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Viewpoint

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Helping Chemists Discover New Antibiotics

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Supporting Information

ABSTRACT: The world is facing a crisis in treating infectious diseases, with a scarcity of new antibiotics in development to treat the growing threat of drug-resistant "superbugs". We need new strategies to reinvigorate the antibiotic pipeline. In this Viewpoint we discuss one such approach, encouraging the community of synthetic chemists to participate in testing chemical diversity from their laboratories for antimicrobial potential. CO-ADD, the Community for Open Antimicrobial Drug Discovery, offers free screening against five bacteria and two fungi with follow up hit confirmation and validation, all with no strings attached.

Antibiotics are precious resources that have been mined from nature with great effort, but they are misused and undervalued. Considered a miracle of modern medicine when discovered in the 1940s, antibiotics are now treated as an everyday commodity, with health maintenance organization (HMO) and government formulary committees balking at paying even a fraction of the yearly fees that highly profitable anticancer drugs can attract. This is despite the fact that antibiotics are some of the very few drugs actually able to cure a disease, often saving lives with a few weeks' treatment. Despite this enormous social benefit, they fail to attract a price premium anywhere near oncology drugs that sometimes extend life for only a few months. Our failure to appropriately value antibiotics, along with industry and government complacency, is catching up with us and we are at the beginning of an ominous rise in microorganisms resistant to all antibiotics. This is accompanied by a steep decline in the number of new antibiotics being approved and the number of major pharmaceutical companies involved in antimicrobial research.[1] Fortunately, there is increasing recognition of this dire global health threat, with two well-publicized and timely reports[2,3] advocating greater research and development efforts. In turn, this has triggered a significant new policy report[4] by the U.S. Obama administration that has the potential to change the future of antibiotic research in the United States. In recent years it appeared that even the major pharmaceutical companies might be coming to the party, with Roche buying up a number of innovative small biotech companies and Merck spending nearly $10 billion in 2015 for arguably the most successful antibiotic company of the past decade, Cubist. Unfortunately these hopes have been quickly dashed, with Merck subsequently laying off the entire 120-member discovery team at Cubist. Astra-Zeneca, another of the few large pharma companies still with skin in the game, also decided to spin out its anti-infectives group after failing to sell it to anyone at the right price. The damage to antibiotic research by dismantling the collective wisdom assembled within these research teams is immeasurable. Antibiotic drug development is a specific discipline, and we are losing the specialized knowledge needed for lead optimization, drug candidate selection, clinical trial design and dosing, and of course the invaluable learning that comes with failures.

We need fresh ideas and new tactics to reinvigorate the antibiotic pipeline. One approach is to return to how antibiotics were originally discovered, from natural products. A recent paper[5] describing the isolation of 'resistance-free' teixobactin from a soil sample cultured using a new technique received much media attention. While arguably overhyped (teixobactin is active only against Gram-positive bacteria, and other antibiotics that target nonprotein membrane components, such as the polymyxins, do in fact eventually lead to resistance in the laboratory and the clinic) the article did reveal an innovative method of expanding the pool of natural products that can be accessed. In contrast, companies such as MerLion Pharmaceuticals (with the assets of GSK's natural products division) found little success in applying more modern analytical techniques to traditional natural product sampling, predominantly rediscovering the same antibiotics again and again. Other companies and academic groups have taken different approaches to expanding the natural product repertoire, assessing extremophiles or marine organisms growing under unusual conditions (e.g., the Marine Bio-products Engineering Center, MarBEC) or utilizing genomic screening technologies to look for specific sequences in gene expression libraries from DNA extracted from environmental samples that may be manipulated to produce novel antimicrobials (e.g., most recently by Warp Drive Bio, previously by Diversa, now Verenium Corporation, and TerraGen Discovery Inc., taken over by Cubist in 2000). However, even here there is a paucity of compounds that have entered the clinical pipeline.

We believe an alternate yet complementary approach is possible. One reason that antibiotic research has been unsuccessful in recent years is because of the growing focus on druglike property rules to assemble screening libraries, such as the Lipinski "rule of five"[6] for oral bioavailability. This and similar leadlike rules focusing on physicochemical properties of a molecule essentially remove most potential antibiotics before

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they are even tested. The problem is exacerbated by screening out "reactive" and other undesirable moieties. Many approved antibiotics are chemically reactive and act as suicide inhibitors (Figure 1). GSK's HTS campaign described several years ago[7]

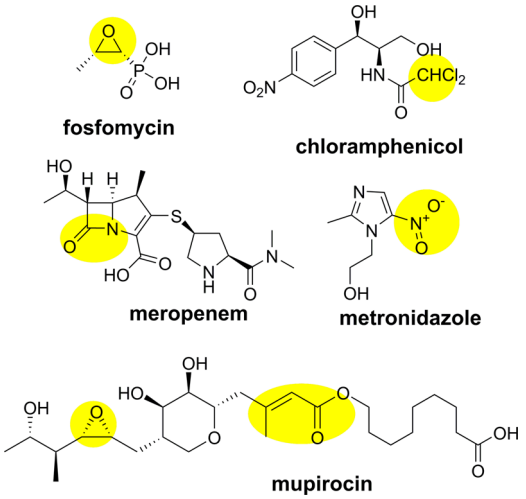

Figure 1. Antibiotics often possess reactive or undesirable functionalities that would eliminate them from further development in most drug-development programs.

attested to the limitations of such approaches, in particular, with respect to target-based screening. Antibiotics are not druglike; the majority of them fail leadlike filters designed to enable oral availability (Figure 2). Although antibiotic oral availability is commercially desirable, particularly for "switch" therapies or for Gram-positive infections where more treatment options are available, for highly drug-resistant life-threatening infections, intravenous treatment is accepted and effective.

So where do we find this additional unfiltered chemical diversity if not from natural products? There are currently 94 million compounds deposited in the CAS registry with 15 000 added each day; 80 million of these are organic compounds with no associated metal ion and a molecular weight of <1500 Da. Using an antibacterial-like filter[8] (log *P* between −10 and 2 and MW < 1200 Da) gives 29 million compounds with the theoretical potential for antibacterial activity, with more than half of these (15.5 million) from academic chemists. Every day, around the world, organic chemists are making thousands of new molecules with an incredible diversity of structures. Moreover, there are thousands of vials of compounds prepared over decades in every academic chemistry laboratory, encased in ice in freezers or collecting dust on shelves, the forgotten products of Ph.D. theses and eclectic research programs from years past. These compounds have been made for a range of reasons, from series of analogues for synthetic methodology development to intermediates and final products from total syntheses to bioactive compounds made for other disease areas. What they have in common is that their creators never considered testing them for antimicrobial activity or were not able to conveniently access such testing. For example, ChEMBL, a large public database of bioactive molecules, contains 1.3 million unique structures; however, only 14% are reported as being tested in any bacterial screening assay.

So how do we motivate the chemistry community to submit compounds for testing? National compound collections have been in existence in several countries for a number of years, but

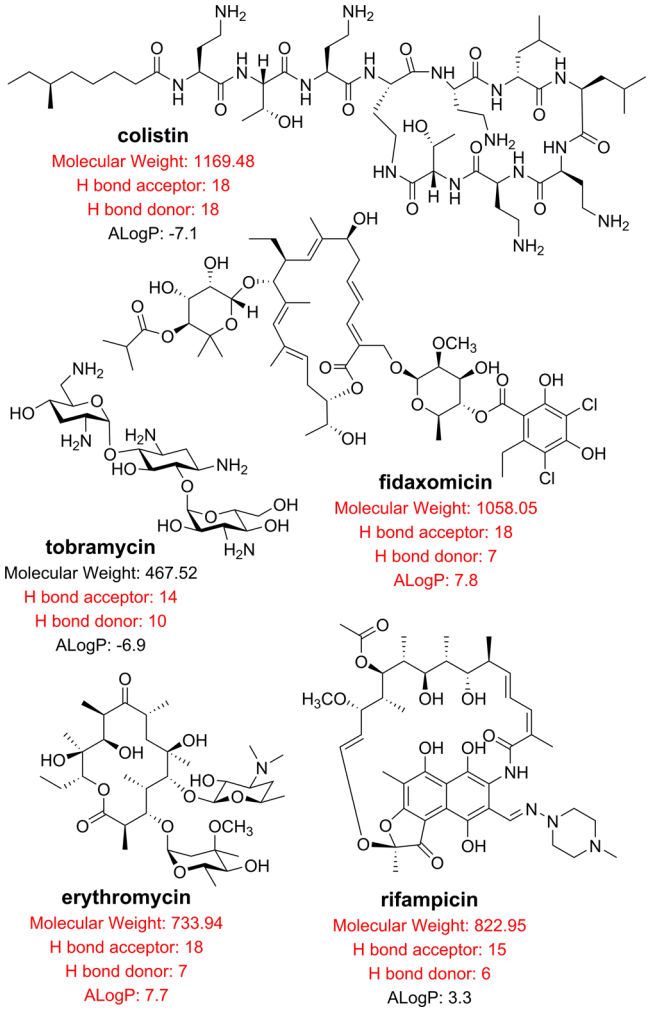

Figure 2. Antibiotics are not druglike and do not obey druglike rules for oral availability, e.g., the Lipinski Rule of Five parameters:[6] molecular weight ≤ 500, H-bond acceptor ≤ 5, H-bond donor ≤ 5, log *P* ≤ 5.

participation has generally been poor, presumably due to a lack of compelling feedback, or some nebulous screening results promised in the distant future. One answer is to lower the barrier to submission by providing an easily accessible and well-publicised screening service, at no cost, with no lengthy legal agreements, and ensure that the provider retains all rights to their compounds, with sufficient time to file patents, publish, obtain grant funding, or partner using the screening results. There is also a tangible appeal to the public good; a chemist may be able to make a small but very real contribution toward addressing the severe threat to human health from superbugs.

To enable all of this to happen, we have launched CO-ADD, the Community for Open Antimicrobial Drug Discovery (www.co-add.org). CO-ADD is supported by the Wellcome Trust, a global charitable foundation established in 1936 with legacies from pharmaceutical magnate Sir Henry Wellcome to fund research to improve human and animal health. Further fiscal and in-kind support comes from the University of Queensland, where CO-ADD is led by a group of passionate and dedicated academics with antibiotic R&D experience. The CO-ADD team noted that open collaboration drove the discovery of most antibiotics during the golden era of antibiotics. We now want to go "back to the future" to a more collaborative and collegial

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environment, unfettered by CDAs and MTAs and commercialization offices that can stifle open discussion, subjugate perspicacity, and thwart opportunities for serendipitous breakthroughs.

CO-ADD is asking for chemists to submit 1 to 2 mg of pure compound, which must be chemically stable and soluble in either water or DMSO. These are to be shipped as dry material or DMSO solutions in appropriate containers such as 1 to 2 mL Eppendorf tubes, although for larger collections that are already formatted, 96-/384-well plates with as little as 50 μL of 10 mM DMSO solution can be accepted. Compounds undergo a primary screen in duplicate at a single concentration (32 μg/mL) in 384-well format to test their killing ability against broth solutions of key ESKAPE bacterial pathogens *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (MRSA) as well as fungal pathogens *Cryptococcus neoformans* and *Candida albicans*.[9] We will also test against membrane-deficient and efflux pump-impaired *E. coli* mutants in order to further develop rules for antibiotic activity, penetration, and inactivation. Any active compounds then undergo hit confirmation profiling, including dose response antimicrobial assays to confirm their activity, LCMS analysis for identity and purity, and counter-screening for adverse effects using cytotoxicity, critical micelle concentration, and membrane depolarization assays. If still promising, the next step of the CO-ADD screening cascade is hit validation by testing the compound against a broader panel of microbes and in the presence of serum and lung surfactant. The hit validation will also include initial assessments of suitability to become drugs, testing hemolysis, microsomal and plasma stability, and protein binding. This last stage of the free screening will be done in collaboration with the submitting research group because additional material might be required, and if structural analogues are available, this will help to eliminate singleton hits and provide early structure−activity relationship data.

A key aspect of this initiative is the generation of a publically accessible database that will allow for antibiotic researchers to query what properties predispose antimicrobial activity. Initially, researchers are asked to provide only compound molecular weight and if possible a fingerprint (which does not reveal structure but confirms that the structure is unique). After a grace period of 18 months subsequent to receiving assay results (sufficient time to publish or patent), they are asked to provide structures for all compounds tested, whether active or not. This will provide an invaluable resource for the global research community because all compounds will have been tested under standardized conditions against seven reference pathogens, allowing for comparisons between compound properties/activities. Gathering and collating such data is currently not possible by mining literature data sets because compounds are tested against different strains using a range of different testing techniques.

CO-ADD is fundamentally asking two questions: can the community work together to address a global threat, and are there antimicrobial compounds within our collectively diverse chemistry? It remains to be seen whether this approach will translate into the discovery of novel classes of antibiotics, but given where we are today, any new strategic initiative to encourage a community response to this crisis should be encouraged, and we hope supported.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.5b00044.

Chemical structures of colistin, tobramycin, fidaxomicin, erythromycin, and rifampicin (CDX)

Chemical structures of fosfomycin, chloramphenicol, meropenem, metronidazole, and mupirocin (CDX)

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Notes

The authors declare no competing financial interest.

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