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Structure-based drug design meets the ribosome

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ARTICLE INFO

Article history:

Received 22 September 2005

Accepted 13 December 2005

Keywords:

Antibacterials

Antibiotics

Structure-based drug design

Ribosomes

X-ray structure

Computational chemistry

ABSTRACT

The high-resolution structures of the bacterial ribosomal subunits and those of their complexes with antibiotics have advanced significantly our understanding of small-molecule interactions with RNA. The wealth of RNA structural data generated by these structures has allowed computational chemists to employ a drug discovery paradigm focused on RNA-based targets. The structures also show how target-based resistance affects antibiotics acting at the level of the ribosome. Not only are the sites pinpointed where different classes of antibiotics inhibit protein synthesis, but their orientations, relative dispositions, and unique mechanisms of action are also revealed at the atomic level. Both the 30S and the 50S ribosomal subunits have been shown to be “targets of targets”, offering several adjacent, functionally relevant binding pockets for antibiotics. It is the detailed knowledge of these validated locations, or *ribofunctional loci*, plus the mapping of the resistance hot-spots that allow the rational design of next-generation antibacterials.

When the structural information is combined with a data-driven computational toolkit able to describe and predict molecular properties appropriate for bacterial cell penetration and drug-likeness, a structure-based drug design approach for novel antibacterials shows great promise.

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1. Introduction

The discovery and development of new antibacterial agents has been for decades a major topic in pharmaceutical companies. Nonetheless, the antibacterial field has witnessed the exodus of many pharmaceutical companies from the front lines. This exodus is disturbing, especially at a time when bacterial resistance to current therapies is high and increasing rapidly, in both, hospital and community settings [1]. Yearly, approximately 90,000 hospital patients in the United States die as a result of a bacterial infection. This death rate increased almost an order of magnitude since 1992, when it was 13,300 [1]. In over 70% of these bacterial infections, the bacteria are resistant to at least one antibacterial drug. Often, they are resistant to multiple antibacterial drugs. Importantly, in the last 43 years only two new classes of antibiotics were

introduced [1–4], and in both cases clinical resistance was observed shortly after their debut [4–7]. Thus, a new approach towards novel antibiotic discovery seems essential.

A drug discovery paradigm that has come of age is structure-based drug design (SBDD) [8–11], where atomic-level details of both the molecular target and locations of key resistance mutations are known and drive the rational design of improved drug candidates. The power of SBDD has been demonstrated most clearly in the AIDS arena, where structural knowledge of HIV-1 protease fueled the successful design and development of five protease inhibitors as marketed drugs [12–14]. Recently, and owing to significant advances in structural biology around one particularly valuable bacterial target—the bacterial ribosome—SBDD has become a suitable and indeed powerful tool for antibacterial drug discovery.

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doi:10.1016/j.bcp.2005.12.026

2. The target: bacterial ribosomes

Ribosomes are the universal ribonucleoprotein complexes (2.5 MDa) responsible for protein synthesis in all cells and organelles. Bacterial ribosomes (70S) are built of two subunits (30S and 50S) of unequal size. These subunits associate upon the initiation of protein biosynthesis.

The ribosome is a particularly well-validated antibacterial target. Ribosomes are the target for about half of the total number of antibiotics characterized to date [15,16]. These antibiotics can be broken down into three classes: (a) inhibitors acting on the 30S; (b) inhibitors acting on the 50S; and (c) inhibitors acting on the 70S, the fully assembled ribosome. Although the structure of the 70S ribosome has been solved [17], increasing our understanding of the way ribosomes work, the resolution of this structure does not allow its use for SBDD.

The 30S subunit is responsible for genetic decoding and thus incorporation of the proper amino acid in the growing polypeptide chain. Several antibiotic classes, including tetracycline and the aminoglycosides, bind to disrupt this function. The 50S subunit encompasses the main enzymatic activity of the ribosome, peptide bond formation. Structurally distinct antibiotic classes such as the macrolides (e.g., erythromycin and azithromycin), lincosamides, streptogramins, phenyl propanoids (e.g., chloramphenicol) and oxazolidinones (e.g., linezolid) bind and disrupt polypeptide synthesis by binding to one of several distinct sites on the 50S subunit.

Although the general mechanisms of action by which the 30S and the 50S subunits work have been known for many years, this knowledge was not sufficient to afford new classes of antibiotics. Now, structural data allows us to delineate the binding of antibiotics to the ribosomal subunits and explains much of the biochemical literature concerning ribosomal antibiotics. Additionally, the structures provide insights to understand ribosome-based resistance mutations. Interestingly, the antibiotic structures solved shows that they primarily (and in most cases exclusively) bind to the RNA component of their respective ribosomal subunits, as it had been inferred from biochemical experiments [18]. Different antibiotic classes occupy adjacent, although not identical, sites in the ribosome. Fig. 1 shows this multivalency in the peptidyl transferase region of the 50S ribosomal subunit. These functionally validated sites can be considered *ribofunctional loci*: critical sites where antibiotics act. The structures provide the details of these sites, highlight their adjacencies, and therefore create opportunities for optimizing interactions in and between these sites.

One interesting feature of the *Haloarcula marismortui* 50S ribosomal subunit is that some of the key target-based resistance mutations in pathogenic bacteria are native to this archaeobacteria. For example, nucleotide A2058 (*Escherichia coli* numbering), a major determinant for macrolide binding and a site known to confer resistance upon mutation, is a guanine in *H. marismortui*. In the A2058G ribosomes, azithromycin does not enjoy the optimal dispersion interactions shown in A2058 because the exocyclic amine causes a

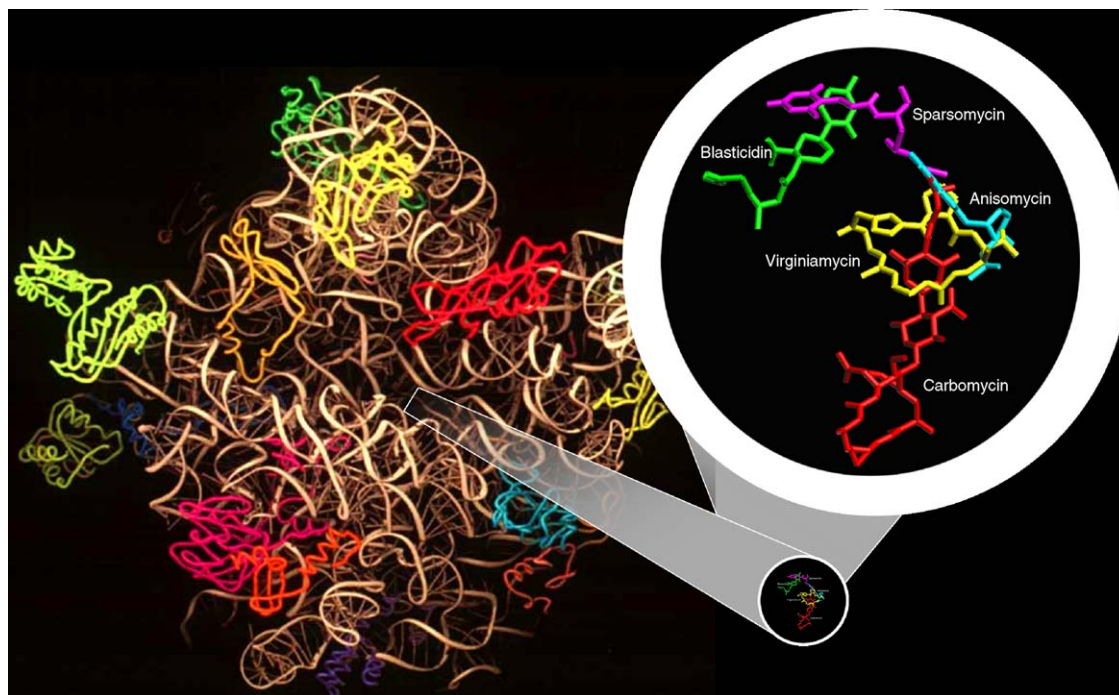


Fig. 1 – Relative bound orientations of diverse antibiotic classes in the complex with the peptidyl transferase region of the *Haloarcula marismortui* 50S ribosomal subunit. The 50S ribosome is shown on the left, the ribosomal RNA is shown as brownish ribbons, and the ribosomal proteins are shown in bright color ribbons. The upper right circle shows a zoom-in view of the relative bound orientations of several diverse antibiotics in the complex with the peptidyl transferase region of the *Haloarcula marismortui* 50S ribosomal subunit. The small circle in the lower right gives an idea of the approximate location and size of the peptidyl transferase region to where this antibiotics bind.

clash with the macrocyclic ring. Biochemically, this translates to a 10^4 reduction in binding affinity [19]. It is from these vantage points that structure-based design is poised to deliver new and important therapies in the antibacterial drug discovery arena.

3. The technological advance: a menu of ribosomal crystal structures

Challenging problems in crystallization, data collection, and phasing had to be solved to gain structural information from a macromolecular complex of the size and complexity of the ribosome. The atomic structures of the 30S [20,21], and the 50S subunits [22,23], as well as those of several antibiotic-ribosome complexes (Tables 1a and 1b) marked a turning point in the understanding of the way antibiotics interact with ribosomes. Further, these structures nearly doubled the world's information on small-molecule complexes with RNA: a query of the Nucleic Acid Database (NDB) [24], focused on RNA:ligand structures, yields 137 hits; of these, 51 are structures with a ribosomal subunit. Such information is critical to the prospective design of new antibacterials, where detailed understanding of the types, geometries, and strengths of interactions serve as important benchmarks.

Some aminoglycoside structures have been solved not with the intact 30S subunit but using an RNA-oligonucleotide that approximates their ribosomal binding site (Table 1a). Comparison of the paromomycin structures determined in the complex with the 30S subunit to that of the structure of paromomycin in complex with RNA-oligonucleotides shows excellent agreement [25,26], indicating that in some cases it is possible to use a ribosomal RNA fragment to obtain structural information on small molecule binding.

For practical reasons of crystallization, most ribosomal structures derive from either halophilic archaeobacteria (*H. marismortui*) or extremophilic bacteria (*Thermus thermophilus* and *Deinococcus radiodurans*). Although functionally important regions of the ribosome are highly conserved across all evolutionary kingdoms, some discomfort has existed in the community concerning drug design targeting pathogenic

bacteria based on structures derived from “extremophilic” ribosomes. As such, researchers at Rib-X Pharmaceuticals, Inc. have screened the ribosomal subunits of several prototypical bacteria for their ability to produce well-diffracting crystals. The efforts of Rib-X scientists resulted in the crystallization and solution of the 50S structure of a novel prototypical bacterium in less than two years' time. Although these crystals were derived from a new bacterial species, analysis of the 16S and 23S rRNA shows clearly that this bacterium is related closely to several known Gram-positive pathogens. Moreover, ribosome crystals of this prototypical Gram-positive bacterium grow in a few days, in sharp contrast to the weeks needed to grow crystals delivering the previously published structures [20,22,23,27]. The speed and reproducibility in growing well-diffracting crystals, has allowed Rib-X scientists to industrialize the generation of complex structures in this new Gram-positive ribosomal platform.

Initial diffraction of the new Gram-positive 50S subunit structure was to 4.1 Å resolution. The current structure is complete and was solved by molecular replacement using the 50S coordinates from *H. marismortui*. Refinement is ongoing. The excellent quality of the electron density maps allowed the placement of all the nucleotides of both the 23S and 5S rRNA. Encouraged by these results and the quality of the diffraction data, Rib-X scientists turned their attention to the crystallographic solution of complexes of antibiotics with this new Gram-positive 50S ribosome. These complexes have yielded important atomic-level insights into subtleties of small-molecule complexes with different bacterial genera, emphasizing that small changes can have a large impact on structure-based design. This impact is seen clearly from an overlap of the three complex structures of azithromycin (Fig. 3): the macrolide antibiotic adopts an orientation in the *D. radiodurans* 50S structure that is qualitatively different from that in either the *H. marismortui* or Gram-positive 50S structures. On one hand, this observation supports the use of complex structures obtained with *H. marismortui* crystals. On the other hand, it suggests that the difference in the azithromycin orientation in *Deinococcus* could be influenced by ribosomal protein L32. In *Deinococcus* and *Thermus*, this protein is extended and protrudes in the binding pocket (not featured in Fig. 2) and therefore

Table 1a – Available structures of antibiotics targeting the small ribosomal subunit (30S)

Proposed mechanism of action	Antibiotic class	Antibiotic	Refs.	PDB ID	System used for structural determination
Bind to A- or P-sites and affect decoding.	Aminoglycosides	Apramycin	[66]	1YRJ	RNA fragment
		Geneticin	[67]	1MWL	RNA fragment
		Hygromycin B	[68]	1HNZ	<i>T. thermophilus</i>
		Paromomycin	[26]	1FJG	<i>T. thermophilus</i>
		Paromomycin	[48]	1IBK	<i>T. thermophilus</i>
		Paromomycin	[25]	1J7T	RNA fragment
		Tobramycin	[50]	1LC4	RNA fragment
		Streptomycin	[26]	1FJG	<i>T. thermophilus</i>
Block binding of A-site tRNA	Tetracyclines	Tetracycline	[68]	1HNW	<i>T. thermophilus</i>
		Tetracycline	[69]	1197	<i>T. thermophilus</i>
Inhibit translocation	Various	Edeine	[69]	1195	<i>T. thermophilus</i>
		Pactamycin	[68]	1HNX	<i>T. thermophilus</i>
		Spectinomycin	[26]	1FJG	<i>T. thermophilus</i>

Table 1b – Available structures of antibiotics targeting the large ribosomal subunit (50S)

Proposed mechanism of action	Antibiotic class	Antibiotic	Refs.	PDB ID	System used for structural determination	
Block peptide bond formation by interfering with A-site or P-site tRNA and/or prevent the elongation of the nascent peptide	Macrolides	Azithromycin	[70]	1M1K	<i>H. marismortui</i>	
		Azithromycin	[71]	1NWY	<i>D. radiodurans</i>	
		Azithromycin	[19]	1YHQ	<i>H. marismortui</i> (G2058A)	
		Erythromycin	[72]	1JZY	<i>D. radiodurans</i>	
		Carbomycin	[70]	1K8A	<i>H. marismortui</i>	
		Erythromycin	[19,79]	1YI2	<i>H. marismortui</i> (G2058A)	
		Clarithromycin	[72]	1J5A	<i>D. radiodurans</i>	
		Roxithromycin	[72]	1JZZ	<i>D. radiodurans</i>	
		Spiramycin	[70]	1KD1	<i>H. marismortui</i>	
		Troleandomycin	[73]	1OND	<i>D. radiodurans</i>	
		Tylosin	[70]	1K9M	<i>H. marismortui</i>	
		Ketolides	ABT-773	[71]	1NWX	<i>D. radiodurans</i>
			Telithromycin	[74,79]	1P9X	<i>D. radiodurans</i>
			Telithromycin	[19]	1YIJ	<i>H. marismortui</i> (G2058A)
	Streptogramins	Dalfopristin	[75]	1SM1	<i>D. radiodurans</i>	
		Quinupristin	[75]	1SM1	<i>D. radiodurans</i>	
		Quinupristin	[19]	1YJW	<i>H. marismortui</i> (G2058A)	
		Virginiamycin S	[19]	1YTT	<i>H. marismortui</i> (G2058A)	
		Virginiamycin M	[76]	1N8R	<i>H. marismortui</i>	
	Lincosamides	Clindamycin	[72,79]	1JZX	<i>D. radiodurans</i>	
		Clindamycin	[19]	1YJN	<i>H. marismortui</i> (G2058A)	
	Pleuromutilins	Tiamulin	[77]	1XBP	<i>D. radiodurans</i>	
	Phenyl propanoids	Chloramphenicol	[72]	1K01	<i>D. radiodurans</i>	
		Chloramphenicol	[76]	1NJ1	<i>H. marismortui</i>	
	Oxazolidinones	Linezolid	[61]	Not available	<i>H. marismortui</i>	
	Various	Puromycin	[78]	1FFZ	<i>H. marismortui</i>	
		Sparsomycin	[76]	1M90	<i>H. marismortui</i>	
Anisomycin		[76]	1K73	<i>H. marismortui</i>		
		Blasticidin S	[76]	1KC8	<i>H. marismortui</i>	

The PDB ID refers to the Protein Data Bank (PDB) identification code of each structure. The atomic coordinates for each structure can be downloaded from <http://www.pdb.org> using their respective PDB IDs.

potentially impacts the orientation and/or conformation of the macrocycle. Such a true structural difference across genera raises the awareness of how structural differences are able to impact the ability of SBDD to design next-generation antibacterials targeting pathogenic bacteria.

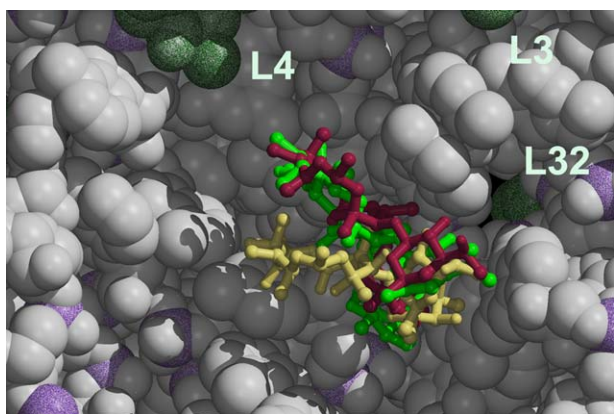


Fig. 2 – Complexes of azithromycin bound to the *D. radiodurans* (yellow), *H. marismortui* (maroon) and new Gram-positive (cyan) 50S ribosomes. The structures have been superimposed upon the new Gram-positive 50S rRNA, represented as Van der Waals surface and colored grey (rRNA), and green (proteins).

4. The design aspect: structure-based drug design

Among the tools for computational design are those that are ligand-based (e.g., pharmacophore-searching methods) and those that are target-based (e.g., docking or grow-search-score methods) [28–30]. In both cases, these aim to cut the drug discovery timeline by computationally rank-ordering compounds that show the most promise for lead molecules, reducing the numbers of compounds that require synthesis and subsequent biological profiling. Before the structures of the ribosomal subunits and their complexes with known antibiotics were solved, computational design for novel antibacterial drugs focused on the ligands.

The goal of ligand-based approaches is to identify a set of features or molecular properties that describe well a pre-defined subset of active molecules but that are not consistent with a set of inactive compounds. Such models are then used in the evaluation of new ideas or in the screening of commercial databases to identify new leads for synthesis and testing. Critical to the success of these approaches are a broad range of biological responses (3–4 orders of magnitude) and healthy diversity in the compounds such that meaningful differences can be discerned. For pharmacophore methods, a geometric relationship among features (e.g., hydrophobic site, aromatic site, hydrogen bond donor site, positive-ionizable

site, etc.) is sought for the active compounds, and limits can be set requiring a certain number of features to be satisfied for the pharmacophore to be robust. This requires an alignment of ligand molecules that best satisfies the model and therefore works best when there is a single, well-defined binding pocket where active ligands bind. Given that this is not the case for the ribosome—which behaves as a “target of targets” and where various classes of antibiotics position themselves in adjacent, sometimes non-overlapping regions—it should come as no surprise that ligand-based computer-aided design did not generate a wealth of novel, next-generation antibacterials. To be sure, and referring once more to Fig. 1, asking for an optimal alignment of and features common among sparsomycin, anisomycin and carbomycin that reflect their relative binding affinities to the 50S ribosome would be misleading at best and unlikely to be helpful in prospective use. This is because sparsomycin and carbomycin share no space in the binding pocket, while anisomycin positions itself between their terminal positions in the pocket.

Once the structures of the ribosomal subunits as well as that of their complexes with antibiotics were determined, structure-based design approaches were possible. Indeed, SBDD seemed the appropriate tool for the job given the successes it has shown in the launching of drugs like Viracept and Agenerase for AIDS therapy, Relenza for the flu, and the controversial COX-2 inhibitors Celebrex and Vioxx [31]. While there are many variations on the themes, the most popular structure-based approaches are docking [32,33] and grow-search-score methods [30,34]. Both approaches have been used by RNA-focused companies; the results will be described later in this article.

Docking requires generally the identification of a binding pocket plus boundaries; it then attempts to optimize the position of ligands in the site through a molecular mechanics calculation. The pocket may be delineated through complex structures of ligands with the target or hypothesized based on shape in an apo structure and biochemical data such as cross-linking, footprinting, or point mutations. For the latter case, particularly with a large target such as the ribosome, a well-designed screening paradigm must be in-place to assess the functional relevance of the putative new site.

Grow-search-score methods start with the positioning and evaluation of a template, or core molecule, in a known binding pocket. As with docking, these approaches rely on scoring functions to assess the binding. Here, the process is iterative, building on the core in a manner that mimics a medicinal chemistry lead optimization program. Although these approaches can be and have been used without knowledge of a bound ligand, their strength is in the ability to ‘grow’ from or add functionality to a fragment or molecule with known orientation in the target site.

Due to the paucity of structural data describing RNA:small-molecule interactions and to a lack of emphasis on RNA targets in the pharmaceutical sector—the scoring functions described herein have been developed exclusively for protein-ligand systems. Owing to a number of key differences between protein and RNA-centric targets, extrapolation to RNA-based systems is neither straightforward nor advised. Certainly the types of interactions that small molecules make with RNA, as well as complexities relating to the charge, solvent, and ion considerations with an RNA target are not well-captured in

protein-ligand systems [35–37]. Thus, off-the-shelf tools are of limited utility for RNA systems, unless the scoring functions can be modified. Companies like Anadys, Vernalis and Rib-X have reported significant advances in RNA-centric scoring functions, due to their emphasis on structure-base design and/or screening of small molecule inhibitors of RNA targets. The advances constitute generally the identification of molecular features important for binding to such complex systems, and while details are few it is clear that the molecular themes are indeed distinct from those seen in protein-ligand systems. As these companies test the transferability of their models to distinct chemical classes of antibacterials, it is hoped that they will become available to the general public for use in interrogating nucleic-acid-based targets.

In recent years the design approaches mentioned above have been coupled with an assessment of molecular properties, so that ligands with high affinity for the target site can be tuned for whole-cell activity and for drug-likeness [38–42]. The importance of the latter has received much attention, beginning with the publication of Lipinski’s Rule of Five [39], which prescribed that molecules with molecular weights less than 500, octanol:water partition coefficients less than 5, 5 or fewer hydrogen bond donor sites, and 10 or fewer hydrogen bond acceptor sites were more likely than others to be successful oral drugs. In brief, using these or more sophisticated absorption, distribution, metabolism and excretion (ADME) filters helps to balance compounds for affinity, activity, and efficacy.

5. Putting the parts together for antibacterial drug discovery

5.1. SBDD and the 30S

Aminoglycosides serve as an excellent template for designer antibiotics because they bind with high affinity to the bacterial decoding site (30S subunit, A-site) and have broad-spectrum antibacterial activity. However, owing to the multiple resistance mechanisms present in pathogenic bacteria and to significant toxicities associated with this class of molecules, their clinical use has been limited [36,43–46].

There is plenty of structural data explaining how aminoglycoside antibiotics such as paromomycin and tobramycin bind to the 30S and disrupts mRNA-decoding fidelity [25,47–50]. Such information provides structure–function relationships important for SBDD efforts.

Several attempts have been made to produce designer aminoglycosides by using ribosomal complex structures and SBDD techniques, described above. While they crested the first hurdle of intrinsic affinity (which was judged by increased inhibition of ribosomal translation), the “designer analogs” reported by Anadys Pharmaceuticals were less potent than the parent aminoglycosides against both *E. coli* and aminoglycoside-susceptible *Staphylococcus aureus* strains [51–55]. These results emphasize the need for additional tools that allow consideration of molecular properties that drive bacterial cell penetration and/or avoidance of potent efflux pumps.

Aside from these “designer analogs,” neamine analogs were pursued in collaboration between Wayne University and

the company formerly known as RiboTargets. These designer antibiotics showed considerably enhanced antibacterial activities against several important pathogenic bacteria, including organisms expressing resistance enzymes to aminoglycosides [56,57]. However, to date, these discovery attempts have not produced a clinical candidate.

Some of the same researchers, now at Vernalis Ltd., developed an RNA-centric scoring function for an in-house docking approach [58]. Their target of interest was the decoding site of the 30S subunit. Using this target, they screened in silico over one million compounds, identifying 129 that showed good shape complementarity for the ribosome. Once synthesized, 34 of them were shown biochemically, through a FRET-based assay, to bind on-target in the A-site, illustrating the power of a well-tuned scoring function for identifying new leads for an antibacterial, RNA-targeting discovery program.

5.2. SBDD and the 50S

Researchers at the Nucleic Acid Design Center (University of Denmark) used the published structure of chloramphenicol bound to the *D. radiodurans* bacterial ribosome as well as the published structure-activity relationships among chloramphenicol analogs in the design of next-generation phenyl propanoids [47]. Their aim was to optimize linking elements between chloramphenicol and dinucleotides that would afford enhanced binding to P-loop nucleotides in the 23S rRNA. Through a series of molecular dynamics simulations of hypothetical constructs, they identified a small subset of molecules for synthesis. Of the six compounds synthesized, one was confirmed through biochemical experiments, e.g., RNA-footprinting, to act on-target and to be more potent by one order of magnitude than the parent chloramphenicol. The rest were shown not to bind, though the authors hypothesize that this is due to suboptimal linker selection.

Scientists at Rib-X Pharmaceuticals succeeded in designing a family of enhanced, or designer, oxazolidinones effective against drug-resistant bacteria in community and hospital settings [59,60]. At the outset, the goal was to broaden the spectrum of linezolid by boosting its intrinsic affinity for the ribosome, thereby overcoming resistant strains and conquering major causative agents in the community, namely streptococci as well as *Moraxella catarrhalis* and *Haemophilus influenzae*. The program used detailed knowledge at the atomic level of the juxtaposition of the ribofunctional loci of linezolid and sparsomycin [61] (Fig. 3).

The overall strategy was: (1) establish that the two ribofunctional loci can be used simultaneously to deliver a potent antibacterial compound; (2) identify the features appropriate for broadening the spectrum into the community arena; and (3) balance for properties consistent with intravenous and oral administration. A limited set of probe molecules was synthesized starting from the complex crystal structures and using a grow-search-score tool (AnalogTM) plus a molecular properties calculator, QikProp [61,62], to prioritize bridging-elements as well as to suggest surrogates for the tightly bound thymine cap of sparsomycin. The original hypothesis was confirmed three ways: (1) these probes inhibited *E. coli* ribosomal translation on-par with linezolid

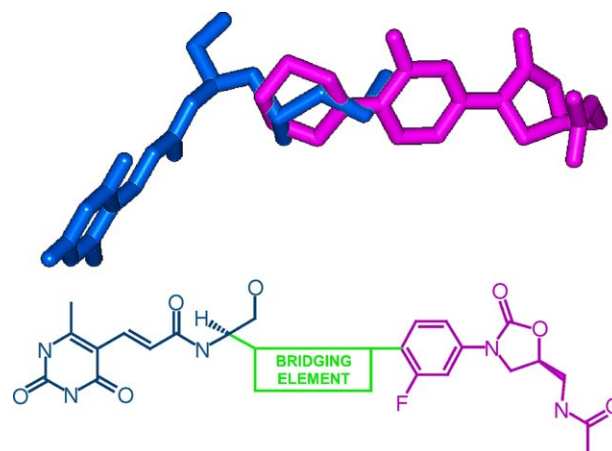


Fig. 3 – (Top) Relative binding orientations of sparsomycin (magenta) and linezolid (blue) in the 50S ribosomal subunit, with the rRNA stripped away for clarity. (Bottom) Original design hypothesis.

(IC₅₀ = 5 μM); thymine replacements showed target selectivity when comparing their relative abilities to inhibit eukaryotic translation (measured using rabbit reticulocytes); (2) the probes showed modest to good antibacterial activity across a panel of nosocomial and community pathogens, although the bridging element was not optimal for overcoming linezolid-resistant enterococci and (3) a complex crystal structure was solved to 2.9 Å resolution, confirming the predicted binding orientation and configuration of the initial probe molecules (Figs. 4 and 5, Table 2) [61].

Scientists at Rib-X identified an optimal bridging element between these molecules by prioritizing interactions and shape complementarity with the ribosome. Based on the

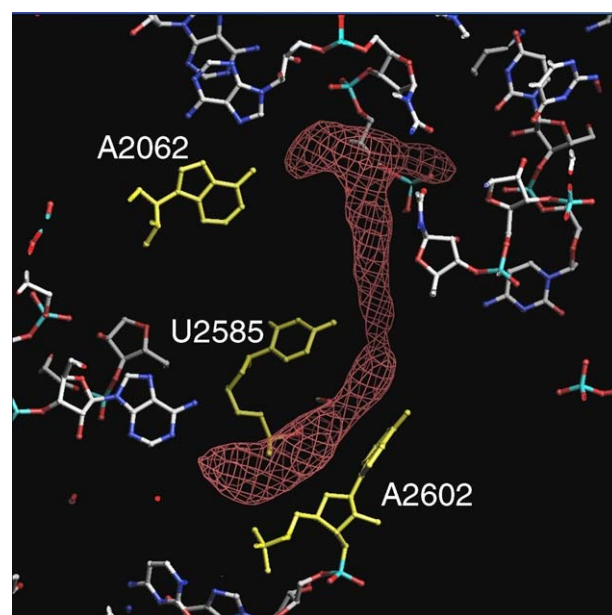
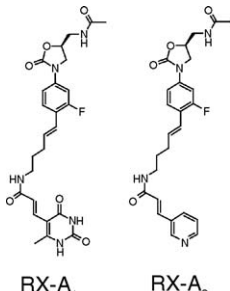


Fig. 4 – Difference density of RX-A₁ revealing its site of interaction with the A-site of *H. marismortui* 50S. Nucleotides numbered according to *E. coli* 23S rRNA.



Chemical structures of RX-A₁ and RX-A₂ are shown on the left. RX-A₁ features a biaryl scaffold with a 4-fluorophenyl group and a 4-pyridyl group. RX-A₂ features a biaryl scaffold with a 4-fluorophenyl group and a 4-pyridyl group, with a different biaryl linkage.

Strain MIC (in µg/ml)	RX-A ₁	RX-A ₂
<i>S. aureus</i> QC	32	64
<i>S. pneumoniae</i> 1175 (<i>mefa</i>)	1	8
<i>S. pyogenes</i> Msr610 (<i>ermB</i>)	1	4
<i>E. faecalis</i> P5 (LNZ-R G2576U)	32	128
<i>H. influenzae</i> parent strain RD1	>128	>128
<i>H. influenzae</i> 895 (<i>acrB</i> KO)	32	1
Translation IC ₅₀ (µM) in prokaryotes	0.92	14.6
Translation IC ₅₀ (µM) in eukaryotes	0.23	>200

Fig. 5 – (Left) 2-D representations of RX-A₁ and RX-A₂. (Right) Biochemical and microbiological results, indicating the original bridging hypothesis was correct but required optimization.

Table 2a – MICs in (µg/ml) of RX-A₆₆₇, a typical designer oxazolidinone from Rib-X's first program

Community RTI strains MIC (µg/ml)	RX-A ₆₆₇	Linezolid	Azithromycin	Ketek
Pathogens responsible for community respiratory track (RTI) infections				
<i>S. pneumoniae</i> (<i>ermB</i>) GRRX003	≤0.25	1	>128	0.03
<i>S. pyogenes</i> 6735-99	0.25	4	0.06	0.03
<i>H. influenzae</i> A1929	0.5	16	4	1
<i>M. catarrhalis</i> A1413	0.5	2	0.03	0.25

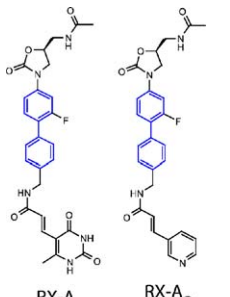
Table 2b – MICs in (µg/ml) of RX-A₆₆₇, a typical designer oxazolidinone from Rib-X's first program

Nosocomial strains MIC (µg/ml)	RX-A ₆₆₇	Linezolid	Vancomycin
Nosocomial pathogens			
<i>S. aureus</i> (MS) A9606	2	4	1
<i>S. aureus</i> (MR) A8557	4	4	1
<i>S. aureus</i> (Coag. Neg.) C211	1	2	2
<i>E. faecalis</i> Van-S A418	1	4	2
<i>E. faecalis</i> Van-R A401	1	2	>128
<i>E. faecalis</i> P5 (Lin-R)	1	32	2
<i>E. faecium</i> Van-R A5138	1	2	>128
<i>E. faecium</i> Van-S A492	0.25	2	2

structures and an energy analysis, it became clear that a biaryl scaffold would best take advantage of a cascade of pi stacking interactions offered to the "B" ring (*m*-fluorophenyl) of linezolid. These biaryl-containing oxazolidinones improved qualitatively the biological profile of these designer oxazolidinones, including activity against the linezolid-resistant enterococci [63] (Table 2b). Moreover, the oxazolidinones corroborated earlier results suggesting the feasibility of replacing the thymine in sparsomycin, which is held tightly

in a web of interactions in a highly conserved region of the A-site, to gain bacterial specificity (Fig. 5).

From these proof-of-concept studies, Rib-X's program focused on optimizing the biaryl oxazolidinone (Fig. 6) to increase potency against the important community pathogen *H. influenzae*, to show antibacterial activity against the linezolid-resistant enterococci, while retaining excellent activity versus hospital G+ positive pathogens, and to marry these in a molecular package with attractive pharmacoki-



Chemical structures of RX-A₇ and RX-A₈ are shown on the left. RX-A₇ features a biaryl scaffold with a 4-fluorophenyl group and a 4-pyridyl group. RX-A₈ features a biaryl scaffold with a 4-fluorophenyl group and a 4-pyridyl group, with a different biaryl linkage.

Strain MIC (in µg/ml)	RX-A ₇	RX-A ₈
<i>S. aureus</i> QC	0.25	4
<i>S. pneumoniae</i> 1175 (<i>mefa</i>)	0.25	0.5
<i>S. pyogenes</i> Msr610 (<i>ermB</i>)	0.25	0.5
<i>E. faecalis</i> P5 (LNZ-R G2576U)	16	16
<i>H. influenzae</i> parent strain RD1	>128	>128
<i>H. influenzae</i> 895 (<i>acrB</i> KO)	0.25	2
Translation IC ₅₀ (µM) in prokaryotes	<0.02	6.8
Translation IC ₅₀ (µM) in eukaryotes	1.5	>100

Fig. 6 – (Left) 2-D representations of RX-A₇ and RX-A₈. (Right) Biochemical and microbiological results, emphasizing significant improvement with an optimized biaryl element.

netics for once- or twice-daily dosing. As compounds and data were generated, three key computational models were validated and placed in-stream of the design efforts: a model pointing in the direction of improved *H. influenzae* activity, a Caco-2-cell prediction (surrogate for oral absorption) from QikProp [62], and a rat oral bioavailability model [61]. Using this integrated SBDD approach, and fewer than 700 compounds, Rib-X scientists identified a family of compounds that balanced all features desired for this series, potency and properties [64,65]. Table 2 shows their superiority (MICs in $\mu\text{g}/\text{ml}$) over existing therapies. These designer oxazolidinones form the base of Rib-X's first program. The first of these is being assessed in First-in-Human studies that began in December 2005.

6. Conclusion

The solution of the structures of the ribosomal subunits and their complexes with several classes of known antibiotics has ushered in a new era in antibacterial drug discovery. When combined with robust computational methodologies and a well-designed screening panel that evaluates all aspects pertinent to drug discovery, ribosomal drug design offers the promise of the next generation of antibiotics important for treating the growing problem of resistance plaguing the field.

Acknowledgments

The authors would like to thank the members of the SBDD, Discovery Biology and Medicinal Chemistry groups at Rib-X Pharmaceuticals, Inc., for generating some of the data and ideas that make possible this analysis, and for their encouragement in preparing this manuscript. We thank Dr. A. Hofmann for critical reading of the manuscript.

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