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Design at the atomic level: Design of biaryloxazolidinones as potent orally active antibiotics

Jiacheng Zhou [b,¶¶], Ashoke Bhattacharjee [b], Shili Chen [b], Yi Chen [b,‡‡], Erin Duffy [a], Jay Farmer [b,†], Joel Goldberg [b,‡], Roger Hanselmann [b], Joseph A. Ippolito [a], Rongliang Lou [b], Alia Orbin [b,§], Ayomi Oyelere [b,¶], Joe Salvino [b,‖], Dane Springer [b,††], Jennifer Tran [b,‡‡], Deping Wang [a,§§], Yusheng Wu [b], Graham Johnson [a,b,*]

[a] Department of Structure-Based Drug Design, Rib-X Pharmaceuticals Inc., 300 George Street, Suite 301, New Haven, CT 06511, USA
[b] Department of Medicinal Chemistry, Rib-X Pharmaceuticals Inc., 300 George Street, Suite 301, New Haven, CT 06511, USA

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ABSTRACT

We have developed a first generation of hybrid sparsomycin–linezolid compounds into a new family of orally bioavailable biaryloxazolidinones that have activity against both linezolid-susceptible and -resistant Gram-positive bacteria as well as the fastidious Gram-negative bacteria *Haemophilus influenzae* and *Moraxella catarrahalis*. The convergent synthesis of these new compounds is detailed.

In the preceding letter[1] we outlined how the X-ray structures of linezolid and sparsomycin bound to the 50S ribosome were used to design a new family of oxazolidinones. Specifically, we described how the previously unknown proximity and juxtaposition of the linezolid and sparsomycin-binding sites led us to the design of new compounds to bridge and occupy the binding sites of both of these antibiotics. In this letter, we describe how these extended and somewhat complex hybrid molecules were transformed into potent and selective next generation biaryloxazolidinone antibiotics with an expanded antibacterial spectrum.

Compound **1** described in our companion letter[1], was prepared using an alkenyl bridge element to connect the desired thymine ring of sparsomycin and the phenyloxazoldinone element of linezolid. Close inspection of the structure of compound **1** in complex with the 50S ribosomal subunit[1] suggested that the placement of the terminal thymine ring could be further optimized. As shown in Figure 1, the condensed heteroaryl face of the key interacting partner of the 50S ribosomal subunit, Adenine 2602 (colored white; *Escherichia coli* numbering of 23S rRNA), participates in an offset π stack interaction with the terminal thymine element in Compound **1**. Computational studies indicated that this is not the optimal relative orientation for a π–π interaction. As such, we prepared three analogs (**2, 3, and 4**) on the biaryl scaffold (Fig. 2) in which we had sought to optimize the π overlap between the terminal heterocycle and the ribosomal base. Specifically we focused our attention on both shortening the chemical functionality bridging the terminal

* Corresponding author. Tel.: +1 203 848 6934; fax: +1 203 624 5627.
E-mail address: gjohnson@rib-x.com (G. Johnson).
† Present address: 1517 Caywood Road, Lodi, NY 14860, USA.
‡ Present address: Wyeth Research, CN 8000, Princeton, NJ 08543, USA.
§ Present address: 4890 Briarwood Drive, Macungie, PA 18062, USA.
¶ Present address: Georgia Tech, 3305 IBB Building, GA 30332, USA.
‖ Present address: Cephalon Inc., 383 Phoenixville Pike, Malvern, PA 19355, USA.
†† Present address: Goodwin and Procter, 53 State Street, Boston, MA 02109, USA.
‡‡ Present address: 91 Golden Hill Drive, Guilford, CT 06437, USA.
§§ Present address: Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142, USA.
¶¶ Present address: Incyte Corporation, Experimental Station, E336, Route 141 and Henry Clay Road, Wilmington, DE 19880, USA.

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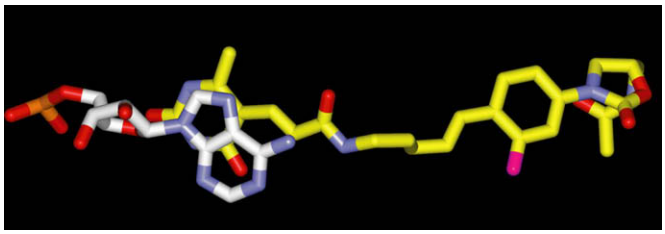

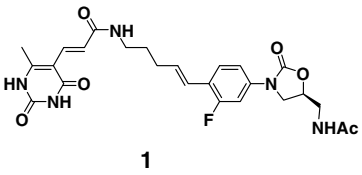

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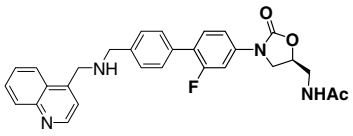
Figure 1. Key interaction of compound **1** (yellow) with Adenine 2602 (A2602) (white) of the 50S ribosomal binding site.



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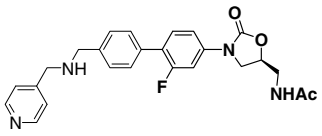

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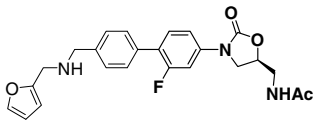

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Figure 2.

aromatic group to the biaryloxazolidinone and on modifying the nature of the terminal aromatic group.

Table 1 shows the results from our efforts to optimize antibiotic–ribosome interactions. In all cases, a simple benzylic amine functionality delivered the aromatic rings for productive interactions with A2602, and simple heterocycles such as **3** were as potent as condensed heterocycles. Exquisite potency across the streptococci was maintained by all three compounds (**2, 3**, and **4**). In addition, all were potent against the linezolid-resistant enterococcal strain. Importantly, all analogs showed potency against *Haemophilus influenzae* RD1, a moderately susceptible clinical strain, although their MIC_{90}s (not shown) were not optimal (8–16 μg/ml). Their molecular properties, in particular the predictions for Caco-2 cell permeability, a surrogate for oral bioavailability, were significantly worse than linezolid and therefore were expected to have limited oral bioavailability. This liability was confirmed in the comparatively poor in vivo efficacy of these compounds in a murine peritonitis model (data not shown).

Balancing the biaryl scaffold for candidate compounds: In conjunction with a series of amino acid biaryl oxazolidinones (details to be published separately), these simplified analogs formed the base for a predictive computational model that described activity against *H. influenzae*.[†††] The model is a simple six-descriptor model that requires a balance between factors that contribute to both affinity and those that speak to drug-like properties (data not shown). Combining this model with accurate Qikprop[2] predictions for Caco-2 cell permeability and aqueous solubility as well as being guided by our structural knowledge within the ribosome, we initiated an optimization program. Our goals were to maintain or improve activity across the Gram-positive bacteria, boost activity against *H. influenzae*, and balance properties required for in vivo efficacy. In some cases this design strategy led us away from the incorporation of a terminal heterocycle (compounds **9** and **11**). In order to be effective, we devised synthetic routes and key intermediates that would allow us to probe several lines of analoging in a parallel and efficient manner. Using these synthetic approaches we designed and generated analogs **5–11** (Fig. 3).[‡‡‡] As noted earlier[1] the highest energy interaction with the ribosome are associated with the biaryl and oxazolidinone residues. From our computational analysis, the diversity of remaining residues incorporated in compounds **5**–**11** are readily accommodated by the ribosome but do not provide for specific interactions. Their major influence is therefore on modifying the various molecular properties associated with cell penetration.

Table 1
Optimization of terminal aromatic interactions

	Linezolid	**2**	**3**	**4**
Intrinsic affinity				
E. coli translation IC_{50} (μM)	4.6	<0.02	0.05	0.04
Selectivity	Y	Y	Y	Y
MIC (μg/ml)				
S. pneumoniae 02J1175 *mef* (A)	2	0.5	0.2 5	0.25
S. pyogenes Msr 610 *erm* (B)	1	0.5	0.5	0.2 5
E. faecalis P5 (linezolid®)	32	2	2	4
H. influenzae parent strain (RD1)	16	8	8	4
H. influenzae 895 (AcrB-KO)	8	1	0.5	0.5

All minimal inhibitory concentration (MIC) determinations were carried out under NCCLS conditions.[3] Ribosomal translation was carried out as described in our companion letter.[1] All compounds were greater than 100-fold selective for *E. coli* over rabbit reticulocyte enzymes.

As shown in Table 2, these compounds showed superior activity across the MIC panel, which included clinically relevant and challenging Gram-positive strains and were >100-fold selective for bacterial ribosomes over those of rabbit reticulocyte (data not shown). Moreover, they showed consistent and significant activity against *H. influenzae* strains, with most MIC_{90}s against *H. influenzae* of 4 μg/ml or better. Additionally, all compounds showed oral efficacy in a *Streptococcus pneumoniae*

[†††] Full details of the structure-based approach, described above will be published elsewhere. Briefly, from iterative solutions of new oxazolidinone-containing compounds bound to their ribosomal target, we ranked new analogs on how they improved the interaction energies and shape complementarity (or "fit") to the binding pocket. This was accomplished using our proprietary suite of computational tools, AnalogTM, which combines a grow-search-and-score algorithm with a highly accurate molecular properties calculator, QikProp. These ideas were filtered based on mathematical models that point in the direction of improved biological activity. MIC data from a broad range of substituted bis-aryloxazolidinones were incorporated as thetraining sets for several computational models of *H. influenzae* inhibition. These models were based on either a linear or neural network approach and were used to generate a target list of substituted alkyl and heteroalkylamino bis-aryl oxazolidinones.

[‡‡‡] Although derived here from first principles by use of the ribosomal structure, several examplesof substituted biaryl oxazolidinones have been previously reported.[5–8] However, the importanceof the therapeuticaly valuable heterocyclic-substituted aminoalkyl variants reported hererepresent a new advance to this important compound class.

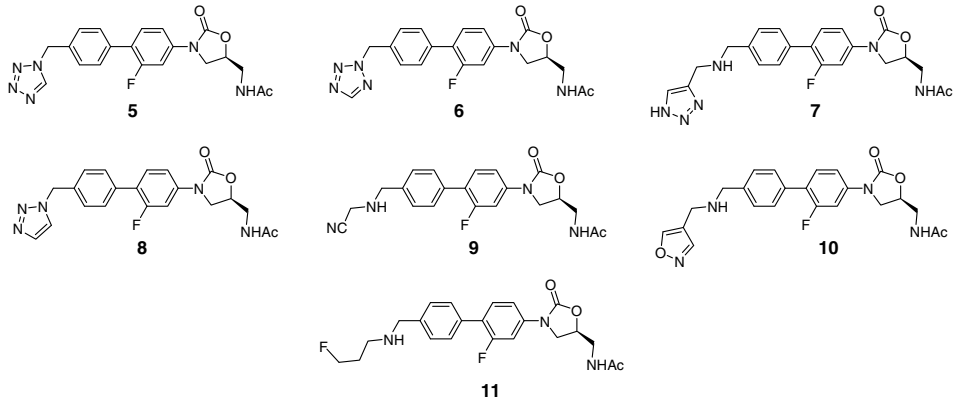
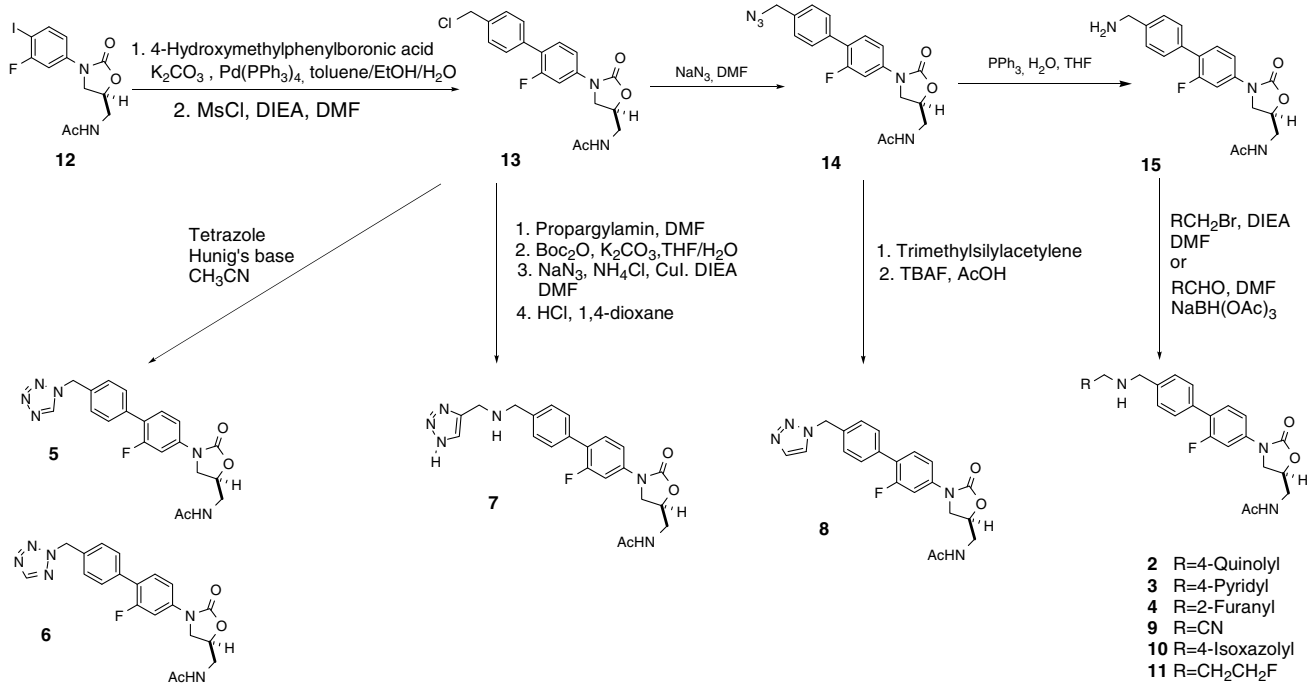


Figure 3.

Table 2
Potent, orally efficacious designer oxazolidinones

Bacterial strain (MIC in μg/ml)	5	6	7	8	9	10	11	Linezolid
E. faecalis ATCC29212-P5 (linezolid^R)	1	1	1	1	1	2	2	32
E. faecalis 1069 *vanB*	0.5	⩽0.25	⩽0.25	0.5	⩽0.25	0.5	0.5	2
E. faecium A6349 *vanA* + (linezolid^R)	1	0.5	0.5	1	1	1	1	16
S. aureus 67-0 MRSA	1	0.5	1	0.5	0.5		1	2
S. pneumoniae 02J1175 *mef (A)*	0.25	0.125	0.25	0.5	0.25	0.125	0.125	1
S. pneumoniae 02J1258 *erm* (B) + L4	0.25	0.25	0.25	0.25	0.25	0.125	0.125	2
S. pyogenes msr610 *erm* (B)	0.5	0.125	0.125	0.5	0.25	0.125	0.125	1
H. influenzae RD1 parent	2	4	0.5	4	4	2	2	16
H. influenzae 1100	2	2	0.25	2	4	1	2	16
H. influenzae A1950	8	8	1	8	8	8	4	32
MIC_{90} *H. influenzae*	4	8	1	4	8	4	4	32
PD_{50} (mg/kg/day) *S. pneumoniae* 02J1175 *mef (A)*	2.50	<0.8	10.70	1.30	2.00	28.40	1.70	5.8

All minimal inhibitory concentration (MIC) determinations were carried out under NCCLS conditions.[3]



Scheme 1.

mouse peritonitis model (Table 2). These values were generally better than the highly orally-bioavailable linezolid, whose oral PD_{50} in this model is 5.8 mg/kg/day.

Synthesis: Compounds **2–4** (Fig. 2) and **5–11** (Fig. 3) were prepared as shown in Scheme 1. Palladium-mediated coupling of hydroxymethylphenyl boronic acid with intermediate **12**[4] followed

by mesylation and chloride displacement afforded compound **13** in excellent overall yield. Displacement of chloride from **13** by tetrazole afforded both possible tetrazole regioisomeric products **5** and **6**. Displacement of chloride from **13** with propargylamine followed by copper-catalyzed 2 + 3 cycloaddition of azide afforded the aminomethyltriazole **7**. Reaction of **13** with sodium azide in DMF gave intermediate **14**). Reaction of **14** in a copper-mediated 2 + 3 cycloaddition of trimethylsilylacetylene followed by deprotection afford the N-linked triazole **8**. Finally, phosphine-mediated reduction of **14** gave the amine **15**. Using either alkylation or reductive amination afforded target compounds **2**–**4**, **9**, **10**, and **11**.

Acknowledgments

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