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Perspective

Antibiotics: A New Hope

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Antibiotic resistance is one of the most significant challenges to the health care sector in the 21st century. A myriad of resistance mechanisms have emerged over the past decades and are widely disseminated worldwide through bacterial populations. At the same time there have been ever fewer new antibiotics brought to market, and the pharmaceutical industry increasingly sees antibiotics as a poor investment. Paradoxically, we are in a Golden Age of understanding how antibiotics work and where resistance comes from. This knowledge is fueling a renaissance of interest and innovation in antibiotic discovery, synthesis, and mechanism that is poised to inform drug discovery to address pressing clinical needs.

A Critical Need for New Antibiotics

The Infectious Diseases Society of America, representing the infectious disease specialists, who are in the front line of antibiotic use, has called for the delivery of ten new antibiotic drugs by the year 2020 (Infectious Diseases Society of America, 2010). This "10 x '20 initiative" is in response to the growing threat of antibiotic resistant pathogens that are increasingly causes of death and debilitating disease, not to mention a massive financial and logistical burden on health care sectors across the globe (Choffnes et al., 2010). Once confined to health care institutions, antibiotic resistant pathogens are now frequently found in the community, making containment and treatment highly challenging. Drug resistance in the so-called ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) has been highlighted as especially concerning (Boucher et al., 2009), but to this list we can add foodborne diseases caused by, for example, *Escherichia coli* and *Salmonella* (DuPont, 2007), *Clostridium difficile* (Clements et al., 2010), *Mycobacterium tuberculosis* (Cegielski, 2010), and increasingly *Neisseria gonorrhea* (Allen et al., 2011). This is a problem that is increasing in scope, with new concerns emerging regularly.

Multidrug resistance (MDR) in these pathogens is now the norm. Pathogenic strains are accumulating large numbers of resistance elements, greatly limiting therapeutic options. The genome of an epidemic MDR strain of *A. baumannii* revealed a large 86 kb resistance island that included 45 genes able to confer resistance to a broad spectrum of antibiotics (Fournier et al., 2006). The recent outbreak of hemolytic uremic syndrome in Europe in 2011 caused by *E. coli* O104:H4 is also instructive (Bielaszewska et al., 2011). The reference strain had been previously identified as a rare β-lactam antibiotic sensitive isolate in 2001; when it re-emerged in a 2011 outbreak, it had acquired a plasmid conferring resistance to a broad spectrum of β-lactam antibiotics (so-called ESBL phenotype). Sequencing of the genome of this strain identified a 90 kb plasmid that includes the extended spectrum β-lactamase CTX-M15, as well as the TEM-1 β-lactamase (Mellmann et al., 2011). In order to avoid induction of virulence toxins, antibiotics are not generally used to treat enterohemorrhagic *E. coli* infection, nevertheless this example demonstrates the ability of strains to rapidly acquire resistance genes and is a testimony to their movement through bacterial populations by horizontal gene transfer. Similarly, the recent, highly publicized emergence of isolates of *Enterobacteriaceae* harboring the NDM carbapenemases were resistant to all available antibiotics, with the exception of colistin, a polycationic peptide associated with significant human toxicity (Kumarasamy et al., 2010). Resistance is pervasive and only increasing in scope and impact.

It is increasingly evident that the origin of the resistance elements circulating in pathogens is the vast collection of non-infectious microbes that populate the globe, the antibiotic resistome (Wright, 2007). Antibiotic resistance genes have been identified in bacteria from soil (D'Costa et al., 2006), water (Zhang et al., 2009), the deep-subsurface (Brown and Balkwill, 2009) and the deep ocean (Toth et al., 2010). Some of the wide distribution of resistance genes in environmental organisms may be the result of human activity, for example, high-level resistance is found in microbes downstream from antibiotic production plants (Li et al., 2009, 2010); however, the majority of resistance in the environment is naturally occurring (Allen et al., 2010). This is supported by the discovery of a plethora of resistance genes in permafrost dating from the Pleistocene era 30,000 years ago (D'Costa et al., 2011). These findings contrast with the resistance profiles of pathogens that pre-date the antibiotic era or have been collected from antibiotic naive populations, in which resistance is relatively rare but in which gene-mobilizing elements, such as R-plasmids, are common and poised to integrate and disseminate resistance genes in the face of appropriate selection pressure (Davis and Anandan, 1970; Hughes and Datta, 1983; Thaller et al., 2010).

Human use of antibiotics has provided the selective pressure for capture of resistance elements existing in the environment for millennia by pathogens. This holds true not only for natural product antibiotics but also for drugs of completely synthetic origin, as evidenced by the emergence of horizontally transferred resistance to the fluoroquinolone antibiotics, such as ciprofloxacin (Strahilevitz et al., 2009). Here the *qnr* genes, likely originating in waterborne bacteria of the *Shewanella* genus (Poirel et al., 2005), have been captured on plasmids that are now widely circulating in *Enterobacteriaceae*. The rapid emergence

Table 1. Recent Innovation in Antibiotics

	Class	Target	Compound Type
Approved antibiotics			
Linezolid	Oxazolidinone	Ribosome	Synthetic
Daptomycin	Lipopeptide	Cell Membrane	Natural product
Retapamulin	Pleuromutilin	Ribosome	Semisynthetic
Tigecycline	Tetracycline	Ribosome	Semisynthetic
Fidaxomicin	Macrolide	RNA polymerase	Natural Product
Telavancin	Glycopeptide	Peptidoglycan	Semisynthetic
Antibiotics in clinical trials			
Omadacycline	Tetracycline	Ribosome	Semisynthetic
ACHN-490	Aminoglycoside	Ribosome	Semisynthetic
Cethromycin	Macrolide	Ribosome	Semisynthetic
AN3365	Oxaborole	Leu-tRNA synthase	Synthetic

Partial list, see Butler and Cooper (2011) for a more extensive survey.

of resistance and its transfer among bacterial species in the antibiotic era is a reflection of the vast genetic diversity of microbes on the planet after 3.5 billion years of evolution and the capacity to mobilize genes vertically and horizontally through populations. This is the lens through which we must try to understand and manage antibiotic discovery and resistance in the future.

New Antibiotics: Successes and Challenges

In the face of growing drug resistance in pathogens, new antibiotics are required. Over the past decade several antibiotics have been brought to market (see ref. Butler and Cooper, 2011; Table 1). However, the vast majority represent relatively minor alterations to well-known chemical scaffolds, in particular the β-lactams and fluoroquinolones. Whereas these classes have been deep wells from which to draw new drugs over the past decades, we may be reaching the limits of their safe and effective chemical diversity. It is sobering to realize that only four of these new drugs represent chemical scaffolds new to human use: linezolid, which is a completely synthetic drug, and the natural products daptomycin, a lipopeptide; fidaxomicin, a macrocyclic polyketide; and the diterpene pleuromutilin, retapamulin (Figure 1). None of these four represent truly novel classes of drugs though having been discovered in decades past and either repurposed for human use or resurrected from failed discovery campaigns.

Nevertheless, they all represent important successes in innovative re-visiting of antimicrobial chemical matter with fresh eyes with resulting clinical benefit. The oxazolidinone scaffold of linezolid was first explored by DuPont in the 1980s to treat plant disease but was eventually elaborated by researchers, first at Pharmacia, then at Pfizer, by classical medicinal chemistry efforts guided by biological activity with attention to pharmacology into the first-in-class drug Zyvox that was FDA-approved in 2000 (Barbachyn and Ford, 2003). Similarly, daptomycin was first explored and then abandoned by Eli Lilly in the 1980s because of toxicological concerns; it was then acquired in the mid-1990s by Cubist, re-deployed with alternate dosing, and received FDA approval in 2003 (Baltz et al., 2005). The pleuromutilins were discovered 60 years ago and used for over 30 years in veterinary medicine but revisited by GlaxoSmithKline in the form of the semisynthetic retapamulin for topical human use, gaining FDA approval in 2007 (Novak and Shlaes, 2010). Fidaxomicin has a similar history, separately identified by groups at Lepetit in 1975 and then at Abbott again in the mid-1980s from actinomycetes as a poorly absorbed antibiotic. It was eventually brought to market in 2011 by Optimer, who took advantage of the poor solubility to selectively target infections caused by the serious intestinal pathogen *C. difficile*, which is responsible for a number of recent outbreaks of nosocomial disease (Hardesty and Juang, 2011).

Despite these great examples of the value of revisiting the deep antimicrobial catalogs of drug companies, none of these antibiotics are panaceas to the broader challenge of resistance. Indeed all of these drugs have limited antimicrobial activity: all are confined to Gram positive pathogens, retapamulin is available for topical use only, and fidaxomicin is narrowly focused on *C. difficile*. The only new antibiotic with significant utility to treat infections caused by Gram negative pathogens, a growing clinical problem approaching crisis levels (Arias and Murray, 2009; Peleg and Hooper, 2010), is the third generation semisynthetic tetracycline, tigecycline. This antibiotic was approved by the FDA in 2005 following years of classical semisynthetic medicinal chemistry effort by the infectious disease group at Wyeth (Sum, 2006).

The lessons in bringing these antibiotics to market are significant and reveal the importance of deep experience in antibiotic drug discovery in the pharmaceutical industry, as well as tenacity, serendipity, and the value of "old" scaffolds in bringing new antibiotics to market. This is especially important as the track record in using target-based approaches guided by genomics and using libraries of synthetic molecules, often tailored for oral bioavailability in campaigns for a variety of clinical indications, has proven ineffective so-far in antibiotic drug discovery (Gwynn et al., 2010; Payne et al., 2007). Lynn Silver has characterized the result as a "Discovery Void" with no new antibiotic scaffolds successfully identified since daptomycin in 1987 (Silver, 2011). Indeed, the selection of what constitutes the "best" chemical matter for antibiotic drug discovery is a significant challenge that needs to be addressed.

Natural products produced by bacteria have been the most successful source of antibiotic drug scaffolds over the past decades. Most of these are from soil-derived members of the actinomycete class. Fungal natural products include the penicillins and cephalosporins (though these can also be produced by bacteria) and the pleuromutilins. No plant sourced antibiotics have been clinically approved and synthetic scaffolds are limited to the quinolones, sulfonamides, the diaminopyridine trimethoprim, and the oxazolidinone linezolid. There has been much discussion about whether we have picked all the "low hanging fruit" and if continued investigation of actinomycetes will be profitable in the search for new antibiotics (Baltz, 2008); indeed, the lack of successful new scaffolds and the repeated rediscovery of known molecules from this source has been cited as a major reason for the closing of natural products divisions in many large pharmaceutical companies. Similarly, the lack of new scaffolds from synthetic molecules, despite 20 years of screening, has frustrated target-based approaches for new antibacterial drug discovery.

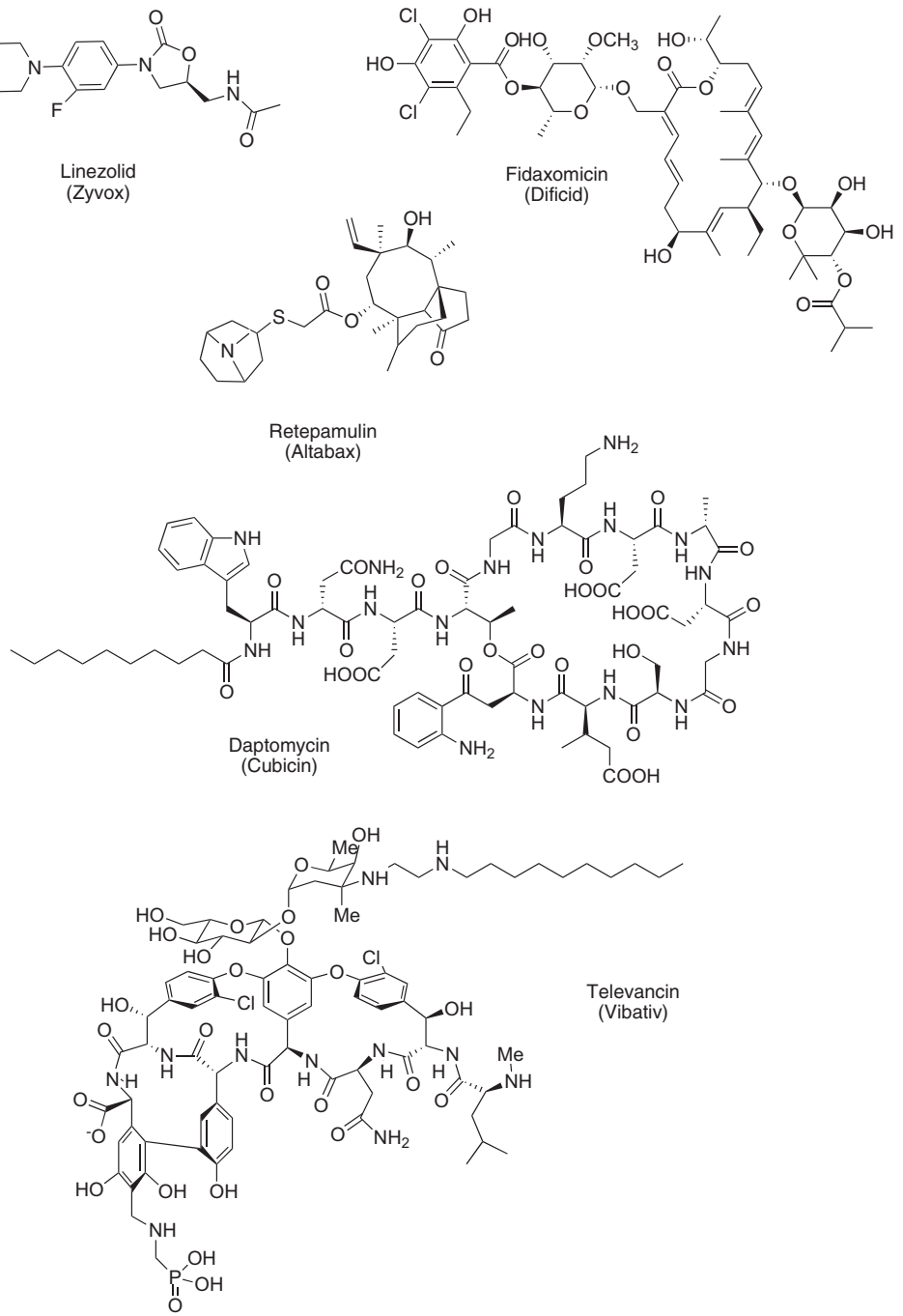

Figure 1. New Antibiotics Approved for Human Use over the Past Decade
These represent new scaffolds for medical application.

Defining good antibacterial targets has been an additional challenge. Leonard Katz has said that "A good antibiotic target is one which there is (or will be) a drug" (Katz, 2000). This statement reflects the difference between identifying enzyme inhibitors and antibacterial compounds in vitro, which is relatively easy, and actually advancing molecules into the clinic and successfully deploying a new drug, which is very hard. The antibiotic scaffolds in current use were discovered using cell-based assays with inhibition of growth as the assay. Subsequent biochemical identification of the molecular targets of these antibiotics revealed a remarkably limited number that include the ribosome, enzymes of peptidoglycan biosynthesis, enzymes of thymidine synthesis, RNA polymerase, DNA topoisomerases, and the bacterial membrane. Analysis of microbial genomes suggests a much larger number of potential targets, even using the strict criteria of essentiality for cell growth, conservation

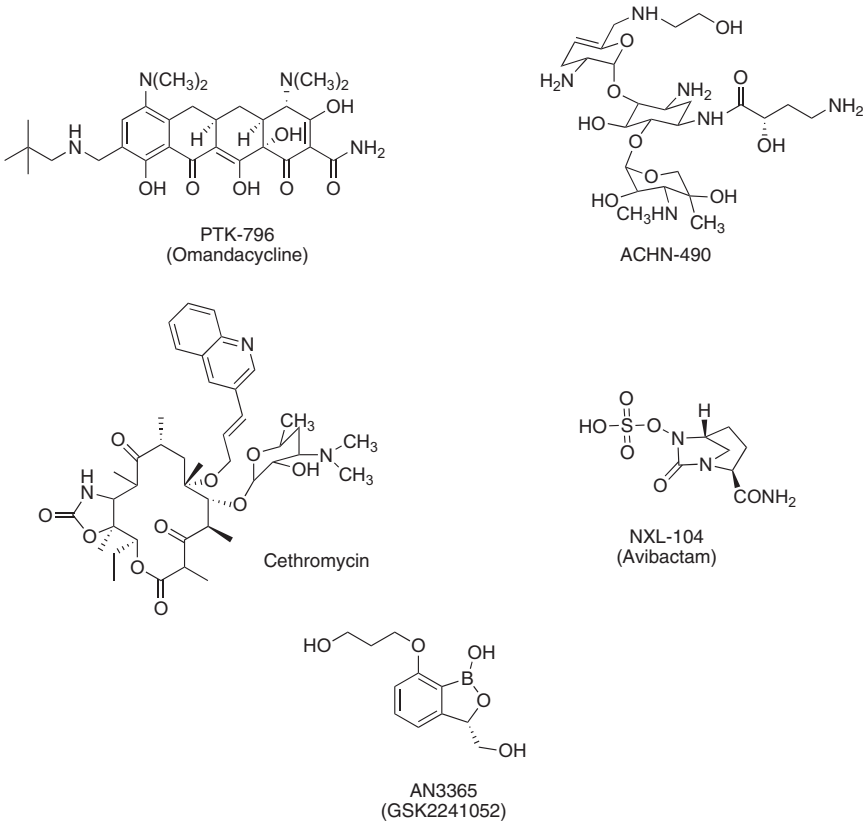

Figure 2. Antibiotics in Development
Representative new agents in phase 2- and phase 3-stage clinical trials. NXL-104 is not an antibiotic per se but an inhibitor of β-lactmases.

across most important pathogens, absence of similar targets in humans, and the ability to bind and be blocked by small molecules. Nevertheless, this apparent richness in targets has failed to generate any new drugs, despite extensive effort by the pharmaceutical industry over 20 years.

At the same time, changing regulatory guidelines for new antibiotics have vexed the antibiotic drug industry (Choffnes et al., 2010; Projan, 2003; Projan and Shlaes, 2004). Whereas the global antibiotics market is substantial (US $42 billion in 2009; Hamad, 2010), the cost of developing new antibacterial drugs, in particular the high costs of clinical trials, has also risen and negatively impacted the return on investment for antibiotics in particular (Choffnes et al., 2010; Cooper and Shlaes, 2011). Furthermore, since antibiotics are given for short periods of time and clinicians are increasingly reluctant to use new agents to preserve their long-term effectiveness, it is not surprising that the for-profit pharmaceutical sector sees diminishing returns in antibiotics.

All of these challenges have conspired to dramatically reduce investment in antibacterial drug discovery. Many large pharmaceutical companies have greatly reduced or abandoned antibiotic discovery all together. The result is a lack of attention (and associated innovation) and a growing unmet clinical need.

Where Will the New Antibiotics Come from?

In order to face the challenge of resistance and address the pressing clinical need for new drugs, many approaches must be considered. It is evident that new investment will be required and that creative methods to incentivize both the academic and pharmaceutical sectors are needed to overcome the unique economic challenges faced by drugs whose obsolescence is virtually guaranteed. There are no irresistible antibiotics. Several important strategies from funding public-private initiatives to extending patent life of antibiotics have been proposed, and these efforts are worthy of serious consideration (e.g., refs. Laxminarayan and Powers, 2011; Spellberg et al., 2011). Even if such policy changes were enacted immediately, the scientific challenges of identifying new antibiotics remain formidable. If we have picked all the low hanging fruit, where do we look for new antibiotics?

One obvious strategy is the continued mining of known scaffolds. Elaboration of bioactive synthetic scaffolds, such as the oxazolidinones and diaminopyrimidines, have resulted in new compounds in late-stage clinical trials, and eight quinolone and fluroquinolone derivatives have come to market since 2000 (Butler and Cooper, 2011). Similarly, several β-lactams have been introduced to the clinic in the last decade or are in clinical trials. Semisynthetic modification of natural products remains a highly viable approach. Telavancin, the first semisynthetic glycopeptide antibiotic approved for clinical use was launched in 2009 (Corey et al., 2009), and at least two additional glycopeptides, oritavancin and dalbavancin, are in late-stage trials. Other new semisynthetic agents in clinical trials include the tetracycline omadacycline (PTK-796; Nat. Rev. Drug Discov., 2009), the aminoglycoside ACHN-490 (Endimiani et al., 2009), and the ketolide cethromycin (Rafie et al., 2010; Figure 2). All of these have broad spectrum antimicrobial activity, including against Gram negative pathogens, which are an increasingly urgent clinical need. Tellingly, all of these are being pursued not by big pharma but by small- and medium-sized pharmaceutical firms.

We certainly have not exhausted the potential for exploiting the natural chemical diversity of known antibiotic scaffolds. Our understanding of the molecular mechanisms underlying natural product biosynthesis, in particular of polyketide and nonribosomal peptide synthetase assembly lines, has greatly matured over the past decade (Walsh and Fischbach, 2010). The simultaneous growth of synthetic biology techniques and reagents, in particular the plummeting cost of DNA synthesis, has moved the in vivo manipulation of chemical scaffolds into cost-effective reality (Keasling, 2010). The heroic pioneering work on the heterologous overexpression (Pfeifer et al., 2002) and engineering (McDaniel et al., 1999) of the 6-deoxyerythronolide B macrolide

antibiotic scaffold demonstrates the chemical diversity accessible by genetic manipulation of biosynthetic pathways and the accessibility to gram quantities of chemically complex compounds through fermentation. Antibiotics, their scaffolds or fragments thereof, that otherwise would be too challenging or costly to access on an industrial scale using total synthesis can be now conceivably accessed using engineered heterologous hosts. By modifying antibiotic scaffolds combinatorially using biosynthetic modifying enzymes and semisynthesis, antimicrobial chemical space can be increased significantly. Furthermore, chemical expansion can be performed directly on nonpurified natural product extracts followed by activity-guided purification, a process that has the potential to further expand chemical space (López et al., 2007; Ramallo et al., 2011).

Revisiting abandoned scaffolds is a proven route to new antibiotics as evidenced by the histories of linezolid, daptomycin, retapamulin, and fidaxomicin. Berdy has estimated that by the year 2,000 approximately 20,000 naturally derived antibiotics were already known (Bérdy, 2005). This is a remarkably rich vein to mine for new leads in clinically useful antibiotics. Most of these are surely unsuitable for direct entry into clinical use; however, they do represent largely untapped chemical matter that, if revisited using a combination of synthetic biology and semisynthesis, could be a source of a number of new antibiotics. Furthermore, even if these do not lead to drugs themselves, they can be very useful in identifying new targets, which can then be pursued using target-based approaches.

Another route is to seek completely novel compounds. There are an estimated 200,000 to 250,000 bioactive natural products ([Bérdy, 2005]; though this number is based on the rate of purification and characterization of compounds in the literature, and the number may be significantly higher). Even if only a small fraction of these have antibiotic activity, this represents a stunning region of untapped chemical space to explore. Synthetic chemicals also represent a potential valuable source of new antibiotics. Here efforts to understand what constitutes suitable chemical matter, which can penetrate and be retained by bacterial cells, in particular the difficult Gram negative pathogens with their lipopolysaccharide outermembrane and panoply of efflux pumps, is needed. There are no simple guidelines akin to Lipinski's "Rule of 5" to guide drug discovers in this area, though some efforts have been made to quantify the physical chemical properties of antibiotics (O'Shea and Moser, 2008). The results of these analyses have been to underline that antibacterial agents occupy unique chemical space apart from drugs directed to human targets and that this information needs to inform library preparation for antibacterial screens. One notable success currently in phase II trials for treatment of infections caused by Gram negative pathogens is the novel oxaborole compound AN3365 (GSK2251052) from Anacor (Figure 2; http://www.anacor.com/). This compound emerged from an antifungal lead that blocked Leu-tRNA synthetase function. The compound is a slow-tight-biding inhibitor of the enzyme that forms an adduct between the boron atom with the geminal *cis*-2′3′-dihydroxy groups of the ribose from the terminal adenine nucleotide of the tRNA, thereby trapping the enzyme-tRNA complex in a form that is incapable of catalysis (Rock et al., 2007). This scaffold offers the first promising new class of synthetic anti-Gram negative drug leads since the introduction of fluoroquinolones over 40 years ago.

Another route to expand chemical space applies the knowledge rising from systems biology studies over the past decade. Here the emerging view of the cell is not one comprised of the linear pathways familiar to biochemistry textbooks but rather as a complex web of genetic interactions, rife with redundancy and populated by a collection of key nodes that link cellular networks (Costanzo et al., 2010). The result is that most genes (>80%) in an organism are dispensable, that is, they can be deleted or otherwise inactivated under common laboratory settings. These would typically be regarded as poor drug targets, though they may be very "druggable", that is, potently inhibited by small drug-like molecules. However, in a genetic background in which such nonessential genes have been deleted, the number of "essential" genes increases substantially (Sharom et al., 2004). The importance of these so-called synthetic lethal interactions to antibiotic discovery is evidenced by the dramatic increased sensitivity to antibiotics in bacterial strains in which nonessential genes have been inactivated (Alvarez-Ortega et al., 2010; Breidenstein et al., 2008; Liu et al., 2010; Tamae et al., 2008). These genes (the intrinsic resistome; Fajardo et al., 2008) therefore offer an orthogonal set of molecular targets for compounds that can enhance antibiotic activity: antibiotic adjuvants (Kalan and Wright, 2011). Target-based discovery of inhibitors of the products of these genes are predicted to discover molecules that potentiate antibiotic action. This approach could breathe new life into old antibiotics and discarded scaffolds. It is worth also noting that the majority of members of our current antibiotic arsenal either block multiple targets, for example, the β-lactams that covalently inhibit a collection of cell-wall metabolism enzymes, the fluoroquinolones that block multiple type II topoisomerases, (in particular, DNA gyrase and topo IV), or target large macromolecular machines (ribosomes) or structures (membranes) with pleiotropic downstream effects.

Such combination drugs would be familiar to infectious disease clinicians as current anti-infective therapy makes extensive use of combinations, including the antibacterials trimethoprim+sulfamethoxazole (Bactrim), quinupristin+ dalfopristin (Synercid), and the co-formulations of anti-HIV antivirals. The use of combinations is also well established to decrease the emergence of resistance and increase efficacy in tuberculosis treatment and during antifungal therapy. Therefore, multicomponent therapy in the form of antibiotic adjuvants offers a powerful approach to extend the life of current antibiotics and decrease the impact of resistance.

We recently reported the successful outcomes of screens to identify such antibiotic adjuvants in both bacteria (Ejim et al., 2011) and yeast (Spitzer et al., 2011). In this work, we explored combinations of known antimicrobials (minocycline for bacteria, fluconazole for yeast) with off patent non-antimicrobial drugs. We reasoned that these compounds offer a privileged source of bioactive compound space; though not themselves antibiotics, they may have cryptic activity towards the intrinsic resistome and synergize with antibiotics at sub-MIC concentrations. This work identified a number of candidate molecules that synergized with known antibiotics. One pair, minocycline + the μ-opioid receptor agonist loperamide (Immodium), a well know drug used to treat diarrhea, demonstrated broad activity versus Gram negative pathogens and was active in a mouse model of salmonellosis. In yeast, the fungistatic drug fluconazole was

potentiated by a variety of neuro-active compounds, including the antidepressant sertraline (Zoloft), and here the combination was fungicidal and active in an insect model of fungal infection. Importantly, in many cases, genus and even species specificity was observed for many combinations. This offers the opportunity to use the combinations stratagem to direct therapy to specific pathogens, thereby sparing the larger microbiome and perhaps reducing opportunity for complications, such as antibiotic-associated colitis, often linked to infection by virulent *C. difficile*.

These studies demonstrate the power of multicomponent versus single molecular therapy. By combining bioactive compounds, the cellular network can be impacted at multiple sites, thereby dramatically increasing effective antimicrobial chemical space and enhancing potency. Given the fact that cryptic antimicrobial activity may be revealed using combinations, one need not be restricted to combining antibiotics with nonantibiotics as described in these examples. A matrix of a thousand compounds systematically assayed in pairs gives rise to the equivalent of almost 10^6 unique combinations, higher order mixtures (well-precedented, e.g., in tuberculosis, HIV, and cancer therapies) scale accordingly, thereby greatly expanding chemical space (Spitzer et al., 2011).

The concept of compound combinations can also be applied to direct blockade of antibiotic resistance. Combinations of β-lactam antibiotics and inhibitors of Ser β-lactamases have been clinical and commercial successes for decades (Drawz and Bonomo, 2010). These inhibitors (clavulanic acid, sulbactam, and tazobactam) are β-lactams themselves that covalent modify the active site Ser but undergo deacylation very slowly, effectively inactivating the resistance enzyme on a clinical timescale. A new cyclic urea β-lactamase inhibitor, NXL-104 (avibactam), has been advanced to phase 2 clinical trials by Novexel, in combination with the cephalosporin ceftazidime demonstrating that there remain opportunities to innovate in this area. Similarly, inhibitors of efflux pumps have been pursued both in the academic and pharmaceutical arenas (Lomovskaya and Bostian, 2006; Nikaido and Pages, 2011; Pagès and Amaral, 2009). A growing understanding of the molecular details of efflux pump mechanism and structure, in particular the tripartite RND systems of Gram negative bacteria that are leading source of MDR, is framing this approach. The challenges are significant, given the heterogeneity and redundancy of efflux systems in many serious pathogens, but common molecular mechanisms suggest that the approach is valid.

Conceptually, any resistance mechanism that requires an active cellular response can be similarly targeted, thereby rescue "old" antibiotics. The challenge of this approach is the fact that in most cases of resistance, there are many mechanisms circulating in pathogens, for example, aminoglycoside resistance can occur by a variety of drug modifications (phosphorylation, acetylation, and adenylylation), efflux, and target modification (methylation of rRNA). Identifying a single molecule able to block all these activities is highly improbable. These challenges could be mitigated through careful attention to resistance epidemiology to identify prominent modes of resistance perhaps in addition to the use of molecular diagnostics to match the specificity of inhibitors of resistance with genes circulating in specific clinical settings. The success of the Ser β-lactamase inhibitors is instructive here, as these have been very useful drugs, despite the heterogeneity of β-lactam resistance. Specific targets, in which one or only a few resistance mechanisms dominate in the clinic, are worthy of such campaigns, including the elements responsible for resistance to vancomycin, Erm-mediated resistance to macrolides, type B streptogramins and lincosamides, Ser-based ESBL and metallo β-lactamases, and aminoglycoside-modifying enzymes.

Another significant opportunity is targeting the bacterial gene products and associated physiology responsible for infection. Such inhibitors of microbial virulence have been touted for some time as orthogonal approaches to antibiotics (Clatworthy et al., 2007; Stanley and Hung, 2009); however, they could also be used in combination with these drugs, likely to great effect. Such molecules include modulators of quorum sensing, biofilm formation (or dispersal), type III secretion, virulence gene regulation, etc. By including virulence, the target vista for antibiotics greatly increases. The effect of these molecules though is often not readily noticeable in changes in in vitro MIC, a standard that drives antimicrobial drug discovery. However, high-throughput methods to screen infection in animal models, such as *Caenorhabditis elegans,* moves this approach into the realm of possibility for more traditional drug discovery approaches (Moy et al., 2006; Mylonakis et al., 2007).

Conclusions

The challenges before infectious disease clinicians and the antibiotic drug discovery communities are formidable. Faced with a growing spread of resistance genes in pathogens in both health care settings and in the community, the ability of drug-resistant bacteria to be carried across the planet in a matter of hours, and our absolute need of antibiotics to maintain modern medical interventions from surgery to cancer chemotherapy, the necessity of a well-stocked arsenal of antibiotics is increasingly critical. The unfortunate retreat of the pharmaceutical industry from this area at the same critical juncture, as a result of economic, regulatory, and scientific challenges, approaches "perfect storm" characterization.

Yet, there are great reasons to be optimistic. The majority of well-validated targets for antibiotics have been characterized in terms of both structure and function. Furthermore, crystal structures of complexes of these targets with antibiotics, from the ribosome to RNA polymerase to DNA gyrase, are known and provide drug-target interaction knowledge that was unheard of even a decade ago. Sources of new compounds from natural product producing actinomycetes to metagenomic libraries that encode the genetic diversity of a myriad of ecological niches can be sequenced in an afternoon yielding the sequence information underlying biosynthetic pathways. Leveraging these to produce new molecules has already been successful (Banik et al., 2010). Furthermore, combining bioactive compounds can dramatically expand antimicrobial chemical space. Knowledge of the molecular mechanism of resistance can also inform new antibiotic design as elegantly shown by the recent rational synthesis of a glycopeptide analog designed to overcome vancomycin resistance (Xie et al., 2011). Whereas overcoming the practical financial and regulatory challenges of the drug discovery process will not be easy, the scientific brain trust is poised to deliver.

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