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Recent contributions of Structure-Based Drug Design to the development of antibacterial compounds

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Abstract

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.

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

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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- 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[i] with 3497 bacterial structures containing ligands larger than 300 Daltons, including drug-like molecules and cofactors such as ATP, NADP, *etc*[ii]. A total of 884 bacterial structures can be identified by searching with the keyword "inhibitor"[iii]. These data suggest that about a third

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of all structure determination is focused on bacterial proteins, of which about 10 to 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.

[i]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)

[ii]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

[iii]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)

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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 3-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 increase affinity, phenotypic activity, and drug-like characteristics.

Fluoroquinolones have been a mainstay of antibacterial treatment for over 40 years by targeting the bacterial DNA gyrase. However, the emergence of antimicrobial resistance has prompted renewed efforts to identify non-quinolone containing compounds, and 5 of the 37 compounds in current clinical trials target this enzyme. Fragment-based discovery efforts have been conducted to "scaffold-hop" away from the quinolone core or to target different parts of the enzyme, for example the ATPase domain. AstraZeneca[18] recently used structure-based development of a lead fragment with an initial IC_{50} of 32 μM to develop a lead compound, which has a final IC_{50} of 10 nm and activity in mouse models. The new compound overcomes resistance mutations in GyrA and ParC enzymes by binding in the *Streptococcus pneumoniae* ParE ATPase domain. Previous work at AstraZeneca also published the development of additional scaffolds through an NMR-based screen and subsequent X-ray structure of fragments bound to the *Staphylococcus aureus* GyrB ATPase domain[19]. Brvar [20] used computational methods to identify fragment molecules based on the structure of *Escherichia coli* GyrB ATPase domain in complex with the natural product clorobiocin. Structural methods were then used to elucidate the mechanism of binding and validate the hypothesized binding mode of lead compounds.

Nuclear Magnetic Resonance (NMR) spectroscopy in antibacterial drug development

The resonance frequencies of certain nuclei naturally present (^{1}H) or easily incorporated (^{13}C,^{15}N) into proteins are extremely sensitive to their local inter- and intramolecular chemical environment [21]. This fundamental property is harnessed in various NMR experiments to provide detailed information on the molecular structure and interactions of proteins [22]. Consequently, NMR is extensively used to assist antimicrobial drug development in various stages of the SBDD process including hit identification, hit validation, and lead optimization [23–25]. Numerous NMR experiments, based on differences in the NMR properties between big (target) and small (fragment) molecules, have been designed to identify and validate lead compounds [24]. These experiments, some of which can be used in high-throughput mode, include WaterLOGSY [26], SLAPSTIC [27], TINS [28], transferred NOEs [29] DOSY-NMR [30] and saturation transfer difference (STD)-NMR [31, 32] For example, STD-NMR was used to identify a series of compounds

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with low-micromolar affinity for the macrophage infectivity potentiator BpML1 from *Burkholderia pseudomallei*, the etiological agent for melioidosis [33]. In terms of lead optimization, the use of NMR to determine structures for protein less than ~25 kDa in size is well established[21, 34]. However, because X-ray crystallography can often determine protein structures at higher resolution much more rapidly, NMR is typically employed to determine structures of proteins that are recalcitrant to forming well-diffracting crystals [34, 35] and is particularly well suited to studying intrinsically disordered proteins, an underexplored area of great interest for new drug design strategies [36]. For proteins under ~30 kDa with a solved structure (*via* XRD or NMR) and an assigned ^{1}H-^{15}N or ^{1}H-^{13}C HSQC spectrum, NMR can be used to obtain structure-activity relationships (SARs) on identified fragment hits to assist the early stages of lead optimization [37] *via* chemical shift perturbation experiments [38].

In eukaryotic cells, targeting the proteins involved in cell division (cytokinesis) has proven highly successful in the discovery and development of anticancer drugs such as the vinca alkaloids (vinblastine and vincristine) and taxanes (docetaxel, paclitaxel, and cabazitaxel), which act by destabilizing and stabilizing, respectively, microtubules [39] [40]. Prokaryotic cells contain a tubulin homologue, FtsZ, which is the most abundant of at least eight proteins involved in prokaryotic cell division [41] and is highly conserved in both Gram+ and Gram- bacteria [42]. Upon binding GTP it polymerizes to form a ring-like structure at the site of cell division and queues the recruitment and assembly of the cell division machinery. Consequently, agents that block Z-ring formation or protein recruitment may represent a powerful new class of antimicrobial drugs [43]. In the quest for such agents, NMR-based methods have been used at a number of stages in the SBDD process. For example, NMR methods were used to determine the solution structure for the C-terminal domain of ZipA [44], an essential component of the cell division complex that forms around FtsZ [45]. Using a library of 850 compounds and the ^{15}N-labelled C-terminal domain of ZipA, two-dimensional ^{1}H-^{15}N HSQC chemical shift perturbation experiments were collected on sets of these compounds to identify seven hits for further SBDD [46]. In another example with cinnamaldehyde, a plant-based small molecule inhibitor of FtsZ, STD-NMR was used to determine the pharmacophoric groups responsible for binding to FtsZ [47]. Traditional small molecule NMR experiments were used to assist the characterization of novel natural antimicrobial products named chrysophaentins, with STD-NMR and NMR competition experiments showing these compounds bind FtsZ at the GTP-binding site [48]. A combination of STD-NMR epitope mapping and transfer NOE (trNOESY) experiments were used to deduce differences in the recognition mode of *Methanococcus jannaschii* and *Bacilus subtilis* FtsZ for C8-substituted guanine nucleotides [49], information that may facilitate the design of FtsZ inhibitors based on GTP analogs.

Structures from orthologous species can be used as surrogate for SBDD

The discovery of new drugs and novel chemical scaffolds can be assisted by structure-guided efforts; however, the corresponding structures are often not available in the PDB. If structure determination of the target of interest fails, it is possible to use the structure of the target from an orthologous species as a surrogate model. For example, a structure of *Mycobacterium smegmatis* served as a surrogate for the target from *M. tuberculosis*.

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Recently, Baugh et al [50] conducted an extensive comparison of structures from *M. tuberculosis* to other non-TB mycobacterial (NTM) species by comparing 106 pairs of Mtb and NTM structures. NTM structures with >55% sequence identity were shown to share similar active site conformation and >85% identity within the active site of the enzyme. Kling *et al*. [51] solved ligand-bound structures of both *M. tuberculosis* and *M. smegmatis* showing that the griselimycins bound to DnaN in identical conformation. Shirude *et al*. [52] utilized the structure of GyrB from *M. smegmatis* to validate hits from a high-throughput screen (HTS) and develop lead inhibitor molecules with activity against *M. tuberculosis*.

NIAID Structural Genomics Centers provide access to structural biology methods

The NIAID has funded two structural genomics centers, which offer structure determination services to the infectious disease research community and deposit all structures to the PDB. The Seattle Center for Structural Genomics (SSGCID; www.ssgcid.org) and the Center for Structural Genomics for Infectious Disease (CSGID; www.csgid.org) have together determined and deposited >1500 structures to the PDB from Category A, B, and C Biodefense organism list [11, 53]. In addition to structure determination services for researchers, the NIAID structural genomics centers determine structures of potential drug targets, essential genes, as well as proteins of unknown function to assist in greater understanding of the proteome of infectious disease organisms. Baugh et al. [54] reported the structures of 88 proteins from *Burkholderia* species derived from a genomic essentiality screen. Forty-nine gene families were covered by at least one structure from an initial pool off 406 putative essential gene families providing a resource for future drug development

Conclusion

Protein structure-based methods will continue to contribute to the discovery and development of anti-infective compounds and will remain an important tool in the arsenal of drug discovery scientists. Advancements in structural biology techniques have reduced the primary bottleneck of structure determination to be protein expression and solubility, rather than technical methodology. The remaining challenge of structural biology is to address the significant challenge of integral membrane proteins, which include many important drug targets.

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Highlights

New beta-lactamase inhibitor with novel mechanism of action is reviewed.

Methods of targeting bacterial gyrase compounds are discussed.

Structures of proteins from orthologous species can be used for drug design and discovery.

Applications of Nuclear Magnetic Resonance to drug discovery are discussed.

Structural genomics programs funded by NIAID provide support to independent investigators.

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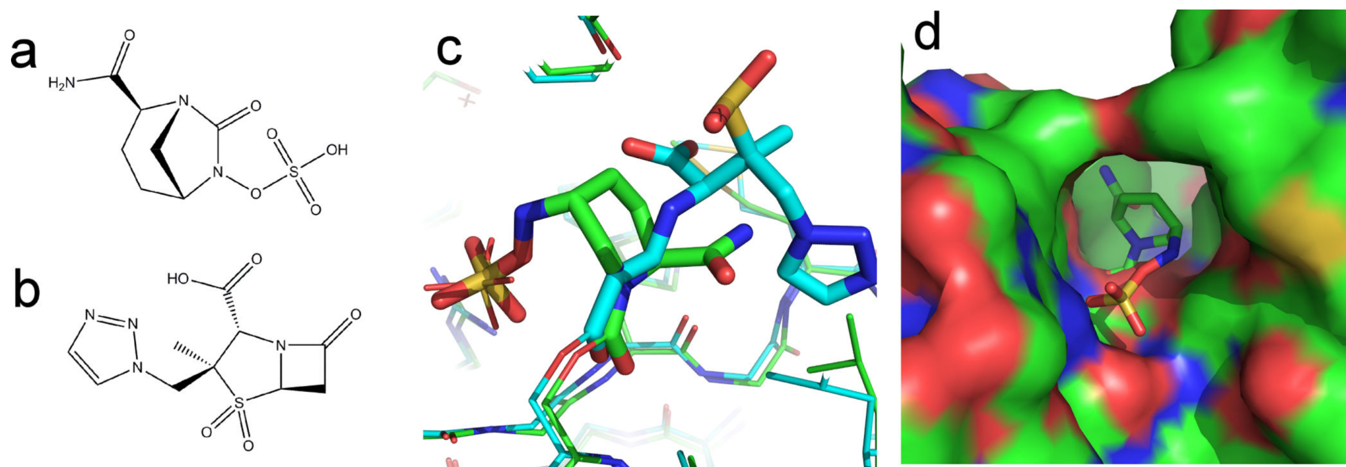

Figure 1. New β-lactamase inhibitors

(**a**) Chemical diagram of Avibactam. (**b**) Chemical diagram of Tazobactam. (**c**) Overlay of the protein structure OXA-24 from *Acinetobacter baumannii* bound to Avibactam (PDB: 4WM9) and Tazobactam (PDB: 3ZNT). Avibactam is a non β-lactam containing compound which binds OXA- 24 in similar ring-open conformation to the β-lactam containing compound Tazobactam. Avibactam structures shown with green carbons. Tazobactam structures shown with cyan carbons. (**d**) Surface of OXA-24 from *A. baumannii* bound to Avibactam. A hydrophobic bridge in Class-D β -lactamases covers the active site thus restricting access. Surface colored by atom (blue=nitrogen, red=oxygen, green=carbon).