Towards the sustainable discovery and development of new antibiotics

ROADMAP** article for **Nat. Rev. Chem.

Towards the sustainable discovery and development of new antibiotics

~~Future Antimicrobial Therapy in the Spotlight: Concepts for Sustainable Drug Discovery and Development~~

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Abstract

The ever-increasing demand for novel therapeutics to treat life-threatening infections caused by the global spread of multidrug-resistant bacterial pathogens is in stark contrast to the largely insufficient investments in future antimicrobials, especially in the fields of synthetic and natural product-based small molecules. New agents displaying innovative chemistry and modes of action are desperately needed worldwide to tackle the public health menace imposed by antimicrobial resistance (AMR). Our consortium presents a strategic blueprint to substantially improve the current situation of antibiotic drug discovery and early development. We propose both short-term and long-term solutions to overcome the most urgent limitations in the various sectors of research and funding, aiming to bridge the gap between academic, industrial and political stakeholders, and to join interdisciplinary expertise for an efficient fueling of the translational pipeline in the interest of future generations.

Introduction

The present article is

aimed as a general roadmap with the central aim to promote and accelerate translational science in the early stages of novel antibiotic discovery towards lead candidate development. Over- and mis-use of antibiotics in healthcare and agriculture, together with inappropriate waste management and environmental transmission, have substantially increased antimicrobial resistance (AMR)[1–5] and associated bacterial persistence[6,7]. This is of major public concern, since most areas of modern medicine are not conceivable without effective antimicrobial treatment[8]. The estimated number of people dying from drug-resistant infections worldwide due to bacterial pathogens, HIV and malaria is at least 700,000 per year, which will potentially rise to 10 million by 2050 if AMR is not controlled[9,10].

The anticipated death toll caused by drug-resistant infections over the next years and decades may be compared with the global fatality rate of the current SARS-CoV-2 (COVID-19) pandemic (https://coronavirus.jhu.edu/), which has already led to multi-billion dollar investments for vaccines, repurposing existing drugs and development of antivirals. Another concerning aspect of the COVID-19 pandemic is the observed high number of secondary infections, often with multidrug-resistant (MDR) bacteria, occurring especially in hospitalized patients and those with compromised immune system[11,12]. Associated with this problem is the massive use of antibiotics as a COVID-19 (co)-treatment worldwide[13–24], which is predicted to add to the ongoing emergence of AMR[25–29]. This multiplying effect of COVID-19 on the spread of bacterial resistance will most likely have further negative clinical, economic, and societal consequences in the near future[30,31].

Unfortunately, the dramatic worldwide rise of bacterial pathogens resistant to agents [32] cannot be counteracted by the current low pace of developing novel therapeutics with new mode(s) of action [MoA(s)]. While there are nearly 4,000 immuno-oncology agents in development[33], only about 30 - 40 new antibacterial compounds are currently in clinical trial phases, and notably those candidates targeting WHO priority pathogens are derivatives of existing classes[34,35]. Indeed, less than 25% of current drugs in the clinical development pipeline represent a novel class or act through a novel mechanism, and none of these are potentially active against Gram-negative ESKAPE or WHO critical threat pathogens[34,36]. In fact, only a small fraction of the antibiotics approved over the past forty years represents new compound classes, while the majority were derived from already known chemical structures, the “latest” new class of antibiotic was discovered during the 1980s[37].

Thus, strategic investment in new therapeutic options to fight AMR is required to urgently address unmet patient need and, additionally, to counterbalance the exponentially increasing

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financial burden on global health systems[38]. Consequently, the research field should aim to leverage hit identification and hit-to-lead optimization programs to ensure a sustainable flow of new antibacterial drug candidates into the development pipeline. For this purpose, the initial stages of drug discovery and development need to be strengthened, since they are essential to identify and validate novel therapeutic candidates effective to fight antibacterial resistance. However, for many years, such early-stage projects have been mainly conducted by academia and are generally underfunded, while increased allocation of funding into early- and mid-stage research and development (R&D) has been recommended to make the pipeline more robust[39–42]. Our network has identified major funding gaps especially for the academic sector as well as for small and medium-sized enterprises (SMEs), which are mainly associated with the early hit discovery and hit-to-lead phases as well as with late lead optimization prior to preclinical candidate nomination (FIG. 1). Large pharmaceutical companies across the globe are extremely hesitant to fund early antibiotic R&D, and particularly new classes of compounds, since the return on investment (ROI) in this area is generally low or even negative. Further, the costs of developing entirely new scaffolds are much higher than for derivatives of established compound classes, while the attrition rate in antibacterial drug discovery has been particularly high in the past decades, reflected by the fact that no new class of Gram-negative antibiotics has been launched for more than 50 years[43,44]. This leaves the field of innovation in the commercial sector basically to SMEs, which have to deal with high attrition mainly in the early phases of discovery and optimization[39,43,45–48], and the associated huge capital risks[49,50].

Generally, new economic models particularly designed for this sector need to be applied to make future advancement[51–54]. A recent initiative that supports SMEs in the late-stage development of new antibiotics is the AMR Action Fund, which was launched by more than 20 leading biopharmaceutical companies to push mainly phase II and III trials of advanced candidates[55], unfortunately without funding the early research stages. In addition, several countries are implementing new pull incentive programs with different priorities. While the Swedish model aims at securing sustained access to relevant antibiotics that have already been approved[56], plans in the UK[57,58] as well as in the US (*e.g.* PASTEUR[59] and DISARM[60] acts) strive to stimulate the development of new antibacterial products by using subscription or de-linkage models[51]. Such initiatives present a promising sign by introducing much needed market entry rewards; however, they might fall short on a global scale if they do not include “critical mass” of the world's largest economies.

Innovation in the early stages of antibiotic drug discovery can also be driven by the academic sector. However, from the academic perspective, partnering with external funders such as the pharmaceutical industry is in many cases only realistic after the nomination of extensively

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validated preclinical candidates, and often even requires phase I clinical data. Typically, this cannot be achieved by research-driven funding and infrastructure alone. Several global health organizations and public-private partnerships (PPPs) including GARDP, CARB-X, IMI and others started to support, at least partially, the mid-to-late lead optimization through to clinical proof of concept[61–63], possibly accompanied by stakeholders associated with the BEAM Alliance or the REPAIR Impact Fund[64,65]. However, even the growing diversity of such push incentives are in many cases insufficient and primarily focused on companies. In addition to these approaches, a strategy is required which helps academic researchers to advance their project portfolio to a level that facilitates early interaction and possibly partnering with pharmaceutical companies in the interest of a successful, cross-sectoral development pipeline[66]. Hence, creating new incentive models in the field is an essential process that can only be moved forward if the public, academic and industrial sectors join forces[39,67–69].

In this respect, our position paper provides an overview of the early phases of antibacterial drug discovery, including hit and lead identification, optimization and development to the (pre)clinical stages by summarizing current limitations, relevant approaches and future perspectives, as well as by presenting selected case studies. In terms of a principal guidance for researchers in the field, we suggest possible solutions for a number of obstacles to improve both quality and quantity of antibacterial hits and leads. To strengthen and emphasize these early stages as a "sine qua non" for a sustained generation of novel antibiotics, we are recommending a new level of interaction between the various stakeholders and academic disciplines in the area of antibiotic drug research. The strengths and opportunities that small-molecule therapeutics offer can help address antibiotic resistance more successfully during the coming years, both in the interest of patients and investors, provided that the multiplicity of hurdles along the translational path will be overcome (TABLE 1). Altogether, our aims are in line with the "One Health Action Plan against Antimicrobial Resistance" introduced by the European Commission[70], as well as the WHO program to fight the rising number of bacterial priority pathogens with steadily growing impact on global public health[71].

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1) Synthetic hit compounds

This chapter addresses ideas to develop profitable strategies for identifying and prioritizing antibacterial hit compounds with a particular focus on synthetic small molecules. As a basis, we introduce three main pillars reflecting key aspects of general hit discovery programs on which novel strategies for fruitful hit identification should be implemented.

1.1) Hit definition, chemical libraries and medicinal chemistry

The concept of "hit compound"[72] as it is widely accepted today needs to be expanded to address the needs imposed by the threat of antibacterial resistance. A hit compound is a molecule with reproducible activity, which corresponds to a defined chemical structure (or set of structures), against one or more bacterial target(s). While selectivity and cytotoxicity of initial hits are seen as important characteristics, their improvement should remain tasks for the hit-to-lead optimization phase (see *chapter* ***3***). Activity of hits against (selected) pathogens must be proven in relevant assays, initially *in vitro* (*e.g.* by using exposed/isolated targets or a whole-cell approach), which can be complemented by animal models of infection later on in the discovery process to evaluate pharmacokinetic and pharmacodynamic properties (see *chapter 3*). In any event, the chemical identity and integrity of a hit must be demonstrated, whereas the actual target and the concise MoA may still be unknown and can be identified at a later stage, thus the initial activity read-out for a hit can be either on the molecular or cellular level (BOX 1).

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It is important to consider not only a single molecule addressing one particular target as a valuable hit, but also a compound hitting multiple defined targets (polypharmacology[73], see *section* ***3.1***), or a combination of molecules as used in combination therapy[74]. Depending on the targets, such hit combinations may act synergistically, preferably with different MoAs, or in an additive fashion. Combinations can be valuable to: potentiate the activity (reflected by the main MoA) of existing antibiotic(s); slow down the onset of resistance; and restore the activity of antibiotics that have become inefficient through resistance.

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One major approach to identify novel hit compounds is by high-throughput screening of chemical libraries. It is important to select the correct set of compounds for each screen, *e.g.* a (large) diverse set, a target-focused set or a fragment library. The make up of a library should be based on specific characteristics or property space The make up of a library should be based on specific characteristics or property space requirements including chemical, structural and physico-chemical aspects (BOX 2); these may be taylored to a particular disease area [75,76].

We believe that carefully designed, and possibly even pre-selected ("biased") chemical libraries, which shall allow to screen a suitable chemical space against the bacterial target(s) of interest, represent an important first step to start a reliable hit identification campaign towards treating a specific bacterial infection. The design, assembly, curation and constant updating or expansion of such libraries are skilled and very costly processes, outside the range of most academic groups, and indeed also for many small companies. Models need to be found to grant access to the correct libraries or compound collections for hit discovery, which should be facilitated at least for non-profit research entities (see *section* ***2.2***).

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Interaction between academia and pharmaceutical companies can accelerate hit discovery, *e.g.* by using the high-throughput infrastructure of companies for screening to interrogate novel academic targets or screens. Or the pharmaceutial partner might search for close analogs of hits identified initially in academia, possibly together with already available biological and chemical property profiles. Such analog series and accompanying data sets can be extremely valuable to allow for early improvement of antibacterial potency as well as hit series validation, and to start profiling the most promising hits in terms of absorption, distribution, metabolism, excretion and toxicity (ADMET) parameters, thus accelerating the early hit discovery and validation process. Sharing the relevant information will reinforce the efforts of medicinal chemistry and enhance its reliability and robustness. This in turn allows programs to reach "Go"/ "No-Go" decisions quicker and to enhance the chances for external funding or early partnering based on the medical need that can be addressed.

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Notably, medicinal chemistry is the key discipline for the subsequent optimization of hits (see case studies in BOX 1 - 4). Lack of sufficient funding and expertise to support medicinal chemistry at this early stage is highly detrimental for the whole translational process. Encouragingly, a larger number of AMR-related pharmaceutical companies signed the AMR Industry Declaration[77] several years ago, in which they jointly committed to support antibiotic R&D processes at virtually all stages. This has led to the formation of the AMR Industry Alliance (https://www.amrindustryalliance.org/). Upon the recent implementation of new AMR-specific capital resources, *e.g.* through the REPAIR Impact and AMR Action Funds, as well as involvement (not only commitment) of PPPs like CARB-X in hit-to-lead campaigns, an intensified collaboration

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between the industrial sector and academia is required to drive the chemical optimization of hits and leads through to new preclinical candidates.

Those academic groups that have already built the capacity to carry out such optimization efforts including a broad know-how in medicinal chemistry, biological assays and ADMET studies would also benefit from early partnering with biopharmaceutical companies, particularly as their projects will stand a better chance of attracting external investment. Both, not-for-profit initiatives like the European Research Infrastructure Consortium for Chemical Biology and early Drug Discovery (EU-OPENSCREEN; https://www.eu-openscreen.eu/) and collaborative PPP models as implemented by the European Lead Factory (ELF)[78,79], allowing for open drug discovery programs based on Europe-wide screening resources (*e.g.*, the Joint European Compound Library, JECL) and infrastructure, could pave the way for such early cross-sectoral interactions and exchanges for the benefit of all involved partners[80].

1.2) Nature of the target

We recommend that hit identification against bacteria follows two convergent approaches: *(i)*
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We recommend that hit identification against bacteria follows two convergent approaches: *(i)* identification of molecules active against molecular targets that are vital for all stages of the bacterial life cycle ("essential targets"), hence directly promoting clearance of the bacteria from the host/patient; and *(ii)* searching for molecules that inhibit so-called "non-essential targets"[53,81,82]. Those can be defined as bacterial structures that are not vital under standard laboratory growth conditions, but become critical during processes of host colonization and infection, *e.g.*, by regulating virulence development, by evading host immune response or by triggering bacterial defense mechanisms[83]. Molecules hitting such targets may have weak or even no activity toward bacterial cells under noninfectious (*in vitro*) screening conditions, but might display highly synergistic or additive effects when tested in relevant *in vivo* infection models, either alone or in combination with antibacterial agents addressing essential targets. The latter molecules may belong to the current antibacterial arsenal or new chemical entities (NCEs), *e.g.,* identified in regular screens as described above.

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According to the specific setting, compounds interacting with non-essential targets are defined as antibiotic "adjuvants", "potentiators" or "resistance breakers"[84,85]. Examples of non-essential target inhibitors are represented by: *(i)* inhibitors of virulence-conferring factors or pathways (also known as anti-virulence compounds or "pathoblockers"[86] targeting *e.g.* quorum

sensing mechanisms[87], biofilm formation[88], bacterial secretion systems[89,90], enzymes for tissue penetration[91] or intracellular survival[92]); *(ii)* efflux pump inhibitors[93]; *(iii)* suicide substrates such as β-lactamase inhibitors[94,95]; *(iv)* inhibitors of pathways serving as a mechanism of defense, *e.g.* glutathione biosynthesis[96,97]; *(v)* modulators and inhibitors of energy metabolism[98,99]; *(vi)* host/pathogen epigenetic modulators[100,101]. For some of the mentioned targets, such as efflux pumps, it has been demonstrated that their inhibition can reverse resistance to several antibacterials[102]. Therefore, an attractive therapeutic combination might be composed of a bactericidal agent and an adjuvant molecule, with the aim of potentiating the antibacterial effect(s) and significantly reducing resistance (intrinsic or evolved)[103]. Since the pathoblocker approach is anticipated to be less susceptible towards resistance development and, in addition, to preserve the commensal bacteria of the microbiome[86], it represents a non-traditional strategy for a focused disarming of resistant high-priority pathogens, most likely to be used as an adjunctive therapy in addition to antibiotic standard treatment[81] (BOX 3).

1.3) Advanced screening and profiling based on standardized assays

There is a fundamental need for assays to identify hit compounds (either synthetic or natural product-based, see *chapter **2***) for specific and clinically most relevant indications. In addition to using focused libraries that may cover desirable chemical diversity and property space, innovative screens are essential to increase chances for identifying potent hits against most prevalent common infections associated with Gram-positive or Gram-negative pathogens such as hospital-acquired pneumonia (HAP), community-acquired pneumonia (CAP), complicated urinary tract infection (cUTI) or complicated intra-abdominal infection (cIAI)[104]. To set a reliable foundation for future development, library screening procedures must be state of the art in academia and industry by following generally accepted rules and basic concepts of standardization.

It is important that a range of relevant assays is used to thoroughly select and profile novel hit compounds. These assays should have a high physiological significance (such as in biomimetic assays)[105], *e.g.* by using defined culture media such as artificial urine for activity screens with uropathogens[106,107]; iron-depleted media that simulate bacterial growth conditions during bloodstream or wound infections[108,109]; or assaying host-bacteria interactions[110]. Such schemes can further include the screening for new MoA(s), new drug sensitizing modes, non-killing mechanisms (*e.g.* anti-virulence factors like pathoblockers), compounds acting against biofilms and molecules acting synergistically even with existing antimicrobials to overcome drug resistance[111–114]. Likewise, as hits generated by conventional biochemical assays or screens often fail to become whole-cell active leads, alternative

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phenotypic assays such as novel target-based whole-cell screening[115] are a more promising foundation for the release of useful hits.

A further aim of the consortium is to design and develop informative assays, which can provide A further aim of the consortium is to design and develop informative assays, which can provide A further aim of the consortium is to design and develop informative assays, which can provide A further aim of the consortium is to design and develop informative assays, which can provide A further aim of the consortium is to design and develop informative assays, which can provide information about the desired antibacterial effect together with further characteristics such as target engagement, bacterial penetration characteristics (*e.g.* kinetics of compound permeation through Gram-negative cell envelope models[117,118]), and potential cytotoxicity (the IC_{50} of a hit compound toward eukaryotic cells should preferably not overlap with the range of its antibacterial potency, *e.g.* MIC, against a panel of relevant pathogens).

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In addition to devising standardized panels of assays according to contemporary technology, developing the respective standard operating procedures (SOPs) is mandatory to meet the requirements for Good Research Practices (GRP), which facilitate the transfer of compounds with potential to become new drugs from academia to non-profit or private organizations for continued development. By utilizing standardized proof-of-concept assays under predefined SOPs, more robust hit series will emerge, increasing their potential for late-stage development and decreasing reproducibility issues. For example, minimum inhibitory concentrations (MICs), and possibly also minimum bactericidal concentrations (MBCs), should always be evaluated in a screening campaign, *e.g.* by using the EUCAST (https://eucast.org/) or CLSI (https://clsi.org/meetings/ast/) guidelines. In addition, selected hits from standard screening panels should be consequently tested against contemporary clinical isolates, e.g. to prove if they may overcome existing resistance mechanisms.

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Due to the high attrition rates from early hit discovery to advanced hits and leads, it is very important especially in the field of antibacterials to diversify and generate multiple hit series, and to characterize them thoroughly regarding all features that appear relevant to the intended therapeutic use. This includes explorations to expand scaffold diversity in the context of understanding the target-based chemical and physico-chemical requirements as well as potential liabilities like ADMET.

A summary of early target hit profiles is essential to nominate the most valuable hit series acting against the pathogen(s) or medical indication(s) of interest. The selection of hit series

for lead generation follows the Target Candidate Profile (TCP), which is pre-defined at the outset of the development program according to the desired Target Product Profile (TPP) (see *section **3.4*** and FIG. 2). Thus, the optimization of hits should generally be driven by TCPs and compound progression criteria that, in turn, are driven by chosen TPPs. If several TPPs have been selected or outlined for a campaign, *e.g.* based on different indications, together with their corresponding TCPs, it has to be decided which TCP should be used as a base to aim at for a given chemical series or possibly natural-product-based hit that emerges from mining of biological sources (see *chapter **2***).

Compound progression criteria consist of a standardized list of essential compound properties required for successful transfer of hits and early leads into the subsequent discovery and development stages. Depending on the defined TPP, such a dossier on physico-chemical and biological properties should comprise a set of minimal criteria (compound progression criteria, see *section **3.4***) based on selected, standardized assays or attributes with clear benchmarks for transit (*i.e.* within the program and/or to industrial partners) and for continued development according to ICH guidelines (https://www.ich.org/page/ich-guidelines). Such parameters of relevance may include: potency/cellular activity (e.g. based on MICs and MBCs), chemical and metabolic stability, solubility, permeability (e.g. based on logP or, for ionizable compounds, logD, or complex membrane partitioning), distribution, efflux avoidance, selectivity/ off-target avoidance (e.g. receptor panel, hERG, etc.), acid/base properties based on pKa, cytotoxicity (especially human cell lines), lack of reactive metabolites, phototoxicity, protein binding, in vivo efficacy and human dose prediction, (oral) bioavailability, genotoxicity (e.g. based on AMES or mouse micronucleus), drug-drug interactions, PK linearity, safety (in vivo toxicity), compound access (e.g. synthetic feasibility and scaling up to gram or kilogram), achievable degree of purity, formulation.

Once the hit discovery transitions into the hit-to-lead and lead optimization phases (see *chapter **3***), it is necessary to enlarge the scope of studies including bacterial killing kinetics, MoA, frequency of resistance (FoR), mechanism of resistance (MoR) and pharmacokinetic/pharmacodynamic (PK/PD) analyses, which will deliver valuable parameters to assess the compound's *in vivo* efficacy, *e.g.* based on sufficient free drug exposure in a relevant animal model with acceptable tolerability, and to further guide its path to (pre)clinical candidates. At this level, it is once more important to acquire a substantial amount of structurally related analogs by extensive medicinal chemistry efforts (*e.g.* in collaboration with PPPs or the pharmaceutical industry as suggested above) in order to rapidly generate a solid body of structure-activity relationship (SAR) and structure-property relationship (SPR). These

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data are essential to consistently improve all the required parameters as a base for nomination of lead structures and their further development into (pre)clinical stages. Computational methods based on machine learning techniques like pQSAR can help to build predictive models regarding activity, selectivity, toxicity, MoA and further parameters for specific compound classes, hence providing valuable *in silico* input for advantageous hit discovery and lead design[119,120].

2) Natural product-based hit compounds

Historically, microbial natural products are the most important source of antibiotic lead compounds; during the last forty years, about 60% of all new chemical entities in the field of antibacterials were based on or derived from natural products[121]. Hence, complementary to key aspects described above, major requirements are outlined here that need to be addressed specifically to make identification and prioritization of antibacterial natural product hits more efficient and, in particular for the academic sector, achievable in all technological and financial demands.

2.1) Identification of new chemotypes from natural sources

Most of the known natural products with antibiotic activity were identified in phenotypic screening campaigns by determining antibacterial activity against panels of test organisms in standardized assays. While these screens, which build the basis for bioactivity-guided isolation of natural products from complex mixtures, efficiently retrieve bioactive compounds when libraries of crude extracts are evaluated, a high rediscovery rate of already known molecules associated with pre-existing resistance mechanisms as well as a great proportion of hits that show significant cytotoxicity or poor ADMET properties severely limit novelty.

We emphasize that there is a general lack of efficient tools and strategies to increase the number of new chemotypes and to reduce the rediscovery rates in antibacterial screening approaches. Even on a global scale, the number of newly discovered chemotypes, especially novel scaffolds acting against Gram-negative bacteria, is consistently low. Several aspects can be relevant to improve this situation:

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(i) One main possibility to identify new antibacterial chemistry is to limit screening of broadly characterized groups of secondary metabolite producers like actinomycetes and to expand efforts on identifying new types of producers by extensive biodiversity mining. This can be achieved by moving the focus to the potentially 99.999% of microbial taxa of the earth

microbiome that remain undiscovered[122], including yet underexplored taxa of human or animal microbiomes[123–126]. Emerging innovative isolation and cultivation techniques such as diffusion bioreactors (also on microscale like iChip), microfluidics, elicitors and various co-cultivations[127–135] will contribute to accessing rare and less-studied groups of microorganisms from diverse habitats[136–138]. Further, molecular (co-)evolution acting to generate novel metabolites for efficient microbial warfare could be exploited[139,140], *e.g.* by sampling from environments heavily contaminated with antibiotics (like sewage in Southeast Asia or South America) known to contain highly resistant microbes[141,142], as well as by laboratory exposure of potent producers to sub-inhibitory antibiotic concentrations[143], or by co-culturing them together with drug resistant (pathogenic) strains[144]. Beyond microbial producers, a great variety of plants[145,146], macroscopic filamentous fungi (*e.g.* Basidiomycota)[147] and animals[148] bear the potential to deliver useful compounds as a base for novel antimicrobials. Altogether, the exploration of untapped biological resources representing a major reservoir for future therapeutics should be generally extended within the academic and industrial sector.

(ii) Upon genome mining of novel microbial isolates or metagenome-driven discovery of novel natural products[149–152], selected biosynthetic gene clusters (BGCs) that potentially produce unknown secondary metabolites should be systematically expressed in specialized host strains[153–155] that allow a straightforward detection and isolation of the new compounds, particularly if their BGCs remain “silent” in the native host. Such host strains or “chassis” can be based on microbial species that commonly produce a large variety of natural products, but have been made devoid of their own secondary metabolite BGCs and/or have been further optimized to efficiently express BGCs originating from “non-common” sources (*e.g.* rare actinomycetes or fungi)[153,156,157]. However, only a limited set of such specialized host strains is available so far, and a much more diverse array of microbial chassis needs to be developed to fit the demands of a growing arsenal of BGCs that potentially produce novel chemistry. Since BGC expression is often most successful in strains closely related to the native producer, standardized heterologous expression of novel BGCs in selected host strains with desirable properties but not yet domesticated for the use as regular chassis could be an alternative strategy[158].

(iii) Another way to enlarge the chemical space is to utilize emerging synthetic biology approaches for medium-to-high-throughput genome editing and pathway engineering (based on CRISPR/Cas9[159,160] and diverse recombination, assembly and integrase systems[161–163]), followed by advanced analytics and screening of the potentially modified natural products, which may be produced only in trace amounts. This technology involves the extensive use of information on genome sequences, enzyme activities and compound structures collected by

publications, databases and web tools (such as MIBiG[164], antiSMASH[165], PRISM[166]) over the last decades. In many cases, the modularity of the BGC composition, *e.g.* coding for polyketide synthases or non-ribosomal peptide synthetases can be used to implement a bioinformatics-supported "plug and play" diversification strategy allowing to exchange and recombine core units as well as modifying enzymes[167–170]. A concomitant refactoring of BGCs, especially from rare microbial sources, often allows high-level heterologous production of the antibiotic compounds in suitable hosts[171–174]. However, these methods are still in their infancy and require wider testing with different classes of antimicrobials to define general principles of feasibility and scalability, which furthermore requires a better understanding of the complex biosynthetic machineries and their modular evolution.

(iv) Advances in analytical chemistry techniques, *e.g.* regarding mass spectrometry-based metabolomics and its enhancement by molecular networking and a variety of machine learning applications, support the process of dereplication[175] during (secondary) metabolome mining for novel antimicrobials[176–180]. Known compounds produced in reasonably high yields can be easily identified via their high-resolution mass, MS/MS fragmentation pattern or structural data (based on NMR, crystallography, etc.), *e.g.* from secondary metabolite databases[137,181–186]. However, the remaining bottleneck is to highlight and annotate novel antibiotic compounds, particularly those with low production titers, as soon as possible in the discovery process (*i.e.* directly from crude extracts, without small-scale fractionation and enrichment). This can be supported by innovative extraction methods prior to bioactivity-guided isolation of novel compounds[187].

(v) Additionally, the revisiting of known potent antibiotics, neglected due to previously unacceptable and non-addressable properties such as lack of stability, high FoR or cytotoxicity, can be a valuable strategy to provide novel leads and candidates. The reassessment of such scaffolds can be based on a variety of efforts including the improvement of production and purification[188], reconsideration of application and effective dose for natural derivatives[189], or advantageous scaffold modification by biosynthetic engineering and semisynthetic approaches[190,191] (BOX 4).

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(vi) Further opportunities remain to improve the discovery and development of agents for combination therapy as indicated above, *i.e.* synergistically acting compounds against MDR and/or high priority pathogens[192,193], and to address the difficulty to discriminate them from non-specific antibiotic activities during the discovery process.

(vii) Improving bacterial target access, enhancing potency and broadening the antimicrobial spectrum of known and novel antibiotic scaffolds by using drug-conjugate strategies, *e.g.* linking of pathogen-specific antibodies[194,195], siderophore moieties[196,197] or positively charged peptides[198,199]
. Though these approaches have been proven effective in many ways, some of them may also bear the risk for undesired effects like spontaneous rise of FoR, which can be problematic, *e.g.* in the case of the "Trojan Horse" approach[200].

Altogether, a variety of innovative and complementary technologies is required to improve access to novel natural product scaffolds. Computational methods can provide powerful assistance at different levels in many of the areas indicated above, as recent efforts show[201,202]. In this context, artificial intelligence might play a game-changing role in the future. The general power of neural networks for detecting new antimicrobial candidates has already been demonstrated[201]. By using a computational model that screens over hundreds of millions of chemical compounds in a few days, potential antibiotics even with new MoA(s) could be proposed rapidly. Given the recent advances in artificial intelligence, these and other models will likely add to the future identification of new candidate drugs.

Interestingly, when looking at compound properties, it appears that there is often more flexibility in the selection of "successful" natural product scaffolds compared to synthetics, *e.g.* regarding Lipinski's Rule of Five (Ro5)[203–205], which natural products frequently "disobey" (such as cyclosporine or macrolides like azithromycin). Thus, antimicrobial drug discovery in "beyond Rule of Five" (bRo5) chemical space is an opportunity when using natural compound collections or when assembling libraries of *de novo* designed compounds[206–208], though the general need for optimizing key pharmacological properties of such hits remains beyond question.

Another major challenge for natural products can be the generation of structurally diverse analogs (if they are not accessible through biosynthesis), since many scaffold positions are often hard to access by means of semisynthesis. Hence, broad derivatization of natural product-based hit and lead compounds is much more labor-intensive, and establishing synthetic access to these scaffolds with the perspective to systematically diversify their chemical space can require large amounts of resources[209]. Nevertheless, the modification of natural scaffolds with ligands that are often easier to incorporate by (semi)synthetic or chemoenzymatic approaches, such as halogens that allow the modulation of solubility, permeability, selectivity, target affinity etc.[210,211], proves that a multitude of opportunities arises when combining synthetic and biological chemistry.

2.2) Required access to biological and chemical material and data

Many scientists regularly experience difficulty in accessing and sharing research material from third parties such as microbial strains, cultivation extracts, pure compounds, genome or gene cluster sequences and further background data (of published or even unpublished results). As an example, an interesting BGC is identified in the publicly accessible databases, but the strain is not specified or not available from the indicated source. Similarly, access to industrial antibiotic overproducers can be impossible, even when a company no longer has a commercial interest in the resulting molecule. This may have various reasons, including legal restraints (*e.g.* imposed by the Nagoya Protocol[212]) or IP claims on strains, compounds, biologics, (re)profiling data of already known structures, etc., either via involved third parties or by own IP shares of the material and data.

In the public interest, standardized procedures are necessary to facilitate access to research materials and to solve IP conflicts, at least within the field of academia, which is supposed to share research materials with colleagues by negotiating appropriate cooperation agreements. Further, the access to in-house compound libraries of pharmaceutical companies (at least subsets of them and especially those that are not intended for antibiotic-related screening) could be very valuable for academic partners who are eager to identify novel antibacterial hits (see *section* ***1.3***), which could lead to joint drug development programs. Enabling access to materials can also be extended to strain collections, including clinical isolates representing the diversity of pathogens associated with a certain clinical indication, and advanced compound information based on pre-existing characterization and profiling campaigns. An increased availability of these resources will be of great benefit to the antimicrobial research community worldwide.

Furthermore, comprehensive databases and data-sharing platforms can provide another valuable resource for present and future antibiotic R&D projects and, hence, should be implemented and maintained with care[213]. There is a growing body of recently initiated and publicly available web-based tools and archives that support accumulation and exchange of data regarding antibacterial compounds in different stages of discovery or therapeutic development, known or predicted antibiotic targets and the diversity of antimicrobial resistance determinants (BOX 5). Further connection and integration of such databases is desirable to achieve an optimum of relevant output for a specific search request. In addition, initiatives comparable to the European Commission's manifesto to maximize the public accessibility of research results in the fight against COVID-19[214] are also highly recommended to support AMR-related scientific research at all levels, including facilitated access to online resources.

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2.3) Prediction of antimicrobial structure and function from genome sequence data of antibiotic producing microbes

Driven by the breakthrough in sequencing technologies and genome mining, the identification of BGCs encoding the biosynthesis of natural products has matured to complement the chemistry- and bioactivity-driven screening processes for natural product hits. Computational methods are developed and continuously improved to identify novel biosynthetic pathways in (meta)genomic sequence data[149,150]. Recently, 3rd generation genome sequencing techniques such as PacBio and Oxford nanopore have been developed that provide high-quality full genome data even for complex microorganisms like filamentous fungi at reasonable cost, which is an ideal prerequisite for large-scale genome mining approaches[215].

However, linking obtained sequence information to possible structural or functional features of the encoded molecules certainly remains a great challenge. Prediction of chemical structures directly from genome data would help to sort out known scaffolds from potentially unknown ones during a very early stage of dereplication; the training of machine-learning algorithms with sufficient amount of genome data from microbial producers could ultimately lead to fairly accurate predictions of chemical structures linked to specific BGCs, and possibly even their biological activities[166].

Furthermore, a successful strategy to decipher antibacterial targets of new natural products, even without the necessity of initially isolating those, is a directed search for known resistance factors in the genomes of producer organisms[216,217], *e.g.* coding for resistant variants of the molecular target(s) or conserved class-specific transporters, which recently led to the discovery of novel antibiotic scaffolds[218]. However, most BGCs do not contain apparent or specific drug-resistance genes that could straightforwardly indicate a compound's function. In the majority of cases, very limited predictions based on genomic data concerning function and potential target(s) of a natural product are currently possible, although advanced automated tools for target-directed genome mining are available[219]. Thus, there is a high demand for innovative methods to predict the molecular function or target of a natural compound based on genomic data. Such data would be extremely valuable in order to prioritize biosynthetic gene clusters (BGCs) for experimental characterization. In the future, artificial intelligence-based approaches, either based on classical machine learning methods (extracting new knowledge from pre-processed data sets) or on deep learning (drawing conclusions from raw data such as representative examples, often by using multi-layer neural networks) may deliver such predictions with increasing accuracy[220]. However, existing algorithms still have to be improved and new ones have to be developed to specifically address the question of how to

assign target-based functions to natural products during the early stages of discovery and prioritization, and they require a huge amount of validated training data[221].

3) Optimization of hits and leads to the (pre)clinical stages

Regardless of whether antibacterial hits emerge from rationally designed synthetic molecules or from the pool of natural products, the subsequent hit-to-lead and lead-to-candidate optimization phases are very similar for compounds irrespective of origin ("Y-Model", see FIG. 2). In this chapter, we discuss the most critical obstacles and requirements for delivering those advanced leads that may eventually become the next generation of (pre)clinical candidates.

3.1) Drug-target interaction studies as a base for hit development

For hits arising from phenotypic assays, cellular MoA(s) or specific molecular target(s) may not be known at the hit-to-lead stage, and sometimes the precise MoA is elucidated years after the approval of a drug, as in the case of daptomycin[222]. However, detailed insight in the mechanism(s) by which compounds exert their pharmacological activity is highly desirable for further rational optimization of chemical scaffolds, particularly when structurally enabled approaches can be used, for a convincing presentation of preclinical candidate dossiers, and for regulatory requirements. Since universally applicable methods for characterizing the MoA(s) of antibiotics do not exist, a full suite of expertise in genetics, genomics, microbiology, chemical biology and biophysics is required. Identification of the molecular target can be achieved by targeted screens of indicator or mutant strains, whole-genome sequencing upon focused resistance development[223,224], pattern recognition techniques based on transcriptomics[225], imaging[226,227], metabolomics[228], macromolecular synthesis[229,230] or mutant fitness profiles[231,232], which can be coupled with machine-learning approaches for directed predictions[224,232], or chemoproteomics[233,234]. The latter will be used especially in the case of non-essential target inhibitors like pathoblockers, since they may not generate resistant mutants (at least under standard laboratory conditions). Additional techniques for MoA studies may include crystallography, a diverse set of spectroscopic and calorimetric analyses[235–239] as well as the use of functionalized derivatives ("tool compounds")[240,241], which can support both target identification and validation and may provide in-depth information of drug-target interaction to drive the rational hit-to-lead optimization process forward. Alternatively, identification of drug-target (or ligand-protein) interactions formed under native ("unbiased") conditions by using specialized proteomic approaches is becoming increasingly successful[242–245]. Current bioinformatic tools can also combine genome-mining approaches with the prediction of potentially innovative MoA(s) based on the presence of resistant target genes in

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BGCs encoding novel antibiotics[219], and a diverse set of emerging learning methods will steadily enhance the predictability of drug-target interactions[246,247].

In addition to the specific molecular target(s), it is important to understand the impact of the antibiotic compound on the general physiology of the bacterial cell. This includes the sequence of events leading to bacterial death, the time-point when killing occurs (based either on individual bacterial cells or their population/colonization level) and the conditions that might enhance or preclude it. Such characterizations may require the application or development of a range of secondary assays. For compounds acting on intracellular bacterial targets (i.e., targets located in the cytoplasm), the processes of compound influx and prevention of efflux (especially in Gram-negative bacteria due to their complex cell envelope and presence of numerous multi-drug efflux pumps) are both critical optimization parameters to ensure sufficient target engagement[248–251]. These can be addressed by suitable compound design, which is generally still rather empirical[252–255]. Other possibilities to address this key area would be to use these compounds in combination with outer membrane permeabilizing agents[256,257] or efflux inhibitors[93,258]. Alternative approaches addressing extracellular virulence factors, *e.g.* extracellular lectins required for attachment and biofilm formation or secreted proteolytic enzymes do not suffer from a possible lack of bacterial uptake[259]. Often antibiotics, and particularly natural products, have more than one target and disturb bacterial physiology in different ways, referred to as polypharmacology[73,260,261], which is beneficial for inflicting severe damage on the bacterial cell and slowing down target-mediated resistance development. Information related to such effects should be acquired for all bacterial species within the spectrum of activity of the potential drug, and it may significantly deviate across phylogenetically distant species.

Apart from the desired biological effects on bacterial pathogens, knowledge about undesired adverse effects on eukaryotic cells ("off-target effects"[262–267]) should be acquired early, since toxicity is a major issue for attrition in the drug development process. However, whilst *in vitro* cytotoxicity screens are useful during the early discovery process, they are often not predictive of toxicological effects that can become most significant during *in vivo* studies (see *section **3.4***). Furthermore, "collateral damage" to the microbiome needs to be considered[268–271], which can be modulated by selective drug design[272]. For compounds with a novel or particularly complex MoA, it often takes several years to achieve a detailed molecular understanding and the cellular consequences of exposure. Therefore, acquiring this knowledge as early as possible is a key aspect for further rational drug optimization including SAR studies. We recommend investing resources into expanded MoA studies already during the initial stages of the drug development process and, furthermore, to build a network of experts who can

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provide MoA analyses that fulfill the requirements of a preclinical candidate dossier. While these aspects are standard for drug development projects in pharmaceutical industry, academia usually suffers from insufficient funding to appropriately address such requirements, hence acquisition of additional resources has to be secured.

3.2) Limited resources to move from hit into lead stage

Once a hit validation has been accomplished, the resources needed to advance the selected compound series into hit-to-lead and lead optimization greatly increase. These stages require a diverse scientific team covering analytical, computational and medicinal chemistry, biochemistry, microbiology, bioinformatics (ideally including machine learning and artificial intelligence methods), drug metabolism and pharmacokinetics as well as, specifically for natural product-based compounds, biotechnology and genetic engineering. In industrial projects, typically five to fifteen medicinal chemists work on the optimization of a hit (depending on how complex the chemistry of a certain compound is) to create promising leads or preclinical candidates, essentially by generating, testing and advancing SAR-based analog series in an iterative manner. The challenge is to simultaneously optimize all properties necessary for the drug to be most effective and least toxic. This includes potency, selectivity, physico-chemical parameters, cytotoxicity as well as pharmacokinetics and pharmacodynamics (see *section **3.4***, and FIG. 2). The multi-parameter optimization can usually be achieved within a timeframe of about two to four years, but remains dependent on the human, technological and financial capacities, as well as the particular challenges represented by the chemical series. Such resources are difficult to acquire through classical academic funding schemes, which usually reward new discoveries in fundamental science rather than subsequent steps of time- and resource-consuming optimization, where there is no guarantee of success.

Therefore, academia must find new ways to provide suitable resources for early-stage translational research. Furthermore, few academic institutions possess the relevant expertise and facilities to carry out lead optimization. Therefore, in the majority of cases, they require access to high-quality expertise and/or capacities through partnering with pharmaceutical companies/SMEs or via contract research organizations (CROs), which can only be achieved through additional funding or partnerships, *e.g.* for improving a particular molecular scaffold over several years with the risk of limited outcome (see *section **3.5***). A possible strategy to increase the opportunities for acquiring appropriate resources could be the endorsement of alternative reward schemes for evaluation of academic project funding, not only based on high-impact publications, but also on verifiable dedication to health research such as contribution to a global antibacterial portfolio. The emergence of centers for translational

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science in many countries (*e.g.* the German Center for Infection Research; https://www.dzif.de/en) could be an opportunity to develop and implement such measures, possibly at an international level.

3.3) The bottleneck of compound supply

The enhanced biological profiling that is mandatory in lead finding and optimization programs requires a considerable amount of sufficiently pure compounds to be tested. While this constitutes an issue for chemists in general even with respect to synthetic hits and leads (especially when considering massive scale-up of typical laboratory reactions)[273,274], the problem of re-supplying increasing amounts of natural products originating from bacteria, fungi or plants is particularly challenging. Indeed, academic projects are often concluded when natural compounds or biotechnologically generated variants thereof are identified at small scale (often <10 mg), rudimentarily profiled and published. In many laboratories, there are no additional resources to increase the yields of natural product hits or initial leads, or to scale-up production in a pre-pilot plant environment that is capable of carrying out the fermentation (possibly by using heterologous production hosts to achieve attractive yields[275,276]). In addition, downstream processing has to be established and optimized for every new compound to ensure sufficient supply and purity for the following stages including scaffold optimization by medicinal chemistry or extended biological profiling. The fact that sufficient amounts of compounds (multigram-to-kilogram scale) cannot be produced in many cases severely decreases the chances of developing novel therapeutics from natural products. This is particularly unfortunate in the antibiotics field, because about two-third of all antibiotic drugs in therapeutic use are derived from natural products (see *chapter **2***). Regrettably, fermentation-independent supply, *e.g.* via total synthesis of complex natural compounds, can only be achieved for a low percentage of novel hits and leads and requires tremendous additional capacities[277–280].

Thus, suitable funding instruments are important to cover the essential process of natural compound supply by biotechnological procedures including fermentation scale-up and efficient downstream processing[281–283], allowing the provision of sufficient source material of high purity for semisynthesis and further studies. In addition, a robust method for large-scale production and downstream processing of the candidate molecule is a prerequisite for process transfer to GMP manufacturing before entering (pre)clinical stages. Generally, further scientific and technological development is required to make the provision of compound material from various sources a more routine and affordable task, particularly in the non-industrial research environment.

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3.4) Requirements for in vivo studies and project transfer

The primary assays in most programs usually address biochemical, biophysical and/or microbiological functionality of newly generated compounds. In order to convert a molecule with *in vitro* activity into a drug, sufficient exposure at the infection site *in vivo* must be achieved. To analyze this, a full suite of ADME(T) assays is required[284,285], followed by pharmacokinetic (PK) experiments in animals (usually starting with rodent models)[286,287], which could be combined with physiologically-based pharmacokinetic (PBPK) modeling to predict *in vivo* ADME and PK behavior in other animal species or in humans[288,289]. As stated above (see chapter 1), it is important to implement physico-chemical and *in vitro* ADME(T) profiling at the start of hit optimization, to make sure that any pharmacokinetic issues are identified early and can be addressed through the entire chemistry program.

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A sufficient correlation between *in vitro* and *in vivo* data, which is not always achievable for all antimicrobial compounds, *e.g.* LpxC inhibitors[290,291], should generally be pursued as early as possible in the program, otherwise continued lead design might be based on irrelevant or misleading data points. Further, the availability of pharmacodynamic (PD) models[292,293] of high translational relevance, *i.e.* reliably predicting a minimal efficacious dose in humans, is a critical factor of success in order to generate the optimal drug candidate during lead and lead-to-candidate optimization. Particularly in the field of antibiotics, preclinical PK/PD relationships are generally predictive and have a high relevance for regulatory dossiers[294,295], *e.g.* for human PK/PD target attainment (PTA) at therapeutic doses and drug formulation development; hence, they have to be evaluated carefully at the earliest possible stages[296–298]. Typically, PTAs for antibiotics require relatively high doses compared to other drug classes, limiting the successful application of existing formulation and delivery technologies. This is especially true for oral medications that may cause further challenges, *e.g.* regarding adequate bioavailability of the drug. Hence, a broader array of potential delivery systems should be tested systematically, which may include conventional permeation enhancers[299] as well as sophisticated nanoformulations[300,301]. The latter, however, can only be produced based on expert knowledge and infrastructure, which is often not available in academia, thus specialized CROs or SMEs may be approached based on available funding.

Another obstacle is the need to perform (initially) rather extensive studies in laboratory animals to understand the PK/PD relationship of a novel compound, which at subsequent stages allows the number of animal experiments to be minimized according to the "3Rs principle"[302]. However, these studies are generally associated with ethical concerns, high costs and administrative burden. Likewise, these matters are relevant for the *in vivo* evaluation of toxicology, toxicokinetics and safety pharmacology to cover safety aspects before entering

clinical trials[303,304]. Here, exploratory or early-stage predictive assays using computational models as well as *in vivo* systems with minimal ethical concerns (*e.g.* in vertebrates like *Danio rerio*, insects like *Galleria mellonella*, or worms like *Caenorhabditis elegans*) are an opportunity to estimate both efficacy and potential toxicity risks before considering standard *in vivo* experiments in rodents and other mammals[305–307].

Ultimately, the demonstration of efficacy in a relevant animal model, associated with convincing exposure at the site of infection and a rough estimation of a reasonable safety margin is often a prerequisite to attract an investor's interest; typical minimum requirements are a tolerance/ dose-range finding study in one or two animal species as well as human dose prediction based on a solid set of PK/PD data, *e.g.* by testing systemic efficacy in the neutropenic thigh infection model in mice[308].

Generally, TPPs and the corresponding TCPs should continue to be the base for all further optimization attempts, especially when including *in vivo* studies, and hence should be thoroughly compiled before the development program starts with the help of subject matter experts. It will guide the strategies and decisions for all chemical and biological development processes during the optimization phases, mainly with respect to one (or more) clinical indication(s). In order to specify robust finishing lines, it should define sets of minimum acceptable criteria for each phase, *e.g.* for biochemical assays during early stages and (pre)clinical endpoints at later stages. Such compound progression criteria (see also section 1.3) should be defined for a validated hit, entry into lead optimization, a late lead and a preclinical candidate. There are different TPPs for different bacterial infections. As projects evolve, they may encounter serendipitous discoveries, unsurmountable hurdles, or important findings from other groups or competitors, which may affect the TPP that they target. Therefore, the TPP can be critically reviewed and possibly refined or adapted throughout the project, *e.g.* at each transition into the next development stage. Ideally, a pool of commonly accepted TPPs (*i.e.*, approved by pharmaceutical industry as well as the public health sector) should be available for the multitude of clinical indications to serve as a base for each discovery and development program of novel therapeutics. These TPPs need to be regularly reviewed, and where necessary updated, to make sure that they reflect the current clinical situation; for example, TPPs addressing indications caused by bacterial infections may be affected by the latest emerging (or anticipated) drug-resistant pathogens of critical relevance. It is important to note that only convincing TPPs together with comprehensive preclinical candidate dossiers (highly informative TCPs) and reliable SOPs for scalable compound supply will allow early partnering and a smooth transfer of the project to an industrial stakeholder to move into (pre)clinical development (BOX 6).

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3.5) The management challenge in hit and lead optimization programs

As the development of antibacterials is a multidisciplinary approach, knowledge of a diverse set of techniques and domains (*e.g.* assay development, high-throughput screening, medicinal and computational chemistry, ADMET, PK/PD, drug delivery, clinical background of disease processes, etc.) is required in order to develop a compound to the level of a preclinical candidate. Single principal investigators (PIs) will usually not possess the broad base of expertise that is necessary, since academia largely focusses on early-stage discovery and compound optimization at laboratory scale. Hence, research groups that do not possess the extensive skill-set for drug development in its various stages should pursue a team approach by collaborating with organizations that have the relevant experience, be it within the academic or industrial sector. There is also the possibility of calling on specialized consultancy or outsourcing packages of work (for example ADMET) to CROs that possess relevant expertise and experimental capabilities. However, apart from the relatively high costs of such services, PIs often struggle with remaining questions once a CRO assignment ends, and sufficient resources for tailor-made optimizations are often lacking. Moreover, the need to interpret results and devise a clear path forward towards the TPP from multiple data packages remains with the project teams. Hence, partnerships and collaborations are essential if relevant in-house expertise or infrastructure is missing. Therefore, we propose the following solutions for efficient translational project management:

(i) Aligning and collaborating with suitable partners from various sectors or disciplines is crucial for groups with limited know-how in drug discovery and development. Generally, larger project teams can provide or identify expertise much faster to sufficiently resolve emerging knowledge gaps. Additionally, project consultants or CROs can be approached at different levels to fulfill remaining tasks, *e.g.* data evaluation or processing defined and highly specific work packages, e.g. in pharmacokinetic and toxicological studies.

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(ii) Databases of experts should be available for relevant research areas or services, and the various technical and IP-related aspects need to be elaborated on a case-by-case basis. Unbiased partners have to be identified to host and curate such databases on a regular basis, which could fall into the remit of non-profit health organizations such as JPIAMR or GARDP.

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(iii) Training of PIs on a frequent basis is required to broaden their knowledge and to ensure a high-level understanding of potential barriers and pitfalls at least until projects reach the (pre)clinical stages. A number of renowned institutions already offer regular workshops and seminars (often as interactive webinars, e.g. GARDP REVIVE; https://revive.gardp.org/) as

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well as extended training programs (*e.g.* the International Course on Antibiotics and Resistance; https://www.icarecourse.org/; hosted by Institut Pasteur, France), and these are increasingly popular. In addition to PIs from academia, also non-academic experts from industry, different health sectors and politics should share their perspectives on current research and funding aspects more regularly within interdisciplinary settings.

Overall, it is important that the necessary financial and legal frameworks for efficiency-oriented project management are established as early as possible to avoid loss of time and resources during the course of a development program. The required settings can be implemented either individually at a certain project level, or management models (*e.g.* available in the industrial sector or in translational research centers) could be used or adopted for the specific purpose.

Conclusions and Outlook

To ensure a healthy and vibrant antimicrobial pipeline, considerable efforts are needed not only to develop the next generation of antibacterial drugs but also to safeguard and foster profound expertise in antibiotic drug discovery and development. In short and medium terms, such capacity building must be performed as a collaborative and iterative process between academia and industry to ensure that the necessary skills are available to translate validated hits into potential drug products. The development of joint initiatives for education in translational sciences will require specific funding, as they are not part of most universities' standard curricula. Many experienced scientists in the pharmaceutical industry are eager to share their translational and regulatory knowledge, often after retirement or due to change of operations. Thus, pharmaceutical companies could serve as a valuable "training ground" for acquiring and developing specific skills in the antimicrobial sector. However, limited funding (especially for SMEs) and economic uncertainties negatively affect this premise by *a)* leading to business closures; *b)* inducing high turnover rates of employees; *c)* preventing the recruitment and training of inexperienced staff; *d)* deterring scientists from starting a career in SMEs. In order to achieve transfer of vital expertise, "academia meets industry" workshops, symposiums and exchanges for students and advanced researchers are required and need financial support. However, there are hardly any market-driven initiatives for such events and therefore, a connection to already existing education and training programs, e.g. those supported by IMI, ESCMID or BSAC, can be a valuable option as long as the transition into an era of mutually sustained knowledge transfer between industry and academia continues.

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Another critical aspect for all future antibiotic R&D projects is the implementation of a legal framework for IP ownership at project commencement. The multidisciplinary and collaborative nature of antibiotic drug discovery often results in collaborations between different institutions on a national or international level. This creates challenging ownership structures with increasing complexity of such consortia, especially when an antibacterial program is out-licensed, for example, to an SME. Negotiating ownership agreements among inventor institutions can be lengthy and may discourage industry from in-licensing valuable assets for further development. The increased collaboration between academia and industry requires fair and justifiable guidelines for knowledge and compound transfer outlined in appropriate agreements. The creation of such guidelines should be supported, *e.g.* in form of templates to settle ownership agreements between project partners or third parties, to facilitate processes for the benefit of researchers with limited experience in these matters. Such a framework will accelerate potential technology and compound transfer towards industrial drug developers, will make the counterpart of the commitment for each participant clearer and their gains more attractive.

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Finally, we believe that AMR research requires diligent lobbying at the national and international level to create entry points for large funders. Many scientists working on antimicrobials in either academia or SMEs are outside the few existing networks that involve decision makers within commercial funding sources such as venture capitalists including the newly announced AMR Action Fund, philanthropic organizations, national or regional governments or international bodies. This leads to a continued situation in which the challenges of antimicrobial drug developers are either not heard or are even ignored, though public awareness of AMR steadily increases. It is evident that a strong lobbying position will lead to changes, which has recently been shown by the BEAM Alliance and their interaction and negotiations with diverse political bodies in Europe, leading to increased recognition of the challenges for antibacterial drug developers by the European Commission and Europe's national governments[309–311].

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For the above reasons, we recommend that an international group of experienced AMR lobbyists should be formed that together can campaign for funding of early antibacterial drug discovery research along the principles set out in this article. Such a group should include national, regional and global scientific and industry associations that have practice in interacting with relevant stakeholders connected to national parliaments, EC, G7, G20 and further decision-making entities[312]. The Global AMR R&D Hub (https://globalamrhub.org/) could be a crystallization point to pioneer such developments, which can be supported by consortia such as presented by the authors of this white paper:

The International Research Alliance for Antibiotic Discovery and Development (IRAADD; https://www.iraadd.eu/), which we have recently established with the support of the JPIAMR Virtual Research Institute (https://www.jpiamr.eu/jpiamr-vri/), identifies itself as a part of the mission that is addressed by the current roadmap. IRAADD aims to improve the situation of novel antibiotic discovery and development by joining together experts for early drug research from the academic and industrial sectors. Although IRAADD currently has only a short-term funding perspective, it is one of our main goals to help define and implement interdisciplinary innovative antibiotic development projects based on sustainable research funding, in order to refill the translational pipeline with new drug candidates in the foreseeable future. In this respect and as a possible long-term vision, the creation of internationally operating Antibiotic Research Hubs, which may emerge from already existing "pre-stage" platforms such as IRAADD, can be a major step forward to engage as many members as possible from academia, industry and public health organizations in antimicrobial R&D collaborations, and to create a strong and path-breaking position that cannot be overlooked. Only a responsible connection of thought leaders and dedicated experts from all relevant sectors of society, joining together now and for the future, will allow the facilitation of suitable rapid responses to globally emerging pathogens, or even future pandemics caused by multi-to-pan drug-resistant ("superbug") bacteria. This aim deserves our undivided attention.

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References

1. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. *P & T : a peer-reviewed journal for formulary management* **40,** 277–283 (2015).
2. Byrne, M. K. *et al.* The drivers of antibiotic use and misuse: the development and investigation of a theory driven community measure. *BMC public health* **19,** 1425; 10.1186/s12889-019-7796-8 (2019).
3. Nadimpalli, M. L. *et al.* Urban informal settlements as hotspots of antimicrobial resistance and the need to curb environmental transmission. *Nature microbiology* **5,** 787–795; 10.1038/s41564-020-0722-0 (2020).

4. World Health Organization. WHO Report on Surveillance of Antibiotic Consumption 2016 - 2018 Early implementation. https://www.who.int/medicines/areas/rational_use/oms-amr-amc-report-2016-2018/en/ (2018).
5. Schrader, S. M., Vaubourgeix, J. & Nathan, C. Biology of antimicrobial resistance and approaches to combat it. *Science translational medicine* **12**; 10.1126/scitranslmed.aaz6992 (2020).
6. Conlon, B. P. *et al.* Persister formation in Staphylococcus aureus is associated with ATP depletion. *Nature microbiology* **1**; 10.1038/NMICROBIOL.2016.51 (2016).
7. Harms, A., Maisonneuve, E. & Gerdes, K. Mechanisms of bacterial persistence during stress and antibiotic exposure. *Science* **354**; 10.1126/science.aaf4268 (2016).
8. Antibiotics Special Issue: Challenges and Opportunities in Antibiotic Discovery and Development. *ACS infectious diseases* **6,** 1286–1288; 10.1021/acsinfecdis.0c00331 (2020).
9. O'Neill, J. Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance (2016).
10. AMR Industry Alliance. Progress Report. https://www.amrindustryalliance.org/wp-content/uploads/2020/01/AMR-2020-Progress-Report.pdf (2020).
11. Zhou, P. *et al.* Bacterial and fungal infections in COVID-19 patients: A matter of concern. *Infection Control and Hospital Epidemiology,* 1–2; 10.1017/ice.2020.156.
12. Chen, X. *et al.* The microbial coinfection in COVID-19. *Applied microbiology and biotechnology,* 1–9; 10.1007/s00253-020-10814-6 (2020).
13. Zhou, F. *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* **395,** 1054–1062; 10.1016/S0140-6736(20)30566-3 (2020).
14. Nori, P. *et al.* Emerging Co-Pathogens: New Delhi Metallo-beta-lactamase producing Enterobacterales Infections in New York City COVID-19 Patients. *International journal of antimicrobial agents,* 106179; 10.1016/j.ijantimicag.2020.106179 (2020).
15. Getahun, H., Smith, I., Trivedi, K., Paulin, S. & Balkhy, H. H. Tackling antimicrobial resistance in the COVID-19 pandemic. *Bulletin of the World Health Organization* **98,** 442-442A; 10.2471/BLT.20.268573 (2020).
16. Oldenburg, C. E. & Doan, T. Azithromycin for severe COVID-19. *The Lancet* **396,** 936–937; 10.1016/S0140-6736(20)31863-8 (2020).

17. Yates, P. A. *et al.* Doxycycline treatment of high-risk COVID-19-positive patients with comorbid pulmonary disease. *Therapeutic Advances in Respiratory Disease* **14**; 10.1177/1753466620951053 (2020).

18. Sodhi, M. & Etminan, M. Therapeutic Potential for Tetracyclines in the Treatment of COVID-19. *Pharmacotherapy* **40,** 487–488; 10.1002/phar.2395 (2020).

19. Baron, S. A., Devaux, C., Colson, P., Raoult, D. & Rolain, J.-M. Teicoplanin: an alternative drug for the treatment of COVID-19? *International journal of antimicrobial agents* **55,** 105944; 10.1016/j.ijantimicag.2020.105944 (2020).

20. Contou, D. *et al.* Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU. *Annals of intensive care* **10,** 119; 10.1186/s13613-020-00736-x (2020).

21. Sharifipour, E. *et al.* Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. *BMC infectious diseases* **20,** 646; 10.1186/s12879-020-05374-z (2020).

22. Langford, B. J. *et al.* Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*; 10.1016/j.cmi.2020.07.016 (2020).

23. Rawson, T. M. *et al.* Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*; 10.1093/cid/ciaa530 (2020).

24. Vaughn, V. M. *et al.* Empiric Antibacterial Therapy and Community-onset Bacterial Co-infection in Patients Hospitalized with COVID-19: A Multi-Hospital Cohort Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*; 10.1093/cid/ciaa1239 (2020).

25. Reardon, S. Antibiotic treatment for COVID-19 complications could fuel resistant bacteria. *Science*; 10.1126/science.abc2995 (2020).

26. Rawson, T. M., Ming, D., Ahmad, R., Moore, L. S. P. & Holmes, A. H. Antimicrobial use, drug-resistant infections and COVID-19. *Nature reviews. Microbiology* **18,** 409–410; 10.1038/s41579-020-0395-y (2020).

27. Bengoechea, J. A. & Bamford, C. G. SARS-CoV-2, bacterial co-infections, and AMR: the deadly trio in COVID-19? *EMBO molecular medicine* **12,** e12560; 10.15252/emmm.202012560 (2020).

28. Huttner, B. D., Catho, G., Pano-Pardo, J. R., Pulcini, C. & Schouten, J. COVID-19: don't neglect antimicrobial stewardship principles! *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **26,** 808–810; 10.1016/j.cmi.2020.04.024 (2020).

29. Buehrle, D. J. *et al.* Antibiotic Consumption and Stewardship at a Hospital outside of an Early Coronavirus Disease 2019 Epicenter. *Antimicrobial agents and chemotherapy* **64**; 10.1128/AAC.01011-20 (2020).

30. Nieuwlaat, R. *et al.* COVID-19 and Antimicrobial Resistance: Parallel and Interacting Health Emergencies. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*; 10.1093/cid/ciaa773 (2020).

31. Bassetti, M. & Giacobbe, D. R. A look at the clinical, economic, and societal impact of antimicrobial resistance in 2020. *Expert opinion on pharmacotherapy,* 1–5; 10.1080/14656566.2020.1802427 (2020).

32. Magiorakos, A.-P. *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **18,** 268–281; 10.1111/j.1469-0691.2011.03570.x (2012).

33. Xin Yu, J., Hubbard-Lucey, V. M. & Tang, J. Immuno-oncology drug development goes global. *Nature reviews. Drug discovery* **18,** 899–900; 10.1038/d41573-019-00167-9 (2019).

34. The Pew Charitable Trusts. Tracking the Global Pipeline of Antibiotics in Development. https://www.pewtrusts.org/en/research-and-analysis/issue-briefs/2020/04/tracking-the-global-pipeline-of-antibiotics-in-development (2020).

35. Beyer, P. & Paulin, S. The Antibacterial Research and Development Pipeline Needs Urgent Solutions. *ACS Infect. Dis.* **6,** 1289–1291; 10.1021/acsinfecdis.0c00044 (2020).

36. World Health Organization. 2019 ANTIBACTERIAL AGENTS IN CLINICAL DEVELOPMENT: an analysis of the antibacterial clinical development pipeline. https://apps.who.int/iris/bitstream/handle/10665/330420/9789240000193-eng.pdf (2019).

37. The Pew Charitable Trusts. A Scientific Roadmap for Antibiotic Discovery. https://www.pewtrusts.org/en/research-and-analysis/reports/2016/05/a-scientific-roadmap-for-antibiotic-discovery (2016).

38. OECD Publishing. Stemming the Superbug Tide: Just A Few Dollars More. https://doi.org/10.1787/9789264307599-en (2018).

39. Årdal, C. *et al.* DRIVE-AB Report: Revitalizing the antibiotic pipeline: Stimulating innovation while driving sustainable use and global access. http://drive-ab.eu/wp-content/uploads/2018/01/CHHJ5467-Drive-AB-Main-Report-180319-WEB.pdf (2018).

40. Kim, W., Prosen, K. R., Lepore, C. J. & Coukell, A. On the Road to Discovering Urgently Needed Antibiotics: So Close Yet So Far Away. *ACS infectious diseases* **6,** 1292–1294; 10.1021/acsinfecdis.0c00100 (2020).

41. Theuretzbacher, U., Outterson, K., Engel, A. & Karlén, A. The global preclinical antibacterial pipeline. *Nature reviews. Microbiology* **18,** 275–285; 10.1038/s41579-019-0288-0 (2020).

42. O'Neill, J. SECURING NEW DRUGS FOR FUTURE GENERATIONS: THE PIPELINE OF ANTIBIOTICS. The review on antimicrobial resistance. https://amr-review.org/sites/default/files/SECURING%20NEW%20DRUGS%20FOR%20FUTURE%20GENERATIONS%20FINAL%20WEB_0.pdf (2015).

43. Hutchings, M. I., Truman, A. W. & Wilkinson, B. Antibiotics: past, present and future. *Current opinion in microbiology* **51,** 72–80; 10.1016/j.mib.2019.10.008 (2019).

44. Lewis, K. The Science of Antibiotic Discovery. *Cell* **181,** 29–45; 10.1016/j.cell.2020.02.056 (2020).

45. Coates, A. R. M., Halls, G. & Hu, Y. Novel classes of antibiotics or more of the same? *British journal of pharmacology* **163,** 184–194; 10.1111/j.1476-5381.2011.01250.x (2011).

46. Hughes, D. & Karlén, A. Discovery and preclinical development of new antibiotics. *Upsala journal of medical sciences* **119,** 162–169; 10.3109/03009734.2014.896437 (2014).

47. Jackson, N., Czaplewski, L. & Piddock, L. J. V. Discovery and development of new antibacterial drugs: learning from experience? *The Journal of antimicrobial chemotherapy* **73,** 1452–1459; 10.1093/jac/dky019 (2018).

48. Payne, D. J., Gwynn, M. N., Holmes, D. J. & Pompliano, D. L. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nature reviews. Drug discovery* **6,** 29–40; 10.1038/nrd2201 (2007).

49. BEAM Alliance. Supporting financial investments on R&D to de-risk antimicrobial development. https://beam-alliance.eu/wp-content/uploads/2019/10/the-beam-push-memo.pdf (2019).

50. Shlaes, D. M. Antibacterial Drugs: The Last Frontier. *ACS infectious diseases* **6,** 1313–1314; 10.1021/acsinfecdis.0c00057 (2020).

51. Rex, J. H. & Outterson, K. Antibiotic reimbursement in a model delinked from sales: a benchmark-based worldwide approach. *The Lancet Infectious Diseases* **16,** 500–505; 10.1016/S1473-3099(15)00500-9 (2016).

52. Outterson, K. A shot in the arm for new antibiotics. *Nature biotechnology* **37,** 1110–1112; 10.1038/s41587-019-0279-8 (2019).

53. Rex, J. H., Fernandez Lynch, H., Cohen, I. G., Darrow, J. J. & Outterson, K. Designing development programs for non-traditional antibacterial agents. *Nature communications* **10,** 3416; 10.1038/s41467-019-11303-9 (2019).

54. Årdal, C. *et al.* Antibiotic development - economic, regulatory and societal challenges. *Nature reviews. Microbiology* **18,** 267–274; 10.1038/s41579-019-0293-3 (2020).

55. *New AMR Action Fund steps in to save collapsing antibiotic pipeline with pharmaceutical industry investment of US$1 billion.* (IFPMA press release, 2020).

56. The Public Health Agency of Sweden. Availability of antibiotics: Reporting of Government commission. https://www.folkhalsomyndigheten.se/contentassets/ab79066a80c7477ca5611ccfb211828e/availability-antibiotics-01229-2017-1.pdf (2017).

57. Mullard, A. UK outlines its antibiotic pull incentive plan. *Nature reviews. Drug discovery* **19,** 298; 10.1038/d41573-020-00070-8 (2020).

58. Mahase, E. UK launches subscription style model for antibiotics to encourage new development. *BMJ (Clinical research ed.)* **369,** m2468; 10.1136/bmj.m2468 (2020).

59. Sen. Michael Bennet and Sen. Todd Young. Pioneering Antimicrobial Subscriptions to End Upsurging Resistance (PASTEUR) Act. https://www.bennet.senate.gov/public/_cache/files/b/5/b5032465-bf79-4dca-beba-50fb9b0f4aee/C1FD8B8C4143F1332DC6A5E5EC09C83B.bon20468.pdf (2020).

60. Danny K. Davis. Developing an Innovative Strategy for Antimicrobial Resistant Microorganisms (DISARM) Act. https://www.congress.gov/116/bills/hr4100/BILLS-116hr4100ih.pdf (2019).

61. Balasegaram, M. & Piddock, L. J. V. The Global Antibiotic Research and Development Partnership (GARDP) Not-for-Profit Model of Antibiotic Development. *ACS Infect. Dis.* **6,** 1295–1298; 10.1021/acsinfecdis.0c00101 (2020).

62. Alm, R. A. & Gallant, K. Innovation in Antimicrobial Resistance: The CARB-X Perspective. *ACS Infect. Dis.* **6,** 1317–1322; 10.1021/acsinfecdis.0c00026 (2020).

63. Innovative Medicines Initiative (IMI) AMR Accelerator Programme. Collaboration for Prevention and Treatment of Multi-Drug Resistant Bacterial Infections (COMBINE). https://amr-accelerator.eu/project/combine/ (2019).

64. BEAM Alliance. BEAM Alliance and global partners call for urgent action on new reimbursement models for life-saving antibiotics. https://beam-alliance.eu/wp-content/uploads/2020/09/post-amr-conference-paper.pdf (2020).

65. Engel, A. Fostering Antibiotic Development through Impact Funding. *ACS Infect. Dis.* **6,** 1311–1312; 10.1021/acsinfecdis.0c00069 (2020).

66. Zuegg, J., Hansford, K. A., Elliott, A. G., Cooper, M. A. & Blaskovich, M. A. T. How to Stimulate and Facilitate Early Stage Antibiotic Discovery. *ACS infectious diseases* **6,** 1302–1304; 10.1021/acsinfecdis.0c00163 (2020).

67. van Hengel, A. J. & Marin, L. Research, Innovation, and Policy: An Alliance Combating Antimicrobial Resistance. *Trends in microbiology* **27,** 287–289; 10.1016/j.tim.2018.12.005 (2019).

68. Simpkin, V. L., Renwick, M. J., Kelly, R. & Mossialos, E. Incentivising innovation in antibiotic drug discovery and development: progress, challenges and next steps. *The Journal of antibiotics* **70,** 1087–1096; 10.1038/ja.2017.124 (2017).

69. BEAM Alliance. Incentivising a sustainable response to the threat of AMR. https://beam-alliance.eu/wp-content/uploads/2019/11/the-beam-pull-memo.pdf (2019).

70. European Commission. A European One Health Action Plan against Antimicrobial Resistance (AMR). https://ec.europa.eu/health/amr/sites/health/files/antimicrobial_resistance/docs/amr_2017_action-plan.pdf (2017).

71. World Health Organization. Global Action Plan on Antimicrobial Resistance. https://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/ (2015).

72. Hughes, J. P., Rees, S., Kalindjian, S. B. & Philpott, K. L. Principles of early drug discovery. *British journal of pharmacology* **162,** 1239–1249; 10.1111/j.1476-5381.2010.01127.x (2011).

73. Reddy, A. S. & Zhang, S. Polypharmacology: drug discovery for the future. *Expert review of clinical pharmacology* **6,** 41–47; 10.1586/ecp.12.74 (2013).

74. Tyers, M. & Wright, G. D. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. *Nature reviews. Microbiology* **17,** 141–155; 10.1038/s41579-018-0141-x (2019).

75. O'Shea, R. & Moser, H. E. Physicochemical properties of antibacterial compounds: implications for drug discovery. *Journal of medicinal chemistry* **51,** 2871–2878; 10.1021/jm700967e (2008).

76. Reck, F., Jansen, J. M. & Moser, H. E. Challenges of antibacterial drug discovery. *Arkivoc* **2019,** 227–244; 10.24820/ark.5550190.p010.955 (2020).

77. AMR Industry Alliance. AMR Industry Declaration. https://www.amrindustryalliance.org/wp-content/uploads/2017/12/AMR-Industry-Declaration.pdf (2016).

78. Karawajczyk, A., Orrling, K. M., Vlieger, J. S. B. de, Rijnders, T. & Tzalis, D. The European Lead Factory: A Blueprint for Public-Private Partnerships in Early Drug Discovery. *Frontiers in medicine* **3,** 75; 10.3389/fmed.2016.00075 (2016).

79. Morgentin, R. *et al.* Translation of innovative chemistry into screening libraries: an exemplar partnership from the European Lead Factory. *Drug discovery today* **23,** 1578–1583; 10.1016/j.drudis.2018.05.007 (2018).

80. Besnard, J., Jones, P. S., Hopkins, A. L. & Pannifer, A. D. The Joint European Compound Library: boosting precompetitive research. *Drug discovery today* **20,** 181–186; 10.1016/j.drudis.2014.08.014 (2015).

81. Theuretzbacher, U. & Piddock, L. J. V. Non-traditional Antibacterial Therapeutic Options and Challenges. *Cell host & microbe* **26,** 61–72; 10.1016/j.chom.2019.06.004 (2019).

82. Fleitas Martínez, O., Cardoso, M. H., Ribeiro, S. M. & Franco, O. L. Recent Advances in Anti-virulence Therapeutic Strategies With a Focus on Dismantling Bacterial Membrane Microdomains, Toxin Neutralization, Quorum-Sensing Interference and Biofilm Inhibition. *Frontiers in cellular and infection microbiology* **9,** 74; 10.3389/fcimb.2019.00074 (2019).

83. Kumar, A., Ellermann, M. & Sperandio, V. Taming the Beast: Interplay between Gut Small Molecules and Enteric Pathogens. *Infection and immunity* **87**; 10.1128/iAI.00131-19 (2019).

84. Laws, M., Shaaban, A. & Rahman, K. M. Antibiotic resistance breakers: current approaches and future directions. *FEMS microbiology reviews* **43,** 490–516; 10.1093/femsre/fuz014 (2019).

85. Annunziato, G. Strategies to Overcome Antimicrobial Resistance (AMR) Making Use of Non-Essential Target Inhibitors: A Review. *International journal of molecular sciences* **20**; 10.3390/ijms20235844 (2019).

86. Calvert, M. B., Jumde, V. R. & Titz, A. Pathoblockers or antivirulence drugs as a new option for the treatment of bacterial infections. *Beilstein journal of organic chemistry* **14,** 2607–2617; 10.3762/bjoc.14.239 (2018).

87. Schütz, C. & Empting, M. Targeting the Pseudomonas quinolone signal quorum sensing system for the discovery of novel anti-infective pathoblockers. *Beilstein journal of organic chemistry* **14,** 2627–2645; 10.3762/bjoc.14.241 (2018).

88. Sommer, R. *et al.* Glycomimetic, Orally Bioavailable LecB Inhibitors Block Biofilm Formation of Pseudomonas aeruginosa. *Journal of the American Chemical Society* **140,** 2537–2545; 10.1021/jacs.7b11133 (2018).

89. Boudaher, E. & Shaffer, C. L. Inhibiting bacterial secretion systems in the fight against antibiotic resistance. *MedChemComm* **10,** 682–692; 10.1039/C9MD00076C (2019).

90. Duncan, M. C., Linington, R. G. & Auerbuch, V. Chemical inhibitors of the type three secretion system: disarming bacterial pathogens. *Antimicrobial agents and chemotherapy* **56,** 5433–5441; 10.1128/AAC.00975-12 (2012).

91. Schönauer, E. *et al.* Discovery of a Potent Inhibitor Class with High Selectivity toward Clostridial Collagenases. *Journal of the American Chemical Society* **139,** 12696–12703; 10.1021/jacs.7b06935 (2017).

92. Paolino, M. *et al.* Development of Potent Inhibitors of the Mycobacterium tuberculosis Virulence Factor Zmp1 and Evaluation of Their Effect on Mycobacterial Survival inside Macrophages. *ChemMedChem* **13,** 422–430; 10.1002/cmdc.201700759 (2018).

93. Marshall, R. L. *et al.* New Multidrug Efflux Inhibitors for Gram-Negative Bacteria. *mBio* **11**; 10.1128/mBio.01340-20 (2020).

94. Bush, K. & Bradford, P. A. Interplay between β-lactamases and new β-lactamase inhibitors. *Nature reviews. Microbiology* **17,** 295–306; 10.1038/s41579-019-0159-8 (2019).

95. Tooke, C. L. *et al.* β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *Journal of molecular biology* **431,** 3472–3500; 10.1016/j.jmb.2019.04.002 (2019).

96. Smirnova, G. V. & Oktyabrsky, O. N. Glutathione in bacteria. *Biochemistry. Biokhimiia* **70,** 1199–1211; 10.1007/s10541-005-0248-3 (2005).

97. Chen, N. H. *et al.* A glutathione-dependent detoxification system is required for formaldehyde resistance and optimal survival of Neisseria meningitidis in biofilms. *Antioxidants & redox signaling* **18,** 743–755; 10.1089/ars.2012.4749 (2013).

98. Lu, P. *et al.* The anti-mycobacterial activity of the cytochrome bcc inhibitor Q203 can be enhanced by small-molecule inhibition of cytochrome bd. *Scientific reports* **8,** 2625; 10.1038/s41598-018-20989-8 (2018).

99. Iqbal, I. K., Bajeli, S., Akela, A. K. & Kumar, A. Bioenergetics of Mycobacterium: An Emerging Landscape for Drug Discovery. *Pathogens (Basel, Switzerland)* **7**; 10.3390/pathogens7010024 (2018).

100. Fol, M., Włodarczyk, M. & Druszczyńska, M. Host Epigenetics in Intracellular Pathogen Infections. *International journal of molecular sciences* **21**; 10.3390/ijms21134573 (2020).

101. Ghosh, D., Veeraraghavan, B., Elangovan, R. & Vivekanandan, P. Antibiotic Resistance and Epigenetics: More to It than Meets the Eye. *Antimicrobial agents and chemotherapy* **64**; 10.1128/AAC.02225-19 (2020).

102. Venter, H. Reversing resistance to counter antimicrobial resistance in the World Health Organisation's critical priority of most dangerous pathogens. *Bioscience reports* **39**; 10.1042/BSR20180474 (2019).

103. Rezzoagli, C., Archetti, M., Mignot, I., Baumgartner, M. & Kümmerli, R. Combining antibiotics with antivirulence compounds can have synergistic effects and reverse selection for antibiotic resistance in Pseudomonas aeruginosa. *PLoS biology* **18,** e3000805; 10.1371/journal.pbio.3000805 (2020).

104. Strich, J. R. *et al.* Needs assessment for novel Gram-negative antibiotics in US hospitals: a retrospective cohort study. *The Lancet Infectious Diseases* **20,** 1172–1181; 10.1016/S1473-3099(20)30153-5 (2020).

105. Lakemeyer, M., Zhao, W., Mandl, F. A., Hammann, P. & Sieber, S. A. Thinking Outside the Box-Novel Antibacterials To Tackle the Resistance Crisis. *Angewandte Chemie (International ed. in English)* **57,** 14440–14475; 10.1002/anie.201804971 (2018).

106. Hennessen, F. *et al.* Amidochelocardin Overcomes Resistance Mechanisms Exerted on Tetracyclines and Natural Chelocardin. *Antibiotics (Basel, Switzerland)* **9**; 10.3390/antibiotics9090619 (2020).

107. Sarigul, N., Korkmaz, F. & Kurultak, İ. A New Artificial Urine Protocol to Better Imitate Human Urine. *Scientific reports* **9,** 20159; 10.1038/s41598-019-56693-4 (2019).

108. Ganz, T. & Nemeth, E. Iron homeostasis in host defence and inflammation. *Nature reviews. Immunology* **15,** 500–510; 10.1038/nri3863 (2015).

109. Martins, A. C., Almeida, J. I., Lima, I. S., Kapitão, A. S. & Gozzelino, R. Iron Metabolism and the Inflammatory Response. *IUBMB life* **69,** 442–450; 10.1002/iub.1635 (2017).

110. Krismer, B., Weidenmaier, C., Zipperer, A. & Peschel, A. The commensal lifestyle of Staphylococcus aureus and its interactions with the nasal microbiota. *Nature reviews. Microbiology* **15,** 675–687; 10.1038/nrmicro.2017.104 (2017).

111. Ayaz, M. *et al.* Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. *Chemico-biological interactions* **308,** 294–303; 10.1016/j.cbi.2019.05.050 (2019).

112. Mahomoodally, M. F. & Sadeer, N. B. Antibiotic potentiation of natural products: A promising target to fight pathogenic bacteria. *Current drug targets*; 10.2174/1389450121666200924113740 (2020).

113. Parkinson, E. I. *et al.* Deoxynybomycins inhibit mutant DNA gyrase and rescue mice infected with fluoroquinolone-resistant bacteria. *Nature communications* **6,** 6947; 10.1038/ncomms7947 (2015).

114. King, A. M. *et al.* Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* **510,** 503–506; 10.1038/nature13445 (2014).

115. Park, S. W. *et al.* Target-based identification of whole-cell active inhibitors of biotin biosynthesis in Mycobacterium tuberculosis. *Chemistry & biology* **22,** 76–86; 10.1016/j.chembiol.2014.11.012 (2015).

116. Verma, S. & Prabhakar, Y. S. Target based drug design - a reality in virtual sphere. *Current medicinal chemistry* **22,** 1603–1630; 10.2174/0929867322666150209151209 (2015).

117. Graef, F. *et al.* In Vitro Model of the Gram-Negative Bacterial Cell Envelope for Investigation of Anti-Infective Permeation Kinetics. *ACS Infect. Dis.* **4,** 1188–1196; 10.1021/acsinfecdis.7b00165 (2018).

118. Richter, R. *et al.* A hydrogel-based in vitro assay for the fast prediction of antibiotic accumulation in Gram-negative bacteria. *Materials Today Bio,* 100084; 10.1016/j.mtbio.2020.100084 (2020).

119. Martin, E. J. *et al.* All-Assay-Max2 pQSAR: Activity Predictions as Accurate as Four-Concentration IC50s for 8558 Novartis Assays. *Journal of chemical information and modeling* **59,** 4450–4459; 10.1021/acs.jcim.9b00375 (2019).

120. Durrant, J. D. & Amaro, R. E. Machine-learning techniques applied to antibacterial drug discovery. *Chemical biology & drug design* **85,** 14–21; 10.1111/cbdd.12423 (2015).

121. Newman, D. J. & Cragg, G. M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of natural products* **83,** 770–803; 10.1021/acs.jnatprod.9b01285 (2020).

122. Locey, K. J. & Lennon, J. T. Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America* **113,** 5970–5975; 10.1073/pnas.1521291113 (2016).

123. Mousa, W. K., Athar, B., Merwin, N. J. & Magarvey, N. A. Antibiotics and specialized metabolites from the human microbiota. *Natural product reports* **34,** 1302–1331; 10.1039/c7np00021a (2017).

124. Zipperer, A. *et al.* Human commensals producing a novel antibiotic impair pathogen colonization. *Nature* **535,** 511–516; 10.1038/nature18634 (2016).

125. Imai, Y. *et al.* A new antibiotic selectively kills Gram-negative pathogens. *Nature* **576,** 459–464; 10.1038/s41586-019-1791-1 (2019).

126. Adnani, N., Rajski, S. R. & Bugni, T. S. Symbiosis-inspired approaches to antibiotic discovery. *Natural product reports* **34,** 784–814; 10.1039/c7np00009j (2017).

127. Lodhi, A. F., Zhang, Y., Adil, M. & Deng, Y. Antibiotic discovery: combining isolation chip (iChip) technology and co-culture technique. *Applied microbiology and biotechnology* **102,** 7333–7341; 10.1007/s00253-018-9193-0 (2018).

128. Kealey, C., Creaven, C. A., Murphy, C. D. & Brady, C. B. New approaches to antibiotic discovery. *Biotechnology letters* **39,** 805–817; 10.1007/s10529-017-2311-8 (2017).

129. Ma, L. *et al.* Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa. *Proceedings of the National Academy of Sciences of the United States of America* **111,** 9768–9773; 10.1073/pnas.1404753111 (2014).

130. Mahler, L. *et al. Highly parallelized microfluidic droplet cultivation and prioritization on antibiotic producers from complex natural microbial communities. doi: 10.1101/2019.12.18.877530* (2019).

131. Jiang, C.-Y. *et al.* High-Throughput Single-Cell Cultivation on Microfluidic Streak Plates. *Applied and environmental microbiology* **82,** 2210–2218; 10.1128/AEM.03588-15 (2016).

132. Ling, L. L. *et al.* A new antibiotic kills pathogens without detectable resistance. *Nature* **517,** 455–459; 10.1038/nature14098 (2015).

133. Pishchany, G. *et al.* Amycomicin is a potent and specific antibiotic discovered with a targeted interaction screen. *Proceedings of the National Academy of Sciences of the United States of America* **115,** 10124–10129; 10.1073/pnas.1807613115 (2018).

134. Seyedsayamdost, M. R. High-throughput platform for the discovery of elicitors of silent bacterial gene clusters. *Proceedings of the National Academy of Sciences of the United States of America* **111,** 7266–7271; 10.1073/pnas.1400019111 (2014).

135. Chaudhary, D. K., Khulan, A. & Kim, J. Development of a novel cultivation technique for uncultured soil bacteria. *Scientific reports* **9,** 6666; 10.1038/s41598-019-43182-x (2019).

136. Masschelein, J., Jenner, M. & Challis, G. L. Antibiotics from Gram-negative bacteria: a comprehensive overview and selected biosynthetic highlights. *Natural product reports* **34,** 712–783; 10.1039/c7np00010c (2017).

137. Hoffmann, T. *et al.* Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. *Nature communications* **9,** 803; 10.1038/s41467-018-03184-1 (2018).

138. Hughes, C. C. & Fenical, W. Antibacterials from the sea. *Chemistry (Weinheim an der Bergstrasse, Germany)* **16,** 12512–12525; 10.1002/chem.201001279 (2010).

139. Abrudan, M. I. *et al.* Socially mediated induction and suppression of antibiosis during bacterial coexistence. *Proceedings of the National Academy of Sciences of the United States of America* **112,** 11054–11059; 10.1073/pnas.1504076112 (2015).

140. Granato, E. T., Meiller-Legrand, T. A. & Foster, K. R. The Evolution and Ecology of Bacterial Warfare. *Current biology : CB* **29,** R521-R537; 10.1016/j.cub.2019.04.024 (2019).

141. Kraemer, S. A., Ramachandran, A. & Perron, G. G. Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy. *Microorganisms* **7**; 10.3390/microorganisms7060180 (2019).

142. Ju, F. *et al.* Wastewater treatment plant resistomes are shaped by bacterial composition, genetic exchange, and upregulated expression in the effluent microbiomes. *The ISME journal* **13,** 346–360; 10.1038/s41396-018-0277-8 (2019).

143. Sierra-Zapata, L. *et al.* Inducible Antibacterial Activity in the Bacillales by Triphenyl Tetrazolium Chloride. *Scientific reports* **10,** 5563; 10.1038/s41598-020-62236-z (2020).

144. Charusanti, P. *et al.* Exploiting adaptive laboratory evolution of Streptomyces clavuligerus for antibiotic discovery and overproduction. *PloS one* **7,** e33727; 10.1371/journal.pone.0033727 (2012).

145. Cowan, M. M. Plant products as antimicrobial agents. *Clinical microbiology reviews* **12,** 564–582 (1999).

146. Newman, D. J. & Cragg, G. M. Plant Endophytes and Epiphytes: Burgeoning Sources of Known and "Unknown" Cytotoxic and Antibiotic Agents? *Planta medica* **86,** 891–905; 10.1055/a-1095-1111 (2020).

147. Hyde, K. D. *et al.* The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity* **97,** 1–136; 10.1007/s13225-019-00430-9 (2019).

148. Silva, O. N. *et al.* Repurposing a peptide toxin from wasp venom into antiinfectives with dual antimicrobial and immunomodulatory properties. *Proceedings of the National Academy of Sciences of the United States of America* **117,** 26936–26945; 10.1073/pnas.2012379117 (2020).

149. Medema, M. H. & Fischbach, M. A. Computational approaches to natural product discovery. *Nature chemical biology* **11,** 639–648; 10.1038/nchembio.1884 (2015).

150. Katz, M., Hover, B. M. & Brady, S. F. Culture-independent discovery of natural products from soil metagenomes. *Journal of industrial microbiology & biotechnology* **43,** 129–141; 10.1007/s10295-015-1706-6 (2016).

151. Crits-Christoph, A., Diamond, S., Butterfield, C. N., Thomas, B. C. & Banfield, J. F. Novel soil bacteria possess diverse genes for secondary metabolite biosynthesis. *Nature* **558,** 440–444; 10.1038/s41586-018-0207-y (2018).

152. Milshteyn, A., Schneider, J. S. & Brady, S. F. Mining the metabiome: identifying novel natural products from microbial communities. *Chemistry & biology* **21,** 1211–1223; 10.1016/j.chembiol.2014.08.006 (2014).

153. Myronovskyi, M. *et al.* Generation of a cluster-free Streptomyces albus chassis strains for improved heterologous expression of secondary metabolite clusters. *Metabolic engineering* **49,** 316–324; 10.1016/j.ymben.2018.09.004 (2018).

154. Gomez-Escribano, J. P. & Bibb, M. J. Engineering Streptomyces coelicolor for heterologous expression of secondary metabolite gene clusters. *Microbial biotechnology* **4,** 207–215; 10.1111/j.1751-7915.2010.00219.x (2011).

155. Komatsu, M. *et al.* Engineered Streptomyces avermitilis host for heterologous expression of biosynthetic gene cluster for secondary metabolites. *ACS synthetic biology* **2,** 384–396; 10.1021/sb3001003 (2013).

156. Sherwood, E. J., Hesketh, A. R. & Bibb, M. J. Cloning and analysis of the planosporicin lantibiotic biosynthetic gene cluster of Planomonospora alba. *Journal of bacteriology* **195,** 2309–2321; 10.1128/JB.02291-12 (2013).

157. Ahmed, Y. *et al.* Engineering of Streptomyces lividans for heterologous expression of secondary metabolite gene clusters. *Microbial cell factories* **19,** 5; 10.1186/s12934-020-1277-8 (2020).

158. Wang, G. *et al.* CRAGE enables rapid activation of biosynthetic gene clusters in undomesticated bacteria. *Nature microbiology* **4,** 2498–2510; 10.1038/s41564-019-0573-8 (2019).

159. Tong, Y., Weber, T. & Lee, S. Y. CRISPR/Cas-based genome engineering in natural product discovery. *Natural product reports* **36,** 1262–1280; 10.1039/c8np00089a (2019).

160. La Fuente-Núñez, C. de & Lu, T. K. CRISPR-Cas9 technology: applications in genome engineering, development of sequence-specific antimicrobials, and future prospects. *Integrative biology : quantitative biosciences from nano to macro* **9,** 109–122; 10.1039/c6ib00140h (2017).

161. Wang, H. *et al.* ExoCET: exonuclease in vitro assembly combined with RecET recombination for highly efficient direct DNA cloning from complex genomes. *Nucleic acids research* **46,** e28; 10.1093/nar/gkx1249 (2018).

162. Zeng, F. *et al.* AFEAP cloning: a precise and efficient method for large DNA sequence assembly. *BMC biotechnology* **17,** 81; 10.1186/s12896-017-0394-x (2017).

163. Snoeck, N. *et al.* Serine integrase recombinational engineering (SIRE): A versatile toolbox for genome editing. *Biotechnology and bioengineering* **116,** 364–374; 10.1002/bit.26854 (2019).

164. Kautsar, S. A. *et al.* MIBiG 2.0: a repository for biosynthetic gene clusters of known function. *Nucleic acids research* **48,** D454-D458; 10.1093/nar/gkz882 (2020).

165. Blin, K. *et al.* antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic acids research* **47,** W81-W87; 10.1093/nar/gkz310 (2019).

166. Skinnider, M. A. *et al.* Comprehensive prediction of secondary metabolite structure and biological activity from microbial genome sequences. *Nature communications* **11,** 6058; 10.1038/s41467-020-19986-1 (2020).

167. Alanjary, M., Cano-Prieto, C., Gross, H. & Medema, M. H. Computer-aided re-engineering of nonribosomal peptide and polyketide biosynthetic assembly lines. *Natural product reports* **36,** 1249–1261; 10.1039/C9NP00021F (2019).

168. Cummings, M. *et al.* Assembling a plug-and-play production line for combinatorial biosynthesis of aromatic polyketides in Escherichia coli. *PLoS biology* **17,** e3000347; 10.1371/journal.pbio.3000347 (2019).

169. Hwang, S., Lee, N., Cho, S., Palsson, B. & Cho, B.-K. Repurposing Modular Polyketide Synthases and Non-ribosomal Peptide Synthetases for Novel Chemical Biosynthesis. *Frontiers in molecular biosciences* **7,** 87; 10.3389/fmolb.2020.00087 (2020).

170. Bozhüyük, K. A. J. *et al.* Modification and de novo design of non-ribosomal peptide synthetases using specific assembly points within condensation domains. *Nature chemistry* **11,** 653–661; 10.1038/s41557-019-0276-z (2019).

171. Luo, Y., Enghiad, B. & Zhao, H. New tools for reconstruction and heterologous expression of natural product biosynthetic gene clusters. *Natural product reports* **33,** 174–182; 10.1039/c5np00085h (2016).

172. Tan, G.-Y. & Liu, T. Rational synthetic pathway refactoring of natural products biosynthesis in actinobacteria. *Metabolic engineering* **39,** 228–236; 10.1016/j.ymben.2016.12.006 (2017).

173. Zhang, J. J., Tang, X. & Moore, B. S. Genetic platforms for heterologous expression of microbial natural products. *Natural product reports* **36,** 1313–1332; 10.1039/C9NP00025A (2019).

174. Huo, L. *et al.* Heterologous expression of bacterial natural product biosynthetic pathways. *Natural product reports* **36,** 1412–1436; 10.1039/c8np00091c (2019).

175. Ito, T. & Masubuchi, M. Dereplication of microbial extracts and related analytical technologies. *The Journal of antibiotics* **67,** 353–360; 10.1038/ja.2014.12 (2014).

176. Quinn, R. A. *et al.* Molecular Networking As a Drug Discovery, Drug Metabolism, and Precision Medicine Strategy. *Trends in pharmacological sciences* **38,** 143–154; 10.1016/j.tips.2016.10.011 (2017).

177. Ernst, M. *et al.* MolNetEnhancer: Enhanced Molecular Networks by Integrating Metabolome Mining and Annotation Tools. *Metabolites* **9**; 10.3390/metabo9070144 (2019).

178. Liebal, U. W., Phan, A. N. T., Sudhakar, M., Raman, K. & Blank, L. M. Machine Learning Applications for Mass Spectrometry-Based Metabolomics. *Metabolites* **10**; 10.3390/metabo10060243 (2020).

179. Hubert, J., Nuzillard, J.-M. & Renault, J.-H. Dereplication strategies in natural product research: How many tools and methodologies behind the same concept? *Phytochem Rev* **16,** 55–95; 10.1007/s11101-015-9448-7 (2017).

180. Li, Y., Kuhn, M., Gavin, A.-C. & Bork, P. Identification of metabolites from tandem mass spectra with a machine learning approach utilizing structural features. *Bioinformatics (Oxford, England)* **36,** 1213–1218; 10.1093/bioinformatics/btz736 (2020).

181. Zani, C. L. & Carroll, A. R. Database for Rapid Dereplication of Known Natural Products Using Data from MS and Fast NMR Experiments. *Journal of natural products* **80,** 1758–1766; 10.1021/acs.jnatprod.6b01093 (2017).

182. van Santen, J. A. *et al.* The Natural Products Atlas: An Open Access Knowledge Base for Microbial Natural Products Discovery. *ACS central science* **5,** 1824–1833; 10.1021/acscentsci.9b00806 (2019).

183. Wang, M. *et al.* Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature biotechnology* **34,** 828–837; 10.1038/nbt.3597 (2016).

184. Guijas, C. *et al.* METLIN: A Technology Platform for Identifying Knowns and Unknowns. *Analytical chemistry* **90,** 3156–3164; 10.1021/acs.analchem.7b04424 (2018).

185. Kuhn, S. & Schlörer, N. E. Facilitating quality control for spectra assignments of small organic molecules: nmrshiftdb2--a free in-house NMR database with integrated LIMS for

academic service laboratories. *Magnetic resonance in chemistry : MRC* **53,** 582–589; 10.1002/mrc.4263 (2015).

186. López-Pérez, J. L., Therón, R., del Olmo, E. & Díaz, D. NAPROC-13: a database for the dereplication of natural product mixtures in bioassay-guided protocols. *Bioinformatics (Oxford, England)* **23,** 3256–3257; 10.1093/bioinformatics/btm516 (2007).

187. Bader, C. D., Neuber, M., Panter, F., Krug, D. & Müller, R. Supercritical Fluid Extraction Enhances Discovery of Secondary Metabolites from Myxobacteria. *Analytical chemistry*; 10.1021/acs.analchem.0c02995 (2020).

188. Nass, N. M. *et al.* Revisiting unexploited antibiotics in search of new antibacterial drug candidates: the case of γ-actinorhodin. *Scientific reports* **7,** 17419; 10.1038/s41598-017-17232-1 (2017).

189. Ishikawa, M. *et al.* Lower ototoxicity and absence of hidden hearing loss point to gentamicin C1a and apramycin as promising antibiotics for clinical use. *Scientific reports* **9,** 2410; 10.1038/s41598-019-38634-3 (2019).

190. Lešnik, U. *et al.* Construction of a new class of tetracycline lead structures with potent antibacterial activity through biosynthetic engineering. *Angewandte Chemie (International ed. in English)* **54,** 3937–3940; 10.1002/anie.201411028 (2015).

191. Grandclaudon, C. *et al.* Semisynthesis and biological evaluation of amidochelocardin derivatives as broad-spectrum antibiotics. *European journal of medicinal chemistry* **188,** 112005; 10.1016/j.ejmech.2019.112005 (2020).

192. Omollo, C. *et al. Developing synergistic drug combinations to restore antibiotic sensitivity in drug-resistant Mycobacterium tuberculosis* (2019).

193. Tan, J. H. *et al.* In Vitro and In Vivo Efficacy of an LpxC Inhibitor, CHIR-090, Alone or Combined with Colistin against Pseudomonas aeruginosa Biofilm. *Antimicrobial agents and chemotherapy* **61**; 10.1128/AAC.02223-16 (2017).

194. Mariathasan, S. & Tan, M.-W. Antibody-Antibiotic Conjugates: A Novel Therapeutic Platform against Bacterial Infections. *Trends in molecular medicine* **23,** 135–149; 10.1016/j.molmed.2016.12.008 (2017).

195. Koenig, S. G. & Pillow, T. H. Discovery and Development of the First Antibody–Antibiotic Conjugate Linker-Drug. In *Complete Accounts of Integrated Drug Discovery and Development: Recent Examples from the Pharmaceutical Industry Volume 2,* edited by J. A. Pesti, A. F. Abdel-Magid & R. Vaidyanathan (American Chemical Society, Washington, DC, 2019), Vol. 1332, pp. 85–105.

196. Negash, K. H., Norris, J. K. S. & Hodgkinson, J. T. Siderophore-Antibiotic Conjugate Design: New Drugs for Bad Bugs? *Molecules (Basel, Switzerland)* **24**; 10.3390/molecules24183314 (2019).

197. Schalk, I. J. Siderophore-antibiotic conjugates: exploiting iron uptake to deliver drugs into bacteria. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **24,** 801–802; 10.1016/j.cmi.2018.03.037 (2018).

198. Blaskovich, M. A. T. *et al.* Protein-inspired antibiotics active against vancomycin- and daptomycin-resistant bacteria. *Nature communications* **9,** 22; 10.1038/s41467-017-02123-w (2018).

199. Umstätter, F. *et al.* Vancomycin Resistance Is Overcome by Conjugation of Polycationic Peptides. *Angewandte Chemie (International ed. in English)* **59,** 8823–8827; 10.1002/anie.202002727 (2020).

200. Kim, A. *et al.* Pharmacodynamic Profiling of a Siderophore-Conjugated Monocarbam in Pseudomonas aeruginosa: Assessing the Risk for Resistance and Attenuated Efficacy. *Antimicrobial agents and chemotherapy* **59,** 7743–7752; 10.1128/AAC.00831-15 (2015).

201. Stokes, J. M. *et al.* A Deep Learning Approach to Antibiotic Discovery. *Cell* **180,** 688-702.e13; 10.1016/j.cell.2020.01.021 (2020).

202. La Fuente-Nunez, C. de. Toward Autonomous Antibiotic Discovery. *mSystems* **4**; 10.1128/mSystems.00151-19 (2019).

203. Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings 1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23 (1997) 3–25. 1. *Advanced Drug Delivery Reviews* **46,** 3–26; 10.1016/s0169-409x(00)00129-0 (2001).

204. Lipinski, C. A. Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. *Advanced Drug Delivery Reviews* **101,** 34–41; 10.1016/j.addr.2016.04.029 (2016).

205. Benet, L. Z., Hosey, C. M., Ursu, O. & Oprea, T. I. BDDCS, the Rule of 5 and drugability. *Advanced Drug Delivery Reviews* **101,** 89–98; 10.1016/j.addr.2016.05.007 (2016).

206. DeGoey, D. A., Chen, H.-J., Cox, P. B. & Wendt, M. D. Beyond the Rule of 5: Lessons Learned from AbbVie's Drugs and Compound Collection. *Journal of medicinal chemistry* **61,** 2636–2651; 10.1021/acs.jmedchem.7b00717 (2018).

207. Doak, B. C., Over, B., Giordanetto, F. & Kihlberg, J. Oral druggable space beyond the rule of 5: insights from drugs and clinical candidates. *Chemistry & biology* **21,** 1115–1142; 10.1016/j.chembiol.2014.08.013 (2014).

208. Tyagi, M., Begnini, F., Poongavanam, V., Doak, B. C. & Kihlberg, J. Drug Syntheses Beyond the Rule of 5. *Chemistry (Weinheim an der Bergstrasse, Germany)* **26,** 49–88; 10.1002/chem.201902716 (2020).

209. Wright, P. M., Seiple, I. B. & Myers, A. G. The evolving role of chemical synthesis in antibacterial drug discovery. *Angewandte Chemie (International ed. in English)* **53,** 8840–8869; 10.1002/anie.201310843 (2014).

210. Agarwal, V. *et al.* Enzymatic Halogenation and Dehalogenation Reactions: Pervasive and Mechanistically Diverse. *Chemical reviews* **117,** 5619–5674; 10.1021/acs.chemrev.6b00571 (2017).

211. Wilcken, R., Zimmermann, M. O., Lange, A., Joerger, A. C. & Boeckler, F. M. Principles and applications of halogen bonding in medicinal chemistry and chemical biology. *Journal of medicinal chemistry* **56,** 1363–1388; 10.1021/jm3012068 (2013).

212. United Nations: Secretariat of the Convention on Biological Diversity. NAGOYA PROTOCOL. https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf (2011).

213. Sorokina, M. & Steinbeck, C. Review on natural products databases: where to find data in 2020. *J Cheminform* **12**; 10.1186/s13321-020-00424-9 (2020).

214. European Commission. MANIFESTO FOR EU COVID-19 RESEARCH: MAXIMISING THE ACCESSIBILITY OF RESEARCH RESULTS IN THE FIGHT AGAINST COVID-19. https://ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/coronavirus-research-and-innovation/covid-research-manifesto_en (2020).

215. Wibberg, D. *et al.* High quality genome sequences of thirteen Hypoxylaceae (Ascomycota) strengthen the phylogenetic family backbone and enable the discovery of new taxa. *Fungal Diversity*; 10.1007/s13225-020-00447-5 (2020).

216. Kling, A. *et al.* Antibiotics. Targeting DnaN for tuberculosis therapy using novel griselimycins. *Science* **348,** 1106–1112; 10.1126/science.aaa4690 (2015).

217. Johnston, C. W. *et al.* Assembly and clustering of natural antibiotics guides target identification. *Nature chemical biology* **12,** 233–239; 10.1038/nchembio.2018 (2016).

218. Panter, F., Krug, D., Baumann, S. & Müller, R. Self-resistance guided genome mining uncovers new topoisomerase inhibitors from myxobacteria. *Chemical science* **9,** 4898–4908; 10.1039/c8sc01325j (2018).

219. Mungan, M. D. *et al.* ARTS 2.0: feature updates and expansion of the Antibiotic Resistant Target Seeker for comparative genome mining. *Nucleic acids research* **48,** W546-W552; 10.1093/nar/gkaa374 (2020).

220. Emmert-Streib, F., Yang, Z., Feng, H., Tripathi, S. & Dehmer, M. An Introductory Review of Deep Learning for Prediction Models With Big Data. *Front. Artif. Intell.* **3**; 10.3389/frai.2020.00004 (2020).

221. Schneider, P. *et al.* Rethinking drug design in the artificial intelligence era. *Nature reviews. Drug discovery* **19,** 353–364; 10.1038/s41573-019-0050-3 (2020).

222. Grein, F. *et al.* Ca2+-Daptomycin targets cell wall biosynthesis by forming a tripartite complex with undecaprenyl-coupled intermediates and membrane lipids. *Nature communications* **11,** 1455; 10.1038/s41467-020-15257-1 (2020).

223. Cardona, S. T., Selin, C. & Gislason, A. S. Genomic tools to profile antibiotic mode of action. *Critical reviews in microbiology* **41,** 465–472; 10.3109/1040841X.2013.866073 (2015).

224. van Camp, P.-J., Haslam, D. B. & Porollo, A. Bioinformatics Approaches to the Understanding of Molecular Mechanisms in Antimicrobial Resistance. *International journal of molecular sciences* **21**; 10.3390/ijms21041363 (2020).

225. O'Rourke, A. *et al.* Mechanism-of-Action Classification of Antibiotics by Global Transcriptome Profiling. *Antimicrobial agents and chemotherapy* **64**; 10.1128/AAC.01207-19 (2020).

226. Nonejuie, P., Burkart, M., Pogliano, K. & Pogliano, J. Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules. *Proceedings of the National Academy of Sciences of the United States of America* **110,** 16169–16174; 10.1073/pnas.1311066110 (2013).

227. Lamsa, A. *et al.* Rapid Inhibition Profiling in Bacillus subtilis to Identify the Mechanism of Action of New Antimicrobials. *ACS chemical biology* **11,** 2222–2231; 10.1021/acschembio.5b01050 (2016).

228. Ribeiro da Cunha, B., Fonseca, L. P. & Calado, C. R. C. Metabolic Fingerprinting with Fourier-Transform Infrared (FTIR) Spectroscopy: Towards a High-Throughput Screening Assay for Antibiotic Discovery and Mechanism-of-Action Elucidation. *Metabolites* **10**; 10.3390/metabo10040145 (2020).

229. Eustice, D. C., Feldman, P. A. & Slee, A. M. The mechanism of action of DuP 721, a new antibacterial agent: effects on macromolecular synthesis. *Biochemical and biophysical research communications* **150,** 965–971; 10.1016/0006-291x(88)90723-1 (1988).

230. Sugimoto, A., Maeda, A., Itto, K. & Arimoto, H. Deciphering the mode of action of cell wall-inhibiting antibiotics using metabolic labeling of growing peptidoglycan in Streptococcus pyogenes. *Scientific reports* **7,** 1129; 10.1038/s41598-017-01267-5 (2017).

231. Cacace, E., Kritikos, G. & Typas, A. Chemical genetics in drug discovery. *Current opinion in systems biology* **4,** 35–42; 10.1016/j.coisb.2017.05.020 (2017).

232. Santiago, M. *et al.* Genome-wide mutant profiling predicts the mechanism of a Lipid II binding antibiotic. *Nature chemical biology* **14,** 601–608; 10.1038/s41589-018-0041-4 (2018).

233. Wright, M. H. & Sieber, S. A. Chemical proteomics approaches for identifying the cellular targets of natural products. *Natural product reports* **33,** 681–708; 10.1039/C6NP00001K (2016).

234. Drewes, G. & Knapp, S. Chemoproteomics and Chemical Probes for Target Discovery. *Trends in biotechnology* **36,** 1275–1286; 10.1016/j.tibtech.2018.06.008 (2018).

235. Hoerr, V. *et al.* Characterization and prediction of the mechanism of action of antibiotics through NMR metabolomics. *BMC microbiology* **16,** 82; 10.1186/s12866-016-0696-5 (2016).

236. Medeiros-Silva, J. *et al.* High-resolution NMR studies of antibiotics in cellular membranes. *Nature communications* **9,** 3963; 10.1038/s41467-018-06314-x (2018).

237. Tietze, L. F., Krewer, B., Major, F. & Schuberth, I. CD-spectroscopy as a powerful tool for investigating the mode of action of unmodified drugs in live cells. *Journal of the American Chemical Society* **131,** 13031–13036; 10.1021/ja902767f (2009).

238. Altharawi, A., Rahman, K. M. & Chan, K. L. A. Towards identifying the mode of action of drugs using live-cell FTIR spectroscopy. *The Analyst* **144,** 2725–2735; 10.1039/C8AN02218F (2019).

239. Cirnski, K., Coetzee, J., Herrmann, J. & Müller, R. Metabolic Profiling to Determine Bactericidal or Bacteriostatic Effects of New Natural Products using Isothermal Microcalorimetry. *JoVE*; 10.3791/61703 (2020).

240. Arrowsmith, C. H. *et al.* The promise and peril of chemical probes. *Nature chemical biology* **11,** 536–541; 10.1038/nchembio.1867 (2015).

241. Wang, Y. *et al.* Evidence-Based and Quantitative Prioritization of Tool Compounds in Phenotypic Drug Discovery. *Cell chemical biology* **23,** 862–874; 10.1016/j.chembiol.2016.05.016 (2016).

242. Franken, H. *et al.* Thermal proteome profiling for unbiased identification of direct and indirect drug targets using multiplexed quantitative mass spectrometry. *Nature protocols* **10,** 1567–1593; 10.1038/nprot.2015.101 (2015).

243. Schopper, S. *et al.* Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry. *Nature protocols* **12,** 2391–2410; 10.1038/nprot.2017.100 (2017).

244. Piazza, I. *et al.* A Map of Protein-Metabolite Interactions Reveals Principles of Chemical Communication. *Cell* **172,** 358-372.e23; 10.1016/j.cell.2017.12.006 (2018).

245. Dai, L. *et al.* Horizontal Cell Biology: Monitoring Global Changes of Protein Interaction States with the Proteome-Wide Cellular Thermal Shift Assay (CETSA). *Annual review of biochemistry* **88,** 383–408; 10.1146/annurev-biochem-062917-012837 (2019).

246. Bagherian, M. *et al.* Machine learning approaches and databases for prediction of drug-target interaction: a survey paper. *Briefings in bioinformatics* **22,** 247–269; 10.1093/bib/bbz157 (2021).

247. Pliakos, K. & Vens, C. Drug-target interaction prediction with tree-ensemble learning and output space reconstruction. *BMC bioinformatics* **21,** 49; 10.1186/s12859-020-3379-z (2020).

248. Davin-Regli, A. *et al.* Membrane permeability and regulation of drug "influx and efflux" in enterobacterial pathogens. *Current drug targets* **9,** 750–759; 10.2174/138945008785747824 (2008).

249. Louw, G. E. *et al.* A balancing act: efflux/influx in mycobacterial drug resistance. *Antimicrobial agents and chemotherapy* **53,** 3181–3189; 10.1128/AAC.01577-08 (2009).

250. Chitsaz, M. & Brown, M. H. The role played by drug efflux pumps in bacterial multidrug resistance. *Essays in biochemistry* **61,** 127–139; 10.1042/EBC20160064 (2017).

251. Weston, N., Sharma, P., Ricci, V. & Piddock, L. J. V. Regulation of the AcrAB-TolC efflux pump in Enterobacteriaceae. *Research in microbiology* **169,** 425–431; 10.1016/j.resmic.2017.10.005 (2018).

252. Richter, M. F. *et al.* Predictive compound accumulation rules yield a broad-spectrum antibiotic. *Nature* **545,** 299–304; 10.1038/nature22308 (2017).

253. Jukič, M., Gobec, S. & Sova, M. Reaching toward underexplored targets in antibacterial drug design. *Drug development research* **80,** 6–10; 10.1002/ddr.21465 (2019).

254. Manchester, J. I., Buurman, E. T., Bisacchi, G. S. & McLaughlin, R. E. Molecular determinants of AcrB-mediated bacterial efflux implications for drug discovery. *Journal of medicinal chemistry* **55,** 2532–2537; 10.1021/jm201275d (2012).

255. Hevener, K. E. *et al.* Chapter Eighteen - Special Challenges to the Rational Design of Antibacterial Agents. *Annual Reports in Medicinal Chemistry* **48,** 283–298; 10.1016/B978-0-12-417150-3.00018-1.

256. Zavascki, A. P., Goldani, L. Z., Li, J. & Nation, R. L. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *The Journal of antimicrobial chemotherapy* **60,** 1206–1215; 10.1093/jac/dkm357 (2007).

257. Ferrer-Espada, R. *et al.* A permeability-increasing drug synergizes with bacterial efflux pump inhibitors and restores susceptibility to antibiotics in multi-drug resistant Pseudomonas aeruginosa strains. *Scientific reports* **9,** 3452; 10.1038/s41598-019-39659-4 (2019).

258. Schweizer, H. P. Understanding efflux in Gram-negative bacteria: opportunities for drug discovery. *Expert opinion on drug discovery* **7,** 633–642; 10.1517/17460441.2012.688949 (2012).

259. Wagner, S. *et al.* Novel Strategies for the Treatment of Pseudomonas aeruginosa Infections. *Journal of medicinal chemistry* **59,** 5929–5969; 10.1021/acs.jmedchem.5b01698 (2016).

260. Zhang, W., Bai, Y., Wang, Y. & Xiao, W. Polypharmacology in Drug Discovery: A Review from Systems Pharmacology Perspective. *Current pharmaceutical design* **22,** 3171–3181; 10.2174/1381612822666160224142812 (2016).

261. Proschak, E., Stark, H. & Merk, D. Polypharmacology by Design: A Medicinal Chemist's Perspective on Multitargeting Compounds. *Journal of medicinal chemistry* **62,** 420–444; 10.1021/acs.jmedchem.8b00760 (2019).

262. Cunningham, M. L., Kwan, B. P., Nelson, K. J., Bensen, D. C. & Shaw, K. J. Distinguishing on-target versus off-target activity in early antibacterial drug discovery using a macromolecular synthesis assay. *Journal of biomolecular screening* **18,** 1018–1026; 10.1177/1087057113487208 (2013).

263. Singh, R., Sripada, L. & Singh, R. Side effects of antibiotics during bacterial infection: mitochondria, the main target in host cell. *Mitochondrion* **16,** 50–54; 10.1016/j.mito.2013.10.005 (2014).

264. Young, M. J. Off-Target Effects of Drugs that Disrupt Human Mitochondrial DNA Maintenance. *Frontiers in molecular biosciences* **4,** 74; 10.3389/fmolb.2017.00074 (2017).

265. Moullan, N. *et al.* Tetracyclines Disturb Mitochondrial Function across Eukaryotic Models: A Call for Caution in Biomedical Research. *Cell reports* **10,** 1681–1691; 10.1016/j.celrep.2015.02.034 (2015).

266. Pasternak, B., Inghammar, M. & Svanström, H. Fluoroquinolone use and risk of aortic aneurysm and dissection: nationwide cohort study. *BMJ (Clinical research ed.)* **360,** k678; 10.1136/bmj.k678 (2018).

267. Fief, C. A., Hoang, K. G., Phipps, S. D., Wallace, J. L. & Deweese, J. E. Examining the Impact of Antimicrobial Fluoroquinolones on Human DNA Topoisomerase IIα and IIβ. *ACS omega* **4,** 4049–4055; 10.1021/acsomega.8b03428 (2019).

268. Becattini, S., Taur, Y. & Pamer, E. G. Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. *Trends in molecular medicine* **22,** 458–478; 10.1016/j.molmed.2016.04.003 (2016).

269. Bhalodi, A. A., van Engelen, T. S. R., Virk, H. S. & Wiersinga, W. J. Impact of antimicrobial therapy on the gut microbiome. *The Journal of antimicrobial chemotherapy* **74,** i6-i15; 10.1093/jac/dky530 (2019).

270. Wipperman, M. F. *et al.* Antibiotic treatment for Tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Scientific reports* **7,** 10767; 10.1038/s41598-017-10346-6 (2017).

271. Xu, L. *et al.* The effect of antibiotics on the gut microbiome: a metagenomics analysis of microbial shift and gut antibiotic resistance in antibiotic treated mice. *BMC genomics* **21,** 263; 10.1186/s12864-020-6665-2 (2020).

272. Evans, L. E. *et al.* Exploitation of Antibiotic Resistance as a Novel Drug Target: Development of a β-Lactamase-Activated Antibacterial Prodrug. *Journal of medicinal chemistry* **62,** 4411–4425; 10.1021/acs.jmedchem.8b01923 (2019).

273. Hong, J. Natural product synthesis at the interface of chemistry and biology. *Chemistry (Weinheim an der Bergstrasse, Germany)* **20,** 10204–10212; 10.1002/chem.201402804 (2014).

274. Bauer, A. & Brönstrup, M. Industrial natural product chemistry for drug discovery and development. *Natural product reports* **31,** 35–60; 10.1039/C3NP70058E (2014).

275. Sucipto, H., Pogorevc, D., Luxenburger, E., Wenzel, S. C. & Müller, R. Heterologous production of myxobacterial α-pyrone antibiotics in Myxococcus xanthus. *Metabolic engineering* **44,** 160–170; 10.1016/j.ymben.2017.10.004 (2017).

276. Pogorevc, D. *et al.* Production optimization and biosynthesis revision of corallopyronin A, a potent anti-filarial antibiotic. *Metabolic engineering* **55,** 201–211; 10.1016/j.ymben.2019.07.010 (2019).

277. Sun, C. *et al.* A robust platform for the synthesis of new tetracycline antibiotics. *Journal of the American Chemical Society* **130,** 17913–17927; 10.1021/ja806629e (2008).

278. Hogan, P. C. *et al.* Large-scale preparation of key building blocks for the manufacture of fully synthetic macrolide antibiotics. *The Journal of antibiotics* **71,** 318–325; 10.1038/ja.2017.116 (2018).

279. Hüttel, S. *et al.* Discovery and Total Synthesis of Natural Cystobactamid Derivatives with Superior Activity against Gram-Negative Pathogens. *Angewandte Chemie (International ed. in English)* **56,** 12760–12764; 10.1002/anie.201705913 (2017).

280. Zong, Y. *et al.* Gram-scale total synthesis of teixobactin promoting binding mode study and discovery of more potent antibiotics. *Nature communications* **10,** 3268; 10.1038/s41467-019-11211-y (2019).

281. Crater, J. S. & Lievense, J. C. Scale-up of industrial microbial processes. *FEMS microbiology letters* **365**; 10.1093/femsle/fny138 (2018).

282. McDaniel, R., Licari, P. & Khosla, C. Process development and metabolic engineering for the overproduction of natural and unnatural polyketides. *Advances in biochemical engineering/biotechnology* **73,** 31–52; 10.1007/3-540-45300-8_3 (2001).

283. Pham, J. V. *et al.* A Review of the Microbial Production of Bioactive Natural Products and Biologics. *Frontiers in microbiology* **10,** 1404; 10.3389/fmicb.2019.01404 (2019).

284. Balani, S. K., Miwa, G. T., Gan, L.-S., Wu, J.-T. & Lee, F. W. Strategy of utilizing in vitro and in vivo ADME tools for lead optimization and drug candidate selection. *Current topics in medicinal chemistry* **5,** 1033–1038; 10.2174/156802605774297038 (2005).

285. Chung, T. D.Y., Terry, D. B. & Smith, L. H. *Assay Guidance Manual. In Vitro and In Vivo Assessment of ADME and PK Properties During Lead Selection and Lead Optimization – Guidelines, Benchmarks and Rules of Thumb* (Bethesda (MD), 2004).

286. Reichel, A. & Lienau, P. Pharmacokinetics in Drug Discovery: An Exposure-Centred Approach to Optimising and Predicting Drug Efficacy and Safety. *Handbook of experimental pharmacology* **232,** 235–260; 10.1007/164_2015_26 (2016).

287. Singh, S. S. Preclinical pharmacokinetics: an approach towards safer and efficacious drugs. *Current drug metabolism* **7,** 165–182; 10.2174/138920006775541552 (2006).

288. Lüpfert, C. & Reichel, A. Development and application of physiologically based pharmacokinetic-modeling tools to support drug discovery. *Chemistry & biodiversity* **2,** 1462–1486; 10.1002/cbdv.200590119 (2005).

289. Espié, P., Tytgat, D., Sargentini-Maier, M.-L., Poggesi, I. & Watelet, J.-B. Physiologically based pharmacokinetics (PBPK). *Drug metabolism reviews* **41,** 391–407; 10.1080/10837450902891360 (2009).

290. Krause, K. M. *et al.* Potent LpxC Inhibitors with In Vitro Activity against Multidrug-Resistant Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy* **63**; 10.1128/AAC.00977-19 (2019).

291. Cohen, F. *et al.* Optimization of LpxC Inhibitors for Antibacterial Activity and Cardiovascular Safety. *ChemMedChem* **14,** 1560–1572; 10.1002/cmdc.201900287 (2019).

292. Felmlee, M. A., Morris, M. E. & Mager, D. E. Mechanism-based pharmacodynamic modeling. *Methods in molecular biology (Clifton, N.J.)* **929,** 583–600; 10.1007/978-1-62703-050-2_21 (2012).

293. Lepak, A. J. & Andes, D. R. Animal Models to Evaluate Anti-infective Pharmacodynamics. In *Antibiotic Pharmacodynamics,* edited by J. C. Rotschafer, D. R. Andes & K. A. Rodvold (Springer New York, New York, NY, 2016), Vol. 191, pp. 59–87.

294. van Peer, A., Snoeck, E., Huang, M. L. & Heykants, J. Pharmacokinetic-pharmacodynamic relationships in phase I/phase II of drug development. *European journal of drug metabolism and pharmacokinetics* **18,** 49–59; 10.1007/BF03220008 (1993).

295. Zhao, M., Lepak, A. J. & Andes, D. R. Animal models in the pharmacokinetic/pharmacodynamic evaluation of antimicrobial agents. *Bioorganic & medicinal chemistry* **24,** 6390–6400; 10.1016/j.bmc.2016.11.008 (2016).

296. McGinnity, D. F., Collington, J., Austin, R. P. & Riley, R. J. Evaluation of human pharmacokinetics, therapeutic dose and exposure predictions using marketed oral drugs. *Current drug metabolism* **8,** 463–479; 10.2174/138920007780866799 (2007).

297. Zou, P. *et al.* Applications of human pharmacokinetic prediction in first-in-human dose estimation. *The AAPS journal* **14,** 262–281; 10.1208/s12248-012-9332-y (2012).

298. Page, K. M. Validation of Early Human Dose Prediction: A Key Metric for Compound Progression in Drug Discovery. *Molecular pharmaceutics* **13,** 609–620; 10.1021/acs.molpharmaceut.5b00840 (2016).

299. Maher, S., Brayden, D. J., Casettari, L. & Illum, L. Application of Permeation Enhancers in Oral Delivery of Macromolecules: An Update. *Pharmaceutics* **11**; 10.3390/pharmaceutics11010041 (2019).

300. Ma, X. & Williams, R. O. Polymeric nanomedicines for poorly soluble drugs in oral delivery systems: an update. *Journal of Pharmaceutical Investigation* **48,** 61–75 (2018).

301. Pridgen, E. M., Alexis, F. & Farokhzad, O. C. Polymeric nanoparticle technologies for oral drug delivery. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **12,** 1605–1610; 10.1016/j.cgh.2014.06.018 (2014).

302. Russell, W.M.S. and Burch, R.L. *The Principles of Humane Experimental Technique* (London: Methuen & Co. Limited, 1960).

303. Jaeger, A., Sauder, P., Kopferschmitt & Dahlet, M. Toxicokinetics in clinical toxicology. *Acta clinica Belgica. Supplementum* **13,** 1–12; 10.1080/17843286.1990.11718122 (1990).

304. Goehl, T. J. Toxicokinetics in the National Toxicology Program. *NIDA research monograph* **173,** 273–304 (1997).

305. Low, Y. S., Sedykh, A. Y., Rusyn, I. & Tropsha, A. Integrative approaches for predicting in vivo effects of chemicals from their structural descriptors and the results of short-term biological assays. *Current topics in medicinal chemistry* **14,** 1356–1364; 10.2174/1568026614666140506121116 (2014).

306. Anthony Tony Cox, L., Popken, D. A., Kaplan, A. M., Plunkett, L. M. & Becker, R. A. How well can in vitro data predict in vivo effects of chemicals? Rodent carcinogenicity as a case study. *Regulatory toxicology and pharmacology : RTP* **77,** 54–64; 10.1016/j.yrtph.2016.02.005 (2016).

307. Zhang, X. *et al.* Zebrafish and Galleria mellonella: Models to Identify the Subsequent Infection and Evaluate the Immunological Differences in Different Klebsiella pneumoniae Intestinal Colonization Strains. *Frontiers in microbiology* **10,** 2750; 10.3389/fmicb.2019.02750 (2019).

308. Herlich, J. A. *et al.* The non-GLP toleration/dose range finding study: design and methodology used in an early toxicology screening program. *Proceedings of the Western Pharmacology Society* **52,** 94–98 (2009).

309. BEAM Alliance. Position Paper 2017: Key Guidelines to implement effective measures toward SMEs to revive the antibacterial R&D field. https://beam-alliance.eu/wp-content/uploads/2019/03/2017-11-16-beam-alliance-position-paper.pdf (2017).

310. BEAM Alliance. A new vision for AMR innovation to support medical care. https://beam-alliance.eu/wp-content/uploads/2019/10/beam-alliance-a-new-vision-to-support-amr-innovation.pdf (2019).

311. BEAM Alliance. Reflexion paper on the EU Pharmaceutical Strategy Roadmap. https://beam-alliance.eu/wp-content/uploads/2020/07/roadmap-reflexion-paper.pdf (2020).

312. ReAct Europe. AMR STAKEHOLDER MAPPING. https://www.reactgroup.org/uploads/Stakeholder%20Analysis_ReActForWHO.pdf (2016).

313. Fisher, J. F. & Mobashery, S. Endless Resistance. Endless Antibiotics? *MedChemComm* **7,** 37–49; 10.1039/C5MD00394F (2016).

314. DiMasi, J. A., Grabowski, H. G. & Hansen, R. W. Innovation in the pharmaceutical industry: New estimates of R&D costs. *Journal of health economics* **47,** 20–33; 10.1016/j.jhealeco.2016.01.012 (2016).

315. The Boston Consulting Group. Breaking through the Wall - A Call for Concerted Action on Antibiotics Research and Development. https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/5_Publikationen/Gesundheit/Berichte/GUARD_Follow_Up_Report_Full_Report_final.pdf (2017).

316. Access to Medicine Foundation. Antimicrobial Resistance Benchmark 2018. https://accesstomedicinefoundation.org/media/uploads/downloads/5bc5edd8367eb_Antimicrobial-Resistance-Benchmark-2018.pdf (2018).

317. Paul, S. M. *et al.* How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nature reviews. Drug discovery* **9,** 203–214; 10.1038/nrd3078 (2010).

318. Pieroni, M., Wan, B., Cho, S., Franzblau, S. G. & Costantino, G. Design, synthesis and investigation on the structure-activity relationships of N-substituted 2-aminothiazole derivatives as antitubercular agents. *European journal of medicinal chemistry* **72,** 26–34; 10.1016/j.ejmech.2013.11.007 (2014).

319. Azzali, E. *et al.* Substituted N-Phenyl-5-(2-(phenylamino)thiazol-4-yl)isoxazole-3-carboxamides Are Valuable Antitubercular Candidates that Evade Innate Efflux Machinery. *Journal of medicinal chemistry* **60,** 7108–7122; 10.1021/acs.jmedchem.7b00793 (2017).

320. Bohacek, R. S., McMartin, C. & Guida, W. C. The art and practice of structure-based drug design: a molecular modeling perspective. *Medicinal research reviews* **16,** 3–50; 10.1002/(SICI)1098-1128(199601)16:1<3::AID-MED1>3.0.CO;2-6 (1996).

321. Hartwig, J. F. Evolution of a fourth generation catalyst for the amination and thioetherification of aryl halides. *Accounts of chemical research* **41,** 1534–1544; 10.1021/ar800098p (2008).

322. Abdel-Magid, A. F. & Mehrman, S. J. A Review on the Use of Sodium Triacetoxyborohydride in the Reductive Amination of Ketones and Aldehydes. *Org. Process Res. Dev.* **10,** 971–1031; 10.1021/op0601013 (2006).

323. Lovering, F., Bikker, J. & Humblet, C. Escape from flatland: increasing saturation as an approach to improving clinical success. *Journal of medicinal chemistry* **52,** 6752–6756; 10.1021/jm901241e (2009).

324. Zender, M. *et al.* Discovery and biophysical characterization of 2-amino-oxadiazoles as novel antagonists of PqsR, an important regulator of Pseudomonas aeruginosa virulence. *Journal of medicinal chemistry* **56,** 6761–6774; 10.1021/jm400830r (2013).

325. Zender, M. *et al.* Flexible Fragment Growing Boosts Potency of Quorum-Sensing Inhibitors against Pseudomonas aeruginosa Virulence. *ChemMedChem* **15,** 188–194; 10.1002/cmdc.201900621 (2020).

326. Ahmed, A.S.A. *et al. WO2020007938A1: PqsR INVERSE AGONISTS* (2020).

327. Schütz, C. *et al.* A New PqsR Inverse Agonist Potentiates Tobramycin Efficacy to Eradicate Pseudomonas aeruginosa Biofilms. *Adv. Sci.,* 2004369; 10.1002/advs.202004369 (2021).

328. Oliver, T. J. & Sinclair, A.C. *US3155582: Antibiotic M-319* (1964).

329. Mitscher, L. A. *et al.* Structure of chelocardin, a novel tetracycline antibiotic. *Journal of the American Chemical Society* **92,** 6070–6071; 10.1021/ja00723a049 (1970).

330. Petkovic, H., Raspor, P. & Lesnik, U. *US8361777B2: Genes for biosynthesis of tetracycline compounds and uses thereof* (2013).

331. Lukežič, T. *et al.* Identification of the chelocardin biosynthetic gene cluster from Amycolatopsis sulphurea: a platform for producing novel tetracycline antibiotics. *Microbiology (Reading, England)* **159,** 2524–2532; 10.1099/mic.0.070995-0 (2013).

332. Molnar, V., Matković, Z., Tambić, T. & Kozma, C. Klinicko-farmakolosko ispitivanje kelokardina u bolesnika s infekcijom mokraćnih putova. *Lijecnicki vjesnik* **99,** 560–562 (1977).

333. Lukežič, T. *et al.* Engineering Atypical Tetracycline Formation in Amycolatopsis sulphurea for the Production of Modified Chelocardin Antibiotics. *ACS chemical biology* **14,** 468–477; 10.1021/acschembio.8b01125 (2019).

334. Flores-Mireles, A. L., Walker, J. N., Caparon, M. & Hultgren, S. J. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews. Microbiology* **13,** 269–284; 10.1038/nrmicro3432 (2015).

335. Irschik, H., Jansen, R., Höfle, G., Gerth, K. & Reichenbach, H. The corallopyronins, new inhibitors of bacterial RNA synthesis from Myxobacteria. *The Journal of antibiotics* **38,** 145–152; 10.7164/antibiotics.38.145 (1985).

336. O'Neill, A. *et al.* RNA Polymerase Inhibitors with Activity against Rifampin-Resistant Mutants of Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **44,** 3163–3166 (2000).

337. Belogurov, G. A. *et al.* Transcription inactivation through local refolding of the RNA polymerase structure. *Nature* **457,** 332–335; 10.1038/nature07510 (2008).

338. Schäberle, T. F. *et al.* Insights into Structure-Activity Relationships of Bacterial RNA Polymerase Inhibiting Corallopyronin Derivatives. *Journal of natural products* **78,** 2505–2509; 10.1021/acs.jnatprod.5b00175 (2015).

339. Schiefer, A. *et al.* Corallopyronin A Specifically Targets and Depletes Essential Obligate Wolbachia Endobacteria From Filarial Nematodes In Vivo. *The Journal of Infectious Diseases* **206,** 249–257; 10.1093/infdis/jis341 (2012).

340. Pfarr, K. M. *et al. US9687470B2: Compounds for use in the treatment of filariasis* (2017).

341. Pfarr, K. M. *et al. EP2704708B1: Compounds for use in the treatment of filariasis* (2017).

342. World Health Organization. Ending the neglect to attain the Sustainable Development Goals – A road map for neglected tropical diseases 2021–2030. https://www.who.int/neglected_diseases/resources/who-ucn-ntd-2020.01/en/ (2020).

343. Loeper, N. *et al.* Elaborations on Corallopyronin A as a Novel Treatment Strategy Against Genital Chlamydial Infections. *Frontiers in microbiology* **10**; 10.3389/fmicb.2019.00943 (2019).

344. Kock, F. *et al.* Orientia tsutsugamushi Is Highly Susceptible to the RNA Polymerase Switch Region Inhibitor Corallopyronin A In Vitro and In Vivo. *Antimicrobial agents and chemotherapy* **62**; 10.1128/AAC.01732-17 (2018).

345. Shima, K. *et al.* Effective inhibition of rifampicin-resistant Chlamydia trachomatis by the novel DNA-dependent RNA polymerase inhibitor corallopyronin A. *International journal of antimicrobial agents* **52,** 523–524; 10.1016/j.ijantimicag.2018.07.025 (2018).

346. Sucipto, H. *et al. EP2994535A1: Production of myxopyronin and of its derivatives* (2018).

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Competing interests

M. H. Medema is a co-founder of Design Pharmaceuticals and a member of the scientific advisory board of Hexagon Bio, and S. Donadio is a cofounder and shareholder of NAICONS owning IP on antibacterial compounds.

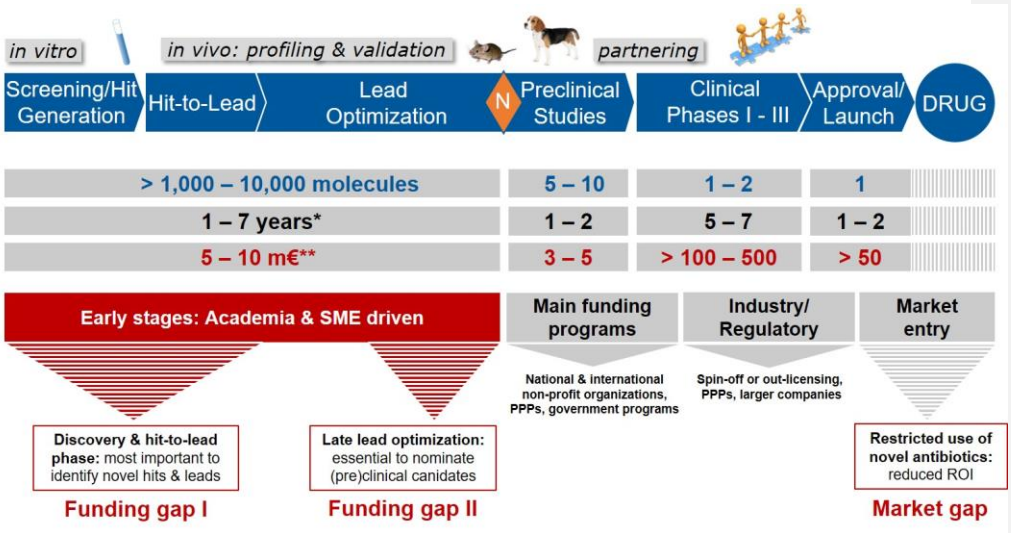


Fig. 1: Current situation of antimicrobial drug development with large funding gaps in the stages of discovery as well as hit and lead optimization associated mainly with academic research and SMEs. Indicated figures are representative numbers of typical broad-spectrum antibiotic development programs leading from several thousands of initial hits to the approval of at least one marketable candidate[72,313–316]. *Timelines (indicated with estimated sub-periods for the different stages) can vary largely based on all factors relevant for straightforwardness and success, assuming a minimum to

maximum span of 8 – 18 (on average 13 – 14) years for the complete development process until market entry. **Cost per molecule/candidate (in million EUROs, m€) does not include extended costs for attrition (failed programs) and lost opportunities associated with increased cycle time until reaching the next development phase; such extensions can increase the required budget for the early stages up to 50 – 100 m€[39,48,317]. N (orange diamond), nomination of (pre)clinical candidate(s); ROI, return on investment; SME, small and medium-sized enterprises; PPPs, public-private partnerships.

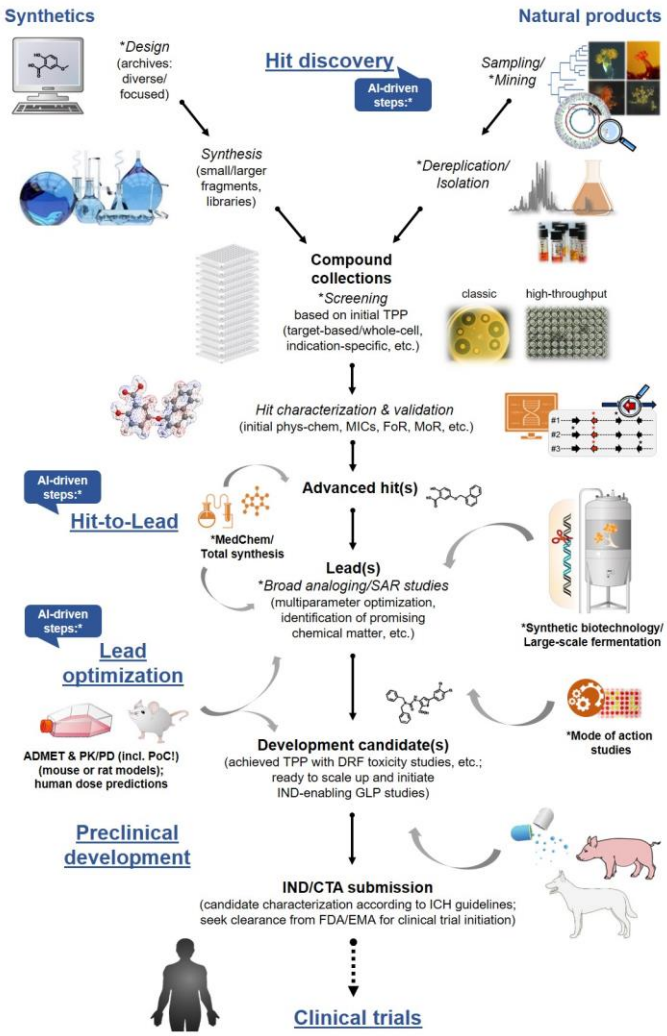


Fig. 2: Summary of major steps and processes in antibacterial drug discovery and development (details given in the text). Approaches marked with * can be linked with emerging artificial intelligence (AI)-

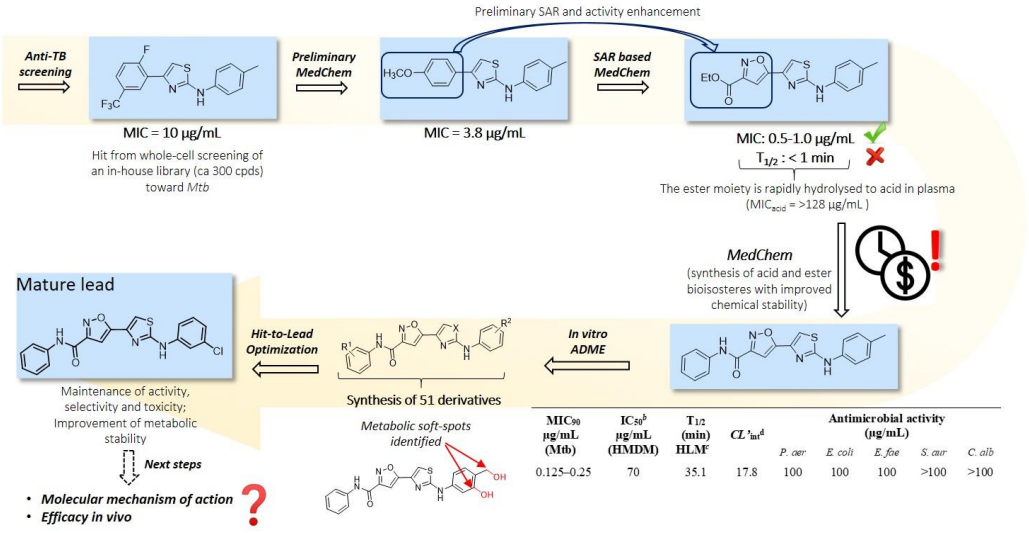
based technology, *e.g.* for advanced data mining, screening or property predictions, to increase efficiency and outcome. Phys-chem, Physico-chemical properties; MICs, minimal inhibitory concentrations; FoR, frequency of resistance; MoR, mechanism of resistance; SAR, structure-activity relationship; TPP, target product profile; GLP, Good Laboratory Practice; ADMET, absorption, distribution, metabolism, excretion, toxicity; PK/PD, pharmacokinetics/pharmacodynamics; PoC, proof of concept; DRF, dose-range finding; IND, investigational new drug; CTA, clinical trials application; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency.

Table 1: SWOT analysis summarizing a path forward in the development of novel antibiotic (AB) drugs

Strengths	**Weaknesses** (current limitations)	**Opportunities** (possible solutions)	**Threats**
● Successful clinical proof of concept for ABs since ~100 years ● Critical need for intensive care units ● Novel ABs are last resort against MDR pathogens ● Knowledge and technologies for AB R&D established and steadily advancing ● High chance of finding new AB classes due to largely under-explored biodiversity ● Large and increasingly diverse chemical libraries available for screening ● Increasingly large and diverse online databases on antibiotic compounds, targets and resistance genes available ● Predictive models available (*via* integrative chem-bioinformatics, *e.g.* PBPK modeling)	● Unavoidable resistance development with classic ABs („vicious circle“) ● Use of ABs in agri- and aqua-culture not globally regulated ● Paucity of novel ~~agents~~ AB classes against Gram-negative~~s~~ priority pathogens ● Lack of innovative assays for hit discovery ● Capacities in academia (MedChem, PK/PD etc.) insufficient for R&D project expansion ● High attrition rates until market entry, especially in the early stages of	● New incentives (push & pull factors) to attract industrial stakeholders: → grants (from governments, health foundations etc.) for innovative programs, *e.g.* early R&D with academia (PPPs) → alliances of physicians, patients and politics (WHO, EU, etc.) advocating for novel ABs → market entry rewards like transferable exclusivity extensions/vouchers (*e.g.* prolonged IP protection of new AB classes or extended protection of other products) → Delinkage models for novel ABs (benchmark-based to insure innovation-driven development)~~models for supply continuity (price-sales delinkage)~~ ~~→ limitations or taxes on generic (“old”) ABs~~ → patent buyouts or payer licenses in return for public control over pricing and distribution → long-term benefits through public prestige, advertisement, etc. ● Advancing cooperation between academia, health foundations and industry (sharing of libraries, data, discovery and translational know-how, IP, etc.) ● Emerging national or international antibiotics research networks, virtual centers and innovation funds ● Academic entrepreneurship (foundation of spin-outs, etc.)	● Rising death toll per year due to AMR ● Loss of expertise in AB R&D in both academia and industry ● Disconnect between early R&D (left to academia) and clinical stages (industry-dependent) ● Disconnect between researchers and regulatory agencies ● Low-cost structure of generic ABs impedes the development of novel classes ● High cost to society (socio-economic burden, increasing patient mortality,

● Loss of efficacy controllable by antibiotic stewardship ● Increasing public awareness of AMR demands for and facilitates national and transnational solutions	discovery and optimization ● High capital risk and negative ROI mainly for industrial sector	● Multiple innovative concepts for non-traditional antibiotics (virulence inhibitors etc.) ● Overcoming existing resistances and/or increase efficiency of ABs by using molecules with synergistic action or innovative conjugates ● Hit discovery becoming more efficient by emerging artificial intelligence technologies ● Innovative concepts can improve: *(i) in vivo* drug delivery, *(ii)* ABinflux in bacterial cells, *(iii)* AB efflux inhibition ● Careful evaluation of regulatory guidelines based on regional discrepancies (high need *vs.* safety)	etc.) over the next decades ● Risk of empty development pipeline if problems (*e.g.* lack of R&D funding) are ignored

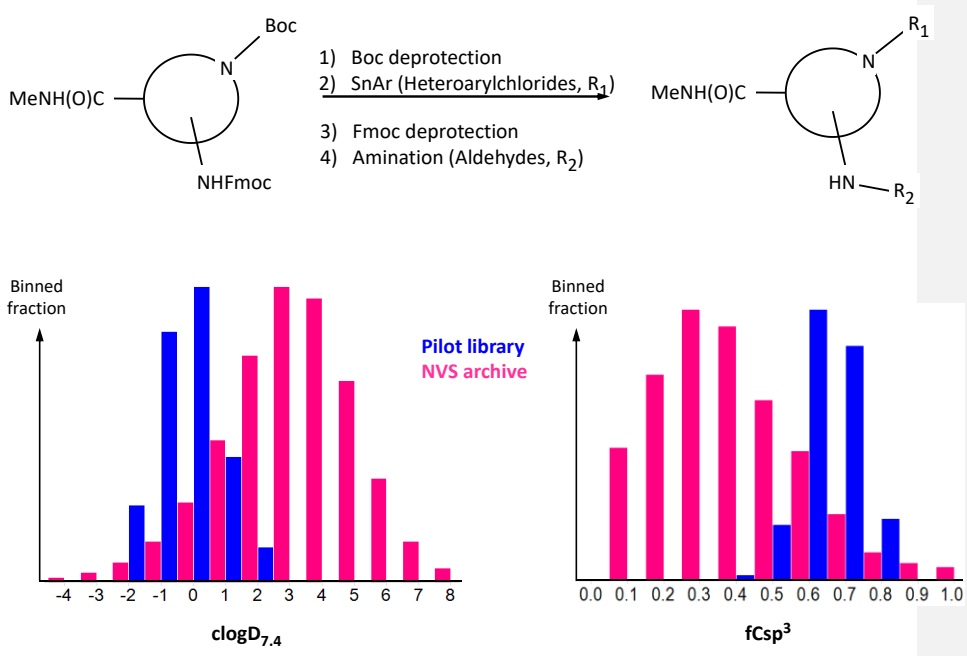
BOX 1: Early stage development of a synthetic antibiotic against *M. tuberculosis*



Historically, the whole-cell assay has fully outclassed target-based methods as the main approach to discover novel antimicrobial drugs. In particular, this applies to antitubercular drugs, where the peculiar cellular structure of *Mycobacterium tuberculosis* (Mtb) is responsible for the lack of correspondence between the biochemical and the phenotypic assays. In this case study, a small in-house chemical library was evaluated via a phenotypic screening against Mtb to identify novel antitubercular chemotypes. A few 2-aminothiazoles were found to be moderately active, and the initial hit series was expanded to investigate the SAR by iterative medicinal chemistry (MedChem) efforts[318], leading to

highly potent derivatives (MICs in the submicromolar range) toward susceptible Mtb. To further promote the advancement of these compounds, additional biological assays were carried out to investigate the activity against multidrug-resistant (MDR) and extensively drug-resistant (XDR) Mtb strains, the selectivity over other bacterial species and eukaryotic cells, and the susceptibility to the action of efflux pumps[319]. The next research step was focused on a hit-to-lead optimization based on the convergent analysis of the SAR and Structure-Metabolism Relationships (SMR). Two metabolic soft-spots were identified, and these findings were instrumental for the design of compounds that escaped rapid clearance by human liver microsomes and, at the same time, maintained good antitubercular activity against both drug-susceptible and drug-resistant strains. At this stage, determination of the mode of action at a molecular level and assays in animal model(s) of infection represent the next research progressions. Generally, academic drug discovery can suffer from long timescales and limited resources which, in turn, make the research process difficult to move forward. For instance, academic chemical libraries are unlikely to yield a significant number of hits from a whole-cell screening, despite the intrinsic chemical novelty that characterizes their creation. Partnership with industrial stakeholders should fill the funding gap and add further expertise, *e.g.* on advanced compound design and *in vivo* studies, to overcome the limitations mentioned above.

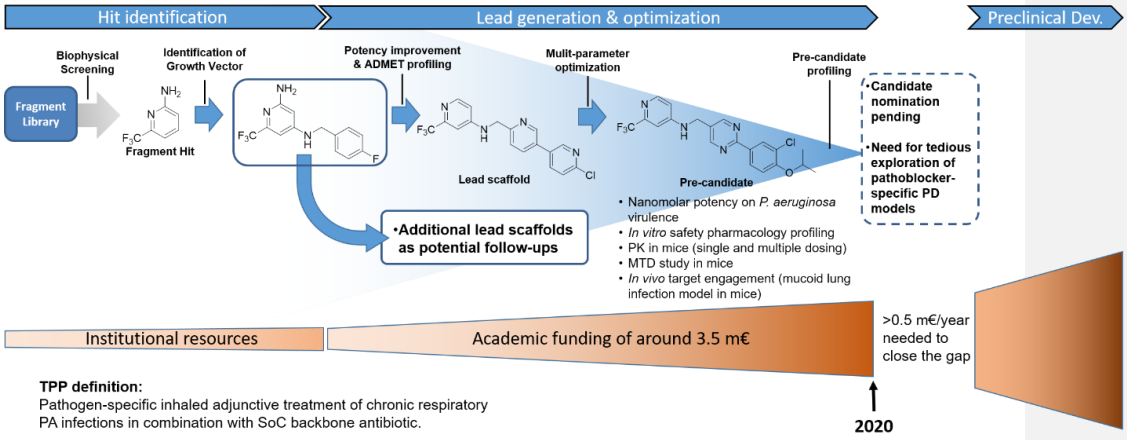
BOX 2: Focused library design generating NCEs within a preferred property space



The chemical drug space has been described as almost infinitely large with an estimated 10^{60} compounds[320]. To exemplify the ease of accessing novel chemical matter within a desired property space[75,76], we designed a focused small library based on commercially available building blocks. The central building block was kept

constant and two substituents were added by nucleophilic heteroaromatic substitution with the secondary amine[321], followed by reductive amination on the primary amine[322]. The *in silico* design was driven by diversity, clogD (pH 7.4) between -2 and 2, molecular weight (Mw) below 450 Dalton, and increased sp^3 content (*i.e.*, level of heavy atom saturation)[323]. For this hypothetical pilot library we chose 15 aldehydes and 15 heteroarylchlorides to provide the 225 compound library shown in comparison to the Novartis (NVS) archive based on polarity ($clogD_{7.4}$) and fraction of sp^3 hybridized carbon atoms (fCsp3). All 225 compounds were unknown in the public domain (Reaxys, https://www.elsevier.com/solutions/reaxys; last accessed in April 2021) and absent from the Novartis archive (April 2018).

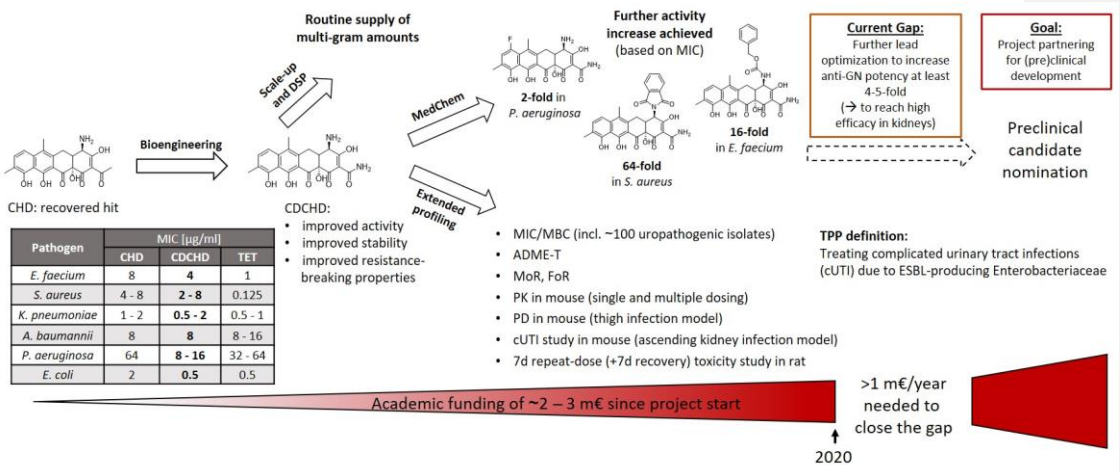
BOX 3: Development of an anti-virulence therapeutic ("pathoblocker") against *P. aeruginosa*



The concept of interfering with the *Pseudomonas* Quinolone Signal (PQS) quorum sensing (QS) system for the discovery of pathoblockers against *Pseudomonas aeruginosa* (PA) has been explored in detail by multiple research groups[87]. Target validation of the bacterial signal molecule receptor PqsR, which functions as a global virulence regulator, has been achieved using mainly acute murine infection models. A target-driven medicinal chemistry campaign tackling this transcriptional regulator has achieved pre-candidate status starting from a fragment-based approach[324–326]. After biophysical screening, initial hit selection was guided by selection of enthalpy-driven binders (as determined by isothermal titration calorimetry; ITC). Successful growth vector identification enabled the detection of qualified hits with cellular anti-virulence activity and potential for advancement to the lead generation and optimization stages[325]. Hit identification was achieved with institutional resources. However, cost-intensive medicinal chemistry and compound profiling work towards a preclinical profiling candidate was only possible via a non-dilutive joint funding, which amounted to approx. 3.5 m€ (see Acknowledgements). The chosen TPP is defined as a pathogen-specific inhaled adjunctive treatment of chronic respiratory PA infections in combination with a standard-of-care (SoC) backbone antibiotic. Resulting pre-candidates have nanomolar on-target and cellular efficacy, potentiate tobramycin efficacy

against PA biofilms, show high exposures *in vivo* (various routes i.t., i.v., s.c., p.o.) and no overt findings in safety pharmacology screens[327]. While demonstration of *in vivo* target engagement by means of signal molecule quantification was achieved swiftly in a mucoid acute murine lung infection model, assaying *in vivo* treatment efficacy related to the pathoblocker-specific activities remains a considerable challenge. Candidate nomination is therefore pending on tedious and expansive exploration of suitable PD models. Currently, this milestone is pursued via further public funding.

BOX 4: Reassessing chelocardin for improved lead development toward cUTI therapy



Pathogen	MIC [µg/ml]		
	CHD	CDCHD	TET
E. faecium	8	**4**	1
S. aureus	4 - 8	**2 - 8**	0.125
K. pneumoniae	1 - 2	**0.5 - 2**	0.5 - 1
A. baumannii	8	**8**	8 - 16
P. aeruginosa	64	**8 - 16**	32 - 64
E. coli	2	**0.5**	0.5

The natural product chelocardin (CHD), a member of the atypical tetracyclines that was first described about 60 years ago[328,329], has recently been recovered to generate a novel lead scaffold, amidochelocardin (2-carboxamido-2-deacetyl-chelocardin, CDCHD), by rational biosynthetic engineering[330]. For this purpose, the CHD biosynthetic gene cluster in *Amycolatopsis sulphurea*[331] was combined with genes from the oxytetracycline biosynthesis pathway of *Streptomyces rimosus*, and production peak titers of the novel hybrid compound CDCHD up to 400 mg/L were achieved[190]. CDCHD represents a new broad-spectrum antibiotic active against pathogens of the ESKAPE panel (including a large number of clinical isolates)[106], which can be routinely supplied at multi-gram scale with >95% purity by using large-scale in-house fermentation at HZI (~100 L batch cultures) and optimized downstream processing. Due to the lack of cross-resistance to known antibiotics (*e.g.*, preserved activity against pathogens carrying multiple tetracycline [TET] resistance determinants), the good production yield and the fact that efficacy for CHD treatment was already shown in a small phase II study[332], CDCHD was chosen to enter a lead optimization program (see Acknowledgements). Optimization of CDCHD includes further bioengineering and medicinal chemistry approaches for extensive SAR profiling, which is currently based on >70 generated analogs with modifications achieved

at about ten different scaffold positions[191,333]. Extended CDCHD profiling by ADMET, PK/PD, toxicity studies and validation of therapeutic efficacy in an ascending kidney infection model indicated the use of CDCHD for treatment of complicated urinary tract infection (cUTI) caused by ESBL-producing Enterobacteriaceae according to the selected TPP[334]. However, further increase in potency is required to achieve higher efficacy in kidneys against clinically most relevant uropathogens, which is essential for preclinical candidate nomination in this project. To achieve this goal, funding limitations in the academic sector shall be overcome by partnering with an industrial stakeholder.

BOX 5: Examples of public databases and tools related to antimicrobial compounds, targets and resistance

(i) Discovery of antibacterial compounds and development into (potential) therapeutics:

https://db.co-add.org/downloads/
https://globalamrhub.org/dynamic-dashboard/
https://chemdb.niaid.nih.gov/DrugDevelopmentTB.aspx
https://coconut.naturalproducts.net/
https://zinc.docking.org/
https://revive.gardp.org/resources/
https://go.drugbank.com/
https://www.antibioticdb.com/
https://www.pewtrusts.org/en/research-and-analysis/articles/2018/09/21/the-shared-platform-for-antibiotic-research-and-knowledge
https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2014/antibiotics-currently-in-clinical-development
https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2017/nontraditional-products-for-bacterial-infections-in-clinical-development
https://www.who.int/research-observatory/monitoring/processes/antibacterial_products_preclinical/en/
https://www.who.int/research-observatory/monitoring/processes/antibacterial_products/en/

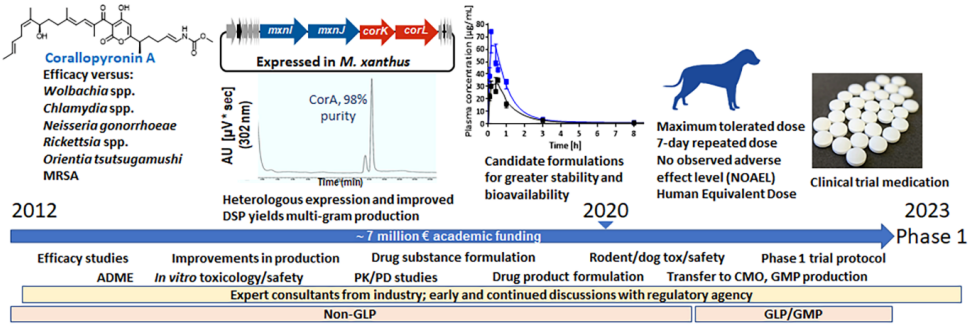
(ii) Antimicrobial target search and prediction:

https://pypi.org/project/targetDB/
http://bioinf.uab.es/cgi-bin/apgnr-cgi/antibactr.pl
https://arts.ziemertlab.com/

(iii) Antimicrobial resistance:

https://card.mcmaster.ca/
https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/
https://bench.cs.vt.edu/deeparg

BOX 6: Late preclinical development of corallopyronin A to first-in-human trial



The bacterial DNA-dependent RNA polymerase inhibitor corallopyronin A (CorA), produced by the soil-dwelling myxobacterium *Corallococcus coralloides*, is active against Gram-negative and Gram-positive bacteria, including MRSA[335,336], by targeting the hinge (switch) region of the holoenzyme[337]. Structure-Activity-Relationships demonstrated that the initial natural product hit was the most effective compound[338], allowing for its development without extensive medicinal chemistry. The essential intracellular *Wolbachia* symbionts (Gram-negative) of human filarial nematodes, which cause the neglected tropical diseases lymphatic filariasis and onchocerciasis (river blindness), are also targets of CorA[339]. Currently, the compound is being developed to clinical phase I to support elimination of these nematode infections[340,341], an aim of the United Nations' Sustainable Development Goal 3 (UN-SDG)[342]. CorA also has activity against the human pathogenic bacteria *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Rickettsia* spp., and *Orientia tsutsugamushi*[343–345], which are included in the UN-SDG or WHO/CDC lists for priority antibiotic discovery and development. Heterologous expression of the CorA biosynthetic cluster in *Myxococcus xanthus*, yielding >100 mg/L[275,276,346], allow consistent multi-gram scale production with a purity of 90-95%. This preliminary product specification has been formally accepted by the German Federal Ministry for Drugs and Medical Devices (BfArM). Most standard *in vitro* and *in vivo* non-GLP ADMET studies have been successfully completed. Compared to rifampicin, the expression of CYP450s is not altered and CYP3A4 induction is eight-fold lower. CorA is stable in plasma >240 min. Its metabolism in human and dog microsomes is $t_{1/2}$ >45 min, resulting in oxidation metabolites and minimal glucuronidation. Off-target profiling resulted in three hits (inhibition/activation), but the EC_{50} are 170-1500-fold higher than the *in vitro* EC_{50} against *Wolbachia*. CorA does not inhibit hERG, and no genotoxicity was observed. These results indicate that CorA is non-toxic and pharmacologically safe (awaiting further *in vivo* validation). The project is publicly funded to finalize preclinical studies including formulation development and *in vivo* toxicity in rodents and dogs (see Acknowledgements). In parallel, the manufacturing protocol for heterologous production and optimized, up-scalable DSP will be transferred to a GMP-certified CMO to produce cGMP-grade material for the GLP and phase I studies. Completing the clinical studies will require a Product Development Partnership. Provision of CorA to countries endemic for filarial infections is envisaged via PPP to achieve the UN-SDG. After regulatory approval, provision of CorA for treating sexually transmitted infections (STIs) and as a reserve antibiotic for MRSA shall be achieved through licensing.