1B shows this same overlap without the ribosomal context. This relationship had been revealed from previous crystallographic studies.[5,6] Both compounds bind within the peptidyl transferase center of the ribosome, an area that we have defined as an important *ribofunctional loci* for targeting inhibition of ribosomal activity.

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doi:10.1016/j.bmcl.2008.10.014

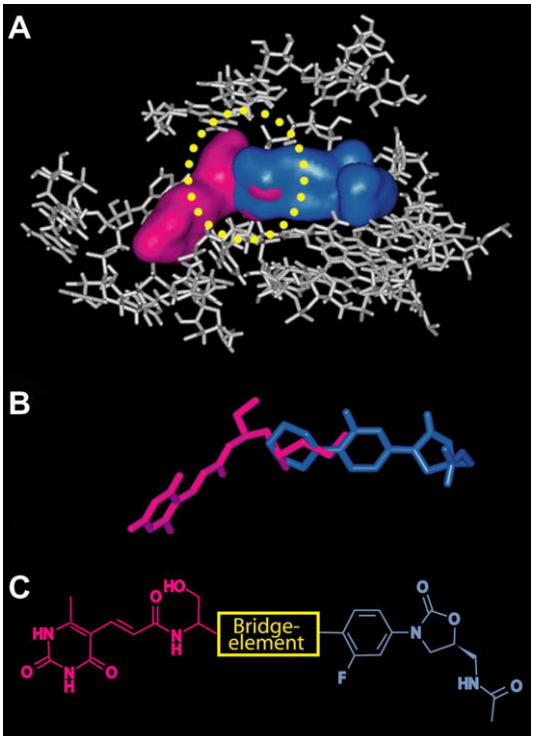


Figure 1. Initial design for enhanced oxazolidinones (sparsomycin is shown in pink, linezolid in blue, and ribosomal RNA bases in gray).

As reviewed previously,[7] our discovery strategy for the design of these enhanced oxazolidinones can be summarized by the following five steps: (I) establish that different ribofunctional loci defined by adjacent or overlapping antibiotics binding to the 50S can be exploited simultaneously to deliver a potent antibacterial compound; (II) identify molecular features important for driving bacterial selectivity within the sparsomycin-binding region; (III) optimize the bridge elements spanning these loci and identify the minimal core and optimal bridging element required for increasing potency (Fig. 1C); (IV) identify the features necessary for enhancing potency against Gram-negative bacteria; and (V) balance the resultant compounds for properties consistent with both intravenous and oral administration.

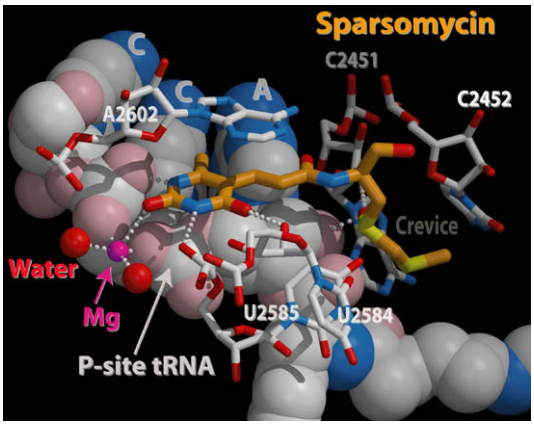


Figure 2. Web of interactions made by the thymine head group of sparsomycin (shown in orange) with RNA bases (shown as white stick figures). A bound P-site tRNA analog is represented as a space-filling surface. The thymine head group is to the left of the sparsomycin structure.

Results and discussion: The atomic coordinates for the structure of the 50S-sparsomycin complex have been previously determined by Hansen and colleagues.[5] The structure shows the thymine head group of sparsomycin to be involved in an extensive network of interactions involving key residues within the core peptidyl transferase region as well as the CCA end of the P-site tRNA substrate (Fig. 2). The fact that these interacting elements are very highly conserved across both prokaryotic and eukaryotic ribosomes may explain why sparsomycin lacks bacterial selective activity.

Although our primary target molecules would maintain the essential molecular elements of both linezolid and sparsomycin, our ultimate plan was to design into our new structures bacterial specificity by selectively modifying portions of the sparsomycin thymine head group involved in making the non-discriminatory interactions with the ribosome. To jump-start the program, a pool of potential bridging elements between the two antibiotics, linezolid, and sparsomycin, were identified via an in-house implementation of Caveat[8] (data not shown). We prioritized the synthesis of several of the best combinations featured therein, based on optimized interactions with the ribosome. Three chemically-distinct templates (Fig. 3, compounds **1–3**) were prepared retaining the key structural features of the phenyloxazolidinone fragment of linezolid and the thymine ring of sparsomycin with different bridge elements. The first of these molecules (**1**) incorporated a piperidine ring bridge element. The second construct (**2**) utilized a simplified linear alkenyl bridge that was designed to maintain the relative position of the thymine head group of sparsomycin and the phenyloxazolidinone of linezolid in an extended conformation. The third composite molecule (**3**) incorporated an aromatic-rich phenyl bridge element. This third molecule was expected to take advantage of π stacking to key ribosomal bases, a feature frequently found within the ribosome.

The ribosomal selectivity for these constructs was evaluated through inhibition of translation of ribosomes derived from both *Escherichia coli* and rabbit reticulocyte[9] (Table 1). Pursuing the notion that simplifying the thymine head group of sparsomycin and hence reducing its network of hydrogen bonds with the RNA backbone might improve selectivity away from eukaryotes, we also prepared compounds **4** and **5**. As can be seen in Table 1 compounds 1 and **2** were potent inhibitors of *E. coli* translation and were as expected, non-selective. Compound **3** showed greater selectivity probably reflecting the influence of the additional aryl ring on enhancing binding to the bacterial ribosome. Nonetheless, given the large size of these hybrid structures the observed level of translation activity was reassuring. We were also gratified to obtain a crystal structure of compound **2** (Fig. 4) bound to the ribosome which clearly confirmed both our computational projection and overall structural hypothesis.[10]

Compounds **4** and **5**, where the thymine group had been simplified to a pyridine ring, were less potent against *E. coli* but clearly demonstrated improved selectivity for bacterial ribosomes over those of rabbit reticulocyte (Table 1). Of these first five compounds, the two which incorporated a phenyl linking element (compounds **3** and **5**), demonstrated both superior translation and antimicrobial inhibition. In addition to reducing entropy, this result confirmed our hypothesis that the biaryloxazolidinone scaffold (Fig. 5) could take advantage of an energetically favorable π stacking interaction with the ribosome. Their refinement into a new family of biaryloxazolidinones is described in the companion letter.[18] Although derived here from first principles by use of the ribosomal structure, several examples of substituted biaryl oxazolidinones have been previously reported.[12–15]

Synthesis: Our initial hybrid probe molecules **1–5** (Fig. 3) were prepared as shown in Schemes 1–3. The common thymine acrylic

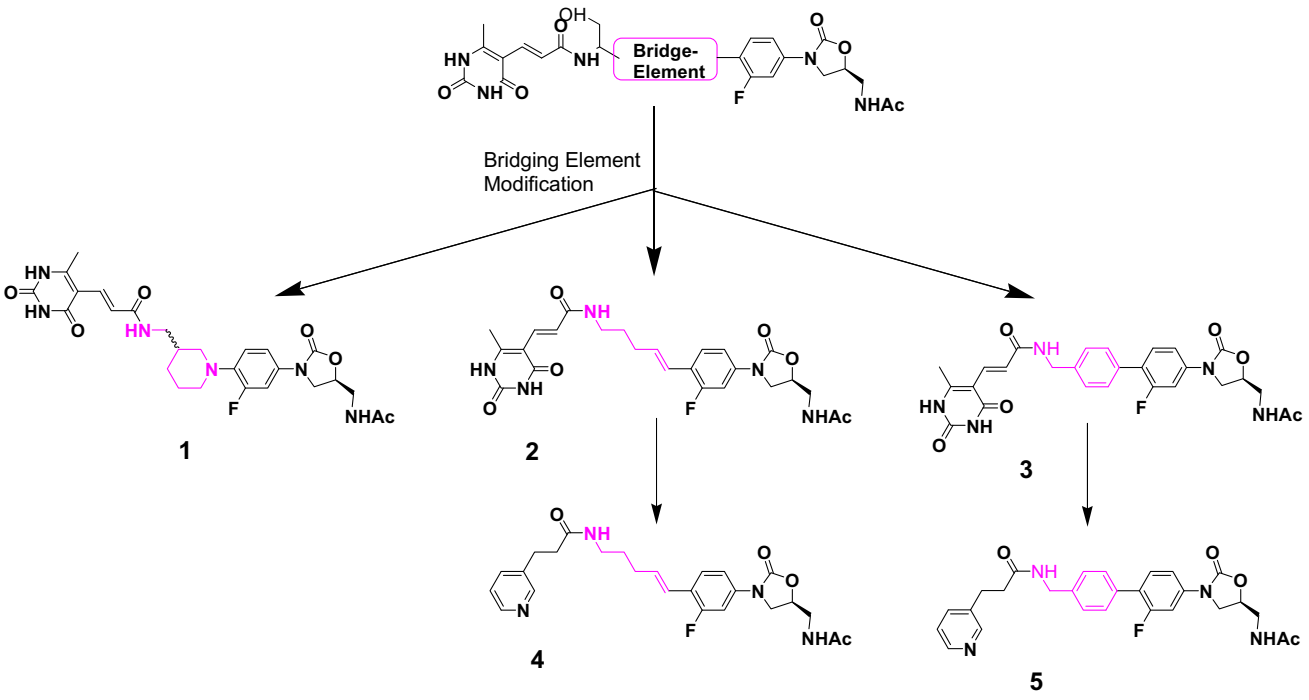


Figure 3.

Table 1
Initial biological results

	Linezolid	Sparsomycin	1	2	3	4	5
Intrinsic affinity							
E. coli translation IC_{50} (μM)	4.6	⩽ 0.02	0.26	0.03	0.03	16	0.58
Selectivity	Y	N	N	N	Y	Y	Y
MIC (μg/ml)							
S. pneumoniae 02J1175 *mef*(A)	2	2	4	1	⩽0.25	2	0.5
S. pyogenes Msr 610 *erm*(B)	1	2	4	1	⩽0.25	2	0.5
E. faecalis P5 (linezolid®)	32	>128	>128	32	16	32	16
H. influenzae parent strain (RD1)	16	8	>128	>128	>128	>128	>128
H. influenzae 895 (AcrB-KO)	8	8	8	1	⩽0.25	8	2

All minimal inhibitory concentration (MIC) determinations were carried out under NCCLS conditions.[11]

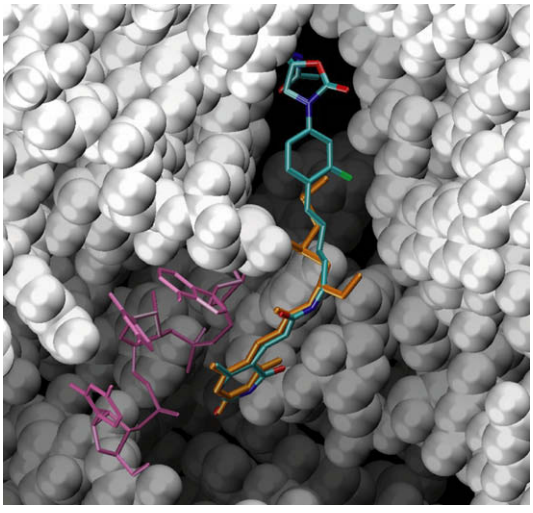


Figure 4. Crystal structure of Compound **2** (cyan) bound within the 50S rRNA pocket (depicted as white van der Waals surface). The tRNA mimetic (CCA) (magenta) is also shown bound within the ribosomal P Site. The crystal structure of sparsomycin (orange) is superimposed for reference.

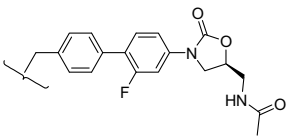
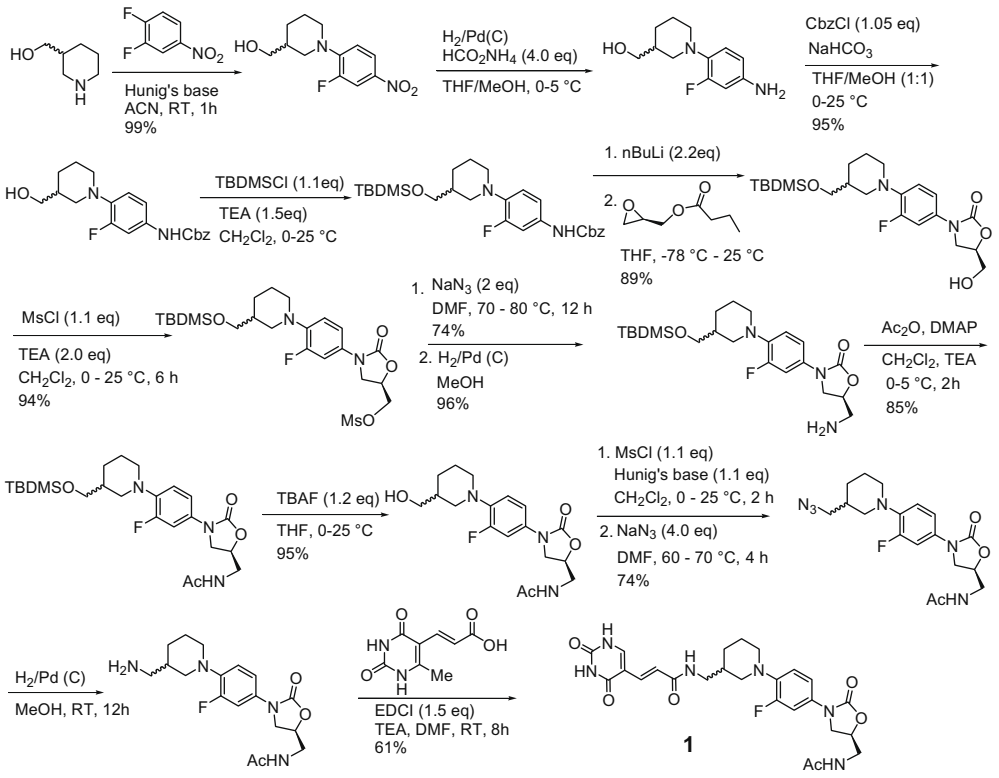
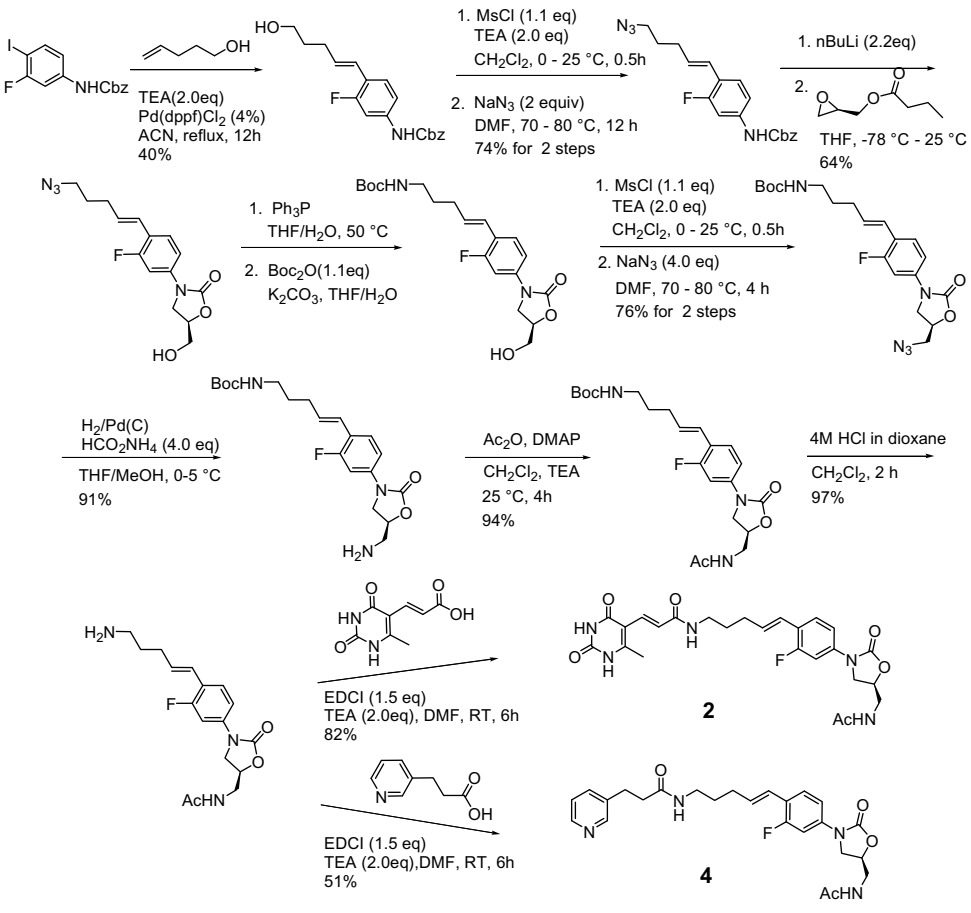


Figure 5. Biaryloxazolidinone scaffold.

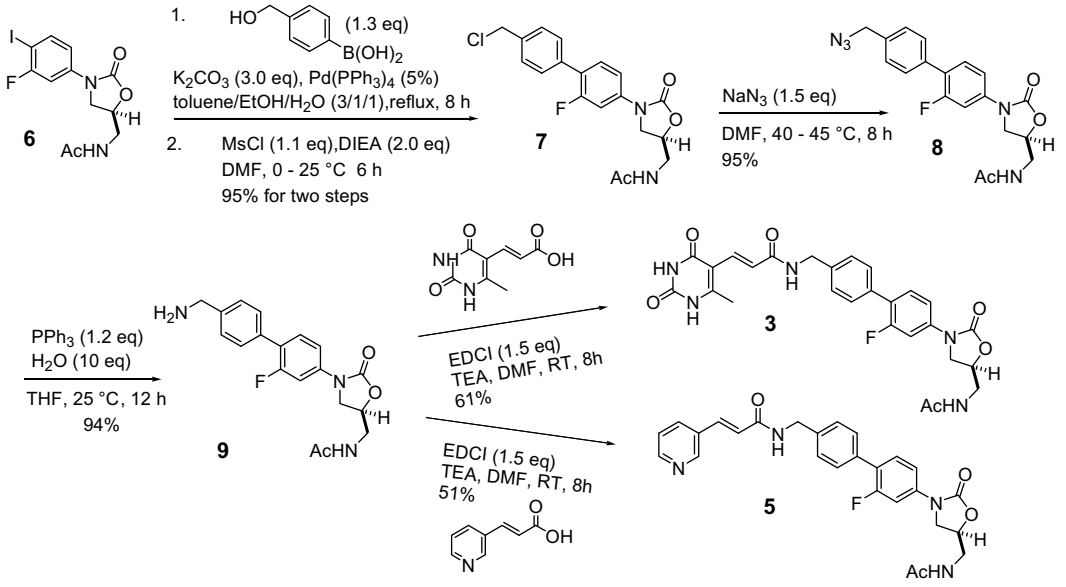
acid head group was prepared by a literature method.[16] In each case this element was attached to the target scaffold via an EDCI (1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide) mediated amide coupling. 4-Aminomethylpiperidine substituted 3-fluorophenyl oxazolidinone, the precursor for compound **1** was generated in a multistep synthesis as shown in Scheme 1. Compounds **2** and **4** were prepared as shown in Scheme 2 starting with carbobenzyloxy (Cbz)-protected 4-iodo-3-fluoroaniline. Compounds **3** and **5** were generated from the versatile intermediate 4-iodo-3-fluorophenyl oxazolidinone acetamide (**6**)[17] as shown in Scheme 3. The more advanced intermediates **7, 8**, and **9** generated in this scheme were later incorporated into the synthesis of more advanced molecules[18].



Scheme 1.



Scheme 2.



Scheme 3.

Acknowledgments

We thank Laura Lawrence, Timothy McConnell, Bradford King, and Xiang Luo, for the biological data incorporated in this paper.

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