Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Recent contributions of structure-based drug design to the development of antibacterial compounds

Bart L Staker[1,2], Garry W Buchko[1,3] and Peter J Myler[1,2,4,5]

According to a Pew Research study published in February 2015, there are 37 antibacterial programs currently in clinical trials in the United States. Protein structure-based methods for guiding small molecule design were used in at least 34 of these programs. Typically, this occurred at an early stage (drug discovery and/or lead optimization) prior to an Investigational New Drug (IND) application, although sometimes in retrospective studies to rationalize biological activity. Recognizing that structure-based methods are resource-intensive and often require specialized equipment and training, the NIAID has funded two Structural Genomics Centers to determine structures of infectious disease species proteins with the aim of supporting individual investigators' research programs with structural biology methods.

Addresses
[1] Seattle Structural Genomics Center for Infectious Disease, United States
[2] Center for Infectious Disease Research (formerly Seattle Biomedical Research Institute), 307 Westlake Ave N, Suite 500, Seattle, WA 98109, United States
[3] Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99354, United States
[4] Department of Global Health, University of Washington, Seattle, WA 98195, United States
[5] Department of Biomedical Informatics and Health Education, University of Washington, Seattle, WA 98195, United States

Corresponding author: Staker, Bart L (bart.staker@cidresearch.org)

Current Opinion in Microbiology 2015, **27**:133–138

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

Edited by **Paul M Dunman** and **Andrew P Tomaras**

For a complete overview see the Issue and the Editorial

http://dx.doi.org/10.1016/j.mib.2015.09.003

Introduction

The primary use of protein structure for the development of drug compounds is to determine the structure of a protein in complex with a tool compound (a known ligand or lead inhibitor) for the purpose of suggesting a new chemical hypothesis in order to improve inhibitor affinity by suggesting new chemical modifications. These are usually guided by the three dimensional scaffold of the protein surrounding the ligand, including hydrogen bond donors or acceptors, hydrophobic patches, and neighboring pockets near the compound binding site. Medicinal chemists use this information to design and synthesize variants of the tool compound, which are then tested for inhibitory activity. This approach, known as structure-based drug design (SBDD), is the traditional and most well-known use of protein structure and often occurs in an iterative cycle where new molecules are synthesized, tested and crystallized with the target protein. In addition to traditional SBDD there are numerous other methods and variations that utilize protein structure in the discovery and development of new drug entities, including X-ray crystallography-based and NMR-based fragment screening, and virtual (*in silico*) screening [1,2]. Several previous reviews have discussed the techniques and technology of SBDD, as well as the application of SBDD methods towards the development of new drug molecules [3,4]. Here we will summarize recent applications of structure-based methods for the development of antibacterial agents.

A recent Pew Research study [5] identified 37 antibacterial molecules currently in active clinical trials. Analysis of the PDB identified 34 of these compounds as having protein complex structural data available for the compound or a similar compound derivative. The three compounds (Brilacidin, Surotomycin, and SMT19969) without direct structural data have unknown mechanisms of action or act on the cell membrane and thus no target structure is available. Brilacidin is a defensin-mimetic that is proposed to act through depolarization of the membrane [6]. Surotomycin is a lipopeptide derivative of daptomycin that also acts through a membrane depolarization mechanism [7]. The mechanism of action of SMT19969 is unknown, but it has been suggested to inhibit DNA synthesis and is structurally similar to Hoechst dyes which bind in the minor groove of double-stranded DNA [8]. The remaining 34 compounds in clinical trials can be grouped into several broad classes with different mechanisms of action, and include fluoroquinolones, oxazolidinones, and β-lactams. Published structural data is available for some specific compounds directly, but usually structural information is available indirectly through a published protein structure bound to a close chemical derivative of the specific clinical trial compound. Understanding the true impact structural data has during the development cycle can be difficult to determine from published literature because the work is often done in commercial laboratories that don't always publish structural coordinate files [9]. However, published retrospective studies, academic investigations

and the Protein Data Bank (PDB) provide a wealth of structural information.

Bacterial protein structures in the Protein Data Bank

The Protein Data Bank (PDB) is the primary worldwide location where structural data is deposited [10], and many scientific journals require authors to submit structural coordinates as a condition of publication. In addition, US government-funded structural genomics centers are required to deposit structural coordinates regardless of publication status or intent [11]. In 2013, 10 566 structures were deposited in the PDB, while in 2014, 10 367 structures were deposited. As of the end of the third quarter of 2015, PDB depositions are on track to reach a similar 10 000 per year rate with 7381 deposited as of September 8th, 2015 (http://www.wwpdb.org/stats/deposition). Of 25 196 structural coordinates released between January 1st, 2013 and August 31st, 2015, 9387 were from a bacterial source,[6] with 3497 bacterial structures containing ligands larger than 300 Daltons, including drug-like molecules and cofactors such as ATP, NADP, among others.[7] A total of 884 bacterial structures can be identified by searching with the keyword 'inhibitor'.[8] These data suggest that about a third of all structure determination is focused on bacterial proteins, of which about 10–20% of bacterial structure determination is directly related to structure-based small molecule development. A few examples of how structure-based drug design has been used recently are explored in more detail below.

Avibactam, a new β-lactamase inhibitor

The most widely used antibiotics are β-lactam containing compounds, which inhibit bacterial cell wall synthesis and include penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. The development of antimicrobial resistance (AMR) has initiated a search for new molecules that overcome resistance. A primary mechanism of β-lactam resistance is over-expression of β-lactamases that degrade the pharmaceutical compounds. One strategy to overcome resistance caused by β-lactamases is to co-administer a β-lactamase inhibitor along with traditional β-lactam-containing antibiotic in order to prevent degradation of the drug [12]. New combinations of β-lactam/β-lactamase inhibitor are under development and six of the 37 new drugs reported by the Pew Foundation contain β-lactamase inhibitors. Prominent among these is Avibactam, a bicyclic diazobicylcooctane (Figure 1a), with a mechanism of action that was recently shown in a series of papers describing the X-ray structures of Avibactam bound to Class A [13••], C [14•], and D [15•] β-lactamases. Avibactam binds the highly conserved active site of β-lactamases with a conformation in which the bicyclic ring mimics the β-lactam ring (see Figure 1c). In the high resolution Class C structure the sulfamite moiety of Avibactam is seen to displace a water molecule responsible for β-lactam hydrolysis. Structures in the ring-opened and ring-closed conformation of Avibactam show that the open ring maintains close positioning to the reactive center allowing re-cyclization and release of re-activated drug from the enzyme. Hydrolysis of β-lactam inhibitors are deactivated upon hydrolysis. However, re-cyclization of Avibactam releases an active drug molecule that can return and inactivate the enzyme. This mechanism accounts for the observed efficiency and long half-life of Avibactam [16,17].

Avibactam has broad activity against Class A and Class C β-lactamases, as well as activity against some Class D β-lactamases. The structure of Avibactam with Oxa-24 and Oxa-48 Class D β-lactamases allowed the identification of the structural features responsible for this selectivity. A hydrophobic bridge at the entrance of the Class D enzymes was identified that restricts entry into the active site (Figure 1d). A series of structure-based sequence alignments of 310 known Class D β-lactamases found the residues that form the hydrophobic bridge can rationalize and predict the activity of Avibactam against Class D enzymes. Larger residues in this conserved region block entry into the active site acting as a thermodynamic barrier to entry and reduced inhibitory activity.

Fragment-based discovery of new gyrase inhibitors

Fragment-based drug discovery is an alternative to high throughput screening for the identification of new compounds active against a target protein. Fragment screening uses biophysical methods, such as Surface Plasmon Resonance (SPR), Nuclear Magnetic Resonance (NMR), or mass spectrometry (MS), to detect binding of small (<300 Da) compounds to a protein. Once a small molecule is identified, a three-dimensional structure of the molecule in complex with the target protein is used to visualize the precise binding mode. The small molecules identified by these binding studies may not show inhibitory activity in enzymatic or phenotypic assays due to low affinity. The 'fragment' provides a starting point for development of a new chemical series by subsequent chemical modification and expansion of the molecule to

[6] *DepositDateQuery: database_PDB_rev.date_original.comparator=between database_PDB_rev.date_original.min=2013-01-01 database_PDB_rev.date_original.max=2015-08-31 database_PDB_rev.mod_type.comparator=< database_PDB_rev.mod_type.value=1 and TAXONOMY is Bacteria (eubacteria).*

[7] *DepositDateQuery: database_PDB_rev.date_original.comparator=between database_PDB_rev.date_original.min=2013-01-01 database_PDB_rev.date_original.max=2015-08-31 database_PDB_rev.mod_type.comparator=< database_PDB_rev.mod_type.value=1 and TAXONOMY is Bacteria (eubacteria) and Ligand Search : Has free ligands=yes.*

[8] *DepositDateQuery: database_PDB_rev.date_original.comparator=between database_PDB_rev.date_original.min=2013-01-01 database_PDB_rev.date_original.max=2015-08-31 database_PDB_rev.mod_type.comparator=< database_PDB_rev.mod_type.value=1 and Text Search for: inhibitor and TAXONOMY is Bacteria (eubacteria).*