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Targeting virulence in *Mycobacterium tuberculosis* and nontuberculous mycobacteria: Focus on anti-infective drug design

POST-SCREEN (GREY)

Laurent Robert Chiarelli [1], Alexander Lepioshkin [2], Maria Rosalia Pasca [1,3,*], Vadim Makarov [2]

[1] Department of Biology and Biotechnology 'Lazzaro Spallanzani', University of Pavia, Pavia, Italy
[2] Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences, Moscow, Russia
[3] Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Antimicrobial resistance is currently one of the ten most serious threats to human health worldwide, and mycobacteria are one of the key factors in this threat. There is a general understanding that new approaches to antimicrobial therapy are needed; one of them is the inhibition of bacterial virulence. Unlike the traditional model of antibiotic discovery, which targets the functions necessary for bacterial growth, the virulence suppression approach aims to neutralize bacteria rather than destroy them. This review analyzes the successes, failures, and prospects of research into both bacterial virulence itself and the possibility of creating drugs that affect it.

Keywords: antimicrobial resistance; virulence; Mycobacterium tuberculosis; nontuberculous mycobacteria

Introduction

Antimicrobial resistance (AMR) has rapidly evolved from a distant concern to a pressing challenge and now stands among the top ten global health threats. In 2021, bacterial drug resistance was responsible for an estimated 4.71 million deaths, including 1.14 million deaths directly linked to AMR.(p1)

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB), is one of the major contributors to AMR. The biggest concern in current global TB management is the resistance to rifampicin (RR–TB), the most powerful first-line TB drug. TB that is resistant to isoniazid (INH), another first-line TB drug, or to both INH and rifampicin, is defined as INH-resistant TB (HR-TB) or multidrug-resistant TB (MDR–TB), respectively. These TB-resistant cases require treatment with more toxic second-line drugs, to which resistance is also gradually developing. Worldwide, an estimated 400 000 cases of MDR/RR–TB were reported in 2024. Among new cases, the estimated proportion of people with MDR/RR–TB was 3.2% and 16% among those previously treated with TB medications.(p2)

The TB drug portfolio appears to be larger than for other bacteria, but most of the drugs were developed a long time ago. As for other bacteria, they are aimed at targeting one of the five essential cellular functions, such as cell wall biosynthesis (INH, ethambutol, ethionamide, pretomanid), protein synthesis (aminoglycosides, macrolides), DNA replication (ciprofloxacin), DNA-dependent RNA synthesis (rifamycins), or metabolic pathways (bedaquiline).(p3) To be effective, these traditional antibiotics must be taken daily for 4, 6, or 9 months, depending on the regimen and drug susceptibility.(p3) Such a prolonged treatment reduces patient adherence and contributes significantly to AMR development. Because these antibiotics exploit old targets, the development of drug resistance is almost inevitable. Therefore more alternative options to tackle mycobacterial infections need to be explored.

According to the Working Group for New TB Drugs and TB Alliance pipelines, around 13 regimens that include new investigational drugs are currently being evaluated in clinical trials.(p4) The development of new, effective, and safe drug combination

* *Corresponding author. Pasca, M.R. (mariarosalia.pasca@unipv.it)*

https://doi.org/10.1016/j.drudis.2025.104501

regimens for TB management is a lengthy and costly process as it requires addressing numerous factors. These include the potential for shortened treatment duration, detailed safety and toxicity profiles, potential drug–drug interactions, suitability for specific patient populations, drug formulation development, and the final cost of the pill.[p5]

In addition to *Mtb*, non-tuberculous mycobacteria (NTMs) are another important public health threat.[p6],[p7] NTMs are a heterogeneous group of mycobacteria comprising over 200 species that are ubiquitous in the environment. NTM infections include pulmonary disease, particularly in patients with chronic lung disease or cystic fibrosis; superficial lymphadenitis; disseminated disease in severely immunocompromised patients; and infection of the skin, soft tissues, bones and joints. NTM are divided into slow-growing (>7 days) and fast-growing (<7 days) mycobacteria. Fast-growing mycobacteria belong to *Mycobacterium abscessus* complex (MABC), *Mycobacterium fortuitum* complex and *Mycobacterium chelonae*, whereas the most common and clinically important slow-growing species are *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium ulcerans*.[p7]

The diagnosis and treatment of NTM infections are challenging because of their nonspecific clinical presentation, which varies widely depending on the site of infection and the species involved, as well as the pattern of resistance to the most commonly used antimicrobials. In addition, NTM infections are often misdiagnosed as drug-resistant TB because of a lack of awareness and diagnostic facilities, making management even more challenging.[p7]

Inhibiting bacterial virulence as an approach to control infection is a relatively recent concept in antibacterial drug discovery. This idea originated early in the so-called Golden Age of antibiotic discovery (1940 s to 1960 s). It was then abandoned, and began to regain attention in the late 1990 s when the threat of AMR caused by, at least, the misuse and/or overuse of traditional antibiotics became increasingly evident. Virulence factors can be targeted by a range of agents including biologics, peptides, and small molecules that inhibit specific mechanisms essential for bacterial persistence and pathogenesis. Consequently, targeting mycobacterial virulence factors relies on the idea of disarming bacteria by acting on nonessential stages of the infection cycle. This approach, instead of directly killing bacteria, might reduce the selection pressure for antibiotic resistance and thus extend antibiotic efficacy when used in combination.[p8] Furthermore, because virulence factors are often specific to pathogens and absent in human gut microbiota, targeting them can lead to more selective, narrow-spectrum antibacterial action, minimizing microbiota dysbiosis.[p8]

Nevertheless, bacterial virulence is a highly complex process, involving numerous factors and pathways. Developing specific inhibitors requires an in-depth understanding of bacterial pathogenesis and host-pathogen interaction. Additionally, modulating virulence factors might not completely eliminate the pathogen from the infected host, which could lead to persistent infections or relapses. Virulence-targeting agents clearly have limited utility alone, for instance, in acute bacterial infections, where rapid bacterial killing is critical, but could potentiate the efficacy of the antibiotics when used in combination.

How to find molecules that affect mycobacterial virulence?

A regular antibiotic can either directly kill bacteria, known as being bactericidal, or prevent their growth, which is termed bacteriostatic; this is clearly defined in routine whole-cell assays. However, traditional *in vitro* growth inhibition screening approaches are ineffective for identifying antivirulence agents because most virulence factors are not required for *in vitro* bacterial growth. The modern approach to discovering such compounds involves the use of a suite of assays to inhibit specifically selected enzymes *in vitro* (i.e. a target-based screen). However, although this approach is effective in identifying inhibitors, there is no guarantee that these compounds will have antivirulence activity *in vivo*. In this regard, assays in *ex vivo* infection models might be more reliable. Indeed, several infection models have been developed to date, for example in macrophages (i.e. in mouse bone-marrow-derived macrophages, J774A.1 macrophages, THP-1 macrophages, and human peripheral blood macrophages), which have proven successful in identifying molecules with effective antivirulence activity.[p9],[p10] Undoubtedly, the most reliable approach remains that of *in vivo* assays. In this context, simpler and cheaper approaches have been developed using *Galleria melonella*[p11] or zebrafish,[p12] for example, as opposed to animal infection models such as those involving mice, rabbits or non-human primates. Finally, artificial intelligence-based methods have recently made a significant contribution to drug discovery, revolutionizing the ability to predict the properties and structures of biomolecules and enabling the generation of new active compounds. Machine learning-based modeling can therefore also make an important contribution to the field of antivirulence drugs by offering a way to circumvent the limitations associated with traditional drug discovery methods.[p13],[p14]

Small molecules targeting mycobacterial virulence factors: is there any progress?

Although antivirulence small molecules for *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been extensively studied, compounds targeting mycobacterial virulence factors are in the early stages of the drug discovery process. In this review, we have highlighted selected interesting molecules that have been identified through high-throughput assays. We have not covered bacteriophages, vaccines or liposomes as antivirulence approaches.

Type VII secretion ESX-1 inhibitors

The ESX VII secretion systems represent the main virulence factor in pathogenic mycobacterial species. In *Mtb*, ESX-1, ESX-3, and ESX-5 are crucial components for virulence, whereas less is known about the role of ESX-2 and ESX-4 in *Mtb*-driven infections. Furthermore, ESX-1 is responsible for the secretion of the most important *Mtb* antigens: ESAT-6 (EsxA) and CFP-10 (EsxB). In this way, ESX-1 facilitates phagosomal evasion and host cell lysis, while suppressing host cellular defense mechanisms. This is essential for bacterial survival in host immune cells.[p15]

To date, only a few chemical compounds are known to inhibit the type VII secretion machinery. ESX/type VII secretion is regulated by at least two two-component regulatory systems, with the

first one being PhoPR.[p16] To identify possible agents targeting the PhoPR regulon, Johnson *et al*. developed a whole-cell high-throughput screen (HTS) of a 220 000-compound library using an acidic-pH-inducible PhoPR-dependent fluorescence reporter.[p17] They identified ethoxzolamide, which is a sulfonamide carbonic anhydrase inhibitor used in glaucoma treatment as well as a diuretic (Figure 1a). Ethoxzolamide inhibits 90% of *Mtb* PhoPR-dependent green fluorescent protein (GFP) reporter cells in infected murine bone marrow-derived macrophages and also reduced *Mtb* growth in both macrophages and infected mice.[p17]

Another two-component system that regulates ESX-1 is MprAB. Rybniker *et al*. identified two hit molecules: BTP15, a benzothiophene-based molecule previously described as a protein kinase G (PknG) inhibitor, and BBH7, derived from a lung fibroblast-based HTS.[p9] Both compounds blocked the secretion of EsxA, a major virulence determinant, and ESX-1 substrate at nanomolar concentrations and promoted phagosome maturation in THP-1 macrophages, thereby reducing bacterial loads. Target identification studies revealed that BTP15 inhibited also histidine kinase MprB (Figure 1a).

Jia *et al*. developed a further HTS assay using a luciferase-fused CFP-10 reporter to identify small molecules inhibiting ESX-1 secretion without impairing bacterial growth *in vitro*. One of the selected hits, IMB–BZ, was found to reduce virulence in an ESX-1-dependent manner (Figure 1b). Originally, IMB–BZ (nitromide) was an antiparasitic agent used for the prevention and treatment of coccidiosis in poultry (chickens and turkeys).[p18] The metabolism of this compound in animals is well documented; in rats, it was rapidly reduced to metabolites, and it was not detected in the rat plasma as early as 1 h after oral or intraperitoneal administration.[p19],[p20] This raises the question of investigating these metabolites in the developed assay, which is not addressed in the paper.

More recently, dual-active ethionamide (ETH) boosters that distinctly inhibit the ESX-1 secretion system were discovered.[p21] In addition to antivirulence molecules, they enhance the activity of mycobacterial prodrugs like ETH whose resistance can be reversed by specific inhibitors of the repressor (EthR) of the activator (EthA). These inhibitors facilitate the therapeutic effect of ETH without requiring high doses.[p21] Screening of Specs World Diversity Set 3 using both fibroblast survival and resazurin microtiter assays yielded the compound S3, which protected human lung fibroblast with an IC_{50} value of 8.6 μM (Figure 1c). As expected, S3 did not inhibit mycobacterial growth at concentrations above 50 μM. It was shown that S3 functions as both an ESX-1 inhibitor and an ETH booster. Subsequent structure–activity relationship (SAR) studies allowed them to separate these properties and to obtain S3_106 as an ESX-1 inhibitor. Although these molecules show promising *in vitro* and *ex vivo* results in preventing mycobacterial pathogenesis, their efficacy in animal models remains to be determined.

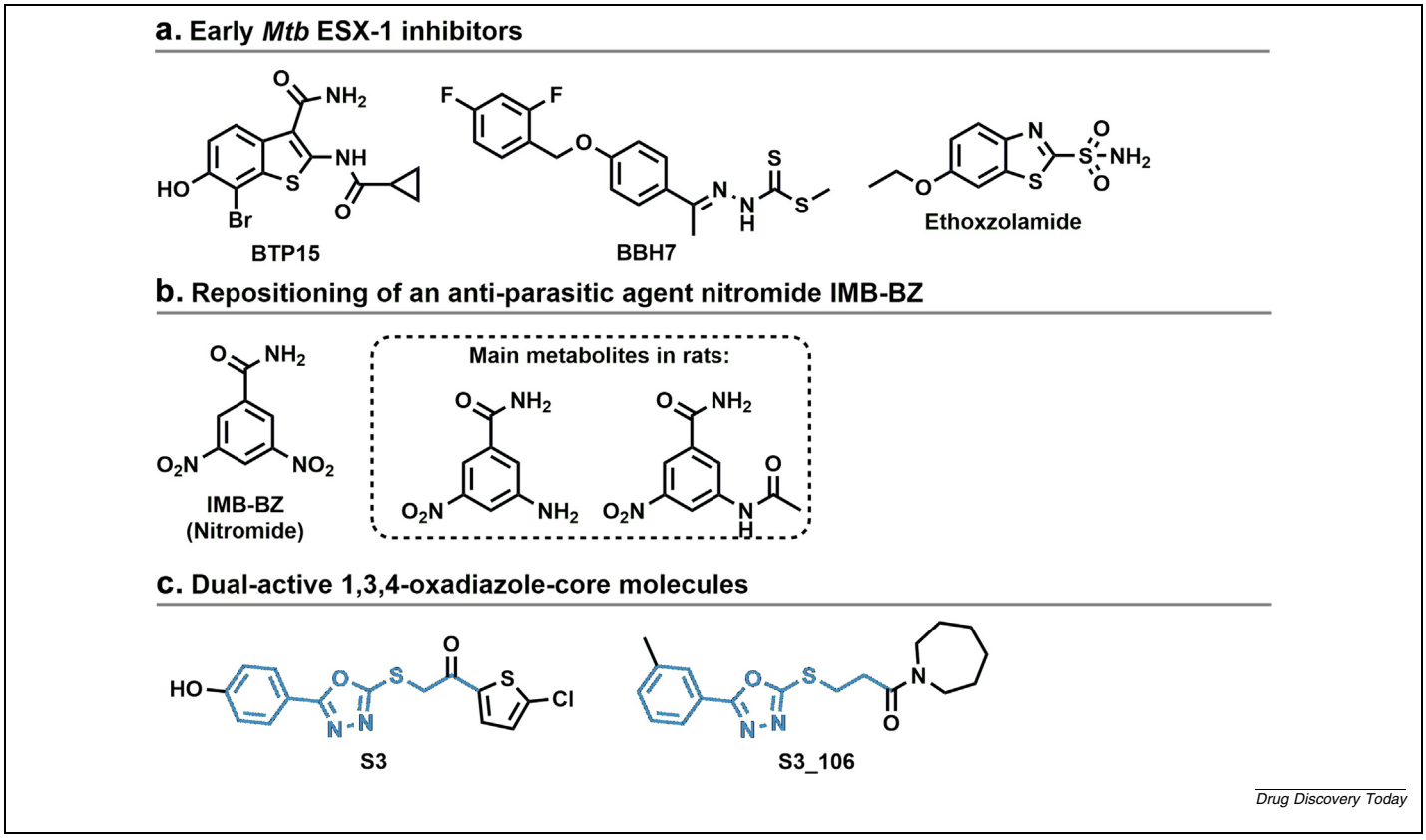

FIGURE 1

Inhibitors of Mtb ESX secretory systems. **(a)** The first *Mtb* ESX-1 inhibitors to be discovered. **(b)** The ESX-1 inhibitor IMB–BZ and its main identified metabolites, derived from repositioning studies. **(c)** Dual-active compounds that act as ethionamide boosters and ESX-1 inhibitors.

Interestingly, *Mycobacterium abscessus* (*Mab*) possesses only the ESX-3 and ESX-4 systems and lacks the ESX-1 system, but it has been proposed that the ESX-4 can be considered as a surrogate of ESX-1.[p22] Furthermore, it was shown that ESX-3 is related to iron metabolism and plays an important role in both biofilm formation and the intracellular survival of *Mab*.[p22],[p23] This suggests the potential of also targeting these proteins and pathways in NTMs. In this context, it should be noted that salicylate synthase has been successfully targeted both in *Mtb* and *Mab*, leading to a significant reduction in siderophores.[p24],[p25]

Two-component regulatory system DosRS–DosST inhibitors

The DosRS/DevRS regulatory system is related to *Mtb* virulence and survival during hypoxia, enabling it to sense host immune signals. In particular, DosS is a sensor histidine kinase, and DosR is a response regulator. This system is induced by hypoxia, nitric oxide and carbon monoxide stimulating nonreplicating persistence. In this state, the bacteria become tolerant to several antimicrobial drugs *in vitro*, and are believed to be responsible for the prolonged course of anti-TB therapy.[p26]

Six inhibitors of the DosRST two-component regulatory system from distinct classes were identified that had minimal effects on *Mtb* growth.[p26] Among these, the antimalarial drug artemisinin directly inhibited *Mtb* DosS and DosT kinases by targeting the sensor-kinase heme. Looking for a better understanding of its mechanism of action (MoA), it was proposed that its peroxide bond undergoes reductive activation by heme to produce radicals that alkylate heme and parasite proteins, suggesting a versatile mode of action. Similar to artemisinin, HC106A directly targeted the sensor kinase DosS heme, via a different mechanism (Figure 2a).[p27] Preliminary SAR studies identified the compound MSU-39446, which inhibits whole-cell DosRST inhibitory activity with an EC_{50} in the submicromolar range.

Following the discovery of the DosRS regulatory system in *Mab*, Belardinelli *et al*. evaluated other synthetic antimalarial peroxides on the *Mab* DosRS system.[p28] Among these, OZ439, a synthetic next-generation derivative of artemisinin, targets the sensor kinase heme of the *Mab* DosS protein and emerged as the most interesting molecule (Figure 2b). Indeed, OZ439 (200 mg/kg) reduced mycobacterial burden in the lungs and liver of an acute severe combined immunodeficiency (SCID) mouse model of *Mab* infection. Also, OZ439 in combination with standard-of-care antibiotics enhanced their efficacy.[p28],[p29]

Serine/threonine protein kinase PknA/PknB/PknG inhibitors

Serine/threonine (Ser/Thr) protein kinases, like tyrosine protein phosphatases, play an important role in the regulation of mycobacterial physiology, particularly virulence. Two of these kinases, PknA and PknB, have been shown to be essential. PknG, in contrast, is not essential for *Mtb* growth *in vitro*, but is fundamental for survival in macrophages and infection in animal models.[p30] The catalytic domain of these bacterial enzymes is homologous to that of eukaryotic protein kinases. Consequently, the number of hits for mycobacterial Ser/Thr protein kinase drug discovery campaigns is expected to be derived from available kinase chemical libraries that share common structural features required for enzyme binding (Figure S1 in the supplementary material online). However, these similarities raise concerns about potential off-target toxicity.[p31],[p32]

PknB was the first *Mtb* Ser/Thr protein kinase to be investigated for its therapeutic potential. The possibility of targeting PknB by small molecules was described in pioneering work by Drews *et al*.,[p33] with 1-(5-isoquinolinesulfonyl)-2-methylpiperazine H7 compound (Figure S1), which is active against both *Mtb* PknB at micromolar concentrations and *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) and *Mycobacterium smegmatis*

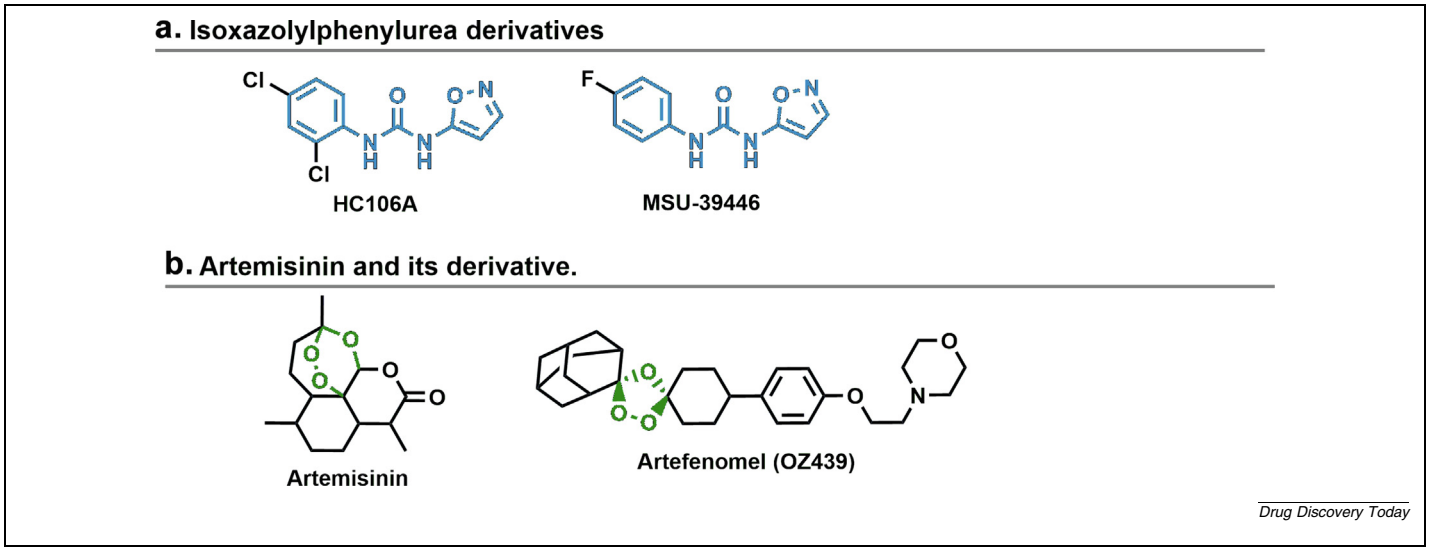

FIGURE 2
Inhibitors of the DosRS–DosST two-component system. **(a)** Isoxazolyl phenylurea compounds targeting the DosS sensor kinase. **(b)** The structure of the antimalarial drug artemisinin and its synthetic derivative OZ439.

growth. However, although several PknA and PknB inhibitors with antimycobacterial activity have been identified, including natural products (NPs) such as K252a (Figure S1), most of them have shown poor selectivity towards mammalian kinases, leading to cytotoxic effects.[p34],[p35]

Nevertheless, in 2004, Walburger *et al.* discovered that another *Mtb* Ser/Thr protein kinase, PknG, plays a role in mycobacterial survival within macrophages by inhibiting phagosome–lysosome fusion.[p36] They identified AX20017 from the Axxima Pharmaceuticals chemical library; this small molecule selectively inhibited PknG activity, with no effect on the other mycobacterial Ser/Thr kinases as well as human kinases (Figure 3a).[p37] However, this hit compound displayed developmental liabilities, such as poor metabolic stability, off-target activity, and heavily patentability, necessitating further fine-tuning. Medicinal chemistry optimization cycles led to the identification of some leads with improved PknG inhibition, which were further optimized. Moreover, the molecule was found to moderately reduce the *Mtb* colony-forming units (CFUs) in macrophages without showing THP-1 toxicity.[p38]

Other molecules that act on PknG include R406,[p39] NU-6027,[p40] RO9021,[p41] and L2W[p42] (Figure 3b).

Zinc metalloprotease-1 inhibitors

Mycobacterial zinc metalloprotease-1 (Zmp1) is an essential enzyme for the intracellular survival and pathogenicity of *Mtb* but its function remains still unclear.[p43] In recent years, significant efforts have been made to develop molecules targeting the mycobacterial zinc metallopeptidase Zmp1. Generally, the design of all zinc-metalloprotease inhibitors implies the use of so-called zinc-binding groups that chelate the catalytic Zn^{2+} within the active site, including thiolate (S^-), carboxylate (COO^-), and hydroxamic acid (HONH-CO) (Figure S2 in the supplementary material online).[p44]

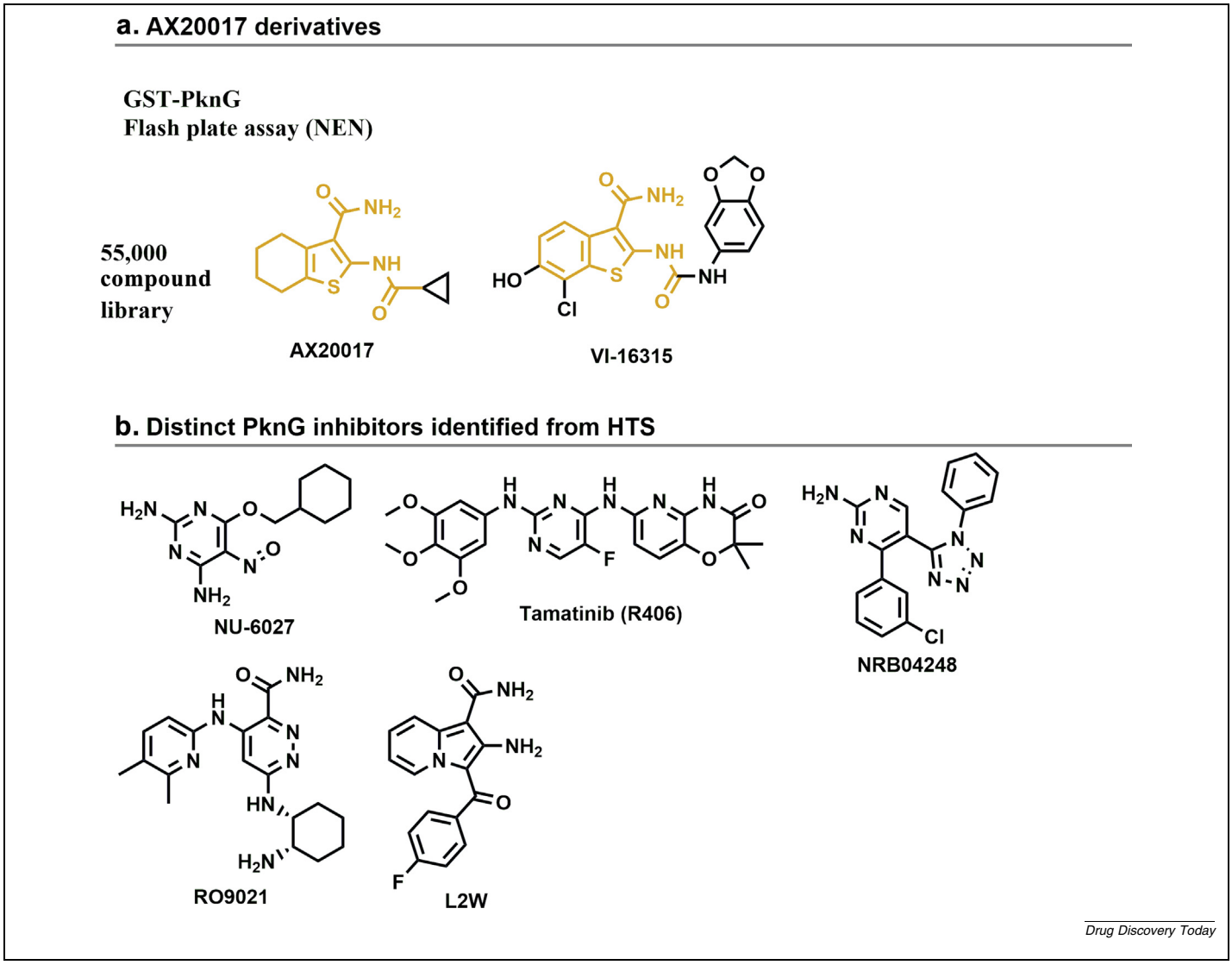

FIGURE 3

Inhibitors of the Ser/Thr protein kinase PknG. **(a)** The structure of the first PknG inhibitor, AX20017, and its derivative, VI-16315. **(b)** Further PknG inhibitors that have been developed. Abbreviation: HTS, high-throughput screening.

The potential for *Mtb* Zmp1 inhibition was evaluated in the pioneering work by the Botta group, which, by combining *in silico* structure-based analysis and biochemical evaluation, identified the weak inhibitor ZTB12. The second round of virtual screening led to the selection of ZTB23, showing K_i in the nanomolar range of concentrations, with the ZTB23(S) enantiomer inhibiting *Mtb* growth in THP-1 infected macrophages, whereas the *R*-enantiomer surprisingly showed no *ex vivo* activity (Figure S2).[p43]

Further studies have been performed by hybridizing well-known zinc-binding moieties, such as hydroxyquinoline and hydroxamate,[p45] thiazolidinedione and hydroxamate (Figure S2),[p46,p47] but all compounds exhibited only modest *ex vivo* activity against *Mtb*. These findings raise questions about the druggability of Zmp1 and its potential as a valuable target for therapeutic application.

Protein tyrosine phosphatase MptpA/MptpB inhibitors

The role of both MptpA and MptpB tyrosine phosphatases in *Mtb* virulence was first revealed in 2000.[p48] They are protein tyrosine phosphatases (PTPs) secreted by *Mtb* into the cytoplasm of macrophages and are required for survival and infection within the host. Because of the fundamental importance of signaling events mediated by PTPs for cell lifespan, the development of inhibitors for these enzymes has received significant attention.[p49] The tyrosyl phosphate (pTyr) residue is a key component in PTP ligand recognition. Thus developing small molecules containing a non-hydrolysable pTyr mimetic is a common approach to designing specific inhibitors.[p49] Typically, most human tyrosine phosphatase inhibitors contain such pTyr mimetics, like phosphonodifluoromethyl phenylalanine (F_2Pmp), salicylic acid, or sulfamic acid, to improve target binding. This structural approach has also been adopted for inhibitors of mycobacterial PTPs as well.[p50]

The possibility of targeting MptpA and MptpB with small molecules was reported five years later by Waldmann and colleagues,[p51] who employed various NP core structures to identify tyrosine phosphatase inhibitors.[p52]

The first evidence that the mycobacterial enzyme MptpB is a druggable target comes from the work of Zhou and colleagues, who evaluated a library of benzofuran salicylic acid-based compounds capable of interacting with both the active and peripheral sites of this enzyme,[p53] and leading to the identification of the first potent inhibitor I-A09 (IC_{50} 1.26 μM) (Figure 4a). Despite its high activity in infected J774A.1 macrophages and the potency against the enzyme, its selectivity (~10-fold) against a panel of human PTPs was relatively modest, requiring further optimization. The parental phenylbenzofuran-5-carboxylic acid core 1 was thus modified by introducing less bulky substituents at position 3,[p54] leading to the compound L01–Z08 (Figure 4a). This compound demonstrated nanomolar potency towards MptpB, improved selectivity against human PTPs, and good efficacy in an *Mtb*-infected J774A.1 macrophage model.[p55] However, the compound was inactive in a guinea pig model of chronic TB infection, probably because of insufficient bioavailability.

Next, Zhang and colleagues identified cefsulodin, a third-generation narrow-spectrum cephalosporin antibiotic active against *P. aeruginosa*, as a hit compound with weak inhibitory activity against the enzyme (IC_{50} 16 μM).[p56] Because of its bulky structure and chemical liabilities, the researchers divided it into three structural moieties to identify the MptpB pharmacophore. Only the α-sulfophenylacetic amide (SPAA) (Figure 4b) fragment exhibited activity (IC_{50} 180 μM); the other two fragments did not show any MptpB inhibition. Guided by the docking data, the amide modification led to the highly selective competitive MtptB inhibitor cpd9 (Figure 4b), with an IC_{50} against MptpB at nanomolar range. Curiously, further fragment-based optimization of SPAA led to the discovery of the competitive inhibitor of MptpA L335–M34 (Figure 4b), which displayed an IC_{50} of 0.16 μM for MptpA and over 32.0 μM for MptpB.[p55] L335–M34 decreased the bacterial load in *Mtb*-infected macrophages, but the therapeutic value in a chronic model of TB infection in guinea pigs was unclear because of the modest reduction in lung CFUs.

More recently, building on scaffolds previously reported as non-hydrolyzable phosphotyrosine (pTyr) mimetics for targeting mammalian PTPs, Ruddraraju and colleagues recognized oxanilic acid as a good starting point for the generation and screening of improved MptpB inhibitors, identifying phenylethynyl-containing compounds (Figure 4c) that exhibited submicromolar potency against the enzyme with more than 4500-fold higher selectivity over mammalian enzymes.[p57] However, no *ex vivo* activity has been reported yet.

Using a similar approach, Beresford *et al*. identified C1 from a focused library of molecules originally developed against human tyrosine phosphatase. This molecule was a modest MptpB inhibitor, with the ability to reduce the mycobacterial burden in J774A.1 macrophages.[p58] Further molecular docking and rational hit optimization led to the identification of C13 (Figure 4d), which showed high activity against MptpB and against *Mtb* in a J774A.1 macrophage infection model.[p59] Moreover, assessment as monotherapy in acute and chronic guinea pig models of TB infection revealed that treatment with C13 was able to reduce the bacterial burden in the lungs. Interestingly, C13 treatment was recently demonstrated to be effective against the NTM *M. avium* by reducing the intracellular burden in RAW264.7 macrophages and increasing the efficacy of bedaquiline and rifampicin in a *G. melonella* infection model.[p60]

Additionally, the use of *in silico* methods has successfully identified new MptpB inhibitors. For instance, Zhang *et al*. (Figure 4e), from a structure-based virtual screening, identified cmpd 15, active against the enzyme and able to reduce intracellular mycobacterial growth in infected J774A.1 macrophages.[p61] SAR investigation of this compound afforded the optimized cmpd 13, which exhibited a 20-fold improvement in the efficacy.[p62]

Secretory acid phosphatase SapM inhibitors

The secreted phosphatase SapM dephosphorylates the phosphatidylinositol-3-phosphate, a regulatory lipid that plays an essential role in phagosomal maturation. It is therefore essential for the pathogenesis of *Mtb*.[p63] To date, only a few articles

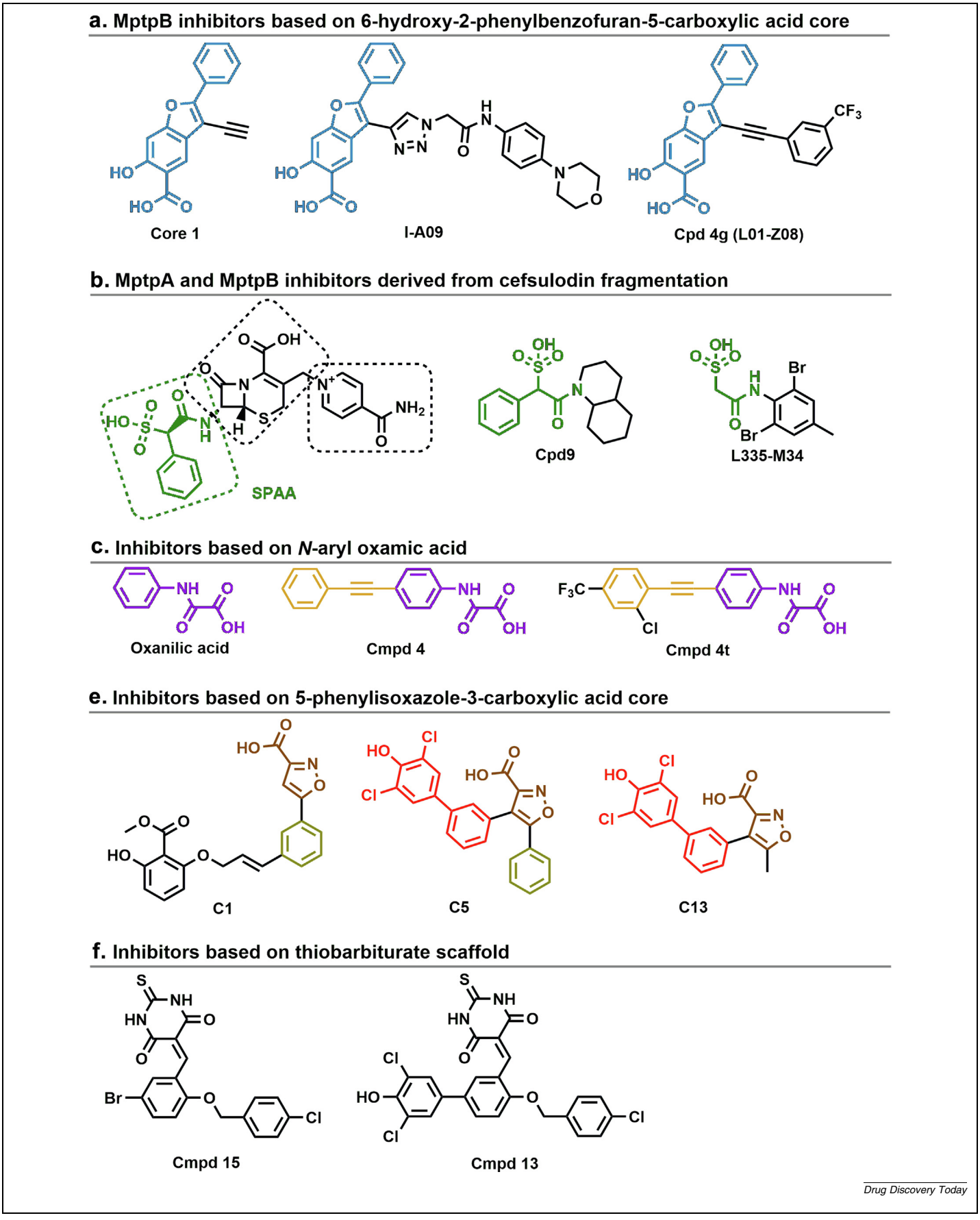

FIGURE 4

MptpA/MptpB inhibitors. **(a)** The structure of benzofuran salicylic acid-based compounds that were initially identified as potent MptpB inhibitors. **(b)** Inhibitors of MptpA and MptpB derived from cefsulodin fragmentation. Further protein tyrosine phosphatase inhibitors are based on **(c)** oxamic acid, **(d)** 5-phenylisoxazole-3-carboxylic acid and **(e)** thiobarbiturate scaffold.

have focused on the discovery and development of SapM inhibitors, which indicate the druggability and clinical importance of this target. Among these, Fernández Soto *et al*. identified tyrphostin AG183 and its isomer (originally developed as inhibitors of epidermal growth factor tyrosine kinase) as good SapM inhibitors, able to inhibit *Mtb* burden in THP-1 macrophages. However, the fact that the tyrphostin family is patented makes further development difficult.[(p64),(p65)]

Concluding remarks and future challenges

The antivirulence approach is the opposite of the traditional antibiotic discovery model: although available drugs target essential functions for bacterial growth, antivirulence compounds affect nonessential bacterial functions. This strategy aims to disarm rather than kill bacteria and diminish their pathogenicity. This approach potentially avoids or minimizes the development of antibiotic resistance because the predicted selection pressure is weaker than that of bactericidal or bacteriostatic compounds. Furthermore, depending on their virulence target and how conserved they are among species, antivirulence compounds have the potential to be species-specific, which would prevent the inhibition of commensal bacteria. However, the fact that antivirulence compounds do not kill pathogens consequently might not clear infections by compromising their clinical application. Nevertheless, combining antibiotics with antivirulence compounds can have synergistic effects and has been shown to be potentially effective in treating infections and limiting the spread of antibiotic resistance.[(p66)] Moreover, compared with antibiotics, the development of antivirulence drugs will likely be more labor-intensive and significantly more costly. There are also no or few standardized, high-throughput assays available to evaluate anti-virulence compounds. This disadvantage slows down early-stage drug discovery and development and makes it more difficult. In 2013, Lewis noted, 'Antivirulence programs were put in place at several large pharmaceutical companies 15–20 years ago but are no longer in existence. This approach continues to be popular in academia, but a compound has yet to reach clinical trials.'[(p67)] Years later, the situation has modestly improved. Big pharma still focus on other therapeutic areas, whereas the development of nontraditional antibiotics is driven by the efforts of academic research institutes and small biotech companies. An analysis by the Working Group for New TB Drugs, a major TB nonprofit organization, revealed that fewer than 10% of all TB preclinical projects aim to develop antivirulence compounds, and none have yet entered clinical trials.[(p2)] There are several interrelated reasons for this situation. First, there are critical gaps in research and development investments, complicated by a lack of cost recovery. Second, although distinct mycobacterial virulence factors have been proven to be targeted by small molecules, the translatability of preclinical discoveries into clinical practice remains unclear. Finally, the clinical implications as well as the integration of such agents into TB drug regimens are also not fully understood and require more complex investigations than traditional antibacterial drug discovery.

In conclusion, although the antivirulence strategy represents a promising and conceptually innovative approach to combating bacterial infections such as TB, its clinical translation remains limited. The current landscape highlights a significant gap between academic interest and pharmaceutical investment, compounded by scientific, economic, and regulatory challenges. Bridging the translational gap is essential to unlock the full potential of antivirulence therapies in the fight against drug-resistant pathogens.

Author contributions

All authors were involved in study conceptualization, searching for literature, drafting the first draft, and reviewing the final draft.

Acknowledgements

We thank Dr Anna Egorova (former employee of the Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences, Russia) for working with an initial version the manuscript as part of her official duties. This research was supported by the Italian Ministry of University and Research (MUR): Design of new precision nanomedicine tools against intracellular mycobacteria pathogens-PRIN20 Pasca (no. 20205B2HZE_003) and by the NextGenerationEUMUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (project no. PE00000007, INF-ACT).

Declarations of interest

No interests are declared.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.drudis.2025.104501.

Data availability

No data was used for the research described in the article.

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