STRUCTURE-BASED ANTIBIOTIC DESIGN

ESKAPE velocity: total synthesis platforms promise to increase the pace and diversity of antibiotic development

Iboxamycin (IBX) is a new oxepanoprolinamide antibiotic based on clindamycin. Crystal structures of IBX in complex with bacterial ribosomes uncover the structural mechanism of its activity against multidrug-resistant pathogens and reveal key interactions with tRNAs and 23S rRNA, including resistance-conferring rRNA methylations.

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Antibiotic resistance is a growing threat to public health. Drug-resistant microbial infections now result in 700,000 deaths per year globally and are projected to cause up to 10 million deaths annually by 2050 should no new antibiotics be identified[1]. Historically, the development of new antibiotic compounds has depended upon semisynthesis, whereby natural products are modified to yield new drugs[2]. However, semisynthesis presents challenges, particularly its limited ability to generate new chemical scaffolds. The development of new antibiotic compounds has slowed over the past few decades[3,4], and there is little financial incentive for pharmaceutical companies to develop the next line of antibiotics. The growing clinical threat of antibiotic-resistant 'ESKAPE' pathogens[5] (encompassing several pathogenic species from *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas* and *Enterobacter*) combined with the limited number of new antibiotics has allowed the increasing spread of resistance. Specifically, some clinical strains have gained resistance to important antibiotics active against the large 50S ribosomal subunit, including the MLS_B phenotype (resistance to macrolides, lincosamides and class B streptogramins) and $PHLOPS_A$ phenotype (resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and class A streptogramins). Resistance is achieved through the expression of 23S ribosomal RNA (rRNA) methyltransferases from the Erm and Cfr families[6–8].

To combat this problem, total syntheses of important antibiotic classes promise to improve both the scope and pace of new antibiotic development, as they greatly expand the chemical space available for testing against multidrug-resistant bacterial strains. In a recent *Nature* study, Mitcheltree et al. do precisely this and report the total synthesis of oxepanoprolinamide antibiotics, a new broad-spectrum antibiotic class structurally related to the lincosamide antibiotic clindamycin[9]. The authors further demonstrate oxepanoprolinamide activity against Gram-positive and -negative bacteria and their robust in vivo efficacy in a mouse model of ESKAPE pathogen infection. Finally, detailed structural and biochemical analyses reveal the molecular basis for their activity against wild-type and multidrug-resistant bacteria carrying resistance determinants.

Drawing from prior experience in designing platforms for the total synthesis of antibiotics including macrolides, tetracyclines and streptogramins[10–12], the authors report a pathway for synthesizing oxepanoprolinamides. Using the aminooctose moiety of clindamycin as a starting point, they succeeded in synthesizing and testing a variety of bicyclic scaffolds that possess underlying substituted proline structures similar to that of clindamycin, but are more rigid and were purported to make additional contacts within the lincosamide-binding site on the 50S ribosomal subunit[13,14] (Fig. 1a). The authors report a method for synthesizing multi-gram quantities of one compound, iboxamycin (IBX), which showed particularly potent activity against a variety of antibiotic resistant Gram-positive and -negative bacterial strains (including several ESKAPE pathogen strains) in minimum inhibitory concentration (MIC) assays. The activity of IBX against Gram-negative pathogens was unexpected, as lincosamides typically have low activity against these bacteria[8]. The mechanism for IBX's evasion of Gram-negative intrinsic antibiotic resistance remains an interesting open question, the answer to which may guide further antibiotic development. The synthesis platform used to discover IBX yielded more than 500 candidate compounds; this and similar platforms thus have potential to dramatically accelerate the development of new antibiotics by making it possible to design, synthesize and test a large variety of potential new drugs.

After producing IBX using their novel oxepanoprolinamide synthesis platform and discovering its activity in MIC screens, the authors sought to better understand its mechanism of action through biochemical studies. Because of its structural similarity to clindamycin, the authors anticipated that IBX would possess a similar mechanism of action. Toeprinting experiments, which map the mRNA position on bacterial ribosomes, indicate that IBX inhibits translation at start codons, like clindamycin[15]. In fact, at equal drug concentration, IBX caused greater inhibition than clindamycin. Subsequent X-ray crystallographic studies of IBX in the context of both wild-type bacterial ribosomes and ribosomes possessing a resistance-associated 23S rRNA dimethylation modification revealed the mechanism by which IBX retains activity against multidrug-resistant bacteria. Like clindamycin, IBX binds the 50S subunit near the peptidyl transferase center, likely preventing peptidyl transfer activity (Fig. 1b). However, unlike clindamycin, IBX makes hydrophobic contacts with the ribosome's A-site cleft. These additional interactions appear to allow IBX binding even in contexts such as resistance-associated 23S dimethylation, where drugs such as clindamycin cannot, and permit IBX to more potently interfere with bacterial ribosome activity (Fig. 1c). IBX also appears to promote mobility in the acceptor end of the A-site tRNA,

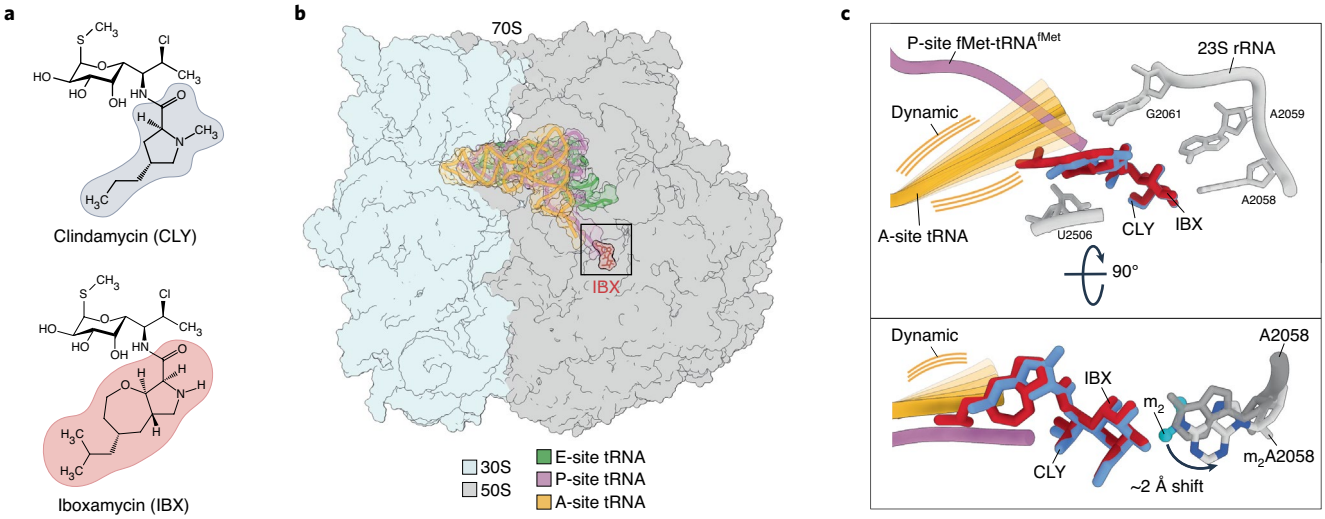

Fig. 1 | New antibiotic IBX inhibits bacterial protein synthesis and appears to overcome resistance mechanisms. a, Chemical structures of clindamycin (CLY) and iboxamycin (IBX). The 'southern hemispheres' of CLY and IBX are highlighted in blue and red, respectively. **b**, Structure of IBX-bound bacterial ribosome containing P-site fMet-NH-tRNA^{fMet}, A-site tRNA and E-site tRNA (PDB 7RQ8). **c**, Superimposed ribosome-bound IBX (red, from PDB 7RQ8) and CLY (blue, from PDB 4V7V) with 23S rRNA. Both CLY and IBX interfere with A-site tRNA acceptor-end positioning (gold, from PDB 6XHW), but IBX projects farther into the ribosomal A-site cleft (upper panel). Methylated m_2A2058 (light gray, CH_3 groups in turquoise) undergoes a ~2 Å shift in position in the presence of IBX compared to unmethylated A2058 (dark gray), disrupting drug–rRNA interactions.

as evidenced by these nucleotides being unresolved in crystal structures (Fig. 1c). Interestingly, these studies reveal an underlying plasticity in the structure of the 23S rRNA, as the dimethylated A2058 undergoes a ~2 Å shift in position relative to its location in unmethylated ribosomes when IBX is bound (Fig. 1c). Although this shift in nucleotide position is sufficient to disrupt antibiotic–rRNA hydrogen bonding and likely contributes heavily to resistance to lincosamides such as clindamycin, Mitcheltree et al. speculate that IBX's additional contacts with the A-site cleft permit it to remain accommodated despite the resistance methylations. These structures are critical for our understanding of how IBX can evade resistance via 23S rRNA methylation, reveal important information about the underlying biology of translation and lay the foundation for further refinement of this new class of antibiotics.

Looking forward, total synthesis platforms like the one used to produce IBX are likely to play an important part in a multi-pronged approach in the race against antibiotic resistance. As the authors note, there is still tremendous value in the rational modification of existing drug scaffolds, and their development of a total synthesis platform for oxepanoprolinamides has clearly already expanded the scope of compounds that are available to be tested for antibiotic activity. Unexpected findings, such as the notable potency of IBX against Gram-negative and lincosamide-resistant bacteria, speak to the importance of testing a broad range of candidate compounds. Findings such as these will likely continue to fall out of screens as the number and diversity of testable antibiotic candidates continues to increase. However, there is also value in seeking new classes of drugs with activity against bacteria-specific cellular machinery. It will be interesting to observe the interplay between synthesis systems such as this, which improve the scope of testable hypotheses, and research seeking compounds with new mechanisms of action. As new total synthesis platforms are developed, the ability of researchers to discover and refine the design of antibiotics that work in new ways is sure to increase. The work performed by Mitcheltree and colleagues in developing oxepanoprolinamides gives reason for optimism that, despite a slowing in the rate of new antibiotic development in recent decades, we will continue to discover new antibiotic compounds to outpace the rapid spread of resistance. ❐

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Competing interests

The authors declare no competing interests.