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Chemical philanthropy: a path forward for antibiotic discovery?

“It remains to be seen if the premise adopted by the Community for Open Antimicrobial Drug Discovery will aid in reinvigorating the antibiotic pipeline.”

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Antibiotic researchers, clinicians and regulators are all too familiar with the concept of antibiotic resistance, with scientific debate generating an ever-increasing number of annual publications on the topic. Despite overwhelming scientific evidence in support of an imminent ‘superbug crisis’ that may have catastrophic results for human health, the continued reporting of ‘doomsday’ messages by the media fails to convince the general public, in part due to a general misunderstanding of what antibiotic resistance actually means and how it relates to the individual. One common misconception is the belief that people, rather than the bacteria, become resistant to the antibiotics [1].

Public ignorance is a major driver of antibiotic resistance due to the significant overuse of antibiotics in both humans and animals. Concerning human use, it is ironic that clinicians, the very people who ought to know better, are often complicit in the overprescription of antibiotics [2]. On the other hand, misguided regulatory frameworks and commercial interests have led to the careless misuse of these precious drugs in agriculture, where pigs, poultry and fish are fed sub-therapeutic concentrations of last-resort antibiotics such as colistin to improve yields under intensive farming conditions [3]. If the emergence of antibiotic resistance was not already dire enough, this incredulous misuse of colistin, which represents the last line of defence against carbapenem-resistant *Enterobacteria-*

ceae, has led to the generation of heretofore never observed plasmid-mediated colistin resistance (*mcr-1*) in *Escherichia coli* (*E. coli*), initially in isolates derived from samples of raw meat, animals and humans in China [4], with subsequent identification from multiple sources worldwide [5–11].

Despite the vigorous rate of publications categorizing the spread of antibiotic resistance, the same cannot be said for the numbers of approved new drugs reaching the market [12], especially those that target antibiotic-resistant Gram-negative bacteria. A recent survey of investigational drugs targeting Gram-negative infections that have entered human clinical trials highlights 24 agents from 22 companies [13]. While it is encouraging to see such active company engagement, no doubt stimulated by numerous policy changes such as those made under the Obama administration [14], most of the antibiotics are improved iterations of legacy scaffolds for which there are already underlying resistance mechanisms (aminoglycosides, β -lactams, β -lactamase inhibitors, quinolones and tetracyclines). Although several novel non- β -lactam β -lactamase inhibitors also comprise the lineup (e.g., boronic acids and diazabicyclooctanes); their modes of action are not novel [13]. While there are clear benefits in making incremental improvements to compound classes that possess well-defined safety and pharmacological profiles, there remains a paucity of novel agents active

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against novel targets. Indeed, no drug with a novel mode of action against Gram-negative bacteria has been approved in the last 50 years.

There has been much debate over how to replenish the antibiotic pipeline [15]. The high attrition rates associated with the lead optimization and clinical development phases of antibiotic discovery necessitate a large number of high-quality leads from the outset. In turn, sources of significant chemical diversity are needed to generate such high-quality leads. Most antibacterial drugs in use today are derived from natural product origins. Historically, we have relied on Mother Nature to provide chemical scaffolds of sufficient complexity to enable the development of effective antibiotics optimized for target binding and bacterial penetration. Unfortunately, having mined natural product sources for over 50 years, it has become extremely challenging to identify new privileged scaffolds buried within an insurmountable background of known and/or nuisance compounds. Our once unfettered access to the rich sources of evolutionary chemical diversity that laid the foundations of the golden era of antibiotic discovery has seemingly come to an end.

With upper estimates of finding a novel secondary metabolite class sitting at a probability of around one in every 10^7 micro-organisms [16], a return to traditional natural product discovery remains a challenging task when coupled with conventional screening approaches, especially in the face of declining pharmaceutical R&D efficiency [17]. However, several new approaches to tackle these problems are gaining traction, such as interrogating ‘unculturable’ bacteria [18] and identifying novel targets by retro-biosynthetic analysis [19].

“The lack of chemical diversity in modern-day synthetic compound libraries has been a contentious issue for some time.”

The challenges of chemical diversity

If current technology limits our ability to further profit from Mother Nature, then the collective synthetic chemistry community provides an alternative source of molecular diversity. The pharmaceutical industry and commercial compound vendors have been dutifully amassing large screening collections full of novel chemotypes of broad utility in the drug discovery arena. Unfortunately, the vast bulk of these chemicals have been derived using high-throughput, but synthetically constrained combinatorial chemistry approaches. Technological advances in combinatorial chemistry have not been commensurate with pharmaceutical R&D productivity, and in the context of antibiotic dis-

covery, collections derived from combinatorial efforts have been demonstrated to be ineffectual [20,21].

The lack of chemical diversity in modern-day synthetic compound libraries has been a contentious issue for some time. Man-made compound libraries cover an infinitesimally small area of infinitely large chemical space, and choosing the best regions to explore remains a significant challenge [22]. Before the advent of high-throughput screening (HTS), legacy drug discovery programs adopted a low-throughput approach, which was not driven by metrics inherent in an HTS discovery model. Comparatively small numbers of compounds possessing greater relative complexity were often made using a wealth of chemical reactions that did not benefit from the breadth of commercially available reagents of the modern era. Contrary to this approach, the modern day practice of outsourcing synthetic chemistry combined with the push to produce analogs using predictable chemical methodology has stifled the production of highly diverse screening libraries. A recent comparison [23] of past (1984) and present (2014) chemical reactions favored in medicinal chemistry versus natural products total synthesis revealed a startling observation: in the context of the types of reactions used to make molecules, the landscape of contemporary medicinal chemistry is essentially the same as it was 30 years ago, with a heavy bias toward amide bond formation, Suzuki-Miyaura coupling and S_NAr reactions. The poor uptake of modern synthetic innovations in industrial medicinal chemistry has led to compound collections rich in nitrogen but deficient in both chiral centers and oxygen atoms, resulting in a preponderance of lipophilic, planar scaffolds often overpopulated with a limited number of molecular shapes [24]. Such trends are at odds with the more polar chemistry space occupied by antibiotics [25], molecules that typically possess dense functionality and skeletal diversity, suggesting a limited capacity of *de novo* designed compound collections to modulate diverse binding targets [26]. Indeed, the common practice of curating libraries for ‘rule of five’ [27] compliance often selects for rod-like shaped molecules that preferentially bind to pocket and internal binding sites [28]. Molecules with structural and physicochemical characteristics beyond the ‘rule of five’ preferentially adopt disk- and sphere-like shapes, which increases the possibility of binding to more topographically difficult sites comprised of open, flat and grooved surfaces [28].

Two recent retrospective analyses of the antibiotic discovery efforts at Glaxo-SmithKline (GSK) and AstraZeneca highlight the above points in the context of antibiotic lead finding. Tommasi *et al.* [20] described the outcome of the discovery efforts from AstraZen-

eca's target-based and phenotypic antibacterial screening efforts between 2001 and 2010. Their commercial focus during this time was to develop agents with broad-spectrum activity against both Gram-positive and Gram-negative organisms. Notably, little difficulty was encountered in identifying hits against most targets during their HTS biochemical screens. However, by their own admission, their hit-to-lead triage strategy to prioritize synthetically tractable candidates devoid of risky chemical features may have led to the premature elimination of viable leads. Biochemical potency was often inextricably linked to hydrophobicity, resulting in lead compounds that were approximately 3–4 log units (clogP) more hydrophobic than typical antibacterial agents. Such compounds could not be engineered to display whole cell activity in Gram-negative bacteria, emphasizing the disconnect between the HTS active starting point and the physicochemical space necessary for efficient cell permeation.

In 2007, the team at GSK reflected on their antibacterial program between 1995 and 2001 [21]. Adopting a strong genomics/target focused approach, GSK devoted significant resources to validate numerous biochemical targets. Upward of 500,000 compounds from the SmithKline Beecham compound collection were screened over 67 HTS runs using a target-based approach, and three HTS runs using whole-cell screens. Although this led to five lead candidates against five distinct targets, none could be progressed to developmental candidate stage. Compounds identified during the biochemical screens lacked whole-cell activity following lead optimization, whereas those identified during the phenotypic screens possessed only Gram-positive activity, and were comprised predominantly of lipophilic nonspecific membrane-active agents. The team concluded that a paucity of biologically relevant chemical diversity in their screening set was a major contributor to the poor outcome.

Chemical philanthropy: CO-ADD

To address the issue of limited access to chemical diversity for generation of novel antimicrobial drug leads, we launched the Community for Open Antimicrobial Drug Discovery (CO-ADD) in early 2015 [29–31]. CO-ADD operates as an open-access facility within the University of Queensland (UQ), led by an academic research team with antibiotic R&D expertise. With financial support from both UQ and the Wellcome Trust, CO-ADD has two interdependent aims – to search for antimicrobial activity among compounds sourced from chemists anywhere in the world, and to generate a dataset from which it might be possible to examine the influence of chemotype on antimicrobial activity, cell penetration and drug efflux. Such data, used in combination with

emerging methods to assay intracellular drug penetration [32], might assist in the formulation of empirical rules to guide antibiotic design.

So, how does CO-ADD propose to address the chemical diversity problem? We believe that the broader chemical community may hold at least part of the answer. Globally, chemists are creating thousands of molecules each day, often constrained only by their imaginations. Without the commercial pressures often associated with drug discovery programs, academic chemists have a virtually unlimited synthetic toolbox; molecules with varying degrees of molecular complexity are being made to test new methodologies, address hypotheses concerning disease targets or to reach a total synthesis end game. Such molecules, once tested or analyzed, usually end up being stashed away in vials alongside the intermediates used to produce them. As the years go by, their final resting place is usually on a shelf in some freezer, and through the ebb and flow of projects in the research group, it is not uncommon for such compounds to eventually be forgotten or discarded. Importantly, very few of these compounds will have ever been tested for antimicrobial activity, as many groups have never considered this possibility, while those who may look to repurpose their compounds in some capacity may not have the resources and/or necessary collaborations to explore this option. It is our contention that such compound collections, unconstrained by metrics that drive commercial drug discovery programs, may have high-quality antimicrobial hits residing amongst them. Indeed, approximately one third [29] of the approximately 100 million unique compounds deposited in the Chemical Abstracts Service registry reside within the physicochemical space of antibacterial compounds (MW <1200 Da and log P between -10 and 2) [25].

In light of this, we ask chemists engaged in synthetic campaigns to adopt the mindset of setting aside 1–2 mg of pure compound for submission to CO-ADD for free antimicrobial testing as part of their routine workflow. The compound must be chemically stable and soluble in either water or DMSO, and there is no restriction on the number of compounds one can submit. A single contribution from one individual may seem insignificant, but with the collective efforts of the global chemistry community, the bigger picture becomes apparent as we strive to build the world's first antimicrobial-focused open access database of unique molecules tested for antimicrobial activity under standardized conditions. The endeavor will generate a powerful knowledge repository of structure–activity and structure–toxicity data that will be freely available to the worldwide research community. Thus, chemists engaged in R&D not necessarily related to antibiotic discovery may be able to make a small but nonetheless significant contribution toward

the discovery of new antibiotic molecules.

Whilst global compound collections and open framework drug discovery initiatives are not new, intellectual property, conflict of interest and licensing policies often stifle collaborative innovation and frustrate early adopters. CO-ADD differentiates itself from existing initiatives in several important ways: its core focus is to find novel starting points for antimicrobial discovery; CO-ADD offers a truly open-access approach to compound screening – compounds are screened free-of-charge, are not preselected on the basis of ‘lead-like’ filtering criteria, and are accepted from anywhere in the world; there is no encumbrance on intellectual property; the provider of the compound retains all rights to the compound, assay results and IP; for the first time, the collective screening results for both active and inactive compounds will be made publicly available in a central repository to assist researchers in understanding what physicochemical properties are important for antimicrobial development (CO-ADD participants have 18 months to patent and develop their compound before they are asked to make structures and results available to the open-access database). We emphasize that curation of existing literature datasets to obtain reliable standardized data for comparison is highly challenging due to the multitude of bacterial strains and assay conditions used across different studies.

So how does it work? Compounds submitted to CO-ADD undergo a primary screen at a single concentration (32 µg/ml) against a select panel of key ESKAPE bacterial pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* [MRSA]), and the two fungal pathogens *Cryptococcus neoformans* and *Candida albicans*. Membrane-deficient and efflux pump impaired *E. coli* mutants are also screened to provide additional information pertaining to bacterial cell penetration and efflux. Notably, screening is conducted using well-defined Clinical and Laboratory Standards Institute (CLSI)-compliant protocols with standard ATCC reference strains to enable the direct comparison of thousands of screening results independent of compound source. Any actives identified from the primary screen are then funneled into a hit confirmation cascade to rule out nuisance compounds by way of dose response antimicrobial assays, QC analysis and cytotoxicity/critical micelle concentration/hemolysis assays. Results from the initial screening and hit confirmation steps are then disclosed to the submitter for their evaluation. Promising results at this stage can trigger the hit validation cascade if sufficient material is available, which will enable further testing against a broader panel of multidrug-resistant clinical isolates, including assessment in the presence/absence of serum and lung surfactant.

Finally, an initial investigation into drug-like properties, including microsomal and plasma stability, and drug–plasma protein binding will deliver a data package suitable to assess the suitability of a candidate for further chemistry optimization. To rule out singleton hits, promising compounds are resynthesized alongside several structural analogs.

Community response

Since its launch in February 2015, CO-ADD has devoted significant resources toward raising awareness to the plight of antibiotic resistance. The program is gaining traction and support through global participation at scientific conferences in Europe, Russia, Asia-Pacific and the USA, and CO-ADD team visits to chemistry departments at numerous academic institutions. Community engagement has been encouraging, with 104 groups from 34 countries participating in the scheme at the time of writing, each submitting anywhere from 10 to 150,000 compounds for screening. By reaching out to the chemical community through organizations such as the Royal Society of Chemistry, the American Chemical Society, the Royal Australian Chemical Institute, Gesellschaft Deutscher Chemiker and MedChemNet, we are looking to engage individuals, laboratories and institutions and build a network of collaborators that will collegially support the arduous process of antibiotic discovery.

It remains to be seen if the premise adopted by CO-ADD will aid in reinvigorating the antibiotic pipeline. With global strategies currently failing to provide us with a much needed arsenal of new antibiotic drug candidates, we hope that the open access approach offered by CO-ADD will encourage the chemical community to join us in a long-term internationally coordinated approach toward antibiotic discovery.

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