Bioorganic & Medicinal Chemistry 24 (2016) 6227–6252

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

New antibiotics from Nature's chemical inventory

Timothy A. Wencewicz *

Department of Chemistry, Washington University in St. Louis, One Brookings Drive, St. Louis, MO 63130, United States

ARTICLE INFO

Article history:
Received 19 July 2016
Accepted 7 September 2016
Available online 13 September 2016

Keywords:
Antibiotics
Resistance
Natural products
Biosynthesis
Metabolomics
Genome mining
Essential genes
Smart screens
Drug discovery

ABSTRACT

The doom and gloom of antibiotic resistance dominates public perception of this drug class. Many believe the world has entered the post-antibiotic era. Classic and modern approaches to antibacterial drug discovery have delivered a plethora of lead molecules with a great majority being natural products of ancient microbial origin. The failure of antibiotics in the resistance era comes from an inability to develop new leads into clinical candidates, which is a costly and risky endeavor for any therapeutic area, especially when resistance is at play. The world needs new antibiotic molecules to replace the exhausted pipeline and the second 'golden era' is certain to come from Nature's chemical inventory once again.

1. Introduction

Antibiotics link the ancient microbial world to modern microbiomes through an evolving web of resistance.[1–5] The field of antibiotics was born out of this premise during the golden era of antibiotics (1940s–mid 1960s) which gave the world its most important natural product scaffolds of clinical significance—β-lactams, aminoglycosides, tetracyclines, glycopeptides, macrolides, chloramphenicols, ansamycins, and streptogramins.[6,7] Discovery during the golden era was dominated by the Waksman platform – the practice of growing environmental microbes, both bacteria and fungi, under laboratory conditions to produce bioactive metabolites, also known as the Waksman platform.[8] Microbes are innately resistant to the antibiotics they produce.[9,10] The genetic and molecular self-protection mechanisms of producing organisms represent resistance mechanisms for competing organisms that acquire these abilities through horizontal or vertical gene transfer.[2,11] Natural ecosystems maintain a balance of antibiotic susceptibility and resistance in the microbial world, but human overuse of broad-spectrum antibiotics in hospital and agricultural venues applies strong selective pressure favoring resistant populations.[4,12]

The widespread use of the Waksman platform slowed as scaffold redundancy, conditional expression of metabolites, and resistance increased. The medicinal chemistry era emerged (mid 1960s–2000) to combat clinical resistance through semi-synthetic variation of natural product scaffolds.[13] Successive generations of modified golden era antibiotics dominated this 40-year period and solidified the transition into the resistance era (2000–present day).[6,14] Continuous attack of conserved targets with related generations of antibiotics led to the rise of multi-drug resistant (MDR) super bugs.[15] It is now commonplace to isolate MDR bacterial pathogens from hospital and community settings, some of which are resistant to all FDA-approved antibiotics.[16–21]

High-throughput, target-based approaches commonly employed in modern pharmaceutical drug discovery have failed to replenish the depleted antibiotic pipeline in the resistance era (2000–present).[17] It is now widely accepted that most commercial and pharmaceutical compound collections primarily contain molecules that lack the physiochemical properties required to penetrate bacterial cell envelopes.[22,23] Natural products coevolve a high affinity for a biological target and the ability to reach the cellular site of action; two distinct advantages of starting from a natural product during lead optimization.[24,25]

Nearly 75 years of Waksman-like screening methods has produced~20,000 microbial metabolites in the chemical and patent literature.[26–28] The vast majority of these compounds were isolated from soil-dwelling bacteria and fungi. It is predicted that less than 1% of all environmental microbes have been cultured in laboratory settings.[29] Disregarding genetic redundancy, which is indubitable, this supports the possibility that greater than 99% of antibiotics

* Tel.: +1 314 935 7247; fax: +1 314 935 4481.
E-mail address: wencewicz@wustl.edu

http://dx.doi.org/10.1016/j.bmc.2016.09.014
0968-0896/

are yet to be discovered.[30] Modern natural products chemistry has largely focused on accessing this 99% of 'dark' chemical matter. Several decades of work on elucidating the genetic and molecular mechanisms of biosynthetic pathways culminated in the virtual prediction of antibiotic structures from genetic sequence alone and directed modular cluster evolution for producing designer natural product scaffolds.[31–34] The constantly growing amount of DNA sequencing data has confirmed the existence of the predicted 'dark' chemical matter revealing that many biosynthetic gene clusters are kept silent under laboratory conditions.[35] New insights and methods for gene regulation, transcription factor engineering, promoter refactoring, and heterologous gene expression in bacteria have enabled new methods for turning on silent antibiotic gene clusters to produce cryptic metabolites.[36] New methods in bacterial cell separation and culturing techniques using diffusible membranes has created the opportunity to grow bacteria in a simulated native environment, enabling laboratory growth of up to 50% of the entire microbial population in some cases.[30,37] Advancements in cell culture and genome mining have expanded the search for antibiotics to new environments, including the human microbiome, which is already yielding new molecules.[38] The field of metagenomics is contributing to natural product discovery as capabilities grow for capturing inserts of genetic material large enough (20–30 kilobases) to harbor entire antibiotic biosynthetic gene clusters.[39,40] Metagenomics allows genetic access to all microbes in an environment, independent of culturability, further expanding access to antibiotic 'dark' matter. Progression from the genomics era (1972–2003) to the proteomics era (2001–present) and now the metabolomics era (2002–present) completes the connection of genes to molecules with assistance from powerful new analytical tools, such as instruments coupling uHPLC with high-resolution NMR and tandem MS, and data mining software. Coupling bioinformatics with metabolomics enables computational prediction and structure elucidation of natural products in complex samples expanding access to once cryptic metabolites.[41]

The stage is now set in the resistance era for the most ambitious campaigns in natural product discovery of all time in both the public and private sectors.[42] Companies like Warp Drive Bio (http://www.warpdrivebio.com/index.php) are using advancements in high-throughput DNA sequencing to mine the genomes of >340,000 Actinomycetes, Nature's most prolific antibiotic producers. As of August 2015, Warp Drive survey-sequenced and assembled ~135,000 genomes, completed 157 genomes, and logged ~3.5 million antibiotic biosynthetic clusters into a proprietary database.[43] A genomic survey of this size is certain to reveal novel structural variants of known natural product families and completely new classes of previously unknown or extremely rare natural products. NovoBiotic Pharmaceuticals, in collaboration with Northeastern University, is the company behind the highly publicized discovery of teixobactin, a potent lipid II binder with low resistance probability.[44,45] Teixobactin is produced by a previously unculturable Gram-negative bacteria *Eleftheria terrae* that was isolated using the iChip device, which is designed for high-throughput microbial cultivation in natural microbial environments.[46] Combining high-throughput genome sequencing with high-throughput microbial cultivation and synthetic biology might be the kick-start needed to transition from the resistance era to the platinum era of antibiotic discovery.[42]

In all eras of antibiotic discovery, natural product lead compounds must still progress through the tedious traditional drug development pipeline (Fig. 1).[6,47] This is the bottleneck of the entire pharmaceutical industry and represents the translational valley of death for academics and small biotechs.[48] Many promising compounds get stuck at this stage, including antibiotics, and without backing from Big Pharma and a cooperative drug development infrastructure translational to the clinic is unlikely.[49] Large pharma companies have mostly abandoned early stage antibiotic discovery and development, although government incentives and growing concerns over the global spread of antibiotic resistance have sparked murmurings of a potential return during this era of crisis and resistance.[50] Of the >20,000 known natural products with antibacterial activity only a tiny fraction have been rigorously investigated as drug candidates.[28,51] Thus, finding new molecules that act in new ways is not the immediate answer to the antibiotics crisis, although this is the best long-term solution that mitigates the risk of an innovation gap in antibiotics research. The most pressing need is to reengage the pharmaceutical industry in antibiotic development and create collaborative interactions with academic, non-profit, and small biotech labs to seriously evaluate forgotten antibiotic leads as new drug candidates.[49] This will take a change of tactic that challenges common practice and beliefs of the traditional antibiotic industry. Traditional antibiotics from the golden era were biased towards having a broad-spectrum and bactericidal mechanism of action taking a blind eye to the identity of the pathogen causing infection.[7] We no longer have the luxury of using such agents in the resistance era so it is time to consider neglected compounds that are narrow-spectrum, apply less selective pressure for resistance development, and preserve the human microbiome.[52,53] It all starts by revisiting Nature's molecules with lessons learned during the resistance era.[54]

2. Finding homes for orphaned antibiotics

The golden era of antibiotics delivered thousands of potent antibiotic lead compounds from all families of natural products including non-ribosomal peptides (NRPs), polyketides (PKs), ribosomal polypeptides (RiPPs), fatty acids, terpenoids, alkaloids, oligosaccharides, non-proteinogenic amino acids, and molecular hybrids of various structural classes.[7,28] Only a small fraction of these molecules were seriously considered for preclinical development with the vast majority left buried in patents and the *Journal of Antibiotics*.[51] Historically antibiotic drug development has been more cost effective and successful compared to other therapeutic areas because of highly predictive in vitro and in vivo efficacy models. However, the financial return on investment has dwindled with the steady rise of antibiotic resistance and inflated drug developments costs.[5] Natural product scaffolds evolve under selective pressure to cross bacterial cell envelopes and interact with the intended cellular target with exquisite potency; both tremendous advantages for a lead compound.[24] Many natural products were never thoroughly investigated as clinical candidates because of toxicity, undesirable physical properties, unknown biological target, limited supply, lack of broad-spectrum bactericidal activity (desirable during the golden era, now less desirable due to increased pressure for resistance development), or lack of financial interest. Addressing toxicity and pharmacokinetic/pharmacodynamic issues using modern medicinal chemistry and identifying the ideal therapeutic application for the drug candidate is a proven method to fast-track the development of these forgotten antibiotic scaffolds (Fig. 2). This tactic was used by Steven Brickner and his colleagues at Pfizer to bring linezolid to market in 2000 as the first new structural class of antibiotics approved in~40 years.[57] The same model has been applied more recently to neglected natural products such as daptomycin and fidaxomicin validating that a careful development plan, proper formulation, and the correct indication can lead to a successful clinical antibiotic.