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PERSPECTIVE

Antibacterial medicinal chemistry – what can we design for?

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ABSTRACT

Introduction: The need for new antibacterial agents continues to grow, but success in development of antibiotics in recent years has been limited. To improve the chances that new compounds will progress into clinical trials and beyond, it is vital that we consider as early as possible in the process the various challenges that discoverers and developers of new antibiotics will face.

Areas covered: The author looks at the factors that affect medicinal chemistry aimed at providing successful antibacterial agents. Target selection, target inhibition, accumulation in bacteria, and pharmacokinetics are all discussed, with a particular emphasis on how our current understanding should impact design and optimization strategies.

Expert opinion: From the perspective of a medicinal chemist, the primary question when considering the various aspects of antibacterial drug discovery should be 'what can I design for?' It is important to be aware of the limitations of our understanding, and also the constraints and challenges that arise due to the diversity of the bacteria we try to address. Progress is needed to simplify approval pathways and to increase return on investment for the next generations of clinically useful agents to succeed.

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Antibiotics; design strategy; medicinal chemistry

1. Introduction

A reminder that there is an urgent need for safe effective medicines to tackle the increasing problem of antibiotic resistance seems unnecessary. Although increasing resistance has been accompanied by increasing publicity surrounding the problem, the broken business model for rewarding discoverers, inventors, and developers of new treatments for bacterial infections has resulted in a continuing decline in industrial research in this area [1]. With this decrease in investment and in the participation of pharmaceutical companies in research into novel antibiotics comes a potential loss of vital expertise [2]. Many former pharma employees now staff the Biotech companies making up the bulk of the industrial research so desperately needed [3], and there are efforts to retain a community of expertise through nonprofit organizations, notably through the Global Antibiotic Research and Development Partnership (GARDP) [4]. Much early research is being conducted in Universities across the world, and while excellent collaborations and consortia help to broaden the capabilities of these groups there are few organizations left where novel treatments can be discovered and developed under one roof [5,6]. With these factors in mind, it is important that we continue to share learnings about antibiotic design.

The holy grail of antibiotic design is a small molecule that can treat a wide range of infections caused by both Gram-positive and Gram-negative pathogens, reaching infection sites throughout the body. The molecule would be cheap, safe, active against known resistant (multi-drug resistant, MDR and extensively drug-resistant, XDR) pathogens and not prone to rapid resistance development. While this is what we may aim for, the reality and experience of antibiotic research over many years has been that such 'ideal' molecules are unattainable [7,8]. There are many reasons for the failure to deliver a continuous pipeline of such drugs, but foremost among them are the scientific challenges that must be overcome.

It is vital when considering the design of new antibiotics that we try to take into account the full range of problems that will assail us during this process. There have been several excellent reviews addressing antibacterial discovery and development [9–11]. The purpose of this piece is to focus on the hit-to-lead and lead optimization phases (figure 1), where molecular design is most important, and to discuss how the challenges particular to antibacterial drug discovery should affect our design strategies.

We start with an overview of the challenges that a small molecule faces when its goal is to enable clearance of bacterial infections. The molecule must first reach the bacteria, then penetrate the bacterial cell envelope, before finally inhibiting its target (Figure 2). For each of these challenges, we consider how medicinal chemistry can approach the problem, what data are available to enable rational design, and how the many challenges require a holistic approach to enable development of successful antibacterial small molecules.

2. Firstly, how will my molecule kill the bacteria?

Starting points for medicinal chemistry can come from a number of different approaches [12]. Target-based screening is performed on libraries of molecules, either through

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Article highlights

- Antibacterial drug discovery is under-valued by society as a whole, and consequently is under-resourced in the pharmaceutical industry.
- Expertise is being lost, and it is vital that we continue to share knowledge and experience to maintain a functioning research base.
- Design and optimization of new small-molecule antibiotics continue to pose a very significant challenge in drug discovery.
- Killing bacteria is easy, but doing so safely in humans while minimizing propensity for resistance development requires careful target selection or a good deal of luck.
- We are beginning to understand the factors that control accumulation of molecules within bacteria, but these will likely be species-specific and so generalized rules may not be achievable.
- The difficulty in designing for a PK profile to match particular infections may mean that optimizing for unbound plasma concentration is the most sensible approach during lead optimization in the majority of cases.

This box summarizes key points contained in the article.

biochemical assays or in silico in virtual screens. These libraries often comprise collections of synthetic compounds, but in antibacterial research natural products have also proved an important source of chemical matter [13,14]. Phenotypic screening, where whole bacteria are treated with libraries of molecules, enables us to screen against all targets simultaneously in their physiological context in the pathogen of interest, and can ensure hits demonstrate antibacterial activity [15]. Finally, target agnostic machine learning approaches have recently identified molecules with antibacterial activity [16]. While antibacterial drug discovery can be pursued without knowledge of the mechanism of action, identifying the cellular target of a new molecule can give an early indication of likely toxicity or propensity for resistance [17].

The question of how a molecule will kill bacteria remains an important one, and a huge amount of work over many decades has identified molecular processes that are vital to the healthy growth of bacteria. More recently computational approaches have been used to identify new potential targets for antibiotics [18]. The cell wall, cell membranes, metabolism, protein synthesis, and processes involved in cell growth and division have all been successfully targeted by small molecule drugs (Figure 3). From a medicinal chemist's perspective data from assays testing activity against isolated proteins, binding assays, and structural data (through X-ray crystallography or other techniques) enables optimization of target potency. This can either be through iterative structure–activity relationship (SAR) building through (to some extent) unbiased synthesis and testing of analogues of active hit molecules, or through 'rational' design whereby structural information about how molecules bind to their targets is used to increase potency through optimization of binding interactions.

The design of the molecule will affect its binding to the target, but other factors also affect whether this will result in death or inhibition of growth of the bacterial cell. For reversible inhibitors, the binding event is an equilibrium and the kinetics of binding can determine whether the binding interaction is effective in inhibiting a vital cellular process and thereby resulting in the desired effect on the cell (usually cell death). There are targets for which minimal inhibition is sufficient to produce rapid effects on cellular processes (e.g., gyrases [19]) whereas for other targets 100% irreversible occupancy of the binding site may be required. The need for complete irreversible inhibition is suggested for MurB-F, possibly because partial inhibition could lead to upregulation of the pathway [10]. While it is generally preferable to identify potent molecules with slow dissociation from the target, for example, LpxC inhibitors with slow-binding kinetics have demonstrated extended cellular and in vivo responses [20], designing for slow off-rate is not straightforward. As off-rate depends on the free energy of both the drug-target complex and that of the transition state between the dissociated and bound states, a good understanding of the interactions in both transition state and complex would be necessary, but maybe not sufficient to design for control of off-rate [21].

It is also important to consider whether a target is essential for bacterial growth. This is usually determined by knocking out specific genes, if the organism can survive without the gene-product then the target is non-essential and therefore not a viable target for antibacterial drug discovery. However, it is possible for growth conditions to affect whether a target is essential [22], meaning that what might be essential in vitro may not be essential in vivo, and vice-versa. Apparently, attractive targets can also be essential in some species, but non-essential in others, for example WalK, which is essential in *S. aureus* and *B. subtilis* [23,24], but not *S. pyogenes* [25], and FabI, which is essential in many species such as *E. coli* and *S. aureus* [26,27], but not in *S. agalacticae* [28]. While homologs of the target may vary sufficiently across species to limit the spectrum achievable, this diversity is compounded when there is significant variation in the target among strains of the same species, as resistant strains could constitute a significant proportion of the bacterial population. It may therefore be important to get an early indication of MIC_{90} (the concentration required to inhibit the growth of 90% of strains in a representative panel, usually a set of clinical isolates).

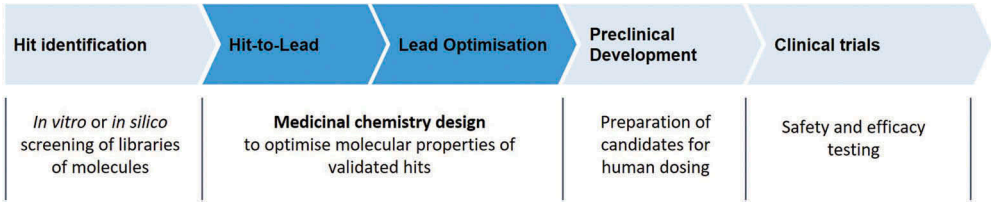


Figure 1. Schematic representation of the drug discovery process. This perspective focuses on hit-to-lead and lead optimization phases.

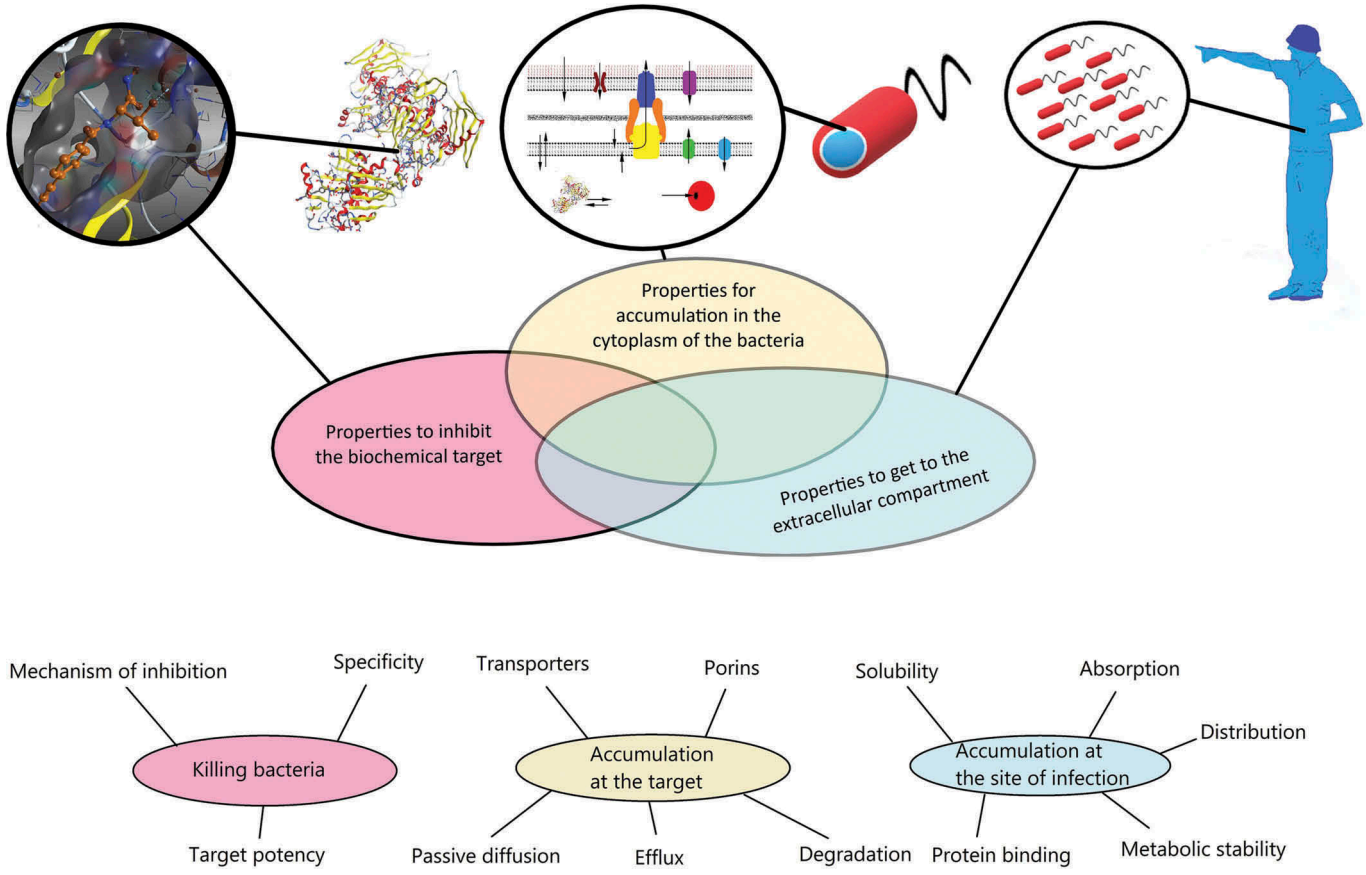


Figure 2. A molecule must first reach bacteria, then penetrate to the site of the target before interacting with the target to exert a biological effect. Each aspect has implications for medicinal chemistry design.

Finally, while considering how to kill bacteria it is important to avoid killing the host (the patient) and so identifying targets without close human homologs, or having a clear strategy for selectivity is vital. In a perspective discussing how to choose appropriate antibacterial targets Silver concluded that, along with essentiality, selectivity, and safety, development criteria should emphasize low resistance-potential and accessibility to inhibitors [17]. For a further exploration of the importance of resistance, the reader is directed to the review by Silver. It is to accessibility that we now turn.

3. How will my molecule access the target?

In this question, we come to probably the greatest hurdle to the development of new antibacterial agents. In contrast to mammalian cells, bacteria, and in particular the troublesome Gram-negative bacteria regarded as the greatest risks to

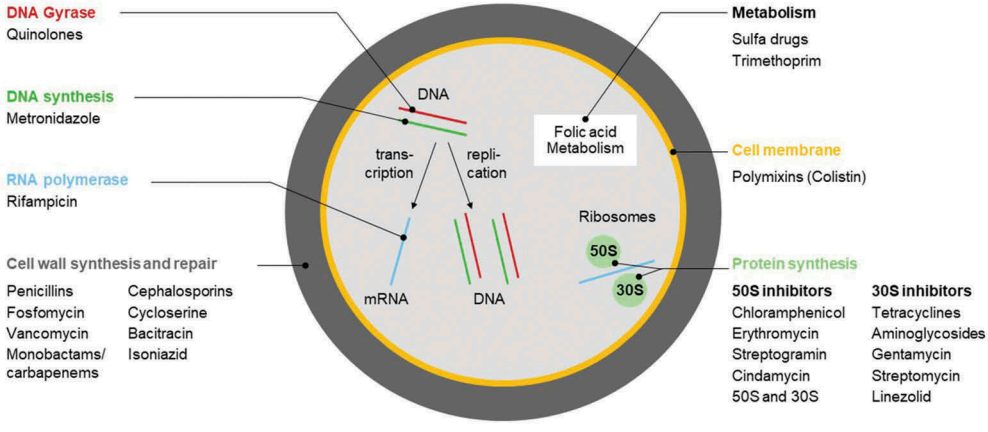


Figure 3. The location of the targets of many known antibiotics in a Gram-positive bacterial cell.

humans in the ongoing fight against antibiotic resistance, have extra layers of defense against harmful small molecules. Along with the cytoplasmic membrane, which provides some barrier to polar molecules accessing targets such as those involved in metabolism, protein synthesis, and DNA synthesis, Gram-negative bacteria also have an asymmetric outer membrane (OM) capable of preventing influx of unwanted lipophilic molecules. In addition to the cell membranes both Gram-positive and Gram-negative bacteria have a cell wall composed of a peptidoglycan mesh. This cell wall provides structural integrity, allowing bacteria to operate at higher osmotic pressure than their surroundings, but does not pose a significant barrier to the entry of small molecules. In order to acquire vital nutrients bacteria have developed mechanisms to enable small molecules to enter, while also having machinery to remove unwanted or toxic materials. This leads to a complex barrier with interplay between membrane structure, porins, uptake transporters, efflux transporters, and metabolizing/degrading enzymes affecting the accumulation of small molecules at the various sites in the Gram-negative bacterial cell (Figure 4). Overcoming this multifaceted barrier to enable the accumulation of an antibiotic at sufficient concentration to kill the bacteria is the defining challenge of small molecule antibacterial drug discovery. For although several successfully exploited targets lie outside the inner membrane (IM), and so are readily accessible in Gram-positive species (see Figure 3), even these targets are protected in Gram-negatives. For cytoplasmic targets, which represent a significant number of attractive unexploited targets, the full spectrum of defenses must be overcome.

3.1. Traversing the outer and inner membranes

The outer portion of the OM of Gram-negative bacteria is formed of lipopolysaccharide (LPS), with an anchor, Lipid A, forming a monolayer that interacts with an internal layer of phospholipid [29,30]. The layer of LipidA is supplemented with further sugars in the core and O-antigen repeat regions, providing a highly polar outer shell through which lipophilic molecules cannot easily pass. Due to its important role in the structural integrity of Gram-negative bacteria, LPS has been targeted by disrupters such as polymyxins, with colistin being the most prominent example used in clinical practice [31]. However, general membrane disruption properties result in toxicity to mammalian cells. Recent advances in specificity have enabled the development of novel agents such as

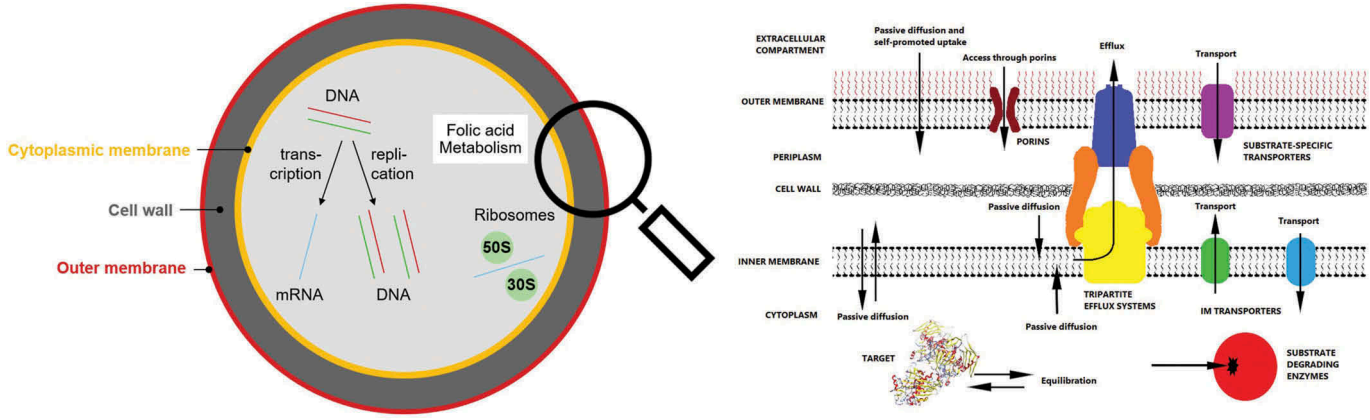
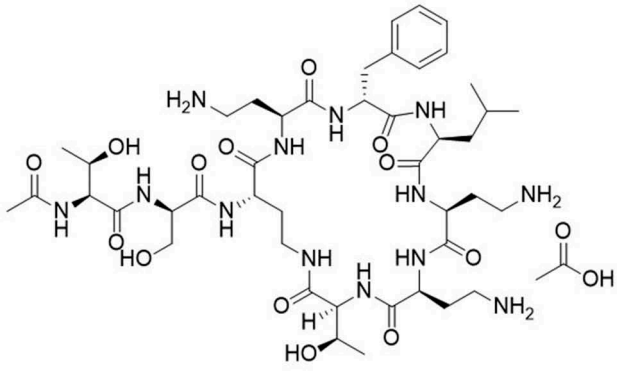
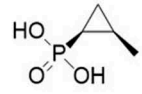


Figure 4. Barriers to accessing targets of small molecule antibiotics in Gram-negative bacteria.



SPR741



Fosfomycin

Figure 5. Structures of SPR741 and fosfomycin.

SPR741 (Figure 5), which cause damage to the OM but not the cytoplasmic membrane [32]. Such agents can act as potentiators of other antibiotics by effectively weakening this outer barrier [33,34]. In general OM disrupters are lipopeptides displaying multiple positive charges, which interact with anions in the LPS. This somewhat limits the chemical space available for agents that act to disrupt the OM, meaning that compounds addressing enzyme targets are unlikely to gain access to the periplasm or cytoplasm in this way. However, such molecules can be potentiated through co-treatment with membrane-disrupters [34].

It is thought that the mode of entry for many small molecule drugs is through OM porins, small water-filled polar channels that allow entry of specific nutrients including a variety of small, highly polar sugars [30,35]. These channels have been extensively studied, and guidelines for entry for a number of these have been established [36–38]. However, the size and expression levels of porins differ between species and even between individual strains of the same species of bacteria, hampering the generality of any rules designed to identify chemical space where permeation through porins is favored.

Alternative modes of uptake are possible. We have discussed above the OM disruption caused by polymyxins, whereby cationic amphiphilic molecules are able to disrupt LPS. Cationic hydrophilic molecules such as aminoglycosides are also able to penetrate the OM through 'self-promoted uptake.' Mg^{2+} ions that stabilize the OM through bridging adjacent LPS chains are disrupted, weakening the OM and allowing the molecules to pass through [39]. A further example of self-promoted uptake involves bacteriocins, antibacterial peptides produced by bacteria to suppress rival species. Bacteriocins hijack nutrient transporters by binding to OM receptors and triggering assembly of translocons, which enable the uptake of large folded proteins into the cell [40]. Some of the nutrients mimicked by the binding regions of bacteriocins are of small-molecule size, such as siderophores and vitamins, suggesting a possible role for transporters in the uptake of other small molecules. Both siderophores [41] and vitamins [42] have been employed in conjugates to facilitate transport of non-penetrant molecules into bacteria. There are many OM receptors targeted by bacteriocins, and binding to these can trigger translocation through specific proteins, or allow concentration of the agents at the OM. Whereas bacteriocins require further transporters to cross the IM small molecules will find this barrier less troublesome, but while passive diffusion may provide a component of passage, IM proteins regulate the majority of traffic both into and out of the cytoplasm [43]. Comparison of accumulation measurements between wild-type *E. coli* and *E. coli* protoplasts (lacking the OM and cell wall) suggests that for small molecules the OM is the main barrier to entry [44].

3.2. Staying inside the cell

Along with orthogonal barriers to entry, Gram-negative bacteria employ a range of efflux pumps to remove unwanted metabolites and to protect against substances in the environment such as bile salts [45]. These efflux pumps can be substrate-specific, or promiscuous and able to transport a wide variety of diverse compounds [46]. IM pumps can export compounds from the cytoplasm into the periplasm, and can act in concert with outer membrane channels to remove these compounds from the cell. Multicomponent systems such as AcrAB-TolC in *E. coli* and MexAB-OprM in *P. aeruginosa* capture molecules from the IM or the periplasm and export them directly to the extracellular medium [47]. A huge amount of effort has gone into understanding the mechanisms and specificities of these pump systems, and while this research has increased our understanding of the impact of efflux on both susceptibility and resistance to antibiotics, at the time of writing we have no general tools to aid design with respect to avoiding efflux. As with other areas of antibacterial drug discovery, the sheer variety and promiscuity of efflux systems between different strains and species of bacteria may make generally applicable design guidelines for avoidance of efflux unrealistic.

As well as efflux from the cell, antibiotics need to survive bacterial metabolism to accumulate at their site of action in sufficient concentration. When bacteria colonize a host they thrive in a niche that provides the necessary nutrients and energy sources [48]. We will see that the location of this niche can have important impacts on the profile and therefore the design of antibiotics needed to treat infection. To utilize nutrients and energy sources bacteria employ a wide range of metabolizing enzymes, and through perhaps billions of years [49] bacteria have evolved a further arsenal of complementary enzymes to degrade toxic molecules. The most well-studied example of these are the beta-lactamases, which effectively enable bacteria to resist some of the most widely used antibiotics such as penicillin [50].

Of the factors described above, guidelines have been proposed for the design of molecules to permeate porins [36], and for oxazolidinones to evade efflux in *E. coli* [51]. Many of the rational approaches to improve accumulation of antibiotics have involved combination with inhibitors of efflux pumps or metabolizing enzymes [52]. While efflux pump inhibitors have yet to make an impact in the clinic, beta-lactamase inhibitors have been one of the major success stories of recent antibacterial research [53,54].

Rather than try to provide guidelines for each of the specific aspects contributing to the accumulation of antibiotics in the cytoplasm of Gram-negative bacteria, Hergenrother's group have worked on a more general approach. In a seminal paper in 2017, the group described the development of rules for predicting accumulation in *E. coli* [44], and showed how these rules could be applied to broaden the spectrum of the Gram-positive only agent based on deoxynybomycin, 6DNM (Compound 1). By measuring accumulation of a diverse set of molecules using an adapted MS-based assay, the group were able to build a dataset that was interrogated to uncover the parameters most predictive for accumulation in *E. coli*. They found that: 'compounds are most likely to accumulate if they contain a non-sterically encumbered amine, some non-polar functionality, they are rigid and have low globularity.' This paper brought the consideration of globularity to the wider antibacterial medicinal chemistry community, and Hergenrother described it as 'a term used to provide information

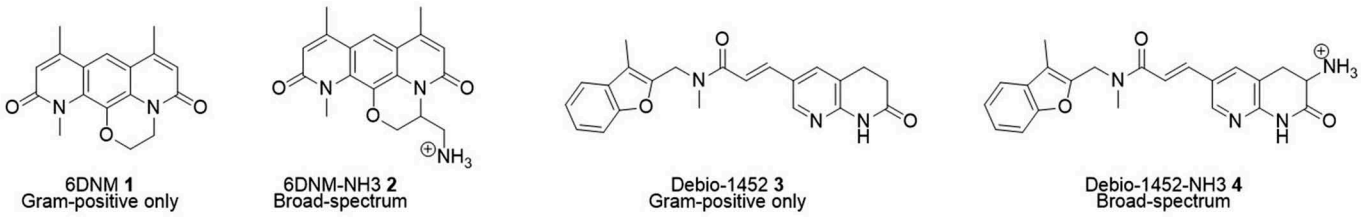


Figure 6. Broadening the spectrum of Gram-positive only agents using eNTRyway rules.

on the three-dimensionality of compounds, where a completely flat compound (for example, benzene) has a globularity of 0 and a spherical compound (for example, adamantane) has a globularity of 1.' Addition of a primary amine to 6DNM gave 6DNM-NH3 (Compound 2), which gave activity against both Gram-positive and Gram-negative organisms. In a follow-on publication, a web-based application eNTRyway was shared [55]. The system uses computational chemistry calculations to predict accumulation in *E. coli* using the refined rules that compounds should have an ionizable nitrogen, low three-dimensionality (globularity) and low numbers of rotatable bonds. Following on from the modification of deoxynybomycin, Hergenrother's team showed how Debio-1452 (Compound 3), another Gram-positive only antibiotic, was altered to give a broad-spectrum compound Debio-1452-NH3 (Compound 4). For both deoxynybomycin and Debio-1452, the key modification was the addition of an unencumbered primary amine (Figure 6). While inclusion of an ionizable nitrogen was successful in these cases, the starting molecules already met two of the three rules and so it should not be concluded that accumulation in *E. coli* can be achieved for all molecules in this way.

4. Getting to the extracellular compartment

While the difficulties outlined above provide a very significant challenge, achieving sufficient potency at a biochemical target and endowing the molecule with the properties that allow it to pass through the cell envelope and accumulate at the subcellular location of the target may not be sufficient to enable the molecule to progress as a drug candidate. To exert an effect in vivo the compound must first reach the extracellular aqueous compartment surrounding the bacterial cell in sufficient unbound concentration to provide a concentration gradient leading to accumulation at the site of the biochemical target.

Using tissue distribution data from pharmacokinetic (PK) experiments, we can optimize for accumulation in various settings, but whole-tissue concentrations are unlikely to be a good indicator of activity in a particular setting [56]. The relevant parameter is free drug concentration in the aqueous medium surrounding the bacterial cells, usually the interstitial fluid in the tissue. In addition, bacterial infections sometimes cause non-homogeneity in tissues, e.g., non-replicating *Mycobacterium tuberculosis* (TB) residing in sanctuary sites. Measuring total drug concentrations in these sites could give a misleading impression of their likely effectiveness, even when coupled with apparently convincing efficacy data. For example when cultured under conditions that mimic their nonreplicating caseum environment, TB bacteria are 10-fold more resistant to clinically used drugs compared with testing in replicating conditions [57]. This casts some doubt on the idea that effectively optimizing for tissue binding (to increase total drug concentration at an infection site) will be beneficial [58].

An exception may be for urinary tract infections (UTI), where the physicochemical properties needed to enhance probability of renal clearance of molecules have been established [59–61]. To illustrate this we can consider fosfomycin, an agent used to treat lower UTI. Fosfomycin (Figure 5) is a small hydrophilic molecule with minimal plasma protein binding (PPB) and is almost exclusively renally cleared [62]. However, critically ill patients may exhibit pathophysiological changes that can affect pharmacokinetic/pharmacodynamic (PK/PD) properties of drugs and in particular kidney function can be strongly affected, complicating the selection of dosing regimens for hydrophilic drugs [63].

So what is the best PK profile to optimize for when designing an antibacterial agent? As noted above, antibiotic distribution depends on many factors including barriers specific to infection sites and changes caused by the disease state [64]. In a clinical setting microdialysis data on tissue penetration, measuring unbound concentrations in interstitial fluid (ISF), can give valuable information as to the likely effectiveness of a drug [65,66]. Using microdialysis Marchand et al. have shown that the unbound concentration of imipenem in thigh interstitial fluid is equal to the unbound concentration in plasma for healthy rats [67], confirming basic PK principles and also findings for several other antibiotics [68]. There can be delays in equilibration to all aqueous compartments, particularly in critically ill patients, meaning ISF concentration vs time curves are shifted when compared with plasma concentration vs time. The shift in the curves means that although full area under the concentration–time curve profiles (AUCs) should be the same for ISF and plasma, using partial curves can give rise to misleading conclusions about the extent of distribution into tissues [69]. Due to species differences and the unpredictable effects of disease state, linking tissue distribution to predicted efficacy is unwise in early preclinical research. While unbound drug concentration in plasma is only sometimes a good surrogate for unbound concentration at the site of action [64], the difficulty in designing for a PK profile to match particular infections may mean that optimizing for unbound plasma concentration is the most sensible approach during lead optimization in the majority of cases.

5. Conclusion

Like all areas of drug discovery, designing new compounds to treat bacterial infections requires the medicinal chemist to balance many (often competing) parameters. None of target potency, cellular accumulation at the site of the target, or ideal PK and safety are sufficient in isolation to deliver useful medicines. New potential candidates must be endowed with properties to occupy the zone where the chemical space for these parameters overlaps. With so many factors to address it is important that medicinal chemists work closely with computational chemistry, biochemists, microbiologists, in vivo, and DMPK scientists. A multi-layered problem requires the efforts of a multidisciplinary team to have the best possible chance of success.

6. Expert opinion

There are many factors that hamper our ability to supply novel antibiotics that will enable us to tackle the increasing levels of resistance to current treatments being found in the clinic. Huge efforts from many large pharmaceutical companies have not delivered the numbers of approved drugs that might have been expected, given the resources and expertise committed. A lack of diagnostic technologies enabling the rapid identification and antibiotic susceptibility profiling of bacteria causing infections mean that broad-spectrum agents are desirable as a first–line treatment. The effectiveness of current options for most infections means that superiority trials are usually not viable. Where current options fail, such as in resistant infections, recruiting patients in sufficient numbers to generate convincing efficacy data is difficult.

Current approval pathways push antibacterial research toward striving for an ideal antibiotic, an agent that is broad-spectrum, with a novel target not prone to fast resistance development, suitable for a switch from IV infusion to once-daily oral dosing, and cheap to produce. In contrast, the realities of infectious diseases, with infections in different settings that may require specific compound properties or dosing strategies, along with the scientific challenges of producing compounds capable of treating a broad spectrum of bacteria, push us toward narrow-spectrum or even single-pathogen agents.

From the perspective of a medicinal chemist, the primary question when considering the various aspects of antibacterial drug discovery should be 'what *can* I design for?' The answer usually depends on the amount of data available. For target potency, structural data can give a head start, but is not vital as SAR can be determined through rounds of hypothesis-driven design, synthesis, and testing. Cellular potency or minimum inhibitory concentration (MIC) depends on the combination of target potency and factors affecting the accumulation of compounds at the site of the target. Understanding mechanism of action is important, as without a link between target potency and antibacterial activity structure-based optimization of target potency would be futile. A good understanding of mechanism of action also helps to avoid antibacterial activity arising as a consequence of nonspecific toxicity. One aspect of design not discussed above is the need to avoid known toxicophores, but this consideration should not be neglected in antibacterial programs. The many and varied factors affecting accumulation mean that designing for any one aspect alone may not be advisable. However, our increasing understanding of the molecular determinants of permeation, degradation, and efflux mean that probabilistic loose guidelines can start to be developed. It seems likely that these will be largely pathogen-specific, but as more species are studied we may see guidelines emerge that identify high probability chemical space covering an expanding spectrum of bacteria. Finally, it is important to consider PK, and while trying to design for disease-specific PK profiles may seem attractive, in hit-to-lead and lead-optimization phases, optimizing free plasma concentration seems the most sensible approach.

Like all drug discovery, antibacterial research involves trying to balance many different and sometimes seemingly opposing pressures on design strategy. Success will come when we can find pathways through both the scientific and nonscientific mazes that currently hamper progress. We hope that work under way to guide us through the scientific aspects, to simplify approval pathways and to increase return on investment will deliver the necessary advances before it is too late.

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Reviewer disclosures

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