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Review

A Review of Antibacterial Candidates with New Modes of Action

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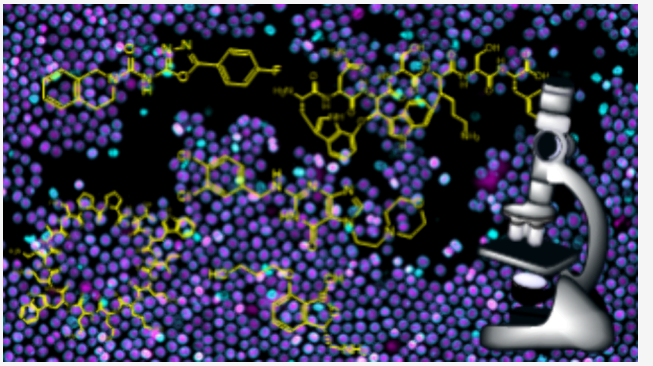
ABSTRACT: There is a lack of new antibiotics to combat drug-resistant bacterial infections that increasingly threaten global health. The current pipeline of clinical-stage antimicrobials is primarily populated by "new and improved" versions of existing antibiotic classes, supplemented by several novel chemical scaffolds that act on traditional targets. The lack of fresh chemotypes acting on previously unexploited targets (the "holy grail" for new antimicrobials due to their scarcity) is particularly unfortunate as these offer the greatest opportunity for innovative breakthroughs to overcome existing resistance. In recognition of their potential, this review focuses on this subset of high value antibiotics, providing chemical structures where available. This review focuses on candidates that have progressed to clinical trials, as well as selected examples of promising pioneering approaches in advanced stages of development, in order to stimulate additional research aimed at combating drug-resistant infections.



KEYWORDS: *antibacterial, antibiotic, mode of action, mechanism, resistance, pipeline, drug development*

INTRODUCTION

The fragility of antibacterial drug research and development has been widely recognized for many years. Discussions have mostly focused on unfavorable market conditions,[1−3] the inevitable emergence of drug resistance,[4] and the difficulty of identifying new drug leads.[5−7] All of these factors can lead to "leaks in the pipeline".[7] There is increasing awareness of the critical medical need for antibacterial drugs, which are essential for maintaining and improving healthcare outcomes. Consequently, there has been a move to incentivize antibacterial drug development through various push and pull mechanisms.[8−13] These initiatives appear to have helped boost the number of antibacterial drug candidates in the early stages of clinical development (phase 1 and 2 clinical trials), from 34 in 2011 to 51 at the end of 2022.[14,15] The antibacterial pipeline has been boosted in the past few years by an increased number of "nontraditional" antibacterials[16−18] in active development,[14,15] which currently account for around half of CARB-X's (Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator) preclinical development portfolio.[10] Nontraditional antibacterials primarily affect bacteria growth or virulence indirectly via a variety of mechanisms that include toxin binding, reducing cell adherence, inhibition of antivirulence targets, and drug resistance modification that can be small molecules, monoclonal antibodies (mAbs), proteins, or live biotherapeutics such as bacteria and bacteriophages. A comprehensive review on Gram-negative (G−ve) antibacterial agents in clinical trials was published in March 2024,[19] which described 28 small molecules, nearly half of which were new β-lactamase inhibitors combined with existing β-lactams, and 21 nontraditional antimicrobial agents.[19] Despite these pipeline advances, there are still obstacles to obtaining funding to evaluate and progress new targets and antibacterials through phase 3 trials, regulatory approval, and post-approval activities.[6,13,19,20] A 2021 World Health Organization review[21] highlighted that only 12 new antibiotics entered the market from 2017 to 2021; with only a few more expected to be approved in the near future. Of those under development only 27 targeted "critical" pathogen microbes, of which only six addressed antibiotic resistance.[22,23]

An ideal antibacterial drug should kill or inhibit bacterial growth at the site of infection approaching 100% sensitivity in target bacterial species, while causing no or very minimal side effects to humans, and be selective for the targeted pathogens but not the beneficial commensal microbiome. While pathogen-specific treatments are desirable, improved rapid

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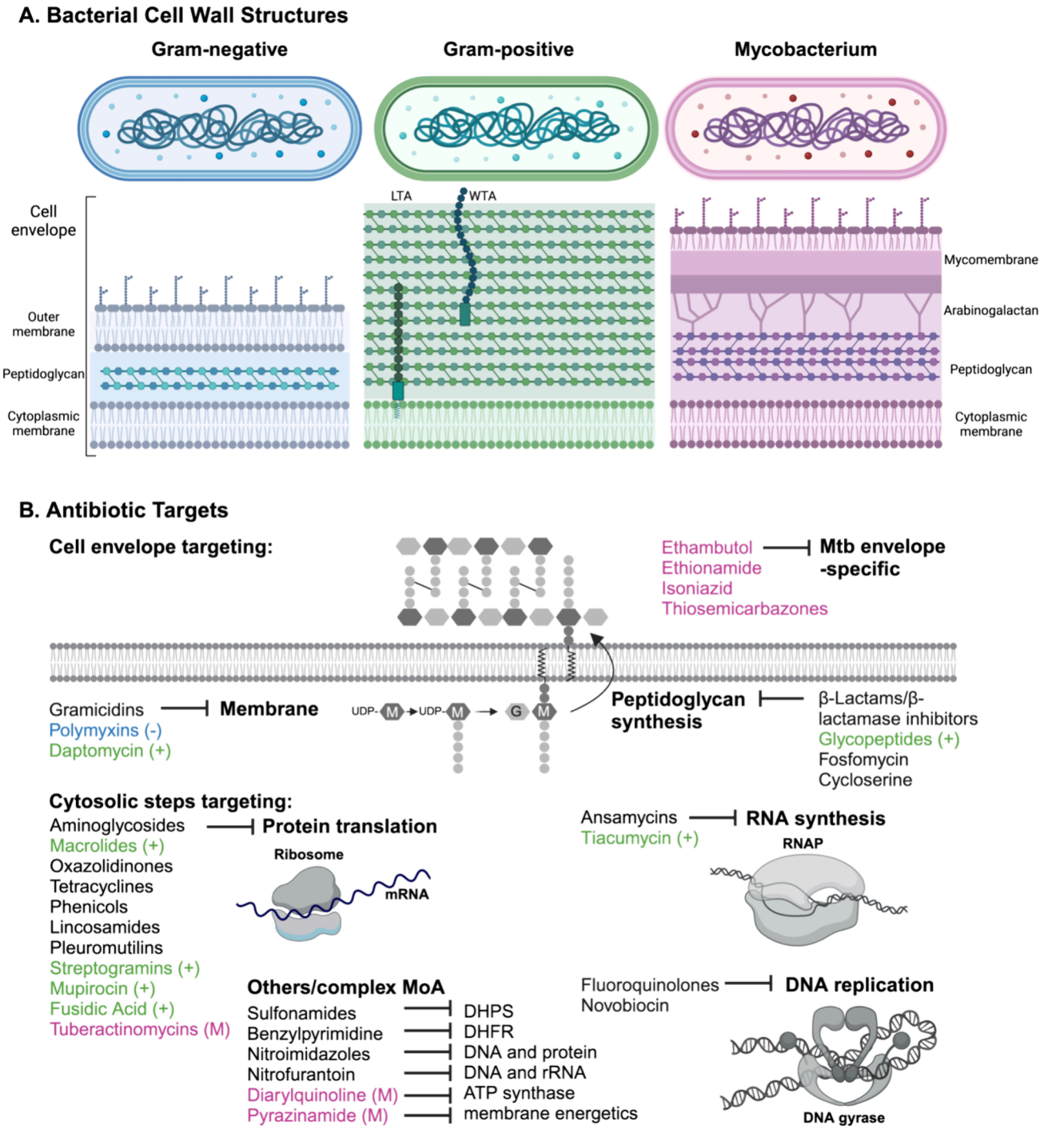

Figure 1. (A) Comparison of cell wall structure of Gram-positive (G+ve) bacteria, Gram-negative bacteria (G−ve), and mycobacteria (Mb). (B) Targets of clinically used antibiotics (names in green with (+) = G+ve only, in blue with (−) = G−ve only, in black = G+ve and G−ve, in magenta with (M) = *Mycobacterium* only.

diagnostics may be required to make clinical trials and therapeutic treatments more feasible. In addition, it should exhibit a low propensity for resistance development both to itself and other antibacterial agents, as well as have minimal cross resistance to other antibiotics. New antibacterials also need to overcome bacterial efflux and entry barrier mechanisms, which have evolved to repel many types of exogenous compounds. Penetration through bacterial membranes is further complicated by the different cell wall types found in Gram-positive bacteria (G+ve), G−ve bacteria, and mycobacteria (Figure 1). Additionally, there are the standard challenges associated with drug development to overcome, which include optimizing pharmacokinetics and pharmacodynamics and avoiding undesirable metabolism and toxicity.

While continually refining existing antibacterial drugs has led to improved drugs with significant clinical utility, the identification of new antibacterials that act on new or underexploited targets (even in the existing major categories) is also desirable. A combination of both strategies is required to fuel a healthy pipeline. Early attempts to screen for new

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Table 1. Approved Antibacterial Drug Classes (Drug Examples), Modes of Action (MoA), and Susceptible Bacteria[a]

drug class (examples)	mode of action (MoA)	susceptible bacteria
	Cell Envelope	
β-lactams (amoxicillin, cefepime, meropenem)	penicillin binding protein (PBP)	G+ve, G−ve, Mb
glycopeptides (vancomycin, teicoplanin, telavancin)	D-Ala-D-Ala binding (peptidoglycan precursor)	G+ve
cyclic lipopeptide (daptomycin)	membrane disruption[30−33]	G+ve
gramicidin (gramicidin S, tyrothricin)	membrane disruption, pore formation, delocalizes peripheral membrane proteins involved with cell wall division and synthesis[34]	G+ve, G−ve
polymyxin (colistin, polymyxin E)	membrane disruption, lipid A binding[35]	G−ve
ethionamide (ethionamide, prothionamide)	enoyl-ACP reductase (InhA)	Mb
ethambutol (ethambutol)	arabinogalactan biosynthesis	Mb
isoniazid (isoniazid)	mycolic acid synthesis	Mb
fosfomycin (fosfomycin)	UDP-*N*-acetylglucosamine-3-enolpyruvyltransferase (MurA)	G+ve, G−ve
cycloserine (cycloserine, terizidone)	Ala racemase and D-Ala:D-Ala ligase	Mb
thiosemicarbazone (thiacetazone, perchlozone)	FASII dehydratase complex HadABC	Mb
	Protein Synthesis	
aminoglycoside (streptomycin, amikacin, tobramycin)	30S ribosome	G+ve, G−ve, Mb
macrolide (erythromycin, azithromycin)	50S ribosome	G+ve
oxazolidinone (linezolid, tedizolid)	50S ribosome	G+ve, G−ve, Mb
tetracycline (oxytetracycline, minocycline, tigecycline)	30S ribosome	G+ve, G−ve, Mb
phenicol (chloramphenicol, thiamphenicol)	50S ribosome	G+ve, G−ve
lincosamide (lincomycin, clindamycin)	50S ribosome	G+ve, G−ve
pleuromutilin (retapamulin, lefamulin)	50S ribosome	G+ve
streptogramin (quinupristin−dalfopristin)	50S ribosome	G+ve
mupirocin (mupirocin (**70**))	isoleucine-t-RNA synthetase	G+ve
fusidic acid (fusidic acid)	elongation factor G (EF-G) (binding after GTP hydrolysis)	G+ve
tuberactinomycin (capreomycin, viomycin)	70S ribosome	Mb
	DNA Synthesis	
fluoroquinolone (ciprofloxacin (**46**), moxifloxacin)	DNA gyrase (GyrA) and topoisomerase IV (ParE)	G+ve, G−ve, Mb
novobiocin (novobiocin (**47**))	DNA gyrase (GyrB) and topoisomerase IV (ParE)	G+ve, G−ve, Mb
sulfonamide (sulfamethoxazole, *para*-aminosalicylic acid)	dihydropteroate synthase (DHPS)	G+ve, G−ve
benzylpyrimidine (trimethoprim)	purine and DNA synthesis−dihydrofolate reductase (DHFR)	G+ve, G−ve
	RNA Synthesis	
ansamycin (rifamycin, rifampicin)	RNA polymerase	G+ve, G−ve, Mb
tiacumicin (fidaxomicin)	RNA polymerase	G+ve
bicyclomycin (bicyclomycin)	Rho (RecA-type ATPase) transcription termination factor	G−ve
	Other Mechanisms and Complex MoAs	
clavulanic acid (clavulanic acid, tazobactam)	β-lactamase inhibition	
diazabicyclooctane (DBO) (avibactam, durlobactam)	β-lactamase inhibition	*b*
boronate (vaborbactam)	β-lactamase inhibition	
nitroimidazole (metronidazole, pretomanid, delamanid)	disrupts DNA and protein synthesis	G+ve, G−ve, Mb
nitrofurantoin (nitrofurantoin)	disrupts rRNA, DNA and other intracellular components	G+ve, G−ve
diarylquinoline (bedaquiline (**86**))	adenosine triphosphate (ATP) synthase	Mb
pyrazinamide (pyrazinamide)	membrane transport and energetics	Mb

[a]Abbreviations: G−ve, Gram-negative; G+ve, Gram-positive; Mb, mycobacteria; MoA, mode of action. [b]Some DBO β-lactamase inhibitors also have antibacterial activity against selected Enterobacteriaceae.[14]

compounds against new targets in the 1980s and 1990s were largely unsuccessful.[24,25] Although potent *in vitro* "hits" were identified, translating these into compounds capable of killing bacteria proved problematic, largely due to issues with cell penetration and efflux.[24,25] Most of the antibacterial drugs with new modes of action (MoAs) were discovered using whole cell screening techniques. Fortunately, some of the earlier challenges in obtaining whole-cell activity (inhibition of growth) from target-active compounds have been overcome over time, and now there are several antibacterial drug candidates in development with new pharmacophores and MoAs.[26]

For the purpose of this review, the modes of action (MoAs) of antibacterial drugs are classified into five major categories:[27,28] cell envelope synthesis, protein synthesis, DNA synthesis, RNA synthesis, and "other" and complex mechanisms (Table 1). In some cases, the MoA can be complex and include additional actions beyond the primary target, such as effects on metabolism or proton motive force.[29] Within these classes, there is a limited repertoire of targets that antibiotics act upon, with the majority discovered during the "golden age" of antibiotic discovery in the 1940s to 1970s.

Here we review small molecule antibacterial drugs with new MoAs that are currently being evaluated in clinical trials,[14] separated by overall classification, and including "nontraditional" small molecules.[15] It is important to note that the term "new MoA" has varying meanings for different people, and thus it affects their interpretation of what constitutes a "new

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Table 2. Antibacterial Compounds in Clinical Trials (See Figure 2) That Target the Cell Envelope with New MoAs Not Found in Approved Antibacterial Drugs[a]

name	compound class (lead source)	MoA	administration; development phase (status), indication (developer)
TXA709 (**3**) (prodrug); TXA707 (**2**)	FtsZ benzamide (S)	FtsZ inhibition	po; phase 1 (completed), G+ve infections (TAXIS Pharmaceuticals)
afabicin (**7**) (prodrug); AFN-1252 (**6**)	benzofuran naphthyridine (S)	FabI inhibition	iv/po; phase 2, *S. aureus* bone or joint infections; phase 2 (completed), ABSSSI (Debiopharm)
murepavadin (**8**)	protegrin I (P)	β-barrel protein LptD inhibition	inhalation; phase 1 (completed) pseudomonal infections (G−ve) (Spexis AG)
zosurabalpin (**9**)	macrocyclic peptide (S)	$LptB_2FGC$ complex inhibition	iv, phase 1, *A. baumannii* infections (Roche)
BTZ-043 (**10**)	benzothiazinone (S)	DprE1	po; phase 2, TB (Ludwig-Maximilian University of Munich)
macozinone (**11**)	benzothiazinone (S)	DprE1	po; phase 2 (completed), TB (Innovative Medicines for Tuberculosis Foundation/ Nearmedic Plus)
quabodepistat (**12**)	3,4-dihydrocarbostyril (S)	DprE1	po; phase 2, TB (Otsuka Pharmaceutical)
TBA-7371 (**13**)	azaindole (S)	DprE1	po; phase 2, TB (TB Alliance/Foundation for Neglected Disease Research/Bill & Melinda Gates Medical Research Institute)
cannabidiol (**14**)	cannabidiol (NP)	membrane disruption	topical, phase 2 (completed), *S. aureus* infections (Botanix Pharmaceuticals)
exeporfinium chloride (**15**)	porphyrin (NP)	membrane-perturbing activity	topical; phase 2b (completed), postsurgical nasal decolonization (Destiny Pharma)
R327	acrolein polymer (S)	disruption of cellular bioenergetics	topical, phase 1/2 (completed), burn wound infections; phase 1/2, diabetic foot ulcers (Recce Pharmaceuticals); also, an iv phase 1 trial
peceleganan (**16**)	cationic peptide (P)	membrane disruption	topical, phase 3, wound infections (ProteLight Pharma)
OMN6 (**17**)	cationic peptide (P)	membrane disruption	iv, phase 2, HABP or VABP caused by *A. baumannii* complex (Omnix Medical)
PL18 (**18**)	cationic peptide (P)	membrane disruption	topical (suppository), phase 1, G−ve and G+ve, bacterial vaginosis (ProteLight Pharma)
PLG0206 (**19**)	cationic peptide (P)	membrane disruption	topical; phase 1, G−ve and G+ve, PJI (Peptilogics)/iv administration in phase 1 trial

[a]Abbreviations: ABSSSI, acute bacterial skin and skin structure infections; DprE1, decaprenylphosphoryl-β-D-ribose 2′-oxidase; G−ve, Gram-negative; G+ve, Gram-positive; HABP, hospital-acquired bacterial pneumonia; FabI, enoyl reductase; FtsZ, filamenting temperature-sensitive mutant Z; iv, intravenous; LptD, lipopolysaccharide assembly complex protein; MoA(s), mode(s) of action; NP, natural product-derived; P, peptide-derived; po, oral (*per os*); PJI, prosthetic joint infections; S, synthetic; TB, tuberculosis; VABP, ventilator-associated bacterial pneumonia.

antibiotic". For example, degrees of innovation for "new or novel" antibiotics can range from (1) chemical classes that bind to exactly the same target site as an existing antibacterial drug (e.g., gyrase enzyme known pocket) but have a different chemical structure to (2) compounds acting on a different binding site within the same target molecule, (3) compounds acting on another target component of the same mechanistic pathway (e.g., DNA transcription via DNA polymerase sliding clamp instead of gyrase), and finally (4) compounds that disrupt a completely new target in a new mechanistic pathway (e.g., LepB target in the protein secretion pathway). Most compounds in clinical trials fall into the first two categories, but this review highlights efforts in the latter two approaches. Furthermore, several of the more promising or advanced preclinical approaches are outlined. The examples discussed in this review are not exhaustive of all new approaches and have been chosen at the author's discretion; however, the choice was influenced by compounds and approaches funded by CARB-X after their peer review process. Compounds being developed as topical treatments have been included, as further development of these compounds and/or targets may lead to systemic analogs. It is hoped that this review will shed light on these new MoAs and inspire further innovation.[26]

COMPOUNDS WITH NEW ANTIBACTERIAL MODES OF ACTION

Cell Envelope: Compounds in Clinical Trials. The disruption of the cell envelope via peptidoglycan, cell membranes, and cytoplasmic related targets has historically been a successful strategy for antibacterial drug development (e.g., β-lactams and glycopeptides). β-Lactam resistance has been circumvented for many antibiotics through coadministration of β-lactamase inhibitors, which belong to several broad classes: clavulanic acid, diazabicyclooctane (DBO), and boronate (Table 1).[14,15] Natural product-derived membrane targeting cyclic lipopeptides (e.g., colistin, polymyxin B, daptomycin) and a cyclic peptide ionophore (gramicidin) have also been used clinically. Antimicrobial peptides (AMPs), which are produced by most animals as part of their innate immune response, are another class of membrane targeting antibacterials that have been investigated in clinical trials. However, no AMPs have obtained regulatory approval to date, mostly due to a lack of selectivity between mammalian and bacterial membrane damage. Compounds that target the cell envelope with new MoAs that are not represented in approved antibacterial drugs currently in clinical trials are listed in Table 2, their structures are provided in Figure 2, and the pathways involved are illustrated in Figure 3.

Bacterial cell division is orchestrated by FtsZ, which forms dynamic filaments that move around the division plane through treadmilling. Initially, filaments are stabilized by membrane anchors FtsA and ZipA, which localize peptidoglycan synthases to future division sites. This proto-ring then recruits more than 15 other cell division proteins, forming a divisome complex that divides the cell. FtsZ might provide a constricting force at the beginning of division[36] but becomes dispensable in deeply constricted division sites.[37] FtsZ is a bacterial homologue of tubulin, which is a protein superfamily that polymerizes into microtubules which are major components of eukaryote cytoskeletons. Although tubulin

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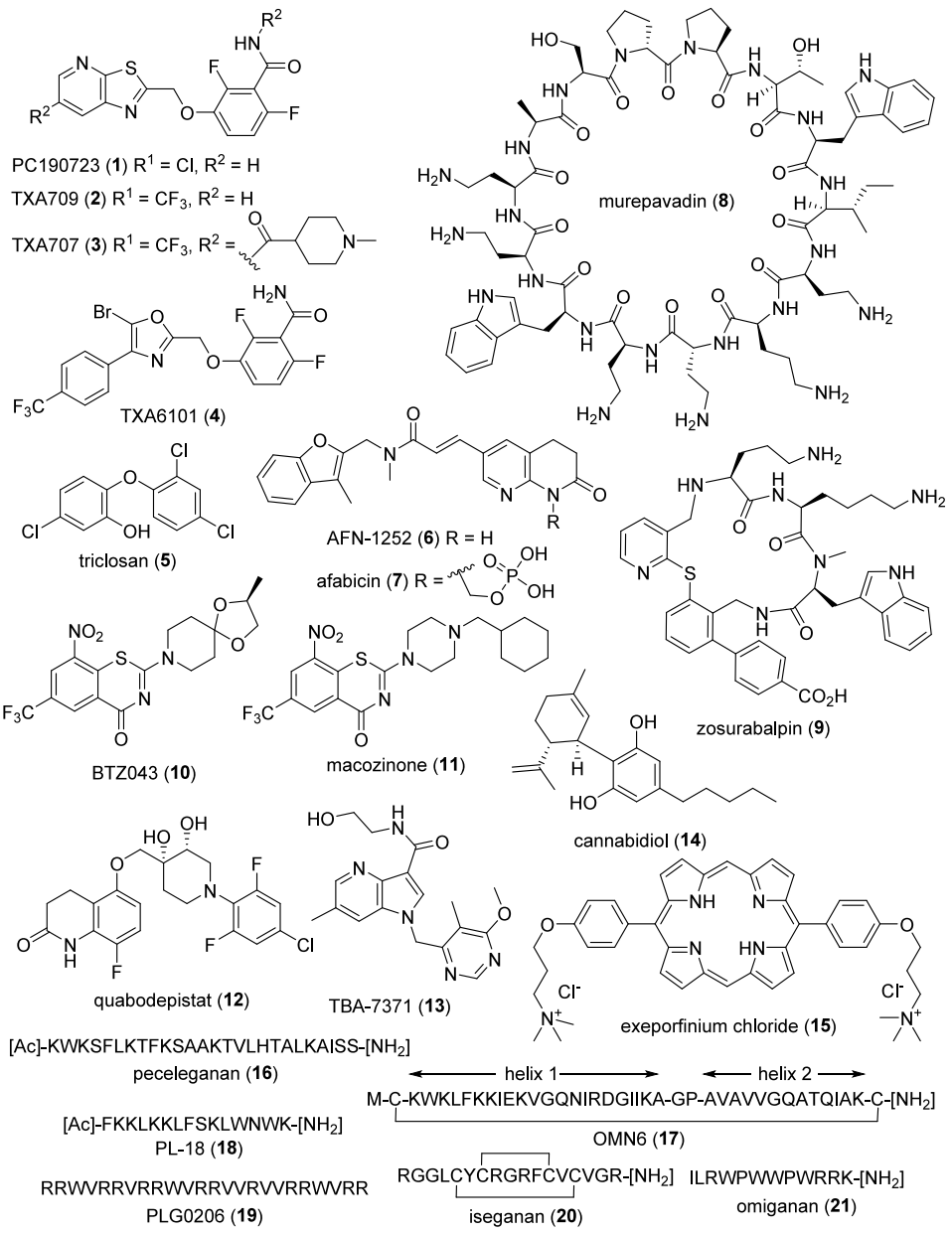

Figure 2. Structures of cell envelope targeting antibacterials and related compounds being evaluated in clinical trials with new MoAs not found in existing drugs.

has been successfully targeted in anticancer drugs (e.g., paclitaxel, ixabepilone, and vincristine), there are no antibacterial drugs targeting FtsZ.[38] The first FtsZ inhibitor with *in vivo* activity was PC190723 (**1**) (Figure 1), which was discovered from optimization of 3-methoxybenzamide.[39−41] Replacement of the Cl in PC190723 (**1**) with a CF_3 group led to the identification of TXA707 (**2**) and its prodrug, TXA709 (**3**),[42−44] which has completed a phase 1 trial as a potential MRSA treatment.[43,45] It was recently shown that more flexible analogs such as TXA6101 (**4**) can overcome resistance induced by TXA707 (**2**) and X-ray crystal structures of **2** and **4** bound to *Staphylococcus aureus* FtsZ and **4** with a G196S mutant were reported.[46,47] The flexibility of **4** allowed it to adapt to the steric interaction introduced by the G196S mutation.

Phospholipids and fatty acids are the principal components of membranes and given that bacteria need to produce their lipids *de novo*, the bacterial fatty acid biosynthesis pathway (FASII) is an under-exploited antibacterial strategy outside of TB.[48,49] FASII pathway intermediates are also used in the synthesis of cofactors like biotin and lipoic acid. A 2022 review summarized FASII biochemistry and the antibacterial potential of FASII inhibitors.[50] Notably, FASII is targeted by the consumer product triclosan (**5**) that forms a noncovalent NAD^+ complex that inhibits enoyl reductase (FabI), which is the final step of the fatty acid elongation cycle.[48] A high throughput screening campaign at GSK against bacterial enzymes[24] identified and then optimized active compounds against *S. aureus* FabI.[51] These compounds had potent activity against staphylococci but did not possess broad spectrum activity, as many pathogens had salvage pathways or isoforms of FabI, such as FabK, that could undertake the biochemical function of the enzyme.[24,51] A new analog, AFN-1252 (**6**) (Debio 1452) and its prodrug afabicin (**7**) (Debio 1450, AFN-

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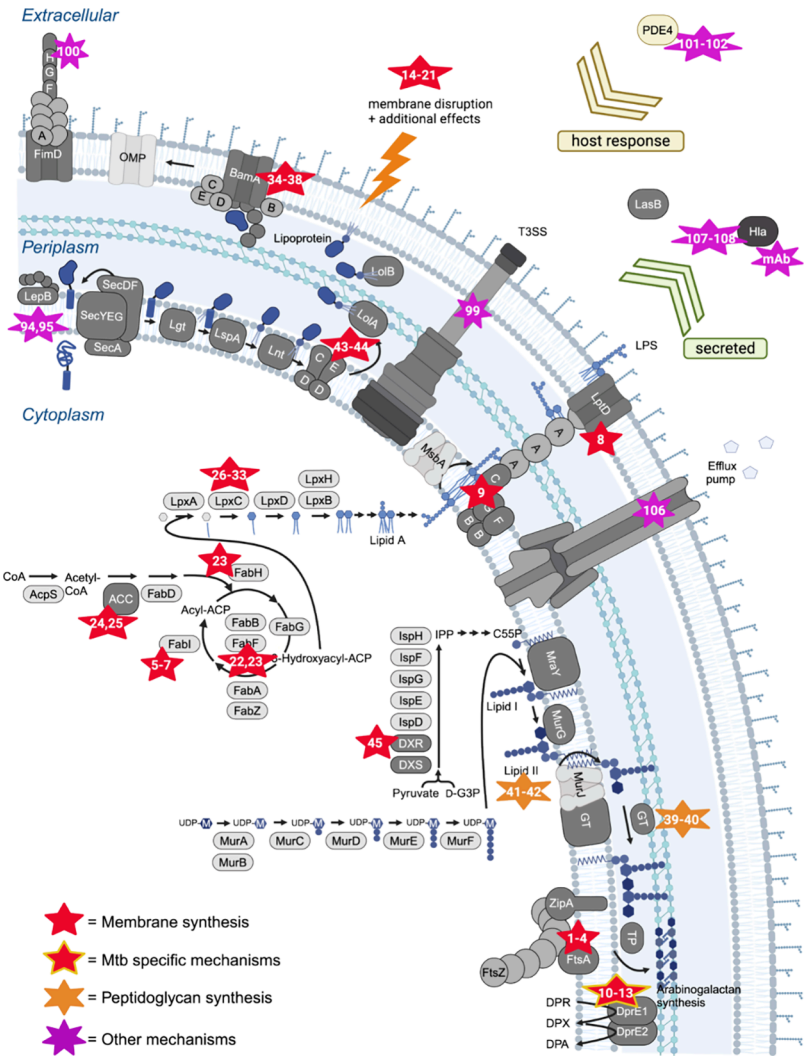

Figure 3. Cell wall, membrane protein, and extracellular pathways targeted by antibiotics with new MoAs not found in existing drugs. Abbreviations: CoA, coenzyme A; ACC, acetyl-CoA carboxylase; ACP, acyl-carrier protein; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DXR, 1-deoxyxylulose-5-phosphate reductoisomerase; IPP, isopentenyl diphosphate; C55, undecaprenyl phosphate; G3P, glyceraldehyde-3-phosphate; GT, glycosyltransferase; TP, transpeptidase; T3SS, type 3 secretion system; LPS, lipopolysaccharide.

1720), were later discovered.[52−54] Crystal structures of *S. aureus* and *Burkholderia pseudomallei* FabI−AFN-1252 complexes showed that AFN-1252 (**6**) bound noncompetitively at the active site but independently of NADPH.[52,55] Afabicin (**7**) is currently being evaluated in a phase 2 trial (NCT03723551) using an iv/oral switch strategy for the treatment of *S. aureus* bone or joint infections[56] and has previously completed a phase 2 trial (NCT02426918) for staphylococcal ABSSSI infections.[57]

Investigation of β-hairpin-shaped peptidomimetics stabilized by a D-proline−L-proline template based on the host-defense peptide protegrin I led to the identification of murepavadin (**8**) (POL7080, RG7929) which has potent activity against *Pseudomonas* spp. and low hemolytic activity, but weak or no activity against other pathogenic bacteria.[58,59] The genome sequencing of resistant mutants, as well as the use of a photoaffinity probe, led to the identification of an outer membrane protein involved with lipopolysaccharide (LPS) assembly, LptD, as the target of murepavadin (**8**).[58] A crystal structure of the *Shigella flexneri* LptD−LptE complex showed that LptD forms a 26-stranded β-barrel with LptE located inside this barrel and that distortion in the first two β-strands could facilitate lateral LPS transport to the outer leaflet of the outer membrane.[60] A murepavadin analog was shown to bind to the LptD β-jellyroll domain of the *Pseudomonas aeruginosa* LptD−LptE complex and block LPS transport.[61] A phase 1 trial investigating inhaled murepavadin (**8**) for cystic fibrosis (CF) has been completed, and there are future development plans to also investigate non-CF bronchiectasis.[62] Previously, two phase 3 trials examined iv administration of **8** for the treatment of *Pseudomonas* nosocomial pneumonia (NCT03582007) and ventilator-associated pneumonia (VAP) infections (NCT03409679), but increase in serum creatinine and acute kidney injury in the pneumonia trial led to discontinuation of both trials.[63] It has been shown that the combination of murepavadin (**8**) with the LPS-binding colistin is synergistic, with improved activity in a murine *P. aeruginosa* model,[64] but cross-resistance has been observed.[65] A tethered murepavadin and colistin derivative had broad spectrum G−ve activity via binding to both LPS and BamA, a component of the β-barrel folding complex (BAM), which is essential for the folding and insertion of β-barrel proteins into the outer membrane.[66]

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Recently, it was disclosed that several tethered macrocyclic peptides (MCPs) were discovered using phenotypical whole cell screening with selective *in vitro* and *in vivo* activity against *Acinetobacter baumannii*.[67] Structure optimization led to the identification of a zwitterionic MCP, zosurabalpin (**9**) (Abx MCP, RG6006, RO7223280), which is currently being evaluated in a phase 1 trial by Roche for the treatment of *A. baumannii* infections. Whole genome sequencing of resistant *A. baumannii* mutants generated using a dynamic culture model showed that mutations were primarily located in genes that encode components of the $LptB_2FGC$ complex, lipid A synthesis and efflux. Biochemical studies showed that **9** inhibited the $LptB_2FGC$ complex of *Acinetobacter baylyi*, but not *Escherichia coli*. In another study, cryogenic electron microscopy (cryo-EM) was used to demonstrate that MCPs formed extensive contacts with both *A. baylyi* $LptB_2FG$ and bound LPS, trapping LPS within the $LptB_2FG$ complex.[68] It was also proposed that there is a toxic accumulation of LPS biosynthesis intermediates, which causes cell death. The selectivity for *Acinetobacter* over other G−ve bacteria was proposed to be due to $LptB_2FG$ complex sequence differences. *A. baumannii* can survive without LPS in the outer membrane,[69] and it was shown that MCPs could not inhibit the growth of LPS-deficient strains.[68] However, there are significant fitness and virulence costs associated with not having LPS in the outer membrane.

Another cell envelope target is decaprenylphosphoryl-β-D-ribose (DPR) 2′-oxidase (DprE1), a critical enzyme in the biosynthesis of arabinogalactan and lipoarabinomannan, which are key components of the mycobacterial cell wall. The extracytoplasmic location of DprE1 enhances the ability of inhibitors to reach their target.[70,71] In 2006, a series of dialkyldithiocarbamate derivatives were reported to have antimycobacterial activity,[72] and further optimization led to the identification of a benzothiazinone, BTZ-043 (**10**),[73−75] with *in vivo* TB activity.[76] MoA studies showed that the BTZs underwent *in vivo* reduction of the nitro group, then formed a semimercaptal covalent bond with cysteine-387 of DprE1.[76−78] A phase 2 trial (NCT05926466) evaluating three BTZ-043 (**10**)-containing TB drug regimens started in September 2023. Macozinone (**11**) (PBTZ169) is another BTZ that acts via the same MoA but possesses improved physicochemical properties.[79,80] It was evaluated in a phase 2 early bactericidal activity (EBA) TB trial (NCT03334734) that started in 2016 but was terminated in 2018 after enrolling only 16 patients. Evidence suggests that BTZs can be dearomatized through the formation of a Meisenheimer complex, reducing their *in vivo* half-lives.[81−83]

There are two further DprE1 inhibitors being evaluated in clinical trials, quabodepistat (**12**) (OPC-167832) and TBA-7371 (**13**). Quabodepistat (**12**) belongs to the new carbostyril class, which was discovered using phenotypic screening, has activity against several *Mycobacterium* species, and non-covalently binds to the active pocket of DprE1.[84,85] Quabodepistat (**12**) started a phase 2 trial (NCT05221502) in April 2022 in combination with other TB drugs. TBA-7371 (**13**) (AZ-7371) is a substituted 1,4-azaindole that was discovered via scaffold morphing efforts with potent TB activity via noncovalent DPrE1 inhibition.[86−89] TBA-7371 (**13**) completed a phase 2 EBA trial (NCT04176250) in October 2022.

Several other clinical candidates act via less well-defined mechanisms that include membrane penetration. Cannabidiol (**14**, CBD), the major non-psychoactive component of the cannabis plant was first reported to have G+ve antimicrobial activity in 1976[90] which was confirmed in 2008,[91] with more comprehensive studies in 2020−2021.[92,93] Botanix Pharmaceuticals has evaluated topical CBD (**14**) (BTX 1801) in phase 2 trials for the clearance of nasally colonized *S. aureus* (ACTRN12620000456954) and has tested topical treatment for bacterial-associated skin diseases including acne (BTX 1503, NCT03573518) and atopic dermatitis (BTX 1204, NCT03824405). The specific antibacterial MoA of **14** has not been fully elucidated, but cell membrane permeation is involved.[93]

S. aureus nasal decolonization via topical treatment has also been targeted by XF-73 (**15**) (exeporfinium chloride),[94,95] under development by Destiny Pharma, with a phase 2b study (NCT03915470) completed in 2021.[96] XF-73 (**15**) is a dicationic porphyrin derivative active against G+ve and select G−ve bacteria. Again, disruption of membrane integrity, followed by loss of ATP and inhibition of DNA, RNA, and protein synthesis, was proposed as the MoA, with a secondary light-activated release of reactive oxygen species.[94,97]

R327 is a synthetic polymer under development by Recce Pharmaceuticals,[98,99] which is composed of a mixture of polyethylene−polyacrolein copolymers in the presence of poly(ethylene glycol) and water. Recce has proposed in a poster presentation that R327 permeabilizes the cell membrane and enters the cell where it disrupts bacterial cellular energetics, protein synthesis, and cell division.[100,101] A phase 1 trial (ACTRN12621001313820) was successfully completed in 2023, while topical treatment of infected skin and implant wounds has also been explored under an Australian TGA special access scheme. A phase 1/2 trial for the treatment of burn wound infections was completed in August 2023 (ACTRN12621000412831). A phase 1/2 trial for diabetic foot ulcers (ACTRN12623000056695) and a phase 1 iv administration study (ACTRN12623000448640) are ongoing.

Several cationic AMPs, working via membrane disruption, have progressed into clinical trials. Peceleganan (**16**) (PL-5) is a chemically synthesized α-helical cationic 26-residue AMP derived from cecropin A and melittin B.[102] A successful phase 2b trial conducted by ProteLight Pharma compared the efficacy and safety of topical peceleganan (**16**) spray, as compared with silver sulfadiazine, in patients with skin and wound infections;[103] a phase 3 trial has been initiated in China (ChiCTR2300071255). OMN6 (**17**) is a 40-amino acid cyclic disulfide peptide with two independent helices linked by a proline hinge based on cecropin A, which has activity only against Gram-negative bacteria such as *A. baumannii* (most potent), *Klebsiella pneumoniae*, and *E. coli*.[104,105] OMN6 (**17**) shows no observable cross-resistance with current Gram-negative antibiotics and is in phase 2 (NCT06087536) for the treatment of hospital-acquired and ventilator-associated bacterial pneumonia (HABP/VABP) caused by *A. baumannii* complex infections. PL18 (**18**) (HPRP-A1), a 15-mer α-helical cationic peptide derived from the N-terminus of the *Helicobacter pylori* ribosomal protein L1 (RpL1),[106−108] is also being investigated as a suppository by ProteLight Pharma for bacterial and fungal vaginosis (phase 1, NCT05340790). Peptilogics Inc. has developed PLG0206 (**19**), a 24-residue helical peptide, as a potential treatment for prosthetic joint infections (PJIs).[109] It has completed a phase 1 iv infusion trial[110] and is undergoing another phase 1 trial in PJI patients with an estimated 2024 completion (NCT05137314).

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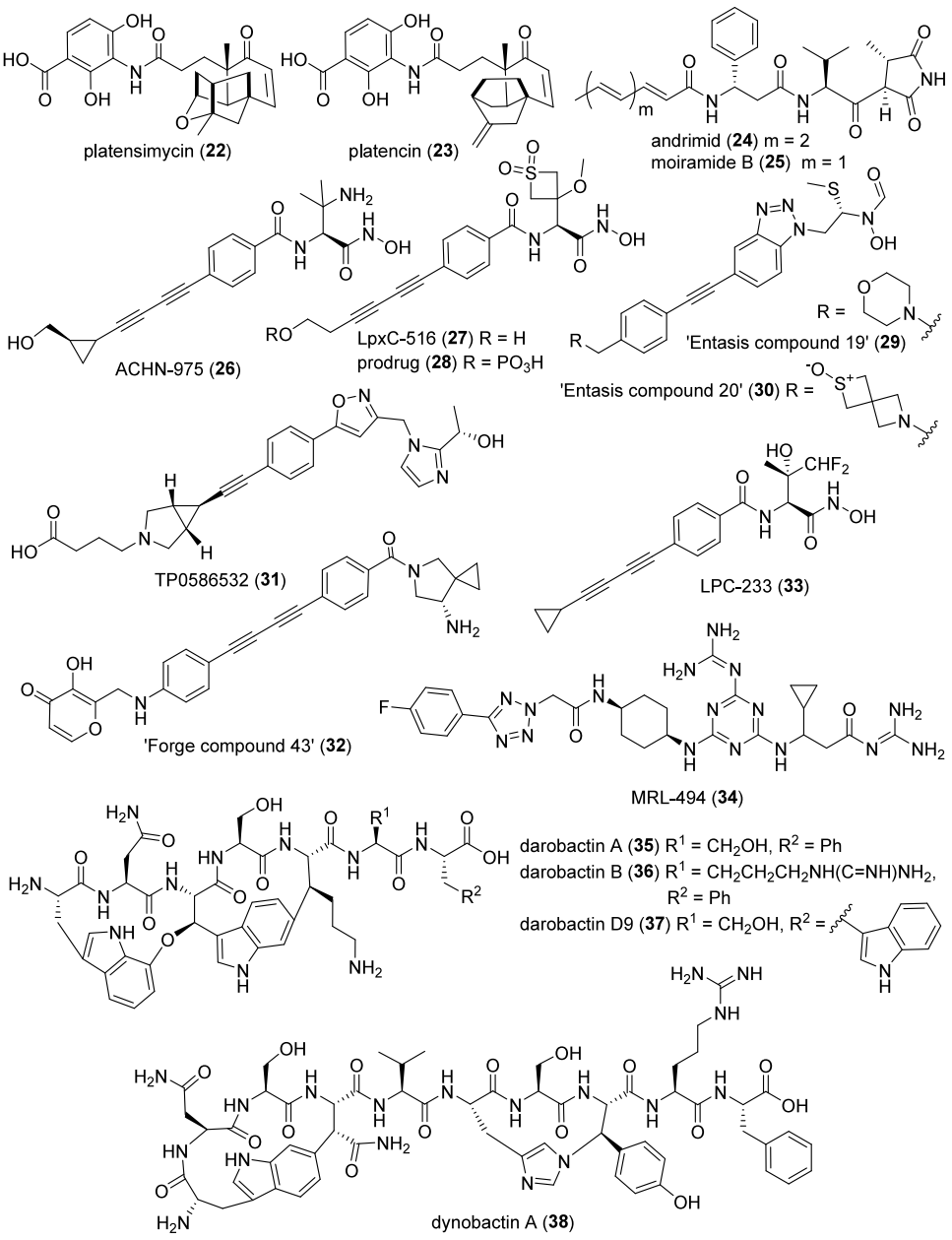

Figure 4. Structures of preclinical cell wall targeting antibacterials and related compounds with new MoAs not found in existing antibacterial drugs (Part 1).

Previous examples of clinical-stage AMPs include the protegrin I analog iseganan (**20**) (IB367).[111] A phase 3 trial for oral mucositis (ClinicalTrials.gov Identifier: NCT00022373) appeared to show efficacy,[112] but a VAP trial (NCT00118781) was terminated in 2004. Omiganan (**21**) (CLS-001, MBI 226) is a synthetic indolicidin analog 12-residue cationic AMP that has completed a range of phase 2 and phase 3 trials, most recently in atopic dermatitis, where it failed to improve clinical symptoms in patients.[113]

Cell Envelope: Other Selected Compounds. In addition to FabI,[48,49] FabH, the initiation-condensing enzyme, and FabF/B, the elongation-condensing enzyme, are also essential FASII pathway enzymes and highly conserved among key pathogens. Platensimycin (**22**) (FabF inhibitor)[114,115] and later platencin (**23**) (dual FabF/FabH inhibitor) (Figure 4)[116] were isolated from two strains of *Streptomyces platensis* using a FabF/FabH antisense two-plate agar diffusion assay,[117,118] and their activity was confirmed using a FASII biochemical assay. Platensimycin (**22**) and platencin (**23**) were active against several pathogenic G+ve bacteria, such as MRSA, vancomycin-resistant enterococci (VRE), *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis* (Mtb),[119] but lacked activity against G−ve bacteria, likely due to membrane penetration and/or efflux issues.[114,116] However, it was later reported that, due to rapid clearance, poor *in vivo* efficacy was observed in a murine model of infection when dosed conventionally (po, sc, and ip), but efficacy was obtained using continuous infusion.[120,121] Their unique structures and promising biological activity spurred research to improve the biological activity and development of SAR from total synthesis, semisynthesis, and the isolation of naturally occurring analogs, which has been reviewed.[122,123]

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Andrimid (**24**) was first reported in 1987 as an antibiotic from an intracellular symbiont, *Enterobacter* sp., of *Nilaparvata lugens* (brown planthopper),[124] while an active analog, moiramide B (**25**), was isolated along with andrimid (**24**) in 1994 from the marine-derived bacterium *Pseudomonas fluorescens*.[125] Both **24** and **25** have broad spectrum low μM minimum inhibitory concentrations (MICs) against pathogens such as *S. aureus*, *S. pneumoniae*, *E. coli*, and *P. aeruginosa*.[124,125] In 2004, it was shown that **24**, **25**, and synthetic analogs inhibited the carboxyltransferase subunit (β-subunit) of the bacterial enzyme acetyl-CoA carboxylase (ACC) with nM affinity.[126−129] In 2016, an X-ray crystal structure of moiramide B (**25**) bound *S. aureus* carboxyltransferase showed that the valine-methyl pyrrolidinedione subunit interacted with the enzyme oxyanion holes via an enol form, which mimics the enolate present in biotin.[130] Recent work has suggested that the sorbamide group in **25** and analogs with aromatic acyl tails can enhance enzyme binding, as well as demonstrating activity against *Mycobacterium*.[131]

The outer membranes of G−ve bacteria contain an asymmetrical bilayer of LPS on the outside and phospholipids on the inside. The biosynthetic pathway for the lipid A portion of LPS is essential and conserved in most G−ve bacteria but is absent in humans, making it an attractive target for antimicrobial intervention[132,133] The lipid A biosynthesis pathway includes a range of targets, such as LpxC (uridine diphosphate-3-*O*-(hydroxymyristoyl)-*N*-acetylglucosamine deacetylase),[134] LpxA (UDP-*N*-acetylglucosamine acyltransferase),[135−137] LpxD (acyl-ACP-dependent *N*-acyltransferase),[135,138,139] and LpxH (UDP-2,3-diacylglucosamine pyrophosphate hydrolase).[134,140−142] Only those targeting LpxC have progressed to clinical trials, with the first LpxC inhibitors described by Merck scientists in 1996.[143] LpxC is a metalloenzyme needed for the first committed step in lipid A biosynthesis. Many LpxC inhibitors incorporate a hydroxamate "warhead" to bind the Zn^{2+} found in the enzyme active site, but the hydroxamate can be a metabolic liability as it can be metabolized to a potentially harmful hydroxylamine.

Two different inhibitors have advanced into clinical trials, Achaogen's ACHN-975 (**26**) (NCT01597947, NCT01870245) and Recida Therapeutics' RC-01 (structure not publicly disclosed, T-1228) (NCT03832517). Unfortunately, further development of both inhibitors was terminated due to safety concerns, with ACHN-975 (**26**) exhibiting dose-limiting cardiovascular toxicity of transient hypotension without compensatory tachycardia.[144] Further work at Achaogen indicated that the "much-maligned hydroxamic acid" was not entirely responsible for the cardiovascular side effects as an amide analog was equally toxic.[145] A new derivative, LpxC-516 (**27**), with improved properties was identified, along with a phosphate prodrug **28** to improve solubility, but unexpectedly, the prodrug (but not the parent) also showed rat cardiovascular toxicity.[145] Entasis Therapeutics reported LpxC inhibitors with an *N*-hydroxyformamide zinc-binding moiety in place of the hydroxamic acid group, and **29** showed activity in a neutropenic mouse *E. coli* infection model.[146] Another analog **30** with an improved profile was found to have a hypotensive effect in a rat hemodynamic assay,[146] which showed that changing the zinc binding motif to an *N*-hydroxyformamide failed to eliminate this cardiovascular toxic liability.

The strong interest in LpxC as an antibacterial target is demonstrated by the continued work in the area: Taisho Pharmaceutical Co has identified TP0586532 (**31**), a non-hydroxamate LpxC inhibitor, which inhibits toxic LPS release during *in vivo Klebsiella pneumoniae* infections.[147] Vernalis has applied a fragment-based approach to identify non-hydroxamate LpxC inhibitors based on a 2-(1*S*-hydroxyethyl)-imidazole core, with low nanomolar inhibition of LpxC and a MIC of 4 μg/mL against *P. aeruginosa*.[148] Forge Therapeutics (now Blacksmith Medicines) has applied a non-hydroxamate metal-binding pharmacophore chemistry platform to develop LpxC inhibitors and has received both CARB-X[149] and NIH/NIAID[150] funding. Their compounds appear to be based on a pyrone structure (e.g., "Forge compound 43" (**32**)[151]) and are proposed to form a bidentate complex with the zinc ion.

Recently, a slow, tight-binding picomolar LpxC inhibitor, LPC-233 (**33**), was reported by researchers from Duke University and Valanbio Therapeutics.[152] It exhibited activity against a wide range of G−ve clinical isolates *in vitro*, was orally bioavailable, and was efficacious in murine soft tissue, sepsis, and urinary tract infection models. Notably, preclinical studies demonstrated a promising safety profile, with no detectable adverse cardiovascular toxicity in dogs when dosed at 100 mg/kg. Note that when comparing cardiovascular effects caused by different molecules, the actual exposure levels achieved are the critical parameter, rather than the dose delivered.

The G−ve outer membrane is decorated with integral outer-membrane proteins (OMPs), including peripherally associated lipoproteins. The OMPs are folded into the membrane by a protein complex called the β-barrel assembly machine (BAM), comprised of 5 proteins, BamA−E. It is essential for viability, in part for its role in inserting/assembling LptD in the outer membrane, and therefore has recently been explored as an antibacterial target. Most efforts have focused on the essential component BamA, with some also targeting the variably essential BamD.[153,154] New assays have been developed to identify and evaluate BAM inhibitors.[155,156]

The first reported BAM inhibitor, MRL-494 (**34**), was discovered while screening for compounds that disrupted the OM barrier at sublethal concentrations, as well as having activity against wild-type *E. coli* and an efflux pump mutant (*tolC* deletion).[157] A series of experiments including cellular thermal shift assays (CETSA), showed that **34** bound to BamA but not the other Bam protein components. MRL-494 (**34**) was also found to thermally stabilize an E470K resistant mutant,[157] and it was recently reported that strains bearing a $BamA^{E470K}$ mutation could bypass the essential requirement for BamD in β-barrel outer membrane protein assembly.[158] MRL-494 (**34**) was also reported to inhibit the growth of G+ve bacteria through cytoplasmic membrane disruption, likely caused by the cationic nature of the guanidine groups.[157] This was supported by reduced activity of guanidine to amine analogs in the SAR study.[159]

Soon after the report on MRL-494 (**34**), darobactin A (**35**) was identified from *Photorhabdus khanii* (a nematode gut symbiont) and was selectively active against G−ve bacteria via inhibition of BamA. It displayed *in vivo* activity against *E. coli*, *P. aeruginosa*, and *K. pneumoniae* in mouse infection models.[160] The darobactins were shown to bind to an open form of the BamA lateral gate by mimicking a β-strand[161−163] which was at an earlier assembly stage compared to MRL-494 (**34**) and the tethered murepavadin and colistin derivative.[164] Mutasynthetic work has led to the identification of more active analogs such as darobactin B (**36**) and D9 (**37**).[165−170] Recent total syntheses of darobactin A (**35**) have also laid the groundwork

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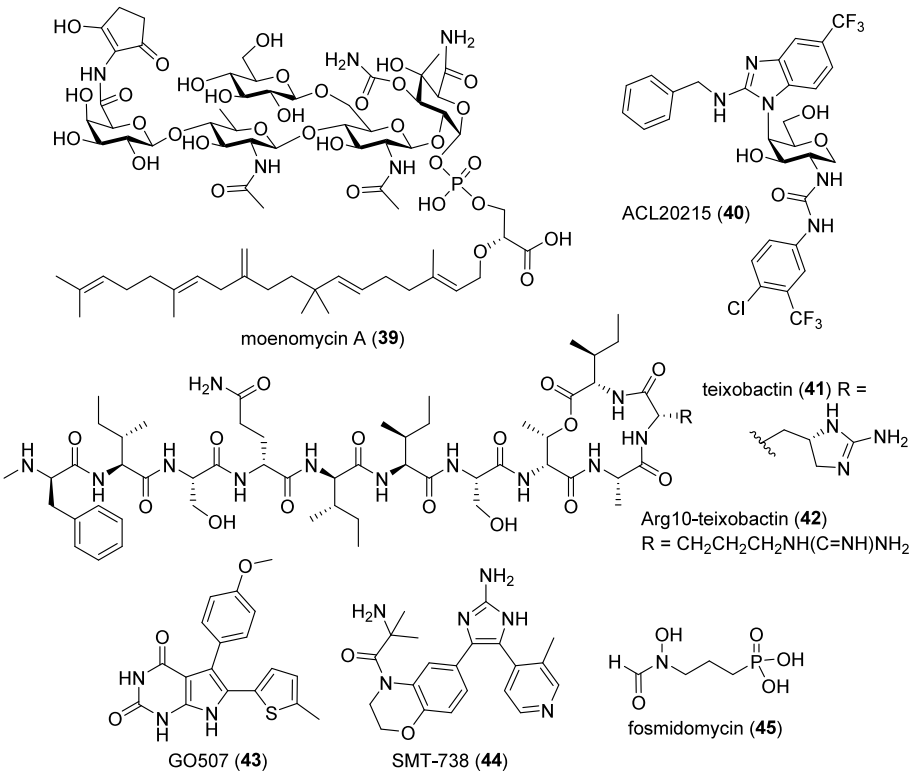

Figure 5. Structures of preclinical cell envelope targeting antibacterials with new MoAs not found in existing antibacterial drugs (Part 2).

for access to new analogs.[171−173] Finally, a computational approach that searched for genes related to the darobactin operon, facilitated the identification of another new BamA inhibitor, dynobactin (**38**), from *Photorhabdus australis*.[174]

There are several inhibitors of peptidoglycan glycosyltransferases, which polymerize the cell wall glycan chain. The moenomycins (e.g., moenomycin A (**39**), Figure 5) are a family of phosphoglycolipid antibiotics isolated from *Streptomyces* that mimic the natural substrate. Bambermycin (flavomycin), a veterinary antibiotic used in animal feed, contains moenomycins A (**39**) and C.[175] A range of structural classes of molecules have been investigated as inhibitors.[176,177] For example, a carbohydrate scaffold produced a set of small molecules, including ACL20215 (**40**), that were efficacious in *in vivo* infection models.[178]

Peptidoglycan synthesis is also one of the targets of teixobactin (**41**), a cyclic undecapeptide that received significant attention when first reported as it was discovered using the "iChip" approach to screen uncultured bacteria from soil samples.[179] Only active against G+ve bacteria, it binds multiple undecaprenol phosphate-coupled cell wall precursors, including peptidoglycan precursor lipid II and wall teichoic acid precursors.[180] The original discovery highlighted a lack of detectable resistance development, but a 2020 study showed *S. aureus* could develop significant resistance to a teixobactin analog, Arg_{10}-teixobactin (**42**), and to moenomycin, on clinically meaningful time scales.[181] However, the levels were 2500-fold and 8-fold less than similarly evolved resistance to rifampicin, and resistance was rapidly lost without antibiotic selection. Recent progress in the development of teixobactin (**41**) analogs was reviewed in 2022.[182,183]

The LolCDE ABC transporter is the inner membrane component of the Lol system, which traffics lipoproteins from the cytoplasm to the outer membrane and is essential for bacterial viability. An AstraZeneca screening campaign uncovered pyridineimidazole and pyrazole inhibitors in 2015,[184,185] while Genentech reported a pyrrolopyrimidinedione compound, G0507 (**43**), in 2018.[186] Summit Therapeutics received CARB-X funding in 2021 for preclinical work on SMT-738 (**44**), a first-in-class LolCDE targeting carbapenem-resistant Enterobacteriaceae (CRE).[187,188]

1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR or IspC) is an enzyme in the bacterial isoprenoid biosynthesis pathway, which differs from mammalian isoprenoid synthesis. Inhibitors have been under development since 1980, when an active site divalent metal binding hydroxamate, fosmidomycin (**45**), was identified. Basilea Pharmaceutica International received CARB-X funding in 2021 for hit-to-lead work on a DXR inhibitor.[189,190]

DNA Synthesis/Transcription: Compounds in Clinical Trials. During cell division, the bacterial DNA replisome acts by first unwinding double-stranded DNA and then copying DNA from one of the original DNA molecules. The replisome consists of 13 protein subunits, many of which are essential for survival. Several of these proteins have been investigated as potential antibacterial targets[191] with inhibition of the type-II topoisomerases DNA gyrase and topoisomerase IV by (fluoro)quinolone antibiotics[192−195] being the best known examples. These essential bacterial enzymes, which are not present in eukaryotes, wind and unwind DNA to reduce topological stress.[196] The A_2B_2 heterotetrameric units of DNA gyrase consist of 2 × GyrA/GyrB subunits, while topoisomerase IV has pairs named ParC/ParE or GrlA/GrlB in G−ve and G+ve bacteria, respectively. Generally, DNA gyrase is the predominant target of quinolones in G+ve bacteria and topoisomerase IV in G−ve bacteria, but this can vary depending upon the bacteria and antibacterial agent. In some bacteria, such as mycobacteria and *H. pylori*, topoisomerase IV

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Table 3. Antibacterial Compounds in Clinical Trials with New MoAs Not Used by Antibacterial Drugs That Target DNA Synthesis[a]

name	compound class (lead source)	mode of action	administration; development phase (status), indication (developer)
zoliflodacin (**48**)	spiropyrimidinetrione (S)	DNA gyrase (GyrB)	po; phase 3, gonorrhea (Innoviva/GARDP)
gepotidacin (**49**)	triazaacenaphthylene (S)	DNA gyrase (GyrA) — different to quinolones	po; phase 3 (completed), UTI; phase 3 (completed), gonorrhea (GSK)
fobrepodacin (**50**) (prodrug); SPR719 (**58**)	"ethyl urea benzimidazole" (S)	DNA gyrase (GyrB) and Topo IV (ParE)	po; phase 2, pulmonary NTM (Spero Therapeutics)
BWC0977 (**51**)	oxazolidinone containing NBTI (S)	DNA gyrase (GyrA) and topoisomerase IV	iv/po, phase 1, G−ve and G+ve, but being developed for G−ve (Bugworks Research)
ibezapolstat (**59**)	dichlorobenzyl guanine (DCBG) (S)	DNA polymerase IIIC	po topical; phase 2, CDI (Acurx Pharmaceuticals)
MGB-BP-3 (**60**)	distamycin A (NP)	DNA minor groove binding	po topical; phase 2 (completed), CDI (MGB Biopharma)

[a]Abbreviations: CDI, *C. difficile* infections; G−ve, Gram-negative; G+ve, Gram-positive; iv, intravenous; NP, natural product-derived; NTM, non-tuberculosis mycobacteria; po, oral (*per os*); S, synthetic; UTI, urinary tract infections. po topical, delivered orally for non-systemic gut topical use.

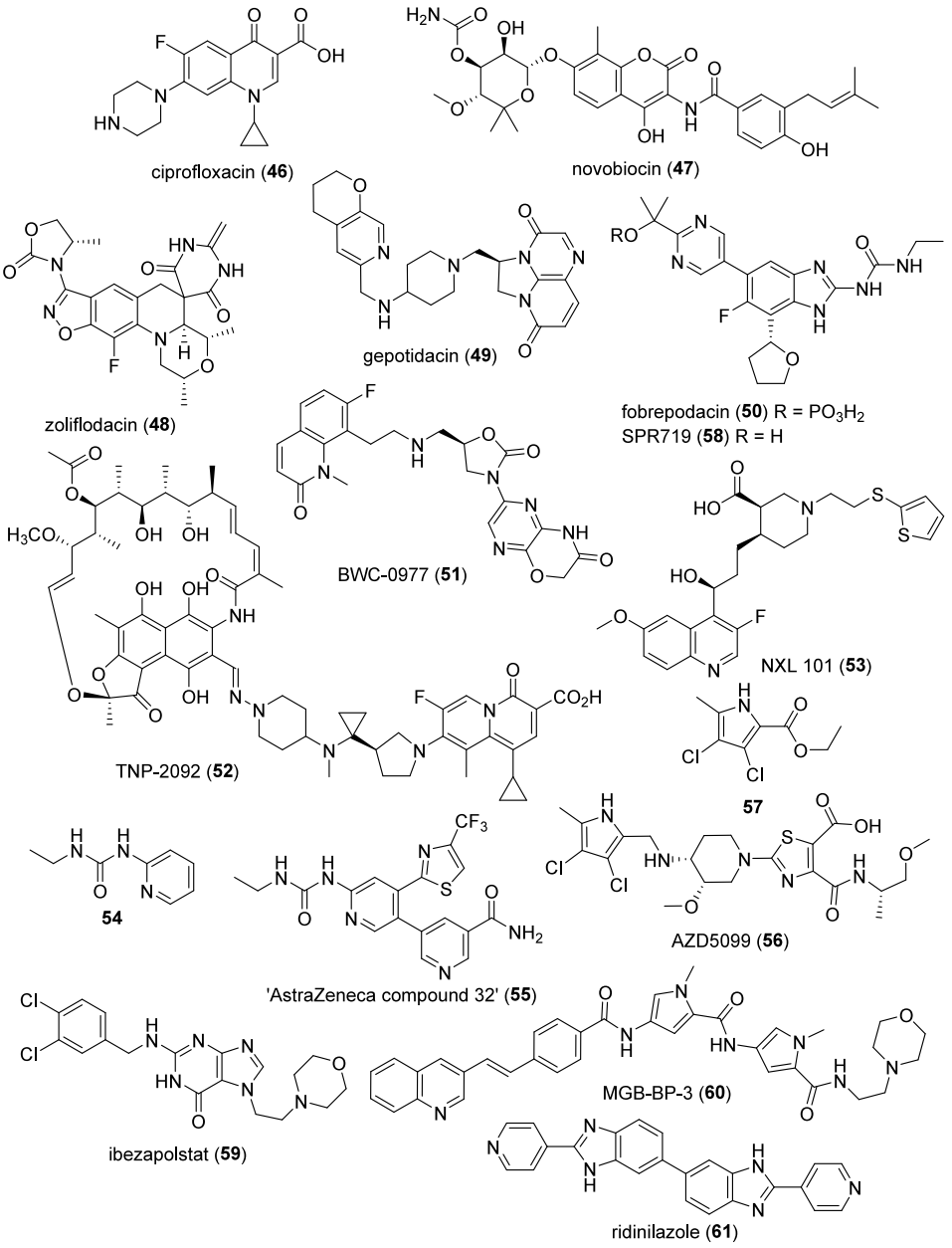

Figure 6. Structures of DNA synthesis targeting antibacterials currently being evaluated in clinical trials and related compounds with new MoAs not used by antibacterial existing drugs.

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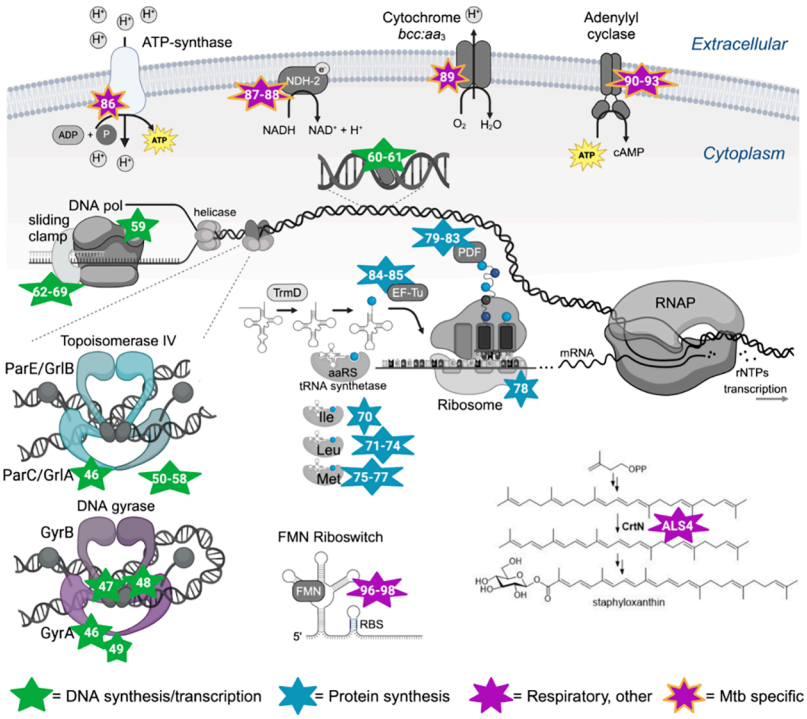

Figure 7. Intracellular pathways targeted by antibiotics with new MoAs not found in existing drugs. Abbreviations: aaRS, aminoacyl-tRNA synthetase; FMN, flavin mononucleotide; EF, elongation factor; PDF, peptide deformylase; RBS, ribosome binding site.

is not present and DNA gyrase is the target.[197,198] These topoisomerases alter DNA topology via a double-strand break, passing a second duplex, and then resealing the break. Quinolones such as ciprofloxacin (**46**) form complexes with either the GyrA subunit of DNA gyrase or ParC/GrlA of topoisomerase IV via a water−metal ion bridge, which is called DNA gyrase poisoning. Another target site is an ATP binding pocket in GyrB that is targeted by novobiocin (**47**), which was used as mostly a G+ve antibacterial agent from the mid-1950s and late 1960s but fell out of favor due to resistance and toxicity issues.[199,200] A list of compounds that target DNA synthesis undergoing clinical trials with new MoAs that are not represented in approved antibiotics are listed in Table 3, their structures are shown in Figure 6, and their pathway targets are illustrated in Figure 7.

There are four new antibacterial classes being evaluated in clinical trials[14] that inhibit DNA gyrase and/or topoisomerase IV, zoliflodacin (**48**), gepotidacin (**49**), fobrepodacin (**50**), and BWC0977 (**51**), which are collectively known as novel bacterial topoisomerase inhibitors (NBTIs).[201] There is also a rifamycin and quinolizinone (quinolone) hybrid, TNP-2092 (**52**), in clinical development that inhibits both RNA polymerase and DNA gyrase/topoisomerase IV.[202]

Zoliflodacin (**48**) (ETX0914, AZD0914)[203−205] belongs to the new spiropyrimidinetrione class and binds to the same DNA cleavage site as the quinolones, but with a different mode of interaction. Two molecules of zoliflodacin (**48**) block access to the same DNA cleavage site on GyrB as the quinolones but did not use the water−metal ion bridge to GyrA that is employed by the quinolones.[206] In *Neisseria gonorrhoeae*, it was demonstrated that **48** was more active against DNA gyrase compared to topoisomerase IV and that most resistant strains had mutations in GyrB.[204] In addition, **48** was shown to induce an SOS response to DNA damage in *E. coli* at levels similar to ciprofloxacin and exhibited minimal inhibition of human type II topoisomerases α and β.[204] Zoliflodacin (**48**) was evaluated in a phase 3 gonorrhea trial supported by Entasis/Innoviva in collaboration with the Global Antibiotic Research and Development Partnership (GARDP). The study tested 930 people with gonorrhea in South Africa, Thailand, the United States, Belgium, and The Netherlands (NCT03959527),[207] with positive results reported in Nov 2023.[208]

Gepotidacin (**49**) (GSK-2140944, triazaacenaphthylene class[209,210]) inhibits GyrA by binding between the two GyrA subunits,[211,212] which is a different binding site from the quinolones.[196] Binding induces single-stranded DNA breaks, which is unlike the quinolones where no double-stranded DNA breaks were observed.[211] Gepotidacin (**49**) met the primary end points of two uncomplicated UTI (uUTI) phase 3 trials (NCT04020341 and NCT04010539)[213] and is also being evaluated in a phase 3 trial (NCT04010539) as a treatment for gonorrhea.[214] This new class of non-quinolone bacterial type II topoisomerases was first exemplified by NXL-101 (**53**),[215] which entered phase 1 but was discontinued due to QT interval concerns.[216]

Other earlier bacterial topoisomerase inhibitors include two discovered using fragment-based screening. 1-Ethyl-3-(2-pyridyl)urea **54** was found to bind to the ATP binding domain of bacterial topoisomerases, and structure optimization led to the identification of **55**, which inhibited GyrB and ParE and had promising activity against G+ve pathogens in a neutropenic mouse thigh *S. aureus* infection model.[217] AZD5099 (**56**) was discovered using the same fragment-based approach starting with ethyl 3,4-dichloro-5-methyl-1*H*-pyrrole-2-carboxylate **57**.[218] AZD5099 (**56**) was evaluated in phase 1, but its development was halted due to variable exposure levels and concerns about mitochondrial changes observed in species during preclinical safety evaluation.[200]

A high-throughput GyrB ATPase assay was used to identify a series of benzimidazole carbamate containing compounds,[219]

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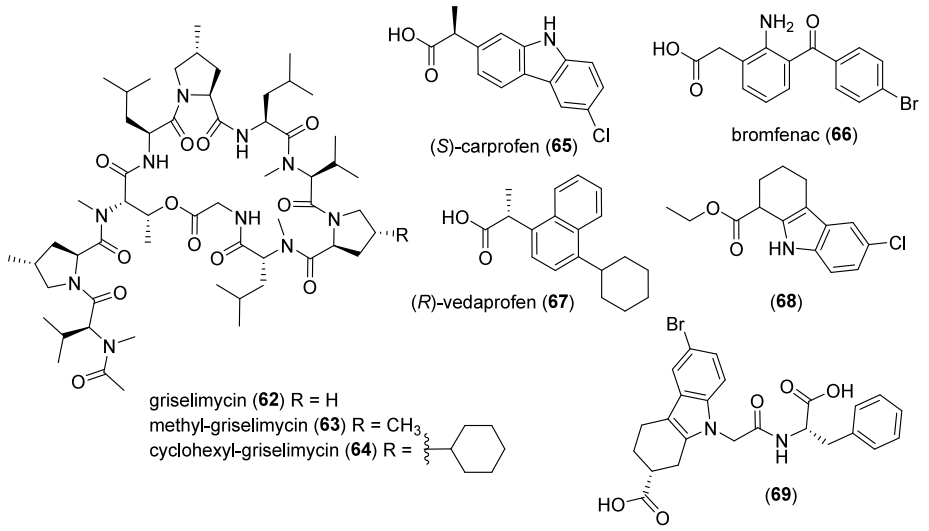

Figure 8. Structures of preclinical DNA synthesis targeting antibacterials with new MoAs not used by existing antibacterial drugs.

with structure optimization leading to the discovery of SPR719 (**58**) (VXc-486) and its phosphate prodrug fobrepodacin (**50**) (SPR720, pVXc-486; "ethyl urea benzimidazole" class).[220,221] SPR719 (**57**) has *in vitro* and *in vivo* activity against G+ve bacteria, some G−ve bacteria, and mycobacteria.[220−224] Members of this series have been shown to target both ATP binding sites of GyrB and ParE in G+ve bacteria[225] and GyrB in mycobacteria (ParE orthologue not present),[224] which is the same as novobiocin (**47**).[199] Fobrepodacin (**50**) is currently being evaluated in a phase 2 clinical trial (NCT03796910) as a potential treatment for non-tuberculosis mycobacteria (NTM) pulmonary disease.

A new NBTI, BWC0977 (**51**),[201,226] has been developed by the Indian company Bugworks to treat critical care G−ve infections including *A. baumannii*, *E. coli*, *K. pneumoniae*, and other Enterobacteriaceae.[227,228] A 2019 conference poster disclosed that **51** equally inhibited both DNA gyrase GyrA and topoisomerase IV, bound to the DNA gyrase GyrA dimer interface, and also triggered an SOS response.[229] In addition, BWC0977 (**51**) was found to stabilize single-stranded DNA breaks, which is different from quinolones such as ciprofloxacin that stabilize double-strand breaks.[229] BWC0977 (**51**), with development supported by CARB-X since 2017, completed a phase 1 trial in 2023 (NCT05088421), and a collaboration with GARDP was announced in July 2023 to advance BWC0977 through phase 2 and 3 studies.[230]

Another class of DNA synthesis targets are bacterial DNA polymerases (C-family), which are structurally distinct from eukaryotic DNA polymerases (B-family). G+ve bacteria with low guanine and cytosine content, which include pathogenic species such as *Clostridioides*, *Enterococcus*, *Mycoplasma*, *Staphylococcus*, and *Streptococcus*, have a subtype of DNA polymerase IIIC.[231,232] Ibezapolstat (**59**) (ACX-362E) is an N7-substituted guanine derivative that inhibits DNA polymerase IIIC. Its structure includes a base pairing domain that binds to cytosines in the DNA template strand, an aryl binding domain, and an N3 substitution that enhances both activity and solubility (Figure 6).[232−236] Ibezapolstat (**59**) is being evaluated in a phase 2 CDI trial (NCT04247542) as an oral (e.g., gut topical) treatment for *Clostridioides difficile* infections (CDIs).[237] Acurx is also developing a preclinical compound based on the same scaffold with the same DNA polymerase IIIC target. ACX-375C (structure not disclosed) is active against all G+ve bacteria, including vancomycin-resistant enterococci (VRE), MRSA, and penicillin-resistant *S. pneumoniae* (PRSP).[238]

MGB-BP-3 (**60**) is a polypyrrole derivative that binds to AT-rich sequences in the minor groove of double stranded DNA, resulting in disruption of DNA transcription, gyrase (supercoiling action), and topoisomerase IV (relaxation and decatenation) in *S. aureus* and *E. coli*[239] *in vitro* assays. MGB-BP-3 (**60**) is being developed as an oral (gut topical) treatment of *C. difficile*-associated diarrhea (CDAD) and completed a phase 2 trial (NCT03824795) in May 2020. Although **60** is active against a range of G+ve bacteria such as *S. aureus* and *Enterococcus faecalis*, limited intracellular accumulation abrogates its activity against G−ve bacteria.[239] It was also reported in a preprint that **60** bound to and inhibited several essential promoters on the *S. aureus* chromosome, potentially indicating multiple MoAs.[240] Another minor groove binder is ridinilazole (**61**), a symmetrical head-to-head bis-benzimidazole, which was also previously in development for CDI.[241,242] Again, multiple MoAs might be in effect, as RNA-seq data indicated that **61** also had broad effects on multiple *C. difficile* compartments, particularly pathways involved in energy generation.[241] Ridinilazole (**61**) completed two phase 3 trials (NCT03595553, NCT03595566) in 2021, but a prespecified superiority threshold was not met, and Summit has halted further development.[243]

DNA Synthesis/Transcription: Other Compounds of Interest. Griselimycin (**62**, Figure 8) belongs to depsipeptide complex isolated from *Streptomyces* sp. in the 1960s with activity against *Mycobacterium*,[244] but its development was halted due to a combination of poor PK/PD and the introduction of rifampin.[245] Investigation into its metabolic stability identified that Pro-8 was a metabolic liability.[245] Two Pro-8 derivatives, methyl-griselimycin (**63**) and cyclohexyl-griselimycin (**64**), were synthesized that were more active and had superior *in vivo* exposure in a TB mouse model compared to griselimycin (**62**). During this study, it was shown that the producing *Streptomyces* was naturally resistant to griselimycins through mutations in its *dnaN* gene, which encodes the bacterial sliding clamp (DNA polymerase III β subunit), that serves as a mobile tether on DNA for several essential partner

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Table 4. Antibacterial Compounds in Clinical Trials with New MoAs Not Used by Antibacterial Drugs That Target Protein Synthesis[a]

name	compound class (lead source)	mode of action	administration; development phase (status), indication (developer)
epetraborole (**72**)	oxaborole (S)	leucyl-tRNA synthetase (LeuRS)	po; phase 2/3, NTM with a focus on the *M. avium* complex (AN2 Therapeutics)
ganfeborole (**73**)	oxaborole (S)	leucyl-tRNA synthetase	po; phase 2, TB (GSK)
CRS3123 (**77**)	"diaryldiamine" (S)	methionyl-tRNA synthetase (MetRS)	po topical, phase 2, CDI (Crestone)
gallium nitrate (Ganite)	metal	iron-dependent synthetic and metabolic pathway inhibition	iv, phase 1 NTM, phase 2, *P. aeruginosa* infected CF subjects (University of Washington)
gallium citrate (AR-501)	metal	iron-dependent synthetic and metabolic pathway inhibition	inhalation, phase 1/2, *P. aeruginosa* infected CF subjects (Ardis)

[a]Abbreviations: CF, cystic fibrosis; NTM, non-tuberculosis mycobacteria; po, oral (*per os*); S, synthetic. po topical, delivered orally for non-systemic gut topical use.

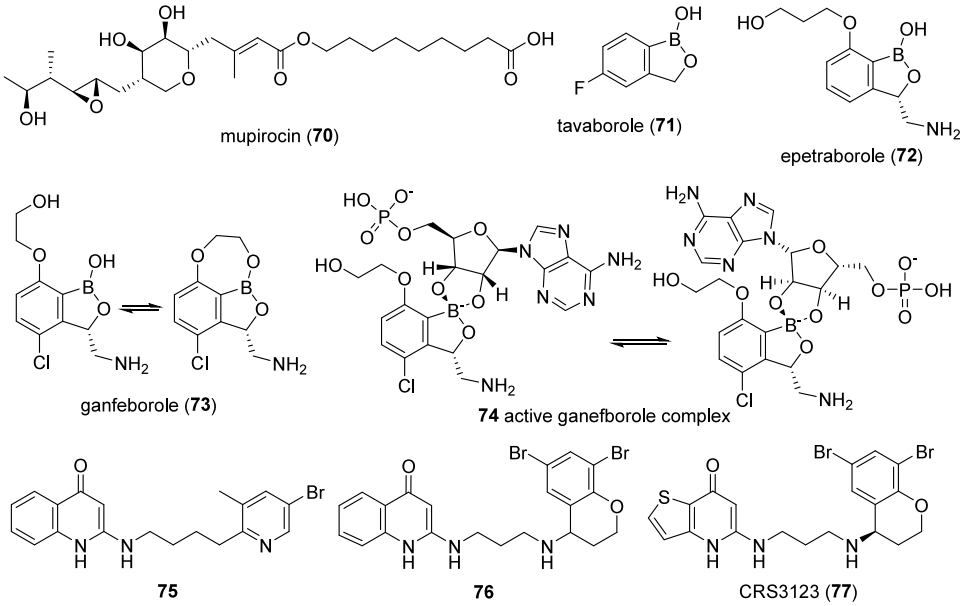

Figure 9. Structures of protein synthesis targeting antibacterials currently being evaluated in clinical trials and related compounds with new MoAs not represented in existing antibacterial drugs.

proteins necessary for the replication and repair of DNA.[246,247] Cyclohexyl-griselimycin (**64**) also induced resistance to DnaN *in vitro* with *Mycobacterium smegmatis* and *in vivo* with Mtb, and DnaN binding was shown using SPR and X-ray crystallography. Cyclohexyl-griselimycin (**64**) was also found to be active *in vitro* and *in vivo* against *Mycobacterium abscessus*.[246,248] Other active methyl-proline containing griselimycin analogs have been produced using heterologous expression and gene inactivation.[249]

The DNA sliding clamp has also been previously shown to be the antibacterial target of nonsteroidal anti-inflammatory drugs such as (*S*)-carprofen (**65**), bromfenac (**66**), and (*R*)-vedaprofen (**67**),[250] as well as inhibitors derived from a fragment-based approach such as **68** and **69**.[251,252]

RNA Synthesis: Compounds in Clinical Trials or Other Compounds of Interest. There are no new antibacterial RNA synthesis inhibitors currently being investigated at advanced stages of testing or in clinical trials.

Protein Synthesis: Compounds in Clinical Trials. Protein synthesis is an integral function of cell survival and replication, proceeding through different aminoacyl-tRNA synthetases that ligate AMP to individual amino acids, which are then delivered to the ribosome to be utilized in protein synthesis. A range of well-established antibiotic classes target the subunits of the ribosome, including aminoglycosides and tetracyclines (30S ribosome) and macrolides, oxazolidinones, pleuromutilins, streptogramin, and phenicols (50S ribosome). Efforts are underway to develop improved versions of many of these classes, as recently reviewed for oxazolidinones.[253] Antibacterial protein synthesis inhibitors currently in clinical trials with new MoAs not represented in approved antibacterial drugs are listed in Table 4, and their structures are illustrated in Figure 9.

Other components of the protein synthesis pathway may also be viable targets for new antibacterials. Aminoacyl-tRNA synthetases have received significant attention in recent years.[254] They can be divided into two classes, I and II,[255] and can be inhibited through individual or multiple modulation of the amino acid binding site, the ATP binding site, the tRNA recognition site, an allosteric site, and the editing domain. Although both eukaryotic and prokaryotic organisms use aminoacyl-tRNA synthetases, there has been enough enzyme divergence to allow for some selectivity.[256,257] There are specific tRNA synthetases for different amino acids. The only marketed tRNA synthetase inhibitor antibacterial drug is mupirocin (**70**),[256−258] an isoleucyl-tRNA synthetase inhibitor that occupies both the isoleucine and ATP binding sites of isoleucyl-tRNA synthetase.[259,260] Mupirocin (**70**) has

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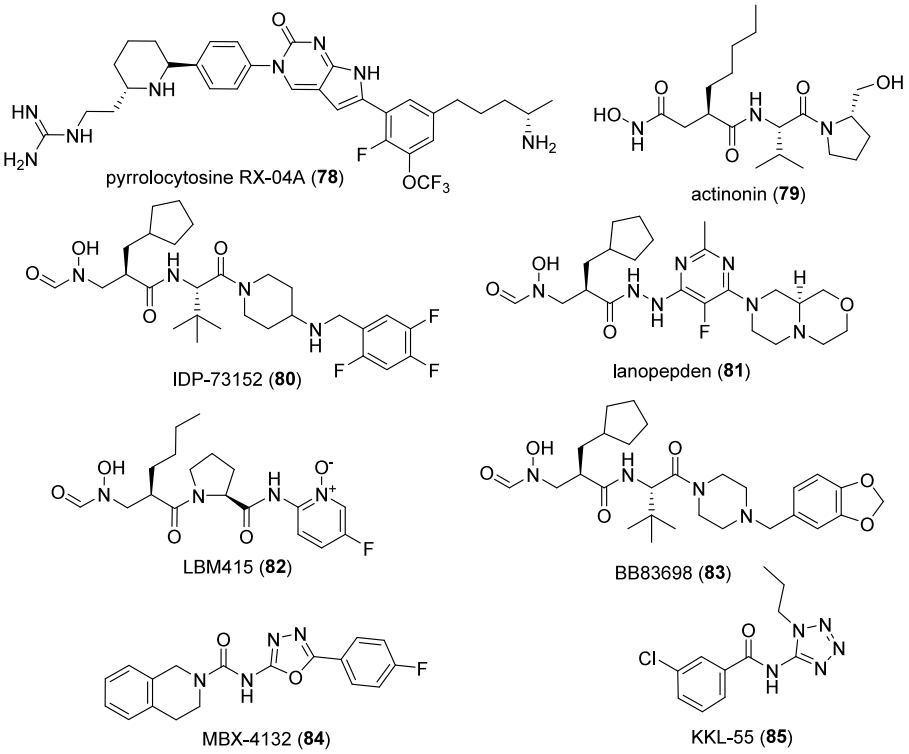

Figure 10. Structures of other preclinical antibacterials with new MoAs not used by antibacterial drugs.

been used topically for G+ve bacterial skin infections since 1985. Aminoacyl-tRNA synthetase inhibitors also have promise for the treatment of other infectious diseases such as fungi (tavaborole (**71**), an FDA approved benzoxaborole leucyl-tRNA synthetase inhibitor), mycobacteria,[255] malaria,[261] leishmaniasis, African trypanosomiasis, and Chagas disease,[262] as well as other human diseases.[257,263,264]

Currently, there are two benzoxaborole-type leucyl-tRNA synthetase inhibitors in clinical development, epetraborole (**72**) (GSK2251052, AN3365 and BRII-658) and ganfeborole (**73**) (GSK3036656). Boron-containing drugs and drug leads have recently been reviewed.[265] Epetraborole (**72**)[266] was first evaluated in phase 2 trials as an oral treatment for G−ve complicated UTI (cUTI) (NCT01381549) and complicated intra-abdominal infections (cIAI) (NCT01381562), but these studies were halted after indications of rapid development of resistance.[267,268] Over a decade later, epetraborole (**72**) started a phase 2/3 trial (NCT05327803) in April 2022 for patients with treatment-refractory *M. avium*[269] complex (MAC) lung disease. Epetraborole (**72**) also has activity against *M. abscessus*,[270] which is another NTM involved in lung infections. Ganfeborole (**73**) (GSK3036656, GSK656) is a chloro-substituted analog[271−273] of **72** with a 1,3-propanediol side chain replaced with a 1,2-ethanediol side chain that exists in both ring opened and closed conformations.[274] Ganfeborole (**73**) is being developed to treat TB, has completed a phase 2a trial (NCT03557281) where the highest dose (30 mg per day) showed measurable treatment responses in lesion types as detected by ^{18}F-fluorodeoxyglucose positron emission tomography,[275] and is currently being investigated in a phase 2 trial (NCT05382312) in combination with the TB drugs delamanid and bedaquiline.

The boron center of both tavaborole (**71**)[276] and epetraborole (**72**)[266] forms a tetrahedral spiroborate−tRNA adduct with the 2′,3′-hydroxy groups of the terminal adenosine ribonucleotide. Tavaborole (**71**) also forms an adduct with AMP, which was shown to occupy a noncognate amino acid-binding pocket in the editing site of leucyl-tRNA synthetase.[276] This has been called the oxaborole tRNA trapping (OBORT) mechanism and shows that benzoxaboroles are actually prodrugs. The binding of tavaborole (**63**) alone to the editing site is not as strong compared to analogs and aminoacyl-AMP with amine groups and explains why AMP or tRNA is required for activity. The additional *O*-propanol and aminomethylene groups present in epetraborole (**72**) compared to tavaborole (**71**) enhance its binding to bacterial leucyl-tRNA synthetases and its antibacterial activity.[266,273] There has also been a recent report investigating the OBORT mechanism for ganfeborole (**73**), which showed that it could form a highly specific and reversible LeuRS inhibition adduct with ATP, AMP, and the terminal tRNA adenosine.[277] The study employed NMR, X-ray crystallography, and SPR to show that one of the two ganfeborole−AMP adducts (**74**) preferentially bound to the active site. In addition, it was proposed that the different benzoxaborole−adenosine adducts could also aid in drug biodistribution and cell entry in various species.[277]

Methionyl-tRNA synthetase can be inhibited by diaryldiamines identified using a methionyl-tRNA synthetase high-throughput screening (HTS) assay. The initial hit compound **75**, which did not exhibit antibacterial activity despite enzymatic inhibition, was optimized to give analogs such as **76**, with potent *S. aureus* methionyl-tRNA synthetase activity (IC_{50} 80 nM) and a MIC_{90} of 0.5 μg/mL against *S. aureus*.[24,278,279] Further work led to the identification of CRS3123 (**77**) (REP3123), which predominantly inhibits Class I bacterial methionyl-tRNA synthetases present in many G+ve bacteria but is not active against the Class II methionyl-tRNA synthetases present in G−ve bacteria. CRS3123 (**77**)

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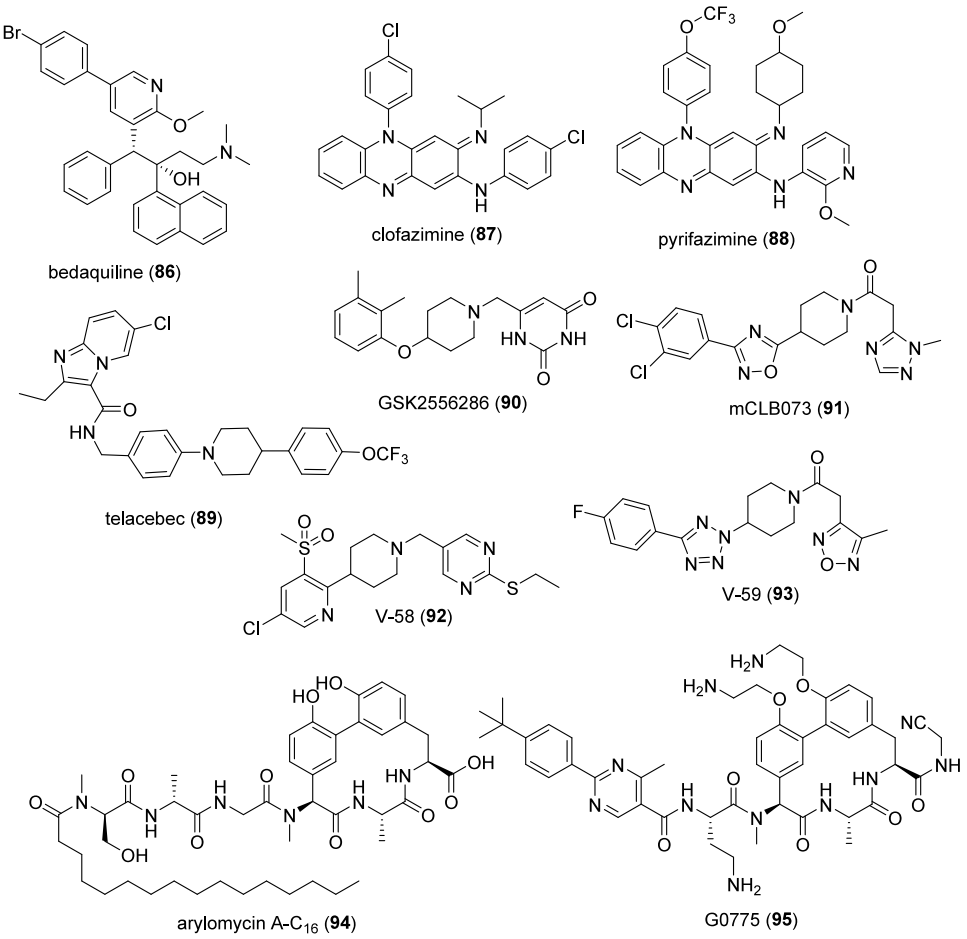

Figure 11. Structures of other antibacterials in clinical trials with new MoAs and related compounds not used by existing antibacterial drugs.

shows activity against *C. difficile*,[280,281] while also preventing sporulation, reducing toxin production, and sparing most normal gut flora.[282] The stereochemistry of the amine in the chromane is important with only the *R*-enantiomer exhibiting antibacterial activity.[280] X-ray cocrystallography of a Class I methionyl-tRNA synthetase showed that 77 occupied the amino acid-binding site and a surrounding auxiliary pocket implicated in tRNA acceptor arm binding.[283] In addition, ITC was used to determine that 77 has considerably less affinity for a Class II methionyl-tRNA synthetase, although binding was observed in an X-ray structure, and that their affinity was substantially increased in the presence of ATP.[283] There are different residues in the methionine and auxiliary pockets that reduce Class II binding, which is consistent with mutant Class I methionyl-tRNA synthetases with reduced binding.[283] CRS3123 (77) is currently in a phase 2 trial (NCT04781387) for CDI, via oral (gut topical) dosing.[284,285]

Gallium(III) has a similar ionic radius to iron(III) and is actively transported into the bacterial cell by some uptake systems where it can disrupt iron-associated metabolic processes,[286−290] for example, binding instead of iron in siderophores and disrupting redox reactions as Ga^{3+} cannot be reduced like Fe^{3+} to Fe^{2+}. Gallium nitrate (Ganite), approved by the FDA to treat symptomatic hypercalcemia secondary to cancer in 2003,[291] has been evaluated in a phase 2 trial (NCT02354859) by The University of Washington using iv administration for people with CF infected with *P. aeruginosa*. There is a phase 1 trial ongoing for CF patients with NTM infections (NCT04294043) with iv gallium nitrate. Aridis Pharmaceuticals is evaluating nebulized (inhalation) gallium citrate (AR-501) in a phase 1 trial (NCT03669614) in healthy adults and *P. aeruginosa* infected CF subjects.[292]

Protein Synthesis: Other Compounds of Interest. In 2021, CARB-X funded lead optimization work for a novel chemical class called the pyrrolocytosines, which target a new site in the ribosome.[293] The compounds were first developed by Melinta (e.g., RX-04A (**78**)) (Figure 10) then taken over by BioVersys (BV300).

Peptide deformylase (PDF) is a metallo-hydrolase enzyme that catalyzes the removal of formyl groups from N-terminal methionines following ribosomal translation in bacteria and is essential for bacterial growth.[294−296] For many years it was thought that there were no functional human orthologs; however, in late 2003 a human PDF (HsPDF) was identified in human mitochondria,[297,298] with inhibitors under development as potential anticancer agents.[294,299] The *Streptomyces*-derived actinonin (**79**) was identified by virtual searching for NPs that possessed a hydroxamate metal-chelating group and methionine-like structures.[300] There have been four PDF inhibitors inspired by actinonin (**79**) that have entered clinical development but were halted: IDP-73152 (**80**) (phase 1 2014, NCT01904318), lanopepden (**81**) (GSK1322322, phase 2 ABSSSI, 2011, NCT01209078), LBM-415 (**82**) (phase 1, 2004),[294] and BB83698 (**83**) (phase 1, 2002).[294] All of these PDF inhibitors have the metal binding hydroxamic acid replaced with an *N*-formyl-*N*-hydroxyl-amine group, presum-

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Table 5. Antibacterial Compounds in Clinical Trials with Other MoAs Not Used by Existing Antibacterial Drugs[a]

name (synonym)	compound class (lead source)	mode of action	administration; development phase (status), indication (developer)
telacebec (**86**)	imidazo[1,2-*a*]pyridine amide (S)	respiratory cytochrome bc1 complex	po; phase 2 (completed), TB (Qurient Co)
GSK2556286 (**90**)	"uracil aryloxypiperidine" (S)	adenylyl cyclase (Cya) Rv1625c	po, phase 1, TB (GSK)
RG6319	"arylomycin" (NP)	type I signal peptidase (LepB) inhibitor	iv; phase 1, G−ve, cUTI (Genentech)

[a]Abbreviations: cUTI, complicated urinary tract infections; G−ve, Gram-negative; NP, natural product-derived; po, oral (*per os*); S, synthetic; TB, tuberculosis.

ably as an attempt to reduce binding to other metalloproteins. It has been disclosed that further development of lanopepden (**81**) was halted due to potentially reactive metabolites found in nonclinical studies, which created an unfavorable risk−benefit profile for a community agent.[301]

During translation in bacteria, ribosomes often stall at the 3′-end of mRNA molecules lacking a stop codon. *Trans*-translation is a quality control mechanism used by bacteria to rescue stalled ribosomes, and since it is absent in eukaryotes, it is a potentially selective target. It utilizes a complex between transfer-mRNA (tmRNA) and a small protein, Smp.[302] Microbiotix, with CARB-X funding, conducted hit-to-lead optimization of acylaminooxadiazoles such as MBX-4132 (**84**) as an oral antibiotic to treat multidrug resistant (MDR) *N. gonorrhoeae*. Cryo-EM studies indicate it binds to a unique site near the peptidyl-transfer center of nonstop ribosomes.[303] The same Emory University and Penn State University academic groups that identified MBX-4132 (**84**) (Figure 11) recently published another trans-translation inhibitor, tetrazole-KKL-55 (**85**), that binds to Elongation Factor Thermo-unstable (EF-Tu), preventing binding between EF-Tu and tmRNA.[304]

Other MoAs: Compounds in Clinical Trials. In addition to the four major bacterial processes affected by most antibiotics, there are several other pathways that do not readily fall under the main classifications. These compounds are listed in Table 5, and their structures are depicted in Figure 11.

Bedaquiline (**86**), an F_1F_O ATP synthase inhibitor, was the first new TB drug launched in 40 years when it was approved in 2012, highlighting the potential of cellular respiration as a target. Respiration is mediated by membrane-embedded protein complexes that produce energy by redox reactions via the electron transport chain and ATP production via ATP synthases. There has been enough divergence between mycobacterial respiration and other respiratory systems in eukaryotes and bacterial mitochondria to enable the discovery of selective inhibitors. Clofazimine (**87**), which is a riminophenazine-type antimycobacterial discovered in the 1950s and used in the treatment of leprosy (*Mycobacterium leprae*), as well as being a component of some TB regimens,[305] has been shown to compete with menaquinone in the respiratory chain enzyme NDH-2 and reduce ATP production.[306−308] However, **87** also likely has other MoAs.[305] An analog, pyrifazimine (**88**), finished a TB phase 2 trial (NCT04670120) a few years ago.

A phenotypic high-content screen was used to identify two imidazopyridine amide leads that were active against Mtb replicating inside macrophages and had low μM MICs in culture broth medium.[309] Medicinal chemistry and screening led to identification of telacebec (**89**) (Q203), which was shown to target the QcrB subunit of the Mtb terminal respiratory oxidase cytochrome *bcc*:aa_3 complex.[309,310] Telacebec (**89**) binds to the Q_O site of QcrB and inhibits the oxidation of menaquinol to menaquinone. Several recent cryo-EM studies have shown **89** bound to Mtb cytochrome *bcc*:acc_1[311,312] and *M. smegmatis* $CIII_2CIV_2$.[313] Although **89** is bacteriostatic due to the presence of a salvage oxidase, cytochrome *bd* oxidase,[314−316] there is a strong synthetic lethal interaction between these two oxidases that could be exploited, as well as the potential for synergy with other antibacterials that target oxidative phosphorylation.[317−319] Telacebec (**89**) completed an early bactericidal activity (EBA) phase 2a trial (NCT03563599) in September 2019,[320] and future clinical studies would likely involve other TB drug combination partners.[321] There is considerable QcrB sequence similarity between Mtb, *M. ulcerans* (Buruli ulcer),[322,323] and *M. leprae* (leprosy),[324] potentially expanding the future clinical utility of telacebec (**89**). Although there is also QcrB sequence overlap with other pathogenic mycobacteria,[311] care needs to be taken as it has been demonstrated that naturally occurring polymorphisms abrogate the activity of **89** in *M. abscessus*.[325]

Mtb uses a specialized set of metabolic pathways when residing in macrophages to derive energy from host-derived nutrients, such as cholesterol and lipids.[326,327] An assay that measured intracellular growth of Mtb within human (THP-1) macrophage-like differentiated monocytes enabled the identification of GSK2556286 (**90**) (GSK-286), a substituted uracil derivative that showed no cross-resistance to known TB drugs and inhibited the growth of Mtb in cholesterol media.[328] GSK2556286 (**90**) is currently being evaluated in a phase 1 trial (NCT04472897). Resistance mutations to **90** were mapped to a Mtb adenylyl cyclase (Cya) Rv1625c,[328] which is a class-III adenylyl cyclase 6-transmembrane homodimer (cryo-EM structure) that converts cellular ATP into 3′,5′-cyclic AMP (cAMP).[329] Further studies[330] showed that **90** was a Cya agonist that induced cAMP production, which inhibited cholesterol catabolism, and cross-resistance was found with a previously reported Cya agonist, mCLB073 (**91**).[331] mCLB073 (**91**) and the analogs V-58 (**92**) (sCEB942) and V-59 (**93**)[331−333] also stimulated cAMP production in Mtb, which was also shown for **91** to dampen TNF-α production in infected macrophages.[332] In February 2023, Scripps Research announced that the Bill & Melinda Gates Medical Research Institute has licensed mCLB073 (**91**) for further development.[334]

Proteins synthesized in the cytoplasm that then operate inside the cytoplasmic membrane or outside the cytoplasm are secreted into or through the cytoplasmic membrane via two major pathways, the Sec-translocase complex or the twin-arginine translocation (Tat) pathway, which differ in transporting folded (Tat) or unfolded (Sec) proteins.[335] Type I signal peptidases (SPases) are serine-lysine proteases associated with both complexes that hydrolyze N-terminal signal peptides from secreted proteins.[336] In contrast to the more common Ser-His-Asp catalytic triad serine proteases, SPases

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function via an unusual Ser-Lys catalytic dyad with a unique nucleophilic attack on the *si*-face of the substrate. In a search for SPase inhibitors, new analogs of the arylomycin type biaryl-bridged lipopeptides (e.g., arylomycin A-C_{16} (**94**))[337,338] were identified in 2004,[339] which also had modest activity against G +ve and G−ve pathogens. A crystal structure of arylomycin A_2 with an *E. coli* SPase was reported in 2004.[340] Interestingly, naturally occurring resistance was shown to reduce arylomycin susceptibly in wild-type strains of *S. aureus*, *E. coli*, and *P. aeruginosa*.[341−343] In a series of studies, arylomycin analogs were synthesized and their SAR was explored,[344−348] eventually leading to Genentech's G0775 (**95**) that had broad spectrum activity against G−ve bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*; MIC range 0.125−2 μg/mL) and a low propensity for resistance against LepB, which is an *E. coli* SPase.[349] These improvements were achieved by (1) shortening the linear D-*N*-Me-Ser-D-Ala-Gly in arylomycin A-C_{16} (**94**) to a single diaminobutyric acid and replacing the aliphatic side-chain with 2-(4-(*tert*-butyl)phenyl)-4-methylpyrimidine-5-carboxylic acid, (2) formation of an amide with 2-aminoacetonitrile, and (3) 2-aminoethoxy derivatization of the two phenolic groups.[349] A crystal structure of G0775 (**95**) bound to LepB showed that the 2-amino-acetonitrile group covalently bound to the catalytic Lys (K146) and not the catalytic Ser (S91), which had not been previously observed.[349] The same Genentech publication reported that membrane penetration of G0775 was porin-independent, potentially a form of self-promoted uptake enhanced by positive charge. Efficacy was demonstrated against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* thigh infections in neutropenic mice, as well as a MDR *K. pneumoniae* lung infection model and peritonitis sepsis model (all subcutaneous dosing). Genentech subsequently described the optimization of the aryomycin lipid tail, assessing its critical role in both crossing the outer membrane and anchoring in the bacterial inner membrane, as well as its effect on drug-like properties (plasma protein binding).[350] Nanopore sequencing recently showed that heterogeneous cell populations can overexpress LepB in response to treatment with G0775 (**95**).[351] Genentech is currently evaluating the LepB inhibitor RG6319 in a phase 1 clinical trial for cUTI (ISRCTN15259645).[352] Although the structure of RG6319 has not been disclosed, arylomycin LepB inhibitors such as G0775 (**95**) have been patented.[353]

Other MoAs: Compounds of Interest. Riboflavin (vitamin B12) is an essential molecule as it forms two coenzymes, flavin mononucleotide and flavin adenine dinucleotide, involved in cellular respiration. The enzymes involved in its synthesis in bacteria represent an intriguing target as they are not found in mammalian cells, which import riboflavin rather than synthesize it. Possible targets include GTP cyclohydrolase II (GCH II), lumazine synthase (LS), riboflavin synthase (RFS), FAD synthetase (FADS), and FMN riboswitch, as reviewed in 2023.[354] A range of early stage inhibitors are described in this review, including the example of ribocil C (**96**) (Figure 12), which is a highly selective inhibitor of the FMN riboswitch.[355−357] The Hergenrother eNTRy rules[358,359] were applied to attempt to improve G−ve uptake, producing more active ribocil C-PA (**97**).[360] Ribocil C has also been tested in combination with the natural compound roseoflavin (**98**), simultaneously targeting two autonomous riboswitches.[357,361]

Nontraditional Small Molecule MoAs: Compounds in Clinical Trials. Most antibacterials attempt to kill pathogens

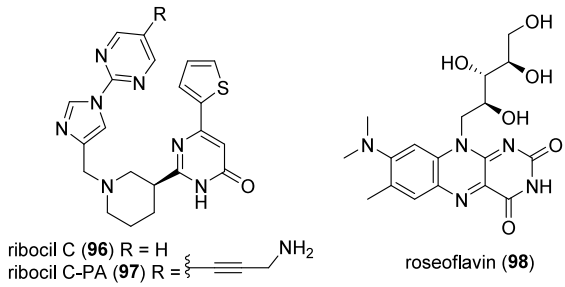

Figure 12. Structures of other preclinical antibacterials with new MoAs not represented in existing antibacterial drugs.

or at least inhibit their growth. However, there is growing interest in targeting bacterial virulence factors, pathways that contribute to pathogenicity but are not essential for survival.[362−364] The hypothesis is that resistance will be less likely to arise, as the bacteria's existence is not directly threatened; however, selection pressure is still very likely to lead to increased growth rates of strains that are less affected by the intervention. Other strategies target bacterial resistance mechanisms or the host response to infections. Nontraditional small molecules currently being evaluated in clinical trials are listed in Table 6, and their structures are in Figure 13.

Type III secretion systems (T3SS) are multiprotein complexes that translocate effector proteins directly from the bacterial cytosol into a host cell via a needle-like mechanism that spans the G−ve cell envelope.[365] The T3SS plays a central role in the virulence of many G−ve human pathogens. Fluorothiazinon (**99**) (ftortiazinon, fluorothyazinon, C-55) is a T3SS inhibitor that has been shown to have *in vivo* antivirulence activity (reduced bacterial loads and inflammation) against *Chlamydia trachomatis*,[366,367] *Salmonella*,[368,369] *P. aeruginosa*,[370] *A. baumannii*,[371] and uropathogenic *E. coli* (UPEC).[372] In these studies,[366−372] fluorothiazinon (**99**) had no direct antibacterial activity and displayed no toxic effects, including mutagenicity and carcinogenicity.[368] Specific details of the MoA of **99** have not yet been reported. A combination of fluorothiazinon (**99**) and the direct acting lactam antibiotic cefepime has been evaluated in a phase 2 trial (NCT03638830) for cUTI caused by *P. aeruginosa*, but no results have been reported. Antibodies have also been used to target T3SS.[373] As of September 2023, Microbiotix was actively funded by CARB-X to conduct lead optimization for a T3SS inhibitor against *P. aeruginosa*.

Uropathogenic *E. coli* (UPEC) strains, which are the cause of most urinary tract infections (UTIs),[374] use their type 1 pili to adhere to the surface of the host cells; therefore, disruption of this interaction is an antivirulence approach to the treatment of UTIs.[375−379] Type 1 pili are protein complexes that consist of FimD in the outer membrane, a pilus rod of between 500 to 3000 FimA subunits, and a tip fibrillum (FimF−FimH), which is constructed by protein-folding chaperone FimC. The critical interaction is the lectin domain of the FimH subunit which binds to host epithelia via oligomannosides in uroplakin Ia. GSK3882347 (structure not publicly disclosed[380]) is a FimH inhibitor being evaluated in a Phase 1b trial (uUTI; NCT05138822).[381] FimH inhibitors also have potential to help treat Crohn's Disease, and sibofimloc (**100**) (EB-8018, TAK-018) has been clinically evaluated.[376,382]

The golden color of >90% of *S. aureus* clinical isolates is due to the carotenoid staphyloxanthin, which also acts as an antioxidant that helps to neutralize reactive oxygen species

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Table 6. Nontraditional Antibacterial Compounds in Clinical Trials with New MoAs Not Used by Existing Antibacterial Drugs[a]

name (synonym)	compound class (lead source)	mode of action	administration; development phase (status), indication (developer)
fluorothiazinon (**99**) + cefepime	thyazinone (S) + cephalosporin (NP)	bacterial type III secretion system (T3SS) (antivirulence)	po; phase 2 (completed), G−ve virulence (Gamaleya Research Institute of Epidemiology and Microbiology)
GSK3882347	mannose-derived (S)	type 1 fimbrin D-mannose specific adhesin (FimH) antagonist (antivirulence)	po, phase 1, G−ve, UTI (GSK/Fimbrion Therapeutics)
ALS-4	not disclosed	dehydrosqualene desaturase (antivirulence)	po; phase 1 (completed), *S. aureus* infections (Aptorum Therapeutics)
dovramilast (**101**)	"3-oxo-1*H*-isoindol-4-yl" (S)	phosphodiesterase 4 (PDE4) inhibitor (host immune response)	po, phase 2, erythema nodosum leprosum; phase 2 (completed), adjunctive TB (Medicines Development for Global Health)
alpibectir (BVL-GSK098) (**103**) + ethionamide (**104**)	spiroisoxazoline (S)	inactivation of TetR-like repressor (EthR2) (resistance breaker)	po, phase 2, TB (BioVersys/GSK)

[a]Abbreviations: G−ve, Gram-negative; NP, natural product-derived; NTM, non-tuberculosis mycobacteria; po, oral (*per os*); S, synthetic; TB, tuberculosis; UTI, urinary tract infections.

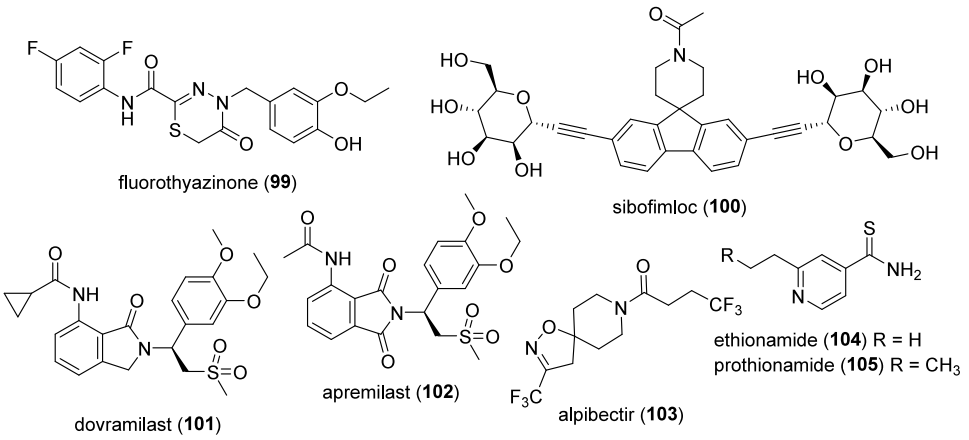

Figure 13. Structures of nontraditional antibacterials in clinical trials and related compounds with new MoAs not used by existing antibacterial drugs.

(ROS) secreted by neutrophils; therefore, disrupting staphyloxanthin biosynthesis should lead to reduced bacterial virulence.[383−386] ALS4 (structure not disclosed) is an inhibitor of 4,4′-diapophytoene desaturase (CrtN), which converts dehydrosqualene to 4,4′-diaponeurosporene in the staphyloxanthin biosynthetic pathway.[387,388] ALS4 completed a phase 1 trial (NCT05274802) in January 2022.

Other therapeutics target the host, rather than the pathogen or pathogen−host interface. Dovramilast (**101**) (CC-11050, AMG-634) is a phosphodiesterase type 4 (PDE4)[389,390] inhibitor based on an isoindole-1-one core that is closely related to the isoindole-1,3-dione structure of apremilast (**102**), a PDE4 inhibitor[391] approved for psoriatic arthritis and psoriasis (USA in 2014 and the EU in 2015). PDE4 hydrolyses cyclic adenosine monophosphate (cAMP) to AMP and its inhibition leads to increased cAMP levels in the host cells, which has an anti-inflammatory effect by reducing levels of the cytokine TNF-α.[392,393] Dovramilast (**101**) is being developed as an adjunctive TB therapy, and it was shown in a phase 2 trial (NCT02968927) that combinations of rifabutin with dovramilast (**101**) or everolimus (mTOR inhibitor) enhanced the recovery of one second forced expiratory volume (FEV_1) in patients with pulmonary TB.[394,395] Dovramilast (**101**) is also being evaluated in a phase 2 trial (NCT03807362) for patients with erythema nodosum leprosum, which is an inflammatory disorder triggered by leprosy.

One of the most significant advances in antibacterial treatments was the development of β-lactamase inhibitors to rescue β-lactam activity. Similar to β-lactamase inhibitors, spiroisoxazolines such as alpibectir (**103**) (BVL-GSK098) have been shown to reverse resistance to ethionamide (**104**),[396−398] which along with the closely related prothionamide (**105**), are second line drugs used to treat MDR TB infections. Ethionamide (**104**) is a prodrug that is activated by Baeyer−Villiger monooxygenases such as EthA, initiating formation of an adduct with NADH, which inhibits the enoyl acyl carrier protein reductase InhA leading to disruption of mycolic acid biosynthesis and cell death.[399−401] However, high concentrations of ethionamide (**104**) are usually required, which can lead to side effects, as the expression of *ethA* is negatively regulated by transcriptional repressor EthR.[402] It was shown that spiroisoxazolines inhibit the repressor EthR, which led to increased levels of EthA and activated ethionamide (**104**).[396,397] EthA is also involved in the bioactivation of the thiourea thiocarlide (isoxyl), and the thiosemicarbazones, thioacetazone and perchlozone.[399,400,403] The spiroisoxazoline alpibectir (**103**) is currently being evaluated in a phase 2 trial (NCT05473195) in combination with ethionamide (**104**) compared to **104** alone in participants with rifampicin- and isoniazid-susceptible pulmonary TB.

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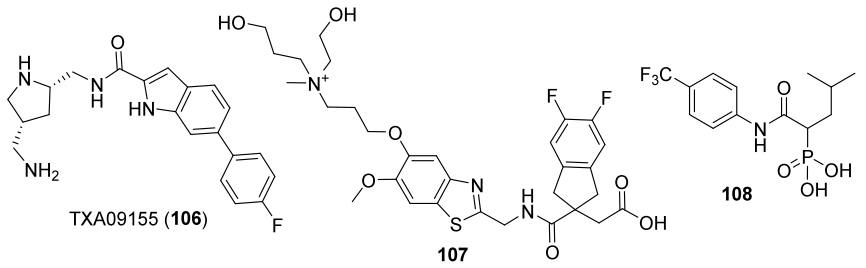

Figure 14. Structures of preclinical nontraditional antibacterials with new MoAs not represented in existing antibacterial drugs.

Nontraditional Small Molecule MoAs: Compounds of Interest. *S. aureus* produces a number of toxins to increase its virulence,[404] including α-hemolysin (Hla). The small β-pore-forming α-toxin perforates the plasma membrane of mammalian cells, causing an uncontrolled flux of ions and water. While several antibodies have progressed into clinical trials[405] (with Aridas Pharmaceutical's AR-301 (tosatoxumab) showing promise in the first (NCT03816956) of two phase 3 trials[406]), a number of small molecule inhibitors have been discovered, including one funded by CARB-X for hit-to-lead development by the Helmholtz Centre.[407]

Efflux pumps are a key resistance mechanism to many antibiotics. Taxis Pharmaceuticals received CARB-X funding for a hit-to-lead project examining a conformationally constrained indole carboxamide targeting *P. aeruginosa* efflux pumps.[408] TXA09155 (**106**) (Figure 14) potentiated multiple antibiotics with efflux liabilities against wild-type and multidrug-resistant *P. aeruginosa*, with ≥8-fold potentiation of levofloxacin, moxifloxacin, doxycycline, minocycline, cefpirome, chloramphenicol, and cotrimoxazole.

A metalloprotease secreted by *P. aeruginosa*, elastase (LasB),[409] plays a key role in pseudomonal infection. Release at the site of infection results in proteolysis of host tissue and immune components. Both Antabio and the Helmholtz Institute have received CARB-X funding to advance LasB inhibitors, potentially based on published indane carboxylates such as **107**,[410,411] and phosphonates such as **108**,[412] respectively.

CONCLUSIONS

Of the approximately 50 small molecular antibacterial drugs approved since 2000,[14,15,19] only six belong to drug classes that have not been previously approved: linezolid (2000, oxazolidinone, G+ve), daptomycin (2003, lipopeptide, G+ve), fidaxomicin (2011, tiacumicin, G+ve), bedaquiline (**86**) (2012, diarylquinoline, Mtb), avibactam (2015, DBO β-lactamase inhibitor), and vaborbactam (2017, boronate β-lactamase inhibitor). Although none of these six have clinically relevant activity against G−ve bacteria, the β-lactamase inhibitors are used in combination with β-lactams to treat these types of infections. While most of the other approved antibacterial drugs are improvements on existing antibiotics, there is a limit to how far these classes can be further explored after 40 to 70 years of intense research. In addition, some relatively recently approved antibacterial drugs have failed to provide an adequate return on investment, due to several reasons that include underwhelming sales, a ready supply of generic antibiotic substitutes, and additional costs to undertake new clinical and Phase 4 trials. Consequently, there has been significant concern about the paucity of innovative antibiotics under development, particularly those that are based on new chemical scaffolds and/or those that target novel bacterial processes.

As this review illustrates, there are actually a number of promising approaches at various stages of development, with others at earlier conceptual stages not discussed here.[413] Rather than a lack of innovative ideas, it could be argued that the gap in antibacterial development is driven by the lack of sufficient financial and structural resources. Research teams or small start-up companies that have identified a novel compound/target combination rarely have the capacity and/or specialized antibiotic development experience to adequately progress their ideas. This is in stark contrast to the deployment of thousands of researchers in large pharmaceutical companies that supported extensive infrastructure during the "golden age" of antibiotics, with the decline in antibiotic researchers highlighted in a recent report by the AMR Industry Alliance.[414] It is imperative that information on the benefits and pitfalls of innovative projects are shared with the global community to avoid wasting precious resources by reinventing a "broken wheel". Ideally, increasing global awareness of the need for improved antibiotic reimbursement strategies that are substantial and globally consistent will re-invigorate commercial interest in these early stage programs and lead to the approval of innovative antibacterial drugs that address future medical requirements.

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