



HAL
open science

Structure-Based Drug Design for Tuberculosis: Challenges Still Ahead

Eduardo Bruch, Stéphanie Petrella, Marco Bellinzoni

► **To cite this version:**

Eduardo Bruch, Stéphanie Petrella, Marco Bellinzoni. Structure-Based Drug Design for Tuberculosis: Challenges Still Ahead. Applied Sciences, 2020, 10 (12), pp.4248. 10.3390/app10124248 . pasteur-03259522

HAL Id: pasteur-03259522

<https://pasteur.hal.science/pasteur-03259522v1>

Submitted on 14 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Review

Structure-Based Drug Design for Tuberculosis: Challenges Still Ahead

Eduardo M. Bruch, Stéphanie Petrella and Marco Bellinzoni * 

Unité de Microbiologie Structurale, Institut Pasteur, CNRS, Université de Paris, F-75015 Paris, France; eduardo.bruch@pasteur.fr (E.M.B.); stephanie.petrella@pasteur.fr (S.P.)

* Correspondence: marco.bellinzoni@pasteur.fr

Received: 30 May 2020; Accepted: 18 June 2020; Published: 20 June 2020



Abstract: Structure-based and computer-aided drug design approaches are commonly considered to have been successful in the fields of cancer and antiviral drug discovery but not as much for antibacterial drug development. The search for novel anti-tuberculosis agents is indeed an emblematic example of this trend. Although huge efforts, by consortiums and groups worldwide, dramatically increased the structural coverage of the *Mycobacterium tuberculosis* proteome, the vast majority of candidate drugs included in clinical trials during the last decade were issued from phenotypic screenings on whole mycobacterial cells. We developed here three selected case studies, i.e., the serine/threonine (Ser/Thr) kinases—protein kinase (Pkn) B and PknG, considered as very promising targets for a long time, and the DNA gyrase of *M. tuberculosis*, a well-known, pharmacologically validated target. We illustrated some of the challenges that rational, target-based drug discovery programs in tuberculosis (TB) still have to face, and, finally, discussed the perspectives opened by the recent, methodological developments in structural biology and integrative techniques.

Keywords: tuberculosis; structure-based drug design; target-based drug design; PknB; PknG; DNA gyrase; antibiotic

1. Introduction

Although the drug discovery process has historically relied on high-throughput screening (HTS) to identify hits to be developed into drug candidates, either on a given target (in most cases, an essential enzyme) or through whole-cell screenings, it still remains an experimentally laborious, expensive, and time-consuming process. Unlike traditional drug discovery, however, drug design is not necessarily based on the screening of large libraries, which is intrinsically a trial and error process, but builds on the available knowledge for a given biological target. A particular, well-known case of drug design is called structure-based drug design (SBDD), which uses the three-dimensional structural knowledge of the target to find or optimize molecules that can bind to the target with high affinity and selectivity. The potential of using structural information for discovering candidate drugs was apparent from the early days of structural biology [1], but it took several years to achieve the first successful examples, i.e., human immunodeficiency virus (HIV) protease inhibitors [2] and carbonic anhydrase inhibitors for the treatment of glaucoma [3].

A three-dimensional model of the target is, therefore, a prerequisite for SBDD. The structure can be obtained experimentally in different ways, in most cases by X-ray crystallography, although nuclear magnetic resonance (NMR) and, more recently, cryo-electron microscopy (cryo-EM) have also attracted attention [4], especially following the spectacular increase in the resolution capabilities of single-particle cryo-EM, which is commonly referred to as the resolution revolution [5,6]. For targets whose experimental structure is elusive, in silico structure prediction is also routinely performed and

can be achieved, whenever suitable models are available, through homology modeling from a closely related homologous protein [7].

The identification of potential drug binding sites can be obvious when ligand-bound target structures are available. However, the capability to detect binding sites for substrates or modulators becomes highly relevant not only for targets for which no ligand-bound three-dimensional structure is available, but also to detect allosteric sites or protein–protein interaction surfaces that might be specifically targeted [8], and for these, dedicated libraries are now available [9]. Considering that binding site identification is not always straightforward, tools have been developed to infer “druggable” pockets from the identification of concave regions—that could accommodate drug-size molecules—by screening for appropriate binding properties, such as volume, hydrophobicity, hydrogen bonding, energy potential, solvent accessibility, and desolvation energy [10].

The search for potential ligands on a given target can then follow two different approaches, commonly referred to as ligand-based drug design (LBDD) or target-based drug design (TBDD). LBDD does not require, a priori, direct structural knowledge of the target, but relies on the identification of hits, usually congeneric compounds, with an established biological effect; hit-to-lead optimization can then proceed through the definition of appropriate chemical descriptors of the series, quantitative structure-activity relationship analysis (QSAR), and pharmacophore modeling [11], all followed by experimental validation. QSAR-based virtual screening approaches, alone or in combination with HTS screenings, can also be used to enlarge the panel of bioactive compounds against the target or pathway of interest, increasing the overall hit rate [12]. TBDD, in contrast, uses the physical-chemical constraints from the target three-dimensional structure, and possibly a well-defined binding pocket, to perform virtual screening of libraries, either of natural or synthetic compounds, usually applying appropriate filters like compliance to Lipinski’s rules or QSAR models, or to design molecules de novo in a step-wise manner. A particular case of de novo molecule design is fragment-based drug design (FBDD), in which low-affinity target binding molecules are identified from appropriate libraries, using a variety of biophysical approaches, and then merged or linked together using the available three-dimensional information to achieve larger binders with improved properties [13,14].

It is common knowledge in the field that although SBDD approaches have proven to be successful for non-transmissible diseases and viral diseases, they have proven to be much less effective in the antibiotic discovery field [15,16]. Unfortunately, tuberculosis represents no exception to this trend [17], despite the considerable progress achieved during the last twenty years in the understanding of the pathogen molecular physiology, starting from the seminal publication of the *M. tuberculosis* genome sequence in 1998 [18], the extensive structural genomics campaigns during the following years [19–21], the advances in understanding host-pathogen interactions and the development of the disease [22,23], and notwithstanding the development of genetic and biochemical tools to allow the in vivo and in vitro validation of targets [24,25].

The purpose of this review was not to provide an exhaustive overview of the capabilities now offered by in silico approaches for antibiotic development against *M. tuberculosis*, already reviewed elsewhere [26,27], nor to make a survey of the current state of structural knowledge of the pathogen proteome and the experimental structures relevant for drug discovery, for which we point the reader to very recent, extensive work [28]. Rather, we focused here on three emblematic case studies of *M. tuberculosis* targets that attracted most efforts for anti-tuberculosis compound development by HTS campaigns, computer-aided, and structure-driven compound identification: the serine/threonine (Ser/Thr) kinases—protein kinase (Pkn)B and PknG—two amongst the most known, supposedly promising new targets offered by the post-genomic era, and the DNA gyrase, the ‘old’ but the well-proven target of fluoroquinolones.

2. Protein Ser/Thr Kinases as Drug Targets

Protein phosphorylation is a well-known, widespread mechanism for signal transduction and regulation of several biological functions. Protein kinases have long been known as major drug

targets [29], especially for the treatment of cancer, where the deregulation of signaling mechanisms is a hallmark of the disease [30]. Protein kinases are not only pharmaceutical targets in cancer chemotherapy but also for the treatment of parasitic infections, ranging from the ones caused by Trypanosomatids or *Leishmania* [31] to malaria [32]. Since the sequence of *M. tuberculosis* H37Rv genome was first reported in 1998 [18], the pathogen has been known to possess eleven genes coding for Hanks-type Ser/Thr kinases [33], named from *pknA* to *pknL* (but no *pknC*), providing one of the first challenges to the paradigm of prokaryotic cell signaling as being entirely driven by two-component systems. One gene coding for a transmembrane Ser/Thr phosphatase, *pstP*, was also identified as lying on the same cluster as *pknA* and *pknB*, forming a putative operon [34], in addition to two genes (*ptpA* and *ptpB*) coding for Tyr phosphatases [35]. Nine out of eleven Ser/Thr kinases (all but PknG and PknK) were predicted to be integral membrane proteins, all sharing the same topology with an N-terminal catalytic domain in which the archetypal Hanks motifs could be identified [33], a single transmembrane segment and a very variable extracellular, C-terminal domain, hypothesized to be acting as a signal sensing domain. The role of such C-terminal domains is, in most cases, still puzzling, although, in some kinases like PknB, it has been suggested to be involved in the kinase activation process, possibly by controlling the kinase oligomerization state as a function of the external signal [36]. Work from Tom Alber's group in Berkeley indeed demonstrated the role of the 'back-to-back' dimerization to promote an active kinase conformation, not only for PknB [37,38] but also for PknD [39], notwithstanding the observation that mutations in the dimerization interface do not abolish kinase activity, as reported by separate groups [37,40]. PknB was also the first *M. tuberculosis* kinase for which the crystal structure of the catalytic domain was described in 2003 [41,42]. Indeed, this first crystal structure confirmed the overall conservation of the bi-lobed protein kinase fold and the initial assumptions about the presence of the known structural features of eukaryotic protein kinases, as predicted by the detection of the Hanks motifs [42], thus underlining the common origin of eukaryotic and prokaryotic kinases. Following these first milestones and the genetic proof of the in vitro essentiality of *pknB* coming both from transposon mutagenesis [43] and targeted studies [44,45], PknB then attracted the attention of tuberculosis (TB) research community as an ideal target for structure-based drug design. The community's interest rise when considering the increasing evidence, over the years, of its role in the control of peptidoglycan synthesis and cell division (recently reviewed in [46]). The first description of the potential use of PknB inhibitors as anti-mycobacterial agents reported the compound H-7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine; Table 1), well before any bacterial protein kinase structure was available [47]. Once the PknB catalytic domain structure was solved, virtual screening carried out on a library of about 40,000 compounds for hits into the PknB adenosine triphosphate-binding (ATP-binding) pocket was performed. The screening led to identifying mitoxantrone, a chemotherapeutic agent known for its DNA intercalating properties, as a sub-micromolar PknB inhibitor [48], similar to staurosporine, K-252-a, and K-252-b (Table 1), who were identified as hits after testing a few commercially available eukaryotic kinase inhibitors [44]. The crystal structure of the PknB-mitoxantrone complex was the first showing the kinase catalytic domain in complex with a non-ATP analog, kinase inhibitor. Two crucial, hydrogen bonding interactions were evidenced between the mitoxantrone hydroxyl groups and main chain atoms from the PknB hinge region that connects the two kinase lobes, i.e., the carbonyl oxygen of Glu93 and the amino group of Val95 [48], opening the way to compound optimization (Figure 1A,B). Several other PknB inhibitors were proposed in the course of the following years, starting from hits developed either from known kinase inhibitors like staurosporine analogs [37], 2-aminopurine and its derivatives, including organometallic compounds [49]), or from hits obtained from HTS on public or proprietary libraries using GarA as the substrate [50–53], or phytocompounds [54] (Table 1). Most of the published work deals with compounds inhibiting the kinase in the micro and sub-micromolar range but with limited activity on mycobacteria. The exception to this trend is IMB-YH-8 (Table 1), a compound that, despite showing an IC₅₀ (half maximal inhibitory concentration) on PknB in the 20 μM range, shows good selectivity for mycobacterial PknB and PknA and a MIC (minimal inhibitory concentration) in

the sub-micromolar range [53]. Compounds highly active on both PknB and PknA have also been reported recently by others in the form of substituted quinazolines, and, for a compound derived from this series (a pyrimidine analog), the crystal structures of the respective complexes with both kinases have been described [55] (Figure 1, Table 1). Both structures underline the common binding mode in the ATP pocket and the crucial interaction with the hinge region (Figure 1B). Despite the inhibition constant for both kinases falls in the nanomolar range, these dual-targeting inhibitors display promising but yet limited antibacterial effect with MIC on *M. tuberculosis* in the lower micromolar range [55]. A different PknB inhibitor for which a crystal structure in complex with the target is available is GSK690693 (Figure 1C, Table 1), also identified through a virtual screening approach on known kinase inhibitors [56]. This compound, member of the imidazopyridine aminofurazans class, also displays conserved features in its binding mode to the PknB hinge region (Figure 1B), and sub-micromolar affinity to the kinase, but no significant antimycobacterial activity on *Mycobacterium smegmatis* or *Mycobacterium bovis* BCG (bacillus Calmette-Guerin). However, the MIC is significantly lowered if the compound is associated with a sub-MIC50 concentration of meropenem, suggesting a synergistic action between PknB inhibitors and β -lactams [56].

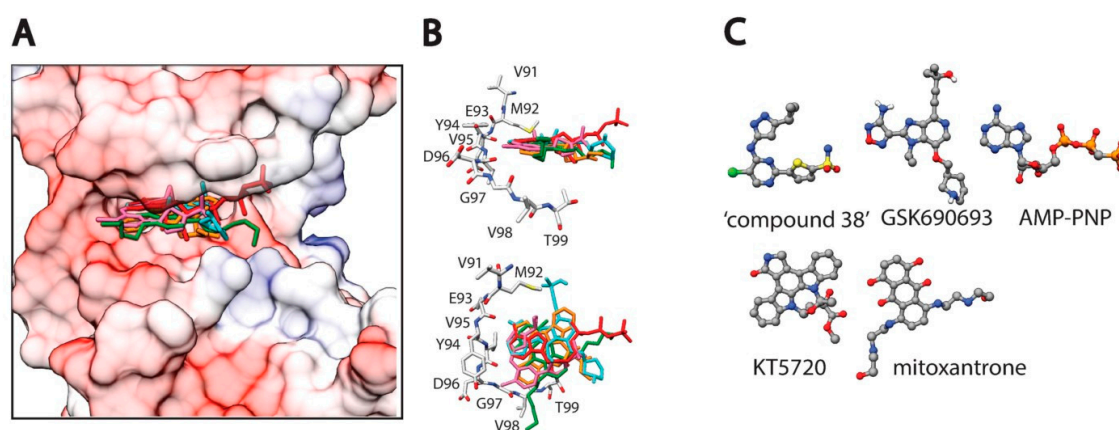


Figure 1. Experimental structures of protein kinase B (PknB)-inhibitor complexes. (A) Electrostatic surface from the X-ray structure of PknB in complex with AMP-PCP (β,γ -methyleneadenosine 5'-triphosphate) in red (pdb: 1O6Y), superimposed with the coordinates of four other PknB complexes with, respectively, mitoxantrone in green (pdb: 2FUM), KT5720 in orange (pdb: 3F69), GSK690693 in cyan (pdb: 5U94), and 'compound 38' in pink (pdb: 6B2P). This compound has been reported as active on both PknA and PknB [55]. (B) Side and top view of the superimposed ligands with the hinge region from the pdb entry 6B2P. Kinase residues are indicated. (C) Ball-and-stick representation of the ligands kept in the same relative binding orientation as in (B) and colored by element.

Table 1. *M. tuberculosis* PknB inhibitors.

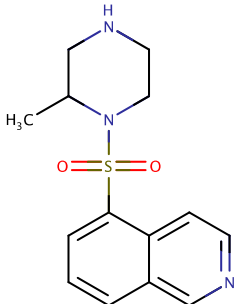
Family	Name	Structure	IC ₅₀ (μ M)	Reference
Isoquinolines	H-7		ND	[47]

Table 1. Cont.

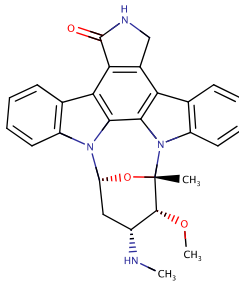
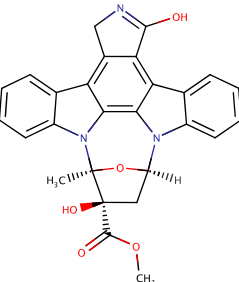
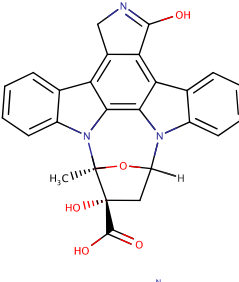
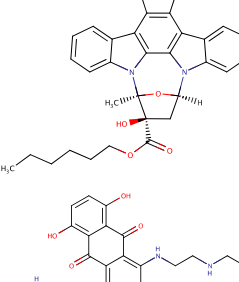
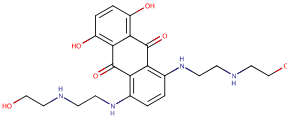
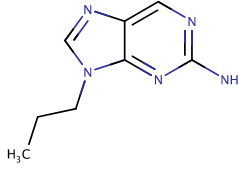
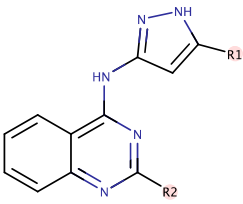
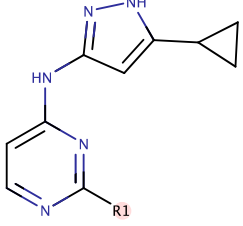
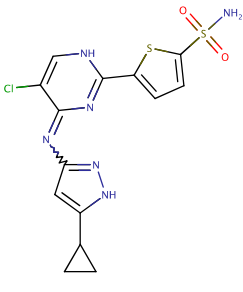
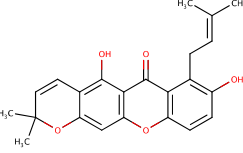
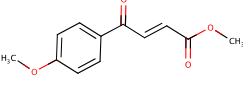
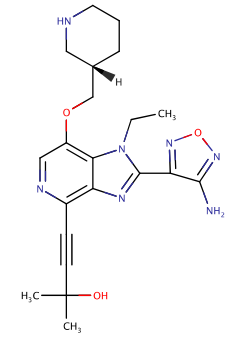
Family	Name	Structure	IC ₅₀ (μM)	Reference
Staurosporine analogues	Staurosporine		0.6	[44]
	K-252-a		0.096	
	K-252-b		0.106	
	KT5720		~1 ^a	[37]
Anthracenediones	Mitoxantrone		0.8	[48]
Aminopurines	2-A9P		1300	[49]

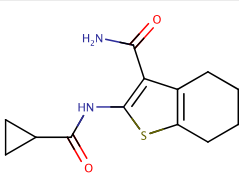
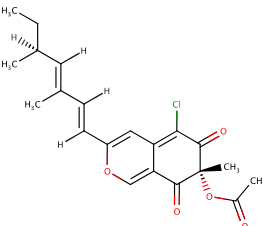
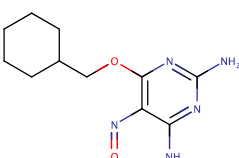
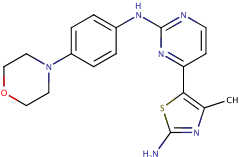
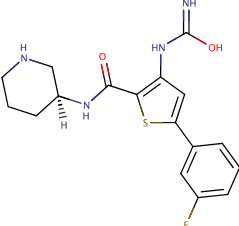
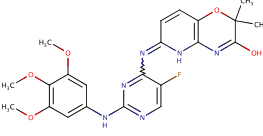
Table 1. Cont.

Family	Name	Structure	IC ₅₀ (μM)	Reference
Quinazolines	Disubstituted series		≤ 1.1	[51]
Pyrimidines	Disubstituted (-R1 also as -NHR1)		≤ 0.4	[51]
	‘Compound 38’		Ki ~ 1 nM	[55]
Phytocompounds	Demethylcalabaxanthone		ND	[54]
4-oxo-crotonic acid derivatives	IMB-YH-8		20.2	[53]
Imidazopyridine aminofurazans	GSK690693		0.34	[56]

ND: not determined. ^a measured on a PknB mutant (M145L, M155V) [37].

On the other hand, another *M. tuberculosis* kinase that attracted as much attention for its potential druggability is PknG, a soluble Ser/Thr kinase that was described, by Jean Pieters and coworkers, as a virulence factor secreted into the human macrophage, where it would inhibit the phagosome-lysosome fusion [57]. Most notably, in the same work, the authors showed that chemical inhibition of PknG by the compound AX20017 (a tetrahydrobenzothiophene identified by HTS and found to inhibit PknG with IC₅₀ in the sub-micromolar range; Table 2) led to the accumulation of *M. tuberculosis* inside lysosomes [57]. Noteworthy, the 2.4 Å resolution crystal structure of an N-terminal truncated form of PknG in complex with AX20017, published later [58], shows a great similarity between the AX20017 binding to PknG and the binding of ATP and mitoxantrone to PknB. Not only the compound occupies the adenine binding pocket but also makes similar interactions with the main chain atoms of residues Glu233 and Val235 (Figure 1). It was only a few years later that further structural work allowed to elucidate the binding mode of ATP to PknG and suggested a regulatory role of the rubredoxin-like domain by a reversible occlusion of the active site entrance [59]. These initial findings paved the way to further structure-based lead optimization and undoubtedly generated excitement for the potential chemical targeting of this kinase, which would allow preventing the arrest of phagosome maturation, directing *M. tuberculosis* to lysosomes. Attempts to develop more potent compounds starting from AX20017 yielded sub-micromolar inhibitors, some of which had no activity in macrophage assays [50], and no other structure of a PknG-inhibitor complex has so far been reported. It is, however, worth noting that, more than ten years later, the role of PknG in arresting the phagosome maturation is still elusive. Although progress has been made in identifying the SecA2 system as responsible for the export of PknG outside *M. tuberculosis* [60,61], and interference by PknG on the host Rab711 signaling pathway has been reported [62], it is largely accepted that several mechanisms, and not a single virulence factor, contribute to the *M. tuberculosis* capability to escape the phagocytic route and survive into macrophages [22,63]. Moreover, an increasing amount of evidence has since validated PknG as a key signaling element in the control of central metabolism, as first shown in *Corynebacterium glutamicum* [64,65], then in mycobacteria [66,67], where genetic and metabolomic evidence point to a role of PknG in regulating the 2-oxoglutarate node according to nutrient availability [68]. Alternative roles of PknG in biofilm formation, adaptation to oxidative stress [69], and hypoxia [70] have also been proposed. Therefore, the observed effects of the lack of PknG on the phagosome-lysosome fusion, and the decreased viability of a *pknG*-deprived *M. tuberculosis* mutant, both in vitro and in the mouse model [71], may not necessarily indicate a direct interference of the kinase on the host signaling pathways but could be ascribed to metabolic alterations caused by the depletion of PknG [68,72]. During the last years, further search for PknG inhibitors has allowed identifying sclerotiorin, an azaphilone derivative isolated from *Penicillium* sp. ZJ27 (Table 2), a known inhibitor of other unrelated enzymes like lipoxygenase, that, despite showing an IC₅₀ on PknG around 76 μM and no inhibition on *M. tuberculosis* growth in vitro, displays capability to partially reduce the growth of *Mycobacterium bovis* BCG inside macrophages, and to enhance the effect of rifampicin [73]. On the other hand, the recently described PknG inhibitor NU-6027 (2,6-diamino-4-cyclohexylmethoxy-5-nitrosopyrimidine; Table 2) was identified from phenotypic screening as having an MIC₉₉ value of 1.56 μM on *M. bovis* BCG and shown to partially inhibit PknG and PknD autophosphorylation in vitro [74]. Another series of four compounds, all inhibiting PknG in the micromolar range, was also recently reported after the screening of an 80-compound, commercially available kinase inhibitor library (Table 2); three of them were found to promote the transfer of mycobacteria to lysosomes, and two to inhibit *M. bovis* BCG growth in macrophages [75]. None of the reported compounds, however, issued from a structure-based screening approach.

Table 2. *M. tuberculosis* PknG inhibitors.

Family	Name	Structure	IC ₅₀ (μM)	Reference
Tetrahydrobenzothiophenes	AX20017		0.39	[57]
			5.5	[75]
Azaphilones	Sclerotiorin		76	[73]
Pyrimidines	NU-6027		ND	[74]
	CYC116		35.1	[75]
Thiophenes	AZD7762		30.3	[75]
Methoxybenzenes	R406 (benzenesulfonate) and its free base R406f (also known as Tamatinib)		8.0 (R406) 16.1 (R406f)	[75]

ND: not determined.

3. DNA Gyrase: A Validated Drug Target

Topoisomerases are enzymes that participate in the overwinding or underwinding of DNA. They are commonly split into two classes, depending on the number of DNA strands cut during a single catalysis cycle: class I topoisomerases, which include eukaryotic topoisomerase I, prokaryotic topoisomerase IIIa, or reverse gyrase among others, and class II topoisomerases, whose members include (but are not restricted to) topoisomerase IV and DNA gyrase [76]. In *M. tuberculosis*, DNA gyrase is a heterotetrameric nanomachine, consisting of two GyrB and two GyrA subunits, and is essential for DNA replication, transcription, and repair in living cells [77].

M. tuberculosis is an exception in the prokaryotic world because of the presence of only one type I and one type II topoisomerase, whereas most other eubacteria have two enzymes of each type. In the canonical situation, DNA gyrase and topoisomerase IV, both type IIA topoisomerases, have distinct specific activities: DNA gyrase removes positive supercoils that accumulate ahead of

replication forks [78], whereas topoisomerase IV decatenates replication intermediates [79]. Thus, in *M. tuberculosis*, DNA gyrase being the unique topoisomerase IIA, it must be able to carry out both activities in vivo [80]. Furthermore, with no other topoisomerase IIA in *M. tuberculosis*, the DNA gyrase is the unique target of antibiotics from the fluoroquinolones (FQ) family (quinolones with higher cellular penetration efficiency), which bind and stabilize the DNA–DNA gyrase complex. Stabilization of this ternary complex leads to the cytotoxic accumulation of double-strand cleaved DNA fragments within the cell, inducing bacterial death [81]. Crystallographic structures of the isolated domain and the cleavage core, either in its *apo* form or co-crystallized with several FQ, brought crucial information to elucidate the mode of binding of these drugs [82–87]. The ratio FQ/DNA–DNA gyrase in the ternary complex is two FQ molecules per complex, with one molecule binding in each cleavage site, wedging between the two ends of the cleaved DNA strands on both halves of the complex, in a drug-binding pocket whose walls are formed by DNA base stacking (Figure 2A) [82]. The emergence of strains with multidrug resistance (MDR) phenotype, or extensively-drug resistance (XDR) phenotype in which drug resistance includes this family of antibiotics, has made improving the activity of this family of drugs by using structural data to be of utmost importance. The main goals of these studies are to increase specific drug/enzyme interactions, but also to find new molecules that inhibit this validated target without cross-resistance with FQ [88].

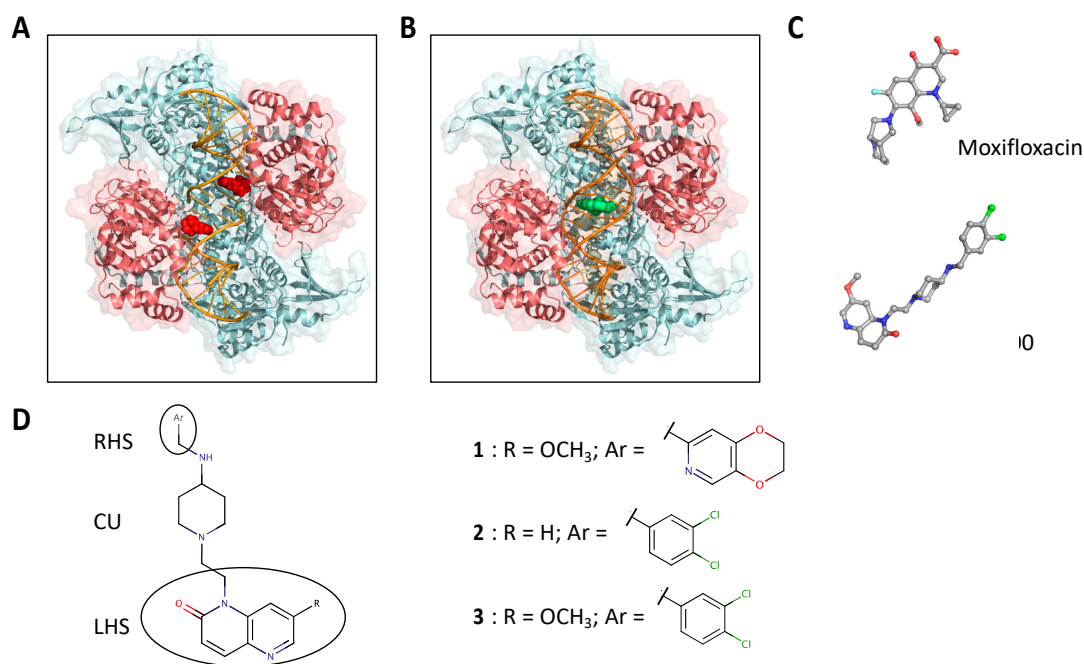


Figure 2. Inhibitors of DNA gyrase. (A) Top view of the X-ray structure of *M. tuberculosis* gyrase cleavage core in complex with DNA and moxifloxacin. Protein is shown in cartoon representation with transparent surfaces, with GyrB in blue and GyrA in pink. DNA is shown in orange, with moxifloxacin as red spheres (pdb: 5BS8). (B) Top view of the model of a complex of GSK000 (green spheres) with *M. tuberculosis* gyrase cleavage core and uncleaved DNA (in orange), based on the crystal structure of GSK299423 with *Staphylococcus aureus* gyrase (pdb: 2XCR). The same representation, as in panel A, was used for the protein. (C) Ball-and-stick representation of the ligands colored by element. (D) Generic chemical structure of the novel bacterial topoisomerase inhibitor (NBTI) series of compounds. Analog 1 is a classical NBTI, whereas analogs 2 and 3 combine the three favorable features for *M. tuberculosis* DNA gyrase inhibitor (MGI).

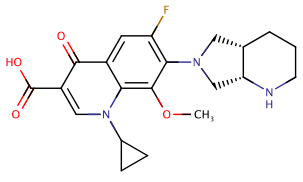
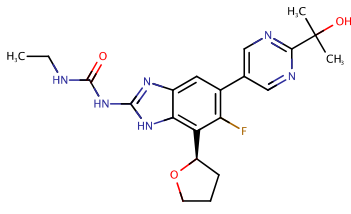
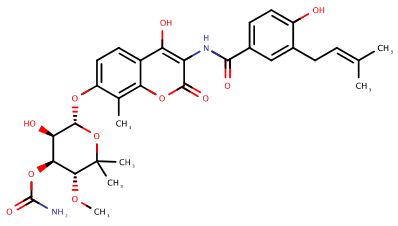
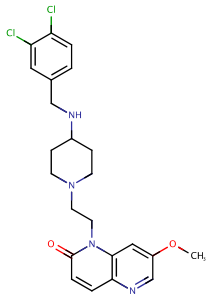
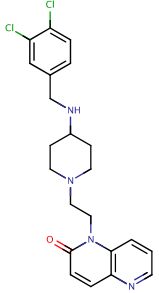
A significant amount of effort has thus been invested in discovering new *M. tuberculosis* gyrase inhibitors, mostly centered on the ATP- and DNA-binding sites, and particularly using in silico methods based on the emerging gyrase structural information, as extensively reviewed by Nagaraja

and coworkers [89]. Although the ATP binding sites have been less successfully exploited as antibacterial targets, with the exception of the natural products, such as coumarins [90,91], it is worth noting that a candidate drug emerged from these studies. SPR720 (Table 3) is an orally bioavailable prodrug of SPR719, an aminobenzimidazole inhibitor of both gyrase and topoisomerase IV. Indeed, this novel class of antimicrobials targets the ATPase subunits of gyrase and topoisomerase IV, and it was optimized using SSBD and structure-activity relationship (SAR) studies of potency against both Gram-positive and some Gram-negative bacterial species [92,93]. Crystal structures of novobiocin (Table 3) bound to *Escherichia coli* GyrB subunit served as a starting point for ligand optimization [94], while further optimization of the metabolic profile led to the identification of SPR720 (formerly VXc-486) [95], whose suitability to be a drug candidate for the treatment of tuberculosis and non-tuberculosis mycobacterial infections was thoroughly evaluated [96,97]. The compound completed Phase I clinical trials in 2019 (study ID NCT03796910, sponsored by Spero Therapeutics, LLC), aimed at evaluating the safety, tolerability, and pharmacokinetics (PK) profile in healthy volunteers. Moreover, an in vivo combination of SPR720 with rifampicin and pyrazinamide (two first-line drugs used in TB treatment) showed comparable efficacy to the combination of three drugs, including moxifloxacin, rifampicin, and pyrazinamide [98].

Work on the DNA-binding site has also been productive, as GlaxoSmithKline (GSK) created a new class of type IIA topoisomerase inhibitors, called novel bacterial topoisomerase inhibitors (NBTIs) (Table 3) [99]. The crystallographic structures of the DNA-bound *Staphylococcus aureus* DNA gyrase with either NBTI or FQ, all obtained at high resolution, revealed that the binding sites for each drug are different [100]. Indeed, the NBTI 'bridges' the DNA and a transient non-catalytic pocket on the two-fold axis at the GyrA dimer interface, remaining close to the active sites and FQs binding sites (Figure 2B). As NBTIs display relatively poor activity against the *M. tuberculosis* DNA gyrase, the antitubercular profiles of 3000 compounds, representative of the chemical diversity of this family, were evaluated by high-throughput phenotypic screenings in vitro and in vivo [101]. MIC determination on *M. tuberculosis* H37Rv showed a high hit rate (68% of compounds with MIC values lower than 10 μ M), with the most potent derivatives matching or even improving the MIC values for currently used TB drugs, including last-generation FQs. Overall, 29% of the compounds had MICs of < 1 μ M, and 18% had MICs of < 0.1 μ M. By using structural data provided by the work of Bax et al., the general structures of these TB-active compounds were divided into three different regions, each of them interacting with one of the three topologically important target-gyrase interacting regions [100]. While a left-hand side (LHS) is responsible for key contacts with the gyrase DNA substrate, a right-hand side (RHS) is embedded into the enzyme, potentially contributing to the protein target selectivity (Figure 2D). Last, a central linker unit (CU) establishes key interactions with the gyrase and offers the opportunity to modulate key physicochemical properties (Figure 2D). These SAR observations helped to schematize synthesis and to rationalize how to balance antimycobacterial potency with oral exposure, safety, and synthetic complexity, leading to the identification of the 7-substituted-1,5-naphthyridin-2-one core as a privileged LHS binder, the N-ethyl-4-aminopiperidines as a linker, and monocyclic aromatic rings with different substitution patterns as the best RHS binding option, in contrast to NBTIs bearing bicyclic rings as the RHS (Figure 2D, analogs 2 and 3) [101]. This work led to the identification of a subclass of naphthyridone/aminopiperidine-containing compounds that displayed activity against *M. tuberculosis*, both in vitro and in the mouse model, known as '*M. tuberculosis* DNA gyrase inhibitors' (MGIs) due to structural and activity differences with respect to NBTIs [101]. More recently, the mechanism of action of two such compounds, i.e., GSK000 and GSK325 (Figure 2C; Table 3), was assessed on the *M. tuberculosis* gyrase, showing that MGIs greatly enhanced DNA cleavage mediated by the bacterial enzyme, but they induced only single-stranded DNA breaks [102]. Their mechanism of action involves stabilizing covalent gyrase-cleaved DNA complexes and appears to suppress the ability of the enzyme to induce double-stranded breaks. Furthermore, these compounds maintained activity against mutant versions of DNA gyrase, bearing the three most commonly observed FQs resistance mutations, but displayed no activity against human topoisomerase II α [102], suggesting good potential

as candidate drugs, especially in the presence of FQ resistance. Modeling studies were carried out by the authors using the crystal structure of the *M. tuberculosis* gyrase cleavage core and the NBTI crystal structure complex of *S. aureus* gyrase [100] (Figure 2B). Most importantly, these studies allowed to confirm that FQs and MGIs do not share the same binding site and bind in a mutually exclusive manner (Figure 2), considerably reducing the risk of developing cross-resistance phenotypes.

Table 3. *M. tuberculosis* DNA gyrase inhibitors.

Family	Name	Structure	IC ₅₀ (μM)	Reference
Fluoroquinolones	Moxifloxacin		1	[102]
			2.5	[101]
SPR	SPR720 (VXc-100, VXc-486)		ND	[92,93]
Coumarins	Novobiocin		0.5	[80]
NBTI	GSK000		0.5	[102]
			5.4	[101]
	GSK325		10.98	[101]

ND: not determined.

4. General Remarks

Several challenges need to be overcome for therapeutic molecules to be active against *M. tuberculosis*. Due to its metabolic adaptability and its known capabilities to occupy different niches inside the human host, from free aerobic bacteria to granulomas, an ideal molecule should be able to act against bacteria in very different, environmentally adapted states. The unique structure and composition of the *M. tuberculosis* cell wall are also well known to act as a barrier for potentially active compounds. To be active against latent and active infections, an ideal drug candidate should thus be able to reach bacteria in all the tubercular lesions and niches inside the human host [103], permeate the mycobacterial cell wall or be actively internalized, and deliver the desired bioactivity in bacteria under different metabolic states. To overcome these limitations and reduce the risk of further resistance development, TB drugs are delivered in combinations, starting from the standard DOTS (directly observed therapy short-course). Drug combinations, however, have to take in due account the bioavailability and pharmacokinetic properties of the single components and their associations, as extensively treated in this same journal issue [104].

In the course of the last ten years, it has become increasingly clear that some of the criteria commonly used to classify a given *M. tuberculosis* biological process, in many cases, an enzymatic step in a pathway, as a 'good' target for drug development, needed to be revised. This is particularly true for in vitro genetic essentiality. Even targets confirmed to be essential by the construction of specific conditional mutants do not necessarily show a good 'druggability', even when highly potent hits become available. The case of PknB is an emblematic example: despite the gene was early shown to be essential for *M. tuberculosis* growth, first by transposon mutagenesis [43], then through attempts to generate a knock-out mutant [44], yet several research groups, including ours, had to face the problem of the lack of correlation between hit potency in vitro and efficacy in vivo [50–52]. Similar issues were also reported for other *M. tuberculosis* kinase inhibitors, in addition to the cross-reactivity sometimes reported towards eukaryotic kinases, and the cytotoxicity shown by some compounds on human cell lines [105]. In addition, although the availability of crystal structures of both PknB and PknG in complex with hits issued from medium-throughput screens (mitoxantrone and AX20017, respectively) has looked as a promising 'proof-of-concept' [48,58], further structure-based work on these kinases has, so far, failed to produce suitable drug candidates. Many reasons might be evoked to explain the lack of *M. tuberculosis* kinase inhibitors that display significant antibacterial activity, ranging from the poor capacity to penetrate the mycobacterial cell and reach their target to the kinase redundancy in *M. tuberculosis* and their, relatively poor, substrate specificity [46], which prompted groups to seek for multiple kinase inhibition [105]. Yet, the main causes should perhaps be looked for in our, still limited, knowledge of mycobacterial molecular physiology and the regulatory networks in *M. tuberculosis*, in which Ser/Thr kinases are actors of a complex interplay [46,106]. Indeed, the activation mechanisms of both PknB and PknG are still a matter of speculation, and changes to *pknB* expression, either as depletion or overexpression, have been reported to alter the bacterial growth significantly [34]. Given the known *M. tuberculosis* metabolic plasticity, whose complexity has only recently started to be elucidated, thanks to the development of genetic and 'omics' tools [107], and the adaptability of the pathogen in the course of infection, it is now largely accepted that genetic essentiality of a putative target in laboratory conditions is not necessarily an indication of chemical vulnerability [15], and even more in clinical conditions. For these reasons, phenotypic screens have increasingly been employed in TB drug development to the detriment of target and structure-based methods, including for lead optimization [15], and the vast majority of candidate TB drugs that have been able to enter clinical trials in the last years were issued from this kind of screens [17,108].

Nevertheless, structure-based and computer-aided drug design maintain a clear potential for the future development of new anti-tubercular drug candidates. For instance, a recent, very promising success of structure-based and fragment-based approaches in TB drug discovery is the development of the so-called ethionamide boosters directed against the EthR repressor, one of which has been shown to be active both in vitro and in vivo [109]. In addition, the recent developments in X-ray

crystallography [110], cryo-EM [4], and integrative structural biology methods [111] will all contribute to increasing the number of tools available to tackle the challenges that lay ahead. These opportunities are well exemplified by the case of mycobacterial DNA gyrase that, considering our capacity to obtain high-resolution structures of an almost full-length form of the *M. tuberculosis* enzyme [112], and the recent, high-resolution cryo-EM structure of *E. coli* gyrase in complex with NBTI [113], let us believe that SBDD will deliver a key contribution to developing new compounds against this 'old' but validated target. High-resolution snapshots of the complete mycobacterial gyrase machinery, especially if in complex with representative members of each known family of inhibitors, might revolutionize our knowledge of this key target and substantially increase chances of improving our therapeutic bullets. For instance, combining crystallographic and cryo-EM data could allow to perform structure-guided drug design to target these flexible complexes and identify new conformations of mycobacterial gyrase that could not, otherwise, be obtained by conventional structural methods. More generally, the integration of biophysical and structural biology data, with the notable contribution of high-resolution EM, will allow the drug discovery pipelines to work on a higher complexity level that was previously not achievable, now looking at targets in their larger biological context (e.g., complexes or cellular compartments). It is, therefore, foreseeable that, despite the technical challenges, target-based and structure-based approaches will have increasing relevance in future drug discovery and will give significant contributions in the search for new tuberculosis drugs.

Author Contributions: All authors contributed to conceptualization, the preparation of the draft, and the review of this work. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by institutional grants from the Institut Pasteur and the CNRS.

Acknowledgments: We are grateful to all our past and present colleagues from the Structural Microbiology Unit, as well as collaborators from the former MM4TB Consortium, for so many fruitful exchanges and insightful discussions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Perutz, M.F. Fundamental research in molecular biology: Relevance to medicine. *Nature* **1976**, *262*, 449–453. [[CrossRef](#)] [[PubMed](#)]
2. Wlodawer, A. Rational approach to AIDS drug design through structural biology. *Annu. Rev. Med.* **2002**, *53*, 595–614. [[CrossRef](#)] [[PubMed](#)]
3. Greer, J.; Erickson, J.W.; Baldwin, J.J.; Varney, M.D. Application of the three-dimensional structures of protein target molecules in structure-based drug design. *J. Med. Chem.* **1994**, *37*, 1035–1054. [[CrossRef](#)] [[PubMed](#)]
4. Renaud, J.-P.; Chari, A.; Ciferri, C.; Liu, W.-T.; Rémy, H.-W.; Stark, H.; Wiesmann, C. Cryo-EM in drug discovery: Achievements, limitations and prospects. *Nat. Rev. Drug. Discov.* **2018**, *17*, 471–492. [[CrossRef](#)] [[PubMed](#)]
5. Kuehlbrandt, W. The Resolution Revolution. *Science* **2014**, *343*, 1443–1444. [[CrossRef](#)]
6. Mitra, A.K. Visualization of biological macromolecules at near-atomic resolution: Cryo-electron microscopy comes of age. *Acta Crystallogr. F Struct. Biol. Commun.* **2019**, *75*, 3–11. [[CrossRef](#)]
7. Muhammed, M.T.; Aki-Yalcin, E. Homology modeling in drug discovery: Overview, current applications, and future perspectives. *Chem. Biol. Drug. Des.* **2019**, *93*, 12–20. [[CrossRef](#)]
8. Kuenemann, M.A.; Sperandio, O.; Labbé, C.M.; Lagorce, D.; Miteva, M.A.; Villoutreix, B.O. In silico design of low molecular weight protein-protein interaction inhibitors: Overall concept and recent advances. *Prog. Biophys. Mol. Biol.* **2015**, *119*, 20–32. [[CrossRef](#)]
9. Bosc, N.; Muller, C.; Hoffer, L.; Lagorce, D.; Bourg, S.; Derviaux, C.; Gourdel, M.-E.; Rain, J.-C.; Miller, T.W.; Villoutreix, B.O.; et al. Fr-PPIChem: An academic compound library dedicated to protein-protein interactions. *ACS Chem. Biol.* **2020**. [[CrossRef](#)]
10. Yuan, Y.; Pei, J.; Lai, L. Binding site detection and druggability prediction of protein targets for structure-based drug design. *Curr. Pharm. Des.* **2013**, *19*, 2326–2333. [[CrossRef](#)]

11. Acharya, C.; Coop, A.; Polli, J.E.; Mackerell, A.D. Recent advances in ligand-based drug design: Relevance and utility of the conformationally sampled pharmacophore approach. *Curr. Comput. Aided. Drug. Des.* **2011**, *7*, 10–22. [[CrossRef](#)] [[PubMed](#)]
12. Neves, B.J.; Braga, R.C.; Melo-Filho, C.C.; Moreira-Filho, J.T.; Muratov, E.N.; Andrade, C.H. QSAR-based virtual screening: Advances and applications in drug discovery. *Front. Pharmacol.* **2018**, *9*, 1275. [[CrossRef](#)] [[PubMed](#)]
13. Davis, B.J.; Hubbard, R.E. Fragment-Based Ligand Discovery. In *Structural Biology in Drug Discovery Methods, Techniques, and Practices*; Renaud, J.-P., Ed.; Wiley: Hoboken, NJ, USA, 2020; Volume 10, pp. 79–98.
14. Mendes, V.; Blundell, T.L. Targeting tuberculosis using structure-guided fragment-based drug design. *Drug Discov. Today* **2017**, *22*, 546–554. [[CrossRef](#)] [[PubMed](#)]
15. Kana, B.D.; Karakousis, P.C.; Parish, T.; Dick, T. Future target-based drug discovery for tuberculosis? *Tuberculosis* **2014**, *94*, 551–556. [[CrossRef](#)] [[PubMed](#)]
16. Payne, D.J.; Gwynn, M.N.; Holmes, D.J.; Pompliano, D.L. Drugs for bad bugs: Confronting the challenges of antibacterial discovery. *Nat. Rev. Drug Discov.* **2007**, *6*, 29–40. [[CrossRef](#)] [[PubMed](#)]
17. Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. The challenge of new drug discovery for tuberculosis. *Nature* **2011**, *469*, 483–490. [[CrossRef](#)]
18. Cole, S.T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S.V.; Eiglmeier, K.; Gas, S.; Barry, C.E.; et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **1998**, *393*, 537–544. [[CrossRef](#)]
19. Holton, S.J.; Weiss, M.S.; Tucker, P.A.; Wilmanns, M. Structure-based approaches to drug discovery against tuberculosis. *Curr. Protein Pept. Sci.* **2007**, *8*, 365–375. [[CrossRef](#)]
20. Terwilliger, T.C.; Park, M.S.; Waldo, G.S.; Berendzen, J.; Hung, L.W.; Kim, C.Y.; Smith, C.V.; Sacchettini, J.C.; Bellinzoni, M.; Bossi, R.; et al. The TB structural genomics consortium: A resource for *Mycobacterium tuberculosis* biology. *Tuberculosis* **2003**, *83*, 223–249. [[CrossRef](#)]
21. Ehebauer, M.T.; Wilmanns, M. The progress made in determining the *Mycobacterium tuberculosis* structural proteome. *Proteomics* **2011**, *11*, 3128–3133. [[CrossRef](#)]
22. Huang, L.; Nazarova, E.V.; Russell, D.G. *Mycobacterium tuberculosis*: Bacterial Fitness within the Host Macrophage. *Microbiol. Spectr.* **2019**, *7*, PMC6459685. [[CrossRef](#)] [[PubMed](#)]
23. Sia, J.K.; Rengarajan, J. Immunology of *Mycobacterium tuberculosis* Infections. *Microbiol. Spectr.* **2019**, *7*, PMC6636855. [[CrossRef](#)] [[PubMed](#)]
24. Woong Park, S.; Klotzsche, M.; Wilson, D.J.; Boshoff, H.I.; Eoh, H.; Manjunatha, U.; Blumenthal, A.; Rhee, K.; Barry, C.E.; Aldrich, C.C.; et al. Evaluating the sensitivity of *Mycobacterium tuberculosis* to biotin deprivation using regulated gene expression. *PLoS Pathog.* **2011**, *7*, e1002264. [[CrossRef](#)]
25. Wei, J.-R.; Krishnamoorthy, V.; Murphy, K.; Kim, J.-H.; Schnappinger, D.; Alber, T.; Sasseti, C.M.; Rhee, K.Y.; Rubin, E.J. Depletion of antibiotic targets has widely varying effects on growth. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4176–4181. [[CrossRef](#)]
26. Aleksandrov, A.; Myllykallio, H. Advances and challenges in drug design against tuberculosis: Application of in silico approaches. *Expert Opin. Drug Discov.* **2019**, *14*, 35–46. [[CrossRef](#)] [[PubMed](#)]
27. Waman, V.P.; Vedithi, S.C.; Thomas, S.E.; Bannerman, B.P.; Munir, A.; Skwark, M.J.; Malhotra, S.; Blundell, T.L. Mycobacterial genomics and structural bioinformatics: Opportunities and challenges in drug discovery. *Emerg. Microbes Infect.* **2019**, *8*, 109–118. [[CrossRef](#)]
28. Pedelacq, J.D.; Nguyen, M.C.; Terwilliger, T.C.; Mourey, L. A Comprehensive Review on *Mycobacterium tuberculosis* Targets and Drug Development from a Structural Perspective. In *Structural Biology in Drug Discovery Methods, Techniques, and Practices*; Renaud, J.-P., Ed.; Wiley: Hoboken, NJ, USA, 2020; Volume 3, pp. 545–566.
29. Cohen, P. Protein kinases—the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.* **2002**, *1*, 309–315. [[CrossRef](#)]
30. Knapp, S. New opportunities for kinase drug repurposing and target discovery. *Br. J. Cancer* **2018**, *118*, 936–937. [[CrossRef](#)]
31. Schoijet, A.C.; Sternlieb, T.; Alonso, G.D. Signal Transduction Pathways as Therapeutic Target for Chagas Disease. *Curr. Med. Chem.* **2019**, *26*, 6572–6589. [[CrossRef](#)]

32. Lima, M.N.N.; Cassiano, G.C.; Tomaz, K.C.P.; Silva, A.C.; Sousa, B.K.P.; Ferreira, L.T.; Tavella, T.A.; Calit, J.; Bargieri, D.Y.; Neves, B.J.; et al. Integrative Multi-Kinase Approach for the Identification of Potent Antiplasmodial Hits. *Front. Chem.* **2019**, *7*, 773. [[CrossRef](#)]
33. Av-Gay, Y.; Everett, M. The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. *Trends Microbiol.* **2000**, *8*, 238–244. [[CrossRef](#)]
34. Kang, C.-M.; Abbott, D.W.; Park, S.T.; Dascher, C.C.; Cantley, L.C.; Husson, R.N. The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: Substrate identification and regulation of cell shape. *Genes Dev.* **2005**, *19*, 1692–1704. [[CrossRef](#)] [[PubMed](#)]
35. Wehenkel, A.; Bellinzoni, M.; Graña, M.; Duran, R.; Villarino, A.; Fernandez, P.; Andre-Leroux, G.; England, P.; Takiff, H.; Cerveñansky, C.; et al. Mycobacterial Ser/Thr protein kinases and phosphatases: Physiological roles and therapeutic potential. *Biochim. Biophys. Acta Proteins Proteom.* **2008**, *1784*, 193–202. [[CrossRef](#)] [[PubMed](#)]
36. Alber, T. Signaling mechanisms of the *Mycobacterium tuberculosis* receptor Ser/Thr protein kinases. *Curr. Opin. Struct. Biol.* **2009**, *19*, 650–657. [[CrossRef](#)]
37. Mieczkowski, C.; Iavarone, A.T.; Alber, T. Auto-activation mechanism of the *Mycobacterium tuberculosis* PknB receptor Ser/Thr kinase. *EMBO J.* **2008**, *27*, 3186–3197. [[CrossRef](#)]
38. Lombana, T.N.; Echols, N.; Good, M.C.; Thomsen, N.D.; Ng, H.-L.; Greenstein, A.E.; Falick, A.M.; King, D.S.; Alber, T. Allosteric activation mechanism of the *Mycobacterium tuberculosis* receptor Ser/Thr protein kinase, PknB. *Structure* **2010**, *18*, 1667–1677. [[CrossRef](#)]
39. Greenstein, A.E.; Echols, N.; Lombana, T.N.; King, D.S.; Alber, T. Allosteric activation by dimerization of the PknD receptor Ser/Thr protein kinase from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **2007**, *282*, 11427–11435. [[CrossRef](#)]
40. Wagner, T.; Andre-Leroux, G.; Hindie, V.; Barilone, N.; Lisa, M.-N.; Hoos, S.; Raynal, B.; Vulliez-Le Normand, B.; O'Hare, H.M.; Bellinzoni, M.; et al. Structural insights into the functional versatility of an FHA domain protein in mycobacterial signaling. *Sci. Signal.* **2019**, *12*, eaav9504. [[CrossRef](#)]
41. Young, T.A.; Delagoutte, B.; Endrizzi, J.A.; Falick, A.M.; Alber, T. Structure of *Mycobacterium tuberculosis* PknB supports a universal activation mechanism for Ser/Thr protein kinases. *Nat. Struct. Biol.* **2003**, *10*, 168–174. [[CrossRef](#)]
42. Ortiz-Lombardía, M.; Pompeo, F.; Boitel, B.; Alzari, P.M. Crystal structure of the catalytic domain of the PknB serine/threonine kinase from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **2003**, *278*, 13094–13100. [[CrossRef](#)]
43. Sasseti, C.M.; Boyd, D.H. Genes required for mycobacterial growth defined by high density mutagenesis. *Mol. Microbiol.* **2003**, *48*, 77–84. [[CrossRef](#)] [[PubMed](#)]
44. Fernandez, P.; Saint-Joanis, B.; Barilone, N.; Jackson, M.; Gicquel, B.; Cole, S.T.; Alzari, P.M. The Ser/Thr protein kinase PknB is essential for sustaining mycobacterial growth. *J. Bacteriol.* **2006**, *188*, 7778–7784. [[CrossRef](#)] [[PubMed](#)]
45. Chawla, Y.; Upadhyay, S.; Khan, S.; Nagarajan, S.N.; Forti, F.; Nandicoori, V.K. Protein kinase B (PknB) of *Mycobacterium tuberculosis* is essential for growth of the pathogen in vitro as well as for survival within the host. *J. Biol. Chem.* **2014**, *289*, 13875. [[CrossRef](#)] [[PubMed](#)]
46. Bellinzoni, M.; Wehenkel, A.M.; Duran, R.; Alzari, P.M. Novel mechanistic insights into physiological signaling pathways mediated by mycobacterial Ser/Thr protein kinases. *Genes Immun.* **2019**, *20*, 383–393. [[CrossRef](#)] [[PubMed](#)]
47. Drews, S.J.; Hung, F.; Av-Gay, Y. A protein kinase inhibitor as an antimycobacterial agent. *FEMS Microbiol. Lett.* **2001**, *205*, 369–374. [[CrossRef](#)] [[PubMed](#)]
48. Wehenkel, A.; Fernandez, P.; Bellinzoni, M.; Catherinot, V.; Barilone, N.; Labesse, G.; Jackson, M.; Alzari, P.M. The structure of PknB in complex with mitoxantrone, an ATP-competitive inhibitor, suggests a mode of protein kinase regulation in mycobacteria. *FEBS Lett.* **2006**, *580*, 3018–3022. [[CrossRef](#)]
49. Bais, V.S.; Mohapatra, B.; Ahamad, N.; Boggaram, S.; Verma, S.; Prakash, B. Investigating the inhibitory potential of 2-Aminopurine metal complexes against serine/threonine protein kinases from *Mycobacterium tuberculosis*. *Tuberculosis* **2018**, *108*, 47–55. [[CrossRef](#)]
50. Székely, R.; Waczek, F.; Szabadkai, I.; Németh, G.; Hegymegi-Barakonyi, B.; Eros, D.; Szokol, B.; Pato, J.; Hafenbradl, D.; Satchell, J.; et al. A novel drug discovery concept for tuberculosis: Inhibition of bacterial and host cell signalling. *Immunol. Lett.* **2008**, *116*, 225–231. [[CrossRef](#)]

51. Chapman, T.M.; Boulou, N.; Buxton, R.S.; Chugh, J.; Loughheed, K.E.A.; Osborne, S.A.; Saxty, B.; Smerdon, S.J.; Taylor, D.L.; Whalley, D. Substituted aminopyrimidine protein kinase B (PknB) inhibitors show activity against *Mycobacterium tuberculosis*. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3349–3353. [[CrossRef](#)]
52. Loughheed, K.E.A.; Osborne, S.A.; Saxty, B.; Whalley, D.; Chapman, T.; Boulou, N.; Chugh, J.; Nott, T.J.; Patel, D.; Spivey, V.L.; et al. Effective inhibitors of the essential kinase PknB and their potential as anti-mycobacterial agents. *Tuberculosis* **2011**, *91*, 277–286. [[CrossRef](#)]
53. Xu, J.; Wang, J.-X.; Zhou, J.-M.; Xu, C.-L.; Huang, B.; Xing, Y.; Wang, B.; Luo, R.; Wang, Y.-C.; You, X.-F.; et al. A novel protein kinase inhibitor IMB-YH-8 with anti-tuberculosis activity. *Sci. Rep.* **2017**, *7*, 5093–5110. [[CrossRef](#)] [[PubMed](#)]
54. Appunni, S.; Rajisha, P.M.; Rubens, M.; Chandana, S.; Singh, H.N.; Swarup, V. Targeting PknB, an eukaryotic-like serine/threonine protein kinase of *Mycobacterium tuberculosis* with phytomolecules. *Comput. Biol. Chem.* **2017**, *67*, 200–204. [[CrossRef](#)] [[PubMed](#)]
55. Wang, T.; Bemis, G.; Hanzelka, B.; Zuccola, H.; Wynn, M.; Moody, C.S.; Green, J.; Locher, C.; Liu, A.; Gao, H.; et al. Mtb PKNA/PKNB Dual Inhibition Provides Selectivity Advantages for Inhibitor Design To Minimize Host Kinase Interactions. *ACS Med. Chem. Lett.* **2017**, *8*, 1224–1229. [[CrossRef](#)] [[PubMed](#)]
56. Wlodarchak, N.; Teachout, N.; Beczkiewicz, J.; Procknow, R.; Schaenzer, A.J.; Satyshur, K.; Pavelka, M.; Zuercher, W.; Drewry, D.; Sauer, J.-D.; et al. In Silico Screen and Structural Analysis Identifies Bacterial Kinase Inhibitors which Act with β -Lactams To Inhibit Mycobacterial Growth. *Mol. Pharm.* **2018**, *15*, 5410–5426. [[CrossRef](#)] [[PubMed](#)]
57. Walburger, A.; Koul, A.; Ferrari, G.; Nguyen, L.; Prescianotto-Baschong, C.; Huygen, K.; Klebl, B.; Thompson, C.; Bacher, G.; Pieters, J. Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. *Science* **2004**, *304*, 1800–1804. [[CrossRef](#)] [[PubMed](#)]
58. Scherr, N.; Honnappa, S.; Kunz, G.; Mueller, P.; Jayachandran, R.; Winkler, F.; Pieters, J.; Steinmetz, M.O. Structural basis for the specific inhibition of protein kinase G, a virulence factor of *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 12151–12156. [[CrossRef](#)]
59. Lisa, M.-N.; Gil, M.; Andre-Leroux, G.; Barilone, N.; Duran, R.; Biondi, R.M.; Alzari, P.M. Molecular Basis of the Activity and the Regulation of the Eukaryotic-like S/T Protein Kinase PknG from *Mycobacterium tuberculosis*. *Structure* **2015**, *23*, 1039–1048. [[CrossRef](#)]
60. van der Woude, A.D.; Stoop, E.J.M.; Stiess, M.; Wang, S.; Ummels, R.; van Stempvoort, G.; Piersma, S.R.; Cascioferro, A.; Jiménez, C.R.; Houben, E.N.G.; et al. Analysis of SecA2-dependent substrates in *Mycobacterium marinum* identifies protein kinase G (PknG) as a virulence effector. *Cell Microbiol.* **2014**, *16*, 280–295. [[CrossRef](#)]
61. Zulauf, K.E.; Sullivan, J.T.; Braunstein, M. The SecA2 pathway of *Mycobacterium tuberculosis* exports effectors that work in concert to arrest phagosome and autophagosome maturation. *PLoS Pathog.* **2018**, *14*, e1007011. [[CrossRef](#)]
62. Pradhan, G.; Shrivastva, R.; Mukhopadhyay, S. Mycobacterial PknG targets the Rab711 signaling pathway to inhibit phagosome-lysosome fusion. *J. Immunol.* **2018**, *201*, 1421–1433. [[CrossRef](#)]
63. Bussi, C.; Gutierrez, M.G. *Mycobacterium tuberculosis* infection of host cells in space and time. *FEMS Microbiol. Rev.* **2019**, *43*, 341–361. [[CrossRef](#)] [[PubMed](#)]
64. Schultz, C.; Niebisch, A.; Gebel, L.; Bott, M. Glutamate production by *Corynebacterium glutamicum*: Dependence on the oxoglutarate dehydrogenase inhibitor protein OdhI and protein kinase PknG. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 691–700. [[CrossRef](#)] [[PubMed](#)]
65. Niebisch, A.; Kabus, A.; Schultz, C.; Weil, B.; Bott, M. Corynebacterial protein kinase G controls 2-oxoglutarate dehydrogenase activity via the phosphorylation status of the OdhI protein. *J. Biol. Chem.* **2006**, *281*, 12300–12307. [[CrossRef](#)] [[PubMed](#)]
66. Ventura, M.; Rieck, B.; Boldrin, F.; Degiacomi, G.; Bellinzoni, M.; Barilone, N.; Alzaidi, F.; Alzari, P.M.; Manganelli, R.; O’Hare, H.M. GarA is an essential regulator of metabolism in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **2013**, *90*, 356–366.
67. O’Hare, H.M.; Duran, R.; Cerveñansky, C.; Bellinzoni, M.; Wehenkel, A.M.; Pritsch, O.; Obal, G.; Baumgartner, J.; Vialaret, J.; Johnsson, K.; et al. Regulation of glutamate metabolism by protein kinases in mycobacteria. *Mol. Microbiol.* **2008**, *70*, 1408–1423. [[CrossRef](#)]
68. Rieck, B.; Degiacomi, G.; Zimmermann, M.; Cascioferro, A.; Boldrin, F.; Lazar-Adler, N.R.; Bottrill, A.R.; Le Chevalier, F.; Frigui, W.; Bellinzoni, M.; et al. PknG senses amino acid availability to control metabolism and virulence of *Mycobacterium tuberculosis*. *PLoS Pathog.* **2017**, *13*, e1006399. [[CrossRef](#)]

69. Wolff, K.A.; de la Peña, A.H.; Nguyen, H.T.; Pham, T.H.; Amzel, L.M.; Gabelli, S.B.; Nguyen, L. A redox regulatory system critical for mycobacterial survival in macrophages and biofilm development. *PLoS Pathog.* **2015**, *11*, e1004839. [[CrossRef](#)]
70. Khan, M.Z.; Bhaskar, A.; Upadhyay, S.; Kumari, P.; Rajmani, R.S.; Jain, P.; Singh, A.; Kumar, D.; Bhavesh, N.S.; Nandicoori, V.K. Protein kinase G confers survival advantage to *Mycobacterium tuberculosis* during latency-like conditions. *J. Biol. Chem.* **2017**, *292*, 16093–16108. [[CrossRef](#)]
71. Cowley, S.; Ko, M.; Pick, N.; Chow, R.; Downing, K.J.; Gordhan, B.G.; Betts, J.C.; Mizrahi, V.; Smith, D.A.; Stokes, R.W.; et al. The *Mycobacterium tuberculosis* protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth in vivo. *Mol. Microbiol.* **2004**, *52*, 1691–1702. [[CrossRef](#)]
72. Chao, J.; Wong, D.; Zheng, X.; Poirier, V.; Bach, H.; Hmama, Z.; Av-Gay, Y. Protein kinase and phosphatase signaling in *Mycobacterium tuberculosis* physiology and pathogenesis. *Biochim. Biophys. Acta Proteins Proteom.* **2010**, *1804*, 620–627. [[CrossRef](#)]
73. Chen, D.; Ma, S.; He, L.; Yuan, P.; She, Z.; Lu, Y. Sclerotiorin inhibits protein kinase G from *Mycobacterium tuberculosis* and impairs mycobacterial growth in macrophages. *Tuberculosis* **2017**, *103*, 37–43. [[CrossRef](#)] [[PubMed](#)]
74. Kidwai, S.; Bouzeyen, R.; Chakraborti, S.; Khare, N.; Das, S.; Priya Gosain, T.; Behura, A.; Meena, C.L.; Dhiman, R.; Essafi, M.; et al. NU-6027 Inhibits Growth of *Mycobacterium tuberculosis* by Targeting Protein Kinase D and Protein Kinase G. *Antimicrob. Agents Chemother.* **2019**, *63*, 39. [[CrossRef](#)] [[PubMed](#)]
75. Kanehiro, Y.; Tomioka, H.; Pieters, J.; Tatano, Y.; Kim, H.; Iizasa, H.; Yoshiyama, H. Identification of Novel Mycobacterial Inhibitors Against Mycobacterial Protein Kinase G. *Front. Microbiol.* **2018**, *9*, 1517. [[CrossRef](#)] [[PubMed](#)]
76. Schoeffler, A.J.; Berger, J.M. DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. *Quart. Rev. Biophys.* **2008**, *41*, 41–101. [[CrossRef](#)] [[PubMed](#)]
77. Gellert, M.; Mizuuchi, K.; O’Dea, M.H.; Nash, H.A. DNA gyrase: An enzyme that introduces superhelical turns into DNA. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 3872–3876. [[CrossRef](#)]
78. Sissi, C.; Palumbo, M. In front of and behind the replication fork: Bacterial type IIA topoisomerases. *Cell. Mol. Life Sci.* **2010**, *67*, 2001–2024. [[CrossRef](#)]
79. Peng, H.; Marians, K.J. Decatenation activity of topoisomerase IV during oriC and pBR322 DNA replication in vitro. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8571–8575. [[CrossRef](#)]
80. Aubry, A.; Fisher, L.M.; Jarlier, V.; Cambau, E. First functional characterization of a singly expressed bacterial type II topoisomerase: The enzyme from *Mycobacterium tuberculosis*. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 158–165. [[CrossRef](#)]
81. Hooper, D.C.; Jacoby, G.A. Topoisomerase Inhibitors: Fluoroquinolone Mechanisms of Action and Resistance. *Cold Spring Harb. Perspect. Med.* **2016**, *6*, a025320. [[CrossRef](#)]
82. Blower, T.R.; Williamson, B.H.; Kerns, R.J.; Berger, J.M. Crystal structure and stability of gyrase–fluoroquinolone cleaved complexes from *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 1706–1713. [[CrossRef](#)]
83. Fu, G.; Wu, J.; Liu, W.; Zhu, D.; Hu, Y.; Deng, J.; Zhang, X.-E.; Bi, L.; Wang, D.-C. Crystal structure of DNA gyrase B’ domain sheds lights on the mechanism for T-segment navigation. *Nucleic Acids Res.* **2009**, *37*, 5908–5916. [[CrossRef](#)] [[PubMed](#)]
84. Tretter, E.M.; Schoeffler, A.J.; Weisfield, S.R.; Berger, J.M. Crystal structure of the DNA gyrase GyrA N-terminal domain from *Mycobacterium tuberculosis*. *Proteins* **2010**, *78*, 492–495. [[CrossRef](#)] [[PubMed](#)]
85. Agrawal, A.; Roue, M.; Spitzfaden, C.; Petrella, S.; Aubry, A.; Hann, M.; Bax, B.; Mayer, C. *Mycobacterium tuberculosis* DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion. *Biochem. J.* **2013**, *456*, 263–273. [[CrossRef](#)] [[PubMed](#)]
86. Bouige, A.; Darmon, A.; Piton, J.; Roue, M.; Petrella, S.; Capton, E.; Forterre, P.; Aubry, A.; Mayer, C. *Mycobacterium tuberculosis* DNA gyrase possesses two functional GyrA-boxes. *Biochem. J.* **2013**, *455*, 285–294. [[CrossRef](#)] [[PubMed](#)]
87. Piton, J.; Petrella, S.; Delarue, M.; Andre-Leroux, G.; Jarlier, V.; Aubry, A.; Mayer, C. Structural Insights into the Quinolone Resistance Mechanism of *Mycobacterium tuberculosis* DNA Gyrase. *PLoS ONE* **2010**, *5*, e12245. [[CrossRef](#)]

88. World Health Organization. Global Tuberculosis Report 2019. Available online: https://www.who.int/tb/publications/global_report/en/ (accessed on 19 June 2020).
89. Nagaraja, V.; Godbole, A.A.; Henderson, S.R.; Maxwell, A. DNA topoisomerase I and DNA gyrase as targets for TB therapy. *Drug Discov. Today* **2017**, *22*, 510–518. [[CrossRef](#)]
90. Vanden Broeck, A.; McEwen, A.G.; Chebaro, Y.; Potier, N.; Lamour, V. Structural Basis for DNA Gyrase Interaction with Coumermycin A1. *J. Med. Chem.* **2019**, *62*, 4225–4231. [[CrossRef](#)]
91. Mizuuchi, K.; O’Dea, M.H.; Gellert, M. DNA gyrase: Subunit structure and ATPase activity of the purified enzyme. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 5960–5963. [[CrossRef](#)]
92. Grossman, T.H.; Bartels, D.J.; Mullin, S.; Gross, C.H.; Parsons, J.D.; Liao, Y.; Grillot, A.-L.; Stamos, D.; Olson, E.R.; Charifson, P.S.; et al. Dual targeting of GyrB and ParE by a novel aminobenzimidazole class of antibacterial compounds. *Antimicrob. Agents Chemother.* **2007**, *51*, 657–666. [[CrossRef](#)]
93. Charifson, P.S.; Grillot, A.-L.; Grossman, T.H.; Parsons, J.D.; Badia, M.; Bellon, S.; Deininger, D.D.; Drumm, J.E.; Gross, C.H.; LeTiran, A.; et al. Novel dual-targeting benzimidazole urea inhibitors of DNA gyrase and topoisomerase IV possessing potent antibacterial activity: Intelligent design and evolution through the judicious use of structure-guided design and structure-activity relationships. *J. Med. Chem.* **2008**, *51*, 5243–5263. [[CrossRef](#)]
94. Holdgate, G.A.; Tunncliffe, A.; Ward, W.H.; Weston, S.A.; Rosenbrock, G.; Barth, P.T.; Taylor, I.W.; Pauptit, R.A.; Timms, D. The entropic penalty of ordered water accounts for weaker binding of the antibiotic novobiocin to a resistant mutant of DNA gyrase: A thermodynamic and crystallographic study. *Biochemistry* **1997**, *36*, 9663–9673. [[CrossRef](#)] [[PubMed](#)]
95. Grillot, A.-L.; Le Tiran, A.; Shannon, D.; Krueger, E.; Liao, Y.; O’Dowd, H.; Tang, Q.; Ronkin, S.; Wang, T.; Waal, N.; et al. Second-generation antibacterial benzimidazole ureas: Discovery of a preclinical candidate with reduced metabolic liability. *J. Med. Chem.* **2014**, *57*, 8792–8816. [[CrossRef](#)] [[PubMed](#)]
96. Brown-Elliott, B.A.; Rubio, A.; Wallace, R.J., Jr. In Vitro Susceptibility Testing of a Novel Benzimidazole, SPR719, against Nontuberculous Mycobacteria. *Antimicrob. Agents Chemother.* **2018**, *62*, 545. [[CrossRef](#)] [[PubMed](#)]
97. Locher, C.P.; Jones, S.M.; Hanzelka, B.L.; Perola, E.; Shoen, C.M.; Cynamon, M.H.; Ngwane, A.H.; Wiid, I.J.; van Helden, P.D.; Betoudji, F.; et al. A Novel Inhibitor of Gyrase B Is a Potent Drug Candidate for Treatment of Tuberculosis and Nontuberculosis Mycobacterial Infections. *Antimicrob. Agents Chemother.* **2015**, *59*, 1455–1465. [[CrossRef](#)]
98. Shoen, C.M.; DeStefano, M.; Pucci, M.; Cynamon, M.H. Evaluating the Sterilizing Activity of SPR720 in Combination Therapy against Mycobacterium tuberculosis Infection in Mice. In Proceedings of the Conference of ASM Microbes 2019, San Francisco, CA, USA, 20–24 June 2019.
99. Coates, W.J.; Gwynn, M.N.; Hatton, I.K.; Masters, P.J.; Pearson, N.D.; Rahman, S.S.; Slocombe, B.; Warrack, J.D.; SmithKline Beecham Ltd. Quinoline Derivatives as Antibacterials. European Patent EP1051413, 4 June 2003.
100. Bax, B.D.; Chan, P.F.; Eggleston, D.S.; Fosberry, A.; Gentry, D.R.; Gorrec, F.; Giordano, I.; Hann, M.M.; Hennessy, A.; Hibbs, M.; et al. Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* **2010**, *466*, 935–940. [[CrossRef](#)]
101. Blanco, D.; Perez-Herran, E.; Cacho, M.; Ballell, L.; Castro, J.; González del Río, R.; Lavandera, J.L.; Remuiñán, M.J.; Richards, C.; Rullas, J.; et al. Mycobacterium tuberculosis Gyrase Inhibitors as a New Class of Antitubercular Drugs. *Antimicrob. Agents Chemother.* **2015**, *59*, 1868–1875. [[CrossRef](#)]
102. Gibson, E.G.; Blower, T.R.; Cacho, M.; Bax, B.; Berger, J.M.; Osheroff, N. Mechanism of Action of Mycobacterium tuberculosis Gyrase Inhibitors: A Novel Class of Gyrase Poisons. *ACS Infect. Dis.* **2018**, *4*, 1211–1222. [[CrossRef](#)]
103. Tanner, L.; Denti, P.; Wiesner, L.; Warner, D.F. Drug permeation and metabolism in Mycobacterium tuberculosis: Prioritising local exposure as essential criterion in new TB drug development. *IUBMB Life* **2018**, *70*, 926–937. [[CrossRef](#)]
104. Van Wijk, R.C.; Ayoun Alsoud, R.; Lennernäs, H.; Simonsson, U.S.H. Model-Informed Drug Discovery and Development Strategy for the Rapid Development of Anti-Tuberculosis Drug Combinations. *Appl. Sci.* **2020**, *10*, 2376. [[CrossRef](#)]
105. Mori, M.; Sammartino, J.C.; Costantino, L.; Gelain, A.; Meneghetti, F.; Villa, S.; Chiarelli, L.R. An Overview on the Potential Antimycobacterial Agents Targeting Serine/Threonine Protein Kinases from Mycobacterium tuberculosis. *Curr. Top. Med. Chem.* **2019**, *19*, 646–661. [[CrossRef](#)]

106. Priscic, S.; Husson, R.N. *Mycobacterium tuberculosis* Serine/Threonine Protein Kinases. *Microbiol. Spectr.* **2014**, *2*. [[CrossRef](#)] [[PubMed](#)]
107. Ehrst, S.; Schnappinger, D.; Rhee, K.Y. Metabolic principles of persistence and pathogenicity in *Mycobacterium tuberculosis*. *Nat. Rev. Microbiol.* **2018**, *16*, 496–507. [[CrossRef](#)] [[PubMed](#)]
108. Sala, C.; Hartkoorn, R.C. Tuberculosis drugs: New candidates and how to find more. *Future Microbiol.* **2011**, *6*, 617–633. [[CrossRef](#)] [[PubMed](#)]
109. Villemagne, B.; Machelart, A.; Tran, N.C.; Flipo, M.; Moune, M.; Leroux, F.; Piveteau, C.; Wohlkönig, A.; Wintjens, R.; Li, X.; et al. Fragment-Based Optimized EthR Inhibitors with in Vivo Ethionamide Boosting Activity. *ACS Infect. Dis.* **2020**, *6*, 366–378. [[CrossRef](#)]
110. Maveyraud, L.; Mourey, L. Protein X-ray Crystallography and Drug Discovery. *Molecules* **2020**, *25*, 1030. [[CrossRef](#)]
111. Rout, M.P.; Sali, A. Principles for Integrative Structural Biology Studies. *Cell* **2019**, *177*, 1384–1403. [[CrossRef](#)]
112. Petrella, S.; Capton, E.; Raynal, B.; Giffard, C.; Thureau, A.; Bonneté, F.; Alzari, P.M.; Aubry, A.; Mayer, C. Overall Structures of *Mycobacterium tuberculosis* DNA Gyrase Reveal the Role of a *Corynebacteriales* GyrB-Specific Insert in ATPase Activity. *Structure* **2019**, *27*, 579–589.e5. [[CrossRef](#)]
113. Vanden Broeck, A.; Lotz, C.; Ortiz, J.; Lamour, V. Cryo-EM structure of the complete *E. coli* DNA gyrase nucleoprotein complex. *Nat. Commun.* **2019**, *10*, 4935. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).