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Confronting the challenges of discovery of novel antibacterial agents

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ABSTRACT

Bacterial resistance is inevitable and is a growing concern. It can be addressed only by discovery and development of new agents. However the discovery and development of new antibacterial agents are at an all time low. This article broadly examines the historical as well as current status of antibacterial discovery and provides some perspective as how to address some of the challenges.

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Bacterial infections and the fight against them have been a focus of mankind since the dawn of time with application of interventions including mercury salts and herbs. The earliest known chemotherapeutic antibacterial discovery began in the 20th century by screening of compounds from the dye industry leading to discovery of salvarsan (arsenic derivative of hydroxy aniline, 1910) and the sulfa drugs (1930's).[1] With understanding of the mechanism of action of sulfa drugs as inhibitors of folate pathway, targeted screening and lead optimization of the pyrimidine class of compounds led to discovery and development of trimethoprim (early 1960's). However, the true revolution of the antibacterial discovery did not begin until the discovery of penicillin in 1928 from *Penicillium notatum* by Sir Alexander Fleming followed by purification, production and clinical treatment in 1940s. This discovery led to a revolution of not only antibacterial discovery but also the field of microbial natural products. Empirical screening of microbial natural product fermentation broths led to the discovery of the antibacterial natural products in next 20 years (1940–1962) designated as 'Golden-Age' of antibacterial discovery.[1,2]

Microbial derived antibiotics (β-lactams, aminoglycosides, tetracyclines, macrolides, glycopeptides, streptogramins) and synthetic quinolones discovered during Golden Age served as drugs or chemical platforms for drug leads for medicinal chemists. For the next five to seven decades, optimization of these leads produced new antibiotics with incrementally improved potency and properties.[3] Improved chemistry, target identification and availability of ligand-bound 3D structure, and increased understanding of resistance mechanisms led to the discovery and development of as many as six generations of antibiotics of most important classes.[3] Convergent total synthesis of tetracyclines by Myers and coworkers is the most notable development of new chemistry published in a long time.[4,5] The new chemistry allowed for the efficient design of tetracycline analogs not possible before, leading to new classes of structures including pentacyclines.[4,5] While iterative modifications to old classes of chemical leads produced new antibiotics with improved potency, drug properties, and resistance profiles this process is not limitless and may have run its course. Despite these challenges, a limited number of compounds from the established classes of antibiotics are in various stages of development.[6]

While the antibacterial field is grappling with these challenges, bacterial resistance continues to grow to all antibiotics regardless of class and mechanism. Some classes and mechanisms are more prone to resistance selection than other classes. Bacterial resistance is inevitable. It is not if but when it will occur. Therefore, to combat resistance, new antibiotics with new mechanisms or new classes of compounds that bind to new binding sites of the established targets are needed.

After a significant innovation gap[3] post 'Golden Age' linezolid, a new synthetic oxazolidinone class of Gram-positive antibiotic was approved by the FDA in 2000.[1] Since then a few other new classes of Gram-positive antibiotics (daptomycin-2003, retapamutilin-2007, fidaxomicin-2011) were approved for clinical use.[1] Empirical screening was used to discover these classes of compounds in 1980's or before.[1]

So what happened during the last five decades since the 'Golden Age' of antibiotic discovery that led to discovery void[1] of *novel*

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classes of antibiotics? Is it truly an innovation gap due to complacency, application of wrong discovery strategies (apparently prudent at the time), de-emphasis of natural products, not enough emphasis or resources for antibiotic research and development?

Arguably, it is a result of the combination of all the factors described above. First, after a great success during the Golden Age of antibiotic discovery, it was assumed that the antibiotic classes already discovered would be sufficient for the treatment of bacterial infections and it was not sufficiently appreciated that bacterial resistance was inevitable under the selective pressure of antibiotics. Penicillin resistance was known at the time and therefore it was not a surprise. This oversight clearly led to complacency, likely poor funding and lack of innovation, until it was recognized otherwise.

All antibiotics during the Golden Age were discovered by empirical screening using inhibition-of-growth assays. Mechanism of action was determined much later, sometimes many decades later.[1] The advent of molecular biology, expression and production of enzymes and receptors made large-scale in vitro enzyme and receptor based screening routine. This turned out to be highly successful approach for chemical lead finding against a variety of mammalian targets followed by optimization leading to drugs. Unfortunately this approach of in vitro cell free screening was utterly unsuccessful for bacterial targets as reported by GSK in 2007.[7] At the time, in comparison to traditional empiric screening, the in vitro MOA based cell-free approach was very attractive due to its 'obvious' rationality. With that in mind, it is likely that most of the companies applied similar cell-free screening approaches for the discovery of antibacterial agents without much success at the end. Historically, natural products have been a most prolific source for providing novel antibiotics. However, in the intervening period most of the companies terminated or reduced their efforts on screening of natural products due to various reasons not least due to repeated rediscovery of known compounds. After spending huge resources for screening without finding *bona fide* tractable leads for chemical optimization to new drugs resulted in tremendous frustration. This led to de-prioritization of the antibacterial programs in many Pharmaceutical Companies.

The lack of success of the discovery of a good chemical lead for building a medicinal chemistry program for discovery and development of novel antibacterial agents with a novel mechanism of action could be attributed to two main factors. First and perhaps most critical is the lack of novel chemical diversity of antibacterial screening libraries and de-emphasis of natural products, and the second, the screening approach.

Chemical diversity, and lack thereof, for drug discovery space is a topic for much debate. Within the confines of chemical diversity the antibacterial agents appear to occupy unique property space compared to other drugs as demonstrated by O'Shea and Moser[8] from the analysis of the CMC database. These findings show that antibacterial agents: are significantly more polar (Gram-negative agents are much more polar, $\log D_{7.4}$ negative 2.8, than Gram-positive agents, $\log D_{7.4}$ negative 0.2 vs average CMC data set $\log D_{7.4}$ positive 1.6), do not obey Lipinski rule of five for oral bioavailability (some orally active drugs do obey these rules), display wide molecular weight (102–1449) ranges (MW: average CMC data set 338 Da, average Gram-positive antibacterial 813 Da and average Gram-negative antibacterial 414 Da).[1] Most of the corporate chemical collections and screening libraries were designed and built up with oral bioavailability and human targets in mind, leading to compounds that are much more lipophilic and unlikely to fill the property and diversity space suitable for antibacterials.[9]

Failure of the cell free screening approach (wet lab and virtual) of the bacterial targets is generally due to lack of cellular activity and lack of understanding of the characteristics required to endow molecules with cellular entry properties. Unlike mammalian cells, bacterial cells are protected by cell wall (Gram-positive) and by an outer-membrane in addition to cell wall (Gram-negative). Most of the bacterial targets with the exception of cell wall/outer membrane targets are intracellular. Therefore, in order to engage with their biological targets, compounds have to cross these strong protective barriers often with opposite physical properties.[1] Bacteria do express and use active transports to transport nutrients. Fortunately, sometimes these active transporters also help transport some of the drugs to periplasm and cytosol. Unfortunately transporters have not been exploited for drug entry with the exception of iron transporters (vide infra) perhaps due to lack of clear understanding of the structure and function of these transporters and also due to the rapid loss of the transporters, leading to resistance. Transporters often do play a role in transporting natural product antibiotics and show exclusive selectivity for some compounds and not for others even within the same class of antibiotics. Unfortunately, even if the drugs pass the membrane entry barrier efflux pumps expressed in bacteria, pump them out from periplasm/cytosol.

Improvement of potency of a chemical lead against an enzyme target by standard medicinal chemistry approaches, with or without structural information, can be achieved rapidly (e.g., LpxC, vide infra). However they may fail to kill bacteria because of their failure to reach the intracellular target (e.g., certain LpxC inhibitors, vide infra). While some structure-function knowledge exists on efflux pumps, not much is known on cell permeability other than highly acidic compounds are not as permeable as neutral, zwitterionic, and basic compounds. Balancing of permeability and efflux, the two *yin–yang* phenomenon, is critical for designing successful antibiotics. If balancing the target activity, cell penetration and efflux were not challenging enough, their diversity and differential expression in different bacterial species make the discovery and development of broad-spectrum antibiotics even more challenging. Therefore lead optimization of a broad-spectrum antibacterial program is akin to running a dozen single-target mammalian programs simultaneously. This is likely one of the reasons for the failure of the programs originating from cell-free screening efforts even after good tractable enzyme inhibitors were discovered. Despite these challenges empirical screening has allowed for the discovery of many great broad-spectrum antibiotics in the past. Detailed understanding of cell penetration and efflux can make this field wide open for new inventions.

Until resolution of the entry barrier, phenotypic assays represent the best approach for screening for antibacterial leads. A good target-based phenotypic screen can help eliminate/reduce the unwanted detergent-like or poisonous hits. This approach could focus the lead optimization resources to cell active leads. A few examples of target based whole cell phenotypic assays that have been applied for the discovery of new leads are: antisense assays[10] (e.g., platensimycin/platencin[11,12] and kibdelomycin[13]), and Wall Teichoic Acid (WTA) pathway assay.[14] Various other target based whole cell based assays have been reviewed.[1,15,16] However, paramount to the success of the antibacterial discovery is a structurally diverse screening library covering antibacterial property space. Without diverse antibacterial chemical libraries with requisite antibacterial drug properties no screening method would produce the desired outcome.

There are approximately 265–350 genetically validated antibacterial targets. Of these about 60% are broad-spectrum targets.[17] Astonishingly, no more than 20 of these are targeted by currently marketed drugs[2] and thus provide tremendous opportunity for the discovery and development of new agents with novel mode of action without cross-resistance, particularly multi-target mechanisms (chemical leads that interact with more than one biological target). With this kind of analysis and information in hand, the time is ripe for focusing resources and applying scientific acumen

for designing new antibacterial compound libraries using all modern chemistry approaches including DOS, BIOS and natural products. This would revitalize lead generation that could be converted into novel antibacterial drugs with new mode of action or new mode of binding to existing targets and low potential for cross-resistance. Meanwhile a narrow range of activity has continued towards new discoveries. A few vignettes of recent antibacterial drug discovery and development programs have been summarized here. Discovery of recent natural product leads was recently described in a BMCL digest article[18] and is not covered here.

Inhibitors of topoisomerases II. A clear pattern emerges upon careful examination of well-established antibacterial lead/target pairs. Targets that are highly privileged and bind to only a very specific compound classes (e.g., D-ala-D-ala → vancomycin and PBP → β-lactams)[2,19] and targets with promiscuous binding sites (e.g., ribosome and DNA topoisomerases II). The target promiscuity provides opportunity to discover novel classes of inhibitors that bind to a different binding site than targeted by existing clinical agents and thus avoiding cross-resistance. A wide range of structural classes/drugs target ribosome, from low MW (e.g., linezolid) and large MW (e.g., thiostrepton) and structures in between including chloramphenicol, clindamycin, aminoglycosides (e.g., streptomycin), macrolides (e.g., erythromycin, tylosin), streptogramins, and tetracyclines.[20]

While natural products dominate binding to ribosomal targets (oxazolidinone is exception), synthetic compounds dominate binding to bacterial DNA topoisomerases II (gyrase and topoisomerase IV), which is a target for quinolones and novobiocin (1) and is clinically validated. Significant efforts have been made to understand mechanism of action of the gyrase and topo IV, including solution of multiple inhibitor bound X-ray crystal structures. While the mechanism of enzyme inhibition is complex, availability of many crystal structures made it possible to apply structure based drug design principles to advance certain screening cell active leads. The topoisomerase II is an enzyme complex comprising four subunits: GyrA/ParC, GyrB/ParE. Each pair of subunits is quite similar leading to inhibition of the pair by many inhibitors hence dual targeting. Quinolones target GyrA/ParC whereas coumarins target the catalytic ATP binding site of the GyrB/ParE. Since these targets represent two different and independent mechanisms various screening approaches have been adopted, for example, ATPase based enzyme and empirical whole cell assays followed by MOA determination. Several reports of virtual screening has been also reported.[21]

Screening of a small library by the Vertex group using an ATPase assay led to the identification of benzimidazole carbamate lead (2, *Staphylococcus aureus* serum shifted MIC >16 μg/mL, *S. aureus* gyrase K_i 2 μM, topo IV K_i >60 μM, Fig. 1), which was systematically optimized using novobiocin bound *Escherichia coli* GyrB. Multiple iterations of structure-based design led to an optimized benzimidazole urea lead VRT-752586 (3, *S. aureus* MIC 0.5 μg/mL, gyrase K_i 0.014 μM) with significantly improved potency and Gram-positive spectrum including ParE (topo IV K_i 0.023 μM) activity.[22] Unfortunately enzyme potency improvement did not result in activity against critical Gram-negative pathogens due to permeability and efflux. VRT-752586 showed in vivo efficacy in murine thigh model of *S. aureus* infection (dosed iv) and murine lung pneumonia model of *Streptococcus pneumoniae* infection (dosed po) at less than 50 mg/kg.[22] The dual targeting of 3 likely leads to low spontaneous frequency of resistance of less than $<1.3 \times 10^{-10}$ in *S. aureus*.[23] Recently a thiazolopyridine (4) was reported as an optimized gyrase B inhibitor (*Mycobacterium smegmatis* GyrB IC$_{50}$ <0.5 nM and Mtb MIC 0.5 μM).[24]

Tari et al. reported a novel tricyclic gyrase B inhibitor (6) based on a fragment screening hit (5) followed by structure-guided lead optimization.[25] This compound showed potent inhibition of *Enterococcus faecalis* GyrB and ParE each with IC$_{50}$ <0.3 nM. It showed potent antibacterial activity and spectrum including *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa.* It was efficacious in various murine models of infections at single doses of 5–15 mg/kg. The activity was bactericidal and showed low (2×10^{-11}) spontaneous frequency of resistance.[25]

Likewise the AstraZeneca group used a fragment-based lead generation approach incorporating known gyrase inhibitor fragments, such as pyrrole, and NMR screening using the 24 kDa N-terminal ATP binding domain of *E. coli* GyrB, and they identified a pyrrolamide fragment 7.[26] A structure-guided lead optimization approach using the *E. coli* gyrase crystal structure led to identification of a potent (MIC sub to low μg/mL) and broad-spectrum Gram-positive agent 8 with activity against selected Gram-negative bacteria with in vivo efficacy along with 150-fold improvement of activity over compound 7. Compound 8 competes with ATP for binding at the ATP binding site of the gyrase B subunit. It is a bactericidal agent. It is not clear whether these compounds are dual targeting, though the frequency of resistance is low ($<10^{-9}$). Cross-resistance to novobiocin was observed but not to quinolones consistent with binding to ATP site.[26]

A series of non-fluoroquinolone novel bacterial type II topoisomerase inhibitors (NBTIs) have been recently described.[27] These inhibitors bind to gyrase A and ParC subunits, at a site different from the fluoroquinolones (e.g., ciprofloxacin, 9, Fig. 2) and aminocoumarin-binding site. They show no cross-resistance to fluoroquinolones and aminocoumarins.[28–38] The distinct-binding site of these inhibitors was demonstrated by X-ray crystal structures of several inhibitors (e.g., GSK299423,[28] 10, Fig. 2 and AM8191, 11)[39] bound to gyrase-DNA complex of *S. aureus.* NBTIs comprise three structural motifs: A left hand site (LHS) bicyclic aromatic heterocycle, a right hand side (RHS) aromatic heterocycle connected by a 8-atom central linker contacting a basic nitrogen at position-7. The X-ray crystal structure exhibited a salt-bridge interaction between the basic nitrogen atom at position-7 and the Asp83.[28,39] A wide-range of linkers are tolerated as long as linker length and position of the basic nitrogen is maintained. These compounds generally impart potent and broad-spectrum activity including Gram-negative activity but suffer from hERG activity. The hERG activity is highly dependent on overall polarity, which negatively correlates with the Gram-negative activity. For example, addition of a hydroxy group in NBTIs improves hERG signal with concomitant loss of activity against *P. aeruginosa.*[39] These compounds were originally discovered in 1999 by whole cell screening.[40] After lead optimization several of the NBTIs (e.g., NXL101, 12, Fig. 2) entered clinical development but were discontinued due to hERG binding and associated Qtc prolongation.[31] Recently GSK2140944 (13, *S. aureus* MIC$_{90}$ 0.5 μg/mL, *S. pneumoniae* MIC$_{90}$ 0.25 μg/mL) entered into clinical development with significantly reduced hERG activity (IC$_{50}$ 1.4 mM).[41] However it has only weak Gram-positive spectrum. Representative examples of NBTIs from AstraZeneca (14, hERG IC$_{50}$ 233 μM)[38] and actelion (15, hERG IC$_{50}$ 19% hERG block at 10 μM) are presented in Figure 2.[42] It appears less likely for this lead series to deliver a broad-spectrum agent due to negative correlation of hERG and Gram-negative activity.

Barbiturate analog QPT-1 (PNU286607, 16, Fig. 2) was discovered by empirical whole cell screening at Pharmacia Upjohn and was subsequently determined as an inhibitor of DNA gyrase by reverse genomics.[43] It showed broad-spectrum Gram-positive antibacterial activity with coverage of *Haemophilus influenzae* and in vivo efficacy. It shows no cross-resistance to quinolones and novobiocin. It targets the β-subunit of bacterial topoisomerase II but the binding site is likely different from quinolones and coumarins due to lack of cross-resistance. Lead optimization of this class of compounds at AstraZeneca led to the identification of isoxazole

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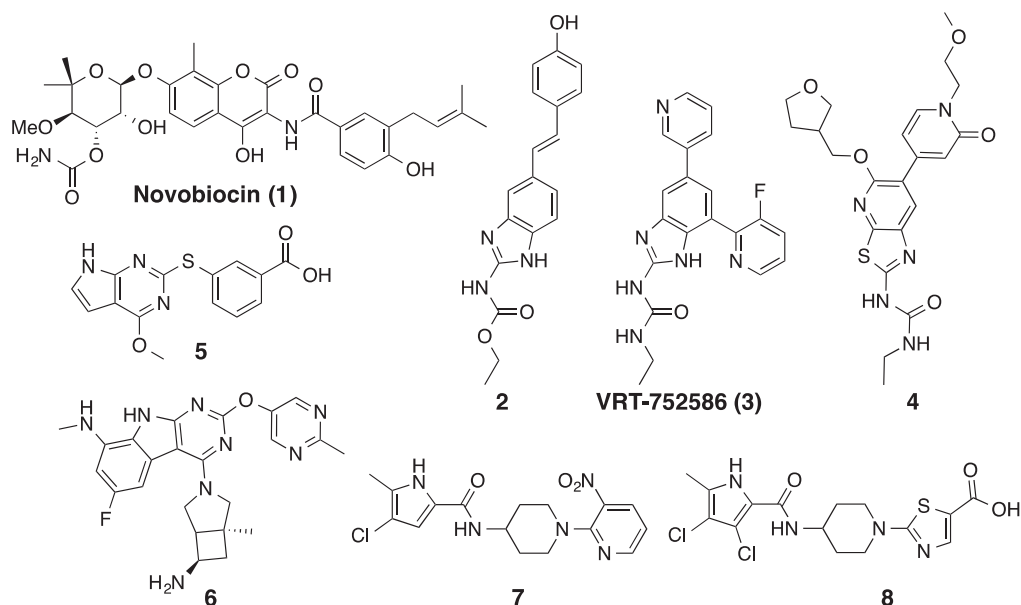
Figure 1. Chemical structures of gyrase B/ParE inhibitors interacting at ATP binding site.
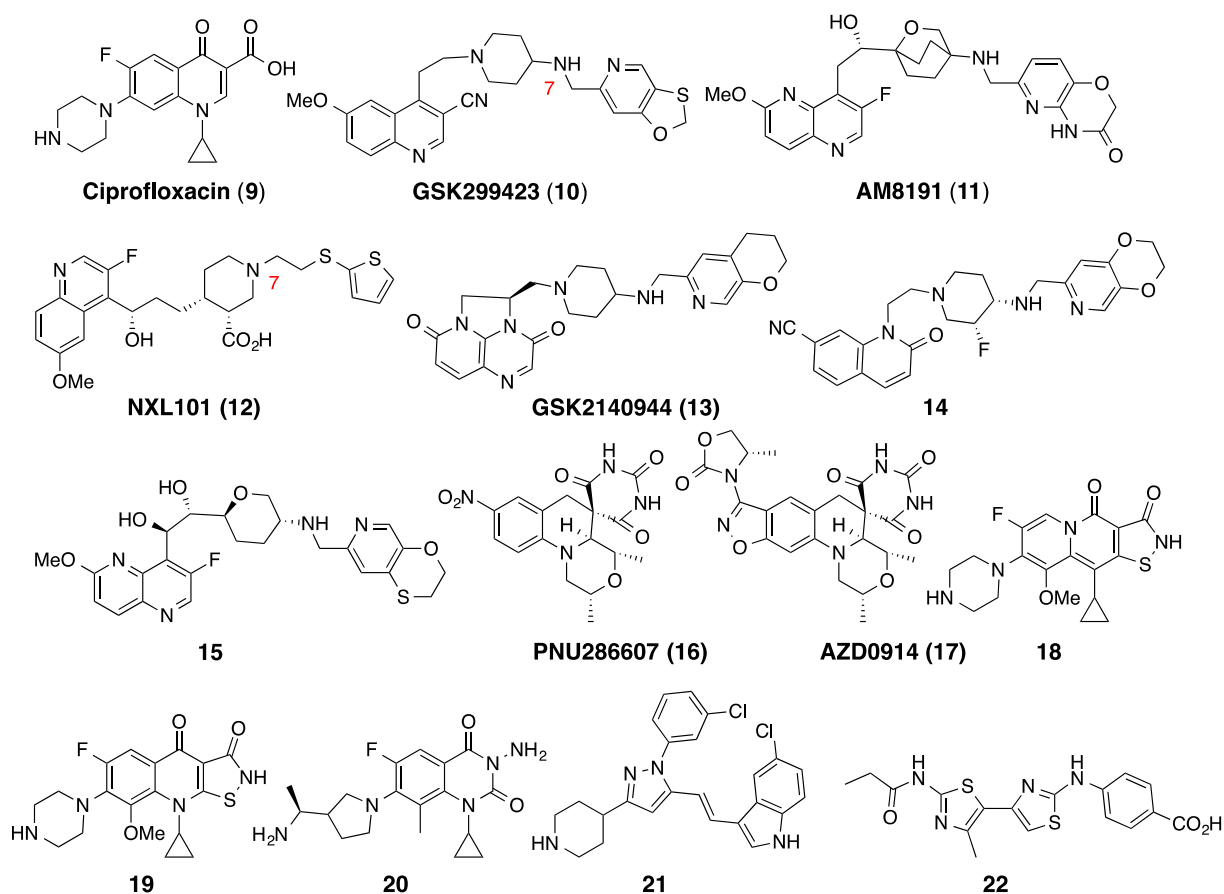
Figure 2. Chemical structures of gyrase A/ParC inhibitiors.

analog AZD0914 (**17**).[44] This compound showed Gram-positive spectrum along with activity against fastidious Gram-negative bacteria, and anaerobes[45] including *Neisseria gonorrhoeae*[46] with low resistance potential.[47] AZD0914 entered in phase I clinical development in 2013.

The gyrase inhibitors reported in Figure 2 are representative examples selected from a large structural variety[27] including 2-pyridines and isothiazolopyridones (**18**),[48] quinolones and isothiazoloquinolones (**19**),[49–51] aminoquinazolinediones (**20**),[52] piperidyl-pyrazoles (**21**),[53] 4,5′-bisthiazole (**22**),[54] and natural

products quercetin glycosides,[55] kibdelomycin.[13] These inhibitors bind to different part of the gyrase enzyme eliciting inhibitory response akin to mammalian receptors.

Tetracycline derivatives. With the development of the new convergent total synthesis of the tetracycline class of compounds,[4,5] the group at Tetraphase synthesized eravacycline (**23**, Fig. 3) a new C-7 fluoro analog of 70 year-old natural product tetracycline by total synthesis. The compound overcame common tetracycline-specific efflux and ribosomal protection mechanisms and therefore show no cross-resistance with tetracycline and maintains broad-spectrum activity.[56] Eravacycline entered phase 3 clinical trials for complicated urinary tract infections (cIUI) and complicated intra-abdominal infections (cIAI).[56]

Hybrid antibiotics. In order to modify the mechanism of action of existing classes of antibiotics, efforts have been expended to make chimeric antibiotics in which two mechanically distinct antibacterial classes are covalently attached to produce a new hybrid antibiotic. Cadazolid (**24**), a fluoroquinolone-oxazolidinone hybrid, represents a prototypical example.[6] It entered in phase II clinical development for *Clostridium difficile* infection with a profile superior to vancomycin.[6] Similar chimeric approaches have been taken with other classes (e.g., quinolizine-rifamycin[57] quinolone-DHFR,[58] quinolone-aminoglycoside[59]). Unfortunately, the challenge inherent to this approach is increased MW which will affect oral absorption, if oral treatment are needed. The group at Actelion took advantage of this inherent flaw for the development of cadazolid for CDAD indication.

β-Lactamase inhibitors. Inhibition of β-lactamase to restore Gram-negative activities of β-lactam antibiotics continue to play substantial role in combating resistance caused by expression of a variety of β-lactamases. Avibactam (**25**, Fig. 3) and MK-7655 (**26**), two members of a novel bridged diazabicyclo[3.2.1]octanone class, are in advanced stages of clinical development. Avibactam and MK7655 are potent inhibitors of class A, C and ESBL β-lactamases and are partnered with ceftazidime and imipenem, respectively, for restoring the activity against organisms producing those β-lactamases.[60] However, they do not provide coverage of MRSA and metallo β-lactamases. RPX7009 (**27**) is a new class of boronic acid based β-lactamase inhibitor with potent activity (IC$_{50}$ 9–11 nM) and is in early stages of clinical development in combination with biapenem.[60]

Efflux pump inhibitors. Like β-lactamase inhibition, inhibition of efflux pumps is another strategy, which merits attention. Various efflux pump inhibitors have been reported including reserpine that inhibits NorA efflux pump and improves MIC values of a number of fluoroquinolones.[61] Many efflux pump inhibitors have been reported[62,63] including boronic acids[64] that synergizes antibiotics in vitro but none have advanced to clinical validation. While

development of β-lactamase inhibitors has been proven, significant development challenges remain for the efflux pump inhibitors.[1,62]

Entry promoters. In order to improve entry in *Pseudomonas* a few groups have taken advantage of its iron uptake mechanism, which is essential for bacterial survival and growth. The iron uptake takes place by binding to siderophores. The groups at Baselia and Pfizer have successfully conjugated a hydroxypyridone siderophore moiety to Gram-negative monobactams to produce BAL30072 (**28**, Fig. 4)[65] and MC-1 (**29**), respectively.[66] These conjugates hijack bacterial siderophore transport system and trick the cells into importing the active drug as if it was nutritionally beneficial compound and thus showed better permeability in *P. aeruginosa*. As a result, these compounds showed significantly improved in vitro activity compared to non-siderophore containing parents. Although siderophore strategy for MC-1 demonstrated reasonable in vitro resistance frequencies, these did not correlate in vivo and have raised concerns about this as a viable approach. In addition, how this mechanism will work in the clinical setting where variable levels of iron and indigenous siderophores, with different iron affinity, are present is unclear and could be answered only from the clinical studies of one of these compounds?[67] While development status of MC-1 is unknown, BAL30072 is in clinical development in part funded by BARDA.

LpxC inhibitors. Significant efforts has been expended on the discovery and development of inhibitors of LpxC, an essential enzyme for the synthesis of lipid A in Gram-negative bacteria particularly after the discovery of hydroxymic acid inhibitor L-161240 (**30**). These efforts led to the discovery of potent inhibitors of LpxC including CHIR-090 with potent (sub μg/mL) MIC against *E. coli* and *P. aeruginosa*.[1,68] While many hydroxymic acid based inhibitors with potent LpxC activity have shown potent cellular activity (perhaps entry is helped by metal transporters) many potent non-hydroxymic acid LpxC inhibitors (e.g., hydantoins, phosphonic acids) do not show cellular activity.[68] ACHN-975 entered clinical development but was halted due to injection site problems and thus clinical outcome of LpxC inhibitors remains unclear.[69]

Antibiotic discovery is predominantly a molecule centric approach[3] and currently suffering from discovery of new lead/target pairs. Relatively straightforward processes exist for the target identification of a cell active antibacterial lead as has been performed throughout the history of antibiotic discovery. Therefore, it is paramount to focus on transforming the chemical diversity with special emphasis on antibiotic-like physicochemical properties for the discovery of novel antibacterial leads. Natural products have been major sources of antibacterial drugs. They have been endowed with better entry (often using active transports),[16] binding properties with soluble enzymes of intracellular targets.[70] Natural products have been deemphasized for the last couple of decades due to many real or perceived challenges, which could
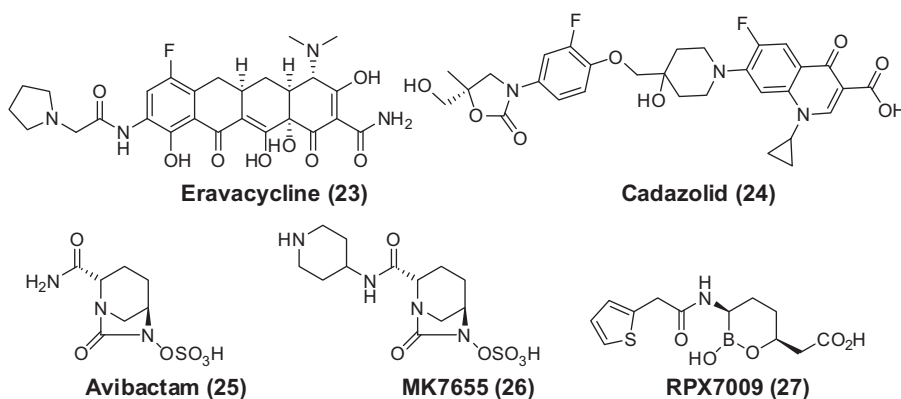

Figure 3. Chemical structures of new tetracycline, hybrid structure, and β-lactamase inhibitors.
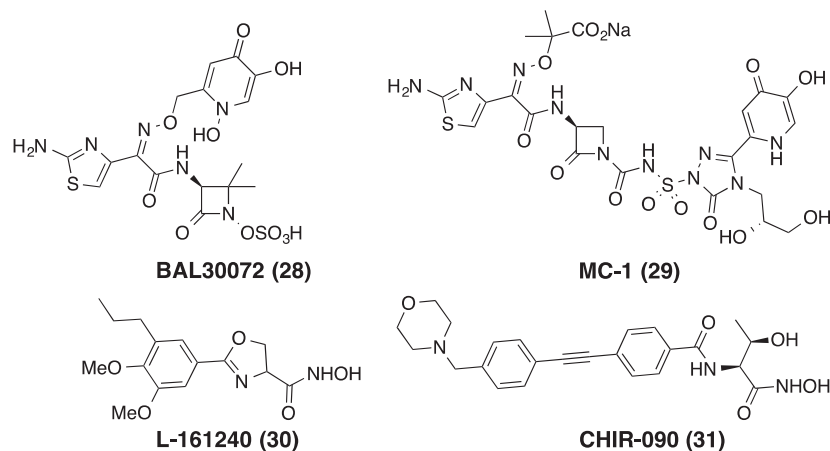
Figure 4. Chemical structures of siderophores and LpxC inhibitors.

be rapidly addressed today with highly sensitive analytical technologies (dereplication), genome sequencing (awakening silent cryptic biosynthetic pathways and predictive chemistry) and combinatorial biosynthesis and synthetic biology (complex structure modification, new structure generation). Significant advances have been also made in the development of target/pathway based phenotypic screens, which must be employed for screening regardless of sources of compounds.[71,72] Unfortunately, without improvement of the quality of compounds for screening other improvements including great phenotypic screens will not improve the odds of success for the discovery of novel antibacterial agents with novel mode of action. In order to improve the odds and reduce cost, it might be an opportune time for discussion of development of a consortium approach for design and synthesis of antibiotic specific libraries. Various models could be envisaged for this approach such as sharing of libraries and pairing that with proprietary assays. If we continue with current trajectory of failed attempts, the consequences could be dire, particularly if infected by resistant untreatable Gram-negative pathogens. We cannot return to pre-penicillin days. Fortunately, initiatives such as IMI's new drugs for bad bugs (www.translocations.eu) and public discussions[73] have started to catalyze antibiotic discovery and incentivize the antibiotic discovery and development. This would in turn help in improved return on investment leading to increased investment by for-profit organizations.

Acknowledgment

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